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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 CO2 reduction

Jiajia Li a,b,d,1, Leilei Xiaoa,b,⁎, Shiling Zheng a,b, Yuechao Zhang a,b,d, Min Luo c, Chuan Tong c, Hengduo Xua,b, Yang Tan a, Juan Liao e, Oumei Wang f, Fanghua Liu a,b,⁎

a Key Laboratory of Coastal Biology and Biological Resources Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China
b Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China
c Key Laboratory of Humid Subtropical Eco-geographical Process, Ministry of Education, Fujian Normal University, Fuzhou 350007, China
d University of Chinese Academy of Sciences, Beijing 100049, China
e College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China
f Binzhou Medical University, Yantai 264003, China

HIGHLIGHTS
• Carbon cloth stimulated CH4 production in anaerobic wetland soil.
• Carbon cloth significantly promoted electron transfer.
• Carbon cloth did not promote CO2 reduction to produce methane.
• Acetoclastic methanogenesis potentially contributed to CH4 production.

GRAPHICAL ABSTRACT

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 (CH3F) for acetoclastic methanogenesis indicated that carbon cloth obviously promoted acetoclastic methanogenesis instead of CO2 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 CO2 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: H₂/CO₂̂ 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 CO₂̂ 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 CO₂̂. Interspecies H₂ 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 CO₂̂ reduction. Direct electron transfer in natural environments is still unclear. It well known that there are plentiful differences between an anaerobic digestor 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 (CH₃F). This study suggested that carbon cloth robustly triggered methane production by potentially promoting a route for acetoclastic methanogenesis instead of CO₂̂ 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 N₂ 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 N₂ beforehand, and incubated statically at 30 °C.

The electrochemical active surface area of carbon cloth was conducted. It was calculated to be 21.64 cm² through measuring cyclic voltammogram curves, according to the Randles-Sevck equation (Bard and Faulkner, 2001):

\[ i_p = 4.464 \times 10^n F A C^{1/2} D^{1/2} v^{1/2} \]

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 (cm²), \( C \) is the initial concentration of K₂Fe(CN)₆/K₄Fe(CN)₉ (mol cm⁻³), \( R \) denotes the gas constant (8.314 J mol⁻¹ K⁻¹), \( D \) is the diffusion coefficient (0.76 microm² 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 CO₂ 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
\[ \delta^{13}C \text{-values of methane and CO}_2. \text{ The supernatant was collected to determine pH value and acetate concentration.} \]

Additional batch experiments were also carried out to explore contributions from different methanogenic pathways (\( \text{CO}_2 \text{ reduction or acetoclastic methanogenesis} \)). \( \text{CH}_3\text{F} \) was added into the headspace of the vials (1.5%) to inhibit acetoclastic methanogenesis pathway (Conrad and Klose, 1999; Janssen and Frenzel, 1997). Theoretically there was no longer an acetoclastic methanogenesis pathway in the reaction system when \( \text{CH}_3\text{F} \) was added. Therefore, we were able to analyse the contribution of acetate-based methanogenic pathway to total methane production in different treatments. It should be noted that the use of inhibitor (\( \text{CH}_3\text{F} \)) and carbon isotope fractionation were two different methods for the analysis of the contribution of the acetate-based methanogenic pathway. Culture and determination were consistent with the descriptions given above.

### 2.2. Chemical analyses

Gas sample (200 μL) was taken from the headspace using a sterilised syringe with a two-way valve. Concentrations of methane and \( \text{CO}_2 \) were analysed using a gas chromatograph (GC; Agilent 7820A, USA) equipped with a flame ionisation detector. The retention time of methane was about 1.15 min and the minimum detectable limit was about 7.14 μg L\(^{-1} \).

