Article

The Biological Performance of a Novel Electrokinetic-Assisted Membrane Photobioreactor (EK-MPBR) for Wastewater Treatment

Maryam Amini [1,*] , **Eltayeb Mohamedelhassan** [2] **and Baoqiang Liao** [3]

1   Department of Biotechnology, Lakehead University, 955 Oliver Road, Thunder Bay, ON P7B 5E1, Canada
2   Department of Civil Engineering, Lakehead University, 955 Oliver Road, Thunder Bay, ON P7B 5E1, Canada; emohamed@lakeheadu.ca
3   Department of Chemical Engineering, Lakehead University, 955 Oliver Road, Thunder Bay, ON P7B 5E1, Canada; bliao@lakeheadu.ca
*   Correspondence: mamini@lakeheadu.ca

Abstract: Developing an effective phycoremediation system, especially by utilizing microalgae, could provide a valuable approach in wastewater treatment for simultaneous nutrient removal and biomass generation, which would help control environmental pollution. This research aims to study the impact of low-voltage direct current (DC) application on *Chlorella vulgaris* properties and the removal efficiency of nutrients (N and P) in a novel electrokinetic-assisted membrane photobioreactor (EK-MPBR) in treating synthetic municipal wastewater. Two membrane photobioreactors ran in parallel for 49 days with and without an applied electric field (current density: 0.261 A/m^2). Mixed liquid suspended soils (MLSS) concentration, chemical oxygen demand (COD), floc morphology, total phosphorus (TP), and total nitrogen (TN) removals were measured during the experiments. The results showed that EK-MPBR achieved biomass production comparable to the control MPBR. In EK-MPBR, an over 97% reduction in phosphate concentration was achieved compared to 41% removal in the control MPBR. The control MPBR outperformed the nitrogen removal of EK-MPBR (68% compared to 43% removal). Induced DC electric field led to lower pH, lower zeta potential, and smaller particle sizes in the EK-MPBR as compared with MPBR. The results of this novel study investigating the incorporation of *Chlorella vulgar* is in an electrokinetic-assisted membrane photobioreactor indicate that this is a promising technology for wastewater treatment.

Keywords: electrokinetic-assisted membrane photobioreactor; nutrient removal; wastewater treatment; phycoremediation

1. Introduction

Wastewater treatment is a growing concern because wastewater contains pollutants such as nitrogen and phosphorus, which in excess can threaten wildlife and marine life [1,2]. Algae can assimilate these nutrients from wastewater and prevent eutrophication [3–6]. Phycoremediation, or biological treatment that utilizes algae for nutrient removal from wastewater, is one of the recent technologies gaining attention due to its low cost and environmental footprint [5,7,8]. Membrane photobioreactor technology (MPBR), as one of the biological wastewater treatment systems, is widely used for simultaneous wastewater treatment and microalgae production [9]. This technology has gained momentum due to promising nutrient removal and the high quality of effluent, together with the production of concentrated microalgae [10,11]. The biomass production of MPBRs has industrial applications, including biofuel, foods, and feeds [12–14].

Several studies on MPBR have been conducted by various research groups with different microalgae species and MPBR configurations [15–18]. These studies have demonstrated the advantages of MPBR systems for nutrient removal and microalgae biomass production compared to the conventional microalgae system [19].

Chlorella vulgaris (*C. vulgaris*) is an extensively used microalgae in MPBRs for wastewater treatment [8,20,21]. It is also produced for human nutrition and biodiesel feedstock applications [12,14,22]. *C. vulgaris* can grow in diverse environments [23–25] such as high temperature, e.g., up to 40 °C [14], acidic and alkaline (pH from 3 to 11.5) [20–22], light intensity [22], and high salinity. The most studied condition affecting their growth is light, but few studies focus on the effect of a low-voltage continuous electric field on photosynthesis and growth efficiency [14].

There are several parameters that affect the phycoremediation process [8]. Wastewater characteristics are one of the factors that have been studied. One recent study investigated the implications of urban wastewater concentration and induced stress on the growth of *Chlorella fusca* [26]. Using real wastewater instead of synthetic wastewater is another recent research focus on the growth study of *C. vulgaris* and its bioremediation of primary (PE) and secondary (SE) urban effluents [27]. Some reported researchers have focused on operating conditions such as hydraulic retention time (HRT), solid retention time (SRT), and turbulent pulsation [28–30].

Some studies investigated the effect of a low-voltage electric field on the growth and nitrogen and phosphorus removal efficiency of *C. vulgaris* [31,32]. Other research groups have studied the application of moderate and short-term electric fields in stimulating the growth and metabolism of *C. vulgaris* [13,33]. They found that in batch culture, a short-term applied electric field could improve the biomass growth of microalgae [13,33]. A study using an applied pulse electric field recently revealed the potential for increased lipid content from *C. vulgaris* [34].

Studies that incorporate electric fields in membrane bioreactors (MBR) have found improved chemical oxygen demand removal (COD) as well as nitrogen and phosphorus removal were observed by applying a short-term electric field in membrane bioreactors (MBR), where activated sludge was used [35–38]. To date, only one study has integrated an electric field into an MBR with algae and activated sludge [2]. However, the combination of an electric field with a membrane photobioreactor utilizing algae as biomass has not yet been investigated.

The current study examines the effect of a low-voltage continuous electric field on the microalgae growth rate, biomass quality, and overall nutrient removal (N and P) of an MPBR with *C. vulgaris* in treating synthetic municipal wastewater. An MPBR and an electrokinetic-assisted MPBR (EK-MPBR) with *C. vulgaris* were operated in parallel for 49 days to investigate the electric field effects on biomass production, biomass productivity, COD removal, and nutrient (N and P) removals. This is the first study on EK-MPBR, and the results demonstrate that it is a promising technology that simultaneously removes nutrients and reproduces microalgae.

2. Materials and Methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich (Merck, Darmstadt, Germany). Deionized water was prepared in the laboratory.

2.2. Microalgae and Culture Conditions

The microalgae *C. vulgaris* was purchased from the Canadian Phycological Culture Centre of the University of Waterloo, ON, Canada. The medium solution was prepared with the following composition and amounts in 1 L of solution [39,40]:

0.66 g NH_4Cl, 0.625 g $MgSO_4$, 0.1105 g $CaCl_2 \cdot 2H_2O$, 0.1142 g H_3BO_3, 0.0498 g $FeSO_4 \cdot 7H_2O$, 0.0882 $ZnSO_4 \cdot 7H_2O$, 0.0144 g $MnCl_2 \cdot 4H_2O$, 0.0118 g $Na_2MoO_4 \cdot 2H_2O$, 0.0157 g $CuSO_4 \cdot 5H_2O$, 0.004 g $CoCl_2 \cdot 6H_2O$, 0.64 g ethylenediamine tetraacetic acid (EDTA)-2 Na·$2H_2O$, 0.6247 g KH_2PO_4, 1.3251 g K_2HPO_4. All components were added to deionized (DI) water and insulated to avoid contamination. The cultivation continued for 25 days to reach the desired concentration of 1.2 g/L of dried biomass under continuous aeration and light illumination at room temperature.

