Int. J. Environ. Res., 4(4): 849-854, Autumn 2010
ISSN: 1735-6865

Effect of the Ammonium Chloride Concentration on the Mineral Medium Composition – Biodegradation of Phenol by a Microbial Consortium

Hamitouche, A.[1,2], Amrane, A.[3,4*], Bendjama, Z.[1] and Kaouah, F.[1]

[1] Laboratoire des Sciences du Génie des Procédés Industriels, Faculté de Génie Mécanique et de Génie des Procédés, Université des Sciences et de la Technologie Houari Boumediene, BP 32, El-Alia, 16111, Bab ezzouar, Alger, Algeria

[2] Centre de Recherche scientifique et technique en Analyses Physico-Chimiques, BP 248, CRAPC, Alger, Algeria

[3] Ecole Nationale Supérieure de Chimie de Rennes, Université Rennes 1, CNRS, UMR 6226, Avenue du Général Leclerc, CS 50837, 35708 Rennes Cedex 7, France

[4] Université européenne de Bretagne, 35000 Rennes, France

Received 20 March 2010; Revised 15 July 2010; Accepted 25 July 2010

ABSTRACT: Phenol and its homologues are aromatics containing hydroxyl, methyl, amide and sulphonic groups attached to the benzene ring. These molecules are both anthropogenic and xenobiotics. Phenols are environmental pollutants discharged through wastewaters from various industries. Phenols are toxic to several biochemical reactions**.** However biological transformation of phenols to non-toxic entities exists in specialized microbes, owing to enzymatic potential involving enzymes of aromatic catabolic pathways. In this study, a series of experiments were performed to examine the effects of the mineral medium composition and the pH on phenol removal. In this purpose, phenol biodegradation was carried out in a batch reactor containing mixed bacteria; the temperature (30°C), the stirring velocity (200 r /min) and the phenol concentration (125 mg/L) were kept constants. The initial pH was varied in the range 5 – 9 and the mineral components were tested in the following concentration ranges: 0 – 2 g/L for NH_4Cl, 0 – 4 g/L for KH_2PO_4, 0 – 4 g/L for NaH_2PO_4 and 0 – 0.2 g/L for $MgSO_4$. Their effects on phenol biodegradation and specific growth rate were examined. All experiments were carried out at a given initial bacterial concentration of 0.08 g/L (based on optical density determination, 0.079). The shorter biodegradation time of phenol was 20.6 h for NaH_2PO_4, KH_2PO_4 and $MgSO_4$ concentrations of 2 – 4, 3 and 0.2 g/L respectively. Maximum specific growth rate (0.65 h^{-1}) and total phenol removal (99.99 %) were recorded for an optimal pH value of 8 and the following mineral medium concentrations (g/L): 1, 4, 3 and 0.1 for NH_4Cl, KH_2PO_4, NaH_2PO_4 and $MgSO_4$ respectively.

Key words: Biodegradation, Phenol, Microbial consortium, Kinetic

INTRODUCTION

Phenol is a very toxic and hazardous chemical compound. Indeed, many phenol-based substances are confirmed or suspected human carcinogens (Gupta *et al.,* 1998; Dabhade *et al.,* 2009 ; Onwurah, 2007). Worldwide, phenols are present at different concentrations (0.002 – 2.6 mg/L) caused by the development of industrialization, more and more industrial wastewater containing phenolic compounds are discharged from industrial processes such as oil refineries, chemical plants and coke ovens (Nemerow, 1978; Patterson, 1985; Berkowitz, 1988; Sittig, 1997; Sa & Boaventura, 2001; Ho *et al.,* 2009; Abduli *et al.,* 2007; Hassani *et al.,* 2009).

*Corresponding author E-mail: abdeltif.amrane@univ-rennes1.fr

A variety of techniques have been used for the remediation of phenol. Conventional methods of treatment are largely chemical or physical, but these processes lead to secondary effluent problems which increase the global cost of the process in a non negligible way. Besides, biological treatment is an effective method which shows an increasing number of industrial applications, since a wide range of microorganisms can assimilate phenol as the sole source of carbon (Shawabkeh *et al.,* 2007).

