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Enhancement of methanogenesis via direct interspecies electron transfer between *Geobacteraceae* and *Methanosaetaceae* conducted by granular activated carbon

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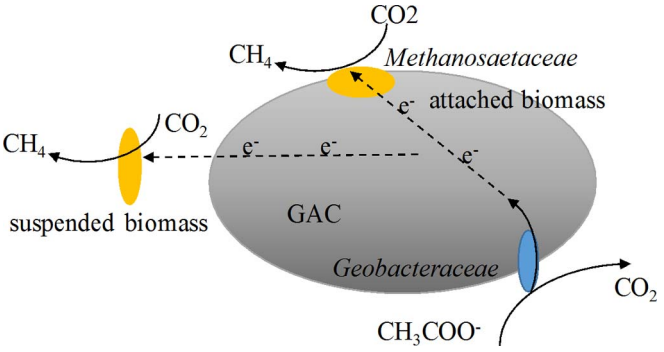
GRAPHICAL ABSTRACT



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ABSTRACT

To understand how granular activated carbon (GAC) promotes methanogenesis, batch tests of CH_4 production potential in anaerobic serum bottles with addition of GAC or not were conducted. Tests showed that GAC promoted methanogenesis remarkably, but the non-conductive zeolite did not. The qPCR demonstrated that the biomass on GAC contributed little to the promotion. High-throughput sequencing data implied that promotion was related with direct interspecies electron transfer between *Geobacteraceae* and *Methanosaetaceae*. According to the c-type cytochromes (*c*-Cyts) response to the supplement of GAC, it was speculated that GAC may play the role of *c*-Cyts' substitution. In the undefined cultures, the phenomenon that *c*-Cyts were repressed by GAC was first observed. This research provided new evidence to microbial mechanism of promoting methanogenesis via GAC.

1. Introduction

Anaerobic digestion is a significant process for biological conversion of organic waste to bioenergy in the form of CH_4 (Batstone & Virdis, 2014; Liu et al., 2016; McCarty et al., 2011). However, the low efficiency and time-consuming start-up limit its application. Generally, anaerobic digestion is a multi-step biological process including hydrolysis, acetogenesis and methanogenesis, of which the last step is crucial

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for the whole process (Batstone & Virdis, 2014; Gou et al., 2016). The enhancement of methanogenesis is necessary for the promotion of anaerobic digestion.

Recently researchers found that the granular activated carbon (GAC) as conductor can stimulate methanogenesis in anaerobic digesters by facilitating direct interspecies electron transfer (DIET) between fermenting bacteria and methanogens. Direct interspecies electron transfer to methanogenesis (called DIET-M in this article) is an unconventional syntrophic metabolism in which electrons flow from exoelectrogenic bacteria to another electrotrophic methanogen via cell components like cytochrome *C* (*c*-Cyts), pilin, etc. (Dube & Guiot, 2015; Lohner et al., 2014; Lovley, 2017). Previously, it was generally accepted that syntrophic methanogenesis metabolism was mainly mediated by the shuttling of hydrogen or formate (Bryant et al., 1967; Liu & Whitman, 2008). While considering with the diffusion limitation of substrate, the DIET-M performs distinctively faster than methanogenesis via hydrogen or formate (Cruz Viggi et al., 2014). Till now, it has been discovered that bacteria belonged to *Geobacteraceae* and archaea affiliated with *Methanosaetaceae* and *Methanosarcinaceae* are mainly involved in DIET-M process, of which GAC may provide conduits of electrons for the enhancement (Liu et al., 2012; Rotaru et al., 2014a) but the reactive groups in GAC does not work (Liu et al., 2012). In both defined pure co-cultures and environmental samples like paddy soil and anaerobic sludge, enhancement of methanogenesis by GAC via DIET-M has already been detected (Lee et al., 2016; Rotaru et al., 2014a). Besides, other conductive materials like carbon cloth (Chen et al., 2014a), biochar (Chen et al., 2014b; Zhao et al., 2015), nanographene (Tian et al., 2017) and magnetite (Cruz Viggi et al., 2014; Jing et al., 2017; Li et al., 2015) were also reported to promote CH_4 production by DIET-M.

As a common filling carrier with porous structure, GAC has long been routinely utilized in anaerobic digesters to increase biomass and enhance efficiency of the reactor (Kuroda et al., 1988; Kindzierski et al., 1992). Until recently, the conductivity character of GAC has been highlighted in terms of enhancement of methanogenesis (Lee et al., 2016; Liu et al., 2012; Rotaru et al., 2014a; Zhao et al., 2017). However, the recent study by Xu et al. didn't detect the existence of exoelectrogenic bacteria in the UASB reactor, even though the addition of GAC indeed enhance methanogenesis (Xu et al., 2015). Thus it is still confused whether GAC promote methanogenesis by increasing biomass or facilitating DIET-M.

