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Impact of CO₂ Flow Rate and Nutrient Augmentation on the Production of Bioelectricity in a Photosynthetic Microbial Carbon Capture Cell

Maryam N. Aljabory *, Naseer A. Alhaboubi

Department of Chemical Engineering, University of Al-Nahrain, Baghdad, Iraq.

Keywords:

CO₂ capturing; Cyanobacteria; Microbial carbon capture cell; Electricity production.

Highlights:

- Possibly use recycled alumina bricks as a powder to produce refractory concrete.
- Possibly use waste alumina bricks as aggregate to produce refractory concrete.
- Different replacement percentages from recycled alumina bricks as powder (0, 10, and 25) % were used.
- The results encourage using recycled alumina bricks as powder as a replacement material for partial replacement in producing high refractory concrete.

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*Corresponding author:

Maryam N. Aljabory

Department of Chemical Engineering, University of Al-Nahrain, Baghdad, Iraq.



Abstract: This study investigates the potential of microbial carbon capture cells (MCCs) as a promising solution for energy generation and carbon capture. MCCs comprise two cylinders of plexiglass cells that serve as anode and cathode compartments (both utilize platinum mesh electrodes) and are separated by an ion exchange membrane (AMI-7001). *Synechococcus* is used as a photosynthetic microorganism in the cathode compartment, which plays a role in carbon dioxide (CO₂) sequestration. Various factors, such as CO₂ flow rate, nutrient augmentation, and light-dark cycles (16:8) (16 hours of light: 8 hours of darkness), were investigated for their impact on MCC performance. The results demonstrate that the high performance of MCC, presenting in a maximum output voltage, was obtained at a CO₂ flow rate of 1.5 L/h as optimal conditions with 539 mV compared to 469 and 407 mV at a flow rate of 3 L/h and 4.5 L/h, respectively. Increasing the CO₂ flow rate positively influenced power generation, with the highest power density of 22 mW/m² achieved at a flow rate of 4.5 L/h. Nutrient augmentation was critical in enhancing power density, leading to an impressive outcome of 43.87 mW/m². Enough illumination has been discovered as an important component in boosting higher voltage and power output. The study also looked at the development of microalgae growth in MCCs, employing absorbance to determine concentration. It was revealed that the kind of *Synechococcus* had considerable expansion in growth over time. This investigation provides helpful information about the factors affecting voltage output and biomass growth within MCCs. Optimizing carbon dioxide (CO₂) flow rates, nutrition supplementation, and exposure to light appear to be essential requirements for enhancing power generation performance and permitting a long-term capture of carbon in microbial carbon capture cells.

تأثير معدل تدفق ثاني أكسيد الكربون وزيادة المغذيات على توليد الكهرباء الحيوي داخل خلية احتجاز الكربون الميكروبي الضوئي

مريم نجم عبيد، نصير عبود عيسى

قسم الهندسة الكيميائية/ كلية الهندسة / جامعة النهرين / بغداد، العراق.

الخلاصة

تركز هذه الدراسة على التحقيق في إمكانيات خلايا احتجاز الكربون الميكروبية (MCCs) كحل واعد لكل من توليد الطاقة واحتجاز الكربون. تتكون MCCs من أسطوانتين من الخلايا الزجاجية التي تعمل كحجرات الأنود والكاثود (كلاهما يستخدم أقطاب شبكية بلاتينية) ويفصلها غشاء تبادل أيوني (AMI-7001). في حجرة الكاثود، يتم استخدام *Synechococcus* ككائنات دقيقة ضوئية تلعب دوراً في عزل ثاني أكسيد الكربون. تم فحص عوامل مختلفة، مثل معدل تدفق ثاني أكسيد الكربون، وزيادة المغذيات، ودورات الضوء والظلام (١٦:٨)، لتأثيرها على أداء (MCC). وتبين النتائج أن الأداء العالي لخلية احتجاز الكربون الميكروبية، الذي يظهر في جهد إخراج أقصى، الذي تم الحصول عليه بمعدل تدفق لثاني أكسيد الكربون قدره ١,٥ لتر/ساعة كظروف مثلى مع ٥٣٩ مللي فولت مقارنة مع ٤٦٩ و ٤٠٧ مللي فولت بمعدل تدفق ٣ لتر/ساعة و ٤,٥ لتر/ساعة. و وجد أن زيادة معدل تدفق ثاني أكسيد الكربون تؤثر بشكل إيجابي على توليد الطاقة، مع تحقيق أعلى كثافة طاقة ٢٢ ملي واط/متر مربع بمعدل تدفق يبلغ ٤,٥ لتر/ساعة. لعب زيادة المغذيات دوراً مهماً في تعزيز كثافة الطاقة، مما أدى إلى نتيجة رائعة بلغت ٤٣,٨٧ ملي واط/متر مربع. تم الاعتراف بوجود ضوء كاف كعنصر محوري في تحفيز زيادة الجهد وتوليد الطاقة. علاوة على ذلك، بحثت الدراسة في نمو الكتلة الحيوية للطحالب الدقيقة في MCCs، باستخدام الامتصاص كمؤشر للتركيز. والجدير بالذكر أن الأنواع *Synechococcus* أظهرت نمواً كبيراً في الكتلة الحيوية بمرور الوقت. بشكل عام، تقدم هذه الدراسة رؤية قيمة حول العوامل التي تؤثر على إنتاج الجهد ونمو الكتلة الحيوية في (MCCs). يبدو أن تحسين معدلات تدفق ثاني أكسيد الكربون، والمكملات الغذائية، والتعرض للضوء تعتبر عوامل حاسمة لتعزيز أداء توليد الطاقة ودعم احتجاز الكربون المستدام في خلايا احتجاز الكربون الميكروبية.

