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Degradation of Pentachlorophenol by Non-Immobilized, Immobilized and Co-Immobilized *Arthrobacter* Cells

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Non-immobilized, immobilized and co-immobilized *Arthrobacter* (ATCC 33790) cells were examined for their ability to degrade PCP in a mineral medium. Non-immobilized cells could completely remove PCP from the aqueous phase and mineralize 77% of the added PCP within 135 h. Alginate-encapsulated cells mineralized 86% of the PCP with a similar profile as free cells. Use of co-immobilized cells (which co-immobilized cells and activated carbon in hydrogel capsules) resulted in rapid removal of PCP from the aqueous phase and extensive PCP mineralization.

Pentachlorophenol (PCP) and its sodium salt (Na-PCP) are widely used as a wood preservative, fungicide, bactericide, molluscicide, algicide, and insecticide (1). Contamination by PCP has been frequently found in water and soil (2, 3). Along with other chlorinated phenols, PCP has been placed on U. S. Environmental Protection Agency list of priority pollutants (1).

Several pure and mixed cultures, including *Arthrobacter* (4), *Flavobacterium* (5), *Rhodococcus chlorophenolicus* (6), *Phanerachaete chrysosporium* (7, 8), aerobic activated sludge (9) and anaerobic sludge (10) and granules (Bhatnagar, L. *et al.*, Abstr. ACS Nat. Meet., Dallas, Texas, USA, 1989), have been shown to be able to degrade PCP. Stanlake and Finn (4) reported the isolation of a strain of *Arthrobacter* (ATCC 33790) that is capable of degrading PCP. Some rate data for using this bacterium and application results of this strain to a soil test were also presented (4, 11). Most of the published data on this strain were based on the removal of PCP that was determined by spectrophotometric analyses. Removal of PCP may be related to several factors, such as physical adsorption, as well as biodegradation. Therefore, more detailed experiments, such as mineralization of ^{14}C-labeled PCP, may be needed to better understand the kinetics of PCP degradation by this strain.

Cell immobilization has been extensively utilized for the degradation of toxic compounds in wastewater treatment processes. Common immobilization methods include matrix entrapment and encapsulation (12). Recently, we proposed a co-immobilization concept in which cells and adsorbents were co-immobilized in a hydrogel matrix (13). The purposes of co-immobilization are to reduce retention time of toxic compounds in the mobile phase, avoid inhibition of high-concentration toxic chemicals on the cells, and provide a suitable microenvironment for biodegradation. Studies on characteristics of using *Arthrobacter* cells in an immobilization matrix and in the co-immobilized system to degrade PCP may offer some insight in developing practical remediation processes.

In this work, *Arthrobacter* cells were used in the non-immobilized form, alginate-encapsulated capsules, and co-immobilized (with activated carbon) capsules to degrade PCP. Mineralization of PCP, as a major parameter, by various preparations of *Arthrobacter* cells was determined.

Arthrobacter (ATCC 33790) was grown in a medium containing Na-PCP (0.1 g), $NaNO_3$ (0.5 g), K_2HPO_4 (0.6 g), KH_2PO_4 (0.2 g), $MgSO_4 \cdot 7H_2O$ (0.1 g), NH_4Cl (0.5 g), $CaCl_2 \cdot 2H_2O$ (0.02 g) in a liter of distilled water (pH 7.0). This seed culture was incubated in 100 ml of medium contained in 500 ml flasks and shaken (200 rpm) at 30°C. After 4 d of incubation, the culture broth (OD_{560} of 0.2) was used as inoculum. To quantify the bacterial cells, aliquots of the culture broth were spread onto tryptic soy agar plates (11). After 3 d of incubation at 30°C, colonies of *Arthrobacter* on the agar plates were counted. One ml of the broth with OD_{560} of 0.2 contained approximately 6×10^5 cells.

