International Biodeterioration & Biodegradation 125 (2017) 86–96

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Biodegradation of aniline by a novel bacterial mixed culture AC

Daizong Cui, Da Shen, Congrong Wu, Cong Li, Dongjin Leng, Min Zhao*

College of Life Science, Northeast Forestry University, Harbin, 150040, China

ARTICLE INFO

Article history:
Received 13 July 2016
Received in revised form
6 July 2017
Accepted 22 August 2017
Available online 7 September 2017

Keywords:
Bacterial mixed culture
Aniline
Degradation
Microbial community
Cell immobilization

ABSTRACT

In this study, a novel bacterial mixed culture AC, capable of degrading aniline, was developed by us. The mixed culture presented better aniline degradation abilities comparing with different pure strains. Aniline at high concentrations could be tolerated and degraded by the mixed culture. An almost complete degradation of aniline at the concentration of 1500 mg L^{-1} was observed within 60 h. To provide further insight into the microbial diversity of the bacterial mixed culture, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and high-throughput sequencing analyses were performed. PCR-DGGE profiles revealed that the microbial community had changed with varying initial concentrations of aniline. The taxonomic classification of the bacterial communities showed that the acclimated mixed culture was mainly composed of *Serratia*, *Escherichia/Shigella*, *Bacillus* and *Acinetobacter*. The mixed culture was immobilized by entrapment in different polumeric matrices. The immobilized bead exhibited good activity for aniline degradation and kept stable during successive repeated experiments. The effects of physico-chemical parameters on the aniline degradation were determined. Comparing with the free cells, the immobilized bead exhibited better degradation ability in wide range of pH, temperature and salinity. HPLC analysis showed that the mixed culture could convert aniline to catechol, and then catechol was further biodegraded to *cis, cis*-muconic acid. Ames test showed that the metabolic transformation products of aniline had no inherent toxicity. All these results showed that the mixed culture had a great potential to be used in the treatment of wastewater containing aniline.

1. Introduction

Aniline is an important synthetic intermediate which is widely used in the synthesis of various chemical products such as dyes, drugs, pesticides, explosives, and herbicides (O'Neill et al., 2000). The manufacture, transport and use of aniline have generated a serious disposal problem. It has therefore been released into the environment through the discharge of industrial wastewater, urban sewage, and agricultural runoff (Zhu et al., 2012). Unfortunately, this chemical exhibits significant toxic, carcinogenic and mutagenic effects on both wildlife and humans. Moreover, it can persist in nature for a long time and can further accumulate in living organisms. Considering all these, aniline was classified as a persistent organic pollutant by the USA and China (Wang et al., 2007).

Many physicochemical methods have been adopted to eliminate organic pollutants, such as adsorption, filtration, coagulation, precipitation and chemical oxidation. However, they have not been widely applied because they have low efficiency, high costs and intensive energy requirements (Elisangela et al., 2009). Biodegradation is considered as an economic, efficient and environmental-friendly alternative and has drawn an increasing interest in recent decades (Tan et al., 2009).

In recent years, some researchers had studied on the biodegradation of aniline and a few species of aniline biodegrading bacteria, including *Pseudomonas*, *Moraxella*, *Comamonas*, *Rhodococcus*, *Frateuria* and *Nocardia*, were successfully isolated from environment (Bachofer et al., 1975; Zeyer et al., 1985; Boon et al., 2000; Murakami et al., 2003; Zhuang et al., 2007; Tanaka et al., 2009). As for the biodegradation pathway of aniline, it had shown that aniline could firstly be converted to catechol using aniline dioxygenase under aerobic conditions, and then catechol would be further biodegraded to cis,cis-muconic acid with catechol 1,2-dioxygenase catalyst (the ortho-cleavage pathway) or to 2-hydroxymuconic semialdehyde with catechol 2,3-dioxygenase catalyst (the meta-cleavage pathway) (Aoki et al., 1984).

It was widely accepted that the treatment systems with mixed microbial populations were more effective to degrade pollutants than that of pure cultures (Khehra et al., 2005). Therefore, it is

* Corresponding author.
E-mail address: 82191513@163.com (M. Zhao).

http://dx.doi.org/10.1016/j.ibiod.2017.08.010
0964-8305/

preferable to utilize mixed cultures in real wastewater treatment plant. The mixed cultures have stronger degradation ability and can utilize wider ranges of substrates than pure strain does. Moreover, the complementary role of the different bacterial isolates in the mixed cultures might lead to an increase in degradation efficiency. In spite of the importance of the mixed cultures for pollutants treatment, the information on microbial population structures and dynamic changes in aniline degradation systems has been quite limited. The performance of pollutants treatment largely depends on the bacterial diversity presented in the mixed degradation system (Saikaly et al., 2005). Therefore, the understanding of the microbial community structure and its response to different concentrations of pollutants entering the wastewater are desirable for stable and efficient wastewater treatment.

Immobilization is a method that increases the stability and ensures there reusability of the cells (Sheldon, 2007). In recent years, immobilized cell systems have valuable application in the degradation of variety of toxic organics, such as azo dyes, phenol, quinoline and naphthalene (Manohar and Karegoudar, 1998; Stolz, 2001; Wang et al., 2001; Karigar et al., 2006).

