

Article

Efficiency of Photosynthetic Microbial Fuel Cells (pMFC) Depending on the Type of Microorganisms Inhabiting the Cathode Chamber

Marcin Zieliński , Paulina Rusanowska , Magda Dudek , Adam Starowicz , Łukasz Barczak and Marcin Dębowski * 

Department of Environment Engineering, University of Warmia and Mazury in Olsztyn, Warszawska 117, 10-720 Olsztyn, Poland; marcin.zielinski@uwm.edu.pl (M.Z.); paulina.jaranowska@uwm.edu.pl (P.R.); magda.dudek@uwm.edu.pl (M.D.); adam.starowicz@uwm.edu.pl (A.S.); lukasz.barczak@uwm.edu.pl (Ł.B.)

* Correspondence: marcin.debowski@uwm.edu.pl

Abstract: Photosynthetic microbial fuel cells (pMFCs) are hybrid systems that enable simultaneous wastewater treatment under anaerobic conditions and the generation of electricity by utilizing the potential difference in the anaerobic anode chamber and the oxygenated cathode chamber. Dairy wastewater with a concentration of 2000 mg COD/L was treated in the anode of a batch pMFC. In the cathode chamber, *Chlorella vulgaris* or *Arthrospira platensis* was cultivated in synthetic medium, and next in diluted effluent from the anode chamber. The highest power density of 91 mW/m² was generated by the pMFC with the cultivation of *Arthrospira platensis*. Higher values of dissolved oxygen remained during the dark phase in the cathodic medium with *Arthrospira platensis* cultivation than with *Chlorella vulgaris*. This depletion of oxygen significantly decreased voltage generation, which during the light phase increased again to the maximum values. The COD removal achieved in the anodic chamber was 87%. The efficiency of nitrogen removal in the cathode chamber during the cultivation of *Arthrospira platensis* and *Chlorella vulgaris* was about 78% and 69%, respectively. The efficiency of phosphorus removal in the cathode chamber with the cultivation of *Arthrospira platensis* and *Chlorella vulgaris* was 58% and 43%, respectively. This study has shown that the introduction of *Arthrospira platensis* into the cathode chamber is more effective than that of *Chlorella vulgaris*.

Keywords: photosynthetic microbial fuel cells; *Chlorella vulgaris* cultivation; *Arthrospira platensis* cultivation



Citation: Zieliński, M.; Rusanowska, P.; Dudek, M.; Starowicz, A.; Barczak, Ł.; Dębowski, M. Efficiency of Photosynthetic Microbial Fuel Cells (pMFC) Depending on the Type of Microorganisms Inhabiting the Cathode Chamber. *Energies* **2024**, *17*, 2296. <https://doi.org/10.3390/en17102296>

Academic Editor: Jun Li

Received: 11 April 2024

Revised: 7 May 2024

Accepted: 8 May 2024

Published: 10 May 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The increase in global energy demand and the depletion of fossil fuel reserves have led to a global energy crisis, prompting scientists and researchers to embark on a search for renewable energy sources that are both environmentally friendly and economically feasible. In addition to the energy crisis, problems such as increasing urbanization, overpopulation, and rapid industrialization have led to severe environmental pollution that threatens human, animal, and plant life. One possible solution to these problems is the use of microalgae in conjunction with a microbial fuel cell (MFC). An MFC is a bioelectrochemical system that generates electrical energy through the degradation of chemical compounds by microorganisms. The microbes in the anode chamber oxidize reduced substrates, generating electrons and protons. The electrons are absorbed by the anode, which acts as an artificial electron acceptor, and then transported to the cathode via an external circuit [1]. The electrons reach the cathode chamber and reduce the electron acceptor, while the protons generated at the anode are exchanged to the cathode through a membrane separator [2,3], or an electrolyte in a membrane-less cell [4]. Oxygen is an economical and environmentally friendly electron acceptor. Instead of mechanical aeration, microalgae could be introduced into the cathode chamber of photosynthetic microbial fuel cells (pMFCs). Microalgae

act as O₂ generators in situ and facilitate the reactions in the cathode chamber. The generation of a high power density is related to the high efficiency of electron transfer to the anode through the anodic biofilm and the high photosynthetic activity of the microalgae developed in the cathode chamber. Therefore, searching for microorganisms with higher oxygen production and faster growth capacity in the cathode chamber will help increase the efficiency of pMFCs.

In microalgae biocathodes, the cost of aeration is reduced by replacing the mechanical aeration device with an oxygen supply provided by the microalgae, which can also reduce CO₂ emissions. Thus, when exposed to light, a photosynthetic culture at the cathode utilizes CO₂ as a carbon source for photosynthesis and produces oxygen, which serves as an electron acceptor for electricity generation. Yang et al. reported that the maximum power density of a pMFC with a microalgae biocathode was 18% higher than the maximum power density of a conventional MFC [5]. *Chlorella vulgaris* is a xenobiotic microorganism that can adapt to different environmental conditions. It is considered as the reference microorganism with the greatest potential for use in the bioeconomy [6,7]. Previous studies have shown that in intensive microalgae cultivation, about 1.83 kg of CO₂ should be supplied to produce 1 kg of microalgae biomass, and therefore, the carbon dioxide content in the culture medium is often a factor limiting the achievement of high biomass growth [8]. It has been demonstrated that even low levels of photosynthetic oxygen produced by microalgae and cyanobacteria increase the efficiency of electrogenesis [9]. *Arthrospira platensis* is a planktonic, filamentous cyanobacterium that is cultivated worldwide both as a source of healthy food and as a source of the blue pigment phycocyanin, which is used in cosmetics and food. The protein content of *Arthrospira platensis* can reach up to 70% of its dry mass, and it is also a source of polyunsaturated fatty acids (especially ω -6), vitamins, and minerals (such as iron) [10]. The growth medium for *Arthrospira platensis* contains inorganic salts with a high concentration of bicarbonate, which keeps the pH between 9 and 10.

The concentration of dissolved oxygen depends on the microalgae in the cathode chamber, or the mechanical aeration. CO₂ also plays an indirect role in this process. If the dissolved oxygen concentration is too low, the electron acceptors of the cathode are not sufficient to continue producing electricity. In a cathode without additional aeration, the consumption of dissolved oxygen is generally higher than the dissolution of dissolved oxygen from air. In contrast, at a high dissolved oxygen concentration, oxygen can diffuse from the cathode chamber to the anode chamber due to osmotic pressure and oxygen gradients, which impairs the growth of anode microorganisms and destroys electron release at the anode, thus reducing the power density [11]. Bazdar et al. observed that increasing the dissolved oxygen concentration in the cathode chamber from 7.8 to 9.5 mg/L decreased the power density by 53.4% [12]. The high photosynthetic efficiency of *Chlorella vulgaris*, resulting in a high concentration of dissolved oxygen, makes it a frequently selected species in pMFCs [13]. In a previous study, the dissolved oxygen concentration obtained with *Chlorella vulgaris* ranged between 12 and 25 mg/L; without microalgae, it was 2–6 mg/L [14].

