

Article

Effects of the Leached Plastic Additive (Bisphenol-A) on *Chlorella vulgaris* and Wastewater Bioremediation

Paulo M. S. Sousa ¹, Inês Moreira ¹ , Manuel Simões ¹  and Cátia A. Sousa ^{1,2,3,*} 

¹ Laboratory for Process Engineering, Environment, Biotechnology and Energy (LEPABE), Associate Laboratory in Chemical Engineering (ALiCE), Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal; up201206729@edu.fe.up.pt (P.M.S.S.); up202004710@edu.fe.up.pt (I.M.); mvs@fe.up.pt (M.S.)

² ISEP/P.PORTO, School of Engineering, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida, 431, 4249-015 Porto, Portugal

³ CIETI, ISEP/P.PORTO, School of Engineering, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida, 431, 4249-015 Porto, Portugal

* Correspondence: fas@isep.ipp.pt; Tel.: +351-22-83-40-500

Featured Application

This study highlights the potential application of *Chlorella vulgaris* in sustainable wastewater (WW) treatment systems for the bioremediation of bisphenol A (BPA)-contaminated effluents. The findings contribute to the development of microalgae-based technologies capable of simultaneously removing emerging contaminants and nutrients from WW, supporting more environmentally friendly and circular treatment strategies.

Abstract

Bisphenol A (BPA) is a common plastic additive found in wastewater (WW) due to its extensive use in industrial and consumer products. As a known endocrine disruptor, BPA poses serious ecological and human health risks, yet its removal remains inefficient in conventional WW treatment plants. This novel study investigates the impact of high environmental BPA concentrations (5 mg/L and 25 mg/L) on *Chlorella vulgaris* in a WW-mimicking environment, assessing microalgal growth, metabolic activity, nutrient removal, and BPA degradation. Exposure to BPA led to a significant reduction in esterase activity and an increase in intracellular reactive oxygen species (ROS) levels, indicating cellular oxidative stress and metabolic disruption. Despite these effects, *C. vulgaris* maintained stable photosynthetic pigment levels, demonstrating a resilient photosynthetic function. The bioremediation potential of *C. vulgaris* was also compromised, with nitrogen and phosphorus removal efficiencies decreasing by up to 38% and 34% in the presence of 5 and 25 mg BPA/L, respectively. Nevertheless, the microalga exhibited the ability to degrade BPA, with removal efficiencies of 34% for 5 mg/L and 21% for 25 mg/L after 168 h, while abiotic degradation was minimal. These findings confirm the potential of *C. vulgaris* as a promising sustainable approach for BPA bioremediation but also highlight critical challenges at high contaminant concentrations.

Keywords: bisphenol-A; *Chlorella vulgaris*; microalgae-based systems; plastic additives; wastewater bioremediation; water pollution



Academic Editor: Amir M. Motlagh

Received: 28 May 2026

Revised: 27 June 2026

Accepted: 30 June 2026

Published: 1 July 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons](https://creativecommons.org/licenses/by/4.0/)

[Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Emerging contaminants (ECs) in wastewater (WW) environments pose a significant environmental and public health challenge due to their persistence, toxicity, and widespread distribution [1]. Among these contaminants, microplastics (MPs) are of particular concern [2]. In fact, WW treatment plants (WWTPs) represent a main source for introducing MPs into aquatic ecosystems, alongside surface runoff and maritime anthropogenic activities [3]. In WWTPs, MPs can be present in high influent concentrations, reaching up to 5.5×10^5 particles per m^3 [4].

While advancements in tertiary treatments have improved MPs removal [4], a significant fraction continues to be released into receiving water bodies [5]. Despite the efforts to mitigate their presence in marine systems, MPs' persistence and global dispersion continue to raise concerns regarding their ecological and human health risks [6]. Their presence has been linked to organ dysfunction, neurotoxicity, and cellular damage in fish and invertebrates [7], with potential bioaccumulation along the food chain [8]. Moreover, human exposure to MPs—primarily through ingestion—has been associated with cytotoxic effects, DNA damage, and metabolic disorders [9,10]. On the other hand, MPs contain chemical additives, including plasticizers and stabilizers, which can be leached into the water, having the potential to exacerbate ecotoxicological risks through potential synergistic effects with other pollutants [11]. Among these additives, bisphenol-A (BPA) is a widely used plastic additive with high environmental relevance [12], leading to increased concern within the scientific community, requiring regulation for its use and commercialization (Commission Regulation (EU) 2024/3190), as well as directives for drinking water systems (Drinking Water Directive (2020/2184)). Strongly used in polycarbonate plastics and epoxy resins, BPA enhances durability and transparency in consumer products, such as food and beverage packaging [13,14]. However, BPA is a known endocrine disruptor with carcinogenic potential, affecting multiple organ systems [13]. Despite its common detection in WW influents at concentrations between 0.33 and 910 $\mu\text{g/L}$ [14,15], BPA removal remains inefficient in conventional WWTPs [16] and may accumulate in treated effluents at concentrations up to 5 mg/L [17]. In addition, MPs can act as persistent secondary sources of BPA, continuously releasing into WWTP matrices and sustaining chronic exposure even when influent concentrations are comparatively low (can be leached from plastic waste at levels up to 139 $\mu\text{g/g}$, with reported leachability reaching up to 55%) [18,19]. These characteristics challenge conventional WWTP removal efficiency, as they are not adequately designed to remove these contaminants, given their reliance on complex multi-stage processes and chemical treatments [4,20,21]. Moreover, WWTPs are associated with modest removal efficiency, as they are not adequately designed to remove these contaminants, given their reliance on complex multi-stage processes and chemical treatments [4,20,21].

Given these limitations, the search for alternative strategies capable of improving the removal of emerging contaminants from wastewater has intensified [22]. Among these, advanced oxidation processes (AOPs), including photocatalytic technologies, have demonstrated high degradation efficiencies for a wide range of emerging contaminants and are increasingly being investigated as complementary WW treatment options [23,24]. Nevertheless, growing attention has been directed toward microalgae-based systems due to their ability to simultaneously remove contaminants and nutrients while contributing to resource recovery and biomass production [21,25–27]. Microalgal-based systems offer several advantages over conventional treatments, including low energy requirements, carbon sequestration potential, and the capacity to remove nutrients, metals, pathogens, and emerging contaminants under diverse environmental conditions [21,28–37]. Notably, microalgae have demonstrated high efficiency in removing ECs, such as BPA, at environmentally relevant concentrations (0.01 to 50.00 mg/L) [12,38–40]. Compared to conventional WW

treatment methods, microalgae-based systems provide lower capital and operational costs, rapid growth cycles, and resilience to toxic WW conditions [4]. Among the various microalgae species, *C. vulgaris* has gained particular attention from the scientific community for its application in WW polishing. This microalga is characterized by its high resilience, efficient nutrient removal capability, and rapid growth under stress conditions, as well as its notable tolerance to elevated concentrations of heavy metals and other toxic compounds, and is not associated with harmful algal blooms [21,41]. Additionally, *C. vulgaris* exhibits remarkable metabolic flexibility, thriving under autotrophic, heterotrophic, and mixotrophic growth conditions [33], enhancing its adaptability and effectiveness in diverse WW treatment scenarios.

In this context, this study aims to assess the effects of different concentrations of BPA on the microalga *C. vulgaris* in terms of intracellular response, including metabolism, accumulation of reactive oxygen species (ROS), and photosynthetic pigment concentration, as well as cellular growth and WW polishing performance, after 168 h. The tested concentrations: 5 mg BPA/L corresponds to the highest BPA level reported in treated effluents from WWTPs [17], whereas 25 mg/L was intentionally chosen as an extreme worst-case exposure scenario. Although the concentration of 25 mg/L may not reflect typical environmental levels, it simulates potential situations of exceptional contamination, such as industrial discharges, accidental releases, or localized accumulation hotspots [42,43], where BPA concentrations may exceed those commonly reported in the literature, which generally range from 0.33 to 910 µg/L in WW influents [15]. This approach allowed the evaluation of microalgal physiological responses and system robustness under severe chemical stress rather than environmentally typical conditions. Additionally, the ability of the microalga to remove BPA from synthetic WW (SWW) was evaluated. By elucidating the interactions between *C. vulgaris* and BPA, this study seeks to contribute to the development of sustainable microalgae-based WW treatment strategies to mitigate the presence of ECs. To the best of the authors' knowledge, this study is novel, simultaneously evaluating the impact of high BPA concentrations on *C. vulgaris* under a scenario of accumulation in SWW, as well as its bioremediation efficiency (removal of nutrients, carbon source, and the EC) and the associated physiological responses.

2. Materials and Methods

2.1. Bisphenol-A Stock Solution

A stock solution of BPA (purity > 97%; Alfa Aesar, Ward Hill, MA, USA) at a concentration of 10 g/L was prepared in methanol (VWR Chemicals, Radnor, PA, USA). The solution was subsequently sterilized one time by UV-C radiation exposure in the flow chamber (Phillips UV-C G23 lamp, North Holland, The Netherlands, 11 W) for 15 min, and stored in the dark at 4 °C until use.

2.2. Microalga Cultures

The microalga *C. vulgaris* UTEX 2714 was provided by the Culture Collection of Algae from The University of Texas at Austin (TX, USA) (UTEX—<https://utex.org/>, accessed on 1 September 2023). The microalga was maintained on solid medium, in accordance with the Organization for Economic Co-operation and Development (OECD) guidelines [44]. Weekly axenic *C. vulgaris* cultures were prepared by following the methodology previously described by Vale et al. [34]. Briefly, microalgal pre-cultures were prepared by inoculating *C. vulgaris* into 40 mL of OECD medium and incubating for 3 days at 25 °C under continuous cool white fluorescent illumination on an orbital shaker (AGITORB 200 ICP, Lisbon, Portugal, 100 rpm). Subsequently, the axenic cultures were established by transferring the pre-culture cells to fresh 200 mL of OECD medium at an initial density of 1×10^6 cells/mL

and incubating for 6 days under the same conditions. After incubation, microalgal cells were harvested by centrifugation (Centrifuge 5810 R, Hamburg, Germany, $4000 \times g$, 5 min).

2.3. Cellular Growth Evaluation

To assess the effects of BPA on microalgal growth, *C. vulgaris* cells in the exponential growth phase were exposed to BPA at concentrations of 5 mg/L and 25 mg/L. Experiments were conducted under continuous fluorescent light (24 h artificial photoperiod [21]), at an initial cell density of 1×10^6 cells/mL in 125 mL of SWW (representing an average WW composition [34]). As a control, microalgal cells were incubated under the same conditions in the absence of the EC. Also, organic solvent control assays (containing the same concentration of methanol without BPA; namely, 0.05% (v/v) and 0.25% (v/v), respectively) were performed to ensure the inhibition is caused by the test substance and not the solvent used [44]. Controls and assays were incubated for 168 h under the same temperature, agitation, and light conditions described above.

