Potential Of Microbial Species In Biodegradation Of Volatile Organic Compounds From Waters

Luiza Jecu[1], Amalia Gheorghe[1], Florina Popea[1], Andreea Rosu[1], Anicuta Stoica[2], Marta Stroescu[2]

[1] - National Research and Development Institute for Chemistry and Petrochemistry – ICECHIM, Spl. Independentei 202, Bucharest, Romania; 004-021.316.30.63, ljecu@icf.ro; amalia_ghe@yahoo.com
[2] - University Politehnica of Bucharest, Faculty of Applied Chemistry and Material Science, Str. Polizu nr. 1, Bucharest, Romania; 004-021.402.38.70, a_stoica@yahoo.com. marta_stroescu_yahoo.com

Keywords: bioremediation, volatile organic compounds,

Environmental contamination by volatile organic compounds (VOCs) from petrochemical and energy-producing industries is continually increasing. Among VOCs, aromatic compounds such as benzene, toluene and xylenes are the most severe contaminants because of their increasing use as gasoline, aircraft fuel and solvent. Due to their low water solubility, acute toxicity and genotoxicity, these compounds are classified as priority pollutants by European Environment Agency. Bioremediation exploits the ability of microorganisms to reduce the concentration and/or toxicity of a large number of pollutants. It is an economical, versatile, environment-friendly efficient treatment, which has a greater public acceptance and support. In this study we investigated growth patterns of microbial strains during volatile organic compounds degradation in order to select organisms for most efficient biodegradation. The aerobic batch tests were performed with *Pseudomonas putida*, *Candida membranes* and *Penicillium sp*, strains screened previously from various microbial species. A minimal liquid medium containing volatile organic compounds as carbon source, single substrate or in binary and tertiary mixtures was used to evaluate degradation activity of microbial strains. The content of organic compounds was analyzed with gas chromatograph coupled with mass spectrometry. As summarized, the selected strains were able of growing and transforming the volatile organic compounds tested. It was shown that benzene is the most resistant towards microbial degradation, the substitution of aromatic ring in toluene and xylene have facilitated the microbial attack.

Introduction

The environment is continuously polluted by a large array of hazardous chemicals with different structures and different toxicity levels that are released from several sources; the main sources of pollution can be identified as industrial activities, munitions waste and agricultural practice. The explosive development of chemical industries has produced a large variety of chemical compounds that include pesticides, fuels, alkanes, polycyclic aromatic compounds, dyes and more. Although these compounds have contributed to modernize lifestyle, several of them may accumulate in soil, water and air. Among volatile organic compounds resulted from petrochemical and energy-producing industries, aromatic compounds such as benzene, toluene and xylenes are severe contaminants. Due to their low water solubility, acute toxicity and genotoxicity, these compounds are classified as priority pollutants by European Environment Agency.

Bioremediation, technique that utilize the microbial ability to degrade and/or detoxify chemical substance, is relatively low cost, with low-technology level, and a high public acceptance. Much work has been done on the degradation of single pollutants (Machnicka and Suschka, 2001; Jn-Gyung J. and P. Chang-Ho, 2004).

Microbial growth on pollutant mixtures is an important aspect of bioremediation and wastewaters treatment (Burbak and Perry, 1993; Deeb and Alvarez-Cohen, 1999; Lee *et al.*, 2002; Attaway *et al*., 2001; Reardon *et al.*, 2002). Recent studies have reported that the presence of benzene inhibited toluene and xylene degradation, irrespective of whether the microorganism grew in two or three components mixtures (Otenio *et al*, 2005).

Our previous studies have shown that among our microbial collection there are microorganisms able to grow and transform volatile organic compounds. Screening activities performed have allowed the selection of three microbial strains, such as *Pseudomonas putida*, *Candida membranes* and *Penicillium sp* (Jecu *et al*., 2007). In present paper, the above mentioned strains were cultivated in medium containing as carbon source, individual, bi- and tertiary mixtures of volatile organic compounds. In order to evaluate the microbial degradability of organic compounds containing aromatic ring and substituted benzene derivates, the experiments were performed with benzene, toluene and o-xylene.

