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Effect of feeding time on the performance of a sequencing batch reactor treating a mixture of 4-CP and 2,4-DCP

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Abstract

This paper investigated the biodegradation kinetics of 4-chlorophenol (4-CP) and 2,4-dichlorophenol (2,4-DCP) separately in batch reactors and mixed in sequencing batch reactors (SBRs). Batch reactor experiments showed that both 4-CP and 2,4-DCP began to inhibit their own degradation at 53 and 25 mg l^{-1}, respectively, and that the Haldane equation gave a good fit to the experimental data because r^2 values were higher than 0.98. The maximum specific degradation rates (q_m) were 130.3 and 112.4 mg g^{-1} h for 4-CP and 2,4-DCP, respectively. The values of the half saturation (K_s) and self-inhibition constants (K_i) were 34.98 and 79.74 mg l^{-1} for 4-CP, and 13.77 and 44.46 mg l^{-1} for 2,4-DCP, respectively. The SBR was fed with a mixture of 220 mg l^{-1} of 4-CP, 110 mg l^{-1} of 2,4-DCP, and 300 mg l^{-1} of peptone as biogenic substrate at varying feeding periods (0–8 h) to evaluate the effect of feeding time on the performance of the SBR. During SBR operation, in addition to self-inhibition, 4-CP degradation was strongly and competitively inhibited by 2,4-DCP. The inhibitory effects were particularly pronounced during short feeding periods because of higher chlorophenol peak concentrations in the reactor. The competitive inhibition constant (K_{ii}) of 2,4-DCP on 4-CP degradation was 0.17 mg l^{-1} when the reactor was fed instantaneously (0 h feeding). During longer feedings, increased removal/loading rates led to lower chlorophenol peak concentrations at the end of feeding. Therefore, in multi-substrate systems feeding time plus reaction time should be determined based on both degradation kinetics and substrate interaction. During degradation, the *meta* cleavage of 4-chlorocatechol resulted in accumulation of a yellowish color because of the formation of 5-chloro-2-hydroxymuconic semialdehyde (CHMS), which was further metabolized. Isolation and enrichment of the chlorophenols-degrading culture suggested *Pseudomonas* sp. and *Pseudomonas stutzeri* to be the dominant species.

Keywords: 4-Chlorophenol; 2,4-Dichlorophenol; SBR; Biodegradation kinetics

1. Introduction

Widespread use of chemicals such as chlorophenols has a potentially negative impact on public health and the ecosystem (Mangat and Elefsiniotis, 1999). Chlorophenols are introduced into the environment through various human activities such as waste incineration, uncontrolled use of wood preservatives, pesticides, fungicides, and herbicides, as well as via bleaching of pulp with chlorine and the chlorination of drinking water (Contrerasa et al., 2003). In 1999, 56,000 tons of waste phenol and 1900 tons of waste chlorophenols were generated by industries in the United States (Tarighian et al., 2003). Some typical values of phenolic compounds reported for chemical industry wastewaters have been 400 mg l^{-1} for phenolic resin production, 50 mg l^{-1} for refineries, 12 mg l^{-1} for naphthalenic acid production and 200 mg l^{-1} for shale dry distillation (Chen et al., 1997). The discharge of these compounds into the environment is thus of great concern because of the compounds' toxicity and suspected carcinogenicity.

Despite the recalcitrance of chlorophenols, efforts are still being made to treat them biologically for economic reasons and in expectation of few byproducts. However, chlorinated compounds have been reported as being in general useless as carbon and energy sources for microbial growth, and should thus be rather biodegraded by cometabolism (Wang and Loh, 1999, 2000; Bali and Şengül, 2002). So far, the major kinetic studies of

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chlorophenols have focused on the degradation by special strains of 4-chlorophenol (4-CP) as a model non-growth substrate (Saéz and Rittmann, 1991, 1993; Hill et al., 1996; Kim and Hao, 1999; Wang and Loh, 2001; Hao et al., 2002). However, mixed cultures are particularly important if we aim at complete mineralization of toxic organics to CO_2. Many pure-culture studies have shown that toxic intermediates accumulate during biodegradation, because a single organism may be incapable of fully mineralizing the xenobiotics (Buitron and Gonzalez, 1996). Therefore, treating chlorophenols with activated sludge that contains an active mixed culture but lacks a special growth substrate would be more meaningful, informative, and practical. The advantage in using a microbial consortium formed by activated sludge is that the species present in the flocks would interact. Furthermore, the degrading capabilities of an activated sludge system can be enhanced by acclimation (Buitron et al., 1998; Kim et al., 2002). For example, Buitron et al. (1998) reported that acclimated activated sludge degraded a chlorophenol mixture up to two orders of magnitude faster than pure strains obtained from an acclimated consortium. Previously, we demonstrated that acclimated activated sludge degraded 2,4-dichlorophenol (2,4-DCP) (Sahinkaya and Dilek, 2002) and 4-CP (Sahinkaya and Dilek, 2005). Wiggings et al. (1987) suggested that during acclimation, physiological transformations in the metabolism of microorganisms, such as changing enzyme levels, regulation and production, and mutations, lead to selection and multiplication of specialized microorganisms. In aerobic microbial communities, acclimation periods range typically from several hours to several days. Thus by definition, acclimation is any response that ultimately leads the community to eliminate stress or find a way to maintain its function despite the stress (Rittmann and McCarty, 2001).

