Développement d'une technologie de biorémediation efficace pour l'élimination des composés monoaromatiques volatils des eaux contaminées

Farhadian Esfahani Mehrdad

N° d'ordre : D. U 1843
E D S P I C : 404

UNIVERSITE BLAISE PASCAL- CLERMONT II

ECOLE DOCTORALE
SCIENCES POUR L'INGENIEUR DE CLERMONT-FERRAND

T h è s e

Présentée par

MEHRDAD FARHADIAN ESFAHANI

pour obtenir le grade de

DOCTEUR D'UNIVERSITE

SPECIALITE : GENIE DES PROCEDES

Development of an effective bioremediation technology for volatile monoaromatics removal from contaminated water

Soutenue publiquement le 11 Juillet 2008 devant le jury :

M.	**Claude-Gilles DUSSAP**, Professeur, Université Blaise Pascal	Président
Mme	**Corinne CABASSUD**, Professeur, INSA Toulouse	Rapporteur et examinateur
M.	**Philippe JACQUES**, Professeur, Université Lille I	Rapporteur et examinateur
M.	**Ashok PANDEY,** Professeur, NIIST (CSIR), Trivandrum (Inde)	Examinateur
M.	**Julien TROQUET**, Directeur Biobasic Environnement	Examinateur
M.	**Christian LARROCHE,** Professeur, Université Blaise Pascal	Directeur de thèse

N° d'ordre : D. U 1843
E D S P I C : 404

UNIVERSITE BLAISE PASCAL- CLERMONT II

ECOLE DOCTORALE

SCIENCES POUR L'INGENIEUR DE CLERMONT-FERRAND

T h è s e

Présentée par

MEHRDAD FARHADIAN ESFAHANI

pour obtenir le grade de

DOCTEUR D'UNIVERSITE

SPECIALITE : GENIE DES PROCEDES

Développement d'une technologie de biorémediation efficace pour l'élimination des composés monoaromatiques volatils des eaux contaminées

Soutenue publiquement le 11 Juillet 2008 devant le jury :

M.	**Claude-Gilles DUSSAP**, Professeur, Université Blaise Pascal	Président
Mme	**Corinne CABASSUD**, Professeur, INSA Toulouse	Rapporteur et examinateur
M.	**Philippe JACQUES**, Professeur, Université Lille I	Rapporteur et examinateur
M.	**Ashok PANDEY,** Professeur, NIIST (CSIR), Trivandrum (Inde)	Examinateur
M.	**Julien TROQUET**, Directeur Biobasic Environnement	Examinateur
M.	**Christian LARROCHE,** Professeur, Université Blaise Pascal	Directeur de thèse

"Whatever we know is only a drop."

"What we don't know is an ocean."

Isaac Newton

(missy.reimer.com/wisdom.html)

Acknowledgments

I would like to express my sincere gratitude to my supervisor Professor Christian LARROCHE together with Mr. David DUCHEZ and Mr. Cédric VACHELARD. Without their constructive supervision and insight this work would never have been possible. I wish to appreciate their dedication, motivation, guidance, support, and encouragement throughout this research.

I would also like to give my sincere thanks to Prof. Mehdi BORGHEI (BBRC, Sharif University of Technology, Tehran, Iran) for fruitful discussions and Mr. Julien TROQUET (Biobasic Environnement, Clermont-Ferrand, France) for providing supports to work.

I would like to give my sincere thanks to the following people for their guidance, help and supports during the course of this work:

- Prof. Claude Gilles DUSSAP (LGCB, Université Blaise Pascal)
- Prof. Gholamreza DJELVEH (LGCB, Université Blaise Pascal)
- Dr. Geneviève GAUDET (Département de Génie Biologique,Université Blaise Pascal)
- Dr. Pierre FONTANILLE, Dr. Hélène de BAYNAST, Dr Laurent POUGEON (LGCB)
- Dr. Cédric DELATTRE (GreenTech *Naturally*, BIOPOLE St Beauzire)
- Prof. Philippe MICHAUD, Dr. Céline LAROCHE, Dr. Marrièle BRIAND (LGCB)
- Mme. Béatrice LOISEAU, Mme. Christine GARDARIN, Mme. Mariline THEVENIOT (LGCB, PolyTech 'Clermont-Fd, Université Blaise Pascal)
- Mr. Fréderic BRUN (ENSCCF, Clermont-Fd., Université Blaise Pascal)
- Mme. Sumitra RAMACHANDRAN, Mr. Juliano LEMOS BICAS, Mr. Denis LINARES, Mr. Wahid JATOI (Université Blaise Pascal)
- Dr. Hossein M.M. SADEGHI, Mr. A. Majid HAJIAN and my colleagues in Isfahan high education and research institute (IHEARI, Esfahan, IRAN)
- French embassy in Tehran and Institute for Energy and Hydro technology (Tehran, IRAN) for a thesis fellowship at Université Blaise Pascal (Clermont-Fd., France)

Throughout my academic endeavors, my family has been supportive. Best wishes and special thanks to them. Finally, I would like to extended my everlasting appreciation to my wife Mojgan and my sons Ali and Youssef whose love are manifested in so many ways, but particularly in their unstinting patience, understanding, encouragement and supports.

TABLE OF CONTENTS

List of Tables

List of Figures

Abbreviations:

B	Benzene
BOD	Biological oxygen demand
BTEX	Benzene Toluene Ethylbenzene Xylenes
BTX	Benzene Toluene Xylenes
COD	Chemical Oxygen Demand
DCM	Dichloromethane
DO	Dissolved Oxygen
E	Ethylbenzene
EC	Electrical Conductivity
EPA	Environmental Protection Agency
F/M	Food / Microorganism
FAS	Fixed film Activated Sludge
FBR	Fluidized Bed Reactor
GAC	Granular Activated Charcoal
GC/FID	Gas Chromatography / Flame Ionization Detector
GC/MS	Gas Chromatography / Mass Spectrometry
GC/PID	Gas Chromatography / Photo Ionization Detector
HAIB	Horizontal flow Anaerobic Immobilized Biomass
HD	Hexadecane
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time
LAS	Linear Alkylbenzene Sulfonate
MBBR	Moving Bed Biological Reactor
MTBE	Methyl *T*er-Butyl Ether
OLR	Organic Loading Rate
PAH	Poly Aromatic Hydrocarbon
PE	Polyethylene
ppb	part per billion
ppm	part per million
PVC	Poly Vinyl Chloride
PTFE	Polytetrafluroethylene
SBR	Sequencing batch reactor
SDE	Simultaneous distillation–extraction

SPME	Solid Phase Microextraction
STR	Stirred tank reactor
T	Toluene
TBA	*T*ert-Butyl Alcohol
TPPB	Two-phase partitioning bioreactor
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
USEPA	United State Environmental Protection Agency
UV	Ultra Violet
VOC	Volatile Organic Compounds
VVM	Volume of gas per volume of liquid per min.
X	Xylenes

Nomenclature:

C	The solute concentrations in the liquid (mol/L)
C_0	The initial solute concentration in the liquid (mol/L)
C_G	The solute concentrations in the gas (mol/L)
C_H	The hexadecane concentration in the liquid (mol/L)
C_S	The equilibrium concentrations of a solute in an solvent (mol/L)
C_W	Water concentration in the aqueous phase (mol/L)
G	The gas flow rate (mol/min)
K	The reaction rate constant ($time^{-1}$)
K and n	The Freundlish isotherm dimensionless constants (-)
m	The mass of the adsorbent (mg GAC)
M (O_2)	Molecular weight of oxygen (g/ mol)
P	The total pressure in the system (mmHg)
P^0	The solute vapor pressure (mmHg)
R	The universal gas constant (L.hPa mol^{-1} K^{-1} or L.mmHg mol^{-1} K^{-1})
T	The absolute temperature (K)
Tm	The measuring temperature (K)
V	The phase volume (mL)
V_G	The gas volume (mL)
V_L	The organic volume (L),
V_l	The liquid phase volume (mL)
V_{tot}	The bottle volume (mL)
x_{BTX}	The mass of the adsorbate (mg)
x	The solute mole fractions in the liquid (-)
y	The solute mole fractions in the gas (-)
Δp (O_2)	The difference in partial oxygen pressure (mbar)
α	The Bunsen absorption coefficient constant (-)
γ	The activity coefficient of the solute dissolved in the liquid (-)
γ^{∞}	The limiting activity coefficient of the solute (-)
γ_T^{∞}	The limiting activity coefficient of the toluene in hexadecane (-)
λ	Wavelength (nm)

Introduction

Important monoaromatic hydrocarbons that can be found at polluted sites of oil production facilities and industries are benzene, toluene, ethylbenzene and isomers xylenes (BTEX) (Kao et al. 2006). These organic compounds are toxic and contaminate water sources (An 2004). Water resources get polluted by monoaromatic compounds due to release of petrol, gasoline, diesel, petrochemical products from storage tanks and wastes from oil industries (Andreoni and Gianfreda 2007). These hydrocarbons have higher water solubility than other organic compounds that are present in gasoline such as aliphatics. Generally, solubility of benzene, toluene, ethyl benzene, xylenes and gasoline in water are respectively 18, 25, 3, 20, 50-100 ppm when gasoline is introduced into water (Kermanshahipour et al. 2005). Percent volume of benzene, toluene, ethylbenzene and xylenes in gasoline, are 1, 1.5, <1-1.5 and 8-10, respectively (An, 2004). Groundwater contaminated by toxic pollutant is a very serious problem because many communities in the world depend upon groundwater as sole or major source of drinking water. Maximum level for monoaromatic compounds in potable water are 0.05, 1, 0.7and 10 ppm for benzene, toluene, ethylbenzene and isomers of xylenes, respectively (USEPA 2006).

There are different methods for monoaromatic compounds removal from groundwater such as physical techniques (electro remediation, air sparging, carbon adsorption, filtration, adsorption by zeolites) (Daifullah and Girgis 2003, Ranck et al. 2005, Yang et al. 2005), chemical methods (chemical oxidation, photo catalysis remediation) (Tiburtius et al. 2005, Mascolo et al. 2007) and biological processes (bioremediation, biodegradation in reactors, phyto-remediation, wetland) (Rozkov et al. 1999, Langwaldt and Puhakka 2000, Vidali 2001, Lynch and Moffat 2005, Wallace and Kadlec 2005, Martínez et al. 2007) methods. These methods can be applied alone or with other systems mainly for polishing. All the above mentioned methods can be divided into in-situ and ex-situ (pump and treat) remediation technologies. In situ remediation is treatment of the contaminated material in place. Among all remediation technologies for treating xenobiotics or monoaromatic compounds from contaminated water, researchers reported that biological technique appears to be an efficient and economical process and environmental friendly approach (Vidali 2001, Dobson et al. 2004, Maliyekkal et al. 2004, Lynch and Moffat 2005).

In recent decades, researchers showed that bioremediation processes could play a major role in the treating of water polluted by monoaromatic compounds. But generally some challenge related to the possibility of pollutants loss through volatilization and stripping, and

also ability of these hydrocarbons to adsorb onto media is not addressed properly in literature reviews. For example, using of conventional aerobic biological treatment processes to treat contaminated waters by monoaromatic compounds, as is commonly practiced in industries, is debatable since the volatile nature of these compounds can lead to stripping of these compounds from the aqueous phase into the atmosphere.

The work presented in this document corresponds to data and knowledge needed for the design of a biofilter-type reactor used in the biological degradation of BTEX compounds. The basic idea is that contaminants present in water will be stripped by air and adsorbed on a support such as activated carbon with biomass colonization. This last feature means that other possible support treatments, such as calcination, will not be considered here.

Main problems addressed are:
- Building of an accurate analytical protocol for BTEX quantitative measurement in an aqueous phase. This protocol needs to avoid any solute loss during handling a liquid sample.
- Characterization of phase transfer phenomena, mainly those involving the gas phase.
- Studies on the behaviour of both aerobic and anaerobic biomass for BTEX biodegradation. This part is carried out with two main objectives, which are i) maximization of reaction rates, ii) minimization of compound losses in the gas.

The document is made of five chapters. The first is a comprehensive review on literature data available on monoaromatic compounds bioremediation, while the second describes the methods used for experiments performance. The three last chapters report results obtained during this work. Chapter three deals with the method that has to be used to properly handle an aqueous sample of monoaromatic compounds. Chapter four give results on solute phase transfers, while chapter five gives experimental results obtained during aerobic and anaerobic bioremediation tests.

Chapter I

Literature survey

I.1 Introduction

I.1.1. Outcome

Benzene, toluene, ethylbenzene and mixture of xylenes (BTEX) are monoaromatic hydrocarbon compounds. Water contamination by monoaromatic compounds is a very serious problem as these compounds are toxic and often classified as carcinogen for human (Pohl et al., 2003; An, 2004; Reineke et al., 2006; Paixão et al., 2007). Important sources of water contamination by monoaromatics are due to industrial waste, leakage, spills, improper disposal and accidents during transportation in oil industries (oil refinery, petrochemical companies). Release of petroleum products from storage tanks (gasoline, diesel fuel, lubricating oil), gas work sites, airports, paint manufactures, chemical industries (pesticides, plastics, synthetic fibers) and railway yards are also sources of contamination (Vidali, 2001; Andreoni and Gianfreda, 2007).

These organic chemicals make up a significant percentage (about 10 to 59 % by weight) of gasoline (Vieira et al., 2007). Monoaromatic compounds are highly soluble when compared to the other hydrocarbons present in gasoline, such as aliphatic. BTEX compounds may comprise mote than 50-60% of the mass that goes into solution when gasoline is mixed with water (Kermanshahi pour et al., 2005). Monoaromatic components are mobile and present in contaminated water as they are not strongly adsorbed by soil (Zytner, 1994; Langwaldt and Puhakka, 2000).

There are different methods for monoaromatic removal from water contaminated such as physical, chemical and biological treatment techniques. These methods can be further subdivided into ex-situ and in-situ remediation technologies. Table I.a provides a summary of several remediation technologies for organic compounds removal. Ex-situ remediation is also referred to as “pump and treat” method. In this technique, the underground contaminated water is pumped to the surface using a series of extraction wells, treated and then reused or reinjected.

Table I.a: Summary of remediation methods for waters contaminated by oil (Holliger et al., 1997; Nyer Evan, 1998; Daifullah and Girgis, 2003; Khan et al., 2004; Arvin et al. 2005; Tiburtius et al., 2005; Ranck et al., 2005, Wallace and Kadlec, 2005; Farhadian et al., 2006)

Category	In-situ technologies	Ex-situ technologies
Physical	Electro remediation Capping Barrier Hydraulic containment Air sparging	Electro remediation (electro dialysis) Air stripping Surfactant-modified zeolite Carbon adsorption Filtration (membranes)
Chemical	Injection of chemical oxidation compounds (O_3, O_2, H_2O_2, Cl_2)	Photo Catalysis remediation (Nano particle TiO_2/UV) Coagulation, flocculation and precipitation Application of chemical oxidation compounds (O_3, O_2, H_2O_2, Cl_2)
Biological	Phyto remediation In-situ Bioremediation (Natural and engineered bioremediation)	Ex-situ bioremediation (Aerobic and anaerobic bioreactors)

In-situ remediation is the treatment of the pollutant in place. Physical methods such as air stripping without air emission control represent the most common approach for volatile organic compounds (VOC) removal including BTEX , but is not a green technology as monoaromatic hydrocarbons transfer from liquid to gas phase (Shah et al., 2004). Also, aromatic adsorption by granular activated carbon (GAC) is technically feasible for water purification, but replacement and disposal of activated carbon as hazardous waste is a major expense in GAC adsorption water treatment systems (Daifullah and Girgis, 2003; Shih et al., 2003; Ayotamuno et al., 2006; Yin et al., 2007). Also ultrafiltration, nanofiltration and reverse osmosis are excellent post-treatment processes, requiring adequate pretreatment for cost reduction. Chemical methods include coagulation, flocculation, precipitation and oxidation (either chemical or photochemical) (Tiburtius et al., 2005). However, most of these processes are poorly efficient for aromatic compounds while chemical oxidation alone or photooxidation are not cost-effective and may even give undesirable residuals. Among the

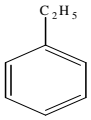
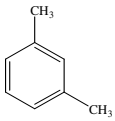
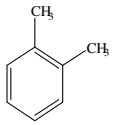
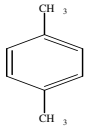
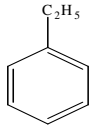
various remediation technologies available for treating monoaromatic contaminated water, biological methods or bioremediation processes appear to be a potentially economical, energy efficient and environmentally sound approach (Vidali, 2001; Shim et al., 2002).

I.1.2 Properties of monoaromatic compounds

Petroleum derivatives such as benzene, toluene, ethyl benzene and mixed xylenes (BTEX) are classified into the group of most dangerous compounds to the environment because of their large migration abilities and toxicity (Coates et al., 2002; An, 2004). Generally, percent volume of benzene, toluene, ethyl benzene and mixed xylenes in gasoline, are 1, 1.5, <1-1.5 and 8-10, respectively (An, 2004; Azev et al., 2004). Then, the presence of light aromatic hydrocarbons in water is an indicator of the presence of oil products. The physical and chemical properties of monoaromatic compounds are important characteristics which help in predicting the fate of these chemicals in the environment, they are summarized in Table I.b (Plyasunov and Shock, 2000; Lide 2002-CRC Handbook; Kermanshahi pour et al., 2005; Farhadian et al., 2006). The relatively high water solubility of monoaromatic hydrocarbons demonstrates their great tendancy to spread in contaminated water (Lipson and Siegel, 2000).

It should be noticed that maximum levels of monoaromatic compounds allowed in the United States for drinking water are 0.005, 1, 0.7 and 10 ppm for benzene, toluene, ethyl benzene and mixed xylenes, respectively (USEPA, 2006). Generally, high vapor pressure and low molecular weight mean that the compound is more likely to volatilize out of water solution (Lee et al., 2004). Monoaromatic compounds define as volatile organic compounds (VOCs) because vapor pressure these hydrocarbons greater than 0.1 mmHg at 20°C (Muñoz et al., 2007). Henry's law constant helps to predict the behavior of pollutants in remediation procedures such as air stripping processes. As data show, ethylbenzene> xylenes> toluene> benzene can diffuse and volatilize through air stripping from water in this order (Langwaldt and Puhakka, 2000). Also, the decimal logarithm of octanol-water partition coefficients is consistent with the already mentioned fact that monoaromatic pollutants are hydrophilic, soluble in water and that they can be easily removed from water by evaporation and stripping (Zytner, 1994; Lipson and Siegel, 2000; Plyasunov and Shock, 2000; Lee et al., 2004; Lin and Chou, 2006).

Table I.b: Monoaromatics characteristics and properties

Properties(unit)	Benzene	Toluene	m-Xylene	o-Xylene	p-Xylene	Ethylbenzene
Chemical Structure						
Chemical Formula	C_6H_6	C_7H_8	C_8H_{10}	C_8H_{10}	C_8H_{10}	C_8H_{10}
Molecular Weight (g/mol)	78.11	92.14	106.17	106.17	106.17	106.17
Some of Trade Name and Synonyms	Benzol90 Pyrobenzol Coalnaphtha Phene	Phenylbenzene Methylbenzene Methacide Toluol	m-Xylol metaxylene 1,3dimethyl - benzene	o-Xylol orthoxylene 1,2dimethyl - benzene	p-Xylol paraxylene 1,4dimethyl - benzene	Ethylbenzol Phenyl – ethane EB
Some of applications	Solvent in chemical industry, LAB, fuel	Solvent, TNT, Urethane, Benzene, fuel	Solvent, p- and o-xylene, fuel	Solvent, PA, fuel, Plasticizers	Solvent, DMT, Polyester fiber, fuel	Styrene, Solvent, fuel
Water Solubility (mg/lit)@ 25°C	1785.5	532.6	161.5	171.5	181.6	161.5
Boiling point Temp. (°C)	80.0	110.6	139.1	144.5	138.3	136.1
Vapor Pressure (mmHg)@ 20°C	95.19	28.4	8.3	6.6	6.15	4.53
Melting point Temp. (°C)	5.50	-94.9	-47.8	-25.2	13.2	-94.9
Specific density @°C	0.8765^{20}	0.8669^{20}	0.8642^{20}	0.8802^{10}	0.8611^{20}	0.8670^{20}
Octanol-Water Partition Coeff. 25 °C (log *P*)	2.13	2.73	3.20	3.12	3.15	3.15
Henry's Law Constant@ 25°C (kPa.m^3/mole)	0.557	0.660	0.730	0.551	0.690	0.843
Solubility of BTEX in water through(as ppm) Gasoline Diesel fuel	 18 7.89	 25 22.8	 20 13.95	 20 13.95	 20 13.95	 3 -
For 1 mg of substance in water : ThOD TOC (as ppm)	3.076 0.923	3.13 0.913	3.17 0.906	3.17 0.906	3.17 0.906	3.17 0.906

Other parameters often used to characterize aqueous solutions of these compounds are theoretical oxygen demand (ThOD), which corresponds to the stochiometric amount of oxygen required to fully oxidize a given molecule and total organic carbon (TOC), which is the total amount of organic carbon in an aqueous sample.

I.1.3. Quantitative determination of monoaromatic compounds

There are several methods that can be used to determine the content of monoaromatic hydrocarbons in a water sample. Techniques such as gas chromatography (GC) and liquid chromatography (LC) can provide quantitative, constituent-specific analysis of volatile hydrocarbons. In particular, gas and liquid chromatography can resolve key constituents for evaluating risk and determining corrective action criteria. Several reports have shown that preferred methods for determination of monoaromatic in water are gas chromatography (GC)/flame ionization detector (FID) (Wang et al., 2002; Pruden et al., 2003; de Nardi et al., 2005, 2006), GC/photo ionization detector (PID) (Cunningham et al., 2001; Dórea et al., 2007), GC/mass spectrometry (MS) (USEPA standard method, 8260B; Disdier et al., 1999; Guerin, 2002; Wang et al., 2002).

The determination of monoaromatic compounds in water samples at the μg per liter level requires the preconcentration of aromatics before analysis if a GC technique has to be used (Demeestere et al., 2007). These preconcentration techniques can be classified into three families: solute concentration in a gas phase, a liquid one, or on a solid. The first approach makes use of techniques such as headspace analysis or the purge and trap process. Both are based on vaporization of solutes from liquid water samples, the first involves direct analysis of the resulting gas phase, while the second allows analyte adsorption onto a porous support followed by desorption prior to GC injection. These techniques are recommended methods for volatile organic compounds analysis in water contaminated as they are rapid, cost efficient and can be automated (Kuran and Sojak, 1996; Ohlen et al., 2005; Gusmão et al., 2006, 2007).

Also, GC/MS, GC/FID, GC/PID through purge and trap for detection and determination of all forms of monoaromatic compounds in contaminated water are recommended by standard methods (USEPA standard method, 8260B; USEPA standard method, 5030C; USEPA standard method, 5030B; Environment Canada, 2005; Rosell et al., 2003; Rosell et al., 2005; Zein et al., 2006).

The second way to preconcentrate volatile compounds from water samples is solvent extraction. The potential disadvantages of solvent extraction are possible solvent peak overlapping with some analytes and, at low concentration levels, the need for highly purified material. Numerous studies on detection and determination of BTX compounds in water by GC/MS indicate that sample preparation through extraction with solvents such as dichloromethane (DCM), pentane, hexane, octane, n-C_9H_{20}, n-$C_{10}H_{22}$ and n-$C_{11}H_{24}$ give bad results, while accurate detection of BTX compounds extracted with n-$C_{12}H_{26}$ and n-$C_{16}H_{34}$ is possible (Farhadian et al., 2006). Recently, solid phase microextraction (SPME) is developed as the third approach. Some researchers claim that it should be the preferred method for monoaromatic analysis in water samples. This technique is a solvent free sample preparation process and combines sampling, isolation and enrichment in one step. In this procedure, a small diameter optical fibre coated with polymeric phase is placed in an aqueous sample. The analytes partition into the stationary phase and are then thermally desorbed, on column, in the injector of a gas chromatograph (Theodoridis et al., 2000). The main difficulty here is to gain an accurate quantitative knowledge of partition phenomena between the adsorbent and the liquid phase (Eisert and Levsen, 1996; Djozan et al., 2004; Ji et al., 2006; Ouyang and Pawliszyn, 2006). General methods used in this area are based on treatment of standard solutions. However it is widely accepted that adsorption characteristics depend on the composition of the liquid medium, which can give rise to potentially high errors during solute quantitation.

As already stated, aromatic compounds can also be detected by high performance liquid chromatography (HPLC) fitted with an UV detector (Yadav and Reddy, 1993; Kelly et al., 1996; Zepeda et al., 2006; Vogt et al., 2000; Kim et al., 2006). These methods can measure constituent concentrations in the part per billion (ppb) ranges, the lower detection limit being between 1 and 500 micrograms per liter (μg/L), depending on the method and equipment. It should be emphasized that present columns that are available for HPLC (see Table I.c) are not able separate all forms of monoaromatic hydrocarbons, since ethybenzene and xylene isomers have usually similar retention times. A summary of the various techniques available for the detection of monoaromatic hydrocarbons in water and typical conditions used is given in Table I.c.

Table I.c: Summary of important methods used for monoaromatic hydrocarbon analysis from contaminated water

Test method	Classification (Standard methods)	Sample preparation (Standard methods)	Typical conditions (Reference)	Recommended analytical methods, detection limit and time for analysis	Remark (s)
Gas chromatography (GC)	FID PID MS (EPA 8260B)	Purge and trap- (EPA 5030C, EPA5030 B) Head space SPME Solvent extraction	Injector: purge and trap (O.I.A. 4560A) , Trap: BTEX (Supelco) at 50 °C during purge, column: 30m length × 0.45 mm, I.D. 2.25 µm , Carrier gas: He at 109 cm/sec, Oven program: 40°C for 2 min, 40-200°C at 12°/min, 200°C for 5 min, Detector A: PID (OIA 4430), 10 eV lamp, 200°C, Detector B: FID, 250°C (Agilent, 2007).	Purge and Trap through GC/FID or GC/PID or GC/MS, Minimum detection limit: 1 µg/L, time analysis: 10-20 min depending to instrument, column and oven program	-In general, benzene, toluene, ethylbenzene and all form of xylenes can be detected and analysed in water pollutant through purge and trap, head space, SPME. - Samples may require dilution to prevent exceeding maximum range of detector.
Liquid chromatography (LC)	HPLC-UV detector	Directly- no filtration	BTX analysis: Column C18, solvent water/methanol (40/60 volume %), flow rate 1 mL/min, λ= 254 nm, injection loop 50 or 100 µL (Kelly et al. 1996) Toluene analysis: Column RP-18, eluant methanol-0.1% phosphoric acid (60:40), UV detector set 208 nm (Yadav and Reddy, 1993)	HPLC-UV vis detector, Minimum detection limit: Benzene 30 µg/L , toluene 31 µg/L, xylenes 50 µg/L Time analysis<20 min (Kelly et al., 1996)	- Generally, water contaminated sample can to inject in instrument, directly. - Ethybenzene and xylenes isomers have similar retention time and normally they can not be separated, together by this method.

Water samples are usually collected from water sources with decontaminated containers such as disposable bottles. Sterile disposable gloves and other clean sterilized sampling equipment should be used. Also, standard key points about sampling, preservation, handling and analysis of monoaromatic in contaminated water have been defined such as (Kuran and Sojak, 1996; USEPA standard method, 8260B; USEPA, 1997; Environment Canada, 2005):

1- Amber glass bottle with teflon lined cap and volume 40 mL is recommended as container.

2- All samples should be collected in duplicates.

3- Sample jars and volatile organic analysis vials should be filled to the top so that no air space (headspace) is present.

4- Samples should be kept chilled at 4°C until analyzed in the laboratory.

5- Samples for analysis should not be filtered

6- The water samples should be transported and analyzed within 1 day if no stabilizer is added.

7- Sodium azide (1 g/L) as a stabilizer and microbial inhibitor can be used for aqueous samples.

8- Hydrochloric acid is also commonly used as a preservative to decrease the pH to about 2.

Other methods such as chemical extraction (benzylsuccinate, trimethylbenzene, catechol 2, 3 dioxygenase), physical methods (depletion of dissolved oxygen, nitrate and sulfate or production of dissolved ferrous iron, sulfide and carbon dioxide), biological (bioassay tools) or numerical, physical and kinetic models can be used for on-line monitoring of monoaromatics degradation during the course of in situ bioremediation (Nadim et al., 2000; Lin et al., 2002; Reusser et al., 2002; Beller, 2002; Johnson et al., 2003; Hua et al., 2003; Schulze and Tiehm, 2004; Kuster et al., 2004; Dobson et al., 2004; Maurer and Rittmann, 2004; Mesarch et al., 2004; Bekins et al., 2005; Gödeke et al., 2006; Hendrickx et al., 2006; Hu et al., 2006; Huang et al., 2006; Kao et al., 2006; Atteia and Guillot, 2007; Biggerstaff et al., 2007; Morasch et al., 2007).

I.2. Bioremediation of monoaromatic compounds

I.2.1. Introduction

Bioremediation is defined as the elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes (Lynch and Moffat, 2005). Bioremediation technologies use microorganisms to treat contaminants by degrading

organic compounds to less toxic material, such as CO_2, methane, water and inorganic salts (Iwamoto and Nasu, 2001). These technologies include intrinsic or enhanced bioremediation and can be performed in-situ or ex-situ under aerobic or anaerobic conditions. Classification of bioremediation options and important factors affecting on water bioremediation processes are shown in Figures I.1 and I.2 (Kampbell et al., 1996; Boopathy, 2000; Schreiber and Bahr, 2002; Bittkau et al., 2004; Farhadian et al., 2006; Atteia and Guillot, 2007).

I.2.2. Microbiology, metabolism and biodegradability

Microorganisms such as bacteria, fungi and microalgae play a key role in monoaromatic removal through in-situ bioremediation processes (Holliger et al., 1997; Semple et al., 1999; Jindrova et al., 2002; Schulze and Tiehm, 2004; Nikolova and Nenov, 2005). Monoaromatic pollutants act as carbon source for microorganisms. Also, they require macro nutrients (nitrogen and phosphorus), micro nutrients (Ca^{+2}, Mg^{+2}, Na^{+}, K^{+}, S^{-2}, co-factors such as heavy metals), electron acceptor (oxygen is the electron acceptor for aerobic metabolism and nitrate, sulfate, ferric, manganese and carbon dioxide in anaerobic processes) and optimum environmental conditions for growth (temperature, pH, salinity, presence of inhibitors and of a nitogen source) (Holliger et al., 1997; Langenhoff et al., 1997 ; Mandelbaum et al., 1997; Fiorenza and Ward, 1997; Lovley, 1997; Salanitro et al., 1997; Field, 2002; Lin et al., 2002 ; Villatoro-Monzon et al., 2003; Van Hamme et al., 2003; Schulze and Tiehm, 2004; Chakraborty and Coates, 2004; Jahn et al., 2005; Dou et al., 2007). Therefore, the rate of bioremediation of fuel contaminants such as monoaromatic hydrocarbons can be enhanced by increasing the concentration of electron acceptors and nutrients in groundwater.

In aerobic respirometry after degradation of light aromatic hydrocarbons, microorganisms produce carbon dioxide, water, sludge, etc. In anaerobic bioremediation, end products are compounds such as methane, CO_2, mineral salts. Biomass has also to be taken into account even if, as already stated, its production remains usually quite low. The overall reactions for benzene, toluene, ethylbenzene and xylenes isomers biodegradation stoechiometries in aerobic and anaerobic conditions are given in Table I.d (Langwaldt and Puhakka, 2000; Cunningham et al., 2001; Villatoro-Monzon et al., 2003; Roychoudhury and Merrett, 2006). The electron transfers which occur during biochemical reactions release energy which is further utilized for growth and cell maintenance.

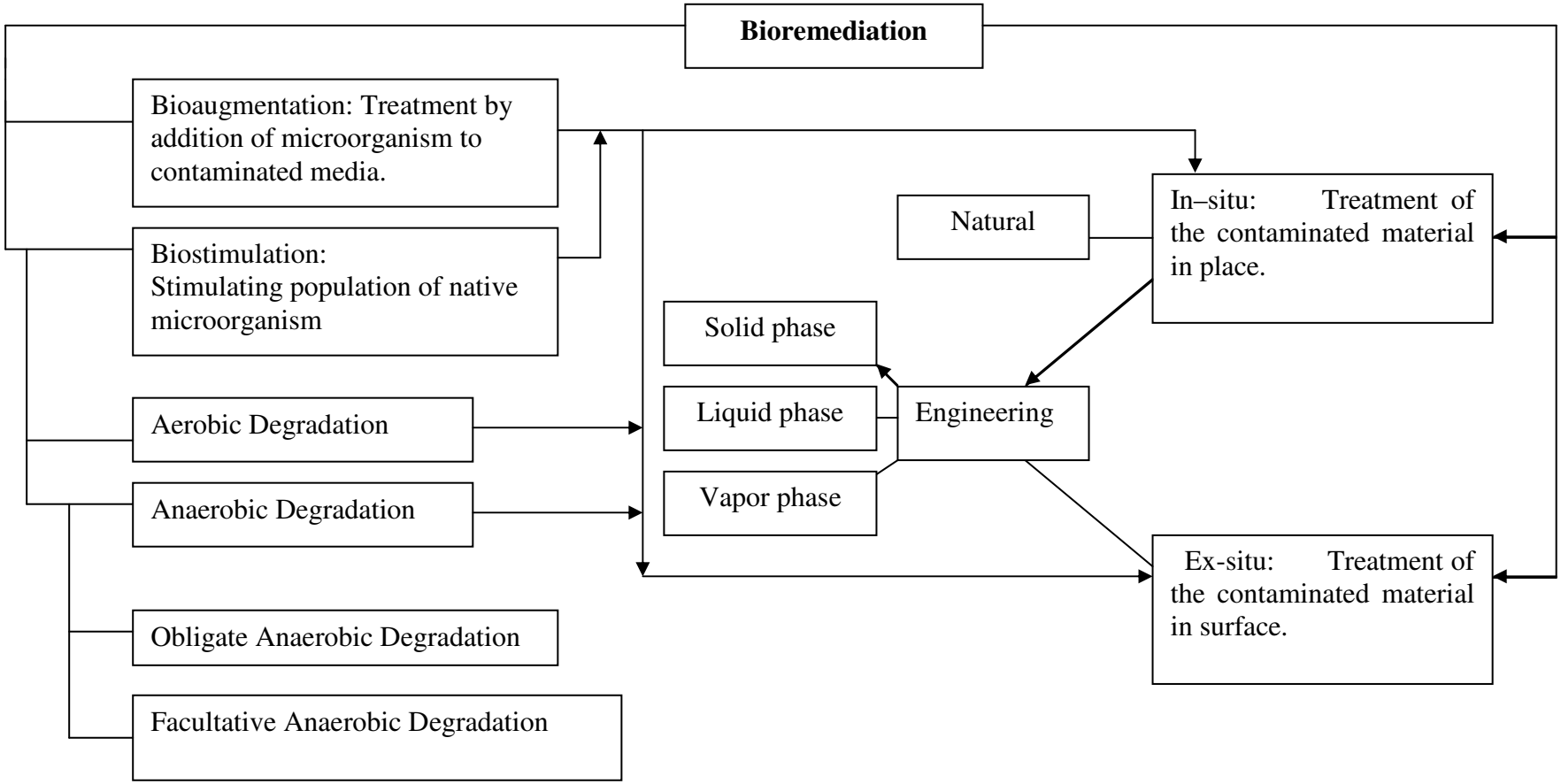


Figure I.1: Classification of bioremediation options

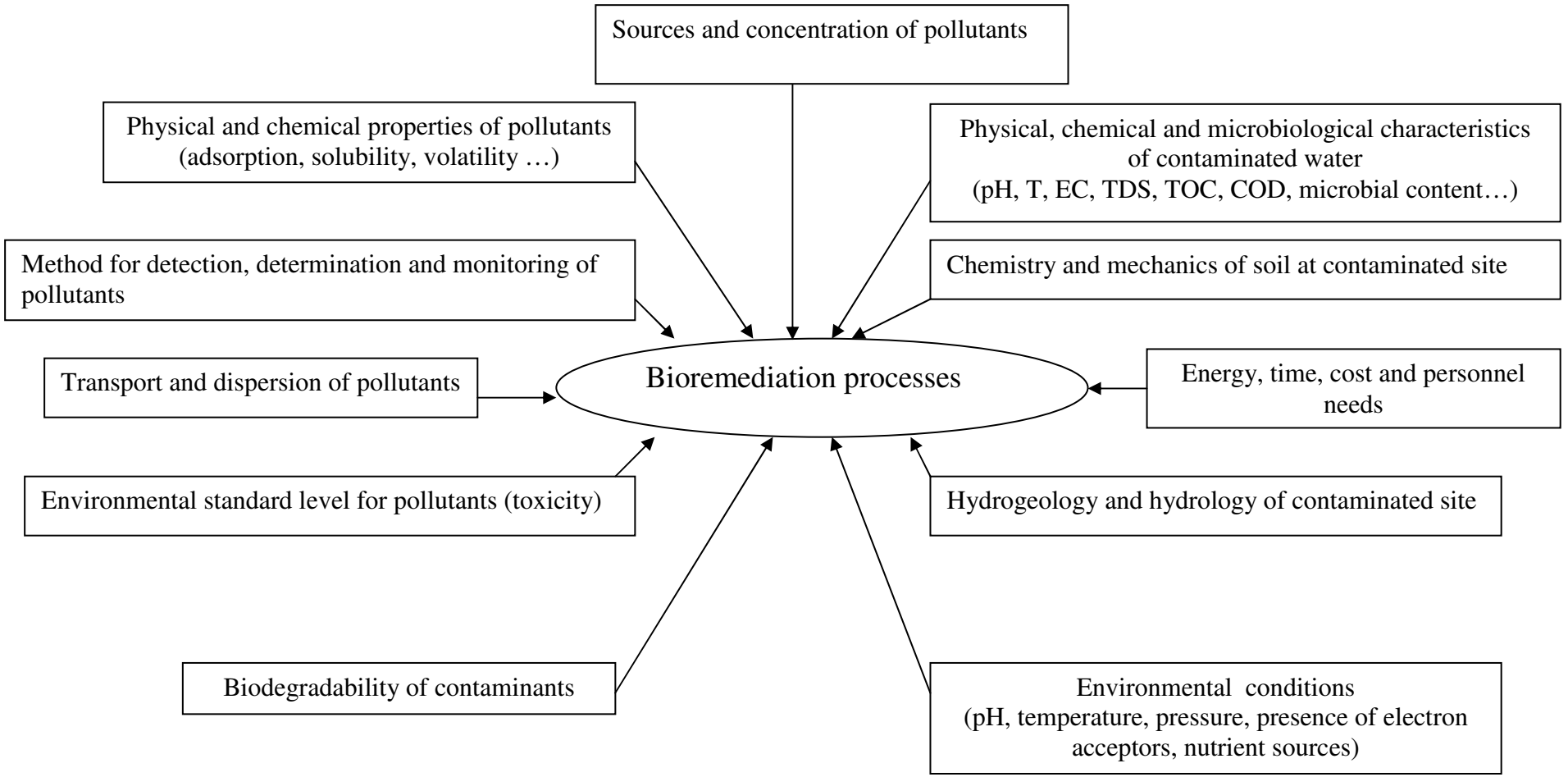


Figure I.2: Important factors that have to be taken into account to establish a water bioremediation process

Table I.d: Monoaromatic degradation stochiometry

Compound	Overall reaction
Benzene	$C_6H_6 + 7.5O_2 \rightarrow 6CO_2 + 3H_2O$
	$C_6H_6 + 6NO_3^- + 6H^+ \rightarrow 6CO_2 + 6H_2O + 3N_2$
	$C_6H_6 + 15\ Mn^{4+} + 12H_2O \rightarrow 6CO_2 + 30H^+ + 15Mn^{2+}$
	$C_6H_6 + 30Fe3^+ + 12H_2O \rightarrow 6CO_2 + 30H^+ + 30Fe^{2+}$
	$C_6H_6 + 3.75\ SO_4^{2-} + 7.5H^+ \rightarrow 6CO_2 + 3.75H_2S + 3H_2O$
	$C_6H_6 + 4.5\ H_2O \rightarrow 2.25\ CO_2 + 3.75CH_4$
Toluene	$C_7H_8 + 9O_2 \rightarrow 7CO_2 + 4H_2O$
	$C_7H_8 + 7.2NO_3^- + 7.2H^+ \rightarrow 7CO_2 + 7.6H_2O + 3.6N_2$
	$C_7H_8 + 18\ Mn^{4+} + 14H_2O \rightarrow 7CO_2 + 36H^+ + 18Mn^{2+}$
	$C_7H_8 + 36Fe3^+ + 14H_2O \rightarrow 7CO_2 + 36H^+ + 36Fe^{2+}$
	$C_7H_8 + 4.5\ SO_4^{2-} + 9H^+ \rightarrow 7CO_2 + 4.5H_2S + 4H_2O$
	$C_7H_8 + 5\ H_2O \rightarrow 2.5\ CO_2 + 4.5CH_4$
Ethylbenzene and isomer Xylenes	$C_8H_{10} + 10.5O_2 \rightarrow 8CO_2 + 5H_2O$
	$C_8H_{10} + 8.4NO_3^- + 8.4H^+ \rightarrow 8CO_2 + 9.2H_2O + 4.2N_2$
	$C_8H_{10} + 21\ Mn^{4+} + 16H_2O \rightarrow 8CO_2 + 42H^+ + 21Mn^{2+}$
	$C_8H_{10} + 42Fe3^+ + 16H_2O \rightarrow 8CO_2 + 42H^+ + 42Fe^{2+}$
	$C_8H_{10} + 5.25\ SO_4^{2-} + 10.5H^+ \rightarrow 8CO_2 + 5.25H_2S + 5H_2O$
	$C_8H_{10} + 5.5\ H_2O \rightarrow 2.75\ CO_2 + 5.25CH_4$

Maximum concentration of electron acceptor compounds that can be added to contaminated groundwater, for oxygen, hydrogen peroxide, nitrate, sulfate and iron are 9-10, 100-200, 80-100, 100-250 and 1 as mg/L, respectively. These values are due to practical limitation, aqueous solubility, drinking water standards and microbial activities (Cunningham et al. 2001).

The average elemental composition of microbial cells on a dry weight basis is given in Table I.e while common organisms which are recognized for their ability to metabolize monoaromatic compounds are shown in Table I.f.

Table I.e: Average elemental composition of microbial cells (Suthersan, 1996)

Element	Percentage of dry weight	Element	Percentage of dry weight
Carbon	50	Sodium	1
Oxygen	20	Calcium	0.5
Nitrogen	14	Magnesium	0.5
Hydrogen	8	Chlorine	0.5
Phosphorus	3	Iron	0.2
Sulfur	1	All others	0.3
Potassium	1	-	-

Table I.f: Microorganisms involved in the degradation of monoaromatic pollutants

Organism	source of pollutant	Reference(s)
Rhodococcus rhodochrous	BTEX	Deeb and Alvarez-Cohen (1999)
Pseudomonas sp. ATCC 55595	BT (p-) X	Collins and Daugulis (1999)
Pseudomonas putida *Pseudomonas fluorescens*	BTE(o-)X	Shim and Yang (1999)
Sphingomonas aromaticivorans strain F199	T , naphthalene	Romine et al. (1999)
Rhodococcus sp. RR1 and RR2	BTE (m-/p-) X	Deeb and Alvarez-Cohen (2000)
Pseudomonas putida F1	BTE, TCE	Parales et al. (2000)
Ralstonia picketii PKO1	T	
Burkholderia cepacia G4	T	
Klebsiella sp.	B	Yeom et al. (2000)
Pseudomonas putida	BTEX	Attaway and Schmidt (2002)
Rhodococcus sp. Strain DK17	BTE (o-) X	Kim et al. (2002)
Pseudomonas putida strain mt-2	T (m-/p-) X	Morasch et al. 2002
Ralstonia pickettii strain PKO1	T	
Cladophialophora sp. strain T1	BTEX	Prenafeta- Boldú et al. (2002 and 2004)
Blastochloris sulfoviridis ToP1	T	Van Hamme et al. (2003)
Denitrifying bacteria		
Azoarcus sp. strain EB1	E	
Azoarcus sp. strain T	T (*m*-)X	
Azoarcus tolulyticus Td15	T (*m*-) X	
Azoarcus tolulyticus To14	T	
Dechloromonas sp. strain JJ	BT	
Dechloromonas sp. strain RCB	BT	
Thauera aromatica K172	T	
Thauera aromatica T1	T	
Fe (III)-reducing bacteria		
Geobacter grbiciae TACP-2T	T	
Geobacter grbiciae TACP-5	T	
Geobacter metallireducens GS15	T	
Sulfate-reducing bacteria		
Desulfobacula toluolica To12	T	
Desulfobacterium cetonicum	T	

Table I.f (cont'nd): Microorganisms involved in the degradation of monoaromatic pollutants

Organism	source of pollutant	Reference(s)
Pseudomonas putida F1	BT, phenol	Abu Hamed et al. (2003, 2004) Abuhamed et al. (2004)
Pseudomonas putida *Pseudomonas fluorescens*	BTEX	Shim et al. (2003 and 2005)
Pseudomonas putida PaW1 *Pseudomonas putida* F1	Aromatic compounds and choloroaliphatics	Leahy et al. (2003)
Pseudomonas aeruginosa	B	Kim et al. (2003)
Rhodococcus pyridinovorans PYJ-1	BT (m-) X	Jung and Park (2004)
Pseudomonas aeruginosa KCCM-40269 *Pseudomonas fluorescens* KCCM-11362 *Pseudomonas putida* KCTC-1769	B B B	Kim et al. (2005) and Mahendran et al. (2006)
Achromobacter xylosoxidans	BTEX	Nielsen et al. (2006)
Geobacteraceae	BTX	Botton et al. (2007)
Thauera aromatica K172 *Geobacter sulfurreducens* *Desulfococcus multivorans*	TEX, chlorinated phenols and aliphatic alcohols	Duldhardt et al. (2007)
Pseudomonas putida F1 Thauera *aromatica* T1 *Ralstonia pickettii* PKO1	T T T	Kim and Jaffé (2007)
Pseudomonas fluorescens-CS2	E	Parameswarappa et al. (2008)
Pseudomonas putida F1	T	Díaz et al. (2008)

Studies on metabolic pathways for BTEX removal in aerobic conditions have indicated that each of these compounds can be degraded through at least one pathway leading to a substituted catechol (Andreoni and Gianfreda 2007). For example, benzene is degraded to catechol (Tsao et al. 1998, Johnson et al. 2003) while toluene and ethylbenzene are degraded via several separate pathways leading to the production of 3-methylcatechol and 3-ethylcatechol, respectively. The xylenes are metabolized to mono-methylated catechols (Jindrova et al. 2002, Stephens 2006). Aerobic pathway for monoaromatic degradation is shown in Figure I.3.

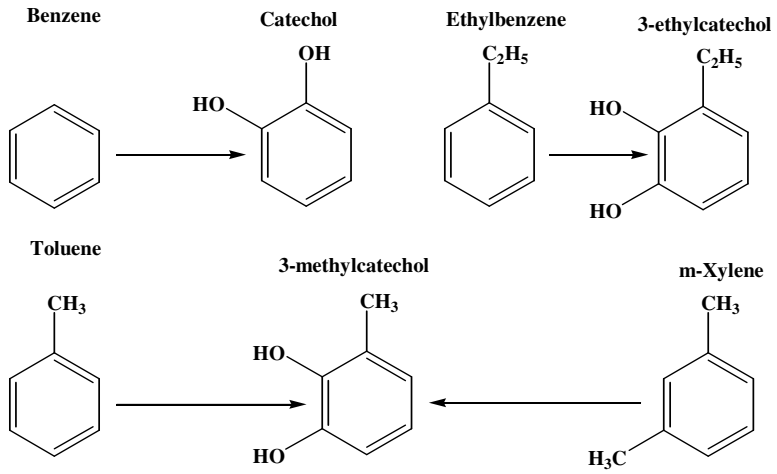


Figure I.3: Aerobic pathway for monoaromatics degradation (Stephens 2006)

A mixed culture derived from gasoline-contaminated aquifer has been shown to degrade all BTEX compounds into CO2 (Deeb and Alvarez-Cohen 2000). Also, some enzymes involved in aerobic metabolism, such as catechol 2, 3- dioxygenase, are used for monitoring BTX bioremediation (Mesarch et al. 2004).

Metabolism of monoaromatic degradation in anaerobic condition, are shown in Figure I.4. Degradation of benzene in anaerobic conditions by mixed populations have been investigated (Coates et al. 2002). Details of the biochemical pathways for toluene and ethylbenzene for anaerobic biodegradation are known (Heider et al. 1999, Heider 2007). Chakraborty and Coates 2004, have shown that for toluene, ethylbenzene and xylene isomers (ortho and meta), it exists a common intermediate metabolite, which is benzoyl-CoA. This compound appears to be the most common central intermediate for anaerobic breakdown of aromatic compounds (Boll et al. 2002, Boll 2005, Zhang and Bennett 2005). Benzoyl-CoA is further reduced and can be converted into acetyl-CoA, finally giving carbon dioxide. It must be emphasized that the pathways for para xylene metabolization under anaerobic conditions are not completely elucidated (Lin et al. 2002, Chakraborty and Coates 2004, Stephens 2006). In most cases, electron balances show a complete anaerobic oxidation of these aromatic compounds to CO2 (Jahn et al. 2005). Some intermediates compounds such as benzylsuccinate and methylbenzylsuccinic acid isomers have been proposed as distinctive indicators for the monitoring of anaerobic monoaromatic biodegradation in fuel contaminated aquifers (Reusser et al. 2002, Beller 2002, Zhang and Bennett 2005, Gödeke et al. 2006).

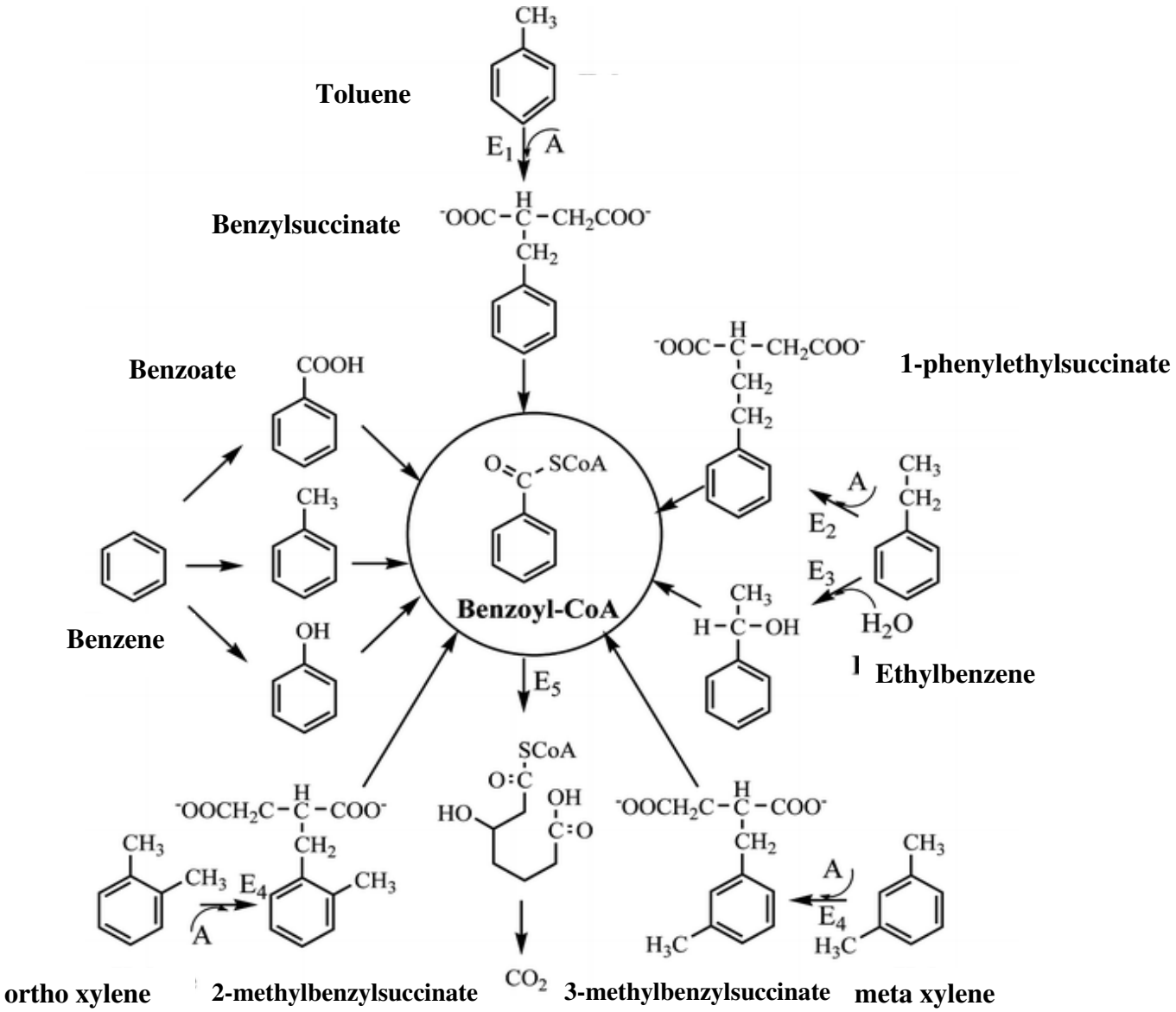


Figure I.4: Anaerobic pathway for monoaromatic degradation (Boll et al. 2002; Chakraborty and Coates, 2004; Zhang and Bennet, 2005)

Also, pathway of anaerobic toluene catabolism by denitrifying bacterium *Thauera aromatica* is good established and some intermediate products such as succinate, benzylsuccinate, furmate, citrate and benzoyl- CoA has been detected during biodegradation (Lochmeyer et al. 1992, Seyfried et al. 1994, Boll 2005, Duldhardt et al. 2007). This strain is readily oxidizes toluene anaerobically to carbon dioxide (Boll et al. 2002).

