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EFFECT OF TEMPERATURE AND CARBON SOURCE ON PHENOL DEGRADATION BY *PSEUDOMONAS AERUGINOSA* (NCIM 2074) AND *PSEUDOMONAS DESMOLYTICUM* (NCIM 2028) AND THEIR COMPARISON

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ABSTRACT

Phenol is a quite 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 and carbon source. The purpose of this investigation was to study the effect of temperature and the influence of carbon source on phenol degradation by *Pseudomonas aeruginosa* (NCIM 2074) and *Pseudomonas desmolyticum* (NCIM 2028) in batch reactor and their comparison.

The optimum process conditions for maximizing phenol degradation (removal) were similar for both *P. aeruginosa* and *P. desmolyticum* and were identified as: Temperature 32^0C and carbon source 0.5 g/L. The ability to degrade phenol by *P. aeruginosa* and *P. desmolyticum* in batch cultures was compared. Though both microorganisms behave in a similar way, *P. aeruginosa* seems to be better for removing phenols as compared to *P. desmolyticum*.

Key words: Phenol, Biodegradation, *Pseudomonas aeruginosa, Pseudomonas desmolyticum*, Temperature, Carbon.

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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[1]. An example of such a compound is phenol. Phenol is released into the environment from industrial discharges[2-5] and spills[6]. According to Prasad and Ellis[7], 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[8]. Hence, removal of phenol from industrial aqueous effluents is an important practical problem. Their presence in sewage and river water has also been confirmed[9]. Because of their high reliability and water solubility, phenols impart taste and odour problems to drinking water supplies even at parts per billion levels[10]. Phenols are protoplasmic poisons and 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[11,12]. 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[13]. 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[14,15]. Researchers are studying pollutant-degrading microorganisms, which inhabit polluted environments[16-22] as well as uncontaminated environment[23,24]. Harnessing the potential of microbes[25-30] to degrade phenol has been an area of considerable study to develop bioremediation approaches, which has been considered as a "green-option" [31] 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. *Pseudomonas* sp. have demonstrated the ability

to mineralize phenol[32-37]. The biodegradation of phenol by *P. aeruginosa* and *P. desmolyticum*, potential degradants of phenol have been investigated for their degrading potentials under different operating conditions and were compared. Two variables of temperature and carbon source were used to identify the significant effects and interactions in the batch studied. The study is aimed at finding the most suitable microorganism for removing phenolic compounds.

EXPERIMENTAL

Chemicals

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

Source of organism

The microorganisms *P. aeruginosa* and *P. desmolyticum* 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 32^0C 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* and *P.desmolyticum* individually. The experimental studies were carried out in shake flasks with agitation at a rate of 120 rpm and temperature 32^0C. Bacterial growth was determined in terms of cell mass by measuring optical density at a wavelength of 500 nm.

Influence of temperature of the medium on phenol degradation

P. aeruginosa and *P. desmolyticum* were grown in MS medium with 500 mg/L of phenol at different temperatures (30^0C, 32^0C, 33^0C and 34^0C) at pH 7, inoculum size 5% v/v[38] and at pH 6, inoculum size 4% v/v[39], respectively. This mixture was contained in 250 mL Erlenmeyer flasks individually. The cultures were placed on a shaker (120 rpm) at the above mentioned temperatures. At different times, growth and phenol degradation were measured.

Effect of carbon source on phenol degradation

The effect of carbon (0.0, 0.5, 1, 2, 3 and 4 mg/L) on phenol degradation of *P. aeruginosa* and *P. desmolyticum* was tested at temperature 32^0C, pH 7, inoculum size 5% v/v and at temperature 32^0C, pH 6, inoculum size 4% v/v, respectively. Cells were grown as shake cultures in MS medium supplemented with 500 mg/L phenol in 250 mL Erlenmeyer flask individually. 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-aminoantipyrine as the color reagent (λ_{max}: 500 nm) according to standard methods of analysis[40].

Growth determination

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

RESULTS AND DISCUSSION

Influence of temperature of the medium on phenol degradation

Temperature exerts an important regulatory influence on the rate of metabolism. However, some work has been done on the microbiological activity of the organisms present in the water treatment plants operating at lower temperatures. But conventional biological waste treatment processes can operate at low temperature, provided sufficient time is allowed for these organisms to degrade organic wastes. Microbiological degradation of phenol in industrial wastewater is affected by temperature in an unexpected manner. The efficiency of treatment by microbiological activity on phenol and other contaminants were significantly good.

