Rhamnolipid-Enhanced Remediation of Styrene-Contaminated Soil Followed by Anaerobic Biodegradation

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ABSTRACT

Rhamnolipid-Enhanced Remediation of Styrene Contaminated Soil Followed by Anaerobic Biodegradation

Yuan Guo, Ph.D.
Concordia University, 2009

Styrene has been used widely in the manufacture of polystyrene, plastics, resins and copolymers. Styrene is classified in the EPA's Toxic Release Inventory (TRI) as a carcinogen. Batch and column adsorption experiments were performed to study the adsorbability of styrene onto soil. Soil type 1(10.2% silt and clay, 5.3% organic matter content) presented the highest styrene adsorbability 1190 mg/kg and bentonite presented the lowest adsorbability 385 mg/kg in batch adsorption tests. The sorption capacity is found to be strongly dependent on the amount of organic matter and clay ratio present in the soil.

Batch and continuous flow washing experiments were used to evaluate the feasibility of using surfactants for the removal of styrene from contaminated soil. Higher styrene removal was achieved from batch experiments compared to column experiments. The highest styrene removal (98.4%) was achieved at 1% rhamnolipid in the batch test. Maximum styrene removal occurred at the highest rhamnolipid concentration in both batch and continuous washing tests. After rhamnolipid adsorption to soil, the two mechanisms of surfactant-aided soil washing are mobilization and solubilisation. The mobilization mechanism occurs at a rhamnolipid concentration below critical micelle concentration (CMC). In this situation, rhamnolipid reduce the surface and interfacial tension between air/water, oil/water, and oil/soil systems. When rhamnolipid concentration is above CMC, the solubility of styrene increased dramatically due to the aggregation of surfactant micelles.

Leachate from soil washing experiments was biodegraded in an UASB reactor before release to the environment. The comparison between the results from batch and continuous experiments led to the conclusion that anaerobic biomass could biodegrade styrene and styrene in rhamnolipid under anaerobic conditions. More than 96% of styrene (co-metabolite with acetic acid, styrene initial concentration was 75 mg/L, 150 mg/L and 250 mg/L) was biodegraded with the 16 hour retention time in the UASB reactor with COD removal up to 82%.

Rhamnolipid has proven its ability as a washing agent in styrene removal from soil. Anaerobic biodegradation of styrene with rhamnolipid achieved high styrene removal but low biogas production. More research is required to improve the performance of the rhamnolipid and styrene biodegradation before scale-up.

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TO MY PARENTS AND MY HUSBAND

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Acronyms and Abbreviations

ABS:	Acrylonitrile-butadiene-styrene
AD:	Anaerobic digestion
APHA:	American Public Health Association
ASTM:	American Society for Testing and Materials
ATSDR:	Agency for Toxic Substances and Disease Registry
BCS:	Benzene, chlorobenzene and styrene
CEPA:	Canadian Environmental Protection Act
CMC:	Critical micelle concentration
COD:	Chemical oxygen demand
DEP:	Department of Environmental Protection
DRE:	Destruction removal efficiency
EEGs:	Electroencephalogram
EPA:	Environmental Protection Agency
GAC:	Granular activated carbon
HRT:	Hydraulic retention time
IARC:	International Agency for Research on Cancer
IC_{50}:	50% inhibitory concentration
IPCS:	International Programme on Chemical Safety
K:	Linear adsorption coefficient
K_f:	Freundlich isotherm coefficient

Kow	Octanol-Water Partition Coefficient
MEK:	Methyl ethyl ketone
MIBK:	Methyl isobutyl ketone
NAPL:	Non-Aqueous Phase Liquid
NSC:	National Safety Council
OSHA:	Occupational Safety and Health Administration
PAAL:	Phenylacetaldehyde
PAA:	Phenylacetic acid
PCBs:	Polychlorinated biphenyls
PCE:	Tetrachloroethene
SAN:	Styrene-acrylonitrile
SBR:	Styrene-butadiene rubber
SDS:	Sodium dodecyl sulfate
SVOC:	Semivolatile organic compounds
TCE:	Trichloroethene
UASB:	Upflow anaerobic sludge blanket
UST:	Underground storage tank
UV:	Ultraviolet
VOCs:	Volatile organic compounds
VSS:	Volatile suspended solids
VFA:	Volatile fatty acid

Chapter 1

Introduction

1.1. General Remarks

Styrene has been manufactured in the United States since 1938; it has been used widely in the manufacture of polystyrene plastics, resins and copolymers such as styrene-acrylonitrile (SAN), acrylonitrile-butadiene-styrene (ABS), and styrene-butadiene rubber (SBR). It is also used to make consumer products such as packaging, electrical, and thermal insulation materials, pipes, automotive components, drinking tumblers, other food use utensils, and carpet backing (ATSDR, 1992).

Styrene is classified in EPA's Toxic Release Inventory (TRI) as a carcinogen; it has been stated that releases of more than 0.454 kg of styrene into the air, water, or land must be reported annually and entered into the TRI (NSC, 2004). In 2006, styrene production reached 7 million tonnes in North America and 26 million tonnes in global (NOVA Chemicals, 2007). Styrene enters the environment during the manufacture, use, and disposal of styrene-based products. Mostly, soil and sediments may become contaminated with styrene by chemical spills, landfill disposal of styrene-containing wastes or discharges of styrene-contaminated water (ATSDR, 1992). From the Ministry of Environment Quebec, the Land Protection and Rehabilitation Regulation, the limit value of styrene in land is 5 mg/kg soil (in dry matter), which is the maximum acceptable limit for residential, recreational and institutional sites and commercial sites located in a residential district (Environment Quebec, 2003). Short-term exposure to styrene leads to mucous membrane and eye irritations, whereas long-term exposure affects the central nervous system, increasing the risk of leukemia and lymphoma. It is pointed out as a possible human carcinogen compound (Araya *et al.*, 2000). Styrene release to soil is subject to biodegradation. Degradation of 87% to 95% has been observed in sandy loam and landfill soil within 16 weeks in incubation (Fu and Alexander, 1996). However, styrene is also quite stable in soil. Styrene that leaked into surrounding soil from buried drums persisted in the soil up to two years. It also has been demonstrated that styrene buried in soil can leach into underlying groundwater. Regarding all of these facts, developing a new method, alternate material and technology is becoming more important.

Surface active agents (surfactants), consist of two parts with opposing characters, one part is hydrophilic and the other is hydrophobic (Tsujii, 1998). Biosurfactants are surfactants produced by bacterial and yeasts that are potentially more biodegradable than synthetic ones. By reducing interfacial tension and forming micelles, many environmental applications demonstrated surfactants or biosurfactants could improve oil recovery, remove heavy metals from contaminated soil, and enhance remediation of hydrophobic organic compounds from soil (Mulligan and Eftekhari 2003, Mulligan, 2005; Urum and Pekdemir 2004, Wang and Mulligan 2004). There are some methods that focus on the use of surfactants in washing formulations to enhance remediation of contaminated soil (Chu, 2003; Mulligan, 2002).

Among the available methods and technologies, biotreatment processes are less expensive than other waste treatments and energy requirements for biotreatment are less (Mulligan 2001). Furthermore, treatment can be done onsite, wastes can be degraded completely, and other chemical and physical treatments can enhance the process. Using anaerobic treatment to treat leachate has become a promising technology due to the low quantities of sludge generated from the treatment, methane production and the high quality of the treated effluent (Mulligan, 2001). Since the activity of microorganisms is vital to the process, the success of this treatment is strongly influenced by the concentration of styrene in the influent, which has a major affect on the bacterial population inside the system. In-situ remediation techniques have considerably lower costs and maintenance requirements compared to ex-situ techniques. They also remove the contamination or immobilize the contaminants in-situ without the need to transfer the affected material from the site, thus eliminating the risk of phase transformation of volatile compounds (Yerushalmi *et al.*, 1999). Due to the limited supply or complete lack of oxygen under in-situ conditions, remediation of leachate or groundwater under anoxic or anaerobic conditions is very important (Yerushalmi *et al.*, 1999). Up-flow Anaerobic Sludge Blanket columns were used in this study. The system contains and degrades styrene leachate that is generated from the rhamnolipid removal test by forming up-flow anaerobic sludge and nutrients are added to enhance the performance.

This research will focus on the use of rhamnolipid for removal of styrene, which has been adsorbed to soil, then to use anaerobic technology to remove or reduce styrene in the leachate which been generated from soil washing from batch or continuous tests. It is expected to improve current methods and technology by enhancing the understanding of the removal of styrene from soil.

1.2. Objectives of the research

The objectives of the research are:

- To evaluate the feasibility of using rhamnolipid for styrene removal from soil, using batch and continuous flow tests.
- To determine the mechanisms for styrene removal from the soil by the rhamnolipid.
- To determine the effect of various parameters on styrene removal in the batch tests and continuous flow tests.
- To determine the feasibility of using an Up-flow Anaerobic Sludge Blanket to treat the styrene-contaminated leachate generated from batch and continuous flow removal test.
- To develop an Up-flow Anaerobic Sludge Blanket to biodegrade the styrene contamination in the leachate from the rhamnolipid removal test.

- To determine the role of rhamnolipid in reducing or removing styrene in the Up-flow Anaerobic Sludge Blanket.

1.3. Organization of the Thesis

The thesis includes six chapters and four appendices as follows:

- Chapter one is the introduction to the problem. The definition of the problem, the objectives of this research, and organization of the thesis are the major concepts discussed in this chapter.
- Chapter two is devoted to the literature review and background information. Previous studies on styrene, the surfactants, available technologies to remove styrene from soil and anaerobic technology are discussed in this chapter.
- Chapter three includes the description of the materials, methods, equipment, and procedures used in the preliminary experiments.
- Chapters four and five deal with the results of the experiments for soil and surfactant characterization, soil washing study and anaerobic treatment of the collected soil washing leachates.
- Chapter six includes conclusions for this study, the scientific contributions and recommendations for the future work.
- Appendix A shows the styrene standard curve from HPLC.
- Appendix B presents the reference curve for measuring methane content of the biogas.

- Appendix C shows the chemical oxygen demand (COD) standard curve.
- Appendix D shows typical methane and carbon dioxide peaks from gas chromatography.

Chapter 2

Literature Review

In this chapter, a review will be made on styrene in the environment, its usage and applications. Available technologies have been used on removal of styrene from water and soil; specifically biotreatment processes will be presented. A brief background on biobarriers, Up-flow Anaerobic Sludge Blanket reactors, and their applications will be presented. Several types of surfactants and their applications in remediation technologies will be also reviewed in this chapter.

2.1. Styrene

Styrene is primarily a synthetic chemical. It is also known as vinylbenzene, ethenyl benzene, cinnamene, or phenylethylene. Styrene is a colorless liquid and has a high volatility (ATSDR, 1995). It dissolves in some liquids, for example alcohol, ether, methanol, acetone and carbon disulfide but doesn't easily dissolve in water. Styrene reacts violently with chlorosulfonic acid, oleum, H_2SO_4, and alkali metal graphite. It reacts vigorously with oxidizing materials and readily undergoes polymerization when heated or exposed to light or a peroxide catalyst (IPIS_INCHEM, 1996).

Styrene is normally produced from industrial synthesis, by dehydrogenating ethylbenzene with iron catalyst to produce styrene (Figure 2-1). Reactions occur at high temperatures (600-700 °C) (Gibbs and Mulligan, 1997). Since ethylbenzene is a common contaminant, it is also produced by oxidation of ethylbenzene to its peroxide, which then reacts with propylene to produce propylene oxide and alpha-methylphenyl carbinol. The carbinol is then further dehydrated to produce styrene (ATSDR, 1992). Also, the cinnamic acid could produce styrene by decarboxylation (Figure 2-2) (Warhurst and Fewson, 1994). Low levels of styrene also occur naturally in a variety of foods such as fruits, vegetables, nuts, beverages, and meats (ATSDR, 2002). Table 2-1 summarizes the properties of styrene.

$$C_6H_5CH_2CH_3 \rightleftarrows C_6H_5CHCH_2+H_2$$

Figure 2-1. Styrene production (adapted from Huntsman, 2001)

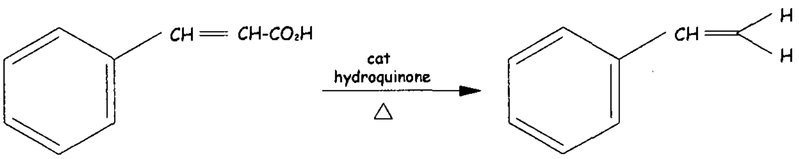


Figure 2-2. Decarboxylation of cinnamic acid to produce styrene (adapted from Warhurst and Fewson, 1994)

Table 2-1. Properties of Styrene (EPA-OPPT, 1994)

Trade Names and Synonyms	Vinylbenzene Phenethylene Cinnamene Diarex HF 77 Styrolene Styrol Styropol
Molecular Formula	C_8H_8
Chemical Structure	$C_6H_5\,CH{=}CH_2$
Physical State	Colorless to yellowish oily liquid
Molecular Weight	104.14
Melting Point	-30.6°C
Boiling Point	145-146°C
Water Solubility	310 mg/L at 25°C
Density	0.9059 g/ml at 20°C
K_{OC}	920
Log Kow	2.95
Vapor Pressure	573.14 Pa at 15°C 666.45 Pa at 20°C 1266.25 Pa at 30°C 1332.89 Pa at 35°C
Flash Point	31°C
Henry's Law Constant	275 Pa. m^3/mol
Odor Threshold	0.036 mg/m^3

2.1.1 Applications and uses

Styrene has been manufactured in the United States since 1938. The last few decades have seen a steady increase in the global production and utilization of styrene. Its production has increased 1.8% in the decade between 1978 and 1988 and with a 4.8% increase in production during the 5-year period between 1983 and 1988. Production of styrene for 1987 was over 3.6 billion kilograms (ATSDR, 1992). In 2004, the total styrene production in the United States was 5.5 billion kilograms, the production in Canada was 0.9 billion kilograms and in Mexico was 150 million kilograms (Chemical profile, 2004). In 2006, the total styrene production in North America was 7 billion kilograms and 26 billion kilograms globally (NOVA Chemicals, 2007).

Styrene has been used predominantly in the manufacture of polystyrene plastics and resins (62%). Some of these resins are used for construction purposes such as in insulation or in the fabrication of fiberglass boats. Styrene is also used as an intermediate, in the synthesis of materials used for ion exchange resins and to produce copolymers such as styrene-acrylonitrile (SAN), acrylonitrile-butadiene-styrene (ABS), and styrene-butadiene rubber (SBR)(ATSDR, 1992). Styrene also been used to make consumer products such as packaging, electrical and thermal insulation materials, pipes, automotive components, drinking tumblers, other food-use utensils, and carpet backing (ATSDR, 1992).

Table 2-2. Distribution of global capacity for styrene production (Gibbs and Mulligan, 1997)

Location	1990 (10^6 kg)	1991 (10^6 kg)	1992 (10^6 kg)
US	4, 035	4,610	4,770
Western Europe	4,025	3,922	4,185
Japan	2,043	2,238	2,398
East Asia	1,070	1,475	1,860
Eastern Europe	1,411	1,451	1,451
Canada	795	800	725
Latin America	562	575	575
Middle East	360	369	360
South Asia	165	165	218
Total	14,466	15,596	16,542

2.1.2. Styrene in the environment

The US EPA offices regulating styrene are the Water Regulations and Standards, Emergency and Remedial Response, Solid Waste, and Toxic Substances. The Food and Drug Administration regulates styrene as a food additive-synthetic flavoring substance, as an indirect food additive, as a component of polymers in paper in contact with dry food, and with a residual styrene monomer limit in polystyrene intended for use in contact with food (NSC, 2004).

Styrene enters the environment during the manufacture, use, and disposal of styrene-based products. Most industrial releases are into the atmosphere. According to the National Safety Council (USA) (1998), a total of 25,612 tonnes of styrene have been released into the environment (Table 2-3), and most into the air (NSC, 2004). Compared to 1988 (Table 2-4), the US increased styrene emissions by 54% in 1998 (NSC, 2004).

Table 2-3. US styrene release in 1998 (NSC, 2004)

	Amount (kg)	Ratio (%)
Total styrene release	25,612,122	100
Air	24,316.366	94.94
Surface water	6,102	0.02
Underground injection	230,281	0.90
Land	153,162	0.60
Transfer off-site for disposal	906,210	3.53

Table 2-4. US styrene release from 1988 to 1998 (NSC, 2004)

Year	1988	1995	1996	1997	1998
Styrene Release (kg)	16,608,595	20,113,675	20,174,676	21,087,616	25,612,122

From Environment Canada, regarding the on-site releases of the 2002 NPRI Pollutants reported for 2001, there was a total of 1883.038 tonnes of styrene released into the environment. A quantity of 1883.037 tonnes of styrene was released into air and 0.001 tonnes of styrene were released to the land. Compared to 2000, the total release increased by 7.8% (CEPA, 2001).

2.1.2.1. Styrene degradation

2.1.2.1.1 Aerobic styrene biodegradation.

The two main pathways for the aerobic degradation of styrene have been determined as shown in Figure 2-3. The first is an initial oxidation of the vinyl side-chain and the other is a direct attack on the aromatic ring. The second way oxidation is styrene direct ring cleavage as shown in Figure 2-4.

2.1.2.1.2 Anaerobic styrene biodegradation

Degradation of styrene under anaerobic conditions was observed in an anaerobic consortium enriched with styrene and ferulic acid. The biodegradation of styrene under anaerobic biodegradation is proposed as (O'Leary *et al.*, 2002):

Styrene → 2-phenylethanol→ phenylacetaldehyde (PAAL) → phenylacetic acid (PAA)

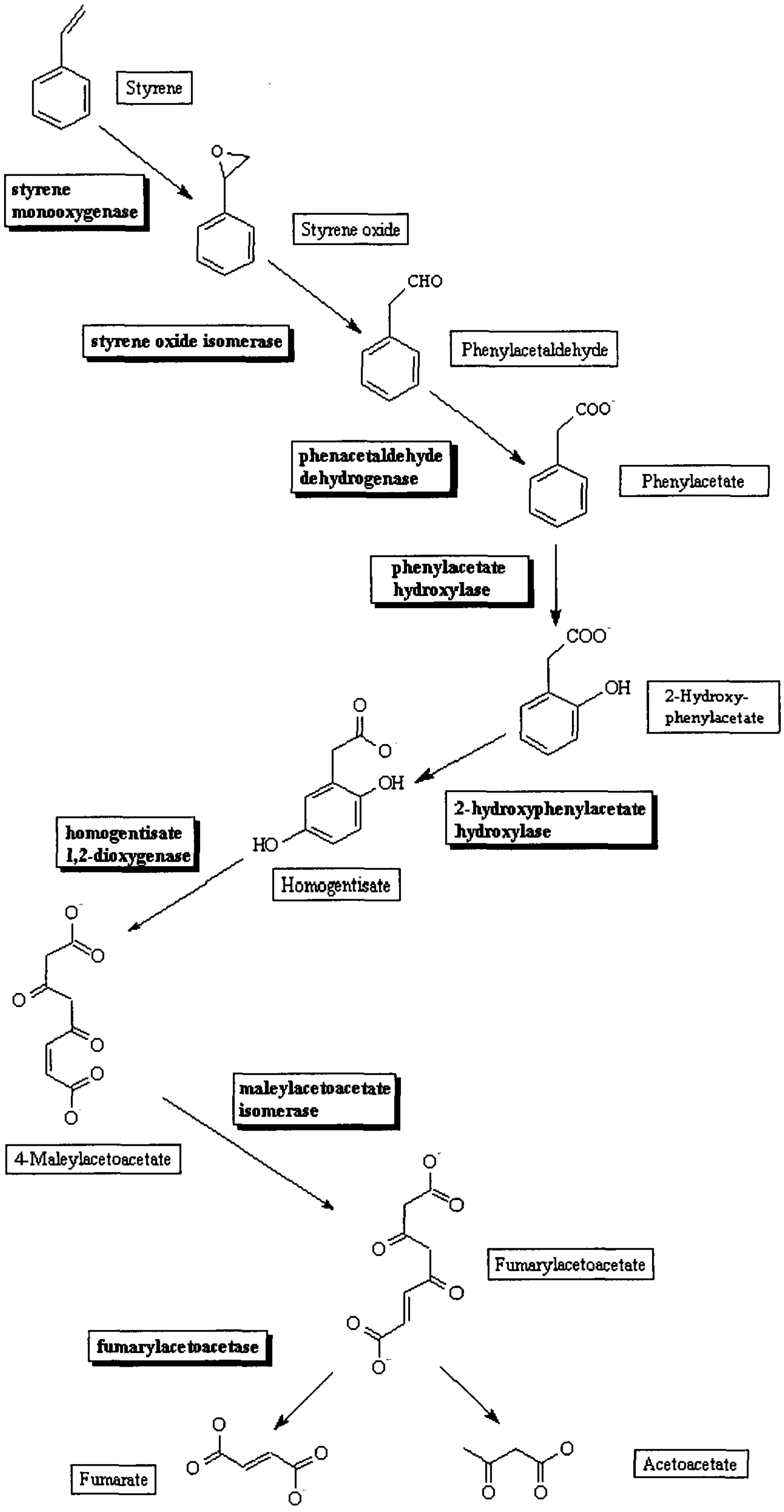


Figure 2-3. Styrene side chain oxidation (Kraus *et al.*, 2008)

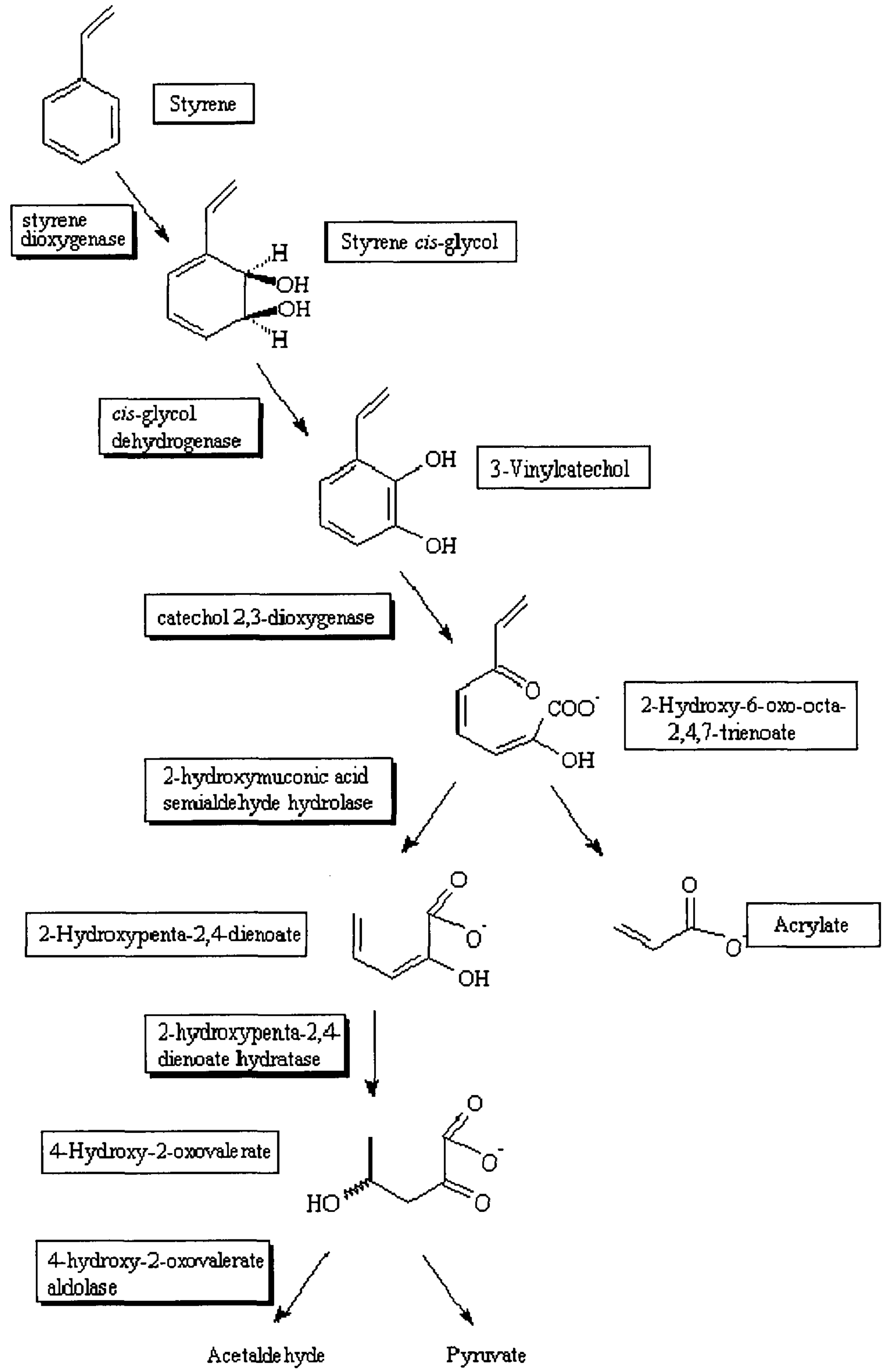


Figure 2-4. Styrene direct ring cleavage (Kraus *et al.*, 2008)

2.1.2.2. Styrene in air

When styrene is released into the atmosphere, it will react rapidly with both hydroxyl radicals and ozone with a combined, calculated half-life between 3.5-9 hours (CEPA, 1993). Styrene is also degraded in the presence of NOx and natural sunlight (EPA_OPPT, 1994).

2.1.2.3. Styrene in water

The principal sources of styrene releases to water are industrial effluents. Styrene has been detected in effluents from chemical, textile, latex, and coal gasification plants. Styrene was also identified in one of 63 industrial effluents at a concentration of < 10 μg/L (ATSDR, 1992).

Volatilization and biodegradation are the dominant transport and transformation processes for styrene in water. Styrene is rapidly lost from surface waters by volatilization, with the half-life ranging from 1 to 60 hours (CEPA, 1993), depending on the depth of the water body and degree of turbulence. On the basis of computer models, the half-life of styrene is 3 days in a pond and 13 days in an oligotrophic lake. Styrene could be biodegraded quite rapidly in water under aerobic conditions. The biodegradation half-life of styrene in water was estimated to be less than 5 days (CEPA, 1993). Very low concentrations of styrene (less than 10 μg/L) were almost completely degraded in 20 minutes in an aerobic biofilm reactor after acclimation, but the chemical was persistent in a methanogenic biofilm column. It was found that the rate of styrene biodegradation in groundwater was slow. The half-life of styrene in groundwater was estimated to be between 6 weeks and 7.5 months (ATSDR, 1992).

Araya *et al.* (1999) used anaerobic treatment of effluents from an industrial polymers synthesis plant. The composition of the wastewater includes acrylates, styrene, detergents, with a minor amount of silicates and a significant amount of ferric chloride. An upflow anaerobic sludge blanket (UASB) was used in this study. The chemical oxygen demand (COD) removal rate fluctuated between 55 and 75% until reaching a constant value of 75%. After 3 months, the methane percentage increased until it reached again a level between 70% and 80%. The alkalinity relationship (relationship between the volatile fatty acid alkalinity and the total alkalinity) was between 0.5 and 0.75. Anaerobic digestion seems to be appropriate for the treatment of the residual waters of a chemical polymer's synthesis plant. After a period of adaptation of the microbial populations, it was able to reduce COD by 75%.

2.1.2.4. Styrene in soil

Soil and sediments may become contaminated with styrene by chemical spills, landfill disposal of styrene-containing wastes or discharge of styrene-contaminated water (ATSDR, 1992). From Environment Quebec Land Protection and Rehabilitation Regulation, the limit value of styrene in land is 5 mg/kg soil (in dry matter), which is the maximum acceptable limit for residential, recreational and institutional sites and commercial sites located in a residential district (Environment Quebec, 2003). Sediments may also contain styrene. For example, 4.2 μg/kg was found in sediment from the Lower

Tennessee River in Kentucky and the chemical was also tentatively identified but was not quantified in sediment from Tobin Lake in Saskatchewan. Styrene was present in the leachates that were collected in 1981 to 1983 from a landfill that received industry and chemical wastes in 1971 to 1979. In a report in 1986 of a survey of 455 hazardous waste sites, styrene was found in 3.5% of soil samples (Alexander, 1997).

Styrene spills have occurred quite frequently in the last decade. On January 26, 1992, two barges collided on the Intercostals waterway close to Wax Lake in Louisiana. One barge carrying a cargo tank with 340,000 litres of styrene started leaking after the collision. The styrene reached the soil on the south embankment. Some of it also polymerized and formed clumps with silt and other solids, causing it to sink (National Oceanic and Atmospheric Administration, 2002).

On February 12, 1990, a fire broke out at a tire dump in Hagersville, Ontario. The fire burned intensely for a total of seventeen days, consuming a total of 12.6 million tires. The incident created 20,000 m^3 of solid waste and contaminated 4.5 hectares of land with fire water containing 12 to 50 m^3 of liquid residue (benzene, toluene, xylene, styrene, oils, etc.). Between 12,000 and 15,000 liters of oil were estimated to have reached the water (Environment Canada, 1984-1995).

On September 5, 2001, from The Maritime and Port Authority of Singapore (MPA), a container vessel *'BLUE SEA'* while proceeding to the Pasir Panjang Container Terminal suffered engine failure and contacted a chemical tanker, *'FUJIGAWA'* that was berthed alongside at the Shell Pandan Jetty. At the time of the incident, the tanker MT *'FUJIGAWA'* was carrying on-board 5 different types of chemical cargo, including 2,100 metric tonnes of styrene. The contact between the two vessels resulted in a 0.8 meter crack in the cargo tank of the chemical tanker causing spillage of styrene into the sea. Subsequently, a fire occurred at the No. 2 Starboard tank and in the sea close to the tanker (Chemical Spill, 2001).

On July 20, 2001, a Canadian National/Illinois Central (CNIC) freight train derailed and spilled a total of 84,721 L of styrene. The response operation was divided into four main scopes of work that include air monitoring, spill area operation, road construction and environment assessment. The case was closed in August 2001 (EPA, 2001).

On March 14, 2005, a styrene release occurred at the Shell Chemicals Scotford site, in Strathcona County, Saskatchewan, Canada. An over-pressured tank contained by product from the styrene monomer manufacturing process (styrene residue). This residue was recycled as a fuel for plant boilers to produce steam. Site remediation include soil excavation of the tank containment area and surface water ditches, collection and removal of the surface water and melting snow to prevent potential contamination from the property; remediation of road surfaces and parking lots on site, power washing of the site building and grass mowing and removal of clippings (Shell Chemicals, 2005).

On September 5, 2006, a truck was carrying approximately 25,740 liters of styrene flipped over at the intersection of a highway in Connecticut (DEP, 2006). Styrene was released through two gashes in the trailer. Connecticut State Police pumped the remaining styrene and the truck was lifted and removed from the highway. The Connecticut Department of Environmental Protection (DEP) found that some of the styrene entered New Haven harbor because the spill carried it into a nearby storm drain that empties into those waters. To capture this styrene, the storm drain system was flushed with water that will run into an area of the harbor lined with containment devices. Then the surface of the water was vacuumed off. Also, the contaminated soil was excavated and replaced (DEP, 2006).

Styrene released to soil is subject to biodegradation. Degradation of 87-95% has been observed in sandy loam and landfill soil over a 16-week period (Fu and Alexander, 1992). It has been demonstrated that styrene buried in soil can leach into underlying ground water. Styrene that leaked into surrounding soil from buried drums persisted in the soil for up to two years. The persistence of styrene under waterlogged condition may be of environmental importance because many waste sites are anoxic (Fu and Alexander, 1996).

Styrene in water may also partition to soils and sediments. The extent of adsorption of sparingly water-soluble compounds is often correlated with the organic carbon content of the adsorbent. In surface soils, where the amount of organic carbon will be highest, the movement of styrene will be retarded by adsorption. In deeper subsurface environments where the amount of organic carbon may be low, adsorption may not be as significant. Based on field measurements, the rate of movement of styrene in an aquifer was about 80 times slower than that of the groundwater, which is attributed to adsorption (ATSDR, 1992).

2.1.2.5. Health effects

Styrene has one of the highest blood/gas phase solubility ratio of the common industrially produced chemicals (Table 2-5) which means most of it is transferred to the blood from the lungs during each respiratory cycle. The rate of its absorption will be greatly increased as the rate and depth of respiration increases (Gibbs and Mulligan, 1997).

Table 2-5. Blood /Gas solubility ratio for selected solvents

(Gibbs and Mulligan, 1997)

Gas/solvent	Blood/gas phase solubility ratio
Ethylene	0.14
Toluene	3.8
Benzene	7.2
Chloroform	15.0
Styrene	32.0

In studies of mammals, styrene is distributed among the kidney, liver, pancreas, and brain and accumulates in the fat tissue. It can also be transmitted to the offspring from the mother via the placenta and the milk. Male Wister rats which were exposed for 5 hours at six different levels of styrene concentration from 227 to 9408 mg/m^3 were reported by Withey and Collins (1979). The distribution within the organs differed with dose but the levels in increasing order were heart and spleen, blood, brain, kidney and liver. Styrene is mostly excreted in the urine of animals and humans. However, if metabolism saturated, unmetabolized styrene is excreted via respiration (Gibbs and Mulligan, 1997).

In humans, styrene is taken up rapidly through the alveoli and into the arterial blood. The distribution of styrene has been examined in the blood, expired air, and adipose tissue. Concentration of 3.6 mg/kg of styrene in the adipose fat was detected 21 hours after exposure. It also found that styrene remained in the tissue for 3 days. In addition, its metabolites were found in the urine and styrene was exhaled for periods of up to 16 hours following exposure (Gibbs and Mulligan, 1997). Acute health effects after accidental exposure to styrene from drinking water in Spain were studied (Alberto *et al.*, 2003). As styrene in water reached concentrations up to 900 μg/L, various symptoms were reported by 46 persons of the 84 persons interviewed (attack rate 54.8 %). The most frequent symptoms were irritation of the throat (26%), nose (19%), eyes (18%) and the skin (14%). General gastrointestinal symptoms were observed with 11% reporting abdominal pain and 7% diarrhea.

For an acute effect, exposure levels of 100 mg/L and above cause irritation of the mucous membranes of the eyes and the upper respiratory tract in humans. Epidemiological studies on workers with long-term occupational exposure to styrene have shown an increased prevalence of abnormal EEGs (electroencephalogram) associated with urinary mandelic acid concentrations of 700 mg/L or more; at 1600 mg/L a reduction in psychomotor performance and visuomotor accuracy in psychological tests has been observed (IPCS, 1983). In Eastern Europe countries, it is assumed that more than 100,000 workers were exposed to styrene (Sperlingova *et al.*, 2004). The major metabolites of styrene are mandelic (MA) and phenylglyoxylic acids (PGA). Legislative regulation on occupational welfare has been set by the International Labour Organization Convention NO.161/1985 (Sperlingova *et al.*, 2004). The workers exposed to styrene have to undergo a periodical medical survey which consists of a basic examination and determination of creatinine and urea in their urine once a year. Determination of MA and PGA in urine is also performed 2-3 times a year. The Occupational Safety and Health Administration (OSHA) regulates styrene on the basis of avoidance of narcosis in the work place. In 1987, the International Agency for Research on Cancer (IARC) in Lyon, France upgraded styrene's classification from a Group 3 "not classifiable" to a Group 2B "possibly carcinogenic to humans" (Styrene Forum, 2005).

2.2. Surfactants

Materials exhibiting the characteristic of modifying interfacial interactions by way of enhanced adsorption at interfaces are referred to as surface-active agents or surfactants. Materials that possess chemical groups leading to surface activity are generally referred to as being "amphipathic" or amphiphilic (Figure 2-5) (Myers, 1992).

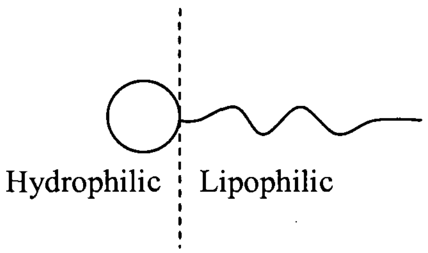


Figure 2-5. Schematic representation of a surfactant molecule

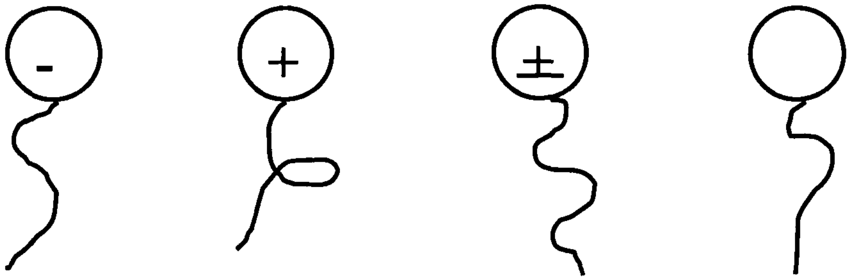
The applications of surfactants in science and industry are numerous. They range from primary processes such as the recovery and purification of raw materials in the mining and petroleum industries to enhancing the quality of finished products such as paints, cosmetics, pharmaceuticals and food. Table 2-6 lists the major applications of surfactants. The most useful chemical classification of a surfactant is based on the nature of the hydrophile, with subgroups being based on the nature of the hydrophobe. Figure 2-6 presents the four basic classes of surfactants.

- Anionic: with the hydrophilic group carrying a negative charge.
- Cationic: with the hydrophile bearing a positive charge.
- Nonionic: where the hydrophile has no charge.
- Amphoteric: where the molecule contains or can potentially contain both a negative and a positive charge.

The effectiveness of a surfactant is determined by its ability to lower the surface tension, which is a measure the surface free energy per unit area required to bring a molecule from the bulk phase to the surface. Due to the presence of a surfactant, less work is required to bring a molecule to the surface and the surface tension is reduced (reviewed by Mulligan, 2005). The surface tension is correlated with the concentration of the surface-active compound until it reaches the CMC. The CMC is defined as the minimum concentration necessary to initiate micelle formation. Figure 2-7 shows the arrangement of surfactant molecules in the two cases; below and above the CMC. Figure 2-8 presents how other parameters vary as a function of surfactant concentration.

