scientific reports

OPEN

Pseudomonas aeruginosa promoted microbial fuel cells for cytidine acid production wastewater treatment

Xiafei Yin[1,4]✉, Feifei Wang[1,4], Mingmin Ge[2], Feng Zhang[2] & Guobin Liang[3]

Microbial fuel cell (MFC) can convert the chemical energy of organic matter in wastewater into electrical energy with high degradation efficiency. In this study, a type of specialized microorganism, *Pseudomonas aeruginosa*, was screened and added to an MFC to promote the degradation of wastewater generated during the production of cytidine acid while improving the performance of the MFC. The MFC achieved a maximum voltage of 57.7 ± 4.4 mV and a maximum power density of 1.9 ± 0.1 mW/m^2; moreover, the degradation efficiencies of chemical oxygen demand (COD), total phosphorus (TP), and phosphate reached 76.3 ± 2.8%, 80.3 ± 3.3%, and 85.3 ± 3.5%, respectively. Gas chromatography-mass spectrometry (GC–MS) and metabolomics analysis revealed that *Pseudomonas aeruginosa* could effectively degrade organic matter in wastewater. Additionally, the metabolic pathways involved may have been pyrimidine metabolism, arginine and proline metabolism, and taurine and hypotaurine metabolism.

Keywords Microbial fuel cell, *Pseudomonas aeruginosa*, Wastewater treatment, Power density, Metabolic pathways

Cytidylic acid is extensively used in various applications within the food and pharmaceutical industries[1]. The production of cytidylic acid involves a biological fermentation or chemical synthesis step[2]. This generates a large amount of organic substances, including incompletely reacted raw materials, intermediates, by-products, and microbial cytoplasm, which increase the chemical oxygen demand (COD) of the wastewater. In the process of producing cytidine acid, the use of phosphorus containing compounds as raw materials or catalysts will produce organic phosphorus intermediates and other substances, resulting in a much higher phosphorus concentration in the wastewater than normal wastewater[3]. Thus, the weakly acidic (pH 6.0–6.5) wastewater generated also contains a large amount of phosphorus, especially in the form of organic phosphorus. High content of COD and phosphorus complicate the wastewater treatment process[5]. Furthermore, these substances cause the eutrophication of water bodies and damage the ecological environment when directly discharged without treatment[6]. Currently, the commonly used treatment methods for this type of wastewater with high phosphorus content and COD are physical, chemical, and biological methods. Acelas et al.[8] utilized porous anion exchange resins in a study to selectively absorb and remove phosphate ions from phosphorus-containing wastewater with a degradation efficiency of 86% for phosphate. The selective removal ability of this method is, however, limited under the environment of multiple ion coexistence and may increase the salinity of wastewater, producing secondary pollution and consequently affecting the safety of discharge and the environment. In a study by Nayeem et al.[9], roasted shellac was used as an adsorbent to degrade phosphorus-containing wastewater, achieving a phosphate degradation efficiency of 62.37%. However, this method has several limitations, including a restricted adsorption capacity, a high dependence on pH for treatment efficiency, and the potential release of pollutants during the roasting process[10] degraded triethyl phosphate (TEP) using a UV/O_3 digestion system with a degradation efficiency of 98.3%. However, the UV/O_3 design process is complicated and requires large monetary investment and high energy consumption, which are not suitable for engineering applications. Pan et al.[11] employed the O_3/CaO_2 advanced oxidation process to degrade sulfate in wastewater, achieving a degradation rate of 90.5%. However, the direct oxidation of phosphate by O_3/CaO_2 is limited, the reaction conditions are harsh, and the process is costly. The biological treatment of phosphorus and COD in wastewater is commonly used in anaerobic–anoxic–oxic (AAO) processes. There are many species of denitrifying bacteria in

[1]School of Resources and Environmental Engineering, Jiangsu University of Technology, Changzhou 213001, People's Republic of China. [2]Jiangsu Kangda Testing Technology Co., Ltd., Suzhou 215000, People's Republic of China. [3]School of Chemistry and Chemical Engineering, Jiangsu University of Technology, Changzhou 213001, People's Republic of China. [4]Xiafei Yin and Feifei Wang contributed equally to this work. ✉email: yinxiafei@jsut.edu.cn

the denitrification stage of the AAO process, with the largest number of species capable of denitrification found within the genus *Pseudomonas*, such as *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas stutzeri*, and *Pseudomonas mendocina*[12]. The AAO process can efficiently remove COD and phosphorus from water and is more energy-efficient and environmentally friendly compared to physical or chemical treatment methods as it reduces the use of chemicals. The technology of the process is mature, and the operation method of the equipment is reliable as it is simple and easy to manage and maintain[13]. However, the activity of microorganisms decreases over time, which reduces the efficiency of treating wastewater.

The addition of an applied electric field stimulates the action of microorganisms, which thus increases their activity. In a study by Hu et al., the relative abundance of bacteria increased by 4.63% at 1.5 V compared with that without applied voltage[14]. However, additional applied voltage will increase costs. Microbial fuel cells (MFCs) can produce electricity spontaneously by degrading organic matter[15], forming an electric field. Some researches have suggested that the electric field generated by MFC can increase the activity and abundance of microbial communities by stimulating cell dehydrogenase activity, increasing the relative permeability of cell membranes, promoting the production of highly conductive and rigid cytochrome OmcZ nanowires in biofilms, enhancing electron transfer, and other methods[16,17], thereby improving the treatment efficiency of wastewater and reducing the cost of treating wastewater. Zhang et al.[18] used MFC to degrade the backflow water generated during shale gas extraction, the closed-circuit MFC had a COD degradation efficiency of 72%, while the open-circuit MFC had a COD degradation efficiency of only 64%. Al-Ansari et al.[19] utilized an MFC to degrade antibiotics in wastewater; the total phosphorus (TP) removal was 89.9 ± 2.8% and the highest COD removal was 91.9 ± 2.3% after treatment with the MFC system. However, MFCs use conventional activated sludge, resulting in a slow degradation efficiency.

In this study, the specialized microorganism was first screened to assess its effectiveness in degrading phosphorus and COD in wastewater generated during cytidine acid production. The selected microorganism was then injected into the MFC to evaluate the treatment performance of the bacteria-supplemented MFC on the wastewater. Subsequently, metabolomics analysis was performed to identify the degradation pathway of phosphorus in the wastewater. The Venn network was used to visualize differences in microbial community composition, which manifested in the form of operational taxonomic unit (OTU). This study expands the application potential of MFC technology and provides scientific and technical support for addressing current challenges in industrial wastewater treatment.

Materials and methods

Materials

The wastewater generated during the production of cytidine acid used in this experiment was collected from a chemical company in Jiangsu, China, with a COD content of 55,600 ± 320 mg/L, a TP content of 5530 ± 122 mg/L, and a phosphate content of 1173 ± 88 mg/L.

Luria–Bertani (LB) medium, agar powder, H_2SO_4, HCl, and NaOH were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents utilized were of analytical grade.

Isolation of a denitrifying bacterium from activated sludge of a wastewater treatment plant using gradient dilution and plate scribing method[20]. The 16S rDNA gene was amplified by polymerase chain reaction (PCR) using universal primers F27 and R1492, and sequenced by Shanghai Sangon Company. The sequence alignment was conducted in GenBank by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A neighbor-joining phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA) 11.0 software. The phylogenetic tree of the strain is shown in Fig. S1, indicating that the obtained strain is *Pseudomonas aeruginosa*.

The activated sludge was collected from the laboratory's conventional MFC. The concentrations of total suspended sludge (TSS) was 3.3 ± 0.2 g/L, and the volatile suspended solid (VSS) concentrations were around 2.8 ± 0.3 g/L.

Experimental methodology

Degradation of wastewater by Pseudomonas aeruginosa

The *Pseudomonas aeruginosa* was inoculated at 2% v/v into 100 mL of LB medium and cultured to a logarithmic phase of growth. The strain was centrifuged and added to 100 mL of wastewater generated during the production of cytidylic acid and incubated for 72 h.

Construction and operation of single-chamber MFC

In this study, an air cathode single-chamber MFC reactor with an working volume of 700 mL was used, with a non-watertight carbon cloth (HCP 330N, Shanghai Hesen Electric Co., Ltd.) as the anode. The air cathode was prepared according to the method of Huang et al.[21],it was made by applying four diffusion layers (polytetrafluoroethylene, PTFE) on 30 wt% watertight carbon cloth (HCP 330P, Shanghai Hesen Electric Co., Ltd.). The cathode and anode were the same size (50 mm × 40 mm × 2.0 mm). The two electrodes were parallel to each other at a distance of 4 cm. In a closed loop, the anode and cathode electrodes were connected to an external resistor (100 Ω) through a titanium wire. The reactor was filled with 500 mL of wastewater and 200 mL of activated sludge. All MFC reactors were operated in an intermittent mode and the temperature was maintained at 30 ± 3℃. A complete cycle ended when the output voltage dropped below 30 mV[22] (Fig. 1).

Four MFCs were constructed: MFC-1, MFC-2, MFC-3, and MFC-4. MFC-1 and MFC-2 were open-circuit, with no strain added to MFC-1. *Pseudomonas aeruginosa* (60 mL) was added to MFC-2 as a control. MFC-3 and MFC-4 were closed-circuit with the cathode and anode connected to a resistor (100 Ω) using titanium wires. No strain was added to MFC-3, and *Pseudomonas aeruginosa* (60 mL) was added to MFC-4 as a comparison.

