International Biodeterioration & Biodegradation 93 (2014) 138–144

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Treatment of high salinity phenol-laden wastewater using a sequencing batch reactor containing halophilic bacterial community

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ARTICLE INFO

Article history:
Received 22 February 2014
Received in revised form
28 March 2014
Accepted 16 April 2014
Available online 13 June 2014

Keywords:
Phenol-laden wastewater
High-salinity
Sequencing batch reactor
Moderate halophiles
Biodegradation

ABSTRACT

Sequencing batch reactors (SBRs) were used to degrade phenol compounds efficiently; however, most investigations on such reactors have focused on fresh rather than saline wastewaters, even though saline effluents containing phenolic compounds are generated by many industries. This study assessed the performance of the aerobic SBR process in the removal of phenol as the sole substrate under conditions of high salinity. A flexible concentration of phenol could be completely degraded in SBR process, varied from 400 mg l^{-1} to 1200 mg l^{-1} at the existing of 80 g l^{-1} NaCl. The value of specific oxygen uptake rate (SOUR) suggested that the exiting bacteria could adapt to the phenol and salt conditions well at each stage. Cloning and sequencing of the 16S rRNA showed that the acclimated active sludge included two dominant genera: *Pseudomonas* and *Alcaligenes*. PCR detection of the functional genes suggested that phenol hydroxylase (*Lph*), catechol 1,2-dioxygenase (*C12O*), and catechol 2,3-dioxygenase (*C23O*) were active in the phenol-degradation process. Real-time PCR showed that the phenol-degrading bacteria comprised 63.3% of the total bacterial community.

1. Introduction

Vast amounts of phenol and phenolic compounds are discharged into the environment through effluents from a variety of industries, including the leather processing, textiles, pharmaceutical, phenol-formaldehyde resin, and oil plants industries (Gianfreda et al., 2006). This has become a major focus of efforts directed at environmental and health pollution control. In order to protect the environment against the adverse effects of phenol, wastewaters containing such toxic compounds need to be treated using an effective and environmentally benign process before the wastewater can be discharged into the environment. Among the various types of biological treatment systems, sequencing batch reactors (SBRs) can be used to degrade phenol compounds efficiently, as the SBR confers greater advantages, including flexibility, robustness, single basin operation, better control of shock loads, simplicity of operation and relatively low cost (Shengquan et al., 2008). However, most investigations that have reported on phenol removal have focused on its removal from fresh wastewaters, while few studies have focused on the removal of phenol from saline wastewaters, despite the fact that saline effluents containing phenolic compounds are generated by a number of industries, such as oil refineries, petrochemical, pharmaceutical, and pesticide production plants (Azbar et al., 2004). High salinity can inhibit the effectiveness of aerobic and anaerobic biological treatment of wastewater markedly, even in the SBR.

Salt has numerous effects on conventional biological treatment cultures. Conventional treatment cultures are sensitive to changes in ionic strength, so that increased salt concentrations tend to disrupt normal metabolic functions and reduce degradation kinetics. Increased salinity also decreases the rate of settlement, so that treatment systems invariably have high concentrations of effluent suspended solids, which may be caused by cell lysis and reduction of the populations of protozoa and filamentous organisms required for proper flocculation (Woolard and Irvine, 1995). Thus, the biological removal of organic compounds from hypersaline wastes without dilution requires specialized microbes. The moderate halophiles are organisms that grow optimally in medium containing 3–15% (0.5–2.5 M) NaCl. These organisms are

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http://dx.doi.org/10.1016/j.ibiod.2014.04.010
0964-8305/

predominantly bacteria, like *Deleya*, *Pseudomonas*, *Vibrio*, *Flavobacterium*, and *Alteromonas* spp. isolated from solar salterns, deep-sea sediments, saline soil, and so on (Li et al., 2004; Cabrera et al., 2007). To tolerate the desiccating osmotic forces present in hypersaline environments, moderate halophilic organisms accumulate compatible solutes to balance the ionic strength of the cytoplasm with that of the external environment. The solutes accumulated by moderate halophiles for such osmotic regulation are a mixture of inorganic cations (K^+, Na^+) and organic compounds (sugars, amino acids, polyols, betaine, and ectoines) (Brill et al., 2011). Although not required for osmotic equilibrium, moderate halophiles also have a specific requirement for Na^+, such as nutrient uptake, regulation of cytoplasmic pH, and potential maintenance all require Na^+ (Hamaide et al., 1983; Quinn et al., 2012). Moreover, halophile enzymes are able to function in hypersaline environments because of the unique adaptations in their primary protein structure. In general, proteins of halophilic microbes have an excess of acidic amino acids and few non-polar residues. Excess acidic residues require cations to shield closely spaced negative charges that would otherwise disrupt the protein.

Many studies have reported the degradation of phenol under high salt conditions using moderate halophiles, such as a strain of *Penicillium chrysogenum* that was isolated from samples of soil from the salt mine of Clona in Portugal, which could degrade more than 300 mg l^{-1} phenol as the sole source of carbon and energy (Leitão et al., 2007). A *Halomonas* sp. strain PH2-2 has also been reported to be capable of degrading up to 1100 mg l^{-1} of phenol in media containing 18% NaCl (Haddadi and Shavandi, 2013). However, few reports have focused on the application of moderate halophiles in SBR process with mixed bacterial cultures, as most of these studies have involved either single microbial species or low inlet phenol concentrations; both of which may have limitations in field application due to the presence of different and/or high concentrations of contaminants in the targeted wastewater.

