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Effect of S_0/X_0 ratio and acclimation on respirometry of activated sludge in the cometabolic biodegradation of phenolic compounds

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ABSTRACT

Aerobic batch biodegradation experiments and respirometric analysis were performed in order to investigate the effect of S_0/X_0 (substrate/biomass) ratio and preliminary acclimation on bi-solute biodegradation of phenolic compounds. It was shown that 2-chlorophenol (2-CP) and 2-nitrophenol (2-NP) could be cometabolically biodegraded only with acclimated biomass in the presence of phenol as growth substrate. Acclimation resulted in domination of phenol oxidizing bacteria which could induce the necessary enzymes for cometabolic transformation of 2-CP and 2-NP. Biodegradation of the cometabolic compounds occurred even after depletion of phenol at resting cell conditions. Both compounds could be successfully biodegraded by the acclimated biomass at initial substrate concentrations as high as 300 mg/L. Respirometric analysis showed that the optimum S_0/X_0 ratio ranged between 1.5 and 5.5 mg COD_{eq}/mg MLSS for cometabolic substrates 2-CP and 2-NP, whereas it was as high as 8.5 mg COD_{eq}/mg MLSS for phenol which corresponds to a phenol concentration of about 1500 mg/L.

1. Introduction

Chlorophenols and nitrophenols may exist in industrial wastewaters because of the wide use of chlorophenols in the production of preservers, pesticides and biocides and nitrophenols in the production of dyes, photochemicals, pesticides, wood preservatives, explosives and leather treatments. However, chlorophenols and nitrophenols are usually poorly degraded in conventional biological treatment systems. Under aerobic conditions, some chlorophenols and nitrophenols can serve as sole carbon sources supporting growth of some isolated specific microbial cultures (Aleksieva et al., 2002; Field and Sierra-Alvarez, 2008). However xenobiotic phenolic compounds (both growth and non-growth substrates) often co-exist in effluents and they usually do not support microbial growth.

Cometabolism has emerged as a way for biotransformation of non-growth substrates (Wang and Loh, 2000). Cometabolism is the biological transformation of a non-growth substrate by non-specific enzymes of bacteria. Synthesis of these enzymes in microbial cells can only be induced by a growth-substrate, which provides energy for cell growth and maintenance of bacteria. Cometabolism can be accomplished by growing cells in the presence of a growth substrate or by resting cells in the absence of the growth substrate (Criddle, 1993).

Studies in literature show that biodegradation of chlorophenols as the sole carbon source is often very difficult and requires special conditions such as the use of special cultures. For example, 2-chlorophenol, up to a concentration of 300 mg/L, could be biodegraded by a special culture *Alcaligenes* sp. at aerobic conditions with removal efficiencies above 97% (Gallego et al., 2001). On the other hand, biodegradation of monochlorophenols could be successfully enhanced in the presence of growth substrates such as glucose and phenol (Basu and Oleszkiewicz, 1995; Farabegoli et al., 2008; Li and Loh, 2005; Wang et al., 2003). Particularly, phenol serves as an ideal growth substrate to induce necessary enzymes due to its structural similarity with chlorophenols.

Cometabolic transformation of chlorophenols could be accomplished either by special cultures (De Los Cobos-Vasconcelos et al., 2006; Kim and Hao, 1999; Loh and Wu, 2006) or mixed cultures of activated sludge (Chiavola et al., 2004). A *Pseudomonas putida* strain could cometabolically remove 2- and 4-chlorophenol in the presence of phenol although it could not remove these compounds as the sole carbon source (Loh and Wu, 2006). In another study, an isolated culture of *Burkholderia tropicolis* cometabolically biotransformed 20 mg/L of 2- and 4-chlorophenol in the presence of 200 mg/L phenol with efficiencies of 93% and 100%, respectively (De Los Cobos-Vasconcelos et al., 2006). However, a pure strain can very rarely metabolize chlorophenols as sole carbon source, e.g. metabolical transformation of 2,4-dichlorophenol by *Achromobacter* sp. (Quan et al., 2004). On the other hand, an acclimated

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activated sludge could completely remove very high concentrations (up to 700 mg/L) of 3-chlorophenol cometabolically in the presence of phenol (Chiavola et al., 2004). In another study, initial concentration of 40 mg/L of 2-CP was cometabolically biodegraded by a mixed culture of microorganisms attached to a biofilm with efficiencies exceeding 99% in the presence of phenol (Farabegoli et al., 2008).

Nitrophenols are usually not growth substrates and may require cometabolic biodegradation, although some special cultures (Kulkarni and Chaudhari, 2006; Tomei et al., 2006) or acclimation of a regular activated sludge (Janeczko and Oleszkiewicz, 1993) may result in metabolic removal of these compounds. A special culture of *Trichosporon cutaneum R57* was able to grow and utilize 2,6-dinitrophenol and partly degrade 3-nitrophenol, but was not able to degrade 4-nitrophenol as sole carbon and energy source and cometabolism of these compounds in the presence of phenol did not further improve degradation rates (Aleksieva et al., 2002).

The ratio of initial substrate concentration to biomass (S_0/X_0 ratio) is an important parameter in batch activated sludge reactors. The importance of this parameter has been pointed out in several previous literature studies (Chudoba et al., 1991, 1992; Ellis et al., 1996; Liu, 1996). These studies showed that the initial concentrations were particularly important in the biodegradation of refractory and xenobiotic organic compounds because of their inhibitory effects. In the cases of cometabolic degradation of xenobiotic phenolic compounds, the ratio of both metabolic substrate and cometabolic substrate to biomass are important.