Table 2-6. Major applications of surfactants (Myers, 1992)

Industrial	Consumer Goods
Agricultural crop applications	Adhesives
Building material	Dry-cleaning fluids
Cement additives	Goods and beverages
Coal fluidization	Household cleaning and laundering
Coating and leveling additives	Pharmaceuticals
Electroplating	Photographic products
Emulsion polymerization	Soaps, shampoos, creams
Industrial cleaning	Water proofing
Leather processing	
Lubrication	
Mold release agents	
Ore flotation	
Paper manufacture	
Petroleum recovery	
Surface preparations	
Textiles	



Anionic surfactant	Cationic surfactant	Amphoteric surfactant	Nonionic surfactant

Figure 2-6. Types of surfactants (Adapted from Myers, 1992)

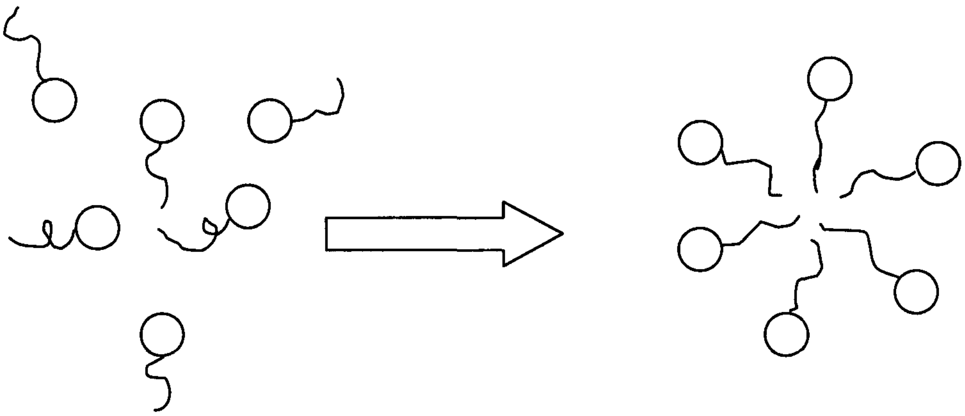


Figure 2-7. Surfactant micellization (Adapted from Becher, 1965)

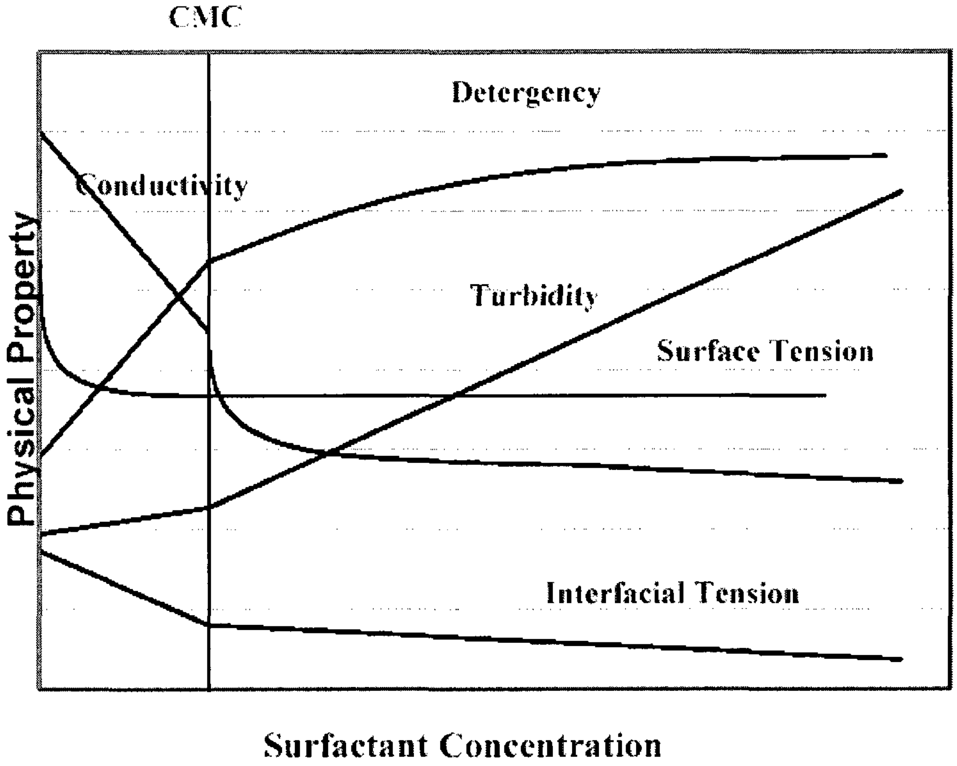


Figure 2-8. Variation of some important physical properties as a function of surfactant concentration (CMC) (Myers, 1992).

2.2.1. Biosurfactants

Some surfactants, known as biosurfactants, are biologically produced from yeast or bacteria from various substrates including sugars, oils, alkanes and wastes. Biosurfactants are categorized mainly by their chemical composition and their microbial origin (Table 2-7). Biosurfactants are grouped as glycolipids, lipopeptides, phospholipids, fatty acids and neutralipids. Most of these compounds are either anionic or neutral. Only a few are cationic such as those containing amine groups (Mulligan *et al.*, 2001).

Table 2-7. Types and microbial origin of biosurfactants (Mulligan, 2005).

Type of surfactant	**Microorganism**
Trehalose lipids	*Arthrobacter paraffineus Corynebacterium spp., Mycobacterium spp., Rhodococus erythropolis, Nocardia sp.*
Rhamnolipids	*Pseudomonas aeruginosa Pseudomonas sp., Serratia rubidea*
Sophorose lipids	*Candida apicola, Candida bombicola Candida bogoriensis, Candida lipolytica*
Glycolipids	*Alcanivorax borkumensis, Arthrobacter sp., Corynebacterium sp., R. erythropolis, Serratia marcescens, Tsukamurella sp.*
Cellobiose lipids	*Ustilago maydis*
Polyol lipids	*Rhodotorula glutinus, Rhodotorula graminus*
Diglycosyl diglycerides	*Lactobacillus fermentii*
Lipopolysaccharides	*Acinetobacter calcoaceticus (RAG1), Pseudomonas sp., Candida lipolytica*
Arthrofactin	*Arthrobacter sp.*
Lichenysin A, Lichenysin B	*Bacillus licheniformis*
Surfactin	*Bacillus subtilis, Bacillus pumilus*
Viscosin	*Pseudomonas fluorescens*
Ornithine, lysine peptides	*Thiobacillus thiooxidans, Streptomyces sioyaensis, Gluconobacter cerinus*
Phospholipids	*Acinetobacter sp.*
Sulfonylipids	*T. thiooxidans, Corynebacterium Alkanolyticum*
Fatty acids (corynomycolic acids, spiculisporic acids, etc.)	*Capnocytophaga sp., Penicillium spiculisporum, Corynebacterium lepus, Arthrobacter paraffineus, Talaramyces trachyspermus, Nocardia erythropolis*
Alasan	*Acinetobacter radioresistens*
Streptofactin	*Streptomyces tendae*
Particulate surfactant (PM)	*Pseudomonas marginalis*
Biosur PM	*Pseudomonas maltophilla*

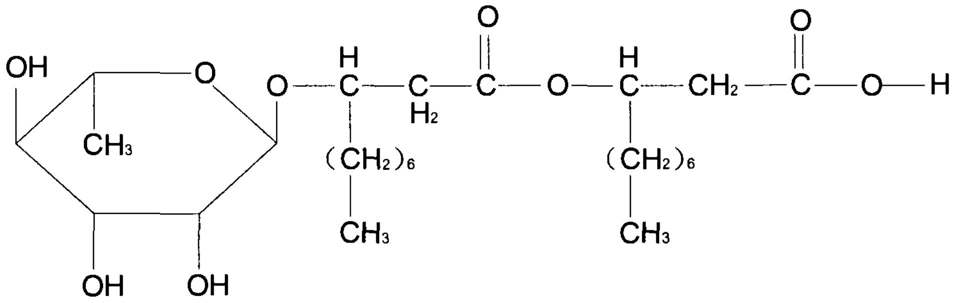
Biosurfactants have several advantages over the chemical surfactants, such as lower toxicity, smaller molecular size (less than 1500), higher biodegradability, better environmental compatibility, higher foaming, high selectivity and specific activity at extreme temperatures, pH, and salinity, and the ability to be synthesized from renewable feedstocks. Earlier studies on biosurfactants centered mainly on the properties, biosynthesis, and chemistry and have been reviewed by many people (Wang and Mulligan, 2004; Mulligan, 2005; Dahr Azma, 2002). However, in the last two decades, significant work on the fermentative production, genetics, and commercial applications of biosurfactants has been done (Dahr Azma, 2002).

2.2.1.1. Rhamnolipid

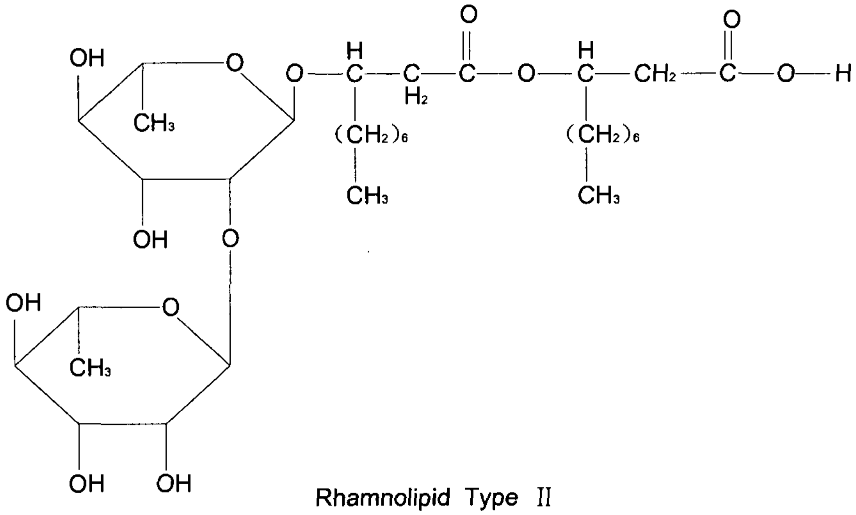
Rhamnolipids, used in this study, are biosurfactants from the glycolipid group made by *Pseudomonas aeruginosa* (reviewed by Mulligan, 2005). The surface tension of rhamnolipid of 29 mN/m has been determined. *Pseudomonas aeruginosa* can produce rhamnolipids from substrates including C11 or C12 alkanes, succinate, pyruvate, citrate, fructose, glycerol, olive oil, glucose and mannitol. Composition and yields depend on the fermentor design, pH, nutrient composition, substrate and temperature used (Mulligan, 2005).

There are four types of rhamnolipids as in Figure 2-9. Rhamnolipid type I and type II are suitable for soil washing and heavy metal removal while type III is for metal processing, leather processing, lubricants, pulp and paper processing. Type IV is usually used in textiles, cleaners, foods, inks, paints, adhesives, personal care products, agricultural adjuvants, and water treatment (Jeneil, 2002).

Several studies have examined the effects of rhamnolipid on the biodegradation of organic contaminants; especially focussing on various hydrocarbons with low solubility. It has been shown that rhamnolipid addition can enhance biodegradation of hexadecane, octadecane, n-paraffin and phenanthrene in liquid systems, in addition to hexadecane, tetradecane, pristine, creosote and hydrocarbon mixtures in soil (Noordman *et al.*, 1998; Mulligan *et al.*, 2001). A couple of possible mechanisms are found to increase the biodegradation. The first is that the substrate solubility increased for the microbial cells and another is the interaction with the cell surface, which increases the hydrophobicity of the surface and allows hydrophobic substrates to associate more easily. It has been determined that rhamnolipid at a concentration of 300 mg/L has increased the mineralization of octadecane to 20% from originally 5%. It also increases the solubility of hexadecane from 1.8 μg/L to 22.8 μg/L. (Mulligan, 2005). In Figure 2-10, it is illustrated that different organic compounds were located in the micelle at their most preferential positions (Tsujii, 1998).

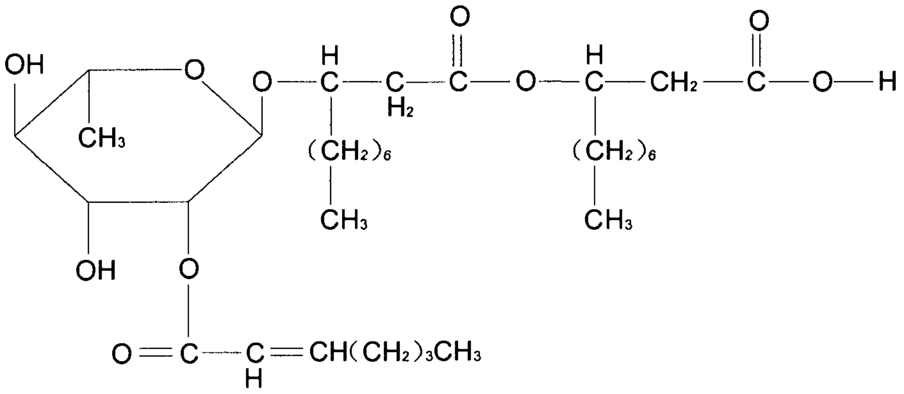


Rhamnolipid Type I

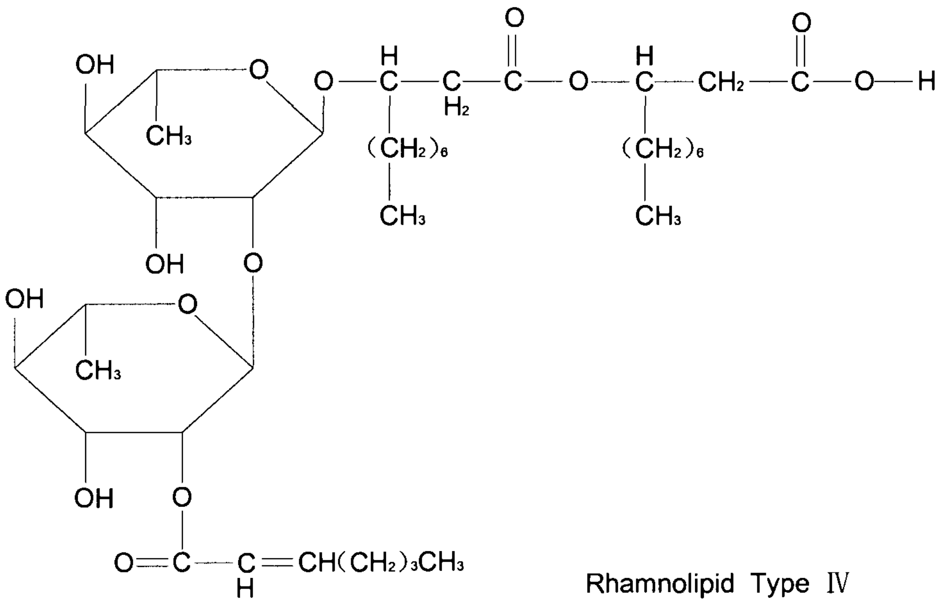


Rhamnolipid Type II

Figure 2-9. Different types of rhamnolipids (Adapted from Tsujii, 1998)



Rhamnolipid Type III



Rhamnolipid Type IV

Figure 2-9-cont'd Different types of rhamnolipids (Adapted from Tsujii, 1998)

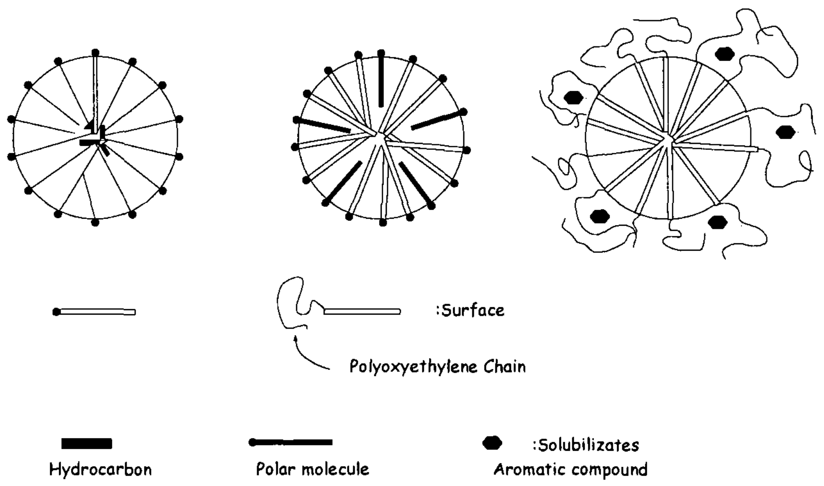


Figure 2-10. Illustration of the solubilized location of some organic compounds in the micelle. Nonpolar, polar, and aromatic compounds are solubilized in their most preferential positions in the micelle (Tsujii, 1998).

2.3 Styrene with surfactant

Doong and Lei (1996) examined the effect of anionic and non-ionic surfactants on sorption and micellar solubilization of monocyclic aromatic compounds include benzene, chlorobenzene and styrene (BCS). Compared to the non-ionic surfactant Triton X-100, the anionic surfactant increased solubilization of aromatic hydrocarbons linearly with increasing concentration. The solubilization of aromatic hydrocarbons was enhanced about 20% to 43% with SDS. Also, surfactant and BCS sorption conformed to the Langmuir isotherm and the adsorption capacity was increased as the hydrophobicity of the organic compounds increased.

2.3.1 Use of surfactants in soil washing and soil flushing

Chemical surfactant and biosurfactants are amphiphilic compounds which can reduce surface and interfacial tensions by accumulating at the interface of immiscible fluids and increase the solubility and mobility of hydrophobic or insoluble organic compounds (Singh *et al.*, 2007). Mechanisms to remove hydrocarbons from soil include micellular solubilization, lowering of interfacial tension, formation of emulsions and displacement of NAPL by increasing viscosity.

Urum *et al.* (2003) studied the surfactant applications in enhancing ex-situ soil washing. Several surfactants including rhamnolipid were used for crude oil contaminated soil. They determined that rhamnolipid had crude oil removal over 80%, temperature and concentrations of surfactant solutions were the most influential parameters. Singh *et al.* (2007) reported that biosurfactants can enhance removal of alkanes and polycyclic aromatic hydrocarbons (PAHs) from contaminated soils. Furthermore, biosurfactants appeared to be more effective in increasing the apparent solubility of PAHs by up to five times as compared to the chemical surfactants.

In general, high permeability soil is favored for surfactant flushing since numerous pore volumes can be passed through the contaminated soil. Layered systems are difficult to remediate because of limited contact in less permeable areas. Kyung and Kyound (2004) illustrated that in soil flushing studies, rhamnolipid removed as much as 70% of the phenanthrene and 60% of the diesel in the sand.

2.4 Available technologies of remove styrene from soil

Most soil and sediments may become contaminated with styrene by chemical spills and landfill disposal of styrene-containing wastes or discharge of styrene-contaminated water (ATSDR 1992). Styrene release to soil is subject to biodegradation. Degradation of 87% to 95% has been observed in the sandy loam and landfill soil within 16 weeks in incubation (Fu, 1992). But styrene is also quite stable in soil; styrene that leaked into surrounding soil from the buried drums persisted in the soil up to two years. It also has been demonstrated that styrene buried in soil can leach into the underlying groundwater (Fu and Alexander, 1996).

2.4.1 Physical and chemical treatment

- **Landfill**

A landfill is defined as a system designed and constructed to contain discarded waste so as to minimize release of contaminants to environment (LeGrega and Buckingham, 2001). Isolating contaminated soil and waste in a landfill is the oldest means of reducing exposure. A 8,094 m^2 abandoned landfill located at Houston, Harris County contained over 550 tons of liquids and sludges from local chemical industries since 1958. In 1984, the investigation found that the contamination included styrene tars, and their degradation products, substituted benzenes, substituted biphenyls, polynuclear aromatic and monoaromatic hydrocarbons. EPA (2004) decided to excavate the contaminated soils and transport them to a hazardous waste landfill regulated under Subtitle C of the Resource Conservation and Recovery Act.

- **Incineration**

Incineration of solid wastes and contaminated soils is a very affective means of destroying organic wastes. Efficiencies of destruction of much greater than 99% of most organic compounds are routinely observed by maintaining temperatures above 1100°C. Some problems are often cited for incineration such as the products of incomplete combustion, the generation of toxic emissions due to the presence of other contaminants, the residue of non-combustible contaminants and high cost of treatment (Reible, 1999). In 1990 in Texas City, an on-site incinerator has operated with contaminants including styrene tars, volatile organic compounds (VOCs), polychlorinated biphenyls (PCBs) and metals. The maximum concentration of styrene was 22,000 mg/kg. The DRE (destruction removal efficiency) reached the goal of 99.99%. The whole operation lasted for 16 months and cost 110 million US dollars and treated a total of 23,021 tons of materials (EPA MOTCO, 2001). The result showed this technology met the requirement from the EPA.

- **Soil vapor extraction (SVE)**

SVE is one of the most efficient and cost-effective methods of removing VOCs from contaminated soil. In the United States, SVE is an accepted technology that has been used at landfill sites and leaking underground storage tank (UST) sites since the 1970s. In 1993, the U.S. Environmental Protection Agency (U.S. EPA) approved SVE as a presumptive remedy for treating soils contaminated with VOCs (EPA, 2006). In general, chemicals that can be treated by SVE systems include halogenated and nonhalogenated

VOCs, some semivolatile organic compounds (SVOC), and certain other polar and nonpolar compounds.

Examples of organic compounds treated by SVE include: gasoline, kerosene, jet fuels, styrene, chlorobenzene, chloroform, carbon tetrachloride, tetrachloroethene (PCE), trichloroethene (TCE), methylene chloride, acetone, methyl ethyl ketone (MEK), and methyl isobutyl ketone (MIBK). Depending on the site and specific remediation goals, a typical SVE system operates for six months to several years. Off-gas streams generated from these remediation systems are generally low- to medium-flows containing dilute concentrations of VOCs. Treatment technologies for off-gas treatment are categorized into the following four groups as below:

1. Thermal – An oxidation process in which the temperature is increased to destroy vapor-phase contaminants.
2. Adsorption – A process separating contaminants using a medium or matrix.
3. Biological – Use of living organisms that consume or metabolize chemicals in the off-gas.
4. Emerging technologies – Including photocatalytic and non-thermal plasma treatment which destroy contaminants using ultraviolet (UV) light and electrical energy.

Because styrene has the potential to polymerize on the GAC bed, biofiltration has been demonstrated to effectively treat VOC waste streams. It had been shown that DRE (destruction and removal efficiency) of greater than 95 percent for the following compounds when the influent concentrations of these constituents were below 300 mg/L:

styrene, methanol, ethanol, formaldehyde, ethyl acetate, ammonia esters, ethers, and phenol (EPA, 2006).

2.4.2 Bioremediation and Biodegradation

Biotreatment processes are less expensive and require less energy requirements than other waste treatment processes. Furthermore, the treatment can be done on site, waste can be degraded completely and other chemical and physical treatments can enhance the processes (Mulligan, 2001). Styrene can have a very high biodegradability. Within 16 weeks in a landfill soil and a sandy loam, 87% to 95% of styrene was converted to CO_2 respectively (Fu and Alexander, 1992). Using bioremediation to treat styrene in soil has significant potential.

Styrene was able to biodegrade through the addition of water to the unsaturated side-chain using a methanogenic consortium isolated from anaerobic sludge. The biodegradation products include 2-phenylethanol and phenylacetic acid (Grbic-Galic *et al.*, 1990). This compound was readily biodegraded under aerobic conditions. First-order rate constants for all anaerobic groundwater studies ranged from 0 to 0.016/day (Fu and Alexander, 1992). Fu and Alexander (1996) using aquifer material, which had never been exposed to styrene, reported that more than 40% of the initial concentration remained after 260 days. No metabolites were detected over this time. The aquifer sediment was collected under aerobic conditions. The loss of styrene in waterlogged soils was also measured; rapid initial loss was most likely due to the presence of oxygen, followed by a much slower rate of degradation. Nearly 30% of the initial styrene remained after 260 days. It was shown that styrene can be anaerobically biodegraded (Grbic-Galic *et al.*, 1990) which indicates that biodegradation of this compound does not absolutely require the presence of oxygen. However, the persistence of styrene in both anaerobic soils and aquifer sediments suggests that toxic products from the biodegradation of this compound may build up and inhibit further metabolism (Fu and Alexander, 1996).

- **Biopiles**

Biopiles are facilities that use the bioremediation process to economically cleanup hydrocarbon-contaminated soils containing gasoline, diesel and jet fuels. These systems consist of an aeration system to provide oxygen to the microbes, an irrigation/nutrient injection system to provide nutrients and moisture after pile construction, and a leachate collection system for controlling excess moisture in the pile. A liner, berm, and cover protect the soil piles from storm events and prevent the spread of contaminants (Mulligan, 2002). Remediation time varies depending on hydrocarbon concentrations, soil, nutrients, temperature, and microbial conditions. A biopile is a variation of landfarming that can be used if there is not enough space for landfarming. The piles are usually between 100 and 1,500 m^3 and costs are between $25 and $85 per 1000 kg for contaminated soil (Mulligan, 2002). Benaxon and Belanger (1994) used a bio-pile to treat styrene-contaminated soil from a polystyrene plant in Canada. After 80 days of treatment, styrene concentration dropped from 325 mg/kg to 0.01 mg/kg, which is a removal efficiency of 96.9% (Benaxon and Belanger, 1994).

2.5 Anaerobic digestion

Anaerobic digestion is a complex series of biological processes that take place in the absence of oxygen and by which organic matter is decomposed and produces a gas principally composed of methane (CH_4) and carbon dioxide (CO_2) otherwise known as biogas. Anaerobic digestion (AD) has been used as the treatment of choice for domestic and industrial wastewater in treatment plants for many years, due to its advantages such as the production of bioenergy for sustainable development, the conservation of ammonia in the digested material, and the low sludge yield. The process is often a net energy producer, which makes the operating costs significantly lower than those of an aerobic digester (Liu *et al.*, 2004). Anaerobic decomposition of complex organic matter into methane and carbon dioxide is a complex, multi-step process involving a well-organized community of various microbial species. The main degradation steps of an anaerobic process as shown in Figure 2-11 include hydrolysis, acid production and methane production (Mulligan, 2001). Anaerobic digestion reduces odor and liquid waste disposal problems and produces a biogas fuel that can be used for process heating and/or electricity generation.

Like any other microbial systems, anaerobic digestion is very sensitive to environmental changes within the system and requires some conditions to maintain its satisfactory functioning (Mulligan, 2002). These conditions include:

- Temperature: 30 to 40 °C for mesophilic methane-producing bacteria and 50 to 60 °C for thermophilic bacteria.

- pH: the optimal pH range for methanogenic bacteria is between 6 and 8, while other anaerobic bacteria prefer close to 7. Because of the production of organic acid and carbon dioxide, alkalinity and pH control must be maintained in the system.
- Ammonia and sulfide control: ammonia concentrations of 50 to 200 mg/L are beneficial, high concentrations of ammonia can inhibit anaerobic treatment process. Sulfate serves as an electron acceptor in anaerobic processes. Sulfide is produced which can inhibit methane production.

DiBacco *et al.* (1997) studied the anaerobic degradation of styrene. Various concentrations of styrene were degraded anaerobically. The bacteria were successfully acclimatized to styrene as indicated by the gas production and pH decrease. The surfactant S-405LF was used to improve the styrene degradation. 75% styrene removal was achieved with using surfactant and 30% styrene removal without surfactant.

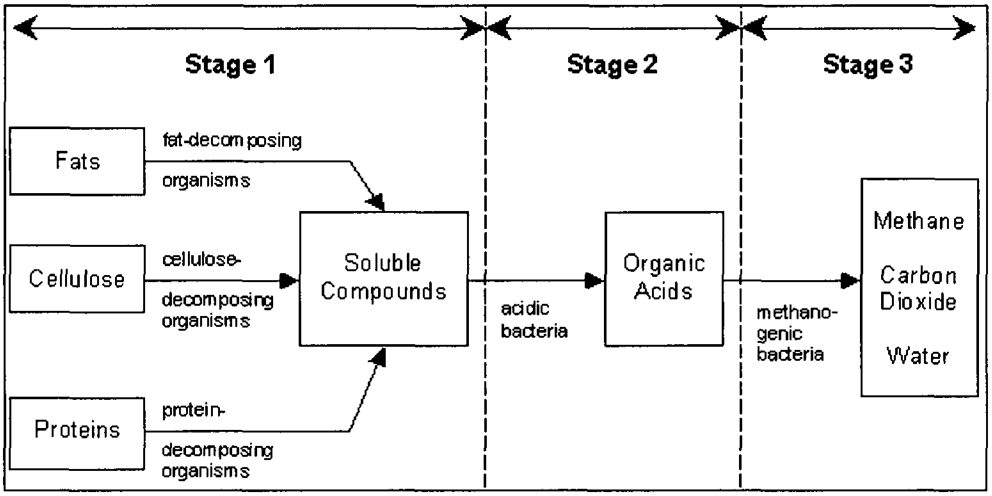


Figure 2-11. Schematic of the pathway of anaerobic digestion

(www.eere.energy.gov/de/biomass_power.html)

2.5.1 Up-flow Anaerobic Sludge Blanket (UASB)

An up-flow anaerobic sludge blanket reactor was first developed in the Netherlands. In the reactor, granules consisting of a high concentration of biomass are produced during the degradation of the easily degraded organic matter. These granules are formed and remain in the reactor. The wastewater enters the bottom of the reactor and passes through the biomass. The organic matter is converted to methane and carbon dioxide which leads to the formation of bubbles that can provide proper mixing and wastewater/biomass contact. In this reactor (Figure 2-12), gas and solid separation is very important since the solid loss could significantly decrease the performance of the reactor (Mulligan, 2001). UASB reactors are suitable for organic loads of 0.5 to 20 kg COD/m^3-day and the hydraulic retention time (HRT) can be less than 24 h.

Li *et al.* (1995) have studied the use of UASB reactor to treat highly concentrated benzoate wastewater at 37°C, pH 7.5, a HRT of 9.8 h, and loading rates up to 30.6 g-COD/(L·day) based on the reactor volume. About 95.2% of the total COD removed was converted to methane; 0.034 g of volatile suspended solids (VSS) was yielded for each gram of COD removed. No carboxylic acids except acetate were found in the effluent and the granules showed very limited butyrate-degrading capability and could not degrade propionate.

The feasibility of sewage treatment by a UASB reactor was studied using actual sewage at a fixed HRT of 4.7 h, and at temperatures in the range of 25-13 °C for six months (Uemura and Harada, 2000). The average total COD removal was 70%, 80% of total solid removal was achieved. Total COD removal rate depended on influent strength, especially solid COD concentration. Particulate organic matter in the influent was effectively removed by entrapment in the sludge bed. The hydrolysis rate of the entrapped organics was significantly affected by temperature, that is, 58% of the entrapped particulate organics was liquefied at 25 °C, decreasing to 33% at 13 °C. Microscopic observation of the morphology of the sludge revealed that even though the sludge remained in granular form as a whole, the granules tended to be partly broken in a state of autolysis.

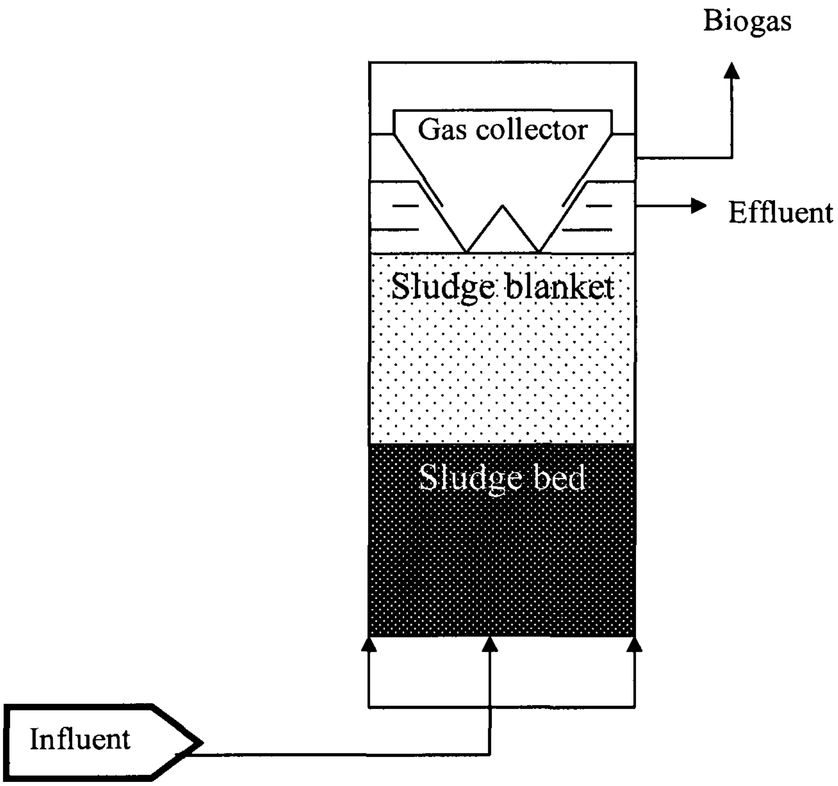


Figure 2-12. Schematic of a UASB reactor (adapted from Mulligan, 2002)

Zhou and Fang (1997) have reported co-degradation of phenol and m-cresol in a UASB reactor that operated at 37°C, one-day hydraulic retention plus effluent recycle, 98% of phenol and 20% of m-cresol without a carbohydrate co-substrate have been degraded in wastewaters containing up to 900 mg/l of phenol and 320 mg/l of m-cresol. Further increases of phenol and m-cresol concentration in wastewater impaired the phenol-degrading of the biomass activity. In treating a wastewater containing 600 mg/l of phenol, m-cresol had a threshold toxicity of 600-800 mg/l in a continuous reactor; but in a batch reactor the toxicity was progressive with an IC_{50} value of 330 mg/l.

2.6 Summary

Styrene contaminated soil presents a serious environmental problem. It may persist in soil over two years and reach the groundwater. Biosurfactants have been widely used in oil recovery, degradation of organic compounds and many other applications. It has been shown that rhamnolipid addition can enhance biodegradation of hexadecane, octadecane, n-paraffin and phenanthrene in liquid system, in addition to hexadecane, tetraadecane, pristine, creosote and hydrocarbon mixtures in soil (Noordman *et al.*, 1998; Mulligan *et al.*, 2001).

Compared to ex-situ techniques, in-situ remediation has lower costs and maintenance, and also eliminates the risk of phase transformation. The greatest disadvantage of in-situ bioremediation is the difficulty of aeration. An Up-flow Anaerobic Sludge Blanket (UASB) reactor was widely used in degradation of organic compounds, with up to 85% of COD removal efficiency (Mulligan, 2001., Zhou and Fang, 1997., Li *et al.*, 1995). In this study, a UASB reactor was used to treat leachate from washing of styrene contaminated soil.

Chapter 3

Materials and Methods

3.1. General Remarks

This chapter is devoted to describing the materials used in this study, the configuration of the experiments and the methods to determine different parameters such as styrene concentration, and chemical oxygen demand (COD). The commonly used materials such as lab distilled water are not mentioned here. The scope of work for this study is detailed in Figure 3-1.

Batch and column adsorption tests were performed to determine the styrene adsorbability on soil. Rhamnolipid was chosen in this study to evaluate its ability to assist in styrene removal from soil. A batch removal test was designed to determine the effect of rhamnolipid concentration, pH, time and clay ratio. Based on the batch test, column leaching experiments were run to further examine the effect of rhamnolipid on styrene remediation by simulating *in-situ* conditions in the experiment. A UASB reactor was also used in this study to evaluate biodegradation ability of styrene with rhamnolipid under anaerobic conditions.

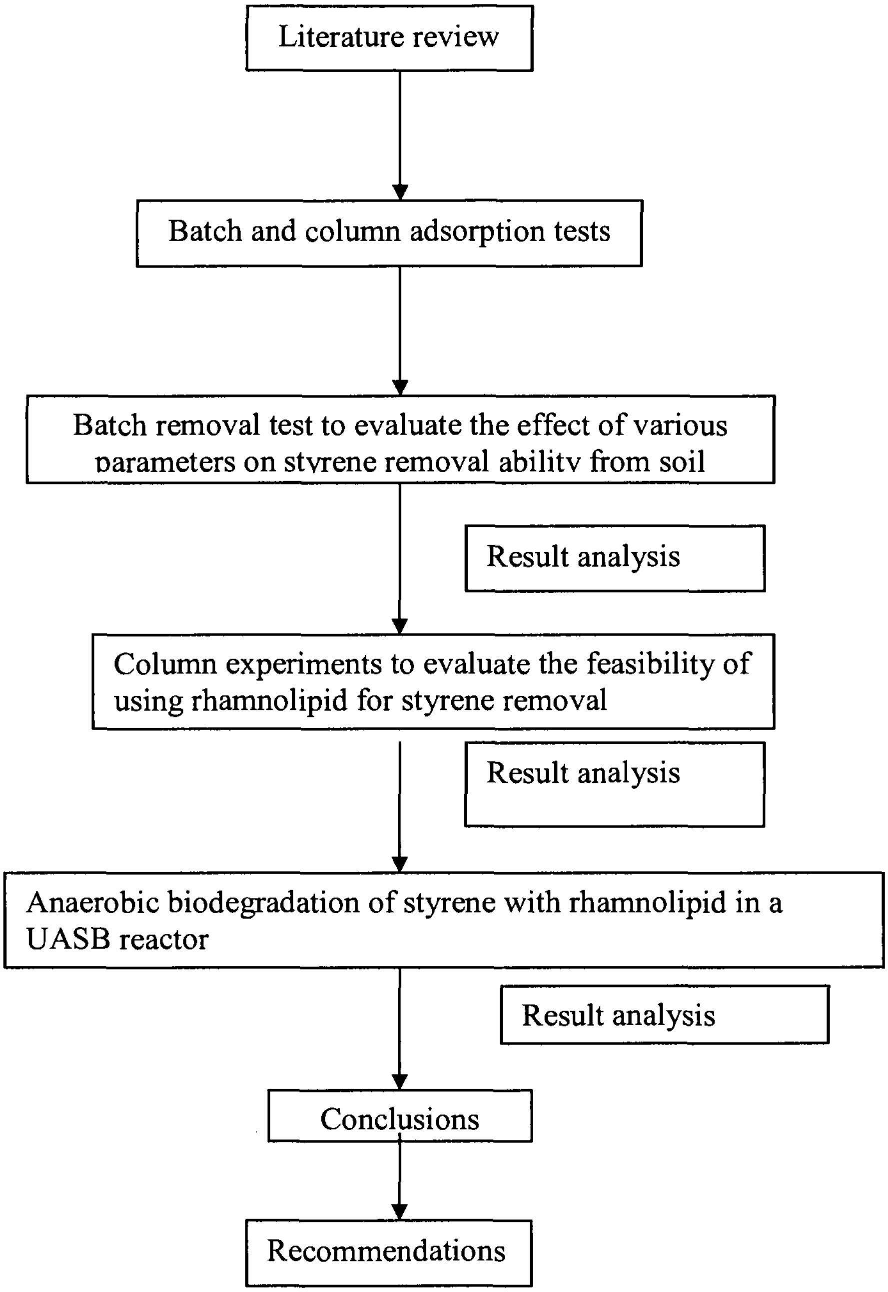


Figure 3-1. Configuration of experiments

3.2. Experimental materials

3.2.1. Styrene

The styrene used in this study was purchased from Fisher Scientific Canada Ltd, is 99%, stabilized with 10-15 ppm p-tert–butylcatechol and was kept in the fridge at 4°C at all times in an amber bottle.

3.2.2 Soils

Three types of soil were used in this study. Soil 1, as a dominant soil sample in this study, was collected from a construction site located on Rue Saint Antoine, Montreal, Quebec. Soils 2 and 3 were also collected from the Montreal area. After collection, all soil samples were dried at 105°C for 24 h and passed through a sieve with a diameter of 2 mm to remove the coarse materials.

3.2.3. Rhamnolipid (JBR-425)

The biosurfactant (JBR-425) used was rhamnolipid with the trademark JBR-425 from the “JENEIL BIOSURFACTANT CO., LLC” 2002. JBR-425 is an aqueous solution of rhamnolipids at 25% concentration. It is produced from a sterilized and centrifuged fermentation broth that has had most of the protein removed. Two major rhamnolipids, RLL (R1) and RRLL (R2) are present (Jeneil, 2002). Chemically, rhamnolipids are glycosides of rhamnose (6-deoxymannose) and β-hydroxydecanoic acid as shown in Figure 2-9. Table 3-1 presents the physical and chemical properties of JBR-425.

Table 3-1. Physical and Chemical properties of JBR-425 (Jeneil, 2002)

Physical/ Chemical property	Description
Appearance	Dark reddish-brown solution
Concentrations	Viscous suspension
Odor	Soapy
Specific Gravity	1.05-1.06
pH	6.5 – 7.5
Solubility in water	Soluble at neutral pH
Suitable diluents	Water and most common alcohols
Surface tension	26 mN/m
Toxicity	• 68.4% on day 10 of the 28 day test, OECD 301D† • EC50 > 1000 mg/L, OECD209† EC50 of 36.1 mg/L, OECD 202†

†Toxicity tests reported by Jeneil Biosurfactant (Jeneil Biosurfactant Co. 2002) as accomplished in accordance with Organization for Economic Cooperation and Development (OECD): OECD 301D for ready biodegradability, OECD 209 for activated sludge respiration inhibition (ASRIT), and OECD 202 for aquatic toxicity to daphnia.

