# STUDIES ON BIODEGRADATION OF VOLATILE ORGANIC COMPOUNDS FROM INDUSTRIAL WASTE GASES

## A DISSERTATION

*Submitted in partial fulfillment of the requirements for the award of the degree of*

**INTEGRATED DUAL DEGREE**

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**in**

**CHEMICAL ENGINEERING**

**(With Specialization in Hydrocarbon Engineering)**

**By**

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## CANDIDATE'S DECLARATION

I hereby declare that the work, which is being presented in this report entitled **"STUDIES ON BIODEGRADATION OF VOLATILE ORGANIC COMPOUNDS FROM INDUSTRIAL WASTE GASES"** in partial fulfillment of the requirement for the award of the degree of **Integrated Dual Degree** in Chemical Engineering with specialization in **Hydrocarbon Engineering** and submitted in the Department of Chemical Engineering of Indian Institute of Technology Roorkee, India, is an authentic record of my own work carried out during the period of May 2011 to June 2012, under the supervision of **Dr. C. Balomajumder**, Associate Professor, Department of Chemical Engineering, Indian Institute of Technology Roorkee India.

The matter embodied in this project work has not been submitted by me for the award of any other degree in any other Institute/University.

Date: 15 June, 2012

**(PRABHAT KUMAR THAKUR)**

Place: Roorkee

## CERTIFICATE

This is to certify that the above statement made by the candidate is correct to the best of my knowledge and belief.

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# ABSTRACT

Benzene, toluene and o-xylene are few of hazardous chemicals whose emission to the environment needs to be monitored and controlled. They are released from motor vehicle exhaust, motor vehicle evaporative losses, petroleum refining, petroleum storage and dispensing facilities, surface coating, use of solvents, biomass burning, and smoking. The various treatment technologies available to reduce the content of emission of BTX in atmosphere are condensation, scrubbing, incineration, catalytic oxidation, adsorption, biological oxidation. Biological treatment is an attractive alternative for low concentration gas streams because of its low energy consumption, relatively moderate operating costs and minimal by-products generation.

Using the above information it was decided to study biodegradation of single substrate i.e Benzene, Toluene and o-Xylene in batch reactor by *A. chroococcum,* estimate kinetic parameters for growth of *A.chroococcum* using BTX as substrate and use of biotrickling filter for treatment of air stream containing BTX.

The optimum pH and temperature for individual biodegradation of BTX and simultaneous BTX was found to 7 and 30 °C respectively. It was found that *A. chroococcum* can successfully degrade BTX. Kinetic parameters were found.

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# NOMENCLATURE

| | |
|---|---|
| $X_{1,2}$ | Biomass concentration at the starting and ending of log phase respectively (mg $L^{-1}$) |
| t | Time (h) |
| $\mu$ | Specific cell growth rate ($h^{-1}$) |
| $\mu_{max}$ | Maximum specific cell growth rate ($h^{-1}$) |
| S | Substrate concentration ($mgL^{-1}$) |
| $K_S$ | Saturation constant ($mgL^{-1}$) |
| $K_I$ | Self-inhibition constant ($mgL^{-1}$) |
| EBRT | Empty bed residence time (min) |
| LR | Inlet Loading Rate ($gm^{-3}h^{-1}$) |
| EC | Elimination capacity ($gm^{-3}h^{-1}$) |

# CHAPTER 1

# INTRODUCTION

## 1.1. Introduction

With the increasing human population, there has been increase in demand for various products for human consumption. The production of these demands has increased the industrialization which in turn has led to increase in emission of industrial pollutants. Many of these compounds have high stability and therefore difficult to be easily degraded or removed by microorganisms and therefore they tend to accumulate in environment. Some of them are highly toxic whose accumulation can cause severe environmental problems. Volatile organic compounds (VOCs) are one such category of compounds whose accumulation in environment can cause serious health issues for humans. Among VOCs, benzene, toluene and o-xylene (BTX) are major pollutants released from various sources and are of particular concern and therefore their emissions needs to be monitored and controlled.

## 1.2. Definition of VOCs

WHO defines VOC as any organic compound whose boiling point is in the range of (50 °C to 100 °C) to (240 °C to 260 °C) corresponding to having saturation vapor pressures at 25 °C greater than 102 kPa (ISO, 2011).

According to European Union, a VOC is any organic compound having an initial boiling point less than or equal to 250 °C measured at standard atmospheric pressure of 101.3 kPa (Directive 2004/42/CE, 2004).

According to California Specification 01350 (California Specification 01350, 2010), VOC refers to carbon containing compounds (excluding carbon monoxide, carbon dioxide, carbonic acid, metallic carbides and carbonates and ammonium carbonate) with vapor pressures at standard conditions approximately ranging between those for n-pentane through n-heptadecane.

In India, VOC refers to compounds having a boiling point range with lower limit between 50 °C and 100 °C and an upper limit between 240 °C and 260 °C (CPCB, 2009).

According to EPA, 62 VOCs have been categorized as Hazardous Air Pollutants. These 62 VOCs are shown in Table 1.1(CPCB, 2009)

**Table 1. 1: List of VOCs categorized as Hazardous Air Pollutants**

| | | |
|---|---|---|
| Acetone | Ethyl dibromide (1,1-Dibromoethane) | Ethanol |
| Benzene | 4-Ethyltoluene | Ethyl acetate |
| Benzyl chloride | Trichlorofluoromethane (Freon 11) | Ethyl benzene |
| Bromoform | Dichlorodifluoromethane(Freon 12 ) | 1,4-Dioxane |
| Bromomethane | 1,1,2-trichloro-1,2,2-trifluoroethane (Freon 113) | Propylene |
| Bromodichloromethane | 1,2-Dichlorotetrafluoroethane (Freon 114) | Styrene |
| 1,3-Butadiene | Hexachloro-1,3-butadiene | Carbon disulfide |
| 2-Butanone (MEK) | 2-Hexanone (MBK) | Carbon tetrachloride |
| Dibromochloromethane | 4-Methyl-2-pentanone (MIBK) | Chlorobenzene |
| 1,2-Dichlorobenzene | Methylene chloride | Chlorethane |
| 1,3-Dichlorobenzene | Methyl-tert-butylether (MTBE) | Chloroform |
| 1,4-Dichlorobenzene | 2-Propanol | Cyclohexane |
| 1,1-Dichloroethane | 1,1,2,2-Tetrachloroethane | Chloromethane |
| 1,2-Dichloroethane | Tetrachloroethene | Heptane |
| 1,1-Dichloroethene | Tetrahydrofuran | Toluene |
| cis-1,2-Dichloroethene | 1,1,1-Trichloroethane | o-Xylene |
| trans-1,2-Dichloroethene | 1,1,2-Trichloroethane | m-Xylene |
| 1,2-Dichloropropane | Trichloroethene | p-Xylene |
| cis-1,3-Dichloropropene | 1,2,4-Trichlorobenzene | Vinyl acetate |
| trans-1,3-Dichloropropene | 1,2,4-Trimethylbenzene | Vinyl chloride |
| Hexane | 1,3,5-Trimethylbenzene | |

CPCB has classified above 62 VOCs into 15 high priority, 17 medium priority and 20 low priority VOCs for India. The high priority VOCs are

**Table 1. 2: High priority VOCs**

| |
|---|
| 1,3-Butadiene |
| Toluene |
| Vinyl Chloride |
| Acrylonitrile |
| Maleic anhydride |
| Toluedine isocynate |
| Epichloro hydrine |
| Phosgene |
| Benzene |
| Ethylene oxide |
| Propylene oxide |
| Caprolactum |
| Phthalic anhydride |
| Carbon tetrachloride |
| Hydrogen cyanide |

Benzene has been classified as high priority VOC due to its high toxicity and carcinogenic property whereas toluene and o-xylene are classified as high and medium priority respectively on the basis of toxicity and photochemical ozone creation potential.

**1.3.Properties of BTX**

Table 1.3 table shows the properties of benzene, toluene and o-xylene.

**Table 1. 3: Properties of benzene, toluene and o-xylene (BTX)**

| | Benzene | Toluene | o-Xylene |
|---|---|---|---|
| Molecular Weight (g $mole^{-1}$) | 78 | 92 | 106 |
| Density (g $ml^{-1}$) | 0.88 | 0.87 | 0.88 |
| Boiling Point (°C) | 80.1 | 110.8 | 144.4 |
| Water solubility ($mgl^{-1}$) | 1780 | 535 | 175 |
| Vapor Pressure (mm of Hg) | 76 | 22 | 5 |

### 1.4. Sources of VOC

VOCs are emitted from both biogenic and anthropogenic sources. Biological sources emit an estimated 1150 Tg of carbon per year in form of VOCs (Goldstein and Galbally, 2007). The majority of total VOC emissions from biogenic sources are from forests, grasslands, shrub-lands and croplands.

Around 142 Tg of carbon per year in form of VOCs are released from anthropogenic sources worldwide (Goldstein and Galbally, 2007). The major anthropogenic sources are motor vehicle exhaust, motor vehicle fuel evaporative losses, petroleum refining, petroleum storage and dispensing facilities, surface coating, use of solvents, biomass burning, and smoking. Fujita (2001) found vehicular exhaust to be greatest emission source of VOCs especially in urban area. Shrivastava et al. (2005) have reported vehicular exhaust and refueling emissions to be major source in Delhi and evaporative emissions to be major contributor in Mumbai.

Table 1.4 shows the concentration of benzene, toluene and o-xylene (BTX) concentration in various cities of world (Kumar and Viden, 2007)

**Table 1. 4: Concentration of BTX (ppb) in various cities of world**

| City | Benzene | Toluene | o-Xylene |
|---|---|---|---|
| Yokohama, Japan | 0.88 | 4.43 | 0.13 |
| Linan site, China | 1.01 | 1.81 | 0.15 |
| PolyU station, Hongkong | 1.52 | 7.66 | 0.66 |
| Seoul, South Korea | 1.60 | 12.80 | 1.50 |
| Ulsan, South Korea | 1.10 | 3.90 | 0.90 |

| | | | |
|---|---|---|---|
| Karachi, Pakistan | 5.20 | 7.10 | 1.10 |
| Bangkok, Thailand | 5.60 | 48.90 | 6.60 |
| Manila, Philipines | 3.90 | 44.20 | 3.80 |
| Taiwan | 1.30 | 7.30 | 1.10 |
| Izmir | 17.50 | 27.80 | 19.50 |
| Delhi, India | 3.92 | 10.88 | 1.29 |
| Munich, Germany | 3.10 | 5.30 | - |
| Lille, France | 2.43 | 5.12 | 0.99 |
| Czech Republic | 0.35 | 0.28 | - |
| UC, London | 1.87 | 3.62 | 0.80 |
| Rome, Italy | 11.14 | 26.52 | 5.80 |
| Athens, Greece | 5.00 | 14.30 | 3.70 |
| Copenhagen, Denmark | 6.20 | 13.50 | - |
| Sydney, Australia | 2.60 | 8.90 | 1.50 |
| Perth, Australia | 1.44 | 2.56 | 0.63 |
| New York, USA | 0.80 | 1.73 | 0.35 |
| Minnesota, USA | 0.57 | 1.01 | 0.18 |
| Chicago, USA | 2.40 | 3.80 | 0.40 |
| Santiago, Chile | 6.00 | 21.80 | 3.80 |
| SP, Brazil | 2.60 | 9.00 | 1.50 |
| SP, Brazil | 2.50 | 15.10 | 3.50 |
| Quito, Ecuador | 1.60 | 4.00 | 0.50 |
| Caracas, Venezuela | 4.40 | 7.60 | 1.30 |

Chattopadhyay et al. (1996) measured the concentration of benzene, toluene and xylene in three steel plants namely Indian Iron and Steel Company, Bokaro Steel Limited and Durgapur Steel plant and the nearby residential areas. The mean concentration of benzene inside the three plants was found to be 717 $\mu gm^{-3}$, 918 $\mu gm^{-3}$ and 576 $\mu gm^{-3}$. For toluene, the concentrations were 183 $\mu gm^{-3}$, 258 $\mu gm^{-3}$ and 198 $\mu gm^{-3}$. For o-xylene, the concentrations measured inside the plant were 6 $\mu gm^{-3}$,124 $\mu gm^{-3}$, and 32 $\mu gm^{-3}$.

### 1.5. Maximum Permissible Limits

Table 1.5 shows the recommended guidelines for water by WHO (WHO 2008) and USEPA (USEPA, 2003).

**Table 1. 5: Water guidelines for BTX in ppb (μg/L)**

| | Benzene | Toluene | Xylene |
|---|---|---|---|
| WHO | 10 | 700 | 500 |
| USEPA | 5 | 1000 | 10000 |

Table 1.6 shows the air quality guidelines recommended by The National Environment Protection Measure (EPHC, 2004).

**Table 1. 6: Air quality guidelines recommended by The National Environment Protection Measure (ppb)**

| | Benzene | Toluene | Xylene |
|---|---|---|---|
| Annual Average | 3 | 100 | 200 |

### 1.6. Effects of BTX

Brief exposure (5–10 minutes) to very high levels of benzene in air (10,000–20,000 ppm) can result in death. Lower levels (700–3,000 ppm) can cause drowsiness, dizziness, rapid heart rate, headaches, tremors, confusion, and unconsciousness. Eating foods or drinking liquids containing high levels of benzene can cause vomiting, irritation of the stomach, dizziness, sleepiness, convulsions, rapid heart rate, coma, and death. People who breathe benzene for long periods may experience harmful effects in the tissues that form blood cells, especially the bone marrow. These effects can disrupt normal blood production and cause a decrease in important blood components. A decrease in red blood cells can lead to anemia. Reduction in other components in the blood can cause excessive bleeding. Long-term exposure to benzene can cause cancer of the blood-forming organs. Exposure to benzene may be harmful to the reproductive organs (ASTDR, 2007a).

Toluene can cause headaches and sleepiness. Low to moderate, day-after-day exposure in your workplace can cause tiredness, confusion, weakness, drunken-type actions, memory loss,

nausea, and loss of appetite. Repeated exposure to toluene can cause problems with speech, vision, hearing, loss of muscle control, loss of memory, poor balance and decreased mental ability. Some of the above changes may be permanent (ASTDR, 2000).