Then, microalgal cell density was monitored every 24 h intervals by measuring the optical density at 750 nm, according to the methodology previously described by Vale et al. [34], following Equation (1). Based on these measurements, the specific growth rate (μ , d^{-1}) was calculated. The doubling time (t_d , h) and maximum growth rate (μ_{max} , d^{-1}) were subsequently determined using the Monod equation, as previously reported in our work by Sousa et al. [21]. Finally, the microalgal total biomass was also quantified following the same methodology reported in Vale et al. [34].

$$OD_{750nm} = \left(3.92 \times 10^{-8}\right) \times C_{cellular} \left(\frac{\text{cells}}{\text{mL}}\right) + 1.34 \times 10^{-2} \quad (1)$$

2.4. Microalga Metabolism

To evaluate the metabolic activity, two key parameters were quantified at 72 h and 168 h: esterase activity and intracellular reactive oxygen species (ROS) accumulation. Esterase activity was measured using fluorescein diacetate (FDA; Sigma-Aldrich, St. Louis, MO, USA), at 5×10^5 cells/mL in SWW after BPA exposures and stained with 20 μmol FDA/L; and ROS accumulation was determined using 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA; Sigma-Aldrich), at 1×10^6 cell/mL stained with 20 μmol H₂DCFDA/L, as described in detail in the previous work Vale et al. [34]. Samples were prepared in quadruplicate in sterile 96-well flat microplates (VWR, Radnor, PA, USA), and incubated for 40 min and 90 min at room temperature in the dark for esterase activity and ROS accumulation, respectively. Fluorescence was measured using a Fluostar Omega microplate reader (Baden-Württemberg, Germany; excitation: 485/14 nm, emission: 535/25 nm). Readings were corrected by subtracting cells, culture medium (SWW), and dye autofluorescence, and normalized considering the cell concentration. The effect of BPA concentrations on esterase activity and intracellular ROS accumulation was expressed as the fluorescence ratio of BPA-exposed cells relative to control cells.

2.5. Photosynthetic Pigments Quantification

At 168 h, the microalgae cells were harvested, and photosynthetic pigments were extracted by immersing the biomass in 90% (v/v) acetone (VWR Chemicals, PA, USA) at 4 °C for 24 h, as described in the previous work [34]. After this extraction process, the concentrations of chlorophyll (Chl *a* and *b*), as well as carotenoids, were determined by UV/Vis spectrometry in quartz cuvettes, according to Strickland et al. [45].

2.6. Nutrients and Organic Carbon Source Removal

At 168 h, culture supernatants were collected after centrifugation (Centrifuge 5810 R, Hamburg, Germany, 4000× *g*, 5 min) for nitrogen (N), phosphorus (P), and glucose quantification. For N quantification, supernatant absorbance was measured at 220 nm in quartz cuvettes [46], and nitrate (NO_3^-) was converted to N using molar mass ratios, with a calibration curve between 0.5 and 500 mg/L, $R^2 = 0.997$, as described in Vale et al. [34]. For P quantification, P was determined using the ascorbic acid–molybdate method (Baird and Eaton 1998 [47]), reading absorbance at 820 nm, and converting phosphate (PO_4^{2-}) to P; the calibration curve was prepared from KH_2PO_4 (with concentrations ranging between 0.5 and 60 mg/L, $R^2 = 0.998$), as described in Vale et al. [34]. The glucose concentration was measured at 0 h and at the end of the experiments (168 h) to determine its consumption by the microalga cells, using the high-pressure liquid chromatography technique with a refractive index detector (HPLC-RI). An Altech OA 1000 organic sugar column (Des Plaines, IL, USA) and 5 mM H_2SO_4 eluent at a flow rate of 0.5 mL/min were used [48]. The calibration was done using D-Glucose (Merck, Hessen, Germany) at concentrations between 5 and 500 mg/L, for a retention time of 15 min, with an R^2 of 0.9997.

2.7. BPA Quantification in Wastewater

To evaluate BPA degradation by *C. vulgaris*, the supernatants were collected at 0 h and 168 h and analyzed using high-performance liquid chromatography (HPLC). BPA quantification was performed using a Shimadzu LC-2040C 3D system equipped with a photodiode array (PDA) detector (Kyoto, Japan) and a column NUCLEODUR 100-5 C18 EC (5 μm × 250 mm × 4 mm). The mobile phase consisted of methanol/ultrapure water (70:30 *v/v*), under an isocratic elution model, at a flow rate of 1 mL/min, an oven temperature of 25 °C, and a pump pressure of 45 MPa. BPA elution was detected at 275 nm, with a retention time of 5 min [49]. A calibration curve was established for BPA (Equation (2)) and validated with concentrations ranging from 0.50 to 50 mg/L, with $R^2 = 0.9994$. The limit of detection (LOD) and quantification (LOQ) are 0.51 and 3.02 mg/L, respectively.

$$\text{Area} = 7843.5 \times C_{\text{BPA}} \left(\frac{\text{mg}}{\text{L}} \right) + 216.3 \quad (2)$$

2.8. Reproducibility of the Results and Statistical Analysis

All experiments were independently performed at least three times, with duplicates ($n \geq 6$). Statistical differences were assessed by one-way ANOVA followed by the Tukey–Kramer multiple comparison test, considering p values < 0.05 as statistically significant.

3. Results

3.1. Impact of BPA on Microalgal Metabolism and Photosynthetic Activity

MPs have been studied concerning their negative physiological effects on different microalgal species (e.g., *Scenedesmus obliquus* [50], *C. vulgaris* [51], *Chlorella pyrenoidosa* [52], *Chlamydomonas reinhardtii* [53], among others), and more recently in the WW environment, involving polypropylene (PP), polyethylene terephthalate (PET), and rayon [54]. Moreover, MPs accumulation in WW can lead to a stronger presence of several chemical additives in the environment that are leached from the MPs [55], potentially compromising the performance of microalgae in bioremediating WW. Thus, in this study, the interaction between BPA (a common plastic additive) and the microalga *C. vulgaris* was studied, assessing its effects on metabolism, namely esterase activity and intracellular ROS production. These metabolic functions were analyzed after 168 h of exposure to BPA at concentrations of 5 and 25 mg/L. Esterase activity serves as a bioindicator of cellular metabolism, as these enzymes are present in metabolically active cells and are commonly used to evaluate the toxic effects

of ECs [34,56]. Additionally, ROS are byproducts of essential metabolic processes such as respiration and photosynthesis [57], but their excessive accumulation can damage lipids, proteins, and DNA, ultimately leading to cell death [34].

As shown in Figure 1a, BPA exposure at both tested concentrations significantly reduced esterase activity in *C. vulgaris* compared to the control ($p < 0.05$), indicating metabolic reduction.

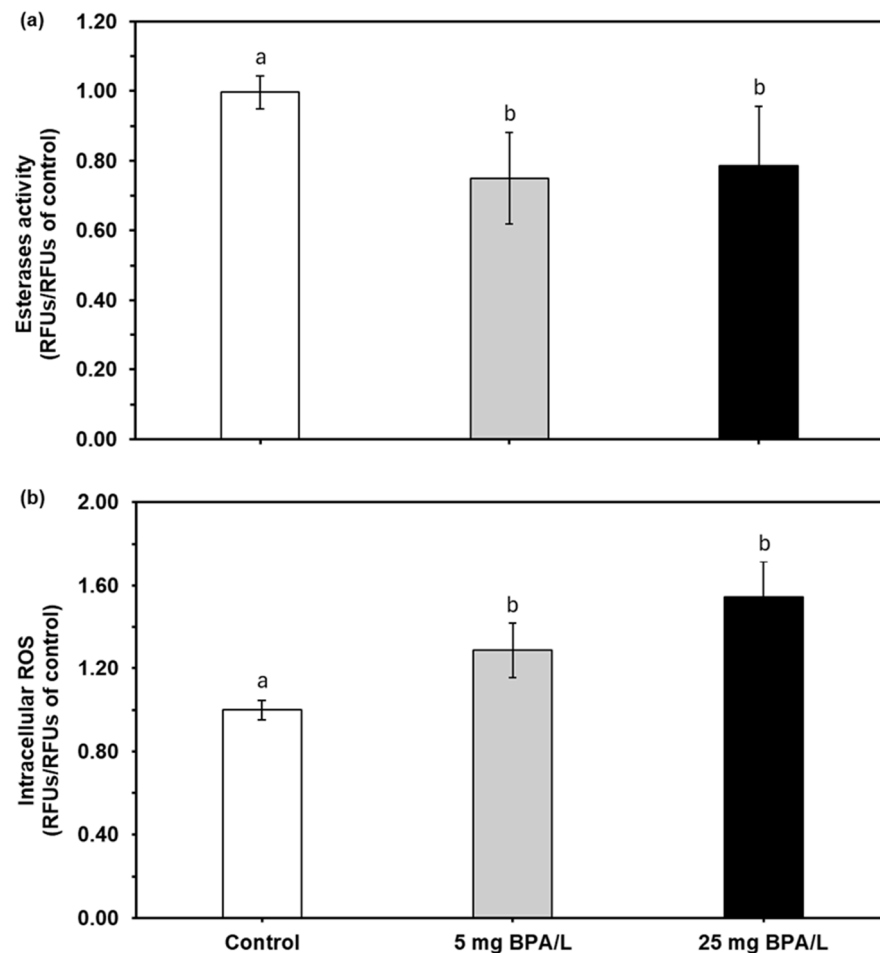


Figure 1. Impact of BPA concentrations on *C. vulgaris* esterase activity and ROS accumulation. (a)—Esterase activity. (b)—Intracellular ROS production. The data are presented as mean values from at least three independent experiments performed in duplicate ($n \geq 6$); in each experiment, four fluorescent readings (in relative fluorescent units, RFUs) were performed ($n \geq 24$). SD are presented (vertical error bars). Statistical differences were subjected to ANOVA followed by the Tukey–Kramer multiple comparison method. The mean values with different letters are significantly different ($p < 0.05$).

These obtained results are aligned with previous studies of Esperanza et al. [58], exposing the microalga *C. reinhardtii* to 30 mg BPA/L for 24 h, which exhibited similar metabolic reduction. This suggests that BPA-induced metabolic inhibition can occur over both long-term (in the present study, 168 h) and short-term (as 24 h [58]) exposure periods. In another study by Duan et al. [59], a transcriptomic analysis of the microalga *C. pyrenoidosa* was performed after exposure to 0.1 and 10 mg BPA/L. The authors revealed that exposure to higher BPA concentrations (at 10 mg/L) repressed genes associated with the tricarboxylic acid (TCA) cycle, glycolysis, and mitochondrial electron transport, suggesting that BPA negatively impacts the metabolic regulation of microalgal cells [59]. Despite no significant effects on metabolic regulation observed at lower BPA concentrations (0.1 mg BPA/L), BPA

acts as a toxic agent for microalgae, exerting a strong inhibitory effect on metabolic activity and impairing *C. pyrenoidosa* growth [59].