الكلمات الدالة: عزل ثاني أكسيد الكربون، البكتيريا الزرقاء، غاز المداخن، احتجاز الكربون الميكروبي، توليد الطاقة.

1. INTRODUCTION

The increase in atmospheric carbon dioxide (CO₂) concentration is mainly due to burning fossil fuels such as petroleum, coal, and natural gases, which results in global warming and climate change [1]. To avoid environmental problems due to CO₂ emission, CO₂ sequestration technologies, especially for energy utilization and conversion, are widely and urgently needed [2]. Microorganisms are microalgae and cyanobacteria that grow through the fixation and consumption of CO₂, also the most efficient in converting CO₂ into oxygen and biomass products. Microalgal CO₂ removal is affected by various factors, including microalgae species, culture system type, nutrition ratio, light intensity, temperature, pH, CO₂ gas flow rate, and CO₂ concentration [3]. Recently, photosynthetic MCC, which combines CO₂ fixation through photosynthetic microorganisms with a bio-electrochemical system, has been viewed as a promising and environmentally friendly approach for CO₂ sequestration and energy production [4]. Microbial carbon capture cell (MCC) is a budding technology that sequesters carbon using photosynthetic microorganisms and can recover electrical energy during wastewater treatment. In an MCC, the CO₂ generated during oxidative degradation of organic matter using anaerobic electrogenic microorganisms in the anodic chamber of MCC can be simultaneously used by photosynthetic microorganisms for electricity generation, CO₂ sequestration, and biomass synthesis in the cathodic chamber [2]. MCCs' effectiveness is substantially impacted by two crucial development parameters: carbon and nitrogen. A notable demonstration of this was given by Chen et al. [5], cultivating the microalga *Chlorella Zofingiensis* using

different glucose concentrations as the principal carbon source. Within the glucose range of 20 to 50 g/l, the microalga showed an ideal growth rate of 0.031 h⁻¹. In 2016, Hu et al. [6] studied the impacts of the intensity of light on the efficiency of an air-lift-type microbial carbon capture cell with an algae-assisted cathode. They grew *Chlorella vulgaris* under various light intensities (2.4, 5.0, 8.9, and 11.4 W/m²) and discovered that the air-lift-type microbial carbon capture cell is light-sensitive. The result found that the highest electricity outputs (972.5 mW/m³) and CO₂ fixing rate (887.8 mg l⁻¹ d⁻¹) were demonstrated under a light intensity of 8.9 W/m². In 2012, Zhao et al. [7] created new MCCs by incorporating immobilized microalgae (*Chlorella vulgaris*) into the cathode compartment to remove negligible carbon dioxide. This operation can remove 84.8 % COD while achieving the highest power density of 2485.35 mW m³ than with suspended *C. Vulgaris*, which are 88 % and 57.7 % greater, respectively. Pandit et al. [8] developed MCC using *Anabaena* sp. with CO₂-air mixture supply and generated a power density of 57.8 mW/m². The present study used two cylindrical plexiglass chambers to grow microbial carbon capture cells. *Synechococcus* sp. PCC 6803 was grown in a cathode chamber to decrease CO₂ emission from the carbon dioxide cylinder and generate electricity. The cathode chamber has been subjected to various CO₂ flow rates to identify the ideal growth conditions. Furthermore, crucial parameters such as CO₂ flow rate, food inputs, and photoperiods of 16 hours of light and 8 hours of darkness were tested to see how they affected the effectiveness of microbial carbon capture cells in power generation. The

goal was to discover the best circumstances for maximum power generation.

2. MATERIALS AND EXPERIMENTAL PROCEDURE

2.1. Microbial Species, Growth Medium, and Cultivation Environment

2.1.1. Anolyte Preparation and Inoculation for Anodic Process

The anolyte solution used throughout the experimental phase was generated using a nutritious broth composition. The composition per liter of this solution consisted of 1.5 grams of (beef extract and yeast extract) and 5.0 grams of (sodium and peptone). To achieve ideal conditions, the pH level of the solution was precisely set to 7.2.

The incubation process was carried out under controlled conditions, maintaining the temperature from 20 °C to 42 °C. A Shaker Incubator laboratory operating at a speed of 150 revolutions per minute was utilized to facilitate thorough mixing while deliberately avoiding any introduction of aeration.

2.1.2. Catholyte Preparation and Inoculation for Cathodic Process

The *Synechococcus* strain was grown in an illuminated Erlenmeyer flask that was autoclaved. After that, *Synechococcus* cells were placed in 1000 mL Erlenmeyer flasks with 500 mL of blue-green BG11 medium and kept at a temperature range of 20 °C to 35 °C. The composition of the medium utilized to stimulate *Synechococcus* growth in the cathodic compartment consisted of the compounds outlined in Table 1.

Table 1 Formulation of BG11 Medium.

Compounds	Amount(g/liter)
K ₂ HPO ₄	4.0
MgSO ₄ ·7H ₂ O	7.5
CaCl ₂ ·2H ₂ O	3.6
citric acid	0.6
ferric ammonium citrate	0.6
Na ₂ MG EDTA	0.01
Na ₂ CO ₃	1.5
trace metal mix A5	1.0 ml/l

The medium's pH level was 7.1, and the growth of *Synechococcus* was over the 14 days. During the growth phase, the cells were subjected to a light regimen encompassing two 16-hour periods of illumination followed by 8 hours of darkness, thereby enabling the cells to acclimate to the light-dark cycle.