At acute-duration inhalation concentrations as low as 50 ppm, xylenes produce irritant effects on the eyes, skin, and mucous membranes; impaired respiratory function; and mild central nervous system effects, including headache and dizziness. Contact of eye with xylene vapor or with xylene liquid can cause photophobia, redness of the conjunctiva, and partial loss of the conjunctival and corneal epithelia. Exposure to airborne xylene concentrations of 100–400 ppm can cause retardation of response times and impairments in memory and body balance. Acute exposure to an estimated 10,000 ppm xylenes elicited tremors, mental confusion, and depressant effects (narcosis) on the central nervous system. Dermal exposure of humans to xylene causes skin irritation, dryness and scaling of the skin (ASTDR, 2007b).

# CHAPTER 2

# LITERATURE REVIEW

## 2.1. VOC Control Technologies

To control the release of benzene, toluene and o-xylene into the environment, various options are available. The various treatment technologies available to reduce the content of emission of BTX in atmosphere are condensation, scrubbing, incineration, catalytic oxidation, adsorption, biological oxidation. Figure 2.1 shows the classification of various control technologies available for VOC emission.

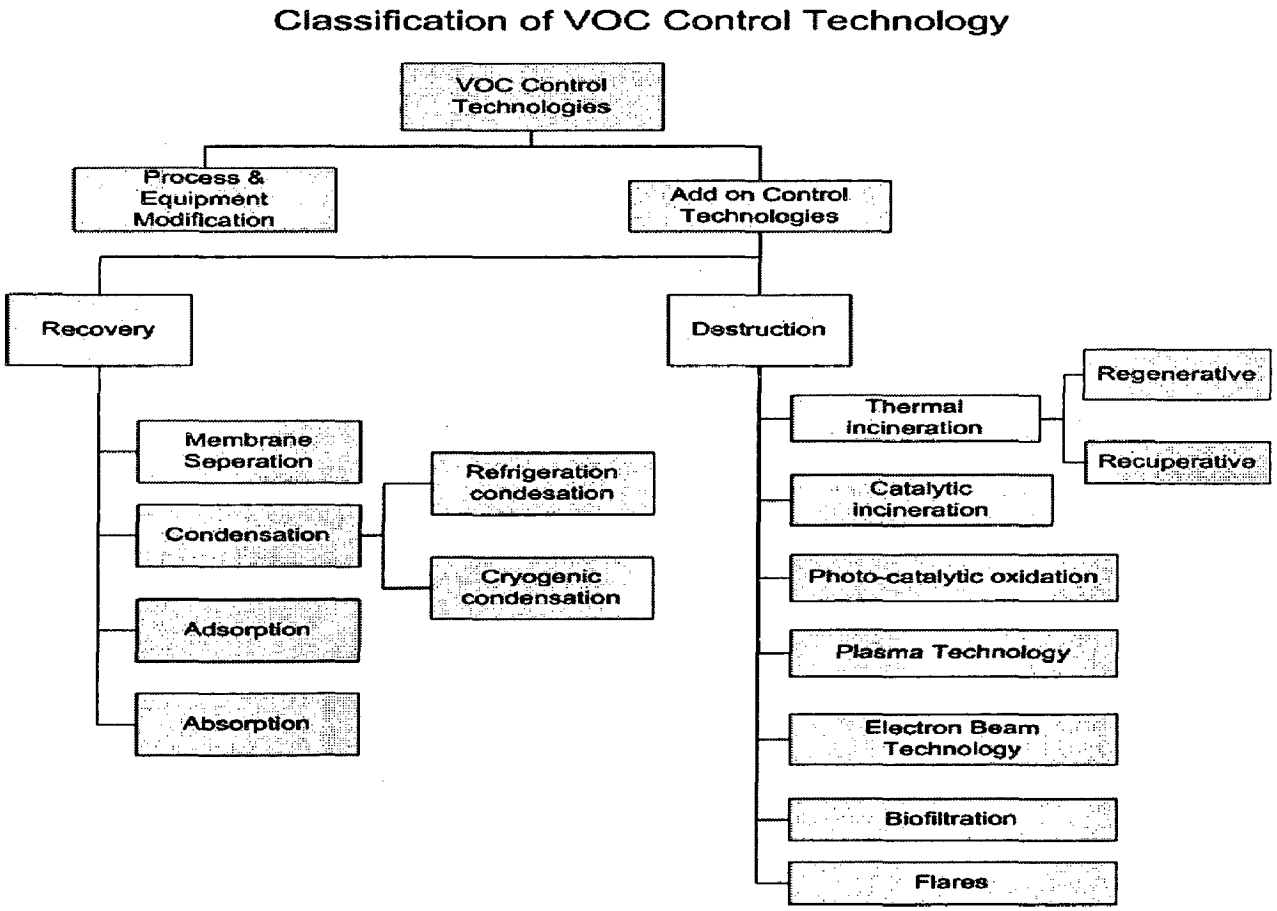



**Figure 2. 1: Various treatment technologies available for VOC emissions**

Table 2.1 shows the properties and operational characterstics of various control technologies available for control of VOC emission.

**Table 2. 1 Properties and operational characteristics of various control technologies available for control of VOC emission**

| Methods (Conventional and upcoming) | Technology involved | Operational characteristics | | | Advantages | Limitations |
|---|---|---|---|---|---|---|
| | | Gas flow ($m^3h^{-1}$) | Temperature $^0F$ | VOC ($gm^{-3}$) | | |
| Adsorption | Activated carbons, zeolites | 5-50000 | <130 | < 10 | Proven and efficient | Specific adsorbent requirement and can saturate fast; Risk of pollutant reemission |
| Incineration | Thermal oxidation at 760- 1200$^0$C | >10000 | 700 | 2- 90 | Efficient | Not cost effective, incomplete mineralization and release of secondary pollutants. |
| Catalytic oxidation | Thermal catalysts (Pt, Al, ceramics) | >10000 | 300 | 2-90 | Efficient, conserves energy | Catalyst deactivation and disposal, by-product formation |

| | | | | | | |
|---|---|---|---|---|---|---|
| Absorption | Washing gas with contaminated water | 100-60000 | Normal | 8-50 | Possible recovery of VOC | Not suitable for low concentrations, generates wastewater |
| Condensation | Liquefaction by cooling or compression | 100-10000 | Ambient | >60 | Possible recovery of VOC | Requirement of further treatment, Applicable in high concentrations only |
| Filtration | Air passed through fibrous material coated with viscous materials | 100-10000 | 50-105 | >60 | Efficient for particle removal, compact and commonly used | Unable to remove gases, fouling, particle reemission can occur due to microbial growth. |
| Electrostatic precipitator with Ionization | Electric field is generated to trap charged particles | - | - | - | Efficiently removes particles and are compact | Generates hazardous byproducts |
| Ozonation | Strong oxidizing agent | - | - | - | Removes fumes and gaseous pollutants | Generates unhealthy ozone and degradation products. |
| Photolysis | UV radiations to oxidize air pollutants and kill pathogens | - | Normal | - | Removes fumes and gaseous pollutants | Release of toxic photoproducts, UV exposure may be hazardous and energy |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | consuming. |
| Photocatalysis | High energy UV radiation used along with a photocatalyst | - | - | - | Energy intensive popular method suitable for broad range of organic pollutants | Exposure to UV radiation may be harmful |
| Membrane separation | Separation through semi permeable membranes | 5-100 | Ambient | >50 | Recommended for highly loaded streams | Membrane fouling and need for high pressure |
| Enzymatic oxidation | Use of enzymes for treatment of air pollutants | - | 95-130 | - | Promising | Requirement of new enzymes periodically |
| Phytoremediation | Use of plants and microbes for the removal of contaminants | - | - | - | Cost effective, pollution free and complete mineralization occurs | Large as compared to other technologies |
| Microbial abatement | Microbes are used for oxidation of contaminants into less harmful products | 200-1500 | - | <5 | Cost effective, more efficient, eco-friendly. | Requirement of large area, need for control of biological parameters |

In spite of the fact there are numerous technologies for control of volatile organic compounds (VOCs) emission, all are not applicable everywhere. All technologies have its own applicability depending upon the source, type and concentration of the VOC (Bohn, 1992). The conventional methods such as thermal incineration, adsorption, absorption, condensation and some recent techniques such as membrane separation, electronic coagulation are very effective at reducing emission of VOCs from various industrial operations (Binot et al., 1994; Janni et al., 2001; Kumar et al., 2008). But they generate undesirable byproducts (Ottengraf, 1987). These are energy intensive and may not be cost-effective for treating high flow air streams contaminated with low concentrations of pollutants. Biological treatment is an attractive alternative for low concentration gas streams because of its low energy consumption, relatively moderate operating costs and minimal by-products generation.

## 2.2. Biological treatment in VOC Control

The biodegradation is done by microorganisms, which are either supported on media or maintained in suspension. Supported microorganisms are immobilized on organic media or inorganic structures, while suspended microorganisms are maintained in a liquid phase such as activated sludge. In all instances, VOCs and odour are biodegraded by microorganisms into carbon dioxide and water. Organic compounds serve as the substrate or source of carbon and energy. These compounds provide the food supply, which allows the microorganism to function and multiply (Jatschak et al., 2004).

Biological waste air treatment technology makes use of several types of bioreactors. There are mainly four types of related biological treatment units: biofilter, biotrickling filter, membrane bioreactor and bioscrubber. These systems have differences in their complexity, process design, equipment dimensions and working parameters, but all of these operated based on the same principle of biological removal (Van Groenestijn et al., 1993; Adler, 2001; Jatschak et al., 2004).

### 2.2.1. Biofilters

Biofilters (BFs) are reactor in which polluted air stream is passed through a porous packed bed on which a mixed culture of pollutant-degrading organisms is immobilized. Biofiltration uses microorganisms fixed to a porous medium to break down pollutants present in an air stream. The microorganisms grow in a biofilm on the surface of a medium or are suspended in the water phase surrounding the medium particles. The filter-bed medium consists of relatively inert substances

like compost, peat, etc. which ensure large surface attachment areas and additional nutrient supply. As air passes through the bed, the contaminants in the air phase sorb into the bio film and onto the filter medium. The contaminants are biodegraded on biofilm (Morales et al., 2003). Biofilters usually incorporate some form of water addition for control of moisture content and addition of nutrients. In general, the gas stream is humidified before entering the bio filter reactor.

The overall effectiveness of a biofilter is largely governed by the properties and characteristics of the support medium, which include porosity, degree of compaction, water retention capabilities, and the ability to host microbial populations. Critical biofilter operational and performance parameters include the quantity of microbial inoculum, pH, temperature, moisture and nutrient content.

Biofiltration is a general term applied to the biodegradation of chemical compounds in gas phase to the carbon dioxide, water, and inorganic salts. Biofiltration is the oldest and the simplest method of the four biological technologies for the removal of contaminated components from waste gases (Van Groenestijn et al., 1993; Devinny et al., 1999; Adler, 2001). The contaminated off-gas is passed through a preconditioner for particulate removal and humidification (if necessary). The conditioned gas stream is then passed from the bottom of a filter bed of soil, peat, composted organic material (such as wood or lawn waste), activated carbon, ceramic or plastic packing, or other inert or semi-inert media. The media provides a surface for microorganism's attachment and growth. The bed and air stream are kept moist to encourage microbial activity. Humidification is generally the most influential parameter affecting the sorptive capacity of a biofilter, especially at lower inlet concentration, where Henry's Law controls mass-transfer rates within the biofilter. Nutrient could be mixed with the packing material either before biofilter installation or after construction (Van Groenestijn et al., 1993; Devinny et al., 1999; Adler, 2001; Jatschak et al., 2004).

#### 2.2.1.1. Biofilter Operations

The operations of biofilters involve a series of steps beginning with the transfer of the pollutant air to the aqueous phase. These are:-

- Transfer of pollutant from air to aqueous phase
- Adsorption onto the medium or absorption into the biofilm

- Biodegradation of VOCs withn the biofilm

The most important physical, chemical and biological parameters affecting the biofiltration process are described below:

#### 2.2.1.1.1. Biofilm

In the biofiltration system, the pollutants are removed due to the biological degradation rather than physical straining as is the case of normal filters. Biofilm is a group of microorganisms (aerobic, anaerobic, and facultative type bacteria, fungi, algae and protozoa) which attach themselves on the surface of the packing media and forms a biological film or slim layer of a viscous, jelly like structure (Peavy et al., 1985). The development of biofilm may take few days or months depending on the microorganisms' concentration. There are three main biological processes that occur in the biofiltration systems

**(i)** Attachment of microorganisms

**(ii)** Growth of microorganisms and

**(iii)** Decay and detachment of microorganisms

Since the microorganisms are attached to the surface, the supply of organics or substrate (food) to the microorganisms in a biofilm is mainly controlled by the bulk and substrate transport phenomena. The substrate must be transported from the bulk fluid to the biofilm's outer surface where it is metabolised after diffusion. The factors which influence the rate of substrate utilization within a biofilm are (i) substrate mass transport to the biofilm, (ii) diffusion of the substrate into the biofilm, and (iii) utilization kinetics of the biofilm.

Biomass detachment is one of the most important mechanisms that can affect the maintenance of biomass in the biofilter (Durgananda et al., 2003). Several forces (i.e. electrostatic interaction, covalent bond formation and hydrophobic interactions) are involved in microbial attachment to a surface. The strength of the attachment and the composition of forces are dependent on various environmental conditions viz gas flow rate, pollutant concentration, oxygen supply, nutrient availability, type of microbial species and their surface properties (Peavy et al., 1985; Cohen, 2001).

Generally a rapid flow rate through the biofilter will hinder the growth of bacterial film resulting in thin film formation. Microorganisms form thinner layers upon smooth surfaces in comparison to those upon porous materials and each treatment system has a typical biofilm

thickness. The biofilm thickness usually varies from 10 micro meters to 10,000 micro meters, although an average of 1,000 micro meters or less is usually observed. However, whole of the biofilm is not active. The activity increases with the thickness of the biofilm up to a level termed the 'active thickness'. Above this level, diffusion of nutrients becomes a limiting factor, thus differentiating an 'active' biofilm from an 'inactive' biofilm (Cohen, 2001).

