INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

Volume-1, Issue-2 :2011 *www.ijpaes.com* *ISSN 2231-4490*

BIODEGRADATION OF PHENOLIC COMPOUNDS BY USING HALOTOLERANT MICROBES

S. Ravikumar*. P. S. Parimala and R. Gokulakrishnan

*School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi – 623409, Ramanathapuram District, Tamil Nadu, India.

Corresponding author: ravibiotech201321@gmail.com

ABSTRACT: The present study made an attempt to find out the biodegradation of phenol by using halophilic microbes isolated from coconut retting water. Thirty two morphologically different strains were isolated and 3 identified strains were selected for the phenol degradation. Among them, *Pseudomonas putida* showed the maximum degradation of phenol up to 2000 $mg.l^{-1}$ without any supplement. The maximum removal of phenol was observed by the three bacterial species at neutral pH, low glucose level and NaCl concentration.

Key words: *Bacillus* sp, Coconut retting water, Phenol degradation, *Pseudomonas putida, Pyrococcus horikoshii*

INTRODUCTION

The environment has been contaminated by variety of toxic compounds through industrial effluents. Phenol is one of the major aromatic pollutants among the variety of toxic compounds derived from pulp mills, coal mines, gasoline, petrochemicals, wood preservation plants, pesticides, insecticides, herbicides, domestic waste, agricultural run-off and chemical spills [1, 2, 3, 4]. Phenol is also designated as hydroxyl benzene, phenic acid etc. It may be colorless solid or pink colour with low melting point. But, the boiling point is high because of the presence of hydrogen bonds. Phenol causes irritation of eye, swelling and finally blindness and high concentration of phenol can cause chronic exposure such as, hepatic damage, vomiting and nervous disorder. It is also suspects that, exposure to phenol may cause paralysis and cancer [5]. However, it is very lethal to fish at the concentration of 5 $mg.l^{-1}$ and 20 $mg.l^{-1}$ for human being [6, 7].

Several methods have been developed for the phenol degradation such as precipitation, coagulation, ion exchange, ultra filtration but expensive and inefficient. The by products from the processes posses toxic compounds [8]. Today biodegradation as an emerging tool to remove the environmental pollutions [9]. Several microbes including bacteria (*Pseudomonas* sp.), fungi (*Fusarium flucciferum* and *Aspergillus fumigates*), yeast (*Candida tropicalis, Trichosporium cutaneum*) have been isolated for the phenol degradation from different sources. Among the microbes, bacteria are the important one because their degrading capacity is higher than the other microbes [10]. The addition of supplements could increase the degradation of phenol at certain level. The author [11] reported that, the rate of degradation was increased with the addition of glucose. However, studies related with the halophilic microbes on phenol degradation are too limited. Application of these developed technologies for the removal of phenol in the coastal and marine based small scale industries like coir retting industry is not possible due to high saline water used for the coconut retting process before the reeling of coir rope. The retting water released from the coconut retting pond has high concentration of phenol, sugar and low pH which causes several damages to the brackish and marine water animals (microorganisms to fishes). Sometimes the fish commit mass mortality which came nascent smell along the coastal villages and also the loss of productivity of the water leads to anoxia in water and surrounding atmosphere. Hence, the present study was made an attempt to find out the halotolerant microbes to be effectively used for the biodegradation of phenol in coconut retting water.

MATERIALS AND METHODS

Water samples were collected from coconut retting ponds in Rajakkamangalam coast (Lat. 8° 07'N; Long. 77° 23' E), Kanyakumari District, Tamil Nadu, India, by using plastic container. Water samples were serially diluted and plated in sterilized nutrient agar media. Phenol (100 $mg.l^{-1}$) was added as a substrate for the isolation of phenol degrading bacteria. After 24 hrs of incubation at 34˚C, colonies appeared on the solid media were counted and the colonies were selected based on the morphological characters and further restreaked on the same nutrient agar medium for purification and species level identification was performed by following the method of Holt et al. [12].

Biodegradation of phenol was carried out by using shake flask method. A loopful of 32 pure bacterial strains were inoculated into 100 ml basal minimal medium [$(NH_4)_2SO_4$-325 mg, $MnSO_4$-8.5 mg, $MgSO_4.7H_2O$-65 mg, $FeCl_3.6H_2O$-2 mg, $CaCl_2.2H_2O$-9 mg, K_2HPO_4-2.62 g and KH_2PO_4-1.43 g per litre] containing various concentration of phenol in 250 ml Erlenmeyer flasks. These flasks were incubated at room temperature with continuous shaking at 150 rpm in a thermostat incubator. The biodegradation of phenol was assessed by the following the method of yang and Humphrey [13].

