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Short Communication

Enhanced elementary sulfur recovery in integrated sulfate-reducing, sulfur-producing rector under micro-aerobic condition

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ABSTRACT

Biological treatment of sulfate-laden wastewater consists of two separate reactors to reduce sulfate to sulfide by sulfate-reducing bacteria (SRB) and to oxidize sulfide to sulfur (S^0) by sulfide oxidation bacteria (SOB). To have SRB + SOB in a single reactor faced difficulty of low S^0 conversion. This study for the first time revealed that dissolved oxygen (DO) level can be used to manipulate SRB + SOB reactions in a single reactor. This work demonstrated successful operation of an integrated SRB + SOB reactor under micro-aerobic condition. At DO = 0.10–0.12 mg l^{-1}, since the activities of SOB were enhanced by limited oxygen, the removal efficiency for sulfate reached 81.5% and the recovery of S^0 peaked at 71.8%, higher than those reported in literature. At increased DO, chemical oxidation of sulfide with molecular oxygen competed with SOB so conversion of S^0 started to decline. At DO > 0.30 mg l^{-1} activities of SRB were inhibited, leading to failure of the SRB + SOB reactor.

1. Introduction

Sulfate-laden wastewaters are yielded in production of sugar, alcohol, pharmaceutical products and monosodium glutamate. Anaerobic treatment of sulfate-laden wastewaters would produce H_2S, which is corrosive to metals and is toxic to living species (Celis-Garcia et al., 2007). A sulfide removal step is essential to proper treatment of sulfate-laden wastewaters. Conventional biological process to treat sulfate-laden wastewater consists of two processes, sulfate reduction to sulfide by sulfate-reducing bacterial (SRB) and sulfide-oxidation to sulfur (S^0) by sulfide oxidation bacterial (Wang et al., 2005). A common drawback of these processes is the need of additional unit that increases capital and operational costs (Villaverde et al., 2001).

Biological sulfide oxidation process is accomplished by respiratory activity of various chemolithotrophic sulfide oxidizing bacteria (SOB), such as colorless sulfide-oxidizing bacteria, using a reduced sulfur compound as the electron donor, oxygen as the electron acceptor and CO_2 as the carbon source. Janssen et al. (1995, 1997) demonstrated that the molar oxygen/sulfide ratio to produce S^0 in a fed-batch reactor was 0.6–1.0. Krishnakumar et al. (2005) studied the sulfide oxidation by *Thiobacillus denitrificans* in a reverse fluidized loop reactor and revealed that under pH-controlled condition 95% of the fed sulfide was converted to S^0.

The concomitant sulfate reduction and biological sulfide oxidation in a single reactor is desired (Celis-Garcia et al., 2008). The SRB and SOB can coexist in hydrothermal vents, microbial mats, marine sediments and wastewater biofilms as a response of high organic input and low dissolved oxygen (DO) concentration (Okabe et al., 2005). van den Ende et al. (1997) succeeded in obtaining mixed cultures of *Desulfovibrio desulfuricans* (SRB) and *Thiobacillus thioparus* (SOB) in a chemostat with limited quantities of lactate and oxygen and excess sulfate. These authors showed that *D. desulfuricans* provided H_2S and CO_2 in need of autotrophic growth of *T. thioparus* to produce S^0. Celis-Garcia et al. (2008) studied the single SRB + SOB reverse fluidized bed reactor under oxygen-limited condition, but, somehow discouraging, noted an unsatisfactorily low S^0 recovery.

Effective conversion of sulfate to S^0 in a single SRB + SOB reactor is of great practical interests. Marazioti et al. (2003) argued that adding low levels of oxygen can effectively reduce the sulfide concentration in mixed liquor in an SOB reactor. Jenicek et al. (2009) dosed a limited amount of air into an anaerobic digester that reduced H_2S levels in the biogas, thereby enhancing methanogenic reactions. Chen et al. (2010) demonstrated that denitrifying sulfide removal process in an SOB reactor was enhanced under micro-aerobic condition (DO = 0.8 mg l^{-1}) and effectively promoted sulfide tolerance by the functional bacteria. Lohwacharin and Annachhatre (2010) investigated biological sulfide oxidation in

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SOB reactor under oxygen-limited conditions (0.2–1 mg l^{-1}) and found that 90% of the sulfide in the influent was converted to elemental sulfur.

The objective of this study was to demonstrate the feasibility of using DO level (0.02–0.35 mg l^{-1}) to regulate S^0 productivity in an expanded granular sludge bed (EGSB) reactor for concomitance of sulfate reduction and sulfide oxidation reactions. Batch assays were used to illustrate the effects of DO on activities of SRB and of SOB for interpreting the experimental findings. To the authors' best knowledge, this is the first study to use DO as a controlling factor to regulate integrated SRB + SOB process in a single reactor.

2. Methods

2.1. Reactor set-up

A continuously fed system consisting of an aeration tank and an EGSB reactor was used. The EGSB reactor has a working volume of 4 l (height of 120 cm and internal diameter of 50 mm) (Fig. S1 in Supplementary data). The temperature of all reactors was maintained at 30 ± 1 °C. A separated 5-l vessel was used as the aeration unit to control the influent DO level. The influent was fed to the EGSB bottom using peristaltic pump. The pH and dissolved oxygen (DO) concentration were monitored, with the latter being controlled by adjusting aeration flow rate. A gas-washing device collected the H_2S gas generated at the column top. Internally circulating fluid with a reflux ratio of 10:1 was applied to suspend granules in the reactor.

