Research Journal of Environmental Sciences

ISSN 1819-3412

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Environmental Sciences 6 (6): 238-244, 2012
ISSN 1819-3412 / DOI: 10.3923/rjes.2012.238.244
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Biodegradation of 4-Chlorophenol by *Pseudomonas putida* NCIM sp. 2650 under Aerobic Conditions

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ABSTRACT

Biological treatments for degradation of chlorophenols are one of the promising treatment techniques as they are relatively less expensive and result in complete mineralisation. Degradation experiments were conducted using *Pseudomonas putida* (NCIM sp. 2650) under aerobic conditions in two reactors, connected in series. The effectiveness of *Pseudomonas putida* to degrade synthetic 4-chlorophenols (4-CP) as well as industrial effluent was tested along with glucose as a carbon source. The synthetic 4-CP initial concentrations were varied from 60, 80 and 100 ppm and it was observed that an equilibrium degradation of 66, 68 and 93% was achieved for reactor 1 and 95, 97 and 99% degradation for reactor 2 at 60, 80 and 100 ppm of 4-CP initial concentrations, respectively. The reactor 2 effluent was also analyzed for Biological Oxygen Demand (BOD_3) and Chemical Oxygen Demand (COD) and an average BOD_3 reduction of 95% and COD reduction of 94% was achieved for all three initial 4-CP concentrations. Equilibrium degradation of 69% and 98% was achieved for reactor 1 and 2 for industrial effluent with a BOD_3 reduction, 96% and COD reduction, 91% showing that *Pseudomonas putida* possessed the strong capacity to degrade 4-CP.

Key words: *Pseudomonas putida*, 4-chlorophenol, chlorophenols degradation, glucose, industrial effluent, oxygen demand

INTRODUCTION

The continual growth of agricultural as well as industrial activities have resulted in the synthesis of chlorinated organic compounds, the most important groups of xenobiotic chemicals (Sahinkaya and Dilek, 2005; Wen *et al*., 2006). Chlorophenols representing xenobiotic chemicals are widely used in many industries such as leather, pesticides, wood and pharmaceutical preservatives, textiles and pulp-paper industries etc. The effluents of these industries containing chlorophenols on discharge to the environment pollute soil and groundwater (Zouari *et al*., 2002; Sahinkaya and Dilek, 2005; Monsalvo *et al*., 2009; Tobajas *et al*., 2012). It was reported that some of the industrial effluents contained chlorophenols in the near range of 150 μg L^{-1} (Valo *et al*., 1990) to 100-200 mg L^{-1} (Ettala *et al*., 1992). Biological degradation techniques are considered as the most effective for treatment of chlorophenols, they are relatively inexpressive and result in complete mineralization as compared with adsorption/chemical oxidation (Armenante *et al*., 1999; Atuanya *et al*., 2000; Jung *et al*., 2001; Li *et al*., 2011). Many microorganisms have proved

successful for the partial or complete degradation of 4-CP up to 300 ppm concentration and to name a few: *Pseudomanas* spp. (Knackmuss and Hellwig, 1978), *Azotobacter* sp. (Wieser *et al*., 1994), *Rhodococcus* sp. (Finkelshtein *et al*., 2000) and *Alcaligenes* sp. (Hill *et al*., 1996).

In the present study, the effectiveness of *Pseudomonas putida* (NCIM sp. 2650) for the biodegradation of 4-CP of 60, 80 and 100 ppm of initial concentrations and an industrial effluent with 176 ppm of 4-CP were tested under aerobic conditions. It has been observed that most of the experiments were performed in shake flasks for 4-CP biodegradation. In this study, an attempt has been made to imitate industrial scenario and use two reactors in series, in order to improve the efficiency and reduce the overall size of reactors. It is known that in experimentations with two reactors are connected in series, the first reactor operates at a higher concentration and the rate and conversion are greater. The second reactor in series builds on the conversion in the first reactor. The experimental data were analyzed and reported.