Figure 1b shows that BPA exposure in a WW environment leads to an increase in intracellular ROS production in *C. vulgaris* compared to the control ($p < 0.05$). These findings align with previous studies showing high ROS levels in microalgae following BPA exposure [58,60–63]. For instance, Li et al. [63] reported a 1.5-fold increase in ROS levels in *C. pyrenoidosa* after 6 d of exposure to 11 mg/L BPA. Similarly, Esperanza et al. [58] observed a significant ROS accumulation in *C. reinhardtii* cells after 24 h of exposure to 30 mg/L BPA, resulting in a significant increase ($p < 0.05$) in the intracellular level of the superoxide anion radical (O_2^-). The marine diatom *Phaeodactylum tricornutum* also exhibited a significant ROS increase after 24 h at 1 mg/L of BPA [61], accompanied by an increase in cell size, likely due to impaired cell division leading to oxidative stress [61]. On the other hand, in this study, a concentration-dependent effect is observed, where higher BPA concentration leads to higher intracellular ROS accumulation, potentially triggering oxidative stress. In fact, ROS overproduction can damage cellular organelles and disrupt metabolic and physiological processes [64]. Moreover, it is known that the presence of BPA can induce the excessive intracellular accumulation of ROS, leading to cellular oxidative stress, promoting lipid peroxidation of the cell membranes [64], altering the fatty acid profile of microalgae, affecting both membrane functionality and metabolic activity [65]. This is mainly due to the fact that BPA preferentially accumulates in cellular lipids, making polyunsaturated fatty acids highly susceptible to free radical attack [61,64]. The increase of malondialdehyde (MDA) levels in several microalgal species exposed to BPA confirms this mechanism [60,64,66]. Also, BPA can interfere with electron transport between Photosystem II (PSII) and Photosystem I (PSI), causing excess electron accumulation and subsequent ROS formation via oxygen reduction [62,64].

Exposure to BPA can also result in other intracellular impacts on microalgae by affecting the content of photosynthetic pigments, which are essential for the microalgae's growth [61,64]. Looking at Figure 2, it can be seen that exposure to 5 mg/L and 25 mg/L of BPA did not significantly affect the concentrations of photosynthetic pigments synthesized by *C. vulgaris*, compared to the control ($p > 0.05$).

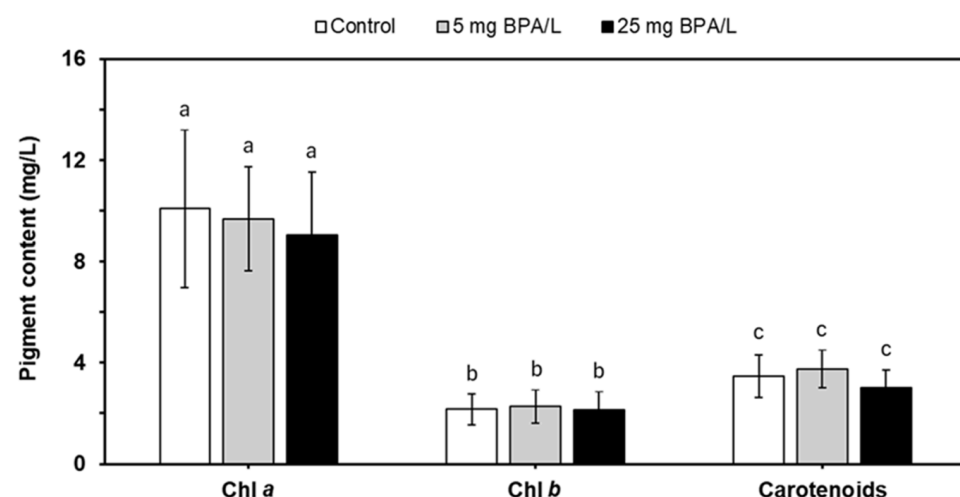


Figure 2. Impact of different BPA concentrations on *C. vulgaris* photosynthetic pigment content. The data are presented as mean values from at least three independent experiments, performed in duplicate ($n \geq 6$). SD are presented (vertical error bars). Statistical differences were subjected to ANOVA, followed by the Tukey–Kramer multiple comparison method. The means with different letters are significantly different ($p < 0.05$).

The synthesis of Chl *a* and *b*, which play essential roles in capturing light for photosynthetic activity [67], was not significantly affected by the presence of BPA (Chl *a* and *b*, $p > 0.05$), suggesting a tendency to resist exposure of this EC in a WW environment. The same intracellular behavior was observed for carotenoids ($p > 0.05$), in which the absence of significant changes may indicate that *C. vulgaris* maintained its photosynthetic integrity. Indeed, several studies have demonstrated the capacity of certain microalgal species to tolerate BPA exposure, maintaining stable pigment levels [59,61,68]. For instance, *P. tricornutum* showed no significant changes in total chlorophyll or carotenoid content at 0.300 mg/L of different BPA analogues (BPAF, BPF, and BPS) during both exponential and stationary growth phases [61], noting that the analogues' concentrations were much lower than the concentrations applied in this study. Similarly, *Stephanodiscus hantzschii* exhibited resistance to BPA concentrations up to 5 mg/L until the 8th day [68], a similar response to *C. vulgaris* in the present study (5–25 mg/L, $p > 0.05$). Likewise, *Navicula incerta* and *C. pyrenoidosa* maintained pigment stability at 1–5 mg/L and 2 mg/L, respectively, without oxidative damage [63,69]. BPA exposure did not significantly affect the MDA content inside the cells, suggesting no oxidative damage to the photosynthetic pigments, and the activity of the antioxidant enzyme peroxidase (POD) remained unchanged, indicating no need for H₂O₂ decomposition as an intracellular antioxidant response, showing that the microalgae were capable of maintaining their photosynthetic integrity [63].

On the other hand, *C. vulgaris* Chl *a* content was reported to decrease above 10 mg/L of BPA [38,70] due to an intracellular stress response [70]. This BPA effect on *C. vulgaris* Chl *a* was observed in two different growth media (BG-11 [38] and Bold's Basal Medium (BBM) [70]) with two different photoperiods, respectively (day/night cycle of 14/10 h, under white light [38] and continuous illumination of white fluorescent light [70]). In contrast, the present study observed stronger resistance, which is in contrast to the fact that, in some studies involving low concentrations of BPA (5 to 25 mg/L), a stimulating effect on algal photosynthesis was observed [59,63]. This effect is possibly caused by: i) changes in membrane permeability caused by BPA, which favors the absorption of nutrients or precursors necessary for pigment synthesis [64], or ii) a hermetic effect, which can be interpreted as a temporary toxic excitation or a defense strategy of the microalgae to overcome intracellular stress [59,64]. This means that the microalga temporarily increases pigment production as a way of optimizing photosynthesis under BPA-stress conditions [64]. This is supported by the results obtained for the overproduction of ROS (Figure 1b). Indeed, Atengueño-Reyes et al. [71] studied a microalgae consortium (*S. obliquus* and *Desmodesmus* sp.) exposed to EC₅₀-96 h of 17 mg BPA/L and found that their exposure led to a stimulation of the Chl *a* content. An over 40% increase in Chl *a* content was observed compared to the control, reinforcing microalgal resilience in WW environments [71].

In summary, the results of this study demonstrate that BPA exerts a clear inhibitory effect on the metabolic activity of *C. vulgaris*, as evidenced by the significant reduction in esterase activity and the increase in intracellular ROS levels after 168 h of exposure to 5 and 25 mg/L of BPA. These findings indicate that BPA can compromise essential cellular functions and support previous studies on its interference with key metabolic pathways. However, despite the induced oxidative stress, *C. vulgaris* maintained stable levels of photosynthetic pigments (chlorophyll *a*, *b*, and carotenoids), suggesting preservation of its photosynthetic integrity under BPA exposure. This contrast between metabolic inhibition and pigment stability highlights the physiological resilience of the microalga in the presence of this EC, which may influence its performance in WW bioremediation processes.

3.2. *C. vulgaris* Growth in the Presence of BPA

The interaction between BPA and microalgae can also have an impact on their growth [38,71]. Microalgae are metabolically flexible microorganisms and can tolerate different WW conditions (which contain various essential properties, such as nutrient levels, carbon source, pH, among others), but the presence of ECs in WW can significantly affect their growth [21]. As shown in Figure 3a,b, exposure to 5 mg/L of BPA did not significantly affect the growth of *C. vulgaris* compared to the control ($p > 0.05$). In contrast, at 25 mg/L, a marked inhibition of approximately 44% was observed ($p < 0.05$).

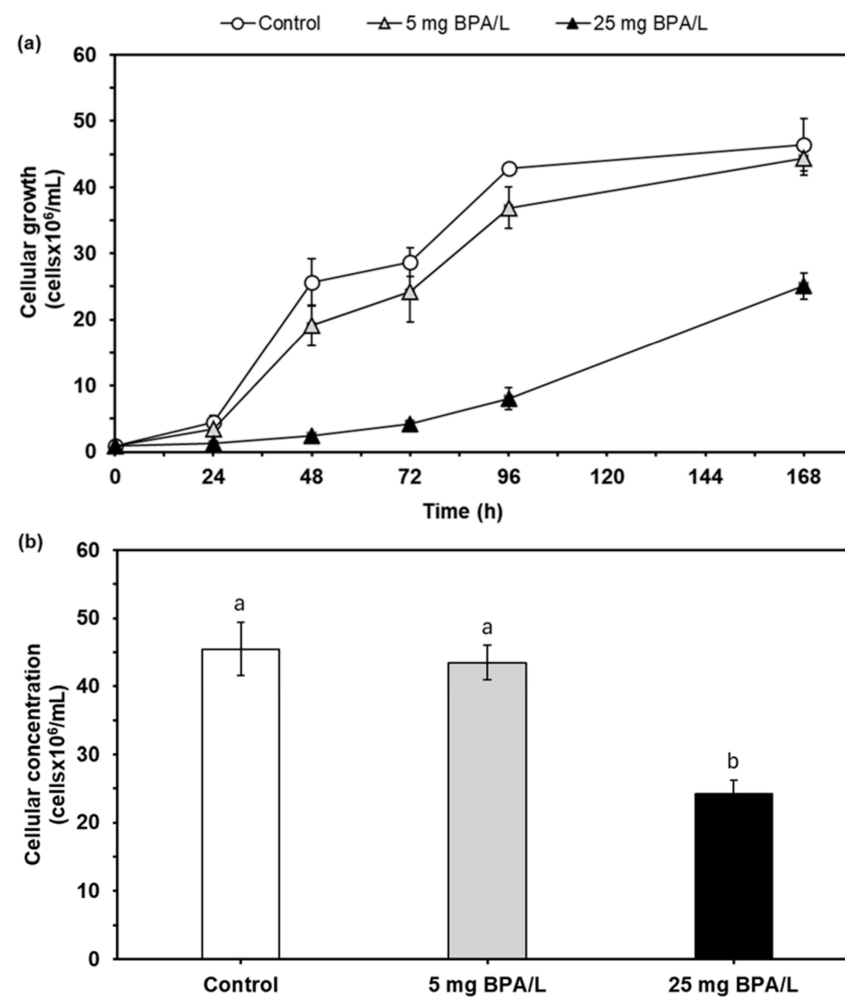


Figure 3. *C. vulgaris* growth in SWW exposed to different BPA concentrations. (a)—Microalga growth. (b)—Cellular concentration after 168 h. The data are presented as mean values from at least three independent experiments performed in duplicate ($n \geq 6$); SD are presented (vertical error bars). Statistical differences were subjected to ANOVA followed by the Tukey–Kramer multiple comparison method. The mean values with different letters are significantly different ($p < 0.05$).

