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Bacterial and archaeal guilds associated with electrogenesis and methanogenesis in paddy field soil

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ABSTRACT

It is tempting to develop green energy biotechnology to supplement the depletion of fossil fuel resources and to reduce the greenhouse gas emissions. It has been recently demonstrated that paddy soil can be used to construct the sediment-type microbial fuel cells (MFCs). The mechanisms involved in electricity generation and its effects on methanogenesis, however, remain poorly understood. In the present experiment, we constructed paddy soil MFCs to evaluate the effect of MFC on CH_4 emission and to determine the bacterial and archaeal populations associated with MFC anodes. We found that electrical current in MFCs increased rapidly in three weeks, while only minor current was detected in MFCs without rice plants. Methane emission decreased in closed circuit MFCs compared with open circuit MFCs that did not have electricity output. Molecular approaches revealed that the upstream fermentation bacterial community was not significantly affected by MFCs. However, the relative abundances of *Geobacteraceae* increased markedly in MFCs; and concurrently the classical syntrophs including *Syntrophaceae*, *Syntrophorhabdaceae*, *Syntrophobacteraceae* and *Syntrophomonadaceae* increased. Among methanogenic archaea, the relative abundance of *Methanosaetaceae* increased significantly in closed circuit MFCs compared with open-circuit MFCs. Collectively, our study suggests that the activities of electron-transferring bacteria and archaea are significantly promoted in paddy soil MFCs.

1. Introduction

The extracellular electron transport (EET) has been found prevailing in anoxic environments (Reimers et al., 2001; Tender et al., 2002). It has been demonstrated that anaerobes not only use this mechanism for metal like iron respiration, but also use it to transfer electrons to electrodes for electricity generation (Bond and Lovley, 2003).

Paddy soils are flooded for most of the period of rice cultivation. The wet–dry cycles, owning to alternate irrigation and drainage management and the natural aeration during the fallow period, make the paddy soil a perfect environment for diverse microorganisms performing aerobic and anaerobic respirations, fermentation, methanogenesis and homoacetogenesis (Conrad, 1999; Stams, 1994; Zinder, 1993; Liesack et al., 2000). The sediment-type microbial fuel cell (MFC) can be developed in paddy soil (De Schamphelaire et al., 2010; Kaku et al., 2008) and this can be a very tempting eco-biotechnology not only for green energy production but also for mitigation of methane emission from paddy fields (Ishii et al., 2008; Rizzo et al., 2013).

Microorganisms involved in paddy soil MFC however are poorly understood. The entire microbial community comprises the upstream fermenters that generate electron donors and the downstream electron consumers that produce methane and/or electricity as end products. Diverse bacteria including *Clostridiales*, *Bacteroidetes*, *Bacillus*, *Acidobacteria*, *Verrucomicrobia*, *Planctomycetes* and *Actinobacteria* could be involved in fermentation (Lynd et al., 2002; Rui et al., 2009). For electricity production, *Geobacter* spp. and *Shewanella* spp. have been considered most important in various MFCs (Bond and Lovley, 2003; Holmes et al., 2004; Nevin and Lovley, 2002). A recent study revealed that different *Geobacter* species are activated in paddy soil MFCs when supplied with different substrates (Kouzuma et al., 2013). The functioning of *Geobacter* spp. in paddy soils however can be diverse. Kato and colleagues have proposed the occurrence of direct interspecies electron transfer between *Geobacter* and methanogens via the conductive iron oxides (Kato et al., 2012). It was also revealed that *Geobacter* were active during the syntrophic oxidations of propionate and butyrate in paddy soils (Gan et al., 2012; Li et al., 2015) and could outcompete methanogens for acetate even in the absence of soluble iron (Hori et al., 2010, 2007). Furthermore, direct interspecies electron transfer between *Geobacter* and *Methanosaeta* occurred in anaerobic bioreactors stimulating the production of CH_4 (Rotaru et al., 2014). Therefore, the functioning of *Geobacter* for electricity or methane in paddy soil will depend on environmental conditions.

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In the present study, we constructed paddy soil MFCs in a greenhouse pot experiment with the soil collected from a paddy field in southeastern China where rice is widely cultivated. Graphite felts were inserted into the rice rooted zone and at the soil–water interface serving as MFC anode and cathode respectively. MFCs were operated under different settings including: closed circuit and open circuit, shifting from closed to open or from open to closed circuit. We hypothesized that competition for electrons occurred between the electricity production (electrogenesis) and methanogenesis. Different MFC operations were used to test this hypothesis. No external substrates or inoculants were applied to MFCs, thus representing natural conditions of paddy soil. Our objectives therefore were to determine: i) the effect of electrical current production on methane emissions; and ii) the effect of different MFC operations on bacterial and archaeal communities in anode-associated paddy soil.

2. Methods and materials

2.1. Preparation for paddy soil MFC

Soil sample was collected from the plow layer (0–20 cm) of a rice field in spring 2009 at China National Rice Research Institute in Hangzhou, China. The soil sample was air dried, sieved (2-mm mesh size) and stored at room temperature. The soil had the following characteristics as measured by the standard methods (Page, 1982): pH 6.7, CEC of 14.4 Cmol kg^{-1}, organic C of 24.2 g kg^{-1}, total N of 2.3 g kg^{-1} and a texture of clay loam.