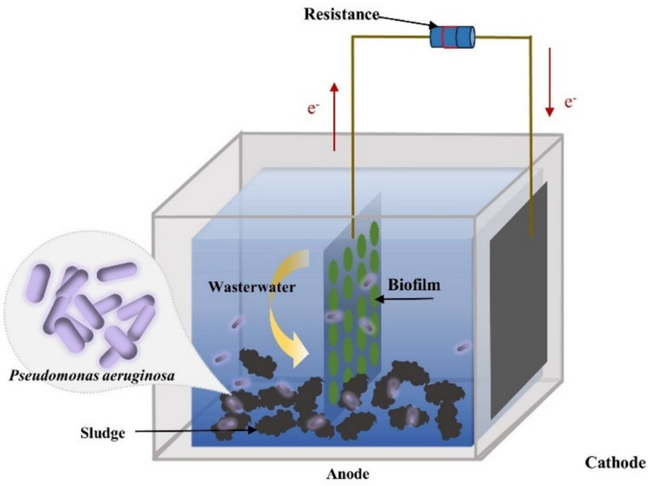


Fig. 1. The experimental setup schematic diagram.

Analytical methods

The changes in the concentrations of COD, TP, and phosphate were measured according to the Chinese State Environment Protection Agency (SEPA) standard methods[23]. Gaschromatography-mass spectrometry (GC–MS, 5977C, Agilent Technologies Inc, USA) was used to analyze the composition of pollutants in wastewater. A data collector (34972A, Agilent Technologies Inc, USA) was used to record the voltage of the MFC system. The steady-state discharge method was used to measure the power density curve of the system. The coulombic efficiency (C_E) was used to characterize energy recovery. The C_E defined as the fraction of electrons extracted for conversion into electricity versus that in the starting organic material, was calculated by estimating the substrate removal efficiency using the COD. The formula for C_E is given below:

$$C_E = \frac{\mathrm{M}\sum I(A)t(s)}{F \times b \times \nu \times \Delta COD}$$

M is the relative molecular mass of oxygen, 32 g/mol; *I* is the current, A; *t* is the time, s; *F* is Faraday's constant, 96.485 C/mol; *b* is the number of electrons transferred by reduction per mole of oxygen, 4 e^- mol/mol; ν is the effective volume of the substrate, L; and Δ *COD* is the difference in COD concentration between the inlet and outlet anolyte, mg/L.

An electrochemical workstation (CHI660D, Shanghai Chenhua Instrument Co., Ltd., China) was used to measure the cyclic voltammetry (CV) curve. Scanning electron microscopy (SEM, SIGMA300, Zeiss, Germany) was employed to illustrate the physical characteristics and morphology of the bacteria on the electrode surface. High-throughput sequencing was used for the measurement of the microbial community. The OTU was identified using Usearch software with a 97% sequence identity cutoff value. Alpha diversity can reflect the abundance [represented by Chao 1, abundance-based coverage estimator (ACE)], evenness (represented by Shannoneven) and diversity (represented by Shannon) of microbial communities. Beta diversity can reflect the similarity [represented by principal coordinates analysis (PCoA)] of microbial communities. The metabolomics were used to analyze the metabolites in the biodegradation process.

Results and discussion

Degradation of wastewater by Pseudomonas aeruginosa

The degradation effect of *Pseudomonas aeruginosa* on COD, TP, and phosphate in wastewater is shown in Fig. 2.

It can be observed in Fig. 2 that the degradation efficiencies of COD, TP, and phosphate all increase with reaction time. In Fig. 2a, there is a significant increase in COD degradation efficiency over time. During the initial phase, microorganisms are exposed to a higher concentration of organic matter, which promotes COD breakdown. As the reaction time extends to 48–72 h, the COD degradation efficiency levels off, likely due to the depletion of organic matter, which limits substrate availability for microbial metabolism[24]. As the dosage of *Pseudomonas aeruginosa* increases from 20 to 60 mL, COD degradation efficiency increases significantly, reaching 50.88 ± 1.82%. This improvement is likely due to the larger microbial population, which can more effectively utilize available carbon sources, thereby accelerating COD degradation. This may be attributed to the fact that when the strain dosage was low, an appropriate increase in the strain dosage could more fully utilize the carbon source in the MFC, thus accelerating the growth and reproduction of the microorganisms[25]. However, when the dosage is further increased to 100 mL, COD degradation efficiency decreases. This could be due to an excess of microorganisms, which leads to rapid consumption of available carbon, ultimately limiting microbial growth and metabolism[26]. In Fig. 2b, the TP degradation efficiency follows a trend similar to that of COD, increasing over time and stabilizing between 48 and 72 h. TP degradation efficiency also rises with increasing *Pseudomonas aeruginosa* dosage, peaking at 45.12 ± 3.0% at a 60 mL dosage. However, similar to COD, when the

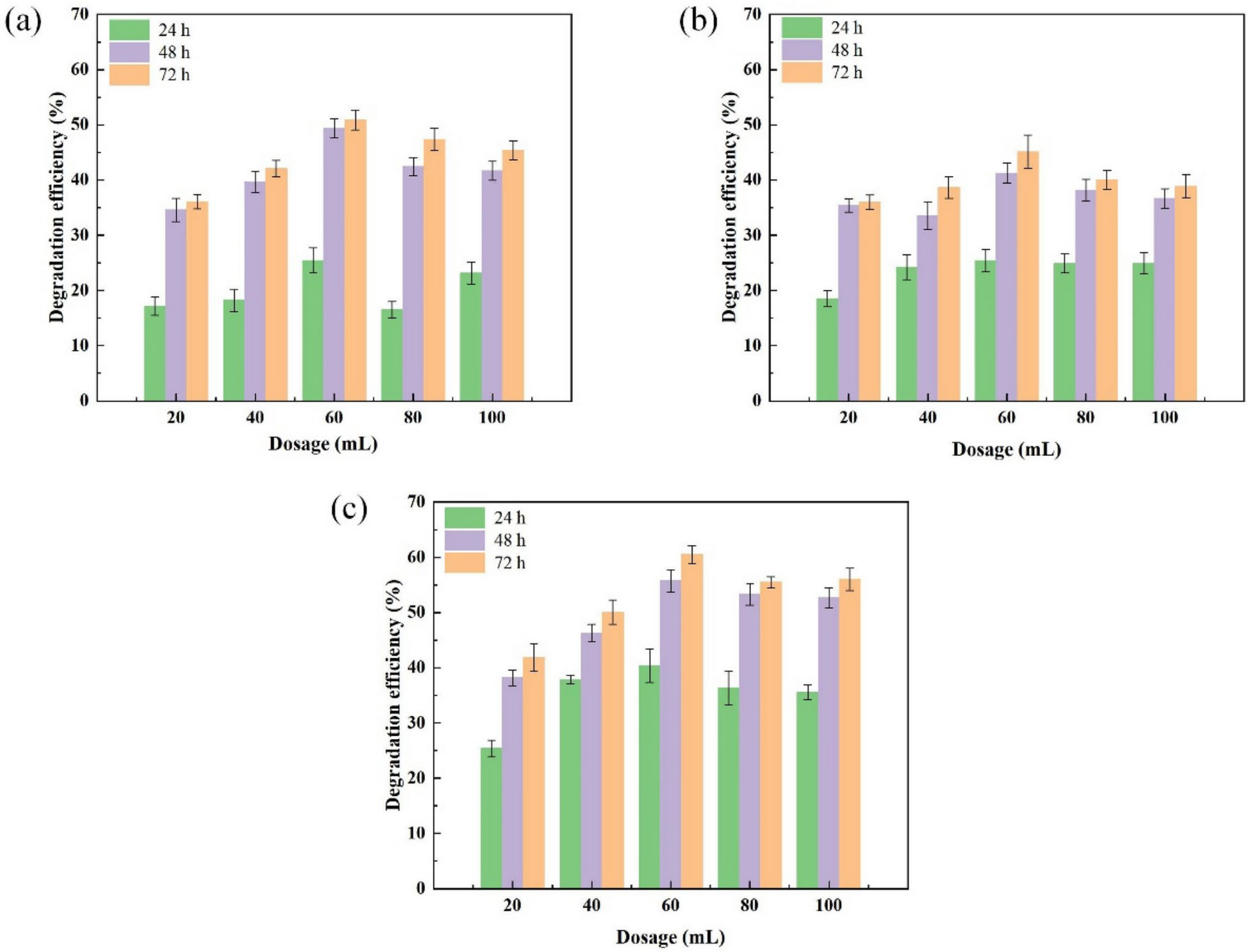


Fig. 2. Degradation efficiency of (**a**) COD, (**b**) TP and (**c**) phosphate in wastewater with different dosages of *Pseudomonas aeruginosa.*

dosage exceeds 60 mL, TP degradation efficiency begins to decline, likely due to the rapid consumption of carbon sources, which limits microbial metabolic activity. The metabolic efficiency of the microorganisms slowed down, leading to a decrease in degradation efficiency[27]. This result suggests that TP degradation follows a pattern closely resembling that of COD, both showing similar dependencies on microbial dosage. In Fig. 2c, phosphate degradation efficiency shows a similar trend to that of TP, gradually increasing over time and stabilizing between 48 and 72 h, reaching 60.52 ± 1.6%. As with the other pollutants, phosphate degradation efficiency increases with the dosage of *Pseudomonas aeruginosa*. At a 60 mL dosage, phosphate degradation efficiency peaks at 60.52 ± 1.6%. Differently from COD and TP, phosphate degradation efficiency remains stable even at higher dosages. This may be due to the sufficient reaction time, along with the high COD concentration, which provides continuous carbon sources for microbial activity, thus maintaining high phosphate removal efficiency[28].

