

Enhanced biomass production and bioelectricity generation in microalgae culture medium using electrode pairings

Amina Lami Mohammed^{1,2}, *Uganeeswary* Suparmaniam^{1,2}, *Man Kee* Lam^{1,2*}, *Jun Wei* Lim^{2,3}, *Peck Loo* Kiew⁴, *Inn Shi* Tan⁵, *Bridgid Lai Fui* Chin⁵, and *Sie Yon* Lau⁵

¹Chemical Engineering Department, Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak, Malaysia

²HICoE-Centre for Biofuel and Biochemical Research (CBBR), Institute of Sustainable Energy, Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak, Malaysia

³Fundamental and Applied Sciences Department, Universiti Teknologi PETRONAS, 32610, Seri Iskandar, Perak, Malaysia

⁴Department of Chemical and Environmental Engineering, Malaysia - Japan International Institute of Technology, Universiti Teknologi Malaysia, Jalan Sultan Yahya Petra, 54100, Kuala Lumpur, Malaysia

⁵Department of Chemical and Energy Engineering, Faculty of Engineering and Science, Curtin University Malaysia, CDT 250, 98009, Miri, Sarawak, Malaysia

Abstract. Microalgae-based biofuel production offers promising avenues for sustainable energy generation. However, optimizing microalgae culture conditions for enhanced biomass production and bioelectricity generation remains challenging. This study addresses this problem by investigating the efficacy of different electrode pairings immersed in a microalgae culture medium. Two electrode pairs, Al-Fe and Cu-Zn were evaluated for their impact on microalgae growth and bioelectricity production. The optimum biomass concentration obtained was 1.204 g/L of Al-Fe electrodes with a lipid content of 15.1% and bioelectricity generation of 0.36V. The results demonstrated that Al-Fe electrodes induced high biomass concentration and lipid content in microalgae culture, facilitating biodiesel production. Conversely, Cu paired with Zn electrode promoted microalgae growth with elevated bioelectricity generation with 0.95V. This research sheds light on the potential of tailored electrode pairings to optimize microalgae culture conditions for dual-purpose biofuels and bioelectricity production, contributing to the advancement of sustainable energy technologies. It was recommended, among others, that further exploration of diverse electrode pairings within microalgae cultured medium could unveil additional effective strategies for optimizing biomass production and bioelectricity generation.

*Corresponding author: lam.mankee@utp.edu.my

1 Introduction

Microalgae have garnered significant attention as a sustainable and renewable source for biofuel and bioenergy production [1]. Their rapid growth rates, high lipid content, and ability to thrive in various environmental situations makes them an attractive alternative to traditional energy sources [2]. As concerns about climate change and the exhaustion of fossil fuel reserves intensify, exploring microalgae-based biofuel and bioenergy technologies becomes increasingly imperative [3]. However, despite extensive research, optimizing microalgae culture conditions to achieve maximal biomass production and bioelectricity generation remains complex and challenging [4]. The intricate interplay of various factors in studying the system environment, including nutrient availability, light intensity, pH levels, and temperature control, underscores the multifaceted nature of microalgae cultivation [5]. Amidst the growing pursuit of microalgae-based biofuel production, a notable research gap exists concerning the role of electrode pairings in modifying microalgae culture dynamics and bioenergy production efficiency [6]. Despite significant efforts to optimize traditional cultivation factors such as nutrient composition and light exposure, the effects of electrode materials and configurations on microalgae growth and bioelectricity generation have been largely neglected [7].

This knowledge gap holds back the comprehensive understanding of microalgae culture systems. It limits the development of approaches to maximize their bioenergy production potential. Addressing this gap is crucial for expanding the area and unlocking the full promise of microalgae-based biofuel and bioenergy technologies. The existing literature on microbial fuel cell systems underscores the significance of understanding the interaction between microalgae and electrodes in electrochemical systems [8]. Few studies have systematically explored how electrode pairing enhanced microalgae's biomass production and bioelectricity generation. This research gap is key, as optimized electrode configurations could improve microalgae culture conditions and boost bioenergy production efficiency. This study aims to fill this gap by investigating the impact of electrode materials and placements on microalgae growth and bioelectricity output, contributing to sustainable biofuel technologies

