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Biodegradation of Trichloroethylene (TCE) in the Presence of Phenolic Compound

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Abstract: Experimental bioreactors operated as closed recirculation systems were inoculated with aerobic bacterial cultures that utilized tryptone-yeast extract as carbon and energy sources. These were inoculated with the bacterial culture, which degraded trichloroethylene (TCE) and was observed after 5 days of incubation. Each bioreactor consisted of an expanded bed column through which the liquid phase was recirculated. TCE degradation was also observed with the metabolism of aromatic hydrocarbons established for indigenous microbial population in soil and ground water, in which TCE removal has been shown to be stimulated by the addition of phenol. So co-metabolism occurred when a non-specific enzyme or co-factor was used to transform the growth supporting carbon source, also capable of degrading non-growth supporting compounds. Gas chromatography was use to monitor TCE and their metabolites which compare to run their standards and to check their retention time (t_r) values. The retention time (t_r) values of phenol, catechol, TCA, TCE were 7.22, 8.82, 8.55 and 2.25.

Key words: TCE, bioreactor, phenolic compound degradation, co-metabolism, *Pseudomonas putida CEMB 10124*, gas chromatography, GC-ECD

Introduction

Trichloroethylene (TCE) a suspected carcinogen is the ground water contaminant (Richmond ***et al.***, 2001 and Ma ***et al.***, 2002). TCE and other chloroalkenes pose serious pollution problems. The combination of high usage and disposal methods has resulted in many subsurface aquifers being contaminated with chlorinated ethenes. The diversity in catabolic potential of microorganisms for the development of bioremediation strategies and contaminated aquifers is a formidable task (Watanabe ***et al.***, 2002). TCE was once considered to be non biodegradable, however it has been shown that TCE and other chlorinated organics can be degraded biologically through co-metabolism which is an enzymatic transformation process that requires a supplemental growth substrate for effective microorganisms. Simple aromatic compounds (toluene and phenol) and short chain hydrocarbons (methane, propane, butane) have been used as growth substrates because of their ability to stimulate the production of oxygenases in indigenous microorganisms (Chang and Alvarez-cohen, 1995). Non-specific oxygenases are responsible for co-metabolic TCE degradation. Phenol has been used exclusively as a growth substrate in this research with hollow-fiber membrane bioreactor because these select the

rapidly growing, biofilm forming microorganisms that reliably yield an acceptable rate of aerobic TCE transformation (Segar and Spietal, 1995; Pressman ***et al.***, 2000). Heterotrophic enrichment culture of ***Pseudomonas stutzeri OX1*** degraded TCE aerobically when induced with certain aromatic compounds such as toluene or phenol (Ryoo ***et al.***, 2001). ***Pseudomonas putida*** F_1 has been implicated in TCE metabolic activities. Both methanotrophes and heterotrophes were inhibited at TCE concentration greater than (10 mg l^{-1}). Heterotrophic consortia degrade TCE at concentration exceeding (100 mg l^{-1}) with propane, methanol or yeast extract as the substrate. In these studies TCE degrading bioreactors containing resilient consortia were operated under various conditions of energy source, pH and nutrient level (Phelp, 1990).

