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Microbial community shifts in sequencing batch reactors for azo dye treatment*

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Abstract: Microbial community structures in sequencing batch reactors (SBRs) for azo dye wastewater treatment were analyzed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). Effects of dye concentration and salinity were investigated for the decolorization of both reactive brilliant red K-2G and KE-3B. The results showed that the microbial community exhibited high color removal efficiency with high concentration of K-2G (1100 mg/l) and moderate KE-3B (400 mg/l), respectively. Under high-salt conditions, decolorization of KE-3B was partially inhibited, while little effect was observed on decolorization of K-2G. The results of similarity analysis demonstrated that the DGGE banding patterns of microbial communities in K-2G and KE-3B decolorization systems were clustered into two independent groups. And it was suggested that the microbial diversities shifted proportionally with the decolorization rates for both dyes. The similarity index (Cs) of the bacterial communities under higher-salt conditions was more than those cultured with lower salinity. It was also found that microbial diversities turned out to be more abundant for both dyes with the increase of NaCl concentration. These results suggested that not only the stability but also the adequate dynamics and diversity of the microbial community structure are important for the stable performance of the SBRs treating hyper-salinity azo dye wastewater.

Keywords: azo dyes; decolorization; microbial communities; polymerase chain reaction-denaturing gradient gel electrophoresis; similarity analysis.

INTRODUCTION

With the increasing demand of textile products, printing and dyeing wastewaters have been increasing rapidly, which becomes one of the main sources of severe pollution problems worldwide [1]. Biological treatment of azo dyes is considered as an environment-friendly and cost-competitive method compared with physical and chemical decomposition process [2]. Until now, it has been reported that various microorganisms such as fungi, bacteria, yeast, and algae possess the potential to decolorize or even mineralize azo dyes [3–5]. In fact, bacterium is the most widely studied and used on azo dye decolorization due to its widespread high growth rate and adaptability. However, the efficiency and stability of individual strain could not meet the requirements of field application for variable and harsh component

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in the wastewater [6]. Acclimatized microbial community is more appropriate for biological treatment of azo dye wastewater.

Wastewater from textile and other dye-stuff industries usually contained significant amounts of synthetic dyes and salt [7,8]. Moreover, a large increase of dye concentration and salinity would result in temporary or even permanent failure in techniques during the biological treatment of azo dye wastewater [9,10]. Therefore, in order to develop an efficient and stable biological treatment process, the effects of these factors should be investigated in detail. With the rapid development of molecular techniques, it is possible to monitor the complex microbial system and determine the relationship between microbial-population dynamic shifts and systematic functions [11]. 16S rDNA-based molecular techniques provide a valuable tool for the characterization of the microbial population diversity in biological wastewater treatment systems. For instance, molecular analysis of activated sludge had revealed the effects of the feed strength and operating conditions on the population structure of the biomass [12,13]. However, the dynamics of microbial evolution during the biological treatment process of azo dye wastewater under different organic-loading and salinity conditions remain to be investigated.

The objective of this study is to evaluate the microbial community dynamics in sequencing batch reactors (SBRs) for decolorization of two different azo dyes (reactive brilliant red K-2G and KE-3B) treatment using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) under different initial dye concentration and salinities.

MATERIALS AND METHODS

Dyes and chemicals

Azo dyes reactive brilliant red K-2G and KE-3B (Fig. 1) used in this study were purchased from Dye Synthesize Laboratory, Dalian University of Technology. All other reagents were analytical grade. Biochemical reagents were purchased from TaKaRa Biotechnology Co., Ltd., Dalian, China.

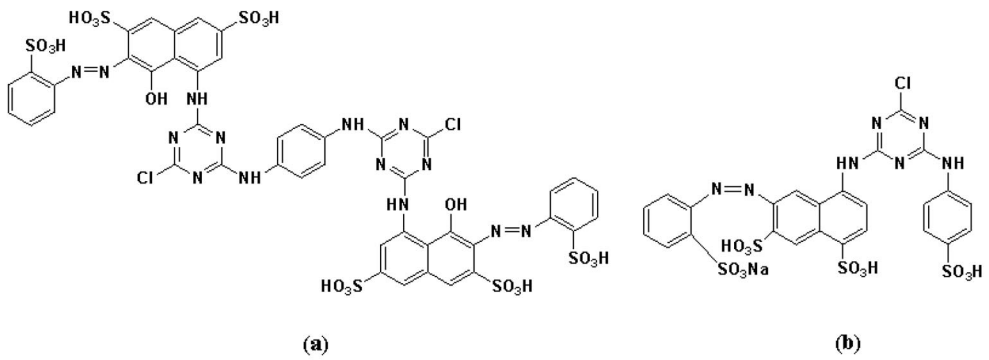

Fig. 1 Chemical structures of (a) reactive brilliant red K-2G and (b) KE-3B.

