Effects of Bioelectricity Generation Processes on Methane Emission and Bacterial Community in Wetland and Carbon Fate Analysis

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Abstract

Wetlands are an important carbon sink for greenhouse gases (GHGs), and embedding microbial fuel cell (MFC) into constructed wetland (CW) has become a new technology to control methane emission. Rhizosphere anode CW-MFC was constructed by selecting rhizome-type wetland plants with strong hypoxia tolerance, which could provide photosynthetic organics as alternative fuel. Compared with non-planted system, methane emission flux and power output from the planted CW-MFC increased by approximately 0.48 ± 0.021mg/(m^2·h) and 1.07 W/m^3, respectively. The methane emission flux of the CW-MFC operated under open-circuit condition was approximately 0.46 ± 0.024 mg/(m^2·h) higher than that under closed-circuit condition. The results indicated that plants contributed to the methane emission from the CW-MFC, especially under open-circuit mode conditions. The methane emission from the CW-MFC was proportional to external resistance, and it increased by 0.669 + 0.012 mg/(m^2·h) when the external resistance was adjusted from 100 Ω to 1000 Ω. High throughput sequencing further showed that there was a competitive relationship between electrogenic bacteria and methanogens in the system. The flora abundance of electricity producing bacteria was high, while methanogens mainly consisted of *Methanothrix*, *Methanobacterium* and *Methanolinea*. The form and content of element C were analysed from solid phase, liquid phase and gas phase. It was found that a large amount of carbon was consumed in each phase by microbial migration and conversion, the relative proportions of liquid phase, solid phase and gas phase accounted for 40.28%, 36.2% and 20.1% respectively. In conclusion, caron transformation in the CW-MFC could be properly regulated via competition of microorganisms driven by environmental factors, which provides a new direction and idea for the control of methane emission from wetland.

Highlights

1.Effects of different operating conditions on methane emission.

2.The competitive relationship between electrogenic bacteria and methanogens was analysed.

3.The morphology and content of C element in different phases were discussed.

4.The bacterial population structure under different experimental conditions was analysed

5.The mechanism of methane emission from CW-MFCwas described in detail.

1. Introduction

In recent years, the generation and releaseof greenhouse gases (such as CO_2, CH_4 and N_2O) has led to a sharp rise in global temperature. The annual growth rate of $CO_2$is 2ppm, which is expected to rise to 800-1000ppm by the end of the 21st century (Yu and Chen 2019, Lopez-Pacheco, Rodas-Zuluaga et al. 2021).In the carbon cycle, the warming effect of CH_4 is 28-36 times greater than that of CO_2, which makes it urgent to control CH_4 emissions(Riddick, Mauzerall et al. 2019).CH_4 emission sources mainly come from wetlands (including natural wetlands and constructed rice field wetlands), which account for

approximately 20-40% of global methane emissions(Bloom, Palmer et al. 2010, Oshita, Okumura et al. 2014).Therefore, it is significant to study the characteristics and influence mechanisms of methane emissions from wetland ecosystems, and then formulate corresponding GHGs (mainly CH_4) emission reduction measures on this basis to alleviate the global warming effect.

Constructed wetlands (CWs) are very mature wastewater treatment technology that removes pollutants from wastewater through microbial degradation, plant absorption, substrate adsorption, and sedimentation and filtration(Wu, Zhang et al. 2017).Due to low cost, strong decontamination and less secondary pollution, CWs have become a popular wastewater treatment method and are used on a large scale(Mohammed, Mutar et al. 2021).However,the use of CWs for sewage treatment may increase CH_4 discharge and lead to "pollution exchange", researchers have taken great interest in managing CWs and flooded rice fields to minimize CH_4 emissions(Pangala, Reay et al. 2010).At this stage, a large number of studies have focused on various factors such as irrigation, seasonality, fertilization and crop rotation affecting CH_4 emission in paddy wetlands and found that the average CH_4 emission fluxes reached approximately25-300 Tg/year (Wang, Gu et al. 2018, Xu, Zhou et al. 2020).GHGs from wetlands are mainly affected by anaerobic microorganisms in the bottom layer of wetlands,and methanogenic bacteria have easy access to root cellulose for their own needs, resulting in large amounts of CH_4 production, which is the direct cause of large amounts of CH_4 production in wetlands(Liu, Feng et al. 2017, Zhang, Wang et al. 2021).So far, the mechanism of CH_4 emission and the impact of microorganisms on methane production have rarely been reported. Moreover, it is urgently necessary to explore new strategies to control CH_4 emissions in the CW wastewater treatment process.

Microbial fuel cells (MFCs) are devices that use "microorganisms" as catalysts to degrade organic pollutants in wastewater and convert chemical energy into electrical energy(Catal, Kul et al. 2019).The degradation of organic pollutants in wastewater by MFCs has become a reality and is gradually maturing. In bio-electrochemical methods, methane can be collected from the biocatalyst of microbial electrolysis cells (MECs) not only by CO_2 electro-conversion methanogenesis, but also by enriching microorganisms on the anode in anaerobic digestion by microbial electrosynthesis (applied voltage) to increase CH_4 production(Zhang, Song et al. 2019, Flores-Rodriguez and Min 2020).It has been reported in the literature that CH_4 production reduces the coulomb efficiency (CE) and thus the sensing accuracy of MFCs, but the significance of this suppression can only be specific to the application(Kaur, Boghani et al. 2014).Wetlands are the main source of long-lived greenhouse gases,and it is possible to couple CWs with MFCs to control CH_4 emissions, which provides a technological advantage in suppressing CH_4 emissions. Most of previous studies have been conducted by controlling the operating conditions of the reactor or adding inhibitors (e.g., antibiotics) to observe the CH_4 emission flux(Xu, Song et al. 2021).However, the real reasons for controlling the CH_4 emission should be analyzed synthetically from the mechanism of gas emission and bacterial community, which need to be further explored.Plant roots play vital roles in CH_4 emission,and it can not only give microbial nutrients (such as cellulose) for the growth of microorganisms without the addition of carbon source, but also discharge gases into the

environment through its own vascular tissue.Plants planted on anodes are not only easy to produce methane, but also easier to release carbon dioxide gas(Liu, Feng et al. 2017).Nevertheless, the bio-electrochemical mechanisms of controlling methane emission are not fully understood, which needs further in-depth discussion from the perspective of mechanism and bacterial community analysis. Furthermore, it is crucial to explore microbial competition strategies driven by environmental factors to regulate methane emissions.