3.2.4. Biomass

The anaerobic biomass (granules) used in this study was obtained from an anaerobic reactor that treats cheese whey generated from a cheese plant " Agropur" located in Notre Dame Du Bon Conseil, Quebec, Canada. The biomass was stored in the refrigerator at 4°C until further usage. Before using the biomass in the experiments, the biomass was incubated at 35°C for 2 weeks with simulated wastewater for acclimation. The simulated wastewater was prepared from chemicals of analytical reagent grade from the lab and purchased from Fisher Scientific Ltd. Tables 3-2 to 3-4 show the composition of the simulated wastewater.

Table 3-2. Wastewater component per liter (Alimahmoodi, 2004)

Compound	**Concentration (g/L)**
NaCl	0.6
KH_2PO_4	0.44
$MgCl_2$	0.2
$CaCl_2$	0.3
NH_4Cl	1.2
Trace mineral solution	10 ml

Table 3-3. Peptone and yeast extract added in solution to the substrate (Alimahmoodi, 2004)

Solution	Concentration
Sodium acetate	0.5 g/L
Acetic acid	1.0 g/L
Yeast extract	0.8 g/L
Peptone	0.4 g/L

Table 3-4. Trace mineral solution components (Alimahmoodi, 2004)

Minerals	Concentration (g/L)
$MnSO_4H_2O$	0.1
$CoCl_2$	0.0131
$CaCl_2$	0.076
$CuCl_2$	0.02
$ZnCl_2$	0.1
NaCl	1.0
$NiCl_2.6H_2O$	0.12
$FeCl_3.6H_2O$	1.34

3.2.5 Chemicals

All chemicals used in this study were analytical agents and purchased from *Fisher Scientific Canada Ltd* and *Sigma Aldrich Canada Ltd.* The distilled water was prepared using a *Barnstead* Model A1011 distillation unit.

3.2.6 Instrumentation

The following instruments were used in this study:

- UV/VIS Spectrophotometer, "*Perkin Elmer* model *Lambda* 40" for measurement of COD.
- Fisher Scientific Isotemp Incubator Model 304 for carrying anaerobic biodegradation experiments.
- Thermolyne/Barnstead adjustable orbital shaker model M66025 for carrying batch experiments.
- Gas Chromatograph (GC), Varian Model "CP-3800", for analysis of CH_4 and CO_2 gas.
- Masterflex model 7553-70 pump for carrying continuous soil washing experiments.
- *Beckman-Coulter* System Gold HPLC (High Pressure Liquid Chromatography) analyzer for analysis of styrene, rhamnolipid and styrene biodegradation products.
- Denver Instrument M-220 analytical balance for measurement of chemicals mass.
- Lindbreg /Blue M gravity oven for assisting in experiments require high temperature.
- Mettler Toledo PB 1502-S top loading balance for analysis of chemicals mass.

- Zeta Meter System 3.0 (Zeta-Meter Inc., USA) for measurement of soil Zeta potential.
- Particle & Surface Sciences FLOWSORB II 2300 surface area analyzer for measurement of soil surface area.
- Fisher Scientific AR25, dual channel pH/Ion meter for measurement of pH.

3.3 Methods

In this section, a brief description of methods used for determining soil, styrene, chemical oxygen demand (COD), biogas (CH_4 and CO_2) and biomass characterization are presented.

3.3.1 Soil characterization

The soil samples were air-dried and well mixed in the laboratory. The main physicochemical characteristics of the soil such as particle size distribution, specific surface area, zeta potential, and organic matter content were measured following standard or well-established methods. They are described in the following subsections.

3.3.1.1 Particle size distribution

The particle size distribution of the three samples was examined following the ASTM method (1998) ASTM D 422-63; sieve analyses were performed by using a set of USA standard testing sieves. The sieves ranged from 0.075-2 mm.

3.3.1.2 Specific surface area

Specific surface area (in m^2/g) was measured by a Flowsorb II 2300 (instruction manual, 1985) using the single point surface area method. The measuring steps were as below:

- Place 1 g of soil sample into sample holder.
- Adjust gas flow rate if necessary.
- Adjust level of liquid nitrogen about cold trap.
- Depress DET, push button.
- When air pulse passes (indicator returns to 0.02 or less), depress SURFACE AREA push button.
- Place liquid nitrogen about the sample and adjust its level to desired point.
- Depress CLEAR DISPLAY push button to clear display of adsorption result when counting ceases.
- Be sure the gas flow rate returned to normal.
- $S=V_m AN/M$ [3-1]

S: The surface area (m^2/g)

Vm: Volume of gas required to form an adsorbed monomolecular layer.

A: Avogadro's number which expresses the number of gas molecules in a mole of gas at standard conditions.

M: Molar volume of the gas

N: Area of each adsorbed gas molecule

3.3.1.3 Organic matter content

The organic matter content of the three samples was examined following the ASTM method (1993) ASTM D2974-87; Organic matter content was measured from the weight loss after calcinations at 550 °C.

- 5g of oven-dried soil were weighed and then placed in the beaker
- The beaker was placed into the furnace for 550°C for 2 hours.
- After two hours, turn off the furnace, and allow to cool overnight. Re-weigh the samples.
- Calculate the weight of the organic matter combusted, and calculate percent of organic matter (OM).
- $$\%\ \text{Organic Matter} = \frac{\text{Total weight before test - Total weight after test}}{\text{Total weight before test}} \times 100\% \quad [3\text{-}2]$$

3.3.1.4 Zeta potential measurement

Zeta potential is a physical property exhibited by any particle in suspension. Zeta potential of the soil samples was determined by a Zeta Meter System 3.0 (Zeta-Meter Inc., USA) with an Automatic Sample Transfer System in this study. A standard type GT-2 electrophoresis cell along with a molybdenum anode and a platinum cathode was used. The unit automatically calculates the electrophoretic mobility of the particles and converts it to zeta potential via Smoluchowski's equation (Sze *et al.*, 2003). An amount of 0.2 g of dried soil (passing through a 200 mesh sieve) was added to 250 ml of distilled water. The suspension was stirred on a magnetic stirrer. The applied voltage during the measurements varied in the range of 50 to 200 mV. The average of 10 to 50 measurements was taken to represent the measured zeta potential.

3.3.2 Biomass solid characterization

Total suspended solids (TSS) is a measure of the total suspended solid in water, includes both organic and inorganic. Total volatile suspended solids (TVSS) is the organic portion of the TSS; this is lost after ignition. Both are presented in mass per volume as g/L or mg/L. These tests were done according to standard method 2540-D and standard method 2540-E methods (APHA, 1999). A 3 ml sample was filtered into a pre-dried and weighed gooch crucible by vacuum filtration. The crucibles were dried to constant weight at 105°C. The increase in weight of the filter represents TSS. The crucibles were then put in a furnace at 550°C for 3 hours and allow it to cool down in a desiccator for 30 minutes. The weight loss presents TVSS. Both TSS and TVSS determinations were performed in triplicate.

3.4 Analytical methods

3.4.1 Styrene concentration determination

In this study, styrene, rhamnolipid and styrene biodegradation products (phenol, benzoic acid, 2-ethylphonel, phenolacetaldehyde, 2-phenylethanol) were analyzed by Beckman-Coulter System Gold HPLC (high pressure liquid chromatography) analyzer. A SUPELCOSIL LC-PAH Modular column of 15 cm x 4.6 mm dimensions was used. The solvent was 40% water (HPLC grade) and 60% acetonitrile (HPLC grade) at 1 ml/min. Detection was by a UV detector Model 168-4 at a wavelength of 254 nm. Table 3-5 shows the chemical elution times from the HPLC.

Table 3-5. Chemical retention times by HPLC

Chemical name	Time (min)
Styrene	4.60
Acetic acid	1.24
Butyric acid	1.78
Propionic acid	1.65
Phenol	1.87
Benzoic acid	1.42
2-Ethylphenol	2.71
Phenolacetaldehyde	2.47
2-Phenylethanol	2.08

3.4.2 Carbon dioxide and methane measurement

This method was adapted from the USEPA (1994). A gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) was used to separate and quantify both carbon dioxide and methane gases in the biogas sample. The sample was injected into a Varian type 1041 On-Column injector, fitted with a *Valco Instruments Co. Inc.* (VICI) pressurized valve delivery system. The first peak to appear was CH_4, which had an average retention time of 7.5 minutes. The second peak to appear was CO_2, which had an average retention time of 12.5 minutes. Method conditions are given in Table 3-6. Appendix A presents a typical chromatogram with peaks for methane and carbon dioxide. A standard curve was made by injecting known compositions of pure carbon dioxide and methane into the GC. The peak ratio of CH_4/CO_2 was measured and plotted against the methane content.

Table 3-6. Conditions used for GC determinations

Parameter	Specification
Carrier gas	Helium
Detector	TCD
Detector temperature	220 °C
Sample delivery	VICI pressurized valve system
Gas sample volume	2 mL
Column flow	5 mL/min
Column oven program temperature	50-100 °C, 5 °C per minute for 15 minutes
Column type	SUPELCO-Carboxen 1010 Plot
Column dimensions	30 mm x 0.53 mm
Injector	1041 On-column
Injector temperature	225 °C

3.4.3 Chemical oxygen demand (COD) determination

The chemical oxygen demand test measures the chemical oxidant required to break down organics. COD is an indicator of the concentration of organics in the solution. The Closed Reflux, Colorimetric Method from American Public Health Association (1995) was used.

A 2.5 ml sample was added to the standard tube which contains 7.5 ml of the digestion solution (commercially available pre-measured solution containing a 7.5 ml mixture of sulfuric acid, potassium dichromate, silver sulfate, and mercuric sulfate in twist cap digestion vials, and potassium hydrogen phthalate (KHP) standard (425 mg KHP/L distilled water; having a theoretical COD of 500 mg O_2/L). The samples were digested for two hours at 150 ^{o}C and then cooled. The test color was measured using a Perkin-Elmer Lambda Spectrophotometer at 600 nm.

3.5 Experimental Design

3.5.1 Styrene adsorption tests

Styrene in soil may permeate through the layer and reach the groundwater system. Since styrene will interact with soil, knowing the fate and transport of styrene is necessary. Two types of styrene adsorption tests were conducted to investigate different parameters involved in the adsorption test, batch adsorption tests and column adsorption tests. Adsorption isotherms were plotted after the batch results in order to present the amount of styrene adsorbed to a particular amount of soil. The batch adsorption test is the most used way to obtain the adsorption isotherm. Column experiments were used to simulate the migration of styrene through soils under anaerobic or anoxic conditions after a long term interaction to produce more representative site-specific results.

3.5.1.1 Batch adsorption test

Regard ASTM D4646-03 (2003), Batch adsorption tests were performed by adding two grams of soil to 45 mL of solution resulting in a soil to liquid ratio of 1:22.5. The mixture was placed in a glass centrifuge tube and covered with aluminum foil to prevent light exposure. Four different styrene concentrations were chosen (40 mg/L, 80 mg/L, 160 mg/L, and 270 mg/L) and tests performed in triplicate. All tubes were put in a container and put on a Thermolyne/Barnstead adjustable orbital shaker model M66025 that was set at 105 rpm for 24 hours.

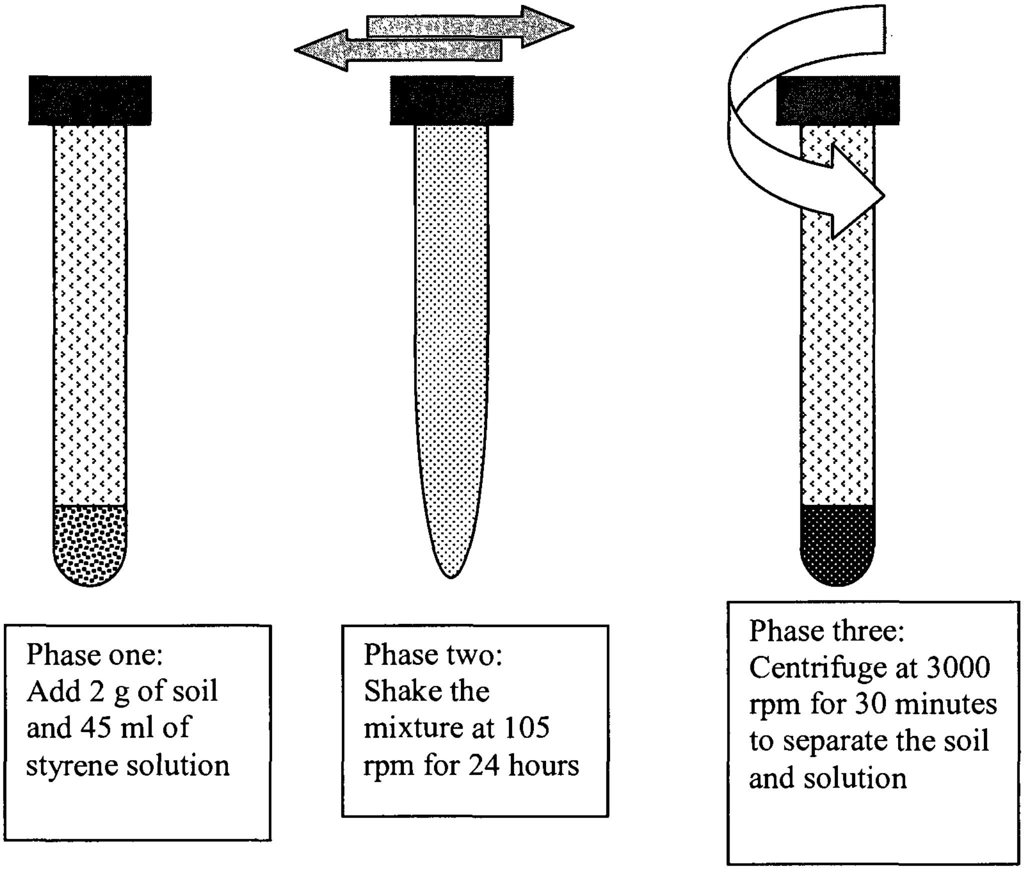


Figure 3-2. Batch adsorption test configuration

After 24 h, all the centrifuge tubes were put in an IEC HN-SII centrifuge for 30 min at 2500 rpm. The supernatant was removed and filtered with a 0.45 μm filter and the concentration of the supernatant was measured with the HPLC. Styrene was extracted by adding 10 mL of methanol (HPLC grade) to the soil (Fu and Alexander, 1992). All tubes were put in a container and put on a Thermolyne/Barnstead adjustable orbital shaker model M66025 that was set at 105 rpm for 30 minutes. After 30 minutes, all the tubes were put in an IEC HN-SII centrifuge for 10 min at 2500 rpm. The supernatant was removed and filtered with a 0.45 μm filter and the concentration of the supernatant was measured with the HPLC. After two times extraction, the styrene was measured with the HPLC. To determine how styrene adsorbed to different materials, batch tests were also conducted with two other soils and bentonite using the same procedure in triplicate.

3.5.1.2 Column adsorption tests

A long term styrene adsorption test was investigated. Three glass columns (30 cm long and 5.3 cm ID) were filled with 450 g of soil (soil 1, soil 2 and soil 3), 12.5 mL of pure styrene and 100 mL of distilled water (styrene equal to concentration 113.75 g/L) was added. The whole column was covered with aluminum foil after sealing both ends with rubber stoppers. The columns were put in the fume hood for 90 days and 16 months. After, a soil sample was taken from each 2 cm of the column, and methanol (HPLC grade) was added to extract styrene from soil. Using two extractions, the supernatant was filtered with a 0.45 μm filter and the concentration of styrene was measured with the HPLC.

3.5.2 Styrene solubilization in rhamnolipid

Styrene solubility in rhamnolipid was studied according to standard method ASTM E-1148. Saturated styrene solution was prepared by mixing 1 mL of styrene and 45 mL of the rhamnolipid solution in a glass centrifuge tube. The rhamnolipid solutions were prepared at varying concentrations ranged from 10 mg/L to 3000 mg/L. All tubes were put in a container and put on a Thermolyne/Barnstead model M66025 adjustable orbital shaker that was set at 105 rpm for 24 hours. After 24 h, all centrifuge tubes were put in an IEC HN-SII centrifuge for 30 min at 2500 rpm. The supernatant was filtered with a 0.45 μm filter and the concentration of the supernatant was measured with the HPLC.

3.5.3 Octanol/water partitioning studies

Octanol partitioning studies were performed using a modified procedure of Mulligan (1998) by adding 35 mL of octanol per 7 mL of styrene/rhamnolipid solution (250 mg/L of styrene with varying rhamnolipid concentrations) in centrifuge tubes which were shaken overnight. The octanol and water fractions were separated by centrifugation (3000 rpm for 30 min), followed by HPLC analysis.

3.5.4 Critical micelle concentration (CMC)

The critical micelle concentration (CMC) can be defined as the minimum concentration of surfactant at which micelle formation initiates. According to standard method ASTM D1331-89 (2001), by increasing the surfactant concentration, the surface tension decreases until it reaches CMC. Beyond this level of surfactant concentration the surface tension remains approximately constant. For optimal performance, the concentration of the biosurfactant needs to be of at least equal to its CMC value. In this experiment, the

CMC value of the biosurfactant was determined indirectly using surface tension measurements of solutions of various dilutions. First, thirteen concentrations of JBR-425 (0.1 mg/L, 0.5 mg/L, 1 mg/L, 5 mg/L, 10 mg/L, 30 mg/L, 50 mg/L, 100 mg/L, 500 mg/L, 1000 mg/L, 2000 mg/L, 3000 mg/L, and 4000 mg/L) were prepared. Then the surface tension was measured using a FISHER SCIENTIFIC Surface Tensiomat (Model 21). Subsequently the surface tension measurements were plotted against the rhamnolipid concentration using a semi log curve.

3.5.5 Interfacial tension study

Consistent with ASTM D 971-99a, (2004), interfacial tension was measured with Fisher Scientific Surface Tensiomat. A 100 mL beaker was cleaned by rinsing with hexane followed by a rinse with acetone. A quantity of 50 mL of styrene of various concentrations was added into the beaker. Carefully layering the styrene on the surface of the rhamnolipid solution (with the ring submerged) until a depth of at least 10 mm was reached. The styrene-rhamnolipid interface was allowed to rest for 30 seconds, and then the ring was slowly raised until the breaking point was reached.

3.5.6 Soil washing studies

Soil was contaminated by styrene and sealed in an amber jar for 30 days before use in the soil washing test. A modified procedure was used in this study to contaminate soil with styrene (Urum *et al.*, 2005). A fixed mass (100 g) of the dry soil was measured and placed in an amber glass jar for contamination with styrene. 1.1 mL, 0.55 mL, 0.35 mL and 0.11 mL of styrene were added to contaminate the soil to achieve styrene concentrations at 10.0 mg/g, 5.0 mg/g, 3.2 mg/g and 1.0 mg/g. The contamination process was carried out by completely mixing styrene with soil and putting the mixture on a Thermolyne/Barnstead adjustable orbital shaker model M66025 that was set at 200 rpm for 12 hours (Urum *et al.*, 2005).

3.5.6.1 Batch soil washing studies

According to standard method ASTM D4646 -03(2003), batch soil washing studies were performed by varying the rhamnolipid concentration, styrene concentration, pH values and washing time in glass centrifuge tubes containing 22.5:1 w/w solution to soil ratios. A series of washings was performed by washing the soil for 24h, removing the supernatant by centrifugation (2500 rpm, 30 min). The supernatants were analyzed for styrene concentration with HPLC as previously described. From the batch washing studies, preliminary results were gathered to analyze the possibility of using rhamnolipid as a washing agent to removal styrene from the soil. Furthermore, various parameters were chosen for the later column experiments.

3.5.6.2 Continuous flow soil washing studies

According to batch soil washing tests, it was possible to use rhamnolipid to remove styrene from soil. The continuous flow soil washing method was adapted from Dahr Azma (2005). The reason for using continuous flow tests is to simulate in-situ soil treatment. To perform the continuous flow tests, a 47 mm long and 47 mm diameter Teflon PFA Holder cylindrical test section was used as a column. Figures 3-5 and 3-6 presented the configuration of the continuous flow tests. Both inlet and outlet tubings had the same diameter. A Masterflex model 7553-70 pump was used to pump rhamnolipid into column from the bottom. The column was filled from bottom to top by: 0.5g Pyrex Brand Wool-Filter Fiber, 30 g 4-mm Pyrex Brand solid glass beads, 0.3 g glass wool, 10 g of soil and 0.5 g glass wool (Figure 3-4). The flow rate (ranging from 0.5 to 20 ml/min) was controlled by a flow meter. Continuous flow washing tests were performed by varying the rhamnolipid concentrations, styrene concentrations and pH values. Ten grams of styrene contaminated soil were placed in a column, after 750 pore volumes washing, the remaining adsorbed styrene from soil was extracted twice by methanol and the styrene concentration in the supernatant was measured by HPLC.

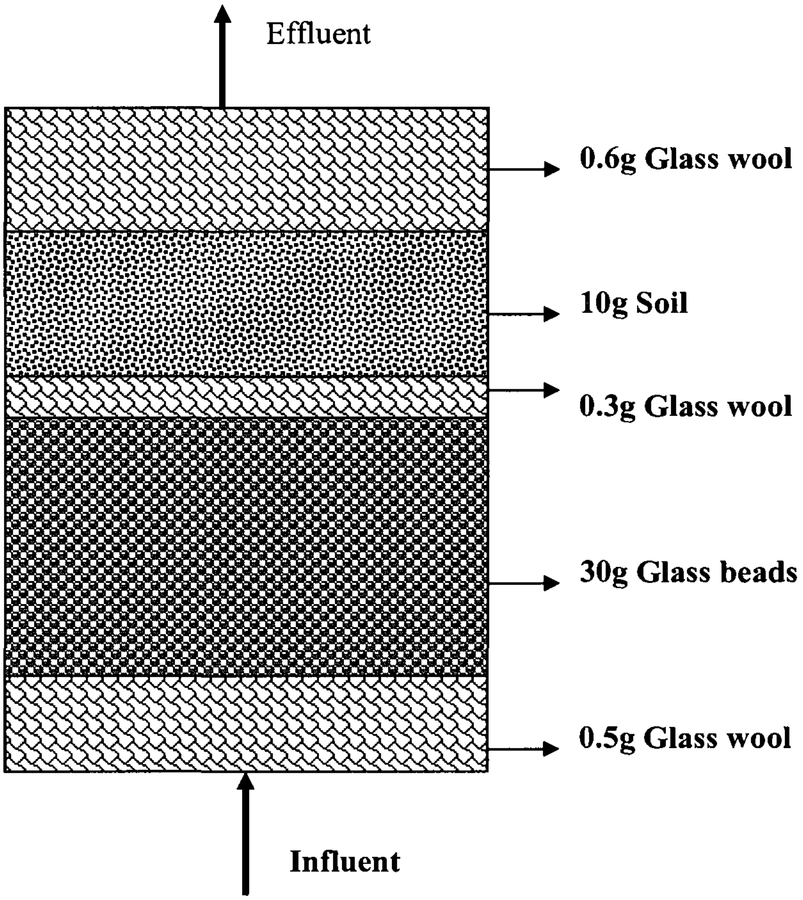


Figure 3-4. General schematic of the continuous flow configuration

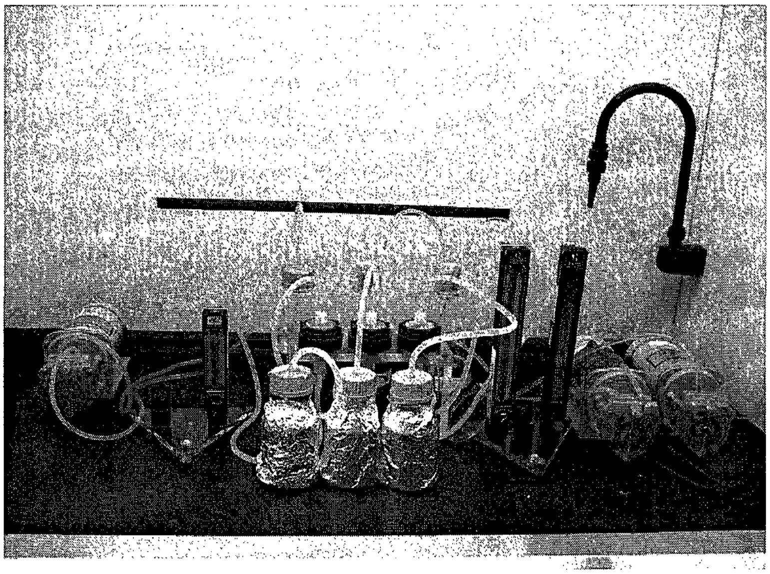


Figure 3-5. Continuous flow configuration

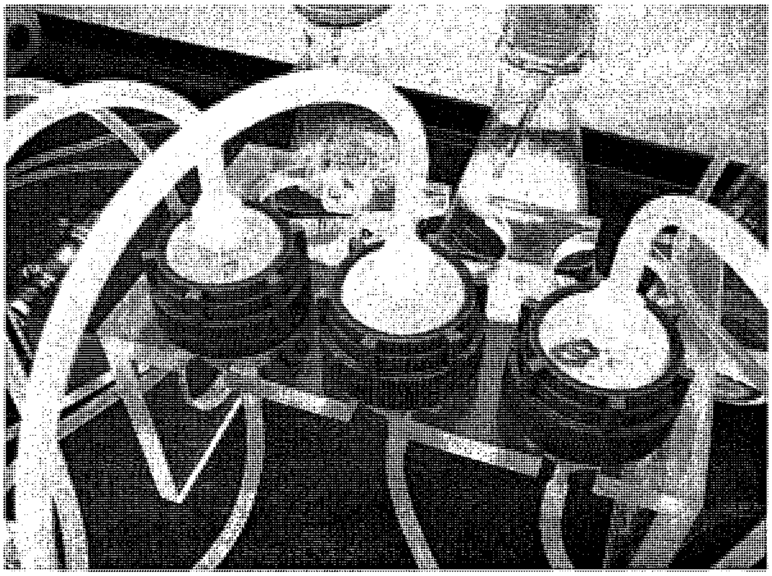


Figure 3-6. Columns in a continuous flow configuration

3.5.7 Styrene biodegradation tests

After soil flushing studies, the extraction fluid which contains mainly styrene with rhamnolipid has to be treated or recycled before it is released into the environment. Anaerobic biodegradation was chosen due to its low cost and energy requirements. Styrene biodegradation tests were prepared to determine the biodegradation rate under anaerobic conditions with or without biomass. Furthermore, since rhamnolipid may have an inhibitory effect on styrene biodegradation, a styrene with rhamnolipid biodegradation test was also prepared under anaerobic conditions with or without biomass. Water used in biodegradation tests was purged with N_2 to ensure the experiments were under anaerobic conditions.

3.5.7.1 Acclimation period

The reactor sludge was first acclimated in batch runs using acetate based aqueous solutions made with de-oxygenated tap water. The objective was to acclimate the biomass in order to start consuming COD and producing biogas. The substrate composition is in Tables 3-2 to 3-4. Two reactors of 4L were used in the acclimation study. Each of the reactors contained 1.5 L biomass and 1.5 L simulated wastewater. This experiment was run in a Fisher Scientific Isotemp Incubator Model 304 at 35±2°C. During the acclimation process, both reactors were fed with the corresponding wastewater with acetic acid. The initial concentration of acetic acid was 1.0 g/L (approximately of 1.07 g COD/L) and later it was increased step-wise until a concentration of 13.5 g COD/L was achieved. During this period, biogas production and

CH_4 content increased and the substrate was fed in only when 80% of COD removal was achieved and a retention time of a day was used as the set point for the next loading.

3.5.7.2 Effect of biomass and rhamnolipid on styrene biodegradation test

Styrene solutions of 75 mg/L, 150 mg/L and 250 mg/L concentrations were prepared. Forty mL of styrene solution alone or 40 mL of styrene in 300 mg/L rhamnolipid were placed in the amber vials and put in the incubator at 35 °C. All vials were covered with screw caps to ensure that no oxygen transferred, to simulate anoxic or anaerobic conditions. Another set of 75 mg/L, 150 mg/L and 250 mg/L styrene solutions were prepared with distilled water and 300 mg/L rhamnolipid. Quantities of 15 mL of solution and 10 ml of biomass were placed in the serum bottle sealed by a rubber septum and aluminum cap and put in the incubator at 35 °C. From preliminary experiments, the period of study was chosen as 4 days when more than 80% of styrene was degraded in styrene with biomass samples.

3.5.7.3 Specific methanogenic activity (SMA) test

The specific methanogenic activities (SMA) of the biomass were measured for over 7 days in 35 ml serum vials (Ince *et al.*, 1995). The substrate-dependent SMA represents the maximum methane production rate by the biomass treating a given substrate. In each test, 10 ml of biomass and 15ml of substrate solution were added in the serum vials. The exact amount of volatile suspended solids (VSS) in each vial was later measured after the SMA test was completed. As soon as biomass and the feed solution were added, each vial was flushed with N_2 and then sealed by a rubber septum and aluminum cap. The vials were then placed in an incubator at 35°C. Both the volume and composition of the biogas were monitored for 7 days: by then the biogas production was nearly exhausted. The SMA values were expressed as L (CH_4)/g VSS.

3.5.7.4 Batch experiments

Styrene with rhamnolipid and biomass biodegradability batch tests were performed using a 1 L glass bottle with a screw cap (Figure 3-7). Each bottle contained 300 ml of biomass and 300 ml of wastewater. Tests using acetic acid as the only substrate and acetic acid with 300 mg/L rhamnolipid as co-substrate were carried out as controls. From preliminary results, using acetic acid as co-substrate could enhance experiment activity. The styrene concentration was increased from 75 mg/L to 250 mg/L. A phase optimization period for the batch experiment was established by changing the substrate every 2 days in order to have a maximum styrene removal and COD reduction. For each styrene concentration, the optimization period was repeated 7 times to achieve the steady state. The initial and final COD and styrene concentration in the effluent were determined after each retention time. Biogas production and biogas components were also measured after a complete retention time. TSS and VSS were analyzed after each styrene level. Guo and Mulligan (2006) reported that no styrene was adsorbed onto biomass after batch experiments. The removed styrene from experiment was biodegraded by the biomass in batch tests.

Table 3-7. Test and time schedule for batch experiments

Test	Frequency
COD	Every 2 days
Styrene	Every 2 days
Biogas rate	Every 2 days
Methane content of the biogas	Every 2 days
pH	Every 2 days
VSS and TSS	Every 2 weeks

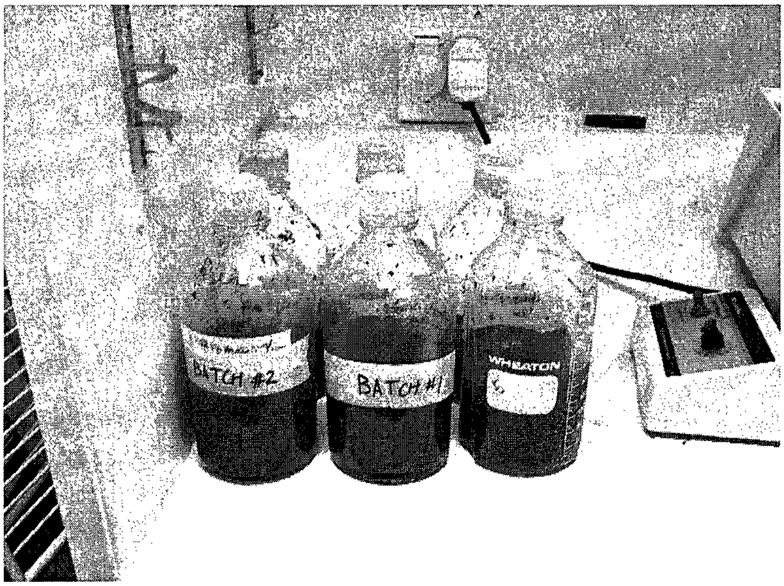


Figure 3-7. Batch biodegradation experiments

3.5.7.5 Continuous experiments

Two acrylic custom-made UASB reactors of 45 cm in height and 8.2 cm inner diameter (ID) were used for this study (Figure 3-8). The volumes used for each reactor were 500 ml of biomass and 500 ml of wastewater. The reactors had four sampling ports along its height: the lowest one for biomass sampling, and the one on the second top for effluent sampling. In addition, the reactors had one opening at the bottom of the reactor as the wastewater inlet, and a gas sampling port at the top of the rubber stopper which served to collect the gas for its qualitative and quantitative measurements. To avoid the escape of biomass and any channeling due to the flow, the bottom of the reactors was covered with a mesh made from fiberglass with openings of 1.0 cm, and glass beads of 4.0 – 5.0 mm. Also, each reactor was carrying a gas-solid separator, made from a plastic funnel, which was attached to the reactor. A peristaltic pump Masterflex L/S model 7519-15 with 6 parallel lines purchased from Cole Parmer was used to inject the substrate in continuous mode. The whole system was put in an incubator with the temperature set at 35 °C.

According to batch experiments, styrene concentrations of 75 mg/L, 150 mg/L and 250 mg/L were chosen for continuous experiments. A phase optimization period for UASB-reactor was established by changing the substrate daily. Reducing the retention time maximize the conversion of formed VFAs to methane. A phase optimization period for UASB-reactor was established by changing the substrate every 16 h in order to maximize styrene removal and COD reduction. For each styrene concentration, the optimization period was repeated 7 times to achieve the steady state. The initial and final COD and styrene concentrations in the effluent were determined after each retention time. Biogas production and biogas component were also measured after a complete retention time. Figure 3-9 shows the continuous flow test run in the incubator. The three concentrations of styrene (75 mg/L, 150 mg/L and 250 mg/L) with co-substrate (300 mg/L rhamnolipid and 5 mL/L acetic acid) as wastewater was pumped from bottom of the reactor into the system. The pH was adjusted to 6.3 to 6.5 in the influent with KOH. TSS and VSS were analyzed after each styrene level.

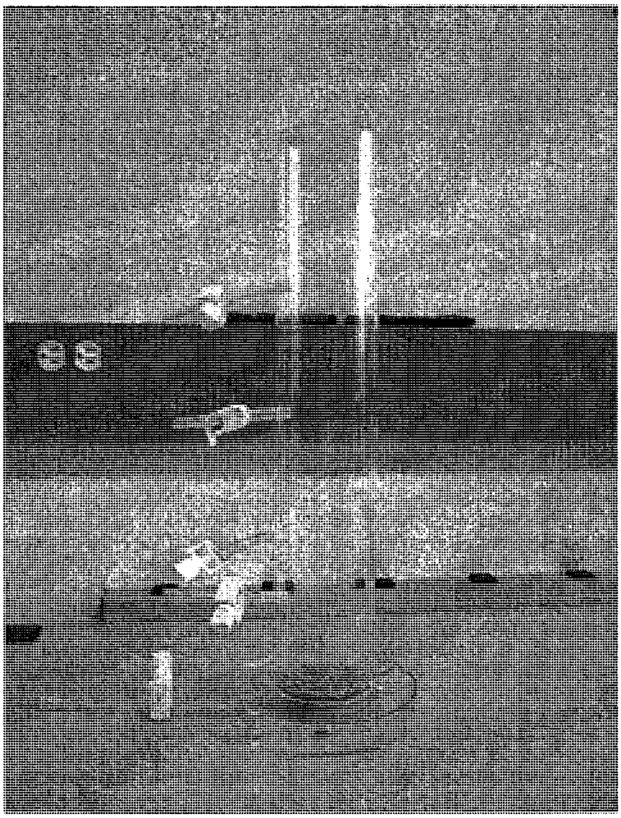


Figure 3-8. Lab scale UASB reactor

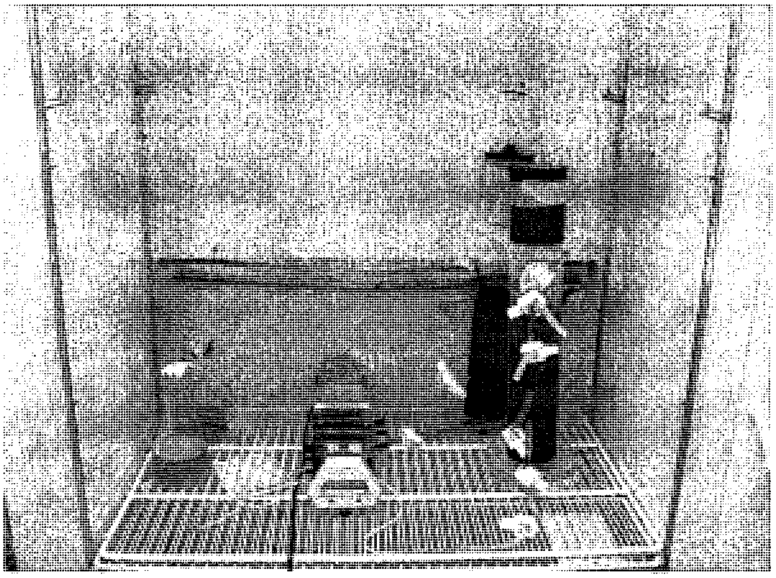


Figure 3-9. Continuous test set-up

Table 3-8. Test and time schedule for continuous experiments

Test	Frequency
COD	Every day
Styrene	Every day
Biogas rate	Every day
Methane content of the biogas	Every day
pH	Every day
VSS and TSS	Every week

Chapter 4

Experimental Results for Soil Adsorption Studies and Soil Washing Experiments

4.1 General Remarks

Styrene contaminated soil is a complex matter for remediation in the sense of choosing the right techniques and reliable washing agents. Before performing soil washing experiments, adsorption tests were conducted to investigate the effects of different parameters on soil adsorbability. A series of soil washing experiments was followed to investigate the functionality of rhamnolipid in styrene removal. In this chapter, the results of soil batch adsorption tests and long term adsorption tests are presented. The results of soil washing experiments using rhamnolipids both in batch and continuous flow configurations are also presented. Various parameters were studied and the effect of each on the removal of styrene from soil was determined.

4.2 Physicochemical characteristics of the soil

A series of measurements was performed to determine the characteristics of the soil. The physicochemical characteristics of the soil are listed in Table 4-1. All three samples have a sand content of more than 80%. 10.2% of soil type 1, 19.5% of soil type 2, and 11.3% of soil type 3 were silt and clay. These results indicate that all three samples can be classified as sandy soils, according to the particle size definition by the American Society for Testing and Materials (ASTM). Particle size distribution is the key physical parameter for determining feasibility of using a soil washing technology (Freeman and Harris, 1995). Although particle size distribution should not count as the sole reason for choosing or eliminating soil washing as a candidate technology for remediation, it can provide an initial means of screening for the potential use of soil washing. Particle sizes ranging from 0.25 to 2 mm are effective for soil washing. Particle sizes ranging from 0.063 to 0.25 mm are limited for soil washing and finer than 0.063 mm are difficult for soil washing. According to Freeman and Harris (1995), the three types of soil used in this study are suitable for soil washing technology.

The pH values of the three types of soil are close to pH 7 which can be categorized into the neutral range. The specific surface areas of the three types of soil are measured and results are listed in Table 4-1. Specific surface area is an important factor related to the adsorption capacity of the materials. The measured specific surface areas of the soils ranged from 2.5 to 11.0 m^2/g for soil type 1 and for soil type 3 have values below 10 m^2/g, conforming to the typical range of the specific surface area for fine sandy particles

(Brady and Weil, 2002). Soil type 2 has a higher specific surface area value partially due to its high silt and clay ratio, since clay usually has a larger specific surface area than silt and sand.

Soil organic matter plays an important role in soil stability; process associated with contamination, formation of maintenance of good soil structure, and improved water retention (Yong and Mulligan, 2004). The organic matter contents of soil types 1 and 2 are 5.3% and 4% by weight, which fall into the range of reported organic matter content in soils (0.5 – 5%, Yong and Mulligan, 2004). Soil type 3 has a higher organic matter content value than soil type 1 and soil type 2, which may result from a previous unknown process for soil type 3 such as agricultural production.

