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Accelerated methanogenesis from effluents of hydrogen-producing stage in anaerobic digestion by mixed cultures enriched with acetate and nano-sized magnetite particles

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HIGHLIGHTS

- The paddy soil enrichments degrading acetate were obtained via magnetite addition.
- Magnetite significantly accelerated methane production from acetate.
- *Rhodocyclaceae*-related species were selectively enriched with magnetite addition.
- Enrichments can rapidly convert the effluents of hydrogen-producing stage to methane.

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ABSTRACT

Potential for paddy soil enrichments obtained in the presence of nano-sized magnetite particles (named as PSEM) to promote methane production from effluents of hydrogen-producing stage in two-stage anaerobic digestion was investigated. The results showed that the addition of magnetite significantly accelerated methane production from acetate in a dose-independent manner. The results from high-throughput sequencing analysis revealed that *Rhodocyclaceae*-related species were selectively enriched, which were likely the key players for conversion of acetate to methane in PSEM. Compared to the paddy soil enrichments obtained in the absence of magnetite (named as PSEC), the maximum methane production rate in PSEM was significantly higher (1.5–5.5 times higher for the artificial medium and 0.2–1.7 times higher for the effluents). The accelerated methane production from the effluents indicated remarkably application potential of PSEM for improving performance of anaerobic digestion.

1. Introduction

Anaerobic digestion (AD) is a commonly employed bioenergy technology to produce methane from organic solid wastes. However, low efficiency and long retention times (30–200 days), which usually occur in traditional one-stage AD, prevent its practical applicability (Kwietniewska and Tys, 2014; Ruile et al., 2015). In tackling these questions, researchers have focused on development of a two-stage AD to separate acidogenesis (hydrogen-producing stage) and methanogenesis (methane-producing stage) (Merlino et al., 2013; Yang et al., 2011). This body of works demonstrate the potentially significant role of the two-stage AD in enhancing biogas yield and improving stability of the process. Despite of these advantages, application of the two-stage AD has been restricted due to long retention times (20–36 days) and low methane production rate (Dareioti and Kornaros, 2014; Massanet-Nicolau et al., 2013). It is known that considerable volatile fatty acids (VFAs) (e.g. propionate and butyrate) produced in the hydrogen-producing stage were converted slowly to the methanogenic substrate (e.g. acetate and H_2) in the methane-producing stage (Wang et al., 2009). Thus, enhancing the degradation rate of VFAs might provide a reduction of long retention times during the two-stage AD.

Effective interspecies electron transfer (IET) is a vital process in AD, constructing basis of syntrophic relationship between fermentative bacteria and methanogen (Sieber et al., 2012). It has been reported that conductive materials (e.g. iron oxides) were capable of enhancing methane production rate via facilitating IET between bacteria and methanogens (Kato et al., 2012; Zhou et al., 2014).

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This led to a central hypothesis that promoting IET might accelerate methane production from VFAs in the methane-producing stage of the two-stage AD.

Nano-sized magnetite particles added to paddy soil resulted in a significant increase in methane production rate (over 30%) from acetate, concomitant with specific enrichment of *Geobacter* and *Methanosarcina* (Kato et al., 2012; Zhou et al., 2014). These findings all demonstrate potentially value of the paddy soil enrichments obtained in the presence of nano-sized magnetite particles (named as PSEM) on accelerating methane production in AD. However, to best of knowledge, no attempts have been made to identify the potential of PSEM for application on the methane-producing stage of the two-stage AD. In this work, the methanogenic characteristics of PSEM were assessed in detail, which led to draw a conclusion that PSEM can serve as improving methane-producing rate for the methane-producing stage of the two-stage AD.

2. Methods

2.1. Paddy soil samples and preparation of nano-sized magnetite particles

Paddy soil samples collected from a rice field (Guangzhou, China) were used as inocula. Total solids (TS) content of the soil samples was 61% (w/w), which contained 8.12% of volatile solids (VS). Nano-sized magnetite particles (8–10 nm) were prepared by slowly adding an acidic solution containing Fe(II) and Fe(III) into NaOH solution under anaerobic conditions (Kang et al., 1996). X-ray diffraction (XRD) analysis showed the resulted products as magnetite.

2.2. Methane production

Batch experiments were carried out at 30 °C in 120 mL anaerobic bottles with 50 mL of working volume, except for experiments 2 and 3, where 20 mL of working volume in 60 mL bottles was used. Each experimental group was performed in triplicate without shaking. Both the inocula and medium were loaded into the bottles, which were then purged with 99.99% of N_2 and closed with butyl rubber stoppers. The initial pH values for all experiments were adjusted to 7.0. The medium (per liter) consisted of 1 g $NaH_2PO_4{\cdot}H_2O$, 0.55 g $Na_2HPO_4{\cdot}H_2O$, 3 g $NaHCO_3$, 275 mg $CaCl_2$, 310 mg NH_4Cl, 330 mg $MgCl_2$, 130 mg KCl, 5 mg $MnCl_2$, 10 mg $FeCl_3{\cdot}7H_2O$, 0.1 mg $CuCl_2{\cdot}5H_2O$, 1 mg $CoCl_2{\cdot}5H_2O$, 28 mg $NiCl_2$, 1 mg $ZnCl_2$, 0.1 mg H_3BO_3, 0.25 mg Na_2MoO_4, 0.24 mg $NiCl_2{\cdot}6H_2O$, and 1 mg EDTA.