2.3. Operating Conditions

The feed of the reactors was a synthetic municipal wastewater effluent (after biological chemical oxygen demand (COD) removal). The amount of the trace element in one liter feed summarized in Table 1 included 0.0025 g NaCl, 0.082 g $MgSO_4 \cdot 7H_2O$, 0.005 g $CaCl_2 \cdot 2H_2O$, 0.02490 g $FeSO_4 \cdot 7H_2O$, 0.00044 $ZnSO_4 \cdot 7H_2O$, 0.00022 g $MnCl_2 \cdot 4H_2O$, 0.00126 g $Na_2MoO_4 \cdot 2H_2O$, 0.00039 g $CuSO_4 \cdot 5H_2O$, 0.00041 g $CoCl_2 \cdot 6H_2O$, 0.09553 NH_4Cl, 0.01537 g KH_2PO_4, 0.3 g $NaHCO_3$ and 0.01874 g Glucose. All the components were added to distilled water. The reactors were fed semi-continuously with a liquid level controller sensor and a peristatic pump, and the feed was kept in a refrigerator at 4–5 °C. The level of the suspensions in the reactors was kept constant by a level sensor (LC40, Flowline Inc., Los Alamitos, CA, USA), and feed pumped by a peristaltic pump (Model 77122-12, Masterflex®C/L®PWR, Cole-Parmer, Vernon Hills, IL, USA). The permeation pumps were programmed to operate in 3-min-on and 2-min-off modes for permeation and relaxation to control membrane fouling. The reactors were operated for 49 days, and hydraulic retention time (HRT) and solid retention time (SRT) were maintained at 2.5 days and 30 days, respectively. During the operation, HRT was controlled by applied membrane flux. Once the transmembrane pressure (TMP) of the MPBRs reached about 30 kPa, physical cleaning was used to remove the foulant layer of the membrane module. The pH of the feed for the MPBR was adjusted using NaOH and HCl solutions.

Table 1. Characteristics of synthetic wastewater.

Water Quality Index	Average Value (mg/L)
Nitrogen	25 ± 2
Phosphorus ($PO_4 - P$)	3.5 ± 0.3
Chemical Oxygen Demand	20 ± 2.5

2.4. Experimental Set-Up

The experiments were conducted using two lab-scale submerged MPBRs. The reactors were filled with 10 L of culture medium. White LED lamps were used to provide illumination. The reactors were put in a magnet stir to mix the algal solution slightly. The schematic diagram of the experimental set-up is shown in Figure 1. Table 2 summarizes the basic parameters of the membrane module and the operating conditions. Two flat sheet rectangular graphite electrodes and two stainless steel meshes were placed in the reactors. The EK-MPBR is connected to a DC power supply system, and the MPBR has the same configuration but without DC power supply. The graphite and stainless steel sheets were connected to positive and negative poles of a DC power supply (B & K precision's, Taiwan), respectively. A constant DC electric field was applied to the microalgae for the entire operation for the EK-MPBR.

Table 2. Specification of the membrane module and operating condition.

Membrane Module	MPBR	EK-MPBR
Total membrane surface area	0.03 m^2	0.03 m^2
Membrane materials	Polyvinylidene fluoride (PVDF)	Polyvinylidene fluoride (PVDF)
Membrane type	Flat sheet	Flat sheet
Mean membrane pore size	0.4 μm	0.4 μm
Operational Parameter		
Working volume	10 L	10 L
Temperature	25 ± 0.8 °C	25 ± 0.8 °C

Table 2. *Cont.*

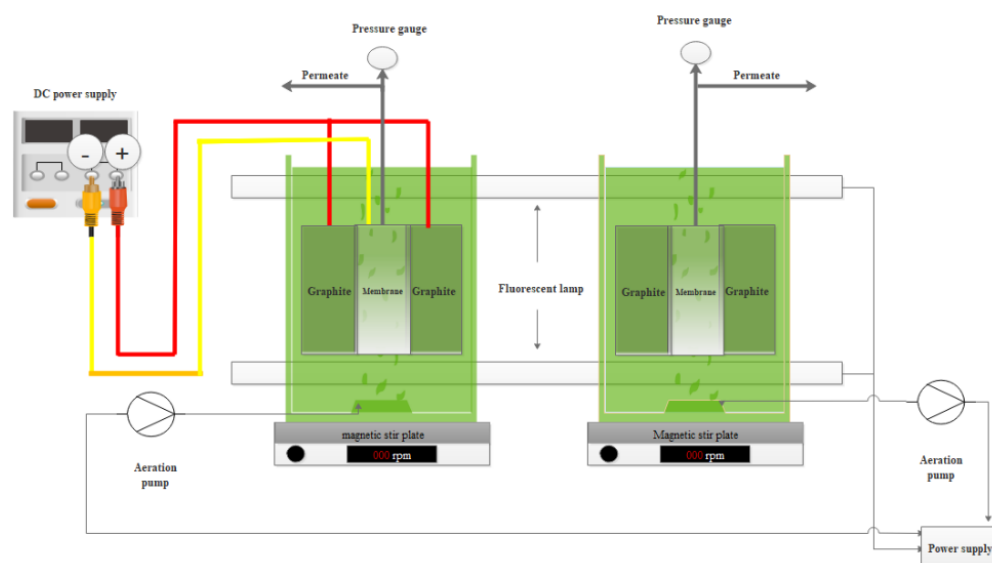| Membrane Module	MPBR	EK-MPBR
pH	8.46 ± 0.5	8.46 ± 0.5
Aeration rate	2.16 ± 0.10 L/min	2.16 ± 0.10 L/min
Illumination intensity	8400 lux	8400 lux
Voltage gradient		0.62 ± 0.02 V/cm
Current density		0.261 A/m^2
Electrodes surface area		0.015 ± 0.008 m^2
Electrodes distance		0.03 m

Figure 1. Schematic of lab-scale MPBR and EK-MPBR set-up.

2.5. Zeta Potential and Routine Analysis

The zeta potential of the flocs was determined by using a NanoBrook ZetaPlus (Brookhaven, NY, USA). The samples were diluted in 1 mM KCl solution. Each sample was tested at least twice to confirm the zeta potential value. Smoluchowski's equation was used to determine the zeta potential [41]. A dissolved oxygen (DO) meter (Model 407510, Extech, Nashua, NH, USA), a pH meter (pH 700, Oakton, VA, USA), and a thermometer were used to measure the DO, pH, and temperature of the suspension in the reactor.

2.6. Determination of Biomass Characteristics

The MLSS and COD were measured using the standard method [42]. Biomass productivity was calculated based on the following equation [43]:

$$r_x = X \times \frac{Q_{waste}}{V_{MPBR}} = \frac{X}{SRT} \tag{1}$$

where, r_x is biomass productivity (mg/L·d); X is the average biomass concentration (g/L), which in this study equals to average MLSS; Q waste is the reactor biomass wasting rate (L/d), and VMPBR is its working volume (L).

Total nitrogen and phosphorus (TN, TP) were monitored on samples taken every other day. Each sample was duplicated, and the values reported are the averages for each sample. Both TN and TP were measured by spectrophotometry using the alkaline potassium persulfate digestion-UV spectroscopy method [39,44] and the ammonium molybdate spectroscopy method, respectively [44].

2.7. Particle Size Distribution and Microalgae Structure

The particle size distribution was measured by a Malvern Mastersize 2000 instrument (Worcester, UK) with a detention of 0.02–2000 μm. Each sample was measured in triplicate. The range of laser obscuration was 0.1–0.4%.

The structure of the algal cell was studied by an inverted microscope (Olympus IX51, Tokyo, Japan). For each picture, the samples were dropped onto a slide, followed by dispersion with a cover slide. To gather images, each sample was randomly photographed with a digital camera connected to a microscope.

2.8. Statistical Analysis

The statistical difference of the parameters in MPBR and EF-MPBR was determined by two-sample t-tests, with the alpha significance level at 0.05 ($p = 0.05$).

3. Results and Discussion

In this study, the performance of the electrokinetic-assisted photobioreactor on phycoremediation is investigated and compared with a control photobioreactor. The effect of the electric field is classified in terms of biomass production, nutrient removal, zeta potential, pH, and floc morphology.