A gas chromatograph combustion isotope ratio mass spectrometer (GC–C–IRMS) system (Thermo Fisher MAT253, Germany) was used to test isotope composition of methane and \( \text{CO}_2 \). For the determination of methane, the isotopes were quantified in a Finnigan MAT253 IRMS. Separation of \( \text{CH}_4/\text{CO}_2 \) was performed in a Finnigan Precon. Each sample (1–2 mL) was injected into a sample container (100 mL), which had been filled with helium gas (99.999% purity). The \( \text{CO}_2 \) was scrubbed from the sample using a chemical trap (filled with Mg(ClO\(_2\))\(_2\) and ascarite). The \( \text{CH}_4 \) was then purified by cold trap, which was filled with Ni wires. The \( \text{CH}_4 \) was then oxidised in a combustion reactor at 960 °C and converted to \( \text{CO}_2 \) and water. The combusted \( \text{CO}_2 \) was enriched by two liquid nitrogen cold traps and transferred into the IRMS for determination. The precision of repeated analyses was ±0.2‰ when 1.3 nmol methane was injected. The abundance of \( ^{13} \text{C} \) in a sample is given relative to a standard using the \( \delta \) notation:

\[
\delta^{13}C = \left[ \frac{^{13}C}{^{12}C} \right]_{\text{sample}} / \left[ \frac{^{13}C}{^{12}C} \right]_{\text{PDB}} - 1 \times 1000
\]

where PDB refers to the Pee Dee Belemnite carbonate that is used as standard which has a \( ^{13} \text{C}/^{12} \text{C} \) ratio of 0.0112372. A similar method was used to test \( ^{13} \text{C}- \text{values of CO}_2 \). The chemical trap was replaced by a water trap.

Acetate was measured using high-pressure liquid chromatography (HPLC; Agilent 1260 Infinity). Separation was accomplished using a Hi-Plex H column at 60 °C with a refractive index detector at 55 °C. The mobile phase used was 5 mM H\(_2\)SO\(_4\) at a flow rate of 0.5 mL/min.

### 2.3. Electrochemical measurements

To characterise the electron transfer during anaerobic reactions, an experiment was conducted for CV measurement using a single-chamber three-electrode electrochemical cell. The three electrodes were: the reference electrode (Ag/AgCl), a graphite plate electrode (3.0 cm × 2.5 cm × 0.3 cm, as a working electrode), and a platinum electrode (the counter-electrode), respectively. Amounts of 70 mL slurry taken from reactors were poured into the electrochemical cell. The cell was purged with \( \text{N}_2 \) for 20 min. After static incubation at 30 °C for 3 days when methane production differed between the two treatments in presence of cotton cloth or carbon cloth, CVs were measured using an electrochemical workstation (CHI660e, Chenhua, China). The scanning voltage of the working electrode was in the range of −1.2 to 1.2 V (versus Ag/AgCl) with scan rates of 10–140 mV/s.

The apparent electron transfer rate constant (\( k_{\text{app}} \)) was calculated according to the Laviron equation (Laviron, 1979). Simply, according to the following equations, we can calculate \( \alpha \):

\[
\ln \left( \frac{\text{RT}_{c}}{\text{RT}_{\alpha}} \right) = \ln \left( \frac{\text{canF}_{\alpha}}{\text{RT}_{\alpha}} \right) \text{ and } \ln \left( \frac{\text{RT}_{\alpha}}{\text{RT}_{\text{app}}} \right) = \ln \left( \frac{\text{canF}_{\text{app}}}{\text{RT}_{\text{app}}} \right)
\]

where \( \alpha \) was the transfer coefficient, \( E_{pc} \) was the potential of the reduction peak, \( E_{pa} \) was the potential of the oxidation peak, \( \nu \) was the sweep rate, and \( R, T, \) and \( F \) had their usual meanings (\( R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}, T = 298 \text{ K}, \text{ and } F = 96,483 \text{ C mol}^{-1} \)). The slopes of the linear portions of the \( \text{RT}_{\alpha} - \text{E}^0 \) versus \( \nu \) plots were \( \text{RT}/(1 - \alpha) \text{ nF} \) and \( \text{RT}/\text{canF} \) for the oxidation and reduction peaks, respectively. Values of \( k_{\text{app}} \) can be calculated by using the following equation:

\[
\lg k_{\text{app}} = \alpha \lg(1 - \alpha) + (1 - \alpha) \lg \alpha - \frac{\text{RT}}{\text{canF}}(1 - \alpha)\alpha n\text{F}_{\alpha} E / 2.303RT
\]

where \( \alpha E \) was potential difference between the reduction, and oxidation, peaks, other parameters were as defined above.