Zeta potential was used to determine the electro physicochemical interaction at soil-liquid interface. The zeta potential of the three types of soil was measured and the results are listed on Table 4-1. Soil type 1 has the lowest zeta potential at -32.9 mV and soil type 3 presented the highest value at -19.8 mV. Zeta potential was affected by many factors such as electrolyte concentration (ionic strength), ion species, pH, dielectric permittivity of the medium, viscosity, temperature, particle size and density and the shape of the suspended particles (Aydin *et al.*, 2004).

Table 4-1. Main physicochemical properties of the soil

Physicochemical properties	Soil type 1	Soil type 2	Soil type 3
Partial size distribution	10.2% silt and clay	19.5% silt and clay	11.3% silt and clay
Organic matter content	5.3%	4.0%	17.5%
pH	7.05	7.01	7.12
Zeta potential (mV)	-32.9	-21.8	-19.8
Specific surface area (m^2/g)	4.0	11.0	2.5

4.3 Styrene adsorption studies

Two types of styrene adsorption tests were conducted to investigate the effects of different parameters involved in the adsorption tests. Batch adsorption tests were conducted with three types of soil and bentonite. The contact time was 24 hours and the solid to liquid ratio was 1:22.5. Long term column adsorption tests were performed with three types of soil for 90 days and 16 months.

4.3.1 Batch adsorption tests

To describe the distribution of the solute in the solid phase and the liquid phase at equilibrium condition, it is necessary to express the amount of solute adsorbed per unit weight of sorbent, X/m, as a function of the residual equilibrium concentration, C_f, of solute remaining in solution. The expression of this relationship is termed an adsorption isotherm.

From Figures 4-1 to 4-4, it can be seen that for adsorption of styrene on the three types of soil initially the uptake capacity increased in a linear way with rising equilibrium concentration. Uptake capacity of bentonite also increased with rising equilibrium concentration and eventually was limited by the fixed number of uptake active sites on bentonite resulting in a plateau.

A mass balance study was conducted in the batch adsorption test. Table 4-2 presented the percentage recovered styrene from batch adsorption tests. Approximately 85% of styrene was recovered when the styrene concentration was 160 and 273 mg/L in the batch test.

Table 4-2. Mass balance results (% recovered) in batch adsorption tests

Initial styrene concentration (mg/L)	Soil 1	Soil 2	Soil 3
40	99.0	89.5	104.2
80	101.0	100.0	100.0
160	87.2	81.6	87.3
273	84.6	85.2	81.3

The loss of styrene during experiments may be the result of the high volatility of styrene. The main chemical properties that affect NAPL transport may include: (1) volatility; (2) relative polarity; (3) affinity for soil organic matter or organic contaminants, and (4) density and viscosity (Yong, 1992).

From Figures 4-1 to 4-4, it was found that the $(X/m)_{max}$ for styrene for the three types of soil and bentonite were 1190 mg/kg, 920 mg/kg, 1090 mg/kg and 385 mg/kg respectively. Soil type 1 presented the highest styrene adsorbability and bentonite presented the lowest styrene adsorbability. Soil 3, which has the highest organic matter content (17.5%), presented the second highest styrene adsorbability. Soil type 2, which has the highest silt and clay ratio (19.5%) among three types of soil, presented the lowest styrene adsorbability. Bentonite, which have the higher clay ratios do not present high styrene adsorbability. Guo and Mulligan (2006) have found sand presented low styrene adsorbability, the maximum styrene adsorbed onto Ottawa sand was 24 mg/kg in batch experiment. The sorption capacity is found to be strongly dependent on the amount of organic matter and clay rations present in the soil. Different conceptual sorption mechanisms are shown in Table 4-3. The possible sorption mechanisms include styrene absorbing on to the organic material of the soil. Styrene adsorbs by hydrogen bonds to mineral and organic soil surfaces and styrene adsorbs by van der Waals interactions to organic and mineral soil surfaces (Thomas, 2005).

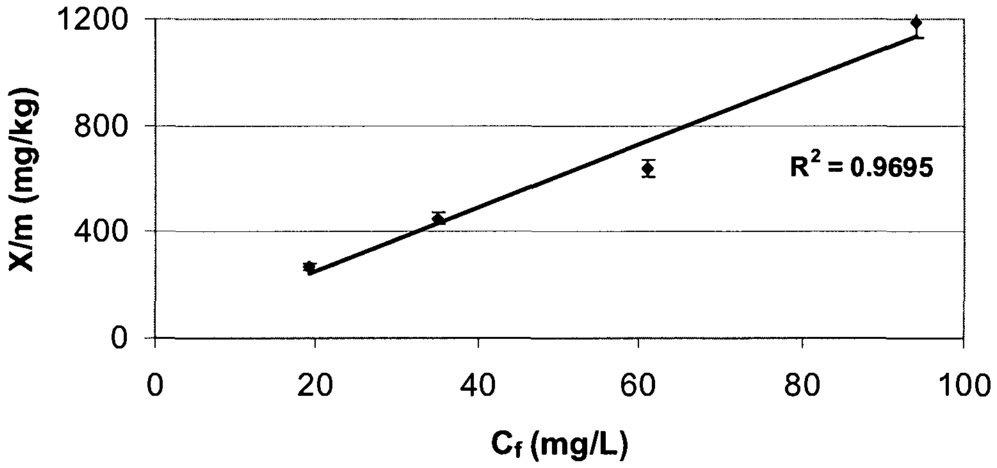


Figure 4-1. Adsorption isotherm for styrene on soil type 1

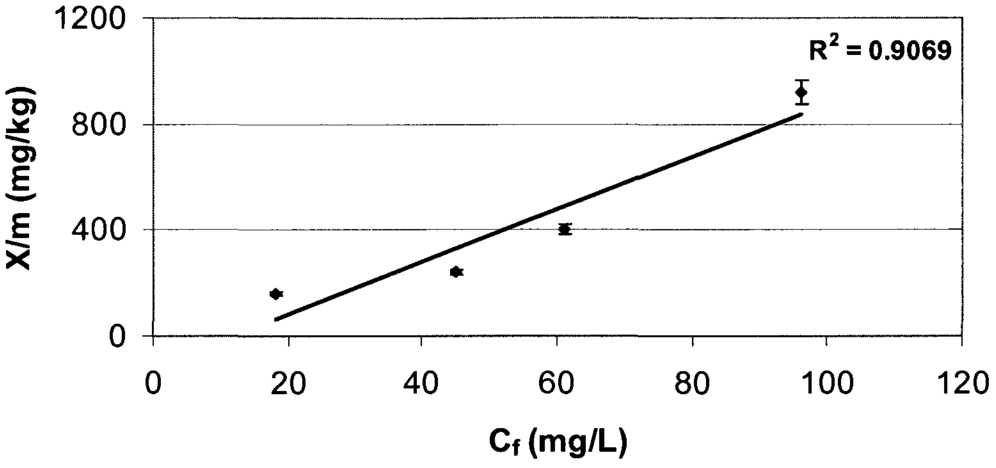


Figure 4-2. Adsorption isotherm for styrene on soil type 2

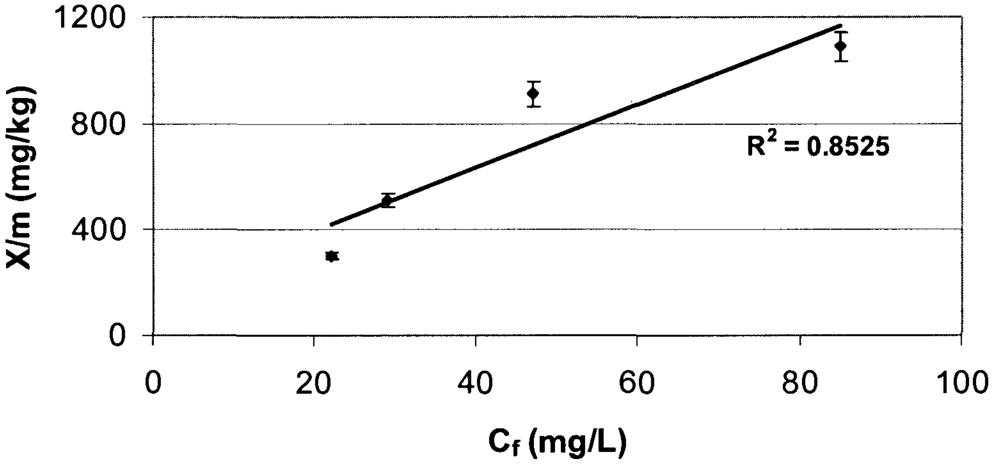


Figure 4-3. Adsorption isotherm for styrene on soil type 3

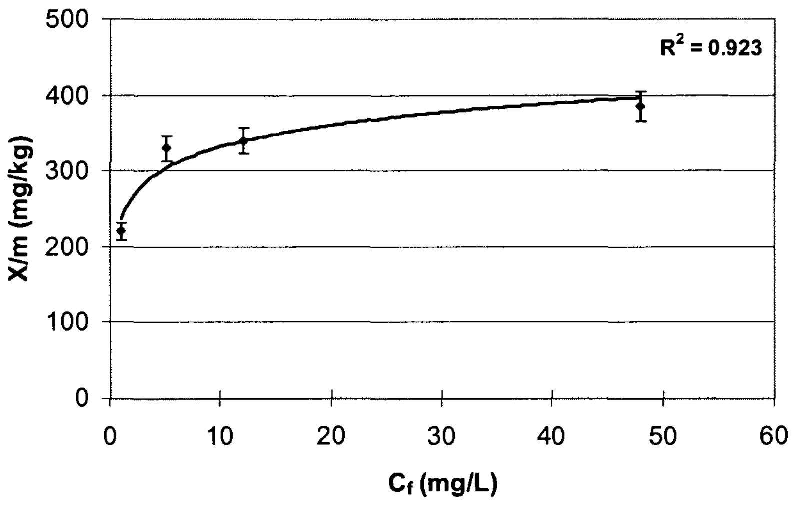


Figure 4-4. Adsorption isotherm for of styrene on bentonite

The various sorption processes that contribute to bonding between organic pollutants and soil fractions include partitioning (hydrophobic bonding) and accumulation through adsorption mechanisms involving the clay minerals and other soil particulates such as carbonates, organic and amorphous materials. The more prominent properties affecting the fate of organic molecules by soil fractions include the following (Yong and Mulligan 2004):

- Soil fractions: surface area, nature of surfaces (composition of surface fractions), configuration of reactive surface.
- Organic chemical molecules: functional groups, structure, charge, size, shape, flexibility, polarity, volatility, water solubility, hydrophobicity.
- Soil environment: temperature, inorganic/organic ligands available, pH, salinity and physical gradients

Table 4-3. Different conceptual sorption mechanisms (Thomas, 2005)

Sorption mechanism	**Co-varying sorbate and sorbent properties**
Neutral sorbate escapes water and absorbs in to the organic material of the soil	Octanol water partition coefficient Aqueous solubility Molecular weight / size
Neutral sorbate adsorbs by van der Waals interactions to organic and mineral soil surfaces	Surface of sorbate
Neutral sorbate adsorbs by hydrogen bonds to mineral and organic soil surfaces	Hydrogen accepting and / or hydrogen donating properties of sorbent and sorbate
Charged sorbate adsorbs by non-specific electrostatic attraction (outer sphere complexation) or specific (ionic) bonding (inner sphere complexation) to mainly mineral soil surfaces with to oppositely charged surface groups	Charge of sorbate Charge of sorbent Ionic strength Specific ionic sites on sorbate and Specific ionic sites on sorbent
Reactive moiety of sorbate covalently bonds with reactive moiety of mineral or organic sorbent	Reactive groups on sorbate Reactive groups on sorbent

Several mathematical models have been developed to quantitatively express the relationship between the extension of sorption and the residual solute concentration. The most widely used models are the Freundlich adsorption isotherm and the Langmuir adsorption isotherm model.

- Freundlich adsorption isotherm model

The Freundlich isotherm, which is an empirical formulation, is expressed as:

$$X/m = KC_f^{1/n} \tag{4-1}$$

Where,

X/m: amount of styrene adsorbed per unit weight of the soil, (mg/kg)

C_f: styrene equilibrium concentration (mg/L)

K and n: experimental constant, K is an indication of the adsorption capacity of the soil; n indicates the effect of concentration on the adsorption capacity and represents adsorption intensity. If the data conforms to the Freundlich model, a plot of log C versus log (X/m) should give a straight line. $\mathrm{Ln}(X/m) = \mathrm{Ln}\,K + (1/n)\,\mathrm{Ln}\,C_f$ [4-2]

As shown in Figures 4-5 to 4-8, the Freundlich isotherm model did fit batch test results very well except bentonite. The Freundlich isotherm model does not indicate a finite uptake capacity of the sorbent, the surface concentration of adsorbate does not approach a saturation value as C increases, and therefore it will frequently represent the adsorption equilibrium over a limited range of solute concentration.

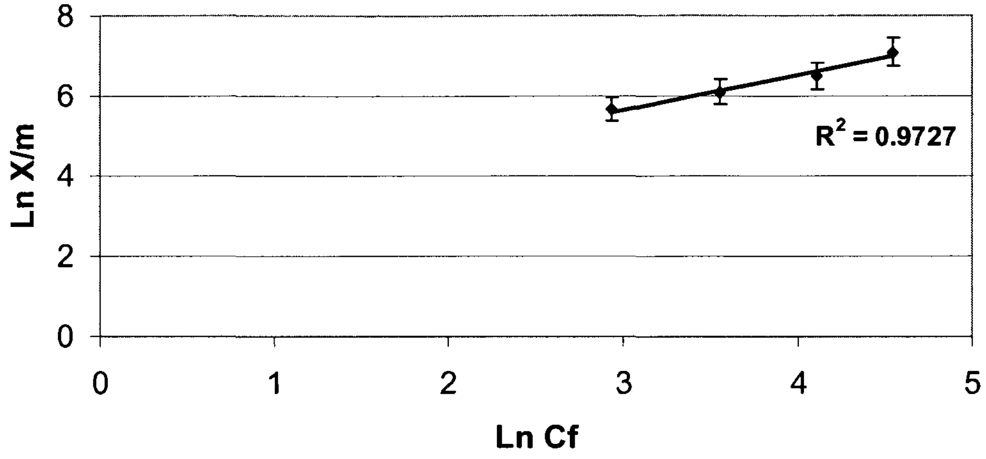


Figure 4-5. Freundlich isotherm for styrene with soil type 1

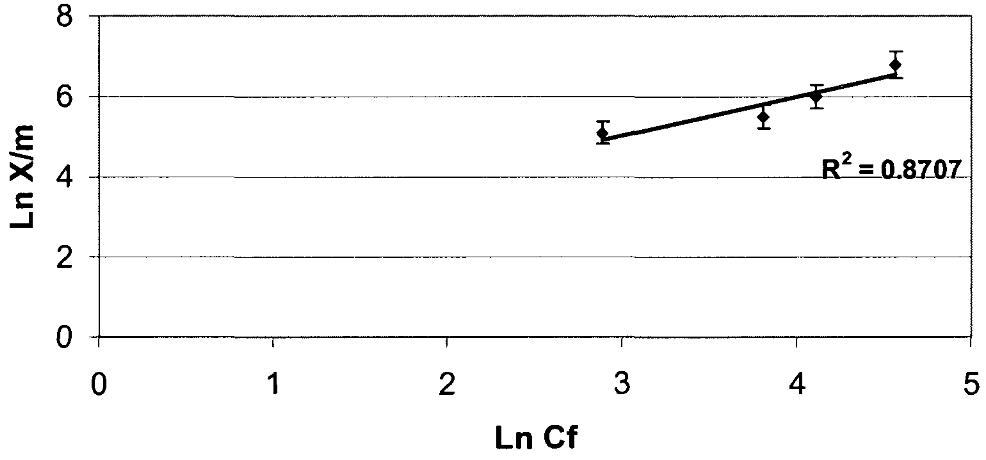


Figure 4-6. Freundlich isotherm for styrene with soil type 2

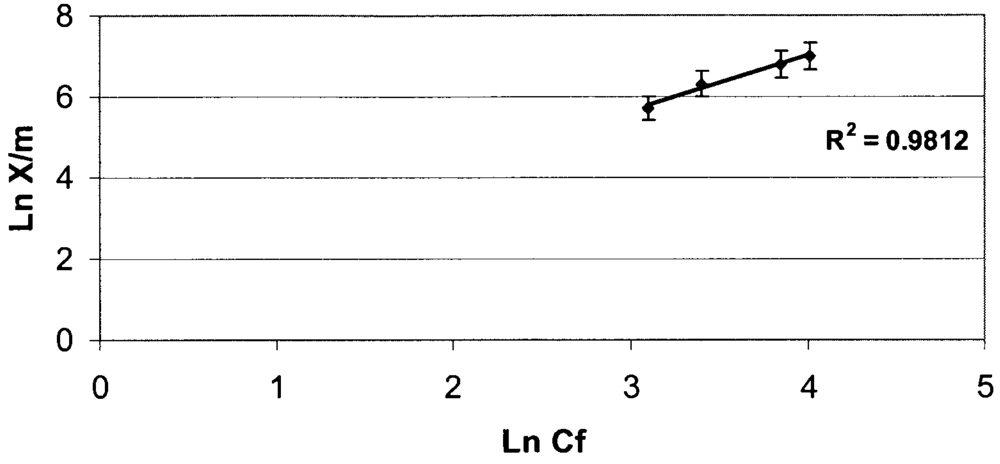


Figure 4-7. Freundlich isotherm for styrene with soil type 3

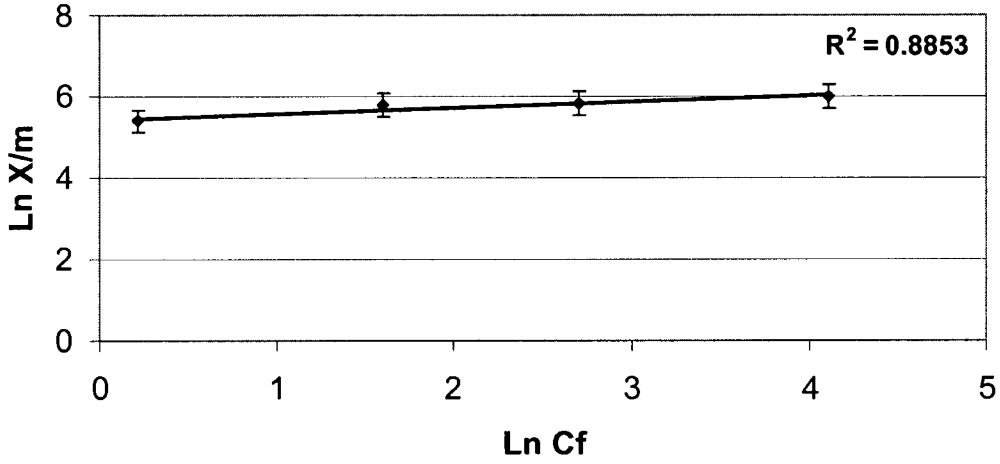


Figure 4-8. Freundlich isotherm for styrene with bentonite

- Langmuir adsorption isotherm

The Langmuir isotherm was constructed by plotting C_f /(x/m) against C_f and if the data are linear, the Langmuir model can be considered to be appropriate. The model could be presented as $C_f/(X/m) = 1/b(X/m)_{max} + Cf/(X/m)_{max}$ [4-3]

Where:

X/m: amount of styrene adsorbed per unit weight of soil, (mg/g)

C_f: styrene equilibrium concentration.

$(X/m)_{max}$: maximum uptake capacity of the soil, (mg/g)

b: adsorption equilibrium constant ($K_{adsorption}/K_{desorption}$)

Figures 4-9 to 4-12 present the Langmuir adsorption isotherms for three types of soil and bentonite. The Langmuir adsorption isotherm model provides a simple mechanistic picture of the adsorption process and gives rise to a relatively simple mathematical expression. The Langmuir model assumes that the rate of adsorption is proportional to the concentration of the solute in the fluid phase and to the adsorbent surface.

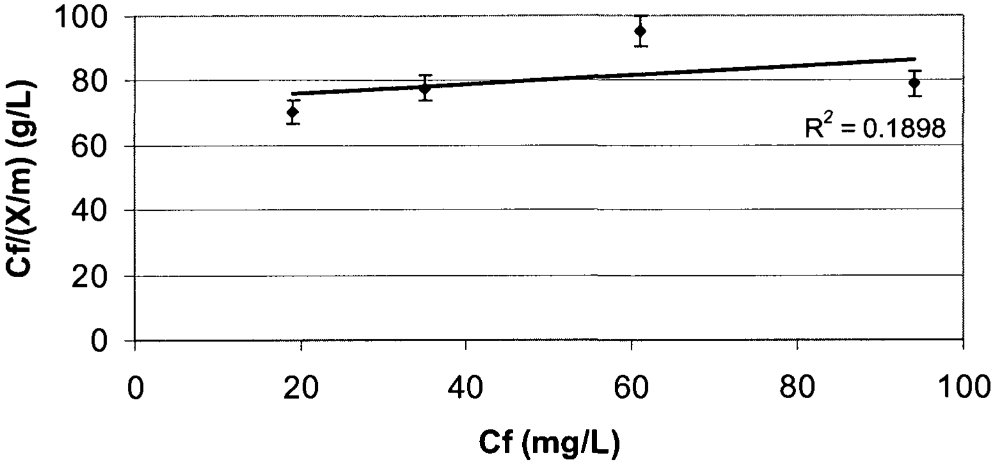


Figure 4-9. Langmuir isotherm for styrene with soil type 1

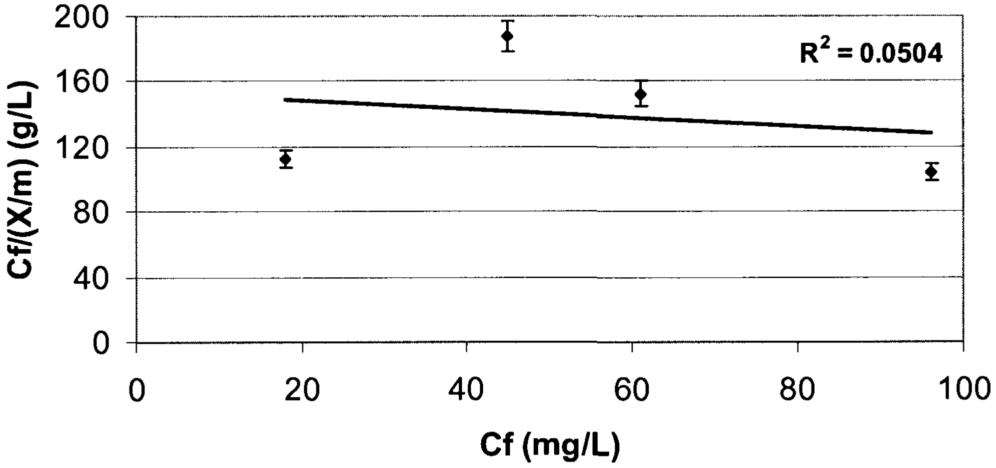


Figure 4-10. Langmuir isotherm for styrene with soil type 2

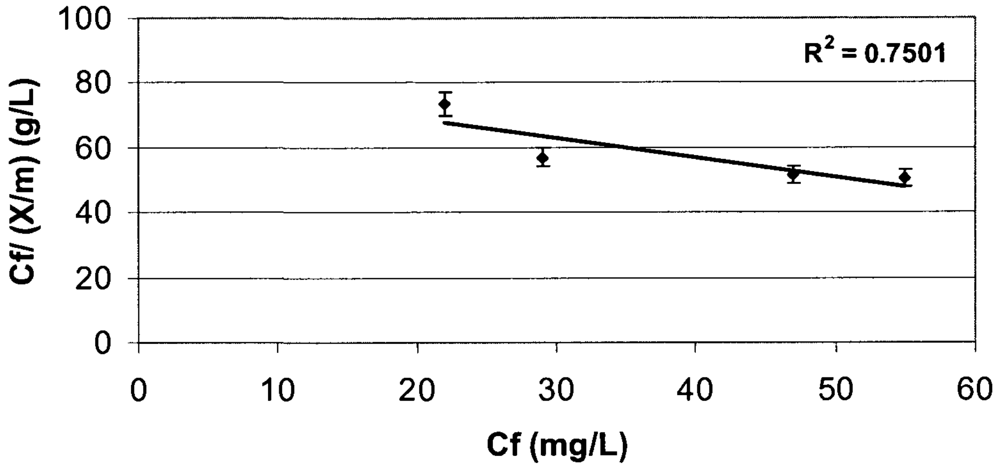


Figure 4-11. Langmuir isotherm for styrene with soil type 3

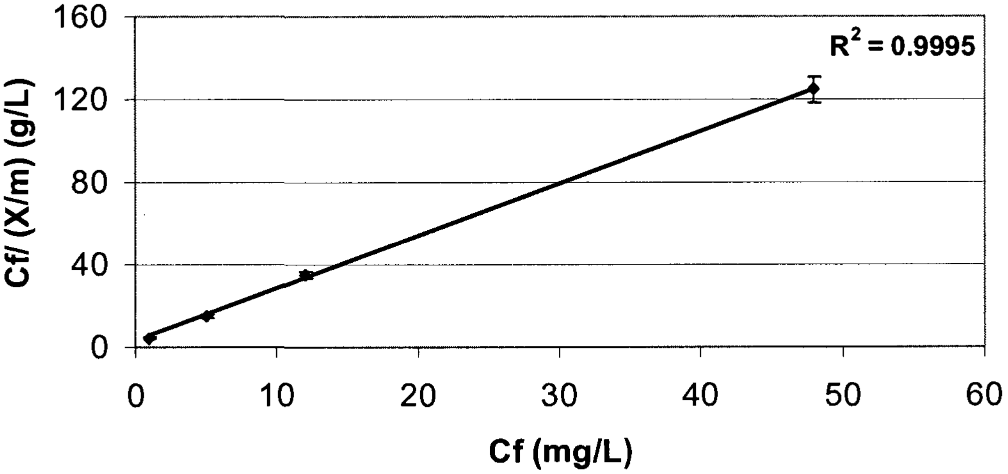


Figure 4-12. Langmuir isotherm for styrene with bentonite

Although the Langmuir and Freundlich isotherms were first introduced about 70 years ago, they still remain the two most commonly used adsorption isotherm equations. Adsorption coefficients of three types of soil and bentonite are listed in Table 4-4. As can be seen, for soil type 1, the Freundlich equation gave the best fit to the adsorption data with a goodness-of-fit (R^2) equal to 0.9727, as compared to 0.1898 and 0.9695 for the Langmuir and linear equations, respectively. This is because linear and Langmuir adsorptions typically occur only at low sorbate concentrations in solution and low loading onto sorbent surfaces. For Freundlich isotherms, n indicates the effect of concentration on the adsorption capacity and represents adsorption intensity (Yong and Mulligan, 2004). Values of $n < 1$ are usually termed as unfavourable sorption since there are greater amounts of solute in the solution than are adsorbed. Likewise, a system with $n > 1$ is said to be favourable sorption. This isotherm with high n value reflecting a relatively high affinity between the solute and the soil and is usually indicative of chemisorption (Taha *et al.*, 2003). The experimental constant K_F is an indication of the adsorption capacity of the adsorbent. Soil type 1 has the highest K_F (16.7 L/mg), and thus presents the highest styrene adsorption capacity among the three types of soil.

For the linear isotherm, soil type 1 has the highest K value (11.96 L/mg) and soil type 2 has the lowest K value (9.98 L/mg). It confirmed that soil type 1 has the highest styrene adsorbability since the higher K value indicates stronger adsorption capacity.

Table 4-4. Linear (*K*), Langmuir, and Freundlich (K_F) adsorption coefficients with different types of soil

Soil type	Linear isotherm		Freundlich isotherm			Langmuir isotherm
	K (L/mg)	R^2	K_F (L/mg)	1/n	R^2	R^2
Soil type 1	11.96	0.9695	16.7	0.95	0.9727	0.1898
Soil type 2	9.98	0.9069	3.7	2.32	0.8707	0.0504
Soil type 3	11.84	0.8525	4.1	1.45	0.9812	0.7501
Bentonite	N/A	0.9230	212	0.19	0.8853	0.9995

Bentonite behaved differently than the sandy soils in the adsorption tests. Langmuir isotherms cannot adequately describe the adsorption data particularly with poor R^2 values (from 0.0504 to 0.7501) for three types of soil but fit for bentonite. The Langmuir isotherm fit for bentonite with a R^2 equal to 0.9995. The Langmuir isotherm is based on the concept that solid surfaces have finite adsorption sites. When all the adsorption sites are filled, the surface will no longer be able to adsorb more of the solute from solution (Yong and Mulligan, 2004). As shown in Figure 4-4 for the adsorption of bentonite, the initial uptake increases were linear. Uptake is eventually limited by the fixed number of active sites and a resulting plateau can be observed at a higher concentration.

From the batch tests results, soil type 1 presented the highest styrene adsorbability and bentonite presented the lowest styrene adsorbability. Soil 3, which has the highest organic matter content (17.5%), presents the second high styrene adsorbability. Soil type 1 has 1.6 time bigger specific surface area (4.0 m^2/g) then soil type 3 (2.5 m^2/g). As clay presented the biggest surface area, soil type 1 has higher clay ratio then soil type 3. Bentonite and soil type 2, which have the higher clay ratio and lower organic matter content do not present high styrene adsorbability. The sorption capacity is found to be strongly dependent on the amount of organic matter and silt and clay ratio presented in the soil. Headley *et al,* (2001) conclude that the sorption capacity is found to be strongly dependent on the amount and type of organic matter present in the soil. For adsorption of benzene and toluene, the organophilic clay (organic carbon content f_{oc}=0.31) presented higher adsorbability then Sarnia clay (f_{oc} =0.005-0.008). The organophilic clay was 15-20 times more adsorptive than Ottawa sand (Headley *el al*, 2001).

4.3.2 Column adsorption test

In the column test, all the soil materials were placed in the glass column and both ends covered with rubber stoppers. A quantity of 450 g of soil was transferred into the column; 12.5 mL styrene and 100 ml distilled water were added. The columns were covered with aluminium foil to prevent styrene exposure to light. This test was used to study the adsorbability of the three types of soil after 90 days and further studies were done for soil 1 over a period of 16 months.

From Figure 4-13, it is obvious that the clay ratio and organic matter played important roles in the styrene adsorption test. According to the mass balance study, soil 2 presented the highest adsorbability, 37% of styrene remained in the soil system. 10% of styrene remained in the soil 3 and 34% styrene remained in soil 1. For soil 1, styrene remained in the 4-8 cm zone, at a high concentration from 700 mg/kg to 47,000 mg/kg (Figure 4-12). A mass balance study was made for soil type 1 and styrene concentration at 47,000 mg/kg. 0.1% of styrene from 47,000 mg/L was dissolved in water phase, 2.5% of styrene was adsorbed onto soil type 1 and the rest 97.4% of styrene was stay as pure phase. For soil 2, styrene remained in the 2-10 cm zone, at a high concentration from 3140 mg/kg to 35,436 mg/kg. For soil 3, styrene remained in the column quite evenly from the top to 14 cm at a concentration from 1540 mg/kg to 3967 mg/kg. Soil 1 in column adsorption test presented the highest styrene adsorbability that was also shown in the batch tests. However, what was different from the batch test was that soil 3 in the column test presented the lowest styrene adsorbability.

The possible mechanisms responding to the loss of styrene include, first, volatilization of styrene to the column headspace. According to the CEPA, the half-life for volatilization of styrene from soil surfaces was estimated to be approximately 1 minute, with the rate of volatilization decreasing with increasing depth (CEPA, 1993). Secondly, styrene may also have biodegraded within the soil system. Thirdly, the rubber stopper used in column test may adsorb styrene as well.

Figure 4-14 presents the styrene with soil 1 in the column adsorption test after 16 months. There was 0.1% of the styrene still remaining in the soil. The highest styrene concentration in column was 79 mg/kg in the 3 cm from the top. The styrene concentration in column was quite even, ranging from 60 mg/kg to 79 mg/kg. All columns were sealed at both ends with rubber stoppers to prevent oxygen transfer into the columns. This column study tried to simulate anaerobic conditions to determine the concentration of styrene at different depths. Both column adsorption tests showed that styrene may persist in the soil system for a long time.

Fu and Alexander (1992) determined that styrene buried in soil can leach into the underlying ground water. Styrene that leaked into the surrounding soil from buried drums persisted in the soil for up to two years. "The extent of adsorption of sparingly water-soluble compounds is often correlated with the organic carbon content of the adsorbent. In the surface soil, where the amount of organic carbon will be highest, the movement of styrene will be retarded by adsorption. In deeper subsurface environments where the amount of organic carbon may be low, adsorption may not be as significant. Based on field measurements, the rate of movement of styrene in an aquifer was about 80 times slower than that of the groundwater, which is attributed to adsorption" (ATSDR, 1992).

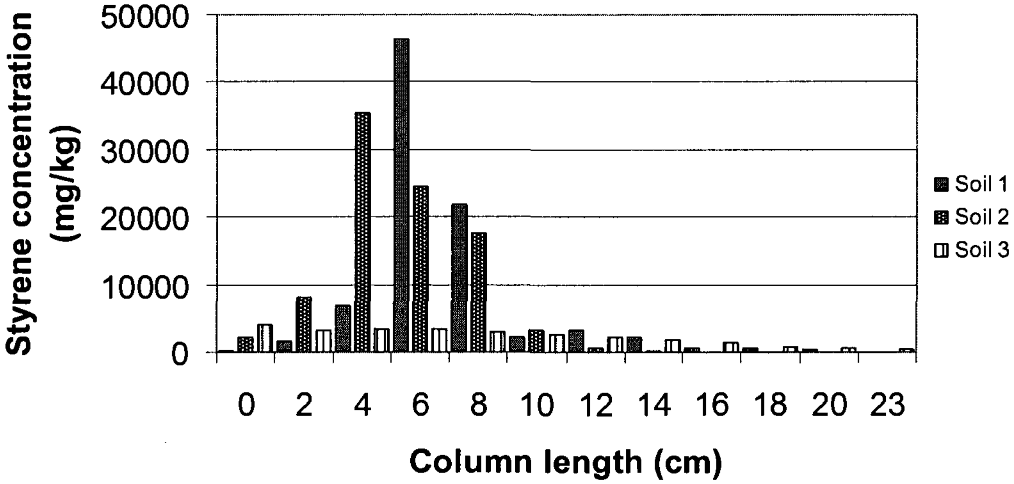


Figure 4-13. Styrene concentrations in a test column after 90 days

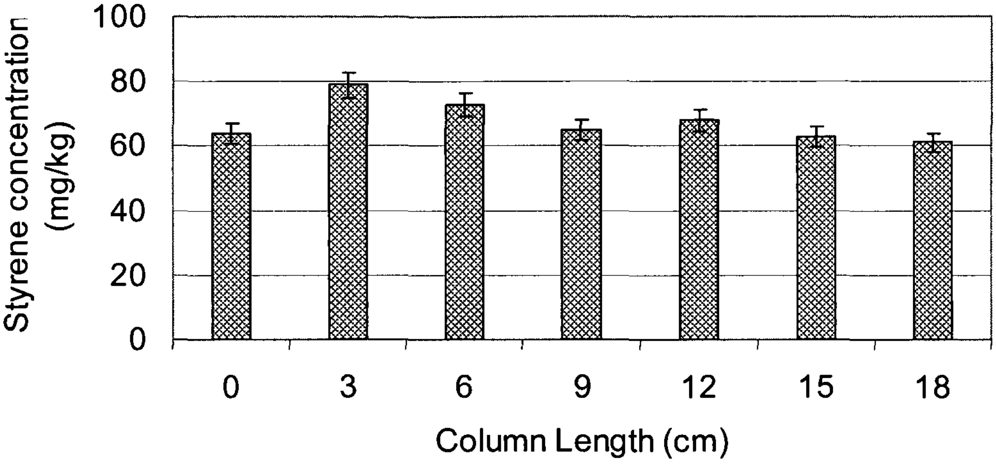


Figure 4-14. Styrene concentrations in a test column after 16 months

4.4 Critical Micelle Concentration (CMC)

Prior to deciding on the biosurfactant concentration, it is vital to identify the critical micelle concentration (CMC) of the biosurfactant. By increasing the biosurfactant concentration, the surface tension decreases until it reaches the CMC. The CMC refers to the minimum concentration of the biosurfactant required for the formation of micelles. The CMC value for rhamnolipids was found to be 20 mg/L (Figure 4-18). The result was close to the previously reported rhamnolipid CMC value by Zhang and Miller (1992) and Wang and Mulligan (2004) of 30 mg/L.

4.5 Styrene solubilization in rhamnolipid

The solubilization of styrene for various concentrations of rhamnolipid was determined and the results are presented in Figure 4-15. Rhamnolipid concentrations ranged from 10 mg/L to 3000 mg/L. Styrene solubility increased as the rhamnolipid concentration increased. At a 3000 mg/L rhamnolipid solution, styrene solubility reached 8427 mg/L, which is 27 times higher than in water (310 mg/L, EPA-OPPT, 1994). The results indicated that rhamnolipid could enhance styrene solubility.

4.6 Octanol/water partitioning study

The octanol-water partition coefficient is the ratio of the concentration of a chemical in octanol and in water at equilibrium. In general, smaller molecules can be more polar molecules that dissolve more rapidly in water, have lower K_{ow} values, and have less tendency to sorb to solids; large molecules and less polar molecules are less soluble, have higher K_{ow} values and are more likely to sorb to solids.

$$K_{ow} = \frac{C\text{octanol}}{C\text{water}} \quad [4\text{-}4]$$

The K_{ow} values for styrene in rhamnolipid are shown in Figure 4-16. Rhamnolipid concentrations ranged from 50 mg/L to 300 mg/L. The reason for not choosing higher concentrations of rhamnolipid was to avoid emulsions from being formed during the experiment. When emulsions form, this decreased the ability of rhamnolipid to transfer styrene from water to the octanol phase. More styrene was transferred to the octanol phase as the rhamnolipid concentration decreased.

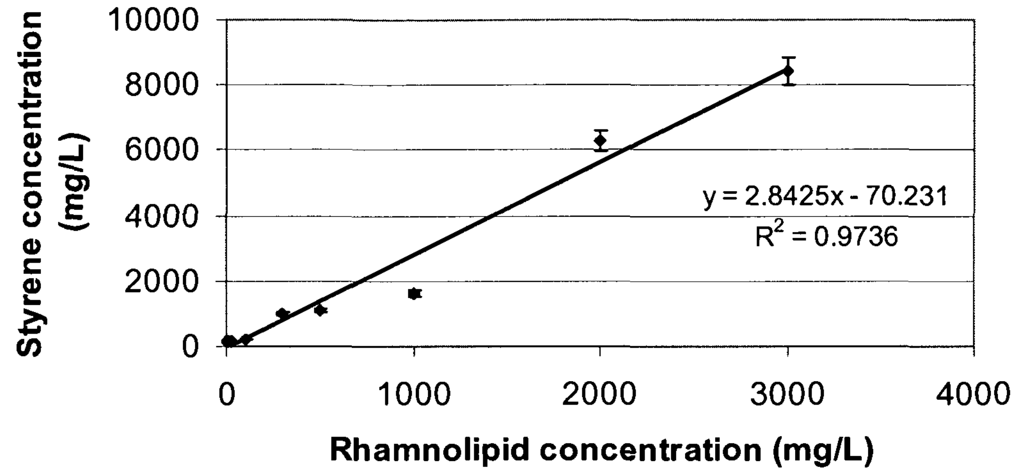


Figure 4-15. Solubilization of styrene in rhamnolipid solution

The rhamnolipid structure was introduced in Chapter 3. The rhamnolipids used in this study were a mixture of R1 and R2 (R2/R1=1.1). The structural formulas of the pure Components R1 [_α-l-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoate] and R2 [2-O-α-l-rhamnopyranosyl-α-l-rhamnopyranosyl-β-hydroxydecanoyl-β hydroxydecanoate] are shown in Figure 2-9. The molecular weight (MW) of R1 is 504 g /mol and that of R2 is 650 g /mol (Ozdemir and Sezgin, 2006). HPLC was used in this study to determine the correlation of R1 and R2 with the rhamnolipid concentration. R2, which appears at 1.90 min from HPLC, proportionally increased with the rhamnolipid concentration. R1 was shown at 0.85 to 1.3 min from the HPLC and had no correlation with the total rhamnolipid concentration.