This growth inhibition aligns with the intracellular effects previously reported, where the same concentration caused a significant decrease in esterase activity and a notable increase in ROS levels, reflecting both metabolic and oxidative stress. Despite these intracellular effects, the stability of photosynthetic pigment content suggests that the growth reduction observed is primarily associated with metabolic impacts rather than damage to the photosynthetic system. To exclude potential solvent-derived effects, control experiments were performed using methanol at the same concentrations used with BPA (0.05% and 0.25%, v/v). As shown in Figure S1a,b (Supplementary Material), methanol alone did not significantly affect *C. vulgaris* growth under WW-mimicking conditions ($p > 0.05$). The

growth curve followed a similar trend to the control throughout the 168 h exposure period, with no statistically significant differences in final cell concentration (Figure S1a,b, $p > 0.05$). Also, the final generated biomass remained comparable among treatments, indicating that methanol at concentrations up to 0.25% does not exert inhibitory effects on *C. vulgaris* under the tested conditions (Table S1, $p > 0.05$). These results confirm that the growth inhibition observed at 25 mg BPA/L can be attributed to BPA itself rather than to solvent interference, ensuring the reliability of the negative impacts. The obtained results regarding BPA effects on microalga growth follow other studies, showing that BPA at lower concentrations (up to 10 mg/L [61,62,71]) may not significantly affect the growth of *C. vulgaris*, however, at higher concentrations (between 17 and 100 mg/L [58,63,70,71]), it can lead to growth inhibition, particularly over extended exposure periods (a concentration-dependent effect) [70,71]. Nevertheless, Figure 3a shows a slight recovery of the microalga growth after 72 h of exposure to 25 mg BPA/L, a behavior also reported by Ji et al. [38] for *C. vulgaris* exposed to the same concentration of BPA, with partial recovery by the end of the 10-day experiment (240 h). On the other hand, the growth inhibition at higher BPA concentrations (25 and 50 mg/L) was linked to a decrease in Chl *a* content, which likely impaired photosynthesis and contributed to growth suppression. At the highest tested BPA concentration of 50 mg/L, severe inhibition (85%) was observed after 240 h. Another study by Czarny-Krzyżmińska et al. [70] examined a broader range of BPA concentrations over 336 h. The study found that at low-moderate concentrations, including near 5 and 25 mg/L, there was an initial stimulation of *C. vulgaris* growth (a hormesis effect, as was observed for the chlorophyll content). However, by the end of 336 h, inhibition at 25 mg/L of BPA ranged between 15% and 20%. The inhibition was associated with a reduction in Chl *a* content, likely due to stress responses such as PSII complex degradation or thylakoid lipid peroxidation. Atengueño-Reyes et al. [71] found that in a microalgal consortium (*S. obliquus* and *Desmodesmus* sp.) exposed to BPA in SWW, 5 mg/L caused less than 50% growth inhibition after 96 h, while 25 mg/L caused more than 50% inhibition. The $EC_{50-96\text{ h}}$ was determined as 17 mg/L. However, using 17 mg/L ($EC_{50-96\text{ h}}$), growth was unexpectedly stimulated, suggesting that consortium responses in WW conditions differ from short-term toxicity experiments.

The BPA effects on *C. vulgaris* growth are reflected in the amount of biomass formed (Table 1). It is possible to observe a significant reduction in the amount of biomass formed by the microalga once exposed to 25 mg BPA/L (489.33 ± 36.90 mg) compared to the control (911.39 ± 78.21 mg), the concentration at which the growth was most inhibited (Figure 3b, $p < 0.05$).

Table 1. Total biomass, specific growth rates (μ), maximum specific growth rates (μ_{max}), and duplication time (t_d) of *C. vulgaris* for 168 h, under different BPA concentrations. Statistical differences were subjected to ANOVA followed by the Tukey–Kramer multiple comparison method. The means with different letters are significantly different ($p < 0.05$).

Culture	μ (d^{-1})	μ_{Max} (d^{-1})	t_d (d)	Total Biomass (mg/L)
Control	0.48 ± 0.03^a	2.07 ± 0.07^d	1.44 ± 0.05^g	911.39 ± 78.21^j
5 mg BPA/L	0.42 ± 0.01^b	2.35 ± 0.02^e	1.55 ± 0.02^h	871.42 ± 50.78^j
25 mg BPA/L	0.18 ± 0.02^c	5.67 ± 0.85^f	3.61 ± 0.23^i	489.33 ± 36.00^k

Regarding the specific growth rate of *C. vulgaris* (μ , Table 1), the value observed for the control ($0.48 \pm 0.03 d^{-1}$) is within the range of values reported for the growth of microalgae in WW ($0.1\text{--}0.95 d^{-1}$) [72–76]. When exposed to 5 and 25 mg BPA/L, the microalga showed statistically significantly different values from the control ($0.42 \pm 0.01 d^{-1}$ and $0.18 \pm 0.02 d^{-1}$, $p < 0.05$, respectively). Compared with the literature, Ji et al. [38] found

that, under the experimental conditions assessed, the specific growth rate of *C. vulgaris* decreased progressively as the concentration of BPA increased, as was shown in this study (Table 1). However, it is possible to see that for BPA concentrations of 5 mg/L and 25 mg/L, the specific growth rates for *C. vulgaris* (0.59 d^{-1} and 0.54 d^{-1} , respectively) reported in BBM differ notably from those obtained in the present study (Table 1), likely due to the contrasting conditions between the two environments—BBM being a nutrient-balanced, contaminant-free medium optimized for algal growth. In contrast, SWW represents a complex environment that better simulates real WW conditions.

In sum, the exposure of *C. vulgaris* to high concentrations of BPA has an impact on the growth of the microalga and subsequent biomass formation. Despite there being no significant effects on its photosynthetic activity, the growth reduction may be associated with the intracellular metabolic impacts caused by the reduction of esterase activity and the overproduction and accumulation of ROS (Figure 1a,b), possibly inducing a cellular oxidative state.

3.3. Microalgal Wastewater Polishing Ability

As the demand for more efficient and sustainable methodologies for WW polishing increases, microalgae-based systems have progressively received more attention, based on their proven ability to effectively remove nutrients and, more recently, ECs [28,29,34]. However, the effects of exposure to different concentrations of BPA can compromise the microalgal bioremediation potential and their removal mechanisms, for example, by affecting their growth [38,58,62,63,70]. Indeed, WW treatment aims to reduce effluent N and P levels to below acceptable limits to avoid eutrophication of the receiving water bodies [77]. According to European legislation, the limit for the N concentration in effluents is established at 15 mg/L for an equivalent population of 10,000–100,000 inhabitants (EP), or 10 mg N/L for more than 100,000 EP, or the removal of more than 70–80% (Ministry of the Environment 1997; European Commission 1998; European Parliamentary Research Service 2024 [78–80]). For P concentrations, the values must be less than 2 mg/L for 10,000–100,000 PE, or 1 mg P/L for more than 100,000 PE, or removal of more than 80% (Ministry of the Environment 1997; European Commission 1998; European Parliamentary Research Service 2024 [78–80]). This means that it is important to understand if nutrient removal can be compromised in the presence of BPA. In the present study, N and P concentrations were quantified in the SWW at the end of 168 h.

The capacity of the microalga *C. vulgaris* to polish WW appears significantly affected by exposure to both concentrations of BPA, compared to the control (control— $80.7 \pm 1.9\%$ of N removal and $66.3 \pm 8.3\%$ of P removal, Figure 4a,b, $p < 0.05$).

Figure 4a shows that the N consumption by *C. vulgaris* was affected by the dosage of BPA, with an estimated reduction of around 20% and 38% for 5 mg/L and 25 mg/L of BPA, respectively. It should be noted that for the highest concentration of BPA, at which the strongest inhibition of *C. vulgaris* growth (Figure 3a,b) and the highest metabolic impacts (Figure 1a,b) were found, there was also the highest reduction of N consumption in WW (Figure 4a). Consequently, after exposure to 5 mg/L and 25 mg/L of BPA, more than the legal limit of 10 mg N/L (Ministry of the Environment 1997; European Commission 1998; European Parliamentary Research Service 2024 [78–80]) remained in the WW (around 24 mg N/L). This reduction effect may be due to a higher metabolic impact on *C. vulgaris* cells in the presence of BPA, reducing esterase activity (Figure 1a) and increasing the production and accumulation of ROS (Figure 1b), compared to the control ($p < 0.05$). For P, BPA also showed significant impacts on its efficient removal compared to the control (Figure 4b, $p < 0.05$). A 29% and 34% reduction in P uptake inhibition was observed after exposure to 5 mg/L and 25 mg/L of BPA, respectively, resulting in WW P concentrations

remaining above the legal limit (Ministry of the Environment 1997; European Commission 1998; European Parliamentary Research Service 2024 [78–80]). Similarly, in the study by Ji et al. [38], the increase in the BPA concentration in the environment (up to 50 mg/L) reduced the nutrient absorption capacity (for total N and total P) by *C. vulgaris*, but specific percentages of this inhibition were not provided for comparison to the present study. In Atengueño-Reyes et al. [71], exposing the consortium of microalgae *S. obliquus* and *Desmodium* sp. to 17 mg BPA/L (as EC₅₀-96h), it was observed that, for an initial inoculum of 300 mg of biomass/L, higher and more significant nitrate removal was obtained (2.51 times more removal) compared to the control in SWW. However, it should be noted that the nitrate concentration used in SWW by the authors was 42 mg/L, a value much lower than the concentration used in the present study (250 mg/L). *C. vulgaris* has demonstrated high nutrient removal efficiency, even under exposure to different ECs, such as pharmaceuticals, personal care products (PPCPs), and endocrine-disrupting chemicals (EDCs), like parabens [34,72,81,82], with studies reporting removal rates of 92.3% for total N and 82.2% for total P in WW after 168 h [81], compared to values above 70% for N and $\geq 83\%$ for P observed in SWW in the study by Vale et al. [34]. On the other hand, the normalized data revealed no statistically significant differences among treatments for either N or P consumption ($p > 0.05$, Table 2). Specifically, N consumption was 45.58 ± 4.71 , 40.62 ± 3.51 , and 50.32 ± 6.52 μg of N per mg of biomass for the control, 5 mg BPA/L, and 25 mg BPA/L exposure, respectively. Similarly, P consumption was 10.67 ± 1.86 , 8.50 ± 1.67 , and 12.95 ± 2.84 μg of P per mg of biomass, respectively. These findings indicate that the decrease in total N and P removal under BPA exposure was primarily a consequence of the reduced biomass production caused by BPA-induced growth inhibition rather than a reduction in the intrinsic nutrient uptake efficiency of the biomass produced.

