Microbial degradation of BTEX in the vadose zone and its implications in the soil matric potential

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Thank you very much.

Dedicated

To my family and friends

Abstract

Benzene, toluene, ethyl benzene and xylene collectively known as BTEX, is a well-known industrial solvent which enters the subsurface through leakages from underground storage tanks, pipelines, accidental spills and leaching landfills. Fate of BTEX is mainly encountered by the microbial activity in the subsurface. Microorganisms may significantly influence the fate and transport of these compounds in the vadose zone by utilizing them as metabolite or co-metabolites. Meantime the growth of bacteria in vadose zone results in the modification of physical and hydraulic properties. The present study involves the microbial degradation of BTEX under batch and continuous mode and also the estimation of soil matric potential due to microbial growth in the unsaturated soil.

Firstly, BTEX resistant cultures are isolated from the petroleum contaminated soil and cultured on pseudomonas isolating agar. And then degradation studies are carried out in batch and continuous mode. Batch studies are performed at various initial concentrations 100mg/L, 200mg/L, 300mg/L and 400mg/L of BTEX as sole carbon source. Lab scale soil column study is then performed at inlet concentration of 500mg/L of each of the BTEX compound. The batch experiment shows the degradation rate of BTEX as 0.191 ppm/hr,0.234ppm/hr,0.350ppm/hr and 0.382ppm/hr respectively. In the batch experiment xylene is found to be degrading faster followed by ethyl benzene, toluene and benzene.

Secondly, Soil is packed at some initial moisture content and the flow experiment is started. Once the steady state is attained under unsaturated flow condition the final moisture content of the soil is noticed. For the moisture content observed during the biodegradation in the soil column, the matric potential values of the soil are then determined. The column experiment shows the degradation rate of BTEX as 0.285 ppm/hr,0.305ppm/hr,0.310ppm/hr and 0.217ppm/hr respectively. Ethyl benzene is found to be degrading faster followed by toluene, benzene and xylene. The suction values developed are found to be lesser in the top 0-15 cm depth of the column. Similar range of soil suction is developed in the middle (15-30 cm depth) and bottom region (30-50 cm) of the column. The result shows that microbial growth has a significant change in the matric potential of the unsaturated soil. The microbial growth has increased the suction value by 34% on an average along the column height. The increase in the matric potential is found to increase with the applied suction value. Higher the suction applied in the sand table apparatus, higher is the developed suction in the soil sample.

Nomenclature

$C_{i, p}$ = Concentration of compound ‘i’ in the phase ‘p’.

$C_{i, g}$ = Concentration of compound ‘i’ in the gaseous phase, atm or ppmv.

$C_{i, s}$ = Concentration of compound ‘i’ in the solid phase following sorption, mg kg^{-1}

$C_{i, s}^{max}$ = Maximum amount of adsorbate that can be adsorbed, mg kg^{-1}.

$C_{i, w}$ = Concentration of compound ‘i’ in the aqueous phase, mg L^{-1}.

D_{air} = Free air diffusion coefficient, cm^2s^{-1}.

D_{aq} = Aqueous phase diffusion coefficients, cm^2s^{-1}

$D_{e, p}$ = Effective diffusion coefficient in phase ‘p’(w = aqueous, g = gaseous),cm^2s^{-1}

f_i = Mass fraction of component ‘i’ in the mixture

$F_{i, d, p}$ = Diffusive flux of compound ‘i’ through phase ‘p’, cm $m^{-2}d^{-1}$

G_s = Specific gravity of soil sample, kg m^{-3}

H = Henry's law constant, dimensionless

K = First order decay rate, d^{-1}

K_b = BET adsorption coefficient, variable units

K_d = Distribution coefficient, mL g^{-1}

K_F = Freundlich adsorption coefficient, variable units

K_H = Henry's law constant, kPa m^3 $mole^{-1}$

K_h = Hydraulic conductivity, cm s^{-1}

K_L = Langmuir adsorption coefficient, variable units

K_m = Saturation coefficient for Monod kinetic reaction, mg L^{-1}

K_v = Volatilization mass transfer coefficient, variable units

M_{air} = Molecular mass of air, g $mole^{-1}$

M_i = Molecular mas of the compound i, g $mole^{-1}$

M_i, g = Molecular mass of gas, g $mole^{-1}$

M_{mix} =Molecular mass of the mixture, g $mole^{-1}$

m_s = Mass of soil, g

m_w = Mass of water, g

r = Retardation factor

S_i = Water solubility of pure compound i, g L^{-1} or mole L^{-1}

T = Temperature, °K

X = Biomass concentration, mg L^{-1}

X_i = Mole fraction of compound i in pure phase mixture

dh/dl = Hydraulic gradient, m m^{-1}

β = Function of η

δ = Dimensionless factor that accounts for constrictivity of pores

ε_t = Effective transport-through porosity

μ = Specific growth Rate

μ_{max} = Maximum specific growth rate, per day

S = Substrate concentration

θ = Total porosity

θ_v = Vapour phase porosity

θ_w = Pore water and air porosity of soils

ρ_b = Bulk soil density, g cm^{-3}

τ = Dimensionless factor that accounts for tortuosity through porous media

a, n, m = Fitting parameters

ψ = Total suction

C (Ψ) =Correction factor

U_w =Porewater pressure

U_a =Pore air pressure

π = Osmatic suction

$U_w - U_a$ =Matric suction

Contents

Chapter 1

Introduction

1.1 General introduction

Hydrocarbon contamination in the subsurface is found to be one of the major environmental threats in the recent scenario. Roughly about 600,000 metric ton per year of natural crude oil is seeping into the subsurface during exploration, processing, transporting and storing of petroleum and its derived byproducts [1]. Petroleum based hydrocarbons which enter the subsurface environment mainly due to leakage and accidental spills primarily consist of gasoline and diesel fuel. Gasoline is a refined petroleum product which is a mixture of hydrocarbons, additives, and blending agents. Among the hydrocarbons, aromatic groups constitute of about 30 percent by weight. The finished gasoline typically contains more than 150 individual aromatic compounds. Benzene, toluene, ethyl benzene and xylene collectively known as BTEX are the primary aromatic hydrocarbon which constitutes of about 50 percent by weight. BTEX is of more concern because of their relatively higher solubility in water and carcinogenic effect in humans. Fate of BTEX in the subsurface is understood through the study of (1) soil characteristics like grain size distribution, porosity, soil permeability, cation exchange capacity and organic content (2) composition, physical and chemical characteristics of BTEX which include solubility, boiling point, air diffusion co-efficient, vapor pressure and Henry's law constant (3) BTEX distribution in the subsurface (4) BTEX transport and transfer mechanism in the subsurface.

The transport of BTEX is caused by diffusion, advection and dispersion. During the course of transport BTEX in the subsurface may undergo the following processes. (1) Volatilize as soil vapor (2) adsorb to the soil particle (3) dissolution in the pore water (4) get trapped as LNAPL in the pore spaces (5) float on the watertable (6)

Dissolve in groundwater (7) abiotic chemical reactions (8) microbial degradation. Microbial degradation especially catalyzed by bacteria is considered as an important cleanup technology that one can adopt to remediate the subsurface environment contaminated with the hydrocarbons. Bacteria can degrade the BTEX compound by using them as source of carbon and energy.BTEX degradation can undergo aerobic pathway (oxygen as electron acceptor) and anaerobic pathway (sulfate, nitrate as electron acceptor).

Aerobic degradation of BTEX undergoes the following pathways. (1) In benzene it involves the addition of two atoms of molecular oxygen into the aromatic ring to form dihydrodiol with cis-configuration which is then dehydrogenated to form catechol. (2) In toluene, ethyl benzene and xylene it is either by oxidizing methyl group to carboxylic group and the formation of dihydrodiol by the attack of dioxygenase and the finally the formation of catechol or by the direct attack of the aromatic ring similar to that of benzene. Hence the formation of catechol is found common in the aerobic degradation of BTEX. The behavior of microbes in the variably saturated soil is different from that of fully saturated soil because of the presence of air-liquid-solid interfaces in the unsaturated soil. The microbial behavior may cause changes in the porosity, permeability, interfacial tensions values of the unsaturated soil. These changes are found to affect the soil matric potential which in turn affects the soil-water characteristic curve (SWCC) or soil-water retention curve.

SWCC is a graphical relationship between water content and soil suction. It primarily governs the engineering behavior of unsaturated soil. The SWCC is found to vary with several factors, such as soil type, texture, gradation, mineral composition, void ratio, water content, stress history and compacted level of the soil among which the water content and the stress history affect the SWCC to a greater extent. Hence understanding the effect of microbial activity in the hydraulic properties of the unsaturated soil is of prime importance in the recent times.

1.2 Objectives of the research

The primary objectives are

1. To isolate BTEX resistant species from the contaminated soil.
2. To study the growth pattern of the isolated BTEX resistant species in the presence of BTEX as the sole carbon source under batch mode.
3. To estimate the bio kinetic parameters for the isolated BTEX resistant species under batch mode.
4. To study the microbial degradation of BTEX resistant species under batch mode.
5. To study transport of BTEX in presence of microbial culture under unsaturated soil condition in continuous mode.
6. To estimate soil matric potential of the unsaturated soil under various conditions this includes Soil + water, Soil+water+BTEX with and without biotransformation.

1.3 Organization of the thesis

Chapter 1-Introduction. This chapter briefly discuss about the hydrocarbons and its entry into the subsurface environment. Gasoline is a hydrocarbon which consists of BTEX is given prime importance of the study. BTEX is found to be the compound of interest for this research owing to its behavior in the subsurface and its hazardous nature. The role of bacteria in attenuating the BTEX compound is discussed. Also a brief introduction about the changes in the unsaturated soil hydraulic properties is discussed. The role of microbes in altering such soil hydraulic properties is put forwarded. The objectives of the research study are then decided.

Chapter 2-Literature review. This chapter briefly discuss about the followings.

- The entry of aromatic compounds into the subsurface environment.
- The sources of such contaminants and the mode of distribution in the subsurface. The properties influencing the distribution, fate, transport and transfer of the hydrocarbons the transport phenomena like advection, diffusion.

- The sorption modes and the sorption models available for the hydrocarbon.
- The factors influencing the biodegradation of hydrocarbons in the subsurface. The mode of biodegradation and the degradation pathway in aerobic condition. The importance of matric potential and the impact of soil matric potential in the soil-water characteristic curve.
- The mathematical forms available for SWCC and the methods available for the determination of SWCC of the soil.
- The procedure involved in the determination of soil matric potential using sand table apparatus.

Chapter 3-Research Methodology. This chapter discusses the various experimental methods used for the study. The experimental methods include soil characterization, sample collecting and isolation of degraders, batch experiments and column experiments for degradation, fabrication of the experimental setup for column study, and determination of soil matric potential.

Chapter 4-Results and discussions. This chapter discusses about the results obtained in the soil characterization study, degradation study under batch and column modes, soil matric potential determined in laboratory.

Chapter 5-Conclusion. This chapter gives a thorough interpretations of the results obtained in the experiments. Conclusions drawn out of the experiments are also discussed in detail.

Chapter 6-Future scope. This chapter suggests the future scope of the research work.

Chapter 2

Literature review

2.1 Aromatic hydrocarbons in the subsurface

Petroleum hydrocarbon contamination is one of the major threats in recent scenario. The sources of petroleum hydrocarbon in the subsurface include leakage from underground storage tanks, accidental spills, residues from refineries, coal tar sites, and wood treating sites. Commonly seen hydrocarbons in petroleum are alkanes, cycloalkanes or naphthenes, alkenes, and aromatics [2]. Aromatics are the hydrocarbon which has alternate single and double bond between the carbon atoms. They form a closed ring structure. They are acutely toxic because of their carcinogenic and chronic effects in humans. Aromatics with more than one ring present in it are called as polycyclic aromatic hydrocarbon. Benzene, toluene, ethyl benzene and xylene collectively known as BTEX are the aromatic hydrocarbon seen in gasoline which constitutes of about 50 percent by weight of aromatic fractions .BTEX is of more concern because of their relatively higher solubility in water and carcinogenic effect in humans

2.1.1 Contamination,source and distribution

The level of hydrocarbon contamination in the subsurface depends on the volume and duration of hydrocarbon release, properties of gasoline, physical properties of the subsurface media and the flow conditions in the subsurface. Once the hydrocarbon enters the subsurface it will possibly contaminate vapour/gas, solid and liquid phases seen in the subsurface [3]. The major entry of hydrocarbons into the subsurface is due to leakages and accidental spills.

The other sources include infiltration of gasoline which results from vehicle accidents, effluents with gasoline diesel fuel and lubricant coming from service stations, residues from refineries, coal tar sites, and wood treating sites [4].

Gasoline released into the subsurface has the potential to influence all the zones of subsurface which includes vadose zone, capillary fringe zone and groundwater aquifer zone. Gasoline released in a larger quantity for a longer time, it forms a pool of free product at the capillary fringe zone or it may reach the watertable when they are capable of overcoming the capillary effect in the soils. Gasoline released in lesser quantity they are found trapped within the soil pores in the vadose zone [5]. Gasoline when present in the subsurface will start move under the influence of gravity towards the watertable. In case of less gasoline spills, it will move until the residual saturation level of soil. Residual saturation is the condition at which the flow of the fluid is made discontinuous. During large and continuous spills, gasoline will move till the capillary fringe zone and it takes the path along the capillary fringe associated with the watertable. As the consequence a pool of free product is found floating on the water table. The pool so formed will create some pressure head and will depress the watertable. Lower density levels of gasoline and the restricted pore volume in the subsurface will limit the vertically downward flow and causes the horizontal spreading [6]. When the free pool of gasoline found along the capillary fringe gets dissolved in the water, the capillary forces are altered in the capillary fringe zone and hence the fringe height is also changed [7].

Soil characteristics also found to have a greater role in the spread or distribution of the hydrocarbon in the subsurface. The interaction of charges between the infiltrating fluid and soil particle results in shrinking and swelling of the soil. Fluids with high dielectric constant like water (dielectric constant=78.5) causes shrinkage in clays which results in decreased permeability whereas the fluids with low dielectric constant value like benzene (dielectric constant=2.3) results in increased permeability values. Experiments showed that water based permeability value of clayey soil ranges from 10^{-7} to 10^{-11} cm s^{-1} whereas the same clay had the permeability value ranges from 10^{-4} to 10^{-7} cm s^{-1} in presence of benzene [8].

Residual saturation of the soil is a parameter which influences the partitioning of contaminant between various phases seen in the porous medium. It is defined by the degree of saturation at which gasoline flow becomes discontinuous and is made immobilized by capillary forces. Soil type, soil moisture and relative permeability of various fluids flowing in the porous medium are the factors which determine the

degree of residual saturation of the soil. A relative proportion of fine fractions (silt and clay) in the soil also affect the degree of residual saturation of the soil [9]. It is neither water nor the gasoline which fills the pore spaces of the soil in vadose zone. And the filling was decided based on the wettability of the fluids flowing in it. There exists a preferential spreading on the solid surface by one fluid over the other fluid in a multiphase fluid flow system. Generally the wetting phase will tend to coat the soil grain whereas the non-wetting phase will flow through large pore openings of the porous medium. There exists a condition such that the gasoline may act as wetting phase or non-wetting phase. When gasoline is made to behave as non-wetting phase (in dry soil condition) its tendency to retain in the pore spaces are more in the porous medium.

2.1.2 Composition and Properties

The properties of gasoline and chemical composition of gasoline varies considerably based on the origin of crude oil and the refining process methodology Generally it is a mixture of C-4 to C-10 hydrocarbon with the boiling point ranges from 23° C to 204 °C. Commonly seen hydrocarbons in gasoline are alkanes or paraffins, cycloalkanes or naphthenes, alkenes or olefins, and aromatics. Figure 2.1 shows the molecular structures of common hydrocarbons present in petroleum [10], [11].

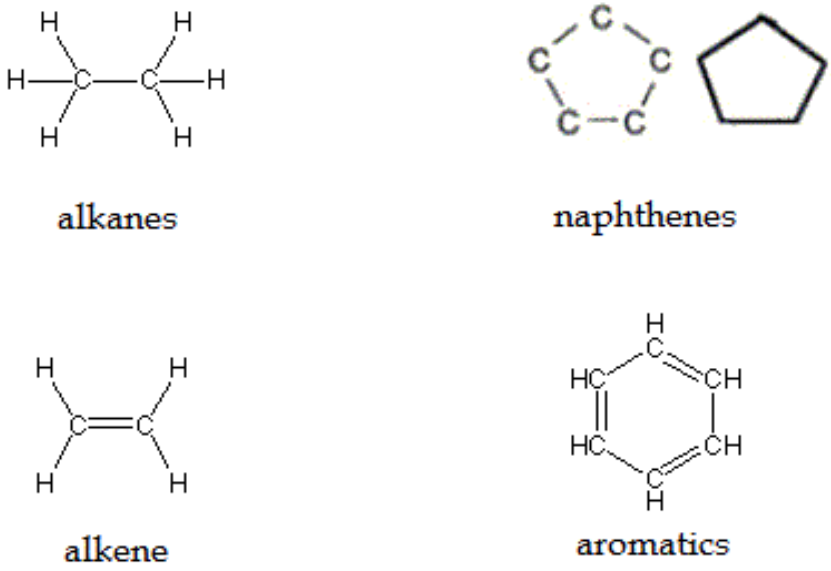

Figure 2.1 Molecular structures of common hydrocarbons present in gasoline

Table 2.1 Hydrocarbon components present in gasoline [11].

S.no	*Compound*	*Percent in weight*
1	Alkanes or Paraffins	
	n-alkanes	17
	Branched-alkanes	32
2	Alkenes or olefins	2
3	Naphthenes or cycloalkanes	5
4	Aromatics	30
5	Other components like octane enhancers, antioxidants, metal deactivators, icing inhibitors, corrosion inhibitors.	14

Aromatics are the hydrocarbon which has alternate single and double bond between the carbon atoms. They form a closed ring structure. They are acutely toxic because of their carcinogenic and chronic effects in humans. Aromatics with more than one ring present in it are called as polycyclic aromatic hydrocarbon. Benzene, toluene, ethyl benzene and xylene collectively known as BTEX are the aromatic hydrocarbon seen in gasoline which constitutes of about 50 percent by weight of aromatic fractions (Table 2.2).

Table 2.2 Aromatic fractions of gasoline [11].

S.no	*Compound*	*Percent in weight*
1	Benzene	3.2
2	Toluene	4.8
3	Ethyl benzene	1.4
4	Xylene	6.6
5	Other aromatic compounds	14

BTEX compounds are found to be lighter and lesser dense than water .They are commonly referred to as “light non aqueous-phase liquids,” or LNAPLs.They do not

readily mix with the groundwater; instead, they remain as a separate phase. This results in the multiphase flow condition in the subsurface. BTEX compounds are found slightly soluble in water. They will readily volatilize to vapour phase. Their viscosity is marginally comparable to water and hence the gravity induced flow through soil is only retarded by sorption. BTEX compounds are found to be biodegradable also. Changes in the physical properties of gasoline have its own impact on the attenuation process of gasoline [12] (Table 2.3).

Table 2.3 Important properties of BTEX compounds [13].

Properties	*Benzene*	*Toluene*	*Ethyl benzene*	*o-xylene*	*m-xylene*	*p-xylene*
Molecular weight (g/mole)	78.11	92.13	106.16	106.16	106.16	106.16
Boiling point (° C)	80.10	110.60	136.20	144.4	139.3	137
Melting point(° C)	5.5	-95	-94.97	-25	-47.4	13
Vapor pressure (mm Hg)	95.19	28.4	4.53	6.6	8.3	3.15
Density (g/mL)	0.87	0.87	0.87	0.88	0.87	0.86
Solubility (mg/L)	1791	535	161	175	146	156
Henry law constant (kPa m^3/mol)	0.557	0.66	0.843	0.551	0.730	0.690

Hydrocarbons generally undergo phase partitioning because of which they get transformed from one phase to another phase. The transfer between the phases is commonly due to dissolution, sorption and volatilization. Once the contaminant is transferred to its possible phases, transport will occur from higher concentration to lower concentration zone through advection and diffusion. Transport processes will be seen in both soilwater and in soil-gas.

Solubility of the compound determines the maximum extent to which it can get dissolved in the solvent. The solubility level depends on polarity and also the structure of the compound. Polar compounds are miscible with water where as non-polar are immiscible [14]. Gasoline is a LNAPL which has mixture of hydrocarbons

and the solubility of individual component is described by Raoult's law. Raoult's law in its simplified form is given as follows

$$C_{i,\,w} = X_i\, S_i$$

Volatilization describes the transformation of a contaminant either in it pure phase or in aqueous phase to gaseous phase. It causes a chemical potential gradient between two phases which results in molecular diffusion. When the vapour phase and the pure phase of the contaminant are at equilibrium, the pressure contained in the system is called vapor pressure. When the vapor pressure of the compound is attained in the system, volatilization will be ceased. Difference in the vapor pressures of the individual compound results in difference in the volatilization rates [15].

Henry's law coefficient describes the equilibrium concentration ratio between the aqueous phase and the vapour phase. Henry's law gives the relation between mole fractions of a compound in aqueous phase at equilibrium and the atmospheric partial pressure of the same compound in gaseous phase. In other words it is the ratio between concentrations of a compound in liquid, in molar units to the partial pressure of the compound in vapor phase connected with the dimensionless Henry's law constant (H).

$$H = C_{i,\,g} / C_{i,\,w}$$

Henry's law is valid for dilute concentrations and is more sensitive to temperature [16].Hence the environmental factors which influence the transformation of the compound are those factors which influence the partitioning of the compound between solid, aqueous and vapor phase.

2.2 Fate of hydrocarbons in the subsurface

The common abiotic processes which determine the fate of BTEX compounds in the subsurface are dissolution, volatilization, sorption and chemical oxidation/mineralization. Biodegradation also plays a vital role in the fate of BTEX compounds in the subsurface [17].

2.2.1 Sorption

Sorption is a physio-chemical process in which one substance is made attached to another substance. It is the common term used for both absorption and adsorption processes. The attachment of a substance in one state into another substance of different state is called absorption. The bonding of components a phase onto the surface of another phase is called as adsorption.i.e the process by which the compounds of a fluid phase are transferred onto the surface of the solid particles is called adsorption. Transferred compounds are called as adsorbate or simply sorbate and the solid particle to which adsorption occurs is called as adsorbent or simply sorbent. The process is dependent upon the tendency of one component in the mixture to get adsorbed more readily than the other and also the relative amount of organic content and mineralogical composition of the sorbents. The process is found reversible. The reversible process Sorption-Desorption will occur till the equilibrium concentration of the compound is achieved in both the phases [18].

2.2.2 Sorption mechanism

The common bonding mechanisms seen in the adsorption of hydrocarbon are π-bond and bonding due to weak physical forces. Aromatic compound like BTEX undergo π-bond mechanism which results in the immobilization of aromatic compounds around the soil organic matter. Π bonding occurs as a result of overlapping of π orbitals between aromatic and soil organic compounds [19]. Also the week Van Der Waal's force of attraction causes sorption of BTEX onto the soil organic matter. When the molecules are very close, Van Der Waal's force of attraction will be high and the hydrocarbons are sorbed onto the surface [14].An additional factor which causes the sorption of hydrocarbon is its hydrophobicity. Entropy driven reaction between soil surface and hydrocarbon is created due to the weak bonding force called hydrophobic bonding.

Several conceptual models have been developed to explain the sorption activity. One of the models explains that the sorption occurs as layers. Monolayer is formed onto the sorbent sites by direct contact of the molecule to be sorbed onto the surface. A second layer of molecules are then sorbed onto the primary layer. Another model for sorption explains that sorption process is the result of diffusive limitation [20].Sorption of hydrocarbons onto the soil particle is highly influenced by the

organic content. This is because the presence of organics increases the sorption sites for the organic compounds. Changes in the temperature also affect the rate of sorption levels. In general an increase in the temperature will decrease the sorption rate [21].

2.2.3 Sorption Isotherms

Sorption of hydrocarbons onto the soil is explained with various sorption isotherms which include linear approximation model, Freundlich, Langmuir, and BET isotherm models. Each isotherm model is described below and their applicability for sorption of hydrocarbons onto soil is then discussed. An inherent assumption is that in all the isotherm models equilibrium state is attained in the system.

Linear approximation model states that the amount of the sorbed material is directly proportional to the concentration of that material in the solution. This model assumes that there is no limit for the maximum concentration of the material to be sorbed. Linear approximation model is approximated with the following equation

$$C_{i,\,s}=K_d\,C_{i,\,w}$$

Investigations have been done for sorption of toluene in clayey soil. The linear equilibrium coefficient K_d is found in the range of 11.3 mLg^{-1} to26 mLg^{-1} [22].

Freundlich isotherm assumes that with the increase in the coverage of adsorbent surface the adsorption energy will decrease logarithmic. This isotherm is mostly applied to the sorption of contaminants from the dilute solutions. Isotherm is presented in equation as follows

$$C_{i,\,s}=K_F\,C_{i,\,w}^{\,(1/n)}$$

The coefficients n and K_F are determined plotting the sorbed concentration and solute concentration in log scale. Slope of the line will give the value of (1/n) while the intercept gives K_F. Sorption of benzene and o-xylene onto a fine sandy soil is investigated using this isotherm approximation and the coefficients are obtained as follows [23] (Table 2.4).