2.2. Configuration of Microbial Carbon Capture Cell

A bioreactor of two cylindrical plexiglass chambers with a diameter of 6 cm was employed for the experiment. The anode chamber, standing at a height of 15 cm and containing a working volume of 423.9 cm³, was positioned above the cathode chamber, which had a height of 20 cm and a working volume of 565.2 cm³ (as depicted in Fig. 1). An ion exchange membrane (AMI-7001) was utilized to segregate the chambers. Illumination of the cathode chamber was achieved through

artificial light sources of 53 lux. The working electrodes, made of platinum, were positioned at a fixed distance of 3 cm from the chamber walls. External resistance was connected using copper wires, thereby completing the circuit. To create an anaerobic environment, stainless-steel screws were used to secure the two compartments. A porous sparger was installed in the cathode compartment's base to facilitate gas exchange. This sparger received gas from the gas mixer, supplied by a carbon dioxide cylinder, thereby promoting the desired gas flow. In the operation of the microbial carbon capture, both open-closed-circuit modes were employed, as shown in Fig. 2. The open-circuit mode allowed the MCCs to achieve their peak voltage potential, while in the closed-circuit mode, an external resistance of 1000 ohms was introduced into the circuit. The detailed specifications of the MCC configuration are provided in Table 2.

Table 2 Details the Two-Chambered MCC's Design Requirements and Operational Requirements.

Microorganism	Anode Chamber	Cathode Chamber
Species	<i>Escherichia coli</i>	<i>Synechococcus</i>
Growth conditions	(20°C to 42°C), 150 rpm, Anaerobic	(20°C to 35°C), mixing, aeration
MCC reactor	Anode chamber	Cathode chamber
Dimensions (L×D)	15cm×6cm	20cm×6cm
Working volume	423.9 cm ³	565.2 cm ³
Operating mode	Anaerobic	Aerobic
Gas, flow rate	None	1–5 l/hr CO ₂ flow rate
Initial pH	7.2	7.1
Electrodes Material	platinum mesh electrode	platinum mesh electrode
Projected surface area	39.76 cm ²	565.2 cm ³
Membrane		
Type	(AMI-7001)	
Structure	Gel polystyrene crosslinked with divinylbenzene	
Color	Beige	
Standard Thickness (mm)	0.45	

2.3. Operation of Microbial Carbon Capture Cells and Experimental Variations

In the experimental realm, the anode chamber underwent a meticulous sealing process, enclosing a nutrient broth of 420 cm³ that hosted a cultured *Escherichia coli* population. In contrast, the cathode chamber was filled with an autoclaved BG11 medium inoculated with *Synechococcus* sp. The operation of the MCCs encompassed two distinct modes: open-circuit, allowing the cells to attain their maximum voltage and closed-circuit mode, facilitated by a 1000-ohm resistance. A pH meter was employed to carefully monitor and regulate the system's pH levels, ensuring optimal environmental conditions.

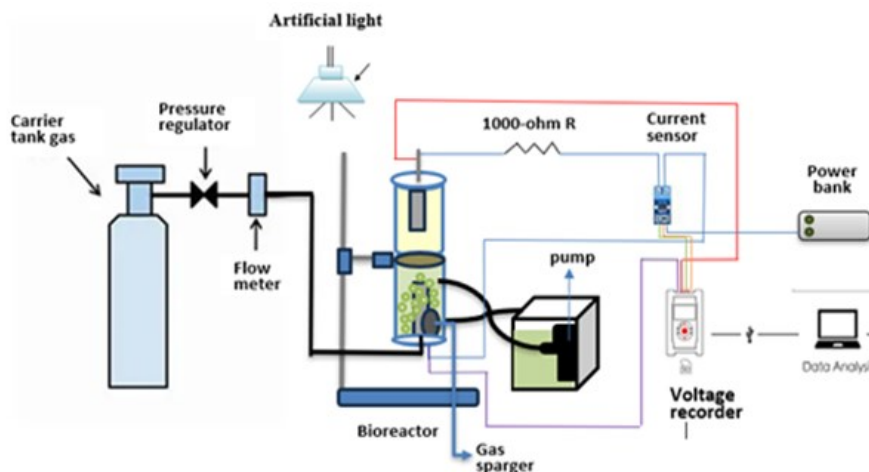


Fig.1 Microbial Carbon Capture Cells (MCCs) Equipment Schematic Design.



Fig. 2 Microbial Carbon Capture Cell while Operating Both in an Open-Closed Circuit.

2.4. Evaluation and Procedure

The voltage disparity between the anode and cathode, about the reference electrode, was meticulously gauged through an MCR-4V Voltage Data Logger. This logger was seamlessly linked to a personal computer via MCR for Windows and T&D graphs software. Regular voltage readings of the Microbial Carbon Capture Cells were conducted daily.

