ISSN (online): 2250-141X
Vol. 2 Issue 2, April 2012

Assessment of Pentachlorophenol (PCP) Degrading Bacterial strains Isolated from the Tannery Effluent Sludge of Jajmau (India)

Prakash Chandra Tewari* and Siddhartha Shukla
***Corresponding author:** Lecturer, Department of Environmental Sciences, Kamla Nehru Institute of Physical & Social Sciences, Sultanpur, (U.P.), India.

Abstract:
Pentachlorophenol is a toxic compound mainly use as preservative in leather and wood industries and also as disinfectant in various sectors such as agriculture, food, wood, oil and paints industries. In present investigation fifteen bacterial strains were isolated from sediment core of tannery effluent sludge (Kanpur, U.P., India). Eight strains were screened on mineral salt agar medium, containing sodium pentachlorophenate (Na-PCP) as a sole source of carbon and energy and bromothymol blue (0.1%) as screening agent. The screened strains were collectively enriched in lab scale chemostat at different concentration of PCP for 30 days and then were characterized morphologically as well as biochemically. The strains showed similarities with Pseudomonas species, Flavobacterium species (three strains of each), Proteus species and Bacillus species (one strains of each). PCP degrading potential of each individual species was performed in lab scale bioreactor for 40 hrs. It was observed that Flavobacterium species has highest PCP degrading potential as it degraded 55% PCP within 42 hours followed by Pseudomonas species and Bacillus species (47% and 44% respectively). The Proteus species showed lowest degradation potential (38%) within the same time whereas in control reactor 20% reduction was observed. Further, combination of different strains were designed for the assessment of consortia based comparative degradation of PCP in which combination of Flavobacterium species with Pseudomonas species showed significant reduction of 73% in PCP concentration as compared to other possible combinations.
Key words: *- Pentachlorophenol, Tannery effluent, Screening, Bacterial biodegradation, Bioreactor.*

Introduction

In Indian economy tannery industries occupy place of pride due to its higher potential for employment, export and growth. The major portion, i.e. 70 to 80 percent of processing occurs at small cottage – scale sectors. Export of leather goods has reclaimed new height of $2.8 billion (Rs.14,000 corers) in 2007-08 comparing to 1965-66 which was $65.5 million (Rs.32

ISSN (online): 2250-141X
Vol. 2 Issue 2, April 2012

corers) (1). To mitigate huge demand, rapid growth of tanneries took place around the nation. There are of about 3000 major tanneries in India, which are mainly located at Kanpur (U.P.), Punjab, Maharashtra, Kolkata (W.B.) and Chennai (Tamil Nadu) and these are discharging their improperly treated effluent in nearby water bodies causing collateral damages to aquatic ecosystem (2-4). The production of leather goes through a process known as tanning. In this process variety of chemicals are used at different stages. Due to biocidal property, PCP is used for curing and preservation of leather in tannery industries (5, 6, 4). Pentachlorophenol is highly recalcitrant xenobiotic chlorinated hydrocarbon. It has ubiquitous occurrence, from ambient air of mountains to rural areas (0.25-0.93 mg/m^3) from urban areas (5.7-7.8 mg/m^3) to groundwater (3-23 mg/l) and surface water (0.07-31.9 mg/l). The maximum level of pentachlorophenol contamination has been set at 0.001 mg/l for drinking water (7, 8).

Pentachlorophenol has both acute and chronic effect on human beings as well as on aquatic environment. The major sites of action are liver, kidneys, plasma protein, brain and spleen. In acute toxicity, pentachlorophenol causes elevated temperature, profuse sweating, dehydration, loss of appetite, decreased body weight, nausea and neurological effect such as tremor, leg pain, muscle twitching and coma. In chronic response, pentachlorophenol inhaled by workers at the working place causes abdominal pain, fever, respiratory irritation as well as eye, skin and throat irritation. In high concentration PCP causes obstruction of circulatory system in lungs, heart failure and damage to central nervous system (9).