Bacteria are a class of microorganisms actively involved in the degradation of organic pollutants from contaminated sites. A number of bacterial species are

known to degrade phenolic compounds. Most of them, showing high biodegradation efficiency, are isolated from contaminated soil or sediments (Haritash *et al.,* 2009). The identified organisms belonged to several genera, like *Pseudomonas*, as well as *Agrobacterium*, *Bacillus* (Gurujeyalakshmi & Oriel, 1989), *Burkholderia*, *Sphingomonas* (Berkowitz, 1988), *Rhodococcus* (Margesin *et al.,* 2005) species and mixed culture.

The main goal of this paper was to investigate the biodegradation of phenol by mixed bacteria; the effect of pH and the mineral medium composition were optimized to determine the best conditions for phenol removal, especially the mineral salts supplementation and the optimal mineral nitrogen (ammonium chloride) concentration.

MATERIALS & METHODS

The mixed bacteria used in this work were obtained from activated sludge from the hazardous wastewater plant of Boumerdès (Algeria). The stock cultures were stored at 4 °C. The mixed bacteria were activated for 24 h at 30 °C in the nutrient medium (NB) containing (g/ L): peptone, 15, yeast extract, 3, sodium chloride, 6, and (D+)-glucose, 1.

After 24 h, when cells were grown, the biomass was harvested by centrifugation. The microorganisms collected after centrifugation (3000 r/min) for 30 min were suspended in NaCl 0.5 % and re-centrifuged. After the third washing, The microorganisms collected after centrifugation were re-suspended in NaCl 0.5 % to determine the concentration of the mixed bacteria. This solution (mixed bacteria and NaCl 0.5%) was analyzed by measuring OD at 600 nm using a Vis spectrophotometer (HACH DR2800); the OD value was then converted to dry cell mass using a dry weight calibration curve. The dry cell mass density (g/ L) was found to follow the following regression equation x (g/L) = $1.044 \times OD_{600}$.

Specific growth rate was determined in the exponential growth phase (Dagley & Gibson, 1965; Stanier *et al.,* 1966; Chiam & Harris, 1982; Worden & Donaldson, 1987). For each flask, the specific growth value was determined from linear semi logarithmic plot of cell concentration versus time during the exponential growth phase, namely when specific growth rate became nearly constant (D'Adamo *et al.,* 1984).

As the OD value of adapted cells reached 2.7 – 2.9, an aliquot of the culture was centrifuged at 3000 rpm for 30 min. To wash the biomass, it was re-suspended in NaCl 0.5% and centrifuged. The cells (1 ml) were then transferred and inoculated in Erlenmeyer flasks (250 mL) to yield an initial OD of 0.078, and containing 100 mL of medium containing nitrogen source (NH_4Cl) and the following mineral salt supplementation (MSS), namely NaH_2PO_4, KH_2PO_4 and $MgSO_4$ at the required concentrations, and 125 mg/ L of phenol. The cells were cultivated at 30 °C and 200 rpm. Samples were withdrawn at suitable time-intervals and the concentration of cells was deduced from optical density measurement and phenol was measured as described below.

Phenol was colorimetrically estimated using a Vis spectrophotometer (HACH DR2800) according to the method previously described by Yang & Humphrey (1975) and based on rapid condensation with 4-aminoantipyrine followed by oxidation with alkaline potassium ferricyanide and absorbance read at 510 nm.

RESULTS & DISCUSSION

The effect of mineral salt supplementation of culture medium on phenol degradation was shown for instance for NaH_2PO_4 (Fig.1). The lag phase was at least 10 h, and in the range of NaH_2PO_4 concentrations tested, total phenol removal (125 mg/L) was recorded in less than 22.5 h.

For NaH_2PO_4 concentrations in the range 2 – 4 g/ L, no really effect of this component was recorded and total phenol removal was observed in about 20.6 h.The similar evolution of cell concentration and residual phenol concentration as function of time for the concentration of KH_2PO_4, the concentration of $MgSO_4$, the concentration of NH_4Cl and initial pH solution were observed, complete phenol degradation (125 mg/L) by the mixed cultures was achieved in a range of time of 20.6 to 33.2 h.