MFCs have become a promising technology for sustainable energy production and wastewater treatment, e.g., to reduce chemical oxygen demand. Wastewater from the food industry often contains large amounts of organic matter that can be used as a substrate for the microorganisms in the MFC, e.g., carbohydrates in wastewater from the beet sugar industry, and starch and proteins in wastewater from starch processing [15,16]. The pMFC has also been used to treat diluted swine wastewater [17], landfill leachate [18], and kitchen wastewater [19]. However, dairy wastewater has never been tested in pMFCs to compare energy production with *Chlorella vulgaris* or *Arthrospira platensis* in the cathode chamber. Dairy wastewater is characterized by large variations in pH, total suspended solids, chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP), as well as fat, oil, and grease [20,21]. Due to their high organic content, which consists mainly of rapidly assimilable carbohydrates and slowly degradable proteins [22] and fats,

dairy wastewaters are characterized by high BOD and COD values, ranging from 0.1 to 100 g/L [21,23]. Due to this characteristic, it could be assumed that dairy wastewater is a suitable substrate for anaerobic treatment [23]. The aim of the study was also to analyze effluent from an anode chamber as a substrate for the cultivation of microalgae, which will additionally lower the cost of this technology. The cultivation of microalgae in the cathode chamber based on synthetic nutrients could be very costly. The culture media cost constitutes about 50% of the total cost of microalgae biomass production [24,25]. The effluent from the anode chamber might be contaminated by anaerobic bacteria and suspended organic compounds that increase the water turbidity. Therefore, dilution of the effluent is often recommended, and was applied in the study. Microalgae growth is possible under autotrophic conditions as well as under heterotrophic or mixotrophic conditions. Some species of microalgae can assimilate and oxidize organic carbon molecules for energy extraction. The mixotrophic microalgae with high photosynthetic capacity adapt quickly and grow in different types of wastewater, removing pollutants and recovering important nutrients such as phosphorus and nitrogen [26]. In many studies, anode effluent has been used for the cathodic cultivation of microalgae, allowing synergistic treatment of wastewater with anode bacteria and cathodic microalgae. Don and Babel have shown that the anodic effluent of the pMFC in the cathode chamber can be used as a growth medium for microalgae if it is fed with a suitable HRT in the anode [27]. This solution additionally helps to utilize the remaining nutrients from the anodic effluent for microalgae growth. Zhang et al. treated swine wastewater, using undiluted swine wastewater for the anode chamber and a low concentration of swine wastewater for the cathode chamber to supplement the nutrients for the microalgae [17]. Consortia of microalgae and bacteria, or individual microalgae such as *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Chlamydomonas* sp., and *Scenedesmus obliquus*, etc., have been utilized by researchers by culturing them on municipal, industrial, and synthetic wastewater in order to produce biodiesel and remediate wastewater [28–32].

Although there are data on pMFC performance with *Chlorella* sp. or *Arthrospira platensis*, there exists a lack of publications comparing both species under the same conditions. This type of study will help decide which of these species is more effective and should be used in pMFCs. Therefore, the aim of the study was to determine the power density of pMFCs with *Arthrospira platensis* or *Chlorella vulgaris* cultivated in the cathode. Dairy wastewater was treated in the anode chamber. The tested pMFC used a synthetic medium and the effluent from the anode chamber as a substrate for microalgae growth. The control MFC with mechanical aeration was also investigated. The efficiency of the wastewater treatment was evaluated.

2. Materials and Methods

2.1. Organization of the Experiments

The experiment began with the start-up phase, which was used to enrich exoelectrogens. Subsequently, three configurations of the MFCs were tested, which differed in their cathode chamber contents. One of the MFCs served as a control in which the cathode chamber was constantly aerated (MFC0). In the other cathode chambers, *Arthrospira platensis* (pMFC1) and *Chlorella vulgaris* (pMFC2) were cultivated. The experiments were performed in duplicate, so six sets of the MFC were used.

2.2. MFC Configuration

Dual-chamber fed-batch MFCs with an H-shape were prepared using 1000 mL glass bottles (Figure 1). The anodic and cathodic chambers were separated by the membrane Nafion™ 117 (C7HF13O5S.C2F4) Sigma-Aldrich, St. Louis, MO, USA) with dimensions 5 cm × 5 cm. The anodic component was prepared from a 5 cm × 5.5 cm piece of carbon cloth. The cathodic component was prepared from a carbon rod with a diameter of 0.4 cm. The anode surface area was 28 cm², and the cathode surface area was 17.84 cm². Both chambers were mixed with a magnetic stirrer to maintain the anode and cathode medium in

suspension and improve matter transfer. The process was kept at an ambient temperature of 20–22 °C. The external circuit consisted of a copper wire and a 1 Ω resistor. The electric current generated by the connection of the electrodes was measured with an ammeter.

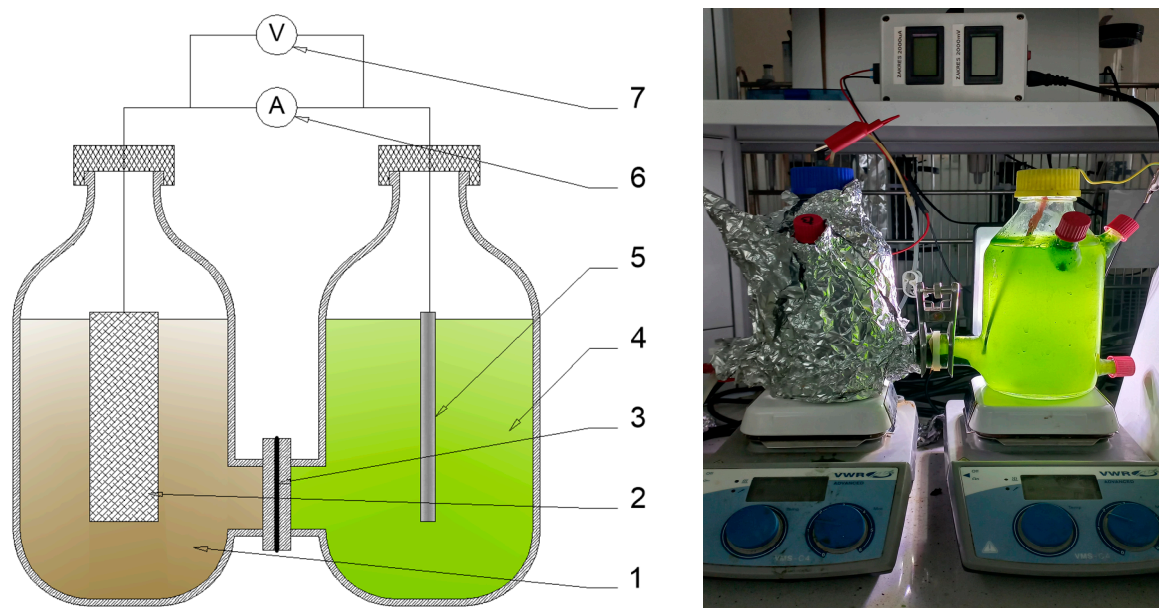


Figure 1. The scheme of the experiment: 1, anode chamber; 2, carbon felt anode; 3, membrane Nafion; 4, cathode chamber; 5, carbon cloth cathode; 6, ammeter; 7, voltmeter.

2.3. Start-Up and Condition of the Anode Chamber

In all MFCs, the anode chamber was filled with anaerobic sludge from the wastewater treatment plant (WWTP) “Łyna” in Olsztyn, Poland. The anaerobic sludge was placed in the anode chamber for one day and mixed so that the sludge settled on the electrode. Then, the remaining sludge was replaced with fresh wastewater. Once a week, the wastewater was removed and replaced with fresh wastewater. The anode chamber was purged with a mixture of 80% N₂ and 20% CO₂ for 10 min with every medium replacement to maintain anaerobic conditions. Dairy wastewater was obtained by dissolving milk powder. The desired COD concentration was 2000 ± 156 mg COD/L, resulting in a nitrogen concentration of 278 ± 12 mg TN/L and 35 ± 14 mg TP/L. During start-up, the cathode chamber was filled with phosphate buffer and aerated at a flow rate of 120 dm³/h (later referred to as control MFC0). Phosphate buffer is used in biological and biochemical experiments to maintain a stable pH in a solution. Phosphate buffer contains ions like potassium, sodium, and phosphate that enhance the conductivity of a solution. The buffer solution was prepared by dissolving powder (Sigma-Aldrich) in 1 L of distilled water. During the entire experiment, dark conditions prevailed in the anode chamber, as it was covered with aluminum foil.