Biodegradation kinetics parameters for monoaromatic removal are commonly obtained from cultivation parameters (substrate residual concentration,…) in batch or continuous conditions and fitting the data with the well known Monod equation (Kelly et al. 1996, Bekins

et al. 1998, Bielefeldt and Stensel 1999, Lovanh et al. 2002, Okpokwasili and Nweke 2005, Zepeda et al. 2006). Kelly et al. 1996 reported that substrate disappearance in discontinuous operations were 1.32, 1.42 and 0.833, as mmol / L. h for benzene, toluene and xylene, respectively. Also, maximum growth specific rate (μmax) value for biomass degrading monoaromatic compounds has been reported to be in the range of 0.046-0.383 h^{-1}. Many kinetic studies, giving parameters for BTX biodegradation in aerobic batch and column systems have been reported. Experimental data given by Bielefeldt and Stensel (1999) show that the kinetic coefficient values for the individual BTEX compounds are affected by the operating solids retention time (SRT) in the reactor and the combination of growth substrates.

Review studies by Suarez and Rifai (1999) indicate that the rate of biodegradation of fuel hydrocarbons follows first order kinetics with rate constants up to 0.445 day^{-1} under aerobic conditions and up to 0.522 day^{-1} under anaerobiosis. Also, an average reaction rate close to 0.3% day^{-1} for benzene was estimated from all published data, while the corresponding values for toluene, ethylbenzene, and xylenes were estimated to be 4, 0.3, and 0.4% day^{-1}, respectively.

Biodegradability is a major aspect for the evaluation of the ecological behaviour of chemical pollutants in the environment (Guhl and Steber, 2006; Lapertot and Pulgarin, 2006). Also, the estimation of biodegradation rate is important for final fate of chemical compounds in water source. It has a key role for pollutant removal by biological processes. There are different aspects to analyse the biodegradability of chemical compounds based on the measurement of organic carbon, carbon dioxide production or oxygen consumption. These methods are including dioxide carbon evolution, carbon dioxide head space, closed bottle and respirometric test (Hoffmann et al., 1997; Tremier et al., 2005). Procedures of standard tests for biodegradability have been defined by organization of economic cooperation and development (OECD) and international organization for standardisation (ISO) (Pagga 1997, Ahtianen et al. 2003).

In the last few years there has been a considerable interest for manometric respirometric method or OxiTop test in the biodegradability of hydraulic oil, creosote oil, linseed oil, rapeseed and tail oil in water polluted as it is very simple and reliable (Kuokkanen et al. 2004, Vahaoja et al. 2005). Also this method is utilised for degradation of chemical components such as dibutyl phthalate, dioctyl adipate, diethylene glycol, 2-ethylhexylacrylate,

cyclohexanone, aniline, pesticides, chlorinated solvents, nonylphenol ether carboxylates, octylphenol ether carboxylates, nonylphenol and aromatic hydrocarbons (Staples et al., 1999; Gotvajn and Zagorc-Koncan 1999, Reuschenbach et al. 2003, Ahtianen et al. 2003, Lapertot and Pulgarin 2006). The bioavailability of contaminants is an important factor in bioremediation processes. The bioavailability of a chemical may be described by its mass transfer rate relative to its uptake and degradation rates by microorganisms (Bosma et al., 1997). The efficiency of hydrocarbon degradation will also depend on the characteristics of contaminated material, environmental conditions, and abilities of the microbial population (Liu et al., 2000; Van Hamme et al., 2003). If the capacity for hydrocarbon degradation is present and environmental conditions are amenable, the microorganisms must have access to the contaminants for degradation.

I.2.3. In-situ bioremediation

I.2.3.1. Main features

In-situ bioremediation has been successful for the treatment of groundwater contaminated with mixtures of chlorinated solvents such as carbon tetrachloride (CT), tetrachloroethylene (TCA), trichloroethylene (TCE), or pentachlorophenol (PCP) (Dyer et al., 1996; Klecka, 1998; Kao and Prosser, 1999; Schmidt et al., 1999; Ferguson and Pietari, 2000; Goltz et al., 2001; Beeman and Bleckmann, 2002; Antizar and Galil, 2003; Kao et al., 2003; Delvin et al., 2004; Widdowson, 2004). Also, contaminants such as gasoline or fuel (Curtis and Lammey, 1998; Yerushalmi et al., 1999; Cunningham et al., 2001; Bhupathiraju et al., 2002 ; Sublette et al., 2006), methyl *t*ert-butyl ether (MTBE) (Fortin et al., 2001; Azadpour-Keeley and Barcelona, 2006; Bradley and Landmeyer, 2006), petroleum hydrocarbons (Hunkeler et al., 1998 and 1999 ; Bolliger et al., 1999 ; Chapelle, 1999 ; Kao et al., 2006), alkylbenzene (Beller ,2000), alkylpyridines (Ronen et al., 1996), oily wastes(Guerin, 2000), synthetic lubricants (Thompson et al., 2006), coal tar contaminated site (Durant et al., 1997) , nitroaromatics (Holliger et al., 1997) and inorganic compounds such as uranium (Wu et al , 2006) have been successfully removed by in-situ bioremediation techniques. These technologies have also been widely used for the treatment of xenobiotic compounds (Olsen et al., 1995), monoaromatic hydrocarbons (Gersberg et al., 1995; Wilson and Bouwer, 1997) or BTEX from groundwater (Olsen et al., 1995; Kao and Borden, 1997; Cunningham et al., 2001; Atteia and Franceschi, 2002; Schreiber and Bahr, 2002; Maurer Rittmann, 2004; Reinhard et al., 2005).

In-situ bioremediation is known as long term technology since there is less certainty about the uniformity of treatment because of the variability of aquifer and soil characteristics. However, this process has advantages such as relative simplicity, low cost, and potentially remarkable efficiency in contamination removal. In in-situ bioremediation, organic pollutants are completely destroyed, therefore no secondary waste stream is produced (Dott et al. 1995).

In-situ bioremediation is a biological process where microorganisms metabolize organic contaminants to inorganic material, such as carbon dioxide, methane, water and inorganic salts, either in natural or engineered conditions. When naturally occurring metabolic processes are used to remediate pollutants without any additional alteration of site conditions, the process is called as intrinsic or natural attenuation. Present results indicate that biodegradation is the best method for BTEX removal (Kao and Prosser, 2001; Kao et al., 2006). When working conditions at the site are engineered, i.e. designed to accelerate the bioremediation of contaminants, the process is referred to as engineered or enhanced bioremediation (Scow and Hicks, 2005).

Main factors affecting in-situ bioremediation of contaminated groundwater have been widely described in the literature (Kampbell et al., 1996; Mac Donald et al., 1999; Boopthy, 2000; Iwamoto and Nasu, 2001; Schreiber and Bahr, 2002; McGuire et al., 2005; Farhadian et al., 2006; Andreoni and Gianfreda, 2007). Some of the main points (as showed in Figure I.2) include:

1- Source and concentration of pollutant
2- Chemistry and toxicity of contamination
3- Solubility, transport, adsorption, dispersion and volatility of pollutant compounds
4- Detection, determination and monitoring of pollutants
5- Chemistry, physics and microbiology of groundwater
6- Chemistry and mechanics of soil at contaminated site
7- Hydrogeology and hydrology of contaminated site
8- Limitations of environmental standards for water and soil
9- Environment conditions, nutrient sources and presence of electron acceptors
10- Biodegradability of contaminants, and the presence of a competent biodegrading population of microorganisms.

In in-situ bioremediation, anaerobic biodegradation plays a more important role than that of aerobic processes. Aerobic bioremediation process requires expensive oxygen delivery systems and process maintenance is often high due to biofouling in subsurface. But anaerobic processes have advantages such as low biomass production and good electron acceptor availability. Anaerobic processes are sometimes the only possible solution to remove pollutants (Holliger et al. 1997) as it is often difficult to inject oxygen into underground waters.

Natural bioremediation is the main method for monoaromatic degradation and results indicate that up to 90% of the BTEX removal by this approach can be attributed to the intrinsic biodegradation process (Kao and Prosser, 2001). However, natural attenuation is often limited by either the concentration of an appropriate electron acceptor or a nutrient required during the biodegradation (Hunkeler et al., 2002). Enhanced degradation accelerates the natural process by providing nutrients, electron acceptors, and competent degrading microorganisms (Scow and Hicks, 2005).

Contamination of groundwater with monoaromatic compounds is often accompanied by other oxygenated molecules such as methyl tert-butyl ether (MTBE), tert-butyl alcohol (TBA), methanol, ethanol (Corseuil et a., 1998 ; Deeb and Alvarez-Cohen, 2000 ; Deeb et al., 2001; Pruden et al., 2001 ; Lovanh et al., 2002; Olivella et al., 2002; Fiorenza and Rifai, 2003; Shih et al., 2004; Pruden and Suidan, 2004; Niven, 2005; Chen et al., 2007). These compounds have been added to gasoline as octane enhancers and stabilizers at levels close to 10-20 percent by volume (Corseuil and Alvarez, 1996; Niven, 2005). Generally, alcohols and oxygenated derivatives have a relatively high solubility in water and high mobility in the subsurface (Table I.g).

Table I.g: Fuel oxygenates characteristics and properties (Lide, 2000 and 2001; Moran et al. 2005)

Compound	Chemical Formula	Molecular Weight (g/mol)	Water Solubility (mg/L) at 25 °C	Specific Density at 25 °C	Vapor Pressure (mmHg)	Boiling Point (°C)	Drinking Water Standard (ppm)
MTBE	$C_5H_{12}O$	88.15	51260	0.744	245-256	53.6-55.2	0.02-0.04
TBA	$C_4H_{10}O$	74.12	miscible	0.791	40-42	82.9	-
Methanol	CH_3OH	32.04	miscible	0.796	121.58	64.7	-
Ethanol	C_2H_5OH	46.07	miscible	0.794	49-56.5	78-79	-

Methanol and ethanol increase the solubility of petroleum constituents such as monoaromatic compounds in the water. For example studies indicate that ethanol in petrol increases the solubility of BTEX from 30 to 210 percent by volume (Corseuil and Alvarez, 1996; Niven, 2005). The biodegradation of methanol or ethanol in groundwater would first deplete the oxygen and then the anaerobic electron acceptors that potentially reduce the rate of monoaromatic pollutant. Also, high concentration of these alcohol spills can inhibit the biodegradation of petroleum contaminants, especially monoaromatic compounds. Thus, the presence of methanol and ethanol in gasoline is likely to hinder the natural attenuation of BTEX, which would contribute to longer BTEX biodegradation processes and a greater risk of exposure (Corseuil et al., 1998; Lovanh et al., 2002). It must be emphasized that MTBE and TBA are difficult to remove from groundwater because they have high water solubility and low biodegradation rates (Fiorenza and Rifai, 2003; Rosell et al., 2005). Present results demonstrate that MTBE is the most recalcitrant compound, followed by TBA (Shih et al., 2004).

I.2.3.2. Engineered bioremediation

Oxygen is the main electron acceptor for aerobic bioprocesses. Aerobic in-situ bioremediation of monoaromatic pollutants is often limited by the dissolved oxygen tension. As a result, various methods such as air sparging, injection of oxygen-releasing compounds (hydrogen peroxide, magnesium peroxide, etc…) and trapped gas phase have been used to increase dissolved oxygen concentrations in ground water (Fry et al., 1996; Fiorenza and Ward, 1997; Johnston et al., 2002; Landmeyer and Bradley, 2003; Bittkau et al., 2004; Waduge et al., 2004; Yang et al., 2005). Oxygen can be applied by air sparging below the water table, which has been shown to enhance the rate of biological degradation of monoaromatic or petroleum pollutants (Johnston et al., 1998 and 2002 ; Waduge et al., 2004 ; Yang et al., 2005). For oxygen generating compounds, a dilute solution is circulated through the contaminated groundwater zone in order to increase its oxygen content and enhance the rate of aerobic biodegradation. However, some researchs have shown that significant difficulties, such as toxicity and microbial inhibition may be encountered when using inorganic nutrients and high concentration of hydrogen peroxide (Morgan and Watkinson, 1992).

Monoaromatic pollutants in groundwater can be removed by anaerobic in-situ bioremediation. Important electron acceptors that are used to accelerate the rate of anaerobic monoaromatic biodegradation are chemical components such as Fe^{+3}, nitrate and sulfate

(Cunningham et al., 2001; Schreiber and Bahr, 2002; Da Silva et al., 2005; Sublette et al., 2006). Electron acceptors can be injected alone (which may even selectively speed up the biodegradation of monoaromatic compounds), or in combination with other activating compounds (Wilson and Bouwer, 1997; Cunningham et al., 2001; Da Silva et al., 2005). Some results and reports for monoaromatic removal from contaminated groundwater through enhanced in-situ bioremediation are summarized in Table I.h.

Table I.h: Engineering of in-situ bioremediation for monoaromatic pollutant

Source of Pollutant	Electron Acceptor(s)	Result(s)	Reference(s)
BTEX (gasoline)	Sulfate (anaerobic)	-Sulfate injection was shown to increase the rates of biodegradation of BTEX. - The subsurface microbial community became more anaerobic in character as sulfate utilization increased as evidenced by its depletion in the aquifer.	Sublette et al. 2006
BTEX and Ethanol	Sulfate, Chelated - ferric ion, Nitrate (anaerobic)	- The addition of sulfate, iron or nitrate suppressed methanogenesis and significantly increased BTEX degradation efficiencies. - The addition of anaerobic electron acceptors could enhance BTEX degradation not only by facilitating their anaerobic biodegradation but also by accelerating the mineralization of ethanol or other substrates that are liable under anaerobic conditions. -The rapid biodegradation of ethanol near the inlet depleted the influent dissolved oxygen, stimulated methanogenesis, and decreased BTEX degradation efficiencies from > 99% in the absence of ethanol to an average of 32% for benzene, 49% for toluene, 77% for ethylbenzene, and about 30% for xylenes.	Da Silva et al. 2005
Benzene, toluene and MTBE	MgO_2 (aerobic)	- Different results can be related to differences in hydrologic and geochemical conditions that characterized the two locations prior to oxygen addition. - The results indicated the important role of pre-existing hydrologic, geochemical, and microbiologic conditions have on the outcome of oxygen-based remediation strategies, and suggested that these properties should be evaluated prior to the implementation of oxygen-based remedial strategies.	Landmeyer and Bradley 2003

Table I.h (cont'nd):

Source of Pollutant	Electron Acceptor(s)	Result(s)	Reference(s)
BTX	Hydrogen peroxide (aerobic)	- Results indicated that first order biodegradation rate coefficients for benzene, toluene, and ortho xylene varied from 0.3 to 0.81, 0.24 to 0.72, and 0.21 to 0.63 d^{-1}, respectively. - At 10 mg/L BTX concentration, the specific first-order coefficients increased with peroxide dose. But, at the 50 mg/l BTX concentration and a peroxide dose of 1020 mg/l, at 30–70% reduction in specific first-order biodegradation coefficients was observed. - First-order biodegradation coefficients decreased with ground water velocity, and increased with hydrogen peroxide dose and BTX concentration.	Nakhla 2003
BTEX (petroleum)	Nitrate (anaerobic)	-Addition of nitrate resulted in loss of TEX after an initial lag period 9 days. -Losses of benzene were not observed over the 60 day monitoring period.	Schreiber and Bahr 2002
BTEX (petroleum)	Nitrate and Sulfate (anaerobic)	-Acceleration of BTEX removal after injection nitrate and sulfate as compared with natural attenuation. - By combining injection of both NO_3^- and SO_4^{-2}, the total electron acceptor capacity was enhanced without violating practical considerations that limit the amount of nitrate or sulfate that can be added individually. - Degradation of total xylenes appears linked to sulfate utilization, indication another advantage versus injection of nitrate alone.	Cunningham et al. 2001
BTEX (gasoline)	Nitrate, Sulfate, ferric and Methanogenic Conditions (anaerobic)	- Results indicated that the fate of the different BTEX components in anoxic sediments is dependent on the prevailing redox conditions as well as on the characteristics and pollution history of the sediment.	Phelps and Young 1999

Table I.h (cont'nd):

Source of Pollutant	Electron Acceptor(s)	Result(s)	Reference(s)
Benzene (petroleum)	Sulfate and Fe^{+3} (anaerobic)	- These results demonstrated that addition of sulfate may be an effective strategy for enhancing anaerobic benzene removal in some petroleum-contaminated aquifers. - In short-term (< 2 weeks) incubations, addition of sulfate slightly stimulated benzene degradation and caused a small decrease in the ratio of methane to carbon dioxide production from benzene. - In longer-term ($\gg$ 100 days) incubations, sulfate significantly stimulated benzene degradation with a complete shift to carbon dioxide as the end product of benzene degradation. -The addition of Fe (III) and humic substances had short-term and long-term effects that were similar to the effects of the sulfate amendments.	Weiner et al. 1998
BTEX (gasoline)	Oxygen (through diffusion from silicon tubing)	- Oxygen delivery from diffusion silicon tubing created a zone of sustained high dissolved oxygen (39 mg/L) in ground water around the injection well and changed the dominant ground water conditions from anaerobic to aerobic. - The oxygen enhanced zone was able to biodegrade benzene and ethylbenzene, which had been relatively resistant to natural attenuation in the plume under the initial anaerobic conditions. - Under study conditions, iron precipitation was observed at the oxygen injection well but did not clog the well screen.	Gibson et al. 1998
BTEX (gasoline)	Sulfate and Nitrate (anaerobic)	- Anaerobic biodegradation of toluene and meta xylene and para xylene were measured (as a summed parameter) occurred at a rate of 7.2 and 4.1 µg /L.h, respectively, with 80 mg/L sulfate as the apparent electron acceptor. -Addition of nitrate stimulated nitrate reducing conditions and increased rates of toluene and xylenes (meta and para) biotransformation to 30.1 and 5.4 µg /L.h, respectively. -However, the data suggested that by nitrate addition enhanced the rate and extent of anaerobic BTEX biotransformation.	Ball and Reinhard 1996

Table I.h (cont'nd):

Source of Pollutant	Electron Acceptor(s)	Result(s)	Reference(s)
BTEX (fuel)	KNO_3 (electron acceptor) and ammonium polyphosphate (nutrients) (anaerobic)	- The data indicated that the BTEX in nitrate-enriched aquifer was biodegraded in-situ under denitrifying conditions. -BTEX declined by 78% in water from the monitoring well which was most contaminated initially and by nearly 99% in water from one of the extraction wells. -At one of the extraction wells, down-gradient of the monitoring well, nitrate appeared in significant concentrations after week 124; this appearance coincided with a marked decline (> 90%) in monoaromatic concentration.	Gersberg et al. 1995

I.2.3.3. Natural processes

Intrinsic bioremediation, which is also known as natural attenuation or passive bioremediation, is an environmental site management approach that relies on naturally occurring microbial processes for petroleum hydrocarbon removal from groundwater, without the engineered delivery of nutrients, electron acceptors or other stimulants (Curtis and Lammey, 1998; Clement et al., 2000; Kao and Wang, 2000; Kao and Prosser, 2001; Widdowson, 2004; Maurer and Rittmann, 2004; Reinhard et al., 2005; Kao et al., 2006). Natural bioremediation removes and decreases organic pollutants from many contaminated sites (Röling and Verseveld, 2002). It is more cost effective than engineered conditions but it takes more time for organic biodegradation (Kao and Prosser, 1999; Andreoni and Gianfreda, 2007).

Mineralization of organic compounds in groundwater under natural bioremediation is, just like with engineered situations, connected to the consumption of oxidants such as oxygen, nitrate and sulfate and the production of reduced species such as Fe^{+2}, Mn^{+2}, H_2S, CH_4 and CO_2 (Lovley, 1997; Bolliger et al., 1999). Some studies of BTEX removal from contaminated groundwater through natural in-situ bioremediation are summarized in Table I.i.

Table I.i: Natural in-situ bioremediation for monoaromatic pollutants

Source of Pollutant	Electron Acceptor(s)	Result(s)	Reference(s)
Benzene	Sulfate	- The degradation of benzene under sulfate-reducing conditions was monitored in a long-term column experiment under close to in-situ conditions and data indicate a high potential for natural attenuation. - Stoichiometric calculations indicate that benzene was mineralized with sulfate as electron acceptor.	Vogt et al. 2007
BTEX (gasoline)	Iron and sulfate	- In an aquifer contaminated by a gasoline spill in South Africa, data showed widespread evidence of anaerobic degradation of BTEX. - At the studied site, results indicated that the majority of the BTEX biotransformation is coupled to sulfate reduction and occurs in winter when the aquifer is replenished by rainwater having a predominantly marine signal. -Iron reduction, although widespread, plays only a minor role in the BTEX degradation process.	Roychoudhury and Merrett 2006
BTEX (Petroleum)	Fe (III) and methanogenic condition	- Data from two research sites contaminated with petroleum hydrocarbons showed that toluene and xylenes degrade under methanogenic conditions, but the benzene and ethylbenzene plumes grow as aquifer Fe (III) supplies are depleted.	Bekins et al. 2005
BTEX (gasoline)	Methanogenic conditions (Natural attenuation)	-BTEX removal rates were rapid for toluene *o*- and *m*-xylenes(< 30 day) and slow for benzene, ethylbenzene and p-xylene degrading (50% removal in 60-90 day). -Results indicated that the presence of electron acceptors (O_2, NO_3^- , Fe^{+3}, SO_4^{-2}) is not a precondition for natural attenuation to occur.	Reinhard et al. 2005

Table I.i (cont'nd):

Source of Pollutant	Electron Acceptor(s)	Result(s)	Reference(s)
BTEX and PAH (Tar oil)	Nitrate, sulfate, ferric, methanogenic for anoxic and oxygen aerobic condition	- In microcosm studies, the autochthonous microflora utilised toluene, ethylbenzene, and naphthalene under sulfate- and Fe (III)-reducing conditions. Additionally, benzene and phenanthrene were degraded in the presence of Fe (III). - Under aerobic conditions, all BTEX and PAH were rapidly degraded. - The microcosm studies in particular were suitable to examine the role of specific electron acceptors, and represented an important component of the multiple line of evidence concept to assess natural attenuation.	Schulze and Tiehm 2004
BTEX (petroleum)	……..	- The mass flux calculation showed that up to 87% of the dissolved total benzene, toluene, ethylbenzene, and xylene (BTEX) isomers removal was observed via natural attenuation - Results revealed that biodegradation was the major cause of the BTEX mass reduction among the natural attenuation processes, and approximately 88% of the BTEX removal was due to the natural biodegradation process. - The calculated total BTEX first-order attenuation and biodegradation rates were 0.036 and 0.025% per day, respectively. - Results suggested that the natural attenuation mechanisms can effectively contain the plume, and the mass flux method is useful in assessing the occurrence and efficiency of the natural attenuation process.	Kao and Prosser 2001

Table I.i (cont'nd):

Source of Pollutant	Electron Acceptor(s)	Result(s)	Reference(s)
BTEX (gasoline)	Nitrate, iron reduction, methanogenic and oxygen	- Results revealed that the mixed intrinsic bioremediation processes (iron reduction, denitrifcation, methanogenesis, aerobic biodegradation) have effectively contained the plume, and iron reduction played an important role on the BTEX removal. - The mass flux calculations showed that up to 93.1% of the BTEX was removed within the iron-reducing zone, 5.6% of the BTEX was degraded within the nitrate spill zone and the remaining 1.3% was removed within the oxidized zone at the downgradient edge of the plume. - Under iron-reducing conditions, toluene and ortho xylene declined most rapidly followed by meta and para xylene, benzene, and ethylbenzene. -Within the denitrifying zone, toluene and meta and para xylene degraded rapidly, followed by ethylbenzene, o-xylene, and benzene.	Kao and Wang 2000
Toluene, Para xylene, naphtalene (diesel - fuel)	Sulfate and CO_2 (anaerobic)	- Results indicated that toluene, p-xylene and naphthalene are degradable through SO_4^{-2} and CO_2 as externally supplied oxidants. - A carbon mass balance revealed that 65% of the hydrocarbons removed from the column were recovered as dissolved inorganic carbon, 20% were recovered as CH_4, and 15% were eluted from the column.	Hunkeler et al. 1998
BTX and ethanol (fuel)	……..	- Studies showed that ethanol can enhance the solubilization of BTX in water, and it might exert diauxic effects during BTX biodegradation.	Corseuil and Alvarez 1996
BTEX (gasoline)	Aerobic respirometric and Methanogenic Conditions	- Assimilation capacities of dissolved oxygen, ferrous iron, and methane distributions when compared to BTEX concentrations showed that the ground water has sufficient capacity to degrade all dissolved BTEX before the plume moves beyond 250 m downgradient. - Evidence obtained from loss of contaminants, geochemistry, and microbial breakdown chemicals showed that intrinsic bioremediation technology would be a viable option to restore the site.	Kampbell et al. 1996

I.2.4. Ex-situ bioremediation

I.2.4.1. Main features

Ex-situ bioremediation through biological reactors, both under aerobic and/or anaerobic conditions, has been successfully used in the treatment of water contaminated with chemical pollutants including percholorate (Min et al., 2004), bromate (Butler et al., 2006), chlorinated hydrocarbons such as trichloroethylene (TCE) (Ohlen et al., 2005), phenol (Kryst and Karamanev, 2001), methyl *t*ert-butyl ether (MTBE) and other oxygenated compounds (Kharoune et al., 2001; Vainberg et al., 2002; Zein et al., 2006), alkylate (Cho et al., 2007), polycyclic aromatic hydrocarbon (PAH) (Guieysse et al. 2000), monoaromatic compounds (Alemzadeh and Vossoughi, 2001; Pruden et al., 2003; Kermanshahi et al., 2006; de Nardi et al., 2006), fuel hydrocarbons (oil, gasoline, and diesel) and other organic compounds (Massol-Deya et al., 1995; Zein et al., 1997; Langwaldt and Puhakka, 2000). The term bioreactor refers to a vessel where the biological degradation of contaminants is performed in fully controlled conditions, i.e. parameters such as temperature, pH, aeration and stirring rates are known and controlled. Bioreactors have also been widely applied for treatment of VOC (such as monoaromatic hydrocarbons) contaminated gases (Pedersen and Arvin 1995, Bielefeldt and Stensel, 1999; Lu et al., 2002).

Research works already reported indicate that ex-situ bioremediation can be successfully applied for organic compounds removal from water. They can be considered as the best technology in this area, even if drawbacks such as the need for water pumping, power supply, energy consumption, sludge production, VOC stripping, and BTEX adsorption on solids are reported.

Microorganisms have important role in biological process. Globally speaking, microbial activity not only depends on medium composition, but also on environmental conditions such as temperature, pH, salinity, pressure (Holliger et al., 1997; Mandelbaum et al., 1997; Fiorenza and Ward, 1997; Lovley, 1997; Granger et al., 1999; Field, 2002; Lin et al., 2002; Villatoro-Monzon et al., 2003; Van Hamme et al., 2003; Schulze and Tiehm, 2004; Chakraborty and Coates, 2004; Jahn et al., 2005).

Several mixed and pure cultures of microorganisms have been recognized as able to metabolize monoaromatic hydrocarbons as carbon and energy sources (Table I.f). Mixed cultures found suitable in this area are often bacterial consortia from domestic or industrial

sludge, soil polluted by oil products and polluted groundwater polluted (Solano-Serena et al., 1999, 2000; Guerin, 2002; Pruden et al., 2003; de Nardi et al., 2002, 2005; Cattony et al., 2005; Ohlen et al., 2005; Kermanshahi pour et al., 2005, 2006; Zein et al., 2006). Table I.j lists some process factors to be considered for bioreactors.

Table I.j: Process control parameters for monoaromatic degradation by bioreactors (Tchobanoglous, 1998; Langwaldt and Puhakka, 2000; Guerin, 2002; Farhadian et al., 2006)

Parameter	Description / Importance
Electron acceptor	Aerobic condition: O_2 Anaerobic condition: chemical compounds such as NO_3^- and SO_4^{-2}
Packing materials	Surface area, porosity and nature of the packing material affect the efficiency of biofilm reactors. Also, evaluation of adsorption and desorption capacity of media and bioregenerability of packing must be considered in biofilm reactors.
Gas control	Gas exiting from bioreactors must be controlled. In aerobic bioreactors, diffusion and stripping of volatile organic compounds by aeration must be considered. In anaerobic conditions monoaromatic compounds can be evaporated and stripped by the biogas produced.
Recycle ratio (Q_R /Q)	Recycle flow may be necessary for feed dilution or biomass recycling.
Temperature	In general, rate of biological reaction in mesophilic conditions increase by a factor of 2 each 10 °C rise in temperature. However, an increased temperature induces an enhanced volatilization rate of monoaromatic hydrocarbons.
Hydraulic retention time (HRT) = V/Q	Flexible handling of influent flow rates is required for effective control.
Influent quality and flow direction (Such as: monoaromatic concentration, pH, EC, TSS, COD, Alkalinity, Salinity, inhibitors, etc)	Variations in influent quality affects the efficiency of bioreactors; selection of flow direction (upflow or downflow) could influence mixing and degree of contamination removal. Volatilization rates of VOC compounds may be accelerated by mixing and thermal energy and inhibited by dissolved chemicals such as organic compounds, salts and etc.

Table I.j (cont'nd)

Parameter	Description / Importance
Flow regime	A bioreactor may be setup as a completely mixed stirred tank or plug-flow bioreactor. Flow regime affects the efficiency of bioreactors.
Biomass	Potential problems arise by the attachment and detachment (sloughing) of biomass; biomass can build up and large oxygen demands associated with aerobic biotreatment process may occur; potential problems with foaming (also dependent on type of chemicals in influent), important factor for biomass control in biological bioreactor are mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS). On the other hand quantities and qualities of biomass applied as starter affect start-up and efficiency of bioreactors.
Nutrient requirements	Varies with contaminants ; provide a macro nutrient (such as N, P) and micronutrient (such as trace element) on basis of TOC in contaminated water and elemental biomass composition
pH	pH control is critical and water contaminated may not have adequate buffer capacity.
OLR=QS/V	Organic loading rate can determine capacity of bioreactor for organic pollutant removal.
F/M=QS/VX	Ratio of food to microorganism is important and influential on efficiency of biological system.

I.2.4.2. Aerobic bioreactors

I.2.4.2.1. Monophasic systems

Aerobic bioreactors for monoaromatic removal from water usually use the fixed film approach with technologies already established with activated sludge reactors, such as moving bed biological reactor (MBBR), fludized bed bioreactor (FBR), submerged fixed film reactor (SFFR) and fixed film activated sludge (FAS) (Lodaya et al., 1991; Voice, 1994; Zhao et al., 1999; Guerin, 2002; Pruden et al., 2003; Ohlen et al., 2005; Zein et al., 2006). Fixed film reactors exhibit high surface area available for microbial growth. Also, these systems present a higher potential for use than suspended growth biomass reactors since the former can retain a higher concentration of biomass with higher metabolic activity when operated with continuous processes. Furthermore, attached biomass is sometimes claimed to be more

resistant to toxicity; it has been reported that biofilm cells can be up to 500 times more resistant to antibacterial agents than freely suspended ones (Pedersen and Arvin, 1995).

Biofilm reactors that have been constructed using different packing materials, such as granular activated carbon (GAC), polyurethane, kaolin, polystyrene, wood chips, soil sand, ceramic saddles, poly vinyl chloride (PVC) and polyethylene (PE) (Guerin, 2002; Pruden et al., 2003; Ohlen et al., 2005; Kermanshahi pour et al., 2005). Actived carbons are special supports, since they are also able to adsorb pollutants (Xing and Hickey 1994, Zhao et al., 1999; Guerin, 2002; Pruden et al., 2003).

If aerobic biofilm reactors are very compact, enable a rapid start-up, have a high biomass retention capacity, and are suitable for use at a low hydraulic retention time (Langwaldt and Puhakka, 2000; Tchobanoglous, 1998), they have also disadvantages such as the need for high power supply, and energy consumption.

From a general point of view, aerobic processes give rise to significant sludge production and volatile compounds loss by volatilization and stripping during aeration. Some reports and results for monoaromatic removal from contaminated water in aerobic bioreactors are summarized in Table I.k. Data show that aerobic biological treatment can play a major role in the remediation of water contaminated by oil derivatives, including monoaromatic hydrocarbons and treatment efficiencies up to 99% and above, in term of monoaromatic disappearance in the liquid, can be achieved.

Most of studies reported do not take into account the role of stripping of volatile organic compounds due to aeration and sorption by packing media.

Table I.k: BTEX removal from contaminated water through aerobic bioreactors

Source of pollutant(s)	Type of bioreactor(s) and condition(s)	Analytical method(s)	Result(s)	Remark(s)	Reference (s)
p-Xylene and Naphtalene (simulated diesel fuel contaminated groundwater)	- Immobilized cell airlift bioreactor -Media: Soil, Semi permeable membrane-geotextile. -Bench Scale study (under batch with volume 0.4L and continue 0.83 L conditions) - The amount of biomass was nearly constant at 460 mg/L during the batch experiment. - Inoculum: Soil contaminated with oil products	Para xylene and naphthalene by GC-FID (solvent extraction-DCM), DO	-Complete biodegradation of p-xylene and naphthalene; the obtained volumetric biodegradation rates at biomass density of 720 mg/L were 15 and 16 mg/L.h respectively. -Rate constants for the continuous regime were determined based on the biomass growth and the amount of substrate utilization, resulting in following values: maximum specific growth rate (μ_{max}) 0.0047 h^{-1}, half saturation constant (K,) 3.9 mg/ L and yield coefficient (Y) 0.05 mg biomass /mg substrate. For batch operation, a similar yield coefficient was assumed and the experimental data was fitted by the Monod equation. Values of 0.0047 h^{-1} for μ_{max} (similar to continuous) and10 mg /L for K_s were obtained.	- There is no discussion about adsorption of pollutants on soil. - Headspace recirculation minimized the loss of VOC through gas phase. - In batch reactor, VOC compounds in the exhaust gas were captured by activated carbon but there are no quantitative data on the efficiency of this system. - In continuous conditions, out let gas was connected to DCM and then VOC in gas phase measured by GC-FID	Kermansh -ahi pour et al. (2005 and 2006)
PAH , MTBE and BTEp-X (groundwater contaminated with gasoline)	-Laboratory biomass retaining bioreactor (BCR) , volume=8L ,Temperature =18°C, DO>3 mg/L -Seed: mixed culture adapted on pollutants.	MTBE and BTEX by GC-FID (Purge and trap), PAH through GC-MS (SPME), pH, DO, VSS	-High removal efficiencies were achieved for PAH, BTEX and MTBE in bioreactors, more than 99%, 99.7% and 99.7%, respectively. -During the study, all the PAHs, such as naphthalene, acenaphtene, methyl-naphthalene and BTEX were detected at concentrations less than 1 µg/L.	-Biomass remains in bioreactor through poly ethylene (PE) porous pot with pore size 18-28 µm. -There is no discussion about adsorption of pollutants by porous pot. - Stripping of volatile organic compounds is considered through effluent gas analysis.	Zein et al. (2006)

Table I.k (cont'nd): BTEX removal from contaminated water through aerobic bioreactors

Source of pollutant(s)	Type of bioreactor(s) and condition(s)	Analytical method(s)	Result(s)	Remark(s)	Reference(s)
BTEX , phenols PAH (coal tar)	-Two bioreactors were applied at laboratory scale (D:8 cm, L:48 cm) and temperature 23°C. - Submerged fixed-film reactor (SFFR) - Fluidized bed bioreactor(FBR) - Media: activated carbon - Initial microbial seed was tacked from water polluted by coal tar.	pH, phenols by HPLC, BTEX by GC-MS (purge and trap), COD, TSS, PAH, NH_3,	-Results showed that the two bioreactor configurations were effective, with high efficiencies of contaminant removal (typically>90%) over a range of hydraulic retention time (3-26 hr). - The FBR was only marginally less effective than the SSFR for the same groundwater contaminants.	-There are no data about stripping of aromatic compounds by aeration and adsorption of pollutants through activated carbon.	Guerin (2002)
BTEX & MTBE	- Aerobic fluidized bed reactor with volume 7.88 L at temperature 20 °C, pH ranges: 7.4-7.9 and DO: 2 mg/L. - Media: granular activated carbon. - Inoculum was used from a mixed culture adapted with MTBE as the sole carbon source. .	pH, DO, COD, TOC, BTEX and MTBE by GC-FID (purge and trap)	- The average influent of each of the BTEX compounds was about 2 mg/L and the range of the average effluent concentration was 1.4 -2.2 mg/L. -This study demonstrates that MTBE contaminated water can be biologically treated using a fluidized bed reactor with and without BTEX addition. - Results indicated that monoaromatic compounds did not inhibit MTBE degradation.	-There are no data and discussion about stripping of volatile organic compounds by aeration and adsorption of pollutants through granular activated carbon.	Pruden et al. (2003)

Table I.k (contn'd): BTEX removal from contaminated water through aerobic bioreactors

Source of pollutant(s)	Type of bioreactor(s) and condition(s)	Analytical method(s)	Result(s)	Remark(s)	Reference(s)
TEX and chlorinated ethylene (paint contaminated site)	-Two stage fluidized bed reactor (FBR) with effective volume 5 L for each reactor at temperature 30°C and dissolved oxygen 5-6 mg/L. -Media: polyurethane foam cubes (surface area 1000m^2/m^3 and density 1100 kg/m^3). - The sludge was taken from a pilot plant treating contaminated groundwater.	GC-FID (head space)	-Results showed that one stage FBR pilot plant could remove all pollutants in the treatment of groundwater from a paint contaminated site. -At HRT= 12 hr in one stage FBR with toluene influent 1200µg/L ethyl benzene 650µg/L m- and p-xylene 1750µg/L and o-xylene 170µg/L , efficiency of bioreactor was more than 98% .	- Pure oxygen was supplied to bioreactors to minimize the gas flow, in order to decrease pollutant loss by stripping. This is not quantified - Adsorption of pollutants onto solid supports is not studied.	Ohlen et al. (2005)
		…….			
BTEX (groundwater contaminated)	- Aerobic fluidized bed bioreactor with capacity 4320 gallons/day. - Media: active carbon. -This system contained secondary suspended solids filtration and activated carbon polishing.		- Over a three-month period of operation for treatment of groundwater plums of BTEX contamination, the unit demonstrated more than 98% consistent reduction of BTEX components in the main bioreactor.	-There is not information about volatile organic compounds in outlet gas. - There are no results about adsorption by activated carbon.	Spectrum-environmental services, Inc. (1996)

Table I.k (cont'nd): BTEX removal from contaminated water through aerobic bioreactors

Source of pollutant(s)	Type of bioreactor(s) and condition(s)	Analytical method(s)	Result(s)	Remark(s)	Reference(s)
Toluene (synthetic)	-Completely mixed (CM) aerobic bioreactor with silicon tubing that was immersed in bioreactor -Bioreactor was a baffled impeller agitated type with volume 1L. -Toluene as a model solvent was circulated within the silicon tubing - *Pseudomonas putida* was used as pure culture.	Toluene by GC-FID	- In this work, the biodegradation of toluene was efficiently carried out using *Pseudomonas putida* in the bioreactor developed. -Operation parameters such as dilution rate and toluene transfer rate were effective on toluene removal.	- In this research for determination of toluene in the culture and separation of biomass from polycarbonate filter (0.45 µm pore size) that generally it is not recommended for another monoaromatics. -Toluene analysed in exit gas.	Choi et al. (1992)
Toluene (synthetic)	- Aerobic- fluidized bed bioreactor (FBR) with volume 14.7 L at pH=8 and temperature 20 °C. - Media: Granular activated carbon (particle size 0.9 mm). - Initial mixed culture inoculums were obtained from a laboratory bioreactor that had been supplied with toluene during one year.	Toluene by GC-FID, biofilm tickness through stereo microscope	-In this study, the adsorption capacity of biofilm coated activated carbon at aerobic-FBR which treated toluene (< 10 mg/L) was evaluated. -Results of adsorption capacity showed greater than 70% of initial levels during first two months and after 6 months operation the remainig capacity was 40, 52 and 57% of the initial value for equilibrium toluene concentration of 0.1, 3 and 3 mg/L, respectively. -Results indicated that there was no direct relationship between the amount of the thickness of the biofilm on the carbon and the residual adsorptive capacity.	-Stripping of toluene at this study was minimized as dissolved oxygen was supplied to the reactor by oxygenating the reactor feed water with pure oxygen. -Adsorption of pollutant by GAC was studied.	Zhao et al. (1999)

I.2.4.2.2. Biphasic media

In recent years an aerobic reactor that incorporates a two phase partitioning bioreactor (TPPB) was applied for treatment of monoaromatic hydrocarbon contaminants (Collins and Daugulis, 1999; Yeom and Daugulis, 2001; Malinowski, 2001; Daugulis, 2001; Davidson and Daugulis, 2003 a,b; Daugulis and Janikowski, 2002; Acuna-Ashkar et al., 2003; Abu Hamed et al., 2004; Muñoz et al., 2007). Also, this approach (TPPB) has been successful for the biodegradation of polychlorinated biphenyls (Rehmann and Daugulis, 2007, 2008), hexane (Muñoz et al., 2006; Arriaga et al., 2006), polycyclic aromatic hydrocarbons (Janikowski et al., 2002; Daugulis and McCracken, 2003; MacLeod and Daugulis, 2003; MacLeod and Daugulis, 2005; Vandermeer and Daugulis, 2007; Eibes et al., 2007; Mahanty et al. 2008), phenol (Collins and Daugulis, 1997; Cruickshank et al., 2000; Maliowski, 2001; Prpich and Daugulis, 2005, 2006), trichlorophenol (Ascon-Cabrera and Lebeault, 1995), nitroaromatics (Pudge et al., 2003) and styrene (Dumont et al., 2006; Muñoz et al., 2007).

Two phase partitioning system or biphasic bioreactors contain a non-aqueous phase (e.g. hexadecane, oleyl alcohol, silicone oil, polymer beads) in addition to an aqueous phase (Malinowski, 2001). The non-aqueous phase improves the transfer of hydrophobic compounds to the organisms and can reduce the exposure of microorganisms to inhibitory substances by lowering their concentration in the aqueous phase. Thus, this system can be applied to the controlled delivery of monoaromatic toxic substrates (Muñoz et al., 2007).

In such a two-phase aqueous–organic system, the substrate is solubilized in the immiscible organic phase and allowed to transfer into the aqueous phase. The microorganisms degrade or transform the substrate at the aqueous/organic interface and/or in the aqueous phase. Thus, the substrate concentration in the aqueous phase can be maintained below the inhibitory level. The partition process itself is controlled to some extent by the metabolic activity of microorganisms (Daugulis, 2001). This process is based on the fundamental principle of thermodynamic equilibrium due to partition coefficient of substance in the ternary system (gas, water and organic phase). This configuration, generally referred to as water-organic solvent, two phase system, is widely used in the area of biotransformations (Dordick et al., 1998; Parales et al., 2002). Performance of biphasic bioreactors that applied for degradation of monoaromatic hydrocarbons are summarized in Table I.l.

Table I.l: Monoaromatic hydrocarbons degradation through two-phase partitioning bioreactors

Toxic compound(s)	Organic phase and condition (s)	Organism(s)	Result(s)	Remark(s)	Reference(s)
Bezene, toluene, phenol	2-undecanone, Organic phase: 2.5 mL, aqueous phase: 40 mL T=30°C, pH= 7, shaker rate: 200 rpm, bench scale, batch feeding	*Pseudomonas putida* F1 (ATCC 700007)	- This study demonstrated that the maximum overall biodegradation rates of benzene, toluene and phenol were obtained as 183, 197 and 18 mg l^{-1} h^{-1}, respectively. - In the biodegradation experiments using mixtures, the presence of phenol did not change the biodegradation times of benzene and toluene and, the presence of benzene and toluene decreased the biodegradation times of phenol. - Results indicated that at serum bottles with total volume 320 ml at organic/ aqueous phase ratios (v/v) 0.5 and 1, no biodegradation was observed that the thickness of the organic phase layer may have prevented the oxygen transfer from the gas phase to the aqueous phase.	- Although at in this research reported that volatilization of monoaromatic compounds during experiments and adsorption of these hydrocarbons by cell is negligible but there are not any related data during studies. - Analytical procedure for aqueous- organic phase analysis and biomass separation from samples is not clear.	Abu Hamed et al. (2004)
Benzene	Hexadecane, T= 30°C, aqueous phase: 1L , organic phase: 0.5 L, Aeration was either by air at 0.25 vvm (based on aqueous volume, Step-1) or by pure oxygen at 0.1 vvm (Step-2), mixing rate:350 rpm, batch feeding	*Alcaligenes xylosoxidans* Y234	- A feed of 7g of benzene was loaded into the organic phase (0.5L), which gave an initial equilibrium aqueous phase concentration of 100 mg/l. -Over the course of 1 day, 63.8% of the benzene was degraded by the microorganism, and 36.2% was stripped through aeration. - By installing a condenser and using a lower gas flow of pure oxygen to reduce stripping, more than 99% of a subsequent 7g benzene addition was degraded by the organisms within one day. - The overall degradation rate of benzene in this batch experiment was in range of 186-291 mg/L.h - The cell yield coefficient range for this system was estimated to be 0.353-0.412 g cells/g substrate.	- A condenser was installed on the bioreactor off-g as port to decrease benzene loss due to stripping. - A lower gas flow of pure oxygen was used to reduce monoaromatic stripping. - Exit gas benzene concentration was monitored to allow calculation of benzene stripping.	Yeom and Daugulis (2001)

Table I.l (cont'nd): Monoaromatic hydrocarbons degradation through two-phase partitioning bioreactors

Toxic compound(s)	Organic phase and condition (s)	Organism(s)	Result(s)	Remark(s)	Reference(s)
Benzene, toluene and phenol	Oleyl alcohol (industrial grade – Adol 85NF) Organic phase: 0.5 L, aqueous phase: 1 L, air flow: 0.5 L/min, T = 30°C, pH= 6.8, mixing rate: 250 rpm, the organic phase loaded with 10.15 g toluene, 2 g benzene or 2.1 g p-xylene.	*Pseudomonas sp.* (ATCC 55595)	- The results of this work have demonstrated the potential applications of this bioreactor system to the degradation of two-component mixtures of benzene, toluene and p-xylene. - The simultaneous fermentation of benzene and toluene consumed these compounds at volumetric rates of 24 and 67 (as mg/L.h), respectively. Also, the toluene and p-xylene are consumed at rates of 66 and 18 (as mg/L.h), respectively. - The use of a sequential feeding strategy reduced the stress on the microorganisms, and promoted increased degradation rates for all compounds present. At this strategy (sequential fermentation) showed that degradation rates of benzene and toluene, were 56 and 79 (as mg/L.h), respectively. The biodegradation rates of 74 and 25 as mg/L.h, was observed for toluene and p-xylene, respectively. - Operational challenges, such as oxygen limitation and wall growth, occurred in all fermentations, and these issues will be addressed in subsequent experiments. - The cell yield coefficient range for this system was estimated to be 0.40-0.42 g cells/g substrate.	-There is not information about volatile organic compounds in outlet gas. - lag phase about two days, may be related to high initial concentration of monoaromatic compounds in aqueous phase (~ 60 for toluene, ~ 25 for benzene, ~ 10 for p-xylene as mg/L). - Possibility of monoaromatic adsorption by inactive biomass did not report.	Collins and Daugulis (1999b)

Table I.l (cont'nd): Monoaromatic hydrocarbons degradation through two-phase partitioning bioreactors

Toxic compound(s)	Organic phase and condition (s)	Organism(s)	Result(s)	Remark(s)	Reference(s)
Toluene	Oleyl alcohol (industrial grade – Adol 85NF) Organic phase: 0.5 L, aqueous phase: 1 L, air flow: 0.25 L/min, T = 30°C, pH= 6.8, mixing rate: 250 rpm	*Pseudomonas sp.* (ATCC 5595)	- In consuming the toluene to completion, the organisms were able to achieve a volumetric degradation rate of 115 mg/L.h . - This study reported that researchers did not observe any losing of monoaromatic compounds during 4 days air sparging and mixing in the abiotic fermentor conditions.	- lag phase about two days, may be related to high initial concentration of toluene in aqueous phase (~ 60 mg/L). -There is no information about adsorption of VOC hydrocarbons by the cells. – Analytical protocol described is not very clear. - Monoaromatic compounds are volatile organic compounds and may strip and volatilize during mixing and aeration of biphasic bioreactor although at this study reported that there was not any losing related to air stripping.	Collins and Daugulis (1999a)

The organic phase in the biphasic bioreactors is a hydrophobic solvent that traditionally need to posses a variety of important properties including low aqueous solubility, low volatility, non biodegradability, non hazardous, chemical stability, available in bulk quantity, biocompatibility, and inexpensive (Malinowski, 2001; Amsden et al., 2003; Heipieper et al., 2007).

Also, this method can be applied as a new technology platform for destroying toxic organic compounds (xenobiotics) from air, water and soil (Daugulis, 2001). The key for using biphasic systems for the remediation of contaminated gas streams is the presence, and use, of the organic solvent to trap the monoaromatic contaminants (see Table I.m) (Hekmat and Vortmeyer, 2000; Daugulis, 2001; Davidson and Dagulis, 2003a,b ; Daugulis and Boudreau, 2003; Nielsen, et al., 2006; 2007a; 2007b). This process can be accomplished using an absorption column (Yeom et al.; 2000) or monoaromatic hydrocarbon can achieved directly by the liquid contents of TPPB as a single-stage system (Davidson and daugulis, 2003). When monocyclic compounds trapped by the organic solvent in adsorption column, it is sent to the biphasic bioreactor where it is transferred from the solvent to the cells in the aqueous phase and the regenerated solvent is recirculated back the absorption column (Yeom et al., 2000; Daugulis and Boudreau, 2003; Davidson and Daugulis, 2003 a,b; Nielsen et al., 2006). Also, this process has been applied successfully to the remediation of xenobiotics from contaminated soil (Daugulis, 2001). Normally, the direct and indirect processes have been recommended for treating of soil polluted through TPPB. Direct method content of the soil washing with an organic solvent that is subsequently used as the organic phase of a biphasic system and an indirect technology in which the organic contaminant is removed by volatilization and subsequently captured by the organic solvent in the TPPB and mineralized.

Table I.m: The use of two phase partitioning bioreactors for monoaromatic removal from waste gas

Pollutant (s)	Microorganism(s)	Organic solvent	Author(s)
Benzene	*Klebsiella* sp.	1-Octadecene	Yeom et al. (2000)
Benzene	*Alcaligenes xylosoxidans* Y234	n- hexadecane	Davidson and Daugulis (2003a)
Benzene and Toluene	*Alcaligenes xylosoxidans* Y234	n- hexadecane	Davidson and Daugulis (2003b)
Toluene	*Alcaligenes xylosoxidans*	n-hexadecane	Daugulis and Boudreau (2003)
Toluene	*Pseudomonas putida* GJ40	perfluorocarbon PFC40	Dumont et al. (2006)
Toluene	Mixed culture	silicone oil	Dumont et al. (2006)
Benzene	*Achromobacter xylosoxidans* Y234	n-hexadecane	Nielsen et al. (2006, 2007 a,b)

I.2.4.3. Anaerobic bioreactors

Anaerobic process is a well established treatment technology, suited to treat high-strength wastewaters (Farhadian et al., 2007). Numerous studies on monoaromatic removal from contaminated water by anaerobic bioreactors have been carried out in recent years (de Nardi et al., 2006; Martínez et al., 2007). In these processes, fermentative bacteria transform aromatic hydrocarbons into volatile fatty acids (VFA) which are further converted to biogas, a mixture of methane and carbon dioxide (de Nardi et al., 2005). Anaerobic bioreactors have several potential advantages over aerobic processes, which include the need for less energy due to omission of aeration and the conversion of organic matter to methane which is an energy source by itself which can be used for temperature control (this temperature can be near 35 °C in the mesophilic range, or 55-60 °C in the thermophilic one). They also give rise to lower production of sludge, which reduces the disposal cost (Farhadian et al., 2007).

However, as always, no system is perfect and some disadvantages exist, such as the need for a longer start-up period time to develop necessary biomass and the fact that they are much more sensitive to the temperature value. This last feature means that heating is generally

necessary, at least under temperate climates when working in the thermophilic range (55-60°C) (Farhadian et al., 2001).

Several configurations of anaerobic bioreactors, such as horizontal anaerobic immobilized bioreactors (HAIB) (de Nardi et al., 2002; Cattony et al., 2005; de Nardi et al., 2005, 2006; Gusmão et al., 2006, 2007) and upflow anaerobic sludge blankets (UASB) have been used for bioremediation of waters contamined with monoaromatic compounds (Martínez et al., 2007). Here also, anaerobic biofilm reactors exhibit a higher potential for use than suspended growth biomass reactors. Reasons for that are the same as those claimed for aerobic systems (see above, Pedersen and Arvin, 1995).

Results and outcomes of reports for monoaromatic removal in anaerobic bioreactors are summarized in Table I.n. Data generated indicate that anaerobic biofilm processes are able to remove up to 95% - 99% monoaromatics from contaminated water. However, just like with aerobic processes, there is no discussion on pollutant sorption by solid supports (such as polyurethane) or on possible evaporation and stripping of these volatile organic compounds with the biogas produced.

