Effect of pH and inoculum size on pentachlorophenol degradation by *Pseudomonas* sp.

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Abstract

Pentachlorophenol (PCP) is a toxic compound which is used as a fungicide, bactericide, herbicide and chemical intermediate. Because of its toxicity, there is a need to decontaminate the PCP-laden soils and bioremediation is a very useful alternative to conventional clean-up methods. The success of this depends on finding strains able to degrade PCP in a changeable environment.

The aim of this work was to study the influence of pH of the medium and the effect of inoculum size on pentachlorophenol degradation by *Pseudomonas* sp. A study of PCP degradation kinetics was performed to assess such effects.

PCP was degraded rapidly at pH values from 6.3 to 8, but the maximum rate of PCP degradation by *Pseudomonas* sp. was at pH 6.3. In contrast, the PCP degradation kinetics at pH 5.5 were significantly lower, although PCP was totally depleted. These results show the broad range of pHs for PCP degradation for this strain.

PCP was degraded at every inoculum size tested and PCP degradation increased with the increasing inoculum size, but cultures inoculated with the lowest inoculum showed the highest specific consumption rate. This reveals a lower consumption of PCP per CFU at a high population density.

These results are useful to understand the physiological and biochemical properties of *Pseudomonas* sp. before its optimum use in environmental application and these data will assist in choosing the right PCP-degrader for a changeable environment.

Keywords: biodegradation, bioremediation, inoculum size, pentachlorophenol, pH, *Pseudomonas* sp.

Introduction

Pentachlorophenol (PCP) has been used as a wood preservative, insecticide, and herbicide and was introduced into the environment by waste streams of several industrial operations. A large amount of PCP is used by the wood-preserving industry (Hoos, 1978; Cirelli, 1978) and PCP contamination in soil represents a serious problem to the environment that surrounds several wood treatment plants and sawmills (Rao, 1978; Valo et al., 1985). PCP is considered a priority pollutant by various regulatory agencies. This polychlorinated aromatic compound is toxic to numerous aquatic organisms at a concentration of as low as 0.5 $mg \cdot \ell^{-1}$ (Borthwick and Schimmel et al., 1978) and adversely affects flora and fauna (Chu and Kirsch, 1973; Liu et al., 1982). It is also suspected to be a human carcinogen (Mc Allister et al., 1996). Because of its toxicity, there is a need to decontaminate the PCP-laden soils.

Several decontamination techniques are available for the removal of contaminants from water, although not all are efficient enough to destroy the contaminant. The use of micro-organisms for bioremediation of PCP-contaminated sites may prove to be a viable alternative to conventional clean-up methods. 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). Biological treatment of chlorophenols attracts more attention than physical and chemical methods, because a variety of micro-organisms are known to utilise chlorophenols as their sole carbon or energy source and because the reaction products are Cl^- ions, CO_2 and biomass. Many species of soil bacteria have been isolated from contaminated soil samples. PCP-degrading bacteria include species of *Arthrobacter* (Stanlake and Finn, 1982), *Flavobacterium* (Saber and Crawford, 1985), *Pseudomonas* (Radehaus and Schmidt, 1992), *Rhodococcus* (Apajalahti and Salkinoja-Salonen, 1986) and *Corynebactrium* (Chu and Kirsch, 1972). Although several of them do not degrade PCP completely, others produce toxic metabolites and some do not tolerate changes of physical and chemical environmental factors.

In previous work published (Murialdo et al., 2003) a *Pseudomonas* sp. strain was isolated from a consortium that degrades PCP. It was shown that glucose and glutamate have positive effects on its population density. This micro-organism could be used very effectively for *in situ* bioremediation in an environment which is highly contaminated with PCP, other chlorinated phenols and hexadecane (Murialdo et al., 2003).

The success of bioremediation may depend on the availability of microbial strains that can mineralise high levels of PCP and withstand adverse conditions to compete under *in situ* conditions. An effective bacterial inoculum should be able to tolerate high levels of PCP while maintaining a level of activity to provide efficient mineralisation (Shaw et al., 1997). Understanding the physiological and biochemical properties of PCP-degrading bacteria is required before optimum use of bacteria in environmental applications.

In order to find a strain able to degrade PCP in a changeable environment, we studied the effect of inoculum size and the influence of the pH of the medium on PCP degradation by a PCP-degrading bacterium, *Pseudomonas* sp., isolated from soils contaminated with PCP.

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