



Special Issue Reprint

Advanced Biotechnologies for Water and Wastewater Treatment, 2nd Edition

Edited by
Yung-Tse Hung, Tsuyoshi Imai, Rehab O. Abdel Rahman, Issam A. Al-Khatib
and Qaisar Mahmood

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Guest Editors

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Preface

Water and wastewater management remain among the most critical challenges facing global sustainability, public health, and environmental protection. Rapid population growth, industrialization, and climate change have intensified pressure on water resources, necessitating innovative and effective treatment solutions. In this context, biotechnology has emerged as a transformative tool, offering environmentally friendly and efficient approaches to pollutant removal, resource recovery, and system optimization. This reprint of Special Issue, *Advanced Biotechnologies for Water and Wastewater Treatment*, 2nd Edition, brings together cutting-edge research and comprehensive reviews that highlight recent advances in biological, chemical, and hybrid treatment technologies. The contributions in this reprint reflect the integration of molecular biology, nanotechnology, and data-driven approaches such as artificial intelligence to improve treatment performance and sustainability. Topics covered include microbial processes, advanced oxidation, membrane technologies, and resource recovery systems. Collectively, these works demonstrate the multidisciplinary nature of modern water treatment research and provide valuable insights for researchers, engineers, and policymakers. We hope this reprint will stimulate further innovation and collaboration in advancing sustainable water and wastewater treatment technologies worldwide.

**Yung-Tse Hung, Tsuyoshi Imai, Rehab O. Abdel Rahman, Issam A. Al-Khatib, and
Qaisar Mahmood**
Guest Editors

Editorial

Advanced Biotechnologies for Water and Wastewater Treatment, 2nd Edition

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1. Introduction

The use of biotechnology in water and wastewater treatment has been advancing rapidly in recent years, offering innovative solutions to urgent environmental challenges. Biological processes are essential for bioremediation, allowing the breakdown of organic pollutants and nutrient recovery. Recent innovations in genome editing have enhanced microbial resilience and specificity for pollutant removal [1]. Fungal biotechnology, such as engineered *Aspergillus niger*, has shown promise in removing heavy metals such as hexavalent chromium from wastewater [2]. Biogenic palladium nanoparticles have accelerated the degradation of persistent antibiotics and reduced pharmaceutical wastewater pollution [3]. Broader engineering innovations have improved scalability and cost-effectiveness in wastewater treatment systems [4]. Microalgae-based technologies offer the benefits of purification of wastewater and biomass valorization [5]. These developments in recent years indicate a shift toward incorporating molecular biology, nanotechnology, and environmental engineering to improve water and wastewater treatment. This Special Issue is the continuation of a previous Special Issue on “Advanced Biotechnologies for Water and Wastewater Treatment” [6].

2. Summary of Contributions

This Special Issue includes ten papers—six original research articles and four review papers—covering a wide range of biotechnological innovations in water and wastewater treatment processes. Each paper presents novel strategies aimed at enhancing the efficiency, resilience, and sustainability of water and wastewater treatment processes.

Gebrehiwet and Krishnamurthy (C1) investigated abiotic and redox processes in wastewater systems, studying lactate oxidation under circumneutral anaerobic conditions with varied electron acceptors. The results indicated the role of ferric iron and light in influencing dissolved inorganic carbon formation, advancing the understanding of abiotic redox mechanisms relevant to wetland systems in wastewater treatment.

Mahmood et al. (C2) used artificial intelligence to analyze anaerobic sulfide oxidation bioreactors, identifying the sulfide-to-nitrite ratio and hydraulic retention time as key

operational parameters concerning sulfur and nitrogen removal. Their work combined data-driven modeling with biological wastewater treatment process optimization.

Mallah et al. (C3) developed antifouling nanocomposite membranes using polyvinylidene fluoride and cellulose acetate for membrane bioreactor systems. The composite membranes demonstrated improved hydrophilicity and reduced fouling, illustrating their potential for improving wastewater treatment efficiency.

Makara et al. (C4) studied a Fenton-based process for the treatment of meat and bone meal wastewater, examining the influence of $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ ratios on microbiological communities. The process effectively reduced microbial loads, illustrating the disinfection and oxidation capacity of advanced Fenton treatment for high-strength industrial wastewaters.

Merabti et al. (C5) evaluated hybrid coagulation–membrane filtration systems for soap-industry wastewater treatment. The optimized integration of ferric chloride coagulation and membrane polishing accomplished high reductions in COD and turbidity, demonstrating a cost-effective and sustainable approach for industrial wastewater treatment.

Keffala et al. (C6) conducted a case study on a combined Oxylag and high-rate algal pond system (COHRAP) implemented at a Tunisian university campus. The study assessed nutrient removal, biomass production, and compliance with reuse regulations, illustrating COHRAP as a promising low-energy option for sustainable wastewater reuse.

Four comprehensive review papers complement these research studies. Wang et al. (C7) provided a systematic review on ferrate-based advanced oxidation processes, clarifying the multiple roles of Fe(VI) species and highlighting strategies for optimizing oxidation pathways. Gonçalves et al. (C8) reviewed agricultural residue-derived biosorbents, demonstrating innovative thermal and chemical activation techniques to enhance pollutant removal. Hossain et al. (C9) reviewed lignocellulosic waste-derived nanomaterials, linking their multifunctional capabilities to circular bioeconomy principles. As a fourth review paper, Guliy and Bunin (C10) provided an extensive review of optical sensor systems for antibiotic detection in water, expanding the scope of biosensing technologies.

3. Conclusions

This second edition of this Special Issue illustrates the important role of biotechnology in water and wastewater treatment. The contributions in this volume, spanning redox chemistry, artificial intelligence, and advanced nanomaterials, demonstrate the multidisciplinary nature of water and wastewater treatment research. The integration of biological, chemical, and data-driven methodologies continues to strengthen water and wastewater treatment efficiency, enhance system resilience, and minimize the effects of environmental pollution. The editors of this Special Issue would like to thank all of the authors involved in the project, and encourage further scientific activity in this area.

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Optical Sensor Systems for Antibiotic Detection in Water Solutions

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Abstract

Antibiotics are persistent organic pollutants that pose a serious problem for water resources, ultimately having a detrimental effect on human and animal health. The most important aspect of controlling and preventing the spread of antibiotics and their degradation products is continuous screening and monitoring of environmental samples. Optical sensing technologies represent a large group of sensors that allow short-term detection of antibiotics in non-laboratory settings. This article reviews the advances in optical sensing systems (colorimetric, fluorescent, surface-enhanced Raman spectra-based, surface plasmon resonance-based, localized surface plasmon resonance-based, photonic crystal-based, fiber optic, molecularly imprinted polymer-based and electro-optical platforms) for the detection of antibacterial drugs in water. Special attention is paid to the evaluation of the analytic characteristics of optical sensors for the analysis of antibiotics. Particular attention is paid to electro-optical sensing and to the unique possibility of its use in antibiotic determination. Potential strategies are considered for amplifying the recorded signals and improving the performance of sensor systems. The main trends in optical sensing for antibiotic analysis and the prospects for the commercial application of optical sensors are described.

Keywords: antibiotics; detection systems; optical sensors; molecular imprinting

1. Introduction

Evaluation of pollution of water resources by antibacterial drugs and their metabolic products is vitally important, because it allows monitoring and degradation of toxic substrates and assessment of possible consequences. Today, the entry of antimicrobial drugs into water is almost inevitable. Undoubtedly, drugs have brought and continue to bring strong assistance in the fight against severe infectious diseases, because they prevent pathological processes in animals and contribute to the long-term preservation of food products during transportation, thereby improving the quality of human life [1].

Under environmental influences, antibiotics alter bacterial populations and their activity in sedimentary water, thus affecting biodegradation, nutrient cycling, and water quality. Epidemiological studies have shown that the misuse of antimicrobials can become a serious problem for human health and the environment [2,3]. By disrupting physiological, metabolic, or genetic pathways, toxic substances can kill, temporarily disable, or permanently harm people, animals, and plants. These include insufficient amounts of every chemical, irrespective of where it comes from or how it is made. According to

recent research, even brief exposure to certain harmful compounds can result in learning difficulties, cancer, infertility, and other severe health issues [4].

The overuse of antibiotics has led to the emergence of antibiotic-resistant microbes, which can contaminate food, enter the food chain, and ultimately penetrate the human body, in which they can cause a variety of health problems.

Monitoring of antimicrobials at various stages of industrial and processing processes, in effluents and wastewater, and in industrial, agricultural, and urban areas has recently become necessary owing to the growing concerns about the toxicity of these substances to the environment and the food industry. Antibiotics are determined by standard microbiological, analytic, and biosensor methods. Microbiological methods of analysis are historically the first and are still used today to determine the content of antibiotics in biological environments. They are highly sensitive but take much more time. Because their broad specificity does not allow individual antibiotics to be identified, they are used mainly for quality control. Enzymatic methods are fairly fast (analysis time is about 20 min) and are based on the specific inhibition of the activity of certain enzymes in the presence of antibiotics [5–7].

The two categories of analytic techniques for antibiotic detection are screening and confirmatory.

- For detection of analyte concentrations, confirmatory techniques use mass spectrometry and liquid chromatography. Another detection method is to combine liquid chromatography with UV or electrophoresis-based techniques.
- The primary purpose of screening is to make semi-quantitative measurements. This method is used because it is less likely to produce false-positive results, simple to use, rapid, affordable, and highly selective [8].

Traditionally, antibiotics are determined by microbiological methods; chromatography, including high-performance liquid chromatography and chromatography–mass spectrometry; stripping voltammetry; electroanalytical determination with modified electrodes; immunological approaches, including the enzyme-linked immunosorbent assay (ELISA); and spectrophotometric, fluorimetric, and chemiluminescent methods. High-performance liquid chromatography, gas chromatography, and mass spectrometry are used widely in the laboratory owing to their high sensitivity and accuracy [9,10]. However, these methods are often limited by complex sample preparation, long analysis times, and expensive equipment, which makes them unsuitable for rapid field studies. Moreover, the environmental risk associated with antibiotic residues primarily depends on their bioavailability (the fraction that can be absorbed by organisms). Only bioavailable antibiotics pose a hazard to organisms and contribute to the spread of resistance. Traditional methods aimed at total extraction may overestimate environmental risks by identifying bioinaccessible fractions. Thus, there is an urgent need to develop rapid, cost-effective, and environmentally friendly detection methods that can specifically measure the bioavailability of antibiotics.

In antibiotic determination, it is often necessary to analyze a fairly large number of samples. To this end, biosensor methods for rapid analysis are being intensely developed. Biosensor systems are highly sensitive and selective, provide rapid results, and can work outside the laboratory. However, biosensors have several limitations associated with both the stability of operation and the sterilization of samples [5,11–14].

Optical systems that use various types of photodetectors with visual analysis deserve special attention. Despite the variety of available optical methods for the visual determination of antimicrobial drugs in water bodies, general strategies for their application in biosensorics have not been fully reviewed. Here, we provide detailed information on the use of biosensors for antibiotic determination with visual analysis results and we show the prospects for their application in the environmental monitoring of water resources.

2. Antibiotics and Their Role in Environmental Pollution

Antibiotic pollution has become one of the most serious global environmental and public health issues. The irrational use and disposal of antibiotics has led to their widespread penetration into water, soil, and sediments, posing serious risks to human health and ecosystem stability [15,16].

Antibacterial medications and their metabolites are present in ecosystems, particularly in aquatic environments, as a result of the ever-increasing use of antibiotics [17]. Food, groundwater, and even drinking water have been found to contain antibiotic residues and metabolites [16]. Figure 1 shows the primary routes of antibiotic entry into groundwater [18]. Antibiotics are present in aquatic environments at ng/L to µg/L [19].

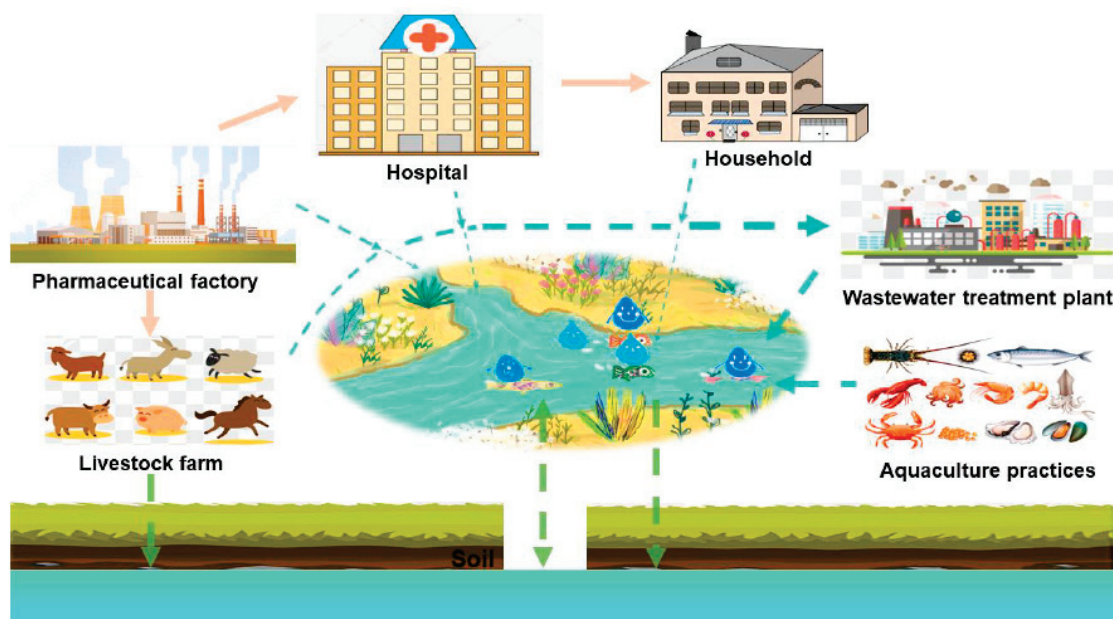


Figure 1. Sources of antibiotics in groundwater (Reprinted from [18]. Creative Commons CC-BY license).

The world's leading consumer of antibiotics is China (about 45% of the world's total antibiotic consumption). In the United States, antibiotics are widespread in groundwater in most regions of the country. The antimicrobials sulfadiazine and sulfamethazine have been detected in groundwater at up to 360 and 1100 ng/L, respectively [20]. In Europe and Africa, concentrations of some antibiotics exceeded 10 and 50 µg/L, and clarithromycin concentrations in Austrian waters reached 12,000 ng/L. In the Asia-Pacific region, concentrations as high as 450 µg/L have been recorded [21,22].

Mashile et al. [23] presented the dynamics of sulfonamides and β -blockers in African and Asian water bodies for the past five years and showed that sulfonamide concentrations are high in Kenya (13,800 ng/L) and in Vietnam (3508 ng/L).

During comprehensive 2-year monitoring (2015 and 2016), 17 of the 53 antibiotics analyzed were detected at least once in wastewater treatment plants in 7 European countries (Portugal, Spain, Ireland, Cyprus, Germany, Finland, and Norway). Figure 2 shows the main groups of antibiotics.

The different antimicrobials that belong to these groups include cephalexin, clindamycin, metronidazole, ampicillin, tetracycline, enrofloxacin, ofloxacin, orbifloxacin, azithromycin, clarithromycin, sulfapyridine, sulfamethoxazole, trimethoprim, nalidixic acid, pipemidic acid, oxolinic acid, cephalexin, clindamycin, ampicillin, and tetracycline. The northern nations (Norway, Finland, and Germany) and Cyprus had low overall antibi-

otic concentrations in wastewater, whereas Ireland and the southern nations (Portugal and Spain) had the highest average values. Three antibiotics—ciprofloxacin, azithromycin, and cephalixin—were proposed as indicators of antibiotic pollution, because they occasionally endanger the environment [24].

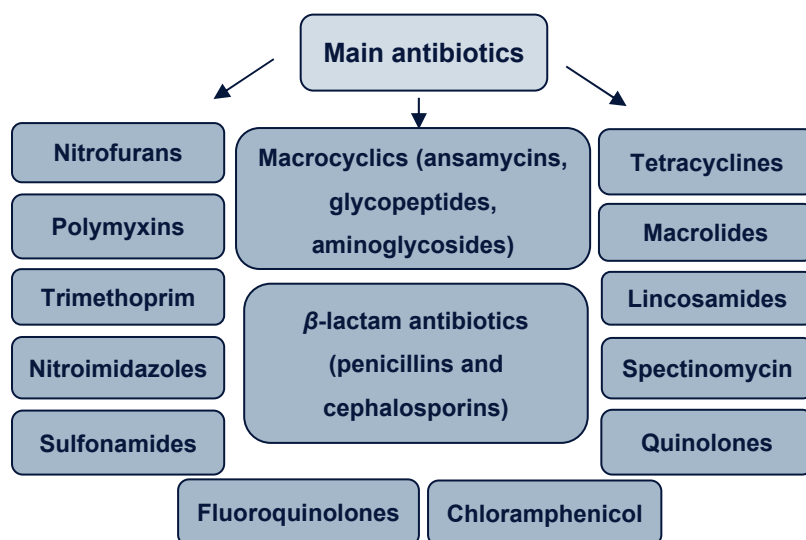


Figure 2. Main groups of antibiotics.

On the basis of 10-year research, Zeng et al. [18] identified 47 types of antibiotics (mainly fluoroquinolones and sulfonamides) that harm the biological environment of ground water and induce the production of resistance genes. Also discussed were the origins, migration, and transformation of antibiotics, pollution levels, and potential hazards to the biological environment in groundwater systems.

Twenty most commonly used antibiotics, including tetracyclines, fluoroquinolones, sulfonamides, and macrolides, were found at 212–4035 ng/L in reclaimed water and at 19–1270 ng/L in groundwater, according to the data from a nationwide survey on water pollution assessment in China (in various humid, semi-humid, and semi-arid regions) [25].

Of particular concern is the development of antibiotic resistance in microbial cells, which can be triggered by even sublethal drug concentrations in the environment. The rapidly evolving antibiotic resistance is becoming a global crisis, because the range of microbial resistance to antibiotics in clinical settings expands and possibilities of developing new antibiotics are reduced [26–29]. At less than 10 g/L, some antibiotics present in surface waters affect microbial cells. Antibiotics (such as ciprofloxacin) that are most prevalent in streams and rivers at these quantities are the strongest. Although prokaryotes may not be killed by sublethal quantities, laboratory investigations have shown that they can change the makeup of single-cell communities and boost bacterial resistance [21].

Antibiotics are often found in surface water and wastewater, usually at 0.01 to 1.0 µg/L [30]. They may persist for several months and cannot be completely removed by traditional disinfection technologies during water purification for drinking water. Relatively little information is available on the antibiotic content of drinking water. Quinolones, chloramphenicols, sulfonamides, and macrolides appear the main antibiotics present at high concentrations in drinking water, with the highest concentration being that of ciprofloxacin (up to 679.7 ng/L) [31].

The overuse and abuse of antibiotics leads to life-threatening consequences in the human body, including allergies, hepatotoxicity, blood disorders, kidney toxicity, nerve toxicity, hearing loss, and increased skin sensitivity [32]. Most antibiotics are excreted as parent drugs or metabolites in feces or urine. On entering the environment, they contribute

to soil and water pollution owing to their complex molecular composition. Antibiotic pollution is exacerbated by waste from pharmaceutical factories, hospitals, and livestock farms. Antibiotic residues in water sources and the environment strongly contribute to the development of resistance in certain microorganisms. Moreover, excessive antibiotic residues in water sources pose serious threats to human health, which include childhood obesity, gastrointestinal and reproductive disorders, bone marrow toxicity, mutagenicity, anaphylactic shock, and cancer [33].

The main consequences of infection with antibiotic-resistant bacteria are: increased duration of illness, increased risk of mortality or invasiveness of the disease, and increased healthcare costs [34,35].

Direct contact with animal blood, saliva, milk, semen, feces, or urine can spread antimicrobial resistance across the food chain. A more complicated method of transmission is indirect contact, which occurs when contaminated eggs, meat, and dairy products are consumed. It is crucial to keep in mind that food trade, foreign travel, and population increase can all contribute to the global spread of antimicrobial resistance through the food chain. When meat, vegetables, and fish are cooked in a variety of bacterial conditions, large amounts of antibiotics are employed. Additionally, this causes resistant bacteria to arise [36].

Owing to concerns about the development of antibiotic resistance, the use of antibiotics in animal feed as growth promoters is banned in the European Union. Therefore, food producers use biocides to control and remove colonies of microorganisms that cause spoilage and disease [37].

The ranking list of bacterial pathogens and their population risk were revised by the World Health Organization (WHO) in 2024 [38]. Antibiotic resistance is predicted to overtake all other causes of death in humans by 2050 if prompt action is not taken. The population-weighted average use of systemic antibiotics in the European Union (EU) in 2023 (community and hospital sectors combined) increased by 1% over 2019. In 2023, the EU's population-weighted average consumption share of WHO Access antibiotics was 61.5% (country range, 41.7–75.1%), which was 3.5% below the 2030 target of over 65% and 0.4% higher than in 2019. The need to increase efforts to stop the overuse and misuse of antibiotics at all levels of healthcare is highlighted by the continuous increase in the use of WHO reserve and broad-spectrum antibiotics [22]. One of the tactics used to prevent the spread of antibiotics in the environment and reduce antibiotic resistance is the global monitoring and detection of antibiotics in the environment, especially in water resources.

Food and drinking water safety is a critical aspect of the food supply chain, as it determines the quality of the final product and, consequently, its acceptance by consumers. The Food Safety Management Program adopts a new strategy for food safety that takes into account every facet of food processing. Accidental or inadvertent microbiological or chemical contamination of a product during the production process is a typical contamination risk. However, the prospect of adulteration or purposeful contamination, which is more difficult to detect, is usually not taken into account by food and drinking water safety procedures. Antibiotic residues in food are becoming a major risk and an illustration of food fraud. They have been discovered in processed foods that are openly sold on the market as well as in a number of common products, most of which are derived from animals.

Antibiotic residues in food have become a serious hazard and an example of food fraud. Antibiotic residues are present in several everyday products, primarily of animal origin, and have also been found in processed foods sold openly on the market [39]. Therefore, research is active into antibiotic determining methods.

The effective monitoring of antibiotic concentrations requires rapid, accurate, and reliable detection methods. These needs are met by sensor systems such as optical biosensors, which will be covered in what follows.

3. Biosensors for Antibiotic Detection

Antibiotics can be successfully analyzed by biosensor techniques. Biosensors are analytic instruments based on a biological sensing element (bioreceptor) complexed with a physical transducer [40–45].

The first biosensor was developed in 1950 by the American biochemist L.L. Clark and is the most well-known example of a commercial biosensor that is still in use for detecting blood oxygen levels (a Clark electrode, or oxygen electrode). This blood glucose biosensor breaks down blood glucose by using the glucose oxidase enzyme. The primary phases of development of the many generations of biosensors currently available on the market are described in [46].

In the first kind of biosensor, glucose oxidation leads to an electric reaction. For a better response, the second type of sensor incorporates specific buffers between the sensor and the reaction. In the third form, there is no middleman engaged; instead, the sensor alone initiates a reaction. With the introduction of groundbreaking technology in the late 1980s, biosensors saw a surge in popularity. Third- and fourth-generation sensors emerged as a result, enabling real-time monitoring of human physiological indicators [46].

With the development of modern technologies, no-label biosensors become increasingly widespread, thanks to which it is possible to screen for interactions with the substrate and obtain information on the presence of antibiotics in the sample being studied [47,48]. This type of sensor has only one sensitive component, and this greatly simplifies the analysis scheme and reduces the time of research. Such systems decrease the economic costs of research by reducing the number of necessary reagents, and they allow quantitative measurements in real time [49,50].

Optical, (piezo)electric, and (micro)mechanical transducers hold promise for the label-free detection of the interaction of a sensor component with the substrate being analyzed. The use of these transducers is supplemented by methods based on the surface plasmon resonance (SPR) [50], surface Raman spectroscopy (SERS) [51–53], quartz crystal microbalance [54], and micro cantilever sensors [50,55]. In what follows, we discuss the main optical sensor systems for antibiotic detection.

4. Optical Sensor Systems for Antibiotic Detection

Optical sensors use the optical concept of signal measurement by transforming light beams into electric signals. They determine the actual light level and transform it into a format that the device can understand. A light source, a measuring device, and an optical sensor are the usual components of an optical sensor. The sensor is often connected to an electric trigger that responds to signal changes in the sensor. The light sensor functions as a photoelectric trigger, increasing or lowering the electric output signal in response to a change. Signals in fiber optic cables or integrated optical circuits can be selectively switched between circuits thanks to an optical switch. In addition to electro-optical and magneto-optical effects, an optical switch can also function mechanically.

In optical biosensors, the analytic signal is not caused by the chemical interaction of the component being determined with the sensitive element. Instead, it is caused by the measured physical variables, including the intensity of absorption and reflection of light, the intensity of luminescence of the object, and other variables. The fundamental phenomena underlying the operation of optical sensors with visual recording of results are as follows:

- Absorption (the ability of a substance to absorb optical radiation).
- Reflection (when a light flux falls on the interface between two media, and part of its radiation is reflected back).
- Luminescence (the glow of a substance that occurs after it absorbs excitation energy, which is excess radiation, as compared with the thermal radiation of the body at a given temperature).
- Photoluminescence (the emission of photons by a substance that occurs when the substance is excited by electromagnetic radiation in the ultraviolet, visible, and infrared wavelength ranges).

Photoluminescence is characterized by absorption and luminescence spectra, polarization, energy yield (the ratio of the energy emitted by a substance in the form of luminescence to the absorbed energy), quantum yield (the ratio of the number of emitted quanta to the number of absorbed ones), and kinetics [56,57].

The basic idea behind optical biosensors is that the crosstalk between the analyte and receptor molecules on the substrate surface alters optical characteristics such as absorption, reflection, transmission, fluorescence, and scattering. The light output signal can be used for quantitative analysis and detection of the target pathogen. The sensitivity of optical devices is achieved through labeling or label-free methods. The label-free approach means that the analyte simply interacts with the transducer's surface to facilitate signal detection; by contrast, the label-based approach uses special optical tags to label the target analyte, generating various optical phenomena to produce a subsequent signal. These two approaches are used widely in optical biosensing to convey information about the interaction between the biosensor's element and the target and highlight the versatility of optical biosensors, as compared with alternative platforms. Optical biosensors are generally classified into colorimetric, fluorescent, surface-enhanced Raman spectra (SERS)-based, surface plasmon resonance (SPR)-based [58], localized surface plasmon resonance (LSPR)-based, photonic crystal-based, fiber optic, molecularly imprinted polymer (MIP)-based, and electro-optical. We now shift to discuss how all these sensors can be useful in antibiotic detection.

4.1. Colorimetric Optical Sensors

Colorimetric sensing is a quantitative method based on the relationship between the color change of a solution and the content of a target object. Colorimetric detection uses special indicators (nanozymes or nanomaterials) that change color when interacting with the molecules of interest. The color information can be recorded by the device, and the resulting signal can be processed by an image processor to obtain final images [59].

In sensors designed for antibiotic detection, the most important factor regulating color changes is the presence or absence of the target analyte in the sample. Colorimetric measurements typically track the change in attenuation caused by a change in the analyte concentration at a specific wavelength. The principle of antigen–antibody interaction is used widely in sensor systems for antibiotic detection [60]. Importantly, the type and quality of enzymes will have a certain effect on the detection efficacy of the biosensor [61–63].

Colorimetric and optical biosensors are often combined, which expands their application in environmental monitoring, food safety, biomedicine, and clinical diagnostics [64,65]. Recently, colorimetric methods for the determination of antibiotics have attracted increasing research attention owing to such characteristics as naked-eye recognition, absence of need for modern instruments, ease of use, short analysis time, and low cost [66].

In some antibiotic-detecting platforms, various types of colorimetric reagents are used, including metal nanoparticles, visible dyes, enzymes, and metal ions [67,68]. Such platforms, combining D-amino acid-modified gold nanoparticles with antibiotics, can

be used to examine the sensitivity of psychrophilic bacteria to antimicrobials and select antibiotic treatment [69].

In another study, kanamycin and its derivatives (kanamycin B and tobramycin) were detected with a single-stranded DNA aptamer and gold nanoparticles [70]. Table 1 summarizes the main achievements of colorimetric sensors in antibiotic detection.

Table 1. Main colorimetric sensors for antibiotic detection.

Detection Method	Analyte	Detection Range/Limit	Ref.
Colorimetric detection	Kanamycin (also kanamycin B and tobramycin)	25 nM	[70]
Smartphone-assisted sensing platform	Ampicillin	0.0500 to 100 µg/mL/12 ng/mL	[71]
Nanozyme-based aptasensor	Ampicillin	70 pM to 10 nM/21.7 pM	[72]
Cu-doped-g-C ₃ N ₄ /POD	Tetracycline	0.1~50 M/31.51 nM	[73]
MIL-101(Fe/Co)/POD	Tetracycline	1–8 M/0.24 M	[74]
NiCo@CHCs/POD	Enrofloxacin	0.1 ng/mL to 50 µg/mL/0.09 ng/mL	[75]
Nickel iron-layered double oxide/POD	Kanamycin	0.01 fM to 0.1 nM/3 aM	[76]
Boron nitride quantum dots supported by porous CeO ₂ nanorods	Kanamycin	0.01 nM to 100 nM/4.6 pM	[77]
WS ₂ /POD nanosheets	Kanamycin	0.1–0.5 M/0.6 µM	[78]
Colorimetric assay based on the aptamer-regulated peroxidase-mimicking activity of Co ₃ O ₄ nanoparticles	Kanamycin	0.1–30 µM/44.2 nM	[79]
FO@ZMFO@FMMOG/OXD POD CAT	Norfloxacin	0.415–6.21 M/52 nM	[80]
Co ₃ V ₂ O ₈ –TiO ₂ nanozyme colorimetric sensing platform	H ₂ O ₂ Neomycin	10–80 µM/1.98 µM 20–90 µM/8.82 µM	[81]
Nanozyme-based sensor	Norfloxacin (NOR) Ciprofloxacin (CIP) Enrofloxacin (ENR)	0.05–2.5 µM and 200–650 µM/0.78 nM for NOR, 0.05–2.5 µM and 350–750 µM/0.80 nM for CIP, 0.05–2.5 µM and 300–650 µM/7.18 nM for ENR	[82]
Nanozyme-based sensor	H ₂ O ₂ Amikacin	20–100 µM/3.04 µM 50–100 µM/1.88 µM	[83]
Colorimetric nanozymes-based sensor	Tetracycline	615.38 nM	[84]
Colorimetric sensor with dual catalytic system of the nanoenzyme and G-quadruplex/hemin DNzyme	Tetracycline	0.333 ng/mL	[85]
Colorimetric detection	Oxytetracycline Doxycycline Tetracycline	19.7 µg/L 23.1 µg/L 35.9 µg/L	[86]

Table 1. Cont.

Detection Method	Analyte	Detection Range/Limit	Ref.
AuNPs/POD	Streptomycin	0.1 μ M to 0.5 μ M/86 nM	[87]
AuNPs/POD	Sulfadimethoxine	0.01–1000 μ g/mL/10 ng/ml	[88]
AuNPs/POD	Tetracycline	0.01 ng/mL to 10 ng/mL/2.7 pg/mL	[89]
Au nanoclusters/POD	Tetracycline	1–16 μ M/46 nM	[90]
Graphene/ AuNP hybrid/POD	Oxytetracycline	0.17 to 0.50 μ M/91 nM	[91]
Colorimetric chemosensor based on the aggregation of ammonium thioglycolate (ATG)-functionalized gold nanoparticles (ATG-AuNPs)	Moxifloxacin Ciprofloxacin	0~200 μ M/1.57 μ M 0~100 μ M/1.30 μ M	[92]
Amino-functionalized Fe(III)-based MOF colorimetric detection	Tetracycline	50–1000 nM/46.75 nM	[93]
Aptamer-capped HRP@MOFs with a colorimeter readout	Streptomycin	0.005–6 ng mL ⁻¹ / 0.51 pg mL ⁻¹	[94]
(MOF)-aptamer-3,3',5,5'-tetramethylbenzidine (TMB)-H ₂ O ₂ -based sensing platform	Chloramphenicol Oxytetracycline Tetracycline Ampicillin	50–200 nM/25 nM (8.1 ng/mL) 50–100 nM/20 nM (9.9 ng/mL) 50–100 nM/18 nM (8.0 ng/mL) 50–100 nM/13 nM (5.2 ng/mL)	[95]
Dual functional Zn(II) MOF for colorimetric detection	Norfloxacin Nitrofurantoin	0.323 ppm 0.590 ppm	[96]

Nanozymes, as artificial enzymes, are commonly used as receptor components of antibiotic-detecting colorimetric sensor systems [10]. Nanozyme sensors have been used for the colorimetric determination of ampicillin [71,72], tetracycline [73,74], enrofloxacin [75], kanamycin [76–79], norfloxacin [80], and neomycin [81].

Iron-based nanozymes doped with iron ions (e-doping) were used to make a simple multichannel colorimetric sensor array for the identification and detection of quinolone antibiotics [82].

Cobalt(II) 5,10,15,20-tetrakis(4-carboxylphenyl)porphyrin (CoTCPP) self-assembled nanoaggregates served as soft templates for the preparation of TiO₂-based nanozymes with enhanced peroxidase-like activity. The sensing platform was tuned by light illumination to measure H₂O₂ and amikacin in lake water (straight forward and practical colorimetric sensor platform) [83].

Another study showed the potential of nanozymes for tetracycline detection [84]. A colorimetric biosensor with a dual catalytic system consisting of a nanoenzyme and G-quadruplex/hemin DNAzyme was constructed for ultra-sensitive detection of tetracyclines [85].

The promise was shown of innovative multifunctional catalysts by depositing α -Fe₂O₃ quantum dots (QDs) on titanium silicalite-1 and creating artificial nanozymes capable of simultaneously detecting, adsorbing, and photodegrading tetracyclines in water (19.7 μ g/L for oxytetracycline, 23.1 μ g/L for doxycycline, and 35.9 μ g/L for tetracycline) [86]. The peroxidase-mimicking activity of the nanozyme was enhanced by tetracyclines and caused a visible color change in 3,3',5,5'-tetramethylbenzidine. The artificial nanozyme showed exceptional adsorption properties and photocatalytic activity under sunlight, achieving an overall removal rate of about 70%.

Gold nanoparticles stand out among other metal-based preparations because of their special optical, electric, and catalytic qualities. These characteristics help create a microenvironment that is conducive to the immobilization of biomolecules while preserving their biological activity, thereby prolonging the biosensor's lifespan [68,97]. Colorimetric sensor systems using gold nanoparticles have been helpful in detecting streptomycin [87], sulfadimethoxine [88], tetracycline [89,90], and oxytetracycline [91].

A simple, rapid, and ultra-sensitive colorimetric chemosensor was developed for the simultaneous detection of moxifloxacin, ciprofloxacin, and Cr(III). It is based on the aggregation of ammonium thioglycolate (ATG)-functionalized gold nanoparticles (ATG-AuNPs) [92].

High porosity, large surface areas, and structural tenability are characteristics of metal-organic frameworks (MOFs), which are made up of metal ions or clusters connected by organic ligands. Therefore they are very promising for use in antibiotic-detecting sensors. Through processes such as electrochemical redox reactions, colorimetric responses via peroxidase-like activity, and fluorescence quenching, MOF-based sensors provide quick, portable, and extremely selective detection. The construction of fluorescent and colorimetric sensors reflects this. MOF-based colorimetric sensors are especially appealing for field research, because they offer an instrument-free, visual approach to antibiotic identification [98,99]. MOF-based colorimetric approaches to antibiotic detection remain understudied, because most MOFs are inherently fluorescent and hence are used primarily through fluorescence quenching mechanisms. Comparatively few MOF-based colorimetric sensors for antibiotic detection have been described in [98].

Examples of MOF-based colorimetric sensors for antibiotic detection have been summarized in [100]. Such sensors have been used to detect tetracycline [93], streptomycin [94], chloramphenicol, oxytetracycline, tetracycline, ampicillin [95], and norfloxacin [96].

The main advantages of colorimetric analysis include ease of use, rapidity of measurement, equipment availability, and ability to analyze low concentrations of antibiotics. Disadvantages include lower accuracy, as compared with that of other methods; the influence of a number of factors on the analysis results; the subjectivity of visual methods, whose accuracy depends on the researcher's experience; and the need to prepare a series of standard solutions, which makes the analysis more lengthy and costly.

Colorimetry allows visual identification of detection results with the naked eye, but disadvantages such as the low sensitivity and the high background signal limit the scope of its application.

However, the complex composition of various samples and the low concentrations of analytes require additional developments for the application of colorimetric sensor systems, the main ones being those that detect antibiotics at the point of analysis in real time. These include colorimetric sensors on paper, colorimetric detection sensors based on portable smartphones, dual-mode colorimetric sensors, and colorimetric sensors with a dual response [65,101]. A separate R&D area is the integration of artificial intelligence and machine learning into colorimetric biosensors, including their potential for technological progress in the near future [102].

4.2. Fluorescence-Based Optical Sensors

Fluorescence is a process characterized by a very brief period of radiation attenuation after turning off the excitation source. This is the short-term absorption of a quantum of light by a fluorophore (a substance that can fluoresce), followed by the rapid emission of another quantum with properties different from the original. Fluorescence is based on the principle that molecules emit light differently, depending on their state [103].

Fluorescence methods are based on changes in the fluorescence intensity, wavelength, or lifetimes that are caused by the interaction of target analytes with sensitive materials. Fluorescence probing methods are based on changes in the fluorescence spectrum caused by contact between probe and detector.

Fluorophore-containing reagent interactions can be easily and quickly recorded by excitation with plane-polarized light and measurement of the degree of polarization of the light emitted (Figure 3 [104]).

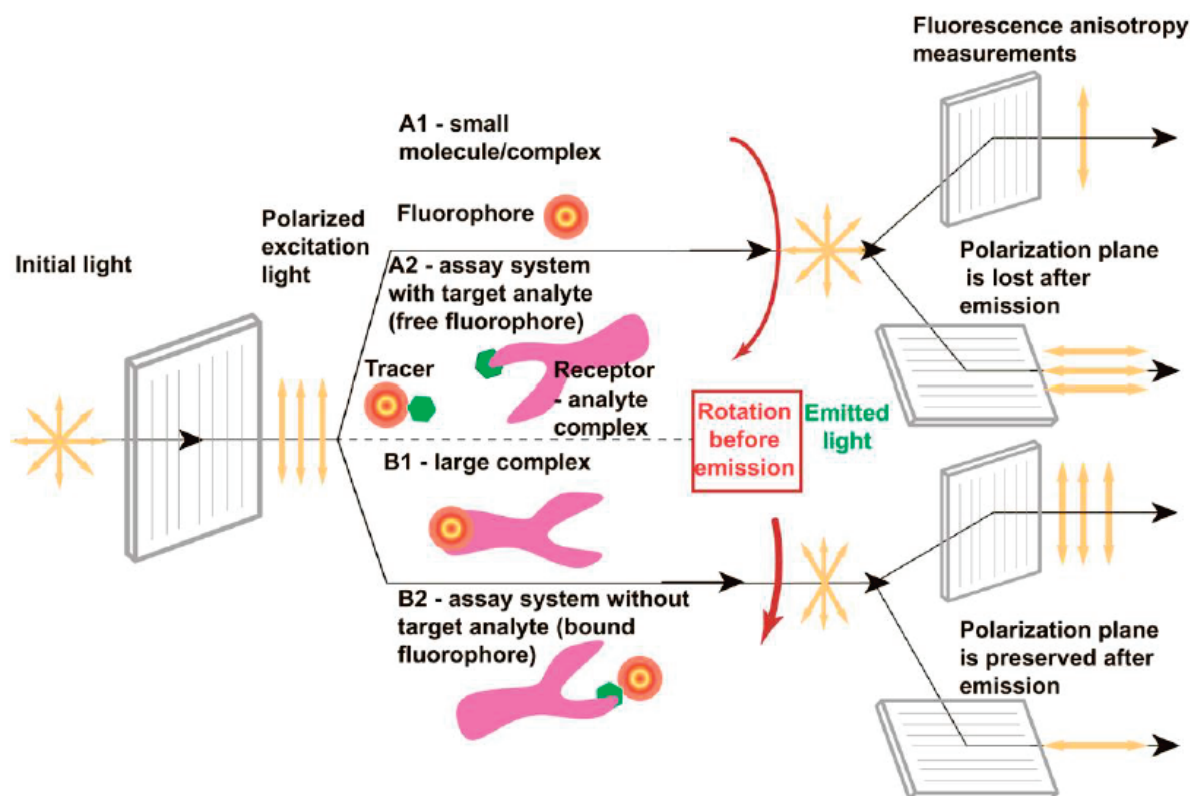


Figure 3. Effect of rotating motion on the anisotropy of fluorescence released after plane-polarized light stimulation (fast rotation is A1 and A2, and slow rotation is B1 and B2) (Reprinted from [104]. Creative Commons CC-BY license).

The polarization plane of the exciting light is not maintained during emission, because the fluorophore or the complex containing it rotates chaotically in solutions owing to Brownian motion. The degree of depolarization can be estimated by measuring emission in planes parallel and perpendicular to the stimulation plane. The emitting light loses the polarization of the exciting light when the fluorophore-containing complex becomes more mobile in the solution (Figure 3, A1). The complex's spin slows down and the polarization of the light it emits increases when a tiny fluorophore binds to a big compound (Figure 3, B1) [104].

Scientists can determine how much of the fluorescent chemical is present in a sample by measuring the light released. Additional advantages of fluorescence sensing over conventional detection techniques include excellent sensitivity and speed in identifying antibiotics at a comparatively low cost. Fluorescence sensors are beneficial in bioanalysis, because they operate on well-defined principles and are unaffected by suspended particles or by the sample solution color. Therefore, combining the principles of colorimetric and fluorescence methods will highlight the advantages of each method to better adapt to various complex detection requirements to achieve highly sensitive detection [58].

However, most fluorescence-based methods require continuous excitation by an external light source to obtain a fluorescence signal. They are, therefore, susceptible to interference from autofluorescence of the sample matrix, which seriously affects the detection accuracy [104]. Compared with colorimetric methods, fluorescence-based methods show a relative advantage in terms of very high sensitivity [105,106].

For example, tetracycline contains hydroxyl and carbonyl groups in its structure, which can form coordination compounds with metal ions [107].

Tetracycline has very weak fluorescence, but when coordinated with certain metal ions (such as Ca^{2+} , Mg^{2+} and Zn^{2+}), it shows intense green luminescence [108]. For example, when tetracycline is combined with Eu^{3+} , the color of the fluorescence changes to red [109].

The advent of nanoparticles and the advancement of nanotechnology have led to their active application in analytic measurements. QDs are nanoparticles with sizes similar to the electron wavelength (1–10 nm). By altering their size, it is simple to change their fluorescence or their absorption wavelength. QDs have favorable fluorescence properties, including a long luminescence duration, a high fluorescence intensity, and an adjustable emission wavelength [110].

Therefore, QDs are more suitable for use as fluorescent markers than organic dyes. Antibiotic concentrations in samples can be ascertained by measuring variations in the fluorescence intensity of QDs, which bind to immunorecognition materials. Specific molecules including DNA, enzymes, antibodies, and antigens might be considered active biomolecules. The principle of antigen–antibody interaction is actively used to develop optical sensor systems, particularly fluorescence immunoassays. A multiplex enzyme immunoassay sensor in a “lab-on-a-chip” format was described for the detection of antibiotics (sulfonamides, fluoroquinolones, and tetracyclines) [111]. It is based on a sensitive optical sensor and a polymer self-contained microfluidic cartridge. Pre-filled microfluidic cartridges were used as binary rapid tests for the simultaneous detection of antibiotics in raw milk [111].

Sulfamethazine (SM2) in chicken muscle tissue can be detected with an indirect competitive fluorescence immunoassay (cFLISA), by using QDs as a fluorescent marker connected to a secondary antibody [112].

A fluorescent sensitive probe based on Cr(III)–metal–organic framework (MOF) was designed for the rapid detection of tetracycline (response time, 1 min). When tetracycline is added, the blue emission at λ_{em} 410 nm changes owing to the interaction of the antibiotic with the Cr(III)–MOF material [113]. Table 2 summarizes the main achievements of fluorescence-and luminescence-based sensors in antibiotic detection.

Table 2. Main fluorescence and luminescence-based sensors for antibiotic detection.

Detection Method	Analyte	Detection Range/Limit	Ref.
Lab-on-a-chip multiplexed biosensor	Sulfonamides, fluoroquinolones, and tetracyclines	100 $\mu\text{g L}^{-1}$	[111]
Indirect competitive fluorescence enzyme-linked immunosorbent assay (cFLISA)	Sulfamethazine	1.0 ng/mL	[112]
Fluorescent sensing probe based on chromium(III)–MOF	Tetracycline	5–45 ng/mL/ 0.78 ng/mL	[113]

Table 2. Cont.

Detection Method	Analyte	Detection Range/Limit	Ref.
Multicolor QDs–antibodies	Dexamethasone Medroxyprogesterone acetate Gentamicin Ceftiofur Clonazepam	0.13 ng/mL 0.16 ng/mL 0.07 ng/mL 0.06 ng/mL 0.14 ng/mL	[114]
Multicolor QDs–antibodies	Sulfonamides, tetracyclines, penicillin G	5 pg/mL	[115]
Hybrid immunosorbent assay	Quinolones Sulfonamides Melamine	0.18 ng mL ^{−1} 0.17 ng mL ^{−1} 7.5 ng mL ^{−1}	[116]
QDs–dsDNA; antibodies–dsDNA	Chloramphenicol	2 pg/mL	[117]
QDs–SSB–DNA	Streptomycin	30 pg/mL	[118]
GOQDs–aptamer	Sulfamethazine	5 pg/mL	[119]
Label-free fluorescent aptasensor	Streptomycin	54.5 nM	[120]
Aptamer-based fluorescence biosensor	Chloramphenicol	0.01 to 1 ng mL ^{−1} /down to 0.01 ng mL ^{−1}	[121]
Multi-color fluorescence sensing platform	Norfloxacin	0.33 nM and 7.3 µM	[122]
Ratiometric fluorescence sensor based on lanthanide-functionalized MOFs (Ag ⁺ /Eu ³⁺ @UiO-66(COOH) ₂ , AEUC)	Tetracycline	Detection limit, 12.8 nmol/L; quantification limit, 38.6 nmol/L	[123]
Ratiometric fluorometric sensor	Norfloxacin	7.3 nM	[124]
Fluorescent zinc-based MOF nanodots for ratiometric detection	Tetracycline	Ratiometric peak intensities of F520/F440, with detection limit of 5 nM Smartphone integrated with theratiometric visual platform visually detected LOD of 10 nM	[125]
CQDs as a label in FPIA	Amikacin Streptomycin Gentamicin	3 ng/mL 10 ng/mL 20 ng/mL	[126]
Fluorescence with molybdenum disulfide nanoplates	Tetracycline	0–50 µM/0.032 µM	[127]
Fluorescent nanosized rod-shaped europium MOF (Eu-MOF)	Ciprofloxacin and chloramphenicol	0.0136 and 3.16 µM	[128]
Ratiometric fluorescent sensor array based on three Eu ³⁺ and Tb ³⁺ co-doped MOFs	Norfloxacin Minocycline	2–200 mM/5.6 mg L ^{−1} 0.4–20 mM/19.1 mg L ^{−1}	[129]

Table 2. Cont.

Detection Method	Analyte	Detection Range/Limit	Ref.
Ratiometric luminescent sensor	Nitrofurans, nitroimidazoles, sulfamethoxazole	98 mg L ⁻¹ and 196 mg L ⁻¹	[130]
Luminescent lanthanide MOF test	Oxytetracycline, tetracycline	1.95 and 2.77 nM	[131]
MOF-based fluorescent sensing	Tetracycline, chlorotetracycline, and oxytetracycline	0.28–0.30 µM	[132]
Europium MOFbased on fluorescence enhancement	Doxycycline	LOD 47 nM	[133]
Colorimetric/fluorescent dual-signal mode sensor (with apt-NiCoFe-MOF-74)	Tetracycline	-	[134]
Cu-BL-based colorimetric/fluorescent dual-mode sensor	Tetracycline Chlortetracycline Oxytetracycline	0.5–80 µM/0.27 µM 1–80 µM/0.22 µM 0.25–80 µM/0.26 µM	[135]
Dual-mode assay based on a colloiddally stable mixture of specific aptamer stabilized AuNPs (Apt-stabilized AuNPs) and graphite carbon nitride nanosheets (g-C ₃ N ₄)	Tetracycline	5.0×10^{-16} – 5.0×10^{-11} M/ 1.0×10^{-16} M	[136]
Smartphone-assisted dual-mode fluorescent/colorimetric biosensors based on Fe ₃ O ₄ -CDs nanozyme	Oxytetracycline	1.8 nM for the colorimetric mode and 7.9 nM for the fluorescence mode	[137]
An oligonucleotide-based switch-on luminescent sensor	Kanamycin	0.2–150 µM/ 143 nM	[138]
Bimetallic ratiometric fluorescent detection	Oxytetracycline Doxycycline	0–60 µM/8.6 nM (visual detection); with smartphones/9.8 nM 0–172 µM/4.8 nM (visual detection); with smartphones/14.2 nM	[139]
Bioluminescent bacteria-based biosensor	Doxycycline Chlortetracycline Tetracycline, oxytetracycline	5 ng g ⁻¹ 7.5 ng g ⁻¹ 25 ng g ⁻¹	[140]
Whole-cell biosensor, LumiCellSense (LCS)	Ciprofloxacin	7.2 ng/mL	[141]

Note: LOD—Limit of Detection.

In another study, an immune complex was formed, with avidin as a bridge to link QDs coated with biotinylated denatured bovine serum albumin and antibodies. This complex was used to simultaneously detect five chemical residues in porcine muscle tissue [114]. Similar studies were conducted in [115].

Other studies have used antibodies that are specific not only to a particular antibiotic but to an entire class of antimicrobial drugs. Zhu et al. [116] used two types of antibodies

for the simultaneous detection of six types of sulfanilamide antibiotics and eleven types of fluoroquinolone antibiotics.

Double-stranded DNA is used as an antibiotic aptamer, one of whose strands can identify chloramphenicol in milk. First, QDs bind to the double-stranded DNA antibody and then contact the double-stranded DNA. Thus, the recognition of antigens and antibodies causes the QDs carrying double-stranded DNA antibodies to assemble with one another, and the fluorescence intensity of the QDs weakens. However, when the antibiotic is added, the recognition of antigens and antibodies causes the QDs carrying double-stranded DNA antibodies to assemble with one another, and the fluorescence intensity of the QDs weakens [117].

A sensor for the detection of streptomycin in milk was made by combining single-stranded DNA, capable of recognizing antibiotics, with a protein. The resulting sensitivity was higher than that of the SPR method [118].

He et al. [119] took a different approach to detecting sulfamethazine residues in milk. It is based on a fluorescent QD immunoassay in which graphene oxide QDs are labeled with an aptamer to sulfamethazine.

Combining fluorescence with aptamers to make aptasensors is common owing to the excellent sensitivity and selectivity of the assay. Taghdisi et al. [120] detected streptomycin in milk and serum on the basis of exonuclease III, SYBR Gold, and an aptamer's complementary strand. In the absence of the antibiotic, the fluorescence intensity was low, but when it was added, the aptamer bound to the target, which led to a high fluorescence intensity. In Wu et al. [121], a fluorescent biosensor with aptamer-conjugated magnetic nanoparticles was used to detect low chloramphenicol concentrations.

More than 10 years ago, an approach was proposed based on elucidating the effect of antibiotics on bacteria by cell staining followed by cell examination by fluorescence microscopy [142]. This approach was tested on *Escherichia coli* cells and is still used in the scientific community [143]. The essence of the method is to compare the cytological profiles of the compounds under study with the profiles obtained for bacteria under the influence of known antibiotics. Another study described a quantitative method of time-lapse fluorescence imaging, called dynamic bacterial morphology imaging (DBMI) [144].

In bacterial imaging, the main direction in fluorescence imaging is to improve image quality by image-processing methods. For example, a system of phenotypic screening at minimal doses was proposed that uses cytological profiling and machine learning. The system allows identification of weak antibacterial effects, improving image quality [145,146]. Further application of artificial intelligence methods may be a promising direction for future developments.

The red-emitting $\text{Ru}(\text{bpy})_3^{3+}$ acts as a steady red fluorescence backdrop in a fluorescence sensor system that uses a lanthanide organic framework (Tb@COF-Ru) as a receptor component with imaging capacity for the detection of fluoroquinolone antibiotics. The green fluorescence of Tb^{3+} grew progressively as the concentration of norfloxacin increased, which ultimately resulted in a multicolor fluorescence shift from red to green [122].

A ratiometric fluorescence sensor based on lanthanide-functionalized MOFs ($\text{Ag}^+/\text{Eu}^{3+}@\text{UiO-66}(\text{COOH})_2$, AEUC) with intrinsic dual-emission bands was fabricated for the determination of tetracycline residues. ($\text{UiO-66}(\text{COOH})_2$, UC) was used as the reference units, Eu^{3+} was used as the recognition units, and Ag^+ was used as the fluorescence enhancer [123], with conspicuous fluorescence color gradation from red to blue. Ratiometric fluorescent detection of norfloxacin was described in [124].

In bioanalytical assays, the color tonality, which is established by own fluorescent MOFs, is quite desirable. Strong blue emission at 440 nm was achieved by synthesizing ultra-small, extremely fluorescent zinc-containing MOFs (FMOF-5) that were excited

at 350 nm. With a notable boost owing to aggregation-induced emission, tetracycline specifically adjusted the blue emission of FMOF-5 to greenish-yellow emission (520 nm). Tetracycline was analyzed fluorimetrically by using F520/F440 ratiometric peak intensities, with a 5 nM detection limit. A smartphone with a detection limit of 10 nM and an integrated ratiometric visual platform was made for point-of-care quantitative analysis based on color tonality [125]. In fluorescence analysis, a TbMOF probe was developed for the determination of tetracycline by using a simple one-step hydrothermal assay. The probe was characterized by a fast response (2 min) and recyclable luminescence sensing during antibiotic detection [147].

The modern level of antibiotic analysis includes polarization fluoroimmunological analysis, which allows results to be obtained immediately after addition of the sample to the immunochemical reagent. The method is based on the competitive binding of the antigen with specific antibodies and the antigen labeled with a fluorescent label (tracer). Polarization of the tracer's fluorescence is of little importance, and when the tracer binds to the antibody molecule, the polarization of the fluorescence of such a complex increases sharply. This effect is observed only for low-molecular-weight compounds (e.g., antibiotics). The magnitude of fluorescence polarization in the reaction mixture reflects the ratio of the bound and free fractions of the tracer and decreases when an unlabeled antigen is introduced into the medium [148]. Of note, it is possible to use QDs as labels in polarization fluorescence immunoassay (PFIA) to determine aminoglycoside antibiotics (amikacin, streptomycin, and gentamicin) in dairy products [126].

Nitrogen-doped carbon dots showed favorable photostability, serving as a multifunctional nanosensor for the detection of three tetracycline antibiotics based on a dual-mode fluorescence sensing strategy [127].

The fluorescence response (both enhancement and quenching) results from either energy transfer or absorption overlap between the MOF and the target antibiotic [149], as shown in fluorescent sensor systems for the determination of ciprofloxacin and chloramphenicol [128]; norfloxacin and minocycline [129]; nitrofurans, nitroimidazoles, and sulfamethoxazole [130]; oxytetracycline and tetracycline [131]; tetracycline, chlorotetracycline, and oxytetracycline [132]; and doxycycline [133].

A separate application of fluorescence methods is to combine them with colorimetric methods. A dual-mode colorimetric and fluorescence strategy was developed by integrating two optical components into the sensor [134]. This minimized environmental influences and potential errors and, when integrated with smart devices (smartphones), facilitated rapid detection of analytes, as shown with tetracyclines [135].

A similar approach to tetracycline determination on the basis of dual-mode colorimetric and fluorescent signals was proposed by Yang et al. [136]. The authors were able to control the tetracycline content in actual samples (drinking water and milk with added antibiotics). The same dual approach was proposed for oxytetracycline detection [137].

The main strong points of fluorescence-based sensors are high sensitivity, good selectivity, and ability to distinguish between the parameters of many fluorescent substances. Weak points are as follows: many substances do not fluoresce; there are many interfering factors (photolysis, oxygen quenching, and light contamination); the chromophore's fluorescence intensity depends on pH; and there is no relationship between fluorescence and structure of a compound.

Luminescence is the glow of a substance that occurs after it absorbs excitation energy and is excess radiation, as compared with the thermal radiation of the body at a given temperature. Photoluminescence, whose source is light, is of greatest importance for determining the composition of the medium. Photoluminescence is characterized by absorption and luminescence spectra, polarization, energy yield (the ratio of the energy

emitted by the substance in the form of luminescence to the absorbed energy), quantum yield (the ratio of the number of emitted quanta to the number of absorbed ones) and kinetics. The most widely used analysis is based on photoluminescence excited by UV radiation, whose source is mercury-quartz and xenon lamps and also lasers. Luminescence is recorded visually and photoelectrically (with a spectrophotometer). Photoluminescence characteristics allow conclusions to be drawn about the presence of certain substances in the samples under study and their concentration. Quantitative analysis is based on the dependence of luminescence intensity on the amount of the luminescent substance [62,63].

The development of extremely accurate and sensitive fluorescence chemosensors for antibiotic monitoring must be mentioned separately. A chemical of abiotic origin that reversibly forms a complex with an analyte and transmits a fluorescence signal along with it was the initial definition of a fluorescent chemosensor. These days, the terms “fluorescent probe” and “probe” are used interchangeably; that is, a probe/chemosensor is any chemical that has a binding/reactive site, a luminophore, and the mechanism of interaction between these two sites. This guarantees that following the probe’s interaction with the target analyte, a measurable result will be obtained. The primary advancements in the creation of these sensor systems were detailed in [150,151].

Fluorescent systems often generate a colorimetric response in addition to the fluorescence output. This is because a change in UV absorption (color) usually correlates with a change in fluorescence emission for any chemosensor. Notwithstanding these achievements, major technological advancements are still needed to address existing problems in this industry. These include: (1) increasing the signal-to-noise ratio to make it easier to obtain a measurable signal; (2) modifying the chemosensor’s solubility and output wavelength to ensure optimal compatibility across a range of applications; and (3) increasing the sensitivity and selectivity to the analyte to increase the accuracy of chemosensors for any desired purpose [150].

Chemi- and bioluminescence-based sensors record light radiation at different wavelengths that is emitted by enzymatic-reaction products in an excited state. These sensors are extremely sensitive and allow femtomolar quantities of a substance to be determined. The last decade of the 20th century saw intense development of a new group of luminescent sensors, using “luminescent markers” (specially synthesized macromolecules including two important functional links). One of them has a pronounced luminescent activity; i.e., it glows brightly under the influence of exciting light (or another exciting factor; this link is called a reporter). The other link is made selectively sensitive to the analyte—the chemical or biochemical substance whose presence must be detected and its concentration determined. This link is called a recognition element. Molecules of natural biochemical enzymes (enzymes, nanozymes) are often used for this purpose. But specially synthesized recognition molecules are also used. These two connections work together as a macromolecule, such that when a particle of the regulated analyte attaches to the recognizer, the luminescence of the signaling device is either quenched or, on the other hand, stimulated. On the basis of changes in luminescence intensity, the sensor ascertains the analyte’s presence and concentration or the properties of a certain external factor.

The relationship between luminescent radiation intensity and light emission wavelength is known as the luminescence spectrum. The most basic are atomic spectra, in which the atom’s electron structure alone determines the aforementioned dependence. Because different valence vibrations and deformations are achieved in molecules, their spectra are considerably more complex. Fluorophores with aggregation-enhanced emission (aggregation-induced emission luminogens) are also used as luminescent agents in nanozyme-based sensors. The advantage of such materials is the ability to control their pho-

tophysical properties by introducing oxidants into the system and changing the solvent's polarity. An example is tetraphenylethylene [152].

An aptamer was transformed from a randomly coiled structure into a particular shape with a hairpin region in a luminous technique based on oligonucleotides for the detection of kanamycin in aqueous solution [138].

A fluorescent chemosensor based on lanthanide MOFs was proposed for the visual determination of oxytetracycline and doxycycline in milk, beef, and pork by using a smartphone [139]. Virolainen et al. [140] described a luminescent biosensor for the rapid determination of residual amounts of tetracycline. The sensor is based on *E. coli* cells that can be stored in lyophilized form and can self-bioluminesce upon recognition of tetracycline.

For ciprofloxacin detection in whole milk, the LumiCellSense (LCS) smartphone-based whole-cell biosensor was proposed [141] that consists of a 16-well biochip with an oxygen-permeable covering and bioluminescent *E. coli* cells attached to the sensor surface.

Of note, luminescent sensors often have higher sensitivity than colorimetric ones because of their lower background signal. Colorimetric and luminescent sensor systems for antibiotic detection, as shown in the presented examples, have great potential for further development, because in colorimetric sensors, visible color changes occur owing to changes in the plasmon resonance frequency of metal nanoparticles, and these changes are visually observable with the naked eye. Given the simplicity of the analytic method and the interpretation of results for antibiotic detection, further development in this area is expected.

4.3. Surface Plasmon Resonance (SPR)-Based and Localized Surface Plasmon Resonance (LSPR)-Based Sensors

Among the various sensor technologies, sensors based on the localized surface plasmon resonance (LSPR) and the surface plasmon resonance (SPR) stand out as systems ensuring accurate, real-time detection of low concentrations of antibiotics [153].

The SPR is the basis for optical biosensors that alter the direction of light flux propagation through an optical fiber or a triangular prism covered in a thin metal sheet [154,155]. The basic operating principle of SPR-based sensors is to detect changes in the refractive index of the chip surface during immunological recognition. A layer of immunorecognition material located outside the SPR chip (antibodies, antigens, enzymes, and molecularly imprinted films) can increase the specificity and sensitivity of the sensors [156].

LSPR sensors rely on the size and shape of nanoparticles, whereas SPR sensors rely on reactions with chemicals. Figure 4 shows a scheme for the two typical technologies (LSPR and SPR) used in real-time analysis [153].

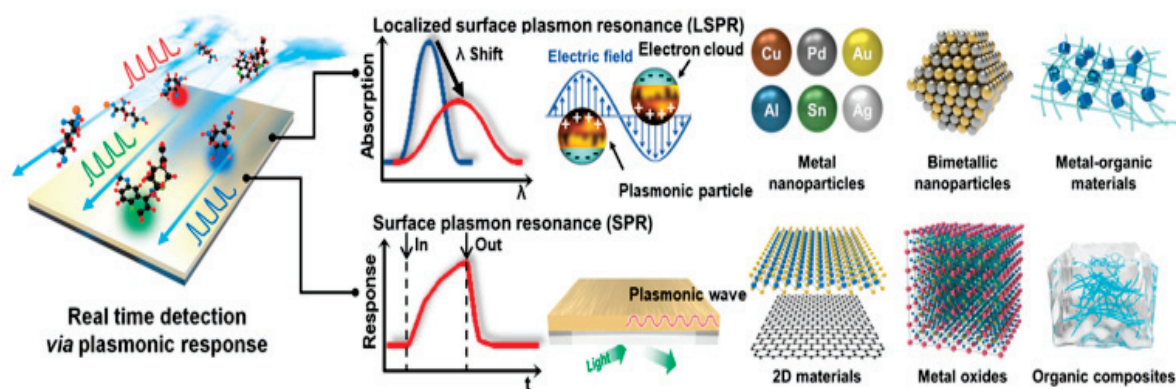


Figure 4. LSPR and SPR detection technologies and the materials used for each technology (Reprinted from [153]. Creative Commons CC BY-NC 3.0).

The food sector frequently uses SPR-based biosensors to identify and measure antibacterial or antimicrobial substances. The SPR is used about 2.5 times more often than any other optical biosensing technique. The SPR is the recommended option because of its great sensitivity, high throughput, dependability, and affordability.

Using competitive inhibition for immobilization on an SPR chip allows a high SPR signal intensity at low concentrations and a lower detection limit for antibiotics, as shown for β -lactams in milk. With this approach, the antibiotic concentration was determined on the basis of the concentration of the remaining antibodies [157]. Polyclonal antibodies were developed against two peptides to measure the amount of enzyme product formed (dipeptide assay) or the amount of remaining enzyme substrate (tripeptide assay), respectively [158].

For detection of glycopeptide antibiotics in milk, molecularly imprinted nanostructures were developed and high-affinity synthetic receptors were linked to an SPR sensor [159]. An SPR-based sensor system enabled the simultaneous analysis of eight antibiotics [160].

LSPR-based sensors have attracted attention owing to their high sensitivity and to the simplicity of analytical procedures. Depending on the nature of the LSPR change, they can be divided into aggregation-based and refractive index-based sensors [102]. LSPR measurements provide a reliable, label-free, and cost-effective analytical tool for the detection of nucleic acids and enable antimicrobial resistance determinants to be monitored [161]. An LSPR sensor system was made by using aptamers in the development of a colorimetric aptasensor for the detection of oxytetracycline on the basis of aggregation of aptamer-functionalized AuNRs [162].

Another study showed the possibility of using a silicon nanopillar metasurface coupled with LSPR for the real-time detection of cephalexin. The metasurface was coupled to BSA-coated gold nanospheres to ensure a red shift in the peak resonance wavelength values, which occurs only in the presence of an antibiotic linker. The fabricated device showed a strong wavelength shift of 22 nm, driven by a change in the local refractive index in the presence of the antibiotic linker [163]. Table 3 summarizes the main achievements of SPR-based optical sensors in antibiotic detection.

Table 3. Main SPR-based and LSPR-based optical sensors for antibiotic detection.

Detection Method	Analyte	Detection Range/Limit	Ref.
SPR-based optical sensors	Streptomycin Dihydrostreptomycin	30 $\mu\text{g kg}^{-1}$	[157]
SPR biosensor (Biacore SPR biosensor)	Penicillin G	2.6 $\mu\text{g kg}^{-1}$	[158]
SPR-based optical sensors	Vancomycin	4.1 ng mL^{-1} to 17.7 ng mL^{-1}	[159]
SPR imaging	Neomycin	1500 ng/mL	[160]
	Gentamicin	100 ng/mL	
	Kanamycin	150 ng/mL	
	Streptomycin	200 ng/mL	
	Enrofloxacin	100 ng/mL	
	Chloramphenicol	0.3 ng/mL	
	Sulfadiazine in milk	100 ng/mL	
LSPR sensor	Oxytetracycline	0.1 nM to 100 nM/0.04 nM	[162]
LSPR sensor	Cephalexin	0.3 $\mu\text{g/mL}$	[163]
LSPR/SPR	Enrofloxacin	0.11 ng/mL	[164]
Multichannel SPR immunosensor	Enrofloxacin	0.30 ng/mL	[165]
	Sulfadiazine	0.29 ng/mL	
	Chloramphenicol	0.26 ng/mL	

Table 3. Cont.

Detection Method	Analyte	Detection Range/Limit	Ref.
SPR-based hybrid sensor	Ampicillin	-	[166]
SPR immunosensor	Penicillin G	1.2 µg/kg and 1.5 µg/kg	[167]

The LSPR effect of gold nanoparticles made it possible to amplify SPR signals, lowering the enrofloxacin detection limit by a factor of 14. Gold nanospheres are frequently used as colorimetric indicators because of their SPR characteristics [68,164,168,169]. Besides the traditional SPR analysis, a substrate diffraction grating can be used to determine antibiotics [165].

By combining of SPR plastic optical fiber (SPR-POF) with electrochemical (bio) sensor, a sensor for ampicillin detection in water was developed [166]. Two SPR biosensor tests were developed to detect β -lactams in milk. These assays rely on the activity of carboxypeptidase, which converts 3-peptide to 2-peptide, and β -lactams inhibit this process. Antibodies in the sensor were used to measure the amount of enzyme product formed or the amount of enzymatic substrate left behind [167].

More possibilities of using SPR-based biosensors for antibiotic detection were shown in [170]. SPR-based sensors permit the detection and study of biochemical reactions at the level of individual biomolecules without additional functionalization of the sensor surface. The main limitations of these sensors are: surface plasmon penetration depth, contribution of the solution volume to the signal, and optical dispersion of the sample, which can reduce the accuracy of estimation of the refractive index.

4.4. Surface-Enhanced Raman Spectra

Because of the unique structure of antibiotic molecules, light waves are absorbed, reflected, and scattered differently when they pass through them. This is the basic idea behind molecular spectroscopy-based antibiotic detection. As a result, molecular spectroscopy offers crucial details regarding the molecular makeup of extremely particular materials. The techniques used in molecular spectroscopy include terahertz spectrum detection, room-temperature phosphorescence, resonant Rayleigh scattering, and surface-enhanced Raman scattering. Surface-enhanced Raman spectroscopy (SERS) is a highly sensitive technique that enhances the Raman scattering of molecules supported by certain nanostructured materials [171].

Most of the recent applications in SERS sensing have been aimed at the determination of one or more specific analytes. Such targeted assays most often use an indirect detection strategy (label-based SERS), in which the observed SERS signal originates not from the targets themselves but from SERS tags (consisting of molecular reporters or tags on a plasmonic nanostructure) that are selectively conjugated to the target through a recognition element [172].

The schematic design of a SERS tag (A) and the main formats of SERS immunoassays (B) are presented in Figure 5 and are described in [173].

Numerous studies have determined the susceptibility of bacteria to antibiotics by SERS-based methods [174–177] and have used SERS to analyze the activity of antimicrobials [178]. An equally progressive area of research is the use of SERS to determine antibiotics (quinolones, tetracyclines, sulfonamides, aminoglycosides, amphenicols, β -lactams, nitrofurans, macrolides, and nitroimidazoles) in foodstuffs, water, and biological matrices [179].

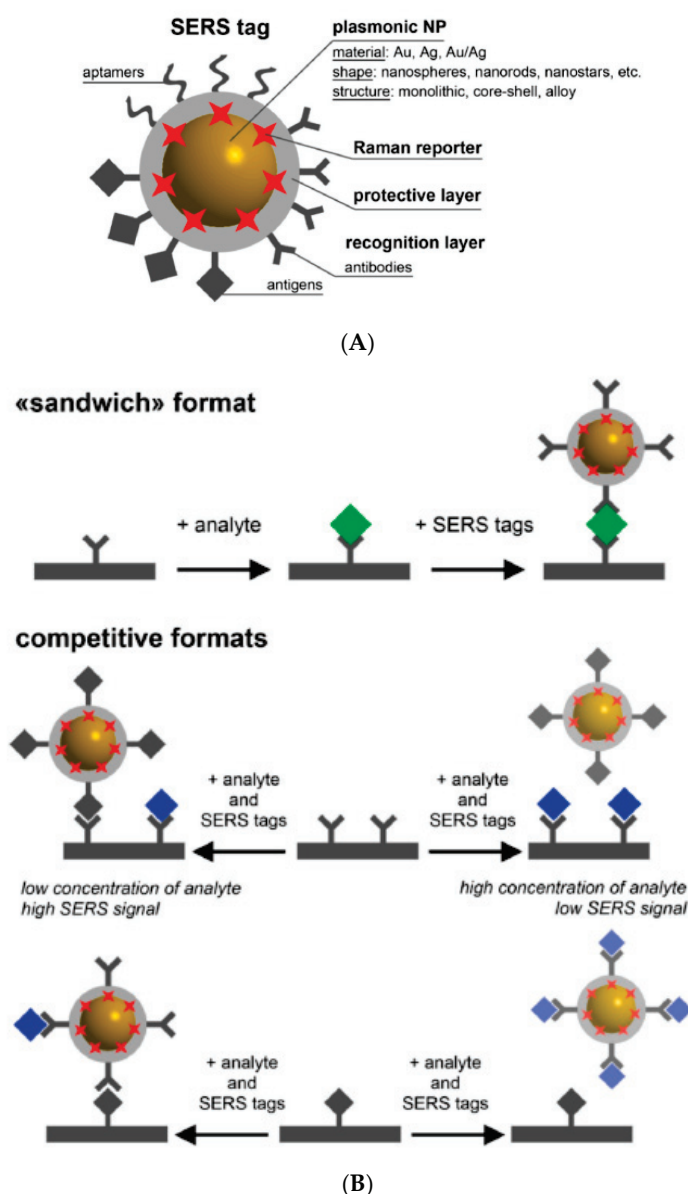


Figure 5. The architecture of a SERS tag (A) and the main formats of SERS immunoassays (B) (Reprinted from [173]. Creative Commons CC BY license).

An important aspect in the development of SERS systems is to increase their antibiotic-detecting sensitivity. One approach to improving detection limits is to use metal nanoparticles [10,68]. For example, the use of silver nanosubstrates coated with colloidal gold made it possible to enhance the recorded analytic SERS signal [180]. The results of these studies facilitated the application of SERS-based sensor substrates modified with metal nanoparticles. In another study, a silver film was deposited both on the surface of aluminum oxide and within its adjustable pores. Because the pore size of the aluminum oxide film is adjustable, the resonant wavelength of the silver films is adjustable too. This allows the aluminum oxide pore size to be tuned so that the resonant wavelength is within the range of the light source and the optical spectrum analyzer. This method maximizes Raman signal amplification and has been tested for the determination of chloramphenicol in milk [181]. By ensuring the ability of the analyte to interact with colloidal silver and by optimizing the aggregation of silver nanoparticles, the SERS intensity and sensitivity of ampicillin detection were increased [182]. For high-throughput analysis, a second method based on LSPR was proposed for tobramycin detection in human serum. The method

uses functionalized nanoparticles and SERS analysis [183]. Table 4 summarizes the main achievements of SERS-based optical sensors in antibiotic detection.

Table 4. Main SERS-based optical sensors for antibiotic detection.

Detection Method	Analyte	Detection Range/Limit	Ref.
Metal nanoparticles combined (LSPR effect to enhance SERS signals)	Enoxacin Chloramphenicol Ciprofloxacin	20 ng/mL 15.79 ng/mL 20 ng/mL	[180]
SERS nanofilm-coated porous Al ₂ O ₃ (tunable pore size)	Chloramphenicol	LOD: 7.5 ng/mL (aqueous) 50 ng/mL (milk) MRL: 0.3 ng/mL (milk)	[181]
SERS AgNPs, samples, salt microfluidic junction	Ampicillin	LOD: 10 ng/mL (milk) MRL: 4 ng/mL (milk)	[182]
Dual detection of LSPR and SERS	Tobramycin	-	[183]
SERS Ag–AgNPs	Penicillin G	LOD: 0.85 ng/mL MRL: 4 ng/mL	[184]
SERS-LFA	Neomycin Norfloxacin	0.37 pg/mL 0.55 pg/mL	[185]
SERS	Quinoline	5.01 ppb	[186]
SERS with AgNPs–graphene oxide	Methotrexate Ampicillin trihydrate 6-aminopenicillanic acid Penicillin G	0.27 ng/mL 0.32 ng/mL 0.15 ng/mL 0.11 ng/mL	[187]
SERS AgNPs/CNTs–GO	Oxytetracyclinehydrochloride Ampicillin trihydrate Tetracycline	0.38 ng/mL 1.29 ng/mL 0.77 ng/mL	[188]
SERS with PVA–CD–AgNPs	Sulfamonomethoxine Sulfadiazine Sulfadimidine	10 ng/mL	[189]
SERS with metal optical nanograting	Fluoroquinolone Enrofloxacin Ciprofloxacin	-	[190]
SERS chips	Ciprofloxacin, amoxicillin, and cefazolin in water Enrofloxacin in milk	-	[191]

Note: LOD—Limit of Detection; MRL—maximum residue limit.

In a different investigation, penicillin G residues in an actual milk sample were found by the SERS approach in conjunction with two-step sample preparation, which prevents interference from other sample components [184].

By using two immunonanoprobe (one for neomycin and the other for quinolones), a SERS detection device, and a portable lateral flow analyzer (LFA), neomycin and quinolone antibiotics were detected in a single step. The two probes were made up of monoclonal antibodies against the matching antibodies and gold nanoparticles coupled to the Raman-active compound 4-aminothiophenol [185].

For the label-free detection of quinoline, two gold nanostructures were proposed as SERS substrates as presented in [186].

Li et al. [187] investigated a disposable Ag-graphene sensor that uses electrophoretic preconcentration with SERS to detect polar antibiotics in water. The sensor promoted the adsorption of molecules owing to the weak π - π interactions between graphene and antibiotics. This increased the sensitivity of SERS detection of antibiotics in aqueous samples without their preliminary separation.

Because of the strong ability of silver nanoparticles and carbon nanotubes intercalated into graphene oxide (AgNPs/CNT-GO) to be enriched through π - π stacking and electrostatic interactions of GO with antibiotic molecules, the use of laminar membranes composed of AgNPs/CNT-GO allowed the determination of sub-nanomolar concentrations of antibiotics [188].

Trace amounts of sulfonamides were determined with great sensitivity and repeatability by a new method combining surface modification with Ag nanoparticle gelation [189]. In Hong et al. [190], SERS nanogratings (platforms supporting the SERS effect) were made by laser interference lithography.

The concept of using SERS to detect residues of antibiotics (ciprofloxacin, amoxicillin, cefazolin, and enrofloxacin) in wastewater and tap water was further developed with the aid of high-performance SERS chips based on self-assembled hollow Ag octahedra. These chips expanded the scope of in situ sensitive detection and identification of antibiotic residues [191].

The limitations of the method are related to the need for pretreatment in the analysis of complex matrices and for modification of the molecular sensor. Analysis of the recorded SERS spectra requires powerful artificial intelligence and machine learning tools, because the complexity of SERS spectra in actual matrices is high owing to different background compositions or higher concentrations of the matrix components, as compared with those of the target molecules. Thus, further development of SERS sensors for antibiotic detection is aimed at improving the analytic SERS signals; the reproducibility, repeatability, and stability of the sensors; and the adaptation of the sensors to work with actual samples [173,192].

4.5. Photonic Crystal Biosensors

Brightly colored photonic crystals (PCs) are novel materials that are frequently used in biological sensors. Because they alter their structural color as they interact chemically with organic solvents, they represent perfect sensor materials for detection by the naked eye. Compared with conventional biochemical sensors, PC-based ones have shown great sensitivity and promise in point-of-care diagnostics and in quick screening [193].

In order to stop light from propagating in particular directions at particular frequencies, PCs are made of components with alternating permittivities on a length scale similar to the wavelength of light. When PCs are used as sensing elements for high-performance detection, their unique structural color can function as a label-free photonic code. The size of the PC building blocks can precisely control this photonic code. Main information on PC sensors can be found in [194]. Optical sensors using PCs are distinguished by their structure, which allows precise control of the optical properties of the sensor and ensures accurate analysis without the use of labels with the possibility of conducting multiplex studies [194].

Biosensors embedded in PC structures are promising for the rapid and precise detection of target substrates. The optical waves measured in these sensors are tangible manifestations of Maxwell's equations, and the wavelength frequency plays a direct part in the characteristics of the medium through which the light (or, more generally, electromagnetic waves) propagates. Information and energy transfer in a PC is controlled by the total group velocity of the Bloch modes at a given frequency of the electromagnetic wave (i.e., light at optical frequencies) incident on the PC medium [195]. Because the PC

has a strong detection response, it is an ideal substrate for sensing and has good potential for rapid qualitative and quantitative detection of antibiotics. For example, kanamycin can be detected with a two-dimensional SiO₂-Au-ssDNA PC, formed from PCs, gold nanoparticles (AuNPs), and aptamers [196]. Table 5 summarizes the main achievements of PC biosensors in antibiotic detection.

Table 5. Main PC sensors for antibiotic detection.

