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Short Communication

A novel bioaugmentation strategy to accelerate methanogenesis via adding *Geobacter sulfurreducens* PCA in anaerobic digestion system

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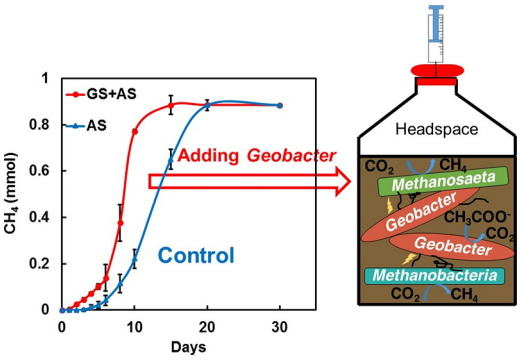
HIGHLIGHTS

- Amendment of *Geobacter* species accelerated CH_4 production remarkably.
- *Methanosaetaceae* and *Methanobacteriaceae* were bioaugmented by adding *Geobacter* species.
- Promoting methanogenesis probably was related with syntrophic associations between *G. sulfurreducens* and methanogens.

GRAPHICAL ABSTRACT



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ABSTRACT

Based on the new syntrophic methanogenesis route via direct interspecies electron transfer (DIET), a novel bioaugmentation method by adding exoelectrogenic *Geobacter* species to accelerate methanogenesis was developed in this study. *Geobacter sulfurreducens* PCA, type exoelectrogenic strain of *Geobacter* species was chosen for the research. To clarify the effect of *G. sulfurreducens* on methanogenesis, batch tests of CH_4 production were carried out. Acetate, the most typical precursor of methanogenesis was chosen as the substrate of batch tests. Amendment of *G. sulfurreducens* accelerated CH_4 production remarkably. The lag phase of CH_4 production was shortened, and the maximum CH_4 production rate was increased by 78%. Fluorescence in situ hybridization showed that *G. sulfurreducens* closely gathered with methanogens. For the archaeal communities, the high-throughput sequencing results demonstrated that *Methanosaetaceae* and *Methanobacteriaceae* were potential bioaugmented methanogens. We speculated that the accelerated methanogenesis by adding *G. sulfurreducens* may result from the syntrophic association between *G. sulfurreducens* and methanogens affiliated with *Methanosaetaceae* and *Methanobacteriaceae*. This research provides a new route to enhance methanogenesis through the utilization of *G. sulfurreducens*. Through this study, the role of *Geobacter* in the anaerobic engineering and carbon cycling of nature should be paid more attention.

1. Introduction

Anaerobic digestion (AD) is a significant process for conversion from organic waste to bioenergy (Batstone and Virdis, 2014; McCarty et al.,

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2011). However, the low efficiency and time-consuming start-up limit its application. AD is a multi-step biological process including hydrolysis, acetogenesis and methanogenesis, of which the last step is usually the rate-limiting step (Batstone and Virdis, 2014). The enhancement of methanogenesis is necessary for promoting AD.

Recently an unconventional syntrophic methanogenesis via direct interspecies electron transfer (DIET) was discovered in which electrons flow from exoelectrogenic bacteria to electrotrophic methanogen via cell components like cytochrome C (*c*-Cyts), pilin, etc. (Dube and Guiot, 2015; Lohner et al., 2014; Lovley, 2017). However, it was accepted that syntrophic methanogenesis metabolism was mainly mediated by the shuttling of hydrogen or formate for a long time. Considered with the diffusion limitation of electron carriers, the DIET performs distinctively faster than methanogenesis via hydrogen or formate (Cruz Viggi et al., 2014). The fast syntrophic methanogenesis may resist acidic impact in complex anaerobic digestion environments (Zhao et al., 2017). Till now, it has been discovered that exoelectrogenic bacteria belonged to *Geobacteraceae* were mainly involved in DIET process (Liu et al., 2012; Rotaru et al., 2014a). *Geobacteraceae* exists in diverse environments such as wetland and aquatic sediments, rice paddy or other soils (Lovley et al., 2011). Recently *Geobacteraceae* has also been detected in methanogenic digesters (Shrestha et al., 2014). That makes the addition of *Geobacter* species to AD system a potential bioaugmentation strategy for enhancing methanogenesis.

But there is no bioaugmentation case by adding *Geobacter* species. Though DIET has been intensively studied, there is no direct evidence to clarify that adding *G. sulfurreducens* accelerates methanogenesis through DIET. So it is significant to validate the enhancement effect of adding *Geobacter* species on methanogenesis.

The aim of the current study was to develop a novel bioaugmentation method by adding *Geobacter* species to accelerate methanogenesis. *G. sulfurreducens* PCA, one type strain of exoelectrogenic *Geobacter* species was chosen in this study (Reguera et al., 2006). To clarify the effect of adding *G. sulfurreducens* on methanogenesis, batch tests of CH_4 production were carried out. Acetate, the most typical precursor of methanogenesis was chosen as the substrate of batch tests. Fluorescence in situ hybridization (FISH) was performed to determine the integration of introduced *G. sulfurreducens* and bioaugmented methanogens. High-throughput sequencing based on 16S rRNA gene was applied to explore the response of methanogens. This research provides a new route to enhance methanogenesis.