Indigenous microbial populations and medium

The microbial populations were obtained from the surface soil of a pharmaceutical factory in Shulan, Jilin, China. Before inoculation, soil was filtrated with a 30-mesh screen and mixed adequately. The medium culture was (per liter): 4.0 g peptone, 1.0 g NH_4Cl, 1.0 g $NaHCO_3$, 0.2 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, and 15.0–150.0 g NaCl, pH 6.5.

SBR operation

SBR operation for azo dye decolorization was simulated in 125-ml rubber-stopped serum bottles. To each serum bottle was added 15 g pretreated soil, and then the bottle was fully filled with medium containing certain concentration of dye and salt and then plugged tightly with rubber plug to prevent possible contamination by oxygen. To evaluate the efficiency of decolorization, the batch decolorization experiments were performed at different initial dye concentrations (K-2G 500–1100 mg/l and KE-3B 200–800 mg/l) and salinity (15–150 mg/l). Other incubation conditions were: 30 °C, pH 6.5, static-incubation.

Genomic DNA extraction and PCR

The genomic DNA of the soil samples was extracted by the method described previously [14]. PCR was carried out to amplify a variable region (V3 region) of the bacterial 16S rDNA gene with the GC-clamp forward primer GC341f (5'-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GCC TAC GGG AGG CAG CAG-3') and the reverse primer 519r (5'-ATT ACC GCG GCT GCT GG-3'). Amplification was done in a standard reaction mixture: 25 µl containing 2.5 µl 10 × PCR buffer (100 mmol/l Tris, 15 mmol/l $MgCl_2$, 500 mmol/l KCl, 1 % Triton); 2 µl standard dNTP mixture; 0.5 µl rTaq polymerase (250 U); 1.0 µl of each primer (0.02 mmol/l) and DNA template; and 17 µl sterile deionized water. The PCR was run with an initial denaturation at 94 °C for 4 min, and 20 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C (first 10 cycles), 55 °C (last 10 cycles) for 30 s, and extension at 72 °C for 2 min, with a final extension at 72 °C for 8 min, and at last remaining at 10 °C. The reaction was conducted in a PCR thermal cycler Dice (Bio-Rad Ltd., Tokyo, Japan).

DGGE analysis

The GC-clamp PCR products were separated according to their sequences with a DCode Universal Mutation System (Bio-Rad Ltd., Tokyo, Japan). The samples were applied onto 8 % (w/v) polyacrylamide gels in a running TE buffer containing 20 mmol/l Tris-acetate and 0.5 mmol/l EDTA-2Na (pH 8.5). The sample volumes were varied from 20 to 40 µl according to fluorescent intensities determined by Goldvicwnal (BioDev Ltd., Beijing, China) staining. The gels were prepared with a denaturing gradient from 30 to 65 % of urea and formamide (7 mol/l urea and 40 % formamide as 100 % denaturants). Electrophoresis was conducted at a constant voltage of 200 V for 5 h at 60 °C. After electrophoresis, the gels were stained with about 120 ml 0.5 × Tris-acetate EDTA-2Na (TE) buffer containing 12 µl GeneFinder (BIO-V Ltd., Xiamen, China) and photographed by a gel imaging instrument (Bio-Rad Ltd., Tokyo, Japan). The profiles (gel image) were transferred and preserved to the connected computer and then analyzed by the attached software "Quantity One".

RESULTS AND DISCUSSION

Performance of SBRs for azo dye decolorization

The effects of dye concentration on color removal for both K-2G (500–1100 mg/l) and KE-3B (200–800 mg/l) were shown in Fig. 2. Nearly 100 % of 1100 mg/l K-2G (Fig. 2a) could be removed within 12 h, and the maximal decolorization rate was about 2185.38 mg/(l•d). As for KE-3B (Fig. 2b), complete color removal was observed after 12 h when the initial concentration was less than 400 mg/l and the maximal decolorization rate was only 392.05 mg/(l•d). When the initial concentration of KE-3B was increased, the color removal rates decreased rapidly. The results suggested that the acclimatized microbial consortium was highly promising for applications involving biodecolorization of high concentration of K-2G. It was reported that charged groups approximate to azo bond [e.g., 1-(2'-sulfophenylazo)-2-naphthol] could significantly hinder the decolorization efficiency and biodecolorization

efficiency would be decreased with the increase of substituent [9]. It was shown that there were two azo linkages and two sulfonic groups in KE-3B, and one hydroxy group charged in the proximity of each azo linkage. As for K-2G, the molecule contained only one azo linkage and two sulfonic groups (Fig. 1). Therefore, K-2G should be more easily decolorized than KE-3B for the view of the molecular structure characteristics.