Detection Method	Analyte	Detection Range/Limit	Ref.
Two-dimensional PC (SiO ₂ -Au-ssDNA 2D PC) that combines PCs, gold nanoparticles (Au NPs), and aptamers	Kanamycin	5 pg·mL ⁻¹ to 5 µg·mL ⁻¹ /1.10 pg·mL	[196]
Two-dimensional PC hydrogel biosensor	Penicillin G	1 µM and 0.1 µM	[197]
Silicon on-chip PC biosensor	Gentamicin	0.1 nM to 1 µM	[198]
Photonic sensor	Chloramphenicol	2 ng mL ⁻¹ to 512 ng mL ⁻¹	[199]

For colorimetric detection of penicillin G and penicillinase inhibitors, a two-dimensional PC hydrogel-containing penicillinase biosensor was made [197]. Progress in photonic biosensors has been shown by the development of the corresponding chips for the detection of antibiotics (gentamicin) by binding to the corresponding antibody pre-immobilized on the sensor surface [198]. PC microcavities formed by two-dimensional air holes on silicon substrates and antibodies immobilized on the sensor surface led to highly specific detection of gentamicin [198]. The authors showed that a silicon photonic biosensor platform can be implemented as a portable lab-on-a-chip sensor system, in which the integration of the sensor chip with microfluidic components enables automated real-time testing of samples. A photonic sensor using a self-cross-linked imprinting close-packed opal as a recognition element for chloramphenicol detection was described in [199]. Photonic sensor systems deserve special attention in connection with advances in molecular imprinting, which will be discussed below.

In summary, basic research has confirmed that the detection spectrum can be enhanced by adjusting the size of the building blocks to shift the optical response to the mid-infrared region of the spectrum. However, the main limitation of PC-based sensors is associated with their use to detect antibiotics in actual samples.

4.6. Fiber-Optic Biosensors

Because optical fibers are inexpensive, respond quickly, and are simple to analyze, their application in bio- and immunosensors shows promise. The fiber typically transmits a range of wavelengths of infrared light. By using the total internal reflection of light as it travels through a waveguide, fiber-optic biosensors produce an attenuated wave boundary on the waveguide's surface. Compared with conventional immunoassay techniques, fiber-optic sensors based on antibodies or immunoassays provide higher sensitivity, selectivity, and speed [200].

Fiber-optic sensors have some advantages over traditional electronic sensors, such as resistance to radiation, fire, radio frequency and other electromagnetic interference, and leakage. They do this by using optical signals as the transmission channel to detect a range of physical characteristics. They may be used over long distances and perform well in distributed sensing systems. Because of the characteristics of nanofibers and the U-shaped optical fiber, the sensitivity at the resonant wavelength increases as the nanofiber electrospinning process lasts longer. The bend radius causes a shift in the sensor's peak signal. Total reflection at the core-cladding interface is not constant as light reaches the bend

zone, and a leaky mode forms in the U-shaped optical fiber's bend region. A waveguide model is made by reflections that take place at the contact between the external medium and the cladding [201].

The constant progress in the development of fiber-optic sensors opens new possibilities for antibiotic detection owing to the advantages of low cost, ease of operation, and high sensitivity. Optical fibers have important properties, including compact size, light weight, integrability, remote sensing and high sensitivity [202,203].

Fiber optics provides several benefits, including ease of installation and operation, corrosion resistance, superior biocompatibility, and resistance to electromagnetic and electrostatic interference. Compound detection is made possible by the optical qualities of fiber. Immobilizing biological components in a coating is the most basic fiber optic biosensor concept; additional layers can be added to the coating to enhance the sensor's functionality, as described in [204].

A U-shaped fiber immunosensor was used for the ultra-sensitive detection of ciprofloxacin in wastewater. Immunoglobulin was mounted on the surface of a silicon fiber functionalized with polymeric polyaniline [205]. Optical and electrochemical sensors that can detect ciprofloxacin in water rapidly, sensitively, and in situ were described in [206].

The chelation interaction between antibiotic and Cu^{2+} allows the measurement of the relative change in Cu^{2+} . The entire electrochemical reaction may be tracked in real time by the plasmonic fiber sensor owing to its high sensitivity to interfacial interactions. The built-in fiber sensor also boasts great specificity, quick detection (255 s), and remarkable sensitivity (fM level) [206]. A U-shaped lossy fiber optic resonant mode structure coated with indium tin oxide for ciprofloxacin detection that is sensitive was reported on in [207].

A separate area of research is optical sensors based on a label-free, time-resolved method called reflectometric interference spectroscopy. The use of various types of interferometers (mainly Mach–Zehnder and Michelson) in optical sensors allows the size of the sensor itself to be reduced without loss of sensitivity. Such biosensors operate by measuring the phase shift between the measured and reference signals. This detection principle was first described for penicillin detection. The method relies on white light interference in thin layers to observe molecular interactions, and studies can be conducted in a buffer by using commercial antibodies [208]. Another study reported on an optical biosensor method that is based on biolayer interferometry and uses a kinetic competitive binding assay. The method can determine less than 1 ppm ($\sim 33 \mu\text{M}$) of amoxicillin. Similarly to the surface plasmon resonance, the method enables the detection of changes occurring upon binding of the antibody molecule to amoxicillin [209]. Table 6 summarizes the main achievements of optical-fiber biosensors in antibiotic detection.

Table 6. Main optical-fiber sensors for antibiotic detection.

Detection Method	Analyte	Detection Range/Limit	Refs.
Immunosensor based on a silica optical-fiber sensor	Ciprofloxacin	0.01 to 10,000 ng L^{-1} / $3.30 \times 10^{-3} \text{ ng L}^{-1}$. The quantification limit of 0.01 ng L^{-1}	[205]
Optical & electrochemical fiber-optic sensor	Ciprofloxacin	$2.69 \times 10^{-14} \text{ M}$	[206]
U-Shaped Fiber Optic Biosensor	Ciprofloxacin	0.001 to 0.029 mol dm^{-3}	[207]
Optical sensor based on interferometer	Penicillin G	250 ppb	[208]
Optical sensor based on interferometer	Amoxicillin	1 ppm ($\sim 33 \mu\text{M}$)	[209]

Table 6. Cont.

Detection Method	Analyte	Detection Range/Limit	Refs.
An all-fiber system biosensor	Amoxicillin	0.01–100 nM/ 0.04 nM	[210]
Chitosan-functionalized porous silicon optical sensor	Ibuprofen	1000 ppb	[211]
Fiber-optic surface plasmon resonance sensor	Ciprofloxacin Enrofloxacin	0.81 ng/mL 1.20 ng/mL	[212]
Optofluidic in-fiber integrated Ag nanoparticle sensor	Ciprofloxacin Norfloxacin	10^{-10} – 10^{-11} M	[213]

A fiber-optic system for rapid determination of antibiotic concentrations was experimentally validated. It is based on an optical enzyme biosensor with a microfiber interferometer and variation in fiber grating power. During the experiment, β -lactamase is immobilized on a polyaniline-coated optical fiber through covalent cross-linking with glutaraldehyde. β -Lactamase can hydrolyze β -lactam antibiotics, forming acidic byproducts that convert polyaniline from its emerald base form to its emerald salt. This leads to a change in the surface refractive index, which is recorded with a microfiber interferometer during the determination of the concentration of β -lactam antibiotics [210].

A chitosan/porous silicon substrate exposed to liquids containing different doses of ibuprofen was used for optical interferometric measurements. In comparison to the unfunctionalized porous film, the inclusion of chitosan increased the sensitivity of the porous film by more than an order of magnitude [211].

The immunosuppressive principle was used to construct a fiber-optic surface plasmon resonance sensor for the detection of ciprofloxacin and enrofloxacin in milk [212].

The potential of a compact optical fiber-based nanomotion sensor was shown for monitoring of dynamic nanoscale cantilever vibrations associated with the viability of the microorganisms whose antibiotic sensitivity was examined—*Escherichia coli* and *Candida albicans*. The analysis took several minutes [214].

A micro-structured optofluidic in-fiber Raman sensor was developed for ciprofloxacin and norfloxacin detection in aqueous media [213].

An innovative approach (Lab-Around-Fiber), which consists of biofunctionalization of silicon optical fibers by grafting antibodies to their outer surface, was described for the identification of target molecules [215].

In recent years, microfiber has been gradually adopted as a tool for antibiotic detection owing to its extremely low optical transmission loss, high refractive index difference between the core and cladding, and high evanescent wavelength transmission. The method of combining materials with optical fiber to form a fiber sensor is simple to operate, efficient, and easy to implement. It can be substance-specific and provides a new platform for antibiotic detection. Further optimization of optical wave sensor systems and their integration with other methods holds great promise for detecting antimicrobial residues in water and beverages.

4.7. Optical Sensors Based on Molecularly Imprinted Polymers

Recognition elements (antibodies, antigens, immunoglobulins, enzymes, and aptamers), most frequently used to form the receptor layer of sensors, are highly specific and enable the acquisition of information about the progress of biochemical reactions in solutions in near real time. When biological objects are used as receptors, it is important to increase the service life of sensors based on them. The complexity of obtaining natural

receptors, their instability during storage, exposure to organic solvents, and high electrolyte concentrations promote the search for synthetic antibodies. These can be developed through the use of molecularly imprinted polymers (MIPs), which offer several undeniable advantages over biomolecules. These advantages include methodological simplicity of production (in contrast, e.g., to the production of antibodies to low-molecular-weight compounds, which is a multi-stage and time-consuming process), and high reproducibility of the synthesis. In addition, MIPs are stable in aggressive environments and under abrupt changes in operating conditions and can be synthesized for virtually any substance, including inorganic ions, drugs, nucleic acids, proteins, cells, and even supertoxic compounds for which, for example, polyclonal antibodies cannot be obtained.

Molecular imprinting is a method for obtaining “molecular imprints” based on the polymerization of functional monomers in the presence of specially introduced target template molecules. When combined with suitable sensor platforms, MIPs are very attractive for the routine environmental monitoring of antibiotics, with account taken of their low production cost, high stability, and high selectivity. Consequently, MIP-based sensors for antibiotics have been reported in the past decade [216].

The most common method for synthesizing MIPs is bulk polymerization. Molecular imprinting is dominated by nonlinear polymers with a macroscopic lattice, which provide the binding sites with the necessary rigidity and mechanical stability. The final structure of such polymers is determined by the crosslinking coefficient, equal to the percentage molar ratio of the crosslinking agent to the monomer, and also by the volume and nature of the pore-forming solvent in which the polymerization occurs [217].

Surface imprinting, nanoimprinting, living/controlled radical polymerization technology, multi-template, multifunctional monomer, and dummy template imprinting strategies are some of the MIP modes that have been developed and applied for antibiotic detection [218–220]. By creating MIPs as a layer on solid particles, surface imprinting technology produces recognition sites with a significant affinity for the substrate surface. Because evenly distributed sites increase the MIPs’ adsorption capacity and the rate at which the recognition sites rebind to the imprinted molecules, they improve the adsorption and separation efficacy of the imprinted material. Eluting and recovering template molecules are facilitated by the concentration of all binding sites on the surface. The size of the impression cavity on the polymer surface can be effectively controlled thanks to this technology. Fast resolution, fast processing speed, high throughput, compatibility with a variety of materials, and low cost are all benefits of using nanoimprinting technology to create nanostructured MIPs.

Therefore, this process is appropriate for producing antimicrobial surfaces based on different polymer surfaces on a wide scale. It is anticipated that nanoimprinted materials will enhance the MIPs’ site accessibility, rebinding kinetics, and adsorption capacity. MIPs for the detection of antibiotic residues and other environmental toxins, such as nitroxide-mediated free radical polymerization, atom transfer radical polymerization, and reversible addition-fragmentation reaction, are increasingly being prepared using LCRP technology [170,171]. The main achievements of MIPs, including prospects and applications concerning new production technologies and strategies, can be found in [221].

Sensors based on MIPs are an important development in the field of biosensor-aided detection of antibacterial medication residues in food products [222–224]. Recent advances in optical sensing systems have focused on their integration with molecular imprinting. When integrated with an optical sensor-transducer, MIPs show high potential for environmental monitoring, including antibiotic detection. Researchers have taken notice of MIPs because of their strong, precise affinity for the template molecule. The MIP method

achieves great selectivity for the analyte under determination by creating extra template imprints. Figure 6 is a schematic diagram of MIP synthesis [225].

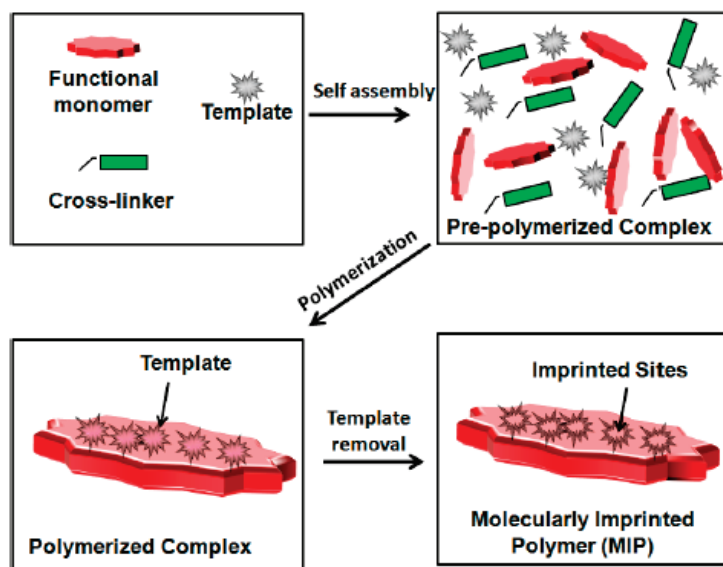


Figure 6. Schematic diagram of MIP synthesis (Reprinted from [225]. Creative Commons CC BY license).

Optical sensing platforms use various optical phenomena arising from the interaction of the analyte with the MIP layer to obtain analyte-correlated sensor responses. Optical sensors are usually very sensitive; therefore, their combination with target-selective MIPs is important in label-free molecular analysis. The most commonly used optical platforms in molecular imprinting are SPR and SERS [216].

Despite the high sensitivity of the SPR method, its versatility was initially limited to the analysis of large biomolecules owing to difficulties in working with biologically and environmentally important small analytes, because the induced change in the refractive index is usually too small to obtain adequate responses [226]. Owing to their robustness, which allows them to withstand harsh chemical conditions of regeneration without noticeable loss of activity, MIPs are adaptive and selective, and they easily integrate with the SPR, as compared with antibodies [227].

Sensors that use MIPs in conjunction with the SPR or LSPR offer extremely sensitive, real-time detection with quick reaction times [225]. A succinct overview of the construction, characteristics, and possible uses of screen-printed electrodes in bacterial, antibiotic, and antibiotic susceptibility testing can be found in [228].

Another study reported on tetracycline detection by using MIPs in conjunction with an optical-fiber sensor [229]. Table 7 summarizes the main achievements of MIP-based optical sensors in antibiotic detection.

Table 7. Main MIP-based optical sensor systems in antibiotic detection.

Detection Method	Analyte	Detection Range/Limit	Ref.
Optical fiber sensor using the SPR and molecular imprinting	Tetracycline	0–0.96 μM	[229]
Surface plasmon resonance sensor with MIP	Azithromycin Dopamine Penicillin	10^{-11} – 10^{-7} mol L ⁻¹	[230]

Table 7. Cont.

Detection Method	Analyte	Detection Range/Limit	Ref.
SPR analysis with MIPs	Neomycin Kanamycin Streptomycin	2.00 ± 0.21 pM 1.00 ± 0.10 pM 200 ± 30 fM	[231]
MIP film with SPR-based sensor	Amoxicillin	73 pM	[232]
MMIPs NPs with SPR	Tetracycline	5.0–100 pg/mL/1.0 pg/mL	[233]
SPR with high-affinity nanoparticles (nanoMIPs)	Ciprofloxacin Moxifloxacin Ofloxacin	36.2 nM 54.7 nM 34.6 nM	[234]
SPR based fiber optic sensor using MIP	Tetracycline	10 ^{−8} M to 10 ^{−5} M	[235]
SPR with MIP/NPs	Ciprofloxacin	3.21 ppb in ultrapure water 7.1 ppb in synthetic wastewater	[236]
Sandwiched silver microsphere/TiO ₂ nanoparticles/MIP structure with SERS	Sulfamethazine	3.6 × 10 ^{−9} mol/L	[237]
Fe ₃ O ₄ @Ag@MIPs with SERS	Enrofloxacin hydrochloride	0.012 nM	[238]
Magnetic MIP with SERS	Cloxacillin	7.8 pmol	[239]
SERS with bAu@mSiO ₂ @MIP	Enrofloxacin	1.5 nM	[240]
SERS with MIPs on membrane materials	Spiramycin	0.027 nM	[241]
Optical nanocrystalline cellulose	Sulfanilamide	-	[242]
MIP with colorimetric detection	Tetracycline	0.4 µM	[243]
MIP with fluorescence detection	Metronidazole	1 to 200 µM/53.4 nM	[244]
U-bend optical fibers with MIP	Bisphenol A	30 ng/mL to 10 µg/mL	[245]
Ratiometric fluorescence biomimetic sensor based on Mg,N-CDs/Eu-MOFs@MIP	Tetracycline	0.56 µM	[246]
Fluorescent sensor based via a facile encapsulation of Mg,N-CDs into MIP	Tetracycline	5–100 ng mL ^{−1} /0.79 ng mL ^{−1}	[247]
Mg,N-CDs/Eu-MOFs@MIP fluorescence sensor	Oxytetracycline	0.02–50 µg/mL/ 6.6 ng/mL	[248]
Fluorescent sensor based on molecularly imprinted magnetic halloysite nanotubes (referred to as MHNTs@FMIPs)	Oxytetracycline	10–300 nM/ 8.1 nM	[249]
Molecularly imprinted fluorescent optical fiber sensor (MIFOFS)	Ciprofloxacin	/6.86 µM	[250]
Molecularly Imprinted Two Dimensional Photonic Crystal Hydrogel Sensor	Levofloxacin	10 ^{−10} to 10 ^{−4} M	[251]
Photonic Crystal Hydrogel Sensor (SMZ–MIPCH) with MIP	Sulfamethoxazole	10 ^{−16} mol L ^{−1} to 10 ^{−10} mol L ^{−1} /10 ^{−16} mol L ^{−1}	[252]
CsPbBr ₃ quantum dots and MIPs	Tetracycline	0.2–5.0 µM/28 nM	[253]

MIPs overcome the shortcomings of antibodies, associated with their short lifespan and difficulty in storage. They are formed by fixing a functional monomer (a molecule of

a specific antibiotic) on a specific substrate, thus forming a specific component for detecting the antibiotic.

For improving control over the formation of MIP films, photopolymerization was used, and MIP films were made that were uniform and controlled in thickness. For the specific detection of ciprofloxacin and its structural counterpart ofloxacin, an SPR-based sensor was created in conjunction with a nanoscale MIP film as a recognition element [230].

The determination of neomycin, kanamycin, and streptomycin in milk by using MIP films and gold nanoparticles for signal enhancement was described in [231].

For real-time antibiotic monitoring, an analytic method combining a label-free sensor platform with a MIP as a potent recognition component was helpful. A highly selective hybrid organic–inorganic MIP film was incorporated into an SPR sensor. This film showed a binding affinity for the antibiotic that was about 16 times greater than that of the same reference non-imprinted polymer. Rebinding and regeneration were carried out in multiple cycles with good repeatability. The stability of the sensor can be maintained at room temperature for up to six months [232].

An SPR-based sensor was combined with MIPs for the detection of tetracycline [233], ciprofloxacin, moxifloxacin, and ofloxacin [234].

MIPs were combined with an SPR-based fiber optic sensor for the determination of tetracycline in food products [235]. The sensor was made by depositing layers of silver film, silver nanoparticles, and a MIP film (made using the tetracycline molecule as a template) onto the bare core of a multimode optical fiber. After light was directed from one end of the fiber using a polychromatic light source, the absorption spectra for a certain concentration of the tetracycline solution around the probe at the other end of the fiber were measured using a spectrometer that was connected to a computer. The peak attenuation wavelength shifted by 102 nm due to the sensor. The sensitivity of the sensor was about double that of a sensor based on LSPR and molecular imprinting.

Another study described a nanosensor based on the SPR in combination with molecularly imprinted nanoparticles (MIP/NPs) for the sensitive and selective detection of ciprofloxacin in ultrapure water and synthetic wastewater. The sensor could be reused with high reproducibility of results [236].

A key step in the development of bioanalytical methods is to find the optimal method for sample preparation. Molecularly imprinted SERS is a potential analytical method for antibiotic detection, thanks to the combination of the matrix MIP, a “smart” material capable of increasing sample preparation selectivity, with SERS substrates (Au, Ag, CuNPs) to make a new material capable of overcoming the existing limitations of SERS detection [237,238,254].

For SERS-based detection of porcine serum cloxacillin, magnetic molecularly imprinted polymer (MMIP)-based sample preparation was combined with a silicon nanopillar-based biosensor [239]. Gold–silica molecularly printed polymer nanoparticles (bAu@mSiO₂@MIP) with a specific ability to recognize enrofloxacin were prepared and used as label-free nano-sensors for the specific SERS detection of enrofloxacin [240].

The potential of combining SERS with MIPs was shown for the detection of spiramycin [241]. An optical sensor based on MIP resin, with nanocrystalline cellulose being used as a template, was developed for the detection of sulfonamides. The synthesized composite film showed a color response to sulfanilamide, visible to the naked eye. This is associated with the reassembly of imprinted regions within the chiral structure, which results in the formation of a yellow reflective film. When exposed to various antibiotics, it can selectively respond to three sulfonamides simultaneously [242].

As shown in the previous section, nanozymes are commonly used for antibiotic detection [10]. Despite all the advantages of nanozymes, they have one drawback, which is

the lack of substrate-specific catalytic specificity. It is difficult to achieve highly selective detection of nanozymes without the use of biological components (aptamers, antibodies, etc.). For solving this problem, a colorimetric assay for the highly accurate determination of tetracycline was developed by adding molecularly imprinted sites to the surface of the Fe_3O_4 nanozyme. The proposed Fe_3O_4 @MIP had multiple channels for substrates to reach the Fe_3O_4 core and showed peroxidase mimetic activity, catalyzing the oxidation of colorless 3,3',5,5'-tetramethylbenzidine to blue. The detection limit of tetracycline ($0.4 \mu\text{M}$) was tested in environmental water. The molecularly imprinted nanozyme is easily extracted and recovered for further use because of its magnetic properties [243].

Polydopamine was self-polymerized onto the MIL-53(Fe) surface by using MIP technology. The MIL-53(Fe)@MIP composite was chosen to develop a fluorescence sensor for metronidazole detection owing to its increased peroxidase-like activity and precise target recognition function. An extra luminous substrate was not needed, because the terephthalic acid substrate was integrated into the MIL-53(Fe)@MIP framework. This prevented substrate interference in the enzymatic detection system and increased analytic precision [244].

An alternative method for detecting bisphenol A in water combined localized surface plasmon sensing with in situ molecular imprinting on gold nanoparticles mounted on U-shaped optical fibers. Direct formation of a MIP layer occurred on top of gold nanoparticles [245]. In [246], a novel concept was proposed for the dual detection of tetracycline, by using the internal filter effect and the detection of heat transfer resistance at the solid–liquid interface.

A sensitive fluorescent nanosensor was developed that is based on the encapsulation of magnesium–nitrogen co-doped carbon dots (Mg,N-CD) within a molecularly imprinted polymer (Mg,N-CD@MIP). The polymer had excellent optical stability and good recyclability [247]. The capabilities of MIPs have been shown for the determination of oxytetracycline in actual environmental samples when combined with fluorescence sensors [248] and when MIPs were combined with magnetic halloysite nanotubes (called MHNTs@FMIPs) [249].

A multi-mode fluorescent optical fiber sensor in combination with replaceable molecularly imprinted nanoparticles composite polyethyleneglycol diacrylate hydrogel was fabricated for the rapid and highly selective detection of ciprofloxacin in environmental water samples. The resulting sensor had the ability of being detachable and replaceable, which makes it promising for use as a portable, handheld device for the rapid on-site detection of antibiotics in water [250].

A molecularly imprinted two-dimensional photonic crystal hydrogel sensor was used for the visual monitoring of levofloxacin [251]. A sulfamethoxazole molecularly imprinted two-dimensional photonic crystal hydrogel sensor was described by [252].

First discovered in the 1980s, QDs have quickly emerged as one of the most promising classes of nanomaterials owing to their exceptional optical tunability, quantum confinement effects, and surface modification capabilities. One way to improve the performance and specificity of QD-based sensors is to combine them with MIPs and develop dual-mode fluorescent–colorimetric systems [255]. The potential of combining QDs with MIPs was shown with the example of a fluorescent sensor for the determination of tetracycline [253].

Attention to MIPs stems from their unique properties, which enable highly selective molecular recognition of one type of target molecule in the presence of multiple other compounds with similar structures. Their undoubted advantages include methodological simplicity of preparation (in contrast, for example, to the preparation of antibodies to low-molecular-weight compounds, which is a multi-step and lengthy process) and high reproducibility of synthesis. MIPs are stable in aggressive environments and can be synthesized for virtually any substance—inorganic ions, drugs, nucleic acids, proteins,

cells, and even highly toxic compounds for which, for example, polyclonal antibodies cannot be developed. Despite the increasing body of research in this area, MIP-based sensors for antibiotics are not yet fully available commercially.

The main challenges in preparation are incomplete template elution and the coupling of homogeneous MIP layers to the sensor. MIPs have found wide application for pre-treatment of complex samples in a chromatography column before additional detection that is not suitable for MIPs.

It should be noted that when optical sensors are combined with MIPs, the latter can serve as an excellent sensor component, opening wide possibilities for the development of analytic methods. The use of MIPs strongly improves the characteristics of sensor analysis. MIPs are a suitable material for eliminating matrix interference for the effective determination of trace concentrations of antibiotics in the presence of several interfering and contaminating components [216,256,257].

4.8. Electro-Optical Approach for Antibiotic Detection

One of the newest methods for assessing the effects of antibacterial drugs on microbial cells is electro-optical monitoring. It involves measurement of the electrophysical and morphometric parameters of suspended microbial cells. This method combines the informative nature of the primary data obtained, the absence of special sample preparation, and the relative simplicity of the required instrumentation. The effect of the electric field on the cells during the measuring procedure is minimal and does not result in their death, and the influence of the supporting environment on the accuracy of measurement is negligible [56,258,259].

In biosensors based on the electro-optical effect, the primary analytic signal is generated not by the chemical interaction of the component being determined with the sensing element but by changes in the physical properties of cellular structures exposed to antibacterial drugs. Various theories of drug action are based on the idea that the substance binds to a specific receptor (often a membrane protein), causing a biochemical response that accelerates or decelerates a specific metabolic reaction and changes membrane permeability. When the electro-optical effect is used, an analysis is made of both the changes in the electrophysical properties of individual cellular structures and the overall effect of these changes owing to changes in cellular metabolism after exposure to an antibacterial drug.

The term electro-optical analysis implies a symbiosis of two methods: changing the electric properties of cellular structures and determining their changes by an optical method. The effect of an alternating electric field is of a probing nature and ensures the manifestation of the polarizability of cellular material at the interfaces of cellular structures: cytoplasm–membrane, membrane–cell wall, and cell wall–interface with the external environment. The magnitude of polarizability is determined by the ratio between the electrophysical parameters of adjacent structures. Because specific electric conductivity has a frequency dependence, polarizability will also have it. The integral parameter of polarizability of all structures for the mechanism of volume polarizability is the polarizability tensor α , which has a frequency dependence $\alpha(\omega)$. The difference between the longitudinal (along the cell axis length) and transverse (along the transverse axis) components of polarizability is available for measurements, which is called the polarizability anisotropy $\Delta\alpha(\omega)$ [56]. This parameter depends on the specific electric conductivity of cellular structures, their permittivity, and their size. Often, there is no need to decipher signals at interfaces. It is sufficient to study the $\Delta\alpha(\omega)$ deviation before and after exposure to an antibacterial drug and correlate it with changes in the most probable parameters of one or more cellular structures, such as cytoplasmic conductivity or membrane permittivity.

Optical measurements in electro-optical analysis focus on the recording of changes in polarizability. When cellular structures become polarizable after an electric field interacts with anisotropic cells, their orientation changes. The integral effect of a slight change in the orientation of a cell ensemble in a sample is a change in its optical properties. Electro-optical analysis most often measures changes in the attenuation of a suspension as a result of changes in light scattering parameters during cell orientation and disorientation. Figure 7 shows a diagram of a device for measuring electro-optical signals.

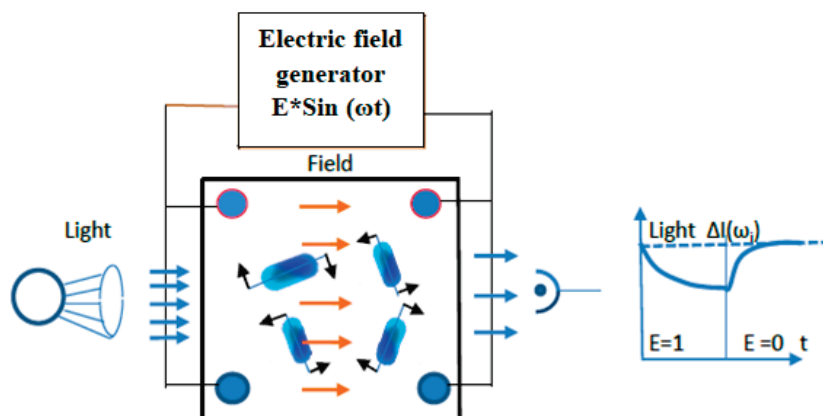


Figure 7. Diagram of a device for measuring electro-optical signals. Blue arrow- Light beam; Red arrow- vector of electric alternating field; Black arrow- direction of rotation force; $\Delta I(\omega_i)$ -change of light beam as function of electric field $E \cdot \sin(\omega t)$ action on the frequency ω_i .

In the absence of a field, cells are randomly oriented. After the action of an electric field at a frequency ω_i , partial orientation occurs owing to the generation of a torque that rotates the cells. The intensity of the transmitted light decreases, and the attenuation of the sample increases. After the electric field is turned off, the cells return to a chaotic state. Figure 8 shows an additional scheme for the chain of events on which is based the electro-optical measurements of antibiotic effect on cells.

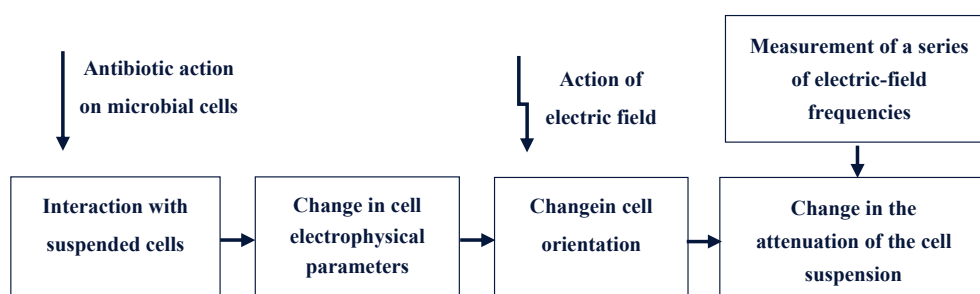


Figure 8. Schematic diagram of the device for measuring photoelectric signals.

There are several typical scenarios for analyzing the effect of antibacterial drugs on cells:

1. Surface adhesion, without changing cellular structure, facilitates shielding of the electric field and reduces the overall polarizability of the cells.
2. Surface adhesion contributes to changes in the electric characteristics and thickness of the cell wall.
3. Penetration of the antibacterial drug into the cell cytoplasm leads to a radical change in the recorded polarizability of the cell.
4. The effect of the antibacterial drug on the membrane contributes to changes in its electric properties or leads to active transport of ions from the cytoplasm and a change in its specific electric conductivity.

Each scenario manifests itself in different parts of the frequency dispersion $\Delta\alpha(\omega)$.

An electro-optical sensor was used to record the effects of antibiotics on bacteria and determine antibiotics in aqueous solutions [56,260–262]. The measurement was based on the inhibitory effect of antibiotics on microbial cells sensitive to the analyte. In [263], an electro-optical sensor was used for ampicillin detection with the help of *Pseudomonas aeruginosa* TSh-18 and a photoelectric sensor.

Electro-optical analysis can be used to identify antimicrobial agents in aquatic environments. Its main advantage is the ability to conduct studies without the use of appropriate labels in short periods without the need for lengthy sample preparation procedures.

5. Discussion and Prospects

Of the available antibiotic detection methods, optical biosensors offer a big advantage owing to their speed of analysis, as they provide virtually instantaneous information on the presence of the analyte in real time. Recent research into the development of optical sensors has shown their heavy demand for antibiotic detection, as evidenced by statistical data from an analysis of published papers. Fluorescence, interferometers, SERS, and SPR-based sensors clearly have the advantage among optical sensor systems (accounting for 83% of all published papers on optical sensors). Fluorescence and SPR sensors allow the detection of residual antibiotic concentrations and are very easy to use. The use of interferometers permits the sensor's size to be reduced without loss of detection sensitivity. A strong advantage of SPR sensors is their reusability [204].

The sensing element in the great majority of SPR spectroscopy-based biosensors is a prism with a base covered with a noble metal layer. The sensitivity of the biosensor can be improved by modifying the structure of this metal sheet. On the other hand, using diffraction gratings or optical fiber as the detecting element's base makes the sensor not only smaller but also more sensitive. SPR spectroscopy-based sensors may operate in a broad concentration range and detect biomolecules at concentrations as low as 100 pM thanks to the different plasmon-excitation schemes and architectures. The ability to get an analytic signal in the presence of interfering components, the ability to do repeated studies in real time without losing sensitivity, and the mobility of the sensor itself are the primary challenges in the development of optical sensors.

Because synthesized MIPs show exceptional stability at high temperatures and pressures in acidic and alkaline environments, and because they are easily stored, optical sensors in conjunction with MIPs have attracted special attention as a solution to these issues. This makes them extremely promising for the analysis of complex samples. MIPs offer ultra-sensitive antibiotic identification in complicated matrices when paired with signal amplification. However, the application of novel nanotechnologies can help standardize MIP production and increase the detection rate. One potential remedy that could enhance the future development and commercialization of biosensors is advancements in MIP development.

A separate area of optical sensor development is electro-optical technology, which enables analysis based not on the chemical interaction of the component being determined with the sensing element but on changes in the physical properties of cellular structures exposed to antimicrobial agents. This technology is promising for antibiotic detection, because it requires no special sample preparation, is easy to use, and provides results within minutes. Furthermore, the sensor itself can be reused multiple times without loss of sensitivity. This promising area of research highlights the potential of electro-optical sensors for antibiotic detection.

A major advance in sensor portability and miniaturization is the use of optical components from smartphones and their integration into biosensors. For example, a sensor

was developed for detecting bisphenol A in water samples by using fluorescence, with an unfiltered detection limit of 4.4 μM from unprocessed samples, suitable for waste leachate and industrial samples [264]. This sensor represents the first approach to the mobile-phone assisted detection of contaminants in water.

A key development in optical sensors is the implementation of machine learning, which can detect patterns in the analysis of complex biological systems. Machine learning will improve optical biosensor performance by (1) simplifying the analysis of raw biosensor output data for data classification, prediction, and prevention of hazardous events and also by (2) identifying data groups with common characteristics [265]. Thus, optical biosensor signals can be analyzed in real time to develop meaningful output paths in algorithms that differentiate between high- and low-quality images [266].

6. Conclusions

This paper has presented the main types of optical biosensors (colorimetric, fluorescence, luminescence, SERS, SRS, photonic crystal, fiber, MIP, and electro-optical) and their potential application in antibiotic detection. The advantages and disadvantages of each of the described sensor systems have been discussed in relation to the task at hand, which involves antibiotic detection. Prospects for the further development of optical sensor systems have been outlined.

Optical biosensors are promising diagnostic tools that combine rapidity of detection of specific molecular markers, simplicity, ease of use, efficiency, accuracy, and cost-effectiveness with the trend toward portable platforms. These qualities exceed generally accepted standards for microbiological and immunological diagnostics and open up broad prospects for the use of these analytic systems in antibiotic detection. When developing new biosensors for practical use, numerous biosensor properties should be considered, such as reliability, reproducibility, simplicity, and shelf life. Further standardization and automation of optical sensor systems will expand their application for antibiotic detection in environmental protection.

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Review

A Comprehensive Review of Agricultural Residue-Derived Bioadsorbents for Emerging Contaminant Removal

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Abstract: The increasing presence of ECs in aquatic environments has drawn significant attention to the need for innovative, accessible, and sustainable solutions in wastewater treatment. This review provides a comprehensive overview of the use of agricultural residues—often discarded and undervalued—as raw materials for the development of efficient bioadsorbents. Based on a wide range of recent studies, this work presents various types of materials, such as rice husks, sugarcane bagasse, and açai seeds, that can be transformed through thermal and chemical treatments into advanced bioadsorbents capable of removing pharmaceuticals, pesticides, dyes, and in some cases, even addressing highly persistent pollutants such as PFASs. The main objectives of this review are to (1) assess agricultural-residue-derived bioadsorbents for the removal of ECs; (2) examine physical and chemical modification techniques that enhance adsorption performance; (3) evaluate their scalability and applicability in real-world treatment systems. The review also highlights key adsorption mechanisms—such as π - π interactions, hydrogen bonding, and ion exchange—alongside the influence of parameters like pH and ionic strength. The review also explores the kinetic, isothermal, and thermodynamic aspects of the adsorption processes, highlighting both the efficiency and reusability potential of these materials. This work uniquely integrates microwave-assisted pyrolysis, magnetic functionalization, and hybrid systems, offering a roadmap for sustainable water remediation. Finally, comparative performance analyses, applications using real wastewater, regeneration strategies, and the integration of these bioadsorbents into continuous treatment systems are presented, reinforcing their promising role in advancing sustainable water remediation technologies.

Keywords: agricultural residues; ECs; adsorption mechanisms; activated biochar; environmental pollutants; circular economy; regeneration

1. Introduction

In recent years, research focused on the removal of ECs (ECs) has gained prominence due to the potential risks these compounds pose to human health and ecosystems on a global scale. These chemical substances are commonly detected at trace concentrations, ranging from nanograms to micrograms per liter, which makes their removal through

conventional treatment methods a significant challenge [1,2]. Among ECs, various types of chemical substances are present, including microplastics, dyes, pharmaceuticals, pesticides, personal care products, heavy metals, and industrial additives, among others [3–6].

Given the diversity and structural complexity of ECs, many of these compounds are non-biodegradable and pseudo-persistent, which significantly hinders their removal through conventional water treatment methods. In addition to their resistance to degradation, these pollutants exhibit environmental behaviors that are difficult to detect, with toxicological effects and removal mechanisms that remain not fully understood after their release into aquatic environments [7,8].

Although several advanced technologies have been investigated to mitigate the presence of these compounds, their application is still mainly limited to laboratory scale due to high operational costs, energy consumption, technical complexity, and the potential formation of toxic by-products. In this context, adsorption emerges as an effective, environmentally safe, and operationally simple alternative, especially when combined with the use of agricultural residues. These residues serve as precursors for adsorbent materials, enabling both contaminant removal and the valorization of agricultural by-products within a sustainable framework [9].

Globally, agricultural activity generates nearly 5 billion tons of waste annually, representing an immense resource that remains largely underutilized [10]. The challenge lies in converting these waste materials into valuable resources through sustainable processes. Recent studies have demonstrated that agro-industrial waste can be effectively upcycled into activated carbons, especially for wastewater treatment applications involving complex pollutants such as dyes [11].

Currently, research efforts are focused on developing innovative materials from agricultural by-products such as rice husks, cassava peels, nut shells, açai seeds, and other plant-based residues. These adsorptive materials have garnered considerable attention due to their high potential for removing environmental pollutants, particularly when converted into activated carbon or biochar [9]. Carbonization followed by physical or chemical activation significantly enhances surface area, as demonstrated by [12], who used coconut shells to produce activated carbon with specific surface areas reaching up to 2500 m²/g. Lignocellulosic materials, which account for approximately 45% of the raw materials used in activated carbon production, are characterized by high volatile matter and low inorganic contents, properties that promote the development of well-defined porous structures [13]. Therefore, in addition to possessing intrinsic properties favorable for adsorption, these materials contribute to the valorization of agro-industrial waste and help mitigate the environmental impacts associated with improper waste disposal.

2. Agricultural Residues

Agricultural residues are biomass-rich by-products generated throughout the agricultural production chain, including land preparation, harvesting, consumption, and agro-industrial processing. They can be classified in different ways: harvest residues (straw, leaves, cobs), industrial processing residues (sugarcane bagasse, fruit peels), livestock waste, and fruit residues. Among the most used precursors for adsorbent materials are rice husks, sugarcane and grape bagasse, corn stalks, cassava peels, peanut shells, and açai seeds [14].

It is estimated that thousands of tons of these residues are discarded into the environment every year, raising concerns about their proper disposal and the mitigation of their environmental impacts. Therefore, to reduce this surplus and promote its valorization, the use of agricultural and industrial residues for pollutant removal has gained increasing attention, especially considering the growing intensification of agricultural activities

over recent decades [10,15]. In this context, utilizing such materials as adsorbents represents a promising and sustainable alternative, provided that their chemical and structural composition is well understood. Understanding the physicochemical characteristics and properties of these residues is crucial for developing effective and selective adsorbents that can efficiently remove ECs [16].

Their lignocellulosic composition makes these residues valuable precursors for the development of sustainable adsorbents (Figure 1). Cellulose, estimated to account for 35–50% of residue biomass, is the main structural component, providing rigidity and stability to the plant cell wall. It is widely present in materials such as corncobs, pineapple fibers, rice straw, and cotton stalks. Hemicellulose (20–35%), composed of a heterogeneous mix of sugars, acts as a binder between cellulose and lignin, exhibiting an amorphous structure and high susceptibility to chemical degradation [17]. Lignin, on the other hand, contributes to mechanical strength and rigidity, and its complex aromatic structure favors π - π interactions with aromatic compounds—making lignin-rich residues particularly effective in the adsorption of pharmaceuticals and dyes [18]. Therefore, the combined presence of lignin, cellulose, and hemicellulose provides a complex matrix rich in functional groups ($-\text{OH}$, $-\text{COOH}$, $-\text{OCH}_3$), enabling interactions with a wide range of ECs [19].

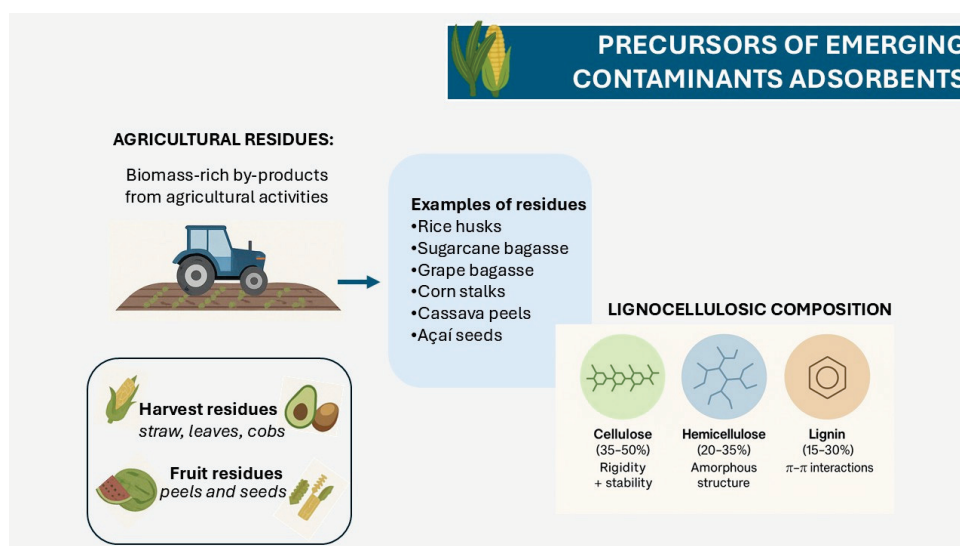


Figure 1. Schematic representation of agricultural residues and their lignocellulosic composition as precursors for adsorbents targeting ECs.

3. Preparation and Modification of Adsorbents

Agricultural residues have attracted increasing attention as sustainable precursors for bioadsorbent development due to their abundance, renewability, and inherent surface functionality. The performance of these materials in removing contaminants from wastewater is directly linked to their physicochemical properties, which are influenced by the methods used in their preparation and modification. Therefore, this section presents the main approaches employed in the synthesis and functionalization of agricultural-residue-based adsorbents, with a focus on pyrolysis, chemical activation, and surface magnetization.

Before delving into the more advanced modification techniques, it is essential to highlight that raw or minimally treated agricultural residues also exhibit promising adsorption capacities for a wide range of emerging contaminants, including compounds of high environmental persistence. For instance, perfluoroalkyl substances (PFASs), notably perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), have been effectively removed using low-temperature pyrolyzed biochars derived from materials such as peach stones, spent coffee grounds, and sawdust. These materials demonstrate

adsorption via hydrophobic partitioning and electrostatic interactions, even without full carbonization [20,21]. Similarly, pinewood-derived and reed straw biochars produced under moderate pyrolysis conditions achieved removal efficiencies of up to 169 mg/g and 80%, respectively [22,23]. These findings underscore that non-activated or mildly activated biochars can serve as viable adsorbents in scenarios where simplicity and cost-effectiveness are essential. Thus, the valorization of agricultural residues through low-energy conversion pathways remains a complementary and attractive strategy to the more intensive modification routes explored in the following subsections.

3.1. Pyrolysis Techniques: Conventional Pyrolysis vs. Microwave-Assisted Pyrolysis

Pyrolysis is a thermochemical conversion technique widely applied for the valorization of lignocellulosic biomass, carried out in the absence of oxygen or the presence of a controlled amount of air. During this process, components such as cellulose, hemicellulose, and lignin are thermally decomposed, yielding three primary products: biochar, bio-oil, and non-condensable gases [24,25]. This conversion enables the production of biochar with high energy density and potential applications as an adsorbent, solid fuel, soil conditioner, or precursor for carbon-based materials. Meanwhile, the generated gases (mainly H_2 , CO, CO_2 , and light hydrocarbons) can be used for energy generation or upgraded into higher-value compounds, provided they exhibit an appropriate $H_2:CO$ ratio [8,9]. Bio-oil, in turn, is a complex and viscous mixture of oxygenated compounds, with high water content and a higher heating value than the original biomass, making it a promising alternative source of liquid energy [26,27].

Pyrolysis can be classified into four main categories based on heating rate, temperature, and residence time: slow, intermediate, fast, and flash pyrolysis [28]. Slow pyrolysis is characterized by low temperatures (300–400 °C), heating rates of 5–7 °C/min, and prolonged residence times, favoring high biochar yields. Intermediate pyrolysis operates between 400 and 500 °C with moderate heating rates (1–70 °C/min) and generates both liquid products and biochar in a balanced ratio. Fast pyrolysis (400–700 °C, heating rate of ~200 °C/min for 2 min) and flash pyrolysis (>500 °C, >1000 °C/min, <2 s) prioritize the production of bio-oil and gases, resulting in lower biochar production. Thus, the higher the heating rate, the greater the formation of liquids and gases; lower rates favor biochar [29].

Microwave-assisted pyrolysis (MAP) emerges as a promising alternative to conventional pyrolysis, employing electromagnetic radiation to heat the biomass rapidly, volumetrically, and selectively [29,30]. In this process, microwaves interact directly with the polar molecules of the biomass, promoting more uniform internal heating, in contrast to conventional heating based on conduction, convection, and radiation [31]. The main advantages of MAP include the higher energy efficiency, precise temperature control, reduced residence time, lower tar formation, and greater development of the porous structure of biochar [32,33]. Additionally, microwave reactors do not require physical contact with heated surfaces, which reduces thermal losses and facilitates the processing of moist or viscous biomass.

However, the performance of MAP is conditioned by the dielectric permittivity of the biomass, which determines its ability to absorb microwave energy. Biomass with low permittivity requires auxiliary absorbers, such as metal oxides, graphite, or silicon carbide, to enable efficient heating [33]. Other technical challenges include the formation of hotspots (overheating zones), greater system complexity, and high infrastructure and operating costs, factors that still limit the large-scale feasibility of MAP [34,35]. Table 1 compares the main operational aspects of conventional pyrolysis and MAP.

Table 1. A comparison between the main operational and structural characteristics of conventional pyrolysis (CP) and microwave-assisted pyrolysis (MAP), highlighting the advantages and limitations of each approach in the conversion of lignocellulosic biomass.

Criterion	Conventional Pyrolysis (CP)	Microwave-Assisted Pyrolysis (MAP)
Heating mode	External, by conduction or radiation	Volumetric and selective (direct interaction with biomass molecules)
Heating rate	Slow	Fast
Heating uniformity	Lower uniformity (temperature gradients)	More uniform heating
Energy efficiency	Lower efficiency	High energy efficiency
Residence time	Long	Short
Process control	Simpler control, less precise	Easier automation and precise control
Interaction with material	Independent of dielectric constant	Dependent on dielectric constant (requires suitable biomass or absorber)
Need for physical contact	Yes (heat transferred via contact with hot surfaces)	No (contactless heating)
Main products	Biochar (high stability), bio-oil, non-condensable gases; lower syngas yield, higher tar formation	Biochar with higher surface area, less tar, H ₂ -rich gas
Industrial application	Broad application and well-established technology	Still limited due to equipment cost and dielectric restrictions

Recent studies emphasize the advantages of microwave-assisted pyrolysis (MAP) over conventional pyrolysis (CP), particularly in energy efficiency, processing time, and the control of biochar porosity. Durán-Jiménez et al. [36] showed that MAP combined with H₃PO₄ activation produced activated carbons with surface areas over 1100 m²·g^{−1} in only 2 min, with yields up to 64% and high CO₂ selectivity (4.1 mmol·g^{−1} at 0 °C). Although both methods resulted in similar surface areas—1260 m²·g^{−1} for CP and 1145 m²·g^{−1} for MAP—the MAP sample had superior CO₂ adsorption due to a higher proportion of ultrafine micropores (<0.7 nm). MAP also favored the formation of hierarchical porosity and reduced structural collapse, attributed to rapid energy absorption by the H₃PO₄-impregnated biomass, which increased dielectric permittivity and internal heating efficiency, resulting in improved textural properties and energy savings of 2.4 GJ·ton^{−1}.

Zhou et al. [37] compared conventional and microwave-assisted pyrolysis (MAP) for the treatment of pharmaceutical sludge and found significant differences in product properties. Under the same temperature (600 °C), MAP biochars exhibited higher surface area (146 vs. 92 m²·g^{−1}), attributed to more efficient volumetric heating, which enhances porosity and prevents structural collapse. MAP also resulted in better adsorptive performance, achieving 99% tetracycline (TC) removal, superior to that of conventional biochar. In terms of pyrolytic liquids, MAP produced oils with fewer oxygenated compounds and more aromatic hydrocarbons, and significantly increased combustible gas production (LHV: 21.45 vs. 11.62 MJ·Nm^{−3}).

While both CP and MAP are applicable for the thermochemical conversion of lignocellulosic and organic residues, they differ in operational, energetic, and structural characteristics. CP offers simplicity, lower equipment costs, and broad biomass applicability, but relies on slower external heating, has a lower energy efficiency, and may cause structural collapse [37]. MAP, by contrast, provides a higher energy efficiency, shorter processing time, and improved functional selectivity due to its volumetric and dielectric heating, though it requires biomass dielectric compatibility and faces challenges such as hotspot formation and high infrastructure costs [36].

Despite the advances highlighted in recent studies, the application of microwave-assisted pyrolysis (MAP) still faces significant limitations for its large-scale consolidation.

Meng et al. [38] demonstrated that increasing microwave power can improve the structure of the produced biochar and its adsorptive capacity for leachate treatment, but it also reduces the yield (from 50% to 35%) and requires the strict control of parameters such as temperature and residence time. Even in systems with more uniform heating, the specific surface area remained relatively low (max. $23 \text{ m}^2/\text{g}$), indicating the need for additional activation strategies.

Additionally, high infrastructure and operating costs impact MAP's economic feasibility. Its sustainability relies on the durability and regeneration of biochar across multiple cycles. These challenges highlight the importance of standardizing operational conditions and optimizing energy use to establish MAP as a scalable and environmentally viable solution. Furthermore, biochar properties are also directly influenced by process parameters such as temperature, residence time, atmosphere, biomass composition, and particle size, as discussed in the next section.

3.1.1. Key Influencing Factors

Temperature, residence time, and reaction atmosphere have a decisive influence on the efficiency and selectivity of pyrolysis, directly affecting the yield and quality of pyrolytic products. In conventional pyrolysis, the gradual increase in temperature and longer residence times favor the formation of biochars with greater thermal stability. In microwave-assisted pyrolysis (MAP) systems, heating is volumetric and extremely rapid, promoting selective reactions in a shorter time, but at the risk of hotspot formation and structural collapse. The atmosphere, in turn, defines the oxidizing or reducing nature of the environment, potentially acting as a physical activating agent or negatively influencing the composition of the products.

In addition to these classic parameters, other operational factors also significantly affect process performance, especially in MAP. These include biomass composition, particle size, applied power, and the use of absorbers or catalysts.

Biomass composition is a key factor in the yield and quality of pyrolytic products, especially biochar. Lignocellulosic biomasses with higher lignin contents tend to produce more stable and higher amounts of biochar due to lignin's greater thermal resistance. In contrast, cellulose and hemicellulose contribute mainly to the volatile fraction, favoring bio-oil formation [35]. Moreover, chemical composition can vary depending on soil type, plant age, and climatic conditions, which underscores the importance of the proper characterization of the feedstock [39].

At higher temperatures ($>600^\circ\text{C}$), there is an increase in porosity and the formation of gaseous and aromatic compounds, with a lower biochar yield [28]. How this temperature is reached also influences the resulting products: rapid heating to temperatures below 650°C followed by abrupt cooling favors the formation of liquid products; on the other hand, slow heating to high temperatures maximizes biochar production [35]. In MAP, the applied power determines the heating rate, and excessive temperatures may lead to the secondary pyrolysis of volatiles, reducing bio-oil yield and favoring the formation of non-condensable gases [28,35].

While conventional pyrolysis is less sensitive and predominantly uses N_2 , MAP is highly influenced by the reaction medium, since the electromagnetic field interacts directly with the dielectric permittivity of the environment. The presence of CO_2 or steam can act as a mild physical activating agent, modifying the porosity of the biochar and affecting its yield [28,38,40].

Other factors, such as biomass particle size, directly influence the thermal efficiency of microwave-assisted pyrolysis. Smaller particles ($<0.25 \text{ mm}$) promote uniform heating and reduce the formation of hotspots—overheated regions capable of causing thermal stress

and the structural collapse of the biochar [40,41]. In contrast, larger particles travel through zones with varying electromagnetic field intensities, generating multiple localized heating points [42]. In addition to particle size, the position of the sample within the reactor is also critical, as lower regions of the cavity concentrate higher electric field intensities and promote more efficient heating [41].

The use of absorbers and catalysts is essential in MAP for biomasses with low dielectric constants. Absorbing materials are crucial for such biomasses because they enable effective coupling with the electromagnetic field, promoting the conversion of microwave energy into heat. These absorbers are classified into two main types: (i) dielectric loss absorbers, such as graphite, activated carbon, and silicon carbide, which function through mechanisms like interfacial polarization, dipolar polarization, and electrical conduction; (ii) magnetic loss absorbers, such as metallic ferrites and Fe, Ni, or Co particles, which operate via magnetic resonance, hysteresis, and eddy currents.

In addition, many of these materials also act as catalysts, influencing product selectivity and increasing yield [40]. In conventional pyrolysis, although less common, metallic catalysts like Fe and Ni are also used to modulate secondary reactions and enhance the formation of gases.

Table 2 summarizes these comparative effects, allowing an integrated view of the operational differences between the two systems and guiding the selection of the most suitable conditions according to the type of biomass and the desired product.

Table 2. Comparative influence of operational parameters on conventional pyrolysis (CP) and microwave-assisted pyrolysis (MAP), highlighting general effects on process efficiency, product quality, and biochar performance.

Parameter	Conventional Pyrolysis (CP)	Microwave-Assisted Pyrolysis (MAP)	General Effects
Biomass composition	Less sensitive to dielectric constant; cellulose and lignin yield more stable biochar [35]	Highly dependent on dielectric permittivity; affects energy absorption [43]	Biomass rich in cellulose and lignin results in more aromatic and stable biochar
Particle size	Larger particles (>0.25 mm) require more time for complete carbonization [29]	Smaller particles (<0.25 mm) favor uniform heating and avoid hotspots [40]	Smaller particle size enhances conversion efficiency and increases surface area
Temperature	Gradual increase (up to >600 °C); favors biochar graphitization and carbonization [37]	Rapid and intense; forms micropores and aromatic compounds in minutes (>600 °C) [36]	Higher temperatures reduce biochar yield while increasing porosity and production of gases and bio-oil
Residence time	Long (minutes to hours); progressive carbonization [28]	Short (seconds to minutes); risk of hotspots [44]	Longer residence time improves structural stability but decreases yield
Atmosphere	Less sensitive; commonly uses N ₂ ; CO ₂ can act as mild activating agent [40]	Atmosphere affects microwave absorption by changing the dielectric permittivity; vapors (e.g., steam or CO ₂) can alter thermal field and promote mild physical activation of biochar [28,38]	Atmospheric conditions influence oxidation, activation mechanisms, and product composition

Table 2. Cont.

Parameter	Conventional Pyrolysis (CP)	Microwave-Assisted Pyrolysis (MAP)	General Effects
Pyrolysis power	Indirectly applicable via temperature and residence time [40]	Defines heating intensity; directly linked to hotspot formation [45,46]	Increased power accelerates reaction rate but increases risk of thermal collapse
Absorbers/catalysts	Less common use; catalysts like Fe and Ni applied to control temperature and increase combustible gas production	Essential for low-dielectric-constant biomass; includes dielectric loss absorbers (e.g., silicon carbide, graphite, charcoal) and magnetic loss absorbers (e.g., ferrites and metal particles like Fe, Ni, Co); influences yield, activation, and selectivity [40]	Catalysts and absorbers enhance product yield, activation efficiency, and selectivity

Thus, it can be observed that the operational parameters of pyrolysis—such as temperature, residence time, atmosphere, biomass composition, applied power, and particle size—act interdependently, directly affecting thermal efficiency, yield, and the quality of the generated products. The choice between these routes should be based on the type of biomass, the desired characteristics of the products, and the technical and economic limitations of each application. Additionally, the post-synthesis modification of adsorbents is a crucial step to enhance their structure and functionality. Techniques such as chemical activation and surface functionalization have been widely employed to increase surface area, introduce active functional groups, and improve performance in adsorption processes. The main approaches applied for this purpose are discussed below.

3.1.2. Energy Efficiency, Economic Feasibility, and Environmental Performance of MAP

Techno-economic assessments (TEAs) and life cycle analyses (LCAs) have recently advanced the understanding of microwave-assisted pyrolysis (MAP) performance at an industrial scale. Zhou et al. [47] reported that 2 kWh/kg of electricity was required to process wood pellets at 800 °C in a continuous MAP reactor, producing 13.7 MJ of syngas, 4.4 MJ of biochar, and 1.0 MJ of tar. This resulted in an overall energy efficiency of 73.3%, which exceeded that of conventional gasification (typically 40 to 65%). Similarly, the MAP of sewage sludge under optimal conditions (500 °C) achieved 87% energy recovery based on the higher heating value (HHV) of the feedstock [48]. However, energy consumption varies significantly depending on reactor design, feedstock characteristics, and operational parameters. Gao et al. [49] reported electricity demands ranging from 0.8 to 2.6 kWh/kg for textile sludge pyrolysis, while Mao et al. [50] observed values from 0.5 to 3.78 kWh/kg for furfural residue, depending on temperature and screw speed. These variations underscore the importance of optimizing process configurations.

Economically, MAP-derived biochars still face limitations. Haeldermans et al. [51] estimated the minimum selling price (MSP) of MAP biochar at EUR 564 to EUR 979 per ton, higher than that of conventional pyrolysis (CP) biochar (EUR 436 to EUR 863 per ton). However, MAP biochars generally exhibit higher surface areas, enhanced porosity, and lower contaminant levels, attributes that may justify premium pricing. Strategies to improve MAP feasibility include the use of microwave absorbers such as SiC and biochar, advanced thermal insulation, energy recovery from pyrolysis gases and char, and the use of low-frequency magnetrons for better scalability.

Additional evidence from Foong et al. [29] confirms that continuous MAP systems, when optimized with energy recovery and dielectric coupling, can achieve a net energy ratio (NER) greater than 0.9, surpassing that of CP. Moreover, the global warming potential (GWP) of MAP was reported to be 62% lower than that of CP under similar operating conditions. These findings highlight MAP's potential to reduce environmental impacts while enhancing energy performance.

Nonetheless, the industrial-scale deployment of MAP still faces technical and economic challenges, including high capital costs for efficient magnetrons, the need for microwave-compatible biomass, and difficulties in managing heat losses and reactor scale-up. According to Foong et al. [29], integrating heat recovery systems, adopting distributed processing models, and valorizing by-products such as upgraded bio-oil or hydrogen-rich gas are crucial strategies to improve MAP's techno-economic feasibility.

Therefore, although microwave-assisted pyrolysis offers clear advantages in terms of energy efficiency, process selectivity, and environmental impact, its large-scale implementation depends on reactor optimization, operational stability, and reduced energy and equipment costs. Addressing these factors in future studies will be essential to position MAP as a sustainable and economically viable alternative for biomass valorization.

3.2. Chemical Activation and Functionalization

Chemical activation primarily aims to increase surface area, develop porosity, and introduce active functional groups on the surface of adsorbents, significantly enhancing their ability to capture and remove contaminants from wastewater [52,53]. This process involves treating the precursor biomass with activating agents such as KOH, NaOH, CaCl_2 , H_3PO_4 , K_2FeO_4 , Na_2CO_3 , ZnCl_2 , or FeCl_3 , followed by heating to between 500 and 900 °C under an inert atmosphere. After pyrolysis, washing and drying steps are essential to remove excess activating agents and stabilize the carbonaceous structure [54,55].

Among chemical activating agents, KOH stands out for promoting high surface areas, microporosity, and reactivity, enabling activation at lower temperatures compared to physical methods [56]. In addition, chemical activation can introduce groups such as carboxyls, hydroxyls, and sulfonic groups, which enhance the adsorption of specific contaminants, such as heavy metals or organic dyes [52].

Experimental studies support these effects. According to Shang et al. [57], biochar activated with ZnCl_2 (ZABC-5) showed high efficiency in removing Cr(VI) (98.68%), outperforming material activated with H_3PO_4 (<40%). This performance was attributed to its surface area (34.58 m^2/g) and the presence of micro- and mesopores and functional groups ($-\text{OH}$, $\text{C}=\text{O}$, $\text{C}-\text{O}$) which acted through synergistic mechanisms such as pore filling, electrostatic attraction, ion exchange, and redox reactions. The material also exhibited high stability after four desorption cycles. However, the environmental impact of the process was considered significant (12.70 kg $\text{CO}_2\text{-eq}/\text{kg}$).

Kaya et al. [58] investigated the use of KOH-activated biochar derived from pine cones for the adsorption of Congo red dye, obtaining a material with an extremely high surface area (1714.5 m^2/g) and a removal efficiency of up to 94.62%. In the study, KOH activation played a crucial role in increasing the material's porosity and promoting the formation of functional groups, including $-\text{OH}$, $\text{C}=\text{O}$, and $\text{C}-\text{H}$, which facilitated electrostatic interactions, hydrogen bonding, and $\pi-\pi$ stacking with dye molecules.

Zhang et al. [59] investigated the production of KOH-activated biochar from wood modified with phenol-formaldehyde resin, obtaining a specific surface area of 2301.61 m^2/g and a pore volume of 1.205 cm^3/g , attributed to its hierarchical porous structure and the presence of structural defects introduced by the chemical modification of the precursor. The maximum adsorption capacities reached 3472.22 mg/g for Congo red

and 1112.35 mg/g for methylene blue. The adsorption mechanisms involved pore filling, electrostatic interactions, hydrogen bonding, and π - π interactions, with the adsorption best described by pseudo-second-order kinetics and the Langmuir isotherm, indicating chemisorption on a homogeneous surface. The prior modification of the biomass contributed to the generation of a greater number of active sites, demonstrating that precursor engineering, combined with the use of KOH, enhances performance.

Hasan Basri et al. [60] and Astuti et al. [61] explored the production of activated carbon from bulrush straw using microwave-assisted pyrolysis (MAP) and ZnCl_2 activation, aiming at the removal of crystal violet (CV) dye. In the first study, Hasan Basri et al. [60] investigated the influence of lignocellulosic composition, and the resulting activated carbon exhibited a surface area of 1141.9 m^2/g and a maximum adsorption capacity of 200.7 mg/g, highlighting the role of cellulose and lignin in pore formation and in generating active functional groups such as $-\text{OH}$ and $\text{C}-\text{O}$. In the second study, Astuti et al. [61] applied multivariate modeling and process optimization, identifying impregnation ratio, pyrolysis time, and ZnCl_2 concentration as key variables that directly influenced adsorption efficiency. Kinetic and isothermal analyses showed the good fit of the pseudo-second-order model and Temkin isotherm, indicating chemisorption with a uniform distribution of binding energies.

In both studies, the use of ZnCl_2 was essential for generating hierarchical porosity and surface functionalization, enhancing the adsorptive capacity of the materials. The application of MAP contributed to rapid and uniform heating, higher yield, and reduced formation of undesired by-products. However, limitations such as high equipment costs, difficulties in heating certain biomasses, and the need for the proper treatment of ZnCl_2 residues were noted. These results indicate that the combination of MAP and chemical activation with ZnCl_2 is effective for producing high-performance activated carbons designed to remove cationic dyes, such as crystal violet.

In addition to the structural improvements promoted by chemical activation, functionalization with groups such as amines ($-\text{NH}_2$), carboxyls ($-\text{COOH}$), and hydroxyls ($-\text{OH}$) enhances both selectivity and adsorption efficiency. These groups enable specific interactions with contaminants through hydrogen bonding, electrostatic forces, and surface complexation. The density of these functional groups depends on the activating agent, pyrolysis conditions, and possible post-synthesis modifications, such as liquid-phase, gas-phase, or ball-milling treatments, as reported by Liu et al. [62].

Despite these advances, gaps remain in the standardization of experimental protocols, which hinder systematic comparisons between different activating agents, pretreatments, and thermal conditions. The variability among studies limits the establishment of universal correlations between structure, functionality, and adsorption performance. Few studies address long-term stability, synergistic effects between functional groups, and behavior in the presence of complex pollutants. Therefore, standardized comparative approaches and the development of multifunctional adsorbents with properties tailored for specific environmental applications are needed [62].

3.3. Surface Engineering and Magnetization

Despite the numerous benefits of adsorbents such as biochar and activated carbon, challenges remain in the efficient recovery of these materials after the adsorption process. To overcome this limitation, magnetic adsorbents have recently been synthesized as an alternative to conventional separation techniques such as centrifugation, sedimentation, and filtration. The incorporation of magnetic properties enables the recovery of the adsorbent using magnetic fields—either by electromagnets or permanent magnets—promoting greater practicality and operational cost savings [63].

Among the most used magnetic materials are iron oxide nanoparticles, such as maghemite ($\gamma\text{-Fe}_2\text{O}_3$) and magnetite (Fe_3O_4), due to their high surface area and ability to interact with a wide variety of contaminants [64]. These oxides exhibit a spinel-type crystalline structure (AB_2O_4), with the distribution of metal cations in tetrahedral and octahedral positions. In the case of magnetite, the inverse structure favors the presence of Fe^{3+} ions in both positions, while Fe^{2+} ions preferentially occupy the octahedral sites [65,66].

In addition to their structural properties, magnetic nanoparticles possess a negative surface charge, which favors the adsorption of cationic pollutants. The combination of these particles with materials containing different ligands increases their affinity for specific compounds, such as heavy metals, dyes, microplastics, and antibiotics. Nanotechnology combined with magnetic separation has proven effective in producing regenerable and selective adsorbents, with applications in the treatment of contaminated water [63,65].

Several studies have explored magnetization strategies applied to different biomasses. Hao et al. [67] produced a porous magnetic biochar from corn straw lignocellulosic residue, modified with Fe_3O_4 via co-precipitation using K_2FeO_4 . The resulting material, FeCS800, exhibited high adsorption capacities for Orange G dye (327.87 mg/g) and tetracycline (243.90 mg/g), maintaining stable performance after five regeneration cycles. Its specific surface area and pore volume reached $866.44 \text{ m}^2/\text{g}$ and $0.44 \text{ cm}^3/\text{g}$, respectively—132 and 22 times greater than the unmodified biochar. The adsorption mechanisms involved electrostatic interactions, π - π stacking, ion exchange, and pore filling, supported by FTIR analysis ($-\text{OH}$, $\text{C}=\text{O}$, $\text{C}-\text{O}$ groups) and magnetic hysteresis (64.08 emu/g), indicating easy magnetic recovery. A high removal efficiency was also observed in natural water, attributed to the formation of abundant oxygen-containing functional groups that form inner-sphere complexes. Additionally, the decreased Fe peak intensities after adsorption suggested that iron oxides acted as extra-active sites, highlighting the importance of magnetic functionalization in adsorption performance.

Peng et al. [68] used corn straw biochar biomodified with phosphate-solubilizing strains and magnetically functionalized with Fe_3O_4 to investigate Pb^{2+} ion adsorption, achieving removal rates above 97%. The specific surface areas of the modified materials ranged from 35.70 to $46.70 \text{ m}^2/\text{g}$, representing a significant increase compared to the unmodified biochar ($\text{BC-0} = 8.56 \text{ m}^2/\text{g}$). The adsorption mechanisms included ion exchange, surface complexation, and phosphate precipitation, facilitated by surface functionalization with oxygen-containing groups. Microbial modification, by increasing the density of functional groups, played a key role in enhancing adsorption efficiency and metal ion passivation, providing a synergistic approach between chemical and biological functionalization.

Duan et al. [69] evaluated the removal of polystyrene microplastics using magnetic biochar derived from rice straw. The material had a specific surface area of $355.4 \text{ m}^2/\text{g}$ and showed surface-functionality-dependent selectivity. FTIR analysis identified hydroxyl and carboxyl groups as key to adsorption via hydrophobic interactions, π - π stacking, Van der Waals forces, hydrogen bonding, and electrostatic attraction. The biochar demonstrated high regeneration stability and maintained performance across multiple cycles. Microplastics with higher surface functionalization were more efficiently removed, confirming the role of oxygen-containing groups. Even positively charged species (APS) were adsorbed, suggesting additional mechanisms such as π - π conjugation and specific surface interactions. Economic analysis estimated a production cost of USD 0.43/kg and a total cost of USD 2.37/kg, comparable to other biomass-based adsorbents. Magnetic separation and biochar renewability (M-WB) could further reduce wastewater treatment costs, with regeneration estimated at USD 0.40/kg.

Srinadh and Remya [70] produced magnetic biochar from industrial hemp via microwave-assisted pyrolysis (MAP) and FeCl₃ impregnation for Congo red (CR) removal. The material had 10.05 wt% iron, a surface area of 17.64 m²/g, and a 4.84 µm average pore size. CR removal reached 92%, remaining at 87% after three cycles. FTIR confirmed the presence of –OH and C=O groups linked to electrostatic interactions and π – π stacking. The biochar showed low iron leaching and was considered environmentally safe. The authors proposed its reuse as a ceramic additive when mixed with clay, bentonite, or sludge at 1100–1300 °C, enabling the stabilization of oxyanions and reducing environmental impacts.

Despite the advances achieved, significant technical challenges remain. Although Hao et al. [67], Peng et al. [68], Duan et al. [69], and Srinadh and Remya [70] reported excellent adsorption performance, regeneration stability, and cost-effectiveness, most studies were limited to lab-scale tests with synthetic matrices. Applications with real effluents—characterized by complex contaminant mixtures and variable physicochemical conditions—remain scarce. Additionally, the long-term stability of magnetic functional groups and risks of metal ion leaching (e.g., Fe³⁺) pose concerns for safe environmental use. The need for microbial modification [68], high-purity reagents, or MAP [70] can also raise production costs and limit scalability. Furthermore, while adsorption mechanisms like π – π stacking, electrostatic interactions, ion exchange, and complexation are well-documented, studies addressing their synergistic effects in complex systems are still lacking.

To enable the large-scale adoption of magnetic biochars, future research must validate their performance in real wastewater treatment, ensure their long-term functional stability, and develop more cost-effective, sustainable production routes. These steps are critical for establishing magnetic biochar as an efficient, regenerable, and environmentally viable technology.

4. Types of ECs and Adsorption Mechanism

The applicability of agricultural-residue-based bioadsorbents has been widely demonstrated across several categories of ECs, particularly those characterized by persistence, low degradability, and complex molecular structures. Due to their structural diversity and varied physicochemical properties, the effective removal of ECs often requires adsorbent materials with a high surface reactivity, tailored functional groups, and tunable selectivity [2].

Among ECs, pharmaceuticals are particularly concerning due to their continuous discharge from municipal, hospital, and industrial sources. Compounds such as ibuprofen, diclofenac, chloroquine, ciprofloxacin, acetaminophen, and metronidazole are frequently detected in natural water bodies, often persisting through conventional biological treatments. Bioadsorbents derived from lignocellulosic materials such as *Parthenium hysterophorus* and corncob have demonstrated high efficiency in pharmaceutical removal. The presence of oxygen-containing functional groups and aromatic domains enhances interactions such as hydrogen bonding and π – π stacking. In batch systems, *Parthenium*-derived biochars exhibited significant sorption capacities for metronidazole and acetaminophen, with synergistic removal effects in multipollutant mixtures. Similarly, corncob biochar functionalized with bimetallic Fe–Cu nanoparticles showed high efficiency in removing ciprofloxacin, not only through adsorption but also via Fenton-like catalytic degradation—an innovative dual-function strategy that integrates adsorption with advanced oxidation [71,72].

Synthetic dyes, extensively used in the textile, paper, and plastic industries, represent another critical class of endocrine-disrupting chemicals (ECs). Their complex aromatic structures confer resistance to light, heat, and microbial degradation, while also exhibiting mutagenic and carcinogenic properties [73]. Agricultural-waste-derived biochars—such as those from peanut shells, pomelo peel, mandarin peels, and corncobs—have been

employed for dye removal. Functionalization with metal oxides (e.g., ZnO, TiO₂, CuO, Fe₃O₄) not only improves dye affinity but also imparts photocatalytic properties, enabling hybrid systems capable of simultaneous adsorption and photodegradation. Some of these materials have demonstrated dye removal efficiencies exceeding 95% and outstanding regeneration capacities. Notably, magnetic composite bioadsorbents have been shown to adsorb both dyes (e.g., methylene blue) and pesticides (e.g., acetamiprid) with maximum adsorption capacities exceeding 350 mg/g, highlighting their versatility and applicability in treating complex effluents [74].

Pesticides and herbicides, such as atrazine, glyphosate, 2,4-D, and acetamiprid, are frequently introduced into surface- and groundwater through agricultural runoff. Due to their mobility and toxicity, they pose serious threats to human health and aquatic ecosystems. Biochars and activated carbons produced from sugarcane bagasse, olive pomace, cassava peels, and pistachio shells have been explored as adsorbents for these contaminants. Through chemical activation and magnetic modification, these materials exhibit improved porosity, increased surface functionality, and enhanced binding affinity. In addition to their high adsorption capacities, some systems incorporate catalytic features that promote the degradation of target compounds, further expanding their removal capabilities [74].

Finally, perfluoroalkyl substances (PFASs)—such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS)—represent a particularly challenging class of ECs due to their extreme resistance to thermal, chemical, and biological degradation. While the literature on PFAS removal using agricultural-residue-based adsorbents remains limited, the inherent versatility of biochar surfaces suggests a strong potential for application. Surface functionalization, particularly with nitrogen, oxygen, or iron species, may facilitate interactions with PFASs through electrostatic attraction and hydrophobic partitioning. Moreover, tailoring the point of zero charge (pH_{pzc}) of the bioadsorbent can improve affinity depending on the contaminant's ionic state, paving the way for future innovations in PFAS-targeted water treatment [75].

4.1. Adsorption Mechanisms in ECS Removal

The adsorption efficiency of bioadsorbents derived from agricultural residues in the removal of ECs (ECs) is intrinsically governed by the physicochemical interactions between the functional groups of the adsorbent surface and the molecular characteristics of the contaminants. Owing to their lignocellulosic origin and the variety of thermal or chemical modifications they undergo, these bioadsorbents often exhibit a heterogeneous surface with multiple binding sites, allowing the simultaneous occurrence of distinct adsorption mechanisms—including physical adsorption, chemisorption, electrostatic interactions, ion exchange, and even catalytic-assisted transformations. This multifunctionality differentiates agricultural-residue-derived adsorbents from conventional materials such as pristine activated carbon, providing not only versatility but also a tunable surface chemistry adaptable to various classes of pollutants [76].

4.1.1. π - π Interactions and Hydrophobic Forces

π - π interactions represent a fundamental mechanism in the adsorption of ECs onto biochars, acting as a primary link between the adsorbent surfaces and the π -electron systems of the contaminants, such as the aromatic rings present in many pharmaceuticals, pesticides, and dyes. Biochars derived from agricultural residues, rich in lignocellulosic structures, exhibit an abundance of graphitic aromatic rings after pyrolysis, which serve as sites for these interactions. In addition to π - π interactions, hydrophobic forces play a complementary role, facilitating the partitioning of less polar ECs to the hydrophobic

surface of biochars. However, it is crucial to acknowledge that the nature and extent of π – π interactions are highly dependent on the specific composition of the precursor biomass. Lignin, a complex and heterogeneous polymer, varies structurally among different plant species, presenting distinct proportions of guaiacyl, syringyl, and *p*-hydroxyphenyl units. This variability directly influences the electron density, spatial orientation, and degree of aromatic condensation of the resulting biochar. Consequently, biochars derived from different lignin sources (e.g., rice straw versus sugarcane bagasse) may exhibit aromatic sites with distinct characteristics, leading to variations in affinity and selectivity for different ECs via π – π interactions [77,78].

While the presence of π – π interactions is widely established, the direct and universal quantification of these interactions for different lignin sources in biochars remains a complex challenge. Advanced characterization methods, such as solid-state Nuclear Magnetic Resonance (NMR) spectroscopy, X-ray photoelectron spectroscopy (XPS), and X-ray Diffraction (XRD), can provide insights into the graphitic structure and surface composition of biochars, indirectly correlating with π – π interaction capacity. Additionally, computational approaches, such as Density Functional Theory (DFT), can model the interaction energies between the biochar's π -systems and specific ECs, offering a theoretical quantification at the molecular level. Nevertheless, the practical application and generalization of these methods across a wide range of biochars and ECs are still active areas of research, requiring rigorous experimental validation. A profound understanding of this variability is essential for the rational design of optimized biosorbents for specific EC removal [77,78].

4.1.2. Hydrogen Bonding and Surface Functional Groups

Hydrogen bonding plays a central role, especially when the bioadsorbent retains or acquires surface functionalities such as hydroxyl (–OH), carboxyl (–COOH), and amine (–NH₂) groups through low-temperature pyrolysis, acid/base activation, or heteroatom doping (e.g., N or O). These groups facilitate selective interactions with polar and moderately polar ECs, including pharmaceuticals and pesticides, by forming reversible, site-specific bonds. The strategic introduction of such groups during functionalization processes (e.g., ZnO/N,O-doped biochars) not only enhances adsorption but also allows tailored selectivity toward certain classes of contaminants, which remains underexplored in most large-scale adsorbent applications [72,78].