In this experiment, emergent plants with strong hypoxia tolerance were planted on the anode, and the static box method was used to cover the wetland to collect greenhouse gases.The objectives were:1) to explore the different operating conditions and configurations on GHG emissions, 2) to analysis the interrelationship between gas emissions and electricity production, 3) to summarize the minimum control conditions for methane emissions, 4) to explore the final fate of the carbon,5) to further analysis of the bacterial community structure under different influences, 6)to further analyse the mechanism of methane emission.

2. Methods

2.1. Reactor construction

A CW-MFC made of acid- and alkali-resistant polypropylene plastic columns was constructed. Four groups of reactors were designed for this experiment, as shown in Fig. 1, namely, closed-circuit/with plants group (group A, CCP), open-circuit/with plants group (group B, OCP), closed-circuit/without plants group (group C, CCN), and open-circuit/without plants group (group D, OCN).

The total height of the system body is 100 cm. The main reactor is 55 cm in height, while the height of the reaction chamber is 45 cm and the height of the static box is set at 45 cm. The total volume of water stored in the reactor is 6.9 L, and the effective water storage volume is 6.4 L. The lower part of the reactor is equipped with a conical inlet chamber with a water distributor, and the lower part of the chamber is filled with 5 cm thick gravel (particle size: 1–3 cm) as a support layer. The bioanode material is carbon fiber felt (CFF, thickness: about 2.5 cm) with stainless steel wire mesh (SS, wire diameter: 2 mm, pore size: 0.5 mm) sandwiched between the carbon felt to collect electrons. The intermediate layer was filled with gravel to a height of 10 cm. The electrode material of the air cathode layer is the same as that of the anode, and the anode and cathode are connected by titanium wire leading out of the reactor and connected by alligator clip wires, with a 1000 Ω resistance wire connected at both ends as the starting condition, and the open circuit reactor must be tested after the start-up is completed. The upper side is equipped with an overflow tank, the purpose of which is to put in the static box and pour in deionized water to achieve the condition of water seal and avoid exchange with the outside atmosphere. In this experiment, a new type of inter-root-anode CW-MFC was constructed by selecting a water-holding plant (Acorus calamus) with high decontamination ability, high anoxic tolerance and cold tolerance to be planted in the bioanode.

2.2. Inoculation and system operation

The sludge was taken from a wastewater treatment plant and incubated anaerobically for 2–3 days. The cultured anaerobic sludge is inoculated in the anode and then started by a peristaltic pump entering the culture from the lower inlet. The nutrient solution was configured as follows: 5 mM phosphate buffer solution (PBS), 0.2 g·L^{-1} $C_6H_{12}O_6$, 0.15 g·L^{-1} NH_4Cl, 0.13 g·L^{-1} KCl, 3.13 g·L^{-1} $NaHCO_3$, and 1 mL·L^{-1} micronutrient solution. The components of the micronutrient solution (1 L contains) were: 5.6 g $(NH_4)_2SO_4$, 2 g $MgSO_4 \cdot 7H_2O$, 200 mg $MnSO_4 \cdot H_2O$, 3 mg H_3BO_3, 2.4 mg $CoCl_2 \cdot 6H_2O$, 1 mg $CuCl_2 \cdot 2H_2O$, 2 mg$NiCl_2 \cdot 6H_2O$, 5 mg $ZnCl_2$, 10 mg $FeCl_3 \cdot 6H_2O$, 0.4 mg $Na_2MoO_4 \cdot 2H_2O$. After inoculation, both ends of the reactor are led by wires to the voltage acquisition board and the data is read out and recorded by the computer (data is recorded once in 600s). The start-up time is about 15–30 days, if the voltage drops the nutrient solution needs to be replaced, the maximum voltage and stable operation means the start-up is completed.

2.3. Water quality and bioelectricity generation performance determination

All samples were filtered through 0.45μm filter membrane to remove suspended solids. COD was measured using the potassium dichromate method (APHA method 5220). The COD removal rate (RCOD) was calculated according to Eq. (1).

$$R_{COD} = \frac{COD_{In} - COD_{Eff}}{COD_{In}} \times 100\%$$

where COD in and COD eff represent the influent COD concentration and the effluent COD concentration, respectively. The unit of COD concentration is mg/L.

Total organic carbon is tested by combustion oxidation - non-dispersive infrared absorption method (HJ 501–2009), which responds to the total amount of carbon contained in dissolved and suspended organic matter in water bodies. There are differential subtraction and direct methods for the measurement of total organic carbon, and the differential subtraction method was chosen for the calculation of this experiment, with the following equation.

TOC = TC-TIC

where TOC, TC and TIC represent the total organic carbon, total carbon and the total inorganic carbon, respectively.

Once the system is running and stable, voltage measurements are mainly recorded by the acquisition board (DAQ3323, 6-digit sensitivity) and delivered to the computer. Power density curves and polarization curves are measured using different external resistance methods, i.e., using a resistance box (XJHS1000) to turn on the maximum resistance of 9000Ω, measuring the voltage every 5min and dropping to 100Ω in turn.

The coulomb efficiency (CE) reflects the ratio of the actual cell yield to the actual yield of the utilized organic material, and it can evaluate the superiority of the MFC power production performance. The specific formula is as follows:

$$CE = \frac{I}{F(4 \div 32)\Delta COD \times Q_{In}}$$

where CE is coulombic efficiency of the MFC-CW (%), I is current (A), F is the Faraday constant (96,485 C/mol), Q_{In} is flow rate (L/s), ΔCOD is the difference of COD between influent and effluent (g/L), 4 is the number of electrons obtained by oxidation-reduction of 1mol oxygen, 32 is the molar mass of oxygen, 32 g/mol.