The correlation of R2 with K_{ow} was shown in Figure 4-17 in the styrene octanol/water partitioning study. The K_{ow} value decreased as the R2 concentration increased which means more styrene has been transferred to the water phase as the rhamnolipid concentration increased. It showed that rhamnolipid could enhance styrene solubility in the water phase. Furthermore, working with styrene, R2 is the main functional component in the rhamnolipid.

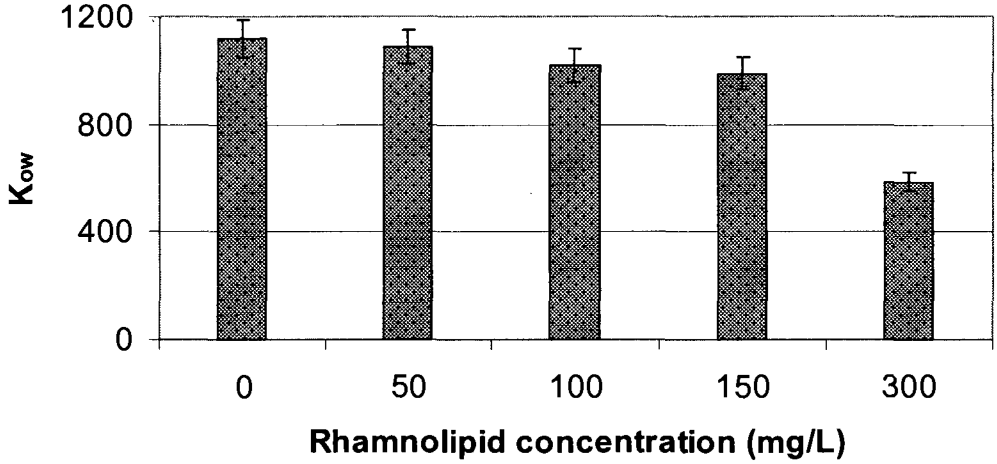


Figure 4-16 K_{ow} values for styrene in rhamnolipid

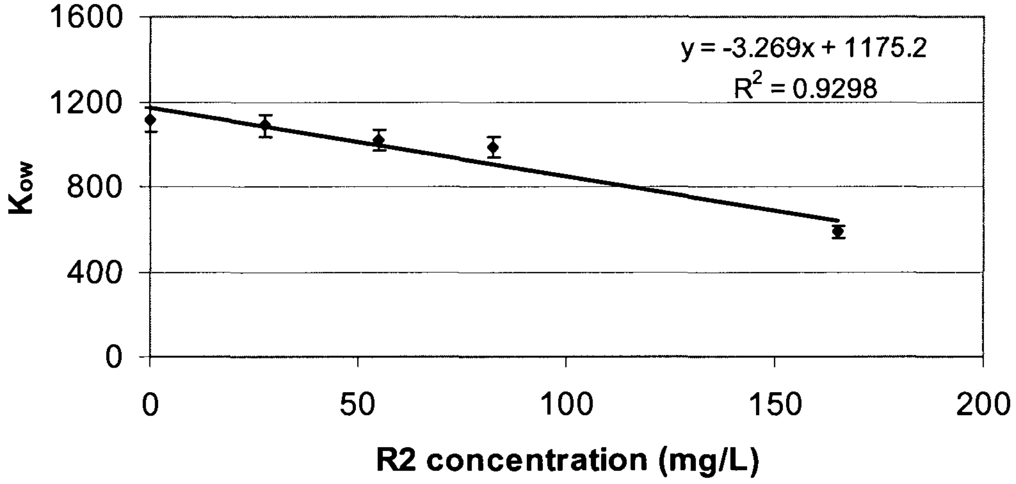


Figure 4-17. Rhamnolipid R2 concentration with K_{ow}

4.7 Interfacial tension

The interfacial tension between styrene and distilled water was measured as 19.5 mN/m. as the rhamnolipid solution was introduced. This value was shown in Figure 4-18. The reduction of interfacial tension indicates the ability of rhamnolipid to remove styrene from soil. As the interfacial tension between styrene and water is reduced, the capillary force holding the styrene and soil is equally reduced. Therefore, this reduction will increase the contact angle and the reduction of the capillary force holding styrene and soil together which result in the mobilization of styrene. Since interfacial tension is concentration dependent on the rhamnolipid concentration increases, the interfacial tension between styrene and rhamnolipid also reduces. Uram and Pekdmir (2004) reported that rhamnolipid could also reduce the interfacial tension between crude oil and distilled water. According to this characteristic, rhamnolipid shows potential for styrene or oil removal from the soil.

Correlation of surface and interfacial tensions of rhamnolipid in styrene was presented in Figure 4-19 and a similar decreasing trend was observed before the CMC. They are two possible mechanisms existing for surfactant treatment of contaminated soils. At low concentrations, surfactants are present as individual molecules (monomers) and concentrate at interfaces. The interface can be at the free solution surface (liquid/gaseous interface), at the container wall (solid/liquid interface), or at the solution and an immiscible fluid boundary (liquid/liquid interface) (Zajic and Seffens, 1984). By replacing the bulk molecules at the interfaces, surfactants reduce surface and interfacial tensions, and facilitate the formation of emulsions between liquids of different polarities

(Mulligan, 1998). An emulsion is the dispersion of a liquid in another immiscible liquid in the form of droplets with diameters of at least 0.1 μm (Mulligan and Gibbs, 2004). The reduction in interfacial tension also lowers the capillary forces, reduces the contact angle, and increases the mobility of the contaminant (Urum and Pekdemir, 2004; Mulligan *et al.*, 2001). The reduction in the surface tension is proportional to the increase in the concentration of the surfactant up to a level known as the critical micelle concentration (CMC) (Mulligan, 1998). Increasing the concentration of surfactants above the CMC has no effect on the surface tension of the solution, instead surfactants monomers start to form aggregates know as micelles (Mulligan, 2005). Thus, the CMC is also referred to as the maximum concentration of surfactant monomers (Mulligan and Gibbs, 2004). Formation of micelles (concentration above CMC) results in the partitioning of the hydrophobic compounds into the hydrophobic core of the micelle. Hydrophobic contaminants are thus dispersed in the aqueous phase above their solubility limits (Pennell *et al.*, 1993) and increasing their bioavailability. Surfactants may also enhance the mobility and removal of heavy metals from soil by complexation (Dahr Azma, 2005; Mulligan, 1998).

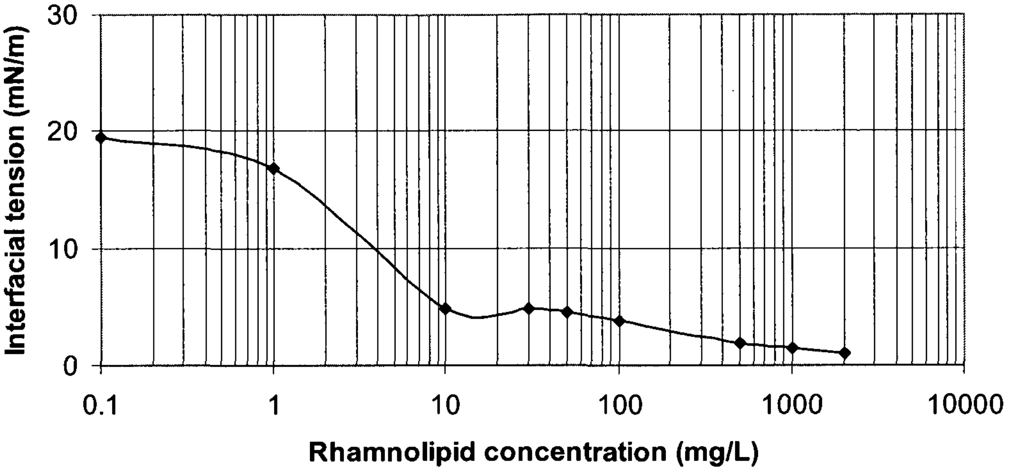


Figure 4-18. Interfacial Tension of styrene in rhamnolipid solution

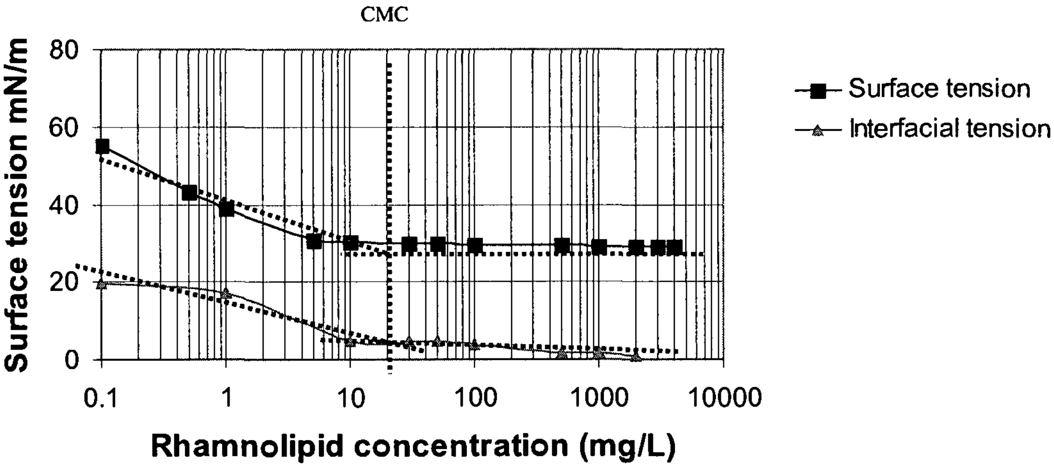


Figure 4-19. Correlation of surface and interfacial tension of rhamnolipid in styrene solution

4.8 Zeta potential measurements of soil with rhamnolipid

Zeta potential is the electrical potential that exists at the "shear plane" of a particle, which is some small distance from its surface. Zeta potential was used to determine the interaction of the rhamnolipid and the soil inter-phase. The zeta potential of soil is negative as seen in Figure 4-20. Measurements were then made on the soil in the presence of rhamnolipid at a concentration above the CMC. Zeta potential presented a significant decrease when the rhamnolipid concentration was increased (Figure 4-20). This would indicate that the adsorption of rhamnolipid is occurring onto the soil. As mentioned in Chapter 2 and Chapter 3, rhamnolipid is an ionic surfactant, if a small amount of rhamnolipid is added into the distilled water, the potential of the shear plane increases. With increased rhamnolipid concentration, more rhamnolipid was adsorbed at the soil surface. The accumulated rhamnolipid molecules over the surface form a shear plane at a further distance relative to the actual surface. Since the potential is inversely proportional to the square of the distance, the zeta potential decrease is observed (Dahr Azma 2005)

Mulligan *et al.* (2001) have postulated that surfactant adsorption is essential for removal of soil contaminants since surfactants that adsorb at the solid–water or soil–water interphases are better detergents. Sorption of the biosurfactants onto the soil interphase followed by metal desorption by complexation with the biosurfactants would be the first step in the metal removal mechanism. In the case of styrene, sorption of rhamnolipid onto the soil interface followed by increased styrene solubility and a lower interfacial tension due to the rhamnolipid would be the first step in the styrene removal mechanism.

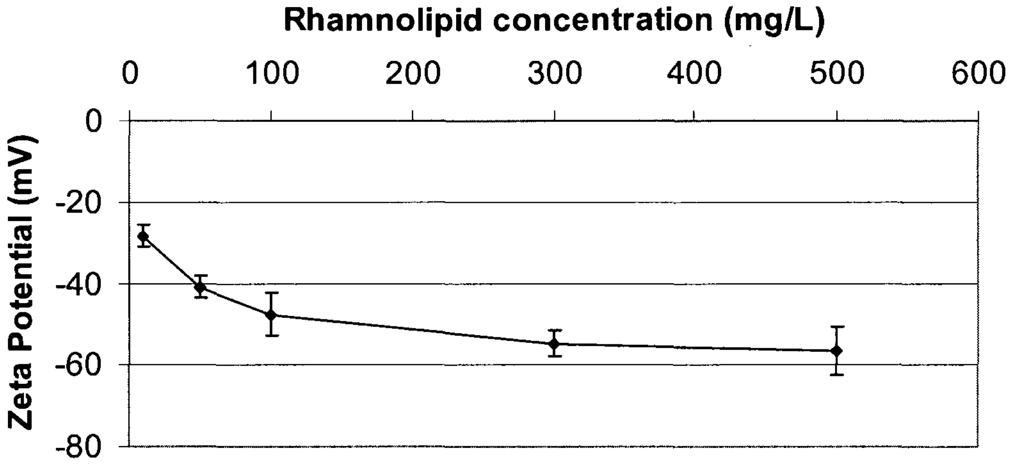


Figure 4-20. Zeta potential of soil with rhamnolipid

4.9 Batch removal experiments

In this study, the pH was set at 7.2 (except for the pH study) because the rhamnolipid used for this study has a pH close to 7.2. Moreover, rhamnolipid precipitates at a pH less than 5.5. All samples were placed on the orbital shaker set at 60 rpm for 24 hours to provide full contact between the soil and washing solution (Wang, 2007). The initial styrene concentration was chosen to be 4440 mg/kg for this study (except for the styrene concentration study test). A styrene distribution study was made according to batch adsorption tests. 27% of styrene from 4440 mg/L was adsorbed onto soil type 1, 1.4% of styrene was dissolved and the 71.6% of styrene was stay as pure phase in the soil system. The rhamnolipid concentration of 300 mg/L was chosen. The preliminary tests showed that although a higher rhamnolipid concentration may provide better removal, the viscosity of the rhamnolipid solution is impractically high at a 5000 mg/L concentration. Therefore to economize the amount of biosurfactant used, 300 mg/L rhamnolipid was chosen in this study. All samples in this study were analyzed after 24 hours except for the time study based on the preliminary tests.

4.9.1 Effect of rhamnolipid concentration

The result of styrene removal by rhamnolipid at different concentrations is illustrated in Figure 4-21. In this washing test, the initial styrene concentration was 4440 mg/kg; the styrene removal increased with increased rhamnolipid concentrations and the highest removal of 94% was achieved with 1% rhamnolipid. Figure 4-22 presents the amount of removed styrene from soil with added rhamnolipid in solution. For each gram of rhamnolipid added in solution, 0.1 gram of styrene was removed from soil. A 1 to 10 relation was achieved from batch washing experiments. From previous study, the interfacial tension between rhamnolipid and styrene decreased as the rhamnolipid concentration increased. As the interfacial tension between styrene and rhamnolipid is reduced, the capillary force holding the styrene and soil is reduced. Therefore, this reduction will increase the contact angle and the reduction of the capillary force holding styrene and soil together which results in the mobilization of styrene. It was determined that to avoid adsorption, biosurfactant concentrations higher than the CMC should be used to limit interfacial hydrophobic adsorption of surfactant aggregates such as hemimicelles to the soil.

4.9.2 Effect of pH

It can be observed that in Figure 4-23, as the pH value increased from 6 to 10, batch washing removal results ranged from 73.9% to 62.6% when using 300 mg/L rhamnolipid as washing solution and styrene concentration at 4440 mg/kg in soil. The best styrene removal of 73.9% was achieved at pH 6. Little difference of styrene removals were achieved from four different pH values. It indicated that pH is not a main factor in styrene removal experiments. Unlike metal removal with rhamnolipid, styrene has no charge period in rhamnolipid solution. Styrene solubility doesn't vary with pH values. Heavy metals such as copper, rhamnolipid removal copper from contaminated sediment curve follows the pattern as the variation of solubility of metal hydroxide with pH. The minimal removal of copper was achieved where the solubility of dissolved copper is at its minimum point (pH 9) (Dahr Azma *et al.* 2007).

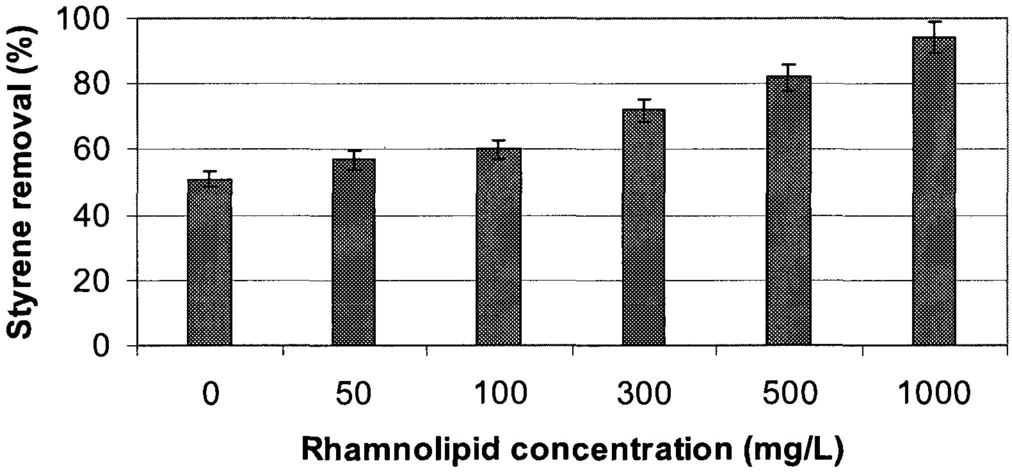


Figure 4-21. Styrene removal at different concentrations of rhamnolipid in batch tests

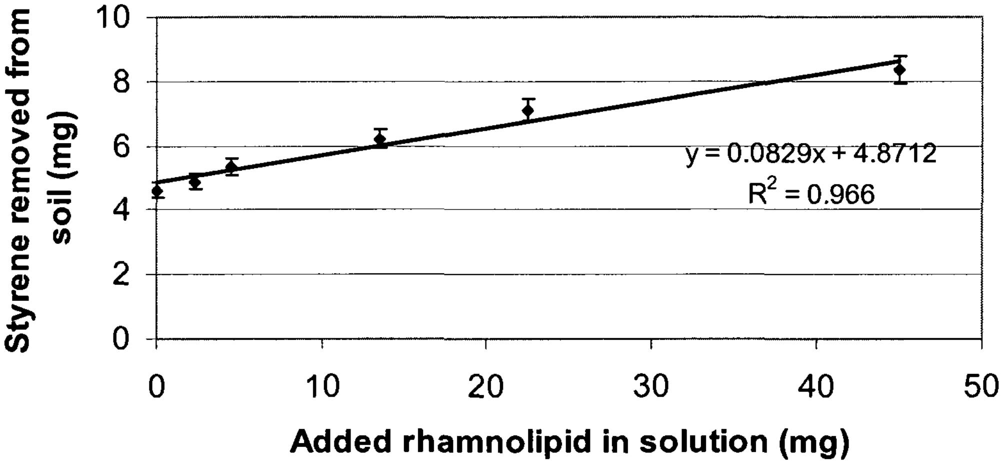


Figure 4-22. Amount of styrene removed from soil with varying amount of rhmanolipid added in batch washing experiments.

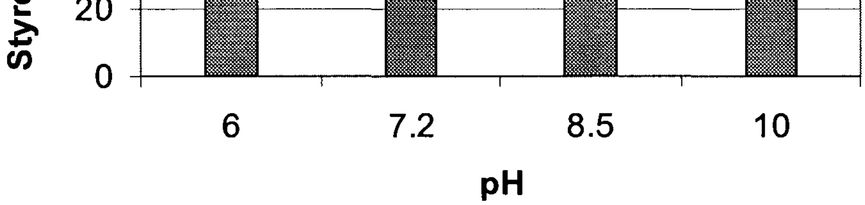


Figure 4-23. Styrene removal at four different pH values in batch tests

4.9.3 Effect of styrene concentration

A series of experiments was performed using 300 mg/L rhamnolipid with different styrene concentrations (480 to 9080 mg/kg). With an increase in the styrene concentration, the rhamnolipid removal ability decreased (Figure 4-24). Styrene at 480 mg/kg has lower percentage removal than 2620 mg/kg styrene. This may be due to readsorption of styrene from the solution on to the soil. The amount of styrene removed with four different initial styrene concentrations is shown in Figure 4-25. As the initial styrene concentration increased, the amount of styrene removed from soil increased as well. 0.63 mg of styrene was removed from soil at an initial styrene concentration of 480 mg/kg. 11.4 mg of styrene was removed from soil at an initial styrene concentration of 9080 mg/kg. When initial styrene concentration increased 19 times, the amount of removed styrene also increased 18 times. A linear relationship was achieved at this time.

4.9.4 Effect of time

The effect of time was studied in batch tests. Daily washes preformed by changing the washing solution daily and single washes performed without changing the rhamnolipid solution. Daily washes and single washes were conducted in a batch washing study to evaluate the time factor on styrene removal efficiency. With a daily wash, removal increased to 93.5% starting at day 2 and the highest removal was achieved at 98.4% on day 3. After day 3, styrene removal became stable. A single wash achieved the highest removal at 79.1% (Figure 4-26) on day 3 and styrene removal decreased after day 3. This may be because styrene readsorbed to the soil in single washes with longer contact times.

A longer contact time did not increase removal efficiency in a single wash test. The rhamnolipid concentration was measured after both experiments. Rhamnolipid was adsorbed onto soil during the removal tests and the results of adsorbed rhamnolipid (%) along with time were illustrated at Figure 4-27. For single wash experiments, maximum rhamnolipid adsorption (15.3 %) was achieved from day 4. The trend of single washing illustrated the adsorption and desorption of rhamnolipid during the 5 day experiment.

For the daily wash experiment, the highest rhamnolipid adsorption (16.2%) was achieved from day 2. After day 2, the adsorption rate was decreased. Rhamnolipid showed adsorbability on soil in the daily wash experiment. Within five days of constant washing with a fresh rhamnolipid solution, more than 10% of rhamnolipid was adsorbed on to the soil daily. These results explained the removal of styrene from soil began with rhamnolipid adsorption onto the soil. No relationship was found between the adsorbed rhamnolipid versus styrene removal rate in both washing experiments.

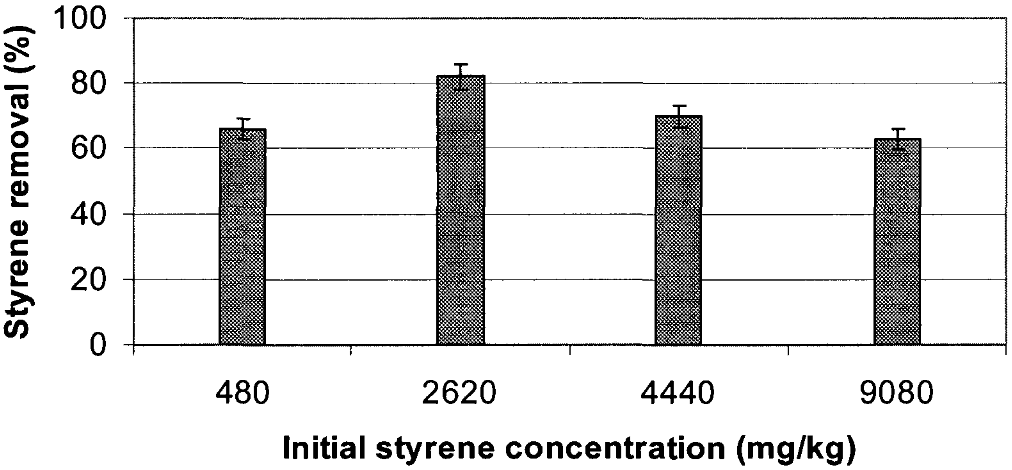


Figure 4-24. Styrene removal at four different styrene concentrations with 300 mg/L rhamnolipid in batch tests

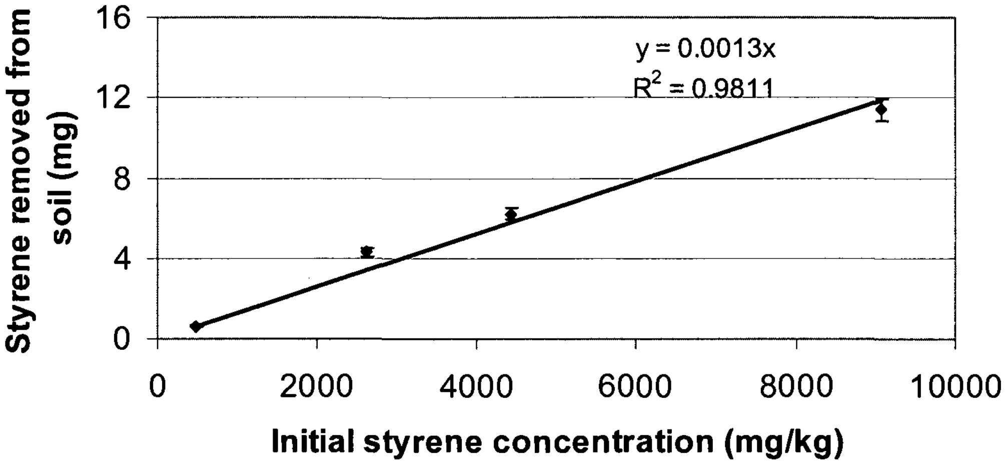


Figure 4-25. Amount of styrene removed from soil at different initial styrene concentration with 300 mg/L rhamnolipid in batch washing tests

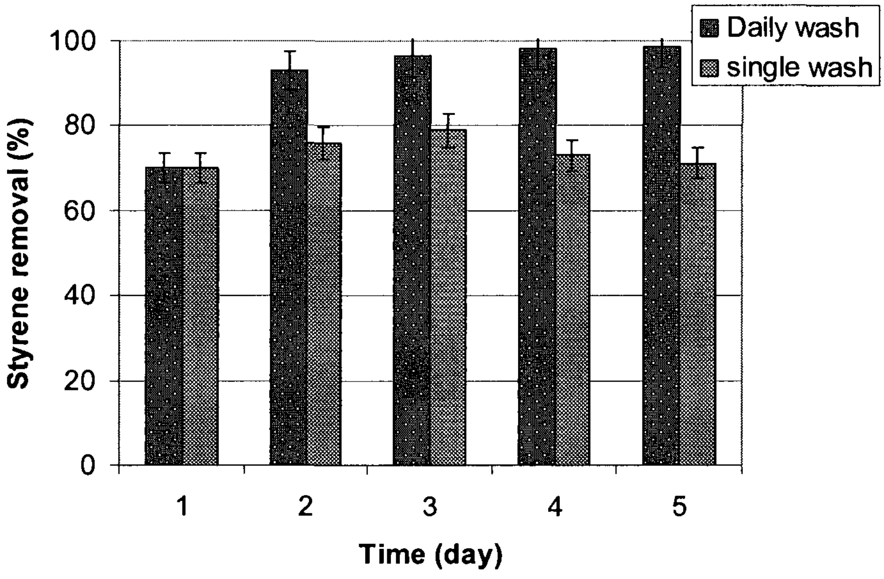


Figure 4-26. Styrene removal from contaminated soil (4440 mg/kg) with 300 mg/l rhamnolipid using a daily wash and a single wash versus time

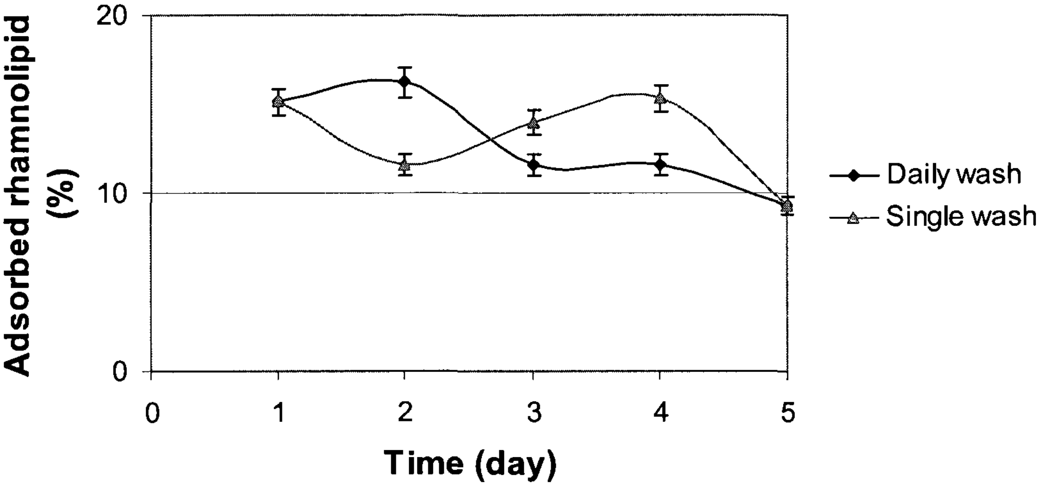


Figure 4-27. Adsorbed rhamnolipid onto soil in styrene removal tests (initial styrene concentration is 4440 mg/kg) with 300 mg/l rhamnolipid in a daily wash and a single wash versus time

4.9.5 Styrene removal with three types of surfactant

In this study, three surfactants were used to compare styrene removal efficiency. JBR-425 is an aqueous solution of rhamnolipid at 25% and JBR-215 an aqueous solution of rhamnolipid at 15% concentration. Rhamnolipids are able to remove metals and ions such as cadmium, copper, lanthanum, lead and zinc due to their complexation ability (Mulligan, 2004). By decreasing interfacial tension and increasing solubility, rhamnolipid are able to remove crude oil, pentachlorophenol (PCP) from soil by soil washing technology (Mulligan, 2004; Urum, 2004). S-405LF alkoxylated linear alcohol which has been widely used in mechanical dishwashing, metal cleaning, dairy equipment cleaning and pulp and paper additives is produced by BASF Corporation. Dibacco *et al.* (1997)

have reported that by adding S-405LF in the styrene degradation test, 75% of styrene could be degraded at 1000 mg/L of the initial concentration and 30% styrene was degraded without S-405LF. In this experiment, two surfactant concentrations were chosen at 300 mg/L and 1000 mg/L. Figure 4-28 illustrate the comparison results. JBR-425 performs the best styrene removal efficiency in both concentrations. It is interesting to find that at the same rhamnolipid concentration, JBR-425 with JBR-215 presented different styrene removals. S-405LF could remove styrene from soil, compared to JBR-425, while JBR-425 presented a better removal under the same conditions. JBR-425 could significantly enhance styrene solubility (Chapter 4.5) and lower the interfacial tension between styrene and rhamnolipid solution (Chapter 4.7). As the interfacial tension between styrene and rhamnolipid is reduced, styrene mobilization increases. Compared to JBR-215 and S-405 LF, JBR-425 performed the best styrene removal in washing experiments.

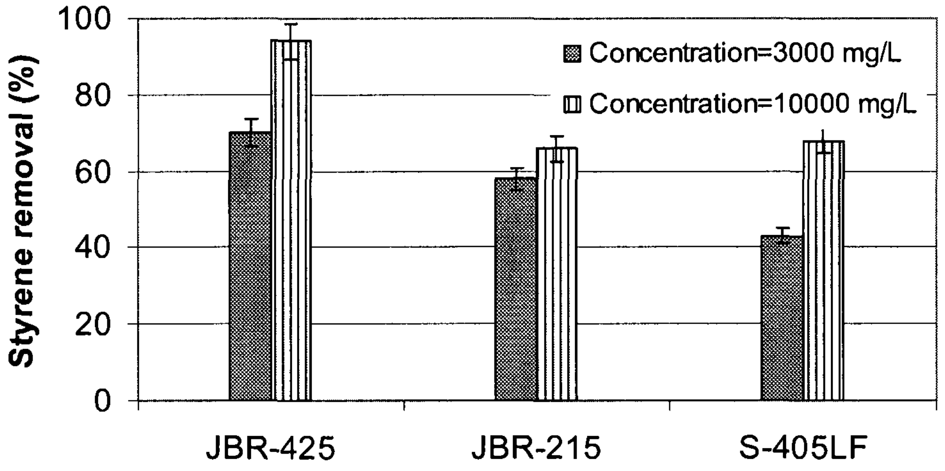


Figure 4-28. Comparison of styrene removal with three surfactants in batch tests

4.9.6 Effect of soil type

The effect of soil type on styrene removal is presented in Figure 4-29. Three types of soil were contaminated with styrene. The initial styrene concentration for soil 1is 4400 mg/kg (27% of styrene adsorbed onto soil, 1.4% of styrene dissolved and 71.6% of styrene stayed as pure phase), for soil 2, it was 4190 mg/kg (22% of styrene adsorbed onto soil, 1.6% of styrene dissolved and 76.4% of styrene stayed as pure phase) , for soil 3; it was 4540 mg/kg (24% of styrene adsorbed onto soil, 1.5% styrene dissolved and 74.5% of styrene stayed as pure phase). Soil 1 presented the highest styrene removal rate. Soil 2 has the highest clay ratio (19.5%) among the three soils and soil 3 presented the highest organic matter content (17.5%). Obviously, the clay ratio and organic matter content played important roles in the styrene removal tests. According to Table 4-2, the various sorption processes that contribute to bonding between organic pollutants and soil fractions include partitioning (hydrophobic bonding) and accumulation through adsorption mechanisms involving the clay minerals and other soil particulates such as carbonates and organic and amorphous materials (Thomas, 2005). When removing styrene from the soil system, all these factors would affect removal efficiency as they did during the styrene adsorption process. Moreover, high contents of clay and organic matter may adsorb more rhamnolipid in the batch washing test. The adsorption of rhamnolipid on soil clays or soil organic matter may result in the loss and reduction of rhamnolipid concentration, which may render them less efficient or ineffective in styrene removal (Urum and Pekdemir, 2004).

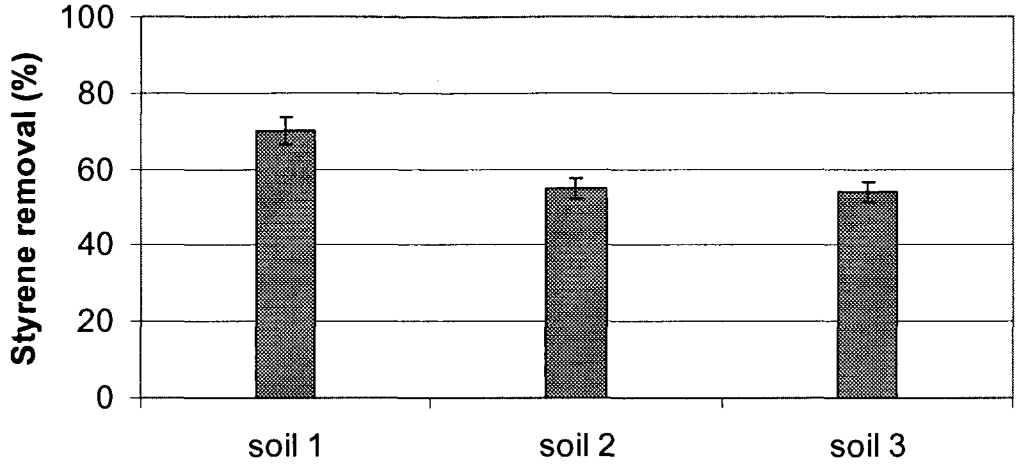


Figure 4-29. Comparison of styrene removal from three types of soil

4.10 Summary of batch tests and preliminary discussion

Results from the batch removal experiments presented the possibility of using rhamnolipid as washing agent to removal styrene from the soil system. The styrene removal increased with an increase in the rhamnolipid concentration. The highest styrene removal (98.4%) was achieved at 1000 mg/L rhamnolipid in the batch test. Daily and single washes were conducted in a batch washing study to evaluate the time factor on styrene removal efficiency. With a daily wash, removal increased to 93.5% starting at day 2 and the highest removal was achieved at 98.4% on day 3. A single wash achieved the highest removal 79.1% on day 3 and styrene removal decreased after day 3. This may be because styrene readsorbed to the soil in single washes with longer contact times. Higher styrene removal was also achieved with lower styrene levels of contaminant soil.

4.11 Continuous flow experiment

Based on the results from batch removal tests, column experiments were run with rhamnolipid as the washing solution to remove styrene from the soil. Distilled water alone was flushed through the columns as a control. Figures 3-6 and 3-7 present the column experimental configuration. The pore volume of the compacted columns was measured to be around 2 mL at a porosity of 0.8. The hydraulic conductivity with water was 2.5 x10^{-2} cm/s which indicates that the soil media is fine sand and the degree of permeability is high to medium (ASTM D-5084). Effluents from the columns were collected according to the number of pore volumes.

Collection of effluents was stopped when the styrene concentration was less than 0.5 mg/L. The results from the column experiments were compared with those from the batch tests. Figure 4-30 shows distilled water and rhamnolipid (300 mg/L and 1000 mg/L), and the styrene average concentration in the effluent. Based on the batch tests, 300 mg/L rhamnolipid was used as the washing solution in continuous flow tests (except rhamnolipid concentration study). The styrene mobilization reached a relatively stable level after 750 pore volumes of flushing. Flushing with distilled water, after a 450 pore volume flushing, indicated that no styrene was detected in the effluent. For the 1000 mg/L rhamnolipid flushing experiment, after 800 pore volumes, styrene became stable in the effluent. It showed that a higher rhamnolipid concentration increased styrene mobilization.

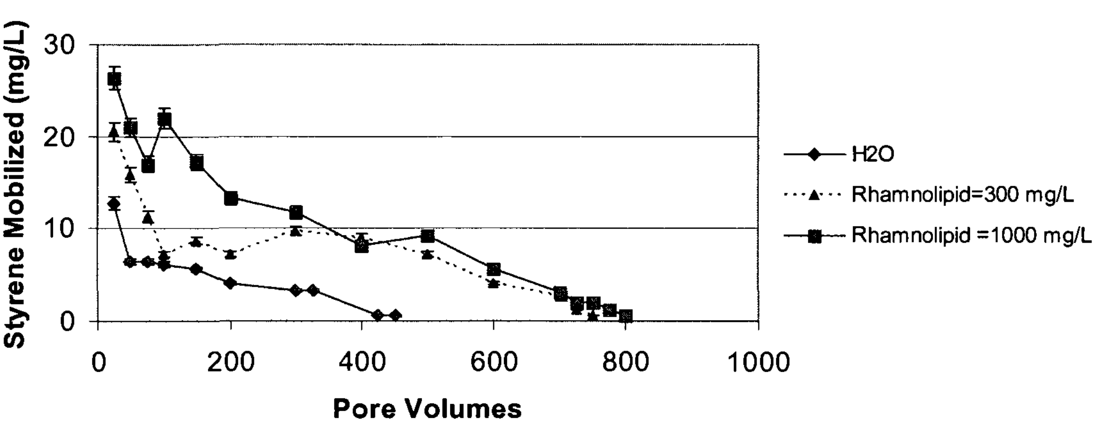


Figure 4-30. Average styrene concentration in the effluent (initial rhamnolipid solution pH was 6.5)

4.11.1 Effect of flow rate

Flow rate is a very important factor in a continuous flow washing test. Figure 4-31 illustrates that at 1 mL/min, a maximum styrene removal was achieved at 60.4% and the lowest removal was achieved at 22.9% with a 20 mL/min flow rate. Variation of flow rate changes the contact time between styrene and rhamnolipid. Higher flow rates result in lower contact times, and thus styrene removal decreased with decreased contact time (Figure 4-31). The contact time was determined as 4.0, 2.0, 0.4, 0.2, and 0.1 minutes for flow rates of 0.5, 1, 5, 10, and 20 mL/min. It should be noted that during a soil washing experiment, when a chemical reaction occurs some bonds are broken enabling new bonds to form. This process needs time and energy (Dahr Azma, 2005). Rhamnolipid should leave the contact area after the production of the new bonds to free the space for non-bonded rhamnolipid in the solution. Thus, increasing the flow rate in some domain can also enhance the rate of removal in the case of styrene where flow rates changed from 0.5

to 1 mL/min. Increasing the contact time from 0.1 to 2.0 minutes, styrene removal increased 3 times. Therefore, the best flow rate is 1 mL/min and time to complete the soil washing is 1500 minutes. Dahr Azma (2005) studied the relationship of flow rate and heavy metal removals in a continuous configuration. Flow rate was presented as an effective parameter in the removal of heavy metals from sediment. Increasing the contact time from 0.2 minutes to 4.3 minutes, heavy metals removal was drastically increased, 3 times for copper, 10 times for zinc and 4 times for nickel.