#### 2.2.1.1.2. Biofilter bed

Biofilter bed is the vital part of the biofiltration process as it provides the main support for microbial growth. A list of characteristics that are necessarily needed for an ideal biofilter reactor is established by Bohn (1992). The most anticipated characteristics of the BF bed comprises of:

- Optimum specific surface area for development of microbial biofilm and gas-biofilm mass transfer
- High porosity to expedite homogenous distribution of gases
- High-quality water retention capacity to preclude bed drying
- Manifestation and availability of intrinsic nutrients and
- Presence of a dense and diverse indigenous microflora.

The most habitually used materials in BF beds are peat, soil, compost, and wood chips. These materials are generally abundant and economical as well. They satisfy most of the desirable criteria and has their own merits and demerits (Maestre et al., 2007). The main advantage of soil is that it has a rich and varied microflora. But it contains restricted amount of intrinsic nutrients, gives low specific area and spawn high-pressure drops (Swanson and Loehr, 1997). Peat contains high amount of organic matter, has high specific area, good water retaining capacity and good permeability. But peat comprises neither high levels of mineral nutrients nor a dense indigenous microflora as that of soil or compost. Compost employs a dense and varied microbial system, good water holding capacity, good air permeability and contains large amounts of intrinsic nutrients. That's why composts are most widely employed in biofiltration.

Furthermore, the consumption of compost in BFs represents an effective way of recycling and utilizing waste residual organic matter, specifically activated sludge from waste water treatment plants, forest products (branches, leaves, barks), domestic residues, etc.( Arnold et al., 1997;Alexander, 1999; Arnold et al., 2000). However, composts are frequently less stable than

that of soil or peat and have the propensity to collapse and become compact and prominent to increase in pressure drop in BF beds. This among other reasons is ascribed to their high water holding capacity. The study of biofiltration using wood chips or barks as packing material has already been carried out by some authors (Smet et al., 1996a; Smet et al., 1996b; Hong and Park, 2004).

Certainly, to preclude bed pulverization and compression, most authors proposed materials that furnish the bed with good structure, easy maintenance and rigidity, thereby hindering the clogging phenomena ultimately enhancing the bed lifespan. For example wood chips or barks (Luo, 2001), perlite (Woertz et al., 2002), vermiculite (Pineda et al., 2000), glass beads (Zilli et al., 2000), polyurethane foam (Moe, 2000), polystyrene (Arulneyam and Swaminathan, 2000), lava rock (Chitwood and Devinny, 2001), etc. Ibrahim et al. (2001) prepared a filter bed composed of activated sludge immobilized on gel beads (Ibrahim et al., 2001). Christen et al. (2002) and Sene et al. (2002) used technologically advanced sugarcane-baggase based bed, for the treatment of ethanol and benzene. Some bed structuring agents also hold interesting chemical characteristics as they contribute to bed properties such as pH-buffering capacity (limestone), or general adsorbing capacity (activated carbon) (Abumaizar et al., 1998). The efficiency of a BF material with respect to the pollutant for treatment is a function of its adsorption coefficient or partition coefficient. The partition coefficients of toluene is 1.43 mg $g^{-1}$ with compost, 2.0 mg $g^{-1}$ with diatomaceous earth, and 0.89 mg $g^{-1}$ with chaff (Tang and Hwang, 1997).The adsorption coefficients for toluene was calculated to be approximately 10–20 times greater than that of granulated activated carbon (Tang and Hwang, 1997; Acuna et al., 1999).

#### 2.2.1.1.3. Nutrients

The pollutants brought into the biofilters form the major carbon and energy source for microbes. The presence of macronutrients (N, P, K and S) and the micronutrients (vitamin and metals) is partly delivered by the filtering materials used in BF. Compost is considered to be a suitable material since it contains various nutrients. From the literature it has been confirmed that irrespective of the filtering material used, steady addition of nutrients is essential to withstand microbial degradation activity (Lee et al., 2002). Progressive exhaustion of the intrinsic nutritive resources occurs when there is long-term utilization of compost based beds (Morgenroth et al., 1996). This eventually leads to nutrient deficiency which ultimately becomes a limiting factor for

the long-term biofiltration performance (Delhomenie et al., 2001a; Delhomenie et al., 2001b). Nutrients for microbial growth are supplied either in the solid form which is directly inserted into the filter bed (Gribbins and Loehr, 1998), or as aqueous solutions, which is the most frequently used method. Compounds such as $KH_2PO_4$, $KNO_3$, $(NH_4)_2SO_4$, $NH_4Cl$, $NH_4HCO_3$, $CaCl_2$, $MgSO_4$, $MnSO_4$, $FeSO_4$, $Na_2MoO_4$, and vitamins are the most commonly used nutrient solutions in BFs. Models of biofiltration performance as a function of nutrient supply, of nitrogen in particular have been developed and experimentally validated (Alonso et al., 2001; Delhomenie et al., 2001a; Delhomenie et al., 2001b).

#### 2.2.1.1.4. pH

For several biological processes, pH has a significant impact on biofiltration efficiency. Microbial activity is severely disturbed by any deviation from an optimum pH range. Due to neutrophilic (optimum pH is 7) behavior of the microorganism, maximum degradation observed for benzene, toluene, ethyl benzene, o-xylene between pH values of 7.5 and 8.0 (Lu et al., 2002). Veiga et al. (1999) studied the effect of pH on alkyl benzene degradation between pH 3.5 - 7.0 and found that alkyl benzene degradation increased with pH.

#### 2.2.1.1.5. Microorganisms

Microorganisms are considered as the catalysts for the biodegradation of VOCs. Heterotrophic microorganisms such as bacteria and fungi are used for the degradation of VOCs. The bed vaccination is completely influenced by the nature of the filtering material and the biodegradability level of the VOC to be treated. By taking the ecological advantages, the most defiant population to the toxic VOC is naturally selected and a microbial hierarchy is formed in the bed (Mohseni and Allen, 2000; Delhomenie et al., 2001a; Delhomenie et al., 2001b; Delhomenie et al., 2002). The inoculation of BF beds with consortia extracted from sewage sludge, or strains derived or isolated from previously operated BF is done for the intractable VOCs. Within BFs, the degrading species comprises between 1 and 15% of the total microbial population (Pedersen and Arvin, 1995; Pedersen et al., 1997; Delhomenie et al., 2001b). Typically the biomass density of BF should contain $10^6$ and $10^{10}$ cfu of bacteria and actinomycetes, respectively per gram of bed (Krailas et al., 2000).

#### 2.2.1.1.6. Oxygen Level

Oxygen level is one of the most important parameter which governs the performance of BFs. The oxygen dependency on biofiltration has been studied by various researchers. Oxygen

was found to be a limiting factor even at low inlet VOC concentration for hydrophilic solvents (Zarook et al., 1997). The concurrent removal of a mixture of methyl ethyl ketone (MEK) and methyl isobutyl ketone (MIBK) did not have any significant improvement with the increase in oxygen content in air. Deshusses et al. (1996) suggested that the cross –inhibition of MEK and MIBK biodegradation occurred. They also found that the kinetic effects were more important than diffusion effects. In transient experiments it has been verified that when either of the compounds were inoculated into BFs, both cross and self-inhibition was observed.

#### 2.2.1.1.7. Moisture Content

The moisture content of the filter bed plays an important role in biofilter performance because microorganisms need water to carry out their metabolic activity. Deficiency of water in microorganisms leads to substantial reduction in the biodegradation rate. On the other hand, availability of excess water to the microorganisms hinders the transfer of oxygen and hydrophobic pollutants to the biofilm, leading to the development of anaerobic zones within the bed. This leads to reduction in reaction rates, foul smell, increased back pressure due to reduced void volume and channelling of the gas within the bed. Partial optimization of moisture levels leads to drying of bed and growth of fissures which eventually cause channelling and short-circuiting (Shareefdeen and Singh, 2005). Moisture content for optimal operation of the biological filter should be within 30–60% by weight, depending on the filtering medium used. Depending on medium, surface area and porosity, optimal water levels vary with different filtering material. Moisture levels are maintained by the pre-humidification of the inlet gas stream which is also necessary to provide direct water to the bed through nozzle system at the top of the bed. Sakuma et al. (2009) stated that biofilters tend to experience drying at the air inlet port, which causes decrease in pollutant removal over time. Drying of the packing material can lead to localized dry spots, non uniformed gas distribution and reduction in the activity of microorganisms (Shareefdeen and Singh, 2005).

### 2.2.1.2. Mechanisms

The biodegradation of pollutants in the biofilm of a biofiltration sytem is a combination of physico-chemical and biological phenomena. Basically following three mechanisms are responsible for the transfer and subsequent biodegradation within the bed (Swanson and Loehr, 1997; Adler, 2001; Mathur et al., 2006) (Figure 2.2).

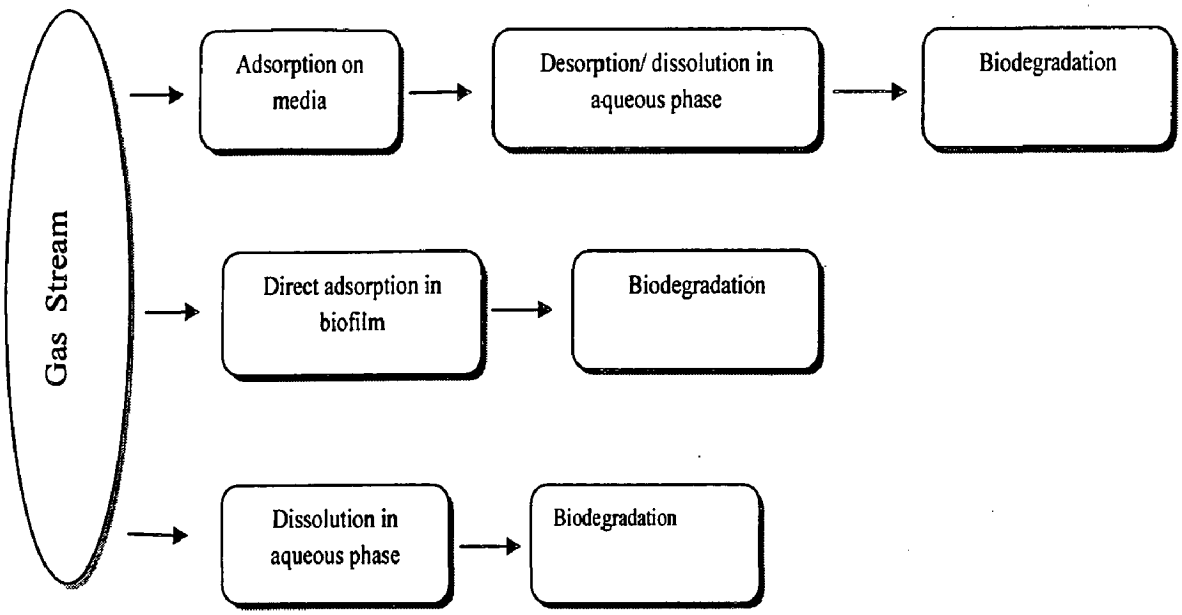



**Figure 2. 2: Mechanism of biodegradation**

Once the pollutants are adsorbed on the biofilm or dissolved in the water layer surrounding the biofilm, the contaminants are available to the microorganisms as a food source to support the microbial life and growth. Air, which is free, or nearly free, of contaminants is then exhausted from the biofilter. Figure 2.3 shows the mechanism of mass transfer occurring during biofilter process. As the gas stream passes through the packing, contaminants are transferred from the gas stream to the water in the biofilm. A number of researchers have worked on the measurement of concentration of contaminants by GC-FID (Sherif et al., 1992). The contaminants diffuse into the depth of the biofilm, where they are absorbed by the microorganisms in the biofilm and biodegraded. Contaminants may also be adsorbed at the surface of the packing. The greater majority of reactors utilize aerobic respiration, so that oxygen and nutrients must also be dissolved in the water or biofilm and degraded by the microorganisms. During operation at moderate-to-high concentration of contaminant, the biofilm will gradually grow thicker. At some point, diffusion will no longer provide all the needed compounds to the deeper portions of the biofilm, and microorganisms will become inactive. Because the pores within the packing are highly irregular in shape, the growing biofilm will change the pore size distribution.

