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Full Length Article

A high-rate sulfidogenic process based on elemental sulfur reduction: Cost-effectiveness evaluation and microbial community analysis

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ABSTRACT

Biological sulfur reduction is an attractive technology for the treatment of metal-laden wastewater, but its efficiency and cost have been questioned due to the insolubility of sulfur. In this study, a laboratory-scale sulfur-reducing bioreactor was constructed to investigate the long-term feasibility of high-rate sulfur reduction. Our results show that 316 ± 46 mg S/L sulfide was produced within 3 h, corresponding to a high sulfide production rate of 32 ± 5 mg S/L-h, significantly higher than those reported in sulfate-reducing systems. Sulfur reduction processes can significantly reduce the total operational cost compared to sulfate reduction processes. Moreover, long-term sulfur feeding significantly shaped the microbial communities. The predominance of sulfate-reducing genera (24.1%) diminished continuously during the 110 days of operation, and sulfur reducers such as *Geobacter* and *Clostridium* became dominant at the end of experiment. All of these findings suggest that high-rate sulfur reduction processes driven by sulfur reducers can be a cost-effective alternative for metal-laden wastewater treatment.

1. Introduction

Microbial treatment of metal-laden wastewater using sulfate-reducing bacteria (SRB) is a promising approach compared to traditional precipitation [1–3]. Sulfidogenic systems based on SRB have been successfully demonstrated to be efficient for the treatment of metal-laden wastewater [1–3]. However, metal-laden wastewater, such as acid mine drainage, is generally deficient in organic matter and thus requires an external organic carbon source to support high-rate biological sulfate reduction. The addition of an organic carbon source increases operational costs and limits the applicability of sulfate reduction processes in the treatment of metal-laden wastewater.

Elemental sulfur is a promising alternative to sulfate as elemental sulfur reduction only requires two electrons per sulfide compared to the eight electrons required for sulfate reduction (Eqs. (1) and (2), taking acetate as an example). Florentino et al. suggest that sulfur reduction processes can potentially be used to treat metal-contaminated wastewater [4]. Based on their theoretical calculations [4], a total cost reduction of approximately 73% can be achieved in sulfur-reducing processes compared to sulfate-reducing processes after taking the cost of sulfur addition into account.

$$\frac{1}{4}Acetate^- + S^0 + \frac{1}{2}H_2O \rightarrow \frac{1}{2}CO_2 + HS^- + \frac{3}{4}H^+ \quad (1)$$

$$Acetate^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^- \quad (2)$$

However, the cost-effectiveness of sulfur reduction processes remains unproven due to the lack of evidence from long-term tests of sulfur-reducing bioreactors. The extremely low water solubility of elemental sulfur (5 μg/L at 25 °C and neutral pH) creates a significant bottleneck in the sulfur reduction process [5]. The low bioaccessibility of sulfur to sulfur reducers may be insufficient for high-rate sulfur reduction and can thus limit the cost-effectiveness of sulfur reduction processes for treating metal-laden wastewater. In a previous study, we found that high-rate sulfur reduction can be achieved in a sulfur-reducing anaerobic fluidized-bed reactor by using sulfur particles as biofilm carriers to enhance sulfur bioaccessibility [6], enabling its cost-effectiveness to be evaluated through long-term experiments.

On the other hand, the sulfur-reducing microbial community has yet to be revealed. Many microrganisms such as *Wollinella succinogenus*, *Geobacter*, *Sulfurospirillum*, *Desulfurella* and *Desulfomicrobium* can biologically reduce sulfur to sulfide using organic

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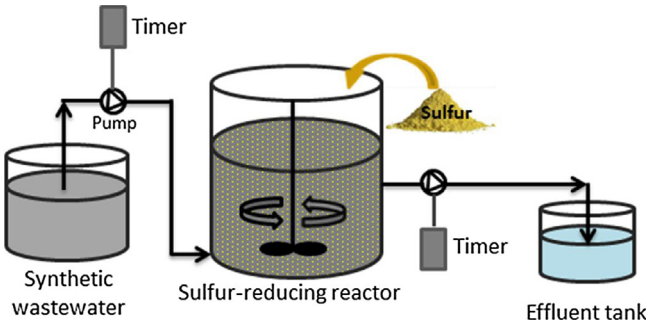

Fig. 1. A schematic diagram of the sulfur-reducing system.

carbons as electron donors [7]. This suggests that sulfur reducers are highly diverse in the environment and differ from sulfate-reducing bacteria. Only a few SRB can grow with elemental sulfur [7]. To our knowledge, the sulfur-reducing microbial community structures in sulfur-reducing bioreactors remain largely unknown and should be elucidated because the performance of the sulfur reduction process highly depends on microbial community composition. Long-term sulfur feeding can significantly shape the sulfidogenic communities in a sulfur-reducing bioreactor using sulfate-reducing sluge as seeding [8].

We therefore aimed to investigate the efficiency in sulfide production of a laboratory-scale sulfur-reducing bioreactor in long-term tests and evalute the cost of the sulfur reduction process using the experimental data. Additionally, the microbial community in the sulfur-reducing bioreactor was characterized using an Illumina Miseq sequencer to provide further insight into the sulfur-reducing microbial community.

