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Influence of discontinuing feeding degradable cosubstrate on the performance of a fluidized bed bioreactor treating a mixture of trichlorophenol and phenol

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ABSTRACT

The purpose of our research was to evaluate the effect of eliminating supplementation of sucrose to the reactor influent on the performance of a lab scale partially-aerated methanogenic fluidized bed bioreactor (PAM–FBBR). Two operational stages were distinguished: in the first stage the influent contained a mixture of 120/30/1000 mg/L of 2,4,6-trichlorophenol/phenol/COD-sucrose (TCP/Phe/COD-sucrose); in the second stage only the xenobiotic concentrations were the same 120/30 mg/L of TCP/Phe whereas sucrose addition was discontinued. Removal efficiencies of TCP, Phe, and COD were very high and close for both stages; i.e., η_{TCP}: 99.9 and 99.9%; η_{Phe}: 99.9 and 99.9%; $\eta_{COD} = 96.46$ and 97.48% for stage 1 and stage 2, respectively. Traces of 2,4,6 dichlorophenol (0.05 mg/L) and 4-chlorophenol (0.07–0.26 mg/L) were found during the first 15 days of operation of the second stage, probably due to the adaptation to no co-substrate conditions. Net increase of chloride anion Cl^- in effluent ranged between 59.5 and 61.5 mg Cl^-/L that was very close to the maximum theoretical concentration of 62.8 mg Cl^-/L. PCR–DGGE analysis revealed a richness decrease of eubacterial domain posterior to sucrose elimination from the influent whereas archaeal richness remained almost the same. However, the bioreactor performance was not negatively affected by discontinuing the addition of co-substrate sucrose. Our results indicate that the application of PAM–FBBR to the treatment of groundwaters polluted with chlorophenols and characterized by the lack of easily degradable co-substrates, is a promising alternative for on site bioremediation.

1. Introduction

Chlorophenols are ubiquitous pollutants in aquifers and wastewaters and considerable research has been devoted to their removal, because they are toxic, recalcitrant, and they accumulate in the tropic chain (Haggblom, 1990). They are also recognized to be carcinogenic to animals and potentially carcinogenic to humans (WHO, 1989). Particularly, 2,4,6-trichlorophenol is also a precursor of carcinogenic substances such as dibenzo *p*-dioxins (WHO, 1989). Chlorinated phenols are extensively utilized as pesticides, fungicides and disinfectants, and are important chemicals in a variety of industrial processes (Androulaki et al., 2000). Several chlorophenol residues are released via chemical or biological transformation or degradation of several other groups of pesticides (for example pentachlorophenol, chlorinated phenoxy herbicides, etc.) as well as compounds present in a variety of industrial wastewaters, such as bleaching effluents from the pulp and paper industry or effluents from the cleaning and disinfectant-manufacturing industry (Carvalho et al., 2001; Leuenberger et al., 1985; Poggi-Varaldo and Rinderknecht-Seijas, 1996). Soils contaminated with wood preservatives very often show high contents of 2,4,6-trichlorophenol (TCP), among other chlorophenols, and also pose a threat to aquifer quality (Perkins et al., 1994). In the manufacture of some domestic cleaners and pesticides, for instance 2,4-dichlorophenoxiacetic acid, a mixture of TCP and phenol (as well as other mono and di-chlorophenols) is commonly found in the effluents (Buenrostro-Zagal et al., 2000). In wood preserving practice in the second half of the 20th century, concentrated

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solutions of polychlorophenols were used. For instance, a formulation used in Finland for wood preserving is a 2% w/v solution, with pH 10 to 11, and contains 80% of 2,3,4,6-tetrachlorophenol, up to 15% of 2,4,6-trichlorophenol, and the remainder is made up of other chlorophenols and phenol (Valo et al., 1984, 1985). In some developing countries such as Mexico, chlorophenol solutions containing TCP are still used as fungicide (Ríos-Leal, E., private communication, 2010).

When these effluents are inadvertently released or intentionally discharged to the environment, they eventually infiltrate the subsurface soil and the mixture of chlorophenols could reach the aquifer (Salkinoja-Salonen et al., 1995). Furthermore, when groundwater is contaminated with TCP or higher chlorinated phenols, reductive dechlorination that might occur in anoxic niches of aquifers could lead to the formation of mono chlorophenols and phenol via reductive dehalogenation (Annachhatre and Gheewala, 1996; Mohn and Tiedje, 1992). Choi et al. (2007) found that only phenol was detected from the transformation of 100 mg/L 2,4,6-TCP in a laboratory-scale sequential barrier system consisting of a chemical and biological (anaerobic) reactive barrier at retention times between 21 and 30 h. Two conclusions were drawn: first, results indicated complete dechlorination of 2,4,6-TCP; second, there was a coexistence of TCP and phenol in selected times and niches of soil and the biobarrier. Further operation of the biobarrier at 7 d retention time achieved complete removal of *ca.* 100 μM phenol.

Lampi et al. (1990) and Salkinoja-Salonen et al. (1995) reported groundwater contaminated by chlorophenols in the range 56–190 mg/L. Chlorophenols leached from a saw mill in southern Finland. Nearly 2000 inhabitants were subjected to a long-term exposure with polluted groundwater as well as consumption of contaminated fish from a nearby lake (fish with up to 5 mg of 2,4,6-trichlorophenol/kg fish fat, among other chlorophenols).