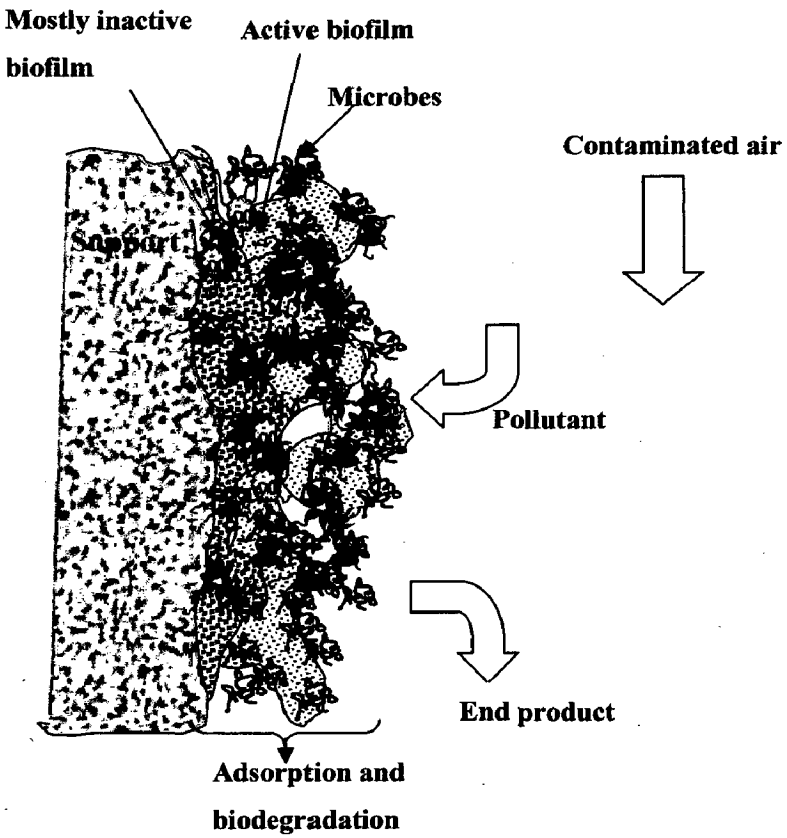



**Figure 2. 3: Phenomena involved in the operation of biofilters (Devinny and Ramesh, 2005)**

### 2.2.2. Biotrickling filter

The biotrickling filter (BTF) works on a similar principle, but liquid nutrient medium is continuously trickled through the bed, allowing improved control over the reaction condition such as pH and nutrient concentration (Cesario et al., 1992; Cai et al., 2004). In this type of filter, the gas is passed through a packed bed, which is continuously wetted with an aqueous solution containing essential nutrients necessitate for the biological system. The growth of microorganisms takes place on the packing material of the biofilter as biofilm, leading to biodegradation within biofilm. The filtering material used to accelerate the gas and liquid flows through the bed, enhancing the growth of microflora, and should endure crushing and compaction. Centinkaya et al. (2000) reported that inert materials such as resins, ceramics, celite, polyurethane and foam can be

used for the BTF packing. Polyurethane based beds have the exceptional specific surface area greater than 1000 $m^{-1}$, but for the other materials, this value lies between 100 to 300 $m^{-1}$. Cox and Deshusses (1999) suggested that the choice of a flow pattern (co or counter) of the liquid and gaseous phase does not have any impact on the biodegradation performance. Biofiltration processes are more preferable over other conventional processes due to the perceptual trickling mechanism for the abolition of water soluble VOCs. However, as the contact between microorganisms and the pollutants occur simultaneously (Cox and Deshusses, 1999), the solubility specifications are less stringent than for bioscrubbers (Henry coefficient < 0.1) (Van Groenestijn and Hesselink, 1993). Generally the VOC inlet concentrations are less than 0.5 g $m^{-3}$ for BTFs. The incessant circulation of nutrient solution expedites the control of the biological operating parameters (viz. pH etc.). VOC diffusion occurs in the liquid film when there is a proper contact between microorganisms and the pollutants, where liquid flow rate and the recycling rate are important parameters (Pedersen et al., 1995). From the literature it has been established that an increase in the liquid flow results in increase in active exchange surface for gas-liquid mass transfer and then an increase in the liquid flow rate should result in proportional increase in the active exchange surface for gas–liquid mass transfer, and then improve the degradation rate (Centinkaya et al., 2000). Lu et al. (2002) verified that maintaining minimum water and nutrient supply is sufficient to achieve good performance. Due to the better control of pressure drop across the bed, pH and nutrient feed, biotrickling filter facilitates more consistent operation than do natural media biofilters. Furthermore, they do not suffer from the effects of aging as do natural media (Mathur et al., 2006).

The biotrickling filter has no height limitation, and hence could be designed as a tower, with a reasonable diameter. The problem of support media drying, as in compost biofilters, is inapplicable to biotrickling filters, since there is a constant aqueous nutrient stream trickling down the surface of the synthetic media.

In some cases, when air contaminants contain nitrogen or sulfur or oxygen, intermediate products of biodegradation (biotransformation) may be acidic. This causes the pH in the biofilter bed to decrease. E.g- when hydrogen sulphide is biotransformed to sulfate, the pH of the bed decreases. In compost beds, decline in pH results in decreased bioactivity, which eventually causes the entire bed to acidify and shut-down. In biotrickling filters, the pH can be controlled by adding buffer in the nutrient flow. In the case of hydrogen sulphide, the sulphate formed is continuously

removed from the process by the flowing nutrient stream, and this sulfate is neutralized before the nutrients are recycled back to the biotrickling filter bed. The biodegradation rate in a biotrickling filter is much higher than the compost beds. This is mainly due to high surface area and increased concentration of immobilized microorganisms in biotrickling filters when compared to the compost media. Hence, the volume of biotrickling filter bed is much smaller than the volume of the compost required for the same destruction efficiency. In this type of biofilters, the inlet gases have to be humidified to prevent bed drying. Since 100% humidity is difficult to achieve in practice, some bed drying always occurs, and this results in reduced bioactivity. In some compost beds, water is also sprayed from the top of the bed, although this is kept to a minimum to prevent bed settling. In compost beds, gas channelling is a big problem, especially since compost beds are shallow with large cross-sectional areas. As biomass growth begins to plug the bed, gas begins to bypass compost regions which have increased biomass concentrations. Since biotrickling filter beds have smaller diameter and are taller, gas channelling is not a major issue. A potential disadvantage of the biotrickling filter operation however is the clogging of the pore space. It occurs when the biotrickling filter treats high VOC loads and is provided with excess nutrients (Cox and Deshusses, 1998; Webster and Devinny, 1998; Devinny et al., 1999).

### 2.2.3. Membrane bioreactor

Membrane bioreactor technology is one of the most emerging technology now a days. Earlier it was only used for the treatment of industrial, domestic and specific wastewaters in which small footprint, water reuse, or stringent discharge standards are required (Roberts et al., 2006). Membrane bioreactor (MBR) can be used for the treatment of waste gas, in which gaseous pollutants diffuse through the membrane and are subsequently degraded by the microorganisms in the biofilm attached to the membrane surface (Ergas et al., 1999; Dolasa et al., 2000; Bo et al., 2002; Fitch et al., 2003; Langenhove et al., 2004). The surface of the membrane forms the contact area (Reij et al., 1998; Clapp et al., 1999). Biomass may also be suspended in the liquid phase. MBR are especially favourable for poorly water-soluble compounds.

Membrane materials are usually dense, microporous, porous or composite. Dense materials are more selective, while microporous materials are more permeable but susceptible to plugging by biomass (Reij et al., 1998). Passage of the pollutants contaminated air across the membrane allows passive diffusion of contaminants through the membrane into the liquid biofilm phase on the other side driven by the concentration gradient (Reij et al., 1995). The mass transfer coefficient through

a dense membrane depends on the solubility and diffusivity of the contaminant with selective membrane materials particular type of pollutants can be extracted from gas phase (Crank and Park, 1968).

#### 2.2.3.1. Configurations and Mechanisms

Various membrane modules such as spiral-wounded, flat sheet, tubular (i.d. > 10mm), capillary (0.5mm < i.d. < 10mm) and hollow fibre (i.d. < 0.5mm) are used in lab scale (Mulder, 1996). In the membrane separation, the solute is taken to the membrane surface by the main solution and is accumulated by the membrane, so the concentration of solute on the membrane surface has become much higher than the main concentration of the original solution. This phenomenon is called concentration polarization (Stern, 1996). For flat plate and spiral wounded modules the mechanisms are as follows:

- Bulk mixing of the contaminants in air entering the bioreactor
- Air boundary layer transport
- Transport through the membrane
- Transfer from the membrane, dissolution and diffusion into the biofilm
- Diffusion through and degradation within the biofilm
- Boundary layer transport through the liquid phase
- Subsequent mixing and degradation within the cell suspension

#### 2.2.3.2. Physical Transport

Membrane bioreactors (MBRs) are generally employed for gaseous pollutants which diffuses through a membrane and are subsequently degraded by micro organisms on the biofilm attached membrane surface. MBRs are generally used for low water soluble compounds. The materials of membrane may be dense, microporous, porous or composite. Dense materials are more widely used while microporous materials are permeable but susceptible to be plugged by biomass. The pollutants present in the contaminated air are passed though the membrane which allows passive diffusion of contaminants into the liquid biofilm phase on the other side which is driven by the concentration gradients. The factors affecting the mass transfer coefficients in dense

membrane are solubility and diffusivity of pollutant. As compared to the traditional biological waste gas treatment such as biofiltraion, bio trickling filter, and bio scrubber etc., MBRs have several advantages like: optimal moistening of biomass and removal of degraded products due to the presence of discrete water which avoids immobilisation of biomass. The flow behaviour of gas and liquid flow can be of wide-ranging without problems of flooding, loading or foaming. A schematic representation of a flat composite membrane bioreactor for the treatment of waste gas is illustrated in the Figure 2.4.

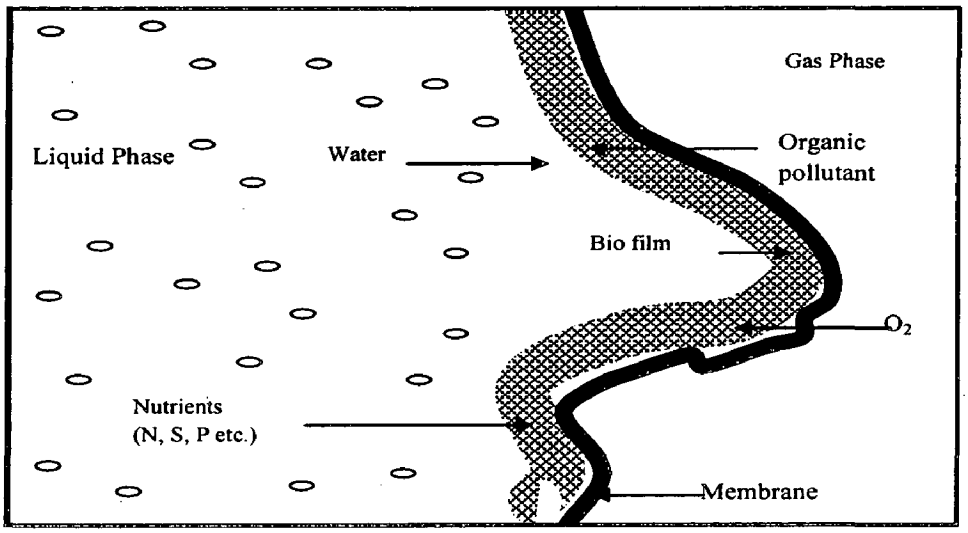



**Figure 2. 4 : Schematics of a membrane bioreactor containing microporous hydrophobic membrane, a biofilm and suspended cells**

In this model, the dried side of the membrane acts as a surface for uptake of pollutants from the air flowing along the membranes, whereas the other side is kept submerged in the nutrient solution which is flowing and covered by a biofilm (Ho and Sirkar, 1992; Lewandowski, 1994; Aziz et al., 1995).

### 2.2.3.3. Mechanisms of Membrane based Biological Waste Gas Treatment

Membrane can be defined as an interphase between two bulk phases of a system by allowing the selective transport of compounds from one phase to other (Lewandowski, 1994). Polluted gases passed through the lumen of the membrane materials are diffused to the liquid phase of the shell side of membrane. Difference in permeability of the species flowing through the membrane leads to the gas separation in membrane.

Membranes can be classified in to porous, dense and composites having reasonable mechanical strength, high permeability and selectivity (Stephenson et al., 2000). Mechanism of membrane based biological waste gas treatment is based upon:

- Bulk mixing of the contaminant in the air entering the bioreactor
- Air boundary layer transport
- Transport through the membrane
- Transfer from the membrane, dissolution and diffusion into the biofilm
- Diffusion through and degradation within the biofilm
- Boundary layer transport through the liquid phase
- Subsequent mixing and degradation within the cell suspension

### 2.2.3.4. Membrane Material

Four types of membrane materials have been used to prevent mixing of the gas and liquid phases and simultaneous transfer of volatile components.

#### 2.2.3.4.1. Microporous Membrane

Hydrophobic microporous membranes are mostly used due to high gas permeability of the membrane, which eventually does not allowing transport of water across the membrane. The pore diameter of the membrane generally lies between 0.1-1.0 μm with a typical viable membrane containing 30-85% pore space (Hartmans et al., 1992). Since the membrane material is hydrophobic, the pores are filled with gas. The membrane pores remain gas filled and compounds transfer from the gas stream through the membrane pores by gaseous diffusion (Hartmans et al., 1992; Attaway et al., 2002; Fitz et al., 2003), usually the ratio between gas and liquid diffusivity is about $10^4$. Water does not enter the pores unless a certain critical pressure at the liquid side is exceeded. When water enters the pore of the membrane it significantly decreases mass transfer rates (Ergas and Reuss, 2001). Bubbles are formed in the liquid phase when the gas side pressure is greater than the bubble point (Semmens et al., 1999). At the critical pressure range the gas-liquid

interface is immobilized at the opening of the membrane pore on the liquid side (Ho and Sirkar, 1992).

Mass transfer rate increases when the transferred components disappears by chemical reaction and also enhanced by microorganisms (Prasad and Sirkar, 1992). If the microorganisms are present as a biofilm on the membrane, liquid flow close to the membrane is absent and as a consequence the mass transfer coefficient does not apply at all. In this case (biofilm), simultaneous diffusion and reaction in a stagnant layer need to be calculated.

#### 2.2.3.4.2. Porous Membrane

Porous membranes have well-defined static pore structures that are aligned with highly connected, non-connected or straight. As per pore size distribution membranes are categorized into macroporous with pore diameter greater then 0.05μm and microporous diameter ranges between 0.05-0.002 μm (Karger and Ruthven, 1992; Min et al., 2002). If the pores are relatively large from 0.1 to 10 μm gases permeate the membrane by convective flow, and no separation occurs. If the pores are smaller than 0.1 μm, then the pore diameter is the same size as or smaller than the mean free path of the gas molecules. Diffusion through such pores is governed by Knudsen diffusion, and the transport rate of any gas is inversely proportional to the square root of its molecular weight (Mulder, 1996; Attaway et al., 2001; Attaway et al., 2002). This relationship is called Graham's law of diffusion. Finally, if the membrane pores are extremely small, of the order of 0.0005–0.002 μm, then gases are separated by molecular sieving. Transport through this type of membrane is complex and includes both diffusion in the gas phase and diffusion of adsorbed species on the surface of the pores (surface diffusion). These very small-pore membranes have not been used on a large scale, but ceramic and ultra microporous glass membranes with extraordinarily high selectivities for similar molecules have been used (Baker, 2004).

