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A gradual change between methanogenesis and sulfidogenesis during a long-term UASB treatment of sulfate-rich chemical wastewater

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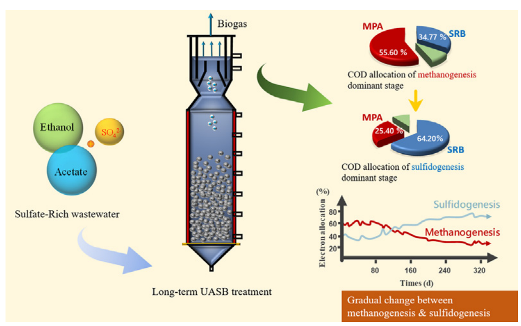
HIGHLIGHTS

- UASB performed well in 330 operation days at a COD/SO_4^{2-} ratio of 1.0.
- Competition between SRB and MPA changed during long-term competition.
- A species of acetate utilizing SRB, *D. acetoxidans*, was significantly observed after a long-term operation.
- All added sulfate were reduced by SRB after 300 days of operation.
- After a long-term competition, COD was firstly utilized by SRB, and the rest converted to methane.

GRAPHICAL ABSTRACT



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ABSTRACT

The competition between methane-producing archaea and sulfate-reducing bacteria is an important topic in anaerobic wastewater treatment. In this study, an Up-flow Anaerobic Sludge Blanket Reactor (UASB) was operated for 330 days to evaluate the treatment performance of sulfate-rich wastewater. The effects of competition change between methane production and sulfate reduction on the organic removal efficiency, methane production, and electrons allocation were investigated. Synthetic wastewater was composed of ethanol and acetate with a chemical oxygen demand $(COD)/SO_4^{2-}$ of 1.0. As a result, the COD removal efficiency achieved in long-term treatment was higher than 90%. During the initial stage, methane production was the dominant reaction. Sulfate-reducing bacteria (SRB) could only partially oxidize ethanol to acetate, and methane-producing archaea (MPA) utilized acetate for methane production. Methane production declined gradually over the long-term operation, whereas the sulfate-reducing efficiency increased. However, UASB performed well throughout the experiment because there was no significant inhibition. After the complete reduction of the sulfate, MPA converted the remaining COD into methane.

1. Introduction

Organic wastewater is now considered a resource of water and energy rather than waste (McCarty et al., 2011; Nagpal et al., 2000). Currently, various processes are being utilized to convert and store different forms of energy produced from organic compounds in wastewater. One example is methane, which can be obtained from anaerobic treatment. Methanogenesis involves the conversion of organic substrates, such as acetate, methanol and even H_2/CO_2, to methane by several different species of methane-producing archaea (MPA) (Hedderich and Whitman, 2006; Kelleher et al., 2002). As a typical anaerobic

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treatment process, the Up-flow Anaerobic Sludge Blanket Reactor (UASB), has been utilized since the 1970s, and is employed in 80% of all anaerobic wastewater treatment around the world (Abbasi and Abbasi, 2012; Visser et al., 1993). Nevertheless, there are problems that may limit the application of anaerobic treatment. One of the most well-known problems is caused by high concentrations of sulfate.

Wastewater from vinasse, pulp and chemical plants is rich in sulfate and organic matter (Barrera et al., 2014; Kamali et al., 2016). Sulfate-reducing bacteria (SRB) are anaerobic microorganisms, which employ organic matters or H_2 as electron donors and reduce sulfate to sulfide in the anaerobic condition. Moreover, SRB contains highly diverse groups with broad metabolic capabilities. When using the anaerobic process to treat sulfate-containing wastewater, MPA and SRB are always competing for a carbon source. The specific problems that need attention in the treatment of this kind of sulfate-rich organic wastewater are as follows: (1) SRB show a higher affinity to organic matter and H_2 for sulfate reduction, and since only a small quantity of electrons are obtained by methanogens while competition occurs, a decline in biogas production occurs; (2) As a reduction product, free (unionized) H_2S is toxic to methanogens as well as to other microorganisms (Li et al., 2015; Velasco et al., 2008); and (3) The toxicity resulting in the buildup of metabolic intermediates such as the volatile fatty acid (VFA) leads to reactor acidification, a sharp decrease in chemical oxygen demand (COD) removal efficiency, and eventually a collapse in the process. In previous studies, our aim was long-term performance using the anaerobic treatment of synthetic sulfate-rich wastewater containing acetate and ethanol discharged from a chemical plant (Jing et al., 2013). It was confirmed that UASB treatment removed more than 80% of COD, where less than 30% of sulfate was reduced with an organic loading rate (OLR) below 12.3 g-COD/L/d and a sulfate loading rate (SLR) below 12.0 g-SO_4^{2-}/L/d, respectively.