As shown in Table 1, compared to the study of Li et al.[29], the removal efficiencies of COD and TP in this study are relatively low. Comparing with the study of[7], the phosphate degradation efficiency in this study is relatively high. Yang et al.[4] found that as the concentration of phosphate increased, the degradation efficiency decreased, probably because of the shortage of reaction time and carbon source during the incubation. However, the degradation efficiency of this study remained stable at 60.52 ± 1.6% under high phosphate concentration, which may be attributed to the longer reaction time and higher COD content of this study.

Notably, *Pseudomonas* was responsible for degrading wastewater generated during the production of cytidine acid, but it is difficult to have a comparative assessment since the initial pollutant concentration and degradation conditions varied across the studies.

In order to further analyze the degradation changes of the substances in the wastewater, we measured the wastewater before and after the degradation process via GC–MS (Tables S1–S2). Table S1 presents the original wastewater, and Table S2 presents the wastewater after treatment with 60 mL of bacterial solution dosage. As can be seen from Table S1, the raw wastewater contained 96 substances, of which the highest levels of several substances were 3,4-dimethylbenzaldehyde, tricyclopentadeca-3,7-dien[8.4.0.1(11,14)], α-patchoulene, triethyl phosphate, ethyl hexadecanoate, and 2,4-di-tert-butylphenol, among others. The degraded wastewater contained 90 substances, of which the most abundant were (sec-butylsulfanyl)cyclohexane2,4-di-tert-butylphenol, triethyl

Bacteria	Initial wastewater	Degradation time (h)	rpm	Temperature (°C)	Degradation efficiency	References
Pseudomonas mendocina A4	Salinity of 10% and 15%, PO_4^{3-}–P concentration of 18.84 mg/L	48	160	30	PO_4^{3-}–P removal efficiency of approximately 40%	30
Pseudomonas aeruginosa	COD concentration of 138.5 ± 15.25 mg/L, TP concentration of 2.07 ± 0.18 mg/L	26	150	28	COD and TP removal efficiencies of 68.23% and 51.21%	29
Pseudomonas stutzeri ADP-19	PO_4^{3-}–P concentration of 20 mg/L	36	150	30	PO_4^{3-}–P removal efficiency of 52.2%	7
Pseudomonas putida strain NP5	PO_4^{3-}–P concentration of 20, 50 and 100 mg/L, respectively	48	160	30	PO_4^{3-}–P removal efficiencies of 89.61%, 46.09% and 25.67%, respectively	4
Pseudomonas aeruginosa	COD, TP and PO_4^{3-}–P concentration of 55,600 ± 320 mg/L, 5,530 ± 122 mg/L and 1,173 ± 88 mg/L, respectively	72	140	40	COD, TP and PO_4^{3-}–P removal efficiencies of 50.88 ± 1.82%, 45.12 ± 3.0%, and 60.52 ± 1.6%, respectively	This study

Table 1. Efficiency of phosphorus-containing wastewater degradation by different *Pseudomonas spp*.

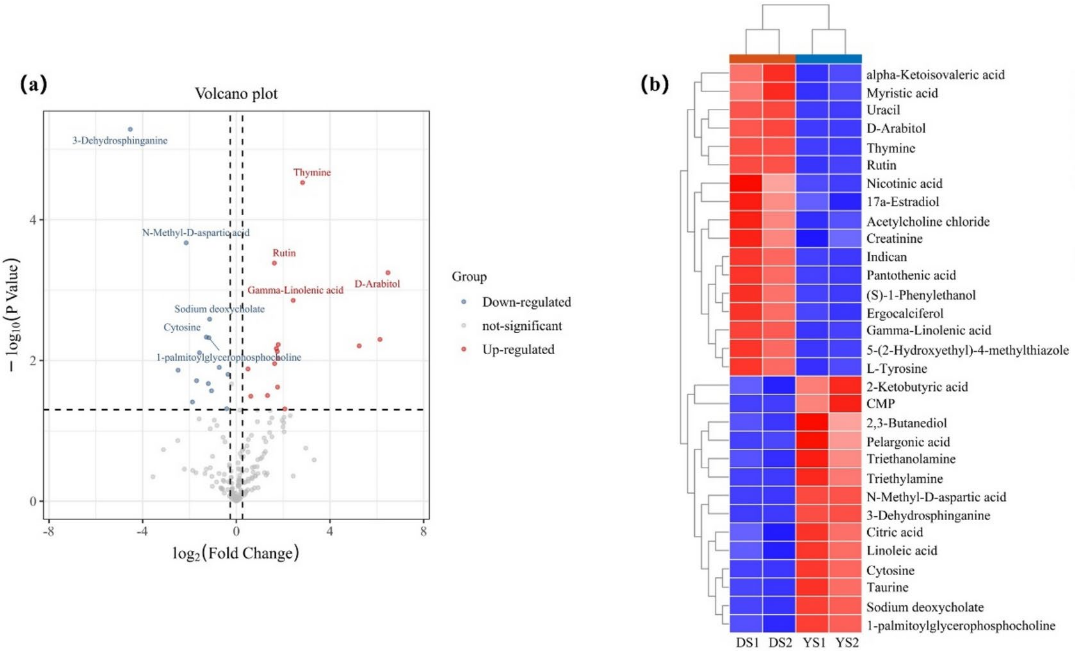


Fig. 3. Degradation of wastewater by *Pseudomonas aeruginosa* alone (**a**) volcano diagram, (**b**) heatmap. DS: wastewater degraded by *Pseudomonas aeruginosa* alone, YS: raw wastewater).

phosphate, tetradecamethylcycloheptasiloxane, and 1-undecanol, among others. By comparing Table S1 and Table S2, it is evident that 3,4-dimethylbenzaldehyde, α-patchoulene, 4-hydroxyneoisomenthol, tricyclopentadeca-3,7-dien[8.4.0.1(11,14)], 3,5,5- trimethylhexyl methylphosphonofluoridate, dichloroacetic acid, 2-(1-adamantyl) ethyl ester, and triethyl ethyl ester were present in the raw wastewater, but these substances disappeared after the degradation process. The contents of 3,5,5-Trimethylhexyl methylphosphonofluoridate, dichloroacetic acid, 2-(1-adamantyl)ethyl ester, and triethyl phosphate decreased. This indicates that *Pseudomonas aeruginosa* can effectively degrade organic matter in wastewater, including phosphorus-containing compounds.

It was evident through metabolomics that, after the degradation of wastewater by *Pseudomonas aeruginosa*, uracil, pantothenic acid, thymine, nicotinic acid, acetylcholine chloride, creatinine, and other metabolites were up-regulated, while cytidine monophosphate (CMP), citric acid, cytosine, taurine, pelargonic acid, and other metabolites were down-regulated (Fig. 3). Nicotinic acid increased during metabolism, which is produced by the metabolism of nicotinic acid salts and nicotinamide. Nicotinic acid promoted cellular metabolism, enhanced microbial activity, thus improving the degradation of wastewater[31]. The down-regulation of CMP indicates that phosphorus-containing substances in the wastewater were degraded by the microorganisms, which led to a decrease in the TP content of the wastewater.

In the Kyoto Encyclopedia of Genes and Genomes (KEGG) repository, all the differential metabolites of the different control groups were matched with the KEGG repository to obtain information on the pathways in which the metabolites were involved[32]. As shown in Fig. 4, during the degradation of wastewater by *Pseudomonas aeruginosa*, nicotinate and nicotinamide metabolism, citric acid cycle, and taurine metabolism are the main metabolism processes of organic matter. sample (Fig. 3) is consistent with the metabolism of nicotinic acid and nicotinamide to generate products, which suggests that nicotinic acid is produced during the degradation

