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Stable degradation of catechol by *Pseudomonas* sp. strain NGK1 encapsulated in alginate and polyurethane foam

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Catechol is a terminal metabolite formed during the degradative pathways of various aromatic compounds, generally pollutants. *Pseudomonas* sp. strain NGK1 (NCIM 5120), a soil microbe, is capable of utilizing catechol as the carbon and energy source. This bacterium was encapsulated in alginate and polyurethane foam (PUF). The degradation rate of 20 and 40 m*M* of catechol in shaken batch cultures, repeated batch cultures, and continuous degradation in a packed bed reactor by free cells was compared with the degradation rate by alginate-and PUF-immobilized cells. The degradation for 72 hrs incubation in batch cultures was: free cells, 6 and 4; alginate-encapsulated cells, 15 and 18; and PUF-encapsulated cells, 18 and 30 m*M* catechol. Further, the alginate- and PUF-encapsulated cells were used in repeated batch degradation of catechol. Alginate- and PUF-encapsulated cells were found more efficient for the degradation than free cells. Continuous degradation in a packed bed reactor was also investigated. The efficiency of both the immobilized systems for the degradation of catechol was examined.

Keywords: immobilization, degradation, catechol, alginate, polyurethane foam, *Pseudomonas* sp. strain NGK1

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Introduction

Naturally occurring hydroxylated aromatic compounds are derived from decaying plant materials. Catechols, intermediates in the degradation of most of the aromatic compounds by aerobic bacteria[1-3], are used as industrial reagent in the manufacturing of dyes, rubber, plastics, pharmaceuticals, and cosmetics, in the production of insecticides, in metal plating and in coal refining[4,5]. They are present in cigarette smoke and has been detected at low levels in ambient air and water. Catechol causes gene mutation in mammalian cells *in vitro*, and may cause carcinogenicity in experimental animals[6]. Irritant action of catechol can lead to dermatitis and other dermal lesions. From a variety of potential degrader microorganisms of catechol[1,5,7,8], *Pseudomonas* sp. strain NGK1 is capable of utilizing catechol as the sole source of carbon and energy[9]. However, degradability of the free bacteria decreases in liquid broth medium when used for long period. Therefore, cell immobilization is one of the most attractive alternatives.

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Whole cell immobilization is gaining considerable attention in biotechnology ranging from biological production of useful chemicals to the degradation of xenobiotics[10]. The main advantages in the use of immobilized whole microorganisms are their higher operational stability, ease of use in continuous reactors, high-cell density and ability to scale-up[11,12]. This communication reports biodegradation of catechol by free and immobilized cells of *Pseudomonas* sp. strain NGK1 in alginate and polyurethane foam (PUF). The rates of degradation of catechol by immobilized cells in these matrices were compared with that of free cells in batches, repeated batches and continuous degradations. The immobilized cells were reused for the efficient degradation of catechol in shaken cultures. The impact of dilution rate in a continuous system on degradation of catechol has also been studied.

Materials and Methods

Chemicals and Bacterial Strain

Catechol was procured from Aldrich, USA and sodium alginate from S D Fine Chemicals, Mumbai, India. PUF was purchased from local supplier. The foam was elastic and its low density (15 kg m^{-3})

provided a large surface area. Other chemicals were of analytical grade.

Pseudomonas sp. strain NGK1 (NCIM 5120) was originally isolated in this laboratory by the naphthalene enrichment culture method[3]. It was maintained on the slants of a medium containing naphthalene (0.05% w/v) and mineral salts (MM1) solidified with 2% agar (w/v).

Media and Culture Conditions

The bacterium was cultivated in MM1 broth medium (*p*H 7.0) containing: K_2HPO_4, 6.3; KH_2PO_4, 1.83; $(NH_4)_2NO_3$, 1.0; $MgSO_4.7H_2O$, 0.1; $MnSO_4.H_2O$, 0.1; $CaCl_2.2H_2O$, 0.1; $FeSO_4.7H_2O$, 0.1 and $NaMoO_3.2H_2O$, 0.005 g l^{-1}. The medium was autoclaved, supplemented with 0.05% (w/v) catechol, which was dissolved in water and filter sterilized, and inoculated with bacterial strain.

The MM2 medium (*p*H 7.0) contained: K_2HPO_4, 0.38; $MgSO_4.7H_2O$, 0.1; KNO_3, 1.0 and $FeSO_4.7H_2O$, 0.05 g l^{-1}. Catechol (20 and 40 m*M*) was added after sterilization of the medium.

Immobilization of Whole Cells

Pseudomonas sp. strain NGK1 cells, harvested during the exponential phase of growth by centrifugation at 5000 × *g* for 10 min at 5°C, were washed twice with potassium phosphate buffer (50 m*M*, *p*H 7.0) and then encapsulated in either PUF or alginate[13].