Fig. 2 shows the effect of culture medium components on specific growth rate. Maximum specific growth rate was 0.34 h^{-1} recorded for 3 g/L NaH_2PO_4 (Fig.2a). This amount was in agreement with the mineral supplementation considered by other workers, since Luo et al. (2009) and Nakano et al. (1999) supplemented with 2.544 and 1 g/L NaH_2PO_4 to biodegrade phenol, respectively; while 4 g/L was used by Zilouei et al. (2006) to biodegrade chlorophenols (2-chlorophenol, 4-chlorophenol, 2, 4-dichlorophenol and 2, 4, 6-trichlorophenol).

The highest maximum specific growth rate was 0.58 h^{-1} recorded for 4 g/L KH_2PO_4 (Fig.2b) concentration, in agreement with other findings (dos Santos *et al.,* 2009) used 4.3 g/L of KH_2PO_4 in mineral salt medium to biodegrade phenol by *Aureobasidium pullulans* FE13 isolated from industrial effluents.

At 30°C and pH 7, the evolution of specific growth rate versus $MgSO_4$ concentration (Fig.2c) shows maxi-

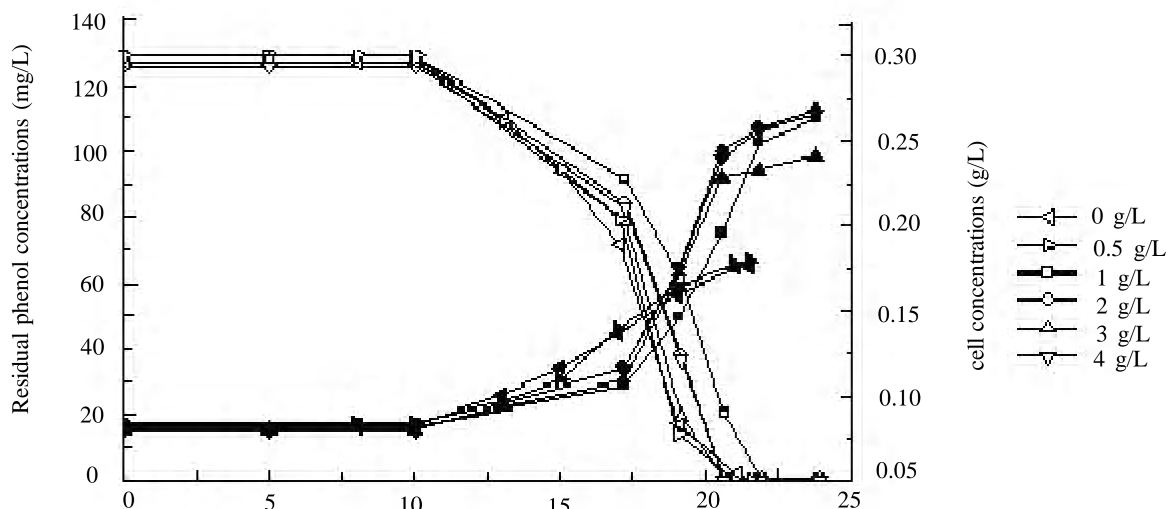

Fig. 1. Time-courses of the residual phenol and biomass concentrations for different initial NaH_2PO_4 concentration. Medium composition and physico-chemical parameter values were [NH_4Cl] = 1 g/L, [KH_2PO_4] = 3 g/L, [$MgSO_4$] = 0.1 g/L, [Phenol] = 125 mg/L, Temperature = 30 °C, stirring velocity = 200 r/min and pH = 7

