

# Influence of dye type and salinity on aerobic decolorization of azo dyes by microbial consortium and the community dynamics

Liang Tan, Shuxiang Ning, Ying Wang and Xiangyu Cao

## ABSTRACT

In this research, aerobic decolorization of different azo dyes by a microbial community was studied. The results showed that more than 80% of four azo dyes (100 mg/L) could be aerobically decolorized by the microbial consortium, however, the time needed was obviously different. Kinetic data indicated that the processes were well described by zero-order kinetics, and the chemical structures of dyes had obvious influence on decolorization rates. On the other hand, effects of salinity on decolorization were also investigated. There was still 40% dye removal for Acid Brilliant Red GR when the salinity increased to 250 g/L. And the microbial community structures with different salinity were detected by PCR-DGGE. It was shown that the same two bacteria were dominant in all decolorization systems, and some typical halophilic microorganisms were found under higher-salt conditions.

**Key words** | aerobic decolorization, azo dye, kinetics, PCR-DGGE, salinity

**Liang Tan** (corresponding author)  
**Shuxiang Ning**  
**Ying Wang**  
School of Life Science,  
Liaoning Normal University,  
Liushunan Street No. 1,  
116029 Dalian, Liaoning,  
China  
Email: [tanl\\_innu@yahoo.com.cn](mailto:tanl_innu@yahoo.com.cn)

**Xiangyu Cao**  
Key Laboratory of Industrial Ecology and  
Environmental Engineering (Ministry of  
Education),  
School of Environmental Science and Technology,  
Dalian University of Technology,  
Linggong Road No. 2,  
116023 Dalian, Liaoning,  
China

## INTRODUCTION

As the largest and most versatile dyes, azo dyes are widely used in many industries including textile, printing, cosmetics, pharmaceutical, food, etc. (Stolz 2001). It was reported that about 4–12% of azo dyes were lost in municipal and industrial wastewaters (Davies *et al.* 2006). Generally, azo dyes are recalcitrant xenobiotics and many of them are toxic to aquatic life and humans (Ozturk & Abdullah 2006). Compared with chemical and physical processes for decolorization of azo dyes, biological methods have been an efficient alternative because they are low cost and environment friendly (He *et al.* 2008).

A combined anaerobic/aerobic system has been regarded as the most effective strategy for azo dye degradation (O'Neill *et al.* 2000). The reductive cleavage of azo bonds under anaerobic conditions resulted in the formation of aromatic amines, which would be further degraded in an aerobic unit (van der Zee & Villaverde 2005). However, anaerobic decolorization might be affected by many factors, such as, environmental conditions (e.g. pH, temperature and salinity) or chemical structure of dyes (Hsueh & Chen 2008; Tan *et al.* 2009a), thus inhibiting the aerobic process. In addition, operation of a combined process was always complicated. Therefore, novel techniques for more simple, economic and efficient treatment of azo dyes should be exploited. It was pointed out that

the azo bonds of Acid Red 151 (AR151) were biochemically cleaved via processes yielding metabolites other than aromatic amines by the aerobic microbial community (Buitrón *et al.* 2004), which suggested that aerobic biodegradation of azo dye was feasible. Additionally, the microbial resources (pure or mixed cultures) which could decolorize azo dyes aerobically were exploited (Pourbabae *et al.* 2006; Sarayu & Sandhya 2010), and it was also found that the aerobic processes were catalyzed by aerobic or oxygen-insensitive azoreductases (Ooi *et al.* 2007; Pandey *et al.* 2007). All the reports suggested that aerobic biological technology would be an effective alternative for azo dyes treatment.

Except for organic pollutants, salt concentration is another important factor to be considered for azo dye decolorization (Tan *et al.* 2009a). High salinity could inhibit the aerobic or anaerobic biological processes by causing plasmolysis and/or loss of activity of microbial cells (Peyton *et al.* 2002). The effects of high salinity on microbial activity have been resolved by a combined adsorption/bio-regeneration system (Gu *et al.* 2008), however, it was costly and complicated to operate. Salt-tolerant microorganisms are a proper alternative, which could produce and accumulate some substance to resist exterior pressure (Bremer & Krämer 2000). And halophilic microbial communities could

also be obtained through acclimatization and isolation from environmental samples (Tan *et al.* 2009a, b).

In this study, aerobic decolorization of azo dyes by a microbial community was investigated. The effects of salinity on decolorizing Acid Brilliant Scarlet GR were also evaluated. The kinetics of decolorization was studied for evaluating the effects of chemical structure and salinity on these processes. Meanwhile, microbial community structures under these conditions were monitored by polymerase chain reaction followed by denaturing gradient gel electrophoresis (PCR-DGGE) and dominant species were detected by sequence analysis of DGGE bands.

## MATERIALS AND METHODS

### Dyes and chemicals

Azo dyes used in this study (Figure 1) were purchased from Dye Synthesize Laboratory, Dalian University of Technology. Biochemical reagents were purchased from TaKaRa Biotechnology Co., Ltd., Dalian, China. Other chemical reagents are of analytical grade.