2. Materials and methods

2.1. Seeding sludge enrichment, system setup and operation

A laboratory-scale sulfur-reducing bioreactor (cylindrical reactor) with an effective volume of 2.38 L (10 cm diameter × 30.3 cm height) was constructed (Fig. 1). The total height of the sulfur-reducing bioreactor was 35 cm, and the effluent height was set at 21.2 cm. The bioreactor was seeded with approximately 2.7 g MLVSS/L activated sludge taken from the Shatin Sewage Treatment Works (STSTW) in Hong Kong. The activated sludge in the STSTW contains abundant SRB due to the treatment of saline sewage [8]. As some SRB can reduce elemental sulfur [7], we speculated that the activated sludge in the STW used as the seeding sludge could shorten the cultivation period. In addition, sublimated and chemical sulfur from sublimation or the Claus process, as inexpensive and common sulfur sources, are less soluble than colloid sulfur and bio-sulfur and thus less accessible to sulfur reducers. Therefore, we used sublimated sulfur as a representative in this study. The sulfur-reducing bioreactor was fed with synthetic wastewater and sublimated sulfur (Damao, China, purity >99.5%, size in 20–40 μm). The stock synthetic wastewater was prepared following Jiang et al. [9], as shown in Table S1.

The sulfur-reducing reactor was a sequencing batch reactor operated with a cycle time of 3 h over the entire period (110 days), consisting of 15 min feeding, 120 min stirring, 30 min settling and 15 min decanting periods. Three different peristaltic pumps were used to control the three different steps in the operational cycles, respectively (Fig. 1). During each cycle, 0.71 L of synthetic wastewater was fed into the reactor. It can be calculated that the actual hydraulic retention time (HRT) was 10 h. The entire period was divided into two phases. Phase I (days 0–50) was the cultivation period, during which the stock synthetic wastewater was diluted to approximately 300 mg/L total organic carbon (TOC) before being fed into the sulfur-reducing bioreactor. After the cultivation period, the sulfide production tended to be stable and then synthetic wastewater had higher dilution factor to achieve lower TOC (100 mg/L) in Phase II (days 51–110) to obtain stable sulfide production with low organic carbon consumption. In addition, in both Phase I and II, 840 mg/L sodium bicarbonate ($NaHCO_3$) used as buffer solution was added into the influent. Sublimated sulfur was supplemented directly through the top opening into the reactor based on the amount of sulfide production every day. According to the results (see Section 3.1), approximately 2.5 g/day and 2.0 g/day sublimated sulfur was required in Phase I and II, respectively.

2.2. Analytical methods

The sulfide, sulfate, thiosulfate and TOC were analyzed regularly after filtration (0.45 μm pore size). Dissolved sulfide (H_2S, HS^- and S^{2-}) was measured using the methylene blue method [10], while TOC concentration was determined using a TOC analyzer (Shimadzu TOC-5000A). The sulfate and thiosulfate concentrations were analyzed with an ion chromatograph (DIONEX-900). pH was measured with a pH meter (HQ40D). Elemental sulfur was quantified with a high-performance liquid chromatograph (HPLC, Shimadzu LC-16, Japan) equipped with a Kromasil column (C_{18}, 5 μ, 100 Å) and a UV detector at 254 nm. Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were measured following APHA standard methods [10].

2.3. DNA extraction, PCR amplification, sequencing and analysis

The seeding sludge (day 0) and the cultivated sulfur-reducing sludge (day 110) collected from the sulfur-reducing reactor at the end of the experiment were retained for microbial community analysis. The total genomic DNA was extracted from collected biofilm samples using the MO BIO PowerSoil DNA isolation kit (Carlsbad, CA) according to the manufacturer's instructions. The 16S rRNA gene was amplified using a primer set (338F/806R) targeting the V3 and V4 hypervariable regions of both the bacteria and archaea domains [11]. The Illumina MiSeq sequencing service (Illumina Inc., San Diego, CA) was provided by Meige Bio. Tech. Inc. (Guangzhou, China).

The obtained paired-end raw 16S rRNA gene sequences were aligned using Mothur [11]. The aligned sequences were checked for chimera using USEARCH 6.1 in QIIME and classified into operational taxonomic units (OTUs) within a 97% similarity range using the de novo OTU picking workflow in QIIME [12]. Heatmap analysis was conducted using the pheatmap package in R (version 3.3.1, http://www.r-project.org/).

3. Results and discussion

3.1. The performance of the sulfur-reducing bioreactor

During Phase I (days 0–50), the sulfide production rate was 41 ± 15 mg S/L-h in the cultivation period (Fig. 2), indicating that sulfur-reducing bacteria were well cultivated. In Phase II (days 51–100), the influent organic carbon concentration was reduced to 100 mg C/L to provide carbon-limited conditions. The performance of the sulfur-reducing bioreactor was then kept stable throughout the entire period, in which 32 ± 5 mg S/L-h sulfide production rate was achieved within each cycle (3 h) (Fig. 2). The influent organic matter was efficiently removed (99% on average) under the carbon-limited conditions in Phase II (Fig. 3a). The sulfide production rate was 1.3 times higher than those (6–44 mg S/L-h with an average of 25 mg S/L-h) reported in the sulfate reduction processes employed in the treatment of metal-contaminated wastewater (Table 1)