To prepare MFC, 4.5 kg of soil (d.w) was weighed into PVC pots (height, 33 cm; inner diameter, 16 cm). The basal fertilizers were mixed with soil at the rates of 30 mg N kg^{-1} in urea and 12.5 mg K and 4.95 mg P kg^{-1} soil in K_2HPO_4. This fertilization represented the local farmer's management practice. During the preparations, one or two graphite felts (10 cm by 10 cm, Beijing Sanye Carbon Co. Ltd., Beijing, China) were buried in each pot serving as anodes according to the experimental treatments (see below). Another graphite felt was placed at the soil–water interface serving as cathodes. All felts were interwoven with a copper wire (diameter, 0.5 mm), which was later attached to the electrical circuitry through an insulated connection during MFC operation. MFCs were flooded with demineralized water maintaining a water layer depth of 3–5 cm above the soil surface. A rice cultivar (*Oryza sativa* var. Jinzao 47, type indica) that was widely cultivated in the southeastern China was chosen for planting. Rice seeds were germinated under dark for 96 h and grown in a soil plate for 15 days. Four rice seedlings were transplanted to each MFC on June 4, 2010. The electrical circuits were closed according to the treatments through the use of a variable external resistance (500 Ω) per MFC. The current across the resister was recorded using a digital ammeter (KJ9205, Shenzhen Kejie Instrument Co. Ltd., Shenzhen, China). Eight treatments were prepared by changing the anode graphite felt number and external circuit connection (Fig. 1): 1) one graphite felt placed at 6 cm with closed circuit (CC1); 2) two graphite felts placed at 6 cm and 14 cm with closed circuit (CC2); 3) one graphite felt with the circuit not closed until 15 days (OC15); 4) one graphite felt with the circuit not closed until 30 days (OC30); 5) one graphite felt with the circuit opened during entire period (OC); 6) one graphite with the circuit opened after day 15 (CC15); 7) one graphite with the circuit opened after day 30 (CC30); and 8) similar to CC2 but without rice planting (CC0), this treatment served as no-plant control. The experiment was carried out with four biological replicates.

2.2. CH_4 emission

For CH_4 flux measurement a closed chamber approach was used (Ma et al., 2010). Briefly, MFC pots were transferred to a large container filled

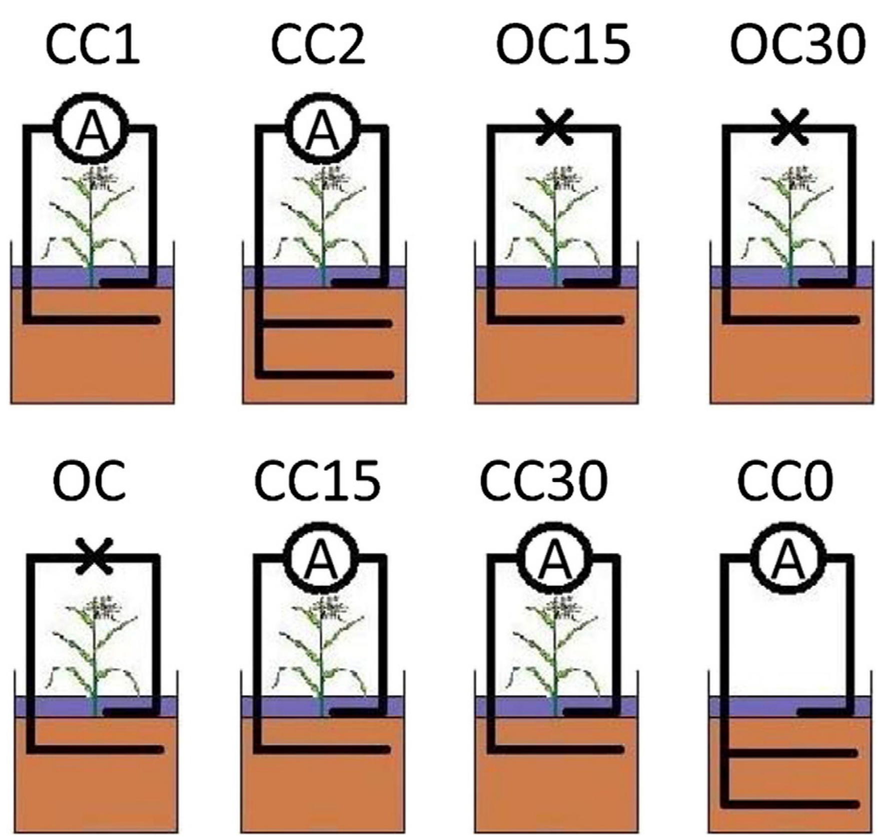

Fig. 1. Diagram of paddy soil MFC treatments prepared. CC1, one graphite felt, closed circuit; CC2, two graphite felts, closed circuit; OC15 and OC30, one graphite felt with the circuit not closed until 15 and 30 days, respectively; OC, one graphite felt, open circuit; CC15 and CC30, one graphite with the circuit opened after day 15 and day 30, respectively.