The acquisition of polarization curves was achieved by systematically altering the external resistance used in the closed-circuit setup was load resistance. Corresponding voltage drops were then precisely measured. To determine the current, an ACS712 current sensor module was utilized. This entailed calculating the current based on the nature of the input current (AC or DC) and measuring the resultant output voltage. The current amount was ascertained using the following relationship: $\text{Current} = ((\text{Voltage} - \text{Voltage offset}) / \text{mV per Amp})$ [9]. The power density (P) was measured by utilizing the following equation:

$$P = (V \times I) / A_{\text{Electrode}} \quad (1)$$

where P is power density (W), I is Current (A), and V is Voltage (V). The growth of

cyanobacteria was measured using a UV-visible spectrophotometer (S1205, UNICO, USA) set at 600 nm (A600). This wavelength was used to determine the sample's optical density (OD).

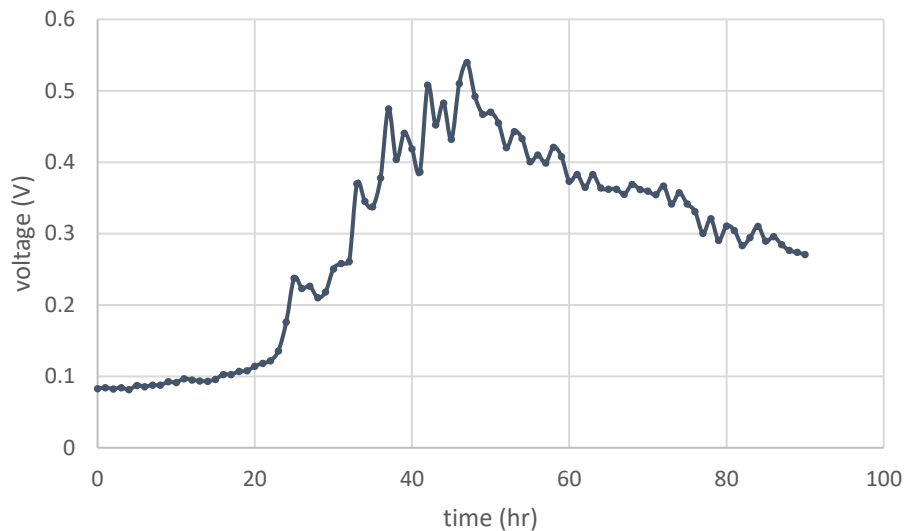
3. RESULTS AND DISCUSSION

3.1. Effects of CO₂ flow rates on the Output Voltage

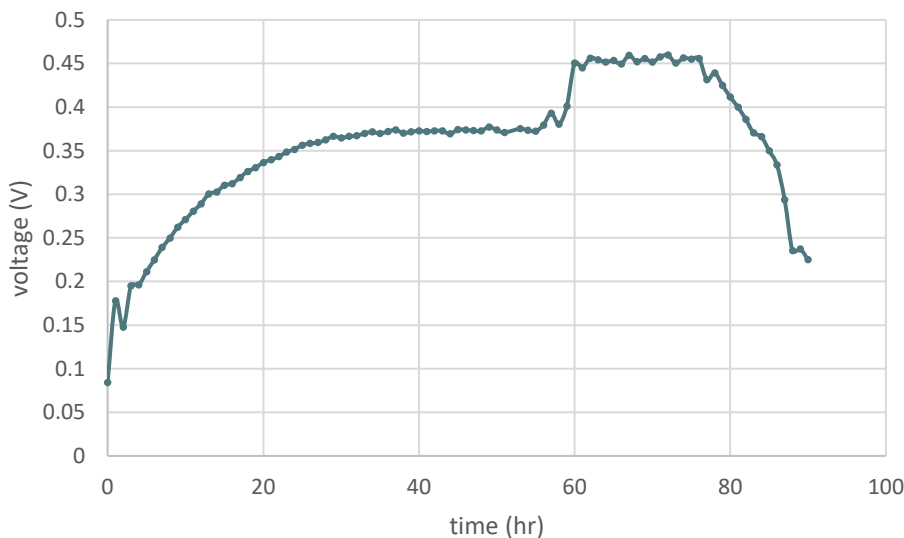
This investigation explains the influence of diverse CO₂ flow rates on the output voltage in open-circuit conditions. The cathode chamber was consistently aerated with a continuous flow of carbon dioxide, serving as the carbon source for the growth of *cyanobacteria*. The research investigations were carried out in batch mode, and the potential of the cell was studied under three different flow rate circumstances. In Figure 3 (a), when operating at a flow rate of 1.5 L/h, it was noticed that the potential gradually developed within the initial 24 hours following startup. Subsequently, the potential continued to rise progressively and ultimately reached its peak value of 539 mV. However, as the flow rate was increased to 3 L/h and 4.5 L/h, the output voltage decreased to 469 mV and 407 mV, respectively, as shown in Figs. 3 (b) and (c). The decrease in output

voltage with increasing CO₂ flow rate may be due to multiple factors, including microalgae activity, pH changes, temperature effects, and high oxygen production. In the absence of CO₂ flow rate or concentration sparging in the catholyte, pH gradually increased, moving towards alkalinity and causing pH splitting, finally decreasing the total open circuit potential (OCP). This phenomenon might be explained by *Anabaena* removing carbonate/bicarbonate from the catholyte [8]. The supplementing of single anodic off-gas to the cathode was insufficient to promote microalgae growth. However, a significant improvement in the performance of the Microbial Carbon Capture (MCC) system was achieved by combining internal anodic CO₂ with external CO₂ pumping at the cathode. This combined approach demonstrated a more