4.1.3. Electrostatic Interactions and pH Dependence

Electrostatic attraction or repulsion between charged contaminants and the adsorbent surface is strongly influenced by the point of zero charge (pH_{pzc}) of the material. Below the pH_{pzc}, the surface is positively charged and favors the adsorption of anionic compounds such as Congo red or PFOS; above it, the surface becomes negatively charged and preferentially adsorbs cationic species like methylene blue or ciprofloxacin. Thus, pH not only modulates surface charge but also alters the ionization state of contaminants, making it a critical parameter in the design of adsorption systems. While this mechanism is often cited, few studies have systematically optimized adsorbent synthesis to tailor the pH_{pzc} toward specific contaminants—a gap that presents a valuable direction for future innovations [74].

4.1.4. Ion Exchange and Metal–Ligand Complexation

Bioadsorbents naturally enriched with exchangeable cations (e.g., Ca²⁺, K⁺, Mg²⁺), or those modified with metal oxides such as Fe₃O₄ or CuO, can remove ionic pollutants via ion exchange and surface complexation. This is particularly effective for pollutants like glyphosate and heavy-metal-coordinating pharmaceuticals. The formation of inner-sphere complexes between functional groups (–COOH, –OH) and metal-active sites in

magnetically modified biochars adds a layer of specificity and strength to adsorption, often resulting in chemisorption-type interactions with high energy bonds [72].

4.1.5. Photocatalytic and Fenton-Assisted Hybrid Mechanisms

One of the most promising innovations involves the integration of bioadsorbents with photocatalytic (e.g., TiO_2 , ZnO) or Fenton-like catalytic systems, yielding the synergistic removal of persistent contaminants. In these hybrid materials, adsorption not only concentrates pollutants near catalytic sites but also enhances degradation by facilitating charge transfer, radical generation, and localized oxidation. This dual functionality—adsorption and in situ degradation—enables the partial or complete mineralization of ECs, especially dyes and antibiotics, and represents a cutting-edge frontier in sustainable water treatment technologies [77,79].

4.1.6. Influence of Ionic Strength and Competing Species

In real wastewater matrices, high ionic strength and the presence of natural organic matter (NOM), heavy metals, and co-contaminants can alter adsorption performance. Competing ions may reduce efficiency by blocking active sites or altering the electrical double layer surrounding the adsorbent. However, some bioadsorbents—especially those with meso- and microporous structures—have shown resilience under competitive conditions, a property that could be enhanced through strategic surface engineering. These aspects remain underexplored in long-term or multi-cycle adsorption studies, highlighting the need for pilot-scale investigations of complex matrices [9].

4.2. Adsorption Studies

A comprehensive understanding of the adsorption behavior of ECs (ECs) onto agricultural-residue-based bioadsorbents requires the integration of equilibrium, kinetic, and thermodynamic analyses. These three pillars offer critical insights into the capacity, rate, and feasibility of adsorption under varying environmental and operational conditions. When evaluated in concert, these models not only allow for the optimization of adsorption systems but also elucidate the dominant mechanisms at play—whether physisorption, chemisorption, or complex hybrid interactions—thus enhancing the predictive power and scalability of the process. Kinetic investigations involving ZnO -doped biochar for tetracycline removal and magnetically activated rice husk biochar for 2,4-D herbicide adsorption have demonstrated multi-stage adsorption behavior, characterized by fast initial surface adsorption followed by slower pore diffusion, suggesting that both external and internal mass transfer mechanisms are involved [77,80].

Recent studies on the adsorption of pharmaceuticals and pesticides onto bioadsorbents derived from pistachio shells, wheat straw, and corncobs have consistently shown negative ΔG° and positive ΔH° and ΔS° values, indicating that the processes are spontaneous, endothermic, and accompanied by an increase in entropy. These findings highlight the predominance of specific interactions such as hydrogen bonding and surface complexation and support the use of moderate heating to enhance adsorption performance in practical applications [74].

Together, isothermal, kinetic, and thermodynamic analyses serve as essential tools not only for describing adsorption mechanisms but also for optimizing system design, scaling up applications, and predicting behavior under real-world conditions. However, despite the widespread use of these models, comparative studies using multiple contaminants under dynamic (e.g., continuous-flow) conditions remain scarce and represent a promising avenue for future investigation and innovation in bioadsorbent research. An in-depth analysis of the literature reveals a wide array of agricultural-residue-derived adsorbents that exhibit promising removal efficiencies for structurally diverse ECs. The diversity

in precursor materials, activation methods, and functionalization strategies underscores the versatility and tunability of bioadsorbents—yet also highlights the need for comparative evaluations under standardized conditions. For instance, corncob-derived magnetic biochars, modified with Fe–Cu bimetallic nanoparticles, have demonstrated superior performance in removing ciprofloxacin, achieving adsorption capacities exceeding 300 mg/g, and exhibiting additional oxidative degradation through Fenton-like reactions. This dual mechanism significantly enhances pollutant removal, especially in antibiotic-contaminated waters [72].

Similarly, ZnO/N,O-doped biochars synthesized from peanut shells effectively removed both methylene blue and tetracycline, with removal efficiencies exceeding 95%. These systems benefit from a combination of π – π interactions, hydrogen bonding, and photodegradation, illustrating the advantage of adsorption–photocatalysis hybrid systems [77]. Bioadsorbents derived from *Parthenium hysterophorus*, an invasive weed, offer a low-cost, eco-friendly alternative and have been successfully applied in the adsorption of multiple pharmaceuticals. These materials exhibit high affinity for aromatic compounds and function well in both batch and fixed-bed systems, confirming their scalability and economic potential [81].

In the context of pesticides, magnetically activated rice husk biochar has been employed for the removal of 2,4-D herbicide, where adsorption proceeded via a multistep process, including rapid surface interaction and slower intraparticle diffusion. Despite its lower surface area compared to synthetic adsorbents, its high removal efficiency under optimized pH and ionic strength demonstrates the potential of engineered lignocellulosic adsorbents [80]. Thermodynamic studies across these systems consistently point to spontaneous ($\Delta G^\circ < 0$) and often endothermic ($\Delta H^\circ > 0$) processes, suggesting the prevalence of chemisorption and entropy-driven mechanisms, particularly in systems with strong functional group interactions. This aligns with findings for bioadsorbents derived from wheat straw, pistachio shells, and olive pomace, where performance improved with temperature, indicating the relevance of thermal activation during operational application [74].

To visually synthesize and compare the performance of various agricultural-residue-based bioadsorbents, a radar chart was constructed (Figure 2). This multi-criteria analysis evaluates key parameters—such as adsorption capacity, versatility toward different classes of ECs, surface functionalization potential, regeneration ability, scalability, and cost-effectiveness—based on the recent literature. Such a comparative framework highlights not only the diversity of functional attributes across materials but also the potential trade-offs and priorities in adsorbent design depending on specific water treatment goals.

The radar chart provides a multidimensional assessment of selected agricultural-residue-derived bioadsorbents, evaluated across six core parameters: adsorption capacity, pollutant versatility, surface functionalization, regeneration potential, scalability, and cost-effectiveness. This comparative framework highlights both the functional diversity and trade-offs inherent in bioadsorbent design and application.

Corn-cob-based Fe–Cu biochar exhibited the most balanced and robust profile. Its high adsorption capacity ($q_{\max} = 253.7$ mg/g for ciprofloxacin) and broad-spectrum performance are largely due to its dual adsorption–oxidation functionality. The material combines a high surface area with catalytic activity, enabling Fenton-like degradation in the presence of H_2O_2 . The process was confirmed to be endothermic ($\Delta H^\circ = +31.1$ kJ/mol) and spontaneous (ΔG° up to -9.5 kJ/mol), indicating chemisorption with increased entropy ($\Delta S^\circ = +127.5$ J/mol·K) [72]. Additionally, its magnetic nature enables easy recovery, reinforcing its suitability for scalable treatment systems.

COMPARATIVE PERFORMANCE OF AGRICULTURAL BIOADSORBENTS

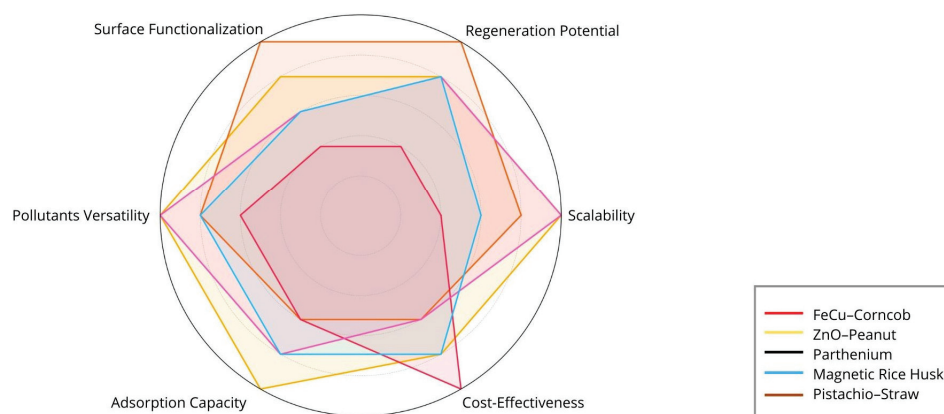


Figure 2. A comparative radar chart illustrating the performance of selected bioadsorbents derived from agricultural residues: corncob–FeCu biochar, ZnO/N,O-doped peanut shell biochar, *Parthenium hysterophorus* biochar, magnetically modified rice husk biochar, and pistachio/wheat straw-based biochar. Note: This chart visually assesses bioadsorbents across six key parameters: Adsorption Capacity, Pollutant Versatility, Surface Functionalization, Regeneration Potential, Scalability, and Cost-Effectiveness. The performance in each criterion is depicted by the extent of the polygon along its respective axis, where a point further from the center indicates a higher relative performance. The overall area encompassed by each bioadsorbent's polygon reflects its general balanced performance across all evaluated aspects, highlighting the functional diversity and inherent trade-offs in bioadsorbent design and application.

ZnO/N,O-doped peanut shell biochar showed superior performance in surface functionalization and multi-contaminant removal. With $q_{\max}$ values of 350.8 mg/g for methylene blue and 250.1 mg/g for tetracycline, this material demonstrated fast kinetics (pseudo-second-order) and effective binding via hydrogen bonding and π – π interactions. The system also benefits from photocatalytic activity, enhancing degradation under light exposure. Thermodynamic parameters indicated spontaneous ($\Delta G^\circ \approx -4.2$ to -7.8 kJ/mol) and endothermic ($\Delta H^\circ = +25.4$ kJ/mol) behavior, aligned with the increased structural disorder during adsorption ($\Delta S^\circ = +84.1$ J/mol·K) [77].

Parthenium-derived biochar, though less functionalized than the engineered composites, achieved significant performance considering its low-cost origin. It showed $q_{\max}$ values of 48.72 mg/g (metronidazole) and 32.10 mg/g (acetaminophen), with pseudo-second-order kinetics and a strong fit to the Langmuir model. Adsorption was confirmed to be endothermic ($\Delta H^\circ = +23.7$ kJ/mol) and spontaneous ($\Delta G^\circ < 0$), with a moderate entropy increase, supporting the feasibility of its application in decentralized or low-resource treatment systems [81].

Magnetic rice husk biochar demonstrated intermediate scores, particularly in regeneration and versatility. It removed 2,4-D herbicide with $q_{\max} = 88.3$ mg/g, following pseudo-second-order kinetics and a Freundlich isotherm model, suggesting heterogeneous surface interaction. Thermodynamic data ($\Delta H^\circ = +19.5$ kJ/mol, $\Delta G^\circ \approx -3.9$ to -5.2 kJ/mol) pointed to spontaneous, moderately endothermic adsorption with entropy-driven interactions [80].

The pistachio-shell-based magnetic biochar achieved the highest adsorption capacity, with $q_{\max} = 370.4$ mg/g for methylene blue and 357.1 mg/g for acetamiprid. The adsorption process, governed by the Langmuir isotherm and pseudo-second-order kinetics, showed strong spontaneous behavior ($\Delta G^\circ < -9$ kJ/mol) and high enthalpy ($\Delta H^\circ = +29.2$ kJ/mol), confirming chemisorption. Its excellent regeneration and magnetic separation properties make it ideal for high-load or complex wastewater systems [74].

In summary, the comparative analysis highlights the multidimensional nature of bioadsorbent performance, where adsorption capacity, selectivity, reusability, and cost must be balanced according to specific treatment objectives. Although each material presents distinct advantages, its effectiveness is also shaped by operational conditions and contaminant complexity. To further understand their applicability, it is essential to examine the performance of case studies involving agricultural-residue-based adsorbents in both controlled and practical scenarios.

5. Application of Adsorbents from Agricultural Residues

Table 3 summarizes biochar adsorbents produced from different agricultural residues, including cereal-derived straws, fruit and nut shells, agro-industrial by-products, and fibrous biomass. These adsorbents have been applied to remove pollutants including pharmaceuticals and personal care products, pesticides, dyes, inorganic pollutants, and emerging micropollutants. Moreover, various surface modification strategies have been investigated to enhance the physicochemical properties and adsorption capacity of biochar adsorbents. These approaches encompass chemical activation, metal and metal oxide functionalization, physical and thermochemical processes, and the development of composite and new materials. Therefore, this section discusses the effect of different operational parameters during the production of biochar adsorbents from agricultural residues, as well as the influence of subsequent surface modification methods on their physicochemical properties.

Liao et al. [82] evaluated the effect of different pyrolysis temperatures (300–600 °C) on the physicochemical properties of corncob and rice husk biochar adsorbents for Pb^{2+} and Cu^{2+} removal. The maximum adsorption capacities were obtained at 500 °C for corncob biochar (61.07 mg/g for Pb^{2+} and 15.29 mg/g for Cu^{2+}) and rice husk biochar (60.26 mg/g for Pb^{2+} and 17.35 mg/g for Cu^{2+}). The adsorption capacity results for corncob biochar were primarily attributed to the improved pore morphology and increased active sites at 500 °C. These characteristics could have improved mass transfer and the accessibility of Pb^{2+} and Cu^{2+} ions to the active surface. Moreover, the adsorption capacity results for rice husk biochar were mainly related to the increased ash content (28.16–48.31%), with an enhanced pyrolysis temperature (0–600 °C). The minerals within ash could have provided additional inorganic binding sites for the adsorption of the heavy metals.

Qi et al. [83] studied the effect of microwave-assisted pyrolysis with activated carbon on the physicochemical properties of a wheat straw biochar adsorbent for Pb^{2+} , Cd^{2+} , and Cu^{2+} removal. The power level of 500 W revealed the highest adsorption capacities, which were 139.44 mg g⁻¹, 52.92 mg g⁻¹, and 31.25 mg g⁻¹ for Pb^{2+} , Cd^{2+} , and Cu^{2+} , respectively. This achievement could be associated with the enhancement in the specific surface area of the wheat straw biochar (from 2.58 to 150.79 m² g⁻¹) with increasing power levels (100–500 W). Moreover, the adsorption mechanism involved complexation with oxygen-based functional groups (–OH and –COOH), ion exchange, and electrostatic interactions between the Pb^{2+} , Cd^{2+} , and Cu^{2+} and functional groups of the biochar, in addition to the precipitation of metal carbonates (CdCO_3 , PbCO_3) on the biochar's surface.

Nguyen et al. [84] studied the effect of phosphoric acid activation on the physicochemical properties of a sunflower seed husk biochar adsorbent for tetracycline, ciprofloxacin, ibuprofen, and sulfamethoxazole removal. The maximum adsorption capacities for tetracycline, ciprofloxacin, sulfamethoxazole, and ibuprofen using non-activated biochar were 228.3, 80.8, 129.1, and 135.8 mg/g, respectively, whereas for the activated biochar the values increased to 429.3, 361.6, 251.3, and 251.1 mg/g, respectively. These improvements were attributed to the enhanced specific surface area (from 6.2 to 378.8 m² g⁻¹) and total pore volume (from 0.012 to 0.206 cm³ g⁻¹) following activation. Furthermore, phosphoric acid

activation introduced additional functional groups on the surface, such as $-\text{COOH}$, $-\text{OH}$, and phosphorus-containing species. For instance, the adsorption mechanisms involved external diffusion, intraparticle diffusion, and chemisorption.

Xiang et al. [85] studied the effect of lignin impregnation on the physicochemical properties of corn straw and wheat stalk biochar adsorbents for tetracycline hydrochloride removal. The maximum adsorption capacities reached 12.61 mg/g for corn straw biochar, 19.08 mg/g for wheat stalk biochar, 15.77 mg/g for lignin-impregnated corn straw biochar, and 32.21 mg/g for lignin-impregnated wheat stalk biochar. These improvements were attributed to the lignin-based impregnation treatment, which increased the specific surface area of the corn straw biochar from 211.55 to 334.19 m^2/g and that of the wheat stalk biochar from 123.84 to 478.39 m^2/g . Furthermore, the total pore volume was enhanced from 0.1370 to 0.1998 cm^3/g for the corn straw biochar and from 0.1349 to 0.3087 cm^3/g for the wheat stalk biochar. These changes suggest that lignin-based impregnation treatment fills larger biochar pores and generates smaller ones, resulting in an increased total pore volume and internal surface area. This structural modification resulted in a more mesoporous biochar (mean pore diameter of 2.58 nm), which may have enhanced the accessibility of functional groups and facilitated the diffusion of tetracycline hydrochloride molecules into the biochar pores. Moreover, the adsorption performance of tetracycline hydrochloride on the modified biochar adsorbents can be attributed to physical adsorption mechanisms, including hydrogen bonding, pore filling, π - π interactions, and electrostatic attraction. Furthermore, the Na^+ , K^+ , Mg^{2+} , and Al^{3+} ions showed competition with tetracycline hydrochloride for adsorption sites, although Ca^{2+} enhanced the adsorption of tetracycline hydrochloride, resulting in tetracycline- Ca^{2+} complexes.

Abdelfatah et al. [86] studied the effect of nitrogen and sulfur co-doping with a copper zinc ferrite composite on the physicochemical properties of a *Beta vulgaris* leaf-based biochar adsorbent for reactive black 5 dye removal from simulated and real wastewater effluents. The maximum adsorption capacity of reactive black 5 dye in the simulated effluent was 276.57 mg/g. XPS (X-ray photoelectron spectroscopy) revealed the involvement of sulfur, nitrogen, oxygen, and metal ions within the adsorption process. The interactions included sulfur interactions, with the formation of sulfate (SO_4^{2-}) on the biochar surface after adsorption, suggesting the binding of sulfur-containing functional groups from reactive black 5 dye; metal complexation between Cu^{2+} , Fe^{3+} , and Zn^{2+} from the biochar and the sulfur-containing functional groups in reactive black 5 dye; and coordination complex formation involving oxygen from the reactive black 5 dye and the metals in the biochar. In addition, the adsorption of reactive black 5 dye onto the biochar was favored by hydrogen bonding, caused by the high number of OH groups, and the π - π stacking between the aromatic rings of the biochar and the reactive black 5 dye. Therefore, the synergetic interaction of nitrogen and sulfur co-doping with the copper zinc ferrite composite in the biochar played an important role in the adsorption of reactive black 5 dye. Furthermore, the application of the biochar in real wastewater resulted in a UV-absorbance decrease, in addition to reductions in TDS (430 to 3.5 ppm), turbidity (127 to 63 NTU), and conductivity (860 to 7 $\mu\text{S}/\text{cm}^3$). Therefore, the modified biochar was also effective in removing reactive black 5 dye from wastewater effluent, despite the presence of competing ions for binding sites.

Zhang et al. [87] investigated the impact of KHCO_3 activation, Fe_3O_4 magnetization, and $\text{La}(\text{OH})_3$ loading on the physicochemical properties of a corn straw biochar adsorbent for phosphate removal from simulated and real wastewater effluents. The modified biochar showed a maximum adsorption capacity of 116.08 mg/g in the simulated effluent. The mechanisms attributed to phosphate adsorption onto the modified biochar encompassed electrostatic attraction, inner-sphere complexation, ligand exchange, and weak precipitation.

The point of zero charge (pH_{pzc}) was reduced after the adsorption of phosphate, which was attributed to the electrostatic attraction of the negatively charged phosphate and positively charged functional groups from the biochar. XPS indicated a change in the electron density of La after the adsorption of phosphates, suggesting inner-sphere complexation between the La^{3+} and phosphates involving direct coordination and electron transfer. Moreover, the dissolution of surface La $(\text{OH})_3$ could have provoked the increase in C–OH intensity from 34.71% to 45.42%, which could have been due to the release of OH-groups in the solution. These functional groups may have facilitated ligand exchange or complexation with phosphate, as verified by an increase in $\text{OH}^-/\text{O–P}$ intensity from 27.02% to 78.88%. The authors also studied the effect of the modified biochar in the adsorption of phosphates present in river water (Songhua River) and real wastewater (Taiping sewage effluent). The phosphate concentration in the river water was reduced from approximately 190 $\mu\text{g/L}$ to approximately 10 $\mu\text{g/L}$ over 20 min using 1.5 g/L of modified biochar. In addition, the phosphate concentration in the real wastewater was reduced from approximately 1.86 mg/L to approximately 0.16 mg/L over 10 min using 5 g/L of modified biochar. For instance, the modified biochar was effective in removing phosphates even under real operational conditions, such as in river water and real wastewater effluent.

Therefore, future studies should not only simulate the adsorption of target pollutants in the presence of competing compounds but also validate these findings using real wastewater samples. In addition, analytical techniques, such as scanning electron microscopy (SEM), nitrogen adsorption–desorption isotherms (BET), point of zero charge (pH_{pzc}), Fourier-transform infrared spectroscopy (FTIR), and X-ray photoelectron spectroscopy (XPS) are important for understanding the morphology of, porosity of, and interactions between the functional groups of pollutants and biochars. Indeed, this data can provide information for the development of new surface modification methods to enhance the adsorption capacity of biochar adsorbents.

Table 3. Summary of biochar adsorbents derived from agricultural residues.

Type of Effluent	Target Adsorbate	Feedstock Type	Surface Modification	Maximum Adsorption Capacity (mg/g)	References
Pharmaceuticals and Personal Care Products	Tetracycline (TC), ciprofloxacin (CIP), ibuprofen (IBP), and sulfamethoxazole (SMX)	Sunflower seed husk	H_3PO_4	429.3, 361.6, 251.3, and 251.1 for TC, CIP, SMX, and IBP, respectively	[84]
	Tetracycline hydrochloride	Corn straw and wheat stalk	Lignin impregnation	31.48	[85]
	Sulfamethazine (SMT), oxytetracycline hydrochloride (OTC), and amoxicillin (AMX)	Rice straw	KOH	6.47, 87.8, and 7.67 for SMT, OTC and AMX, respectively	[88]
	Ciprofloxacin	Tea waste	MnSO_4	121.42	[89]
	Chloroquine	Cassava residue	NaOH and microwave-assisted pyrolysis	39	[71]
	Methyl paraben (MPB), carbamazepine (CZP), ibuprofen (IBP), and triclosan (TCS) from simulated and real wastewater	Empty palm bunch	H_2SO_4	60.2, 51.7, 38.8, and 35.4 for MPB, CZP, IBP, and TCS, respectively	[87]
	Ciprofloxacin from real wastewater	Pomegranate peels	H_3PO_4	142.86	[90]

Table 3. Cont.

Type of Effluent	Target Adsorbate	Feedstock Type	Surface Modification	Maximum Adsorption Capacity (mg/g)	References
Pesticides	Chlorpyrifos	Pomegranate peels	H ₃ PO ₄	100	[91]
	Imidacloprid	Sugarcane bagasse	KOH with iron/zinc	313	[92]
Dyes	Methylene blue	Wheat straw	Ball-milled biochar with fly ash	854.75	[93]
	Methylene blue	Rice straw	Ammonium phosphate	156.36	[94]
	Methylene blue	Walnut shell	Supercritical CO ₂ pretreatment and potassium hydroxide activation	80.4	[95]
	Methylene blue	Fronds and leaves from date palm	FeSO ₄ ·7H ₂ O	85.1	[96]
	Food Red 17 and Acid Blue 9	Cassava bagasse	NH ₄ Cl	131 and 150 for Food Red 17 and Acid Blue 9, respectively	[97]
	Reactive black 5 dye from simulated and industrial wastewater	Beta vulgaris leaves	Nitrogen and sulfur co-doping with copper zinc ferrite composite	276.57	[86]
Inorganic Pollutants	Fluoride in simulated and glass wastewater	Coconut husks	MnFe ₂ O ₄ magnetization/Al-La metal-organic framework in chitosan/ β -cyclodextrin aerogel	38.59	[98]
	Pb ²⁺ and Cu ²⁺	Rice husk and corn cob	-	61.07 and 17.35 for Pb ²⁺ and Cu ²⁺ , respectively	[82]
	Cd ²⁺	Rice straw	Fe-Mn oxides	120.77	[99]
	Pb ²⁺ , Cd ²⁺ , and Cu ²⁺	Wheat straw	Microwave-assisted pyrolysis with activated carbon	139.44, 52.92, and 31.25 for Pb ²⁺ , Cd ²⁺ , and Cu ²⁺ , respectively	[83]
	Cd ²⁺	Corn stalks	Amino modification	375.58	[100]
	Cd ²⁺	Sesame straw	Alkaline hydrogen peroxide pretreatment	87.13	[101]
	Cd ²⁺ and As ³⁺	<i>Pennisetum</i> sp. straw	Fe-Mn oxides	141.1 and 31.8 for Cd ²⁺ and As ³⁺ , respectively	[102]

Table 3. *Cont.*

Type of Effluent	Target Adsorbate	Feedstock Type	Surface Modification	Maximum Adsorption Capacity (mg/g)	References
Pesticides	Phosphate from simulated, river, and sewage wastewater	Corn straw	KHCO ₃ activation, Fe ₃ O ₄ magnetization, and La(OH) ₃ loading	116.08	[103]
	Phosphate	Orange peels	CaCO ₃ /ZnO	52.96	[104]
	Phosphate	Corn cob, sugarcane bagasse, rice straw, and sawdust	Fe ²⁺ and La ³⁺ impregnation	27.49	[105]
	Phosphate	Papaya leaves	Lanthanum-organic framework coating	47.5	[106]
	Tris-(1-chloro-2-propyl) phosphate	Corn straw	-	2.34	[107]
Emerging Micro-Pollutants	Microplastics	Corn cob	Fe(NO ₃) ₃ and FeSO ₄	1737	[108]

6. Regeneration and Reusability

Although adsorption offers several advantages, one major challenge is disposing of adsorbent materials loaded with pollutants. Improper handling of these materials can lead to secondary pollution, particularly soil and water contamination. Therefore, developing strategies for treating, regenerating, and reusing adsorbents is critical from environmental and economic perspectives, aligning with sustainability and circular economy principles. Reused adsorbents can be employed in subsequent adsorption processes or for alternative applications such as catalysts or biofertilizers. It is important that the adsorbed contaminants can be rapidly released back into solutions, allowing the adsorbent to be reused. The most effective adsorbents offer high potential for regeneration or repeated use.

Various regeneration methods have been studied, but no single technique has proven universally effective for all adsorbents. In some cases, combining two or more regeneration methods has improved performance and could offer a practical solution for restoring spent adsorbents. The choice of method depends on both adsorbent and adsorbate characteristics, including toxicity, combustibility, corrosiveness, radioactivity, and whether the adsorption mechanism is physical (physisorption) or chemical (chemisorption) [109–111]. An ideal regeneration technique should fulfill multiple criteria: it should be effective in desorbing pollutants, non-toxic, environmentally sustainable, economically feasible, and operationally simple. Furthermore, the method should ensure that the regenerated material retains its adsorption capacity, especially for applications in water treatment.

Activated carbon typically undergoes 6 to 12 regeneration cycles, depending on the type of adsorbate and the regeneration method employed. In contrast, biosorbents (derived from biological materials) generally exhibit limited regeneration potential. Their mechanical and thermal instability often restricts them to two to six reuse cycles. Despite their low cost and environmental benefits, their lower reusability remains a key limitation in practical applications [112].

6.1. Regeneration of Spent Adsorbents

Regeneration refers to the process of restoring spent adsorbents for reuse in subsequent adsorption cycles. As previously mentioned, it plays a crucial role in determining the practical viability of an adsorbent for large-scale industrial applications, as it directly influences economic feasibility. In this context, several regeneration methods have been proposed in the literature to desorb pollutants from adsorbent materials, including chemical regeneration, thermal regeneration, microwave-assisted regeneration, and techniques based on magnetic separation and recovery.

6.1.1. Chemical Regeneration

Chemical regeneration involves using solvents or reagents to desorb target species from spent adsorbents. Commonly used chemical regenerants include sodium hydroxide, hydrochloric acid, acetone, and ethanol. These agents modify the solution pH to influence the ionization states of the adsorbent surface and the adsorbates, facilitating desorption. The effectiveness of chemical regeneration depends on several factors such as the concentration of the solvent, the solubility of the adsorbates, the surface charge of the adsorbent, and the pH of the solution. Generally, acidic conditions favor the desorption of cationic heavy metals, as these positively charged ions adsorb more effectively in acidic environments. Moreover, pH variations can alter the behavior and binding characteristics of organic pollutants or other organic compounds adsorbed during remediation [109].

Chemical regeneration can be economically advantageous in some cases, particularly when reagents are inexpensive and recovery systems are in place. However, its practical application is often limited by drawbacks such as secondary pollution and potential adsorbent degradation [109]. Therefore, selecting an appropriate eluent is critical for optimizing desorption efficiency. Key considerations include the chemical compatibility of the eluent with both the adsorbent and adsorbate, its ability to disrupt adsorbate–adsorbent interactions, and its pH, which governs ionization and electrostatic forces. Additionally, the potential for complex formation and the presence of organic modifiers can influence desorption performance and must be carefully evaluated during eluent selection [112].

Despite the widespread use of chemical desorbents such as acids, alkalis, chelating agents, and organic solvents, their application presents several challenges. These include secondary pollution, toxicity issues (particularly with solvents like methanol and benzene), the structural degradation of the adsorbent, the potential leaching of nanomaterials, and gradual declines in regeneration efficiency over multiple cycles. Moreover, many chemical regenerants are expensive and require recovery and recycling to maintain economic viability. Nonetheless, chemicals like nitric acid, alkalis, salts, and chelating agents remain prevalent in adsorbent regeneration due to their effectiveness [112,113].

Table 4 summarizes the main studies on the chemical regeneration of adsorbents derived from agro-industrial residues. It is noted that, generally, higher removal efficiencies are achieved, maintaining removal efficiencies above 70% across multiple cycles. Prabakaran et al. [114] evaluated the reuse of palm kernel shell biochar using 0.1 N HCl and 0.1 M NaOH solutions. They reported that the adsorbent showed good reusability for methylene blue removal, maintaining its effectiveness over four successive adsorption–desorption cycles with only a 2.56% decrease in efficiency. Similarly, activated carbons derived from rubber seed and its shell removed Congo red and methylene blue with high efficiency (97–98%) over seven cycles, indicating strong structural stability and solvent compatibility [115].

Table 4. Removal efficiency and number of effective regeneration cycles of various agro-industrial residue-derived adsorbents for different adsorbates, with regeneration performed using chemical methods.

Adsorbent	Adsorbate	Solvent	Removal Efficiency (%)	Number of Effective Re-generation Cycles	Reference
Palm kernel shell biochar	Methylene blue	0.1 N HCl and 0.1 M NaOH solutions	97.5	4	[114]
Corn cob and luffa sponge activated carbons	Ciprofloxacin	Methanol	80.7% and 78.3%	5	[116]
Activated carbon made from rubber seed and rubber seed shell	Congo red and methylene blue	NaCl (0.01–0.3 mol/L)	97–98%	7	[115]
Sugarcane bagasse	Pb (II) and Ni (II)	0.1 M HNO ₃ , HCl, and NaOH	45–55% for NaOH; 75–79% for HCl; 90–96% for HNO ₃	-	[117]
Coconut shell and coir	CR (IV)	Solutions of HCl, H ₂ SO ₄ , NaOH (0.2 N), and distilled water	60–100%	4	[118]
Peanut husk with iron oxide biochar	Cr(IV)	0.1 M NaOH	56–96%	4	[119]
Corn cob	CR (III) and Cr (IV)	0.1 M NaOH	80–96%	5	[120]
Olive leaves	Cr (IV)	NaOH (0.1 mol/L), NaCl (0.1 mol/L), water	70–100% for NaOH; 60–100% for NaCl; and 30–90% for water	5	[121]
Sweet lime peel biochar	Cr(IV)	1 M HCl, 1 M NaOH, and 0.1 M HCl	80–95% for 1 M HCl; 30–80% for NaOH; 15–80% for 0.1 M HCl	3	[122]
Biochar from rice husk and sewage sludge	Alizarin Red S	Acetone, ethanol, methanol, and subcritical ethanol	60–80% for acetone, ethanol, and methanol; 80–100% for subcritical ethanol	5	[123]
Soybean biochar	Ofloxacin	Ethanol	60–85%	6	[124]
Bermuda-grass-derived biochar	Sulfamethoxazole	0.1 M NaOH	50–100%	4	[125]
Seed hulls with iron nanoparticles	Ivermectin	-	83–100%	5	[126]
Cassava-derived activated carbon	Chloroquine	Hydrochloric acid solution (0.2 mol/L)	70–100%	5	[71]
Cassava-derived biochar	Food Red 17 and Acid Blue 9 dyes	NaOH (2 mol/L)	66–100% for Acid Blue 9; 20–100% for Food Red 17	12	[97]

Organic solvents also showed promising results. El-Bendary et al. [116] reported desorption efficiencies of 80.7% and 78.3% for ciprofloxacin using methanol on activated carbons derived from corn cob and luffa sponge, respectively, with reusability maintained over five cycles. Even more effective was the use of subcritical ethanol in the regeneration

of biochars from rice husk and sewage sludge, achieving 80–100% removal efficiency for Alizarin Red S [123]. This suggests that employing solvents under specialized conditions, such as subcritical states, can significantly enhance desorption performance.

The impact of electrolyte concentration on adsorbent reuse was highlighted in studies using NaCl solutions to desorb dyes from rubber-seed-derived activated carbons [115]. Higher salt concentrations led to decreased adsorption efficiencies due to competitive binding and electrostatic repulsion effects between sodium ions and dye molecules on the adsorbent surface. Although a decline was observed after seven cycles, the reduction was relatively small (3% for Congo red and 2% for methylene blue), suggesting reasonable stability under these conditions.

For heavy metal recovery, acid-based desorption methods, particularly nitric acid, have proven most effective. Desorption efficiencies of nearly 90% for lead and over 96% for nickel were reported for sugarcane bagasse adsorbents treated with 0.1 M HNO₃ [117]. This enhanced desorption under acidic conditions is attributed to surface protonation facilitating metal ion release. These results underscore the necessity of coupling high adsorption capacity with efficient desorption and reusability for sustainable metal remediation.

It is also important to highlight the role of adsorbate chemistry and interaction mechanisms in solvent selection. For instance, sweet lime peel biochar showed better regeneration with 1 M HCl (80–95%) compared to NaOH (30–80%) [122]. In contrast, iron oxide-modified biochars and olive leaf biosorbents, where 0.1 M NaOH proved efficient in desorbing Cr(VI), maintained high removal rates over four to five cycles [121].

Regarding reusability, most adsorbents could be effectively reused over at least four cycles while maintaining acceptable removal efficiency. Soybean-derived biochar retained 60–85% of its ofloxacin removal capacity over six ethanol-based regeneration cycles [124], and iron-nanoparticle-coated seed hulls achieved 83–100% ivermectin removal across five cycles [126].

6.1.2. Thermal Regeneration

Thermal regeneration involves heating spent adsorbents to break the interactions between the adsorbent and the adsorbate, restoring the material's adsorption capacity. This method is typically performed at high temperatures ranging from 300 to 900 °C and often includes oxidative treatment in furnaces, where adsorbed pollutants are decomposed and oxidized. The process generally occurs in two main stages under an inert atmosphere: drying at approximately 105 °C to remove moisture, and pyrolysis and oxidation at elevated temperatures (300–900 °C), which vaporize organic compounds and degrade carbonaceous residues. Despite its effectiveness, thermal regeneration often leads to a 5–15% loss in adsorbent mass per cycle due to structural changes and active site blockage at high temperatures. This material loss limits the number of feasible regeneration cycles and can affect the long-term performance of the adsorbent [127].

Traditional regeneration techniques commonly involve the use of steam or hot inert gases, while non-traditional methods utilize microwave or ultraviolet (UV) radiation. Steam regeneration is particularly effective for activated carbon. The high latent heat of steam leads to a rapid increase in adsorbent temperature, facilitating desorption. Additionally, water molecules can compete with adsorbates for pore space, further enhancing desorption efficiency [109,127].

Pollutant adsorption and desorption efficiency under thermal treatment is closely linked to the activation energy required for these processes. While thermal regeneration offers a robust desorption performance, it is often considered cost-intensive due to its high energy demands and need for specialized equipment and gases (e.g., steam or inert atmo-

spheres). Furthermore, thermal processes can release harmful gases, posing environmental risks such as air pollution [109].

Table 5 summarizes key studies on the thermal regeneration of adsorbents derived from agro-industrial residues. The data indicates that the regeneration temperatures for these materials are generally moderate, typically not exceeding 500 °C, which is considerably lower than the temperatures reported for activated carbon regeneration, sometimes reaching up to 950 °C [128]. Additionally, it is noteworthy that removal efficiency significantly declines after three [129] to four regeneration cycles [125,130], highlighting the limited durability of these biosorbents under repeated thermal treatment. For instance, a sulfamethoxazole-saturated adsorbent regenerated at 300 °C for 3 h achieved nearly 100% efficiency, while lower temperatures between 100 °C and 200 °C were ineffective. However, repeated regeneration cycles at 300 °C led to a decline in performance, with efficiency dropping to 87% after two cycles and 46% after four cycles. This decrease is likely due to structural damage and changes in the surface of the adsorbent. Increased carbon–oxygen vibrations observed after four cycles suggested enhanced surface hydrophilicity, which weakened hydrophobic interactions (the primary adsorption mechanism at pH 3) [125].

Table 5. Removal efficiency and number of effective regeneration cycles of various agro-industrial residue-derived adsorbents for different adsorbates, with regeneration performed using thermal methods.

Adsorbent	Adsorbate	T (°C)	Time (h)	Gas Flow	Removal Efficiency (%)	Number of Regeneration Cycles	Reference
Durian peel biochar	Ciprofloxacin	400	2	Air	33–72%	3	[129]
Rape straw biochar	Tetracycline	400	1	Air	97%	6	[131]
Bermuda-grass-derived biochar	Sulfamethoxazole	275	3	Air	50–100%	4	[125]
Coconut shell activated carbon	Basic Blue 9 and Acid Blue 93	500	0.5	Nitrogen	42–100%	4	[130]
Magnetic sugarcane bagasse activated carbon	Methylene blue	300	0.5	Air	85–100%	4	[132]
Magnetic biochar from snake fruit peel	Rhodamine B	200	-	Air	99%	5	[133]

Chemical regeneration, although often effective, may result in secondary pollution and adsorbent degradation, whereas thermal regeneration tends to offer greater desorption efficiency at the cost of higher energy consumption [112]. In a comparative study, alkali, thermal, and hydrothermal regeneration methods were employed to restore ciprofloxacin-saturated durian peel biochar [129]. For alkali regeneration, a mixture of 0.1 M NaOH and ethanol (1:1, *v/v*) was used. Thermal regeneration involved heating the spent adsorbent from room temperature to 400 °C at a rate of 10 °C/min in air, with a 2 h residence time. Hydrothermal regeneration was performed by treating the used adsorbent with 180 mL of deionized water in a 250 mL autoclave at 300 °C for 3 h. After each treatment, the regenerated adsorbent was filtered and rinsed with deionized water. Thermal regeneration showed the highest initial regeneration rate (72%), followed by the alkali (66%) and hydrothermal methods (39%). However, adsorption capacity declined with successive cycles, with regeneration rates dropping to 33%, 23%, and 13%, respectively, after the third cycle. Thermal regeneration was most effective, likely due to its ability to mineralize

adsorbed ciprofloxacin and restore active sites. Further characterization of the regenerated adsorbent was performed to investigate adsorbent deactivation mechanisms. The textural analysis revealed that all regeneration methods resulted in a decline in surface area and pore volume over successive cycles. Thermal regeneration retained the highest surface area ($666.71 \text{ cm}^2/\text{g}$ after the third cycle), followed by the alkali and hydrothermal methods. The decline was attributed to pore collapse, ash accumulation, and structural degradation, varying by regeneration technique.

6.1.3. Microwave-Assisted Regeneration

Conventional thermal regeneration methods have notable limitations, including being time-consuming and energy-intensive, requiring elevated temperatures, prolonged residence times, and gradual energy transfer. Repeated heating and cooling cycles can lead to the deterioration of the adsorbent's pore structure, ultimately reducing its adsorption capacity [130]. In contrast, microwave-assisted regeneration presents a more efficient alternative. Unlike conventional heating, in which heat is transferred from the surface inward, often leaving the core cooler, microwave irradiation heats materials volumetrically, generating higher internal temperatures while maintaining relatively lower surface temperatures. This inside-out heating profile accelerates desorption and promotes the more effective breakdown of adsorbed contaminants, making microwave regeneration a promising substitute for traditional thermal methods [127].

Carbon-based adsorbents are particularly well-suited for microwave-assisted regeneration due to their excellent microwave absorption properties, which arise from their interfacial polarization mechanisms. Under microwave irradiation, these materials rapidly achieve high temperatures, thereby increasing their micropore volume and enhancing their adsorption capacity. Additionally, the generation of microplasmas and localized hotspots within the carbon matrix can significantly accelerate the degradation of pollutants trapped in the pores [134]. Therefore, the main advantages of microwave-assisted regeneration are the reduced regeneration time and the tendency to better preserve the original structure of the adsorbent.

Despite these advantages, fewer studies have evaluated microwave-assisted regeneration for adsorbents derived from agro-industrial residues. Wheat straw biochar was regenerated using both a 0.01 mol/L NaOH solution and microwave irradiation [135]. The chemical regeneration with NaOH achieved a regeneration efficiency of 55%. In comparison, the microwave-assisted regeneration demonstrated significantly higher efficiency, reaching up to 98% after treatment at 320 W for 5 min. Over three regeneration cycles at 320 W, the yields were 98.0%, 88.7%, and 85.1%, respectively. The impact was minimal, while repeated cycles slightly reduced microporosity and regeneration efficiency.

Microwave and conventional regeneration methods were compared for recovering dye-saturated activated carbons derived from coconut shells [130]. Microwave regeneration was performed using a single-mode microwave cavity operating at 2.45 GHz under a nitrogen atmosphere, treating 2 g of spent carbon placed in a quartz tube. Conventional thermal regeneration was conducted in a tubular furnace with nitrogen flow, applying a heating rate of $3 \text{ }^\circ\text{C}/\text{min}$ and maintaining the target temperature for 30 min. The best results were obtained with conventional heating at $500 \text{ }^\circ\text{C}$ for 11 s and microwave treatment at 200 W for 180 s. Although Basic Blue 9 (BB9) dye removal was slightly lower with microwave regeneration, this method was significantly faster than the conventional approach. For the dye Acid Blue 93 (AB93), the adsorption capacity was lower than that of BB9 on fresh carbon, but the microwave-regenerated samples generally showed higher AB93 uptake than BB9. In contrast, conventional regeneration at $500 \text{ }^\circ\text{C}$ resulted in much lower AB93 adsorption.

6.1.4. Magnetic Separation and Recovery

The development of magnetic adsorbents represents an innovative approach in wastewater treatment, achieved by incorporating magnetic components, such as Fe_3O_4 or CoFe_2O_4 , into the adsorbent matrix. These magnetic inclusions allow for the rapid separation of the adsorbent from aqueous solutions using an external magnetic field. Thanks to their high magnetic responsiveness, such materials can achieve effective solid–liquid separation in under 10 s, significantly decreasing the operational time and energy requirements compared to conventional separation techniques, including filtration and centrifugation [136]. Moreover, this functionalization also enhances the adsorption performance of the materials, particularly for heavy metals, dyes, and organic pollutants.

Various authors have shown magnetic adsorbents derived from agro-industrial residues to enable rapid and efficient separation from aqueous solutions using an external magnetic field [126,132,137]. The separation efficiency of the adsorbents from liquid solutions is typically demonstrated through visual documentation (e.g., photographs) and supported by magnetometry analysis. In one study, a magnetic biochar composite demonstrated effective recyclability and ease of separation using an external magnetic field [119]. When regenerated with 0.1 M NaOH, the material retained a high Cr(VI) removal efficiency across multiple cycles. After the first regeneration, the removal efficiency slightly declined from 96% to 90%. By the third cycle, the efficiency remained relatively stable at 72%, but a more pronounced reduction was observed by the fourth cycle, with efficiency falling to 56%. This decline was likely due to partial pore blockage or the loss of active adsorption sites.

Photocatalyst regeneration was performed by magnetically separating biochar powder made from snake fruit peel, followed by washing with ethanol and re-calcination at 200 °C after each cycle [133]. The degradation efficiency remained consistently above 99% through five cycles, indicating excellent material stability and reusability. The effectiveness of recovery was directly related to the magnetic properties of the composite, as confirmed by vibrating sample magnetometry analysis.

Despite the many advantages of magnetic biochars, concerns remain regarding their long-term environmental stability, particularly the potential leaching of Fe^{3+} ions from embedded magnetic particles [138,139]. This phenomenon may occur under acidic or redox-active conditions, posing risks of secondary contamination. Therefore, when considering applications, it is essential to assess the chemical stability of magnetic composites through long-term leaching studies and explore surface modifications that can mitigate iron release while preserving magnetic recovery performance [140,141].

6.2. Reuse and Final Disposal of Spent Adsorbents

Beyond regeneration, managing spent adsorbents requires consideration of both environmental sustainability and economic feasibility. While disposal methods such as incineration and landfilling remain common, reuse strategies aligned with circular economy principles, like soil amendment or catalyst applications, offer promising alternatives but require rigorous toxicity assessments [142]. Overall, various strategies have been employed for managing, disposing of, and reusing spent sorbents, including reuse, incineration, and landfilling.

Several reuse strategies have been explored, including applications in agriculture, capacitor components, and catalyst support materials, while incineration and landfilling remain common and established disposal methods [137]. For instance, in the study by Priya et al. [143], the authors proposed that their adsorbent could be reused as a slow-release fertilizer. The adsorbent was cellulose derived from rice straw for phosphate removal from secondary-treated wastewater. According to the authors, the material was biodegradable

and was used as fertilizer, enhancing plant growth, similarly to the growth improvement promoted by commercial fertilizer. Other authors have produced adsorbents from agro-industrial residues and evaluated their reuse for latent fingerprint detection [114,144,145]. In the study by Prabakaran et al. [114], spent biochar powder derived from palm kernel shells was utilized for latent fingerprint detection. The powder was applied to fingerprints previously deposited on glass slides, and excess material was carefully removed using a hairbrush. This process revealed well-defined ridge patterns under natural light. The fingerprints were subsequently documented using a smartphone camera, demonstrating the potential of this spent adsorbent for low-cost and effective forensic applications. In the study by Adeiga et al. [109], a spent macadamia nutshell composite adsorbent was utilized for the photocatalytic degradation of ciprofloxacin, resulting in 86% of the pharmaceutical being efficiently degraded within 80 min.

Once the adsorbent can no longer be regenerated or reused, its safe disposal becomes critical. Incineration and landfilling remain the most common disposal methods, although each has significant limitations [137]. Incineration, often regarded as a waste-to-energy strategy, allows spent adsorbents to serve as alternative fuels to coal, offering reduced corrosiveness and lower toxic gas emissions. However, this method may require pre-treatment and does not eliminate the risk of toxic emissions. Landfilling also requires the prior assessment of adsorbate concentration to determine its suitability for disposal, and often mandates pretreatment steps for hazardous materials [146]. Advanced stabilization and solidification techniques can enhance the safety of landfilled materials [147]. For example, zirconium-based compounds have been employed to stabilize arsenic-laden adsorbents [148]. Cementitious binders are also frequently employed to reduce the mobility of contaminants [149]. Other disposal methods include physical separation techniques such as washing, leaching, and chlorination, as well as thermal processes like sintering (including plasma, microwave, and flash sintering), melting, and vitrification. While these methods can produce stable waste forms that comply with regulatory standards, they are often limited by low waste-loading capacities, high energy demands, long processing times, and risks such as hydrogen generation. In this context, cold sintering has emerged as a promising low-temperature alternative. This technique densifies inorganic powders using a transient liquid phase under moderate pressure and temperatures below 300 °C. Cold sintering has shown success in processing a wide range of inorganic materials and has recently been extended to the immobilization of radioactive isotopes such as Cs, I, Ni, and Co in various host matrices [150].

7. Integration of Bioadsorbents into Water Treatment Systems

Although several studies have demonstrated the efficiency of bioadsorbents derived from agricultural waste under laboratory conditions, their practical application depends on additional factors, such as integration into real treatment systems, scalability, operational stability, economic viability and regulatory compliance, as illustrated in Figure 3. The practical incorporation of bioadsorbents into operational water treatment units requires evaluating real water systems on a laboratory scale, expanding their use to large-scale operations. In addition, the verification of operational stability is crucial in order to ensure stability under various operating conditions. Economic and regulatory perspectives verify the financial viability of applications by legal standards and guidelines [150–154].

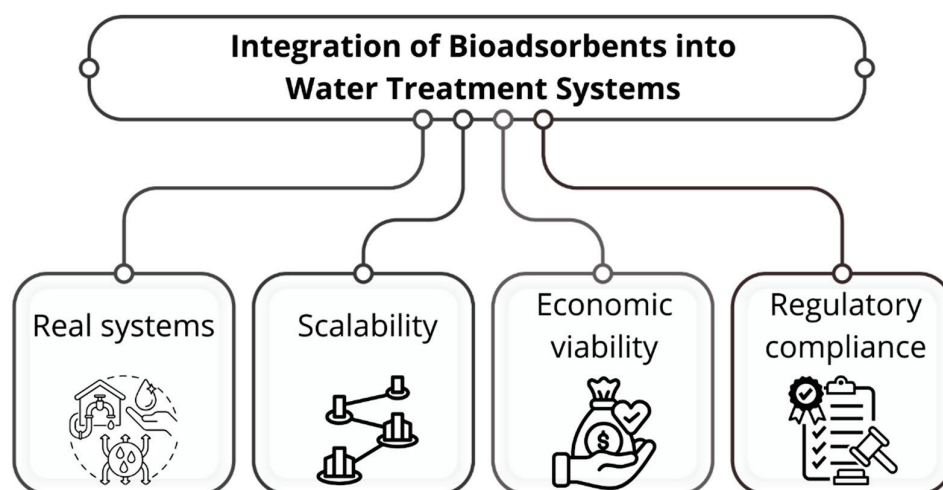


Figure 3. Illustration of factors necessary for integration of bioadsorbents into water treatment.

Although bioadsorbents show promising performances, the transition from laboratory-scale to industrial applications still represents a major challenge. Variability in raw material availability, irregularity in the physicochemical characteristics of adsorbents, and the absence of standardized production protocols are the factors that limit scalability. Furthermore, real wastewater matrices present complexity due to the competition between ions, variations in pH, and variable contaminant loads, aspects that are often not considered in laboratory analyses [155–158]. Future projects should focus on continuous-flow systems, performing technical–economic analyses (TEAs) and life cycle assessments (LCAs) to determine cost-effectiveness and environmental impact, ensuring sustainability and economic competitiveness [159,160]. In addition, it is necessary to evaluate the performance and stability of long-term regeneration under operational conditions, as well as establish quality control standards for bioadsorbent production, considering aspects such as surface area and porosity [155,156,158].

Ferreira Junior et al. [161] carried out the prototyping and economic evaluation of the use of an anionic bioadsorbent from sugarcane bagasse in the treatment of drinking water contaminated by arsenic. Technological innovation was technically feasible, with an 80-fold increase in synthesis scale and level 4 technological readiness. Regarding the financial analysis of the proposed business model (180,000 units in 5 years in a semi-industrial process, 4% of the Latin American market, and a selling price of USD 6.95 per unit), the authors found that the cash flow showed a return on investment above 20% per year, which is attractive for investments.

Another limiting factor for the use of biosorbents is regulatory barriers, due to the lack of official classification and standardization for these materials. In most countries, environmental agencies do not yet recognize biosorbents as approved materials for tertiary or advanced treatment stages, especially when derived from residual biomass. In the European Union and the United States, materials used in water treatment must comply with strict guidelines, such as the EU Drinking Water Directive (2020/2184) and the EPA’s National Primary Drinking Water Regulations [162,163]. In Brazil and Latin America, similar concerns are governed by national frameworks, such as CONAMA Resolution 430/2011 [164], which focuses on the quality of treated effluents but does not yet specify standards for biosorbents.

7.1. Fixed-Bed and Continuous-Flow Systems

The adsorption operation in fixed-bed columns is simple, provides high yields, and can be easily expanded through a laboratory procedure. Under continuous-flow conditions,

liquid-phase adsorption analysis can be performed using breakthrough curves in a column system. The consistency between the results obtained in batch and fixed-bed systems suggests that batch data can serve as a basis for designing and optimizing continuous water treatment systems. With the adequate optimization of operational parameters, it is possible to transpose batch data to applications in continuous systems, promoting sustainable and efficient solutions for the treatment of contaminated water [81,155]. Some studies have used sugarcane bagasse [155], palm oil residues [165], *Peltophorum pterocarpum* tree pods [80], and industrial lignin obtained from pulping and paper manufacturing processes [166] in fixed beds as bioadsorbents.

Although laboratory column adsorption research has shown efficient results, it is essential to conduct studies on larger-scale columns to understand how the parameters of the fixed bed (such as adsorption capacity, breakthrough time, saturation time, and the size of the mass transfer zone, among others) are impacted by increasing the scale. This will allow us to understand the true potential of this technology and the possibility of applying it on an industrial scale [155]. Ramola et al. [167] evaluated the effectiveness of sugarcane bagasse and pruned bamboo biochars modified with Fe in the adsorption of Pb^{2+} and Cu^{2+} ions in fixed-bed systems, aiming at practical applications in the treatment of contaminated waters. The modified biochars demonstrated high efficiency in removing Pb^{2+} and Cu^{2+} , with adsorption capacities superior to those of unmodified biochars. The sugarcane bagasse biochar modified with Fe was more effective in removing Cu^{2+} . Comparing the results with the batch performance, the iron-modified biochars also maintained considerable efficiency in the fixed bed, although with a slight reduction in capacity due to hydrodynamic limitations.

7.2. Hybrid and Integrated Technologies

Combining adsorption with additional technologies can enhance treatment efficiency, especially in complex wastewater systems. This is because each pollutant removal process is distinct, unique, and best suited to the removal of a particular type of pollutant. Thus, by combining processes, different mechanisms work together to improve the removal of a broader range of pollutants. Pollutants that are not effectively removed by one process are efficiently removed by another process [168]. Table 6 presents recent studies of photocatalytic systems, Fenton-type processes, and membrane filtration associated with adsorption which have been effective in removing a variety of pollutants, such as dyes, antibiotics, pesticides, heavy metals, and nutrients.

The combination of adsorption and photocatalysis balances the advantages and disadvantages of both techniques, allowing the more efficient degradation and removal of contaminants from water. The isolated use of each process can result in high costs for adsorbent regeneration and the generation of secondary pollution. In synchronized applications, adsorption can improve the contact between photocatalysts and pollutants, while adsorbents with good charge transfer properties can accelerate the separation and transport of photoinduced electron–hole pairs, increasing photocatalytic efficiency. In this context, the integration of adsorption and photocatalysis has emerged as a promising hybrid approach in which the adsorbent acts as a support for the photocatalyst, promoting the concentration of contaminants on its surface and facilitating their exposure to the photocatalytic effect [79,169].

Metal oxide semiconductors are used due to their non-toxicity, self-cleaning, harmlessness, and high photocatalytic activity [79,169]. In the photocatalytic–adsorbent systems in Table 6, the high removal efficiencies for different classes of dyes and antibiotics stand out, with contact times ranging from 30 to 140 min. These systems combine the efficient adsorption of pollutants with photochemical degradation, promoting not only physical

removal but also the partial or total mineralization of contaminants. The incorporation of semiconductors such as ZnO, TiO₂, and CuO into the modified biochars significantly improves the separation of photoinduced charges as well as the catalytic activity.

Table 6. Technologies for the use of bioadsorbents with integrative technologies.

Technologies	Agricultural Residues	Composites	Degradation	Main Results	Reference
Photocatalytic-adsorptive systems	Peanut shell	ZnO/N,O-containing biochar	Dye (methylene blue) and antibiotics (tetracycline hydrochloride)	Removal efficiency of 96.0 and 97.1% and contact time of 70 and 140 min for methylene blue and tetracycline hydrochloride, respectively	[77]
	Fallen sycamore leaves	Lamellar sycamore leaf TiO ₂ /biochar	Dye (methyl orange) and antibiotics (ciprofloxacin)	Removal efficiency of 95.7 and 80.8% and degradation of 74.2 and 50.2% for methyl orange and Ciprofloxacin, respectively	[79]
	Pomelo peel	Sn quantum dot-loaded N- and O-containing biochar	Dye (methylene blue, malachite green, and rhodamine B)	Removal efficiency of >90% in 60 min after 5 cycles	[78]
	Corn plants	Corn plant biochar and manganese (Mn)-composited copper oxide (CuO)	Dye (Congo red and Eriochrome Black T)	Removal efficiency of 98 and 95%, and degradation of 92 and 88% for Congo red and Eriochrome Black T, respectively	[170]
	Mandarin peels	TiO ₂ /mandarin waste peels (cellulose source)	Dye (methyl orange)	Removal efficiency of 98.9%, contact time of 30 min, and adsorption capacity of 104.2 mg/g	[169]
Fenton-like processes	Pistachio shell	Biochar-based Fe ₃ O ₄ nanoparticles and ascorbic acid	Dye (methylene blue) and pesticides (acetamiprid)	Adsorption capacity of 370.4 and 357.1 mg/g for methylene blue and acetamiprid, respectively	[74]
	Corn cob	Biochar-based magnetic Fe–Cu bimetallic	Antibiotic (ciprofloxacin)	Removal efficiency of 93.6%, contact time of 360 min, and degradation of 66%	[72]
	Rubber tree bark and coconut shell	Biochar with FeCl ₃ and H ₂ O ₂ solutions	Dye (methylene blue) and hexavalent chromium (Cr (VI))	Adsorption capacity of 335.6 and 258.1 mg/g for methylene blue and chromium, respectively	[171]

Table 6. Cont.

Technologies	Agricultural Residues	Composites	Degradation	Main Results	Reference
Photocatalytic–adsorptive systems	Wheat straw	Iron tailings and wheat straw blends	Dye (methylene blue)	Removal efficiency of 84.0%	[172]
	Laurel leaves and watermelon peels	Hydrochars	Dye (anionic—reactive red 180; cationic—basic red 18)	Removal efficiency of 99.8 and 98.8% for reactive red 180 and basic red 18, respectively	[173]
Membrane filtration pretreated with adsorption	Corn stover	Biochar and ceramic membrane filtration	Nitrogen, phosphorus, and organic matter	Removal efficiency of 91.42%, 91.49%, 89.54%, and 76.34% for total nitrogen, total ammonia nitrogen, total phosphorus, and soluble chemical oxygen demand, respectively	[174]

The use of Fenton-type catalysts is crucial in wastewater treatment, as current catalysts lack antiparasitic properties and exhibit reduced activity at high generation rates. This is due to the intrinsic contradiction between the slow sorption rate and the rapid and inefficient utilization of free radicals [175]. Hybrid systems that combine adsorption and Fenton or Fenton-like processes, using agricultural residues as precursors for bioadsorbents, increase their effectiveness in eliminating ECs. In Fenton/biochar processes, the efficiency and removal characteristics are improved due to the movement of electrons between the biochar and the active sites of the iron or redox cycles. Furthermore, the existence of unequal electrons in persistent free radicals in biochar can accelerate the exchange of electrons and intensify the degradation rate of the contaminated pollutant [172].

The choice of residue and appropriate modification are crucial to optimizing system efficiency. The effects of several operational factors, such as the solution pH, catalyst quantity, hydrogen peroxide (H₂O₂) concentration, initial concentration of the emerging contaminant, and structure of the biosorbent, must be considered when combining these systems [173]. The combination of adsorption and Fenton processes focuses on dye removal and the use of agro-industrial shells (Table 6). Fenton-like processes also show promising results, with high adsorption capacities for organic and inorganic contaminants. The presence of metallic nanoparticles (such as Fe₃O₄ or Fe–Cu) activated by reducing agents or peroxides enhances the generation of hydroxyl radicals, which are highly reactive in the degradation of pollutants. These systems benefit from the porous and functionalized nature of biochars derived from waste such as pistachio shells, wheat straw, and corncobs.

Organic micropollutants (MPOs) are efficiently removed by membrane filtration. Although this technique has advantages such as not needing chemicals, high efficiency, and selective rejection, fouling is a significant limitation that can be reduced through pretreatment using adsorption [176]. As shown in Table 6, studies using bioadsorbents as a pretreatment for filtration are still scarce. Lin et al. [174] developed a high-performance ammonia removal, biochar adsorption, and ceramic membrane filtration process for the treatment of kitchen waste biogas sludge. The authors found that integrating bioadsorbent with filtration increased phosphorus removal and reduced membrane contamination. In addition, the by-products of the process were reusable (ammonium sulfate, biochar, and concentrated sludge), which could offset costs through agricultural applications such as fertilizers and soil amendments.

Wang et al. [177] performed a synergistic removal of ultrafine coal particles and $\text{Ca}^{2+}/\text{Mg}^{2+}$ from mine wastewater by two-stage adsorption before membrane filtration. The authors found that the first-stage adsorption was driven by ultrafine coal particles within the bulk solution, and the cake layer formed on the membrane surface initiated the second-stage adsorption. Thus, suspended solid removals of greater than 99.9% and $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratios of more than 20% were achieved.

As evidenced, technologies used in integrated ways improve the efficiency of contaminant removal. In this sense, Mu et al. [178] studied a process combining Fenton oxidation and adsorption followed by filtration through a ceramic membrane. The authors improved the treatment efficiency and operational cycle of refinery effluents. Activated carbon was used in the adsorption process, demonstrating the potential for future studies to assess the efficiency of alternative bioadsorbents.

Although the hybrid systems listed in Table 6 have demonstrated high removal efficiency, it is important to highlight that most of these results were obtained using synthetic or simplified model solutions. Real wastewater matrices typically present more complex compositions, including competing ions, organic matter, and fluctuating pH, which can significantly influence adsorption and catalytic behavior. Therefore, further validation in real wastewater scenarios is crucial in order to confirm the practical applicability and robustness of these hybrid systems.

8. Conclusions

This work presented the wide range of materials that can be developed from agricultural residues to produce advanced bioadsorbents aimed at removing ECs from aqueous media. Biochars obtained through thermal treatments, such as conventional and microwave-assisted pyrolysis, demonstrated increased porosity, high surface area, and partial graphitization—features that enhance π - π interactions with aromatic contaminants like diclofenac and chloroquine. These effects were even more pronounced in systems using biochar derived from *Parthenium hysterophorus* or corncob functionalized with metallic nanoparticles.

Chemical modifications, including activation with ZnCl_2 , KOH, or H_3PO_4 and functionalization with metal oxides (ZnO , Fe_3O_4 , CuO), further improved the adsorbents by increasing their surface acidic group density and introducing catalytic properties. These hybrid materials not only enhanced adsorption performance but also enabled the partial degradation of contaminants, as seen in the Fe-Cu-modified biochars used for ciprofloxacin removal via adsorption and Fenton-like reactions.

The main interaction mechanisms between adsorbents and adsorbates included hydrogen bonding (favored by $-\text{OH}$ and $-\text{COOH}$ groups), ion exchange (necessary for compounds like 2,4-D and glyphosate), and pH-dependent electrostatic forces relative to the materials' point of zero charge. Surface-charge-tuned materials demonstrated selective affinity toward different ionic species such as PFOS and methylene blue.

High adsorption capacities (>300 mg/g) were reported across a broad spectrum of contaminant classes. Several materials also exhibited regeneration potential and have shown promise in preliminary continuous system applications. However, it is crucial to recognize that many findings are still limited to laboratory-scale studies. While fixed-bed columns and real wastewater trials have provided encouraging results, challenges such as adsorbent fouling, regeneration efficiency, and long-term stability remain underexplored at the pilot and industrial scales.

Thus, adsorbents derived from modified agricultural residues represent a promising and environmentally sound alternative to conventional materials. Beyond their high performance and low cost, these materials contribute to circular economy practices and

sustainable water treatment. Further efforts in upscaling, durability assessment, and integration into real treatment systems are essential to ensuring their practical viability.

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Article

Chemical and Isotopic Investigation of Abiotic Oxidation of Lactate Substrate in the Presence of Varied Electron Acceptors and Under Circumneutral Anaerobic Conditions

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Abstract: Abiotic processes have ramifications in wastewater treatment, in situ degradation of organic matter, and cycling of nutrients in wetland ecosystems. Experiments were conducted to investigate abiotic oxidation of organic compounds (lactate) as a function of electron acceptors (ferric citrate and hydrous ferric oxide (HFO)), media composition, and pH under anaerobic conditions, using sodium bicarbonate as the buffering agent. Dissolved inorganic carbon (DIC) was used as a proxy for the oxidation of substrates. HFO media generated more DIC compared to ferric citrate containing media. Light and pH had major roles in the oxidation of lactate in the presence of ferric iron. Under dark conditions in the presence or absence of Fe(III), the DIC produced was low in all pH conditions. Inhibition of DIC production was also observed upon photo exposure when Fe (III) was absent. Isotopically, the system showed initial mixing between the bicarbonate and the carbon dioxide produced from oxidation later being dominated by carbon isotope value of lactate used. These redox conditions align with previous studies suggesting cleavage of organic compounds by hydroxyl radicals. The slower redox processes observed here, compared to previous studies, could be due to the scavenging effect of chloride ion on the hydroxyl radical.

Keywords: photochemistry; sensitizing agents; iron reduction; organic compounds Fenton chemistry

1. Introduction

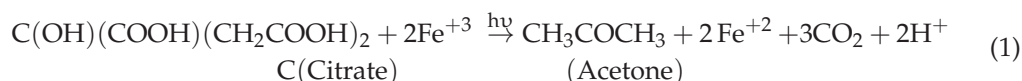
The fate, mobility, and bioavailability of inorganic and organic compounds in the environment are heavily influenced by various geochemical processes, including sorption, precipitation, dissolution, and electron transfer [1–3]. Investigating the different geochemical processes and their reaction mechanisms would add to the understanding of natural systems and the fates of inorganic and organic compounds in pristine and contaminated environments. Electron transfer processes could be either enzymatically mediated microbial metabolism or abiotically controlled.

For studies related to biotic redox processes, strains (e.g., *Shewanella putrefaciens*) are cultured in the laboratory under favorable conditions of media, pH, buffering agents, etc., and suitable indicators of redox processes are analyzed. Since the redox process generates CO₂, an analysis of Dissolved Inorganic Carbon (DIC) is a convenient method to study this when the redox process is allowed to take place in vitro.

Abiotic electron transfer processes have drawn much attention in many fields, including wastewater treatment, enhancing in situ degradation of organic and inorganic

compounds, cycling of nutrients in wetland ecosystems, and in atmospheric chemistry for investigating sources and sinks of chemicals in the troposphere [1–13].

Direct photolysis is an abiotic process in which organic compounds absorb light, and, as a result, they undergo photochemical transformation. With some organic compounds, exposure to a light source alone does not result in electron-transferring reactions [4,7,8]. Indirect photolysis takes place either due to energy transfer from another excited species (sensitized photolysis) or chemical reactions (e.g., Fenton like reactions) of nonexcited compounds. Non-excited compounds possess short-lived but very reactive species (e.g., OH- and peroxy- radicals, singlet oxygen) formed in the presence of light due to reactions of excited compounds (e.g., citrate, lactate). Sensitizing agents (e.g., Fe(III) and Zn) act as geocatalysts (acting as catalyzing agents) in natural ecosystems assisting photochemical reactions to transform pollutants [2,11]. One of these catalysts is ferric iron, which acts as a photosensitizer and participates in the formation of oxidants via photo-initiated transformations [2,11]. Under our experimental conditions discussed here, Fe^{+3} forms complexes with polycarboxylic acids (e.g., ferric citrate) and oxyhydroxides (e.g., HFO). Photochemical dissociation of ferric citrate complexes in aqueous solutions have been reported to involve the reduction of ferric iron to ferrous iron, coupled with the oxidation of citrate-producing acetone and carbon dioxide represented by the general reaction (1), shown below [5,6,13]. Along with microbial metabolism, photochemical processes and Fenton-like reactions enhance the reduction of metals and oxidation of organic compounds [14–16].



where $h\nu \rightarrow$ photons or quanta, $h =$ Planck constant, 6.63×10^{-34} J.s, and $\nu =$ frequency.

Recent studies have reported that the photochemical reaction of ferric citrate and ferric oxyhydroxides form hydroxyl radicals via an intermediate process involving a modified Fenton-like reaction [6,16]. The redox potential and thermodynamic properties make ferric citrate and hydrous ferric oxide excellent electron acceptors to study biotic as well as abiotic oxidation of organic substrate [8]. Fenton chemistry ($\text{Fe}^{+2} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{+3} + \cdot\text{OH}$) plays a role in photochemical transformations of organic compounds in two ways. One is producing a hydroxyl radical that, in turn, attacks organic compounds, such as citrate and lactate, mineralizing them to lower-molecular-weight carbon compounds, such as carbon dioxide, both aerobically and anaerobically [2,6,10–13]. Second, Fenton chemistry oxidizes ferrous to ferric iron, an excited species, for further sensitized photo-oxidation of the organic compounds. Under anaerobic conditions, the presence of anions such as chloride inhibits abiotic reduction–oxidation couplings by forming halogenated compounds and by scavenging the hydroxyl radicals [17,18].

Iron is very reactive in near-surface environments, and its oxyhydroxides are common on the earth's crust and can function as geocatalysts in speciation of organic and inorganic pollutants in the subsurface environment [2,19]. Recent studies have shown the importance of dissolved organic compounds (DOC) and iron ions involved in the photochemical formation of hydrogen peroxide and other photooxidants in degrading natural and anthropogenic compounds [1,9,11,13].

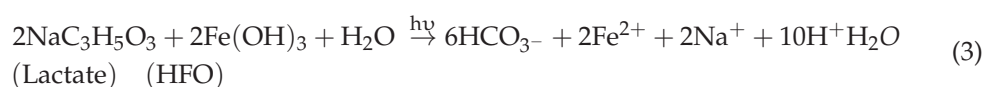
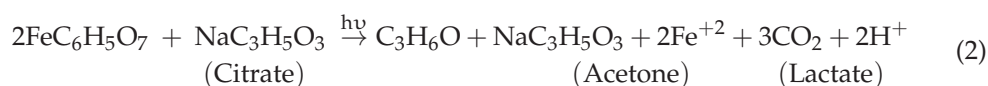
Carboxylic acids (e.g., oxalate, citrate) possess one of the most common functional groups (RCOO) of dissolved organic compounds present in natural waters and form strong complexes with ferric iron [6]. The photooxidation of these carboxylic acids could be sensitized by the presence of Fe(III) undergoing rapid photochemical reactions upon irradiation according to reaction 1 [20]. Deng et al. [6] used Fe(III)–citrate complexes as a photochemical sensitization material to promote the discoloration of dyes,

forming more reactive species (e.g., H_2O_2 and $\cdot\text{OH}$) compared to Fe(III)–hydroxyl and Fe(III)–oxalate complexes at acidic pH conditions. Deng et al. [6] reported that at a pH of 2, the photodegradation rates of organic compounds increased for varying citrate-to-Fe(III) ratios in the order of 4:1 > 2:1 > 1:4 > 1:1 > 1:2. They also pointed out that at neutral-to-alkaline conditions, Fe(III)–hydroxyl dominates the photoreactivity compared to Fe(III)–citrate complexes. Hydroxyl radicals produced in systems containing Fe(III)–ligands complexes could be responsible for the oxidation of organic compounds coexisting in aqueous solutions [6]. Deng et al. [6] concluded that under near-UV light, the efficiency of the Fe(III)–citrate complexes induced photoreduction reaction in aqueous solutions that depended on the pH and the initial citrate-to-Fe(III) ratio.

Several variables, including photo-formation of $\cdot\text{OH}$ radicals, $\cdot\text{OH}$ radicals attacking target groups, or bonds in the respective organic compounds, could result in different photodegradation rates for different organic compounds [9]. The latter might be a rate-controlling step in the photodegradation system. Wu and Deng [9] concluded that the photochemical nature of organic compounds coexisting in photo-reactive complexes containing solutions is strongly influenced by the pH, Fe(III) concentration, wavelength, and energy of the irradiation source of the solution. This observation concurred with the observation made by [4] that organic compounds dissolved in solutions rich in ions easily become oxidized upon illumination.

Understanding abiotically controlled electron transfer processes in a laboratory-based anaerobic system is the focus of this study. This can be carried out *in vitro* exactly the same manner as with biotic redox processes, with the difference being that no microorganisms are introduced into the system, and any DIC produced is expected to be from abiotic oxidation.

A literature review of such studies showed that photo-assisted oxidation of polycarboxylics (e.g., citrate, oxalate) and other organic compounds in the presence of ferric iron, other metals (Mn, Cd, and Ni), and semiconductors (TiO_2) is a well-documented phenomenon [1,5,6,13]. Most of these previous studies were performed under acidic and aerobic conditions. Our study was completed under anaerobic conditions, in the presence or absence of light, and at varying pH (5 to 9) conditions. Here, the oxidation of lactate and citrate with and without ferric iron was used to investigate the effect of (1) electron acceptor (ferric citrate vs. HFO) in a carbonate-buffered system; (2) media components; (3) buffers under neutral pH condition (4); pH conditions; and (5) fluorescent light. Most of these factors have environmental implications in natural and engineered remediation strategies of contaminated sites. It will also be important for adding data to the new and exciting ongoing research in the abiotic degradation of organic pollutants. Moreover, to the best of our knowledge, there are no reported abiotic studies on the oxidation of lactate. Here, we report the possible mechanisms of the DIC produced during the abiotic oxidation of lactate and citrate under strict anaerobic conditions, circumneutral pH, and a temperature of 30 °C. We also report carbon isotopic signatures associated with the abiotic electron transfer processes. The general schematic ferric-iron-sensitized photocatalytic processes in this study are represented by the reactions (Equations (2) and (3)):



2. Materials and Methods

2.1. Media Composition

Details of media composition and experimental setups are discussed elsewhere in detail [21,22]. Briefly, a defined circumneutral pH medium was prepared with components containing NH_4Cl , $\text{NaH}_2\text{PO}_4\text{H}_2\text{O}$, KCl , vitamins, Wolfe's minerals, sodium lactate, ferric citrate, and sodium bicarbonate. Ferric citrate stock solution was prepared using autoclaved double-deionized water ($R \sim 17.6 \text{ M}\Omega \text{ cm}$) amended with reagent-grade ferric citrate (Fischer Chemical[®], Pittsburgh, PA, USA) and 10 N NaOH [22,23].

An HFO (70 mM)-based experiment was performed using solid HFO prepared using the method discussed by Schwertmann and Cornell [19]. The solid HFO was added into each vacutainer tube. $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (80 g/L) was dissolved in DDIW (Double Distilled Ionized Water) with 330 mL/L of 1 M KOH, and the pH was adjusted to ~ 7.7 . The solution was stirred for 1 h and centrifuged repeatedly at 5 min intervals at 1500 rpm to remove any impurities. Then, the solution was filtered using $0.2 \mu\text{m}$ sized filters before freeze-drying. HFO loses its structure on heating above 80°C , so to preserve its structure, HFO was added in the anaerobic chamber after autoclaving the rest of the media components.

2.2. Media Preparation

Eight 500 mL/L Pyrex bottles were autoclaved and filled with defined salt media by omitting one media component at a time. Each bottle was set to a pH of ~ 7.1 by adding HCl dropwise. All respective media components, with the exception of minerals and vitamins, were added before autoclaving. For each experimental setup, six vacutainer tubes of 15 mL/L volume were loaded with 8 mL/L of the media solution within the anaerobic chamber after applying nitrogen to each for an hour. All samples were taken in duplicates, and a pair of samples from each Pyrex bottle was taken before starting to shake to know the starting values. This set of experiments continued for about 2900 h (~ 121 days).

A set of experiments with ferric citrate or HFO as Fe(III)-containing compounds were used to examine the effect of ferric iron–complex compounds on the oxidation of the organic compounds under the experimental conditions discussed above.

The effect of two buffering compounds, NaHCO_3 on the generation of carbon dioxide and soluble ferrous iron, was compared under both dark (wrapped by aluminum foil) and fluorescent light conditions. Each sample was treated similarly, and the experiments were carried out for pH 5 and 7 to check the relative importance of two of the controlling factors, pH and fluorescent light.

The effect of pH on the photo-oxidation of ferric citrate and sodium lactate was investigated in the presence of light for pH varying from acidic (pH ~ 5) to alkaline pH (pH ~ 9) conditions for the bicarbonate buffered system.

Photooxidation of organic compounds were investigated in the presence and absence of light and Fe(III) in the acidic (pH ~ 5) to neutral pH (pH ~ 7) conditions. This portion of the experiment was conducted to compare and contrast the effect of pH, Fe(III), and light on redox processes.

2.3. Chemical Analyses

Details of chemical analyses are explained in detail in earlier publications [21,22], and repetition is deemed unnecessary and beyond the scope of this paper. The iron concentration was determined using UV/VIS spectrophotometer absorbance wavelength of 562 nm via the ferrozine method under anaerobic conditions and is reported in mg/L of Fe (II)/Fe(III) [24]. The pH of each sample was determined using a portable pH/mV meter.

2.4. Stable Carbon Isotope Analyses

The carbon isotopic composition of sodium lactate, sodium acetate, citric acids and ferric citrate was determined from CO₂, as discussed in Gebrehiwet & Krishnamurthy [21]. The stable carbon isotopic ratio of sodium bicarbonate used as a buffering agent was analyzed by reacting with 85% phosphoric acid [25], and DIC was analyzed via the gas evolution method [26]. Carbon isotope measurements were made using a VG IRMS (Isotope Ratio Mass Spectrometer), and the results are reported in the traditional δ notation relative to Vienna PeeDee Belemnite (V-PDB) standard: (Equation (4)).

$$\delta^{13}\text{C}_{\text{‰}} = \frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 10^3 \quad (4)$$

where R_{Sample} and R_{Standard} represent the ¹³C/¹²C ratio of the sample and standard (VPDB), respectively. The overall precision of the analytical processes are better than 0.1‰ for $\delta^{13}\text{C}$, $\delta^{13}\text{C}_{\text{org}}$, and $\delta^{13}\text{C}_{\text{Inorg}}$ and 1‰ for carbon dioxide measurements. All abiotic samples were treated with cupric oxide at ~900 °C to purify other gases produced during the photochemical reactions before subsequent isotope ratio measurements. This purification resulted in much cleaner isotope ratio traces.

3. Results and Discussion

3.1. Extent of Iron Reduction and Organic Compound Oxidation

Soluble iron and DIC measurements indicated that, in all experimental conditions, except when covered with aluminum foil (not exposed to light), both ferrous iron and CO₂ yield increased as a function of the time of reaction, as shown in Figures 1 and 2. Figure 1 shows the ferrous iron concentration of the ferric citrate media exposed to fluorescent light at pH~7.