Table 2. Nutrient consumption normalized per mg of biomass produced. Statistical differences were subjected to ANOVA followed by the Tukey–Kramer multiple comparison method. The means with different letters are significantly different ($p < 0.05$).

Culture	N Consumption (μg of N per mg of Microalgal Biomass)	P Consumption (μg of P per mg of Microalgal Biomass)
Control	45.58 ± 4.71^a	10.67 ± 1.86^b
5 mg BPA/L	40.62 ± 3.51^a	8.50 ± 1.67^b
25 mg BPA/L	50.32 ± 6.52^a	12.95 ± 2.84^b

Microalgae are microorganisms with a remarkable ability to adapt to different WW environments, capable of adopting mixotrophic or heterotrophic nutritional modes, which allows them to use both light and organic carbon matter available in the environment for their development in WW [28,33]. In this study, glucose was also evaluated as a source of organic carbon in SWW, and Figure 4c shows that axenic *C. vulgaris* culture achieved high performance in glucose consumption from the WW environment, with no statistically significant difference observed at 5 mg of BPA/L compared to the control (96.4% and 98.6%, respectively; $p > 0.05$). However, at the highest BPA concentration tested, glucose uptake by the microalga was significantly inhibited, with only $79.6 \pm 9.2\%$ removal compared to both the control and the exposure of 5 mg/L BPA ($p < 0.05$, Figure 4c). According to the European Wastewater Treatment Directive (UWWTD), the chemical oxygen demand (COD) must be reduced to 125 mg/L, or a minimum reduction of 75% for secondary treatment needs to be achieved [79,80]. Based on the glucose removal observed in this study, the remaining glucose concentrations were in agreement with the EU regulatory standards [80], with a minimum reduction of 75% in both the control and exposure to 5 mg/L and 25 mg/L of BPA (remaining less than 5 mg/L and 100 mg/L of residual glucose in SWW, respectively).

Similarly, Salgueiro et al. [83] reported that *C. vulgaris* was able to assimilate the carbon source in WW to aid rapid growth, achieving over 70% organic matter removal within 9 d.

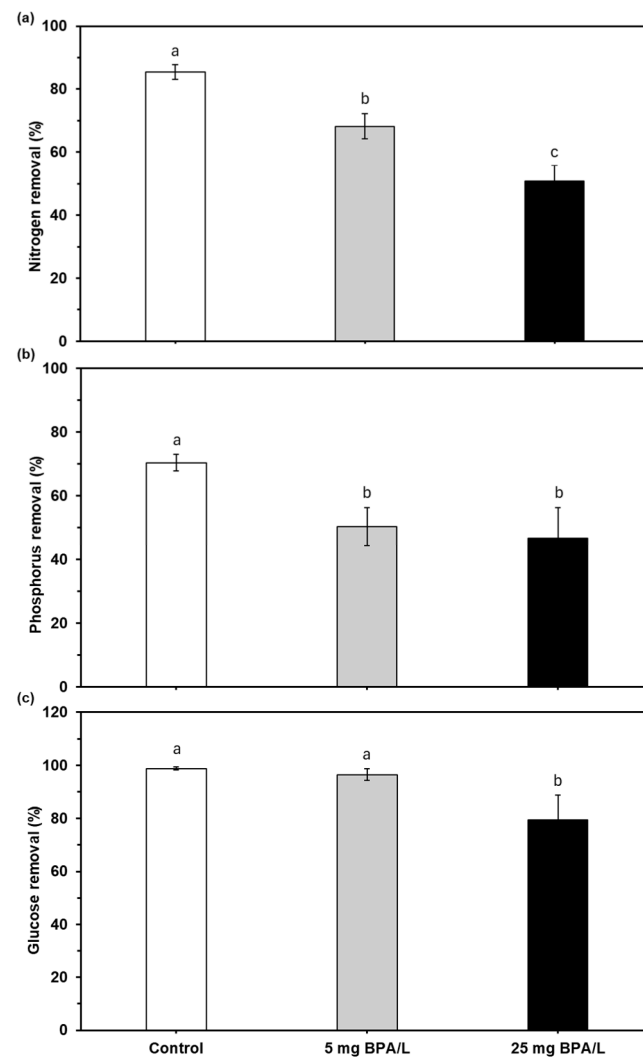


Figure 4. Nutrients and glucose removal from SWW supplemented with two concentrations of BPA, after 168 h. (a)—Nitrogen removal. (b)—Phosphorus removal. (c)—Glucose removal. The data are presented as mean values from at least three independent experiments performed in duplicate ($n \geq 6$); SD are presented (vertical error bars). Statistical differences were subjected to ANOVA followed by the Tukey–Kramer multiple comparison method. The mean values with different letters are significantly different ($p < 0.05$).

The results obtained highlight the problem of BPA accumulation at higher levels in WW systems, compromising their polishing performance by microalgae-based systems, and reducing nutrient consumption. Also, it is noteworthy that exposure to higher concentrations of BPA compromises the ability of the microalga to consume the organic carbon source present in the WW, thereby affecting their mixotrophic behavior.

3.4. BPA Removal from Wastewater

Recent studies have shown that microalgae-based systems are capable of efficiently removing polluting organic compounds from WW [84], such as antibiotics [85], pesticides [86], and parabens [34], using them as a secondary carbon source for their growth [87]. To understand the ability of microalgae to mitigate the presence of plastic additives leached into the WW system, the capacity of *C. vulgaris* to remove BPA from the WW environment for its growth and metabolic activity was analyzed in this study.

Looking at Figure 5, after 168 h of exposure, *C. vulgaris* was able to remove circa 34% and 21% of the amount of BPA present in the SWW when exposed to 5 mg/L and 25 mg/L of BPA, respectively.

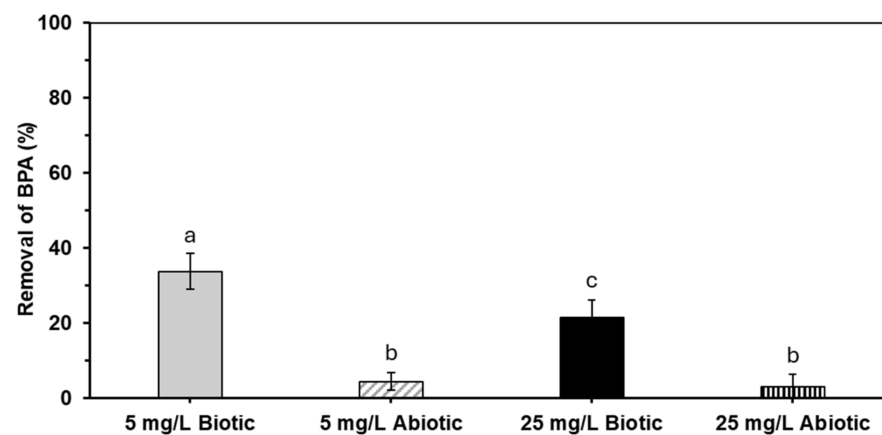


Figure 5. Removal of BPA in the presence or absence of microalga cells. Two different initial concentrations of BPA in SWW were tested in the presence or absence of *C. vulgaris* for 168 h. The data are presented as mean values from at least three independent experiments performed in duplicate ($n \geq 6$); SD are presented (vertical error bars). Statistical differences were subjected to ANOVA, followed by the Tukey–Kramer multiple comparison method. The mean values with different letters are significantly different ($p < 0.05$).

BPA removal was very effective by the microalga, with values higher than those observed in the study by Ji et al. [38] for the same exposure concentrations (25% and 14%, respectively, after 240 h), to study the ability of *C. vulgaris* to biodegrade and/or bioaccumulate BPA in a BBM medium. In Gulnaz and Dincer [12], *C. vulgaris* was exposed for 216 h to a concentration of 20 mg/L of BPA in a Jaworsky medium, showing that most of the contaminant was degraded after 96 h ($\geq 50\%$) and decreased below the detection limit after 144 h (the removal percentage and the detection limit are not mentioned). Other microalgae species were studied for their capacity to remove BPA, at concentrations ranging from 1 mg/L to 50 mg/L, in growth media (such as BBM or mineral salts medium—MSM) for 48 to 240 h. *Chlorella sorokiniana* showed the ability to remove 43.9% of the initial concentration of 20 mg/L of BPA and 39.5% of the concentration of 50 mg/L in MSM [88]. The same efficiency was observed for two extremophilic species (*Picocystis* sp. and *Graesiella* sp.), which were able to remove 39.9% and 34.8%, respectively, for 25 mg BPA/L in BBM medium [60]. Also, *Chlamydomonas mexicana* in BBM could remove up to 38% and 16%, after 240 h exposure, for 5 mg/L and 25 mg/L of BPA, respectively [38].

It is possible to infer that increasing the concentration of BPA reduces its removal percentage by the microalgae, as also observed in this study (Figure 5), but to emphasize that in terms of the removal rate, the microalgae achieve more when exposed to higher concentrations of BPA compared to the exposure to 5 mg/L, after 168 h (0.798 ± 0.040 mg/L.d and 0.264 ± 0.035 mg/L.d respectively, $p < 0.05$). This may be due to the higher bioavailability of BPA in the SWW environment, competing with glucose as an organic source, which can be consumed by microalgae for their growth and metabolic activity [38]. This competition can be observed in Figure 4c, which shows that at a concentration of 25 mg/L of BPA, there was a decrease in the glucose consumption by microalgal cells as a carbon source in SWW, due to the presence of a higher amount of BPA. Furthermore, Figure 3a shows that from 72 to 96 h of exposure to 25 mg BPA/L, *C. vulgaris* begins to recover its growth, corroborating Ji et al. [38]. This means that, with the consumption of BPA ($21 \pm 3\%$), its concentration is reduced in the SWW, allowing the microalga to adapt to the toxic effects of

BPA caused by esterase reduction and the intracellular accumulation of ROS (Figure 1a,b). However, to better understand whether this reduction is solely due to microalgal activity or influenced by external factors, the role of photodegradation potentiated by microalgae-based systems [34] must also be considered. For this reason, in this present work, the influence of photodegradation was also assessed by the exposure of abiotic controls to BPA (at 5 and 25 mg/L), cultivated for 168 h, with the same exposure to light, temperature, and agitation as the assays with the *C. vulgaris* culture. Figure 5 shows that BPA was minimally reduced in the abiotic controls ($p < 0.05$), with only 5% and 3% estimated for 5 mg/L and 25 mg/L of BPA, respectively. Similar results were reported by Gulnaz and Dincer [12] and Gatullo et al. [89], in which the authors observed that physicochemical abiotic degradation of BPA was absent or was not statistically significant for BPA. In fact, previous studies have demonstrated that BPA can be highly persistent in sterile or abiotic environments, with no significant changes in concentration observed in artificial water over 150 d [90]. In natural surface waters, reported BPA half-lives vary widely, from around 66 h to 160 d, depending on physicochemical and biological conditions [91].