2 Methodology

2.1 Microalgae cultivation enriched with compost

A high-lipid *Chlorella vulgaris* strain was cultured in an optimized nutrient medium with electrodes integrated for direct interaction with the microalgae. A fertilizer solution was prepared by mixing 10 g of local granular chicken compost with 600 mL of water, stirred for 24 hours, and filtered. 400 mL of this solution was added to a photobioreactor containing 5 liters of tap water, with the pH adjusted to 3-3.5. Subsequently, 400 mL of *C. vulgaris* seed culture was introduced. The system was continuously aerated and illuminated with cool-white, fluorescent light at 60–70 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

2.2 Experimental setup

2.2.1 Microalgae cultivation and electricity production

The experimental setup involved multiple replicates in a photobioreactor for each electrode pairing to ensure robust statistical analysis and reproducibility. Cultures were maintained under controlled conditions to monitor cell density and bioenergy production. Each

photobioreactor contained 400 mL of liquid, 32 mL of *Chlorella vulgaris* seed culture, and 32 mL of nutrients from chicken compost. The culture, maintained at a specific pH, was aerated at 0.4 L/min with compressed air for CO₂ supply and illuminated with cool-white, fluorescent light at 60-70 μmol m⁻² s⁻¹. pH was monitored every 2 days and microalgae growth was tracked using a UV-Vis spectrophotometer with an initial concentration of 0.502 g/L. Electricity production was measured daily using a multimeter. Aluminium, copper, and iron electrodes, selected for their EMF potential difference, were placed 4.0 cm apart, with copper as the cathode and aluminium as the anode. Voltage readings were recorded daily over 7 days, as illustrated in Figure 1.

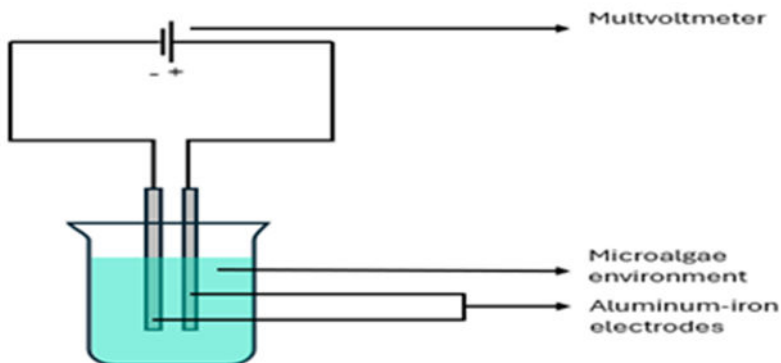


Fig. 1. Schematic diagram of the electrode-integrated photobioreactor system.

2.2.2 Harvesting and collection of microalgae biomass

During the 7-day cultivation period, once the microalgae got to the stationary phase of development, aeration in the cultured system was discontinued. The microalgae biomass was then let to naturally settle at the bottom of the photobioreactor for 2 days. This process resulted in the formation of two distinct layers: an upper layer of water containing suspended microalgae cells and a lower layer of concentrated microalgae biomass. The water from the upper layer was carefully decanted, leaving behind the microalgae biomass, which was subsequently dried in an oven at 80°C for 24 hours. The dried biomass was then collected, transferred to a sterile dried container, and stored for further biochemical analysis.

2.2.3 Extracting lipids from microalgae biomass

Lipid extraction from microalgae biomass with a modified Bligh and Dyer (1959) method by [9]. Specifically, 0.1 g of finely ground biomass was mixed with 15 mL of a 2:1 methanol-chloroform solution and stirred for 1 hour at 200 rpm. The mixture was then filtered, and the filtrate was air-dried for 24 hours in a fume hood, followed by oven-drying at 80°C for another 24 hours. The vial's weight before and after drying was measured to determine the lipid content percentage on a dry weight basis.

3 Results and discussions

3.1 Microalgae growth and electricity generation

The investigation into the efficacy of different electrode pairings immersed in microalgae cultures yielded compelling insights into their impact on biomass production and bioenergy generation, as shown in Figure 2. According to the figure, Al-Fe electrodes significantly improved biomass concentration. However, the Cu-Zn-treated biomass exhibited the lowest growth rate and final biomass concentration, suggesting that the Cu-Zn treatment inhibited microalgae growth more than the Al-Fe treatment. This finding concurs with an earlier study [10] that copper ions can impede microalgae growth by disrupting cellular processes and photosynthesis. Furthermore, the Al-Fe treatment supported microalgae growth better than the Cu-Zn treatment. However, it was not as effective as the control samples. The control condition showed a high growth rate of microalgae, indicating stable and optimal growth without any mineral disturbances from electrodes. This result aligned with the findings of [11], which also demonstrated that iron supplementation can enhance microalgae growth by serving as a cofactor for various enzymes involved in photosynthesis and nitrogen fixation.

