IMMOBILIZED MUTANT STRAIN OF *PSEUDOMONAS PICTORUM* FOR THE DEGRADATION OF PHENOL IN WASTEWATER

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Phenols present in the wastewater discharged from many phenol based industries act as antimicrobial agents and hence biological treatment of phenol laden wastewater to a standard level prescribed by the Environmental Protection Agency is difficult. In the present investigation, a biocatalyst was developed by immobilizing cells of a mutant strain of *Pseudomonas pictorum* (MU 174) onto rice bran-based activated carbon. Phenol in wastewater was observed to be degraded up to 3 g/l under batch studies. A removal of 99.9% of phenol in domestic wastewater was achieved at a hydraulic retention time (HRT) of 24 h with a biocatalyst packed reactor under continuous flow studies. The biocatalyst had a reasonable shelf life after its preparation and had the desirable recycle capacity.

Accumulation of alien chemicals that are unable to be assimilated creates toxicity both for flora and fauna and for human beings who eat fish which feed on food containing these chemicals. Phenol is one such chemical that causes alarm by this process of accumulation (17). Phenols are among the most common water pollutants, since it is one of the most widely used organic compound in existence (1). Phenol is the raw material for a variety of synthetic organic compounds like dyes, perfumes, pesticides etc. Phenols and substituted phenols are reported to be present in the wastewater from industries like textile, timber, paper and pulp etc. (9, 14). Wastewater containing phenol needs careful treatment before it is discharged into the receiving water bodies especially if such water is used for drinking purposes.

Phenol in wastewater can be removed either by physical, chemical or biological methods. Biodegradation using micro-organisms is versatile, inexpensive and can

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potentially turn a toxic material into harmless end products. If properly designed and operated, biological processes can realize total oxidation of organic matter so that there are no sludges that must be eradicated as a result of treatment. This is in contrast to physical or chemical methods that usually produce residues that must be eradicated by encapsulation or other similar methods (3). The versatility and adaptability that microbial degradation of hazardous wastes have demonstrated, makes it tempting to promise potentially too much in finding immediate solutions to our pollution problems and in meeting tighter effluent discharge standards. Aerobic Gram negative *Pseudomonas* (4) are the most widely used bacterial strains for treating various organic compounds. In addition to these strains various other microbial strains have also been studied with regard to their capacities to degrade phenol or phenolic compounds. These include *Bacillus* (5,11), *Acinetobacter* (12), *Candida* (15,18), *Trichosporon* (10,16), *Rhodotorula* (13), and *Fusarium flociferum* (2).

In the present investigation, five *Pseudomonas* species were screened from which *P. pictorum* was selected due to its higher tolerance limit. *P. pictorum* was treated with NTG and a mutant strain designated as MU 174 was selected after screening. To improve the degradation potential as well as to prevent washout of cells, MU 174 was immobilized onto rice bran-based activated carbon. Phenol degradation by the mutant strain of *P. pictorum* (MU 174), and the biocatalyst prepared by immobilizing MU 174 onto activated carbon, was carried-out in nutrient medium. Economically viable and feasible sewage medium replaced the nutrient medium under continuous column studies. The storage of the biocatalyst and repeated usability of the biocatalyst were also studied.

MATERIALS AND METHODS

Source of micro-organism. Five *Pseudomonas* species were obtained from the culture collection, NCL, Pune, India. Namely, 1) *P. resinovorans* (NCIM 2599), 2) *P. aeruginosa* (NCIM 2074), 3) *P. desmolyticum* (NCIM 2028), 4) *P. cruciviae* (NCIM 2004), and 5) *P. pictorum* (NCIM 2077). Among these, *P. pictorum* showed greater resistance to very high concentrations of phenol and thus was selected for further studies (Table 1).

Table 1. Selection of *Pseudomonas* strains for phenol degradation.

Serial No.	Organism	Phenol concentration in mg/1,000 ml								
		250	500	750	1,000	1,250	1,500	1,750	2,000	>2,000
1.	<i>Pseudomonas pictorum</i> (NCIM 2077)	+	+	+	+	+	+	+	+	+
2.	<i>P. cruciviae</i> (NCIM 2004)	+	+	—	—	—	—	—	—	—
3.	<i>P. desmolyticum</i> (NCIM 2028)	+	+	—	—	—	—	—	—	—
4.	<i>P. resinovorans</i> (NCIM 2599)	+	+	+	+	—	—	—	—	—
5.	<i>P. aeruginosa</i> (NCIM 2074)	+	+	+	+	—	—	—	—	—

+, presence of growth; —, absence of growth.

Selection of mutant. After extensive screening, a mutant strain of *P. pictorum* MU 174 was selected according to the method of Chitra et al. (7).

Preparation of "biocatalyst." The biocatalyst was prepared by immobilizing cells of strain MU 174 onto rice bran-based activated carbon according to the method of Chitra et al. (6). The optimum conditions needed for the immobilization of MU 174 onto activated carbon are shown in Table 2.

Batch studies. The composition of the nutrient medium used for batch studies was the same as that used by Chitra et al. (7). Phenol was estimated after removing the cells by filtration using 4-amino-antipyrine according to the standard method of analysis (8). Bacterial growth in terms of cell mass was determined by measuring the optical density at a wavelength of 600 nm.