Table 2.4 Sorption coefficients for benzene and o-Xylene

compound	K_F $mg\ g^{-1}$	*n*
Benzene	2.33×10^{-3}	1.08
o-xylene	6.17×10^{-3}	0.87

The Langmuir adsorption model was developed to describe the adsorption of gas onto the solids. It assumes that the adsorbate will get attached to the sorbent at some definite homogeneously localized sites to form a monolayer of the sorbate. The process is then stopped once the monolayer has been formed. Another assumption of this model is that the heat of adsorption is constant over the entire monolayer and there is no lateral interaction between the sorbed species. The isotherm model explains that the system attains equilibrium once the surface is completely covered. Furthermore sorption will occur only after an equal number of sorption sites are formed due to desorption [15], [24]. Langmuir isotherm is presented in equation as follows

$$C_{i,s}=K_L \ C_{i,s}^{max} \ C_{i,w} / (1+ C_{i,s}^{max} \ C_{i,w})$$

BET isotherm was developed by Brunauer, Emmett and Teller for the adsorption of gas components onto the solid surfaces [14]. It is an extension of Langmuir isotherm theory as the physical adsorption of molecule cannot be limited to monolayer adsorption. Hence this theory explains that there exists a multilayer adsorption of molecules onto the solid surface [20]. The theory is based on the balancing rate of evaporation and condensation for various adsorbed layers. The theory describes a multilayer adsorption which is characteristics of bonding by weak Van der Waal's force [24]. The equation describing the BET model is given as

$$C_{i,s}=K_b \ C_{i,s}^{max} \ C_{i,w} / (S_i- C_{i,s}) (1+ (K_b -1) \ C_{i,w}/ S_i)$$

The figure below is to express the various sorption isotherm models for organic compounds (Fig 2.2).

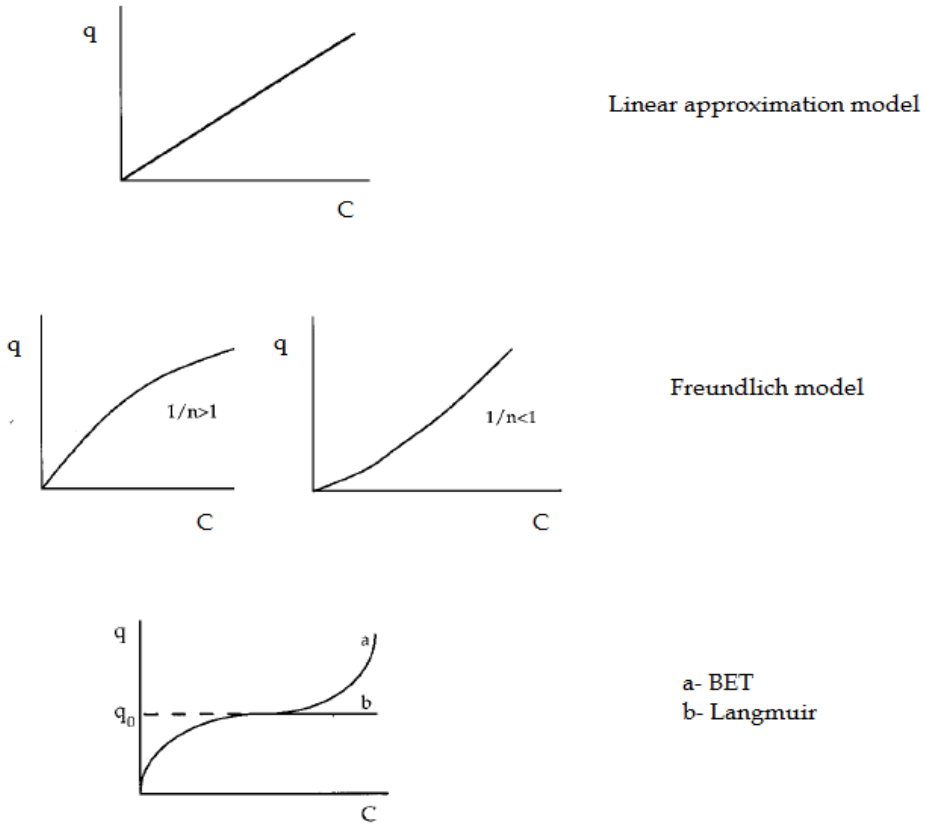

Figure 2.2 Typical isotherm models describing sorption of organic compounds

2.2.4 Importance of Sorption in contaminant transport in the subsurface.

The mass transport of the contaminant in the porous medium is influenced due to sorption because of the removal of the contaminant from the bulk solution [14], [24], [25]. The removal of these contaminants causes retardation of the both fluids and gases which get transport through the soil matrix due to diffusion or by advection [14]. The retardation of the contaminant occurs in three stages [24]. Each stage is an attempt to bring equilibrium in the concentration between various phases present in the system. When the contaminant in these phases are found to have different solubility and vapor pressure values no true steady state will be attained. Because the component fractions present in various phases will be continuously changing [24].

2.3 Transport of hydrocarbons in the subsurface

Bulk transport of BTEX also occurs in the subsurface due to diffusive and advective transport processes.

2.3.1 Diffusion

The transport of contaminants seen in gas phase in the subsurface is primarily through diffusive transport mechanism. Also the aqueous phase of the contaminant will be subjected to diffusion under low hydraulic conductivity conditions, as seen in clays. Hence it is inferred that irrespective of the phases diffusion is more important in low permeability soils. The term diffusion refers to the random molecular walk of molecules which makes the mass flux to flow from higher concentration to lower concentration area. This eventually results in the spreading of concentration peak with time. Diffusion is found to be dependent on the mass and volume of the molecules and the medium through which diffusion occurs [14].

Diffusion through liquid and gas phases is mainly due to the Brownian movement of the molecules which means the random erratic zigzag movement of the molecules that occur without any external cause like stirring or convection. In other words diffusion is due to the result of thermal motion of the molecules, which is caused due to constant collision of the particles with the neighboring particles [26].

Diffusion is described through Fick's first law as

$$F_{i,d,p} = - D_{e,p} . d/dx(C_{i,p})$$

Conservation of mass will give us Fick's second law which describes the change in the concentration of solute with respect to time. The equation is given as follows

$$d/dt\ (C_{i,p}) = D_{e,p}\ \ d^2/dx^2(C_{i,p})$$

Estimation of diffusion coefficient is important in predicting the mass transport in the subsurface region. The value is different for different phases flowing through the soil matrix. Studies showed that the diffusion of water is 10^5 times slower than gases. Palmer found that the water phase diffusion and vapor phase diffusion values are in the following ranges [7] (Table 2.5).

Table 2.5 Diffusion coefficients for various phases

Phase types	*Diffusion coefficient* $m^2 s^{-1}$
Water phase diffusion	10^{-9}
Vapor phase diffusion	10^{-5}

An empirical relation to predict the diffusion coefficient of vapor phase in the porous media was given by Millington and Quirk. The relation is based on the total porosity and air filled porosity of the medium. The equation to describe the diffusion coefficient as per Millington and Quirk is given as below [5] [14] [27].

$$D_{e,g} = D_{air} \left[\theta^{(10/3)} / \theta\right]$$

For accounting the soil properties in the transport the free air diffusivity is converted to an effective diffusivity of the soil matrix using the properties like porosity (θ) and tortuosity (τ) [28].

$$D_{e,g} = D_{air} \left[\theta / \tau\right]$$

The effective diffusive coefficient for aqueous phase is described as follows [14].

$$D_{e,w} = D_{aq} \left[\varepsilon_t / \delta\right]$$

Where D_{aq} is the diffusion rate through water and ε_t, δ are dimensionless factors that account for tortuosity and constrictivity of the pores respectively. Effective aqueous diffusion coefficient for benzene in clay is obtained using the equation. The value is found to be in the range of $2x10^{-7}$ cm^2 s^{-1} to $2x10^{-8}$ cm^2 s^{-1}[14]. Effective diffusion coefficient of BTX in clay with total porosity of 0.54 was determined and tabulated as below (Table 2.6).

Table 2.6 Diffusion coefficients if BTEX in air and in aqueous phases

Compound	D_{aq} $x\ 10^{-5}\ cm^2\ s^{-1}$	D_{air} $cm^2\ s^{-1}$
Benzene	1.02	0.094
Toluene	0.906	0.084
o-xylene	0.819	0.0735
m-xylene	0.819	0.0735
p-xylene	0.819	0.0735

2.3.2 Advection

Subsurface pressure gradient is the factor responsible for advective transport of the BTEX vapor in the subsurface. When the soil vapor has different composition of gases in sufficient concentration compared to atmospheric air subsurface pressure gradient will be developed in the vadose zone. Subsurface pressure gradient will be developed in response to barometric pressure changes also.

When hydrocarbons enter the subsurface, the density of the soil gas will be increased and it has the potential to spread the vapor plume by density driven flow. However sufficient differentials in density must exist for the process to occur. Presence of chlorinated compounds has significant potential to produce density driven flow [29] [30]. Among BTEX it is found that only benzene may be subjected to density driven flow. The other compounds like toluene, ethyl benzene and xylene may not have affected by this density driven flow because of their tendency to partition to aqueous phase [30].

Out gassing which occurs due to high wind and low barometric pressure has the influence in the transport in subsurface region [11]. Horizontal soil vapor transport is found to occur up to 10 m d^{-1} in the sandy soils if there is a barometric pressure change of less than 3kPa. This is because a change in the barometric pressure results in entry of atmospheric air into the subsurface [31].

2.4 Biodegradation of hydrocarbons

Partitioning does not cause the removal of the contaminant from the system. It results only in the transformation of the phases. Degradation is the process which results in the loss or decrease of the contaminant from the system. Degradation activity carried out by microorganisms is called as biodegradation. BTEX degradation studies were extensively carried out with the bacterial consortia from sewage sludge, groundwater microorganisms, and indigenous soil [53].

Soil bacterium includes Alcaligenes, Arthrobacter, Acidovorax, Agrobacterium, Aquaspirillum, Brevibacterium, Bradyrhizobium, Variovorax and Stenotrophomonas. Common BTEX degraders from sewage and fresh water are Ralstonia [54], Microbacterium, Mycobacterium, Azoarcus [55], Thauera [56] Burkholderia [57] and Sphingomonas [58]. It is considered as one of the most

significant natural attenuation process of the contaminant from the environment. The biodegradation ends up with the formation of carbon dioxide and water is called as mineralization process [15].

2.4.1 Factors influencing biodegradation

The *chemical properties* like solubility, degree of branching in the hydrocarbon, nature of the substituting compound and saturation level of hydrocarbon will govern the preferential use of hydrocarbon for the biodegradation. For instance linear chain hydrocarbons are easily biodegradable when compared to branched hydrocarbons. Solubility is important because the microorganisms obtain their nutrients from the solution only.

High volatility and higher tendency of the compound for sorption will decrease the availability for microbes. Microorganisms first prefer the most available nutrient compound and moves onto the next preferred compound. The order of preferential use of hydrocarbon by microorganism in the subsurface has been proposed as follows [15]. The compounds are listed in decreasing order of degradability by the microorganisms

- Linear alkanes
- Hydrocarbon gases
- Alkanes
- Branched alkanes
- Alkenes
- Branched alkenes
- Aromatics
- Cycloalkanes

Several *environmental factors* are influencing the biodegradation of the compounds in the subsurface. This includes changes in the temperature, pH value, soil moisture content, nutrient availability, oxygen availability. Any unfavorable condition of these factors makes the microorganisms dormant or even dies in some situations.

The *optimum soil temperature* range required for the microorganism in the subsurface is from 5°C to 40° C [15]. However it varies from species to species also. Some microbes function effectively even in extreme condition of temperature ranging from -2°C to 70° C. the bacterial population developed in colder regions

will have more resistant to the temperature fluctuations. An increase of 10° C will result in double the biodegradation rate [11].

The *pH level* required for the soil bacteria for efficient biodegradation ranges between 7 and 8. However pH value from 6 to 9 is found tolerable for the microbes. When pH value decreases it causes increased anionic adsorption. Any changes in the sorption-desorption level of the contaminant onto the soil surface will affect the nutrient availability for the microbes [15].

Nutrient availability and *the moisture content* are directly in relation with respect to the impact on biodegradation. Because the nutrients required for the microorganisms are obtained from the aqueous solution available in the soil. The common nutrients required for the microorganisms include nitrogen, phosphorous, sulfur and carbon [11] [15]. The optimized C: N: P ratio for biological activity has been proposed after several studies are 100:10:1 [40]. The necessary carbon to nitrogen to phosphorous ratio is (100:15:3). Even with the periodic supply of oxygen for the degradation process, the microbial activity is found limited because of the absence of nutrients [41]. Also the addition of nutrients makes the degradation of gasoline components faster [42]. Although all the bacteria need nitrogen in some form to maintain the cell growth the choice of nitrate form added to the system depends on abiotic factors of the environment.

When the *microbial population* of specific catabolic abilities are introduces into the environment, the degradation activity will be enhanced .Microbes with the specific catabolic abilities are obtained because of the acclimation of the species to their compounds of interest. The possible ways by which the acclimation will occur in the microbial community are as follows [32].

- Induction of enzymes
- Genetic changes

Acclimation due to enzyme induction usually occurs in an hour. Some species will get acclimatize for several days. Microbial population in the soil is found to affect the degradation rate. This becomes a limiting factor only during the lag phase of acclimatization and during the start of the growth phase [15]. After the growth phase is reached the limiting factor of the system is found to be availability of nutrients to the biomass. Mass of the active microorganisms responsible for the degradation rate

is obtained as the product of microbial mass concentration and the degradation rate coefficient [11].

The *effect of inoculation of the enriched indigenous microbes* in the biodegradation of BTEX contaminated soil was also studied. The test was done in a laboratory soil column apparatus containing sand as soil medium. BTEX compounds were decided as the targets for the indigenous species. The technique involves a small biologically active carbon reactor with low volume pumping arrangement system to make the microbes acclimatized under the flow condition in the column apparatus. The microbes are then sloughed from the reactor, grown in the nutrient medium and again transferred to the column apparatus.

The experiment showed that the indigenous culture and the inoculated enriched culture had wide range of changes in the degradation in terms time scale. For the given concentration of BTEX and the population adopted to $4x10^6$ microorganisms/g of dry soil , the enriched culture utilizes all the electron acceptor in 10 hours ,while the indigenous species could able to utilize the electron acceptor in 10 days [43].

Compounds like oxygen, nitrate act as *electron acceptors* in the metabolic process. They transfer the energy in form of electrons. Hence a fair amount of electron acceptor is needed for the complete mineralization of the organic compounds. Under aerobic condition where oxygen is used as electron acceptor, the oxidation reactions of BTEX without cell growth are given as follows [32] (Table 2.7).

Table 2.7 mg of oxygen required for oxidation per mg of BTEX

Compound	*reaction*	*mg of O_2 required per mg of compound*
Benzene	$C_6H_6+7.5O_2$----------$6CO_2+3H_2O$	3.08
Toluene	$C_7H_8+9O_2$----------$7CO_2+4H_2O$	3.13
Ethyl benzene	$C_8H_{10}+10.5O_2$--------$8CO_2+5H_2O$	3.50
Xylene	$C_8H_{10}+10.5O_2$--------$8CO_2+5H_2O$	3.50

Hence unavailability of oxygen will become as a limiting factor in the biodegradation. Research works have been done to understand the effect of oxygen concentration in the aerobic degradation of BTEX [33] [34].In conditions where oxygen if found to be depleting in the environment, the feasibility of degradation of

BTEX is carried out by using hydrogen peroxide as supplemental oxygen source [35] [36]. Aerobic degradation study of BTX was also studied using activated sludge immobilized in calcium alginate gel. The study shows 60% of benzene was degraded by the immobilized biomass in batch test after 24 hours with 100 mg/L as initial concentration [35].

Degradation study under anoxic condition (electron acceptor other than oxygen) was also carried out in presence of Fe (III) oxides [36], sulfate [37] and nitrates [38] as the source electron acceptors. Benzene which is refractory in absence of oxygen, found to be degraded easily in presence of Fe (III) oxide as electron acceptor. Studies revealed that the use of nitrates as electron acceptors will aid the remediation activity by increasing the electron acceptor pool and decreasing the total biological oxygen demand (BOD) of the system. Laboratory tests were done to get the optimum conditions for BTEX biodegradation using indigenous microorganisms with nitrate as electron acceptors. Toluene, ethyl benzene and xylene compound were degraded to the concentration level of below 5 mg/L, however benzene was not degraded with nitrate as electron acceptor in the degradation activity [39].

Other possible factor influencing the biodegradation rate in the soil is the presence of competitive or inhibitory substrate for the microorganism and the accumulation of toxic byproducts in the environment [11].

2.4.2 Aerobic biodegradation pathway for BTEX

Bacteria degrade the BTEX compound by using them as source of carbon and energy.BTEX degradation is done via aerobic pathway (oxygen as electron acceptor) and anaerobic pathway (sulfate, nitrate as electron acceptor). Aerobic degradation of BTEX is carried out in the following pathways.

(1) In benzene it involves the addition of two atoms of molecular oxygen into the aromatic ring to form dihydrodiol with cis-configuration which is then dehydrogenated to form catechol. Catechol then further enters the mineralization process either by ortho fission or Meta fission pathways (Fig 2.3).

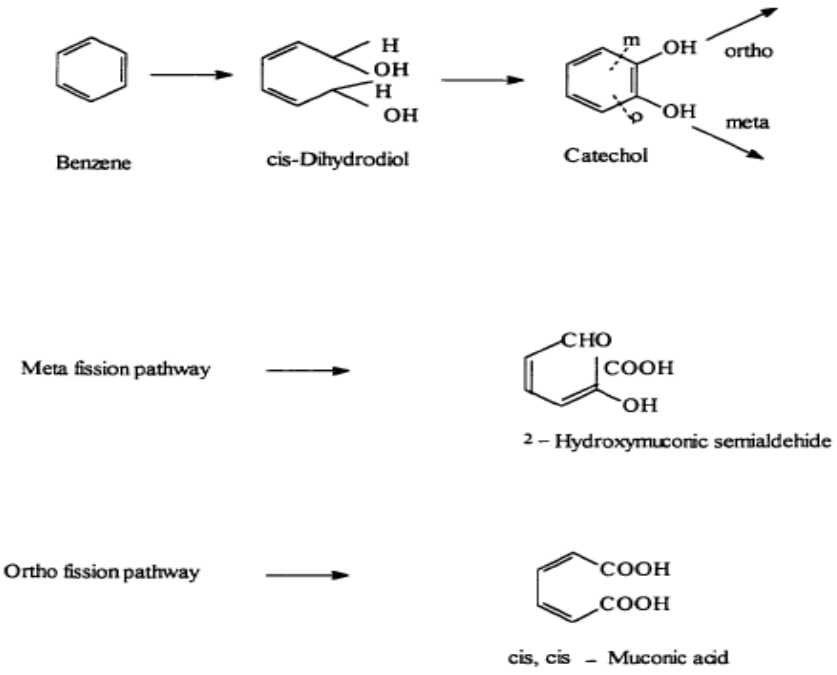

Figure 2.3 Aerobic biodegradation of benzene

(2) In toluene, ethyl benzene and xylene it is either by oxidizing methyl group to carboxylic group and the formation of dihydrodiol by the attack of dioxygenase and the finally the formation of catechol or by the direct attack of the aromatic ring similar to that of benzene (Fig 2.4 (a) & 2.4 (b))..

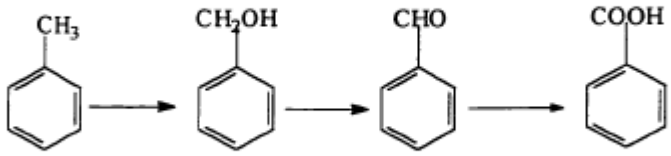

Figure 2.4 (a) Attack of the substation group (methyl)

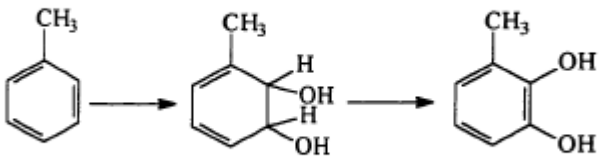

Figure 2.4 (b) Direct attack of the benzene ring

Hence the formation of catechol is found common in the aerobic degradation of BTEX.

2.4.3 Biodegradation modeling

Biodegradation modeling in the subsurface is a complex activity because of the large nurnber of interrelated processes that occurs in the subsurface. Several studies have been done to understand the biodegradation kinetics by considering BTEX as single substrates and also as a mixture. Degradation models help in the prediction of the time required for the substance to reduce to certain concentration in the environment, design of bioremediation schemes to remove chemical contaminant to a designed concentration, predict the amount of biomass production achievable at a given time. The common types of models generally used to describe the biodegradation process are as follows. *Monod kinetic model* was found to dominate the biodegradation of organic contaminants in the subsurface is presented in equation as below [52].

$$\mu = (\mu_{max}).\ S / (Ks + S)$$

The Monod reaction kinetic expression is found to be more reliable in describing the biodegradation when the concentration of organic compound varies in the system. It expresses the microbial growth rate as a function of the nutrient that limits the growth of the microbes. The assumption of the model is that only one limiting substrate is present in the system, and a variation of its concentration causes alterations of the microbial growth. When the concentration of substrate (S) in the system is sufficient to saturate the biodegrading abilities of the microbes, the saturation coefficient (K_s) becomes negligible, and the reaction approximates a zero-order reaction. Once the substrate concentration decreases to below the saturation

concentration for the rnicrobial activity, the kinetic expression approximates a first-order reaction. Monad's model is found unsatisfactory when the substrate is found inhibitory for its degradation. Hence several inhibitory models were developed. Among the inhibitory models *Andrew's model* is widely used in the studies. Equation for Andrew's model is given as below

$$\mu = (\mu_{max}).\ S / (Ks + S + (S^2/K_i))$$

Also a system is generally found to have more than one substrates present in it. Hence several studies were done to understand the kinetic parameters under multiple substrate condition. The sum kinetics interaction parameter model (SKIP) is widely accepted form for the kinetic study with multiple substrates. The generalized equation of SKIP model with 'N' no of substrates is given as follows.

$$\mu = (S1, S_2 \ldots S_N)$$

$$= \sum \quad (\mu_{max,\,i}).\ S_i / (K_{si} + S_i + (\sum S_j\ I_{j,\,i})) \qquad \text{for} \quad i, j = 1 \ldots N$$

This equation also helps to understand the effect of one substrate on the degradation of other substrate by the term $S_j\ I_{j,\,i.}$ [60]

2.5 Unsaturated soil

The soil profile which is seen above the groundwater table is called as vadose zone. The soil in the vadose zone will have negative pore water pressure in it. The properties of unsaturated soil are highly non-linear and are usually expressed in terms of unsaturated soil property functions. One of the possible independent stress state variables seen in the unsaturated soil is the matric suction which is given as $(U_w - U_a)$. Total soil suction (Ψ) of the soil comprises of two major components namely (1) matric suction and (2) osmatic suction (π) and is given as below.

$$\Psi = (U_w - U_a) + \pi$$

Any impact in the surrounding environment of the soil particle will reflect some changes in the matric suction value. Osmatic suction is related to the salt content present in the porewater in the soil. Its effect is considered only for soils in saline or marine areas. In rest of the study, the effect of osmatic suction is negligible and it can be ignored. When a change in the surrounding environment occurs, for instance moisture content of the soil is found to be changing; the change in the total suction of the soil can be equaled to the change in the matric suction itself.

2.5.1 Soil water characteristic curve (SWCC)

Soil water characteristic curve is also called as water retention curve is highly important in understanding the behavior of unsaturated soil. It has been applied in the fields like soil physics, soil science, agronomy and agriculture. It is considered as the water phase constitutive relation in the field of geotechnical engineering. This curve is useful in predicting the unsaturated soil property functions. SWCC is the graphical relationship of water content and soil suction value. It reflects the water holding capacity of the soil under the influence of matric suction. Water content will be plotted as a function of matric suction. The typical features of SWCC are as shown in the figure below (Fig 2.5).

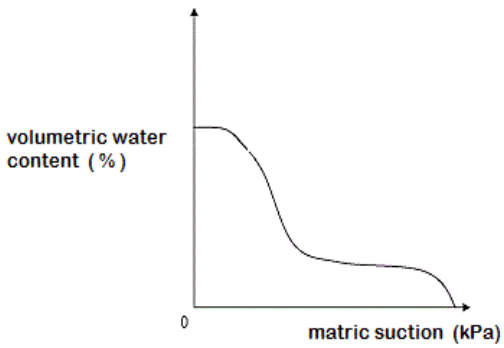

Figure 2.5 Typical soil water characteristic curves (SWCC)

2.5.2 Mathematical forms of SWCC

Defining SWCC mathematically is more important in the analysis of various engineering problems. Summary of various equations proposed for soil water characteristic curve is as shown below (Table 2.8). Most of them do not properly model the entire suction range in the soil. Fredlund and Xing proposed an empirical equation for the wider range of soil suction value ranging from 0 to 1000 000 kPa. It is derived on the basis of assumption that soil has the interconnected pores that are distributed randomly.

