Identification of Chlorophenols in Wastewater and Selective Removal using Pseudomonas and Bacillus Bacterial Microbial Strains

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Abstract

The present study is aimed at biodegradation of 4-Chlorophenol and 2,4-Dichlorophenol using Pseudomonas aeruginosa and Bacillus subtilis in the form of mixed and pure cultures. Optimum degradation conditions of 30°C temperature and 7 pH for maximum removal rate of chlorophenols have been established in this study. The mixed culture consisting of 50/50 Pseudomonas aeruginosa and Bacillus subtilis cultures shows maximum chlorophenol removal efficiency as compared to pure culture. After about 130 hours of contact, mixed culture could degrade the chlorophenols completely. Finally considering the difficulties related to segregating pure cultures and then using them for degradation, the use of mixed culture for chlorophenols removal has been proposed.

Keywords: *- Biodegradation; 4-Chlorophenol; 2,4-Dichlorophenol; Pseudomonas aeruginosa; Bacillus subtilis.*

INTRODUCTION

Various chemical, biochemical and petrochemical industries produce a wide range of highly toxic pollutants in the environment. Amongst these industrial pollutants organic compounds are the most hazardous in general. The effluents often consist of organic compounds, which have developed a resistance towards biological degradation, and hence they remain existing in the environment. Hence, they have a capability of getting accumulated and stay in animal and plant tissues for a longer period of time. Many organic pollutants in the effluent can definitely cause serious hazards to the human life [1]–[4]. Many organic aromatic compounds such as phenols and phenolic compounds pose a serious threat to water bodies, soil; has caused a great concern worldwide. Different phenol derivatives are considered as the most omnipresent types of synthetic toxins in modern wastewater, produced for the most part from oil refineries, coal plants, polymer industries, petrochemical industries and pharmaceutical industries [5]–[8].

Phenolic compounds are severely toxic compounds to human life, aquatic life and others [9]–[11]. Consumption of ~1 g of phenol is considered to be deadly to humans [12].

Phenolic compounds are considered to be very hazardous pollutants, and they are most difficult to be eliminated [13]. They are not agreeable to traditional treatment forms and, within the sight of chlorine, can respond to deliver chlorophenols, which are cancer-causing and considerably more impervious to degradation than phenol itself. [14], [15]. The Environmental Protection Agency (USA) has made it mandatory to lower the content of phenol in wastewater below 1 mg/L [16].

Chlorophenols are the most common foreign impurities, which widely exist in wastewater from many industrial conglomerates based on biocides, plastics, paper and pulp, petrochemicals, oil refineries [3], [17], [18]. Some of them get produced while chlorinating of water bodies, pesticides and chlorinated aromatic compounds use [18]–[20]. Because of the high virulence, exceptionally solid odour, ingenuity in the environment and as they are cancer-causing to living lifeforms, chlorophenols represent a genuine biological issue as natural contaminators [18], [19], [21].

2, 4 dichlorophenols, 2 chlorophenol (2 CP) and other phenolic compounds have been incorporated into EPA norms rundown of priority pollutants. Also, 2 CP, 4 chlorophenol (4 CP) and 2, 4 DCP are the most significant phenolic compounds produced after water chlorination [15], [22], [23]. Worldwide, the highest tolerable concentration of total content of phenol in the water bodies is set to be less than 1 mg/L [24].

In any case, higher fixations were often found polluted, where the prescribed levels of chlorophenols could range from 150 - 200 mg/L and beyond [25]. Ecological enactment in as far as possible, the aggregate phenolic compounds in water bodies released into the marine environment ranges beyond 0.1 mg/L [26].

A biofilm reactor was used in continuous and batch operation to determine the chlorophenol degradation potential. It was concluded that, in continuous flow biofilm reactor (CFBR) a gradation of biomass happened, while biomass in the sequencing batch biofilm reactor (SBBR) grew consistently because of the utilization of a propelled blade technique.

Under the shock loading conditions, degradation in SBBR was superior to in CFBR. Be that as it may, even the CFBR demonstrated a high adaptability, i.e. it performed superior than anticipated [27], A biofilm reactor consisting of activated granular carbon was utilized for the degradation of 4-CP and was explored under persistent stream task utilizing a feed ranging from 20– 50 mg/L with a living arrangement time of 1020 sec over 180 days and the reactor showed 4-CP expulsion efficiency ranging from 69–100% [28].

