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Biodegradation of *p*-cresol by immobilized cells of *Bacillus* sp. strain PHN 1

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Abstract The *Bacillus* sp. strain PHN 1 capable of degrading *p*-cresol was immobilized in various matrices namely, polyurethane foam (PUF), polyacrylamide, alginate and agar. The degradation rates of 20 and 40 mM *p*-cresol by the freely suspended cells and immobilized cells in batches and semi-continuous with shaken cultures were compared. The PUF-immobilized cells achieved higher degradation of 20 and 40 mM *p*-cresol than freely suspended cells and the cells immobilized in polyacrylamide, alginate and agar. The PUF- immobilized cells could be reused for more than 35 cycles, without losing any degradation capacity and showed more tolerance to pH and temperature changes than free cells. These results revealed that the immobilized cell systems are more efficient than freely suspended cells for degradation of *p*-cresol.

Keywords Degradation · Immobilization · *p*-Cresol · Polyurethane foam · *Bacillus* sp. strain PHN 1

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Introduction

Phenolic pollution is characteristic of industries manufacturing pesticides, pharmaceuticals and plastics. *p*-Cresol is component of the total phenolic fraction of toxic waste water from petroleum hydrocarbon refinery. *p*-Cresol is used in disinfectants and fumigants, in the manufacture of synthetics resins, photographic developers and explosives. It is highly toxic, corrosive and causes nervous system depression. *p*-Cresol has been reported to be environmental pollutant because of its widespread use and toxicity.

There are several reports on the degradation of *p*-cresol by free cells of microorganisms (Bayly et al. 1966; Hopper and Taylor 1975; Jones et al. 1993; Tallur et al. 2006). However, there are very few reports on the degradation of *p*-cresol by immobilized cells of microorganisms (O'Reilly and Crawford 1989; Hutchinson and Robinson 1988). Microorganisms could be immobilized in various matrices by entrapment and adsorption methods. Immobilized microbial cells are used for production of useful chemicals and for the degradation of xenobiotics (D'Souza 2002). The main advantages of using immobilized cells of microorganisms are their higher operational stability, increased rate of degradation, high cell density and can be used in continuous reactors (Cassidy et al. 1996). In the present study, the degradation of *p*-cresol by free and immobilized cells of *Bacillus* sp., strain PHN 1 in alginate, agar, polyacrylamide and polyurethane foam (PUF) has

been investigated. The rate of degradation of *p*-cresol by immobilized cells in various matrices were compared with that of free cells in batch and semi continuous degradation.

Materials and methods

Chemicals

p-Cresol and sodium alginate were obtained from SD Fine chemicals, India. Agar, acrylamide, bis-acrylamide, ammonium persulphate were obtained from Himedia, India.

PUF was obtained from local supplier. All other chemicals used in these studies were of analytical grade.

Organism

A *Bacillus* sp. strain PHN 1, originally isolated in our laboratory by *p*-cresol enrichment culture method was used in this studies (Tallur et al. 2006). The organism was maintained on the slants of *p*-cresol mineral salts medium solidified with 2% agar (w/v).

Media and growth conditions

The bacterium grown in Seubert's mineral salt medium (MM 1) supplemented with 0.2% *p*-cresol (w/v) as described previously (Tallur et al. 2006), was used for inoculation. The medium (MM2) used for degradation studies contained K_2HPO_4, 0.38; $MgSO_4 \cdot 7H_2O$, 0.12; KNO_3, 1.0 and $FeSO_4 \cdot 7H_2O$, 0.05 g l^{-1}. The pH was adjusted to 7.0 and *p*-cresol (20 and 40 mM) was added after sterilization of the medium.

Immobilization of whole cells

Bacillus sp. strain PHN 1 was grown in the MM 1 medium containing 20 mM of *p*-cresol. The cells were harvested during the mid.-logarithmic growth phase by centrifugation at 8,000×*g* for 10 min at 4°C and washed twice with 50 mM phosphate buffer, pH 7.0. The cells were immobilized in different matrices namely; Polyurethane foam (PUF), polyacrylamide, alginate and agar. The PUF immobilization was carried out by the method of Hall and Rao (1989). The alginate entrapment of the cells was performed according to the method of Bettman and Rehm (1984). The agar and polyacrylamide entrapment procedure were performed according to the Jonathan (1988).

Degradation conditions

Batch degradations

The batch degradations were performed for both freely suspended cells and immobilized cells in various matrices. The batch degradation with high cell population was performed. For freely suspended cell culture, exponentially growing cells were added to 500-ml Erlenmeyer flasks containing 100 ml of MM 2 medium containing *p*-cresol (20 and 40 mM) along with suitable controls. The degradation process was carried out at 30°C on a rotary shaker at 150 rpm. The samples from the culture broth were withdrawn under sterile conditions at different incubation period for the analysis of residual *p*-cresol.