Table 2.8 Available mathematical equations for soil matric potential

References	*Equation*	*Soil parameters*
Gardner (1958)	$\theta_w=\theta_s /[1+(\Psi/a_g)^n{}_g]$	a_g , n_g
Van Genutchen (1980)	$\theta_w=\theta_s /[1+(\Psi/a_{vg})^n{}_{vg}]^m{}_{vg}$	a_{vg} , n_{vg} , $^m{}_{vg}$
Mualem (1976)	$\theta_w=\theta_s /[1+(\Psi/a_m)^n{}_m]^m{}_m$	a_m , n_m , $^m{}_m$
Burdine (1953)	$\theta_w=\theta_s /[1+(\Psi/a_b)^n{}_b]^m{}_b$	a_b , n_b , $^m{}_b$
Fredlund & Xing (1994)	$\theta_w=C(\Psi)\ \theta_s /\{\ln [e+(\Psi/a_f)^n{}_f] \}^m{}_f$	a_f , n_f , $^m{}_f$, $^\Psi{}_f$

Where a, n, m are the fitting parameters which can determine by the non-linear regression.

2.5.3 Estimation methods to develop SWCC

Several estimate methods were developed to form the SWCC of the soils. Some of them attempted to correlate the fitting parameters of the equation for SWCC with the plasticity and grain size distribution properties of the soil. Commonly used approaches to determine the soil water characteristic curves of the unsaturated soil are as follows.

- Matching measured soil-water characteristic curve with soil-water characteristic curves already available in the databases.
- Matching measured classification properties such as plasticity index with the classification properties already available in the databases.
- Use of measured grain-size distribution curve to directly compute SWCC Fredlund & Xing (1994).

These approaches are found encouraging for sandy and silty soils, but further researches are needed to follow this approach for clayey soils.

2.5.4 Laboratory measurements of soil suction

Some of the commonly used instruments used to measure the total suction of the soil are listed below.

- Psychrometers
- Filter paper technique
- Pressure plate apparatus

- Axis translation technique
- Tensiometers
- Using thermal conductivity sensors.
- Sand table apparatus

Sand table apparatus

A variety of techniques are needed to measure the soil water characteristics over the range of possible matric suctions (which can exceed 15 bars). The sand table is one technique which is used at the wet end of the range, typically for suctions less than 0.1 bars.

Fig 2.5.a Sand table apparatus

Fig 2.5.b Sand table apparatus

A sand table consists of a column of saturated fine sand to which suction can be applied. Samples are placed on the surface of the sand and the suction transmitted to them. By leaving the samples to reach equilibrium and then weighing them, the soil water contents corresponding to various suctions can be found. It is particularly suitable for undisturbed samples and has the advantage that several samples can be tested at the same time.

2.5.5 Significances of soil-water characteristic curves

The soil water characteristic curve is useful in predicting the unsaturated soil property functions. Some of the important unsaturated soil property functions are listed below.

- Hydraulic conductivity function
- Water storage function
- Shear strength function
- Volume change function

2.5.6 Factors influencing soil-water characteristic curves

The features of soil-water characteristic curve depends on several factors like soil type, soil texture, mineralogy, particle size distribution, void ratio, porosity, initial water content , stress history and compaction level of the soil. Among the factors mentioned above, stress history and initial water content of the soil have greater influence on the soil structure and in turn have greater dominance over the nature of soil-water characteristic curve.

The behavior of microbes in the variably saturated soil is different from that of fully saturated soil because of the presence of air-liquid-solid interfaces in the unsaturated soil. The decrease in hydraulic conductivity due to microbial growth has been investigated in column experiments. The significant reduction of hydraulic conductivity is due to bioclogging. Cunningham et al. (1991) used a column inoculated with bacteria results in the decrease of more than 90% of hydraulic conductivity and 50–90% of porosity. Thus microbial behavior may cause changes in the porosity, permeability, interfacial tensions values, surface tension values, and density and viscosity values. These changes due to the biomass are found to have significant effect the on soil-water characteristic curve (SWCC) or soil-water retention curve [68].

Chapter 3

Materials and methods

3.1 Introduction

The experimental study is divided into the following phases.

- Soil sampling and characterization.
- Isolation of the BTEX degrading species from the BTEX contaminated soil.
- Bio-kinetic study under batch mode.
- BTEX transport study under continuous mode.
- Soil hydraulic properties under unsaturated condition

3.2 Soil sampling and characterization

Soil characteristics largely influence the contaminant fate and transport phenomena. Therefore, soil obtained from the site was characterized for moisture content, organic content, inorganic content and grain size as these factors contribute. For the characterization study the soil sampling was done at various locations in the IITH kandi campus and the following experiments were carried out.

3.2.1 Soil Moisture Content:

It is an important parameter for expressing the relation between the behavior of the soil and it is expressed as a percentage of the mass of porewater in given mass of the soil to the mass of the dry solids of the soil. The test was performed as per the Standard Test Method ASTM D 2216. The test was performed using simple lab instruments such as Hot air drying Oven, Weighing balance, Moisture can, Heat resistant gloves, and a spatula. The procedure adopted is as follows.

- Weigh the initial weight/ mass of the well cleaned and dry empty moisture can with its lid and record the mass as "M_C".

- Place the moist soil obtained from the field in the moisture can and close with lid and weigh the mass of the soil filled moisture can. Record the mass as "M_{CMS}".
- Heat dry the moist soil placed in moisture can without the lip in a hot air oven at 105°C for overnight.
- Remove the dried moisture can and weigh the mass with its lid. Record the mass as "M_{CDS}"
- Determine the soil moisture content using the following formula: Mass of soil solids, $M_S = M_{CDS} – M_{SC}$; Mass of pore water, $M_W = M_{CMS} – M_{CDS}$; Water content, $(M_W/M_S)*100$.

3.2.2 Soil Organic Content:

The soil organic test was performed to determine the organic matter of the soils. It is the ratio which is expressed as percentage of the mass of organic matter, present in soil to the mass of the dry solids of soil. Knowing the organic content is important because it influences the physical, chemical and biological properties of the soil. Some physio-chemical biological properties include Soil structure, its compressibility and shear strength, the water holding capacity and nutrient content for biological activity. The soil organic content test was performed as per the Standard Test Method ASTM D 2974. The test was performed using simple lab instruments such as Muffle furnace, Weighing balance, porcelain dish, spatula and tongs. The procedure adopted is as follows.

- Weigh the mass of a clean, dry, and empty porcelain dish. Record the mass as "MP".
- Place the oven-dried soil sample in the porcelain dish and weigh the mass. Record the mass as "MPDS".
- Place the porcelain dish in muffle furnace and increase the temperature gradually to 440°C and allow it to stay for overnight.
- Remove the porcelain dish with the help of tongs and allow it to cool. Weigh its mass of ash and record as "MPA".

- Determine the soil moisture content using the following formula: Mass of the dry soil, MD = MPDS-MP; Mass of the ash, MA = MPA-MP; Mass of organic content Mo = MD – MA.
- The Soil Organic content, Oc = (Mo/MD)*100.

3.2.3 Soil grain size analysis:

The soil grain size analysis was performed to determine the percentage of different grain sizes contained within the soil. The mechanical or sieve analysis was performed to determine the distribution of coarser, larger-sized particles and also to classify the soil. The test was performed as per the Standard Test Method ASTM D 422. The test was performed using simple equipment such as Balance, Set of sieves, Cleaning brush, and Sieve shaker.

The procedure adopted is as follows.
The weight of each sieve is for each sieve diameter is recorded. The initial weight of the dry soil should be recorded. The sieve should be arranged from #4 at the top to #200 sieves at the bottom. The pan should be placed below the #200 sieve. Place the soil sample at the top at the sieve #4. And shake it well to pass through the stack of different sieve sizes. Weights of the each sieve along with the soil retained and record their values and calculate the percentage passing and percentage retained for the overall weight of the soil.

3.2.4 Saturated hydraulic conductivity

The saturated hydraulic conductivity test is done on the basis of ASTM D2434 - 68(2006) Standard Test Method for Permeability of Granular Soil. The constant head permeability test involves flow of water through a column of cylindrical soil sample under the constant pressure difference. The test is carried out in the permeability cell, or permeameter, which can vary in size depending on the grain size of the tested material. The soil sample has a cylindrical form with its diameter being large enough in order to be representative of the tested soil. As a rule of thumb, the ratio of the cell diameter to the largest grain size diameter should be

higher than 12 (Head 1982). The usual size of the cell often used for testing common sands is 75 mm diameter and 260 mm height between perforated plates.

The testing apparatus is equipped with a adjustable constant head reservoir and an outlet reservoir which allows maintaining a constant head during the test. Water used for testing is de-aired water at constant temperature. The permeability cell is also equipped with a loading piston that can be used to apply constant axial stress to the sample during the test. Before starting the flow measurements, however, the soil sample is saturated. During the test, the amount of water flowing through the soil column is measured for given time intervals.

Knowing the height of the soil sample column L, the sample cross section A, and the constant pressure difference Δh, the volume of passing water Q, and the time interval ΔT, one can calculate the permeability of the sample as

$$k_{sat} = Q.l / h.A.t.$$

3.3 Microbial isolation and bio kinetic study

Reagents

The target monoaromatic compounds BTEX were purchased. The purity level and some of the properties of the purchased compounds are tabulated below (Table3.1).

Table3.1 Properties of the reagents

Compound	*Benzene*	*Toluene*	*Ethyl benzene*	*xylene*
Purchased from	SRL	SRL	SDFCL	MERCK
Purity level	Min.99.7	Min.99.5	Min.98	Min.98
Molecular weight (g/mole)	78.11	92.13	106.16	106.17
Boiling point (° C)	80.10	110.60	136.20	138
Density (g/mL)	0.87	0.87	0.87	0.86
Solubility (mg/L)	1791	535	161	146

Nutrient solution

Nutrient salt solution called as mineral salt media (MSM) will contain all the necessary micro and macro nutrients. MSM is to be supplied for healthy microbial population. Nitrogen, phosphorous like elements are to be added to meet the biosynthesis need. The composition of the nutrient medium used for the study is tabulated as below (Table3.2).

Table3.2 Composition of MSM used in the research

Salt	*Concentration ,mg/L*
$(NH_4)_2\ SO_4$	500
K_2HPO_4	500
KH_2PO_4	500
$MgSO_4 \cdot 7H_2O$	500
$CaCl_2 \cdot 2H_2O$	9.8
$MnSO_4 \cdot H_2O$	10
$Fe(NH4)_2\ (SO4)_2 \cdot 6H_2O$	8
$ZnSO_4 \cdot 7H_2O$	2
nitrilotriacetic acid	20

Culture

BTEX degradation studies were extensively carried out with the bacterial consortia from sewage sludge, groundwater microorganisms, and indigenous soil [53]. Indigenous culture is used in this research purpose. Among the various BTEX degrading bacteria, Pseudomonas group is wide intensively used in the biodegradation studies. Hence Pseudomonas bacteria are isolated from contaminated soil collected for the study purpose using Pseudomonas isolation agar.

Soil

The soil is collected at various points from the site. Soil is then characterized for the following properties as per the method of soil analysis [59]. The soil obtained is classified with the grain size distribution. GSD parameters were obtained from sieve

analysis test. Percentage fines and Organic content of the soil are determined as .02 and 0.001 respectively. .

Methods

Species isolation

The pseudomonas species is solely used as BTEX degrader in this research. The required bacterial strain is obtained from the contaminated soil collected from petrol and diesel fuel contaminated site. The soil sample is collected and stored at 4°C. About 17 g of the soil is added to 100 ml of deionized water and rigorously mixed to form a homogeneous solution. The isolation technique for species isolation is spread plate technique.

The spread plate technique is practiced to get evenly distribute bacteria across the plate. Each colony is developed in the plate is considered "pure". Nutrient agar is prepared in complete sterile condition. The agar medium is then transferred aseptically to the petri dishes inside UV chamber. The medium is then allowed to solidify. From the homogeneous soil solution about 1ml is pipetted out and is made up to 10ml. Serial dilution is then done. About 0.1ml from each dilution is transferred to the petri dishes and it is made spread over the solidified agar evenly using the spreader. (Fig 3.1 & 3.2)

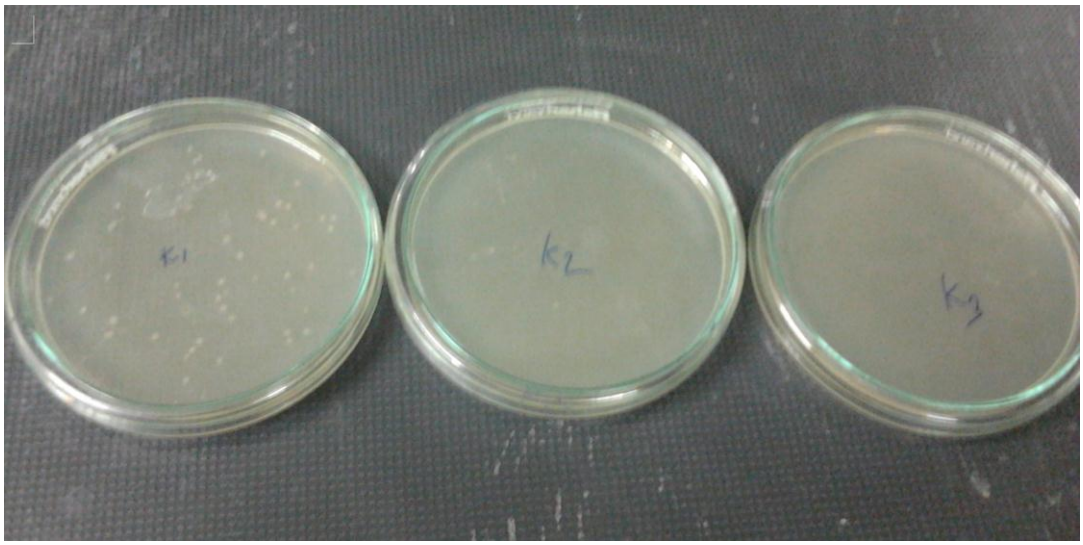

Figure 3.1 Pseudomonas species isolated using spread plate technique

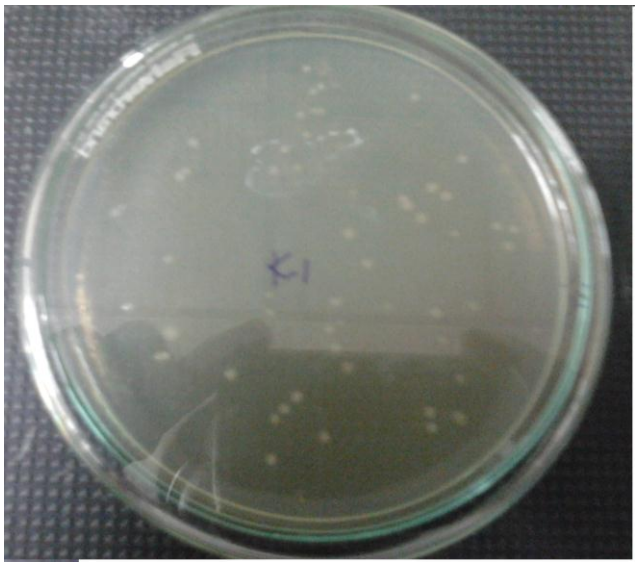

Figure 3.2 isolated colonies of Pseudomonas species in the first dilution

The pseudomonas species isolated from spread plate technique are then transferred to the nutrient broth solution. The broth is then kept for incubation in the BOD incubator, maintained at 25°C, 110 rpm. After 24 hours, the broth solution is taken into the vials for centrifuge. The slimy pellets of the cultures will get settled at the bottom of the vials. The pellet is then transferred to the vial containing deionized water and is mixed thoroughly to get a uniform bacterial solution. Optical density (OD) of the bacterial solution in then determined using UV spectrophotometer. It is found that the maximum absorbance value for the bacterial solution is measured at the wavelength of 725λ.

Degradation and growth kinetic study

Batch biodegradation experimental study of BTEX is carried out in the 500 mL reagent bottles with screwed septa caps. The stock solution was prepared as follows. Based on the solubility levels of each substrate (B, T, E, X), a single standard stock solution is prepared. The concentration of each compound in the single standard stock is tabulated as below (Table3.3).

Table3.3 Concentration of BTEX in the stock solution

Compound	*Concentration level in the stock, mg/L*
Benzene	500
Toluene	500
Ethyl benzene	100
Xylene	100

From the stock solution, further dilutions are made to get the solutions of desired final concentration. The BTEX standards are taken in an exact quantity and the dilution to desire concentrations are achieved by making up with MSM solution. The MSM used for preparing the initial samples has to be preadjusted for the pH value of 7.Concentration of the B, T, E, X in the single standard stock solution and in the diluted solutions at the initial condition is tabulated below (Table3.4).

Table3.4 Concentration of BTEX in the serial dilution samples

Dilutions	*Benzene*	*Toluene*	*Ethyl benzene*	*o-xylene*	*m-xylene*	*p-xylene*	*Samples*
Stock	500	500	113	30	40	17	Sample1
Dilution1	250	250	56.5	15	20	8.5	Sample2
Dilution2	200	200	45.2	12	16	6.8	Sample3
Dilution3	100	100	22.6	6	8	3.4	Sample4
Dilution4	50	50	11.3	3	4	1.7	Sample5

To every sample about 5mL of the bacterial solution whose OD value is already known is then added. Sample 6 is considered as the control sample is made of without bacterial. It is also taken for the batch studies to understand any abiotic loss of BTEX. All the samples bottles are then incubated in the BOD incubator shaker maintained at 25°C, 110 rpm. Liquid sample aliquots are collected at regular intervals to measure OD, pH and the concentration levels of BTEX. Concentration levels in the control bottle (sample 6) are also measured to assess the abiotic loss. OD values are obtained using UV spectrophotometer and the concentration values of B, T, E, X are determined using GC-FID.

Analysis using GC-FID

The set of analytical procedure necessary for the comprehensive analysis of BTEX compounds using GC-FID is discussed below. The method involves a direct aqueous injection of the sample containing the target compounds. Analytical conditions used in the experimentation are tabulated as below (Table3.4).

Table3.5 Chromatographic conditions used in GC FID analysis

A-Chromatographic conditions	
Gas chromatograph	
Column	ZB-Zebron wax column, [30 meters x 0.32 mm x 0.50 μm]
Carrier gas	Helium

B-Oven temperature program	
Initial temperature	60°C
Initial time	2.5 minutes
Final temperature	90°C
Final time	1.5 minutes

C- Injector	
Injector mode	PTV mode, Split 20:1
Injection volume	0.2 μL
Injector temperature	150°C

D- Detector	
Detector temperature	220°C
Make up gas	Nitrogen
Air flow rate	300 ml/min

The calibration curve for the BTEX standards purchased is then done. The method developed results in the peaks of each compound is obtained as per the following retention time (Table3.5).

Table3.5 Retention time of BTEX obtained in GC FID analysis

Compounds	*Retention time*
Benzene	2.11
Toluene	2.82
Ethyl benzene	3.59
o-xylene	4.22
m-xylene	3.74
p-xylene	3.68

Calibration details for each of the BTEX compound are discussed below. The chromatograms obtained for each of the BTEX compound is also given. Method linearity is also shown with the regression coefficients for each of the BTEX compound. (Fig 3.3)

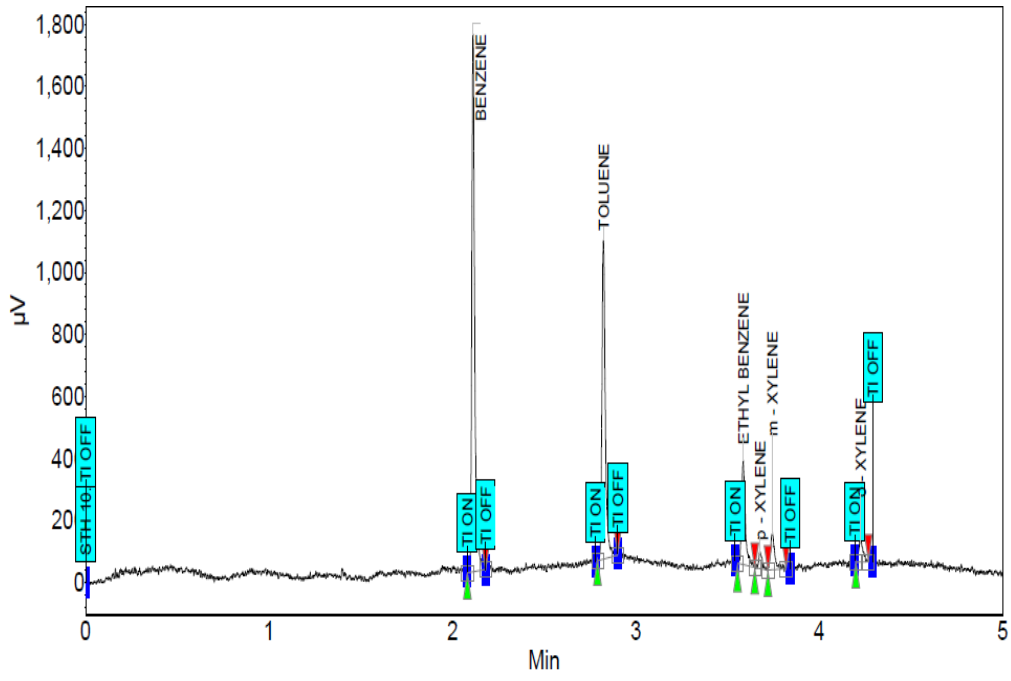

Figure: 3.3 Chromatogram obtained for each of the BTEX compound

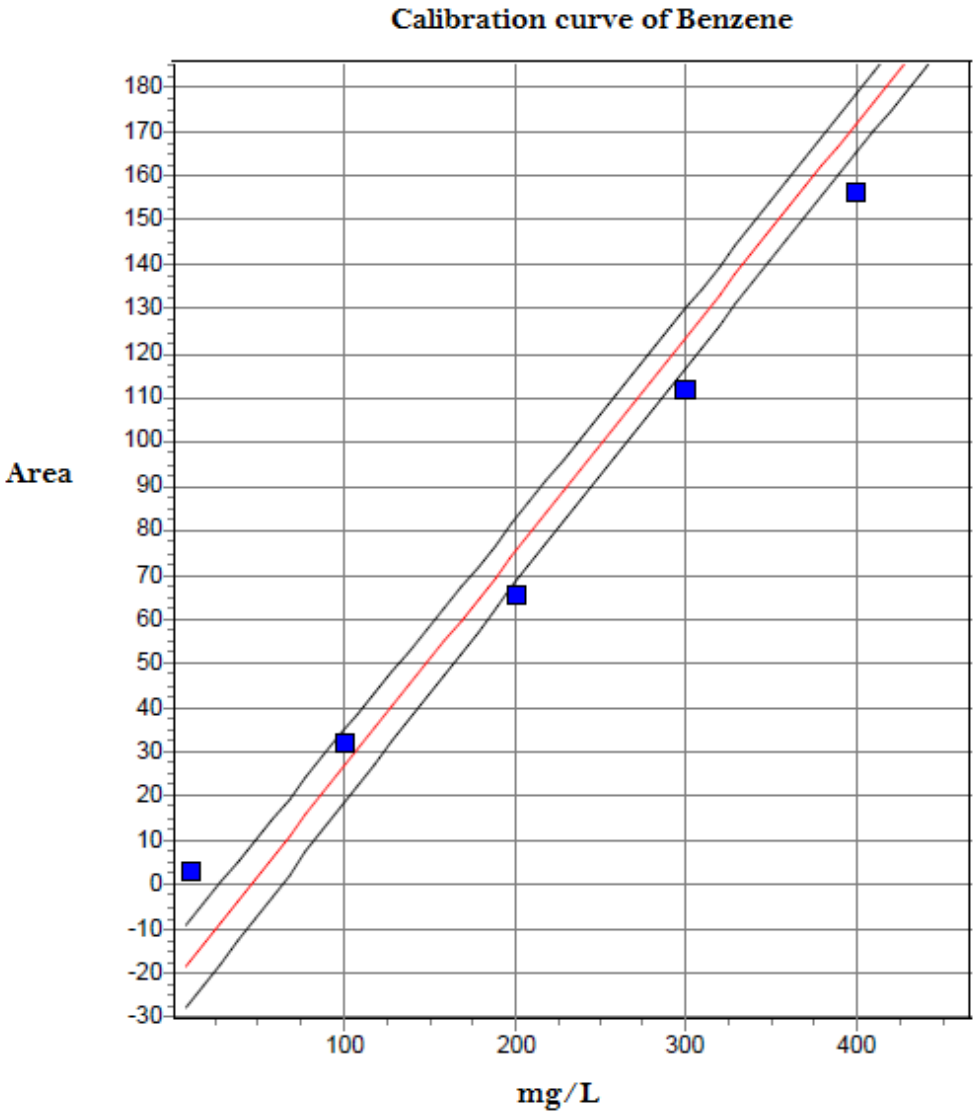

Fig 3.4 Calibration curve for benzene.

