

Aerobic Bioremoval of CAHs and BTEX from Contaminated Soil

by

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Doctor of Philosophy in Civil Engineering

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Faculty of Science and Technology

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Abstract

Chlorinated aliphatic hydrocarbons and monoaromatic hydrocarbons are major soil and water pollutants as a result of their widespread anthropogenic use. Soil contamination is of particular concern as it results in offsite contamination of groundwater and surface waters by migration of contaminants through and with soil materials. Co-contamination of soil with chlorinated aliphatic hydrocarbons and monoaromatic hydrocarbons allows for the application of aerobic bioremediation to achieve the simultaneous mineralization of both types of compounds.

Bench scale laboratory experiments were run to select and enrich indigenous microbial isolates capable of cometabolizing two representative chlorinated aliphatic hydrocarbons, *cis*-1,2-dichloroethylene (*cis*-DCE) and trichloroethylene (TCE), mixed with benzene, toluene, ethylbenzene, and three isomers of xylene (BTEX) provided as substrates. The well-characterized toluene-oxidizing bacteria, *Pseudomonas plecoglossicida*, as well as 7 unidentified strains of bacteria isolated from potentially contaminated soil and sludge samples, were examined for their ability to degrade mixtures of BTEX, TCE or *cis*-DCE in liquid culture. This process was used as an initial screen to select the best bacterial isolate for further investigation. Based on results of these experiments, *Pseudomonas plecoglossicida*, showing higher mineralization extent of the mixture, especially TCE and *cis*-DCE, was chosen to investigate further.

The aerobic simultaneous bioremoval of the mixture of TCE, *cis*-DCE, and BTEX was studied under different conditions through laboratory-scale batch experiments in 6-day incubation. Studies were performed on selected parameters (soil physicochemical properties—pH, temperature, and moisture; nutrient concentrations—nitrogen and phosphorus; contaminant properties—contaminant

concentration, ratio of growth substrate (BTEX) to nongrowth substrate (chloroethenes); microbial inoculation amount) and optimization carried out to improve TCE/*cis*-DCE/BTEX mixture removal. The central composite design (CCD) together with the response surface methodology (RSM) was applied to evaluate the interactive effects of the parameters, as well as the optimal bioremoval conditions.

The bioremoval of the mixture varied, depending on the conditions, and interactions among these compounds, especially growth and nongrowth substrate, were complicated. The presence of toluene, nonetheless, enhanced the bioremediation of other compounds unanimously.

Overall, the methods and technologies presented here can be good candidates for bioremediation of the mixed wastes-contaminated sites.

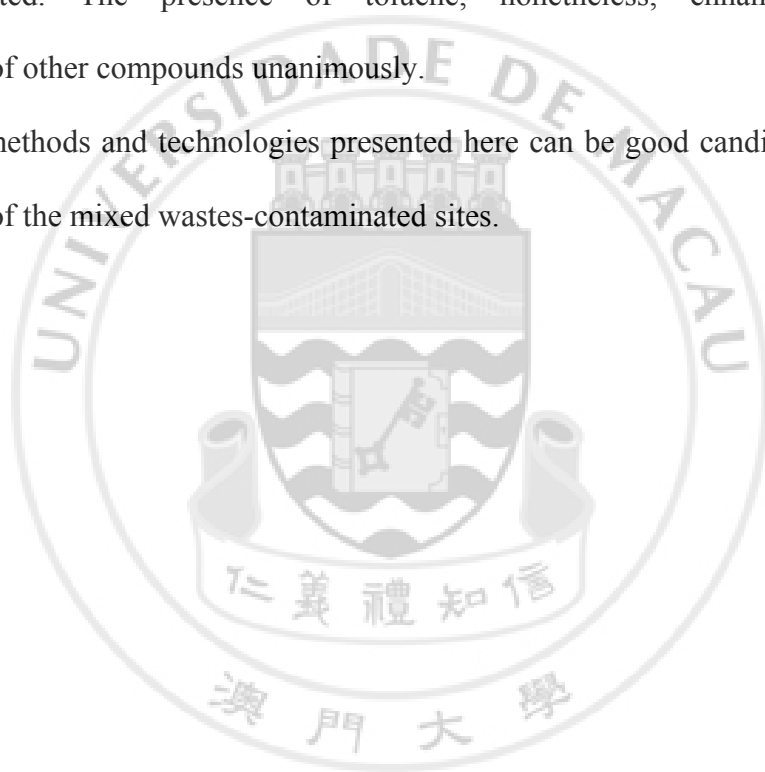


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Glossary

Abiotic losses: Refers to contaminant losses that do not result from biological.

Bioaugmentation: a technique for improvement of the degradative capacity of contaminated areas by introduction of specific competent strains or consortia of microorganisms (Mrozik & Piotrowska-Seget, 2010).

Bioremediation: is defined as a process that uses microorganisms, green plants or their enzymes to treat the polluted sites for regaining their original condition.

Biostimulation: involves the modification of the environment to stimulate existing bacteria capable of bioremediation. This can be done by addition of various forms of rate limiting nutrients and electron acceptors, such as phosphorus, nitrogen, oxygen, or carbon (e.g., in the form of molasses).

Biotic losses: Contaminant losses that are due to microbial breakdown or complete mineralization.

Co-metabolism: the transformation of a non-growth substrate in the obligate presence of a growth substrate or another transformable compound (Dalton & Stirling, 1982).

Natural attenuation: is the reduction in toxicity, mass and/or mobility of a contaminant without human intervention owing to both physical (e.g., dilution, sorption and precipitation) and biological processes (biodegradation) (Röling & van Verseveld, 2002).

Subculture: Refers to a new cell or microbiological culture made by transferring some or all cells from a previous culture to fresh growth medium.

List of Abbreviations

1,1-DCE	1,1-dichloroethylene
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
BTEX	Benzene, Toluene, Ethylbenzene, and three isomers (<i>ortho</i> -, <i>meta</i> -, and <i>para</i> -) of Xylene
CAHs	Chlorinated Aliphatic Hydrocarbons
CCD	Central Composite Design
<i>cis</i> -DCE	<i>cis</i> -1,2-dichloroethylene
C:N:P	Carbon : Nitrogen : Phosphorus
DCEs	Dichloroethylenes
DMF	Dimethylformamide
DNA	Deoxyribonucleic Acid
(D)NAPL	(Dense) Non-aqueous Phase Liquids
DO	Dissolved Oxygen
EPA	Environmental Protection Agency
FWC	Field Water-holding Capacity
FID	Flame Ionization Detector
GC	Gas Chromatography
GEMs	Genetically Engineered Microorganisms
HPLC-MS	High-performance Liquid Chromatography-Mass Spectrometry
IC	Ion Chromatography
μL	Microliter
MCL	Maximum Contaminant Level
mL	Milliliter

mol	Molarity
MSCCs	Maximum Soil Contaminant Concentration Levels
MSM	Mineral Salts Medium
NA	Nutrient Agar
NB	Nutrient Broth
N.C.	North Carolina
NMR	Nuclear Magnetic Resonance
NPL	National Priority List
OD	Optical Density
p	Probability
PCE	Perchloroethylene
ppb	part per billion
ppm	part per million
rpm	Revolutions Per Minute
RSM	Response Surface Methodology
SAR	Special Administrative Region
SVOCs	Semivolatile Organic Compounds
TCE	Trichloroethylene
TPH	Total Petroleum Hydrocarbons
UST	Underground Storage Tank
v/v	volume/volume
VC	Vinyl Chloride
VOCs	Volatile Organic Compounds
w/w	weight/weight
WHC	Water Holding Capacity