It is rapidly absorbed through the gastrointestinal tract following ingestion, with a biological half-life of only 10 hours and its bioaccumulation may result significant. Several species of fish, invertebrates and algae have high levels of pentachlorophenol that were significantly higher (up to 10,000 times) than the concentration in the surrounding waters. Accumulation is not common, but if it does cause teratogenic, mutagenic, carcinogenic (10).

The excessive use of this chemical has resulted in environmental nuisance and immensely demands its remediation. Several physico-chemical methods are available for the degradation of PCP but the most feasible way is the bioremediation technique. A number of aerobic and anaerobic cultures of fungi and bacteria have been applied for the degradation of pentachlorophenol by different workers at nation as well as international level (4, 11-18). The workers have got effective results during their study but complete mineralization of this xenobiotic compound is still unstated.

In present investigation the bacterial strains were isolated from their indigenous place i.e. sediment core of tannery effluent, their stable consortia was developed, enriched and applied for effective removal of PCP from tannery effluent

Materials and Methods

The present investigation was conducted on tannery effluent released from tannery

ISSN (online): 2250-141X
Vol. 2 Issue 2, April 2012

industries of Jajmau, Kanpur (U.P.) India. Here 402 tanneries are situated on both sides of the road. The effluent of these industries is discharged into river Ganga through a main channel. Samples were collected randomly from the main channel and sediment along with the effluent in the ratio of 1:10 (w/v) was collected. The samples were then brought to laboratory for further analysis.

For the isolation of bacterial strains, the sample after filtration with the help of muslin cloth was serially diluted upto10^{-10} and each dilution was spread over nutrient agar plates and incubated overnight at 29^0C. The screening of PCP degrading bacterial strains was performed by culturing the isolates on mineral salt agar medium. The medium consist of (L^{-1}): $Na_2HPO_4.2H_2O$, 7.8 g; KH_2PO_4, 6.8 g; MgSO4, 0.02 g; Fe $(CH_3COO)_3$ NH_4, 0.01 g; Ca $(NO_3)_2$, $4H_2O$, 0.05 g; $NaNO_3$, 0.085 g; Agar 14 g; PCP 0.5 g; Bromothymol blue 0.1% and pH was maintained at 7 and 1 ml trace element solution was added to the medium (4). The culture plates were incubated for three days at 29^0C. The results were observed on the basis of change in color of medium. The screened colonies were re-cultured on mineral salt agar medium alternatively for standardizing the process.

Pentachlorophenol was extracted by acidifying 10ml effluent sample with 5N HCL. Then PCP from sample was extracted three times by Dichloromethane (10 ml). The organic phase was re-extracted with 0.5N NaOH. Now, aqueous phase was taken and optical density of pentachlorophenol was analyzed by spectrophotometer at 320nm (19).

The strains showing PCP degrading capability were enriched with different concentration of Na-PCP (5, 10, 15 and 20 mg/l) in a laboratory scale chemostat (Fig.-1) for 120 days. The chemostat was fabricated with three autoclaveable plastic jars connected with plastic tubing. The first chamber (Jar I) contained mineral salt medium, from outlet at its base the media was supplied to second culture chamber (Jar II). The upper-most part of this chamber was provided with three openings for stirring, aeration and an inlet for mineral salt medium, the stirring was made possible by fixing a motor and oxygen was provided by passing sterile air through aerator. The used media was transferred to third chamber (Jar-III) from an outlet at the middle of culture chamber. The temperature of the culture chamber was maintained by keeping it in an incubator and monolayer formation was prevented by using stirrer. The growth of bacterial strains was measured at different time intervals (0, 1, 2, 3, 4, 5, 6, 12, 18, 24 and 30 days) by taking absorbance at 540 nm on UV-Visible Spectrophotometer (4).

The enriched bacterial colonies were cultured on nutrient agar plates for morphological characterization depending upon their shape, size, color, opacity, texture, elevation, spreading nature and margin (20). The biochemical characterization of the strains was performed by the methods described by Edgehill and Finn, (1983) (21).