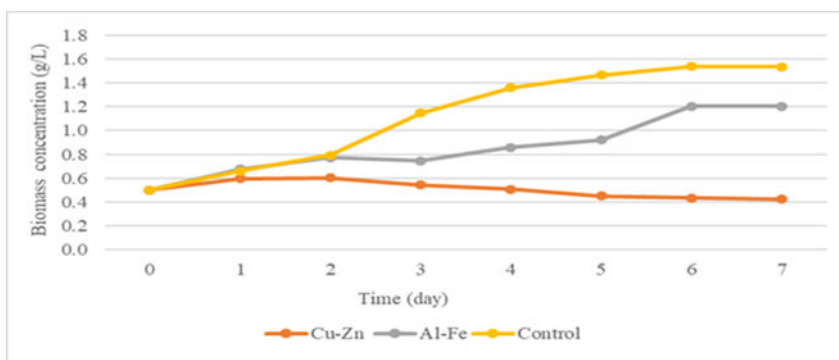


Fig. 2. Biomass concentration (g/L) obtained from different electrode pairing and control for 7 days of cultivation.

Electrochemical reactions in electrode-integrated systems boost microalgae growth by enhancing nutrient availability, stimulating metabolic activity, and optimizing pH and redox potential. This approach increases biomass yield and generates bioelectricity, offering dual benefits [12]. Although the pairing of Cu with Zn electrodes impeded microalgae growth, this pairing generated elevated bioelectricity due to the high conductivity of Cu-Zn electrodes, as depicted in Figure 3. This finding aligned with the electrochemical properties of copper, zinc, and graphite, as elucidated in a prior study [13].

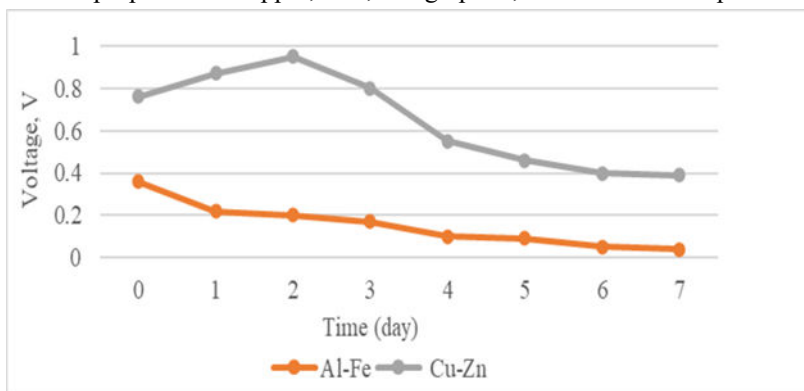


Fig. 3. Electricity profile of Al-Fe and Cu-Zn electrode.

The study examined the impact of copper-based electrodes in microbial fuel cells (MFCs) across different configurations, comprising single chamber (SC), double chamber (DC), and series of chambers (SC) setups, with various anode-cathode combinations such as Cu-Cu, Zn-Cu, and graphite-Cu. The highest efficiencies recorded were 0.936 V for Cu-Zn and 0.86 V for Cu-graphite, with the Cu-Cu combination reaching 0.50 V. These metals facilitated electron transfer processes in microbial fuel cells. The enhanced bioelectricity generation observed with Cu-Zn electrodes underscored their potential for integrating microalgae cultivation with bioelectricity production systems. Therefore, leveraging the inherent electrochemical properties of Cu and Zn, these electrode pairings offered a promising avenue for enhancing bioenergy generation alongside biomass production within microalgae cultures, addressing another critical objective of this study.

3.2 Lipid content

Figure 4 shows lipid content in microalgae biomass for different electrode pairings compared to the control. The Al-Fe electrode produced the highest lipid content, followed by the control and Cu-Zn. This could be ascribed to the stress effect induced by the Al-Fe electrodes, thereby enhancing lipid accumulation. These findings confirmed previous studies conducted by [14] and [15], which observed comparable lipid accumulation patterns when using an integrated digital microfluidic bioreactor for automated screening of microalgae growth and stress-induced lipid accumulation in cultivation systems. The capacity of Al-Fe electrodes to stimulate lipid biosynthesis in microalgae cultures carried significant implications for biodiesel production, considering that lipids served as a critical precursor for biofuel synthesis.