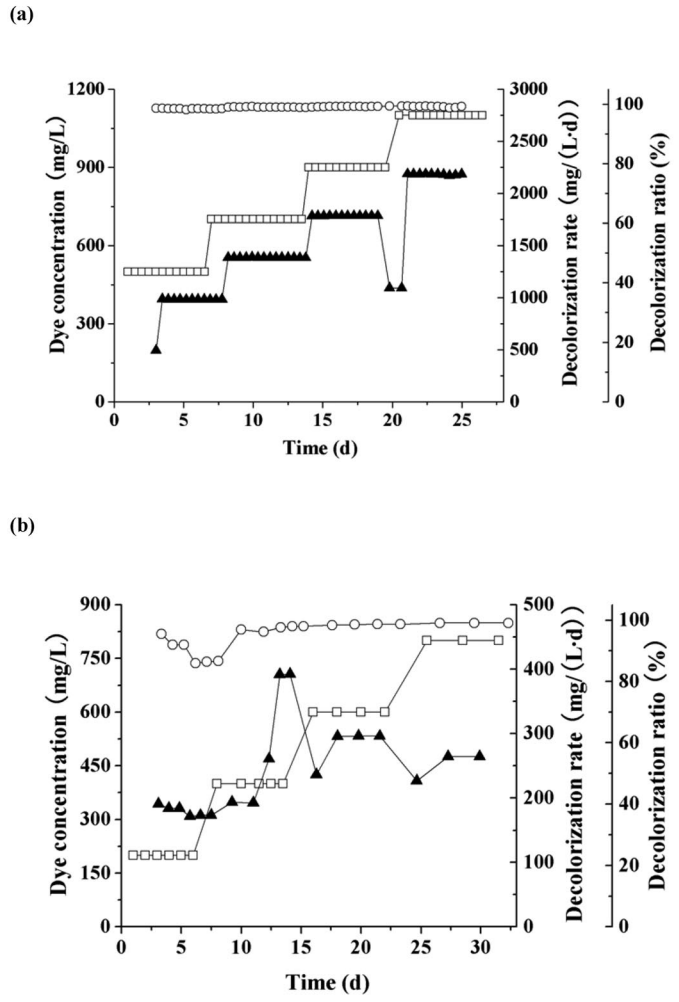

Fig. 2 Decolorization process with different initial concentrations of (a) K-2G and (b) KE-3B and 1.5 % (w/v) salinity. Symbols: (□) initial dye concentration; (○) decolorization ratio; (▲) decolorization rate; (△) initial salinity.

Shifts of microbial community structures with different azo dyes

DGGE banding patterns in Fig. 3 displayed the influence of initial concentration for both dyes. Degrees of similarity between populations were summarized by a dendrogram constructed using the unweighted pair-group method with arithmetic mean (UPGMA). It was shown that the DGGE patterns of the microbial communities for K-2G were distinct from those for KE-3B. And the Dice index of similarity analysis (Cs) between the two separate groups is 0.53. The microbial diversity (band number) increased gradually with the initial K-2G concentration increased (Fig. 3, lanes S1–S4). However, there were few

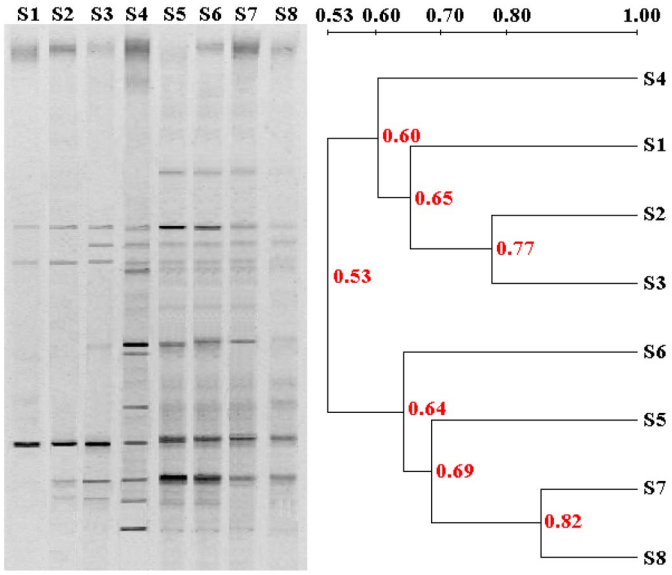

Fig. 3 DGGE fingerprints and dendrogram of samples under 1.5 % (w/v) salinity and different initial dye concentration: (S1–S4) 500, 700, 900, 1100 mg/l K-2G; (S5–S8) 200, 400, 600, 800 mg/l KE-3B.