#### 2.2.3.4.3. Dense Membrane

Dense membranes consist of a dense film through which pollutants are transported by diffusion under the driving force of a pressure, concentration or electrical potential gradient ( Kesting and Frtizsche, 1993; Koros and Fleming, 1993). The separation of various components of a mixture is related directly to their relative transport rate within the membrane, which is determined by their diffusivity and solubility in the membrane material. Thus, nonporous, dense

membranes can separate permeates of similar size if their concentration in the membrane material (that is, their solubility) differs significantly. Most gas separation, pervaporation, and reverse osmosis membranes use dense membranes to perform the separation (Sirkar, 1992; Wickaramsinghe et al., 1992). Usually these membranes have an anisotropic structure to improve the flux. Dense membranes are limited to polymeric materials, such as latex, silicon rubber, polypropylene, and polyethylene, etc. These can be operated at high gas pressure, and have good resistance to chemical as well as mechanical abrasion. Dense membranes have also been susceptible to be more resistant to biofouling than porous membranes, because of the hydrophobic nature of membranes resists attachment of microorganisms (Cote et al., 1988; Freitas et al., 1995; Fitch and England, 2002).

#### 2.2.3.4.4. Composite Membrane

Composite membrane holds both the properties of dense and porous material and therefore has better interface and mass transfer. In a composite membrane bioreactor, porous layer is used as support while the thin dense layer is used to obstruct the microbial growth through the membrane. The internal diameter of the hollow fiber type membrane is of the range of 200-400 μm which provides surface area to volume ratio lies between 30-100 $cm^{-1}$ (Karoor and Sirkar; 1993).

Various types of composite membranes have been fabricated to improve the membrane performance (Kreulen et al., 1993). For the removal of toluene, a flat sheet composite membrane comprised of a dense polydimethylsiloxane having internal pore size 1.0 - 2.5 μm placed over a supported layer of polyvinylfluoride of pore size 210 μm has been used (Parvatiyar et al., 1996a; Parvatiyar et al., 1996a; Reij and Hartmans, 1996; Pressmann et al., 2000). Similarly Bo et al. (2002), successfully removed dimethylsulphide using a flat sheet composite membrane consisting of a porous zirfon (polysulfone membranes containing $ZrO_2$ filters support layer (175 μm) coated with a thin dense polydimethylsiloxane top layer (17 μm)).

### 2.2.4. Bioscrubber

Bioscrubbers consist of two units: the contaminated air is contacted with an aqueous phase into which the VOC is transferred; the aqueous phase then flows into a separate bioreactor where microbial degradation of the compound occurs. Bioscrubbers allow even closer control over reaction conditions than biotrickling filters, at the expense of reduced surface area for mass

transfer and more complex operation. Both bioscrubbers and biotrickling filters are more complex to operate than biofilters, requiring increased capital and operating expenditure for the supplementary equipment and disposal of the increased amount of biologically active waste generated.

In a bioscrubber, after initial contaminant absorption occurs, the degradation of the contaminants is performed by a suspended consortium of microbes in a separate vessel. Absorption may be achieved in a packed column, spray tower, or a bubble column. The water laden with contaminants is transferred to a separate vessel where optimal environmental conditions for degradation are maintained.

In a bioscrubber, the pollutant is absorbed in an aqueous phase in an absorption tower. The aqueous phase containing the dissolved compound is then treated in a separate AS unit. The effluent of this unit is circulated over the absorption tower in a co or counter current direction to the gas stream, which results in excellent cleaning of highly soluble pollutants. The pollutants are dissolved and degraded by the naturally occurring microorganisms in the AS unit (Kennes and Thalasso, 1998). Bioscrubbers have several advantages over media-based filtration. For example, the process is more easily controlled because pH, temperature, nutrient balance and removal of metabolic products can be altered in the liquid (Smet et al., 1999). One disadvantage of the bioscrubber is that the pollutant must be dissolved into the liquid phase during its short residence time in the absorption column, so it is best suited to contaminants with high aqueous solubility which is a major drawback in the treatment of many air pollutants and odorants which are highly volatile and exhibit low aqueous solubility. Obviously, this would not be a problem if the process was going to be used to treat a liquid phase waste stream.

### 2.3. Brief Review of recent works

**Monero et al., (2003): Batch kinetics of *Pseudomonas* sp. growth on benzene. Modeling of product and substrate inhibitions**

**Objective** : batch tests of benzene degradation were performed in this work at relatively high biomass level ($X_0$ ) 220-270 $mg_X/L$) and variable starting benzene concentration ($30 < S_0 < 200$ $mg_S/L$), and the experimental data were then used to calculate the volumetric and specific rates of biomass production and substrate consumption. These results were finally used to propose and check a new overall kinetic model that takes into account simultaneous uncompetitive-type

substrate inhibition and competitive-type product inhibition, the latter being likely due to 2-hydroxymuconic semialdehyde (2-HMS) accumulation in the medium.

**Results and Discussions**: The cell growth was found to decelerate with increasing benzene concentration from 30 to 200 mg/L suggesting a possible growth inhibition. The specific growth rate also decreased with increasing benzene concentration confirming the occurrence of growth inhibition due to substrate even at low substrate concentration which would be consistent with benzene toxicity. The degradation rate increased rapidly at low benzene concentration and slowly at high concentration with increasing benzene concentration demonstrating that cell growth is by far more sensitive to benzene inhibition that substrate degradation. It was justified that the 2-HMS is an intermediation rather that n the final product of benzene metabolism in *Pseudomonas* sp. Its accumulation within the cell would be ascribed to the kinetic control of the meta-pathway by the enzyme responsible to 2-HMS degradation, while the observed inhibition could be the macroscopic result of its competition with another intermediate for the active site of the enzyme limiting the same pathway.

**Kwon and Cho, (2009): A comparative, kinetic study on cork and activated carbon biofilters for VOC degradation**

**Objective**: Two bio-filters packed with cork, an organic macroporous material and activated carbon, an inorganic microporous one were compared in terms of operative performance on biological degradation of VOC, which was a mixture of benzene, toluene, ethylbenzene and xylene (BTEX) vapors.

**Results and discussions**: In general, the biofilter with packed cork performed obviously better than the one with bacterial activated carbon (BAC). The same was true for both total BTEX mixture and each individual compound. For the very initial times, the BAC bio-filter with its much greater adsorption capacity of activated carbon showed nearly perfect removal though. The maximum elimination capacities turned out to be 86 g/($m^3$ h) for the cork and 67 g/ ($m^3$ h) for the BAC around 94 g/($m^3$ h) of inlet load. No nutrient input even for a short time was likely to damage the microbial consortia in both filters thus resulting in less amount of $CO_2$ generated; the worse for the BAC filter. Curiously, the higher biomass in the cork column was counted even if specific surface area on BAC media was 12 times larger, which was further supported by the model calculation (based on Otengraf's) in which the effective surface area of cork bed turned out to be

33% more than that of BAC. The derived relationship between consumption of BTEX and growing cell mass was found to be logarithmically proportional to each other, which was confirmed experimentally in this work.

**Robledo-Ortiz et al., (2011): Benzene, toluene and o-xylene degradation by free and immobilized *P.putida* F1 of postconsumer agave-fiber/polymer foamed composites**

**Objective:** The objective of this work was to study the degradation of BTX by suspended and immobilizedP. putida F1 of agave-fiber/polymer foamed composites produced from waste materials (recycled polyethylene and wasted agave fiber).

**Results and Discussions:** In general, the immobilized bacteria degraded the chemicals faster than did the suspended cells. The inhibitory effect due to catechol accumulation, usually present in suspended systems when degrading high benzene concentrations, was not observed with immobilized bacteria. The biodegradation kinetics of benzene and toluene were adequately described by the Monod equation. However, the biodegradation kinetics of o-xylene were not described with the above-mentioned model, because it was only partially degraded. Scanning electron microscope observations and FTIR-ATR analysis showed that the *P. putida* F1 was capable of developing biofilms on the composite surface using an organic compound as sole carbon source.

**Zilli et al., (2005): Laboratory scale experiments with a powdered compost biofilter treating benzene polluted air**

**Objective:** The main objective of this work was to study the effect of powdered compost as high superficial area packing medium on the removal rate of benzene vapours in a laboratory biofilter.

**Results and Discussions:** A maximum removal capacity of 20.1 g $m_{packing\ material}^{-3}$ $h^{-1}$ was achieved at a benzene-loading rate of 24.8 g m−3 h−1. Benzene and biomass concentrations profiles along the column indicated that the distribution of biomass depended on the pollutant mass loading and that a linear relationship existed between biomass concentration and specific elimination rate. A biofilm model incorporating zero-order kinetics was applied to interpret and characterize the process macrokinetics. At low benzene inlet concentration and superficial gas velocity, the system performance was well described by a diffusion limitation model, whereas

possible reaction limitation took place under harder conditions. It was found that the structure and composition of the support should exert no appreciable influence on biofilm maintenance

**Abuhamed et al., (2003): Substrate interaction during the biodegradation of benzene, toluene and phenol mixtures**

**Objective**: The objectives of the study was biodegradation of benzene, toluene and phenol at high initial concentrations individually and in mixture using P.putida F1 and obtain information on interactions among aromatics during the biodegradation at high initial concentrations.

**Results and Discussions**: 250 mg/L of benzene, 225 mg/L toluene and 200 mg/L phenol were degraded individually in 19, 14 and 35 h respectively. The specific growth rate were found to be 0.530/h for 30 mg/L benzene, 0.410/h for 28 mg/L toluene and 0.037/h for 50 mg/L of phenol. Toluene and benzene were found to be better as carbon source than phenol in individual, binary and tertiary mixtures. The presence of toluene, phenol and toluene-phenol mixture increased the biodegradation of benzene. The presence of benzene and/or phenol did not affect significantly the biodegradation time of toluene. The presence of benzene, toluene and benzene-toluene mixture decreased the biodegradation time of phenol.

**Yang et al., (2011): Performance of biotrickling filters packed with structured or cubic polyurethane sponges for VOC removal**

**Objective**: The performance of biotrickling filter packed with either structured polyurethane sponge plugs (BTF 1) or cubes (BTF 2) for toluene removal from waste gas streams were evaluated and compared under different gas empty bed contact time and toluene loading rates.

**Results and Discussions**: VOC removal efficiency increased with increasing gas empty bed residence time for both biotrickling filters (BTF). BTF 1 and BTF 2 could startup successfully and exceeded 99% in removal efficiency after 19 and 27 days, respectively and recovered in 3-7 days after excessive biomass was removed from the BTFs by washing. BTFs packed with polyurethane sponges could effectively remove toluene from waste gas streams at various VOC loading rates and gas EBCTs. However BTF 2 displayed higher removal efficiency even under shorter EBCT or higher loading rate than BTF 1 when other operating conditions were similar, while showed lower pressure drop than BTF 1 during the whole period of operation. Therefore it

was concluded that proper shape selection of packing materials in BTF systems could affect performance of BTFs for VOC removal significantly.

Table 2.2 shows the summary of research works reported on biodegradation of VOCs

**Table 2. 2: Summary of previous works reported on biodegradation of volatile organic compounds**

| Reference | Substrate | Type of biofilter | Type of packing material | Microorganisms used | Critical Loading rate ($gm^{-3}h^{-1}$) | Average Removal efficiency (%) | Maximum Elimination capacity ($gm^{-3}h^{-1}$) | pH | Temp $^0C$ |
|---|---|---|---|---|---|---|---|---|---|
| Singh et al., ( 2010) | Toluene | Biofilter | Wood charcoal | *Pseudomonas putida* | 1104.5 | 95 | 872.5 | 20-30 | |
| Jeong et al., (2009) | o-Xylene, m-Xylene, p-Xylene, Ethyl benzene | Biofilter | Biosol | *Pseudomona* sp. NBM21, *Rhodococcu* sp.BT061 | 250 | >90 | 220 | - | 20 |
| Kwon and Cho (2009) | BTEX | Biofilter | Cork<br>GAC | | 110<br>95 | >90<br>75 | 86<br>67 | - | - |
| Lee et al., ( 2009) | Benzene<br>Toluene | Biofilter | Polyurethane | *Rhodococcus* sp. EH831 | - | 95 | - | - | - |

| Reference | Substrate | Type of biofilter | Type of packing material | Microorganisms used | Critical Loading rate ($gm^{-3}h^{-1}$) | Average Removal efficiency (%) | Maximum Elimination capacity ($gm^{-3}h^{-1}$) | pH | Temp $^{0}C$ |
|---|---|---|---|---|---|---|---|---|---|
| Chan and Su. ( 2008) | Ethyl acetate<br>Amyl acetate | Biofilter | GAC + Peat | | 250<br>220 | - | 81<br>34 | - | - |
| Garci-Pena et al., (2008) | BTEX | Biofilter | Vermiculite | *Paecilomyces variotii* | - | 30 | 110 | - | 25 |
| Jeong et al., (2008) | o-Xylene | Biofilter | Biosol | *Rhodococcus*sp. BT062 | 230 | 90 | 180 | - | 30 |
| Moussavi and Mohseni ( 2008) | Phenol | Biotrickling filter | Polyurethane cubes | - | 330 | >96 | 642 | - | 25 |
| Pandey et al., (2006) | Pyridine | Biofilter | Compost and wood chips | *Pseudomonas pseudoalcaligenes-KPN* | 434 | > 99% | 0.03-1103.36 | 7 ± 0.1 | 30 ± 2 |

| Reference | Substrate | Type of biofilter | Type of packing material | Microorganisms used | Critical Loading rate ($gm^{-3}h^{-1}$) | Average Removal efficiency (%) | Maximum Elimination capacity ($gm^{-3}h^{-1}$) | pH | Temp $^{0}C$ |
|---|---|---|---|---|---|---|---|---|---|
| Moe and Qi (2005) | Paint VOCs<br>Acetone, MEK, Toluene, Ethyl benzene, *p*-xylene | Biofilter | Polyurethane foam cubes (Honey well-PAI), 1.25 cm per side | Mixture of compost wood waste + compost of municipal wastewater | 80.3 | >99% | - | 7 | 23 ± 2 |
| Atoche and Moe (2004) | MEK<br>Toluene | Sequencing batch biofilter<br>Continuous flow biofilter | Polyurethane foam cubes 1.25 cm per side + Powdered activated carbon | Mixed culture | 225<br>450 | 57% (CFB)<br>87% (SSB) | - | - | 23 ± 2 |