The stabilized and enriched bacterial strains were applied for PCP removal in a lab scale bioreactor (Fig.-2) fabricated by using a

ISSN (online): 2250-141X
Vol. 2 Issue 2, April 2012

glass column of 5 L with effective volume of 2 L**.** The first chamber contained tannery effluent (Chamber 1), from outlet at its base the effluent was supplied to the second reactor chamber (Chamber II). The uppermost part of the reactor chamber was provided with three opening for stirring, aeration and inlet for effluent, the stirring was made possible by fixing a motor, oxygen was provided by passing sterile air through aerator. 20ml bacterial culture was added in 2 L effluent of reactor chamber and 12 hr retention time was set. A layer of gravel (150 g) and sand (100 g) was placed in the lower portion of the reactor. The lowermost part of the reactor chamber was connected to Chamber III. The samples were collected at different time intervals (0, 4, 8, 12, 16, 20, 24, 28, 32, 36 and 40 hrs) for PCP estimation.

Result and Discussion

The bacterial communities were isolated from depth of 1mm sediment core from effluent sludge of tannery industries Jajmau, Kanpur. Fifteen bacterial colonies were isolated from tannery effluent sludge. As the effluent of tannery industries contain chlorinated phenols, so there is high probability of getting PCP degrading bacterial strains. Several workers have also isolated PCP degrading bacterial strains from different sources (4, 22-24). Isolated strains were cultured on mineral salt agar medium containing sodium pentachlorophenol (0.5 g/l) as sole source of carbon and energy and bromothymol blue (0.1%) as indicator and incubated for three days at 29^0 C. No supplementary co-substrate was provided in the medium. After three days of incubation, out of fifteen isolated strains, eight strains showed growth on the medium determined on the basis of color change. The color of bromothymol blue remains blue in basic medium and changes to yellow in acidic medium. Due to the degradation of PCP, chlorine was released resulting change in the pH of the medium and hence yellow colouration. Thus, the bacterial strains showing color change of the medium indicated there ability for PCP degradation without any co-substrate. Reports are available which show that PCP is utilized as sole source of carbon and energy by different bacterial strains (4, 24, 25).

The screened bacterial strains were further enriched in the lab scale chemostat at different concentration of 5, 10, 15 and 20 ppm (Fig.-3). The morphological characterization of the isolates after enrichment was performed according to cell morphology observed under microscope (Table-1). Samples were picked from agar culture onto a glass cover slip and fixed with buffer containing glutaraldehyde. Fixed samples were dehydrated by passing through series of ethanol solutions with concentration 30%, 60%, and 90% of ethanol for 10 min each and strains were observed under bright field microscope (1000X). The strains were further biochemically characterized (Table-2) and it was observed that eight phenotypically different bacterial strains shared their characteristics with different genus of *Bacillus (one species)*, *Proteus* (one species), *Flavobacterium* (three species) and

ISSN (online): 2250-141X
Vol. 2 Issue 2, April 2012

Pseudomonas (three species).

Pentachlorophenol degrading capabilities of these strains is also reported by different workers. Stanlake and Fine, (1982) (11) isolated and characterized *Arthrobacter* species from soil that showed degradation of PCP. Utilization of PCP as sole source of carbon by *Pseudomonas species* and *Arthrobacter species* was also reported (4, 24, 26). Sharma and Thakur, (2008) (27) isolated *Pseudomonas species* from paper mill and studied the potency of the isolated strains for PCP reduction in sequential bioreactor. Kotresh and Vidyasagar, (2008) (28) also reported the PCP reduction by *Pseudomonas* species. PCP degrading *Proteus species* and *Bacillus species* were isolated from rhizosphere and characterized by Munazza et al., (2004) (29). Tripathi and Garg (2010) (18) isolated and characterized *Bacillus species* form the tannery effluent.

