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Enhancing sludge methanogenesis with improved redox activity of extracellular polymeric substances by hematite in red mud

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ABSTRACT

Different conductive materials have been employed to stimulate direct interspecies electron transfer (DIET) during methanogenesis, but few studies have been concerned with the interaction between conductive materials and extracellular polymeric substances (EPS) such as the effect on sludge aggregation and redox activity of EPS. This study aims to systematically investigate the role of red mud with 45.46 wt% hematite on methanogenesis during the anaerobic digestion of waste activated sludge. The results showed that the multivalent cations from hematite effectively promoted the formation of large and compact aggregates, which might contribute to the rapid direct electron exchange during the DIET process. Meanwhile, more redox-active mediators including *c*-type cytochromes (*c*-Cyts) and humic substances, particularly in tight-bound EPS (TB-EPS), and more redox-active metals such as Fe introduced by red mud could take part in the interspecies electron transfer process between syntrophic bacteria and methanogenic archaea, which also promoted methane production ($35.52 \pm 2.64\%$ increase compared with the control). This study provided initial scientific evidence to comprehensively assess the role of conductive materials during methanogenesis, with important implications for the biogeochemical redox processes of conductive minerals in natural and engineered environments.

1. Introduction

Interspecies electron transfer between secondary fermenting bacteria and methanogenic archaea plays a key role in anaerobic methanogenesis (Stams and Plugge, 2009). Direct interspecies electron transfer (DIET), cell-to-cell transfer of electrons between species through shared physical electrical connections, is increasingly recognized as an important interspecies electron transfer pathway (Lovley, 2017). Microorganisms that take part in DIET can establish biological electrical contacts with partners and act as the foundation for the development of novel biotechnologies (Summers et al., 2010). Compared with the best-known interspecies H_2/formate transfer, DIET has biogeochemical significance and practical applications with unique advantages such as high electron transfer efficiency and energy utilization efficiency (Smith et al., 2015).

Extracellular polymeric substances (EPS) could store electrochemically active substances such as *c*-type cytochromes (*c*-Cyts) (Xiao et al., 2017), which has been reported to be able to improve the DIET process (Zhang et al., 2017b). EPS are natural high-molecular-weight polymers excreted by microorganisms, accounting for 80% of the mass of activated sludge (Sheng et al., 2010). Because of the functional groups such as carboxyl, phosphoric, and hydroxyl groups, EPS can promote the formation of microbial aggregates, which is beneficial to cooperative actions such as DIET involved in the syntrophic anaerobic metabolism (Mostafa, 2012; Morita et al., 2011). In a complex environment such as an anaerobic sludge treatment system, the excretion of EPS could be influenced by the external conditions (Badireddy et al., 2008; Priester et al., 2006).

In recent years, conductive materials have been demonstrated to act as the solid conduits for stimulating direct electron transfer and methane production (Zhao et al., 2017; Liu et al., 2012a).

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However, most studies focused mainly on improving the conductivity of anaerobic systems, and the effects of conductive materials on the excretion of EPS in the complex matrix remain unclear. Furthermore, little is known about the interactions between conductive materials and EPS such as the effects of conductive materials on the redox activity of EPS and sludge aggregation. Better understanding of these interactions will help with comprehensive assessment of the role of conductive materials during methanogenesis.

A representative conductive material of interest to DIET is iron-based material such as hematite (Fe_2O_3), which is a major constituent in red mud with a content ranging from 7 wt% to 65 wt% (Freire et al., 2012). Red mud is a by-product generated from the alumina refining of bauxite ore (Ye et al., 2014, 2015). The annual worldwide growth rate of red mud has significantly increased, leading to severe environmental disasters such as river pollution, haze generation, and land contamination (Hua et al., 2017). The reuse of red mud has drawn considerable research attention (Liu et al., 2012b; Smičiklas et al., 2014). Our previous study demonstrated that methanogenesis was enhanced by red mud with the simultaneous improvement of hydrolysis-acidification and electron transfer processes. Furthermore, the micro- and macronutrients in red mud such as manganese and sodium were beneficial for the growth of microorganisms. The relatively abundances of *Geobacter*, *Methanosaeta* and *Methanosarcina* increased after the addition of red mud (Ye et al., 2018). Previous researches demonstrated the occurrence of DIET process during the growth of syntrophic bacteria (*Geobacter*) and methanogens (*Methanosaeta* and *Methanosarcina*) (Rotaru et al., 2014a, b; Tang et al., 2016; Chen et al., 2014; Liu et al., 2015). Therefore, DIET process might also exist in the reactor with red mud. This study aimed to further systematically explore the role of red mud in methanogenesis such as the formation of bioaggregates and the composition and redox properties of EPS. The critical roles of different components of EPS and red mud in the interspecies electron transfer and methane production were explored. This work is expected to provide a new insight into the functions of EPS in anaerobic digestion and the possible application of red mud to improve sludge digestion.