2.4. pMFCs1 with *Arthrospira Platensis*

The cathodic chamber was filled with *Arthrospira platensis* and a suitable medium (modified Aiba and Ogawa medium [33]). The medium included 13.61 g NaHCO₃, 4.03 g Na₂CO₃, 0.5 g K₂HPO₄, 2.5 g NaNO₃, 1.0 g K₂SO₄, 1 g NaCl, 1.0 g MgSO₄·7H₂O, 0.04 g CaCl₂·2H₂O, 0.01 g FeSO₄·7H₂O, 0.08 g Na₂EDTA, and 1 mL A₅ and B₆ oligoelement solutions per liter of distilled water. The cyanobacteria were purchased as UTEX 3086 from the Culture Collection of Algae from the University of Texas, Austin, TX, USA, and then cultured in 2 L photobioreactors placed in a closed thermostatic chamber at a temperature of 30 °C. After two weeks of pMFC operation, half of the medium was replaced by the effluent from the anode chamber, and every two weeks thereafter, half of the medium was replaced by a new portion of effluent from the anode chamber. This procedure was

continued for the next 10 weeks; in summary, experiments lasted 84 days. The cathode chamber was illuminated in a day–night cycle (16:8 h) with white light at an intensity of 100 mmol/photon/m² using fluorescent lamps. The chamber was equipped with a heater to ensure an appropriate temperature for the cultivation of cyanobacteria.

2.5. pMFCs2 with *Chlorella Vulgaris*

The cathode chamber was filled with *Chlorella vulgaris* and a suitable medium. The medium for microalgae growth was prepared based on the synthetic medium 3N-BBM+V (Bold Basal Medium with 3-fold Nitrogen and Vitamins, modified) [34]. The medium included: 0.75 g NaNO₃, 0.025 g CaCl₂·2H₂O, 0.075 g MgSO₄·7H₂O, 0.075 g K₂HPO₄·3H₂O, 0.175 g KH₂PO₄, 0.025 g NaCl, 4.5 mg Na₂EDTA, 0.582 mg FeCl₃·6H₂O, 0.246 mg MnCl₂·4H₂O, 0.03 mg ZnCl₂, 0.012 mg CoCl₂·6H₂O, 0.024 mg Na₂MoO₄·2H₂O, 1.2 mg thiamine hydrochloride, and 0.01 mg cyanocobalamin per liter of distilled water. *Chlorella vulgaris* was purchased as UTEX 2714 from the Culture Collection of Algae, University of Texas, Austin, TX, USA, and cultivated in the laboratory photobioreactors on synthetic medium (2 L). After two weeks of pMFC operation, half of the medium was replaced by the effluent from the anode chamber, and for every two weeks thereafter, half of the medium was replaced by a new portion of effluent from the anode chamber. This procedure was continued for the next 10 weeks; in summary, experiments lasted 84 days. The cathode chamber was illuminated in a day–night cycle (16:8 h) with white light at an intensity of 100 mmol/photon/m² using fluorescent lamps.

2.6. Analytical Methods

Reference electrodes comprising Ag/AgCl (saturated KCl solution, +197 mV against a standard hydrogen electrode, SHE) were used to record electrode potentials. Current (I) and potential (V) measurements were recorded once in 2 h of operation using an auto-range digital multimeter by connecting a 1 Ω external circuit. The power (W) was calculated using the relationship $P = I \cdot V$, where I and V represent the current (A) and voltage (V), respectively. The power density (mW/m²) was calculated by dividing the power obtained by the anode surface area (m²).

The concentrations of COD, TN (total nitrogen), N-NH₄⁺ (ammonium), and TP (total phosphorus) were determined in the influent, effluent from the anode chamber, and effluent from the cathode chamber using LCK Hach-Lange cuvette tests, and a DR-5000 spectrophotometer with HS-250 mineralizer (Hach-Lange GmbH, Düsseldorf, Germany). Dissolved oxygen was measured with a portable dissolved oxygen meter with a dissolved oxygen electrode (Hach-Lange GmbH, Düsseldorf, Germany). Changes in taxonomic biomass and chlorophyll-a content were monitored at microscope magnifications of 1.25 × 10 × 40 or 1.25 × 10 × 100 and with the algae analyzer (bbe Moldaenke GmbH, Schwentinal, Germany).

2.7. Statistical Analyses

The statistical analysis of the results was performed using the STATISTICA 10.0 PL package with the W Shapiro-Wilk test and one-way analysis of variance (ANOVA). After testing for homogeneity of variance with Levene's test, the significance of differences between the variants was tested with Tukey's HSD test ($p < 0.05$).

3. Results and Discussion

3.1. Power Generation

Irrespective of the experimental variant carried out, the density of the power generated depended primarily on the availability of the substrate in the anode chamber (Figure 2). Around the 5th day, the substrate was used up, which led to a decrease in the power density. After the 7th day, the substrate was replaced with fresh wastewater. After 2 days following the replacement of the wastewater, the power density returned to the maximum value before the wastewater was replaced. From this, it can be concluded that the crucial part of the MFC is the anode chamber. The availability of the substrate, which is required for the exoelectrogens and the production of electrons, determines the efficiency of the cell.

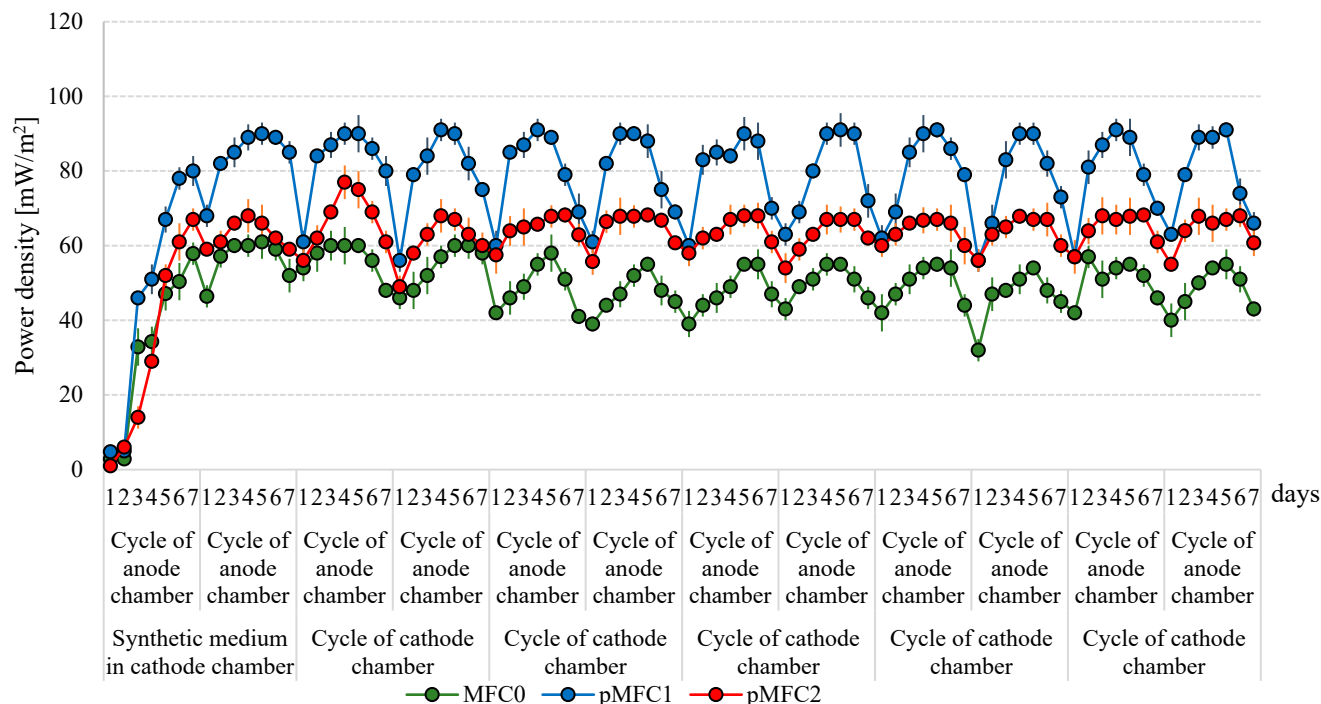


Figure 2. The power density of the control MFC, pMFC1 (with *Arthrospira platensis*), and pMFC2 (with *Chlorella vulgaris*).