Materials and Methods

Co-metabolism of TCE using phenolic compound degradation

Co-metabolism occurs when a non-specific enzyme or co factor used to transform the growth supporting carbon source (phenol) is also capable of degrading the non-growth supporting compound TCE (Hyman, 1995). The ***Pseudomonas putida*** has ability to use aromatic compound like that of phenol as a carbon and energy source and in the presence of phenol degrade aliphatic/linear compound TCE (Baldwin ***et al.***, 2000). ***Pseudomonas putida CEMB 10124*** was tested for their degradation potential against phenol (Muhammad Ferhan ***et al.***, 2002). The strain was first grown in M-9 medium, which contained per l: 6.0 g Na_2HPO_4, 3.0 g KH_2PO_4, 0.5 g NaCl, 1.0 g NH_4Cl, Adjust pH to 7.4, autoclave and then add: 1 M $MgSO_4$ 2 ml and 1 M $CaCl_2$ 0.1 ml, the above solutions should be sterilized separately by filtration or autoclaving, to check their growth behaviour on a single carbon source, i.e; 0.5% glucose. The mid log phase comes after 300-360 minutes. Four reaction flaks were taken, one of the four reaction flasks the bacterial culture was grown with 0.5% glucose in M-9 medium. In second one after reaching on the mid log phase, the cells were harvested and resuspended into fresh M-9 medium with defined dose (200 mM) of phenol. In the third flask, which was an experimental flask, while the other three were control flasks, the cell harvested on mid log phase and remove glucose from the medium then again resuspended into M-9 medium with defined dose of phenol (200mM) and TCE (0.2 mM) and O.D at 600nm was taken. Basically optical density (O.D) tells us about the per unit density of bacterial culture at specific wavelength which was 600nm, In the fourth reaction flask the cells were harvested at mid log phase and give induction with 0.2 mM TCE and O.D at 600nm was checked. Procedure remains the same while the dose of phenol and concentrations of TCE were changed shown in Fig. 1(a-e).

Bioreactor experiments

Reactor was inoculated with test culture, growth in LB (Luria-Bertani) medium, circulated for overnight. After overnight circulation of test culture, induction was done with the known concentration of TCE induced with phenol, the bacterial consortia use phenol and degrade TCE, and the term is used "Co- metabolism". TCE treated with bacterial consortia where no substrate was added to test reactors, the term is used "Starvation", defined as" The non growth supporting compound (TCE) is treated without addition of any substrate or growth supporting

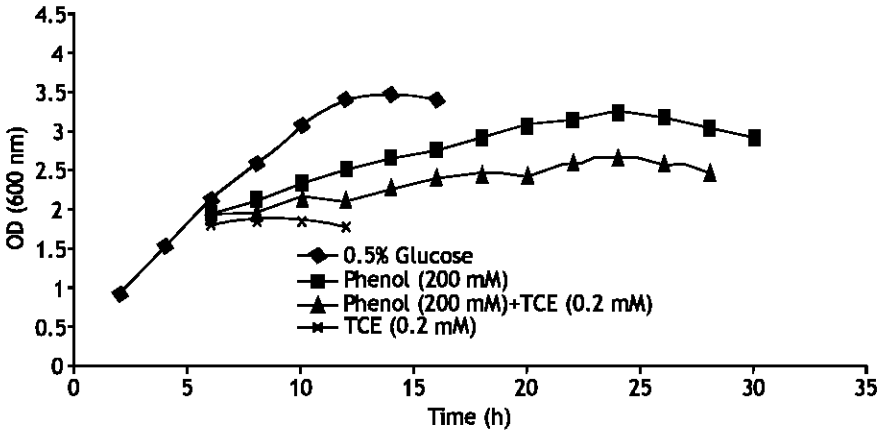

Fig. 1a: Dose of phenol (200 mM) TCE (0.2 mM)

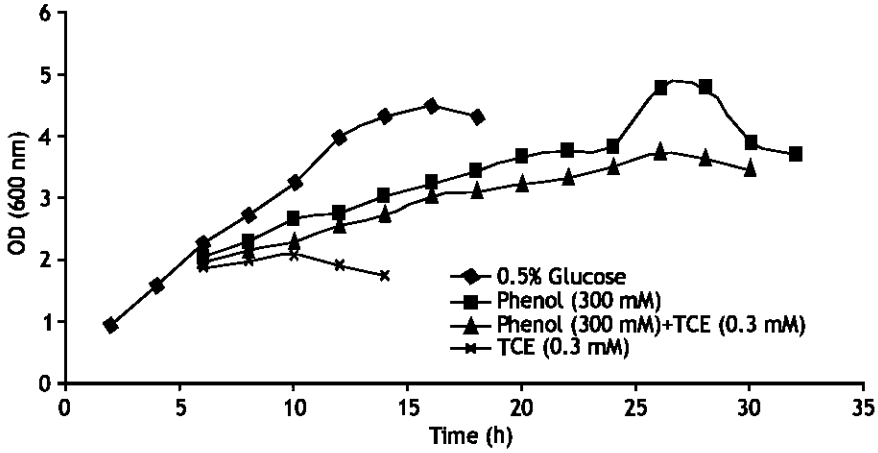

Fig. 1b: Dose of phenol (300 mM) TCE (0.3 mM)

