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Comparative pentachlorophenol degrading potential of two bacterial consortia isolated from tannery and pulp and paper mill effluent sludge

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Abstract: *Pentachlorophenol (PCP) is highly toxic and recalcitrant compound which is frequently and extensively being used in a variety of industries (wood, cardboard, leather etc.) and agriculture as a preservative and biocide. The PCP degrading bacterial consortia isolated from tannery and pulp and paper mill effluents were enriched in a chemostat containing mineral salt media. The bacterial strains obtained from chemostat were characterized as two species each of Pseudomonas and Arthrobacter and one species of Sphingomonas from Tannery effluent and three species of Pseudomonas and one species of Flavobacterium from pulp and Paper mill effluent. The enriched bacterial consortia were applied for the removal of PCP in a lab-scale bioreactor and it was observed that bacterial consortium isolated from tannery effluent have more capability to degrade PCP as it removed 75% PCP within 9 days than the bacterial consortium isolated from pulp and paper mill effluent showing only 60% removal within same time.*

Key Words: Bioreactor, Chemostat, Consortium, Enrichment, PCP removal, Tannery effluent.

Introduction

Pentachlorophenol (PCP) is a highly chlorinated recalcitrant and xenobiotic compound. It is being frequently used as a preservative in leather, wood and cardboard industries and has a variety of applications in agricultural and domestic fields (Yu and Ward 1996; Dercova *et al.*, 2004). The average annual worldwide production of PCP has been estimated to be 57,000 tons (Wang *et al.*, 2000) and its contamination limit has been set at 0.001 mg/l for drinking water (Nagyun *et al.*, 2002). The USEPA has listed PCP as priority pollutant and the soils contaminated with PCP are considered as hazardous (Dercova *et al.*, 2004). PCP is toxic in low concentrations affecting flora and fauna and poses threat to human health (Thakur *et al.*, 2001; Habash *et al.*, 2002; Wolski *et al.*, 2006). It is widely believed that PCP exerts its toxic effects, at least in part, by uncoupling mitochondrial oxidative phosphorylation, thereby causing accelerated aerobic metabolism and increased heat production (Weinbach and Garbus, 1965). The major target organs of PCP toxicity are liver, kidney with toxic effects occurring at low doses.

Due to its abundant use and improper disposal, PCP has contaminated the environment and has become a pollutant of high concern (Wang *et al.*, 2000; Miller *et al.*, 2004). Thus the current need is to remove this pollutant from the environment. Since several physico- chemical techniques are available, but no one among these methods is feasible in mineralizing the compound completely (Murialdo *et al.*, 2003; Wolski *et al.*, 2006). Studies have been conducted by different workers on bacterial degradation of PCP but there is no common consensus over the reaction mechanism involved in its complete degradation (Thakur *et al.*, 2001).

Therefore objectives of the present study at initial stage were to develop stable bacterial consortia by continuous enrichment in the chemostat, characterize the members of the bacterial consortia and evaluation of utilization potential of PCP. The study could help in developing strategies for enhancing degradation and large scale removal of PCP from the environment.

Materials and Methods

Sediment samples and culture conditions

Two sites selected for the present study were tannery industries situated at Jazmau area of Kanpur city and pulp and paper mill located at Darshannagar about eight kilometers away from Faizabad city (U. P), india. Two litre effluent sample along with sediment (10:1) v/w were collected randomly from the main channels at each site. The homogenized sample was filtered and the filtrate was diluted with phosphate buffer saline (1:100 w/v). Small fractions of this sample were incubated over night (29°C) on nutrient agar plates (Shukla *et al.*, 2001).

Cultures from nutrient agar plates were screened by plating them on mineral salt agar medium with a composition of (g/l): $Na_2HPO_4.2H_2O$, 7.8; KH_2PO_4, 6.8; $MgSO_4$, 0.2; ammonium ferric citrate, 0.01; $Ca(NO_3).4H_2O$, 0.05; $NaNO_3$, 0.085 and trace element solution, 1ml/l containing sodium pentachlorophenol (1 mg/l) as the sole source of carbon and energy and 0.1% Bromothymol blue as an indicator for PCP degradation (Thakur, 1995). The culture plates were then kept for 4-5 days incubated at 29° C and results were observed on the basis of change in colour of medium.

The bacterial colonies were morphologically and biochemically characterized as per the methods described by Seelay and Van de Mark (1974) and Aneja (2001) respectively.

Enrichment of bacterial consortia

The enrichment of the isolates was performed in a two liter laboratory scale chemostat. The chemostat was fabricated by using three autoclavable glass bottles connected with plastic tubing. An aerator was used for aeration and a peristaltic pump to regulate the flow rate in culture flask. The temperature of the culture flask was maintained by keeping it in an incubator and monolayer formation was prevented by using a stirrer. The screened bacterial strains were enriched using mineral salt broth medium with different concentrations of sodium pentachlorophenol (1, 5 and 10 mg/l). The samples from the chemostat were collected in triplicate and the growth of bacterial consortia was determined by measuring the cell density at 540 nm at different time intervals (Thakur *et al.*, 2001).