### 2.4. DNA extraction and 16S rRNA gene sequencing

Methane production differed on day 3, so the slurry sampled from the cotton-, or carbon cloth, surface was used to analyse the communities of bacteria and archaea at this point in time. Two duplicate assays were carried out for each type of treatment. DNA was extracted and PCR amplification of 16S rRNA gene using primers of bacterial (Bac515F and Bac926R) and archaeal (Arch519F and Arch915R) was performed. An Amplicon library was built following by sequencing using an Illumina Miseq platform from Tiny Gene Bio-Tech (Shanghai) Co., Ltd. The bioinformatics analysis was conducted with reference to previously described methods (Edwards et al., 2015). In brief, low quality sequences, clustering operational taxonomic units (OTUs), and taxonomic classifications were processed. The raw fastq files for all samples were demultiplexed on the basis of their barcode. Trimmmomatic (ver. 0.35) was used to remove low-quality base pairs from the paired-end reads (parameters used: SLIDINGWINDOW = 50:20; MINLEN = 50). The trimmed reads were then further merged using the FLASH program (ver. 1.2.11) with default parameters. The low quality contigs were removed using the ‘screen.seqs’ command (filtering parameters: maxambig = 0, minlength = 200, maxlength = 580, and maxhomop = 8). A combination of various software packages: mothur (ver. 1.33.3), UPARSE ([usearch ver. 8.1.1756, http://drive5.com/uparse/]), and R (ver. 3.2.3) were applied to analyse the 16S sequences. The demultiplexed reads were clustered at 97% sequence identity into OTUs using the UPARSE pipeline (http://drive5.com/usearch/manual/uparse.cmds.html). The OTU representative sequences were used for taxonomy assignment against the Silva 119 database (confidence score ≥ 0.8) using the ‘classify.seqs’ command in mothur. The OTU taxonomies (from phylum to species) were determined based on the NCBI database.

### 2.5. Statistical analysis

Data are presented as mean ± standard deviation of triplicate cultures. All statistical analyses were performed with SPSS 19.0 (SPSS Inc., Chicago, USA) and Origin 8.5 (Origin Lab Corporation, USA) software. A one-way analysis of variance with HSD’s test was used to analyse the significance level, and a P value of < 0.05 was considered statistically significant.
3. Results

3.1. Methane production in the presence of carbon cloth

Methane concentration in the vials containing cotton cloth was lower than that in unamended control group samples. The rate of methane production in the vials with the addition of carbon cloth was higher than that in the unamended control and cotton cloth groups (Fig. 1). Methane production was maintained a relatively steady rate for treatments involving cotton- and carbon cloth at 0.175 and 0.564 mmol L\(^{-1}\) h\(^{-1}\), respectively. Compared with cotton cloth, the apparent effect of carbon cloth was to increase the rate of methane production by 222.3%.

3.2. The effect of carbon cloth on electron transfer

To confirm that the increased methane production was related to the higher conductivity of carbon cloth, an analysis of the dependence of the peak currents on the scan rates was undertaken. The peak currents increased linearly with an increase in scan rates for peaks 1 and 2 in both treatments (Fig. 2A and C, insets). Further, the dependence of peak potential on the scan rate was investigated by plotting \(E_p\) versus \(\ln v\) (Fig. 2B and D). Herein, slopes were the same as those obtained when plotting \(E_p - E^0\) or \(E_p\) versus \(v\). Significantly, peak potentials were presented as a function of the logarithm of the scan rates. Based on Laviron theory, values of \(k_{app}\) calculated from two redox peaks (peaks 1 and 2) with cotton- and carbon cloth were 0.0017 ± 0.0003 and 0.0056 ± 0.0015 s\(^{-1}\), respectively: the value of \(k_{app}\) was thus improved by the use of carbon cloth.

