Jr. of Industrial Pollution Control 25 (2) (2009) pp 195-198

EFFECT OF TEMPERATURE AND CARBON ON PHENOL DEGRADATION BY *PSEUDOMONAS AERUGINOSA* (NCIM 2074)

M.V.V. CHANDANA LAKSHMI* AND V. SRIDEVI [a]

Department of Chemical Engineering (Biotechnology), College of Engineering,
Andhra University, Visakhapatnam 530 003, Andhra Pradesh, India

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

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. Hence, bioremediation is a very useful alternative to conventional clean-up methods. The aim of this work was to study the effect of two variables – temperature (30^0C, 32^0C, 33^0C, 34^0C) and carbon (0.5, 1, 2, 3, 4 gm/L) to identify the significant effects and interactions in the batch studies. It was found that the degrading potential of *Pseudomonas aeruginosa* (NCIM 2074) was strongly affected by the variations in carbon and temperature. Optimum conditions of the variables for the growth of *P. aeruginosa* (NCIM 2074) and for maximum biodegradation of phenol are temperature (32^0C) and carbon (0.5gm/L). These results are useful to understand the physiological and biochemical properties of *P. aeruginosa* (NCIM 2074) before its optimum use in environmental application and these data will assist in choosing the right phenol degrader for a changeable environment.

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 (Singleton, 1994). 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 homology are aromatic molecules containing hydroxyl group attached to the benzene ring structure. 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). The presence of phenol in water imparts carbolic odor to receiving water bodies and can cause toxic effects on aquatic flora and fauna (Ghadhi and Sangodkar, 1995). It is lethal to fish even at relatively low concentrations of 5-25 mg/L (Nuhoglu and Yakin, 2005). Phenols are toxic to human beings and effects several biochemical functions (Saha *et al*. 1999).The concentration of phenols in waste waters varies from 10 to 300 mg/L. Phenol is also a priority pollutant and is included

***Address for correspondence - Email** : mahantilakshmi@yahoo.com ; vellurusridevi@yahoo.co.in

in the list of EPA (1979) (Indu Nair *et al*. 2008). As a result, phenol - containing effluents have to be properly treated prior to discharge (Keith, 1976; Jungclaus *et al*. 1978; Parkhurst *et al*. 1979; Pfeffer, 1979; Delfino and Dube, 1976). 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 (Santiago Esplugas *et al*. 2002), adsorption, solvent extraction, coagulation, flocculation, reverse osmosis, ozonation, photo catalysis, and electrolytic oxidation (Arutchelvan *et al*. 2006), 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 (Kang and Park, 1997; Hugues and Cooper, 1996; Wang *et al*. 1996; Ha *et al*. 2000; Chirwa and Wang, 2002. Several studies have been carried out with the bacterium *P. aeruginosa* in pure cultures (Agarry *et al*. 2008) in which phenol is degraded via the meta-pathway (Sala-Trepat *et al*.1972). 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 (Shaw *et al*. 1997). 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 biodegradent of phenol has been investigated for its degrading potential under different operating conditions. Chandana and Sridevi (2009) identified the optimum conditions on phenol degradation by *P. aeruginosa* (NCIM 2074): pH 7, inoculum size 6% v/v. This is a continuation of previous studies. Two variables of temperature, glucose (as carbon source) were used to identify the significant effects and interactions in the batch studied.

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. aeruginosa* (NCIM 2074) 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^{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 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. aeruginosa* (NCIM 2074). 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 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^{0}C, 32^{0}C, 33^{0}C, 34^{0}C) at pH 7 and inoculum size 5%v/v (Chandana and Sridevi, 2009). This mixture was contained in 250 mL Erlenmeyer flasks. The cultures were placed on a shaker (120rpm) at the above temperatures. At different times, growth and phenol degradation were measured.

Effect of glucose on phenol degradation

The effect of glucose (0.5, 1, 2, 3, 4gm/L), on phenol degradation was tested. Cells were grown as shake cultures at 32^{0}C in MS medium supplemented with 500 mg/L phenol at pH 7 and inoculum size 5%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 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. aeruginosa* (NCIM 2074) was the most 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 waste water is affected by temperature in an unexpected manner. The efficiency of treatment by microbiological activity on phenol and other contaminants were significantly good. Four temperature values from (30^{0}C, 32^{0}C, 33^{0}C, 34^{0}C) were investigated in Fig.1. Phenol was degraded rapidly at 32^{0}C. At this temperature value, phenol degradation was high compared to other values. However, the phenol degradation at temperature 30^{0}C, 33^{0}C, 34^{0}C was slower. These results showed that *P. aeruginosa* (NCIM 2074) degraded more phenol per day at 32^{0}C than at any other temperature value.

Effect of carbon on phenol degradation

Phenol was degraded by *P. aeruginosa* (NCIM 2074), at different concentrations of glucose (0.5, 1, 2, 3, 4gm/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. aeruginosa* (NCIM 2074) is able to tolerate higher levels of phenol when supplemented with glucose as additional sources. The optimum level of glucose was 0.5gm/L. This value shows that higher concentration of glucose however had no effect on

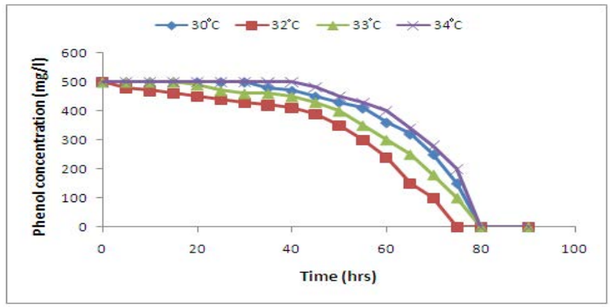

Fig. 1 Effect of Temperature on phenol degradation

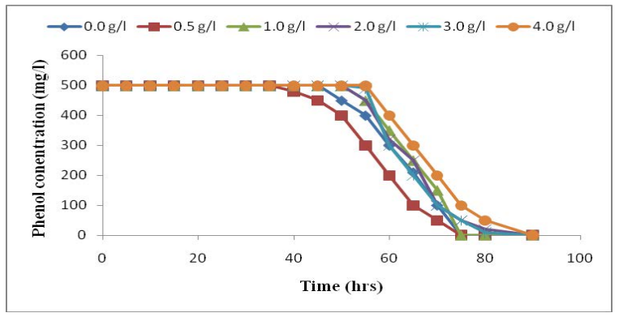

Fig. 2 Effect of glucose on phenol degradation

phenol degradation. In addition, the rate of phenol degradation was tested.

