Biosciences, Biotechnology Research Asia Vol. **6**(2), 593-598 (2009)

A comparative and optimization study on phenol degradation by *Pseudomonas aeruginosa* (NCIM 2074) and *Pseudomonas desmolyticum* (NCIM 2028)

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(Received: March 10, 2009; Accepted: April 29, 2009)

ABSTRACT

Phenol is a reasonably common wastewater contaminant, which has been found to be either toxic or lethal to fish, and most types of organisms at relatively low concentrations. Studies on microbial means of treating or removing phenols date back to atleast three decades. It was found that degrading potential of *Pseudomonas* sp., was strongly affected by the variations in pH, temperature, inoculum size. The purpose of this investigation was to study the effect of inoculum size and the influence of pH on phenol degradation by *Pseudomonas aeruginosa* (NCIM 2074) and *Pseudomonas desmolyticum* (NCIM 2028) in batch reactor and their comparison. The maximum process conditions for maximizing phenol degradation (removal) were recognized as follows -*P. aeruginosa*: pH 7, inoculum size 5% v/v and *P. desmolyticum* : pH 6, inoculum size 4% v/v. *P. aeruginosa* had better degradation rate than *P. desmolyticum*. *P. aeruginosa* at the optimum conditions (pH 7, 5% v/v) degraded 1000 mg/l of phenol concentration and *P. desmolyticum* at the optimum conditions (pH 6, 4% v/v) could not tolerate 1000 mg/l of phenol concentration and could degrade upto 700 mg/l. Hence, maximum removal efficiency of phenol was achieved at the optimum process conditions by *P. aeruginosa* (pH 7, 5% v/v).

Key words: Phenol, Biodegradation, *Pseudomonas aeruginosa, P. desmolyticum*, pH, Inoculum size.

INTRODUCTION

Environmental pollution has been considered as a side effect of industrial society. Soil, lakes, rivers and seas are highly contaminated with different toxic compounds (Alexander, 1981). An example of such compound is phenol. Phenol is released into the environment from industrial discharges (Keith, 1976, Jungclaus *et al,* 1978, Parkhurst *et al*, 1979, Pfeffer, 1979) and spills (Delfino and Dube, 1976). According to Prasad and Ellis (1978), phenols and its derivatives are among the most frequently found pollutants in rivers, industrial effluents and landfill run-off waters. Hence, populations residing near waste disposal sites, landfill sites or phenol spills may be at risk for higher exposure to phenol than other populations. The origin of phenol in the environment is both natural and industrial. Natural sources of phenol include forest fire, natural run off from urban area where asphalt is used as the binding material and natural decay of lignocellulosic material. Industrial sources such as oil refineries, chemical, petrochemical, pharmaceutical, metallurgical, pesticide products, paint and varnish industries, textile and also in the polymer industries like phenolic resins, bisphenol A, alkylphenols, caprolactums and adipic acid (Paula *et al,* 1998). Hence removal of phenol from industrial aqueous effluents is an important practical problem. Their presence in sewage and river water has also been confirmed (Thomas, 1973). Because of their high reliability and water solubility, phenols impart taste and odour problems to drinking water supplies even at parts per billion levels (Gosselin *et al,* 1984). Phenols are protoplasmic poisons, hence damage all kinds of cells. They are alleged to have caused an astonishing number of poisonings since they have come into general use. Fatal doses of phenols can be absorbed by the skin.

The removal of phenol from industrial effluents has attracted researchers from different fields (Yang and Humphrey, 1975, Shingler, 1996). The increasing awareness on the environment in

both developed and developing countries has initiated more studies of possible solutions for treating phenol.

Environmental biotechnology relies on the pollutant - degrading capacities of naturally occurring microorganisms (Liu and Suflita, 1993). It has been reported to be advantageous over physical and chemical treatments due to its relatively low cost and has less ecological impact to the environment (Head, 1998, Edington, 1994). Researchers are studying pollutant-degrading microorganisms which inhabit polluted environments (Kumaran, 1980, Kapoor *et al,* 1998, Yap *et al*, 1999, Heinaru *et al,* 2000, Komarkova *et al,* 2003, Santos and Linardi, 2004, Margesin *et al,* 2005) as well as uncontaminated environment (Bastos *et al,* 2000a, Kounty *et al,* 2003). Harnessing the potential of microbes (Ahmed, 1995, Fulthorpe and Allen, 1995, Bastos *et al,* 2000b, Ruiz-Ordaz *et al,* 2001, Vojta *et al,* 2002, Paca Jr. *et al,* 2003) to degrade phenol has been an area of considerable study to develop bioremediation approaches which has been considered as a "green-option " (Singleton, 1994) for treatment of environmental contaminants.