Soybean peroxidase and hydrogen peroxide were utilized for removal of 4-CP from aqueous solutions in a continuous tank reactor. The impact of enzyme and substrate concentrations and contact time, on the removal effectiveness of 4-CP was contemplated. The reactor display was fathomed and the hypothetical estimations of the unfaltering state change were ascertained utilizing inborn dynamic parameters.

Forecast of enduring state conduct of the reactor was affirmed utilizing the motor condition and the design model, and in addition the intrinsic kinetic parameters by making a decent estimation between the test and ascertained estimations of the consistent state transformation [29].

In light of expanding political and social interest regarding the environmental research on purification of water which has been developing widely over the most recent couple of years. As quality of water and directions against unsafe poisons have turned out to be stricter in numerous nations [14], [30].

The administration of wastewater containing high convergences of phenolic compounds speaks to a noteworthy financial and ecological test to generally businesses. This investigation goes for building up an incorporated framework for biodegradation of chlorophenols, thinking about 4-chlorophenol (4-CP) and 2, 4-dichlorophenol (2,4-DCP) as model contaminants.

2. EXPERIMENTAL

2.1 Methods

2.2.1 Stock solution of chlorophenol

The stocks of 800, 1000, 1200 and 1500 mg/L, 4-Chlorophenol and 2,4-Dichlorophenol were prepared by dissolving chlorophenol in deionized water. All compositions were prepared by diluting the stock solution with deionized water, and pH was changed in accordance with a particular incentive with 1 M NaOH or HCl.

2.1.2 Wastewater containing Phenols

The synthetic wastewater (SWW) and Mineral salt medium utilized for the batch assays was preparedusing phosphate buffer (50 mmol)at 7.5 pH (Table I & II).

Table I. Composition of Phosphate buffer and mineral salt medium

Phosphatebuffer		Mineral salt medium	
Constituent	**Concentration (g/L)**	**Constituent**	**Concentration (g/L)**
Peptone	0.16	K_2HPO_4	0.5
Meat extract	0.11	$MgSO_4$	0.2
Urea	0.03	$CaCl_2.2H_2O$	0.01
Sodium chloride	0.007	NH_4NO_3	3
Calcium chloride	0.004	$FeSO_4.7H_2O$	1.36
Magnesium sulfate	0.002	$Na_2MoO_4.2H_2O$	0.24
		$CuSO_4.5H_2O$	0.25
		$ZnSO_4.7H_2O$	0.58
		$NiSO_4.6H_2O$	0.11
		$MnSO_4.H_2O$	1.01
		H_2SO_4	1 mL/L

Table II. Composition of synthetic wastewater

Constituent	Quantity (mg/L)
Glucose	1000
Magnesium Sulphate	100
Di Potassium hydrogen phosphate	1100
Potassium di-hydrogen phosphate	550
Urea	250
Calcium Chloride	1

2.1.3 Batch experiments of chlorophenol biodegradation

The biomass was generated by incubating the cultures. The SWW was utilized for the development of microbial cultures. Batch cultivation experiments were performed utilizing chlorophenols as single restricting substrate for blended and unadulterated culture. Loopfull of biomass is added to the manufactured wastewater containing 4-Chlorophenol and 2,4-Dichlorophenol independently. The degree of chlorophenol degradation utilizing these diverse initial concentrations was explored for a few times

by discontinuous inspecting at each twelve hours interim.

2.1.4 Continuous tubular reactor

A tubular glass bioreactor (0.4 cm i.d.; 5 mL working volume) was utilized for the present study. The mixed culture was immobilized onto sodium alginate beads and the same were sandwiched in between alternate glass wool packings inside the reactor. Continuous flow of mixed chlorophenols was maintained from the bottom of the reactor using peristaltic pump at very low flow rates. The effluent chlorophenol stream was collected from the top of the reactor and the samples were analyzed accordingly.

The degradation of Chlorophenols(R), was most commonly utilized as a measure of biodegradation.

$$R = (C_i - C_f) * D \quad (1)$$

where C_i= influent chlorophenol concentration, C_f =steady state concentration of effluent chlorophenol, and D = dilution rate.

Like numerous studies in the past on chlorophenol removal, the unit utilized widely for R is mg/(L.h). The unit is capable to be scaled-up to kg/(m.day) (the degradation occurring per unit time per unit volume) by multiplying a specific factor.

