Degradation of phenol and chlorophenols by mixed and pure cultures

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Abstract

The enrichment of mixed cultures for species capable of degrading phenol and chlorophenols, as well as the isolation of pure cultures are investigated. The cultures obtained are capable of degrading phenol and chlorophenols (pentachlorophenol 2,3,5,6 tetrachlorophenol and 2,4,6 trichlorophenol) but not 2,4,5 trichlorophenol. The results suggest the feasibility of the use of toxic chemicals as phenols, hexadecane and other chlorophenols as co-substrates in field decontamination processes. The inhibitory effect of PCP is shown, and the influence of a readily degradable ancillary carbon source on the performance of pure cultures is reported, as well as the preliminary identification of the bacteria that showed higher PCP degrading activity.

Keywords: phenol degradation, chlorophenols, biodegradation

Introduction

Chlorophenols and phenols are introduced in the environment in the waste streams of several industrial operations, through its use as biocides or as by-products of other industrial operations, such as pulp bleaching with chlorine, water disinfection or even waste incineration. Chlorophenols and phenols have also been used as general purpose disinfectants, and it has been found that they can also appear as degradation products of other chlorinated xenobiotics (Bollag. et al., 1986). Because of their toxic effects, phenol and chlorophenols tend to accumulate and in some cases the contamination of soil and water is of concern (Keither and Tellard, 1979, Moos et al., 1983; Borthwick and Schimmel, 1978).

Several decontamination techniques are available for the removal of contaminants from water, although not all (such as adsorption or ion exchange) actually destroy the contaminant. Some techniques, such as incineration have recently come under heavy criticism. Although not exempt from potential implementation problems, biodegradation is a technique which could potentially degrade these contaminants to innocuous products (mainly CO_2 and H_2O; also Cl^- in the case of chlorinated phenols). Microbial and fungi degradation of phenol and chlorophenols have been reported by several groups (Baker et al., 1980; Pignatello et al., 1983; Saber and Crawford, 1985; Rozich and Colvin, 1986; Apajalahti and Salkinoja-Salonen, 1986; Radehaus and Schmidt, 1992; Ramos et al., 1995; Haggblom and Valo, 1995; McBain et al., 1995; Colores et al., 1995. Lee et al., 1998; Toumela et al., 1999; Reddy and Gold, 2000; Cortés et al., 2002). Other works are reported on chlorophenol degradation by mixed cultures (Kirsch and Etzel, 1973; Liu et al., 1981; Klecka and Maier, 1985, Puhakka et al., 1995). Although PCP degrading activity is in some cases higher when using pure cultures, the ability of mixed cultures to survive in a non-sterile environment is a key issue in field applications of biodegradation. A potential problem regarding PCP-degrading bacteria in soil is the high concentrations of PCP at some contaminated soil sites, where PCP concentrations as high as 9000 $mg \cdot kg^{-1}$ have been reported. An effective bacterial inoculum needs to tolerate high levels of PCP while maintaining a level of activity to provide efficient mineralisation (Shaw et al., 1997). The addition of organic substrates stimulates the dechlorination of chloroaromatic compounds (Hendriksen et al., 1992). Therefore the purpose of this report is to describe the effect of the presence of nontoxic organic compounds as glucose on the biodegradation of pentachlorophenol. Investigations concerned with pentachlorophenol removal pattern performed on the toxic waste components phenol, chloropehnols and hexadecane were also conducted since inevitably toxic components will be found in mixtures with nontoxic, or conventional wastes (Rozich and Colvin, 1986).

We report on our work on the enrichment of mixed cultures capable of degrading phenols and chlorophenols, usually the most recalcitrant (Neilson et al., 1985; Sittig, 1981) and introduce steps for the isolation of pure cultures and their preliminary identification.

Materials and methods

Chemicals and reagents

Pentachlorophenol (PCP 99% pure) was obtained from Sigma Chemical Co. (St. Louis MO 63178 USA). Phenol and all chlorophenols were of the highest purity available from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals used were of the highest purity available commercially.

Culture conditions and media

Samples were collected using non-sterile procedures from soil with a history of phenol contamination, wood chunks that had been exposed to formol and chlorophenol solutions, and soil containing pentachlorophenol near a wastewater discharge site. All enrichments were done in a mineral salts medium (MS base) containing (in grams of ingredient per liter): $NaNO_3$, 0.5; K_2HPO_4, 0.65; KH_2PO_4, 0.17; and $MgSO_4$, 0.1. The mineral salts (MS) medium for the lower buffer PCP medium, usually used for the incubation of PCP-degrading

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