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Full Length Research Paper

Bacterial removal of toxic phenols from an industrial effluent

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Chlorinated phenols, widely used in industries, are of growing concern owing to their high toxicity, carcinogenicity and wide distribution in industrial wastes. In the present study, one *Pseudomonas* isolate, identified as *Pseudomonas fluorescens*, was obtained using the enrichment process with 2,4,6-trichlorophenol (2,4,6-TCP) as a sole carbon source. This isolate was found to be able to degrade various highly chlorinated phenolic compounds such as pentachlorophenol, 2,4,5-TCP, 2,4,6-TCP as well as phenol, 2,4-dibromophenol and 2,4-dichlorophenol (2,4-DCP). The ability of *P. fluorescens* isolate to remove phenol from a resin producing industrial effluent was tested by scanning the spectrum with a UV-VIS spectrophotometer. The results indicated that this isolate metabolized phenol in the *meta*-pathway. The optimal phenol degradation conditions of *P. fluorescens* isolate were at pH 7 and 30°C. At the 480 mg/l of phenol concentration, the highest specific degradation rate of was observed. Further increases in phenol concentration slowed down the degradation ability of the isolate. However, *P. fluorescens* isolate still has the ability of degrading phenol at the concentration of 3.2 g/L. The supplementation of 1% glucose stimulated the growth of *P. fluorescens* isolate and enhanced the ability to utilize phenol from the effluent sample. GC-MS results show that 85.4% of phenol in the effluent sample was metabolized after 40 days. In conclusion, *P. fluorescens* isolated in this study has the ability of utilizing various chlorophenolic compounds and demonstrates its potentials of degrading high concentration of phenol in industrial effluents.

Key words: Bioremediation, *Pseudomonas fluorescens*, industrial effluent, chlorophenols.

INTRODUCTION

Chlorinated phenols are important chemicals widely used in industries for the manufacture of products such as dyes, insecticides, disinfectants, wood preservatives, as chemical products in building, agriculture and hospital (Hoos, 1978; Haggblom, 1992; Agostini et al., 2003) and in effluents from pulp and paper mills (Watanabe et al., 1996). Because of its broad application in industrial and medical settings, it has become one of environmental contaminants especially in the underground water (Radchaus and Schmidt, 1992; Breining et al., 2000). Chlorophenols stick to soil and to sediments at the bottom of lakes, rivers, or streams and rapidly enter the body through the skin and the gastrointestinal tract (Exon, 1984). Owing to their high toxicity, carcinogenicity and wide distribution in industrial wastes leading to great harm to human being and marine organisms (Exon, 1984; Ping et al., 2003), they are of growing concern (Alexander, 1981; Sittig, 1981).

Although there have been many studies regarding the environment fate of chlorinated phenols, including photochemical degradation and sequential aerobic and anaerobic degradation. The bioremediation means have been generally accepted by the public because they lead innocuous tailings, are non-invasive to environments and are cost-efficient compared physiochemical approaches (Gallizia et al., 2003). Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds. The organic pollutants are used as sole source of carbon and energy. Growth process results in a complete degradation (mineralization) of organic pollutants. Generally chlorinated phenols are transformed

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via oxidative dechlorination (aerobic biodegradation) (Steinle et al., 1998), while in anaerobic conditions via reductive dechlorination (Annachhatre and Gheewala, 1996).

In the present study, we report the characteristics of *Pseudomonas fluorescens* isolate capable of degrading various chlorophenolic compounds and the potentials to remove the phenolic compounds from a local resin producing industrial effluent.

MATERIALS AND METHODS

Isolation and identification

Soil samples were collected from Botany nursery (garden) at the Westville campus of the University of KwaZulu Natal. 10g of garden soil was added into 100 ml of Bushnell Haas (BH) medium, pH7.2, containing NH_4NO_3 (1 g/L), K_2HPO_4 (1 g/L), KH_2PO_4 (1 g/L), $CaCl_2.2H_2O$ (0.02 g/L), $MgSO_4.7H_2O$ (0.2 g/L), $FeCl_3$ (0.0 5g/L) (Atlas, 1994). 200 µM of 2,4,6-trichlorophenol (2,4,6-TCP) was used as sole carbon source. These flasks were shaken at 30°C and 160 rpm for four weeks with 1 ml of enriched media transferred into freshly prepared enrichment media each week. Serial dilutions (1/10) of final enriched media were spread-plated on BH agar supplemented with 2,4,6-TCP incubated at 30°C. The single colonies were streaked onto nutrient agar plates, incubated at 30°C overnight and the pure isolates were stored at 4°C until further use.