Materials and Methods

Organism. The microorganisms applied under aerobic conditions were *Pseudomonas putida, Candida membranes, Penicillium sp.* Fungal strains and yeasts were maintained on dextrose/glucose/agar medium; the bacteria strains were maintained on gelose.
*Culture conditions.*The experiments performed in batch cultures used 150 ml glass vials sealed with teflon-coated rubber septa and aluminum caps. Vilas were rotary shaken (28^{o}C, 200 rpm). pH media was set at 7.0 and during experiments no significant modifications were observed. The inoculum was represented by microbial biomass obtained from cultures

grown on peptonate water and Czapek-Dox medium. The culture medium contained mineral salts (per liter of distilled water): a) bacteria - NH_4NO_3 (1.0); K_2HPO_4 (1.0); KH_2PO_4 (1.0); $MgSO_4$. $7H_2O$ (0.2); b) fungi and yeasts: $NaNO_3$ (2.0); KCl (0,5); K_2HPO_4 (1.0). The organic compounds were added after autoclaving to minimize losses from volatilization. The concentration of organic contaminant, the sole carbon and energy source was finally 0.1 % (v/v). For binary systems, the individual component concentrations were equal, the total being 0.1 % (v/v). In tertiary mixtures, the individual concentrations were 0.035 % (v/v). The samples performed in duplicate on rotary shaker to avoid volatilization losses, were taken at time intervals and analyzed for organic compound level, after adequate solvent extraction.

Analytic methods. The content of organic compounds was analyzed with gas chromatograph coupled with mass spectrometry. GC/MS analysis was accomplished with FOCUS GC DSQIL produced by THERMO ELECTRON CORPORATION, equipped with capillary column TR 5MS (5% phenyl methyl siloxane). The oven temperature was isothermal at 60^o C for 2 minute, programmed to 300 ^{o} C at 10o C/min and kept at 300^o C for 10 min. Injector temperature was 250^o C. Each measurement was done in triplicate. Aqueous samples were extracted with chloroform, metylene cloride or carbon tetrachloride. Samples were stored at 4^o C in screw cap vials with teflon-lined rubber septa, until analysis.

Results and Discussion

The yield of organic compound extraction from aqueous culture media depends on the solvent used; i.e. for carbon tetrachloride extraction yield was 89%, for methylene chloride 72-74 %, and tetrachloride 75-79 %. It should be noted that all the efforts were done to minimize the losses due to volatilization of organic compounds.

The present work was focused to evaluate the ability of microbial strains to grow on volatile organic compounds, therefore the level of contaminant was kept constant, at 0.1 % (v/v), for all experiments on single or mixture substrates.

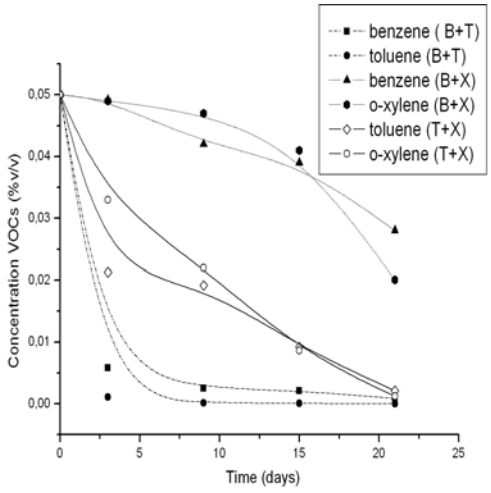
The first step was to cultivate microbial strains on a medium containing single volatile organic compound. The better results were obtained in cultures with *Candida membranes* (Table 1.), despite our expectations as regarding *Pseudomonas putida*, known to be the most versatile in metabolizing aromatic substrates.

The cultures showed that benzene was the most resistant to microbial degradation, while o-xylene and toluene presented differences, with preference for o- xylene. After 21 days of culture, the xylene degradation yields were 10% for *Pseudomonas*, 23% for *Candida* and 14% for *Penicillium*; the yield values for toluene degradation were 28.4%, 34,4% and 15 %, respectively. Similar results were presented by Jn-Gyung J. and P. Chang-Ho (2004).