Table I.n: BTEX removal from contaminated water through anaerobic bioreactors

Source of pollutant(s)	Type of bioreactor(s) and condition(s)	Analytical method(s)	Result(s)	Remark(s)	Reference(s)
BTEX and Ethanol (synthetic)	-Horizontal flow anaerobic immobilized biomass reactor (HAIB) with volume 2 L and at temperature range 28-32°C. -Media: polyurethane foam (5 mm in size and density 23 Kg/m^3) - Initial inoculum source was obtained from an UASB treating poultry slaughterhouse wastewater.	COD, pH, Alkalinity, NO_3^-, VFA BTEX through GC (head space), Microbial consortium (biofilm)	- Ethanol, was removed with an average efficiency of 83% at a mean influent concentration of 1185 mg/L. - A concomitant removal of 97% of nitrate was observed for a mean influent concentration of 423.4 mg/L. - BTEX removal efficiencies were of 97% at an initial concentration of benzene 41.4, toluene 27.8, ethylbenzene 31.3, m-xylene 28.4, o-xylene 28.5, p-xylene 32.1 as mg/L. -Hydrocarbons removal efficiencies were of 99% at an initial concentration of benzene 26.5 mg/L , toluene 30.8mg/L, m-xylene 32.1 mg/L , ethylbenzene 33.3 mg/L and BTEX 26.5 mg/L. - This system was shown to be an alternative for treating water and wastewater contaminated with nitrate, ethanol and monoaromatic hydrocarbons.	- There is not any result about gas analysis and evaluation of evaporation of BTEX at conditions 30°C and HRT 12hr. -There is not any discussion about adsorption of pollutant(s) by packing.	Gusmão et al. (2006 and 2007)
Toluene and Ethanol (synthetic)	- HAIB (length 1m , diameter 5 cm) at temperature range 28-32°C. - Media: Polyurethane (5 cm, density=23 Kg/m^3) -Media was previously inoculated with sludge taken from UASB reactors treating a poultry slaughterhouse wastewater.	COD, SO_4^{2-}, pH, VSS, HCO_3^-, VFA, CH_4, toluene by GC	- Organic matter removal efficiency was close to 95%with a maximum toluene degradation rate of 0.06 mg toluene/mg VSS.day and sulfate reduction was close to 99.9% for all nutritional amendments. Toluene and ethanol were added in range 2-9 mg/L and 170-960 mg/ L, respectively at HRT=12 hr, T=30 ± 2°C. - HAIB reactors under sulfate reducing conditions are a potential alternative for aromatics bioremediation.	-There is not any discussion about adsorption of pollutant(s) by packing and evaluation of toluene evaporation at 30°C and a HRT of 12h .	Cattony et al. (2005)

Table I.n (cont'nd): BTEX removal from contaminated water through anaerobic bioreactors

Source of pollutant(s)	Type of bioreactor(s) and condition(s)	Analytical method(s)	Result(s)	Remark(s)	Reference(s)
BTEX & Ethanol (synthetic)	-Two bench scale horizontal anaerobic immobilized bioreactor (length=100 cm, diameter=5 cm) at temperature range 29-31 °C. - Media: polyurethane foam matrices (size= 5mm , density=23 kg/m^3, porosity=0.95) - Initial inoculums source was obtained from an UASB treating poultry slaughterhouse wastewater.	COD, pH, Alkalinity, TVA, CH_4, CO_2, BTEX by GC-FID (Head space)	- For synthetic substrate with BTEX concentration 3-15 mg/L , COD removal percent was 96% at HRT=11.4hr , T=30 ± 1°C and BTEX removal efficiency varying from 75-99% were achieved during this experimental period. - At simulated actual field conditions BTX compositions were gradually raised to 15mg/L. -The best COD and BTX removal efficiencies achieved were about 99% and 95% respectively, with a HRT exceeding 12 hr. -A first-order kinetic model with correlation 0.994 fitted the experimental data.	-There is no discussion about adsorption of pollutant(s) by packing and no data about evaluation of BTEX evaporation at conditions 30°C and retention time11.4hr.	de Nardi et al. (2005 and 2006)
BTEX & Ethanol & LAS (synthetic)	- HAIB (volume 138mL) at temperature range 27-33 °C and pH 7.5- 8.2. -Media: polyurethane foam (density 23 kg/m^3) -Initial mixed culture taken from UASB reactors treating recycled paper industry wastewater, domestic sewage and poultry slaughterhouse wastewater.	COD, pH, Alkalinity, TS, VS, VFA, MPN, microbial morphology, BTEX by GC	- The inlet BTEX concentration ranged from 1.3-2.7mg/L of each compound and outlet concentrations were lower than 0.1mg/L for both the experiments with ethanol and LAS at HRT range 5- 13.5 hr.. - Methanogenic Archae were found to represent less than 0.5% of the total anaerobic organisms in the biomass inside the reactor. - The results indicated the viability of using the HAIB reactor for treatment of wastewater and groundwater contaminated with BTEX and gasoline.	-There is no discussion about adsorption of pollutant(s) by packing and no information about evaporation of BTEX at conditions 30°C and high retention time in bioreactor.	de Nardi et al. (2002)

Table I.n (cont'nd): BTEX removal from contaminated water through anaerobic bioreactors

Source of pollutant(s)	Type of bioreactor(s) and condition(s)	Analytical method(s)	Result(s)	Remark(s)	Reference(s)
Toluene and acetate (synthetic)	-Up flow anaerobic sludge blanket (UASB) with volume 1.4 L at temperature 30°C. - Initial inoculums of denitrifying sludge was used with two synthetic media contained the nitrogen and acetate and toluene as electron sources.	NO_3^-, toluene by GC-FID, acetic, N_2, CH_4, CO_2, N_2O	-The results indicated that simple UASB denitrifying reactor systems have promising applications for complete conversion of nitrate, toluene and acetate into N_2 and CO_2 with a minimal sludge production. -When acetate-C was the only electron source a dissimilative denitrifying process resulted as indicated by bicarbonate yield Y_{HCO3}, mg HCO_3^- produced/mg carbon consumed) of 0.74 and denitrifying yield (Y_{N2}, mg N_2 produced/mg NO_3^- -N consumed) of 0.89 .	- Bioreactor feed was kept at 5°C, but there is no data about toluene concentration in exhaust gas	Martínez et al. (2007)

I.2.4.4. Alternative systems

Use of peroxides

An alternative method to supply oxygen without aeration in an aerobic process is the injection of hydrogen peroxide into the medium (Shim et al., 2002; Vogt et al., 2004; Kulik et al., 2006; Menendez-Vega et al., 2007). Research carried out showed that this reagent was widely applied as a chemical oxidant (Ferguson et al., 2004; Okawa et al., 2005; Poulopoulos et al., 2006; Ferrarese et al., 2008) or also as an oxygen releasing source (Nam et al., 2001; Qi et al., 2004; Fischer and Hahn, 2005; Farré et al., 2006).

Hydrogen peroxide is soluble in water and can decompose to oxygen and water. The resulting dissolved oxygen should then be made available for microbial respiration (Vogt et al., 2004). Another kind of reaction is direct oxidation of organic compounds by this chemical compound in the presence of enzymes (peroxidases) or metal-based catalysts. In this case molecular oxygen is not evolved since it is consumed in the chemical reaction (Ferguson et al., 2004; Kulik et al., 2006; Ferrarese et al., 2008). When H_2O_2 is added to the polluted waters, as oxygen source in bioremediation processes or as chemical oxidation, it decomposes according to several mechanisms and reaction products can affect both biological and chemical processes (Buyuksonmez et al., 1998; Watts et al., 1999; Howsawkeng et al., 2001; Watts et al.; 2003).

The most common application of an abiotic transformation in water remediation has been the injection of hydrogen peroxide and catalysts ($FeSO_4$, $KMnO_4$). Iron (II) promotes the generation of hydroxyl radicals (OH·) through so-called Fenton-like reactions (1):

$$H_2O_2+Fe^{2+}\rightarrow Fe^{3+}+OH^-+OH\cdot \qquad (1)$$

Hydroxyl radicals are more effective in oxidizing of trichloroethylene (TCE), aromatic compounds, halogenated alkenes and petroleum hydrocarbons (Walling, 1975; Ferguson et al., 2004; Ferrarese et al., 2008). Also, H_2O_2 when catalysed by manganese oxide, generates superoxide anion ($O_2\cdot^-$), a highly reactive radical that seems to be able to degrade organic pollutants (Watts et al., 2003; Ferrarese et al., 2007).

In this remediation technique it may also be necessary to minimize hydrogen peroxide toxicity, as this reagent is known to be inhibitor of bacteria activity at concentration higher than 100-200 mg/ in bioremediation processes (Cunningham et al. 2001; Watts et al., 2003).

Fiorenza and Ward, 1997 reported that with microbial adaptation to hydrogen peroxide, concentration of this reagent can be increased to 500 mg/L for monoaromatics biodegradation.

Use of a solid adsorbent : activated carbon

Bioregeneration of activated carbon can be defined as a favorable alternative for pollutant removal from contaminated waters since in this process, pollutants can be adsorbed on the surface of carbon particles and then stabilized and bioregenerated by microorganisms. This technique provides a combined adsorption, desorption and bioremediation process, using activated carbon, to deal with a wide variety of pollution problems (Ivancev-Tumbas et al., 1998; Ha et al., 2000, Klimenko et al., 2004; Aktaş and Çeçen**,** 2007). Bioregeneration of activated carbons in aerobic biphasic bioreactors (containing solid waste and aqueous phase) has been well documented (Aktaş and Çeçen**,** 2007). Efficiency of this technique is dependent on several factors including biodegradability, characteristics of activated carbons, adsorbability and desorbability of sorbate, presence of nutrients and optimum environmental conditions for biomass metabolism, solute stripping, analytical methods for monitoring of pollutants and process configuration, and it can be optimized by varying the operational conditions. Aktaş and Çeçen**,** 2007 reported that future research is still required to optimum conditions (such as activated carbon types, nature of the microbial community, validity and efficiency of exo-enzymatic activities) for an efficient bioregeneration process.

I.3. Conclusion

Monoaromatic pollutants in groundwater are threatening drinking water resources and therefore have, when presented, to be removed. The analysis presented here suggests that in some case, naturally-occurring aerobic biodegradation phenomena can take place at a rate high enough to reach environmental standard limits in a reasonable time. However, the most common situation is that it is necessary to artificially improve the performances of this process. This approach corresponds to the so-called engineered in-situ bioremediation, which is most often really able to increase the rate of organic pollutant biodegradation.

It is also possible to make use of anaerobic approaches, since anaerobic microbial pathways able to fully decompose aromatic hydrocarbons do exist. Present data demonstrate that enhanced anaerobic bioremediation is already successfully applied in some areas contaminated with oil products. Data reported shows that some challenges related to

monoaromatics removal from contaminated groundwater by in situ bioremediation deal with the possibility of pollutant loss through volatilization and stripping (such as VOC stripping during air sparging in engineering bioremediation), dilution of contaminants in groundwater resources, and also ability of these hydrocarbon compounds to adsorb onto soil organic matter and soil mineral surface and that generally, these parameters are not addressed properly by researchers.

This review indicated that biological treatment can play a major role in the remediation of contaminated water by gasoline. Treatment efficiencies up to 99% and above, in terms of BTEX removal from the liquid phase, can be achieved by aerobic biofilm processes. Researches carried out up to now indicate that, generally, the maximum concentration of monoaromatic hydrocarbons in contaminated water by oil products is lower than 50 mg/L (Sauer and Costa, 2004). Therefore, initial concentrations of this pollutant for a bioremediation process are low when compared to industrial wastewater. The feasibility of microbial degradation of compounds such as monoaromatics can be considered as now well established. Results published up to now show that the productivities achieved for monoaromatic compounds removal from an aqueous phase are similar in aerobic and anaerobic processes and close to 20 mg/L.h and in an biphasic bioreactor, degradation rate can increased to 200 mg/L.h.

The chemical properties of monoaromatic compounds demonstrate that phenomena such as stripping and volatilization can significantly contribute in a non-negligible amount to their removal from the liquid phase. It is important to realize that this phenomenon is likely to occur both in aerobic and anaerobic processes. Hence, simple calculations from data in Table I.b show that, for example, a gas phase at equilibrium with a saturated aqueous solution of benzene would contain a concentration of this compound as high as 5.1 mol/m^3 (1.5 mol/m^3 for toluene). It must be emphasized that mass balances corresponding to phenomena taking place during bioremediation processes are often incomplete, which means that their efficiency, which is generally expressed as pollutant loss from the liquid, must be re-evaluated in terms of global depollution, i.e. by taking into account all phases of the system (liquid, gas and solid). Hence, a non-negligible part of the pollutant is in fact only displaced from the liquid to the gas and thus still present in the environment.

Chapter II

Material and Methods

II.1. Chemicals and materials

Monoaromatic compounds such as benzene (99%) and xylenes (mixture of isomers) were purchased from Sigma-Aldrich (France) and toluene (99.5%) was obtained from Merck. Mineral salts and other chemical compounds in analytical grade were provided from Acros Organics (Belgium). The deionized water (EC<2 µS/cm) used for preparing working solutions was purified by a Millipore (Elix 5, USA) water purification system.

Syringe microfilters used for filtration experiments were all disposable, hydrophilic, non sterile, 13 mm in diameter and had a porosity of 0.45 µm. Three materials were tested, nitrocellulose (NC, Minisart, Sartorius, France), polyvinylidene fluoride (PVDF, Millex HV Syring Filter unit, Millipore, USA) and polypropylene GH Polypro membrane (GHP, Acrodisc Syringe Filters, Pall Life Sciences, USA). The volume filtered was 10 mL.

Granular activated charcoal (GAC) with particle size 4-8 mm and surface area 950-1050 m^2/g from Fluka (France) was rinsed with distilled water to remove fine powder prior to use.

II.2. Apparatus

II.2.1. pH meter

pH and temperature of samples were measured using a combined probe (Mettler Toledo Lab 412) connected to a digital pH meter Jenway 3310 (UK).

II.2.2. Spectrometer

A Safas UV mc^2 (Monaco) spectrophotometer was used for the determination of the optimum wavelength for detection of monoaromatic compounds and optical density of biological samples.

II.2.3. Centrifugation

Separation of microorganism and BTX behaviour were performed using a Sigma type 3K30 (Bioblock Scientific, France) centrifuge operated in various conditions (such as temperature, time and rotation speed).

II.2.4. Microscope

The basic morphological characteristics of inocula in liquid medium were examined by phase-contrast microscopy. Microscope (OLYMPUS BX41TF, Japan) was equipped with a CCD camera (*Kappa Opto-Electronics GmbH*, Germany). A commercial software package

(Image-pro plus, version 4.1.0.0) was used for CCD image acquisition. Approximately 10 µl of inoculm solution was applied to the interface of a cover glass and a glass slide.

II.3. Methods

II.3.1. Aqueous phase

II.3.1.1. Monoaromatics analysis by HPLC

Monoaromatic hydrocarbon compounds in aqueous phase were analysed through HPLC. Liquid chromatography was performed on a Waters system (USA). Chromatographic separation of BTX compounds was carried out on a Supelco Discovery C8 (4.6×150 mm, 5 µm, Intact) silica based reverse phase column. Column temperature was kept at 35 °C through a column heater module. The mobile phase reservoirs contained methanol and water with volume percent 60/40 and the flow rate was 1 ml/min. Compounds were detected using a UV/Vis detector (Waters 2487 dual λ absorbance detector). The volume of the injection loop was 50 µL (RHEODYNE, USA). The system was also consisting of a Waters 515 high pressure pump, a degasser for solvent (Waters In line model IDL) and a HPLC guard cartridge system (Security Guard Phenomenex, Analytical KJO-4282). Typical HPLC operating pressure was approximately 105 bars at the oven temperature. A personal computer with Agilent HPChem software (Chem station) was used to control the system and record data.

Sterile amber glass bottles with Teflon lined cap and 500 mL total volume were used as containers for standard samples preparation. They were completely filled with water (pH adjusted to 2 by 37% w/w hydrochloric acid) in order to eliminate any gas phase in the system and kept chilled at 4°C for 2 hr. The solutes were then added and the resulting solutions were homogenized by a magnetic mixer during 30 min. All standard samples were prepared in duplicate and were analyzed within 2 hr by HPLC.

II.3.1.2. Organic acids quantification

Organic acids were analyzed using a Hewlett-Packard HPLC (LC-1100 Series, USA) equipped with auto sampler (G1329A), quaternary pump (G1311A), vacuum degasser (G1322A) , and refractive index detector (RID-G1362A). The column used was Phenomenex (Rezex ROA-Organic acid H^+, USA) with 8 µm porosity (300mm × 7.8 mm ID) and it protected by a Security Guard Cartridge (Phenomenex, Inc., USA). Solvent was sulfuric acid (0.005 M) and flow rate of mobile phase was 0.7 mL/min. Temperature of column was kept in 50°C, and injection volume was 10 µL. Typical HPLC operating pressure was approximately

62 bars at the oven temperature. A personal computer with Agilent HPChem software (A.08.03) was used to control the system and record data. Organic acid standards were purchased from Fluka (France), and individual organic acids were quantified on the basis of the external standard method.

II.3.1.3. DO, pH, temperature and EC analysis

Analysis of dissolved oxygen (DO as mg/L), pH, temperature and electrical conductivity (EC as µS/cm) of water samples were measured by universal pocket meter (WTW MultiLine P4, Germany). pH electrode (SenTix 41) was combined with integrated temperature probe. Also, conductivity cell and DO probe were TetraCon® 325 and CellOx 325, respectively.

II.3.1.4. Nitrate and nitrite analysis

Nitrate and nitrite concentrations were determined using a colorimetric assay (Hach DR/890 colorimeter, Hach Company, Loveland**,** USA) which requires a sample volume of 10 ml (Figure II.1). Nitrate was quantified using the cadmium reduction method (Hach Method 8039) and nitrite, using the ferrous sulfate method (Hach Method 8153). The detection ranges for nitrate and nitrite were 0-132 and 0-150 mg/L, respectively.

Figure II.1: Portable colorimeter instrument (Hach-DR/890)

Method 8039 utilizes cadmium to reduce nitrate present in a sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt which couples to 2,5-dihydroxybenzoic acid to form an amber-colored product. Method 8153, ferrous sulfates in an acidic medium reduces nitrite to nitrous oxide. Ferrous ions

combine with the nitrous oxide to form a greenish-brown complex in direct proportion to the nitrite present.

II.3.1.5. Hydrogen peroxide analysis

The total hydrogen peroxide content present in the solution after the reaction was measured by a test kit (Hach, Model HYP-1, Cat. no. 22917-00)**.** The detection ranges was 0.2-10 mg/L.

The sample was acidified and the reaction was catalyzed by the addition of the ammonium molybdate solution. The reagent was added to provide iodide and starch. The hydrogen peroxide was reduced by the iodide to produce water and free iodine. Sodium thiosulfate ($Na_2S_2O_3$) was then used to titrate the iodine to a colorless end point. Starch enhances the determination of the end point by producing a color change from dark blue to colorless. The amount of hydrogen peroxide in the sample is calculated from the quantity of sodium thiosulfate added. The reactions involved are:

$$H_2O_2 + 2\,I^- + 2H^+ \longrightarrow I_2 + 2H_2O$$

$$I_2 + 2\,Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2\,NaI$$

II.3.1.6. Volatilization tests

Monoaromatic solutions were poured into glass vessels that were placed under controlled temperature in the laboratory. The vessels were glass backer (Pyrex type) with 10.5 cm in diameter and with 1 L volume, and the liquid depth was 6.5 cm. The volatilization rates of the monoaromatic hydrocarbons in water samples were determined by analyzing the residual concentrations of BTX compounds in the solution by HPLC during two days.

II.3.1.7. Stripping experiments

Studies on stripping of monoaromatic hydrocarbons from synthetic polluted water were carried out using a mechanically stirred, thermostatted bioreactor of 2 L working volume (Biostat MD, B. Braun, Melsungen, Germany). The gas flow rate was monitored by a mass flow controller.

II.3.2. Organic phase: monoaromatic analysis by GC/MS

Identification and quantification of toluene in n-hexadecane (organic phase) was done by GC (6890, Agilent Technologies) equipped with a MS detector (5973, Agilent

Technologies) and auto sampler (7683 Series, Agilent Technologies, USA) and separated on an polar capillary column (HP-5MS, 30 m × 0.1 mm i.d., 0.25 μm coating thickness).The carrier gas was ultra-purified helium (99.999%) and the oven temperature was kept at 80 °C for 3 min, then raised with rate 30 °C/min to 300 °C and held for 1min. The injector and detector temperatures were 250 °C for both; the split ratio for injection was 1:5. Injection volume was 1μL. Mass spectrometry conditions involved an electron-impact ionization energy of 70 eV, an accelerating voltage of 1.1 kV, an emission current of 35 μA, and quadrupole, ion source and interface (line transfer) temperatures of 150, 230 and 280 °C, respectively. Data were analyzed using MSD ChemStation software (G1701CA, Agilent Technologies). Identification of monoaromatic compound was confirmed by comparison of collected mass spectra with those of authenticated standards and spectra of the National Institute for Standards and Technology (NIST) mass spectral library (version 1.7a, 2000).

II.3.3. Solid phase

II.3.3.1. Adsorption isotherm studies

The equilibrium isotherm for monoaromatic adsorption from an aqueous solution on GAC was determined under controlled temperature conditions. Different amount of activated carbon (in range 0.1-1 g) were weighted and added into each bottle (1 L). The bottles were then filled with monoaromatics solution, covered with Teflon lined caps and stirred with magnetic stirrers at the maximum speed. Based on preliminary results, the duration of the equilibrium experiments in the isotherm bottles was selected to be 1 day. In order to avoid any solute volatilization during the course of experiments, no head space was left above the liquid.

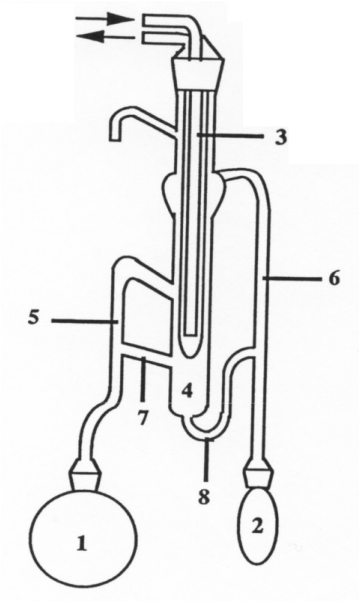
II.3.3.2. Desorption isotherm studies

Desorption isotherm experiments were conducted at 24 °C to determine the degree of monoaromatic adsorption irreversibility on granular activated charcoal. GAC were loaded with different amounts of monoaromatic compounds in glass bottles (1 L) covered with Teflon lined caps following the procedure described earlier for the adsorption isotherm studies. Upon equilibrium, and measurement of the liquid-phase monoaromatic concentration in each bottle, the activated carbon was separated from the solution and rinsed into the empty bottle using distilled water. The bottles were then filled with pure water leaving no headspace and covered with Teflon lined caps. The slurry was stirred for minimum 1 to 2 days to ensure equilibration and liquid phase monoaromatic concentration was measured again.

II.3.3.3. Monoaromatic extraction from GAC

II.3.3.3.1. Simultaneous distillation-extraction

The monoaromatic hydrocarbons were extracted by simultaneous distillation–extraction (SDE) using a modified Likens–Nickerson apparatus (Nikelson and Likens, 1966). For SDE extraction, 0.5g of GAC loaded with BTX compounds (according to procedure described par. II.3.3.1) and 20 ml of distilled water (or in preliminary tests 20 mL monoaromatic solutions) were placed in the large distilling flask and in second flask 2 ml dichloromethane (DCM) was used as the organic phase (Figure II.2). The extractions were carried out at atmospheric pressure. The sample was kept at 100 ± 2 °C, the solvent at 70 ± 2 °C and the condensation system at -4.0 ± 0.5 °C. Period of SDE was 2 h. These operating conditions were defined after optimization. Then BTX compounds in DCM were analyzed by GC/FID. The gas chromatographic analyses were carried out on a HP 4890A (Hewlett Packard, USA) equipped with a flame ionization detector (FID). The GC was equipped with a SupelcoWax 10 capillary column (30 m × 0.32 mm × 0.5 μm). The oven temperature was initially kept at 27°C for 2min, then programmed at 10 °C/min up to 110°C and held for 0.5min and finally increased to 230°C at 15 °C/min and kept for 15 min. The injector and detector temperatures were set at 210 and 300°C, respectively. Both carrier and make-up gases were nitrogen (99.99995 % purity) at 2 and 8 mL/min, respectively. Split ratio and injection volume were 5:1 and 1μL, respectively. Also, internal standard was cyclooctane (5μL in 20 mL DCM). The compounds were identified by comparison with the retention time of the standards and, to avoid any doubts, the standard addition method was employed for peak verification.



1- Aqueous and solid phase
2- Organic phase
3- Condensation system
4- Organic and aqueous phase separation
5- Vapor tube for aqueous phase
6- Vapor tube for organic phase
7- Return of aqueous phase
8- Return of organic phase

Figure II.2: Simultaneous distillation–extraction apparatus

II.3.3.3.2. Soxhlet extraction

The Soxhlet apparatus (Büchi Extraction System B-811, 1250 W, Switzerland) with a standard procedure (Soxhlet standard) was applied in solid-liquid extraction. Extraction in this apparatus (Figure II.3) was selected with two main steps involving a boiling and a rinsing step. In the first step, 75 mL dichloromethane (DCM as solvent) was evaporated (in 40°C) and condensed in the condenser (-4.0 ± 0.5°C). This procedure was programmed for 5 cycles. During this step, DCM was passed into the sample holder containing 0.5 gram activated carbon loaded with monoaromatic compounds (see II.3.3.1.). Then in rinsing step, when the solvent level reached the optical sensor the glass valve opened and the solvent containing dissolved analyte returned into the solvent cup.

Figure II.3 : Soxhlet apparatus

II.3.3.3.3. Ultrasonic extraction

After adding 40 mL of DCM to 0.5 gram GAC loaded by toluene hydrocarbons (described par. II.3.3.1.) in a glass vial, the resulting mixture was ultrasonicated using an ultrasonic probe (IKA LaborTechnic, U 50control, 230V, 50/60 Hz, 50W, Germany). The sample was sonicated 3 times of 3 min each with DCM as solvent in continuous power mode (Llompart et al., 1997). Then supernatant obtained from extraction sample was analysed by GC/FID (described par. II.3.3.3.1.).

II.3.3.3.4. Pressurized microwave-assisted extraction

Extraction using a pressurized microwave assisted (MARS system, XP-1500 plus™, USA) was performed using 0.5 GAC loaded by toluene hydrocarbons (described par.

II.3.3.1). The samples were irradiated for 10 min with 30 mL dichloromethane (DCM) as solvent. Microwave energy output and temperature range used were 400W and 115 °C, respectively. These operating conditions were obtained after 3 series tests. This system (Figure II.4) allowed up to 14 extraction vessels to be irradiated simultaneously. Also, it is equipped with a solvent alarm to call attention to an unexpected release of flammable and toxic organic solvent. Solvent losses were checked in several experiments and were found below 1%. All the sample vessels were held in a carousel that was located within the microwave cavity. Each vessel has a vessel body and an inner liner. The liner is made of polytetrafluroethylene (Teflon-PTFE).

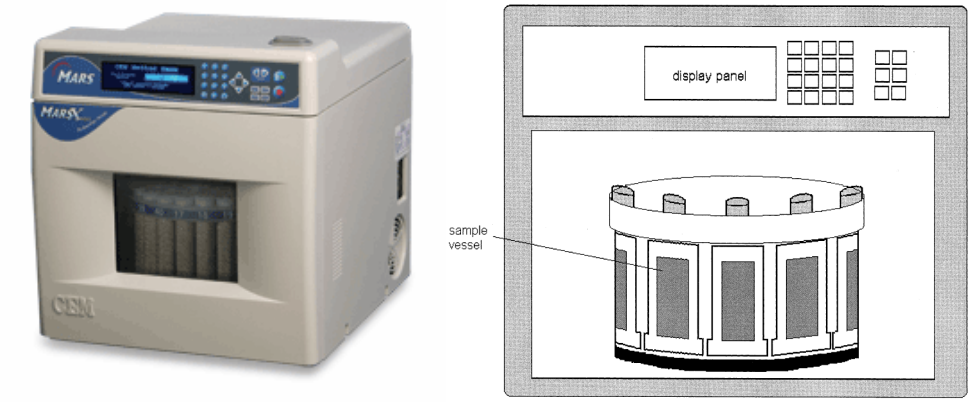


Figure II.4: Schematic of a pressurised microwave assisted extraction

II.3.3.3.5. Extraction by carbon disulfide

NIOSH standard technique (method 1501, 2003) was used for desorption of monoaromatic compounds from granular activated charcoal using disulfide carbon as solvent extraction. Granular activated charcoals (within the limits of 0.5g) were loaded with different concentrations of toluene in glass bottles (1 L) covered with Teflon lined caps (following the procedure described par. II.3.3.1.). After equilibrium, and toluene analysis in the aqueous phase in each bottle, the activated carbon was separated from the solution and transferred into the empty vials (40 mL). The vials were then filled with CS_2 without headspace and covered with Teflon lined caps. The slurry was homogenized by a magnetic stirrer for 30 minute at 20°C. Then 17 mL of supernatant (containing toluene and carbon disulfide) was transferred into glass tube (Corex tubes, USA) with Telfon caps and 5µL cyclooctane, used as internal standard. Monoaromatic compound in carbon disulfide was measured by GC/MS (using the system described par. II.3.2.1.). The oven temperature program was: initial temperature isothermal at 40 °C for 10 min, then from 40 to 230°C at 10°C/ min. Injector and detector

temperature were 250 and 300°C, respectively. Also, injection volume was 1.0 µL with split ratio 5:1.

II.4. Biological process

II.4.1. Biomass concentration

Biomass concentration was measured as optical density (OD) at 600 nm using a spectrophotometer (described par. II.2.2). The dry mass measurement was obtained by 5-20 mL of well mixed samples on to a predried (24h in a drying oven 105°C) Millipore membrane filter (0.45 µm pore size, diameter 4.5 cm, HA, hydrophilic, France), positioned in a vacuum filtration apparatus (Knf NEUBERGER, NSE 800, Germany). After filtration, the filter and solids were replaced in the drying oven, and dried to constant weight (24h at 105°C). Then the filters were allowed to cool to room temperature in a desiccator and reweighed. The dry weight of the residual solids was calculated as the difference between the weight of the filter before and after use. Cell dry weight was conducted according to Standard laboratory procedure (Clesceri et al., 1998). Also, biomass concentration was estimated from a correlation between OD and cell dry weight.

II.4.2. Samples treatment in biological processes

Filtration tests (see par. II.1 for filters used) were performed with 10 mL of sample. Centrifugations were performed using either centrifuge glass tubes (Corex tubes, USA, total volume 15 mL) or 10 mL poly tetra fluoro ethylene tubes (PTFE, FEP Oak Ridge tubes fitted with a screw top, Nalgene, USA).

II.4.3. Aerobic bioreactors

II.4.3.1. SBR biological process

Aerobic sequencing batch reactors (SBR) were incubated with an adapted biomass. It was obtained from an activated sludge collected in the domestic wastewater treatment plant of Riom, France. It was grown in the presence of BTX with successive transfers in a fresh medium each week during 3 months. The working volumes of SBR bioprocesses in Pyrex-glass bottles were 250 mL and total volume was 500 mL. A Gerhardt LABOSHAKE LS 500/RO 500 rotating shaker was used at room temperature and 150 rpm. Nutrient solution contained KH_2PO_4 85, $Na_2HPO_4.2H_2O$ 334, NH_4Cl 5, $CaCl_2.2H_2O$ 36.4, $MgSO_4.7H_2O$ 22.5, $FeCl_3.6H_2O$ 0.25 as mg/L according to OECD standard method. Initial BTX and dry biomass concentration used in aerobic bioreactors were in the range of 50-150 and 50-1000 mg/L,

respectively. Hydraulic retention time of bioreactors was between 8-30 hr and pH of samples was in the range of 7.45-7.55.

II.4.3.2. Biphasic bioreactor

II.4.3.2.1. Microorganism

Pseudomonas fluorescens (NCIMB 11671) was preserved in LGCB laboratory as a frozen suspension in liquid medium at -75 °C using the Protect Bacterial Preserver system (Technical Service Consultants Ltd., Heywood, Lancashire, UK) which is made of porous ceramic beads immersed. It was grown on Tryptic Soy Agar (Tryptone Soya Agar, Fluka) poured in Petri dishes held at 30 °C for 24 h. Successive replications were performed every 3 days.

II.4.3.2.2. Preculture

Precultures were prepared in Erlenmeyer flask of 500 mL volume filled with 250 mL "*Pseudomonas* Basal Medium" (Cohen-Bazire et al., 1957) containing 0.25g $(NH_4)_2SO_4$, 5mL of Hutner solution, 10mL of solution A , 235mL dionized water and with 4 g/L of glucose as carbon source (See Table II.a). Flasks were inoculated with individual colonies picked out an agar dish. After 24 h growth at 30 °C in a rotary shaker (Infors HT, Switzerland) operated at 200 rpm the optical density at 600 nm (OD_{600}) of the media was close to 4.0.

Table II.a: *Pseudomonas* Basal Medium

Solution A		Basal medium	
KH_2PO_4	33.12 g	$(NH_4)_2SO_4$	0.25g
K_2HPO_4	26 g	Hutner solution	5 mL
Distilled water	1000 mL	Solution A	10 mL
		Distilled water	235 mL
Trace element solution		**Hutner solution**	
Na_2EDTA	250 mg	$CaCl_2.2H_2O$	4.92 g
$FeSo_4 . 7H_2O$	356 mg	$Na_2MoO_4.2H_2O$	15.6 mg
$Zn\ So_4 . 7H_2O$	680 mg	$FeSO_4. 1.5H_2O$	78.4 mg
$MnSo_4 . H_2O$	154 mg	$MgSO_4.7H_2O$	33 mg
$CoCl_2 . 6\ H_2O$	20.3 mg	Nitrilotriacetic acid	10 g
$CuSo_4 . 5\ H_2O$	39.2 mg	Trace element solution	55.6 mL
$Na_2\ B_4O_7 . 10\ H_2O$	17.7 mg	Distilled water	1 L
Distilled water	100 mL		

II.4.3.2.3. Culture

Twenty milliliters of preculture were used to inoculate 500 mL Erlenmeyer flasks. The medium was made of 250 mL "*Pseudomonas* Basal Medium" with 12.5 mL of n-hexadecane. Then monoaromatic substrates were added to the organic phase as energy and carbon source. The agitation rate was maintained at 200 rpm, the temperature at 30°C.

II.4.4. Anaerobic bioreactor

II.4.4.1. Inoculum

Thauera aromatica K172 (DSM 6984) was obtained from the German Resource Centre for Biological Material (Braunschweig, Germany).

II.4.4.2. Biomass production

II.4.4.2.1. Preculture

This strain was cultivated in a mineral medium (see Table II.b), with 0.72 g/L sodium benzoate as carbon and energy source and 2 g/L KNO_3 as terminal electron acceptor. According to DSMZ protocol (http://www.dsmz.de) for medium preparation, solutions A and B (Table II.b) were adjusted to pH= 7.2, then autoclaved separately and combined after cooling. After mixing of solutions, 10 ml of sterile trace elements and 5 ml vitamin solution added (see Table II.b).

II.4.4.2.2. Culture in the batch systems

Culture was grown to optical density at 600 nm (OD_{600}) of 0.8 at temperature 30°C. Then cells were harvested by centrifugation (Jouan KR 22i, France) for 10 min at 10000 ×*g* in 10 °C. The resulting cell pellets were resuspended in the mineral salts medium (containing KH_2PO_4 0.816, K_2HPO_4 5.92, NH_4Cl 0.53, $MgSO_4$.$7H_2O$ 0.2, $CaCl_2.2H_2O$ 0.02 as g/L) with pH 7.2 to an OD_{600} range of 4.5-10 (biomass concentration close to 1.5- 3.5 g/L). This biomass was immediately used for Infors anaerobic bioreactors (HT Multifors, Switzerland).

Table II.b: *Thauera aromatica* medium

Solution A		Solution B	
KH_2PO_4	0.816 g	NH_4Cl	0.53 g
K_2HPO_4	5.92 g	$MgSO_4 . 7 H_2O$	0.2 g
Distilled water	500 mL	KNO_3	2 g
		$CaCl_2 . 2 H_2O$	0.025 g
		Na-benzoate	0.72 g
		Distilled water	500 mL

Trace element solution		Vitamin solution	
HCl (25%; 7.7 M)	10 mL	Vitamin B_{12}	50 mg
$FeCl_2 . 4 H_2O$	1.5 g	Pantothenic acid	50 mg
$ZnCl_2$	70 mg	Riboflavin	50 mg
$MnCl_2 . 4 H_2O$	100 mg	Pyridoxamine-HCl	10 mg
H_3BO_3	6 mg	Biotin	20 mg
$CoCl_2 . 6 H_2O$	190 mg	Folic acid	20 mg
$CuCl_2 . 2 H_2O$	2mg	Nicotinic acid	25 mg
$NiCl_2 . 6 H_2O$	24 mg	Nicotine amide	25 mg
$Na_2MoO_4 . 2 H_2O$	36 mg	α-lipoic acid	50 mg
Distilled water	990 mL	p-aminobenzoic acid	50 mg
		Thiamine-HCl . $2 H_2O$	50 mg
		Distilled water	1 L

II.4.4.2.3. Culture in the bioreactor

Biomass production in the anaerobic bioreactor was carried out in Biostat ED (B.Braun, Melsungen, Germany) equipped with pH, temperature and dissolved oxygen sensors and 4 L working volume (Figure II.5). *Thauera aromatica* medium (See Table II.b) was added to bioreactor and after degassing of medium by nitrogen (200 mL/min, 15 min), it was inoculated with 400 mL of preculture (10 % v/v). The temperature of bioreactor was controlled at 30°C and one day after of inoculating, the stirring rate was maintained at 50 rpm, and pH was controlled at 7.2 using NaOH (1 M) and H_2SO_4 (1M) solutions. In this process other parameters such as biomass productions, intermediate organic acid products, and nitrate and nitrite consumption by sampling were controlled. Also, the gas exhaust of the bioreactor was passed from a condenser (controlled at 4°C) connected to the reactor and CO_2 production rate were monitored by a gas analyzer (Servomex Xentra 4100, Servomex Company Inc., Nerwood, USA). Then effluent gas was bubbled into an 800 ml, magnetically stirred, 0.5 M KOH solutions for quantification of CO_2 production.

The reaction involved was:

$$2KOH + CO_2 \longrightarrow CO_3^{-2} + H_2O + 2K^+$$

The carbonate content of the mixture was periodically determined using HCl (0.127 N) in a titration method (Vogel, 1961). In this process, biomass concentration was increased to 1.5 g/L during 40 h by feeding of carbon source (Na-benzoate, 100g/L) and nitrate source (KNO_3, 100g/L) under controlled conditions step to step (fed-batch).

Figure II.5: Biomass production in anaerobic bioreactor (Biostat ED)

II.4.4.3. Monoaromatic bioremediation

Toluene biodegradation in anaerobic bioreactors by *Thauera aromatica* K172 was investigated under different conditions containing monophasic and biphasic media. Monophasic bioreactor was containing synthetic water contaminated by toluene, nitrate source and biomass where in biphasic media an organic phase (hexadecane) or solid phase (GAC) was presented in the bioreactor.

II.4.4.3.1. Monophasic bioreactor

In this experimental tests, two types of bioreactor containing Infors (500 mL, HT Multifors, Switzerland) and Biostat ED (4 L, B.Braun, Melsungen, Germany) equipped with pH, pO_2 and temperature sensors and condenser for exhaust gasses (Julabo LABORTECHNIK, EC, Germany) were used. In infors bioreactors (Figure II.6) concentrated bacteria (described par. II.4.4.2.2.) was used and then nitrate solution (50-100 g/L) was added by pump and toluene injected to bioreactor using micropipette (25µL, Hamilton-Bonaduz, Switzerland) through Teflon septum.

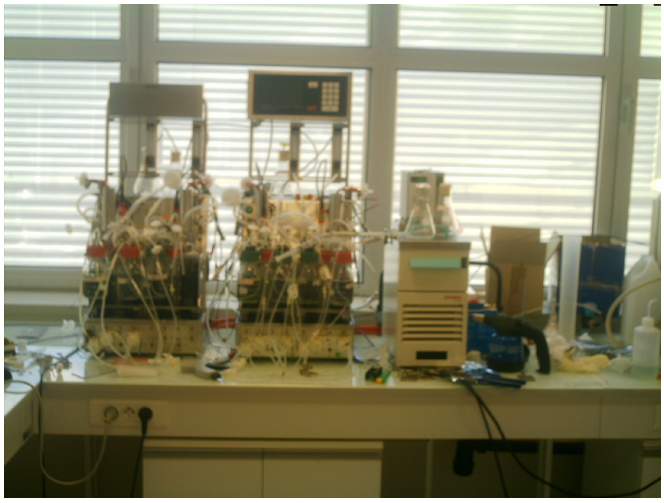


Figure II.6: Monoaromatic removal in anaerobic bioreactor (HT Multifors)

Also, a series studies was carried out in Biostat bioreactor after biomass production in bioreactor. Also, toluene was injected into bioreactor in controlled conditions by HPLC micro pump (100µL/min, Water 501, USA).

II.4.4.3.2. Two phase partitioning bioreactor

In this study an anaerobic reactor (Biostat ED, B.Braun, Melsungen, Germany) with two phase partitioning bioreactor (TPPB) was applied for treatment of monoaromatic hydrocarbon contaminants. Hexadecane (800 mL) was added to reactor after biomass production and toluene was injected into by micro pump. Organic phase was also well mixed by a devoted Rushton turbine.

II.4.4.3.3. GAC bioregeneration

Two anaerobic bioreactors (HT Multifors, infors, Switzerland) with 500 mL working volume containing 500 mL of concentrated biomass and 2.5 g GAC loaded with toluene hydrocarbon (100 mg toluene/g GAC) were used. For monitoring of toluene consumption in GAC, particles were separated in five Teflon bags (5 × 0.5g) with porosity 100µm and size 1 cm × 2 cm. The pH at 7.2 was controlled in experiments. Also, temperature and stirring rate were regulated at 30 °C and 50 rpm, respectively.

II.4.5. Biodegradability tests through OxiTop assay

Manometric respirometric tests were carried out with the WTW OxiTop Control system (Figure II.7). The respirometric method is based on pressure measurement in a closed bottle under constant temperature. Oxygen was consumed during the degradation of the

organic matter and CO_2 gas released was adsorbed from the gas space by caustic soda (NaOH) so that the resulting pressure decline is a measure of the biological oxygen demand. Also, samples in the bottles were sealed with a cap containing an electronic pressure indicator and continuously mixed. The BOD value was calculated from the following equation (1):

$$BOD\ (mg/\ L) = M\ (O_2)/RTm \cdot [(V_{tot}-V_l)/V_l + \alpha Tm/T_0] \cdot \Delta p\ (O_2) \qquad (1)$$

where M (O_2) is the molecular weight of oxygen (32000 mg/ mol), R the gas constant (83.144 L.hPa /mol. K), Tm is the measuring temperature (K), T_0 is 273.15 K, V_{tot} is the bottle volume (mL), V_l is the liquid phase volume (mL), α is the Bunsen absorption coefficient for oxygen (0.03103) and Δp (O_2) is the difference in partial oxygen pressure (hPa) as given by OxiTop. The nutrient solution used for these assays contained KH_2PO_4 85, $Na_2HPO_4.\ 2H_2O$ 334, NH_4Cl 5, $CaCl_2\ .2H_2O$ 36.4, $MgSO_4\ .7H_2O$ 22.5, $FeCl_3\ .\ 6H_2O$ 0.25 as mg/L in accordance with the OECD standard method. Also, mixed culture biomass after adaptation with BTX compounds was taken from aerobic sequencing batch bioreactor and used as inoculums for OxiTop tests (described par. II.4.3.1.).

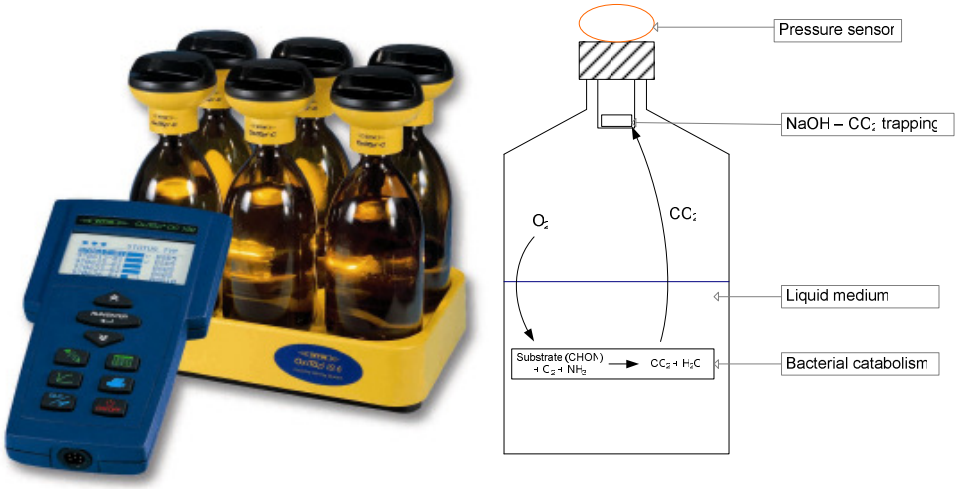


Figure II.7: OxiTop system

II.5. Data analysis

Standard errors on means and slops were calculated using the standard Microsoft Excel spreadsheet routines.

Chapter III

Accurate monoaromatics quantification in aqueous samples containing biomass

III.1. Objectives

HPLC with UV or UV-visible detection is characterized by its high efficiency, high speed, high sensitivity and wide application range (Vogt et al., 2000; Kim et al., 2006). In this method, BTX contaminated water can be directly injected without any pre-treatment. It has been reported that wavelengths (λ) such as 206 nm (Morasch et al., 2002), 230nm (Yadav and Reddy, 1993) and 254nm (Kelly et al., 1996; Zepeda et al., 2006; Enright et al., 2007) could be used for detection and analysis of monoaromatic components in aqueous samples. For example, Kelly et al. reported that BTX compounds could be analyzed by HPLC with the following operating conditions: C18 column, solvent water/methanol (40/60 volume %), flow rate 1 mL/min, λ= 254 nm, injection loop 50 or 100 µL.

Samples taken from bioremediation process can not be injected directly to analysis instrument as biomass cause obstruction of injection module, guard cartridge system or column. Different techniques such as filtration and centrifugation are usually used for biomass separation from biological samples. But the main problems were related to monoaromatic loss due to evaporation, stripping and adsorption during the performance of these separation techniques. Physical and chemical properties of volatile aromatic compounds (Table I.b) are important characteristics which help predict the behaviour of these compounds during biomass separation. This section reports a protocol which enables to obtain the true quantitative content of volatile and easily adsorbable solutes in a liquid suspension of solids.

The objective of this work was to build a protocol enabling the separation of biomass from an aqueous suspension with minimal solute loss. A search for the minimization of the detection limit for benzene, toluene and xylenes using HPLC equipment is also reported. Globally speaking, the aim of this research is to define a HPLC-based protocol for accurate sampling, detection and determination of BTX compounds during a bioremediation process.

III.2. Wavelength selection for HPLC analysis

A wavelength of 254 nm has been routinely reported to be used for detection of BTX compounds by HPLC (Kelly et al., 1996; Zepeda and Texier, 2003; Spima et al., 2004; Zepeda et al., 2006; Enright et al., 2007). Curves in Figure III.1 clearly indicated that maximum absorbances of benzene, toluene and mixed xylenes in aqueous phase were in fact 205, 208 and 210 nm, respectively. A second peak of absorption could be evidenced at 254 nm, but with a much lower signal.

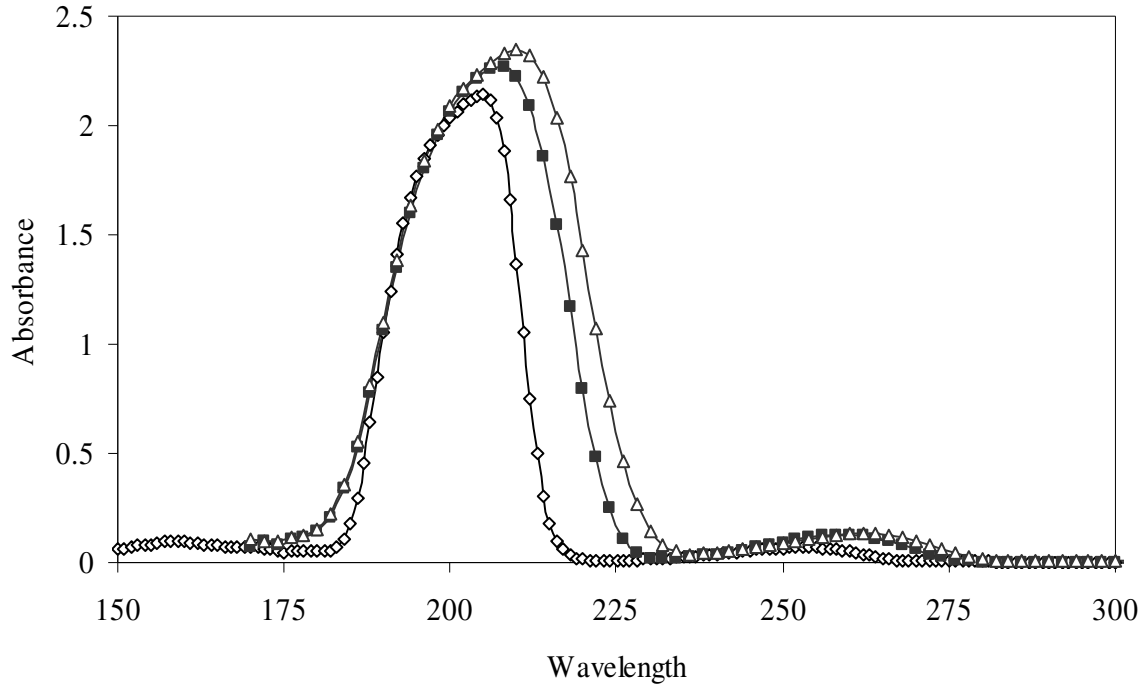


Figure III.1: UV Absorbance vs. wavelength for synthetic BTX contaminated water (Benzene= 32.2, toluene= 38.6 and mixed xylenes= 47.7 mg/L, temperature= 23 °C, pressure= 965 mbar) (-◊- : benzene; -■-: toluene; -Δ-: xylenes)

This test showed that a wavelength of 208 nm could be considered as an optimal value to get a maximal sensitivity for detection of BTX compounds in water samples by HPLC. This was clearly seen on chromatograms of Figure III.2 and calibration curves presented in Figure III.3 showed that sensitivity of detection at 208 nm was 37, 63 and 43 times higher than at 254 nm for benzene, toluene and mixed xylenes, respectively. It was also possible to demonstrate a linear relationship (with regression coefficient greater than 0.99) between results obtained at the two wavelengths (Figure III.4).

Minimum detection limits using 208 nm wavelength were 5, 4 and 10, as µg/L, for benzene, toluene and mixed xylenes, respectively. These values were significantly lower than literature data which reported minimum detection limits for monoaromatic compounds through HPLC close to 30, 31 and 50 µg/L for benzene, toluene and xylenes, respectively (Kelly et al., 1996).

