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EFFECT OF pH AND INOCULUM SIZE ON PHENOL DEGRADATION BY *PSEUDOMONAS AERUGINOSA* (NCIM 2074)

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ABSTRACT

Phenolic compounds are hazardous pollutants that are toxic relatively at low concentrations. Accumulation of phenol creates toxicity both for flora and fauna. Because of its toxicity, there is a need to decontaminate the phenol-laden soils. Here, bioremediation is a very useful alternative to conventional clean-up methods. The aim of this work was to study the effect of inoculum size and the influence of pH on phenol degradation by *Pseudomonas aeruginosa*. Phenol was degraded rapidly at pH (6 to 9), but the maximum rate of phenol degradation by *P. aeruginosa* was at pH 7. In contrast, the phenol degradation at pH (6, 8, and 9) was significantly lower, although phenol was totally depleted. Phenol was degraded at every inoculum size tested (1-10% v/v) but the maximum rate of phenol degradation was observed at 5% v/v in batch experimental system.

These results are useful to understand the physiological and biochemical properties of *P. aeruginosa* before its optimum use in environmental application and these data will assist in choosing the right phenol degrader for a changeable environment.

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

INTRODUCTION

The massive increase in the synthesis of organic chemicals by man has led to the production of wide variety of compounds, some of which are xenobiotic. Their xenobiotic character means that their structures are not easily recognized by existing degradative enzymes and as a result, they accumulate in the environment[1]. As they persist in the environment, they are capable of long-range transportation, bioaccumulation in human and animal tissue and biomagnifications in food chain. Phenol and its higher homologous are aromatic molecules containing hydroxyl group attached to the benzene ring structure. The

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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. Industries like oil refineries, chemical, petrochemical, pharmaceutical, metallurgical, pesticide products, paint and varnish industries, textile and polymer are the source of phenolic resins, bisphenol A, alkylphenols, caprolactums and adipic acid[2]. The presence of phenol in water imparts carbolic odor to receiving water bodies and can cause toxic effects on aquatic flora and fauna[3]. It is lethal to fish even at relatively low concentrations of 5 - 25 mg/L [4]. Phenols are toxic to human beings and effects several biochemical functions[5]. The concentration of phenols in waste waters varies from 10 to 300 mg/L. Phenol is also a priority pollutant and is included in the list of EPA (1979)[6]. As a result, phenol – containing effluents have to be properly treated prior to discharge[7-11]. Efficient treatment methods are necessary to reduce phenol concentration in waste water to acceptable level, which is 5 ppm (USEPA).

Conventional methods of treatment for phenolic wastes have been largely chemical or physical methods like chlorination, advanced oxidation process[12], adsorption, solvent extraction, coagulation, flocculation, reverse osmosis, ozonation, photocatalysis, and electrolytic oxidation[13], but these processes have led to secondary effluent problems. Biological treatment for the bulk removal of these pollutants is therefore generally preferred. Biological degradation of phenol has been extensively studied using pure and mixed cultures[14-18]. Several studies have been carried out with the bacterium *P. aeruginosa* in pure cultures[19] in which phenol is degraded via the meta-pathway[20]. The success of bioremediation may depend on the availability of microbial strains that can mineralize high levels of phenol and withstand adverse conditions to compete under *in situ* conditions. An effective bacterial inoculum should be able to tolerate high levels of phenol while maintaining a high level of activity to provide efficient mineralization[21]. Understanding the physiological and biochemical properties of phenol degrading bacteria is required before optimum use of bacteria in environmental applications.

The biodegradation of phenol by *P. aeruginosa* (NCIM 2074), a potential biodegradant of phenol has been investigated for its degrading potential under different operating conditions. Two variables of pH and inoculum size were used to identify the significant effects and interactions in the batch studied.

EXPERIMENTAL

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) was obtained from culture collection (NCL) Pune, India. The microorganism was maintained 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 32^0C 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) .The experimental studies were carried out in shaking flasks with agitation at a rate of 120 rpm and at temperature 32^0C. Bacterial growth was determined in terms of cell mass by measuring optical density at a wavelength of 500 nm.