| Reference | Substrate | Type of biofilter | Type of packing material | Microorganisms used | Critical Loading rate ($gm^{-3}h^{-1}$) | Average Removal efficiency (%) | Maximum Elimination capacity ($gm^{-3}h^{-1}$) | pH | Temp $^{0}C$ |
|---|---|---|---|---|---|---|---|---|---|
| Elmrini, et al., (2004) | Xylene | Biofilter | Peat | *Microbial activated consortium* | - | >98% | 12-61 | - | 26-28 |
| Moe and Qi (2004) | Paint VOCs *n*-Butyl acetate, MEK, MPK, Toluene | Biofilter | Polyurethane foam cubes (Honey well-PAI), 1.25 cm per side | *Cladosporium sphaerospermum (Fungus)* | 94.3 | >98% | 92 | 5 | 23 ± 2 |
| Moe and Qi (2004) | Paint VOCs *n*-Butyl acetate, MEK, MPK, Toluene | Biofilter | Polyurethane foam cubes (Honey well-PAI), 1.25 cm per side | *C sphaerospermum, P. brevicompactum, Exophiala jenselmei, Fusa-rium oxysporum* | 94.3 | >98% | 92 | 5 | 23 ± 2 |

| Reference | Substrate | Type of biofilter | Type of packing material | Microorganisms used | Critical Loading rate ($gm^{-3}h^{-1}$) | Average Removal efficiency (%) | Maximum Elimination capacity ($gm^{-3}h^{-1}$) | pH | Temp $^{0}C$ |
|---|---|---|---|---|---|---|---|---|---|
| Davidson and Daugulis (2003) | Benzene + Toluene | Partitioning bioreactor | Aqueous phase | *Alcaligenes xylosoxidans* | - | 99% (Benzene), 94% (Toluene) | 62.4 (Benzene), 47.9 (Toluene) | 6.6 | 30 |
| Namkoong et al., (2003) | Gasoline and BTEX | Biofilter | Compost | - | <7.8<br><br><1.6 | 80% (TPH)<br>85% (BTEX) | 40 (TPH)<br>5.3 (BTEX | 8.8 | 20 |
| Torkian et al., (2003) | Aromatic compounds (BTEX) | Biofilter | Compost, wood chips | Municipal solid waste | - | 90% | - | - | 30 |

| Reference | Substrate | Type of biofilter | Type of packing material | Microorganisms used | Critical Loading rate ($gm^{-3}h^{-1}$) | Average Removal efficiency (%) | Maximum Elimination capacity ($gm^{-3}h^{-1}$) | pH | Temp $^{0}C$ |
|---|---|---|---|---|---|---|---|---|---|
| Delhomenie et al., (2002) | Toluene | Biofilter | Matured Compost and organic binder (90:10) | Mixed culture | - | 80% | 90–95 | 7.8 | 20 |
| Lee, et al., (2002) | BTEX | biofilter | - | *Stenotrophomonas maltophilia* T3-c | - | 89% | - | 5-8 | 20-40 |
| Liu, et al., (2002) | Ethyl acetate + Toluene | Compost Biofilter | Compost and soil | Bacteria and Fungi | - | More than 90% | 400 | 5.5-7.08 | 20-25 |
| Lu et al. (2002) | BTEX | Biotrickling filter | Coal particles | Activated sludge | 143 | 80% | - | 5-8.5 | 25 ± 2 |
| | | | | | | | | | |

| Reference | Substrate | Type of biofilter | Type of packing material | Microorganisms used | Critical Loading rate ($gm^{-3}h^{-1}$) | Average Removal efficiency (%) | Maximum Elimination capacity ($gm^{-3}h^{-1}$) | pH | Temp $^0C$ |
|---|---|---|---|---|---|---|---|---|---|
| Lu, et al., (2002) | Acrylonitrile + Styrene | Biotrickling filter | Coal particles | *Activated sludge* | - | >80% | <22 | - | - |
| Sene, et al., (2002) | Benzene | Biofilter | Sugarcane bagasse + Glass beads (4:1) | *Pseudomonas species* NCIMB 9688 | - | 78-97% | 3.5-3.8 | 7 | 20-22 |
| Shim et al. (2002) | BTEX | Batch and Fibrous bed bioreactor | Porous wire cloth + Ceramic saddles | *Pseudomonas putida and Pseudomonas fluorescens* | 1000 mg/l | - | - | 7 ± 0.1 | 25 ± 1 |
| Van Groenestijna et al., (2002) | Alpha Pinene | Biofilter | Perlite,compost, Polyurethane foam cubes | *Fungi (mycelia)* | - | 50-90% | 24-38 | 4.0-6.8 | 25 |

| Reference | Substrate | Type of biofilter | Type of packing material | Microorganisms used | Critical Loading rate ($gm^{-3}h^{-1}$) | Average Removal efficiency (%) | Maximum Elimination capacity ($gm^{-3}h^{-1}$) | pH | Temp $^{0}C$ |
|---|---|---|---|---|---|---|---|---|---|
| Cox and Deshusses, (2001) | $H_2S$ + Toluene | Biotrickling filter | Pall ring | - | - | 90% | 70 - Toluene<br>20 - $H_2S$ | 7 – 4.5 | - |
| Lu et al., (2001) | Ethyl acetate, Toluene, xylene | Trickle bed air biofilter | Coal + Activated Sludge | *Mixed Culture* | 77 (EA)<br>8 (T)<br>10 (X) | >80% | 75 | 7.3-8.1 | 27-30 |
| Zilli, et al., (2001) | Toluene<br><br>Styrene | Biofilter | Peat + Glass Beads (4:1) | *Acinetobacter* sp. NCIMB 9689, *Rhodococcus rhodochrous AL* NCIMB 13259 | - | >90% | 242<br><br>63 | 6.8 | 25 |

| Reference | Substrate | Type of biofilter | Type of packing material | Microorganisms used | Critical Loading rate ($gm^{-3}h^{-1}$) | Average Removal efficiency (%) | Maximum Elimination capacity ($gm^{-3}h^{-1}$) | pH | Temp $^{0}C$ |
|---|---|---|---|---|---|---|---|---|---|
| Kim et al., (2000a) | Paint VOCs *n*-Butanol, MEK, Butyl cellosolve, Toluene | Bioreactor | Powdered activated carbon + Dewatered sludge | Mixed culture | - | 74 - 93% | - | 7-8.5 | 15-30 |
| Lu et al., (1999) | BTEX | Biotrickling filter | Coal particles | *Activated sludge* | 52 (low) 143 (high) | 89% | - | 7-8 | 15–50 |
| Shim and Yang (1999) | BTEX | Batch and Fibrous bed bioreactor | Porous wire cloth + Ceramic saddles | *Pseudomonas putida and Pseudomonas fluorescens* | >1000 mg/l | - | - | 7 ± 0.1 | 25 ± 1 |

| Reference | Substrate | Type of biofilter | Type of packing material | Microorganisms used | Critical Loading rate ($gm^{-3}h^{-1}$) | Average Removal efficiency (%) | Maximum Elimination capacity ($gm^{-3}h^{-1}$) | pH | Temp $^0C$ |
|---|---|---|---|---|---|---|---|---|---|
| Abumaizar et al., (1998) | BTEX | Biofilter | Compost + Activated carbon | *Mixed culture* | <200 ppm of each compound | >90% | - | - | 22-25 |
| Peixoto, et al., (1998) | Toluene | Trickling filter | Raschig rings | *Pseudomonas putida* | - | 94% for inlet conc. <400ppm | - | 6.8 | 25-30 |
| Abumaizar et al., (1997) | BTEX | Biofilter | Compost + Activated carbon | *Mixed culture* | - | - | - | - | 22-25 |
| Sorial, et al., (1997) | BTEX | Biotrickling filter | R-635 Celite pellets | *Mixed culture* | 258.33 | 80% | 87.5 | 7.2-7.6 | 32 |

## 2.4.Objective

Based on the above literature review following objectives were formulated in consultation with my supervisor

- Biodegradation of single substrate i.e Benzene, Toluene and o-Xylene in batch reactor by *A. chroococcum*
- Estimation of kinetic parameters for growth of *A.chroococcum* using BTX as substrate
- Use of biotrickling filter for treatment of air stream containing BTX

# CHAPTER 3

# EXPERIMENTAL SETUP AND INSTRUMENTATION

The following section describes the various instruments used for achieving the objectives described in Chapter 2.

## 3.1.Work Plan

The objective of this work as described in Chapter 2 were

- Biodegradation of single substrate i.e Benzene, Toluene and o-Xylene in batch reactor by *A. chroococcum*
- Estimation of kinetic parameters for growth of *A.chroococcum* using BTX as substrate
- Use of biotrickling filter for treatment of air stream containing BTX

For achieving the above objectives, following work plan was devised. Figure 3.1 shows the work plan of this research work.

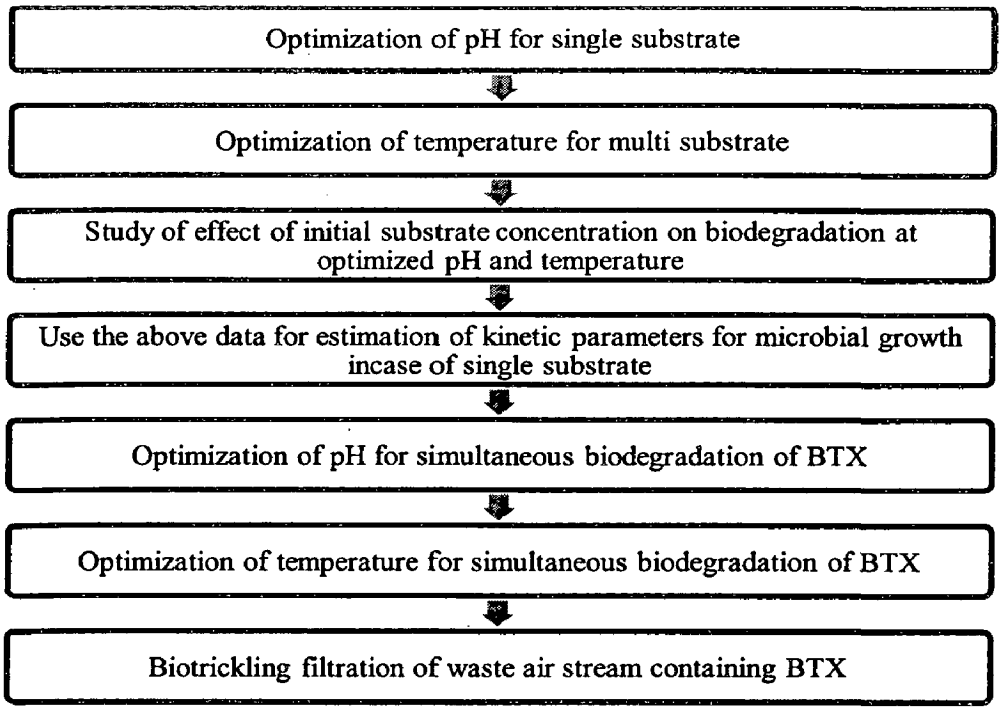



**Figure 3. 1 : Work plan of the research work**

### 3.2.Batch Studies

Biodegradation of benzene, toluene and o-xylene was carried out individually in batch reactor by *A. chroococcum.* The batch reactor was 500 mL Schott Duran Laboratory glass bottle whose screw cap was modified to have a sampling port sealed with septum to collect samples of liquid and gas at regular intervals. Figure 3.2 shows the bottle used for conducting batch experiments

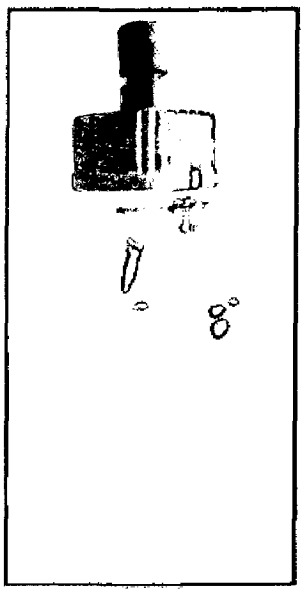


**Figure 3. 2: Batch Reactor**

The bottles were agitated in Metrex Orbital Shaking Incubator at 120 rpm. The details of operating parameters for batch studies of biodegradation of benzene, toluene, o-xylene and simultaneous BTX is given in Table 3.1, Table 3.2, Table 3.3 and Table 3.4 respectively.

**Table 3. 1 Operating parameter for batch biodegradation of benzene**

| pH | Temperature (°C) | Initial Concentration of Benzene (mg/L) | Contact time (hours) | Agitation Speed (rpm) |
|---|---|---|---|---|
| 1-12 | 30 | 50 | 48 | 120 |
| 7 | 15-45 | 50 | 48 | 120 |
| 7 | 30 | 50-500 | 0-96 | 120 |

**Table 3. 2 Operating parameter for batch biodegradation of toluene**

| pH | Temperature (°C) | Initial Concentration of Toluene (mg/L) | Contact time (hours) | Agitation Speed (rpm) |
|---|---|---|---|---|
| 1-12 | 30 | 50 | 48 | 120 |
| 7 | 15-45 | 50 | 48 | 120 |
| 7 | 30 | 50-500 | 0-96 | 120 |

**Table 3. 3 : Operating parameter for batch biodegradation of o-xylene**

| pH | Temperature (°C) | Initial Concentration of o-Xylene (mg/L) | Contact time (hours) | Agitation Speed (rpm) |
|---|---|---|---|---|
| 1-12 | 30 | 50 | 48 | 120 |
| 7 | 15-45 | 50 | 48 | 120 |
| 7 | 30 | 50-250 | 0-96 | 120 |

**Table 3. 4 : Operating parameter for simultaneous batch biodegradation of benzene, toluene and o-xylene**

| pH | Temperature (°C) | Initial Concentration of Benzene + toluene + o-xylene (mg/L) | Contact time (hours) | Agitation Speed (rpm) |
|---|---|---|---|---|
| 1-12 | 30 | 60 | 48 | 120 |
| 7 | 15-45 | 60 | 48 | 120 |

### 3.3.Biotrickling filtration

Biodegradation of contaminated air stream containing benzene, toluene and o-xylene was performed in biotrickling filter at the optimized pH and temperature determined from simultaneous removal of benzene, toluene and o-xylene in batch reactor. The operational parameters of biotrickling filtration is shown in Table 3.5.