The bacterial strains were applied for PCP removal from tannery effluent in a lab- scale bioreactor. *Flavobacterium* species showed highest PCP degrading potential as it degraded 55% PCP within 42 hrs followed by *Pseudomonas species* and *Bacillus species* (47% and 44% respectively). The *Proteus* species showed lowest degradation potential (38%) within the same time whereas in control reduction was 38% which was not so significant (Fig.-4). Further, combination of different strains were designed for the assessment of consortia based comparative degradation of PCP in which combination of *Flavobacterium* species with *Pseudomonas* species showed significant reduction of 73% in PCP concentration (Fig.-5) as compared to other possible combinations. Brown et al., (1986) (12) studied consortium of *Flavobacterium* and *Arthrobacter* in a fixed film bioreactor and reported that 60-80% of PCP reduction in 120 days. The consortium of *Pseudomonas* and *Arthrobacter* in a sequential bioreactor showed 79.2% of PCP reduction in 9 days (24). In another sequential bioreactor study, 65% of PCP reduction was reported in 300 hrs (26).

Acknowledgements

Authors are immensely thankful to Department of Environmental Sciences, Kamla Nehru Institute of Physical & Social Sciences, Sultanpur for rendering the support to conduct this work.

References

1. Natesh, S., Leather Goes Green, Biotechnology News, 4, 3-7, **(2009)**.
2. Hammer, E., Krowas, D., Schafer, A., Specht, M., France, W. and Scaver, F., Isolation and Characterization of a Dibenzofuran- Degrading Yeast: Identification of Oxidation and Ring Cleavage product, Applied Environmental Microbiology, 64, 2215-2219 **(1998)**.
3. Elisa, M., Angelo, D and Reddy, K. R., Aerobic and anaerobic transformation of

ISSN (online): 2250-141X
Vol. 2 Issue 2, April 2012

pentachlorophenol in wetland soils, Soil Science Society J., 64, 933-943, **(2000)**.

4. Shukla, S., Sharma, A. and Thakur, I. S., Enrichment and Characterization of Pentachlorophenol Degrading Microbial Community for the Treatment of Tannery Effluent, Pollution Res., 20, 353-363, **(2001)**.

5. Fi**s**her, B., Pentachlorophenol: Toxicology and Environmental Fate, J. of Pesticide reformation, 11, 2-5, **(1991)**.

6. Premlata, A. and Rajkumar, G. S., Pentachlorophenol degradation by Pseudomonas aeruginosa. World J. of Microbiology and Biotechnology, 10, 334-337, **(1994)**.

7. McAllister, K. A., Lee, H. and Trevors, J. T., Microbial degradation of Pentachlorophenol, Biodegradation, 7, 1-40, **(1996)**.

8. Yang, C. F., Lee, C. M., Wang, C. C., Isolation and physiological characterization of the Pentachlorophenol degrading bacterium Sphingomonas chlorophenolica, Chemosphere, 62, 709-714, **(2006)**.

9. U.S. Department of Health and Human Services (Public Health Service), Public Health Statement, In: Toxicological Profile for Pentachlorophenol, Prepared by Syracuse Research corporation, 1-11, **(2001)**.

10. Jain, R. K., Kapur, M., Labana, S., Lal, B., Sarma, Priyangshu, M., Bhattacharya, D. and Thakur, I.S., Microbial diversity: Application of micro-organisms for the biodegradation of xenobiotics, Current Science, 89, 101-112, **(2005)**.

11. Stanlake, G. J. and Fine, R. K., Isolation and characterization of Pentachlorophenol degrading bacterium, Applied Environmental Microbiology, 44, 1421-1427, **(1982)**.

12. Brown, E. J., Pignatello, J. J., Martinson, M. M. and Crawford, R. L., Pentachlorophenol degradation-A pure bacterial culture and an epilithic microbial consortium, Applied Environmental Microbiology, 1, 92-97, **(1986)**.

13. Schie, M. P. and Young, Y. L., Isolation and Characterization of Phenol- Degrading Denitrifying Bacteria, Applied Environmental Microbiology, 64, 2432-2438, **(1998)**.

14. Nagyun, A., Martin, M., Shukla, S., Margrave, J. L., Parga, J., Pentachlorophenol degradation by the bacteria Enterobacter cloacae, Res. J. of Chemical and Environment, 6, 53-57, **(2002)**.

15. El- Syed, W. S. I, Mohamed, K., Shady, M., El-Beith, F., Ohmura, N., Saiki, K. and Ando, Kikazu, Isolation and Characterization of Phenol Catabolizing Bacteria from

ISSN (online): 2250-141X
Vol. 2 Issue 2, April 2012

a Coking Plant, Bioscience Biotechnology Biochemistry, 67, 2026 – 2029, **(2003)**.