Styrene has a high volatility. During the continuous flow experiment, some styrene was volatilized after mobilization from the soil to the aqueous phase. When the flow rate is as slow as 1 mL/min, styrene remains longer in the transportation phase, more styrene volatilizes from the aqueous phase to the gas phase. Styrene is less volatilized when the flow rate reaches 20 mL/min and 30% of the styrene volatilizes when the flow rate reached 1 mL/min. Compared to styrene remaining in soil at 1 mL/min, the result was found at a flow rate of 0.5 mL/min. 59% of styrene remained in soil at a 0.5 mL/min flow rate and 40% remained at 1mL/min. The lower styrene removal may result from readsorption of the mobilized styrene back to the soil since the contact time is longer than for the 1 mL/min flow rate soil washing.

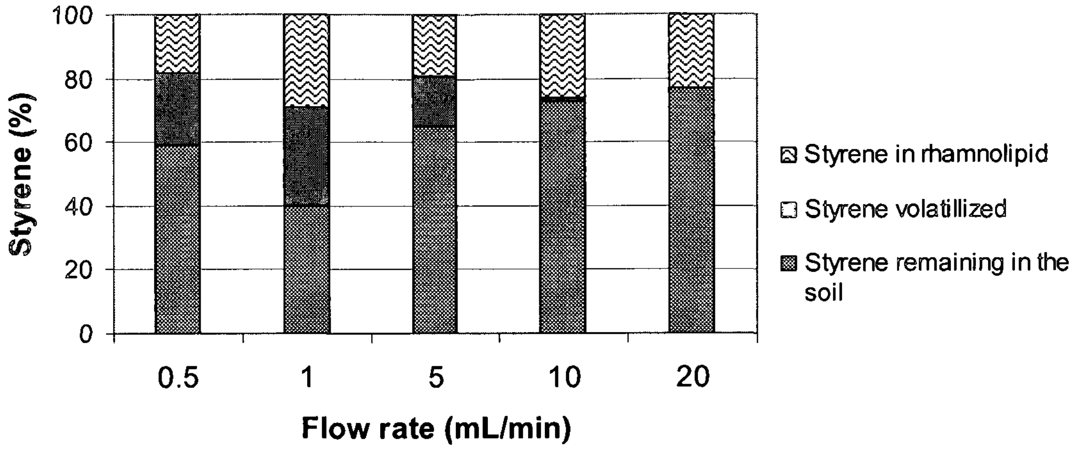


Figure 4-31. Styrene removal with different flow rates in a continuous flow washing test with 300 mg/L rhamnolipid

4.11.2 Effect of rhamnolipid concentration

The results of styrene removal by rhamnolipid at different concentrations are illustrated in Figures 4-32 and 4-33. In this washing test, the initial styrene concentration is 4440 mg/kg; the styrene removal increased with increased rhamnolipid concentrations and the highest removal of 68% was achieved with 1000 mg/L of rhamnolipid. From the data reported in section 4.5 and Figure 4-15, styrene solubility increased as the rhamnolipid concentration increases. As the styrene solubility increased, more styrene transferred into rhamnolipid from soil system thus styrene removal increases. An interesting observation was found from the continuous washing experiment. A higher ratio of styrene has been volatilized in the system when a high concentration of styrene was found in the aqueous phase.

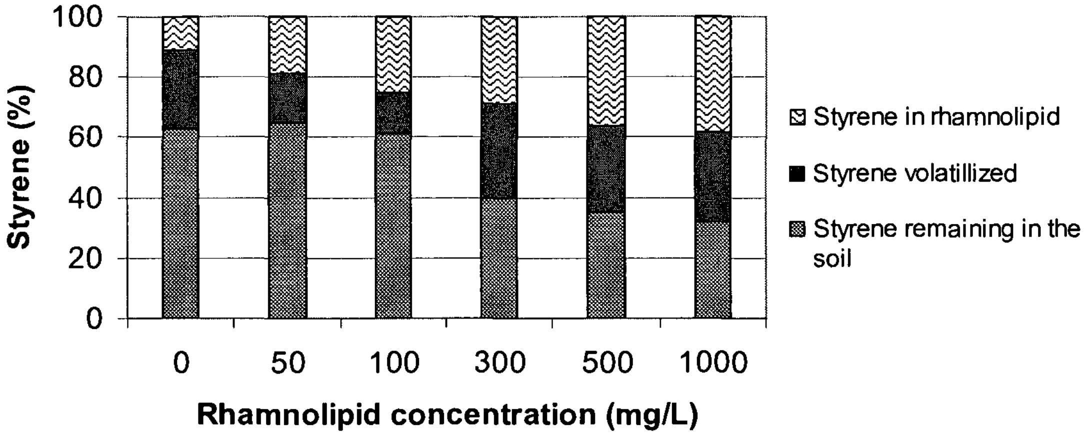


Figure 4-32. Variation of styrene content in the effluent during the soil washing study with different rhamnolipid concentrations

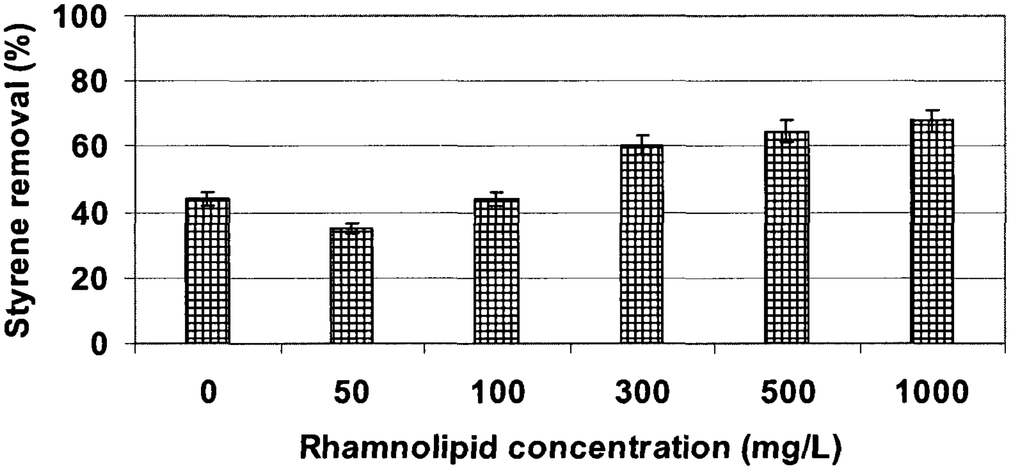


Figure 4-33. Variation of styrene removal from soil with different rhamnolipid concentrations after continuous flow experiments

Styrene has high volatility in a water system. CEPA (1993) published that styrene is rapidly lost from surface water by volatilization with the half-life ranging from 1-60 hours, depending on the depth of the water body and degree of turbulence. Regardless of the amount of removal, batch and continuous flow configurations showed the same patterns of styrene removal with a variation of the rhamnolipid concentration.

4.11.3 Effect of styrene concentration

A series of experiments was performed using 300 mg/L rhamnolipid with different initial styrene concentrations (480 mg/kg to 9080 mg/kg). With an increase in the initial styrene concentration, the rhamnolipid removal ability decreased substantially from 87.3% to 43.5% (Figure 4-34). Styrene at 480 mg/kg had the highest removal of 87.3% and the lowest removal of 43.5% occurred when the initial concentration of styrene was 9080 mg/kg. Even the styrene removal decreases as styrene concentration increased, the total styrene removal was increased at higher initial concentration. 39.5 mg of styrene was removed from 9080 mg/kg initial styrene concentration compared to 4.2 mg of styrene removal at 480 mg/kg initial styrene concentration. When the initial styrene concentration increased 19 times (9080/480=18.9) in washing experiments, the total styrene removal was only increased 9 times (39.5/4.2=9.4). This may due to the limited contact area and time during the continuous flow washing tests. If we assume the styrene was uniformly adsorbed on the soil surface, only part of the adsorbed styrene will be contacted with rhamnolipid and removed from soil according to limited styrene solubility (978 mg/L) at 300 mg/L of rhamnolipid. A high proportion of styrene will be solubilized when the initial styrene concentration is low, a low proportion of styrene will be solubilized when the initial styrene concentration is high. This may be the reason why the initial styrene concentration increased 19 times that only 9 times the amount of styrene was removed.

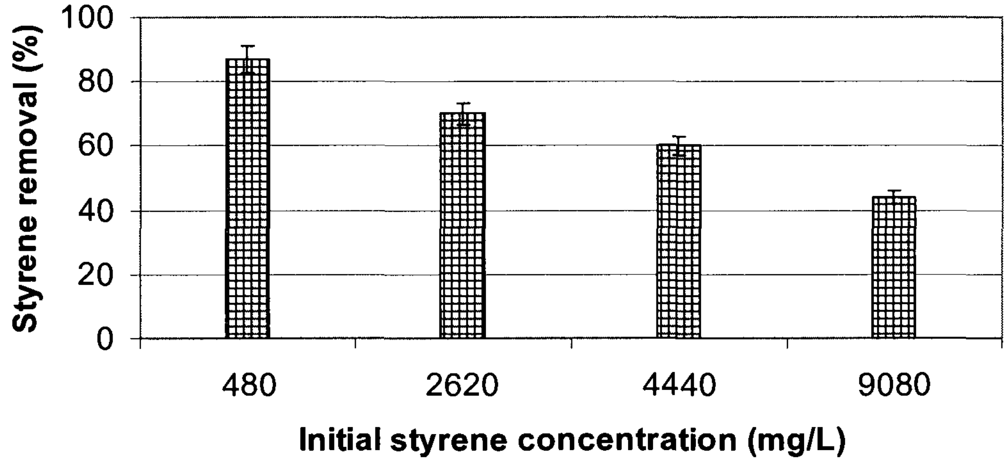


Figure 4-34. Variation of styrene removal from soil with different initial styrene concentrations after continuous flow experiments

4.11.4 Effect of pH

It can be observed that in Figure 4-35, as the pH value increased from 6 to 10, the continuous flow washing removal results ranged from 56.0% to 61.5% when using 0.3% rhamnolipid as the washing solution and the initial styrene concentration of 4440 mg/kg in the soil. The results are similar to the batch removal results. pH had very little effect on the continuous flow test. This is because styrene is not a pH sensitive compound since styrene is no charge with varying pH values. Styrene does not change performance by changing pH.

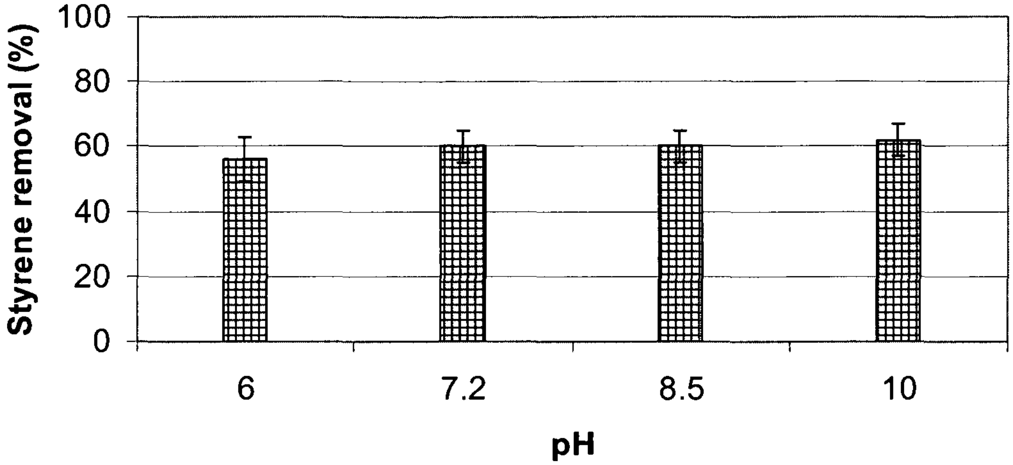


Figure 4-35. Variation of styrene removal from soil with different pH values after 750 pore volumes in continuous flow experiments

4.11.5 Effect of soil types

The effect of soil type on styrene removal is presented in Figure 4-36. Three types of soil were contaminated with styrene at an initial styrene concentration for soil 1 of 4400 mg/kg, for soil 2, it was 4190 mg/kg and for soil 3, it was 4540 mg/kg. Soil 1 presented the highest styrene removal rate. Soil 2 has the highest clay ratio (19.5%) among the three soils and soil 3 presented the highest organic matter content (17.5%). Similar removal results were achieved in continuous flow experiments as in batch experiments.

4.11.6 Styrene removal with three types of surfactant

Figure 4-37 illustrate the results of styrene removal with three different surfactants (JBR-425, JBR-215, and S-405LF). S-405LF could remove less styrene from soil, compared to JBR-425. JBR-425 showed the best styrene removal efficiency among the three types of surfactant. It is interesting to find that at same rhamnolipid concentration, JBR-425 with

JBR-215 presented different styrene removal results. JBR-425 has 25% rhamnolipid and JBR-215 has 15% rhamnolipid. JBR-425 has a higher purity than JBR-215, which could improve the ability for removal of styrene from the soil. Moreover, according to Figure 4-17, R2 is the main functional component in rhamnolipids. The R2 concentration increases as the total rhamnolipid concentration increases. Styrene removal tests show that dirhamnolipid (R2) in rhamnolipid solution is concentration dependent. For JBR-215, R1 and R2 concentrations changed randomly and no main functional component was found with rhamnolipid concentration increases. When both biosurfactants were at the same rhamnolipid concentration for the styrene removal experiments, the R2 concentrations were not equal in JBR-425 and JBR-215, thus showing different styrene removal abilities.

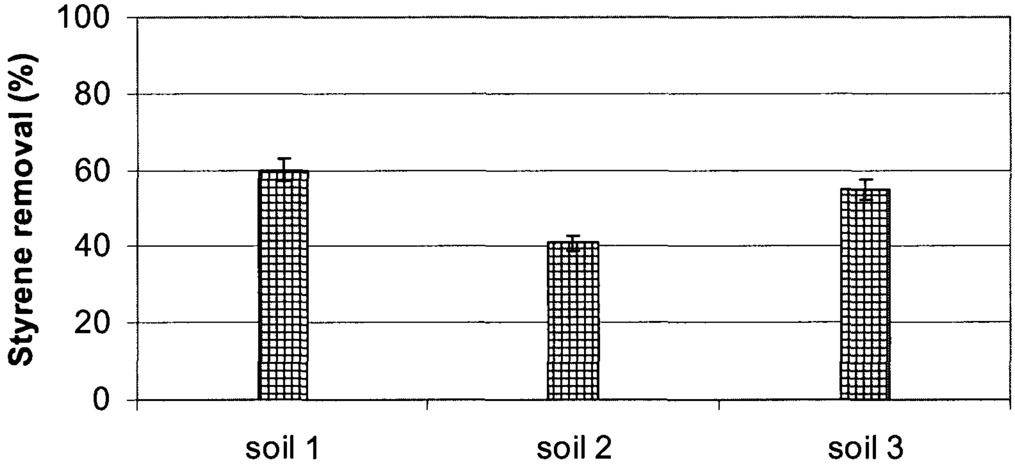


Figure 4-36. Variation of styrene removal from different soil types for continuous flow experiments

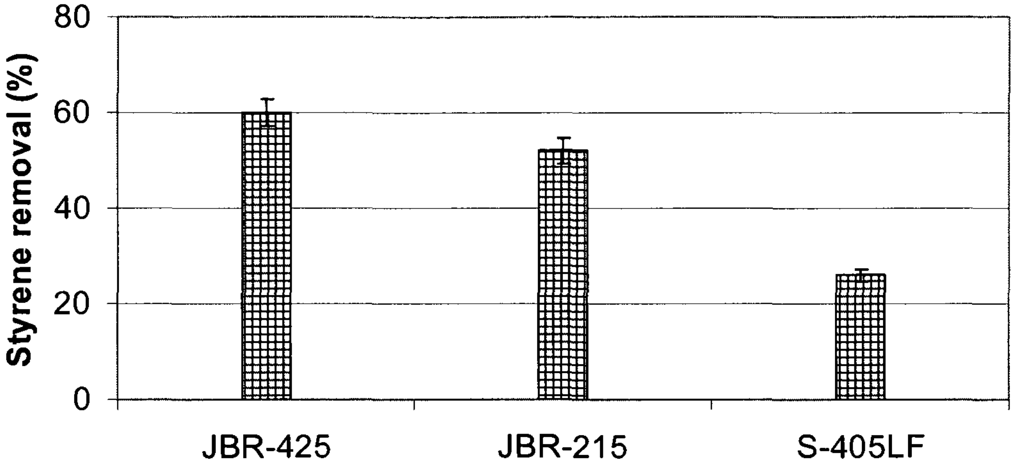


Figure 4-37. Variation of styrene removal from soil with different surfactants during continuous flow experiments

4.12 Comparison of the results from batch and continuous removal configuration

A comparison of styrene removal for both batch and continuous configurations is discussed in this section. The importance of the configuration (batch and continuous flow) of a wide range of parameters on the same soil was studied. The advantage of the comprehensive study of various parameters that affect the removal of styrene in different configurations enables one to evaluate the degree of importance for each parameter or configuration for engineering applications in industry soil washing techniques.

Figures 4-38 to 4-44 present the comparison results according to each characteristic. In this study, both configurations (batch and continuous flow) showed that it was feasible to use rhamnolipid as a washing agent to remove styrene. Rhamnolipid was chosen as the washing agent in soil washing studies due to its capability of removal for heavy metals and organic contaminants from soil and sediment (Mulligan, 1998; Dahr Azma, 2005; Mulligan and Gibbs, 2004; Urum and Pekdemir, 2004; Chu and Chan, 2003; Urum *et al*, 2006). The highest styrene removal (98.4%) was obtained by a batch washing test with 1% rhamnolipid. Maximum styrene removal occurred at the highest rhamnolipid concentration in both batch and continuous washing tests. The same trend was also reported by Urum and Pekdemir (2004) for crude oil removal. This trend was expected, since the force of attraction between soil and oil would be reduced due to the increase in contact angle and change in wettability of the system in the presence of rhamnolipid.

The results showed that more than 70% of the removal could be achieved for an initial styrene concentration of 4440 mg/kg of styrene after one day and 98.4% removal after four days with a daily wash (Figure 4-26). A longer contact time with daily fresh rhamnolipid solution will achieve higher styrene removal efficiency. An amount of 13.5 mg of rhamnolipid was added in single wash experiments and 67.5 mg rhamnolipid was added in daily wash experiments. Compared to single wash results, daily wash styrene removals increased from 79.1% to 98.4% by adding 5 times more rhamnolipid. When fresh rhamnolipid solution was supplied, it allowed improved contact for the soil sorbed styrene to make contact with the unoccupied micelles, and therefore the styrene will be transferred to the water phase. When a prolonged contact time during a single wash was used in Figure 4-26, 79.1% of styrene was removed at day 3 since the unoccupied micelles were limited in rhamnolipid solution (Chu and Chan, 2003).

The highest removal efficiency was achieved from the lowest styrene concentration (480 mg/kg) at 87.3% in a continuous flow washing test with 300 mg/L rhamnolipid. The maximum amount of styrene removed from soil in batch and continuous flow tests were achieved at the highest initial rhamnolipid added in solution (Figure 4-40). High removal efficiency was achieved at either high rhamnolipid concentration or low initial styrene concentration. With constant rhamnolipid concentrations, low initial styrene concentration or high rhamnolipid concentration both result in better contact of sorbed styrene with the unoccupied micelles. Therefore the sorbed styrene was removed from the soil phase and the removal efficiency increased under these situations.

The efficiency and success of biosurfactant in facilitating removal of organic and inorganic contaminants from soil systems will depend largely on the amount of rhamnolipid present in the aqueous phase. A study by Ochoa-Loza *et al.* (2006) has illustrated that the sorption of monorhamnolipid (R1) is concentration dependent. Illite presented the most sorption at high R1 concentration; hematite has the most sorption when R1 concentration is low. Among the three soils used in this study, soil 1 has the best styrene removal in both batch and continuous flow experiments. Compared to soil type 2 and soil type 3, soil type 1 has a lower clay ratio and organic matter content. Soil organic matter content, type of organic matter and clay ratio within the soil are important factors from styrene adsorption tests and styrene removal tests.

An interesting result was observed that styrene removal correlates with the R2 concentration (Figure 4-39). From batch removal tests, styrene removal is increased in a linearly with increasing R2 concentration. From the continuous flow removal test, styrene removal increased logarithmically with increasing R2 concentration. A plateau was observed when the R2 concentration reached 550 mg/L rhamnolipid. Compared to batch washing tests, continuous flow tests have a limited contact time. With insufficient contact time, less styrene removal was achieved than batch washing tests. The result indicated that contact time is an important factor in styrene removal experiments.

The pH has little effect on styrene removal efficiency. Regardless of the removal rate, batch washing tests performed better for styrene removal than the continuous flow tests. Batch experiments supplied complete contact between styrene and rhamnolipid, which allowed the rhamnolipid to enhance the styrene solubility and reduce the interfacial tension. Styrene removal stays at same trend in both batch experiments and continuous flow experiments. Compared with JBR-215 and S-405LF, JBR-425 performs the best styrene removal efficiency in both experiments since R2 concentration is increased as rhamnolipid concentration increase. Styrene removal tests show that dirhamnolipid (R2) in rhamnolipid solution is concentration dependent. The R2 is the main functional component in rhamnolipid.

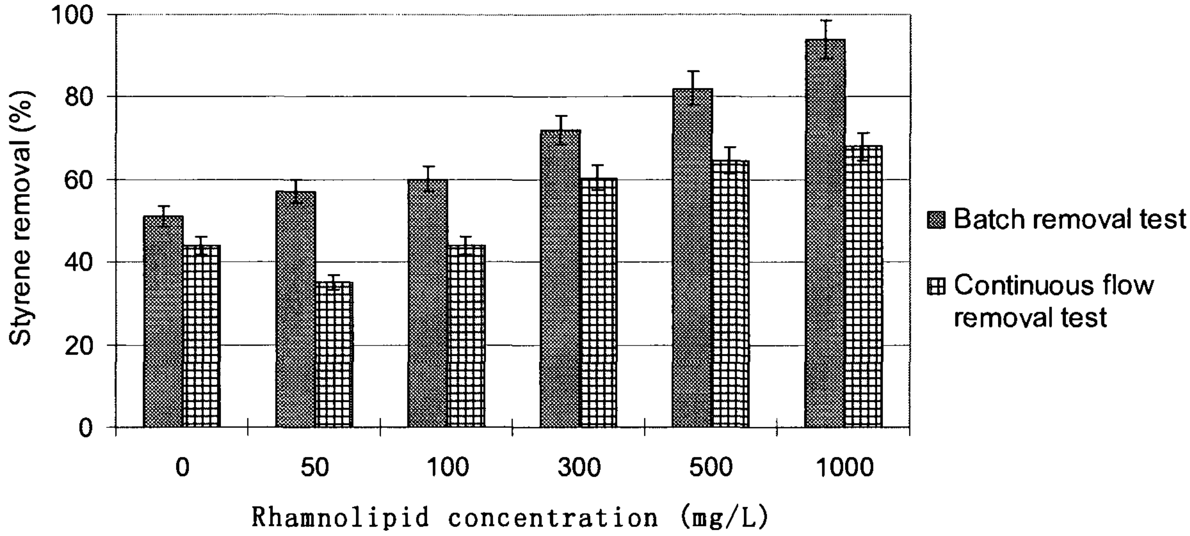


Figure 4-38. Styrene removal at different concentrations of rhamnolipid

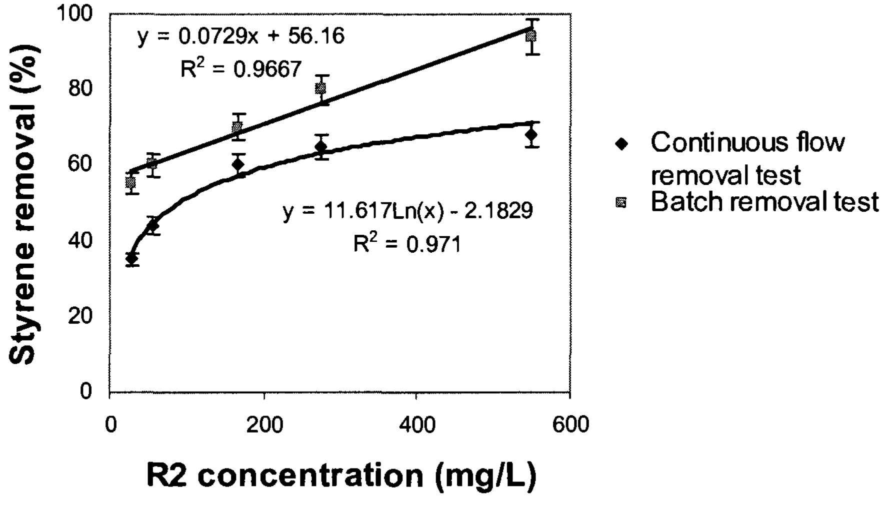


Figure 4-39. Styrene removal at different concentrations of R2

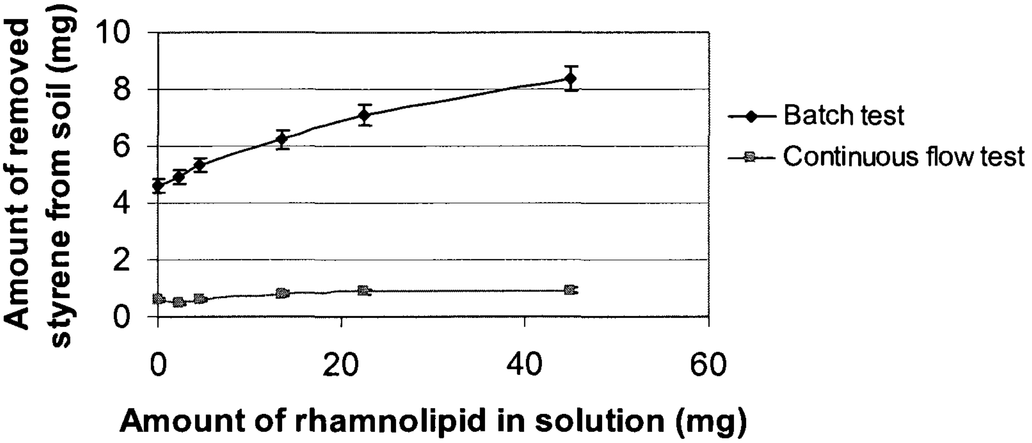


Figure 4-40 Amount of removed styrene at different amounts of rhamnolipid

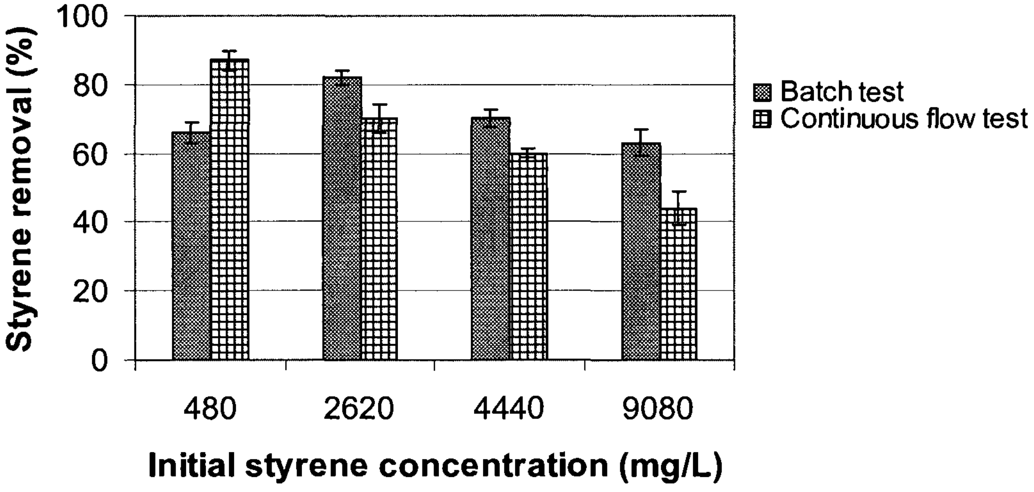


Figure 4-41. Styrene removal at four different styrene concentrations with 300 mg/L rhamnolipid in batch and continuous washing tests

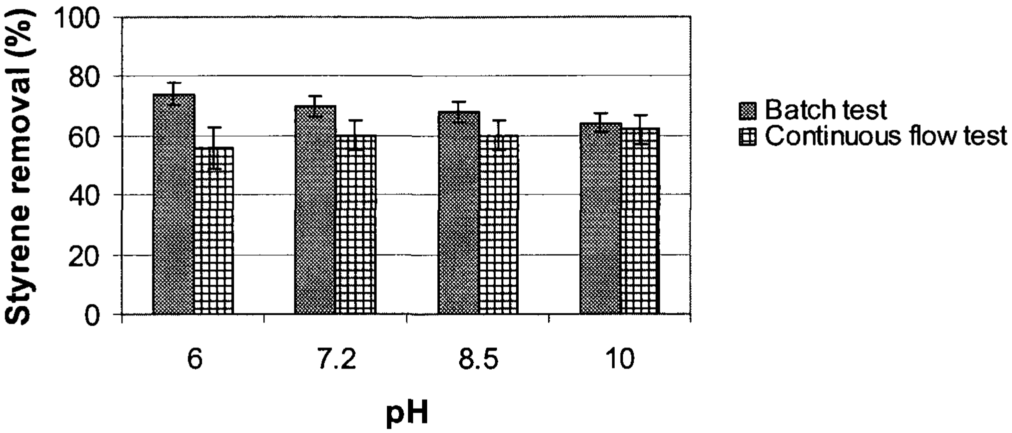


Figure 4-42. Styrene removal at different pH values in batch and continuous washing tests

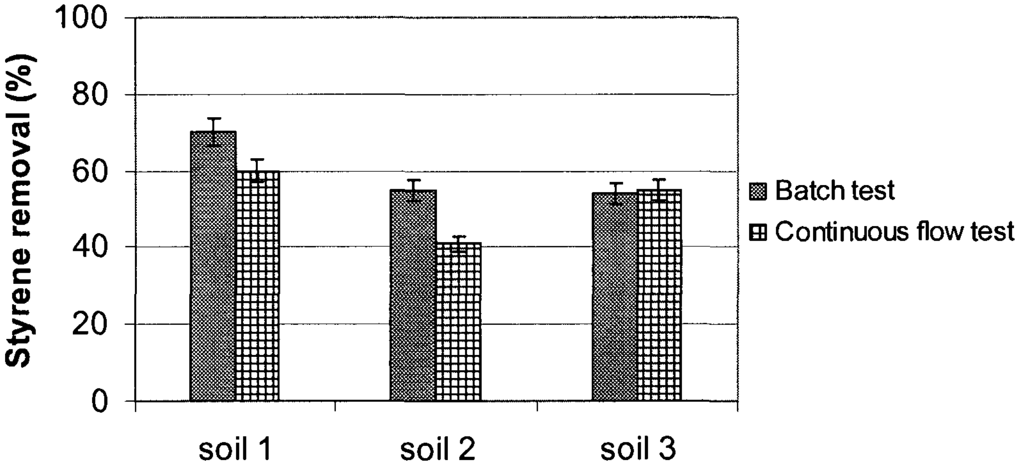


Figure 4-43. Styrene removal for different soil types with 300 mg/L rhamnolipid in batch and continuous washing tests

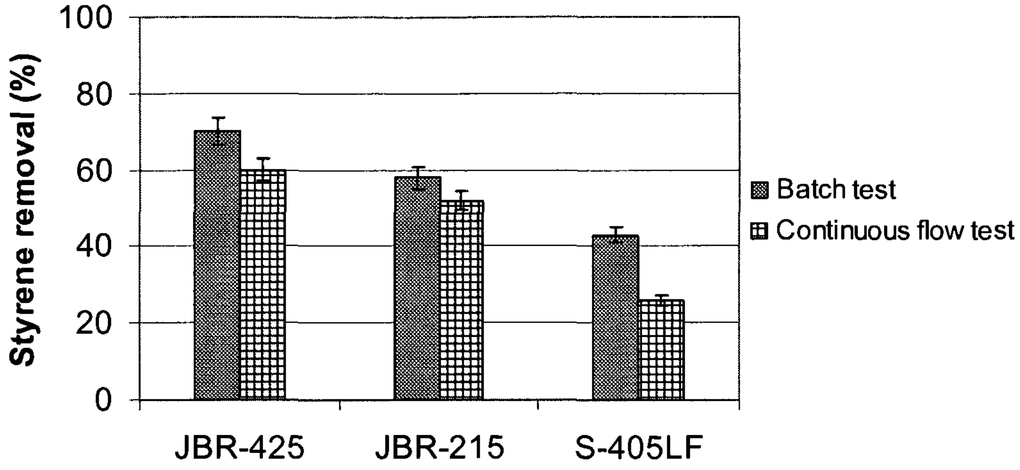


Figure 4-44. Styrene removal with different surfactants in batch and continuous washing tests

4.13 Styrene removal mechanism study

By collecting information from the batch adsorption tests, column adsorption tests, batch removal experiments and continuous flow washing experiments, the final step in this chapter was to find the possible mechanisms of styrene removal from soil. Understanding the mechanism of rhamnolipid in contact with soil and styrene, choosing the right strategy for styrene removal, and providing a guide to choose the supplementary removal method to complete the removal processes are essential.

4.13.1 Biosurfactant adsorption

Rhamnolipid has shown its promising washing ability in styrene removal tests and has achieved high styrene removal efficiency (Figures 4-36 to 4-44) in both batch and continuous flow experiments. The efficiency and success of biosurfactants in facilitating removal of organic and metal contaminants from soil systems will depend largely on the amount of rhamnolipid present in the aqueous phase (Ochoa-Loza *et al.*, 2006). Zeta potential (Figure 4-20) presented a significant decrease when the rhamnolipid concentration was increased. This would indicate the adsorption of rhamnolipid is occurring onto the soil (Chapter 4.8).

The tests were performed with the procedure explained in Chapter 3. Various rhamnolipid concentrations were applied to the soil samples in a batch tests. The results are shown in Figure 4-45 and a linear sorption isotherm was obtained with a correlation coefficient of 0.99. Rhamnolipid uptake capacity increased linearly with increasing equilibrium concentration. The pattern of the sorption of rhamnolipid was compared with different types of isotherms and it was determined that linear isotherm fitted nicely with a correlation coefficient of 0.9955 (Figure 4-45). Since the adsorption isotherm is linear isotherm, a maximum value for adsorption cannot be determined. The relationship between initial rhamnolipid concentration and the loss of rhamnolipid due to adsorption to the soil in batch experiments was shown in Figure 4-47. Using a higher concentration of rhamnolipid causes a higher adsorption but it should be noted that the increment in the loss is much less than the increment in rhamnolipid concentration. The loss of rhamnolipid due to sorption is also presented in Table 4-5. The possible mechanisms for sorption of the anionic surfactant are the following (Dahr Azma, 2005):

- Cation bridging between sorbed cations in the soil and anionic head of surfactant
- Hydrophobic interactions between the organic matter in soil and hydrophobic portion of surfactant.

Herman *et al.* (1995) pointed out that the sorption of rhamnolipid to the soil depends on the concentration of metals in the soil. The presence of Cd^{2+} in the soil decreased the sorption of rhamnolipid to the soil. A mixture of metals decreased the sorption even further. Dahr Azma (2005) studied the sorption of rhamnolipid to the heavy metal contaminated sediment. 25.1% of rhamnolipid was adsorbed to the sediment with 500 mg/L of rhamnolipid in continuous flow experiments. Since the efficiency of the surfactant may be reduced by the adsorption of rhamnolipid to the soil, it is recommended that a surfactant concentration higher than CMC should be used in soil was recommended.

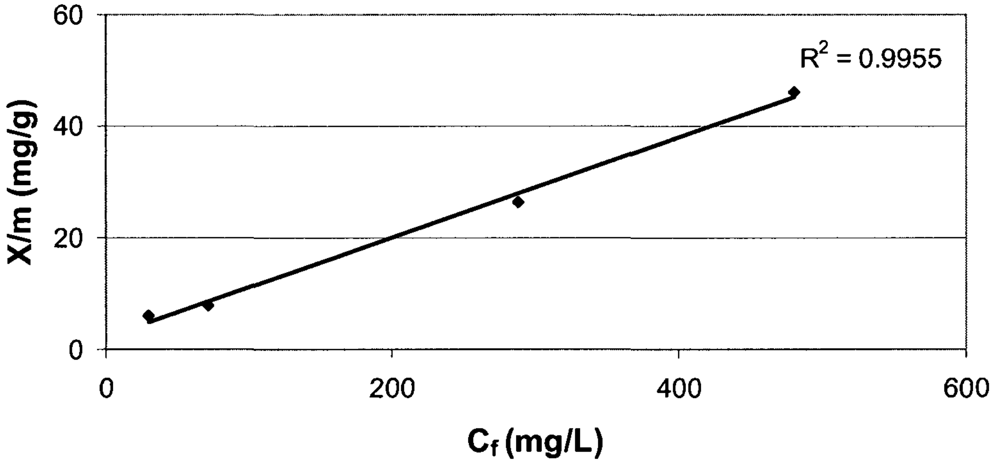


Figure 4-45. Rhamnolipid adsorption isotherm on soil 1 during the batch experiment

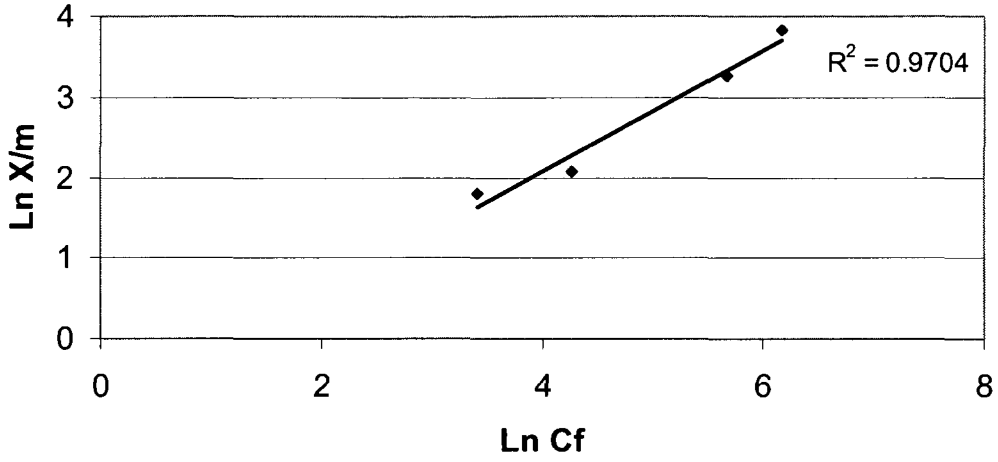


Figure 4-46. Freundlich isotherm for rhamnolipid on soil in the batch experiment

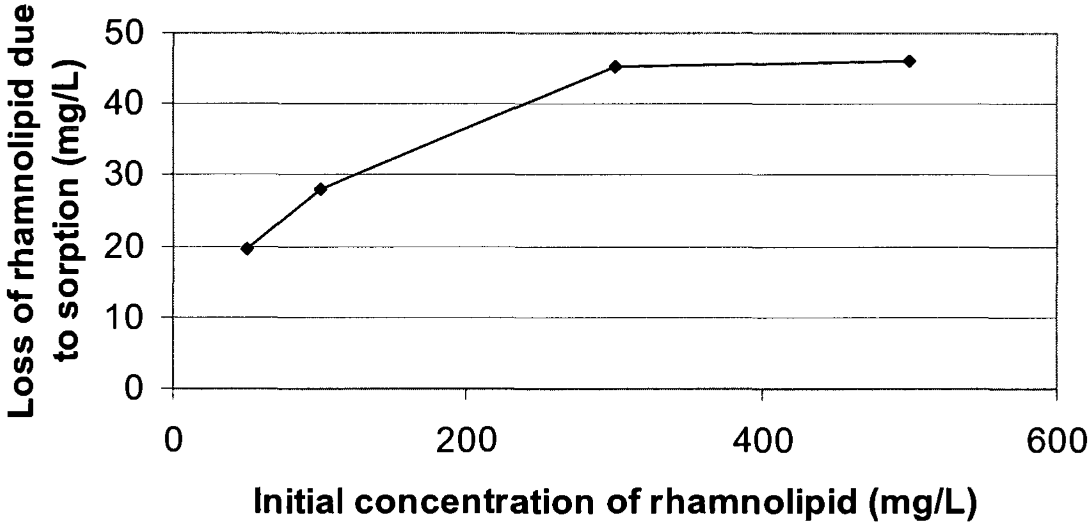


Figure 4-47. Relationship between initial concentration and loss of rhamnolipid due to sorption in the batch test

Table 4-5. Loss of rhamnolipid due to adsorption to the soil

Initial rhamnolipid concentration (mg/L)	Loss of rhamnolipid due to adsorption (%)
50	39.2
100	28
300	15.1
500	9.6

4.13.2 Effect of porosity on styrene removal

Porosity was varied in this set of experiments to evaluate the styrene removal rate. Porosity was determined using the standard relation of $e = 1 - \rho / \rho_s$, where e is the porosity, ρ and ρ_s are bulk density and particle density of the soil, respectively. The variation of porosity was obtained by changing the mass of soil in a constant volume of test column. The ratio of the mass of soil to the volume of the rhamnolipid remained constant in all tests. The removal of the styrene was measured for porosities of 0.2, 0.4, 0.6 and 0.8. The results are shown in Figure 4-48. It is obvious that the removal decreased as the compaction of soil samples increased in lower porosity. 60% of styrene removal was achieved at soil porosity of 0.2 and 81% of styrene removal achieved at soil porosity of 0.8. When the soil porosity increased 4 times, styrene removal only increased by 20%. The increase in styrene removal is non-linearly proportional to the increment in porosity. This can be explained as the decline in the porosity, decreases the wetted contact surface area between soil particles and rhamnolipid solution, which is the first step in the removal of styrene from soil. Similar results were obtained by Dahr Azma (2005). The highest styrene removal was achieved at the highest porosity.