2.4. Methane collection, determination and flux calculation

The gas is collected by the static box method, with gas collection holes on top of each box(Liu, Feng et al. 2017). Samples were taken by introducing one end of the suction pump into the static chamber and the other end into the gas bag. Methane and carbon dioxide were measured using a gas chromatograph (Agilent 6890B, USA) equipped with a FID detector and a TCD detector, and the carrier gas was nitrogen, and the gas was sampled at 8–10 am each day. For the accuracy of the experimental data, the gas was sampled in triplicate each time. In order to calculate the actual gas production, the formula is as follows.

$$J = \frac{dc}{dt}\frac{V}{A}\frac{MP}{RT}$$

where J is the methane emission ($g/m^2 \cdot min$), V is the effective volume of closed chamber for collecting methane (m^3), A is the surface area of the electrode surface (m^2), dC/dt is the concentration change of methane in the closed chamber per unit time (mL CH_4/mL gas/min), T is average temperature (25°C), M is the molar mass of gas (g/mol), P is the atmospheric pressure at the time of measurement (Pa), R is the gas equilibrium constant (8.314 J/mol/k).

2.5. Measurement and calculation of element C

The proportion of carbon content in solid phase, liquid phase and gas phase is obtained by observing and analyzing the trend of carbon content before and after input in the system. The specific distribution is as follows:

$C_{influx} = C_{outflux}$

Where: C_{influx}—Total carbon content in the system

$C_{outflux}$—Total carbon content in the outflow system

$C_{influx} = C_{inlet} + C_{photosynthesis}$

Where: C_{inlet}—Carbon content of liquid phase entering the system

$C_{photosynthesis}$—Carbon content of plant photosynthesis entering the system

$C_{outflux} = C_{effluent} + C_{CH4} + C_{CO2} + C_{matrix\ absorption}$

Where: $C_{effluent}$—Carbon content of liquid phase in outflow system

C_{CH4}—Flux of methane produced in the system

$C\ co_2$—Flux of carbon dioxide produced in the system

$C_{matrix\ absorption}$—Carbon content absorbed by matrix in the system

$C_{inlet} = C_{effluent} + C_{CH4} + C_{CO2} + C_{matrix\ absorption} - C_{photosynthesis}$

2.6. Bacterial and archaeal communities analysis

Illumina sequencing analysis of microbial communities was performed by taking anodic carbon felt substrates from open and closed circuits as well as from conditions with or without plants, after special treatment. After extracting microbial genomic DNA from anodic carbon felts, the upstream primer 338F (this primer sequence: ACTCCTACGGGAGGCAGCA) and the downstream primer 806R (this primer sequence: GGACTACHVGGGTWTCTAAT) were analysed to amplify the standard 16S V3-V4(a) region of bacteria. Then the PCR amplification of methanogenic bacteria was continued, and the mcrA gene of methanogenic bacteria was amplified by the upstream primer mcrA-F (the sequence of this primer is: GGTGGTGTMGGATTCACACARTAYGCWACAGC) and the downstream primer mcrA-R (TTCATTGCRTAGTTWGGRTAGTT).

3. Results And Discussion

3.1. Methane fluxes in closed/open circuit mode CW-MFCs

The open/closed circuit model is one of the most important factors affecting CH_4 emissions. In the opened circuit case, the CW-MFC is equivalent to a CW, so it is important to explore the relationship between the two, the dynamics of the two modes of open/closed circuit during operation are shown in Fig. 2(A). Compared the analysis of with plants, the CH_4 emission flux in the opened circuit mode was higher than closed circuit mode by about 0.46 ± 0.024 mg/(m^2·h). In the system non-plants, the CH_4 emission flux in the opened circuit mode was higher than closed circuit mode by about 0.21 ± 0.012 mg/(m^2·h), which clearly shown that the CH_4 emission from the opened circuit is higher than closed circuit. In previous studies, the CH_4 emission fluxes were 6.37–7.28 mg/(m^2·h) and 7.43–8.36 mg/(m^2·h) for closed circuit and opened circuit, respectively, and the CH_4 emission fluxes in our study was basically consistent with the relevant literature report(Xu, Song et al. 2021). The difference between the

open/closed circuit model is the bioanode. It has been reported in the literature that electrons are more easily produced in closed circuit mode due to electrical stimulation to produce electrochemically active bacteria (EAB), with the same source of carbon and nitrogen, electrogenic bacteria have easier access to food, allowing methanogenic bacteria to be suppressed(Kaur, Boghani et al. 2014). In an anaerobic fermentation environment, no current passes in the open circuit, methanogenic bacteria proliferate and increase the production of CH_4 gas(Ishii, Hotta et al. 2008).

Of course, the effect of substrate concentration on GHG emissions were exceptionally important, the higher the substrate concentration, the more microorganisms would be on the anode(Rahmani, Navidjouy et al. 2022). However, considered the effect of plants on CH_4 gas emissions, i.e., controlled the glucose concentration at 200 mg/L, it was found that plants also increased CH_4 gas emissions. In the closed circuit system, the plant group improved by about 0.48 ± 0.021 mg/(m^2·h), compared to the non-plant group, the plant roots (cellulose) were easily decomposed by microorganisms for their own consumption, increasing the CH_4 emission(Rismani-Yazdi, Carver et al. 2013). Moreover, when plants were planted, GHG emissions were not only in the form of gas spillage and liquid-phase diffusion, but also in the form of gas transport through plant pipes(Gong, Song et al. 2020). Excluding the effect of substrate concentration (i.e., entering substrate concentration of 0 mg/L) as shown specifically in Fig. 3, the CH_4 emission flux of 0.39 ± 0.01 mg/(m^2·h) was found for the plant group under closed circuit conditions. In contrast, the microorganisms on the anode of the plant-free group in the absence of substrate concentration had no nutrients and died. The fact that cellulose can be used as a carbon source by *cellulomonas fimi*, *cellulomonas biazotea*, and c*ellulomonas flavigena*, where *cellulomonas spp*. is a direct cellulose-based microorganism(Takeuchi, Khawdas et al. 2017, Toczylowska-Maminska, Szymona et al. 2018).

