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Simultaneous degradation efficiency of C-MFCs for three pollutants in aquaculture wastewater

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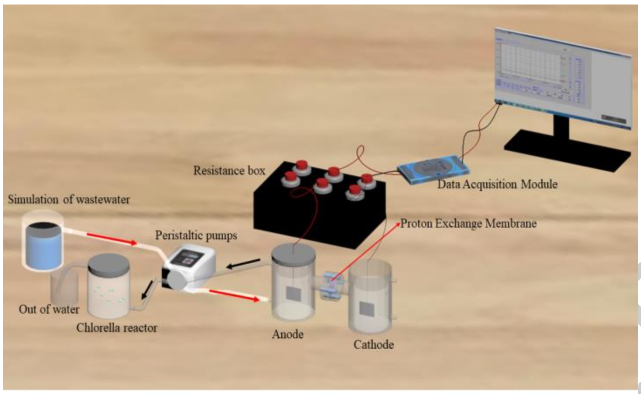
Graphical abstract



Abstract

Chlorella microbial fuel cells （C-MFCs） may be an alternative technology for wastewater treatment. C-MFCs were established using microalgae and activated sludge as raw materials, and the effects of different nitrogen and phosphorus ratios in the feed water on the electrochemical performance of C-MFCs and the removal of NH3-N, COD and TP were investigated. The results show that when N:P=3:1 and the external resistance is 1000 Ω, the removal rates of NH_3-N, COD and TP are 72.48±1.94%, 81.26±4.4% and 65.62%±2.14%, respectively, and the highest chlorophyll a content is 108.82 mg/L. The maximum voltage and maximum power 92.94 mV and 234.01 mW/m^2. The microbial community structure in the anode chamber was analyzed and the results are as follows: At N:P=3:1, the dominant bacteria at the genus level in the anode chamber were *Klebsiella* (32.12%) and *Prevotella* (14.45%). The number of OTUs in the anode chamber changes under different N:P conditions. It can be concluded that N:P affects the power generation capacity of C-MFCs and the removal of NH_3-N, COD and TP. Overall, when N:P=3:1, the C-MFCs had the best power production capacity and the removal of NH_3-N, COD and TP. Regulation of N:P in aquaculture wastewater is an effective way to improve performance of C-MFCs.

Keywords: C-MFCs, nitrogen-phosphorus ratio, aquaculture wastewater, chlorella, microbial community structure

1. Introduction

Nitrogen, phosphorus, and Chemical Oxygen Demand (COD) in aquaculture wastewater are high in content, which can accumulate in the soil, pollute groundwater, cause eutrophication in ordinary water bodies, affect plant growth, and affect human physical and mental health (Chen *et al.*, 2012; Ra *et al.*, 2021). Therefore, in recent years, the treatment of culture wastewater has become more and more common in society. Currently, there are various techniques, including physical, physical and chemical methods, and biological methods, but these methods still have some deficiencies and need to be improved.(Suzuki *et al.*, 2007; He *et al.*, 2014) For example, physical processes, which mainly include mechanical filtration and foam separation technologies, can only remove large particles of pollutants and are very ineffective in removing soluble organic pollutants. The chemical method mainly uses chemical oxidation to remove soluble organic pollutants in water, but the cost is high and it is easy to cause secondary environmental pollution.(Fei and Han, 2004) It is very effective to use algae and microorganisms to absorb and decompose organic pollutants in aquaculture wastewater (Zhao *et al.*, 2019). Common anaerobic and aerobic processes often require a large amount of space and equipment, and the operating costs are very high (Tang *et al.*, 2019). Therefore, several studies have been conducted on bioelectrochemical systems, mainly including microbial fuel cells (MFCs) and microbial electrolysis cells (MECs). MFCs makes up for the shortcomings of traditional biological treatment processes, and is an emerging innovative technology for sewage treatment and synchronous power generation (Liu and Yu, 2020; Raychaudhuri and Behera, 2022). In MFCs studies, domestic wastewater (Puig *et al.*, 2011; James *et al.*, 2020), industrial wastewater (Yu *et al.*, 2015; Abbasi *et al.*, 2016), and swine wastewater (Cheng *et al.*, 2021) were demonstrated for power generation and treatment.

