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The effects of closed circuit microbial fuel cells on methane emissions from paddy soil vary with straw amount

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ABSTRACT

Straw return increases methane emissions from paddy fields due to carbon input. In closed circuit microbial fuel cells (CC-MFCs), because exoelectrogenic bacteria can compete with methanogens for soil organic carbon, CC-MFCs should reduce methane emissions. We aimed to study the effects of CC-MFCs on methane emissions from paddy soil that was amended with different amounts of straw. Paddy soil was packed into the CC-MFCs and flooded after amendments with 0.5%, 1% and 2% straw or without straw addition. The methane flux from the soil was assessed every seven days. Forty-five days after the CC-MFCs were put into operation, the dissolved oxygen concentration in the overlaying water was determined. Soil was collected from the CC-MFCs, the chemical properties of the soil were measured and DNA was extracted. This was followed by the quantification and sequencing of the methanogenic *mcrA* and methanotrophic *pmoA* genes. The results revealed that with 0.5% straw, CC-MFCs generated less methane than open circuit MFCs (OC-MFCs) which were set as references. However, the CC-MFCs generated more methane than the OC-MFCs under 1% and 2% straw conditions. The dissolved oxygen concentration, which was lower in CC-MFCs than in OC-MFCs, decreased as the straw amount increased. The abundance of methanotrophs in CC-MFCs was significantly lower than that in OC-MFCs with 2% straw. *Methylococcaceae*, which belong to type II methanotrophic bacteria, were more abundant in CC-MFCs than in OC-MFCs. Thus, we suggest that the effects of CC-MFCs on methane emissions vary with the straw amount. With 0.5% straw, the competition for organic carbon between methanogens and exoelectrogenic bacteria mitigated the production of methane. However, CC-MFCs with 2% straw decreased the concentration of dissolved oxygen, inhibiting the activity of methanotrophs and increasing the emissions of methane.

1. Introduction

The paddy fields are one of the major sources of methane emissions, contributing approximately 15% to 20% of the global total anthropogenic methane emissions (Li et al., 2011). Straw return is an effective practice to improve the organic content of soil and soil aggregation (Wang et al., 2015), and thus it has been promoted as a sustainable agricultural practice to replace straw burning (Lu et al., 2010). However, straw return significantly increases methane emissions from flooded paddy fields due to carbon input (Hang et al., 2014). Accordingly, various approaches have been applied to mitigate methane emissions from the soil. Water management involving midseason drainage, intermittent flooding and percolation control can achieve the dual goals of sustaining rice productivity and minimizing methane emissions (Sanchis et al., 2012; Zhou et al., 2015). However, these practices are labor intensive and require high amounts of water, while having little effect on methane emissions under flood conditions. Recent studies have found that incorporating biochar into paddy fields decreases methane emissions during the rice growing cycle (Dong et al., 2013; Zhao et al., 2014). However, there are also studies indicating that the adding of biochar has no effect on methane emissions (Xie et al., 2013) or even enhances methane emissions from the rice paddies (Zhang et al., 2012).

Microbial fuel cells (MFCs) are a type of device in which microorganisms degrade organic substrates and generate electrical current. Because soil contains organic carbons and a diversity of exoelectrogenic bacteria, it can generate electrical power in MFCs (Deng et al., 2012, 2014, 2015). Soil exoelectrogenic bacteria on an anode may compete for organic carbons with methanogens. One evidence in support of this was that the affinity (*Ks*) of known exoelectrogenic bacteria *Geobacter sulfurreducens* for acetate (10 μM) is much lower than the *Ks* of *Methanosaetaceae* (160 μM) and *Methanosarcinaceae* (3 mM) (Esteve-Núñez et al., 2005; Qu et al., 2009), although it is unknown whether all exoelectrogenic bacteria have lower *Ks* values than methanogens. Many studies have found that MFCs can reduce methane

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emissions from lake sediment (Jeon et al., 2012) and paddy soil (Rizzo et al., 2013). Since the inhibition of methanogenesis does not involve energy exhaustion or chemical applications, MFCs are thought to be a potentially novel and sustainable method to reduce methane emissions from soil (Rismani-Yazdi et al., 2013). However, one study found that MFCs had no effect on methane emissions from paddy soil (Kaku et al., 2008). Arends et al. (2014) proposed that the contradicting results might be partly attributed to the variations of organic carbon contents in the studies, and it is possible that the methanogenic metabolism might out-compete the current generating metabolism with an excess of organic carbon. However, there is a lack of empirical studies regarding the mechanisms responsible for the contradicting effects of MFCs on methane emissions.

Methane emissions from flooded paddy soil are the net result of methanogenesis and methane oxidation. The methanotrophic bacteria can oxidize a large portion of methane before being released into the air (Zhang et al., 2015); however, their activity can be decreased by decreasing the oxygen content (Hilger et al., 2000). On one hand, MFCs might reduce methane emissions through the competition between exoelectrogenic bacteria and methanogens, whereas on the other hand, the MFCs cathode exhausts oxygen as electron acceptors, thus inhibiting methane oxidation. Considering that the addition of organic carbon can stimulate electricity generation and accelerate oxygen exhaustion, we hypothesize that MFCs might increase methane emissions from soils that contain an excessive addition of organic substrates. To test our hypothesis, we embedded an anode in flooded paddy soil that was amended with varied amounts of straw and operated MFCs for 45 days. The operational MFCs were closed circuit MFCs (CC-MFCs) whose anodes were placed in flooded paddy soil, and the cathode in the overlaying water was connected using conductive wires. References were set using open circuit MFCs (OC-MFCs) whose anodes and cathodes were not connected (Lin and Lu, 2015). Methane emissions were measured during the period. We aimed to understand whether the effect of CC-MFCs on methane emissions from soil varied based on the addition and on the amount of straw.

