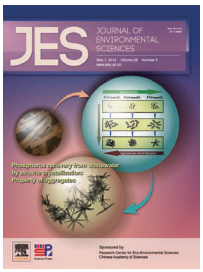
Journal of Environmental Sciences 26 (2014) 1099–1107

Available online at www.sciencedirect.com

Journal of Environmental Sciences

www.jesc.ac.cn



Microbial community functional structure in response to micro-aerobic conditions in sulfate-reducing sulfur-producing bioreactor

Hao Yu[1,2], Chuan Chen[1,*], Jincai Ma[3], Xijun Xu[1], Ronggui Fan[2], Aijie Wang[1,4,*]

1. State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China. E-mail: micro_yh@126.com

2. School of Environmental Science and Engineering, Liaoning Technical University, Fuxin 123000, China

3. Biosciences and Biotechnology Division, Lawrence Livermore National Laboratory, Livermore, California 94550, USA

4. Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

ARTICLE INFO

Article history:
Received 22 September 2013
revised 30 October 2013
accepted 07 November 2013

Keywords:
micro-aerobic condition
elemental sulfur recovery
microbial community
functional gene array

DOI: 10.1016/S1001-0742(13)60589-6

ABSTRACT

Limited oxygen supply to anaerobic wastewater treatment systems had been demonstrated as an effective strategy to improve elemental sulfur (S^0) recovery, coupling sulfate reduction and sulfide oxidation. However, little is known about the impact of dissolved oxygen (DO) on the microbial functional structures in these systems. We used a high throughput tool (GeoChip) to evaluate the microbial community structures in a biological desulfurization reactor under micro-aerobic conditions (DO: 0.02–0.33 mg/L). The results indicated that the microbial community functional compositions and structures were dramatically altered with elevated DO levels. The abundances of *dsrA/B* genes involved in sulfate reduction processes significantly decreased ($p < 0.05$, LSD test) at relatively high DO concentration (DO: 0.33 mg/L). The abundances of *sox* and *fccA/B* genes involved in sulfur/sulfide oxidation processes significantly increased ($p < 0.05$, LSD test) in low DO concentration conditions (DO: 0.09 mg/L) and then gradually decreased with continuously elevated DO levels. Their abundances coincided with the change of sulfate removal efficiencies and elemental sulfur (S^0) conversion efficiencies in the bioreactor. In addition, the abundance of carbon degradation genes increased with the raising of DO levels, showing that the heterotrophic microorganisms (e.g., fermentative microorganisms) were thriving under micro-aerobic condition. This study provides new insights into the impacts of micro-aerobic conditions on the microbial functional structure of sulfate-reducing sulfur-producing bioreactors, and revealed the potential linkage between functional microbial communities and reactor performance.

Introduction

As a common contaminant, sulfate is found in the effluents of many industries, such as food processing, chemical industry, paper manufacturing and petroleum refining. One of the prevailing problems associated with the anaerobic treatment of sulfate-laden wastewater is the large amounts of sulfide generation by sulfate-reducing bacteria (SRB). It is well known that sulfide may result in severe corrosive and toxic effects on sewers, ecosystems and living species (Xu et al., 2012). Biological sulfide removal is a promising strategy to solve this problem since it is environmentally friendly and cost-effective (Chen et al., 2008). Sulfide can be removed coupled to sulfate reduction by sulfide-oxidizing bacteria (SOB), typically colorless sulfur bacteria, which oxidize reduced sulfur compounds using oxygen as the electron acceptor and CO_2 as the carbon source (Sahinkaya et al., 2011). Moreover, sev-

* Corresponding author. E-mail: echo110244@126.com (Chuan Chen); waj0578@hit.edu.cn (Aijie Wang)

eral colorless sulfur bacteria including *Thiobacillus* sp., *Thiomicrospira* sp. *and Thioalkalivibrio* sp. have been proved to be responsible for sulfide oxidation in the bio-desulfurization process (Huang et al., 1996; McComas et al., 2001; Sorokin et al., 2008).

Recently, there has been increased interest in production of elemental sulfur (S^0) in bio-desulfurization processes, because S^0 can be reused as fertilizer after separation from the effluent or biosolids (Celis-García et al., 2008). It has been demonstrated that the sulfide oxidation process is regulated by the oxygen availability, and S^0 is the major final product (Janssen et al., 1995; Kobayashi et al., 2011). Biological sulfide control by introduction of limited amounts of oxygen/air into anaerobic systems has been extensively investigated in various reactors (Lohwacharin and Annachhatre, 2010; Sahinkaya et al., 2011; van der Zee et al., 2007), showing a high conversion efficiency of sulfide to S^0. A conceptual process which integrates SRB and SOB in a single expanded granular sludge bed (EGSB) reactor has been operated under different micro-aerobic conditions (Xu et al., 2012). Their results demonstrated that recovery of S^0 was effectively controlled by the amount of oxygen in the bioreactors.