Considerable research has been published concerning the treatment of chlorophenols-contaminated waters and wastewaters (Annachhatre and Gheewala, 1996). Regarding TCP, most experiments in anaerobic treatment report reasonable removals of higher substituted chlorophenols; however, accumulation of lower-substituted chlorophenol compounds, such as 2,4-dichlorophenol and 4-chlorophenol, occur under anaerobic conditions (Woods et al., 1989; Mohn and Tiedje, 1992; Vallecillo et al., 1999). Complete removal and degradation of TCP under anaerobic conditions by undefined consortia has been demonstrated in only a couple of cases (Kennes et al., 1996).

On the other hand, complete aerobic mineralization of higher substituted chlorophenols such as pentachlorophenol and tetrachlorphenol is more difficult to achieve. Yet, there are several reports on aerobic degradation of TCP by pure strains of bacteria either as cometabolism or as the sole source of carbon and energy (Aranda et al., 2003; Briglia et al., 1996; Clément et al., 1995; Kim et al., 2002; Maltseva and Oriel, 1997; Tiirola et al., 2002) and ligninolytic fungi (Choi et al., 2000; Ehlers and Rose, 2005; Leontievsky et al., 2002; Snyder et al., 2006), as well as aerobic bacterial consortia (Gallego et al., 2009; Gomez-de-Jesus et al., 2009; Kharoune et al., 2002; Kim et al., 2002; Lora et al., 2000) in both flask tests and bioreactor experiments (Atuanya et al., 2000; Eker and Kargi, 2009, 2008, 2007; Gomez-de-Jesus et al., 2009; Kim et al., 2002; Mäkinen et al., 1993; Melin et al., 1998; Zilouei et al., 2006).

Partially-aerated methanogenic (PAM) bioreactors offer an interesting alternative for achieving a more complete removal of toxic compounds and their eventual metabolites, because they could provide more diverse catabolic pathways (Macarie and Guiot, 1996; Garibay-Orijel et al., 2006a). In particular, PAM bioreactors were successfully applied for the removal of *ca.* 100% of up to 68.5 mg/L pentachlorophenol in an upflow anaerobic sludge blanket (UASB) reactor (Macarie and Guiot, 1996), *ca.* 100% of an average of 32 mg/L of 2,4,6-TCP in an upflow reactor (Gardin et al., 2001), and 88% of 42.9 mg/L perchloroethylene in an upflow packed bed reactor (Tartakovsky et al., 1998). The UASB has been the most commonly used bioreactor for the treatment of recalcitrant and toxic compounds in PAM conditions (Garibay-Orijel et al., 2006a). Other systems have utilized biomass immobilized in calcium alginate, k-carrageneen, etc, in upflow reactors (Tartakovsky et al., 1998; Gardin et al., 2001). On the other hand, works based on the application of partially-aerated methanogenic fluidized bed bioreactors for the treatment of 2,4,6-trichlorophenol contaminated waters are very scarce (Garibay-Orijel et al., 2006a).

Previous recent work of our research group has demonstrated the excellent performance of a PAM–FBBR for the removal of a mixture of 2,4,6-triclorophenol (TCP) and phenol (Phe) when continuously supplemented with 1000 mg COD-sucrose/L (Garibay-Orijel et al., 2006a). However, concern has arisen regarding bioreactor performance without supplementation of an easily degradable carbon source (co-substrate sucrose). Thus, the objective of our work was to evaluate the effect of eliminating the supplementation of sucrose to the reactor influent on the performance of a PAM–FBBR.

2. Materials and methods

2.1. Reactor and experimental design

The laboratory-scale, mesophilic, methanogenic-aerated FBBR consisted of a glass column of 4.5 cm internal diameter, 185 cm length and 3 L of working volume (Fig. 1). It was loaded with biocatalysts (a bed of bioparticles, each bioparticle had a core of granular activated carbon colonized by an undefined microbial consortium that consisted of anaerobic, facultative and aerobic microorganisms. At the start-up, a mixture of activated sludge, cow manure, and garden soil was anaerobically enriched in lab scale, mesophilic, semi-continuous, complete mix bioreactors (CMB), intermittently fed with a solution 2 g sucrose/L plus 2 g $NaHCO_3$. Once the CMB became fully methanogenic, they were drawn-and-filled once a day with a synthetic wastewater containing 5 g sucrose/L, 1 g HAc/L plus nutrients at 21 d HRT. When steady state was reached, contents of CMBs were loaded into the FBBRs; the latter were gassed with nitrogen gas to expel any oxygen and to keep anaerobic conditions in the units. Immediately, the

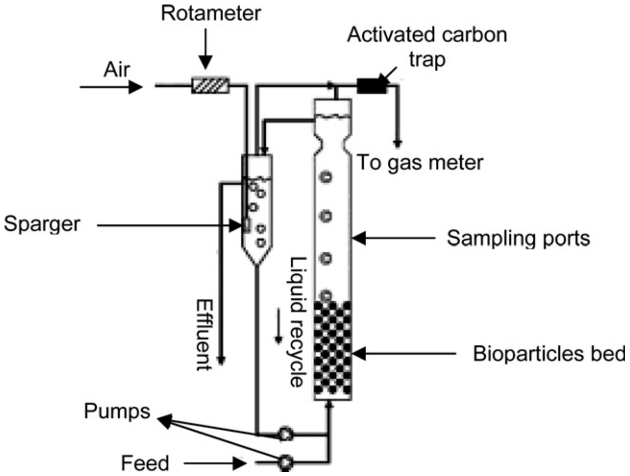

Fig. 1. Scheme of bioreactor set up.