3.1. Effect of EF Treatment on Biomass Production

We investigated the effect of the applied electric field on algae growth in photobioreactors. The MLSS concentration was measured and used as an indicator of algal growth throughout the study. The time course measuring the MLSS concentration of the reactor is shown in Figure 2. Both MPBRs operated in parallel with an initial concentration of 1.16 ± 0.4 g/L of MLSS. The biomass value and productivity of the control reactor (MPBR) varied from 0.97 g/L to 2.12 g/L and 32.33–70.66 g/Ld, respectively. For the EK-MPBR, the corresponding values were lower than the control, with biomass ranging from 0.62 g/L to 1.59 g/L of MLSS and productivity ranging from 18–53 mg/Ld. However, these values were not significantly different ($p > 0.05$).
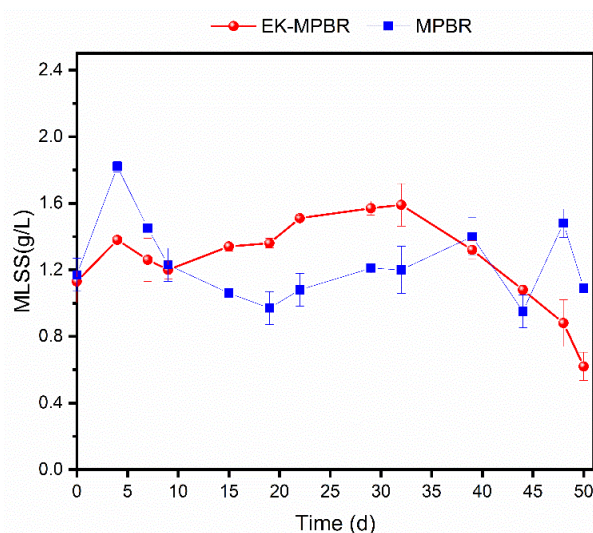

Figure 2. Variation of biomass production for MPBR with and without applied electric field over the experimental period.

In the EK-MPBR, MLSS fluctuation over the first 39 days of the experiment was smoother and relatively higher compared to the control MPBR. This improved productivity suggests that the applied electric field stimulated the growth of microalgae. The present study is consistent with the findings of others that the electric field increased the productivity of *Chlorella vulgaris* by enhancing the transport of substances across the algae

cell membrane [45,46]. The hormetic response of low-dose stimulation and high-dose inhibition seen in Figure 2 is an adaptive cell response that is stimulatory in the short term and inhibitory in long-term exposure [47] was also observed in the pre-treatment of *Chlorella vulgaris* with the application of a short-term moderate electric field [13].

Corpuz et al. studied the effect of a long-term applied electric field bioreactor, where they observed a similar trend in the Algae-Activated sludge bioreactor [2]. In the study of Corpuz et al., it was mentioned that more prolonged exposure to the electric field inhibited microalgae growth starting on day 28. This retardation of microalgae, which is caused mainly by electrochemical reactions around the cathode, was delayed in this study until day 39. Indirect oxidation due to the modified design of the cathode in our study could play a role in growth inhibition caused by hydroxyl radicals. By placing the cathode behind the membrane, the released ions in the permeate could be removed by the permeate pump, and therefore, their accumulation over time is controlled. The indirect oxidation effect on the molecular level in MPBRs could be the focus of future studies.

3.2. Nutrient Removal and Wastewater Treatment Potential

The wastewater treatment performance of EK-MPBR was compared to the control MPBR to determine electric field efficiency in terms of nutrient removal from the wastewater. The efficiency of a phycoremediation system is defined by how well algae can remove nitrogen, phosphorus, and COD from wastewater. Figure 3 shows the percentage of nutrient removal of EK-MPBR and MPBR over time. The concentrations of N, P, and COD in the influent were maintained constant at the levels of $25n \pm 2$ mg/L, 3.5 ± 0.3 mg/L, and 20 ± 2.5 mg/L, respectively. EK-MPBR showed a statistically significant higher phosphorous removal with an overall removal of $97.98n \pm 0.02\%$ ($p < 0.05$). This demonstrates the advantage of EK-MPBR for phosphorus removal compared to the overall removal of $41.81 \pm 0.05\%$ of the control reactor. The main two phosphorus removal mechanisms in algal systems are biomass adsorption and precipitation of phosphorus [2,25]. In an electric field-assisted system, electrochemical oxidation on the surface of the electrodes and electrochemical reactions in the suspension can also contribute to phosphorus removal. Given a pH range of 7.5 to 8.5 for EF-MPBR, phosphorus adsorption on the surface of the anode (graphite) is not the dominant mechanism [48]. The improved phosphorus removal in EK-MPBR can be attributed to the occurrence of electrochemical reactions in the suspension and the overall ion strength in the mixed liquor solution [33,49]. A recent study showed that in biomass combined with activated sludge, the applied electric field improved phosphorus removal by 65% compared to its control reactor and was mainly due to electrochemical reactions [2]. The released aluminum ions from the aluminum anode and the generation of phosphate aluminum complex contribute to the removal of phosphorus [50,51]. Although a number of studies have evaluated the effect of electric field on MBRs [2,33,52,53], its effect on molecular adsorption in MPBRs needs to be verified in future studies.

Limited phosphate concentration in the suspension in EK-MPBR can lower the biomass productivity and removal efficiency caused by a low biomass concentration [54]. However, this is in contrast to the MLSS concentration (Figure 2). As such, it is likely that the stimulating effect of the electric field outweighed the inhibitory effect of P depletion. Furthermore, the limited phosphate concentration in the suspension in EK-MPBR is beneficial for reducing membrane fouling. The correlation between P depletion and biofilm growth has been reported by other studies [55,56]. The low concentration of P, ranging from 0.05 mg/L to 1.09 mg/L in EK-MPBR, is below the concentration needed for biofilm growth compared to the control MPBR, ranging from 1.79 to 2.39 mg/L [49]. This suggests the existing potential of EK-MPBR for enhanced membrane performance.
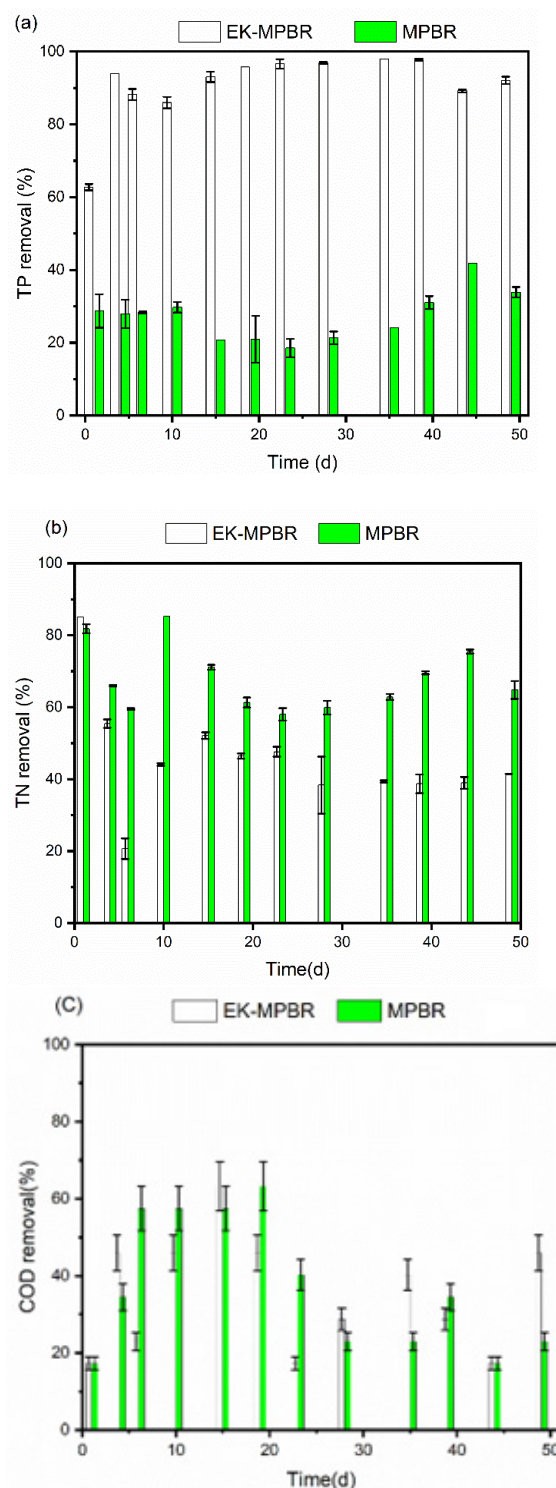
Figure 3. Nutrient removal of MPBR and EK-MPBR: (**a**) comparison of TP removal efficiency, (**b**) TN removal efficiency, and (**c**) COD removal efficiency.

