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A new insight into the strategy for methane production affected by conductive carbon cloth in wetland soil: Beneficial to acetoclastic methanogenesis instead of CO_2 reduction

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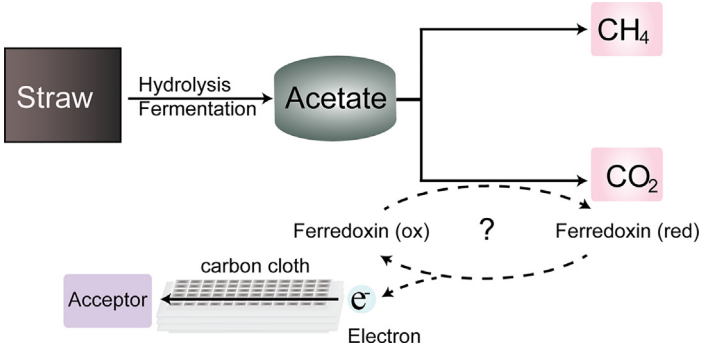
HIGHLIGHTS

- Carbon cloth stimulated CH_4 production in anaerobic wetland soil.
- Carbon cloth significantly promoted electron transfer.
- Carbon cloth did not promote CO_2 reduction to produce methane.
- Acetoclastic methanogenesis potentially contributed to CH_4 production.

GRAPHICAL ABSTRACT



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ABSTRACT

Conductive materials/minerals can promote direct interspecies electron transfer (DIET) between syntrophic bacteria and methanogens in defined co-culture systems and artificial anaerobic digesters; however, little is known about the stimulation strategy of carbon material on methane production in natural environments. Herein, the effect of carbon cloth, as a representative of conductive carbon materials, on methane production with incubated wetland soil was investigated. Carbon cloth significantly promoted methanogenesis. With the application of electrochemical technology, calculation of the apparent electron transfer rate constant showed that carbon cloth significantly increased electron transfer rate (ETR) compared with the control experiment in presence of cotton cloth, from 0.0017 ± 0.0003 to 0.0056 ± 0.0015 s^{-1}. Results obtained from both stable carbon isotope measurements and application of specific inhibitor (CH_3F) for acetoclastic methanogenesis indicated that carbon cloth obviously promoted acetoclastic methanogenesis instead of CO_2 reduction. High-throughput sequencing showed that methane production may stem from the involvement of *Methanosarcina* for both treatments. Our findings suggested that conductive carbon material can promote acetoclastic methanogenesis instead of CO_2 reduction in a natural environment.

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1. Introduction

Methane plays an active part in global carbon and energy cycles as a potent greenhouse gas and a feasible source of renewable energy. Conversion of organic matter to methane occurs widely in anaerobic environments, e.g., soil, sediment and engineered anaerobic ecosystems (Grinham et al., 2018; Murray et al., 2017; Rotaru et al., 2014). Methane production involves a rather sophisticated cooperation between different kinds of microorganisms: primary fermenting microorganisms, secondary fermenting microorganisms, and methanogens (Stams and Plugge, 2009). The mineralization of organic matter to methane by microbial processes contributes to more than two-thirds of all atmospheric methane (Conrad, 2009). Two main types of methanogenic pathways are found: H_2/CO_2 and acetate-dependent methanogenesis, contributing in the ratio of 1:2 to the total amount of methane production (Conrad, 1999).

Acetate is the key intermediate in the anaerobic degradation of organic matter and the primary substrate in methane production. Methanogenic acetate degradation proceeds by means of direct cleavage or syntrophic acetate oxidation (SAO) coupled to hydrogenotrophic methanogenesis. In the direct pathway, methanogens convert acetate into methane and CO_2 by a disproportionation reaction. In the syntrophic pathway, acetate is degraded via the syntrophic interaction between acetate-oxidising bacteria (acetate oxidation process) and methanogens with the ability to reduce CO_2. Interspecies H_2 transfer between non-methanogenic bacteria and methanogens is assumed to be the predominant strategy in syntrophic methanogenic communities ever since the first major breakthrough in the field (Stams and Plugge, 2009). Subsequent findings, however, have shown that there may be an alternative direct interspecies energy transfer mechanism, direct interspecies electron transfer (DIET), for CO_2 reduction. DIET was first found in Lovley's laboratory by analysis of co-cultures of two species of *Geobacter* (Summers et al., 2010). Subsequently, they revealed that DIET was potentially used for methanogenesis between *Geobacter* species and *Methanosaeta* in aggregates (Morita et al., 2011; Rotaru et al., 2014).

Methanogenesis has been found to be stimulated by electrically conductive materials, such as granular activated carbon (GAC), biochar, magnetite, and carbon cloth. In previous research, we proposed that GAC and biochar can serve as a conduit between *Geobacter metallireducens* and *Methanosarcina barkeri* to stimulate syntrophic metabolism (Chen et al., 2014b; Liu et al., 2012). This process was also facilitated by electric syntrophy via magnetite in anaerobic systems (Cruz Viggi et al., 2014; Yang et al., 2015; Zheng et al., 2017). Furthermore, other substances, such as conductive polymers and nano-zero-valent iron, can also enhanced methane production in anaerobic digestion (Hu et al., 2017; Yang et al., 2013). Recently, it has attracted much attention in the study of microbial biogas production with the aid of carbon cloth, which acts as an excellent conductive material. For a defined microbial culture, carbon cloth can promote DIET in syntrophic co-cultures of *G. metallireducens* and *M. barkeri* (Chen et al., 2014a). In anaerobic reactors, syntrophic metabolism in up-flow anaerobic sludge blanket reactors was enhanced with this type of conductive material (Zhao et al., 2015), and potentially shifting from interspecies hydrogen transfer to DIET for syntrophic metabolism to resist acidic impact may be the key (Zhao et al., 2017).

