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Performance of Microbial Fuel Cells Integrated with a Photo-Bioreactor: Effects of Light Regimes and Microalgae (*Chlorella* vs. *Scenedesmus*)

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ABSTRACT

Microbial fuel cells (MFCs) represent a promising technology for wastewater treatment and energy recovery, because of their ability to simultaneously convert organic matter into electricity via electroactive bacteria in anode chambers. This technology can significantly decrease the high energy costs typically associated with conventional wastewater treatment. The incorporation of green microalgae to self-supply oxygen may further enhance the environmental sustainability of the MFC system. This study evaluates the feasibility of integrating a double-chamber MFC with a photo-bioreactor (PBR) using specific microalgae under different light conditions. The experimental results indicated that chemical oxygen demand (COD) removal efficiencies using *Chlorella vulgaris* and *Scenedesmus protuberans* were 76.28% and 86.71%, respectively. A notable variation in cell voltage was observed when shifting from continuous illumination to a 12-hour light/12-hour dark (12/12) cycle. Under the 12/12 light/dark regime, *Chlorella* and *Scenedesmus* produced voltages of 350 mV and 340 mV, respectively, while continuous light resulted in higher voltages of 390 mV and 400 mV. Power densities achieved under continuous and 12/12 lighting were 1,140 mW/m^3 and 975 mW/m^3 for *Chlorella*, and 1,350 mW/m^3 and 1160 mW/m^3 for *Scenedesmus*, respectively. Overall, the system's performance could be substantially improved by stabilizing influent concentration and flow rate. The integration of PBR into MFCs proved effective for simultaneous wastewater treatment and bioenergy recovery, with *Scenedesmus* and continuous illumination yielding superior performance.

HIGHLIGHTS

- MFC-PBR integration treats wastewater and recovers energy.
- Microalgae supply oxygen to boost energy recovery in MFCs.
- The photo-bioreactor optimizes microbial fuel cell power.
- Light regimes significantly impact wastewater treatment.

1. INTRODUCTION

Water pollution and energy scarcity are major global challenges, while urban wastewater remains a significant pollution source, with only about 12.5% treated before discharge. Conventional treatment is also energy-intensive due to aeration, highlighting the need for more energy-efficient technologies. Microbial Fuel Cell (MFC) technology is a promising bioenergy approach that has attracted significant attention in recent years due to its ability to simultaneously generate electricity and treat wastewater (Elangovan et al., 2023; Zhang et al., 2011). First reported over a century ago (Potter, 1911), MFCs convert organic matter in wastewater into electricity via bacterial oxidation in the anode, with electrons transferred to the cathode where oxygen acts as the electron acceptor. Although more efficient than conventional anaerobic systems, MFCs are still

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limited by low power output and aeration energy demand. Microalgae are fast-growing photosynthetic microorganisms with applications in biofuel production, biomass generation, and environmental remediation. Species such as *Chlorella vulgaris* and *Scenedesmus protuberans* are widely studied for their high photosynthetic efficiency, CO fixation, oxygen production or ability to remove pollutants (Abdelfattah et al., 2023; Li et al., 2023). The application of algae in wastewater treatment has also become widespread, particularly when combining wastewater-grown microalgae with activated sludge (Su et al., 2011). Consequently, integrating green microalgae into MFC systems presents a promising approach to enhancing wastewater treatment efficiency, generating electricity, and conserving energy. Incorporating an algal photobioreactor (PBR) into the MFC cathode chamber may further improve cathodic reactions and supply an oxygen-enriched electrolyte to the cathode.

In MFC systems, coupling microalgae with a photobioreactor (PBR) at the cathode can enhance oxygen supplies, improve cathodic reactions, and reduce external aeration requirements. In addition, microalgae contribute to CO_2 sequestration, pH stabilization, biofilm interactions, and nutrient removal, thereby improving both treatment efficiency and energy recovery.

This study evaluates an integrated MFC-PBR system using real wastewater, different light regimes, and two microalgae. Unlike previous work using synthetic wastewater, this study focuses on realistic conditions with varying organic loads. *Chlorella* and *Scenedesmus* were selected due to their distinct physiological traits and reported effectiveness. The novelty lies in the combined evaluation of real wastewater, light regimes, and microalgae in a single system, providing more practical insights for real-world applications.

2. METHODOLOGY

2.1 MFCs reactor configuration

The MFC system was designed with two chambers: an anode chamber (5.5×5.5×8.0 cm) and a cathode chamber (5.5×5.5×4.5 cm). The two reaction chambers were separated by gaskets and a cation exchange membrane (CEM) (CMI-7000S, size: 100 mm × 100 mm; Membrane International, USA).

The electrodes consisted of carbon cloth fibers wrapped around a platinum wire core (1 mm in diameter). The electrodes were connected via cooper wires to an external resistor, forming a closed circuit. Wastewater and microalgae were recirculated from storage tanks into the corresponding electrode chambers by a peristaltic pump (77200-50, Masterflex, USA).

An acrylic tubular photobioreactor (PBR) (d=0.14 m, h=0.37 m) equipped with white LED lamps (1×0.7×4 cm) providing a light intensity of 1285 lux (Model HLN3S, IST, Korea) was utilized to cultivate and supply oxygen-rich microalgae suspension to the cathode chamber. The oxygen-rich suspension from the microalgae reactor was filtered using a 0.01 μm UF filter (IONPIA, Korea) before being pumped into the cathode chamber to prevent algae from entering the electrochemical system. A timer was additionally installed to control the on/off mode of the LED light source, supporting the photosynthetic activity of microalgae in the photobioreactor (Figure 1).

2.2 Inoculation and medium

The system was acclimated with mixed wastewater in two steps. In step 1, synthetic wastewater (2.2 g $Na_2HPO_4 \cdot 12H_2O$; 0.53 g KH_2PO_4; 0.5 g NaCl; 0.03 g $MgSO_4 \cdot 7H_2O$; 0.02 g $CaCl_2 \cdot 7H_2O$; 2.92 g CH_3COONa for 1 L) containing acetate substrate (COD=2,000 mg/L; COD: Total Kjeldahl Nitrogen (TKN)=50:1; Q=1.0 mL/min) was used to acclimate and enrich the electroactive microbial community in the anode chamber. Anaerobic sludge was supplemented 2-3 times / (10 mL each time with MLSS=3,000 mg/L) into the anolyte solution during the first week of acclimation to improve the electrode-attached microbial community. In step 2, the system continued to be acclimated with mixed wastewater WW1 (COD=800 mg/L COD: TKN=3:1, Q=1.0 mL/min) for 14 days and WW2 (COD=1,500 mg/L; COD: TKN=5:1, Q=1.0 mL/min) for the following 14 days, until the cell potential became stable. Data for evaluating experimental performance was collected thereafter.