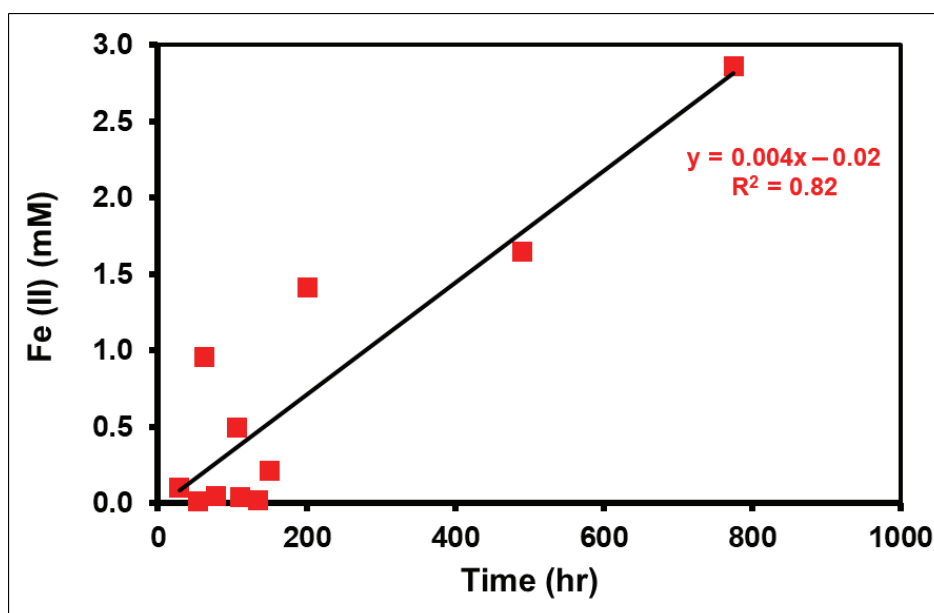


Figure 1. Graph showing the Fe(II) concentration for all abiotic samples exposed to fluorescent light at neutral pH as a function of time.

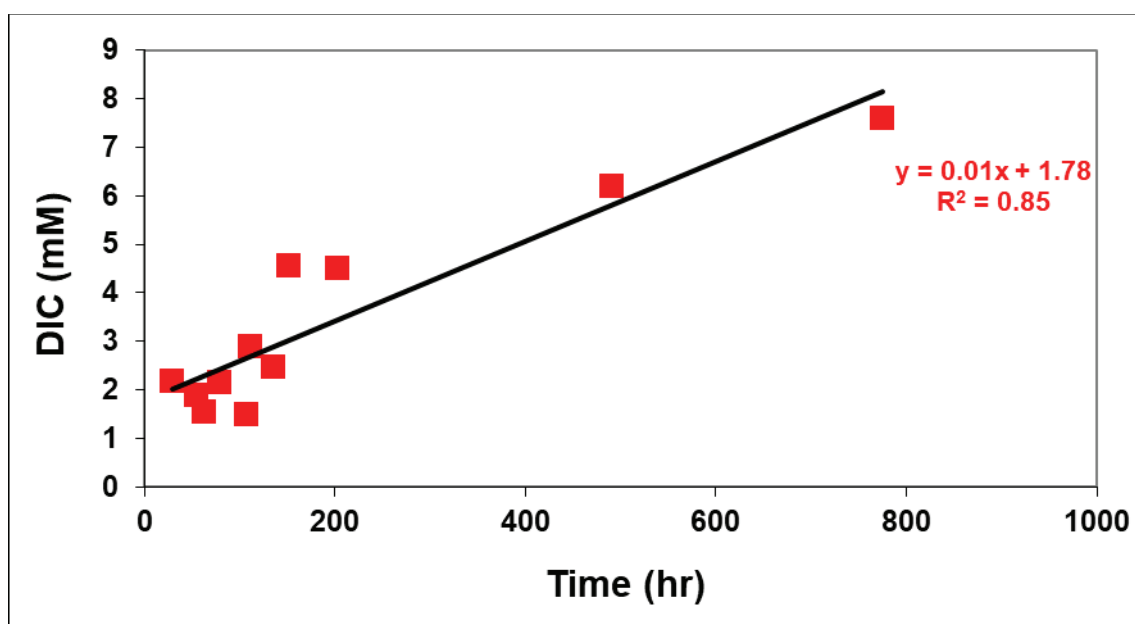


Figure 2. CO₂ yield from all abiotic samples exposed to fluorescence as a function of time.

The DIC production, which is assumed to be a proxy for organic compound oxidation also increased with the reaction time in all experiments except in the combined absence of light or Fe(III)-containing compounds (Figure 2).

The behavior observed in Figures 1 and 2 means there should be a correlation between DIC and ferrous iron concentration. That this indeed is the case is evident from Figure 3. Figure 3 is a cross-plot of ferrous iron as a function of the DIC produced in the bicarbonate buffed conditions in the ferric-citrate-containing media.

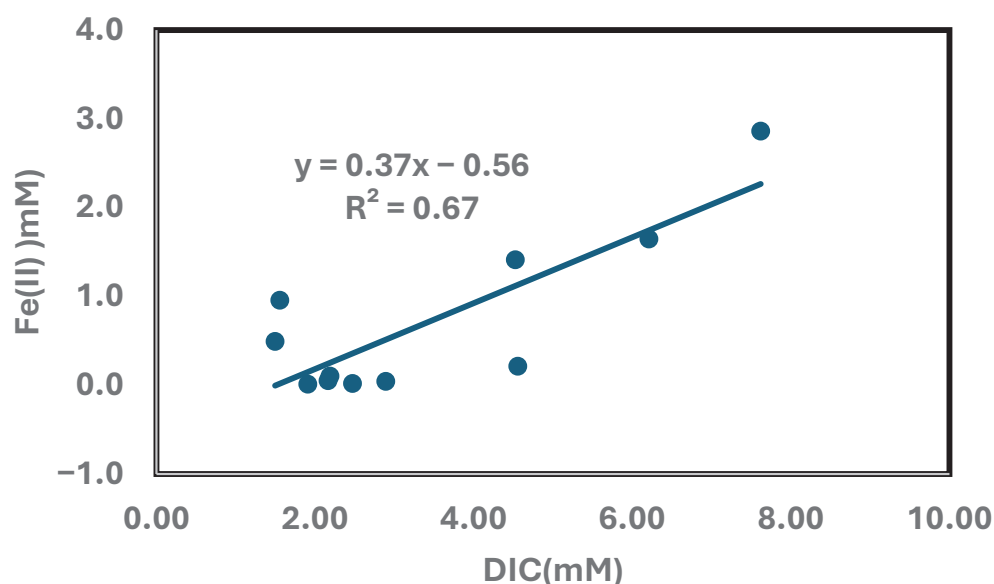


Figure 3. Fe(II) vs. DIC produced for all abiotic samples exposed to fluorescent light at neutral pH in the presence of Fe(III).

There is linear relationship between the concentrations of the reduced form of iron and the production of carbon dioxide, consistent with a photo-induced abiotic redox process. Somewhat low correlation between DIC and Fe(II) values ($R^2 = 0.67$) could be explained by

the possible re-oxidation of ferrous to ferric iron via the Fenton-like reaction (Equation (5)) with increased generation of hydrogen peroxide (H_2O_2) and ferrous iron [6].



3.2. Effects of Media Composition

Experiments were carried out to examine if media components had an effect. For this, one media component was removed from the rest, and the amount of DIC produced was measured. For instance, M-MIN was the media without the minerals, M-VITM was the media without the vitamins, and so on. Generally, the removal of a media component in all the samples was observed to have comparable carbon dioxide generation with time. The significant change occurred when ferric citrate was removed from the media (M-FC). The M-FC samples gave the least amount of DIC from the degradation of lactate, the only significant carbon-containing organic compound. The amount of DIC in the M-FC samples was less by a factor of about 5 compared to the other set of samples in the current experimental setup. Some plausible explanation is provided based on the following experiments.

3.3. Effect of Fe(III)-Containing Compounds

Two sets of experiments with two different iron-containing electron acceptors, ferric citrate and HFO, were carried out to compare the importance of the form of ferric iron in organic carbon oxidation. As shown in Figure 4, HFO in the presence of lactate produced twice as much DIC, compared to ferric citrate in the presence of lactate, and increased with time. The observed difference between the two sets of samples could be attributed to the efficiency of HFO in oxidizing organic compounds at near-neutral pH conditions compared to ferric citrate [4,6].

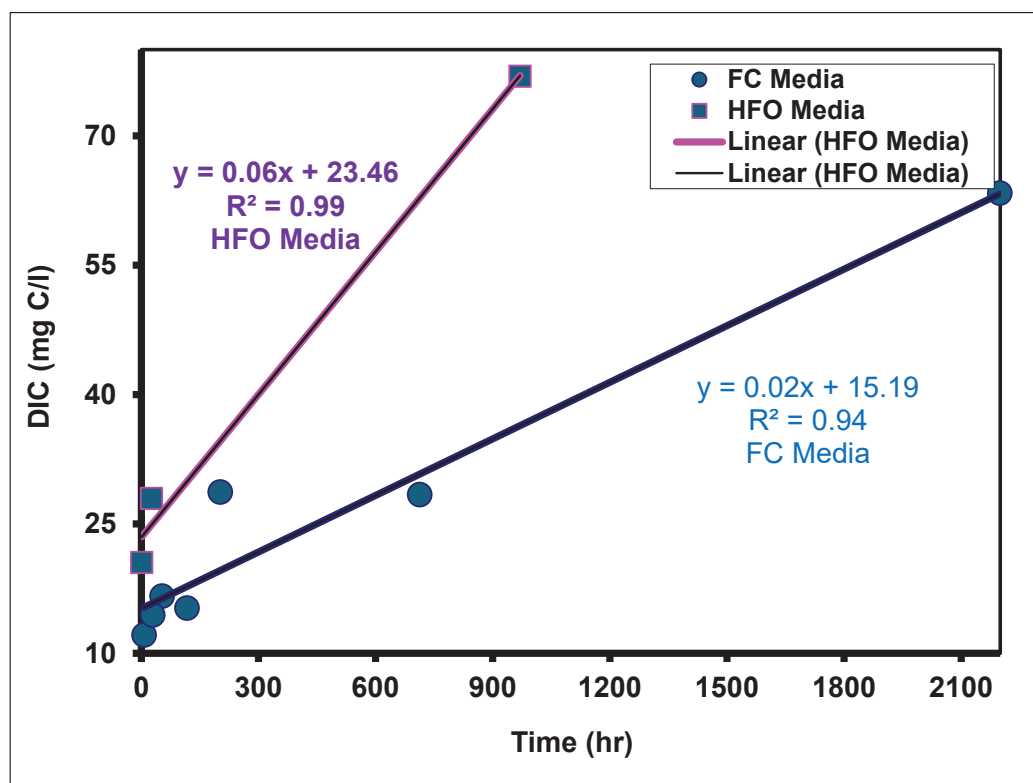


Figure 4. CO_2 yield in experiments with lactate-containing media and ferric citrate compared to those with lactate containing media and HFO (hydrous ferric oxide) at pH 7 as a function of time.

3.4. Effect of pH

The effect of pH on the abiotic oxidation of organic compounds has been investigated by various researchers [6,11,27,28]. Most of these studies have been carried out at low pH conditions (<5). Here, our study focused on the effects of pH, ranging from 5 to 9, on the redox process for the bicarbonate-buffered system. Fe(II), Fe(III), pH, and DIC were determined in each sample. Figure 5 shows the DIC as function of time for the acidic (pH = 5), neutral (pH = 7), and alkaline (pH = 8 and 9) conditions. DIC production was the highest at acidic conditions followed by the neutral and alkaline conditions, respectively. Similarly, the highest Fe(II) accumulation was observed in the acidic condition compared to the neutral and alkaline pH conditions, respectively (Figure 6).

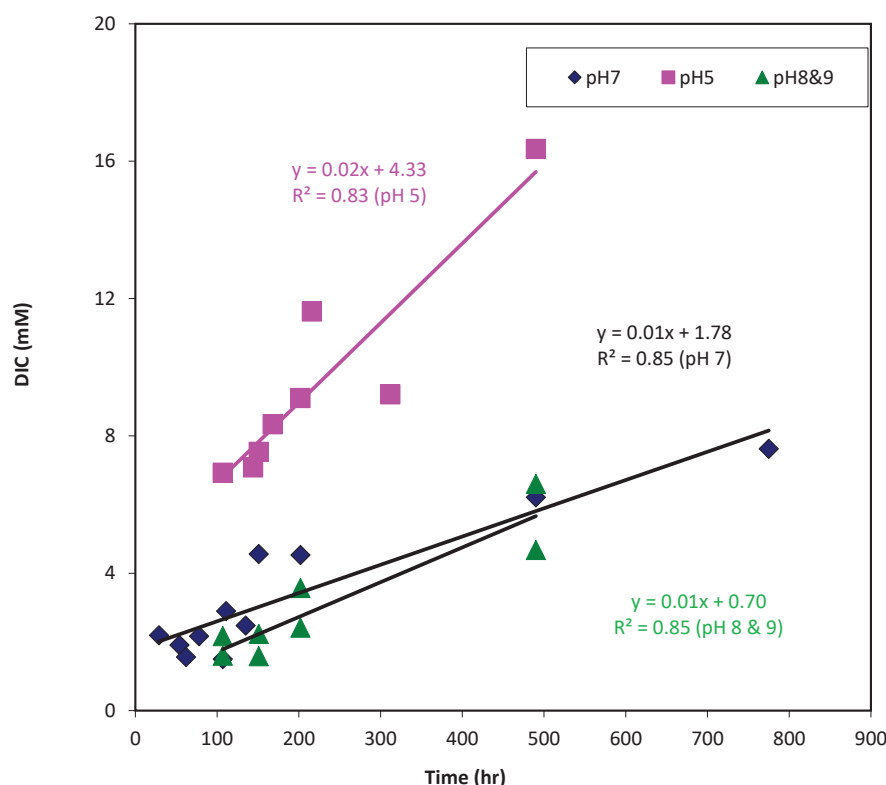


Figure 5. Comparison of DIC yield under different pH conditions (5, 7, or 8 and 9) in the bicarbonate-buffered system as a function of time, indicating increasing DIC production with lowering pH from 9 to 5.

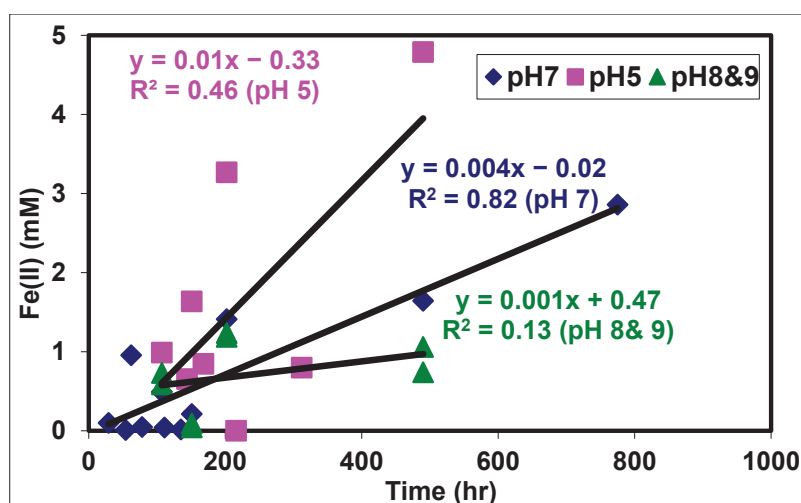


Figure 6. Ferrous iron accumulation at pH of 5, 7, or 8 and 9 in the bicarbonate-buffered system as a function of time, clearly indicating that the lower the pH, the higher the accumulation of Fe(II).

3.5. Effect of Light Under Acidic and Neutral Conditions

A set of experiments were conducted to investigate the effect of light, pH, and ferric iron. Experiments were carried out under fluorescent light and dark conditions, at acidic and neutral pH, and in the presence and absence of ferric iron. In the absence of ferric iron, no DIC generation occurred under any photo-exposure or different pH conditions. Figures 7 and 8 compare the effect of light and pH on the DIC and Fe(II) generations for the bicarbonate buffered condition, respectively, in the presence of ferric iron.

The main conclusion from these experiments is that, under our experimental conditions, fluorescent light had a greater effect on the redox process than pH in the presence of ferric iron. Regardless of the pH conditions, in the absence of light or ferric iron, both DIC and ferrous iron accumulate slowly or not at all, indicating the absence of organic compounds oxidation.

3.6. Stable Carbon Isotopes in Abiotic Systems

Results of isotopic analyses at neutral pH and anaerobic conditions of the extracted DIC are shown in Figures 9 and 10. Figure 9 shows a plot of $\delta^{13}\text{C}_{\text{DIC}}$ as a function of total DIC in the bicarbonate system without correcting for the initially added bicarbonate buffer that had a $\delta^{13}\text{C}$ value of 9.3‰. At lower pH conditions (pH~5), there were no systematic relationships between the DIC and its respective carbon isotopic values. Figure 10 shows graph of the $\delta^{13}\text{C}_{\text{DIC}}$ values as a function of inverse of the total DIC in the bicarbonate system. Such plots are useful in estimating the source of additional carbon dioxide produced from oxidation of organic compounds [29]. The y-intercept of such a plot provides clues regarding the original source of organic carbon involved in the redox process. In this case, the lactate which was the organic carbon source had a $\delta^{13}\text{C}$ value of -25‰ .

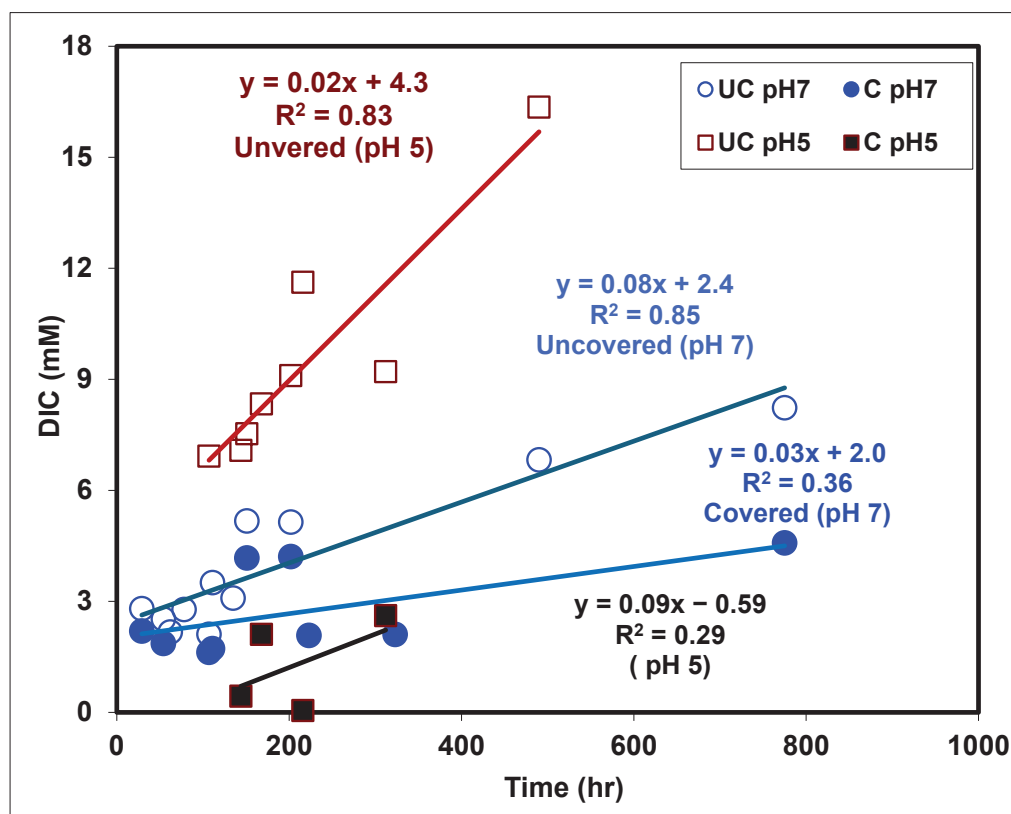


Figure 7. Influence of light under acidic and neutral conditions on DIC production in the bicarbonate-buffered system; UC (open symbols) = uncovered, exposed to light; C (filled symbols) = covered, dark.

3.7. Chemical Factors Controlling Redox Processes

Experiments were carried out in anaerobic conditions under different controlling factors. Our results agreed well with previous studies reporting the importance of pH, light, and ferric iron for the abiotic oxidation of organic compounds coupled to metal reduction. In addition, the concentration of the ferric iron, wavelength, and energy of the irradiation source could influence the rate of redox processes [7]. The type of photo-oxidizing catalysts, the strength of the reducing agents, and the presence of multidentate ligands also affect the rate of photo-assisted redox processes [4]. Moreover, different organic compounds exhibit different photodegradation rates involving several intermediate reactions in aqueous solutions [6,7]. Our results could be affected by a combination of the multiple controlling factors mentioned above and by other researchers.

In the experimental setups containing all media components, both Fe(II) and DIC increased with time. The rate of redox processes observed in our experimental conditions was slower compared to most of the previous studies both at acidic and neutral conditions [6,16]. Deng et al. [6] studied at a lower pH (2) compared to our pH (ranging from 5 to 9), so considering the effectiveness of ferric citrate at lower pH could partially explain the observed discrepancies in the two studies. Northup and Cassidy [16] used modified Fenton mechanism using hydrogen peroxide at a pH of 8. Such a modification could explain the faster rate observed in their study compared to our study. Moreover, in these two studies [6,16], the concentration of chloride ion is low compared to our experimental conditions, where we used KCl (1.4 mM) and NH_4Cl (4.7 mM) in the media preparation. Recent studies [17,18] have shown that the presence of chloride ion in anaerobic conditions inhibits indirect photolysis by scavenging hydroxyl radicals in the systems. Hence, the chloride ions could be partially responsible for the slow rate of oxidation observed.

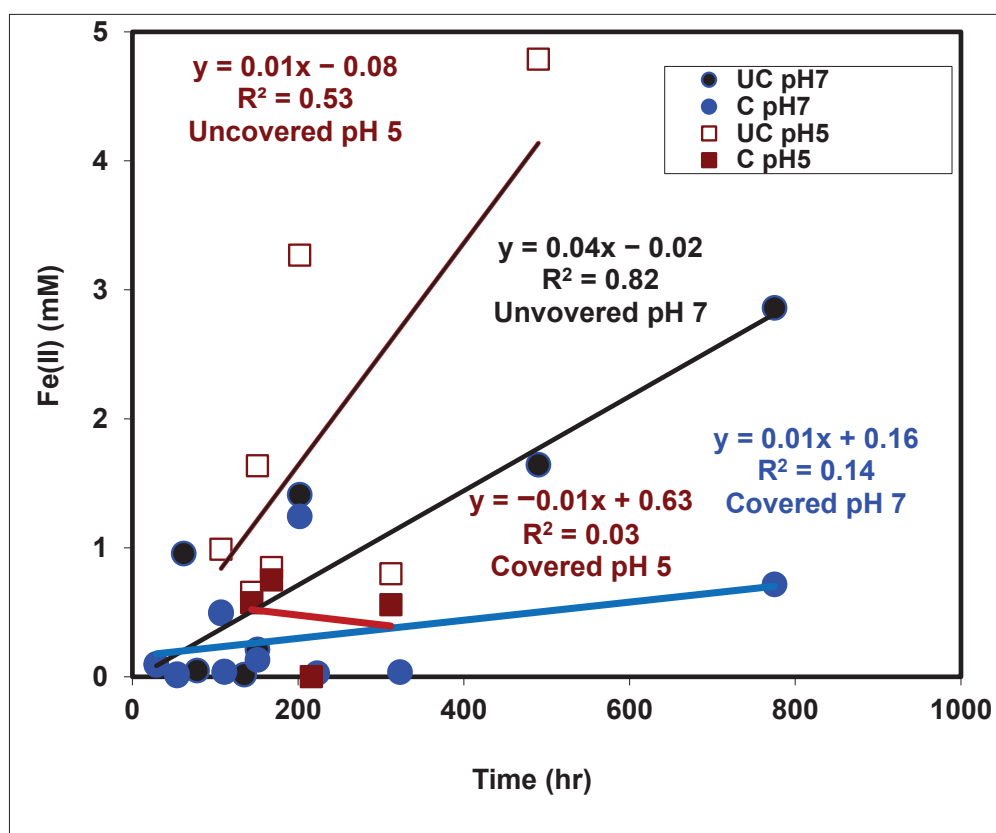


Figure 8. Ferrous iron production in the bicarbonate buffering system in the presence or absence of light at pH 5 and 7 as a function of time. UC (open symbols) = uncovered, exposed to light; C (filled symbols) = covered, dark.

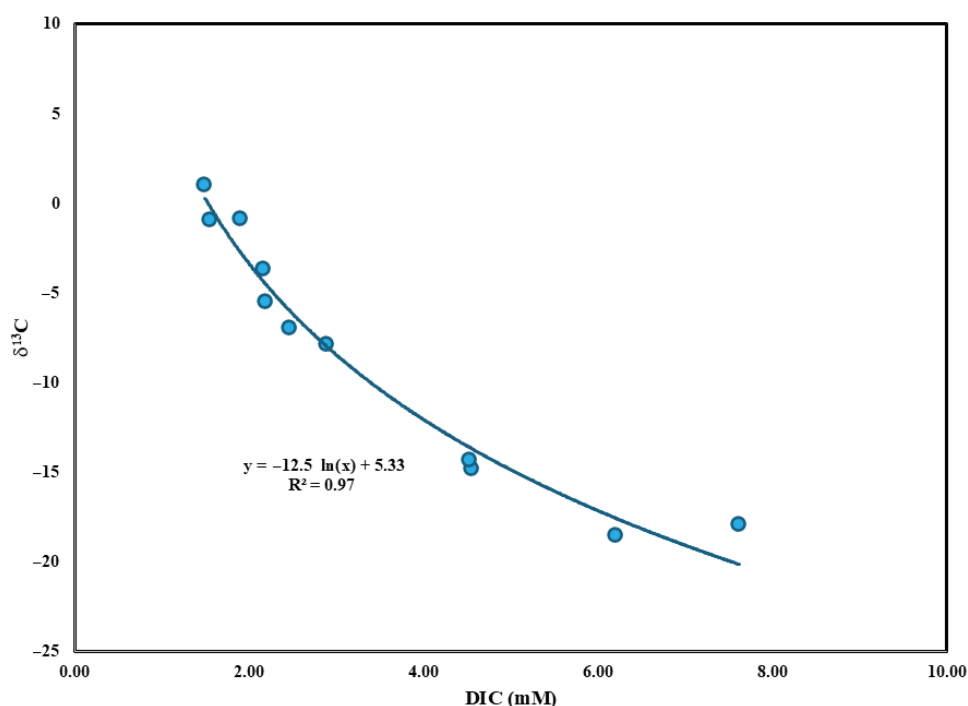


Figure 9. Carbon isotopic values of DIC vs. DIC concentration under neutral pH conditions in the bicarbonate buffered system as a function of total DIC.

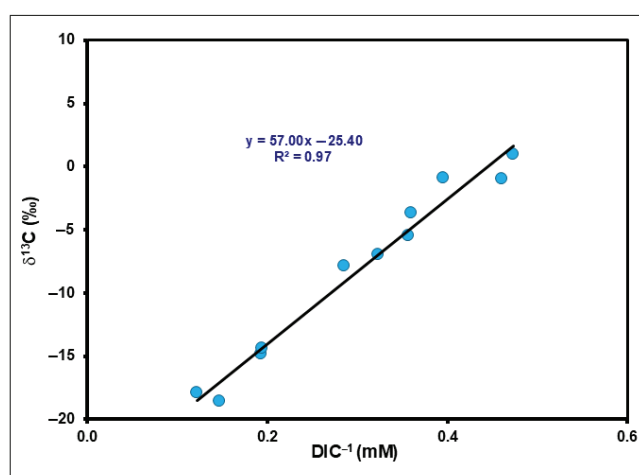


Figure 10. Carbon isotopic values of DIC vs. inverse of DIC concentration relationship of the bicarbonate-buffered system under neutral pH conditions. The y-intercept of the regression of the line provides the estimate of the starting $\delta^{13}\text{C}$ value of the organic compound being oxidized in the bicarbonates system.

The HFO experiments (70 mM total Fe) generated more DIC than the ferric citrate experiments (50 mM total Fe) showing the effect of the concentration of initial Fe(III). The ratio of ligand to Fe(III) is also reported to influence the rate of photodegradation of organic compounds, at least at pH values less than 2 [2]. In general, a larger ligand-to-sensitizing-agent ratio results in a greater rate of organic compound photodegradation. In case of the two ligands (OH and citrate) containing electron acceptors used in our experiments, the ligand-to-Fe(III) ratios are 3:1 in HFO compared to 1:1 in ferric citrate. This could also contribute to the difference in the rates of lactate/citrate oxidation observed for these two media compositions.

No measurable differences were observed when using either KH_2PO_4 or NaHCO_3 as buffering agents in the DIC or soluble ferrous iron production. These observations showed

that as long as the pH conditions are maintained, the type of buffering agent has little effect on the redox process. This was contrary to microbially mediated systems [21,22], where bicarbonate clearly enhanced the oxidation and reduction process at a near-neutral pH. The complex enzymatic reactions in biotic systems could be responsible for the observed difference due to enhanced metabolism in the bicarbonate system. Whereas in abiotic systems, both bicarbonate and phosphate buffers affect the formation of $\cdot\text{OH}$ radical at a neutral pH equally enhancing the effect of Fenton mechanisms.

pH determines the dominance of different metal–ligand complexes influencing their photo reactivity and participation in redox reactions. Under acidic conditions, Fe(III)–citrate complexes photochemically produce more reactive species (e.g., H_2O_2 and $\cdot\text{OH}$) compared to Fe(III)–hydroxyl and Fe(III)–oxalate complexes [4,6]. This observation could explain the greater DIC yield and ferrous iron production measured at a pH of 5 compared to the neutral and alkaline pH conditions. At near-neutral-to-alkaline conditions, Fe(III)–hydroxyl becomes more photoreactive than the Fe(III)–citrate complex [6]. Thus, the greater DIC production observed in HFO media compared to the ferric citrate media at near neutral pH conforms well to the observation of Deng et al. [6].

The effects of light and sensitizing agents (Fe(III) and minor concentrations of transition metals in the Wolfe’s minerals) were clearly demonstrated in our experimental conditions. In the absence of both Fe (III) and fluorescent light, minimal oxidation of lactate and citrate were observed, indicating that, in abiotic conditions, these two organic compounds could only obtain oxidization via indirect (sensitized) photocatalytic processes. Omission of Wolfe’s minerals from the media also affected the DIC yield, indicating that the transition metals (e.g., Mn, Co, Zn, and Cu) might make some contribution to the oxidation of the organic compounds used.

Understanding the importance of microbially mediated and abiotic mechanisms, such as the synergistic combination of microbial–Fenton systems [14,16,27] and sonochemically induced Fenton reactions [10], would help remediation of contaminants. These abiotic mechanisms could be important in the degradation of less microbially degradable compounds (e.g., PCE and TCE) by increasing the production of H_2O_2 , which, in turn, produces more hydroxyl radicals.

3.8. Isotopic Factors Controlling Redox Processes

The trend of isotopic values in Figure 9 indicated the presence of two isotopically distinct carbon sources (e.g., bicarbonate vs. organic compounds) and their sequential time-dependent contribution of carbon, indicating mixing of these isotopically distinct sources. As time progresses, the isotopic contribution of the bicarbonate became exponentially less influential, whereas the DIC coming from the oxidation of organic compounds increased.

The graph of the $\delta^{13}\text{C}_{\text{DIC}}$ of the samples as a function of the inverse of the total DIC (DIC^{-1}) shown in Figure 10 suggests clearly that the source of the starting component in the bicarbonate system is lactate ($\delta^{13}\text{C}_{\text{DIC}} = -25.4\text{‰}$). At lower pH conditions (pH~5), there were no systematic relationships between the DIC and its respective carbon isotopic values. The reason for such a behavior under lower pH conditions is unclear and warrants more quantitative work, including the usage of isotopically tagged organic carbon compounds.

4. Conclusions

Most previous studies were conducted under aerobic and acidic conditions (pH = 2–5), with a few under anaerobic conditions. Such acidic pH conditions are less likely in the natural environment due to the buffering capacity of soils in natural ecosystems. Here, this research reported results from experimental work performed under circumneutral and anaerobic conditions. The effects of light, Fe(III) presence and concentrations, ligand-to-

Fe(III) ratio, and pH on the redox processes involving lactate and citrate as model organic compounds were observed in our study, confirming previous observations made mostly under acidic and aerated conditions. The practical implications of abiotic processes are in relation to totally anaerobic conditions, which are not ubiquitous in nature but do exist. Atmospheric processes can also be included here.

Both lactate and citrate undergo oxidation by indirect photolysis involving sensitizing agents and the modified Fenton reaction. Hydroxyl radicals play a vital role in recycling the oxidized form of the sensitizing agents (e.g., Fe(III)) and could accelerate organic matter mineralization, producing bioavailable intermediate products. The rate of oxidation of the organic compounds in our experimental condition was slowed down due to the presence of inhibiting ions, such as chloride, by the scavenging of the hydroxyl radical produced via the Fenton-like mechanism.

Stable carbon isotopic signatures in bicarbonate-buffered systems in neutral pH conditions were observed to approach $\delta^{13}\text{C}$ values of the lactate used ($\approx -25.0\text{‰}$) with the increasing DIC yield. The isotopic signatures in acidic conditions did not show a similar distinctive pattern to those observed in neutral pH conditions and need more quantitative work.

Author Contributions: This research formed part of the partial requirement for the doctoral dissertation of T.A.G. R.V.K. supervised the research. T.A.G. designed the experiments and conducted isotope measurements and wrote the first version of the manuscript. R.V.K. and T.A.G. interpreted the results and R.V.K. finalized the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Dataset available on request from the authors.

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Conflicts of Interest: The authors declare no conflicts of interest.

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Review

Lignocellulosic Waste-Derived Nanomaterials: Types and Applications in Wastewater Pollutant Removal

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Abstract: Industrial wastewater pollution has reached acute levels in the environment; consequently, scientists are developing new sustainable treatment methods. Lignocellulosic biomass (LB) stands as a promising raw material because it originates from agricultural waste, forestry residues, and energy crop production. This review examines the application of nanomaterials derived from lignocellulosic resources in wastewater management, highlighting their distinctive physical and chemical properties, including a large surface area, adjustable porosity structure, and multifunctional group capability. The collection of nanomaterials incorporating cellulose nanocrystals (CNCs) with lignin nanoparticles, as well as biochar and carbon-based nanostructures, demonstrates high effectiveness in extracting heavy metals, dyes, and organic pollutants through adsorption, membrane filtration, and catalysis mechanisms. Nanomaterials have benefited from recent analytical breakthroughs that improve both their manufacturing potential and eco-friendly character through hybrid catalysis methods and functionalization procedures. This review demonstrates the ability of nanomaterials to simultaneously turn waste into valuable product while cleaning up the environment through their connection to circular bioeconomic principles and the United Nations Sustainable Development Goals (SDGs). This review addresses hurdles related to feedstock variability, production costs, and lifecycle impacts, demonstrating the capability of lignocellulosic nanomaterials to transform wastewater treatment operations while sustaining global sustainability.

Keywords: lignocellulosic waste; lignocellulosic biomass; nanomaterial; wastewater treatment; pollutant removal

1. Introduction

Lignocellulosic biomass (LB) is a renewable resource sourced from energy crops, agricultural residues, and forestry by-products, making it one of the earth's most widespread natural materials [1–3].

This abundant resource holds immense significance due to its role in addressing pressing global challenges, including the dependency on non-renewable energy sources like oil, coal, and natural gas, which contribute to greenhouse gas emissions, climate warming, and substantial environmental destruction [4–6]. LB stands out as a cornerstone of sustainable development, offering a pathway to reduce reliance on fossil fuels and combat environmental pollution, thereby supporting the transition to renewable energy solutions and sustainable waste management strategies [1–3].

LB is widely produced and available, positioning it as a globally accessible feedstock [7,8]. Its abundance is driven by its origins in agricultural waste, forestry residues, and dedicated energy crops, ensuring a steady supply for innovative applications [1–3].

LB serves multiple critical uses, notably in biofuel production, where its composition of cellulose, hemicellulose, and lignin determines the efficiency of conversion processes, with advanced pretreatment methods being required to overcome its recalcitrant nature [9,10]. Beyond biofuels, LB-derived nanomaterials have emerged as a transformative tool in wastewater treatment, leveraging properties such as large surface areas, adjustable porosity, and functional group capabilities [11–14]. These nanomaterials excel in extracting heavy metals, dyes, and organic pollutants from industrial waste streams through mechanisms like adsorption, membrane filtration, and catalysis [15,16]. Additional applications include the use of biochar to recover nutrients and mitigate environmental impacts in wastewater treatment [17], bacteria–algae co-cultures to detoxify pretreatment wastewater while reducing carbon emissions [18], and chemically adjusted biosorbents to remove toxic pollutants [19]. The integration of cellulose nanocrystals (CNCs) further enhances nanomaterial properties for diverse sustainable applications [20], while biopolymers from LB offer eco-friendly alternatives to synthetic materials [21].

Despite these advancements, a comprehensive synthesis of the current research on LB-derived nanomaterials for wastewater treatment remains absent, necessitating this review. The rapid evolution of green nanomaterials and their potential in environmental cleanup operations highlight the urgency to consolidate knowledge and address gaps in understanding their full capabilities. Green nanomaterials, especially lignocellulosic nanomaterials, create a sustainable solution that simultaneously benefits environmental relationships. They also contribute to health protection through the qualities of biocompatibility, biodegradability, and non-toxicity, making them safe for use in the medical sector [22]. Plant-based nanomaterials represent an emerging contemporary antibiotic alternative that provides improved medical outcomes through reduced toxic effects on human cells [23].

The implications of this work are profound, aligning with the United Nations Sustainable Development Goals (SDGs) by promoting clean water access and sustainable industrial practices [4]. By transforming lignocellulosic waste into valuable nanomaterials, this research supports a circular bioeconomy, enhancing resource recovery, carbon neutrality, and carbon sequestration while reducing environmental harm [24,25]. These efforts underscore LB's potential to simultaneously tackle waste management and pollution on a global scale [11,12,26,27].

The scope of this review involves the application of LB-derived nanomaterials in sustainable wastewater treatment, focusing on their extraction, properties, and pollutant removal efficiencies. The review also examines challenges such as production scalability and cost-effectiveness, alongside future prospects. The review is structured to first outline LB's foundational role, followed by detailed discussions on nanomaterial synthesis, their applications in wastewater management, associated challenges, and emerging opportunities for advancing global sustainability.

1.1. Lignocellulosic Biomass

Lignocellulosic biomass is primarily composed of three biopolymers: cellulose (35–60%), hemicellulose (20–40%), and lignin (10–25%) [28,29]. These components provide structural integrity to plants but also present challenges due to their recalcitrant nature. Cellulose is a crystalline homopolymer characterized by strong hydrogen bonding networks that confer mechanical strength and chemical stability. Hemicellulose is an amorphous heteropolymer that contributes flexibility to plant cell walls. Lignin, a complex phenolic polymer with aromatic structures and diverse functional groups such as hydroxyl (-OH)

and carboxyl (-COOH), provides rigidity but is highly resistant to degradation [30,31]. The complex structures of these materials create obstacles for their transformation into valuable products, yet their potential remains high due to their renewable nature [32,33]. The global production of lignocellulosic biomass is estimated to be approximately 200 billion tons annually [34]. Despite its abundance and renewability, the structural complexity of lignocellulosic biomass necessitates advanced pretreatment methods, such as thermal decomposition or gamma-valerolactone (GVL)-based fractionation, to unlock its potential for conversion into high-value products, including biochar, bio-oil, syngas, or nanomaterials [26,35].

1.2. Nanomaterials Derived from Lignocellulosic Biomass

Nanomaterials derived from lignocellulosic biomass include nanocellulose, for instance, cellulose nanocrystals (CNCs), and carbon-based nanostructures such as graphene oxide (GO), carbon nanotubes (CNTs), lignin nanoparticles (LiG NPs), and biochar. These materials exhibit exceptional properties, including high surface area-to-volume ratios, tunable porosity, mechanical strength, chemical reactivity, and functional group diversity. For example,

- i. CNCs possess high adsorption capacities for heavy metals like Pb(II) and Cr(VI) due to their surface charge tunability [29].
- ii. GO effectively removes dyes and organic pollutants via electrostatic interactions [36].
- iii. MnO₂-modified lignin nanoparticles demonstrate adsorption capacities of up to 806 mg/g for methylene blue dye removal [37].

These attributes make lignocellulosic-derived nanomaterials ideal candidates for wastewater treatment applications such as the adsorption of pollutants, the catalytic degradation of contaminants, antimicrobial coatings, and membrane filtration technologies [38,39].

1.3. Nanomaterials in Wastewater Treatment

Water pollution caused by industrial effluents containing heavy metals, dyes, pharmaceuticals, and other hazardous contaminants poses significant risks to human health and ecosystems. Conventional wastewater treatment methods often fail due to inefficiency at low pollutant concentrations, high operational costs, or the generation of secondary waste. Utilizing lignocellulosic biomass for wastewater treatment addresses both environmental pollution and transforms waste into valuable resources, thereby enhancing sustainability through closed-loop systems and recycling waste materials into biofuels [40]. Nanotechnology offers a promising alternative by enabling the development of advanced materials with superior adsorption capacities and catalytic efficiencies [16,41]. Nanomaterials such as magnetite nanoparticles (Fe₃O₄) have unique physicochemical properties, including high surface area and reactivity, which make them ideal for pollutant adsorption and biodegradation processes. Their integration with biological systems enhances the efficiency of wastewater treatment [42]. In Figure 1, the process of utilizing nanomaterials in wastewater treatment is illustrated.

Nanomaterials derived from lignocellulosic biomass effectively address these challenges. Biochar exhibits excellent adsorption properties for heavy metals like Zn(II) through ion exchange mechanisms. Functionalized CNCs enhance selectivity for specific contaminants such as Cr(VI) or methylene blue via carboxylation or phosphorylation processes [29]. Magnetite nanoparticles derived from lignocellulose offer superparamagnetic behavior for easy recovery using magnetic fields while efficiently adsorbing pollutants like Pb(II) and Cu(II) [43].

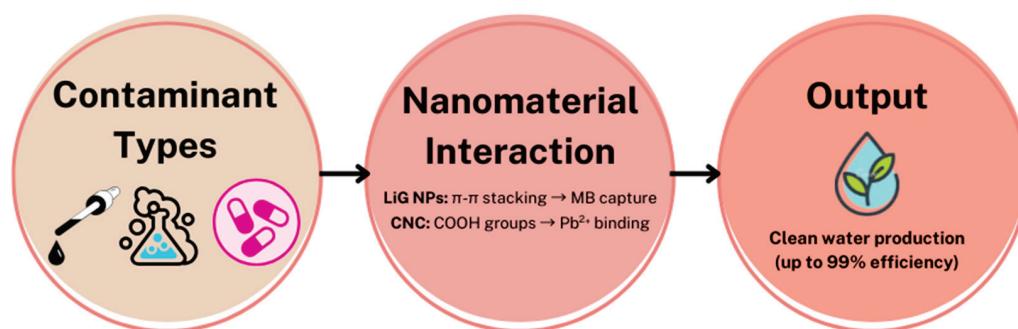


Figure 1. Process of using nanomaterials in wastewater treatment.

This review aims to critically explore the potential of lignocellulosic waste as a pioneer for nanomaterial innovation in advancing sustainable wastewater treatment technologies. It emphasizes key aspects, including extraction methods, material properties, pollutant removal mechanisms, scalability challenges, economic feasibility, and prospects. By integrating insights from diverse studies across the field, this work aims to provide a comprehensive understanding of how lignocellulose-derived nanomaterials can revolutionize wastewater treatment, addressing pressing environmental concerns globally. In Figure 2, a detailed overview of lignocellulosic nanomaterials in wastewater treatment, from feed-stock to application, is presented.

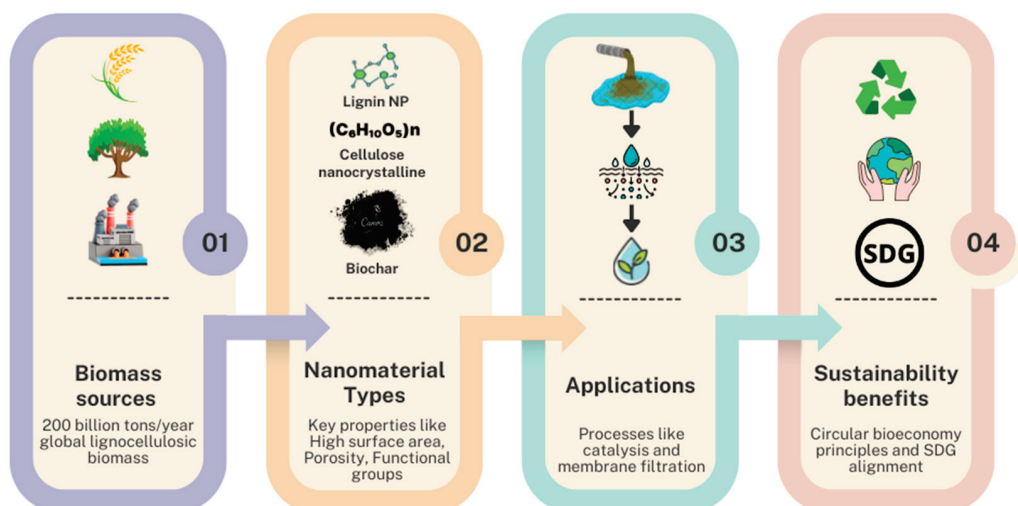


Figure 2. Overview of lignocellulosic nanomaterials in wastewater treatment.

2. Lignocellulosic Biomass: A Renewable Resource

2.1. Sources and Availability

Lignocellulosic biomass (LB) is a widely available renewable resource derived from agricultural residues (e.g., sugarcane bagasse, rice straw, corn stover, and wheat straw), forestry by-products (e.g., sawdust and wood chips), and industrial waste streams (e.g., paper pulp and sewage sludge) [1,2,4]. Dedicated energy crops, such as switchgrass and Miscanthus, also contribute significantly to its supply. The global production of lignocellulosic biomass exceeds 200 billion tons annually, making it an abundant and cost-effective feedstock for bio-based applications [34,37,44–50]. Forestry residues and non-wood sources, such as flowers and leaves, further expand the biomass pool [16,35,51]. Industrial wastes, such as xylose residue (XR) and oil palm trunk fibers, are also promising sources for the synthesis of nanomaterials [52]. Importantly, LB does not compete with food production systems, making it a sustainable second-generation feedstock [34].

2.2. Structural Composition

Lignocellulosic biomass is primarily composed of three biopolymers: cellulose, hemicellulose, and lignin. These components are intricately organized to provide structural integrity to plants.

Cellulose: Cellulose is a linear polymer of β -D-glucose units linked by β -(1 $\rightarrow$ 4) glycosidic bonds. It forms crystalline microfibrils that impart mechanical strength and rigidity [11,26,27]. Its crystalline regions are highly resistant to enzymatic degradation but are valuable for nanocrystal production [29].

Hemicellulose: Hemicellulose is an amorphous heteropolymer composed of pentoses (e.g., xylose) and hexoses (e.g., mannose). It serves as a matrix linking cellulose and lignin but has lower thermal stability compared to cellulose [1,26].

Lignin: Lignin is a complex aromatic polymer composed of monolignols such as p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. It provides rigidity and hydrophobicity but hinders enzymatic hydrolysis due to its recalcitrance [28,53]. Other minor components include extractives, such as terpenes, and ash which contribute to the chemical complexity of LB [3].

2.3. Current Utilization

Despite its abundance, lignocellulosic biomass remains underutilized due to its structural recalcitrance. There are noteworthy traditional applications. LB is extensively used for bioethanol production through enzymatic hydrolysis and fermentation. Other biofuels, such as biodiesel and biohydrogen, are also derived from LB using dark fermentation processes [35,51]. The pyrolysis of LB produces biochar for soil amendment or environmental remediation applications [45]. Cellulose fibers from LB are used in the paper manufacturing process. However, this process underutilizes hemicellulose and lignin components [54].

Emerging applications aim to valorize all components of LB into high-value products. Cellulose nanocrystals (CNCs) and lignin nanoparticles are being explored for their potential applications in composites, aerogels, and membranes, with a focus on water treatment technologies [29]. Hemicellulose and cellulose derivatives are being developed into bio-based plastics with enhanced biodegradability [55]. Lignin is being converted into carbon fibers and activated carbons for the adsorption of pollutants [56].

Advanced pretreatment methods, such as acid hydrolysis or deep eutectic solvents (DES), have enabled the efficient fractionation of LB into its functional components. This has expanded its utility in producing platform chemicals, such as furfural, and specialty materials, including nanocellulose composites [52,57]. Only 10–15% of lignocellulosic waste is currently valorized, primarily for low-value applications such as animal feed or combustion. High-value applications, such as nanocellulose production, remain underexplored [58,59].

3. Nanomaterials from Lignocellulosic Waste

3.1. Types of Nanomaterials

Lignocellulosic biomass serves as a versatile precursor for various nanomaterials, including:

Nanocellulose: This comprises cellulose nanocrystals (CNCs) and cellulose nanofibrils (CNFs), known for their high mechanical strength, biocompatibility, and biodegradability [11,26,60,61]. CNCs are characterized by high crystallinity and functional groups, such as sulfate or carboxyl groups, which enhance adsorption [29,62]. Fe-Cu composites with CNCs adsorb Pb(II) (85.8 mg/g) through chelation [29,62–64]. CNFs exhibit high aspect ratios and tunable surface chemistry [12,38].

Lignin-Based Nanoparticles: These offer antioxidant properties, UV resistance, and hydrophobicity, making them suitable for pollutant adsorption, drug delivery, and environ-

mental remediation [27,28,55]. Functionalized lignin nanoparticles, such as MnO₂-modified variants, enhance adsorption capacities due to hierarchical structures [37].

Carbon-Based Nanomaterials: These include carbon nanotubes, graphene oxide, and biochar-derived nanoparticles produced via pyrolysis or hydrothermal processes [2,26,65]. Carbon quantum dots (CQDs) derived from sugarcane bagasse exhibit photoluminescence and pollutant detection capabilities [36].

Metal/Metal Oxide Nanoparticles: These are synthesized using lignin or cellulose as reducing agents; these nanoparticles exhibit catalytic activity and pollutant binding potential [2,43]. Magnetite nanoparticles (Fe₃O₄) are applied for pollutant adsorption and microbial immobilization [42].

Other Nanomaterials: Magnetic nanoparticles (MNPs) are used for enzyme immobilization and pollutant recovery [12]. Nanohemicelluloses and silica nanoparticles enhance film-forming properties and chemical stability, respectively [16,66]. In Table 1, different types of lignocellulosic biomass-derived nanomaterials and their properties are shown.

Table 1. Types of lignocellulosic biomass-derived nanomaterials and their properties.

Nanomaterial Type	Source Biomass	Key Properties	Applications	References
Cellulose Nanocrystals	Sugarcane bagasse	Surface area: 500 m ² /g; tensile strength: 220 GPa	Heavy metal adsorption, membranes	[29]
Lignin Nanoparticles	Rice straw	Thermal stability: >300 °C; functional groups: -OH, -COOH	Dye removal, catalytic supports	[37]
Biochar	Forestry residues	Porosity: 0.8 cm ³ /g; adsorption capacity: 806 mg/g	Organic pollutant degradation	[11]
Graphene Oxide	Wheat straw	Electrical conductivity: 10 ³ S/m; surface charge: −25 mV	Photocatalysis, sensors	[36]
Magnetic Fe ₃ O ₄ NPs	Corn stover	Superparamagnetic; adsorption capacity: 95% Pb(II)	Magnetic recovery systems	[43]

3.2. Extraction Techniques

Achieving a uniform distribution of nanoparticles in lignocellulosic biomass is critical for maximizing their effectiveness in treatment applications. Emerging green technologies like nanobiotechnology are addressing this challenge while focusing on sustainable processing methods [67]. In Table 2, a comparative overview of pretreatment methods for nanomaterial extraction is provided. The extraction of nanomaterials from lignocellulosic biomass involves a variety of mechanical, chemical, thermal, and biological methods, including the following:

Table 2. Comparative analysis of pretreatment methods for nanomaterial extraction.

Method	Energy Use	Yield (%)	Environmental Impact	Scalability	References
Acid Hydrolysis	High	45–60	Toxic effluent generation	Moderate	[1]
Enzymatic Hydrolysis	Low	30–40	Biodegradable by-products	High	[28]
Pyrolysis	Very High	50–70	CO ₂ emissions	Low	[45]
Microwave-Assisted	Moderate	55–65	Reduced solvent use	High	[52]
Gamma-Valerolactone	Low	60–75	Solvent recyclability	High	[35]

Mechanical Methods: These include high-pressure homogenization, ball milling, and ultrasonication, which are employed to isolate CNFs but are energy-intensive [34,38].

Chemical Pretreatments: Acid hydrolysis dissolves amorphous cellulose regions to yield CNCs with high crystallinity [29]. Alkaline treatments remove lignin to expose cellulose fibers for further processing [26].

Thermal Processes: Pyrolysis and hydrothermal carbonization convert biomass into biochar or carbon-based nanostructures under controlled conditions [4,68].

Biological Methods: Enzymatic hydrolysis using cellulases or fungi like *Aspergillus oryzae* reduces energy consumption while preserving structural integrity [1,28].

Emerging Technologies: Techniques such as microwave-assisted extraction and solvent-based fractionation using green solvents like gamma-valerolactone (GVL) optimize yield while minimizing environmental impact [35,52].

Functionalization Techniques: Methods like TEMPO oxidation introduce carboxyl groups to enhance adsorption capacities for dyes and heavy metals [29].

3.3. Properties of Nanomaterials

Nanomaterials derived from lignocellulosic waste exhibit the following unique properties that make them suitable for diverse applications:

Surface Area and Porosity: High surface area-to-volume ratios ($>500 \text{ m}^2/\text{g}$) and mesoporous structures enhance adsorption capacities for pollutants such as heavy metals and dyes [12,15].

Mechanical Strength: Nanocellulose exhibits tensile strengths of up to $\sim 220 \text{ GPa}$, making it ideal for durable membranes and composites [54].

Functional Group Diversity: Abundant oxygenated functional groups ($-\text{OH}$, $-\text{COOH}$) enable chemical modifications for specific applications like catalysis or pollutant binding [2,11].

Thermal Stability: Lignin-based nanoparticles demonstrate high thermal stability, making them suitable for biomedical applications [28].

Biocompatibility and Biodegradability: These properties minimize secondary pollution while supporting environmental sustainability in wastewater treatment systems [13,16].

Catalytic Activity: Carbon-based nanostructures exhibit excellent electrical conductivity and enhanced catalytic activity in environmental remediation processes [65,69]. In Table 3, a comparison is shown which provides detailed information on the feedstock sources, properties, pollutant removal efficiencies, and cost range of different lignocellulosic-derived nanomaterials.

Table 3. Comparison of different lignocellulosic-derived nanomaterials.

Features	CNC	Lignin NPs	Biochar
Feedstock sources	Sugarcane bagasse	Hardwood	Rice husk
Key properties	High crystallinity (220 GPa)	Antioxidant and UV-resistant	Mesoporous ($500 \text{ m}^2/\text{g}$)
Pollutant removal efficiency	Pb(II) (96% removal)	Methylene blue (806 mg/g)	Zn(II) (85% ion exchange)

4. Applications in Wastewater Treatment

4.1. Nanocellulose-Based Composites

Nanocellulose-based composites have emerged as efficient materials for wastewater treatment due to their high surface area, functional groups, and biodegradability. These composites are widely used for adsorbing heavy metals, such as Pb(II) and Cd(II), as well as organic pollutants like dyes and pharmaceuticals. Functionalized nanocellulose membranes demonstrate high selectivity in removing contaminants through mechanisms such as adsorption, size exclusion, or electrostatic interactions [11,12,26,55]. For example, carboxylated nanocellulose crystals (CNCs) exhibit an adsorption capacity of up to 1237 mg/g for Pb(II), while CNC-based aerogels effectively remove Cu(II), because of

their porous structure [41]. Additionally, nanocellulose composites such as chitosan–CNC multilayers achieve over 99% oil–water separation, demonstrating their versatility [29]. The integration of nanomaterials into wastewater treatment processes enables innovative solutions for industrial effluents containing harmful contaminants. This includes the use of biochar as a catalyst and nanocatalysts to improve energy efficiency and pollutant degradation [67,70]. Nanocellulose composites are also utilized in membrane filtration systems, enhancing mechanical strength and fouling resistance. Modified CNCs improve water flux and chlorine resistance in thin-film composite membranes [16]. Real-world applications include CNC/graphene aerogels used for oil spill remediation and CNC-based adsorbents, which achieve 96% removal of Cr(VI) from industrial effluents [29,71]. Chitosan–CNC multilayers on stainless steel achieve 99.5% oil–water separation [29,62–64]. A study highlights the potential of amino-functionalized lignocellulosic biopolymers as effective and sustainable adsorbents for removing toxic anionic contaminants, such as Congo red and Cr(VI), from wastewater, demonstrating their excellent reusability and eco-friendly nature [72]. Another study highlights that chemically modified cellulosic biofibers, such as those derived from okra, can significantly enhance the adsorption capacity for heavy metal ions, offering a sustainable and cost-effective solution for wastewater remediation [73]. In Table 4, the pollutant removal efficiencies of various lignocellulosic nanomaterials are provided.

Table 4. Pollutant removal efficiencies of lignocellulosic nanomaterials.

Pollutant	Source/Industry	Nanomaterial	Removal Efficiency	Mechanism	References
Pb(II)	Battery manufacturing, metal plating	Carboxylated CNCs	1237 mg/g	Electrostatic interaction	[29]
Methylene Blue	Textile dyeing	MnO ₂ –lignin nanocomposites	806 mg/g	Chemical adsorption	[37]
Cr(VI)	Electroplating, leather tanning	TEMPO-oxidized cellulose	96%	Redox reaction	[29,71]
Tetracycline	Pharmaceutical manufacturing, hospitals	CNC–chitosan membranes	97%	Size exclusion	[16]
Cu(II)	Mining, metal plating	Biochar–ZnO hybrid	92%	Ion exchange	[15]

4.2. Catalytic Applications

Lignin-derived nanoparticles and biochar serve as catalysts or catalyst supports in advanced oxidation processes (AOPs), enabling the degradation of organic pollutants like dyes and phenols. Functionalized biochar enhances the adsorption kinetics of heavy metals, such as Pb(II), Zn(II), and Cu(II), while maintaining a high recycling capacity over multiple cycles [52]. Similarly, MnO₂–lignin nanocomposites exhibit superior adsorption capacities for dyes, such as methylene blue, due to chemical interactions between the MnO₂ nanodots and dye molecules [37]. Biochar derived from lignocellulosic waste exhibits excellent catalytic properties due to its high porosity and environmental sustainability. Current research focuses on producing and activating biochar-based catalysts for wastewater remediation, with significant potential for removing hazardous contaminants [74]. Fe-modified lignocellulosic biochar simultaneously performs wastewater treatment by combining adsorption with catalytic performance, thus boosting treatment efficiency [75]. CNC-supported ZnO/TiO₂ composites degrade organic dyes (e.g., 97% methylene blue removal) via photocatalysis [29,62–64]. Photocatalysis using nanocellulose–metal oxide hybrids or lignin-derived materials is another promising approach. For ex-

ample, CNC-supported ZnO/TiO₂ composites achieve over 97% methylene blue removal under UV light [29]. Fe₃O₄-lignin nanocomposites facilitate Fenton reactions, achieving a 95% reduction in phenol within two hours [36]. Magnetic nanoparticles immobilized with enzymes further enhance catalytic efficiency in pollutant degradation processes [34]. The integration of enzyme-based nanobiocatalysts in the treatment of lignocellulosic biomass wastewater can significantly enhance resource recovery, and it also minimizes environmental pollutants [76].

4.3. Lignin Valorization

Lignin valorization involves converting lignin into value-added products such as activated carbon, biochar, or nanoparticles for wastewater treatment. Lignin-derived biochar exhibits high adsorption capacities for pollutants like methylene blue and phenols due to its porous structure and functional groups [11,15]. Lignin nanoparticles are also utilized as adsorbents for toxic metals, such as Hg(II) and Cd(II), through ion exchange mechanisms or as precursors for activated carbon production [5,56]. Lignin-derived carbon quantum dots exhibit photocatalytic activity for degrading pharmaceuticals, while DES-based flocculants achieve over 99% turbidity reduction and simultaneous sterilization of harmful microorganisms like *E. coli* [52]. Additionally, lignin fractions from gamma-valerolactone processes can be used to produce carbon foams or battery anodes, contributing to environmental sustainability [35].

4.4. Agricultural Waste-Based Membranes for Wastewater Treatment

Agricultural waste-based membranes represent a promising sustainable solution for wastewater treatment applications, offering both environmental benefits and effective pollutant removal capabilities. Cellulose extracted from agricultural residues provides excellent mechanical strength for gas separation, water purification, and mixed matrix membrane applications [77]. Ultra-low-density nanostructured carbon and activated carbon aerogels derived from sugarcane bagasse, rice straw, wheat straw, and cotton stalks exhibit very high porosity (>50%) and surface area, enabling outstanding adsorption of organic dyes (249.6–1078 mg/g capacity), heavy metals (e.g., gold up to 650.08 mg/g), and various toxic chemicals with removal efficiencies of 98–99%. Nanocellulose-based variants also show similar results [77,78]. These bio-based membranes offer greener, cost-effective alternatives to petroleum-derived counterparts while valorizing agro-waste. Pretreatment using ultrafiltration (UF) followed by reverse osmosis (RO) membranes, which are fabricated from agricultural waste-derived materials, achieved nearly complete removal (approx. 100%) of TSS, COD, and BOD from pulp and paper mill wastewater [79]. Incorporating 5 wt.% corn starch into a clay–feldspar–quartz mix and sintering at 1000 °C yields ceramic supports with approximately 40% porosity and a 0.09 µm mean pore size, achieving 558.5 Lh⁻¹m⁻² water permeability. A thin polyamide 6 dip coating provides antifouling (flux recovery ratio 92.3%) and removes 99.97% of heavy metals from real agricultural drainage. Raw materials cost under USD 0.17 per m², demonstrating the economic viability of using locally sourced agro wastes [80].

4.5. Case Studies: Real-World Applications

Numerous case studies highlight the practical efficacy of lignocellulosic nanomaterials in industrial wastewater treatment. In Table 5, the case studies are presented with various types of necessary information.

Table 5. Case studies on the application of lignocellulosic nanomaterials in industrial wastewater treatment.

Source of Waste	Parameter Improved	Type of LB Nanomaterial	Efficiency/Performance	Reference(s)
Dye wastewater (methylene blue)	Dye concentration	Biochar from rice straw	Adsorption capacity: 160.5 mg/g	[11]
Oil–water mixture	Oil content	Nanocellulose-based membranes	Effective separation	[61]
Heavy metal wastewater	Heavy metal concentrations	Sugarcane bagasse-derived nanomaterials	Significant removal efficiencies	[51]
Pharmaceutical wastewater (tetracycline)	Tetracycline concentration	CNC-based membranes	Effective removal via electrostatic interactions	[12]
Industrial wastewater	Overall water quality	Lignin-derived materials	Achieved drinkable water quality	[52]
Wastewater with microbial contamination	Microbial contamination and fouling	CNF-based membranes	Retained bacteria and viruses, antifouling	[38,54]
Lead–zinc mineral processing wastewater	Overall water quality	Lignocellulosic nanomaterials	Achieved drinkable water quality	[52]
Oil spill water (Klang Valley)	Oil content	CNC/graphene aerogels	Restored water quality	[29]
Wastewater with microbial contamination	Microbial contamination	Biogenic silver nanoparticles in membranes	Potent antibacterial activity against <i>E. coli</i>	[16]
Heavy metal wastewater (lead)	Lead concentration	SiO ₂ nanoparticles from rice husk ash	Effective lead removal	[16]
Arsenic-contaminated groundwater	Arsenic concentration	Carbon–silicon nanostructures from rice husk	90% removal efficiency	[36,81,82]
Heavy metal wastewater (Pb(II), Zn(II), and Cu(II))	Heavy metal concentrations	Functionalized biochar	Efficient adsorption, high recycling capacity	[52]

5. Related Challenges

The production and scaling up of nanomaterials from lignocellulosic biomass involve multifaceted challenges across technical, economic, and environmental dimensions. It is crucial to address these challenges to achieve industrial scalability, cost-effectiveness, and sustainability. The primary challenge in utilizing lignocellulosic biomass lies in its recalcitrant structure. Lignin’s resistance to microbial degradation and hemicellulose’s hydrophilicity necessitate innovative pretreatment strategies for the efficient conversion of these materials into value-added products. Additionally, the underutilization of industrially produced lignin, of which only about 2% is currently valorized, represents a significant opportunity for research into high-value applications, such as binders or dispersants [28]. High energy costs for pyrolysis (≥ 700 °C) and enzymatic pretreatment (approximately 20% of the total cost) hinder commercialization [58,59].

5.1. Technical Barriers

Pretreatment methods such as pyrolysis, hydrothermal liquefaction, and acid hydrolysis demand significant energy inputs, raising concerns about scalability [4,44,61]. Moreover, the heterogeneous composition of lignocellulosic biomass complicates the standardization of extraction processes and achieving consistent quality in nanomaterials [31,34,53]. Achieving high yields and purity during extraction remains challenging due to the recalcitrant structure of biomass [32,83]. Additionally, maintaining uniform particle size distribution

and material stability during synthesis is difficult [28,56]. Scaling up laboratory processes to industrial levels is hindered by the complexity of techniques such as microwave-assisted pyrolysis and enzymatic hydrolysis [50,69]. Reusability issues like inefficient regeneration methods for solvents and declining adsorption capacity after multiple cycles pose additional hurdles [29,52].

5.2. Economic Viability

The cost of advanced technologies like ionic liquids, green solvents, enzymatic hydrolysis, and mechanical disintegration limits commercial scalability [12,14,26,29]. High initial capital costs for biorefineries and advanced equipment hinder widespread adoption despite long-term benefits [84,85]. Nanomaterials from lignocellulosic waste must compete with traditional materials like activated carbon, which are significantly cheaper (approx. 1–5 USD/kg vs. approx. 50 USD/kg for CNC production) [29,62]. Developing value-added products like nanocomposites or biofuels is essential to offset production costs [35,86]. In Table 6, the economic and environmental metrics of lignocellulosic nanomaterial production are presented.

Table 6. Economic and environmental metrics of lignocellulosic nanomaterial production.

Parameter	Conventional Materials	Lignocellulosic Nanomaterials	Improvement Factor	References
Production Cost (USD/kg)	1–5	20–50	4–10×	[62]
Carbon Footprint (kg CO ₂ /kg)	8–12	2–4	3–4× reduction	[26]
Adsorption Capacity (mg/g)	100–300	500–1200	2–5×	[41]
Reusability Cycles	3–5	8–12	2–3×	[52]

In addition, the economic viability of producing nanomaterials from lignocellulosic biomass is further complicated by geographical variations in LB availability and processing costs. The type and abundance of LB differ significantly across regions due to climate, agricultural practices, and forestry activities. For instance, tropical regions like Asia and Latin America generate large quantities of agricultural residues (such as sugarcane bagasse and rice straw), while temperate regions like North America and Europe produce more forestry residues (wood chips and corn stover). These differences influence feedstock accessibility and cost. Additionally, the affordability of advanced processing methods, such as enzymatic hydrolysis or microwave-assisted extraction, varies by region and depends on local infrastructure, labor costs, and access to technology. Developed regions may leverage advanced methods, while developing regions may require simpler, cost-effective alternatives. Table 7 illustrates these geographical disparities and their impact on feasibility.

5.3. Environmental Considerations

Chemical pretreatments generate toxic by-products and sulfate-rich wastewater that require effective disposal strategies to avoid secondary pollution [1,29]. The environmental footprint of energy-intensive processes like pyrolysis (approx. 700 °C) must be minimized through renewable energy integration or process optimization [45,61]. The potential ecotoxicity of nanomaterials such as carbon nanotubes necessitates rigorous lifecycle assessments to prevent unintended ecological impacts [36,39]. Transitioning to greener synthesis methods using supercritical CO₂ or recyclable solvents like GVL is critical to ensure sustainability [41,84]. Closed-loop systems for solvent recovery and zero-waste principles are essential for reducing environmental risks during large-scale production [52].

Table 7. Geographical variation in lignocellulosic biomass availability and processing feasibility.

Region	Dominant LB Types	Processing Costs	Feasibility of Advanced Methods	Examples/Notes	Reference(s)
North America	Forestry residues, corn stover	Moderate	High	Well-established infrastructure; pilot projects in U.S. and Canada	[29,51]
Europe	Wheat straw, forestry residues	High	Moderate	Advanced technology but high labor/energy costs; research in Germany	[35]
Asia	Rice straw, sugarcane bagasse	Low to moderate	Varies	Cost-effective in India and China; limited in less-developed areas	[11,51]
Africa	Agricultural residues (e.g., maize and cassava)	Low	Low	Limited access to technology; potential for simpler methods	[11]
Latin America	Sugarcane bagasse, coffee husks	Moderate	Moderate	Abundant LB but variable infrastructure; applications in Brazil	[51]

5.4. Integrated Challenges

The interplay between technical barriers, economic viability, and environmental considerations highlights the complexity of scaling up nanomaterial production. There are several integral challenges; for example, energy-intensive pretreatment methods exacerbate both economic and environmental challenges. High costs associated with advanced technologies limit their adoption despite the potential to reduce environmental impacts. Moreover, the toxicity of inevitable by-products demands innovative solutions that align with sustainability goals while maintaining economic feasibility.

6. Innovations and Future Prospects

6.1. Emerging Technologies

The development of choline-based ionic liquids and deep eutectic solvents as green solvents has revolutionized sustainable LB processing and pretreatment methods by diminishing environmental effects according to [46,87]. The efficiency of producing lignocellulosic nanomaterials has increased via advancements in enzymatic hydrolysis methods, coupled with bio-based solvents, and integrated biorefineries [13,28]. The extraction of nanomaterials using microwave-assisted pyrolysis, combined with ultrasound pretreatments and hybrid catalytic approaches, demonstrates high potential for efficient extraction while simultaneously degrading pollutants, as shown in references [5,26,32]. When lignocellulosic biomass is effectively pretreated, it can serve as a valuable feedstock in microbial fuel cells (MFCs) for both electricity generation and wastewater treatment [88]. Nanotechnology yields three distinct applications in pollutant management: multifunctional nanocomposites serve as pollution removal tools, blended membranes unite biological treatments with nanotechnology, and magnetic nanobiocatalysts provide reusable systems for pollutant degradation [12,58]. The application of microbe-derived enzymes combined with plant-based extracts in green synthetic methods both promotes eco-friendly characteristics and scales up performance [43]. Experts have integrated machine learning (for example, Python 3.7.9 with scikit-learn 0.24.2; XGBoost 1.3.3, Python 3.8.10 with scikit-learn 0.23.2; LIBSVM 3.24 etc.) to enhance the biological reaction pathways of biochar during wastewater treatment [74]. Modern technological applications involving biological and physical treatments demonstrate improved process efficiency. Research on surface modifications with thiol and amine group attachment to lignin nanoparticles has improved the selective pollutant

capture capacity of these nanoparticles for Hg(II) and Pb(II) [41]. Flash Nano Precipitation is a sustainable production technique for generating uniformly sized nanoparticles at large-scale operational levels [28]. Bioaugmentation through engineered microbial consortia shows great promise to synergistically enhance pollutant degradation when combined with nanomaterials according to [57]. In Table 8, innovations in functionalization techniques to improve performance are presented. In Figure 3, a framework for the circular bioeconomy for lignocellulosic nanomaterial applications is presented.

Table 8. Innovations in functionalization techniques for enhanced performance.

Functionalization Method	Nanomaterial	Outcome	Applications	Cost Considerations	References
TEMPO Oxidation	Cellulose nanocrystals	Increased carboxyl groups (-COOH)	Selective Cr(VI) adsorption	Moderate to high; requires TEMPO catalyst and controlled conditions	[29]
MnO ₂ Deposition	Lignin NPs	Hierarchical pore structure	Dye degradation	Moderate; involves manganese precursors and deposition methods	[37]
Fe ₃ O ₄ Coating	Biochar	Magnetic separation capability	Heavy metal recovery	Low to moderate; uses inexpensive iron salts and simple processes	[43]
Chitosan Grafting	CNC membranes	Antifouling properties	Oil–water separation	Moderate; requires chitosan and grafting reagents	[16]
DES (deep eutectic solvents) Modification	Lignin–carbon	Enhanced dispersibility	Flocculation	Low; utilizes cheap DES components and simple processing	[52]

6.2. Circular Bioeconomy

Lignocellulosic waste valorization serves sustainability goals through waste conversion into valuable nanomaterials, along with biofuels, as well as bioplastics, while reducing environmental impacts according to [45,61]. Research shows that the execution of closed-loop recycling systems that utilize solvents such as gamma-valerolactone (GVL), as well as all fractions of biomass, leads to improved resource utilization [35]. Integrated biorefineries produce nanomaterials together with bioenergy or biochemicals while minimizing waste to a degree of 70% according to [36,81]. The conversion of agricultural waste into nanomaterials helps achieve zero-waste targets by addressing global waste management issues and simultaneously producing usable products, such as bioethanol and biogas [57,89]. LB extraction for industrial purposes results in improved resource efficiency combined with better environmental sustainability because it decreases industrial-scale waste disposal quantities [90]. In Figure 4, the lifecycle of lignocellulosic biomass, from feedstock to nanomaterial synthesis, applications, and its role in a circular economy, is illustrated.

6.3. Policy and Industry Support

Government incentives play a key role in facilitating the large-scale deployment of sustainable technologies. Lignocellulosic-based innovations are gaining wider acceptance for wastewater treatment when governments implement renewable resource promotion alongside enhanced effluent regulations [15,55]. The commercialization of green nanotechnology requires critical research and development subsidies to accelerate its development process [29]. Technology transfer, together with industrial adoption, requires the strong support of public–private partnerships. Industry–academia partnerships create conditions that increase the manufacturing scale yet maintain sustainability standards [54,87]. The use of renewable materials in wastewater treatment can accelerate global sustainability

targets when policy frameworks support their utilization [65]. The convergence of modern green technology advancements with circular bioeconomic models and appropriate policy structures demonstrates great potential to transform bioenergy systems that utilize lignocellulosic materials. Advanced technology solutions address vital environmental problems while enabling the development of large-scale, sustainable industrial practices. Future investigations must establish affordable biomass fractionation procedures to enhance the complete utilization of biomass, advance LB-derived nanomaterials in environmental cleanup, and optimize bio-based plastics through improved manufacturing approaches.

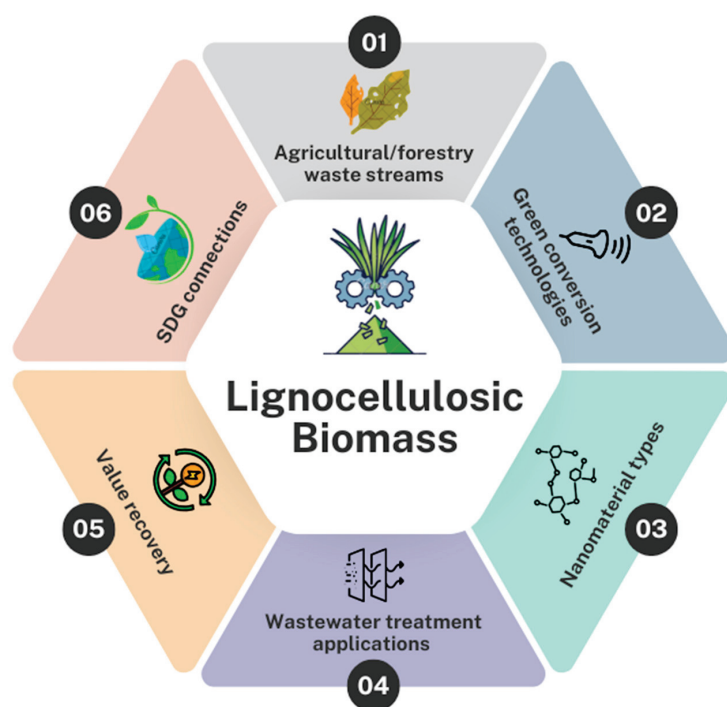


Figure 3. Circular bioeconomy framework for lignocellulosic nanomaterial applications.

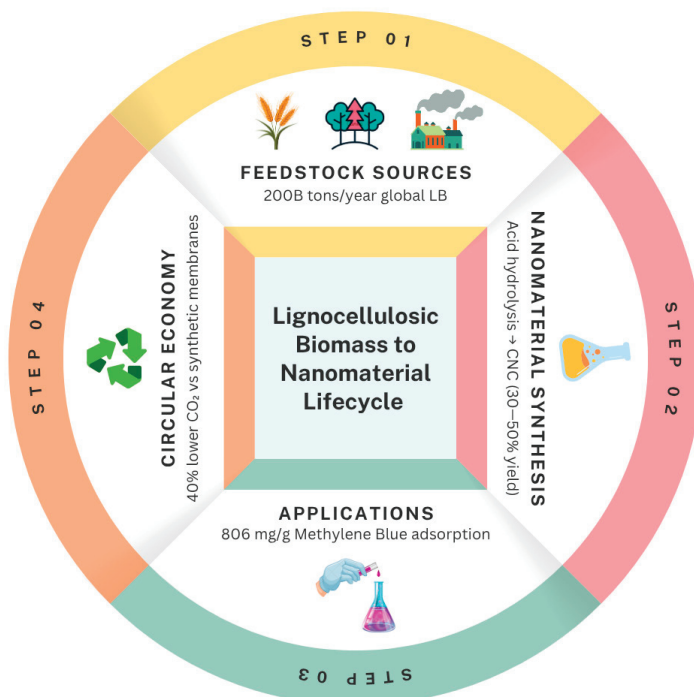


Figure 4. Lifecycle of lignocellulosic biomass to nanomaterials.

7. Conclusions

This review provides in-depth measurements regarding the ability of nanomaterials made from lignocellulosic biomass to transform ecological wastewater management methods. The development of novel wastewater treatment solutions relies on the exploitation of nanomaterials derived from lignocellulosic biomass as these materials offer large surface areas along with adjustable porosity features and various functional groups, enabling the extraction of heavy metals, dyes, and organic pollutants through adsorption, catalytic effects, and membrane separation. Nanomaterials have gained environmental and scalable qualities through the application of green extraction technology combined with hybrid catalysis systems that adhere to bioeconomy principles and support the Sustainable Development Goals (SDGs). The industrial use of these promising nanomaterials presents significant challenges due to inconsistent feedstock requirements, high production costs, and the need for environmental assessments. The combination of policy assistance with green chemistry advancements and process enhancement will create sustainable economic stability that preserves ecological sustainability. Resource efficiency improvements become achievable through functionalized techniques that integrate with hybrid systems and closed-loop biorefineries, enabling environmental footprint reduction. Lignocellulosic nanomaterials represent a significant advancement in wastewater treatment, offering an effective solution to global pollution issues and facilitating waste transformation toward value creation. The future of material and scalability research in water treatment needs functionalization strategies and full industrial integration to establish sustainable treatment systems at all levels worldwide.

