

Application of Bioelectrochemical Technique in Energy Recovery and CH₄ Control From Constructed Wetland (CW) and Associated Biochemical Mechanisms

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Abstract

In the context of global warming, the bioelectrochemical method (microbial fuel cell MFC) was proposed for CH₄ control from CWs. The main focus is to further explore the effect of plant roots location at the electrode, plant species on CH₄ emissions, bioelectricity generation and the mechanism underlying competition between electrogenesis and methanogenesis at the anode. The results showed that the operation of MFC effectively reduced the CH₄ emissions and promoted COD removal rates. CH₄ emission was significantly higher in open circuit (6.2 mg m⁻² h⁻¹) than in closed circuit reactors (3.1 mg m⁻² h⁻¹). Plant roots at the cathode had the highest electricity generation and the lowest CH₄ emissions. The highest power generation (0.49 V, 0.33 w m⁻³) and the lowest CH₄ emissions (2.3 mg m⁻² h⁻¹) were observed in the reactors where *Typha Orientalis* was planted with plant roots at the cathode. The role of plants in strengthening electron acceptor was greater than that of plant rhizodeposits in strengthening electron donors. q-PCR and correlation analysis indicated that the *mcrA* genes and CH₄ emissions were positively correlated ($r=0.98, p<0.01$), while no significant relationship between CH₄ emissions and *pmoA* genes was observed. More nanowires, which are conductive to electron transfer, were found when plant roots were in cathode by scanning electron microscope (SEM). Illumina sequencing revealed that more abundant exoelectrogens and denitrifying bacteria (*Geobacter*, *Desulfobulbu*, *Nitrospira* and *Anaerolinea*) were observed when plant roots located in cathodes. Strictly acetotrophic archae (Methanosaetaceae) were likely main electron donor competitors with exoelectrogens. In addition, plant species played a more important role in CH₄ emissions and electricity generation than the plant roots location at the electrode. Therefore, it is necessary to strengthen plant configuration to reduce CH₄ emissions, so as to promote sustainable development of wastewater treatment.

1. Introduction

In the context of global warming and the increasingly significant impact of human activities on the environment, greenhouse gas emission reduction, low-carbon economy and low-carbon cities are the research hotspots nowadays [20], [34]. Global climate change caused by the increase of greenhouse gas emissions in the atmosphere has become the focus of human concern, and is also a huge challenge facing the sustainable development of the world economy. Methane is an important greenhouse gas, which is considered to be one of the most important greenhouse gases after CO₂. Its greenhouse effect is 20–30 times that of CO₂ [35], which contributes about 20% of the global climate directly drive [27]. Methane has become an important part of global "carbon reduction".

Constructed wetland (CW) is considered as a reliable wastewater treatment technology, which has the advantages of less investment, less operation cost, easy maintenance and stable effluent quality [29], which plays an important role in non-point source pollution control. However, constructed wetlands will release greenhouse gases such as CO₂, CH₄ and N₂O during operation, which will have a negative impact on the environment. Studies showed that constructed wetlands emit 2 to 10 times more greenhouse

gases than natural wetlands ^[15], its role in global warming cannot be ignored. In recent years, scholars have also carried out research on methane emission reduction in CWs. In general, methane emission control in CWs can be divided into two categories: first, macro control, including physical control methods such as aeration^{[17],[24]}, Chemical methods (e.g. REDOX potential control, iron ion control, chemical substance control)^[13], Hydrologic method (influent pattern and water level variation) etc ^[7]. Another method is micro-control, such as molecular biological methods ^[9] and nano-control ^[5]. So far, however, study on the microscopic control of methane emissions is limited. In particular, the literature on methane control by microbial or bioelectrochemical method is quite limited.

Microbial fuel cell (MFC) is a device that using electrogenic microorganisms to directly convert chemical energy of organic matter into electrical energy. In the past 10 years, a lot of work has been done to improve the electrical performance of MFC, wastewater treatment efficiency, reactor configuration and electrode materials ^{[12],[37],[41]}. But it is a pity that its electricity generation efficiency is still very low and its engineering application is limited. Therefore, the development and application of MFC in the academic community caused controversy and doubt ^[26]. In fact, we can change our thinking and use MFC as a tool rather than as a research purpose. Due to the similar operating conditions of microbial fuel cell and constructed wetland, the integration of MFC with CW technology becomes possible. Since CW-MFC integrated technology was first proposed in 2012^[39], it has been proved in recent years that CW-MFC Technology can greatly improve the sewage treatment efficiency and electricity generation performance^{[22],[38]}. Theoretically, there exists a competitive relationship between electrogenic microorganisms and methanogens in the anodes of MFC and CW, which can inhibit methane emission. Our previous study confirmed that methane emission could be inhibited by running MFC ^[43]. However, the research on the regulation of methane emission in CW by bioelectrochemical method (MFC) is still inadequate and very limited. In addition, there are still many scientific problems to be further explored. It is not clear, for example, whether the type of plant and the location of plant root system on the electrode has an effect on methane emissions, how much, and how it works. In the current CW-MFCs studies, the plant roots were mostly placed at the anode^{[21],[33]}, while in some studies, the plant roots were mostly placed at the cathode. Theoretically, placing them at the anode would provide organic matter for electricity generation, while placing them at the cathode would increase the number of electron receptors needed to generate electricity. However, it has not been reported whether the plant roots placed at the anode or the cathode are more conducive to electricity generation and methane emission.

Therefore, this study aims to further explore the influence mechanism of MFC on CW methane emissions, wastewater treatment and electricity generation based on the above problems. The influence of the electrode position of plant root system on methane emission and electrical performance and associated biochemical processes were mainly explored. The mechanism underlying competition between electrogenesis and methanogenesis at the anode was investigated. The systematic elucidate of the biological process involved in the integrated system (CW-MFC) will provide a theoretical basis for CH₄ control in constructed wetlands and promote the sustainable development of wastewater treatment.

2. Materials And Methods

2.1. MFC-CW construction and operation

Eight polypropylene plastic circular columns with the height of 0.55 m and the diameter of 0.18 cm were adopted as the main reactors to simulate the vertical flow CW-MFC system. Two plant species (*Typha orientalis*, *Cyperus alternifolius*) were planted in anodes and cathodes, respectively. In CW-MFCs planted with *Typha Orientalis*, plant roots located at the cathode and anode were named as P1-Ca and P1-An, respectively. In CW-MFCs planted with *Cyperus alternifolius*, plant roots located at the cathode and anode were named as P2-Ca and P2-An, respectively. The other four reactors operated under open circuit as a control, marked as CK1, CK2, CK3 and CK4. From the bottom to the top of the reactor are: the first layer is 10 cm thick gravel, the second layer is 10 cm thick granular activated carbon GAC (anode layer), the third layer is 20 cm thick gravel, and the fourth layer is 5 cm thick granular activated carbon (cathode layer). Stainless steel wire mesh (12 cm in diameter) is placed in the middle of the anode layer and the cathode layer respectively to collect and transfer electrons. The cathode and anode are connected by titanium wire, and the external copper wire is connected with the resistance box to form a closed loop. The schematic diagram of the CW-MFCs is shown in Fig.1, the control reactors in open circuit are not shown. In this study, the selection of electrode materials, the setting of electrode distance and the configuration were according to the reported literature ^[4], which has good power generation, wastewater treatment capacity and economic feasibility.

Activated sludge was taken from a local wastewater treatment plant. The aerobic activated sludge was inoculated at the cathode of CW-MFCs and the anaerobic activated sludge was inoculated at the anode of CW-MFCs. The reactor operated in a continuous flow mode with water inflow at the bottom. The synthetic wastewater was composed as follows: KCl 130.0 mg/L, Na₂HPO₄·12H₂O 275.0 mg/L, NH₄Cl 133.7 mg/L, NaHCO₃ 313.0, MgSO₄·7H₂O 25.0 mg/L, CaCl₂ 15.0 mg/L, NaH₂PO₄·2H₂O 497.0 mg/L, trace elements 0.1 ml/L. The influent COD was controlled at 300 mg/L. The hydraulic residence time was set according to the experimental requirement. The reactors were operated at 26 ± 2 °C.