Catechol Degradation Conditions

Batch Degradations

The free cells (2×10^{11} cfu ml^{-1}) of exponential growth phase were inoculated to 100 ml of MM2 medium contained in 250-ml conical flask at catechol loadings of 20 and 40 m*M*. The batch degradations with the immobilized cells were performed by taking 8 g wet beads of alginate-encapsulated cells (3×10^{11} cfu g^{-1}) and 5 g of PUF-encapsulated cubes (2.8×10^{11} cfu g^{-1}), into 250 ml conical flasks containing 100 ml of MM2 medium loaded with catechol (20 and 40 m*M*). The degradation process was carried out in a mild dark room at ~32°C on a rotary shaker at 150 rpm. Samples from the culture broth were withdrawn under sterile conditions at 12, 24, 36, 48, 60 and 72 hrs of incubation for the analysis of residual catechol.

Repeated Batch Degradations

The long-period stability of catechol degradation by immobilized cells in both the matrices was established by studying repeated batch degradations. After each cycle of incubation (72 hrs/cycle), the used medium was decanted and beads were washed with sterile water and transferred into a fresh sterile MM2 medium containing catechol. The degradation process was carried out and the residual catechol in the spent medium was analyzed.

Continuous Degradation in a Packed Bed Reactor

The degradation of catechol (20 and 40 m*M*) was performed by the supplementation of catechol-MM2 medium into the packed bed reactor at different dilution rates (Fig. 1). Design of packed bed reactor and the conditions were maintained[13]. The reactor was packed with 120 g wet alginate-encapsulated beads (3×10^{11} cfu g^{-1}) and 40 g PUF-encapsulated foam cubes (2.8×10^{11} cfu g^{-1}) to a height of 20 cm with a working volume of 100 ml. The cells (2×10^{11} cfu ml^{-1})were suspended in catechol-MM2 medium and this cell suspension was placed in the reactor. The column was attached to a reservoir of the catechol-MM2 medium, which was supplied through a peristaltic pump (Maclins, India). The effluent was continuously collected from the side arm situated just above the packed bed and analyzed for the residual catechol[14]. The evaporation of catechol from the sterile control was negligible.

The dilution and degradation rates calculated as were:

$$\text{Dilution Rate (D)} = \frac{\text{Flow rate of influent into the packed bed reactor (ml h}^{-1})}{\text{Volume of influent in the packed bed reactor}}$$

Degradation Rate (R) = [(Concentration of substrate in influent) – (Concentration of substrate in effluent)] X Dilution rate

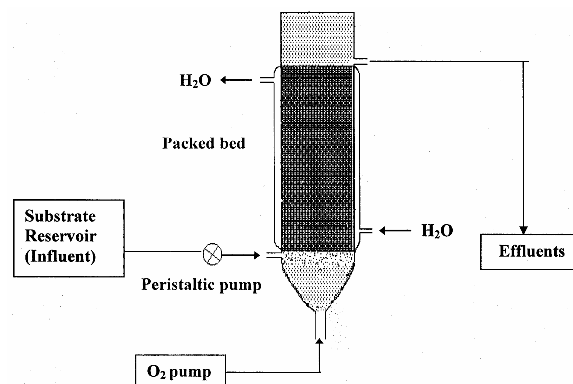

Fig. 1—A schematic representation of packed bed reactor

Results and Discussion

Degradation of Catechol by Free and Immobilized Cells in Shaken Batch Cultures

In all the experiments, the results were the representatives of average of duplicate sets. The free cells degraded 6 m*M* of catechol after 72 hrs of incubation from an initial 20 m*M* load, and only 4 m*M* was degraded when the initial concentration was increased to 40 m*M*. Even after incubating for prolonged periods (>140 hrs), further degradation of catechol had not occurred. The cells encapsulated in alginate degraded 16 and 18 m*M* of catechol, whereas PUF-immobilized cells degraded 18 and 30 m*M* after 72 hrs of incubation from an initial 20 and 40 m*M* load, respectively (Figs 2a and b). The free cells degraded catechol only at the lower initial loading (20 m*M*). The degradation rate decreased at a higher initial catechol loading (40 m*M*). The data obtained from immobilized cells of both the matrices suggested that the rate of degradation of catechol, even at higher loadings (40 m*M*), was higher than that of free cells.