Despite the significant phosphorus removal of EK-MPBR, the nitrogen removal efficiency of EK-MPBR was depressed to some extent. The better phosphorous removal efficiency compared to the nitrogen removal efficiency in EK-MPBR might be because of their different removal mechanisms. At the water-oxide interface, phosphate removal utilizes an inter-sphere adsorption mechanism that is less affected by ionic strength as compared to nitrogen removal, which has an outer-sphere adsorption mechanism [48]. As shown in Figure 3b, EK-MPBR TN removal ranged from 17.82% to 85%, whereas in control

MPBR, TN was removed by 58% to 85% ($p < 0.05$). The concentration of TN in the influent was kept at a constant value of 25 ± 2 mg/L for both reactors. The lower nitrogen removal efficiency agrees with other studies that showed that the electric field might interfere with the nitrogen removal process [33,57,58]. The fluctuation over the 49 days in TN removal in EK-MPBR could be attributed to the change of MLSS concentration (Figure 3a), zeta potential (Figure 4), and ionic strength of the suspension, which may have interfered with nitrification. The lower nitrogen removal in EK-MPBR due to the presence of an electric field agrees with the study by Zhang et al. [33]. The potential changes in ionic properties of the microalgae could be due to the electrochemical reactions and the incorporation of the electric field, which further have an inhibitory impact on the removal of total nitrogen [33]. However, both EK-MPBR and MPBR showed nitrogen removal comparable to other studies (Table 3).
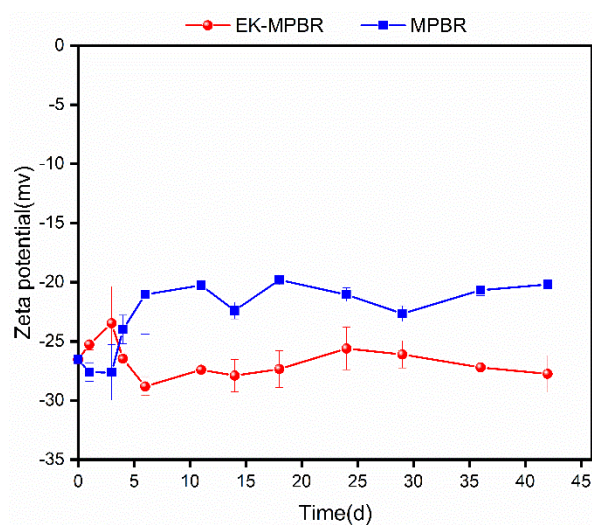

Figure 4. Zeta potential variation during EK-MPBR and MPBR operations.

Table 3. The removal efficiency of MPBRs with *Chlorella vulgaris*.

Source of Water/Wastewater	Type of MPBR	Influent Concentration (mg/L)	Organic Loading Rate (mg/L·d)	HRT and SRT (d)	Membrane Pore Size	Removal Efficiency%	Ref.
Synthetic municipal wastewater effluent	MPBR	TN: 25 ± 2 TP: 3.5 ± 0.4	TN: 10.58 ± 1.02 TP: 1.48 ± 0.2	HRT: 2.5 SRT: 30	0.4 µm	68 ± 3 of TN 41.81 ± 0.05 of TP	This Study
Synthetic municipal wastewater effluent	EK-MPBR	TN: 25 ± 2 TP: 3.5 ± 0.4	TN: 10.58 ± 1.02 TP: 1.48 ± 0.2	HRT: 2.5 SRT: 30	0.4 µm	43 ± 2 of TN 97.98 ± 0.02 of TP	This Study
Synthetic municipal wastewater	MPBR	N/A		HRT: 2.5 d SRT:12.5 d	N/A	50 of TN 50 of TP	[59]
Synthetic municipal wastewater	MPBR	TN: 14.1 ± 0.5 TP: 2.5 ± 0.2		HRT: 1 d SRT: 9 d	0.04 µm	31 of TN 30 of TP	[43]
Synthetic municipal wastewater	MPBR	TN: 14.1 ± 0.5 TP: 2.5 ± 0.2		HRT: 1 d SRT: 30 d	0.04 µm	32 of TN 25 of TP	[43]

Figure 3c represents the COD concentration over the experimental period. The influent COD concentration for both reactors was kept constant at 20 ± 1.8 mg/L. The COD reduction can be attributed to the thickness of the biofilm, electrochemical oxidation of

organic substances, and oxidation of the organic compounds by electrochemically generated oxidants such as hydrogen peroxide [2]. In this study, due to the induced multiple factors, further investigation is needed before highlighting any underlying reasons as the main mechanism of COD removal in EK-MPBR when comparing it with MPBR.

3.3. Effect of EF on the Physiology of Microalgae

Zeta potential, pH, and the morphology of biomass are measured as indicators of changes in physiology under the electric field and their effect on the phycoremedation efficiency [60,61]. In both MPBRs, the zeta potential remained negative over the experimental period. Zeta potential is dependent on factors such as pH and ion type and strength [62]. In alkaline conditions, zeta potential increases (i.e., becomes more negative) with pH increase as particles are surrounded by more negative charge in the suspension. The zeta potential values, represented in Figure 3, along with the pH data depicted in Figure 4, agree with the above statement.

The zeta potential in the control MPBR was higher than that of EK-MPBR. This can be attributed to the higher pH in the reactor compared to EK-MPBR [62]. Furthermore, zeta potential is a function of other factors, such as the composition and concentration of metabolites in the suspension [62]. This could explain the fluctuating behavior, especially over the first days of the operation, when the microalgae have unstable conditions due to adaptation to the new environment.

Surface charge, as represented by the zeta potential here, can contribute to the nutrient uptake efficiency of the system [63]. In alkaline solution, the predominant phosphate ions are HPO_4^{2-} and PO_4^{3-} [64]. The higher the surface charge, the stronger the electrostatic repulsion would interfere with the adsorption of these ions on the algal cell surface [64,65]. Reportedly, the lower surface charge positively affects the adsorption of the orthophosphate by *C. vulgaris* [65].

Sedimentation is another mechanism of phosphorus removal from wastewater that is also affected by the surface charge and electrostatic repulsion [66,67]. The phosphate ions form complex salts in wastewater, such as calcium phosphate in the form of sediments [67]. The lower surface charge would enhance sedimentation and phosphorus removal. Therefore, as shown in Figure 4, the decreased zeta potential in EK-MPBR (-27 compared to -20 mv in MPBR) can be attributed to the better phosphorus removal efficiency in EK-MPBR ($97.98 \pm 0.02\%$ in EK-MPBR compared to $41.81 \pm 0.05\%$ in MPBR).

The nitrogen removal, however, was affected differently by the surface charge. Due to the increased surface charge, the adsorption of negatively charged hydroxyl ions (OH) that are part of the denitrification process could be decreased [68]. The effect of the electric field on this removal pathway can be further verified through electrochemical analysis of the cells in future studies.