2.1.5 Analytical methods

Concentration of chlorophenol was measured using 4-aminoantipyrine spectophotometric method by Shimadzu UV-Vis 1800 model and the absorbance of chlorophenol was measured at a wavelength of 510 nm.

RESULTS AND DISCUSSION

3.1 Batch removal of 4-Chlorophenol

The degradation behaviour of 4-chlorophenol was studied at 800 ppm using microbial cultures of *Pseudomonas aeruginosa* (PsA) and *Bacillussubtilis* (BS) in pure and mixed forms. It is clearly evident (Fig.1) that the mixed culture is able to reduce chlorophenol concentration in less amount of time. The measurements of chlorophenols degraded using various cultures were followed until it was completely degraded. The results of these investigations show that the mixed culture is efficient in biodegradation as compared to the pure ones. At higher concentrations, lag phase was observed, though the well-acclimatized inoculum was utilized in these investigations. Initially there is no chlorophenol reduction until 36 hours of contact. The 4-chlorophenol reduction potential is observed to be similar for both the pure cultures. The mixed culture is able to completely degrade the 4-chlorophenol in a time span 120 hours. It was observed that towards the end of the substrate consumption curve, there is a region of relative reduction in the rate of substrate removal. Two possible explanations may be offered at this stage. One is the deficit in availability of oxygen as these experiments were done in cotton-plugged conical flasks. Second being the reduction in pH levels with the further consumption of the substrate. In addition, the mixed culture must be creating a synergistic effect and hence has a better degradation efficiency towards 4-chlorophenol [6].

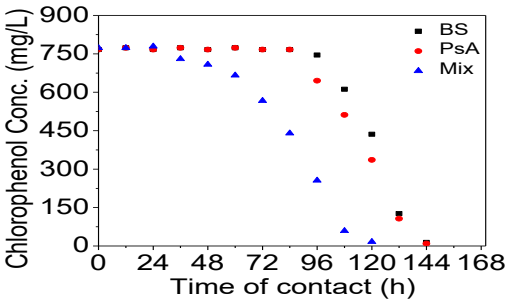

Fig. 1 Effect of various bacterial cultures on biodegradation behaviour of 800 ppm 4-Chlorophenol

3.2 Batch removal of 2,4-dichlorophenol

The degradation behaviour of 2,4-dichlorophenol at 800 ppm concentration utilizing pure and mixed microbial cultures shows a considerable variation as compared to for 4-CP (Fig. 2). The biodgradation potentials of various cultures were assessed for individual chlorophenols to determine their capabilities. The complete degradation of 4 CP takes a longer time duration as compared to for 2,4-DCP, which extendsupto 144h. The degradation activity by mixed culture for 2,4-DCP starts at a bit later stage of about 60h as compared to for 4-CP. Similar results were obtained for biodegradation of chlorophenols using Pseudomonas fluorescensin co- culture with Rhodococcuserythropolis[31].

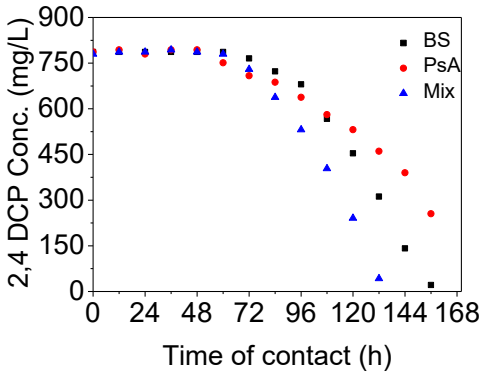

Fig. 2 Effect of various bacterial cultures on biodegradation behaviour of 800 ppm 2,4-Dichlorophenol

3.3 Batch biodegradation of mixed chlorophenols

The bacterial cultures utilized in the present study were significantly able to degrade mixed chlorophenols at 800 ppm (Fig. 3). The degradation was found tobe complete usingall the bacterial cultures. The difference in the degradation using mixed and pure cultures was much lesser while degrading mixed phenols, as compared to 2,4 DCP. The degradation behaviour using mixed culture is almost similar for mixed chlorophenols as compared to 2,4-DCP.A significant amount of degradation started after 30 h of contact time. After about 130 h of incubation mixed culture is significantly able to degrade mixed chlorophenols completely. There is no reduction in degradation potential when mixed chlorophenols are reduced using mixed culture. Similar results were observed for phenol biodegradationutilizingAspergillus awamori cells [11].