Many researchers support the biological treatment of phenols. A number of studies with prokaryotic microorganisms have been carried out for the purpose to improve the technological processes of biodegradation. Some examples are *Pseudomonas* sp., have demonstrated the ability to mineralize phenol (Ehrhardt and Rehm, 1989, Hinteregger *et al,* 1992, Ahmed, 1995, Chitra *et al,*1995, Dapaah and Hill, 1992, Fulthorpe and Allen, 1995, Fava *et al,* 1995, Loh and Wang, 1998).The biodegradation of phenol by *P. aeruginosa* and *P. desmolyticum*, potential degradents of phenol have been investigated for its degrading potential under different operating conditions and were compared. Two variables of pH and inoculum size were used to identify the significant effects and interactions in the batch studied. Phenol degradation was studied by *P. aeruginosa* and *P. desmolyticum* with different phenol concentrations (500 mg/l, 700 mg/l, 900 mg/l and 1000 mg/l) at their optimized conditions. The maximum tolerance limit of phenol for *P. aeruginosa* and *P. desmolyticum* were also tested.

MATERIAL AND METHODS

Chemicals

Phenol (99% pure, chemical grade) 4-amino antipyrine and all other chemicals used were from Merck.

Source of organism

The microorganism *P. aeruginosa* (NCIM 2074) and *P. desmolyticum* (NCIM 2028) were obtained from culture collection (NCL) Pune, India. The microorganisms were maintained separately on a medium containing Beef extract: 1.0 g/l, Yeast extract: 2.0 g/l, Peptone: 5.0 g/l, NaCl: 5.0 g/l and Agar: 20 g/l. The pH of the medium was adjusted to 7.0 by adding 1N NaOH. It was stored at 32^{0}C for further use.

Growth determination

To study the extent of degradation, the cells were grown in a Minimal Salts (MS) medium with the following composition: Phenol 0.500 g/l; K_2HPO_4, 1.5 g/l; KH_2PO_4, 0.5 g/l; $(NH_4)_2SO_4$, 0.5 g/l; NaCl, 0.5 g/l; Na_2SO_4, 3.0 g/l; Yeast extract, 2.0 g/l; Ferrous sulfate, 0.002 g/l; $CaCl_2$, 0.002 g/l in conical flasks containing and inoculated with *P. aeruginosa*(NCIM 2074) and *P.desmolyticum*(NCIM 2028) individually. The experimental studies were carried out in shake flasks with agitation at a rate of 120 rpm, temperature at 32^{0}C. Bacterial growth was determined in terms of cell mass by measuring optical density at a wavelength of 500nm.

Influence of pH of the medium on phenol degradation

Pseudomonas Cells were grown in MS medium with 500 mg/l of phenol at different pH 5 to 9. This mixture was contained in 250 ml Erlenmeyer flasks. The cultures were placed on a shaker (120rpm) at 32°C. At different times, growth and phenol degradation were measured.

Effect of inoculum size on phenol degradation

The effect of inoculum size (1 - 10% v/v) on phenol degradation was tested. Cells were grown as shake cultures at 32°C in MS medium supplemented with 500mg/l phenol at pH 7 in case of *P. aeruginosa* and pH 6 for *P. desmolyticum* in 250 ml Erlenmeyer flask. At different times, growth and phenol degradation were measured.

Estimation of phenol

Phenol was determined quantitatively by the Spectrophotometric method (DR/ 4000 V, Hach) using 4-amino antipyrine as the color reagent (λ_{max}: 500nm) according to standard methods of analysis (APHA, 1989).

Growth determination

Bacterial growth was determined in terms of cell mass by measuring optical density at a wavelength of 500nm.