Relevant studies have shown that MFCs has been successfully applied to remove ammonia nitrogen (NH_3-N), biochemical oxygen demand (COD) and total phosphorus (TP) in aquaculture effluent, indicating that MFCs has great potential in the treatment of aquaculture effluent (Estrada-Arriaga *et al.*, 2015; Cheng *et al.*, 2021). Although MFCs has a good effect on the removal of COD from laboratory environmental wastewater, the removal of NH_3-N and TP needs to be further improved (Estrada-Arriaga *et al.*, 2015; Zhu *et al.*, 2022). A large number of studies have shown that Chlorella has a good removal effect on NH_3-N and TP in wastewater (Xiong *et al.*, 2007; Alazaiza *et al.*, 2022; Lavrinovics *et al.*, 2022). Chlorella can absorb and use organic and inorganic nitrogen compounds in water, and use CO_2 and phosphate as carbon sources for photo-energetic autotrophic growth. At the same time, photosynthesis by Chlorella raises the pH of the water body, and high pH has a disinfecting effect (Abdelfattah *et al.*, 2023). Therefore, C-MFCs can be an effective technology to improve the removal rate of NH_3-N and TP.

Many studies have been devoted to improving the electrochemical performance and wastewater treatment capacity of MFCs (Lin *et al.*, 2023), mainly focusing on the improvement of MFCs electrode materials (Lin *et al.*, 2023; Wang *et al.*, 2023) and proton exchange membranes (Patel *et al.*, 2022), and relatively significant improvements were achieved. In addition, the nitrogen to phosphorus ratio in the anode chamber substrate is very important for the performance of MFCs (Tariq *et al.*, 2021). Importantly, the nitrogen to phosphorus ratio of the effluent from the anode chamber affects not only the structure of the microbial community in the anode chamber, but also the growth of chlorine spheres, which affects the electrochemical performance of MFCs and its effect on NH_3-N, COD and total phosphorus in the wastewater (Xu *et al.*, 2022). Therefore, it is important to investigate different nitrogen and phosphorus ratios to improve the C-MFCs. In this study, we used four nitrogen to phosphorus ratios to observe the electrical properties of C-MFCs and the removal of NH_3-N, COD and TP. In addition, the anode chamber microbial communities of C-MFCs operating under different nitrogen to phosphorus ratios were collected and then sequenced by high-throughput 16S rRNA genes.

2. Materials and methods

2.1. Materials and chemicals

Na_2HPO_4-$12H_2O$, NH_4Cl, KCl and glucose were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China), and all reagents were of analytical grade.

2.2. C-MFCs construction

Two 500 mL Plexiglas bottles were used to construct the double chamber C-MFCs and 500 mL Plexiglas bottles were also used to construct the algae reactor. The same volume of graphite plates (size 4 cm × 5 cm × 0.5 cm) was used for the anode and cathode plates. The wastewater enters the anode chamber, then flows into the Chlorella reactor and is finally discharged. The cathode chamber solution was taken as 0.2 g/L KCl solution. Throughout the experiment, the anode and cathode were connected by an external circuit with a load of 1000 Ω. The protons between the anode and cathode were transferred through the PEM , and the anode chamber was connected to the Chlorella vulgaris reactor through a rubber tube. The experimental setup is shown in Figure 1. The simulated aquaculture wastewater was configured according to the indicators in the aquaculture wastewater and autoclaved as the feed solution of C-MFCs. Then the activated sludge suspension from the secondary sedimentation tank of the wastewater treatment plant and Chlorella vulgaris were inoculated into the anode chamber and Chlorella reactor respectively, both with 20% inoculation ratio. C-MFCs run on a 7-day cycle. Set four gradients of N:P=1:1, N:P=2:1, N:P=3:1 and N:P=5:1, respectively. Three parallel experiments were set up during the study. Throughout the experiment, C-MFCs were placed in an environment with a temperature of 20°C and a pH of about 7~8. The tables in the text were created using Excel and the graphs were drawn using Origin 2022.