Although the S^0 conversion efficiency and sulfide removal efficiency under micro-aerobic conditions had been widely investigated for optimized bioreactor operations, very little is known about the change of diversity, structure of microbial communities, and functional species (e.g. SRB and SOB) in response to introduction of oxygen into anaerobic reactors. In addition, research on the metabolic potential of microbial communities at the gene level is important to assess and improve the overall performance of bioreactors. To date, the information on microbial functional structures in bio-desulfurization systems is limited, which may be due to the complexity of functional species and drawbacks of conventional molecular approaches for microbial ecology detection (Bai et al., 2013; Liu et al., 2010; Zhou et al., 2010). Recently, GeoChip-based metagenomics technology has been proved to be a powerful and high throughput tool for characterizing functional microbial communities in both natural and artificial (bioreactor) environments (Bai et al., 2013; He et al., 2010; Liu et al., 2010). The aim of this work is to investigate the change of the diversity, structure, and abundances of functional genes/species at different dissolved oxygen (DO) levels using a functional gene array (GeoChip) and to reveal the linkage between the abundances of functional gene/species and the performance of the reactor, such as sulfate removal efficiency and S^0 conversion efficiency.

1 Materials and methods

1.1 Reactor setup and working conditions

The continuous experiment was conducted in a 4-L Plexiglas EGSB reactor, which has an internal diameter of 50 mm and a height of 120 cm. The structure and detailed information of the reactor was reported previously (Xu et al., 2012). The bioreactor was operated at a thermal state of (30±1)°C with hydraulic retention time (HRT) of 18 hr. The entire experiment consisted of six stages as listed in **Table 1**. As a single controllable parameter, the concentration range of DO was varied from 0.02 to 0.33 mg/L by adjusting the aeration flow rates.

The bioreactor was initially inoculated with granules from an EGSB reactor described previously (Chen et al., 2008). The sulfate-laden synthetic wastewater containing 1000 mg/L of SO_4^{2-}, 3000 mg COD/L of lactate, 220 mg/L of K_2HPO_4, 100 mg/L of $CaCl_2$ and 100 mg/L of $MgCl_2$ was fed into the bioreactor. The pH was maintained at 8.0 ± 0.3 by using sodium bicarbonate ($NaHCO_3$) solution.

1.2 DNA preparation and hybridization

Twelve sludge samples were collected from stage I (DO: 0.02 mg/L), II (DO: 0.09 mg/L), III (DO: 0.11 mg/L) and VI (DO: 0.33 mg/L). In each stage, samples were continuously collected from the last three operational days as replicates for community analysis and listed as follows: stage I (S1a, S1b, S1c), stage II (S2a, S2b, S2c), stage III (S3a, S3b, S3c) and stage VI (S6a, S6b, S6c). Total

Table 1 Operational conditions and reactor performance

Stage	Operational days of each stage	DO (mg/L)	Influent SO_4^{2-} (mg/L)	COD (mg/L)	Effluent S^{2-} (mg/L)	SO_4^{2-} removal (%)	S^0 recovery (%)	COD removal (%)	TOC removal (%)
I	19	0.02	1000	3000	285.9 ± 12.9	96.0 ± 2.1	20.6 ± 6.7	32.5 ± 3.1	39 ± 3.4
II	29	0.09	1000	3000	24.3 ± 9.2	82.6 ± 1.2	76.9 ± 2.5	35.4 ± 12	44.7 ± 4.4
III	26	0.11	1000	3000	162 ± 14.5	97.8 ± 1.4	56.3 ± 2.2	43.5 ± 3.9	50.8 ± 5.3
IV	20	0.23	1000	3000	180.1 ± 6.9	97.6 ± 0.8	43.2 ± 2.3	58.5 ± 5.5	52.7 ± 4.1
V	22	0.25	1000	3000	197.7 ± 21.1	93.0 ± 2.9	31.2 ± 5.4	69.3 ± 9.4	77.1 ± 5.9
VI	16	0.33	1000	3000	96.5 ± 28.8	48.4 ± 17.8	17.0 ± 9.0	76.1 ± 2.1	95.2 ± 5.7

Performance data are presented as mean ± standard deviation. The performance at the last five time points in each stage was selected to calculate statistical data.