Component: *Benzene*

Polynomial: y = b x +a

a = -22.30308

b = 0.48644

Correlation Coef = 0.992

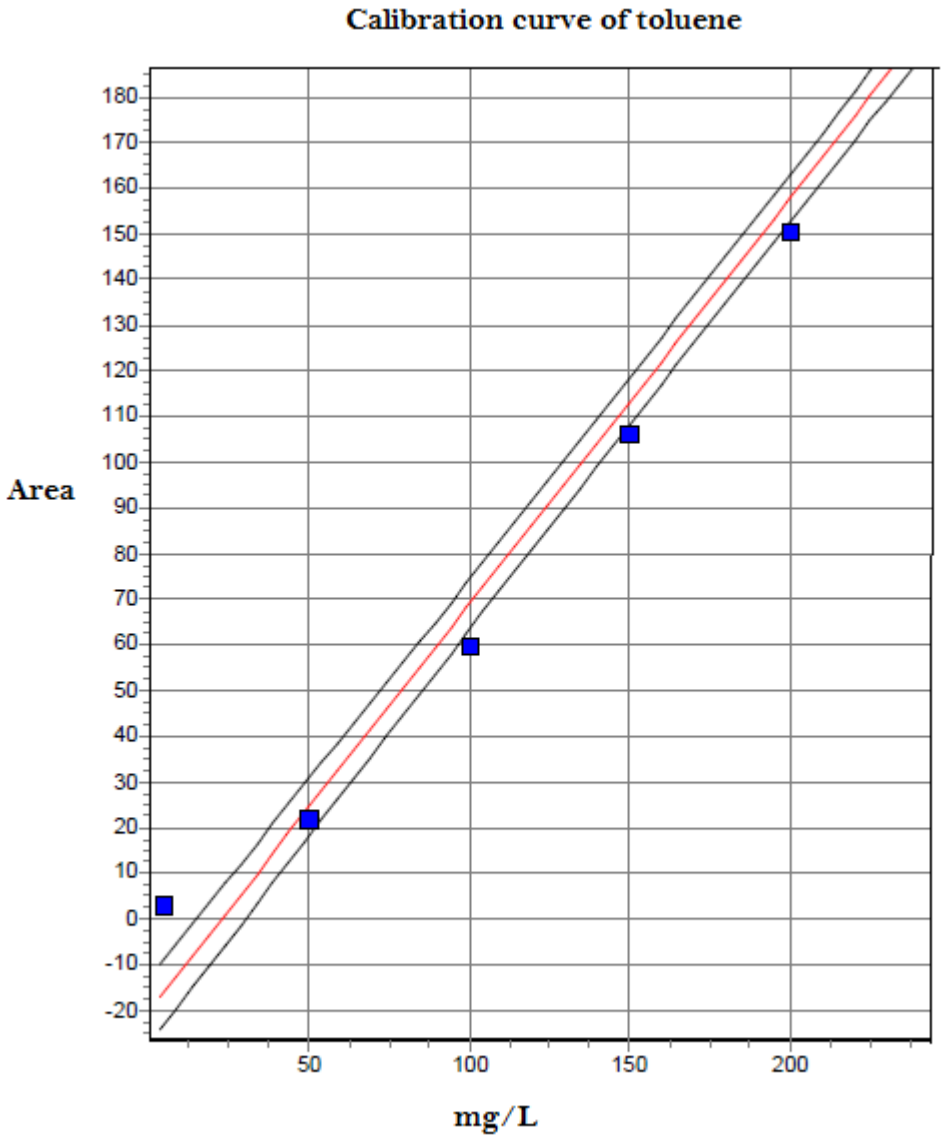

Fig 3.5 Calibration curve for Toluene

Component: *Toluene*

Polynomial: y = b x +a

a = -20.47549

b = 0.89225

Correlation Coef = 0.9950

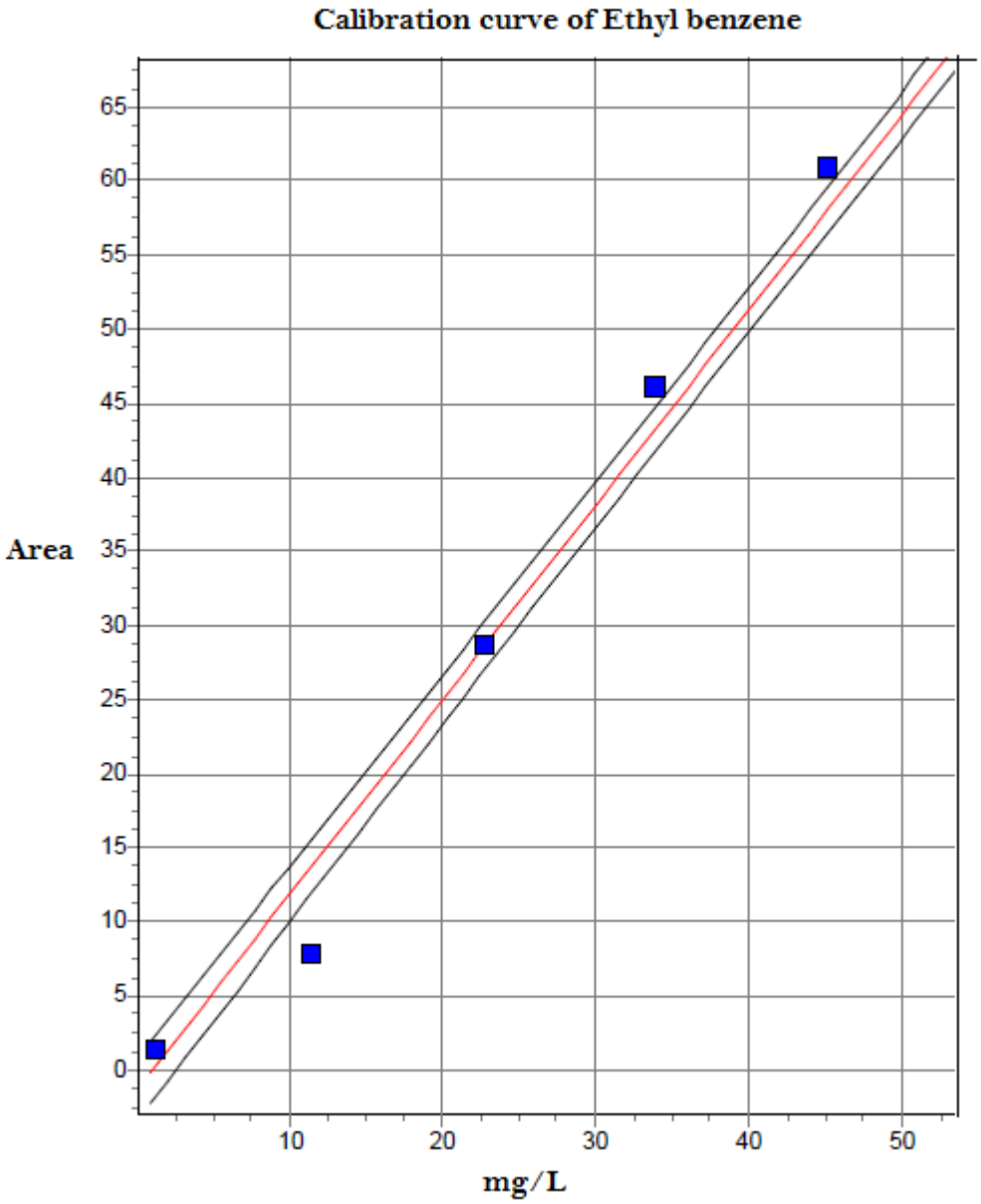

Fig 3.6 Calibration curve for ethyl benzene

Component: Ethyl benzene

Polynomial: y = b x +a

a = -1.33844

b = 1.31649

Correlation Coef = 0.9962

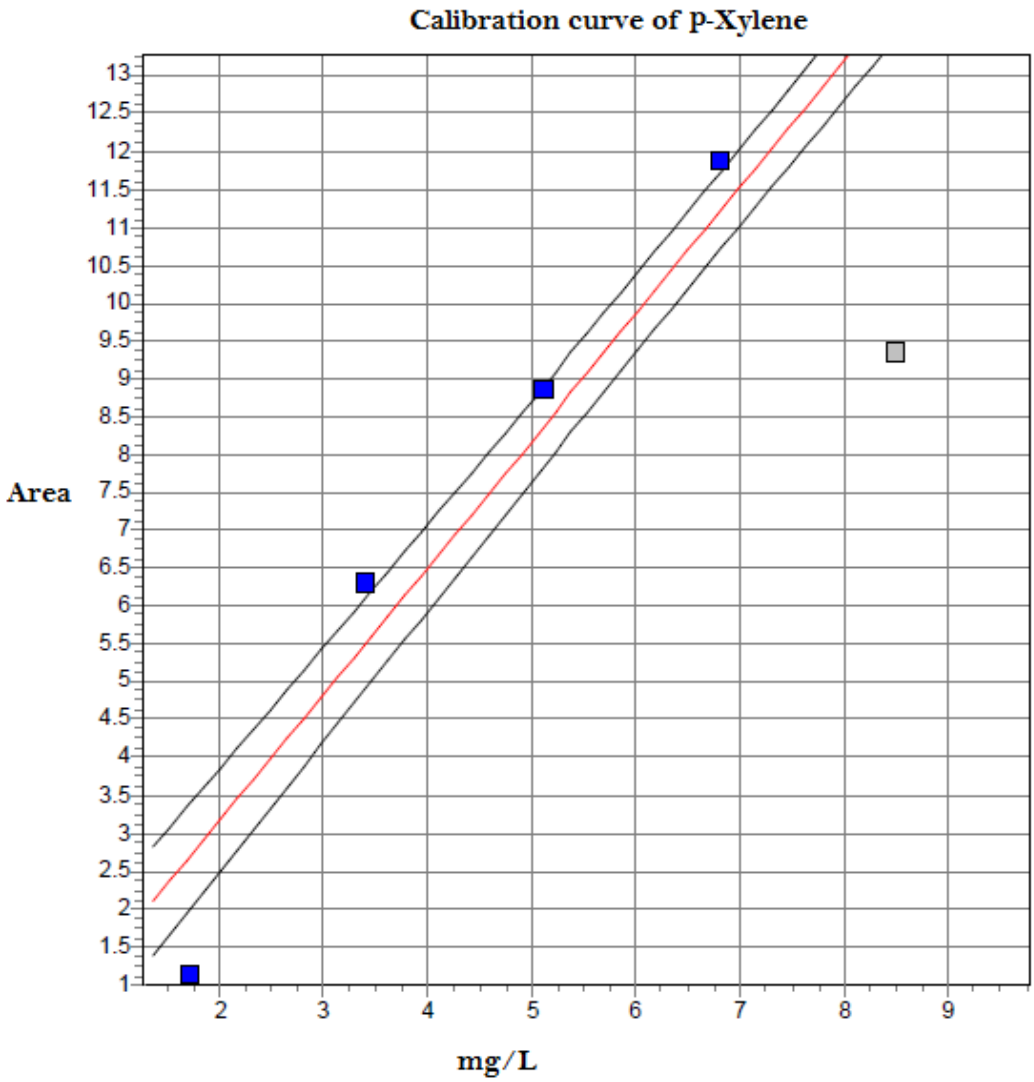

Fig 3.7 Calibration curve for p-xylene

Component: p-xylene

Polynomial: y = b x +a

a = -0.16969

b =1.67147

Correlation Coef = 0.9905

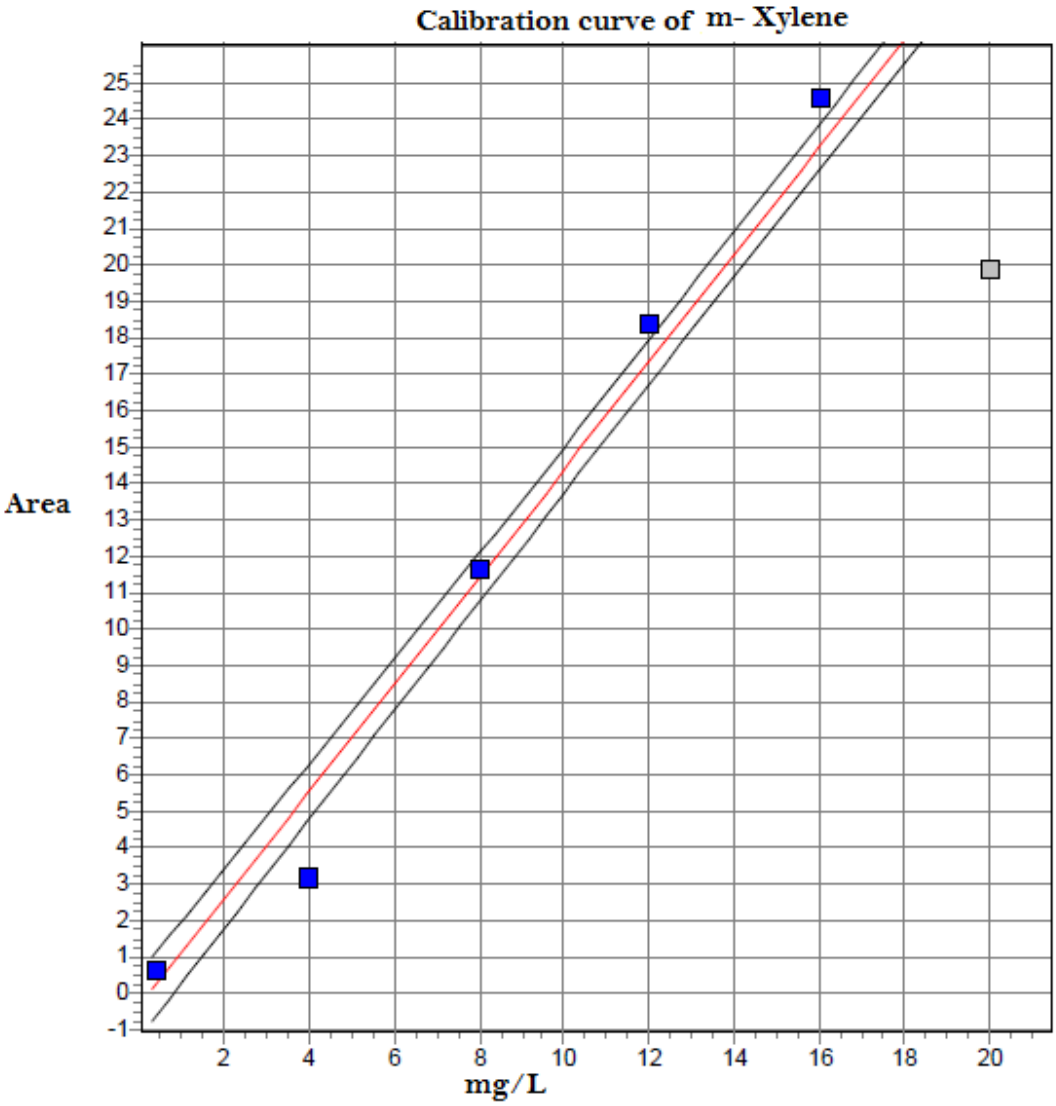

Fig 3.8 Calibration curve for m-xylene

Component: m-xylene

Polynomial: y = b x +a

a = -0.36865

b =1.47687

Correlation Coef = 0.9958

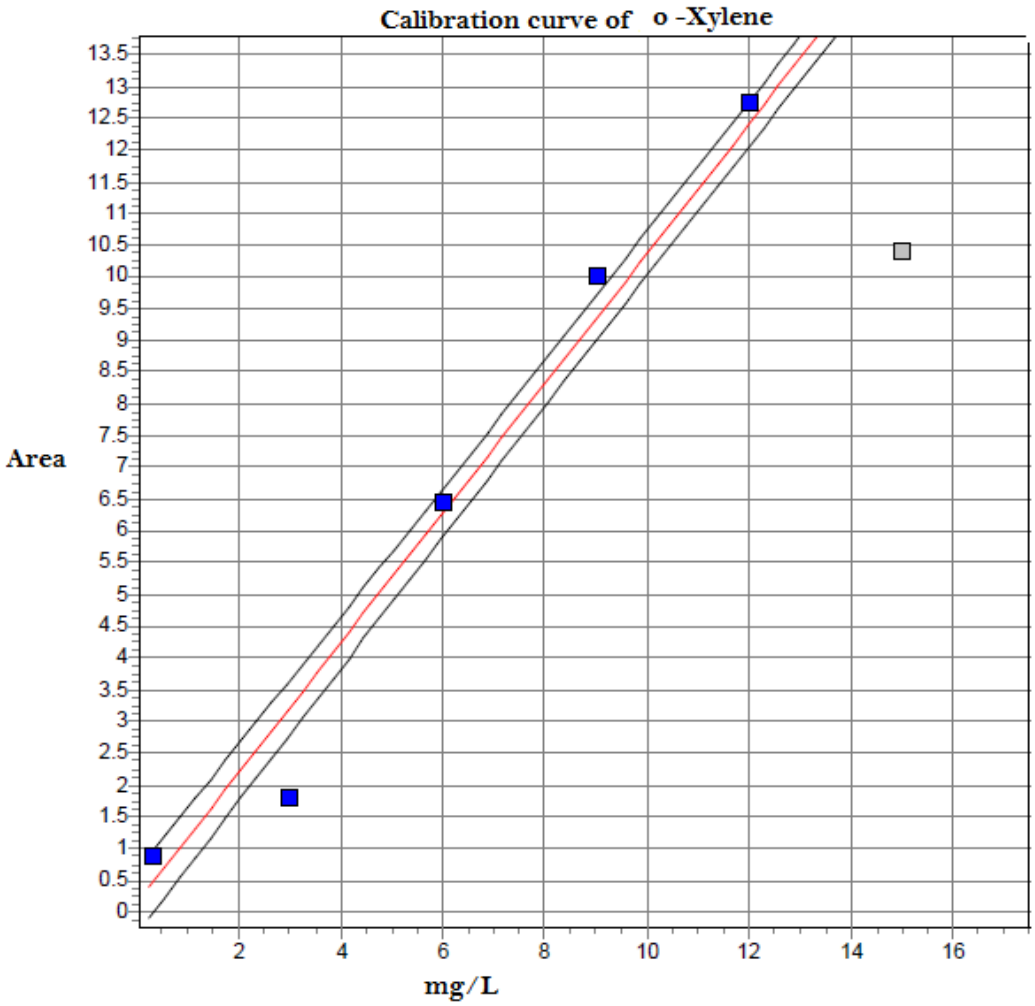

Fig 3.9 Calibration curve for o-xylene

Component: o-xylene

Polynomial: y = b x +a

a = -0.15194

b =1.02134

Correlation Coef = 0.9951

3.4 BTEX transport under continuous mode

The degradation of BTEX in unsaturated soil is understood with the flow experiment carried out in the acrylic column fabricated for the research study (Fig 3.4.5). Also the microbial induced changes in the hydraulic properties in particular the changes in the soil suction value is estimated with the soil used for the flow experiment in the acrylic column.

Materials

Acrylic glass column

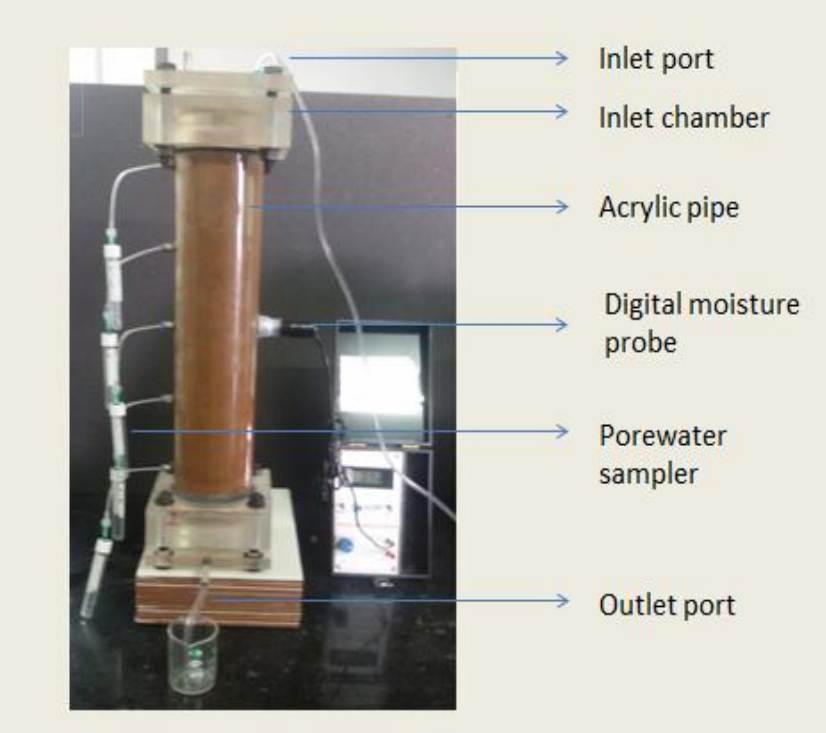

Fig 3.4.1 Acrylic column set up

The acrylic column is fabricated with the diameter of about 10 cm and the height of about 50 cm.The two ends of the glass chamber is given with porous plate having pores of 1mm diameter. The porous plate helps in confining the soil and also in the uniform distribution of water. The column is provided with the inlet and outlet chambers (Fig 3.4.1).

Porewater samplers

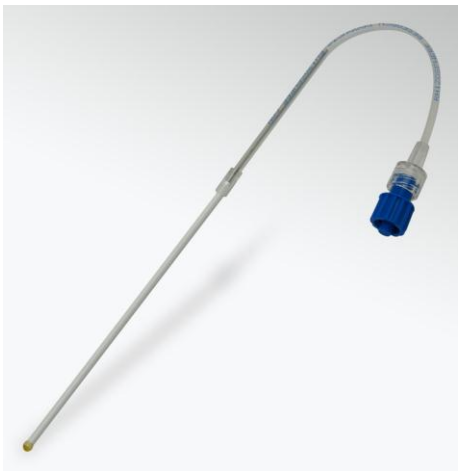

Fig 3.4.2 Porewater sampler

Along the length of the column, pore water samplers were fixed to it. The samplers help in collecting the porewater. The sampler is capable of collecting the soilwater even the moisture content of soil is about 10-20 %. The yield of the sampler is about 4 mL of porewater per minute (Fig 3.4.2)

Moisture content measuring probe

Fig 3.4.3 digital moisture meter

The acrylic column was provided with the moisture content measuring probe (Fig 3.4.3) which measures the moisture content ranging from 0-100%.

Pumping system

Fig 3.4.4 Peristaltic pumping system

The pumping system had the following unit.

It consists of a peristaltic pump which is having the pumping capacity ranging from 5mL/hr to 20L/hr, fluid reservoirs, and tubing of ID (2mm). This pumping unit is used for pumping BTEX solution which is prepared in the mineral salt medium (Fig 3.4.4).

Flow experiment methodology

Fig 3.4.5 Flow experiment setup

The soil was collected from IITH Kandi campus whose physio-chemical characteristics were already. The soil was sieved through 4.75 mm sieve. The fractions passing through the sieve were used in the column apparatus. Also the soil had been washed with sodium acetate (pH=5) to remove carbonates; with hydrogen peroxide (6%) to remove organic matter; and with a mixture of sodium citrate, sodium bicarbonate, and sodium hydrosulfite to remove iron oxides. It was further autoclaved for 90 min shortly before assemblage of the column.

Before filling the soil into the acrylic column, the empty weight was measured accurately. The soil was then placed into the acrylic column in layers. Each layer was given enough compaction so as to reach the field density of the soil inside the acrylic column. The soil was wet-packed into column with the bacterial solution having the initial OD value of 2.118. After the soil was placed the final weight was then measured.

Using the weights, the density to which the soil is placed in the column apparatus was determined. Once the soil is placed the initial moisture content is also measured with the help of soil moisture measuring probe. The pumping systems were operated at the flow rates of 5 mL per minute. The flow in the column was made from top to downwards so as to have the gravity driven flow in the soil column. The flow experiment was continued till the concentration of BTEX is depleted to the maximum level from the initial concentration. The duration of flow was also found sufficient enough for the growth of biomass in the acrylic column.

The soilwater was collected through the pore water samplers fixed at some regular intervals. The sample collected was centrifuged at 6000 rpm for 15 minutes. After that centrifuged samples were then analyzed for the concentration of BTEX using GC-FID. The degradation profile of BTEX along the length of the soil column was obtained. The experiment was also carried out without any wet packing of the soil with the bacterial solution. However the soil is placed at some initial water content which is required to achieve the field unit weight of the soil inside the acrylic column. This experimental run helps in understanding the loss of BTEX in the absence of bacteria in the unsaturated soil column.

After the end of the flow experiment the soil sample was removed and it was processed to measure OD value using UV spectrophotometer to assess the microbial growth in the soil during the flow experiment. Also the soil sample was used to measure the suction value using sand table apparatus. Detailed procedure for the measurement and comparison of the suction values of the soil before and after the flow experiment are discussed in the later section

3.5 Soil hydraulic property under unsaturated condition

Apparatus

The soil suction value was measured with the sand table apparatus. The model used for the research work was FEL4 –Soil Moisture Suction Sand Table from supplied by ARMFIELD-Engineering Teaching & Research Equipment. The apparatus is primarily used for the derivation of soil moisture characteristic curves. Also the FEL4 helps in understanding the principle of water retentivity and its relationship with soil moisture levels. The overall dimension specification details of the device are as follows. Height: 1.3m :Width: 0.4m :Depth: 0.5m.