Temperature values (30^0C, 32^0C, 33^0C and 34^0C) were investigated for *P. aeruginosa* and *P. desmolyticum* as shown in Fig. 1 and Fig. 3, respectively. *P. aeruginosa* and *P. desmolyticum* degraded phenol rapidly at temperature 32^0C. At this temperature, the phenol concentration began to decrease rapidly after 24 hrs inoculation and degraded completely around 60 hrs by *P. aeruginosa* as shown in Fig. 1. Phenol was degraded rapidly by *P. desmolyticum* at 32^0C after 45 hrs and complete degradation was found at around 75 hrs as shown in Fig. 3. Hence, *P. aeruginosa* had better degradation rate than *P. desmolyticum.*

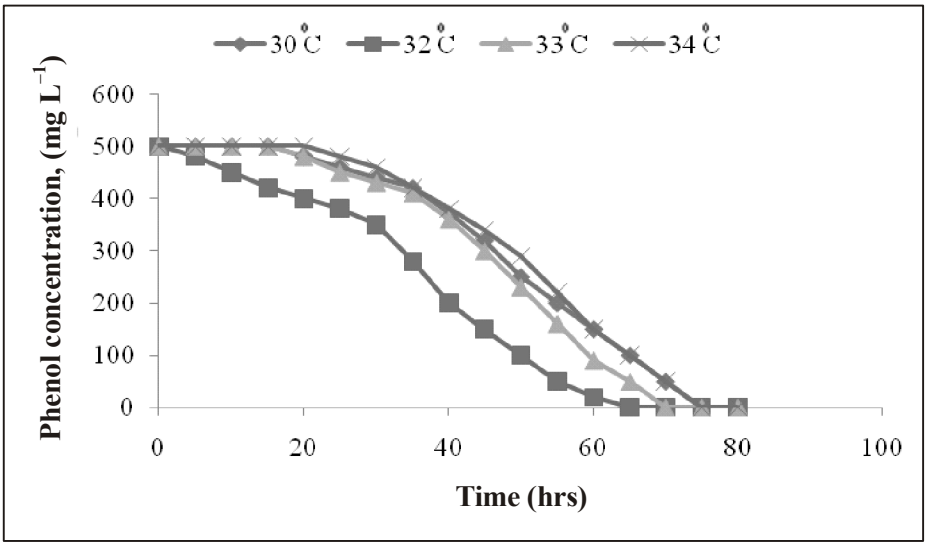

Fig. 1: Effect of temperature on phenol degradation by *P. aeruginosa*

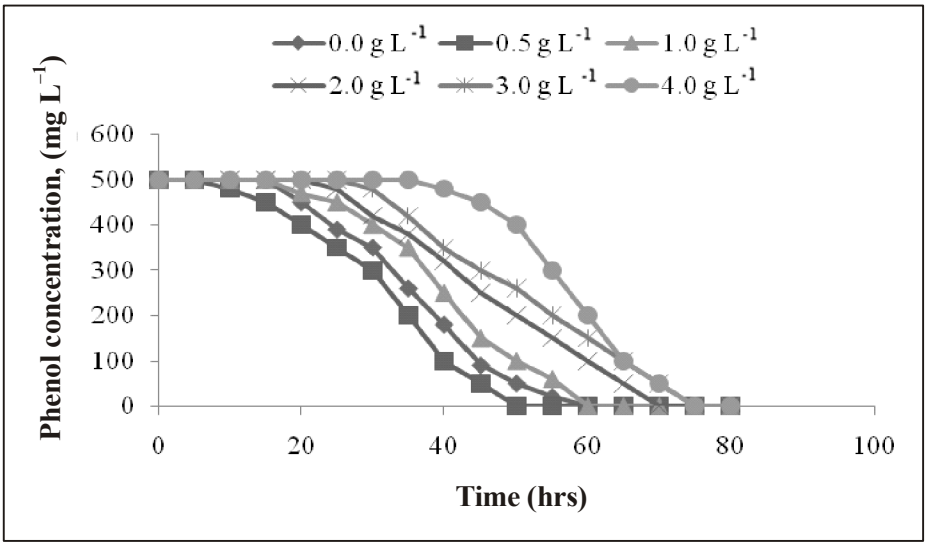

Fig. 2: Effect of glucose on phenol degradation by *P. aeruginosa*

Effect of carbon source on phenol degradation

The presence of glucose in the culture medium increased the tolerance of the organisms to high phenol concentrations by providing a good source readily metabolisable carbon to support cell growth. Hence, it was concluded that glucose on MS medium supported phenol degradation.