This variability highlights that, under sterile and abiotic conditions, BPA tends to remain largely unchanged for extended periods, while in natural environments its fate is strongly modulated by the presence of microbial activity and other degradation pathways.

This study demonstrates the ability of *C. vulgaris* to promote significant BPA removal from SWW, with abiotic controls confirming that BPA attenuation was primarily associated with the presence of the microalga. Although higher concentrations of BPA reduced removal efficiency (%), the removal rates increased. The results confirm the potential of the microalga for the bioremediation of plastic additives in WW systems.

4. Conclusions

This study evaluated the effects of BPA on microalga *Chlorella vulgaris* in a SWW environment and assessed its potential for simultaneous WW polishing and BPA removal. BPA exposure induced a concentration-dependent physiological stress response, characterized by reduced esterase activity and increased intracellular ROS accumulation. These metabolic alterations negatively affected microalgal growth, resulting in a 44% reduction in biomass production at 25 mg BPA/L. Despite these effects, *C. vulgaris* maintained stable chlorophyll and carotenoid contents, demonstrating the resilience of its photosynthetic apparatus under BPA exposure.

The inhibitory effects of BPA were also reflected in the WW polishing performance of the microalga. Nutrient removal efficiencies decreased by up to 38% for N and 34% for P, while glucose consumption was significantly reduced at the highest BPA concentration tested. These findings indicate that BPA-induced metabolic stress compromises the capacity of *C. vulgaris* to assimilate nutrients and organic carbon from WW, directly affecting the overall treatment efficiency.

Nevertheless, *C. vulgaris* significantly reduced BPA concentrations in the WW matrix, achieving removal efficiencies of 34% and 21% at initial concentrations of 5 and 25 mg/L, respectively, whereas abiotic losses remained negligible. These results demonstrate that BPA attenuation was primarily associated with the presence of the microalga and highlight the potential of *C. vulgaris* for the treatment of BPA-contaminated WW. However, the analytical approach employed in this study quantified only the decrease in BPA concentration in the aqueous phase and did not distinguish among biodegradation, biosorption, intracellular bioaccumulation, or transformation into intermediate metabolites.

Importantly, the results provide valuable insights for optimizing microalgae-based BPA remediation systems. Since BPA removal was closely associated with microalgal physiological performance, strategies aimed at maintaining high biomass productivity and

metabolic activity are expected to improve treatment outcomes. The superior performance observed at 5 mg BPA/L suggests that preventing excessive contaminant accumulation is critical for sustaining efficient remediation. Furthermore, increasing biomass density, extending cultivation or hydraulic retention times, and promoting acclimation of microalgal cultures to BPA exposure may enhance both BPA removal and WW polishing performance.

The significant BPA removal achieved by *C. vulgaris* highlights the need for future studies to elucidate the mechanisms involved in contaminant attenuation. A better understanding of the relative contributions of biosorption, intracellular accumulation, biodegradation, and metabolite formation, using complementary analytical techniques, would provide a scientific basis for optimizing microalgae-based wastewater treatment systems and further improving BPA removal under realistic operating conditions.

Overall, this work demonstrates that microalga *C. vulgaris* is a promising candidate for the treatment of BPA-contaminated WW, while identifying key physiological and operational factors that should be considered to optimize the performance of microalgae-based remediation systems.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/app16136578/s1>, Figure S1: *C. vulgaris* growth in SWW exposed to 0.05% and 0.25% of methanol. (a)—Microalga growth. (b)—Cellular concentration after 168 h. The data are presented as mean values from at least three independent experiments performed in duplicate ($n \geq 6$); SD are presented (vertical error bars). Statistical differences were subjected to ANOVA followed by the Tukey–Kramer multiple comparison method. The mean values without different letters are not significantly different ($p > 0.05$); Table S1: Total biomass produced by *C. vulgaris* for 168 h, under 0.05% and 0.25% of methanol concentrations. Statistical differences were subjected to ANOVA followed by the Tukey–Kramer multiple comparison method. The means without different letters are not significantly different ($p > 0.05$).

Author Contributions: P.M.S.S.: Conceptualization, investigation, and writing—original draft. I.M.: investigation, and writing—original draft. M.S.: writing—review and editing, validation, supervision, and funding acquisition. C.A.S.: conceptualization, writing—review and editing, validation, and supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by national funds through Foundation for Science and Technology/The Ministry of Education, Science, and Innovation (FCT/MECI): Laboratory for Process Engineering, Environment, Biotechnology and Energy (LEPABE), UID/00511/2025 (<https://doi.org/10.54499/UID/00511/2025>) and UID/PRR/00511/2025 (<https://doi.org/10.54499/UID/PRR/00511/2025>), and Associate Laboratory in Chemical Engineering (ALiCE), LA/P/0045/2020 (<https://doi.org/10.54499/LA/P/0045/2020>); the grant attributed by Portuguese Foundation for Science and Technology, I.P.—FCT—to Paulo Sousa by project reference 2022.14159.BD (<https://doi.org/10.54499/2022.14159.BD>).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors thank the Faculty of Engineering of the University of Porto and LEPABE/ALiCE for providing access to their laboratory facilities.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Das, S.; Parida, V.K.; Tiwary, C.S.; Gupta, A.K.; Chowdhury, S. Emerging Contaminants in the Aquatic Environment: Fate, Occurrence, Impacts, and Toxicity. In *Bioremediation of Emerging Contaminants in Water*; American Chemical Society: Washington, DC, USA, 2024; Volume 1, p. 1. [\[CrossRef\]](#)
2. Ebrahimi, P.; Abbasi, S.; Pashaei, R.; Bogusz, A.; Oleszczuk, P. Investigating impact of physicochemical properties of microplastics on human health: A short bibliometric analysis and review. *Chemosphere* **2022**, *289*, 133146. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Murphy, F.; Ewins, C.; Carbonnier, F.; Quinn, B. Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. *Environ. Sci. Technol.* **2016**, *50*, 5800–5808. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Sousa, P.M.S.; Kinney, K.A.; Sousa, C.A.; Simões, M. *Microalgae for Microplastic Removal from Water and Wastewater: A Review*; Springer International Publishing: Berlin/Heidelberg, Germany, 2025. [\[CrossRef\]](#)
5. Liu, W.; Zhang, J.; Liu, H.; Guo, X.; Zhang, X.; Yao, X.; Cao, Z.; Zhang, T. A review of the removal of microplastics in global wastewater treatment plants: Characteristics and mechanisms. *Environ. Int.* **2021**, *146*, 106277. [\[CrossRef\]](#) [\[PubMed\]](#)
6. GESAMP. *Sources, Fate and Effects of Microplastics in the Marine Environment: A Global Assessment*; GESAMP: London, UK, 2015.
7. Bhuyan, M.S. Effects of Microplastics on Fish and in Human Health. *Front. Environ. Sci.* **2022**, *10*, 827289. [\[CrossRef\]](#)
8. Saeedi, M. How microplastics interact with food chain: A short overview of fate and impacts. *J. Food Sci. Technol.* **2024**, *61*, 403–413. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Li, Y.; Tao, L.; Wang, Q.; Wang, F.; Li, G.; Song, M. Potential Health Impact of Microplastics: A Review of Environmental Distribution, Human Exposure, and Toxic Effects. *Environ. Health* **2023**, *1*, 249–257. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Winiarska, E.; Jutel, M.; Zemelka-Wiacek, M. The potential impact of nano- and microplastics on human health: Understanding human health risks. *Environ. Res.* **2024**, *251*, 118535. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Maddela, N.R.; Kakarla, D.; Venkateswarlu, K.; Megharaj, M. Additives of plastics: Entry into the environment and potential risks to human and ecological health. *J. Environ. Manag.* **2023**, *348*, 119364. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Gulnaz, O.; Dincer, S. Biodegradation of Bisphenol a by *Chlorella vulgaris* and *Aeromonas Hydrophilia*. *J. Appl. Biol. Sci.* **2009**, *3*, 7.
13. Kataria, N.; Bhushan, D.; Gupta, R.; Rajendran, S.; Teo, M.Y.M.; Khoo, K.S. Current progress in treatment technologies for plastic waste (bisphenol A) in aquatic environment: Occurrence, toxicity and remediation mechanisms. *Environ. Pollut.* **2022**, *315*, 120319. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Mojiri, A.; Zhou, J.L.; Farahani, S. Advancements in photocatalysis and machine learning for bisphenol A (BPA) degradation in aquatic systems: A critical review. *J. Clean. Prod.* **2025**, *518*, 145919. [\[CrossRef\]](#)
15. Tappert, L.; Bunge, M.; Hoehne, D.; Dlugi, I.; Fetters, K.; Fischer, B.; Mueller, G.; Bock, M.; Gestermann, S. Bisphenol A in surface waters in Germany: Part I. Reassessment of sources and emissions pathways for FlowEQ modeling. *Integr. Environ. Assess. Manag.* **2024**, *20*, 211–225. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Hu, Y.; Zhu, Q.; Yan, X.; Liao, C.; Jiang, G. Occurrence, fate and risk assessment of BPA and its substituents in wastewater treatment plant: A review. *Environ. Res.* **2019**, *178*, 108732. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Ferrer-Polonio, E.; Alvim, C.B.; Fernández-Navarro, J.; Mompó-Curell, R.; Mendoza-Roca, J.A.; Bes-Piá, A.; Alonso-Molina, J.L.; Amorós-Muñoz, I. Influence of bisphenol A occurrence in wastewaters on biomass characteristics and activated sludge process performance. *Sci. Total Environ.* **2021**, *778*, 146355. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Dimassi, S.N.; Hahladakis, J.N.; Yahia, M.N.D.; Ahmad, M.I.; Sayadi, S.; Al-Ghouti, M.A. Effect of temperature and sunlight on the leachability potential of BPA and phthalates from plastic litter under marine conditions. *Sci. Total Environ.* **2023**, *894*, 164954. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Xu, S.; Zhang, H.; He, P.; Shao, L. Leaching behaviour of bisphenol A from municipal solid waste under landfill environment. *Environ. Technol.* **2011**, *32*, 1269–1277. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Sadia, M.; Mahmood, A.; Ibrahim, M.; Irshad, M.K.; Quddusi, A.H.A.; Bokhari, A.; Mubashir, M.; Chuah, L.F.; Show, P.L. Microplastics pollution from wastewater treatment plants: A critical review on challenges, detection, sustainable removal techniques and circular economy. *Environ. Technol. Innov.* **2022**, *28*, 102946. [\[CrossRef\]](#)
21. Sousa, C.A.; Sousa, H.; Vale, F.; Simões, M. Microalgae-based bioremediation of wastewaters—Influencing parameters and mathematical growth modelling. *Chem. Eng. J.* **2021**, *425*, 131412. [\[CrossRef\]](#)
22. Sun, J.; Dai, X.; Wang, Q.; van Loosdrecht, M.C.M.; Ni, B.J. Microplastics in wastewater treatment plants: Detection, occurrence and removal. *Water Res.* **2019**, *152*, 21–37. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Trakulmututa, J.; Chuaicham, C.; Shenoy, S.; Sriksaow, A.; Sasaki, K.; Smith, S.M. Effect of transformation temperature toward optical properties of derived CuO/ZnO composite from Cu–Zn hydroxide nitrate for photocatalytic ciprofloxacin degradation. *Opt. Mater.* **2022**, *133*, 112941. [\[CrossRef\]](#)
24. Tan, J.-X.; Chen, Z.-Y.; Chen, C.H.; Hsieh, M.-F.; Lin, A.Y.-C.; Chen, S.S.; Wu, K.C.-W. Efficient adsorption and photocatalytic degradation of water emerging contaminants through nanoarchitectonics of pore sizes and optical properties of zirconium-based MOFs. *J. Hazard. Mater.* **2023**, *451*, 131113. [\[CrossRef\]](#) [\[PubMed\]](#)

