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Original Research

Optimization of CW-MFC System for Fish Wastewater Treatment by Response Surface Methodology

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Abstract

Freshwater fish farming in China is conducted on a large scale with a high degree of intensification, resulting in substantial wastewater production that leads to eutrophication and ecological pollution of surrounding water bodies. This article focuses on a constructed wetland coupled with a microbial fuel cell (CW-MFC) system, utilizing a Box-Behnken design and response surface methodology to optimize operating conditions (hydraulic retention time, external resistance, and aeration rate) and removal efficacy in treating fish farming wastewater. The results indicate that the optimal operating conditions were an HRT of 3.19 days, an external resistance of 704.06 Ω, and an aeration rate of 5.30 L/min. Under these conditions, the actual removal rates for COD, NH_4^+-N, and TN were 95.03%, 95.22%, and 93.48%, respectively. The optimized operating conditions increased the abundance and stability of the microbial community, enhanced the relative abundance of electricity-producing and denitrifying bacteria, and improved pollutant removal efficacy.

Keywords: Constructed wetlands, microbial fuel cells, fish farming wastewater, response surface methodology

Introduction

China is a major global leader in aquaculture, with its production consistently exceeding 60% of the world's total aquaculture output since 2008 [1]. The freshwater fish farming industry has evolved over decades from low-density, extensive practices to high-density, high-yield, intensive, and industrialized modes of production [2]. Intensive aquaculture modes, coupled with increased feed inputs, generate substantial amounts of residual feed and feces from fish and shrimp, leading to eutrophication of the aquaculture

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water bodies. This complicates management and water quality control, affecting the growth of farmed animals [3, 4]. Furthermore, to promote fish growth and control various disease outbreaks, antibiotics are often added to the aquaculture waters. Due to the lack of necessary regulation and treatment, this poses ecological risks to the surrounding environment [5, 6]. Yadav [7] first introduced the CW-MFC concept in 2012, applying it to the treatment of textile wastewater and achieving significant removal efficiencies for COD and methylene blue dye. Tao et al. [8] applied high-density polyethylene packing in a CW-MFC to treat low-nitrogen wastewater, achieving a 97.55% removal of TN and a power density of 2.22 mW/m^2. Liu et al. [9] were pioneers in applying the CW-MFC system to the treatment of marine aquaculture wastewater, focusing not only on the removal of COD, nitrogen, and phosphorus but also on enhancing the removal of sulfamethazine and heavy metals [10]. Currently, CW-MFC treatment mainly targets dye wastewater, heavy metal pollution, antibiotics, and emerging pollutants [11-13], with limited research on freshwater fish farming wastewater. Additionally, the efficiency of CW-MFC treatments can be affected by operational conditions, and studies on how operational conditions affect CW-MFC treatment of freshwater fish farming wastewater are still lacking. Therefore, we attempted to apply CW-MFC for the treatment of freshwater fish farming wastewater, studying the effects of three operational conditions: Hydraulic Retention Time (HRT), external resistance, and aeration rate on the treatment efficiency. Using response surface methodology, we optimized the design to determine the optimal operational conditions for CW-MFC, aiming to achieve the best pollutant degradation results and provide a reference for the promotion of CW-MFC treatment in freshwater fish farming wastewater.

Materials and Methods

CW-MFC Microcosms Design and Operation

A rising-flow CW-MFC wastewater treatment system was constructed using a UPVC pipe with a diameter of 30 cm and a height of 75 cm. The system design includes a water inlet at the bottom and an outlet at the top, located at 65 cm. The system was structured from the bottom up, with a zeolite layer (20 cm in height, with a particle size of 8-10 mm) at the bottom, followed by an anode area (activated carbon, 20 cm in height, with a particle size 2-4 mm), a middle section (ceramic layer, 15 cm in height, with a particle size 10-15 mm), and a cathode area (activated carbon, 10 cm in height, with a particle size 2-4 mm). The anode and cathode were made of a 30cm round stainless steel mesh wrapped with activated carbon. The cathode and anode wires were connected by titanium and copper wires, respectively, and wrapped with epoxy resin. An external resistance box with a resistance range of 0-10,000 Ω was connected. The experimental setup, using plantain as a wetland plant, is shown in Fig. 1.

Synthetic Wastewater Preparation and Water Sampling

The experimental study was conducted at the water station of the Ecological Environment Research Centre, Yanshan Campus, Guilin University of Technology. To simulate the freshwater fish culture wastewater, daily ready-made artificial wastewater was used due to the unavailability of stable wastewater around the test site. Synthetic wastewater consisted of tap water, nutrients (in mg/L), including glucose (112.5), NH_4Cl (10), KH_2PO_4 (12.5), $NaHCO_3$ (62.5), $MgSO_4$ (5.63), $NaNO_2$ (1.86).

Fig. 1. CW-MFC device diagram.

Seeding Anaerobic Granular Sludge

The CW-MFC system requires the activated carbon particles in the anode layer to be inoculated with sludge. The sludge used in this study was taken from the concentrated anaerobic sludge of Liquan Brewery in Guilin. To achieve better domestication, the activated sludge was anaerobically incubated in the laboratory for one week before being introduced into the reactor system. The reactor system was inoculated with sludge and incubated for two months before the start of the experiment.

Water Quality Analysis

Effluent from the CW-MFC system was sampled every three days to determine pollutants. The influent and effluent water samples were filtered by 0.45 µm membrane and analyzed within 24 h. The concentrations of total nitrogen (TN), NH_4^+-N, were determined by the UV spectrophotometry method. The COD was determined by the dichromate method (HJ 828–2017).

Experimental Conditions

Fish effluent was pumped through a peristaltic pump with an HRT of 3 days, an external resistance value of 1000 Ω, aeration of 2 L/min, and a continuous flow inlet method to start the reactor after the construction of the CW-MFC unit was completed. The subsequent experiments were carried out only after the device had been in stable operation for 2 months. Different single-factor experimental conditions were run for 45 days each, and the water was sampled every 3 days to measure concentrations of COD, NH_4^+-N, and TN. After conducting the one-factor experimental conditions, the pollutant removal effect of the CW-MFC system in treating fish farming wastewater was investigated using the Box-Behnken model in Design Expert 13.0, a response surface analysis software, under different operating conditions. The appropriate level values of the three condition factors were screened based on the results of the one-factor test, and the optimal operating conditions of the CW-MFC system were determined accordingly. Three sets of validation tests were conducted to verify the validity of the optimization results based on the predicted conditions.