2. Material and methods

2.1. Soil sampling

Soil was sampled in July 2013 from a paddy field of approximately one hectare in Yujiang County, Jiangxi Province, China (116.89°E, 28.30°N). The climate is subtropical and wet with an average annual precipitation of 1785 mm and an average annual temperature of 17.8 °C. In the paddy field, surface soil samples (0 to 20 cm) from three randomly selected plots (0.5 m × 0.5 m) spaced approximately 20 m apart were collected and mixed. After sampling, the fresh soil was passed through a 2 mm mesh and thoroughly mixed. Part of the sieved soil was air-dried for chemical analysis using routine methods (Page et al., 1982). Briefly, soil pH was measured at 1:2.5 (soil:water); soil organic carbon (SOC) was determined by K_2CrO_4 oxidation; and total nitrogen (TN) was based on Kjeldahl digestion.

2.2. Straw addition

Rice straw collected from soil sampling sites was oven dried at 105 °C for 6 h and ground to pass through a 1 mm mesh. The straw powder was added to sieved fresh soil at three rates, including 5 g straw kg^{-1} dry weight soil (0.5% straw), 10 g straw kg^{-1} dry weight soil (1% straw) and 20 g straw kg^{-1} dry weight soil (2% straw). The three straw return rates were adopted according to agricultural practices (Zhao et al., 2016). Soil without the addition of straw served as the control treatment. The total carbon and total nitrogen contents of the straw were 652.91 mg kg^{-1} and 7.03 mg kg^{-1}, respectively, as determined by using an elemental analyzer (Vario EL III, Elementar, Hanau, Germany).

2.3. MFCs operation with different straw amounts

Twenty-four MFCs were constructed in PVC with dimensions of Ø10 cm × 10 cm (diameter × height) (Fig. 1). The 24 MFCs were randomly assigned to one of four groups (six MFCs in each group) according to straw addition treatments (control, 0.5%, 1% and 2% straw). In each MFC, the anode and cathode were carbon felt with a diameter of 7 cm. The thickness of the carbon felt was 0.5 cm. Each MFC was packed with 400 g of soil (dry weight). The anode was embedded below 5 cm of thick flooded soil. The water was added gently to the soil until it measured 3 cm above the soil surface, and the cathode was submerged into the overlaying water. To study the effect of operating CC-MFCs on methane emissions for each straw addition treatment, three MFCs were randomly selected as OC-MFCs, and the other three MFCs were set as CC-MFCs. Because the circuits of the OC-MFCs were not connected, no electricity was generated. The OC-MFCs were references against the CC-MFCs, whose circuits were connected and thus generated an electrical current (Lin and Lu, 2015).

The anode and cathode of each CC-MFC were connected with a 1000 Ω external load, which was then replaced with a 10 Ω external load after the generated voltage was stable to obtain a higher current. As a result, the 24 reactors were assigned to eight treatments that combined operating MFCs (OC-MFCs or CC-MFCs) with straw additions of 0.5%, 1%, or 2% or with no straw addition. There were three replicates for each treatment. The day that the MFCs started running was assigned as day 0. The 24 MFCs were incubated in our lab from Aug 21st to Oct. 4th, and the daily mean air temperatures during the period were recorded (Supplementary Fig. S1). The voltage was recorded every 10 min from day 9 to day 45 using a data acquisition module because, on day 9, the 1000 Ω external load was replaced with a 10 Ω external load.

2.4. Chemical analysis

To measure the methane flux, gas emitted from the MFC reactors was collected between 14:00 and 16:00 on days 1, 9, 16, 23, 30, 37

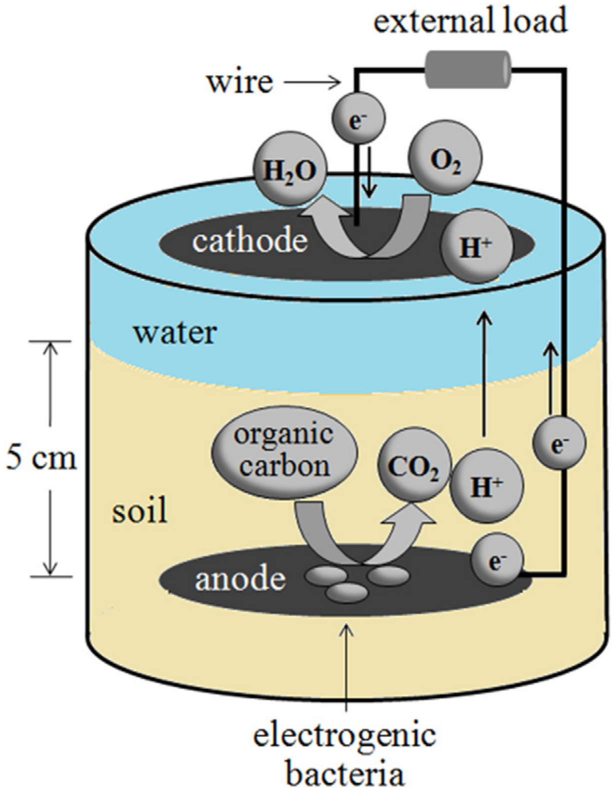

Fig. 1. Diagram of a closed circuit MFC applied in the present study.