RESULTS AND DISCUSSION

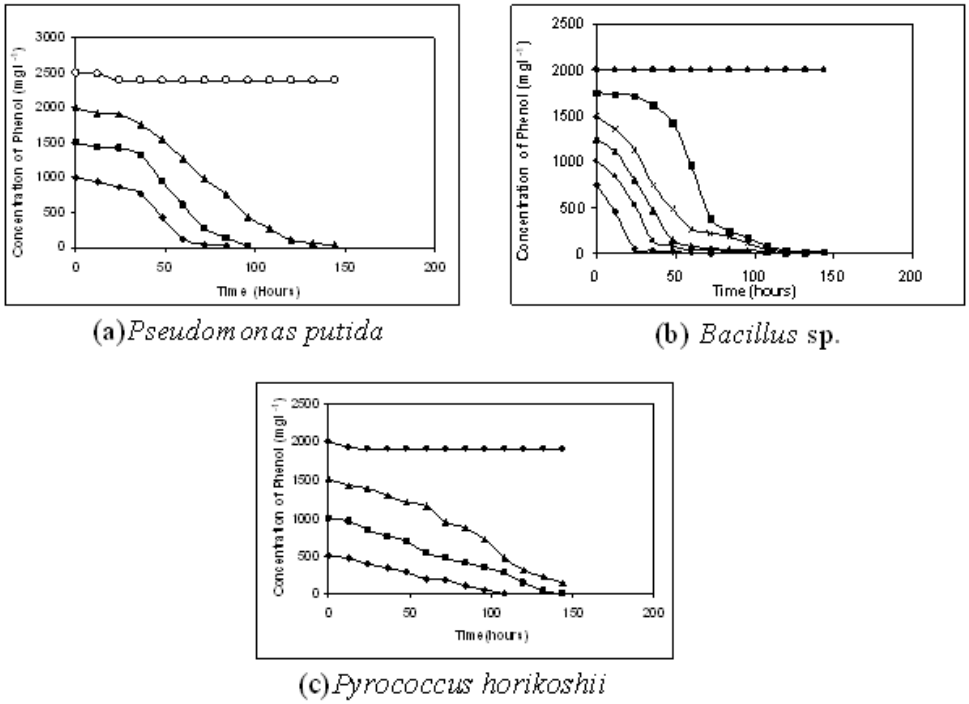
The present study was aimed to remove the phenolic compounds and its derivatives by using halotolerant bacterial species. Thirty two morphologically different strains were isolated from the retting water. Among them, 3 strains showed better degradation. Based on the morphological and biochemical tests the strains were identified as *Pseudomonas putida, Bacillus* sp., *Pyrococcus horikoshii* (Tables 1 & 2). Among them, *Pseudomonas putida* showed the maximum degradation of phenol up to 2000 $mg.l^{-1}$ within 144 hrs, than the other strains (Fig. 1).

Table 1 Morphological Characteristics of the isolated halobacteria

Morphological Characteristics	*Pseudomonas putida*	*Bacillus sp.*	*Pyrococcus horikoshii*
Configuration	Round	Round	Round
Margin	Entire	Wavy	wavy
Elevations	Convex	Convex	flat
Surface	Smooth	Rough	rough
Pigments	-	-	-
Gram-reaction	Negative	Positive	Positive
Shape	Rod	Rod	cocci
Motility	+	+	+