25. Kaur, S.; Kaur, S.; Thakur, B.; Narwat, N. Microalgae-based bioremediation of emerging contaminants: Techniques, recent developments, and future perspectives. *Arch. Microbiol.* **2025**, *208*, 29. [CrossRef] [PubMed]
26. Deepika, C.; Frascaroli, G.; Gowri, A.; Silkina, A. Microalgae-Based Bioremediation: A Sustainable Approach for the Removal of Emergent Pollutants. In *Biotechnological Interventions in the Removal of Emerging Pollutants*; Dey, S., Bhattacharya, S., Eds.; Springer Nature: Singapore, 2025; pp. 101–139.
27. Qin, Y.; Li, T.; Nie, C.; Geng, X.; Sun, X. Microalgae: Promising solutions paving the way toward a greener and more sustainable future. *Front. Fuels* **2025**, *3*, 1643675. [CrossRef]
28. Sutherland, D.L.; Ralph, P.J. Microalgal bioremediation of emerging contaminants—Opportunities and challenges. *Water Res.* **2019**, *164*, 114921. [CrossRef] [PubMed]
29. Agüera, A.; Plaza-Bolaños, P.; Fernández, F.G.A. Removal of contaminants of emerging concern by microalgae-based wastewater treatments and related analytical techniques. In *Current Developments in Biotechnology and Bioengineering*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 503–525. [CrossRef]
30. Rempel, A.; Gutkoski, J.P.; Nazari, M.T.; Biolchi, G.N.; Cavanhi, V.A.F.; Treichel, H.; Colla, L.M. Current advances in microalgae-based bioremediation and other technologies for emerging contaminants treatment. *Sci. Total Environ.* **2021**, *772*, 144918. [CrossRef] [PubMed]
31. Pacheco, D.; Rocha, A.C.; Pereira, L.; Verdelhos, T. Microalgae water bioremediation: Trends and hot topics. *Appl. Sci.* **2020**, *10*, 1886. [CrossRef]
32. Al-Jabri, H.; Das, P.; Khan, S.; Thaher, M.; Abdulquadir, M. Treatment of wastewaters by microalgae and the potential applications of the produced biomass—A review. *Water* **2021**, *13*, 27. [CrossRef]
33. Abdelfattah, A.; Ali, S.S.; Ramadan, H.; El-Aswar, E.I.; Eltawab, R.; Ho, S.H.; Elsamahy, T.; Li, S.; El-Sheekh, M.M.; Schagerl, M.; et al. Microalgae-based wastewater treatment: Mechanisms, challenges, recent advances, and future prospects. *Environ. Sci. Ecotechnol.* **2023**, *13*, 100205. [CrossRef] [PubMed]
34. Vale, F.; Sousa, C.A.; Sousa, H.; Santos, L.; Simões, M. Impact of parabens on microalgae bioremediation of wastewaters: A mechanistic study. *Chem. Eng. J.* **2022**, *442*, 136374. [CrossRef]
35. Liu, S.; Hua, J.; Zhang, Y.; Gao, Y.; Han, X.; Liu, Y.; Daigger, G.T.; Li, Q.; Guo, N.; Song, Z.; et al. A critical review on microalgae-driven wastewater treatment: Dual focus on high-value bioproducts recovery and emerging contaminants removal. *J. Environ. Sci.* **2026**. [CrossRef]
36. Tan, J.; Fang, L.; Li, P.; Zhai, X.; Xu, W.; Zhao, C.; Luan, T.; Yang, Y.; Yang, L. Microalgal resilience under Bisphenol A exposure: Dynamic metabolic reprogramming, deoxynucleotide pool imbalance and DNA damage management. *Environ. Chem. Ecotoxicol.* **2026**, *8*, 2167–2180. [CrossRef]
37. da Silva, P.R.; Mounteer, A.H. Removal of environmental estrogens and estrogenic activity by microalgae and microalgae-bacteria consortia: An integrative review. *Environ. Sci. Pollut. Res.* **2025**, *32*, 22794–22811. [CrossRef] [PubMed]
38. Ji, M.K.; Kabra, A.N.; Choi, J.; Hwang, J.H.; Kim, J.R.; Abou-Shanab, R.A.I.; Oh, Y.K.; Jeon, B.H. Biodegradation of bisphenol A by the freshwater microalgae *Chlamydomonas mexicana* and *Chlorella vulgaris*. *Ecol. Eng.* **2014**, *73*, 260–269. [CrossRef]
39. Hirooka, T.; Nagase, H.; Uchida, K.; Hiroshige, Y.; Ehara, Y.; Nishikawa, J.I.; Nishihara, T.; Miyamoto, K.; Hirata, Z. Biodegradation of bisphenol A and disappearance of its estrogenic activity by the green alga *Chlorella fusca* var. *vacuolata*. *Environ. Toxicol. Chem.* **2005**, *24*, 1896–1901. [CrossRef] [PubMed]
40. García, N.; Rodríguez, R.; Vicente, G.; Espada, J.J.; Bautista, L.F. Comprehensive Study on Endocrine Disruptor Removal from Wastewater Using Different Microalgae Species. *Appl. Sci.* **2025**, *15*, 132. [CrossRef]
41. Maizatul, A.Y.; Radin Mohamed, R.M.S.; Al-Gheethi, A.A.; Hashim, M.K.A. An overview of the utilisation of microalgae biomass derived from nutrient recycling of wet market wastewater and slaughterhouse wastewater. *Int. Aquat. Res.* **2017**, *9*, 177–193. [CrossRef]
42. Martinelli, E. *Occurrence, Fate and Toxicity of Bisphenol A in the Aquatic Environment—A Short Review E02-Final Assignment*; Università di Bologna: Bologna, Italy, 2021.
43. Petrie, B.; Lopardo, L.; Proctor, K.; Youdan, J.; Barden, R.; Kasprzyk-Hordern, B. Assessment of bisphenol-A in the urban water cycle. *Sci. Total Environ.* **2019**, *650*, 900–907. [CrossRef] [PubMed]
44. OECD. *Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test*; OECD: Paris, France, 2011. [CrossRef]
45. Strickland, J.D.H.; Parsons, T.R.; of Canada, F.R.B.; Strickland, J.D.H.A. *A Practical Handbook of Seawater Analysis*; Bulletin: New South Wales, Australia; Fisheries Research Board of Canada: Ottawa, ON, Canada, 1972. Available online: <https://books.google.pt/books?id=0aJExgEACAAJ> (accessed on 4 January 2026).
46. Carvalho, A.P.; Meireles, L.A.; Malcata, F.X. Rapid spectrophotometric determination of nitrates and nitrites in marine aqueous culture media. *Analisis* **1998**, *26*, 347–351. [CrossRef]
47. Baird, R.B.; Eaton, A.D. *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; APHA-AWWA-WEF: Washington, DC, USA, 1998.

