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Phenol degradation by a bacterium *Pseudomonas putida* in the presence of chromium

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Abstract

Phenol and Phenolic compounds are well known components in aqueous waste of many different pollution generating industries. These compounds must be regarded as hazardous to the environment and are resistant to biological degradation. Degradation of Phenol in the presence of hexavalent chromium was studied using phenol degrading bacteria, *Pseudomonas putida*. The bacteria was able to degrade phenol up to 80% in the absence of chromium but the degradation was increased up to 81.67% in the presence of 25mg/l initial concentration of chromium (VI). The degradation was declined further up to 21.67%, when concentration of Chromium (VI) was applied 50mg/l.

Key Words: ***Phenol degradation, Pseudomonas putida, Hexavalent chromium.***

Introduction

Phenol is a common constituent of effluents from polymeric resin production, oil refining, coking plants, textile, pulp and paper mills and tannery industries. The discharged effluents contain phenolic compounds ranging 6-200 mg/L, however their admissible limit is only 3 mg/L in the receiving water bodies (Jossens 1978). Phenol can be toxic to fish at concentrations of 5 mg/L and gives an objectionable taste to drinking water at far lower concentrations (Throop 1975). The persistence of phenolic compounds in the aquatic and terrestrial environment can be injuries to the health of humans and cause allergic dermatitis, skin irritation, cancer and mutation (Bui Kema *et al.* 1979, Zitomer and

Speece 1993 and Sing and Viraraghavan 1966).

Recent literature on the method of degradation of phenol and their compounds from industrial effluents focus on microbial degradation process. *Pseudomonas putida* has an ability to degrade a wide variety of aromatic compounds. The present study has revealed the potential of phenolic compounds degradation through microbial degrading bacterium *Pseudomonas putida* in the presence or absence of Chromium as the wide spread use of chromium and its compounds by various industries has led to the release of this element into environments. Being mutagenic, carcinogenic and teratogenic, hexavalent chromium is about 100-fold more toxic than the trivalent form (Petilli and Flora 1977).

Materials and Methods

A pure culture of *Pseudomonas putida* was obtained from Institute of Microbial Technology, Chandigarh (UT).The bacterial strain (*Pseudomonas putida*) was maintained on nutrient agar medium, stored at 4 ^{o}C and sub cultured every week. The culture media used to perform the growth experiments were LB (Luri Bertani) media containing peptone 10gm/L, NaCl 10gm/L, yeast extract 5 gm/L, agar 2% and LB (Luria broth) having above composition without agar. The complete growing medium were sterilized in an autoclave at 121 ^{o}C for 30 minutes and subsequently cooled. Phenol solution and chromium were added to the sterilized medium to make the solution of desired concentration. In each experiment the initial pH of the medium was raised to 7.0 by addition of concentrated NaOH before sterilization. The LB (Luria broth) media (25 mL) was dispensed into a sterile 150 ml flask fitted with a cotton plug. Aromatic compound (Phenol) and Chromium (VI) as chromate (K_2CrO_4) were added as sole electron donors for chromium (VI) reduction. Harvested cells of *P. putida* were placed into each experiment before incubation in the dark on a rotator shaker at 140 RPM and at 30 ^{o}C temperature.

The viable cells of *P. putida* were counted by using haemocytometer at the desired time intervals. The cell growth was measured by the optical density (OD at 699 nm) taken at regular intervals. Hexavalent chromium was determined calorimetrically by using spectrophotometer at 540 nm by reaction with diphenylcarbazide in acid solution (APHA 1989). The phenol concentration was determined calorimetrically using a spectrophotometer at 500 nm by reaction with 4-amino-antipyrine in the presence of potassium ferricyanide (APHA 1989).

Results and Discussion

In the culture, *Pseudomonas putida* has oxidized the phenol and used Cr (VI) as an electron acceptor. The oxidation of phenol by *P. putida* has initialized the energy flow of the culture and served as a primary energy source for the strain while Cr (VI) reduction occurred only as result of metabolism

(Shen and Wong 1995). The effect of different concentration of chromium (VI) mixed with bacterial culture (*Pseudomonas putida*) growth has shown in Fig. 1 and observation revealed that growth was inhibited up to incubation period of 6 hrs in the culture media containing chromium (VI). The chromium concentration (50 mg/L) in culture medium has exhibited more inhibitory phenomenon than culture of initial chromium concentration (25 mg/L). At the 24 hrs incubation period, the culture of chromium concentration (25 mg/L) was found growth promoters, where as chromium concentration (50 mg/L) has shown considerable inhibitory effect as increasing the incubation periods.