The Sand Table consists of a circular sand box, mounted in a free standing steel frame and connected, via a flexible tube to a constant head cell. The sand box is made of metal and is approximately 330mm in diameter by 300mm deep. During use the sand box is filled with a saturated column of fine sand and suctions in the range 0 to 0.1 bars can be applied to soil samples placed on the surface of the sand. The steel supporting frame stands approximately 1.5m high and can be leveled by means of adjusting screws in the feet.

The constant head cell has a water inlet at the top and a drainage outlet approximately half way up. The cell is mounted on a vertical graduated scale on the side of the support frame. It can be moved up and down the scale and fixed at any point by means of a locking screw. The cell can be used to apply suction to samples placed on the surface of the sand. The graduated scale allows the applied suction to be determined quickly and easily.

Method

The experimental set up used for the measurement of soil suction is as shown below (Fig 3.5.1).

Fig 3.5.1 Sand table apparatus for measuring matric potential

Prior to the start of the experiment the sand table apparatus had to be commissioned as per the procedure. Then turn on the water supply to the constant head cell. Saturate the sand table very slowly raising the constant head cell until there is free water on the surface of the sand. Care has to be taken so that no air bubbles are seen or craters form in the surface of the sand table. The sand table was allowed to equilibrate for 24 hours. During this time the tensiometer probes and flexible tubing should become primed with water.

Connect the flexible tubes to the three manometer tubes. Connect another length of flexible tube to the overflow pipe in the top of the manometer and connect the other end to a suitable drain. Note the height of the mercury column above the mercury reservoir. Record the distance of the sand surface below the zero point on the scale.

Now place the soil sample in the sampler over the saturated sand in the sand box. Then lower the constant head cell until the level is 100mm, below the level of the sand surface. This applies a suction of100 mm to the sand table. The maximum suction that can be applied to the sand table is determined by the maximum pore diameter in the sand. From the literature it is found that the suctions value of 800mm of water (0.08 bars) can be safely applied for the soil used for the research purpose.

Allow the sand table to equilibrate for a few hours then record the mercury column height in all three tensiometer probes. The achieved suction at the tensiometer probe relative to the sand table surface is calculated as follows.

Achieved suction (mm of H_2 O) = 13.6 x (mercury column height at applied suction - mercury column height at zero suction).

Hence suction developed in the soil is then measured.

Chapter 4

Results and Discussions

4.1 Results and Discussion

Experiments for BTEX biodegradation in unsaturated soil condition were performed. Transport and degradation activities in the soil environment were found to have an impact on the soil matric potential. Soil matric potential values were measured for the unsaturated soil subjected to degradation activity. Also the same was measured for the soil where only BTEX transport was carried out. BTEX resistant cultures were isolated from the petroleum contaminated soil and cultured on pseudomonas isolating agar. And then degradation studies were carried out in batch and soil column.

4.2 BATCH STUDIES

4.2.1 O.D and Dry cell weight correlation

BTEX reducing bacteria from oil contaminated soil, collected from Sangareddy, Telengana, India. Bio-kinetic parameters of this microbial culture were then determined. Cell suspension solution of various dilutions was prepared. Each dilution was checked for the optical density value. Correlation graph was then drawn for O.D values and cell suspension

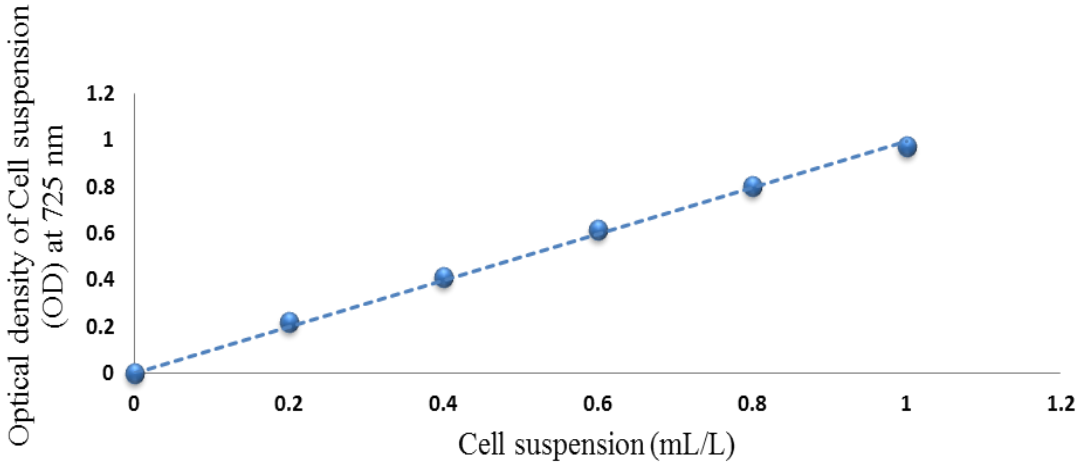

Figure. 1 a. Standard curve of pseudomonas cell suspension at 725 nm. (Wavelength chosen by full wavelength scan on UV - Vis. Spectrometer Lab India)

The O.D values of the culture are to be converted in terms of dry cell weight of the culture to represent the amount of culture present in the respective dilution of the cell suspension solution. Filter paper technique was adopted for arriving the dry cell weight concentration of the culture from the cell suspension solution.

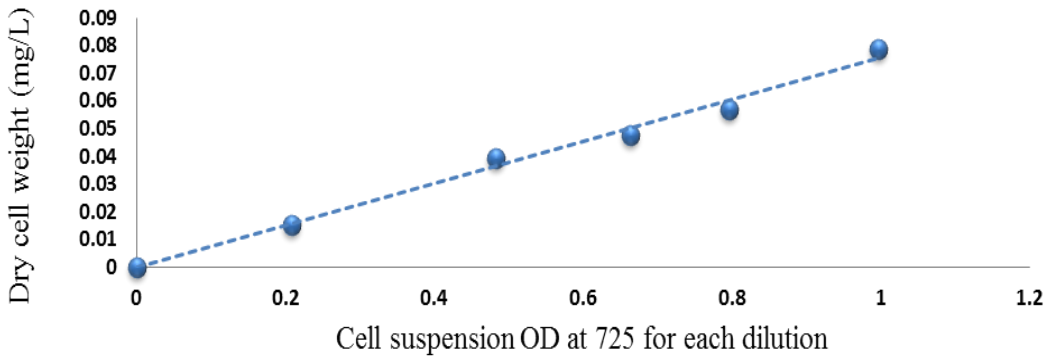

Figure. 1 b. Correlation of Dry cell weight with optical density of cell suspension.

4.2.2 Growth kinetic study of Pseudomonas Sp in BTEX as sole carbon source

Biotransformation studies were conducted for each of the BTEX compounds and each with the initial concentrations of 100,200,300,400 ppm. Bacterial concentration of 0.023 mg/L was added to the mineral salt media solution. Bacterial concentrations and BTEX compound concentrations were measured at regular time interval. Kinetics of microbial growth for different initial concentrations was determined. Growth pattern followed by the specific growth rate are determined for each of the compound and their growth pattern and rate curves are arrived as follows.

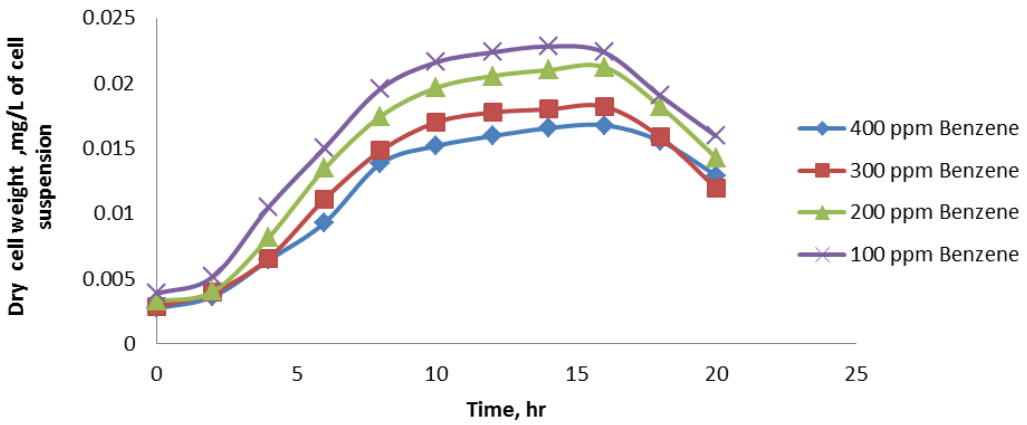

Fig4.1.2 a Growth pattern of Pseudomonas sp. With benzene as sole carbon sorce.

The growth curve for the Pseudomonas sp at different initial concentations of benzene as sole carbon source is given in (Fig4.1.2 a). The initial concentrations used in the batch were as follows 100,200,300,400 ppm. The concentration values are plotted against the amount in microbes expressed in terms of dry cell weight.

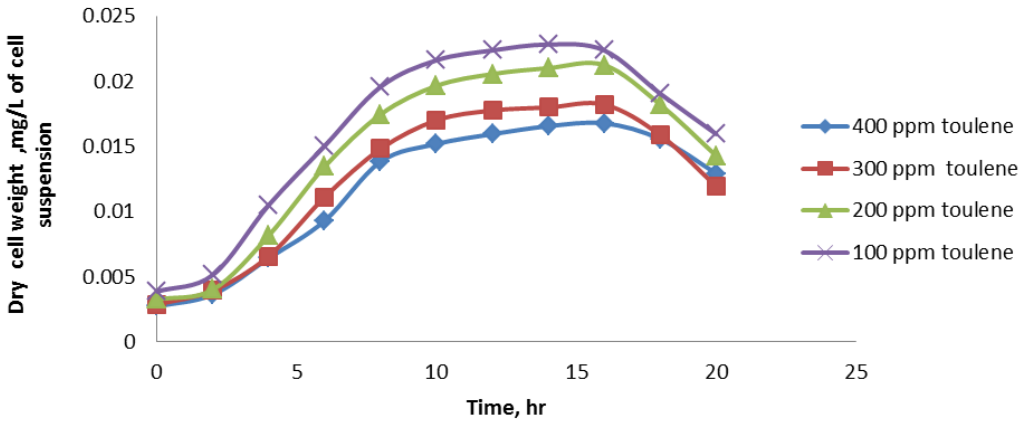

Fig4.1.2 b Growth pattern of Pseudomonas sp. With Toulene as sole carbon sorce

The growth curve for the Pseudomonas sp at different initial concentations of toluene as sole carbon source is given in (Fig4.1.2 b). The initial concentrations used in the batch were as follows 100,200,300,400 ppm. The concentration values are plotted against the amount in microbes expressed in terms of dry cell weight.

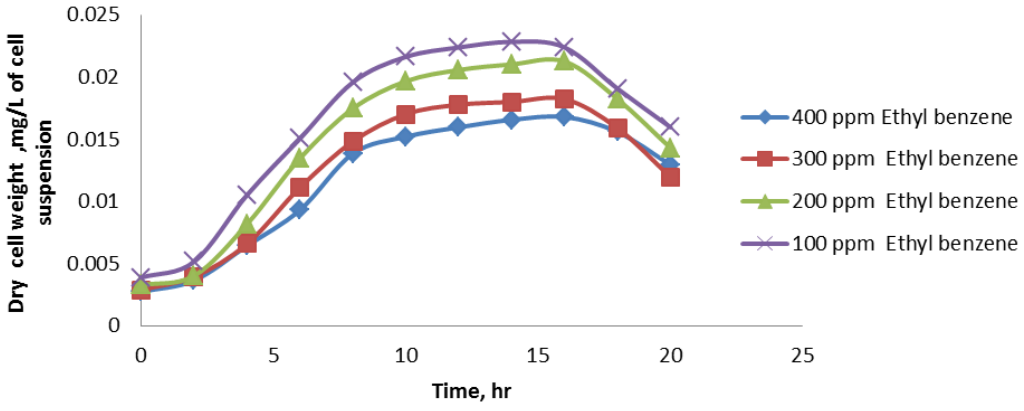

Fig4.1.2c Growth pattern of Pseudomonas sp.with ethylbenzene as sole carbon sorce

The growth curve for the Pseudomonas sp at different initial concentations of ethylbenzene as sole carbon source is given in (Fig4.1.2 c). The initial concentrations used in the batch were as follows 100,200,300,400 ppm. The concentration values are plotted against the amount in microbes expressed in terms of dry cell weight.

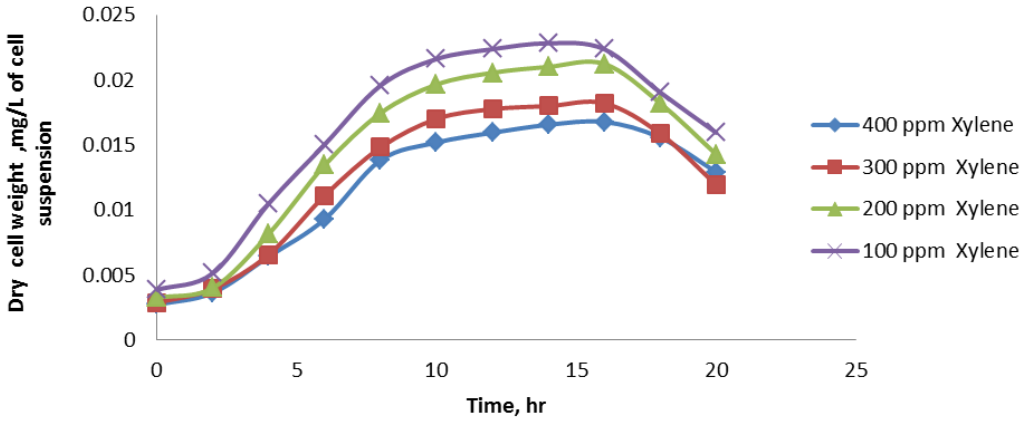

Fig4.1.2 d Growth pattern of Pseudomonas sp. With Xylene as sole carbon sorce

The growth curve for the Pseudomonas sp at different initial concentations of xylene as sole carbon source is given in (Fig4.1.2 d). The initial concentrations used in the batch were as follows 100,200,300,400 ppm. The concentration values are plotted against the amount in microbes expressed in terms of dry cell weight.

From the growth pattern curves, the specific growth rate for each of the BTEX compound at each of their initial concentrations are arrived. The specific growth rate is obtained from the slope of the exponential growth phase of the growth curve. The specific growth rate curve helps to get the concentration at which the growth of the microbe is maximum. The bio kinetic parameters like μ_{max} and K_s are determined (Table 4.2.1) .

The specific growth rate of the microbes in benzene carbon source is obtained as follows. The bio-kinetic parameters namely μ_{max} and K_s are determined as 0.138 and 2.59 respectively (Table 4.2.1) . .

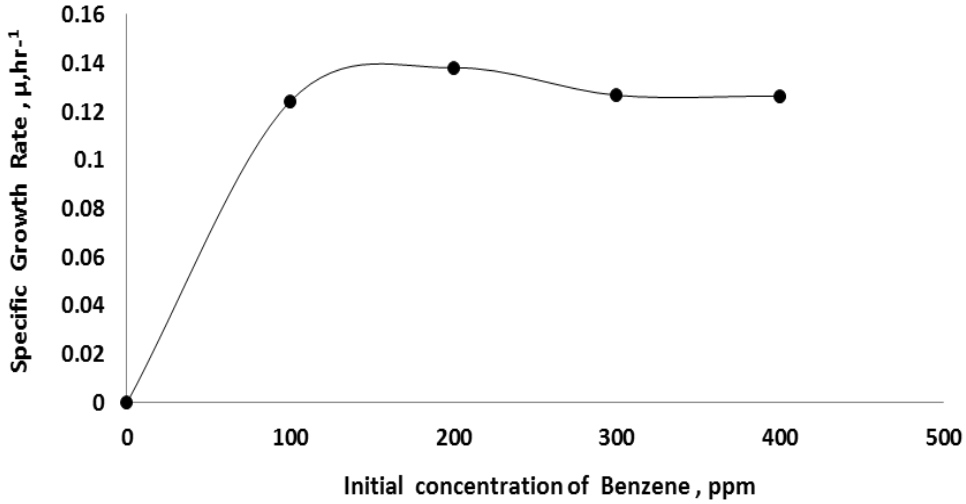

Fig4.1.2 e Specific Growth Rate curve Pseudomonas sp. For varying initial concentrations of benzene as sole carbon sorce

The specific growth rate of the microbes in Toulene carbon source is obtained as follows. The bio-kinetic parameters namely μ_{max} and K_s are determined as 0.114 and 1.65 respectively (Table 4.2.1) .

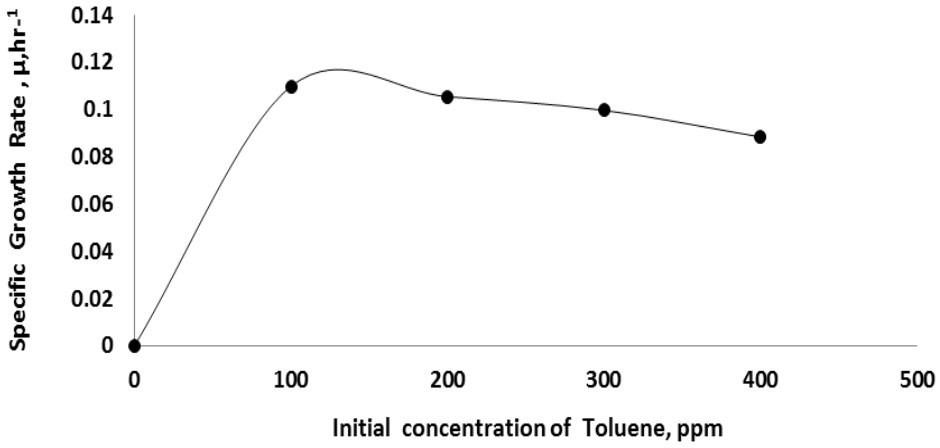

Fig4.1.2 f Specific Growth Rate curve Pseudomonas sp. For varying initial concentrations of toulene as sole carbon sorce.

The specific growth rate of the microbes in ethyl benzene carbon source is obtained as follows. The bio-kinetic parameters namely μ_{max} and K_s are determined as 0.121 and 2.12 respectively (Table 4.2.1) ..

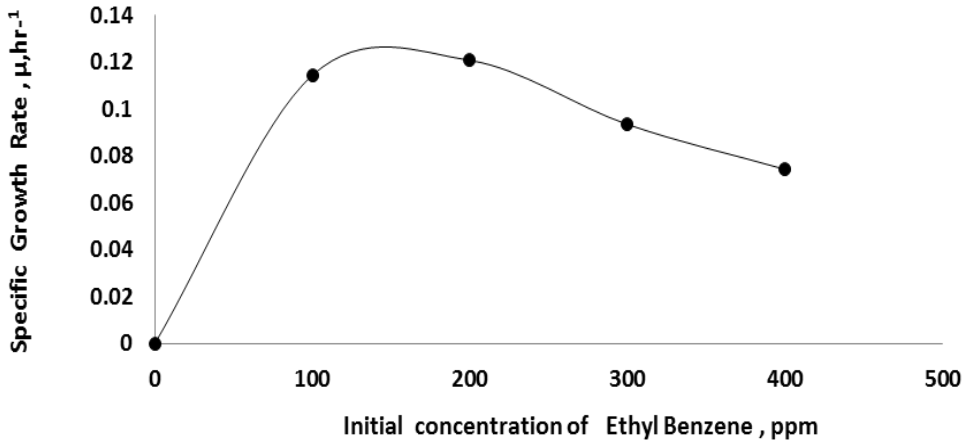

Fig4.1.2 g Specific Growth Rate curve Pseudomonas sp. For varying initial concentrations of ethyl benzene as sole carbon sorce

The specific growth rate of the microbes in Xylene carbon source is obtained as follows. The bio-kinetic parameters namely μ_{max} and K_s are determined as 0.142 and 3.36 respectively (Table 4.2.1) .

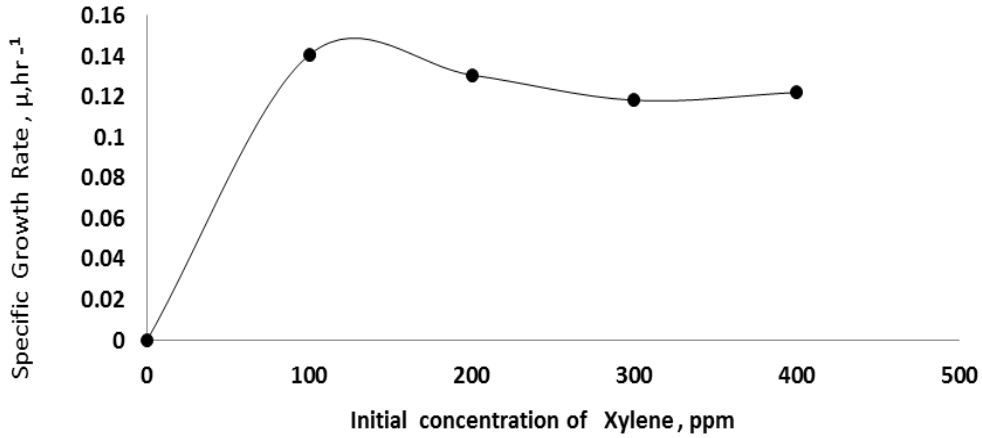

Fig4.1.2 h Specific Growth Rate curve Pseudomonas sp. For varying initial concentrations of xylene as sole carbon sorce.

The specific growth rates of the microbes in each of the BTEX substrate is plotted as follows. The curves on solving yields the bio-kinetic parameters like μ_{max} and K_s . (Table 4.2.1) .

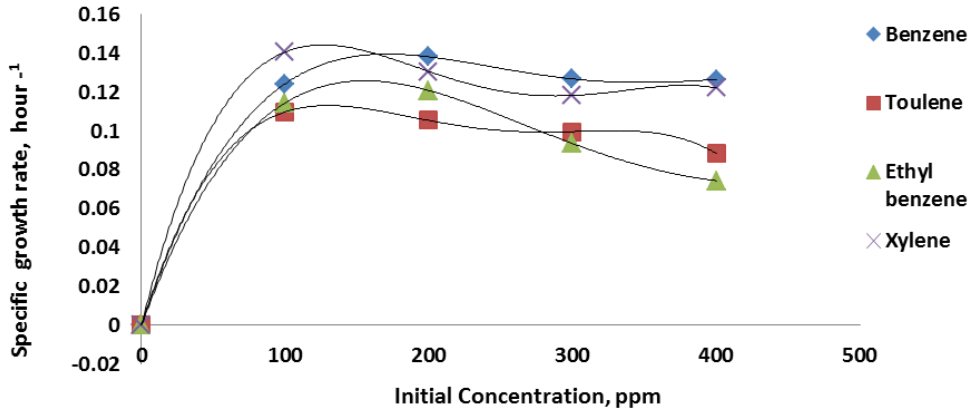

Fig4.1.2 i Specific Growth Rate curve Pseudomonas sp. For varying initial concentrations of BTEX as sole carbon sorce.

The bio-kinetic parameters like μ_{max} and K_s for each of the BTEX compound are calculated and tabulated as follows (Table 4.2.1).

Table 4.2.1 Bio-kinetic parameters μ_{max} and K_s for BTEX compounds

S.no	Compound	Maximum specific growth rate, μ_{max}, hr^{-1}	Half saturation constant , K_s
1	Benzene	0.138	2.59
2	Toluene	0.114	1.65
3	Ethyl benxene	0.121	2.12
4	Xylene	0.142	3.36

The growth rate order of pseudomonas sp. In BTEX compounds are found to follow the order of X >E>T>B.

4.2.3 Batch degradation kinetics of BTEX

Batch biodegradation experimental study of BTEX is carried out in the 500 mL reagent bottles with screwed septa caps. The stock solution preparation is done as follows. From the stock solution, further dilutions are made to get the solutions of desired final concentration.

The BTEX standards are taken in an exact quantity and the dilution to desire concentrations are achieved by making up with MSM solution. The MSM used for preparing the initial samples has to be preadjusted for the pH values of 7.To every sample about 5mL of the bacterial solution whose OD value is already known is then added.

Sample 5 is considered as the control sample is made of only the stock solution. It is not inoculated with the bacterial solution. It is also taken for the batch studies to understand any abiotic loss of BTEX. All the samples bottles are then incubated in the BOD incubator shaker maintained at 25°C, 110 rpm. Liquid sample aliquots are collected at regular intervals to measure OD, pH and the concentration levels of BTEX. Concentration levels in the control bottle (sample 5) are also measured to assess the abiotic loss. The concentration of B, T, E, X are determined using GC-FID (Fig 4.1.3.a).