Microbial Community Analysis

During the operation of the experimental system, the system filler was sampled according to experimental requirements, 16SrRNA gene sequencing was used, and 454 high-throughput sequencing technology was used to analyze the microbial community structure. During sampling, the filler samples taken out were put into sampling bottles, stored in ice packs during transportation to the laboratory, and then stored in a -80°C refrigerator for sequencing by Shanghai Sangon Bioengineering Co., Ltd. After sampling, DNA was first extracted using a kit, then the 16sRNA gene fragment in the V3-V4 region was amplified and purified, and finally 454 high-throughput sequencing was performed. The original sequence is screened using the tag sequence (gene sequences less than 50 bp in length are discarded), and then non-specific amplified fragments, specific primer information, and tag sequences in the sequence are trimmed and removed to obtain effective sequences.

Results and Discussion

Influence of Various Factors on the Treatment Efficiency of the CW-MFC System

HRT

The effect of HRT on pollutant removal in the CW-MFC system is shown in Fig. 2. In Fig. 2a), when the HRT was in the range of 1-3 d, the average COD removal rate increased from 72.15% to 92.92% and the average effluent concentration decreased from 23.17 mg/L to 6.01 mg/L. COD removal is usually based on microbial biodegradation of organic matter [14]. At shorter HRT, the residence time of the wastewater in the CW-MFC system was short, the microbial community could not adequately degrade the organic matter in the wastewater, and the microorganisms needed time to adapt and multiply, the shorter HRT might prevent the microbial community from developing adequately and maintaining the diversity of the microbial community. When the HRT was increased to 3 d, the organic matter in the wastewater came into full contact with the microorganisms in the system, which was conducive to the formation of electrochemically active bacteria (EAB) and their reaction at the electrodes [15], which strengthened the electron transfer and electrochemical degradation process, and microorganisms such as the EAB sufficiently degraded the organic matter and added value through metabolism, and the rate of organic matter degradation was dramatically increased [16].

In Fig. 2b) and 2c), NH_4^+-N removal rate increased from 76.17% to 92.19% and the average effluent concentration decreased from 0.56 mg/L to 0.18 mg/L; TN removal rate increased from 69.74% to 89.75% and the average effluent concentration decreased from 3.22 mg/L to 1.08 mg/L. The shorter HRT may not be conducive to the production of sufficient numbers of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) to oxidize NH_4^+-N to nitrite (NO_2-N) and nitrate (NO_3-N), thus preventing denitrifying bacteria from using NO_3-N as an external electron acceptor to reduce it to nitrogen gas (N_2) or nitrogen oxides [17]. The alternate use of O_2 and N_2 by nitrifying and denitrifying microorganisms requires a certain amount of time, and the microbial community may not be able to adequately adapt to the changing redox conditions within a short period, resulting in limited nitrification and denitrification reactions, which reduces the treatment efficiency of NH_4^+-N and TN. Increasing the HRT to 3 d allowed the microbial community to better adapt and participate in the above transformation processes, and NH_4^+-N and TN removal rates were subsequently increased [18]. However, when the HRT was further increased to 5 d, the COD removal rate decreased to 89.62% with an average effluent concentration of 9.35 mg/L. The NH_4^+-N removal rate decreased to 89.16% with an average effluent concentration of 0.25 mg/L. The TN removal rate decreased to 85.86% with an average effluent concentration of 1.46 mg/L. When the HRT exceeds the critical value (In this experiment, the duration was 3 d to 4 d.), the system's treatment efficiency is slowed down and at the same time, the system's treatment capacity is drastically reduced. In addition, prolonged HRT exacerbates anaerobic conditions in the CW-MFC, which can adversely affect the removal efficiency of organic matter and nitrogen [19].

External Resistance

The effect of external resistance on COD removal by the system is shown in Fig. 3a). When the external resistance was 400 Ω, the CW-MFC system had the best COD removal performance, with an average removal rate of 91.43% and an average effluent concentration of 7.34 mg/L. As the resistance value increased, the removal efficiency decreased, and when the resistance value was 1200 Ω, the average removal rate was the lowest at 80.93% and the average effluent concentration was 16.35 mg/L. With a low resistance value, the electron flow increases, and the active electrode reaction helps to remove organic matter [20]. Too low an external resistance (100 Ω) may result in too many electrons being lost in the system instead of being used efficiently in the organic matter decomposition process [21]. The COD removal efficiency was slightly different (i.e. 2%) when the external resistance was changed from 400 Ω to 750 Ω. As the external

