Original Research Article

Influence of media supplements on phenol biodegradation by

Pseudomonas aeruginosa SPD10

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ABSTRACT

The conventional methods of removing phenols from wastewater are very expensive. Degrading phenols and phenolic compounds by microorganisms is an alternative technology. A variety of microorganisms are known to utilize phenol as the sole carbon or energy source. Exploring the ability of microorganisms to metabolize phenols has received much attention due to the environmental persistence and its toxicity. In the time course study, Pseudomonas aeruginosa was able to completely utilize phenol within 3 days with 0.02 h⁻¹ and 28.19 mg L⁻¹ h⁻¹ specific growth rate and substrate consumption rate, respectively. In the present study we also enhanced biodegradable capacity of P. aeruginosa by its media optimization. Phenol degradable media was supplemented with different organic nitrogen sources (Aspartic acid, Beef extract, Peptone, Tryptone and Yeast extract,) and metal ions (Copper, Iron, Nickel, Selenium, and Zinc). The maximum 89% of phenol degradation was found to be yeast extract and peptone present media and other nitrogen sources also showed the significant percent of biodegradation. The metal ions zinc and selenium contained media showed the maximum 87% of phenol degradation at a constant pH 7.0 and 35°C. This work may be supported to select the best organic nitrogen source and metal ions for the large scale biodegradation of phenol.

Introduction

Phenol is widely distributed as a characteristic pollutant due to its commonly found in waste byproduct of many industries such as oil refineries, petrochemicals, dyeing, textiles, and coal conversion (Bandhyopadhyay et al., 2001; Yemendzhiev et al., 2008). Phenols containing industry effluents are often recalcitrance of phenol compounds. The untreated industrials effluents which are containing phenols compounds may lead to contamination of soil and groundwater, and their toxicity seriously affects living organisms even at a low concentration. The efficient removal of these compounds is necessary and significant for
environmental protection (Kibret et al., 2000; Wang et al., 2009).

In environmental remediation, biological methods have the advantage of reduced capital and operating costs compared with other methods, besides being ecofriendly. Contemporary research underscores the significance of microbial bioconversion as a better strategy for remediation and environmental conservation (Vidali, 2001). Recent literature on the methods of removal of phenol and their compounds from waste water focuses on adsorption and microbial biodegradation process (Battaglia-Brunet et al., 2002; Annadirai et al., 2007; Chakraborty et al., 2010; Abd El-Zaher et al., 2011)

The capability of microbes to remove harmful chemicals from polluted environments strongly depends on the presence of other compounds. The fluctuation in substrate loading also influenced the composition of the microbial community in the biological treatment system. The population shift in microbial community was regarded as one of the causes of the breakdown of those treatment processes (Watanabe, 1999). Certain species like Pseudomonas sp. under very controlled conditions of pH, temperature and in the presence of some specific nutrients can degrade phenol (Rasmussen et al., 2002; Annadirai et al., 2007). Babu et al., (1995) demonstrated that the efficient degradation of phenol or cresols/3-CBA mixtures by a defined mixed culture of two strains of Pseudomonas at appropriate inoculums and substrate ratios.

So many studies showed that optimizing the culture medium could enhance the biodegradation of xenobiotics. A positive impact on biodegradation of some aliphatic chlorinated xenobiotics has been observed when the culture medium supplemented with minerals (Henery and Grbic-Galic, 1995). It was also showed that yeast extracts, vitamins, nitrogen source and trace elements could significantly enhance the aerobic degradation rate of chlorobenzoic acid isomers (Armenante et al., 1995; Fava et al., 1995; Abd-El-Haleem et al., 2003). In the present study we describe the effect of different organic nitrogen sources (Peptone, Beef extract, Yeast extract, Tryptone and Aspartic acid) and metal ions (Iron, Nickel, Selenium, Copper and Zinc) on phenol degradation by newly isolated Pseudomonas aeruginosa SPD10.

Materials and Methods

Microorganism

In the present study newly isolated Pseudomonas aeruginosa SPD10 was used and it was maintained on nutrient agar medium incorporated with yeast extract (1.5 g/l), Beef Extract (1.5g/l), NaCl (5.0g/l), Agar (15g/l), tryptone (5 g/l), KH2PO4 (1 g/l), MgSO4 7H2O (1 g/l), thiamine (1 g/l), glucose (5 g/l) and stored at 4ºC until further use.

Biodegradation of phenol

Phenol degradation experiments were carried out in 500 ml shake flask containing 200 ml of Minimal Salt Medium (MSM) as follows : Na2HPO4 (6 g/l), KH2PO4 (3 g), NaCl (0.5 g/l), NH4Cl (1 g/l), CaCl2.2H2O (1 M/l) and MgSO47H2O (1 M/l). 2500mg l⁻¹ of phenol was added to MS medium as sole carbon source. Culture media pH was adjusted to 7.0 with 1 N NaOH or HCl and incubated at 35ºC in a temperature controlled orbital shaker at 150 rpm for 72 hrs in triplicates.
Phenol degradation and bacterial growth was observed by *P. aeruginosa* SPD10 growing in MS medium containing maximum concentration of 2500 mg L\(^{-1}\) of phenol. For each experiment freshly prepared 5% (v/v) inoculums of OD 0.92 was used. Samples were taken at 6 h intervals and analyzed for bacterial growth and phenol concentration.