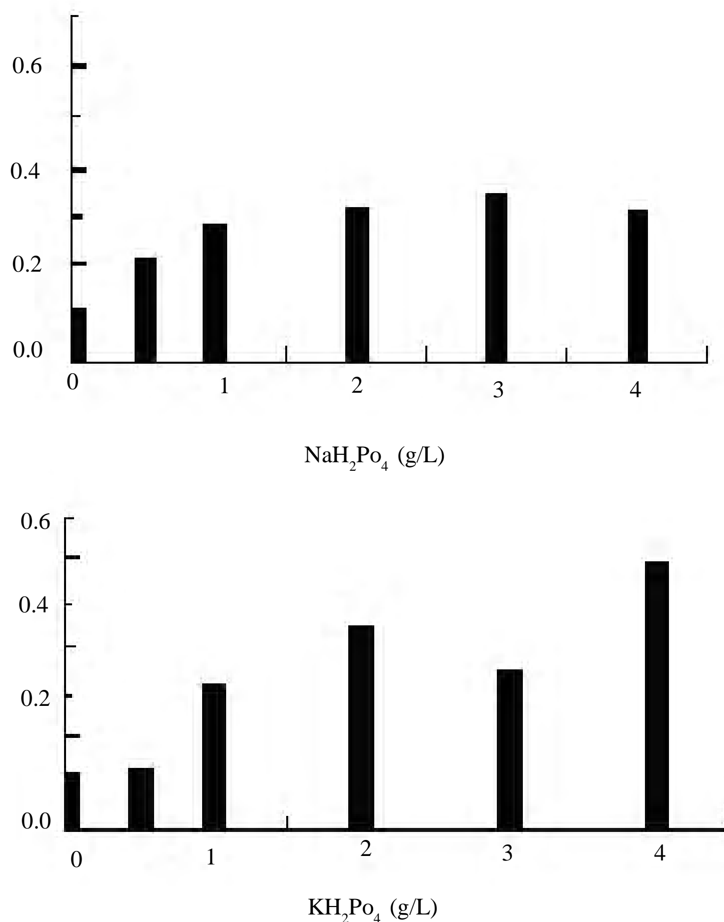

Fig. 2. Specific growth rate as function of initial NaH_2PO_4 (a), KH_2PO_4 (b), $MgSO_4$ (c), NH_4Cl (d) concentrations and pH (e). Except the considered culture medium component or physico-chemical parameter, the other medium parameter values were [Phenol] = 125 mg/L, [NH_4Cl] = 1 g/L, [$MgSO_4$] = 0.1 g/L, [NaH_2PO_4] = 3 g/L, [KH_2PO_4] = 4 g/L, temperature = 30 °C, stirring velocity = 200 r/min and pH = 7 (Continues)