### Microbial community and medium

The microbial community was fed from the sea mud of Heishijiao Beach Park, Dalian, China. Before inoculation, the sea mud was well mixed and filtrated with a 100-mesh screen, and then stored at  $-20^{\circ}\text{C}$ . Before inoculation, the sea mud was firstly acclimatized with the culture medium added with 100 mg/L of corresponding azo dyes for 7 d

(7 cycles, 1 cycle for 24 h) in 250 mL flasks, and then the suspension was reversed after standing for 30 min and most of the water removed through centrifugation (8,000 r/min, 10 min). The inoculum size was about 2.07 g/L of wet cell pellet. The culture medium for decolorization contained (g/L): 10.0 sucrose, 5.0  $(\text{NH}_4)_2\text{SO}_4$ , 1.0  $\text{KH}_2\text{PO}_4$  and 0.5  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ . And the pH of the medium was adjusted to about 7.0.

### Analytical methods

Concentration of azo dyes in the liquid phase was analyzed using a V-560 UV-Vis scanning spectrophotometer (JASCO Co., Ltd., Tokyo, Japan) after centrifugation (8,000 r/min). The standard curve of dye concentration versus absorbance value at corresponding specific wavelengths was drawn, which could be used for the calculation of dye concentration (data not shown).

### Aerobic decolorization processes and kinetic study

Decolorization was performed in 250 mL flasks (the actual reaction volume was 100 mL) in the form of batch tests. Culturing conditions were as follows:  $30^{\circ}\text{C}$ , pH 7.0, rotation speed of 150 r/min and inoculum size of 30% (v/v). The effects of dye types and salinities (0, 30, 50, 100, 150, 200 and 250 g/L of NaCl) on decolorization processes were investigated by kinetic study. The samples were determined once at intervals of 2 h (day time) or 12 h (at night). Curves of dye concentrations versus reaction time were drawn and kinetics equations of decolorization were fitted based on these curves. Additionally, in order to eliminate

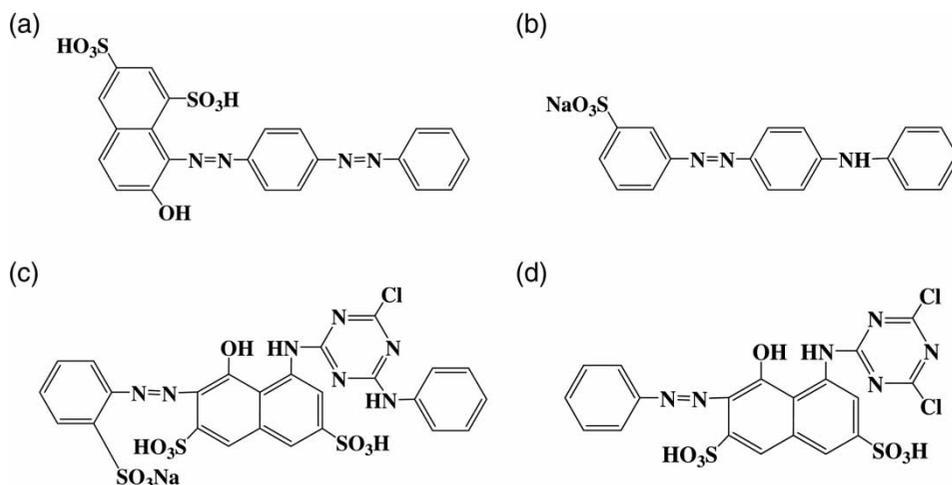


Figure 1 | Chemical structures of the azo dyes used in this study. (a) Acid Brilliant Scarlet GR; (b) Acid Orange G; (c) Reactive Brilliant Red K-2BP; (d) Reactive Brilliant Red X-3B.

decolorization rate from adsorption, control experiments with sterilized sea mud under the same conditions were performed simultaneously.

