Journal of Hazardous Materials 260 (2013) 563–568

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

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

Aerobic biodegradation of a mixture of monosubstituted phenols in a sequencing batch reactor

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HIGHLIGHTS

- Aerobic biodegradation of a mixture of *p*-nitrophenol and *o*-cresol is feasible.
- Simultaneous biodegradation of *p*-nitrophenol and *o*-cresol was achieved at long-term.
- *o*-Chlorophenol caused complete failure of the sequencing batch reactor.
- Biomass had good settling properties although no mature granules were obtained.
- *p*-Nitrophenol is believed to be responsible for granulation failure.

ARTICLE INFO

Article history:
Received 11 February 2013
Received in revised form 3 May 2013
Accepted 28 May 2013
Available online 12 June 2013

Keywords:
Aerobic biodegradation
p-Nitrophenol
o-Chlorophenol
o-Cresol
Sequencing batch reactor

ABSTRACT

A sequencing batch reactor (SBR) was inoculated with *p*-nitrophenol-degrading activated sludge to biodegrade a mixture of monosubstituted phenols: *p*-nitrophenol (PNP), PNP and *o*-cresol; and PNP, *o*-cresol and *o*-chlorophenol. Settling times were progressively decreased to promote biomass granulation. PNP was completely biodegraded. The PNP and *o*-cresol mixture was also biodegraded although some transitory accumulation of intermediates occurred (mainly hydroquinone and catechol). *o*-Chlorophenol was not biodegraded and resulted in inhibition of *o*-cresol and PNP biodegradation and complete failure of the SBR within a few days. The biomass had very good settling properties when a settling time of 1 min was applied: sludge volume index (SVI_5) below 50 mL g^{-1}, SVI_5/SVI_{30} ratio of 1 and average particle size of 200 μm.

1. Introduction

Phenolic compounds play an important role in the chemical industry and are wide spread in the environment. Many phenol derivatives are highly toxic [1] and their co-existence in wastes from industrial plants such as oil refineries, coking plants, chemical-synthesis factories, pharmaceuticals, plastic industries, textiles manufacturing and many others is well established [2]. Within the chemical industry, the most prevalent phenolic compounds are nitrophenols, cresols and chlorophenols, which are commonly used for preparing disinfectants, herbicides and fungicides and as synthesis precursors in the manufacture of paracetamol, mesalazine, 2-benzyl-4-chlorophenol and phenacetin.

Treatments for these organic pollutants are mainly physicochemical, although many have serious drawbacks, such as high installation and running costs, formation of secondary hazardous by-products or incomplete mineralization [3–5]. However, biodegradation has become an emerging technological option to degrade such compounds with effective mineralization and economical processing [6,7]. Bacteria have been used in several studies on the degradation of phenolic compounds [6–8, among others]. Some genus of Gram-negative bacteria: *Pseudomonas*, *Sphingomonas*, *Acinetobacter*, *Ralstonia* and *Burkholderia* and Gram-positive genus *Rhodococcus* have been reported to degrade xenobiotic aromatic compounds [9,10].

Importantly, Martín-Hernández et al. [6] reported the enrichment of an activated sludge able to biodegrade *p*-nitrophenol (PNP) up to 0.26 g PNP g^{-1} VSS d^{-1} through two different metabolic pathways: through hydroquinone and through 4-nitrocatechol. This PNP-degrading sludge was mainly composed of *Acinetobacter* and *Arthrobacter* bacteria [10] and can biodegrade mixtures of dihydroxybenzenes [8]. Aerobic biological processes have also been reported to remove cresols [7,11] and chlorophenols [12,13]. Recently, Nielsen and Ingvorsen [14] demonstrated that a strain of *Citricoccus*, capable of effectively degrading PNP, was also able to degrade (and grow on) *p*-chlorophenol and phenol, but not

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http://dx.doi.org/10.1016/j.jhazmat.2013.05.052

on *o*-nitrophenol, *m*-nitrophenol or *o*-chlorophenol. Few studies have been done on the aerobic biodegradation in continuous reactors of mixtures of phenols with different substituents (nitro, methyl and chloro functional groups). Gallego et al. [7] reported the simultaneous biodegradation of phenol, *o*-chlorophenol and *m*-cresol in an aerobic fluidized-bed reactor with the biomass supported on activated carbon. Recently, Silva et al. [15] reported the simultaneous removal of *o*-cholorophenol, phenol, *p*-cresol and *p*-hydroxybenzaldehyde under nitrifying conditions. In both these studies, high-strength phenolic wastewaters (≈200 mg C L^{-1}) were treated.

Aerobic granular biomass has been employed to remove nitrophenols [16–18], cresols [19] and chlorophenols [20], and shows several advantages relative to activated sludge processes, such as a larger capacity to withstand toxic substrates and stressful loadings, higher biomass retention, no requirement for a settler and enhanced biodegradation at high-loading rates [21]. To the best of our knowledge, aerobic granules have not been used for simultaneous removal of mixtures of phenols with different functional groups.

This study investigates the aerobic biodegradation of a mixture of monosubstituted phenols (≈200 mg C L^{-1} in the influent) by using aerobic granular biomass.