The isolate that was capable of degrading chlorophenolic compounds were identified by Gram stain, biochemical tests (Garrity et al., 2005) and confirmed by 16S rDNA sequencing (Marchesl et al., 1998). For long–term preservation, the bacterial isolate was stored in 40% glycerol at -70°C.

Substrate preferences for *P. fluorescens* isolate

The substrate preference of the isolate was studied by using various chlorophenolic compounds as carbon sources (Table 1) at 30°C and 160 rpm. The cell growth was measured by the optical density at 600 nm.

Degradation of phenolic compounds in an industrial effluent

One industrial effluent containing phenolic compounds from a local resin producing plant was used as the sole carbon source to test the degradation potentials of the bacterial isolate. During the course of the study, phenol (32 g/l) was identified as the main component in the effluent using GC-MS and colorimetric analyses.

The bacterial isolate was grown overnight in nutrient broth and the grown bacteria were centrifuged for 15 min at 3000 rpm. Cells were re-suspended on BH broth. Cell number of each isolate was normalized to optical density (OD_{600}) of 1.

Preliminary degradation studies were carried out with the addition of 1 ml normalized inocula into 100 ml of BH broth containing 160 mg/l of phenol from the industrial effluent. Flasks were shaken at 30°C and 160 rpm. The reactions containing all components but devoid of bacterial inoculums were used as the controls. Optimal conditions for the substrate degradation by bacterial isolate were determined using different pHs (pH 6.0, 7.2 and 9.0), temperatures and different concentrations of substrate. In order to enhance the phenol-degradation ability of bacterial isolate at the high phenol concentration, additional 1% glucose was supplemented in the growth media.

The phenol concentration was determined using a UV-VIS spectrophotometer (Varian Cary 50) at low phenol concentration and using TLC and the colorimetric methods, while the phenol concentration was high, as described below. The cell growth was also monitored by optical density at 600 nm (OD_{600}).

Table 1. The substrate preference of *P. fluorescens* isolate using various halo-phenolic compounds as the sole carbon source in BH media at pH7.2, 30ºC and 160 rpm for 4 days.

Substrate	*P. fluorescens*
Phenol (200 µM)	+ + +
2,4-dibromophenol (200 µM)	+ + + +
2,4-dichlorophenol (200 µM)	+ + + +
4-chloro-3-nitrophenol (200 µM)	+ + + +
2,6-dichlorophenol (200 µM)	+ + + + +
2,4,6-trichlorophenol (200 µM)	+ + + + +
2,4,5-trichlorophenol (200 µM)	+ +
Pentachlorophenol (200 µM)	+ +

+ + + + +: $OD_{600} > 0.700$.
+ + +: $0.700 > OD_{600} > 0.400$.
+ +: $0.400 > OD_{600} > 0.200$.
+: $OD_{600} < 0.200$.

Determination of phenol concentration

1 ml of supernatant after centrifugation at given time point was assayed by scanning its spectrum at 200-400 nm with a Cary 50 UV spectrophotometer (Varian Inc.). The concentration of phenol was determined by OD_{268} with the absorbance coefficient of 2169 (El-Sayed et al., 2003).

Thin Layer chromatography (TLC)

The aliquots of treated effluents were spotted on a TLC plate (Merck Silica gel 60 F254). The solvent system was toluene, acetyl acetate and acetic acid in 7:2:1 ratio (Harborne, 1986). The phenolic compound was visualized using Syngene Genius gel documentation system. The concentrations of phenolic compound was measured based on the total integrated area of the designated spot compared with the control (Rf value of 0.57).

Colorimetric assay of phenolic compounds

The residual amounts of phenolic compounds present in the effluents at different incubation period were also measured by colorimetric assay (Klibanov et al., 1980). In brief, aliquots (5 ml) of sample were reacted with 0.025 ml of 4-aminoantipyrine (2% aqueous solution), 0.025 ml of 6.0 M NH4OH and 0.05 ml potassium ferricyanide (8% w/v). After 5 min, the dye formed was extracted with 2.5 ml of chloroform. The absorbance of the extract was determined at 510 nm.

Gas chromatography- mass spectrometry (GC-MS)

The samples during bacterial treatment were analyzed using GC-MS. Agilent 6890 GC/5973 MS with J and W HP5-MS column (300 x 2.5 mm) was used.