### Genomic DNA extraction, PCR and DGGE

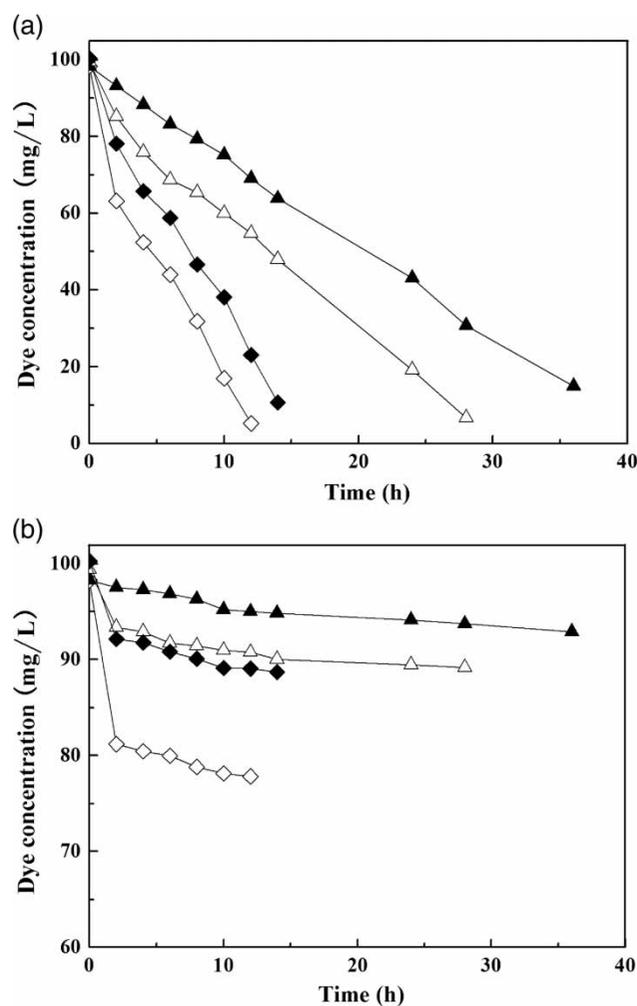
The microbial samples were gathered at the end of batch tests. After centrifugation, the cell pellet was used for extraction of total DNA. Total DNA was extracted and purified by the method described by *Qu et al. (2009)*. The primers for PCR were GM341F-GC and DS907R (*Teske et al. 1996*), and about 500 bp of 16 S r DNA fragment was amplified using a PCR thermal cycler Dice (BioRad Co., Ltd., USA) with a 'touchdown' program (*Tan et al. 2009a*). DGGE was performed with a BioRad Dcode system (BioRad Co., Ltd., USA) and the denaturing gradient ranged from 40 to 55% (100% is 7 mmol/L urea and 40% (v/v) formamide). The conditions for electrophoresis were as follows: 60 °C, 200 V and 5 h. Then the gel was stained with the nucleic acid stain 'GeneFinder' (BIO-V Co., Ltd., Xiamen, China) at 10,000-fold dilution, and the gel's UV transillumination image was captured using a gel imaging instrument (BioRad Co., Ltd., USA).

DGGE profiles were analyzed using 'Quantity One', software for cluster analysis. Besides, dominant DGGE bands were excised and re-amplified by PCR with the same primers without the GC clamp in the forward primer (GM341F/DS907R). The sequencing of PCR products were performed by TaKaRa Biotechnology Co., Ltd., and the sequences were compared with the nucleotide sequences in the GenBank database using the BLAST program.

## RESULTS AND DISCUSSION

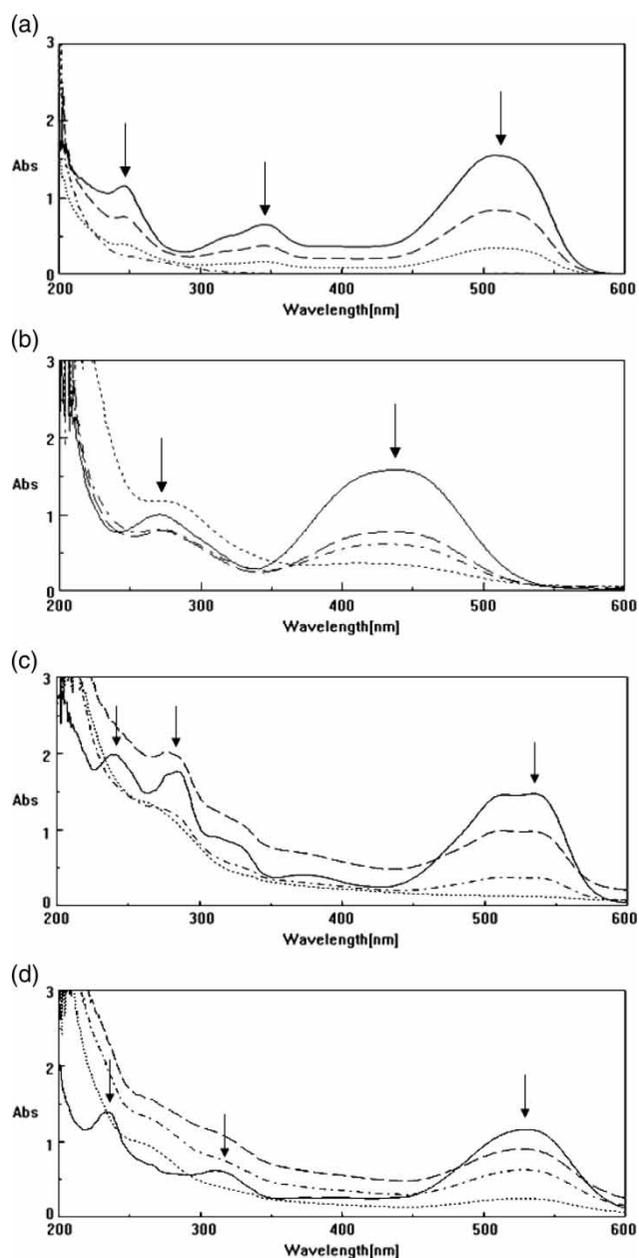
### Aerobic decolorization of different azo dyes and the kinetic study

Aerobic decolorization processes of four azo dyes by the microbial community are shown in *Figure 2*. It was demonstrated that at least 80% of the four dyes were decolorized within 36 h, and slight adsorption was observed within the first 4 h (*Figure 2(b)*), which suggested that decolorization was mainly due to biodegradation. On the other hand, in order to further analyze the probable decolorization pathway, the liquid phases for the four systems were analyzed by spectrophotometric method at certain time intervals. As shown in *Figure 3*, that the absorbance of specific