3.3. Impact of carbon cloth on carbon isotopic fractionation

The isotope discrimination was different between incubated soil containing cotton- and carbon cloth (Fig. 3A). In the incubated soil with cotton cloth, methane was relatively enriched in 12\(^{\text{C}}\), with \(\delta^{13}\text{C}\)-values between −54 and −66‰. Combined with \(\delta^{13}\text{C}\)-values of CO\(_2\), Fig. 3B showed that the production of methane mainly used acetate as a precursor. Methane was steadily depleted in 12\(^{\text{C}}\) with the addition of carbon cloth throughout the experiment, at values of −50 to −54‰, and aceticlastic methanogenesis predominately contributed to the production of methane at all sampling times (Fig. 3B).

For the control (Fig. 4A), the difference was negligible in the concentration of methane generated with, and without, CH\(_3\)F. The contribution of aceticlastic methanogenesis to total methane production decreased gradually, and it was −20% after 5 days’ incubation. Methane in the control group was mainly derived from the reduction of CO\(_2\). The trend was different in the presence of carbon cloth (Fig. 4B). CH\(_3\)F substantially inhibited methane production in the presence of carbon cloth. The percentage of methane produced from aceticlastic methanogenesis remained stable and it was above 85% throughout the experiment. The CH\(_3\)F exerted a greater influence on treatment with carbon cloth compared to the control. Therefore, the methane concentration in samples treated with carbon cloth in the presence of CH\(_3\)F was much lower than that of those in the cotton cloth group. Combined with the results shown in Fig. 1A, methanogenesis was mainly driven by acetate cleavage, i.e., the use of carbon cloth promoted soil methane production by increased aceticlastic methanogenesis in this study.

3.4. Impact of carbon cloth on methane production under the inhibition of aceticlastic methanogenesis

Carbon cloth did not change the abundance of the major bacteria (Fig. 5). Mobilisleae spp. was the most abundant bacteria in both treatment groups. This kind of microbe was able to ferment a variety of mono-, di-, and polysaccharides, including microcrystalline cellulose. Therefore, Mobilisleae spp. may contribute significantly to the fermentation process in this study. According to the results in Fig. 5A, pseudobacteroides spp., the second most abundant bacteria, may also participate in straw degradation.

At genus level, Methanosarcina spp., the most metabolically versatile of the methanogenic archaea, showed the highest abundance of methanogens in both treatments (Fig. 5B). These results indicated that carbon cloth did not appear to alter the community structure of methanogenic archaea to any significant extent, and methanogenesis may stem from the decomposition of acetate by Methanosarcina.

The functional methanogenic archaea was also analysed at family level (Fig. S1). The results showed that the proportion of unclassified archaea was approximately 25%. Fortunately, these unclassified archaea belong to phylum Thaumarchaeota. As the microorganisms actively involved in methanogenesis have a restricted phylogenetic distribution, it was hypothesised that archaeal CH\(_4\) metabolism originated within the phylum Thaumarchaeota (Gribaldo and Brochier-Armanet, 2006). To the best of our knowledge, there is still no experimental evidence to prove that Thaumarchaeota archaea can produce CH\(_4\). Therefore, unclassified archaea may contribute very little to methane production. The production of methane was very likely to have arisen from the 75% of the detected archaea, especially Methanosarcinaeae.

4. Discussion

The carbon cloth used in this study was shown in Fig. S2: it had a relatively high surface area. Previous studies showed that carbon cloth can provide attachment sites for microbes affecting methane production processes (Chen et al., 2014a; Dang et al., 2017). Therefore, cotton cloth was used as a control experiment considering that it also has a certain adsorption effect. If the control group is an experiment without any treatment, then we cannot exclude the effect of carbon cloth on methane production due to the adsorption thereon. Furthermore, a control experiment with no addition of either carbon cloth or cotton cloth (unamended treatment) was also conducted. Experimental results showed that the effects of the addition of cotton cloth and unamended treatment on methane production were comparable (Fig. 1). It suggested that cotton cloth did appear to have a certain influence on the methane production process. This confirmed that the addition of
Exogenous substance may affect the performance of anaerobic systems. To exclude the effect of carbon cloth on the reaction system due to non-conductive properties, the use of cotton cloth may be a better control.