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Article

Explainable Artificial Intelligence Analysis of Simultaneous Anaerobic Nitrogen and Sulfur Removal in Anaerobic Sulfide Oxidation Bioreactor

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Abstract: Biological wastewater treatment systems exhibit a wide range of operating conditions and influent substrate types. Artificial intelligence methods, particularly artificial neural networks (ANNs), are increasingly being employed to manage this complexity. This study uses ANN modeling to identify the key operational parameters that influence the efficacy of anaerobic sulfide oxidation (ASO) biotechnology, which simultaneously treats nitrite and sulfide. The ANN model was further analyzed through SHAP analysis to determine the key operational parameters. The dataset used in this study was derived from previously published operational data of ASO reactors. The results revealed that the sulfide-to-nitrite (S:N) ratio and hydraulic retention time (HRT) had the greatest impact on sulfide removal. In contrast, influent sulfide and nitrite concentrations had no effect on the prediction of effluent pH. While other parameters had a positive effect, HRT had a slight negative impact on effluent pH, with the S:N ratio having the most effect. Furthermore, while other factors contributed to sulfate generation, sulfide influent and HRT had a significant impact. Predicting nitrite-nitrogen (NO_2^- -N) removal is mostly dependent on the S:N ratio and influent pH. To enhance ASO reactor performance for sulfide and nitrite removal, it is recommended to prioritize the optimization of the S:N ratio and HRT, as these parameters have the greatest impact on key treatment outcomes, including sulfide and NO_2^- -N removal and sulfate formation.

Keywords: Anaerobic Sulfide Oxidation (ASO); Artificial Neural Networks (ANN); S:N Ratio; Hydraulic Retention Time (HRT); Nitrite-Nitrogen (NO_2^- -N) Removal; wastewater treatment modeling

1. Introduction

Industrial developments and urban settlements are unavoidable in modern times. However, such unchecked developments have threatened the future of humans, as evidenced by serious environmental consequences including global climate changes, soil contamination, and most importantly the contamination of water resources. Among these

undesirable consequences of non-sustainable development is water pollution. Water pollution caused by toxic pollutants is a global issue that affects the population in numerous ways, including its impact on direct consumption, agriculture, and recreational activities [1]. Various methods for the removal of pollutants from wastewater include chemical, photovoltaic, aerobic, and anaerobic pathways. Anaerobic methods utilize microorganisms to transform organic matter without the presence of oxygen, making them effective for treating high-strength wastewater.

Recent investigations have extensively focused on biotechnology for the concurrent removal of nitrogen and sulfur [2–6]. The use of biotechnology is essential for the well-being of ecosystems. This is because it transforms pollutants into benign substances, generates biodegradable materials from renewable sources, and develops environmentally safe manufacturing and disposal processes [2]. Some of these processes are conducted in the absence of oxygen, referred to as anoxic, such as denitrification for the removal of nitrate [7]. Another one of those technologies is anaerobic sulfide oxidation (ASO). The anoxic sulfide-oxidizing reactor is an up-flow reactor with biomass retention. The reactor is made of Perspex with a working volume of 1.3 L. The synthetic influent is pumped through a peristaltic pump from the 5 L influent vessel to the reactor. The flow rate can vary between 0.6 and 12.5 L per day, which provides the possibility of operating at HRTs between 2.0 and 0.1 days. A recycling pump is used in order to mix the influent (substrate) and sludge (biocatalyst) well and hence to decrease possible substrate inhibition. The process can be controlled by changing the ratio of recycling flow to the influent flow and the temperature of the reactor between [2]. One of the challenges with the employment of ASO technology is the variation in reactor performance due to the intensity of shock loads. The effect of shock loading is directly proportional to the intensity of the shock loads. The reactor performance is observed to be stable at a relatively lower intensity (1.5 times shock load), while it shows considerable disturbance at a higher intensity (higher than 2.0 times shock load). The effluent sulfide-sulfur concentration was also found to be another sensitive parameter affecting the steady state of the reactor [4].

The ASO reactor efficiently managed elevated influent quantities of sulfide (1920 mg/L) and nitrite (2265.25 mg/L). A 0.10-day hydraulic retention time (HRT) yielded peak loading rates of 13.84 kgS/(m³·d) and 16.311 kgN/(m³·d). A stoichiometric analysis indicated incomplete oxidation of sulfide, leading to the formation of elemental sulfur, attributed to a low sulfide-to-nitrite ratio of 0.93 [2]. HRT did not influence sulfide removal but significantly affected nitrite removal. The reactor withstood a 0.10-day hydraulic retention time, but performance declined when it dropped to 0.08 days [2,3].

Mahmood et al. [3] reported that sulfide removal efficiency in ASO was not significantly affected when HRT was lowered from 1.0 to 0.10 days. However, reducing HRT to 0.08 days negatively impacted reactor performance. Nitrite removal was significantly influenced by changes in HRT, indicating the reactor's adaptability to operational adjustments [4]. The importance of aerobic polishing following the ASO reactor for achieving effluent standards was emphasized, with results showing successful compliance after 12 h of aeration at dissolved oxygen (DO) levels below 1.0 mg/L [4].

Artificial neural network (ANN) modeling proved effective for the performance prediction of the ASO reactor, achieving good agreement between predicted and observed values with correlation coefficients of 0.96 for nitrite and 0.98 for sulfide [5]. Further optimization of the ASO reactor via ANN and Genetic Algorithm (GA) models enhanced performance under varying influent concentrations and operational conditions [5]. Similarly, simultaneous removal of nitrogen and sulfur has been investigated in mixed culture bioreactors, demonstrating efficient performance under controlled conditions [6]. Researchers have also applied constructed wetlands (CWs) to treat wastewater containing sulfide and nitrogen

compounds. CWs offer low-cost and sustainable treatment solutions by leveraging natural processes such as microbial activity, plant uptake, and sedimentation [8–11].

In recent years, machine learning (ML) techniques have shown great potential in optimizing wastewater treatment processes, including sulfide and nitrogen removal [12]. Among these, artificial neural networks (ANNs) have proven to be a powerful tool for modeling complex, non-linear relationships inherent in biological treatment systems [13,14].

ANN models mimic the functioning of biological neural systems to learn patterns in data, enabling them to predict system behavior under different operational conditions [13,15]. They have been applied successfully in various domains, including speech recognition [16,17], natural language processing [18–20], autonomous driving [21], medical diagnosis [22], and financial prediction [23].

Recently, researchers have increasingly applied ANNs to environmental engineering problems, particularly in water and wastewater treatment [24,25]. In this study, we utilized ANN modeling to predict the performance of an ASO reactor for the simultaneous removal of sulfide and nitrite under various operational conditions. Additionally, we employed SHapley Additive exPlanations (SHAP) analysis to interpret the contribution of different input variables to the reactor performance prediction. The use of SHAP analysis with ANNs is referred to as Explainable AI or XAI. Its application in the environmental field, despite its popularity in other fields, is rather limited. The limited literature found thus far shows that this approach increases the robustness of the models, as well as their generalizability. Moreover, it improves the interpretability of the results. Consequently, higher accuracy and interpretability aid decision-making ability, which could be vital for wastewater treatment systems [26,27].

The applications of XAI found thus far in the field of water treatment are all recent, which shows the popularity of this approach among modern researchers. More prominently, they deal with the prediction of water quality; such examples include [28–30]. Another field of application is water demand management and prediction [31]. With regard to the use in wastewater treatment, Nasir and Li [32] have used this approach successfully for sludge prediction through employment of various tree-based models. Sludge monitoring was also conducted through image detection by applying deep learning [33]. With regard to the tree models, they were also used in other studies for optimizing the parameters of wastewater treatment plants for cost minimization [34]. One of the very few studies found to focus on the chemical parameters of treated wastewater is by [35].

It is evident from the above that previous research on the use of ANNs and XAI in microbially controlled biotechnologies is scarce. Artificial intelligence techniques are now widely used to reap the benefits of modern technological progress. The artificial neural network model is expected to aid in identifying the important elements influencing the ASO biotechnology that treats nitrite and sulfide at the same time. Based on these findings, this study employs ANN models to predict the removal of pollutants from wastewater subjected to ASO treatment technology. The data for this study was taken from previously published works of [2–6], and the focus of the study was kept on the modeling aspect. The models were subjected to SHAP analysis for determining the key parameters and their impact on pollutant removal. XAI lends itself as an effective and efficient method for obtaining accurate predictions of results as well as for determining the impacts of variables despite limitations of data and prior information about them.

The comparative analysis provided by the SHAP analysis, between different parameters, cannot be achieved through other traditional methods with the above-mentioned limitations. This type of research is non-existent in the field of ASO reactor performance prediction. The prediction model and its findings provide a solid basis for the application

and improvement of the ASO method by exploring the values of operational factors beyond their known ranges.

2. Methods of Analysis

2.1. Data Collection

The ASO reactor is an up-flow mode that retains biomass. The reactor is composed of Perspex with a working volume of 1.3 L. The synthetic influent is pumped from the 5 L influent vessel to the reactor via a peristaltic pump (Longer Pump YZ1515X, Hebei, China). The hydraulic retention time (HRT) of this influent pump is determined by the pump flow rate, as the residence time is defined as the volume–flow rate ratio. The flow rate can vary between 0.6 and 12.5 L per day, allowing for HRTs of 2 to 0.1 days. A recycling pump is employed to ensure proper mixing of the influent (substrate) and sludge (biocatalyst), hence reducing the possibility of substrate inhibition. The recycling flow-to-influent flow ratio is fixed at around 2.5–3. The influent flow rate is, however, identical to the actual effluent flow rate because the withdrawal threshold is 1.3 L. The temperature of the reactor can be regulated using a thermostat between 20 °C and 70 °C, though the normal operational temperature is 35 °C, as is typical of the ASO process. The data obtained from the operation of various aspects of ASO reactors have been published previously [2–6] and were employed for artificial neural network studies.

2.2. Artificial Neural Network

ANN models work with the combination of three types of layers, which are shown in Figure 1 and described as follows:

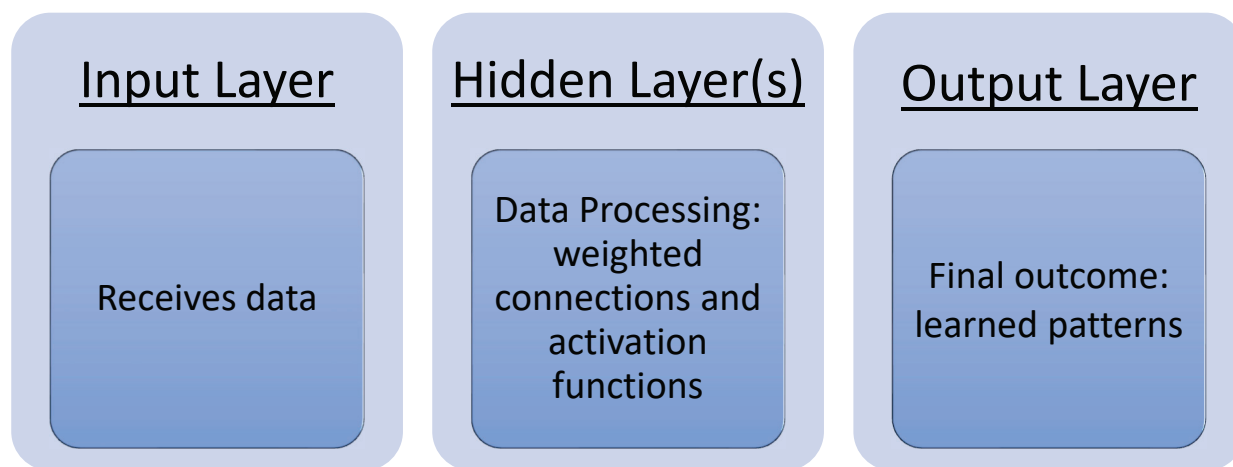


Figure 1. Working flow of artificial neural network models.

1. Input Layer: Receives input data, which is typically normalized or standardized.
2. Hidden Layers: Process the input data through a series of weighted connections and activation functions, capturing complex patterns.
3. Output Layer: Produces the final output based on the learned patterns. The number of nodes in this layer depends on the type of task (e.g., classification, regression) [24].

During training, the network adjusts the weights of connections to minimize the difference between predicted and actual outputs (the loss function). This process, known as backpropagation, involves iteratively updating weights using optimization algorithms like gradient descent. Once trained, the neural network can make predictions on new, unseen data [25].

In conclusion, neural network models are a powerful tool in the field of artificial intelligence, with a wide range of applications and the ability to learn complex patterns

from data. There have been previous studies in which ANN models have been used for the prediction of chemical processes in water treatment, and they have proven to perform better than statistical techniques such as linear regression [26]. Having said that, their application in the field of chemical engineering biology is rather limited. Hence, this study is an attempt to capitalize on the positive aspects of ANN models to improve the prediction ability and understanding of chemical processes associated with water treatment.

2.3. SHAP Analysis

SHAP (SHapley Additive exPlanations) analysis is a powerful method used to explain the output of machine learning models by attributing the prediction to its individual features. It is based on the concept of Shapley values from cooperative game theory, which assigns a value to each feature representing its contribution to the prediction [27].

SHAP begins by calculating Shapley values, which quantify the impact of each feature on the model's prediction. It considers all possible feature combinations and how each feature contributes to the prediction when added to a subset of features. The calculation of SHAP values can be performed using Equation (1):

$$SHAP(v) = \frac{1}{p} \sum_s \frac{[v(s \cup \{m\}) - v(s)]}{\binom{p-1}{k(s)}}, \quad m = 1, 2, 3, \dots, p \quad (1)$$

where v is the function or model for which SHAP values are calculated, m is the member for which the current SHAP value is calculated, s denotes the possible subset of the variables and $s \cup \{m\}$ shows the value of the model with the addition of member m . The Shapley values are then used to rank the features based on their importance. Features with higher Shapley values are considered more important as they have a greater impact on the model's output [36].

SHAP provides a clear and interpretable explanation of the model's predictions, making it easier to understand the model's behavior. It allows for both global and local interpretation, providing insights into how each feature affects individual predictions as well as the overall model behavior. It can also be used to compare the importance of features across different models, helping in model selection and feature engineering. SHAP is robust to correlated features and provides reliable explanations even in complex models like ensemble methods and neural networks [37]. Due to the above advantages, SHAP has been used in a variety of fields such as prediction of skin cancer, identification of manipulations in an image, evaluation of industrial internal security, and many others [38].

The above discussion clearly shows that SHAP analysis is a valuable tool for understanding and interpreting machine learning models, providing insights into feature importance and model behavior, and finding applications across various industries. This is the reason for employing it in the current study.

3. Results

3.1. ANN Models

There is a wide variety of ANN techniques and approaches which have been adopted by researchers. This study limits itself to the use of a multilayer perceptron (MLP), which is a popular type of ANN model. The network weights were optimized using a two-step procedure including backpropagation and conjugate gradient methods applied in a series with the same order. The input and output variables for each model developed in this study are shown in Figures 2–6. They were normalized using Equation (2) [39].

$$X_N = \frac{(X - X_{min})}{(X_{max} - X_{min})} \quad (2)$$

The sum of squared errors was minimized to find the appropriate weights and select the best network architecture. The former included optimizing the number of hidden layers and the number of neurons in each of those layers. The hidden-layer neurons had a hyperbolic activation function, while for the output layer, it was logistic. STATISTICA version 14.1.0 (by STATSOFT Inc., Hamburg, Germany) was used for this analysis. The accuracy of the models was tested using three different parameters, namely, correlation coefficient (CC), root mean square error (RMSE) and mean absolute percentage error (MAPE). The available dataset was divided into three parts; 50% was randomly selected for training the model and 25% was randomly selected for selecting the best model parameters, while the remaining was used for testing the model. The approach is similar to that found in recent research in similar areas [27]. The weights for each ANN model are included in the Appendix A for readers interested in replicating the results. The above methodology of testing the model on an independently selected test dataset, along with the application of various accuracy parameters to all datasets, gives fair evidence about the reliability of the models (see Tables 1–5).

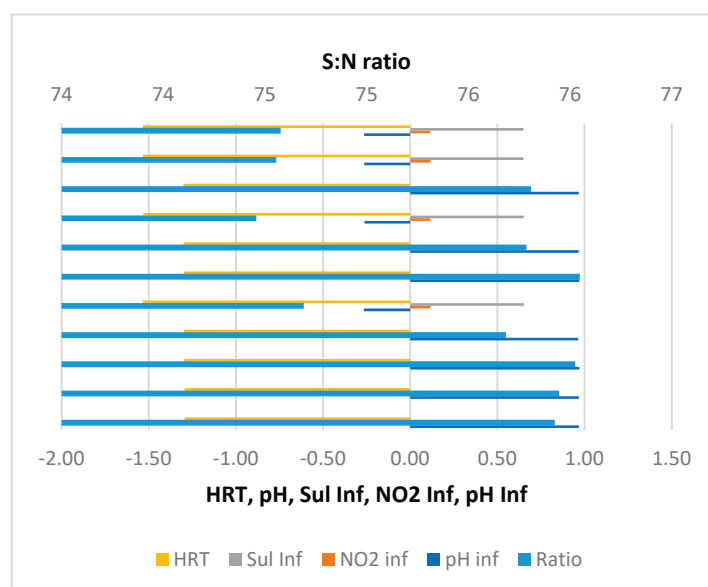


Figure 2. Feature importance for sulfide removal prediction model.

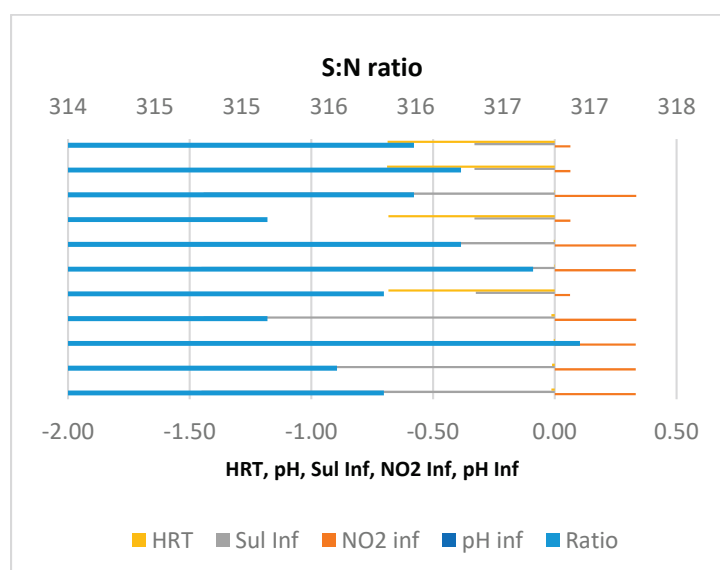


Figure 3. Feature importance for sulfate formation prediction model.

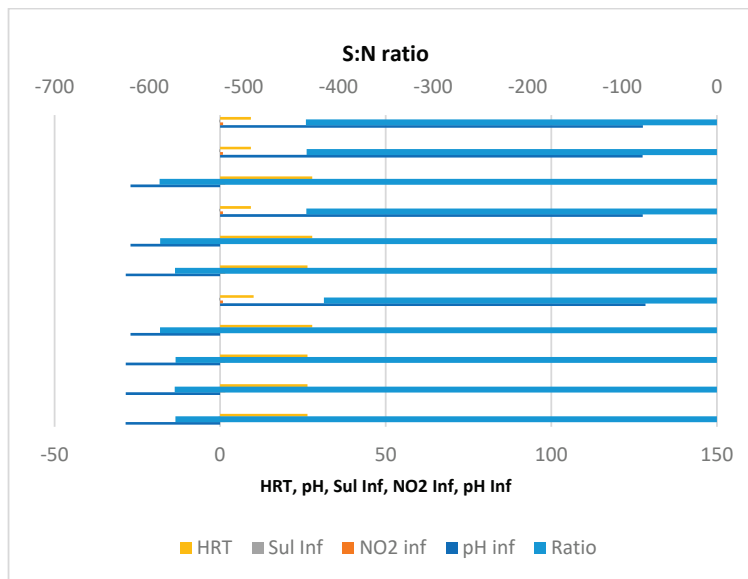


Figure 4. Feature importance for NO₂-N removal prediction model.

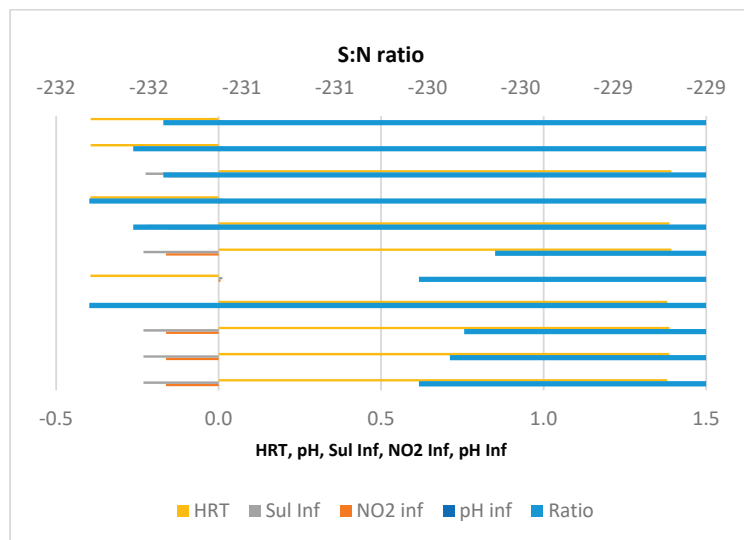


Figure 5. Feature importance for NH₄-N formation prediction model.

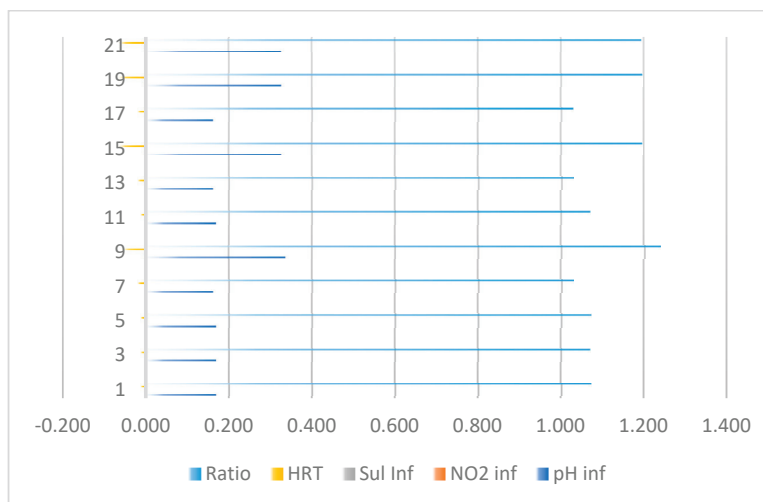


Figure 6. Feature importance for pH effluent prediction model.

Table 1. Accuracy of sulfide removal prediction model.

Dataset	RMSE	MAPE	CC
Training	11.48	2.26	0.99
Selection	20.71	2.79	0.99
Test	14.29	1.36	0.99

Table 2. Accuracy of sulfate formation prediction model.

Dataset	RMSE	MAPE	CC
Training	83.47	106.04	0.80
Selection	95.88	57.26	0.68
Test	111.23	43.46	0.56

Notes: Not good results. Recommended to be modeled using other techniques such as deep learning.

Table 3. Accuracy of NO₂-N removal prediction model.

Dataset	RMSE	MAPE	CC
Training	43.53	4.28	0.99
Selection	63.92	4.10	0.99
Test	53.77	5.01	0.99

Table 4. Accuracy of NH₄ formation prediction model.

Dataset	RMSE	MAPE	CC
Training	20.55	N/A	0.95
Selection	15.52	N/A	0.89
Test	47.70	N/A	0.64

Table 5. Accuracy of effluent pH prediction model.

Dataset	RMSE	MAPE	CC
Training	0.22	1.97	0.93
Selection	0.28	2.50	0.90
Test	0.24	2.14	0.92

Most of the models showed acceptable RMSE and CC values for all dataset types (training, selection and test). More importantly, the models for sulfide and NO₂-N removal provided a high CC across all datasets, which is the most important result of the ASO process. RMSE and CC are more stable parameters as compared to MAPE. It is possible to calculate them for any dataset, even with large ranges or values of 0 magnitude. The sulfide removal prediction model was the best in terms of both the CC between predictions and actual values and MAPE. The models for predicting the formation of sulfate and NH₄ showed a large variation in accuracies between different datasets, which raises a question about their robustness. The accuracies of the present models are generally better than those obtained by Mahmood et al. [2,3], including those models which are mentioned above. The possible reasons could be the implementation of a two-stage learning process and the use of two hidden layers, wherever appropriate. Moreover, the present study evaluates the models in a better way by using it on test datasets and including multiple parameters. In conclusion, it can be said that the models developed in this study are more accurate and

the results are more reliable than previous relevant studies due to testing on independent datasets and employing various accuracy parameters.

3.2. SHAP Analysis Outcome

It was discovered that the S:N ratio and HRT had the greatest influence on predicting sulfide removal (Figure 2 and Table S1). The average SHAP value for the S:N ratio was 75 (Table S1), as depicted in the secondary x -axis of Figure 2. This implies that all variables except HRT could boost sulfide removal, which makes sense because a lower HRT means less time available for the sulfide removal process.

According to Figure 3, the S:N ratio and sulfide influent had the greatest influence on the prediction of sulfide removal. Sulfide influent and HRT both had a detrimental impact on sulfate formation, whilst other variables had a beneficial impact.

As evident in Figure 4, the S:N ratio and influent pH had the highest impact on the prediction of $\text{NO}_2\text{-N}$ removal. The S:N ratio and sulfide influent had negative impacts on the removal of $\text{NO}_2\text{-N}$, while other variables had a positive impact on it.

As shown in Figure 5, the S:N ratio and HRT had the highest impact on the prediction of NH_4^+ formation. The impact of influent pH was almost zero in this case (Table S4). From the other remaining variables, only HRT had a positive impact, which could be linked to the time of the chemical process.

Figure 6 illustrates that sulfide and NO_2^- for the influent had almost no impact on the prediction of effluent pH. HRT had a small negative impact on the predictions, while the remaining variables had a positive impact on it, with the impact of the S:N ratio being the highest. Similar findings have been found for pH levels for gas treatment processes with an impact of the ratio [40].

Table 6 illustrates that the influent substrate ratio significantly affected all forecast types. HRT exerted a more significant impact on most models. Conversely, influent factors like pH and NO_2^- significantly affected the predictions of $\text{NO}_2\text{-N}$ removal.

Table 6. Comparison of SHAP values for all models.

Predictions	pH Influent	NO_2 Influent	Sulfide Influent	HRT	Ratio
Sulfide Removed	0.52	0.10	0.59	−1.38	75.41
Sulfate Formed	0.00	0.24	−1.04	−0.25	315.96
$\text{NO}_2\text{-N}$ Removed	28.73	1.28	−0.40	20.63	−524.81
$\text{NH}_4\text{-N}$ Formed	0.00	−0.10	−0.14	0.74	−230.82
pH Effluent	0.23	0.00	0.00	−0.03	1.11

3.3. Critical Parameters Affecting N and S Removal

Researchers discovered that artificial neural networks can accurately estimate effluents from anoxic sulfide oxidation processes based on influent data. XAI provides accurate predictions coupled with greater interpretability of the model results. Hence, the parameters affecting the performance of the process are known and their impacts are determined. Consequently, it can be used for better decision making. The current technique accurately predicts effluent pH, nitrite, and nitrogen contents in ASO reactors. Nonetheless, the model cannot predict the formation of sulfide and sulfate throughout the ASO process. The observed oscillations during the experiment make the ANN model's erratic behavior regarding sulfate formation understandable.

In our real trial [2,3], HRT had little effect on the percentage of sulfide elimination. When the hydraulic retention duration was reduced from 1.5 to 0.10 days, the effluent sulfide concentration remained continuously below 2 mg/L, with a clearance rate of more

than 99.16%. The process abruptly ended when the hydraulic retention period was reduced to 0.08 days, resulting in a 96.66% fall in sulfide removal efficiency [2]. When reduced, sulfide can be oxidized to sulfate or elemental sulfur in the presence of an electron acceptor. McNeice [41] observed that lower sulfide-to-oxygen ratios resulted in the formation of both sulfur and sulfate; however, in oxygen-limited conditions, thiosulfate emerged as the predominant product. Sun et al. [42] found that sulfide concentration and input sulfide–nitrate ratios were important determinants in sulfide oxidation. The optimal conditions for manufacturing sulfur as the principal product were proposed to be sulfide concentrations below 9 mmol/L and sulfide-to-nitrate ratios of 1.6 and 2.5. Mahmood et al. [2] discovered that higher influent sulfide-sulfur loading resulted in elemental sulfur being the predominant product of sulfide biooxidation when nitrite was used as the electron acceptor. The inhibitory sulfide levels could prompt the growth of sulfur-, iron-, nitrate- and nitrite-metabolizing microorganisms [43]. The experiment, which used an ASO reactor with different hydraulic retention times (HRTs), found that nitrite removal was responsive to changes in HRT. The ANN model properly predicted nitrite removal. The model may be validated using estimates for nitrite elimination. The speeds of biological processes follow laws like those governing pure substances. The reaction rates for biochemical processes vary depending on plant conditions and environmental variables such as temperature and pH.

Dependence is sometimes non-monotonic, with certain processes emerging exclusively at specific dosage levels. Diverse inputs to the ASO reactor, including sulfide and nitrite, can result in sulfate or sulfur (not measured), dinitrogen gas, and trace amounts of ammonium. In ANN analysis, hydraulic retention time (HRT) and pH are considered essential input parameters for predicting reactor output. As a result, four input variables—sulfide, nitrite, pH, and hydraulic retention time (HRT)—can influence the generation of four outputs: sulfate, sulfur, nitrogen, and ammonium. Yuan et al. [43] found no significant difference in nitrate-nitrogen (NO_3^- -N) levels between pH levels 6 and 7.5 and hydraulic retention times (HRTs) of 7.41 to 6.83 h. Nonetheless, lowering the HRT caused an increase in elemental sulfur accumulation. A lower pH, especially near 7, encouraged the buildup of elemental sulfur. A second pH drop resulted in a modest rise [44].

In the current study, HRT had a minor negative effect on outcome prediction, whereas the other variables had a favorable influence, with the impact ratio being the most pronounced. The efficacy of NO_2^- -N removal was significantly affected by the influent's pH and nitrite contents.

The ANN prediction shows that the influent S:N ratio and HRT have the greatest influence on NH_4 production prediction. The technical impediment to the commercially practical adoption of this method for sour waste treatment may be related to substrate and product inhibition, which explains the partial oxidation of sulfide [45]. Sulfide is harmful to *Thiobacillus denitrificans* because it acts as an inhibitory substrate. The practical effect is that reactor systems designed to handle sulfide-laden waste must be sulfide-limited, which ensures that the steady-state concentration of sulfide in the bulk liquid remains below inhibitory limits. Furthermore, reactor systems must be adequately mixed to avoid significant sulfide concentration gradients, which might lead to isolated zones with inhibitory sulfide levels [45]. In the current study, it was discovered that sulfate concentrations more than 250 mg/L, together with a higher reactor pH, may have had inhibitory effects (product inhibition) on the complete oxidation of sulfide to sulfate. This was the case observed in the present study, which shows that the reactor was in the transient state. The higher removal rates observed in this investigation at various nitrite-to-sulfide influent molar ratios suggest that sulfide oxidation is incomplete, resulting in the generation of elemental sulfur. It was found in previous studies that sulfate oxidation

is primarily affected by the liquid-to-solid ratio, followed by the inlet SO_2 concentration, with temperature having the lowest impact among them [46]. In this regard, a wide variety of techniques have been applied to enhance the oxidation process for the removal of unwanted substances. Among them, photo-assisted techniques and electrochemistry have been found to be very effective [47]. However, their integration with the ASO process is still to be explored.

T. denitrificans produces sulfate through the aerobic or anoxic oxidation of sulfides (with nitrate). Sulfate concentrations of more than 250 mg inhibit *T. denitrificans* [48]. This is most likely due to increased ionic strength rather than the inherent inhibition of sulfate. Product inhibition limits the operation of both batch and continuous reactors. Sulfate accumulation in batch systems can have an impact on the batch cycle duration. In a continuous system, the dilution rate determines the sulfate concentration at a steady state; hence, for any given sulfide input rate, the reactor volume or hydraulic throughput is determined by the sulfate concentration needed in the culture [48,49]. Our previous research into concurrent sulfide and nitrite elimination at an influent molar ratio of 1.17 revealed that sulfide oxidation was not fully achieved, with approximately 10–11% of sulfide removal attributed to autooxidation caused by trace amounts of dissolved oxygen in the influent wastewater [3].

The simultaneous oxidation of sulfide and reduction of nitrite is a promising biological method for wastewater treatment, providing a sustainable and cost-effective way to eradicate both sulfur and nitrogen contaminants at once. This method uses microbes to turn sulfide into less harmful forms, like elemental sulfur or sulfate, while also turning nitrite into nitrogen gas. This solves the problem of both sulfurous and nitrogenous pollution in different types of industrial and agricultural wastewater. The sulfide-to-nitrite ratio (S:N ratio), pH, ammonium concentration, and influent sulfide concentration are just a few of the environmental and operational factors that have an impact on this process's efficiency and stability. These factors also have a significant impact on the microbial community structure and metabolic pathways. Understanding these features and their complex inter-relationships is critical for improving the efficacy and dependability of sulfide oxidation and nitrite reduction systems in actual applications. Mahmood [2,3] discovered four input variables (sulfide, nitrite, pH, and HRT) that result in three outputs (sulfate, nitrogen, and ammonium).

4. Conclusions

The present investigation on the SHAP (SHapley Additive exPlanations) analysis of data obtained from an anaerobic ASO reactor treating sulfide and nitrite led to the following key findings.

The efficiency and stability of the ASO biotreatment process are influenced by a complex interaction of environmental and operational factors, including the sulfide-to-nitrite ratio (S:N ratio), pH, ammonium concentration, and influent sulfide concentration, all of which have a significant impact on the microbial community structure and metabolic pathways.

The S:N ratio and HRT were found to have the greatest influence on the prediction of sulfide removal efficiency. The positive impact of the S:N ratio on the removal rates suggests that sulfide oxidation is incomplete, resulting in the generation of elemental sulfur. Sulfide and nitrite concentrations in the influent had minimal effect on predicting the effluent pH, while the S:N ratio had the highest positive impact. The higher removal rates observed in this investigation at various nitrite-to-sulfide influent molar ratios suggest that sulfide oxidation is incomplete, resulting in the generation of elemental sulfur. Sulfide influent and HRT showed a detrimental effect on sulfate formation, whereas other variables contributed positively. The S:N ratio and influent pH were the most influential factors in predicting NO_2^- -N removal.

To optimize reactor performance for sulfide and nitrite removal, it is recommended to prioritize fine-tuning the S:N ratio and HRT, as these parameters significantly influence key performance indicators like sulfide and NO_2^- -N removal, as well as sulfate formation. The SHAP analysis showed that both these parameters have opposite effects on sulfide removal and nitrate formation. However, the impact on nitrate was higher than on sulfide removal. Hence, it could be said that a focus on nitrate removal would have a slightly negative impact on sulfide removal.

This study found that pH and HRT have significant impacts on wastewater treatment plants, but their accuracy varies due to high variation in the data. To improve the results, meta-heuristic or deep learning models may be employed. Future research should include real-world validation and explore microbial dynamics under varying environmental parameters. Advanced oxidation techniques could also be explored to reduce unnecessary substances in the system.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w17131880/s1>, Supplementary Table S1. Shapely Values for Sul Removed Prediction Model. Supplementary Table S2. Shapely Values for Sulfate Formed Prediction Model. Supplementary Table S3. Shapely Values for NO_2^- -N Removal Prediction Model. Supplementary Table S4. Shapely Values for NH_4 -N Formed Prediction Model. Supplementary Table S5. Shapely Values for pH Effluent Prediction Model.

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Appendix A

Table A1. Neural Network Weights for Sul Removed.

Layer	Weight	Neuron 1	Neuron 2	Neuron 3	Neuron 4	Neuron 5
2	Threshold	0.734895	0.14332	0.609806	0.553195	0.734895
	W1	0.125874	−0.01108	−0.123308	−0.079186	0.125874
	W2	−0.184298	−0.42810	0.045927	0.033984	−0.184298
	W3	1.033897	−1.34412	0.546967	0.886106	1.033897
	W4	−0.000431	0.10962	0.073516	−0.002870	−0.000431
	W5	−0.138503	−0.06813	0.118240	0.065655	−0.138503
3	Threshold	0.441433	0.757272	−0.807444	0.331590	
	W1	−0.382654	1.009133	0.122110	−0.342085	
	W2	0.622816	−0.195391	−0.185834	1.268736	
	W3	−0.000892	0.578166	0.226279	0.142720	
	W4	−0.554850	0.874384	0.136965	−0.431479	
4	Threshold	0.24543				
	W1	−1.24951				
	W2	2.17651				
	W3	0.31365				
	W4	−1.24422				

Table A2. ANN Weights for Sul Formed.

Layer	Weight	Neuron 1	Neuron 2	Neuron 3	Neuron 4	Neuron 5	Neuron 6	Neuron 7
2	Threshold	−0.25402	−0.856211	−1.03164	1.283578	−2.47577	−0.252317	−0.177186
	W1	−0.74013	0.388996	0.41232	2.338687	−1.48365	−0.511173	−0.815234
	W2	−1.03677	1.290209	−0.00255	0.512451	0.09562	2.200229	3.938584
	W3	0.59293	−0.259599	0.24495	0.864413	−1.83519	0.006008	0.501213
	W4	0.60005	0.422904	−0.09469	−0.453886	0.74836	0.323768	−0.537115
	W5	−0.25402	−0.856211	−1.03164	1.283578	−2.47577	−0.252317	−0.177186
3	Threshold	−0.33212						
	W1	0.99034						
	W2	−0.46433						
	W3	0.62852						
	W4	2.45765						
	W5	2.68945						
	W6	−1.37689						
	W7	−2.75697						

Table A3. ANN Weights for NO2-N Removed.

Layer	Weight	Neuron 1	Neuron 2	Neuron 3	Neuron 4	Neuron 5	Neuron 6	Neuron 7	Neuron 8	Neuron 9	Neuron 10	Neuron 11	Neuron 12
2	Threshold	0.77412	0.456014	−0.031473	1.181602	0.485206	−0.612913	−0.179313	0.175743	−0.097188	−0.226884	−0.832653	0.315406
	W1	−0.56876	0.218188	−0.370007	0.076601	0.389217	−0.788336	−0.660659	−0.368171	−0.085167	0.579430	−0.104846	−0.306158
	W2	−1.05890	0.881294	−0.426706	0.422553	0.034106	−0.372050	0.987558	−0.399188	0.083581	0.567741	0.968475	0.514185
	W3	0.67022	−0.472024	0.008493	0.797143	−0.066656	0.577456	−0.153783	−0.835811	−0.054092	0.712828	−0.360356	0.792020
	W4	0.15358	0.420844	−0.852411	0.647223	0.388734	0.297250	−0.064015	1.029580	−0.037335	−0.435801	0.572786	−0.270927
	W5	0.72592	−0.688227	−0.644760	0.031604	0.843404	0.737271	0.381358	−0.907832	−0.003515	0.147512	0.164616	−0.826120
3	Threshold	0.070233	0.297925	0.205179	0.572240	0.867277	−0.294691						
	W1	0.515142	−0.922552	−0.958962	−0.618872	−0.064719	0.331149						
	W2	−0.231970	−0.207528	−0.229932	0.100993	−0.220813	0.676270						
	W3	0.706281	−0.999298	−0.016030	−0.575983	0.479162	0.755654						
	W4	1.051252	0.090775	0.161735	−0.345654	−0.324120	−0.585142						
	W5	0.217223	0.351567	0.371642	−0.201453	−0.120096	−0.026625						
	W6	−0.340079	0.637359	0.495911	−0.907844	0.258160	−0.279963						
	W7	−0.295758	−0.764562	0.539758	0.396139	−0.686912	1.006009						
	W8	−0.967223	0.141457	−0.226930	0.673835	−0.118477	0.127589						
	W9	−0.623107	0.569373	0.464128	−0.903109	−0.795511	−0.754020						
	W10	0.949142	0.143367	−0.428181	0.210982	0.444387	0.501235						
	W11	−0.011089	0.547601	−0.160073	0.244322	−0.208659	−0.129049						
	W12	0.595417	0.681863	0.241115	0.849668	0.018242	−0.819467						
4	Threshold	0.756666											
	W1	0.406573											
	W2	0.709643											
	W3	0.599181											
	W4	0.420095											
	W5	−0.735801											
	W6	0.640824											

Table A4. ANN Weights for NH₄-N Formed.

Layer	Weight	Neuron 1	Neuron 2	Neuron 3	Neuron 4	Neuron 5	Neuron 6	Neuron 7	Neuron 8	Neuron 9	Neuron 10	Neuron 11
2	Threshold	7.58530	−0.59720	0.206648	−2.43860	0.540810	−1.39966	1.286364	−1.73442	0.925801	1.053922	0.96235
	W1	4.34586	−1.79148	−0.426081	0.03309	0.295972	−0.23042	−0.211485	0.82877	0.303824	−0.126191	0.97313
	W2	3.12185	−1.72340	0.059979	0.60584	0.176060	−0.17481	−0.116214	1.10853	0.539688	−0.032217	1.15592
	W3	−2.02107	2.56593	0.213490	0.18549	0.839211	0.09246	−0.324752	−0.22160	−0.054978	−0.174338	−0.53851
	W4	1.86011	3.79056	−0.164943	−0.09287	0.878869	0.69389	−0.378472	−0.47720	0.264341	−0.072815	−2.03750
3	W5	7.58530	−0.59720	0.206648	−2.43860	0.540810	−1.39966	1.286364	−1.73442	0.925801	1.053922	0.96235
	Threshold	−0.65140										
	W1	5.68331										
	W2	−2.16740										
	W3	0.31994										
	W4	−1.65635										
	W5	−0.57112										
	W6	1.30191										
	W7	−0.96438										
	W8	1.50757										
	W9	−0.60192										
	W10	−0.69405										
	W11	−2.87544										

Table A5. ANN Weights for pH Effluent.

Layer	Weight	Neuron 1	Neuron 2	Neuron 3	Neuron 4	Neuron 5	Neuron 6	Neuron 7	Neuron 8	Neuron 9	Neuron 10
2	Threshold	1.05823	0.133405	0.897519	−0.928437	0.13166	0.430889	0.496107	0.792288	−0.338700	−1.18038
	W1	−0.17187	−0.647502	−0.279515	−0.130696	−1.23356	−0.318732	1.171366	0.455697	−0.528742	−0.70187
	W2	−1.27256	−0.184953	1.480335	0.304847	−1.25112	2.287030	1.179459	−0.598229	0.184459	−1.15570
	W3	1.77981	0.047005	1.408471	0.736807	0.61838	0.275934	−0.098645	0.840573	−0.026089	−0.35959
	W4	−0.14882	−0.240262	−0.329721	0.468579	1.37652	1.029025	0.532582	0.264717	0.967747	−0.42022
3	W5	1.44024	0.351557	−0.041667	0.495466	0.34486	0.170214	−0.535821	0.777898	−0.297486	1.00858
	Threshold	1.72206									
	W1	−0.43139									
	W2	−1.29787									
	W3	0.55378									
	W4	−1.65740									
	W5	−1.48795									
	W6	1.56548									
	W7	0.91893									
	W8	−0.70073									
	W9	−1.42133									
	W10	1.72206									

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Article

Development of Antifouling Polyvinylidene Fluoride and Cellulose Acetate Nanocomposite Membranes for Wastewater Treatment Using a Membrane Bioreactor

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Abstract: Membrane technology has received great attention in the desalination and water treatment sectors over the last few decades. However, membrane fouling remains a critical issue that affects membrane performance, a phenomenon common in membrane bioreactors (MBRs). This major drawback can be overcome by the preparation of antifouling membranes using an electrospinning technique that generates a hydrophilic modification of membranes. In this study, nanocomposite polyvinylidene fluoride (PVDF) and cellulose acetate (CA) polymer was fabricated to mitigate membrane fouling. Surface and mechanical characterization of the electrospun membrane was performed to assess morphology, chemical composition, and hydrophilic/hydrophobic properties. Anti-fouling performance of the composite PVDF/CA membrane was evaluated versus a neat PVDF membrane through bench-scale experiments. The PVDF/CA nanofiber membrane displayed a more hydrophilic nature, demonstrated by a lower water contact angle (101° vs. 115°) and increased wastewater flux ($190 \text{ L/m}^2 \cdot \text{h}$ vs. $160 \text{ L/m}^2 \cdot \text{h}$), although the composite membrane demonstrated lower tensile strength ($2.0 \pm 0.1 \text{ MPa}$ vs. $1.7 \pm 0.1 \text{ MPa}$). The new material demonstrated greater anti-fouling performance compared to the neat PVDF membrane. Results suggest that this nanofiber material shows promise as an enhanced antifouling membrane that can overcome membrane fouling limitations.

Keywords: polyvinylidene fluoride; cellulose acetate; membrane; electrospinning; anti-fouling; water flux

1. Introduction

Complex contaminant mixtures comprised of concentrated organic matter, inorganic salts, microorganisms, and other constituents necessitate enhanced water treatment methods. Established technologies to enhance water purification, such as distillation, flocculation, and advanced oxidation processes (AOPs), often inadequately treat complex solutions

and/or incur high equipment, operation and energy costs [1]. Membrane separation technology has wide application in wastewater treatment due to its cost-effectiveness, ease of operation, and high separation efficiency [2]. Electrospinning nanofiber membranes have higher interconnected pores, high porosity, narrow pore size distribution, and possibilities of membrane modifications; due to this, they could overcome the conventional membranes [3]. State-of-the-art membrane systems, such as membrane bioreactors (MBRs), can help play a role in fostering a more sustainable water purification paradigm by presenting robust, environmentally friendly, energy-efficient treatment options.

Membrane technology faces several challenges that reduce cost-effectiveness and operational efficiency, such as fouling caused by the accumulation of organic matter, biofilm and inorganic scaling [4,5]. Fouling not only interferes with ion transport through membranes but also requires higher operating pressure and voltage [6]. Colloids, sludge flocs, extracellular polymeric substances (EPSs), and soluble microbial products (SMPs) that adhere to the membrane surface during MBR operation impede the optimization of MBR operating parameters [7].

Higher viscosity feed water allows greater contaminant accumulation on the membrane surface [8]. Meanwhile, microbial activity has been reported to cause more than 45% of membrane fouling during wastewater treatment [9]. Extracellular polymeric substances (EPSs) and soluble microbial products (SMPs) are released by micro-organisms; comprised primarily of proteins, polysaccharides, and humic substances, these constituents represent key foulant agents [10–12]. These complex organic compounds have been shown to induce membrane pore blockage, while high molecular weight EPSs can form suspended solid particles that also contribute to cake fouling [10]. The hydrophobic functional groups inherent to these substances have a propensity to attach to hydrophobic membranes and promote fouling [13]. The strong protein adsorption to membranes makes these contaminants difficult to remove [14]. Moreover, this attachment stimulates biofilm growth, comprised of a mixture of bacterial cells in an EPS matrix that contains proteins, nucleic acids, and polysaccharides [15].

Several beneficial membrane materials limit biofouling impacts. Among these, polyvinylidene fluoride (PVDF), a semi-crystalline polymer, is used for filtration in wastewater treatment due to its chemical resistance and thermal stability in the presence of acids and alkalis, high hydrophobicity, and excellent mechanical strength [16]. The amorphous phase of this polymer accommodates the desired flexibility needed in a variety of applications, while the crystalline phase provides thermal stability similar to that inherent in other polymers [17]. These properties, plus their compatibility with biological substances, make it an attractive option in microfiltration [18,19], ultrafiltration [20,21], and membrane distillation [22,23]. A piezoelectric, hydrophobic PVDF membrane, exhibiting excellent chemical and mechanical strength, can thus be used as a composite material for various environmental applications [24].

Although PVDF provides a beneficial material for membrane separation purposes in MBRs, a major drawback is the hydrophobicity of these membranes, predisposing them to fouling by proteins and pollutants that display hydrophobic properties [25]. These hydrophobic membranes suffer from rapid membrane fouling and flux decline. A hydrophilic membrane overcomes fouling problems because it can form a hydration layer on the membrane surface and reduce pollutant adhesion [25].

Functional nanofibers offer an advantageous material with strong performance characteristics due to uniform nanoparticle distribution. The electrospinning device, which consists of a copper porous spinneret and multiple air inlets, can achieve high yields of these materials [26]. Fouling of hydrophobic materials can be reduced by blending hydrophilic materials with the original membrane material in the form of nanofibers using

electrospinning technology [27]. Surface hydroxyl (–OH) groups are abundant on cellulose fibers, and they form hydrogen bonds with water molecules, allowing water to spread across the surface. By nature, cellulose is a hydrophilic and hygroscopic substance that can absorb water. Despite its intrinsic hydrophilicity, cellulose offers unrivalled benefits as a superhydrophobic material substrate [28]. Cellulose acetate (CA) membranes are widely used for various separation applications due to such advantageous characteristics as low price, moderate chlorine resistance, good biocompatibility, and hydrophilicity [29]. The blending of CA as a major matrix with PVDF to fabricate a completely hydrophilic membrane with suitable mechanical properties for RO application is considered the first investigation [30].

Electrospinning has gained considerable interest due to its ability to produce a wide diversity of nanofiber materials that can be designed to have porous structures with excellent oil/water separation efficiency [31,32]. Nanofiber membranes developed through electrospinning exhibit high surface area, light weight, mechanical strength, good resistance, strong molecular orientation along the fiber axis, high length-to-diameter ratio and enhanced porosity [33,34]. The method of modifying the mechanical characteristics of core/shell composite electrospinning fibers by varying the core diameter may offer additional benefits by extending this approach to different core/shell material systems [35]. These advantages and potential for modification lend these nanofiber materials to fields such as health care, energy, environmental engineering, and defense.

Oil/water separation and stress-strain tests revealed that a composite PVDF/CA/PS material produced using electrospinning exhibited excellent mechanical strength, high oil-water flux, good mechanical strength, and good separation efficiency [36]. Such materials can be used in membrane bioreactors, which are a cost-effective and efficient technology for wastewater treatment that combines bioreactors with membrane technology [37]. Treating wastewater using membranes lowers environmental hazards like groundwater contamination and eutrophication [38].

The research presented here aims to utilize electrospinning technologies to develop a membrane with physicochemical properties that mitigate fouling in membrane bioreactors (MBRs). To enhance the hydrophilicity of a polyvinylidene fluoride (PVDF) matrix, cellulose acetate (CA) was incorporated into the PVDF matrix. This led to the creation of an electrospinning PVDF/CA nanofiber membrane. A pristine, unaltered “neat” PVDF membrane was also produced for comparative analysis. This technique for modifying membrane properties was investigated to enhance overall membrane performance, with the expectation that a hydrophilic composite PVDF/CA material could be produced.

2. Materials and Methods

A cellulose acetate (CA) polymer (m.w. 30 kDa) and a polyvinylidene fluoride (PVDF) polymer (m.w. 534,000 Daltons) were purchased from Merck KGaA (Darmstadt, Germany) and N-N-dimethylformamide (DMF) and acetone as a solvent were purchased from Aldrich Sigma (St. Louis, MO, USA).

2.1. Electrospun Nanofiber Membrane

Sixteen percent PVDF polymer (wt/vol) was dissolved in a binary solvent of acetone and DMF at a 1:1 ratio, as reported in the literature [39]. The mixture was stirred vigorously for 5 h at 80 °C. A 5-mL plastic syringe was used to introduce the PVDF solution into the electrospinner. A pure copper wire was attached to the positive terminal and suspended in the pristine PVDF solution while a ground collector was connected to the negative terminal. An 11 cm distance was maintained as a 20 kV voltage was applied. The neat PVDF sheet

was deposited on a rotating collector up to the desired thickness. The electrospinning parameters are given in Table 1.

Table 1. Electrospinning parameters for composite PVDF/CA membrane development.

Parameters	Value
Distance from the tip of the needle to the collector	128–150 mm
Voltage applied	22–24 kV
Moisture	40–50%
Speed of the metallic collector	300 rpm
Speed of the pump filled with polymer solution	1.6 mL/min
Needle tip diameter	0.006 cm
Membrane drying time	18–24 h
Angle of the syringe with the abscissa	12 degrees

Similarly, an 18% CA solution was dissolved in a DMF-acetone solvent (1:2) at room temperature. The PVDF/CA mixture was blended by a stirrer at an 80:20 ratio and subsequently electrospun using the same procedure as stated above. The electrospinning solution parameters are found in Table 2.

Table 2. Solution parameters of neat and composite PVDF/CA nanofiber membranes.

Solution		Ambient Conditions		Electrospinning Parameters	
Polymer/Solution	Composition	Temperature	Humidity	Applied Voltage	Distance
PVDF	16%	80 °C	40–45%	20 kV	10 cm
CA	18%	25 °C	40–45%	16 kV	11 cm
PVDF/CA	80:20	25 °C	40–45%	20 kV	11 cm

2.2. Dead-End Filtration Experimental Set-Up

Antifouling performance determination of the neat PVDF and composite PVDF/CA nanofiber membranes was conducted using a dead-end filtration experiment using an Amicon Cell 200 mL (Amicon, Miami, FL, USA) experimental setup (Figure 1). The antifouling performance and the filtration tests were performed on a 28.3 cm² piece of membrane that was sized according to the vessel dimensions. Before each experiment, the membranes were soaked in milli-Q water to remove any chemicals. Both membranes were operated under a constant 10 psi pressure in a beaker where the filtrate was collected and placed on a digital balance. Relevant data were collected every 15 s.

The water flux across each membrane was evaluated using an experimental cell with a dead-end mode. To remove any potential chemical contamination, the membranes were pressurized at 0.2 MPa for an hour, after which a clean water filtration test was performed, and the permeation flux was calculated using Equation (1) [40,41].

$$J = V / A \times T \quad (1)$$

where J is total flux (m² h)^{−1}, V is permeate volume (L), A is area (m²), and T is time (h). Nitrogen gas was injected into the Amicon cell during the dead-end filtration test to determine the filtration resistance of clogged membranes. Due to the constant pressure, the membranes fouled, and the permeate declined. During the filtration process, there was almost a 100% rejection of total suspended solids in both the neat and composite PVDF

nanofiber membranes. Equations (2)–(4) were used to form a model series, and resistance was applied to determine the filtration resistance [42].

$$J = \Delta p / \mu R = dVp / A dt \quad (2)$$

$$R = R_m + R_c \quad (3)$$

$$R_c = m\alpha \quad (4)$$

where μ is permeate viscosity, p is membrane pressure, R is total hydraulic resistance (m^{-1}), m is accumulated mass on the membrane surface, J is the water flux, A is membrane surface area, and R_c is the total cake resistance.

A rearrangement of Equations (2)–(4) yields [43]:

$$t / Vp = \mu R_m / A \Delta P + \mu C_0 \alpha / 2A^2 \Delta P \times Vp \quad (5)$$

where C_0 is the initial solute concentration and T is the total time that occurred during the process.

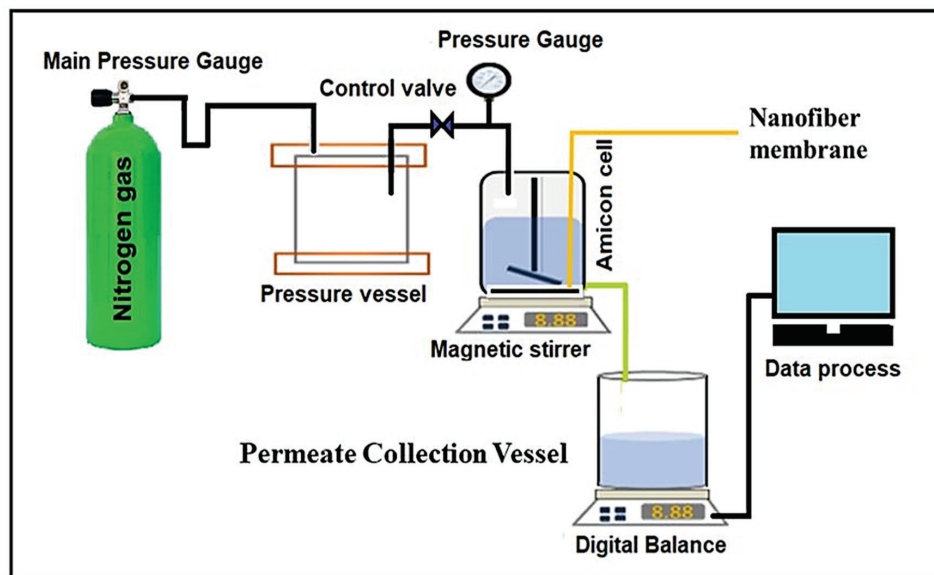


Figure 1. The schematic of the dead-end filtration apparatus used to test water flux and membrane fouling.

2.3. Nanofiber Characterization

The morphology of neat and composite PVDF/CA nanofibers was determined through scanning electron microscopy (SEM). To increase resolution, the membranes were gold-plated by applying a 10 kV voltage at a 5 mm working distance, which yielded a quality SEM image. The average membrane thickness was determined using a digital micrometer screw gauge. To measure the fiber diameter of at least 50 fibers, Image J 1.54d software was used. The apparent porosity and density of the membrane were calculated using Equations (6) and (7) [42].

$$\text{Apparent density of fibers} \left(\text{g/cm}^3 \right) = \frac{\text{Mat mass in (g)}}{\text{Mat thickness (cm)} \times \text{Mat area in (cm}^2\text{)}} \quad (6)$$

$$\text{Porosity of Mat} = 1 - \left(\text{Mat apparent density} \left(\text{g/cm}^3 \right) \times 100\% / \text{Mixture density} \left(\text{g/cm}^3 \right) \right) \quad (7)$$

The contact angle of the membrane surface was calculated using the DataPhysics OCA 15EC (DataPhysics Instruments, Filderstadt, Germany) by applying 3 μm of milli-Q water

at room temperature. After applying the droplet on the membrane surface, the contact angle measurements were carried out immediately. The experiments were replicated six times, and average PVDF and PVDF composite membrane values were calculated. Higher contact angles denote more hydrophobicity, and lower contact angles indicate greater hydrophilicity.

The molecular structure of the neat and composite PVDF/CA nanofiber membranes was determined using Fourier Transform infrared spectroscopy (FTIR). The depth of penetration was approximately 0.44–0.95 μm using a 45° incident angle of light on an ATR element. It is well known that infrared spectroscopy is a useful technique to elucidate the structure, composition, and conformation of polymeric chains, but sufficient information about polymeric membrane structure is not achieved through this technique. Energy dispersive spectroscopy (EDS) was used to determine the nanofiber chemical structure of the membranes. The stress-strain curve indicated the mechanical properties of the composite PVDF/CA and neat PVDF membranes. The strip test method was used to conduct the tensile strength tests with a tensile tester that adhered to ASTM standard D-5035-06 [39]. Membrane subsamples were 4 cm by 2 cm and were 100–120 μm thick, as determined by a digital micrometer. The load cell test's maximum extension was 100 cN. During testing, the maximum strain was determined to be the elongation at the break point.

2.4. Submerged Membrane Use in Bioreactors for Wastewater Treatment

A lab-scale submerged membrane bioreactor was used to simulate wastewater treatment. The composite PVDF/CA and neat PVDF nanofiber membranes (25.4×25.4 cm) were fitted in the submerged membrane bioreactor as shown in Figure 2. The diffuser was used to produce compressed air in the reactor. Due to the hydrostatic pressure, the water was forced across the membrane. To prevent overflow of the reactor, an auto-leveller was installed. Activated sludge from a wastewater treatment plant was used to inoculate the reactor. The operating parameters of the membrane bioreactor are shown in Table 3. The wastewater was added to the reactor, having a pH of 8.3 ± 0.28 , total suspended solids (TSSs) of 78 ± 1.88 mg/L, turbidity of 37 ± 1.2 NTU, and chemical oxygen demand (COD) of 651 ± 22 mg/L (Table 4).

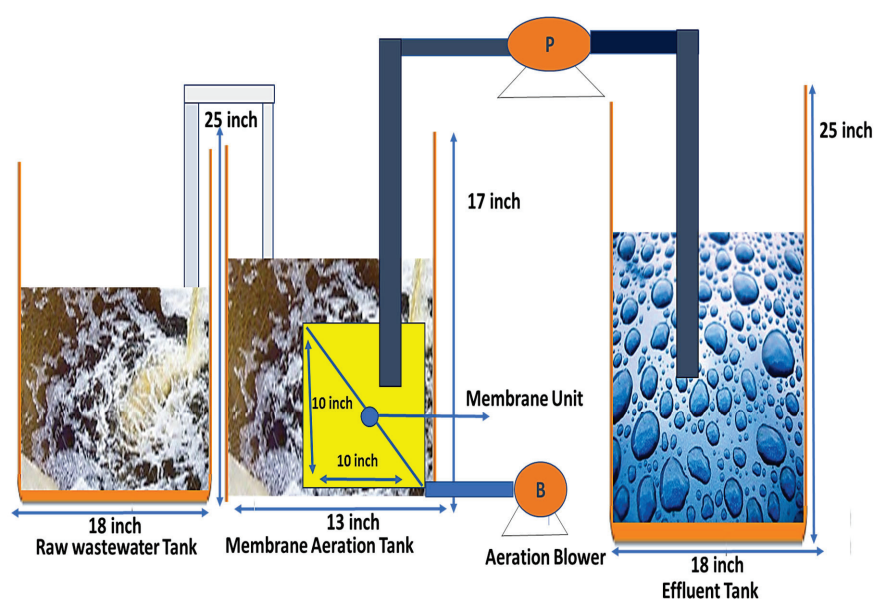


Figure 2. Submerged membrane bioreactor design for simulated wastewater treatment.

Table 3. Working parameters of the membrane bioreactor.

Parameters	Average $\pm$ Std
Total suspended solids of sludge	1700 $\pm$ 49 mg/L
Oxygen concentration	2.5 $\pm$ 0.2 mg/L
Operation time	25 days
Temperature	28 $\pm$ 1 °C
Reactor flowrate	14 $\pm$ 1 mL/min

Table 4. Properties of wastewater used in the membrane bioreactor.

Parameters	Average $\pm$ Std
Total suspended solids	78 $\pm$ 1.88 mg/L
pH	8.3 $\pm$ 0.28
Chemical oxygen demand	651 $\pm$ 22 mg/L
Turbidity	37 $\pm$ 1.2 NTU

3. Results and Discussion

3.1. Surface Morphology of the Nanofiber Membranes

Scanning electron microscope (SEM) images provided insight into the surface morphology of the neat and composite PVDF/CA nanofiber membranes (Figure 3). SEM analysis indicated that the blending of CA into the PVDF increased nanofiber roughness (Figure 3d–f), compared to the uniform and bead-free nanofibers of the neat PVDF membrane (Figure 3a–c). Correspondingly, the CA additions increased the nanofiber diameter, as represented by the accompanying histograms (Figure 3g,h).

Table 5 displays physical properties associated with the two membrane types. The average diameter of the composite PVDF/CA nanofiber was substantially larger than the neat PVDF nanofiber. The larger diameter can be attributed to the higher solution viscosity resulting from the CA amendments [43]. Correspondingly, the composite membrane exhibited a high porosity (91%) due to the well-developed interstices [44]. When the fiber structure becomes more porous, it likewise increases the surface area. Moreover, the fully interconnected porous structure of the composite PVDF/CA membrane allows for increased penetration and a greater water flux. These changes in the nanofiber structure are favorable for various applications, such as ultrafiltration [41,45], absorption [46], and ion-exchange [47].

Table 5. Nanofiber physical properties.

Membrane Type	Nanofiber Diameter (nm)	Pore Size (μ m)	Porosity (%)
Neat PVDF	530 $\pm$ 60	0.51 $\pm$ 3	83 $\pm$ 3
Composite PVDF/CA	860 $\pm$ 83	0.54 $\pm$ 2	91 $\pm$ 2

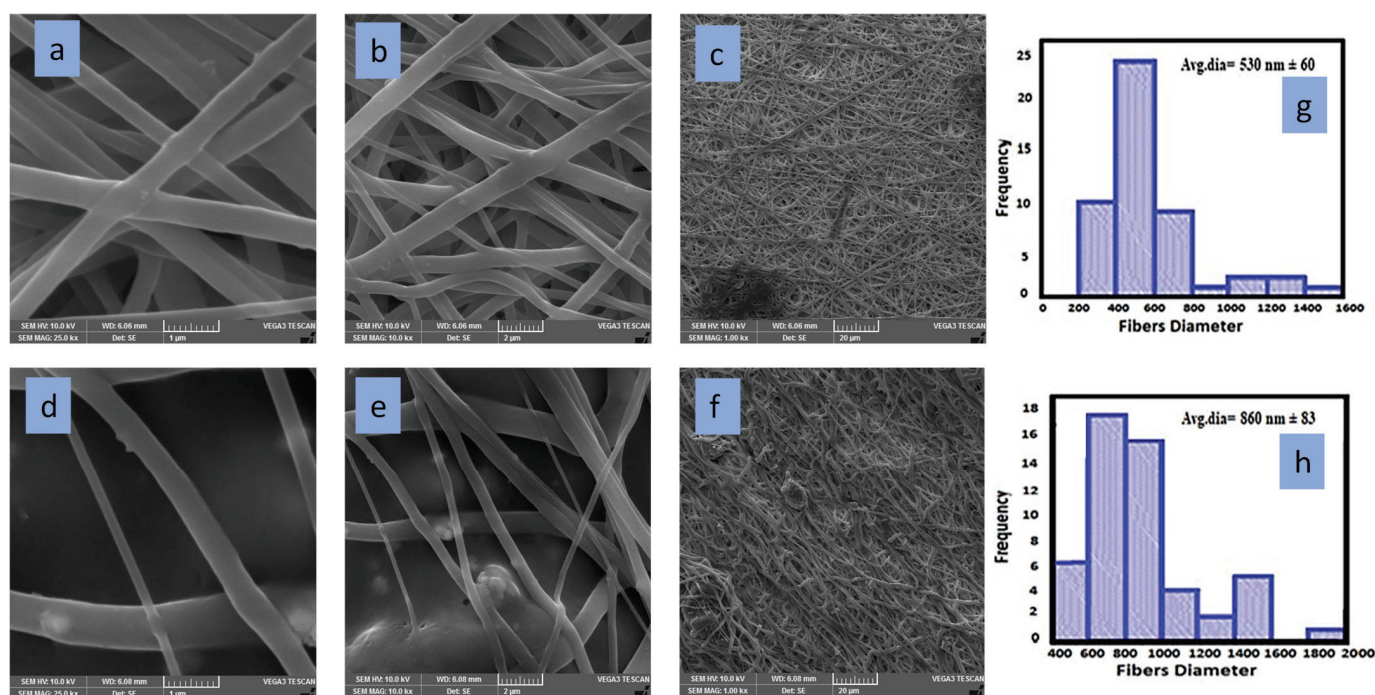


Figure 3. Scanning electron microscope (SEM) images of (a–c) neat PVDF membrane and (d–f) composite PVDF/CA membrane. (g,h) Histograms of nanofiber diameter for the neat PVDF and composite PVDF/CA membranes, respectively.

3.2. Energy Dispersive Spectroscopy (EDS)

Energy dispersive spectroscopy (EDS) results are displayed in Figure 4 and Table 6. The atomic percentage of oxygen in the composite PVDF/CA membrane increased from 4.3% to 10.4%, while the atomic percentage of fluorine decreased dramatically from 70.1% to 59.7%. These findings indicate that when cellulose acetate is mixed with PVDF membrane, it changes the structure of the membrane. The larger oxygen concentration will increase the membrane's hydrophilicity, which will be further strengthened by the lower water contact angle described in the section below.

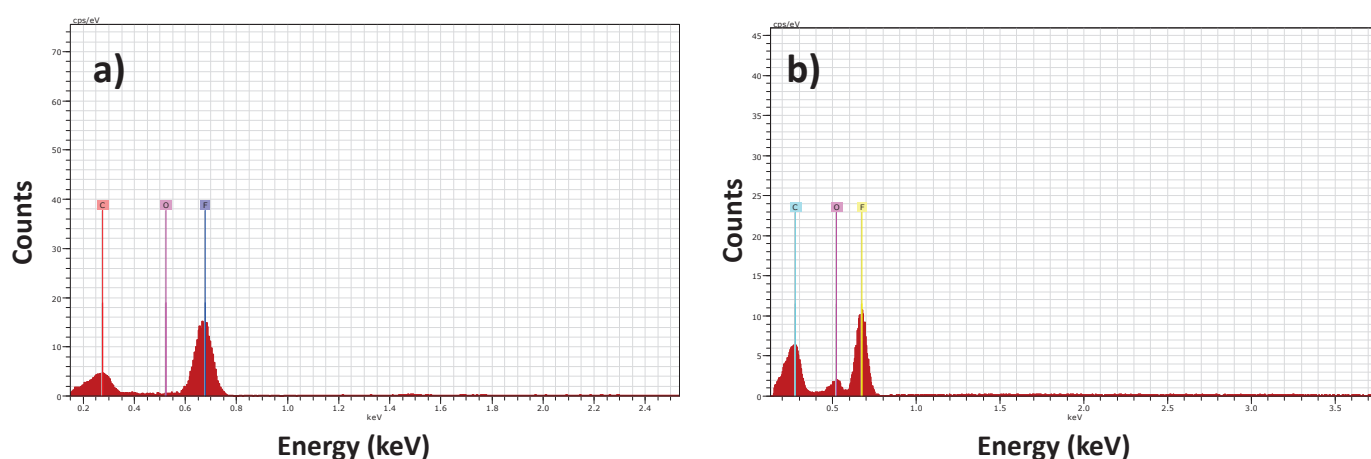


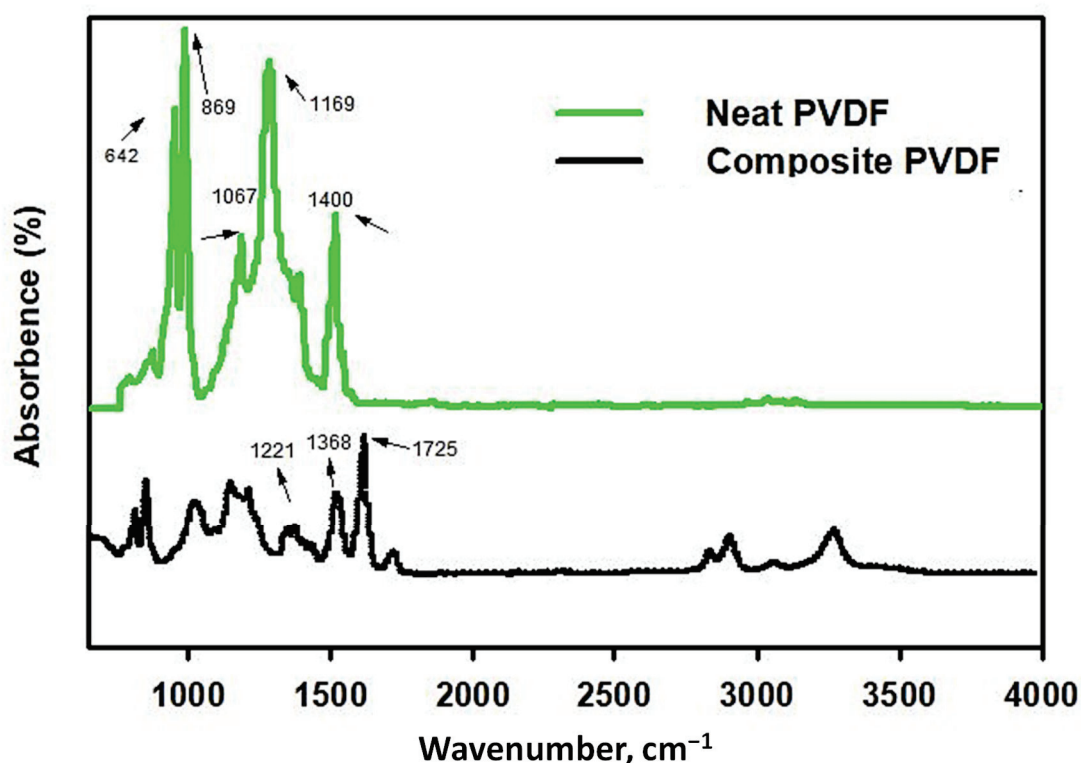
Figure 4. Energy dispersive spectroscopy (EDS) spectrum for the (a) composite PVDF/CA membrane and (b) neat PVDF membrane.

Table 6. Energy dispersive spectroscopy (EDS) elemental data for the composite PVDF/CA and neat PVDF nanofiber membranes.

Element	Composite PVDF/CA		Neat PVDF	
	Weight (%)	Atomic (%)	Weight (%)	Atomic (%)
Carbon	21.6	29.9	18.0	25.6
Oxygen	10.1	10.4	4.0	4.3
Fluorine	68.3	59.7	78.0	70.1

3.3. FTIR Spectra of the Nanofiber Membranes

The Fourier transform infrared radiation (FTIR) of the composite PVDF/CA and neat PVDF membranes is shown in Figure 5. The bands that appear at 642 cm^{-1} , to indicate asymmetric stretching, while the peaks found at 869 cm^{-1} and 1067 cm^{-1} are moieties out-of-plane and in-plane moieties of the membrane. At 1169 cm^{-1} and 1400 cm^{-1} , the two absorption bands observed for the PVDF nanofibers are generated by CF_2 groups [48–50]. The band at 1725 cm^{-1} in the composite membrane results from symmetrical stretching of the C=O carbonyl (likely in the acetyl functional group), while that at 1221 cm^{-1} is caused by the stretching of the ester bond and the peak at 1368 cm^{-1} results from C-H deformation. These phenomena develop from the presence of CA, which is readily incorporated into the PVDF matrix. The spectra shown in Figure 5 indicate that CA is successfully incorporated into the PVDF membrane.

**Figure 5.** Fourier transform infrared spectrum of the pristine and composite PVDF/CA nanofiber membranes.

3.4. Membrane Tensile Strength

The tensile strengths of the composite PVDF/CA and neat PVDF nanofibers were assessed with a universal tensile tester apparatus, using thicknesses ranging from 80 to 100 μm (Figure 6). The neat PVDF membrane's ultimate tensile strength was $2.0 \pm 0.1\text{ MPa}$

and its elongation break was 45%. The composite PVDF/CA nanofibers were examined at various cellulose acetate ratios, which demonstrated a decrease in Young's modulus when increasing the cellulose acetate concentration, an increase in elongation at break, and a reduction in tensile strength. A PVDF/CA ratio of 70:30, 80:20, and 90:10 yielded a tensile strength of 1.3 ± 0.1 MPa, 1.4 ± 0.1 MPa, and 1.7 ± 0.1 MPa, respectively. Correspondingly, the elongation break values of $85 \pm 1\%$, $77 \pm 1\%$, and $62 \pm 1\%$ and Young's modulus of 0.8 ± 0.1 , 1.4 ± 0.1 , and 2.0 ± 0.2 , were measured, respectively.

The neat PVDF nanofibers had smaller average diameters (530 ± 60 nm) and better crystalline and molecular orientations than the composite membrane's fibers, which yield stronger mechanical properties [44,49]. The higher tensile strength of the neat PVDF was a result of the lower nanofiber diameter, but the tensile strength of the composite membrane was lower due to an increase in nanofiber diameter. The general relationship between strength and diameter lends validity to this assumption, according to Equation (8):

$$\text{Stress (Strength)} = \text{Load (N)} / \text{Area} (\pi r^2) \quad (8)$$

Thus, neat PVDF nanofibers with a diameter of 570 nm exhibit higher tensile strength than those found for the PVDF/CA membrane.

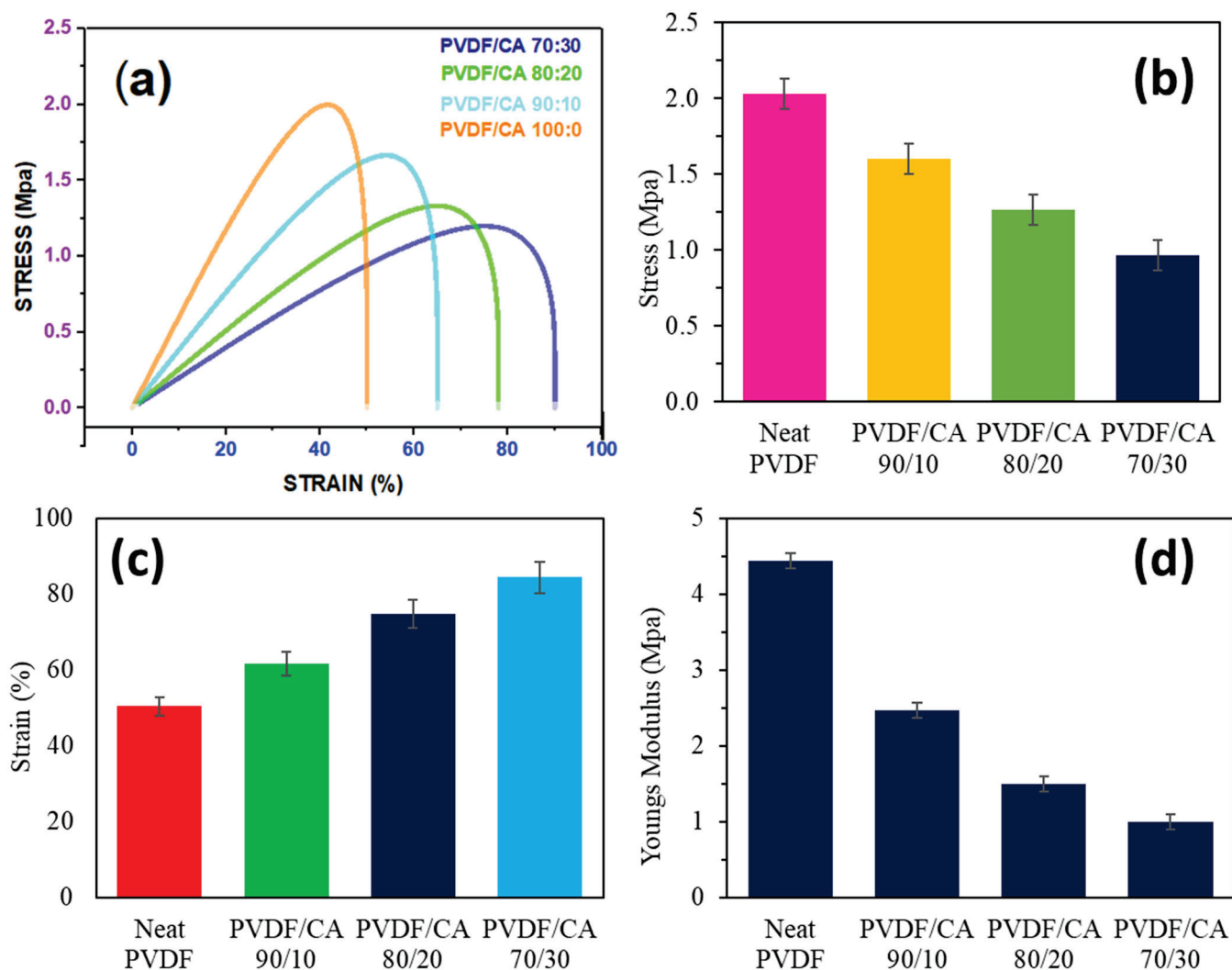


Figure 6. Tensile strength of neat and composite PVDF/CA nanofiber membranes with varying cellulose acetate concentration: (a) stress-strain performance, (b) stress, (c) strain, and (d) Young's modulus.