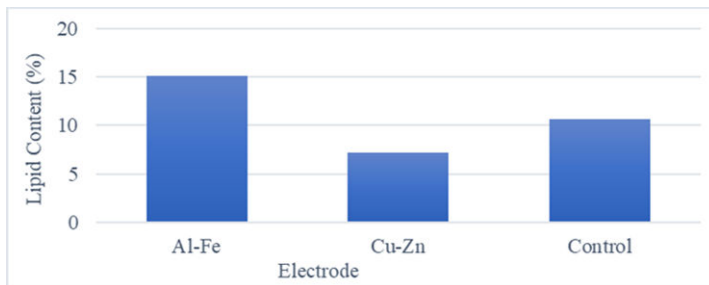


Fig. 4. Lipid content from the electrode and control.

Additionally, an appropriate electrode facilitated establishing an optimal pH level for microalgae growth, consequently promoting lipid accumulation. Earlier studies clarified a similar trend in which mineral stress from salt-induced osmotic pressure disparities within microalgae cells, triggering a stress response and compelling microalgae to adapt to novel environments, thereby changing their metabolism [16]. This phenomenon also impacted microalgae cells' oxidative stress and fatty acid metabolism mechanisms, ultimately augmenting lipid accumulation.

4 Conclusions and recommendations

In conclusion, the study demonstrated that electrode pairings can potentially optimize microalgae culture conditions for bioelectricity and lipid production. Al paired with Fe electrodes enhanced biomass concentration and lipid content. In contrast, Cu with Zn electrodes inhibited biomass growth while improving bioelectricity generation. These findings emphasize the promise of electrode pairings in enhancing bioenergy generation.

Based on the findings of this study, further research is recommended to explore a broader range of electrode pairings within microalgae cultures, varying different parameters such as pH, nutrient concentration, and salinity to optimize biomass production and bioelectricity generation. Valuable combinations can be identified by testing various materials, sizes, and configurations, enhancing stability and productivity, and contributing to more reliable and efficient biofuel and bioenergy production processes.

The authors gratefully acknowledge funding from the Joint Research Project between Curtin-UTP-Nottingham-UMT (Cost Centre: 015MD0-187), the Ministry of Higher Education (MOHE) Malaysia through the HICoE Grant to CBBR (Cost Centre: 015MA0-052), Yayasan Universiti Teknologi PETRONAS (Cost Centre: 015LC0-410), and the Petroleum Technology Development Fund (PTDF) Nigeria.