2.2 COD measurement and CH₄ fluxes

The chemical oxygen demand (COD) was determined by potassium dichromate method. The methane was collected by a static chamber method and analyzed by a gas chromatograph (PerkinElmer GC Clarus 680). The injection temperature was 85°C, the split ratio was 20:1, the column temperature was 75 °C, and the gasification temperature was 250 °C. The content of CH₄ was calculated by the linear relationship between the peak area and the known content. The methane emission flux was calculated by the following formula:

$$E_{\text{methane}} = \rho \times \frac{V}{A} \times \frac{dc_{\text{methane}}}{dt} \times \frac{273}{273+T}$$

In the equation: E_{methane} – methane emission flux, g/m²/h; ρ – The density of methane in standard condition, 714.3 g/m³; V – Closed volume of collected gas, m³; A – Area of electrode surface, m²; $dc \text{ methane}/dt$ – Change in methane gas in a given time, mg/ mL /h; T – average temperature

2.3 Bioelectricity generation monitoring

Bioelectricity generation performance was measured under the resistance of 1000 Ω by data acquisition system (model 2700, Keithley Inc.). The voltage data was recorded every 5 minutes. The current value is obtained according to voltage and resistance ($I=U/R$). Subsequently, power density (P , W/m³) and current density (J , A/m³) were calculated according to the anode volume. The current density and power density were calculated as $J = U/RA$ and $P = IU/A$, respectively. Where, I is the current, U is the voltage, R is the resistance and A is the working volume of the anode. The polarization curve was determined by changing the external electrical resistance from 50 to 90,000 Ω at the end of experiment.

2.4 DNA extraction and Illumina sequencing

The total DNA of samples was extracted by a Power Soil DNA Isolation Kit 178 (MOBIO Laboratories Inc., USA) according to the manufacturer's instructions. Bacterial 16S rDNA and archaeal gene for pyrosequencing were amplified by PCR. The primers BAC27F (5'-AGAGTTTGATCCTGGCTCAG-3'), BAC1492R (5'-GGTTACCTTGTTACGACTT-3') and the primers ARC25F (5'-CYGGTTGATCCTGCCRG-3'), ARC958R (5'-YCCGGCGTTGAMTCCAATT-3') were used for bacterial and archaea, respectively. The products of PCR were loaded on 1.5% agarose gel and the gel was purified with Gel Extraction Kit (OMEGA Bio-tek Inc, USA). The DNA concentration and quality were determined by real-time PCR (FTC-3000™), subsequently the amplicons were further used for Illumina MiSeq sequencing. All the raw reads were analyzed by combining different programs from the quantitative insights into microbial ecology (QIIME) after sequencing. When the low quality adaptors, primers, barcodes and reads were eliminated, the remaining 16S rRNA sequences were clustered into OTUs using UCLUST with the distance of 0.03.

2.5 Quantitative PCR (q-PCR) of functional genes

The function genes for methanogens and methane-oxidizing bacteria were determined by q-PCR on an ABI 7500 real-time PCR system instrument (Applied Biosystems, USA). Primers *mcrA*-f (5'-GGTGGTGTMGATTACACARTAYGCWACAGC-3') and *mcrA*-r (5'-TTCATT GCRTAGTTWGGRTAGTT-3') were used for *mcrA* gene amplification. Primers A189-f (5'-GGNGACTGG GACTTCTGG-3') and mb661-r (5'-CCGGMGCAACGTCYTTACC-3') were used for *pmoA* gene amplification. The reaction volume, the reaction temperature, the reaction procedure of PCR amplification and the plasmid curve were plotted according to our previously reported methods^[42].

2.6 Scanning electron microscope (SEM) analysis of bioanode

The morphology of anode microorganism was imaged using SEM (JSM-5610, Japan). The samples were treated as follows: A small amount of sample was placed in a 5 mL centrifuge tube, 2.5% glutaraldehyde

was added, and fixed in the refrigerator for 1.5 hours. And then rinsing with phosphate buffer solution of 0.1mol/L (pH=6.8) for three times. The samples were dehydrated with 50%, 70%, 80% and 90% ethanol in turn. Rinse with a mixture of anhydrous ethanol and isoamyl acetate at volume ratio of 1:1 for 15 minutes, then rinse with pure isoamyl acetate for 15 minutes. The dried sample was pasted on the sample table of SEM and coated with Au film of 1500 nm thickness on the sample surface. Finally, the samples were observed under SEM.

2.7 Statistical analysis

Statistical analyses were conducted using SPSS software (version 21.0) (SPSS Inc., Chicago, USA). Significance analysis was used to identify differences between experimental groups or control groups by one-way ANOVA at $p < 0.05$. The R software package and Origin (version 8.0) were used for bioinformatic analysis graphical works.

3. Results And Discussion

3.1 CH₄ emissions from CW-MFCs

After four weeks of batch operation, the voltage in all reactors reached a steady state. The reactors then operated in a continuous flow mode and began recording data. During the experiment, the plant death in two reactors led to abnormal data (CK3, CK4), so the data of these two reactors would not be recorded in the subsequent experiment. So there are six reactors running stably. As shown in Fig. 2a, CH₄ emissions from all reactors showed a downward trend with the increase of hydraulic residence time (HRT). CH₄ emission was significantly higher in open circuit than in closed circuit. When HRT = 1, the average CH₄ emissions of CK1 and CK2 reached $6.1 \text{ mg m}^{-2} \text{ h}^{-1}$ and $6.4 \text{ mg m}^{-2} \text{ h}^{-1}$ (Fig. 2b), indicating that MFC operation in constructed wetlands can effectively reduce methane emission. When plant roots were located at the anode, methane emission was significantly higher than that at the cathode (P1-an = $3.9 \text{ mg m}^{-2} \text{ h}^{-1}$, P2-an = $4.5 \text{ mg m}^{-2} \text{ h}^{-1}$). CH₄ emissions was lowest when plant roots were located at the cathode, with an average of $2.3 \text{ mg m}^{-2} \text{ h}^{-1}$ found in P1-Ca. Statistical analysis showed that both plant and root location had an impact on methane emission, but the impact of plant root location on methane emission was more significant ($P < 0.01$). CH₄ emissions from the reactor planted with *Typha Orientalis* were lower than those of the reactor planted with *Cyperus Alternifolius* both at the cathode and anode.

With the increase of HRT, methane emission in CW-MFC tends to decrease, which is consistent with previous research conclusion of CH₄ emissions in CW [16]. Although a lot of work has been done on the role of plants in CWs, consensus has been reached in the academic circle that plants have little impact on the wastewater treatment efficiency of constructed wetlands. The contribution of plants to sewage treatment is about 10%, while the fillers, matrix, operation modes and other factors contribute 90% of sewage treatment efficiency [3],[28]. However, different plant species on the bioelectricity generation and CH₄ emissions in CW-MFCs have not been fully explored. From our results, it can be seen that plants

played an important role in CH₄ in CW-MFCS. Therefore, from the perspective of sustainable development of wastewater treatment, more attention should be paid to plant species selection during CW development and management.