3.5. Membrane Hydrophobicity/Hydrophilicity

Membrane hydrophilicity and hydrophobicity can be inferred from contact angle measurements. The contact angles of the neat PVDF and composite PVDF/CA membranes were 115° and 101° , respectively (Figure 7). These results indicate that the composite PVDF-CA membrane exhibits greater hydrophilicity than the neat membrane. The water contact angle of the composite PVDF/CA membrane significantly decreased by the incorporation of CA into the PVDF matrix. It is well known that membrane hydrophilicity, wettability, and pore size can greatly affect the contact angle of the membrane [50]. When the water drops and encounters the composite PVDF/CA membrane, the water instantly spreads along the surface due to hydrogen bonds and electrostatic interactions. The presence of CA, having an inherent wettability, forms a micro-porous membrane surface when incorporated into the PVDF membrane that facilitates water drop penetration into the membrane matrix. The enhanced hydrophilicity will benefit the fouling resistance of the composite PVDF/CA membrane.

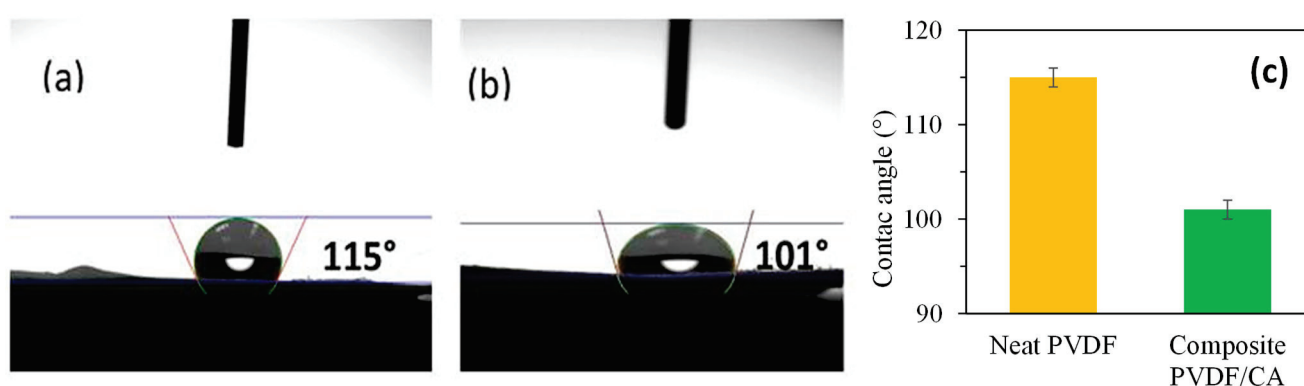


Figure 7. Images of water contact angles for the (a) neat PVDF nanofiber membrane, (b) PVDF/CA nanofiber membrane, and (c) error bar graph of water contact angles.

3.6. Water Flux and Filtration Resistance

The clean water flux and wastewater flux were assessed during the dead-end filtration. Initial water flux increased for the composite membrane due to the inherent properties of cellulose acetate. The clean water and wastewater fluxes for the composite membrane were $650 \text{ L/m}^2 \cdot \text{h}$ and $190 \text{ L/m}^2 \cdot \text{h}$, respectively, and $350 \text{ L/m}^2 \cdot \text{h}$ and $160 \text{ L/m}^2 \cdot \text{h}$, respectively, for the neat PVDF membrane (Figure 8). A higher membrane porosity, as measured, is expected to attract water molecules to the membrane matrix and subsequently make it easier for flow through the membrane (i.e., increasing permeability) [51]. The flux rates all declined as a function of time. Comparing flux rates for the clean water, the ultimate flux rate for the composite PVDF/CA membrane was substantially higher than for the neat PVDF membrane (note the differences in the scale of the Y-axis). While the flux rate of wastewater diminished faster for the composite membrane compared to the neat membrane, the composite material reached an equilibrium state more rapidly that was ultimately greater than that found for the neat material.

Corresponding to the higher flux rates, the PVDF/CA membrane exhibited less resistance compared to the neat PVDF membrane by an order of magnitude for both filtration of clean water and wastewater (Figure 9). A hydration layer is presumably formed on the membrane surface during the filtration process, which mitigates membrane fouling and decreases contaminant adsorption. When pore blocking took place earlier in the filtering process, the fouling resistance (R_t) increased [52,53]. The composite PVDF/CA hydrophilic nanofiber membrane resisted foulants when treated with wastewater. While the wastewater flux results are not definitive, the inherent hydrophilicity of the cellulose

acetate when blended with PVDF membrane improved the water flux and membrane antifouling performance.

When considering flux capacity reduction in relation to volume of permeate filtered, the composite PVDF/CA membrane outperformed the neat PVDF membrane (Figure 10). During filtration at a constant pressure of 10 psi, the flux declined for both membranes rapidly at first, but the composite membrane reached an equilibrium while the neat membrane continued to display a diminished flux. The PVDF/CA membrane continued to perform about four times longer than the neat PVDF membrane. This behavior is presumably due to higher hydrophilicity, higher porosity, and larger pore diameter in the PVDF/CA material. As the hydrophobic surface of a membrane increases, organic hydrophobic constituents become more readily attached to the membrane surface due to hydrophobic-hydrophobic interactions [49].

These findings indicate that the hydrophilicity of the composite membrane played a vital role in the filtration of hydrophobic constituents. The strong affinity of water to the CA material likely provides a layer of water molecules on the membrane, which prevents hydrophobic pollutants from attaching to the PVDF/CA material. The presence of hydrophilic atoms (i.e., O and N) in CA increases the wettability of the grafted and cross-linked cellulose acetate membrane, which improves its hydrophilic characteristics through the formation of hydrogen bonds [54]. The hydrophilicity, freedom of electrostatic charges, and motion of hydrated chains all contribute to repulsive interactions with substances such as humic acid [55]. Therefore, the PVDF-CA blend reduced hydrophobic constituent attachment on the membrane surface, which enhanced the antifouling performance of the composite membrane. These findings highlight the potential beneficial use of PVDF/CA composite nanofiber membranes during wastewater treatment.

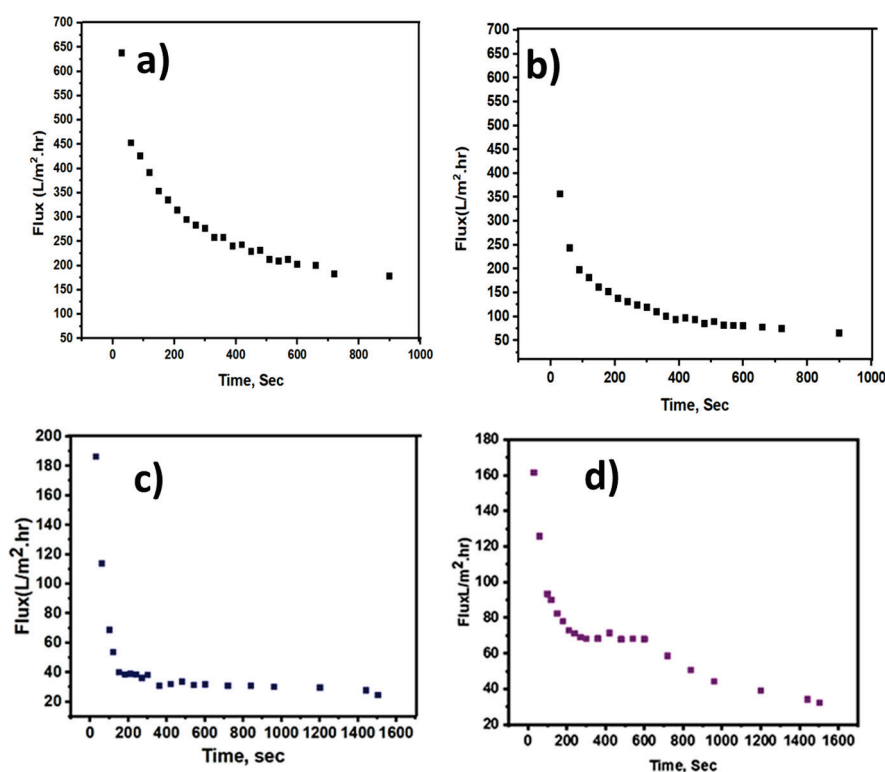


Figure 8. (a) Clean water flux across the composite PVDF/CA membrane. (b) Clean water flux across the neat PVDF membrane. (c) Wastewater flux across the composite PVDF/CA membrane. (d) Wastewater flux across the neat PVDF membrane.

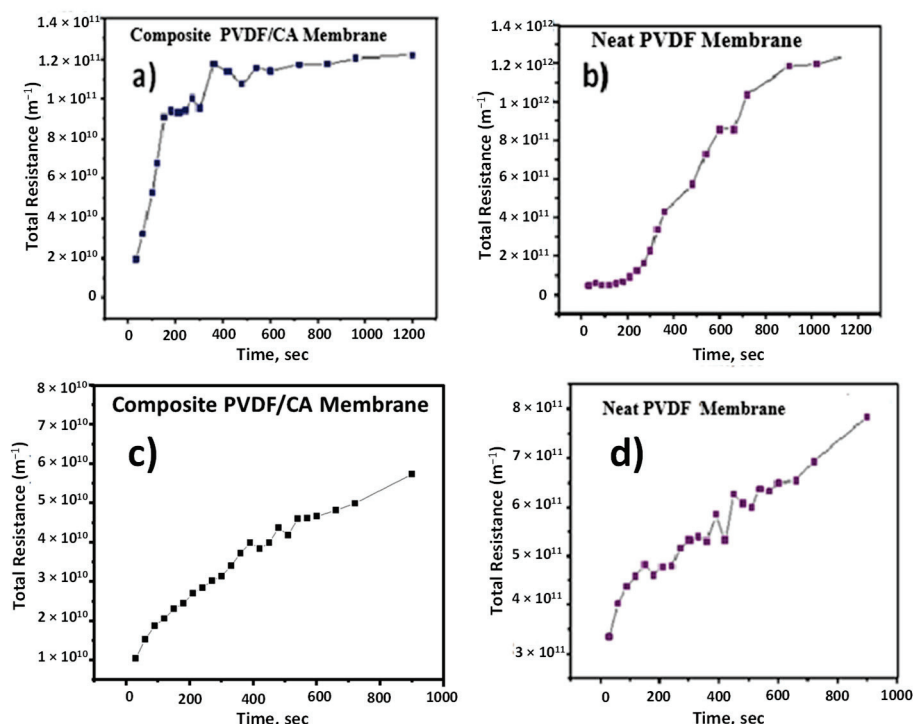


Figure 9. Resistance during the filtration process: (a) Clean water across the composite PVDF/CA membrane. (b) Clean water flows across the neat PVDF membrane. (c) Wastewater across the composite PVDF/CA membrane. (d) Wastewater flows across the neat PVDF membrane.

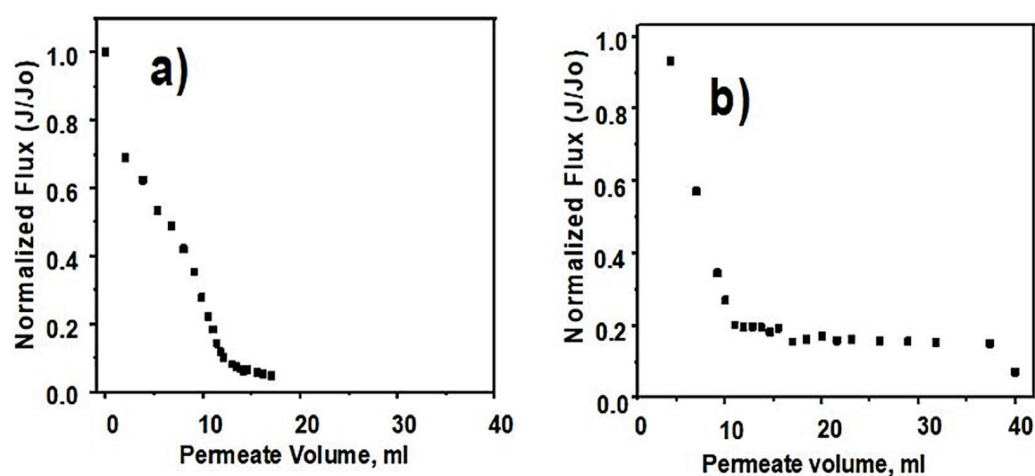


Figure 10. Flux reduction in (a) the neat PVDF nanofiber membrane and (b) the composite PVDF/CA nanofiber membrane.

3.7. Submerged Membrane in a Membrane Bioreactor

Evaluation of the two membrane types in a pilot-scale membrane bioreactor was conducted using the configuration shown in Figure 11. Initial tests found that increasing the membrane thickness to 100 μm provided greater durability to pump pressures and allowed for prolonged testing periods. It was necessary to wash the membranes periodically during wastewater treatment because biomass and particle accumulation clogged the membrane surfaces, resulting in reversible fouling that was easily removed [56]. This was achieved using a 0.1 percent hydrochloric acid and 0.5 percent sodium hypochlorite (NAOCL) solution. The initial mixed liquor volatile suspended solids (MLVSS) was $1014 \pm 26 \text{ mg/L}$ and $1700 \pm 49 \text{ mg/L}$ during the the reactor operation. Using forced aeration, the dissolved

oxygen in the aerobic zone of the reactor was controlled at 2.5 ± 0.2 mg/L, and the temperature of the reactor was maintained at 28 ± 1 °C.

The composite PVDF/CA membrane yielded slightly better wastewater treatment physicochemical parameters than the neat PVDF membrane (Table 7). Notably, chemical oxygen demand (COD) was 92.0 mg/L for the composite membrane (85% reduction), compared to 105.0 mg/L for the neat membrane. Turbidity was reduced by approximately 98% in the bioreactor with the composite membrane, and total suspended solids (TSS) removal approached 100%. The high acidity mixed liquor in the bioreactor resulted in a drop in pH for both nanofiber materials.

A digital transmembrane pressure gauge was installed in the reactor zone to monitor the antifouling performance of the membrane; an increase in transmembrane pressure is a good indicator of increased membrane fouling [57]. Figure 12 demonstrates a substantial difference between the two membrane types, with the composite PVDF/CA membrane exhibiting a lower transmembrane pressure compared to the neat/conventional PVDF membrane. These findings indicate less membrane fouling and better operational efficiency of the composite PVDF/CA membrane.



Figure 11. Photographs of the (a) membrane assembly, (b) bioreactor configuration, and (c) pilot-scale membrane bioreactor installation setup.

Table 7. Physicochemical parameters following wastewater treatment for the composite PVDF/CA and neat PVDF nanofiber membranes (average values $\pm$ standard deviation).

	Neat PVDF	Composite PVDF/CA
Chemical oxygen demand (mg/L)	105.0 ± 8	92.0 ± 5
Total suspended solids (mg/L)	0.00	0.00
pH	7.2 ± 0.1	6.8 ± 0.3
Turbidity (NTU)	1.3 ± 0.6	1.1 ± 0.2

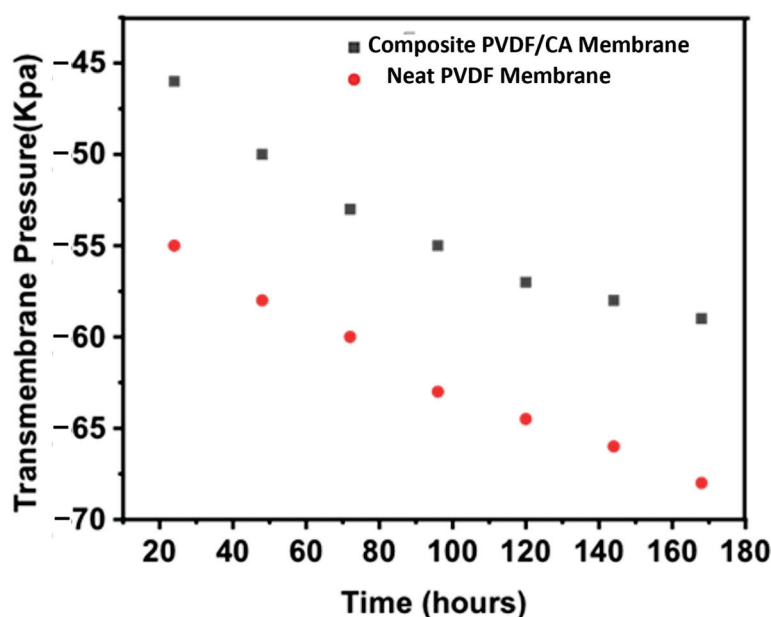


Figure 12. Transmembrane pressure (TMP) of composite PVDF/CA and neat PVDF membranes.

4. Conclusions

A novel PVDF/CA electrospun nanofiber membrane was successfully created to present a hydrophilic composite membrane that displays enhanced anti-fouling properties. The porosity of the composite PVDF/CA membrane, compared to the neat PVDF membrane, increased from 81% to 91.3%, and wastewater flux increased ($160 \text{ L/m}^2\cdot\text{h}$. vs. $190 \text{ L/m}^2\cdot\text{h}$). A diminished water contact angle was observed for the composite PVDF/CA membrane from 115° to 101° , indicating enhanced hydrophilicity. The composite membrane exhibited a reduced overall mass transfer resistance and a higher permeability. The transmembrane pressure of the neat PVDF membrane was $68 \pm 5 \text{ KPa}$ compared to $55 \pm 3 \text{ KPa}$ for the composite PVDF/CA nanofiber membrane, indicating fouling of the neat PVDF membrane, which limits durability and operational lifespan. This study suggests that the composite PVDF/CA has potential use for municipal and industrial wastewater treatment and the possibility of outperforming non-modified nanofiber membranes.

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Article

Fenton-Based Treatment of Meat and Bone Meal Wastewater: Influence of Variable $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ Ratios on Microbiological Abundance and Community Composition

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Abstract: Processing of meat waste into meat and bone meal generates wastewater that must be properly treated to minimize its environmental impact. In addition to its high organic load, it contains microorganisms, including pathogens, that pose a threat to human health. In this study, wastewater from meat and bone meal production was treated using the Fenton process with a variable $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ mass ratio ranging from 1:2 to 1:10, followed by neutralization with lime milk. A microbiological analysis was performed on both the raw wastewater and the liquid fractions obtained after treatment, along with additional analyses of selected physicochemical parameters. The identification of isolated microorganisms was carried out using the MALDI-TOF MS technique. Qualitative analysis of the treated wastewater samples showed the presence of microorganisms belonging to the genera *Staphylococcus*, *Enterococcus*, *Alcaligenes*, and *Pseudomonas*. *Staphylococcus aureus* and *Alcaligenes faecalis* were present in each of the treated samples. The Fenton process effectively reduced the total number of microorganisms, with the lowest counts of 1.3×10^2 CFU/mL and 3.2×10^2 CFU/mL found in wastewater samples treated with $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ mass ratios of 1:8 and 1:10, which correlated with higher doses of hydrogen peroxide.

Keywords: wastewater treatment; Fenton process; microbial contamination; turbidity; MALDI-TOF MS

1. Introduction

The growth of industrial production, an increasing global population, and rising consumer demands result in the generation of billions of tons of wastewater annually [1], which contains large amounts of organic and inorganic chemicals, heavy metals, microorganisms, and microplastics. Wastewater provides a favorable environment for the growth of both pathogenic and non-pathogenic microorganisms. Dangerous pathogens that pose a threat to human health include intestinal bacteria, viruses, protozoa, parasitic worms, and their eggs [2], which can enter wastewater from various sources. Livestock and poultry can be infected with zoonotic enteric pathogens that are discharged into wastewater during processing and represent a potential health risk [3], alongside solid industrial waste generated during animal processing [4]. The degree of contamination in industrial wastewater

can vary significantly depending on the nature of the production process and the raw materials used. Therefore, an individual approach tailored to each specific wastewater type is essential for effective treatment and, in some cases, even recovery of certain components.

The composition of wastewater from meat and bone meal production can be highly variable in terms of its physicochemical and microbiological properties and depends on the type and amount of processed meat waste. The primary raw material is animal carcasses or animal by-products rejected during meat inspection, as well as animals that died naturally or were euthanized. The most common method of processing animal by-products is their conversion into meat and bone meal (MBM) using the hydrothermal method [5]. In the European Union, about 20 million t/year of meat waste is generated, which is mainly processed into meat and bone meal [6], while in Poland, the amount is estimated at approximately 1 million t/year [7]. In the MBM production process, animal waste is crushed into particles of up to 50 mm in diameter and mixed to ensure homogenization and uniform heating. The mixture is then transferred into a jacketed tank, where it is stirred and preheated with steam to 101–104 °C. The next stage is sterilization, carried out in accordance with EU regulations, at a temperature of 133 °C for 20 min and under a pressure of 3 bar. The drying process follows, conducted at atmospheric pressure at temperatures ranging from 100 °C to 120 °C. The fat fraction is separated using a screw press and further purified via centrifugation. The defatted pomace is then cooled and ground into meat and bone meal [5,8,9]. The MBM production process generates wastewater with a high content of organic compounds, with chemical oxygen demand (COD) reaching up to 25 g/m³, and a high level of microbiological diversity, which poses a potential threat to the environment. In Poland, approximately 1 million m³/year of this type of wastewater is produced, which presents considerable challenges and requires a multi-stage purification and neutralization process. Chemical treatment methods, often in combination with biological approaches, are used to manage wastewater from MBM production. The effectiveness of wastewater treatment depends on the policy of the disposal plant, the technologies implemented, and the final management of the treated effluent [10,11].

To reduce the level of pollutants in wastewater from MBM production, it is necessary to combine multiple purification methods, whose synergistic action enables achieving the intended goal. In MBM production, the raw material is animal waste containing numerous microorganisms. Therefore, in accordance with regulations, these materials must undergo thermal treatment to eliminate microbial contamination. Despite the application of high temperatures in the technological process, reaching up to 133 °C, various biological pollutants persist in the wastewater, including pathogens responsible for numerous diseases. This type of wastewater contains a diverse bacterial microflora, including bacteria of the genera *Escherichia*, *Staphylococcus*, *Enterococcus*, *Pseudomonas*, *Corynebacterium*, *Ralstonia*, *Proteus*, *Alcaligenes*, *Providencia*, and *Morganella*. Due to the high microbial diversity in MBM wastewater, effective purification is essential to minimize risks to human health and the environment.

In recent years, advanced oxidation processes (AOPs) have gained significant importance in wastewater treatment. These processes are based on the generation of highly reactive oxygen species, such as hydroxyl radicals, which can destroy organic pollutants and pathogenic microorganisms. AOPs offer advantages over conventional oxidation methods: they do not generate disinfection by-products and present a viable alternative to chlorination [12–14]. AOPs refer to a group of processes that involve the generation of hydroxyl radicals, with ozone and H₂O₂ acting as oxidants, often in the presence of light, catalysts, ultrasound, and/or heat. Various configurations include Fenton, photo-Fenton, and peroxidation combined with ultraviolet light, etc. [15–17].

Advanced oxidation processes (AOPs), including the Fenton process, are widely used for the removal of recalcitrant organic pollutants from industrial and municipal wastewater. The Fenton process employs a reagent consisting of a mixture of hydrogen peroxide and ferrous iron. The oxidation mechanism is based on the generation of reactive hydroxyl radicals, which exhibit rapid and non-selective oxidation of various organic compounds, as well as some inorganic substances. Due to the strong oxidative properties of hydroxyl radicals, organic pollutants in wastewater are effectively degraded, often to the point of complete mineralization into carbon dioxide, water, and inorganic salts. Additionally, iron complexes formed during the reaction act as flocculants [18–20].

One of the advantages of the Fenton process is that it operates under ambient temperature and atmospheric pressure. However, process parameters such as temperature, pH, and the initial concentration of organic substances significantly affect the required amounts of hydrogen peroxide and iron, as well as the reaction time. The oxidation activity of hydroxyl radicals ($\bullet\text{OH}$) is strongly influenced by the pH of the solution. The Fenton process is typically carried out at a pH of around 3. The activity of the Fenton reagent decreases with increasing pH due to the precipitation of inactive iron oxo-hydroxides and iron hydroxide, which limit the availability of active Fe^{2+} . On the other hand, at very low pH values, the presence of iron complexes $[\text{Fe}(\text{H}_2\text{O})_6]^{2+}$ and the stable oxonium ion $[\text{H}_3\text{O}_2^+]$ reduce the reactivity between iron and hydrogen peroxide. Therefore, both excessively low and high pH values reduce the efficiency of the Fenton process [19,21–23]. Hydrogen peroxide concentration also plays a critical role in the process's effectiveness. Generally, as H_2O_2 concentration increases, the degradation of pollutants also increases. However, excessive dosages can lead to reduced efficiency due to scavenging of $\bullet\text{OH}$ radicals by excess H_2O_2 or their recombination into less reactive species [21,22,24]. The application of coagulation after the treatment process leads to the removal of iron hydroxy complexes, residual iron, as well as color [21,25,26].

The Fenton process is a promising alternative to conventional wastewater treatment methods, which, despite their widespread use, have certain limitations, especially when dealing with industrial effluents containing high concentrations of organic and microbiological pollutants. Traditional methods often struggle with persistent pollutants, lack flexibility in adapting to variable wastewater composition, and produce excess sludge that requires further treatment. In contrast, the Fenton process ensures high removal efficiency of pollutants and allows for flexible adjustment of operational parameters to the specific properties and variable composition of the wastewater.

The goal of this study was to treat wastewater generated during meat and bone meal production using the Fenton process, followed by neutralization, and to evaluate the impact of the applied methodology and treatment parameters, particularly the mass ratio of Fenton reagents ($\text{Fe}^{2+}/\text{H}_2\text{O}_2 = 1:2\text{--}1:10$) and their dosages, on the microbiological quality of the treated wastewater. The research focused mainly on the microbiological quality of the treated wastewater samples, as the Fenton process, compared to other treatment methods, is one of the most effective methods for eliminating microorganisms and reducing the risk to humans and the environment. A fixed dose of iron, approximately 2.0 g/L, was used in the treatment process, while the hydrogen peroxide dose varied within the range of 4–20 g/L of treated wastewater. This wide range of hydrogen peroxide dosages was necessitated by the high turbidity and significant microbiological contamination of the wastewater. Additionally, to provide a more comprehensive assessment of the quality of the treated liquid fractions, selected physicochemical parameters were measured, including chemical oxygen demand, color, turbidity, and pH.

2. Materials and Methods

2.1. Wastewater from Meat and Bone Meal Production

The analyzed wastewater was collected from an agricultural and industrial facility located in the Wielkopolska Province of Poland, engaged in the production of meat and bone meal (see Figure 1). The wastewater was characterized by an unpleasant odor, a strongly gray coloration, and high turbidity, resulting from the processing of large quantities of animal by-products such as horns, hooves, and feathers. The treated wastewater was subjected to microbiological analysis and selected physicochemical assessments, as described in Sections 2.4.1 and 2.4.2, respectively.



Figure 1. Wastewater from meat and bone meal production.

2.2. Reagents Used in the Wastewater Treatment Process

For the treatment of wastewater from MBM production using the Fenton process, a 30% solution of hydrogen peroxide (analytical grade), purchased from Avantor Performance Materials Poland S.A., Gliwice, Poland was used as an oxidant. The source of iron was iron(II) sulfate(VI) heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), ACS reagent, 99% purity, from Sigma-Aldrich, St. Louis, MO, USA. In the neutralization reaction of the treated wastewater suspension, a 10% solution of lime milk was used; calcium oxide (pure grade) from Chempur, Piekary Śląskie, Poland (was used for its preparation. To adjust the pH of the wastewater, a 4 molar solution of sulfuric acid(VI), prepared from 98% H_2SO_4 (analytical grade, Chempur), was applied. For the determination of chemical oxygen demand (COD) by the titrimetric dichromate method, analytical grade potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) and ammonium iron(II) sulfate ($(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$) were obtained from Sigma-Aldrich. Analytical grade sulfuric acid (H_2SO_4), silver sulfate (Ag_2SO_4), and mercury(II) sulfate (HgSO_4) were also sourced from Chempur. Analytical grade 1,10-phenanthroline monohydrate ($\text{C}_{12}\text{H}_8\text{N}_2 \cdot \text{H}_2\text{O}$), used in the preparation of ferroin as a titration indicator, was obtained from the former POCH S.A., Gliwice, Poland.

2.3. Media Used in Microbiological Analyses

In microbiological tests, microbiological media were used, purchased from BTL Sp. z o.o. Zakład Enzymów i Peptonów (Łódź, Poland). The TSA (tryptone soy agar) substrate was used to cultivate microorganisms; it is a universal substrate for culturing and multiplying microorganisms with increased nutritional requirements. The composition of the medium ensures optimal bacterial growth. This medium serves as a source of carbon, nitrogen, and minerals, and also contains casein and soy peptone, which support the growth of a wide range of microorganisms. MacConkey agar was also used, which is a selective medium that inhibits the growth of most Gram-positive bacteria due to the presence of bile

salts and crystal violet. In addition, MacConkey agar serves as a differential medium, allowing for the assessment of microorganisms' ability to utilize lactose. Selective Sabouraud agar with chloramphenicol was used for the cultivation and isolation of fungi [27].

To identify *Enterococcus* spp., the Slanetz–Bartley substrate was used. It is a selective medium for Gram-positive bacteria due to the presence of sodium azide, while the presence of 2,3,5-triphenyl tetrazolium chloride (TTC) inhibits the growth of most non-target bacteria. Enterococci reduce TTC to red formazan, resulting in colonies that range in color from pink to deep red [27].

For the isolation and differentiation of staphylococci, particularly *Staphylococcus aureus*, Baird-Parker agar was used. This is also a selective medium due to the presence of lithium chloride and potassium tellurite, which inhibit the growth of accompanying microflora. On Baird-Parker medium, typical *Staphylococcus aureus* colonies appear as black-gray to black, smooth, shiny, and convex, measuring 1.0–1.5 mm in diameter after 24 h of incubation, or 1.5–2.5 mm after 48 h. Due to their proteolytic and lipolytic activities, the colonies are typically surrounded by a clear halo [27].

For the detection and enumeration of *Escherichia coli*, TBX (Tryptone Bile X-glucuronide) selective medium was employed. The composition of this chromogenic medium includes the BCIG complex (5-bromo-4-chloro-3-indolyl- β -D-glucuronic acid), which undergoes enzymatic hydrolysis upon entering the bacterial cell. The chromogen accumulates within the cell, imparting a blue or blue-green coloration to *E. coli* colonies [27].

2.4. Wastewater Treatment Methodology

The treatment of wastewater from MBM production was carried out using the Fenton process, with variable mass ratios of $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ of 1:2, 1:4, 1:6, 1:8, and 1:10, respectively. After adjusting the pH to 3.0, 100 mL of wastewater was transferred into five beakers and placed on a magnetic stirrer. A constant, weighed amount (approximately 1.0 g) of iron(II) sulfate(VI) heptahydrate, the source of Fe^{2+} ions, was then added to each of the five wastewater samples, which were subsequently mixed for 15 min. Next, variable doses of H_2O_2 were added, with the amounts weighed to maintain the appropriate mass ratio of $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ in the range of 1:2–1:10. After adding hydrogen peroxide, the samples were stirred on the magnetic stirrer for 45 min. Then, a 10% solution of lime milk was introduced to adjust the pH of the wastewater suspension to a value between 8.0 and 8.5. The treatment process was conducted in duplicate. Table 1 shows the amounts of all reagents (the mean of two repetitions) used in the wastewater treatment process. In the final stage, the tested wastewater samples were filtered, resulting in the separation of liquid fractions (treated wastewater) and solid sediments. The subject of microbiological and physicochemical analyses was raw and treated wastewater.

Table 1. Amounts of reagents used and parameters of wastewater treatment from meat and bone meal production in the Fenton process (reagent consumption per 100 mL of treated wastewater).

Sample Number	Mass Ratio $\text{Fe}^{2+}/\text{H}_2\text{O}_2$	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (g)	Fe^{2+} (g)	30% H_2O_2 Solution (g)	10% Solution of Lime Milk (g)
1	1:2	1.0006	0.2010	0.4045	4.2744
2	1:4	1.0060	0.2021	0.8023	4.4020
3	1:6	1.0009	0.2011	1.2055	5.2840
4	1:8	1.0030	0.2015	1.6016	5.3420
5	1:10	1.0029	0.2015	2.0049	5.1420

2.4.1. Methodology of Microbiological Analysis of Raw Wastewater and Samples Obtained After the Purification Process

The microbiological analysis of raw wastewater and treated wastewater samples began with a tenfold dilution series in a sterile physiological saline solution. Surface inoculation was used in the studies, which consisted of taking a 0.1 mL sample with a pipette and transferring it to the surface of the microbiological media in a Petri dish, followed by careful spreading with a spreader. The inoculations were performed in duplicate on the following microbiological media: TSA, Baird-Parker, Slanetz–Bartley, TBX, MacConkey, and Sabouraud agar with chloramphenicol. Dilutions and inoculations were carried out in an Alpina laminar flow cabinet. The inoculated medium was incubated under aerobic conditions in a Memmert incubator at 30–35 °C for 72 h for bacterial identification and at 20–25 °C for 7 days for fungal identification. After incubation, the grown microorganisms were counted using the Scan 100 Interscience bacterial colony counter, and calculations were performed. In the case of raw wastewater analysis, the total number of microorganisms per 1 mL of the sample was determined, and the number of *Enterococcus* and *Staphylococcus aureus* bacteria was calculated on selective microbiological media. No growth of characteristic blue-green colonies was observed on the TBX agar, which would confirm the presence of *Escherichia coli* bacteria, and no fungal growth was obtained on Sabouraud agar with chloramphenicol. In quantitative microbiological analyses of treated wastewater samples, the total number of microorganisms per 1 mL of the treated wastewater sample was calculated. All different colonies isolated from wastewater and treated wastewater samples in the Fenton process were subjected to qualitative identification using the MALDI-TOF MS technique, i.e., matrix-assisted laser desorption/ionization mass spectrometry with a time-of-flight analyzer. The MALDI-TOF MS method involves transferring a small amount of the tested material (a colony from the culture) onto a plate for analysis. Then, a solution of an appropriate matrix is applied to the tested material. After drying, the sample is placed in the analyzer chamber, where, under the influence of the laser, the matrix molecules and bacterial proteins undergo desorption and ionization. The matrix strongly absorbs the energy of laser light, contributing to the desorption of the analyte molecules. The measurement result of these molecules is recorded in the form of a mass spectrum, which is automatically analyzed and compared with a database containing spectra characteristic of specific microbial species. The probability of correct identification of a microorganism in the MALDI Biotyper system is expressed in the form of a numerical index, which determines the degree of similarity to the reference spectra [28]. In qualitative analyses, microbial identification based on MALDI-TOF MS technology was used: the Bruker MALDI Biotyper, Billerica, MA, USA, with the MBT IVD LIBRARY mass spectral library (version J, 2022). Microbial identification with an index of ≥ 2.00 was considered, allowing for high certainty.

2.4.2. Methodology of Physicochemical Characteristic Determinations of Raw Wastewater and Samples Obtained After the Purification Process

Selected physicochemical parameters were determined in raw wastewater from the MBM production and in treated wastewater samples: chemical oxygen demand (COD), color, turbidity, and pH. The COD was determined using the dichromate method according to the PN-ISO 6060:2006 standard [29]. This method consists of oxidizing organic and some inorganic compounds found in wastewater using an oxidizing mixture in which potassium dichromate (VI) is the oxidant. The mineralization process was carried out at 148 °C for 2 h in a WSL M9 mineralizer. A digital burette, Titrette, was used for titration. The color of the raw wastewater and its treated wastewater samples was determined according to the PN-EN ISO 7887:2012 standard [30] based on the platinum–cobalt scale. The actual color was determined after removing turbidity from the tested samples by filtering them

through a filter with a pore diameter of 0.45 µm. Color measurements were performed at a wavelength of $\lambda = 436$ nm, using the calibration curve saved in the memory of the Nanocolor UV/VIS spectrophotometer from Macherey-Nagel, Düren, Germany. Turbidity was determined by the nephelometric method at a wavelength of $\lambda = 860$ nm [31]. A nephelometer built into the Nanocolor UV/VIS spectrophotometer from Macherey-Nagel was used for these determinations. pH measurements were performed potentiometrically using a glass electrode included in the Mettler Toledo Five Easy pH meter.

3. Results and Discussion

3.1. Characteristics of Wastewater from Meat and Bone Meal Production

Table 2 presents the results of the microbiological analysis of the raw wastewater, from MBM, which includes a qualitative analysis of isolated microorganisms conducted using the MALDI-TOF MS technique and a quantitative analysis of the total number of microorganisms per 1 mL of wastewater. Table 3 presents data on the number of specific types of bacteria isolated on the selective media used. Table 4 presents the results of the determination of selected physicochemical parameters of the wastewater.

Table 2. Results of the qualitative analysis of microorganisms isolated from the wastewater and identified using the MALDI-TOF MS technique, and the general quantitative analysis. Total number of microorganisms.

Isolated Microorganisms	Total Number of Microorganisms ¹ (CFU/mL) $\pm$ Standard Deviation, SD
<i>Alcaligenes faecalis</i>	$2.4 \times 10^{10} \pm 1.5 \times 10^8$
<i>Pseudomonas oryzae</i>	
<i>Ralstonia pickettii</i>	
<i>Ralstonia</i> sp.	
<i>Corynebacterium</i> sp.	
<i>Enterococcus faecalis</i>	
<i>Staphylococcus aureus</i>	
<i>Lysinibacillus xylanilyticus</i>	

Note(s): ¹ Microbiological media used for the determination of the total number of microorganisms: TSA, Baird-Parker, Slanetz–Bartley, TBX, MacConkey, Sabouraud with chloramphenicol.

Table 3. Quantitative analysis of bacterial species isolated on selective media from meat and bone meal production wastewater.

Name of Selective Substrate	Identified Type of Bacteria	Number of Bacteria in Wastewater (CFU/mL) $\pm$ Standard Deviation, SD
TBX	<i>Escherichia</i>	No growth
Baird-Parker	<i>Staphylococcus</i>	$2.8 \times 10^6 \pm 1.1 \times 10^5$
Slanetz–Bartley	<i>Enterococcus</i>	$4.3 \times 10^4 \pm 1.4 \times 10^3$

Table 4. Physicochemical characteristics of wastewater from meat and bone meal production.

Parameter	Unit	Values
COD	mg O ₂ /L	$4940 \pm 145.5^*$
Turbidity	NTU	$968 \pm 26.8^*$
Color	mg Pt/L	$1630 \pm 42.2^*$
pH	-	8.50–8.72

Note(s): * Values $\pm$ standard deviation, SD.

The qualitative microbiological analysis of the wastewater performed by the MALDI-TOF MS method (Table 2) showed the presence of Gram-negative bacteria belonging

to the genera *Alcaligenes*, *Pseudomonas*, and *Ralstonia*, and Gram-positive bacteria of the genera *Corynebacterium*, *Enterococcus*, *Staphylococcus*, and *Lysinibacillus*. The isolated Gram-negative bacteria were *Alcaligenes faecalis*, *Pseudomonas oryzihabitans*, *Ralstonia pickettii*, and *Ralstonia* sp., and the Gram-positive bacteria were *Corynebacterium* sp., *Enterococcus faecalis*, *Staphylococcus aureus*, and *Lysinibacillus xylanilyticus*. The total number of microorganisms in the raw wastewater was estimated at 2.4×10^{10} CFU/mL.

The bacterial species *Alcaligenes faecalis* belongs to the genus *Alcaligenes*, which was described by Castellani and Chalmer [32]. These bacteria are Gram-negative, strictly aerobic, motile, rod-shaped, or intermediate in shape between a coccus and a rod (coc-cobacilli), have catalase and oxidase activity, and are non-fermentative [33–36]. *Alcaligenes faecalis* commonly occurs in natural aquatic and terrestrial environments as well as in hospital environments, e.g., on respirators or hemodialysis systems [33,37]. It can cause opportunistic infections in humans, which are difficult to treat due to increased resistance to certain antibiotics. The most common sites of infection are the blood, urinary tract, skin and soft tissues, and the middle ear [38]. Literature data also report the positive effects of *Alcaligenes faecalis* bacterial strains and their use in active bioremediation for removing heavy metals, plastics, and xenobiotics [39].

The bacterial species *Pseudomonas oryzihabitans*, belonging to the genus *Pseudomonas*, was isolated from wastewater, which is one of the best-studied bacterial genera and the genus with the largest number of species among Gram-negative bacteria [40]. *Pseudomonas oryzihabitans* is an oxidase-negative, non-fermenting bacterium that does not form endospores and has a rod-like shape with dimensions of 0.8 by 2.0 μm , and rounded ends. The cells occur singly, rarely in pairs, and are motile using a polar monotrichous flagellum [41]. *Pseudomonas oryzihabitans* is a saprophytic microorganism that is considered a potential hospital pathogen responsible for infections in people with a weakened immune system or with catheters or prosthetic materials [42,43].

The genus *Ralstonia* was proposed in 1995, and the type species of the genus is *Ralstonia pickettii* [44]. *Ralstonia pickettii* (formerly *Pseudomonas pickettii* and *Burkholderia pickettii*) is a non-fermentative Gram-negative rod that occurs in soil, purified water, and sewage, and has human associations [45]. *Ralstonia pickettii* is the most common *Ralstonia* species in human medicine [46], and is an opportunistic pathogen in hospital infections, especially in immunocompromised patients [46–48].

The genus *Corynebacterium* has over 110 validated species [49] and is highly diverse, with bacteria described as Gram-positive, but the shape, oxygen requirement, and preferred environment are divergent among species [49–51]. Heterogeneity within the genus includes species that originate from the environment, as well as human, zoonotic, and strictly animal commensals or opportunistic pathogens [52]. *Corynebacterium* can cause opportunistic infections in both humans and immunocompromised domestic animals and can be multidrug resistant [52,53]. Non-pathogenic species have shown strong importance in human, veterinary, and biotechnological medicine [51].

Enterococcus faecalis has been found in wastewater as a Gram-positive commensal member of the gut microbiome of a wide range of organisms [54]. Enterococci are members of the gastrointestinal consortia of humans and other mammals, birds, and insects and are a major cause of multidrug-resistant hospital infections [55]. *Enterococcus faecalis* and *Enterococcus faecium* are primarily responsible for hospital infections [56,57], with *Enterococcus faecalis* having a greater inherent ability to cause infection regardless of resistance [58]. The habitats of enterococci include not only animal hosts but also plants, soil, water, and human-made products. In many of these habitats, antibiotic-resistant strains of enterococci have emerged, and strains with new resistance mechanisms are isolated with alarming regularity [55].

Staphylococcus aureus, a Gram-positive, coagulase-positive bacterium with a spherical shape, has been isolated from wastewater [59]. It is a commensal bacterium and a human pathogen, as it is estimated that about 30% of the human population is colonized by *Staphylococcus aureus* [60]. It causes a wide range of clinical infections, contributing to bacteremia, infective endocarditis, as well as osteoarticular, skin and soft tissue, pleural, and pulmonary infections, and device-related infections. Over the past two decades, an increasing number of healthcare-associated infections and an epidemic of skin and soft tissue infections caused by strains with specific virulence factors and resistance to β -lactam antibiotics have been observed [61].

The genus *Lysinibacillus* was proposed in 2007 by Ahmed et al. [62]. *Lysinibacillus* belongs to the family *Bacillaceae* and has been isolated from various environments, including soil [63], surface water [64], and plants [65]. These bacteria are motile, rod-shaped, and produce spherical or ellipsoidal endospores. Oxidase and catalase tests are positive, while indole and H_2S production, nitrate reduction, and β -galactosidase (ONPG) tests are negative [62]. *Lysinibacillus xylanilyticus* isolated from wastewater from the MBM production is a Gram-positive, aerobic bacterium that, according to literature data, was isolated from forest humus and can degrade xylan [66].

The above-presented short description of selective characteristics of isolated types of bacteria and their species was aimed at showing the diversity of microflora occurring in wastewater from MBM production and the need to use appropriate methods of treating this wastewater type, which will enable the effective reduction of not only physicochemical but also microbiological parameters. It should also be mentioned that the microbiological parameters of wastewater from the production of meat and bone meal are very variable both in terms of quality and quantity, which are determined by the type, quality, and amount of processed raw materials (animal waste) in the production of meat and bone meal, as well as the parameters of the MBM production process.

The composition of the substrates and the ability of specific bacterial types to decompose the components of the substrates or accumulate them result in *Staphylococci*, and in this case, *Staphylococcus aureus*, confirmed by MALDI-TOF MS on Baird-Parker agar, growing as black colonies with a light border. *Enterococcus* bacteria on Slanetz–Bartley agar form round, convex colonies with a beetroot color.

Counting bacteria with a characteristic appearance on selective substrates (Baird-Parker and Slanetz–Bartley agar) allowed for determining the total number of bacteria from the genera *Staphylococcus* or *Enterococcus* (Table 3). From the genus *Staphylococcus*, only *Staphylococcus aureus* bacteria were confirmed by the MALDI-TOF MS technique, which occurred in very large numbers, reaching 2.8×10^6 CFU/mL. The number of bacteria from the genus *Enterococcus* was 4.3×10^4 CFU/mL, and the identified species was *Enterococcus faecalis*. No *Escherichia coli* bacteria were isolated from the wastewater, and they did not grow on any of the microbiological media used, including TBX.

The goal of this study was to examine the biological contamination of raw wastewater and purified wastewater samples; additionally, the basic physicochemical characteristics were determined (Table 4). Physicochemical parameters of raw wastewater from the MBM production have been examined many times by the authors [10,11] and are very variable depending on the type and amount of processed raw material. The COD of the raw wastewater was relatively low and amounted to 4940 mg O_2 /L, and in this type of wastewater, COD values can reach even several thousand mg O_2 /L. The wastewater contained a large amount of suspended solids, which caused a high turbidity of 968 NTU (Nephelometric Turbidity Unit). The actual color of the wastewater after the removal of suspensions by the filter was at the level of 1630 mg Pt/L, and the visual appearance of the wastewater was gray. The pH of the wastewater was alkaline, ranging from 8.50 to 8.72.

3.2. Results of the Analyses of Treated Wastewater Samples Obtained from the Purification of Wastewater from MBM Production Using the Fenton Process

Figure 2 presents images of the appearance of wastewater samples purified using the Fenton process with a variable $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ mass ratio ranging from 1:2 to 1:10. Table 5 presents the results of microbiological analyses of the purified wastewater samples, while Table 6 presents the results of physicochemical analyses.

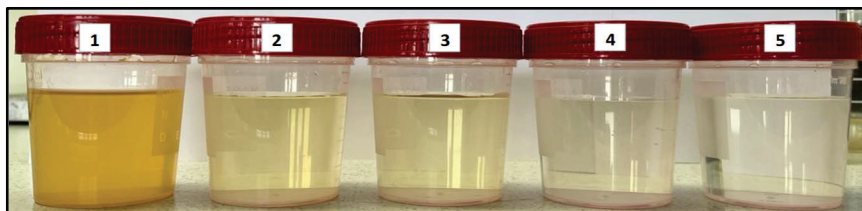


Figure 2. Images of wastewater samples from the production of meat and bone meal purified in the Fenton process (mass ratio of $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ in the purification process: 1—1:2, 2—1:4, 3—1:6, 4—1:8, 5—1:10).

Table 5. Results of qualitative and quantitative microbiological analysis of wastewater samples from meat and bone meal production purified in the Fenton process (qualitative analysis using MALDI-TOF MS, general quantitative analysis).

Sample Number	Mass Ratio $\text{Fe}^{2+}/\text{H}_2\text{O}_2$	Isolated Microorganisms	Total Number of Microorganisms (CFU/mL) $\pm$ Standard Deviation, SD
1	1:2	<i>Staphylococcus aureus</i> <i>Alcaligenes faecalis</i> <i>Pseudomonas oryzihabitans</i> <i>Enterococcus faecalis</i>	$3.3 \times 10^4 \pm 1.4 \times 10^3$
2	1:4	<i>Staphylococcus aureus</i> <i>Alcaligenes faecalis</i> <i>Pseudomonas oryzihabitans</i>	$6.4 \times 10^3 \pm 3.4 \times 10^2$
3	1:6	<i>Staphylococcus aureus</i> <i>Alcaligenes faecalis</i> <i>Pseudomonas oryzihabitans</i> <i>Enterococcus faecalis</i>	$1.6 \times 10^4 \pm 4.8 \times 10^2$
4	1:8	<i>Staphylococcus aureus</i> <i>Alcaligenes faecalis</i>	$1.3 \times 10^2 \pm 1.9 \times 10^1$
5	1:10	<i>Staphylococcus aureus</i> <i>Alcaligenes faecalis</i>	$3.2 \times 10^2 \pm 2.5 \times 10^1$

The results of the microbiological analysis presented in Table 5 show that the microbiological quality of the wastewater treatment samples changes as the doses of Fenton's reagent increase. In the wastewater treatment, a constant dose of Fe^{2+} (2 g/L of wastewater) and variable doses of hydrogen peroxide (ranging from 4 to 20 g/L) were used, resulting in the Fenton process being carried out at mass ratios of $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ of 1:2, 1:4, 1:6, 1:8, and 1:10, respectively. In the final stage of treatment, the wastewater slurry was neutralized with a 10% solution of lime milk, primarily to remove iron from the liquid fraction and to achieve the appropriate pH of the wastewater treatment samples after treatment and filtration.

Table 6. Physicochemical parameters of liquid samples after the purification of wastewater from meat and bone meal production using the Fenton process.

Parameter	Sample Number				
	1	2	3	4	5
Mass ratio $\text{Fe}^{2+}/\text{H}_2\text{O}_2$	1:2	1:4	1:6	1:8	1:10
COD ($\text{mg O}_2/\text{L}$)	$2407 \pm 135.4^*$	$1841 \pm 80.7^*$	$1626 \pm 93.4^*$	$1498 \pm 61.7^*$	$1091 \pm 46.8^*$
COD reduction degree (%)	51.28	62.73	67.09	69.68	77.91
Turbidity (NTU)	$106 \pm 10.4^*$	<1	<1	<1	<1
Turbidity reduction degree (%)	89.05	100	100	100	100
Color (mg Pt/L)	$2092 \pm 94.6^*$	$262 \pm 15.6^*$	$235 \pm 18.1^*$	$91 \pm 10.4^*$	$56 \pm 4.3^*$
Color change (%) (increase $\uparrow$ or decrease $\downarrow$)	$\uparrow 28\%$	$\downarrow 83.93$	$\downarrow 85.58$	$\downarrow 94.42$	$\downarrow 96.56$
pH	7.76–7.95	7.62–8.08	7.81–8.13	7.73–8.15	7.83–8.02

Note(s): * Values $\pm$ standard deviation, SD.

The applied wastewater treatment method and the selected process parameters significantly reduced the total number of microorganisms. Prior to the purification process, the degree of microbiological contamination in the wastewater was estimated at 2.4×10^{10} CFU/mL. After purification, the total number of microorganisms in the purified wastewater samples ranged from 3.3×10^4 to 1.3×10^2 CFU/mL (Table 5). The highest degree of microbiological contamination, 3.3×10^4 CFU/mL, was observed in the wastewater treated with the lowest dose of hydrogen peroxide (4 g/L) and a mass ratio of $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ of 1:2 (sample 1). Further purification of the wastewater using higher amounts of hydrogen peroxide (8 g/L and 12 g/L, with $\text{Fe}^{2+}:\text{H}_2\text{O}_2$ mass ratios of 1:4 and 1:6, respectively) resulted in a fivefold (sample 2) and twofold (sample 3) reduction in the number of microorganisms in the purified filtrates, compared to the purification with the lowest reagent mass ratio (1:2). Using the Fenton process with the highest $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ mass ratios of 1:8 and 1:10 led to the greatest reduction in microorganism numbers in the treated wastewater, with counts of 1.3×10^2 CFU/mL (sample 4) and 3.2×10^2 CFU/mL (sample 5), respectively.

Identification of microorganisms by the MALDI-TOF MS method revealed the types of bacteria or specific species present in the treated wastewater samples and indicated which of them showed the greatest resistance to the applied treatment method and its parameters. In the first three samples, obtained as a result of wastewater treatment with $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ mass ratios in the range of 1:2 to 1:6, nearly identical microbiological compositions were found, as *Staphylococcus aureus*, *Alcaligenes faecalis*, *Pseudomonas oryzihabitans*, and *Enterococcus faecalis* were isolated and identified on the media (Table 5). In sample 2, obtained with a $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ mass ratio of 1:4 (8 g H_2O_2 /L of wastewater), no fecal streptococci were detected, although they were present in sample 3, which had a higher hydrogen peroxide dose (12 g H_2O_2 /L of wastewater). This suggests that, with these purification parameters, fecal streptococci were still resistant to the prevailing environmental conditions. Increasing the amount of hydrogen peroxide, and consequently the $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ mass ratio, to 1:8 and 1:10 resulted in the isolation of only *Staphylococcus aureus* and *Alcaligenes faecalis* from the purified liquid samples. None of the purified liquid samples contained bacteria from the genera *Ralstonia*, *Corynebacterium*, or *Lysinibacillus*, which had been isolated from the wastewater before the purification process.

In summary, the MALDI-TOF MS analysis results clearly indicate that the most resistant microorganisms to the purification parameters were *Staphylococcus aureus* and *Alcaligenes faecalis*, both of which were present in each sample after wastewater purification. Throughout the entire purification process, highly variable conditions were applied, which

influenced the survival of microorganisms in the wastewater sample. The purified wastewater also exhibited high turbidity (968 NTU), caused by suspended solid particles, which may have diminished the effectiveness of the reagents in the Fenton process.

Particles present in wastewater can interfere with treatment processes and significantly increase treatment costs, thereby raising operational expenses (chemical demand, energy consumption) or infrastructure costs by requiring additional treatment steps to achieve the required levels of pathogen inactivation. Many microorganisms (viruses, bacteria, protozoa) bind to particles in wastewater, allowing them to survive disinfection processes and pose a health risk [67]. Particles contained in wastewater can either be physically associated with microorganisms in the form of clumps or be unassociated, but still protect the microorganisms from UV light or contribute to increased demand for disinfectants [68]. The binding of microorganisms to particles can occur through electrostatic attraction, hydrophobic interactions, or physical entrapment [67,69]. In wastewater treatment plant effluents after various treatment steps, turbidity can serve as a measure of treatment efficiency [70].

Hydrogen peroxide is used in wastewater treatment; however, according to the literature, extracellular hydrogen peroxide weakens cell walls, making bacteria more sensitive to oxidative stress, which may explain the inactivity. Despite the high disinfection efficiency of hydrogen peroxide, little is known about its biocidal mechanism. In hydrogen peroxide disinfection, the cytotoxic effect has not been attributed to the oxidative properties of the molecular form, but to the oxidative stress caused by hydroxyl radicals ($\text{HO}\bullet$), singlet oxygen (O_2^*), and superoxide species ($\bullet\text{O}_2^-$) derived from its natural decomposition. The decomposition products of H_2O_2 are active oxygen molecules, which are toxic to cells and can attack organic compounds, including lipids, membrane proteins, and nucleic acids of cells [71–73]. In the Fenton process, reactive oxygen species are generated by the decomposition of hydrogen peroxide mainly to hydroxyl and hydroperoxyl radicals, thanks to the catalyzed homolytic cleavage of the peroxide bond by dissolved metal ions (most often Fe^{2+}) in the homogeneous phase [72–74]. The disinfection efficiency in the Fenton process will depend on the reaction rate between the catalyzing metal ion and hydrogen peroxide and on the formation rate of reactive oxygen species. However, the efficiency of the Fenton process is influenced by many factors, such as the reaction medium, pH, the presence of suspended solids, and chemical and physical interactions between reagents and microorganisms; therefore, each wastewater treatment system should be approached individually.

Literature data show that structural differences between Gram-negative and Gram-positive bacteria may be the main reason for differences in sensitivity to the applied disinfection treatments. For example, *Escherichia coli* (Gram-negative) is more sensitive than *Enterococcus sp.* (Gram-positive), which has a thicker cell wall. However, other factors, such as the internal defense mechanisms of these bacteria, must also be considered [12]. Osmotic stress causes a greater weakening of the *E. coli* cell wall, increasing permeability, making its cells more sensitive than those of *Enterococcus sp.* [12,75]. In our study, *Staphylococcus aureus* (Gram-positive bacterium) and *Alcaligenes faecalis* (Gram-negative bacterium) were present in each sample of treated wastewater. Therefore, the structural differences between these bacteria did not affect their survival, but rather the internal defense mechanisms of these bacteria. *Staphylococcus aureus* can survive in many different environments, including stressful ones, and adapt to them because it has regulatory systems to cope with stress. *Staphylococcus aureus* can form biofilm in response to environmental stressors such as desiccation and oxidative damage [76]. *Alcaligenes faecalis*, commonly found in the natural environment, can survive there thanks to effective adaptation to the environment. Additionally, it has an excellent ability to decompose various pollutants,

from heavy metals to plastic and other hydrocarbons [77]. Some strains of *Alcaligenes faecalis* can cause diseases in humans, birds, and other vertebrates. Most of these strains are resistant to almost all existing antibiotics, and this resistance is conferred by antibiotic resistance genes (ARGs) [78]. Antibiotic resistance genes in *A. faecalis* strains are activated by a wide range of antibiotics currently used to treat infections, and strains living in the open environment are less resistant to antibiotics than clinical strains. The release of large amounts of antimicrobials into natural ecosystems can alter microbial population dynamics, including the selection for resistance, which may have uncertain consequences for human health [79].

The present study focused on comparing the effectiveness of microorganism elimination depending on the concentration of H_2O_2 in a system with a constant amount of Fe^{2+} ions. Although the observed increase in pathogen removal efficiency with higher H_2O_2 concentrations suggests the involvement of radical reactions, the direct effect of hydrogen peroxide on microorganisms cannot be definitively excluded. Therefore, future research will expand the experimental setup to include controls without the iron catalyst, which will allow for a more precise assessment of the contribution of individual oxidation mechanisms to the observed effect.

Table 6 presents the physicochemical parameters of the liquid samples obtained as a result of wastewater treatment using the Fenton process and neutralization with a 10% solution of lime milk. Analyzing the determined parameters, it can be clearly stated that increasing the mass ratio of $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ in the range from 1:2 to 1:10 resulted in a significant effect on the efficiency of the treatment process. The least effective wastewater treatment was achieved with the lowest $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ mass ratio of 1:2 (sample 1), while the most effective treatment was achieved with a 1:10 mass ratio (sample 5). Comparing the turbidity results, sample 1 of the treatment, with the lowest mass ratio of reagents, had a value of 106 NTU (turbidity reduction degree of about 89%). However, the remaining samples, 2–4, showed the value of this parameter below 1 NTU. This showed that using a $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ mass ratio in the range of 1:4 to 1:10 had an equal effect on the removal of pollutants causing turbidity in the wastewater. The color of the purified samples ranged from 2092 to 56 mg Pt/L, with an increased color value in sample 1 compared to the raw wastewater color after filtration. This was due to the addition of too small an amount of oxidant (H_2O_2) relative to the catalyst (Fe^{2+}), resulting in the yellow color of the filtrate caused by iron. In subsequent samples, i.e., 2–5, the mass ratio of the reagents was systematically increased by increasing the doses of hydrogen peroxide. As a result, the color systematically decreased, and in sample 5, purified at the highest mass ratio of the reagents, the highest color reduction degree was obtained, amounting to about 97%.

The content of organic compounds, determined by the COD parameter, systematically decreased with increasing doses of oxidant, varying within the range of 2407–1091 mg O_2/L . This corresponds to a reduction in COD values of approximately 51% to 78%. After the neutralization and filtration stages, the pH of the treated wastewater ranged from 7.62 to 8.15.

The Fenton process can be applied as a supplementary or supporting method for wastewater treatment prior to biological treatment, or as a primary treatment method, depending on the type and concentration of pollutants present in the wastewater, the required treatment efficiency, and the potential for further reuse. The COD values of wastewater generated from meat and bone meal production may range from 4000 mg O_2/L to as high as 25,000 mg O_2/L ; therefore, various treatment strategies must be developed. Wastewater from MBM production, after undergoing mechanical treatment followed by chemical processes (coagulation and flocculation), often supported by pressure flotation in a DAF (Dissolved Air Flotation) system [11], must be adjusted to contain a level of organic

pollutants compatible with the capacity of the on-site biological treatment facility (typically, COD values should not exceed 5000 mg O₂/L) before being discharged into it. However, achieving such levels is not always straightforward. As such, the Fenton process may serve as an effective solution, provided that process parameters are appropriately tailored to the degree of pollution. Additionally, monitoring of microbiological and physicochemical parameters after treatment (including residual hydrogen peroxide levels) is essential, as these influence the feasibility of subsequent treatment stages or the reuse of the treated wastewater within the facility.

A wide range of hydrogen peroxide concentrations (4–20 g/L) was used in this study; therefore, the concentration of residual hydrogen peroxide in the treated wastewater should be monitored, depending on its intended further application. This is particularly important when the next stage involves biological wastewater treatment. Neutralization with lime milk (calcium hydroxide suspension) may support the decomposition of hydrogen peroxide, primarily through pH elevation and potential catalytic effects, but it does not function as a typical neutralizing reagent for H₂O₂. Hydrogen peroxide decomposes spontaneously into water and oxygen, particularly under alkaline conditions (elevated pH), in the presence of trace metals (Fe, Cu, Ni, Mn, Cr) acting as catalysts [80,81], under UV radiation, and in the presence of certain enzymes (catalase) [80]. In the case of storing treated wastewater in tanks (prior to potential reuse for technical purposes within the plant), hydrogen peroxide will gradually decompose over time, with its half-life decreasing as the pH increases [82].

There are no publications in the available scientific literature regarding the study of the impact of the Fenton reagent on the microbiological quality of treated wastewater from meat and bone meal production. Oxidation processes can be applied as an alternative to conventional technologies for removing hazardous pollutants and pathogens from wastewater, as well as for increasing their reuse and reducing risks to human health and the environment. The inactivation of wastewater samples is a complex issue, which requires consideration of the composition of the effluent, including the number and presence of both organic and inorganic substances, as well as the microbial population. The matrix composition plays a crucial role in the effectiveness of removing different types of pollutants, which is why the efficiency of the treatment process, including disinfection, will vary for samples with different levels of contamination.

4. Conclusions

The application of the Fenton process using varying doses of hydrogen peroxide (4–20 g/L) and a constant concentration of ferrous ions (Fe²⁺, 2 g/L) significantly improved both the microbiological and physicochemical quality of wastewater generated during meat and bone meal production. An increase in the Fe²⁺/H₂O₂ mass ratio from 1:2 to 1:10 resulted in a gradual decrease in the total microbial count, which was reduced to a range of 3.3×10^4 – 1.3×10^2 CFU/mL. Despite the considerable reduction in microbial populations, two species—*Staphylococcus aureus* and *Alcaligenes faecalis*—were present in all samples, regardless of treatment intensity, indicating their resistance to oxidative stress and variable environmental conditions. Their persistence is most likely due to intracellular defense mechanisms rather than differences in cell wall structure.

The process was also effective in removing turbidity and color; turbidity values dropped below 1 NTU, and color removal reached up to 97% with higher oxidant doses, demonstrating efficient removal of suspended solids and residual iron. The concentration of organic compounds, expressed as COD, was reduced by up to 78%, confirming the effectiveness of the Fenton process in removing organic pollutants from a complex wastewater matrix. The presence of suspended particles in the wastewater significantly affected treatment efficiency by shielding microorganisms from oxidative agents and increasing

chemical demand, underscoring the importance of their effective removal for process optimization.

Future research should include systems without the iron catalyst to better assess the individual role of hydrogen peroxide and radical-based mechanisms in microbial deactivation. The obtained results highlight the need for further targeted studies, particularly concerning pathogen resistance and the integration of the Fenton process with existing treatment technologies.

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Review

The Effect and Adjustment of Ferrate Species in Ferrate-Based Advanced Oxidation Processes for Wastewater Treatment: A Systematic Review

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Abstract: Interest in the combination of ferrates and advanced oxidation processes (AOPs) for wastewater treatment has increased, as revealed in this systematic review. In this study, the multiple functions of Fe(VI) in ferrate-based AOPs are summarized based on the Fe species. Various enhanced oxidation pathways are achieved through electron capture by Fe(VI), oxidation by Fe(V) and Fe(IV), or the catalytic effects of Fe(III) and Fe(II). The different contributions of high-valent Fe species and general reactive oxidation species are highlighted by analyzing the results of quenching, methyl phenyl sulfoxide probing, and electron paramagnetic resonance analysis. Methods that are used to adjust the Fe species, including changing the reaction pH, oxidant dosage, dosing pattern, and the addition of reducing or complexing additives, can influence the enhancement efficiency of micropollutant treatment from the perspective of determining the transformation from Fe(VI) to Fe(V) and Fe(IV) with higher reactivity or Fe(III) and Fe(II) circulation. Future studies should focus on the in situ production of high-valent Fe and oxidation pathway-based adjustments in Fe(VI)-AOP techniques.

Keywords: ferrate; advanced oxidation processes (AOPs); Fe species adjustment; wastewater treatment

1. Introduction

The removal of emerging contaminants such as pharmaceuticals, personal care products, and endocrine-disrupting chemicals from wastewater has generated great interest in the past few years. Advanced oxidation processes (AOPs) have been reported to effectively remove micropollutants by producing free radicals such as sulfate radicals and hydroxyl radicals with high redox potentials. Non-radical oxygen species such as singlet oxygen (¹O₂) also contribute under certain conditions [1]. AOP techniques using O₃, H₂O₂, peroxy-monosulfate (PMS and HSO₅[−]), and peroxydisulfate (PDS, S₂O₈^{2−}) as precursors and light, carbon-derived materials, and metal ions as activators have been reported [2–4]. However, traditional AOPs depend significantly on the catalytic process [5] and are limited to a narrow pH range [6]. Moreover, concerns have arisen about their non-selective oxidation,

which is attributed to the generation of disinfection byproducts [7] and slow reactions with micropollutants in water with a strong background of natural organic matter [8].

High-valent Fe species (Fe(VI), Fe(V), and Fe(IV)) have drawn wide attention because they have a high oxidation capacity and low risk [9]. Fe(VI) shows great potential for pollutant removal via oxidation [10], coagulation, and disinfection [11], with the resulting Fe(III)/Fe(II) species exhibiting catalytic effects [3]. However, its practical application is limited by its rapid decomposition at a neutral pH and its low reactivity under basic conditions [12]. Different ferrate techniques have been assessed for the treatment of emerging organic pollutants [13] and to clarify the mechanisms and kinetics of pollutant removal [14,15]. The contribution of Fe(VI) to total organic carbon (TOC) removal and the inhibition of disinfection byproducts during water and wastewater treatment has been highlighted [16]. Recent review articles have elucidated Fe(VI) activation methods, focusing on the production of Fe(V) and Fe(IV) by metal ions [17] and inorganic reductants [18]. Iron-based materials are commonly used to activate AOPs owing to their high catalytic performance and environmental compatibility. Consequently, Fe(VI) decomposition products, including Fe(III) and Fe(II) ions, as well as FeOOH, Fe₂O₃, and Fe₃O₄, have been reported to be effective activators of AOPs precursor oxidants [19] and as feedstock for Fe(IV) generation [20]. These Fe species serve as potential connections between Fe(VI) and AOPs, through which the disadvantages of the two individual oxidation techniques can be mitigated [21]. In 2010, Sharma innovatively summarized the Fe(VI)-enhanced photocatalytic oxidation reaction [22], and subsequently highlighted the oxidation contributions of Fe(V) and Fe(IV) to these systems [18]. Further investigations into the oxidation mechanisms of ferrate-AOPs have emerged, focusing on various aspects such as oxidation kinetics, contaminant removal efficacy [23,24], and optimized ferrate-AOP techniques [25,26]. Recently, attention has been drawn to the formation of multiple reactive species in ferrate-AOPs, including high-valence Fe species and radicals [27,28]. However, comprehensive reviews on the reaction mechanisms of different Fe species, their transformation processes, and their adjustment measures are currently lacking.

This review aims to (1) demonstrate the different roles of Fe species under different ferrate-AOP conditions and describe the corresponding mechanisms by which the processes are enhanced and (2) discuss methods to optimize the ferrate-AOP by adjusting the Fe species in the system.

2. Key Fe Species in Ferrate-Based AOP Systems

The self-decay of Fe(VI) is accompanied by the removal of organic pollutants and the production of catalysts, such as Fe(III) and Fe(II), which provide a bridge connecting AOPs to Fe(VI) oxidation. A comprehensive understanding of the behavior of Fe intermediates in ferrate-AOPs will guide the development of improved pollutant removal systems and facilitate the application of this combined system to complex practical wastewater treatment.

Various transient reactive species, including Fe(V), Fe(IV), and free radicals, are generated in the oxidation reaction system. Fe(VI) self-decomposes in pure water to Fe(V) and Fe(IV). The synergistic effects of reducing agents with Fe(VI) could enhance the formation of Fe(IV) and Fe(V). Sulfur-containing compounds could activate Fe(VI) to generate reactive Fe(V) and Fe(IV) intermediates, thereby augmenting the oxidative capacity of the Fe(VI) system via the in situ formation of high-valent iron species. Acting as redox mediators, the phenolic hydroxyl groups can promote the production of Fe(IV) and Fe(V) through the electron-shuttle mechanism with Fe(VI). Recent studies have shown that oxidants (e.g., PMS, H₂O₂, and periodic acid) combined with Fe(VI) can induce the formation of highly reactive Fe(IV) and Fe(V) intermediates [27]. Scavenging experiments were performed to verify whether the active species play a role in the degradation of contam-

inants [29]. Methyl phenyl sulfoxide (PMSO) has been widely employed to confirm the formation of high-valent Fe because it can react with Fe(V)/Fe(IV) to form methyl phenyl sulfone (PMSO₂) as a diagnostic product [30]. PMSO was utilized to identify the roles of Fe(V)/Fe(IV) species in the removal of sulfamethoxazole (SMX) [31] and polycyclic aromatic hydrocarbons [32] in the Fe(VI)-PMS system. The selective conversion of PMSO into PMSO₂ demonstrates the formation of high-valent Fe species (Fe(V)/Fe(IV)) [33]. To quench the reactive species, ethylene diamine tetraacetic acid was used as a photogenerated hole quench agent, and tert-butanol (TBA) was added to eliminate •OH. Sodium azide is a widely used scavenger of ¹O₂, while p-benzoquinone was used to detect O₂•⁻ [33]. Benzoic acid and methanol (MeOH) were utilized to quench SO₄•⁻ and •OH and establish their effects on the removal of SMX in the Fe(VI)/PMS process [31,34]. In the quenching experiment, the results indicated that the photogenerated electrons and •OH radicals play important roles in the degradation of dimethyl phthalate (DMP) [35]. In the Fe(VI) and Fe(VI)/percarbonate systems, PMSO, ethanol (EtOH), and benzoic acid were employed as scavengers to determine the changes in the reactive species after the addition of percarbonate [34]. Radical quenching experiments with EtOH and TBA were conducted to distinguish the contribution of •OH and SO₄•⁻ to the removal of bisphenol A (BPA) in the reaction system [36].

2.1. Photocatalysis

Fe(VI) has a relatively high redox potential, and its reduction products are nontoxic Fe oxides [37]. Fe(VI) can enhance photoreaction processes significantly (Figure 1, Table 1), which is due to the high reactivity between Fe(VI) and the conduction band electrons e⁻ ($k = 2.0 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$) in photocatalytic oxidation systems [38]. This prevents the recombination of e⁻-h⁺ pairs, which improves the quantum efficiency of the UV-TiO₂-Fe(VI) system [35]. The hydrolysis of Fe(VI) can be essentially activated by ultraviolet light, which promotes the formation of Fe(IV) and H₂O₂ and particularly enhances the generation rate of O₂•⁻ and •OH [39]. DMP was barely degraded by Fe(VI), whereas UV-TiO₂-Fe(VI) oxidation showed a 3–11 times higher removal efficiency than the oxidation of Fe(VI) alone. The participation of Fe(VI) in the photoreaction also led to an increase of approximately 83.8% in the degradation rate compared to that in the UV-TiO₂ process alone [35]. The degradation of BPA was similarly enhanced in the order of UV-TiO₂ < Fe(VI) < UV-TiO₂-Fe(VI) based on the scavenging of e⁻ by Fe(VI) [22]. The Fe(VI)-TiO₂-UV system showed high DMP degradation under UV irradiation, because the electron scavenging by Fe(VI) greatly reduced the recombination of the e⁻ and h⁺ [40]. The Fe(VI) concentration decreased rapidly in the presence of a TiO₂ suspension under UV light [41]. The influence of light wavelength on the SMX degradation efficiency in the UVA-Fe(VI)-PMS process was also investigated [42]. Compared to the reaction in the dark, SMX removal increased with the introduction of light at all the examined wavelengths. Therefore, Fe(VI) may affect the photocatalytic oxidation reaction by absorbing light.

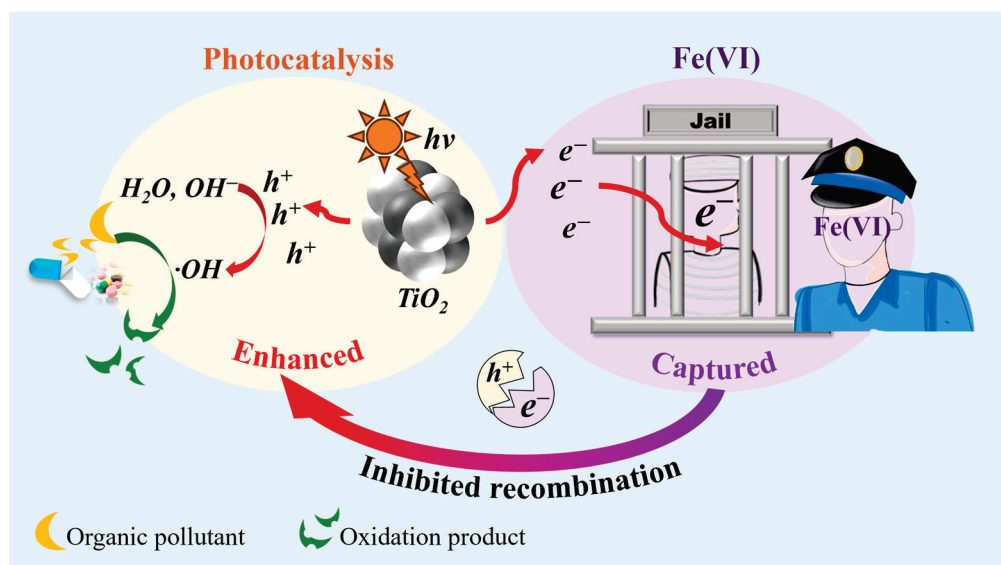


Figure 1. Enhanced mechanism of Fe(VI) in photocatalysis.

Table 1. Removal of organic pollutants in ferrate-AOP systems with the main species of Fe(VI), Fe(V), and Fe(IV).

Experimental System	Experimental Condition	Active Specie	Adjustments	Pollutant	Enhancement Effect	Reference
UV-TiO ₂ -Fe(VI)	[Fe(VI)]: 50.0 µM; [TiO ₂]: 0.5 g/L; light intensity: 0.15 mW/cm ² at 254 nm; pH 9.0	h ⁺ and OH	pH	Sulfadiazine/ Sulfamerazine/ Sulfamethoxazole: 20.0 µM	UV-TiO ₂ - Fe(VI): 89.2%, 83.4%, 82.0%; UV-TiO ₂ : 71.3%, 72.7%, 76.0%; Fe(VI): 65.2%, 66.0%, 71.9%	[43]
Fe(VI)-UV-TiO ₂	[Fe(VI)]: 80.0 µM; [TiO ₂]: 2.0 g/L; light intensity: 4.0 mW/cm ² at 340–420 nm; pH 6.0	Fe(V) and Fe(IV)	pH	Microcystin-LR: 1.0–2.0 mg/L	Fe(VI)-UV- TiO ₂ : 100.0%; UV-TiO ₂ : 63.0%; Fe(VI): 54.0%	[44]
Fe(VI)-UVA- light-emitting diode (LED)	[Fe(VI)]: 75.0 µM; LED bulb: 3.0 W; UVA: 37.0 mW/cm ² ; pH 8.0	Fe(V) and Fe(IV)	pH	Sulfamethoxazole: 5.0 µM	Fe(VI)-UVA- LED: Reacting 2.6–7.2-fold faster than for Fe(VI)	[45]
UV-TiO ₂ -Fe(VI)	[Fe(VI)]: 30.0 mg/L; [TiO ₂]: 0.05 g/L mg/L; light intensity: 9 W at 365 nm; pH 9.0	h ⁺ and •OH	pH and oxidant dosage	Dimethyl phthalate: 0.3 mg/L	UV-TiO ₂ - Fe(VI): 95.2%; UV-TiO ₂ : 51.8%; Fe(VI): 23.5%	[35]
Fe(VI)-UV-TiO ₂	[Fe(VI)]: 9.0 mg/L; [TiO ₂]: 40.0 mg/L; light intensity: 0.4 mW/cm ² at 365 nm; pH 9.0	Holes, OH, and Fe(V)	Oxidant dosage	Dimethyl phthalate: 11.6 mg/L	Fe(VI)-UV- TiO ₂ : 83.0%; UV-TiO ₂ : 68.0%; Fe(VI): negligible	[41]

Table 1. Cont.

Experimental System	Experimental Condition	Active Specie	Adjustments	Pollutant	Enhancement Effect	Reference
Fe(VI)-Visible light-g-C ₃ N ₄	[Fe(VI)]: 100.0 µM; [g-C ₃ N ₄]: 50.0 mg/L; visible light; pH 9.0	Fe(V) and Fe(IV)	Oxidant dosage	Carbamazepine: 5.0 µM	Fe(VI)-Visible light-g-C ₃ N ₄ : 100.0%; Visible light-g-C ₃ N ₄ : 41.0%; Fe(VI)-Visible light: 38.0%	[33]
Fe(VI)-CaO ₂	[Fe(VI)]: 50.0 µM; [CaO ₂]: 50.0 µM; pH 8.0	Fe(V) and Fe(IV)	Oxidant dosage	Sulfamethoxazole: 5.0 µM	Fe(VI)-CaO ₂ : 82.7%; CaO ₂ : negligible; Fe(VI): 35.4%	[46]
Fe(VI)-H ₂ O ₂	[Fe(VI)]: 50.0 µM; [H ₂ O ₂]: 0–30.0 µM; pH 8.0	Fe(V) and Fe(IV)	Oxidant dosage	Caffeine: 5.0 mM	k _{app} between caffeine and Fe(VI): 12.8 to 171.8 M ^{−1} s ^{−1} with H ₂ O ₂ from 0 to 30.0 µM	[47]
Fe(VI)-H ₂ O ₂	[Fe(VI)]: 50.0 µM; [H ₂ O ₂]: 70.0 µM; pH 7.0–9.0	Fe(V) and Fe(IV)	Oxidant dosage	Sulfamethoxazole: 5.0 µM	Fe(VI)-H ₂ O ₂ : 78.0%; H ₂ O ₂ : negligible; Fe(VI): 35.0%	[48]
Fe(VI)-H ₂ O ₂	[Fe(VI)]: 250.0 µM; [H ₂ O ₂]: 2.5 mM; pH 8.0	Fe(V) and Fe(IV)	Oxidant dosage	Bisphenol A: 50.0 µg/L	Fe(VI)-H ₂ O ₂ : 97.7%; H ₂ O ₂ : 23.5%; Fe(VI): 85.5%	[49]
Fe(VI)-UV	[Fe(VI)]: 0.2 mM; light intensity: 26.0 µW/cm ² at 254 nm; pH 7.0	O ₂ • [−] , •OH, Fe(V) and Fe(IV)	Dosing pattern	Lake water	Fe(VI)-UV: UV ₂₅₄ removal increased; TOC removal increased than Fe(VI)	[40]

Fe(V) and Fe(IV) are high-valent Fe species with selective oxidation behaviors that differ from those of free radicals [50]. Fe(V) and Fe(IV) can be produced from Fe(VI) through one- and two-electron transfer, where electrons can be provided by pollutants, H₂O, H₂O₂, and photocatalysis-generated e[−] [27,33]. The observed first-order rate constants of Fe(V) and Fe(IV) with pollutants are approximately 10³ and 10⁵ times that of Fe(VI), respectively [51]. Therefore, the generation of Fe(V) and Fe(IV) usually contributes to a greater removal efficiency of organic pollutants. These ferrate-AOP systems, based on the generation of Fe(V) and Fe(IV), generally show advantages such as low toxicity, low generation of disinfection by-products, and high interference resistance to natural organic matter. In certain Fe(VI) photocatalytic systems, Fe(V) and Fe(IV) produced by the combination of Fe(VI) with photocatalyzed electrons dominate the oxidation of organic pollutants (Figure 2). Scavenging tests and electron paramagnetic resonance analysis were conducted by adding quenching agents of TBA and MeOH and trapping agents of 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). The results revealed a small contribution of free radicals, and Fe(V) and Fe(IV) were determined to play a primary role in the degradation of pollutants [45].