In addition, the relationship between CH_4 gas emissions and output voltage can be further illustrated with polarization curves and power density curves. As shown in Fig. 2(B), the current density of the plant group was 1.07 W/m^3 higher than the plant-free group, corresponding to an internal resistance of 187.02 Ω and257.91.9 Ω, respectively. This also indicated that the addition of plant roots provides more terminal reduction electron acceptors (O_2) and increases the reduction medium on the cathode surface reducing the internal resistance of the system thus increasing the output voltage, however, the plant causes an increase in the CH_4 emission flux(Nandy, Sharma et al. 2019). From a microbiological point of view, electrochemical bacteria (e.g., *Aspergillus*, *Actinobacter*, *Fimicus* and *Acidobacter*) on the anode compete with methanogenic bacteria for nutrients, electrogenic bacteria become the dominant flora resulting in increased voltage and thus inhibiting CH_4 production. The same explains the transport of CH_4 gas in vascular tissues inside the plant, which increases the emission of the gas. Rice as a typical vascular plant, according to the literature, the power density with and without plants was 26 ± 7 mW/m^2 and 3.7 ± 1.8 mW/m^2, respectively, while rice wetlands induced CO_2 as well as CH_4 production(De Schamphelaire, Van den Bossche et al. 2008, Wang, Yu et al. 2018). The current density in the plant group without substrate can reach 1.43 A/m^3 and the power density is 0.5 W/m^3, yet the internal resistance is as high as 233.15 Ω. The reason for this phenomenon may be a decrease in substrate concentration,

microorganisms do not have enough nutrients for their own needs thus reducing the production of electrons and making it difficult for protons to pass from the anode to the cathode. It has been reported in the literature that cellulose can only recharge the microorganisms in MFCs, especially the *Cellulomonas* strain NBRC-15513(Khawdas, Watanabe et al. 2019).

3.2. CH_4 emission fluxes in different external resistances

The external resistance can affect the stable output of voltage and the best treatment of wastewater. Therefore, different external resistances (100 Ω, 200 Ω, 500 Ω and 1000 Ω) are set according to the purpose of the experiment to observe the control of GHG. As shown in Fig. 3(A), with the external resistance increases, the emission trend of CH_4 also increases gradually. Compared external resistance 100 Ω and 1000 Ω respectively, the 1000 Ω increased by about 0.669 + 0.012 mg/(m^2·h). It was enough to show that the external resistance was in positive proportion to CH_4 emission, but it has no statistical significance in practice. It was further understood from the figure that different external resistance has different control over methane, because the electron transfer rate, changes of microbial metabolic activities and kinetics of substrate utilization are different under different external resistance(Picioreanu, Head et al. 2007). Therefore, it was further considered to increase the external resistance and increase the growth of methanogens, which is not the growth of electrogenic bacteria, but the growth of electroactive microorganisms mainly uses the substrate under the action of electrical stimulation(Picioreanu, van Loosdrecht et al. 2008).

As shown in Fig. 3(B), when the external resistance was 1000 Ω, the power density reaches the maximum (the maximum power density was 2.17 A/m^3), but the current gradually decreases, making CH_4 reach the maximum. However, when the external resistance was 100 Ω, the power density reaches 1.38 A/m^3, the current is increasing, and methane was well controlled. This was enough to show that there is a strong competitive relationship between the electrogenic bacteria on the anode and the methanogenic bacteria to produce current by consuming the substrate. Of course, the increase of external resistance reduces the degradation of COD removal rate from 95.71–90.12%. This may be because the high external resistance is not conducive to the consumption of organic matter by anode microorganisms, reduces the ability of microorganisms to produce electrons, and increases the emission flux of CH_4.

3.3. The ultimate fate of "carbon cycle"

The question of the final destination of the wetland "carbon cycle" is to explore the key process of carbon conversion in wetlands, which has important implications for the carbon saving function. The experiments in this section describe the transport and transformation of wetland carbon fractions between different interfaces in the atmospheric vegetation, water bodies and summarize the carbon cycle in terms of the carbon stocks accounted for by the atmosphere, water bodies and plants.

TC (glucose) enters as an aqueous solution (TC ~ 606.9 mg/L), it is transported and transformed under anaerobic and microbial conditions or is also broken down and transformed by cellulosic bacteria in the

plant root system (element [C] is in the form of small organic molecules), the above process has both liquid-liquid transfer and gas-liquid exchange([C] is in the form of gas and organic matter). Of course, the aeration tissue of plant roots also transmits a small amount of gas to the atmosphere, and the total emission fluxes of CO_2 and CH_4 are 62.76 mg and 25.84 mg, and the final TC of the effluent water was 352.19 mg/L. As shown in Fig. 4, a large amount of substrate is consumed by microorganisms in solution, accounting for about 40.28% of the entire system, CH_4 and CO_2 account for 11.16% and 24.6% of the entire graph, respectively, indicating a larger production of gases, the remainder is influenced by other factors. The carbon content in the matrix was complex, accounting for about 10.76% of the pie chart, including microorganisms in the matrix and cellulose shed by plant roots. The carbon content of plants also changed before and after the reaction, accounting for about9.34%.

The substrate concentration only provides nutrients to the microorganisms, and the microorganisms' extracellular electron transfer capacity is enhanced, thus favoring the development of electrogenic bacteria and thus inhibiting methane production, so the substrate concentration occupies a large part. Due to the temporal and spatial variability of greenhouse gas emissions from wetlands, the flux of CH_4 emissions increases as the flux of CO_2 emissions increases, because the increase in CO_2 concentration changes the concentration of oxygen in the root zone and the availability of carbon sources(Kao-Kniffin and Zhu 2013, Zhang, Brodylo et al. 2021). The addition of plants increases the gas transmission and small molecules of organic matter (a few small molecules) are absorbed by the plants to purify the wastewater and increase the voltage of the system. Using carbon (C) and nitrogen (N) isotopic signatures of organic matter (OM) to detect changes in associated plant and microbial processes in aquatic systems(Olaleye, Nkheloane et al. 2014). In contrast, carbon isotopes are variable in wetland plants, including photosynthetic pathways, the nature of the major inorganic carbon sources (atmospheric and dissolved), the form of effective carbon and subsequent assimilation (CO_2, HCO_3^-), and the basis for diffusion limitations by plant life forms or aquatic environmental conditions(Guareschi, Pereira et al. 2014). In summary, it can be seen that the tendency of [C] elements can be roughly divided into those consumed by microorganisms, those produced by liquid-gas conversion and gas-gas exchange, and finally, the nutrient solution coming out of the cathode port before the substrate is consumed.