References

1. G. Ezhumalai, M. Arun, A. Manavalan, R. Rajkumar, K. Heese, A holistic approach to circular bioeconomy through the sustainable utilization of microalgal biomass for biofuel and other value-added products. *Microb. Ecol.* **87**, 1-61 (2024).
<https://doi.org/10.1007/s00248-024-02376-1>
2. K. Hinduja, R. Achar, K.T. Vadiraj, Microalgae as a source of sustainable energy resource for biofuels: A review. *Handb. Emerg. Mater. Sustain. Energy* 467-492 (2024).
<https://doi.org/10.1016/B978-0-323-96125-7.00002-2>
3. S. Sahu, P. Kunj, A. Kaur, M. Khatri, G. Singh, S. K. Arya, Catalytic strategies for algal-based carbon capture and renewable energy: A review on a sustainable approach. *Energy Convers. Manag.* **310**, 118467 (2024).
<https://doi.org/10.1016/j.enconman.2024.118467>
4. H. Li, X. Sun, Y. Sun, L. Ye, H. Xue, F. Gao, Y. Yang, Enhancing lipid production in *Chlorella* under successive stresses of periodic micro-current and salinity: Performance and contribution. *Chem. Eng. J.* **486**, 150409 (2024).
<https://doi.org/10.1016/j.cej.2024.150409>
5. M.Y. Zhang, X.R. Xu, R.P. Zhao, C. Huang, Y.D. Song, Z.T. Zhao, Y.B. Zhao, X.J. Ren, X.H. Zhao, Mechanism of enhanced microalgal biomass and lipid accumulation through symbiosis between a highly succinic acid-producing strain of *Escherichia coli* SUC and *Aurantiochytrium* sp. SW1. *Bioresour. Technol.* **394**, 130232 (2024).
<https://doi.org/10.1016/j.biortech.2023.130232>
6. R. Amaral, D. Duci, F.C. Cotta, F.L. Bacellar, S. Oliveira, F. Verret, K. Asadi, L.K.J. Vandamme, N.M. Reis, L.D. Bryant, D. Tosh, J.-L. Mouget, R. Perkins, P.R.F. Rocha, Ion-driven communication and acclimation strategies in microalgae. *Chem. Eng. J.* **473**, 144985 (2023). <https://doi.org/10.1016/j.cej.2023.144985>
7. J.C. Chin, F.L. Ng, H.S. Kang, B. Li, P.S. Goh, J.W. Lim, A.A. Ghfar, A.A.A. Mohammed, M.K. Shahid, Effect of hydrodynamic aeration on bioelectricity generation from photoautotrophic *Spirulina platensis* in biophotovoltaic devices. *Algal Res.* **77**, 103341 (2024). <https://doi.org/10.1016/j.algal.2023.103341>
8. X. Ning, R. Lin, J. Mao, C. Deng, L. Ding, R. O'Shea, D.M. Wall, J.D. Murphy, Improving the efficiency of anaerobic digestion and optimising in-situ CO₂ bioconversion through the enhanced local electric field at the microbe-electrode interface. *Energy Convers. Manag.* **304**, 118245 (2024).
<https://doi.org/10.1016/j.enconman.2024.118245>

9. Y.L. Kam, K.M. Lee, U. Suparmaniam, M.R. Shamsuddin, J.W. Lim, I.S. Tan, B.L.F. Chin, P.L. Show, M.K. Lam, Potential of using manure in microalgae cultivation for third generation of biofuel production. *Sustain. Mater. Technol.* 85-105 (2023). https://doi.org/10.1007/978-981-19-4120-7_4
10. Y. Jin, Y. Li, Y. Qi, Q. Wei, G. Yang, and X. Ma, A modified cultivation strategy to enhance biomass production and lipid accumulation of *Tetrademus obliquus* FACHB-14 with copper stress and light quality induction. *Bioresour. Technol.* **400**, 130677 (2024). <https://doi.org/10.1016/j.biortech.2024.130677>
11. S. Pandey, I. Narayanan, R. Vinayagam, R. Selvaraj, T. Varadavenkatesan, and A. Pugazhendhi, A review on the effect of blue-green 11 medium and its constituents on microalgal growth and lipid production. *J. Environ. Chem. Eng.* **11**, 109984 (2023). <https://doi.org/10.1016/j.jece.2023.109984>
12. D. Li, Y. Zhao, D. Wei, C. Tang, and T. Wei, Key issues to consider toward an efficient constructed wetland-microbial fuel cell: The idea and the reality. *Environ. Sci. Pollution Res.* **31**, 11559-11575 (2024). <https://doi.org/10.1007/s11356-024-31984-0>
13. Y.A. Kumar, G.R. Reddy, T. Ramachandran, D.K. Kulurumotlakatla, H.S.M. Abd-Rabboh, A.A.A. Hafez, S.S. Rao, S.W. Joo, Supercharging the future: MOF-2D MXenes supercapacitors for sustainable energy storage. *J. Energy Storage*, **80**, 110303 (2024). <https://doi.org/10.1016/j.est.2023.110303>
14. Y. Wang, H. Zhao, X. Liu, W. Lin, Y. Jiang, J. Li, Q. Zhang, G. Zheng, An integrated digital microfluidic bioreactor for fully automatic screening of microalgal growth and stress-induced lipid accumulation. *Biotechnol. Bioeng.* **118**, 294-304 (2020). <https://doi.org/10.1002/bit.27570>
15. C. Tsoi, Microfluidic droplet arrays for high-throughput screening of microalgae, Ph.D. thesis, Hong Kong Polytechnic University (2024)
16. Y. Zhao, Q. Li, D. Gu, L. Yu, and X. Yu, The synergistic effects of gamma-aminobutyric acid and salinity during the enhancement of microalgal lipid production in photobioreactors. *Energy Convers. Manag.* **267**, 115928 (2022). <https://doi.org/10.1016/j.enconman.2022.115928>