2012 International Conference on Future Environment and Energy
IPCBEE vol.28(2012) © (2012)IACSIT Press, Singapoore

Batch and Column Study on 4-cp Removal Using Calcium AlginateImmobilized *Arthrobacter Chlorophenolicus A6*

Sujith P. Abraham[1], N. K. Sahoo[2], K. Pakshirajan[3] and Pranab Kumar Ghosh[1+]

[1] Department of Civil Engineering, Indian Institute of Technology Guwahati, India – 781 039
[2] Centre for the Environment, Indian Institute of Technology Guwahati, India – 781 039
[3]Department of Biotechnology, Indian Institute of Technology Guwahati, India – 781 039

Abstract. The stability of calcium alginate beads immobilized with *Arthrobacter chlorophenolicus A6*, and its performance evaluation on 4-chlorophenol (4-CP) biodegradation in batch shake flasks at different proportion of sodium alginate and calcium chloride with varying initial pH was investigated. Results revealed that, at pH 6.5 and lower the *A. chlorophenolicus A6* encapsulated beads were found stable and 4-CP removal was 96.24% within 20h of culture period. Calcium alginate beads with composition Na.Alg 3.5% and $CaCl_2$ 7.5% immobilized with A. Chlorophenolicus A6 was found stable as well as efficient in complete removal of 4-CP from up to an initial concentration of 150 mgl^{-1} at pollutant loading rate of 1276 $mgl^{-1}d^{-1}$.

Keywords: *A. chlorophenolicus A6*, 4-chlorophenol, calcium alginate beads, substrate inhibition kinetics

1. Introduction

Chlorophenols are listed as priority pollutants (Wild et al.,1993) that can be removed by various physicochemical as well as biological processes (Ra et al., 2008). Arthrobacter chlorophenolicus A6 is an aerobic microorganism that has been demonstrated to degrade various toxic substituted phenols (Westerberg et al., 2000). Immobilization technology via cells entrapment is increasingly used in pollution control due to its several advantages over suspension cultures, such as, high cell concentrations, cell reuse, and elimination of costly processes of cell recovery and cell recycling, resistant to washout of cells even under conditions of negligible cell growth etc. (Aksu and Bulbul, 1999). Many gel-like materials are used as carriers which may be based on natural (alginate, carrageenan, agar, chitosan, etc.) or synthetic (polyacrylamide, polyacrylate, polyurethane etc.) precursors (Adinarayana et al., 2004). Among them, entrapment of cells in alginate is a promising method for microbial degradation of toxic substances. However, there are several reports on dissolution of such beads into the wastewater during experiment (Westmeier and Rehm, 1985). In addition, its stability depends upon various factors such as the proportion of sodium alginate and calcium chloride, as well as the pH and composition of wastewater to be treated (Adinarayana et al., 2004; Tepe and Dursun, 2008). Optimization of pH along with alginate and calcium chloride ratio are highly essential parameters to improve the stability and diffusion limitation for a better biodegradation of pollutants. The purpose of this and 4-CP biodegradation by calcium alginate immobilized *Arthrobacter chlorophenolicus A6*.

2. Materials and Methods

All the chemicals used in this project were either of analytical reagent (AR) grade or laboratory reagent (LR) grade. *Arthrobacter chlorophenolicus A6* was gifted by Prof. Janet K. Jansson, Department of media (Westerberg et al., 2000) with 0.3% yeast extract and 2% agar, pH 7.4. The media used for developing the seed culture for use in biodegradation experiments contained mineral salt media (MSM) (Alexander et. al.,

[+] Corresponding author. Tel.: + (91-3612582418); fax: +(91-3612582440).
E-mail address: (pkghosh@iitg.ernet.in).

1966) with 0.1% yeast extract and 4-CP at a concentration of 150 mg l-1. The seed culture medium (100 ml) taken in a 250 ml Erlenmeyer flask was inoculated with a loop full of the culture freshly grown on agar slants and incubated for 48 h at 28°C and 180 rpm. All 4-CP biodegradation experiments in the study were performed in an optimized MSM (Sahoo et al., 2010) having the composition (g l-1): K2HPO4 2.64, KH2PO4 0.4, NH4NO3 0.58, MgSO4 0.17, CaCl2 0.038, FeCl3 0.002, and containing 300 mg l-1 4-CP as the sole source of carbon and energy. The culture grown in the above mentioned seed culture media were collected by centrifugation, washed in sterile phosphate buffer (pH 7.4) and were grown overnight in 4-CP at 300 mg l-1 as the sole source of carbon and energy. The cells were again centrifuged (5000g, 20 min at 22ºC), washed in 1× phosphate buffer saline (pH 7.4) and were used for alginate bead preparation. The centrifuged and PBS washed cells, as prepared above, were re-suspended in various concentration of Na-alginate solution to a final cell concentration of 5.6 g l-1. The alginate-cell suspensions were then added drop wise using a peristaltic pump, through a needle to well-stirred, sterilized CaCl2 solutions of different concentration (table 1) to get beads of more or less uniform sizes of 2 mm. The beads were immersed in calcium chloride solution at 4°C for at least 2 h to complete gel formation. The beads were then washed with Millipore water.