During the acclimation phase, WW1 and WW2 were prepared by mixing livestock wastewater collected from a cattle farm in Vinh Loc B Commune, Binh Chanh District (Ho Chi Minh City) and domestic wastewater collected from the drainage outlet behind Building B1 (of Ho Chi Minh City University of Technology) at a ratio of 1:10.

The microalgae culture solution was prepared with distilled water containing nutrient components including $MgSO_4$ (2.86 mg/L), $NaNO_3$ (93.4 mg/L),

$Ca(NO_3)_2$ (8.2 mg/L), $K_2HPO_4 \cdot 3H_2O$ (6.26 mg/L), Na_2CO_3 (4.2 mg/L), $FeCl_3$ (1.68 mg/L), EDTA (triplex) (3.705 mg/L), and 1 mL of trace element medium (Graffron solution) with microalgae cultivated in modified Z8 medium. The prepared solution had a COD=3 mg/L, PO_4^{3-}-P (Ortho-phosphate-P)=3 mg/L, and TKN=0.5 mg/L.

Biomass (both anode and cathode) was stored separately and pumped into the system at 1.0 mL/min during acclimation and 1.5 mL/min during operation. No mixing was applied in either chamber. The anode was fed with synthetic and real wastewater, while the cathode received the microalgae culture solution.

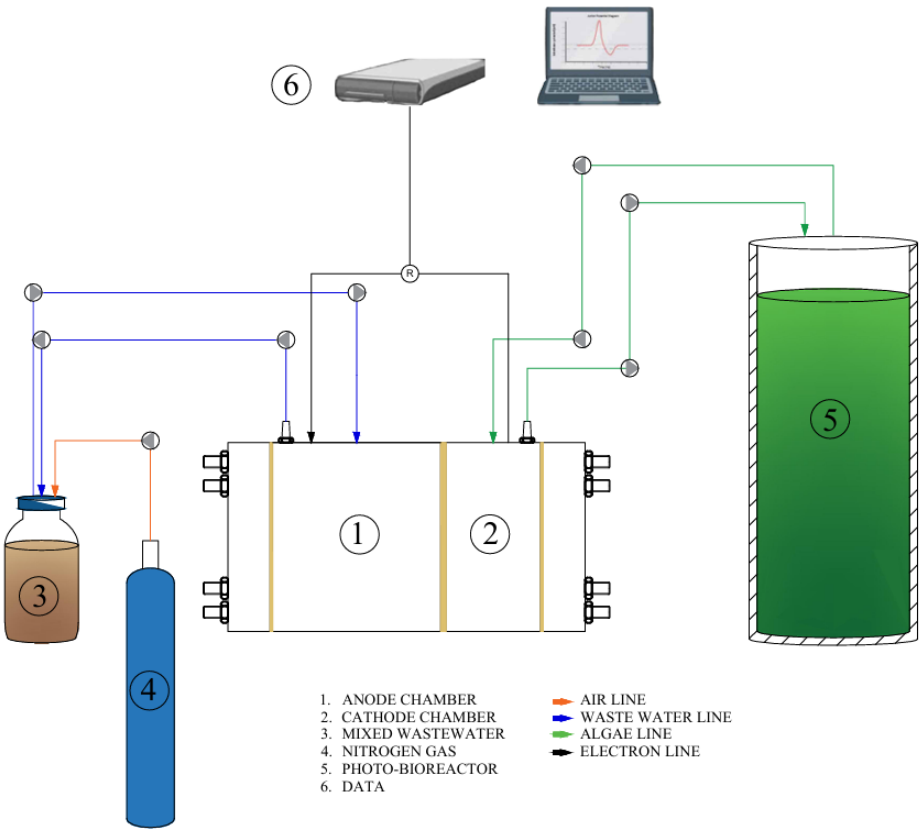

Figure 1. MFC with a PBR reactor setup

2.3 Start-up and operation

After the second acclimation phase, the MFC system entered the experimental stage using real wastewater. In MFC studies, varying external resistance is generally used to determine the open-circuit voltage and maximum power point, thereby balancing electricity generation, microbial growth, and overall system performance. Here, the optimal performance was evaluated using polarization curves. External resistance (Rext) was gradually decreased from 9,000 Ω to 0 Ω in steps of 100 Ω. Cell voltage and current were continuously recorded using a data logger (ADC-20, PicoTech, UK) every 30 min.

Current density normalized to anode volume was calculated as:

$$I = \frac{U}{R_{ext} \times V_{Chamber\ Anode}} \quad (1)$$

Where; I is current density (A/m³), U is voltage (V), Rext is external resistance (Ω), and Vanode is anode volume (m³).

Power density was calculated as:

$$P = U \times I \quad (2)$$

Where; P is power density (W/m³), U is voltage (V), and I is current density (A/m³).

U-I and P-I curves were constructed from measured and calculated data. Internal resistance was determined using a previously reported method (Zhang et al., 2015). Polarization curves were analyzed prior to operation.

The MFC-PBR system was evaluated using wastewater WW3 (COD=3,000 mg/L; COD:TKN= 10:1; Q=1.5 mL/min) with two microalgae under different light conditions (24/0 and 12/12) in the PBR.

The system was operated in batch mode with a flow rate of 1.5 mL/min, corresponding to HRTs of 90 min (anode) and 50 min (cathode). Wastewater was stored in an external reservoir and pumped into the reactor via a bottom inlet, creating an upflow regime. Effluent exited from the top outlet and was recirculated to the reservoir. Each batch lasted three days before being replaced with fresh wastewater.

Chlorella vulgaris and *Scenedesmus protuberans*, provided by the Ecotoxicology Laboratory, Ho Chi Minh City University of Technology, were cultured in sterile nutrient medium under aeration and continuous light. To evaluate illumination effects, the light regime was controlled at 12/12 (6:00 AM-6:00 PM) to support microalgae photosynthesis in the PBR. The microalgae medium and biomass were replaced at regular intervals.