The variation of pH with time is shown in Figure 5. While both reactors started with the same pH, the applied electric field lowered pH over time. The electrochemical reaction around the cathode is a determining factor [69]. In alkaline conditions, the following reaction at the cathode can be expected:

$$2H_2O + 2e^- \ \rightarrow \ H_2(g) + 2OH^- \ (aq) \tag{2}$$
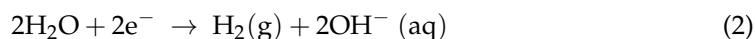
As a result of this electrolysis reaction, the pH near the cathode is expected to increase under the applied electric field. However, the results show that other factors may be involved in pH changes. One of the underlying reasons for pH changes could be the materials used and impurities that arise during the strengthening of the carbon in the manufacturing process, which could be released from the electrode to the suspension when placed under the electric field [70].
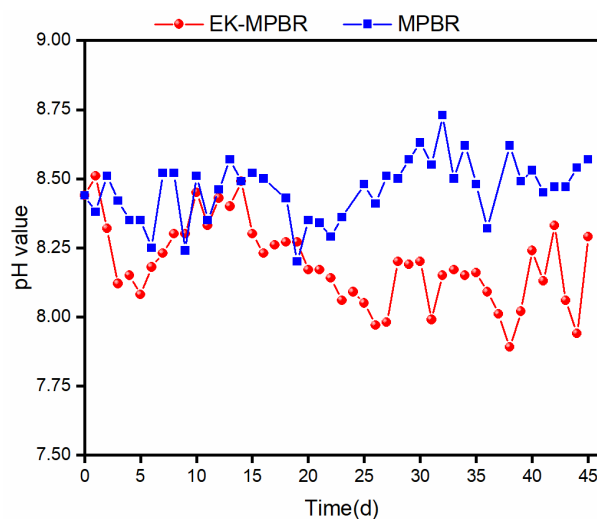
Figure 5. pH variation during EK-MPBR and MPBR operations.

pH is also sensitive to the mechanism of microalgae growth. Considering glucose in the influent, the suspension provides a mixotrophic and/or heterotrophic culture for *C. vulgaris* growth. In both cases, mixotrophic and heterotrophic, pH depends on the microalgae's preferred growth kinetics. While the pH remained unchanged in the mixotrophic condition, heterotrophic culture showed a gradual decrease in the suspension [71]. As shown in Figure 6, the lighter suspension over time under the electric field demonstrates heterotrophic dominancy, and therefore decreased pH.
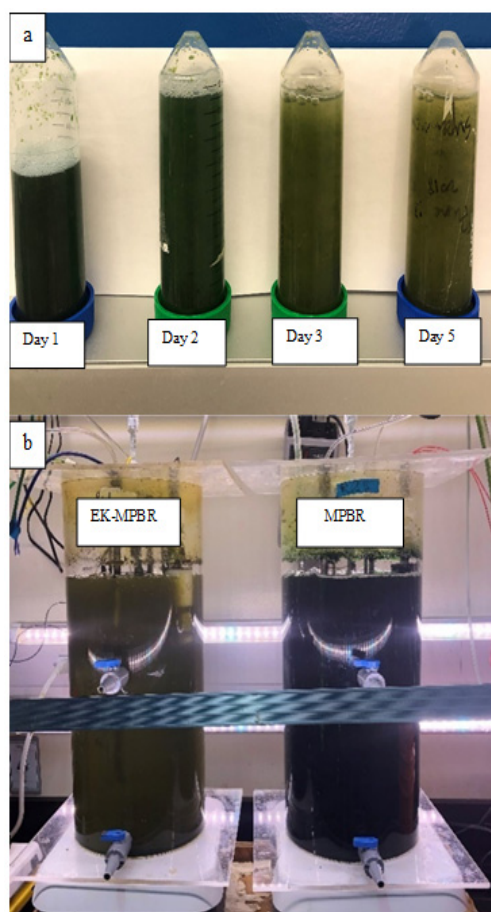

Figure 6. The color of microalgae grown in (**a**) EK-MPBR from day 1 to day 5 and (**b**) EK-MPBR and MPBR at day 20.

Figure 7 shows the morphology of the suspensions in MPBR and EK-MPBR. One of the main objectives of this investigation is to study possible morphology changes due to the applied electric field. As shown in Figure 6, the floc size in the EK-MPBR is smaller than that of the MPBR. This agrees with the higher fraction of smaller particles seen in the particle size distribution (PSD) analysis (Figure 8). The fraction of the smaller particles in EK-MPBR (Figure 8) can be attributed to floc breakage and disintegration due to the applied electric field and the electrophoresis phenomenon. Electrophoresis and movement of the charged particles could result in more breakage of the flocs [49]. In electrophoresis, the charged particles tend to move toward the electrode with the opposite charge, which can cause collisions and smaller particle formation. This potentially explains the formation of smaller flocs in EK-MPBR. The conceptual image of the phenomena is presented in Figure 9.
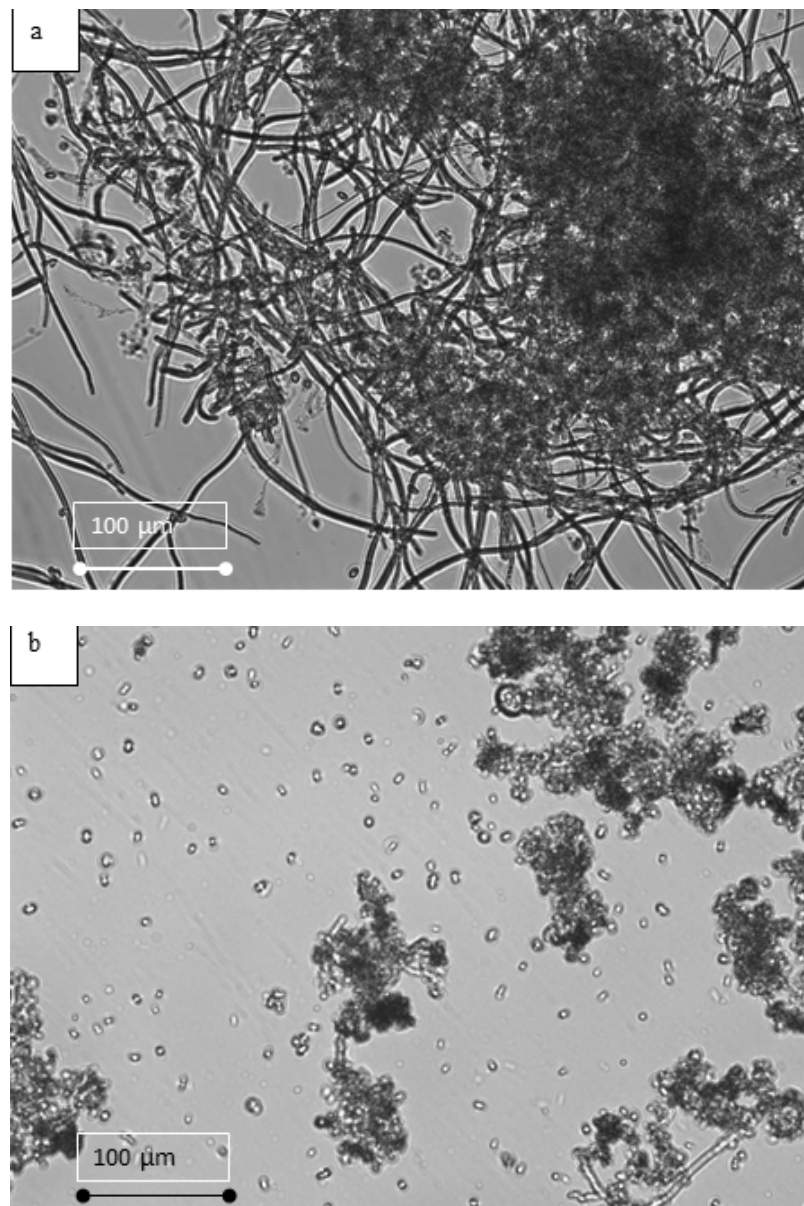
Figure 7. Microscopic images representing the morphology of *C. vulgaris* in (**a**) MPBR and (**b**) EK-MPBR.
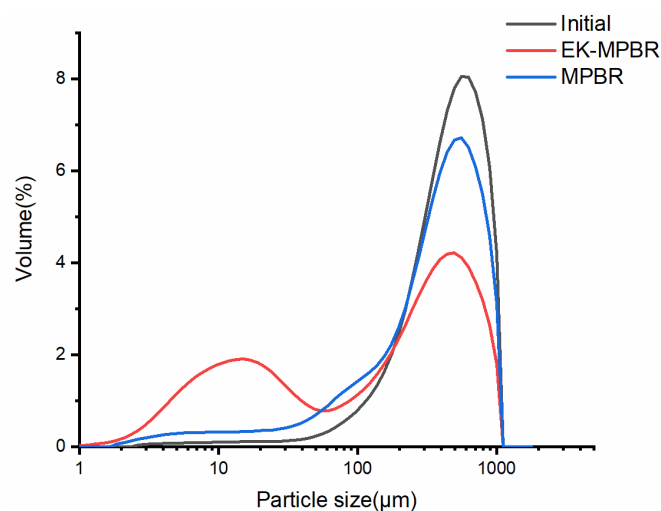
Figure 8. Particle size distribution of the floc suspension in the MPBR and EK-MPBR.
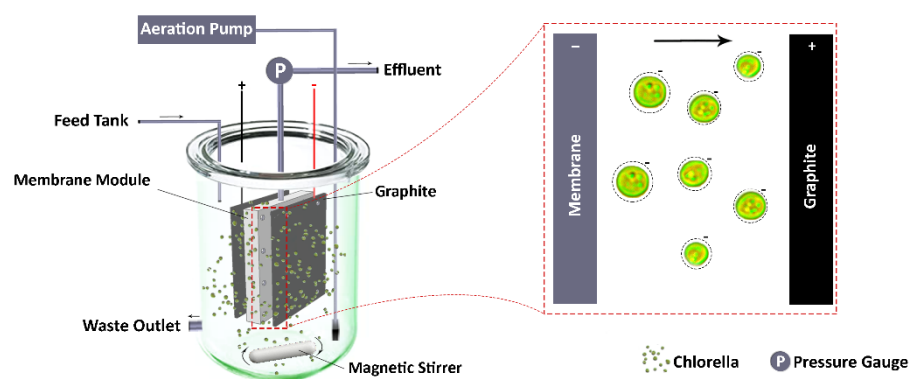

