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Potentially direct interspecies electron transfer of methanogenesis for syntrophic metabolism under sulfate reducing conditions with stainless steel

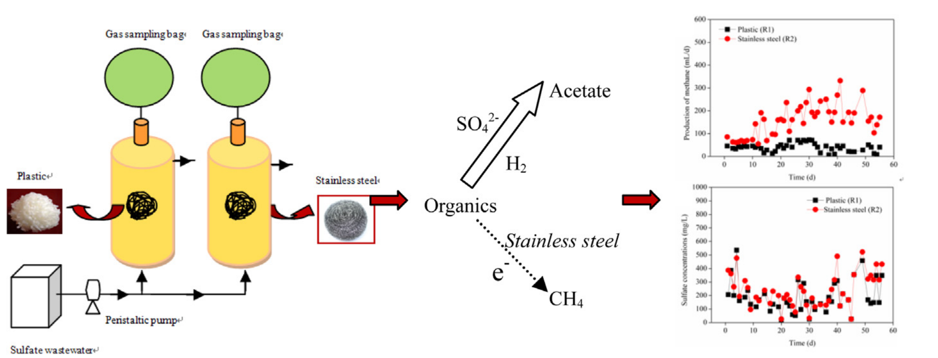
Yue Li, Yaobin Zhang *, Yafei Yang, Xie Quan, Zhiqiang Zhao

Key Laboratory of Industrial Ecology and Environmental Engineering (Dalian University of Technology), Ministry of Education, School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, China

HIGHLIGHTS

- Organic removal and methanogenesis were improved in sulfate-containing wastewater.
- Adding stainless steel enhanced methanogenesis to resist the sulfate impact.
- Potential direct interspecies electron transfer was enriched on the stainless steel.
- Methanogenesis via DIET is faster transfer to electron than sulfate reduction via IHT.

GRAPHICAL ABSTRACT



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ABSTRACT

Direct interspecies electron transfer (DIET) is an alternative to syntrophic metabolism in natural carbon cycle as well as in anaerobic digesters, but its function in anaerobic treatment of sulfate-containing wastewater have not yet to be described. Here, conductive stainless steel was added into anaerobic digesters for treating sulfate-containing wastewater to investigate the potential role of DIET in the response to the sulfate impact. Results showed that adding the conductive stainless steel made the anaerobic digestion less affected by the sulfate reduction than adding insulative plastic material. With adding stainless steel, methane production of the digesters increased by 7.5%–24.6%. Microbial analysis showed that the dissimilatory Fe (III) reducers like *Geobacter* species were enriched on the surface of the stainless steel. These results implied that the potential DIET of methanogenesis was established associating with stainless steel to outcompete the sulfate reduction.

1. Introduction

Anaerobic digestion (AD) is an efficient process for recovering energy from organic wastes (Desloover et al., 2015). However, anaerobic digestion is subject to strict environmental factors like neutral pH, low ORP and appropriate temperature because of the sensitivity of methanogen. Sulfate, a typical component in many wastewaters, can stimulate the growth of sulfate reducing bacteria (SRB) that competes with methanogen for electron (Kristjansson et al., 1982; Yoda et al., 1987). Also, sulfide as a product of the sulfate reduction may significantly inhibit the metabolism of methanogens to decrease the performance of anaerobic digestion and

* Corresponding author.
E-mail address: zhangyb@dlut.edu.cn (Y. Zhang).

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even lead to a failure of the anaerobic process (Liamleam and Annachhatre, 2007).

Traditionally, interspecies hydrogen transfer (IHT) between syntrophs and hydrogen-utilizing methanogens was considered as a key mode of electron transfer in anaerobic methanogenesis (Stams and plugge, 2009). IHT depends on the elaborate collaboration between syntrophs and hydrogen utilizing methanogens. When hydrogen-utilizing methanogens are inhibited by environment factors (Muyzer and Stams, 2008), IHT may be blocked to further hamper the anaerobic respiration.

Recently, direct interspecies electron transfer (DIET) had been found as an alternative to IHT in syntrophic metabolism of anaerobic digestion (Summers et al., 2010; Rotaru et al., 2014a,b; Wang et al., 2016). DIET was believed as the primary mechanism for interspecies electron exchange in anaerobic digesters treating brewery wastewater (Morita et al., 2011; Shrestha et al., 2014). Further studies revealed that DIET could be enhanced to accelerate the anaerobic digestion by the addition of electrically conductive materials, such as carbon materials (Liu et al., 2012) and magnetite (Cruz Viggi et al., 2014). Carbon materials serving as conduit was capable of replacing pili of *Geobacter* species (Liu et al., 2012) that was of metal-like conductivity. Differently, magnetite (Liu et al., 2015) could substitute for the cytochromes associated with pili, but still required pili for DIET. More recently, it was reported that DIET (44.9×10^3 e^-/cp/s) had a higher external electron transfer rates per cell pair (cp) than hydrogen-interspecies electron transfer (IET 5.24×10^3 e^-/cp/s) (Storck et al., 2016).