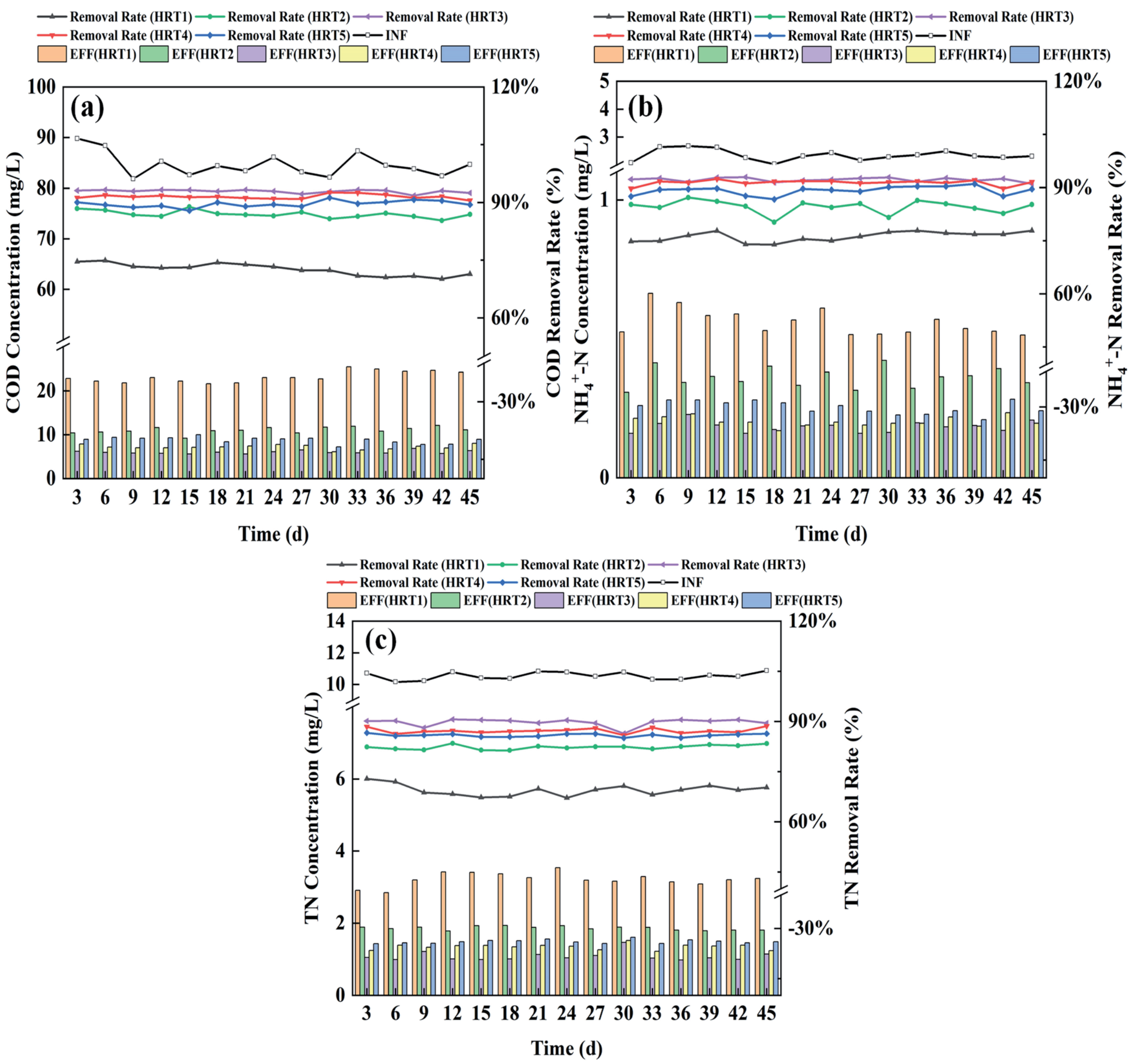

Fig. 2. Effect of pollutants in CW-MFC system under different HRT conditions, a) COD removal rate, b) NH_4^+-N removal rate, c) TN removal rate. INF: Influent, EFF: Effluent.

resistance is further increased, the higher resistance impedes the flow of electrons through the circuit, which is detrimental to the degradation of organic matter by electrogenic microbes in the CW-MFC [22].

As shown in Fig. 3b), the effect of external resistance on NH_4^+-N removal efficiency is smaller than that on COD removal, and the change is relatively small, which is basically consistent with the study of Srivastava et al. [23]. When the external resistance was 750 Ω, the CW-MFC system had the best effect on the removal of NH_4^+-N and TN, and the average removal rate of NH_4^+-N reached 92.72%, and the average effluent concentration was 0.17 mg/L. The average removal rate of TN reached 90.37% and the average effluent concentration was 1.01 mg/L. When the external resistance was further increased to 1200 Ω, the average removal rate of NH_4^+-N decreased to 87.46%, and the average effluent concentration was 0.30 mg/L. The average TN removal rate decreased to 82.36% and the average effluent concentration was 1.83 mg/L (Fig. 3c). The nitrification process requires oxygen as an electron acceptor. Low external resistances will accelerate electron transfer, increasing oxygen supply and promoting nitrification. However, if the external resistance is too low (<100 ohms), the electron transfer may be too fast and the redox reaction rate will be out of balance, limiting the denitrification reaction in the reactor, which may lead to the accumulation of nitrate nitrogen in the system [24, 25]. Therefore, the selection of the optimum external resistor is critical to the removal of nitrogen from wastewater by the CW-MFC system.

Aeration

Dissolved oxygen (DO) is a necessary oxygen supply for microbial respiration and metabolic processes, and aeration of the cathodic region of the CW-MFC system to increase DO had a significant effect on pollutant removal [26]. As shown in Fig. 4a), COD removal efficiency initially increased and then decreased with increasing aeration. When the aeration volume was 4 L/min, the average removal rate was up to 92.87%