Both electrodes (anode and cathode) play an important role in MFCs. The anode is a very important part of the MFC, responsible for bacterial growth, removal rate, electron generation, and electron transfer to a cathode. For this reason, interest in anode configurations, materials, and design for efficient MFC performance has recently increased. Anode materials must have some basic properties, such as good biocompatibility, high conductivity, high chemical stability, good thermal and mechanical stability, and a large surface area. A large number of anode materials have been tested in MFCs, but all of them have some obstacles that make MFC unsuitable for commercial applications [35,36]. The literature has shown that modifying the anode to achieve a large surface area, efficient electron transferability, and bacterial adhesion has become a new research interest in the field of MFCs [37].

The second decisive factor for the power density of the pMFC is the cultivation efficiency of microalgae in the cathode chamber. The power density of the pMFC depends on the amount of oxygen produced by the microalgae, and this depends on the light and the substrate. Aiyer et al. reported that the average power density increased from 175 to 248 mW/m² after adding *Chlorella vulgaris* to the cathode chamber. Colombo et al. also demonstrated that a higher power density of 0.85 mW/m² was observed in a pMFC with *Chlorella vulgaris* in the cathode than in an MFC with water 0.5 mW/m² [14]. Bazdar et al. used *Chlorella vulgaris* cultivated in medium BG-11 in the cathode chamber, while the influent to the MFC was municipal wastewater [12]. An aerator was additionally used to provide inorganic carbon in the cathode. In comparison between mechanical aeration alone and mechanical aeration with algae, the system with algae showed lower resistance, higher energy production, and higher dissolved oxygen content. In the present study, the introduction of microalgae into the cathode chamber increased the power density compared to the control MFC with mechanical aeration. The highest power density obtained from the MFC with mechanical aeration was 57 mW/m², whereas from the pMFC it was about 15% and 35% higher when *Chlorella vulgaris* and *Arthrospira platensis* were cultivated in the cathode chamber.

When *Chlorella vulgaris* was cultivated in the cathode chamber, a lower power density was achieved than with *Arthrospira platensis*. The maximum power density of about

90 mW/m² was found in pMFC1. The pMFC with *Chlorella vulgaris* cultivated in the effluent from the anode generated twice the power density compared to a conventional MFC of 34.2 mW/m² [38]. However, Don and Babel showed that the performance of the biocathode depends on the concentration of COD in the substrate for *Chlorella vulgaris* cultivation [39]. A COD concentration of 100 mg/L in the cathodic medium resulted in mixotrophic conditions, where both organic and inorganic substrates were present. These conditions produce higher power densities compared to autotrophic conditions. However, when the COD concentration increases above 100 mg/L, the duration of power generation decreases due to the favorable conditions for the heterotrophic metabolism of the microalgae. In the present study, the concentration of COD in the anode chamber was about 240 mg/L (data presented below), which according to Don and Babel, favored heterotrophic conditions, which could have limited power generation by *Chlorella vulgaris*.

In the case of *Arthrospira platensis*, cultivation in a cathode chamber with anode effluent increased the power density generated in the pMFC. Rago et al. showed that the use of swine wastewater as a substrate in the anode chamber and a synthetic medium for *Arthrospira platensis* resulted in a pMFC with higher power density, as the concentration of dissolved oxygen in the cathode was higher than in an MFC with a water cathode [40]. *Arthrospira platensis* is a photosynthetic, multicellular, and filamentous cyanobacterium that occurs naturally in tropical regions and inhabits alkaline lakes (pH 11) with a high concentration of NaCl and bicarbonates. *Arthrospira platensis* is a species with a high tolerance for changing environmental conditions, characterized by a relatively simple cultivation methodology, separation, and harvesting [41]. *Arthrospira platensis* tolerates a lack of oxygen in the water, pH fluctuations, and organic contaminants as well as long-term dry periods and high temperatures [42]. However, physicochemical parameters, such as nitrate, phosphate, salt, and pH, are decisive in biomass production and composition [43,44]. The pH fluctuations change the chemistry of the media, as well as the physiology of the organisms and the production of biomass. Nitrogen and NaCl have a significant influence on the biomass and protein production of *Arthrospira platensis* [43–45]. The high salt content in the medium can affect photosystems I and II of cyanobacteria due to its destructive effect on protein degradation [46]. However, since *Arthrospira platensis* is an alkalophilic microorganism, it requires relatively high concentrations of sodium ions for unhindered growth. Therefore, the addition of sodium to cultivation media with low salt content is necessary [47]. Despite this, *Arthrospira platensis* is a species that can adapt very well to difficult conditions, and it has been successfully used to remove nutrients from various types of wastewater [45,48]. Wastewater that contains sufficient quantities of essential nutrients can be used as a sustainable raw material for cost-effective biomass production in accordance with the principles of the circular economy [49,50]. This was also observed in the present study, where *Arthrospira platensis* was successfully cultivated on effluent from the anode chamber.

The pMFC can be used for energy production and wastewater treatment both during the light phase and dark phase. The power density results shown in Figure 2 were recorded at the end of the light period during microalgae cultivation in the cathode chamber. However, the daily changes in the observed voltage were clearly pronounced (Figure 3). During the light phase, the electricity generation was higher than in the dark phase. Photosynthesis is expressed as a redox reaction driven by light energy, in which CO₂ and water are converted into carbohydrates and oxygen molecules are released [51]. This process can be divided into two phases, taking place in the light and the dark. In the light reactions, which take place at the photosynthetic membranes, the light energy is converted into chemical energy, which supplies a biochemical reducing agent NADPH₂ and an energy-rich compound ATP. In the dark phase, which takes place in the stroma, NADPH₂ and ATP are used in the biochemical reduction of CO₂ to carbohydrates.

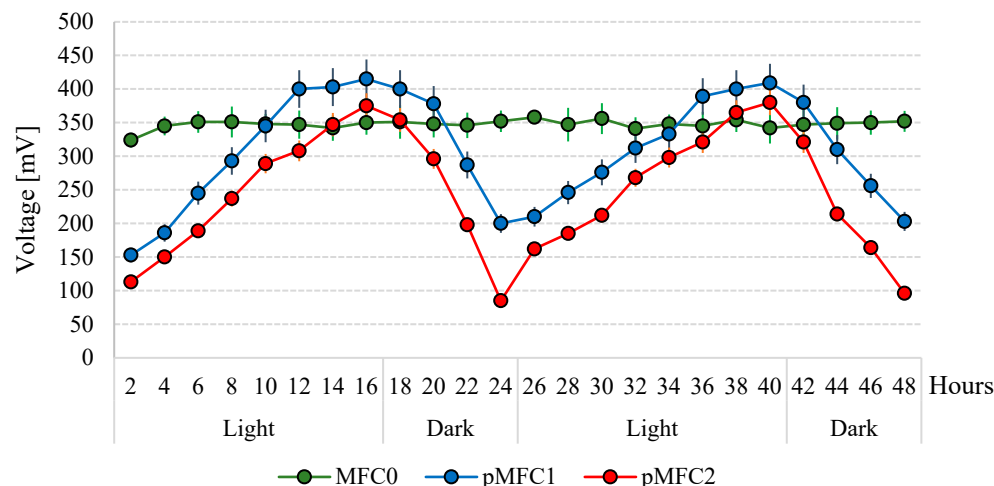


Figure 3. Daily changes in the voltage generated in the control MFC, the pMFC with *Arthrospira platensis* (pMFC1), and the pMFC with *Chlorella vulgaris* (pMFC2).