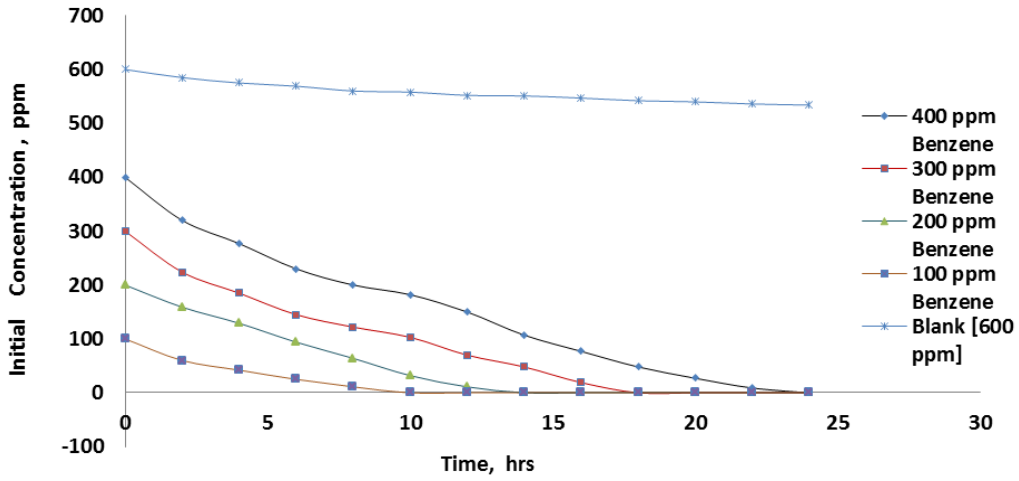

Fig4.1.3 a Biodegradation of benzene of varying initial concentrations with Pseudomonas sp. Inoculated with 0.023 mg/L dry cell weight.

The biodegradation of benzene in the batch is observed in the Fig4.1.3 a . The result shows that the abiotic loss of benzene was 0.004ppm/hr .The total concentration of benzene was found to be degraded in 24 hours.

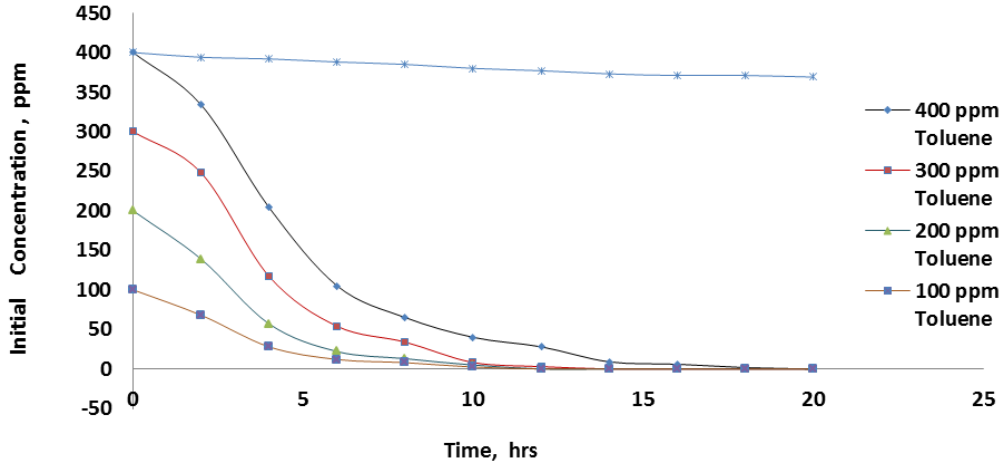

Fig4.1.3 b Biodegradation of toulene of varying initial concentrations with Pseudomonas sp. Inoculated with 0.023 mg/L dry cell weight

The biodegradation of toluene in the batch is observed in the Fig4.1.3 b . The result shows that the abiotic loss of benzene was 0.0036ppm/hr .The total concentration of benzene was found to be degraded in 20 hours.

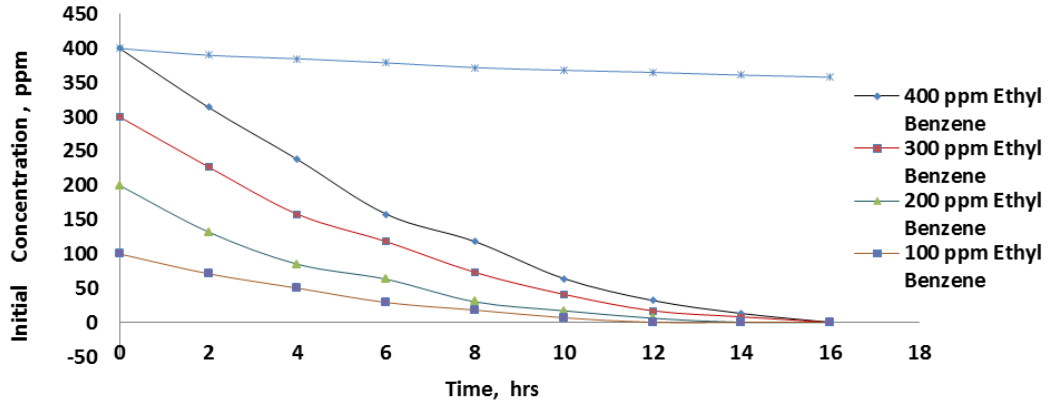

Fig4.1.3 c Biodegradation of ethyl benzene of varying initial concentrations with Pseudomonas sp. Inoculated with 0.023 mg/L dry cell weight.

The biodegradation of ethylbenzene in the batch is observed in the Fig4.1.3 c . The result shows that the abiotic loss of benzene was 0.005ppm/hr .The total concentration of benzene was found to be degraded in 16 hours.

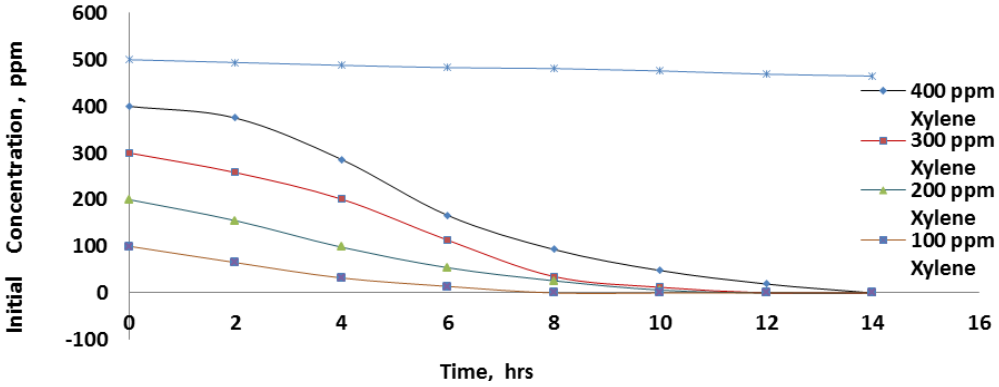

Fig4.1.3 d Biodegradation of Xylene of varying initial concentrations with Pseudomonas sp. Inoculated with 0.023 mg/L dry cell weight

The biodegradation of xylene in the batch is observed in the Fig4.1.3 d . The result shows that the abiotic loss of benzene was 0.0052ppm/hr .The total concentration of benzene was found to be degraded in 14 hours.

From the degradation curves, the degradation rate for each of the BTEX compound is determined from which the maximum degradation rate is also obtained as follows.

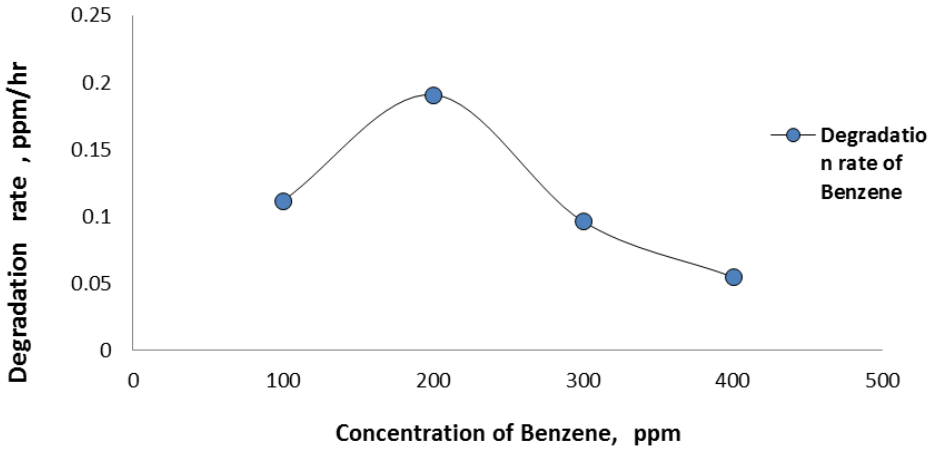

Fig4.1.3 e Biodegradation rate of benzene of varying initial concentrations with Pseudomonas sp. Inoculated with 0.023 mg/L dry cell weight

The degradation rate of benzene for various initial concentration is observed in Fig4.1.3 e. Also the maximum degradation rate of benzene was found to be 0.191 ppm/hr and it was observed for the concentration of 200mg/L (Table 4.2.2).

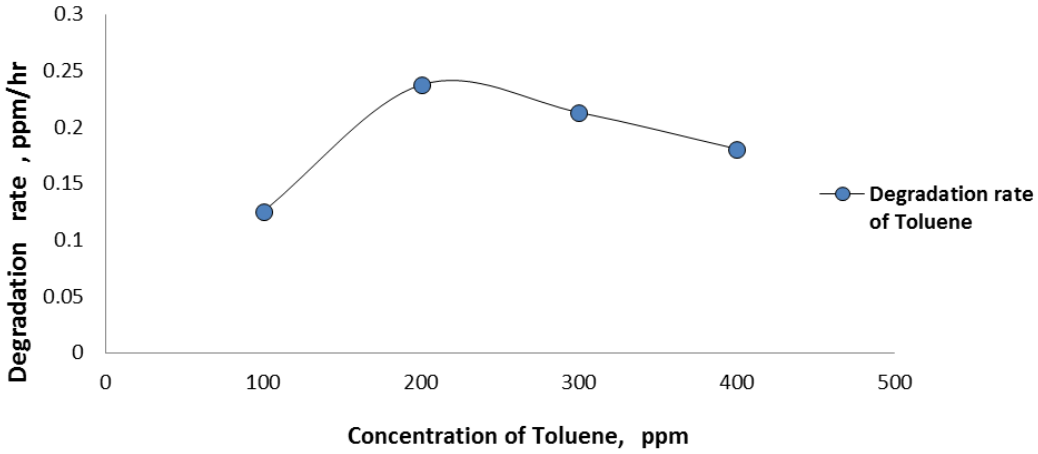

Fig4.1.3 f Biodegradation rate of toulene of varying initial concentrations with Pseudomonas sp. Inoculated with 0.023 mg/L dry cell weight

The degradation rate of toluene for various initial concentration is observed in Fig4.1.3 f. Also the maximum degradation rate of benzene was found to be 0.234 ppm/hr and it was observed for the concentration of 250mg/L (Table 4.2.2).

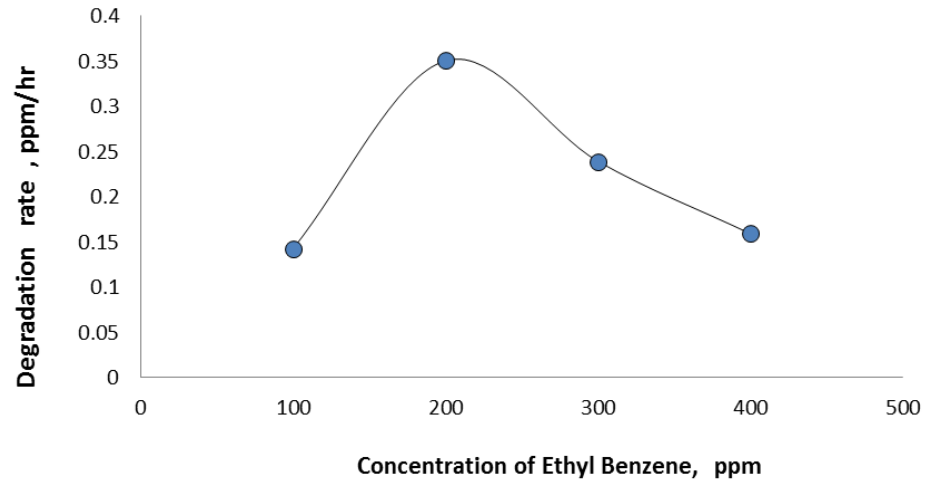

Fig4.1.3 g Biodegradation rate of ethyl benzene of varying initial concentrations with Pseudomonas sp. Inoculated with 0.023 mg/L dry cell weight

The degradation rate of ethylbenzene for various initial concentration is observed in Fig4.1.3 g. Also the maximum degradation rate of benzene was found to be 0.350 ppm/hr and it was observed for the concentration of 200mg/L (Table 4.2.2).

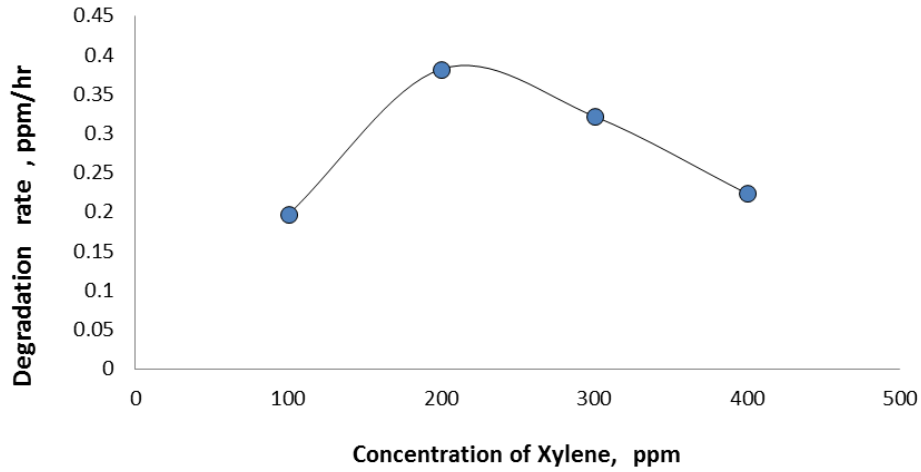

Fig4.1.3 h Biodegradation rate of Xylene of varying initial concentrations with Pseudomonas sp. Inoculated with 0.023 mg/L dry cell weight

The degradation rate of xylene for various initial concentration is observed in Fig4.1.3 h. Also the maximum degradation rate of benzene was found to be 0.382 ppm/hr and it was observed for the concentration of 225mg/L (Table 4.2.2).

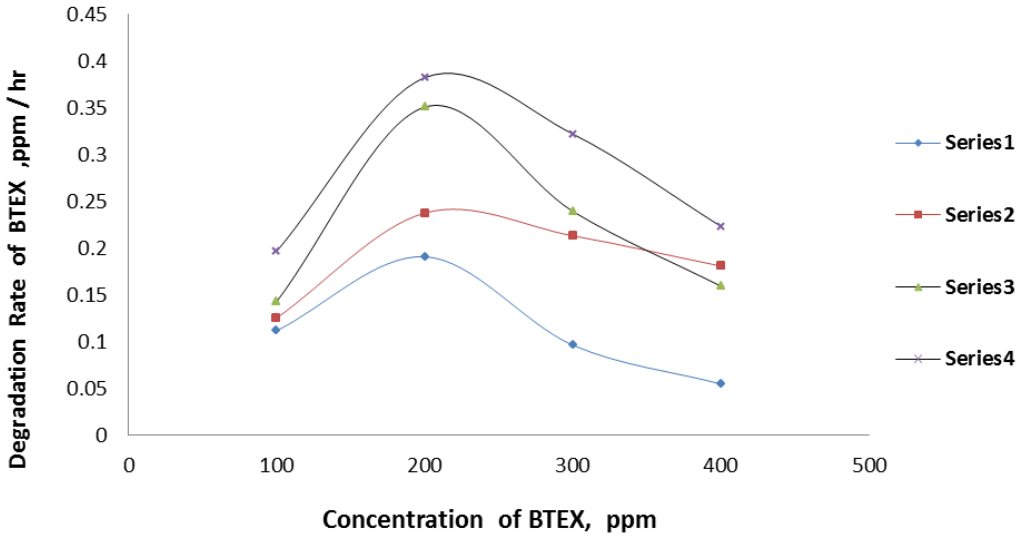

Fig4.1.3 i. Biodegradation rate of BTEX of varying initial concentrations with Pseudomonas sp. Inoculated with 0.023 mg/L dry cell weight

The maximum biodegradation rate for each of the BTEX compound in determined from the degradation rate curve Fig4.1.3 i. The results are then tabulated as follows (Table 4.2.2).

Table 4.2.2 Bio-kinetic parameters μ_{max} and K_s for BTEX compounds

S.no	Compound	Maximum Degradation rate ppm/hr	Observed concentration, ppm
1	Benzene	0.191	~200
2	Toluene	0.234	~250
3	Ethyl benxene	0.350	~200
4	Xylene	0.382	~225

Degradation order of BTEX compound is found to follow the order of X >E>T>B.

4.3 COLUMN STUDIES.

The soil is placed with the initial moisture content which is measured with the help of soil moisture measuring probe. The pumping systems are operated at the flow rates of 5 mL per minute. The flow in the column is made from top to downwards so as to have the gravity driven flow in the soil column. The flow experiment is continued till the concentration of BTEX is depleted to the maximum level from the initial concentration. The duration of flow is also found sufficient enough for the growth of biomass in the acrylic column. The soilwater is collected through the pore water samplers fixed at some regular intervals. The sample collected is centrifuged at 6000 rpm for 15 minutes. After centrifuge samples are then analyzed for the concentration of BTEX using GC-FID. The degradation profile of BTEX along the length of the soil column is then obtained (Fig 4.2.1).

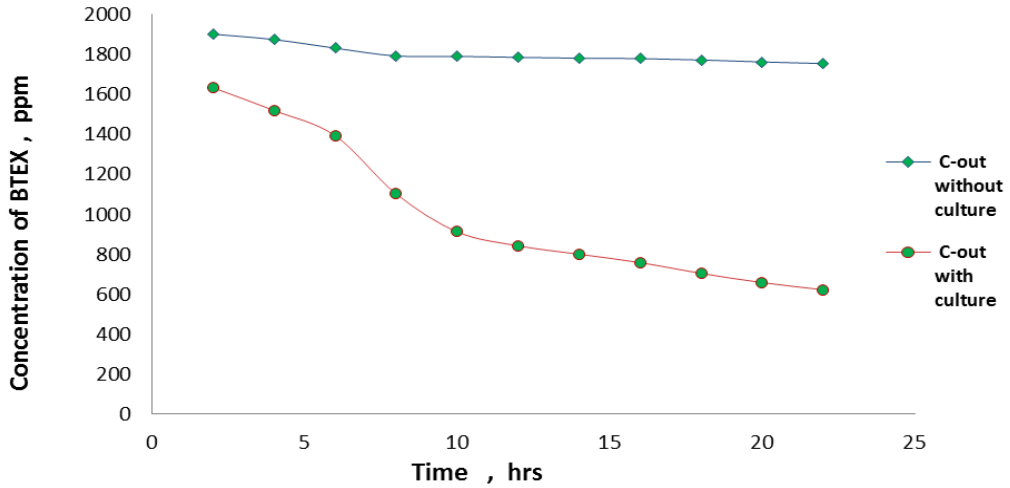

Fig4.2.1 a. Breakthrough curve of BTEX in the port-1,placed at 10 cm from the top of the soil column-with and without biotransformation

The transport of BTEX with and without biotransformation under unsaturated flow condition is observed all along the length of the soil column. Fig 4.2.1.a shows the breakthrough curve of BTEX observed in the sampling port placed at the depth of 10cm from the top of the soil.

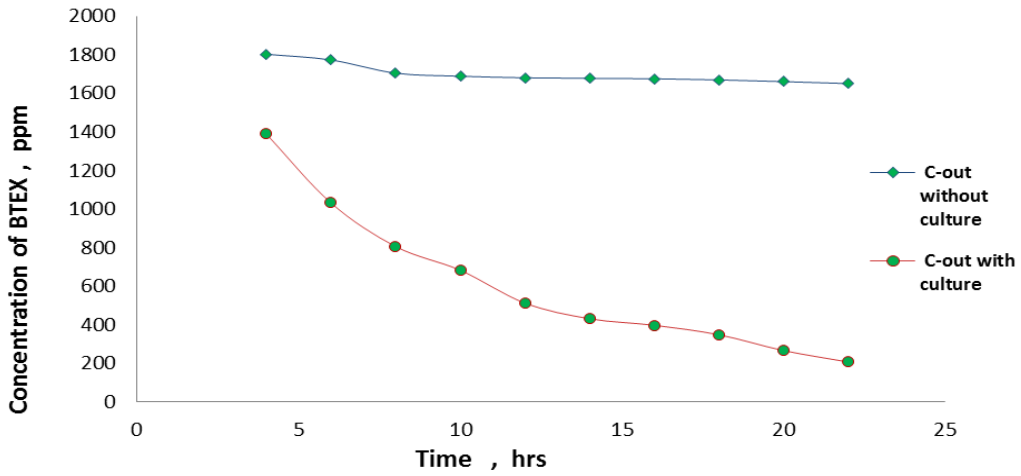

Fig4.2.1 b. Breakthrough curve of BTEX in the port-2,placed at 20 cm from the top of the soil column-with and without biotransformation.

The transport of BTEX with and without biotransformation under unsaturated flow condition is observed all along the length of the soil column. Fig 4.2.1.b shows the breakthrough curve of BTEX observed in the sampling port placed at the depth of 20cm from the top of the soil.

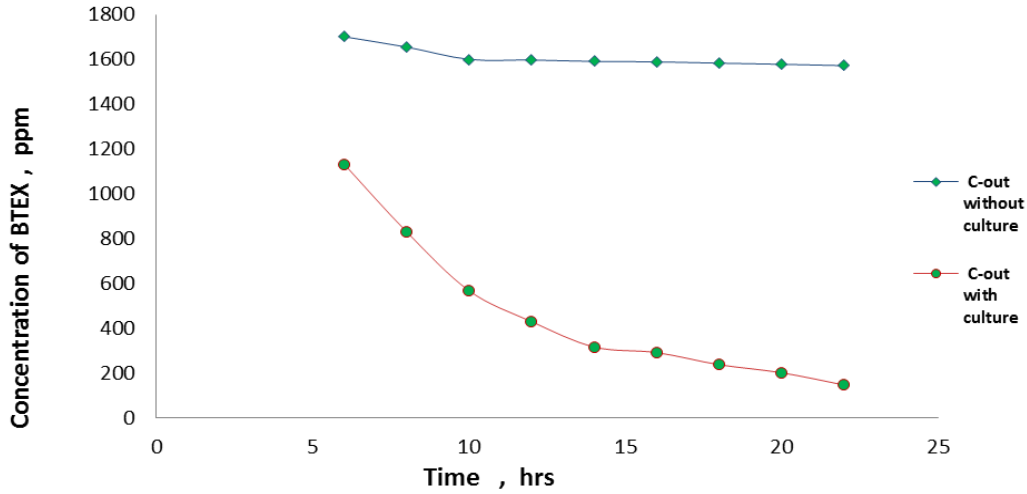

Fig4.2.1 c. Breakthrough curve of BTEX in the port-3,placed at 30 cm from the top of the soil column-with and without biotransformation

The transport of BTEX with and without biotransformation under unsaturated flow condition is observed all along the length of the soil column. Fig 4.2.1.c shows the breakthrough curve of BTEX observed in the sampling port placed at the depth of 30cm from the top of the soil.

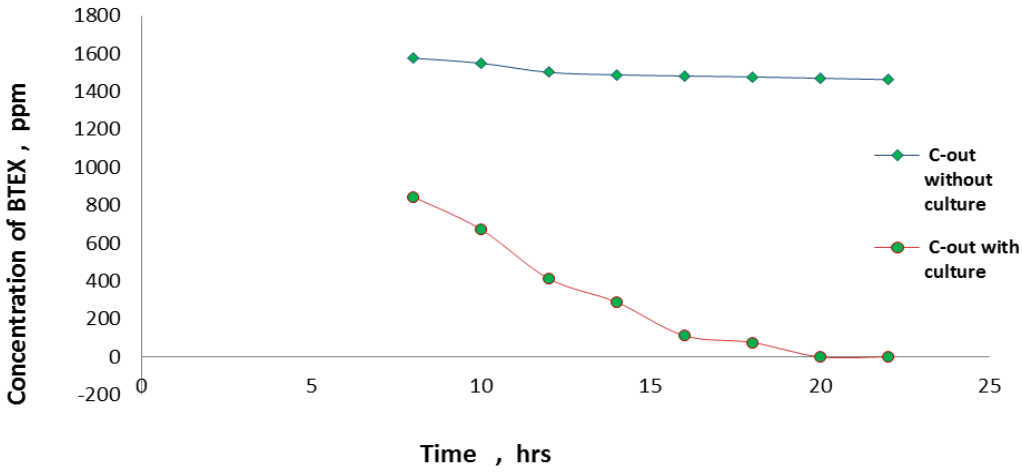

Fig4.2.1 d. Breakthrough curve of BTEX in the port-4,placed at 40 cm from the top of the soil column-with and without biotransformation.

