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Clarifying electron transfer and metagenomic analysis of microbial community in the methane production process with the addition of ferroferric oxide

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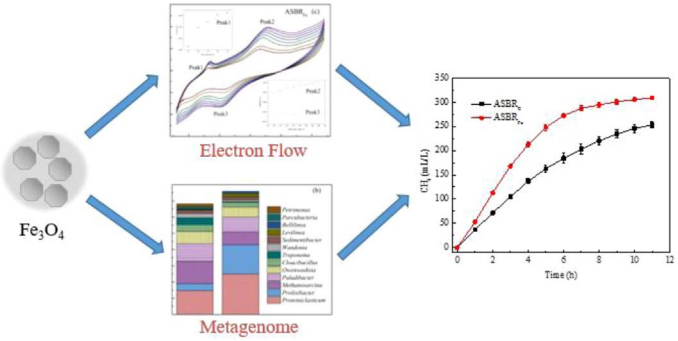
GRAPHICAL ABSTRACT



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ABSTRACT

Direct interspecies electron transfer (DIET) is a new mechanism responsible for syntrophic methane production. Supplementation of conductive material in syntrophic communities can facilitate electron transfer and enhance methane production. Ferroferric oxide (Fe_3O_4) was dosed in an anaerobic sequencing batch reactor (ASBR) fed with tryptone-based synthetic wastewater to examine its effect on the anaerobic treatment process. Long-term dosage of Fe_3O_4 not only enhanced the maximum methane production rate by 78.3% in a reaction cycle but also improved methane production when hydrogen/carbon dioxide or acetate was used as the substrate. The conductivity of anaerobic sludge, activity of the electron transport chain, and extracellular electron transfer ability were enhanced with the addition of Fe_3O_4, inducing enhanced system performance for methane production. *Proteiniclasticum* and *Prolixibacter* were the enriched acidogens responsible for hydrolysis and acidification. With the addition of Fe_3O_4, *Methanosarcina* was the dominant methanogen, and metagenomic analysis further revealed the genes involved in the hydrogenotrophic pathway of methanogenesis. *Methanosarcina* might be involved in DIET, and Fe_3O_4 was responsible for its stimulation.

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1. Introduction

Anaerobic treatment is a reliable and sustainable technology for wastewater treatment [1,2]. Methanogens play a key role in the anaerobic treatment process due to their ability to convert organic waste to methane (CH_4) [3]. However, the relatively slow syntrophic metabolism between acidogens and methanogens limits the efficiency of methanogenesis [4]. In recent years, direct interspecies electron transfer (DIET) has been proposed as a new mechanism for syntrophic CH_4 production [5,6]. In methanogenic communities that are capable of DIET, electrons can be exchanged directly by electrically conductive pili and outer membrane c-type cytochromes [5,7] rather than H_2 or formate. Thus far, *Methanosarcina*, *Methanosaeta* and *Geobacter* have been shown to be able to exchange electrons via DIET [7,8]. Furthermore, conductive materials, such as granular activated carbon (GAC), carbon fiber and ferroferric oxide (Fe_3O_4), have been shown to facilitate CH_4 production by enhancing DIET [9–12].

Conductive materials shorten the lag phase of CH_4 production and enhance the maximum CH_4 production rate [13,14]. In the treatment of wastewater with complex organic substrates, the production and consumption of intermediate products including volatile fatty acids (VFAs) can be accelerated with the addition of conductive materials [12]. Chemical oxygen demand (COD) removal rates are also increased in upflow anaerobic sludge blanket reactors with the addition of conductive carbon materials [11]. Moreover, higher capacity to resist the acidic impacts can be achieved in anaerobic digester supplemented with conductive carbon cloth [15]. However, the primary mechanism for these improvements is still unclear. Liu et al. [9] found that in the presence of granular activate carbon, cells attached to the surface of conductive materials without close aggregation, suggesting that conductive materials might act as pili or c-type cytochromes to exchange electrons between microorganisms. Zhao et al. [16] found that the c-type cytochrome was increased in microbial electrolysis cells with the supplementation of carbon belt. Tian et al. [17] found that nano-graphene significantly induced the coenzyme F420 contents, and stimulation of coenzyme F420 was relevant to the graphene dosage.

Apart from these results, increasing attention has been focused on the characteristics of the electron transfer process. Although electrons are difficult to be detected directly, certain parameters relevant to the electron transfer process can be examined to reflect the electron transport efficiency. For example, Zhao et al. [18] reported that the conductivity was obviously increased for sludge enriched with ethanol compared with other substrates. Furthermore, the 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride-electron transport system (INT-ETS) method was used to measure the activity of the electron transport system (ETS) in microorganisms [12,19]. Cyclic voltammetry (CV) experiments were conducted to characterize the redox reactions induced by biomass enriched with GAC [20], the performance of microbial fuel cells [21], and microbial electrolysis cells [22].