According to the acetate concentration shown in Fig. S3, there was little cotton cloth degradation to provide additional acetate in the 5-day experimental period. The low concentration of acetate (<0.6 mM) may be due to the low abundance or activity of fermenting bacteria and acetogenic bacteria in the soil. Our recently published study showed that the acetate concentration was approximately 1.5 mM when the used soil was collected at the same site but at different times (Xiao et al., 2018). The soil used for previous research was collected in the summer and the soil used this time was collected at a lower temperature in December. The lower temperature may affect the abundance and activity of related microorganisms. Methane production was maintained a relatively steady rate in both treatments, and the addition of carbon cloth was beneficial to methanogenesis. We then investigated whether, or not, the increased methane production rate was due to an increase in the ETR. Many studies showed that the CV experiment could be used to quantify the capacity of extracellular electron transfer between biomass and electrode (Yuan et al., 2011; Zhang et al., 2017). According to recent studies (Lee et al., 2017; Yin et al., 2018), CV was applied to reflect the electron transfer between electron-donating and -accepting partners. For both types of reactors (with cotton- and carbon cloth), currents at peaks 1 and 2 increased linearly with an increase in scan rates (Fig. 2A and C), suggesting that these redox reactions were typical kinetically controlled processes (Yin et al., 2018; Yu et al., 2015). Furthermore, the dependence of peak potential on the scan rate was investigated by plotting $E_p$ versus $\ln v$ (Fig. 3B and D): their relationship followed the zero-order kinetic equation. According to Laviron’s theory, the values of $k_{app}$ calculated from two redox peaks (peak 1 and peak 2) with cotton- and carbon cloth were $0.0017 \pm 0.0003$ and $0.0056 \pm 0.0015$ s$^{-1}$, respectively. The greater value of $k_{app}$ in the reactor with carbon cloth (increased by 229.4% compared to cotton cloth) suggested that a faster ETR was triggered by conductive carbon cloth. Taken together, the conductive property, but not adhesion, may be the causative factor for the stimulation of methanogenic process, i.e., the enhanced methanogenic efficiency was very likely to have been due to the higher ETR.

In subsequent tests the $\delta^{13}C$ values of methane were determined to evaluate underlying methane production pathways. It is widely recognised that the $\delta^{13}C$ value of methane was smaller when CO$_2$ was used as substrate compared to that of methane obtained via the acetoclastic methanogenesis pathway (Conrad, 2005; Conrad et al., 2007). $\delta^{13}C$-values in the vials containing carbon cloth were much heavier than that of cotton cloth (Fig. 3 A). Combined with $\delta^{13}C$-values of CO$_2$, Fig. 3B showed that the production of methane was very likely to use acetate as a precursor, i.e., this is a robust indication that the increase in acetoclastic methanogenesis contributed to the production of methane.
To confirm the results based on carbon isotope determination, CH$_3$F was used to inhibit acetoclastic methanogenesis for clarifying the contributions of acetate cleavage to total methane production. Under these circumstances, the amount of methane produced with the addition of carbon cloth was almost equal to that of cotton cloth (Fig. 4, compared two treatments with CH$_3$F). It suggested that increased methane generation did not arise from CO$_2$ reduction, which was the main pathway for methane production except for acetoclastic methanogenesis, with the presence of carbon cloth. For the control experiment (cotton cloth), the difference was slight with respect to the concentration of methane regardless of the absence, or presence, of CH$_3$F. The contribution of acetoclastic methanogenesis to total methane production decreased gradually, and it was ~20% after 5 days’ incubation. For the possibility of adsorbing CH$_3$F on cotton cloth to affect the production of methane, the detected methane production may be lower if the amount of CH$_3$F was sufficient. For the analysis of the relative contribution of various methanogenic pathways, the adsorption of CH$_3$F may overestimate the proportion produced via the CO$_2$ reduction pathway. In contrast, the percentage of methane produced from acetoclastic methanogenesis remained stable at >85% throughout the experiment.