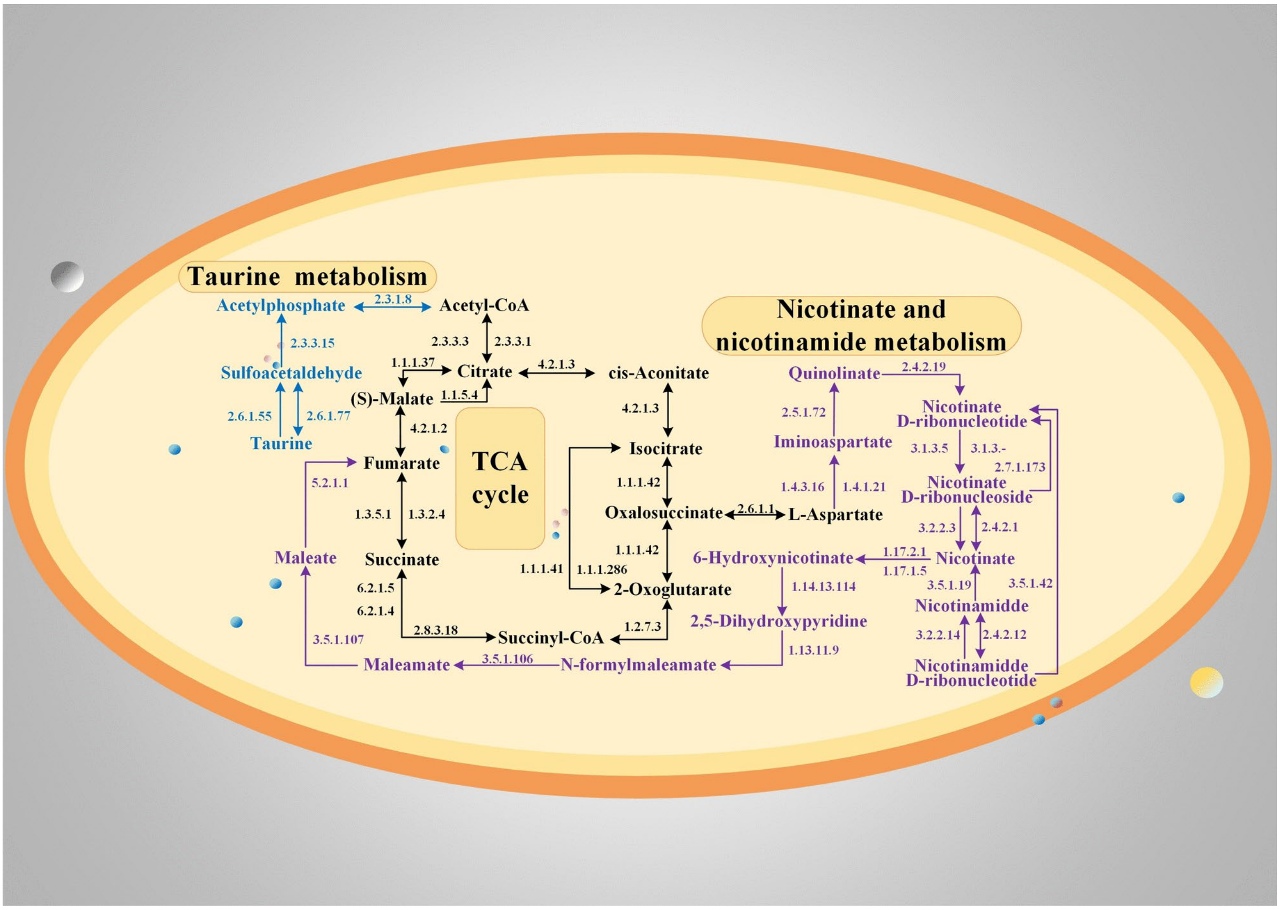


Fig. 4. Metabolic pathway of nicotinate and nicotinamide metabolism, citric acid cycle, taurine metabolic during degradation.

process. Nicotinic acid was obtained from upstream nicotinamide under the metabolism of nicotinic acid salt and nicotinamide, and nicotinic acid can promote cell metabolism, increase microbial activity[33]. Under the metabolism of nicotinate and nicotinamide, nicotinic acid is obtained from upstream nicotinamide, which promotes cellular metabolism and increases microbial activity, thereby accelerating COD and phosphate degradation. The tricarboxylic acid (TCA) cycle is the central metabolic network of organisms, closely associated with the metabolic process of sugar, lipids, and proteins and it provided precursors for energy metabolism[34]. The energy produced by the TCA cycle supports the growth and metabolic activities of *Pseudomonas aeruginosa*, especially involved in the conversion of phosphate into components of the organisms or the precipitation of phosphate minerals, and the TCA cycle is directly involved in the mineralization of organic matter in the wastewater, which breaks down the complex organisms into carbon dioxide and water, resulting in a significant reduction of COD. During taurine metabolism, *Pseudomonas aeruginosa* is able to utilize taurine as a carbon source and energy source to reduce the total organic carbon concentration in wastewater[35], thereby reducing COD.

Degradation of wastewater by MFC containing *Pseudomonas aeruginosa*

Figure 5 shows the effect of the MFC enhancing the degradation of high-phosphorus-containing wastewater after one full cycle of an operation of a single-chamber MFC.

As can be seen in Fig. 5, compared to the degradation of wastewater by *Pseudomonas aeruginosa* alone (Fig. 2), the addition of the bacterial solution to the MFC resulted in higher degradation efficiency. This may be attributed to the abundant microbial community in the activated sludge of MFC, which promotes the degradation of wastewater[36]. Additionally, higher voltage contributes to increased microbial activity, which further supports degradation. Elevated voltage enhances the surface hydrophobicity of microorganisms, promotes the migration of negatively charged sludge, facilitates contact between sludge and substrates, improves the ability of bacteria to extract nutrients from wastewater, and provides more energy for bacterial growth and reproduction. This in turn promotes metabolic activity and degradation efficiency ([37]). It was obvious that MFC with added *Pseudomonas aeruginosa* had higher removal efficiencies for COD, TP, and phosphate than MFC without added *Pseudomonas aeruginosa*, regardless of whether it operates in open or closed circuit. Moreover, closed-circuit operation MFC had higher removal efficiencies than open-circuit operation, regardless of whether *Pseudomonas aeruginosa* was added or not. The high removal efficiency in the closed-circuit operation was due to the higher species

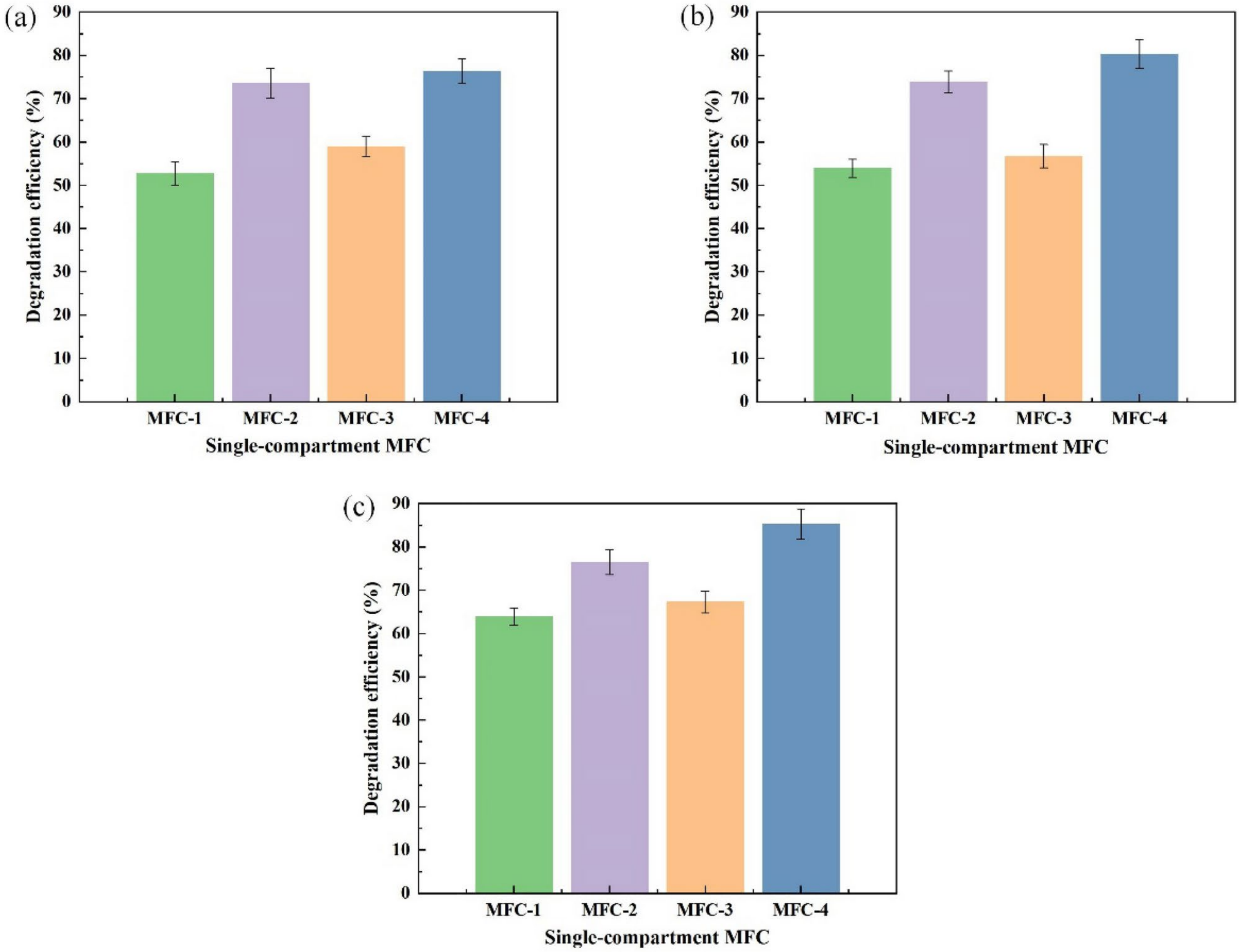
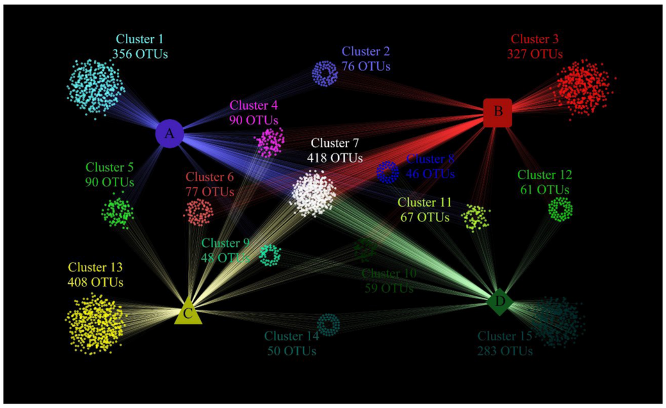


Fig. 5. Degradation efficiency of COD (a), TP (b), phosphate (c).