Calculation of specific degradation rate

The specific phenol degradation rate of *P. fluorescens* isolate under

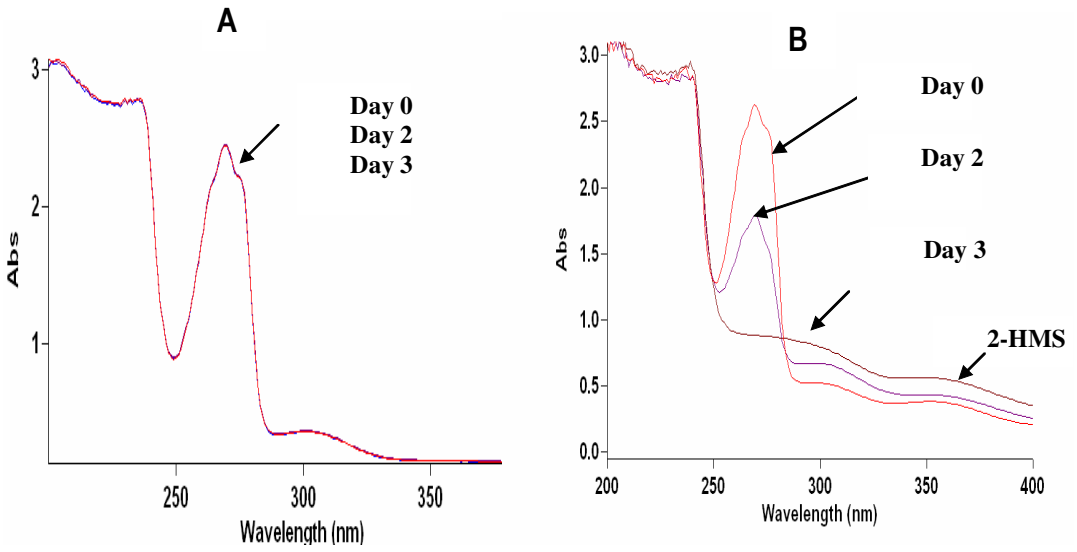

Figure 1. Phenol biodegradation measured by scanning the spectrum with a UV-VIS spectrophotometer at different time intervals in the absence (A) and the presence (B) of *P. fluorescens* isolate.

specific conditions was calculated as the following: δ[phenol]/δt per CFU, where δ[phenol] was the difference of phenol concentrations between two sampling times (mg/L) and δt was time difference in hours (Murialdo et al., 2003).

RESULTS

A cream-white Gram-negative rod was obtained using 2,4,6-TCP as the sole carbon source using the enrichment process. The bacteria isolate was identified as *P. fluorescens* respectively using biochemical tests and confirmed by 16S rRNA identification.

The bacterial isolate was tested for the abilities to utilize various chlorophenolic compounds as the sole carbon source (Table 1). The results show that *P. fluorescens* was capable of degrading several highly halo substituted phenolic compounds such as pentachlorophenol, 2,4,5-TCP and 2,4,6-TCP and 2,6-dichlorophenol (2,6-DCP) as well as phenol, 2,4-dibromophenol or 2,4-DCP. The isolate utilized 2,4,6-TCP and 2,6-DCP more effectively. The optimal growth of *P. fluorescens* using 2,4,6-TCP as the energy source was at 30°C and pH 7-9. When the substrate concentration was higher than 200 µM, the higher the substrate concentration was, the slower the bacterial growth rate. No growth was observed in the case of *P. fluorescens* while the concentration of 2,4,6-TCP was reached 600 µM (Data not shown).

In order to explore the industrial potentials to remove the toxic phenolic compounds from the industrial effluents, one industrial effluent from a local resin producing company was obtained. The effluent was found to contain high concentration of phenol (32 g/L) measured by the colorimetric assay. Figure 1 shows biodegradation of phenol (160 mg/L; 1.7 mM) from an industrial effluent by *P. fluorescens* isolate by scanning the spectrum with a UV-VIS spectrophotometer. In the absence of the bacterial isolate, the phenol concentration remained unchanged throughout the incubation period. The respective decrease and increase in absorption at 268 and 375 nm in the spectra demonstrate the breakdown of the ring of phenol resulting in the formation of 2-hydro-xymuconate semialdehyde (2-HMS). Decrease in the shaking speed stimulated the accumulation of the peak at 375 nm along with the intensity of the yellow color in the solution. These results indicate that this *P. fluorescens* isolate metabolized phenol in the *meta*-pathway through aerobic process. The highest specific degradation rate of 4.29 x 10^{-10} mg/h/cfu was observed when the phenol concentration was at 480 mg/L compared to 1.27x10^{-10} mg/h/cfu at 640 mg/L and 4.76 x 10^{-11} mg/h/cfu at 320 mg/L. Similar to the case of 2,4,6-TCP as the substrate, high phenol concentration had a negative impact on the degradation ability of bacterial isolate.