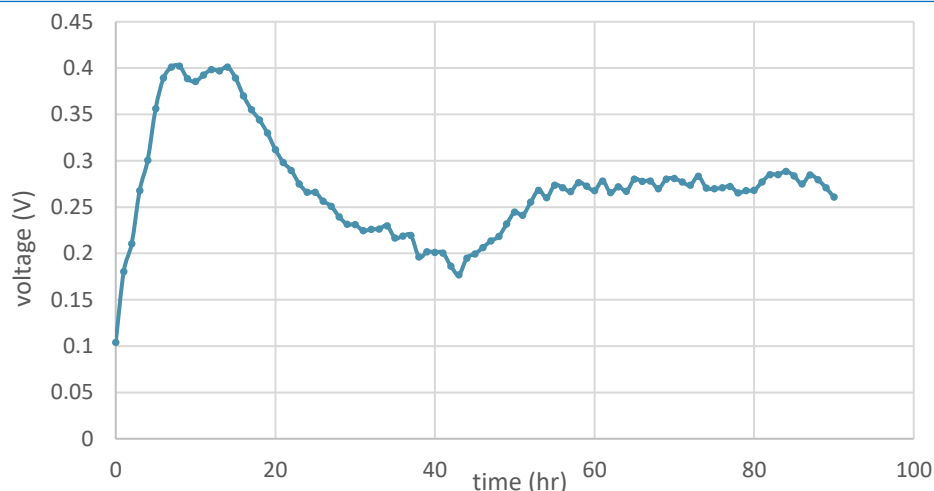
effective and conducive environment for microalgae growth [10]. Nevertheless, an extremely high concentration of CO₂ can lead to a decrease in pH, subsequently reducing cell growth activity [11]. The continuous introduction of a CO₂-air mixture into the catholyte containing *Anabaena* enabled the maintenance of the cathodic pH at approximately 7. The dissolved CO₂ in the catholyte exhibited buffering properties, contributing to stabilizing pH levels [8]. Consequently, the pH imbalance between the anode and cathode chamber was prevented. The cell concentration gradually increased, consequently, the dissolved oxygen in the catholyte increased. CO₂ dissolves rapidly in alkaline circumstances, facilitating biomass formation [2].



(a) 2% CO₂ Concentration with a Flow Rate of 1.5 l/h.



(b) 2% CO₂ Concentration with a Flow Rate of 3 l/h.



(c) 2% CO₂ Concentration with a Flow Rate of 4.5 l/h.

Fig. 3 The Open Circuit Voltages of Microbial Carbon Fuel Cells (MCCs) under 2% CO₂ Concentration Sparging in the Catholyte at Various CO₂ Flow Rates of 1.5 L/h, 3 L/h, and 4.5 L/h.

3.2. Effect of Different CO₂ Flow Rates on Power Generation

The results obtained in these experiments suggest that the highest power generation performance about power density (22 mW/m²) was achieved when the catholyte had a CO₂ flow rate of 4.5 L/hr, compared to the flow rates of 1.5 L/hr and 3 L/hr. This indicates that increasing the CO₂ flow rate within the studied range improved power generation performance, as shown in (Figs. 4 (a)–(c)). The increase in power output can be attributed to the combined impact of elevated dissolved O₂ concentrations caused by *Anabaena* bio photolysis, the buffering effect of CO₂/bicarbonate providing improved proton compensation via the continuous flow of CO₂-air mixture, and the reduction of bicarbonate to cell biomass at the cathode [8]. The continuous CO₂ addition into the catholyte resulted in an enhancement in power generation. This outcome can be attributed to the buffering action provided by the added CO₂ [12]. Power generation was impacted by CO₂ dissolving, which resulted in the development of carbonate/bicarbonate and/or by experiencing CO₂ and N₂ reduction in the cathode chamber during cell biomass synthesis. Given that O₂ is the principal electron acceptor, the saturation of oxygen levels, helped by passive aeration from *Synechococcus* photosynthetic activity at the cathode, most likely led to increased power output. Varanasi et al. (2020) demonstrated that supplementing the cathodic chamber with an external CO₂ supply resulted in improved removal of Chemical Oxygen Demand (COD), increased biomass growth, and enhanced power generation in the Microbial Carbon Capture (MCC) system [12]. Nonetheless, carbon dioxide released in the anode chamber during organic substrate degradation poses no substantial obstacles to power generation. This

is because algae in the cathode chamber constantly lower the carbon dioxide content. Microbial Carbon Capture Cells (MCCs) had the maximum power density of 5.6 W/m³ when carbon dioxide was transferred from the anode chamber to the cathode chamber, where algae consumed it. However, when pure CO₂ was introduced into the cathode, the voltage rise was only 60 mV, which was attributed to a pH change in the solution [2].

3.3. Nutrient Effect

The impact of nutrients was examined by augmenting the quantity of BG11 medium in the bioreactor. In this experiment, the cathode chamber was connected to a tank containing 2.4 liters of medium, with a portion allocated for cyanobacterial growth and the rest to demonstrate its influence on power generation. This factor yielded the most favorable outcome for the microbial carbon capture cell, reaching an impressive value of 43.87 mW/m². Nitrate addition to catholyte in MCC has already been shown to increase power generation [14] (Fig. 5). Nam et al. [13] demonstrated that electricity generation increased from 1884 to 2981 mW/m³ as the substrate loading rate was raised from 1.9 to 3.8 g L⁻¹ d⁻¹. The enhancement of power generation in MFCs by adding nitrate to the catholyte has already been demonstrated, lending validity to our findings. The work by Pandit et al. [8] indicated that substituting the BG11 medium in the cathode compartment with the BG11₀ medium, together with constant sparging of a CO₂-air combination, resulted in a significant increase in the MCC's maximum sustained power density. This enhancement increased the value from 52.8 mW/m² to an astounding 76 mW/m². The influence of nutrition supplementation on power production and electrical current is depicted in Fig. 5.

