International Biodeterioration & Biodegradation 85 (2013) 210–216

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Short communication

Aerobic decolorization and mineralization of azo dyes by a microbial community in the absence of an external carbon source

Liang Tan [a,*], Shuxiang Ning [a], Houzhen Xia [a], Jian Sun [b]

[a] School of Life Science, Liaoning Normal University, Dalian 116081, China
[b] Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

ARTICLE INFO

Article history:
Received 7 February 2013
Received in revised form
20 February 2013
Accepted 21 February 2013
Available online

Keywords:
Aerobic decolorization
Mineralization
Azo dyes
Microbial community
External carbon source

ABSTRACT

In this research, aerobic decolorization and mineralization of azo dyes by a microbial community in the absence of an external carbon source was studied. Effects of conditions on decolorization and mineralization of Reactive Red 3BS were investigated and a possible pathway of decolorization was proposed. Additionally, continuous treatment of Reactive Red 3BS by the consortium was evaluated and the microbial community structures were analyzed by polymerase chain reaction followed by denaturing gradient gel electrophoresis (PCR-DGGE). The results showed that the microbial community could decolorize and partially mineralize six azo dyes (50 mg l^{-1}) in the absence of an external carbon source. The optimal conditions were as follows: inoculation size, 3.81 g l^{-1} (wet cell pellet); rotation speed, 150 rpm; pH, 5.0–7.0; and temperature, 30 °C. Furthermore, the consortium exhibited high levels of stability and adaptability during continuous treatment processes. The community structure was relatively stable with the fluctuation of organic load and the dominant microorganisms were mainly affiliated with six different classes: α-, β-, and γ-proteobacteria, *Bacilli*, *Cytophagia*, and *Nitrospirales*.

1. Introduction

Azo dyes are the largest group of dyes, constituting up to 70% of annual dye production; they are widely used in the textile, pharmaceutical, cosmetics, and food industries (Tony et al., 2009). It has been estimated that about 2% dyes are lost during their manufacture and approximately 10–15% of dyes are released into the environment during dyeing processes (Pearce et al., 2003; Işik and Sponza, 2004). They are generally designed to be chemically and photolytically stable and hence are highly persistent in natural environments (Pandey et al., 2007). Industrial wastewaters containing azo dyes have caused serious environmental pollution because of their high chromaticity and toxicity to aquatic organisms (Supaka et al., 2004). Therefore, removal of azo dyes from industrial wastewaters has been a big challenge. In this case, the application of chemical or biotechnological processes, not only for color removal but also for the complete mineralization of azo dyes, is being continually developed (dos Santos et al., 2007).

Some chemical or photochemical methods, such as advanced oxidation processes (AOPs), are potentially useful for treatment of industrial wastewater containing azo dyes (Shu and Chang, 2005; García-Montaño et al., 2006). However, they have not been widely used because of the high costs associated with reagents and energy consumption. By contrast, biological methods have been seen as better choices because they are more cost-effective and environmentally friendly. Degradation of azo dyes can be achieved under anaerobic (methanogenic), anoxic, or aerobic conditions by different microorganisms. It was once considered that azo dyes were generally resistant to bacterial attack under aerobic conditions; nevertheless, some strains have been isolated that reduce the azo linkage through the catalysis of oxygen-insensitive azoreductases, even using some released byproducts as growth substrates (Nachiyar and Rajakumar, 2005; Ooi et al., 2007; Pandey et al., 2007; Priya et al., 2011). On the other hand, most decolorization intermediates, such as sulfonated aromatic amines, are still difficult to degrade by a single bacterium. Thus, specialized aerobic microbial consortia are required for their mineralization (Feigel and Knackmus, 1993; Noisommit-Rizzi et al., 1996). Considering the recalcitrance of azo dyes, the use of sequential anoxic/aerobic treatment systems or the addition of co-substrates for complete degradation have been suggested (Pandey et al., 2007; dos Santos et al., 2007; Franciscon et al., 2009). However, for simple and economic bioprocesses, both suggestions can be avoided by using selected microbial strains that are capable of utilizing azo compounds as a carbon source and energy.

* Corresponding author. Tel./fax: +86 411 85827068.
E-mail addresses: tanliang19811210@yahoo.cn, tanliang19811210@163.com, tanl_lnnu@yahoo.com.cn (L. Tan).

0964-8305/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ibiod.2013.02.018

In this study, a microbial community was acclimatized to decolorize and mineralize azo dyes under aerobic conditions in the absence of an external carbon source. The effects of initial dye concentration and other parameters (including inoculation size, rotation speed, pH, and temperature) on decolorization and mineralization of Reactive Red 3BS by the consortium were further investigated. In addition, continuous decolorization and mineralization of Reactive Red 3BS were also studied through sequencing batch tests, and the microbial community structures were analyzed by polymerase chain reaction followed by denaturing gradient gel electrophoresis (PCR-DGGE).

2. Materials and methods

2.1. Reagents

Azo dyes used in this study were purchased from the Dye Synthesis Laboratory, Dalian University of Technology, Dalian, China. They (and their characteristic absorption wavelengths) were Reactive Red 3BS (540 nm), Reactive Violet KN-4R (680 nm), Acid Brilliant Scarlet GR (511 nm), Acid Red B (516 nm), and Acid Orange II (484 nm). Biochemical reagents were purchased from TaKaRa Biotechnology Co., Ltd., Dalian, China. Other chemical reagents are analytical grade.