P. fluorescens isolate failed to grow in the media when one part of industrial effluent was mixed with one part of double strength BH media (final phenol concentration of 16 g/L). When the phenol concentration was reduced to 3.2 g/L at the neutral pH, the cell number of *P. fluorescens* isolate was able to increase slowly up to 0.07 ($OD_{600\ nm}$) at days 3 and deceased thereafter in the standard degradation conditions. The addition of 1% glucose as the supplement allowed *P. fluorescens* isolate to sustain a steady growth to 0.6 ($OD_{600\ nm}$) at day 10 (data not shown). The degradation of phenolic compound in the diluted effluent sample was monitored over a period of 40 days using TLC and colorimetric assays. Figure 2 shows the residual phenolic compounds determined by colorimetric assay in the presence of 1% glucose over a 40-day period as well as a TLC at 0 and 25 day (Figure 2 inlet). The results demonstrated that *P.*

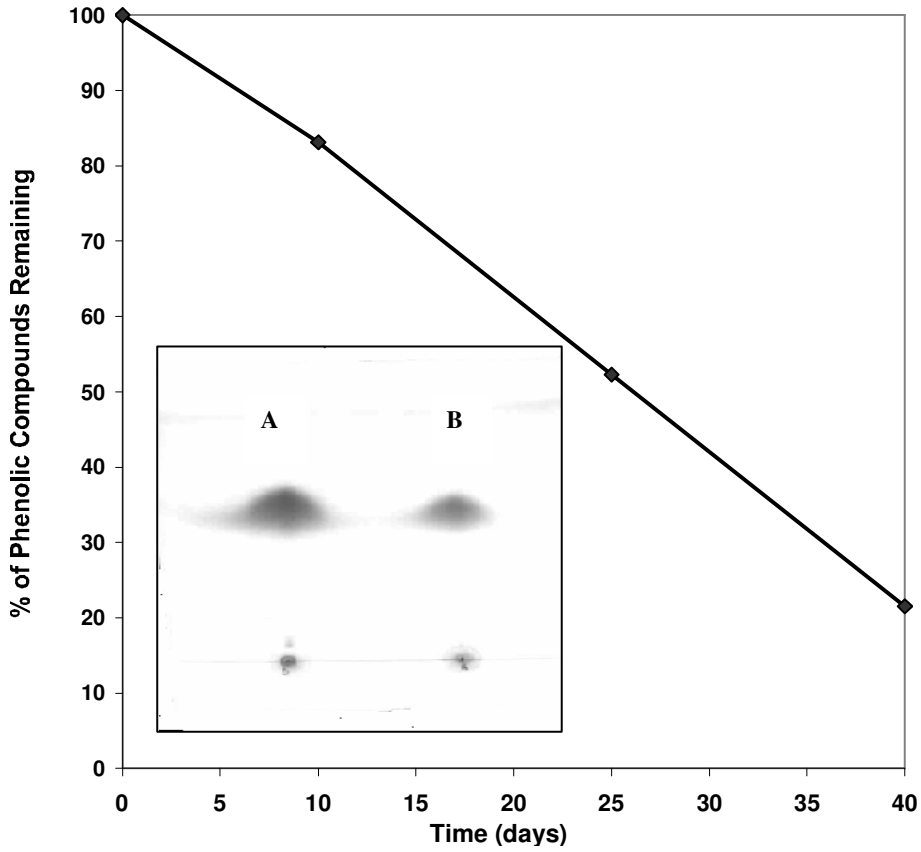

Figure 2. Percentage of phenol remaining (colorimetric analyses) by *P. fluorescens* isolate in the presence of 1% glucose at 3.2 g/L phenol, pH 7.2, 30°C and 160 rpm over a 40-day period. (inlet) TLC results at day 0 (A) and day 25 (B).

fluorescens was able to utilize the phenolic compounds as the carbon source at high substrate concentration. There were 52.3 and 21.5% of residual phenolic compounds remaining at 25 days and at 40 days, respectively using colorimetric assays. Similar value of 56.08% residual remaining at day 25 calculated from TLC was obtained.

GC-MS analyses (Figure 3A) showed that there were two phenolic compounds in the industrial effluent. The major compound was identified as phenol with the elution time at 6.10 min and a minor peak at 7.73 min was found to be 4H-1,3-benzodioxin. At the day 40, 4H-1,3-benzodioxin was completely degraded and only 14.6% of phenol were remained in the effluent sample (Figure 3B), that was corresponding to the value of 21.5% obtained by the colorimetric assay.