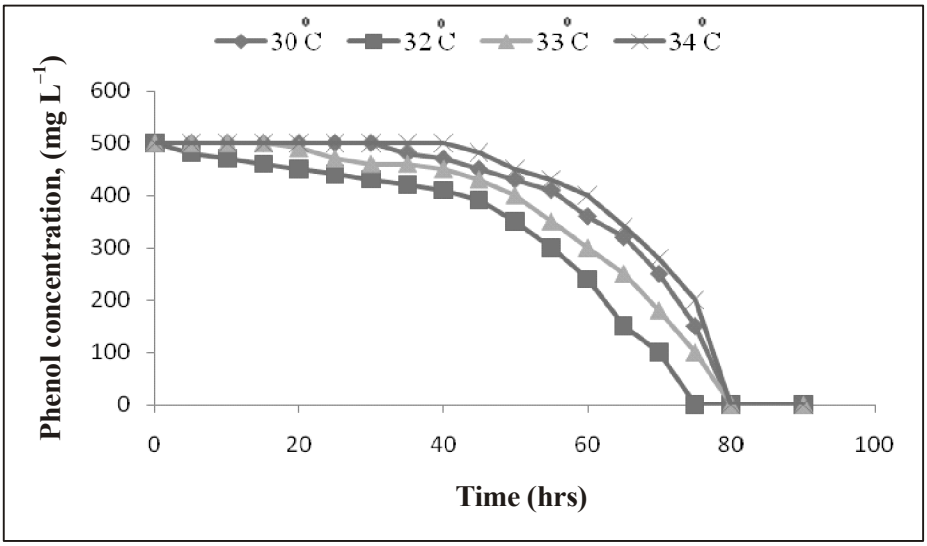

Fig. 3: Effect of temperature on phenol degradation by *P. desmolyticum*

Phenol degradation by *P. aeruginosa* and *P. desmolyticum* at different concentrations of glucose (0.0, 0.5, 1.0, 2.0, 3.0, 4.0 mg/L) was tested (Fig. 2 and Fig. 4, respectively). Cultures inoculated with 0.5 mg/L glucose showed the highest rate of phenol degradation, while the cultures inoculated with the other concentrations showed a decrease in phenol consumption as shown in Fig. 2 and Fig. 4, respectively.

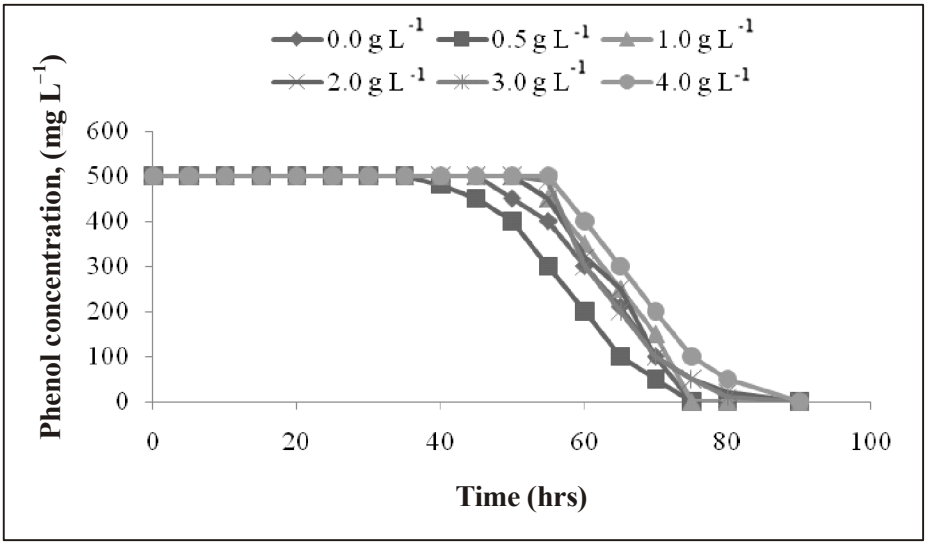

Fig. 4: Effect of glucose on phenol degradation by *P. desmolyticum*

In summary, these results show that *P. aeruginosa* is able to tolerate higher levels of phenol, when supplemented with glucose as additional source compared to *P. desmolyticum.* Time is the limiting factor as the biodegradation of recalcitrant compounds can range from hours to years.

In Fig. 5, the variation of phenol concentration throughout both fermentations are shown. As can be seen, in case of *P. aeruginosa,* phenols removing rate is slightly higher than that of *P. desmolyticum.*

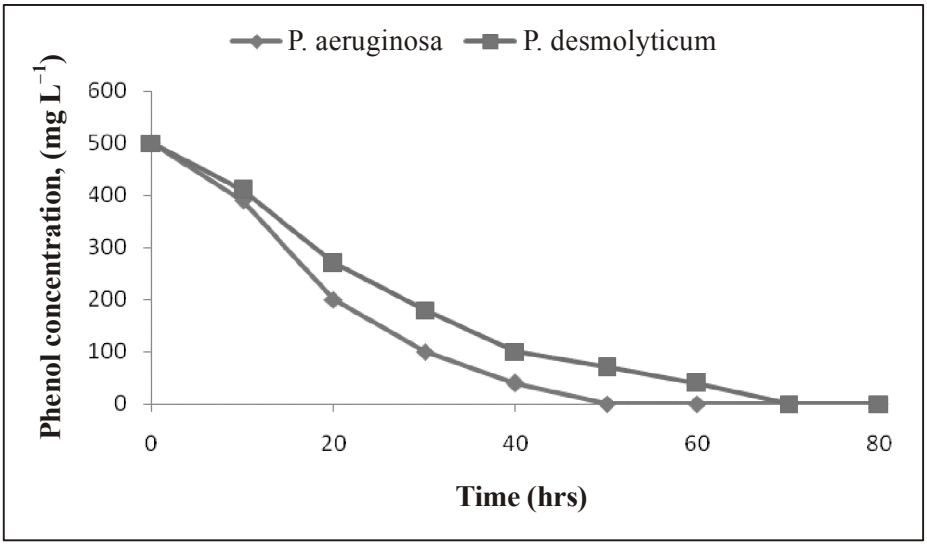

Fig. 5: Experimental values of phenol concentration during fermentations by *P. aeruginosa* and *P. desmolyticum*

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