2.4 Analysis and calculations

Parameters such as COD, PO_4^{3-}-P, and TKN were analyzed according to Standard Methods. COD was determined using the kali dichromate method, PO_4^{3-} was analyzed using the ascorbic acid method, and TKN was measured using the Kjeldahl method. Prior to analysis, samples were filtered through a 0.45 µm paper filter. This study focused on dissolved substrates as they represent the readily bioavailable fraction for microbial activity. The potential contribution of particulate matter, which may undergo hydrolysis and subsequent degradation, was not explicitly considered and should be addressed in future studies.

The organic matter consumption rate (kgCOD/m^3/day) was calculated based on the decrease in COD concentration over a given period and the volume of the anode chamber.

Coulombic efficiency (CE) was calculated based on the amount of COD removed using the following formula:

$$CE\ (\%) = \frac{M \times I}{F \times q \times n \times \Delta COD} \quad (3)$$

Where; M, I, F, q, n, and ΔCOD are respectively the molecular weight of O_2 (32 g/mol), current (A), Faraday's constant (96,485 C/mol), anolyte flow rate (L/s), the number of electrons exchanged per mole of O_2 (4 mol e^-/mol), and the difference in COD concentration between the influent and effluent of the anode chamber (g/L).

The volumetric power density (P_v, mW/m^3) of the MFC system was calculated using the effective volume of the anode chamber:

$$P_v(mW/m^3) = \frac{U \times I \times 10^{-3}}{v} \quad (4)$$

Where; U is the output voltage (V), v is the anode chamber volume (m^3), and I is the current (µA).

COD removal efficiency (%) was calculated as:

$$H\,(\%) = \frac{\Delta COD}{COD_{t0}} \times 100 \quad (5)$$

Where; ΔCOD is the difference in COD concentration between the start and end of the batch (3 days) (mg/L), and COD_{t0} is the influent COD concentration (mg/L).

The TKN removal efficiency (%) was calculated according to the following equation:

$$H\,(\%) = \frac{\Delta TKN}{TKN_{t0}} \times 100 \quad (6)$$

Where; ΔTKN is the difference in TKN concentration between the start and end of the batch (3 days) (mg/L), and TKN_{t0} is the influent TKN concentration (mg/L).

The TKN removal rate (mg/L/day) was calculated as:

$$H\,(mg/L/day) = \frac{\Delta TKN}{t} \quad (7)$$

Where; ΔTKN is the difference in TKN concentration between the start and end of the batch (3 days) (mg/L), and t is the hydraulic retention time or cycle duration (days).

3. RESULTS AND DISCUSSION

3.1 Voltage

Polarization analysis was conducted before operation to evaluate electrochemical performance. The maximum power output within the tested range was observed at around 1,000 Ω (Figure 2), which was therefore selected as the fixed external resistance for all experiments.

Data corresponding to each of the both microalgae (*Scenedesmus* and *Chlorella*) are clearly distinguished throughout the figures and text using consistent labeling and color coding.

3.1.1 Adaptation stage with acetate and mixed wastewater

Before operating with real wastewater, the system was acclimated using acetate (COD=2,000 mg/L) to ensure microbial adaptation. During this stage, COD reduction led to noticeable voltage fluctuations of 150-200 mV (Figure 3). Voltage increased sharply after fresh medium replacement but then decreased again within the following two days, reflecting substrate-dependent system behavior (from 211.96±12.72 mV down to 150.47±16.88 mV).