3.4. Bacterial community analysis

3.4.1 Richness and diversity of the microbial community

The data of bacterial community of wetland microbial dye battery to control GHGs emission are limited, so it is of great significance to analyze the richness and diversity of bacterial community(Lopez, Sepulveda-Mardones et al. 2019). As shown in Table 1, Chao1, Shannon, observed species and Simpson shown that the total number of bacteria and species richness of CCP were higher than those of CCN and of OCP. This indicated that the closed circuit helps the electron-producing bacteria to produce electrons and the plants planted at the anode help to enrich the bacterial community leading to an increase in both electricity and gas production. And Faith-pd and Pielou-e in the table illustrate that the higher their values, the better the genetic diversity of the species and the homogeneity of the community. The above

phenomenon is reflected in 3.1 and 3.2 where the addition of plants contributes to the enhancement of the bacterial community. Of course, plant roots help microorganisms to adhere and obtain nutrients so that bacteria accelerate extracellular electron transfer(Zhang, Liang et al. 2016).

3.4.2 Methanogenic bacteria community

Further investigation of the effect of open and closed circuit and the presence or absence of plants on the bacterial community composition at the anode gate level of the system is shown in Fig. 5. In the plant closed-circuit system, the dominant bacteria are *Acinetobacter*, *Pseudomonas*, *spirochetes* and *Bacteroides*, accounting for 1.85%, 18.77%, 7.54% and 7.79% respectively. In the opened circuit system with plants, the dominant bacteria are *Acinetobacter* and *spirochetes*, accounting for 78.77% and 3.29% respectively. In the non-plant closed circuit, the dominant bacteria were *Acinetobacter*, *Pseudomonas*, *spirochetes* and *Bacteroides*, accounting for 1.25%, 6.16%, 7.28% and 7.68% respectively. Through comparative analysis, under the conditions of open and closed circuit in plants, it can be found that *Acinetobacter* is suitable to survive in the case of open circuit, and the electricity producing microorganism is relatively single. The richness of microbial flora in closed circuit is higher than that in open circuit, which further proves that the diversity of electrogenic bacteria in closed plant system in 3.4.1 is richer, and only *Acinetobacter* was in open circuit. Compared with the situation with or without plants in the closed system, the richness of microbial flora is richer than that without plants, which may be because the addition of plants increases the diversity of flora. From the perspective of relative richness, the richness of the plant group is slightly higher than that of the non-plant group, which also greatly shows that the plant root system improves the nutrients for microorganisms, stimulates its reproduction rate, and makes the power production of the plant group higher than that of the non-plant group.

Methanogenic bacteria are the main genus of methane producing bacteria, as shown in Fig. 6, the main bacteria producing methane were *Methanothrix*, *Methanobacterium* and *Methanolinea*. All of the above bacteria are the main microorganisms producing methane. Comparative analysis shown that under the conditions of plant closed circuit and open circuit, the number of *Methanothrix*, *Methanobacterium* and *Methanolinea* in closed circuit is about 1%, 2.79% and 0.36% higher than that in open circuit, which fully shown that the number of methanogens in open circuit is higher, which is helpful for methanogens to produce CH_4. By comparing the presence or absence of plants in the closed circuit, it can be known that the methane emission flux of *Methanothrix* in the case of plants is about 4.74% higher than that in the non-plant group, which also greatly proves that the CH_4 emission flux of closed circuit non plant in Section 3.1 was the smallest. However, *Methanobacterium* was higher in the closed-circuit plant-free group, with a value of about 2.44%, which may be because *Methanogens* was suitable to take the easily decomposed carbon source as the nutrient, while cellulose is a macromolecular organic matter, which is difficult to decompose. The coexistence of *Methanothrix* and *MethanoRegula* can be concluded from the analysis, which indicated that methane gas production greatly originates from wetlands, and the use of acetate and CO_2/H_2 were two common substrates(Galand, Fritze et al. 2005). The above detection of methanogens at the genus level in 16S does not prove the relative richness of electrogenic bacteria and methanogens at the genus level, which needs to be further explored.

3.5. Mechanism of methane emission

To investigate the mechanism of electricity production by bioanodes, the competition mechanism between electricity-producing bacteria and methanogenic bacteria was further explored from the above study, as well as the analysis of the principle of methane gas production. CH_4 is not produced under all conditions, but rather due to the limited oxygen supply and anaerobic state of the wetland, which creates the prerequisites for wetland methane production(de la Varga, Ruiz et al. 2015). Of course, CH_4 production can be divided into three stages, the first is the anaerobic fermentation and decomposition of complex organic substrates by fermenting bacteria into ethanol and fatty acids, etc., and the production of acetic acid, hydrogen and CO_2 by syntrophic bacteria. Or acetic acid production by specialized acetic acid-producing bacteria, and finally methane production from acetic acid or CO_2/H_2 in the presence of methanogenic bacteria(Wang and Ren 2013). The specific equation is as follows.

$C_6H_{12}O_6 + 2H_2O \rightarrow 2CO_2 + 2CH_3COOH + 4H_2$ (1)

$CH_3COOH \rightarrow CO_2 + CH_4$ (2)

$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O$ (3)

$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$ (4)

$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$ (5)

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ (6)

The contribution of the two CH_4 production methods varies due to differences in microbial families, organic matter species and content, etc. in different wetlands. A study of peat bogs using isotope tracing found that 70% of the CH_4 was produced by fermentation of acetic acid, while only 30% was formed by reduction of CO_2(Chen, Zhao et al. 2021). In contrast, the root system in the plant group, the plant has an enhanced ability to deliver oxygen to the cathode, resulting in an enhanced ability to produce H_2O from the cathode. However, the oxygen-secreting capacity of the root system shows a positive correlation with the aeration tissue, so not only does the concentration of the carbon source increase in the presence of plants, but their aeration tissue also emits a certain amount of methane gas.

4. Conclusions

Nowadays, wetland GHG emissions continue to be severe and climate deterioration is still accelerating, reflecting strongly on environmental factors such as the atmosphere, water bodies, and soils. CW-MFCs provide direction and technical support for the development of carbon sink technologies, making control of GHGs such as methane a reality. The experimental analysis showed that plants (paddy) in wetlands are higher than microbial fuel cells. By comparing different conditions, CH_4 emissions can be effectively controlled without the addition of substrate and closed circuit. High-throughput sequencing showed that

the anode microorganisms differed significantly under different conditions. Changed the experimental conditions put the electrogenic bacteria at an advantage in competing for nutrients in the anode, thus controlling CH_4 emissions. It can be seen that MFCs have practical application to control CH_4 emissions and should be carried out on a large scale.

Declarations

Acknowledgements

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Tables

Table 1
α-diversity indices of bacterial communities in the reactor anode substrate was determined.