The experimental data were analyzed using IBM SPSS Statistics 26.0. The standard deviation and p-value of the experimental data were obtained by one-way ANOVA. Statistical significance was set at $p < 0.05$.

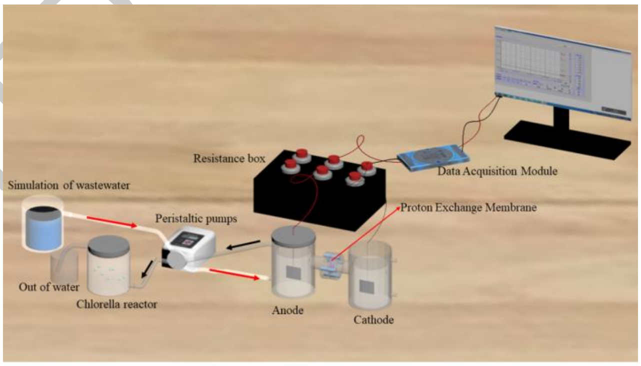


Figure 1. Diagram of experimental setup

2.3. Data collection and analysis methods

Water samples were collected every 24 hours and stored in a refrigerator at 0~4 ℃. The wastewater treatment capacity of C-MFCs was assessed by measuring NH_3-N, COD and TP. The determination of ammonia nitrogen was carried out by the colorimetric method with nano reagent, the determination of TP was carried out by the spectrophotometric method with molybdate, and the determination of COD was carried out by the rapid digestion method. The chlorophyll-a content of Chlorella was determined and extracted by acetone grinding method (Anderson and Calvin, 1962). Determination of TP by ammonium spectrophotometry. The voltage data of C-MFCs were collected every 1 min by the data acquisition module (DAQM-4202). Electron microscopy (SEM, Quanta 250 FEG, FEI, USA) was used to observe the morphological characteristics of the anode biofilm (Zhang *et al.*, 2021).

2.4. Microbial community analysis

Sangon Bioengineering Co., Ltd. (Shanghai, China) was used to analyze the microbial community structure in the anode chamber, including DNA extraction, database construction and sequencing, and data analysis. 16s rRNA sequencing was carried out by the Shanghai Biotechnology Sequencing Department (Shanghai, China) to analyze the relative abundance and diversity of the colonies, and the analysis of microbial function prediction was carried out through the PICRUSt(v1.1.4) software (Sun *et al.*, 2012).

3. Results and discussion

3.1. Power generation of C-MFCs

From Figure 2, it can be seen that C-MFCs have the best electrochemical performance at N:P=3:1 generating maximum voltage and maximum power density of 92.94 mV and 234.01 mW/m2, respectively, and the worst electrochemical performance at N:P=5:1. The R of C-MFCs for different N:P conditions can be obtained by fitting the polarization curves to obtain the effect of different N:P on the internal resistance of microbial fuel cells. Electricity production is one of the important indicators to evaluate the performance of Chlorella and MFCs (Ottoni *et al.*, 2019). In contrast, the electroactivity of electrogenic microorganisms was higher under the operating condition of N:P=3:1.