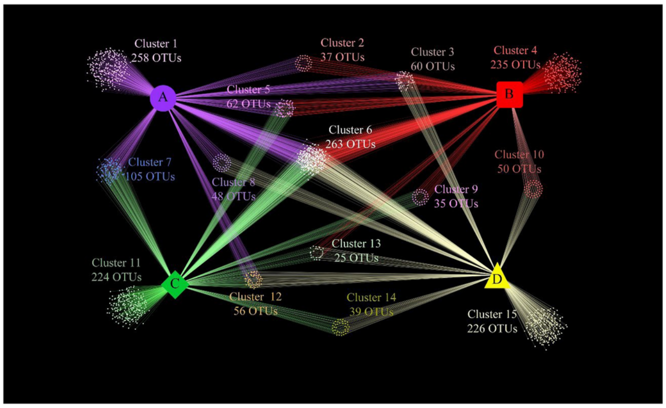
abundance, which had a positive effect on degradation[38]. A total of 1,153,287 high-quality sequences were acquired from the sludge, anode and cathode of the four MFCs. In the sludge of MFC-1 and MFC-2, 356 (Cluster 1) and 327 (Cluster 3) OTUs were unique (Fig. 6a), while 258 (Cluster 1) and 235 (Cluster 3) OTUs were unique to the anode (Fig. 6b), and 26 (Cluster 1) and 221 (Cluster 3) OTUs were unique to the cathode (Fig. 6c). In MFC-3 and MFC-4, 408 (Cluster 13) and 283 (Cluster 15) OTUs were unique to the sludge (Fig. 6a), 224 (Cluster 11) and 226 (Cluster 15) OTUs were unique to the anode (Fig. 6b), and 651 (Cluster 13) and 531 (Cluster 15) OTUs were unique to the cathode (Fig. 6c). These findings indicate that the sludge, anode, and cathode in both open- and closed-circuit MFCs exhibit distinct microbiota compositions. Community diversity showed a high degree of consistency across samples for each tested condition. The species abundance (Chao 1 and ACE, as shown in Table S3) in the sludge, anode and cathode of the closed-circuit MFC was higher than that of the open-circuit MFC. A PCoA (Fig. S2) also exhibited distinctions between the in the sludge, anode and cathode of open-circuit MFC and closed-circuit MFC. And it can be seen that there is a marked separation between the MFCs with added *Pseudomonas aeruginosa* and the MFC without *Pseudomonas aeruginosa*.

Cai et al.[39] also pointed out that electrical current in MFCs shaped microbiomes, especially biofilms, and thus improved pollutant removal efficiency. MFCs were dominated by the phyla *Proteobacteria*, *Firmicutes*, *Actinobacteriota* and *Bacteroidetes* (Fig. 7). The majority of phyla in anode belonged to *Proteobacteria* (41.47–93.81% relative abundance) in closed-circuit MFCs and *Firmicutes* (11.54–54.47%) in open-circuit MFCs. Moreover, as can be seen in Fig. 7, in the classification level of the genus bacteria, there are differences in the dominant populations between the closed-circuit MFCs and open-circuit MFCs. In the cathode of closed-circuit MFCs, *Pseudomonas* showed a higher relative abundance than in open-circuit MFCs, with relative abundances ranging from 3.37% to 9.54%. Additionally, in the anode of closed-circuit MFCs, *Enterobacter* showed a higher relative abundance than in open-circuit MFCs.

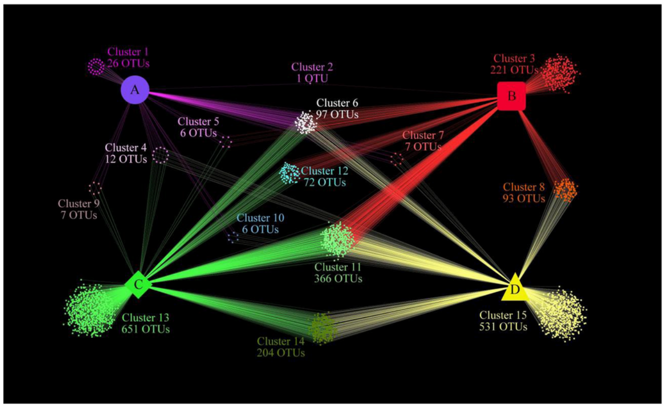
In MFC-4, the removal efficiencies of COD, TP and phosphate are the highest, reaching 76.3 ± 2.8%, 80.3 ± 3.3%, and 85.3 ± 3.5%, respectively. It is clear that the addition of *Pseudomonas aeruginosa* and the device state of a closed-circuit are the best conditions for the degradation of wastewater. MFC-4 was injected with *Pseudomonas aeruginosa*, which, on the one hand, can promote the degradation of wastewater to produce electrons, and, on the other hand, can cause a stable cycle of power production as the bacterium has been shown



(a) Venn network of microbiomes in sludge of MFCs, Nodes A, B, C and D represent the sludge of MFC-1, MFC-2, MFC-3 and MFC-4, respectively.

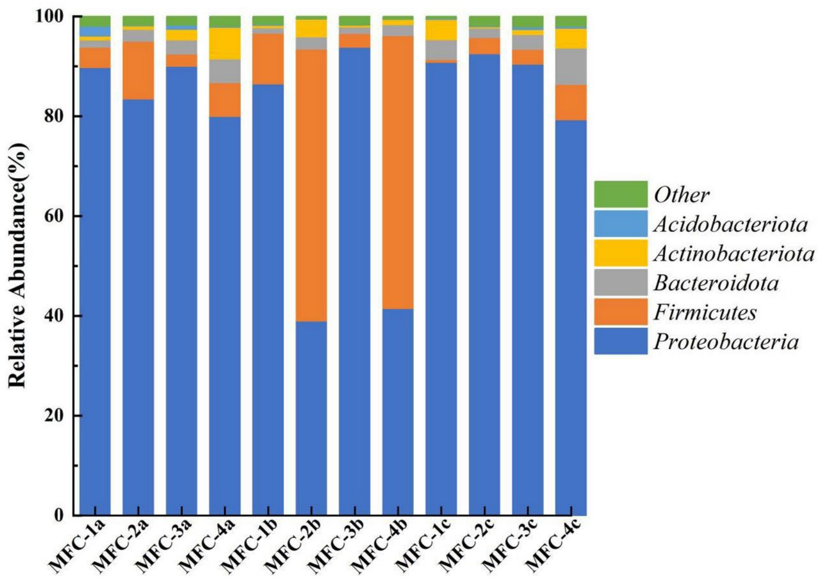


(b) Venn network of microbiomes in anode of MFCs, Nodes A, B, C and D represent the anode of MFC-1, MFC-2, MFC-3 and MFC-4, respectively.

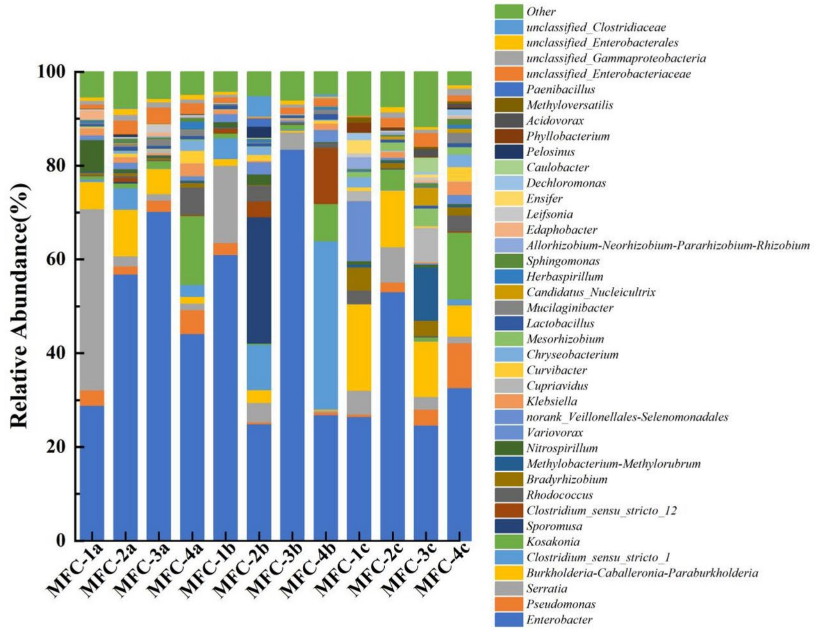


(c) Venn network of microbiomes in cathode of MFCs, Nodes A, B, C and D represent the cathode of MFC-1, MFC-2, MFC-3 and MFC-4, respectively.

Fig. 6. Venn network of microbiomes. (**a**) Venn network of microbiomes in sludge of MFCs, Nodes A, B, C and D represent the sludge of MFC-1, MFC-2, MFC-3 and MFC-4, respectively. (**b**) Venn network of microbiomes in anode of MFCs, Nodes A, B, C and D represent the anode of MFC-1, MFC-2, MFC-3 and MFC-4, respectively. (**c**) Venn network of microbiomes in cathode of MFCs, Nodes A, B, C and D represent the cathode of MFC-1, MFC-2, MFC-3 and MFC-4, respectively.



(a) Composition and abundance of microbiomes at phyla level (a, b and c represent the sludge, anode, cathode, respectively)



(b) Composition and abundance of microbiomes at classification level (a, b and c represent the sludge, anode, cathode, respectively)

Fig. 7. The composition and relative abundance of microbiomes. (**a**) Composition and abundance of microbiomes at phyla level (a, b and c represent the sludge, anode, cathode, respectively). (**b**) Composition and abundance of microbiomes at classification level (a, b and c represent the sludge, anode, cathode, respectively).

to transfer electrons by contacting the electrodes directly or by secreting electronic intermediates[40,41]. As shown in Figs. S3 and S4, the maximum output voltage (57.7 ± 4.4 mV) and power density (1.9 ± 0.1 mW/m^2) of MFC-4 are 15.3% and 90.2% higher than those of MFC-3, respectively. The internal resistance of MFC-4 (360 Ω) has decreased by 86.32% compared to MFC-3 (Fig. S5). This may be due to the addition of *Pseudomonas aeruginosa* improving the power generation performance of MFC.