DISCUSSION

Pseudomons species are regarded as one of the most common species of bacteria degrading phenolic compounds isolated from contaminated sites (Hinteregger et al., 1992; Fava et al., 1995; Hughes and Cooper, 1996; Whiteney et al., 2001; Van Hamme et al., 2003; Collins et al., 2004). Several *Pseudomonas* species demonstrated their ability of degrading 2.4.6-TCP, 2,4-DCP and pentachlorophenol (Kiyohara et al., 1992; Tomasi et al., 1995; Lee et al., 1998; Farrell and Quilty 2002a,b; Wolski et al., 2006). In the present study, *P. fluorescens* isolate was capable of utilizing a wide range of phenolic compounds with different levels of halo-substitution as the carbon sources. *P. fluorescens* isolate possessed a better ability of utilizing 2,4,6-TCP and 2,6-DCP as the substrate based on the growth rate (Table 1). Increasing substrate concentrations enhanced the growth of *P. fluorescens* isolate, but further increases in the concentration retarded the cell growths of the same isolate. Several reports have shown that microbial growth and concomitant biodegradation of phenol are hindered by the toxicity exerted by high concentration of substrate itself (Kargi and Eker, 2005). High concentrations of chlorophenols/phenol are usually inhibitory to the growth of microorganisms. Antizar-Ladislao and Galil (2004) reported that the degree of ionization of phenol/chlorophenol and the surface properties of the biomass were affected by the pH that an increase in pH values led to a lower electrostatic attraction between the substrate and

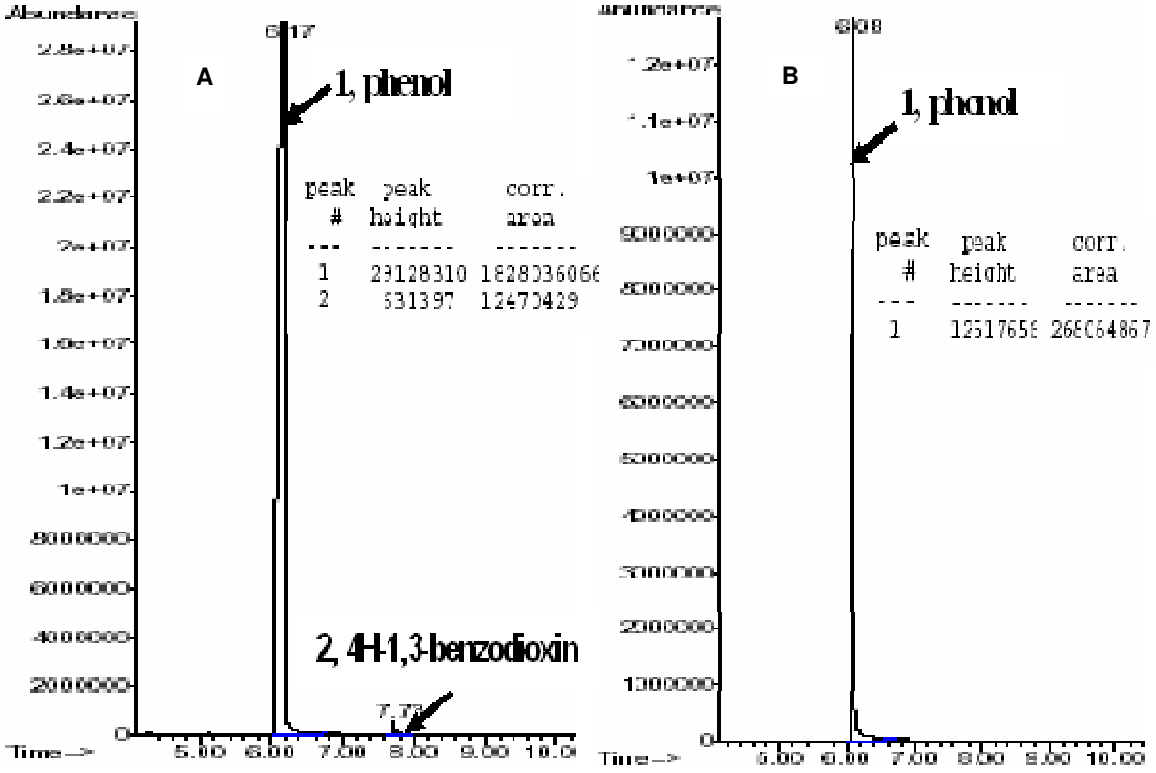

Figure 3. GC-MS analyses of phenol degradation by *P. fluorescens* isolate in the presence of 1% glucose at 3.2 g/L phenol, pH 7.2, 30°C and 160 rpm at day 0 (A) and day 40 (B).