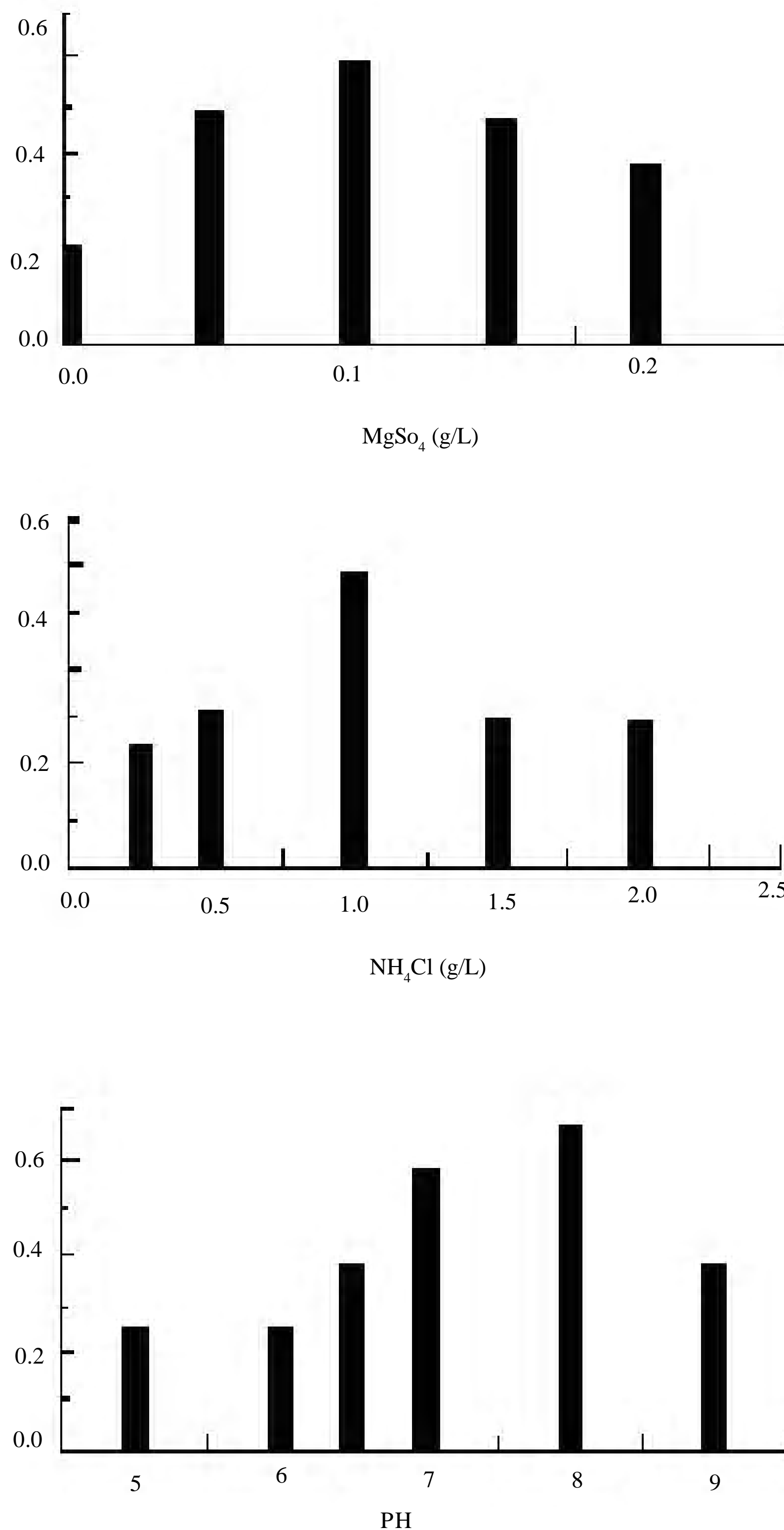

Fig. 2. Specific growth rate as function of initial NaH_2PO_4 (a), KH_2PO_4 (b), $MgSO_4$ (c), NH_4Cl (d) concentrations and pH (e). Except the considered culture medium component or physico-chemical parameter, the other medium parameter values were [Phenol] = 125 mg/L, [NH_4Cl] = 1 g/L, [$MgSO_4$] = 0.1 g/L, [NaH_2PO_4] = 3 g/L, [KH_2PO_4] = 4 g/L, temperature = 30 °C, stirring velocity = 200 r/min and pH = 7 (Continuation)

mum value of 0.58 h^{-1} when $MgSO_4$ for 0.1 g/L $MgSO_4$, in agreement with Zhao et al. (2009) during their studies on phenol biodegradation.

Fig.2d shows that the optimal value of specific growth rate was 0.58 h^{-1}, recorded for 1 g/L NH_4Cl supplementation. The effect of pH on μ_{max} shows an optimum (0.65 h^{-1}) for pH 8 (Fig.2e). Other workers in the field obtained the same optimal pH during phenol and p-nitrophenol degradation (Wang *et al.,* 2007; Ho *et al.,* 2009; Qiu *et al.,* 2009).The optimal values of specific growth rate are in the same order of magnitude than the values reported by other authors for mixed cultures, namely in the range 0.13 to 0.6 h^{-1} (Pawlowsky & Howell, 1973; Hill & Robinson, 1975; Yang & Humphrey, 1975; D'Adamo *et al.,* 1984; Rozich & Colvin, 1986; Sokol, 1987; Allsop *et al.,* 1993; Goswami *et al.,* 2005).