changes observed from the DGGE banding patterns of the first three samples for decolorization of 200, 400, and 600 mg/l KE-3B, and the microbial diversity decreased with further increase of initial KE-3B concentration (Fig. 3, lanes S5–S8). Furthermore, the microbial community diversity was abundant when the initial KE-3B concentration was 400 mg/l, but the diversity seemed relatively low with 800 mg/l of the dye. As mentioned above, the most and least decolorization rates were obtained when initial KE-3B concentrations were 400 and 800 mg/l, respectively (Fig. 2b). Therefore, it could be concluded that the efficiency of the microbial community probably corresponds to the microbial diversity. The results here were consistent with the previous reports about the simultaneous change of organic removal loading and microbial diversity [15,16].

Color removal under different salinity conditions

Effects of salinity on decolorization of both dyes were also studied. As shown in Fig. 4, both microbial communities could keep high decolorization efficiency with 50 g/l NaCl. When salt concentration was further increased to 100–150 g/l, the decolorization rate of K-2G was not decreased (Fig. 4a). However, the color removal rates of KE-3B decreased rapidly under the same higher-salt conditions (Fig. 4b). It was suggested that high salinity could cause different effects on decolorization of the two dyes. The KE-3B decolorization consortium was more sensitive to high concentration of salt. As previous reported, hyper-salinity wastewater usually caused plasmolysis and/or loss of activity of cells and brings negative impacts to the aerobic or anaerobic biological treatment techniques [10]. However, some special microorganisms could keep high efficiency under high-salinity conditions because they had a prominent capacity to produce and accumulate some substance to resist exterior pressure [17]. This type of bacteria could be obtained through cultivation under high-salt conditions for a period of time [18,19]. In addition, though some bacteria could survive under high-salt conditions, their metabolic activity would be inhibited. It was concluded that the microbial community for decolorization of K-2G exhibited higher stability and efficiency under high-salt conditions than the dominant degraders for KE-3B.

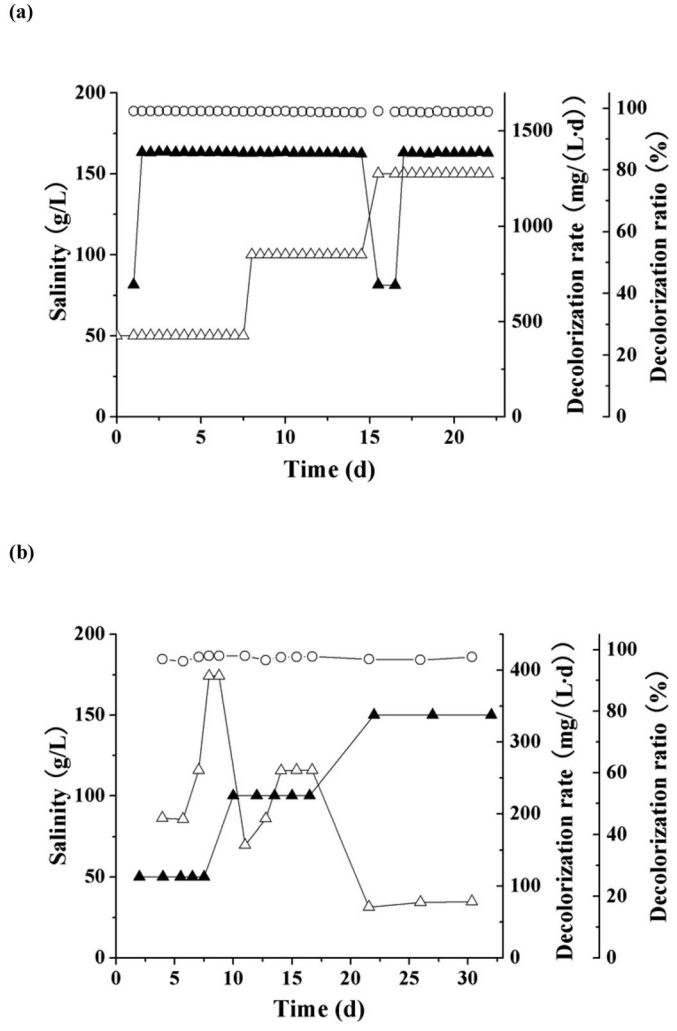

Fig. 4 Decolorization processes of (a) K-2G and (b) KE-3B with different salinities. Symbols: (○) decolorization ratio; (▲) decolorization rate; (△) initial salinity.