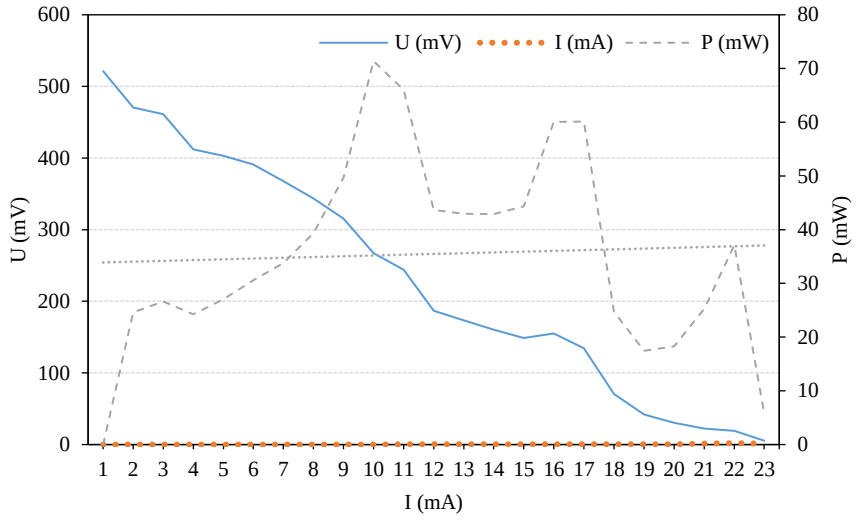

Figure 2. Polarization test

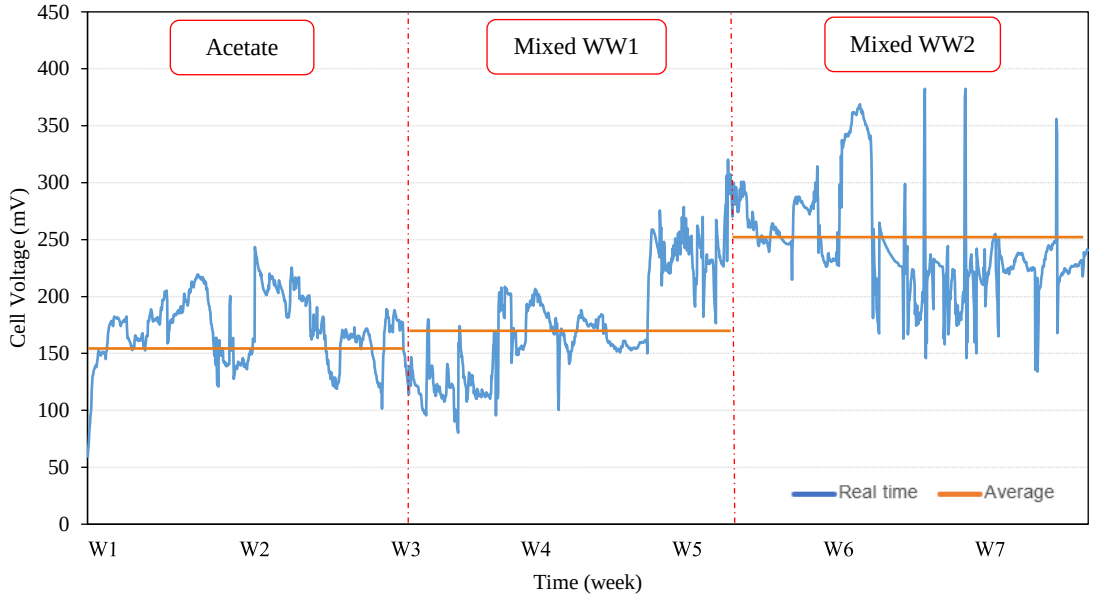

Figure 3. Cell voltage during the adaptation stage

After the adaptation stage with acetate as the substrate, the system was switched to adaptation with mixed wastewater WW1 (COD=800 mg/L; COD:TKN=3:1) for the following two weeks, and then to WW2 (COD=1,500 mg/L; COD:TKN=5:1) for four weeks (Figure 3). Previous studies on MFC systems have shown that low substrate concentrations significantly limit electricity generation. When COD levels decrease below a critical threshold, electron donor availability becomes insufficient, leading to reduced cell voltage and even system instability or shutdown (Logan et al., 2019; Zhang et al., 2015).

For WW1, cell voltage remained relatively high (>150 mV) and stable during the first week, then decreased and stabilized at 130-170 mV. This decline may be due to competing anaerobic processes such as

fermentation and methanogenesis, which consume substrates and reduce electron transfer. The higher COD threshold required for stable operation with real wastewater compared to simple substrates (e.g., acetate) reflects its complex and less biodegradable nature, limiting electroactive microbial activity (Zarabadi et al., 2019).

For WW2, voltage fluctuated between 175-300 mV (244.46±62.78 mV). Sharp drops occurred when COD fell below 140 mg/L, due to insufficient substrates, but recovery was observed after supplementation. Fluctuations compared to acetate-fed operation were attributed to shock loading and incomplete biofilm acclimation. Instability may also be related to microalgae inhibition under continuous illumination and biomass aging or decay (Montoya-Vallejo et al., 2025). Voltage spikes up to ~400 mV likely resulted from accumulation of dead algae, reducing photosynthetic efficiency, and indicating the need for periodic culture replacement.

It was observed that during the second week of the WW2 stage, the system sometimes spiked from about 150 mV up to nearly 400 mV. These spikes are likely attributed to the dynamic response of the anodic biofilm to the shock loading from the increased COD concentration. Specifically, the rapid hydrolysis and consumption of readily degradable organic fractions temporarily boosted electron transfer rates, leading to short-term voltage increases. After these easily degradable substrates were depleted, the voltage dropped again, reflecting that the microbial community had not yet fully stabilized and adapted. The dead microalgae cells settled, aggregated, and hindered the photosynthetic activity of the remaining healthy cells. Therefore, it should be noted that replacing the microalgae culture solution after each experimental run is necessary for system stability.

3.1.2 Operation stage with mixed wastewater

For the cathode chamber, the influent was supplied from the PBR under the conditions of two light/dark cycles: illumination conditions of 24/00 and 12 hours/day (12/12), in combination with *Chlorella*. Over the course of three weeks, the system was operated under 12/12 illumination in the PBR. At this time, the obtained cell voltage was 353.81±39.18 mV, fluctuating between 300-375 mV during the first two weeks, then increasing to 360-420 mV in the final week. The system was then switched to the WW3 (COD=3,000 mg/L; COD:TKN=10:1; Q=1.5 mL/min) *Chlorella* experiment with 24/00 illumination in the PBR. Cell voltage was then maintained at 391.72±47.62 mV and stabilized within 350-450 mV, with the highest value reaching 461.5 mV (Figure 4). At the beginning of each phase, voltage fluctuated due to system adaptation, and changing illumination conditions. After about one week, performance became more stable as both the anodic microbial community and PBR microalgae acclimated, improving overall system operation. Under the 12/12 light/dark regime, clear day-night voltage fluctuations were observed, whereas continuous illumination (24/0) resulted in higher and more stable voltage outputs. Compared to the acclimation phase, overall voltage performance was also higher during operation.

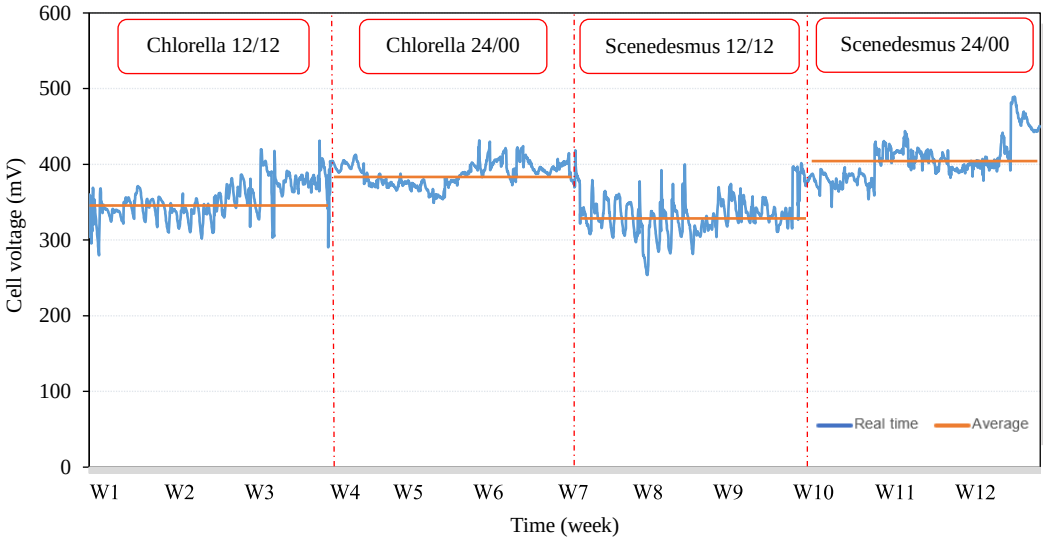

Figure 4. Cell voltage during operation stage with *Chlorella* and *Scenedesmus* microalgae