CONCLUSION

Growth kinetics of the used microbial consortium and its potential for phenol assimilation were investigated leading to high biodegradation activity of the considered mixed culture, with an optimal maximum specific growth rate of 0.65 h^{-1} for a total phenol biodegradation time of 24.2 h. Irrespective of the culture conditions, total phenol biodegradation (125 mg/L) was achieved during times ranging from 20.6 to 33.2 h. The optimal mineral medium concentrations (g/L) were 1, 4, 3 and 0.1 for NH_4Cl, KH_2PO_4, NaH_2PO_4 and $MgSO_4$ respectively, and the optimal pH value were 8.

REFERENCES

Abduli, M. A., Nabi Bidhendi, G. R., Nasrabadi, T. and Hoveidi, H. (2007).Evaluating the Reduction of Hazardous Waste Contact in Tabriz Petrochemical Complex, Focusing on Personal Protective Equipment Method. Int. J. Environ. Res., **1(1),** 14-18.

D'Adamo, P. D., Rozich, A. F. and Gaudy, A. F. Jr. (1984). Analysis of growth data with inhibitory carbon sources. Biotechnol. Bioeng., **26 (4)**, 397-402.

Allsop, P. J., Chisti, Y., Moo-Young, M. and Sullivan, G. R. (1993). Dynamic of phenol degradation by Pseudomonas putida. Biotechnol. Bioeng., **41(5)**, 572-580.

Berkowitz, J. B. (1988). Standard Handbook of Hazardous Water Treatment and Disposal – Hazardous waste recovery processes. (New York: McGraw – Hill).

Chiam, H. F. and Harris, I. J. (1982). A model for noninhibitory microbial growth. Biotechnol. Bioeng., **24 (1)**, 37-55.

Dabhade, M. A., Saidutta, M.B. and Murthy, D.V.R. (2009). Adsorption of Phenol on Granular Activated Carbon from Nutrient Medium:Equilibrium and kinetic Study. Int. J. Environ. Res., **3(4),** 545-556.

Dagley, S. and Gibson, D. T. (1965). The bacterial degradation of catechol. Biochem. J., **95 (2)**, 466-474.

Goswami, M., Shivaraman, N. and Singh, R. P. (2005). Microbial metabolism of 2-chlorophenol, phenol and p-cresol by Rhodococcus erythropolis M1 in co-culture with Pseudomonas fluorecens P1. Microbiol. Res., **100**, 101-109.

Gupta, V. K., Sharma, S., Yadav, I. S. and Mohan, D. (1998). Utilization of Bagasse Fly Ash Generated in the Sugar Industry for the Removal and Recovery of Phenol and p-Nitrophenol from Wastewater. J. Chem. Technol. Biotechnol, **71 (2)**, 180-186.

Gurujeyalakshmi, G. and Oriel, P. (1989). Isolation of phenol degrading Bacillus stearothermophilus and partial characterisation of the phenol hydroxylase. Appl. Environ. Microbiol., **55 (2)**, 500-502.

Haritash, A. K. and Kaushik, C. P. (2009). Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. J. Hazard. Mater., **169 (1-3)**, 1-15.

Hassani, A. H., Otadi, N., Javid, A.H., Khiabani, F.F. and Hoshyaripour, G. (2009). Improving the performance of Pars Oil Refinery Wastewater Treatment System. Int. J. Environ. Res., **3(4),** 653-662.

Hill, G. A. and Robinson, C. W. (1975). Substrate inhibition kinetics: phenol degradation by Pseudomonas putida. Biotechnol. Bioeng., **17 (11)**, 1599-1615.

Ho, K.-L. Lin, B., Chen, Y.-Y. and Lee, D.-J. (2009). Biodegradation of phenol using Corynebacterium sp. DJ1 aerobic granules. Bioresour. Technol., **100 (21)**, 5051-5055.

Luo, H., Liu, G., Zhang, R. and Jin, S. (2009). Phenol degradation in microbial fuel cells. Chem. Eng. J., **147 (2-3)**, 259-264.

Margesin, R., Fonteyne, P. A. and Redl, B. (2005). Low-temperature biodegradation of high amounts of phenol by Rhodococcus spp and basidiomycetous yeasts. Res. Microbiol., v.156, No.1, pp.68-75.