48. Eblagon, K.M.; Arenillas, A.; Malaika, A.; Fernando, R.; Pereira, M.; Figueiredo, J.L. The influence of the surface chemistry of phosphorylated carbon xerogel catalysts on the production of HMF from fructose in water. *Fuel* **2023**, *334*, 126610. [[CrossRef](#)]
49. Banihashemi, B.; Droste, R.L. Trace level determination of bisphenol-A in wastewater and sewage sludge by high-performance liquid chromatography and UV detection. *Water Qual. Res. J.* **2013**, *48*, 133–144. [[CrossRef](#)]
50. Natarajan, L.; Soupam, D.; Dey, S.; Chandrasekaran, N.; Kundu, R.; Paul, S.; Mukherjee, A. Toxicity of polystyrene microplastics in freshwater algae *Scenedesmus obliquus*: Effects of particle size and surface charge. *Toxicol. Rep.* **2022**, *9*, 1953–1961. [[CrossRef](#)] [[PubMed](#)]
51. Xiang, Q.; Zhou, Y.; Tan, C. Toxicity Effects of Polystyrene Nanoplastics with Different Sizes on Freshwater Microalgae *Chlorella vulgaris*. *Molecules* **2023**, *28*, 3958. [[CrossRef](#)] [[PubMed](#)]
52. Cao, Q.; Sun, W.; Yang, T.; Zhu, Z.; Jiang, Y.; Hu, W.; Wei, W.; Zhang, Y.; Yang, H. The toxic effects of polystyrene microplastics on freshwater algae *Chlorella pyrenoidosa* depends on the different size of polystyrene microplastics. *Chemosphere* **2022**, *308*, 136135. [[CrossRef](#)] [[PubMed](#)]
53. Li, S.; Wang, P.; Zhang, C.; Zhou, X.; Yin, Z.; Hu, T.; Hu, D.; Liu, C.; Zhu, L. Influence of polystyrene microplastics on the growth, photosynthetic efficiency and aggregation of freshwater microalgae *Chlamydomonas reinhardtii*. *Sci. Total Environ.* **2020**, *714*, 136767. [[CrossRef](#)] [[PubMed](#)]
54. Afonso, V.; Borges, R.; Rodrigues, B.; Barros, R.; João Bebianno, M.; Raposo, S. Are native microalgae consortia able to remove microplastics from wastewater effluents? *Environ. Pollut.* **2024**, *349*, 123931. [[CrossRef](#)] [[PubMed](#)]
55. Yu, Y.; Kumar, M.; Bolan, S.; Padhye, L.P.; Bolan, N.; Li, S.; Wang, L.; Hou, D.; Li, Y. Various additive release from microplastics and their toxicity in aquatic environments. *Environ. Pollut.* **2024**, *343*, 123219. [[CrossRef](#)] [[PubMed](#)]
56. Xiao, X.; Han, Z.; Chen, Y.; Liang, X.; Li, H.; Qian, Y. Optimization of FDA–PI method using flow cytometry to measure metabolic activity of the cyanobacteria, *Microcystis aeruginosa*. *Phys. Chem. Earth Parts A/B/C* **2011**, *36*, 424–429. [[CrossRef](#)]
57. Wang, J.; Zhu, J.; Liu, S.; Liu, B.; Gao, Y.; Wu, Z. Generation of reactive oxygen species in cyanobacteria and green algae induced by allelochemicals of submerged macrophytes. *Chemosphere* **2011**, *85*, 977–982. [[CrossRef](#)] [[PubMed](#)]
58. Esperanza, M.; Seoane, M.; Servia, M.J.; Cid, Á. Effects of Bisphenol A on the microalga *Chlamydomonas reinhardtii* and the clam *Corbicula fluminea*. *Ecotoxicol. Environ. Saf.* **2020**, *197*, 110609. [[CrossRef](#)] [[PubMed](#)]
59. Duan, L.; Chen, Q.; Duan, S. Transcriptional Analysis of *Chlorella pyrenoidosa* Exposed to Bisphenol A. *Int. J. Environ. Res. Public Health* **2019**, *16*, 1374. [[CrossRef](#)] [[PubMed](#)]
60. Ben Ouada, S.; Ben Ali, R.; Leboulanger, C.; Zaghdien, H.; Choura, S.; Ben Ouada, H.; Sayadi, S. Effect and removal of bisphenol A by two extremophilic microalgal strains (Chlorophyta). *J. Appl. Phycol.* **2018**, *30*, 1765–1776. [[CrossRef](#)]
61. Fabrello, J.; Guidorizzi, S.; Ciscato, M.; Battistuzzi, M.; Moschin, E.; Dalla Vecchia, F.; Moro, I.; Roverso, M.; Bogiatti, S.; Matozzo, V. Ultrastructural changes, pigment responses and bioaccumulation in the microalga *Phaeodactylum tricornutum* Bohlin exposed to BPA analogues. *Aquat. Toxicol.* **2024**, *272*, 106970. [[CrossRef](#)] [[PubMed](#)]
62. Seoane, M.; Cid, Á.; Esperanza, M. Toxicity of bisphenol A on marine microalgae: Single- and multispecies bioassays based on equivalent initial cell biovolume. *Sci. Total Environ.* **2021**, *767*, 144363. [[CrossRef](#)] [[PubMed](#)]
63. Li, J.; Wang, Y.; Li, N.; He, Y.; Xiao, H.; Fang, D.; Chen, C. Toxic Effects of Bisphenol A and Bisphenol S on *Chlorella Pyrenoidosa* under Single and Combined Action. *Int. J. Environ. Res. Public Health* **2022**, *19*, 4245. [[CrossRef](#)] [[PubMed](#)]
64. Azizullah, A.; Khan, S.; Gao, G.; Gao, K. The interplay between bisphenol A and algae—A review. *J. King Saud Univ. Sci.* **2022**, *34*, 102050. [[CrossRef](#)]
65. Li, R.; Liu, Y.; Chen, G.; Tam, N.F.Y.; Shin, P.K.S.; Cheung, S.G.; Luan, T. Physiological responses of the alga *Cyclotella caspia* to bisphenol A exposure. *Bot. Mar.* **2008**, *51*, 360–369. [[CrossRef](#)]
66. Yang, M.; Fan, Z.; Xie, Y.; Fang, L.; Wang, X.; Yuan, Y.; Li, R. Transcriptome analysis of the effect of bisphenol A exposure on the growth, photosynthetic activity and risk of microcystin-LR release by *Microcystis aeruginosa*. *J. Hazard. Mater.* **2020**, *397*, 122746. [[CrossRef](#)] [[PubMed](#)]
67. He, D.; Zeng, Y.; Zhou, G. The influence of microplastics on the toxic effects and biodegradation of bisphenol A in the microalgae *Chlorella pyrenoidosa*. *Aquat. Ecol.* **2022**, *56*, 1287–1296. [[CrossRef](#)]
68. Li, R.; Chen, G.Z.; Tam, N.F.Y.; Luan, T.G.; Shin, P.K.S.; Cheung, S.G.; Liu, Y. Toxicity of bisphenol A and its bioaccumulation and removal by a marine microalga *Stephanodiscus hantzschii*. *Ecotoxicol. Environ. Saf.* **2009**, *72*, 321–328. [[CrossRef](#)] [[PubMed](#)]
69. Liu, Y.; Guan, Y.; Gao, Q.; Tam, N.F.Y.; Zhu, W. Cellular responses, biodegradation and bioaccumulation of endocrine disrupting chemicals in marine diatom *Navicula incerta*. *Chemosphere* **2010**, *80*, 592–599. [[CrossRef](#)] [[PubMed](#)]
70. Czarny-Krzywińska, K.; Krawczyk, B.; Szczukocki, D. Toxicity of bisphenol A and its structural congeners to microalgae *Chlorella vulgaris* and *Desmodesmus armatus*. *J. Appl. Phycol.* **2022**, *34*, 1397–1410. [[CrossRef](#)]
71. Atengueño-Reyes, K.; Velasquez-Orta, S.B.; Yáñez-Nogues, I.; Monje-Ramirez, I.; Mijaylova-Nacheva, P.; Chávez-Mejía, A.; Orta Ledesma, M.T. Microalgal consortium tolerance to bisphenol A and triclosan in wastewater and their effects on growth, biomolecule content and nutrient removal. *Ecotoxicol. Environ. Saf.* **2023**, *262*, 115117. [[CrossRef](#)] [[PubMed](#)]

72. Kong, W.; Kong, J.; Ma, J.; Lyu, H.; Feng, S.; Wang, Z.; Yuan, P.; Shen, B. *Chlorella vulgaris* cultivation in simulated wastewater for the biomass production, nutrients removal and CO₂ fixation simultaneously. *J. Environ. Manag.* **2021**, *284*, 112070. [CrossRef] [PubMed]
73. Matamoros, V.; Uggetti, E.; García, J.; Bayona, J.M. Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: A laboratory scale study. *J. Hazard. Mater.* **2016**, *301*, 197–205. [CrossRef] [PubMed]
74. Tran, T.K.; Truong, N.T.; Nguyen, N.H. Ability of *Chlorella vulgaris* algae for nutrients removal in domestic wastewater and its collection by ferrate. *J. Vietnam. Environ.* **2019**, *11*, 27–32. [CrossRef]
75. Sousa, S.A.; Esteves, A.F.; Salgado, E.M.; Pires, J.C.M. Enhancing urban wastewater treatment: *Chlorella vulgaris* performance in tertiary treatment and the impact of anaerobic digestate addition. *Environ. Technol. Innov.* **2024**, *34*, 103601. [CrossRef]
76. Wang, L.; Min, M.; Li, Y.; Chen, P.; Chen, Y.; Liu, Y.; Wang, Y.; Ruan, R. Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Appl. Biochem. Biotechnol.* **2010**, *162*, 1174–1186. [CrossRef] [PubMed]
77. Liang, Z.; Ge, F.; Zeng, H.; Xu, Y.; Peng, F.; Wong, M. Influence of cetyltrimethyl ammonium bromide on nutrient uptake and cell responses of *Chlorella vulgaris*. *Aquat. Toxicol.* **2013**, *138–139*, 81–87. [CrossRef] [PubMed]
78. Ministério do Ambiente. *Diário Da República—I SÉRIE-A*; Ministério do Ambiente: Lisbon, Portugal, 1997; pp. 4775–4780. Available online: <https://dre.pt/application/conteudo/365343> (accessed on 16 January 2026).
79. European Commission. UWWTD—Amendment 1998 (98/15/EC). *Off. J. Eur. Communities* **1998**, *29–30*. Available online: <https://eur-lex.europa.eu/EN/legal-content/summary/urban-waste-water-treatment.html> (accessed on 27 May 2026).
80. European Parliamentary Research Service. Briefing: EU Legislation in Progress. In *Urban Wastewater Treatment, Updating EU Rules*; European Parliamentary Research Service: Brussels, Belgium, 2024.
81. Zhou, G.J.; Ying, G.G.; Liu, S.; Zhou, L.J.; Chen, Z.F.; Peng, F.Q. Simultaneous removal of inorganic and organic compounds in wastewater by freshwater green microalgae. *Environ. Sci. Process. Impacts* **2014**, *16*, 2018–2027. [CrossRef] [PubMed]
82. Al-Mamun, A.; Amid, A.; Karim, I.A.; Rashid, M.S. Phytoremediation of Partially Treated Wastewater by *Chlorella vulgaris*. 2012. Available online: <http://irep.iium.edu.my/id/eprint/25890> (accessed on 1 February 2026).
83. Salgueiro, J.L.; Pérez, L.; Maceiras, R.; Sánchez, Á.; Cancela, Á. Bioremediation of Wastewater using *Chlorella Vulgaris* Microalgae: Phosphorus and Organic Matter. *Int. J. Environ. Res.* **2016**, *10*, 465–470. [CrossRef]
84. Liu, R.; Li, S.; Tu, Y.; Hao, X. Capabilities and mechanisms of microalgae on removing micropollutants from wastewater: A review. *J. Environ. Manag.* **2021**, *285*, 112149. [CrossRef] [PubMed]
85. Frascaroli, G.; Roberts, J.; Hunter, C.; Escudero, A. Removal efficiencies of seven frequently detected antibiotics and related physiological responses in three microalgae species. *Environ. Sci. Pollut. Res.* **2024**, *31*, 14178–14190. [CrossRef] [PubMed]
86. Nie, J.; Sun, Y.; Zhou, Y.; Kumar, M.; Usman, M.; Li, J.; Shao, J.; Wang, L.; Tsang, D.C.W. Bioremediation of water containing pesticides by microalgae: Mechanisms, methods, and prospects for future research. *Sci. Total Environ.* **2020**, *707*, 136080. [CrossRef] [PubMed]
87. Maia, C.; Sousa, H.; Vale, F.; Sousa, C.A.; Simões, M. The influence of photoperiod and organic carbon levels in parabens removal from wastewater by *Chlorella vulgaris*. *J. Environ. Manag.* **2025**, *375*, 124391. [CrossRef] [PubMed]
88. Eio, E.J.; Kawai, M.; Niwa, C.; Ito, M.; Yamamoto, S.; Toda, T. Biodegradation of bisphenol A by an algal-bacterial system. *Environ. Sci. Pollut. Res.* **2015**, *22*, 15145–15153. [CrossRef] [PubMed]
89. Gattullo, C.E.; Bährs, H.; Steinberg, C.E.W.; Loffredo, E. Removal of bisphenol A by the freshwater green alga *Monoraphidium braunii* and the role of natural organic matter. *Sci. Total Environ.* **2012**, *416*, 501–506. [CrossRef] [PubMed]
90. Kocaman, E.; Ozhan, K. Degradation of Bisphenol A in Natural and Artificial Marine and Freshwaters in Turkey. *Bull. Environ. Contam. Toxicol.* **2019**, *103*, 496–500. [CrossRef] [PubMed]
91. Im, J.; Löffler, F.E. Fate of Bisphenol A in Terrestrial and Aquatic Environments. *Environ. Sci. Technol.* **2016**, *50*, 8403–8416. [CrossRef] [PubMed]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.