MATERIALS AND METHODS

Wastewater: In the present study, synthetic 4-CP prepared in laboratory, raw industrial wastewater collected by Karnataka State Pollution Control Board, were made available for experimentation. The industrial effluent was collected, preserved and transported in accordance with the standard methods for the examination of water and effluent. The effluent was characterized in terms of BOD_3 and COD. The concentration of 4-CP in effluent was measured by 4-amino-antipyrine spectrophotometric analysis method (APHA, 1998). The instrument used was UV-Vis Spectrophotometer Elico-BL 198. The characteristics of industrial effluent are tabulated in Table 1.

Microorganism: The microorganism *Pseudomonas putida* (NCIM sp. 2650) for the biodegradation of 4-CP was obtained from National Chemical Laboratory, Pune. The cultures were periodically sub-cultured on agar slants and stored at 4°C.

Media preparation: Two mineral media were prepared in the laboratory and pH was adjusted to 7 using 0.1 N sodium hydroxide solution. The chemical compositions of both media are summarized in Table 2.

Table 1: Characteristics of industrial effluent

Parameter	Value (ppm)
4-chlorophenol	176
Biological oxygen demand	500
Chemical oxygen demand	1500

Table 2: Chemical compositions of media

Media	Chemicals	Concentration (g L^{-1})
A	Ammonium nitrate	1.000
	Ammonium sulfate	0.500
	Sodium chloride	0.500
	Di-potassium hydrogen ortho-phosphate	1.500
	Potassium di-hydrogen ortho-phosphate	0.500
B	Calcium chloride	0.001
	Magnesium sulfate	0.050

Acclimatization: The *Pseudomonas putida* strain was acclimatized to use 4-CP as the sole source of carbon and energy. The acclimatization was carried out in multiple stages and is described as follows:

Primary acclimatization: Media A of 98 mL was mixed with 1 mL of Media B and sterilized. A volume of 1 mL (10,000 ppm) phenol solution was added to make up the initial phenol concentration to 100 ppm with a total volume of 100 mL. The *Pseudomonas putida* was inoculated to this media and incubated at 30°C and 150 rpm for 48 h.

Secondary acclimatization: The media was prepared as per the previous protocol and 1 mL of primary acclimatized culture was inoculated. This acclimatization procedure was repeated four times taking 100 ppm of phenol concentration.

Acclimatization to 4-CP: The phenol acclimatized *Pseudomonas putida* were further acclimatized to 4-CP of 10, 20, 30, 50, 70 and 100 ppm concentrations.

Experimental set-up and general procedure: The experimental set-up as shown in Fig. 1 consists of feed tank and two PVC reactors of 10 L capacity each, connected in series. The feed tank and reactors were provided with UV lamp source, inlet for medium and co-substrate (glucose) and an aeration system at the top. In between the pipes connecting the reactors, a ball valve was fitted followed by a union having 0.45 μm bacterial filter (Millipore) to avoid the contamination of reactor contents. Sampling ports were provided for both reactor 1 and 2 to collect the biodegraded sample.

The reactor setup was sterilized using alcohol and UV light. The 3 L synthetic 4-CP/industrial effluent along with 30 mL of glucose were added to feed tank and aeration system was switched on. The aerated and sterilized mixture was fed into reactor 1 and 30 mL of 4-CP acclimatized culture was added by means of a sterile injector provided at the top of the reactor. The reaction was allowed to occur and the reactor contents were analyzed for 4-CP concentrations for every 24 h. In order to measure the concentration of 4-CP, the samples were centrifuged at 5000 rpm for 10 min. The supernatant fluid was used to determine the concentration of 4-CP by 4-amino-antipyrine