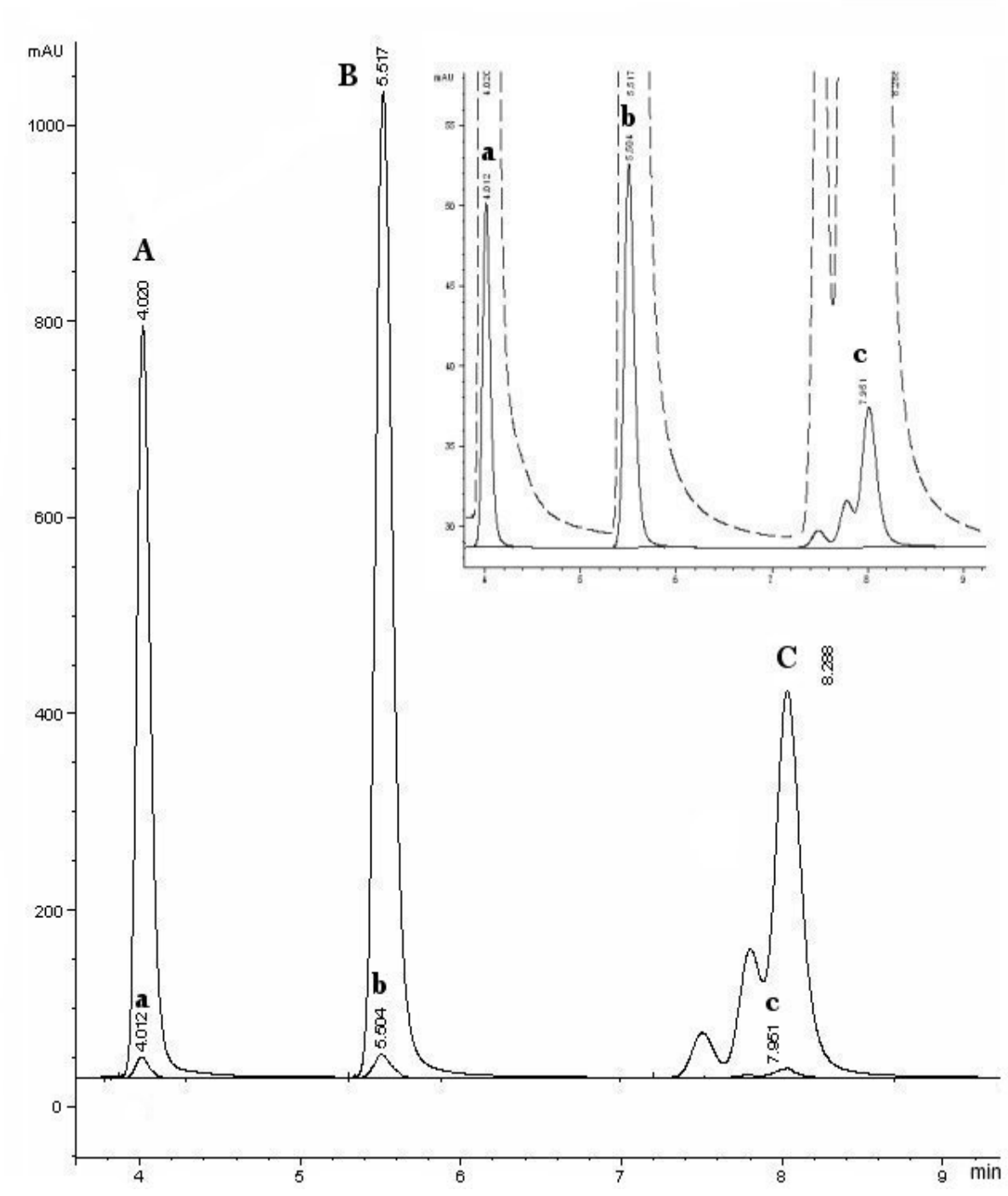
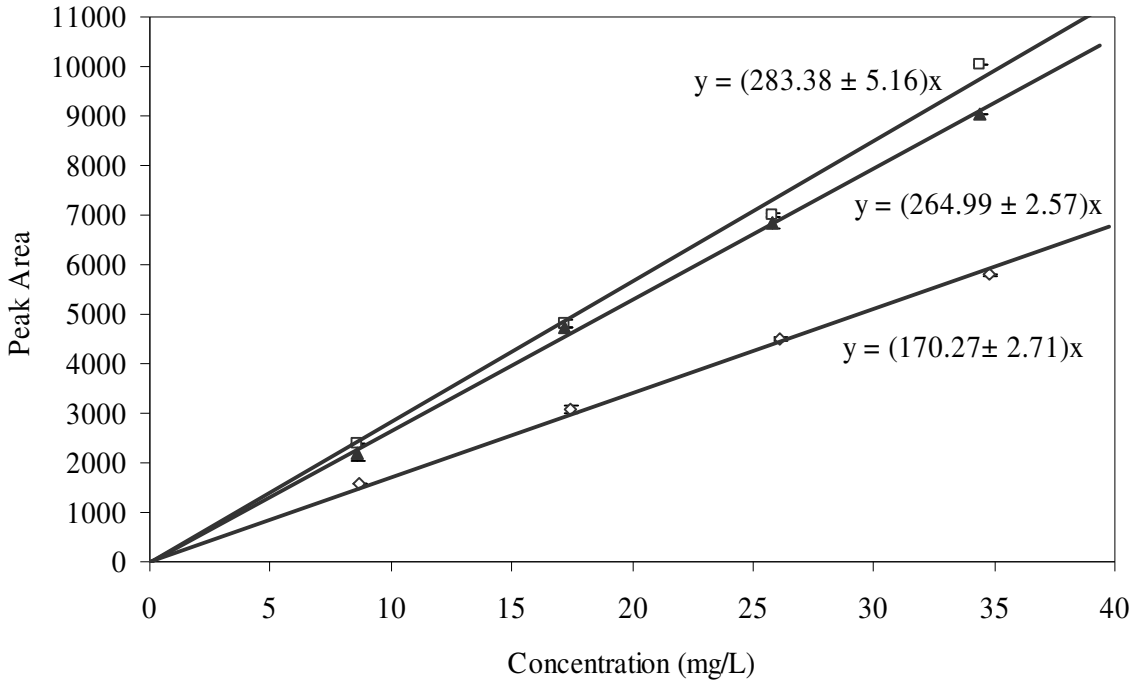


Figure III.2: Typical peaks for BTX detection by HPLC/UV at different wavelengths (A, B, C: 208 nm ;a, b, c: 254 nm) (A, a: benzene; B, b: toluene; C, c: mixed xylenes) (Benzene: 28.120, toluene: 28.470 and xylenes 23.210 as mg/L, pH=2.1)

(a)



(b)

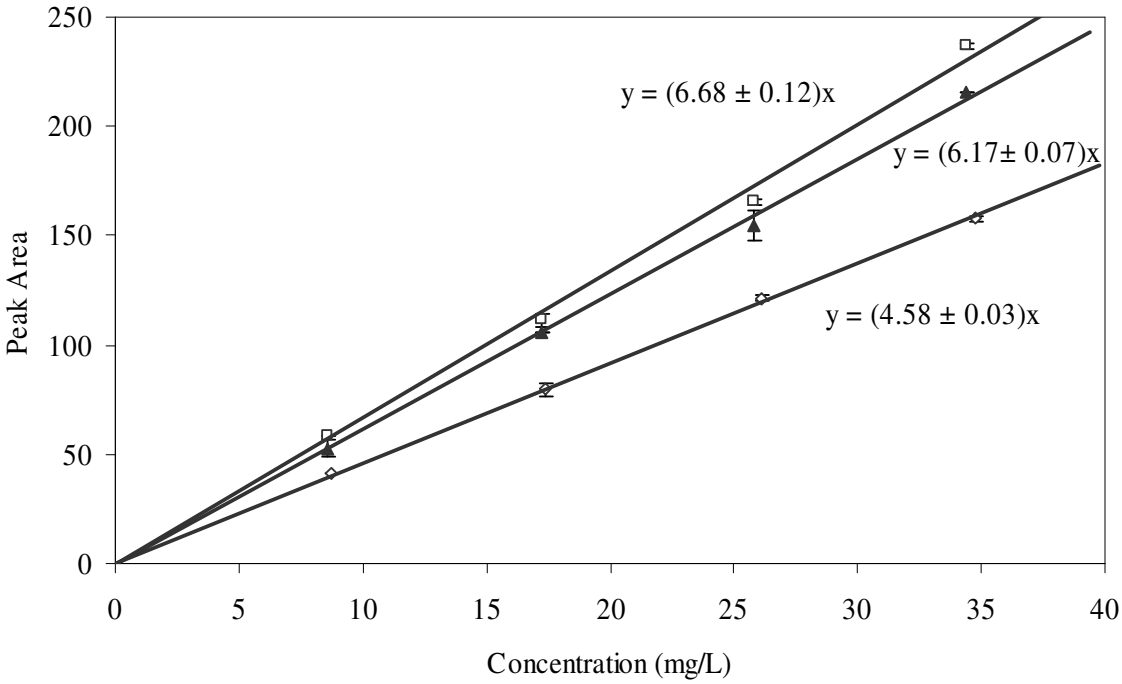


Figure III.3: Calibration curve for BTX determination by HPLC/UV visible at defined conditions (a: 208 nm, b: 254 nm) (-◊- : benzene; - -: toluene; -▲-: xylenes)

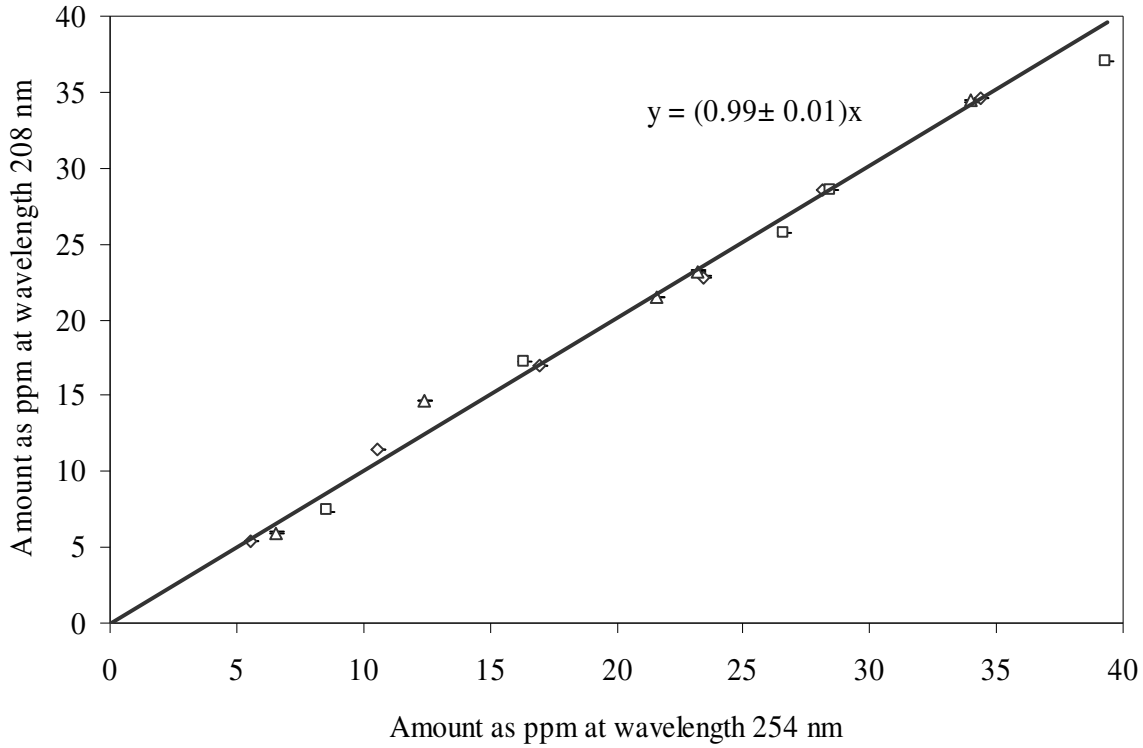


Figure III.4: HPLC results comparison for same samples at different UV wavelength (-◊- : benzene; - -: toluene; -Δ-: xylenes)

III.3. Filter tests

All filters used in this study proved, as expected, their ability to efficiently retain the biomass present in a sample. A series of experiments was carried out with abiotic (biomass free) samples containing varying concentrations of benzene, toluene and xylenes. The solute concentrations were determined both before and after a filtration. Results reported in Figures III.5, III.6 and III.7 demonstrated that this treatment always afforded a solute loss, which could depend on both the nature of the solute and that of the membrane. The nitrocellulose filters exhibited the highest retention with values as high as 12 % for benzene, 27 % for toluene and 47 % for xylenes (Figure III.5). This feature reflected the well-known high and non-specific adsorption of this kind of membrane (technical data at http://www.sartorius.com).

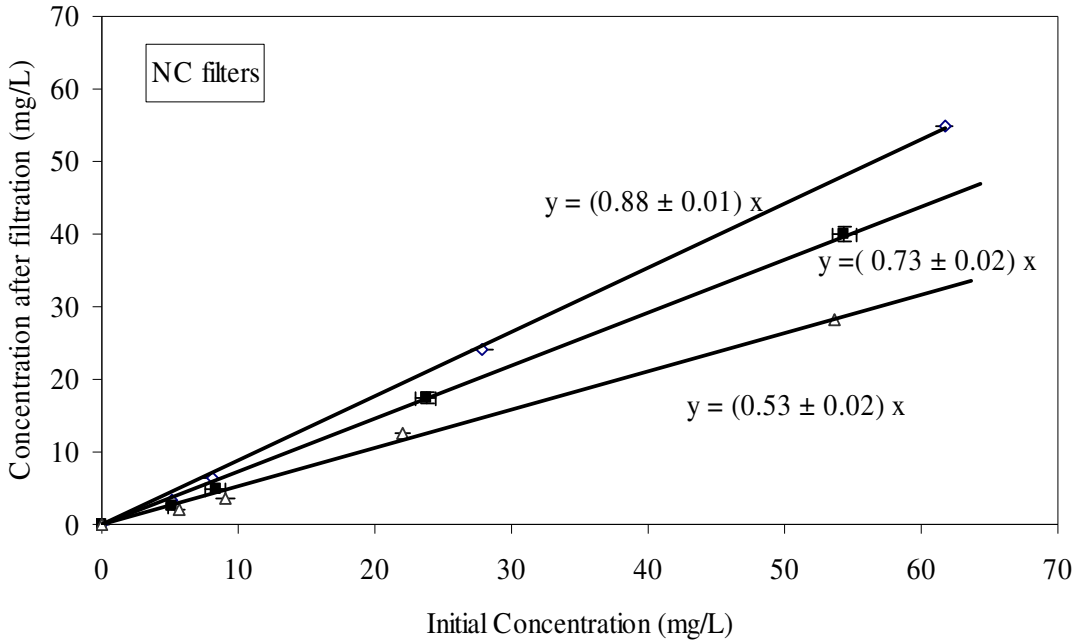


Figure III.5: BTX adsorption by syringe nitrocellulose micro filters (T=20°C, filtration volume =10 ml, pore size= 0.45µm, pH=7.1) (-◊- : benzene; -■-: toluene; -Δ-: xylenes)

The two other materials, which are claimed to have low non-specific retention, actually gave better results since this retention was independent of the solute and took a value of 11% for GH Polypro membranes and 12 % for PVDF (from Figures III.6, III.7). It should be noticed that Choi et al., 1992 used polycarbonate micro filters with a pore size of 0.4 µm. However, according to Advantec (http://www.advantecmfs.com), if this kind of micro filter is suitable for toluene it is not recommended for benzene and xylenes. It is the reason for which this material was not assayed in this study.

It could be concluded that only PVDF or GH Polypro membranes could be used for filtration purposes. However, the significant non-specific retention of solutes had to be taken into account. This led to consider the carrying of experiments devoted to check for the ability of centrifugation to decrease this solute loss during the handling of aqueous samples.

Data highlighted the fact that biomass removal from the medium by membrane filtration must be carried out by taking into account solute adsorption phenomena, which impairs the accuracy of results. Many reports that use this technique apparently pay no attention to this feature (Lovanh et al, 2002).

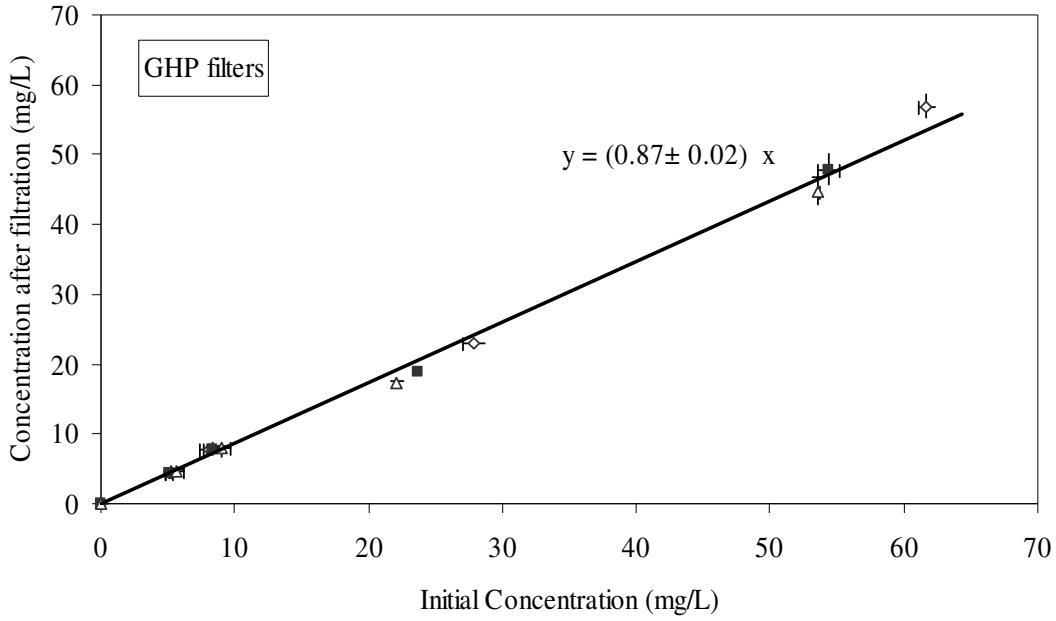


Figure III.6: Monoaromatic adsorption by syringe GHP micro filters (T=20°C, filtration volume =10 ml, pore size= 0.45µm, pH=7.1) (-◊- : benzene; -■-: toluene; -Δ-: xylenes)

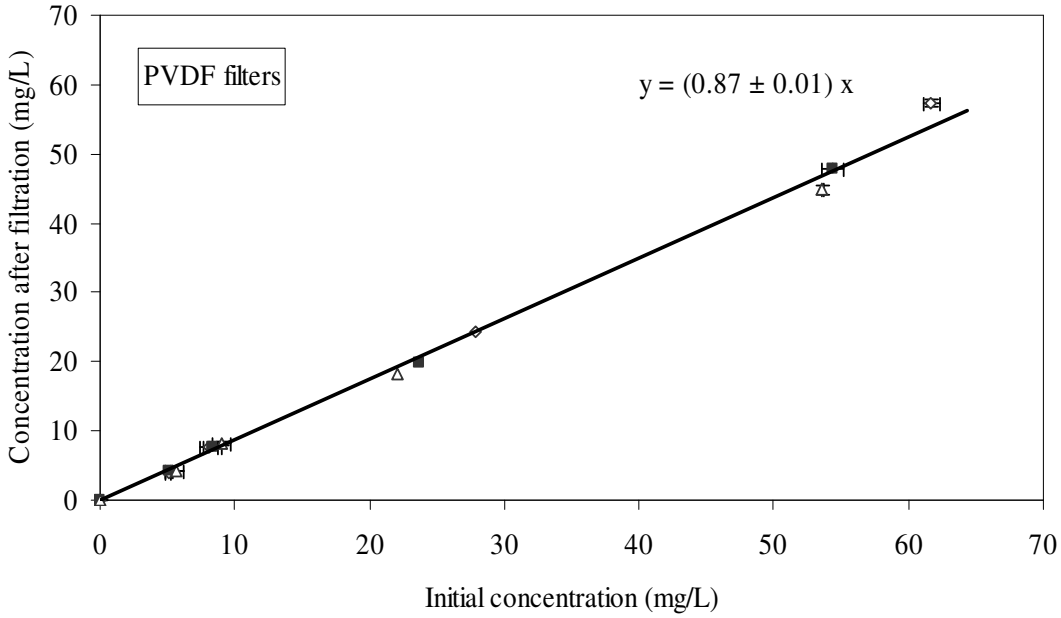


Figure III.7: Solute adsorption by syringe PVDF micro filters (T=20°C, filtration volume =10 ml, pore size 0.45µm, pH=7.1) (-◊- : benzene; -■-: toluene; -Δ-: xylenes)

III.4. Centrifugation experiments

III.4.1. Solute determination after a centrifugation step

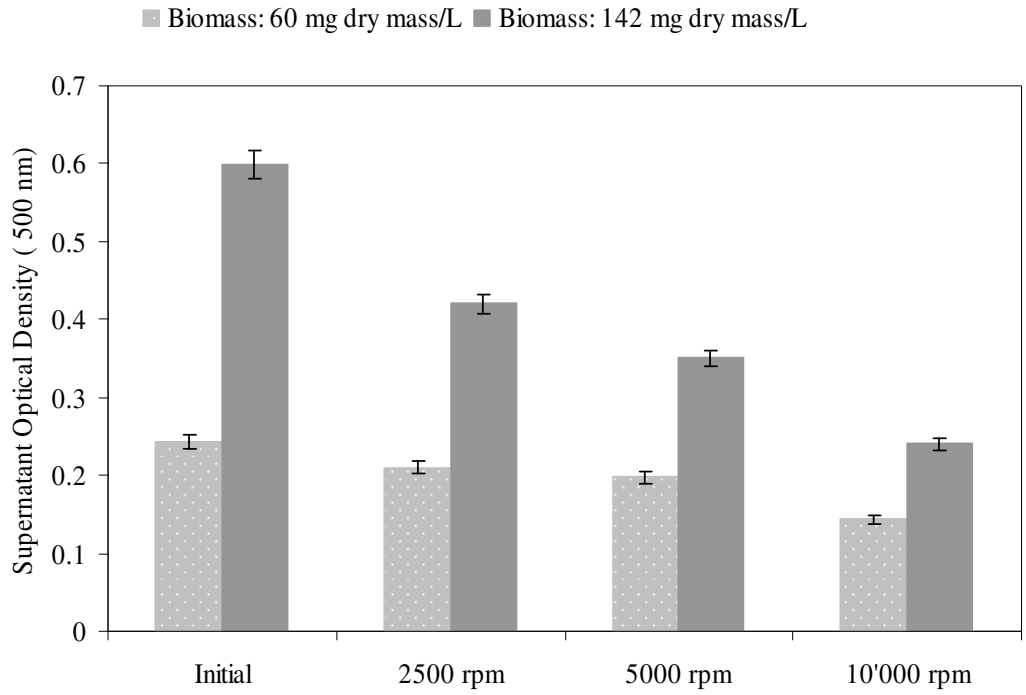
Results presented in Figure III.8 showed that a treatment at a centrifugation speed of 10500 $\times g$, a classical value for the obtention of bacterial pellets, needed duration of at least 10 min. In the mean time, it was demonstrated that centrifugation of aqueous solutions of BTX gave rise to a significant solute loss (Figure III.9). Preliminary results showed that although stripping rate of monoaromatic compounds in temperature 4°C was minimized but discharge of centrifuge glass tubes from rubber keeper is not easy for the reason of rubber contraction. Thus suitable temperature of centrifuge velocity was selected at 10 °C. Data highlighted the fact that at 10 min centrifugation there is a constant BTX stripping rate from water samples to air (Figure III.10).

It was also observed that the ratio of the residual concentration after 10 min operation C to the initial value C_0 was independent of the initial solute content and centrifugation characteristics. These data led us to consider that a significant solute transport from the liquid to the gas phase took place during a centrifugation process. This theory was confirmed by using capped poly tetra fluoro ethylene (PFTE-Teflon) tubes filled with various volume of liquid. Hence, the ratio C/C_0 continuously increased with increasing liquid volume, and solute loss was reduced when the tube was filled with the liquid (Figure III.11). This situation corresponded to the disappearance of the gas phase, which confirmed that solute losses were actually due to liquid gas transfers. This protocol allowed an accurate and sensitive solute determination by HPLC (Figure III.12).

III.4.2. Determination of activity coefficients in water

Preceding results demonstrated that the solute concentration in the liquid phase depended both on the phase volume ratio and operating conditions such as time. This parameter has been shown to reach a limiting value after some time of operation (see Figure III.9). This behaviour led to consider that the system could, at this moment, be considered as being at thermodynamic equilibrium (Figure III.13).

(a)



(b)

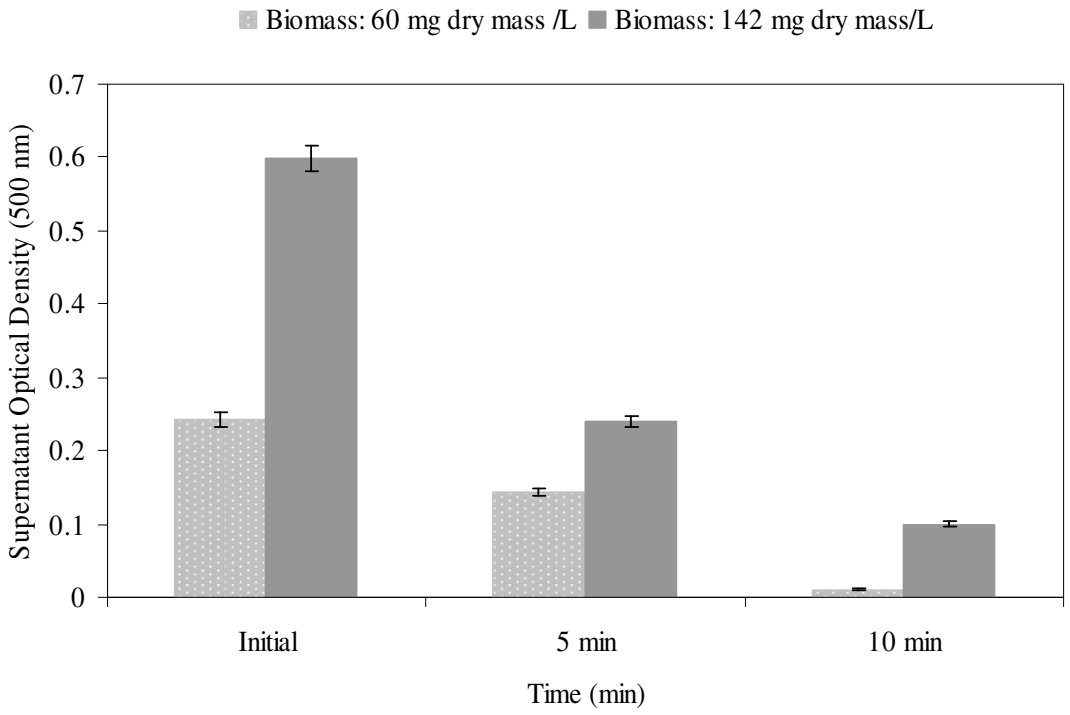


Figure III.8: Effect of centrifugation speed and time on biomass separation (T=20 °C, pH=7.1) (volume sample: 5 mL, Tube volume: 15 mL)

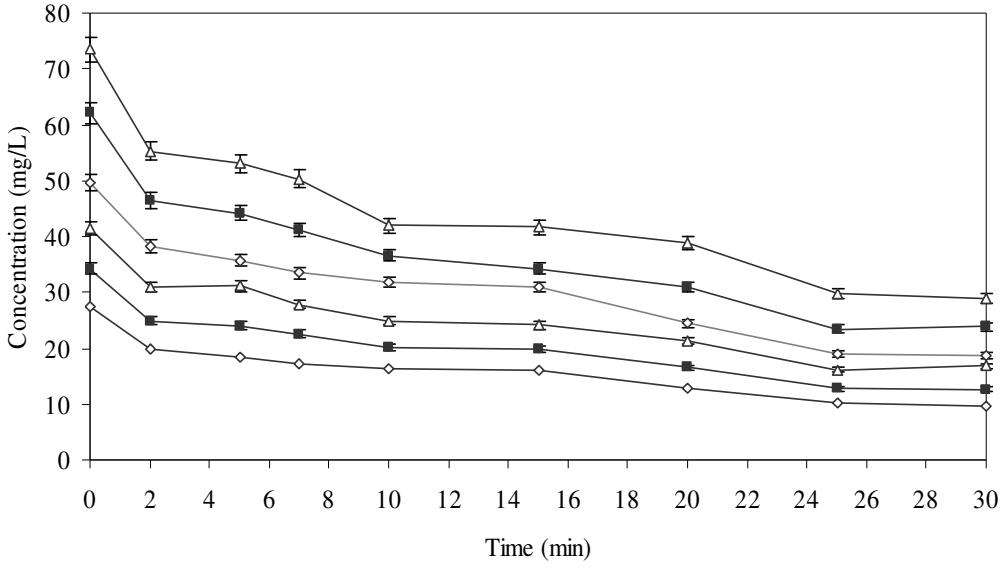


Figure III.9: Time effect on BTX hydrocarbon stripping through centrifuge (10°C, 10500 ×g, pH=7.1) (-◊- : benzene; -■-: toluene; -Δ-: xylenes) (volume sample: 5 mL, Centrifuge glass tubes: 15 mL, Open tubes)

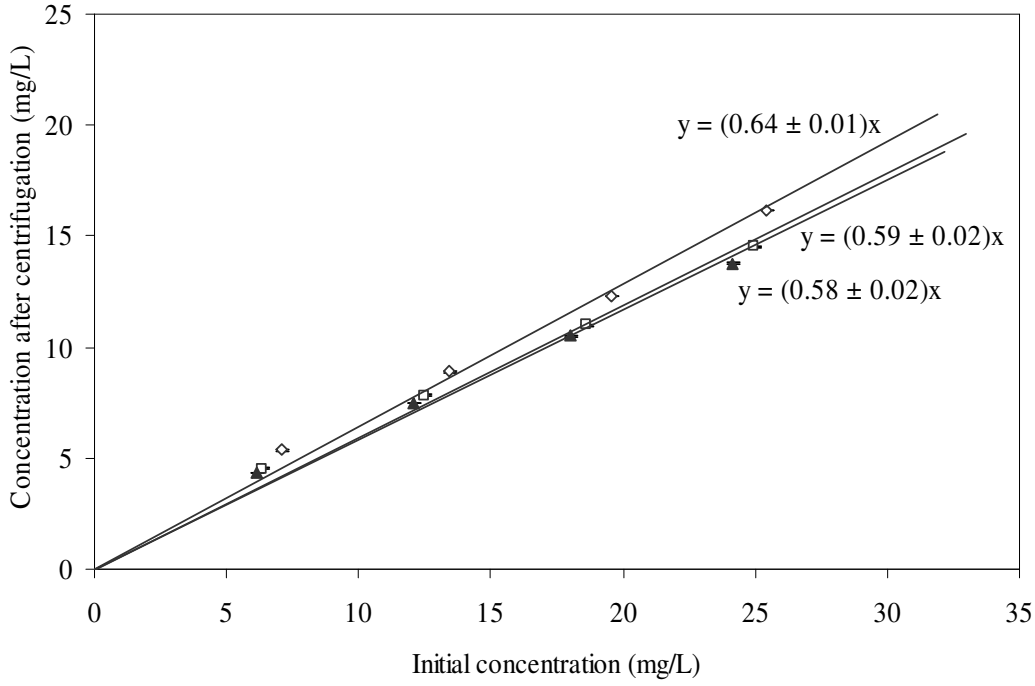


Figure III.10: Effect of centrifugation on BTX compounds at optimum conditions for biomass separation and volatile aromatic stripping (10500 ×g, 10 min, 10°C) (-◊- : benzene; - -: toluene; -▲-: xylenes) (volume sample: 5 mL, Centrifuge glass tubes: 15 mL, Open tubes)

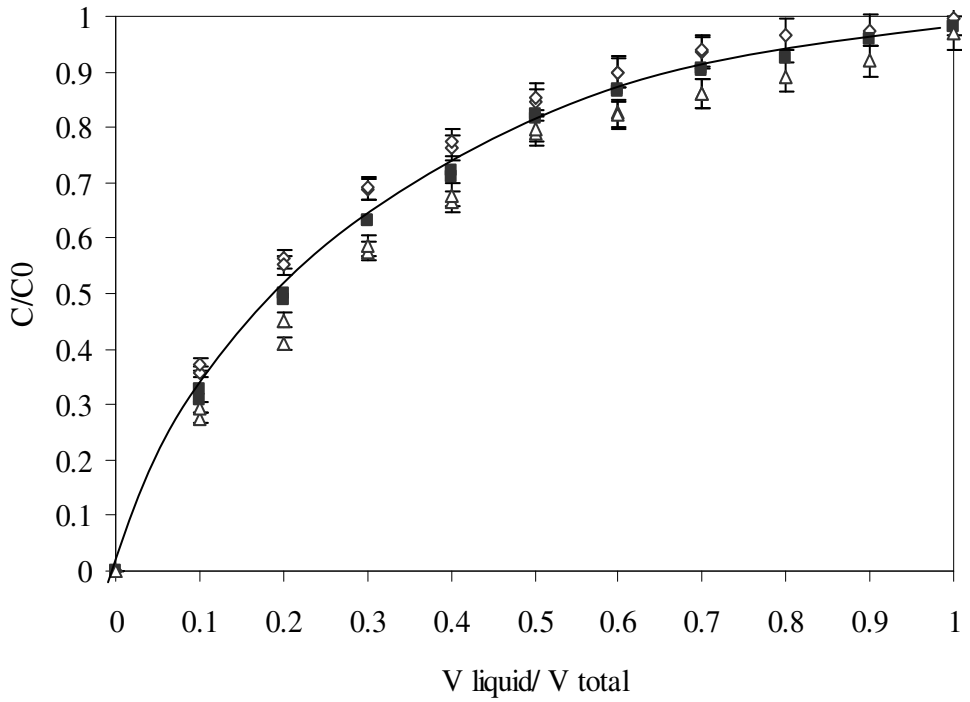


Figure III.11: Ratio of final solute concentration to the initial value plotted against the ratio of liquid to total sample volumes during centrifugations carried out at 10500 ×g, 25 °C for 2 hours (-◊- : benzene; -■-: toluene; -Δ-: xylenes)(Teflon capped tubes).

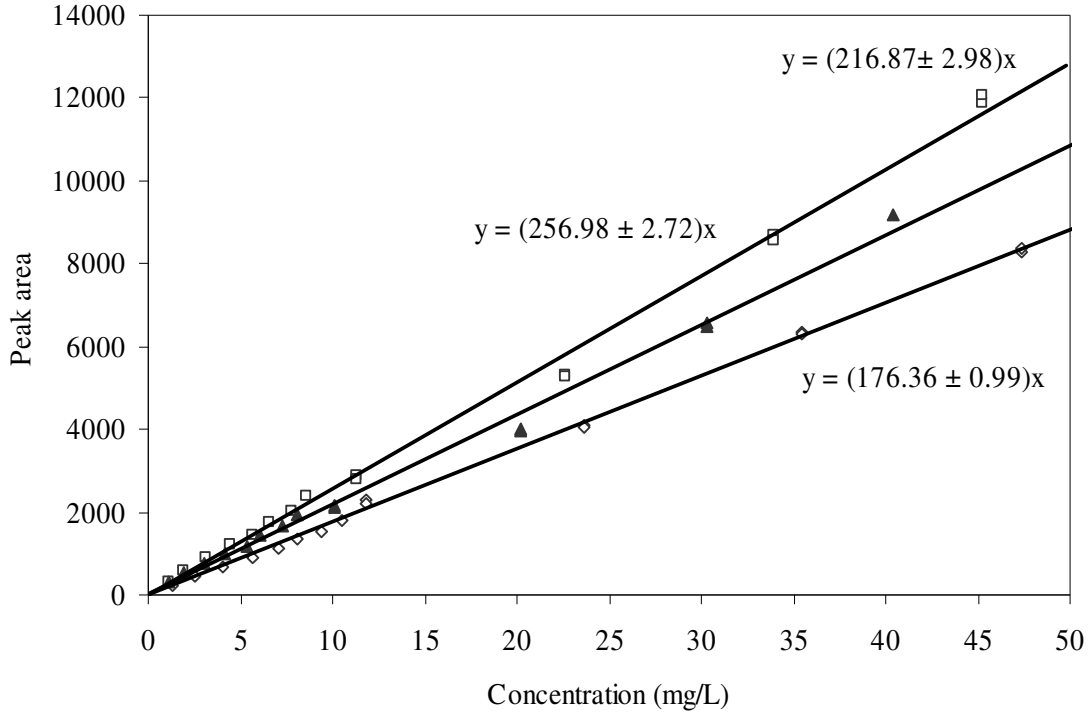


Figure III.12: Calibration curve for BTX analysis in calibration samples after centrifugation (10°C,10500 ×*g*, 10 min) by HPLC/UV visible at defined conditions and λ=208 nm (-◊- : benzene; - -: toluene; -▲-: xylenes) (Teflon capped tubes, 100% volume filled with sample)

A material balance on the solute gave eq. (1):

$$V\,C + V_G C_G = V\,C_0 \qquad (1)$$

where C_0 was the initial solute mole concentration in the liquid, C and C_G were the solute mole concentrations in the liquid and the gas, respectively, and V and V_G the volumes of the corresponding layer.

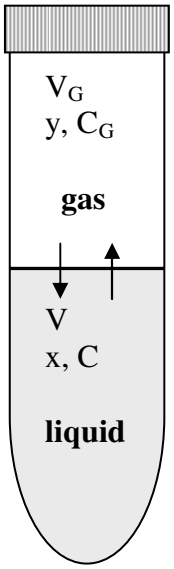


Figure III.13: Description of the system when a liquid phase containing a volatile solute is placed in a test tube plugged with an impermeable cap (The subscript G refers to the gas, V is the phase volume, C the mole concentration of the solute, x and y the mole fraction in the liquid and the gas, respectively).

When both gas and liquid phases were at thermodynamic equilibrium, eq. (2) could be written as:

$$y = \frac{\gamma x P^0}{P} \qquad (2)$$

where y and x were the solute mole fractions in the gas and the liquid, respectively, γ the activity coefficient of the solute dissolved in the liquid, P^0 its vapor pressure and P the total pressure in the system.

Assuming that the gas phase had an ideal behaviour ($y = \frac{RTC_G}{P}$, where R is the universal gas constant, T is absolute temperature) and that the liquid could be considered as dilute ($x = \frac{C}{C_W}$, with C_W being the solvent (water) concentration) gave eq. (3):

$$\frac{V_G}{V} = \frac{(C_0 - C)}{C} \frac{C_W RT}{\gamma P^0} \qquad (3)$$

which showed that plotting $\frac{V_G}{V}$ as a function of $\frac{(C_0 - C)}{C}$ would give a straight line passing through the origin and with a slope enabling γP^0 to be calculated. It should be noticed that C was here the mole concentration of the solute when the system became equilibrated and that γ was the limiting activity of the solute, i.e. the value attained for dilute solutions.

[2]If we consider that the activity coefficients of monoaromatic hydrocarbons are quite dispersed, depending on the source (Kojima et al, 1997), it could be assumed that results in Figure III.14 and Table III.a demonstrated the validity of this approach. It could thus be considered that this protocol could be an efficient alternative to the well-known dilutor method (also sometimes called gas stripping technique) to determine activity coefficients of organic compounds (Fichan et al, 1999). It took benefit from the fact that application of a centrifuge force to a solution enables faster exchanges between liquid and gases. This feature is already exploited in centrifuge evaporators such as Savant Speed Vac®.

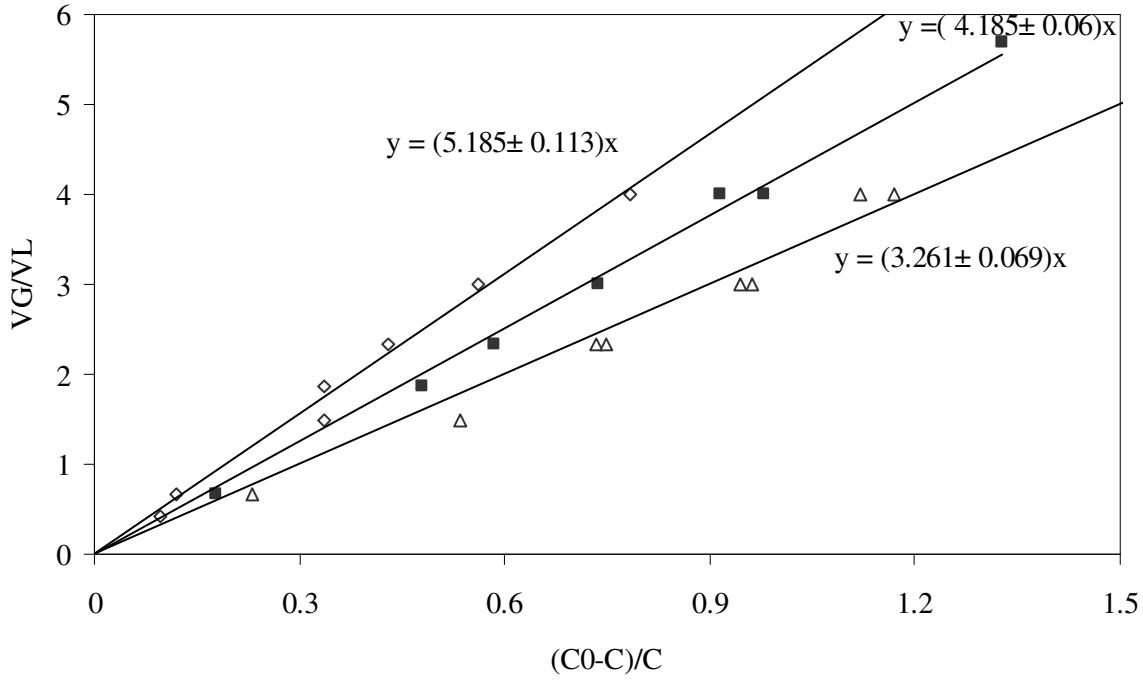


Figure III.14: Plot of $\frac{V_G}{V}$ vs. $\frac{(C_0 - C)}{C}$ for monoaromatics compounds (20°C, Corex glass tubes with Teflon caps, 10500 ×*g* at 4 h)

Table III.a: Solute activity coefficients obtained from centrifugation experiments

Compound	γ (literature)[a)]	P^0 (literature, mmHg)[b)]	γ (this work)[c)]
Benzene	1700-2530	95.19	2058
Toluene	4500-10400	28.4	8546
Xylenes			
o-	30540 - 33000	6.6	
m-	33214-39000	8.3	
p-	33257-37900	6.15	
average[d)]		7.02	44369

a- Kojima et al., 1997 and Hovorka and Dohnal, 2000 in temperature range 20-25°C
b- vapor pressure P^0 from data in Table I.b
c- value from the slope of straight line in Figure III.13 and equation (3); calculations performed with $C_W = 55.56 \times 10^3$ mol m^{-3}, R = 62.3637 L.mmHg mol^{-1} K^{-1},
d- Estimated considering an equimolar mixture of isomers and that there was no interaction between them

III.5. Conclusions

Aromatic hydrocarbons in aqueous samples are solutes that can be easily stripped and that adsorb readily on solids. They can be analyzed to maximum standard levels of potable waters levels through high performance liquid chromatography with UV detector at set point 208 nm and using of C8 reverse phase column. This section demonstrated that the best protocol involved centrifugation using poly tetra fluoro ethylene (PTFE) capped tubes completely filled with the liquid suspension, i.e. without any gas phase inside it. This approach allowed a solute loss lower than 1%. The results indicated also that optimum centrifugation conditions were 10500 ×g and 10°C for 10 min. This approach could further be used for the direct determination of the limiting activity coefficient of a solute, which leads to consider it as an efficient, easy to perform alternative to the well-established gas stripping technique.

This study additionally highlighted the fact that polyvinylidene fluoride micro filters (PVDF) and propylene GH polypro membrane (GHP) with pore size 0.45µm could eventually be used for biomass separation, although 11-12 % monoaromatic adsorption by membrane was still present.

Chapter IV

Gas-liquid and liquid-solid transfers

IV.1. Objectives

There are different methods for monoaromatics removal from polluted water such as physical methods, chemical techniques and biological processes. Past research has indicated that solute removal by biological processes appears to be an economical, efficient, fast and environmentally sound approach. Data reported shows that some challenges related to monoaromatics removal from polluted water by bioremediation processes deal with the possibility of volatile aromatic compounds loss through volatilization and stripping, and also ability of BTX adsorption onto media (packing in fixed film bioreactors or soil organic matter in in-situ bioremediation) are not addressed properly by researchers (Farhadian et al., 2007, 2008a).

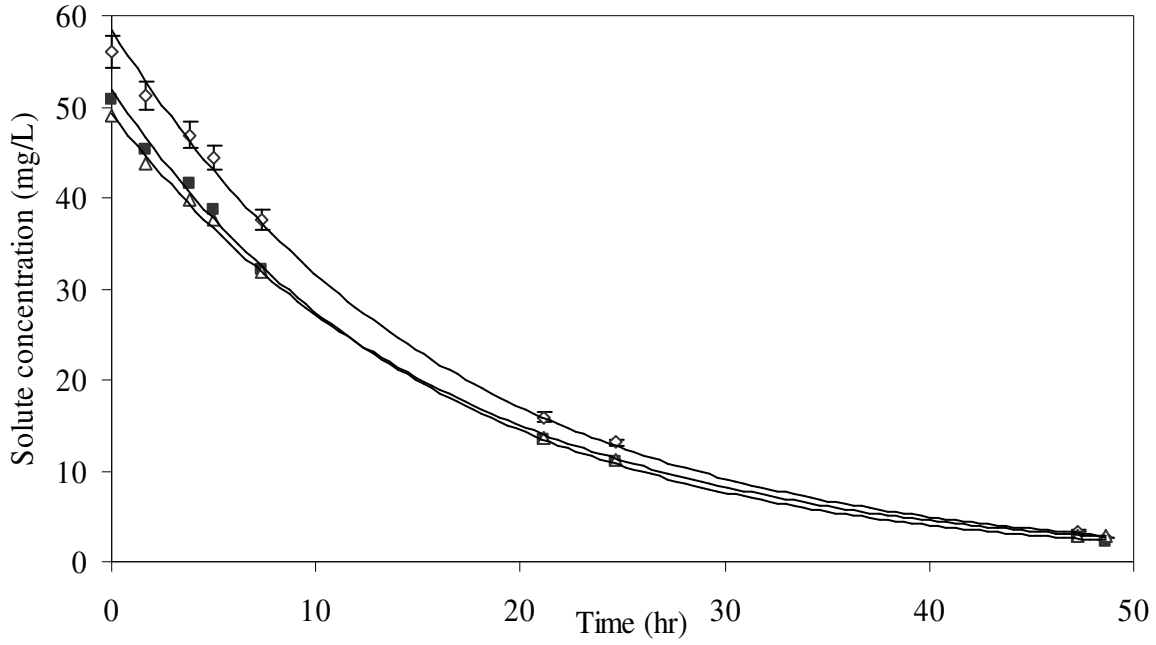
The true efficiency of these processes is still to be correctly evaluated as the physical and chemical properties of monoaromatic compounds demonstrate that phenomena such as stripping and volatilization can contribute in a non negligible amount to their removal from the liquid phase (Farhadian et al., 2008b). It is important to realize that this phenomenon is likely to occur in both aerobic and anaerobic processes.

The aim of this approach was to study monoaromatics evaporation and stripping from synthetically contaminated water under abiotic (biomass free) and open systems. Also, solute adsorption onto granular activated charcoal (GAC), calculation of kinetic parameters and BTX desorption from the solid adsorbents was investigated. Finally, in this research a protocol for analysis and monitoring of monoaromatics compounds in GAC particles was developed.

IV.1. Monoaromatics evaporation from an aqueous phase

Figure IV.1 shows the volatilization rate of BTX compounds from water solutions in abiotic (biomass free) and open systems. Results indicated that more than 95% of BTX was lost from polluted water by evaporation in temperature 20°C and in the period of 2 days. Thus volatilization is a significant process in determining the fate of volatile aromatic compounds in the environment. Also, this experiment showed that solute volatilization rate from polluted water can be described as a first-order reaction (Figure IV.1b).

(a)



(b)

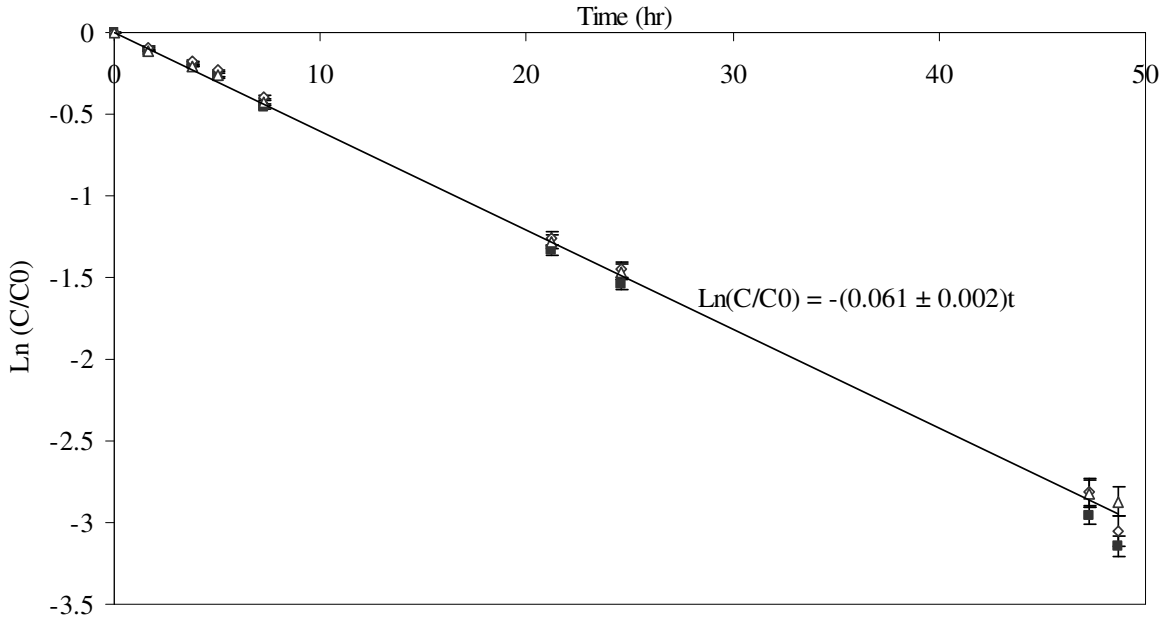


Figure IV.1: Evaporation rate of monoaromatic compounds from contaminated water without stirring (a: BTX concentration vs. time, b: Semi-log plot of C/C_0 vs. time) (-◊- : benzene; -■-: toluene; -Δ-: xylenes) (Temperature: 20°C)

The change in the concentration of the BTX compounds according to the time could be expressed as (equation 1):

$$C = C_0 \exp(-kt) \quad (1)$$

and could be linearized as (eq. 2): $\mathrm{Ln}(C/C_0) = -kt$ (2)

where C_0 is the initial BTX concentration in the bulk-water phase and k is the reaction rate constant ($time^{-1}$).

Also, as depicted in Figure IV.2, turbulent stirring of polluted water could accelerate monoaromatic hydrocarbons removal through a stripping phenomenon. Curves in Figure IV.2 also showed that first order reaction also could describe toluene stripping from synthetic polluted water. Data highlighted the fact that mixing or aeration in open systems could increase toluene loss rate by a factor higher than 80. Similar behaviour of volatile organic compounds (VOC) in the presence of surfactants have been reported by Lee et al. (2004) and Chao et al. (2005, 2007).

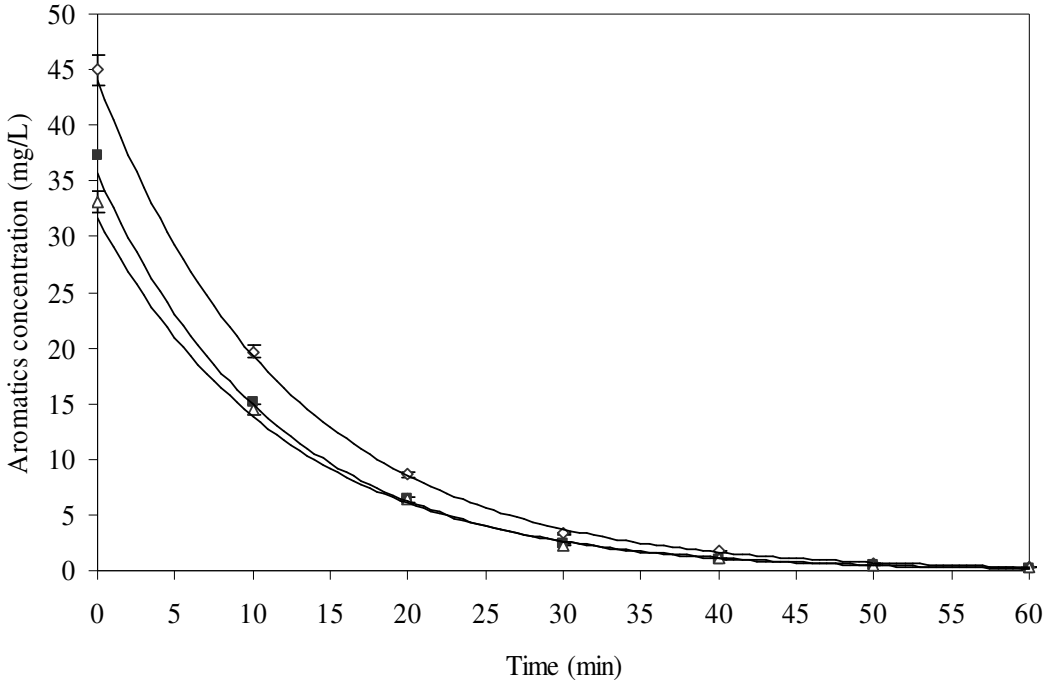
IV.2. Solute stripping by aeration

IV.2.1. Aqueous phase

As depicted in Figure IV.3a, air sparging in polluted water could readily reduce its monoaromatic hydrocarbon content through a stripping phenomenon. Results indicated that air sparging in contaminated water can eliminate BTX compounds from 140 ppm to about 5 ppb in only 1 h processing with a gassing rate of 0.33 VVM. This result was consistent with bibliographical data which have clearly indicated that air sparging has become a popular tool for the remediation of contaminated water by volatile organic compounds (VOCs), particularly dissolved petroleum hydrocarbons (Johnston et al., 1998; Chao et al., 2008). This approach cannot be considered as a green technology as volatile organic compounds are only transferred from the liquid phase to the gas phase.

Also, Figure IV.3b showed that a logarithmic plot of the residual concentration in liquid phase vs. time gave a straight line. This feature was characteristic of the so-called dilutor method (Duhem and Vidal, 1978) and allowed the calculation of the activity coefficient of the solute, provided both liquid and gas phases can be considered as diluted and at thermodynamic equilibrium (Fichan et al., 1999; Cappaert and Larroche, 2004).