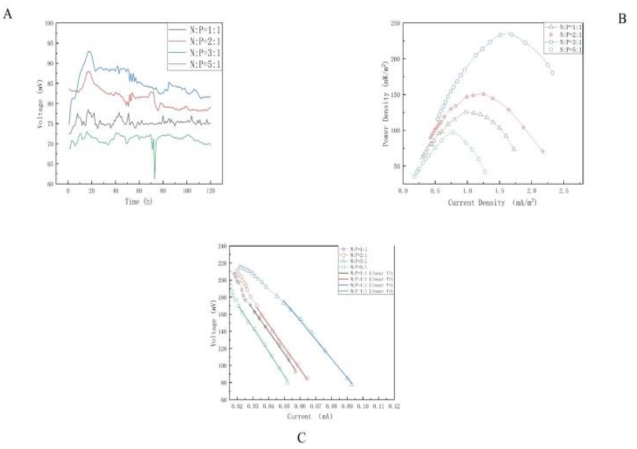


Figure 2. (A) Voltage change curve under different N:P conditions (B) power density curve under different N:P conditions (C) Polarization Curve and Its Linear Fitting Line under different N:P conditions

However, the $R_{R\text{-}\Omega}$ of C- MFCs is very different under the four N:P conditions, which may be due to the impact of different N:P on the electron transfer rate of microorganisms in the anode chamber, resulting in a large difference in the $R_{R\text{-}\Omega}$ of C-MFCs (Liu and Vyverman, 2015). This provides a reference for the indirect enhancement of C-MFCs by increasing the activity of electricity-producing microorganisms. These results effectively confirmed that the appropriate N:P is of great significance to improve the power generation performance of C-MFCs (Huang *et al.*, 2017).

3.2. Pollutants degradation of C-MFCs and changes in chlorophyll-a content

As shown in Table 1 and Figure 3, C-MFCs showed the highest removal rate of NH_3-N and COD at N:P=3:1. The removal rates reached 72.48±1.94%, 81.26±4.4%, respectively. C-MFCs showed the highest removal rate of TP at N:P=1:1. The removal rate was 65.62±2.1% at this time. C-MFCs showed the lowest removal rates of NH_3-N, COD and TP at N:P=5:1. Similarly, chlorophyll-a content was highest at N: P=3:1 and lowest at N: P=5:1.

Studies have shown that Chlorella can easily absorb ammonium ions and nitrogen and phosphorus from wastewater. The wastewater in question is, for example, textile (Wu *et al.*, 2020), sewage (Boonchai and Seo 2015), municipal (Zhou *et al.*, 2016), agricultural (Khalid *et al.,* 2019) and industrial (Osorio *et al.,* 2018). As shown in Figure 3D, the growth rate of chlorophyll content a was the fastest at N:P=3:1. The growth rate was the slowest at N:P=5:1. This corresponds to the optimal and worst conditions for the ability of C-MFCs to treat wastewater. It can be inferred that the removal of NH_3-N, COD and TP from wastewater has an important correlation with Chlorella and MFCs.

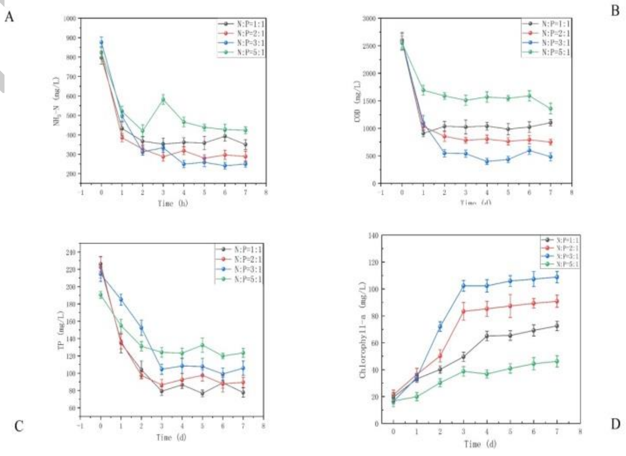


Figure 3. (A) Changes in NH3-N under different N:P conditions (B) Changes in COD under different N:P conditions (C) Changes in TP under different N:P conditions (D) Changes in chlorophyll a content of Chlorella vulgaris under different N:P conditions