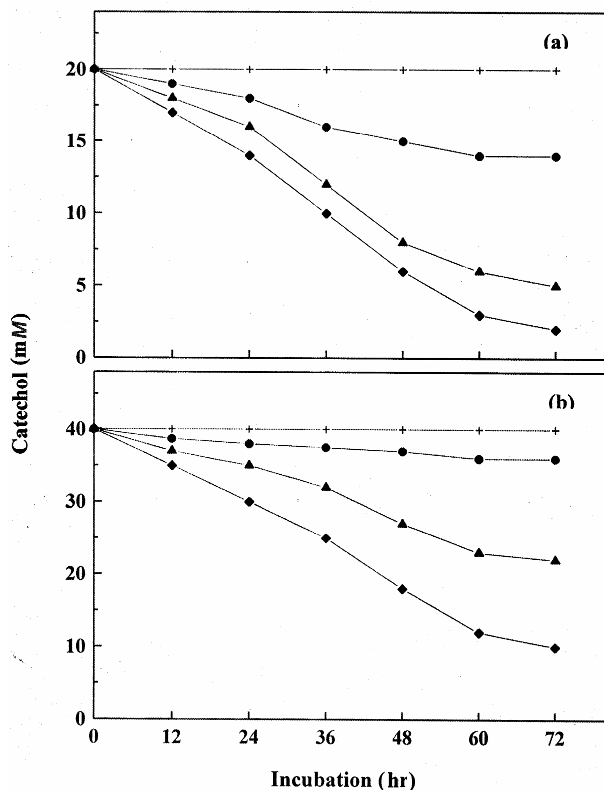

Fig. 2—Degradation of (a) 20 m*M* and (b) 40 m*M* catechol in shaken batch cultures (+), (●), (▲) and (♦) are uninoculated control, free cells (2×10^{11} cfu ml^{-1}), alginate (3×10^{11} cfu g^{-1}) and polyurethane foam (2.8×10^{11} cfu g^{-1}) immobilized cell systems, respectively.

The enhanced degradation by immobilized cells was probably due to the accelerated reaction rates caused by the high local cell density in or on the immobilized matrix. Immobilization also provides a kind of membrane stabilization, which is assumed to be responsible for the protection of cells and better degradation rates in immobilized cells[12,13].

Repeated Batch Degradation of Catechol by Immobilized Cells

For an initial 20 m*M* of catechol, the alginate-encapsulated cells could be reused for a maximum of 12 cycles; a decrease in the degradation capacity was noted thereafter (Fig. 3a). The PUF- encapsulated cells, however, could be reused for more than 20 cycles without losing their degrading capacity. When the initial concentration of catechol was increased to 40 m*M*, the immobilized cells could also be reused but the percentage of catechol degradation was lower (Fig. 3b). The degradation by alginate-encapsulated cells was shown to decline after 9 cycles, but PUF-encapsulated cells retained the degradation capacity. PUF-encapsulated cells retained their degrading

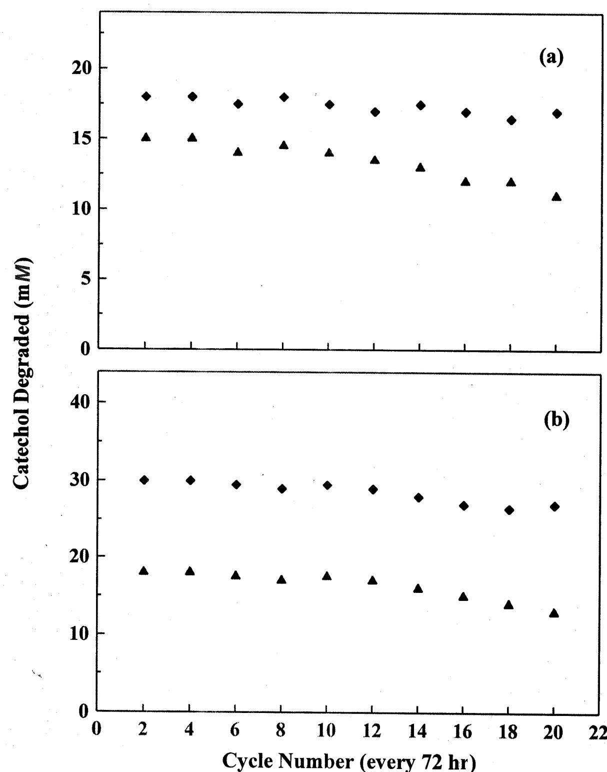

Fig. 3—Repeated batch degradation of catechol at 20 m*M* (a) and 40 m*M* (b) concentration (▲) and (♦) are alginate (3×10^{11} cfu g^{-1}) and polyurethane foam (2.8×10^{11} cfu g^{-1}) immobilized cell systems, respectively.

ability for a longer period (50 days) than the alginate-encapsulated cells. The enhanced degradation of catechol by PUF-immobilization of cells is probably due to porosity, and mechanical strength. The storage stability and microbial activity of encapsulated cells in PUF were better than those of cells encapsulated in alginate[15,16]. The alginate-encapsulated cells showed a lower degradability of catechol with an increased cycle number, even though there was lower (10^{3-4} cfu ml^{-1}) cell leakage. This may be due to the mechanical instability of alginate-encapsulated beads, which finally led to cell leakage from these beads thus decreasing the degradation rate with the increased cycle number[17].