3.2 COD removal efficiency

The removal efficiency of COD was measured under different HRT. The results show that the removal efficiency of COD increases with the increase of HRT (Fig. 3). Except for P1-An, the COD removal efficiency was positively correlated with HRT. The removal rates of COD in close-circuit reactors were significantly higher than that of open-circuit reactors. This result indicated that the operation of MFC effectively promoted the degradation of organic matter. The average COD removal rate was about 85% in the control groups. In contrast, the average COD removal rate is more than 90% in the CW-MFC reactors. Previous studies have also shown that MFC integrated with CW could significantly promote the efficiency of sewage treatment^{[31],[38]}. The probable reason may be that electrochemical processes can promote the efficiency of electron transfer, thus effectively improving the degradation of pollutants^[28]. When plant roots were located at the cathode, COD removal efficiency was higher than that of plant roots at the anode. In the P1-Ca reactor, the average COD removal rate reached 95.1%. Unlike the removal of COD, there was no significant difference between two plant species ($p > 0.05$). This results were consistent with previous studies on the effectiveness of plants in removing pollutants from CWs^{[11],[23]}.

3.3 Bioelectricity performance

The bioelectricity generation performances in four CW-MFCs were monitored. As shown in Fig. 4, the average voltage generated by the four reactors varied significantly. The voltage of plant roots at the cathode was greater than that at the anode (P1-Ca = 490 mV, P1-An = 437 mV). P2-Ca = 354 mV, P2-An = 321 mV). Different from the influence of plants on COD removal efficiency, plant species had a significant influence on voltage. The voltage generated by the reactor growing *Typha Orientalis* was higher than that generated by the reactor planted *Cyperus Alternifolius*, whether the plant roots were at the anode or the cathode. This may be related to the type and amount of exudates and organic matter in the root system of different plant species^[14]. The organics from plant rhizodeposits can provide more electron donors for the electrochemical active bacteria (EAB). Plant roots at the cathode have a better electrical performance than plants at the anode, probably because they have limited electron receptors in the system, and the oxygen produced by plants increases the electron receptors^[25]. Thus, the electrical performance of the reactor is improved. However, the effect of the improved electron acceptor brought by the plant root at the cathode on the electric efficiency was less than that of the plant species.

The polarization curve and the power density curve of four CW-MFCs were obtained by changing the external resistance from 50 Ω to 90 k Ω (Fig. 5). The P1-Ca produced maximum power density of 0.22 W m⁻³. The corresponding current density was 0.4 A m⁻³. The lowest power density was observed in P2-An with 0.058 W m⁻³. In the reactor planted with *Cyperus Alternifolius*, the power density was less than 0.14 W m⁻³. On the contrary, in the reactor where *Typha Orientalis* was planted, the power density was above

0.22 W m⁻³. These results indicated that plant species had a significant effect on power density in CW-MFC systems.

Due to the enhanced performance of wastewater treatment and bioelectricity generation, bioelectrochemical systems (MFC and MEC) integrated constructed wetlands have gained a considerable amount of attention^[10]. The enhancement of wastewater treatment and bioelectricity generation mainly emanated from the electron transfer or flow, particularly in anaerobic areas^[25]. However, there are few studies on the influence of plant roots location on the electrical performance in CW-MFCs system, and the references available are still very limited. Previous study showed that plant roots located at the anode could improve the electrical performance due to root exudates and other reasons^[36]. In our study, it is found that when plant roots were located at the cathode, the role of plant in increasing electron donor is greater. The existence of electron donors is conducive to improving the electrical performance^[28]. However, at present, both CW and CW-MFC have low power generation, which is difficult to be applied in engineering. Inadequate electron transfer may be one of the important reasons for the low efficiency of electricity generation. Therefore, further understanding the electron transfer between electrode-anode and cathode in CW-MFCs is essential to improve the development of this integrated technology.

3.4 q-PCR of *mcrA* genes and *pmoA* genes

To quantify the methanogens and methanotrophs in reactors, samples were collected from the anode and cathode for the determination of these two bacteria, respectively. The quantification of *mcrA* genes and *pmoA* genes, which encode methanogenesis and methanoxase, were investigated by q-PCR. The results showed that the number of *mcrA* genes in open-circuit systems was greater than that in closed-circuit systems (Fig. 6). The number of *mcrA* genes at the anode was greater than that of cathode. Meanwhile, the differences between plant species were observed. In the reactor planted with *Cyperus Alternifolius*, the average number of *mcrA* gene is 3.6×10^4 copies mL⁻¹. In contrast, in the reactor planted with *Typha Orientalis*, the average number of *mcrA* genes is 4.7×10^4 copies mL⁻¹. Correlation analysis showed that there was a significant positive correlation between the number of methanogens and CH₄ production in the reactor ($r = 0.98$, $p < 0.01$). Unlike *mcrA* genes, *pmoA* genes increased slightly in closed-circuit systems. At the same time, the number of *pmoA* genes in the plant root at the cathode was greater than that in the anode. The influence of plant species on the number of *pmoA* genes is not obvious. Correlation analysis showed there was no significant correlation between CH₄ emissions and the number of *pmoA* genes, which suggests that plants not only affect CH₄ emissions by influencing methanotrophs, but also through their physiological functions, such as plant conduction.

Methane emission is the net result of methane production, oxidation and transport^[18]. The production and oxidation of methane are closely related to microorganisms, especially methanogens and methane-oxidizing bacteria (methanotrophs) are important biological factors that determine methane emission. However, from the results of this study, there was no significant correlation between CH₄ emissions and methanotrophs, indicating that other factors may play a key role. Plants play an important role in methane production and transport. On the one hand, the organic matter and plant rhizodeposits provided

by different plants can lead to different CH₄ emissions. On the other hand, plants affect the redox potential in the rhizosphere zone and the rates of oxygen transport via plant aerenchyma from the rhizosphere^{[6],[42]}. In addition, some plants have been reported to release methane under aerobic conditions^[1]. Therefore, a lot of systematic studies about the influence of plant species on CH₄ emissions in the CW-MFCs are still needed, rather than the current studies on phenomena.

3.5 Bacterial and archaeal community analysis

The bacterial community composition at phylum level is shown in Fig. 7a. Bacteroidetes and Planctomycetes were the predominant community in CKs, which accounted for 21% – 29% of total composition. In contrast, their abundances in the other MFC-CWs were low. Chloroflexi and Proteobacteria were dominant community in close circuit MFC-CWs, which was significantly higher than that of CKs. Proteobacteria as the most abundant population in P1-CA reactor accounted for 29%, followed by Chloroflexi (15%). In addition, the abundance of Firmicutes in close circuit MFC-CW was higher than that of CKs. The community abundance of other phyla did not differ significantly in all reactors. At the genus level, the community composition of all reactors also showed significant differences (Fig. 7b). The genera of exoelectrogens (*Geobacter*, *Geothrix* and *Desulfobulbus*) were not detected in CKs. The main community composition of CKs was *Saprospiraceae* and *Arthrobacter*. P1-Ca and P2-Ca had high abundance of exoelectrogens. The highest value (21%) of *Geobacter* was observed in P1-Ca. All the reactors had the nitrification and denitrification community (*Nitrospira*, *Brachymonas* and *Nitrosomonas*). In addition, *Anaerolinea* was found had high percentage in close circuit MFC-CWs.

A high proportion of Proteobacteria was found in the reactor, which may be related to the formation of exoelectrogens and the increase of nitrification and denitrification bacteria^[40]. These bacteria belong to the phylum of Proteobacteria. High abundance of Firmicutes in CC MFC-CW resulted in high electricity generation and COD removal. Firmicutes belongs to cellulolytic bacteria, which can hydrolyze cellulose derived from dead plant roots into smaller compounds like fatty acids^[32]. These acids can then be utilized by EAB to electricity generation. Chloroflexi belongs to fermentative bacteria, can ferment small molecular saccharides to short-chain fatty acids and H₂^[19], which can be used as electron donors for EAB. Therefore, their high proportion in CC MFC-CWs promotes the increase of current in the reactor. The common dephosphorization bacteria (*Accumulibacter phosphatis* and *Tetrasphaera*) in wastewater treatment plants were not detected, which indicated that the removal of phosphorus in the MFC-CWs was mainly through the absorption of plant rather than biological removal. The denitrification bacteria (*Nitrospira*, *Brachymonas* and *Nitrosomonas*), which were widely detected in the municipal sewage treatment plant, were detected in the Closed Circuit MFC-CWs, indicating that they have potential for denitrification. The *Geobacter* was widely reported as exoelectrogen in MFC. While the *Desulfobulbus* and *Geothrix* were rarely reported in MFC-CW. Its role remains to be further studied.