Isotope fractionation plus inhibition experiments confirmed that carbon cloth did exclusively trigger robust acetoclastic methanogenesis in an anaerobic soil with complex microbial communities.

For the studies of complex microbial communities, according to our understanding, previous reports suggested that carbon cloth promoted the production of methane through an increased CO$_2$ reduction process with the stimulation of ETR (Lei et al., 2016; Liu et al., 2017; Lovley, 2017; Zhao et al., 2017; Zhao et al., 2015). Compared with anaerobic digester, there is relatively little research on the effect of conductive matters/materials on methane production in soil/sediment. This research into the impact of carbon cloth on soil methane production is novel. Methanogenesis has been found to be stimulated by other conductive magnetite and carbon nanotube material (Kato et al., 2012; Yang et al., 2016; Zhang and Lu, 2016; Zhang et al., 2018; Zhuang et al., 2015). The promotion of CO$_2$ reduction by increased electron transfer is the key mechanism advocated by those studies. These studies did not take into account the possibility of the acceleration of acetoclastic methanogenesis: in this study, the electron transfer rate was increased upon the addition of carbon cloth. Referencing the redox reaction in the methanogenesis of acetate-derived methanogenic pathway, electrons released during the oxidation of ferredoxin may be more easily transmitted with the aid of carbon cloth (Fig. S4). Of course, we must also consider that the increase in methane-generation in the carbon cloth treatment group may not be related to the conductivity of the carbon cloth. The carbon cloth may be more conducive to providing an attachment site for acetate-utilising methanogens and more prone to stimulating the activity of such methanogens than cotton cloth or a treatment without addition of any extra material. It should be noted that a recent study also showed that syntrophic acetate oxidation and hydrogenotrophic methanogenesis were restricted or inhibited by the presence of β-FeOOH (akaganèite), and akaganèite would promote the conversion of acetate to methane via a disproportionation reaction in a high-temperature petroleum reservoir (Pan et al., 2017). Combining the findings in this study, differently promoted strategies in natural ecosystems may exist.

5. Conclusion

This study showed that carbon cloth robustly triggered methane production by promoting a route for acetoclastic methanogenesis. To the best of our knowledge, it was the first report to mention that conductive carbon material may accelerate acetoclastic methanogenesis instead of CO$_2$ reduction. This expanded our knowledge of the role of conductive material in methane production in natural soil and suggested a potential mechanism for the increase in methane production in engineered ecosystems.
Conflict of interest

The authors declare that they have no competing financial interest associated with this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.06.271.

References

Bard, A.J., Faulkner, L.R., 2001. Electrochemical Methods: Fundamentals and Applications. 2nd ed. John Wiley and Sons, Inc., New York.

Chen, S.S., Rotaru, A.E., Liu, F.H., Phillips, J., Woodward, T.L., Nevin, K.P., Lovley, D.R., 2014a. Carbon cloth stimulating direct interspecies electron transfer in syntrophic co-cultures. Biosens. Bioelectron. 60, 117–127.

Conrad, R., Chan, O.C., Claus, P., Casper, P., 2007. Characterization of methanogenic Archaebacteria and archaea. Nat. Rev. Microbiol. 7, 578–587.

Summers, Z.M., Fogarty, H.E., Leang, C., Franks, A.E., Malvankar, N.S., Lovley, D.R., 2010. Direct exchange of electrons within aggregates of an evolved syntrophic coccus of methanotrophic bacteria. Science 330, 1413–1415.