After the two WW3 experiments using *Chlorella*, the system process was performed with *Scenedesmus* under different illumination regimes of 24/00 and 12/12. During the first three weeks under the 12/12 light/dark cycle, the voltage was 339.47±19.28 mV, fluctuating between 300 and 360 mV. Continuous illumination (24/00) followed, and the cell voltage fluctuated around 400 mV, with an average of 393.96±32.97 mV and peak of 490 mV reached in the final week. Similar to the *Chlorella* experiments, the 12/12 regime showed clear voltage differences between light and dark phases, while continuous illumination (24/00) resulted in higher and more stable voltage due to improved system adaptation. Under 12/12, voltage drops (~250 mV) were linked to substrate depletion but rapidly recovered (>350 mV) after replenishment, indicating strong dependence on organic matter availability.

The superior performance of *Scenedesmus* should be interpreted cautiously, as previous studies have reported higher voltages for *Chlorella*, indicating strong dependence on operating conditions. The improved performance of *Scenedesmus* may be due to its ability to form stable cathodic biofilms, enhancing oxygen transfer and reducing internal resistance, while *Chlorella* may show different oxygen production behavior (Zhang et al., 2024; Argiz et al., 2022). Microalgae-microbial interactions may also influence electroactive communities and redox mediator production, making performance system-dependent.

Comparison of WW3 between the two microalgae species showed that under continuous illumination (24/00), *Scenedesmus* generated higher average and maximum voltages than *Chlorella*, although with greater fluctuations. Under the 12/12 light-dark regime, *Scenedesmus* produced lower voltages than *Chlorella* (Mathimani et al., 2021). In both microalgae systems, continuous illumination maintained higher and more stable voltages, with decreases observed only when COD dropped below 500 mg/L. Voltage rapidly recovered after wastewater replacement, indicating stable activity of acclimated electroactive microorganisms in the anode, consistent with previous studies (Zhang et al., 2024; Argiz et al., 2022). In contrast, intermittent illumination caused clear voltage fluctuations (approximately 50-75 mV), likely due to periodic changes in oxygen availability for cathodic reduction.

These differences can be explained by the influence of light on photosynthetic oxygen production in the cathode chamber. Continuous illumination supports stable cathodic reactions, whereas light/dark cycles may increase internal resistance and reduce system stability. Performance variations between *Chlorella* and *Scenedesmus* may also be related to their distinct physiological characteristics. *Scenedesmus* can form aggregates that potentially improve light utilization and interactions with cathodic biofilms, thereby enhancing oxygen transfer and electron transfer processes.

Compared with conventional MFCs, which rely on passive diffusion or aeration, an integrated PBR enables in situ oxygen production through photosynthesis, in turn reducing energy demands and improving cathodic performance (Logan, 2006; Argiz et al., 2022). However, microalgae-assisted systems also introduce more complex biological interactions and greater sensitivity to environmental conditions such as light regimes and nutrient availability.

3.2 Substrate removal

COD removal mainly depends on microbial activity and wastewater composition. Sequential biodegradation of complex organics also plays a key role (Edmundson and Huesemann, 2015; Sforza et al., 2012). In this study, higher influent COD led to better performance, with the highest efficiency (>85%) at 3,000 mg/L COD and a COD:TKN ratio of 10:1. Synthetic wastewater showed higher removal than real wastewater due to simpler substrates, consistent with typical MFC performance (~80%) (Zhang et al., 2011; Zhang et al., 2015). In the dual-chamber MFC system, osmotic pressure differences between the anolyte and catholyte may induce water transport across the membrane, potentially leading to partial dilution of the anolyte and an overstatement of the biological COD removal efficiency (Ge and He, 2012). However, no noticeable volume changes were observed during the experimental period, suggesting that this effect was limited. Therefore, although osmotic dilution may have contributed to a minor extent, COD removal was mainly attributed to biological degradation and electrochemical activity within the MFC system.

The organic loading rate (OLR) was defined as the mass of COD applied per unit reactor volume per unit time (kgCOD/m³/day). At a WW3 with *Chlorella vulgaris*, the system under continuous illumination (24/00 cycle) showed 0.86±0.05 kgCOD/m^3/day with a removal efficiency of 89.82±1.2%, which was superior to the 12/12 light/dark cycle 0.68±0.03 kgCOD/m³/day and 76.28±4.11%. This improvement can be attributed to the abundant substrate, which

promoted both biomass growth and electron flow. For *Scenedesmus protuberans* under the same condition, both the 24/00 and 12/12 cycles achieved showed slightly higher removal efficiency compared to *Chlorella* with 0.81±0.01 kgCOD/m³/day; 86.71± 2.3% in 12/12 and 0.91±0.08 kgCOD/m³/day; 89.95±1.6% in 24/00 (Figure 5).

Thus, it can be seen that COD removal efficiency is influenced by the illumination conditions in the PBR. Continuous lighting accelerates the COD removal rate, thereby enhancing the overall performance of the system. Compared with other studies which also used domestic wastewater and reported a COD removal efficiency of approximately 86% (Valladares Linares et al., 2019), our study achieved higher removal efficiencies for both microalgae species under continuous illumination (24/00). Only in the case of *Chlorella* under the 12/12 light-dark cycle was the COD removal efficiency slightly lower than that reported in previous studies. Meanwhile, the illumination conditions in the PBR influenced the stability of the cathodic oxygen supply-thereby supporting the overall electrochemical balance. Under continuous illumination (24/00), the system produced higher and more stable voltages, likely due to continuous oxygen supply supporting cathodic reactions and lowering internal resistance. In contrast, the 12/12 regime caused voltage fluctuations from periodic oxygen limitation and system adaptation. Overall, performance is governed by the balance between COD-driven electron supplies and DO-driven cathodic reactions. However, these conclusions remain preliminary due to limited microbial and controlled experimental data.

The relationship between COD removal and voltage was not strictly proportional. Although higher voltages were observed under continuous illumination (~393.96±32.97 mV vs. ~339.47±19.28 mV), the increase in COD removal was moderate. With DO consistently above 8 mg/L, oxygen limitation was unlikely, suggesting that performance differences are more related to system stability and biological interactions. Therefore, the results are interpreted cautiously, emphasizing trends rather than definitive causality.