Figure 9. Conceptual figure of the electrophoresis movement of charged particles toward the opposite electrode in EK-MPBR.

4. Conclusions

A modified membrane photobioreactor that incorporated a low-voltage electric field and the algae *C. vulgaris* was developed. This novel study compared the biomass production and the nitrogen and phosphorus removal efficiency of *C. vulgaris* in the electrokinetic-assisted membrane bioreactor with that of the control.

The biomass production in the EK-MPBR was comparable to that in the MPBR. Nutrient removal was lower and significantly higher in EK-MPBR for total nitrogen and total phosphorus, respectively. This can be explained by electrochemical reactions around the electrodes. The results also showed that increased cell charge and formation of smaller particles under the applied electric field was observed, which may affect biomass production.

The work presented here has implications for future studies of the electric field in MPBRs and may help modify the design of membrane photobioreactors for membrane fouling control. Further research identifying the mortality rate of the algae under different applied currents molecular changes and electrokinetically affected assimilation efficiency of the cells will be beneficial for improving electro-phycoremediation techniques in wastewater treatments.

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

References

1. Touliabah, H.E.-S.; El-Sheekh, M.M.; Ismail, M.M.; El-Kassas, H. A Review of Microalgae-and Cyanobacteria-Based Biodegradation of Organic Pollutants. *Molecules* **2022**, *27*, 1141. [CrossRef] [PubMed]
2. Corpuz, M.V.A.; Borea, L.; Senatore, V.; Castrogiovanni, F.; Buonerba, A.; Oliva, G.; Ballesteros, F., Jr.; Zarra, T.; Belgiorno, V.; Choo, K.-H. Wastewater treatment and fouling control in an electro algae-activated sludge membrane bioreactor. *Sci. Total Environ.* **2021**, *786*, 147475. [CrossRef] [PubMed]
3. Lavrinovičs, A.; Murby, F.; Zīverte, E.; Mežule, L.; Juhna, T. Increasing Phosphorus Uptake Efficiency by Phosphorus-Starved Microalgae for Municipal Wastewater Post-Treatment. *Microorganisms* **2021**, *9*, 1598. [CrossRef] [PubMed]
4. 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]
5. Priyadharshini, S.D.; Babu, P.S.; Manikandan, S.; Subbaiya, R.; Govarthanan, M.; Karmegam, N. Phycoremediation of wastewater for pollutant removal: A green approach to environmental protection and long-term remediation. *Environ. Pollut.* **2021**, *290*, 117989. [CrossRef]
6. Wu, P.; Zhang, X.; Wang, X.; Wang, C.; Faustin, F.; Liu, W. Characterization of the start-up of single and two-stage Anammox processes with real low-strength wastewater treatment. *Chemosphere* **2020**, *245*, 125572. [CrossRef]
7. Karthik, V.; Saravanan, K.; Bharathi, P.; Dharanya, V.; Meiaraj, C. An overview of treatments for the removal of textile dyes. *J. Chem. Pharm. Sci.* **2014**, *7*, 301–307.
8. Verma, R.; Suthar, S.; Chand, N.; Mutiyar, P.K. Phycoremediation of milk processing wastewater and lipid-rich biomass production using *Chlorella vulgaris* under continuous batch system. *Sci. Total Environ.* **2022**, *833*, 155110. [CrossRef]
9. Bilad, M.; Azizo, A.; Wirzal, M.; Jia, L.J.; Putra, Z.; Nordin, N.; Mavukkandy, M.; Jasni, M.; Yusoff, A. Tackling membrane fouling in microalgae filtration using nylon 6, 6 nanofiber membrane. *J. Environ. Manag.* **2018**, *223*, 23–28. [CrossRef]
10. Gao, F.; Li, C.; Yang, Z.-H.; Zeng, G.-M.; Feng, L.-J.; Liu, J.-Z.; Liu, M.; Cai, H.-W. Continuous microalgae cultivation in aquaculture wastewater by a membrane photobioreactor for biomass production and nutrients removal. *Ecol. Eng.* **2016**, *92*, 55–61. [CrossRef]
11. Lee, J.-C.; Baek, K.; Kim, H.-W. Semi-continuous operation and fouling characteristics of submerged membrane photobioreactor (SMPBR) for tertiary treatment of livestock wastewater. *J. Clean. Prod.* **2018**, *180*, 244–251. [CrossRef]
12. Walter, U.; Beyer, M.; Klein, J.; Rehm, H.-J. Degradation of pyrene byRhodococcus sp. UW1. *Appl. Microbiol. Biotechnol.* **1991**, *34*, 671–676. [CrossRef]
13. Nezammahalleh, H.; Ghanati, F.; Adams, T.A., II; Nosrati, M.; Shojaosadati, S.A. Effect of moderate static electric field on the growth and metabolism of *Chlorella vulgaris. Bioresour. Technol.* **2016**, *218*, 700–711. [CrossRef] [PubMed]
14. Serra-Maia, R.; Bernard, O.; Gonçalves, A.; Bensalem, S.; Lopes, F. Influence of temperature on *Chlorella vulgaris* growth and mortality rates in a photobioreactor. *Algal Res.* **2016**, *18*, 352–359. [CrossRef]
15. Zhang, M.; Lee, E.; Vonghia, E.; Hong, Y.; Liao, B. Introduction to aerobic membrane bioreactors: Current status and recent developments. In *Current Developments in Biotechnology and Bioengineering*; Ng, H.Y., Ng, T.C.A., Ngo, H.H., Mannina, G., Pandey, A., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 1–23.
16. Bokhary, A.; Maleki, E.; Hong, Y.; Hai, F.I.; Liao, B. Anaerobic membrane bioreactors: Basic process design and operation. In *Current Developments in Biotechnology and Bioengineering*; Ng, H.Y., Ng, T.C.A., Ngo, H.H., Mannina, G., Pandey, A., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 25–54.
17. Luo, Y.; Le-Clech, P.; Henderson, R.K. Simultaneous microalgae cultivation and wastewater treatment in submerged membrane photobioreactors: A review. *Algal Res.* **2017**, *24*, 425–437. [CrossRef]
18. Bilad, M.; Arafat, H.A.; Vankelecom, I.F. Membrane technology in microalgae cultivation and harvesting: A review. *Biotechnol. Adv.* **2014**, *32*, 1283–1300. [CrossRef]
19. Zhang, M.; Leung, K.-T.; Lin, H.; Liao, B. Membrane fouling in a microalgal-bacterial membrane photobioreactor: Effects of P-availability controlled by N:P ratio. *Chemosphere* **2021**, *282*, 131015. [CrossRef]
20. Katırcıoğlu Sınmaz, G.; Erden, B.; Şengil, İ. Cultivation of *Chlorella vulgaris* in alkaline condition for biodiesel feedstock after biological treatment of poultry slaughterhouse wastewater. *Int. J. Environ. Sci. Technol.* **2022**, *86*. [CrossRef]
21. Kothari, R.; Pandey, A.; Ahmad, S.; Singh, H.M.; Pathak, V.V.; Tyagi, V.; Kumar, K.; Sari, A. Utilization of *Chlorella pyrenoidosa* for Remediation of Common Effluent Treatment Plant Wastewater in Coupling with Co-relational Study: An Experimental Approach. *Bull. Environ. Contam. Toxicol.* **2022**, *108*, 507–517. [CrossRef]
22. Safi, C.; Zebib, B.; Merah, O.; Pontalier, P.-Y.; Vaca-Garcia, C. Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. *Renew. Sustain. Energy Rev.* **2014**, *35*, 265–278. [CrossRef]