Although the studies on effects of conductive materials on artificial anaerobic digestion of complex organic matter to produce methane have boosted in recent years, whether or not these mechanisms, or conclusions, apply equally to natural environment is still unclear. It well known that there are plentiful differences between an anaerobic digester and methanogenic soil (Holmes et al., 2017). Most directly, most of anaerobic digesters have a relatively simple organic composition, and substrates are provided at high rates to support rapid metabolic fluxes. Conversely, much more complex assemblage of polymeric fermentable materials, which are slowly degraded, widely scatters in methanogenic terrestrial ecosystems as the primary source of organic substrates used for methane production. Based on this, compared with artificial anaerobic fermentation system, strategies pertinent to use of conductive carbon materials in microbial methane production require clarification in a natural environment. Some studies showed that methanogenic process was also facilitated by electric syntrophy via magnetite (Kato et al., 2012; Xiao et al., 2018; Zhuang et al., 2015), biochar (Wang et al., 2017), and carbon nanotubes (Zhang and Lu, 2016; Zhang et al., 2018) in natural soil or sediments: however, little is known about the stimulation strategy of carbon cloth on methane production in natural environments.

In the present study, we investigated the effect of carbon cloth on methanogenic processes in wetland soil with the application of electrochemical technology (cyclic voltammetry, CV), carbon isotope fractionation and a methanogenesis pathway inhibitor (CH_3F). This study suggested that carbon cloth robustly triggered methane production by potentially promoting a route for acetoclastic methanogenesis instead of CO_2 reduction in the natural soil with complex communities.

2. Materials and methods

2.1. Microcosm cultivation

Soil was collected from the Yellow River Delta, a sensitive wetland ecosystem (Xiao et al., 2017). The air-dried soil was passed through a plastic sieve (2 mm mesh). Incubated soil was prepared by adding soil (40 g) and 0.4 g dry ground straw of *Phragmites australis*, which is the main vegetation in this region, into a 250 mL bottle (containing 80 mL anoxic sterile water) capped with a rubber stopper as our previous description (Liu and Conrad, 2010). The bottle was flushed with N_2 and incubated statically at 30 °C for 12 days. When paddy soil was used to conduct related research, inoculation with 0.05% straw for >6 weeks, we still detected degradation of the straw resulting in acetate accumulation (Liu and Conrad, 2010). Other studies have also shown that a considerable amount of straw (10 mg dry straw per gramme of dry soil) can be used for incubation cycles up to 4 weeks (Conrad et al., 2012), even 3.5 months (Conrad and Klose, 2011). In this study, 1% straw (by mass) was used over 12 days of incubation to provide excess straw to ensure that the methanogenic substrate was not affected by an insufficiency of straw. Then, 5 mL incubated soil was dispensed into sterile 12-mL serum vials, which were previously evacuated and flushed with N_2 beforehand, and incubated statically at 30 °C.

The electrochemical active surface area of carbon cloth was conducted. It was calculated to be 21.64 cm^2 through measuring cyclic voltammogram curves, according to the Randles-Sevcik equation (Bard and Faulkner, 2001): $i_P = 4.464 \times 10^4 nFAC(\frac{nF}{RT})^{1/2} D^{1/2} v^{1/2}$ where i_P is the peak current (A), n represents the number of electrons, F is Faraday constant (96,487 C mol^{-1}), A is the active surface area (cm^2), C is the initial concentration of $K_3Fe(CN)_6/K_4Fe(CN)_6$ (mol cm^{-3}), R denotes the gas constant (8.314 $J\ mol^{-1}\ K^{-1}$), D is the diffusion coefficient (0.76 $mic^{-6}\ cm^2\ s^{-1}$, at 298 K in 0.1 M KCl solution) and v is the scan rate (V s^{-1}). Three pieces of carbon cloth (about 1 cm × 1 cm × 0.04 cm, electrical resistance: 98 ± 13 Ω; mean ± standard deviation, $n = 4$) or the same size of cotton cloth (electrical resistance: 628 ± 83 kΩ) were added to each vial, respectively.

The vials were sealed with Teflon®-coated septa and treated with five cycles of vacuum/charging nitrogen gas. These vials were incubated statically, in the dark, at a constant temperature of 30 °C. During the preliminary experiments, it was found that the concentration of acetate exhibited a rapid upward trend in the presence of cotton when the duration of the experiment exceeded 7 days. Therefore, the experiment was set to last 5 days in this study. Vials were sacrificed in triplicate for testing the concentration of methane and CO_2 after certain incubation times (0.8, 2, 3, 4, and 5 days). The gas was transferred to 12-mL vacuum borosilicate vials (Labco, UK) for the determination of the