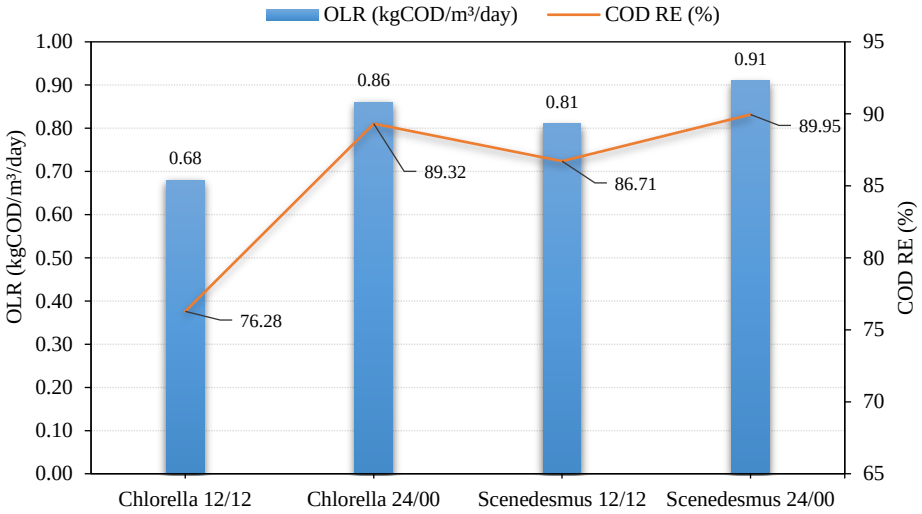

Figure 5. Organic loading rate and COD removal efficiency in operation stage

Coulombic efficiency (CE) reflects the proportion of electrons recovered from organics via electrochemical activity of biofilms dominated by *Geobacter sulfurreducens* (Ewusi-Mensah et al., 2021), and it fluctuates throughout MFC operation depending on the influent substrate. The results from this study show that CE under continuous illumination was higher than under the 12/12 light-dark cycle in both microalgae. Among them, *Scenedesmus protuberans* produced the highest result with 0.57±0.02%, under continuous illumination. Meanwhile, under the 12/12 light-dark condition, both microalgae gave almost similar results (Figure 6). However, compared with previous studies reporting a coulombic efficiency (CE) in the range of 1-10% (Valladares Linares et al., 2019), the energy conversion efficiency in this study was lower, even though the COD removal efficiency was comparable or higher.

The differences in performance between *Chlorella* and *Scenedesmus* should be interpreted cautiously within the specific conditions of this study. Although *Scenedesmus* showed higher voltage outputs (e.g., ~393.96±32.97 mV under 24/00 vs. ~339.47±19.28 mV under 12/12), this trend likely results from the combined effects of biological and operational factors rather than a single mechanism. One possible explanation relates to species-specific characteristics. *Scenedesmus* can form aggregates, which may enhance light utilization and interactions with cathodic biofilms, potentially improving oxygen transfer and system stability.

Overall, the superior performance of *Scenedesmus* in this study appears to be condition-dependent. Variations in factors such as OLR, reactor configuration, and microbial community may also contribute, and therefore the results should be interpreted as indicative trends rather than definitive conclusions.This superior performance is likely due to the higher photosynthetic efficiency of *Scenedesmus protuberans*, which ensured a more abundant oxygen supply for the cathodic reduction reaction, largely because its clustered chain-like cellular morphology enables more efficient light absorption (Min and Logan., 2004).

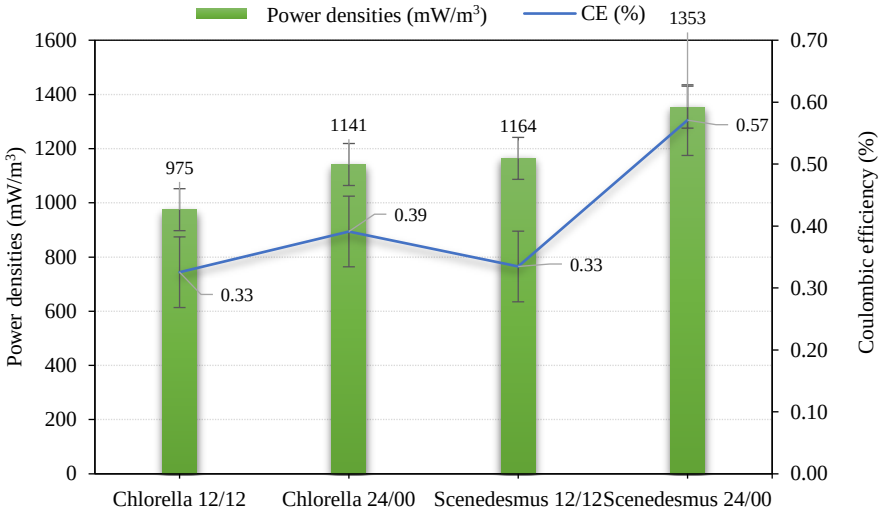

Figure 6. Power densities and coulombic efficiency in operation stage

3.3 Changes in the indicators PO_4^{3-}- P, TKN in the anode chamber

Phosphorus in this study was recorded in the form of PO_4^{3-}- P. In *Chlorella Vulgaris*, the PO_4^{3-}- P level on the first day in anode was 34.12±1.4 mg/L, then decreased to 26.37±2.3 mg/L under 12/12 light/dark condition. Under 24/00 illumination, initial PO_4^{3-}-P concentrations exceeded 43 mg/L. Overall, *Scenedesmus* showed higher phosphate removal efficiency than *Chlorella*, particularly under the 12/12 condition (Figure 7). Higher substrate loading in the anode corresponded to increased PO_4^{3-}-P levels, while differences between 24/00 and 12/12 suggest that PBR illumination may influence phosphorus dynamics in the anode. However, in the *Scenedesmus* system, similar influent phosphate levels led to comparable effluent concentrations, indicating that phosphate removal was more strongly governed by loading conditions than light regime alone. Phosphate removal depends on biological uptake and intracellular storage, which are limited by nutrient availability and biomass activity. Therefore, the effect of light should be interpreted cautiously when influent conditions are not strictly controlled, and further controlled studies are needed.

