Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 20(2008) 897–899
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Profiling of microbial communities in a bioreactor for treating hydrocarbon-sulfide-containing wastewater

LIAO Bo[1,2], JI Guodong[1,2,*], CHENG Liqiu[1,2]

1. The Key Laboratory of Urban Human Residential Environmental Science and Technology, Shenzhen Graduate School, Peking University, Shenzhen 518055, China. E-mail: jiguodong@iee.pku.edu.cn
2. Department of Environmental Engineering, Peking University, The Key Laboratory of Water and Sediment Sciences, Ministry of Education, Beijing 100871, China

Received 5 April 2008; revised 18 April 2008; accepted 28 May 2008

Abstract

A technology of polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was used to profile the structure and dynamic changes of microbial communities in a bioreactor for treating hydrocarbon-sulfide-containing (HSC) wastewater. The results showed that the heterotrophic genus of *Acinetobacter* and the autotrophic genera of *Thiobacillus* and *Thiomonas* could survive well in all of three operating conditions. Some special genera were also observed with changes of micro-ecoenvironment in the reactor, such as the halophilic genus of *Nesterenkonia*. Further, a new genus was found in the reactor, which was likely to have the ability to degrade sulfide and hydrocarbon at the same time. All of these detected and the new found genera have widely applicable potential in the treatment of HSC wastewater.

Key words: hydrocarbon-sulfide-containing wastewater; biological sulfide oxidation; microbial ecology; PCR-DGGE

Hydrocarbon wastewater often containing sulfide is a increasingly arousing worldwide concern due to the great harm it poses to ambient environment and human health (Vaiopoulou *et al*., 2005). So far, among the numerous sulfide treating strategies, biological sulfide oxidation (BSO) which has advantages in high efficiency, economy, with no secondary pollution (Janssen *et al*., 2003), has been widely applied in the treatment of sulfide wastewater and has thus become a research interest within the field of wastewater treatment. However, most of the previous studies focused on the mechanism and influencing factors of BSO (Janssen *et al*., 2003; Krishnakumar *et al*., 2005). Of the available literature, only a small amount of it has been concerned with the microbial diversity during the process. To date, there are no reported investigations about the profiling of microbial diversity by the PCR-DGGE technology in treating hydrocarbon-sulfide-containing (HSC) wastewater. In this study, a bioreactor was used to treat HSC wastewater to obtain the complete oxidation of sulfide. Furthermore, the microbial diversity, community, and dynamic changes of special genera in three different operating conditions were analyzed in the molecular level by the PCR-DGGE technology.

An up-flow reactor made of polymethyl methacrylate was used in this study with an inner diameter of 70 mm, height of 750 mm, having an effective volume of 2.1 L. The inoculated sludge was obtained from a SBR tank of a landfill leachate treatment plant. The simulating HSC wastewater mainly consisted of mineral nutrients, NaCl, Na_2S, and petroleum hydrocarbon, in which the petroleum hydrocarbon was prepared according to the standard hydrocarbon components (SEPA, 2002), China.

The three operating conditions in this study include: operation in fed-batch type (S1); continuous operation with high hydrocarbon and low sulfide concentration (S2); and continuous operation with low sulfide and high hydrocarbon concentration (S3) (Table 1). In each condition, after a stable steady was achieved, water quality parameters were continuously measured daily for 20 d. On day 20, microbial in the second (B#) and forth (D#) layer of the reactor were also collected to detect microbial communities. There were a total of six microbial samples for S1, S2 and S3, marked with 1B, 1D, 2B, 2D, 3B, and 3D in turn. All the six samples were preserved at –20°C for further determination.

Determination data of water quality under three operating conditions (data not shown) suggest that a stable sulfide removal rate was achieved in the reactor, with an average removal rate of about 100% for S1 and S3, and 90% for S2. However, the best TOC removal rates in S1, S2, and S3 were only 25.6%, 15.3%, and 19.3%, respectively, indicating no obvious TOC removal in the reactor.

DGGE results of the microbial samples (Fig.1) show that, under the same condition, the samples from the layers of B# and D# had similar profiles, that is, little difference

* Corresponding author. E-mail: jiguodong@iee.pku.edu.cn.

Table 1 Operating condition

Item	Operating condition		
	S1	S2	S3
Influent sulfide conc. (mg/L)	226.1±8.3	230.1±9.2	120.5±5.4
HRT (h)	–	1.5	1.5
Sulfide volume ($kg/(m^3 \cdot d)$)	0.23±0.01	3.68±0.15	1.93±0.08
Influent pH	7.53±0.02	7.53±0.02	7.54±0.02
Influent hydrocarbon conc. (mg/L)	26.9±0.5	27.4±0.6	48.7±1.5
Aeration (ml/min)	300	300	300

–: Operating condition was 24 h under S1, and 2.1 L of wastewater was fed to the reactor in exchange on a daily basis. Data expressed as mean ± SD.