In the present study, during the dark period, the power generated by the pMFC decreased significantly due to dwindling oxygen supplies ($p < 0.05$). During illumination, the microalgae consumed the nutrients and CO_2 present in the medium during photosynthesis and produced oxygen. The photosynthetic oxygen was used for the reduction reaction in the cathode chamber, which increased the efficiency of voltage generation by the pMFC. During darkness, the remaining oxygen was additionally used by the microalgae/cyanobacteria for respiration, due to organic carbon available in the effluent from the anode chamber. Therefore, there was not enough dissolved oxygen available for the reduction reaction, which led to a decrease in voltage generation by the pMFC. A similar tendency was observed when *Chlorella vulgaris* and *Arthrospira platensis* were cultivated in the cathode chamber. However, during light conditions, dissolved oxygen reached $11.43 \text{ mg O}_2/\text{L}$ (142.9% oxygen saturation) in the cathode medium with *Chlorella vulgaris*, and $18.65 \text{ mg O}_2/\text{L}$ (210.1% oxygen saturation) in the cathode medium with *Arthrospira platensis*. The concentration of dissolved oxygen dropped to $1.1 \text{ mg O}_2/\text{L}$ (15.9% oxygen saturation) and $3.12 \text{ mg O}_2/\text{L}$ (33.9% oxygen saturation) in the cathode medium with *Chlorella vulgaris* and *Arthrospira platensis*, respectively. Similar concentrations of dissolved oxygen in the cathode chamber with *Chlorella vulgaris* of 10–11 $\text{mg O}_2/\text{L}$ have been reported previously [27,38,52]. A larger decrease in voltage generation was noted in the pMFC with *Chlorella vulgaris* than with *Arthrospira platensis*, which probably resulted from oxygen depletion in the cathode chamber. The voltage generation dropped to about 90 mV in pMFC2 with *Chlorella vulgaris*, and to about 155 mV in pMFC1 with *Arthrospira platensis*. Opposite conclusions were presented in the study of pMFC performance with *Scenedesmus* sp. cultivation in the cathodic chamber. Ullah et al. observed a significant drop in voltage during 12/12 h light/dark phases from about 480 mV to 40 mV, whereas dissolved oxygen decreased from about 8.4 mg/L to 5.9 mg/L. The authors concluded that power generation was lowered by anode chamber performance. In summary, more stable oxygen conditions were ensured by *Arthrospira platensis* [53]. The control MFC was constantly aerated so that the observed voltage was stable, but it was lower than in the pMFCs with photosynthetic microorganisms. Similar observations have been reported previously [27,38,52].

3.2. Microalgae Growth

Chlorophyll-a is a green pigment in plants, algae, and photosynthetic bacteria [54,55]. Therefore, chlorophyll-a concentration was used as an indicator of microalgae biomass concentration in the reactor in this study (Figure 4).

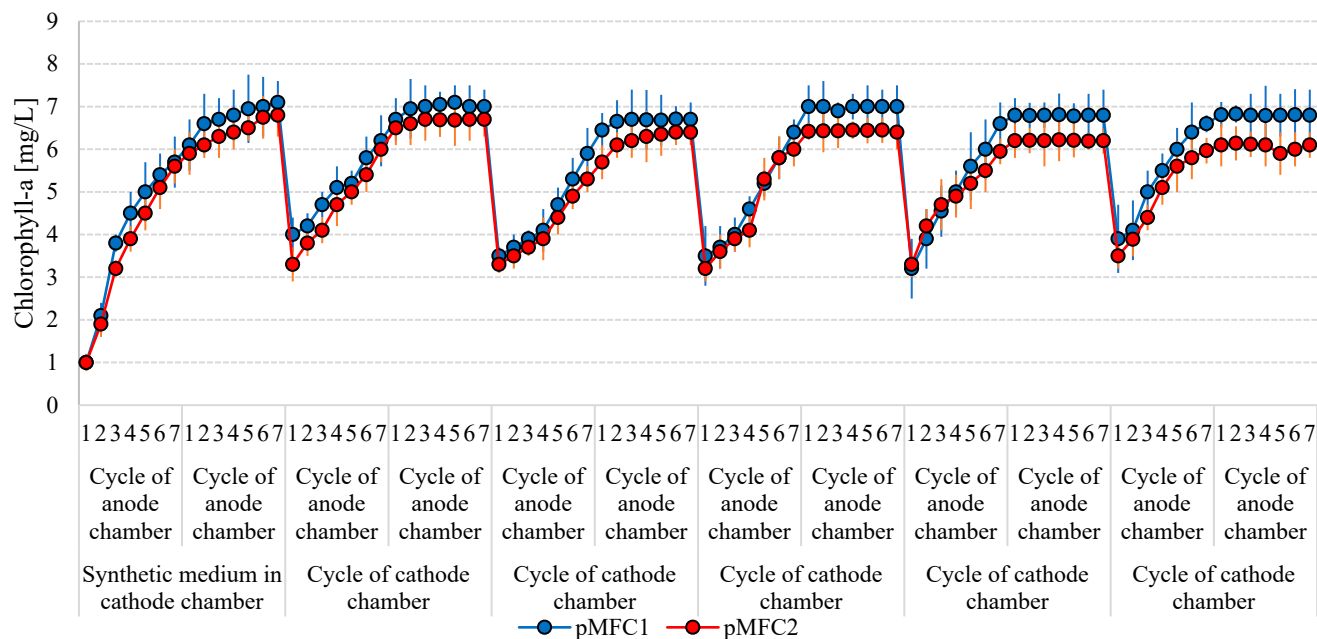


Figure 4. The growth of microalgae *Arthrospira platensis* (pMFC1) and *Chlorella vulgaris* (pMFC2) based on chlorophyll-a content in pMFC.

The maximum chlorophyll-a content was determined during the cultivation of *Arthrospira platensis*. The chlorophyll-a content after two weeks of MFC operation was 7.1 ± 0.6 mg/L and 6.8 ± 0.5 mg/L in the cathode chamber with *Arthrospira platensis* and *Chlorella vulgaris*, respectively. During the replacement of the medium, the chlorophyll-a content decreased by half. In the following operations, the chlorophyll-a content increased to similar values. However, chlorophyll-a content during the cultivation of *Chlorella vulgaris* was slightly lower than in the case of *Arthrospira platensis*. These cyanobacteria are known for their fast growth ability. It is also worth mentioning that the lower growth of microalgae than cyanobacteria might be connected with a lower dissolved oxygen concentration, as noted in the medium with *Chlorella vulgaris* (which was discussed above).

3.3. The Removal of Nutrients and Organics

The efficiency of COD removal in the anode chamber was about 87% for all tested MFC types, and the COD removal rate was 203 mg/(L·d) (Figure 5). The COD in the effluent from the anode chamber was about 221 ± 21 mg/L. This effluent was diluted in the cathode chamber by exchanging half of the medium so that the COD in the anode chamber was about 242 ± 16 mg/L. In the cathode, the COD was mainly removed during darkness, resulting in a final COD concentration of about 205.3 ± 13 mg/L for *Chlorella vulgaris* and 152.3 ± 13 mg/L for *Arthrospira platensis* cultures (Figure 6). A pMFC system treating similar wastewater containing 1500 mg COD/L and 314 mg NH_4^+-N /L achieved up to a 60% reduction in COD during the 10-day treatment in the anode chamber, which increased to 74% during a further 10-day treatment in the cathode chamber. During this period, NH_4^+-N was reduced by 79% in both chambers [56].

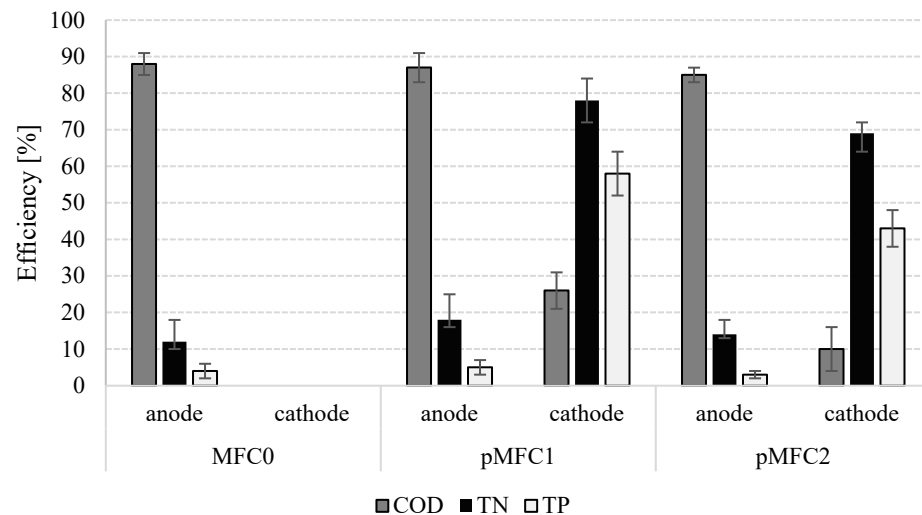


Figure 5. Efficiency of organic compounds and nutrient removal during the experiment.

