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

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ABSTRACT

Phenols are one of many commonly occurring organic pollutants in the environment. These compounds are stable and even at low concentrations they may be toxic towards living organisms and cause unfavorable chemical changes in waters and soils. Among others, biotechnological methods are applied for their removal. These techniques are based on degradation using specifically selected microorganisms, which utilize the pollutants as their energy, carbon, nitrogen and phosphorus or sulfur sources. The biodegradation of phenol by *Pseudomonas desmolyticum* (NCIM 2028), a potential biodegradent has been investigated for its degrading potential under different operating conditions. *P. desmolyticum* (NCIM 2028) can grow using phenol as carbon resource and has ability of phenol degradation. The effect of temperature and carbon addition on biodegradative capacity of *P.desmolyticum* (NCIM 2028) was studied in batch cultures as a function of temperature (30^0C, 32^0C, 33^0C, 34^0C) and carbon (0, 0.5, 1, 2, 3, 4 gm/L). It was found that the degrading potential of *P. desmolyticum* (NCIM 2028) was strongly affected by the variations in temperature and carbon. Optimum conditions of the variables for the maximum degradation of phenol by *P. desmolyticum* (NCIM 2028) are : temperature - 32^0C and carbon - 0.5 gm/L. These results are useful to understand the physiological and biochemical properties of *P. desmolyticum* (NCIM 2028) before its optimum use in environmental application and these data will assist in choosing the right phenol degrader for a changeable environment.

INTRODUCTION

Phenol and its derivatives are the basic structural unit in a wide variety of synthetic organic compounds (Annadurai *et al*. 2000). It is an organic, aromatic compound that occurs naturally in the environment (Prpich and Daugulis, 2005), but is more commonly produced artificially from industrial activities such as petroleum processing, plastic manufacturing, resin production, pesticide production, steel manufacturing and the production of paints and varnish (Mahadevaswamy *et al*. 1997; Banyopadhyay *et al.* 1998). This aromatic compound is water soluble and highly mobile (Collins and Daugulis, 1997) and as such wastewaters generated from these industrial activities contain high concentrations of phenolic compounds (Change *et al*. 1998) which eventually may reach down to streams, rivers, lakes, and soil, which represent a serious ecological problem due to their wide spread use and occurrence throughout the environment (Fava *et al*. 1995).

Phenol is a listed priority pollutant by the U.S. Environmental Protection Agency (EPA, 1979) and

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is considered to be a toxic compound by the Agency for Toxic substances and Disease Registry (ATSDR, 2003). The adverse effects of phenol on health are well documented (Calabrese and Kenyon, 1991) and death among adults has been reported with ingestion of phenol ranging from 1 to 32 gm (Prpich and Daugulis, 2005). The low volatility of phenol and its affinity for water make oral consumption of contaminated water, the greatest risk to humans (Prpich and Daugulis, 2005).

A variety of techniques have been used for the removal of phenol from industrial effluents and contaminated waters with bioremediation receiving the most attention due to its environmentally friendly, its ability to completely mineralize toxic organic compounds and of low-cost (Kobayashi and Rittman, 1982; Prpich and Daugulis, 2005). Microbial degradation of phenol actively studied and these studies have been shown that phenol can be aerobically degraded by wide variety of fungi and bacteria cultures such as *Candida tropicalis* (Ruiz-ordaz *et al*. 2001; Chang *et al*. 1998; Ruiz-ordaz *et al*. 1998). *Acinetobacter calcoaceticus* (Paller *et al*. 1995), *Alcaligenes eutrophus* (Hughes *et al*. 1984; Leonard and Lindley, 1998), *Pseudomonas putida* (Hill and Robinson, 1975; Nikakhtari and Hill, 2006) and *Burkholderia cepacia* G4 (Folsom *et al*. 1990; Solomon *et al*. 1994). For high strength and low volumes of wastewaters, phenol removal by degradation technique using *Pseudomonas* sp., has been adopted (Annadurai *et al*. 1999).

Growth can be inhibited not by just the presence of toxic compounds but by the availability of micro and macro nutrients. Therefore, it was thought worthwhile to consider various environmental factors like pH, inoculum, carbon and temperature that affect the biodegradation of phenol. Sridevi and Chandana, 2009 identified the optimum conditions on phenol degradation by *P. demolyticum* (NCIM 2028) pH 6, inoculum size 4% v/v. In the present investigation the parameters- temperature and glucose affecting the degradation of phenol were identified. Temperature exerts an important regulatory influence on the rate of metabolism (Ghosh and Swamynathan, 2003). Besides, it has been suggested (Roszak and Colwell, 1987; Watanable *et al.* 1998) that other factors, such as the nutrient availability (carbon source), the presence of toxins and physical parameters can affect the bacterial growth.