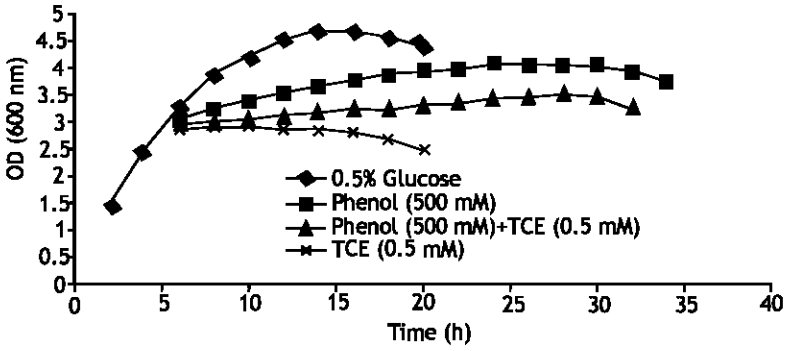

Fig. 1c: Dose of phenol (500 mM) TCE (0.5 mM)

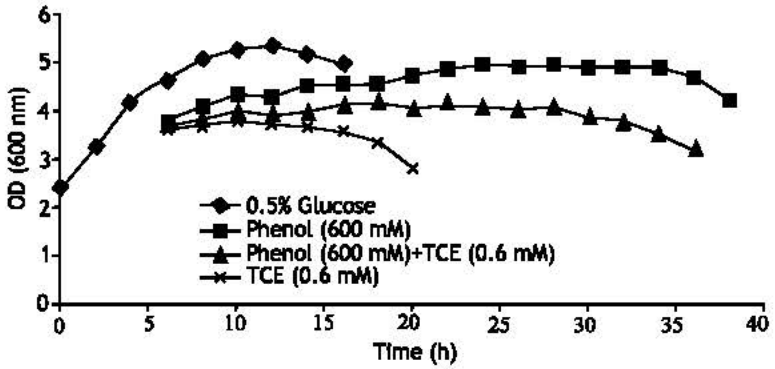

Fig. 1d: Dose of phenol (600 mM) TCE (0.8 mM)

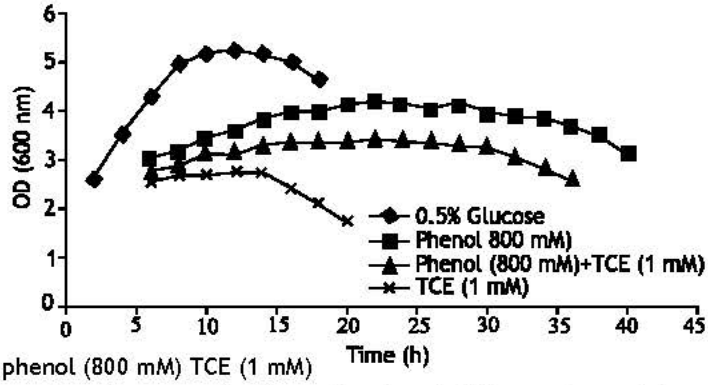

Fig. 1e: Dose of phenol (800 mM) TCE (1 mM)

Fig. 1(a-e): Shows that the co-metabolism utilization of different doses of phenol i.e., (200 mM, 300 mM, 500 mM, 600 mM and 800 mM) with respect to degradation of (0.2 mM, 0.3 mM, 0.5 mM, 0.8 mM and 1 mM) of trichlorothylene (TCE)