**Figure 2** | Decolorization processes of four azo dyes by the (a) aerobic microbial community (sea mud) and (b) control (sterilized sea mud) under the following conditions: 30 °C, pH 7.0, rotation speed of 150 r/min, inoculum size of 30% (v/v) and dye concentration of 100 mg/L. -◇- Acid Brilliant Scarlet GR; -◆- Acid Orange G; -△- Reactive Brilliant Red K-2BP; -▲- Reactive Brilliant Red X-3B.

wavelengths (both of UV and visible) of the four dyes gradually reduced with time. As mentioned in some previous reports, decrease of UV absorbance indicated further degradation of aromatic intermediates (*Buitrón et al. 2004*; *Davies et al. 2006*), some of which were always recalcitrant compounds. And these processes always occurred under aerobic conditions. In contrast, under anaerobic conditions, UV absorbance obviously increased after decolorization, which suggested that azo dye decolorization always stopped at the cleavage of azo bonds and formation of stable aromatic amines (*Xu et al. 2007*). Therefore, it was suggested that most of Acid Brilliant Scarlet GR and its decolorization intermediates (corresponding aromatic amines) were further degraded into small pieces, and part of the other three dyes and their intermediates were also further degraded.



**Figure 3** | UV-Vis spectra of four azo dyes before and after decolorization. (a) Acid Brilliant Scarlet GR: — 0 h; - - 4 h; ..... 8 h; - · - 12 h; (b) Acid Orange G: — 0 h; - - 4 h; ..... 8 h; - · - 12 h; (c) Reactive Brilliant Red K-2BP: — 0 h; - - 12 h; ..... 24 h; - · - 28 h; (d) Reactive Brilliant Red X-3B: — 0 h; - - 12 h; ..... 24 h; - · - 36 h.

Azo dyes are generally resistant to bacterial attack under aerobic conditions, nevertheless, some strains had been found that could reduce the azo linkage by aerobic or oxygen-insensitive azoreductases, and some of them even can use the released byproducts as growth substrates (Ooi *et al.* 2007; Pandey *et al.* 2007). For instance, an oxygen insensitive intracellular azoreductase has been purified

from *Pseudomonas aeruginosa* by Nachiyar & Rajakumar (2005), and the affinity of the enzyme for different azo dyes was studied. In addition, white-rot fungi can degrade a wide variety of recalcitrant compounds by their extracellular enzymatic systems, and the enzymatic reaction mechanism was different from that of bacterium (Ciullini *et al.* 2008). However, there were few reports of the azoreductases based on microbial communities, probably because they were complex and difficult to study.

As shown in Figure 2(a), a linear relationship between dye concentration and time was observed after 4 h. Indeed, the whole curves of biological decolorization approached linear (data not shown) without regard to the impact of adsorption, which suggested that the processes could be described by zero-order kinetics. The kinetic equations and corresponding values of  $R^2$  are shown in Table 1. It was confirmed that the decolorization processes were well described by zero-order kinetics, and the decolorization rates were kinetic constants ( $k'$ , the slope of kinetics equation). The kinetic constants of decolorization processes of Acid Brilliant Scarlet GR and Acid Orange G were 5.8365 mg/(L h) and 5.5064 mg/(L h), respectively, which were higher than those of the other two dyes. This result further proved that Acid Brilliant Scarlet GR and Acid Orange G were more easily decolorized by aerobic consortium than Reactive Brilliant Red X-3B and K-2BP.

It was indicated that the variation of molecular structures would affect the color removal capability of microorganisms (Hsueh & Chen 2008). The position of sulfo groups would affect the decolorization rate due to its strong electron-withdrawing effect. Generally, when sulfo groups were *para* to the azo bond, they would promote the decolorization. However, when they were *ortho* to the azo bond, the decolorization rate significantly decreased compared with the *p*-substituted isomer. Additionally carboxyl and hydroxyl groups which were *ortho* to the azo bond could also inhibit the decolorization processes, but the effects were not as strong as those of sulfo groups. Therefore, since no sulfo

**Table 1** | Kinetic equations of the decolorization processes of different azo dyes

Dyes (100 mg/L)	Kinetic equations <sup>a</sup>	$R^2$
Acid Brilliant Scarlet GR	$C = -5.8365t + 76.4540$	0.9932
Acid Orange G	$C = -5.5064t + 89.9519$	0.9926
Reactive Brilliant Red K-2BP	$C = -2.9149t + 88.7410$	0.9973
Reactive Brilliant Red X-3B	$C = -2.3168t + 97.4350$	0.9986