Continuous tests were conducted with operational parameters listed in Table 1. The aeration rate of the reactor was changed step by step from 10 to 160 ml min^{-1} at period I–VI (day 1–132). In period VII (day 133–153), aeration was ceased to recover the system from serious deterioration.

2.2. Inoculum and influent

The inoculum was collected from the EGSB reactor developed by Chen et al. (2008) which was operating for more than 6 months. The original seed sludge was collected from WenChang wastewater treatment plant in Harbin City, China. The influent was prepared by supplementing anhydrous sodium sulfate to obtain a sulfate concentration of 1000 ± 100 mg l^{-1}, sodium lactate to obtain a chemical oxygen demand (COD) of 3000 ± 200 mg^{-1} and a sodium bicarbonate ($NaHCO_3$) solution to pH 8.0 ± 0.3. Macronutrients were added at 0.22 g l^{-1} K_2HPO_4, 0.1 g l^{-1} $MgCl_2$ and 0.1 g l^{-1} $CaCl_2$. One milliliter of trace element solution was added to 1 l of feed with compositions listed in Chen et al. (2009).

2.3. Batch assays

Batch tests were performed in 1.5-l stirred tank reactor containing 1.0 l of mineral medium described above. In the reactor, sulfate was added to an initial concentration of 1000 mg l^{-1}, and sulfate reduction rates and sulfide oxidation rates were obtained at different DO's (0.02–0.35 mg l^{-1}) (Table 1). Biological sulfide oxidation rates were calculated by subtracting the measured rates in the absence of biomass (abiotic oxidation) from the values measured in the presence of activated sludge (abiotic and biological oxidation rates) (de Graaff et al., 2012).

2.4. Analytical procedures

An ion chromatography (Dionex ICS-3000, USA) measured the concentration of sulfate, thiosulfate in the liquor samples after 0.45-μm filtration. Sample separation and elution were performed using an IonPac AG4A AS4A-SC 4 mm analytic column with carbonate/bicarbonate eluent (1.8 mmol dm^{-3} Na_2CO_3/1.7 mmol l^{-1} $NaHCO_3$ at 1 cm^3 min^{-1}) and a sulfuric regeneration (H_2SO_4, 25 mmol l^{-1} at 5 cm^3 min^{-1}). Sulfide concentration (including H_2S, HS^- and S^{2-}) was determined according to the methylene blue method (Truper and Schlegel, 1964). Gas chromatography (6890, Agilent, USA) was used to measure the compositions of gas (CO_2, CH_4). Both volatile suspended solids and suspended solids were measured according to Standard Methods (APHA, 1995). Measurements for the concentrations of total organic carbon (TOC) and inorganic carbon (IC) were taken by the TOC analyzing instrument (TOC-VCPH, Japan) equipped with platinum catalyst quartz tube. The flow rate of oxygen gas was 130 ml/min and the furnace temperature was 680 °C. The dissolved oxygen in liquid samples was measured by DO meter (pH/Oxi 340i, WTW, Germany). A pH/ORP meter (pHS-25, ShangHai, China) determined the pH and oxidation–reduction potential (ORP) of liquid samples. At steady state conditions, the elemental sulfur concentration was calculated according to the following equation (Eq. (1)) (de Graaff et al., 2012):

$$[S^0] = [\text{Influent S}] - [SO_4^{2-}] - 2^*[S_2O_3^{2-}] - [HS^-] \qquad (1)$$

Table 1
Operating conditions and performances of the SRB + SOB reactor.

Period	I	II	III	IV	V	VI	VII
Time (day)	1–19	20–48	49–74	75–94	95–116	117–132	133–152
HRT (h)	18	18	18	18	18	18	18
AR (ml min^{-1})	0	10	20	40	80	160	0
DO (mg l^{-1})	0.02	0.08–0.10	0.10–0.12	0.20–0.24	0.24–0.26	0.30–0.35	0.02
Influent SO_4^{2-}–S (mg l^{-1})	378	330	342	333	333	346	–
Effluent SO_4^{2-}–S (mg l^{-1})	5.3	61.6	18.4	21.8	17.1	176	–
SO_4^{2-}–S load (kg m^{-3} d^{-1})	2.04	1.86	1.85	1.80	1.80	1.87	1.80
COD (kg m^{-3} d^{-1})	16.0	16.0	15.8	16.2	17.0	17.6	16.8
Sulfate removal (%)	98.6	81.5	94.6	93.5	94.9	10–60[a]	
S^{2-} (mg l^{-1})	300	31.9	110	178	192	118	
${S_2O_3}^{2-}$–S (mg l^{-1})	0	0	0	0	0	0	
H_2S_{gas}–S (mg l^{-1})	<1	<1	<1	<1	<1	7.8	
S^0 conversion (%)	19.2	71.8	62.5	39.9	37.2	14.8	
SRR	17.9	19.7	19.9	20.1	20.2	–	–
SOR	0.25	4.39	3.88	2.98	8.75	–	–

AR: aeration rates; TSO: total sulfide oxidation; ASO: abiotic sulfide oxidation; SRR: sulfate reducing rate, mmol SO_4^{2-} g^{-1} VSS d^{-1}; SOR: sulfide oxidizing rate, mmol S^{2-} g^{-1} VSS d^{-1}.

[a] Not stable.