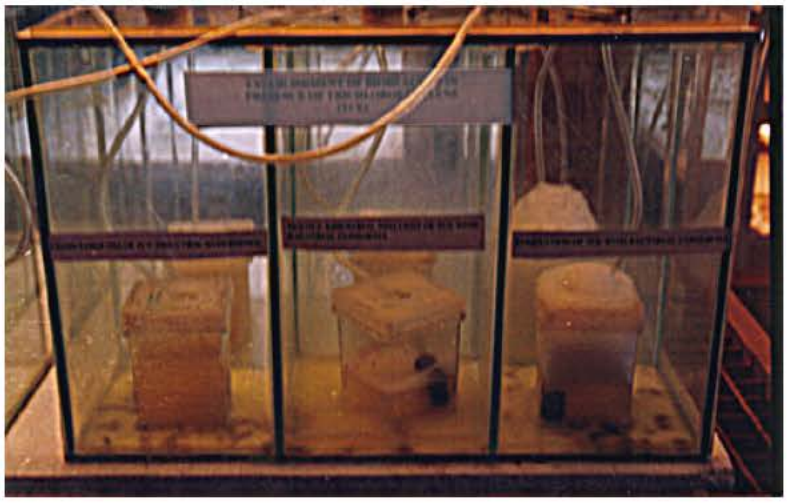

Fig. 2: The establishment of bioreactor for the biodegradation of TCE and textile industrial effluent