(+) indicates positive; (-) indicates negative

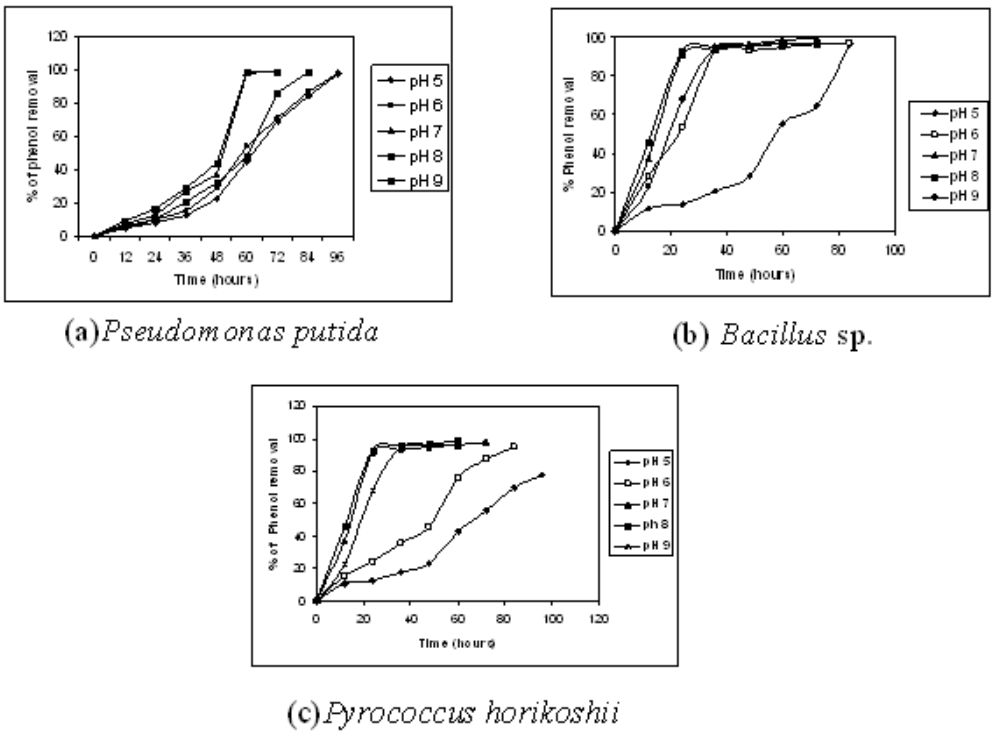
An interesting observation was noted that, with in the short time the isolated strain *Pseudomonas putida* showed the maximum degradation of phenol. This might be due to the production of different enzymes including oxygenases, hydroxylases, etc. Moreover, the purified enzymes oxidized the phenolic compounds very effectively [5]. Generally, the breakdown of the resonance structure of the aromatic compounds is too critical by the microbes. However, in the aerobic system, phenol is first converted into catechol subsequently; it is degraded only through meta fission, because most of the halogenated compounds undergoes through ortho pathway. Similarly, [14] reported that, *Pseudomonas* sp. isolated from the petroleum contaminated soil capable of growing at the concentration of 1200 $mg.l^{-1}$. Moreover, the *Pseudomonas aeruginosa* degrades the phenol up to 1300 $mg.l^{-1}$ within 156 hrs. The author [15] reported that, *Pseudomonas putida* showed maximum degradation of phenol up to 2000 $mg.l^{-1}$ within 156 hrs.



(a) *Pseudomonas putida* (b) *Bacillus* sp.

(c) *Pyrococcus horikoshii*

Fig. 1 Biodegradation of phenol by halotolerant bacterial species at normal condition



(a) *Pseudomonas putida* (b) *Bacillus* sp.

(c) *Pyrococcus horikoshii*

Fig. 2 Biodegradation of phenol by halotolerant bacterial species at different pH levels

Table 2 Biochemical Characteristics of the isolated halobacteria

Biochemical Characteristics	*Pseudomonas putida*	*Bacillus sp.*	*Pyrococcus horikoshii*
Growth on MacConkey Agar	+	-	-
Indole Test	-	-	-
Methyl Red Test	-	-	-
Voges Proskauer Test	+	+	-
Citrate Utilization	+	+	+
Gas Production from Glucose	-	+	+
Casein Hydrolysis	-	+	-
Starch Hydrolysis	-	+	+
Urea Hydrolysis	-	-	-
Nitrate Reduction	+	+	-
Nitrite Reduction	-	-	-
H_2S Production	-	-	-
Cytochrome Oxidase	+	-	-
Catalase Test	+	-	+
Gelatin Hydrolysis	-	+	-
Oxidation/Fermentation	-	-	-
Arginine dihydrolase	+	+	-
Lysine decarboxylase	-	-	-
Ornithine decarboxylase	-	-	-
Acid Production			
Adonitol	+	-	+
Arabinose	+	+	-
Cellobiose	+	+	+
Dextrose	+	+	+
Dulcitol	+	-	+
Fructose	+	+	-
Galactose	+	-	+
Inositol	+	-	-
Lactose	+	+	+
Maltose	+	+	+
Mannitol	+	+	-
Melibiose	+	+	+
Raffinose	+	-	-
Rhamnose	+	+	+
Salicin	+	+	-
Sorbitol	+	-	-
Sucrose	+	+	+
Trehalose	+	-	+
Xylose	+	+	-

(+) indicates positive; (-) indicates negative

Effect of different pH level on the biodegradation of phenol was increased with the increasing time. *Pseudomonas putida* showed the maximum degradation of phenol up to 100% within 72 hrs at pH 7 followed by the *Bacillus* sp. and *P. horikoshii* with in 80 hrs (Fig. 2). When compared with other pH, the neutral pH showed 100% removal of phenol at different time interval. Most of the organisms, cannot tolerate the pH values below 4.0 and above 9.0 as because the acids and bases which can easily entered in to the cell which affect the metabolic pathway and denature the proteins finally leads to lethality [16, 17].