2. Materials and methods

2.1. Batch tests with a PNP acclimated sludge

Batch experiments were performed to assess the possibility of biodegrading different phenolic compounds with activated sludge acclimated to consume PNP [6]. The tested compounds were *o*-nitrophenol, *o*-cresol, *p*-cresol, *o*-chlorophenol and *p*-chlorophenol. Each compound was tested as the sole carbon source in the batch reactor then, binary mixtures of PNP and each of the compounds were used.

The batch reactor consists of a magnetically stirred vessel with a working volume of 1 L. Continuous aeration was achieved from the bottom through a microdiffuser and a non-limiting dissolved oxygen (DO) concentration (above 4 mg O_2 L^{-1}) was maintained. The temperature of the vessel was controlled at 25 ± 0.5 °C with a thermostatic bath. The pH was continuously monitored with a pH probe (WTW-Sentix 81) and maintained (proportional control) at 7.5 ± 0.2 by addition of acid or base with an automatic microburette (Crison MultiBurette 2S). DO concentration was measured with a WTW-CellOx 325 probe.

2.2. Experimental set-up

2.2.1. Reactor and operational strategy for a long-term experiment

The reactor employed for a long-term experiment was a SBR working as a bubble column with a volume of 2 L operated at room temperature (20–25 °C). The pH of the SBR was not adjusted and ranged between 7 and 8. Its height to diameter ratio was 12, which is relatively high to obtain high flow velocities, and facilitate the aerobic granule formation and selection [22]. At start-up, the airflow rate was 0.9 NL min^{-1} and increased to 2.5 NL min^{-1} after 42 d. This flow-rate was constant until the end of the experiment and was enough to maintain high shear-stress in the reactor, which is a key factor for granulation [23].

The exchange volume was fixed at 25% throughout the operational period. For first 54 d, the total cycle time was 6 h and the hydraulic retention time (HRT) was 1 d. From day 55 until the end of the experiment, the total cycle time was increased to 12 h and the HRT was 2 d. Settling time was also modified to select a fast-settling biomass and promote granulation of the activated sludge.

The reactor was inoculated with 1 L of the same enriched PNP-degrading activated sludge previously used for the tests described in Section 2.1. The initial concentration of biomass in the reactor was 4 g VSS L^{-1}.

The feeding medium was a synthetic wastewater prepared with a constant total organic carbon (TOC) concentration of 400 mg C L^{-1}. For the first 148 d, 25% of the TOC was supplied by sucrose, 25% by glucose and 50% by PNP (200 mg C L^{-1}). From days 148 to 162, *o*-cresol was introduced into the feeding composition, supplying 10% of the TOC (40 mg C L^{-1}), whereas the PNP concentration was reduced to 40% of TOC (160 mg C L^{-1}). From days 162 to 265, PNP and *o*-cresol provided 25% TOC each (100 mg C L^{-1} each). Finally, from day 265 to the end of the operation, 5% of the TOC was provided by *o*-chlorophenol (20 mg C L^{-1}), and PNP and *o*-cresol supplied 22.5% each (90 mg C L^{-1} each). Micronutrients were also added to the feed according to Martín-Hernández et al. [6].

2.2.2. Observed and synthesis growth yield calculations

Observed and synthesis growth yields were calculated when stable operation of the reactor had been achieved. Observed growth yield (Y_{obs}) was calculated as the variation of the biomass [measured as volatile suspended solids (VSS)] in the reactor plus the biomass loss in the effluent divided by the total consumption of organic matter (measured as TOC) in the same period. Synthesis growth yield (Y) was calculated by using the following equation:

$$Y_{obs} = \frac{Y}{1 + b_H \cdot SRT} \quad (1)$$

where b_H is the decay rate coefficient for heterotrophs (d^{-1}) and *SRT* is the sludge retention time (d).

2.3. Analytical and microbiological methods

Phenols and their metabolic intermediates were analyzed by HPLC (UltiMate 3000, Dionex Corporation), with an Agilent Zorbax SB-C18 column and a UV detector [6]. TOC was measured with an OI Analytical TOC Analyzer (Model 1020A) equipped with a non-dispersive infrared detector and a furnace maintained at 680 °C. Total suspended solids (TSS) and VSS, as well as, sludge volumetric index after 5 and 30 min of settling (SVI_5, SVI_{30}, respectively) were determined according to Standard Methods [24]. Particle sizes were measured with a Malvern Mastersizer 2000 particle size analyser.

Fluorescence in situ hybridization (FISH) identification was performed according to Suárez-Ojeda et al. [10]. In situ hybridization was carried out over polycarbonate filters and two pre-treatment steps were used to improve permeation through the cell wall. The probes used were UNIV1390, targeting all bacteria; Burkho, for Burkholderiaceae family; KO 02, for *Arthrobacter* sp.; ACA652, targeting the genus *Acinetobacter*; and Ppu, for *Pseudomonas* sp.

3. Results and discussion

3.1. Batch tests with a PNP-degrading activated sludge

A PNP-degrading activated sludge, composed of 26 ± 2% *Arthrobacter* sp. and 31 ± 10% genus *Acinetobacter* [10], was employed in batch tests in which each phenolic compound was the sole organic carbon source. These experiments did not show any significant degradation in the short-term except, for the PNP (data not shown). After that, PNP was supplied in all tests together with one of the other compounds. This strategy would determine if non-specific enzymes were responsible for PNP biodegradation and if they might be able to degrade other phenols to some extent, as well as, providing information about hypothetical inhibitory effects