The transport of BTEX with and without biotransformation under unsaturated flow condition is observed all along the length of the soil column. Fig 4.2.1.d shows the breakthrough curve of BTEX observed in the sampling port placed at the depth of 40cm from the top of the soil.

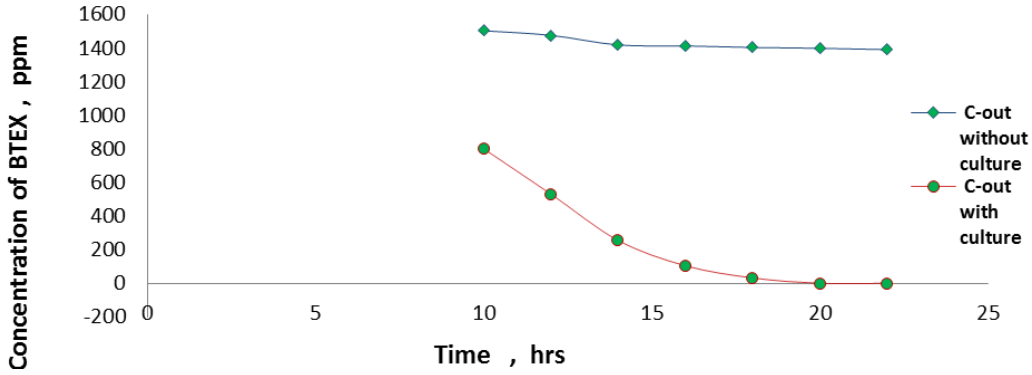

Fig4.2.1 e. Breakthrough curve of BTEX in the port-5,placed at 50 cm from the top of the soil column-with and without biotransformation

The transport of BTEX with and without biotransformation under unsaturated flow condition is observed all along the length of the soil column. Fig 4.2.1.e shows the breakthrough curve of BTEX observed in the sampling port placed at the depth of 50cm from the top of the soil.

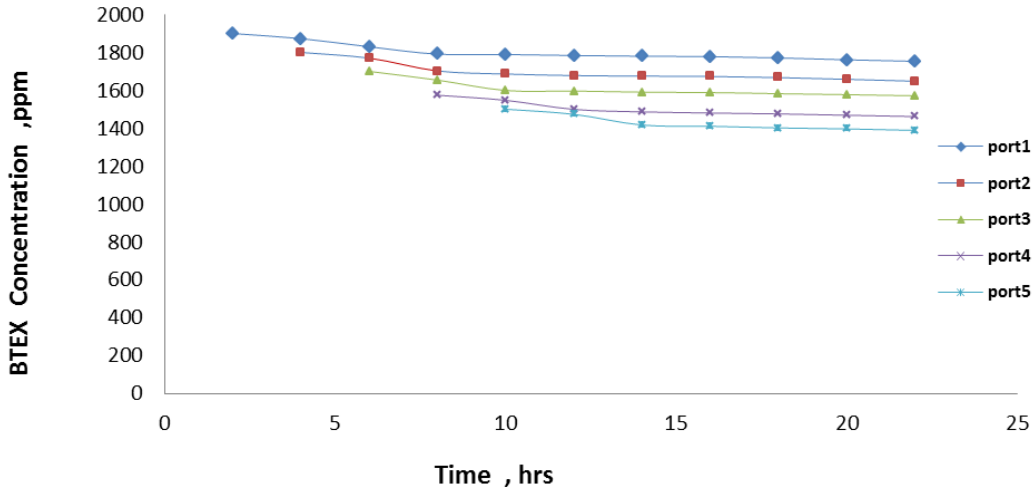

Fig4.2.2.a Breakthrough curve of BTEX along the length of the soil column(ports placed at 10cm interval) without biotransformation.

The transport of BTEX without biotransformation under unsaturated flow condition is observed all along the length of the soil column. Fig 4.2.2.a shows the breakthrough curve of BTEX observed in the sampling port placed at the depth of 10cm interval from the top of the soil.

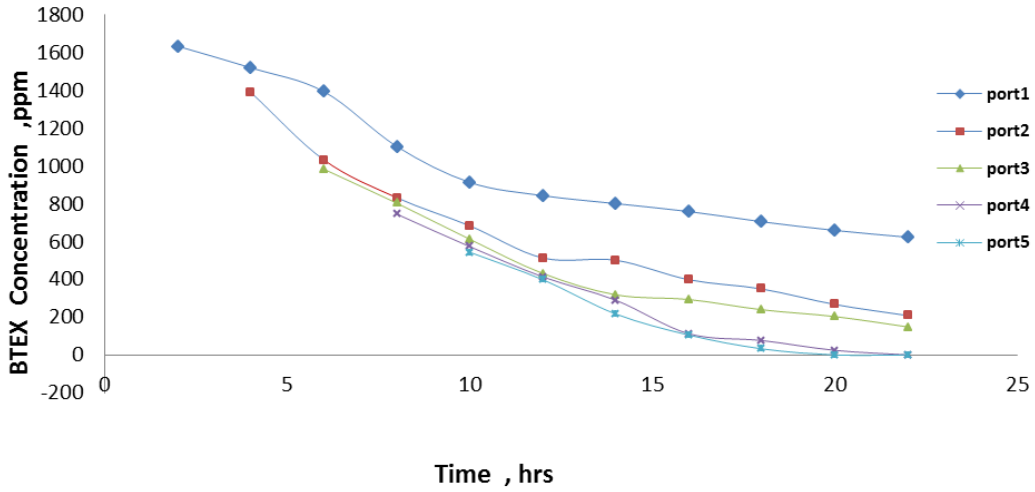

Fig4.2.2.b Breakthrough curve of BTEX along the length of the soil column(ports placed at 10cm interval) with biotransformation

The transport of BTEX with biotransformation under unsaturated flow condition is observed all along the length of the soil column. Fig 4.2.2.b shows the breakthrough curve of BTEX observed in the sampling port placed at the depth of 10cm interval from the top of the soil.

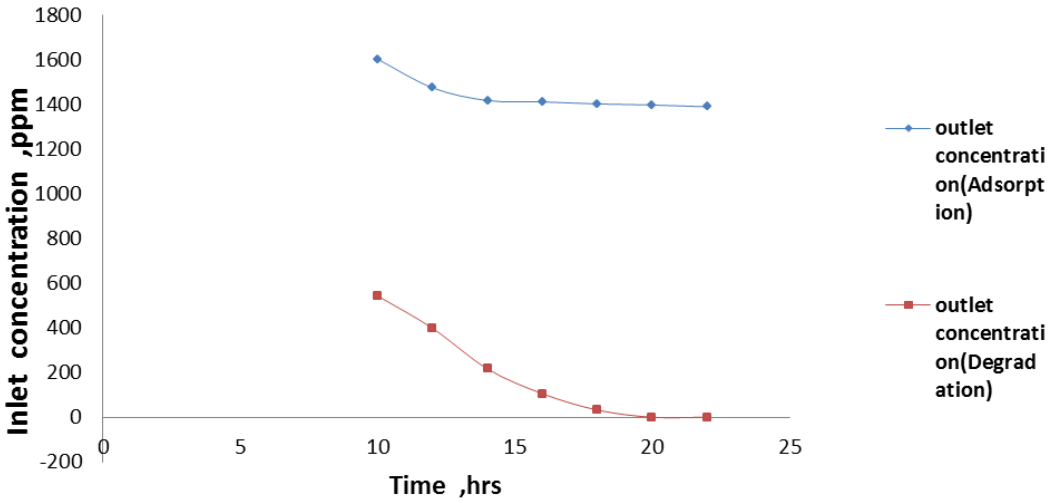

Fig4.2.2.c Breakthrough curve of BTEX –with and without biotransformation over the length of the column.

The transport of BTEX with and without biotransformation under unsaturated flow condition is observed all along the length of the soil column. Fig 4.2.2.c shows the breakthrough curve of BTEX observed with respect to the outlet concentration.

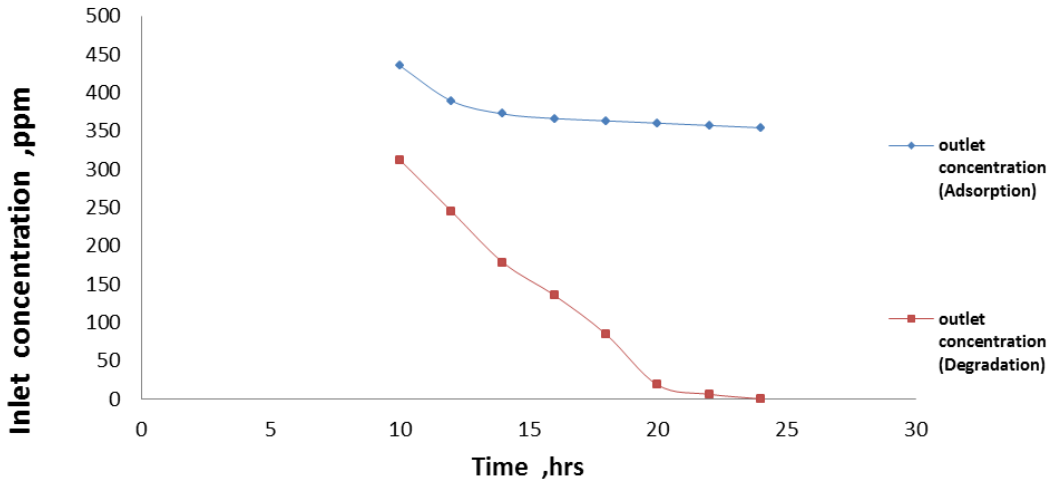

Fig4.2.3.a Breakthrough curve of Benzene –with and without biotransformation over the length of the column.

The transport of benzene with and without biotransformation under unsaturated flow condition is observed all along the length of the soil column. Fig 4.2.3.a shows the breakthrough curve of benzene observed with respect to the outlet concentration.

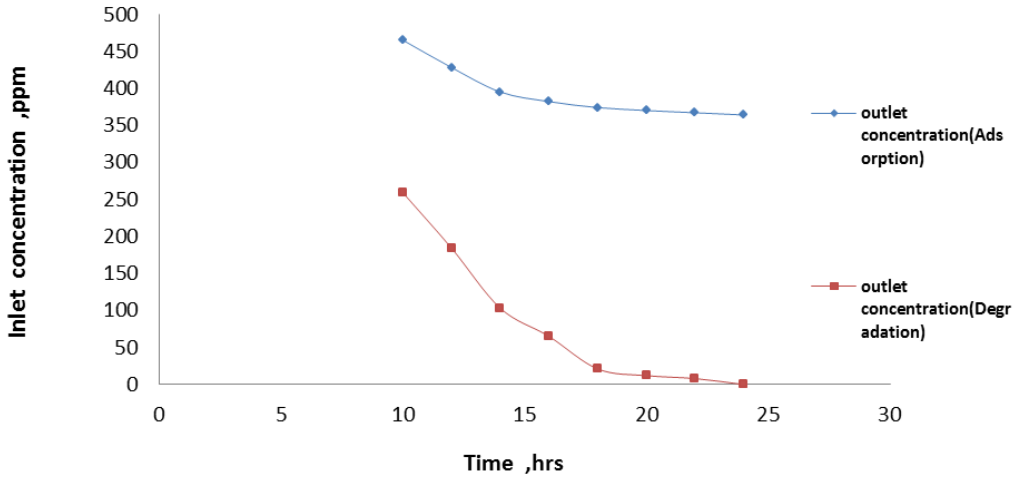

Fig4.2.3.b Breakthrough curve of toulene –with and without biotransformation over the length of the column.

The transport of toluene with and without biotransformation under unsaturated flow condition is observed all along the length of the soil column. Fig 4.2.3.b shows the breakthrough curve of toluene observed with respect to the outlet concentration.

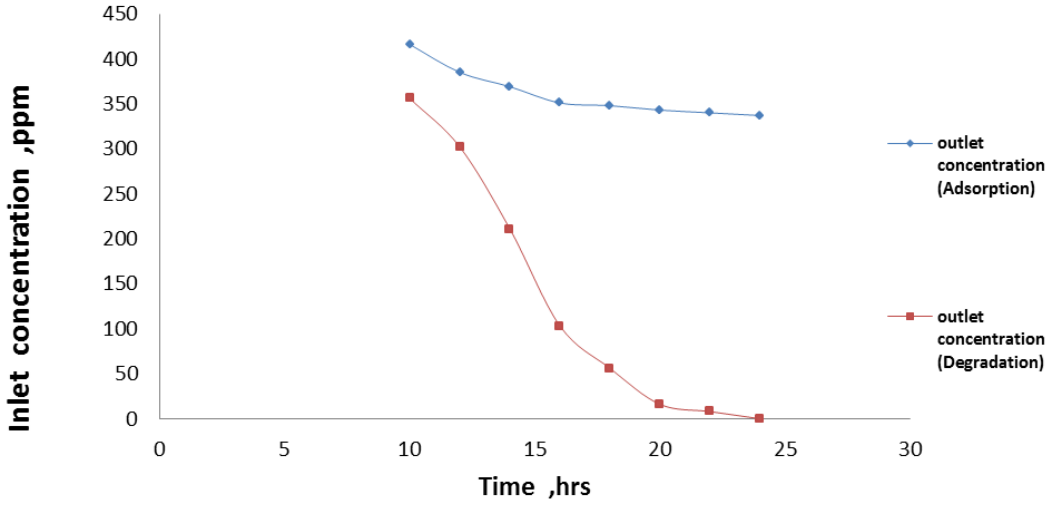

Fig4.2.3.c Breakthrough curve of Ethyl Benzene –with and without biotransformation over the length of the column.

The transport of ethylbenzene with and without biotransformation under unsaturated flow condition is observed all along the length of the soil column. Fig 4.2.3.c shows the breakthrough curve of ethylbenzene observed with respect to the outlet concentration.

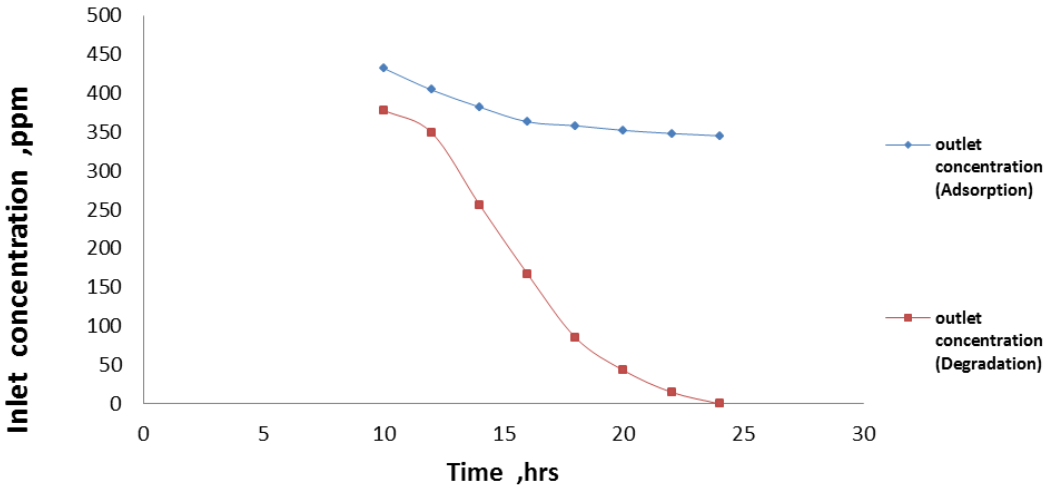

Fig4.2.3.d Breakthrough curve of Xylene –with and without biotransformation over the length of the column.

The transport of xylene with and without biotransformation under unsaturated flow condition is observed all along the length of the soil column. Fig 4.2.3.d shows the breakthrough curve of xylene observed with respect to the outlet concentration.

The degradation rate for each of BTEX compound in determined. The degradation rate is found to follow the order as E>T>B>X whereas the degradation rate observed in the batch experiment was X >E>T>B.

4.4 SOIL SUCTION EXPERIMENT

The soil suction value is measured with the sand table apparatus. The model used for the research work is FEL4 –Soil Moisture Suction Sand Table from supplied by ARMFIELD-Engineering Teaching & Research Equipment. After completing initial equilibrium condition, place the soil sample in the sampler over the saturated sand in the sand box. Then lower the constant head cell until the level is 100mm, below the level of the sand surface. This applies a suction of100 mm to the sand table. Allow the sand table to equilibrate for a few hours then record the mercury column height in all three tensiometer probes. The achieved suction at the tensiometer probe relative to the sand table surface is calculated as follows.

Achieved suction (mm of H_2 O) = 13.6 x (mercury column height at applied suction - mercury column height at zero suction).

The experiment is carried out for different region of soil in the soil column namely the top,middle and bottom portion. The amount of suction developed in the soil corresponding to the applied suction for the soil innoculated with culture and for the soil not innoculated with culture are obtained. The results are tabulated and kept for comparision below.

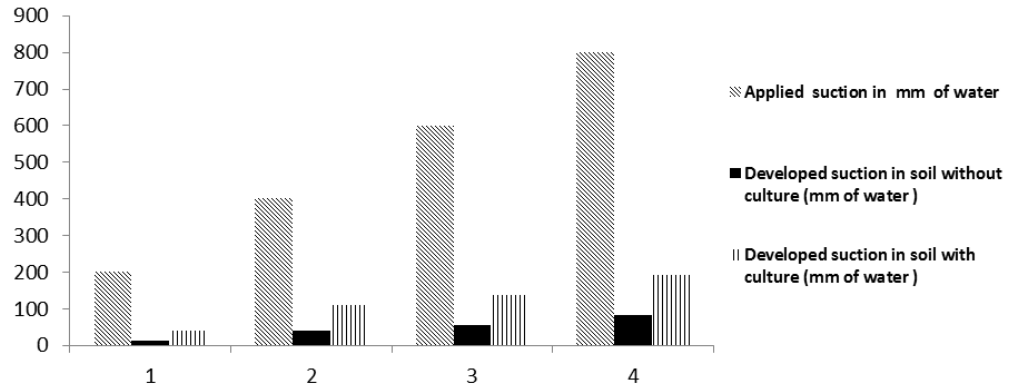

Fig4.3.1.a Suction developed in the top 15 cm of the soil in the soil column apparatus-with and without biotransformation in the soil column.

The suction developed along the column height in the soil with and without biotransformation process is obtained. It is found that the amount of suction developed in the top 15 cm is less (Fig4.3.1.a).

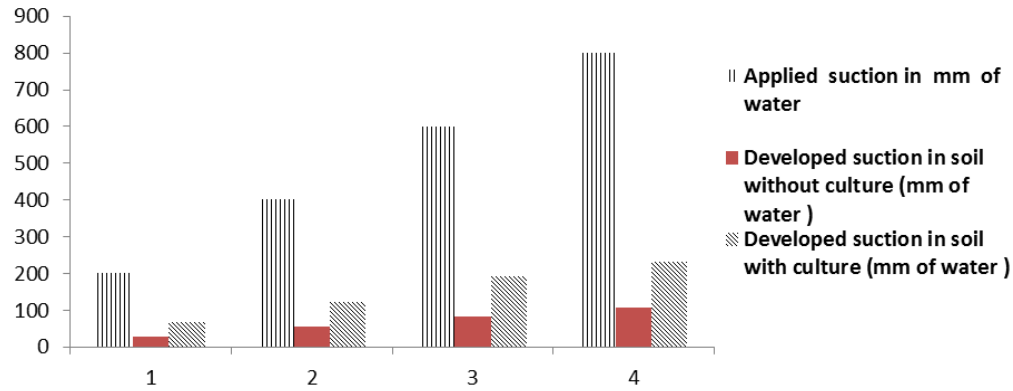

Fig4.3.1.b Suction developed in the middle 15 cm of the soil in the soil column apparatus-with and without biotransformation in the soil column

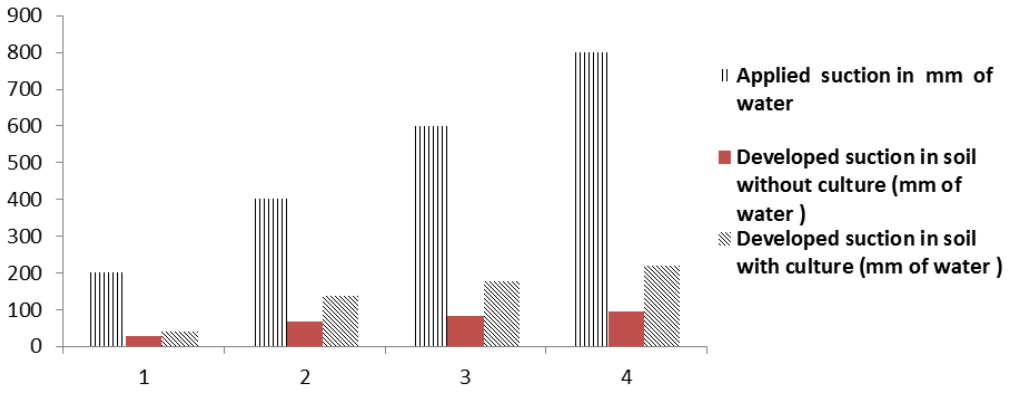

Fig4.3.1.c Suction developed in the bottom 20 cm of the soil in the soil column apparatus-with and without biotransformation in the soil column.

Same amount of suction is developed in the region from 15-30 cm and 30-50 cm of the soil column. Similar pattern of suction values are found to develop along the column height either in presence or in the absence of the microbial growth in the soil column (Fig4.3.1.b and Fig 4.3.1.c).

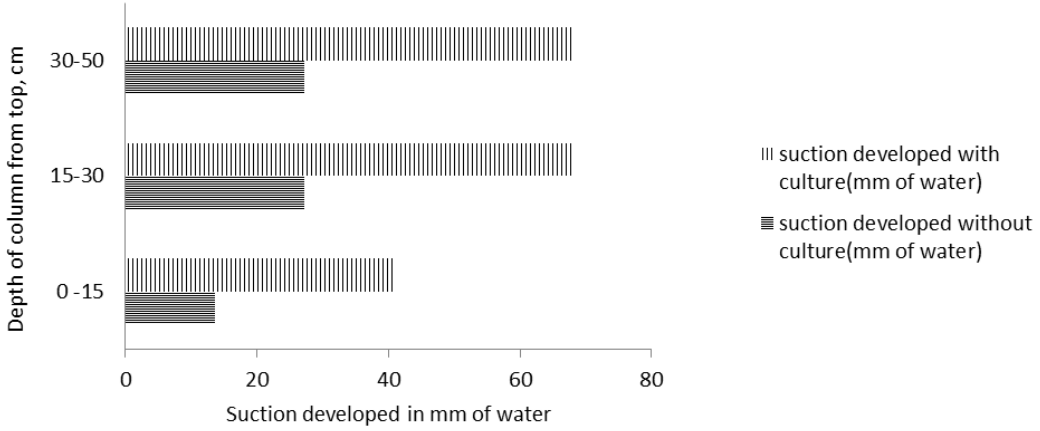

Fig4.3.2.a Suction developed along the height of the soil in the soil column apparatus-with and without biotransformation in the soil column.(when the applied suction is the minimum of of 0.2 bar)

It is found that lesser suction value developed in the top 0-15 cm depth of the column. Also lesser the applied suction lesser is the suction developed along the column height.

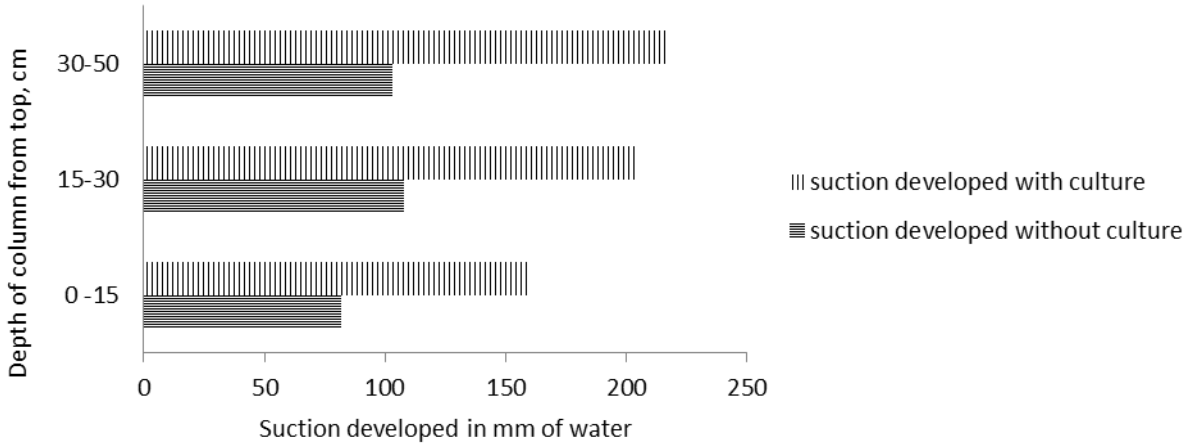

Fig4.3.2.b Suction developed along the height of the soil in the soil column apparatus-with and without biotransformation in the soil column. .(when the applied suction is the maximum of 0.8 bar)

Similar range of soil suction is developed in the middle (15-30 cm depth) and bottom region (30-50 cm) of the column. Also higher the applied suction higher is the suction developed along the column height.