**Table 3. 5 : Operational parameters for biotrickling filtration of air stream containing BTX**

| Operational parameters | Value |
|---|---|
| Temperature | 30 °C |
| pH | 7 ± 0.2 |
| Gas flow rate | 1 - 5 L $min^{-1}$ |
| EBRT | 0.385 - 1.925 min |
| Inlet Loading Rate | 22-627 g $m^{-3}$ $h^{-1}$ |

Figure 3.3 shows the schematics of the biotrickling filter used for biodegradation of BTX in air stream. Figure 3.4 shows the biotrickling filter used for biodegradation of air stream containing benzene, toluene and o-xylene. Various components of bioreactor are as follows:-

### 3.3.1. Air Generation Unit

The BOSS Air compressor supplied by K.G. Khosla & Co Pvt. Ltd., Faridabad, India was used for generation of air stream. The compressor compressed the incoming air and provided the air stream at fixed pressure controlled by automatic pressure regulating switch. The air was filtered to remove oil, water and particulate matters using the filter provided along with compressor. The airflow rate was measured by rotameter supplied by JTM, Jaspin Industrial Instrumentation, India. This rotameter can measure air flow rates between 1 and 10 $Lmin^{-1}$. The air stream from the rotameter was divided into two sections- Major Air Stream and Minor Air Stream. Figure 3.5 shows the Air Generation Unit.

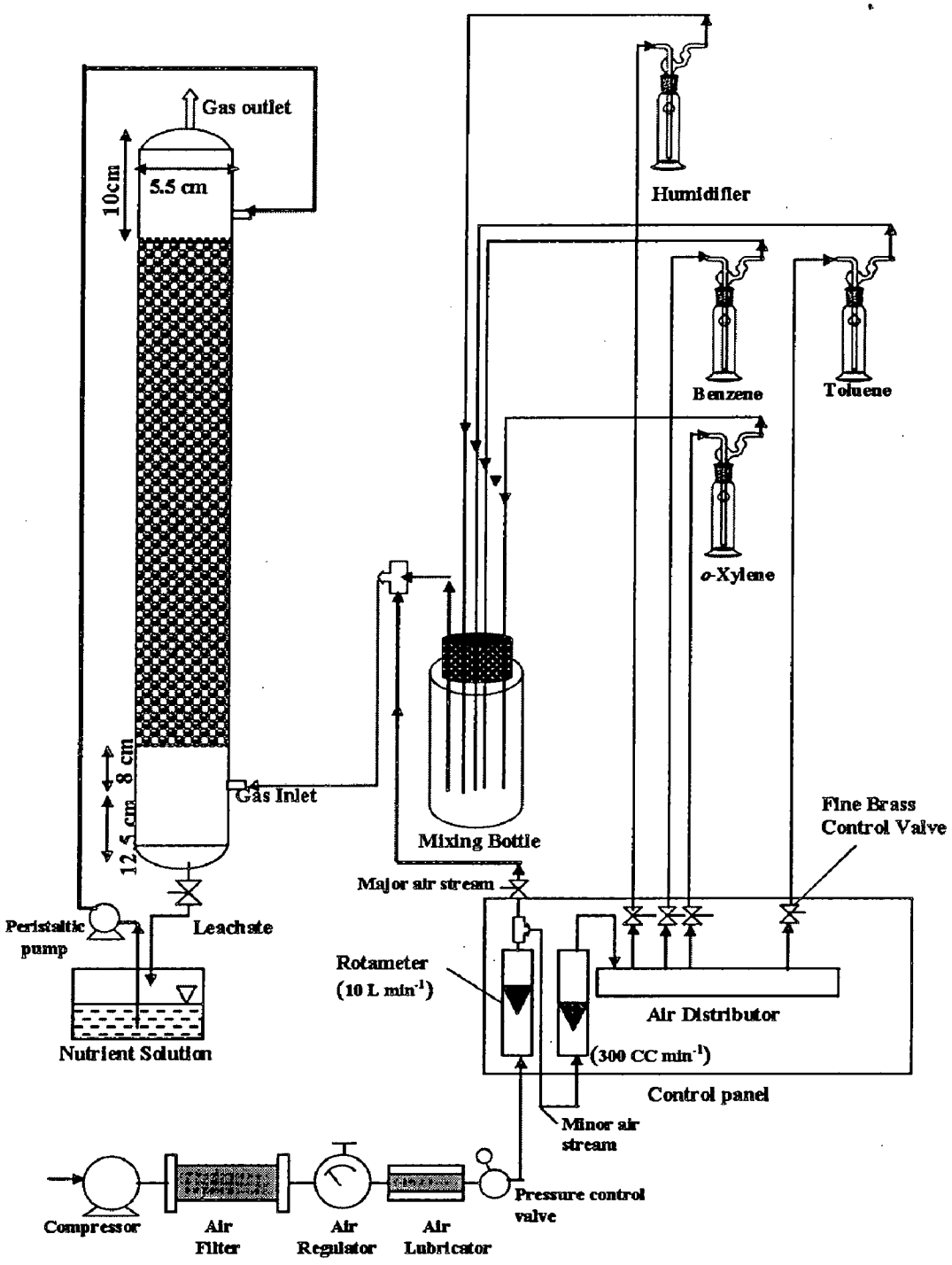



**Figure 3. 3: Schematics of biotrickling filter used for biodegradation of BTX in air stream**


**Figure 3. 4: Biotrickling filter used for biodegradation of air stream containing BTX**


**Figure 3. 5 : Air Generation Unit**

### 3.3.2. VOC Control Unit

VOC Control Unit was used to control the amount of BTX in air stream. It consisted of a plastic cylinder having one inlet and four outlet connection. The minor air stream from rotameter entered the VOC Control Unit. The flow rate in four outlet connection were controlled by fine brass valve. The outlet connections were connected to VOC Generation and Humidification System. Figure 3.6 shows the VOC Control Unit.

### 3.3.3. VOC Generation and Humidification Unit

Figure 3.7 shows VOC Generation and Humidification Unit. It was used for generating humidified air stream containing BTX. It consisted of closed bottles containing benzene, toluene, o-xylene and water. The minor air stream which was divided into 4 parts in VOC Control Unit was passed through three 1000 mL bottles containing BTX respectively and another bottle containing distilled water. These four air stream were again joined in mixing chamber. The mixing chamber consisted of a bottle having four inlet and one outlet connection. The mixing chamber allowed for formation of humidified air stream containing BTX. The outlet from this chamber was later mixed with Major Air Stream to form inlet air stream for the biotrickling filter.

### 3.3.4. Biotrickling filter

The biotrickling filter was used for biodegradation of humidified air stream containing BTX by *A.chroococcum* immobilized on corn cob. The biotrickling filter consisted of perpex pipe having internal diameter 5.5 cm and total height of 111.5 cm. The inlet air stream entered at height of 12.5 cm from the bottom of biotrickling column. The packing consisting of sterilized corn cob supported on the acrylic sieve plate, started at height of 20.5 cm from the bottom. The height of packing was 80 cm. The design parameters of biotrickling filter are shown in Table 3.6. Inlet Air stream containing BTX enters from the bottom of the bed and rises through packing of corn cob on which *A.chroococcum* is immobilized. While passing through bed, BTX in air is removed and the outlet stream from the above of the bed is relatively free from BTX.

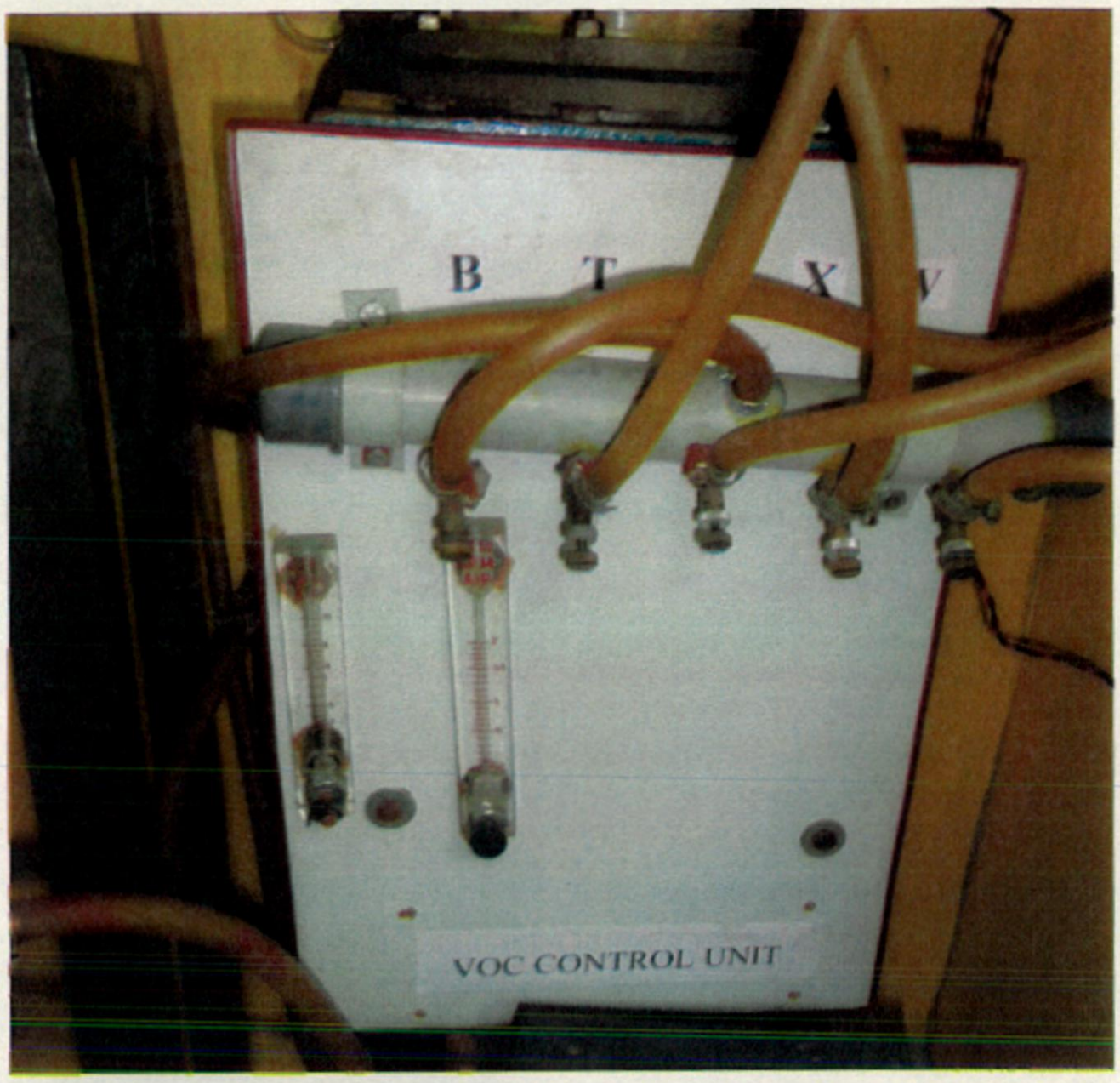



**Figure 3. 6: VOC Control Unit**

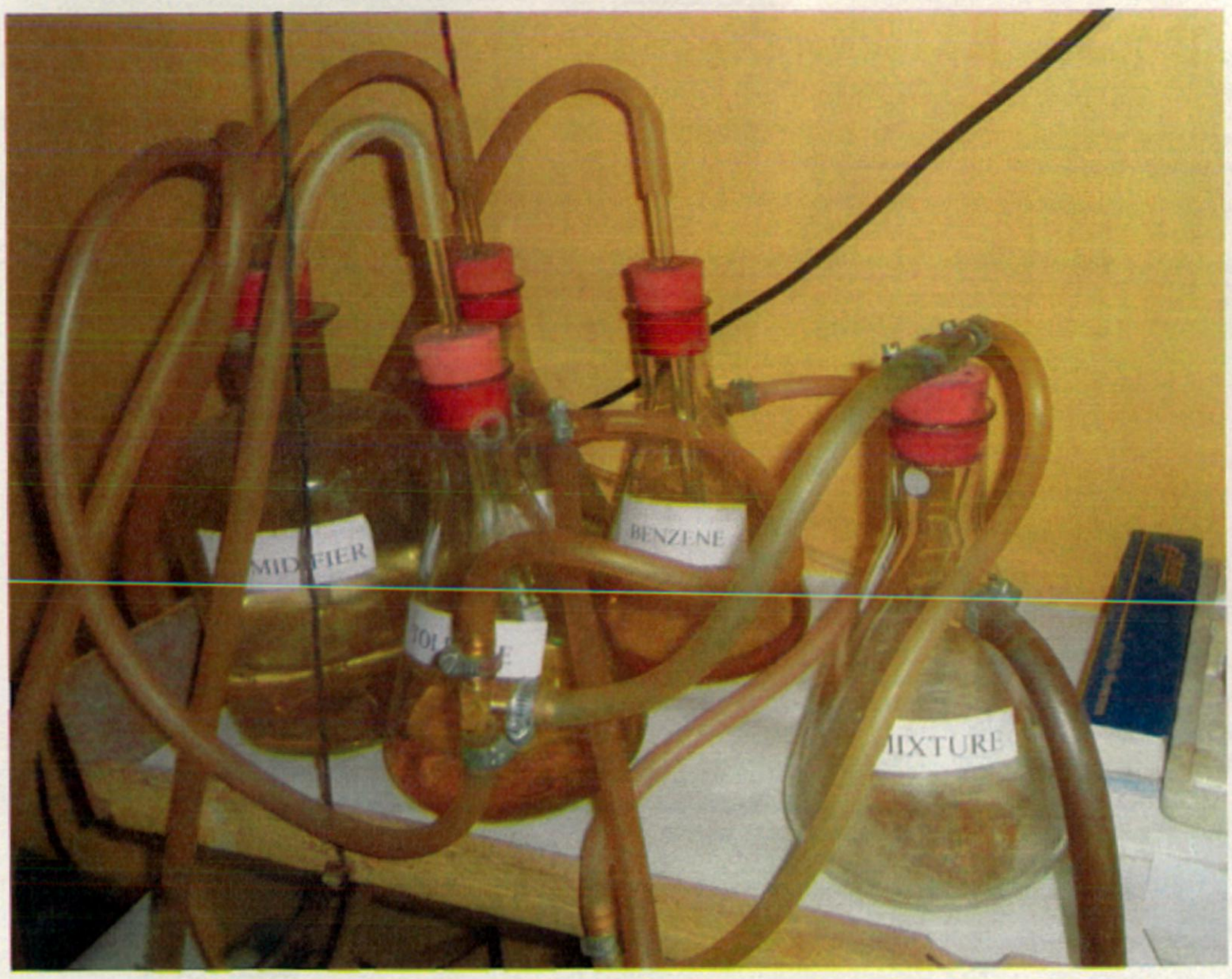



**Figure 3. 7: VOC Generation and Humidification System**

**Table 3. 6 : Design Parameters of biotrickling filter**

| Design parameters | Biotrickling Filter |
|---|---|
| Material of construction | Perspex pipe |
| Internal diameter | 5.5 cm |
| Height of column | 111.5 cm |
| Bed height | 80cm |
| Packing material | Corn cob |
| Volume of the packing | 1.925 L |

### 3.3.5. Temperature Controller

The entire setup is installed in plywood chamber of size 1.2 m x 1.2 m x 1.8 m. The double walled chamber contained glass wool in between the walls for insulation. The temperature was controlled by a digital temperature controller supplied by ESCORT, Japsin Pvt. Ld., New Delhi. The digital temperature controller was connected to hot air blower which supplied hot air when the temperature fell below the set point. The sensor of digital temperature controller was placed near the column.

