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Full Length Article

Response of methane production and microbial community to the enrichment of soluble microbial products in goethite-dosed anaerobic reactors

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HIGHLIGHTS

- Addition of goethite promoted the secretion of SMP in anaerobic digestion reactors.
- SMP enhanced the overall methane production and generation rate.
- Goethite and enrichment of SMP influenced the bacterial community significantly.

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ABSTRACT

Iron oxide facilitated methane production process has recently attracted more and more attention in the worldwide. On the other hand, soluble microbial products (SMPs) was found to play an important role in the wastewater treatment systems. The purpose of this study was to investigate the role of SMPs in the goethite (a typical iron oxide mineral) facilitated methanogenic process, where a dialysis bag was used to enrich the SMPs in the aqueous system. Results showed that both methane yield and production rate were enhanced significantly using the dialysis bag with a molecular weight of 2000 Da compared to the controlled goethite-dosed reactors. Chemical analysis indicated that the increase in contacting time between goethite and microorganisms had insignificant impact on SMPs formation and methane production. It was also found that SMPs especially Flavins secreted from microorganisms could play an important role in the goethite facilitated methane production process. Additionally, enrichment of SMPs not only improved the abundance of electroactive bacteria and but also promoted the growth of aceticlastic methanogenesis.

1. Introduction

Anaerobic digestion has gained increasing attention over the last decades due to the fossil fuel depletion and urgent need for renewable energy [1,2]. To improve the efficient production of methane, a lot of efforts have been made to enhance anaerobic digestion performance. One of the effective ways was the addition of natural and synthetic iron oxides such as akaganeite [3], magnetite [4,5], and goethite [6,7]. As for the iron oxide facilitated methane production process, several possible mechanisms have been suggested. On one hand, Fe (II) can decrease the re-dox potential of cultures which benefited the growth of methanogenic microorganism and enzymatic activity [8,9]. On the other hand, iron oxide might function as an electron conduit to mediate electric syntrophy or promote the syntrophic effect between fatty oxidizing bacteria and methanogenic archaea [10].

Soluble microbial products (SMPs) are compounds excreted by microorganisms and have been shown to act as media and bridges between solid minerals and microorganisms, fulfilling an important role in the interaction between solid mineral phase and microbe [11]. SMPs served as the connection site and made mineral dissolution and precipitation possible during the attachment of microbes to a solid mineral surface [12]. On the other hand, it has been reported that some components of SMPs, such as riboflavin-5′-phosphate (FMN) and riboflavin (RBF), could act as extracellular electron shuttles to participate in the redox process [13–15].

Goethite as a cost-effective and natural mineral has been verified to promote methane production from series of organic biomass [7,10]. Besides for the impact on the microbial communities, it was hypothesized that SMPs generated from the

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microorganism might play an important role on the methane generation. However, few studies were reported to explore the potential role of SMPs on the goethite facilitated anaerobic methanogenic process. Therefore, in the current study dialysis bags were used to improve the SMPs concentrations in the aqueous system and test the influence of SMPs on the methane production and microbial communities. Moreover, SMPs in the goethite dosed system was further characterized.

2. Materials and methods

2.1. Inoculum and goethite

Digester sludge was taken from an anaerobic reactor at China Resources Snow Breweries (Hefei, China). The sludge was passed through a 40-mm sieve to remove solid particles and then used as the inoculum. Hydrothermally synthetic goethite was purchased from the Zhenxin Refinery Chemical Plant (Zhenxin, China).

2.2. Batch tests

Each experiment was conducted in 250-mL serum bottles with a 150-mL working volume. The initial sludge concentration was 0.164 g-VS/L (VS: volatile solids) in each reactor. Feeding medium included 1.64 g/L (20 mM) acetate, 3 g/L $NaHCO_3$ as pH buffer, 1 mL/L microelements and vitamin solutions [16], and 1.64 g/L goethite was also added into the reactor. In the group of reactors using dialysis bag, goethite and sludge were inoculated within the dialysis bag (Spectrum Medical Industries, Los Angeles, CA) to concentrate SMPs in dialysis bags and form a SMPs-dense system. Two kinds of dialysis bags with molecular weights of 10 kDa and 2000 Da were used. The reactors were noted as "Sludge", "Goethite", "10 kDa" and "2000 Da", respectively. The bottom-sealed dialysis bags were suspended in the serum bottles containing 130 mL substrate solution. The culture medium pH was adjusted to 7.0 ± 0.1 using 0.5 M HCl. The bottles were flushed for 5 min with argon gas to remove air, and immediately capped with butyl rubber stoppers and aluminium seal and then cultured in an incubator at 35 ± 1 °C. Each experiment was repeated at least twice.