The archaeal community composition is shown in Fig. 8. *Methanosaeta* and *Methanosarcina* were the dominant archaea in both cathode and anode in CK. *Methanosaeta* were also predominant in all the other reactors, but their relative abundance dropped around 40% in close circuit CW-MFCs. Hydrogenotrophic

methanogens (Methanobacteriales and Methanococcales) showed an increased trend in all the electrical generation reactors. Methanopyrales, Thermococcales and Desulfurococcales were not detected in CKs, but they were in high percentage in electrical generation reactors. The higher values of Methanosarcina were observed in P1-Ca and P2-Ca, with 20% and 15% abundance, respectively. At the same time, about 8% of the archaea were not identified, suggesting a large number of unknown archaea.

At present, there is very limited information about archaea communities and their roles in CW-MFCs. The decrease of strictly acetotrophic methanogens (Methanosetaceae) in close circuit reactors was related to the hydrogen ions formation and competition between acetotrophic archaea and bacteria^[8]. On contrary, the hydrogenotrophic methanogens (Methanobacteriales and Methanococcales) tended to increase, the probable reason was that the formation of hydrogen ions and electrons during electricity generation improved the activity of hydrogenotrophic methanogens^[30]. Thermococcales and Desulfurococcales are the hyperthermophilic archaea, which were seldom reported in previous studies regarding the archaeal community of MFCs. Thermococcales and Desulfurococcales were reported only in plant fuel cells^[14], their role is unclear. Therefore, the role and mechanism of these hyperthermophilic archaea need to be further studied. Methanopyrales has not been reported in archaeal communities of MFCs.

Desulfurococcales were reported to be autotrophic and heterotrophic. Some of them are autotrophic archaea using hydrogen as the electron donor and sulfur, nitrate, nitrite and thiosulfate as the electron acceptor, some of them can also grow through organic fermentation^[14]. They were predominant in P1-Ca and P2-Ca, resulting in high electricity generation. They may participate in fermentation process to produce simple material for electricity generation and methane inhibition.

3.6 SEM analysis of electrode microorganisms

The morphologies of the electrode biofilm collected from reactors were scanned using SEM. As shown in Fig. 9, various forms of microorganisms, including cocci and bacilli, were adsorbed on the electrode surface. In particular, in P1-Ca reactors, numerous types of microbes were found to be tightly attached to the electrodes including spiral, rod and spherical. Microbes on the anode are more diverse than those on the cathode. At present, the morphology of anode microorganisms has been studied intensively, but the morphology and function of cathode microorganisms have been studied less. Nanowires, circled in red, were also found on the microbial surface except P2-An. These wires originated from the cell surface and extend in different directions. Interspecific electron transport, including direct electron transport (DIET) and indirect electron transport, greatly accelerates biochemical reactions^[44]. There are three types of interspecific direct electron transport: REDOX proteins, nanowires, and electron shuttles (riboflavin, anthraquinone). One of the most common is nanowires. The number and mode of nanowires have an important impact on the efficiency of electricity generation^[2]. In this study, the largest number of nanowires was found in the P1-Ca reactor, which may also be related to the high efficiency of electricity generation. How to enhance the interspecific electron transfer and overcome the electrolyte resistance and charge transfer resistance caused by too slow reaction rate on the electrode are the keys to improve the efficiency of CW-MFC. Therefore, more relevant studies are needed in the future.

In this study, we mainly focused on the effects of plants and their roots location on CH₄ emission, electricity generation and COD removal, and did not focus on the effects on electrode materials and electrode distance. In fact, electrode materials and electrode distance may have a great influence on the performance of CW-MFCs^{[25],[28]}. At present, there are few studies on the relationship between the configuration and performance of CW-MFC reactor. The effect of reactor configuration on CH₄ emissions has not been studied. In addition, the current research on CW-MFC is in the experimental scale, and the actual scale of the system is still in the development stage.

4. Conclusion

The results showed that the operation of MFC could not only improve the wastewater treatment efficiency of CW, but also effectively reduce the methane emission, mainly through the competition between electrochemical active bacteria (EAB) and methanogenes at the anode. EAB were more advantageous than methanogens in utility of carbon sources and became the dominant bacteria group of anode. Plant roots located at the cathode are more conducive to electricity generation and CH₄ control. The enhancement effect of plant roots as electron donors in CW-MFC system was greater than that of root exudates as electron donors. At the same time, plants species played a more important role in CH₄ emissions in the CW-MFC system than the location of plant roots at the electrode. Although our study showed that running MFC in CW and plant roots located at the cathode were more conducive to energy recovery and CH₄ control, its durability of CH₄ emissions mitigation over a long period of time is still a challenge.

Declarations

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Authors Contributions **Ke Zhang:** Writing - original draft, Writing - review & editing. **Xiangling Wu:** Methodology, Writing - review & editing. **Wei Wang:** Methodology, Writing - review & editing. **Hongbing Luo:** Writing - review & editing. **Wei Chen:** Formal analysis, Writing - review & editing. **Wei Chen:** Data curation, Formal analysis, Writing - review & editing. **Jia Chen:** Writing - review & editing.

Data availability Not applicable

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to Participate Not applicable

Consent to Publish Not applicable

Conflict of Interest The authors declare that they have no conflict of interest.

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Figures

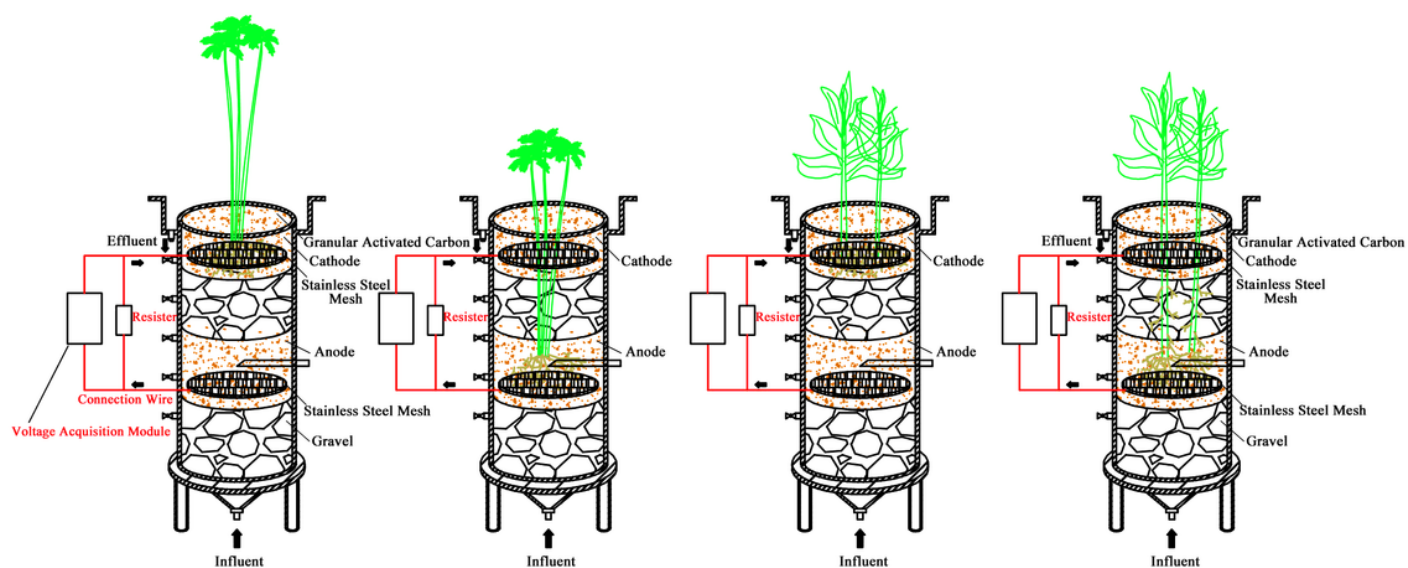


Figure 1

Schematic diagram of different types of CW-MFCs.