Sample	Chao1	Faith-pd	Observed-species	Pielou-e	Shannon	Simpson
CCP	3397.8	197.6	2849.2	0.79	9.05	0.99
OCP	1621.4	89.9	1325.0	0.51	5.23	0.82
CCN	2502.4	142.6	2004.6	0.63	6.87	0.91

Figures

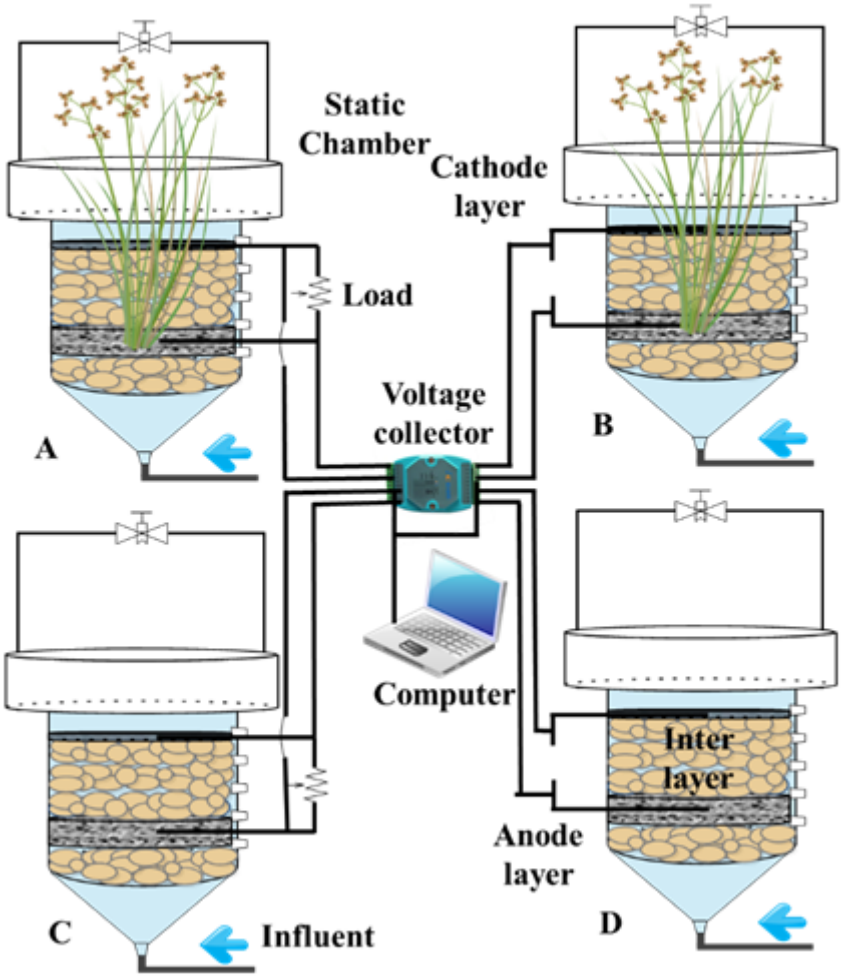

Figure 1

Configuration of the microbial fuel cell-constructed wetland (MFC-CW).

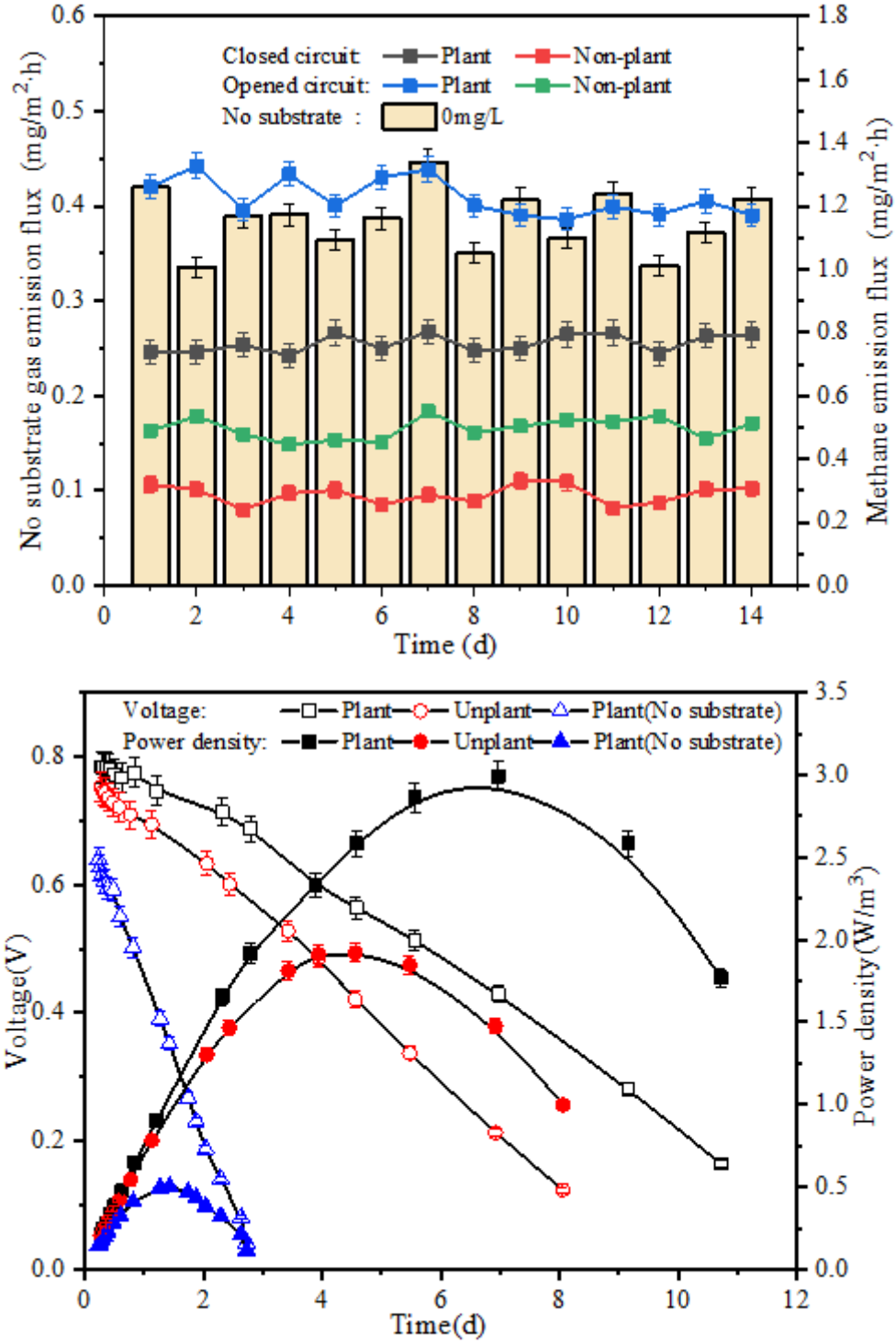

Figure 2

Changes of greenhouse gas emission flux and polarization curve/power density curve in closed/open circuit CW-MFCs.