The phenol removal efficiency was also determined by the addition of different concentration of glucose at neutral pH. It reveals that, the selected strains showed maximum degradation of phenol up to 1500 $mg.l^{-1}$ with the addition of 0.2% glucose. The degradation rate decreased with the increased concentration of glucose (Fig. 3). This might be due to the catabolic respiration by glucose; i.e. the presence of glucose could inhibit the utilization of target substances [18, 19]. The growth and degradation of phenol by *Pyrococcus horikoshii* was totally affected at 0.8% level of glucose.

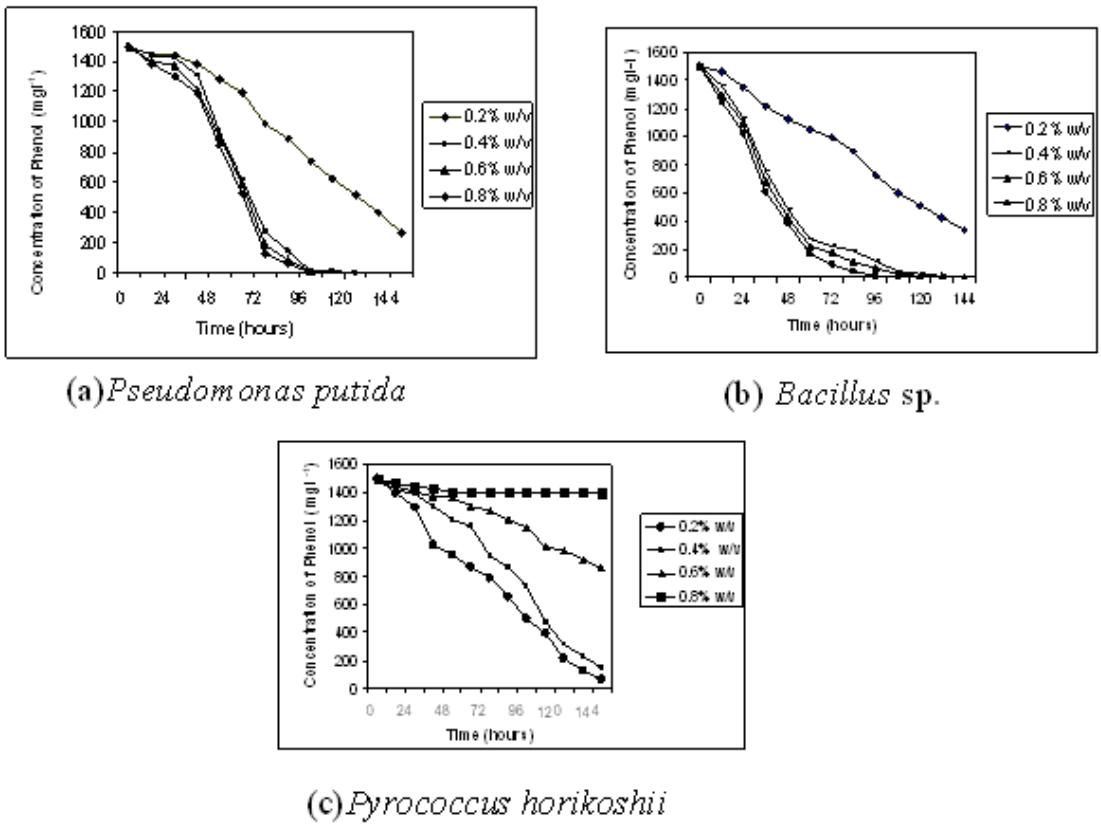


Fig. 3 Biodegradation of phenol at different concentration of glucose level by halotolerant bacteria.

Effect of different concentration of NaCl on the biodegradation of phenol was also carried out. It revealed that, *Pseudomonas putida* and *Bacillus* sp. degrade the phenol maximum at 0.35 M NaCl.