2.2. Preparation of microbial cell pellets

The microbial community was collected from the sea mud of a beach that was close to an industrial harbor zone (38.98° N, 121.89° E) of Dalian, China. The sea mud was first pretreated by filtration with a 100-mesh screen for removing gravel. Second, 50 ml of pretreated sea mud, 50 ml of phosphate buffer solution (pH = 7.0), and 10 g of autoclaved quartz sand were added in a 250-ml flask for separation of microbial cell pellets from the mud. After shaking at 150 rpm for 1 h and static settling for 30 min, the sediment was removed and the suspension was centrifuged at 8000 rpm for 10 min. Then, the centrifugation sediment was preserved and washed with phosphate buffer solution (pH = 7.0) twice and resuspended in the same solution for inoculation.

2.3. Analytical methods

The concentration of azo dyes in supernatant was analyzed using a V-560 UV–Vis scanning spectrophotometer (JASCO Co., Ltd., Japan) after centrifugation (8000 rpm) for 10 min. The weight of sediment divided by the volume of bacterial suspension was the concentration of wet cell pellet (Tan et al., 2012). Total organic carbon (TOC) was analyzed using a TOC-5000 total organic carbon analyzer (Shimadzu Co., Ltd., Japan) to measure the mineralization degree of dyes. All the analytical experiments were done in replicates (four times) and the significance of data was analyzed with the *t*-test method (confidence level of 95%). The average values with standard deviations were plotted.

2.4. Decolorization experiments

Decolorization experiments were performed in 250-ml flasks with 100 ml of medium. The medium for decolorization contained (in grams per liter): 0.25 $(NH_4)_2SO_4$, 0.20 KH_2PO_4, 0.10 $MgSO_4 \cdot 7H_2O$, and 0.02 $CaCl_2$. After adjustment to pH 6.0, corresponding azo dye was added and the culture medium was autoclaved at 121 °C for 20 min before inoculation. Decolorization of azo dyes was investigated under these conditions: 30 °C, 150 rpm, initial dye concentration of 50 mg l^{-1}, and inoculation size of 3.81 g l^{-1} (wet cell pellet). Meanwhile, control experiments that were inoculated with autoclaved cell pellets were performed to investigate the manner of decolorization. Both dye concentration and TOC were monitored during the decolorization processes.

2.5. Effects of different parameters on decolorization and mineralization of Reactive Red 3BS

Reactive Red 3BS was chosen as the target dye for further investigation. Effects of different parameters on decolorization were investigated through batch tests; these parameters included initial dye concentration (50–200 mg l^{-1}), inoculation size (1.27–6.35 g l^{-1} of wet cell pellet), rotation speed (0–200 rpm), pH (3.0–11.0), and temperature (15–45 °C). The time for one batch test was 24–48 h.

2.6. Continuous decolorization of Reactive Red 3BS by the consortium

The performance of the microbial community for continuous decolorization of Reactive Red 3BS was investigated through sequencing batch tests in flasks. The influent dye concentration was 50 mg l^{-1} at the first stage of six cycles (24 h each cycle), and then was increased to 100 mg l^{-1} at the second stage of another six cycles (36 h each). To avoid the loss of biomass with effluent, centrifugation was used for liquid–solid separation instead of immobilization of cells or membrane separation. The liquid phase was separated from the system through centrifugation and removed at the end of each cycle, and fresh medium containing the same initial concentration of the dye was supplied and mixed well with the solid phase for the next cycle of treatment. At the end of each stage, microbial samples were preserved at −80 °C for further microbial community analysis.

2.7. Microbial community analysis with PCR-DGGE

Total DNA of the microbial samples was extracted and purified using the method described by Qu et al. (2009). GM341F-GC (forward primer) and DS907R (reverse primer) were chosen as the universal PCR primers of bacteria following Teske et al. (1996). About 500 bp of the16S rDNA fragment were amplified using a PCR thermal cycler Dice (BioRad Co., Ltd., USA) with a "touchdown" method. The PCR products were separated by DGGE, which was performed with a BioRad Dcode system (BioRad Co., Ltd., USA), and the denaturing gradient ranged from 40 to 55% (100% corresponds to 7 mmol l^{-1} urea and 40% (v/v) formamide). Other electrophoresis conditions were: temperature 60 °C, voltage 200 V, and time 5 h. The gel was stained with "GeneFinder" gene stain (BIO-V Co., Ltd., Xiamen, China) at 10,000-fold dilution, and the image was captured using a gel imaging instrument (BioRad Co., Ltd., USA). The PCR and DGGE experiments were done in replicates.

The DGGE profiles were analyzed with "Quantity One" for cluster analysis using the UPGMA method. Then dominant DGGE bands were excised and re-amplified by PCR with the primers GM341F and DS907R. The sequencing of PCR products was performed by TaKaRa Biotechnology Co., Ltd. (Dalian, China), and the results were identified using the GenBank database BLAST program (Benson et al., 2002).

3. Results and discussion

3.1. Aerobic decolorization and mineralization of azo dyes by the microbial community

Azo dyes were once known as recalcitrant compounds that could not be utilized as carbon sources or energy by microorganisms. As a