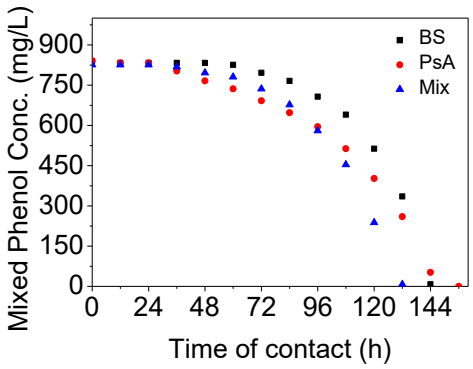

Fig. 3 Effect of various bacterial cultures on biodegradation behaviour of 800 ppm mixed phenols

CONCLUSIONS

It can be concluded that mixed culture bacterial strains were observed to be abetter substitute to pure cultureschlorophenol degraders at an optimum 7 pH and 30°C incubation temperature. Introduction of glucose up to a certain specific low concentration could certainly escalate the rate of degradation, but inhibited the process of degradation at higher concentrations. In addition, few column degradation studies were performed. This study puts a bright light on more cost-effective applications of mixed bacterial strains for chlorophenol removal at large scale specificallyin industries, where it poses alarming difficulties, due to its hazardous effects on environment.

REFERENCES

1. C. I. Nair, K. Jayachandran, and S. Shashidhar, “Biodegradation of phenol,” vol. 7, no. 25, pp. 4951–4958, 2008.
2. L. Yan et al., “Comparative study of different electrochemical methods for petroleum re fi nery wastewater treatment,” vol. 341, pp. 87–93, 2014.
3. J. Peng, J. Liu, X. Hu, and G. Jiang, “Direct determination of chlorophenols in environmental water samples by hollow fiber supported ionic liquid membrane extraction coupled with high-performance liquid chromatography,” vol. 1139, pp. 165–170, 2007.
4. T. Chung, H. Tseng, and R. Juang, “Mass transfer effect and intermediate detection for phenol degradation in immobilized Pseudomonas putida systems,” vol. 38, pp. 1497–1507, 2003.
5. D. Rajkumar and K. Palanivelu, “Electrochemical treatment of industrial wastewater,” vol. 113, no. May, pp. 123–129, 2004.
6. V. R. S. Cheela, G. S. Kumar, D. V Padma, and C. V Subbarao, “Biodegradation of phenol using pure and mixed Culture Bacteria,” e-Journal Sci. Technol., vol. 2, no. 9, pp. 91–95, 2014.
7. A. Kumar, S. Kumar, and S. Kumar, “Biodegradation kinetics of phenol and catechol using Pseudomonas putida MTCC 1194,” vol. 22, pp. 151–159, 2005.
8. M. Edalatmanesh, M. Mehrvar, and R. Dhib, “Chemical Engineering Research and Design Optimization of phenol degradation in a combined photochemical – biological wastewater treatment system,” vol. 6, no. June, pp. 1243–1252, 2008.
9. G. K. Agarwal and A. K. Ghoshal, “Packed bed dynamics during microbial treatment of wastewater : Modelling and simulation,” vol. 99, pp. 3765–3773, 2008.
10. M. Shourian et al., “Efficient phenol degradation by a newly characterized Pseudomonas sp . SA01 isolated from pharmaceutical wastewaters,” DES, vol. 246, no. 1–3, pp. 577–594, 2009.
11. I. Stoilova, A. Krastanov, V. Stanchev, D. Daniel, M. Gerginova, and Z. Alexieva, “Biodegradation of high amounts of phenol , catechol , 2 , 4-dichlorophenol and 2 , 6-dimethoxyphenol by Aspergillus awamori cells,” vol. 39, pp. 1036–1041, 2006.
12. A. Nuhoglu and B. Yalcin, “Modelling of phenol removal in a batch reactor,” vol. 40, no. April 2004, pp. 1233–1239, 2005.
13. M. H. El-naas, S. A. Al-muhtaseb, and S. Makhlouf, “Biodegradation of phenol by Pseudomonas putida immobilized in polyvinyl alcohol (PVA) gel,” vol. 164, pp. 720–725, 2009.
14. V. L. Santos and V. R. Linardi, “Biodegradation of phenol by a filamentous fungi isolated from industrial effluents - Identification and degradation potential,” Process Biochem., vol. 39, no. 8, pp. 1001–1006, 2004.
15. Y. Yavuz, A. S. Koparal, and Ü. Bak, “Treatment of petroleum re fi nery wastewater by electrochemical methods,” vol. 258, pp. 201–205, 2010.
16. O. Abdelwahab, N. K. Amin, and E. Z. El-ashtoukhy, “Electrochemical removal of phenol from oil refinery wastewater,” vol. 163, pp. 711–716, 2009.
17. L. Wang, Y. Li, P. Yu, Z. Xie, Y. Luo, and Y. Lin, “Biodegradation of phenol at high concentration by a novel fungal strain Paecilomyces variotii JH6,” J. Hazard. Mater., vol. 183, no. 1–3, pp. 366–371, 2010.
18. F. Fava, P. M. Armenante, and D. Kafkewitz, “Aerobic degradation and dechlorination of 2 = chlorophenol , 3-chlorophenol and 4-chlorophenol