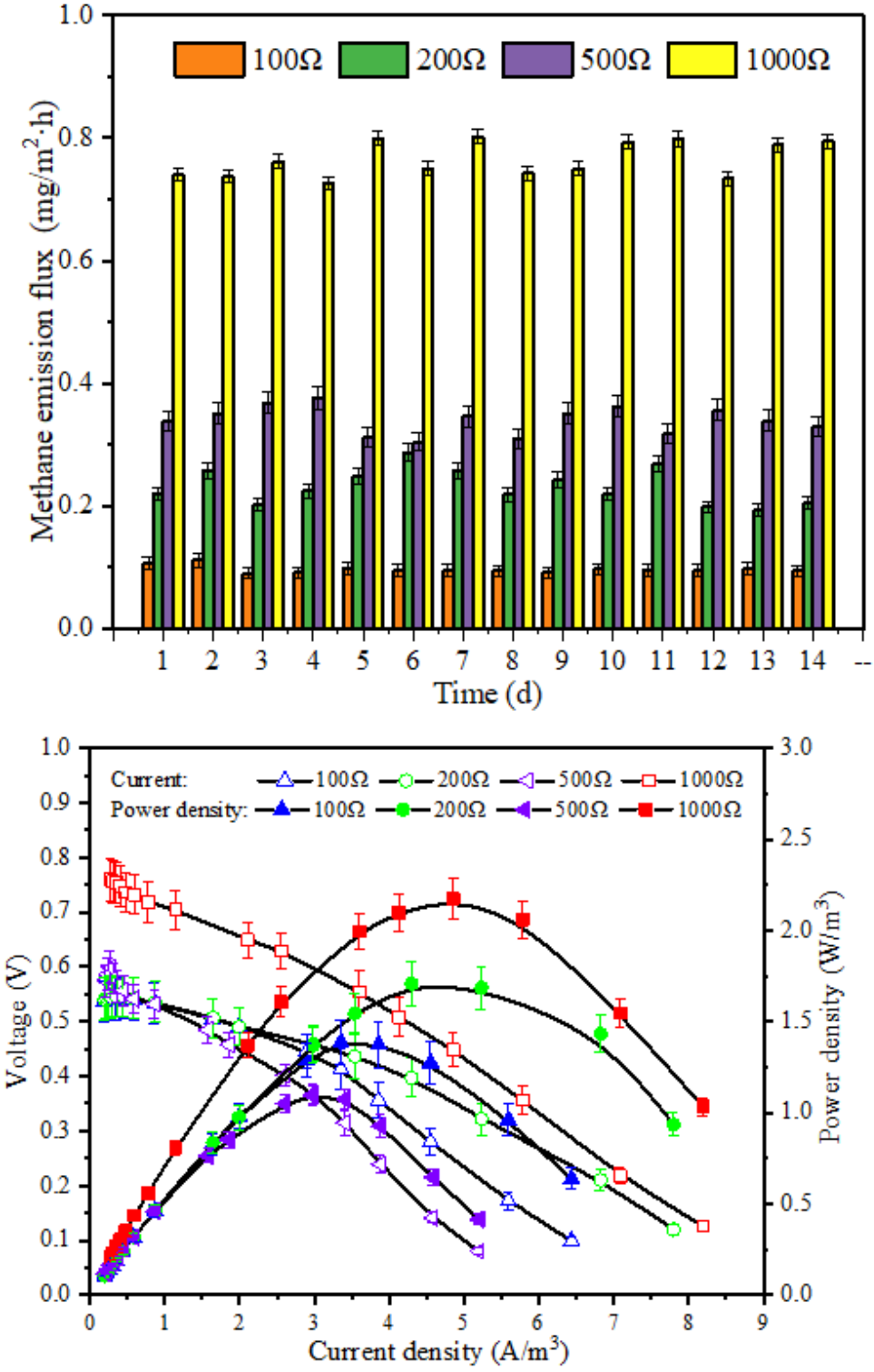

Figure 3

Methane emission fluxes and polarization curve/power density curve of different external resistors.

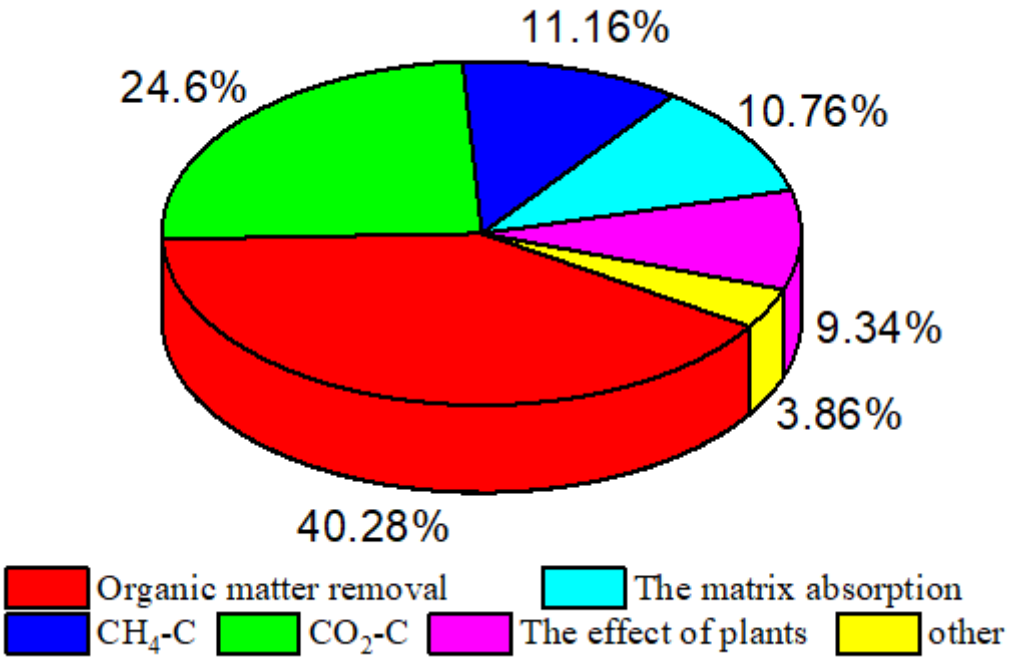

Figure 4

Carbon storage of carbon cycle in atmosphere, water and plants.