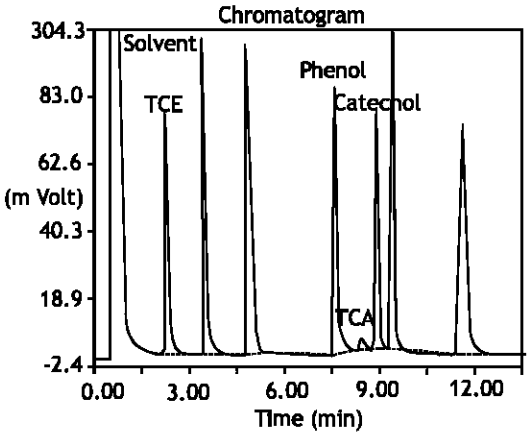

Fig. 3.1: Standard peaks of standard solutions and both industrial effluents

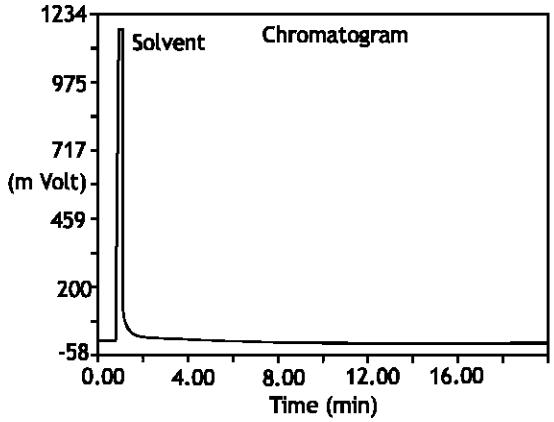

Fig. 3.2: Solvent chromatogram

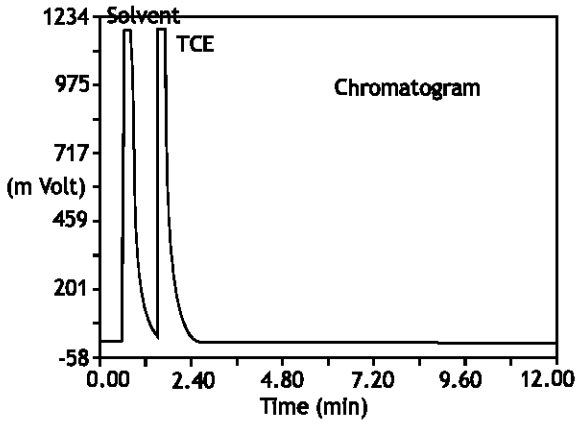

Fig. 3.3: Standard solution of TCE

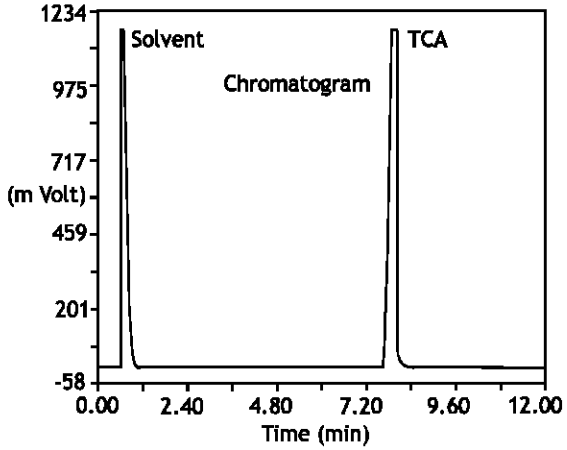

Fig. 3.4: Standard solution of TCA

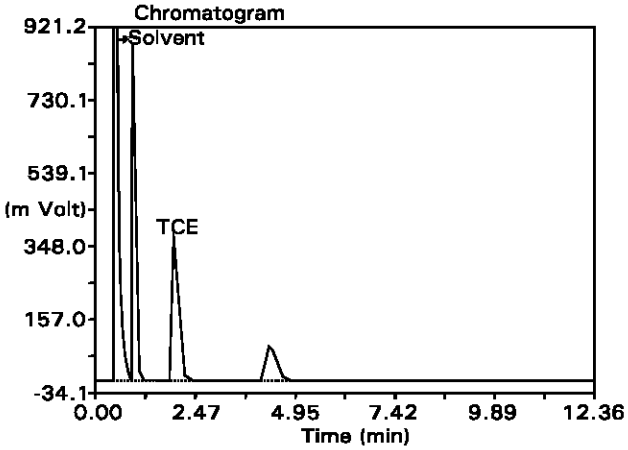

Fig. 3.5: Textile industrial effluent

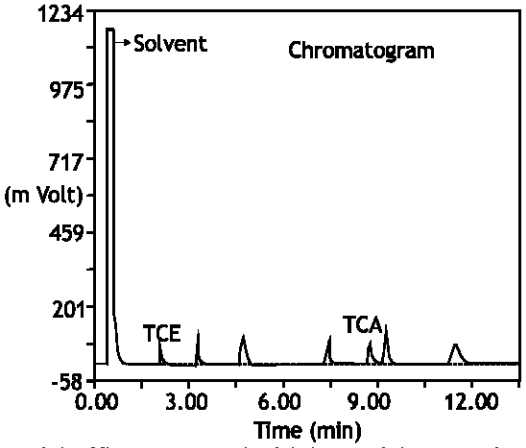

Fig. 3.6: Textile industrial effluent treated with bacterial consortia

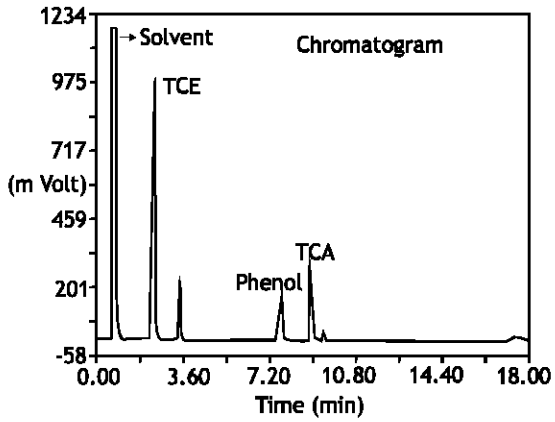

Fig. 3.7: Co-metabolism of TCE in the presence of phenol

Fig. 3(3.1-3.7): Shows different chromatograms of standard solutions and samples treated with bacterial consortia and to check their metabolites run with their standard solutions and the qualitative determination of chromination of chromatograms to compare with their retention time (t_r) values

compound like phenol" the liquid phase was maintained at pH 7.2 throughout the time course. Biomass remained stably attached to the substratum throughout the experiment. Crushed glass (70g of 60 to 80 mesh) served as the substratum, the continuous circulation in the bioreactor with Spurger (Fig. 2). The bacterial consortia was similarly treated with textile industrial effluent because the industrial effluent is a mixture of different compounds, after 5 days of inoculation the supernatants were analyzed by GC-ECD, to identify the metabolites formed, after the treatment by bacterial consortia.