(a)



(b)

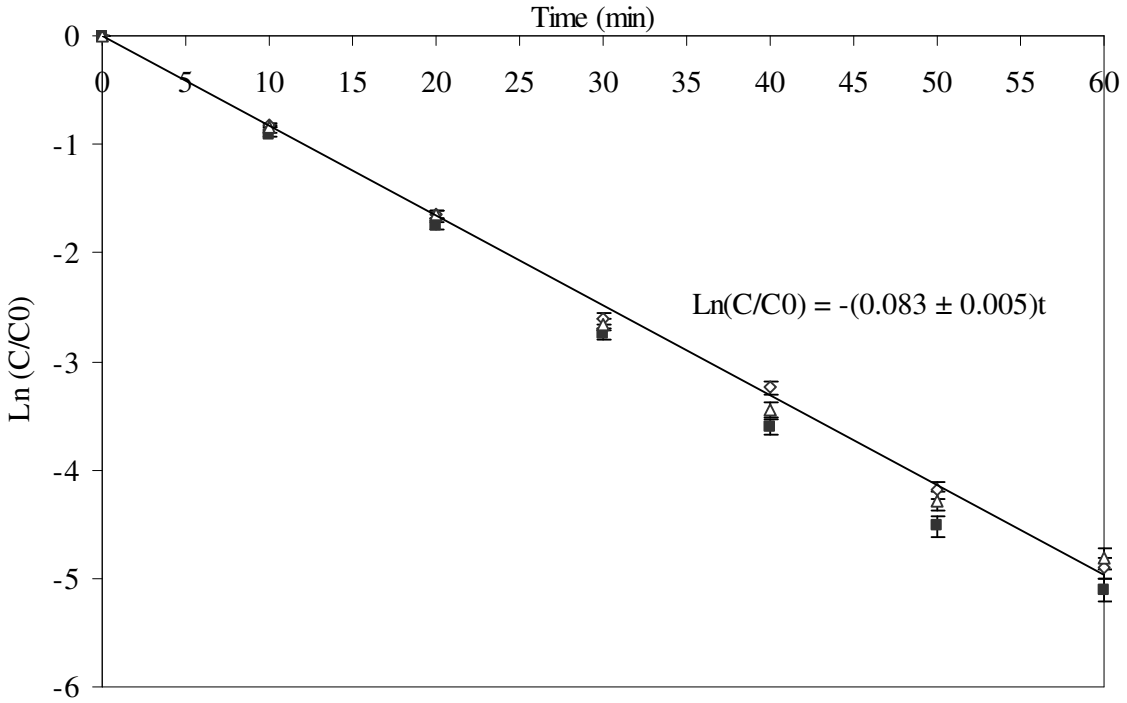
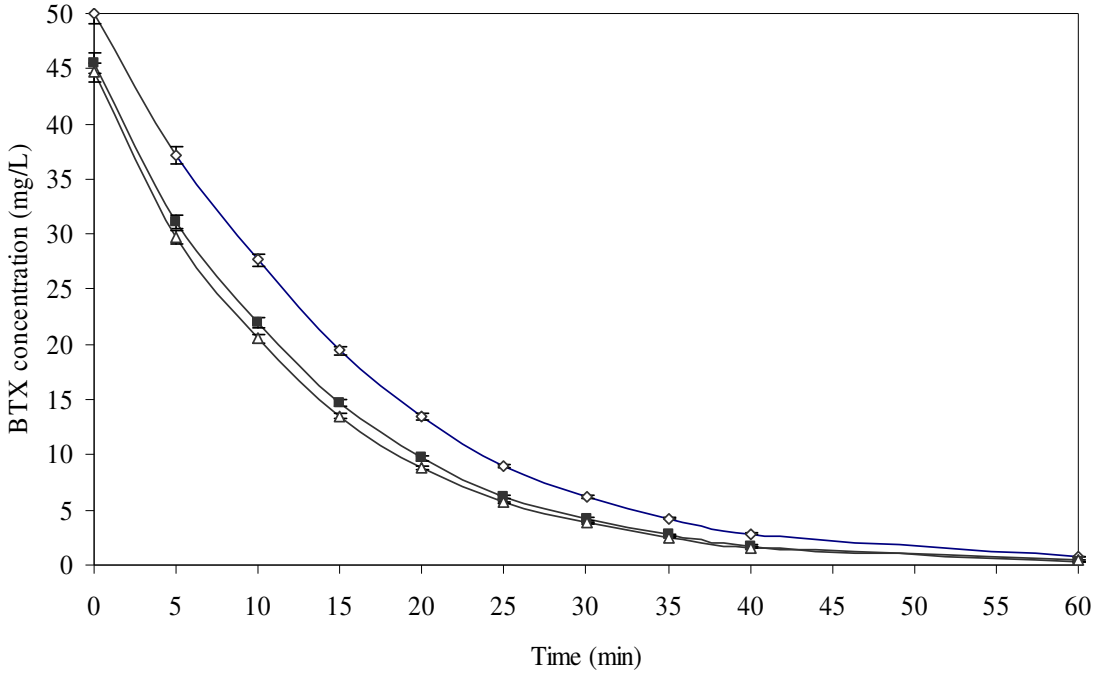


Figure IV.2: Volatilization rate of monoaromatic compounds from contaminated water with mixing (a: BTX concentration vs. time, b: Semi-log plot of C/C_0 vs. time) (-◊- : benzene; -■-: toluene; -Δ-: xylenes) (Temperature: 20°C, Shaker mixing rate: 150 rpm, working volume: 0.5L, bottle volume: 1L, system: open)

(a)



(b)

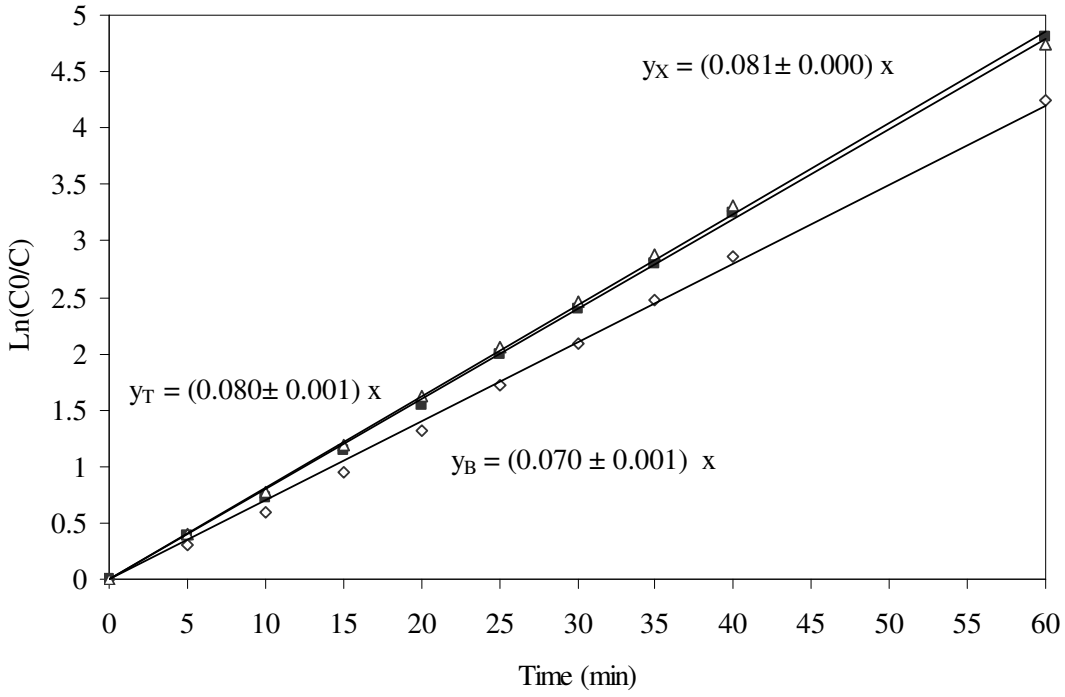


Figure IV.3: Monoaromatic removal from contaminated synthetic water through air sparging (-◊-: benzene; -■-: toluene, -Δ-: xylenes) (a: BTX concentration vs. time, b: Semi-log plot of C/C_0 vs. time)(Liquid volume: 1500 ml, air flow rate: 500 mL/min, 0.33 VVM or volume of gas per volume of liquid per min, stirring rate: 600 rpm, temperature :20°C).

Hence, the following equation (3) resulted from a mass balance on a solute with the preceding assumptions:

$$Ln\frac{C_0}{C} = \frac{G\,\gamma^{\infty}\,P^0}{C_W V_L\,P}\,t \qquad (3)$$

where C_0 and C are the solute concentrations at time 0 and t, respectively. G was the air molar flow rate (0.0207 mol/min), C_W the water concentration in the liquid (55.6 mol/L), V_L the liquid volume (1.5 L), P the total pressure in the vessel (720 mmHg), P° the solute vapor pressure and γ^{∞} the limiting activity coefficient of the solute. If a was the slope of the straight line, then the limiting activity coefficient could be calculated using the equation (4):

$$\gamma^{\infty} = \frac{a\,V_L\,P\,C_W}{G\,P^0} \qquad (4)$$

Table IV.a shows that the results obtained with these assumptions were close to literature data, which meant that the experimental device used enabled interfacial exchanges to be fast enough to allow the two phases to be always near the thermodynamic equilibrium. This data is useful for estimating of the losses of monoaromatic compounds by gas stripping.

Table IV.a: Comparison of literature data and experimental results obtained in this work for limiting activity coefficients of BTX compounds

Compound	Vapour pressure (mm Hg, 20 °C)	Limiting activity coefficient[a)]	Limiting activity coefficient[b)]	Limiting activity coefficient[c)]
Benzene	95.19	2400	2132	2541
Toluene	28.4	9700	8168	12864
Xylenes				
o-	6.6	33100		
m-	8.3	36600		
p-	6.15	36400		
average[d)]	7.02	35400	33455	60656

a- Value at 20°C calculated from solubility data found in the SRC PhysProp Database (http://esc.syrres.com/interkow/webprop.exe). Calculations were made using the equation : $\gamma^{\infty} = \frac{1}{x}$, with $x = \frac{S_W}{MW}$, where S_W was the water solubility (g/l) and MW the molecular weight (g/mol)

b- Experimental value at 20°C, calculated from data in Figure IV.3

c- Predicted by the original Unifac model using the UNIFACAL program (http://www.filewatcher.com/b/ftp/ftp.eq.uc.pt/pub/software/pc/chemistry.0.0.html)

d- Estimated considering an equimolar mixture of isomers, and that there was no interaction between them

Indeed the product G y, where G is the molar gas flow rate and y the mole fraction of the solute in the gas phase (eq. 5), gives the molar loss rate of a BTX compound in the environment.

$$y = \gamma^{\infty} \frac{CP^0}{C_W P} \qquad (5)$$

Also, data presented in Figure IV.3 showed that the rate of BTX stripping in the reactor with aeration and agitation were significantly increased in comparison to volatilization tests. According to eq. 3 and data presented in Table IV.a, it could be defined an "apparent air flow rate" in volatilization experiments, with values of 0.0024 L/min or 0.0043 VVM (Figure IV.1-without agitation) and 0.18 L/min or 0.36 VVM (Figure IV.2-with mixing). This concept would merit further studies to enable full prediction of volatile solutes loss in non aerated systems.

IV.2.2. Monophasic organic phase

Organic solvent such as hexadecane can be applied as an adsorbent for trapping of monoaromatic hydrocarbons from waste gas stream. But as depicted in Figure IV.4, air sparging in hexadecane, can reduce toluene concentration from 2100 to about 500 mg/L in 3 days with a gassing rate of 1 VVM.

According to equation 3 and mass balance on toluene in hexadecane-air system, activity coefficient in this process could be calculated using the equation (6):

$$Ln\frac{C_0}{C} = \frac{G\ \gamma_T^{\infty} P^0}{C_H V_L P} t \qquad (6)$$

where C_0 and C are the toluene concentrations at time 0 and t, respectively. G was the air molar flow rate (0.0414 mol/min), C_H the hexadecane concentration in the liquid (3.413 mol/L), V_L the organic volume (1 L), P the total pressure in the vessel (720 mmHg), P° the toluene vapor pressure (28.4 mmHg) and γ_T^{∞} the limiting activity coefficient of the toluene. Data showed that toluene activity coefficient in organic solvent is closed to 0.748 that it is about 11000 times lower than solute activity in aqueous phase.

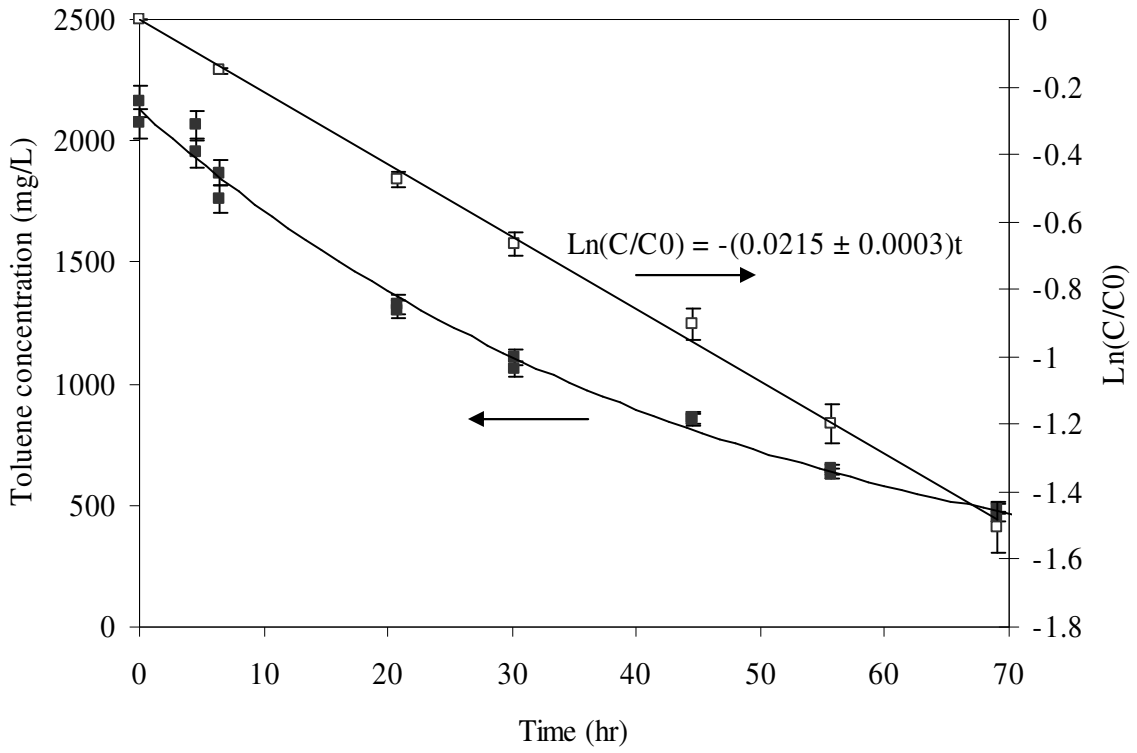


Figure IV.4: Toluene stripping from hexadecane by air sparging at 20 °C (Organic solvent volume: 1 L, air flow rate 1 L/min, 1 VVM or volume of gas per volume of liquid per min, stirring rate: 600 rpm).

IV.2.3. Biphasic media

Actually, in a biphasic media system, aromatic compounds can be solubilized in the immiscible organic phase and allowed to transfer into the aqueous phase (Figure IV.5). The controlled substrate delivery from organic to the aqueous phase is based on the fundamental principle of thermodynamic equilibrium due to partition coefficient of solute in the ternary system (air, water and solvent). This configuration is widely used in the area of bioremediation for monoaromatics treatment (Daugulis, 2001; Malinowski, 2001; Muñoz et al., 2007). In this section aromatic losing in biphasic media in abiotic and aerobic system was studied. Also, partition coefficient for toluene compounds in the ternary system was investigated.

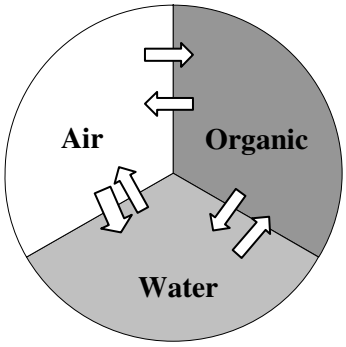


Figure IV.5: Schematic diagram for two-liquid phase partitioning systems

IV.2.3.1. Partition coefficients between air, water and organic phase

Partition coefficient is the ratio of concentration of an aromatic compound in the two phases at equilibrium. Gas/solvent partition coefficient can be defined through equation (7):

$$K_{S/G} = \frac{C_S}{C_G} \qquad (7)$$

where C_S and C_G are the equilibrium concentrations of a solute in an solvent and the gas respectively. The water/organic phase partition coefficients can be converted into other through eq. (8):

$$P_{S/A} = \frac{C_S}{C_A} \qquad (8)$$

where C_S and C_A are the equilibrium concentrations of a solute in an solvent and the aqueous phase, respectively.

Experiments carried out in Teflon caps glass bottles containing 480 mL water, 25 mL hexadecane, 217.5 µL toluene and 620 mL air, under mixing with magnetic agitator showed that after 7 day, partition coefficients in gas/organic and organic/aqueous were according to data presented in Table IV.b, which were also consistent with data in Figure IV.6.

Table IV.b: Partition coefficient for toluene in ternary system

Compound	Log K (Literature)[a]	Log K (this work)[b]	Log P (Literature)[a]	Log P (this work)[b]
Toluene	3.39	3.26	2.74	2.55

a- calculated at 25 °C for toluene in undecane-air-water system (Abraham and Acree, 2004)
b- measured at 30 °C for toluene in hexadecane-air-water system

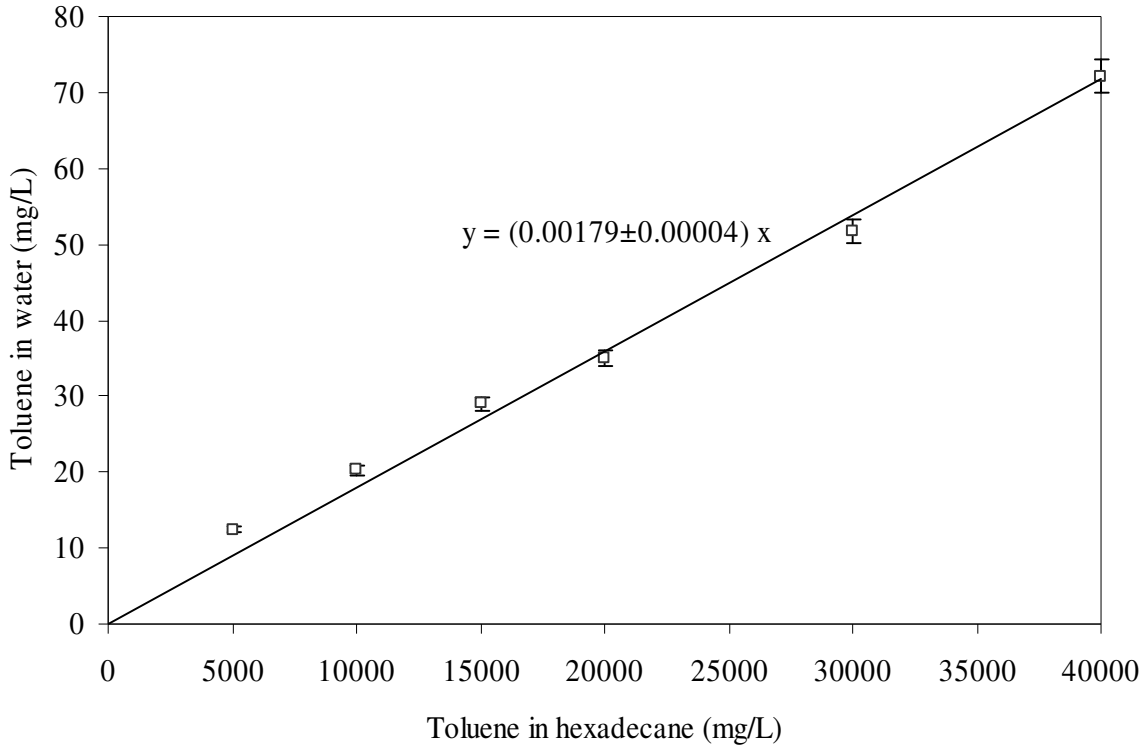


Figure IV.6: Solute transfer in aqueous/organic phase (temperature: 30°C, hexadecane: 2 mL; water: 38 mL; shaker mixing: 250 rpm; glass bottles with Teflon caps: 40 mL; after 7 days)

IV.2.3.2. Activity coefficient for toluene in organic solvent-water-air systems

Generally two phase partitioning reactors (TPPB) are applied at a temperature of 30 degrees centigrade for monoaromatics treatment (See Table I.l). Results presented in Figure IV.7 showed that in a system containing aqueous, organic and air phase, volatile aromatic hydrocarbon are stripped from liquid to gas phase at a rate close to that observed with monophasic hexadecane. This feature emphasized the fact that, since water solubility in hexadecane was very low, it could be considered that the thermodynamic solute activities, which could be regarded as equal in all phases, remained close to those observed in monophasic organic solvent systems (Figure IV.7). The activity coefficient derived from Figure IV.4, 0.748 was found close to the value predicted by UNIFAC (see table IV.a) which was estimated to a value close to 1.035 at 20°C for a dilute toluene solution in hexadecane. It was thus possible to predict liquid-vapor data for benzene, toluene and xylenes for temperatures in the range 10 – 30°C (Table IV.c). It should be noticed that the term γP° allows direct calculation of the gas-liquid partition coefficient, since :

$$\frac{y}{x} = \frac{\gamma P^0}{P} \qquad (9)$$

where y and x were the mole fractions in the gas and liquid phases, respectively, and P the total pressure in the system. The relationship with $K_{S/G}$ (eq. 7) could be written as:

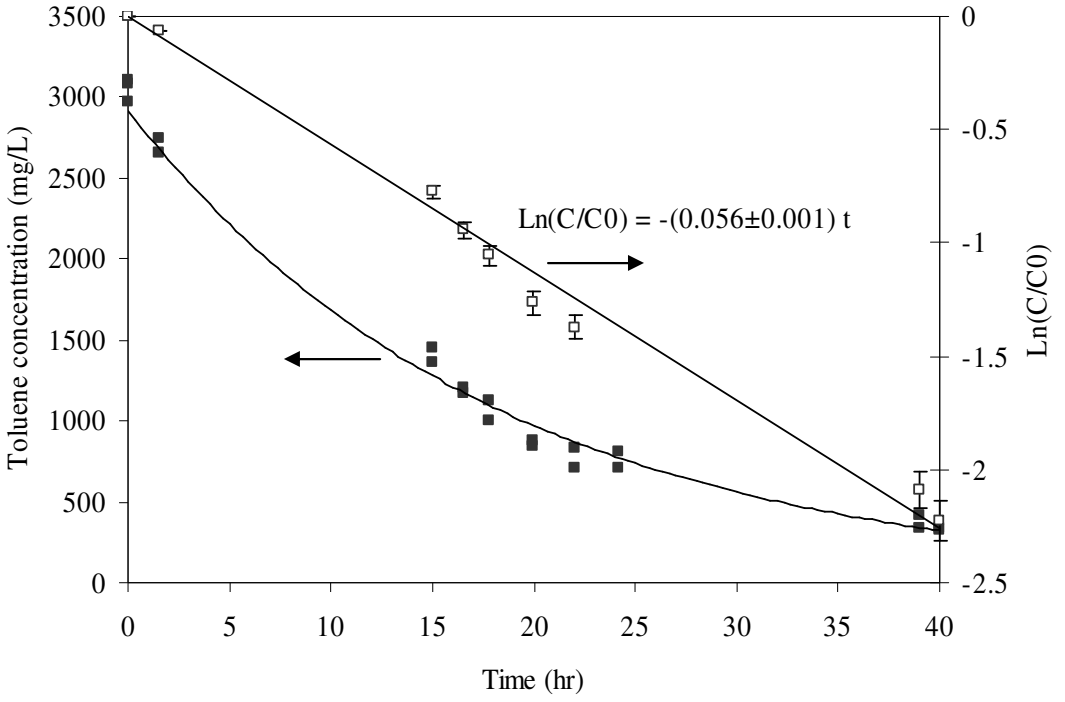
$$\gamma P^0 = \frac{C_{solv} RT}{K_{S/G}} \qquad (10)$$

Table IV.c: Values predicted for liquid-vapor equilibria data of BTX compounds in liquid systems involving hexadecane as organic solvent (γ is the limiting activity coefficient in hexadecane estimated fromUNIFACAL (see table IV.a legend) and P° the vapour pressures)

Compound	Temperature (°C)	γ	P° (mmHg)	γP° (mmHg)
Benzene	10	0.930	50.77[a)]	47.216
	15	0.923	64.33	59.389
	20	0.917	80.85	74.139
	25	0.911	100.84	91.815
	30	0.905	124.86	112.936
Toluene	10	1.052	12.45[b)]	13.097
	15	1.049	16.60	17.314
	20	1.035	21.86	22.625
	25	1.027	28.47	29.239
	30	1.020	36.69	37.424
m Xylene	10	1.103	3.26	3.596
	15	1.096	4.51	4.943
	20	1.089	6.15	6.697
	25	1.082	8.29	8.970
	30	1.075	11.03	11.857

a - from http://www.s-ohe.com/Benzene_cal.html
b - from http://www.s-ohe.com/Toluene_cal.html
c - from http://www.s-ohe.com/Benzene_cal.html (data for m-xylene)

(a)



(b)

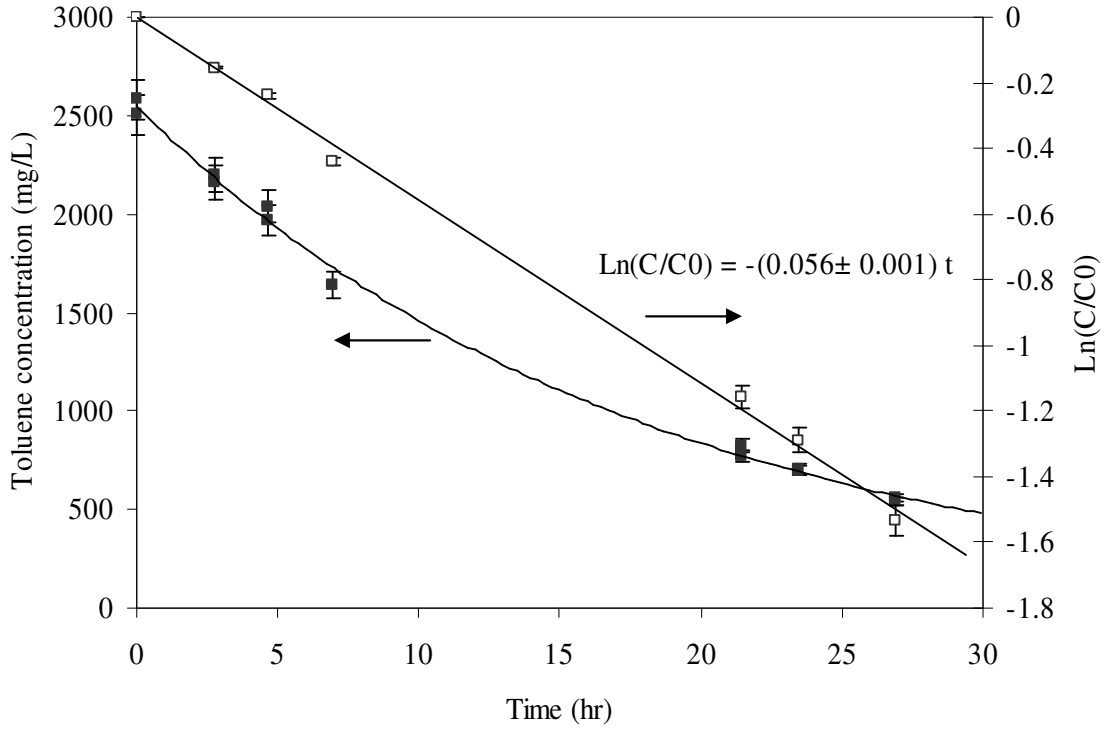


Figure IV.7: Aromatics loss from biphasic media by gas stripping (a: organic solvent-air system; b: organic solvent-water-gas system)(Organic volume: 1 L, aqueous volume: 1L, air flow rate 1 L/min, stirring rate 600 rpm, temperature 30°C).

Data highlighted that presence of organic phase such as hexadecane can be reduced the solubility of xenobiotic compounds in aqueous phase, so delivery of substance can be controlled during in process.

IV.4. Aromatics sorption and desorption

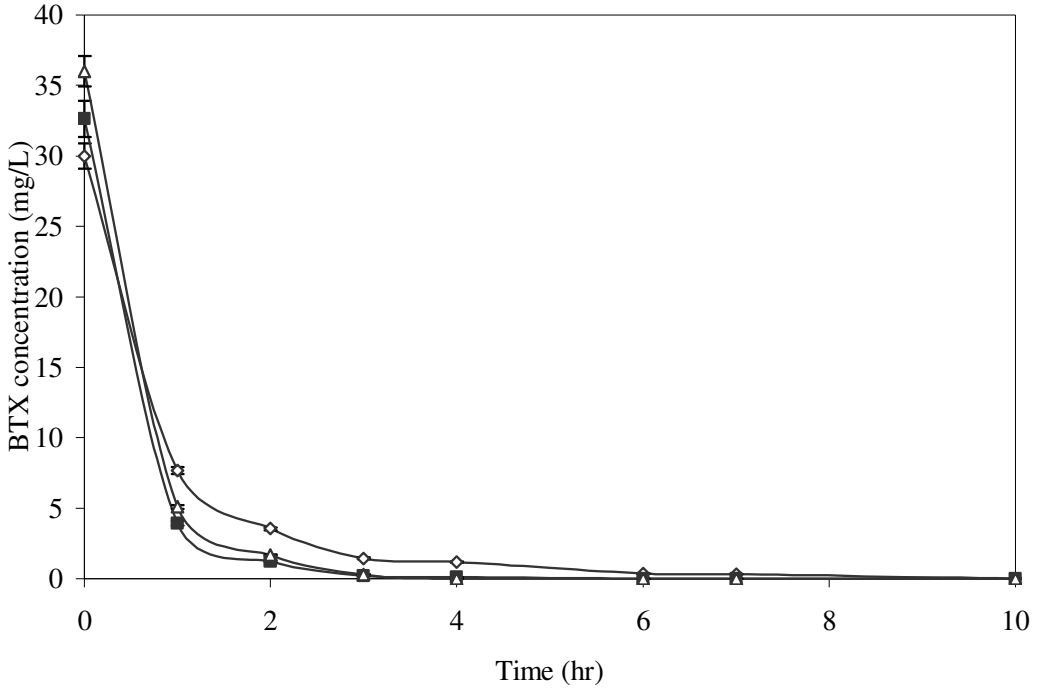
IV.4.1. BTX adsorption on GAC

Experiments shown in Figure IV.8 were carried out in fully filled bottles to avoid solute gas transfers, as depicted in the preceding paragraph. As a result, the lowering of liquid concentrations was actually because of solute adsorption on the solid adsorbent. The curves highlighted the fact that the use of activated carbon was effective in the removal of BTX from contaminated water. Activated carbon used in granular form was indeed able to remove BTX down to residual concentrations as low as 5 µg/l. The kinetics of BTX adsorption was observed to be in the order xylenes> toluene> benzene. Favourable adsorption of compounds in this order has been explained as associated with the decrease in solubility and increase in molecular weight.

Similar observations have been reported in 2003 by Daifullah and Girgis. It should also be remembered that with a solid adsorbent, the contaminants are retained until its adsorptive capacity. This maximum solute retention could be estimated as being close to 350 mg/g GAC for xylenes, 250 mg/g for toluene and 150 mg/g for benzene (Figure IV.9a).

It should be emphasized that an equimolar mixture of these compounds, giving BTX, had a different behavior as the maximal loading could be estimated at a value close to 200 mg/g, instead of the expected value of 145 mg/g (the average of the individual maximal loading capacities). This result indicated that solute interactions were likely to take place in the sorption process.

(a)



(b)

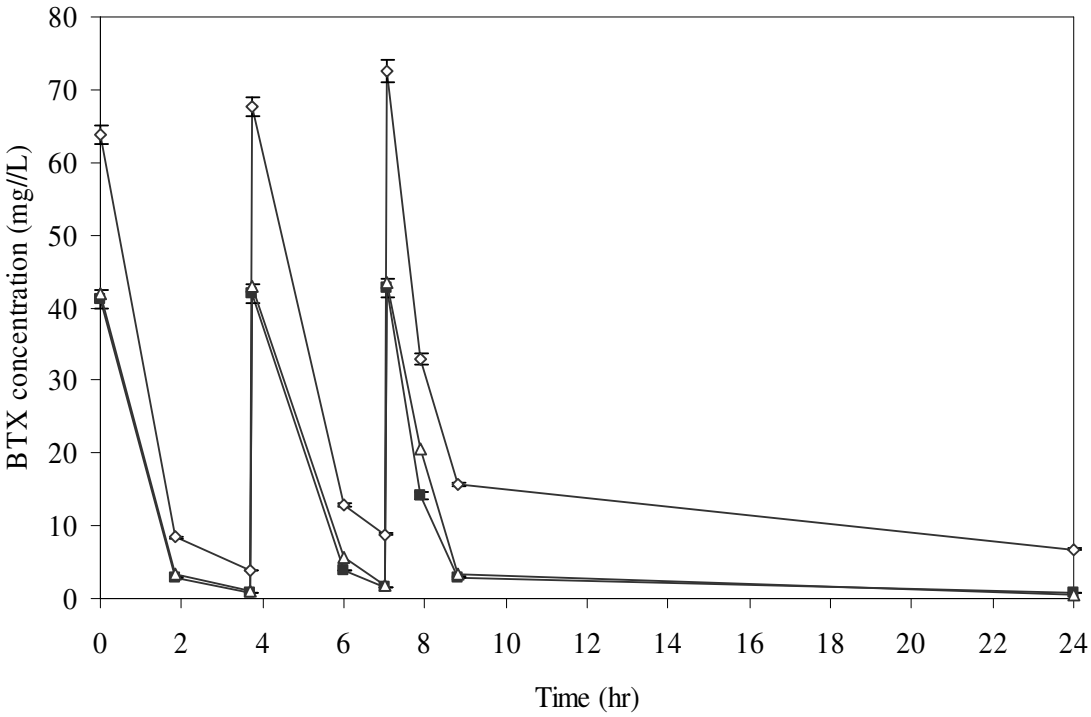
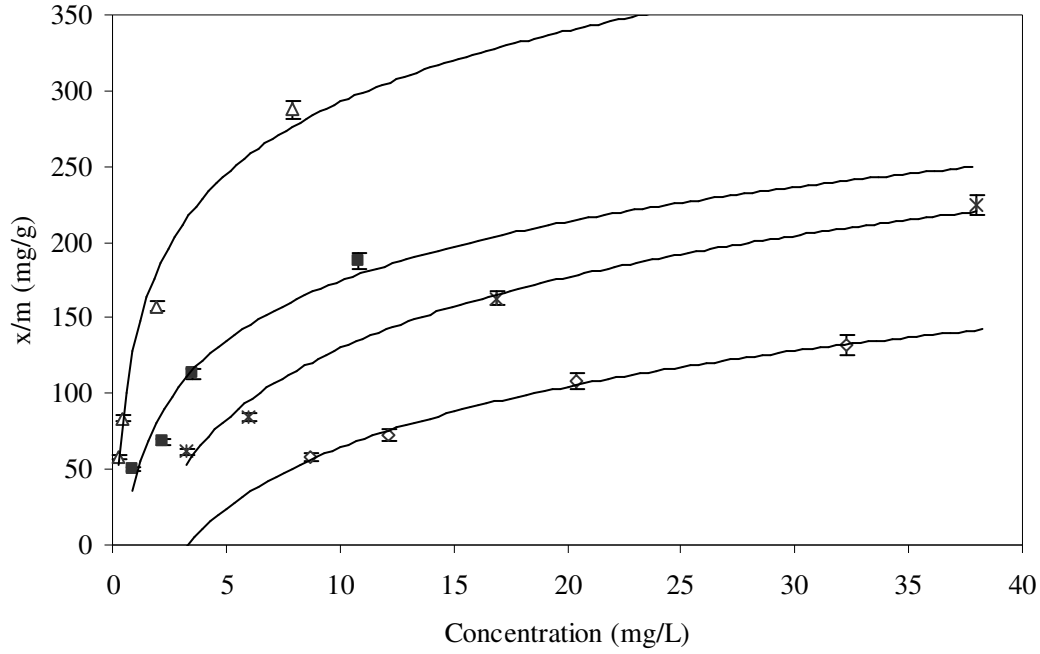
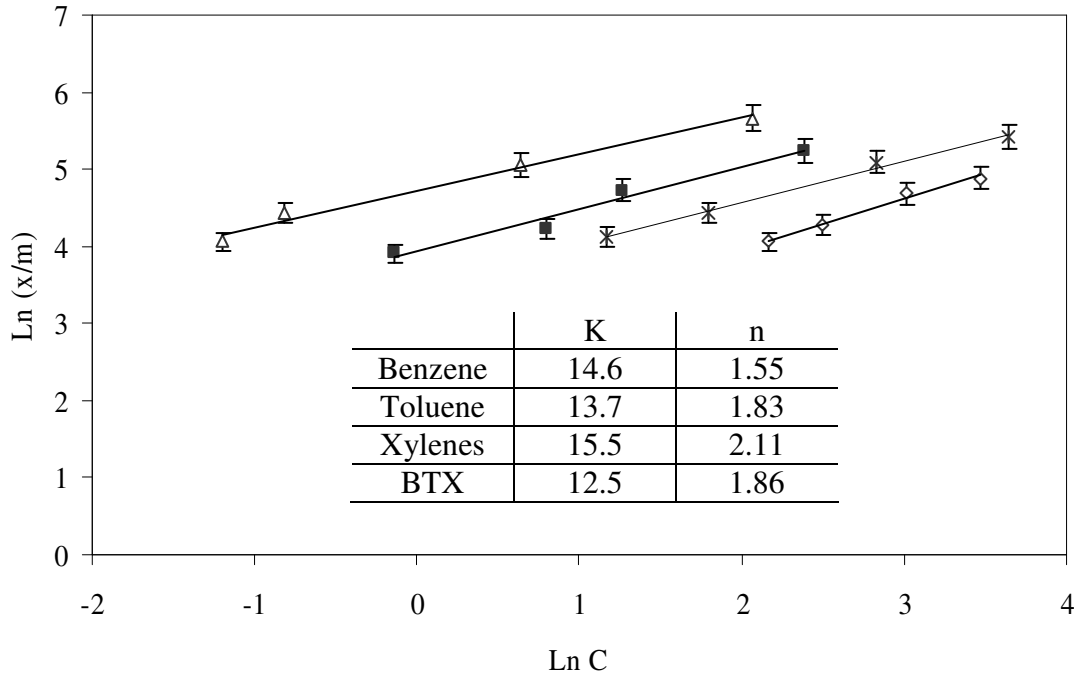


Figure IV.8: BTX adsorption from contaminated water by GAC (temperatue: 24°C, GAC: 2.5 g, working volume: 500mL without air space) (a: BTX adsorption in a single step; b: BTX adsorption in three step)(-◊- : benzene; -■-: toluene; -Δ-: xylenes)

(a)



(b)



	K	n
Benzene	14.6	1.55
Toluene	13.7	1.83
Xylenes	15.5	2.11
BTX	12.5	1.86

Figure IV.9: Freundlich isotherm for monoaromatic adsorption from synthetic contaminated water by GAC (working volume: 500 ml without air space, temperature 21°C) (a: Freundlich isotherm; b: kinetic parameters according to equation (12)) (-◊- : benzene; -■-: toluene; -Δ-: xylenes; -✳- : BTX)

The curves in Figure IV.9b also showed that the Freundlich isotherm could describe BTX adsorption from synthetically polluted water by GAC. It was mathematically expressed as:

$$\frac{x}{m} = K C^{\frac{1}{n}} \qquad (11)$$

and could be linearized as : $$\mathrm{Ln}\frac{x}{m} = \mathrm{Ln}\,K + \frac{1}{n}\mathrm{Ln}\,C \qquad (12)$$

where x was the mass of the adsorbate (mg BTX), m the mass of the adsorbent (mg GAC) and C is the equilibrium concentration of adsorbate in solution (mg/l). K and n are dimensionless constants incorporating all factors affecting the sorption process and were close to 14 for K and 2 for n (Figure IV.9b). Similar behaviour has already been found for monoaromatic compounds and petroleum adsorption by activated carbon (Zynter, 1994; Ayotamuno et al., 2006).

IV.4.2. Solute desorption from GAC

Solute desorption from the solid adsorbent was also studied. After the adsorption equilibrium was reached, the supernatant was removed and its sorbate concentration was measured. The supernatant was then replaced by clean distilled water. Desorption of BTX from activated carbon was allowed to continue until equilibrium, i.e. at least for 24 h. This process was then repeated, Figure IV.10 showed that after eight replicas, less than 8% of contaminants were released in water.

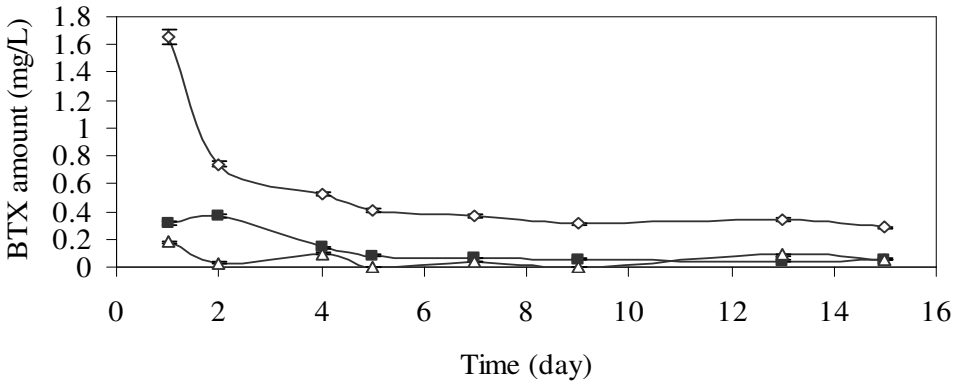


Figure IV.10: BTX desorption from GAC in distillated water (working volume: 1 L without air space, initial benzene, toluene and xylenes adsorbed by GAC were 13.1, 14.1 and 12.4 mg/g GAC, respectively; GAC: 2g, temperature 24°C, pH=6.3) (-◊- : benzene; -■-: toluene; -Δ-: xylenes) (Each point corresponds to an external water change).

This result demonstrated that BTX retention in GAC particles was not a pure, i.e. fully reversible, adsorption phenomenon and that strong interactions were likely to take place between the solutes and the support, in addition to those taking place between solute molecules, evidenced above.

IV.4.3. Monoaromatics extraction from GAC

Table IV.d compares the extraction effectiveness of five different commonly applied extraction techniques for the determination of monoaromatic compounds such as toluene hydrocarbons in GAC. The techniques included are simultaneous distillation-extraction (SDE), Soxhlet, sonication, pressurized microwave assisted extraction and solvent extraction by disulfide carbon. Generally, these methods are applied for organic extraction from contaminated soil (Dean and Xiong, 2000; Eskilsson and Björklund, 2000; Sporring et al., 2005). Results highlighted the fact that solvent losing and VOC stripping is the main problem in some of extraction techniques. For example monoaromatic stripping by sonication from water solution was showed in Figure IV.11.

Although Campos-Candel et al. (2007) reported that accelerated solvent extraction (ASE), a new technology claimed to enable high solute recovery (minimum 95%) could be used for desorption of monoaromatic compounds from activated charcoal during 10 min. However data presented in Table IV.d showed that toluene desorption from GAC by carbon disulfide as extraction solvent was the best protocol for the monitoring of aromatic compounds in activated carbon particles. This technique was recommended by NIOSH (method 1501, 2003) for the measurement of volatile aromatics compounds in air and was found suitable for toluene extraction from GAC particles with 94 ± 1 % (w/w) efficiency (see Figure IV.12).

Table IV.d: Comparison of extraction techniques for determination of toluene in GAC

Extraction technique	Remark(s)	Maximum recovery (%)	Related reference(s)
SDE (Likens-Nickerson)	- Solvent losing was observed during processing and in optimal conditions (described par. II.3.3.3.1) 8.7% (v/v) of solvent stripped from system.	-	Nickerson and Likens (1966); Godefroot et al., 1981
Soxhlet	- This method required large volume (up to 75 mL) of DCM solvent. - Solvent losing was detected during extraction process and in applied operation conditions (described par. II.3.3.3.2) 12.6% (v/v) of solvent stripping was observed.	-	Sporring et al., 2005
Sonication	- Solvent losing was negligible (<1%v/v) in 9 min extraction process according to condition described par. section II.3.3.3.3. - This process was not very efficient for toluene extraction. - Extracted toluene was stripped by sonication from solvent during extraction period as system was open (see Figure IV11).	22.5	Llompart et al., 1997; Choi et al., 2007
Pressurized microwave-assisted	- There are not any solvent losing during this extraction technique (described par. section II.3.3.3.4). - GAC particle was crushed during extraction procedure and sample pre-treatment was necessary (Centrifugation in glass capped Teflon tubes), before sample injection to GC/MS.	59.8	Eskilsson and Björklund, 2000; Dean and Xiong, 2000; Ania et al., 2007
Solvent extraction by carbon disulfide	- This technique is very simple, efficient and relatively fast (30 min). - This system is closed during extraction period (described par. II.3.3.3.5.), then there are not any solvent losing.	94	NIOSH manual of analytical methods, 2003; Campos-Candel et al., 2007

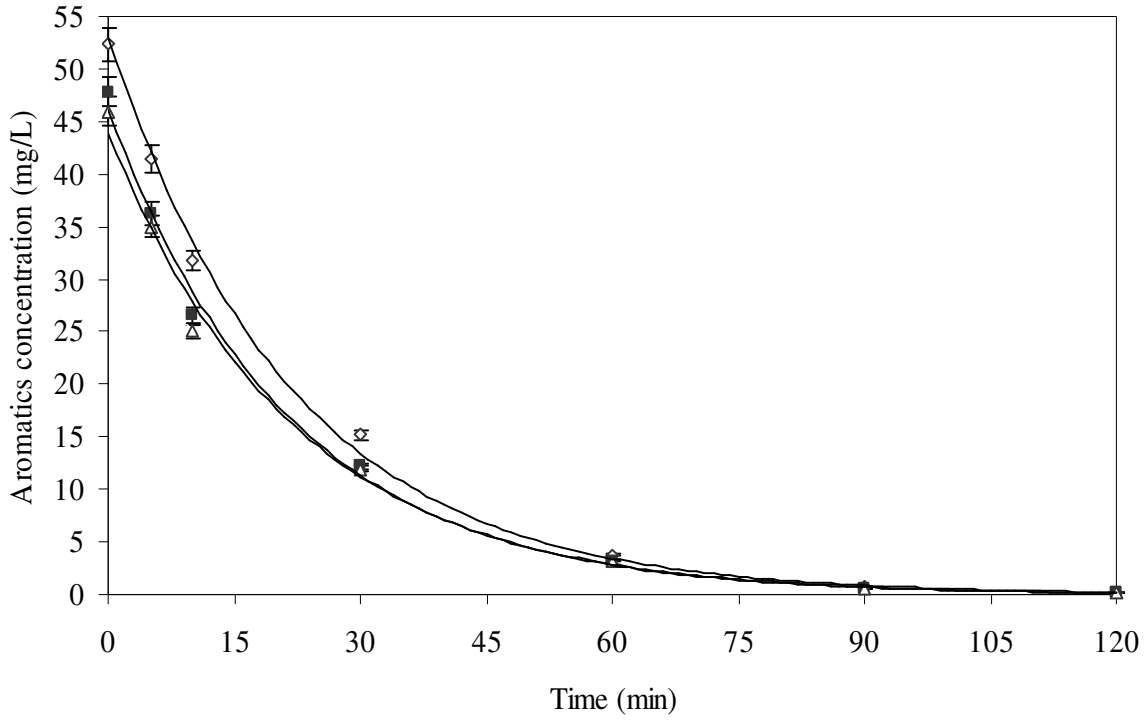


Figure IV.11: Monoaromatics stripping from water solution by sonication (Liquid volume: 40 mL, ultrasonic apparatus described par. II.3.3.3.3. was used with 5 cm probe immersed in the liquid phase, temperature: 22 ± 2 °C).

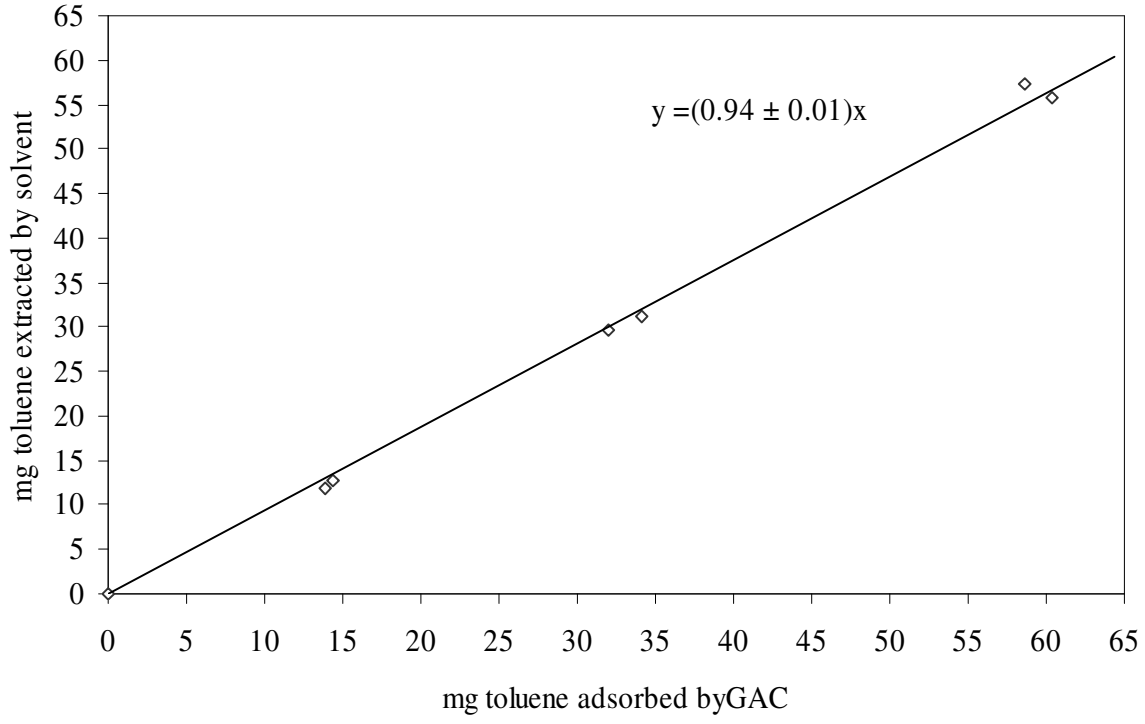


Figure IV.12: Calibration curve for toluene extraction from GAC by carbon disulfide

IV.5. Conclusion

Treatment efficiencies of 99 % and above, in term of BTX disappearance from water polluted could be achieved by aeration and adsorption process. Results showed that air sparging in polluted water can reduce monoaromatic compounds from 140000 to about 5 as µg/L in only 1 h process with a gassing rate of 0.33 VVM. Present data demonstrated that monoaromatics volatilization and stripping phenomena during water remediation processes account for a significative amount.

Also, results obtained indicated that adsorption by GAC was highly successful in the removal of BTX compounds from liquid media till environmentally acceptable levels. Solute adsorption onto granular activated charcoal (GAC) showed that maximum solute retention could be estimated to 350, 250 and 150 (as mg/g GAC) for xylenes, toluene and benzene, respectively.

Chapter V

Ex-situ monoaromatic bioremediation

V.1. Objectives

As already stated, monocyclic aromatic hydrocarbons are major volatile constituents of oil products. Contamination of water by volatile aromatic compounds is a serious environmental problem as these hydrocarbons can cause hazard for human health and they are highly soluble in water in comparison to aliphatic compounds. Results in preceding chapters have shown that aromatic hydrocarbons under abiotic conditions can be reduced from polluted water by evaporation, aeration or adsorption onto a solid adsorbent. These techniques cannot be considered as green technologies as pollutants are transferred from contaminated water to air or solid materials.

Literature survey indicated that biological processes can play a major role in the treatment of water contaminated by monoaromatic hydrocarbons. These studies also highlighted the fact that in biological techniques, volatile organic compounds (VOC) can also be stripped from the system to gas exhaust. Also, these hydrocarbons can be adsorbed by the biomass solid support in biofilm reactors. Thus, the control of these factors during bioremediation processes is still necessary.

The objective of this research was the development of an effective method on the basis of bioremediation technology for monoaromatic removal from contaminated water without any further environment damage. In this section, solute removal from synthetically water polluted under aerobic and anaerobic condition was evaluated. In aerobic systems, solute biodegradation by sequencing batch reactors, aromatics biodegradability by OxiTop assay, and toluene remediation in two-phase partitioning bioreactors was investigated.

Also, toluene treatment from contaminated water by a novel approach involving anaerobic biphasic bioreactors was studied. The objective of this research was to evaluate the efficiency of a strategy involving toluene adsorption by granular activated charcoal and subsequent regeneration of this support by an anaerobic bioleaching process. GAC bioregeneration in anaerobic bioreactor with the denitrifying bacterium *Thauera aromatica* was studied. A search for the biodegradation of toluene at high initial concentration in an organic-aqueous phases (biphasic) bioprocess with nitrate respiration is also reported.

V.2. Aerobic systems

V.2.1. BTX biodegradation in aerobic batch bioreactors

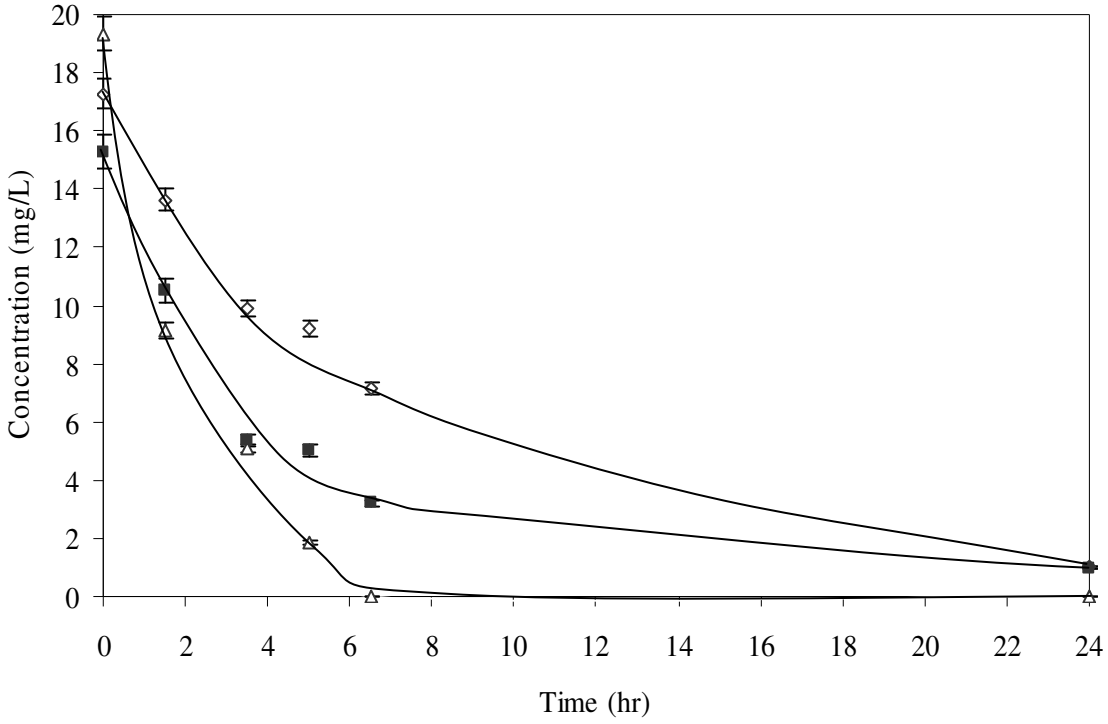
Results presented in Figures V.1, V.2 and V.3 highlighted the fact that BTX compounds were easily biodegradable by the adapted aerobic biomass used in this study (described par. II.4.3.1.). Apparent treatment efficiencies up to 99%, in term of monoaromatics disappearance from the liquid could be achieved. However, comparison with a control experiment carried out without biomass allowed to demonstrate that, as expected, a biological process actually took place together with gas-liquid transfers (Figures V.1b, V.2b and V.3). Data indicated that the sequencing batch bioreactor was also a successful method for BTX degradation but VOC stripping and volatilization can contribute in a non negligible amount to the final result. This behaviour was consistent with the fact that results published up to now have clearly demonstrated that the feasibility of BTX biodegradation through aerobic bioreactors could be considered as well established (Guerin, 2002; Zein et al., 2006).