To clarify whether GAC promote methanogenesis by increasing biomass or facilitating DIET-M, batch tests of CH_4 production were carried out in this study, with the addition of conductive filler GAC or nonconductive filler zeolite. Quantitative PCR (qPCR) was performed to determine the biomass variation pattern in terms of microbial cell number. High-throughput sequencing based on 16S rRNA gene was applied to explore the potential syntrophic microorganisms participating in DIET-M. To further explore the function of GAC, the *c*-Cyts' concentration was also quantified in this study.

2. Materials and Methods

2.1. Batch tests of CH_4 production potential

2.1.1. Source culture

Anaerobic sludge was collected from a mesophili anaerobic digester fed with waste activated sludge from the Xiaohongmen Wastewater Treatment Plant, Beijing, China. To resuscitate methanogenesis-related microbial activity, the collected AS was pre-acclimated using glucose as substrate before experiment. After two weeks' acclimation, the sludge produced CH_4 steadily. At the end of pre-acclimation, the mixed liquor volatile suspended solids (MLVSS) of the sludge was 10.27 ± 0.18 g/L.

2.1.2. Pretreatment of GAC and zeolite

GAC made with coconut were purchased from Heatton Environmental Protection Technology Co., Ltd., Shanghai. Natural zeolite was purchased from Hengtai Co., Ltd, Zhengzhou, Henan, China. Firstly, they were smashed and sieved to 1–2 mm. Secondly, they were washed with the diluted hydrochloric acid (10%) and by the deionized water. Then, they were dried in the oven at 105 °C. Next, the dried GAC and zeolite were divided respectively in the serum bottles (1 g per bottle). Then the adsorption saturation was conducted in the medium (described as below) to eliminate the adsorption effect. The medium should be renewed time and time again until the acetate concentration in the medium did not change any more. At last, they were ready to be used after the adsorbed medium was poured away.

Table 1
Samples profiles in the batch tests.

Number	Abbreviation	Anaerobic sludge	Acetate	GAC	Zeolite
1	GAC + AS	+	+	+	−
2	Zeo + AS	+	+	−	+
3	AS	+	+	−	−
4	CK1	+	−	+	−
5	CK2	+	−	−	+
6	CK3	−	−	+	−
7	CK4	−	−	−	+

"+" represents supplement and "−" represents no supplement. CK1 and CK2 were biotic blank control without The blank CK1 and CK2 were biotic without substrate. The blank CK3 and CK4 were abiotic with substrate. The blank controls were conducted only in batch1. The other experimental groups were conducted in both two batches.

2.1.3. Batch tests

All batch tests were conducted in anaerobic 120 mL serum bottles. Each bottle included 30 mL culture medium and 90 mL headspace. The experimental and control groups were designed as the Table 1. The control treatments of CK1 and CK2 were conducted to eliminate the impact of adsorbed substrate on CH_4 production. The control treatments of CK3 and CK4 were conducted in abiotic. In the batch tests, 3 mL acclimated anaerobic sludge was inoculated in the 30 mL medium. The 0.1 g sodium acetate was utilized as the carbon source because it is one of typical intermediate products in the period of anaerobic digestion and significant methanogenic substrates. Besides, in the 30 mL medium, there are 0.03 g NH_4Cl, 0.0075 g NaCl, 0.003 g $MgCl_2{\cdot}6H_2O$, 0.003 g $CaCl_2{\cdot}2H_2O$, 0.0033 g KH_2PO_4, 0.0066 g $K_2HPO_4{\cdot}3H_2O$, 0.0015 g Na_2SO_4, and 0.036 g $NaHCO_3$. The vitamin and trace element solutions were added as described previously (Morita et al., 2011). These serum bottles were placed in the shaking incubator at 34 ± 2 °C, 170 rpm. To ensure the strict anaerobic conditions, the medium was sparged by N_2/CO_2 (80/20) and then butyl rubber stoppers were applied to seal the serum bottles. When CH_4 production approached to plateau, the first batch finished. Before the second batch began, to release the headspace pressure, gas in the bottle was discharged to atmosphere pressure with syringe. Then the substrate was added without sludge being discharged.

2.2. Chemical analyses

2.2.1. MLVSS

Analysis of MLVSS was conducted according to Standard Methods for the Examination of Water and Wastewater.

2.2.2. CH_4 in headspace

The gaseous samples were regularly collected from serum bottles headspace using pressure-lock analytical syringe (Baton Rouge, LA, USA). 200 μL headspace samples were injected into Agilent 7890 B Gas Chromatograph (TCD detector, porapak Q column and molsieve 5 A 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).