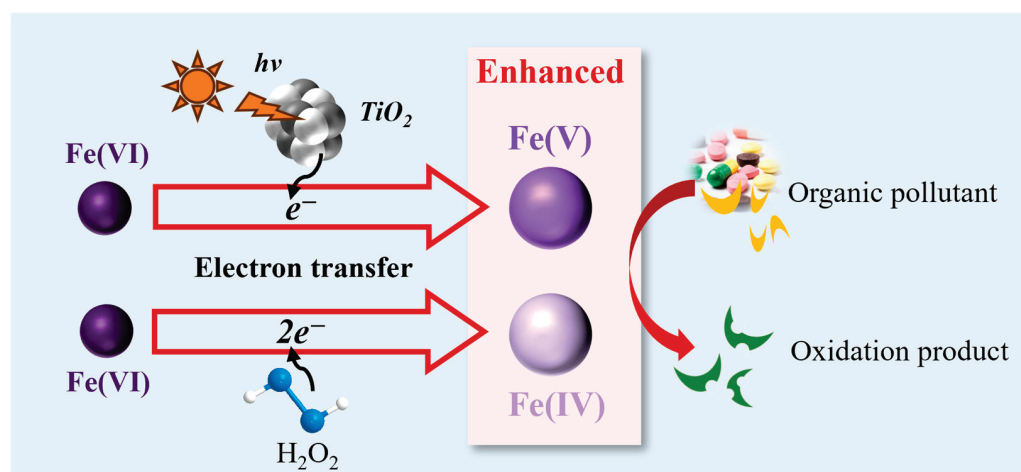


Figure 2. Enhanced mechanism based on Fe(V) and Fe(IV) oxidation.

Fe(V) attacked the alkyl chain of DMP instead of the aromatic rings, following a different degradation path than the $\bullet\text{OH}$ oxidation pathway [41]. Probing the reaction with PMSO revealed that Fe(V) and Fe(IV), which were produced in the oxygen-doped ZnIn₂S₄ nanosheet-visible light-Fe(VI) combined system, were responsible for the high removal of trimethoprim [52]. MeOH and TBA had negligible effects on SMX removal, indicating that $\bullet\text{OH}$ radicals contributed little to SMX oxidation [45]. There was no DMPO-HO $\bullet$ adduct signal observed, suggesting that $\bullet\text{OH}$ played a negligible role in the Fe(VI)-UVA-LED process [45]. PMSO can be oxidized to phenyl methyl sulfone (PMSO₂) specifically by high-valence Fe species [31]. The ratio of produced PMSO₂/reduced PMSO was close to 100% in the photocatalytic Fe(VI)-visible light-g-C₃N₄ systems, indicating that Fe(V) and Fe(IV) were the dominant reactive oxidation species in the degradation of micropollutants [33]. The $\bullet\text{OH}$ generation pathway from ferrate self-decay is inhibited by the remarkable oxidation between Fe(VI) and organic pollutants. The Fe(V) and Fe(IV) generated from Fe(VI) facilitate several orders of reactivity with organic pollutants, reducing the self-decay of Fe(V) and Fe(IV) in water [33].

2.2. Fenton-like Reaction

Electron transfer efficiency in Fe(VI)-H₂O₂ systems is strongly dependent on pH. Under alkaline conditions, H₂O₂ promotes the decomposition of Fe(VI), resulting in highly oxidizing Fe(IV) [53]. Under acidic conditions, Fe(II) and H₂O₂ react to form a Fenton system through a series of chain reactions. The $\bullet\text{OH}$ produced is also actively involved in the oxidation of pollutants [54]. Fe(VI) can be reduced to Fe(IV) by a two-electron transfer with H₂O₂ (Figure 2) and subsequently to Fe(II). Fe(VI) has much higher reactivity with H₂O₂ than with H₂O; therefore, the generation of Fe(IV) is enhanced in the Fe(VI)-H₂O₂ system compared to that in individual Fe(VI) [47]. The conversion tendency of Fe(IV) into Fe(II) is high with H₂O₂ ($k = 10^4 \text{ M}^{-1}\text{s}^{-1}$). The Fe(II) thus generated reacts further with Fe(VI) to produce Fe(V) ($k = 10^7 \text{ M}^{-1}\text{s}^{-1}$) [55]. This explains the higher concentrations of Fe(V) and Fe(IV) for the ferrate-H₂O₂ system, which is consistent with the observed increase in pollutant removal from 35.0 to 78.0% for SMX [48].

Radical-scavenging experiments demonstrated the dominant roles of Fe(V) and Fe(IV) in the degradation of organic pollutants. SMX removal by ferrate-H₂O₂ is barely affected by the addition of TBA ($k_{\bullet\text{OH}/\text{TBA}} = 3.8\text{--}7.6 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$) and MeOH ($k_{\bullet\text{OH}/\text{MeOH}} = 9.7 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$), which rules out the contribution of $\bullet\text{OH}$ radicals. The 100% yield of PMSO₂ confirmed the dominant contribution of high-valence Fe species. Moreover, the contribution of Fe(VI) was ruled out by the strong inhibitory effect of SMX

removal with PMSO, as Fe(VI) has much higher reactivity with SMX than with PMSO. Thus, the main contributions of Fe(V) and Fe(IV) were confirmed [48]. The same phenomenon was observed for SMX oxidation in the ferrate- CaO_2 system, where CaO_2 was used as a sustainable resource for H_2O_2 [46]. The oxidation pathway of BPA in the ferrate- H_2O_2 system differs significantly from that of the H_2O_2 process but is highly similar to pure ferrate oxidation, indicating that the oxidation can be attributed to Fe(V) and Fe(IV) [49].

As the dosage of H_2O_2 ranged from 0 to 30 μM , the removal rates of caffeine and carbamazepine (CBZ) in the ferrate- H_2O_2 system increased [47]. These findings confirmed that the addition of H_2O_2 promoted the decomposition of Fe(VI) and the generation of Fe(V) and Fe(IV) [47]. The enhanced Fe(V) and Fe(IV) production promoted pollutant removal significantly. At pH 8, the SMX removal rate was 1.2 and 1.3 times higher by the Fe(VI)- H_2O_2 and Fe(VI)- CaO_2 systems, respectively, when compared to the removal rate with Fe(VI) alone [46,48]. Similarly, BPA removal was strengthened in the Fe(VI)- H_2O_2 system, with an 11.1% increase in the removal rate when H_2O_2 was added to Fe(VI) [49]. Peracetic acid (PAA) and percarbonate can also act as suppliers of H_2O_2 to enhance the ferrate oxidation process, contributing to the enhanced degradation of CBZ [56] and an increase in fatty acid production via sludge pretreatment [57].

Fe(VI) reduction products, including Fe(III) and Fe(II) and Fe oxides, can be used as alternative Fe catalysts for Fenton-like reactions [58]. Unlike the traditional Fenton reaction, ferrite-based Fenton-like reactions have several advantages, such as low pH dependence and a lower dosage of oxidants [58]. Ferrate-based Fenton-like reactions play an important role in $\bullet\text{OH}$ production from ex situ H_2O_2 under the catalysis of Fe(III) and Fe(II) from Fe(VI), which is a more common sequence than dosing of Fe(VI) followed by H_2O_2 [59,60]. Fe(VI) pretreatment for organic dyes was conducted for 30 min, which caused most of the Fe(VI) to decompose into Fe(III) and Fe(II) species [60]. Ex situ H_2O_2 was then added. The significant increase in the color-reduction rate after H_2O_2 addition indicated the activation of a Fenton-like reaction [60]. The degradation rate of phenanthrene was 10.4% greater than that when only Fe(VI) was added [59]. The degradation rate of DMP was controlled by the concentration of $\bullet\text{OH}$, indicating that a Fenton-like reaction activated by Fe(III) and Fe(II) contributed to oxidation [58].

2.3. O_3

Secondary wastewater treatment using Fe(VI) and ozonation has a synergistic oxidation effect due to the Fe(III)/Fe(II) and $\text{FeOOH}/\text{Fe}_2\text{O}_3$ particles. This enhancement was found to increase dissolved organic carbon by 14.9% and UV_{254} removal by 29.0% [19]. This combined system can also be used for the removal of tetrabromobisphenol A, with the complete removal of contaminants and a higher mineralization rate [61]. The SMX and diclofenac sodium can also be removed efficiently by the ozone/Fe(VI) process. With the increase in Fe(VI) dosage or ozone concentration, the degradation rate of SMX and diclofenac sodium was significantly accelerated, reaching 77.0% and 82.7% [62].

As shown in Table 2, the main enhancing effect stems from the catalytic effect of the Fe(VI) reduction products on ozonation, partly because of the production of high-valent Fe formed from the oxidation of Fe(III)/Fe(II) by ozone [19,61]. On the one hand, Fe(VI) could activate O_3 to produce $\bullet\text{OH}$; on the other hand, O_3 also helps regenerate Fe(VI) [63]. The decomposition of O_3 and Fe(VI) occurs simultaneously, and the Fe(III)/Fe(II) ions and $\text{FeOOH}/\text{Fe}_2\text{O}_3$ particles further promote the decomposition of O_3 . The decomposition of Fe(VI) was reported to produce Fe nanostructures with a $\gamma\text{-FeOOH}$ shell and a $\gamma\text{-Fe}_2\text{O}_3$ core [64], with the dissociation of water molecules on its surface leading to hydroxylation, which further aids the bonding between ozone and surface -OH to produce $\bullet\text{OH}$ [19].

Table 2. Removal of organic pollutants in ferrate-AOP systems with the main species of Fe(III) and Fe(II).

Experimental System	Experimental Condition	Active Specie	Adjustments	Pollutant	Enhancement Effect	Reference
Fe(VI)-PMS	[Fe(VI)]: 0.05 mM; [PMS]: 0.05 mM; pH 5.2	SO ₄ ● [−] and ●OH	pH	Sulfamethoxazole: 0.1 mM	Fe(VI)-PMS: 55.5% in 30 min; PMS: negligible; Fe(VI): 20.0%	[65]
Fe(VI)-PMS	[Fe(VI)]: 2.5 mM; [PMS]: 5.0 mM; pH 6.0	SO ₄ ● [−] and ●OH	Oxidant dosage	Atrazine: 46.5 μM	Fe(VI)-PMS: 81.5% in 60 min; PMS: negligible; Fe(VI): 11.7%	[66]
Fe(VI)-PMS	[Fe(VI)]: 100.0 μM; [PMS]: 500.0 μM; pH 6.0	SO ₄ ● [−] and ●OH	Oxidant dosage	Diclofenac: 5.0 μM	Fe(VI)-PMS: 93.5% in 15 min; PMS: negligible; Fe(VI): 42.2%	[42]
Fe(VI)-PMS	[Fe(VI)]: 30.0 μM; [PMS]: 30.0 μM; pH 7.0	SO ₄ ● [−] and ●OH	Oxidant dosage	Enrofloxacin/ marbofloxacin/ ofloxacin: 30.0 μM	Fe(VI)-PMS: 48.0%, 55.0% and 59.0%; PMS: 19.0%, 21.0% and 18.0%; Fe(VI): 14.0%, 10.0% and 13.0%	[67]
Fe(VI)-PMS	[Fe(VI)]: 10.0 mg/L; [PMS]: 2.0 mg/L; pH 7.0	SO ₄ ● [−] and ●OH	Oxidant dosage	Chlorpyrifos: 2.0 mg/L	Fe(VI)-PMS: 94.8% in 90 min; PMS: 56.5%; Fe(VI): 83.0%	[68]
Fe(VI)-PDS/PMS	[Fe(VI)]: 0.25 mM; [PDS]: 0.5 mM; [PMS]: 0.5 mM; pH 7.0	SO ₄ ● [−] , ¹ O ₂ , ●OH, Fe(V) and Fe(IV)	pH and Oxidant dosage	Ibuprofen: 20 μM	Fe(VI)-PDS: 31.1%; Fe(VI)-PMS: 92.8%	[69]
Fe(VI)-PMS	[Fe(VI)]: 50 mM; [PMS]: 200 mM; pH 7.0	SO ₄ ● [−] , Fe(V) and Fe(IV)	pH and Oxidant dosage	Sulfamethoxazole: 10 μM	Fe(VI)-PMS: >80% in 15 min	[70]
Fe(VI)-PMS-light	[Fe(VI)]: 1.0 mg/L; [PMS]: 5.0 mg/L; Solar irradiance: 550 W/m ² ; T: 35.0 °C; pH 6.5	SO ₄ ● [−] and ●OH	Oxidant dosage	Sulfamethoxazole: 0.1 mg/L	Fe(VI)-PMS-light: 70.0% in 60 min; PMS-light: 20.0%	[71]
Fe(VI)-O ₃	[Fe(VI)]: 300.0 μM; [O ₃]: 13.5 mg/L; pH 7.0	●OH	Dosing pattern	Dissolved organic carbon (DOC): 8.4 mg/L; UV ₂₅₄ : 0.174	Fe(VI)-O ₃ : 53.0% (DOC); Ozonation: 23.0% (DOC); Fe(VI): 21.0% (DOC); Fe(VI)-O ₃ : 70.0% (UV ₂₅₄); Ozonation: 44.0% (UV ₂₅₄); Fe(VI): 35.0% (UV ₂₅₄)	[19]

Table 2. Cont.

Experimental System	Experimental Condition	Active Specie	Adjustments	Pollutant	Enhancement Effect	Reference
Fe(VI)-O ₃	[Fe(VI)]: 150.0 µM; [O ₃]: 10.0 mg/L; O ₃ : 100.0 mL/min; pH 6.3–6.4	•OH	Dosing pattern	DOC: 7.5 mg/L; UV ₂₅₄ : 0.15	Fe(VI)-O ₃ : 70.5% (DOC), 80.5% (UV ₂₅₄); O ₃ : 26.7% (DOC), 60.0% (UV ₂₅₄)	[72]
Fe(VI)-O ₃	[Fe(VI)]: 0.5 µM; [O ₃]: 0.5 µM; pH 7.0	•OH	Dosing pattern	Tetrabromobisphenol A: 1.8 µM	Fe(VI)-O ₃ : 68.9% in 1 min Ozonation: 11.7%; Fe(VI): 32.0%	[61]
Fe(VI)-O ₃	[Fe(VI)]: 25.0 µM; [O ₃]: 52 µM; pH 5–9	•OH	pH	Sulfamethoxazole/diclofenac sodium: 20 µM	Fe(VI)-O ₃ : 98.4% and 94.3%; O ₃ : slightly enhanced than the Fe(VI) alone process; Fe(VI): 65.3% and 85.5%	[62]
Fe(VI)-O ₃	[Fe(VI)]: 0.2 mM; [O ₃]: 15.1 mg/L; pH 5–9	•OH	pH	Atrazine: 5 mg/L	Fe(VI)-O ₃ : 90.1% in 18 min	[63]
Fe(VI)-O ₃ -ceramic membrane	[Fe(VI)]: 150.0 µM; [O ₃]: 10 mg/L; filtration flux: 90 L/(m ² ·h); pH 6.0–6.4	•OH	Dosing pattern	DOC: 6.0 mg/L; UV ₂₅₄ : 0.1; Protein: 3.0 mg/L	Fouling transmembrane pressure decreased 96.7% with Fe(VI)-O ₃	[73]
Fe(VI)-PDS	[Fe(VI)]: 90.0 µM; [PDS]: 90.0 µM; pH 4.0	SO ₄ • [−] and •OH	Reducing and complexing additives	Ciprofloxacin: 30.0 µM	Fe(VI)-PDS: 72.6% in 15 min; PDS: negligible; Fe(VI): 54.2%	[74]

2.4. Persulfate

Persulfate, including PMS and PDS, have received increasing attention as AOP precursors due to their advantages of low cost, stable physicochemical properties, and high redox potential [75]. Moreover, sulfate radicals (SO₄•[−]) from PMS or PDS activation show higher stability and more powerful oxidation ability compared to •OH [76]. Fe-based catalysts are commonly used as effective and environment-friendly activators of PMS and PDS [77].

Fe(II) ions are commonly used as homogeneous activators with higher reactivity with PMS and PDS to produce SO₄•[−] radicals (Figure 3) [3]. The amount of Fe(II) ions in the Fe(VI)-PMS system first increased to 9 µM at 3 min and then decreased continually to less than 2 µM at 15 min, which is consistent with the degradation curve of PMS and diclofenac, where 93.5% of diclofenac was removed within 15 min [42]. A similar trend in Fe(II) ion concentration was observed in the Fe(VI)-PMS system for atrazine degradation, indicating the involvement of Fe(II) ions in the activation of PMS for contaminant degradation [66]. An *o*-dichlorobenzene removal rate of 76.5% was achieved using Fe(VI)-PMS, and the removal efficiency increased to 86.4% when FeS was used as the catalyst. The main contribution of FeS was the provision of additional Fe(II) ions for PMS activation [78]. High SMX degradation in Fe(VI)-PMS system was due to the presence of Fe³⁺ ions from the rapid decomposition of Fe(VI), which increases the rate of production of SO₄•[−] [70].

Heterogeneous materials such as Fe_2O_3 have been used for PMS activation in rhodamine B degradation, showing a 1.5-fold increase in the decolorization rate compared with the reaction with no activator [79]. Fe(VI) residual particles ($\gamma\text{-Fe}_2\text{O}_3$) were separated and shown to have catalytic effects on PMS, causing a 58.6% degradation of atrazine [66]. Compared with the low removal of 31.1% in Fe(VI)–PDS, the Fe(VI)–PMS system provided a synergistic effect for ibuprofen removal of 92.8% [69]. Superior to the use of ferric and polymeric coagulant individually, Fe(II)/PMS treatment was utilized to promote the removal of alkali lignin in wastewater. At the optimal dose of 2.4 mM for Fe(II) and 7.8 mM for PMS, the removal efficiency of alkali lignin (2 g/L) can reach 99% [80].

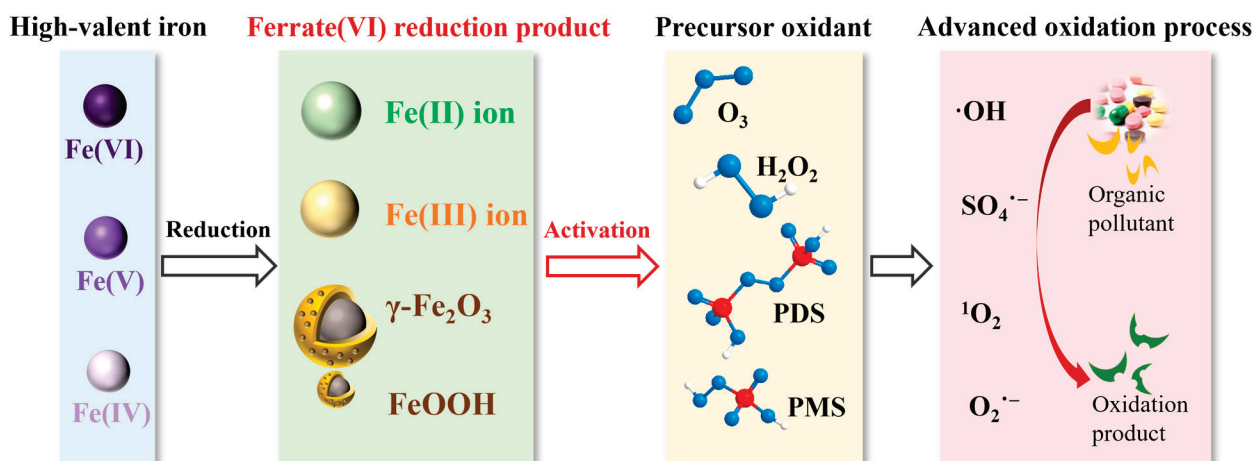


Figure 3. Contributions of Fe(III) and Fe(II) to ferrate-based AOPs and their applications.

The catalytic effect of Fe(III) and Fe(II) on the formation of radicals in the Fe(VI)–persulfate systems was revealed by quenching experiments in which $\text{SO}_4^{\cdot-}$ and $\cdot\text{OH}$ played a dominant role in the removal of organic pollutants [81]. The inhibition rate of diclofenac degradation by either MeOH or tetrabromobisphenol A was more than 50%, and the same suppression effect was observed for atrazine degradation in a similar Fe(VI)–PMS system at pH 6 [42,66] and ciprofloxacin (CIP) removal at pH 4 in the Fe(VI)–PDS system [74]. Fe(III) and Fe(II) can react with PMS or PDS to form $\text{SO}_4^{\cdot-}$, $\text{SO}_5^{\cdot-}$, and $\text{S}_2\text{O}_8^{\cdot-}$ [27,82,83]. Fe(II) showed a reaction constant of $k = 3.0 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ with HSO_5^- , whereas Fe(III) presented limited catalytic reactivity [84].

3. Adjustment of the Fe Species in Ferrate-Based AOP Systems

Ferrate can play the role of (1) oxidant, (2) activator, or (3) electron scavenger in ferrite-based AOP combined systems, depending on the AOP conducted, solution pH, dosage strategy of chemicals, and chemical structure of the contaminants. These roles are not isolated from each other, and Fe(VI) can play two or more roles in one system, causing a synergistic effect with AOPs. By adjusting certain factors, the function of ferrate can be controlled and, more importantly, the total efficiency of ferrite-based AOP systems can be improved (Table 1).

3.1. pH

Solution pH is a key factor that determines the oxidation efficiency of ferrate-based AOP systems [15]. It generally influences the oxidation process in four ways (Figure 4): (1) It determines the concentration and speciation of different valences of iron [65]. (2) It affects the generation and oxidation capability of free radicals [78,85]. (3) It influences the pollutant structure [31]. (4) It influences the oxidant species [60,66]. Among the four

methods, the influence of pH on Fe(VI) is the most significant because of its important role as an oxidant and activator.

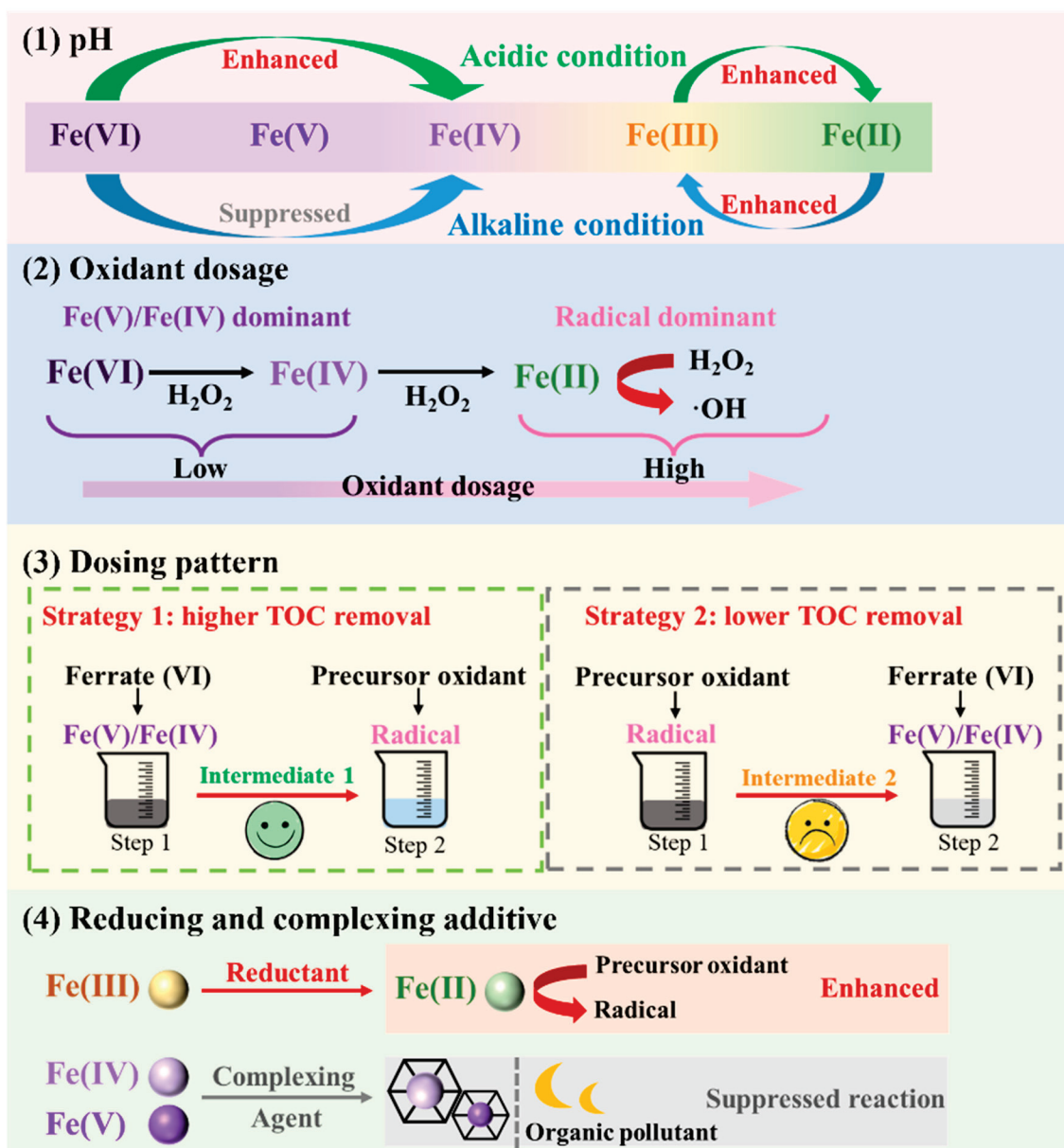


Figure 4. Adjusting the Fe species in ferrate-based AOPs.

Controlling the pH can determine the molecular structure of high-valent Fe species and further influence the oxidation system, owing to the different decomposition pathways of Fe(VI) under various conditions [18]. Fe(VI) is distributed into four different chemical structures depending on the pH of the solution. At an acidic and neutral pH, the dominant HFeO_4^- forms $\text{Fe}^{\text{VI}}_2\text{O}_7^{2-}$ via O–O coupling to produce $\text{Fe}^{\text{IV}}_2\text{O}_6^{2-}$, whereas under base conditions, H_2O molecules attack FeO_4^{2-} to form an O–O bond, followed by the release of Fe(IV). This also leads to different ferrate decomposition rates, which decrease significantly from 4×10^{-3} at pH 7 to below 1×10^{-4} at pH 9 and generally remain stable at pH 9–12 [86]. The self-decomposition of Fe(VI) is significantly dependent on pH. The lower pH increases the oxidizing capacity of Fe(VI), but the decomposition rate becomes faster [27]. The active iron produced has a short service life, and the final decomposition product is Fe(II)/Fe(III) [53]. Therefore, an optimized pH can be used to adjust the Fe species and generate high reaction rates in the AOPs [87].

When high-valent Fe species are the dominant electron acceptors, such as when acting as electron scavengers in UV-TiO₂-Fe(VI) and as the dominant oxidant in ferrate-H₂O₂, the balance between the oxidation capability and stabilization usually lies near pH 7. pH 7 was found to be more effective for the removal of sulfonamides in the UV-TiO₂-ferrate system [43]. A neutral pH is also suitable for SMX degradation in the Fe(VI)-UVA-LED system, with removal efficiencies of 98% and 90% within 2 min at pH 7 and 8, respectively [45]. Increasing the pH enhanced microcystin-LR removal from 65% at pH 2 to 100% at pH 6 [44].

When Fe(III)/Fe(II) is used as an activator for the persulfate and Fenton reactions, acidic conditions are preferred because of the higher Fe²⁺ ion concentrations in this pH range, resulting in a higher activation efficiency. In Fe(VI)-PMS at pH 3.2–11.2, the generation of Fe²⁺ ions was rapid in the first few minutes and then decreased continually during the reaction [65]. The highest concentration of ferrous ions was generated at pH 3.2, which was consistent with the best SMX removal performance [65]. The degradation of SMX decreased at pH from 3 to 11, with the highest removal rate in a Fe(VI)-PMS system without photolysis occurring at pH 3 [31].

3.2. Oxidant Dosage

The dose of H₂O₂ influences the Fe species through electron transfer between H₂O₂ and ferrate. When 50 µM CaO₂ was used as a slow H₂O₂-releasing material, the yield of PMSO₂ in the ferrate-CaO₂ system was nearly 100%, indicating that a low H₂O₂ dosage enhanced oxidation by accelerating the production of Fe(V) and Fe(IV) [46]. While a high H₂O₂ dosage system may initially generate Fe(V) and Fe(IV), a Fenton-like reaction occurs in the later reaction process, catalyzed by Fe(III)/Fe(II) generated from Fe(VI) decay [58]. However, information on the reactive oxidation species in ferrate-H₂O₂ at high H₂O₂ dosages is required. An increase in the H₂O₂ dosage within a certain range is beneficial for the degradation of contaminants; however, an excessive dosage of H₂O₂ reduces the oxidation efficiency. At the initial concentration of Fe(VI) of 50 µM, the degradation of PMSO increased as the H₂O₂ dosage increased from 0 to 30 µM [47]. Similar phenomena were observed for the degradation of SMX, dimethyl phthalate, and BPA [48,49,58]. However, a continual increase in the H₂O₂ dosage inhibits oxidation due to the competition between Fe(V)/Fe(IV) or •OH and H₂O₂ and the contaminants [48,60,85].

The enhanced oxidation effect of persulfate and ferrate is achieved by the activation of persulfate by Fe²⁺, Fe³⁺, γ-FeOOH, and γ-Fe₂O₃ from Fe(VI) decomposition. An insufficient dosage of persulfate leads to wasted Fe(VI) decomposition products, whereas an excess dose of persulfate leads to a quenching effect and competition for radicals [88]. With an increase in the PMS dosage from 50 to 200 µM, the percentage of PMSO oxidized by free radicals increased from 5% to 20%, indicating that the utilization of Fe(II) and Fe(III) by PMS was enhanced [31]. The degradation removal of ATZ increased from 25% to 100% when the molar ratio of Fe(VI) to PMS was increased from 6:5 to 10:1 [66], which is consistent with observations of the degradation of chlorpyrifos, which showed that when the molar ratio was increased from 2:1 to 10:1, the removal efficiency increased from 83.0% to 97.5% [68]. A similar phenomenon was observed in the degradation of diclofenac [42]. The increase in the PMS dosage also enhanced degradation due to the enhanced production of sulfate radicals [67,68]. However, upon increasing the PMS dosage, diclofenac oxidation was inhibited, decreasing to 85% [42]. Response surface methodology was used to analyze the degradation of SMX, and it was found that the dosage of Fe(VI) had a positive effect on SMX removal [89].

3.3. Dosing Pattern

The dosing strategy of Fe(VI) and precursor oxidants influences the degradation efficiency of pollutants because of the interactions between Fe(III)/Fe(II) and the precursor oxidants. The UV₂₅₄ removal efficiency was higher when the treatment involved Fe(VI) followed by ozone than when ozone was followed by Fe(VI) because at a pH of approximately 7.5, the intermediates of the ozone ($E_0 = 2.07$ V) treatment were harder to mineralize using Fe(VI) ($E_0 = 0.72$ V), and the catalytic effect of Fe(III)/Fe(II) on ozone was hindered by the relatively low ozone concentration when Fe(VI) was added [19]. For the same reason, in another combined Fe(VI)-O₃ process, ozone pre-oxidation also inhibited the degradation of tetrabromobisphenol A [61]. The Fe(VI)-ozone system achieved 76.0% and 76.7% removal efficiencies, while the ozone-Fe(VI) system only yielded 49.4% and 54.8% removal for SMX and DCF. The ozone-Fe(VI) system exhibited limited removal efficiency due to rapid ozone depletion by organics and subsequent competitive consumption of Fe(VI) by degradation intermediates [62]. Based on the utilization of Fe(II) in a Fenton-like reaction, the degradation efficiency when H₂O₂ was added at 30 min was the lowest among the cases of the simultaneous addition of the two oxidants and the addition of H₂O₂ at 10 min, owing to the decreasing concentration of Fe(II) with time [85].

The stepwise addition of Fe(VI) slowed the oxidation of SMX when the intermediates competed for radicals, thereby promoting TOC removal [65]. The stepwise addition of PMS caused a radical-scavenging effect via the reaction between the Fe²⁺ and low concentrations of radicals [65]. In a similar case of the TOC removal of CBZ, applying ⁶⁰Co radiation followed by the Fe(VI) process increased the removal rate to 1.8 times the sum of the removal rates of single treatments, which could have resulted from formic, acetic, and oxalic acids generated by the radiation being decomposed by Fe(VI) [90].

3.4. Reducing and Complexing Additives

The conversion between Fe²⁺ and Fe³⁺ is a key factor in controlling the catalytic efficacy of PDS and PMS because Fe²⁺ has a higher catalytic efficiency [36]. Hydroxylamine [74], solar light [71], and sulfites [91] can promote the conversion of Fe³⁺ into Fe²⁺. The addition of hydroxylamine to the Fe(VI)-PDS system to increase the concentration of Fe²⁺ increased the CIP removal efficiency by approximately 20% compared with that in normal Fe(VI)-PDS systems. Reducing agents such as sodium thiosulfate and ascorbic acid have also been reported to enhance CIP degradation in Fe(VI)-PDS systems [74]. Ex situ Fe²⁺ can also be added to enhance the production of Fe(V)/Fe(IV) from Fe(VI) and simultaneously activate PMS [78]. In the Fe(VI)/sulfur reducing agent system, over 90% of PMSO was converted to PMSO₂ at pH 8–10 [92]. At a sulfite concentration of 10 μM, the removal ratio of PMSO was 37.5%, and the molar ratio of PMSO₂ and PMSO was 95.5%, suggesting the presence of Fe(IV) and Fe(V) [45].

Some complexing agents can promote the electron transfer efficiency between oxidants and pollutants. PAA is an oxidant that functions similarly to H₂O₂ and decomposes into H₂O₂ in water. An additional promoting effect on the removal efficiency was observed by comparing the Fe(VI)-PAA system with Fe(VI)-H₂O₂ when the concentration of H₂O₂ was equivalent to that produced in PAA at pH 9 when treated with CBZ [56]. At pH 7 and 8, the combination of PAA with ferrate(VI) demonstrated significantly enhanced CBZ removal efficiency compared to ferrate(VI) alone [26]. Similarly, the addition of PAA to the ferrate(VI) system enabled rapid CBZ removal, reaching 90% efficiency within 1 min [93]. It was assumed that the PAA complexed with Fe(V)/Fe(IV) and that the complex was more oxidative than the common hydroxide Fe complex. The addition of quinone to ferrate systems prominently improves the treatment performance by activating the decomposition of ferrate into Fe(V) and Fe(IV) [21].

4. Effect of Wastewater Composition and Compatibility with Traditional Treatment Methods

Natural organic matter can enhance or inhibit the efficiency of ferrate-based AOPs, depending on its concentration and composition. At a low concentration, NOM can act as a co-oxidant, enhancing the formation of reactive intermediates and improving the oxidation efficiency. However, at a high concentration, NOM can scavenge reactive intermediates such as Fe(V) and Fe(IV), leading to reduced oxidation efficiency [62]. Additionally, NOM can form complexes with ferrate, reducing its availability for decomposition and the generation of reactive species [94]. Inorganic inhibitors can directly react with reactive species like $\bullet\text{OH}$, consuming them and reducing their availability for oxidation reactions [95].

The simultaneous application of Fe(VI) and ozone in on-site wastewater treatment achieved 80.0% and 60.0% aniline removal, respectively [25]. The Fe(VI)-perborate system exhibited lower SMX removal in WWTP influent (35.5–63.9%) compared to that in pure water (75.0%), attributable to radical scavenging by abundant organic matter and ions in actual wastewater that suppress oxidative capacity. In addition, the actual sewage contains relatively high levels of COD and $\text{NH}_3\text{-N}$, which compete with pollutants for active free radicals [95].

Ferrate decomposition forms a negatively charged, porous, and hydrophilic ferric (hydro) oxide prefiltration layer on membranes, which can help to repel hydrophilic dissolved organic carbon and reduce membrane fouling [21]. The oxidative action of ferrate could also degrade aromatic and phenolic constituents in NOM into smaller organic molecules, reducing their membrane fouling potential [39]. However, ferrate can be toxic to certain microorganisms, potentially disrupting the microbial communities essential for biological treatment processes. Ferrate processes offer promising advantages in wastewater treatment. However, more laboratory research and long-term continuous operation results need further exploration.

5. Conclusions and Prospects

The oxidation enhancement in UV-TiO₂-Fe(VI) systems is caused by an improvement in quantum efficiency, with Fe(VI) capturing the photocatalytic electrons. In Fe(VI)-UV-TiO₂ systems, Fe(V) and Fe(IV), which have higher reactivity resulting from Fe(VI) reduction by light-generated electrons, are responsible for the high removal of pollutants. The contribution of Fe(VI) to H₂O₂-based AOPs is similar to that in Fe(VI) photocatalysis. A low H₂O₂ dosage and simultaneous dosing pattern gave Fe(V)- and Fe(IV)-dominated oxidation, where Fe(VI) is activated by electron transfer, whereas the Fenton-like reaction is triggered by a high H₂O₂ dosage and sequencing dosing pattern. The key bridge between Fe(VI) and Fenton-like reactions, ozonation, and sulfate radical-based AOPs is the effect of Fe(III) and Fe(II). Homogeneous catalysis can be realized using Fe(III) and Fe(II), whereas heterogeneous catalysis can be achieved by the successful catalysis of ozone and persulfate by the separated FeOOH/Fe₂O₃ particles.

The role of Fe can be changed by adjusting the operating factors such as pH, oxidant dosage, and dosing pattern. Additives with reducing or complexing abilities may also affect the change of Fe(III) and Fe(II) or the oxidation ability of Fe(V) and Fe(IV). An acidic pH accelerates the production of Fe(V)/Fe(IV) from Fe(VI) and maintains a higher Fe(II) concentration in the solution to activate the oxidants. The oxidant dosage mainly influences the efficiency with which the Fe species are utilized in the ferrate-AOP. The dosage in these systems can be optimized, and even the dominant oxidation contributors can vary due to changes in the Fe species. The dosing strategy includes the dosing sequence of ferrate and mother oxidants, which primarily affects the use efficiency of Fe(III)/Fe(II) by ozone, H₂O₂, and PMS, as well as the contribution of radicals in oxidizing pollutants. Some

reducing agents, such as hydroxylamine, can accelerate the conversion of Fe(III) into Fe(II), which has a higher catalytic ability, and complexing agents such as PAA may promote the oxidation ability of Fe(V)/Fe(IV).

Directions for future work on ferrate-AOP techniques include the following:

- (1) In a practical wastewater process, the stability of reactive Fe species under the condition of variable wastewater composition might limit their large-scale practical application. The relevant research on Fe(VI) regulation was mainly carried out in pure water systems or for specific contaminants, with fewer studies on the removal of organic pollutants in actual wastewater. Based on the selective and nonselective oxidation of Fe(VI) and AOPs, further study of the different oxidation pathways triggered by adjusting the Fe species is required to guide research on natural organic matter resistance reduction during wastewater treatment. It is necessary to strengthen the research on the treatment efficiency of organic pollutants in actual wastewater.
- (2) The development of cost-effective ferrate production methods and stable ferrate formulations is essential for its large-scale application. Combining ferrate-AOP with other treatment methods can address specific limitations and improve overall treatment performance. In situ production of high-valent Fe is required to develop more promising wastewater treatment processes based on ferrate-AOP techniques. Recently, new methods for generating Fe(IV)-based single-atom catalysts and the electrochemical synthesis of high-valence Fe species provide new prospects for generating Fe(VI). More efforts are needed to develop efficient Fe recycling and utilization in ferrate-AOP. While scaling up ferrate AOPs for large-scale applications faces economic and technological challenges, the potential benefits in terms of treatment efficiency and broad applicability make it a promising area for future research and development.
- (3) A detailed characterization of the Fe(VI) decomposition byproducts is required. Although the residue particles from Fe(VI) activate ozone and PMS, their surface morphology and Fe valence are unclear. Moreover, a quantitative analysis of Fe(V) and Fe(IV) is required.

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Article

Diagnosis and Assessment of a Combined Oxylag and High Rate Algal Pond (COHRAP) for Sustainable Water Reuse: Case Study of the University Campus in Tunisia

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Abstract: Universities and other institutes of higher education could be considered as key actors in the implementation of sustainability pillars, such as the adoption of sustainable practices in wastewater management. However, the adoption of such practices is still an emerging issue. This paper discusses the design and operation of the first combined Oxylag and high rate algal pond (COHRAP) constructed at the university campus in Tunisia for irrigation. Performance was evaluated based on the removal efficiencies of nutrients, chemical oxygen demand (COD), biochemical oxygen demand (BOD), heavy metals, coliforms, and biomass productivity. The potential reuse of sludge and algal biomass is discussed based on the Tunisian national standard regulation for sludge reuse in agriculture (NT 106.20) and the European regulation (EC, 2019/1009) for fertilizer products. Effluent phytotoxicity is tested on the germination and growth on *Zea mays* L. The results indicate that the COHRAP performance was globally satisfactory; however, biomass productivity ($1.4 \text{ g m}^{-2} \text{ d}^{-1}$) was low, indicating the need for adjustments in the operational parameters. Despite the effluent limitations for TSS and Hg, no phytotoxic effect was observed. Regarding the heavy metal content in sludge and algal biomass, the results obtained were in compliance with NT 106.20 and EC, 2019/1009, respectively. The energy consumption of COHRAP is 1.05 kWh/m^3 resulting in operational costs of 0.29 euros/m^3 . This study revealed that COHRAP could be a sustainable option to treat wastewater from university campuses with resource recovery. Such a choice can be improved by the implementation of an algae recovery step.

Keywords: high rate algal pond; Oxylag; wastewater reuse; microalgae productivity; energy cost; sustainable practices; wastewater university campus; design criteria

1. Introduction

Tunisia is affected by arid to semi-arid climate conditions and has limited water resources, especially as growing population needs and recent economic development have led to a rapid increase of water demand, resulting in high levels of water stress. In addition, water scarcity is accentuated by factors such as climate change and inefficient

water management. In this context, advancing wastewater management and sanitation, including water reuse, is recognized as a key solution for developing countries, as it reduces the water deficit [1]. Environmental engagement must be integrated across various sectors (e.g., education, agriculture, industry) and sustainable innovative solutions have to be promoted. University campuses can significantly contribute to water conservation efforts because of their high-water consumption. Implementing water treatment and reuse systems reduces demand and promotes environmental responsibility.

Sustainable wastewater treatment technology has become a fashionable trend in recent years due to its economic, social and environmental advantages. These systems could be an interesting alternative in rural areas or in small communities such as university campuses, where traditional treatment plants cannot be installed [2]. Different and extensive technologies exist for wastewater treatment, each with its benefits and drawbacks. Traditional waste stabilization ponds, for example, provide a cost effective and efficient degradation of organic matter but have disadvantages, including large land requirements, variable effluent quality and a deficient nutrient and pathogen removal. More than that, these systems are not intended to recover resources from wastewater. The treatment of domestic wastewater by the aerated lagoon system remains among the most used techniques in countries with arid to semi-arid hot climates. Aerobic bacteria in the aerated lagoon consume dissolved oxygen to oxidize organic matter in the wastewater. Oxygenation is provided by a surface aerator or air insufflation. The operating principle differs from activated sludge in the absence of continuous sludge recycling or removal [3,4]. The research into aerated lagoons has led to the development of a new aerated lagoon system, marketed under the name Oxylag. In this new type of reactor, oxygen transfer and biodegradation volume are separated in order to reduce oxygen supply cost and land requirement [5]. This system includes an oxygen transfer pit, separated into two parts, ensuring both aeration and circulation of water. Several Oxylag municipal wastewater treatment plants have been installed in Morocco and Algeria for populations ranging from 1000 to 15,000 inhabitants.

The use of alternative and sustainable treatment methods (such as algae-based systems) is of interest. These systems, such as high rate algal ponds (HRAP), typically allow for high levels of resource recovery, promote circular economy concepts and reduce greenhouse gas emissions. HRAP is an advanced pond system suitable for arid and semi-arid areas [6] and has many advantages, such as a low land occupation and a low cost investment [7]. Furthermore, HRAP is a suitable solution for wastewater treatment, particularly in small communities [8]. These natural systems provide cost-effective and efficient wastewater treatment where weather conditions are favorable for microalgae growth [9–11]. The latter assimilates nutrients and produces oxygen, which is then consumed by bacteria for the oxidation of biodegradable organic matter [12,13].

HRAP overcomes many of the disadvantages of conventional ponds, including the limited nutrient and pathogen removal. It also provides significant nitrogen and phosphorus removal through assimilation into algae biomass and stripping at high pH [14]. Microalgae are not only restricted to nutrient removal from wastewater, they are also efficient at removing pharmaceutical and pesticide substances [15,16] and metal pollutants [17]. For microalgal cell development, several positive heavy metals, such as Fe, Cu, Mn, Co, Zn, and Mo, are necessary and may be adsorbed [18]. However, negatively charged exopolysaccharides (EPSs) may be released by a number of microalgae [19] and are able to adsorb metals [20]. In addition, HRAP provides significant bacteria removal. Pathogen inactivation within the HRAP is influenced by high pH, dissolved oxygen concentration and UV radiation exposure. These factors damage pathogens' RNA and DNA [21] and promote the photo-oxidation of dissolved organic contaminants [22].

In addition, HRAP technology has been shown to provide more effective wastewater treatment for use in agriculture applications [12,23], with the additional benefit of recovering wastewater nutrients as harvestable algal/bacterial biomass. HRAP has a notable algal productivity, with a mean of $45 \text{ g m}^{-2} \text{ d}^{-1}$, and can thus serve as a source of animal protein [7]. In addition, microalgal biomass allows CO_2 capture while treating wastewater. Indeed, producing 1 kg of microalgal biomass would require 1.83 kg of carbon dioxide [24].

In terms of potential applications, algal biomass from wastewater can be used as biofertilizers, animal feed and valuable compounds such as pigments and lipids [25–27]. However, there is still a lack of knowledge regarding the accumulation of several contaminants into biomass such as heavy metals, which could impair its safe use as biofertilizer [28]. Moreover, algal biomass from wastewater can also be recovered for bioenergy through conversion into biogas, bioethanol, bio-crude oil or biodiesel via lipid trans-esterification [29]. However, recovery and harvest algae meet technical and economic difficulties [30].

In HRAP, more than 3000 microalgae strains have been identified [31], with varying features and capacities to remediate wastewater. Environmental factors like temperature, light, and nutrients can cause temporal shifts in dominant species or community structures.

Several studies have been conducted to optimize HRAP operating conditions, including depth, hydraulic retention time (HRT), and dynamics [32–34]. However, the role and effect of the primary treatment upstream of HRAP remains controversial. Posadas et al. [35] suggest that primary suspended solids removal is probably not an essential step prior to HRAP systems but further research is needed to prove that this configuration is feasible. Tyagi and Couillard [36] have reported that wastewater may include a number of substances that could inhibit microalgal growth and suggest that the pretreatment of wastewater is necessary. According to Couto et al. [37], the HRAP system could be combined with other ponds, such as maturation ponds or anaerobic digestion, to meet effluent requirements prior to effluent discharge.

Despite the operational and environmental benefits of HRAP, as well as its effectiveness in treating domestic or university wastewater, the large-scale deployment with on-site reuse of treated wastewater in agriculture is still relatively limited [38]. Most research studies have been conducted in laboratory-scale systems operating under constant conditions, which will never accurately describe system performance under real conditions. In addition, few studies have focused on the treatment of wastewater generated on university campuses using the HRAP treatment system. These effluents are characterized by their heterogeneity and are generally of an industrial nature [39].

In December 2023, a demonstration plant of a combined Oxylag–HRAP system, pioneered in Tunisia, was constructed at the campus of the High Agronomic Institute of Chott Mariem, to contribute to water conservation efforts, to increase knowledge on this technology among the local community and authorities and to promote sustainable practices and a culture of environmental responsibility among students.

The objectives of this study were to (1) demonstrate the feasibility of sustainable wastewater management practices on a university campus, (2) evaluate the performance of a COHRAP system and verify its compliance with current standards, (3) identify the main limitations and provide possible alternatives and solutions and (4) discuss the potential reuse of treated wastewater, algal biomass and sludge.

2. Materials and Methods

2.1. Study Site

The wastewater treatment demonstration pilot plant (WWTDPP) was built on the campus of the High Agronomic Institute of Chott Mariem (ISA-CM) located in the Sousse region of Tunisia. The plant was part of a research project funded by Wallonie Bruxelles In-

ternational. The institute is built on a 54-hectare site with 450 people, including researchers, students, administrative staff and workers. The operation of the plant started in January 2023. In this region, the climate is semi-arid, the average rainfall is less than 361 mm per year and the average temperature is around 18 °C. Prior to the construction of the plant, the raw wastewater was sampled from the general sewage network of the High Agronomic Institute of Chott Mariem and analyzed weekly for physicochemical characteristics for one month. The daily flow rate of raw wastewater was monitored for one week. Wastewater corresponding to 40 m³ d⁻¹ is produced by laboratories, lecture halls libraries, housing services, restaurant, and academic dormitory, and is discharged into the municipal sewerage system through a unified sewage network.

2.2. Design Criteria

The Oxylag and HRAP designs are based on the raw wastewater characteristics (Table 1), the organic loading rate of the influent was estimated as 100 population equivalents (PE) considering a specific load of 60 g BOD per person per day and a specific flow of 90 L per person per day. Tunisian standards for reuse (NT 106.03) [40], desired BOD percentage of 95% removal, climate (solar irradiation, temperature and precipitation) and available area have also been taken into consideration in the pilot design. Biological and aeration sizing was carried out to estimate the volume of the two ponds, the surface area needed, the amount of dissolved oxygen per hour required and the water velocity in the HRAP. The biological sizing of the Oxylag and HRAP is based on flow rate and BOD concentration of the raw wastewater.

Table 1. Raw wastewater characteristics.

Parameters	Mean Concentration (mg/L)	Mean Loading Rate (kg/d)
COD	483	19.3
BOD	230	9.2
TSS	220	8.8
TN	96	3.8

Note: Data given as average values of the mean (n = 4). COD: chemical oxygen demand; BOD: biological oxygen demand; TSS: total suspended solids; TN: total nitrogen.

The minimum surface area of the Oxylag pond was determined based on the maximum BOD loading rate commonly used for the design of an aerated lagoon while ensuring good performance. The BOD loading rate of 60 g m⁻² d⁻¹ should be supplied to the Oxylag pond to achieve 60% BOD removal. The water depth of 1.5 m was considered taking into account the power of the aeration system designed to supply the two ponds (Oxylag and HRAP) while ensuring correct mixing. The airlift design was based on the amount of oxygen required per day, expressed as (kgO₂ d⁻¹), allowing for the removal of 60% of the BOD loading rate and the performance of partial nitrification and in order to meet the sediment oxygen demand. The parameters used for the Oxylag aeration design are presented in Table 2. The amount of oxygen required per day was converted to standard conditions. Finally, the hourly air flow requirement, expressed as (Nm³ h⁻¹) was determined by dividing the quantity of oxygen requirement, expressed as (kgO₂ h⁻¹), by the oxygenation capacity of perforated membrane air diffuser expressed as (g O₂ Nm⁻³).

Table 2. Parameters values used for airlift sizing of Oxylag.

Parameter	Value
Oxygen required for BOD removal ($\text{kg O}_2/\text{kg BOD}_{\text{removed}}$)	1.5
Oxygen required for nitrification ($\text{kg O}_2/\text{kg N-NH}_4_{\text{removed}}$)	4.57
Sediment oxygen demand ($\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$)	4
Oxygenation capacity of perforated membrane air diffuser ($\text{g O}_2 \text{ Nm}^{-3}$)	30

For the HRAP, the minimum surface area was determined based on the BOD loading rate of $20 \text{ g m}^{-2} \text{ d}^{-1}$ [26,41] and the assumption that 40% of BOD loading rate supplied at the inlet of Oxylag would be treated in the HRAP. The total volume of the HRAP was calculated considering the water depth of 0.5 m and the ratio of length to width of the channel greater than 10. The selection of the BOD loading rate of $20 \text{ g m}^{-2} \text{ d}^{-1}$ builds on our previous studies carried out under climatic conditions similar to those in Tunisia.

Furthermore, the proposed HRT is a minimum of 4 days to allow for the removal of organic material and nutrients [42].

For HRAP airlift sizing, the desired velocity of 0.1 m s^{-1} [43] was assumed and the air flow requirement ($\text{m}^3 \text{ d}^{-1}$) in the HRAP channel airlift was determined.

2.3. Wastewater Treatment Pilot Plant Description

The WWTDPP was established to treat a specific amount of the total wastewater generated by the campus. It was designed for 100 inhabitants with an average daily flow of $10 \text{ m}^3 \text{ d}^{-1}$ and an organic loading rate of 2 kg BOD d^{-1} .

The pilot plant consists of two wastewater treatment stages operated in series (Figure 1)—an aerated lagoon called Oxylag followed by a high rate algal pond (HRAP).



Figure 1. Photograph of ISA-CM combined Oxylag and High rate algal pond wastewater treatment demonstration pilot plant (COHRAP/WWTDPP).

The plant was fed by raw wastewater taken from a 3.4 m^3 storage tank equipped with an automated pumping system. The feeding rate was controlled by a Siemens LOGO programmable logic controller, by considering the desired hydraulic and organic load. The technical characteristics of the two ponds are shown in Table 3.

Table 3. Dimensional specifications of the Oxylag and HRAP.

	Length (m)	Water Surface (m ²)	Depth (m)	Volume (m ³)
Oxylag pond	11	54.3	1.5	57.8
AirLift pit		4.8	2.7	13
HRAP	12.7	86.7	0.5	44.2
AirLift pit		3.2	2.8	9

The Oxylag system has an area of 54.3 m² and an operating depth of 1.5 m, with a capacity of 57.8 m³ due to the shape of the pond (45 degrees slope banks). Its main function is to reduce the organic loading rate of the HRAP feed. The aerated pond is equipped with an air lift aeration system to ensure both the circulation and homogenization of the effluent, in addition to enhancing biological degradation of the organic matter by bacteria. The airlift of the HRAP consists of a 13 m³ pit separated into two communicating parts (Figure 2). Air injection is ensured in the first part of this pit, where six perforated membrane air diffusers OTT 02-Ökonom Magnum Clip IN 1.000 Flexsil 2X500 lg 1200 D67 (OTT Group, Langenhagen, Germany) are installed at 25 cm above the bottom. Water flow velocities measured at the Oxylag and HRAP ponds are 7.86 and 13.14 cm s⁻¹ respectively, ensuring circulation and homogenization of the water column.

**Figure 2.** Photos of the Oxylag pit with air diffusers.

The high rate algal pond (45 degrees slope banks) has a serpentine shape with six channels of 12.7 m in length. It has a surface area of 86.7 m² and an operating depth of 50 cm with an operational volume of 44.2 m³. It is fed from the Oxylag pond. It is equipped with an airlift aeration system to ensure the circulation and homogenization of the effluent, and to maintain the algal/bacterial flocs in suspension. The airlift of the HRAP consists of a 9 m³ pit separated into two communicating parts. Air supply is ensured in the first part where six perforated membrane air diffusers (OTT 02-Ökonom Magnum Clip IN 1.000 Flexsil 2X500 lg 1200 D67) are installed at 25 cm above the bottom of the pond (Figure 2).

The bottom and walls of the ponds (HRAP and Oxylag) are sealed using a membrane (high density polyethylene membrane, HDPE 1.5 mm) fitted on a geo-textile layer.

The two treatment stages (Oxylag and HRAP ponds) are supplied in the air by 4 KW Robuschi Robox type ES 15/1 P blower (Robuschi S.p.A, Parma, Italy) controlled by a

frequency converter. The intermittent operation of the air supply system is performed by a Logo controller.

2.4. Hydraulic Flow and Operational Conditions

Figure 3 shows the flow diagram of the combined Oxylag and HRAP plant. The influent wastewater is taken from a storage tank and then passes through a first venturi device to measure the Oxylag's influent flow rate. The defined influent flow rate is about $9.8 \text{ m}^3 \text{ d}^{-1}$ and corresponds to 5.8 days hydraulic retention time (HRT) in the Oxylag and 4.5 days in the HRAP. After pretreatment within the Oxylag, the HRAP is gravity fed. Finally, the effluent passes through second counting equipment (venturi) before being discharged into a final tank equipped with a submersible pump for on-site water reuse if the water quality is suitable. Alternatively, in the event of unsatisfactory quality, treated water is discharged into the general sewage network of the campus.

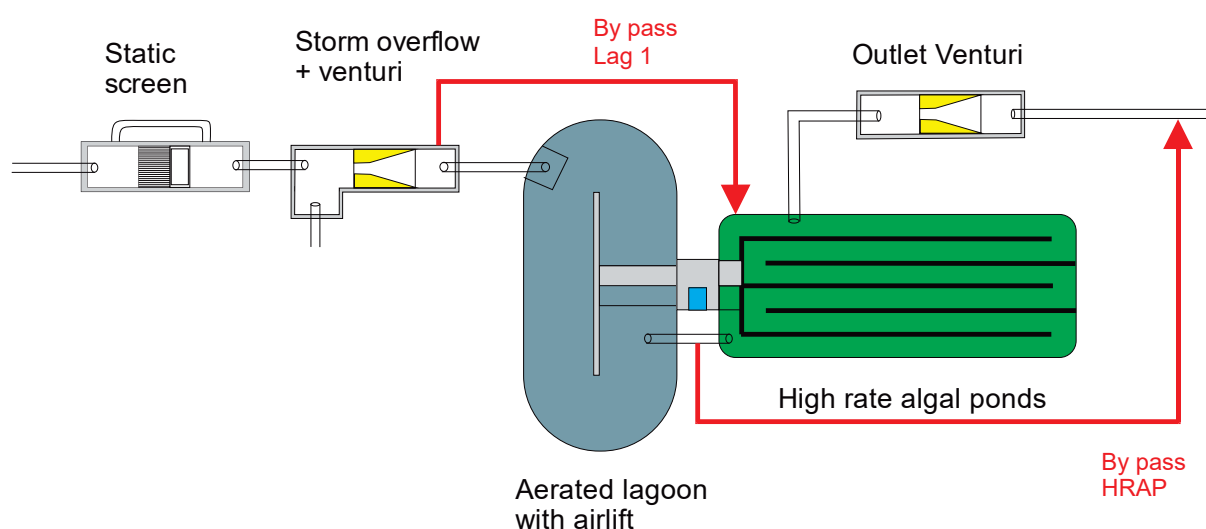


Figure 3. Schematic diagram of a combined Oxylag–HRAP wastewater treatment demonstration pilot plant of the High Agronomic Institute of Chott Mariem.

During the observation period, air flow rates were 34 and $97 \text{ m}^3 \text{ h}^{-1}$ at the Oxylag and HRAP, respectively, with a daily operating time of the blower air system of 6 h (5 min ON/15 min OFF). Air flow rate is determined by multiplying the air velocity by the cross-sectional area of air supply pipes. The air velocity was measured using a TROTEC TA400 pitot tube (TROTEC GmbH, Heinsberg, Germany).

2.5. Sampling and Monitoring

Climate monitoring was assessed by the weather station (WS-GP2, Delta-T Devices Ltd., Cambridge, UK) installed on the campus site, which continuously logged total solar radiation, air temperature, rainfall, humidity, wind speed and direction. During the observation period, the recorded average air temperature was 22°C with a maximum of 48.6°C in July 2023 and a minimum of 5.5 in January 2024, average light intensity during the sun rises was about 500 W m^{-2} .

Water quality parameters were monitored from July 2023 to December 2024. Samples were collected monthly from July to September 2023 for the first campaign and from March to December 2024 for the second campaign. These samples were collected at the inlet and outlet of each pond to assess the operating efficiency of the WWTDPP in the first-year

operation period. Removal efficiency (R%) for water indicator parameters was calculated using Equation (1).

$$R (\%) = \frac{\text{Influent concentration} - \text{effluent concentration}}{\text{Influent concentration}} * 100 \quad (1)$$

Continuous in-situ measurements of pH, temperature (T) and dissolved oxygen (DO) were performed using DO and pH probes Hach LDO 101 and Hach pH C 101 (Hach Company, Loveland, CO, USA) with data logging (Hach HQ40d).

For microalgal species identification, water samples (in triplicate) collected at HRAP were analyzed monthly during the observation period (May, August and November 2024) using a Leica DM 500 light microscope (Leica Microsystems GmbH, Wetzlar, Germany). Microalgae species were identified according to the identification guide books [44]. To determine algae and biomass productivity within the HRAP, water samples were collected weekly, within the pond, from March 2024 to May 2024 for chlorophyll-a and for total suspended solids (TSS) and volatile suspended solids (VSS) analysis. Algae biomass in HRAP was estimated from chlorophyll-a concentration according to Equation (2) [45]. The algal productivity (Pa) was calculated based on Equation (3) [46].

$$\text{Algae biomass} \left(\frac{\text{mg}}{\text{L}} \right) = \text{Chlorophyll } a \left(\frac{\text{mg}}{\text{L}} \right) * \frac{100}{1.5} \quad (2)$$

Equation (2) assumes that the algal biomass has a chlorophyll-a content of 1.5% of dry weight [47]. This method provides a relative measure of algal biomass as chlorophyll content varies with algal species and growth condition. Using a constant ratio to convert chlorophyll a to algal biomass may not always give a correct estimate of algal biomass and may be subject to error (over or underestimation), which may be greater when samples are taken over a long period. Determining the relationship between the chlorophyll-a content and the concentration of algae species is part of our research.

$$\text{Algal productivity} (Pa) = \frac{(Ct - C0) V}{HRT * S} \quad (3)$$

where Pa is algae productivity ($\text{g m}^{-2} \text{d}^{-1}$); Ct and $C0$ are the concentrations of dried algae biomass at t and t_0 , respectively; V is the HRAP volume (44.17 m^3); HRT is hydraulic residence time; and S is the surface area of the pond (86.6 m^2).

In addition, water samples were taken from the HRAP and microalgae was extracted by centrifugation. The dried biomass was then analyzed for heavy metal (HM) content. Likewise, sludge samples were collected from Oxylag and analyzed for HM.

2.6. Analytical Procedures

Composite water samples were collected in 1000 mL polypropylene bottles for water quality analysis and 100 mL sterile bottles for microbiological examination at the following time range: 8 to 9 a.m., 12 to 14 p.m. and 17 to 19 p.m. All analysis were performed on the same day according to the standard methods for the examination of water and wastewater [48]. Physicochemical parameters, including pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), volatile suspended solids (VSS) and total nitrogen (TN), were monitored. As HRAP effluent samples contained algae biomass, the BOD, COD and TN parameters were performed on filtered samples. Ammoniacal nitrogen ($\text{NH}_4\text{-N}$), phosphate ($\text{PO}_4\text{-P}$) and nitrate ($\text{NO}_3\text{-N}$) were measured in filtered samples both at the inlet and outlet of the plant. All samples analyzed were performed in triplicate.

The determination of Chl a was performed using a hot-ethanol extraction technique (90%), as described in ISO 10260 (1992) [49].

The standard most probable number (MPN) method was used for the determination of total and fecal coliforms using McCrady's probability tables.

Heavy metal (HM) analysis was performed on unfiltered samples for the inlet Oxylag and on filtered samples for the HRAP effluent. In the case of algal biomass and sludge, the samples were pretreated according to EPA 3051A method which involves digestion in a closed microwave oven system in the presence of concentrated HNO₃ and HCl at an HNO₃/HCl ratio of 3:1 [50]. Thereafter heavy metals (Cd, Cu, Ni, Pb, Cr, Zn, Fe, Hg, As, Se, Co, B) were analyzed using an inductively coupled plasma mass spectrometer (Thermo Scientific, iCAP RQ, Waltham, MA, USA) according to ISO 17294-2 [51]. All samples analyzed were performed in triplicate.

Heavy metal (HM) analysis was performed on unfiltered samples for raw effluent and filtered samples for the inlet and for water leaving the plant.

2.7. Phytotoxicity Tests of the HRAP Effluent

A growth test was carried out on pre-germinated seeds with the same radicle length (2 mm). These seeds were initially pre-germinated in distilled water. Afterward, they were placed in 9 cm Petri dishes lined with filter paper and exposed to 5 mL of HRAP effluent (treatment) or 5 mL of distilled water (control). The effect of the HRAP effluent on the growth of *Zea mays* seedlings was assessed by measuring the length of roots and shoots seven days after germination. Germination rate (GR) and germination indices (GIs) were calculated following Equations (4) and (5).

$$GR (\%) = \frac{\text{Number of germinated seeds after 7 days}}{\text{Total number of seeds}} * 100 \quad (4)$$

$$GI = (N1 * 1) + (N2 - N1) * \frac{1}{2} + (Nn - N_{n-1}) * \frac{1}{n} \quad (5)$$

where $N1$, Nn , N_{n-1} are the proportions of seeds germinated in the first, second and N_{th} days, GI expresses the delay in germination.

2.8. Energy Consumption and Operational Costs of WWTDPP

The power consumptions of the pump and air blower were measured and recorded using a KWh measurement device. The energy consumption is calculated based on operational time and KWh data recorded.

The annual operating costs of the WWTDPP include electricity, staff and maintenance expenditures. Staff costs were estimated by assuming a need of 100 working h/year with 3 EUR/h, this results in staff costs of 300 EUR/yr. A price of 0.156 EUR/kWh was assumed for the cost of electricity and a price of 16 EUR/m³ was considered for the cost of sewage sludge management.

2.9. Statistical Analysis

Mean values $\pm$ standard deviation (Std) are provided. Statistical analyses on measured physicochemical parameters of the effluents in the inlets and outlets points were performed using statistical software (SPSS: version 18.0). The significance between inlet and outlet series was made by paired *t*-test at the 5% level. The significance between the control and seeds irrigated with HRAP effluent was made by Duncan test.

3. Results and Discussion

3.1. Physicochemical Characteristics of Raw Wastewater

The monitored parameters of ISA-CM effluent have a high variability in terms of organic load. Physicochemical results (Table 4) show an average COD value of $886.5 \pm 576 \text{ mg L}^{-1}$, with a minimum value of 134 mg L^{-1} recorded in July 2023 and a maximum value of 1773 mg L^{-1} recorded in April 2024. The BOD value ranged from 21 and 594 mg L^{-1} with an average value of $284.7 \pm 190 \text{ mg L}^{-1}$. The minimum BOD value was recorded in July 2023. The organic pollution ratio BOD/COD is about 0.3 ± 0.1 , allowing for a poor biodegradability of the effluent and acts as a limitation of the biological treatment. This could be related to the chemical solution discharged from laboratories and effluent from restaurant containing oils and fats.

Table 4. Comparison of raw wastewater characteristics, with values from university campuses in Morocco [52] and Spain [53].

Parameters	Unit	ISA-CM (Tunisia)		Faculty of Science (Morocco)	Las Palmas de Gran Canaria (Spain)
		Range	Mean $\pm$ Std	Range	Range
pH	-	7.4–8.7	7.89 ± 0.4	7.18–8.18	
EC	$\mu\text{S cm}^{-1}$	2480–3070	2834 ± 141	2470–3980	
COD	mg L^{-1}	134–1773	886.5 ± 576	967.4–1151.0	315.1–365.6
BOD	mg L^{-1}	21–594	284.7 ± 190	70.5–119.0	230.7–328.5
SS	mg L^{-1}	54–330	178.1 ± 71	223.6–1659.7	201.3–264.8
VS	mg L^{-1}	24–163	90.7 ± 51	-	
TN	mg L^{-1}	66.9–132.4	91.2 ± 26	-	
NH ₄	mg L^{-1}	0.5–80.3	42 ± 25	3.5–17.5	
NO ₃	mg L^{-1}	0.1–2.2	1.0 ± 0.7	0.6–1.4	
PO ₄	mg L^{-1}	1–10.7	6.5 ± 2	0.7–2.7	

Note: ISA-CM results are presented as mean $\pm$ standard deviation (n = 13).

For particulate suspended solids (TSS), concentration values range between a minimum of 54 (July 2023) and a maximum of 330 mg L^{-1} (March 2023) with an average content of $178.1 \pm 71 \text{ mg L}^{-1}$. The nutrient concentration of ISA-CM effluent is characterized by a mean of total nitrogen (TN) contents of $91.2 \pm 26 \text{ mg L}^{-1}$, an average of ammonium ion (NH₄⁺-N) of $42 \pm 25 \text{ mg L}^{-1}$, and an average value of nitrate $1.0 \pm 0.7 \text{ mg L}^{-1}$. For orthophosphate (P-PO₄), the average content is about $6.5 \pm 2 \text{ mg L}^{-1}$.

Thus, the high variability of the physicochemical parameters is mainly related to the sampling period, which depends on the term time and vacations (July and August). Discharge of wastewater with high complexity due to different uses may also affect the wastewater characteristics, resulting in high variability. Previous studies have also shown a high variability in the characteristics of raw wastewater from university campuses. Melian et al. [53], studying stone filters in combination with a pond–wetland system treating raw wastewater from a university campus in Las Palmas de Gran Canaria in a pilot plant, obtained significant variability of BOD, COD, SS and NH₄, with mean ($\pm$ standard deviation) concentrations of $314 \pm 92 \text{ mg L}^{-1}$, $416 \pm 215 \text{ mg L}^{-1}$, $158 \pm 100 \text{ mg L}^{-1}$, and $139 \pm 74 \text{ mg L}^{-1}$, respectively. Grimah et al. [52] have also reported the same trend of variation in the quality of raw wastewater from the Faculty of Sciences Ben M'Sick (Casablanca, Morocco). Average values ranging between 7.18 and 8.18 for pH, 2.47 and 3.98 mS cm^{-1} for electrical conductivity, 967.4 and 1151 mg L^{-1} for COD, 70.5 and 119 mg L^{-1} for BOD, 223.6 and 1659.7 mg L^{-1} for TSS were obtained. The authors have also reported an orthophosphate (P-PO₄) content varying between 0.7 and 2.7 mg L^{-1} , while ammonium,

nitrite and nitrate concentrations varied between 3.5 and 17.5 mg L⁻¹; 0.17 and 3.6 mg L⁻¹; 0.6 and 1.4 mg L⁻¹ respectively. The authors suggest that the high variability may be slightly influenced by the rainwater when it is collected by the unitary sanitation system, resulting in a variation in pollutant concentration. As the daily climate data for the observation period of the present study indicates a severe rainfall deficit in the region of Sousse, and in the country as a whole, the high variability observed is not thus related to rainy periods.

3.2. Loading Rate, Efficiency and Effluent Quality

Oxylag pond and HRAP were fed with wastewater at 5.9 and 4.5 d HRT, respectively, to acclimate the algae and bacteria. These values are consistent with a typical range of 5–10 d for wastewater treatment in HRAP [29] and of 5–10 d for wastewater treatment by Oxylag [5].

Mean loading rates and effluent concentrations of the main parameters measured during the observation period for the two treatment stages are shown in Table 5.

Table 5. Mean loading rates and effluent characteristics ($n = 13$) compared with limit values for wastewater reuse in Tunisia (NT 106.03) [40].

	Oxylag		HRAP		Limit Values
Parameters	Influent Loading Rate (g m ⁻² d ⁻¹)	Influent Loading Rate (g m ⁻² d ⁻¹)	Effluent Concentration (mg L ⁻¹)		Concentration mg L ⁻¹
	Range	Range	Range	Mean ± Std	
COD	24.3–321.2	3.9–38.6	30.8–98	68.07 ± 23	90
BOD	3.8–107.6	0.5–15.7	2.0–30.0	12.8 ± 11	30
SS	9.8–119.5	4.5–33.8	47.0–1103	343.17 ± 308	30
VS	4.3–29.5	3.7–27.0	36–880	241.5 ± 229	
TN	12.1–32.9	0.6–16.0	3.3–42.2	16.6 ± 15	
NH ₄ ⁺	0.09–26.0	0.01–1.7	0.1–2.1	0.7 ± 0.4	
NO ₃ [−]	0.02–0.4	0.1–1.4	0.1–14.1	3.78 ± 4	
PO ₄ ^{3−}	0.2–3.1	0.09–1.0	0.4–3.9	1.5 ± 1	

The chemical oxygen demand (COD), the biochemical oxygen demand (BOD) and total nitrogen (TN) loading rates applied to the Oxylag pond ranged from 24.3 to 321.2 g COD m⁻² d⁻¹, 3.8 to 107.6 g BOD m⁻² d⁻¹, and 12.1 to 32.9 g TN/m⁻² d⁻¹. Loading rates applied to the high-rate algal pond ranged from 3.9 to 38.6 g COD m⁻² d⁻¹, 0.5 to 15.7 g BOD m⁻² d⁻¹, and 0.6 to 16 g TN/m⁻² d⁻¹. The lowest COD and BOD loads applied to both systems were recorded in July and September 2023, while the highest were recorded in April 2024, which is linked to the campus activities, as explained earlier. It should be noted that, during vacation, the influent loads are reduced, while the influent volume remains constant following the data recorded by the pump's daily operation time.

The loading factor is a ratio between the hydraulic and nominal surface loading rates. Considering the nominal surface loading rates of the Oxylag and HRAP, which are 60 g BOD m⁻² d⁻¹ and 20 g BOD m⁻² d⁻¹ [26], respectively, the load factors calculated varied between 0.02 and 0.78, with a mean of 0.31 for the HRAP. Additionally, the load factors calculated for the Oxylag varied between 0.06 and 1.79 with a mean of 0.86. This means that the inflow rate and hydraulic loads applied to the Oxylag pond were sometimes insufficient as a result of decreasing campus activities. On the other hand, the hydraulic loads applied to the Oxylag could sometimes reach twice the nominal loading rate. In the

case of the HRAP, the hydraulic loads are insufficient because of overtreatment within the Oxylag, which was more efficient than specified in the design.

Therefore, maintaining an optimal hydraulic loading rate depending on the time periods and raw wastewater characteristics is crucial to ensure the efficiency of the WWTDPP and energy consumption. The modelling of the COHRAP could be of great interest to optimize the operating parameters and discover new improvements for this plant. This is part of our ongoing research activities. In addition, continuous assessment and control tools will be installed at the WWTDPP. An ultrasonic flow sensor equipped with a data logger is currently being implemented at the inlet of the Oxylag pond to ensure continuous monitoring of the volumetric flow and thus enables proactive operation of a WWTDPP. At the same time, and to overcome the high variability of the effluent, an automated sampler is intended to be installed to collect 24 h composite samples to cover the temporal variations associated with human activity, thus ensuring more representative and reliable monitoring.

The mean removal rates R (Figure 4) performed by the COHRAP during the monitoring period were 91.2% for BOD, 86.3% for COD, 81.4% for TN, 97.8% for N-NH_4 and 75.5% for orthophosphate (P-PO_4). BOD removal efficiency achieved in Oxylag is the result of organic biochemical oxidation by heterotrophic bacteria. Oxylag pond processes are similar to those of the aerated lagoon, where the conversion of organic matter, carbonaceous nitrogenous, into inorganic compounds occurs [54].

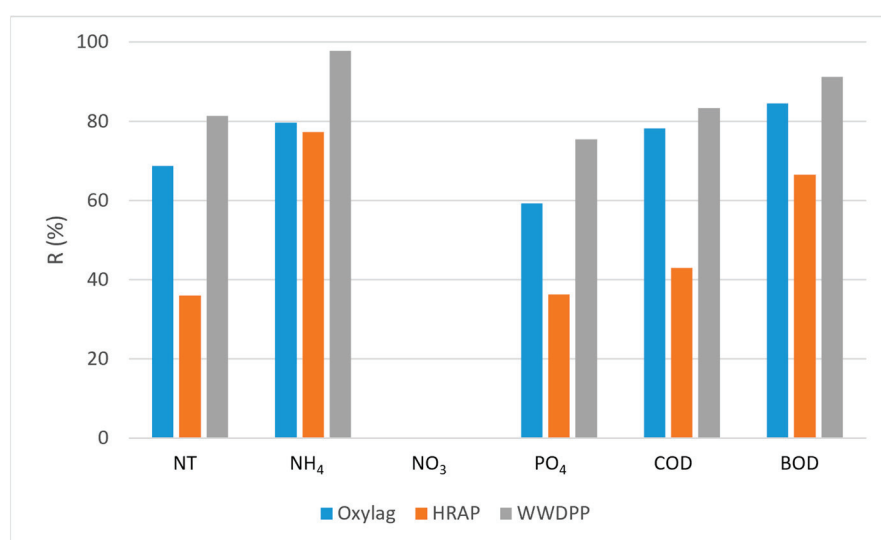


Figure 4. Removal rates (R) of HRAP, Oxylag and WWTDPP.

Despite the large variability of the loading rate, these removals remain high, whatever the quality of the final effluent. Figure 4 shows that most of the COD and BOD reduction takes place in the first treatment stage, with removal efficiencies of $78.3 \pm 15\%$ for COD and $84.5 \pm 6\%$ for BOD in the Oxylag pond, whereas the COD and BOD removal rates in the HRAP are $48.5 \pm 30\%$ and $66.6 \pm 33\%$ respectively. The higher removal efficiency is probably due to the high mean DO concentration (8.2 mg/L) within this pond, in addition to the higher loading rate applied to this first stage, with a mean of $55.9 \text{ g BOD m}^{-2} \text{ d}^{-1}$ and $172 \text{ g COD m}^{-2} \text{ d}^{-1}$, compared with the second stage HRAP ($5.9 \text{ g BOD m}^{-2} \text{ d}^{-1}$, $18.7 \text{ g COD m}^{-2} \text{ d}^{-1}$). This higher efficiency at higher loads is a known characteristic of extensive wastewater treatment systems [55].

Arashiro et al. [25] have reported an average COD removal efficiency between 60 and 67% in a HRAP pilot plant at the university of Catalunya in Spain, with an influent flow rate of 105 L/day and HRT of 4.5 days. In the present study, removal efficiencies were in accordance with Melian et al. [53] on a combined stone filter with a pond–wetland

system treating wastewater from a university campus in Las Palmas (Spain), where removal efficiencies of 95% of BOD and 78% of COD were found.

The study of Abd-Elmaksoud, et al. [56] on an integrated pilot-scale system, including a facultative pond (FP), a rock filter (RF) and a high-rate algal pond (HRAP) with a 5-days hydraulic retention time and a volume 4.3 m³, reported removal rates of 89.39%, 89.26%, and 82.24% for NH₄-N, BOD, and COD, respectively.