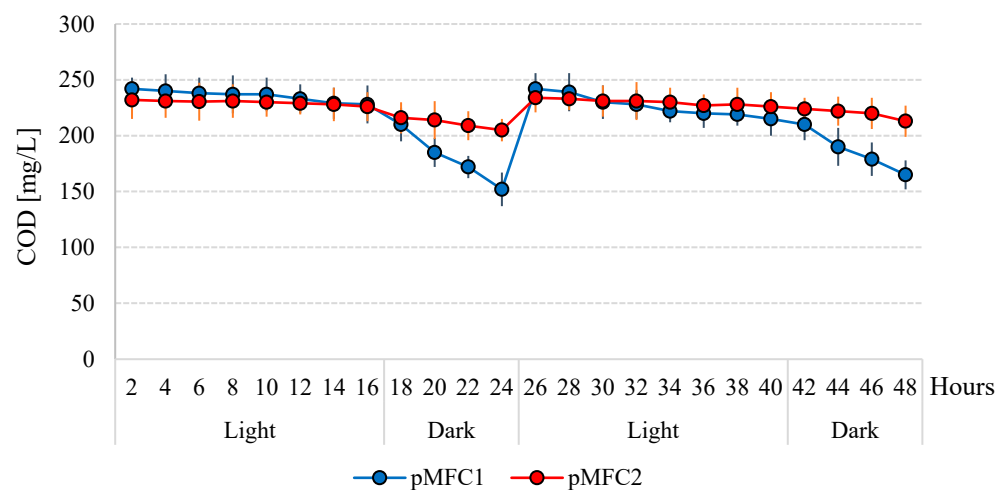


Figure 6. The concentration of chemical oxygen demand during exemplary cycles of light and dark period.

The total nitrogen was poorly removed in the anode chamber in all MFC types tested; overall, only about 14% of the total nitrogen was removed (Figure 5). In dairy wastewater, the nitrogen mainly comes from milk proteins. Approximately 40% of the nitrogen is found in the form of ammonium, and the remaining 60% forms part of the organic matter [57]. During anaerobic reactions, nitrogen changes forms from organic to inorganic ammonium, which increased the concentration in the anode chamber. The concentration of total nitrogen in the effluent from the anodic chamber was about 132 ± 12 mg/L. This effluent was diluted in the cathode chamber by replacing half of the medium. When *Chlorella vulgaris* was cultivated in the cathode chamber, the removal efficiency of total nitrogen was found to be about 69%. The nitrogen removed by microalgae and cyanobacteria was ammonium nitrogen (about 97% efficiency). The total nitrogen removal efficiency did not differ significantly between the experiments with effluent from the anode chamber introduced to the cathode chamber. However, the removal efficiency of total nitrogen was significantly higher when *Arthrospira platensis* was cultured in the cathode chamber in a medium containing the effluent from the anode chamber ($p < 0.05$). A similar trend was observed for the removal of phosphorus (Figure 5). For *Chlorella vulgaris* cultivated in the cathode chamber, a removal efficiency of total phosphorus of about 43% was observed. In *Arthrospira platensis* cultivation, however, this removal was about 58%. In opposition to the presented results, more efficient removal of phosphorus from municipal wastewater was observed in the cultivation of *Chlorella vulgaris* compared to *Arthrospira platensis* and

Scenedesmus quadricauda [58]. However, in the present study, the concentration of the phosphorus in the medium was low (about 21 ± 5 mg/L), and therefore, the reductions were not significant ($p < 0.05$).

As presented in this study, pMFCs can be operated by feeding the anodic effluent into the cathode chamber for further treatment. During treatment in the anode chamber, mostly organic compounds were removed. The previous studies also proved that the effluent from the anode chamber contained significant amounts of nitrogen compounds, phosphate, and dissolved inorganic carbon, which are useful for microalgal growth [38,59]. Microalgae mainly assimilated these nutrients; however, they could further degrade the remaining organic carbon compounds through aerobic respiration [60]. Previous studies have shown that mixotrophic cultivation results in higher biomass concentration compared to autotrophic and heterotrophic growth. A significant content of lipids in cells was also achieved in the mixotrophic cultivation of *Chlorella vulgaris* [61]. When the anode effluent was introduced to the cathode chamber in a pMFC, the removal efficiencies for soluble COD, ammonium nitrogen ($\text{NH}_4^+\text{-N}$), total nitrogen (TN), and phosphate-phosphorus ($\text{PO}_4^{3-}\text{-P}$) were 93.2%, 95.9%, 95.1%, and 82.7%, respectively [62].

4. Conclusions

The presented experiments showed that the introduction of *Arthrospira platensis* into the cathode chamber was more effective than that of *Chlorella vulgaris*. A power density of 91 mW/m^2 was generated by the pMFC with the cultivation of *Arthrospira platensis*. The pMFC with *Arthrospira platensis* not only enabled a higher power generation, but also a higher removal of organic compounds, nitrogen, and phosphorus (reaching about 27%, 78%, and 57%, respectively). Daily observations suggested that the cyanobacteria produced more oxygen, which reduced the oxygen deficit during the dark phase of photosynthesis, allowing for higher, more stable power generation. The study also proved that the introduction of photosynthetic microorganisms into the cathode chamber is more efficient than mechanical aeration. In order to further develop pMFC technology, the continuous mode of operation with microalgae should be tested for stable power generation.

Author Contributions: Conceptualization, M.Z. and P.R.; methodology, M.Z. and P.R.; software, P.R.; validation, M.Z., P.R. and M.D. (Marcin Dębowski); formal analysis, P.R., M.D. (Magda Dudek), A.S. and Ł.B.; investigation, P.R., M.D. (Magda Dudek), A.S. and Ł.B.; data curation, M.Z. and P.R.; writing—original draft preparation, M.Z. and P.R.; writing—review and editing, M.D. (Marcin Dębowski); visualization, P.R.; supervision, M.Z.; project administration, P.R.; funding acquisition, M.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financed by the Polish National Science Center (Grant Number 2021/41/B/NZ9/02225).

Data Availability Statement: Data are contained within the article.

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

References

1. Logan, B.E. *Microbial Fuel Cells*, 1st ed.; Wiley-Interscience: Hoboken, NJ, USA, 2008.
2. Zhao, F.; Harnisch, F.; Schröder, U.; Scholz, F.; Bogdanoff, P. Challenges and constraints of using oxygen cathodes in microbial fuel cells. *Environ. Sci. Technol.* **2006**, *40*, 5193–5199. [\[CrossRef\]](#)
3. Mathuriya, A.S.; Yakhmi, J.V. Microbial fuel cells to recover heavy metals. *Environ. Chem. Lett.* **2014**, *12*, 483–494. [\[CrossRef\]](#)
4. Taşkan, B.; Bakır, M.; Taşkan, E. Enhanced power generation from algal biomass using multi-anode membrane-less sediment microbial fuel cell. *Int. J. Hydrog. Energ.* **2021**, *45*, 2011–2022. [\[CrossRef\]](#)
5. Yang, Z.; Pei, H.; Hou, Q.; Jiang, L.; Zhang, L.; Nie, C. Algal biofilm-assisted microbial fuel cell to enhance domestic wastewater treatment: Nutrient, organics removal and bioenergy production. *Chem. Eng. J.* **2017**, *332*, 277–285. [\[CrossRef\]](#)
6. Shi, X.M.; Liu, H.J.; Zhang, X.W.; Chen, F. Production of biomass and lutein by *Chlorella protothecoides* at various glucose concentrations in heterotrophic cultures. *Process. Biochem.* **1999**, *34*, 341–347. [\[CrossRef\]](#)
7. Liu, C.H.; Chang, C.Y.; Liao, Q.; Zhu, X.; Chang, J.S. Photoheterotrophic growth of *Chlorella vulgaris* ESP6 on organic acids from dark hydrogen fermentation effluents. *Bioresour. Technol.* **2013**, *145*, 331–336. [\[CrossRef\]](#)