2.3. Analytical methods

Methane was determined using a gas chromatography instrument (GC-2010, Shimadzu Co., Ltd., Tokyo, Japan) equipped with a RTX-1 column (30 m × 0.25 mm × 0.25 μm). Liquid samples in the "2000 Da" and "10 kDa" reactors were taken from the inside of the dialysis bags. Each liquid sample was centrifuged at 12,000 rpm for 5 min at 4 °C and then the supernatant was filtered through 0.45-μm membrane. The filtrates were used for analyzing flavins and main components of SMPs. The polysaccharide and nucleic acid contents of SMPs were determined as reported by Sheng et al. [17], while the protein content was measured using the Bradford method [18]. Total organic carbon (TOC) in the SMPs were measured using TOC analysis instrument (Jena C/N 2100, Germany).

The concentrations of both FMN and RBF in the filtrates were measured according to a method reported previously [19], where 50-μL filtrate was injected into high performance liquid chromatograph (U-3000, Dionex, CA, USA) equipped with a WonderSil C18 column (Shimadzu Co., Japan). Flavins were detected with a RF-2000 fluorescence detector (Dionex, CA, USA). To detect riboflavin, the mobile phase was pumped at 0.8 mL/min and consisted of 60% methanol and 40% ammonium acetic acid (0.05 M, pH 7.0) in deionized water; the detector was set at an excitation wavelength of 420 nm and an emission wavelength of 525 nm. For FMN detection, the mobile phase consisted of 80% methanol and 20% ammonium acetic acid at a flow rate of 0.8 mL/min, and the detector was set at an excitation wavelength of 450 nm and an emission wavelength of 520 nm. Standards of FMN and riboflavin were purchased from Sigma Inc., USA. The concentration of flavins refers to the sum of FMN and RBF.

The microbial populations in reactors were analyzed using PCR-DGGE as previously reported [10]. Modified Gompertz equation was adopted to calculate the maximum methane production rate. Appropriate, standard deviations are indicated in this study. Significant differences were determined by a t test, and levels of significance are represented by $p < 0.05$. A data analysis was performed using the SPSS software version 16.0 (SPSS Inc., Chicago, USA).

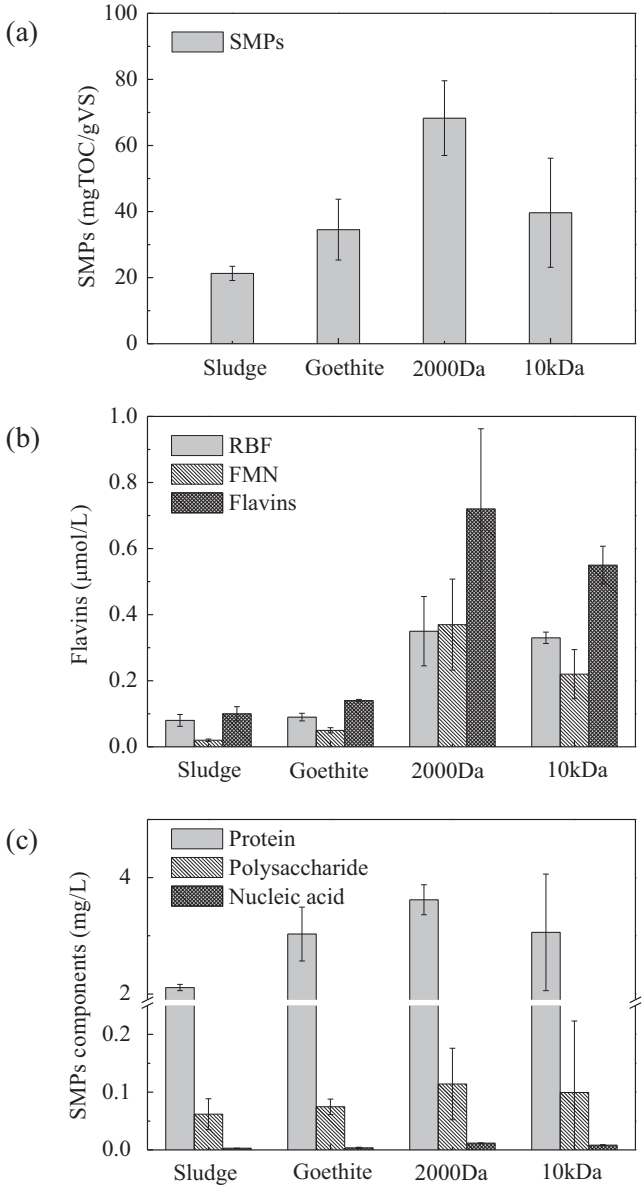

Fig. 1. Production of SMPs (a), flavins (b) and (c) main components of SMPs in the reactors.