16. Chandra, R., Ghosh, A., Jain, R. K. and Singh, Shail, Isolation and Characterization of two potential pentachlorophenol degrading aerobic bacteria from Pulp Paper effluent sludge, J. of General Applied Microbiology, 52, 125-130, **(2006)**.

17. Rahman, R. A. and Anuar, N., Pentachlorophenol Removal via Adsorption and Biodegradation, World Academic of Science Engineering and Technology, 55, 194-199, **(2009)**.

18. Tripathi, M. and Garg, S.K., Studies on selection of efficient bacterial strains simultaneously tolerant to Hexavalent Chromium and Pentachlorophenol isolated from treated tannery effluent, Res. J. of Microbiology, 5, 707-716, **(2010)**.

19. Edgehill, R.U. and Finn, R., Microbial treatment of soil to remove Pentachlorophenol, Applied Environmental Microbiology, 45, 1122-1125, **(1983)**.

20. Seelay, H. W. and Van de Mark, Microbes in Action, 5th Edition, W.H. Freeman and Company, **(1974)**.

21. Aneja, K. R., Experiments in Microbiology, Plant pathology, Tissue culture and Mushroom Production Technology, 3rd Edition, New Age International Publication New Delhi, India, **(2001)**.

22. Visvanathan, C., Thu, L. N., Jegastheesan, V. and Anotai, J., Biodegradation of pentachlorophenol in a membrane bioreactor, Desalinations, 183, 455-464, **(2005)**.

23. Khadijah, O., Lee, K. K. and Abdullah, M. F. F., Isolation, screening and development of local bacterial consortia with azo dyes decolourising capability, Malaysian J. of Microbiology, 5, 25-32, **(2009)**.

24. Andrabi, S.Z., Tewari, P.C., Chaudhary, C.B. and Shukla, S., Biotreatability of pentachlorophenol by aerobic bacterial consortium in tannery effluent, Asian J. of Chemical and Environmental Res., 3, 46-51, **(2010)**.

25. Thakur, I.S., Verma, P.K. and Upadhaya, K.C., Molecular cloning and characterization of Pentachlorophenol degrading Monooxygenase genes of Pseudomonas sp. from the chemostat, Biochemistry Biophysics Res. Communication, 290, 770-774, **(2002)**.

26. Tripathi, M. and Garg, S. K., Study on selection of efficient bacterial strains simultaneously tolerant to hexavalent chromium and isolated from treated tannery effluent, Res. J. of Microbiology, 5, 707-716,

ISSN (online): 2250-141X
Vol. 2 Issue 2, April 2012

(2010).

27. Sharma, A. and Thakur, I. S., Characterization of pentachlorophenol degrading bacterial consortium from chemostat, Bulletin of Environmental Contamination Toxicology, 181, 12-18, **(2008)**.

28. Kotresha, D. and Vidyasagar, G. M., Isolation and Characterization of phenol-degrading Pseudomonas aeruginosa MTCC-4996, World J. of Microbiology Biotechnology, 24, 541-547, **(2008)**.

29. Munazza, Ajaz, Nabeela Noor, Sheikh Ajaz Rasool and Shakeel A. Khan, Phenol resistant bacteria form soil; Identification-characterization and genetic studies, Pakistan J. of Botany, 36, 415-424, **(2004)**.

Table 1 – Morphological characterization of PCP degrading bacterial strains screened from tannery sludge:-

Characteristics	Bacterial strains							
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
Shape	I	C	C	C	C	I	I	C
Size (mm)	0.05	0.25	0.05	0.1	0.1	0.05	0.25	0.05
Color	Y	CR	Y	CR	OR	Y	OR	W
Opacity	T	T	O	T	O	T	O	O
Texture	V	NV	NV	V	NV	V	NV	V
Spreading nature	Y	N	N	Y	Y	N	Y	N
Elevation	F	F	F	CO	CO	F	F	F
Margin	SR	SR	S	SR	SR	S	S	SR

C- Circular, I-Irregular, W-Whitish, CR- Creamish, OR-Orange, P-Pinkish, Y-Yellowish, GW-Grayish white, O-Opaque, T-Transparent, V-Viscous, NV- Not viscous, Y- Yes, N-No, F-Flat, CO-Convex, S-Smooth, SR –Serrated.