The primary objective of this work was to investigate the biological treatment of high concentrations of phenol in saline wastewater, using a mixed culture in SBR. This study acclimated a moderate halophilic bacterial sludge in the SBR which performed biodegradation well under high salt conditions. The microbial community composition and the metabolism pathway were investigated to illustrate the relationship between degradation and the halophilic bacterial populations, which contributed to develop the bio-treatment for the high salinity phenol-laden wastewater effluent in chemical industry.

2. Materials and methods

2.1. SBR setup, operation, and wastewater composition

In this study, a cylindrical bench-scale reactor, the schematic of which is shown in Fig. 1, was constructed and used for the treatment of saline wastewater containing phenol, under laboratory conditions. The reactor was made from glass and consisted of a 2.5-L total volume column [internal diameter (D) × height (H): 13 cm × 18 cm], 2 L of which served as the working volume (Moussavi et al., 2010). The SBR was operated in cyclic mode, so that each operating cycle comprised filling and aerating, settling, decanting, and idling for 10 h, 12 h, 1.5 h, and 20 min, respectively, for a total time of 24 h. The fill volume of the reactor was kept constant at 1.4 L during the entire course of the experiment, so that 30% of the full volume was replaced with new synthetic wastewater during the filling period in each cycle. The synthetic wastewater in this study contained (g l^{-1}): 80 NaCl, 2.65 KH_2PO_4, 0.5 NH_4Cl, 0.01 $FeCl_2 \cdot 4H_2O$, 0.02 $CaCl_2$, 0.2 $MgSO_4 \cdot 7H_2O$; the concentration of phenol ranged from 400 to 1200 mg l^{-1}.

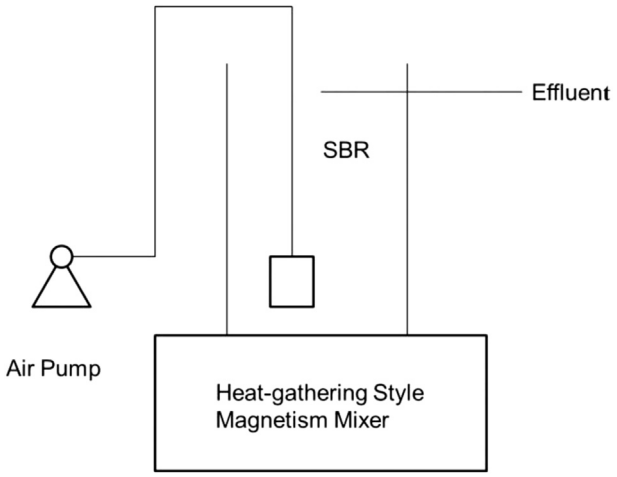

Fig. 1. Schematic representation of the experimental sequence batch reactor (SBR).

2.2. Experimental systems

Samples of fresh activated sludge (AS) were sampled from a biochemical reaction basin of the Sinopec Gaoqiao petrochemical factory, Shanghai, China; these were used as the indigenous populations in the SBR. To obtain regular operation of the SBR in the presence of a NaCl concentration of 80 g l^{-1}, we first operated the SBR in the presence of an NaCl concentration of 30 g l^{-1}; after a period of preliminary domestication, the concentration of NaCl was increased to 50 g l^{-1}. Thereafter, the salt concentration in the SBR was increased to and maintained at 80 g l^{-1} NaCl. The initial concentration of AS was 3200 mg l^{-1} of mixed-liquor suspended solid (MLSS). It was cultured in a basal salts medium with a single carbon compound, phenol, as the sole source of carbon and energy; the concentration of phenol was gradually increased from 400 mg l^{-1} to 1200 mg l^{-1} (Ping et al., 2009).

2.3. Analytical methods

The concentration of phenolic compounds in this study was periodically measured spectrophotometrically. In the decanting phase, a sample was withdrawn from the SBR and filtered through a 0.45-μm filter for subsequent phenol quantification. The colorimetric 4-aminoanitipyrene (4-AAP) technique was used to quantify the phenol content (SEPAC, 2002). Absorbance at 510 nm was determined using a spectrophotometer (UV 1800, Shimadzu). SOUR was detected using a BM-Advance Multi-purpose Respirometer (SURCIS, S.L; Alicante, Spain).

2.4. Total DNA extraction, cloning, and sequencing

The AS samples were obtained from the culture vessel as described in Section 2.3. Two milliliters of AS were used to extract the total DNA from each sample, using Ultraclean Microbial DNA Isolation kits (Mo Bio Laboratories, Solana Beach, CA, USA) according to manufacturer's instructions. The extracted DNA was evaluated on 1% (wt/vol) agarose gel and stored at −20 °C until required for further use. The partial 16S rDNA genes in the extracted samples were PCR-amplified using the universal primers 27F (5′-GAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). PCR amplification was performed using the following cycling program: 1 cycle at 94 °C for 5 min, 30 cycles each consisting of 94 °C for 1 min, annealing at 52 °C for 30 s, and extension at 72 °C for 1 min, followed by an additional