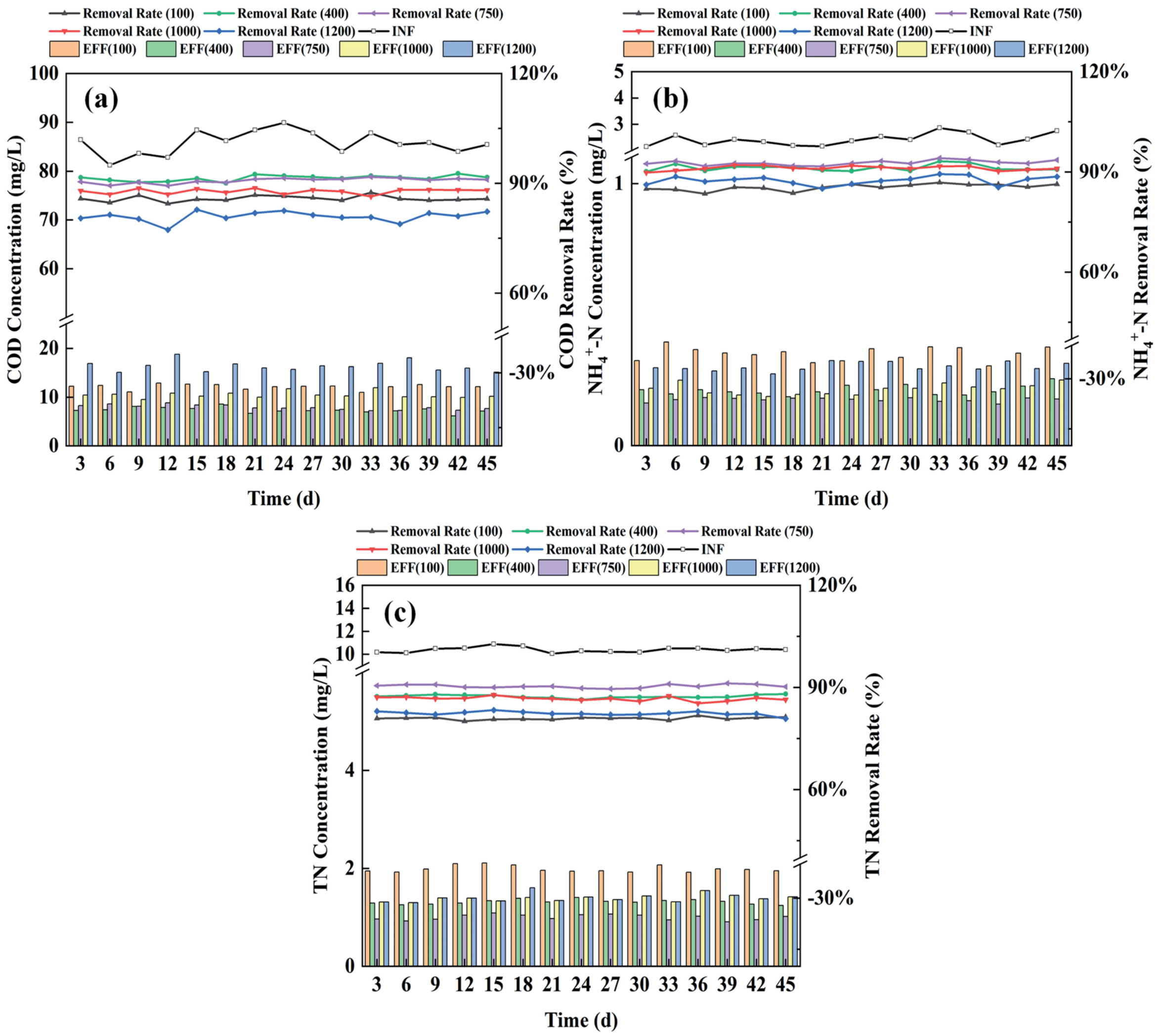

Fig. 3. Effect of pollutants in CW-MFC system under different external resistance conditions, a) COD removal rate, b) NH_4^+-N removal rate, c) TN removal rate. INF: Influent, EFF: Effluent.

and the average effluent concentration was 6.21 mg/L. When the aeration volume increased further to 8 L/min, the average removal rate decreased to 87.81% and the average effluent concentration was 10.62 mg/L. The average COD removal rate of the cathodic section of the CFC system decreased as the aeration volume increased to 8 L/min. Aeration can affect electron transfer processes and microbial activity in the cathodic region. By increasing the supply of oxygen, the efficiency of electron transfer and inter-root microbial activity can be improved [27], which may help to drive the oxidation reaction of organic matter in wastewater more efficiently. COD removal occurs mainly in the anaerobic zone, and some of the unused organic matter may not be completely decomposed after passing through the anaerobic zone. The aerobic zone of aeration provides sufficient oxygen for these residual organics to be further degraded by aerobic microorganisms, effectively reducing the COD content of the effluent [28]. However, excessive aeration can lead to changes in the microbial community. Some anaerobic microorganisms may be suppressed, while aerobic microorganisms may dominate, affecting COD removal efficiency. Therefore, the effect of cathodic aeration on COD removal is limited, and maintaining an appropriate level of aeration will promote COD removal.

Table 1. Response surface experiment factors and levels design.

Level of factors	factors		
	HRT $(X_1)/(d)$	Electrical resistance $(X_2)/(\Omega)$	Aeration $(X_3)/(L\cdot min^{-1})$
-1	2	400	2
0	3	750	4
1	4	1000	6

NH_4^+-N and TN removal efficiencies increased with increasing aeration (4(b), 4(c)). When aeration was increased from 0 L/min to 8 L/min, the average NH_4^+-N removal increased from 86.21% to 93.64% and the average effluent concentration decreased from