23. Gao, Y.; Yang, M.; Wang, C. Nutrient deprivation enhances lipid content in marine microalgae. *Bioresour. Technol.* **2013**, *147*, 484–491. [CrossRef] [PubMed]

24. San Pedro, A.; González-López, C.; Acién, F.; Molina-Grima, E. Marine microalgae selection and culture conditions optimization for biodiesel production. *Bioresour. Technol.* **2013**, *134*, 353–361. [CrossRef] [PubMed]

25. Schmidt, J.J.; Gagnon, G.A.; Jamieson, R.C. Microalgae growth and phosphorus uptake in wastewater under simulated cold region conditions. *Ecol. Eng.* **2016**, *95*, 588–593. [CrossRef]

26. Arrojo, M.Á.; Regaldo, L.; Calvo Orquín, J.; Figueroa, F.L.; Abdala Díaz, R.T. Potential of the microalgae *Chlorella fusca* (Trebouxiophyceae, Chlorophyta) for biomass production and urban wastewater phycoremediation. *AMB Express* **2022**, *12*, 1–14. [CrossRef] [PubMed]

27. Salgado, E.M.; Gonçalves, A.L.; Sánchez-Soberón, F.; Ratola, N.; Pires, J.C. Microalgal cultures for the bioremediation of urban wastewaters in the presence of siloxanes. *Int. J. Environ. Res. Public Health* **2022**, *19*, 2634. [CrossRef]

28. Huang, H.; Zhong, S.; Wen, S.; Luo, C.; Long, T. Improving the efficiency of wastewater treatment and microalgae production for biofuels. *Resour. Conserv. Recycl.* **2022**, *178*, 106094. [CrossRef]

29. Novoa, A.F.; Fortunato, L.; Rehman, Z.U.; Leiknes, T. Evaluating the effect of hydraulic retention time on fouling development and biomass characteristics in an algal membrane photobioreactor treating a secondary wastewater effluent. *Bioresour. Technol.* **2020**, *309*, 123348. [CrossRef]

30. Qiu, S.; Yu, Z.; Hu, Y.; Chen, Z.; Guo, J.; Xia, W.; Ge, S. An evolved native microalgal consortium-snow system for the bioremediation of biogas and centrate wastewater: Start-up, optimization and stabilization. *Water Res.* **2021**, *196*, 117038. [CrossRef]

31. Gordalina, M.; Pinheiro, H.M.; Mateus, M.; da Fonseca, M.M.R.; Cesário, M.T. Macroalgae as Protein Sources—A Review on Protein Bioactivity, Extraction, Purification and Characterization. *Appl. Sci.* **2021**, *11*, 7969. [CrossRef]

32. Buchmann, L.; Böcker, L.; Frey, W.; Haberkorn, I.; Nyffeler, M.; Mathys, A. Energy input assessment for nanosecond pulsed electric field processing and its application in a case study with *Chlorella vulgaris*. *Innov. Food Sci. Emerg. Technol.* **2018**, *47*, 445–453. [CrossRef]

33. Zhang, J.; Satti, A.; Chen, X.; Xiao, K.; Sun, J.; Yan, X.; Liang, P.; Zhang, X.; Huang, X. Low-voltage electric field applied into MBR for fouling suppression: Performance and mechanisms. *Chem. Eng. J.* **2015**, *273*, 223–230. [CrossRef]

34. Canelli, G.; Tevere, S.; Jaquenod, L.; Dionisi, F.; Rohfritsch, Z.; Bolten, C.J.; Neutsch, L.; Mathys, A. A novel strategy to simultaneously enhance bioaccessible lipids and antioxidants in hetero/mixotrophic *Chlorella vulgaris* as functional ingredient. *Bioresour. Technol.* **2022**, *347*, 126744. [CrossRef] [PubMed]

35. Salamati Mashhad, N. Investigation of Activated Sludge Properties under Different Electrical Field and in the Presence of Calcium. Master's Thesis, Concordia University, Montreal, QC, Canada, November 2010.

36. Su, F.; Liang, Y.; Liu, G.; Mota Filho, C.R.; Hu, C.; Qu, J. Enhancement of anti-fouling and contaminant removal in an electro-membrane bioreactor: Significance of electrocoagulation and electric field. *Sep. Purif. Technol.* **2020**, *248*, 117077. [CrossRef]

37. Giwa, A.; Dindi, A.; Kujawa, J. Membrane bioreactors and electrochemical processes for treatment of wastewaters containing heavy metal ions, organics, micropollutants and dyes: Recent developments. *J. Hazard. Mater.* **2019**, *370*, 172–195. [CrossRef]

38. Asif, M.B.; Maqbool, T.; Zhang, Z. Electrochemical membrane bioreactors: State-of-the-art and future prospects. *Sci. Total Environ.* **2020**, *741*, 140233. [CrossRef]

39. Zhang, M.; Leung, K.-T.; Lin, H.; Liao, B. The biological performance of a novel microalgal-bacterial membrane photobioreactor: Effects of HRT and N/P ratio. *Chemosphere* **2020**, *261*, 128199. [CrossRef]

40. Muñoz, R.; Jacinto, M.; Guieysse, B.; Mattiasson, B. Combined carbon and nitrogen removal from acetonitrile using algal–bacterial bioreactors. *Appl. Microbiol. Biotechnol.* **2005**, *67*, 699–707. [CrossRef]

41. Shaw, D.J. *Introduction to Colloid and Surface Chemistry*; Butterworths: Oxford, UK, 1980.

42. Water Environment Federation; American Water Works Association; American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association (APHA): Washington, DC, USA, 2005; Volume 21.