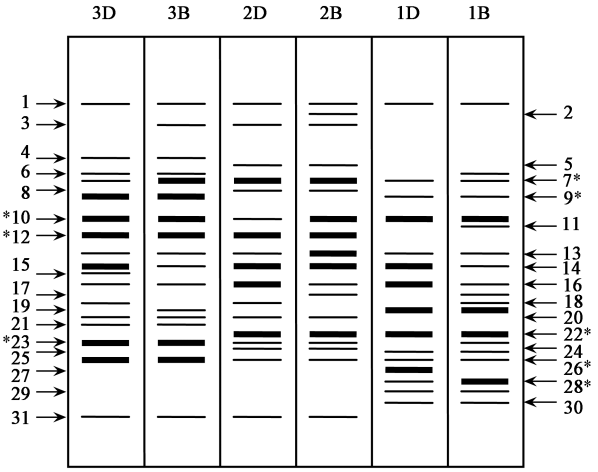

Fig. 1 Schematic diagram of relative intensity of bands in DGGE profiles. * Bands have been successfully sequenced.

in band number and position but some difference in the position of high intensity bands. Thus, it can be concluded that the microbial community structures in different spatial positions of the reactor were similar, whereas the profiles of samples under different conditions had obvious differences. Some bands, such as Band 1, 7, 10, 13, and 26, have been found in all the samples under the three conditions, indicating that microbes corresponding to these bands had a strong adaptability to environmental changes (Fig.1).

Typical bands from the DGGE profiles were excised, re-amplified and sequenced. Eight 16S rRNA sequences were obtained. All of them were submitted to the GeneBank database with the assigned accession numbers of EU545130–EU545137. Sequence identification was performed by use of the Blast facility of the Nation Center, USA for Biotechnology Information.

Table 2 summarizes the sequence comparison results and relative abundance of sequenced bands. Sequence analysis showed that Bands 7, 10, and 26 had similarity levels of 99%, 98%, and 100%, respectively, which was more than the genus similarity level (98%, González-Toril *et al*., 2003). It was therefore considered that sequences of these three bands were from *Acinetobacter*, *Thiobacillus*, and *Thiomonas* genus respectively. Earlier works have found that both *Thiobacillus* and *Thiomonas* belonged to the typical bacterium of chemoautotrophic sulfide oxidation, which were commonly found in the BSO reactors (Janssen *et al*., 2003; Vaiopoulou *et al*., 2005; Ryoki *et al*., 2007). *Acinetobacter* has been reported to have the ability to decompose petroleum hydrocarbon and has generally been isolated from organic wastewater with the high concentration of hydrocarbon (Razak *et al*., 1999). In present study, these three genera were all found in the three operating conditions. Furthermore, autotrophic *Thiobacillus* and *Thiomonas* could maintain metabolic activity under containing organic compounds environment, while *Acinetobacter* was tolerant to sulfide with a certain concentration, suggesting that these three bacteria had a strong tolerance to changes in environment. Thus, a microbial ecosystem predominated by communities of sulfide oxidation and petroleum hydrocarbon degradation had formed in the reactor with great stability under various operating conditions. However, there are remarkable differences in the intensity of these bands in three operating conditions (Table 2). Band 7 had a high intensity in S2, medium in S3 (with deviation of about 50% between two layers), and weak in S1. Band 10 had a high intensity in S1, medium in S3, and weak in S2 (with deviation of about 40% between two layers). Band 26 had a high intensity in S3, but weak in S1 and S2. Taking into consideration of the substance, concentration petroleum hydrocarbon load decreased in the order of S3 > S2 > S1 and sulfide load in the order of S2 > S3 > S1 in the three conditions. Thus, the variety of species abundance observed in this study had a direct relationship with concentration of the two substances and the dimensional positions. However, the quantities relationship among them should be studied further.

Because of the obvious differences of the three operating conditions (Table 1), some different special genera tuned up in the reactor in the three conditions. The sequence corresponding to Band 12, which existed only in S2 and S3, had a 95% similarity with *Xanthomonas* genus, less than the genus similarity level (98%, González-Toril *et al*., 2003). It is most probable that this microbe corresponding to Band 12 was of a new kind. It has been reported that *Xanthomonas* belongs to a kind of heterotrophic sulfide oxidation bacterium that can synthesize cellular substance from organic compounds (Cho *et al*., 1992). Considering the high load of sulfide and hydrocarbon in S1 and S2 conditions and the metabolic characteristics of the closest *Xanthomonas* genus, it can be deduced that this new microbe corresponding to Band 12 might have the double functions of sulfide oxidation and hydrocarbon degradation, thus, having widely applicable potential in treating HSC wastewater. Moreover, Band 12 had a higher intensity in S2 with relative high sulfide loading than S3, indicating that this new microbe was adapted to survive in the high sulfide-loading environment. However, all the above deductions need to be certified by the further studies on the substance's utilizing spectrum of sulfur and carbon and functional genes. Band 28 was only observed in S1 and its corresponding sequence had a high similarity of 100% with *Nesterenkonia* genus. Yoon *et al*. (2006) have already isolated a pure strain of *Nesterenkonia* from

Table 2 Similarity comparison of nucleotide sequences and abundance of sequenced DGGE bands