As is known, *Pseudomonas aeruginosa* is a type of electrogenic bacterium[42–44] that is present on the activated sludge, anode, and cathode of MFC-4 (Fig. 7). The relative abundance (9.54%) of *Pseudomonas aeruginosa* in MFC-4 was higher than that of the other MFCs. Arkatkar et al.[45] demonstrated the ability of *Pseudomonas aeruginosa* to increase the voltage of an MFC by inoculating anaerobic sludge with *Pseudomonas aeruginosa* to enhance the power production of the MFC. It is thus proven that the addition of specific enriched and domesticated bacterial strains is able to increase the power generation of MFCs. These added strains exhibit an ecological advantage within the microbial cell[46], enabling them to adhere more densely to the surfaces of the cathode and anode electrodes (as shown in Fig. S6) as well as facilitating metabolic activity, thus enhancing the MFC's ability to produce electricity. Therefore, with the addition of bacterial strains, which can effectively promote the growth of microbial communities. It is worth noting that the species abundance (Chao 1 and ACE) of MFC-3 is greater than that of MFC-4, which may be due to the fact that the natural microbial community has higher functional redundancy and ecological niche differentiation, as well as greater resilience and adaptability. The introduction of specific strains may disrupt the original ecological balance and lead to competitive disadvantages for certain microorganisms, thus reducing species abundance[47]. However, the microbial diversity (Shannon) and evenness (Shannoneven) of the sludge and anode in MFC-4 were higher than those in MFC-3, indicating that the addition of *Pseudomonas aeruginosa* increased the stability of the microbial community in MFC-4. Therefore, MFC-4 has higher electricity generation and wastewater degradation efficiency.

As shown in Table 2, although the output voltage and power density of MFC-4 were lower than those reported by Taşkan et al.[48] and Hu et al.[49], the degradation efficiencies of COD, TP and PO_4^{3-}-P in this study were relatively high. These results highlight the potential of using MFCs injected with specific bacteria for wastewater treatment, even under suboptimal conditions for power generation. These findings underscore the effectiveness of *Pseudomonas aeruginosa* in enhancing the degradation efficiency of the MFC.

To further contextualize these results, we compared them with previous studies. As shown in Table 2, a notable limitation of this study is the relatively low C_E of the MFC-4, which was only 4.40 ± 0.16%. This value is significantly lower than the C_E of 12.8% and 13% achieved for cottonseed effluent and municipal sludge, respectively, as reported by Taşkan et al.[48,50]). The low C_E of the MFC constructed in this study can be attributed to several factors. Firstly, the air cathode may allow oxygen to infiltrate, disrupting the anaerobic environment and reducing the activity of electroproducing microorganisms, thereby lowering electroproduction efficiency. Secondly, electrons generated by anaerobic microorganisms during organic matter degradation are directly consumed by oxygen, decreasing the number of electrons transferred to the cathode[51]. Thirdly, oxygen oxidizes part of the organic matter that should have been oxidized by microorganisms, resulting in material loss and reduced efficiency[52]. Finally, the presence of non-electroproducing microorganisms (e.g., methanogens) in the MFC, which do not generate electrical energy during COD degradation, further contributing to the low C_E and power density[53]. Despite this limitation, the MFC-4 demonstrated high removal efficiencies for COD, TP and PO_4^{3-}-P, which are comparable to or exceed those reported in previous studies.

Analysis of degradation mechanisms

To further investigate the mechanism of wastewater degradation by closed-circuit MFC with *Pseudomonas aeruginosa*, the main differential metabolites before and after degradation were selected for analysis. As shown in Fig. 8, the percentage of nicotinic acid increased in the sample, which is consistent with the metabolism of nicotinic acid and nicotinamide to generate products, indicating that nicotinic acid was produced in the course of the experiment. Nicotinic acid was obtained from upstream nicotinamide under the metabolism of nicotinic acid salt and nicotinamide; it promotes cellular metabolism, increases microbial activity, and allows microorganisms to attach more tightly to the carbon plate of the single-chamber MFC electrode, which promotes the degradation of cytidylic acid.

The analysis of the metabolic pathways (Fig. 9) allowed us to derive a mechanism of action for the addition of *Pseudomonas aeruginosa* to the single-chamber MFC for the treatment of wastewater generated during the production of cytidylic acid, particularly their capacity in the decomposition and transformation of organic matter, including the effect on hydroxyl-containing, carbonyl-containing, and phosphorus-containing compounds. These changes may reflect the ability of *Pseudomonas aeruginosa* to degrade, transform, or synthesize new compounds of specific organics in wastewater. For phosphorus in wastewater, the following metabolic actions occur primarily in the degradation of CMP, one of the phosphorus-containing pollutants. For example, in the pyrimidine metabolic pathway, CMP is converted to uridylic acid (UMP) by the action of cytidylic acid deaminase, and UMP is hydrolyzed to uridine (Urd) and inorganic phosphoric acid (Pi) by

Wastewater	Voltage (mV)	Power density (mW/m^2)	C_E	Degradation efficiency	References
Cottonseed effluent	193	187	12.8%	COD removal efficiency of 66.6%	[48]
Aquaculture wastewater	32.94	102.38	–	COD, and TP removal efficiencies were 80.30 ± 3.4%, and 54.44 ± 4.5%, respectively	[49]
Municipal sludge and wastewater	–	160	13%	The percentage of total chemical oxygen demand (TCOD), TN and TP were reduced by 67% (4100 mg/L), 45% (30,280 mg/kg) and 52% (7620 mg/kg), respectively	[50]
Wastewater generated during the production of cytidine acid	57.7 ± 4.4	1.9	4.40 ± 0.16%	COD, TP and PO_4^{3-}-P removal efficiencies of 76.3 ± 2.8%, 80.3 ± 3.3% and 85.3 ± 3.5%, respectively	MFC-4 in this study

Table 2. The performance of different MFCs.

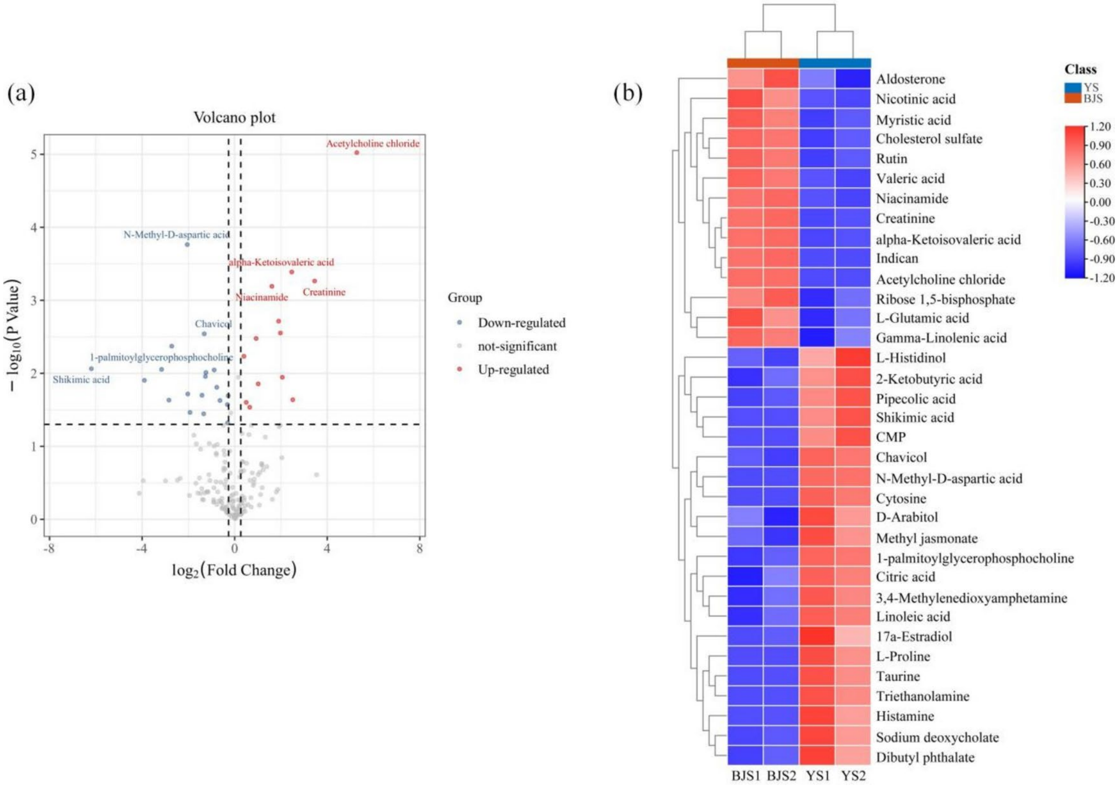


Fig. 8. Degradation of wastewater by MFC-4 (**a**) volcano diagram, (**b**) heatmap. BJ: wastewater degraded by MFC-4, YS: raw wastewater.