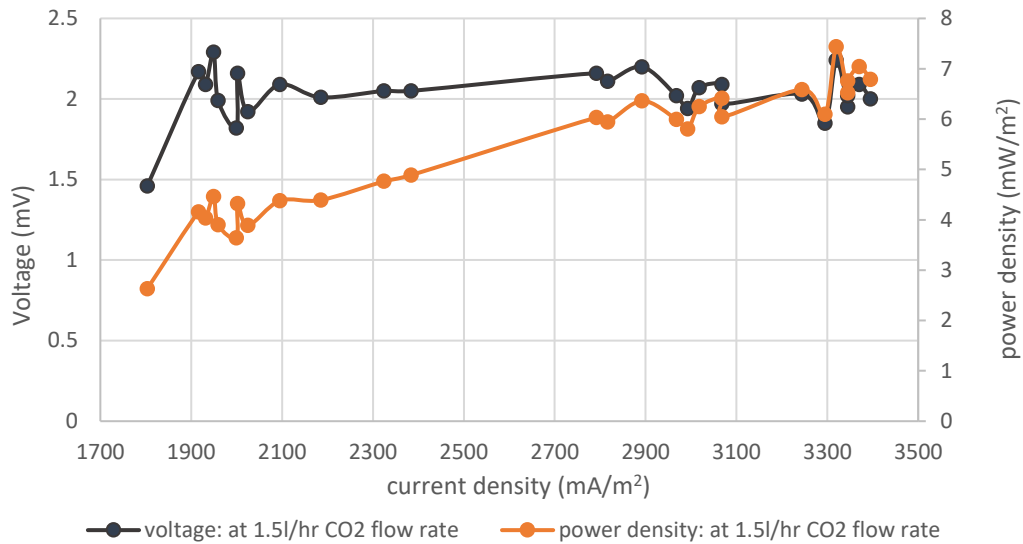
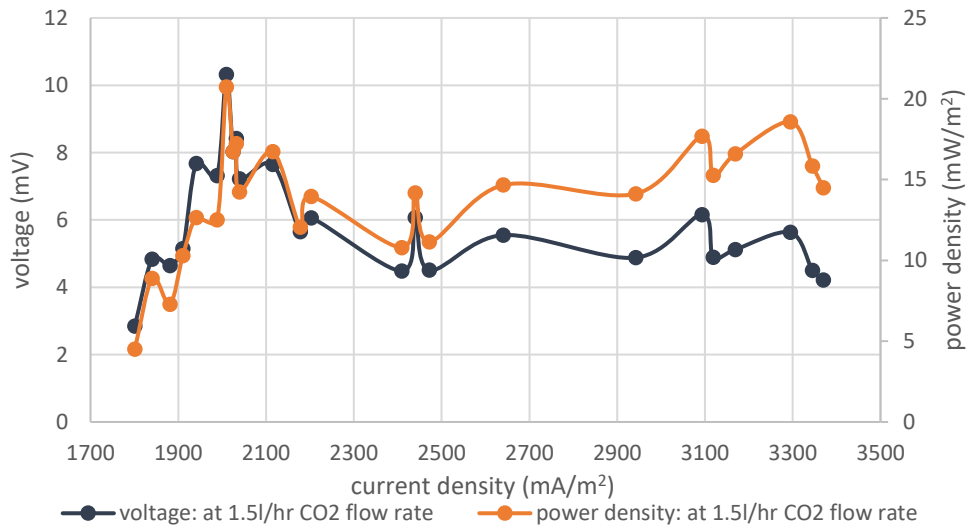
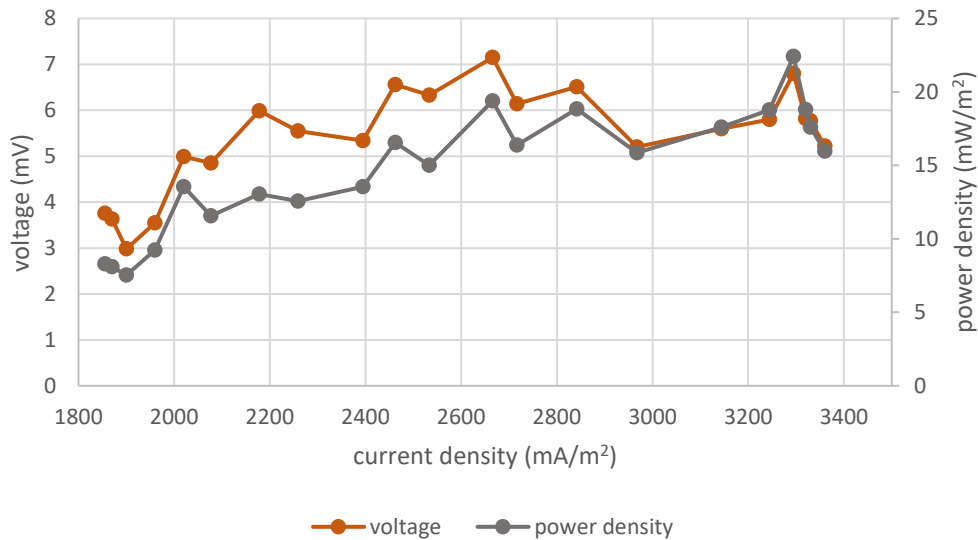
(a) 5% CO₂ Concentration with a Flow Rate of 1.5 l/h.(b) 5% CO₂ Concentration with a Flow Rate of 3 l/h.(c) 5% CO₂ Concentration with a Flow Rate of 4.5 l/h.