Table 1. Removal of NH_3-N, COD and TP under different N: P conditions (All units are mg/L)

N:P=2:1			N:P=3:1			N:P=5:1		
NH_3-N	COD	TP	NH_3-N	COD	TP	NH_3-N	COD	TP
823.45±24.44 227	2604.56± 138.454	222.34± 12.465	876.18±27.88	2575.73± 275.18	214.59 ±8.48	821.75± 31.44	2548.90± 217.95	190.52± 4.01
279.30±23.41	763.45±65.39	92.56±7.23	249.76±4.94	505.37± 83.92	104.20 ±3.76	429.55± 7.72	1497.84± 123.70	125.23± 2.65
66.28±22.64	70.69±23.36	58.37±9.38	72.48±1.94	81.26±4.4	51.44± 1.75	47.73± 4.82	41.24±4.85	46.23±4.17

3.3. Anode microbial community composition analysis

It is well known that anode electrogenic microorganisms have an important relationship with the electricity generation performance of C-MFCs. Based on the above results, it can be seen that the electrochemical performance and wastewater treatment capacity of C-MFCs have different changes under different N:P conditions. The reason may be that the change of N:P caused the electrochemical activity of microorganisms in the anode chamber. In order to reveal the influence of N:P on the microorganisms in the anode chamber, the microbial community in the anode chamber under different N:P conditions was further explored.(Yoo *et al.,* 2010)

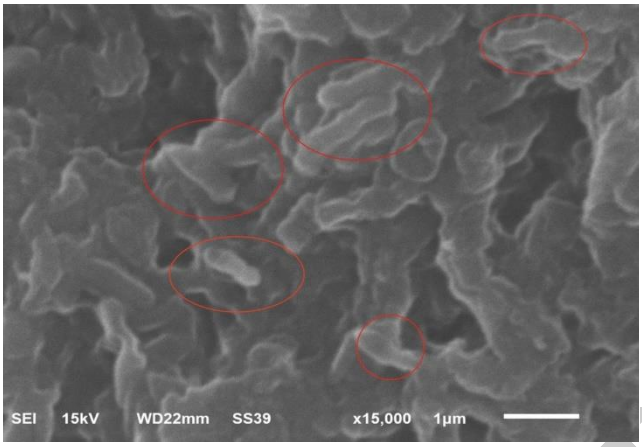


Figure 4. The SEM image of anodic biofilm

3.3.1. Morphology characterization of odic biofilm

The morphology of the anode chamber biofilm of the C-MFCs was characterized by SEM as shown in Figure 4. It is obvious to see that the surface of the anode biofilm is mainly distributed with a large number of rod-shaped bacteria, *Klebsiella* is rod-shaped bacteria, further verifying that the electricity production capacity of the anode chamber may be mainly associated with *Klebsiella*.(Lee *et al.,* 2016; Ramanaiah *et al.,* 2021)

3.3.2. Microbial diversity analysis

To further explore the microbial diversity of the anode chamber under different N: P conditions. Alpha diversity was first analyzed as an index of abundance and homogeneity. The alpha diversity analysis mainly included four indices, Shannon, Chao, Ace and Simpson (Plaisance *et al.,* 2009). As shown in Table 2, the Ace and Chao indices were 153.26±19.32 and 162.93±10.28 for N:P=1:1, he Ace and Chao indices were 175.36±16.31 and 230.51±8.62 for N:P=2:1, 205.99±26.93 and 317.53±28.14 for N:P=3:1, 94.74±9.73 and 87.42±13.27 for N:P=5:1. It is obvious that the Ace index and Chao index are maximum at N:P=3:1 and the power generation performance of C-MFCs is the best at this time. Similarly, for N:P=1:1, the Shannon and Simpson index ratios were 2.50±0.08 and 0.46±0.19. For N:P=2:1, the Shannon and Simpson index ratios were 3.54±1.21 and 0.62±0.12. For N:P=5:1, the Shannon and Simpson index ratios were 1.24±0.52 and 0.36±0.08. For N:P=3:1, the Shannon and Simpson index ratios were 4.98±1.49 and 1.13±0.62. Chao ,Ace ,Shannon and Simpson had p-values of 0.02, 0.01, 0.05 and 0.01 between groups, respectively. The Shannon and Simpson indices were significantly different under different N:P conditions. As shown in Figure 5, The number of OTUs is 304 when N:P=3:1. The number of OTUs is 192 when N:P=5:1. The number of OTUs is directly proportional to the overall performance of C-MFCs. The better the performance, the higher the number of OTUs in the anode chamber. This indicates that the microbial community diversity in the anode chamber rises under the conditions of better power generation performance with N: P = 3:1.