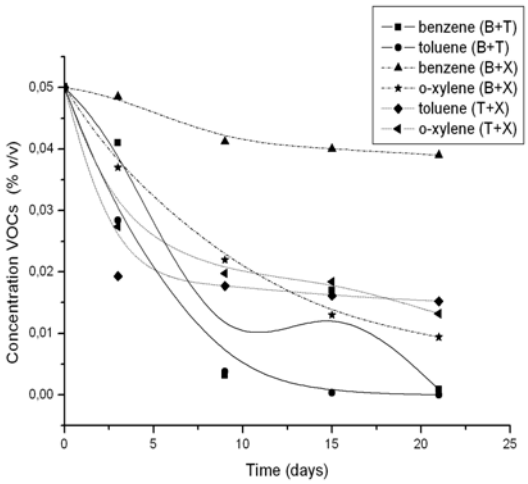
Table 1. Microbial degradation of individual volatile organic compounds (initial concentration 0.1 % v/v)

Strain		Concentration x 10^{-3} (%, v/v)			
		Time (days)			
		3	9	15	21
Pseudomonas putida	B	54.3	13.0	10.14	0.8
	T	9.8	4.34	0.85	0.284
	o-X	12.4	2.3	0.94	0.105
Candida membranes	B	12.43	5.49	1.92	0.87
	T	18.3	6.2	0.55	0.344
	o-X	22.0	4.1	0.49	0.23
Penicillium sp.	B	88.70	85.51	79.51	0.249
	T	10.12	5.6	0.32	0.15
	o-X	14.0	6.1	0.47	0.138

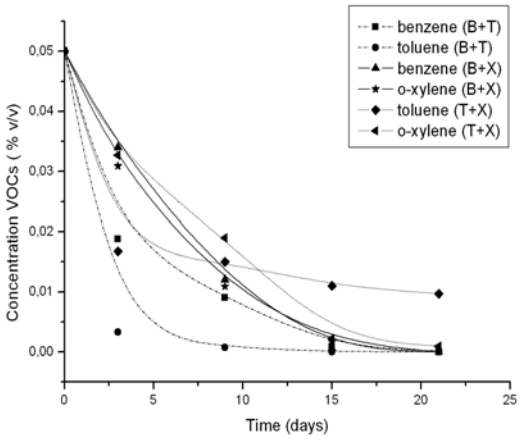
Figure 1 presents the typical degradation curves of different binary mixtures, combination of benzene, o-xylene and toluene.



a) *Candida membranes*



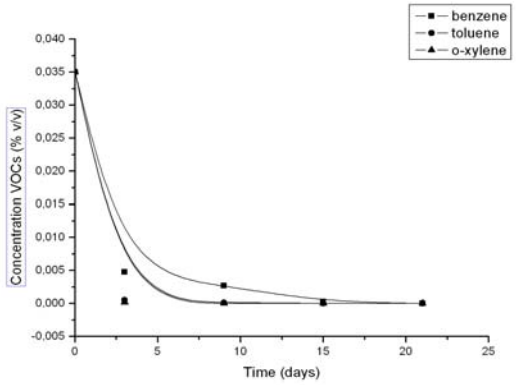
b) *Penicillium sp.*



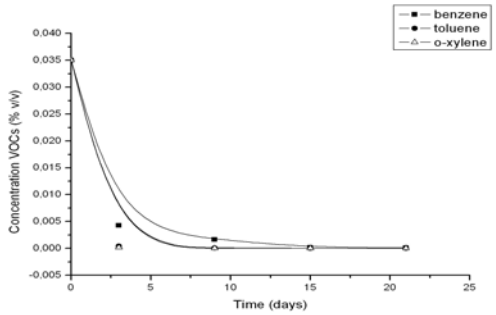
c) *Pseudomonas putida*

Figure 1. Cultivation of microbial strains on binary mixtures of VOCs. (a) *Candida membranes*; b) *Penicillium sp.; c) Pseudomonas putida*