Short-term oxygen uptake rate (OUR) experiments can be successfully used for determination of biological removal performance and biodegradation kinetics in batch reactors (Dang et al., 1989) which provides useful data particularly for the comparison of biodegradation of single organic chemicals such as phenolic compounds (Orupold et al., 2001). An OUR curve determined with respect to time can provide the same information obtained from a substrate removal curve. Previous literature work showed that kinetic parameters may differ if experiments are conducted at different S_0/X_0 ratios (Ellis et al., 1996). When the ratio is sufficiently high ($S_0/X_0 > 20$ mg COD/mg MLSS), biomass conditions itself to limitless growth and the measured kinetic parameters can be independent of the retrospective feeding of the biomass. Kinetic parameters obtained at these conditions show the maximum activity (Pollard et al., 1998). On the other hand, physiological form of biomass is preserved at low S_0/X_0 ratios, since the growth of biomass is prevented. Therefore, kinetic parameters obtained at such conditions are called extant; i.e. these values presently exist and are dependent on the history of biomass (Ellis et al., 1996). At these conditions, biomass growth is assumed to be similar to an original full-scale biodegradation reactor at steady-state which is rather operated under low substrate to biomass ratios. Hence, extant kinetic parameters obtained in a batch reactor can reflect the biomass activity in continuous-flow reactors. Therefore, low S_0/X_0 ratios are preferred in batch studies (Pollard et al., 1998). However, at low S_0/X_0 ratios, it may be difficult to determine the concentrations of specific pollutants; hence respirometric methods are preferred (Ellis et al., 1996).

Interpretation of literature studies mentioned above showed that respirometric experiments at varying S_0/X_0 ratios may provide useful data on biodegradation of xenobiotic organics. However, the effect of S_0/X_0 ratio and acclimation on cometabolic biotransformation has not been previously investigated in literature and needs to be extensively explicated. For this purpose, in the present study, respirometric experiments were performed with acclimated biomass at varying S_0/X_0 ratios for the cometabolic biodegradation of xenobiotic phenolic compounds in addition to analysis of substrate removal in batch experiments.

2. Methods

2.1. Acclimation of activated sludge

Acclimation of activated sludge was performed separately for 2-CP and 2-NP in two batch reactors with volumes of 4 L. Acclimation to 2-CP was performed by feeding the activated sludge with 170 mg/L phenol and 110 mg/L 2-CP starting from phenol as the only carbon source and gradually increasing the concentration of 2-CP. The activated sludge acclimated to this mixture of phenol and 2-CP was called as AS-P + 2-CP throughout the study. Acclimation to 2-NP was performed by feeding with 200 mg/L phenol and 60 mg/L 2-NP in the same way. The activated sludge acclimated to this mixture of phenol and 2-NP was called as AS-P + 2-NP throughout the study. In both batch acclimation reactors, the synthetically prepared solution which contained $(NH_4)_2SO_4$, KH_2PO_4, K_2HPO_4, $MgSO_4{\cdot}7H_2O$, $CaSO_4{\cdot}2H_2O$, $FeSO_4{\cdot}7H_2O$, and $NaHCO_3$ in proper amounts was added in order to obtain the required nutrients and minerals and buffer pH. Details for the synthetic solution were given in previous studies (Aktaş and Çeçen, 2009, 2010). Batch-wise feeding was repeated every 2–3 days. Biological activity was shown respirometrically as measured by oxygen uptake rates as high as 20 mg/L h at the start of each batch. Besides, at the end of every 48–72 h aeration period, it was determined by gas chromatographic analysis that almost all of the phenolic compounds were removed. Environmental scanning electron microscopic (ESEM) analysis of the acclimated sludges showed that bright cocci-shaped bacteria dominated. But large protozoans and particularly filamentous microorganisms seemed to decrease in number compared with the non-acclimated sludge. Scanning Electron Microscopy (SEM) images were obtained by a Philips XL30-FEG Environmental SEM operating at the wet mode at 25 °C and 0.3–0.6 Torr.

For comparison purposes, an activated sludge which was not acclimated to phenolic compounds was also used. Non-acclimated activated sludge was fed batch-wise in every 2–3 days with a synthetically prepared wastewater containing glucose, sodium acetate, peptone and sufficient amounts of nitrogen, phosphorus and required minerals (Aktaş and Çeçen, 2006). The activated sludge which was not acclimated to phenolic compounds was called as Non-Acc.AS throughout the study.

2.2. Batch biodegradation experiments

The preliminary batch biodegradation experiments were performed in 2 L aerated batch reactors (Aktaş and Çeçen, 2006) either with acclimated or non-acclimated activated sludges. Mixed liquor suspended solids (MLSS) concentrations in batch reactors ranged between 1000 and 1900 mg/L in reactors with acclimated biomass, and as high as 2700 mg/L in those with non-acclimated biomass. Concentrations of phenolic compounds were measured with respect to time following the mixing of activated sludge and synthetically prepared wastewater.

Phenol, 2-CP and 2-NP concentrations were determined with an Agilent 6890N gas chromatograph equipped with a FID (Flame Ionization detector) and HP-5 column (length 30 m, ID 0.32 mm, film thickness 0.25 μm). Helium was used as the carrier gas at the splittless mode with a flow rate of 25 cm/s. The inlet temperature was 240 °C and detector temperature was 300 °C. The oven temperature was held at 40 °C for 1 min, increased to 140 °C at 10 °C/min and then increased to 260 °C at 20 °C/min. One milliliter sample was extracted with 0.5 mL methylene chloride in 2 mL closed vials for 3 min and 2 μL of the methylene chloride phase was injected with an auto-injector. MLSS analysis followed the standard methods (APHA-AWWA-WPCF, 1998).