Dynamics of microbial communities under different salinity conditions

The effects of salinity on microbial community structure were shown in Fig. 5. The DGGE banding patterns of the samples under higher-salt conditions (100–150 g/l NaCl) were more abundant than that with relative low salinity (50 g/l NaCl) for both dye solutions. It could be explained that under lower-salt conditions, azo dye was fit for the growth of a few dominant species and played a major role on the microbial community structure. However, when the salinity was increased, it would become one of the major factors and some salt-tolerant and halophilic species would be enriched during the acclimatization. Salt-tolerant bacteria could survive and/or keep high efficiency under high-salt conditions. Meanwhile, they could also survive under lower-salt conditions. However, it was indicated that halophilic bacteria would show the highest growth and metabolic activities with given salinity. In other words, the performances would not be affected under either higher- or lower-salinity conditions [20]. Therefore, it was suggested that four members marked with the arrows in Fig. 5 were probably moderate halophilic bacteria because they were absolutely absent under lower-salt conditions, and they ap-

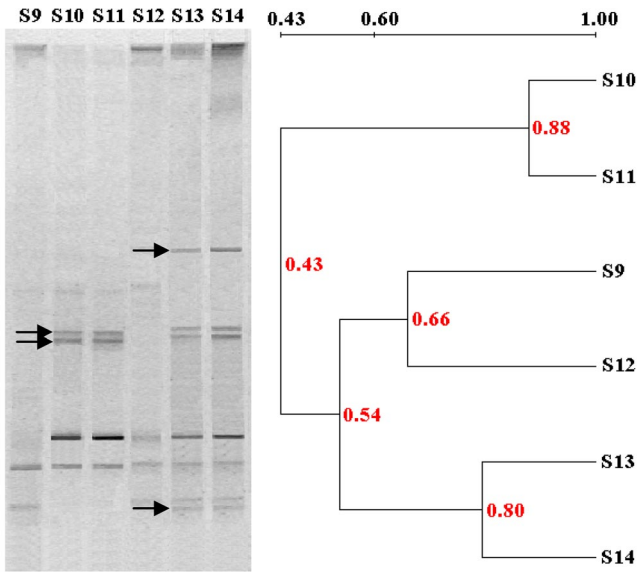

Fig. 5 DGGE fingerprints and dendrogram of samples under different initial salinities: (S9–S11) 5, 10, 15 % (w/v) salinity containing 700 mg/l K-2G; (S12–S14) 5, 10, 15 % (w/v) salinity containing 400 mg/l KE-3B.

peared when salinity was increased to moderate concentration. The other bands were present in all the samples and gradually changed in band intensity, which indicated that they might be salt-tolerant bacteria. In addition, one band at the bottom of lane S9 disappeared when NaCl concentration was increased to higher than 100 g/l. It was probably due to the inhibition of high-salt concentration. The similarity for S10/S11 and S13/S14 was as relatively high as 0.88 and 0.80, respectively. It was suggested that the effects of high-salt concentration on microbial diversity was more than that of lower salinity.

CONCLUSIONS

In conclusion, the adaptation of a microbial population to degrade toxic or recalcitrant compounds is very useful to improve the rate of decolorization process. Microbial consortium was obtained from soils and acclimatized to high concentration of azo dyes K-2G and KE-3B for about 30 days. The results showed that the microbial consortium could effectively decolorize both dyes. And the microbial consortium was also salt-tolerant, that the activity could not be affected even with 150 g/l NaCl for decolorization of K-2G. However, as for KE-3B degraders, the efficiency decreased when salt concentration was higher than 50 g/l NaCl. PCR-DGGE analysis showed that microbial diversity was approximately proportional to dye decolorization rate. And under moderate-salt conditions, some halophilic bacteria were enriched after cultivation for a period of time. These results suggested that adequate dynamics and diversity of the microbial community were important factors for the stable performance of azo dye decolorization under high organic loading and high-salinity conditions.

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