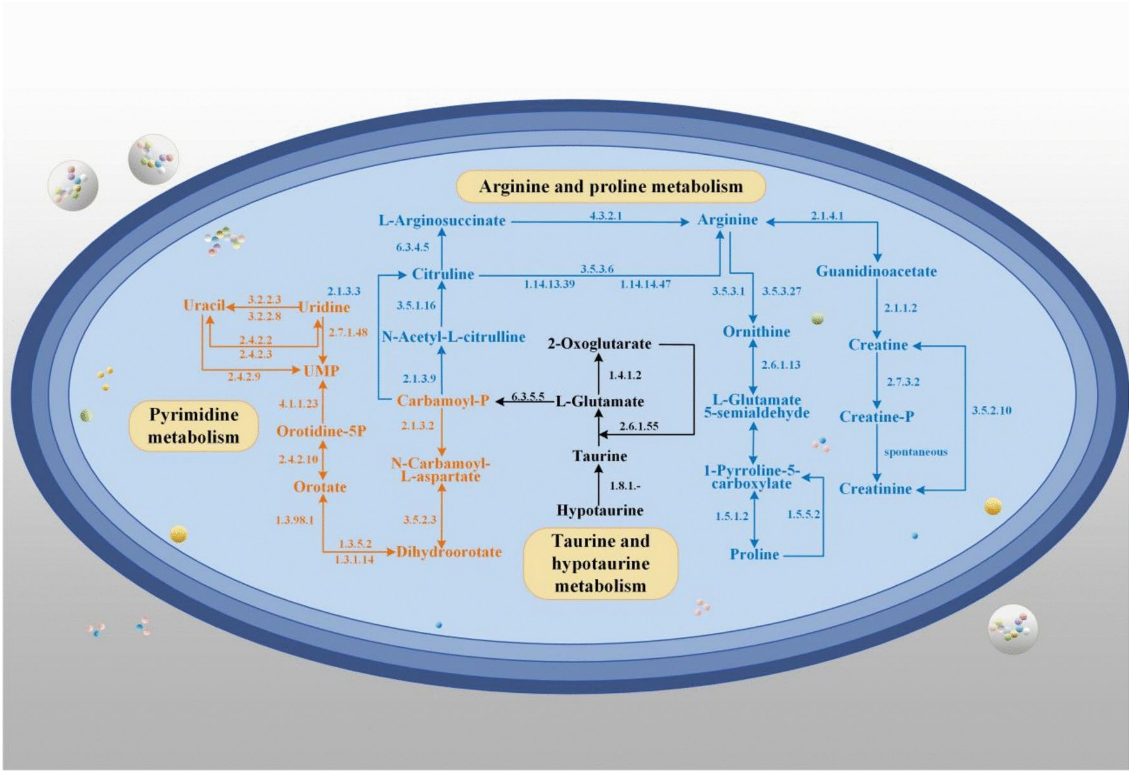


Fig. 9. Metabolic pathway of pyrimidine metabolism, arginine and proline metabolism, Taurine and hypotaurine metabolism during degradation.

uridine diphosphatase and uridine monophosphatase; additionally, uridine's phosphoric acid moiety is removed by uridine phosphorylase, producing uracil. The reaction during the microbial degradation of wastewater is thus demonstrated.

The major metabolic pathways (Fig. 4) relevant in the treatment of wastewater generated during the production of cytidylic acid with *Pseudomonas aeruginosa* alone are not the same as the major metabolic pathways involved in the degradation of wastewater when *Pseudomonas aeruginosa* is added to a single-chamber MFC. When *Pseudomonas aeruginosa* was added to the complex microbial community of the MFC, the population structure of the original sludge colony in the MFC was optimized to a certain extent, thus indicating that the MFC degraded wastewater more efficiently than *Pseudomonas aeruginosa* alone.

This study demonstrates a prospective strategy to combine specific microbial strains with MFC technology to promote the practical application of MFCs in the efficient degradation of wastewater generated during the production of cytidine acid. Through careful screening and cultivation of special strains of microorganisms, not only can they efficiently decompose complex organic pollutants, but they can also significantly enhance the electron transfer process within the MFC, effectively converting chemical energy into electrical energy, thus increasing the activity of the strains and improving the efficiency of wastewater degradation. This innovative approach not only optimizes the resource recovery process and reduces environmental pollution, but also provides a new sustainable and energy self-sufficient pathway for industrial wastewater treatment.

In terms of environmental benefits, MFC technology effectively removes phosphorus from wastewater, mitigates eutrophication, and supports ecological restoration[54]. Additionally, it reduces energy consumption and greenhouse gas emissions associated with traditional wastewater treatment, contributing to green and sustainable development. Economically, MFC technology lowers treatment costs through energy recovery, reduces reliance on external resources, and fosters the growth of green technology industries. As a result, it offers lower long-term operating costs and potential economic returns[55]. The introduction of specific microorganisms can significantly improve the efficiency of MFC in wastewater treatment. Microorganisms are able to degrade organic pollutants while generating electrical energy for the dual purpose of treating wastewater and pollutants. In this way, the MFC not only reduces the energy consumption of the wastewater treatment process, but also has the ability to degrade the wastewater more efficiently. The operation of the MFC contributes fundamentally to the energization of wastewater, which is in line with the concept of a closed-loop economy[56]. The selective addition of microorganisms (such as *Pseudomonas aeruginosa*) with high degradation capacity enables rapid and effective degradation of specific pollutants[57]. The addition of microorganisms that may have a wider range of metabolic pathways and be able to process a wider variety of organics increases the adaptability and capacity of the MFC for complex pollutants. The addition of microorganisms may optimize the microbial community structure in the sludge of the MFC and accelerate the degradation of wastewater by the MFC.

However, a notable limitation of this study is the relatively low power density achieved by the MFC. While the system demonstrated effective wastewater treatment and electricity generation, the power density remains below the threshold required for practical applications. This limitation highlights the need for further advancements in MFC technology. Strategies such as optimizing electrode materials, increasing the surface area of electrodes, enhancing microbial electrochemical activity, and refining operational conditions could potentially address this issue. Future studies should prioritize these improvements to enhance the feasibility and scalability of MFC for real-world applications.

Conclusions

In this study, *Pseudomonas aeruginosa*-supplemented MFC achieved maximum degradation efficiencies of COD, TP, and phosphate at 76.3 ± 2.8%, 80.3 ± 3.3%, and 85.3 ± 3.5%, respectively. The biofilm formed on the electrode enriched electroactive bacteria (*Pseudomonas aeruginosa*), enhancing the electroproduction function of the MFC and increasing microbial activity, thereby improving the wastewater degradation efficiency. Additionally, a Venn network analysis based on OTU interactions revealed that the microbial community on the electrode biofilm in the MFC exhibited high complexity and stability. The metabolic pathways contributing to wastewater degradation by *Pseudomonas aeruginosa* included pyrimidine metabolism and arginine and proline metabolism, which effectively degrade organic pollutants in the wastewater. A limitation of this study is the relatively low power density of the MFC. Despite its ability to treat wastewater and generate electricity, the power density achieved in this system is below the levels required for practical applications. Therefore, future research should focus on enhancing the power density of MFCs, which could be achieved by exploring strategies such as optimizing electrode materials, increasing the surface area of electrodes, improving microbial electrochemical activity, and refining operational conditions.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

Received: 16 November 2024; Accepted: 12 February 2025

References

1. Zhou, M. et al. Systematic engineering of *Escherichia coli* for efficient production of cytidine 5′-monophosphate. *ACS Omega* **9**(6), 6663–6668 (2024).
2. Wu, S. et al. Concomitant use of immobilized uridine-cytidine kinase and polyphosphate kinase for 5'-cytidine monophosphate production. *Sheng wu Gong Cheng xue bao Chin. J. Biotechnol.* **36**(5), 1002–1011 (2020).