This study seeks to optimize the biodegradation rate of phenol by *P. desmolyticum* (NCIM 2028) by supplementing the growth medium with glucose and to study the effect of temperature on the substrate inhibition tolerance of the bacteria.

MATERIALS 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. desmolyticum* (NCIM 2028) was obtained from culture collection (NCL) Pune, India. The microorganism was maintained on a medium containing Beef extract: 1.0 gm/L, Yeast extract: 2.0 gm/L, Peptone: 5.0 gm/L, NaCl: 5.0 gm/L and Agar: 20 gm/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 gm/L; K_2HPO_4, 1.5 gm/L; KH_2PO_4, 0.5 gm/L; $(NH4)_2SO_4$, 0.5 gm/L; NaCl, 0.5 gm/L; Na_2SO_4, 3.0 gm/L; Yeast extract, 2.0 gm/L; Ferrous sulfate, 0.002 gm/L; CaCl2, 0.002 gm/L in conical flasks containing and inoculated with *P.desmolyticum* (NCIM 2028). The experimental studies were carried out in shake flasks with agitation at a rate of 120 rpm, temperature at 32^0C. Bacterial growth was determined in terms of cell mass by measuring optical density at a wavelength of 500nm.

Influence of temperature of the medium on phenol degradation

Pseudomonas cells were grown in MS medium with 500 mg/L of phenol at different temperature values (30^0C, 32^0C, 33^0C, 34^0C) at pH 6 and inoculum size 4% v/v (Sridevi and Chandana, 2009). This mixture was contained in 250 mL Erlenmeyer flasks. The cultures were placed on a shaker (120 rpm) at the above temperatures. At different times, growth and phenol degradation were measured.

Effect of glucose on phenol degradation

The effect of glucose (0, 0.5, 1, 2, 3, 4 gm/L), 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 6 and inoculum size 4% v/v 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 DISCUSSION

Biological treatment using *P.desmolyticum* (NCIM 2028) was the effective method for removal of phenol. It is also a time saving method compared to other conventional methods.

Influence of temperature of the medium on phenol degradation

Temperature exerts an important regulatory influence on the rate of metabolism. However, little 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 in 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. Fig. 1 shows the reduction of phenol concentration during the degradation process at different temperatures (30^0C, 32^0C, 33^0C, 34^0C). Phenol was degraded rapidly at 32^0C. At this temperature value, phenol degradation was high compared to other values and phenol concentration decreased rapidly after 45hrs inoculation. However, the phenol degradation at temperature 30^0C, 33^0C, 34^0C was slower. These results showed that *P. desmolyticum* (NCIM 2028) degraded more phenol per day at 32^0C than at any other temperature value.

Effect of carbon on phenol degradation

Phenol was degraded by *P.desmolyticum* (NCIM

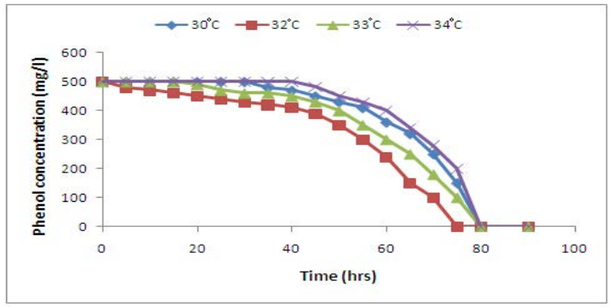

Fig. 1 Effect of Temperature on phenol degradation

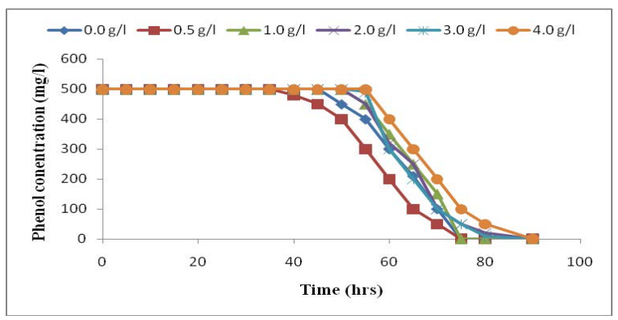

Fig. 2 Effect of glucose on phenol degradation

2028) at different concentrations of glucose (0, 0.5, 1, 2, 3, 4 gm/L) as shown in Fig.2. 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. In summary, these results show that *P.desmolyticum* (NCIM 2028) is able to tolerate higher levels of phenol when supplemented with glucose as additional source. The optimum level of glucose was 0.5 gm/L. This value shows that higher concentration of glucose however had no effect on phenol degradation. In addition, the rate of phenol degradation was tested.

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