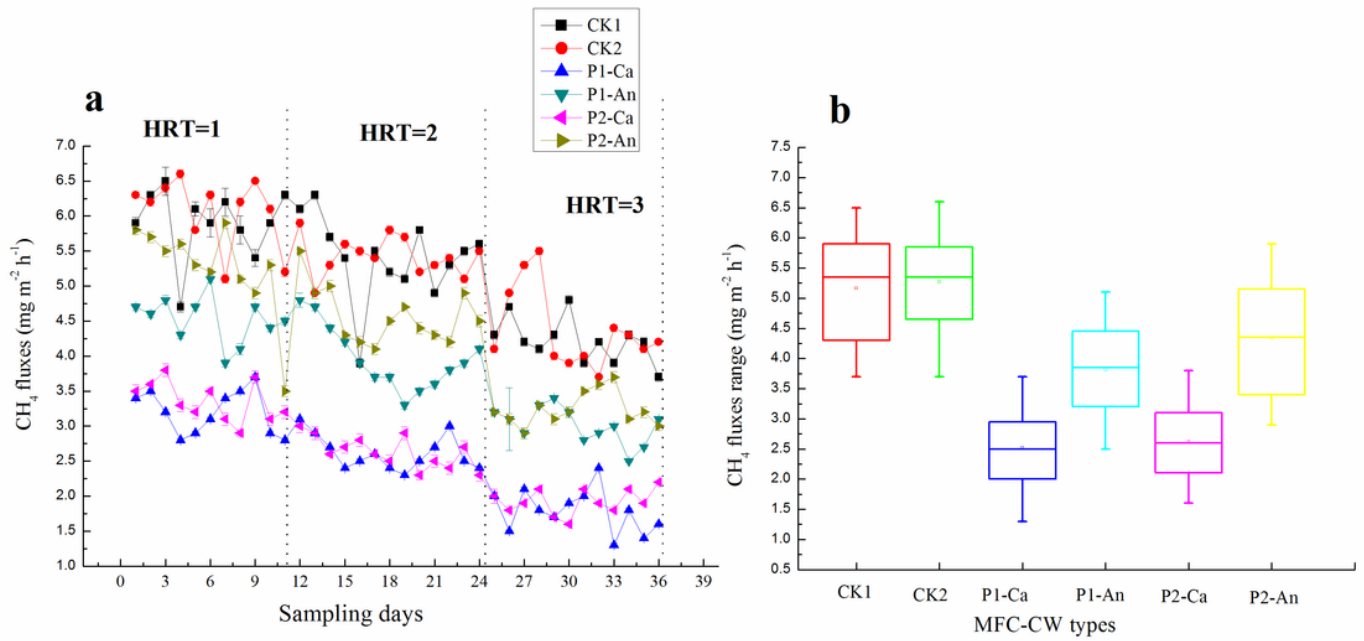


Figure 2

The average CH₄ fluxes from different CW-MFCs under different hydraulic retention time HRT (a), Box plots of CH₄ flux from different CW-MFCs (b). Values are mean $\pm$ standard deviation. Error bars are ± 1 standard deviation from the test mean (n=3).

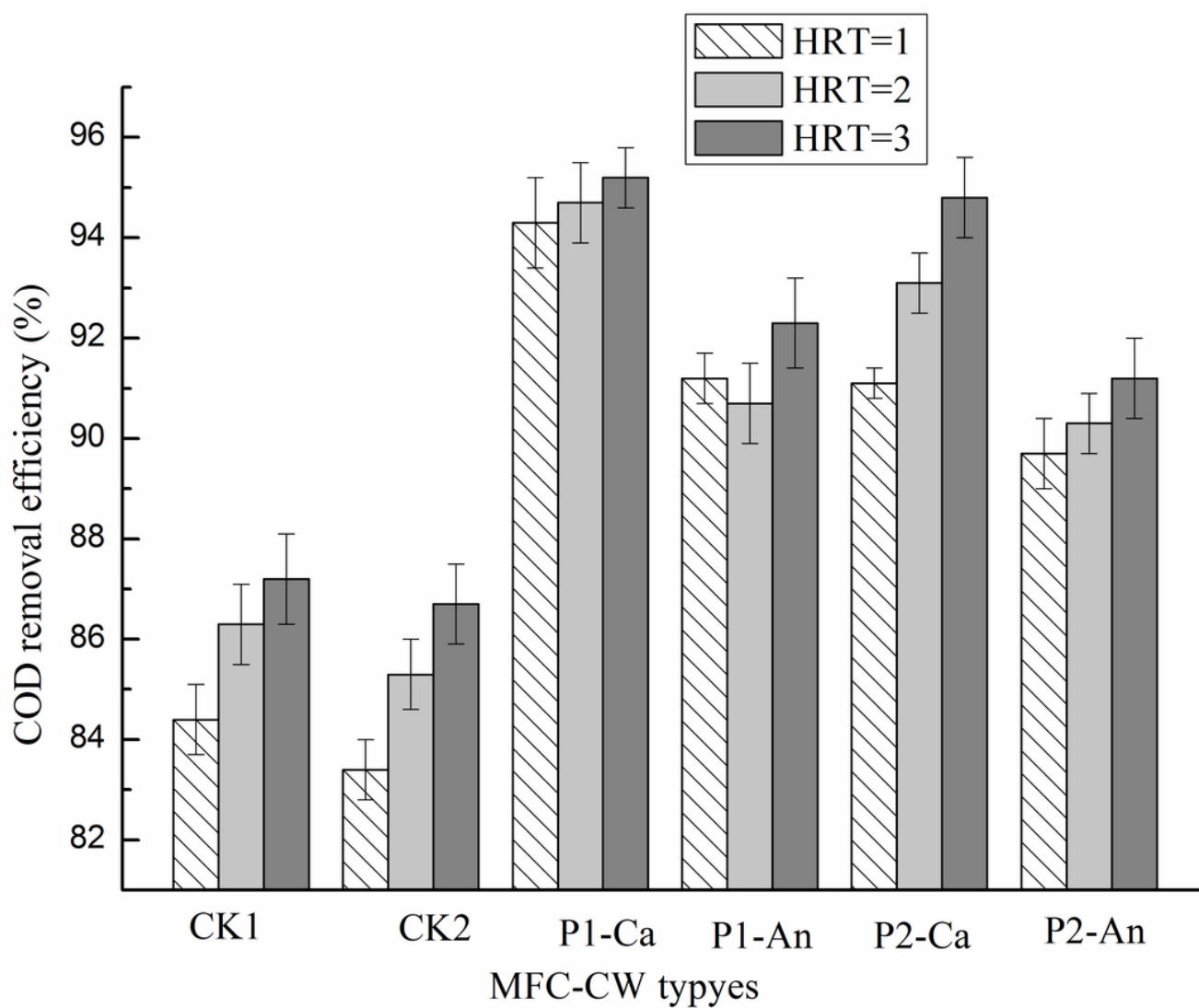


Figure 3

COD removal rates in different MFC-CWs (open-circuit and close circuit conditions) under different HRT. Error bars are ± 1 standard deviation from the test mean (n=3).