the binding sites of biomass surface. Phenolic compounds have the abilities to partition into membranes, disrupted membrane functions and caused cell death. *Pseudomonas* sp. grew better at pH values within a range of 6 to 9 as observed in this study and others (Tsuji et al., 1982; Wolski et al., 2006). Whiteney and coworkers (2001) found that the *P. fluorescens* and *P. putida* isolates were effectively insensitive to phenol and showed virtually no decrease in the respiratory response over the phenol concentration up to 400 mg/L. Our results also demonstrate that *P. fluorescens* isolate achieved the highest specific degradation rate per cell when the phenol concentration reached 480 mg/L. However, further increases in the substrate concentration suppressed the degradation rate. The concentration of phenol seems to play a crucial role of degradation as observed in this study and others (Baker and Mayfield, 1980; Ursin, 1985; Hwang et al., 1989).

The respective decrease and increase in absorption at 268 and 375 nm in the UV-VIS spectra (Figure 1) suggest the cleavage of the extradiol ring of phenol resulting in the formation of 2-hydroxymuconate semialdehyde (2-HMS) as demonstrated by El-Sayed et al. (2003). In the metabolism of phenol degradation under aerobic condition, two different pathways, either the *meta*- or the *ortho*-pathway, have been identified. Decrease in the shaking speed stimulating the accumulation of 2-HMS at 375 nm demonstrated that the degradation process of *P. fluorescens* isolate favors aerobic process and this isolate metabolized phenol using the *meta*-pathway.

As demonstrated above, the concentration of industrial effluent has an impact on the cell growth. Although *P. fluorescens* isolated in this study failed to replicate in the extreme high phenol concentration (16 g/L), this isolate could still be classified as highly phenol-tolerant strain as described by El-Sayed et al. (2003). *P. aeruginosa* AT2 and *B. cepacia* PW3 isolated by El-Sayed and the coworkers could grow aerobically on phenol as a carbon source at 3 g/L. The addition of 1% glucose as nutrient supplement stimulated the growth of *P. fluorescens* isolate and enhanced the bacterial ability to utilize phenol and other compound(s) (Figures 2 and 3). Several studies have reported the effects of additional organic compound on phenol biodegradation either enhancement (Hess et al., 1993; Zaidi and Mehta, 1995; Annachhatre and Gheewala, 1996; Zaidi and Imam, 1996; Loh and Wang, 1997; Ambujom, 2001) or inhibition (Rozich and Colvin, 1986). Similar enhanced degradation of p-nitrophenol by the addition of glucose was observed with a system inoculated with *Pseudomonas* sp. Strain K (Zaidi and Imam, 1996). The results of TLC, colorimetric assays and GC analyses indicate that the enzyme(s) produced by *P. fluorescens* isolate in this study were able to steadily remove phenol and other compounds in the

effluent. Even though high concentrations of chlorophenols are usually inhibitory to microorganisms, if however the microorganisms are adapted to chlorophenols, they improve the biodegradative ability and alleviate the inhibitory effects of chlorophenol to some extent (Singleton, 2004).

In conclusion, *Pseudomonas* isolate in this study demonstrates the potential for the industrial effluent treatments based ob the abilities to utilize various substrates and to sustain in a high substrate concentration. The contribution of bacteria to the overall rate of degradation may be affected by a number of factors such as phenol concentration (Baker and Mayfield, 1980; Ursin, 1985; Hwang et al., 1989), temperature (Baker and Mayfield, 1980; Bak and Widdel, 1986; Hwang et al., 1986; Thornton-Manning et al., 1987; Gurujeyalashmi and Oriel, 1989). The presence of other nutrients such as glucose stimulated bacterial growth (Rubin and Alexander, 1983; Fedorak and Hrudey, 1986; Colvin and Rozich, 1986; Thorton-Manning et al., 1987) and thus enhanced the phenol degradation ability.

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REFERENCES

Alexander M (1981). Biodegradation of chemicals of environmental concern. Science 211: 132-138.

Ambujom S (2001). Studies on composition and stability of a large membered bacterial consortium degrading phenol. Microbiol. Res. 156: 293-301.

Annachhatre AP, Gheewala SH (1996). Biodegradation of chlorinated phenolic compounds. Biotechnol. Adv. 14: 35-56.

Agostini E, Coniglio MS, Milrad SR, Tigier HA, Giulietti AM (2003). Phytoremediation of 2,4-dichlorophenol by Brassica napus hairy root cultures. Biotechnol. Appl. Biochem. 37(2): 139-144.

Antizar-Ladislao B, Galil NI (2004). Biosorption of phenol and chlorophenols by acclimated residential biomass under bioremediation conditions in a sandy aquifer. Water Res. 38: 267-276.