Fig. 4 Polarization and Energy Production in Microbial Carbon Fuel Cells (MCCs) at 5% CO₂ Concentration and 1000 Ω when Sparging in Catholyte at Various CO₂ Flow Rates: a- 1.5 L/h, b- 3 L/h, and c- 4.5 L/h.

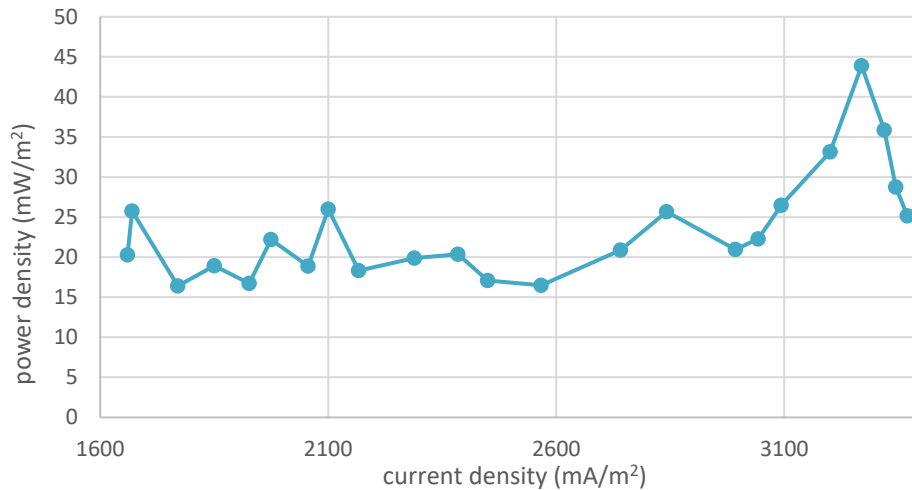


Fig. 5 Microbial Carbon Capture Cells Generate Power Utilizing a Large Volume of BG11 Medium at a CO_2 Flow Rate of 1.5 L/hr.

3.4. Effect of Light

3.4.1. Power Generation

The influence of illumination on Microbial Carbon Fuel Cell performance was investigated. When subjected to a lighting cycle comprising 16 hours of light followed by 8 hours of darkness, notable enhancement in power generation during the illuminated periods, contrasted by a decline during the dark intervals. Fig. 6 highlights a significant peak in power density, reaching 1.559 mW/m^2 , which occurred at the midpoint of the light phase, underscoring the impact of light on the system's performance. Likewise, several studies have documented improved power generation outcomes under the influence of light. For instance, Liu and Cheng [14] reported a significantly higher power density of 187 mW/m^2 under illuminated conditions compared to only 21 mW/m^2 when operating in the absence of light. Furthermore, González Del Campo et al. [15] demonstrated that continuous mode operation produces more electricity in comparison to sequencing-batch mode operation.

3.4.2. MCC Performance

The impact of a 16-hour light and 8-hour dark cycle on voltage generation in a microbial carbon capture cell was investigated. The results revealed interesting findings regarding the relationship between light exposure, bicarbonate utilization, and voltage generation. During the light phase, when the cathode was exposed to light, voltage generation consistently increased over time. This observation suggests that light availability plays a crucial role in promoting higher-voltage generation. Conversely, in the dark phase, the cell voltage gradually decreased from the 16th hour until the end of the day. This decline in voltage over time during the dark period highlights the importance of light as an energy source for sustaining voltage generation in microbial carbon capture cells. As demonstrated in (Fig. 7), the voltage restored its previous value. Several studies have found that photoperiod affects both microalgae's growth and chemical composition [10].

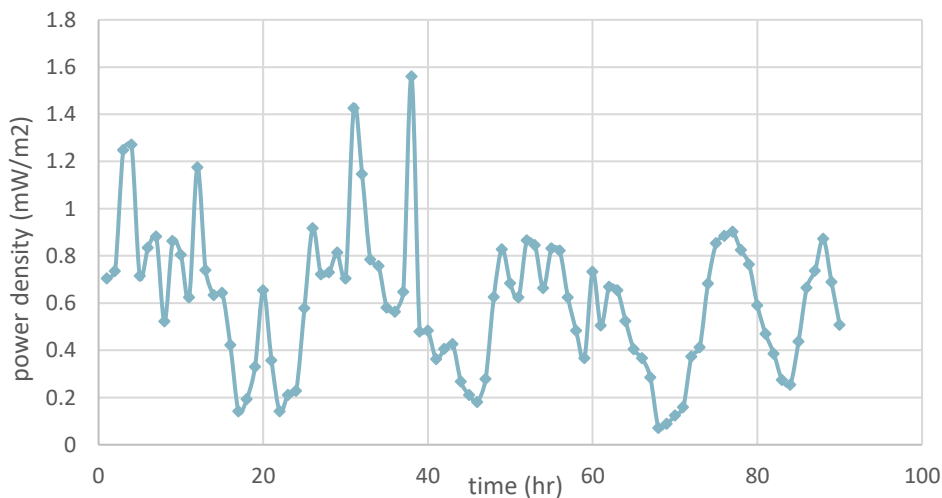


Fig. 6 The Performance of MCCs Influenced by Illumination under 8 Hours of Darkness Followed by 16 Hours of Light During Each Day for Electricity Generation at under 1000 Ω .