Batch experiments were carried out to evaluate the performance and stability of those beads at different pH of wastewater. Column study was also carried out in up flow mode using a 45 cm long and 2.54 cm internal diameter perspex glass column for treatment of synthetic wastewater with initial 4-CP concentration of 50, 100, 150, 200 and 300 mg/l at various EBCTs. 4-CP concentration was measured in reverse phase HPLC (Varian Prostar 210) fitted with C18 column using acetonitrile-water (80:20, v/v) as the mobile phase: retention time = 5.6 min; flow rate = 0.8 ml min^{-1} at 28 ^{0}C.

Table 1. Preparation of *A. chlorophenolicus A6* immobilized alginate bead at different concentration of alginate to calcium chloride.

Bead type	Alginate (%)	Calcium chloride (%)	Remarks
1	3	3	Batch kinetic experiment
2	3	5	Batch kinetic experiment
3	3	7.5	Batch kinetic experiment
4	5	7.5	Batch kinetic experiment
5	7.5	7.5	Batch kinetic experiment
6	3.5	7.5	Column experiment

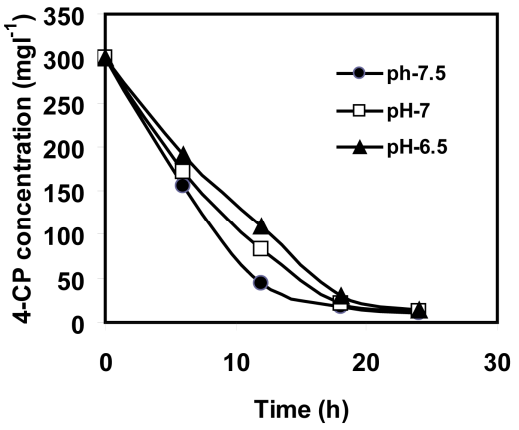
3. Results and Discussions

3.1. Effect of pH, Na-alginate and Calcium chloride concentration on beads performance

Effect of pH on removal of 4-chlorophenol by Ca- alginate beads prepared with 3% Na-alginate and 3% calcium chloride is shown in Figure 1. It is clear from the figure that the initial 4-CP removal efficiency was found higher at pH more than 7. However, towards the end of the degradation curve after 20 h, the 4-CP degradation efficiency was found almost equal in all the pH tested in the study. Although the initial 4-CP degradation at higher pH was found better, however, beads were found dissolved after a long run. Therefore, in the present study pH 6.5 was found more stable and the latter experiments to study the effect of Na-alginate and Ca-chloride concentration on 4-CP removal were carried out at pH 6.5. Figure 2 demonstrated the change in percentage of 4- CP degradation with Na-alginate and Ca-chloride concentration.

From the figure it is revealed that the Na-alginate concentration for the immobilization of cells would play a prominent role in the 4-CP degradation. From the data, it was observed that the 4-CP degradation was reduced from 97% to 92% when the Na-alginate concentration was increased from 3-7.5%. The reduction of 4-CP removal efficiency may be due to reduced porosity of the beads limiting the nutrient supply and oxygen diffusion. On contrary, though the degree of cross-linking of alginate molecules are determined by the concentration of Ca^{+2} ion, however, in the present study the effect of Ca-chloride on the 4-CP degradation

was found insignificant. Therefore, 3% alginate and 3% calcium chloride was found to be the optimum concentration for formulation of spherical and stable beads for enhanced 4-CP degradation.



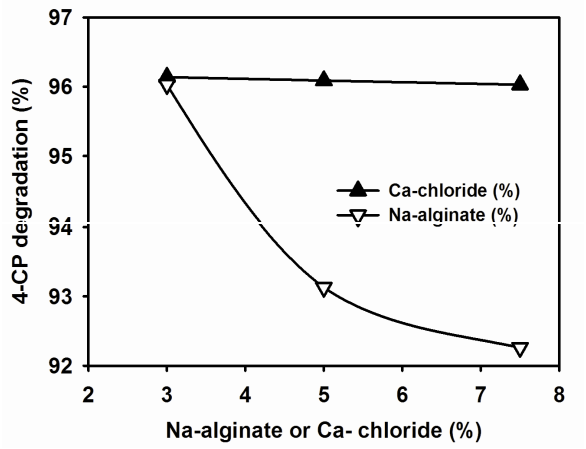


Fig.1 Effect of pH on removal of 4-CP by calciumalginate bead of Type 2 (3% +3%)