Atlas RM (1994). Handbook of Biological Media. Parks Ed. CRC Press, p. 175.

Baker MD, Mayfield CI (1980). Microbial and non-biological decomposition of chlorophenols and phenol in soil. Water Air Soil Pollut. 13: 411-424.

Bak F, Widdell F (1986). Anaerobic degradation of phenol and phenol derivatives by *Desulfobacterium phenolicum*, new species. Arch. Microbiol. 146(2): 177-180.

Breining S, Schilts E, Fuchs G (2000). Genes involved in metabolism of phenol in bacterium *Thauera aromatica*. J. Bacteriol. 182(20): 5849-5863.

Collins G, Foy C, McHugh S, O'Flaherty V (2005). Anaerobic treatment of 2,4,6-trichlorophenol in an expanded granular sludge bed-anaerobic filter (EGSB-AF) bioreactor at 15^0C. FEMS Microbiol. Ecol. 53(1): 167-178.

Colvin RJ, Rozich AR (1986). Phenol growth kinetics of heterogeneous populations in a two-stage continuous culture system. Water Pollut. Contr. Fed. 58(4): 326-332.

El-Sayed WS, Ibrahim MK, Abu-Shady M, El-Beih F, Ohmura N, Saiki H, Ando A (2003). Isolation and Characterization of Phenol-catabolizing Bacteria from a Coking Plant. Biosci. Biotechnol. Biochem. 67(9): 2026-2029.

Exon JH (1984). A review of chlorinated phenols. Vet. Human Toxicol. 26(6): 508-520.

Farrell A, Quilty B (2002a). Substrate-dependent autoaggregation of *Pseudomonas putida* CP1 during the degradation of monochlorophenols and phenol. J. Ind. Microbiol. Biotechnol. 28: 316-324.

Farrell A, Quilty B (2002b). The Enhancement of 2-chlorophenol degradation by a mixed microbial community when augumented with *Pseudomonas putida* CP1. Water Res. 36: 2443-2450.

Fava F, Armenante P, Kafkewitz D (1995). Aerobic degradation and dechlorination of 2-chlorophenol, 3-chlorophenol and 4-chlorophenol by Pseudomonas pickettii strain. Appl. Microbiol. Biotechnol. 43: 171-177.

Fedorak PM, Hrudey SE (1986). Nutrient requirements for the methanogenic degradation of phenol and p-cresol in anaerobic draw and feed cultures. Water Reservoir 20(7): 929-934.

Gallizia I, McClean S, Banat IM (2003). Bacterial biodegradation of phenol and 2,4-dichlorophenol. J. Chem. Technol. Botechnol. 78: 959-963.

Garrity M, Brenner DJ, Krieg NR (2005). Bergey's manual of Systematic Bacteriology, 9th edition. McGraw – Hill Companies, Inc.

Gurujeyalashmi S, Oriel P (1989). Isolation of phenol-degrading *Bacillus stearothermophilus* and partial characterization of the phenol hydroxylase. Appl. Environ. Microbiol. 55(2): 500-502.

Haggblom MM (1992). Microbial breakdown of halogenated aromatic pesticides and related compounds. FEMS Microbiol. Rev. 103: 29-72.

Harborne JB (1986). Biochemistry of phenolic compounds. Academic, London, pp. 511-543.

Hess TF, Silverstein J, Schmidt SK (1993). Effect of glucose on 2,4-dinitrophenol degradation kinetics in sequencing batch reactors. Water Environ. Res. 65: 73-81.

Hinteregger C, Leitner R, Loidi M, Fersh A, Streichsbir F (1992). Degradation of phenol and phenolic compounds by *Pseudomonas putida* EKII. Appl. Microbiol. Biotechnol. 37: 252-259.

Hoos R (1978). Patterns of pentachlorophenol usage in Canada: An overview. In: Rao KR (ed.) Pentochlorophenol. Plenum Press. New York, USA.

Hughes B, Cooper D (1996). Biodegradation of phenol using the self-cycling fermentation process, Biotechnol. Bioeng. 51(1): 112-119.

Hwang HM, Hodson RE, Lee RF (1986). Degradation of phenol and chlorophenols by sunlight and microbes in estuarine water. Environ. Sci. Technol. 20(10): 1002-1007.

Hwang HM, Hodson RE, Lewis DL (1989). Assessing interactions of organic compounds during biodegradation of complex waste mixtures by naturally occurring bacterial assemblages. Environ. Toxicol. Chem. 8(3): 209-214.