Analytical procedures

Gas chromatography (GC) was performed with a Fisons model 8000 series equipped with an electron capture detector (ECD). For analytical purposes, separation was achieved using a capillary column (Methyl silicon gum SE 30), 30.0 m length by 0.35 mm (inside diameter); Flow rate was kept at 1ml/min. The injection volume was 10µl. The initial column temperature was kept at 50°C for 1 minute and then programmed to 280°C at a rate of 7°C/min. The carrier gas was Nitrogen and the pressure adjusted to 100Kpa. The signal of the solvent (acetone) was used an internal reference, the first peak in all chromatograms are solvent peak because it appears first on GC response as shown in Fig. 3.1 to 3.7. This technique was used to measure the qualitative identification of toxicants and substrates. The qualitative identification is based on the retention time (t_r) of the sample.

Results and Discussion

The co-metabolism studies indicated that in the presence of 200mM of phenol and 0.2mM of TCE used bacterial culture 24 h and 300 mM of phenol and 0.3 mM of TCE used consortia 26-28

h, 500 mM of phenol and 0.5 mM of TCE with in 28-30 h, 600 mM of phenol and 0.8 mM of TCE with in 32 h, 800 mM of phenol and 1 mM of TCE with in 34 h Fig. 1(a-e). There was a decline observed in bacterial growth when higher concentration of TCE was added into the culture medium. After 34 h, a stage reached in the presence of phenol and TCE when there was no further improvement in the bacterial growth and bacterial density started to decrease. This experiment was repeated for several times to give induction with different doses of phenol with respect to change the concentrations of TCE. There was possibility that this decline in bacterial growth may be due to some mineral deficiency and formation of metabolites from phenol and TCE.

Microbes have crucial role in the dissimulation of environmental pollutants. Both aerobic and anaerobic bacteria harbor specialized genes, which are capable of detoxifying many of the xenobiotic compounds. Laboratory studies revealed that these genes have ability to oxidize aromatic compounds into less toxic compounds and develop their carbon and energy source.

The research was conducted to study the biodegradation of trichloroethylene (TCE) and phenol by native bacterial strains or naturally occurring microbes (NOM). TCE is a chlorinated solvent used widely in many industries including textile, metal processing, electronic, printing, pulp and paper. TCE is suspect class carcinogen and can be reduced to the known carcinogen vinyl chloride VC (Vanderberg ***et al.***, 1995). The uses of ***Pseudomonas putida*** are capable of using a wide variety of organic compounds as a growth substrate aerobically, ***Pseudomonas*** strains were exposed to different concentrations of phenol, the results showed that only ***Pseudomonas putida CEMB 10124*** was the best degrader of phenolic compounds. The phenol 800mM was consumed within 40 hours (Muhammad Ferhan ***et al.***, 2002), after that there was a declining trend in bacterial growth due to some mineral deficiency as well as the toxic metabolites formed during bacterial growth. Co-metabolism studies show that when 800mM phenol induced the degradation of 1mM TCE within 36h. TCE has been removed in this manner, however some still rewill induce the enzymes, which catalyze catechol metabolism through the meta cleavage pathway (Fujita ***et al.***, 1995). One of these enzymes is catechol 2,3 dioxygenase, which incorporate atmospheric oxygen into organic compounds such as TCE, thus aiding in its degradation (Wilcox ***et al.***, 1995). It has been shown that 60% of the carbon present in TCE is converted to carbon monooxide with 57 to 78% of the remaining carbon being related with cell metabolism (Nelson ***et al.***, 1990). No microorganism has been found that can degrade TCE as a sole carbon source, thus the bioremediation process relies on co-metabolism (Hyman ***et al.***, 1995). Aerobic degradation of TCE can be achieved by ***Methanotrophes***, ***Mycobacterium vaccae***, ***Nitrosomonas europaea***, ***Pseudomonas putida***, ***Propane oxidizing bacteria***, ***type IV actinomycetes***, ***Xanthobacter sp***, ***Rhodococcus sp***. and many more (Hopkins ***et al.***, 1993; Khindaria ***et al.***, 1995; Ewers ***et al.***, 1990; Malachowosky ***et al.***, 1994; Henry and Galic, 1991). The catechol (one of the intermediate compound of phenol degradation) is also a toxic substance like other pollutant that affects the bacterial growth as well as other higher animals and plants. The next step was to degrade the catechol formed during phenolic compound degradation. It has already been reported that when catechol is accumulated in the cell, the catechol is metabolized exclusively by the meta pathway, which involves 2, 3-dioxygenase (Andreyeva ***et al.***, 1993; Nishihara ***et al.***, 1994 and Futamata ***et al.***, 2001) mediated fission of catechol (meta cleavage) result in the production of acetaldehyde and pyruvate at the end of the pathway (Harayama and Rekik, 1993).