Dahr Azma (2005) studied heavy metal removal versus varied porosity of sediment. Copper, zinc and nickel removal from sediment were tested with sediment porosity varying from 0.4 to 0.76. Copper removal was sensitive to the change in porosity and the percentage of copper removal is non-linearly proportional to porosity. 2.25 times more copper removed from porosity at 0.76 than porosity at 0.4. The change in zinc removal was much less compared to copper. When soil porosity was changed in continuous flow experiments, the soil hydraulic conductivity will change, so do the wetted surface area. These three parameters also played a very important role in styrene removal from soil.

4.13.3 Styrene removal with variation of hydraulic conductivity

The hydraulic conductivity for the samples used in the porosity study was measured. The results of styrene removal with variation of hydraulic conductivity were reported in Figure 4-49. Hydraulic conductivity varies proportionally to porosity. Hydraulic conductivity increased with an increase in porosity for the same mass of soil. Styrene removal increased as the hydraulic conductivity or porosity increased. The similarity in the patterns of removal of styrene from soil versus hydraulic conductivity and porosity were expected as seen in Figure 4-49. The same trend of results was presented by Dahr Azma (2005) in removal of the heavy metals from the sediment.

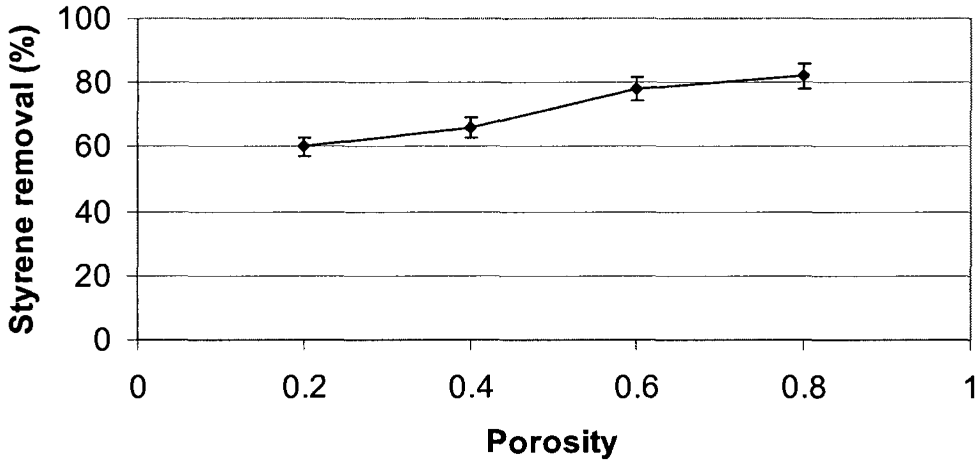


Figure 4-48. Variation of styrene removal with 300 mg/L rhamnolipid for different porosities

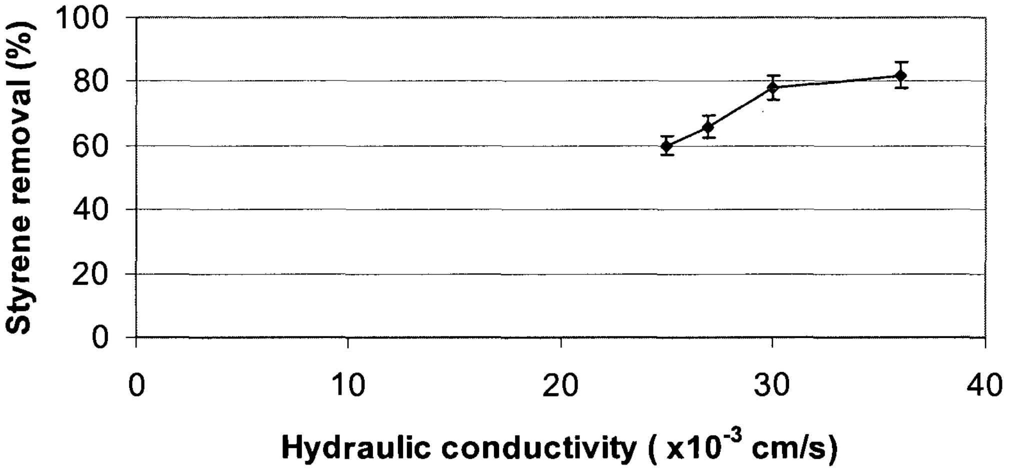


Figure 4-49. Variation of styrene removal with 300 mg/L rhamnolipid for different hydraulic conductivities

4.13.4 Styrene removal with variation of wetted surface area

When the solution contacts with soil in a soil washing study, the wetting process starts as the first step. This process includes coverage of the particle surface by the solution. The specific surface area of a soil is the total surface area of all particles in a specific volume. Wetted surface area is the surface area of the pore channels in the soil through which fluid flow occurs (Yong and Mulligan, 2004). According to the first step in removal of contaminant from the soil is wetting the soil particle surface with the washing agent, it is important to study the relationship between styrene removal and wetted surface area.

To account for the influence of the properties of pore channels defined by structure of a soil, and the fact that the wetted soil particles' surface area is controlled by the structure of the soil, Yong and Mulligan (2004) used a modification of the combined form of the Poiserille and Kozeny-Carman (K-C) model as:

$$k = \frac{Cn^3\gamma}{\eta T^2 S_w^2} \qquad [4\text{-}5]$$

η = viscosity of the permeating fluid (N.s/m^2)

γ = specific gravity of soil (N/m^3)

T= tortuosity, the ratio of effective flow path to the thickness of test sample, usually taken as T^2=2

S_w= wetted surface area per unit volume of soil particles (m^2/m^3)

The other parameters are defined earlier. Yong and Warkentin (1975) suggested C=0.4

The viscosity of the 300 mg/L rhamnolipid solution was measured at 1.43 centipoise (by the method proposed by Shawm 1980). According to the results of the variation of styrene removal with hydraulic conductivity in Figure 4-49, by means of equation 4-4, for a known fluid and specific soil sample, S_w can be determined if the K is known for any specific configuration. The styrene removal versus wetted surface area is presented in Figure 4-50. Higher styrene removal was achieved with a larger wetted surface area. This result is very important for the continuous flow experiment since in the batch experiment, soil specific area equal to the soil wetted surface area (5.1×10^6 m^2/m^3), assuming that all the soil surface particles are wetted.

Compared to 82% of styrene was removed at 4.8×10^4 m^2/m^3 wetter surface area, 60% of styrene was removed with 3700 m^2/m^3 wetted surface area. When wetted surface area increased 13 times, styrene removal only increased 1.4 times. The styrene removal versus wetter surface area is a non-linear relationship. Styrene removal is sensitive to the wetted surface area because when styrene adsorbed on to soil, the soil organic matter content and types of organic matter played very important role. Furthermore, organic materials have the largest surface area among the fractions in the soil (Yong and Mulligan, 2004). A decrease in the wetted surface area affects this fraction more than the others and consequently reduces the styrene removal from the soil more effectively.

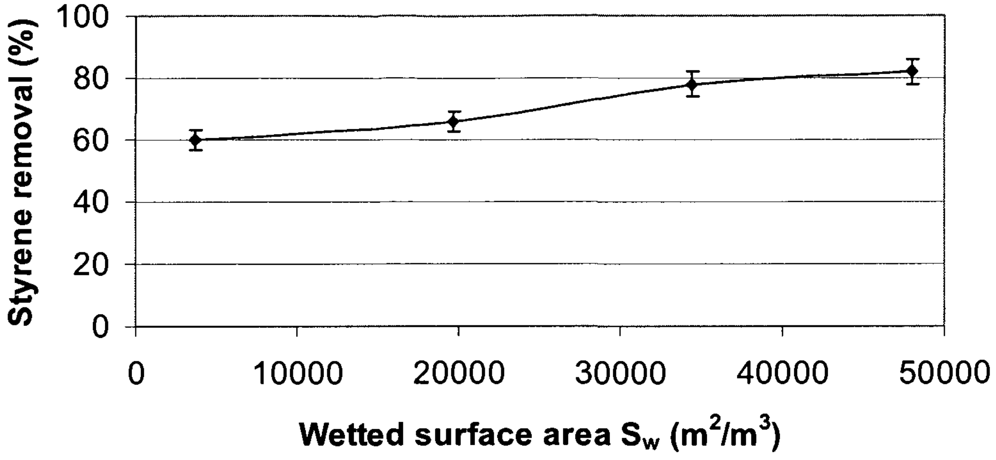


Figure 4-50. Variation of styrene removal with 300 mg/L rhamnolipid of wetted surface area S_w

4.13.5 Discussion

When the rhamnolipid solution comes in contact with styrene contaminated soil, the wetting processes starts with covering soil particles with rhamnolipid. The higher styrene removal was achieved with a larger wetted surface area. With larger wetted surface areas, rhamnolipid has a better chance to contact with styrene and to remove styrene from soil. The wetted surface area, S_w, was inversely proportional to the hydraulic conductivity coefficient and porosity of soil as well as the viscosity of the flowing fluid (Equation 4-5). The wetted surface area of sediment versus heavy metals removal was studied by Dahr Azma (2005). A higher efficiency of heavy metals removal from sediment was achieved at a larger wetted surface area. This finding is also in agreement with the statement made by Yong and Mulligan (2004) that showed that a higher wetted surface area provided a larger contact space for the permeant with the soil particles.

After the rhamnolipid solution wets the surface area, some rhamnolipid then adsorbed onto the soil surface. The efficiency and success of biosurfactants in facilitating removal of organic and metal contaminants from soil systems will depend largely on the amount of rhamnolipid present in the aqueous phase (Ochoa-Loza *et al.*, 2006). From soil in the rhamnolipid zeta potential study and the rhamnolipid adsorption study, rhamnolipid sorption onto the soil is occurring in styrene removal tests. The adsorption of biosurfactant onto soil and sediment has been widely studied in the last decade (Mulligan, 2004; Kuyukina *et al*, 2005; Urum and Pekdemir, 2004; Dahr Azma 2005). The effectiveness of surfactant-based soil washing can be limited by the adsorption of surfactant to the soil, particularly by clay minerals and organic soil matter. Clay and humus chemosorption reduces surfactant effectiveness for in-situ remediation of subsoil and groundwater. Cationic and non-ionic surfactants can be sorbed by negatively charged clay minerals, thereby decreasing the micelle concentration. Anionic surfactants can be precipitated by Ca^{2+} and Mg^{2+} from soil minerals (Kuyukina *et al*, 2005). Since the efficiency of the surfactant may reduced by adsorption of rhamnolipid to the soil, a surfactant concentration higher than the CMC should be used in soil was recommended. Dahr Azma (2005) used 2% of rhamnolipid with additives to removal heavy metals from sediment. Mulligan (1998) used 0.1%, 0.25%, 0.5% and 2% of rhamnolipid with1% NaOH to removal Cu and Zn from contaminated soil.

The two mechanisms of surfactant-aided soil washing are mobilization and solubilization (Chu and Chan, 2003; Kuyukina *et al.*, 2005). The mobilization mechanism occurs at a surfactant concentration below CMC. In this situation, surfactants reduce the surface and interfacial tension between air/water, oil/water, and oil/soil systems. Surfactants in contact with soil/oil system increase the contact angle and reduce the capillary force holding oil and soil together due to the reduction of the interfacial force. The interfacial tension between styrene and rhamnolipid was reduced as rhamnolipid concentration increased. The interfacial tension between styrene and water is 20 mN/m; it drops to 4.6 mN/m when the rhamnolipid concentration reached CMC. Styrene was mobilized from soil due to the reduction of the interfacial force.

When surfactant concentration is above CMC, the solubility of oil increased dramatically due to the aggregation of surfactant micelles. Styrene solubility increased by a factor of 27 times more with 3000 mg/L rhamnolipid compared to water alone (310 mg/L). The hydrophobic end of the surfactant molecules cluster together inside the micelle structure with the hydrophilic end exposed to the aqueous phase on the exterior. Consequently, the interior of a micelle constitutes a compatible environment for hydrophobic organic molecules. The process of incorporation of these molecules into a micelle is known as solubilization (Urum and Pekdemir, 2004). As styrene entered the core of rhamnolipid micelles, the remaining styrene in rhamnolipid is reduced, which allows a better chance for the sorbed styrene to make contact with unoccupied micelles. In this study, solubilization is the main removal mechanism since rhamnolipid concentration was choose above CMC and most of removed styrene was initially distributed as pure phase in styrene contaminated soil. Rhamnolipid in this study has shown great potential in styrene removal due to its low CMC and high sorption to soil. Urum and Pekdemir (2004) concluded that biosurfactant with low CMC values and high degree of sorption to soil may have stronger abilities to remove oil, if the solution concentrations employed during soil washing is greater than the CMC. They also found that removing crude oil from soil was only due to mobilization due to the low solubility of crude oil. For the case of removal of styrene from soil, both mobilization and solubilization occurred during the experiments.

Chapter 5

Experimental Results for Anaerobic Biodegradation of Soil Washing Leachates Using a UASB Reactor

5.1 General Remarks

After styrene was removed from soil, the effluents from soil washing experiments which contain styrene and rhamnolipid need further treatment before release to the environment. Anaerobic biodegradation is the preferred degradation process for wastewater with high organic loadings such as chemical, textile, petrochemical and paper industry wastewater. The advantages of anaerobic biodegradation include low production of sludge and production of methane which is useful for energy purposes (Mulligan, 2001).

This chapter describes the investigation of the performance and functionality of styrene and rhamnolipid under anaerobic biodegradation experiments. The results from both batch and continuous experiments are presented and discussed in this chapter. The results for CO_2 and CH_4 composition in the biogas are provided as a percentage of the pure gas. Styrene, VFA and styrene biodegradation products are provided as mg/L. HPLC and GC operating conditions were previously mentioned in Chapter 3. Styrene removal was determined by the difference between initial and final concentrations for both batch and continuous experiments.

5.2 Acclimation of the sludge

The reactor sludge was first acclimated in batch runs using acetate-based aqueous solutions made with deoxygenated tap water. The objective was to acclimate the biomass in order to start consuming COD and producing biogas. The substrate composition was previously mentioned in Tables 3-2 to 3-4. It was observed that with increasing operating loading rates (OLRs), the COD consumed per day and methane content in biogas increased as well. It took 3 weeks acclimation of biomass with acetate solution to increase the CH_4 content in the biogas from 12% to 78%. A new stock solution was fed when 80% of COD reduction was achieved and the CH_4 content reached 80% in the biogas. The pH values of the substrate increased from 6.5 to 7.6 ±0.2 respectively and biogas production reached 0.134 L CH_4 / g COD. Figure 5-1 shows the organic loading rates profile during the acclimation period.

5.3 Preliminary styrene biodegradation test

Styrene biodegradation tests were prepared to demonstrate the biodegradation rate under anaerobic conditions with or without biomass. Furthermore, since rhamnolipid may have an inhibitory effect on the styrene biodegradation test, a styrene with rhamnolipid biodegradation test was also prepared under anaerobic conditions with or without biomass.

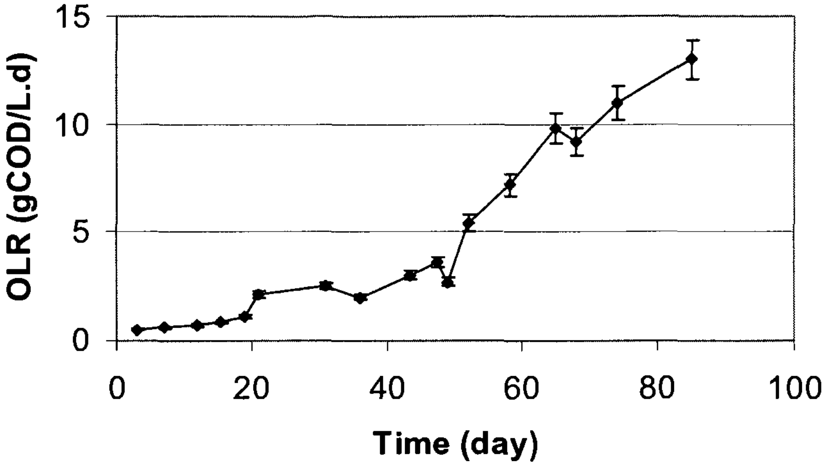


Figure 5-1. Increase in the organic loading rate (OLR) during the acclimation period

Styrene can be biodegraded quite readily in water under aerobic conditions. The biodegradation half-life of styrene in water was estimated to be less than 5 days (CEPA, 1993). But, styrene was persistent in a methanogenic biofilm column (U.S. Public Health Service, 2007). Control experiments in Table 5-1 showed that after 4 days, only 14.2% of the styrene was removed from a 75 mg/L styrene solution, and no styrene removal occurred from styrene in 300 mg/L rhamnolipid after 4 days. It indicated that under anaerobic control conditions, that styrene or styrene with rhamnolipid did not degrade or volatilize. The 14.2% styrene removed from the 75 mg/L styrene solution may be because of styrene volatilization to amber vial's headspace or experimental error since there were no by-products found in the solution.

Table 5-1 Styrene removal for different styrene concentrations with or without biomass

Sample description	Styrene removal (%)
75 mg/L styrene	14.2
150 mg/L styrene	0
250 mg/L styrene	0
75 mg/L styrene with biomass	98.9
150 mg/L styrene with biomass	97.8
250 mg/L styrene with biomass	98.2
75 mg/L styrene with 300 mg/L rhamnolipid	0
150 mg/L styrene with 300 mg/L rhamnolipid	0
250 mg/L styrene with 300 mg/L rhamnolipid	0
75 mg/L styrene with 300 mg/L rhamnolipid with biomass	89.0
150 mg/L styrene with 300 mg/L rhamnolipid with biomass	96.2
250 mg/L styrene with 300 mg/L rhamnolipid with biomass	96.4

Rhamnolipid is an environmentally friendly product ($EC_{50} \geq 1000$ mg/L for activated sludge respiration inhibition) (Jeneil, 2002) and it has been shown that rhamnolipid did not inhibit styrene biodegradation under anaerobic conditions. The initial styrene concentrations were 75 mg/L, 150 mg/L and 250 mg/L. For the 75 mg/L styrene solution, there was a 98.9% styrene removal by the biomass after day 4 and 89.0% of styrene was removed from the styrene rhamnolipid solution. 96.4% of styrene removal was achieved for 250 mg/L styrene in rhamnolipid with biomass and 98.2% of styrene removal was achieved for 250 mg/L styrene with biomass. Compared to the tests without biomass, significant styrene removal was achieved with biomass. These results indicate that the biomass could be used in this anaerobic biodegradation study; rhamnolipid didn't inhibit the anaerobic biodegradation experiments.

5.4 Batch experiments

Batch experiments were carried out using solutions with different concentrations of styrene and mixed with 300 mg/L of rhamnolipid and 5 mL/L acetic acid at different OLRs and HRTs. At the first stage, the substrate solution containing 5 mL/L acetic acid was carried out as a control. Styrene concentrations of 75 mg/L, 150 mg/L and 250 mg/L were used for the batch experiments. The experiment was run for 14 days for each styrene concentration. Figure 5-2 illustrates the variation of methane content in the batch experiment along with time. The methane content increased with longer experimental times and decreased with higher OLRs and higher styrene concentration. According to Galic *et al.* (1990), styrene was extensively degraded but no methane production was observed when styrene was the sole carbon and energy source during the experiments.

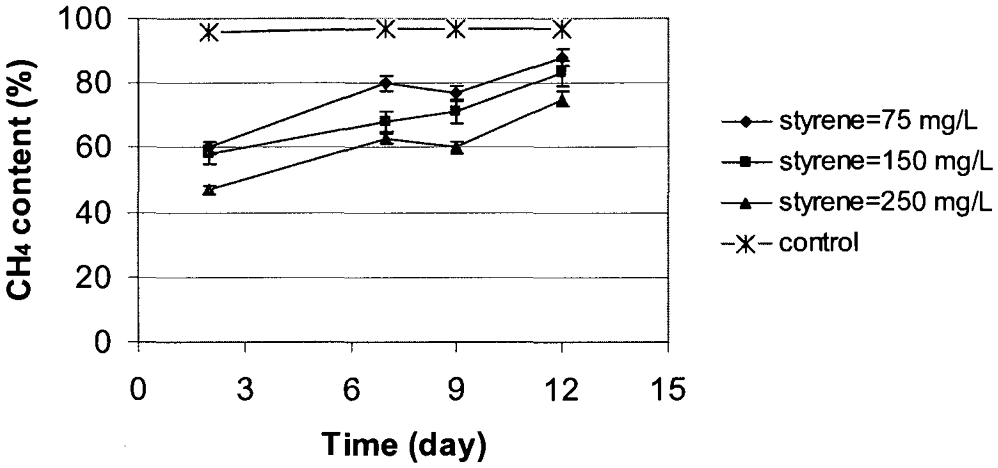


Figure 5-2. **CH_4 content from biogas at different initial styrene concentrations**

Acetic acid was used in the anaerobic experiment to improve methane production. From styrene side chain oxidation (Figure 2-3), styrene is degraded through phenylethanol to phenol and ended with acetic acid. It was 100 years after the discovery of methane by Alessandro Volta in 1776 that acetate was first implicated as substrate for methanogenesis (Ferry, 1993). During the 1960s, it became evident that most of the methane in freshwater environments is derived from acetate. Mulligan (2001) showed that anaerobic digestion can be considered as a three step process. The first step in the degradation process is hydrolysis to break down large molecules and dissolve suspended solids which enable easier transport into the bacterial cell for metabolism. The second step includes acetogenesis in which acetogenic bacteria produce acetic acid and hydrogen. The last step is the formation of methane and carbon dioxide from acetic acid by methanogenesis. Acetic acid is used as the supplemental carbon source to acclimate microorganisms for degradation of organic compounds (Sachs *et al.*, 2003, Flores *et al.*, 1999, Araya *et al.*, 2000, Sharma *et al.*, 1993).

Figure 5-2 illustrates that as time increased the methane content from produced biogas increased as well. Styrene may affect methanogenic bacteria to produce methane but did not inhibit the biomass during the anaerobic degradation process. Figure 5-3 presents a high styrene removal by the biomass. More than 95% of styrene was removed on day 2. At the end of the 12 day experiment, 98.4% of styrene was removed of the initial 250 mg/L. These results confirmed that biomass could be used for anaerobic biodegradation of styrene. A low COD removal (Figure 5-4) was achieved from the biodegradation experiment. Compared to 91% of COD removal by the control study, the 250 mg/L styrene solution showed a decrease to 16% COD removal. The control study was acetate with nutrients and the COD value equals 6.2 g/L. COD of styrene at 75 mg/L, 150 mg/L and 250 mg/L equal to 6.5 g/L, 7 g/L and 7.9 g/L. The low COD removal at 250 mg/L styrene solution indicated that styrene at high concentration may be toxic to the biomass. Araya *et al.* (2000) reported the toxicity limit (IC_{50} or the half maximal inhibitory concentration, represents the concentration of an inhibitor that is required for 50% inhibition of its target i.e, an enzyme, cell, cell receptor or a microorganism) of styrene was 146 mg/L for the acetoclastic activity. The toxicity limit (146 mg/L) was similar to the results from 150 mg/L styrene and 250 mg/L styrene solutions which were both achieved at low levels of COD removal (22% and 16%).

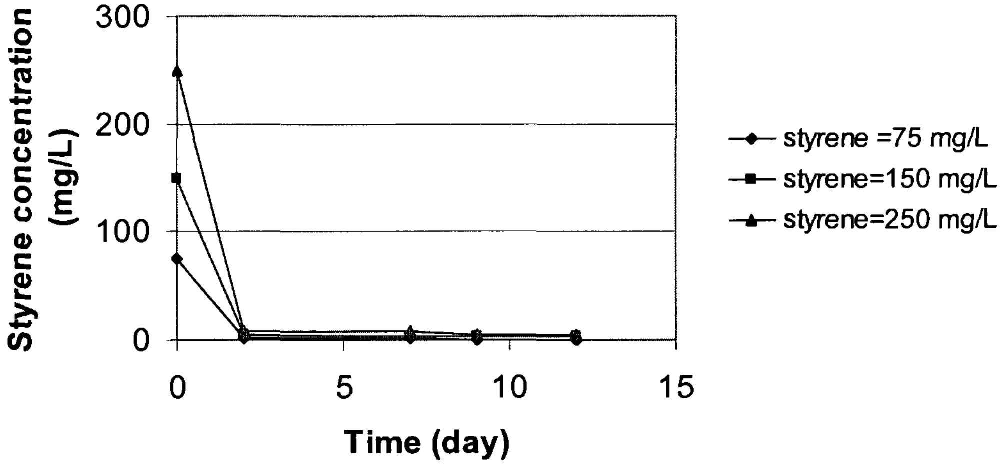


Figure 5-3. Styrene concentration reduction in batch experiments

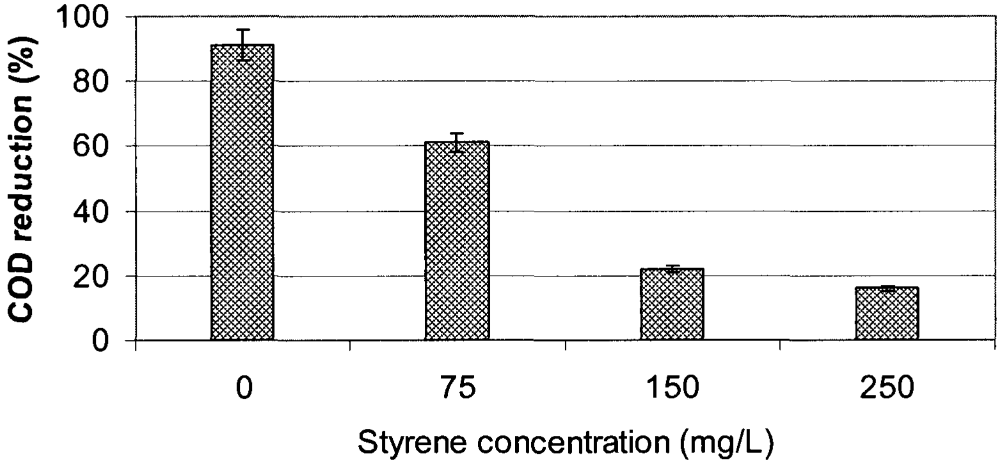


Figure 5-4. COD reduction in the batch experiment

At the second stage, 300 mg/L rhamnolipid was introduced in the batch experiments. 300 mg/L rhamnolipid with 5 mL/L acetic acid and nutrients with biomass were used as control and the styrene concentration was varied from 75 mg/L, 150 mg/L to 250 mg/L in the batch experiments.

Mohan *et al.* (2006) reported the biodegradability of rhamnolipid under aerobic, anoxic and anaerobic conditions. 47.2% of the COD removal was achieved under anaerobic conditions with a biogas production of 0.6 mL/mg COD removed, respectively. It indicated the biodegradability of rhamnolipid under anaerobic conditions. In this study with 300 mg/L rhamnolipid, a 93.9% COD removal was obtained with a 2 day retention time. The methane content was 87% from a total of 320 mL produced biogas in batch experiments (Figures 5-5 to 5-7). Rhamnolipid presented excellent biodegradability in the batch experiments; furthermore, it also improved styrene biodegradation performance in the batch experiments. Higher biogas production rates were collected with styrene in the batch tests, compared with styrene alone; styrene at 75 mg/L with rhamnolipid has produced 154 mL of biogas at a 2 day retention time.

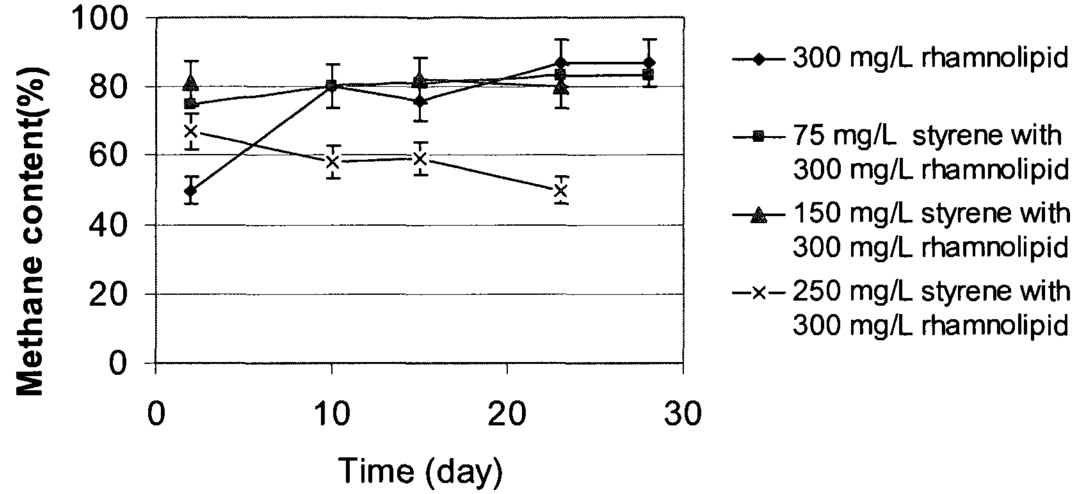


Figure 5-5. **Variation of methane content in the batch experiments with 0.3% rhamnolipid**

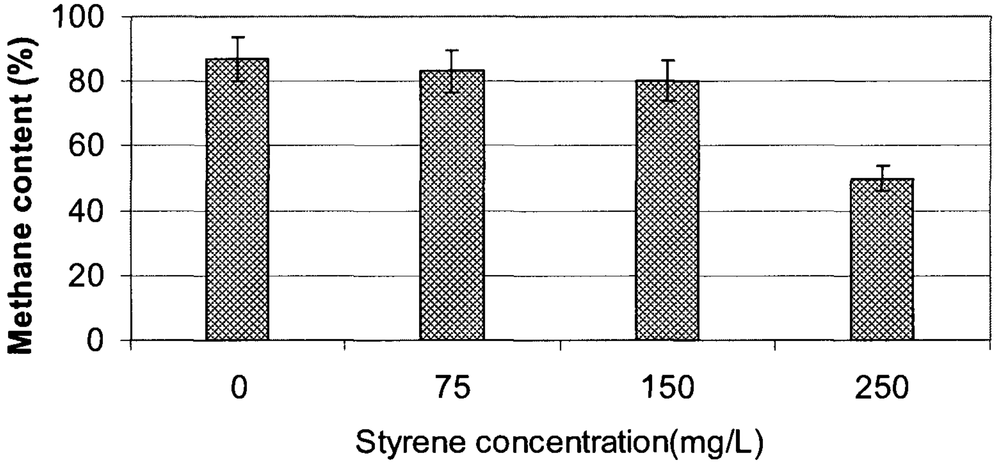


Figure 5-6. **Variation of methane content in batch experiments with rhamnolipid on day 2**

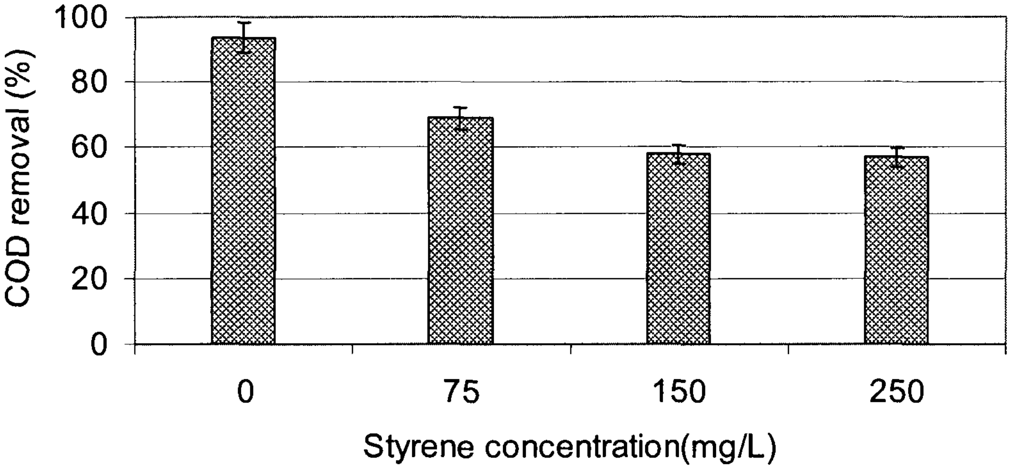


Figure 5-7. Variation of COD removal in the batch experiments with 0.3% rhamnolipid

The biogas production rate decreased as the styrene concentration increased. Low values of methane yield per gram of COD removed at three different styrene concentrations were presented in Figure 5-8. Styrene may affect methanogenic bacteria to produce methane in anaerobic biodegradation experiments. Styrene with rhamnolipid presented a higher COD removal and methane content than the styrene alone in the batch experiments. The variation of COD removal with different styrene concentrations was the same as styrene alone. As the styrene concentration increased, a decrease of COD removal was achieved. At 250 mg/L of styrene, 57% of COD removal achieved with rhamnolipid which is higher than the 16% COD removal with styrene alone. High levels of styrene and VFA removal were achieved in both batch experiments which indicated the biodegradability of styrene under anaerobic conditions (Table 5-3, Figure 5-9). Styrene concentration is a key factor in anaerobic biodegradation experiment. At 250 mg/L styrene, a fast decrease was observed in both biogas production and methane content results (Figures 5-6 and 5-8). Products of styrene degradation were found in both batch experiments (Table 5-2). Compared to styrene alone, higher concentrations of the degradation products were found in styrene with the rhamnolipid experiments. Some of these compounds, especially benzoic acid and phenol, indicate the most likely initial transformation reactions which could happen in anaerobic microbial communities degrading styrene in "the phenylacetate route" (Galic *et al.*, 1990). The styrene is initially transformed through additional water across the double bond in the side chain, with one carbon atom oxidation and reduction of another carbon atom. In the styrene side chain oxidation pathway (Figure 2-6), styrene was transformed from phenylethanol to butyric acid and ended up with acetic acid.

In both batch experiments, styrene alone achieved higher VFA removal than styrene with 300 mg/L rhamnolipid. VFA removal decreased as styrene concentration increased in both batch experiments. Since the VFAs are formed by acidogens and removed by methanogens, the high concentration of acetic acid (3.6±0.1 g/L) appeared in 250 mg/L styrene with rhamnolipid. This explains the low methane production in batch experiments due to the inhibitory effect of VFAs on methane production (Wong *et al.*, 2008). Rhamnolipid presented lower VFAs removal (68.9%) than acetic acid (87.8%) in the batch experiment. Styrene concentration played a key factor in VFAs removal. As methane production decreased, the removal rate for VFAs also decreased in both batch experiments (Tables 5-2 and 5-3, Figure 5-8). High volatile fatty acid concentrations in the system cause the inhibition of methanogenesis. In the presence of inhibitors, methanogenic activity cannot remove hydrogen and VFAs as quickly as they are produced. The result is the accumulation of acids, the depletion of buffering capacity and the depression of pH to levels that inhibit the hydrolysis/acidogenesis phase (Siegert and Banks, 2005). Styrene may affect methanogenic bacteria in the production of methane in anaerobic biodegradation experiments.