Continuous Degradation of Catechol by Free and Immobilized Cells

An initial 20 m*M* catechol in a continuous system by free cells increased degradation rate up to 1.5 h^{-1} but decreased at higher dilution rates (2 h^{-1}). The cells encapsulated in alginate and PUF showed that the degradation of catechol increased with an increase in the dilution rate up to 1.5 h^{-1}, and even at dilution rate 2 h^{-1} there was no significant decrease in the degradation rate (Fig. 4a). When the initial concentration of catechol increased to 40 m*M*, the degradation rate by free cells showed no remarkable increase with an increase in the dilution rate from 0.5 to 1 h^{-1}, and at the dilution rate above 1 h^{-1} the degradation rate did not alter, and showed no remarkable degradation. In contrast, the immobilized cells showed an increased degradation rate with an increase in the dilution rate from 1.0 h^{-1} to 1.5 h^{-1}, while a decrease in the degradation rate at dilution rate 2 h^{-1} was observed in alginate-encapsulated cells. However, the PUF-encapsulated cells, at a dilution rate of 2 h^{-1}, still showed a higher degradation rate than the alginate-encapsulated cells (Fig. 4b). Thus, the increase in the dilution rate increased the degradation rate , but only when it was kept below 1.5 h^{-1} for 20 m*M* and 1 h^{-1} for 40 m*M* of catechol, and beyond these ranges the degradation rate decreased (Figs 3a and b). The immobilized system is better for degrading catechol than the free cells at dilution rates above 1 h^{-1}. Superiority of the immobilized cell systems is more pronounced with the higher dilution rate. This is probably based on the total number of viable cells available for bioreactor compared to free cells. The decrease in the degradation rate of free cells at higher dilution rates was probably due to the formation of flocks at these higher dilution rates and

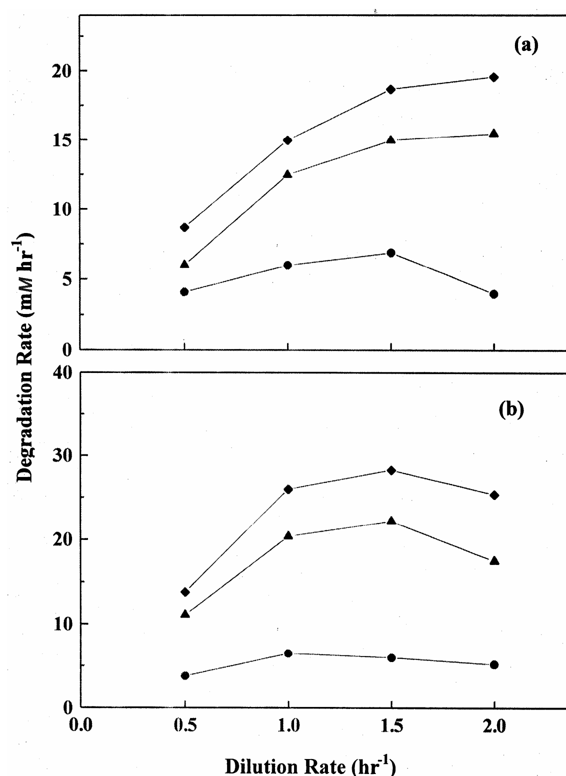

Fig. 4—Continuous degradation of catechol at 20 m*M* (a) and 40 m*M* (b) concentration in a packed bed reactor. (●), (▲) and (♦) are free cells (2×10^{11} cfu ml^{-1}), alginate (3×10^{11} cfu g^{-1}) and polyurethane foam (2.8×10^{11} cfu g^{-1}) immobilized cell systems, respectively.

the washout of unflocculated cells contributing to a loss of degradative capability. Present findings matched with the earlier reports for the degradation of phenol by immobilized *Pseudomonas putida*[18], the degradation of 2-methylnaphthalene[19], and in the degradation of salicylic acid by this bacterium[16].

The results reaffirm the utility of using alginate and PUF-encapsulated cells for degradation of toxic pollutants. Operational stability and longevity of cells encapsulated in PUF was significantly better than alginate. Biodegradation of industrial effluents rich in organic pollutants with immobilized bacterial strains in PUF is an extremely versatile approach that can be used in detoxification of different pollutants for longer periods than with non-immobilized microorganisms, provided the process is made economical and convenient for use on a large-scale.

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