Kargi F, Eker S (2005). Kinetics of 2,4-dichlorophenol degradation by *Pseudomonas putida* in batch culture. Int. J. Biodeterioration Biodegrad. 55: 25-28.

Klibanov AM, Alberti BN, Morris ED, Felsin LM (1980). Enzymatic removal of toxic phenols and anilines from wastewaters. J. Appl. Biochem. 2: 414-421.

Kiyohara H, Hatta T, Ogawa Y, Kakuda T, Yokohama H, Takizawa N (1992). Isolation of *Pseudomonas pickettii* strains that degraded 2,4,6-trichlorophenol and their dechloroination of chlorophenols. Appl. Environ. Microbiol. 58: 1276-1283

Lee SG, Yoon BD, Park Y (1998). Isolation of a novel pentachlorophenol- degrading bacterium, *Pseudomonas* sp. J. Appl. Microbiol. 85: 1-8.

Loh KC, Wang SJ (1997). Enhancement of biodegradation of phenol and a non-growth substrate 4-nitrophenol by medium augmentation with conventional carbon. Biodegradation 8(5): 329-338.

Marchesl JR, Sato T, Martin AJ, Fry JC, Hiom SJ, Wade W (1998). Design and evaluation of useful bacterium – specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl. Environ. Microbiol. 64(2): 795-799.

Ping W, Gui-Peng Y, Zhao X (2003). Sorption behavior of 2,4-dichlorophenol on marine sediment. J. Colloid Interface Sci. 265: 251-256.

Radchaus PM, Schmidt SK (1992) Characterization of a novel *Pseudo-*

monas sp that mineralizes high concentrations of pentachlorophenol. Appl. Environ. Microbiol. 58: 2879-2885.
Rubin HE, Alexander M (1983) Effect of nutrients on the rates of mineralization of trace concentrations of phenol and p-nitrophenol. Environ. Sci. Technol. 17: 104-107.
Rozich AF, Colvin RJ (1986). Effect of glucose on phenol degradation by heterogeneous populations. Biotechnol. Bioeng. 28: 965-971.
Singleton I (2004). Microbial metabolism of xenobiotics: Fundamental and applied research, Chemical Technol. Biotechnol. 59(1): 9–23.
Sittig M (1981). Handbook of toxic and hazardous chemicals, Noyces Publications, Park Ridge, N.J. USA.
Steinle P, Stucki G, Stettler R, Hanselmann W (1998). Aerobic mineralization of 2,6-Dichlorophenol by *Ralstonia* sp. Strain RK1. Appl. Environ. Microbiol. 64(7): 2566–2571
Thornton-Manning JR, Jones DD, Federle TW (1987). Effects of experimental manipulation of environmental factors on phenol mineralization in soil. Environ. Toxicol. Chem. 6: 615-621.
Tomasi I, Artaud I, Bertheau Y, Mansuy D (1995). Metabolism of polychlorinated phenols by *Pseudomonas cepacia* AC1100: determination of the first two steps and specific inhibitory effect of methimazole. J. Bacteriol. 177(2): 307-311.
Tsuji A, Kaneko Y, Takahashi K, Ogawa M, Goto S (1982). The effect of temperature and pH on the growth of eight enteric and nine glucose non-fermenting species of gram-negative rods. Microbiol. Immuno. 26: 15-24.
Ursin C (1985). Degradation of organic chemicals at trace levels in seawater and marine sediment: The effects of concentration of the initial fractional turnover rate. Chemosphere 14(10): 1539-155.
Van Hamme D, Jonathan A, Singh A, Ward OP (2003). Recent advances in petroleum microbiology. Microbiol. Mol. Rev. 67(4): 503-549.
Watanabe K, Himo S, Takahashi N (1996). Responses of activated sludge to an increase in phenol loading. J. Ferment. Bioeng. 82(5): 522-524.
Whiteney AS, Wiles S, Lilley AK, Philp J, Bailey MJ (2001). Ecological and Physiological analyses of Pseudomonad species within a phenol remediation system. J. Microbiol. Methods 44: 79-88.
Wolski EA, Murialdo SE, Gonzalez JF (2006). Effect of pH and inoculum size on pentachlorophenol degradation by *Pseudomonas* sp. Water *SA* 32(1): 1-5.
Zaidi BR, Imam SH (1996). Inoculation of microorganisms to enhance biodegradation of phenolic compounds in industrial wastewater: Isolation and identification of three indigenous bacterial strains. J. Gen. Appl. Microbiol. 42: 249-256.
Zaidi BR, Mehta MK (1995). Effects of organic compounds on the degradation of p-nitrophenol in lake and industrial wastewater by inoculated bacteria. Biodegradation 6: 275-281.