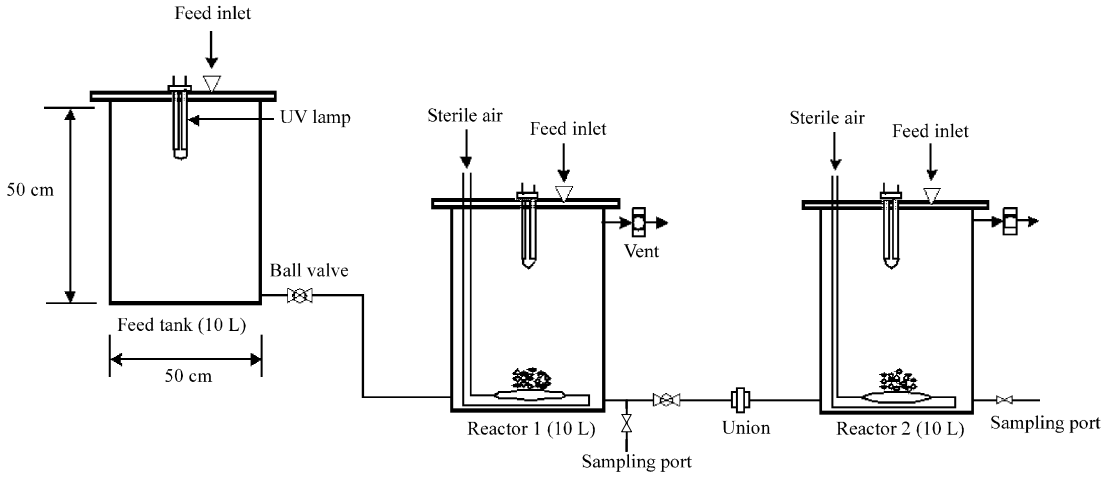

Fig. 1: Schematic diagram of experimental set-up

spectrophotometric analysis method. When decrease in 4-CP concentrations became negligible, contents of reactor 1 were transferred to reactor 2 and additional glucose was added and the reaction was allowed to occur till equilibrium conditions. Reactor 2 effluents were analyzed for 4-CP concentrations for every 24 h and for BOD_3 and COD.

RESULTS AND DISCUSSION

To ensure the degradation of 4-CP is solely due to *Pseudomonas putida*, a dry run was carried out for a period of 7 days without inoculum with 80 ppm of 4-CP and 300 ppm of glucose. After every 24 h of residence time, the extent of 4-CP degradation was measured. At the end of day 7 the concentration of 4-CP was 79.5 ppm. This shows that photo-oxidation has negligible effect on 4-CP degradation.

A series of experiments were further carried out in presence of *Pseudomonas putida* to investigate the effectiveness of *Pseudomonas putida* to degrade 4-CP at different initial concentrations. The initial concentrations of 4-CP were varied from 60, 80 and 100 ppm and glucose concentration maintained constant as 300 ppm. The time course variation of 4-CP degradation during continuous growth of *Pseudomonas putida* for reactor 1 for different initial concentrations of 4-CP were measured and the degradation profiles are shown in Fig. 2. It was observed that a significant biodegradation of 4-CP was obtained on day 2 and increased with increase in contact time. It was also observed that biodegradation increased with an increase in 4-CP initial concentrations and an equilibrium degradation of 66, 68 and 93% was achieved for reactor 1 at initial 4-CP concentrations of 60, 80 and 100 ppm, respectively on day 7 and 8. The effluent sample was transferred to reactor 2 when decrease in 4-CP became negligible. Figure 3 shows the 4-CP degradation profile for reactor 2 for different initial concentrations of 4-CP. A similar trend was observed for reactor 2 effluent. Equilibrium degradation of 99% was achieved on day 11 for 100 ppm of 4-CP initial concentration, whereas equilibrium degradation of 97 and 95% was obtained on day 12, respectively for 80 and 60 ppm of 4-CP initial concentrations. The reactor 2 effluent was also analyzed for BOD_3 and COD at the end of the experiment and an average BOD_3 reduction, 95% and COD reduction, 94% was achieved for all three initial 4-CP concentrations.

The degradation experiments were further carried out utilizing industrial effluent (4-CP of 176 mg L^{-1}). Experimental procedure as carried out for synthetic 4-CP was followed and Fig. 4