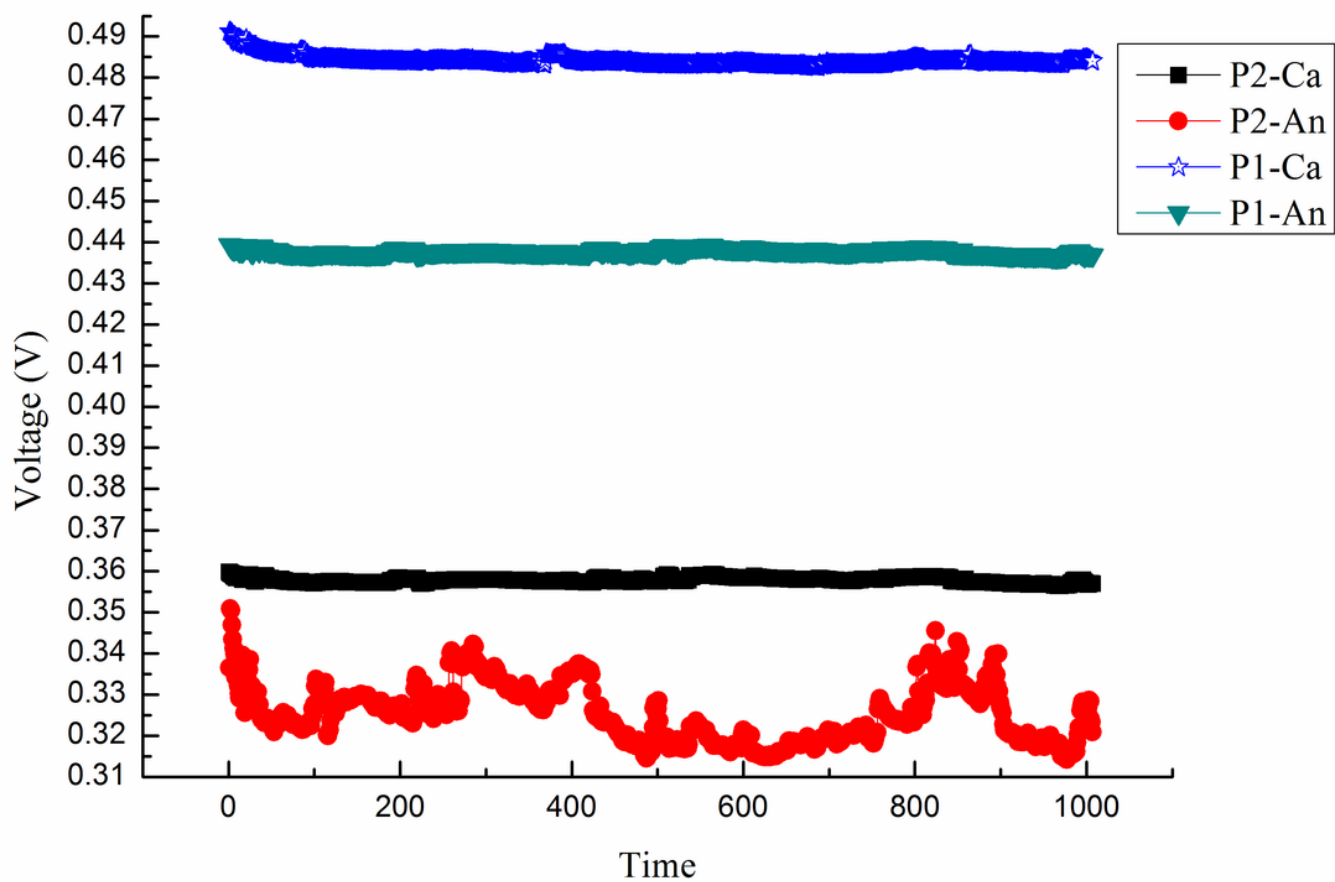


Figure 4

Voltage values of four CW-MFCs under closed - circuit conditions.

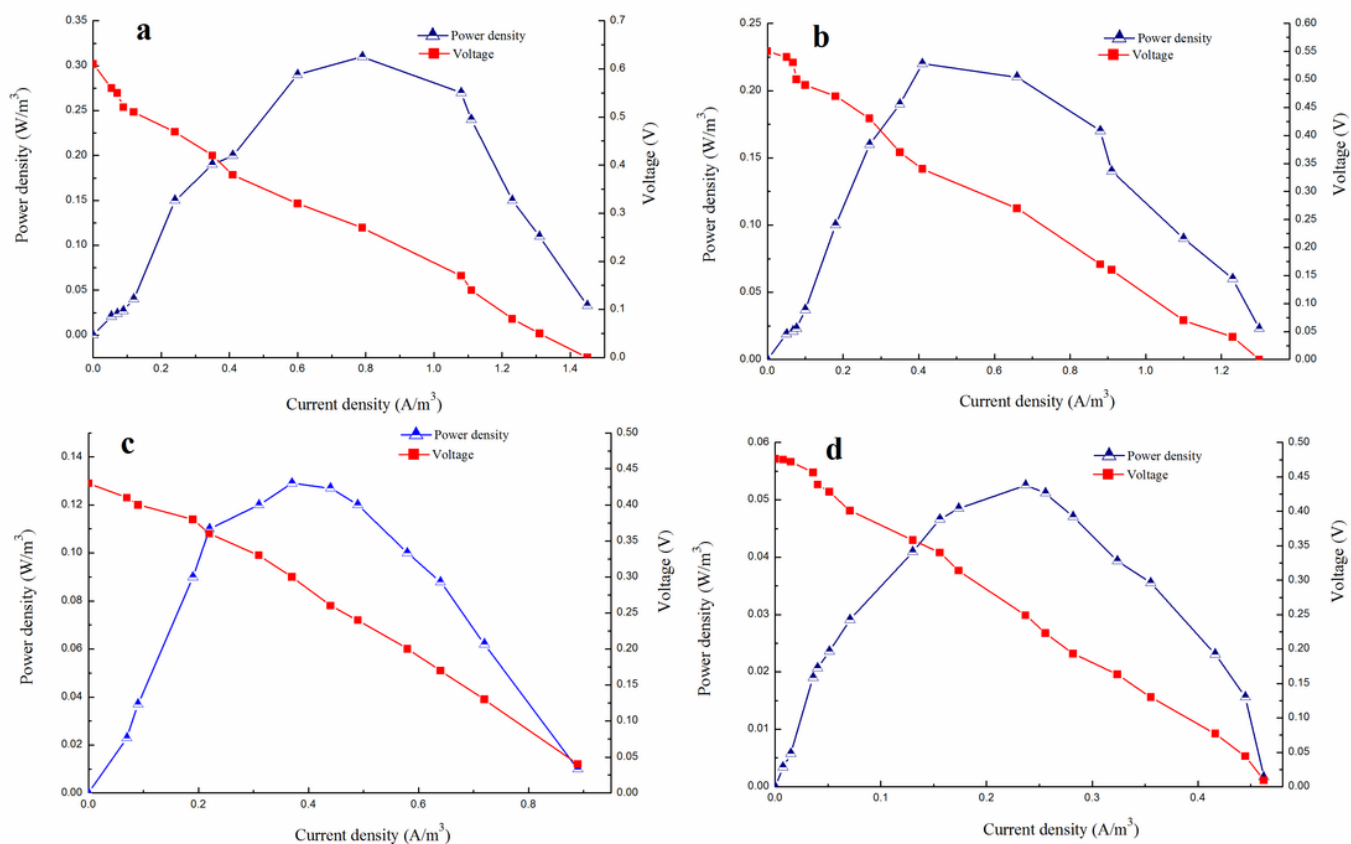


Figure 5

Power density and polarization curves of four CW-MFCs under closed-circuit conditions (the external resistance ranging from 50Ω to $90 \text{ k}\Omega$). (a) p1-ca, (b) p1-an, (c) p2-ca, (d) p2-an.