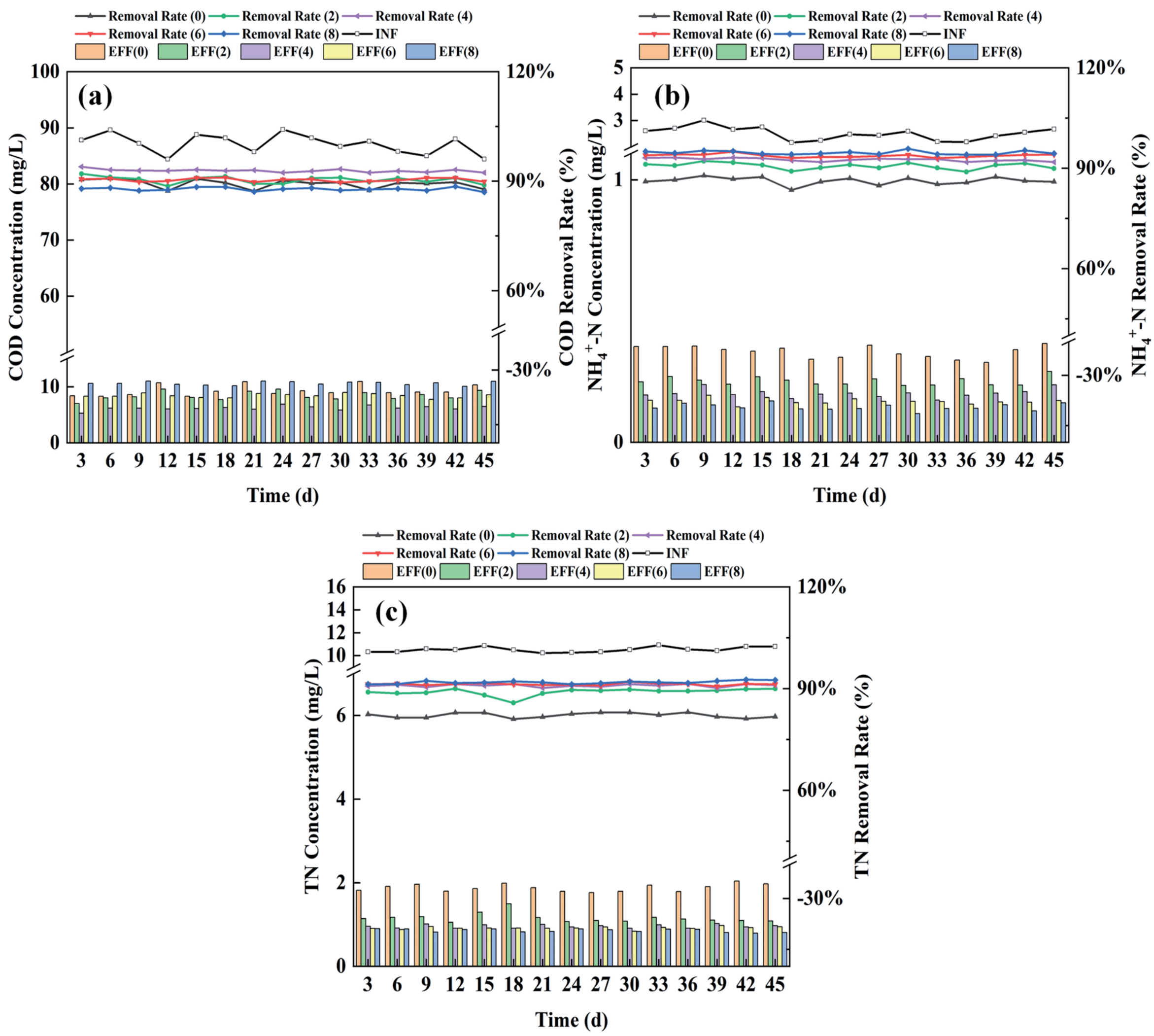

Fig. 4. Effect of pollutants in CW-MFC system under different Aeration conditions, a) COD removal rate, b) NH_4^+-N removal rate, c) TN removal rate. INF: Influent, EFF: Effluent.

0.35 mg/L to 0.13 mg/L. The average TN removal rate increased from 82.14% to 91.90% and the average effluent concentration decreased from 1.88 mg/L to 0.86 mg/L. The aerobic zone of the CW-MFC system is favorable for nitrification. By increasing the aeration in the cathodic zone, the AOB and NOB can be more active under aerobic conditions, accelerating the oxidation process of NH_4^+-N [29]. The redox potential of the reactor is also increased, which improves the removal of nitrogen. However, too much DO can lead to excessive competition for electron transfer in the oxygen reduction reaction in the cathodic region and reduce the efficiency of denitrification [30, 31]. Therefore, a careful balance between aerobic and anaerobic conditions and the intensity and frequency of aeration is required to maximize the effluent treatment efficiency of the system.

Response Surfaces Optimize CW-MFC System Operating Conditions

Response Surface Methodology (RSM) is a powerful mathematical and statistical technique used for model development and optimization, effectively analyzing the influence of multiple variables on one or more response variables [32]. Compared to traditional orthogonal experimental design, RSM not only assesses the impact of individual factors on the outcome but also reveals interactions between different factors and identifies the optimal combination of influencing factors [33]. In this study, a second-order model was employed to determine the interactive effects on the CW-MFC system performance of Hydraulic Retention Time (HRT), external resistance, and aeration amount.

Response Surface Design

A Box-Behnken design with 17 experimental points was used for a 3-factor, 3-level response surface design with HRT (X_1), electrical resistance (X_2), and aeration (X_3) as independent variables and effluent COD removal (Y_1), NH_4^+-N removal (Y_2) and TN (Y_3) as dependent variables, respectively, and the experimental factors and levels are shown in Table 1.

Response Surface Results Analysis

The results of the response surface experiments are shown in Table 2, and the response equation is obtained by fitting the multiple regression to the experimental results using Design Expert 13 statistical software:

$$Y_1 = 64.19279 + 16.21133A + 0.016583B - 0.302445C - 0.000234AB + 0.1925AC + 0.000611BC - 2.6515A^2 - 0.000017B2 - 0.044125C^2 \quad (1)$$

Table 2. Response experiment results.

Experiment number	X_1	X_2	X_3	Y_1	Y_2	Y_3
1	2	400	4	90.19	87.22	84.83
2	4	400	4	92.05	89.86	87.09
3	2	1000	4	87.27	89.01	86.25
4	4	1000	4	88.81	91.43	88.09
5	2	750	2	89.28	87.57	85.87
6	4	750	2	90.42	90.97	88.76
7	2	750	6	90.05	90.63	87.92
8	5	750	6	92.73	92.67	90.74
9	3	400	2	93.61	89.2	87.85
10	3	1000	2	89.12	91.71	89.12
11	3	400	6	94.26	91.98	89.03
12	3	1000	6	91.23	92.79	90.71
13	3	750	4	93.12	94.63	92.24
14	3	750	4	93.62	94.78	92.86
15	3	750	4	93.87	94.93	92.25
16	3	750	4	93.14	93.59	92.37
17	3	750	4	93.49	94.15	93.09

Note: There were a total of 17 trials, of which 12 were analytical factorization trials and 5 were center trials to estimate error

$$Y_2 = 40.52946 + 20.09273A + 0.036300B + 3.44891C - 0.000155AB - 0.170000AC - 0.000668BC - 2.9980A^2 - 0.000022B^2 - 0.2395C^2 \quad (2)$$

$$Y_3 = 37.71408 + 21.98758A + 0.041442B + 1.92481C - 0.000235AB - 0.00875AC + 0.000212BC - 3.4260A^2 - 0.000028B^2 - 0.203375C^2 \quad (3)$$

The results of the response model analysis are presented in Tables 3 and 4. The F-value and P-value given by the software calculations show that in the COD, NH_4^+-N, and TN removal rate models, the P-value is <0.0001, indicating that all three models are significant. The one-way significance shows that in the COD removal rate model, the P value of X_1, X_2<0.0001, indicating that HRT and external resistance have a greater influence on the COD removal process, followed by aeration amount; in the TN removal rate model, the P value of X_1<0.0001, indicating that HRT has a greater influence in the COD removal process, followed by external resistance and aeration amount.