Phenol degradation rate (\(Q_S\), mg L\(^{-1}\) h\(^{-1}\)) and cell yield coefficient (\(Y_{X/S}\), mg cell mg\(^{-1}\) phenol) during the time course study of the biodegradation processes were determined by using the method of Pirt (1975). \(Q_S\) was determined from the maximum slope in plot of phenol concentration (mg L\(^{-1}\)) versus time of incubation.

**Effect of different nitrogen source and metal ions on phenol biodegradation**

Effect of different nitrogen source on the biodegradation of phenol was determined by adding 1% of Peptone, Beef extract, Yeast extract, Tryptone and Aspartic acid separately to MS medium containing 1000 mg L\(^{-1}\) phenol. Medium was adjusted to pH 7.0 and incubated at 35\(^{\circ}\)C in a temperature controlled orbital shaker at 150 rpm for 48 hrs in triplicates. Similarly effect of different metal ions (Iron, Nickel, Selenium, Copper and Zinc) on the biodegradation of phenol was also studied.

**Estimation of phenol**

Phenol was estimated by the spectrophotometric Method 4-amino-antipyrine was used as the colouring agent and the absorbance was measured at 510 nm by UV-Visible spectrophotometer (APHA 1992). The percentage degradation of phenol was calculated by the following equation:

\[
\% \text{ of Degradation} = \left( \frac{P_o - P_f}{P_o} \right) \times 100
\]

**Results and Discussion**

The time course for phenol degradation by *P. aeruginosa* SPD10 was studied in mineral salt medium amended 1000 mg L\(^{-1}\) of phenol and incubated for 4 days. Periodic observations were made on growth and phenol degradation. The results are shown in Figure 1. Phenol degradation began with an initial lag period for about one day. This strain was able to completely utilize phenol within 3 days with 0.02 h\(^{-1}\) and 28.19 mg L\(^{-1}\) h\(^{-1}\) specific growth rate and substrate consumption rate, respectively. Muhammad Afzal et al., (2007) reported that the maximum initial concentration of phenol utilized by *P. aeruginosa* was 2600 mg L\(^{-1}\) with 0.016 h\(^{-1}\) specific growth rate and 26.16 mg L\(^{-1}\) h\(^{-1}\) phenol degradation rate. The previous reports on biodegradation of phenol in batch processes were to a maximum concentration of 1750 mg L\(^{-1}\) (Arutchelyan et al., 2006). In the present investigation we found that the newly isolated *P. aeruginosa* phenol degradation capacity is high. This might be due to the adaptation of *P. aeruginosa* at high phenol concentration of industry effluents from where we isolated.

1% of different nitrogen source such as Peptone, Beef extract, Yeast extract, Tryptone and Aspartic acid were supplemented to MS medium containing 1000 mg L\(^{-1}\) phenol and incubated at 35\(^{\circ}\)C in a temperature controlled orbital shaker at 150 rpm for 48 hrs in triplicates. Maximum 89% of phenol degradation was found to be at yeast extract and peptone present media (significant, <\(F\)) (Figure 2).
**Figure.1** Time course study of phenol degradation by *P. aeruginosa* SPD10

![Graph showing phenol degradation and biomass over time](image1)

**Figure.2** Effect of different nitrogen source on phenol degradation by *P. aeruginosa* SPD10

![Bar chart showing % of phenol degradation](image2)

**Figure.3** Effect of different metal ions on phenol degradation by *P. aeruginosa* SPD10

![Bar chart showing % of phenol degradation](image3)
Other nitrogen source also showed significant phenol degradation. Sourav Bhattacharya et al. (2012) reported that the low concentration of Peptone influences on phenol degradation as well as above 1.0 g/L peptone was inhibitory.

**Effect of different metal ions on phenol degradation by* P. aeruginosa *SPD10**

Effect of different metal ions such Cu, Fe, Ni, Se and Zn on the biodegradation of phenol was determined by adding 1% separately to the culture medium. These study were carried out in MS medium containing 1000 mg L\(^{-1}\) phenol and incubated at 35\(^\circ\)C in a temperature controlled orbital shaker at 150 rpm for 48 hrs in triplicates. Maximum 87% of phenol degradation was achieved by selenium and zinc amended media (significant, <F) (Figure. 3). Other metal ions also showed significant effect on phenol degradation by *P. aeruginosa* SPD10. The essential elements Cu, Fe, Mn and Zn are required in low concentrations by all kinds of life because they play important role in metabolic processes taking place in living cells (Botkin and Keller 2005). However, elevated levels of these elements are toxic to most organisms (Kaplan, 2004).

In the present study, the newly isolated *P. aeruginosa* SPD10 degraded phenol to a maximum initial concentration of 1000 mg L\(^{-1}\) within 72 hours. *P. aeruginosa* SPD10 also proved to be a better strain in terms of degrading higher concentration of phenol and phenol degradation rates. Among different nitrogen source yeast extract and peptone enhanced the phenol degradation. The presence of heavy metals selenium and zinc in MS medium had a significant (significant, <F) effect of phenol biodegradation by *P. aeruginosa* SPD10. The newly isolated *P. aeruginosa* SPD10 could be used as a potential organism for bioremediation of phenol.

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