Configurations of encapsulated immobilized cells and co-immobilized cells are shown in Fig. 1. To prepare immobilized capsules, 1 ml of the seed culture broth was mixed with 0.3 M $CaCl_2$ solution to a total of 2.5 ml (for one flask of degradation mixture). This mixture was then dropped through an air nozzle into a stirred alginate solution (0.5%). Mixing was continued for 2 min. The slurry of the formed capsules and alginate solution was diluted about 5 fold with distilled water to quench the reaction between Ca^{++} and alginate. The capsules were washed with distilled water. Co-immobilized capsules were prepared by the same procedure with the addition of powdered activated carbon (Darco, G-60, 100 mesh; Aldrich Co., USA). The diameter of the immobilized and co-immobilized capsules used in this experiment was 3.0 ± 0.2 mm.

Three preparations of the *Arthrobacter* cells were used to test the PCP degradation: (i) non-immobilized cells (1 ml of the seed culture broth), (ii) immobilized capsules, and (iii) co-immobilized capsules. These three preparations were added to 250 ml flasks containing 20 ml of degradation mixture, respectively. For the experiments to determine PCP removal, the degradation mixture contained 3 ml of unlabeled PCP stock solution (final concentration of 117 μM) and 17 ml of mineral solution (the seed culture medium with Na-PCP omitted). The unlabeled PCP stock solution was prepared by dissolving PCP (99% purity, Aldrich Co., USA) in double distilled water and adjusted the pH to 11 with 1 N NaOH to increase PCP solubility. In

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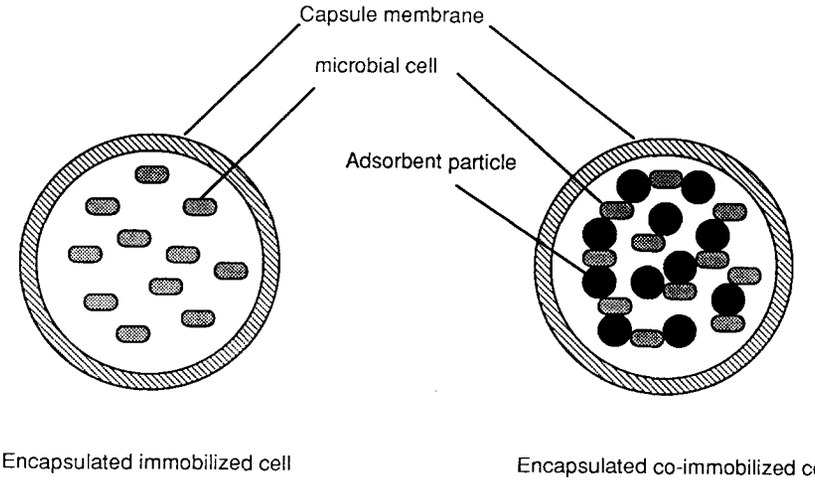

FIG. 1. Schematic diagram illustrating configurations of encapsulated immobilized cells and co-immobilized cells.

the experiments for determination of PCP mineralization, the PCP stock solution was mixed with a required amount of ^{14}C-PCP (Sigma Chemical Co., USA) before being added to the flask. The total radioactivity in each flask was approximately 6.5×10^4 DPM. Flasks containing the degradation samples were maintained in a shaker at 80 rpm and 30°C. PCP concentration in the aqueous phase or $^{14}CO_2$ release was measured at indicated intervals. For the degradation using free cells, optical density of the degradation broth (cell mass concentration) was determined by a spectrophotometer set at 560 nm.

PCP in solution was assayed by HPLC using a modification of the technique of Mikesell *et al.* (14). Separation was accomplished using a Waters Radial-Pak C-18 column. The mobile phase consisted of acetonitrile and 5% acetic acid (8 : 2) and PCP was measured at an absorbance of 300.5 nm (8). Evolved $^{14}CO_2$ was assayed by trapping in 10 ml of an ethanolamine-containing scintillation fluid (15). Degradation mixture was contained in a flask fitted with ports that permitted periodic flushing for $^{14}CO_2$ release. Air was used for flushing at a flow rate of 100–200 ml/min for a total of 20 min. The radioactivity trapped in the scintillation fluid was then determined using a liquid scintillation spectrometer (Beckman LS 3801). Data of $^{14}CO_2$ release were corrected for background radioactivity and for counting efficiency. The latter was determined with an internal standard of ^{14}C-toluene.