by a Pseudomonas pickettii strain," pp. 307–312, 1995.

19. F. Ye, "Acclimation of anaerobic sludge degrading chlorophenols and the biodegradation kinetics during acclimation period," vol. 54, pp. 1573–1580, 2004.
20. E. Sahinkaya and F. B. Dilek, "Effect of biogenic substrate concentration on the performance of sequencing batch reactor treating 4-CP and 2 , 4-DCP mixtures," vol. 128, pp. 258–264, 2006.
21. S. Wang, "Aerobic granulation for 2 , 4-dichlorophenol biodegradation in a sequencing batch reactor," vol. 69, pp. 769–775, 2007.
22. A. O. Olaniran and E. O. Igbinosa, "Chemosphere Chlorophenols and other related derivatives of environmental concern : Properties , distribution and microbial degradation processes," Chemosphere, vol. 83, no. 10, pp. 1297–1306, 2011.
23. P. S. Majumder and S. K. Gupta, "Removal of chlorophenols in sequential anaerobic – aerobic reactors," vol. 98, pp. 118–129, 2007.
24. F. Alshehrei, "Effect of physicochemical factors on the biodegradation of phenol by Pseudomonas putida ATCC 12842 and Pseudomonas fluorescens ATCC 948," vol. 16, no. 39, pp. 1962–1968, 2017.
25. J. Zdarta, K. Antecka, R. Frankowski, A. Zgo, and H. Ehrlich, "Science of the Total Environment The effect of operational parameters on the biodegradation of bisphenols by Trametes versicolor laccase immobilized on Hippospongia communis spongin scaffolds," vol. 615, pp. 784–795, 2018.
26. A. D. Berillo, A. K. A. Al-jwaid, J. L. Caplin, A. Cundy, and I. Savina, "Biodegradation of Chlorophenol Derivatives Using Macroporous Material," vol. 4, no. 5, p. 58858, 2017.
27. H. P. Kaballo, Y. Zhao, and P. A. Wilderer, "Elimination of p-chlorophenol in biofilm reactors - a comparative study of continuous flow and sequenced batch operation," Water Sci. Technol., vol. 31, no. 1, pp. 51–60, 1995.
28. M. F. Carvalho, I. Vasconcelos, A. T. Bull, and P. M. L. Castro, "A GAC biofilm reactor for the continuous degradation of 4-chlorophenol: Treatment efficiency and microbial analysis," Appl. Microbiol. Biotechnol., vol. 57, no. 3, pp. 419–426, 2001.
29. J. L. Gómez, E. Gómez, J. Bastida, A. M. Hidalgo, M. Gómez, and M. D. Murcia, "Experimental behaviour and design model of a continuous tank reactor for removing 4-chlorophenol with soybean peroxidase," Chem. Eng. Process. Process Intensif., vol. 47, no. 9–10, pp. 1786–1792, 2008.
30. D. Suryaman and K. Hasegawa, "Biological and photocatalytic treatment integrated with separation and reuse of titanium dioxide on the removal of chlorophenols in tap water," J. Hazard. Mater., vol. 183, no. 1–3, pp. 490–496, 2010.
31. M. Goswami, N. Shivaraman, and R. P. Singh, "Microbial metabolism of 2-chlorophenol , phenol and q -cresol by Rhodococcus erythropolis M1 in co- culture with Pseudomonas fluorescens P1," vol. 160, 2005.