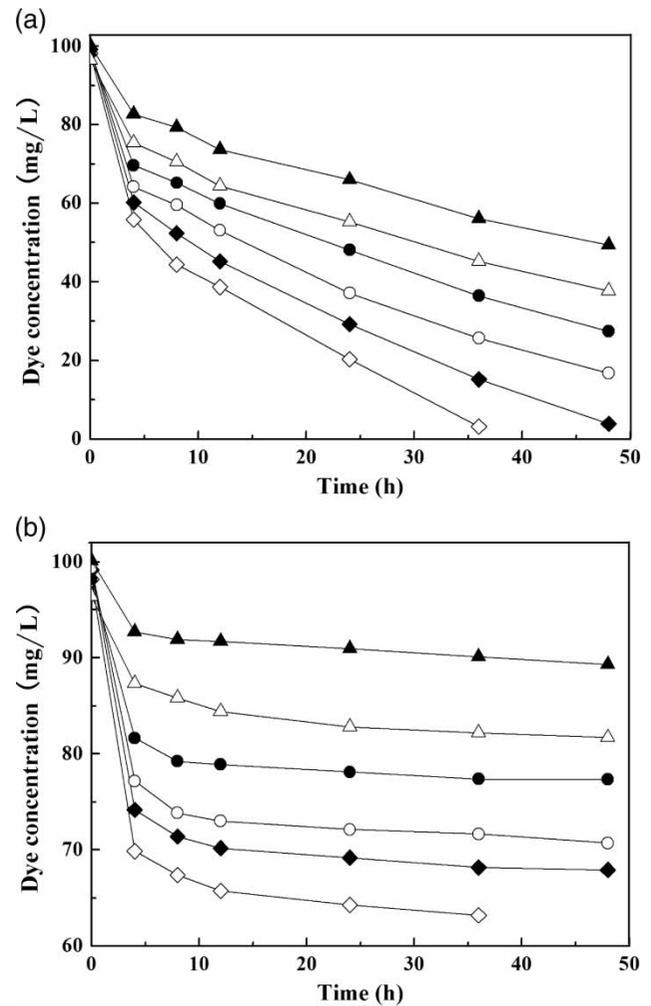
<sup>a</sup> $C$ , concentration of dyes;  $t$ , time.

groups were *ortho* to the azo bonds, the decolorization processes of Acid Brilliant Scarlet GR and Acid Orange G were faster than those of the other two dyes.

### Effects of salinity on aerobic decolorization and the kinetic study

It was indicated that halophilic and halotolerant microorganisms can be the best candidates for a practical biodecolorization process as they are able to grow easily at high concentrations of salts (Amoozegar *et al.* 2011). Meanwhile, the microbial community used here was from saline conditions. Thus, the effects of salinity on azo dye decolorization by the microbial community were investigated, and Acid Brilliant Scarlet GR was chosen as the model compound. The results showed that the decolorization was inhibited under the high-salt conditions and the inhibition effects increased as the salinity increased (Figure 4(a)). It was reported that the efficiency of biological treatment would be impacted by hyper-salinity wastewater because high concentration of salt would cause plasmolysis of microbial cells and lead to loss of activity (Peyton *et al.* 2002). However, even when the NaCl concentration was up to 250 g/L, there was still about 40% of color removal through biodegradation due to the acclimatization under high-salt conditions (Tan *et al.* 2009a). On the other hand, increasing salinity resulted in the decrease of decolorization efficiency by adsorption (Figure 4(b)), which might be caused by the death of some microorganisms possessing strong adsorption capacity or the structural damage of zoogloea under the high osmotic stress conditions (Peyton *et al.* 2002).

Meanwhile, zero-order decolorization kinetics was also presumed for the processes with different salinity according to the curves shown in Figure 4. The kinetic equations and corresponding values of  $R^2$  are shown in Table 2. It was demonstrated that the decolorization processes were also well described by zero-order kinetics according to the values of  $R^2$ . Furthermore, decolorization rates ( $k'$ ) sharply decreased when the concentration of NaCl increased from 0 to 30 g/L. It was suggested that the microbial activity for aerobic decolorization was seriously depressed by high salinity. When NaCl concentration further increased to 50–250 g/L, decolorization rates decreased slightly. All these results suggested that aerobic decolorization of Acid Brilliant Scarlet GR by the community structure was seriously but not completely inhibited by high salinity. Some salt-tolerant microorganisms probably survived and became dominant members



**Figure 4** | Effects of salinity on Acid Brilliant Scarlet GR (100 mg/L) decolorization by (a) microbial community (sea mud); and (b) control (sterilized sea mud). The concentrations of NaCl (g/L):  $\diamond$ - 30;  $\blacklozenge$ - 50;  $\circ$ - 100;  $\bullet$ - 150;  $\triangle$ - 200;  $\blacktriangle$ - 250.

**Table 2** | Kinetic equations of Acid Brilliant Scarlet GR decolorization with different NaCl concentrations

Concentration of NaCl (g/L)	Kinetic equations	$R^2$
0 <sup>a</sup>	$C = -5.8365t + 76.4540$	0.9932
30	$C = -1.5050t + 59.0920$	0.9899
50	$C = -1.2675t + 62.2220$	0.9885
100	$C = -1.1035t + 66.9910$	0.9858
150	$C = -0.9693t + 72.4450$	0.9951
200	$C = -0.8448t + 76.6530$	0.9870
250	$C = -0.7596t + 84.5700$	0.9909