The degradation capacity of the selected strains decreased with the increasing NaCl concentration, which leads to decrease the growth rate and denaturing the proteins. No, degradation was occurred at NaCl concentration above 0.35 M. This might be due to the higher salt concentration which reduces the microbial activity. But, in the case of *Pyrococcus horikoshii*, it degrades the phenol up to 1000 $mg.l^{-1}$ at 0.7 M NaCl (Fig. 4). This might be due to osmoregulatory process [20]. Biodegradation of phenol in the presence of high concentration of sodium chloride has been already reported [21]. The author [22] reported that, 2% NaCl is optimum to degrade the phenol and toluene by *Bacillus subtilis* and *Bacillus laterosporous.*

CONCLUSION

It is concluded from the present study that, the biodegradation of phenol by the halophilic microbes such as *Pseudomonas putida*, *Bacillus* sp. and *Pyrococcus horikoshii* isolated from the coconut retting water are recommended to use for the biodegradation of phenol in saline condition after making appropriate change in the level of pH and sugar concentration in the coconut retting water before the biodegradation process.

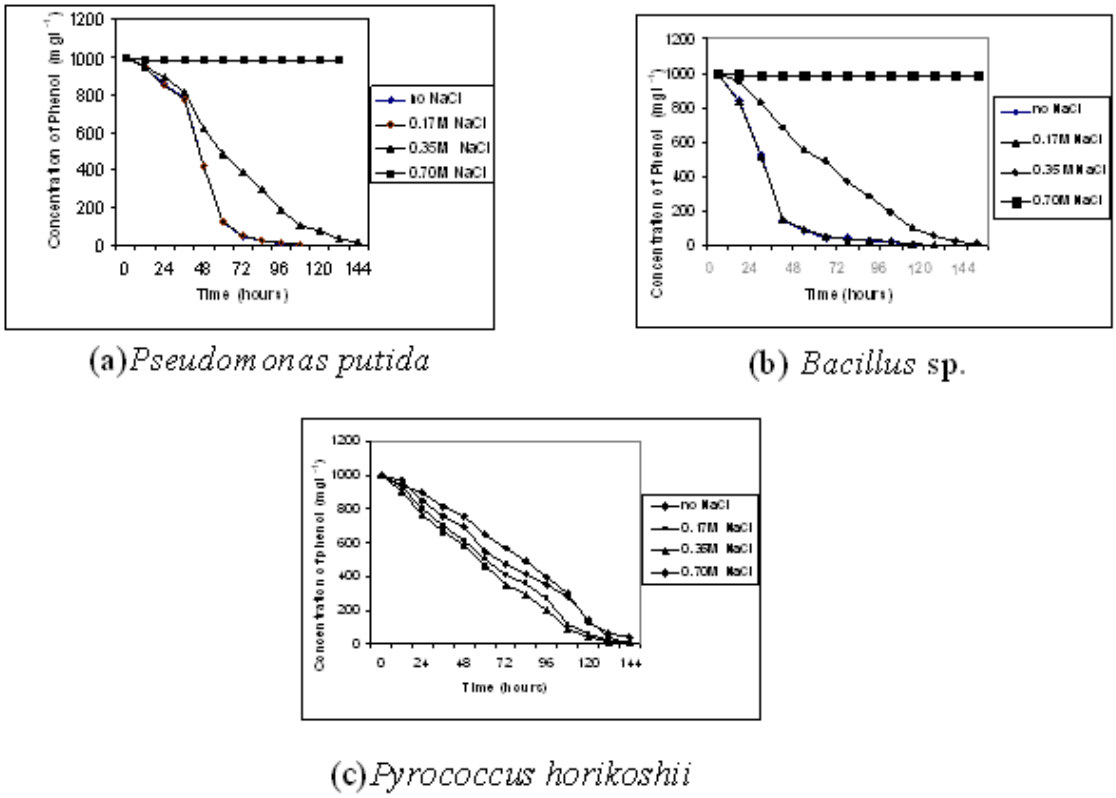


Fig. 4 Biodegradation of phenol at different concentration of NaCl concentration by halotolerant bacteria

Acknowledgement

The authors are grateful to the Ministry of Environment and Forest, New Delhi for providing financial assistance.