8. Chiu, S.-Y.; Kao, C.-J.; Chen, C.-H.; Kuan, T.-C.; Ong, S.-C.; Lin, C.-S. Reduction of CO₂ by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. *Bioresour. Technol.* **2008**, *99*, 3389–3396. [\[CrossRef\]](#)
9. Rosenbaum, M.; He, Z.; Angenent, L.T. Light energy to bioelectricity: Photosynthetic microbial fuel cells. *Curr. Opin. Biotechnol.* **2010**, *21*, 259–264. [\[CrossRef\]](#)
10. Aouir, A.; Amiali, M.; Bitam, A.; Benchabane, A.; Raghavan, V.G. Comparison of the biochemical composition of different *Arthrospira platensis* strains from Algeria, Chad and the USA. *J. Food Meas. Charact.* **2017**, *11*, 913–923. [\[CrossRef\]](#)
11. Logan, B.; Wallack, M.; Kim, K.-Y.; He, W.; Feng, Y.; Saikaly, P. Assessment of Microbial Fuel Cell Configurations and Power Densities. *Environ. Sci. Technol. Lett.* **2015**, *2*, 150803121308002. [\[CrossRef\]](#)
12. Bazdar, E.; Roshandel, R.; Yaghmaei, S.; Mardanpour, M.M. The effect of different light intensities and light/dark regimes on the performance of photosynthetic microalgae microbial fuel cell. *Bioresour. Technol.* **2018**, *261*, 350–360. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Nagendranatha Reddy, C.; Nguyen, H.T.H.; Noori, M.T.; Min, B. Potential applications of algae in the cathode of microbial fuel cells for enhanced electricity generation with simultaneous nutrient removal and algae biorefinery: Current status and future perspectives. *Bioresour. Technol.* **2019**, *292*, 122010. [\[CrossRef\]](#)
14. Colombo, A.; Marzorati, S.; Lucchini, G.; Cristiani, P.; Pant, D.; Schievano, A. Assisting cultivation of photosynthetic microorganisms by microbial fuel cells to enhance nutrients recovery from wastewater. *Bioresour. Technol.* **2017**, *237*, 240–248. [\[CrossRef\]](#)
15. Lu, N.; Zhou, S.G.; Zhuang, L.; Zhang, J.T.; Ni, J.R. Electricity generation from starch processing wastewater using microbial. *Biochem. Eng. J.* **2009**, *43*, 246–251. [\[CrossRef\]](#)
16. Rahman, A.; Borhan, M.S.; Shafiqur, R. Evaluation of microbial fuel cell (MFC) for bioelectricity generation and pollutants removal from sugar beet processing wastewater (SBPW). *Water Sci. Technol.* **2017**, *77*, 387–397. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Zhang, Y.; Zhao, Y.; Zhou, M. A photosynthetic algal microbial fuel cell for treating swine wastewater. *Environ. Sci. Poll. Res.* **2019**, *26*, 6182–6190. [\[CrossRef\]](#)
18. Nguyen, H.T.H.; Ramesh, K.; Booki, M. Algae cathode microbial fuel cells for electricity generation and nutrient removal from landfill leachate wastewater. *Int. J. Hydro Energy* **2017**, *42*, 29433–29442. [\[CrossRef\]](#)
19. Hou, Q.; Cheng, J.; Nie, C.; Pei, H.; Jiang, L.; Zhang, L.; Yang, Z. Features of *Golenkinia* sp. and microbial fuel cells used for the treatment of anaerobically digested effluent from kitchen waste at different dilutions. *Bioresour. Technol.* **2017**, *240*, 130–136. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Britz, J.T.; van Schalwyk, C.; Hung, Y.T. Treatment of dairy processing wastewaters. In *Waste Treatment in the Food Processing Industry*; Wang, L.K., Hung, Y.T., Lo, H.H., Yapijakis, C., Eds.; CRC Press: Boca Raton, FL, USA, 2006; pp. 1–25.
21. Karadag, D.; Köroğlu, O.E.; Ozkaya, B.; Cakmakci, M. A review on anaerobic biofilm reactors for the treatment of dairy industry wastewater. *Process. Biochem.* **2015**, *50*, 262–271. [\[CrossRef\]](#)
22. Janczukowicz, W.; Zieliński, M.; Dębowski, M. Biodegradability evaluation of dairy effluents originated in selected sections of dairy production. *Bioresour. Technol.* **2008**, *99*, 4199–4205. [\[CrossRef\]](#)
23. Demirel, B.; Yenigun, O.; Onay, T.T. Anaerobic treatment of dairy wastewaters: A review. *Process. Biochem.* **2005**, *40*, 2583–2595. [\[CrossRef\]](#)
24. Xia, A.; Murphy, J.D. Microalgal Cultivation in Treating Liquid Digestate from Biogas Systems. *Trends Biotechnol.* **2016**, *34*, 264–275. [\[CrossRef\]](#)
25. Mtaki, K.; Kyewalyanga, M.S.; Mtolera, M.S.P. Supplementing wastewater with NPK fertilizer as a cheap source of nutrients in cultivating live food (*Chlorella vulgaris*). *Annals. Microbiol.* **2021**, *71*, 7. [\[CrossRef\]](#)
26. Hamidian, N.; Zamani, H. Biomass production and nutritional properties of *Chlorella sorokiniana* grown on dairy wastewater. *J. Water Process Eng.* **2022**, *47*, 102760. [\[CrossRef\]](#)
27. Don, C.D.Y.Y.A.; Babel, S. Circulation of anodic effluent to the cathode chamber for subsequent treatment of wastewater in photosynthetic microbial fuel cell with generation of bioelectricity and algal biomass. *Chemosphere* **2021**, *278*, 130455.
28. Abdel-Raouf, N.; Al-Homaidan, A.A.; Ibraheem, I.B. Microalgae and wastewater treatment. *Saudi J. Biol. Sci.* **2012**, *19*, 257–275. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Gao, F.; Yang, Z.H.; Li, C.; Zeng, G.M.; Ma, D.H.; Zhou, L. A novel algal biofilm membrane photobioreactor for attached microalgae growth and nutrients removal from secondary effluent. *Bioresour. Technol.* **2015**, *179*, 8–12. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Gonçalves, A.; Pires, J.; Simões, M. A review on the use of microalgal consortia for wastewater treatment. *Algal Res.* **2016**, *24*, 403–415. [\[CrossRef\]](#)
31. Hena, S.; Fatihah, N.; Tabassum, S.; Ismail, N. Three stage cultivation process of facultative strain of *Chlorella sorokiniana* for treating dairy farm effluent and lipid enhancement. *Water Res.* **2015**, *80*, 346–356. [\[CrossRef\]](#)
32. Kyriakopoulos, G.; Doulia, D.; Eleni, A. Adsorption of pesticides on porous polymeric adsorbents. *Chem. Eng. Sci.* **2005**, *60*, 1177–1186. [\[CrossRef\]](#)
33. Aiba, S.; Ogawa, T. Assessment of growth yield of a blue-green alga: *Spirulina platensis* in axenic and continuous culture. *J. Gener. Microbiol.* **1977**, *102*, 179–182. [\[CrossRef\]](#)
34. Guo, Z.; Phooi, W.B.A.; Lim, Z.J.; Tong, Y.W. Control of CO₂ input conditions during outdoor culture of *Chlorella vulgaris* in bubble column photobioreactors. *Bioresour. Technol.* **2015**, *186*, 238–245. [\[CrossRef\]](#) [\[PubMed\]](#)