In a control experiment in which no inoculum was employed, no decrease in PCP concentration in the mineral medium was found (data not shown). When non-immobilized *Arthrobacter* cells were added to the mineral medium, removal and mineralization of PCP and the cellular growth were observed (Fig. 2). Within 0.5 h, approximately 43% of added PCP was removed from the aqueous phase. Between 0.5 and 22 h, PCP concentration in the medium decreased further but at a relatively low rate. PCP completely disappeared from the aqueous phase at 44 h. Before 22 h, PCP mineralization was slow. The fast PCP mineralization occurred between 22 and 44 h while the removal rate of PCP was also high in this period. When PCP completely disappeared from the aqueous phase at 44 h, about 50% of the PCP was mineralized. The mineralization continued for a much longer period. At 135 h, 77% of the PCP was converted into CO_2. Cellular growth was observed when PCP was degraded. Within 22 h, cell mass concentration increased by two fold, further degradation of PCP led to a little increase in cell mass concentration. At 135 h, cell mass concentration was found to be lower than that at 44 h. These data indicate: (i) *Arthrobacter* cells can readily mineralize PCP, and (ii) there is a significant difference in the kinetic characteristics between PCP removal and PCP mineralization.

To explore characteristics of immobilized *Arthrobacter* cells, experiments using alginate-encapsulated cells for PCP degradation were conducted. Figure 3 shows the profile for PCP mineralization by the encapsulated *Arthrobacter* cells in comparison with that using free cells. The profile characteristics of PCP mineralization by both immobilized cells and non-immobilized cells were similar. PCP mineralization by the encapsulated cells was about 10% higher than that by the free cells for a period of 135 h. The total PCP mineralization by the encapsulated cells for this period was 86%. These results demonstrate that the alginate encapsulation of *Arthrobacter* cells can maintain

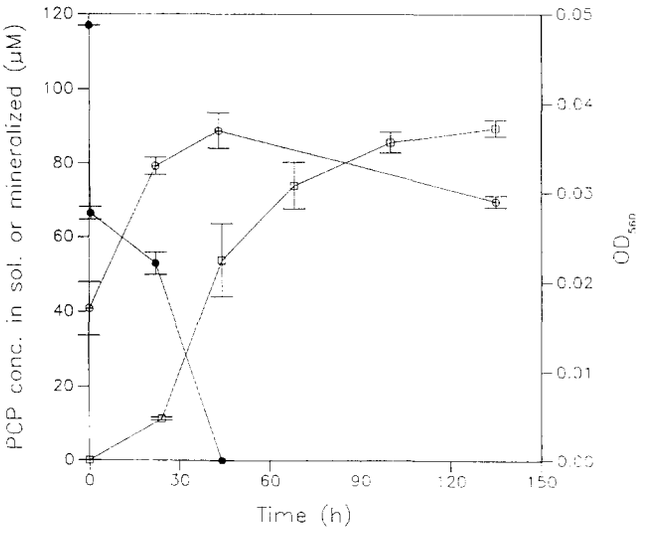

FIG. 2. Time profiles of PCP removal and mineralization and cellular growth in free cell culture. Data are the average of duplicate samples. Symbols: ○, cell growth; □, PCP mineralization; ●, PCP removal.

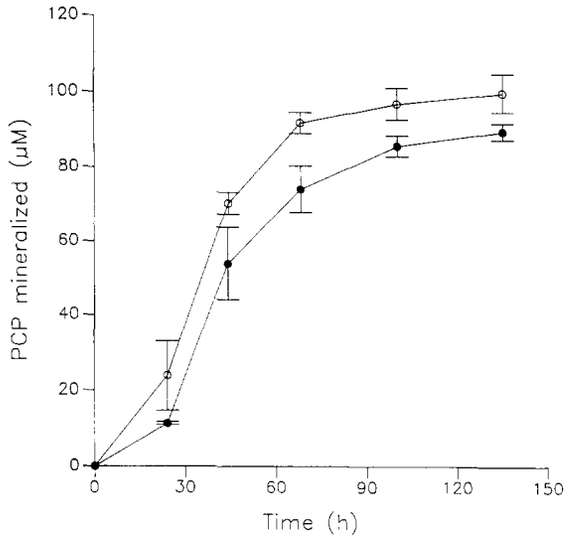

FIG. 3. PCP mineralization by encapsulated cells and free cells. Initial concentration of PCP was 117 μM. Data are the average of duplicate samples. Symbols: ●, free cells; ○, encapsulated cells.

a high biological activity for PCP degradation.