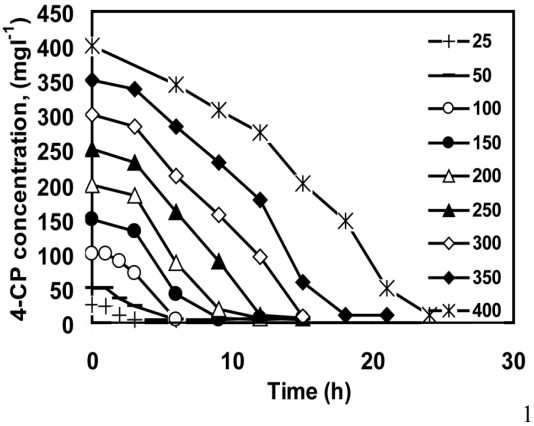
Fig. 2 Effect of Na-alginate and Ca-chloride percentages on removal of 4-CP by *A. chlorophenolicus A6.*

Batch experiment was carried out at these optimum conditions. Results revealed that increasing concentrations of 4-CP were better tolerated and more quickly degraded by immobilized cells than by the free cells. For an instant the immobilized A. chlorophenolicus A6 degraded 4-CP more than 96% at an initial concentration of 400 mgl^{-1} with in 24 h of culture (15.8 $mgl^{-1}h^{-1}$) where as in free cell the maximum degradation concentration was found to be 350 mgl^{-1} with a larger culture time of 30h (Sahoo et al., 2011). Furthermore, in the present study the degradation rate was found superior to many literatures such as 0.79 $mgl^{-1}h^{-1}$, 4.27$mgl^{-1}h^{-1}$ by Alcaligene species and Aspergillus sp. LEBM2 for 4-CP and phenol respectively (Adinarayana et al., 2004). Similarly in another study Tepe and Dursun, 2008 reported that a maximum 68% phenol removal was achieved at an initial phenol concentration of 100 mgl^{-1} by Ca-alginate immobilized Ralstonia eutropha. Furthermore, 4-CP removal due to adsorption and vitalization was found to be below 9%.Therefore, it is evidenced that abiotic 4-CP removal was much lower than the total removal observed due to presence of the microorganism. Hence, removal of 4-CP in the novel PBR can be well said to be due to biodegradation by *A. chlorophenolicus A6.*

3.2. Degradation kinetics of *A. chlorophenolicus A6* for 4-CP biodegradation

Figure 3 shows the time profile of 4-CP degradation by *A. chlorophenolicus A6* at its various initial concentrations. It is clear from the profile that the time taken by the actinomycetes to degrade the compound was dependent upon its initial concentration. The results show that the maximum degradation rate was achieved at 100 mg l^{-1} of 4-CP.

Possible explanations towards this phenomenon may be given based on a fall in pH and depletion of oxygen in the culture medium. Similar reason due to fall in pH of culture has previously been reported for phenol degradation by a mixed culture that mainly composed of Pseudomonadaceae, Vibrionaceae (Lallai and Mura, 1989). In this study the pH was found to drop from an initial value of 6.5 to final 6. The final low values of both oxygen and pH may affect the kinetics of substrate consumption adversely (Blanch and Clark, 1996).



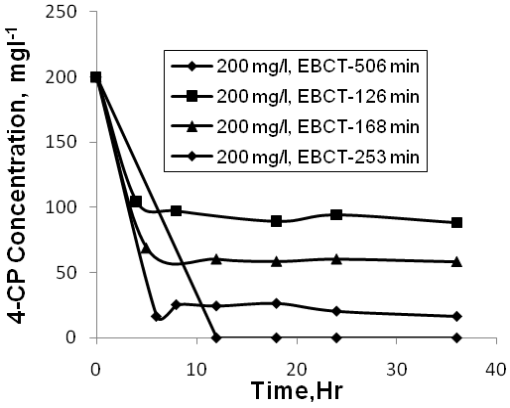


Fig. 3 Time profile of 4-CP degradation by *A.chlorophenolicus A6*

Fig.4 Removal of 4-CP at different EBCTs in PBR having an initial concentration of 200 mgl^{-1}