The results of the confidence analysis of the COD, NH_4^+-N, and TN removal rate response models are shown in Table 5, the R^2 values of the three models are above 0.95, indicating that the correlation of the regression equations is better, and the difference between the Adjusted R^2 and the Predicted R^2 is much less than 0.2, which shows that the regression model can sufficiently explain the effect of different operating conditions on the removal of pollutants and the regression is better. C.V.% (coefficient of variation) <10 and Adep Precision (effective signal to noise ratio) >4 meet the requirements. Therefore, it is reasonable to use the above three models to evaluate the interaction of different operating conditions and the effect of factors on pollutant removal, and the fitted regression equations are in accordance with the above test principles and have good adaptability.

The significance of the interaction term obtained by fitting the two random factors to the response values indicated that the different operating conditions in the CW-MFC system affected the removal of pollutants to different degrees. In the COD removal model, X_1X_3<0.0418, X_2X_3<0.0486, indicating significant interactions between HRT and external resistance, external resistance and aeration, and no significant interactions between the other operating conditions. The response surface plots fitted to each model are shown sequentially in Fig. 5-7.

Validation of the Response Surface Model

The quadratic regression model established using response surface methodology can predict the optimal operational parameters. Optimization performed with Design Expert 13.0 software has determined the best operational parameters for removing pollutants from fish farming wastewater: an HRT of 3.19 d, an external resistance of 704.06 Ω, and an aeration rate of 5.30 L/min. The pollutant removal efficiencies of the CW-MFC system are as follows: COD removal of 94.26%, NH_4^+-N removal of 94.76%, and TN removal of 93.84%. The results were verified through three repetitive experiments, and the average COD removal rate was 95.03%, the average NH_4^+-N removal rate was 95.22%,

Table 3. Analysis of variance for quadratic regression equations -1.

Form	Df	Square sum			Mean-square sum		
		COD	NH_4^+-N	TN	COD	NH_4^+-N	TN
Model	9	73.34	94.46	108.45	8.15	10.5	12.05
X_1	1	8.34	7.76	13.22	8.34	7.76	13.22
X_2	1	16.32	3.53	2.17	16.32	3.53	2.17
X_3	1	4.2	4.68	3.65	4.2	4.68	3.65
X_1X_2	1	0.02	0.0087	0.0201	0.02	0.0087	0.0201
X_1X_3	1	0.5929	0.4624	0.0012	0.5929	0.4624	0.0012
X_2X_3	1	0.545	0.6511	0.0654	0.545	0.6511	0.0654
X_1^2	1	29.60	37.84	49.42	29.60	37.84	49.42
X_2^2	1	9.36	14.96	25.1	9.36	14.96	25.1
X_3^2	1	0.1312	3.86	2.79	0.1312	3.86	2.79
Residual	7	0.6714	139	116	0.0959	0.1985	0.1662
Lack of fit	3	0.2595	0.1942	0.5582	0.0865	0.0647	0.1861
Pure error	4	0.4119	1.20	0.6055	0.1030	0.2989	0.1514
Cor total	16	74.02	95.85	109.62			

Table 4. Analysis of variance for quadratic regression equations -2.

Form	Df	F-Value			P-Value		
		COD	NH_4^+-N	TN	COD	NH_4^+-N	TN
Model	9	84.96	52.86	72.49	<0.0001	<0.0001	<0.0001
X_1	1	86.98	39.11	79.5	<0.0001	0.0004	<0.0001
X_2	1	170.12	17.78	13.04	<0.0001	0.004	0.0086
X_3	1	43.84	23.57	21.94	0.0003	0.0018	0.0023
X_1X_2	1	0.2088	0.0441	0.1212	0.6615	0.8397	0.738
X_1X_3	1	6.18	2.33	0.0074	0.0418	0.1708	0.934
X_2X_3	1	5.68	3.28	0.3934	0.0486	0.1131	0.5504
X_1^2	1	308.62	190.62	297.3	<0.0001	<0.0001	<0.0001
X_2^2	1	97.54	75.36	150.96	<0.0001	<0.0001	<0.0001
X_3^2	1	1.37	19.46	16.76	0.2805	0.0031	0.0046
Lack of fit	3	0.8402	0.2166	1.23	0.5385	0.8803	0.4085

Table 5. Response surface modeling credibility analysis.

Model statistic	COD	NH_4^+-N	TN
R^2	0.9909	0.9855	0.9894
Adjusted R^2	0.9793	0.9669	0.9757
Predicted R^2	0.9318	0.9483	0.9101
C.V.%	0.3383	0.4865	0.4653
Adep Precision	29.7471	21.1675	25.4900
Significance	highly significant	highly significant	highly significant
Goodness of fit	high	high	high
Stability	great	great	great
Accuracy	high	high	high
Ranking of influences	$X_1>X_2>X_3$	$X_1>X_3>X_2$	$X_1>X_3>X_2$

Note: Adjusted R^2-Predicted R^2<0.2, C.V.%<10, Adep Precision>4 [34]

and the average TN removal rate was 93.48%, with relative errors of 0.77%, 0.46%, and 0.64%, respectively. These results indicate that the response surface method is a feasible approach for optimizing the different operating conditions of the CW-MFC system, providing a useful guide for the treatment of fish farming wastewater in the CW-MFC system.

Microbial Community Analysis

Community Diversity Analysis

B1 and B2 represent the CW-MFC unoptimized and optimized operating condition groups (Table 6). Microbial diversity indices were determined for both periods using high-throughput sequencing. Table 6 shows that the coverage index reached 0.99, indicating that the sequencing volume covered all species in the test samples. The number of OTUs generally indicates the presence of more microbial species in the samples. After optimizing operational conditions, the Chao, ACE, and Shannon indices of phase B2 were higher than those of the unoptimized phase B1, indicating that the optimized conditions were effective in enhancing the diversity and richness of the microbial community within the system. Adjusting factors such as HRT, external resistance, and aeration volume has a direct impact on microbial growth and diversity. The Simpson index for the B2 sample was as low as 0.0187, indicating that under optimal operating conditions, the species' dominance at the anode of the CW-MFC system is minimal. This suggests that no few species dominate the community, resulting in higher diversity.