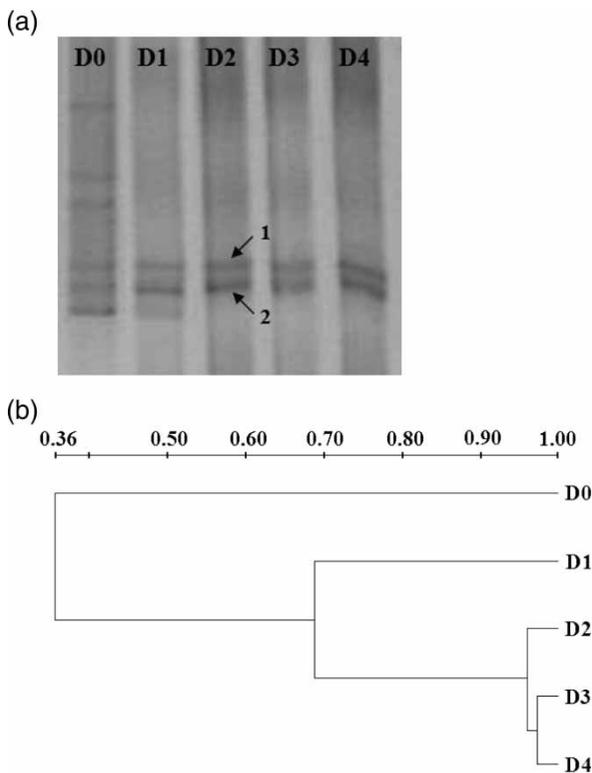
<sup>a</sup>From the decolorization curve of Acid Brilliant Scarlet GR in Figure 2.

of the consortium, which could resist high osmotic stresses and continuously decolorize the azo dye (Tan *et al.* 2009a).

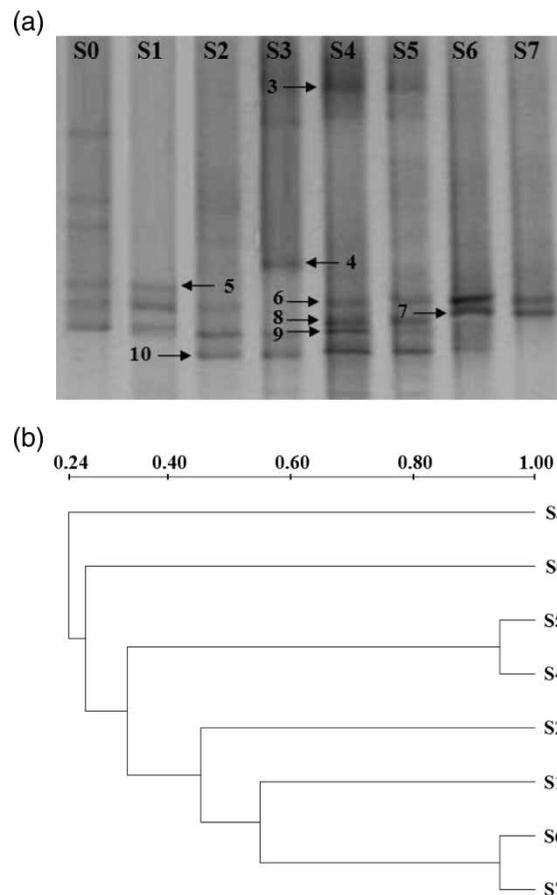
### Microbial community dynamics revealed by PCR-DGGE

To further reveal the impacts of different dyes and salinities on microbial decolorization processes, the dynamics of microbial community were studied by PCR-DGGE, as shown in Figure 5(a) and 6(a), respectively. Dominant bands were labeled with a number. And the results of cluster analysis are presented in Figure 5(b) and 6(b), respectively.

In Figure 5(a), lane D0 was the original microbial community from the sea mud, and the other four lanes were the consortiums for decolorization of different dyes. It was shown that the band patterns of D1–D4 were far different from that of sea mud (D0). The similarity between D0 and the other samples was less than 0.4 (Figure 5(b)). In addition the number of dominant bands sharply decreased, which indicated that the dyes were probably toxic to previously dominant microbes (Qu *et al.* 2006). Bands 1 and 2 were dominant members in all four lanes, which suggested that they were probably efficient degraders of these dyes. The PCR products of the two bands were cloned and sequenced, and the results are listed in Table 3. The



**Figure 5** | DGGE fingerprints of microbial communities for decolorizing different azo dyes (a) and cluster analysis based on 'UPGMA' method (b). Lane D0, the original microbial community; lanes D1–D4, the microbial communities for decolorization of Acid Brilliant Scarlet GR, Acid Orange G, Reactive Brilliant Red K-2BP and Reactive Brilliant Red X-3B, respectively.



**Figure 6** | DGGE fingerprints of microbial communities for decolorizing Acid Brilliant Scarlet GR with different salinities (a) and cluster analysis based on 'UPGMA' method (b). Lane S0, the original microbial community; lanes S1–S7, the microbial communities for decolorization of Acid Brilliant Scarlet GR with 0 g/L, 30 g/L, 50 g/L, 100 g/L, 150 g/L, 200 g/L, 250 g/L of NaCl, respectively.