Band[a]	Accession No.	Closest relative	Similarity (%)	Relative abundance of bands (%)[b]		
				S3	S2	S1
7	EU545130	*Acinetobacter* sp. LZXC8	99	13.8	32.8	2.9
9	EU545131	*Thiobacillus neapolitanus* DSM 581	96	20.8	–[c]	4.6
10	EU545132	*Thiobacillus* sp. UAM-I	98	14.1	3.0	15.5
12	EU545136	*Xanthomonas* sp. 2	95	4.4	12.0	–
22	EU545137	*Rhodobacter vinaykumarii*	100	–	5.6	8.7
23	EU545133	Uncultured β-*proteobacterium*	100	9.4	3.3	1.8
26	EU545134	*Thiomonas* sp. 3As	100	13.0	1.8	3.2
28	EU545135	*Nesterenkonia* sp. EL-30	100	–	–	20.3

[a] Bands are designated as shown in Fig.1; [b] abundance was calculated by the BandScan (Version 5.0) software, and expressed as the mean value of the two samples in the same operating condition; [c] not detected.

seafood, which showed a high activity in culture medium with 2%–5% (*W/V*) NaCl. In this study, the substrate of the reactor in S1 with relative low load rate of sulfide and petroleum hydrocarbon was mainly high concentration of NaCl (with an average concentration of 1.5%) and mineral nutrients, which provided beneficial living conditions for *Nesterenkonia*. The sequence corresponding to Band 22, found in S1 and S2 conditions, showed a 100% similarity with *Rhodobacter*. Srinivas *et al*. (2007) have reported that this bacterium was capable of both chemoheterotrophic and photoheterotrophic growth by using organic compounds as an electron donor and carbon source in a culture medium. However, *Rhodobacter* disappeared in the S3 condition, which may be due to the much more complex wastewater used in our study comparing with the culture medium. Detailed reasons for the disappearance of *Rhodobacter* in S3 need to be studied further.

The results show that a relative stable microbial ecosystem composed of heterotrophic and autotrophic bacterium has formed in a reactor for treating HSC wastewater after selective acclimation. Both sulfide oxidation genera and hydrocarbon degradation genera could survive in the execrable environment with toxic sulfide and petroleum hydrocarbon, and observed a high sulfide oxidation activity in three operating conditions. Furthermore, the structure of microbial communities varied with the change of operating conditions. Some different special genera turned up to form new and stable microbial ecosystems. A new genus was also found likely to have the ability of degrading sulfide and hydrocarbon. All of these results obtained provide stable practical operational data and useful information for artificially forming communities for the treatment of HSC wastewater by a bioreactor. However, in this study, only qualitative analysis was given to the microbial communities. The function of the new and special genera should be studied further by molecular-biological methods such as isolation and expression of functional genes, RT-PCR, FISH, as well as physiological and biochemical tests.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 20507001), the Fok Ying Tong Education Foundation (No. 104004) and the National Key Technology Research and Development Program of China (No. 2006BAD01B08).

References

Cho K S, Hirai M, Shoda M, 1992. Degradation of hydrogen sulfide by *Xanthomonas* sp. strain DY44 isolated from peat. *Appl Environ Microbiol*, 58(4): 1183–1189.

González-Toril E, Llobet-Brossa E, Casamayor E O, Amann R, Amils R, 2003. Microbial ecology of an extreme acidic environment, the Tinto River. *Appl Environ Microbiol*, 69(8): 4853–4865.

Janssen J S, Albert J H, Hulshoff P W, 2003. Development of a novel process for the biological conversion of H2S and methanethiol to elemental sulfur. *Biotechnol Bioeng*, 82(1): 1–11.

Krishnakumar B, Majumdar S, Manilal V B, 2005. Treatment of sulphide containing wastewater with sulfur recovery in a novel reverse fluidized loop reactor (RFLR). *Water Research*, 39: 639–647.

Razak C N A, Wang W F, Rahman S H S A , Basri M, Salleh A B, 1999. Isolation of the crude oil degrading marine *Acinetobacter* sp. E11. *Acta Biotechnologica*, 19(3): 213–223.

Ryoki A, Takako S, Yutaka N, 2007. Isolation and characterization of sulfur oxidizing bacteria from cattle manure compost. *Animal Science Journal*, 78(3): 330–333.

SEPA (State Environmental Protection Administration), 2002. Monitoring and Analytical Methods of Water and Wastewater (4th ed.). Beijing: Environmental Science Press of China.

Srinivas T N R, Kumar P A, Sasikala C, Ramana C V, Imhoff J F, 2007. *Rhodobacter vinaykumarii* sp. nov., a marine phototrophic alphaproteobacterium from tidal waters, and emended description of the genus *Rhodobacter*. *Int J Syst Evol Microbiol*, 57: 1984–1987.

Vaiopoulou E, Melidis P, Aivasidis A, 2005. Sulfide removal in wastewater from petrochemical industires by autotrophic denitrification. *Water Research*, 39: 4101–4109.

Yoon J H, Jung S Y, Kim W, Nam S W, Oh T K, 2006. *Nesterenkonia jeotgali* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. *Int J Syst Evol Microbiol*, 56: 2587–2592.