3.3. Column study result

Complete removal occurred in all the flow rates tested for initial concentration of 100 mgl^{-1} or less. Optimum conditions of PBR operations were observed at an initial concentration of 150 mgl^{-1} operating at 0.75 ml/min flow rate (i.e., HRT = 168.81 min) (Figure 4), which is equal to the 4-CP loading rate of 1275.59 mgl^{-1} d^{-1}. Mordocco et al. (1999) determined the maximum phenol degradation rate in PBR as 1400 mg $l^{-1}d^{-1}$ for P. putida immobilized in calcium-alginate beads. Tepe and Dursun (2008) determined the maximum phenol degradation rate in PBR as 870 mg $l^{-1}d^{-1}$ for ralstonia eutropha immobilized in calcium alginate beads. The maximum degradation rate obtained in this study (1474 mg $l^{-1}d^{-1}$) is comparable to those values obtained in the above mentioned literatures. By increasing either concentration or flow rate more than that of the above mentioned conditions significant amount of 4-CP were found in effluent samples. The beads prepared for column study were found stable for about 88 days of continuous run.

4. Conclusion

The biodegradation of 4-CP by Ca-alginate gel immobilized A. chlorophenolicus A6 was studied as a function of initial 4-CP concentration, and at different proportion of sodium alginate and calcium chloride with varying initial pH. 4-CP was utilized as sole source of carbon and energy by A. chlorophenolicus A6 even at a concentration of 400 mgl^{-1}. Results revealed that the specific degradation rate and the stability of the cell immobilized beads were highly affected by pH and initial 4-CP concentration. The best performance was observed at the pH of 6.5 when tested with the beads containing 3% Na-Alg. and 3% of CaCl2. It was observed that removal of 4-chlorophenol by Ca-alginate beads only due to adsorption was not significant. The column reactor could completely remove 4-CP from an initial concentration of 150 mgl^{-1}, when operated at EBCT of 169 min. The pollutant loading rate at the above mentioned conditions was 1276 $mgl^{-1}d^{-1}$. The beads prepared for column study were found stable for about 88 days of continuous run.

5. Reference

[1] Adinarayana K., Bapi Raju K.V.V.S.N and Ellaiah P. (2004), Investigations on alkaline protease production with B. subtilis PE-11 immobilized in calcium alginate gel beads, *Process Biochemistry.,* 39, 1331–1339.

[2] Aksu Z and Bu¨lbu¨l G. (1999), Determination of the effective diffusion coefficient of phenol in Ca alginateimmobilized P.putida beads, Enzyme and Microbial Technology 25:344–348.

[3] Alexandar M and Lustigman BK (1996) Effect of chemical structure on microbial degradation of substituted benzenes, *J Agric Food Chem.,* 14, 410–413.

[4] Blanch HW and Clark DS. (1996), *Biochemical engineering*. Marcel Dekker, New York.

[5] Lallai A and Mura G. (1989), pH variation during phenol biodegradation in mixed cultures of microorganisms, *Water Res.,* 23,1335–1338.

[6] Mordocco.A, Kuek.C, Jenkins.R (1999), Continuous degradation of phenol at low concentration using immobilized *Pseudomonas putida* Enzyme and Microbial Technology 25, (1999) 530–536.

[7] Sahoo N.K., Pakshirajan K., Ghosh P.K and Ghosh A.(2011), Biodegradation of 4-chlorophenol by *Arthrobacter chlorophenolicus A6*: effect of culture conditions and degradation kinetics, *Biodegradation. 22(2), 275-286.*

[8] Sahoo N.K., Pakshirajan K and Ghosh P.K. (2010), Enhancing the biodegradation of 4-chlorophenol by Arthrobacter chlorophenolicus A6 via medium development, *International Biodeterioration & Biodegradation* 64: 474-480.

[9] Tepe.O and Dursun A.Y. (2008), Combined effects of external mass transfer and biodegradation rates on removal of phenol by immobilized *Ralstonia eutropha* in a packed bed reactor. *Journal of Hazardous Materials.* 151: 9–16.

[10] Ra J.S.,Oh S.Y.,Lee B.C and Kim S.D. (2008),The effect of suspended particles coated by humic acid on the toxicity of pharmaceuticals, estrogens, and phenolic compounds. *Environmental international* 34:184-192.

[11] Westerberg, K., Elvang, A.M., Stackebrandt, E., Jansson, J.K., 2000. *Arthrobacter chlorophenolicus* sp.nov., a

new species capable of degrading high concentration of 4-chlorophenol, International Journal of Systematic and Evolutionary Microbiology 50, 2083-2092.

[12] Westmeier F. and Rehm H.J. (1985), Biodegradation of 4-CP by entrapped Alcaligens sp. A 7-2 . Appl. Microbial Biotechnol (1985) 22:301-305.

[13] Wild SR., Harrad SJ and Jones KC.(1993), Chlorophenols in digested U.K. sewage sludges. *Water Research*. 27:1527-1534.