3. Yang, M. et al. Phosphorus removal and recovery from fosfomycin pharmaceutical wastewater by the induced crystallization process. *J. Environ. Manag.* **231**, 207–212 (2019).
4. Yang, L. et al. Simultaneous removal of nitrogen and phosphorous by heterotrophic nitrification-aerobic denitrification of a metal resistant bacterium *Pseudomonas putida* strain NP5. *Bioresour. Technol.* **285**, 121360 (2019).
5. Sun, C. et al. In vitro biosynthesis of ATP from adenosine and polyphosphate. *Bioresour. Bioprocess.* **8**, 1–10 (2021).
6. Li, Y. et al. Advances in the treatment of phosphorus-containing wastewater. IOP Conference Series: Earth and Environmental Science. *IOP Publish.* **647**(1), 012163 (2021).
7. Li, B. et al. Simultaneous removal of nitrogen and phosphorus by a novel aerobic denitrifying phosphorus-accumulating bacterium, *Pseudomonas stutzeri* ADP-19. *Bioresour. Technol.* **321**, 124445 (2021).
8. Acelas, N. Y. et al. Selective removal of phosphate from wastewater using hydrated metal oxides dispersed within anionic exchange media. *Chemosphere* **119**, 1353–1360 (2015).
9. Nayeem, A. et al. Utilization of cockle shell powder as an adsorbent to remove phosphorus-containing wastewater. *Environ. Res.* **216**, 114514 (2023).
10. Yu, X., Yang, H. & Sun, L. Kinetics study on the degradation of tri-ethyl phosphate in O_3, UV, and UV/O_3 treatments. *Water Air Soil Pollut.* **227**, 1–7 (2016).
11. Pan, S. et al. Simultaneous degradation of antibiotic and removal of phosphate in water by a O_3/CaO_2 advanced oxidation process. *Sep. Purif. Technol.* **312**, 123452 (2023).
12. Tian, J. et al. Aerobic denitrification of *Pseudomonas stutzeri* yjy-10 and genomic analysis of this process. *Appl. Biochem. Microbiol.* **58**(3), 294–301 (2022).
13. He, X. et al. Process energy and material consumption determined by reaction sequence: From AAO to OHO. *Water* **16**(13), 1796 (2024).
14. Hu, Z. et al. Exerting applied voltage promotes microbial activity of marine anammox bacteria for nitrogen removal in saline wastewater treatment. *Water Res.* **215**, 118285 (2022).
15. Logan, B. Microbial fuels for the future. *Nat. Int. Wkly. J. Sci.* **454**(7207), 943–944 (2008).
16. Huang, S. et al. Electric field effect of microbial fuel cells on biological reactions: A review. *Int. Biodeterior. Biodegrad.* **194**, 105886 (2024).
17. Patel, D. et al. Electroactive bacterial community augmentation enhances the performance of a pilot scale constructed wetland microbial fuel cell for treatment of textile dye wastewater. *Bioresour. Technol.* **332**, 125088 (2021).
18. Zhang, X. et al. Simultaneous removal of organic matter and iron from hydraulic fracturing flowback water through sulfur cycling in a microbial fuel cell. *Water Res.* **147**, 461–471 (2018).
19. Al-Ansari, M. M., Benabdelkamel, H. & Al-Humaid, L. Degradation of sulfadiazine and electricity generation from wastewater using *Bacillus subtilis* EL06 integrated with an open circuit system. *Chemosphere* **276**, 130145 (2021).
20. Ren, Y. X., Yang, L. & Liang, X. The characteristics of a novel heterotrophic nitrifying and aerobic denitrifying bacterium, *Acinetobacter junii* YB. *Bioresour. Technol.* **171**, 1–9 (2014).
21. Huang, L. et al. A monolithic three-dimensional macroporous graphene anode with low cost for high performance microbial fuel cells. *RSC Adv.* **6**(25), 21001–21010 (2016).
22. Raad, N. K. et al. Simultaneous power generation and sewage sludge stabilization using an air cathode-MFCs. *Biomass Bioenergy* **140**, 105642 (2020).
23. SEPA. *Water and Wastewater Monitoring Analysis Method,* 4th edn. (China Environmental Science Press, 2002).
24. Gil-Carrera, L. et al. Performance of a semi-pilot tubular microbial electrolysis cell (MEC) under several hydraulic retention times and applied voltages. *Bioresour. Technol.* **146**, 63–69 (2013).
25. Wang, J. et al. Effect of liquid volume and microflora source on degradation rate and microbial community in corn stover degradation. *AMB Express* **11**, 1–12 (2021).
26. Krainara, S. et al. Bioaugmentation of activated sludge with the immobilized 2-mercaptobenzothiazole-degrading bacterial consortium for rubber industrial wastewater treatment. *J. Water Process Eng.* **55**, 104129 (2023).
27. Liu, F. et al. Remediation of petroleum hydrocarbon-contaminated groundwater by biochar-based immobilized bacteria. *Biochem. Eng. J.* **197**, 108987 (2023).
28. Schurer, R., Hijnen, W. A. M. & Van Der Wal, A. The significance of the biomass subfraction of high-MW organic carbon for the microbial growth and maintenance potential of disinfectant-free drinking water produced from surface water. *Water Res.* **209**, 117898 (2022).
29. Li, C. et al. Removal of nitrogen by heterotrophic nitrification–aerobic denitrification of a phosphate accumulating bacterium *Pseudomonas stutzeri* YG-24. *Bioresour. Technol.* **182**, 18–25 (2015).
30. Shu, H. et al. Simultaneous aerobic nitrogen and phosphate removal capability of novel salt-tolerant strain, *Pseudomonas mendocina* A4: Characterization, mechanism and application potential. *Bioresour. Technol.* **393**, 130047 (2024).
31. Ajunwa, O. M. et al. Evaluation of extracellular electron transfer in *Pseudomonas aeruginosa* by co-expression of intermediate genes in NAD synthetase production pathway. *World J. Microbiol. Biotechnol.* **38**(5), 90 (2022).
32. Kanehisa, M. et al. KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Res.* **51**(D1), D587–D592 (2023).
33. Huang, J. et al. The ratio of nicotinic acid to nicotinamide as a microbial biomarker for assessing cell therapy product sterility. *Mol. Ther.-Methods Clin. Dev.* **25**, 410–424 (2022).
34. Gu, X. et al. Influence mechanism of C/N ratio on heterotrophic nitrification-aerobic denitrification process. *Bioresour. Technol.* **343**, 126116 (2022).
35. Duszka, K. Versatile triad alliance: bile acid, taurine and microbiota. *Cells* **11**(15), 2337 (2022).
36. Miran, F. & Mumtaz, M. W. Complex waste acclimatized mixed culture enhances bioelectricity generation and tannery wastewater treatment in microbial fuel cells. *Biomass Convers. Biorefin.* **13**(14), 12467–12473 (2023).
37. Zeyoudi, M. et al. Impact of continuous and intermittent supply of electric field on the function and microbial community of wastewater treatment electro-bioreactors. *Electrochim. Acta* **181**, 271–279 (2015).
38. Feng, L. I. et al. Treatment effect of wastewater containing antibiotic and microbial community response in microbial fuel cell integrated with constructed wetland under different circuit operation mode. *Chin. J. Environ. Eng.* **15**(9), 3038–3048 (2021).
39. Cai, X., Zhou, H., Lou, Y. *et al*. Microbiome and antibiotic resistome in bioelectrochemical toilets for onsite treatment of fecal sludge. *Water Res.* 121956 (2024).
40. Xie, T. et al. Degradation of nitrobenzene-containing wastewater by a microbial-fuel-cell-coupled constructed wetland. *Ecol. Eng.* **112**, 65–71 (2018).
41. Zani, A. C. B. et al. Electrobiochemical skills of *Pseudomonas aeruginosa* species that produce pyocyanin or pyoverdine for glycerol oxidation in a microbial fuel cell. *Chemosphere* **335**, 139073 (2023).
42. Lim, C. E. et al. Predicting microbial fuel cell biofilm communities and power generation from wastewaters with artificial neural network. *Int. J. Hydrogen Energy* **52**, 1052–1064 (2024).
43. Lu, Y. et al. Co-substrate-assisted dimethyl sulfide degradation and electricity generation in a microbial fuel cell. *Energy Fuels* **36**(1), 514–520 (2021).
44. Surti, P., Kailasa, S. K. & Mungray, A. K. Enhancement of electrode properties using carbon dots functionalized magnetite nanoparticles for azo dye decolorization in microbial fuel cell. *Chemosphere* **313**, 137601 (2023).
45. Arkatkar, A., Mungray, A. K. & Sharma, P. Biological modification in air-cathode microbial fuel cell: Effect on oxygen diffusion, current generation and wastewater degradation. *Chemosphere* **284**, 131243 (2021).

46. Narayanasamy, S. & Jayaprakash, J. Improved performance of *Pseudomonas aeruginosa* catalyzed MFCs with graphite/polyester composite electrodes doped with metal ions for azo dye degradation. *Chem. Eng. J.* **343**, 258–269 (2018).
47. Salar-Garcia, M. J. et al. Impact of inoculum type on the microbial community and power performance of urine-fed microbial fuel cells. *Microorganisms* **8**(12), 1921 (2020).
48. Taşkan, E., Özkaya, B. & Hasar, H. Comprehensive evaluation of two different inoculums in MFC with a new tin-coated copper mesh anode electrode for producing electricity from a cottonseed oil industry effluent. *Environ. Progr. Sustain. Energy* **35**(1), 110–116 (2016).
49. Hu, Z. et al. Treatment of aquaculture wastewater by C-MFCs. *Chem. Eng. Process. Process Intensif.* **194**, 109579 (2023).
50. Taşkan, B., Taşkan, E. & Hasar, H. Electricity generation potential of sewage sludge in sediment microbial fuel cell using Ti–TiO_2 electrode. *Environ. Progr. Sustain. Energy* **39**(5), e13407 (2020).
51. Liu, J. D. et al. Integration of bio-electrochemical cell in membrane bioreactor for membrane cathode fouling reduction through electricity generation. *J. Membr. Sci.* **430**, 196–202 (2013).
52. Yao, S. et al. Effect of the membrane electrode assemble design on the performance of single chamber microbial fuel cells. *Energy Proc.* **61**, 1947–1951 (2014).
53. Malamis, S. et al. Assessment of metal removal, biomass activity and RO concentrate treatment in an MBR-RO system. *J. Hazard. Mater.* **38**(1), 23–31 (2012).
54. Hamedani, E. A., Abasalt, A. & Talebi, S. Application of microbial fuel cells in wastewater treatment and green energy production: A comprehensive review of technology fundamentals and challenges. *Fuel* **370**, 131855 (2024).
55. Srivastava, R. K., Boddula, R. & Pothu, R. Microbial fuel cells: Technologically advanced devices and approach for sustainable/renewable energy development. *Energy Convers. Manag. X* **13**, 100160 (2022).
56. Kundu, D. et al. Valorization of wastewater: A paradigm shift towards circular bioeconomy and sustainability. *Sci. Total Environ.* **848**, 157709 (2022).
57. Al-saned, A. J. O., Kitafa, B. A. & Badday, A. S. Microbial fuel cells (MFC) in the treatment of dairy wastewater. IOP Conference Series: Materials Science and Engineering. *IOP Publish.* **1067**(1), 012073 (2021).

Acknowledgements

This work was supported by a grant from Changzhou Longcheng Talent Program (CQ20210092).

Author contributions

X.Y. and F.W. wrote the main manuscript text; X.Y. also contributed to visualization, investigation, software, and writing—original draft. F.W. contributed to software, methodology, and writing—review & editing. M.G. and F.Z. supervised the work and contributed to writing—review & editing. M.G. also contributed to conceptualization. G.L. contributed to validation and supervision. All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-90361-0.

Correspondence and requests for materials should be addressed to X.Y.