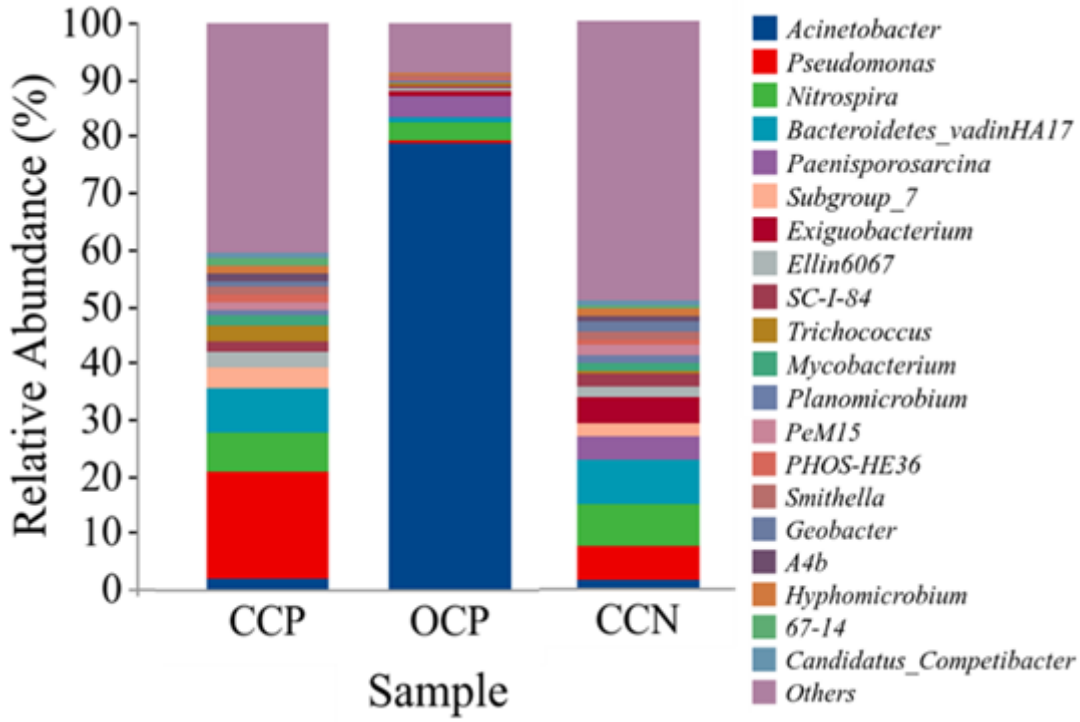

Figure 5

Relative abundance of major genera in CCP, OCP and CCN under 16S primers. Genera with relative abundances above the top twenty are defined as "other".

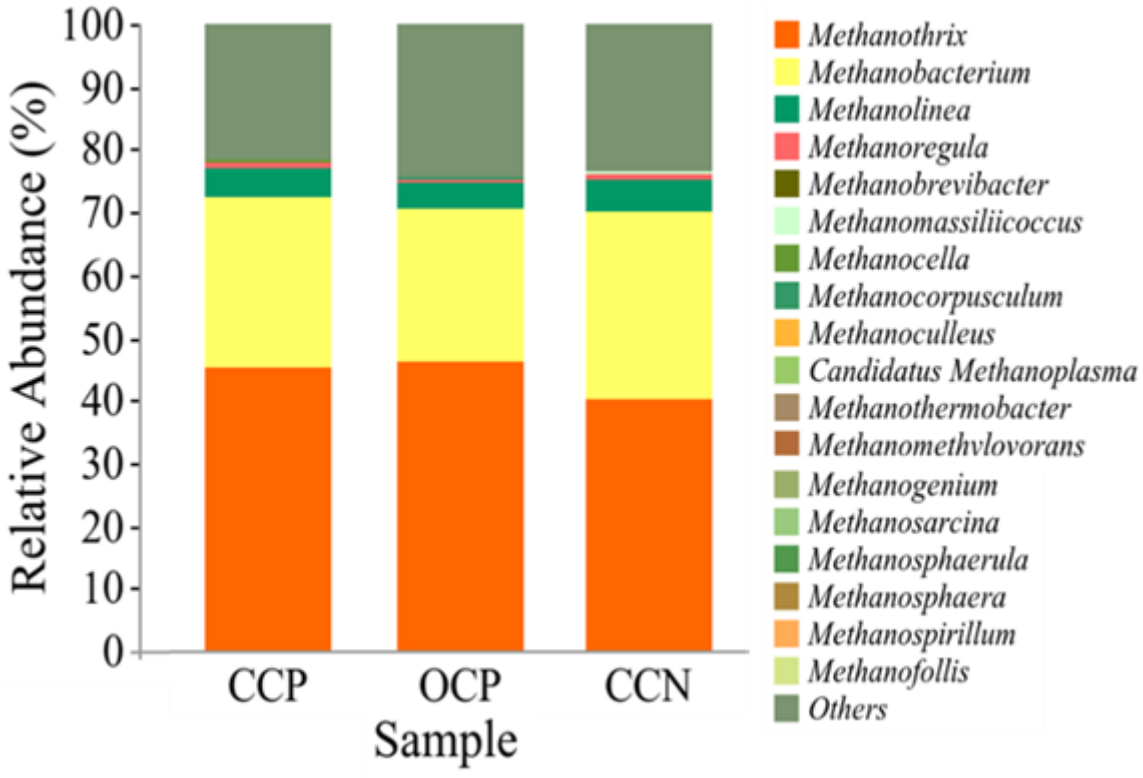

Figure 6

Relative abundance of major genera in CCP, OCP and CCN under methanogenic primers. Genera with relative abundances above the top twenty are defined as "other".

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