Yang, W., Chang, Z., Xue, X., Yu, J., Shi, X., Ma, L., Li, H., 2017. Biocatalyst estimates nitrogen oxide and enhance methane emissions via altering microbial community of anaerobic paddy soil. Sci. Total Environ. 581–582, 689–696.

Whiticker, M.J., 1999. Carbon and hydrogen isotopic systematics of bacterial fermentation and oxidation of methane. Chem. Geol. 161, 291–314.

Xiao, L., Xie, B., Liu, J., Zhang, H., Han, G., Wang, O., Liu, F., 2017. Stimulation of long-term anammox nitrogen deposition on methanogenic Methanobrevibacter. FEMS Microbiol. Ecol. 95, 337–343.

Yang, W., Liu, F., Liu, J., Li, J., Zhang, Y., Yu, J., Wang, O., 2018. Nano-Fe3O4 particles accelerating electromethanogenesis on an hour-time scales in wetland soil. Environ. Sci. Technol. 52, 436–445.

Yang, Y., Guo, J., Hu, Z., 2013. Impact of nano zero valent iron (NZVI) on methanogenic activity and population dynamics in anaerobic digestion. Water Res. 47 (17), 6790.

Yang, Z., Shi, X., Wang, C., Wang, L., Guo, K., 2015. Magnetite nanoparticles facilitate methane production from ethanol via acting as electron acceptors. Sci. Rep. 5, 16138.

Yang, Z., Guo, R., Shi, X., Wang, C., Wang, L., Dai, M., 2016. Magnetite nanoparticles enable a rapid conversion of volatile fatty acids to methane. RSC Adv. 6 (31), 25662–25668.

Yin, Q., Yang, S., Wang, Z., Xing, L., Wu, G., 2018. Clarifying electron transfer and metagenomic analysis of microbial community in the methane production process with the addition of ferrooxidic ore. Chem. Eng. J. 333, 216–225.

Yu, Y.Y., Guo, C.X., Yong, Y.C., Li, C.M., Song, H., 2015. Nitrogen doped carbon nanoparticles enhanced extracellular electron transfer for high-performance microbial fuel cells. Chemosphere 140, 1413–1415.

You, Z., Zhou, S., Xu, N., Zhuang, L., 2011. Electrochemical characterization of anodic microbial fuel cells. Bioresour. Technol. 102 (30), 1415–1420.

Zhang, J.C., Lu, Y.H., 2016. Conductive Fe3O4 nanoparticles accelerate syntrophic methane production from butyrate oxidation in two different Lake sediments. Front. Microbiol. 7.

Zhang, P., Liu, J., Qiu, Y.P., Feng, Y.J., 2017. Enhanced Shewanellae oneidensis MR-1 anode performance by adding fumarate in microbial fuel cell. Chem. Eng. J. 328, 697–702.

Zhang, J., Xia, X., Li, S., Ran, W., 2018. Response of methane production via propionate oxidation to carbohydrate-multiwalled carbon nanotubes in paddy soil enrichments. Environ. Pollut. 250, 1–10.

Zhao, Q.Z., Zhang, Y.B., Woodward, T.L., Nevin, K.P., Lovley, D.R., 2015. Enhancing syntrophic metabolism in up-flow anaerobic sludge blanket reactors with conductive carbon materials. Biosens. Bioelectron. 70, 140–145.

Zhao, Q., Zhang, Y.B., Liu, Y., Dang, Z., Sun, L., Qian, X., 2017. Potentially shifting from interspecies hydrogen transfer to direct interspecies electron transfer for syntrophic metabolism to resist acidic impact with conductive carbon cloth. Chem. Eng. J. 313, 10–18.

Zheng, S.L., Wang, B.C., Liu, F.H., Wang, O.M., 2017. Magnetite production and transformation in the methanogenic consortia from coastal riverine sediments. J. Microbiol. 55, 862–870.

Zhuang, L., Tang, J., Wang, Y.Q., Hu, M., Zhou, S.G., 2015. Conductive iron oxide minerals accelerate syntrophic cooperation in methanogenic benzoate degradation. J. Hazard. Mater. 293, 37–45.