Also, data highlighted the fact that ratio of substrate to microorganism (S/M) is important and influential on efficiency of aerobic batch reactors (Figures V.1 and V.2). Results showed that with increasing this ratio from 0.26 to 0.77 mg BTX per mg dry mass, monoaromatic biodegradation rate decreased from 11.3 to 2.5 mg BTX / L.h, respectively, showing an inhibition phenomenon. This feature was in accordance with the well-known fact that monoaromatic compounds are xenobiotic compounds. Other results also demonstrated that BTX concentrations higher than 150 mg/L were toxic and inhibitory for mixed cultures in aerobic batch bioreactors.

Experiments presented in Figure V.3, also revealed the ability of inactive (autoclaved) biomass to adsorb BTX compounds. Data indicated that an increase in biomass concentration in bioreactors, could result in an increase in the amount of total organic compounds sorption onto biological substances.

Finally, although the amount of BTX stripping from this bioprocess could be minimized through batch feedings, this technique was not as an environmentally friendly approach, since volatile organic compounds were still present in a non negligible amount in exhaust gas.

(a)



(b)

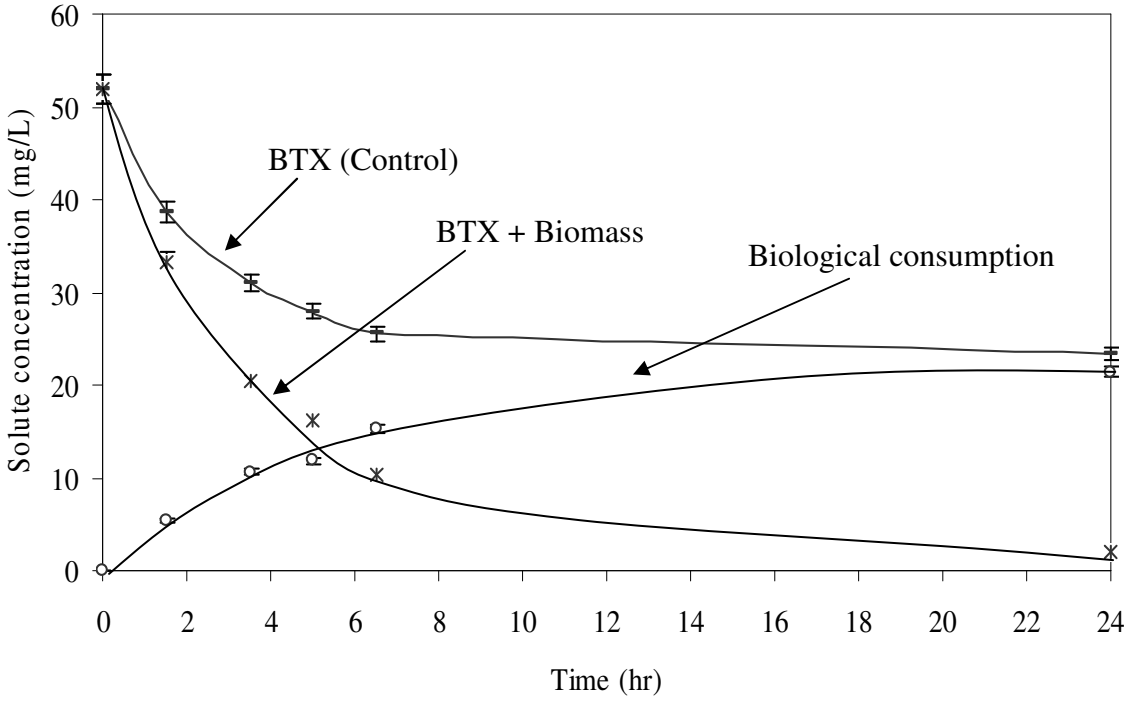
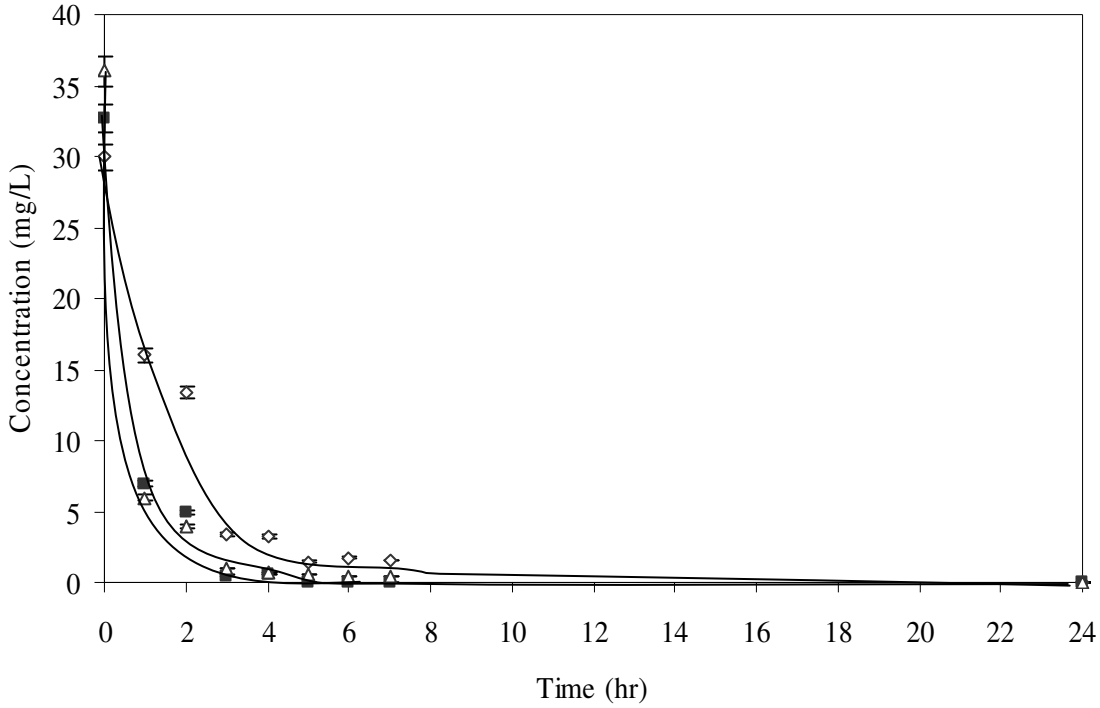


Figure V.1: Monoaromatic removal from polluted water through aerobic batch bioreactor (Working volume 250 mL, temperature 23°C, biomass concentration 65 mg/L, BTX/biomass 0.77 mg/mg)(a: gives the residual concentrations of individual compounds, -◊- : benzene; -■-: toluene; -Δ-: xylenes) (b: gives the overall residual concentrations and the true biological consumption)

(a)



(b)

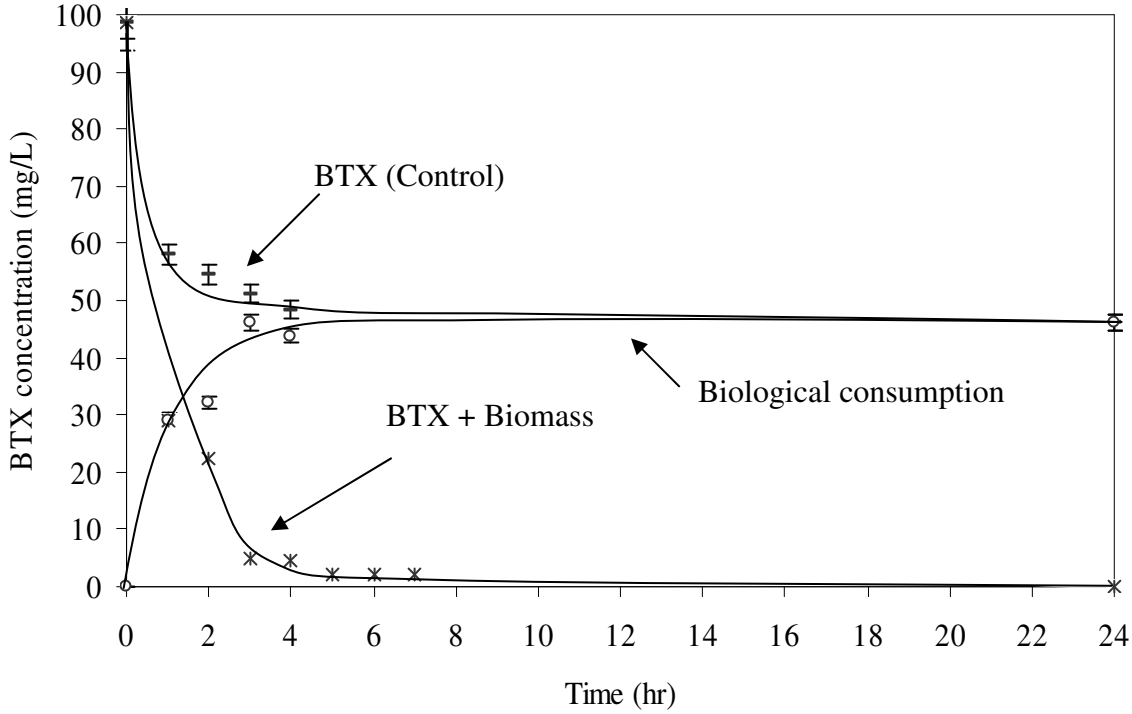
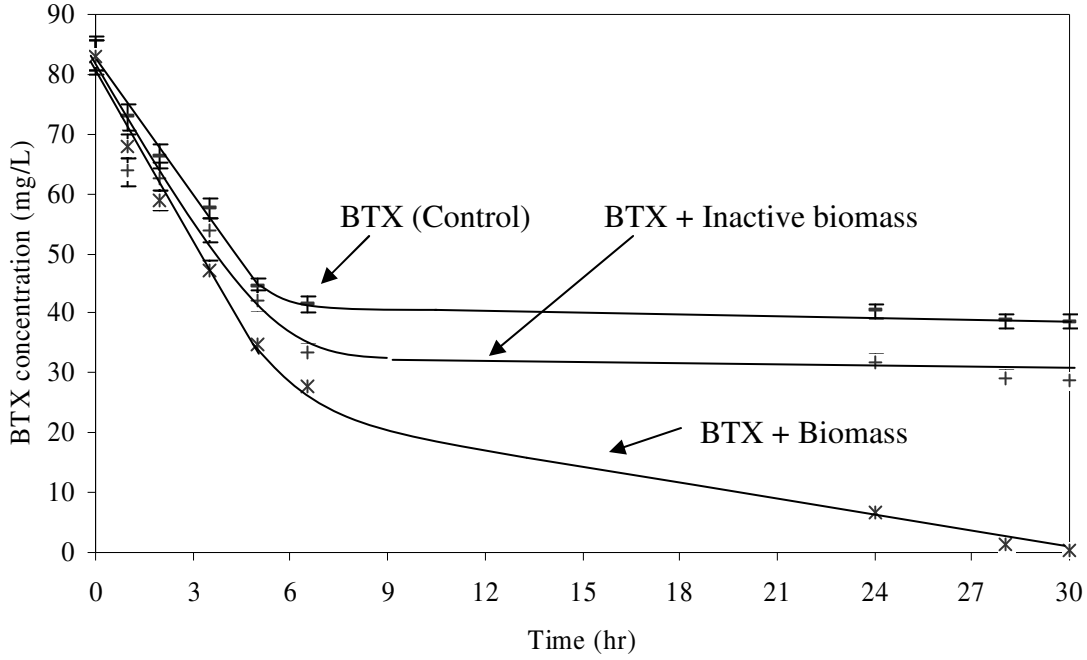


Figure V.2: BTX treatment from contaminated water by aerobic batch bioreactor (Working volume 250 mL, temperature 23°C, biomass concentration 392 mg/L, BTX/biomass 0.26 mg/mg)(a: gives the residual concentrations of individual compounds, -◊- : benzene; -■-: toluene; -Δ-: xylenes) (b: gives the overall residual concentrations and the true biological consumption)

(a)



(b)

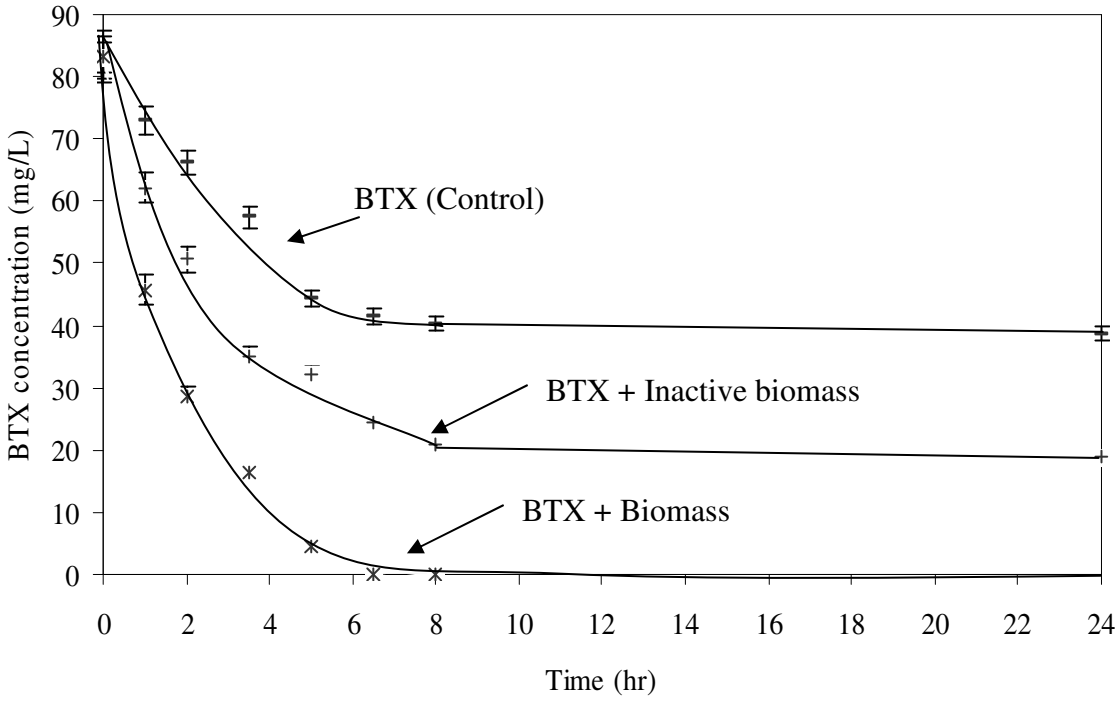


Figure V.3: Ability of adsorption of monoaromatic compounds by inactive biomass in aerobic batch bioreactors (Working volume 250 mL, temperature 25°C)(a: biomass concentration: 65 mg/L, b: biomass concentration: 334 mg/L)

V.2.2. Building of an aerobic biodegradability test based on OxiTop assay

Assessment of a bioremediation process could thus be done by indirect metabolic measurements. They were done using OxiTop tests, based on the measurement of pressure drop as a result of oxygen consumption in a closed vessel where the carbon dioxide produced was trapped by caustic soda. In this section biodegradability of BTX compounds in synthetically contaminated water by the respirometric biological oxygen demand (BOD) was evaluated.

V.2.2.1. Aromatics bioavailability

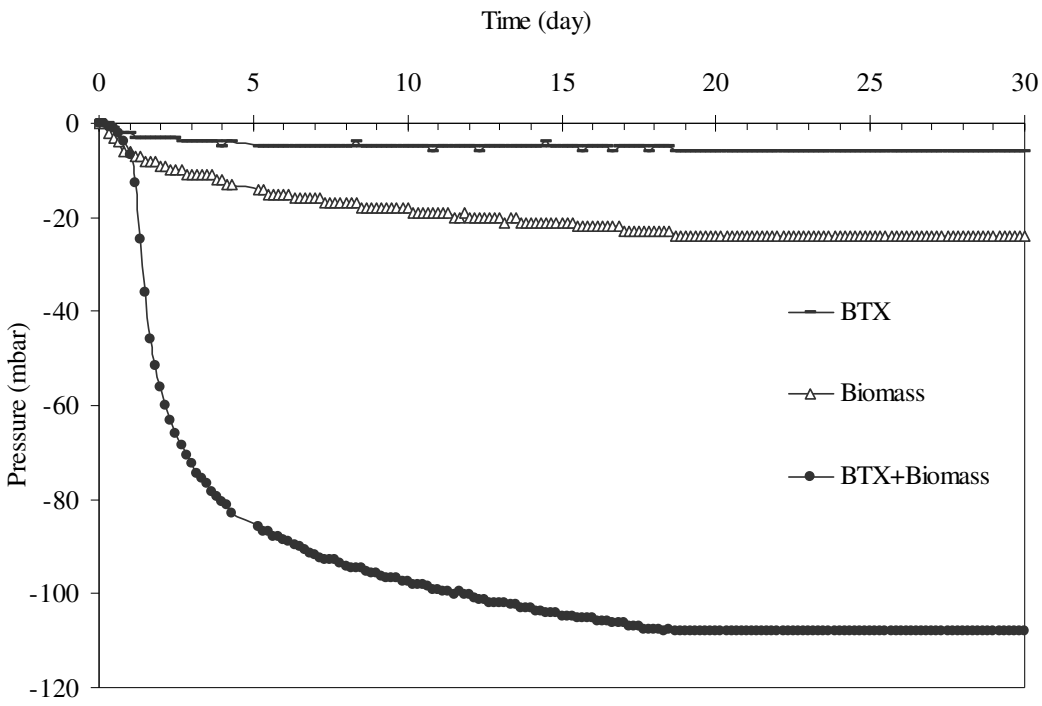
Results presented in Figure V.4 shows the bioavailability of monoaromatic hydrocarbons in OxiTop systems. Data highlighted that BTX compounds were biodegradable by aerobic mixed culture biomass. The produced results in this system showed that maximum of biodegradability measured in batch systems for 60-80 mg BTX/L were in ranges 70-80 % (mg BTX /mg ThoD). Experimental works showed that the reason of this difference to 100 % BTX degradation was connected to oxygen limitation in closed systems and monoaromatic adsorption by rubbers gaskets of OxiTop materials. Actually, in 625 mL of air presented in OxiTop bottles, there was 187.5 mg oxygen that corresponded to 42 mg BTX contaminants that could be metabolized in the aqueous phase (or 88 mg BTX/L), in accordance to the theoretical oxygen demand and oxygen requirement for biomass production. After 30 days, BTX concentration in biological samples taken from OxiTop bottles was 0 mg/L (as analysed by HPLC technique, described par. IV.4.) and no intermediate product could be detected by GC/MS (described par. IV.5).

In this approach biomass could not be used with high initial concentrations to increase the biodegradation rate. Hence, data presented in Figure V.5 showed that in standard conditions (mixed culture without carbon source) aerobic bacteria consumed oxygen during stationary and endogenous phases. Thus biomass concentration had to be less than 50 mg/L for this type of experiments. This work thus allowed to suggest a versatile, efficient methodology to assess the activity of an aerobic biomass to metabolize volatile organic compounds.

V.2.2.2. GAC bioleaching

Bioleaching of granular activated charcoal (GAC) loaded with aromatic hydrocarbons can be thought as a favourable alternative for treatment of BTX contaminated water

(a)



(b)

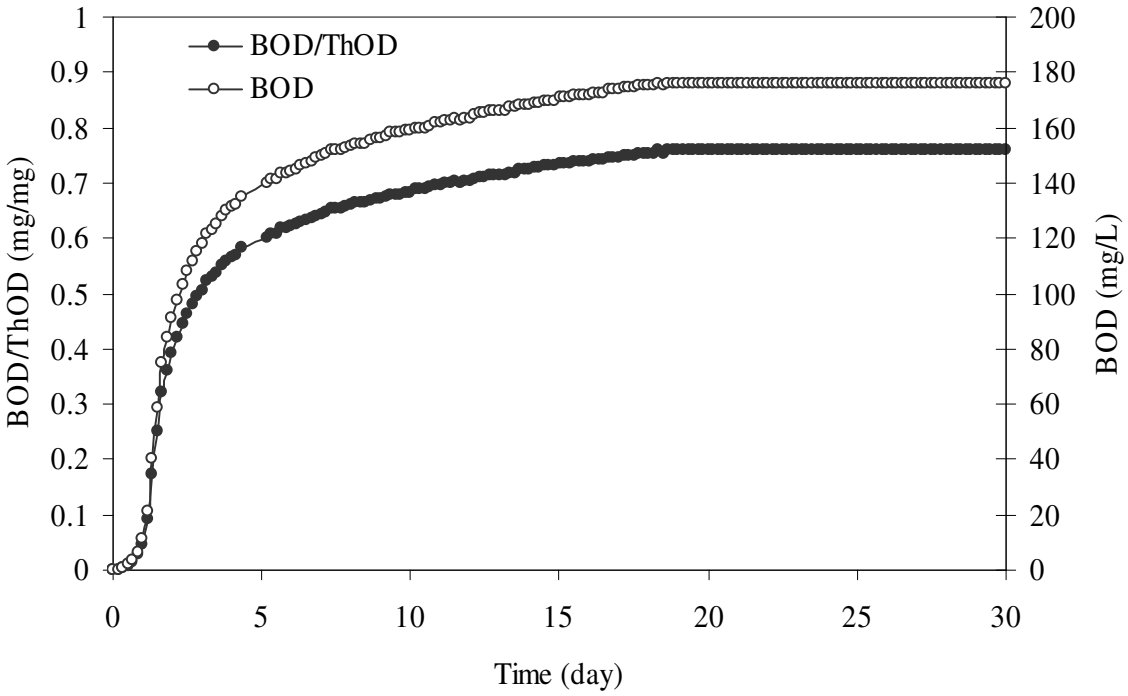


Figure V.4: BTX biodegradability in synthetically water polluted by OxiTop tests (initial biomass concentration: 21 mg/L; temperature: 30°C; BTX concentration: 75 mg/L (25 mg/L for each substances); working volume: 500 mL; bottle volume: 1125 mL)(a: pressure change vs. time; b: BOD and BOD/ThOD vs. time))(The theoretical oxygen demand is presented in Table I.b for monoaromatics compounds)

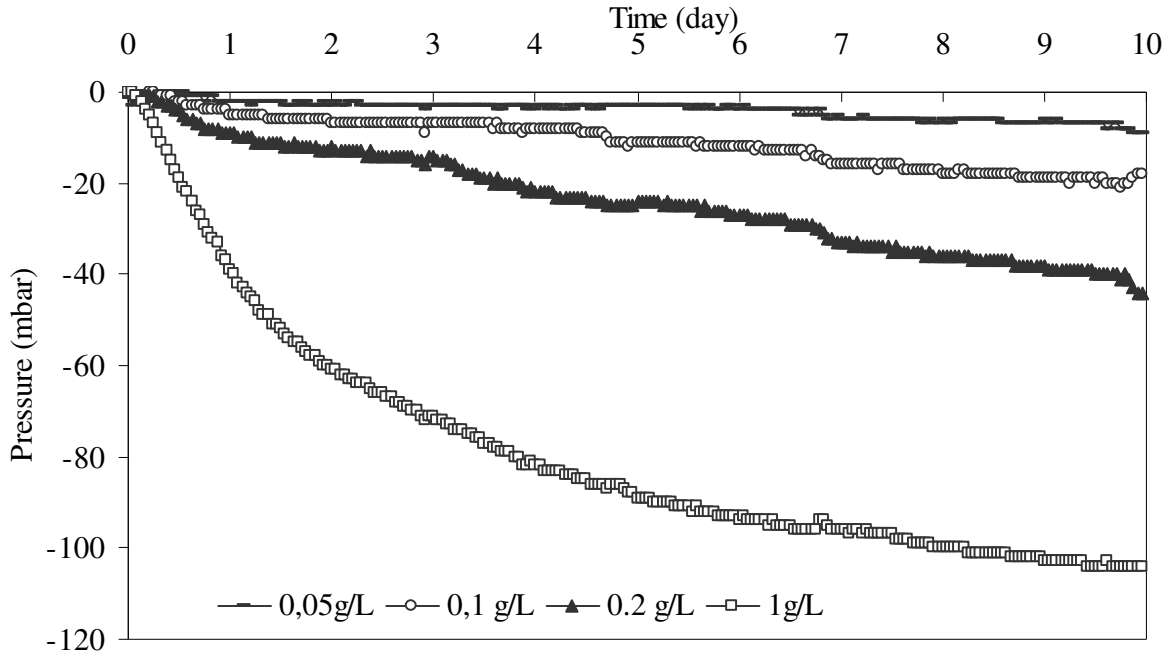
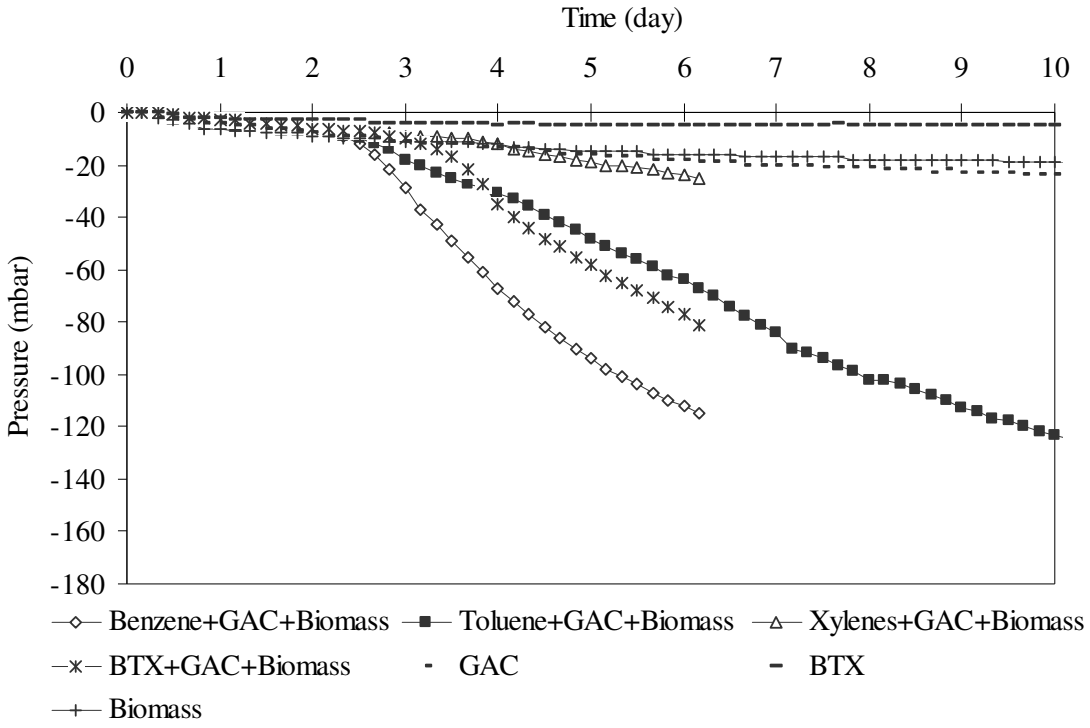


Figure V.5: Effect of initial biomass concentration on pressure drop in OxiTop systems (without any carbon source; Temperature: 30 °C)

In this process, monoaromatic hydrocarbon can be adsorbed on the surface of GAC particles and then stabilized by the biomass at the surface of activated carbon. Then bioleaching process provides a combined biological-degradation and adsorption-desorption method, using activated carbon, to deal with a wide variety of pollution problems.

When the solid adsorbent was used with a low BTX loading (0.02 g/g), the residual pollutant concentration in the liquid was close to zero, as shown in Figure IV.8. Results clearly showed that bioleaching of GAC actually took place, which meant that biomass was able to have an access to BTX retained in the GAC particles (Figure V.6). This process appeared more efficient for benzene, toluene and mixed xylenes in that order, and up to 35% toluene could be removed after 10 days of process with a very low biomass concentration (46 mg/L) and a significant GAC loading (0.5 % w/w).

(a)



(b)

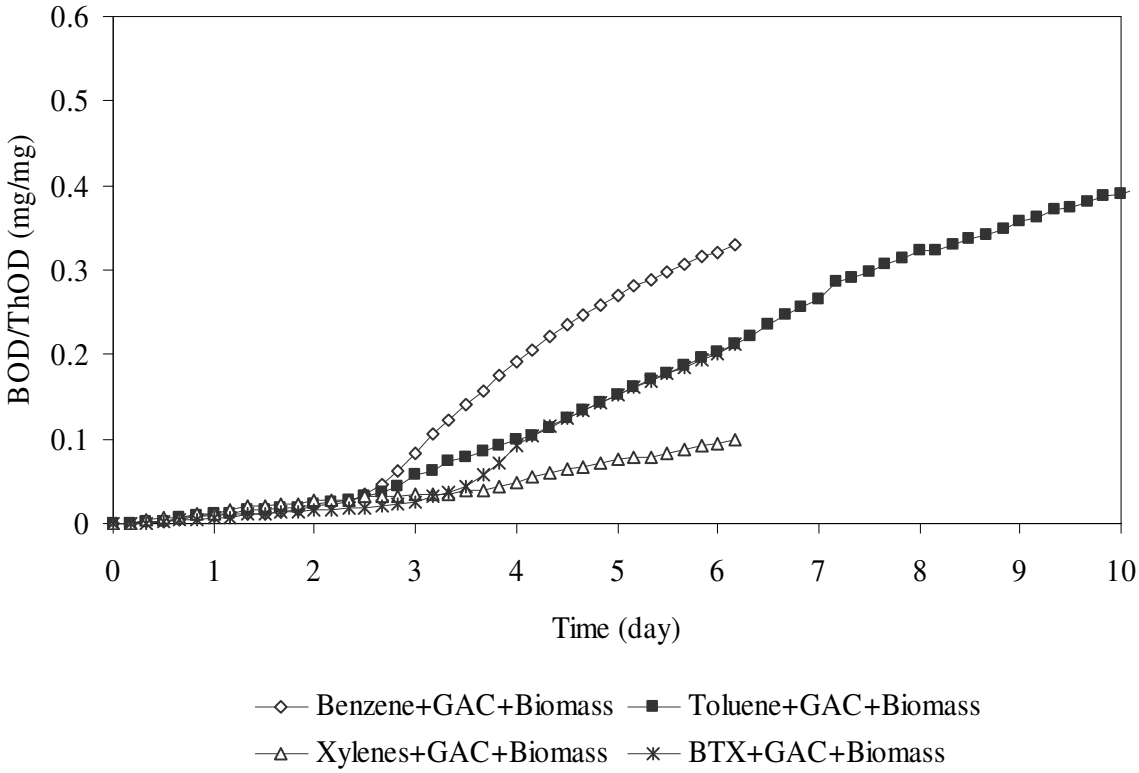


Figure V.6: Bioleaching of activated carbon after monoaromatic adsorption in OxiTop (30°C, biomass concentration= 46mg/L, contaminants/GAC= 22 mg/g, GAC= 2.5 g)(The theoretical oxygen demand is presented in Table I.b for monoaromatics compounds)(The samples containing GAC and biomass correspond to the bioremediation experiments, whereas the others are controls (GAC, BTX or biomass alone in water).

This approach showed that the strategy of BTX removal from contaminated water by trapping of solutes in activated carbon particles followed by biodegradation by a bacterial culture in a separate bioreactor could be explored. Current results were very promising for the building of a process enabling the removal of these compounds from polluted water without any further environmental damage.

V.2.3. Aerobic two- phase partitioning bioreactor

V.2.3.1. Organic-aqueous-air system

As explained in the literature survey, a novel technique entitled two-phase partitioning bioreactor containing of organic, aqueous and gas phases was developed and recommended by researchers for monoaromatic removal from contaminated water. Preliminary experiments in bench scale showed that *Pseudomonas fluorescens* (NCIMB 11671) were capable for toluene biodegradation in biphasic systems (Table V.a.). But as shown by results presented in Figure IV.6, in this system volatile aromatic compounds could still be stripped from the organic phase by aeration. Data obtained showed that generally this phenomenon has not been taken into account by researchers (Collins and Daugulis, 1999a, b; Abu Hamed et al., 2004).

Table V.a: Toluene degradation in aerobic two-phase partitioning bioreactor

<table>
<tr><th>Condition (s)</th><th>Result(s)</th></tr>
<tr><td>- Erlenmeyer flasks: 500 mL
-Temperature: 30 °C
- Shaker: 200 rpm
- Organic phase: hexadecane (12.5 mL) + Toluene (0, 6.25, 12.5, 25, 50 µL) as carbon source
-Aqueous phase: Pseudomonas Basal Medium (230 mL) + Preculture (20 mL)
- pH= 7.2-7.3</td><td>- Initial optical density at 600 nm (OD_{600}) was closed to 0.06 (24 mg dry mass/L).
- after 1 day agitation
<table>
<tr><td>Toluene (µL)</td><td>0</td><td>6.25</td><td>12.5</td><td>25</td><td>50</td></tr>
<tr><td>OD_{600}</td><td>0.21</td><td>0.28</td><td>0.69</td><td>0.67</td><td>0.24</td></tr>
<tr><td>Biomass (mg/L)</td><td>84</td><td>112</td><td>276</td><td>268</td><td>96</td></tr>
</table>
</td></tr>
</table>

Also, as depicted in Figure IV.7, high initial toluene concentration in the organic phase can cause increasing of solute transfer in aqueous phase. Then the lower biomass growth achieved with increased concentrations of xenobiotic compound can be connected to a toxicity of toluene in aqueous phase. Also, a major challenge in this system was still related to toluene stripping from hexadecane, even in Erlenmeyer flasks conditions.

Thus, this technique can be developed using peroxides as an alternative system for oxygen supply without the need for a continuous gassing in biphasic bioreactors.

V.2.3.2. Alternative system

Experimental works in abiotic (biomass free) conditions showed that this hydrogen peroxide did not induce any chemical degradation of monoaromatic compounds in aqueous phase, although it is well known as oxidizer (www.H2O2.com). This series of tests was carried out at 20°C, with BTX concentrations in the range of 100-150 mg/L, while hydrogen peroxide was added to contaminated water in the range of 500-5000 mg/L. The experimental device included fully filled and Teflon capped bottles, the medium being mixed with magnetic stirrer. Data indicated that during 5 days reaction time, BTX concentration did not change.

Also, it is well established that hydrogen peroxide is not chemically stable in water and that among the different factors contributing to its decomposition it is possible to cite: increasing temperature (2.2 factor increase for each 10 °C), increasing pH (especially at pH > 6-8), catalyze increasing (especially transition metals such as manganese and iron), and ultraviolet light (www.H2O2.com). Also, this compound in high concentrations is toxic for microorganisms and could be used as disinfectant in water industries. Preliminarily experiments in bench scale showed that *Pseudomonas fluorescens* (NCIMB 11671) was able to use hydrogen peroxide as oxygen source and toluene compound as carbon source. Shim et al., 2002 reported that *Pseudomonas fluorescens* was known as peroxidase bacteria since it can catalyze the conversion of hydrogen peroxide into water and oxygen.

Tests were carried out in capped, fully filled bottles containing toluene (2µL), hexadecane (2 mL), biomass from a precultivation (1.7 mL), *Pseudomonas* Basal Medium (36.3 mL), hydrogen peroxide (0, 250, 500, 750 and 1000 as mg/L in aqueous phase) at a temperature of 30°C and a shaking rate of 200 rpm. Data showed that hydrogen peroxide in concentrations higher than 500 mg H_2O_2/L was inhibitory for bacterial growth because the optical density at 600 nm was decreased from 0.05 to 0.02 after 24 hours, while the biomass concentration was increased from 20 to 96 and 148 as mg/L, for 250 and 500 mg H_2O_2 /L, respectively. Biomass concentration for standard samples (without hydrogen peroxide) was changed from 20 to 59 as mg/L.

These results demonstrated the feasibility of such an approach. Additionnal work is, of course, needed to optimise this system.

V.3. Anaerobic processes

The feasibility of toluene biodegradation by denitrifying bacteria such as *Thauera aromatica* has been well documented in the last decades (Lochmeyer et al. 1992, Seyfried et al. 1994, Boll et al., 2002; Shinoda et al., 2004; Boll 2005, Zhang and Bennett, 2005; Duldhardt et al. 2007; Kim and Jaffé, 2007). The aims of this study were i) solute removal from organic phase in an anaerobic two phase partitioning bioreactor and ii) bioregeneration of GAC loaded with toluene hydrocarbon in the anaerobic bioreactor. *T. Aromatica* K172 was chosen since it is well known to be able to readily oxidize anaerobically (nitrate respiration) toluene to carbon dioxide (Biegert et al., 1996; Boll et al. 2002).

V.3.1. Biomass production in the bioreactor

No report could be found on the production of large amounts of *Thauera aromatica* K172 cells. Attempts made in this work involved bacteria grown anaerobically in a stirred bioreactor (Biostat ED, 4L) with a mineral medium containing benzoate as energy and carbon source and nitrate as the terminal electron acceptor (described part II.4.4.). The morphology of the cells during mass production (Figure V.7) showed that the bacterial cells behave as coccoid rods, as previous reported during cultivations in flasks (Anders et al., 1995 and Song et al., 1998).

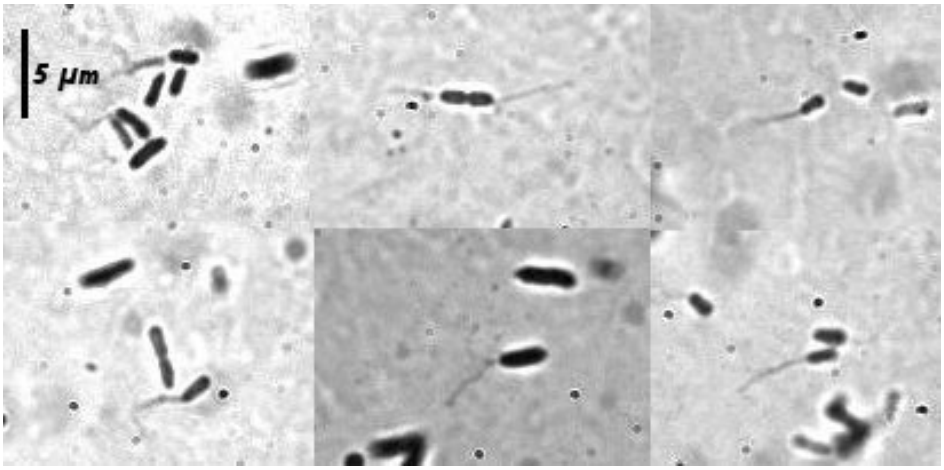


Figure V.7: Strain *Thauera aromatica* K172 grown in liquid medium under denitrifying conditions (Bar: 5 µm, OLYMPUS BX41TF, ×100, oil immersion) (Flagella staining was done according to the method of Mayfield and Kodake (Larpent and Larpent-Gourgaud, 1997)) (30°C, pH= 7.2, stirring rate: 0-50 rpm, volume of growth medium: 4 L)

Figure V.8 shows the production of biomass and carbon dioxide as a function of incubation time in the bioreactor. This process was operated in discontinuous mode, with a sequential feed of the substrate (fed-batch operation) and doubling rate of the cells was close to 2 h 30 min. Duldhardt et al., 2007 reported that the generation times for this strain in the batch system was 3 h1 8 min. Also, results indicated that ratio of carbon dioxide production to sodium benzoate loaded was close to 1 mol CO_2/mol Na-benzoate during biomass production. Figure V.9 shows correlation between optical density and cell dry weight. Data indicated that culture of *Thauera aromatica* K172 were grown in mineral medium with a growth yield close to 0.43 g of dry mass per g sodium benzoate. Also, according to Figure V.8, citrate was detected as intermediate organic acid during biomass production, as also reported by Boll et al., 2002 for the metabolism of anaerobic oxidation of aromatic hydrocarbons.

In this bioreactor, pH of culture had a tendency to increase, it had thus to be maintained at 7.2 with acid adding. It had to be pointed out that ammonium ions were detected as intermediate metabolite during nitrate consumption (Figures V.10 and V.11).

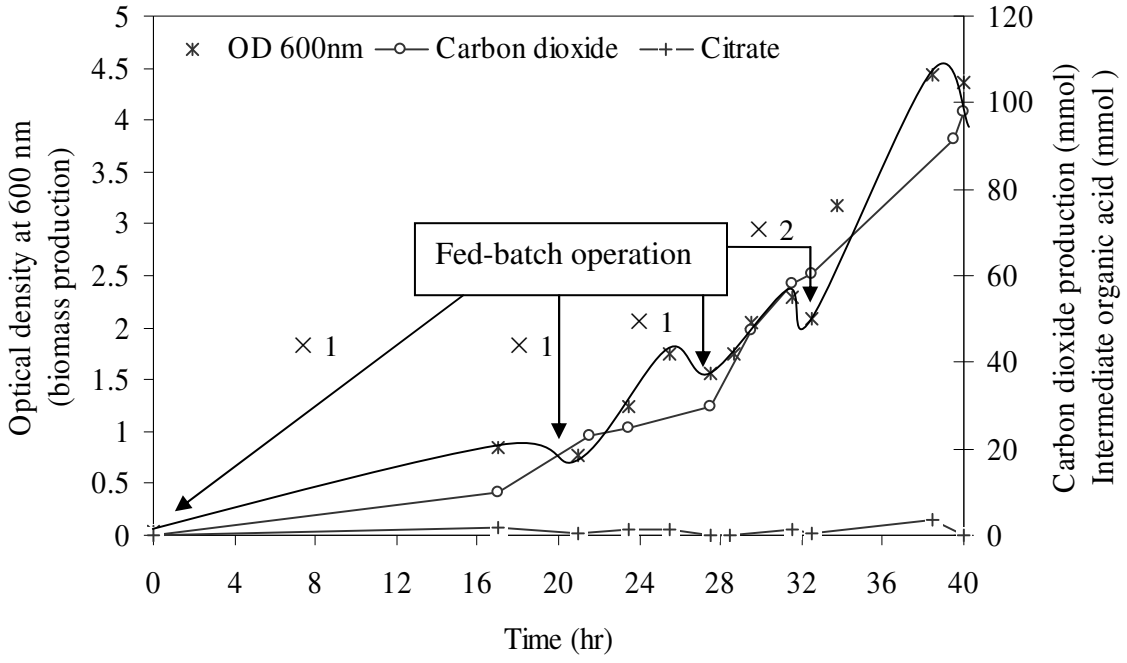


Figure V.8: Biomass and carbon dioxide production vs. incubation time in the bioreactor (*Thauera aromatica* K172, Biostat ED, 4L, 30°C, pH =7.2, Each feeding conditions: Sodium benzoate= 2.88 g, KNO_3= 8 g)(-∗- : OD_{600} , -o- : carbon dioxide, -+- : citrate)

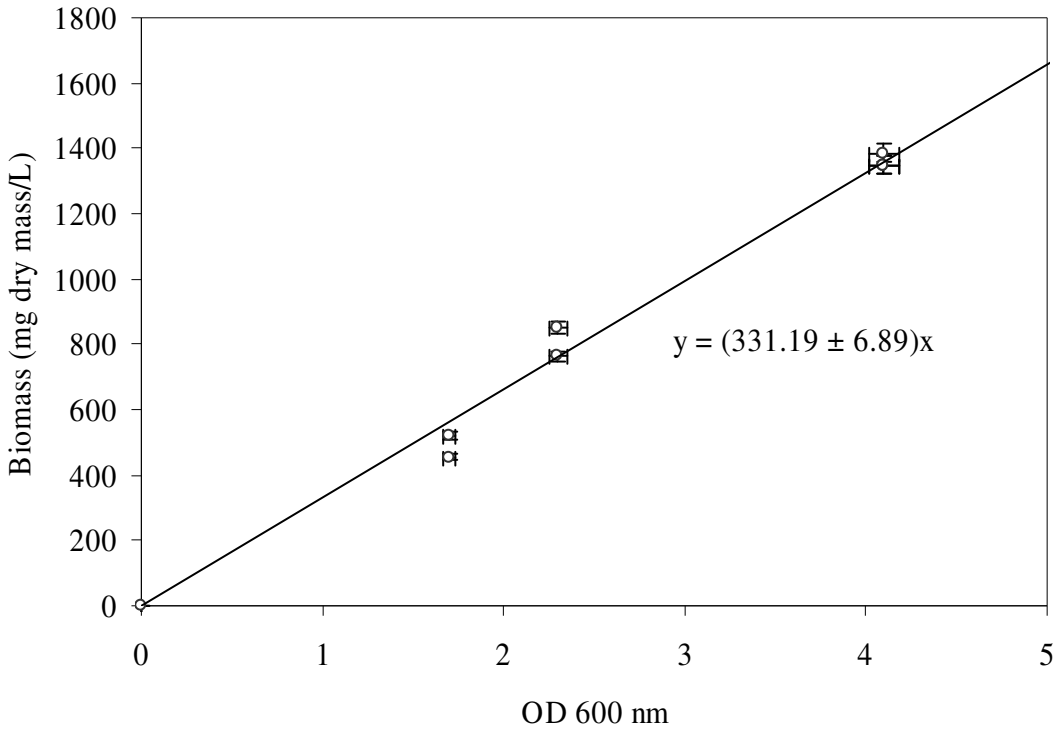


Figure V.9: Correlation of optical density at 600 nm (OD_{600}) and biomass concentration for *Thauera aromatica* in the bioreactor (Biostat ED, 4L, 30°C, pH =7.2)

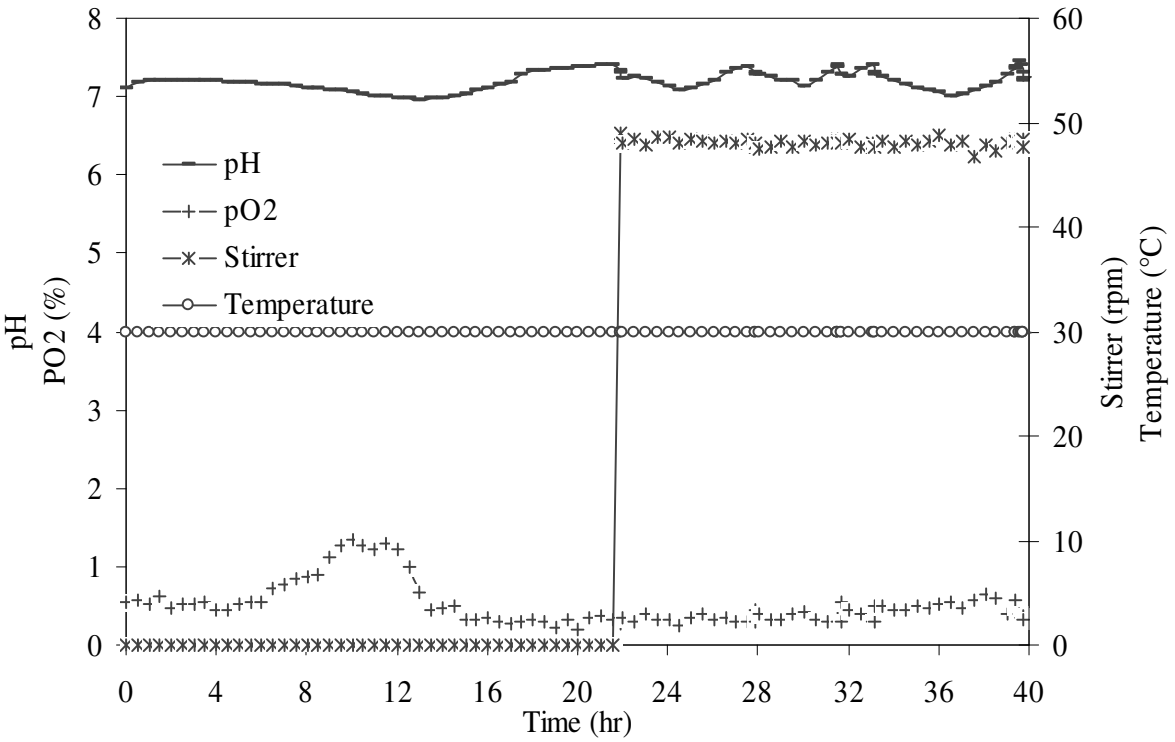
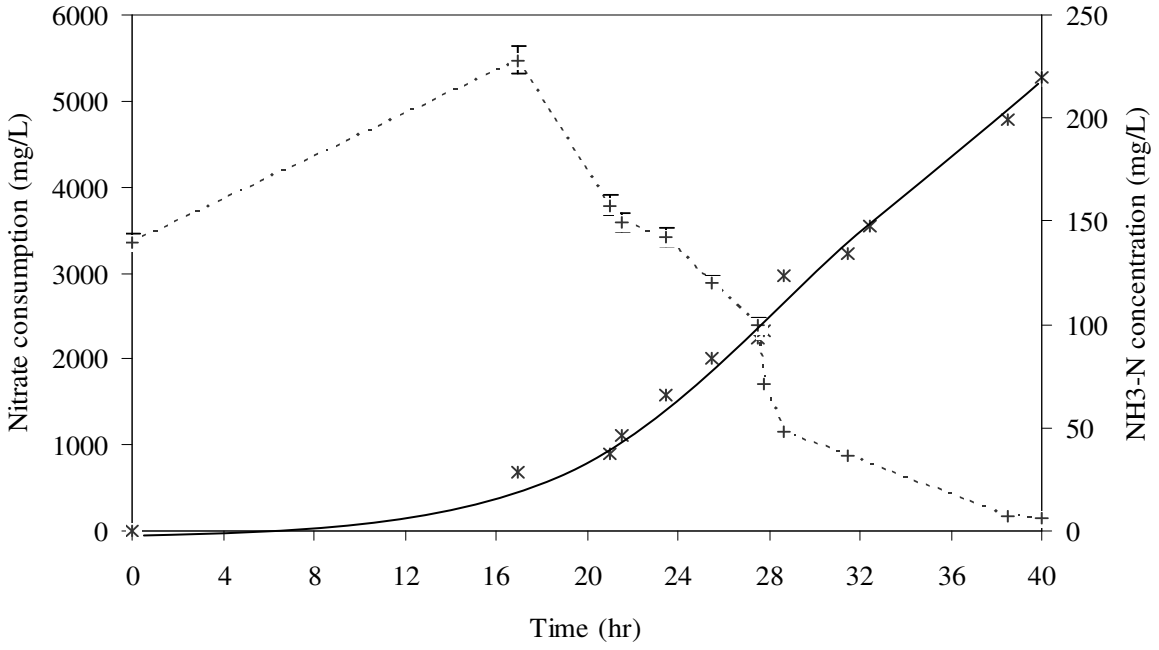


Figure V.10: Process control during biomass production in the anaerobic bioreactor (Biostat ED, volume: 4L, *Thauera aromatica* K172).

(a)



(b)

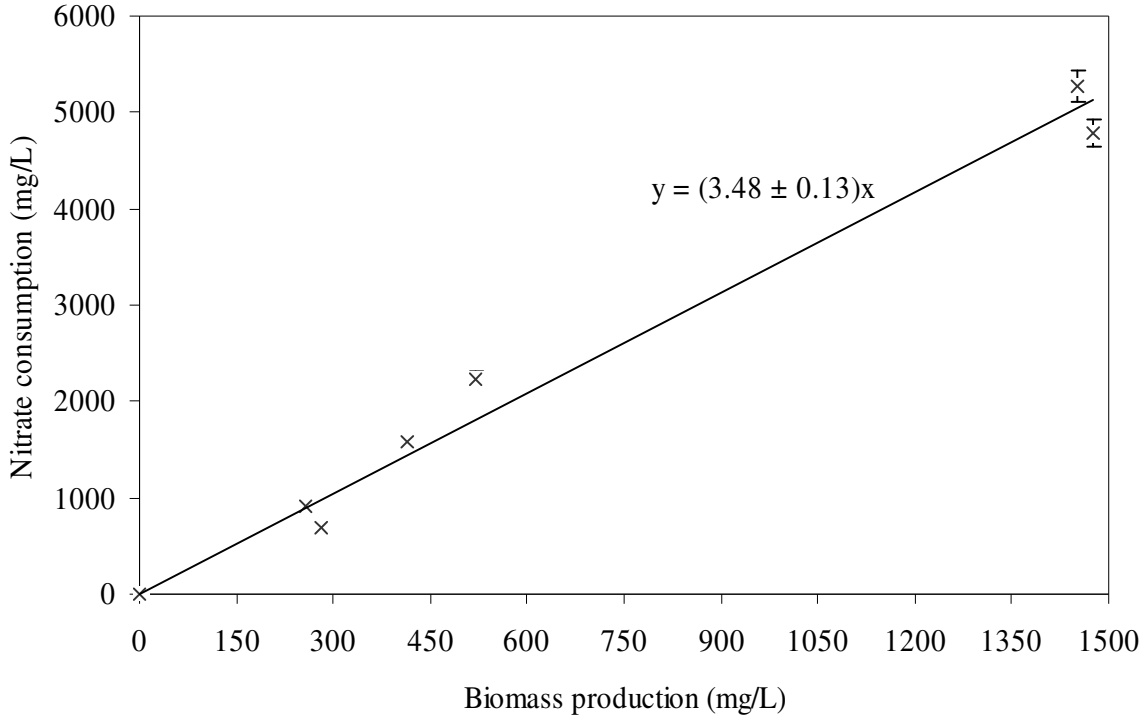


Figure V.11: Calculated nitrate (-∗-) consumption and ammonia concentration (-+-) during biomass production in the anaerobic bioreactor (a: nitrate consumption vs. time; b: nitrate consumption vs. biomass production) (Biostat ED, Volume: 4L, 30°C, pH =7.2)

V.3.2. Monophasic system

Experimental works in Infors micro-bioreactors (as described par. II .4.4.3.1) showed that *Thauera aromatica* K172 was actually able to degrade toluene from synthetically contaminated water with nitrate respiration (Figure V.12) with toluene as sole carbon source. Similar observation has been reported by Seyfried et al., 1994 and Duldhardt et al., 2007 at the bench scale. Results highlighted the fact that in these conditions the utilization of nitrate was linked to toluene biodegradation since it was necessary to add nitrate together with toluene during a process (Figure V.12). Data indicated that the average toluene biodegradation rate in this bioreactor was closed to 8 mg/L.h.

It should be noticed that in abiotic (biomass free) conditions, toluene loss through evaporation and mixing was lower than 1.6 % of total mass of contaminant added to reactor during 3 days, which demonstrated the suitability of this approach to minimize pollutants dissemination in the environment.