DIET also increased the flexibility of anaerobic system to resist to the impacts of the operation. DIET replaced IHT to become the dominant working mode for syntrophic metabolism during the acid inhibition (Adhikari et al., 2016; Zhao et al., 2017). However, till now there was no available information about the effects of potential DIET on sulfate-containing wastewater treatment. Whether DIET could alleviate the inhibition of sulfate on anaerobic methanogenesis remained unknown. In this study, stainless steel serving as a conductive material was added into anaerobic digesters to investigate its effects on the treatment of sulfate-containing organic wastewater. The objective of this study was to explore a simple and useful method to improve anaerobic treatment of the sulfate-containing wastewater.

2. Materials and methods

2.1. Sludge and wastewater

Anaerobic sludge used in this study was collected from a laboratory-scale anaerobic reactor in our laboratory treating sodium lactate wastewater, which was cultivated for six months. The ratio of volatile suspended sludge to total suspended sludge (VSS/TSS) was 0.72 with initial TSS of 15,200 mg/L.

An artificial wastewater was used in this study, in which sodium lactate (COD = 1600 mg/L), Na_2SO_4 (SO_4^{2-} = 1300 mg/L), NH_4CL and KH_2PO_4 (at ratio of COD:N:P = 200:5:1) were used as the organic substrate, sulfate, nitrogen and phosphorus sources respectively. Trace elements were added according to Zhao et al. (2015).

2.2. Experimental setup

Firstly, the experiment was operated in two upflow anaerobic sludge blanket (UASB) reactors (Φ60 mm × 280 mm, working volume of 0.7 L) with a hydraulic retention time (HRT) of 6 h. 18 g plastic threads (0.5–2 mm in width) or 18 g stainless steel (0.5–2 mm in width) were placed into the two UASB reactors, respectively. A gas sampling bag (2 L) was connected at the top of the reactor for collecting biogas. Before experiments, each of the UASB reactors received 350 mL sludge inoculums (TSS = 15,200 mg/L, VSS/TSS = 0.72). The reactors were operated at 37 ± 1 °C.

The effects of stainless steel dosage and H_2 partial pressure were investigated. To clarify the roles of stainless steel, the experiments were operated in serum bottles with a batch mode in triplicate. (1) A series of dosage of stainless steel (0, 0.2, 0.5 and 0.8 g) were added in four 125 mL serum bottles containing 40 mL artificial wastewater (COD = 3200 mg/L, SO_4^{2-} = 2600 mg/L) and 2.5 mL sludge inoculums taken from the stainless steel-added UASB reactors. All the bottles were sealed with Teflon-faced butyl rubber stoppers and then flushed with N_2 for 0.5 h in the headspace. The reactors were operated in the dark at 37 °C.

In the hydrogen partial pressure experiments, a series of dosage of stainless steel (0, 0.2, 0.5 and 0.8 g) were added into four 125 mL bottles in triplicate. 40 mL artificial wastewater (COD = 1600 mg/L, SO_4^{2-} = 1300 mg/L) and 2.5 mL sludge inoculums taken from the mentioned above stainless steel-added UASB reactor were added into serum bottles (125 mL). All the bottles were sealed with Teflon-faced butyl rubber stoppers and then flushed with N_2 for 0.5 h in the headspace. Before experiments 10 mL hydrogen (corresponding to a H_2 partial pressure of 0.12 atm, 298 K) was injected into the headspaces. The reactors were operated in the dark at 37 °C.

2.3. Stainless steel surface characterization

Cyclic Voltammetrys (CVs) were recorded at 10 mV/s to investigate the electrochemical activity of biofilms of the stainless steel collected from the UASB reactor. During the CVs measurement, the stainless steel was used as anodes, and glassy carbon electrode was used as the cathode. The reference electrode was Ag/AgCl. The scanning range was from −0.8 to +0.2 V.

The surface of stainless steel was observed using a scanning electron microscopy (SEM and EDX; S-4800, Hitachi, operated at 10 kV) and a confocal Raman microscopy.

Fluorescence in situ hybridization (FISH) was used to determine the existence of dissimilatory Fe (III) reduction associated stainless steel. FISH was conducted according to the method described by Wu et al. (2001). The genus-specific probes for *Geobacter metallireducens* (GEO 1 - Cy3, AGAATCCAAGGACTCCGT, red) was used in this study. The conductivity of bulk sludge was measured as description in Zhao et al. (2016).

2.4. Theoretical analysis of interspecies electron transfer

1) Methanogenesis via DIET

In the case of DIET, the maximum electron carrier flux was calculated using Omh' law, electric current associated with the electron carrier flux (i) was calculated as follows Eq. (1) (Cruz Viggi et al., 2014):

$$I = \sigma \frac{\text{Sconduit}}{\text{d}} (E_{Met} - E_{Acet}) \quad (1)$$

where i is the electron carrier flux (A), σ is the electrical conductivity of stainless steel (assumed 6.67×10^2 μS/cm), s is the cross-sectional area of the electron conduit (here, assumed 7.85×10^3 nm^2), d is the interbacterial distance (0.5 μm), and E_{Met} - − E_{Acet} is calculated using the following Eq. (2):

$$\Delta E = E_{Met} - E_{Acet} = -\Delta G'/nF \quad (2)$$

ΔE is the maximum redox potential (V), n is the amount of the electrons (24), F is the Faraday's constant (96,485 C/mol), ΔG' is calculated using the following Eq. (3):

$$\Delta G' = \Delta G^{0'} - \Delta G_{diss} \quad (3)$$