Semi-continuous enrichment cultivations (experiment 1) were performed to obtain PSEM. 1.5 g of soil samples were mixed with 50 mL of sterile medium containing 20 mM acetate and 20 mM magnetite. 5 mL of enrichments were transferred into 45 mL of fresh medium amended with 20 mM acetate and 20 mM magnetite when each generation had the highest methane yield. Five generations of subculture were conducted.

In experiments 2 and 3, the resulted enrichments from the fifth generation were used as inocula at the concentration of 0.11 g-VS/L. Experiment 2 was used to detect effects of initial magnetite concentrations (ranging from 20 to 320 mM) on methane production from 20 mM of acetate. The effects of initial acetate concentrations (ranging from 20 to 200 mM) on methanogenesis were evaluated in experiment 3.

Repeated batch cultivation (experiment 4) was conducted to examine the performance of PSEM on degradation of effluent from the hydrogen-producing stage of the two-stage AD. Artificial medium and effluents from hydrogen-producing stage of *Macrocystis pyrifera* biomass residues were used. Total amounts of ethanol and VFAs (270 mM) in the artificial medium consisted of ethanol (40.7%), acetate (22.6%), propionate (19.3%) and butyrate (16.8%). Total VFAs and ethanol (201 mM) in the effluents obtained through centrifugation at 4000 rpm were composed of acetate (48.1%), propionate (10.2%), butyrate (28.8%), valerate (12.2%), caproate (0.7%) and trace amounts of ethanol. In the start-up incubation stage, 0.11 g-VS/L of the resulted enrichments from the fifth generation were grown in the medium containing 20 mM acetate and 20 mM magnetite. When methane production ceased, 20% (v/v) of the medium was withdrawn and replaced with the same amounts of the effluents or the artificial medium. Biogas remained in headspace of bottles was withdrawn using a vacuum pump and replaced with high purity N_2 before start of the next cycle. For the repeated batch experiments, the mixed cultures from the first cycle that had the highest methane production were used as the inocula for the second cycle of cultivation. Two cycles of harvesting and nutrient feeding were performed.

2.3. Analytical methods

The volume of produced biogas was determined using water displacement method. The fraction of CH_4 in the produced biogas was periodically measured using gas chromatograph (SP6890, Shandong, China) equipped with a thermal conductivity detector according to methods of Yang et al. (2011). Concentrations of ethanol and individual VFAs were determined with high performance liquid chromatography (Agilent HPLC 1200 series) equipped with UV detection (210 nm), refractive index detection and Aminex HPX-87P cation exchange column (Bio-Rad Laboratories, Hercules, CA). 5 mM of H_2SO_4 was used as the mobile phase. The flow rate was 0.6 mL/min with the column temperature of 65 °C. According to the standard methods (APHA, 1998), TS of the samples were measured after drying at 105 °C for 12 h in an oven, then VS of the samples was measured after igniting at 550 °C for 2 h in a muffle furnace. The maximum methane production rate (Rm) and the lag-phase time (λ) in each batch experiment were depicted by the modified Gompertz equation (Yang et al., 2011). The methane yield (Ps) was calculated by dividing cumulative methane production with total acetate used.

2.4. DNA extraction, PCR amplification and 16S rDNA sequencing

Samples from the fifth generation of enrichments were collected for DNA extraction. Total genomic DNA was extracted according to a CTAB/SDS method. PCR was conducted on Thermal Cycler (Bio-rad T100) with bacterial primer pair (515f/806r) for V4 region of 16S rRNA (Peiffer et al., 2013). 30 μL of PCR reaction volume consisted of 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers, and about 10 ng of template DNA. Thermal cycles consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s, followed by a final step at 72 °C for 5 min. PCR products were purified using GeneJET Gel Extraction Kit (Thermo Scientific). Then the purified PCR products were used for sequencing libraries preparation using NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA). The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina MiSeq platform.

Raw tags were produced from the merged paried-end reads using FLASH (Magoč and Salzberg, 2011). To obtain clean tags, the low quality sequences and chimeras were filtered, trimmed and removed using the Quantitative Insights into Microbial Ecology (QIIME software v1.3.0) pipeline (Caporaso et al., 2010) and UCHIME Algorithm (Edgar et al., 2011). FASTAQ formatted of clean tags have been submitted to the GeneBank Sequence Read