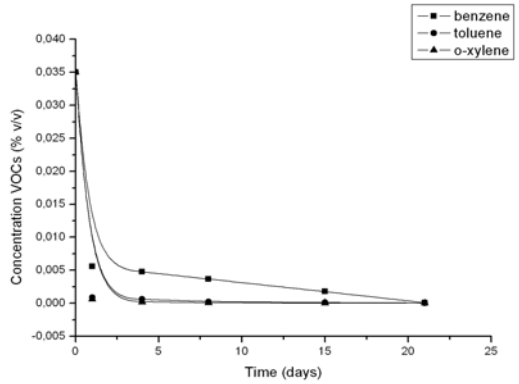
In binary mixtures of benzene and toluene, *Candida* and *Pseudomonas* have presented the same curves shape, a rapid decrease of contaminant concentration after 5 days of cultivation, while *Penicillium sp* needed more time, about 7 days. The more resistant mixtures were, more or less, benzene with o-xylene, microbial strains presenting a different behavior. After 21 days, *Pseudomonas* have reached the total transformation of contaminants, while *Candida* and *Penicillium* have degraded about 40 % (benzene), 50 % toluene), and 15 % (benzene) 80 % (toluene), respectively.



Candida membranes



Penicillium sp.



Pseudomonas putida

Figure 2. Cultivation of microbial strains on tertiary mixtures of VOCs

As regarding the tertiary mixture, the *Candida membranes* was the best microbial agent for organic pollutant degradation. The curves shape for all strains tested were very similar, benzene being most resistant to microbial degradation. But, it should be noticed that, the presence of the other compound have stimulated the benzene degradation, whose concentration decreasing to 0.

The initial concentrations of organic volatile compounds, individual and in mixtures, were different, only the total concentration being constant, therefore it is difficult to evaluate the interactions between compounds. Further researches will be directed towards substrate competitions and inhibitions during microbial degradation of organic compounds.
.

Acknowledgments

The authors gratefully acknowledge financial support from CNMP-Romania through CEEX project No. 127/2006.

References

Attaway, H., C. H. Gooding and M. G. Schmidt, 2001, Biodegradation of BTEX vapors in a silicone membrane bioreactor system, J. Inds. Microbiol. Biotechnol. 26, 316.

Burbak, B. L. and J. J. Perry, 1993, Biodegradation and biotransformation of groundwater pollutant mixtures by *Mycobacterium vaccae*, Appl. Environ. Microbial. 509, 1025.

Deeb, R. A and L. Alvarez-Cohen, 1999, Temperature effects and substrate interactions during the aerobic biotransformation of BTEX mixtures by toluene-enriched consortia and *Rhodoccocus rhodochorus*, Biotechnol. Bioeng. 62, 526.

Goudar, C. T. and K. A. Strevett, 1998, Comparison of relative rates of BTEX biodegradation using respirometry, J. Inds. Microbiol. Biotechnol. 21, 11.

Jecu, L., A. Gheorghe, G, Epure, F. Popea, A. Rosu and A. Roseanu, 2007, A biological treatment for the purification of wastewaters containing volatile organic compounds, Proceedings of Romanian International Conference on Chemistry and Chemical Engineering RICCCE ^{15th}, Sinaia (Romania), Sept 20-22, 2007.

Jn-Gyung J. and P. Chang-Ho, 2004, Characteristics of *Rhodoccocus pyridinovorans* PYJ-1 for the biodegradation of benzene, toluene, m-xylene (BTEX), and their mixtures, J. Biosci. Bioeng. 97, 429.

Lee, E. Y., Y. S. Jun, K. S. Cho and H. W. Ryu, 2002, Degradation characteristics of toluene, benzene, ethylbenzene and xylene by *Stenotrophomonas maltophila*, J. Air Waste Manag. Assoc. 52, 400.

Machnicka A. and J. Suschka, 2001, Activity of selected microorganisms and mixtures in BTX biodegradation, Polish J. Environ. Studies 10, 341.

Reardon, K. F., D. C. Mosteller, J. B. Rogers, N. M. DuTeau and K. H. Kim, 2002, Biodegradation kinetics of aromatic hydrocarbon mixtures by pure and mixed bacterial cultures, Environ. Health Pers. 110, 1005.