Application of bacterial consortia in bioreactor - The stable bacterial consortium was added into a two litre bioreactor designed in the laboratory to remove PCP from tannery effluent. The bioreactor was made from a chromatographic column in which a series of scratched and perforated small compact discs (CDs) were fixed at different levels. The CDs were initially kept for 1 week in bacterial culture enriched in the chemostat. A thin biofilm developed on every CD and then these were fitted in the chromatographic column. Similarly a control bioreactor was made without applying stable bacterial consortia. The effluent with retention time of one day was continuously passed through the column and PCP concentration was assessed in triplicate at different time intervals from all the three reactors.

Estimation of PCP and chloride release - The pentachlorophenol in the effluent was estimated by centrifuging a 10 ml sample at 7000 rpm for 10 minutes to remove the suspended matters. The supernatant was

basified with 0.5N NaOH and dichloromethane was added. After proper shaking in a separating funnel, the aqueous phase of the solution was taken and acidified with 5N HCl. For further purification, dichloromethane was again added to the acidified solution and transferred to the separating funnel. After proper shaking, the organic phase was removed and the PCP from the organic phase was extracted with 0.5 NaOH and its concentration was assessed by taking optical density at 320 nm (Edgehill and Finn, 1983b).

Chloride release in the culture flask was estimated by silver nitrate method described in APHA (2005). In this method, 1 ml potassium dichromate was added to 10 ml sample. The mixture was titrated against silver nitrate until persistent red precipitate was formed.

Results and Discussion

Several PCP degrading bacterial strains have been isolated from different sources for the degradation of PCP (Yu and Ward 1996; Thakur *et al.*, 2001; Yang *et al.*, 2006). In the present study, samples collected from two industrial sites of tannery and pulp and paper mill effluents yielded thirty one strains (Fifteen strains from tannery effluent and sixteen from pulp and paper mill effluent) on nutrient agar. As the effluent of both industries contain chlorinated phenols, so there is high probability of getting PCP utilizing bacterial strains. The isolated bacterial strains were distinguished on the basis of their morphological characterization as all the colonies showed different features regarding their shape, size, color, texture etc. The isolates were then cultured on mineral salt agar media containing sodium pentachlorophenol (1 mg/l) as sole source of carbon and energy and Bromothymol blue (0.1%) as indicator. The bacterial strains possessing the capability to degrade PCP changed the color of media from blue to yellow. The colour of bromothymol blue remains blue in basic condition and changes to yellow in acidic conditions. Due to degradation of PCP, chloride was released which resulted in a lowering of pH and colour of the medium was changed to yellow (Alexander, 1981). Out of thirty one isolated bacterial strains, twenty three strains (Eleven from tannery and twelve from pulp and paper mill) showed the ability to degrade PCP.

The bacterial consortia isolated from the two sites showing the PCP degrading capability were enriched separately in a laboratory scale chemostat. The prior enrichment of bacterial culture enhances its capacity to sustain a shock load of high PCP concentration as well as its degradation (Reyding *et al.*, 1994). Figure 1 and 2 shows the growth pattern of the bacterial consortia enriched in the presence of PCP. The growth pattern of each bacterial consortia at every PCP concentration in the chemostat showed a lag phase of initial fluctuation with exponential growth after the lag phase and onward almost growth stabilization. The increase in the number of cells in the chemostat was due to physiological adaptation of bacterial cells in sodium pentachlorophenol and probably due to nutritional interaction between the members of the bacterial community (Slater and Lovatt, 1984). After growth stabilization at final PCP concentration in chemostat, a sample was removed from each culture media and plated on nutrient agar plates. The bacterial colonies that appeared on the plates were morphologically and biochemically characterized. Only five strains from tannery and four strains from pulp and paper mill were obtained on agar plates, where as the other bacterial strains were unable to tolerate the shock load of high PCP concentrations. The strains from tannery were characterized as two species each of *Pseudomonas* and *Arthrobacter* and one species of *Sphingomonas* whereas pulp and paper mill strains as three species of *Pseudomonas* and one species of *Flavobacterium*. The PCP degradation ability of

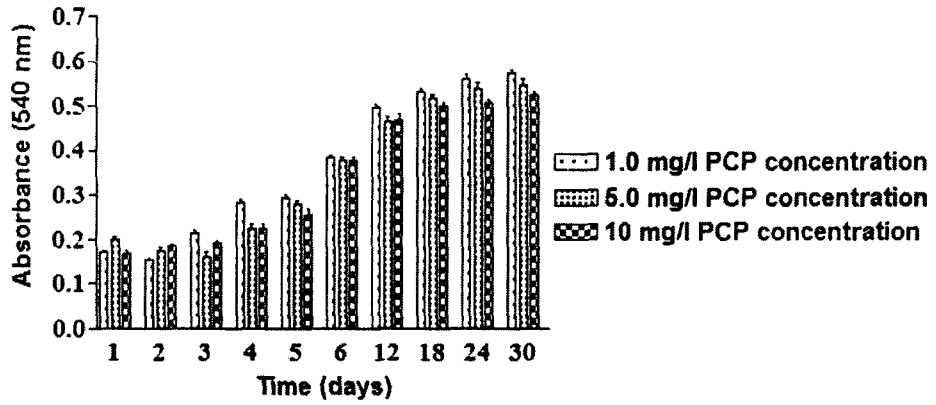

Fig.1 Growth of bacterial consortium isolated from tannery effluent in chemostat.