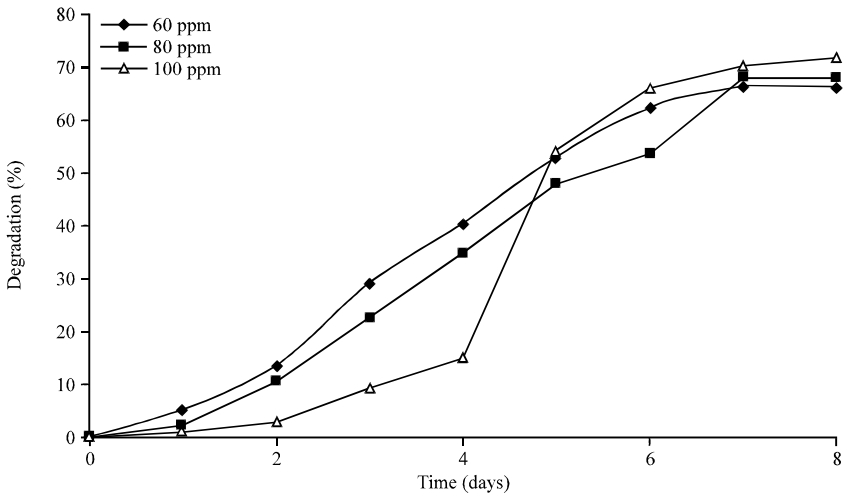

Fig. 2: Time course variation of 4-CP degradation in reactor 1 for synthetic 4-CP solution

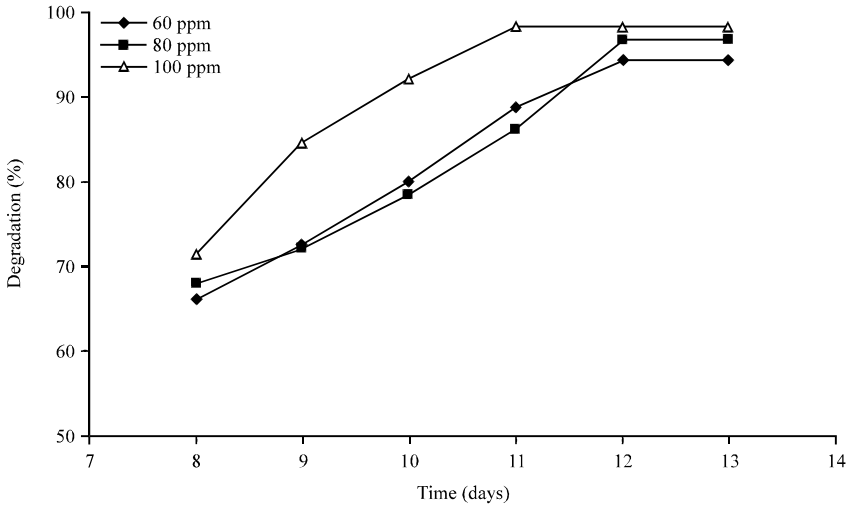

Fig. 3: Time course variation of 4-CP degradation in reactor 2 for synthetic 4-CP solution

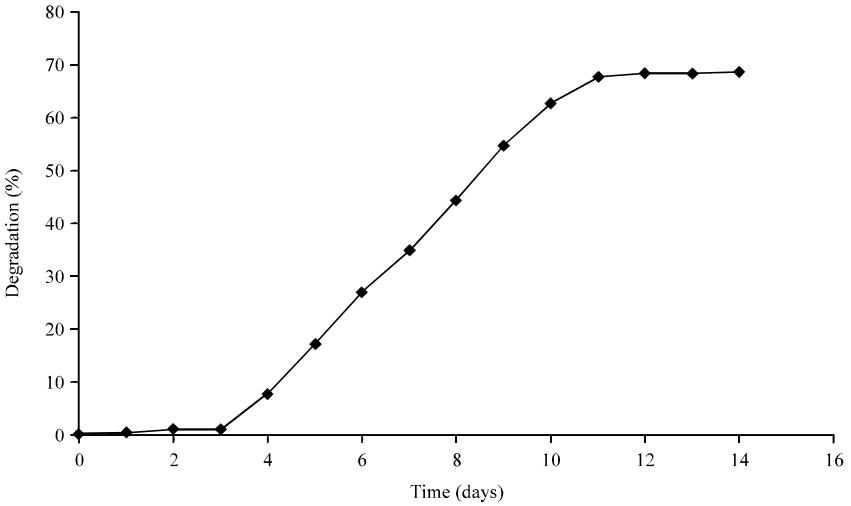

Fig. 4: Time course variation of 4-CP degradation in reactor 1 for industrial effluent