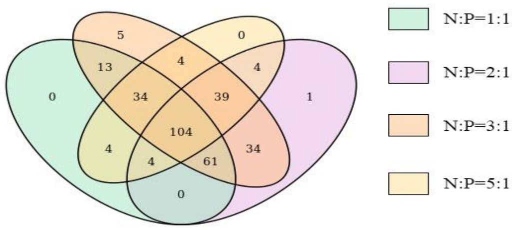


Figure 5. Venn plot at OUT level of Anode

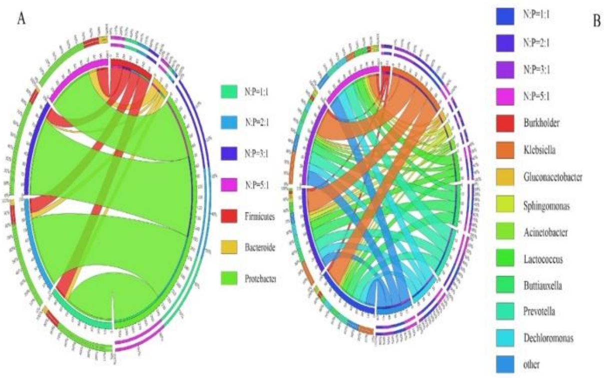


Figure 6. (A) Phylum level colony composition four N:P conditions (B) Genus level colony composition four N:P conditions

Table 2. The Alpha diversity under different N: P conditions

Index	N:P=1:1	N:P=2:1	N:P=3:1	N:P=5:1	P
Shannon	2.50±0.08	3.54±1.21	4.98±1.49	1.24±0.52	0.05
Chao	162.93±10.28	230.51±8.62	317.53±28.14	87.42±13.27	0.02
Ace	153.26±19.32	175.36±16.31	205.99±26.93	94.74±9.73	0.01
Simpson	0.46±0.19	0.62±0.12	1.13±0.62	0.36±0.08	0.01

3.3.3. Species composition analysis

In order to intuitively analyze the microbial community composition in the anode compartment under different N: P conditions, a visual circos plot was used to reveal the changes in the community composition at the phylum and genus levels under the four different conditions. Under different N:P conditions in the anolyte, the dominant bacteria are all Proteobacteria. When N:P=1:1, Proteobacteria accounted for 76.34%, when N:P=2:1, Proteobacteria accounted for 79.45%, when N:P=3:1, Proteobacteria accounted for 83.72%, and when N:P=5:1, Proteobacteria accounted for 68.34%. As shown in Figure 6B, when the anolyte was under the condition of N:P=1:1, the dominant bacteria at the genus level were *Klebsiella* (23.34%) and *Prevotella* (14.45%). When the anolyte was under the condition of N:P=2:1, the dominant bacteria were *Klebsiella* (25.67%) and *Buttiauxella* (14.67%). When the anolyte was under the condition of N:P=3:1, the dominant bacteria were *Klebsiella* (32.12%) and *Prevotella* (14.45%). When the anolyte was under the condition of N:P=5:1, the dominant bacteria was *Prevotella* (17.23%). These results show that under different N:P conditions, there is an important influence on the composition of the microbial community in the anode compartment, with *Klebsiella* having the largest proportion at N:P=3:1, and at N:P=5:1 The lower proportion is the smallest. The obvious change in the proportion of *Klebsiella* indicates that this bacterium may be related to the electricity production performance of C-MFCs. And it is inferred that the formation of anodic biofilm and the electricity production of C-MFCs are closely related to Proteobacteria (Sakr *et al.,* 2021).