The NH₄-N and TN concentrations in the effluent were significantly lower ($p < 0.05$) than the influent concentration with a removal rate of 79.7% and 68.7% in Oxylag, and 77.4% and 36.1% in the HRAP, respectively. Whereas NH₄ and TN concentrations in the Oxylag and HRAP effluents were not significantly different. It is observed that nitrate concentration values in the raw wastewater (Table 4) varied between 0.1 and 2.2 mg L⁻¹. In the subsequent treatment steps, these have higher concentration values that vary between 0.88 and 12 mg N-NO₃ L⁻¹ in the Oxylag effluent and 0.1 and 14.1 mg N-NO₃ L⁻¹ in the HRAP effluent (Table 5). However, nitrate concentration in the raw wastewater, Oxylag and HRAP effluents did not differ significantly ($p > 0.05$).

It seems that the nitrogen removal pathway within the Oxylag is nitrification, as indicated by the significantly lower ammonia and higher nitrate concentrations compared with the raw wastewater. However, nitrate concentrations found in the HRAP and Oxylag effluents are not significantly different compared with the inlet, suggesting that ammonia stripping occurs within the two ponds, where the pH varied between 7.4 and 9.3 in the Oxylag and from 7.6 to 10 in the HRAP system. Nitrifying bacteria have an optimum growth between pH 7.5 and 8.5 and are inhibited below pH 6.5 [57]. Sutherland et al. [58] found that nitrification rates were highest when cultures were maintained between pH 7.0–8.0. In wastewater treatment HRAP, N-NH₄ can be removed by microalgae assimilation, nitrification or volatilization at pH > 9 [59]. In general, NH₄⁺ is the preferred form of nitrogen uptake for most microalgal species, followed by NO₃⁻ [60]. In HRAP, algae biomass assimilation of ammonia and nitrate could occur. However, in this study, it is more plausible to assume that algal assimilation is low considering the low mean algal surface productivity obtained (1.4 g m⁻² d⁻¹), which favors the occurrence of ammonia stripping. In fact, when both nitrification and algal uptake are low, ammonia stripping is promoted [61]. As the present study is the first diagnosis of the WWTDP's operation, a more detailed analysis of the nitrogen removal processes needs to be performed in laboratory scale experiments using the plant effluent.

Likewise, the orthophosphate (P-PO₄) concentrations in the Oxylag and HRAP effluents were significantly lower ($p < 0.05$) compared with the influent, with removal rates of 59.4% and 36.3%, respectively. However, P-PO₄ concentrations in the Oxylag and HRAP effluents were not significantly different. The low P-PO₄ removal efficiency recorded for HRAP could be related to low biomass productivity due to nitrogen and phosphorus limitation or probably to the mixing frequency of HRAP. In fact, the daily operating time of the blower air system is 6 h (5 min ON/15 min OFF). In this condition, the dark period could be increased and most of the algae will passively settle to the bottom of the pond, over time [62]. Conversely, as vertical mixing increases, algal biomass production is enhanced due to more favourable light conditions leading to higher net photosynthesis [58]. Cosgrove [63] suggests that extended darkness could leave algal cells in a chronic photoinhibition state, which may reduce photosynthesis and biomass production.

Finally, the TSS removal rate in the WWTDP was unsatisfactory, with higher values in the effluent compared with the influent found in most cases. This result was expected, as a complementary treatment stage including a harvesting system was not yet installed. Ongoing laboratory research is underway to develop a low cost and sustainable harvesting method that could be effective on a semi-large-scale plant.

3.3. Microalgae Community

Based on three triplicate samples collected over the course of the study (May, August and November 2024), the microalgae group of Chlorophyta was identified. The predominant species were *Scenedesmus*, *Chlorella* and *Closterium* (Figure 5).

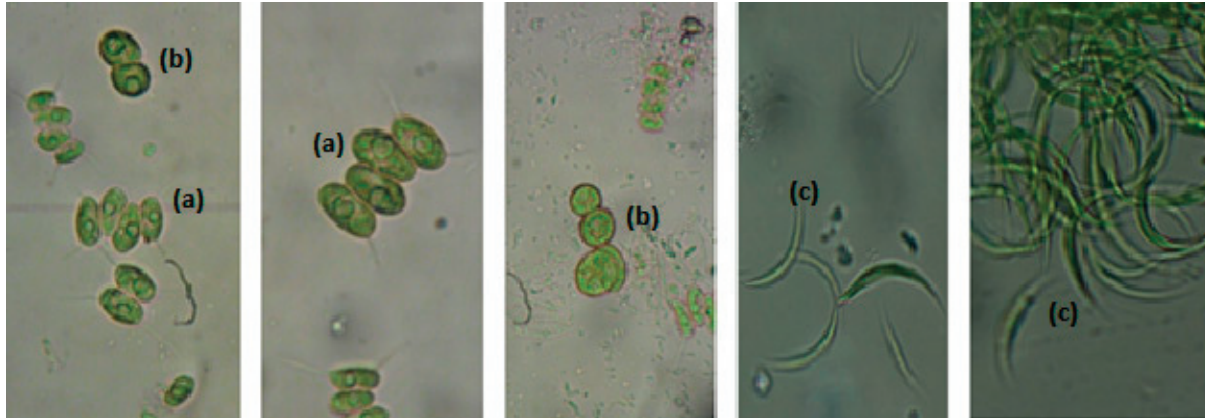


Figure 5. Microalgae community in HRAP. (a) *Scenedesmus*, (b) *Chlorella*, (c) *Closterium*.

Recorded species are known to be commonly identified in the wastewater treatment HRAP with dominant relationships between species. In August, the prevailing microalgae species is *Closterium*, while *Chlorella* and *Scenedesmus* dominate in November and May.

Previous studies of microalgal species in full-scale wastewater treatment in HRAP report that individual species become dominant when they develop adaptations under environmental conditions that give them an advantage over other species or due to biological interactions [64]. Some species are more competitive when water temperature or light are lower or when a major nutrient is poorly supplied. Refs. [30,65] report that influent ammonia concentration strongly influences the dominant species in HRAP. However, the authors note that nutrient removal efficiency in a full scale HRAP is not affected by microalgal species.

3.4. Microalgae Production, SS and VSS Variation in HRAP

During the experimental period, variation of VSS, TSS and algal biomass concentration within the HRAP were monitored from March to May 2024 and are shown in Figure 6. The mean VSS, TSS and algal biomass concentrations for the HRAP were 300.4 g m^{-3} , 468.06 g m^{-3} and 237.52 g m^{-3} respectively. The mean proportion of algae in the volatile suspended solids of the HRAP is 80%. The mean algal productivity in the HRAP varied between 0.2 and $4.1 \text{ g m}^{-2} \text{ d}^{-1}$, with a mean of $1.4 \pm 1 \text{ g m}^{-2} \text{ d}^{-1}$ ($1.75 \text{ g VSS m}^{-2} \text{ d}^{-1}$), which is within the reported range of algal biomass in conventional wastewater treatment ponds, typically less than $2.5 \text{ g VSS m}^{-2} \text{ d}^{-1}$ [23]. However, maximum productivity was recorded in April with a value of $4.1 \text{ g m}^{-2} \text{ d}^{-1}$.

Algal biomass productivity expressed as VSS ($1.75 \text{ g VSS m}^{-2} \text{ d}^{-1}$), is lower than that measured in previous studies of HRAP. Craggs et al. [23] reported a value of mean algal productivity ranging from 4.4 to $11.5 \text{ g VSS m}^{-2} \text{ day}^{-1}$ in four full-scale replicate wastewater treatment HRAPs in New Zealand. Park and Craggs [47] have reported a mean algae biomass production of $20.7 \text{ g VSS m}^{-2} \text{ d}^{-1}$ on a HRAP with an HRT of 4 days and operated with CO_2 addition. A contrast in algal biomass productivity has been observed across HRAP sizes in similar climates [66]. However, the main drivers for increasing algal biomass yields have been explored in the literature. Algal productivity is influenced by several parameters including nitrogen to phosphorus ratio (N:P). In HRAP influent, mean

N:P ratio is about 2.6:1 during the observation period (March to May 2024), which is lower than the range reported in previous studies.

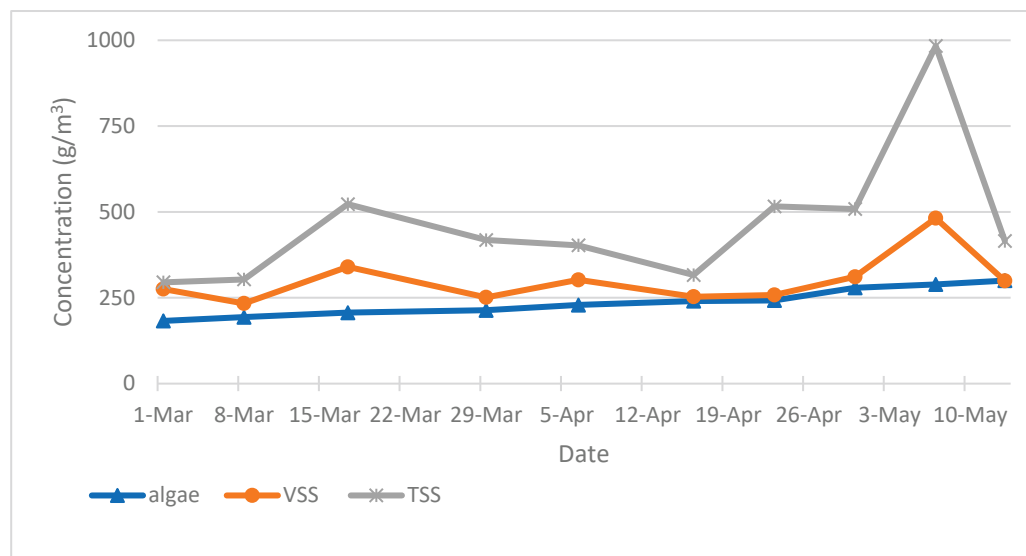


Figure 6. Algal productivity, VSS and TSS variations in HRAP (March, April, May 2024 data).

According to Klausmeier et al. [67], an N:P ratio between 5.5:1 and 17.4:1 was the optimal range. Otherwise, low N:P (<5.5:1) and high N:P (>30:1) imply the limitation of nitrogen and phosphorus, respectively.

Other parameters can affect algal productivity, such as increasing temperature beyond optimal growth conditions, which can reduce algal growth and productivity [35]. Long-term exposure to direct sunlight can also cause photoinhibition, leading to reduced growth cell death in algal cultures [68]. On the other hand, in warm climates, evaporation is high, leading to an increase in salinity that causes osmotic and cellular ionic stress [69].

The lowest algal biomass obtained in the present study could be related to the lack of nutrient in the HRAP. This is probably the reason why ammonia, nitrate and phosphate removals were low in this pond. To increase algal productivity, the operating conditions of Oxylag could be modified to limit the rate of nutrient removal in this pond, making more nutrients available in the HRAP influent. Another option that should be assessed is to discharge some of the raw effluent directly to the HRAP, bypassing the Oxylag.

3.5. Heavy Metal (Hm) Content and Removal in WWTDP

The concentrations of heavy metal (HM) at the inlet and outlet of the WWTDP are shown in Table 6. The concentrations measured in the raw wastewater followed the following order: Cd < Hg < Co < Pb < Cr < Se < Ni < As < Cu < Zn < B < Fe. Those measured in the effluent at the outlet of the WWTDP were in the following order: Cd < Hg < Se < Pb < As < Cr < Cu < Ni < Co < Zn < Fe < B. No significant differences ($p > 0.05$) were observed between the concentrations monitored in the inlet and outlet. Nevertheless, HM concentrations are in accordance with the Tunisian standards (NT 106.03) for treated wastewater reuse, except for Hg content. The effluent Hg concentration varies between 0.001 and 3.71 $\mu\text{g L}^{-1}$, with the highest concentrations of 1.08 and 3.71 $\mu\text{g L}^{-1}$ observed during April and May 2024, respectively. Unlike the findings of previous studies on the efficiency of algae in removing metal pollutants [17,70], the removal efficiency of HM in the HRAP seems to be relatively low. The use of microalgae for heavy metal removal was first proposed by Oswald [71] and has been increasingly continued, with field experiments undertaken regarding the effect of factors affecting the efficiency of heavy metal

removal, including pH, light regime, N:P mass ratio, algae biomass and species, contact time, temperature and ion strength [70]. The lowest HM removal could be linked to the low algal productivity in HRAP as a result of nutrient limitation. Wang et al. [70] suggest that the algal HM removal efficiency depends largely on nutrient availability. A positive correlation between HM removal and algal growth was noted [70]. The HM removal in wastewater treatment plants involves several mechanisms such as adsorption on algal cells and suspended particles, sedimentation and complexation with organic or mineral colloidal elements [72].

Table 6. Heavy metal content in both influent and effluent compared with Tunisian standards (NT 106.03) [40].

Heavy Metal	Mean Concentration $\pm$ Std ($\mu\text{g/L}$)		
	Inlet Raw Wastewater	Outlet HRAP Stage	NT 106.03
Cr	2.19 ± 1	1.01 ± 1	100
Fe	2206.94 ± 1853	243.79 ± 124	5000
Ni	4.39 ± 1.7	8.12 ± 7	200
Cu	18.48 ± 21	7.75 ± 3	500
Zn	55.89 ± 54	87.92 ± 56	5000
Cd	0.15 ± 0.14	0.13 ± 0.1	10
Hg	0.45 ± 0.8	0.95 ± 1	1
Pb	2.09 ± 1.6	1.58 ± 1.4	1000
As	6.90 ± 2.8	4.57 ± 3	100
B	312.02 ± 167	268.67 ± 61	3000
Co	1.06 ± 1	2.21 ± 2	100
Se	4.26 ± 5	1.21 ± 1	50

In our case, the low HM removal in HRAP could also be attributed to the potential release of heavy metals from the sludge accumulated in the Oxylag, whose HM concentration is shown in Figure 7a. Indeed, the mixing conditions within the Oxylag pond results in sediment disturbance, which leads to a remobilization of HM as a result of change in the chemical proprieties of sediment [73]. Therefore, there is an urgent need to identify sources of Hg contamination and take suitable measurements to avoid their discharge in wastewater. The potential source of Hg in the raw wastewater could be related to the activities of the chemical laboratory, where chemical reagents containing mercury compounds are used in research and practical works. However, the full identification of the relationship between the mercury content of the wastewater and the potential source of this metal remains an unquantified assumption.

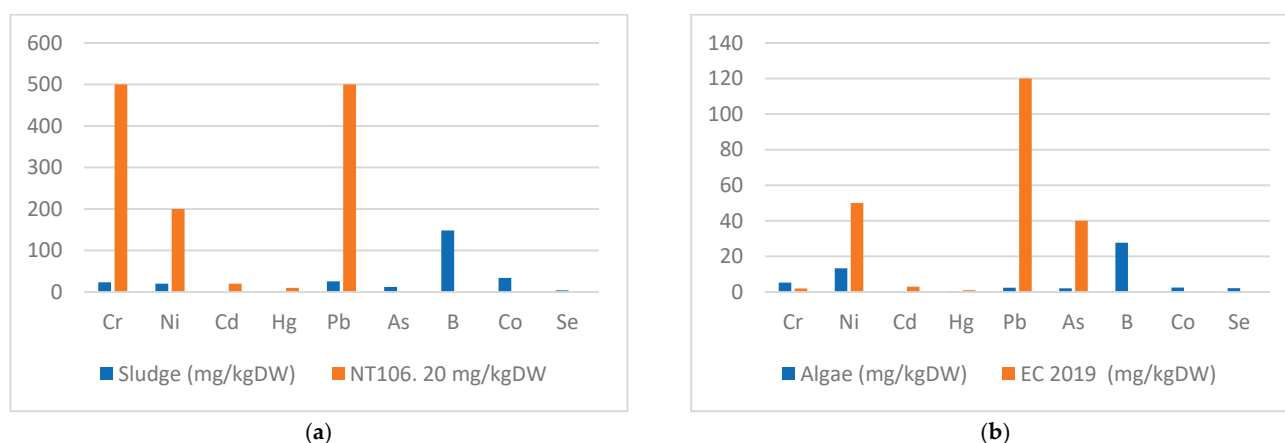


Figure 7. Heavy metal content in sludge (a) and algae (b).

Previous studies have confirmed the high ability of several algae species such as *Chlorella* and *Scenedesmus* in heavy metal removal from wastewater [74–76]. However, most research studies have been conducted in lab-scale systems operated under constant temperature, pH, initial concentration of metal and contact time, which could not provide an accurate description of system performance under outdoors conditions [77].

Mean HM concentration measured on algae samples collected from HRAP showed the following order: $\text{Cd} < \text{Hg} < \text{As} < \text{Se} < \text{Pb} < \text{Co} < \text{Cr} < \text{Ni} < \text{Cu} < \text{B} < \text{Zn} < \text{Fe}$, whereas the mean HM concentrations measured on sludge samples collected from Oxylag pond followed the following trend: $\text{Cd} < \text{Hg} < \text{Se} < \text{As} < \text{Ni} < \text{Cr} < \text{Pb} < \text{Co} < \text{B} < \text{Cu} < \text{Zn} < \text{Fe}$. Iron is the most dominant heavy metal both in the effluent, algae biomass and the sludge with respective mean concentrations of $2206.96 \pm 1853 \mu\text{g L}^{-1}$, $1858 \pm 111 \text{ mg kgDW}^{-1}$, and $28,356 \pm 6805 \text{ mg kgDW}^{-1}$.

The results suggest that HM adsorption on algal cells and suspended particles as well as sedimentation and complexation with organic or mineral colloidal elements occur during the treatment process.

An evaluation of heavy metal content of algae biomass was carried out to assess compliance with the European regulation (EC, 2019/1009) [78] on fertilizer products and to confirm their retention efficiency in the HRAP system. Although the regulation (EC, 2019/1009) does not cover algal biomass harvested from wastewater and used as biofertilizer, it could be an alternative to organo-mineral fertilizers due to its abundance in nitrogen, phosphorus and micronutrients [28]. The algae's HM concentration is in accordance with the European regulation (EC, 2019/1009) for reuse, except for the chromium content of the algae (Figure 7b). Cr concentration varied between 4.6 and 5.8 mg kgDW^{-1} which exceeds the limit of the European regulation (2 mg kgDW^{-1}). Heavy metal contents in wastewater-grown algae have been reported by Markou et al. [79]. The authors report that HM concentrations in algae using agro-industrial wastes were in the ranges of 13.5–44.5 mg kgDW^{-1} , 226–333 mg kgDW^{-1} and 9.2–15.1 mg kgDW^{-1} for Cd, Cr and Hg, respectively. Alvarez-Gonzalez et al. [28] report heavy metal contents in wastewater-grown algae biomass of 3.10 mg kgDW^{-1} for Cd, 1.31 mg kgDW^{-1} for Cr, 46.5 mg kgDW^{-1} for Ni, 0.52 mg kgDW^{-1} for Hg, 46.5 mg kgDW^{-1} for Pb, 279 mg kgDW^{-1} for Cu and 437 mg kgDW^{-1} for Zn. The authors suggest that, despite these results on heavy metal concentrations, algae biomass could be used for agricultural purposes.

Regarding the sludge Hm content, the recorded concentrations are in accordance with the Tunisian standards (NT 106.20) [80] (Figure 7a) for reuse in agriculture as fertilizer, even for both Hg and Cd, with concentrations of 1.12 ± 0.02 and $0.63 \pm 0.01 \text{ mg kgDW}^{-1}$, respectively. The limit values of NT 106.20 for Hg and Cd are 10 and 20 mg kgDW^{-1} , respectively. Therefore, this sludge can be reused as an organic fertilizer where the amount of organic matter in the soil is poor and the price of organic fertilizer is high. These results are consistent with the study reported by Marzougui et al. [81] on sludge produced by five Tunisian WWTPs to promote sustainable sludge use and management for agriculture. The authors report that Hg and Cd concentrations varied from 0.57 and 1.3 mg kgDW^{-1} for Cd and from 0.4 and 1.2 mg kgDW^{-1} for Hg, they have shown that, in terms of cadmium, chromium, copper, mercury, lead, zinc, and nickel, the results comply with Tunisian standards (NT 106.20) with an agricultural reuse for fodder, industrial and food crops.

In conclusion, the WWTDPP of the university campus of Chott Mariem is the only small scale combined HRAP and Oxylag system in Tunisia and is an opportunity of a practical case study of a real scenario where microalgae cultivation systems are exposed to the fluctuating environmental and physicochemical conditions. Therefore, additional monitoring studies with high sampling frequency approaches are needed and caution should be considered for the reuse of treated wastewater on the campus site. However,

we did not directly test whether the sludge was a source and/or sink of heavy metals. In order to optimize the removal of heavy metals and to identify the factors affecting algae adsorption, metal sludge accumulation and release, further studies should be carried out under controlled environmental conditions. The investigation of the potential sources and sinks of harmful compounds remains of great interest.

3.6. Bacteria Removal

In this study, total coliform (TC) concentration ranged from 7.32 to 8.35 log₁₀ in inlet samples and from 2.87 to 4.17 log unit in the effluent samples. The fecal coliform (FC) concentration ranged from 7.07 to 7.53 log units in inlet samples and from 2.65 to 3.07 log units in outlet samples. Furthermore, the average removal rates of TC and FC were 4.16 and 4.36 log units, respectively. It was observed that the removal rate of total and fecal coliforms was about 99.99%.

The reduction in coliform concentration in the effluent indicates the ability of the COHRAP to remove bacteria. HRAP is known for its efficiency in removing bacteria due to ultraviolet light. Algae are known to act as bactericides, thus reducing the ability of pathogenic bacteria to proliferate [82]. Pathogens could also be removed from the liquid fraction by sedimentation after adsorption to solid particles [83]. Pathogen removal in HRAP depends on several factors, such as sunlight, pH, dissolved oxygen (DO) and temperature, as well as predation [84]. However, Chambonniere et al. [85] have mentioned that the reduction of fecal coliforms does not necessarily imply the removal of various pathogens (e.g., viruses, helminth eggs or protozoa) and suggest that it is imperative to investigate the ability of the WWTP to remove different pathogens in order to avoid potential risk in water reuse.

In comparison with previous studies, the average removal rates obtained are similar to those reported by Albornoz et al. [86] for a compact wastewater treatment plant that included biological treatment, a secondary clarifier and a disinfection tank on a university campus in Brazil. The authors report a removal rate reaching 95%. Removal efficiencies of 5.66 and 5.63 log units for TC and FC, respectively, were reported by Abd-Elmaksoud et al. [56] in a wastewater treatment plant in Egypt, including three treatment stages (i.e., facultative pond, HRAP, and rock filter).

Considering the Tunisian standards (NT 106.03) for the reuse of treated wastewater, which defines the list of crops that can be irrigated with treated wastewater (e.g., industrial, cereal and fodder crops, fruit trees) and considers a limit for helminth eggs (<1 egg/L) and not for bacteria, the treated effluent provided by the WWTDPP and intended for irrigation of fruit trees, cereal crops and ornamental plants could be suitable (in terms of microbiological quality) for reuse at the Chott Mariem University campus. On the other hand, these promising results for bacterial removal need to be confirmed for other pathogens such as helminth eggs and *E. coli* pathogens. To prevent potential pathogen risk, selecting appropriate irrigation management (e.g., drip irrigation), regularly monitoring effluent and utilizing preventive measurements to protect workers from wastewater handling can help to minimize the risk. However, TSS compounds in the effluent need to be consolidated by algae recovery before drip irrigation can be implemented.

3.7. Phytotoxicity of Treated Wastewater and Potential Reuse for Agriculture Purposes

Preliminary trials were conducted in Petri dishes to evaluate the effect of treated wastewater irrigation on germination and growth of *Zea mays* L., aiming to ensure the safe use of treated wastewater. The results indicate that treated wastewater had no significant effect on germination, as both germination rate (GR) and germination index (GI) showed no significant differences between the control and seeds irrigated with HRAP effluent ($p > 0.05$,

Duncan test). The GR was 100% for both groups. The GI, expressed as a percentage of the control, was $97.44 \pm 0.16\%$, indicating that the treated seeds exhibited similar germination dynamics to the control. Similarly, no significant differences were observed in growth parameters between the control and the wastewater irrigated seeds. Specifically, the root and shoot lengths for the control were 7.73 ± 0.17 cm and 1.63 ± 0.08 cm, respectively, compared with 7.42 ± 0.21 cm and 1.57 ± 0.04 cm for the treated seeds. These results suggest that treated wastewater is not phytotoxic to the target species. Given the large area of forest plants, fruit trees and fodder crop as well as ornamental plants on the university campus of Chott Mariem, a possible reuse of the treated wastewater appears as an interesting alternative to address the water deficit across the campus area.

However, further experiments should be carried out to confirm this finding. In fact, the effect of the reuse of WWTDP effluent on plant growth, in pots experiments, are part of our ongoing research. Furthermore, field trials could be conducted to assess the potential microbial risk and to ensure the benefits of reusing treated water for plants and soil. Improving the quality of treated water for further non-restrictive crop irrigation could be considered in future research.

Finally, considering the current regulation in Tunisia (NT 106.03) for the reuse of water in agriculture with restrictive crop irrigation, the results obtained in this study have limitations with regard to TSS and Hg, as the limit values are sometimes exceeded (Table 5). However, even if the limits for all parameters were respected, bioaccumulation of HM after long-term irrigation remains a challenge.

3.8. Operational Costs of WWTDP

The annual operating costs of the WWTDP include the costs of personal, electricity and sludge management (Table 7). The results indicate that energy and sludge management are the two units with the highest costs. The energy consumption expressed in kWh per 1 m^3 of treated water is 1.05 kWh m^{-3} , which corresponds to an average operating cost of 0.29 EUR m^{-3} of treated wastewater, assuming the price of 0.156 EUR/kWh . This energy consumption includes both air blower of Oxylag and HRAP and wastewater pumping.

Table 7. Energy consumption and cost categories of WWTDP.

Energy Consumption				Electricity Costs	
kWh d ⁻¹	kWh yr ⁻¹	kWh PE ⁻¹ yr ⁻¹	kWh m ⁻³	EURyr ⁻¹	EURPE ⁻¹ yr ⁻¹
10.5	3832.5	38.32	1.05	601	6.01
Working Hours (h)				Personnel Costs	
100 h yr ⁻¹				300 EUR/yr	
Sewage Sludge Management				Sludge Management Costs	
20 m ³ yr ⁻¹				320 EUR/yr	
TOTAL COST: 1061 EUR yr ⁻¹					

In literature, limited studies dealing with energy consumption are available using a combined HRAP, particularly on small scale treatments plants. Nevertheless, the obtained results are higher than those reported by Kohelhb et al. [87] on a demonstration scale HRAP treating $300 \text{ m}^3 \text{ d}^{-1}$, including with an energy consumption of 0.10 kWh m^{-3} in the presence of submersed mixed technology. Whereas the energy consumption is about 0.17 kWh m^{-3} with a simple paddle wheel mixing system. Robles et al. [88] has reported an energy requirement of around 0.29 kWh per m^3 of treated water on a combined membrane–HRAP system with a working volume range comparable to that of COHRAP.

It should be noted that the installed blower has a higher oxygenation capacity than the required oxygenation, as it is the smallest blower range available on the market. In our

case, lower energy consumption could be achieved by reducing the frequency of blower operation. However, the oxygen concentration in the Oxylag should be maintained at 2.5 mg L^{-1} and the flow velocities in HRAP and Oxylag must be respected.

4. Conclusions

The implementation of WWTDPP on the university campus of Chott Mariem could provide an alternative water resource for irrigation. It would provide a concrete example of innovative water treatment and reuse of water and bio-products within the campus and the wider environmental community. In addition to promoting sustainability and resource recovery, the implementation of the demonstration plant will provide and disseminate scientific knowledge on HRAP and Oxylag technologies.

The studied WWTDPP presents a good performance. However, improvement measures are needed in terms of monitoring, regulatory compliance and energy performance in order to establish the groundwork for future optimization.

In terms of monitoring and control strategies, continuous flow measurement and automated sampling have been suggested and are currently being implemented to ensure reliable monitoring and greater accuracy of the results.

Considering the current regulation in Tunisia (NT 106.03) for wastewater reuse in agriculture with restrictive crop irrigation, the treated effluent provided by the WWTDPP have limitations with regard to TSS and Hg, as the limit values are sometimes exceeded. The implementation of an algae recovery system downstream of the HRAP is necessary to improve TSS and Hg removal. In addition, it is suggested that the frequency setting of the variable speed blower drive and the operating time should be changed in order to reduce energy consumption and the rate of oxygen supply to the Oxylag. Nitrogen and phosphorus removal occurring in the Oxylag should be minimized to make these elements more readily available in the HRAP influent. Thus, nutrients in the HRAP will improve algae productivity, which is a valuable resource for items such as biofertilizer. On the other hand, the operational strategy of flow and retention time adjustment must be integrated, taking into account the different periods of the academic year. A more detailed critical analysis of the effect of loading rate on efficiency removal will be undertaken to identify opportunities for improvement. Regarding heavy metal content in the effluent, there is an urgent need to identify the source of Hg contamination and take appropriate measures to prevent its discharge. In addition, further studies should be performed to investigate the factors affecting heavy metal algae adsorption and release.

Although the levels of TSS and Hg were occasionally higher than the Tunisian standards (NT 106.03), the phytotoxicity tests show no significant differences between the control and the seeds irrigated with HRAP effluent. Hence, reuse of effluent for the irrigation of fruit trees, cereal crops and ornamental plants could be appropriate on the campus site. In addition, the algae biomass and the sludge comply with the requirements of the European regulation on fertilizers (EC, 2019/1009) and the Tunisian national standard (NT 106.20) regarding sludge reuse in agriculture, except for the chromium content of the algae. However, even if the limits for all parameters were respected, the bioaccumulation, persistence, occurrence and toxicity of heavy metals and harmful pollutants after long-term irrigation and reuse of sludge and algae as biofertilizers remains a challenge.

In the context of sustainable development, the combined HRAP-Oxylag system is a viable and promising alternative for wastewater treatment with valuable by-products.

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Abbreviations

COHRAP	Combined Oxylag and high rate algal pond
COD	Chemical oxygen demand
BOD	Biochemical oxygen demand
TSS	Total suspended solids
HRAP	High rate algal pond
PE	Population equivalents
WWTDPP	Wastewater treatment demonstration pilot plant
HDPE	High density polyethylene membrane
HRT	Hydraulic retention time
P_a	Algae productivity
TN	Total nitrogen
HM	Heavy metal
GR	Germination rate
GI	Germination indices
ISA-CM	Higher agronomic institute of Chott Mariem
FP	Facultative pond
RF	Rock filter
DW	Dry weight
TC	Total coliforms
FC	Fecal coliforms

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Hybrid Coagulation–Membrane Filtration Techniques for Sustainable Soap Wastewater Treatment

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Abstract: Wastewater from soap production often contains high levels of organic pollutants, exceeding regulatory discharge limits and posing significant environmental concerns. This study investigates a two-stage treatment approach integrating ferric chloride (FeCl₃)-based coagulation–flocculation with membrane filtration to enhance wastewater purification efficiency. This method is one of the appropriate treatment techniques to reduce water pollution. Thus, numerous Jar test trials have been carried out in order to determine the optimal conditions and parameters that make it possible to reduce suspended solids. Key water quality parameters, including chemical oxygen demand (COD), pH, and turbidity, were monitored to assess process performance. Optimization experiments identified optimal coagulation–flocculation conditions, achieving a substantial COD reduction from 9200 mg/L to 351 mg/L significantly improving water quality. However, the treated effluent still failed to meet reuse standards, necessitating further purification. A subsequent membrane filtration stage was implemented, achieving a significant decrease in turbidity to 0.85 Ntu and a turbidity removal efficiency of 99.97%, indicating high treatment efficiency. The final COD of the collected water was 58 mg/L, well below regulatory limits. This hybrid treatment approach offers a highly effective and sustainable solution for soap wastewater management, supporting environmental protection and resource recovery.

Keywords: wastewater treatment; ferric chloride; soap industry; coagulation; flocculation; membrane filtration; water reuse

1. Introduction

Global water resources are increasingly strained due to population growth, industrial expansion, and agricultural activities, leading to pollution and the urgent need for effective wastewater treatment. Recycling treated wastewater is vital for water conservation

and ecosystem preservation. In Algeria, this is reflected in the implementation of over 137 treatment plants reclaiming approximately 16 million cubic meters annually [1]. Among the major industrial polluters is the soap and detergent industry, which consumes large volumes of water and generates wastewater with high variability and pollution loads, including temperature, color, suspended solids, biochemical oxygen demand (BOD), chemical oxygen demand (COD), and surfactants [2]. These pollutants are difficult to remove due to their colloidal nature, surface charge, and small size, often rendering conventional treatment methods ineffective.

Pollutants are challenging to remove due to their surface charge and small size. Various methods, including coagulation–flocculation, ion exchange, precipitation, adsorption, biological processes, and advanced oxidation, have been employed to eliminate colloidal particles from wastewater [3–5]. However, conventional methods such as biological treatment, sedimentation, or simple filtration often fall short when dealing with complex soap wastewater due to high concentrations of surfactants, oils, and other resistant compounds [4]. Among all treatment methods, coagulation–flocculation is one of the oldest and most essential for most water and wastewater treatments. This process has gained increased attention as it has proven effective in removing various contaminants, including toxic organic matter, heavy metals, and viruses [5–7]. Due to its high efficiency in contaminant removal, operational simplicity, effectiveness, and affordability, this process has been widely applied to the treatment of factory effluents [8,9]. It involves a two-phase process aimed at removing stable colloids from water by forming larger aggregates, separable by sedimentation. The first phase involves adding a coagulant to the water to destabilize the particles by reducing the repulsive forces between the colloids through neutralization of their negative charges. The second phase involves flocculating the destabilized particles, forming larger aggregates separable by sedimentation [10]. Nevertheless, suspended particles vary considerably in terms of source, composition, charge, size, shape, and density. The appropriate selection of coagulants and the correct application of coagulation and flocculation processes depend on understanding the complex interaction between these factors [11]. To overcome these limitations and promote green chemistry within wastewater treatment, hybrid processes integrating coagulation–flocculation with membrane filtration have emerged as promising solutions for enhanced overall performance. This synergy offers several key advantages; it improves pollutant removal by capitalizing on the strengths of each technique but also aligns with the principles of sustainability [12]. Coagulation reduces bulk pollutants and membrane fouling, while membrane filtration ensures high-level removal of residual turbidity, organic matter, and surfactants. It also enhances virus removal and demonstrates significant improvements in treating specific industrial wastewaters like soapy and laundry effluents [12–14]. Notably, the development and implementation of sustainable green solvents in membrane technology and related processes are crucial for minimizing environmental impact and fostering a green environment [15].

Numerous studies have explored coagulation–flocculation for treating soap and detergent wastewater, demonstrating varying degrees of success. Aboulhassan et al. [16] demonstrated that coagulation–flocculation with FeCl_3 can be effectively used to treat soap factory wastewater, eliminating surfactants and COD. The treatment was optimal between pH 7 and 9, achieving 99% surfactant removal and 88% COD reduction. Additionally, the 5-day biochemical oxygen demand BOD_5/COD ratio increased from 0.17 to 0.41. Sharghi et al. [17] optimized FeCl_3 dosage with anionic flocculants, which resulted in 80.8% COD removal and good turbidity removal. The optimal condition was 1000 mg/L of FeCl_3 with an anionic flocculant, improving biodegradability. Maung et al. [18] studied highly polluted soap industry wastewater, finding that it exceeded Indian discharge

standards (BOD 43,000 mg/L, COD 99,200 mg/L). They proposed two treatment plant designs including coagulation–flocculation with alum: one using anaerobic and activated sludge biological treatment, and the other using aerobic biological treatment. Both designs successfully reduced BOD to 347 mg/L, thus meeting discharge standards. Ali et al. [19] tested two flocculants, aluminum chloride–polyacrylamide (AlCl_3 -PAM) and oat grafted with polymethyl methacrylate (OAT-g-PMMA), for treating soapy wastewater. The first one removed 82% of COD and 81.5% of TSS, compared to 63% and 66% for oat grafted with polymethyl methacrylate (OAT-g-PMMA). The treated effluents met Egyptian standards for irrigation.

However, significant variations in wastewater composition and treatment conditions highlight the need for further optimization. Hybrid processes, such as coagulation coupled with membrane filtration, have shown promise in improving treatment efficiency by addressing membrane fouling and enhancing organic matter removal [20–22]. Combining coagulation with membrane filtration enhances virus removal by incorporating them into larger flocs, improving membrane retention and creating a robust hygienic barrier [23]. This hybrid approach also reduces membrane fouling and increases the removal of organic matter that would otherwise permeate the membrane [24]. Louhichi et al. [25] demonstrated that combining coagulation–flocculation (CF) with ultrafiltration (UF) using a 5 kDa membrane significantly enhances the treatment of soapy water effluents. While CF alone achieved 99% turbidity and 27% COD removal, the CF/UF hybrid process resulted in 100% turbidity and 88% COD removal, displaying its strong potential. Huang et al. [26] developed a multi-stage laundry wastewater treatment process, combining 140 mg/L natural coagulant for coagulation–flocculation, 10 h equilibrium activated carbon adsorption and $60.51 \text{ L h}^{-1} \text{ m}^{-2}$ microfiltration. The combined process effectively improved water quality, with removals of 99.9% for color, 80% for COD, 92.9% for surfactants, and 99.4% for turbidity.

This study aims to evaluate the effectiveness of combined treatment processes for wastewater from the soap industry with high surfactant content. These processes include coagulation–flocculation, followed by membrane filtration, with the goal of obtaining high-quality water. Another more specific objective of this work is to determine the appropriate coagulant, its proper dosage, and to study the optimal experimental conditions for the pollutant removal capacity of wastewater from soap production. The industrial effluent addressed here is highly alkaline, rich in surfactants and organic matter, and presents a unique challenge due to its complex chemical matrix. Moreover, we utilize a lab-prepared microfiltration membrane synthesized by the phase inversion technique with a green solvent system. By specifically addressing the intricate characteristics of soap industry wastewater and utilizing a sustainably produced membrane, this research contributes novel insights to the application of hybrid coagulation/flocculation and membrane filtration for this specific industrial effluent, offering practical guidance for optimizing its treatment. This integrated approach, driven by the principles of green chemistry, underscores the importance of innovative solutions in membrane technology for achieving sustainable wastewater treatment.

2. Materials and Methods

2.1. Source of Wastewater

For our study, effluents were collected from a soap production factory “PROLIPOS” located in Ain Mlila in the east of Algeria, producing large quantities of effluent during the production process by saponification. Industrial soap manufacturing wastewater presents a complex chemical profile. Characterized by a highly basic sodium hydroxide solution and saponification byproducts, both contributing to elevated pH levels [27]. The

wastewater also contains a mixture of residual vegetable oils, unconverted fatty acids, and unrecovered soap compounds, indicating incomplete reactions and product wastage, alongside dissolved sodium and chloride ions originating from various stages of the manufacturing process.

Following the physicochemical characterization of raw wastewater (Table 1), a significant chemical pollutant load was identified in the soap effluent. Notably, turbidity exceeded regulatory limits, the pH was alkaline, and the chemical oxygen demand (COD) reached a substantial 9200 mg/L far surpassing the Algerian discharge standard of 120 mg/L [28]. Similarly, the biochemical oxygen demand (BOD₅) was measured at 662 mg/L, also exceeding regulatory standards (35 mg/L) [28], indicating a high concentration of organic pollutants in the raw wastewater.

Table 1. Raw water physicochemical properties.

Temperature (°C)	pH	Salinity (g/L)	Conductivity (ms/cm)	Turbidity (NTU)	COD (mg/L)	BOD ₅ (mg/L)
27.3 °C	12.83	2.6	5.4	3235	9200	662

2.2. Coagulation—Flocculation

The efficiency of coagulation–flocculation is affected by several critical parameters, including temperature, pH, coagulant type, dosage, and mixing intensity [27]. To optimize these parameters for treating our wastewater, a series of Jar test experiments [29] were conducted, focusing on the performance of iron(III) chloride (FeCl₃) as a coagulant. These tests evaluated the impact of varying conditions on chemical oxygen demand (COD) reduction, pH adjustment, and turbidity removal. The primary reagents employed in the coagulation–flocculation process (Figure 1) were Aluminum Sulfate (Al₂(SO₄)₃) and Iron(III) Chloride (FeCl₃); MERCK brand.

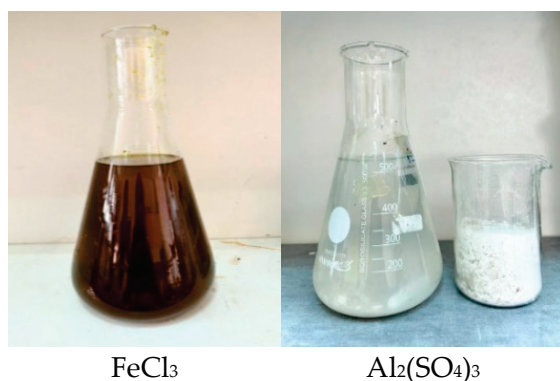


Figure 1. Reagents employed in the coagulation–flocculation process.

A standard Jar test procedure, following the guidelines outlined by Iwuozor [29], was employed to optimize the coagulation–flocculation treatment of soap factory effluent. The experiments were conducted using a six-beaker “Lovibond” model MT 740 Jar tester, with each one-liter beaker equipped with a rotating paddle connected to a common motor, ensuring uniform mixing across all samples (Figure 2) The Jar test procedure involves three distinct stages: coagulation, flocculation, and sedimentation.

1. Coagulation: Rapid mixing was applied at 150 rpm for 5 min immediately following the addition of the FeCl₃ coagulant.
2. Flocculation: Slow mixing was then carried out at 30 RPM for 20 min to promote floc growth.

3. Sedimentation: The samples were allowed to settle without mixing for 30 min.



Figure 2. Jar test apparatus—Lovibond model MT 740.

The coagulants tested in this study were Aluminum Sulfate ($\text{Al}_2(\text{SO}_4)_3$) and Ferric Chloride (FeCl_3), both widely used in industrial wastewater treatment. No additional polymeric flocculants were applied to isolate the performance of each coagulant. The experiments investigated a range of coagulant dosages: 0.8 g/L, 1 g/L, 1.2 g/L, 1.4 g/L, and 1.6 g/L. The initial characteristics of the soap factory effluent were as follows: COD = 9200 mg/L, BOD₅ = 662 mg/L, turbidity = 3235 NTU, and pH = 12.83. All experiments were conducted at an ambient temperature range of 22–25 °C.

After the 30 min sedimentation period, 20 mL supernatant samples was carefully siphoned from the middle depth of each beaker to avoid disturbing the settled sludge. These samples were then analyzed for COD and BOD₅ according to the Standard Methods for the Examination of Water and Wastewater.

2.3. Membrane Filtration

In this study, membrane filtration is implemented as part of a hybrid process, following coagulation–flocculation, to enhance the treatment of the soap factory wastewater collected. This combined approach serves as a polishing step, further improving the characteristics of the pretreated water and ensuring the achievement of our specific treatment objectives.

The selection of membrane type, thickness, and operating pressure are critical parameters for determining the suitability of a membrane for effective water treatment. Membranes are categorized based on their structure, materials, and manufacturing processes [30]. The membrane employed in this study is an organic microfiltration membrane, synthesized by using the phase inversion technique [31]. This method facilitates the production of filtration membranes from a collodion solution. The solution contains triethyl phosphate (TEP) used as an alternative solvent, in which a blend of polymers, including poly(methyl methacrylate) (PMMA), poly(vinylidene fluoride) (PVDF) and other additives such as polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG), are dissolved [32,33]. Triethyl phosphate (TEP) has been used as green and no harmful solvent, in accordance with the concept of sustainable development and green chemistry principles [34]. TEP is not teratogenic, carcinogenic, or mutagenic, and as stated in its safety datasheet “contains no components considered to be either persistent, bioaccumulative and toxic, or very persistent and very bioaccumulative at levels of 0.1% or higher” [35].

The membrane was previously characterized in terms of morphology using Scanning Electron Microscopy (SEM), average pore diameter, contact angle, thickness, and mechanical strength [31]. The results of these characterizations are compiled in Table 2.

Table 2. Membrane Characteristics [32].

Membrane Code	Thickness (mm)	Porosity (%)	Mean Pore Diameter (μm)	Young's Modulus (N/mm^2)	Lengthening at Break (%)	Contact Angle ($^\circ$)
M-0.12	0.063 ± 0.003	79.48 ± 1.03	0.35	114.33 ± 4.88	28.16 ± 1.40	104.6 ± 3.3

The experimental setup used is a laboratory-scale test bench (Figure 3) equipped with a crossflow filtration cell with viewport for photocatalytic application designed by DeltaE SRL (Rende (CS) Italy) (Figure 4). The system consisted of a 2 L capacity feed reservoir, an ESMATEC-IDEX peristaltic pump and valves to ensure the circulation of the water to be filtered, linked together with polymeric flexible pipes. Two pressure gauges are placed at the inlet (P1) and outlet (P2) of the filtration cell. All experiments were carried out at room temperature.

**Figure 3.** Membrane filtration setup.**Figure 4.** Crossflow membrane filtration cell.

Figure 5 illustrates the experimental setup employed in this study. Filtration experiments were conducted with the following process parameters: a feed volume of 1 L, a flow rate of approximately 2 L per minute, and a pressure range of 0.5 to 2 bars. A flat sheet membrane with a 47 mm diameter, providing an effective surface area of 0.0013 m^2 , was supported within the filtration cell by a stainless-steel grid. Before each filtration test, the membrane was cleaned by recirculating purified water through it for 30 min. The feed solution for the filtration experiments consisted of water that had undergone coagulation–flocculation treatment. During filtration, the retentate was recirculated back to the feed reservoir, while the permeate (filtered treated water) was collected and its characteristics measured.

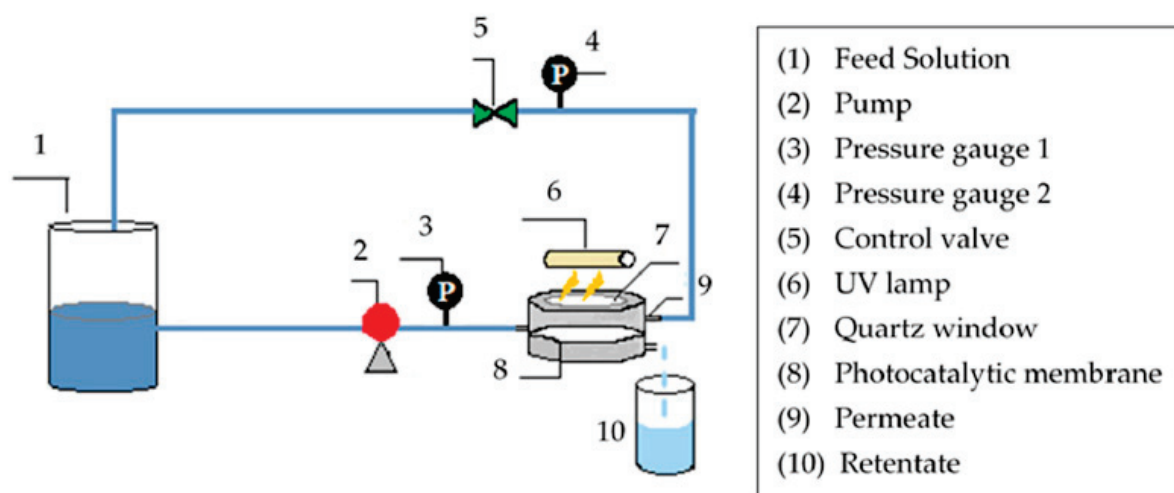


Figure 5. Schematic of the crossflow system [33].

3. Results and Discussion

3.1. Coagulation Flocculation Treatment

3.1.1. Selection of the Appropriate Coagulant

The experimental study is divided into two main parts. The first part focuses on characterizing the soap manufacturing wastewater and conducting experiments using the coagulation–flocculation technique to identify a suitable coagulant for our specific effluent. This will be followed by a parametric investigation of the operating conditions for the coagulation–flocculation process to optimize treatment of our wastewater. The second part presents the results of membrane separation treatment conducted in a crossflow reactor.

Before optimizing the operating conditions by a parametric study, a feasibility test was carried out to allow the selection of the appropriate coagulant. The first preliminary tests were carried out, on the Jar test device, with two types of coagulants $\text{Al}_2(\text{SO}_4)_3$ and FeCl_3 at a concentration of 1 g/L, under the same operating conditions:

- Coagulation speed: 150 rpm, coagulation time: 5 min;
- Flocculation speed: 30 rpm, flocculation time: 20 min;
- Settling time: 30 min.

The evaluation of the coagulation–flocculation performance was carried out by monitoring the turbidity and pH values before and after treatment. The preliminary treatment results for Ferric Chloride (FeCl_3) and Aluminum Sulfate ($\text{Al}_2(\text{SO}_4)_3$) are presented in Figures 6 and 7, respectively. A notable difference in treated water quality is evident between the two coagulants. Specifically, the Ferric Chloride treatment demonstrated a significantly superior outcome. The corresponding turbidity and pH removal efficiencies for both coagulants are summarized in Table 3.

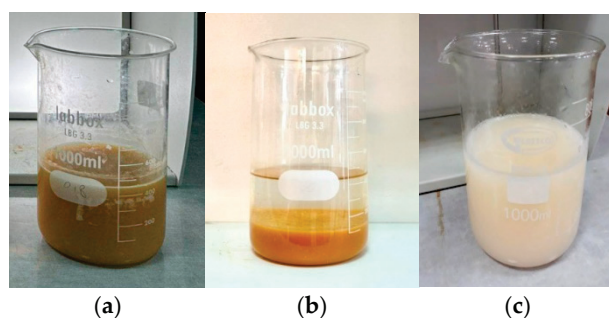


Figure 6. Preliminary treatment results for Ferric Chloride (FeCl_3). (a) Raw water; (b) After coagulation–flocculation; (c) After settling.

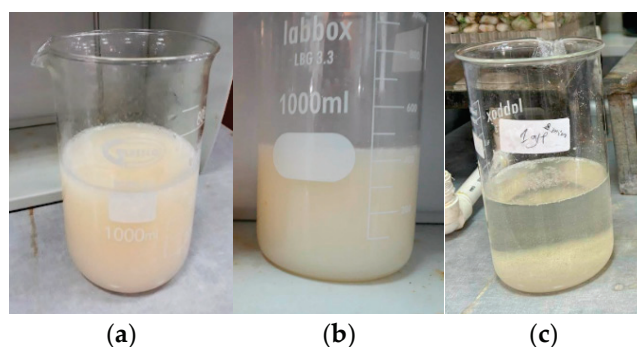


Figure 7. Preliminary treatment results for Aluminum Sulfate ($\text{Al}_2(\text{SO}_4)_3$). (a) Raw water; (b) After coagulation–flocculation; (c) after Settling.

Table 3. Preliminary treatment results.

Coagulants	pH Before	pH After	Turbidity Before (NTU)	Turbidity After (NTU)	Turbidity Removal Efficiency (%)
$\text{Al}_2(\text{SO}_4)_3$	12.83	6.07	3235	1531	32.67%
FeCl_3	12.83	8.80	3235	1025	68%

The removal efficiency was calculated using the following formula [36]:

$$\text{Efficiency}(\%) = [(C_0 - C_1)/C_0] \times 100 \quad (1)$$

where C_0 is the initial concentration and C_1 is the final concentration after treatment.

The data indicate a substantial reduction in turbidity when using FeCl_3 compared to $\text{Al}_2(\text{SO}_4)_3$. Given its superior performance in turbidity reduction, FeCl_3 was selected for subsequent parametric studies.

3.1.2. Parametric Study of Coagulation–Flocculation

To optimize the coagulation–flocculation process for effective pollution removal from soap factory effluent, a comprehensive parametric study was conducted. This involved systematically varying key operational parameters, including coagulant concentration, coagulation speed, flocculation speed, coagulation time, and flocculation time. The resulting measurement data, obtained after treatment with Ferric Chloride (FeCl_3), are presented below.

- **Coagulant dose**

The first parameter is the coagulant dose. Experiments were conducted under consistent coagulation–flocculation operating conditions: a coagulation speed of 150 rpm for 5 min, a flocculation speed of 30 rpm for 20 min, and a Settling time fixed at 30 min.

The results show, in Figure 8a, a significant decrease in turbidity corresponding to an optimal coagulant concentration of $C = 1.2$ g/L. Turbidity initially decreases with decreasing pH due to better charge neutralization of colloids. However, at very low pH, floc destabilization or solubilization may occur, causing turbidity to rise again. In Figure 8b, as the coagulant dose increases, more colloids are neutralized and aggregated. However, beyond 1.2 g/L, overdosing may lead to charge reversal or excess sludge formation, which reduces removal efficiency. The removal rate reached 99.68%, which indicates that the treatment was carried out under optimal conditions.

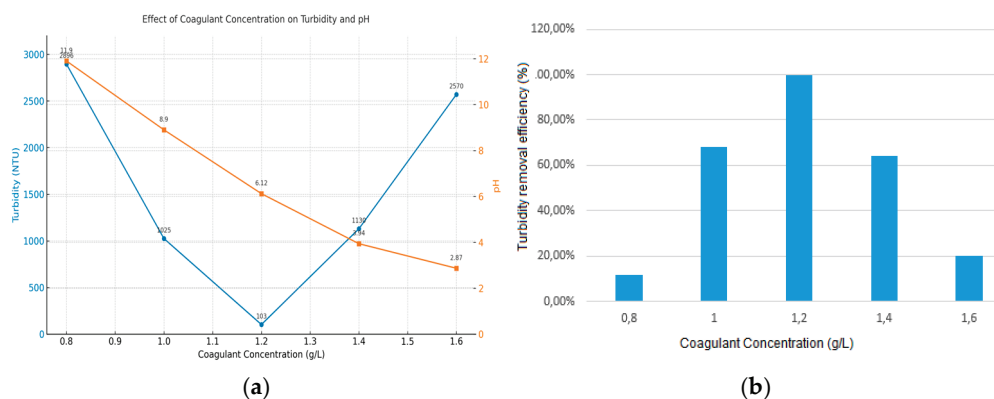


Figure 8. Concentration effect (a) on pH and turbidity (b) on turbidity removals.

We conducted the rest of the experiments using the optimal dosage of 1.2 g/L. We sequentially varied the coagulation time, coagulation speed, and flocculation time parameters while keeping the remaining parameters (coagulation speed, flocculation speed, and flocculation time) constant. Settling time was consistently maintained at 30 min.

• Coagulation time and speed

We conduct these experiments using an optimal coagulant dosage of 1.2 g/L. Initially, we determined the optimal coagulation time. Subsequently, we varied the coagulation time while maintaining the following fixed parameters:

- Coagulation speed: 150 rpm;
- Flocculation speed: 30 rpm;
- Flocculation time: 20 min;
- Settling time was held constant at 30 min.

In Figure 9a, turbidity decreases significantly as pH drops in the initial coagulation phase (2–4 min), suggesting a lower pH promotes coagulation, likely through enhanced coagulant hydrolysis and charge neutralization. Subsequently, turbidity stabilizes at a low level despite further pH fluctuations in the acidic range. This stabilization indicates that optimal coagulation conditions are likely reached, and further pH reduction does not improve removal, possibly due to shifts in coagulant species or exceeding the ideal pH range.

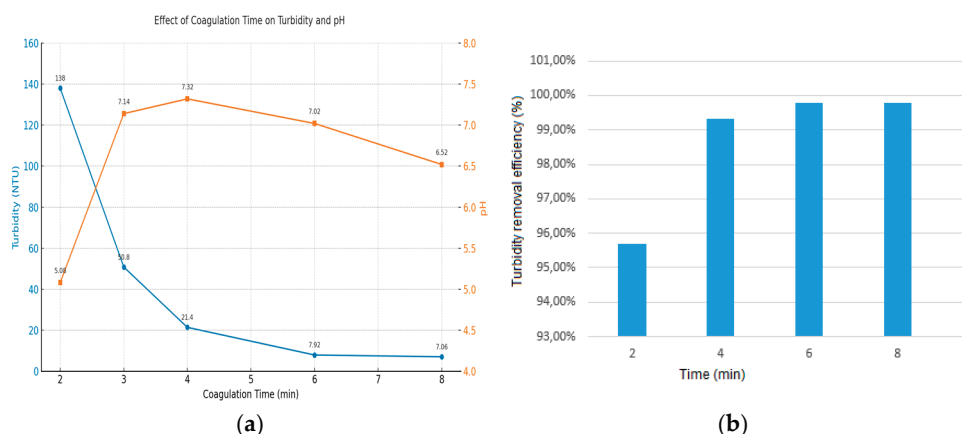


Figure 9. Effect of coagulation time (a) on pH and turbidity (b) on turbidity removals.

Figure 9b shows that turbidity removal efficiency increases with coagulation time, rising from 95.6% at 2 min to a peak of 99.8% at 6 min. Extending the time to 8 min provides no significant further improvement, indicating an optimal coagulation time around 6 min under these conditions.

Optimization of coagulation speed is performed using a fixed coagulant dosage of 1.2 g/L and a coagulation time of 5 min. The coagulation speed parameter is varied while maintaining the following constants:

- Flocculation speed: 30 rpm;
- Flocculation time: 20 min;
- Settling time: 30 min.

The data presented in Figure 10a reveal that a complex interplay between coagulation speed and pH on turbidity. There appears to be an optimal coagulation speed of around 150 rpm in this experiment, which coincides with a pH of around 5.82, resulting in the lowest turbidity. Deviating from this speed, especially increasing it significantly, leads to higher turbidity, possibly due to increased shear forces disrupting floc formation. The changing pH with coagulation speed also plays a role, initially seeming to favor coagulation as it decreases but then potentially hindering it at very low values.

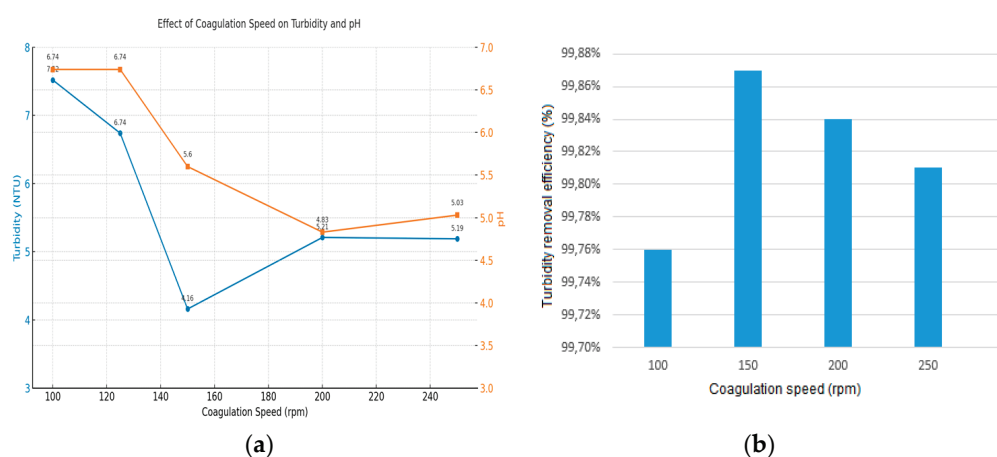


Figure 10. Effect of coagulation speed (a) on pH and turbidity (b) on turbidity removals.

Figure 10b demonstrates that there is an optimal coagulation speed for maximizing turbidity removal. In this case, it appears to be around 150 rpm. Below this speed, mixing might be insufficient for effective coagulant distribution and particle destabilization. Above this optimal speed, the increased shear forces become detrimental, hindering floc growth and reducing the overall removal efficiency. This visually confirms the text's conclusion that the coagulation process has a low sensitivity to variations in agitation rate around the optimal point, and that 150 rpm was selected as a good balance for efficient operation.

• Flocculation time and speed

The impact of flocculation time was assessed by varying the duration between 10 and 40 min. During these experiments, the following parameters that remained constant are as follows:

- Coagulant dosage (1.2 g/L);
- Coagulation speed (150 rpm, 5 min);
- Flocculation speed (30 rpm);
- Settling time (30 min).

Figure 11a clearly shows that for this specific system, there appears to be an optimal flocculation time around 20 min, which resulted in the lowest turbidity. Deviating from this optimal time, either shorter or longer, leads to higher turbidity. Shorter times are likely insufficient for proper floc growth, while longer times, especially beyond 20 min, seem to cause floc breakup due to prolonged mixing, regardless of the pH fluctuations observed.

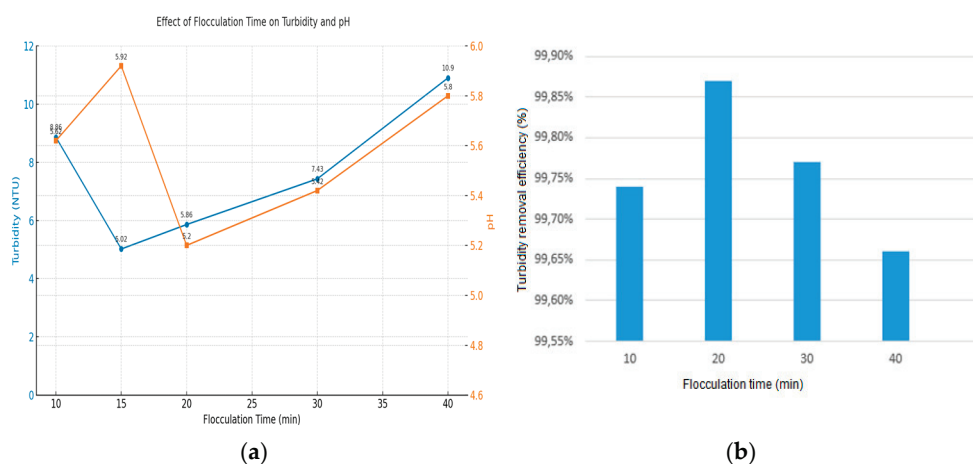


Figure 11. Effect of flocculation time (a) on pH and turbidity (b) on turbidity removals.

Figure 11b demonstrates that a 20 min flocculation period resulted in the peak removal rate of 99.87%, indicating a highly efficient process at this specific duration. This peak performance suggests that at 20 min, the flocs formed were optimally sized and dense, allowing for maximum particle capture and subsequent settling. Consequently, 20 min was selected as the optimal flocculation time for this particular treatment process.

To examine the effect of flocculation speed on the treatment process, a series of experiments were conducted. The optimal coagulant dosage was maintained at a constant 1.2 g/L, ensuring that variations in performance were solely attributable to changes in flocculation speed. To provide a controlled experimental environment, other critical parameters were held fixed at optimal results found in the previous experiments. The coagulation speed was set at 150 rpm, the coagulation time at 5 min, the flocculation time at 20 min, and the settling time at 30 min. By systematically altering the flocculation speed while keeping these parameters constant, we aimed to determine the optimal agitation rate for maximizing floc formation and subsequent removal efficiency.

The data presented in Figure 12a clearly indicate that flocculation speed significantly impacts process results. It reveals a non-linear relationship between flocculation speed, pH, and turbidity. The optimal turbidity is around 30 rpm. Increasing the speed to 40 rpm is very detrimental. However, further increasing the speed to 50 rpm unexpectedly leads to a significant reduction in turbidity, accompanied by a rise in pH. Finally, increasing the speed to 60 rpm shows a slight increase in turbidity again. This complex behavior highlights that the optimal flocculation speed is a balance between promoting floc collision and growth and avoiding excessive shear that can cause floc breakup, and this balance can be influenced by the changing pH conditions.

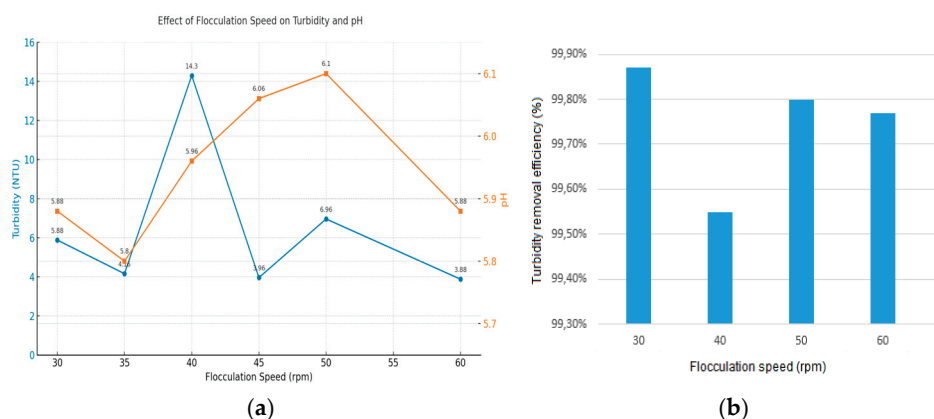


Figure 12. Effect of flocculation speed (a) on pH and turbidity (b) on turbidity removals.

Figure 12b illustrates that a flocculation speed of 30 rpm achieves the peak turbidity removal efficiency. Moving away from this optimum, particularly increasing the speed to 40 rpm, significantly reduces performance. A further rise to 50 rpm leads to a partial recovery in removal, before a subsequent decrease at 60 rpm. This non-linear relationship emphasizes the importance of finding the right balance in flocculation speed to maximize particle aggregation and settling.

Consequently, a speed of 30 rpm was taken as the optimal value, providing ideal agitation, low enough to prevent floc breakage, but sufficient for aggregation. With the optimal parameters for coagulation and flocculation now defined, we proceed to evaluating the sedimentation time, using the previously determined optimal conditions.

• Sedimentation time

Sedimentation is a critical stage in this treatment process, directly impacting the overall process's efficiency. As illustrated in Figure 13a, we observe a significant initial decrease in turbidity from 7.27 NTU at 30 min to 4.16 NTU at 60 min. This indicates that a substantial amount of suspended particles settles out within the first half-hour of sedimentation. Following this sharp decline, there is a slight increase in turbidity to 5.07 NTU at 90 min, which could potentially be due to minor disturbances or re-suspension, although it is followed by a continued gradual decrease. Over longer sedimentation times, the turbidity steadily declines, reaching its lowest observed value of 3.28 NTU at 1440 min (24 h). This highlights that extending the sedimentation period generally leads to clearer water as more particulate matter settles out under gravity.

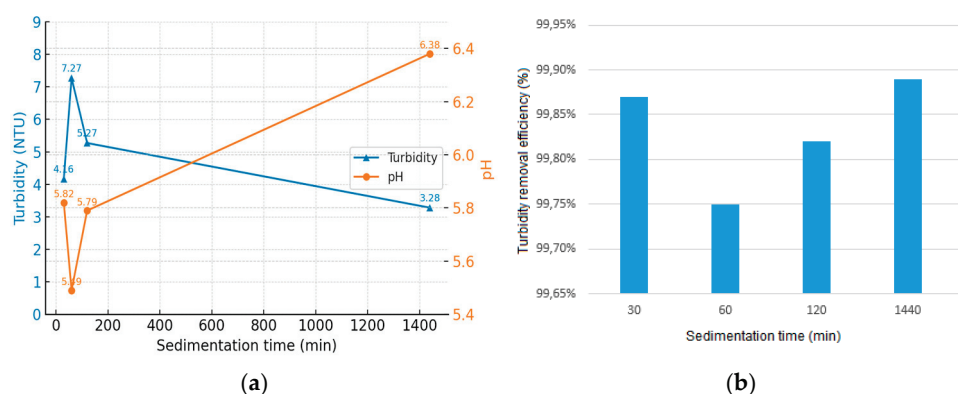


Figure 13. Effect of sedimentation time (a) on pH and turbidity (b) on turbidity removals.

For the evolution of pH with sedimentation time, starting at 5.79 at 30 min, the pH decreases to 5.59 at 60 min. Subsequently, the trend reverses, and the pH gradually increases over time, reaching 6.38 at 1440 min. This suggests that the sedimentation process influences the chemical characteristics of the water, leading to a less acidic condition with prolonged settling. Further investigation is warranted by the initial pH decrease and subsequent increase. This one should focus on the chemical reactions during sedimentation, considering the potential for acidic and alkaline components to settle at different rates.

By examining Figure 13b, we can directly quantify the effectiveness of sedimentation at different time intervals. The high removal efficiency at 30 min supports favorable results at this time, representing a good compromise between treatment effectiveness and operational speed. The subsequent slight dip and then increase in efficiency at longer times provide a more nuanced understanding of the settling dynamics. The eventual plateauing of the removal efficiency suggests that extending the sedimentation time beyond 24 h yields diminishing returns in terms of turbidity removal. Consequently, to maximize process effectiveness, a sedimentation time of 30 min is recommended for practical applications, balancing performance with operational efficiency.

In summary, the coagulation–flocculation treatment demonstrably achieved a significant reduction in turbidity, decreasing from 3235 NTU to 3.28 NTU, and a substantial reduction in chemical oxygen demand (COD), from 9950 mg/L to 351 mg/L. This marked improvement in water clarity, visually confirmed in Figure 14, which compares untreated and treated soapy effluent, indicates a highly successful treatment outcome. The optimal operating conditions, identified by the lowest turbidity values, are detailed in Table 4.



Figure 14. Soap effluent before and after coagulation–flocculation treatment.

Table 4. Characteristics of treated water by coagulation–flocculation.

pH	Turbidity (NTU)	COD (mg/L)
6.38	3.28	351

Furthermore, the use of Ferric Chloride as the coagulant in the process resulted in a high removal efficiency of 99.89%. In addition to the very satisfactory treatment efficiency, the process yielded a visually clear water product. The optimal operating parameters of this outcome, including coagulant dosage, flocculation speed and time, and sedimentation time, are summarized in Table 5.

Table 5. Optimal parameters of coagulation–flocculation treatment.

Coagulant Dose (g/L)	Coagulation Time (min)	Coagulation Speed (rpm)	Flocculation Time (min)	Flocculation Speed (rpm)	Sedimentation Time (min)
1.2	5	150	20	30	30

Our research validates the effectiveness of coagulation–flocculation with FeCl_3 for treating soap manufacturing wastewater, characterized by high turbidity and COD [16]. The selection of FeCl_3 as a superior coagulant to $\text{Al}_2(\text{SO}_4)_3$ aligns with previous observations on surfactant laden wastewater, where iron based coagulants are more effective for colloidal destabilization and floc formation [16,17,25]. Through parameter optimization, we achieved significant reductions, decreasing turbidity by 99.89% to 3.28 NTU and reducing COD to 351 mg/L. These results align with findings from comparable studies on detergent wastewater treatment [16,17] and underscore the potential of this method. Furthermore, the achieved effluent quality supports the use of coagulation–flocculation as a crucial pretreatment step before membrane filtration to minimize fouling [25,26], although subsequent treatment is necessary to address the remaining COD.

3.2. Membrane Filtration Treatment

Although iron chloride coagulation–flocculation effectively improved water clarity and quality, the resulting COD level (351 mg O_2 /L) still exceeds Algerian reuse standards [28]. Consequently, while the optimized parameters from the coagulation–flocculation

will be used in further experiments, membrane filtration is required as a secondary treatment. This additional step will ensure the treated water meets the necessary discharge or reuse standards.

To evaluate the stability and performance of the chosen membrane for this secondary treatment, a permeability test was conducted using a tangential flow filtration cell. Recognizing that membrane flux can be influenced by applied pressure and initial pore compaction, the membrane was first stabilized by pre-filtering pure water. Following this stabilization, the membrane's permeability was determined by measuring the pure water flux (J) at varying working pressures (P) of 0.5, 1.0, and 1.5 bar.

The results of this permeability test are presented in Figure 15, which illustrates the pure water flux as a function of the working pressure across the membrane. The pure water flux exhibits a linear relationship with the applied working pressure, with the trend line passing through the origin. This observed linear behavior is consistent with Darcy's law, which describes the flow of a fluid through a porous medium under pressure. This conformity to Darcy's law indicates stable and predictable performance of the membrane under the tested pressure range.

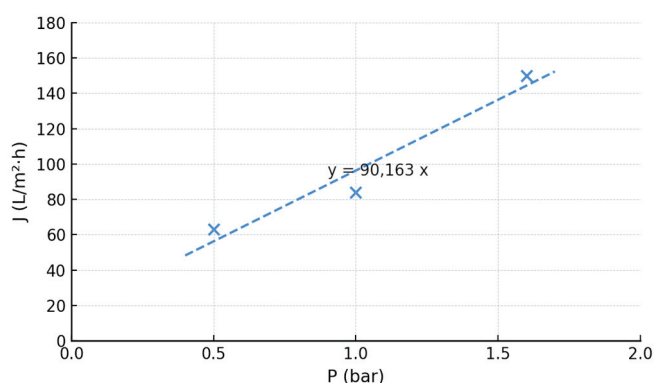


Figure 15. Pure water flux as a function of working pressure.

The calculated average pure water permeability of the membrane was found to be $90.16 L/m^2 \cdot h \cdot bar$ for a $12.5 cm^2$ flat membrane section. This permeability value confirms the membrane's suitability for microfiltration, suggesting an adequate capacity to allow the passage of water at the tested pressures. The stable and predictable performance, as evidenced by the linear relationship between flux and pressure, is a crucial factor in the potential long-term effectiveness of this membrane in the secondary treatment stage.

For the membrane treatment, the pressure was fixed at one bar. We carried out filtration tests of the soap factory water treated by Ferric Chloride coagulation–flocculation. The permeate was collected every 30 min. We measured the pH and turbidity as pollution parameters for each volume collected. The results showed (Figure 16) that starting at approximately 3.3 NTU at the 30 min, the turbidity exhibits a gradual decrease over the filtration period, reaching around 0.9 NTU at 150 min. This suggests that the membrane continues to effectively remove residual suspended solids or colloidal matter that were not entirely eliminated during the preceding coagulation–flocculation stage. The decreasing turbidity indicates an ongoing purification process by the membrane. Regarding pH, the initial measurement at 30 min was approximately 6.4. Following a slight drop to about 6.3 at 60 min, the pH exhibited a further gradual decrease, settling at approximately 6.1 by the end of the 150 min of filtration. This trend suggests that the membrane filtration process has a subtle influence on the pH of the treated water. The gradual decrease in pH could be attributed to the selective passage or retention of certain ionic species by the membrane. Consequently, results highlight the dynamic nature of membrane filtration and emphasize the importance of evaluating its performance over extended operational

durations. The continued reduction in turbidity suggests that longer filtration times under these conditions could lead to even higher permeate quality in terms of clarity. Similarly, the gradual decrease in pH warrants consideration in the context of the intended reuse or discharge standards for the treated water.

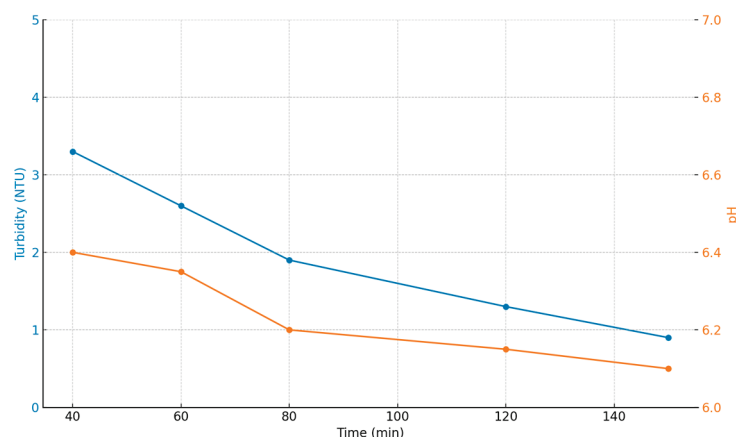


Figure 16. pH and Turbidity as a function of time after membrane filtration.

Table 6 elucidates the substantial turbidity reduction achieved by the hybrid coagulation–flocculation/microfiltration (CF-MF) system. This demonstrates a marked decrease in the pollution load from the initial raw soap factory wastewater, following both coagulation–flocculation and subsequent membrane filtration. The overall turbidity removal efficiency reached 99.97%. The chemical oxygen demand (COD) of the permeate also exhibited a significant reduction, decreasing from 351 mg/L to 58 mg/L after membrane filtration.

Table 6. Results of CF-MF hybrid treatment.

Parameters	Raw Water	CF Treatment	MF Treatment	Standards [20]
Turbidity (NTU)	3235	3.28	0.90	-
Turbidity removal efficiency	-	99.89	99.97	-
pH	12.83	6.38	6.10	6.5–8.5
COD (mg/L)	9200	351	58	120

The resulting chemical oxygen demand (COD) value, achieved after the coagulation–flocculation and membrane filtration processes, now falls within the permissible limits mandated by environmental discharge standards. This compliance signifies a substantial reduction in organic pollutants, ensuring that the treated wastewater meets regulatory requirements and minimizes its potential impact on receiving ecosystems.

Our study employs a coagulation–flocculation (CF) and membrane filtration (MF) hybrid system for treating heavily polluted soap wastewater, highlighting CF as a vital pre-treatment for reducing turbidity and COD similarly to other studies [25]. We further detail the optimization of the CF stage with FeCl_3 . In contrast to more complex treatments [26] for laundry effluent, our simpler CF-MF approach effectively polishes the pretreated effluent, demonstrating the synergy between the two stages in achieving COD levels [25,26]. The study’s findings on membrane permeability and flux behavior are consistent with general MF principles for soap wastewater treatment [25].

Furthermore, as depicted in Figure 17, the membrane filtration stage effectively demonstrated its ability to selectively retain Ferric Chloride, used in the pretreatment process. This selective retention is a crucial attribute of the membrane, particularly in the context of finishing treatment. The presence of residual Ferric Chloride in treated wastewater

can lead to adverse environmental effects, including pH imbalances and increased metal concentrations. By effectively preventing the carryover of Ferric Chloride, the membrane filtration process not only enhances the overall treatment efficiency but also mitigates the risks associated with residual coagulant, thereby contributing to a more sustainable and environmentally sound wastewater treatment solution.



Figure 17. Ferric Chloride Residue on Membrane After Hybrid CF-MF Treatment.

4. Conclusions

This study successfully demonstrated the efficacy of a hybrid coagulation–flocculation (CF) and membrane filtration (MF) system for treating highly polluted wastewater from a soap manufacturing industry. The initial characterization of the wastewater revealed significant levels of turbidity, COD, and BOD, exceeding Algerian discharge standards, necessitating a robust treatment approach. Through systematic optimization of the CF process, using Ferric Chloride as the coagulant, optimal operating parameters were identified, resulting in a 99.89% turbidity removal and a substantial reduction in COD. However, the residual COD still surpassed discharge regulations, highlighting the need for a secondary treatment. The subsequent MF stage, utilizing a microfiltration membrane, further polished the effluent, achieving an impressive 99.97% overall turbidity removal and reducing COD to within acceptable discharge limits. The novelty of this work lies in its successful application of this combined CF-MF system optimized specifically for the challenging characteristics of soap industry wastewater with its high surfactant and alkalinity content to achieve compliance with stringent Algerian discharge standards. Notably, the membrane effectively retained residual Ferric Chloride, demonstrating its capability as a reliable finishing treatment. This work emphasizes the significant potential of tailored combined CF-MF systems for the soap manufacturing industry to achieve high-quality treated wastewater, comply with strict discharge regulations, and contribute to sustainable water resource management within industrial contexts. The findings of this study offer valuable insights for optimizing wastewater treatment processes in similar industries, thereby promoting environmental sustainability and adherence to regulatory requirements. Moving forward, future research will focus on a comprehensive exploration of membrane parameter optimization within this hybrid system. This will involve investigating the influence of various membrane characteristics, such as pore size, material, and operating conditions, to further enhance the system's efficiency, reduce operational costs, and minimize membrane fouling, ultimately striving for even more sustainable and economically viable wastewater treatment solutions.

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