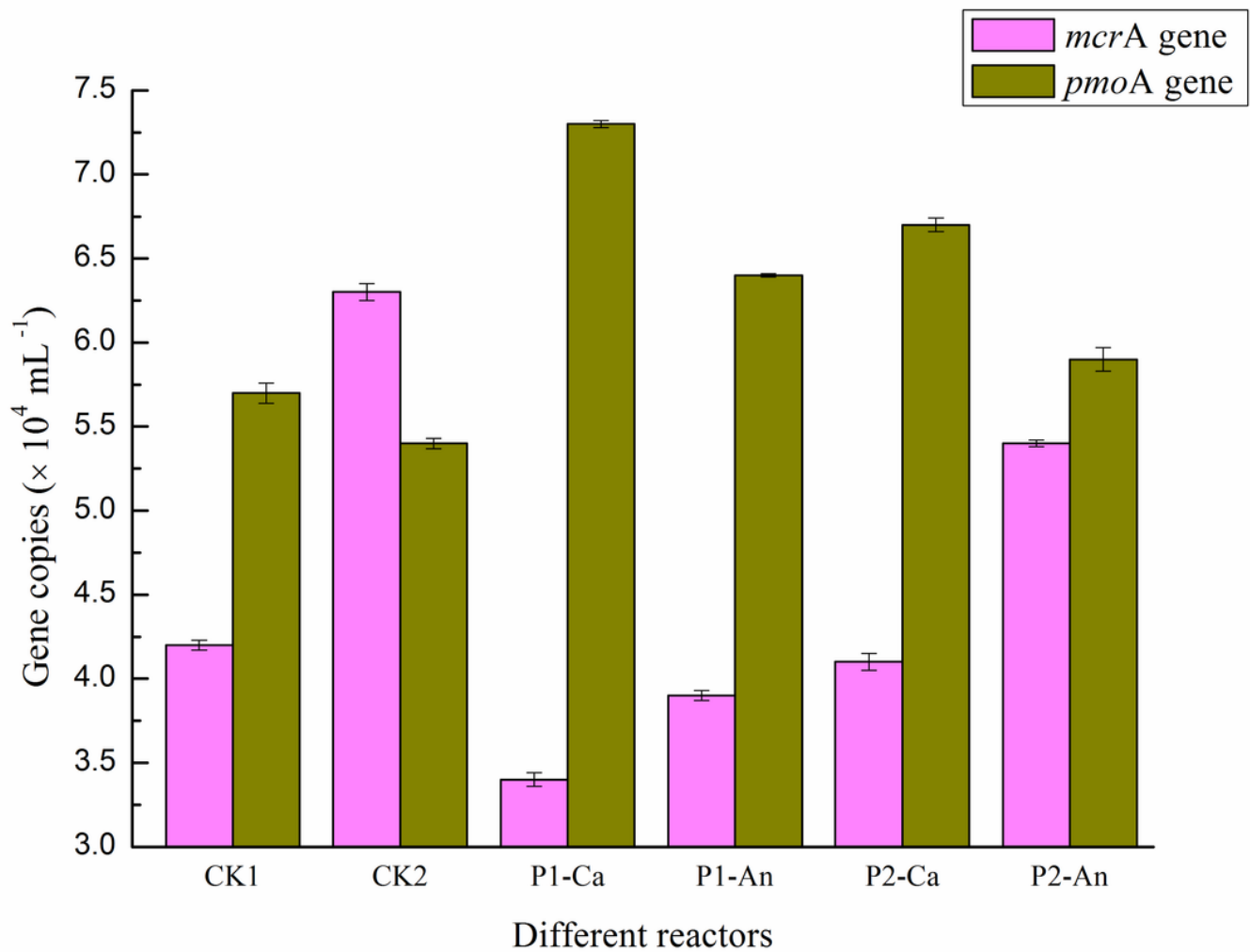


Figure 6

Quantification of methanogens and methanotrophs based on *mcrA* and *pmoA* genes by q-PCR. Error bars are ± 1 standard deviation from the test mean (n=3).

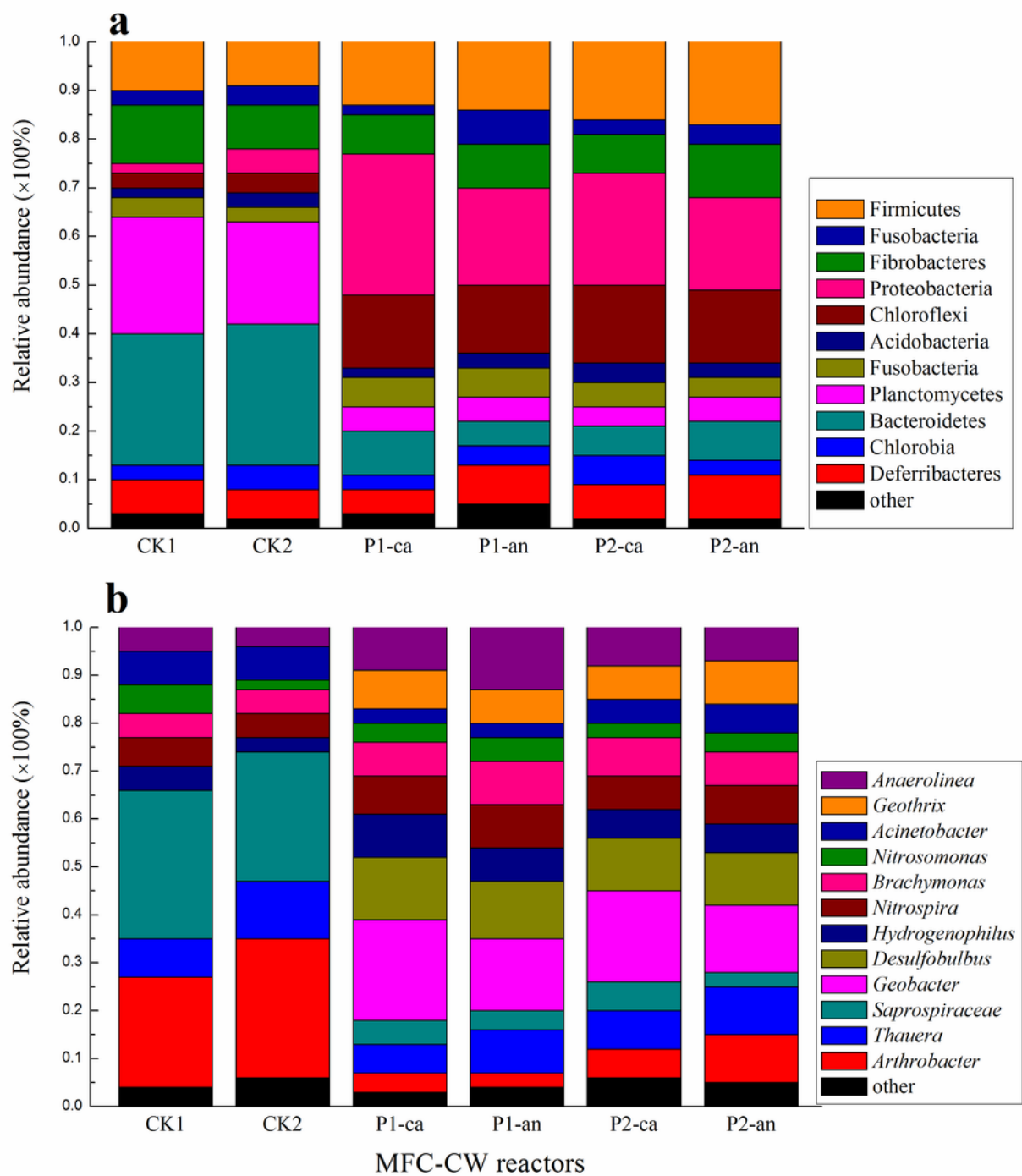


Figure 7

Bacterial community compositions in different CW-MFCs based on 16s gene analysis. (a) at the phylum level, (b) at the genus level.