REFERENCES

1. Bulbul, G. and Aksu, Z. Investigation of wastewater treatment containing phenol using free and Ca-alginated gel immobilized *Pseudomonas putida* in a batch stirred reactor. Turkish Journal of Engineering and Environmental Sciences, 1997, 21:175–181.
2. Aksu, S. and Yener, J. Investigation of biosorption of phenol and monochlorianated phenols on the dried activated sludge. Process Biochemistry, 1998, 33: 649–655.
3. Gupta, V.K. Sharma, S. Yadav, I.S. and Mohan, D. Utilization of bagasse fly ash generated in the sugar industry for the removal and recovery of phenol and P-nitrophenol from wastewater. Journal of Chemical Technology and Biotechnology, 1998, 71: 180–186.
4. Loh, K.C. Chung, T.S. and Wei-Fern, A. Immobilized cell membrane bioreactor for high strength phenol wastewater. Journal of Environmental Engineering, 2000, 126: 75–79.
5. Indu Nair, C. Jayachandran, K. and Shashidhar, S. Biodegradation of phenol. African Journal of Biotechnology, 2008, 7(25): 4951-4958.
6. Saha, N.C. Bhunia, F. and Kaviraj, A. Toxicity of phenol to fish and aquatic ecosystems. Bulletin of Environmental Contamination and Toxicology, 1999, 63:195–197.
7. Wallace, J. Phenol. In: Kroschwitz JI (ed) Kirk-Othmer Encyclopedia of Chemical Technology. Wiley, New York, 1991, pp: 592–602.
8. Rengaraj, S. Moon, S.H. Sivabalan, R. Arabindoo, B. and Murugesan, V. Agricultural solid waste for the removal of organics: adsorption of phenol from water and wastewater by palm seed coat activated carbon. Waste Management, 2002, 22: 543–548.
9. The Environmental Protection Agency (EPA). Collation of toxicological data and intake values for humans. EPA Report. 2004, pp: 44-64.
10. Kafilzadeh, F. Farhangdoost, M.S. and Tahery, Y. Isolation and identification of phenol degrading bacteria from Lake Parishan and their growth kinetic assay. African Journal Biotechnology, 2010, 9(40): 6721-6726.
11. Armenante, P.M. Fava, F. and Kafkewitz, D. Effect of yeast extract on growth kinetics during aerobic biodegradation of chlorobenzoic acid. Biotechnology and Bioengineering, 1995, 47: 227–233.
12. Holt, J.G. Krieg, N.R. Sheath, P.H.A. Stanley, J.T and Williams, S.T. Bergy's manual of determinative bacteriology. 9th Edn. Williams and wilkins, Baltimore, Maryland, 1994, USA,
13. Yang, R.D. and Humphrey, A.E. Dynamic and steady state studies of phenol biodegradation in pure and mixed cultures. Biotechnology and Bioengineering, 1975, 17: 1211-1235.
14. Paraskevi, N.P. and Euripides, G.S. Effect of temperature and additional carbon sources on phenol degradation by an indigenous soil Pseudomonad. Biodegradation, 2005, 16: 403–413.
15. Chung, T.P. Tseng, H.Y. and Juang, R.S. Mass transfer effect and intermediate detection for phenol degradation in immobilized *Pseudomonas putida* systems. Process Biochemistry, 2003, 38: 1497–1507.
16. Bandyopadhyay, K. Das, D. and Maitri, B.R. Kinetics of phenol degradation using *Pseudomonas putida* MTCC 1194'. Bioprocess Engineering, 1998, 18: 373–377.
17. Annadurai, G. Balan, M.S. and Murugesan, T. Design of experiments in the biodegradation of phenol using immobilized *Pseudomonas pictorium* (NICM – 2077) on activated carbon. Bioprocess Engineering, 2000, 22; 101-107.

18. Papanastasious, A.C. Kinetics of biodegradation of 2, 4-Dichlorophenoxyacetate in the presence of glucose. Biotechnology and Bioengineering, 1982, 24: 2001–2011.
19. Satsangee, R. and Ghosh, P. Anaerobic degradation of phenol using an acclimated mixed culture. Applied Microbiology and Biotechnology, 1990, 34: 127–130.
20. Reed, R.H. Microbes in extreme environments, Special publications for the Society for General Microbiology, Herbert RA and Codd GA (Eds.) Academic Press, London, 1986, 17: pp: 51
21. Veenagayathri, K. and Vasudevan, N. Effect of pH, Nitrogen sources and salts on the degradation of phenol by the bacterial consortium under saline conditions. International Journal of Biotechnology and Biochemistry, 2010, 6(5): 783-791.
22. Bayoumi, R.A. and Abul-Hamd, A.T. Optimization of Bacterial Biodegradation of Toluene and Phenol under Different Nutritional and Environmental Conditions. Journal of Applied Science and Research, 2010, 6(8): 1086-1095.