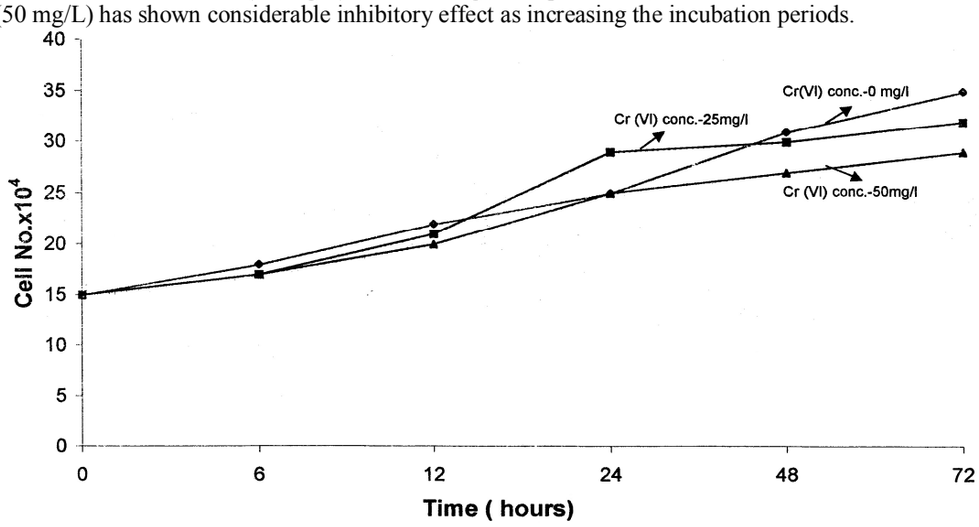

Figure 1. Growth pattern of *Pseudomonas putida* at diferent concentration of Cr (VI)

The sudden change in the growth of *P. putida* at the incubation period of 24 hrs can be explained with changing pH (Fig. 2). In the culture medium, the changing pattern of pH has observed after 12 hours exposure period. This change in pH may be due to the presence of some metabolites released by *P. putida* in the culture (Shen and Wang 1995).

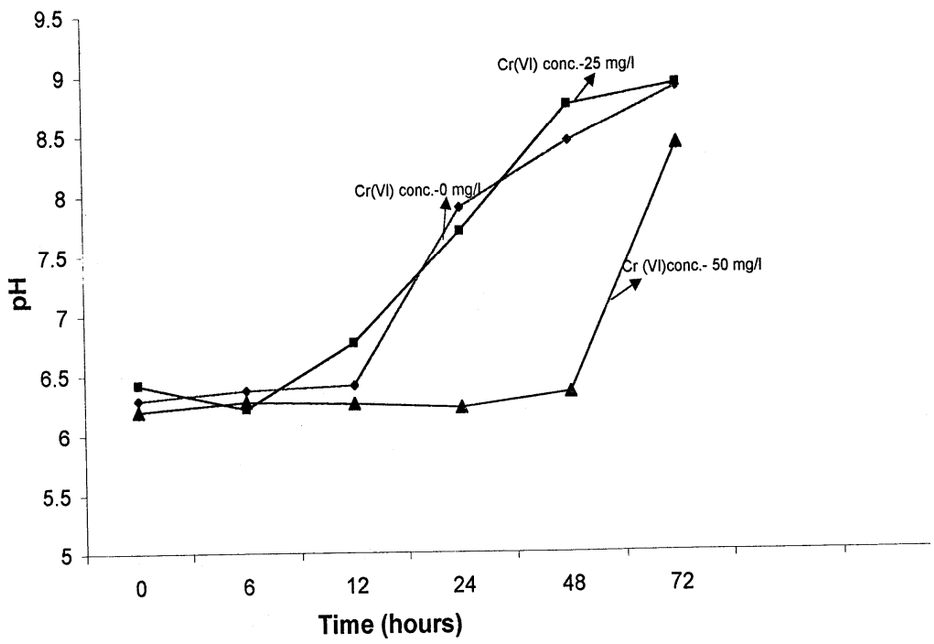

Figure 2. Change in pH at different Cr (VI) concentration with time

In the experiments chromium (VI) reduction occurred in the culture of *P. putida* using phenol as a