2. Materials and methods

2.1. Pure strains cultivation

Wild-type *G. sulfurreducens* PCA (ATCC 51573) was obtained from laboratory stocks frozen at −80 °C. The strains grew under anaerobic conditions at 30 °C in *Geobacter* medium 1957 to reach the late stationary phase. During the cultivation period, OD600 was measured to monitor the growth of strains. Then the cells were collected by centrifugation at 8000*g* for 3 min, after that they were mixed with mineral salts medium and ready for inoculation. The mineral salts medium composition includes 1 g/L NH_4Cl, 0.25 g/L NaCl, 0.1 g/L $MgCl_2 \cdot 6H_2O$, 0.1 g/L $CaCl_2 \cdot 2H_2O$, 0.1 g/L KH_2PO_4, 0.2 g/L $K_2HPO_4 \cdot 3H_2O$, 0.05 g/L Na_2SO_4, 1.2 g/L $NaHCO_3$ and vitamin, trace element solutions were added as described previously (Morita et al., 2011). The concentration of *G. sulfurreducens* PCA was 4–5 × 10^9 cells mL^{-1}.

2.2. Anaerobic sludge

Inoculated anaerobic sludge was collected from a mesophilic anaerobic digester fed with excess activated sludge in a Municipal Wastewater Treatment Plant, Beijing, China. The fresh anaerobic sludge was cultivated without substrate for one week to exhaust the organic carbon. The anaerobic sludge was then centrifuged at 8000 *g* for 3 min and ready for inoculation.

2.3. Batch tests

All batch tests were conducted in anaerobic 100 mL serum bottles. Each bottle included 50 mL culture medium and 50 mL headspace. During the experiment, firstly, 1 g anaerobic sludge and 47 mL medium were inoculated in serum bottles. 60 mg sodium acetate was utilized as the carbon source. Besides, the medium contained 1 g/L NH_4Cl, 0.25 g/L NaCl, 0.1 g/L $MgCl_2 \cdot 6H_2O$, 0.1 g/L $CaCl_2 \cdot 2H_2O$, 0.1 g/L KH_2PO_4, 0.2 g/L $K_2HPO_4 \cdot 3H_2O$, 0.05 g/L Na_2SO_4, 1.2 g/L $NaHCO_3$. The vitamin and trace element solutions were added as described in Section 2.1 (Morita et al., 2011). Secondly, to ensure strict anaerobic conditions, the medium was sparged by N_2/CO_2 (80/20) and then butyl rubber stoppers were used to seal the serum bottles. Thirdly, 3 mL *G. sulfurreducens* PCA strains were injected in experimental bottles or 3 mL mineral salts medium were injected in control. The medium was the same as the 3 mL *G. sulfurreducens* PCA strains medium described in Section 2.1. In addition, the blank control without acetate was conducted to eliminate the effect of residual organic carbon. The experimental and control groups were triplicated and named GS + AS, AS, CK, respectively. "GS + AS" referred to the experimental groups added with *G. sulfurreducens* PCA. AS referred to the control groups without *G. sulfurreducens* PCA. "CK" referred to the blank control groups without *G. sulfurreducens* PCA and acetate. All treatments for the experimental and control groups were identical but the *G. sulfurreducens* PCA strains were added or not. Finally, these serum bottles were placed in the shaking incubator at 35 ± 2 °C, 150 rpm. When CH_4 production approached to plateau, the batch ended. In order to exclude randomness and prove good reproducibility, the repeated experiment was conducted. All of the inoculation conditions kept consisted with the previous batch tests but the sodium acetate dosage was changed to 30 mg to conduct the repeated test as soon as possible.

2.4. Chemical analysis

The gaseous samples were regularly collected from serum bottles headspace using pressure-lock analytical syringe (Baton Rouge, LA, USA). 200 μL headspace sample were injected into Agilent 7890B Gas Chromatograph (TCD detector, porapak Q column and molsieve 5A column; N_2 carrier gas 45 mL/min; He carrier gas 25 mL/min; gas oven temperature 70 °C; injector temperature 250 °C; TCD temperature 200 °C).

Liquid samples were collected during the batch tests and filtered through 0.22 μm filters. The concentration of acetate and possible intermediate product formate were analyzed using Agilent HPLC Model-1100 equipped with Atlantis T3 column and UV flexible wavelength detector. The mobile phase was 0.05% H_3PO_4 and the flow rate was 3 mL/min. The column temperature was 55 °C. The sample size was 20 μL.

2.5. Mathematical model

The CH_4 production experimental data was elaborated by a best-fit procedure, following the modified logistic Eq. (1) known as the Gompertz equation (Mu et al., 2007) to model the biogas production:

$$V_{gas} = V_{max}\left\{-\exp\left[\frac{R_{max} * e}{V_{max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

V_{gas} (mmol) is the cumulative amount of CH_4 product at reaction time t(d). V_{max} presents the potential maximal amount of biogas product. R_{max} (mmo1 d^{-1}) presents the maximum biogas production rate. λ(d) represents the lag time. SPSS 16.0 was utilized to determine the parameters.