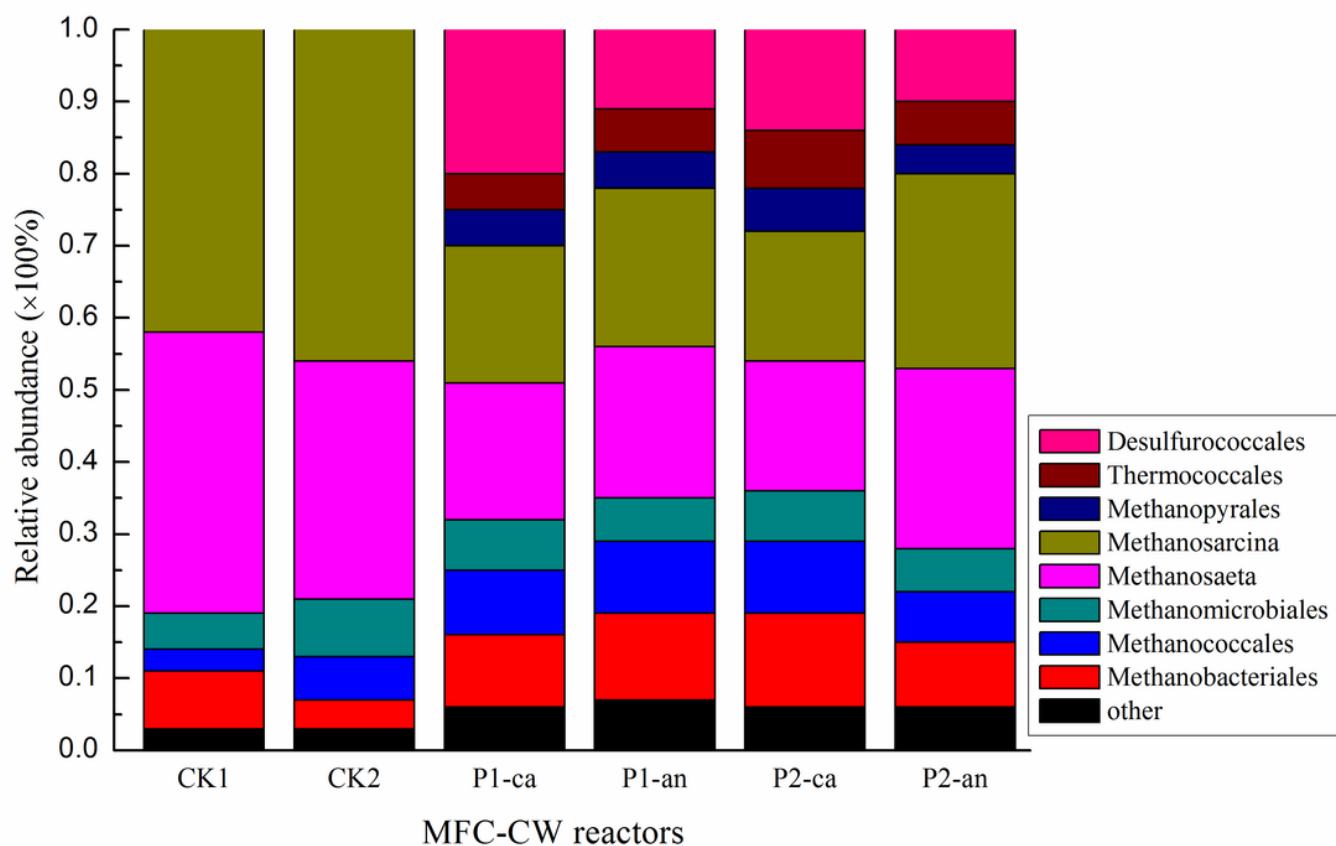


Figure 8

Methanogenic archaea community compositions from different CW-MFCs based on Illumina Miseq sequencing.

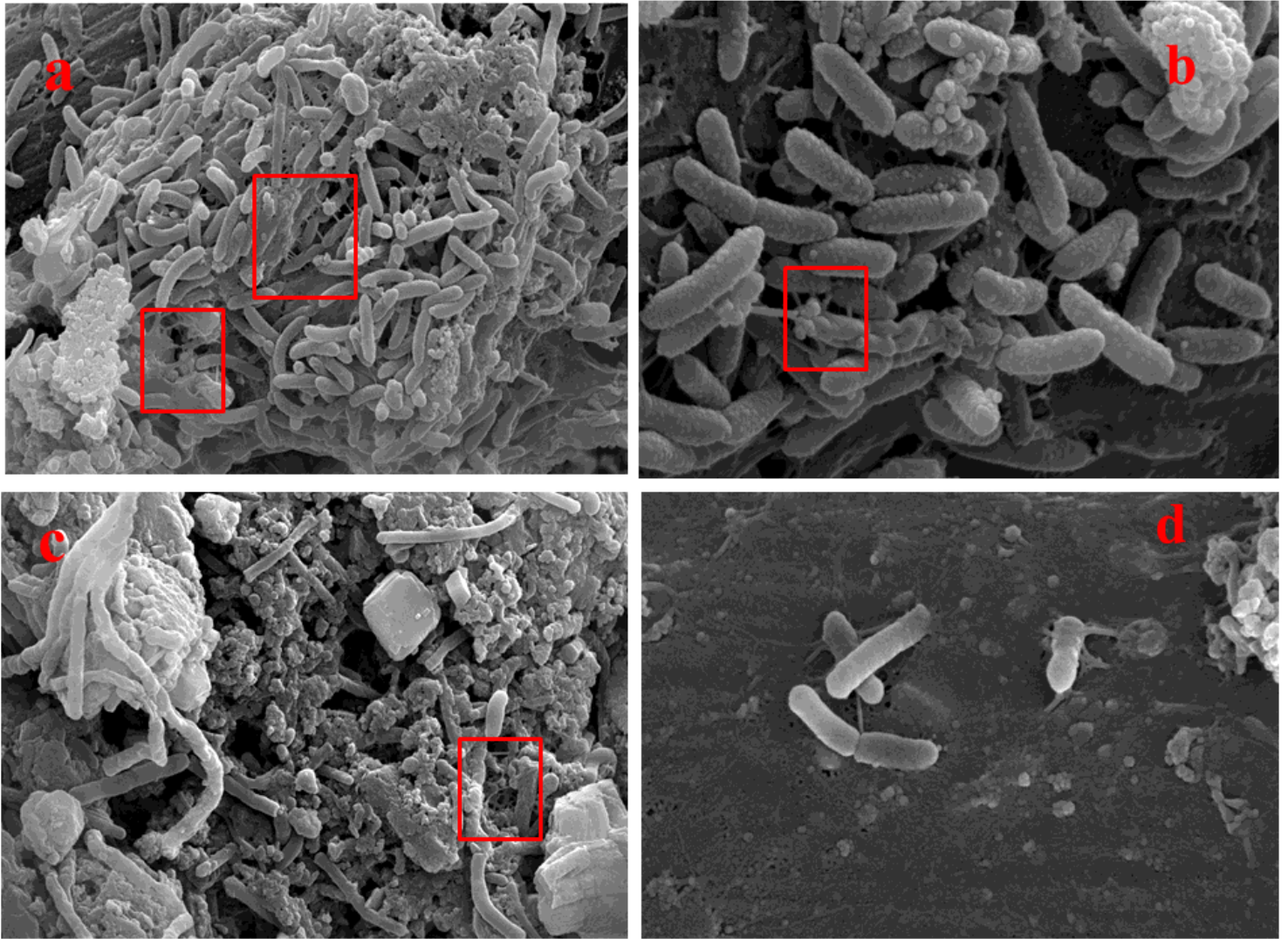


Figure 9

Scanning electron microscope (SEM) micrographs of bioanode from CW-MFCs. (a) P1-Ca $\times 20$ k, (b) P1-An $\times 50$ k, (c) P2-Ca, $\times 20$ k, (d) P2-An $\times 50$ k.