4.4.1 Soil suction developed in undisturbed condition

For determining the soil suction in undisturbed condition, the soil sample in the column is taken from the modified acrylic glass column. The set up is made such a way that the column can be dismantled into slices of 10cm height. Each of the soil sample in the sliced portion is kept in the sand table apparatus to determine the amount of suction developed for various ranges of the appled suction.

The experiment is carried out for the following combination of flow conditions.

- Water + BTEX
- Only water

Case 1- Water + BTEX

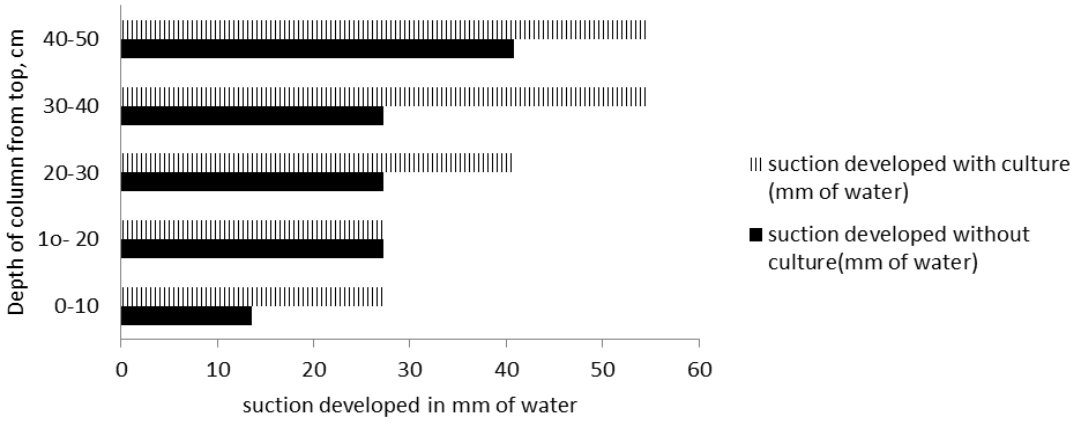

Fig4.3.3.a Suction developed along the height of the soil in the soil column apparatus-with and without biotransformation in the soil column. .(when the applied suction is of 0.2 bar)

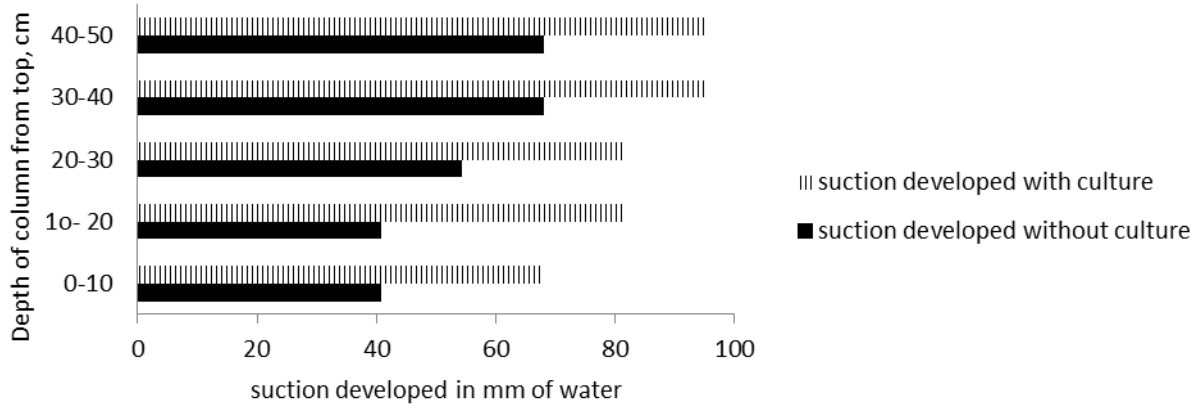

Fig4.3.3.b Suction developed along the height of the soil in the soil column apparatus-with and without biotransformation in the soil column. .(when the applied suction is of 0.4 bar)

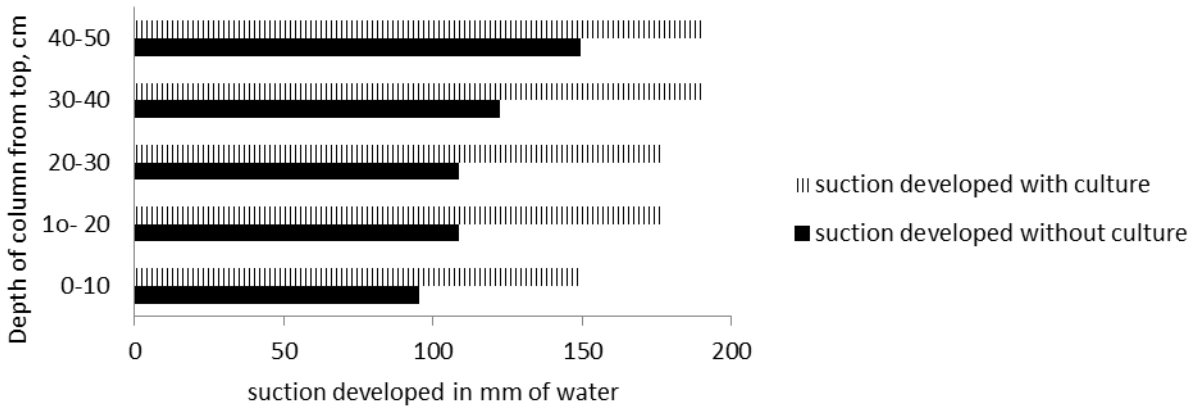

Fig4.3.3.c Suction developed along the height of the soil in the soil column apparatus-with and without biotransformation in the soil column. .(when the applied suction is of 0.6 bar)

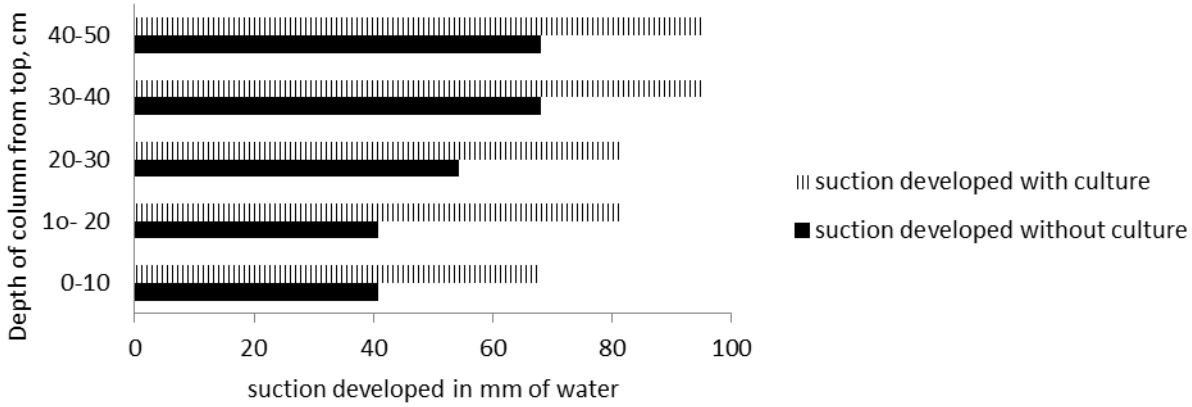

Fig4.3.3.d Suction developed along the height of the soil in the soil column apparatus-with and without biotransformation in the soil column. .(when the applied suction is of 0.8 bar)

Case 2- Only Water

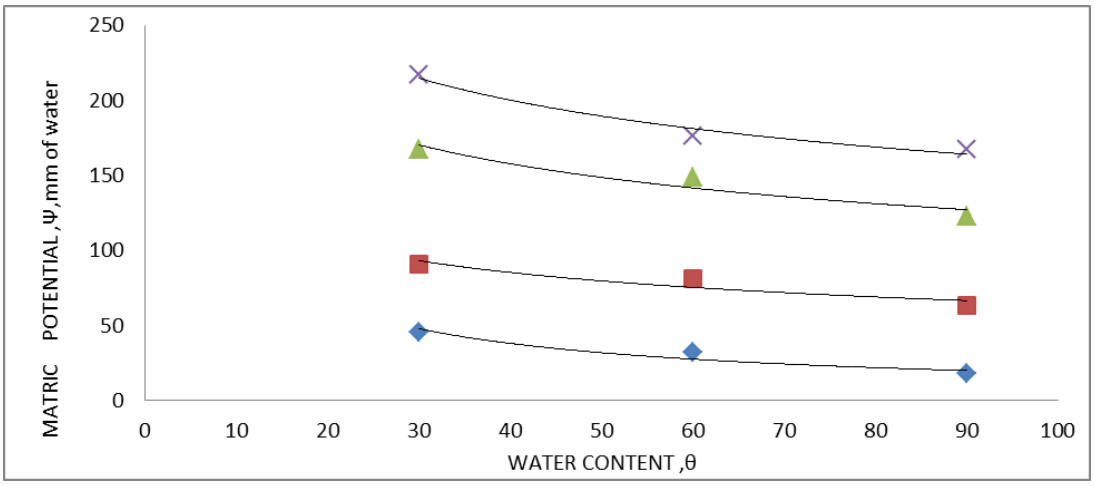

Fig4.3.3.e Suction developed in the soil sample with 30,60,90 percent moisture content for various amount of suction applied

The suction values of the soil are determined for varying saturation and at varying applied suction values. The results are plotted to arrive the soil water characteristic curve (SWCC) (Fig 4.3.3.e) which shows that higher the water content lower is the suction developed in the soil sample.

Chapter 5

5.1 Conclusion

The bio-kinetic study helps to determine the bio-kinetic parameters. The bio-kinetic parameters signify the specific growth rate of BTEX. The observed specific growth rate of the resistant culture for BTEX is found to follow the order of X >E>T>B. The batch biodegradation study helps to know the degradation rate of BTEX compounds. The observed degradation rate of BTEX is of the order X >E>T>B. Degradation study done in the continuous mode also give degradation rate of BTEX. The order is as follows E>T>B>X.

Soil suction test done using sand table apparatus gives the soil suction along the height of the soil column. It is found that lesser suction value developed in the top 0-15 cm depth of the column .Similar range of soil suction is developed in the middle (15-30 cm depth) and bottom region (30-50 cm) of the column. Considering the undisturbed soil sample, the amount of suction developed corresponding to the applied suction is found to be lesser. However the suction developed along the column height follows the similar pattern. Lesser suction values are observed in the top region of the soil sample (0-30 cm depth) when compared to the bottom 30-40 cm depth of the soil column.

The saturated hydraulic conductivity of the soil is found to be 0.000235cm/sec. The suction values of the soil are determined for varying saturation and at varying applied suction values. The results are plotted to arrive the soil water characteristic curve (SWCC). The graph shows higher the applied suction values, higher is the developed suction. And higher the saturation, lower is the developed matric potential.

Chapter 6

6.1 Future study

- The study can be extended to determine the effect of BTEX dynamics in unsaturated hydraulic conductivity and to develop k-Ψ-θ relationship in presence of microbial degradation.

Chapter 7

Figures

List of figures

References

[1] K. A. Kvenvolden and C. K. Cooper (2003) “Natural seepage of crude oil into the marine environment,” Geo-Marine Letters, 23,140–146.

[2] D.K.Scholz, J.H.Kucklick, A.H.Walker, A.Bostrom and P.Fischbeck, (1999), Fate of spilled oil in marine water. Health and Environment Science Department, API publication number 4691, 1-57.

[3] J.W.Mercer, R.M. Cohen, (1990) "A Review of immiscible Fluids in the Subsurface: Properties, Models, characterization and Remediation". Journal of Contaminant Hydrology. 6,107-163

[4] P.Lecomte and C.Mariotti (1997). Handbook of Diagnostic Procedures for Petroleum Contaminated Sites. New York: John Wiley & Sons, Inc.

[5] Devitt, D.A., Evans, R.B., Jury, W.A., and Starks, T.H (1992) Soil Gas Sensing For Detection and Mapping of Volatile Organics. Prepared for Office of Research and Development, U.S.EPA. Law Vegas, Nevada: National Groundwater Association

[6] Hudak, P.F., and Loaiciga, H.A (1999)"Conjunctive Vadose and Saturated Zone Monitoring for Subsurface Contamination”. Environmental monitoring and Assessrnent, 59, 15-29.

[7] Palmer, CD., and Johnson, R.L (1989) Transport and Fate of Contaminants in the Subsurface, Seminar Publication Nurnber. EPA/625/489/019.

[8] Yong, R.N., and Rao, S.M (1991). "Mechanistic Evaluation of Mitigation of Petroleum Hydrocarbon Contamination by soil Medium'. Canadian GèotechnicalJourna1, 28, 84-91.

[9] Hasan, S.E (1996). Geology and Hazardous Waste Management, New Jersey: Prentice Hall,143-166

[10] Patnaik, P (1997). Handbook of Environmental Analysis: Chemical Pollutants in Air, Water, Soil, and Solid Wastes. New York: Lewis Publishers.

[11] Riser-Roberts, E (1998). Remediation of Petroleum contaminated soils: Biological, Physical and Chemical Processes. Boca Raton, Florida: Lewis Publishers.

[12] Wiedemeier, J.T.Wilson, D.H.Kampbell, R.N.Miller, J.E.Hansen, (1995*)* Technical Protocol for Implementing Intrinsic Remediation with Long-Term Monitoring for Natural Attenuation of Fuel Contamination Dissolved in Groundwater, Air force center for Environmental Excellence.

[13] Muftah H. El-Naas, Janice A. Acio, Ayat E. El Telib, (2014). Aerobic biodegradation of BTEX: Progresses and Prospects Journal of Environmental Chemical Engineering 2 , 1104–1122

[14] Grathwohl.P (1998), Diffusion in Natural Porous Media: Contaminant Transport, Sorption/Desorption and Dissolution Kinetics. Boston, Massachusetts: Kluwer Academic Publishers.

[15] Eweis, J.B., Ergas, S.J., Chang, D.P.Y., and Schroeder, E.D (1998). Bioremediation Principles. Boston: McGraw Hill.

[16] Mackay, D. and Shiu, W.Y (1981). "A Critical Review of Henry's Law Constants for Chernicals of Environmental interest". *J. Phys. chem. Ref.Data*.10, 1175-1199.

[17] Lyman W.J, Smoley C.K (1992) Mobility and Degradation of Organic Contaminants in the subsurface Environments, Chelsea, Michigan.

[18] Alessandro Delle Site, (2001), Factors Affecting Sorption of Organic Compounds in Natural Sorbent Water Systems and Sorption Coefficients for Selected Pollutants. A Review, J. Phys. Chem. Ref. Data, 30.

[19] Manahan, S.E. (1994) Environmental Chemistry, Sixth Edition. Boca Raton, Florida: Lewis Publishers.

[20] Morrissey, F.A., and Grismer, M.E (1999). "Kinetics of Volatile Organic Compound Sorption/Desorption on Clay Minerals". Journal of contaminant hydrology, 36,291-312.

[21] Chiou, C.T., and Shoup, T.D (1985). "Soil Sorption of Organic Vapors and Effects of Hurnidity on Sorptive Mechanism and Capacity". Environmental science and Technology, 19, 1196-1200.

[22] Barone, F.S., Rowe, R.K (1992) and Quigley, R.M. "A Laboratory Estimation of Diffusion and Adsorption Coefficients for Several Volatile Organics in a Naturally Clayey Soil". Journal of Contaminant Hydrology, 10,225-250.

[23] English, C.W., and Loehr, R.C. (1991) "Degradation of Organic Vapors in Unsaturated Soils". Journal of Hazardous Materials 28, 53-63.

[24] Yaron.B, Calvet.R and Prost, R (1996).soil Pollution: Processes and Dynamics. Germany: Springer.

[25] Tchobanoglous, G., and Schroeder, E.D. (1987) Water quality Don Mills, Ontario: Addison-Wesley Publishing Company.

[26] Mahan, B-M., and Myers, R.J (1987). University Chemistry. Don W, Ontario: The Benjamin/Cummings Publishing Company, Inc.

[27] Millington, R.J., and Quirk, J.M. (1961) "Permeability of Porous Solids". Trans. Faraday soc, 57.1200-1207.

[28] Logan, B.E (1999) Environmental Transport Processes. Toronto: John WiIey & Sons, hc.

[29] Mendoza, C.A., and Frind, E.O (1990). "Advective-Dispersive Transport of Dense Organic Vapors in the Unsaturated Zone: 2. Sensitivity Analysis". Water Resources Research. 26,388-398.

[30] Falta, R.W., Javandel, I., Pruess, K., and Witherspoon, P.A (1989). "Density-Driven FIow of Gas in the Unsaturated Zone Due to the Evaporation of Volatile Organic Compounds". Water Resources Research.25, 2159-2169.

[31] Massmann, J., and Famer, D.F (1992). "Effects of Atmospheric Pressure Cycles on Gas Transport in the Vadose Zone". Water Resources Research.28, 777-791.

[32] Corseuil H.X (1992),"Enhanced degradation of mono aromatic hydrocarbons in sandy aquifer materials by microbial inoculation using biologically active carbon reactors" Ph.D. Dissertation, University of Michigan.

[33] Morgan P, Lewis S.T, Watkinson R.J. (1993),"Biodegradation of benzene, toluene, ethyl benzene and xylenes in gas-condensate-contaminated ground-water. Environmental pollution, 82, 181-190

[34] C. Y. Chiang, J. P. Salanitro, E. Y. Chai, J. D. Colthart and C. L. Klein,(1989), Aerobic Biodegradation of Benzene, Toluene, and Xylene in a Sandy Aquifer—Data Analysis and Computer Modeling,27,823-834.

[35] Mayur,Lodaya, Fayaz Lakhwala, Emilia Rus, Manjari Singh, Gordon Lewandowski & Sam Sofer (1991)"Biodegradation of benzene and a BTX mixture using immobilized activated Sludge",26,121-137

[36] Derek R. Lovely, Joan C. Woodward, Francis H, (1994)," Stimulated anoxic biodegradation of aromatic hydrocarbons using Fe (III) ligands" 370,128-131.

[37] Franciscka Haag, Martin Reinhard and Perry L. McCarty, (1991)," Degradation of toluene and *p*-xylene in anaerobic microcosms: Evidence for sulfate as a terminal electron acceptor", 10, 1379-1390.

[38] Alvarez PJ, Anid PJ, Vogel TM, (1991), "Kinetics of aerobic biodegradation of benzene and toluene in sandy aquifer material", 2, 43-51.

[39] Stephen R. Hutchins , Guy W. Sewell , David A. Kovacs , Garmon A. Smith,(1991)," Biodegradation of aromatic hydrocarbons by aquifer microorganisms under denitrifying conditions",25,68-73

[40] V. La Rue. (1993),” Bioremediation of petroleum hydrocarbons in soil: activated sludge treatability study”, M.Sc. thesis, the university of Manitoba.

[41] P.Scherer, G.Mille, (1989),” Biodegradation of crude oil in an experimentally polluted peaty mangrove soil”, marine pollution bulletin, 20,430-432.

[42] Enning Zhou, L.Ronald, Crawford, (1995),” Effects of oxygen, nitrogen, and temperature on gasoline biodegradation in soil, 6,127-140.

[43] W.J.Weber, Jr.H.X.Corseuil, (1994),” Inoculation of contaminated subsurface soils with enriched indigenous microbes to enhance bioremediation rates”28, 1407-1414.

[44] R. R. Yarwood, M. L. Rockhold, M. R. Niemet, J. S. Selker and P. J. Bottomley. (2006),”Impact of microbial growth on water flow and solute transport in unsaturated porous media” Water Resour. Res., 42, W10405

[45] R.Brooks, A.Corey (1964). Hydraulic Properties of Porous Media, Hydrology Paper No. 3. Colorado State University, Fort Collins, CO

[46] Liu Yan-hua, Gong Wei-bi and Su Hong (2002) “Research on soil-water characteristic curve of unsaturated soils.” Engineering Investigation, (3), 8-11.

[47] Zhou Jian (2005) “Research summary about main influence factors of unsaturated soil- water characteristic curve.” The second national symposium on unsaturated soil, Hangzhou: Zhejiang University.

[48] Tan Yun-zhi, Wang Shi-mei, and Chen Yong (2005) “Soil-water characteristic curve study.” The second national symposium on unsaturated soil, Hangzhou: Zhejiang University.

[49] S.K.Vanapalli, D.E Pufahl, D.G.Fredlund, (1999). The influence of soil structure and stress history on the soil-water characteristic of a compacted till. Geotechnique, 49(2), 43-159.

[50] D.G.Fredlund, A. Xing, (1994). Equations for the soil-water characteristic curve. Canadian Geotechnical Journal, 31,521-532.

[51] M.P.Suarez, H.S.Rifai, (1999.). "Biodegradation Rates for Fuel Hydrocarbons and Chlorinated Solvent in Groundwater". Bioremediation Journa1, 3(4), 337-362.

[52] D.W. Ostendorf, and D.H.KampbeU, (1991). "Biodegradation of Hydrocarbon Vapors in the Unsaturated Zone". Water Resources Research, 27(4) 453-462.

[53] B. Hendricks, H. Junca, J. Vosahlova, A. Lindner, I. Ruegg, M. Bucheli-Witschel.et al (2006), Alternative primer sets for PCR detection of genotypes involved in bacterial aerobic BTEX degradation: distribution of the genes in

BTEX degrading isolates and in subsurface soils of a BTEX contaminated industrial site, Journal of Microbiological Methods 64 250–65.

[54] M.P. Ryan, J.T. Pembroke, C.C. Adley, Ralstonia pickettii in environmental biotechnology (2007), potential and applications, Journal of Applied Microbiology 103,754–64.

[55] L. Cavalca, E. Dell' Amico, V. Andreoni (2004), Intrinsic bioremediability of an aromatic hydrocarbon-polluted groundwater: diversity of bacterial population and toluene monoxygenase genes, Applied Microbiology and Biotechnology 64,576–87.

[56] Y. Shinoda, Y. Sakai, H. Uenishi, Y. Uchihashi, A. Hiraishi, H. Yukawa, et al (2004), Aerobic and anaerobic toluene degradation by a newly isolated denitrifying bacterium, Thauera sp. Strain DNT-1, Applied and Environmental Microbiology 70,1385–92.

[57] G. Johnson, R. Olsen (1997), multiple pathways for toluene degradation in Burkholderia sp. strain JS150, Applied and Environmental Microbiology 63.

[58] J.K. Fredrickson, D.L. Balkwill, G.R. Drake, M.F. Romine, D.B. Ringelberg, D.C (1995). White, Aromatic-degrading sphingomonas isolates from the deep subsurface, Applied and Environmental Microbiology 61 (5).

[59] A.L. Page, (1982). Methods of soil analysis: chemical and microbiological properties. Soil Science Society of America Inc., 643-703.

[60] G. C.Okpokwasili and C. O. Nweke (2005), "Microbial growth and substrate utilization kinetics," African J. Biotechnology., 5(4) 305–317.

[61] E. Alfnes, G.D.Breedveld, H.K.French, T.S.Pedersen, and P. Aagaard, (2002)," Transport and Degradation of Toluene and o-Xylene in an Unsaturated Soil with Dipping Sedimentary Layers," J. Environ. Qual.1809–1823. [1]

[62] T. Abuhamed, E. Bayraktar, T. Mehmetoglu, and U.Mehmetoglu, "Kinetics model for growth of Pseudomonas putida F1 during benzene, toluene and phenol biodegradation," Process Biochem., vol. 39, no. 8, pp. 983–988, 2004.

[63] M. B. Desta (2013), "Batch sorption experiments: Langmuir and freundlich isotherm studies for the adsorption of textile metal ions onto teff straw agricultural waste," J. Thermodyn.

[64] A. Fasano, M. U. Dini, M. Primicerio, and M. U. Dini (2006), “Modeling changes of hydraulic properties induced by biomass growth in unsaturated,”1, 1–4.

[65] P. Vandeviveret and P. Baveye, “Effect of bacterial extracellular polymers on the saturated hydraulic conductivity of sand Effect of Bacterial Extracellular Polymers on the Saturated Hydraulic Conductivity of Sand Columns,” Appl. Environmental Microbial., vol. 58, no. 5, pp. 1690–1698, 1992.

[66] M. Thullner, J. Zeyer, and W. Kinzelbach, “Influence of microbial growth on hydraulic properties of pore networks,” Transp. Porous Media, vol. 49, no. 1, pp. 99–122, 2002.

[67] P. Vandevivere and P. Baveye, “Saturated Hydraulic Conductivity Reduction Caused by Aerobic Bacteria in Sand Columns,” Soil Sci. Soc. Am. J., vol. 56, no. 1, p. 1, 1992.

[68] M. L. Rockhold, R. R. Yarwood, M. R. Niemet, P. J. Bottomley, and J. S. Selker, “Considerations for modeling bacterial-induced changes in hydraulic properties of variably saturated porous media,” Adv. Water Resour., vol. 25, no. 5, pp. 477–495, 2002.