In addition, several studies also suggested that conductive materials could also affect the microbial community structure. For instance, specific microorganisms were enriched with the addition of conductive materials [14]. Under low-temperature conditions, the archaeal community structure remained constant in the presence of graphene [17]. Although species confirmed with DIET capability are limited, microorganisms enriched in a conductive material-supplemented environment are considered as potential candidates for DIET. Therefore, transcriptome and metaproteomics were further applied to supply direct proof of changes in the metabolic pathways [8,23]. It was suggested that selected genes relating to DIET might be induced by conductive materials and also up-regulated their expression.

In this study, knowledge gap was addressed by investigating the effect of conductive materials on syntrophic methanogenesis. A series of experiments were conducted to characterize not only the methanogenic activity, but also ETS. In addition to identifying the microbial community with 16S rRNA gene analyses, the methanogenic pathway was characterized by the metagenomic analysis to clarify the gene level effect of conductive material. This study clarified the facilitative mechanism for enhanced methane production systematically by analyzing ETS and metagenome with the addition of conductive material.

2. Materials and methods

2.1. Experimental setup and operation

Anaerobic sludge acclimation was conducted in two 2-L anaerobic sequencing batch reactors (ASBRs) at 35 ± 1 °C. The inoculated anaerobic sludge was collected from the secondary sedimentation tank of a wastewater treatment plant in Shenzhen, China. $ASBR_C$ represented the control reactor containing only inoculated anaerobic sludge, and $ASBR_{Fe}$ was dosed with Fe_3O_4 at the optimized concentration of 10 g/L. The suspended sludge (SS) in the seed sludge was 6.29 ± 0.18 g/L, and the volatile suspended sludge (VSS) was 3.96 ± 0.12 g/L. The hydraulic retention time was 24 h, and the sludge retention time was approximately 33 d. Each reaction cycle included 11 h of anaerobic mixing (including 6 min of filling), 54 min of settlement and 6 min of withdrawal. To maintain the Fe_3O_4 concentration of 10 g/L, an equal amount of Fe_3O_4 was supplemented in the $ASBR_{Fe}$ to compensate for that removed during the sludge withdrawal period [12].

The reactors were fed with synthetic wastewater containing the following components: 1818 mg/L tryptone corresponding to COD concentrations of 2000 mg/L, 480 mg/L NH_4Cl, 100 mg/L $CaCl_2$, 200 mg/L $MgCl_2$, 120 mg/L Na_2HPO_4, 200 mg/L $KHCO_3$, and 1 mL/L trace elements (additional component details of the trace elements are described in the Supplementary Material).

To examine the methanogenic activity of enriched cultures from the two reactors, a typical ASBR reaction cycle was investigated under steady-state condition. During the reaction cycle, liquid and gas samples were periodically collected to analyze the concentrations of COD, VFAs, soluble iron ion, CH_4 and the ETS activity, respectively.

2.2. Batch experiments

To further evaluate the methanogenic activity fed with acetate and H_2/CO_2, batch experiments were conducted. Two 125-mL aliquots of sludge were taken from $ASBR_C$ and $ASBR_{Fe}$ after effluent discharge and placed in 250 mL bottles. For the acetate experiment, a 125-mL aliquot of synthetic wastewater was added to these bottles. Sodium acetate was used as the solo carbon source instead of tryptone. Before experiment, nitrogen gas (N_2) was used to remove oxygen from the headspace of the reactors for 3 min, and bottles were sealed with rubber stoppers and mixed in an air bath shaker at 170 r/min and 35 °C. Liquid and gas samples were periodically collected to analyze concentrations of acetate and CH_4 and the ETS activity, respectively. For the H_2/CO_2 experiment, H_2/CO_2 (4:1) was added to the headspace of the bottles in the absence of tryptone. The other conditions were the same as the above experiment. Gas samples were periodically collected to analyze the CH_4 production.

2.3. Analytical methods

The COD, SS, VSS, ferrous ion and ferric ion contents were measured according to standard methods [24], and CH_4 and VFAs were measured according to Yin et al. [12].

The modified Gompertz model [25] was applied to quantitatively analyze the production of CH_4 under various conditions:

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

where P is the cumulative CH_4 production (mL) at time t, P_{max} is the maximum CH_4 potential (mL) at the end of incubation, t is the time (h),