RESULTS AND DISCUSSIONS

P. aeruginosa (All the data is not plotted for clarity)

Influence of pH of the medium on phenol degradation

pH values from 5 to 9 were investigated (Fig. 1). Phenol was degraded rapidly at pH 7. At this pH value, phenol degradation was high compared to other pH values. However, the phenol degradation at pH 5, 6, 8, and 9 was slower and phenol concentration decreased rapidly after 24 hrs inoculation. These results showed that *P. aeruginosa* degraded more phenol per day at pH 7 than at any other pH value.

Effect of inoculum size on phenol degradation

Phenol was degraded by *P. aeruginosa* during all the inoculum sizes (1-10% v/v) tested (Fig. 2). At 5% v/v the phenol concentration began to decrease rapidly after 5 hrs and reached 5mg/l after approximately 70 hrs. Cultures inoculated with 5 % v/v inoculum size showed the highest rate of phenol degradation, while the cultures inoculated with the other inoculum sizes showed a decrease in phenol consumption.

***P. desmolyticum* (All the data is not plotted for clarity)**

Influence of pH of the medium on phenol degradation

pH values from 5 to 9 were examined

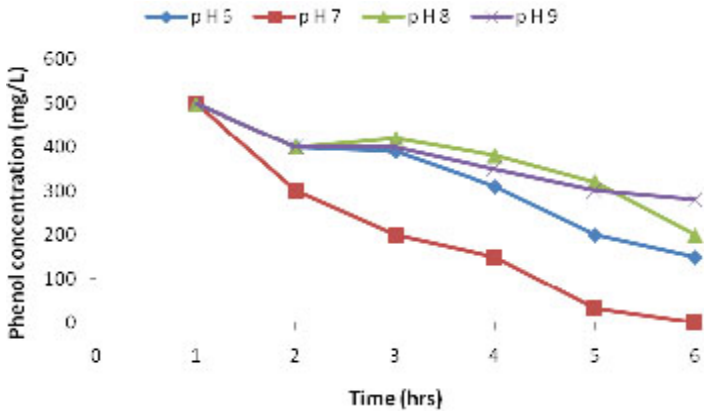

Fig. 1: Effect of pH on phenol degradation

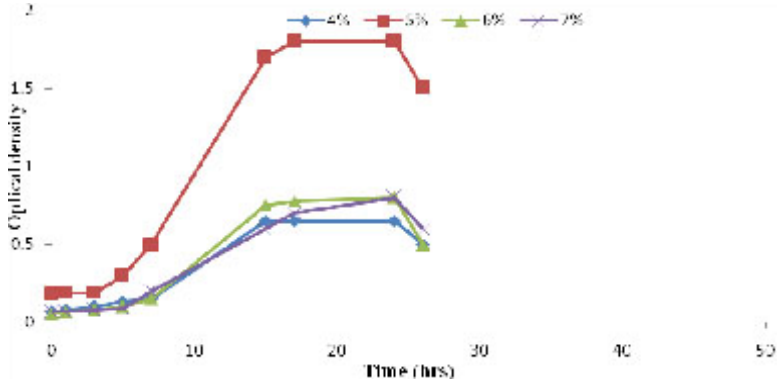

Fig. 2: Effect of inoculum size on phenol degradation

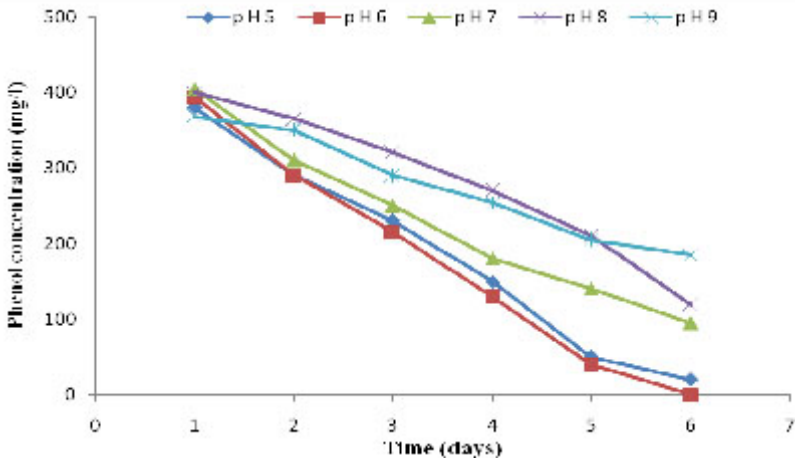

Fig. 3: Influence of pH on phenol degradation