Nakano, Y., Nishijima, W., Soto, E. and Okada, M. (1999). Relationship between growth rate of phenol utilizing bacteria and the toxic effect of metabolic intermediates of trichloroethylene (TCE). Water Res., **33 (4)**, 1085-1089.

Nemerow, N. L. (1978). Industrial Water Pollution, Origin, Characteristics and Treatment. (London: Addison-Wesley).

Onwurah, I. N. E.; Ogugua, V. N.; Onyike, N. B.; Ochonogor, A. E. & Otitoju, O. F. (2007).Crude Oil Spills in the Environment, Effects and Some Innovative Clean-up Biotechnologies. Int. J. Environ. Res., **1(4),** 307-320.

Patterson, J. W. (1985). Industrial Wastewater Treatment Technology. 2nd Edition. (New York: Butterworths).

Pawlowsky, U. and Howell, J. A. (1973). Mixed culture biooxidation of phenol, Determination of kinetic parameters. Biotechnol. Bioeng., **15 (5)**, 889-896.

Qiu, X., Wu, P., Zhang, H., Li, M. and Yan, Z. (2009). Isolation and characterization of *Arthrobacter* sp. HY2 capable of degrading a high concentration of p-nitrophenol. Bioresour. Technol., **100 (21),** 5243-5248.

Rozich, A. F. and Colvin, R. J. (1986). Effects of glucose on phenol biodegradation by heterogeneous populations. Biotechnol Bioeng, **28 (7)**, 965-971.

Sa, C. S. A. and Boaventura, R. A. R. (2001). Biodegradation of phenol by *Pseudomonas putida* DSM 548 in a trickling bed reactor. Biochem. Eng. J., **9 (3)**, 211-219.

Shawabkeh, R., Khleifat, K., Al-Majali, I. and Tarawneh, K. (2007). Rate of biodegradation pf phenol by Klebsiella oxytoca in minimal medium and nutrient broth conditions. Bioremed. J., **11 (1)**, 13-19.

Sittig, M. (1997). How to Remove Pollutants and Toxic Materials from Air and Water, A Pratical Guide. (Park Ridge, NJ: Noyes Data Corporation).

Sokol, W. (1987). Oxidation of an inhibitory substrate by washed cells (oxidation of phenol by Pseudomonas putida). Biotechnol. Bioeng., **30 (8)**, 921-927.

Stanier, R. Y., Palleroni, N. J. and Doudoroff, M. (1966). The aerobic *Pseudomonas* taxonomic study. J. Gen. Microbiol., **43**, 159-275.

dos Santos, V. L., de Souza Monteiro, A., Braga, D. T. and Santoro, M. M. (2009). Phenol degradation by Aureobasidium pullulans FE13 isolated from industrial effluents. J. Hazard. Mater., **161 (2-3)**, 1413-1420.

Wang, Y., Tian, Y., Han B., Zhao, H.-B., Bi, J.-N., Cai, B.-L. (2007). Biodegradation of phenol by free and immbilized Acinetobacter sp. strain PD12. J. Environ. Sci., **19 (2)**, 222-225.

Worden, R. M. and Donaldson, T. L. (1987). Dynamics of a biological fixed film for phenol degradation in a fluidized-bed bioreactor. Biotechnol. Bioeng., **30 (3)**, 398-412.

Yang, P. D. and Humphrey, A. E. (1975). Dynamic and steady state studies of phenol biodegradation in pure and mixed cultures. Biotechnol. Bioeng., **17 (8)**, 1211-1235.

Zhao, G., Zhou, L., Li, Y., Liu, X. ,Ren, X. and Liu, X. (2009). Enhancement of phenol degradation using immobilized microorganisms and organic modified montmorillonite in a two-phase partitioning bioreactor. J. Hazard. Mater., **16(1-3)**, 402-410.

Zilouei, H., Guieysse, B. and Mattiasson, B. (2006). Biological degradation of chlorophenols in packed-bed bioreactors using mixed bacterial consortia. Process Biochem. **41 (5)**, 1083-1089.