**Table 3** | Identity of dominant DGGE bands labeled in Figures 5(a) and 6(a)

Band	Closest relative (Accession number)	Similarity (%)
1, 5	Uncultured prokaryote isolate DGGE band GW1b4–6(S) (AY501825)	99
2, 6	Uncultured bacterium clone EDW07B002_41 (HM066354)	99
3	<i>Thalassobacillus</i> sp. NCCP-58 (AB541110)	99
4	Uncultured bacterium clone B217 (HM459660)	92
7	<i>Halobacillus</i> sp. 399K3-1 (HM222689)	100
8	Uncultured Bacteroidetes bacterium clone QEDN4CB05 (CU926767)	96
9	Activated sludge bacterium GC_R2A_S_2 (GU136495)	98
10	Uncultured bacterium isolate DGGE gel band F5 (DQ536185)	99

cloned 16S rDNA sequence of band 1 most closely related to 'Uncultured prokaryote isolate DGGE band GW1b4-6 (S)', which was detected in a chlorinated solvent-contaminated bedrock aquifer (NCBI database, unpublished). It was suggested that it might be an organics degrading bacterium. And the closest relative of band 2 was 'Uncultured bacterium clone EDW07B002\_41', which was found in a saline water in the Edwards Aquifer, Texas (NCBI database, unpublished). None of the two dominant bands were confirmed as azo dye degraders according to the existing information. Thus, they could be identified as novel bacterium for degradation of azo dyes under aerobic conditions.

Shown in Figure 6(a) are the DGGE profiles of the microbial consortiums for decolorization with different salinities (lanes S1-S7). Obviously differences in DGGE patterns could also be reflected in the results of cluster analysis (Figure 6(b)). However, the similarity between lanes S4 and S5 approached 1.0 (100% similarity), which indicated that the corresponding two consortiums were highly similar in structure. It was suggested that the microbial community structure would be relatively stable when NaCl concentration ranged from 100 to 150 g/L. And the same relationship could also be observed between lanes S6 and S7. Furthermore, it was obviously shown that bands 5 and 6 were dominant species under the low-salt conditions. However, with the increase of salinity, band 5 disappeared and some other bands became new dominant ones, such as bands 3 and 6-10. Among these new bands, bands 9 and 10 appeared when NaCl concentration increased from 30 to 150 g/L, and bands 3 and 8 were dominant when the salinity ranged from 100 to 150 g/L. However, all of them disappeared when NaCl concentration reached 200-250 g/L of NaCl. At the same time, band 6 and another new band 7 became dominant species under these conditions. It was illustrated that some microorganisms could survive in a hyper-saline environment through production and accumulation of some substance for resisting exterior pressure (Bremer & Krämer 2000). Salt-tolerant microorganisms were divided into five categories by Kushner according to their optimal salinity for growth (Kushner 1978). The optimal salinities for moderately and extremely halophilic bacteria were about 29.3-146.3 and 146.3-304.0 g/L of NaCl, respectively. Therefore, it could be speculated that the species of bands 3, 6, 8, 9 and 10 might belong to moderately halophilic bacteria and those of bands 6 and 7 might belong to extremely halophilic bacteria.

All of the dominant bands were cloned and sequenced, and the results were listed in Tables 2 and 3. Bands 5 and 6 were the same with bands 1 and 2, respectively. On the

other hand, the most closely related species of bands 3, 6, 7 and 10 were all salt-tolerant bacteria according to the information on the NCBI database (all were unpublished). Among them, the most similar bacterium of band 6 was found in saline water. And band 7, which was most closely related to 'Halobacillus sp. 399K3-1', was probably a halophilic bacterium. It was suggested that both bands 6 and 7 were similar to typical halophilic bacteria, which was consistent with the results shown in the DGGE profiles. However, the most similar species of bands 4, 8 and 9 were not found in saline environments, suggesting that they might also be moderately salt-tolerant bacteria.

## CONCLUSIONS

The study evaluated a microbial community for aerobic decolorization of azo dyes. The effects of salinity on the decolorization process were also investigated. The results showed that the microbial community could aerobically decolorize 100 mg/L of four azo dyes by effective biodegradation. The processes were well described by zero-order kinetics and the decolorization rates were obviously different. Additionally, the microbial community also exhibited a certain tolerance of high-salt conditions, however, decolorization rate decreased with the increase of salinity. Two dominant bacteria were speculated as being typical azo dye degraders and some halophilic bacteria were detected by PCR-DGGE from high-salt environments.

## ACKNOWLEDGEMENTS

The work was financially supported by the National Natural Science Foundation of China (No. 51108223). And the authors gratefully acknowledge the support of the Chinese Academy of Sciences, Shenyang Institute of Applied Ecology (CAS SIAE).

## REFERENCES

- Amoozegar, M. A., Hajighasemi, M., Hamed, J., Asad, S. & Ventosa, A. 2011 Azo dye decolorization by halophilic and halotolerant microorganisms. *Annals of Microbiology* 61 (2), 217-230.
- Bremer, E. & Krämer, R. 2000 Coping with osmotic challenges: osmoregulation through accumulation and release of compatible solutes in bacteria. In: *Bacterial Stress Responses*