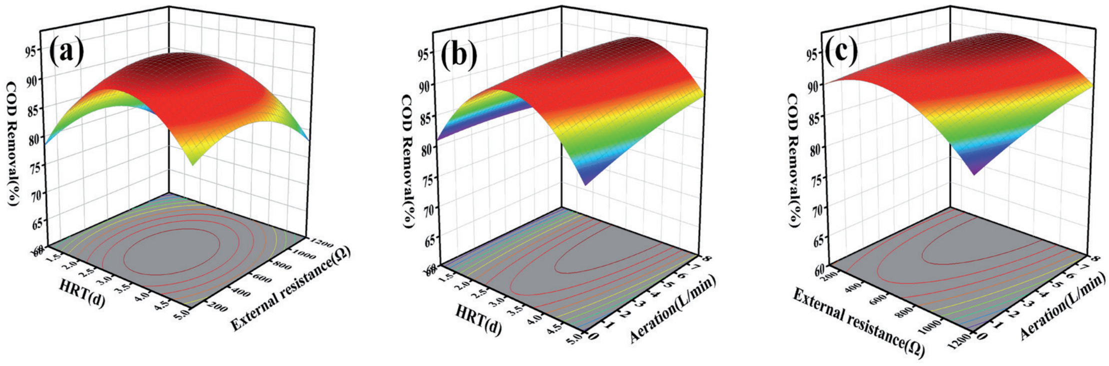

Fig. 5. Surface plot of COD response to the interaction of different operating factors, a) HRT, External resistance interaction, b) HRT, Aeration interaction, c) External resistance, Aeration interaction.

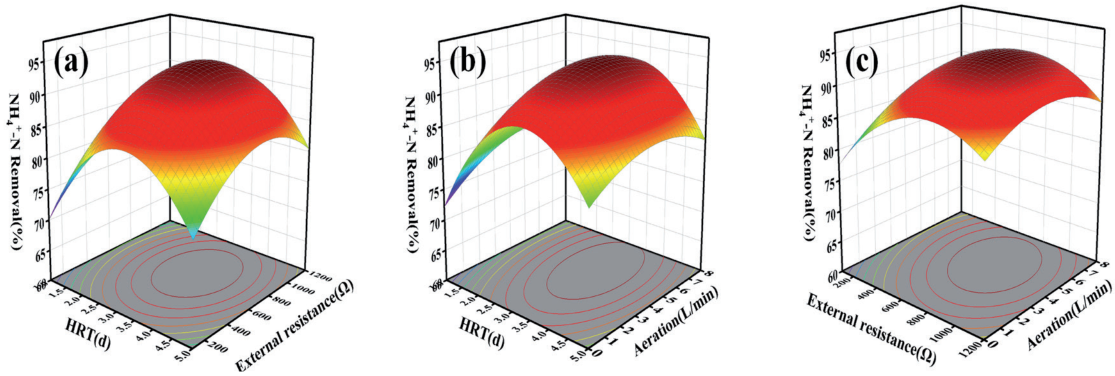

Fig. 6. Surface plot of NH_4^+-N response to the interaction of different operating factors, a) HRT, External resistance interaction, b) HRT, Aeration interaction, c) External resistance, Aeration interaction.

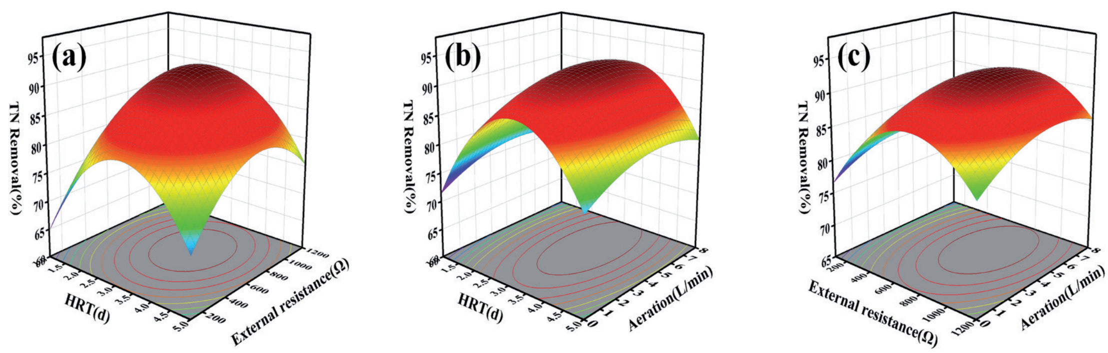

Fig. 7. Surface plot of TN response to the interaction of different operating factors, a) HRT, External resistance interaction, b) HRT, Aeration interaction, c) External resistance, Aeration interaction

Table 6. Statistical table of alpha diversity index.

Sample	OTUs	Shannon	Chao	Ace	Simpson	Coverage
B1	1387	5.42	1511.08	1503.88	0.0229	0.99
B2	1602	5.85	1598.95	1585.91	0.0187	0.99