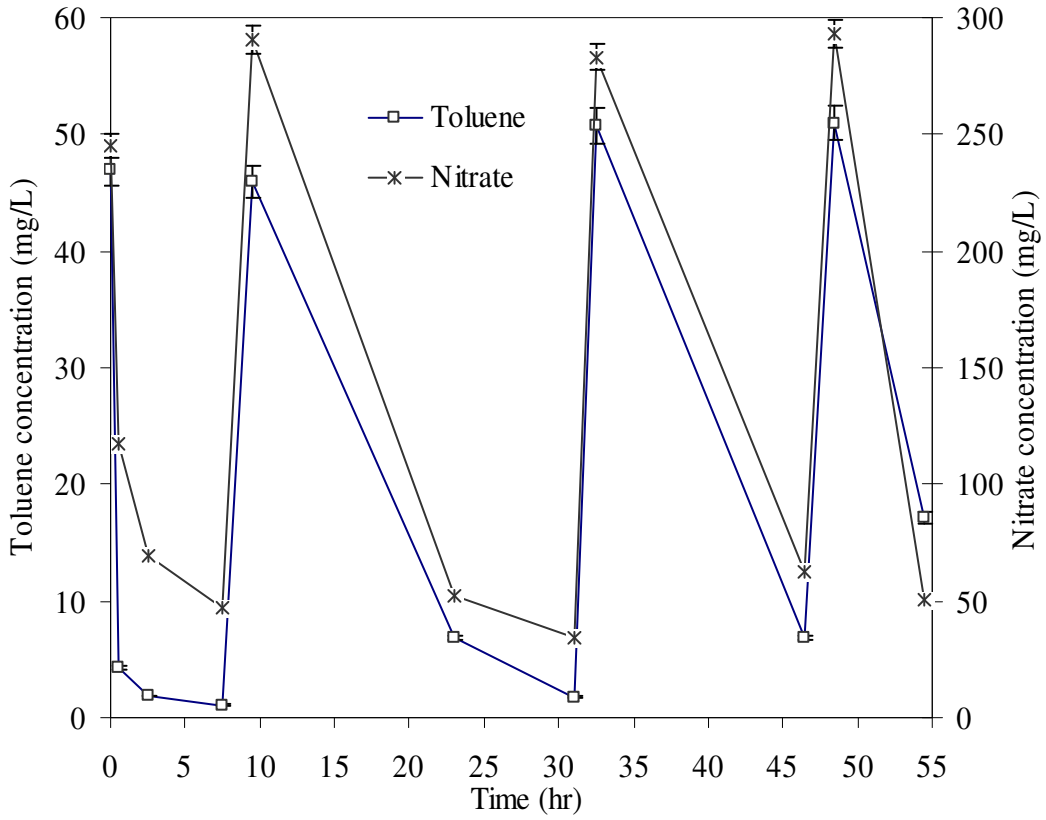


Figure V.12: Toluene degradation (- -) and nitrate (-∗-) consumption in the Infors bioreactor (Temperature: 30 °C, pH=7.2, biomass concentration: 2648 mg dry mass per litre, fed-batch operation).

Below equations (1, 2) demonstrate the stochiometric equation of toluene oxidation coupled to nitrate reduction (Mester and Kosson, 1996; Farhadian et al., 2007; Dou et al., 2008).

$$C_7H_8 + 6\ NO_3^- \longrightarrow 7\ CO_2 + 4\ H_2O + 3\ N_2 + 6\ e^- \quad (1)$$

$$C_7H_8 + 7.2\ H^+ + 7.2\ NO_3^- \longrightarrow 7\ CO_2 + 7.6\ H_2O + 3.6\ N_2 \quad (2)$$

The idealized stochiometric ratios of toluene to nitrate from equation rate are 1:6 and 1:7.2, which are in good agreement with results presented in Figure V.13. A similar behaviour was found using the Bioreactor Biostat ED. Toluene hydrocarbon as sole carbon source was injected into bioreactor by micro pump (described par. II.4.4.3.1.).

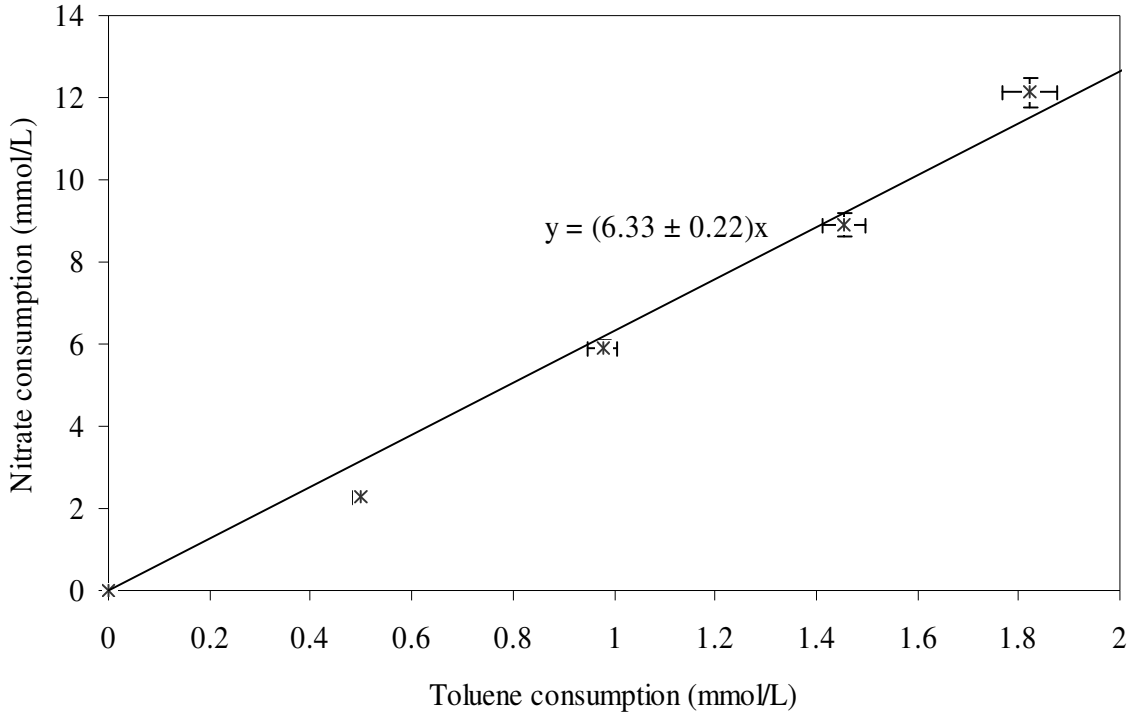


Figure V.13: The molar coupling between the reduction of nitrate and toluene degradation (Infors bioreactor, temperature: 30 °C, pH=7.2, biomass concentration: 2648 mg dry mass per litre, fed-batch operation).

V.3.3. Biphasic media

V.3.3.1. Organic-aqueous system

The concentration of toluene in the abiotic biphasic reactor (described par. II.4.4.3.2.) was monitored for any potential losses due to volatilization and stripping by mixing. After 5 days, no change in the toluene level was detected in either the aqueous or organic phase. In addition, given the high boiling point (287 °C) of the solvent used as the organic phase in this

system, no loss of organic phase was expected. Experimental results showed that a biphasic medium could help in reducing loss of volatile aromatic compounds in a gaseous phase exiting from a bioreactor in comparison to pure aqueous systems. Also, the controlled xenobiotic delivery from hexadecane to the aqueous phase (see Figure IV.6) could open a new area of application of this strategy for monoaromatic anaerobic degradation.

The organic-aqueous system utilized n-hexadecane as organic phase, and the organism *Thauera aromatica* K172 in the aqueous phase to achieve the degradation of toluene. Results presented in Figure V.14 demonstrated that denitrifying bacteria was able to degrade toluene with high initial concentration rate. This was found close to 20 $mg.L^{-1}.h^{-1}$ i.e. 2.5 times higher than in a monophasic anaerobic system. In this system, monoaromatics remediation was also coupled with nitrate reduction (Figure V.15). The molar stoechiometry of toluene degradation to nitrate reduction in biphasic media system was found close to 1:6. During toluene bioremediation, some intermediate organic acids such as formiate, acetate and/or citrate in low level concentration (< 0.0005 mol/L) were detected by HPLC.

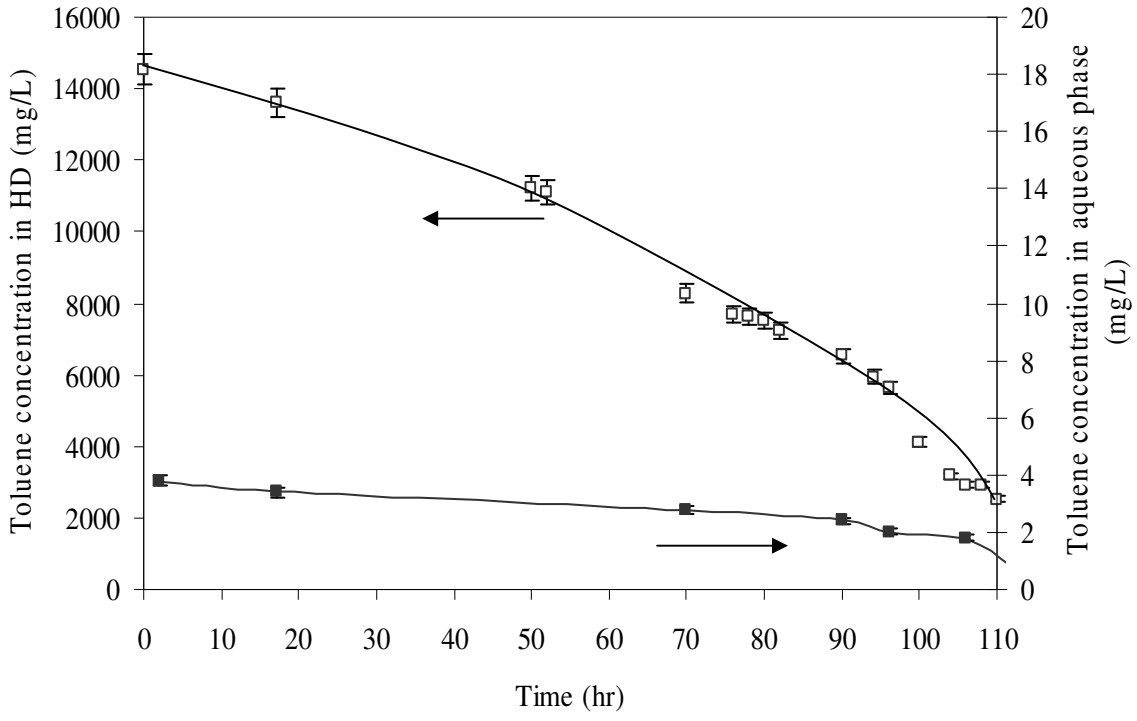


Figure V.14: Toluene monitoring in the two-phase partitioning anaerobic bioreactor. Biostat MD, 30 °C, pH=7.2, biomass concentration: 1325 mg dry mass/L, mixing rate: 50 rpm; -■-, aqueous phase toluene concentration; - -, organic-phase toluene concentration.

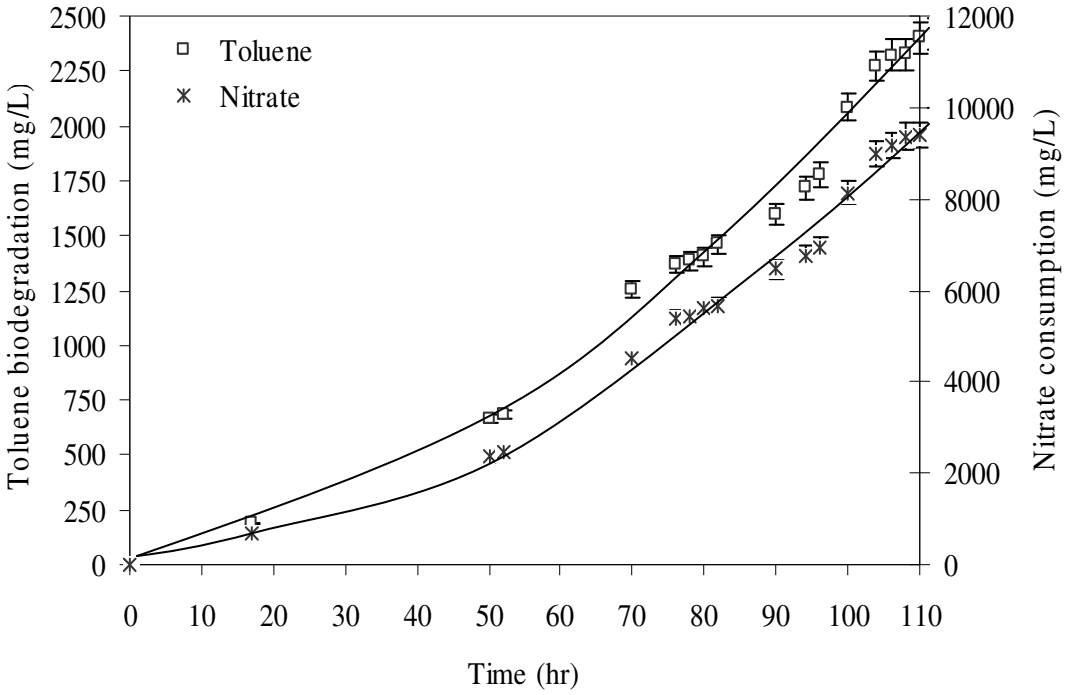


Figure V.15: Toluene biodegradation in aqueous phase (- -) and nitrate reduction (-∗-) in the two-phase partitioning bioreactor. Biostat MD, 30 °C, pH=7.2, biomass concentration: 1325 mg dry mass/L, stirring rate: 50 rpm.

This research showed that the strategy of monoaromatics removal from contaminated water by gas stripping and further trapping of solutes in organic solvent followed by biodegradation by a bacterial culture in a separate anaerobic bioreactor could be explored.

V.3.3.2. GAC bioregeneration

Results presented in Figure IV.8 showed that toluene adsorption onto GAC as solid adsorbent is technically feasible for water treatment. Data indicated that GAC particles is able to remove toluene down to residual concentration as low as 1 mg/L. Kim et al. 2007 and Wibowo et al., 2007 reported that activated carbon is capable for adsorption of monoaromatic compounds from water solution and gas wastes but replacement and disposal of GAC as hazardous waste is a major expense. Also, data highlighted the fact that solute desorption from GAC by pure water in abiotic system is not fully reversible. One potential method for regenerating is off-line anaerobic bioregeneration, in which contaminants are desorbed and biodegraded in a separated bioreactor.

The results presented in Figure V.16 showed that in anaerobic bioreactors fully filled with *Thauera aromatica* K172 and GAC wastes (described par. II.4.4.3.3.), toluene could be extracted from solid while solute concentration in the aqueous phase remained lower than 0.5 mg/L. These experiments showed that 90% of pollutant adsorbed onto solid wastes could be metabolized after 2 days.

According to Figure V.17 the average molar stoichiometry of toluene removal onto GAC by microorganisms to nitrate reduction was 1:6.2. Data clearly showed that biomass was able to have an access to toluene retained in the GAC particles. This feature demonstrated that adsorption by GAC was highly successful in removing toluene down to environmental water levels and that GAC wastes could then be regenerated in separated batch anaerobic bioreactor.

Successful implementation of this approach could reduce costs of GAC replacements and disposal of spent GAC as hazardous waste. Also, this technique could reduce the toxicity, mobility, and the volume of monoaromatic contaminants through bioremediation. Thus, this technique could be considered as favourable and an environmentally friendly approach for removing monoaromatics contaminants.

V.4. Conclusion

Data obtained clearly demonstrate that monoaromatic volatilization and stripping from water decontamination by aerobic ex-situ bioremediation processes must be taken into account, including for the measurement of a microbial acivity. Thus, a biodegradation process in a hogeneous, aqueous system, is not environmentally consistent if used alone. It has been demonstrated that volatile aromatic compounds can be extracted from contaminated water by an adsorbent (such as organic solvent or activated carbons particles) and then these wastes regenerated by anaerobic bacteria in a separated bioreactor without aeration and environmental damage.

This approach justify further developments of off-line anaerobic bioregeneration of solid adsorbents such as GAC as an integrated method for the treatment of water contaminated with monoaromatic hydrocarbons.

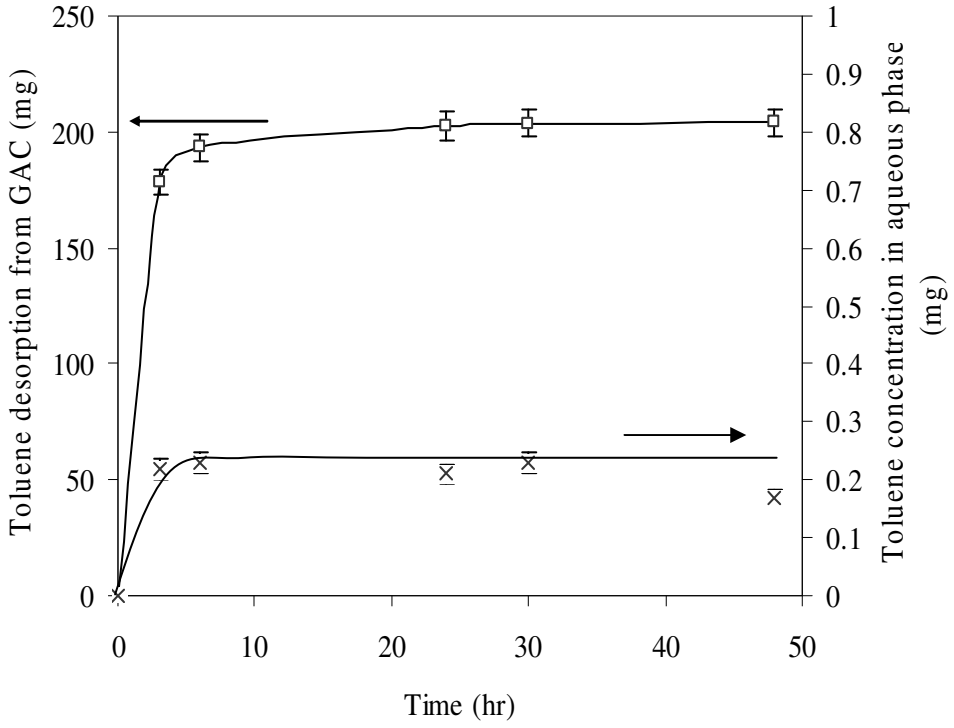


Figure V.16: Toluene monitoring in anaerobic bioreactor. Infors bioreactor, 30°C, pH: 7.2, stirring rate: 50 rpm, biomass concentration: 1500 mg dry mass/L.

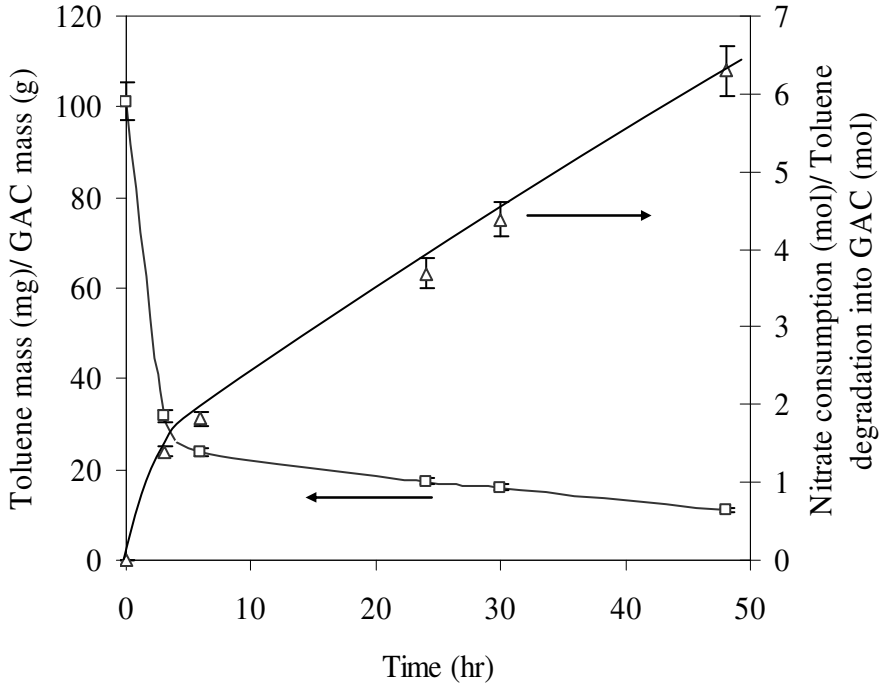


Figure V.17: Nitrate (-Δ-) consumption and toluene biodegradation (-)- vs. time in biphasic bioreactors. Infors bioreactor, 30°C, pH: 7.2, mixing rate: 50 rpm, biomass concentration: 1500 mg dry mass/L.

Conclusions and perspectives

Conclusions

Works performed during studies presented here have focussed on the biodegradation of monoaromatic compounds (benzene, toluene, xylenes, BTX) present in water. A preliminary study of literature data led to consider that in most of published works authors did not properly take into account phase transfers while these phenomena were well known to be important. Hence, some industrial processes already make use of them by simply stripping volatile organic pollutants from water, thus enabling only the displacement of the pollutants from a liquid phase to the atmosphere.

It appeared thus that a critical issue in this area was to be able to quantify these liquid-gas transfers in order to be able to control them. The ultimate goal of the research presented here was thus to make progress towards a bioremediation process avoiding volatile solute dispersal in the atmosphere, i.e. in the environment.

It has been shown that the volatility of compounds such as BTX is high enough be able to interfere with analytical methods used for their determination. Hence, sample preparation, a step which needs separation of biomass from the liquid, was shown to be critical. An optimized protocol has been built, it involves centrifugation with capped tubes completely filled with the liquid sample. This configuration could suppress liquid-gas transfers, enabling to obtain reliable solute concentrations in the sample.

These studies also led to propose a new approach to measure the activity coefficient of a solute. The preferred method for that is the so-called "dilutor" method, which involves solute stripping by a gas in such a way that thermodynamic equilibrium between phases can be assumed. It has been demonstrated that centrifugation of a solution in a closed tube with known volumes of liquid and gas phases could allow to reach the equilibrium. Knowledge of the initial and final compositions of the system enabled calculation of the activity coefficient, provided the vapour pressure was known.

An other key point underlined the difficulty to build an efficient test to measure the true biodegradation activity of an aerobic biomass. Hence, in an open system under abiotic (biomass free) conditions more than 95% mass of monoaromatic pollutants with initial concentration close to 150 mg/L was lost from contaminated water by evaporation in 2 days at 20 °C. Data also highlighted that aeration of contaminated water could eliminate

monoaromatic compounds from 140 to about 0.005 mg/L in only 1 hour processing with a gassing rate 0.33 VVM. These data, consistent with the already pointed out need for careful protocol for analytical purposes, clearly revealed that activity tests in an open, gassed aqueous system could not be considered as a valuable approach. It was shown that a closed vessel with a known volume of gas and fitted with a system enabling the measurement of oxygen consumption (Oxi Top) could, after careful optimization, be an efficient system. This approach allowed to continuously monitor the reaction progress without any sampling, thus avoiding solute loss out of the reaction vessel. This tool allowed to demonstrate that it was actually possible to obtain an active aerobic biomass by training an activated sludge obtained from a domestic water treatment plant.

However, data obtained during aerobic biodegradation processes demonstrated that biphasic liquid-liquid systems, although allowing a strong decrease in liquid-gas transfers, were not efficient enough to prevent significant solute loss from the system. It was thus decided to consider two approachs. One involved oxygen supply through the use of peroxides, the second was about the use of an anaerobic microbial system cultivated in a biphasic liquid-liquid medium.

Experimental investigations were more detailed with the last system. The model choosen was *Thauera aromatica* K172, a bacterium known for its ability to both perform nitrate respiration and metabolize toluene. It has been clearly demonstrated that this system was very promising. Alternatively, the ability of this bacterium to metabolize toluene adsorbed onto granulated activated carbon (GAC) was investigated. It was shown that 90% of contaminant (100 mg toluene/ g GAC) could be biodegraded in 2 days.

These results were very promising for the building of a process enabling the removal of toxic compounds from polluted water without any further environmental damage. This study showed that a biodegradation process had to involve an heterogeneous medium. Also, the strategy of monoaromatic removal from contaminated water by solute trapping in activated carbon particles followed by biodegradation by a bacterial culture in a separate anaerobic bioreactor could be explored.

Recommendations for future work

The following studies, which involve mainly anaerobic processes, are recommended to continue the development and implementation of monoaromatics remediation from contaminated waters.

They involve works on **micoorganisms**,

- Enrichment and screening of monoaromatics degrading bacteria from adapted domestic (or industrial) activated sludge;
- Biomass adaptation to hydrogen peroxide and bioprocess optimization for monoaromatic degradation in fixed film aerobic reactors without aeration;
- Study of monoaromatics mineralization under sulfate or manganese oxide reducing conditions in anaerobic biphasic reactor;
- Biodegradation by mixed cultures with nitrate and sulfate reduction in anaerobic biphasic bioreactor

on the **medium composition,**

- Study of potential interactions between monoaromatic components (benzene, toluene, ethylbenzene and the mixed xylenes) during their biodegradation;
- Effect of gasoline additives (methanol, ethanol, MTBE, etc) on volatile aromatics biodegradation;
- Effect of surfactants, phenol and poly aromatic hydrocarbons on solute biodegradation

investigations on **heterogeneous systems,**

- Elucidation of limiting factors in the monoaromatics biodegradation in anaerobic two-phase partitioning bioreactors;
- Effect of activated carbon type (surface area, type of activation, etc…) on contaminants adsorption and its reversibility;
- Physico chemical parameters affecting bioregeneration of activated carbon loaded with monoaromatic compounds;
- Bioregeneration of GAC loaded with aromatic hydrocarbons by mixed anaerobic bacteria;
- Determination of optimum conditions for increasing of bioregeneration efficiency where activated carbon loaded with xenobiotic compounds;

- Monoaromatic extraction from activated carbon wastes by ethanol (or methanol) and then pollutants treatment in separated anaerobic bioreactor;
- Study of ability of activated carbon in sorption of intermediate and final products in the bioleaching process;

experiments at the **process level**,

- Application of online nitrate and pCO_2 electrodes in the anaerobic biphasic bioreactor for bioprocess control;
- A pilot study for treatment of water contaminated by oil products in anaerobic biphasic reactors.

References

Abraham, MH, Acree Jr., WE. (2004) Correlation and prediction of partition coefficients between the gas phase and water, and the solvents dodecane and undecane. New Journal of Chemistry **28**, 1538-1543.

Abu Hamed, T., Bayraktar, E., Mehmetoglu, T., Mehmetoglu, Ü.(2003) Substrate interactions during the biodegradation of benzene, toluene and phenol mixtures. Process Biochemistry **39** (1), 27-35.

Abu Hamed, T., Bayraktar, E., Mehmetoglu, Ü., Mehmetoglu, T. (2004) The biodegradation of benzene, toluene and phenol in a two-phase system. Biochemical Engineering Journal **19**(2), 137-146.

Abuhamed, T., Bayraktar, E., Mehmetoglu, T., Mehmetoglu, Ü. (2004) Kinetics model for growth of *Pseudomonas putida* F1 during benzene, toluene and phenol biodegradation. Process Biochemistry **39**(8), 983-988.

Acuna-Askar, K., Englande, A.J., Jr., Ramirez-Medrano, A., Coronado-Guardiola, J.E. and Chavez-Gomez, B. (2003) Evaluation of biomass production in unleaded gasoline and BTEX-fed batch reactors. Water Science and Technology **48** (8) 127-133.

Ahtiainen, J., Aalto, M., Pessala, P. (2003) Biodegradation of chemicals in a standardized test and in environmental conditions. Chemosphere **51**(6), 529-537

Alemzadeh, I. and Vossoughi, M.(2001) Biodegradation of toluene by an attached biofilm in a rotating biological contactor. Process Biochemistry **36**(8-9), 707-711.

Amsden, B.G., Bochanysz, J., Daugulis, A.J. (2003) Degradation of xenobiotics in a partitioning bioreactor in which the partitioning phase is a polymer. Biotechnology and Bioengineering **84**(4), 399-405.

An, Y.-J.(2004) Toxicity of Benzene, Toluene, Ethylbenzene, and Xylene (BTEX) Mixtures to Sorghum bicolor and Cucumis sativus. Bulletin of Environmental Contamination and Toxicology **72**(5), 1006–1011.

Anders, H.-J., Kaetzke, A., Kämpfer, P., Ludwig, W., Fuchs, G. (1995) Taxonomic position of aromatic-degrading denitrifying Pseudomonad strains K 172 and KB 740 and their description as new members of the genera Thauera, as Thauera aromatica sp. nov., and Azoarcus, as Azoarcus evansii sp. nov., respectively, members of the beta subclass of the Proteobacteria. International Journal of Systematic Bacteriology **45(**2), 327-333.

Andreoni, V.,Gianfreda, L. (2007) Bioremediation and monitoring of aromatic-polluted habitats. Applied Microbiology Biotechnology **76**, 287-308.

Ania, C.O., Parra, J.B., Menéndez, J.A., Pis, J.J. (2007) Microwave-assisted regeneration of activated carbons loaded with pharmaceuticals. Water Research **41**(15), 3299-3306.

Antizar-Ladislao, B., Galil, N.I. (2003) Simulation of bioremediation of chlorophenols in a sandy aquifer. Water Research **37**, 238–244.

Arriaga, S., Muñoz, R., Hernandez, S.,Guieysse B., Revah, S. (2007) Gaseous hexane biodegradation by *Fusarium solani* in two liquid phase packed-bed and stirred tank bioreactors. Environmental Science & Technolology **40**, 2390–2395.

Arvin, E., Engelsen, P. and Sebber, U.(2005) Biodegradation of gasoline compounds (BTEX) in a water works sand filter. Water Supply **4**(5-6), 29–33.

Ascon-Cabrera, M. A., Lebeault, J.-M. (1995) Cell hydrophobicity influencing the activity/stability of xenobiotic-degrading microorganisms in a continuous biphasic aqueous-organic system. Journal of fermentation and bioengineering **80**(3), 270-275.

Attaway, H.H., and Schmidt, M.G.(2002) Tandem biodegradation of BTEX components by two *P*seudomonas sp. Current Microbiology **45**(1), 30-36.

Atteia, O., and Guillot, C. (2007) Factors controlling BTEX and chlorinated solvents plume length under natural attenuation conditions. Journal of Contaminant Hydrology **90**(1-2), 81-104.

Atteia, O., Franceschi, M. (2002) Kinetics of natural attenuation of BTEX: review of the critical chemical conditions and measurements at bore scale. The Scientific World Journal **16**, 1338-1346.

Ayotamuno, M.J., Kogbara, R.B., Ogaji, S.O.T. and Probert, S.D. (2006) Petroleum contaminated ground-water: Remediation using activated carbon. Applied Energy **83**(11), 1258-1264.

Azadpour-Keeley, A., Barcelona, M.J. (2006) Design of an MTBE remediation technology evaluation. Ground Water Monitoring & Remediation **26**,103–113.

Azev, V.S., Emel'yanov1, V.E., Turovskii, F.V.(2004) Automotive Gasolines Long-Term Requirements for Composition and Properties. Chemistry and Technology of Fuels and Oils **40**(5), 291-297.

Ball, H.A., Reinhard, M., (1996) Monoaromatic hydrocarbon transformation under anaerobic conditions at seal beach, california: laboratory studies. Environmental Toxicolology and Chemistry **15**, 114-122.

Bauer, R.D., Maloszewski, P., Zhang, Y., Meckenstock, R.U., Griebler, C. (2008) Mixing-controlled biodegradation in a toluene plume — Results from two-dimensional laboratory experiments. Journal of Contaminant Hydrology **96,** 150-168.

Beeman, R.E., Bleckmann, C.A. (2002) Sequential anaerobic–aerobic treatment of an aquifer contaminated by halogenated organics: field results. Journal of Contaminant Hydrology **57**, 147– 159.

Bekins, B.A., Cozzarelli, I.M., Curtis, G.P. (2005) A simple method for calculating growth rates of petroleum hydrocarbon plumes. Ground Water **43**, 817-826.

Bekins, B.A., Warren, E., Godsy, E. M. (1998) A comparison of zero-order, first-order, and Monod biotransformation models. Ground Water **36**, 261-268.

Beller, H. R. (2000) Metabolic indicators for detecting *in situ* anaerobic alkylbenzene degradation. Biodegradation **11**,125–139.

Beller, H.R. (2002) Analysis of benzylsuccinates in groundwater by liquid chromatography/tandem mass spectrometry and its use for monitoring in situ BTEX biodegradation. Environmental Science and Technology **36**, 2724-2728.

Bhandare, R., Calabro, M., Coschigano, P.W. (2007) Site-directed mutagenesis of the Thauera aromatica strain T1 tutE tutFDGH gene cluster. Biochemical and Biophysical Research Communications **346** (3), 992-998.

Bhupathiraju, V.K., Krauter, P., Holman, H.Y. N., Conrad, M.E., Daley, P. F., Templeton, A. S.,Hunt, J. R., Hernandez, M., Alvarez-Cohen, L. (2002) Assessment of in-situ bioremediation at a refinery waste-contaminated site and an aviation gasoline contaminated site. Biodegradation **13**, 79–90.

Biegert, T., Fuchs, G., Heider, J. (1996) Evidence that anaerobic oxidation of toluene in the denitrifying bacterium *Thauera aromatica* is initiated by formation of benzylsuccinate from toluene and fumarate. FEBS Journal **238** (3), 661-668.

Bielefeldt, A.R. and Stensel, H.D. (1999) Treating VOC-Contaminated Gases in Activated Sludge: Mechanistic Model to Evaluate Design and Performance. Environmental Science and Technology **33**(18), 3234 -3240.

Biggerstaff, J.P., Le Puil, M., Weidow, B.L., Leblanc-Gridley, J., Jennings, E., Busch-Harris, J., Sublette, K.L., White, D.C., Alberte, R.S. (2007) A novel and in situ technique for the quantitative detection of MTBE and benzene degrading bacteria in contaminated matrices. Journal of Microbiological Methods **68**(2), 437-441.

Bittkau, A., Geyer, R., Bhatt, M. and Schlosser, D. (2004) Enhancement of the biodegradability of aromatic groundwater contaminants. Toxicology **205**(3), 201-210.

Boll, M (2005) Key enzymes in the anaerobic aromatic metabolism catalysing Birch-like reductions. Biochimica et Biophysica Acta (BBA) - Bioenergetics **1707,** 34-50.

Boll, M, Fuchs, G, Heider, J (2002) Anaerobic oxidation of aromatic compounds and hydrocarbons. Current Opinion in Chemical Biology **6,** 604-611.

Bolliger, C., Höhener, P., Hunkeler, D., Häberli, K., Zeyer, J. (1999) Intrinsic bioremediation of a petroleum hydrocarbon-contaminated aquifer and assessment of mineralization based on stable carbon isotopes. Biodegradation **10**, 201–217.

Boopathy, R. (2000) Factors limiting bioremediation technologies. Bioresource Technology **74**, 63-67.

Botton, S., Harmelen, M.V., Braster, M., Parsons, J.R., Röling, W.F.M. (2007) Dominance of *Geobacteraceae* in BTX-degrading enrichments from an iron-reducing aquifer. FEMS Microbiology Ecology **62**, 118-130.

Bradley, P.M., Landmeyer, J.E. (2006) Low-Temperature MTBE Biodegradation in Aquifer Sediments with a History of Low, Seasonal Ground Water Temperatures. Ground Water Monitoring and Remediation **26**(1), 101–105.

Butler, R, Ehrenberg, S., Godley, A.R., Lake, R., Lytton, L., Cartmell, E. (2006) Remediation of bromate-contaminated groundwater in an ex situ fixed-film bioreactor. Science of the Total Environment **366** (1), 12-20.

Buyuksonmez, F., Hess, T.F., Crawford, R.L.,Watts, R.J. (1998) Toxic effects of modified Fenton reactions on Xanthobacter flavus FB71. Applied and Environmental Microbiology **64**, 3759–3764.

Campos-Candel, A., Llobat-Estellés, M., Mauri-Aucejo, A. R. (2007) Desorption of BTEX from activated charcoal using accelerated solvent extraction : evaluation of occupational exposures. Analytical and bioanalytical chemistry **387**(4), 517-1523.

Cappaert, L., and Larroche, C. (2004) Simple calculation for compounds lost by gas stripping in a two-phase liquid system involving dilute substances. Biotechnology Letters **26**, 137-141.

Cattony, E.B., Chinalia F.A., Ribeiro R., Zaiat M., Foresti E., Varesche M.B.(2005) Ethanol and toluene removal in a horizontal-flow anaerobic immobilized biomass reactor in the presence of sulphate. Biotechnology and Bioengineering **91**(2), 244-253.

Chao, H.P., Lee, J.F., Lee, C.K., Huang, F.C., Annadurai, G. (2007) Volatilization reduction of monoaromatic compounds in nonionic surfactant solutions. Chemical Engineering Journal, In Press (doi:10.1016/j.cej.2007.11.020).

Chao, H.P., Lee, J.F., Lee, C.K., Huang, H.C. (2005) An alternative method for predicting organic solute volatilization rates under gas and liquid turbulence. Chemosphere **59,**711–720.

Cesário, M.T., Beverloo, W.A., Tramper, J., Beeftink, H.H. (1997) Enhancement of gas–liquid mass transfer rate of apolar pollutants in the biological waste gas treatment by a dispersed organic solvent. Enzyme Microbial Technololgy **21**, 578–588.

Cesário, M.T., de Wit, H.L., Tramper, J., Beeftink, H.H. (1997) Dispersed organic solvent to enhance the overall gas/water mass transfer coefficient of apolar compounds in the biological waste-gas treatment- Modeling and evaluation. Biotechnology Progress **13**, 399–407.

Chakraborty, R., and Coates, J.D. (2004) Anaerobic degradation of monoaromatic hydrocarbons. Applied Microbiology and Biotechnology **64** (4), 437-446.

Chao, K.P., Ong, S.K., and Huang, M.C. (2007) Mass transfer of VOCs in laboratory-scale air sparging tank. Journal of Hazardous Materials, In Press (doi:10.1016/j.jhazmat.2007.07.087) .

Chapelle, F.H. (1999) Bioremediation of petroleum hydrocarbon-contaminated ground water: the perspectives of history and hydrology. Ground Water **37**, 122–132.

Chen, Y. D., Barker, J. F., Gui, L. (2007) A strategy for aromatic hydrocarbon bioremediation under anaerobic conditions and the impacts of ethanol: A microcosm study. Journal of Contaminant Hydrology, in press. (doi:10.1016/j.jconhyd.2007.09.006)

Cho, J., Zein, M.M., Suidan, M.T., Venosa, A.D. (2007) Biodegradability of alkylates as a sole carbon source in the presence of ethanol or BTEX. Chemosphere **68**(2), 266-273.

Choi, Y.B., Lee, J.Y., Kim, H.S. (1992) A novel bioreactor for the biodegradation of inhibitory aromatic solvents: Experimental results and mathematical analysis. Biotechnology and Bioengineering **40**(11), 1403 – 1411.

Clement, T.P., Johnson, C.D., Sun, Y., Klecka, G. M., Bartlett, C.(2000) Natural attenuation of chlorinated ethane compounds: model development and field-scale application at the Dover site. Journal of Contaminant Hydrology **42**,113–140.

Clesceri, C., Greenberg, A.E., Eaton, A.D. (1998) Standard Methods for the Examination of Water and Wastewater (20th ed.), American Public Health Association, the American Water Works Association and the Water Environment Federation, USA.

Coates, J.D., Chakraborty, R., McInerney, M.J. (2002) Anaerobic benzene biodegradation – a new era. Research in Microbiology **153**, 621-628.

Cohen-Bazire, G., Sistrom, W.H., Stanier, R.Y. (1957) Kinetic studies of pigment synthesis by non-sulfur purple bacteria. Journal of Cellular and Comparative Physiology **44**, 25–68.

Collins, L. D., Daugulis, A. J. (1999) Benzene/toluene/ *p* -xylene degradation. Part II. Effect of substrate interactions and feeding strategies in toluene/benzene and toluene/ *p* -xylene fermentations in a partitioning bioreactor. Applied Microbiology and Biotechnology **2**(3), 360-365.

Collins, L.D., Daugulis, A.J. (1997) Biodegradation of phenol at high initial concentrations in two-phase partitioning batch and fed-batch bioreactors. Biotechnology and Bioengineering **55**, 155–162.

Collins, L.D., and Daugulis, A.J. (1999) Benzene/toluene/p-xylene degradation. Part I. Solvent selection and toluene degradation in a two-phase partitioning bioreactor. Applied Microbiology and Biotechnology **52**(3), 354-359.

Corseuil, H.X., Alvarez, P.J.J. (1996) Natural bioremediation perspective for BTX contaminated ground water in brazil: effect of ethanol. Water Science and Technology **34**, 311-318.

Corseuil, H.X., Hunt, C.S., Ferreira dos Santos, R.C., Alvarez, P.J.J. (1998) The influence of the gasoline oxygenate ethanol on aerobic and anaerobic BTX biodegradation. Water Research **32**, 2065-2072.

Cruickshank, S.M., Daugulis, A.J., McLellan, P.J. (2000) Modeling of a continuous two-phase partitioning bioreactor for the degradation of xenobiotics. Process Biochemistry **35**(9), 1027-1035.

Cunningham, J.A., Rahme, H., Hopkins, G.D., Lebron, C., Reinhard, M. (2001) Enhanced in situ bioremediation of BTEX-contaminated groundwater by combined injection of nitrate and sulphate. Environmental Science and Technology **35**(8), 1663-1670.

Curtis, F., Lammey, J. (1998) Intrinsic remediation of a diesel fuel plume in Goose Bay, Labrador, Canada. Environmental Pollution **103**, 203-210.

Da Silva, M.L., Ruiz-Aguilar, G.M., Alvarez, P.J. (2005) Enhanced anaerobic biodegradation of BTEX-ethanol mixtures in aquifer columns amended with sulfate, chelated ferric iron or nitrate. Biodegradation **16**,105-114.

Daifullah, A.A.M., Girgis, B.S. (2003) Impact of surface characteristics of activated carbon on adsorption of BTEX. Colloids and Surfaces A: Physicochemical and Engineering Aspects **214**(1), 181-193.

Daugulis AJ. (2001) Two-phase partitioning bioreactors: a new technology platform for destroying xenobiotics. Trends in Biotechnology **19,** 457-462.

Daugulis, A.J., Boudreau, N.G. (2003) Removal and destruction of high concentrations of gaseous toluene in a two-phase partitioning bioreactor by *Alcalignes xylosoxidans*. Biotechnology Letters **25**, 1421–1424.

Daugulis, A.J., Janikowski, T.B. (2002) Scale-up performance of a partitioning bioreactor for the degradation of polyaromatic hydrocarbons by *S*phingomonas aromaticivorans , Biotechnology Letters **24**(8), 591-594.

Daugulis, A.J., McCracken, C.M. (2003) Microbial degradation of high and low molecular weight polyaromatic hydrocarbons in a two-phase partitioning bioreactor by two strains of *Sphingomonas* sp. Biotechnology Letters **25**(17), 1441-1444.

Davidson, C.T., Daugulis, A.J. (2003) The treatment of gaseous benzene by two-phase partitioning bioreactors: a high performance alternative to the use of biofilters. Applied Microbiology and Biotechnology **62**, 297–301.

Davidson, C.T., Daugulis, A.J. (2003) Addressing biofilter limitations: a two-phase partitioning bioreactor process for the treatment of benzene and toluene contaminated gas streams. Biodegradation **14,** 415–421.

de Nardi, I.R., Ribeiro, R., Zaiat, M., Foresti, E. (2005) Anaerobic packed-bed reactor for bioremediation of gasoline-contaminated aquifers. Process Biochemistry **40**(2), 587-592.

de Nardi, I.R., Varesche, M.B.A., Zaiat, M., Foresti, E. (2002) Anaerobic degradation of BTEX in a packed-bed reactor, Water Science & Technology **45** (10), 175–180.

de Nardi, I.R., Zaiat, M, Foresti, E. (2006) Kinetics of BTEX degradation in a packed-bed anaerobic reactor. Biodegradation **7**, 1-8.

Dean, J.R., Xiong G. (2000) Extraction of organic pollutants from environmental matrices: selection of extraction technique TrAC Trends in Analytical Chemistry **19**(9), 553-564.

Deeb, R.A., and Alvarez-Cohen, L. (2000) Aerobic Biotransformation of Gasoline Aromatics in Multi Component Mixtures. Bioremediation Journal **4**(2), 171-179.

Deeb, R.A., and Alvarez-Cohen, L. (1999) Temperature effects and substrate interactions during the aerobic biotransformation of BTEX mixtures by toluene-enriched consortia and Rhodococcus rhodochrous. Biotechnology and Bioengineering **62**(5), 526-536.

Deeb, R.A., Hu, H.Y., Hanson, J.R., Scow, K.M., Alvarez-Cohen, L. (2001) Substrate interactions in BTEX and MTBE mixtures by an MTBE-degrading isolate. Environmental Science and Technology **35**, 312-317.

Demeestere, K., Dewulf, J., De Witte, B., Van Langenhove, H.(2007) Sample preparation for the analysis of volatile organic compounds in air and water matrices. Journal of Chromatography A **1153**(1-2), 130-144.

Devlin, J.F., Katic, D., Barker, J.F.(2004) In situ sequenced bioremediation of mixed contaminants in groundwater. Journal of Contaminant and Hydrology **69**, 233– 261.

Díaz, L.F., Muñoz, R., Bordel, S., Villaverde, S. (2007) Toluene biodegradation by *Pseudomonas putida* F1: targeting culture stability in long-term operation. Biodegradation , in press (doi:10.1007/s10532-007-9126-6).

Disdier, B., Arfi, C., Pastor, J., Pauli, A.M., Portugal, H. (1999) Analysis by GC-MS of monocyclic and polycyclic aromatic hydrocarbons in thermolysed waste products. Analusis **27**(3), 235-241.

Djozan, Dj., Pournaghi-Azar, M.H., Bahar, S. (2004) Modified polypyrrole with tetrasulfonated nickel phthalocyanine as a fiber for solid-phase microextraction application to the extraction of BTEX compounds from water samples. Chromatographia **59**, 595-599.

Dobson, R., Schroth, M.H., Schuermann, A., Zeyer, J.(2004) Methods to assess the amenability of petroleum hydrocarbons to bioremediation. Environmental Toxicolology and Chemistry **23**, 929-937.

Dórea, H.S., Bispo, J.R.L., Aragão, K.A.S., Cunha, B.B., Navickiene, S., Alves, J.P.H., Romão, L.P.C., Garcia, C.A.B. (2007)Analysis of BTEX, PAHs and metals in the oilfield produced water in the State of Sergipe, Brazil. Microchemical Journal **85**(2), 234-238.

Dott, W., Feidieker, D., Steiof, M., Becker, P.M., Kämpfer, P. (1995) Comparison of Ex situ and In situ Techniques for Bioremediation of Hydrocarbon-polluted Soils. International Biodeterioration & Biodegradation **35** (1-3), 301-3 16.

Dou, J., Liu, X., Hu, Z., Deng, D. (2007) Anaerobic BTEX biodegradation linked to nitrate and sulfate reduction. Journal of Hazardous Materials, in press (doi:10.1016/j.jhazmat.2007.06.043).

Duhem, P., and Vidal, J. (1978) Extension of the Dilutor Method to Measurement of High Activity Coefficients at Infinite Dilution. Fluid Phase Equilibria **2**, 231-235.

Duldhardt, I., Nijenhuis, I., Schauer, F., Heipieper H.J., (2007) Anaerobically grown *Thauera aromatica* , *Desulfococcus multivorans* , *Geobacter sulfurreducens* are more sensitive

towards organic solvents than aerobic bacteria. Applied Microbiology and Biotechnology **77**(3), 705-711.

Dumont, E., Andrès, Y., Le Cloirec, P. (2006) Effect of organic solvents on oxygen mass transfer in multiphase systems: Application to bioreactors in environmental protection. Biochemical Engineering Journal **30**(3), 245-252.

Durant, N.D., Jonkers, C.A.A., Bouwer, E. J. (1997) Spatial variability in the naphthalene mineralization response to oxygen, nitrate, and orthophosphate amendments in MGP aquifer sediments. Biodegradation **8**, 77–86.

Dyer, M., Heiningen, E.V., Gerritse, J. (2003) A field trial for in-situ bioremediation of 1, 2-DCA. Engineering Geology **70**, 315–320.

Eibes, G., Moreira, M.T., Feijoo, G., Daugulis, A.J., Lema, J.M. (2007) Operation of a two-phase partitioning bioreactor for the oxidation of anthracene by the enzyme manganese peroxidase. Chemosphere **66**(9), 1744-1751.

Eisert, R., and Levsen, K. (1996) Solid-phase microextraction coupled to gas chromatography: a new method for the analysis of organics in water(Review). Journal of Chromatography A **733**, 143-157.

Eskilsson, C.S., Björklund, E. (2000) Analytical-scale microwave-assisted extraction. Journal of Chromatography A **902** (1), 227-250.

Farhadian, M., Borghei, M., Golshan, K., Talebi, H. (2001) The strong wastewater treatment by new methods. The 6th national congress of chemical engineering, Iran, May 2001; p. 63.

Farhadian, M., Borghei, M., Umrania, V.V. (2007) Treatment of beet sugar wastewater by UAFB bioprocess. Bioresource Technology **98**(16), 3080-3083.

Farhadian, M., Duchez, D, Vachelard, C, Larroche, C (2008b) BTX removal from polluted water through bioleaching processes. Applied biochemistry and biotechnology journal, in press (doi: 10.1007/s12010-008-8189-0)

Farhadian, M., Duchez, D.,Vachelard, C., Larroche, C. (2008a) Monoaromatics removal from polluted water through bioreactors—A review. Water Research 42: 1325-1341.

Farhadian, M., Larroche, C., Borghei, M., Troquet, J., Vachelard, C. (2006) Bioremediation of BTEX-contaminated groundwater through bioreactors. 4ème Colloque Franco-Roumain de chimie appliquée, Université Blaise Pascal, Clermont-Ferrand, France, 28 Juin- 2 Juillet 2006 ; p.438.

Farhadian, M, Vachelard, C, Duchez, D, Larroche, C (2007) In situ bioremediation of monoaromatic pollutants in groundwater: A review**.** Bioresource Technology, In Press (doi:10.1016/j.biortech.2007.10.025).

Farré, M.J., Doménech, X., Peral, J. (2006) Assessment of photo-Fenton and biological treatment coupling for Diuron and Linuron removal from water. Water Research **40**(13), 2533-2540.

Ferguson, J.F., Pietari, J.M.H. (2000) Anaerobic transformations and bioremediation of chlorinated solvents. Environmental Pollution **107**, 209-215.

Ferguson, S.H., Woinarski, A.Z., Snape, I., Morris, C.E., Revill, A.T. (2004) A field trial of in situ chemical oxidation to remediate long-term diesel contaminated Antarctic soil. Cold Regions Science and Technology **40**(1-2), 47-60.
Ferrarese, E., Andreottola, G., Oprea, I.A. (2008) Remediation of PAH-contaminated sediments by chemical oxidation. Journal of Hazardous Materials **152**(1), 128-139.

Fichan, I., Larroche, C., and Gros J.B. (1999) Water solubility, vapor pressure, and activity coefficients of terpenes and terpenoids. Journal of Chemical and Engineering Data **44**, 56-62

Fischer, A., Hahn, C. (2005) Biotic and abiotic degradation behaviour of ethylene glycol monomethyl ether (EGME). Water Research **39**(10), 2002-2007.

Field, J.A. (2002) Limits of anaerobic biodegradation. Water Science and Technology, **45**(10), 9–18.

Fiorenza, S., and Ward, C.H. (1997) Microbial adaptation to hydrogen peroxide and biodegradation of aromatic hydrocarbons. Journal of Industrial Microbiology & Biotechnology **18** (2-3), 140-151.

Fischer, A., Werner, P.(2000) Phenylacetylene as a new surrogate standard for the determination of BTEX by GC/FID. Journal of Analalytical Chemistry **368**, 424–425.

Fortin, N.Y., Morales, M., Nakagawa, Y., Focht, D.D., Deshusses, M.A. (2001) Methyl tert-butyl ether (MTBE) degradation by a microbial consortium. Environmental Microbiology **3**, 407-416.

Fraile, J., Ninerola, J.M., Olivella, L., Figueras, M., Ginebreda, A., Vilanova, M., Barcelo, D. (2002) Monitoring of the gasoline oxygenate MTBE and BTEX compounds in groundwater in Catalonia (northeast Spain). Science World Journal **2**, 1235-1242.

Fraile, J., Ninerola, J.M., Olivella, L., Figueras, M., Ginebreda, A., Vilanova, M., Barcelo, D.
Fry, V. A., Istok, J.D., O'Reilly, K.T.(1996) Effect of trapped gas on dissolved oxygen transport - implications for in situ bioremediation. Ground Water **34**, 200-210.

Gersberg, R.M., Korth, K.G., Rice, L.E., Randall, J.D., Bogardt, A.H., Dawsey, W.J., Hemmingsen, B.B. (1995) Chemical and microbial evaluation of in-situ bioremediation of hydrocarbons in anoxic groundwater enriched with nutrients and nitrate. World Journal of Microbiology and Biotechnology **11**, 549-558.

Gibson, T.L., Abdul ,A.S., Chalmer, P.D. (1998) Enhancement of in situ bioremediation of BTEX-contaminated ground water by oxygen diffusion from silicone tubing. Ground Water Monitoring & Remediation **18**(1), 93–104.

Gödeke, S., Richnow, H.H., Weiâ, H., Fischer, A., Vogt, C. , Borsdorf, H., Schirmer, M. (2006) Multi tracer test for the implementation of enhanced in-situ bioremediation at a BTEX-contaminated megasite. Journal of Contaminant Hydrology **87**(3-4), 211-236.

Goltz, M.N., Bouwer, E.J., Huang, J. (2001) Transport issues and bioremediation modeling for the in situ aerobic co-metabolism of chlorinated solvents. Biodegradation **12**, 127–140.