SRB can be traditionally classified into two types depending on their different metabolism pathways for organic matter. Incomplete oxidizing SRB (IO-SRB) convert organic matter to acetate rather than CO_2, and has been reported to use ethanol as an electron donor (Hao et al., 2014; Velasco et al., 2008). The growth of IO-SRB on ethanol for sulfate reduction can be represented by the Eq (1) (Nagpal et al., 2000). The frequently isolated and intensively studied *Desulfovibrio sp.*, was only able to oxidize organic matter to the level of acetate. A complete oxidizer was first demonstrated for *Desulfotomaculum acetoxidans* (Strittmatter et al., 2009). Complete oxidizing SRB (CO-SRB) specialize in the oxidation of organic substrates, mainly acetate, all the way to CO_2 (or HCO_3^-) according to Eq. (2) (Colleran et al., 1995; Kaksonen et al., 2003). Acetate is reported as a quantitatively dominant substrate for methanogenesis (Eq. (3)) (Pan et al., 2016). Both IO-SRB and CO-SRB show thermodynamic and kinetic advantages in anaerobic digestion, but CO-SRB may outcompete MPA for acetate-utilizing sulfidogenesis and lead to a further enriched sulfide concentration in the reactor (McCartney and Oleszkiewicz, 1993; Xu et al., 2012).

$$CH_3CH_2OH + 0.5SO_4^{2-} \rightarrow CH_3COO^- + 0.5HS^- + 0.5H^+ + H_2O; \Delta G^0 = -16 \text{ kJ/mol} \quad (1)$$

$$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^-; \Delta G^0 = -28 \text{ kJ/mol} \quad (2)$$

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-; \Delta G^0 = -33 \text{ kJ/mol} \quad (3)$$

From this perspective, it is necessary to identify the characteristics of MPA and SRB in long-term treatment in an attempt to ensure organic removal efficiency, and therefore determine the feasibility of employing the anaerobic process to treat sulfate-rich wastewater.

Some studies in recent were conducted in the relatively low COD/SO_4^{2-} ratio to understand the anaerobic treatment performance: (Das et al., 2015) through a short-term experiment found that COD removal efficiency only reached 48.6% in a COD/SO_4^{2-} at 8.0, further decreased to 37.1% when the ratio was 4.0. (Li et al., 2012) reported that the total COD removal rate was lower than 50% when set the COD/SO_4^{2-} ratios at the range of 2.1 to 0.5. Although our previous submission confirmed that 58.0% of total COD was converted to methane when the COD/SO_4^{2-} ratio of the wastewater (the organic compound was same with this study) was 0.5 (Hu et al., 2015), the result could not provide an adequate basis for the long-term stable anaerobic treatment, due to this experimental condition with a short operating duration. These known reports were all conducted by gradually changed the COD/SO_4^{2-} ratio, and confirmed the sulfidogenesis shown a significant enhancement in a low COD/SO_4^{2-} ratio. However, few works have conducted a 1-year-experiment (330 days) continuously for anaerobic treatment of a constant substrate contained an extremely low COD/SO_4^{2-}, and tracked the gradual change of methanogenesis and sulfidogenesis during the anaerobic treatment.

The aims of this study were (1) to reveal the transition mechanism of the competitive advantage of methanogens and sulfidogens in UASB reactor via the long-term observations and microbial analysis; (2) by taking a real wastewater as example, to provide a reference and strategy for a long and stable UASB treatment of sulfate-rich wastewater. According to the composition of wastewater from a chemical plant, synthetic wastewater made in the laboratory was used in the following experiment.

2. Materials and methods

2.1. Influent and reactor operating conditions

The substrate investigated in this study was synthesized according to the composition of real wastewater of a fiber manufacturing plant in Japan. The components of the synthetic wastewater were as follows (mg/L), the chemicals purity was given in parentheses: 3000 Sulfate (purity: 99.0%), 1000 Acetate (99.0%), 1000 Ethanol (99.5%). The concentration of other constituents in the artificial wastewater were as follows: 850 NH_4Cl (99.5%), 750 KCl (99.5%), 250 K_2HPO_4 (99.0%), 100 KH_2PO_4 (99.5%), 125 $MgCl_2 \cdot 6H_2O$ (99.0%), 4.2 $NiCl_2 \cdot 6H_2O$ (98.0%), 4.2 $CoCl_2 \cdot 6H_2O$ (99.0%), 15 $CaCl_2 \cdot 2H_2O$ (99.0–103.0%) and 42 $FeCl_2 \cdot 4H_2O$ (99.0–102.0%). Additional NaOH was used to control the pH within a range of 7.0 to 8.0. All the chemicals used in the experiment provided by Wako Pure Chemicals Industries, Ltd.

A lab-scale UASB reactor (Fig. 1) was operated at a hydraulic retention time (HRT) of 6 h. The influent flow rate was 24 L/d, and the theoretical organic loading rate (OLR) was 12 g-COD/L/d. The substrate was stored in a substrate tank with an effective volume 70 L, and the synthetic wastewater was fed to a UASB with a working volume of 6 L. The substrate was refreshed every three experimental days. Seed granular sludge was collected from a beer brewery wastewater treatment plant located in Japan, and its characteristics were described in a study published by (Hu et al., 2015). A water bath was equipped to keep the reactor operating at the mesophilic condition (35 ± 1 °C). The continuous experiment lasted 330 days with a constant composition of substrate as well operational condition of reactor to allow the long-term competition behavior between MPA and SRB under a fed COD/SO_4^{2-} ratio of 1.0.

2.2. Analytical methods

COD_{Cr} and sulfide measurements were carried out according to APHA Standard Methods (Association et al., 1915). Sulfide concentration was not considered when measuring the COD in the effluent. While the total COD and sulfide (T-COD and T-S) concentration were measured without any filtration, the soluble COD and sulfide (S-COD&S-S) concentration was measured after a 0.45 μm filter filtration. The volume of the biogas produced was recorded using a wet gas meter (SHINAGAWA W-NK-0.5). The proportion of CH_4, CO_2 and N_2 in the biogas was analyzed using a gas chromatograph (Shimadzu, GC-8A, Japan). All the measurements of CH_4 were normalized to the