ISSN (online): 2250-141X
Vol. 2 Issue 2, April 2012

Table 2 – Biochemical characterization of PCP degrading bacterial strains screened from tannery sludge:-

Characters	Bacterial strains and their responses							
	I	II	III	IV	V	VI	VII	VIII
Gram staining	-	+	+	-	-	-	+	+
Starch Test	-	+	-	+	+	+	+	+
Casein Test	+	-	+	-	-	+	-	+
Lipid hydrolysis Test	-	+	+	-	+	+	+	+
Citrate Test	-	-	-	+	+	+	+	+
Urease Test	+	-	-	-	-	+	+	-

+ Positive result; – Negative result

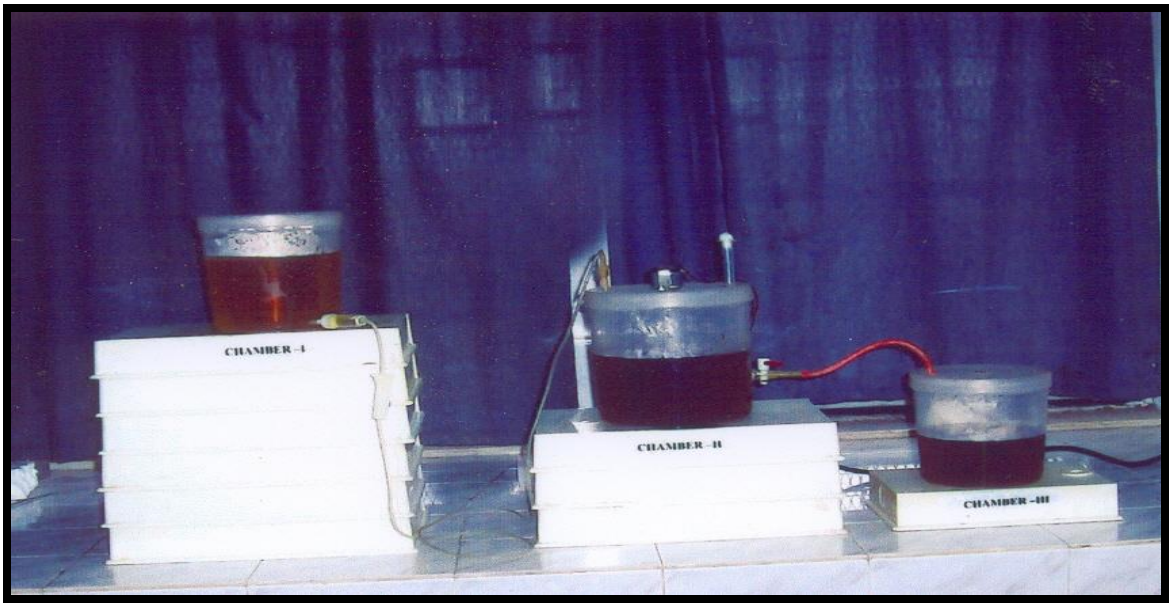

Figure 1- Lab Scale chemostat

ISSN (online): 2250-141X
Vol. 2 Issue 2, April 2012

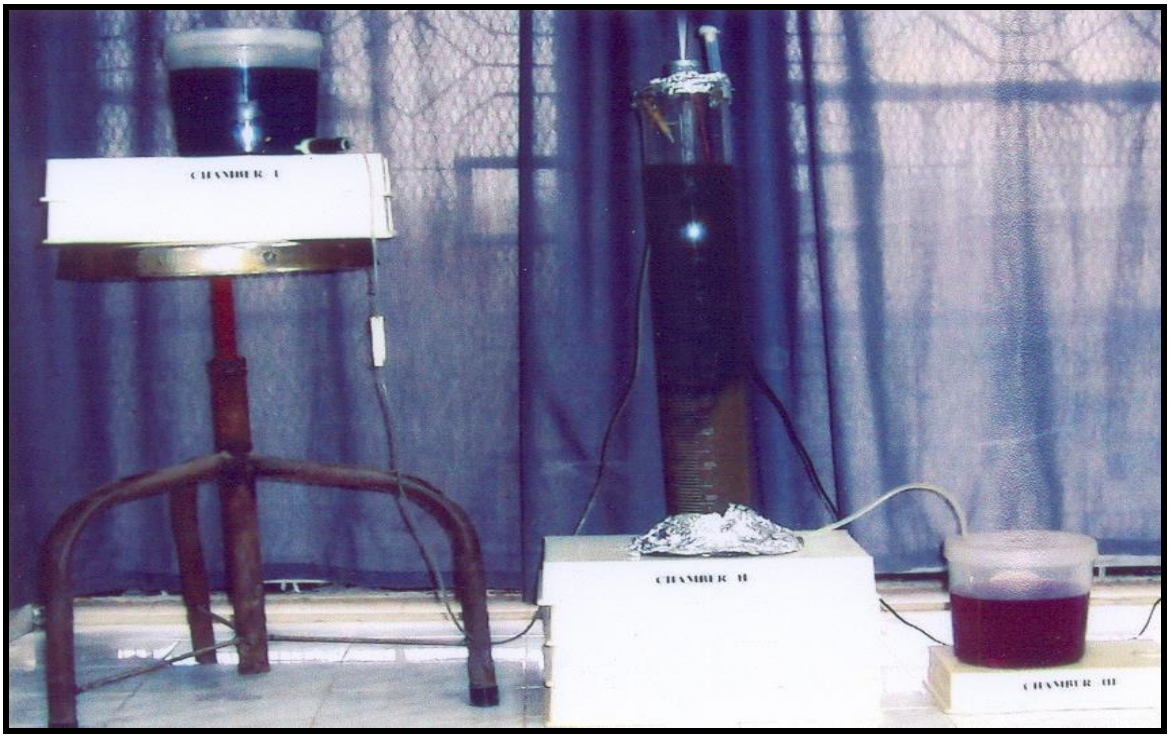

Figure 2- Lab Scale bioreactor

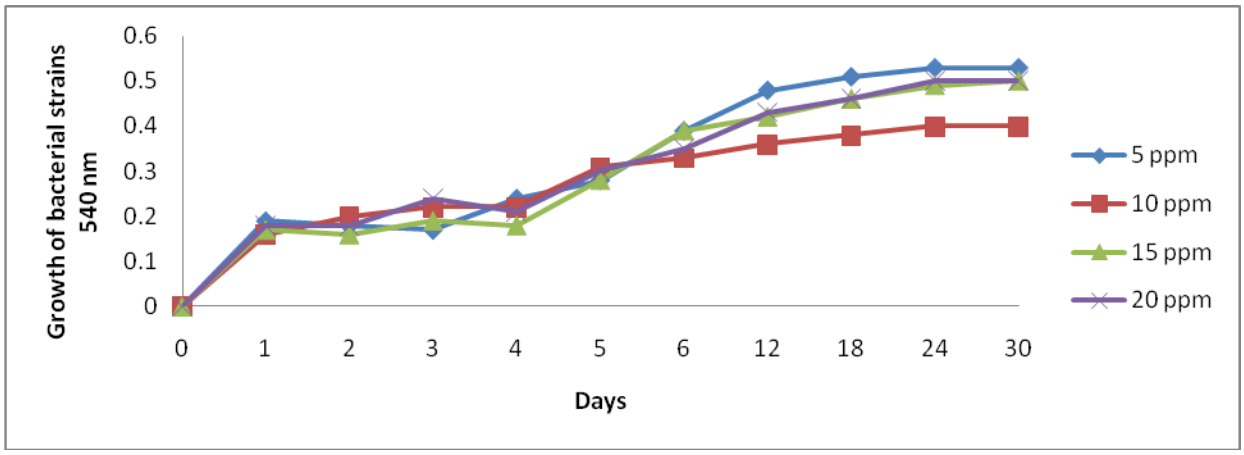

Fig.-3- Growth of bacterial strains in chemostat:

ISSN (online): 2250-141X
Vol. 2 Issue 2, April 2012

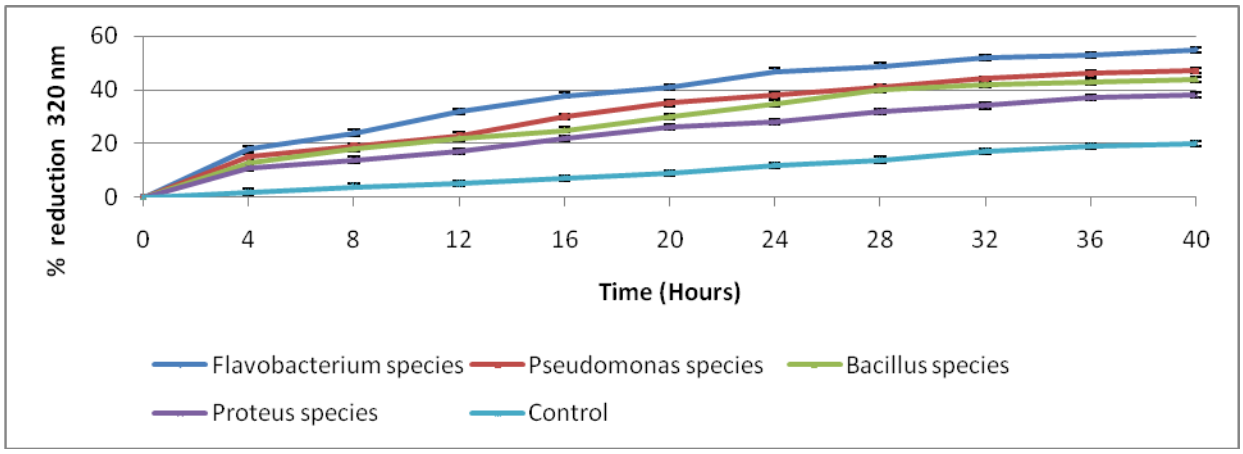

Fig.-4 - Reduction of PCP in bioreactor

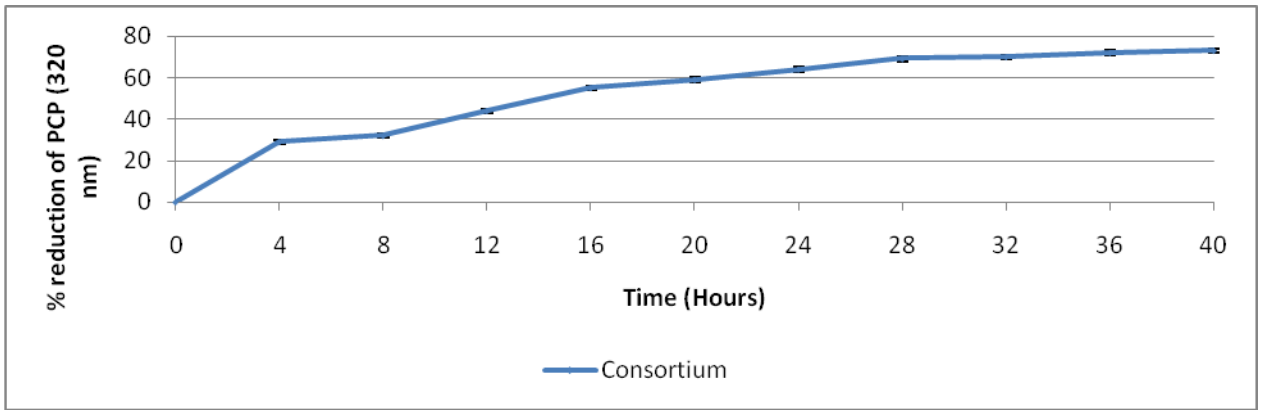

Fig.-5- Reduction of PCP by consortium in bioreactor