2. Materials and methods

2.1. Materials

Bayer red mud with a hematite content of 45.46 wt% (Table S1) was obtained from Shandong Aluminum Industry Corporation in Shandong, China. The primary particles of red mud ranged from 50 to 300 μm with a D_{50} value of 123.2 ± 4.5 μm (Fig. S1A). The inoculum sludge was collected from a laboratory-scale upflow anaerobic sludge blanket (UASB) reactor in our laboratory. Waste activated sludge was obtained from a local municipal wastewater treatment plant (Fuzhou, China) as substrate. Fresh waste activated sludge was thickened by gravity settling and then stored at 4 °C for later use. The characteristics of waste activated sludge were as follows: total chemical oxygen demand (TCOD) of 30.15 ± 0.55 g/L, soluble chemical oxygen demand (SCOD) of 1.43 ± 0.06 g/L, total solids (TS) of 31.92 ± 0.26 g/L, and pH of 7.24 ± 0.05.

2.2. Batch experiments

The experiments were conducted in 250-mL anaerobic serum bottles that contained 10 mL sludge inocula and 90 mL substrate. Red mud was dosed into the bottles in different amounts (0.5, 1.0, and 2.0 g). The bottles were kept at 35 °C for 32 days in a constant temperature incubator. To maintain strict anaerobic conditions, all bottles were sealed with Teflon®-coated rubber and aluminum crimp caps after being bubbled with nitrogen gas for 30 min at a rate of 5 mL/min to remove oxygen (Zhuang et al., 2015). The control reactors without the addition of red mud were operated under the same condition. The samples for EPS analysis were obtained on day 0, day 7, day 14, day 21, and day 28 from the selected reactors, and the methods for EPS extraction including soluble EPS (SEPS), loose-bound EPS (LB-EPS) and tight-bound EPS (TB-EPS) followed the method reported previously (Zhang et al., 2016). All experiments were conducted in biological triplicate. Differences were evaluated using Student's *t*-test, and a p value < 0.01 was considered statistically significant.

2.3. Analytical methods

The X-ray diffraction (XRD) patterns of red mud and sludge samples were detected using an X-ray diffractometer (XRD-6000, Shimadzu, Japan) with Cu K_α radiation at 40 kV and 30 mA and recorded in a 2θ range of 5–70° at a scan speed range of 0.02°/s. The quantification of different minerals was obtained by a relative intensity ratio method. The surface of red mud was imaged by the scanning electron microscopy (SEM) (Phenom-World BV, Eindhoven, The Netherlands) and then mapped by energy-dispersive X-ray spectrometry (EDX 3600B Skyray Instrument Inc., Braintree, MA, USA). The concentrations of TCOD, SCOD, TS and volatile suspended solids (VSS) of sludge were measured following the Standard Methods for the Examination of Water and Wastewater (APHA et al., 2005). The methane content in biogas was determined using an Agilent 7890A Gas Chromatograph equipped with a flame ionization detector (FID).

The size distribution of bioaggregates was measured by a laser particle size analysis system and analyzed with the volume (%) curve (Mastersizer 3000, Malvern, UK). Multiple fluorescent staining and imaging of the samples followed the method described by Chen et al. (2007) with a Zeiss LSM 880 confocal microscope (Carl Zeiss, Jena, Germany). Contributions of different components of EPS to the sludge aggregation were evaluated with the flocculation experiments according to Hou et al. (2015). The zeta potentials of red mud and EPS were measured using a Nano-brook Omni analyzer (Nano-brook Omni, Brookhaven, US).

Polysaccharide and protein concentrations were measured using the anthrone method (Loewus, 1952) and BCA Protein Assay Kit (Thermo Scientific Pierce), respectively. The concentration of total organic carbon (TOC) was quantified with a TOC analyzer (TOC-L CPN, Shimadzu, Japan). The concentration of humic substances was measured using the method reported by Frølund et al. (1995). Three-dimensional excitation emission matrix (3-DEEM) spectra were obtained using a Cary Eclipse Fluorescence Spectrophotometer G9800A (Agilent Technologies, USA) with an excitation range from 210 to 400 nm in 10 nm sampling increments and an emission range from 290 to 550 nm in 10 nm sampling intervals. The spectra were recorded at a scan rate of 1200 nm/min. The spectra were then analyzed using the parallel factor analysis (PARAFAC) algorithm to quantitatively determine the components of fluorophores; the scores from the first loading of PARAFAC model were also obtained. These scores were based on the signal intensities of components presented in each sample and were expressed as F_{max} values.

EPS was electrochemically characterized by cyclic voltammetry (CV) at a scan rate of 0.1 V/s (Yang et al., 2017). A chronoamperometry measurement was performed to quantify the electron-accepting capacity (Q_{EAC}) and electron-donating capacity (Q_{EDC}) of EPS according to Yu et al. (2015). An improved staining procedure was utilized to detect the cytochrome content in different EPS fractions (Thomas et al., 1976). UV/Vis spectroscopy (UV-2600, Shimadzu, Japan) in a diffuse transmission mode was employed to measure the electronic absorption spectra of the