35. Choudhury, P.; Prasad Uday, U.S.; Bandyopadhyay, T.K.; Ray, R.N.; Bhunia, B. Performance improvement of microbial fuel cell (MFC) using suitable electrode and bioengineered organisms: A review. *Bioengineered* **2017**, *8*, 471–487. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Yaqoob, A.A.; Ibrahim, M.N.M.; Rafatullah, M.; Chua, Y.S.; Ahmad, A.; Umar, K. Recent Advances in Anodes for Microbial Fuel Cells: An Overview. *Materials* **2020**, *13*, 2078. [\[CrossRef\]](#) [\[PubMed\]](#) [\[PubMed Central\]](#)
37. Liang, H.; Han, J.; Yang, X.; Qiao, Z.; Yin, T. Performance improvement of microbial fuel cells through assembling anodes modified with nanoscale materials. *Nanomater. Nanotechnol.* **2022**, *12*. [\[CrossRef\]](#)
38. Commault, A.S.; Laczka, O.; Siboni, N.; Tamburic, B.; Crosswell, J.R.; Seymour, J.R.; Ralph, P.J. Electricity and biomass production in a bacteria-Chlorella based microbial fuel cell treating wastewater. *J. Power Sour.* **2017**, *356*, 299–309. [\[CrossRef\]](#)
39. Don, C.D.Y.Y.A.; Babel, S. Effects of organic loading on bioelectricity and micro-algal biomass production in microbial fuel cells using synthetic wastewater. *J. Water Process Eng.* **2020**, *39*, 101699. [\[CrossRef\]](#)
40. Rago, L.; Cristiani, P.; Villa, F.; Zecchin, S.; Colombo, A.; Cavalca, L.; Schievano, A. Influences of dissolved oxygen concentration on biocathodic microbial communities in microbial fuel cells. *Bioelectrochemistry* **2017**, *116*, 39–51. [\[CrossRef\]](#) [\[PubMed\]](#)
41. Bayona-Morcillo, P.J.; Plaza, B.M.; Gómez-Serrano, C.; Rojas, E.; Jiménez-Becker, S. Effect of the Foliar Application of Cyanobacterial Hydrolysate (*Arthrospira platensis*) on the Growth of Petunia x Hybrida under Salinity Conditions. *J. Appl. Phycol.* **2020**, *32*, 4003–4011. [\[CrossRef\]](#)
42. Abdel-Latif, H.M.R.; El-Ashram, S.; Yilmaz, S.; Naiel, M.A.E.; Abdul Kari, Z.; Hamid, N.K.A.; Dawood, M.A.O.; Nowosad, J.; Kucharczyk, D. The Effectiveness of Arthrospira platensis and Microalgae in Relieving Stressful Conditions Affecting Finfish and Shellfish Species: An Overview. *Aquac. Rep.* **2022**, *24*, 101135. [\[CrossRef\]](#)
43. Celekli, A.; Yavuzatmaca, M.; Bozkurt, H. Modeling of biomass production by Spirulina platensis as function of phosphate concentrations and pH regimes. *Bioresour. Technol.* **2009**, *100*, 3625–3629. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Celekli, A.; Yavuzatmaca, M. Predictive modeling of biomass production by Spirulina platensis as function of nitrate and NaCl concentrations. *Bioresour. Technol.* **2009**, *100*, 1847–1851. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Markou, G.; Kougia, E.; Arapoglou, D.; Chentir, I.; Andreou, V.; Tzovenis, I. Production of Arthrospira platensis: Effects on Growth and Biochemical Composition of Long-Term Acclimatization at Different Salinities. *Bioengineering* **2023**, *10*, 233. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Allakhverdiev, S.I.; Murata, N. Salt stress inhibits photosystems II and I in cyanobacteria. *Photosynth Res.* **2008**, *98*, 529–539. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Kandasamy, S.; Nagarajan, P. Effect of salinity on biomass and biochemical constituents of Spirulina platensis (Geitler). *Intern J. Plant Protect.* **2014**, *7*, 71–73.
48. Dębowski, M.; Zieliński, M.; Vdovychenko, A.; Kazimierowicz, J. The Use of the Autotrophic Culture of Arthrospira platensis for CO₂ Fixation from Biogas Combustion. *Processes* **2024**, *12*, 396. [\[CrossRef\]](#)
49. Dudek, M.; Dębowski, M.; Kazimierowicz, J.; Zieliński, M.; Quattrocchi, P.; Nowicka, A. The Cultivation of Biohydrogen-Producing Tetraselmis subcordiformis Microalgae as the Third Stage of Dairy Wastewater Aerobic Treatment System. *Sustainability* **2022**, *14*, 12085. [\[CrossRef\]](#)
50. Ummalyma, S.B.; Sirohi, R.; Udayan, A.; Yadav, P.; Raj, A.; Sim, S.J.; Pandey, A. Sustainable Microalgal Biomass Production in Food Industry Wastewater for Low-Cost Biorefinery Products: A Review. *Phytochem. Rev.* **2022**, *3*, 969–991. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Kisieleska, M.; Zieliński, M.; Dębowski, M.; Kazimierowicz, J.; Romanowska-Duda, Z.; Dudek, M. Effectiveness of Scenedesmus sp. Biomass Grow and Nutrients Removal from Liquid Phase of Digestates. *Energies* **2020**, *13*, 1432. [\[CrossRef\]](#)
52. Abazarian, E.; Gheshlaghi, R.; Mahdavi, A.M. Impact of light/dark cycle on electrical and electrochemical characteristics of algal cathode sediment microbial fuel cells. *J. Power Sour.* **2020**, *475*, 228686. [\[CrossRef\]](#)
53. Ullah, Z.; Sheikh, Z.; Zaman, W.Q.; Zeeshan, M.; Miran, W.; Li, J.; Khan, M.A.N.; Saleem, S.; Shabbir, S. Performance comparison of a photosynthetic and mechanically aerated microbial fuel cell for wastewater treatment and bioenergy generation using different anolytes. *J. Water Proc. Eng.* **2023**, *56*, 104358. [\[CrossRef\]](#)
54. Nio Song, A.; Banyo, Y. Leaf chlorophyll concentration as an indicator of water deficiency in plants. *Sci. J. Sci.* **2011**, *15*, 166. [\[CrossRef\]](#)
55. Zieliński, M.; Kazimierowicz, J.; Dębowski, M. The Possibility of Deploying CO₂ from Biogas Combustion to Improve the Productivity of a Periodical Chlorella vulgaris Culture. *Front Biosci. Elit.* **2023**, *15*, 3. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Don, C.D.Y.Y.A.; Babel, S. Effects of ammonium concentration in the catholyte on electricity and algal biomass generation in a photosynthetic microbial fuel cell treating wastewater. *Bioresour. Technol. Rep.* **2021**, *16*, 100867. [\[CrossRef\]](#)
57. Jones, H.R. *Pollution Control in the Dairy Industry*; Noyes Data Corporation: Park Ridge, NJ, USA, 1974.
58. Jalal, K.C.A.; Alam Md, A.M.; Kamaruzzaman, Y.; Akbar, J.; Hossain, T. Removal of Nitrate and Phosphate from Municipal Wastewater Sludge by Chlorella vulgaris, Spirulina platensis and Scenedesmus quadricauda. *IIUM Eng. J.* **1970**, *12*, 125–132. [\[CrossRef\]](#)
59. Wang, X.; Feng, Y.; Liu, J.; Lee, H.; Li, C.; Li, N.; Ren, N. Sequestration of CO₂ discharged from anode by algal cathode in microbial carbon capture cells (MCCs). *Biosens. Bioelectron.* **2010**, *25*, 2639–2643. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Perez-Garcia, O.; Escalante, F.M.; de-Bashan, L.E.; Bashan, Y. Heterotrophic cultures of microalgae: Metabolism and potential products. *Water Res.* **2011**, *45*, 11–36. [\[CrossRef\]](#) [\[PubMed\]](#)

61. Mohammad, M.; Mohammad, A.; Kalbasi, M.; Mousavi, S.; Ghobadian, B. Investigation of Mixotrophic, Heterotrophic and Autotrophic Growth of *Chlorella vulgaris* Under Agricultural Waste Medium. *Prep. Biochem. Biotechnol.* **2015**, *46*, 150–156. [[CrossRef](#)]
62. Wang, Y.; Lin, Z.; Su, X.; Zhao, P.; Zhou, J.; He, Q.; Ai, H. Cost-effective domestic wastewater treatment and bioenergy recovery in an immobilized microalgal-based photoautotrophic microbial fuel cell (PMFC). *Chem. Eng. J.* **2019**, *372*, 956–965. [[CrossRef](#)]

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.