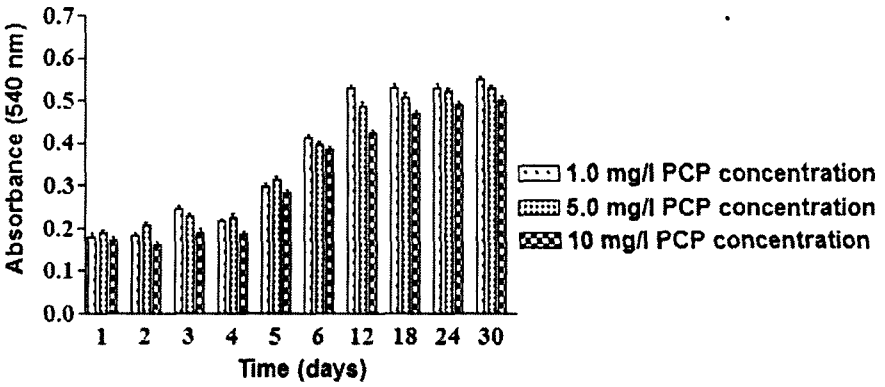

Fig. 2 Growth of bacterial consortium isolated from pulp and paper mill effluent in chemostat.

all these strains was also reported by different workers (Stanlake and Finn 1982; Brown *et al.*, 1986; Yu and Ward 1996; Thakur *et al.*, 2002; Yang *et al.*, 2006).

The enriched bacterial consortia obtained from two different industrial sites were applied separately for the removal of PCP from tannery effluent in a bioreactor. Figure 3 shows degradation of PCP by bacterial consortia investigated in terms of PCP utilization and chloride release. Utilization of PCP was determined by spectrophotometric analysis. Samples taken at different time intervals from the bioreactor after the application of bacterial consortia showed decreasing concentration of PCP. The Fig. 3 clearly depicts that the concentration of PCP in tannery effluent was reduced up to 25% on third day by the bacterial consortium isolated from pulp and paper mill effluent which on day six reached up to 50% and then about 60% on day nine and after that there was very little removal but the bacterial consortium obtained from tannery effluent showed 35% PCP removal on third day reaching about 60% on sixth day and ultimately 75% on day ninth and after that a very little removal was also reported on continuous application. In the control bioreactor, the reduction in PCP concentration was only 28% in nine days. The increased rate of PCP utilization by these bacterial consortia may be due to their prior enrichment using sodium pentachlorophenol. During utilization of pentachlorophenol a stoichiometric amount of chloride was accumulated in the culture broth. The release of chloride was highest in the case of bacterial consortium isolated from tannery

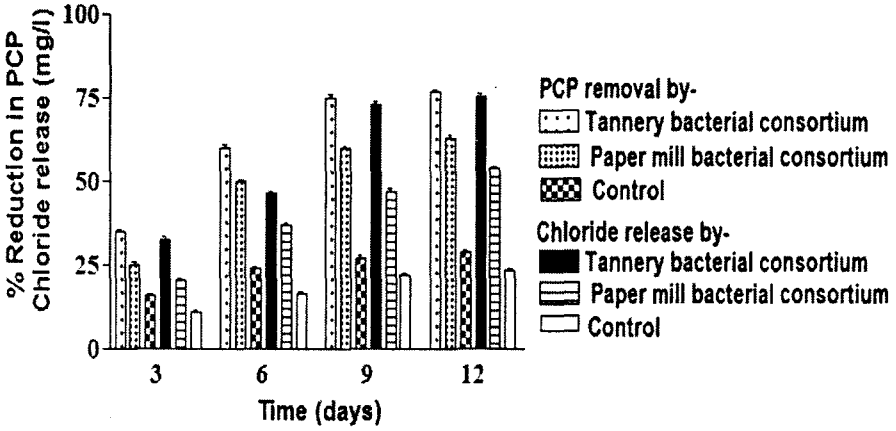

Fig. 3. Removal of PCP by bacterial consortia in bioreactor.

effluent than the pulp and paper mill consortium indicating greater degradation of PCP by tannery consortium. The greater PCP degrading efficiency of the bacterial consortium isolated from tannery effluent can be due to the adaptation of these isolates in high PCP containing tannery effluent. The removal of PCP in an aerobic bioreactor by different bacterial strains was also reported by various workers (Edgehill and Finn, 1983a; Karamanev and Samson, 1998; Melin *et al.*, 1997, 1998a, b). The study reveals that the combination of *Pseudomonas, Arthrobacter* and *Sphingomonas* species can effectively degrade PCP as compared to the combination of *Pseudomonas and Flavobacterium* species without any co- metabolite. This work can help in choosing the right and feasible bacterial consortium for the removal of PCP from the environment.

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