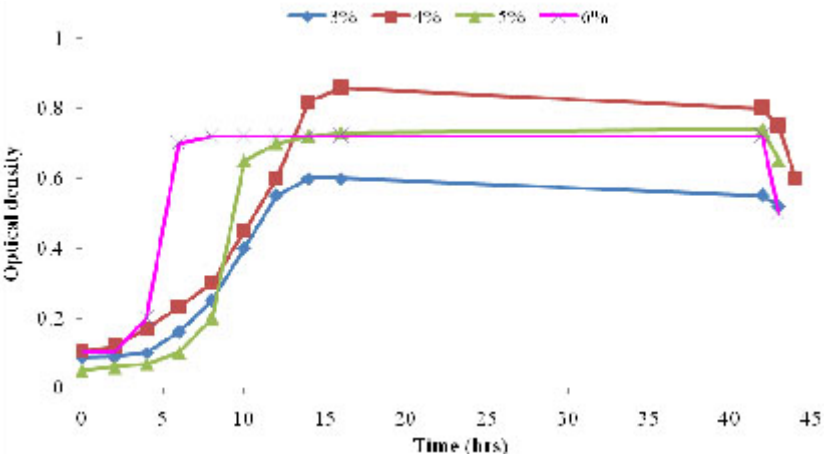

Fig. 4: Influence of inoculum size on phenol degradation

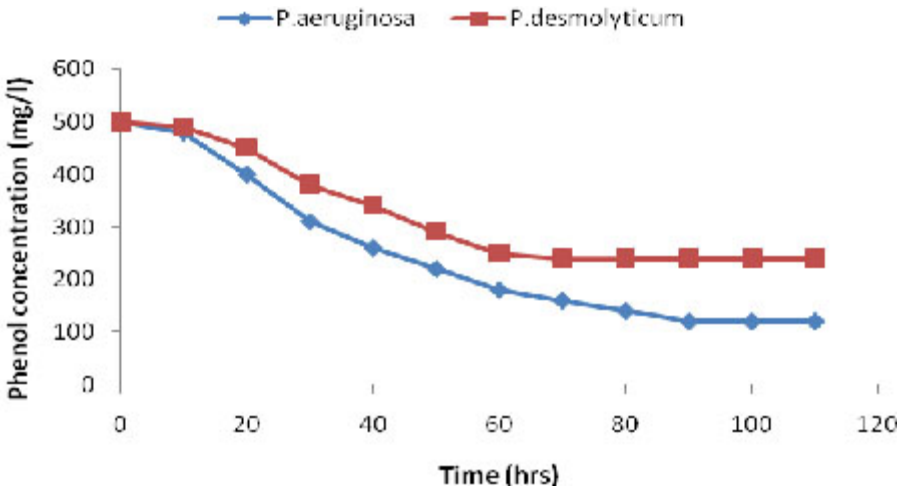

Fig. 5: Experimental values of phenol concentration during fermentations by *P.aeruginosa* and *P.desmolyticum*

(Fig. 3). At pH 6 phenol concentration started decreasing after 24 hrs inoculation when compared to other pH values. These results showed that *P.desmolyticum* degraded more phenol per day at pH 6 than at any other pH values.

Effect of inoculum size on phenol degradation

Phenol degradation by *P. desmolyticum* at inoculum sizes (1-10% v/v) was tested (Fig. 4). The phenol degradation was optimized at 4% v/v and phenol concentration began to decrease rapidly after 5 hrs and reached 50 mg/l after approximately 70 hrs.

At these optimized values of *P. aeruginosa* (pH 7, 5%v/v) and *P.desmolyticum* (pH 6, 4% v/v), the phenol degradation was tested and compared. Fig. - 5, show the experimental variation of the phenol concentration with time. As can be seen, *P. aeruginosa* at its optimum conditions degraded 1000mg/l of phenol concentration and *P. desmolyticum* at its optimum conditions could not tolerate 1000 mg/l of phenol concentration and could degrade only upto 700 mg/l. Hence, maximum removal efficiency of phenol was achieved at the optimum process conditions by *P. aeruginosa*.

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