In addition to suspended growth systems, biofilms have been used to degrade chlorophenols. For example, Shieh et al. (1990) examined the ability of immobilized cells grown under oxic and fluidized-bed conditions to degrade 4-CP and 2,4-DCP and evaluated pseudo-steady-state chlorophenols degradation under different dilution rate conditions, with chlorophenols as the sole organic carbon source. They achieved good and stable TOC removal at empty bed hydraulic retention times (HRTs) as low as 1 h. In another study, Puhakka and Jarvinen (1992) degraded polychlorinated phenols in continuous-flow fluidized-bed reactors, using pure oxygen for aeration and a celite carrier to immobilize the cells.

Sequencing batch reactors (SBRs) offer an attractive alternative to conventional biological wastewater treatment systems, mainly because of their simple and flexible operation and cost effectiveness for small-scale treatment facilities (Mangat and Elefsiniotis, 1999; Chiavola et al., 2004). The key difference is that the reactor volume of SBRs varies with time but remains constant with traditional continuous-flow systems (Mohan et al., 2005). Enforcement of controlled, short-term, non-steady conditions in SBRs may favor induction of enzymes to degrade biorefractory compounds (Tomei et al., 2004). Another significant point for SBRs is that the chlorophenols peak concentration in the reactor, adjustable by changing the feeding time, markedly affects reactor performance. Accumulated compounds may adversely affect both their own degradation via self-inhibition and the degradation of other compounds in the influent via substrate interaction and/or inhibition of the biomass. Therefore, SBR operational parameters should be optimized especially when chlorophenols are present in the mixture.

In light of the above studies, chlorophenols degradation needs to be studied in SBRs with mixtures containing a chlorophenols-degrading enrichment culture to produce data directly applicable to real-scale operations. Therefore, we examined the effect of feeding time on the performance of an SBR treating a mixture of 4-CP and 2,4-DCP and sought to define substrate interaction between 4-CP and 2,4-DCP.

2. Materials and methods

2.1. Biomass selection and enrichment

To start up our SBR and for batch reactor experiments, we obtained a chlorophenol-degrading enrichment culture from a fed-batch reactor receiving 220 mg l^{-1} of 4-CP, 110 mg l^{-1} of 2,4-DCP, and 300 mg l^{-1} of peptone. We used about 200 ml of well-mixed sludge (1200 mg l^{-1} of mixed liquor volatile suspended solids (MLVSS)) as initial seed for the SBR.

As initial seed for the fed-batch reactor, we used primary settling tank effluent from the Greater Municipality of Ankara Domestic Wastewater Treatment Plant. For 2 weeks, the fed-batch reactor was fed with a growth medium devoid of chlorophenol to produce a reasonably concentrated biomass. Then, 4-CP and 2,4-DCP were added to the feed at low concentrations with influent concentrations gradually increased to 220 mg l^{-1} of 4-CP and 110 mg l^{-1} of 2,4-DCP within about 5 months.

2.2. Batch experiments

Batch experiments were conducted in 500-ml Erlenmeyer flasks, stoppered with cotton plugs, with a working liquid volume of 250 ml. All experiments were run in an orbital shaking incubator set at 200 rpm and $26 \pm 1\,^{\circ}C$. The biomass from the fed-batch reactor, used as a parent bioreactor to seed the SBR, was washed two times with distilled water to remove organics adsorbed on the biomass. Then cultures were resuspended in distilled water, and parallel batch reactors were seeded to a biomass concentration of about 150–200 mg l^{-1}. In addition to inorganic components, reactors were supplemented with 4-CP or 2,4-DCP at varying concentrations as sole carbon and energy sources (Table 1). Reactors not receiving biomass were operated as controls under the same