TKN was only recorded in the influent and the final sample of each wastewater batch due to experimental operating constraints. Based on the research results, the TKN of all experiments on the final day was lower when compared to the first day. In *Chlorella vulgaris*, the TKN level on the first day in anode was 316.83±11.2 mg/L, then decreased to 233.89±7.43 mg/L under 12/12 light/dark condition and 332.45±10.1 mg/L, then decreased to 272.43±8.6 mg/L with 24/00 illumination condition. In *Scenedesmus protuberans*, the TKN level on the first day was 331.74±6.2 mg/L, then decreased to 291.08±5.45 mg/L under 12/12 light/dark condition and 336.40±6.6 mg/L, then decreased to 289.58±9.5 mg/L with 24/00 illumination condition (Figure 8).

Chlorella showed higher TKN removal than *Scenedesmus*, especially under the 12/12 regime, likely due to differences in influent conditions affecting system activity. The decrease in TKN in wastewater was partly due to the conversion of ammonium into nitrite and nitrate during the nitrification process. First, ammonium, a primary component of TKN, migrates across the CEM to the cathode chamber to maintain electroneutrality during current generation (Nguyen and Babel, 2024). Once transported to the oxygen-rich environment of the PBR-cathode chamber, ammonium can undergo aerobic nitrification. Second, a portion of the nitrogen is assimilated into cellular biomass by the anodic electroactive biofilm for growth and metabolism. This suggests that nitrogen removal cannot be attributed to a single pathway. The TKN concentrations under the 12/12 and 24/00 illumination conditions for *Scenedesmus* were highly comparable. Therefore, no statistically significant difference in TKN removal was observed between these two lighting regimes for this specific microalgae.

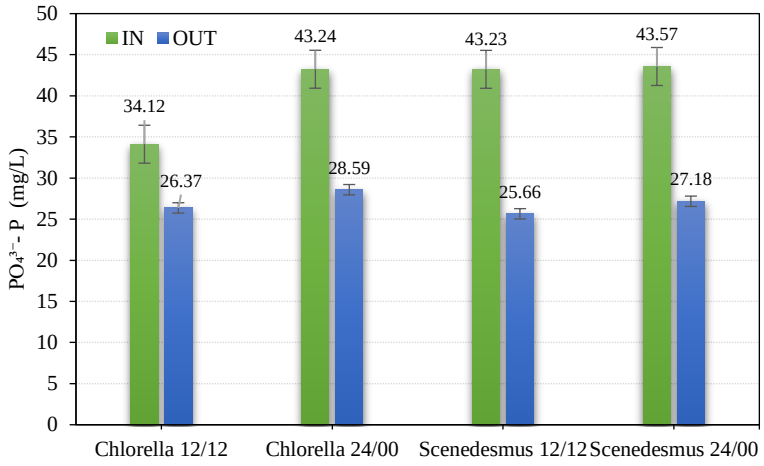

Figure 7. PO_4^{3-}- P in operation stage

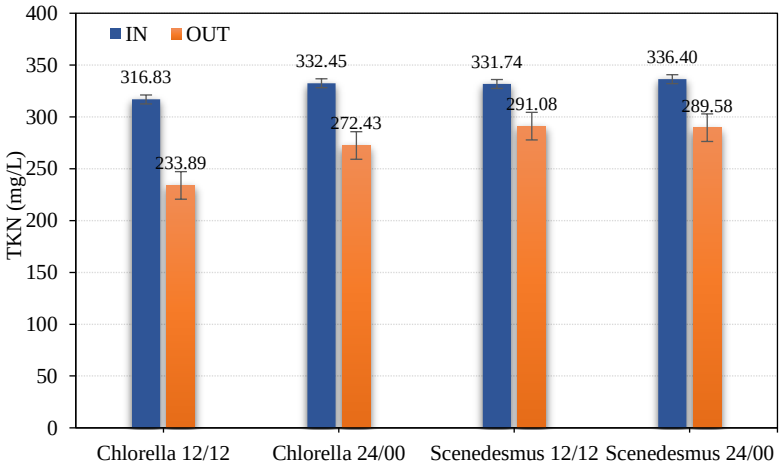

Figure 8. TKN in operation stage

3.4 Effect of light/dark regimes in the photobioreactor on MFC performance

In the photobioreactor, continuous microalgae growth is essential for electricity generation, requiring suitable conditions such as light/dark cycles, carbon sources, aeration, pH, and temperature (Zarabadi et al., 2019). Microalgae metabolism occurs in both light and dark phases, while respiration in the dark phase is important for biomass growth (Edmundson and Huesemann, 2015). Photosynthetic efficiency decreases under non-optimal conditions (Sforza et al., 2012). Therefore, the system was operated under two light regimes (24/00 and 12/12) to evaluate DO and pH using WW3 with *Chlorella* and *Scenedesmus*.

For WW3-*Chlorella*, DO under 12/12 started at 8.26 mg/L, increased above 8.0 mg/L, and then remained stable. Under 24/00, DO started at 8.4 mg/L and stabilized at 8-9 mg/L for two weeks. For WW3-

Scenedesmus, DO under 12/12 started at 9.09 mg/L and stabilized above 9.2 mg/L, while under 24/00 it increased from 6.86 mg/L to 9-10 mg/L. Overall, *Scenedesmus* produced about 1 mg/L higher DO than *Chlorella*, with more stable voltage generation (Figure 9). Although the DO difference (8 vs. 9 mg/L) may not strongly affect cathodic oxygen availability, it could influence microbial community structure and redox mediator production, thereby indirectly affecting electricity generation.

With the same initial conditions in both experiments (200 mL algae in 5 L medium), it was shown that dissolved oxygen fluctuated more under 12/12 (8.26±0.52 mg/L in *Chlorella* and 9.09±0.35 mg/L in *Scenedesmus*) than under 24/00 (8.4±0.26 mg/L in *Chlorella* and 9.3±0.44 mg/L in *Scenedesmus*). This was reasonable since during 12h of darkness, algae undergo respiration, leading to fluctuations in dissolved oxygen. Compared to previous studies (Zhang et al., 2011; Zhang et al., 2015), dissolved oxygen concentrations in this study were lower across all experiments. Despite fluctuations between the two regimes, dissolved oxygen consistently remained above 8 mg/L throughout the experiments.