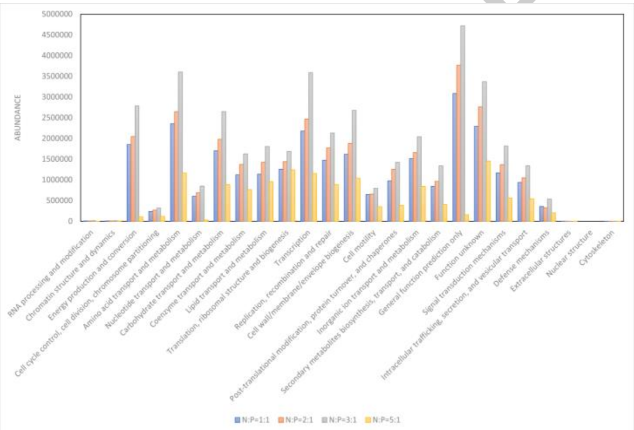


Figure 7. 16S function prediction by PICRUSt based on COG secondary functional classification of different N: P

3.3.4. Functional prediction analysis

Functional prediction analysis of 16S rRNA was performed on the anode chamber electrolyte. The COG functional classification map was obtained using PICRUS software. As shown in Figure 7, Energy production and conversion was the highest contents at N:P=3:1. Energy production and conversion content were lowest at N:P=5:1. This is consistent with the results of microbial community composition under different N:P conditions. Moreover, as shown in Figure 7, amino acid transport and metabolism, nucleotide transport and metabolism. The highest contents of amino acid transport and metabolism, carbohydrate transport and metabolism, coenzyme transport and metabolism translation and ribosomal structure and biogenesis were also found in the anode chamber, which may be the result of the microorganisms in the anode chamber. The organic pollutants in the wastewater were used for their own growth and metabolism. It can be inferred that the anode microbial community is enriched with microorganisms related to the degradation of organic pollutants and the ability to produce electricity in the operation of the C-MFCs.

4. Conclusion

C-MFCs, a commonly used bioelectrochemical system, was successfully applied to the treatment of farm wastewater with good removal rate and good electrical production performance for pollutants in farm wastewater. In addition, N:P=3:1 was determined as the optimum for C-MFCs to treat culture wastewater. Microbial community analysis showed that the content of *Klebsiella* and *Gluconacetobacter* in the anode chamber was positively correlated with the pollutant removal rate and electricity production capacity of C-MFCs. The chlorophyll-a content in the coccolithophore reactor was also positively correlated with the pollutant removal rate from the culture wastewater. In general, the results showed that the combination of Chlorella and MFCs could effectively treat aquaculture wastewater under the condition of N:P=3:1. At N:P=3:1, the removal of NH3-N, COD and TP by C-MFCs was as high as 72.48±1.94%, 81.26±4.4% and 65.62%±2.14%, respectively. And at this time, C-MFCs have the lowest internal resistance, the highest output power, and the best electrochemical performance and pollutant removal effect. Finding the right N:P is of great importance to improve C-MFCs. However, C-MFCs treatment of culture wastewater is carried out as much as possible under laboratory conditions. And the electrochemical performance of C-MFCs needs to be further improved. Therefore, the effect of other external and inter conditions on C-MFCs needs to be analyzed to further improve the performance of C-MFCs.

Conflict of interest

There exists no potential conflict of interest among the authors.

Data Availability Statements

The data that support the funding of this study are available from the corresponding auther upon reasonable request.

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