carbon source, the results have observed that Cr (VI) reduction was greater in the culture containing Chromium concentration (50 mg/L) (Fig. 3). The Chromium reduction suddenly increased up to 60 percent in the culture containing Chromium con. (25 mg/L) after 12 hrs incubation time. The effect of Cr (VI) and phenol concentrations on Cr (VI) reduction and phenol degradation were also investigated by (Shen and Wang 1995 and Annadurai *et al.* 1999). The phenol degradation patterns have shown in Fig-4 and the results occurred as Chromium (VI) with concentration of 25 mg/L have shown maximum rate of phenol degradation in between 24-48 hours incubation time, while the chromium (VI) conc. (50 mg/L) has suppressed the phenol degradation up to many fold in comparison to control.

When *Pseudomonas putida* was grown in the culture to degrade the phenol, the fluids turned yellow transiently and the yellow material accumulated in the culture was obtained maximum with the maximum phenol degradation (Molin and Nilsson 1985).

In the present study, the results have shown that *Pseudomonas putida* has degraded the phenol compound up to 80% in the absence of Chromium (VI). The degradation level has enhanced up to 81.67% in the presence of 25 mg/L initial concentration of Chromium (VI). The simultaneous Chromium reduction was observed 68% and 70% in initial Chromium concentration used as 25.0 and 50.0 mg/L respectively.

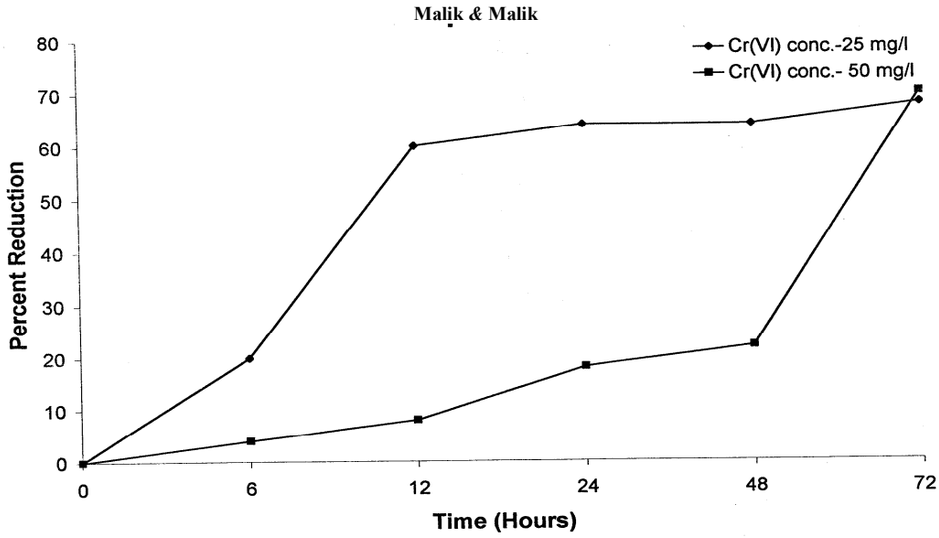

Figure 3. Chromium reduction at different time in presence of phenol

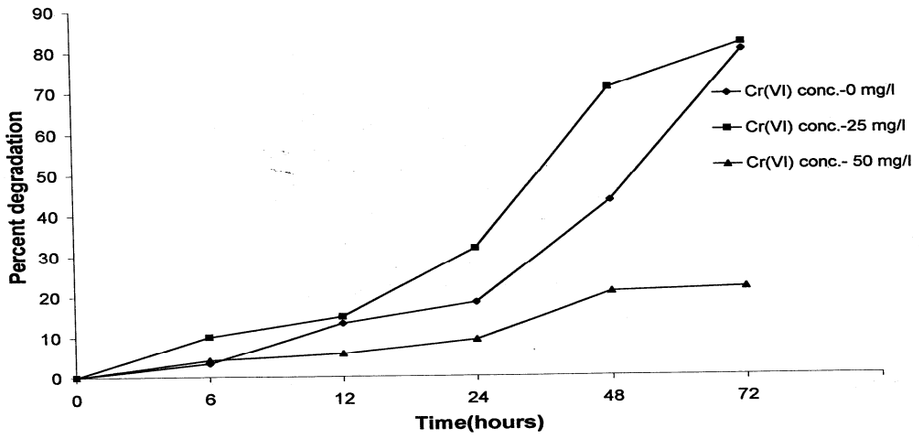

Figure 4. Phenol degradation with different Cr (VI) concentration

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