MFC performance was also influenced by pH in the photobioreactor. Under 24/00, pH started at 8.0 and stabilized at ~8.4, while under 12/12 it remained around 8.6. Overall, pH showed no significant variation between the beginning and end of each batch and was lowest when DO decreased (8.0, DO=6.8 mg/L). Thus, light conditions had little effect on pH, and values in both regimes remained suitable for microalgae growth (Zarabadi et al., 2019) (Figure 10).

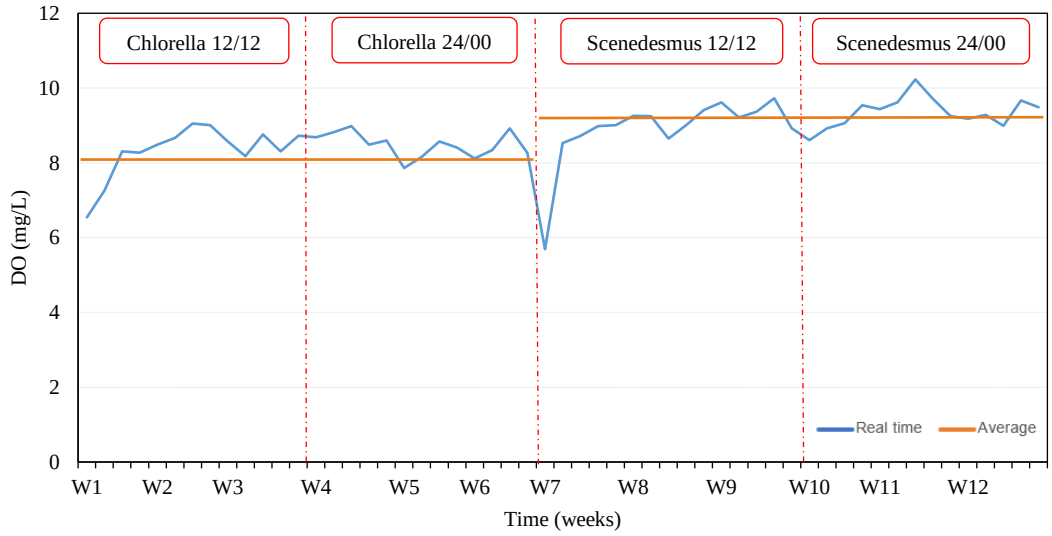

Figure 9. Dissolved oxygen in PBR during operation stage

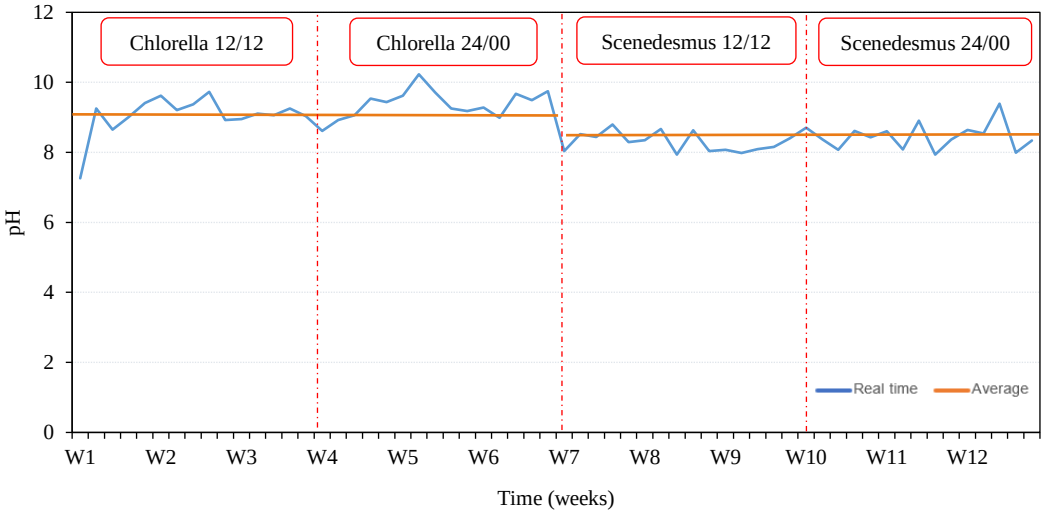

Figure 10. pH in PBR during operation stage

4. CHALLENGES AND FUTURE PERSPECTIVES

From the research results, it is evident that the MFC-PBR system holds great potential for wastewater treatment combined with energy recovery. *Chlorella vulgaris* demonstrated stable performance in both COD removal and energy recovery across two experimental runs under different light conditions. *Scenedesmus protuberans* achieved slightly higher efficiency. The system exhibited more consistent power output over time under the 24/00 condition, suggesting a potential improvement in operational stability. Moreover, the results also indicated that the system is capable of effectively treating wastewater with high concentrations, suggesting the possibility of applying it to various types of wastewater with different characteristics. In addition, testing multiple light regimes could help identify optimal illumination conditions for system development.

5. CONCLUSION

The results confirmed the feasibility of the MFC-PBR system under different light regimes, COD loadings, and microalgae. *Scenedesmus* consistently produced higher voltages than *Chlorella*, and continuous illumination (24/00) led to more stable and higher performance than the 12/12 cycle. For WW3-Scenedesmus, voltages were 339.47±19.28 mV (12/12) and 393.96±32.97 mV (24/00), with fluctuations observed under light/dark conditions. COD removal was higher under continuous light (89.64%) than under 12/12 cycles (81.5%), though differences may also be influenced by loading and dilution effects. DO remained >8 mg/L in both cases, suggesting that microbial interactions and redox mediators may also affect performance. Overall, light regime influences system behavior but cannot be considered independent of other factors. Continuous light improves stability due to sustained oxygen production, while light/dark cycles cause transient limitations. Differences between microalgae species indicate the role of physiological traits and biofilm interactions in system performance.

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AUTHOR CONTRIBUTIONS

Hoang Phuong Tuan Nguyen: Conduct the research, prepare the draft paper and revise the paper based on the comments. Hoang Dung Nguyen: Supervision of research, correction of the submitted and revised manuscript, corresponding author. Nguyen Xuan Que Vo, Thanh Son Dao: revise and finalize the manuscript.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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