The degradative plasmids in ***Pseudomonas putida F1*** comprise a rather unique group of plasmids (Hallier-Soulier ***et al.***, 1999), each of which contains specialized genes involved in the biodegradation of organic compounds (Harayama, 1994). The experiments demonstrated that bioreactors containing aerobic bacterial culture are capable of degrading the TCE, and phenol providing the energy source. The textile industrial effluent and the supernatants after treatment by ***Pseudomonas putida CEMB 10124*** was analyzed by using the analytical method like gas chromatography, the analysis of samples and the metabolites formed after treatment by bacterial consortia were determined according to their retention time (t_r). In this study it was found that phenol were first oxidized into catechol which was further dissimulated into muconic acid, these metabolites were not toxic that's why the bacterial growth for longer period and degrade phenol more efficiently. The GC results were also shows that when phenol induced in the presence of TCE, the catechol and TCA (trichloroacetic acid) formed, which further metabolized into CO_2, and phenol oxidized into catechol. The metabolites were confirmed to run their standards and to check their retention time (t_r) values. The retention time (t_r) values of phenol, catechol, TCA, TCE were 7.22, 8.82 8.55 and 2.25. So the establishment of bioreactor containing aerobic bacterial consortia is capable of degrading phenol and TCE into an industrial scale.

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References

Andreyeva, A.L., AV Slepenkyn and I.I. Starovoytov, 1993. Increased expression of the plasmid-determined 2,3-dihydroxybiphenyl dioxygenase gene in strains of ***Escherichia coli***, ***Pseudomonas putida*** and ***Pseudomonas aeruginosa***. FEMS Microbiol. Lett., 2: 211-216.

Baldwin, B.R., M.B. Mesarch and L. Nies, 2000. Broad substrate specificity of naphthalene- and biphenyl-utilizing bacteria. Appl. Microbiol. Biotechnol., 6: 748-753.

Chang, Alvarez-cohen, 1995. Transformation capacities of chlorinated organics by mixed culture enriched on methane, propane, toluene or phenol. Biotechnol. Bioengin., 5: 440-449.

Ewers, J., D.F. Schroder and H.J. Knackmuss, 1990. Selection of trichloroethylene degrading bacteria that resist inactivation by TCE. Archieves Microbiol., 154: 410-413.

Fujita, M., M.I. Ike, K. Kotaoka and M. Takev, 1995. Degradation by genetically engineered bacteria carrying cloned phenol catabolic genes. J. Ferment. Bioengin., 79: 100-106.

Futamata, H., S. Harayama and K. Watanabe, 2001. Group-specific monitoring of phenol-stimulated trichloroethylene bioremediation. Appl. Environ. Microbiol., 10: 4671-4677.

Hallier-Soulier, S., V. Ducrocq and N. Truffant, 1999. Conjugal transfer of a TOL-like plasmid and extension of the catabolic potential of ***Pseudomonas putida*** F1. Can. J. Microbiol., 11: 898-904.

Harayama, S. and M. Rekik, 1993. Comparison of the nucleotide sequences of the meta-cleavage pathway genes of TOL plasmid pWW0 from ***Pseudomonas putida*** with other meta-cleavage genes suggests that both single and multiple nucleotide substitutions contribute to enzyme evolution. Mol. Gen. Genet., 239: 81-89.