REFERENCES

Agarry, S.E., Solomon, B.O. and Layokun, S.K. 2008. Optimization of process variables for the microbial degradation of phenol by *Pseudomonas aeruginosa* using response surface methodology. *African J. Biotechnol*. 7: 2409-2416.

APHA,1989. American Water Works Association, Water Pollution Control Federation. *Standard Methods for the Examination of Water and Wastewater*. 17th Ed. Washington, D.C. American Public Health Association. 9-55 - 9-62.

Arutchelvan, V., Kanakasabai, V., Elagovan, R., Nagarajan, S. and Muralikrishna, V. 2006. Kinetics of high strength phenol degradation using *Bacillus brevis*. *J. hazardous Mat*. 129 : 216-222.

Chandana Lakshmi, M.V.V. and Sridevi, V. Effect of pH and inoculum size on phenol degradation by *Pseudomonas aeruginosa* (NCIM 2074). *International Journal of Chemical Sciences*. Communicated

Chirwa, E.N. and Wang, Y.T. 2002. Simultaneous Chromium (VI) reduction and phenol degradation in an anaerobic consortium of bacteria. *Wat. Res*. 34 : 2376-2384.

Delfino, J.J. and Dube, D.J.1976. Persistent contamination of ground water by phenol. *Environ. Sci. Health.* A11 : 345-355.

Ghadhi, S.C. and Sangodkar, U.M.X. 1995. Potentials of *Pseudomonas cepacia* PAA in bioremediation of aquatic wastes containing phenol. *Proceedings of National Symposium Frontiers in applied and Environmental Microbiology*. Dec. Cochin. 11-13.

Ha, S.R., Vinitnantharat, S. and Ozqki, H. 2000. Biodegradation by mixed microorganisms of granular activated carbon loaded with a mixture of phenols. *Biotechnol. Lett*. 22 : 1093-1096.

Hugues, S.M. and Cooper, D.G. 1996. Biodegradation of phenol using the self-cycling fermentation (SCF) process. *Biotechnol. Bio.Engg*. 51 : 112-119.

Indu Nair, C., Jayachandran, K. and Shankar Shashidhar. 2008. Biodegradation of phenol. *African J. Biotechnol*. 7 : 4951-4958.

Jungclaus, G.A., Lopez-Avila, V. and Hites, R.A. 1978. Organic compounds in an industrial wastewater.
A case study of their environment impact. *Env. Sci. Technol*. 12 : 88-96.

Kang, M.H. and Park, J.M. 1997. Sequential degradation of phenol and cyanide by a commensal interaction between two microorganisms.*Chem. Technol. Biotechnol*. 69 : 226-230.

Keith, L.H. 1976. Identification of organic compounds in unbleached treated kraft paper mill wastewaters. *Env. Sci. Technol*. 10 : 555-564.

Nuhoglu, A. and Yakin, B. 2005. Modeling of phenol removal in a batch reactor. *Proc. Biochem*. 40 : 233-239.

Paula, M., Van Schei and Young, L.Y. 1998. Isolation and characterization of phenol degrading denitrifying bacteria. *Appl. Environ. Microbiol*. 64 : 2432-2438.

Parkhurst, B.R., Bradshaw, A.S. and Forte, J.L. 1979. An evaluation of the acute toxicity to aquatic biota of a coal conversion effluent and its major components. *Bull. Environ. Contam. Toxicol*. 23 : 349-356.

Pfeffer, F.M. 1979. The 1977 screening survey for measurement of organic priority pollutants in petroleum refinery wastewaters. ASTM Spec. *Tech. Publ.* 181-190.

Saha, N.C., Bhunia, F. and Kaviraj, A. 1999. Toxicity of phenol to fish and aquatic ecosystem. *Bull. Environ. Contam. Toxicol*. 63 : 195-202.

Sala-Trepat, J.M., Murray, J.M. and William, P.A. 1972. The metabolic divergence in the meta- cleavage of catechols by *Pseudomonas putida* NCIB 10015 Physiological significance and evolutionary implications. *Env. Biochem*. 28 : 347-456.

Santiago Esplugas, Jaime Glimenez, Sandra Contreras, Esther Pascual, Miguel Rodriguez. 2002.Comparison of different advanced oxidation processes for phenol degradation. *Water. Res*. 36 : 1034-1042.

Shaw, K.W., Lee, H. and Trevors, J. 1997. Effect of initial cell density, substrate concentration and
temperature on pentachlorophenol degradation by *Pseudomonas* sp., UG-30. *J. Chem. Technol. Biotechnol*. 69 : 107-113.

Singleton, I. 1994. Microbial metabolism of xenobiotics: Fundamental and applied research. *J.Chem. Biotechnol*. 59 : 9-23.

Wang, K.W., Baltzis, B.C. and Lewandowski. 1996. Kinetics of phenol biodegradation in the presence of glucose. *Biotechnol. Bio. Engg*. 51 : 87-94.