Profiles for PCP removal by co-immobilized cells and free cells are shown in Fig. 4a. The PCP removal by the co-immobilized cells was rapid. Approximately 90% of PCP was removed from the aqueous phase by co-immobilized cells within 2 h. In contrast, only 50% of PCP disappeared from the aqueous phase in 22 h when using free cells. At 458 h, 55% of PCP was mineralized by the co-immobilized capsules, although the rate of PCP mineralization was lower than the rate using free cells (Fig. 4b). These results demonstrate that the adsorbent used in the co-immobilized capsules can rapidly remove PCP from the aqueous phase and the adsorbed PCP is subsequently biodegraded. For the co-immobilized capsules, an obvious increase of cell mass inside the capsules could be observed for the first several days of the biodegradation experiment.

The processes occurring in the co-immobilized capsules may consist of adsorption, desorption and biodegradation (16, 17; Lin, J.-E., Ph. D. Thesis, Univ. of Michigan, Ann. Arbor, 1990). Compared with a biological process, physical adsorption of toxic chemicals by an adsorbent is usually much faster (13). This faster adsorption rate results in majority of a toxic compound being adsorbed onto the adsorbent at the initial period of the operation. Since the pore size of many conventional adsorbents (such as activated carbon) is usually smaller than the size of cells, cells can not fit in the micropores of the adsorbent which form the major adsorbing surface area (16). The adsorbed toxic chemical thus needs to desorb in order to be biodegraded. The desorption rate of the adsorbed toxic chemical is determined by its loading in the adsorbent, as well as the properties of the adsorbent and the toxic compound. In this study, increasing the content of activated carbon in the co-immobilized system from 5 mg to 20 mg for a given cell mass and initial PCP concentration resulted in a significant decrease in PCP mineralization (data not shown), indicating the lower PCP loading in per unit adsorbent leads to lower PCP mineralization. In order to enhance the efficiency of co-immobilized system, search for appropriate desorption operations (such as using suitable adsorbent content and adding surfactant to enhance desorption) and for more suitable adsorbents which allow the cells to function without desorption of adsorbed toxic chemicals may be needed.

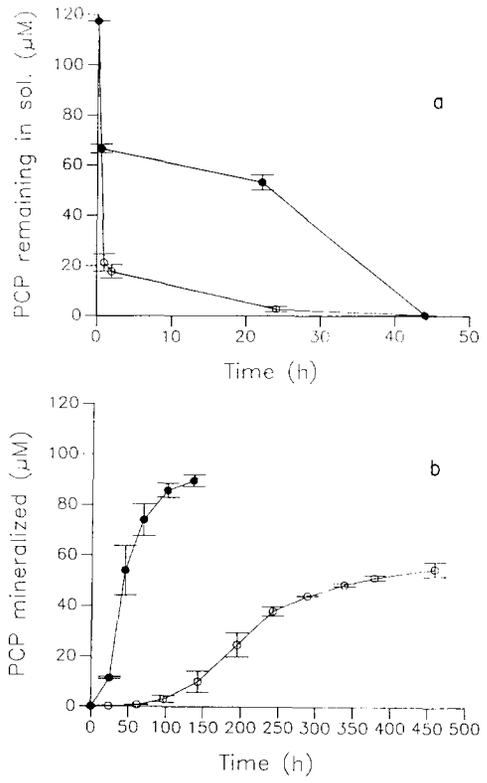

FIG. 4. PCP removal (a) and mineralization (b) by co-immobilized capsules and free cells. Five mg of activated carbon was used in the co-immobilized system. Data are the average of duplicate samples. Symbols: ○, co-immobilized capsules; ●, free cells.

The results from this study have demonstrated the potential of using non-immobilized, immobilized and co-immobilized *Arthrobacter* cells to degrade PCP in aqueous media. Depending on treatment processes, one of these three preparations of *Arthrobacter* cells may be chosen for PCP degradation.

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