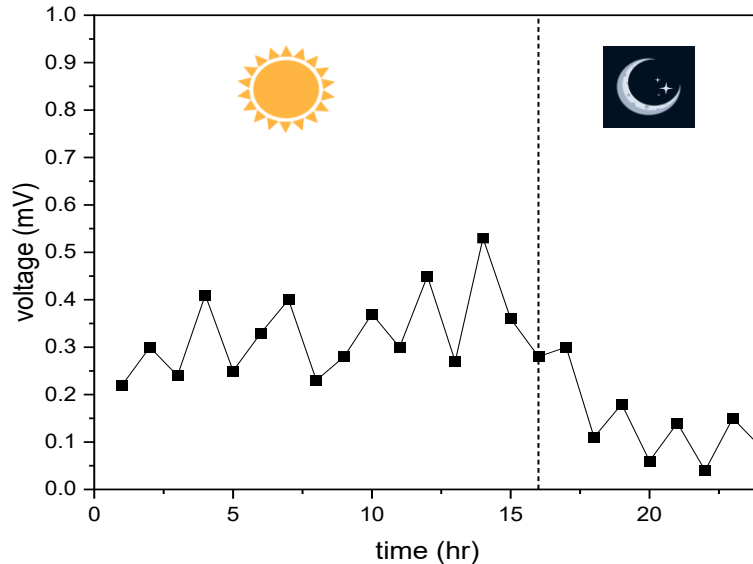


Fig. 7 Cell Voltage's Behavior During Cycles of Light and Darkness in a Closed-Circuit Mode at a CO₂ Flow Rate of 1.5 L/hr.

3.5. Growth Performance

Figure 8 displays the development of microalgae biomass over time in a microbial carbon capture system, as determined by microalgae culture absorbance. The absorbance value may be utilized to predict cyanobacteria cells' concentration and overall

health at 16/8 hours (16 hours of light and 8 hours of darkness) and 2% CO₂ concentration. Initially, the absorbance measurements were insignificant, suggesting that there was little concentration of microalgae in the medium. *Synechococcus* biomass peaked at 38 hours after the procedure began.

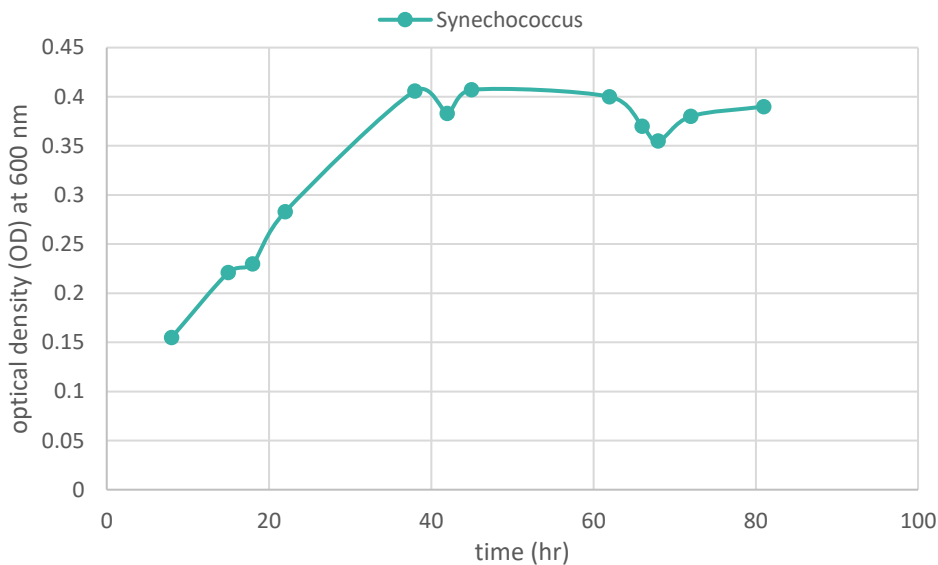


Fig. 8 Growth Measurement Regarding the Optical Density of Cyanobacteria (*Synechococcus*) Grown in BG11 Medium.

4. CONCLUSION

- 1- Carbon dioxide flow rates impacted microbial carbon capture cells' (MCC) performance at various concentrations. The open-circuit output voltage gradually increased at a flow rate of 1.5 L/h.
- 2- The generation of electricity in the MCC was impacted by CO₂ flow rates (1.5, 3, and 4.5 L/h) and concentration levels. The highest power density was attained with a 5% CO₂ concentration and a flow rate of 4.5 L/hr. As a result,

increasing the CO₂ concentration considerably improves power generation. The trials also indicated that increasing the flow rate at varied concentrations significantly increased power production.

- 3- The influence of the presence of nutrients on MCC efficiency was investigated by increasing the amount of BG11 medium, resulting in a substantial increase in power generation. Additionally, a significant increase in voltage production and

power densities with increasing illumination period was observed. Photoperiods of 16/8 hours (16 hours of light: 8 hours of darkness) become most suitable for the cells.

- 4- The growth of microalgae concentrations, particularly *Synechococcus* species, significantly increases.

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