Continuous column studies. A diagram of the glass reactor used for the continuous treatment process is shown in Fig. 1. The activated carbon loaded with the cells was packed to a volume of 250 ml in a glass column. The degradation of phenol by the loaded cells was studied under different hydraulic retention time (HRT) of 6, 12, 24, and 48 h. Four column runs were carried out at each HRT.

The feed for the continuous fermentation reactor was prepared by mixing a

Table 2. Optimum conditions for immobilization.

Cell concentration	12.8×10^4 cells/ml
Temperature	30–34°C
pH	6–9
Specific adsorption	9.5×10^5 cells/g
Glucose	0.01 g/l
Nitrate	0.1 g/l

Fig. 1. Schematic flow diagram of continuous column reactor for the treatment of phenol laden wastewater.

known concentration of phenol in a sewage medium. The preparation of sewage medium was according to that of Chitra et al. (7). The effect of the shelf-life of immobilized cells was tested by washing the column with distilled water.

RESULTS AND DISCUSSION

Batch studies on degradation of phenol by the biocatalyst

Figure 2 shows a phenol concentration-time-degradation diagram of the mutant *P. pictorum* immobilized onto rice bran-based activated carbon, i.e. the biocatalyst. The time required for start of degradation (t_s), time required for 100% degradation (t_f) and time for intermediate percentage of phenol degradation at various phenol concentration can be obtained from the diagram. To the left of the t_s curve, the phenol degradation does not occur and to the right of the t_f curve phenol in the medium is negligibly small, t_{50} corresponds to time for 50% degradation of phenol. The biocatalyst initiated phenol degradation faster and was able to degrade 3,000 mg/l phenol. However, free cells of mutant MU 174 were able to degrade only up to 2,000 mg/l phenol.

Studies on degradation of phenol under the continuous treatment process using biocatalyst

Application of specific cultures under field conditions become impracticable as they need specific nutrient mediums, whose degradation products also contribute to the pollution load of wastewater. Hence, an economically-viable and environ-

Fig. 2. A phenol concentration-time-degradation diagram for immobilized MU 174 cells in nutrient medium at 31°C.

Table 3. Characteristics of phenol-laden wastewater before and after aerobic treatment using immobilized MU 174 cells packed in an up-flow reactor.

Parameter	Before treatment	After treatment	% Removal
pH	8.7	8.2	—
Alkalinity	120	110	—
COD	870	109	87
BOD	270	58	79
Phenol	500	0.5	99.9
Dissolved solids	345	40	88
Volatile solids	163	20	88

Conditions of phenol loading rate (0.5 kg/m^3 , day, hydraulic retention time 24 h, dissolved oxygen in feed solution 8 mg/l) were used. COD, chemical oxygen demand; BOD, biological oxygen demand.

mentally-safe medium such as sewage was used for treating phenolic wastewater under continuous column studies.

A glass column packed with the biocatalyst was used for the continuous flow system at set HRT values. The results presented in Table 3 indicate that about 99.9% phenol degradation was achieved in about 24 h HRT. The removal of chemical oxygen demand (COD) was in proportion to the removal of phenol in the sewage medium. At optimum HRT, the COD reduction was only 87% (Table 3), while phenol removal was about 99.9%. The residual COD in treated wastewater was mainly due to the organic compounds contained in the sewage medium, and partly due to undegradable phenol. Removal of 88% dissolved solids (Table 3) was accompanied with elimination of phenol at optimum HRT.

Storage stability and repeated usability of the biocatalyst

If immobilization of cells was only onto the surface of the carrier material, the cells might be sloughed off by attrition during continuous treatment leading to a decrease in efficiency of the biocatalyst on phenol degradation. It was expected that immobilization of the organism was in pores of the activated carbon, which was explored by washing the column after each run (24 h, set HRT). A negative result was obtained in the biological analysis of wastewater from the column. It is clear from Fig. 3, that the behaviour of the immobilized cell reactor for the degradation of phenol was not altered even with repeated usage. Therefore, the immobilized biocatalyst was subsequently used for phenol removal. The experiment did show that the biocatalyst had maintained its efficiency in percentage removal of phenol in wastewater.

The immobilized cells are advantageous in wastewater treatment due to easy transportation and storage. During storage and transportation, the immobilized organism is likely to be affected due to environmental alteration and thus the efficiency deteriorates. This fact is considered to be important while advocating the immobilized system for biodegradation or bioconversion of organics in wastewater. In the present study, as seen in Fig. 4, the same immobilized cells were able to

Fig. 3. Effect of washing of immobilized MU 174 cells on to activated carbon on degradation of phenol in sewage medium.

Fig. 4. Effect of storage of immobilized MU 174 cells on to activated carbon on degradation of phenol in sewage medium.

maintain their efficiency for three months. The efficiency of the immobilized cells was tested every month and remained the same as shown in the figure.

The storage stability and repeated usability of the biocatalyst, and the economically-viable and environmentally-safe sewage medium used for phenol degradation in a continuous column reactor packed with the biocatalyst, are considered to be the unique points of this method.

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