Gotvajn, A.Z., Zagorc-Koncan, J.(1999) Laboratory simulation of biodegradation of chemicals in surface waters: closed bottle and respirometric test. Chemosphere **38**(6), 1339-1346.

Granger, D.A., Butler, B. J. , Barker, J.F.(1999) A case of phosphorus limiting monoaromatic hydrocarbon biodegradation in groundwater. Bioremediation Journal **3** (3), 213-221.

Guerin, T. F. (2000) Long-term performance of a land treatment facility for the bioremediation of non-volatile oily wastes. Resources, Conservation and Recycling **28**, 105–120.

Guerin, T.F. (2002) A pilot study for the selection of a bioreactor for remediation of groundwater from a coal tar contaminated site. Journal of hazardous materials **89**(2-3), 241-252.

Guhl, W., Steber, J.(2006) The value of biodegradation screening test results for predicting the elimination of chemicals organic carbon in waste water treatment plants. Chemosphere **63**(1), 9-16.

Guieysse, B., Bernhoft, I., Andersson, B.E., Henrysson, T., Olsson, S., Mattiasson, B.(2000) Degradation of acenaphthene, phenanthrene and pyrene in a packed-bed biofilm reactor. Applied Microbiology and Biotechnology **54**(6), 826-831.

Gusmão, V.R., Chinalia, F.A., Sakamoto, I.K., Varesche, M.B.(2007) Performance of a reactor containing denitrifying immobilized biomass in removing ethanol and aromatic hydrocarbons (BTEX) in a short operating period . Journal of Hazardous Materials **139**(2), 301-309.

Gusmão, V.R., Martins, T.H., Chinalia, F.A., Sakamoto, I.K., Thiemann, O.H., Varesche, M.B.A. (2006) BTEX and ethanol removal in horizontal-flow anaerobic immobilized biomass reactor, under denitrifying condition. Process Biochemistry **41**(6), 1391-1400.

Ha, SR, Vinitnantharat, S., Ozaki, H. (2000) Bioregeneration by mixed Use of peroxides microorganisms of granular activated carbon loaded with a mixture of phenols. Biotechnology Letters **22**, 1093-1096.

Heider, J. (2007) Adding handles to unhandy substrates: anaerobic hydrocarbon activation mechanisms. Current Opinion in Chemical Biology **11**(2), 188-194.

Heider, J., Spormann, A.M., Beller, H. R., Widdel, F. (1999) Anaerobic bacterial metabolism of hydrocarbons. FEMS Microbiology Reviews **22**(5), 459-473.

Heipieper, H.J., Neumann, G., Cornelissen, S., Meinhardt, F. (2007) Solvent-tolerant bacteria for biotransformations in two-phase fermentation systems. Applied Microbiology and Biotechnology **74**(5), 961-973.

Hendrickx, B., Dejonghe, W., Faber, F., Boenne, W., Bastiaens, L.,Verstraete, W., Top, E.M., Springael, D. (2006) PCR-DGGE method to assess the diversity of BTEX mono-oxygenase genes at contaminated sites. FEMS Microbiology Ecology **55** (2), 262–273.

Hoffmann, J.,Reznicekova, I., Vanokova, S., Kupec, J. (1997) Manometric determination of biological degradability of substances poorly soluble in aqueous environments , International Biodeterioration and Biodegradation **39** (4), 327-332.

Holliger, C., Gaspard, S. , Glod, G., Heijman, C., Schumacher, W., Schwarzenbach, R.P., Vazquez, F. (1997) Contaminated environments in the subsurface and bioremediation: organic contaminants. FEMS Microbiology Reviews **20**(3-4), 517-523.

Howsawkeng, J., Watts, R.J., Washington, D.L., Teel, A.L., Hess, T.F., Crawford, R.L. (2001) Evidence for simultaneous abiotic-biotic oxidations in a microbial Fenton's system. Environmental Science and Technology **36,** 2961–2966.

Hu, Z., Chan, C.W., Huang, G.H. (2006) Model predictive control for in situ bioremediation system. Advances in Engineering Software **37**, 514–521.

Hua, Z.Y., Huanga, G.H., Chan, C. W. (2003) A fuzzy process controller for in situ groundwater bioremediation. Engineering Applications of Artificial Intelligence **16**, 131–147.

Huang, Y.F., Huang, G.H., Wang, G.Q., Lin, Q.G., Chakma, A. (2006) An integrated numerical and physical modeling system for an enhanced in situ bioremediation process. Environmental Pollution **144**(3), 872-885.

Hunkeler, D., Hohener, P., Zeyer, J. (2002) Engineered and subsequent intrinsic in situ bioremediation of a diesel fuel contaminated aquifer. Journal of Contaminant Hydrology **59**, 231– 245.

Hunkeler, D., Hohener, P., Bernasconi, S., Zeyer, J. (1999) Engineered in situ bioremediation of a petroleum hydrocarbon-contaminated aquifer: assessment of mineralization based on alkalinity, inorganic carbon and stable carbon isotope balances. Journal of Contaminant Hydrology **37**, 201–223.

Hunkeler, D., Jorger, D., Haberli, K., Hohener, P., Zeyer, J. (1998) Petroleum hydrocarbon mineralization in anaerobic laboratory aquifer columns. Journal of Contaminant Hydrology **32**, 41–61.

Iwamoto, T., Nasu, M. (2001) Current bioremediation practice and perspective, Journal of Bioscience and Bioengineering **92**(1), 1-8.

Jahn, M.K., Haderlein, S.B., Meckenstock, R.U.(2005) Anaerobic degradation of benzene, toluene, ethylbenzene, and o-xylene in sediment-free iron-reducing enrichment cultures. Applied and Environmental Microbiology **71**(6), 3355-3358.

Janikowski, T.B., Velicogna, D., Punt, M., Daugulis, A.J. (2002) Use of a two-phase partitioning bioreactor for degrading polycyclic aromatic hydrocarbons by a *Sphingomonas sp.* Applied Microbiology and Biotechnology **59** (2-3), 368-376.

Ji, J., Deng, C., Shen, W., Zhang, X. (2006) Field analysis of benzene, toluene, ethylbenzene and xylene in water by portable gas chromatography–microflame ionization detector combined with headspace solid-phase microextraction, Talanta **69**(4), 894-899.

Jindrova, E., Chocova , M., Demnerova, K., Brenner, V. (2002) Bacterial aerobic degradation of benzene, toluene, ethylbenzene and xylene, Folia Microbiologica (Praha) **47**(2), 83-93.

Johnson, S.J., Woolhouse, K.J., Prommer, H. , Barry, D.A. , Christofi, N. (2003) Contribution of anaerobic microbial activity to natural attenuation of benzene in groundwater. Engineering Geology **70**, 343–349.

Johnston, C.D., Rayner, J. L., Patterson B. M., and Davis G. B.(1998) Volatilisation and biodegradation during air sparging of dissolved BTEX-contaminated groundwater. Journal of Contaminant Hydrology **33**(3-4), 377-404.

Johnston, C.D., Rayner, J.L., Briegel, D. (2002) Effectiveness of in situ air sparging for removing NAPL gasoline from a sandy aquifer near Perth, Western Australia. Journal of Contaminant Hydrology **59**(1-2), 87-111.

Johnston, C.D., Rayner, J.L., Patterson, B.M., Davis, G. B. (1998) Volatilisation and biodegradation during air sparging of dissolved BTEX-contaminated groundwater. Journal of Contaminant Hydrology **33**(3-4), 377-404.

Jung, I.G., and Park, C.H. (2004) Characteristics of *R*hodococcus pyridinovorans PYJ-1 for the Biodegradation of Benzene, Toluene, m-Xylene (BTX), and Their Mixtures. Journal of Bioscience and Bioengineering **97**(6), 429-431.

Kampbell Don, H., Wiedemeier, T.H., Hansen, J.E. (1996) Intrinsic bioremediation of fuel contamination in ground water at a field site. Journal of Hazardous Materials **49**(2-3), 197-204.

Kao, C. M., Wang, C. C. (2000) Control of BTEX migration by intrinsic bioremediation at a gasoline spill site. Water Research **34**(13), 3413-3423.

Kao, C.M. , Borden, R.C. (1997) Site-specific variability in BTEX biodegradation under denitrifying conditions. Ground Water **35**(2), 305-311.

Kao, C.M., Chen, S.C., Wang, J.Y., Chen, Y.L., Lee, S.Z. (2003) Remediation of PCE-contaminated aquifer by an in situ two-layer biobarrier: laboratory batch and column studies. Water Research **37**, 27–38.

Kao, C.M., Huang, W.Y., Chang, L.J., Chen, T.Y., Chien, H.Y., Hou, F. (2006) Application of monitored natural attenuation to remediate a petroleum-hydrocarbon spill site. Water Science and Technology **53**(2), 321-328.

Kao, C.M., Prosser, J. (1999) Intrinsic bioremediation of trichloroethylene and chlorobenzene: field and laboratory studies. Journal of Hazardous Materials **69**, 67–79.

Kao, C.M., Prosser, J. (2001) Evaluation of natural attenuation rate at a gasoline spill site. Journal of Hazardous Materials **82**(3), 275-289.

Karačonji, B., Skender, L., Karačić, V.(2006) Benzene, toluene, ethylbenzene, and isomeric xylenes in various watr samples in Croatia. Bulletin of Environmental Contamination and Toxicology **76**(3), 458-462.

Kelly, W.R., Hornberger, G.M., Herman, J. S., Mills, A.L. (1996) Kinetics of BTX biodegradation and mineralization in batch and column systems. Journal of Contaminant Hydrology **23**(1-2), 113-132.

Kermanshahi pour, A., Karamanev, D., Margaritis, A. (2005) Biodegradation of petroleum hydrocarbons in an immobilized cell airlift bioreactor. Water Research **39**(15), 3704-14.

Kermanshahi pour, A., Karamanev, D., Margaritis, A. (2006) Kinetic modelling of the biodegradation of the aqueous p-xylene in the immobilized soil bioreactor. Biochemical Engineering Journal **27**(3), 204-211.

Khan, F.I., Husain, T., Hejazi, R. (2004) An overview and analysis of site remediation technologies. Journal of Environmental Management **71**(2), 95-122.

Kharoune, M., Pauss, A, Lebeault, J.M, (2001) Aerobic biodegradation of an oxygenates mixture: ETBE, MTBE and TAME in an upflow fixed-bed reactor, Water Research **35**(7), 1665-1674.

Kim, D., Cai, Z, Sorial, GA, Shin, H, Knaebel, K (2007) Integrated treatment scheme of a biofilter preceded by a two-bed cyclic adsorption unit treating dynamic toluene loading. Chemical Engineering Journal **130**, 45-52.

Kim, D. , Kim, Y.S., Kim,S.-K., Kim, S.W., Zylstra, G.J., Kim, Y.M., Kim, E.(2002) Monocyclic aromatic hydrocarbon degradation by *Rhodococcus* sp. Strain DK17. Applied and Environmental Microbiology **68**(7), 3270–3278.

Kim, D.-J., Choi, J.-W., Choi, N.-C., Mahendran, B., Lee, C.-E. (2005) Modeling of growth kinetics for *Pseudomonas* spp. during benzene degradation. Applied Microbiology and Biotechnology **69**(4), 456-462.

Kim, H.-su, and Jaffé, P.R. (2007) Spatial distribution and physiological state of bacteria in a sand column experiment during the biodegradation of toluene. Water Research **41**(10), 2089-2100.

Kim, S.B., Park, C.H., Kim, D.J., Jury, W.A. (2003) Kinetics of benzene biodegradation by *P*seudomonas aeruginosa: parameter estimation. Environmental Toxicology and Chemistry **22**(5), 1038-45.

Kim, W. J., Lee, J. I., Lee, S., Ahn, K.H., Park, J.W. (2006) UV spectroscopic monitoring of vaporized monoaromatic hydrocarbons from petroleum-contaminated soils. Environmental monitoring and assessment **120**(1-3), 527-536.

Klecka, G.M., Carpenter, C.L., Gonsior, S.J. (1998) Biological transformations of 1,2-dichloroethane in subsurface soils and groundwater. Journal of Contaminant Hydrology **34**, 139–154.

Kryst, K., and Karamanev, D.G. (2001) Aerobic phenol biodegradation in an inverse fluidized-bed biofilm reactor. Industrial and Engineering Chemistry Research **40** (23), 5436 - 5439.

Kulik, N., Goi, A., Trapido, M., Tuhkanen, T. (2006) Degradation of polycyclic aromatic hydrocarbons by combined chemical pre-oxidation and bioremediation in creosote contaminated soil. Journal of Environmental Management **78**(4), 382-391.

Kuokkanen, T., VÄhÄoja , P., VÄlimÄki, I., Lauhanen, R. (2004) Suitability of the respirometric BOD oxitop method for determining the biodegradability of oils in ground water using forestry hydraulic oils as model compounds. International Journal of Environmental and Analytical Chemistry **84** (9), 677-689.

Kuran, P., and Sojak, L. (1996) Environmental analysis of volatile organic compounds in water and sediment by gas chromatography (Review). Journal of Chromatography A **733**, 119-141.

Kuster, E., Dorusch, F., Vogt, C., Weiss, H., Altenburger, R. (2004) On line biomonitors used as a tool for toxicity reduction evaluation of in situ groundwater remediation techniques. Biosensors and Bioelectronics **19**(12), 1711-22.

Landmeyer, J.E., Bradley, P.M. (2003) Effect of Hydrologic and Geochemical Conditions on Oxygen-Enhanced Bioremediation in a Gasoline-Contaminated Aquifer. Bioremediation Journal **7**(3-4), 165-177.

Larpent, J.P., and Larpent-Gourgaud, M., 1996. Mémento technique de microbiologie, 3^{éme} édition, TEC& DOC, Paris, page 63.

Langwaldt, J.H., and Puhakka, J.A. (2000) On-site biological remediation of contaminated groundwater: a review. Environmental pollution **107**(2), 187-197.

Leahy, J.G., Tracy, K.D., Eley, M.H. (2003) Degradation of mixtures of aromatic and chloroaliphatic hydrocarbons by aromatic hydrocarbon-degrading bacteria. FEMS Microbiology Ecology **43**, 271-276.

Lee, C.K., Chao, H.P., Lee, J.F. (2004) Effects of organic solutes properties on the volatilization processes from water solutions, Water Research **38**(2), 365-374.

Lee, M.R., Chang, C.M., Dou, J. (2007) Determination of benzene, toluene, ethylbenzene, xylenes in water at sub-ng l−1 levels by solid-phase microextraction coupled to cryo-trap gas chromatography–mass spectrometry. Chemosphere **69**(9), 1381-1387.

Lide D.R.(Editor in chief), 2000-2001. CRC Handbook of Chemistry and Physics, 81th Edition, CRC Press, USA.

Lin, J.H., Chou, M.S. (2006) Henry's law constant variations of volatile organic compounds in wastewater and activated sludge. Aerosol and Air Quality Research **6**(2), 180-192.

Lipson, D., and Siegel, D.I. (2000) Using ternary diagrams to characterize transport and attenuation of BTX, Ground Water **38**(1), 106-113.

Liu, D., Maguire, R.J., Lau, Y.L., Pacepavicius, G.J., Okamura, H., Aoyama, I. (2000) Factors affecting chemical biodegradation. Environmental Toxicology **15**, 476–483.

Llompart, M.P., Lorenzo, R.A., Cela, R., Li, K., Bélanger, J.M.R., Paré, J. R. J. (1997) Evaluation of supercritical fluid extraction, microwave-assisted extraction and sonication in the determination of some phenolic compounds from various soil matrices. Journal of Chromatography A **774** (1-2), 243-251.

Lochmeyer, C., Koch, J., Fuchs, G. (1992) Anaerobic degradation of 2-aminobenzoic acid (anthranilic acid) via benzoyl-coenzyme A (CoA) and cyclohex-1-enecarboxyl-CoA in a denitrifying bacterium. Journal of Bacteriology **174,** 3621-3628.

Lodaya, M., Lakhwala, F., Rus, E., Singh, M., Lewandowski, G., Sofer, S. (1991) Biodegradation of benzene and a BTX mixture using immobilized activated sludge. Journal of Environmental Science and Health- Part A **26**(1), 121-137.

Lovanh, N., Hunt, C.S., Alvarez, P.J. (2002) Effect of ethanol on BTEX biodegradation kinetics: aerobic continuous culture experiments. Water Research **36**(15), 3739-46.

Lovley, D.R. (1997) Potential for anaerobic bioremediation of BTEX in petroleum contaminated aquifers. Journal of Industrial Microbiology & Biotechnology **18**, 75–81.

Lu, C., Lin, M.R., Chu, C. (2002) Effects of pH, moisture, and flow pattern on trickle-bed air biofilter performance for BTEX removal. Advances in Environmental Research **6**(2), 99-106.

Lynch, J.M., and Moffat, A.J. (2005) Bioremediation – prospects for the future application of innovative applied biological research. Annals of Applied Biology, **146**(2), 217-221.

Lapertot, M.E., and Pulgarin, C. (2006) Biodegradability assessment of several priority hazardous substances: Choice, application and relevance regarding toxicity and bacterial activity. Chemosphere **65**(4), 682-690.

MacDonald, T.R., Kitanidis, P.K., McCarty, P.L., Roberts, P.V. (1999) Effects of shear detachment on biomass growth and in situ bioremediation. Ground Water **37**(4), 555-563.

MacLeod, C. T., Daugulis, A. J. (2003) Biodegradation of polycyclic aromatic hydrocarbons in a two-phase partitioning bioreactor in the presence of a bioavailable solvent. Applied Microbiology and Biotechnology **62** (2-3), 291-296.

Mahanty, B., Pakshirajan, K., Venkata Dasu, V. (2008) Biodegradation of pyrene by Mycobacterium frederiksbergense in a two-phase partitioning bioreactor system. Bioresource Technology **99**(7), 2694-2698.

Mahendran, B., Choi, N.-C., Choi, J.-W., Kim, D.-J. (2006) Effect of dissolved oxygen regime on growth dynamics of *Pseudomonas* spp during benzene degradation. Applied Microbiology and Biotechnology **71**(3), 350-354.

Malinowski, J.J. (2001) Two-phase partitioning bioreactors in fermentation technology. Biotechnology Advances **19**(7), 525-538.

Maliyekkal, S.M., Rene, E.R., Philip, L., Swaminathan, T. (2004) Performance of BTX degraders under substrate versatility conditions. Journal of Hazardous Materials **109**(1-3), 201-211.

Mandelbaum, R.T., Shati, M.R., Ronen, D. (1997) In situ microcosms in aquifer bioremediation studies. FEMS Microbiology Reviews **20**, 489-502.

Martínez, S., Cuervo-López, F.M., Gomez, J. (2007) Toluene mineralization by denitrification in an up flow anaerobic sludge blanket (UASB) reactor, Bioresource Technology **98**(9), 1717-1723.

Mascolo, G., Ciannarella, R., Balest, L., Lopez, A. (2007) Effectiveness of UV-based advanced oxidation processes for the remediation of hydrocarbon pollution in the groundwater: A laboratory investigation. Journal of Hazardous Materials, in press (doi:10.1016/j.jhazmat.2007.07.120).

Massol-Deya, A.A., Whallon, J., Hickey, R.F., Tiedje, J.M.(1995) Channel structures in aerobic biofilms of fixed-film reactors treating contaminated groundwater. Applied and Environmental Microbiology **61**(2), 769-777.

Maurer, M., Rittmann, B.E. (2004) Modeling intrinsic bioremediation for interpret observable biogeochemical footprints of BTEX biodegradation: the need for fermentation and abiotic chemical processes. Biodegradation **15**(6), 405-417.

McGuire, J.T., Long, D.T., Hyndman, D.W. (2005) Analysis of recharge-induced geochemical change in a contaminated aquifer. Ground Water **43** (4), 518–530.

Menendez-Vega, D., Gallego, J.L.R., Pelaez, A.I., de Cordoba, G.F., Moreno, J., Muñoz, D., Sanchez, J. (2007) Engineered in situ bioremediation of soil and groundwater polluted with weathered hydrocarbons.European Journal of Soil Biology **43**(5-6), 310-321

Mesarch, M.B., Nakatsu, C.H., Nies, L. (2004) Bench-scale and field-scale evaluation of catechol 2,3-dioxygenase specific primers for monitoring BTX bioremediation. Water Research **38**(5), 1281-1288.

Mester, K.G., Kosson, D.S. (1996) Anaerobic biodegradation of toluene under denitrifying conditions in contaminated groundwater and soil. Journal of Hazardous Materials **45**(2-3), 219-232.

Min, B., Evans, P.J., Chu, A.K., Logan, B.E. (2004) Perchlorate removal in sand and plastic media bioreactors. Water Research **38**(1), 47-60.

Moran, M.J., Zogorski, J.S. , Squillace, P.J. (2005) MTBE and gasoline hydrocarbons in ground water of the United States. Ground Water 43(4), 615-627.

Morasch, B., Höhener, P., Hunkeler, D. (2007) Evidence for in situ degradation of mono-and polyaromatic hydrocarbons in alluvial sediments based on microcosm experiments with ^{13}C-labeled contaminants. Environmental Pollution **148**(3), 739-748.

Morasch, B., Richnow, H.H., Schink, B., Vieth, A., Meckenstock, R.U. (2002) Carbon and hydrogen stable isotope fractionation during aerobic bacterial degradation of aromatic hydrocarbons. Applied and Environmental Microbiology **68**(10), 5191-5194.

Morgan, P., and Watkinson, R.J. (1992) Factors limiting the supply and efficiency of nutrient and oxygen supplements for the *in situ* biotreatment of contaminated soil and groundwater. Water Research **26(**1), 73-78.

Muñoz, R., Arriaga, S., Hernández, S., Guieysse B., Revah S. (2006) Enhanced hexane biodegradation in a two phase partitioning bioreactor: Overcoming pollutant transport limitations. Process Biochemistry **41** (7), 1614-1619.

MacLeod, C.T., and Daugulis, A.J. (2005) Interfacial effects in a two-phase partitioning bioreactor: degradation of polycyclic aromatic hydrocarbons (PAHs) by a hydrophobic Mycobacterium. Process Biochemistry **40** (5), 1799-1805.

Muñoz, R., Villaverde, S. Guieysse B., Revah, S. (2007) Two-phase partitioning bioreactors for treatment of volatile organic compounds. Biotechnology Advances **25**(4), 410-422

Nadim, F., Hoag, G.E., Liu, S., Carley, R.J., Zack, P. (2000) Detection and remediation of soil and aquifer systems contaminated with petroleum products: an overview. Journal of Petroleum Science and Engineering **26**(1), 169-178.

Nakhla, G. (2003) Biokinetic modeling of in situ bioremediation of BTX compounds-impact of process variables and scale up implications. Water Research **37**(6), 1296-1307.

Nam, K, Rodriguez, W, Kukor, J.J. (2001) Enhanced degradation of polycyclic aromatic hydrocarbons by biodegradation combined with a modified Fenton reaction. Chemosphere **45**(1), 11-20.

Nielsen, D. R., Sask, K.N., McLellan, P. J., Daugulis, A.J. (2006) Benzene vapor treatment using a two-phase partitioning bioscrubber: an improved steady-state protocol to enhance long-term operation. Bioprocess and Biosystems Engineering **29**(4), 229-240.

Nielsen, D.R., Daugulis, A.J., McLellan, P.J. (2007) Dynamic simulation of benzene vapor treatment by a two-phase partitioning bioscrubber: Part I: Model development, parameter estimation, and parametric sensitivity. Biochemical Engineering Journal **36**(3), 239-249.

Nielsen, D.R., McLellan, P.J., Daugulis, A.J.(2006) Direct estimation of the oxygen requirements of *A*chromobacter xylosoxidans for aerobic degradation of monoaromatic hydrocarbons (BTEX) in a bioscrubber, Biotechnology Letters **28**, 1293–1298.

Nikolova, N., and Nenov, V.(2005) BTEX degradation by fungi, Water Science and Technology **51**(11), 87-93.

Niven, R.K. (2005) Ethanol in gasoline: environmental impacts and sustainability review article. Renewable and Sustainable Energy Reviews **9**(6), 535-555.

Nyer Evan, K., 1998. Groundwater and soil remediation: practical methods and strategies. Sleeping Bear Press.USA, pages171-205.

Ohlen, K., Chang, Y.K., Hegemann, W., Yin, C.R., Lee, S.T. (2005) Enhanced degradation of chlorinated ethylenes in groundwater from a paint contaminated site by two-stage fluidized-bed reactor. Chemosphere **58**(3), 373-377.

Okawa, K., Suzuki, K., Takeshita, T., Nakano, K. (2005) Degradation of chemical substances using wet peroxide oxidation under mild conditions. Journal of Hazardous Materials **127**(1-3), 68-72.

Okpokwasili, G.C., and Nweke, C.O. (2005) Microbial growth and substrate utilization kinetics. African Journal of Biotechnology **5** (4), 305-317.

Olivella, L., Figueras, M., Fraile, J., Vilanova, M., Ginebreda, A., Barcelo, D. (2002) Fate of MTBE and DCPD compounds relative to BTEX in gasoline-contaminated aquifers. Scientific World Journal **2**, 1108-1114.

Olsen, R.H., Mikesell, M.D., Kukor, J.J., Byrne, A.M.(1995) Physiological attributes of microbial BTEX degradation in oxygen-limited environments. Environmental Health Perspectives **103** (Suppl 5), 49-51.

Ouyang, G., and Pawliszyn, J. (2006) Recent developments in SPME for on-site analysis and monitoring. TrAC Trends in Analytical Chemistry **25** (7), 692-703.

Pagga, U. (1997) Testing biodegradability with standardized methods. Chemosphere **35**(12), 2953-2972.

Painter, H.A. (1995) Detailed review paper on biodegradability testing, Organisation for economic co-operation and development (OECD), Paris, France.

Paixão, J.F., Nascimento, I.A., Pereira, S.A., Leite, M.B.L., Carvalho, G.C., Silveira, Jr., J.S.C., Rebouças, M., Matias, G.R.A., Rodrigues, I.L.P. (2007) Estimating the gasoline components and formulations toxicity to microalgae (Tetraselmis chuii) and oyster (Crassostrea rhizophorae) embryos: An approach to minimize environmental pollution risk. Environmental Research **103**(3), 365-374.

Parales, R.E., Ditty, J.L., Harwood, C.S. (2000) Toluene-degrading bacteria are chemotactic towards the environmental pollutants benzene, toluene, and trichloroethylene. Applied and Environmental Microbiology **66**(9), 4098-4104.

Parameswarappa, S., Karigar, C., Nagenahalli, M. (2008) Degradation of ethylbenzene by free and immobilized *Pseudomonas fluorescens*- CS2. Biodegradation **19**(1), 137-144.

Pedersen, A.R. and Arvin, E. (1995) Removal of toluene in waste gases using a biological trickling filter. Biodegradation **6**(2), 109-118.

Phelps, C.D., and Young, L.Y.(1999) Anaerobic biodegradation of BTEX and gasoline in various aquatic sediments. Biodegradation **10**(1), 15-25.

Plyasunov, A.V., and Shock, E.L. (2000) Thermodynamic functions of hydration of hydrocarbons at 298.15 K and 0.1 MPa . Geochimica et Cosmochimica Acta **64**(3), 439-468.

Pohl, H.R., Roney, N., Wilbur, S., Hansen, H., De Rosa, C.T. (2003) Six interaction profiles for simple mixtures. Chemosphere **53**(2), 183-197.

Poulopoulos, S.G., Arvanitakis, F., Philippopoulos, C.J. (2006) Photochemical treatment of phenol aqueous solutions using ultraviolet radiation and hydrogen peroxide. Journal of Hazardous Materials **129**(1-3), 64-68.

Prenafeta-Boldú, F. X., Ballerstedt, H., Gerritse, J., Grotenhuis, J. T. C.(2004) Bioremediation of BTEX hydrocarbons: Effect of soil inoculation with the Toluene-growing fungus *Cladophialophora* Sp. Strain T1. Biodegradation **15**(1), 59-65.

Prenafeta-Boldu, F.X., Vervoort, J., Grotenhuis, J.T., Van Groenestijn, J.W. (2002) Substrate interactions during the biodegradation of benzene, toluene, ethylbenzene, and xylene (BTEX) hydrocarbons by the fungus *C*ladophialophora sp. strain T1., Applied and Environmental Microbiology **68**(6) ,2660-2665.

Prpich, G.P., and Daugulis, A.J. (2005) Treatment of biogas produced in anaerobic reactors for domestic wastewater: odor control and energy/resource recovery. Biodegradation **16**(4), 329-339.

Prpich, G.P., and Daugulis, A.J. (2006) Biodegradation of a phenolic mixture in a solid–liquid two-phase partitioning bioreactor. Applied Microbiology and Biotechnology **72** (3), 607-615.

Pruden, A., Sedran, M., Cuidan, M., Venosa, A. (2003) Biodegradation of MTBE and BTEX in an aerobic fluidized bed reactor. Water Science & Technology **47** (9), 123–128.

Pruden, A., Suidan, M.T., Venosa, A.D., Wilson, G.J. (2001) Biodegradation of methyl tert-butyl ether under various substrate conditions. Environmental Science and Technology **35**(21), 4235-4241.

Pruden, A., and Suidan, M. (2004) Effect of benzene, toluene, ethylbenzene, and p-xylene (BTEX) mixture on biodegradation of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA) by pure culture UC1. Biodegradation **15**(4), 213-227.

Pudge, I.B., Daugulis, A.J., Dubois, C. (2003) The use of Enterobacter cloacae ATCC 43560 in the development of a two-phase partitioning bioreactor for the destruction of hexahydro-1,3,5-trinitro-1,3,5-s-triazine (RDX). Journal of Biotechnology **100** (1), 65-75.

Qi, L, YingXu, C, Wei, WY, YuanPeng, W. (2004) Study on the possibility of hydrogen peroxide pretreatment and plant system to remediate soil pollution. Chemosphere **57**(10), 1439-1447.

Ranck, J.M., Bowman, R.S., Weeber, J.L., Katz, L.E., Sullivan, E.J. (2005) BTEX removal from produced water using surfactant-modified zeolite. Journal of Environmental Engineering **131**(3), 434-442.

Raymond, J.W., Rogers, T.N., Shonnard, D.R., Kline, AA. (2001) A review of structure-based biodegradation estimation methods. Journal of Hazardous Materials **84**(2-3), 189-215.

Rehmann, L., and Daugulis, A.J. (2007) Biodegradation of biphenyl in a solid–liquid two-phase partitioning bioreactor. Biochemical Engineering Journal **36**(3), 195-201.

Rehmann, L., and Daugulis, A.J. (2008) Bioavailability of PCBs in biphasic bioreactors. Biochemical Engineering Journal **38**(2), 219-225.

Reineke, V., Rullkötter, J., Smith, E.L., Rowland, S.J. (2006) Toxicity and compositional analysis of aromatic hydrocarbon fractions of two pairs of undegraded and biodegraded crude oils from the Santa Maria (California) and Vienna basins. Organic Geochemistry **37**(12), 1885-1899.

Reinhard, M., Hopkins, G. D., Steinle-Darling, E., LeBron, C. A. (2005) In Situ biotransformation of BTEX compounds under methanogenic conditions. Ground Water Monitoring & Remediation **25**(4), 50–59.

Reuschenbach, P., Pagga, U., Strotmann, U. (2003) A critical comparison of respirometric biodegradation tests based on OECD 301 and related test methods. Water Research **37**(7), 1571-1582.

Reusser, D.E., Istok, J.D., Beller, H.R., Field, J.A. (2002) In situ transformation of deuterated toluene and xylene to benzylsuccinic acid analogues in BTEX-contaminated aquifers. Environmental Science and Technology **36**(19), 4127-4134.

Röling, W.F.M. and Verseveld, H.W.V. (2002) Natural attenuation: What does the subsurface have in store?. Biodegradation **13**, 53–64.

Romine, M .F., Fredrickson, J. K., Li, S.-M. W. (1999) Induction of aromatic catabolic activity in *Sphingomonas aromaticivorans* strain F199. Journal of Industrial Microbiology and Biotechnology **23**(4-5), 303-313.

Ronen, Z., Bollag, J.M., Hsu, C.H., Young, J.C. (1996) Feasibility of bioremediation of a ground water polluted with Alkylpyridines. Ground Water **34**(2), 194 – 199.

Rosell, M., Lacorte, S., Forner, C., Rohns, H.P., Irmscher, R., Barcelo, D. (2005) Fate of gasoline oxygenates in conventional and multilevel wells of a contaminated groundwater table in Dusseldorf. Germany Environ Toxicol Chem. **24**(11), 2785-2795.

Rosell, M., Lacorte, S., Ginebreda, A., Barcelo, D. (2003) Simultaneous determination of methyl tert.-butyl ether and its degradation products, other gasoline oxygenates and benzene, toluene, ethylbenzene and xylenes in Catalonian groundwater by purge-and-trap-gas chromatography-mass spectrometry. Journal of Chromatography A **995**(1-2), 171-184.

Roychoudhury, A. N. and Merrett, G.L. (2006) Redox pathways in a petroleum contaminated shallow sandy aquifer: Iron and sulfate reductions. Science of the Total Environment **366**(1), 262-274.

Rozkov, A., Vassiljeva, I., Kurvet, M., Kahru, A., Preis, S. , Kharchenko, A., Krichevskaya, M., Liiv, M., Aèrd, A., Vilu, R. (1999) Laboratory study of bioremediation of rocket fuel-polluted groundwater. Water Research **33**(5), 1303-1313.

Salanitro, J.P., Wisniewski, H.L., Byers, D.L., Neaville, C.C., Schroder, R.A.(1997) Use of aerobic and anaerobic microcosms to assess BTEX biodegradation in aquifers. Ground Water Monitoring & Remediation **17**(3), 210-221.

Schmidt, L.M., Delfino, J.J., Preston, J.F., Laurent, G.S. (1999) Biodegradation of low aqueous concentration pentachlorophenol (PCP) contaminated groundwater. Chemosphere **38**(12), 2897-2912.

Schreiber, M.E., and Bahr, J.M. (2002) Nitrate-enhanced bioremediation of BTEX-contaminated groundwater: parameter estimation from natural-gradient tracer experiments. Journal of Contaminant Hydrology **55**, 29– 56.

Schulze, S., and Tiehm, A. (2004)Assessment of microbial natural attenuation in groundwater polluted with gasworks residues. Water Science and Technology **50**(5), 347-353.

Scow, K.M. and Hicks, K.A. (2005) Natural attenuation and enhanced bioremediation of organic contaminants in groundwater. Current Opinion in Biotechnology **16**, 246–253.

Semple, K.T., Cain, R.B., Schmidt, S. (1999) Biodegradation of aromatic compounds by microalgae. FEMS Microbiology Letters **170**(2), 291-300.

Seyfried, B, Glod, G, Schocher, R, Tschech, A, Zeyer, J. (1994) Initial reactions in the anaerobic oxidation of toluene and *m*-xylene by denitrifying bacteria. Applied and Environmental Microbiology **60**(11), 4047-4052.

Shah, M.R., Noble, R.D., Clough, D.E. (2004) Pervaporation–air stripping hybrid process for removal of VOCs from groundwater. Journal of Membrane Science **241**(2), 257-263.

Shih, T., Rong, Y., Harmon, T., Suffet, M. (2004) Evaluation of the impact of fuel hydrocarbons and oxygenates on groundwater resources. Environmental Science and Technology **38**(1), 42-48.

Shih, T.C., Wangpaichitr, M., Suffet, M. (2003) Evaluation of granular activated carbon technology for the removal of methyl tertiary butyl ether (MTBE) from drinking water. Water Research **37**(2), 375-385.

Shim, H., and Yang, S.T. (1999) Biodegradation of benzene, toluene, ethylbenzene, and o-xylene by a coculture of *P*seudomonas putida and *P*seudomonas fluorescens immobilized in a fibrous-bed bioreactor. Journal of Biotechnology **67**(2-3), 99-112.

Shim, H., Hwang, B., Lee, S.S., Kong, S.H. (2005) Kinetics of BTEX biodegradation by a coculture of *P*seudomonas putida and *P*seudomonas fluorescens under hypoxic conditions. Biodegradation **16**, 319–327.

Shim, H., Shin, E.B., Yang, S.T. (2002) A continuous fibrous-bed bioreactor for BTEX biodegradation by a co-culture of *P*seudomonas putida and *P*seudomonas fluorescens. Advances in Environmental Research **7**(1), 203-216.

Shinoda, Y., Sakai, Y., Uenishi, H., Uchihashi, Y., Hiraishi, A., Yukawa, H., Yurimoto, H., Kato, N. (2004) Aerobic and Anaerobic Toluene Degradation by a Newly Isolated Denitrifying Bacterium, *Thauera* sp. Strain DNT-1. Applied and Environmental Microbiology **70**(3), 1385–1392.

Smith, M.R.(1990) The biodegradation of aromatic hydrocarbons by bacteria, Biodegradation 1(2-3), 191 - 206.

Solano-Serena, F., Marchal, R., Huet, T., Lebeault, J.M., Vandecasteele, J.P. (2000) Biodegradability of volatile hydrocarbons of gasoline. Applied Microbiology and Biotechnology **54**(1), 121-125.

Solano-Serena, F., Marchal, R., Ropars, M., Lebeault, J.M., Vandecasteele, J.P. (1999) Biodegradation of gasoline: kinetics, mass balance and fate of individual hydrocarbons. Journal of Applied Microbiology **86**(6), 1008-1016.

Song, B., Young, L.Y., Palleroni, N.J. (1998) Identification of denitrifier strain T1 as *Thauera aromatica* and proposal for emendation of the genus *Thauera* definition. International Journal of Systematic Bacteriology **48**, 889–894

Staples, C.A., Williams, J.B., Blessing, R.L., Varineau, P.T. (1999) Measuring the biodegradability of nonylphenol ether carboxylates, octylphenol ether carboxylates, and nonylphenol . Chemosphere **38**(9), 2029-2039.

Starodub, N.F. (2007) Hydrogen peroxide: Control of its content in water objects. Journal of Water Chemistry and Technology **29**(6), 269-276.

Suarez, M.P. and Rifai, H.S.(1999) Biodegradation Rates for Fuel Hydrocarbons and Chlorinated Solvents in Groundwater. Bioremediation Journal **3**(4), 337-362.

Sublette, K., Peacock, A., White, D., Davis, G., Ogles, D., Cook, D., Kolhatkar, R. , Beckmann, D., Yang, X. (2006) Monitoring Subsurface Microbial Ecology in a Sulfate-Amended, Gasoline- Contaminated Aquifer. Ground Water Monitoring & Remediation **26**(2), 70–78.

Suthersan, S.S. (1996) Remediation Engineering: Design concepts. CRC Press, USA.

Tchobanoglous G., 1998. Wastewater Engineering, Treatment and Reuse. McGraw-Hill, 3rd edition (Metcalf& Eddy).

Theodoridis, G., Koster, E.H.M., de Jong, G. J. (2000) Solid-phase microextraction for the analysis of biological samples. Journal of Chromatography B: Biomedical Sciences and

Applications **745**(1), 49-82.

Thompson, B.A., Davies, N.W., Goldsworthy, P.M., Riddle, M.J., Snape, I., Stark, J.S. (2006) In situ lubricant degradation in Antarctic marine sediments. 1. Short-term changes. Environ. Toxicol. Chem. **25**(2), 356-366.

Tiburtius, E.R., Peralta-Zamora, P., Emmel, A. (2005)Treatment of gasoline-contaminated waters by advanced oxidation processes. Journal Hazardous Materials **126**(1-3), 86-90.

Tremier, A., De Guardia, A., Massiani, C., Paul, E., Martel, J.L. (2005) A respirometric method for characterising the organic composition and biodegradation kinetics and the temperature influence on the biodegradation kinetics, for a mixture of sludge and bulking agent to be co-composted. Bioresource Technolology **96**(2), 169-80.

Tsao, C.-W., Song, H.-G., Bartha, R. (1998) Metabolism of Benzene, Toluene, and Xylene Hydrocarbons in Soil. Applied and Environmental Microbiology **64**(12), 4924–4929.

USEPA (U.S. Environmental Protection Agency), 1997. Expedited Site Assessment Tools For Underground Storage Tank Sites: A Guide for Regulators. Chapter VI . EPA 510-B-97-001.

USEPA (U.S. Environmental Protection Agency), 2006. Edition of the Drinking Water Standards and Health Advisories, EPA 822-R-06-013, Washington DC.

USEPA standard method 5030C, 2003. Purge- and trap for aqueous samples, Revision 3.

USEPA standard method 8260B, 1996.Volatile Organic Compounds by Gas Chromatography / Mass Spectrometry (GCMS), Revision 2.

Vahaoja, P., Kuokkanen, T., Valimaki, I.,Vuoti, S.,Peramaki, P. (2005) Biodegradabilities of some chain oils in groundwater as determined by the respirometric BOD OxiTop method. Anal Bioanal Chem. **381**(2), 445-450.

Vainberg, S., Togna, A.P., Sutton, P.M., Steffan, R.J. (2002) Treatment of MTBE-contaminated water in fluidized bed bioreactor. Journal of Environmental Engineering **128**(9), 842-851.

Van Hamme, J.D., Singh, A., Ward, O.P. (2003) Recent Advances in Petroleum Microbiology, Microbiology and Molecular Biology Reviews **67**(4), 503–549.

Vandermeer, K.D., Daugulis, A.J. (2007) Enhanced degradation of a mixture of polycyclic aromatic hydrocarbons by a defined microbial consortium in a two-phase partitioning bioreactor. Biodegradation **18**(2), 211-221.

Vidali M. (2001) Bioremediation- An overview. Pure Applied Chemistry **73**(7), 1163-1172.

Vieira, P.A., Vieira, R.B., de França, F.P., Cardoso, V.L. (2007) Biodegradation of effluent contaminated with diesel fuel and gasoline, Journal of Hazardous Materials **140** (1-2), 52-59.

Villatoro-Monzon, W.R., Mesta-Howard, A.M., Razo-Flores, E. (2003). Anaerobic biodegradation of BTEX using Mn(IV) and Fe(III) as alternative electron acceptors, Water Science and Technology **48** (6), 125-31.

Vogl, AI., 1961. Quantitative inorganic analysis including elementary instrumental analysis, 3rd edition, Longmans, London, pages 249-252.

Vogt, C., Gödeke, S., Treutler, H.C., Weiß, H., Schirmer, M., Richnow, H.H.(2007) Benzene oxidation under sulfate-reducing conditions in columns simulating in situ conditions. Biodegradation **18**(5), 625-636.

Vogt, C., Alfreider, A., Lorbeer, H., Hoffmann, D., Wuensche, L., Babel, W. (2004) Bioremediation of chlorobenzene-contaminated ground water in an in situ reactor mediated by hydrogen peroxide. Journal of Contaminant Hydrology 68(1-2), 121-141.

Vogt, F., Tacke, M., Jakusch, M., Mizaikoff, B. (2000) A UV spectroscopic method for monitoring aromatic hydrocarbons dissolved in water. Analytica Chimica Acta **422**(2), 187-198.

Voice, T. (1994) Combined treatment method shows promise for cleaning up groundwater. Centerpoint **2**(1), 11-12.

Waduge, W.A., Soga, K., Kawabata, J. (2004) Effect of NAPL entrapment conditions on air sparging remediation efficiency. J. Hazard. Mater. **110**(1-3), 173-183.

Wallace, S. and Kadlec, R. (2005) BTEX degradation in a cold-climate wetland system, Water Science and Technology **51**(9), 165-171.

Walling, C. (1975) Fenton's reagent revisited. Accounts of Chemical Research **8**, 125–131.

Wang, Z., Li, K., Fingas, M., Sigouin, L., Menard, L. (2002) Characterization and source identification of hydrocarbons in water samples using multiple analytical techniques. Journal of Chromatography A **971**, 173–184.

Watts, R. J., Washington, D., Howsawkeng, J., Loge, F.J., Teel, A.L. (2003) Comparative toxicity of hydrogen peroxide, hydroxyl radicals, and superoxide anion to Escherichia coli. Advances in Environmental Research **7** (4), 961-968.

Watts, R.J., Foget, M.K., Kong, S.H., Teel, A.L. (1999) Hydrogen peroxide decomposition in model subsurface systems. Journal of Hazardous Materials **69**(2), 229-243.

Weiner, J. M., Lauck, T. S., Lovley, D.R. (1998) Enhanced Anaerobic Benzene Degradation with the Addition of Sulfate. Bioremediation Journal **2**(3), 159-173.

Wibowo, N., Setyadhi, L., Wibowo, D., Setiawan, J, Ismadji, S (2007) Adsorption of benzene and toluene from aqueous solutions onto activated carbon and its acid and heat treated forms: Influence of surface chemistry on adsorption. Journal of Hazardous Materials **146,** 237-242.

Widdowson, M.A. (2004) Modeling natural attenuation of chlorinated ethenes under spatially varying redox conditions. Biodegradation **15**, 435–451.

Wilson, L.P. and Bouwer, E. J. (1997) Biodegradation of aromatic compounds under mixed oxygen/denitrifying conditions: a review. Journal of Industrial Microbiology and Biotechnology **18**(2-3), 116-130.

Woo Choi, J., Chung, S.-G., Kim, D.-J., Lee, C.E. (2007) Regeneration of AG–AC beads for adsorption of monoaromatic compounds. Current Applied Physics, in press (doi:10.1016/j.cap.2007.09.007).

Wu, W.M., Carley, J., Gentry, T., Ginder-Vogel, M.A., Fienen, M., Mehlhorn, T., Yan, H., Caroll, S., Pace, M.N., Nyman, J., Luo, J., Gentile, M.E., Fields, M.W., Hickey, R.F., Gu, B., Watson, D., Cirpka, O.A., Zhou, J., Fendorf, S., Kitanidis, P.K., Jardine, P.M., Criddle, C.S. (2006) Pilot-scale in situ bioremedation of uranium in a highly contaminated aquifer. 2. Reduction of u(VI) and geochemical control of u(VI) bioavailability. Environmental Science and Technology **40**(12), 3986-3995.

Xing, J., and Hickey, R. (1994) Response in performance of the GAC-fluidized bed reactor process for BTX removal to perturbation in oxygen and nutrient supply. International Biodeterioration & Biodegradation **33**(1), 23-39.

Yadav, J. S., Reddy, C.A.(1993) Degradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) by the lignin-degrading basidiomycete Phanerochaete chrysosporium, Applied Environment Microbiology **59**(3), 756–762.

Yang, X., Beckmann, D., Fiorenza, S., Niedermeier, C.(2005) Field study of pulsed air sparging for remediation of petroleum hydrocarbon contaminated soil and groundwater. Environmental Science and Technology **39**(18), 7279-7286.

Yeom, S.H., and Daugulis, A.J. (2001) Benzene degradation in a two-phase partitioning bioreactor by Alcaligenes xylosoxidans Y234. Process Biochemistry **36**(8-9), 765-772.

Yeom, S.H., Dalm, M.C.F., Daugulis, A.J. (2000) Treatment of high-concentration gaseous benzene streams using a novel bioreactor system. Biotechnology Letters **22**, 1747–1751.

Yerushalmi, L., Manuel, M.F., Guiot, S.R. (1999) Biodegradation of gasoline and BTEX in a microaerophilic biobarrier. Biodegradation **10**(5), 341-352

Yin, C.Y., Aroua, M.K., Daud, W.M.A.W. (2007) Review of modifications of activated carbon for enhancing contaminant uptakes from aqueous solutions, Separation and Purification Technology **52**(3), 403-415.

Zein, M.M., Pinto, P.X., Garcia-Blanco, S., Suidan, M.T., Venosa, A.D. (2006). Treatment of groundwater contaminated with PAHs, gasoline hydrocarbons, and methyl tert-butyl ether in a laboratory biomass-retaining bioreactor, Biodegradation **17**(1), 57-69.

Zein, M.M., Suidan, M.T., Venosa, A.D. (1997) Bioremediation of groundwater contaminated with gasoline hydrocarbons and oxygenates using a membrane-based reactor, Environmental Science & Technology **40**(6), 1997-2003.

Zepeda, A., Texier, A.-C., Gomez, J. (2003) Benzene transformation in nitrifying batch cultures. Biotechnology Progress **19**, 789-793.

Zepeda, A., Texier, A.-C., Razo-Flores, E., Gomez, J. (2006) Kinetic and metabolic study of benzene, toluene and m-xylene in nitrifying batch cultures. Water Research **40**(8), 1643-1649.

Zhang, C., Bennet, G.N. (2005) Biodegradation of xenobiotics by anaerobic bacteria: Mini-Review. Applied Microbiology and Biotechnology **67**(5), 600-618.

Zhao, X., Hickey, R.F., Voice, T.C. (1999) Long-term evaluation of adsorption capacity in a biological activated carbon fluidized bed reactor system. Water Research **33**(13), 2983-2991.

Zytner, R.G. (1994) Sorption of benzene, toluene, ethylbenzene and xylenes to various media. Journal of Hazardous Materials **38** (1), 113-126.

Internet references

Agilent, http://www.chem.agilent.com/cag/cabu/pdf/c382.pdf

Environment Canada, http://www.on.ec.gc.ca/pollution/ecnpd/tabs/tab05-table2-e.html

Sauer, T.C., and Costa, H.J. (2004) Fingerprinting BTEX Sources in Groundwater, http://ipec.utulsa.edu/Conf2004/Papers/sauer_costa.pdf

Spectrum-environmental services, http://www.pbiosystems.com/products/aerobic_systems.htm

Stephens S. (2006) BTEX Metabolism Metapathway Map, http://umbbd.msi.umn.edu/BTEX/BTEX_map.html

Appendix: Publications

Scientific journals with referees

– M. FARHADIAN, D. DUCHEZ, C. VACHELARD, C. LARROCHE (2008). Monoaromatics removal from polluted water through bioreactors - A review. Water Research, 42, 1325-1341.

– M. FARHADIAN, C. VACHELARD, D. DUCHEZ, C. LARROCHE (2008). In-situ bioremediation of monoaromatic pollutants in groundwater: a review. Bioresource Technology, 99, 5296-5308.

- M. FARHADIAN, D. DUCHEZ, C. VACHELARD, C. LARROCHE (2008). BTX removal from polluted water through bioleaching processes**.** Applied Biochemistry and Biotechnololgy, in press (DOI 10.1007/s12010-008-8189-0)

M. FARHADIAN, D. DUCHEZ, C. VACHELARD, C. LARROCHE (2008). Accurate quantitative determination of monoaromatic compounds for the monitoring of bioremediation processes. Bioresource Technology, in press (DOI: 10.1016/j.biortech.2008.05.046).

Congresses with reviewed papers

- M. FARHADIAN, C. LARROCHE, M. BORGHEI, J. TROQUET, C. VACHELARD (2006). Biorémédiation d'aquifères contaminés par des BTEX à l'aide de bioréacteurs. Actes du congrès COFrRoCA 2006, Clermont-Ferrand, 28 juin – 2 juillet 2006, ed Alma Mater, Bacău, Roumanie, p. 438. ISBN 973-8392-17-9

- M. FARHADIAN, C. VACHELARD, J. TROQUET, D. DUCHEZ, C. LARROCHE, M. BORGHEI (2007). Monoaromatic hydrocarbons removal from contaminated groundwater by biosorption processes. Actes du colloque GRUTTEE 2007, Pau, 29-31 octobre 2007.

Communications to congresses

- M. FARHADIAN, D. DUCHEZ, C. VACHELARD, C. LARROCHE (2008) Development of an effective bioremediation technology for monoaromatic removal from contaminated water. 5ème rencontres des microbiologistes du pôle clermontois, Clermont-Ferrand, 27 Mars 2008.

- M. FARHADIAN, C. LARROCHE, M. BORGHEI, J. TROQUET, C. VACHELARD, D. DUCHEZ (2008). BTX removal from water pollutants through biological activated charcoal. 5th International Chemical Engineering Congress (IChEC 2008), Kish Island, Iran, 2 - 5 January 2008.

- M. FARHADIAN, C. VACHELARD, J. TROQUET, D. DUCHEZ, C. LARROCHE, M. BORGHEI (2008). BTX removal from groundwater pollutants through biological activated carbon**.** UFZ/TNO International Conference on Soil-Water Systems (ConSoil 2008), Milano, Italy, 3-6 June 2008 (communication acceptée)

Miscellaneous

M. FARHADIAN, M. BORGHEI, V. UMRANIA (2007) Treatment of beet sugar wastewater by UAFB bioprocess. Bioresource Technology 98, 3080–3083.

ABSTRACT

The work reported focus on the biodegradation of volatile aromatic compounds (benzene, toluene, xylenes) present in aqueous solutions. The main issue addressed is quantification and control of gas-liquid solute transfers. These transfers lead to organic compounds loss in the gas at each step of handling of an aqueous solution. It has been demonstrated that biomass removal from a liquid suspension must be performed with special care and a protocol based on centrifugation with Teflon capped, filled tubes is proposed. A new protocol for determination of solute activity coefficient at infinite dilution, also based on centrifugation experiments, is presented. Main work at the microbial level reveals that solute stripping in gassed systems cannot be avoided, even with the use of liquid-liquid systems. An anaerobic approach, involving a nitrate-respiring bacterium, is shown to have a great potential when used in combination with heterogeneous media.

Key words: monoaromatic compounds, bioremediation, polluted water, activated carbon, biphasic liquid-liquid media, solute stripping

RESUME

Le travail rapporté porte sur la biodégradation des composés aromatiques volatils (benzène, toluène, xylènes) en solution aqueuse. La principale question abordée est la quantification et le contrôle des transferts gaz-liquide de soluté. Il a été démontré que la séparation de la biomasse d'un liquide doit être effectuée avec une attention particulière et un protocole basé sur la centrifugation de la suspension est proposé. Une nouvelle technique pour la détermination expérimentale du coefficient d'activité à dilution infinie, également basée sur des expériences de centrifugation, est présentée. Le travail principal au niveau microbien révèle que les pertes de soluté par stripping gazeux ne peuvent pas être évitées, même avec l'utilisation d'un système biphasique liquide-liquide. Une approche anaérobie mettant en œuvre une bactérie effectuant la respiration des nitrates a montré que cette technique pouvait présenter un grand intérêt lorsqu'elle est utilisée avec un milieu hétérogène.

Mots clefs: composés monoaromatiques, biorémediation, eau polluée, charbon actif, milieu biphasique liquide-liquide, entraînement des solutés