Harayama, S., 1994. Codon usage patterns suggest independent evolution of two catabolic operons on toluene-degradative plasmid TOL pWW0 of ***Pseudomonas putida***. J. Mol. Evol., 4: 328-335.

Henry, S.M. and D. Grabic-Galic, 1991. Inhibition of trichloroethylene oxidation by the transformation intermediate carbon monooxide. Appl Environ Microbiol., 57: 1770-1776.

Hopkins, G.D., J. Munakata, L. Semprini, P.L. Mc. Carty, 1993. Trichloroethylene concentration affects on the pilot field scale in situ groundwater bioremediation by phenol oxidizing microorganisms. Environ. Sci. Technol., 27: 2542-2547.

Hyman, M.R., 1995. Inhibition Inactivation and recovery of ammonia oxidizing activity in co-metabolism of TCE by Nitrosomonas europea. Appl. Environ. Microbiol., 61: 1480-1487.

Khindaria, A., T.A. Grover and Aust, 1995. Reductive dehalogenation of aliphatic hydrocarbons by lignin peroxidase of phanerochaete chrysosporium. Environ. Sci. Technol., 29: 719-725.

Ma H.W., K.Y. Wu and C.D. Ton, 2002. Setting information priorities for remediation decisions at a contaminated-groundwater site. Chemosphere., 1: 75-81.

Malachowosky, K.J., T.J. Phelps, A.B. Tebolic, D.E. Minnikin and D.C. White, 1994. Aerobic mineralization of TCE, Vinyl Chloride, and aromatic compounds by Rodococcus sp. Appl. Environ. Microbiol., 60: 542-548.

Muhammad Ferhan, Zahoor Ahmed, S. Riazuddin, M.I. Rajoka and A.M. Khalid, 2002. Estimation and removal of phenol in pharmaceutical industrial effluents from paracetamol and aspirin manufacturing units. OnLine J. Biol. Sci., 2: 587-590.

Nelson, M.J., J.V. Kinsella and T. Monotya, 1990. In situ Biodegradation of TCE in contaminated ground water. Environmental Progress., 9: 190-196.

Nishihara, T., T. Yamada, K. Takano, S. Osada, J. Nishikawa, M. Imagawa, 1994. Characterization of the B gene encoding catechol 2,3-dioxygenase. Lett. Appl. Microbiol., 19: 181-183.

Phelp, T.J., 1990. Biodegradation of Trichloroethylene in Continuous recycle expanded bed bioreactor. Appl. Environ. Microbiol., 56: 1702-1709.

Pressman. J.G., G. Georgious and G.E. Spietel Jr, 2000. A hollow-fiber membrane bioreactor for the removal of trichloroethylene from the vapor phase. Biotechnol. Bioeng., 5: 548-556.

Richmond, S.A., J.E. Lindstrom and J.F. Braddock, 2001. Assesment of natural attenuation of chlorinated aliphatics and BTEX in subarctic groundwater. Environ. Sci. Technol., 20: 4038-4045.

Ryoo, D., H. Shim, F.L. Arenghi, P. Barbieri and T.K. Wood, 2001. Tetrachloroethylene, trichloroethylene and chlorinated phenols induce toluene-o-xylene monooxygenase activity in ***Pseudomonas stutzeri*** OX1. Appl Microbiol Biotechnol., 56: 545-549.

Segar and Spietal, 1995. Sustained TCE co-metabolism by Phenol-degrading bacteria in sequencing Biofilm Reactors. Water Environ Research., 5: 674-684.

Vanderberg, L.A., B.L. Burback and J.J. Pery, 1995. Biodegradation of Trichloroethylene by ***Mycobacterium vaccae***. Can. J. Microbiol., 41: 298-301.

Watanabe, K., H. Futamata and S. Harayama, 2002. Understanding the diversity in catabolic potential of microorganisms for the development of bioremediation strategies. Antonie Van Leeuwenhoek., 81: 655-663.

Wilcox, D.J., R.L. Autenrit and J.S. Bonner, 1995. Propane induced biodegradation vapour phase Trichloroethylene. Biotechnol. Bioengin., 46: 333-341.