In this study, we enriched a mixed culture AC, which could degrade aniline under aerobic conditions. Degradation text showed that it had much better efficiency to degrade aniline than pure strains. The microbial community diversity of the mixed culture was evaluated by PCR-DGGE and high-throughput sequencing analysis. To obtain more details about the aerobic degradation process, the degradation characteristics of the free mixed culture and the immobilization beads were studied. HPLC method was used to determine the intermediate products after aniline degradation. Moreover, the mutagenic potential of aniline and its metabolites was determined by Ames test. All these results showed that the mixed culture AC had a high ability to degrade aniline and had a great potential for use in the treatment of effluents containing aniline.

2. Materials and methods

2.1. Chemicals

Aniline used in the study was of industrial grade and was procured from the Guangfu Fine Chemical Research Institute (Tianjin, China). The standards of aniline, catechol and *cis, cis*-muconic acid were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). The rTaq DNA polymerase, dNTPS, PCR purification kit, DNA extraction kit, pMD-18T vector and *E. coli* JM109 strain were purchased from TaKaRa (Dalian, China). *Salmonella typhimurium* tester strains TA97 and TA98, the S9 mix were obtained from iPHASE Biosciences (Beijing, China). All of the other chemicals used were of analytical grade or the highest quality available.

2.2. The degradation medium

The mixed culture was grown in the degradation medium containing the following ingredients in 1 L of distilled water: glucose 2.0 g, NH_4Cl 1 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, KH_2PO_4 0.4 g, K_2HPO_4 0.1 g, $Fe_2(SO_4)_3 \cdot H_2O$ 0.01 g, $MnSO_4 \cdot H_2O$ 0.01 g.

2.3. Mixed culture enrichment

The mixed culture for aniline degradation was enriched from the soil of a chemical industry corporation explosion site in Jilin, China. The sub-surface (10 cm below) soil (2 g) was inoculated into a 100 mL flask containing 50 mL distilled water and shaken (at 120 r/min) for 3 h. 1 mL of the supernatant was inoculated to 50 mL LB culture medium (pH 7.0) and incubated at 37 °C at 120 r/min for 12 h, the mixed culture was withdrawn and used as the enrichment culture. Then 1 mL of cultures was transferred to 50 mL of the degradation culture medium containing 100 mg L^{-1} aniline and incubated at 37 °C at 120 r/min for 36 h. Repeated transfers were performed using fresh media with different initial aniline concentrations (100–1000 mg L^{-1} aniline, increasing 100 mg L^{-1} of aniline for each transfer). The microbial sample for continuously degrading 1000 mg L^{-1} aniline was used as the acclimated culture.

2.4. Strain isolation and identification

To isolate the pure strains from the degradation mixed culture, the LB plates containing 100 mg L^{-1} of aniline were inoculated with the degradation mixed culture. The different isolates with high potential for aniline degradation were isolated and used for further study.

Identification of the isolated strains was performed using 16S rDNA gene sequence analysis. The genomic DNA extraction of the isolated strains was carried out as described previously (Cui et al., 2012). The 16S rDNA gene was amplified by PCR using the specific primers 27f (5′-GAGTTTGATCMTGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′). The PCR amplification used the following protocol: initial denaturation of DNA for 3 min at 94 °C; 30 cycles of 1 min at 94 °C, 1 min at 56 °C and 1 min at 72 °C, followed by a final extension for 10 min at 72 °C. The amplified product was purified and was cloned into the pMD18-T vector. The DNA sequences were determined using the chain-termination method on an ABI 3730 DNA sequencer by a commercial service (Sangon, China).

2.5. Effects of co-substrate on aniline biodegradation

We evaluated several co-substrates to determine if other carbon sources could enhance aniline degradation efficiency. Each 100 mL flask was supplemented with 200 mg L^{-1} aniline, 50 mL degradation medium, and 1 mL of mixed culture with different co-substrates such as 0.2% glucose, 0.2% sucrose, 0.2% mannitol, 0.2% starch, 0.2% lactose, 0.2% peptone and 0.2% yeast powder. Each flask was incubated at 37 °C with shaking. Samples were withdrawn for analysis of residual aniline after 16 h.

2.6. PCR-DGGE analysis

2.6.1. PCR amplification of the 16S rDNA V3 region

For bacterial DGGE analysis, the V3 region of the 16S rRNA gene was amplified using the forward primers F357-GC (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTAC-GGGAGGCAGCAG-3′) and reverse primer R518 (5′-ATTAC-CGCGGCTGCTGG-3′) under the PCR conditions of initial denaturation of DNA for 5 min at 94 °C; 20 cycles of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C; 15 cycles of 1 min at 94 °C, 1 min at 50 °C and 1 min at 72 °C followed by a final extension for 10 min at 72 °C. The PCR products were electrophoresed in 1% (w/v) agarose gel and were run in 1 × TAE buffer to evaluate the extent of amplification.

2.6.2. DGGE analysis

DGGE was performed using the Dcode universal mutation detection system (Bio-Red Co., Ltd., Hercules, CA, USA). The PCR products were electrophoresed in a 10% poly-acrylamide gel in 1 × TAE buffer. The denaturing gradient ranged from 40% to 70% (100% is 7 mmol L^{-1} urea and 40% (v/v) formamide). Electrophoresis was run at 60 °C with a constant voltage of 160 V for 6 h. The gel was stained with $AgNO_3$, and an image of the gel was captured using a bio-imaging system (Bio-Red Co., Ltd., Hercules, CA, USA). DGGE profiles were analyzed using the software Quantity One