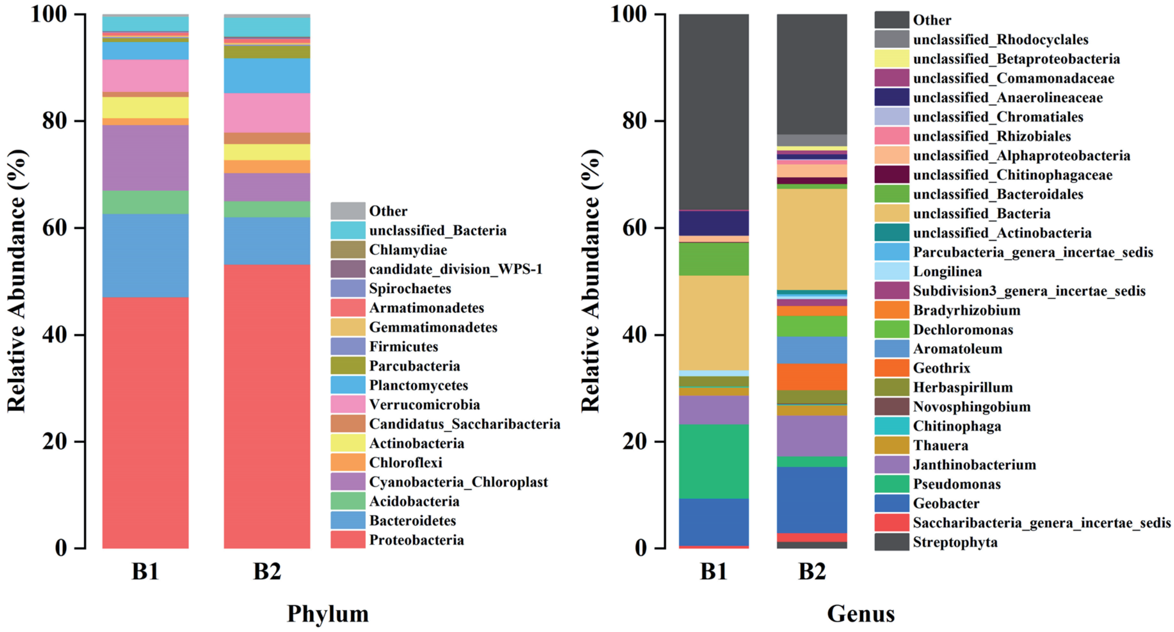

Fig. 8. Relative abundance map of the CW-MFC microbial community composition at the phylum level and the genus level.

Analysis of System Community Composition

In order to improve the analysis of pollutant removal by micro-organisms in the CW-MFC system, we have analyzed the microbial communities before and after the optimization. Fig. 8 shows that *Proteobacteria* (44.87%-53.23%), *Bacteroidetes* (8.85%-15.61%), and *Cyanobacteria_Chloroplast* (1.59%-12.26%) were the main microbial phyla with higher relative abundance in both the unoptimized operating condition CW-MFC system (B1) and the optimized operating condition CW-MFC system (B1). *Proteobacteria* and *Bacteroidetes* are common electrochemically active bacteria (EAB) and denitrifying bacteria play significant roles in extracellular electron transfer [35]. Bacteria within the *Proteobacteria* phylum are capable of processing organic materials and pollutants such as nitrogen and phosphorus in wastewater [36], and participate in the electron transfer processes within CW-MFC, enhancing current production. The anode is the primary site for electron transfer in CW-MFCs, and the highest relative abundance of *Proteobacteria* in B2 suggests that optimized operating conditions enhance the enrichment of *Proteobacteria* in the anode area, improving pollutant removal and electricity generation capabilities of CW-MFC. *Bacteroidetes*, consisting largely of obligate anaerobes, are involved in the hydrolysis of proteins and play a significant role in the breakdown of large organic molecules and the enhancement of nitrogenous material utilization [37]. In the anodic community of the CW-MFC, the relative abundance of *Bacteroidetes* is greater in B2 than in B1. The optimization of operational conditions may have facilitated the growth of bacteria such as *Proteobacteria*, while Bacteroidetes were inhibited, leading to a reduced relative abundance. *Cyanobacteria_Chloroplast* play a crucial role in nitrogen fixation in wetlands and produce intermediate compounds such as acetate and lactate, which are utilized in denitrification and electricity generation [38]. Previous studies have introduced cyanobacteria into MFCs, resulting in the enrichment of heterotrophic denitrifying bacteria at both anodes and cathodes, effectively facilitating simultaneous nitrification and denitrification, and enhancing nitrogen removal and power generation [39]. This aligns with the findings of our experiment, where a higher relative abundance of *Cyanobacteria_Chloroplast* in the anodic region of the CW-MFC aids in wastewater denitrification. However, the relative abundance of Cyanobacteria_Chloroplast in B2 significantly declined compared to B1. This reduction may be attributed to optimized operational conditions increasing oxygen supply, potentially creating competitive pressure on photosynthetic oxygen-producing cyanobacteria, affecting their growth and relative abundance.

Pseudomonas (1.05%-13.86%), *Geobacter* (2.51%-12.35%), *Janthinobacterium* (5.38%-7.61%), *Herbaspirillum* (1.04%-2.51%), and *Thauera* (1.23% -1.90%) are the dominant genera at the anode. *Pseudomonas* and *Geobacter* are the principal electricity-producing microbes at the CW-MFC anode, releasing pyocyanin into the electron mediators, thereby enhancing the power generation by electrochemical bacteria. *Pseudomonas* has the potential for both autotrophic and heterotrophic denitrification, participating in wastewater denitrification and organic pollutant degradation [40]. However, the relative abundance of *Pseudomonas* in the B2 sample may have decreased because it is better adapted to the initial electrochemical conditions, while the optimized conditions might favor the growth of other microbes. For instance, an increase in the relative abundance of *Geothrix*, along with *Pseudomonas* and *Geobacter*, formed the primary EABs in B2 [41], enhancing the electricity production capability of the CW-MFC post-optimization. *Geobacter*, involved in the carbon and nitrogen cycles in anaerobic environments [42], enhances the system's capacity for wastewater degradation. The increase in the relative abundance of *Geobacter* from B1 to B2 in the CW-MFC is related to electron transfer [43]. The elevated anodic potential under optimized conditions suggests that the anode area provided an environment conducive to the growth and metabolic activities of *Geobacter*. Additionally, the relatively high abundance of nitrogen-fixing bacteria (*Herbaspirillum*) and denitrifying bacteria (*Thauera*) in B2 post-optimization facilitated their growth and reproduction, explaining the higher denitrification efficiency of the CW-MFC.

Conclusions

This study successfully optimized the operational conditions for treating fish farming wastewater using a CW-MFC system via the response surface Box-Behnken design, identifying the optimal parameters as follows: an HRT of 3.19 d, an external resistance of 704.06 Ω, and an aeration rate of 5.30 L/min. Under these conditions, the actual removal rates for COD, NH_4^+-N, and TN were 95.03%, 95.22%, and 93.48%, respectively. Furthermore, the optimized conditions enhanced the microbial community and abundance within the CW-MFC, with an increase in the relative abundance of EAB and denitrifying bacteria, thereby improving the pollutant removal efficacy. This research provides a potential direction for effective management of fish farming wastewater, contributing positively to the protection of the aquatic environment and the sustainable development of aquaculture. Future studies may explore different operational factors, such as electrode materials, system configurations, and influent C/N ratios to further refine the application of CW-MFC in treating fishery wastewater.

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Conflict of Interest

The authors declare no conflict of interest.

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