Semi-continuous degradation

To determine the longevity of degrading activity of immobilized cells in various matrices, repeated batch degradations were carried out. After each cycle of incubation (96 h/cycle), the spent medium was decanted and beads/foam cubes were washed with sterile water and transferred into fresh MM2 medium containing *p*-cresol. The degradation process was carried out under identical conditions and the spent medium was analyzed for the residual *p*-cresol.

Analytical methods

p-Cresol concentrations were determined by HPLC using a reversed phase HPLC with a 5 µ spherisorb-ODS (C18) column (25 cm × 4.6 mm). Acetonitrile-phosphate buffer (50 mM pH 7.0) mixture (60:40, v/v) was used as mobile phase, with a flow rate of 1 ml/min. The absorbance of compound was measured at 280 nm.

Results

Degradation of *p*-cresol by free and immobilized cells of *Bacillus* sp. PHN 1 in batch cultures

The degradation of 20 and 40 mM of *p*-cresol was carried out in batch cultures both by freely suspended

cells and cells immobilized in alginate, agar, polyacrylamide and PUF and their results are given in Fig. 1a and b. The free cells degraded 12 mM of *p*-cresol after 120 h of incubation from an initial 20 mM *p*-cresol. But only 8 mM *p*-cresol was degraded when the initial concentration was increased to 40 mM. No further degradation of *p*-cresol was observed even after incubating for prolonged periods (>144 h). The PUF-immobilized cells completely degraded 20 mM *p*-cresol after 120 h of incubation and 38 mM of *p*-cresol was degraded from an initial 40 mM of *p*-cresol. The cells encapsulated in polyacrylamide, alginate and agar degraded 17, 14 and 13 mM, respectively, from the initial concentration of 20 mM *p*-cresol (Fig. 1a). When the initial concentration was 40 mM, the same immobilized cells degraded 30, 28 and 25 mM of *p*-cresol, respectively (Fig. 1b).

Semi-continuous degradation of *p*-cresol by immobilized cells of *Bacillus* sp. PHN 1

The repeated batch degradation of *p*-cresol by cells immobilized in PUF, polyacrylamide, alginate and agar were carried out at two different concentrations of *p*-cresol (20 and 40 mM) for 120 h and their results are shown in Fig. 2a and b. The PUF-immobilized cells can be reused for 35 cycles without losing the *p*-cresol degrading capacity, when the initial concentrations of *p*-cresol were 20 and 40 mM. In contrast, polyacrylamide, alginate and agar immobilized cells could be reused for 25, 18 and 15 cycles, respectively with 20 mM *p*-cresol (Fig. 2a). However, when the initial concentration of *p*-cresol was increased to 40 mM, these immobilized cells could be reused with a decreased rate of degradation of *p*-cresol. These observations suggest that lower concentration of *p*-cresol (20 mM) could

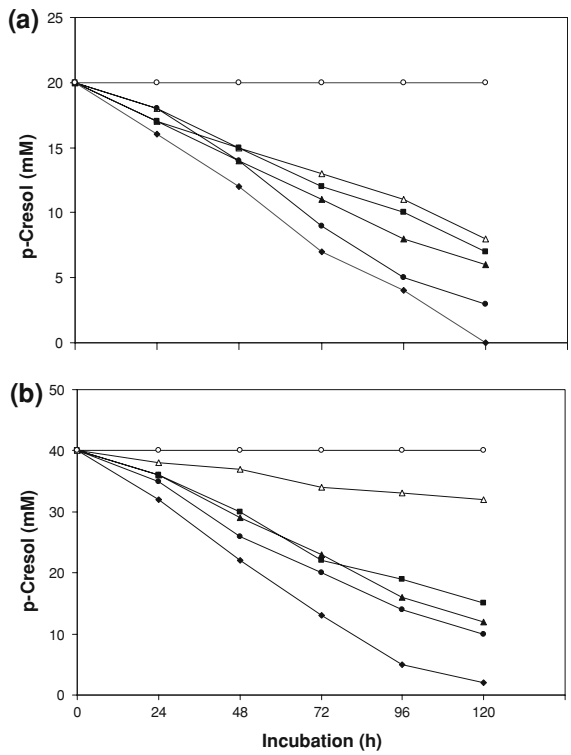

Fig. 1 Degradation of *p*-cresol at 20 mM (**a**) and 40 mM (**b**) concentration in batch cultures by cells of *Bacillus* sp. PHN 1 immobilized on PUF (-◆-), polyacrylamide (-•-), alginate (-▲-), agar (-■-), freely suspended cells (-Δ-) and uninoculated control (-◦-)