- (G. Storz & R. Hengge-Aronis, eds). ASM Press, Washington, D. C., America, pp. 77–97.
- Buitrón, G., Quezada, M. & Moreno, G. 2004 Aerobic degradation of the azo dye acid red 151 in a sequencing batch biofilter. *Bioresource Technology* **92** (2), 143–149.
- Ciullini, I., Tilli, S., Scozzafava, A. & Briganti, F. 2008 Fungal laccase, cellobiose dehydrogenase, and chemical mediators: combined actions for the decolorization of different classes of textile dyes. *Bioresource Technology* **99** (15), 7003–7010.
- Davies, L. C., Pedro, I. S., Novais, J. M. & Martins-Dias, S. 2006 Aerobic degradation of acid orange 7 in a vertical-flow constructed wetland. *Water Research* **40** (10), 2055–2063.
- Gu, X., Zhou, J., Zhang, A., Wang, P., Xiao, M. & Liu, G. 2008 Feasibility study of the treatment of aniline hypersaline wastewater with a combined adsorption/bio-regeneration system. *Desalination* **227** (1–3), 139–149.
- He, Z., Lin, L., Song, S., Xia, M., Xu, L., Ying, H. & Chen, J. 2008 Mineralization of C.I. Reactive blue 19 by ozonation combined with sonolysis: performance optimization and degradation mechanism. *Separation and Purification Technology* **62** (2), 376–381.
- Hsueh, C. C. & Chen, Y. B. 2008 Exploring effects of chemical structure on azo dye decolorization characteristics by *Pseudomonas luteola*. *Journal of Hazardous Materials* **154** (1–3), 703–710.
- Kushner, D. J. 1978 Life in high salt and solute concentrations: halophilic bacteria. In: *Microbial Life in Extreme Environments* (D. J. Kushner, ed.), Academic Press, London, England, pp. 318–358.
- Nachiyar, C. V. & Rajakumar, G. S. 2005 Purification and characterization of an oxygen insensitive azoreductase from *Pseudomonas aeruginosa*. *Enzyme and Microbial Technology* **36** (4), 503–509.
- O'Neill, C., Hawkes, F. R., Hawkes, D. L., Esteves, S. & Wilcox, S. J. 2000 Anaerobic-aerobic biotreatment of simulated textile effluent containing varied ratios of starch and azo dye. *Water Research* **34** (8), 2355–2361.
- Ooi, T., Shibata, T., Sato, R., Ohno, H., Kinoshita, S., Thuoc, T. L. & Taguchi, S. 2007 An azoreductase, aerobic NADH-dependent flavoprotein discovered from *Bacillus* sp.: functional expression and enzymatic characterization. *Applied Microbiology and Biotechnology* **75** (2), 377–386.
- Ozturk, A. & Abdullah, M. I. 2006 Toxicological effect of indole and its azo dye derivatives on some microorganisms under aerobic conditions. *Science of the Total Environment* **358** (1–3), 137–142.
- Pandey, A., Singh, P. & Iyengar, L. 2007 Bacterial decolorization and degradation of azo dyes. *International Biodeterioration and Biodegradation* **59** (2), 73–84.
- Peyton, B. M., Wilson, T. & Yonge, D. R. 2002 Kinetics of phenol biodegradation in high salt solutions. *Water Research* **36** (19), 4811–4820.
- Pourbabae, A. A., Malekzadeh, F., Sarbolouki, M. N. & Najafi, F. 2006 Aerobic decolorization and detoxification of a disperse dye in textile effluent by a new isolate of *Bacillus* sp. *Biotechnology and Bioengineering* **93** (4), 631–635.
- Qu, Y., Zhou, J., Wang, J., Song, Z., Xing, L. & Fu, X. 2006 Bioaugmentation of bromoamine acid degradation with *Sphingomonas xenophaga* QYY and DNA fingerprint analysis of augmented systems. *Biodegradation* **17** (1), 83–91.
- Qu, Y., Zhang, Q., Wei, L., Ma, F., Zhou, J., Pi, W. & Gou, M. 2009 Optimization of metagenomic DNA extraction from activated sludge samples. *Asia-Pacific Journal of Chemical Engineering* **4** (5), 780–786.
- Sarayu, K. & Sandhya, S. 2010 Aerobic biodegradation pathway for Remazol Orange by *Pseudomonas aeruginosa*. *Applied Biochemistry and Biotechnology* **160** (4), 1241–1253.
- Stolz, A. 2001 Basic and applied aspects in the microbial degradation of azo dyes. *Applied Microbiology and Biotechnology* **56** (1–2), 69–80.
- Tan, L., Qu, Y., Zhou, J., Ma, F. & Li, A. 2009a Dynamics of microbial community for X-3B wastewater decolorization coping with high-salt and metal ions conditions. *Bioresource Technology* **100** (12), 3003–3009.
- Tan, L., Qu, Y., Zhou, J., Li, A. & Gou, M. 2009b Identification and characteristics of a novel salt-tolerant *Exiguobacterium* sp. for azo dyes decolorization. *Applied Biochemistry and Biotechnology* **159** (3), 728–738.
- Teske, A., Wawer, C., Muyzer, G. & Ramsing, N. B. 1996 Distribution of sulfate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable-number counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA fragments. *Applied and Environmental Microbiology* **62** (4), 1405–1415.
- Xu, M., Guo, J. & Sun, G. 2007 Biodegradation of textile azo dye by *Shewanella decolorationis* S12 under microaerophilic conditions. *Applied Microbiology and Biotechnology* **76** (3), 719–726.
- van der Zee, F. P. & Villaverde, S. 2005 Combined anaerobic-aerobic treatment of azo dyes – a short review of bioreactor studies. *Water Research* **39** (8), 1425–1440.

First received 7 September 2011; accepted in revised form 25 November 2011