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 values (6, 7, 8 and 9). This mixture was contained in 250 mL Erlenmeyer flasks. The cultures were placed on a shaker (120 rpm) at 32^0C. 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 32^0C in MS medium supplemented with 500 mg/L phenol at pH 7 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}: 500 nm) according to standard methods of analysis[22].

Growth determination

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

RESULTS AND DISCUSSION

Biological treatment using *P. aeruginosa* (NCIM 2074) was the most effective method for removal of phenol. It is also a time saving method as compared to other conventional methods.

Influence of pH of the medium on phenol degradation

Four pH values from 6 to 9 were investigated (Fig. 1). Phenol was degraded rapidly at pH 7. At this pH value, phenol degradation was high as compared to other pH values. However, the phenol degradation at pH 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.

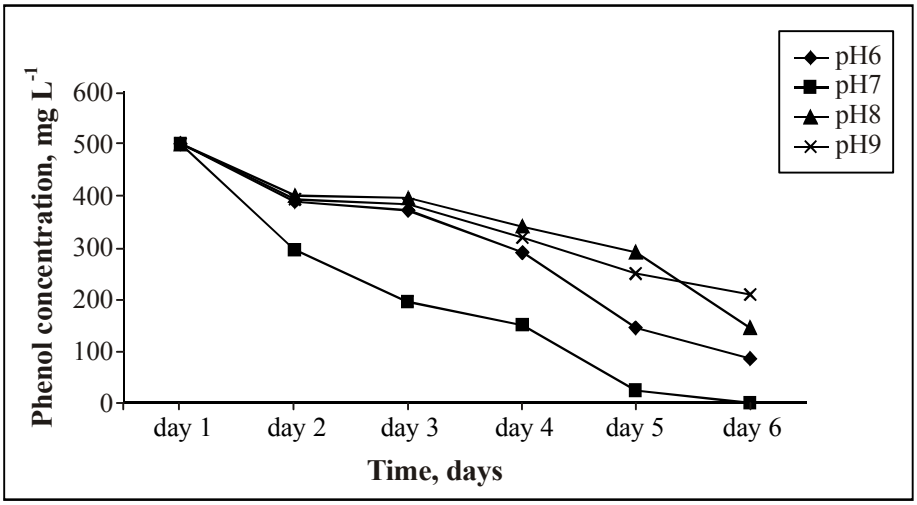

Fig. 1: **Effect of pH on phenol degradation**

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 5 mg/L after approximately 70 hrs. However, in cultures receiving lower inoculum densities, there was a progressive decrease of phenol concentration.

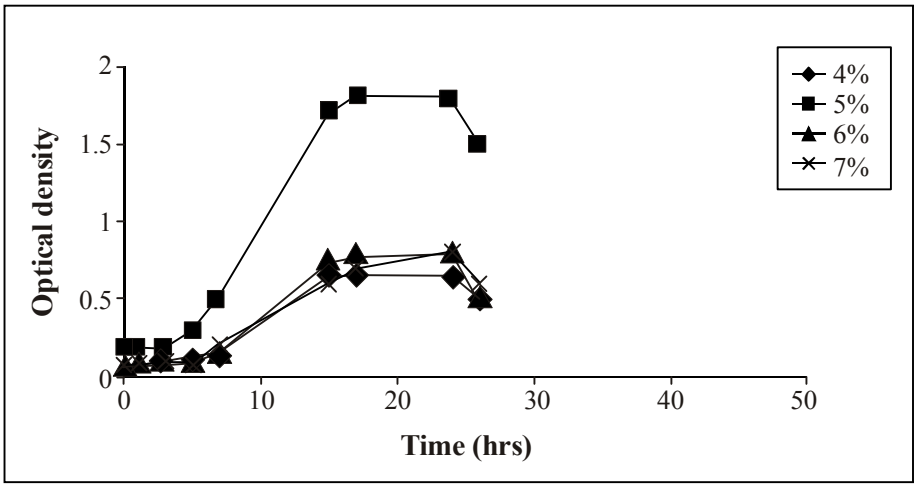

Fig. 2: **Effect of inoculum size on phenol degradation**

In addition, the rate of phenol degradation was also tested. Cultures inoculated with 5% v/v inoculum size showed the highest rate of phenol degradation, while the cultures inoculated with a higher inoculum size showed a decrease in phenol consumption.

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