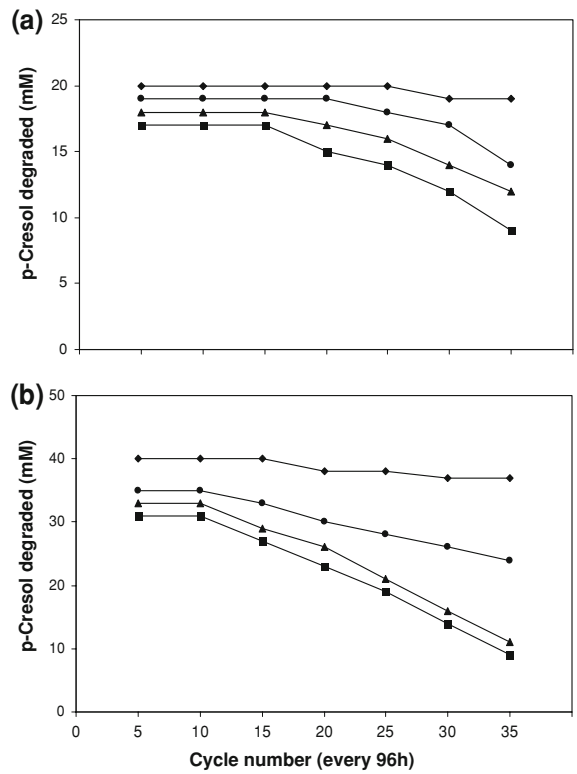

Fig. 2 Semi-continuous degradation of *p*-cresol at 20 mM (**a**) and 40 mM (**b**) concentration by cells of *Bacillus* sp. PHN 1 immobilized on PUF (-◆-), polyacrylamide (-•-), alginate (-▲-), agar (-■-)

be fed at much higher frequency than higher concentrations of *p*-cresol (40 mM).

Effect of pH and temperature on degradation capacity of PUF-immobilized cells

The PUF-immobilized cells can be stored for 5 months at 4°C without decrease in their degrading capacity. The effect of temperature on the rate of degradation of *p*-cresol by PUF-immobilized cells showed activity at temperatures between 20 and 45°C. But, the free cells showed activity between 28 and 30°C. The effect of pH on the degradation of *p*-cresol by immobilized cells showed that variation of initial pH between 5.0 and 10.0 had no effect on degradation. However, the free cells showed activity in the pH between 6.0 and 7.5 only.

Discussion

The rates of degradation of *p*-cresol by immobilized cells of *Bacillus* sp. strain PHN 1 in various matrices, PUF, alginate, polyacrylamide and agar were compared with that of freely suspended cells in batches and semi-continuous with shaken cultures. The data obtained from immobilized cells in all the matrices with batch culture suggested that the rate of degradation of *p*-cresol, even at higher concentration (40 mM) was much higher than that with freely suspended cells. The enhanced degradation by immobilized cells was probably due to the accelerated reaction rates caused by high local cell density in or on the immobilized matrix. Immobilization also provides a kind of membrane stabilization, which may be responsible for the protection of cells and better degradation rates in immobilized cell (Cassidy et al. 1996; Hall and Rao 1989).

The results of semi-continuous degradation suggested that the PUF and polyacrylamide immobilized cells retained the *p*-cresol degradation capacity for a longer period and they could be reused for 35 and 25 cycles, respectively. When the initial concentration of *p*-cresol was increased to 40 mM, the PUF immobilized cells could be reused without losing their degrading capacity. The immobilized cells in other matrices could also be reused but with decreased rate of degradation of higher concentration (40 mM). The enhanced degradation of *p*-cresol by PUF immobilized cells may be due to porosity and mechanical strength. The storage stability and activity of cells encapsulated in PUF were better than those cells encapsulated in other matrices. The alginate and agar encapsulated cells showed lower degradability of *p*-cresol with increased cycle number. The mechanical instability and gradual cell leakage from these beads decreased the degradation rate with increasing cycle number (Trevors et al. 1992). The PUF-immobilized cells showed more tolerance to pH and temperature changes than free cells.

The present study indicated that more effective degradation of *p*-cresol at high concentration could be achieved by immobilized cells of *Bacillus* sp. strain PHN 1 when compared to freely suspended cells. The PUF-immobilized cells have a higher degradation rate than polyacrylamide, alginate and agar. The operational stability and longevity of cells immobilized in PUF is significantly better than the other matrices. Thus the immobilized microbial technology is an extremely versatile approach that can be used for degradation of toxic pollutants from the industrial effluents.

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