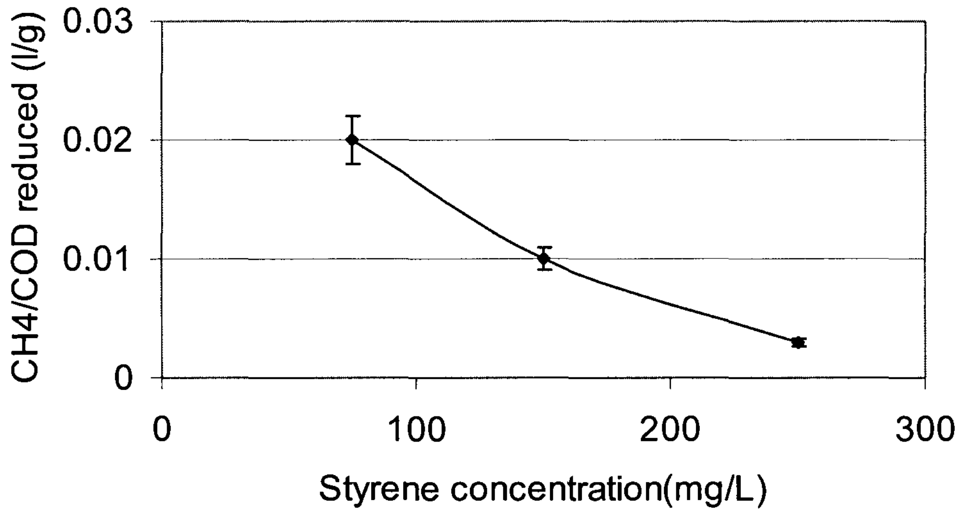


Figure 5-8. Methane yield per g of COD reduced in batch tests

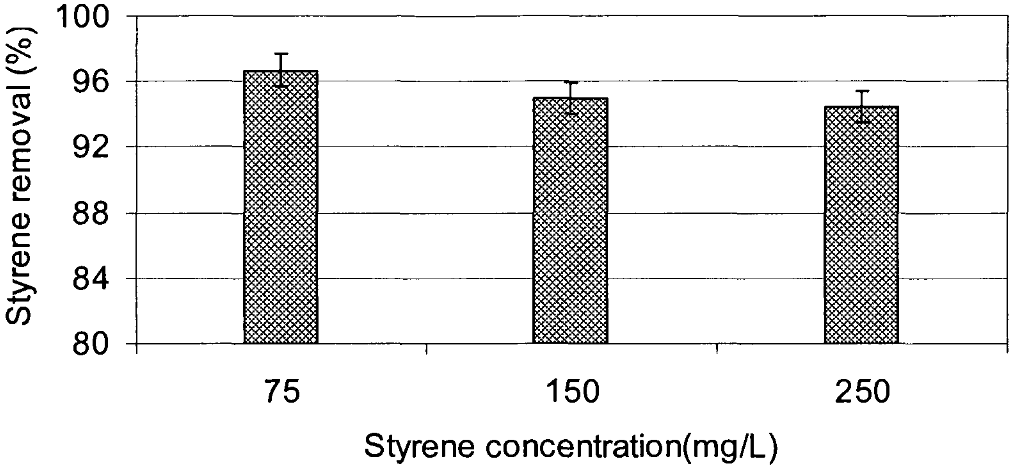


Figure 5-9. Variation of styrene removal in batch tests with rhamnolipid

Table 5-2. Effect of styrene concentrations on the VFA and styrene removal in batch experiments on day 2

	VFA Removal (%)	Styrene Removal (%)
	1st Stage	
5mg/L acetate	87.8	N/A
75 mg/L styrene	84.0	99.2
150 mg/L styrene	72.1	98.4
250 mg/L styrene	62.6	98.4
	2nd Stage	
300 mg/L rhamnolipid	68.9	N/A
75 mg/L styrene	48.7	96.7
150 mg/L styrene	52.0	94.8
250 mg/L styrene	38.0	94.4

Table 5-3. Composition of effluents from batch experiments with rhamnolipid

	1st Stage			2nd Stage		
Styrene (mg/L)	**75**	**150**	**250**	**75**	**150**	**250**
2-Phenylethanol (mg/L)	2.5±0.4			41.4±7.5	24.8±5.9	30.3±2.1
Phenylacetaldehyde (mg/L)		0.2±0.1	0.3±0.1	1.8±0.7	0.7±0.1	3.2±0.8
Benzoic acid (mg/L)	0.3±0.1	0.7±0.1			40.9±6.4	
Phenol (mg/L)		0.3±0.1	0.5±0.1	6.8±2.1		9.5±2.9
2-Ethylphenol (mg/L)					0.3±0.1	
Acetic acid (g/L)	0.8±0.3	1.4±0.2	1.9±0.3	2.6±0.1	2.4±0.4	3.6±0.1

5.5 Continuous flow experiments

One of the most successful types of reactor used for anaerobic treatment of wastewater is the UASB-reactor (Kalyuzhnyi *et al.*, 1996). The high efficiency of this reactor is achieved by maintaining high concentrations of active biomass in the reactor. A phase optimization period for UASB-reactor was established by changing the substrate daily. Reducing the retention time minimizes the conversion of formed VFAs to methane. A phase optimization period for UASB-reactor in this study was established by changing the substrate every 16 h in order to have a maximum styrene removal and COD reduction. For each styrene concentration, the optimization period was repeated for 7 times to achieve the steady state. Figure 5-10 illustrated the variation of pH at different styrene concentrations from the influent and effluent for the UASB-reactor. The pH value in influent was adjusted to 6.5±0.1; the effluent pH increased from 7.0 to 7.3 with styrene concentration increases. The pH value in the reactor was increasing due to VFAs which showed high VFAs removal and CO_2 and H_2 consumption by the methanogenic reactions in continuous experiments.

Variation of effluent COD from UASB-reactor at three different styrene concentrations is presented in Figures 5-11 to 5-13. As mentioned above, after 7 retention times to achieve a steady state, the results were collected and analyzed with standard methods as described in Chapter 3.

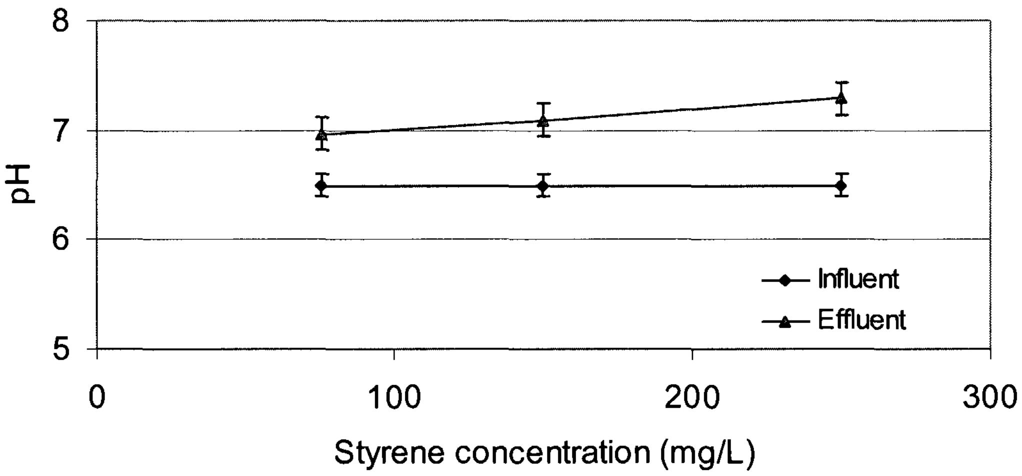


Figure 5-10. Variation of pH values at different styrene concentrations from influent and effluent for UASB-reactor

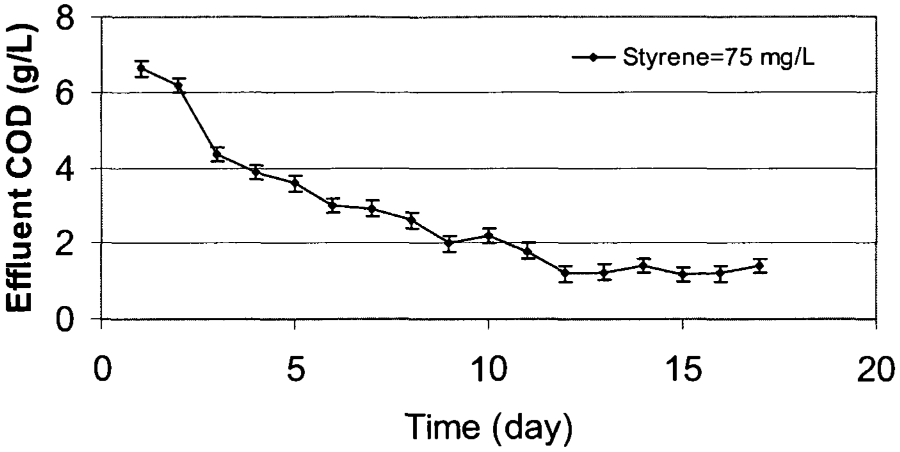


Figure 5-11. Variation of the effluent COD for a styrene concentration of 75 mg/L

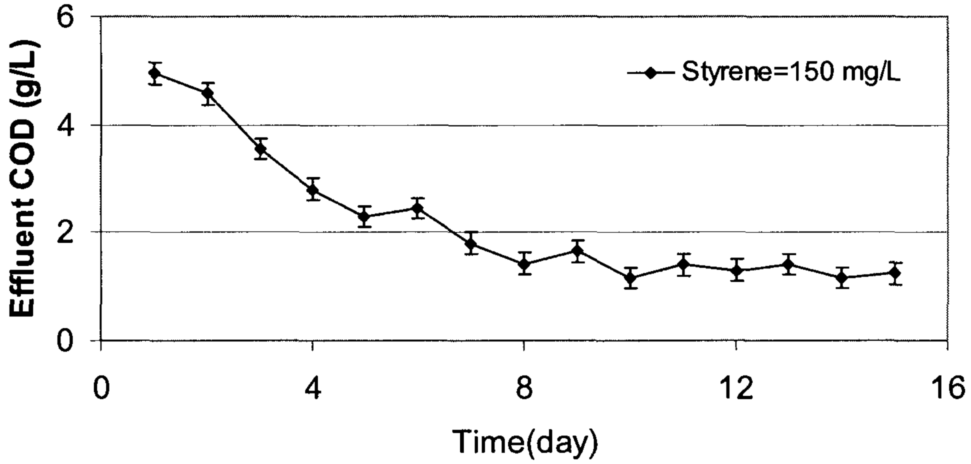


Figure 5-12. Variation of effluent COD for a styrene concentration of 150 mg/L

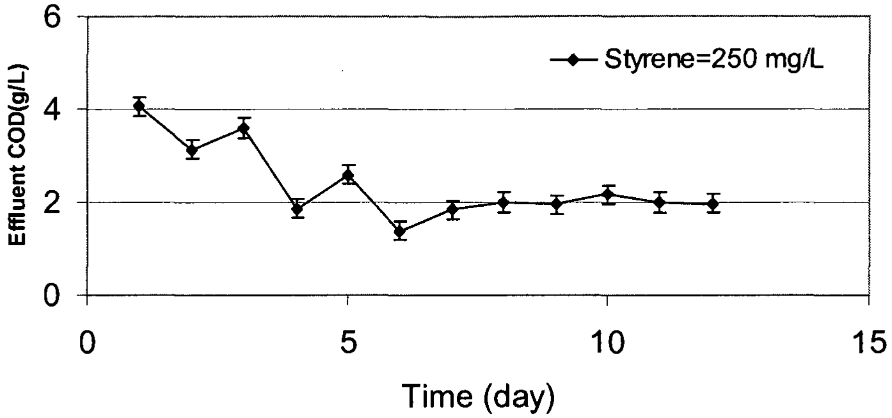


Figure 5-13. Variation of effluent COD for a styrene concentration of 250 mg/L

Results of the UASB-reactor performance under different OLRs are presented in Table 5-4. It is obvious that the results from UASB-reactor are much better than the batch experiments. High styrene removal efficiency was achieved in the continuous experiments. More than 96% of styrene was removed of the initial concentration of 250 mg/L. This result was close to batch results. 82% of COD removal in UASB-reactor was achieved with 84% methane content as styrene at 250 mg/L. Compared to 57% COD removal and 50% methane content in the batch experiment, the results from the UASB-reactor were better than the batch experiments especially with the higher styrene concentrations. VFAs removal rate kept the same trend as in batch experiments which decreased as styrene concentration increases. 38% of VFAs removal was achieved in batch experiment with 250 mg/L styrene in rhamnolipid and 55% of removal was obtained with UASB reactor. For each styrene concentration, the VFAs removal rate in

UASB reactor was higher than the batch experiments.

Biogas production decreased as the styrene concentration increased. The volume of biogas reduction is not proportional to the increment of styrene concentration. When styrene concentration increased 3 times from 75 mg/L to 250 mg/L, biogas production was reduced by 7 times. This reduction in biogas may be due to the toxicity of styrene to the biomass at a high styrene concentration. From Table 5-4, a conclusion could be made that UASB-reactor in this study could successfully biodegrade styrene with rhamnolipid as the wastewater. Styrene concentration is the key factor in performing anaerobic biodegradation experiments in a batch configuration and the UASB reactor.

Table 5-4. UASB-reactor performance

Initial styrene concentration (mg/L)	**75**	**150**	**250**
Styrene removal (%)	>99.9	97±2%	96±2%
VFA removal (%)	64.6±4%	62.2±2%	55.1±1.4%
COD removal (%)	87±9.8%	87±18%	82±8%
CH_4 content (%)	81±3%	82±5%	84±5%
Biogas production (mL/d)	110±15	55±7	16±4
Biogas/COD removed (L/g)	0.02	0.01	0.006

Table 5-5 shows the compositions of the effluent from the UASB-reactor. Degraded styrene as 2-phenylethanol, phenylacetaldehyde, 2-ethylphenol, benzoic acid and phenol were found in the effluents. The hydration of styrene results in the formation of 2-phenylethanol, which is subsequently oxidized to phenylacetaldehyde and phenylacetic acid. “Phenylacetic acid was easily biodegraded by methanogenic consortia degrading aromatic lignin derivatives to be possibly converted to benzyl alcohol and oxidized to benzaldehyde and benzoic acid” (Galic *et al.*, 1990). This side chain oxidation led to acetic acid. The anaerobic reactions were utilizing water as an oxygen source through hydration processes. Another biodegradation product, 2-ethylphenol, which was found in the effluent, indicated another styrene degradation pathway: ring oxidation. Due to the presence of a number of different types of styrene biodegradation products, two possible mechanisms (initial side chain oxidation and initial ring oxidation) were occurring simultaneously in both batch and continuous flow configurations. These results were also supported by Galic *et al.* (1990) who studied styrene transformation by anaerobic consortia. The initial side chain oxidation of styrene or “the phenylacetate route” which was named by Galic *et al.* (1990) is presented in Figure 5-14. Styrene initial ring oxidation is shown in Figure 5-15.

Styrene → Phenylethanol → Phenylacetic acid → Benzyl alcohol
→ Benzaldehyde → Benzoic acid → Phenol → Cyclohexanol

→ Butyric acid → Acetic acid

Figure 5-14. Styrene side chain oxidation (Galic *et al.*, 1990)

Styrene → 2-Ethylphenol → 2-Ethyl-1-hexanol → 2-Ethylhexanoic acid

Figure 5-15. Styrene ring oxidation (Galic *et al.,* 1990)

Table 5-5. Composition of effluent from the UASB-reactor

Styrene concentration (mg/L)	**75**	**150**	**250**
2-phenylethanol (mg/L)	1.1±0.4	5.1±1.8	30.3±4.2
Phenylacetaldehyde (mg/L)		10.9±1.6	
Benzoic acid (mg/L)	8.9±2.4		
Phenol (mg/L)	1.0±0.6	1.3±0.4	9.3±2.9
2-Ethylphenol (mg/L)		3.5±1.2	
Acetic acid (g/L)	1.8±0.5	1.9±0.8	2.2±0.6

A comparison of methane yield per g COD removed in batch and continuous experiments is illustrated in Figure 5-16. In both tests, the methane yield per g COD reduced decreased with styrene concentration increases. The results showed that continuous flow experiments have a better performance than in the batch experiments. A slight improvement of methane yield per g COD removed was observed at 250 mg/L styrene which indicated that the styrene concentration plays a key factor in the biodegradation experiments. Cronin and Lo (1998) studied anaerobic treatment of brewery wastewater using an UASB reactor. With 0.48 to 1.91 g COD/L .d organic loading rate, 91 % COD was removed and the methane yield per gram COD removed is 0.31L which is close to the theoretical value (0.4 L methane/ g COD removed). It should be noted that not all UASB reactors could match this value. Caixeta *et al.* (2002) studied using a UASB reactor for treating slaughterhouse wastewater. The results showed that as the organic loading rate increased from 2.7 to 8.7 kg/m^3.d, the produced biogas per gram of COD removed decreased from 0.84 L to 0.19 L. Hydrophobic functional groups such as aromatic and aliphatic chemicals were very toxic and showed a higher inhibition effect on methanogenesis (Sponza, 2002). Low methane production rate was reported by Araya *et al.* (2000), 7 mL methane per gram VSS per day was produced as 74% of styrene was methanized at an initial styrene concentration at 200 mg/L; however it was not degraded in an industrial wastewater at 6 mg/L which also contained acrylates and detergents.

Galic *et al.* (1990) found that styrene at concentrations ranging from 0.01 to 1.04 g/L was transformed to a variety of intermediaries including VFA and CO_2, with no methane formation. The styrene may present toxicity to the biomass when the concentration is above IC_{50} (146 mg/L for acetoclastic acitivity, Araya *et al.* 2000). The termination of methanization will lead to low methane production during the anaerobic biodegradation.

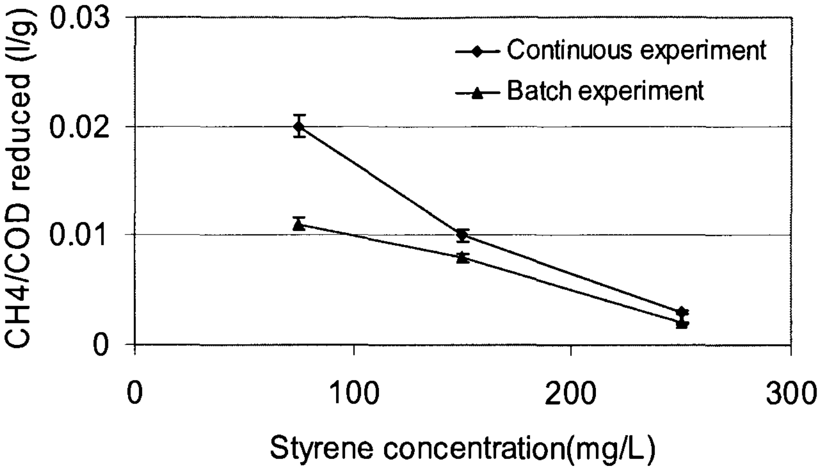


Figure 5-16. Comparison of the methane yield per g COD removed in batch and continuous experiments

Biomass characteristics in batch experiment and continuous experiments were compared in Table 5-6. Each sample was measured in duplicate. A slight decrease of TSS was observed in continuous experiments. The VSS/TSS ratio remained around 56% in the batch test and 57% in the continuous experiments. SMA results for the initial and final biomass were compared in Table 5-7. The entire biodegradation experiment was run for 10 months. The final biomass SMA activity was almost 10 times higher (L CH_4/g TVSS-d) than the initial biomass when the initial styrene was at 75 mg/L. A volume of 18 ml methane produced with a styrene initial concentration of 150 mg/L and it dropped to 3.6 ml methane when the styrene concentration increased to 250 mg/L. Similar results were reported by Araya *et al.* (2000) in which 7 mL methane was produced with an initial styrene concentration of 200 mg/L in the anaerobic experiments. Araya *et al.* (2000) reported that styrene presented a relative greater toxicity than other compounds of similar structure. The toxicity limit (IC_{50}) of styrene was 146 mg/L for acetoclastic activity, 47 mg/L and 166 mg/L for the methanogenic activity in the presence of 2.2 g/L of propionate and ethanol respectively. Styrene presents a relative higher toxicity than other compounds of similar structure. The toxicity limit (IC_{50}) for acetoclastic bacteria are 1.19 g/L, 0.6 g/L and 0.25 g/L for benzene, toluene and xylene. Styrene was toxic to methanogenic activity as was reported in the literature (Galic *et al.* 1990, Araya *et al.* 2000, Castilla *et al.* 2005, Aalam *et al.* 1993).

Table 5-6. Comparison of the biomass characteristics in batch and continuous experiments

	TSS (g/L)	**TVSS (g/L)**	**TVSS/TSS**
Batch experiment			
75 mg/L styrene	50.5	28.2	56%
150 mg/L styrene	51.7	29.4	57%
250 mg/L styrene	51.1	28.3	55%
Continuous experiment			
75 mg/L styrene	47.3	27.9	59%
150 mg/L styrene	48.3	28.3	58%
250 mg/L styrene	49.3	27.1	55%

Table 5-7. Comparison of SMA for the initial and final biomass

Styrene concentration (mg/L)	SMA (L CH_4/g TVSS-d) Initial biomass	SMA (L CH_4/g TVSS-d) Final biomass
75	3.8×10^{-3}	0.035
150	3.3×10^{-3}	0.018
250	2.9×10^{-3}	3.6×10^{-3}

5.6 Discussion

The comparison between the results from batch and continuous experiments led to the conclusion that anaerobic biomass could biodegrade styrene and styrene in rhamnolipid under anaerobic conditions. Batch experiments indicated that anaerobic biodegradation is feasible for treating leachates containing styrene and rhamnolipid. A continuous flow configuration showed better results than in the batch configuration. Rhamnolipid could improve biodegradation performance in both configurations. In the continuous configuration, leachates containing styrene and rhamnolipid were pumped from bottom of the reactor, biomass obtained better contact with styrene because of arising bubble of produced biogas. A steady operation condition was achieved in the continuous experiment. UASB-reactor could successfully biodegrade styrene with rhamnolipid. Styrene breakdown as 2-phenylethanol, phenylacetaldehyde, 2-ethylphenol, benzoic acid and phenol were found in the effluents. The presence of phenol and benzoic acid from experimental effluents indicated the initial transformation of styrene side chain oxidation.

The hydration of styrene results in formation of 2-phenylethanol, which is subsequently oxidized to phenylacetaldehyde and phenylacetic acid (Galic *et al.*, 1990). "The addition of water across the double bond in the styrene side chain could result theoretically in two products: phenylethanol (if the terminal carbon in the side chain is oxidized) and a secondary alcohol (if the subterminal carbon atom is oxidized)" (Galic *et al.*, 1990). Another early transformation intermediate found in effluents was 2-ethylphenol. 2-Ethylphenol can be derived from styrene during hydroxylation of the ring and a reduction of the double bond in the side chain. According to the styrene biodegradtion products that appeared, the two possible mechanisms, styrene side chain oxidation and ring oxidation occurred simultaneously in both batch and continuous experiments. Galic *et al.* (1990) also reported that the toxic effect from phenol and 2-ethylphenol to methanogenic cells rather than only inhibitory because no methanogenic activity revival was observed after the elimination of phenols through degradation. This may explain the low biogas production during the experiments.

Styrene degradability was evaluated by Aalam *et al.* (1993). Silicone oil was used to solubilize styrene and prevent its toxicity toward the microorganisms. The selected bacterial population was able to grow on styrene as a sole carbon source without any additives. The results suggest that at low concentrations in the water phase, the nutritional requirement for styrene degradation is minimal. Treating low styrene concentrations in wastewater using UASB reactor was studied by Castilla *et al.* (2005), Araya *et al.* (1999) and Araya *et al.* (2000). 24 mg/L of styrene in the mixture of methanol, isopropyl alcohol, ethylene glycol, methyl, acrylic acid, butyl and methyl acrylate, xylene and isopropyl acetate was fed to an UASB reactor. After decreasing the HRT to 4 and 3 h yielding 4.8 and 6.5 g COD/L-d, removal efficiencies decreased to 79 and 74% respectively. In this experiment, styrene has no effect on removal efficiency since it is at low concentration. Araya et al. (1999) evaluated the feasibility of the anaerobic treatment of an industrial polymer synthesis plant effluent which contains acrylates, styrene, detergents, a minor amount of silicates and a large amount of ferric chloride. A UASB reactor was used in this study with 4.3 kg COD/m^3-d. Within a 12 hour retention time, more than 75% COD removal was achieved with a 290 L CH_4/kg COD load. These promising results indicated that anaerobic digestion seems to be appropriate for the treatment of the mixed wastewater. UASB reactor was shown to be an efficient technology for the treatment of this wastewater. Araya *et al.* (2000) studied the biodegradability and toxicity of styrene in the anaerobic digestion process. 200 mg/L styrene was treated in batch configuration, 74% of styrene was degraded at a rate of 7 mL methane per gram of volatile suspended solid a day. This result was close to the result in SMA tests for final biomass (Figure 5-7) which showed 18 mL methane yield per gram of VSS when styrene at 150 mg/L. 6 mg/L of styrene with other compounds were treated in UASB reactor. UASB reactor operation was unstable due to other compounds in influent such as acrylates or detergents.

Styrene with rhamnolipid was shown to be feasible for anaerobic biodegradation with the UASB reactor. A high styrene removal rate was achieved for both batch and continuous experiments. Continuous experiments presented a higher COD removal rate and methane content in each styrene concentration.

Chapter 6

Conclusions, Scientific Contributions and Recommendations

6.1 General Remarks

This chapter will provide the conclusions of the results from this research. Soil adsorption tests were carried out to investigate the effect of clay ratio, organic matter content and contact time on the adsorption of styrene onto soils. The feasibility of using rhamnolipid as a washing agent to remove styrene from contaminated soil was studied after the adsorption research. The effects of rhamnolipid concentration, contact time, pH, and soil type were examined in this study. A comparison of batch and continuous washing removal results was made. Furthermore, anaerobic biodegradation was performed in this research to evaluate the biodegradability of styrene and rhamnolipid contaminated water. A UASB reactor was used for the anaerobic biodegradation experiments to biodegrade the leachates from soil washing studies before release to the environment. This chapter presents the main conclusions, expected scientific contributions and recommendations for future research.

Figure 6-1 shows the schematic proposed in-situ system. In this system, rhamnolipid will be injected through an injection well to the contaminated site. Leachate was collected and pump into an UASB reactor that is build above the ground. Monitoring wells placed at both sides in order to screen the washing performance.

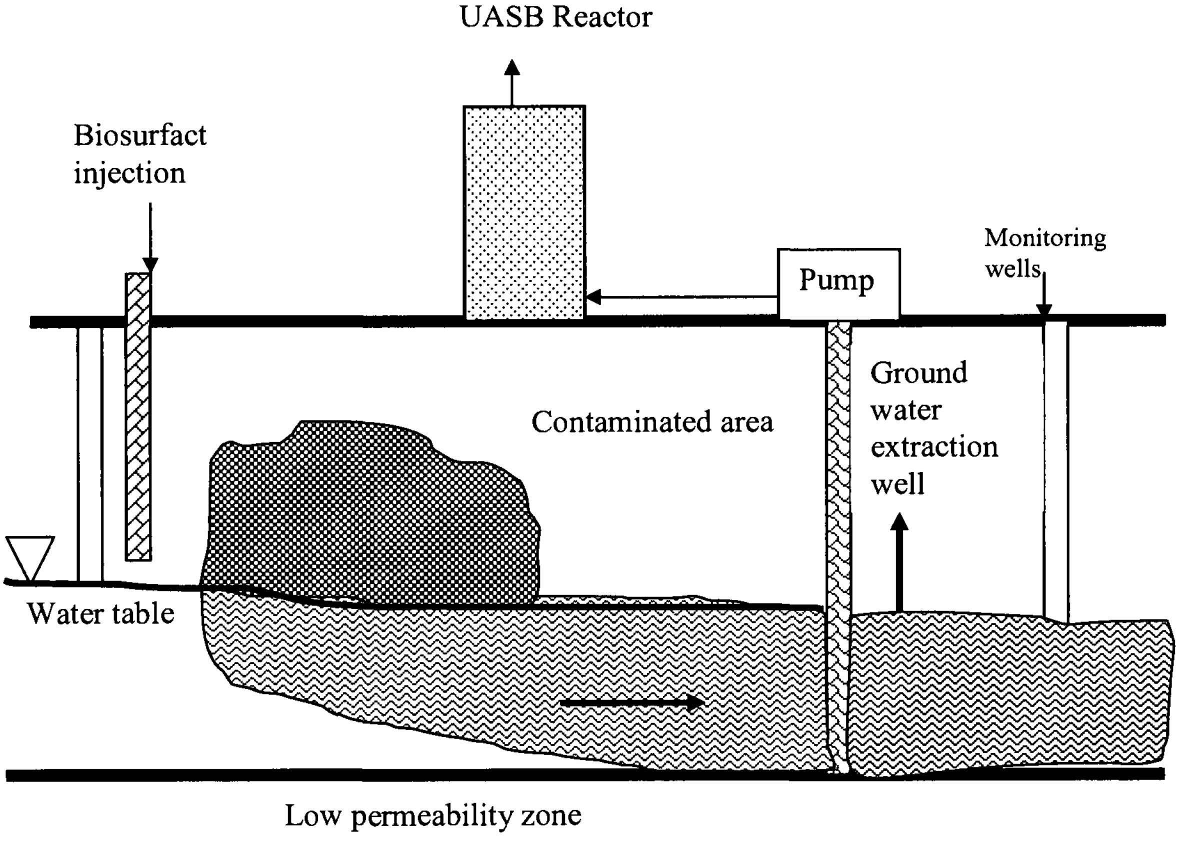


Figure 6-1. Schematic of proposed in-situ system

6.2 Conclusions

Batch adsorption tests and long term column adsorption tests were carried out in this research study to investigate the adsorbability of styrene onto soil system. The effect of clay ratio, organic matter content and contact time was examined in adsorption tests. From the batch tests results, soil type 1 (10.2% silt and clay, 5.3% organic matter content) presented the highest styrene adsorbability and bentonite presented the lowest styrene adsorbability. Langmuir adsorption isotherm and Freundlich adsorption isotherm were plotted to study the styrene adsorption process. The Freundlich isotherm model fit batch test results very well except bentonite. Soil type 1 also presented the highest styrene adsorbability in the column adsorption tests. 37% of styrene remained in the soil system after 90 days and 0.1% of styrene remained in the soil system after 16 months. The column study showed that under anaerobic condition, styrene may persist in the soil system for a long time.

Rhamnolipid was used as the washing agent in batch and continuous flow configurations to remove styrene from contaminated soil. The CMC of rhamnolipid was determined through surface tension measurements to be 20 mg/L. Styrene solubility was increased with an increase in rhamnolipid concentration. The effect of concentration of rhamnolipid, concentration of styrene, pH, time, soil type, and surfactant type were studied in both batch and continuous configurations. For continuous flow configuration, additional parameters including the effect of flow rate, porosity and hydraulic conductivity were also evaluated. The highest styrene removal rate (98.4%) was achieved at 1000 mg/L rhamnolipid in the batch test. A batch configuration presented better removal rate than continuous flow configuration due to the more complete contact of rhamnolipid with styrene. Increasing the concentration of rhamnolipid and decreasing the styrene concentration enhanced the styrene removal rate. A longer contact time did not increase removal efficiency in the single wash test. pH showed little effect on styrene removal in the batch tests. Continuous flow configuration presents the limited contact area between rhamnolipid and soil, increasing the flow rate generally reduced the removal in continuous flow configuration due to the reduction of the contact time between rhamnolipid and soil. Porosity and hydraulic conductivity were also found to be important parameters in the removal process since these parameters are related to the space between particles where the rhamnolipid flows (Chapter 4). Regarding the washing results in Chapter 4, R2 from rhamnolipid was determined as the main functional component in styrene removal tests. Soil type 1 presented the highest styrene removal rate among three types of soil and JBR-425 achieved the best styrene removal compared to JBR-215 and S-LF405.

The mechanism of removal of styrene from soil using rhamnolipid was studied through rhamnolipid adsorption, wetted surface area, zeta potential measurement, styrene solubility in rhamnolipid and interfacial tension studies. After the rhamnolipid solution contacts the soil surface area, some rhamnolipid adsorbs onto the soil surface. The two mechanisms of surfactant-aided soil washing are mobilization and solubilisation. The mobilization mechanism occurs at surfactant concentration below CMC. In this situation, surfactants reduce the surface and interfacial tension between the air/water, oil/water, and oil/soil systems. When surfactant concentration is above CMC, the solubility of styrene increased dramatically due to the aggregation of surfactant micelles. In this research, solubilization is the dominant mechanism since most styrene existed within soil samples as pure phase. Rhamnolipid in this study has shown great potential in styrene removal due to its low CMC and high sorption to soil. In this study, both mobilization and solubilization occurred during the experiments.

After styrene was removed from soil, the effluents from soil washing experiments which contain styrene and rhamnolipid were treated by an UASB reactor. Batch experiments indicated that anaerobic biodegradation is feasible for treating the leachates containing styrene and rhamnolipid. A continuous flow configuration showed better results than in the batch configuration. Rhamnolipid improved biodegradation performance in both configurations. High styrene removal and COD removal were achieved in both configurations. VFAs removal and biogas production were decreased as styrene concentration increases. Styrene breakdown as 2-phenylethanol, phenylacetaldehyde, 2-ethylphenol, benzoic acid and phenol were found in the effluents. According to the appearance of styrene biodegradation products, the two possible mechanisms, styrene side chain oxidation and ring oxidation occurred simultaneously in both batch and continuous experiments. UASB-reactor could successfully biodegrade styrene with rhamnolipid (Chapter 5).

6.3 Scientific contributions

In general, the findings of this research study will be important to advancing our understanding in the fate and transport of styrene in the environment. Use of rhamnolipid in styrene remediation may be developed as an environmental friendly process and cost effective option to reduce styrene contamination and avoid further contamination. Anaerobic digestion will be important in wastewater treatment regarding its low energy cost and less sludge production.

This research has contributed to scientific knowledge in the following aspects:

- First demonstration of using rhamnolipid in batch and continuous configurations to remove styrene from contaminated soil.
- Successful application of using in-situ soil washing technology to treat styrene contaminated soil followed by leachate biodegradation with the UASB reactor.
- Investigation of soil clay ratio and organic matter content are the main factors in adsorption of styrene on soil in both batch and long term column configurations. .
- Evaluation of the feasibility of using rhamnolipid to remove styrene from contaminated soil.
- First study on the solubilization of styrene by rhamnolipid.
- The influence of the soil type, rhamnolipid concentration, pH, styrene concentration and different surfactants on the removal of styrene from contaminated soil was studied.
- Determining the dirhamnolipid (R2) from JBR-425 is the main functional compound in styrene removal tests.

- Demonstration of styrene solubilization as the main mechanism of removal styrene from soil using rhamnolipid.
- Evaluation of the performance of high concentration of styrene contaminated water with rhamnolipid by an UASB reactor.
- Determination of the feasibility of using anaerobic biomass to biodegrade styrene with rhamnolipid wastewater under anaerobic conditions.
- Determining styrene concentration is the key factor affecting the UASB reactor performance.

6.4 Recommendations

The following aspects are recommended for future research work:

- Examination of the type of organic matter that affect styrene adsorption onto soil.
- Investigation of other factors affecting the styrene adsorption process.
- Investigation of the effect of temperature on styrene removal.
- Evaluation of other surfactants for styrene removal.
- Examination of the recovery of the biosurfactants from soil washing leachates.
- Investigation on the low biogas production from styrene anaerobic biodegradation experiments.
- Investigation of the optimization of UASB reactor for the treatment of styrene with rhamnolipid wastewater.

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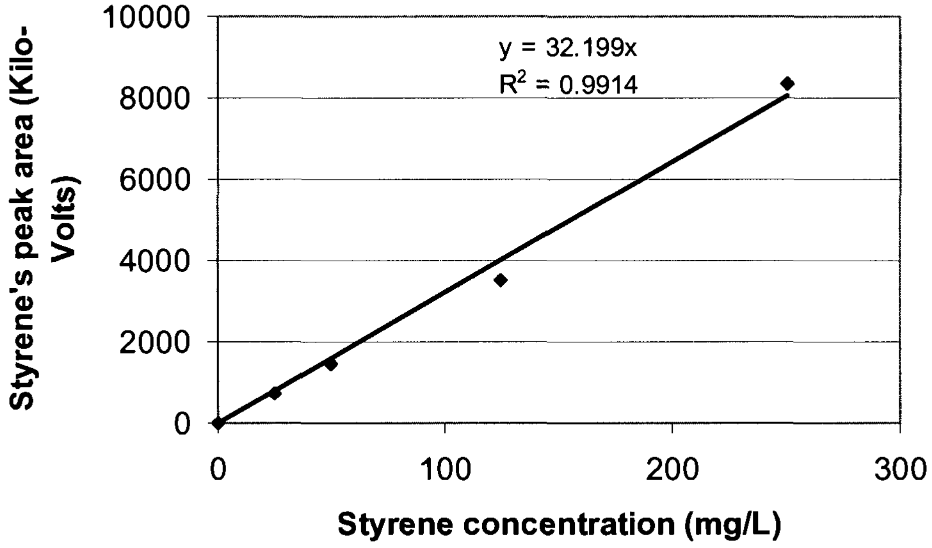
Appendix A

Styrene standard curve from HPLC

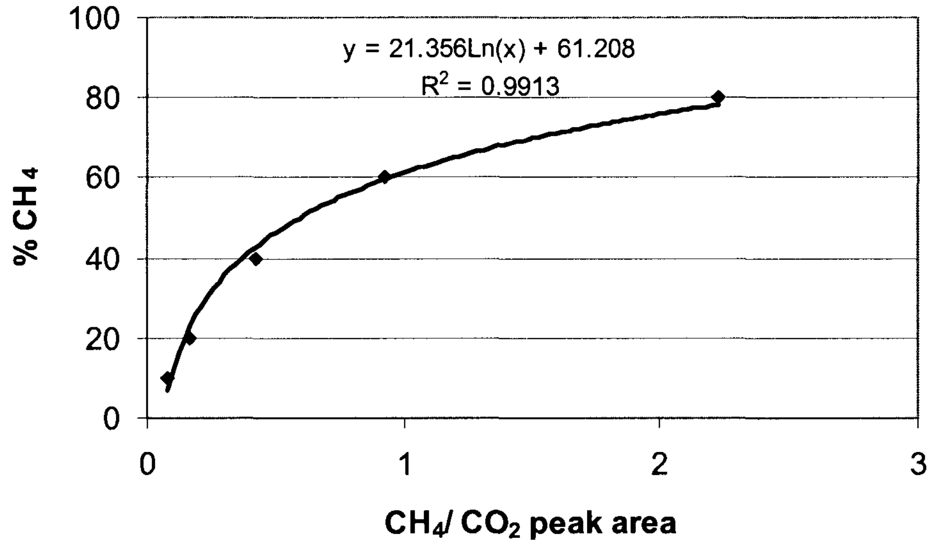
Styrene concentration (mg/L)	Area 1 (Kilo-Volts)	Area 2 (Kilo-Volts)	Area 3 (Kilo-Volts)	Area ave (Kilo-Volts)
25	762	749	733	748
50	1375	1472	1518	1455
125	3451	3506	3549	3502
250	8329	8401	8317	8349



Appendix B

Reference curve for measuring methane content of the biogas

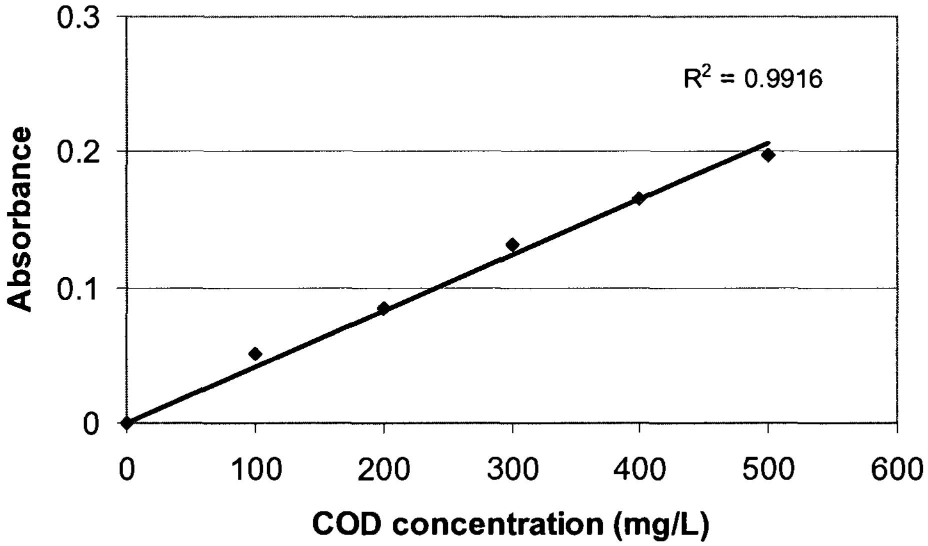
CH_4	CO_2	CH_4/CO_2	CH_4/CO_2	CH_4/CO_2 ave
10	90	0.0851	0.0721	0.078
20	80	0.1615	0.1658	0.163
40	60	0.4192	0.4313	0.425
60	40	0.9201	0.9375	0.928
80	20	2.0964	2.3487	2.222



Appendix C

Chemical oxygen demand (COD) standard curve

COD concentration (mg/L)	Absorbance
100	0.0506
200	0.0843
300	0.1317
400	0.1655
500	0.1976



Appendix D

Typical methane and carbon dioxide peaks from Gas Chromatography