shows the 4-CP degradation profile during the continuous growth of *Pseudomonas putida* for reactor 1. It was observed that for a period of 3 days the degradation was negligible. On day 4 a significant degradation was obtained and increased with increase in contact time. Equilibrium degradation of 69% was achieved on day 11 for reactor 1. The 4-CP degradation profile for reactor 2 is shown in Fig. 5 and 98% degradation was achieved for reactor 2 on day 19. Murcia *et al.* (2012) tested *Pseudomonas putida* strain for a higher 4-CP concentration of 250 mg L^{-1} and obtained only 22% of degradation in presence of glucose. They concluded that 4-CP exerted an inhibitory effect on the strain at 250 mg L^{-1}. Yan *et al.* (2008) and Tobajas *et al.* (2012) used dual substrate containing glucose and phenol and concluded that the presence of phenol lead higher 4-CP biodegradation as compared with glucose using the mutant strain CTM 2 and *Comamonas testosteroni* CECT 326T, respectively. The complete transformation of 4-CP depends on the dose of phenol added as well as the ratio of initial 4-CP concentration to initial biomass

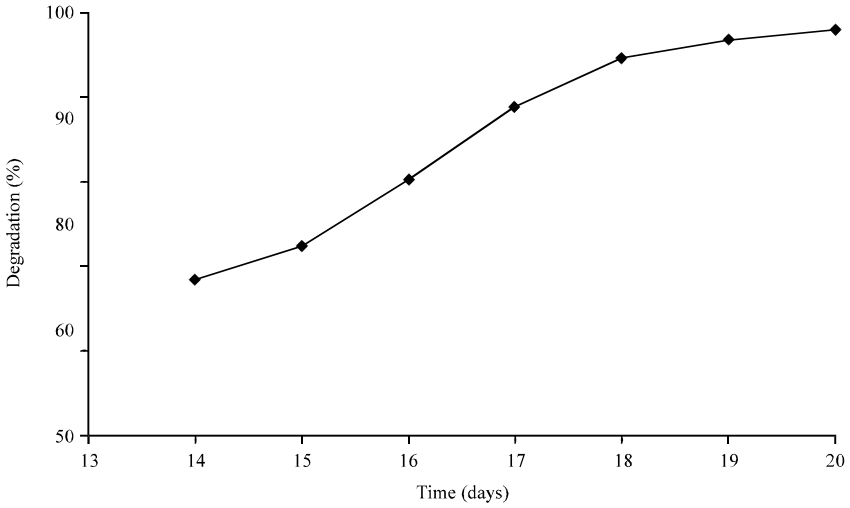

Fig. 5: Time course variation of 4-CP degradation in reactor 2 for industrial effluent

concentration (Lee and Lee, 2007). Sahoo *et al.* (2010) developed a medium containing an optimum combination of 2.62 g L^{-1} of di-potassium hydrogen phosphate, 0.58 g L^{-1} of ammonium nitrate, 0.17 g L^{-1} of magnesium sulfate and 0.04 g L^{-1} of calcium chloride and reported 100% conversion efficiency by *Arthrobacter chlorophenolicus* A6. The industrial effluent after successful degradation using *Pseudomonas putida* was further analyzed for both BOD_3 and COD and BOD_3 reduction, 96% and COD reduction, 91% was achieved.

CONCLUSION

Experiments to degrade 4-CP under aerobic conditions were carried out to study the effectiveness of *Pseudomonas putida* at different concentrations of 4-CP. The effectiveness of *Pseudomonas putida* was also tested for phenolic industrial effluent and *Pseudomonas putida* possessed the strong capacity to degrade 4-CP. The maximum degradation of 99% was achieved for synthetic 4-CP and degradation of 98% for industrial effluent having 4-CP concentration of 176 ppm. The average BOD_3 reduction of 96% and COD reduction of 91% was observed for industrial effluent.

ACKNOWLEDGMENT

The authors of the paper gratefully acknowledge the support and facilities provided by KLE Dr. M.S. Sheshgiri College of Engineering and Technology, Belgaum-590008, Karnataka, India in carrying out this study.

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