43. Luo, Y.; Le-Clech, P.; Henderson, R.K. Assessment of membrane photobioreactor (MPBR) performance parameters and operating conditions. *Water Res.* **2018**, *138*, 169–180. [CrossRef]

44. Jia, G.; Jing, H.; Hu, B.; Ju, Z.; He, S.; Shui, F.; Ce, J.; Xi, F.; Fa, F.; Hui, W. *Monitoring and Analysis Methods for Water and Wastewater*; China Environmental Science Press: Beijing, China, 2002; pp. 232–235.

45. Haberkorn, I.; Buchmann, L.; Hiestand, M.; Mathys, A. Continuous nanosecond pulsed electric field treatments foster the upstream performance of *Chlorella vulgaris*-based biorefinery concepts. *Bioresour. Technol.* **2019**, *293*, 122029. [CrossRef]

46. Buchmann, L.; Mathys, A. Perspective on pulsed electric field treatment in the bio-based industry. *Front. Bioeng. Biotechnol.* **2019**, *7*, 265. [CrossRef]

47. Sharma, A.; Kaur, T.; Singh, H.; Kaur, G. Intermittent fasting–dietary restriction as a biological hormetin for health benefits. In *The Science of Hormesis in Health and Longevity*; Rattan, S.I.S., Kyriazi, M., Eds.; Elsevier: Amsterdam, The Netherlands, 2019; pp. 99–104.

48. Zong, E.; Wei, D.; Wan, H.; Zheng, S.; Xu, Z.; Zhu, D. Adsorptive removal of phosphate ions from aqueous solution using zirconia-functionalized graphite oxide. *Chem. Eng. J.* **2013**, *221*, 193–203. [CrossRef]

49. Wei, C. Nutrient Removal and Fouling Reduction in Electrokinetic Membrane Bioreactor at Various Temperatures. Ph.D. Thesis, University of Manitoba, Winnipeg, MB, Canada, 2009.

50. Guo, M.; Feng, L.; Liu, Y.; Zhang, L. Electrochemical simultaneous denitrification and removal of phosphorus from the effluent of a municipal wastewater treatment plant using cheap metal electrodes. *Environ. Sci. Water Res. Technol.* **2020**, *6*, 1095–1105. [CrossRef]

51. Bani-Melhem, K.; Smith, E. Grey water treatment by a continuous process of an electrocoagulation unit and a submerged membrane bioreactor system. *Chem. Eng. J.* **2012**, *198*, 201–210. [CrossRef]

52. Liu, L.; Liu, J.; Gao, B.; Yang, F. Minute electric field reduced membrane fouling and improved performance of membrane bioreactor. *Sep. Purif. Technol.* **2012**, *86*, 106–112. [CrossRef]

53. Akamatsu, K.; Lu, W.; Sugawara, T.; Nakao, S.-i. Development of a novel fouling suppression system in membrane bioreactors using an intermittent electric field. *Water Res.* **2010**, *44*, 825–830. [CrossRef]

54. Choi, H.J.; Lee, S.M. Effect of the N/P ratio on biomass productivity and nutrient removal from municipal wastewater. *Bioprocess. Biosyst. Eng.* **2015**, *38*, 761–766. [CrossRef]

55. Wu, J.; Chen, F.; Huang, X.; Geng, W.; Wen, X. Using inorganic coagulants to control membrane fouling in a submerged membrane bioreactor. *Desalination* **2006**, *197*, 124–136. [CrossRef]

56. Fan, F.; Zhou, H.; Husain, H. Use of chemical coagulants to control fouling potential for wastewater membrane bioreactor processes. *Water Environ. Res.* **2007**, *79*, 952–957. [CrossRef]

57. Liu, L.; Liu, J.; Gao, B.; Yang, F.; Chellam, S. Fouling reductions in a membrane bioreactor using an intermittent electric field and cathodic membrane modified by vapor phase polymerized pyrrole. *J. Membr. Sci.* **2012**, *394*, 202–208. [CrossRef]

58. Bani-Melhem, K.; Elektorowicz, M. Performance of the submerged membrane electro-bioreactor (SMEBR) with iron electrodes for wastewater treatment and fouling reduction. *J. Membr. Sci.* **2011**, *379*, 434–439. [CrossRef]

59. Marbelia, L.; Bilad, M.R.; Passaris, I.; Discart, V.; Vandamme, D.; Beuckels, A.; Muylaert, K.; Vankelecom, I.F. Membrane photobioreactors for integrated microalgae cultivation and nutrient remediation of membrane bioreactors effluent. *Bioresour. Technol.* **2014**, *163*, 228–235. [CrossRef] [PubMed]

60. Matho, C.; Schwarzenberger, K.; Eckert, K.; Keshavarzi, B.; Walther, T.; Steingroewer, J.; Krujatz, F. Bio-compatible flotation of *Chlorella vulgaris*: Study of zeta potential and flotation efficiency. *Algal Res.* **2019**, *44*, 101705. [CrossRef]

61. Abt, V.; Gringel, F.; Han, A.; Neubauer, P.; Birkholz, M. Separation, characterization, and handling of microalgae by dielectrophoresis. *Microorganisms* **2020**, *8*, 540. [CrossRef]

62. Wei, C.; Huang, Y.; Liao, Q.; Xia, A.; Zhu, X.; Zhu, X. Analysis of the energy barrier between *Chlorella vulgaris* cells and their interfacial interactions with cationic starch under different pH and ionic strength. *Bioresour. Technol.* **2020**, *304*, 123012. [CrossRef] [PubMed]

63. Rashid, M.; Price, N.T.; Pinilla, M.Á.G.; O'Shea, K.E. Effective removal of phosphate from aqueous solution using humic acid coated magnetite nanoparticles. *Water Res.* **2017**, *123*, 353–360. [CrossRef]

64. Gizaw, A.; Zewge, F.; Kumar, A.; Mekonnen, A.; Tesfaye, M. A comprehensive review on nitrate and phosphate removal and recovery from aqueous solutions by adsorption. *AQUA Water Infrastruct. Ecosyst. Soc.* **2021**, *70*, 921–947. [CrossRef]

65. Markou, G.; Depraetere, O.; Vandamme, D.; Muylaert, K. Cultivation of *Chlorella vulgaris* and *Arthrospira platensis* with recovered phosphorus from wastewater by means of zeolite sorption. *Int. J. Mol. Sci.* **2015**, *16*, 4250–4264. [CrossRef]

66. González, L.E.; Cañizares, R.O.; Baena, S. Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. *Bioresour. Technol.* **1997**, *60*, 259–262. [CrossRef]

67. Hu, B.; Min, M.; Zhou, W.; Li, Y.; Mohr, M.; Cheng, Y.; Lei, H.; Liu, Y.; Lin, X.; Chen, P. Influence of exogenous CO_2 on biomass and lipid accumulation of microalgae *Auxenochlorella protothecoides* cultivated in concentrated municipal wastewater. *Appl. Biochem. Biotechnol.* **2012**, *166*, 1661–1673. [CrossRef]

68. Sakakibara, Y.; Kuroda, M. Electric prompting and control of denitrification. *Biotechnol. Bioeng.* **1993**, *42*, 535–537. [CrossRef]

69. Ibeid, S.; Elektorowicz, M.; Oleszkiewicz, J. Modification of activated sludge properties caused by application of continuous and intermittent current. *Water Res.* **2013**, *47*, 903–910. [CrossRef] [PubMed]

70. Luo, S.; Griffith, R.; Li, W.; Peng, P.; Cheng, Y.; Chen, P.; Addy, M.M.; Liu, Y.; Ruan, R. A continuous flocculants-free electrolytic flotation system for microalgae harvesting. *Bioresour. Technol.* **2017**, *238*, 439–449. [CrossRef] [PubMed]

71. Chojnacka, K.; Marquez-Rocha, F.-J. Kinetic and stoichiometric relationships of the energy and carbon metabolism in the culture of microalgae. *Biotechnology* **2004**, *3*, 21–34. [CrossRef]