### 3.3.6. Nutrient Supply System

For growth of *A.chroococcum,* nutrient media was supplied. The nutrient media had the composition as shown in Table 3.7.

**Table 3. 7: Composition of nutrient media**

| Chemical | Concentration (g $L^{-1}$) |
|---|---|
| $Na_2HPO_4$ | 8.8 |
| $KH_2PO_4$ | 1.2 |
| NaCl | 5 |
| $NH_4Cl$ | 1 |
| $MgSO_4.7H_2O$ | 0.25 |

The nutrient media was supplied to the biotrickling filter from the top with the help of peristaltic pump at the rate of 14 mL $min^{-1}$. The leachate collected at the bottom was recirculated to the top. The nutrient media was changed at an interval of every 24 hours.

### 3.3.7. Pressure Drop Measurement

The pressure drop across the bed was measured with the help of U tube manometer with the water being the manometric liquid.

## 3.4.Analytical methods

The amount of BTX in liquid phase in batch reactor was calculated using Henry's partition coefficient based on headspace gas concentration.

The amount of BTX in an air sample was analyzed using MICHRO -9100 Gas Chromatograph supplied by Netel India Ltd.. BTX measurement was done in HP5 type capillary column having flame ionization detector.

The amount of $CO_2$ produced was also measured with the help of the same gas chromatograph. $CO_2$ measurement was done in Porapack Q column with thermal conductivity detector. The operational details of gas chromatograph are given in Table 3.8.

**Table 3. 8 : Operational details of gas chromatograph**

| Parameter | Column | Type of detector | Oven Temp (°C) | Injection Temp (°C) | Detector Temp (°C) | Carrier gas | Fuel Gas + $O_2$ |
|---|---|---|---|---|---|---|---|
| VOC in gas phase | HP 5, Capillary column, 30 m length, 0.249 mm I.D., 0.25 µm film thickness, temperature limits: -60 $^0$C to 325 $^0$C | FID | 60 | 210 | 230 | $N_2$ | $H_2$ |
| $CO_2$ | Porapak Q, Packed Stainless steel column, 2 m Length, | TCD | 60 | 150 | 180 | $N_2$ | - |

Biomass growth was measured turbidometrically at 600 nm using a cuvette with 1 cm light path in Double Beam UV-Vis Spectrophotometer (SHIMAZDU, Japan).

Fourier Transform Infrared Spectroscopy studies were done to determine the type of functional groups present on the surface of corn cob before and after biodegradation of benzene, toluene, o-xylene and BTX simultaneously by *A. chroococcum* using FTIR, Nicolet 6700, USA. Corn cob pellets were made with KBr and FTIR studies were studied for wavelengths from 4000 to 400 $cm^{-1}$.

The Scanning Electron Microscope (SEM) studies were done using Leo electron microscopy, England to observe the surface texture of corn cob before and after biodegradation of benzene, toluene, o-xylene and BTX simultaneously by *A. chroococcum*. The samples were given a metal coating of gold using Sputter Coater Edwards S150 to provide better conductivity to samples and then SEM studies were done at various magnifications.

# CHAPTER 4

# EXPERIMENTAL PROCEDURE

The following section describes the procedure employed for achieving the objectives described in Chapter 2. The preparation of corn cob and *A. chroococcum* for biodegradation in batch reactor and biotrickling filter is discussed in this chapter.

## 4.1. Procurement of Materials

All the chemicals used in this experimental work were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. All the necessary chemicals were of analytical grade. The corn cobs were collected from local agricultural field

## 4.2. Pretreatment of Corn cobs

The corn cobs were crushed with the help of rotary mill. The corn cobs were dried in hot air oven at 50 °C for 4 hours to completely remove moisture, cooled to room temperature and were stored in air tight polybags to form heat treated corn cob. The heat treated corn cobs were further treated with 0.5 M HCl for 24 hours in 1:1 ratio of liquid to solid. The corn cobs were further washed several times with distilled water and dried in hot air oven at 50 °C for 4 hours to completely remove moisture, cooled to room temperature and were stored in air tight polybags to form acid treated corn cob which was used for immobilization of *A. chroococcum* to form acid treated corn cob.

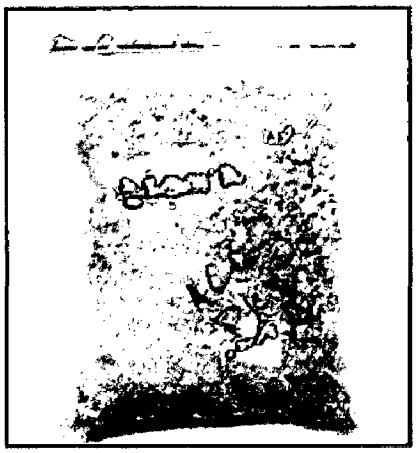


**Figure 4. 1: Air tight polybads used for storage of corn cobs**

### 4.3.Preparation of *A. chroococcum*

Microbial strain of *Azotobacter Chroococcum* (MTCC 446) was obtained from MTCC, Chandigrah, India. The obtained microorganism was initially cultured in nutrient broth and then plated on nutrient agar and preserved. Nutrient Broth and Nutrient agar was obtained from HiMedia Laboratories Pvt. Ltd. Mumbai. From the fresh culture obtained by above procedure plating of the microorganism was done in the following manner.

- A 25 ml of nutrient agar solution was prepared by mixing 1g of nutrient agar in 25 ml of distilled water. The solution was autoclaved at 121 ° C for 15 min.
- The solution was allowed to cool for some time till it was warm and easy to handle. Cooling is also necessary to avoid condensation of vapors on petridish. Then the medium was poured in dish slowly to avoid bubble formation.
- The dish was covered and the gel was allowed to solidify at room temperature.
- The wire loop is then flamed to red hot and was allowed to cool. A very small amount of microorganism was scooped from the nutrient broth.
- The bacteria were spread onto the media in the culture plate. Plates were incubated for 24 h at 30 ° C.
- After incubation period of 24 h, colonies of microorganisms were observed to grow on the plates. These plates were carefully stored at 4 $^0$C until further use.

### 4.4.Acclimatization of *A. chroococcum*

The cultures were acclimatized to benzene, toluene, o-xylene individually as well as simultaneously by exposing the culture to increasing concentrations of substrate in a series of flasks. The startup of acclimatization was obtained by inoculating 100 mL of nutrient media containing 10 mg/L of substrate with cultures from nutrient agar slants under sterile conditions. After a good growth was observed, 5 mL of this culture was added to fresh nutrient media containing substrate as innoculum. The concentration of substrate in fresh nutrient media was increased sequentially to 500, 500, 250 and 600 mg/L in case of benzene only, toluene only, o-xylene only and BTX respectively.

### 4.5.Immobilization of *A. chroococcum*

Cultures were grown overnight and thereafter 2 g of sterilized corn cob was added to the flasks under aseptic condition. The flask was further incubated for 24 hours. Immobilized bacteria was filtered out and used as innoculum for biodegradation of benzene only, toluene only, o-xylene only and BTX.

### 4.6.Batch Studies

The calibration curve was prepared by injecting known amounts of the BTX into a sealed bottle equipped with a Teflon septum according to the standard procedure (Lodge, 1989). The injected amount of BTEX and MTBX was allowed to evaporate in the air space within the bottle at experimental temperature (30 °C). For the calibration, air samples are drawn from the bottle by a 1 mL gas tight syringe (Hamilton-Bonaduz-Schweiz) and analyzed by gas chromatograph.

All the batch experiments were performed in 500 mL sealed bottles with Teflon septum for sampling of air and liquid sample at regular intervals. Only 100 mL working volume of liquid was taken in 500 mL bottles to avoid deficit of oxygen. 24 hour old incubated culture immobilized on 2 g of corn cob was used for biodegradation by *A. chroococcum.* The effect of pH, temperature and initial concentration of substrate on removal efficiency of benzene only, toluene only, o-xylene only and BTX by *A. chroococcum* acclimatized with benzene only, toluene only, o-xylene only and BTX respectively was studied.

### 4.7.Biotrickling Filtration

The continuous studies were done on biotrickling filter packed with corn cob on which *A. chroococcum* was immobilized. The air stream containing BTX was passed through corn cob bed to allow biodegradation of BTX by *A. chroococcum.* The pH of the leachate was monitored and maintained at 7. The temperature of the wooden chamber housing the biotrickling filter was maintained at 30 °C. The inlet and outlet concentration of BTX and $CO_2$ was measured daily. Initial flow rate of air was maintained at 1 L $min^{-1}$. Initial loading rate of 23 g $m^{-3}h^{-1}$ was applied to biotrickling filter. When the removal efficiency of each of BTX reached 99.5% or was within ±2% for 3 days , the loading rate was increased.

# CHAPTER 5

# RESULTS AND DISCUSSIONS

## 5.1.Characterization of corn cob

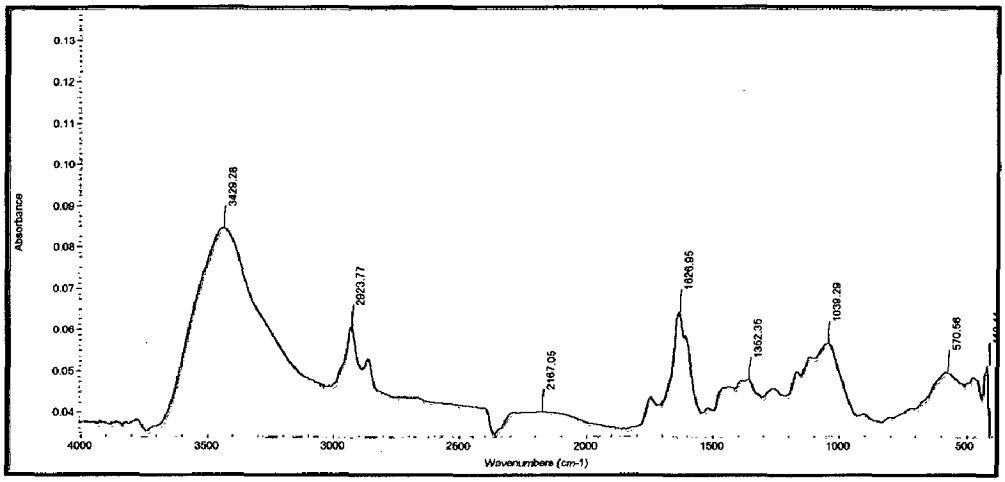
The general characteristic of corn cob used for immobilization of *A. chroococcum* is provided in Table 5.1.

**Table 5. 1: General characteristics of corn cob used for immobilization of *A. chroococcum***

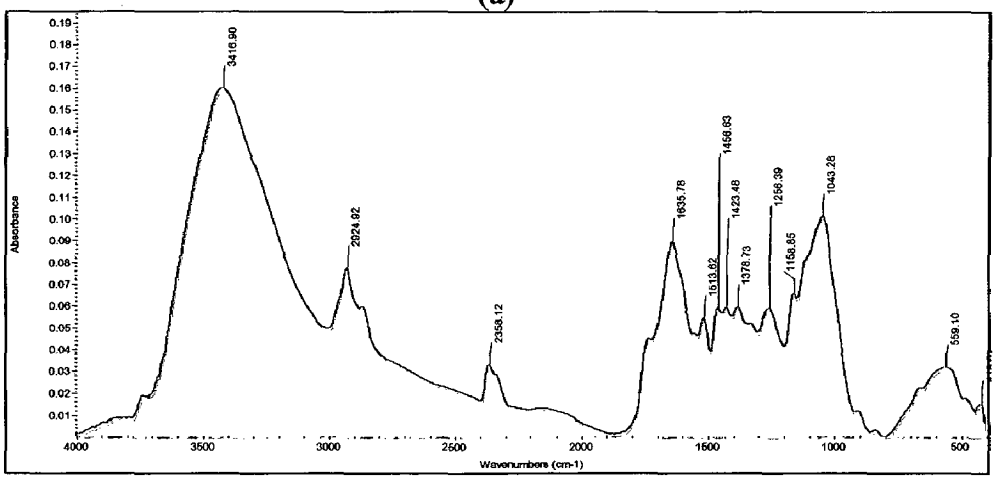
| Parameter | Value | |
|---|---|---|
| | Heat treated corn cob | Acid treated corn cob |
| **Proximate Analysis** | | |
| Fixed Carbon | 14.99 | 13.55 |
| Volatile Matter | 74.79 | 79.35 |
| Moisture | 8.75 | 6.05 |
| Ash | 1.47 | 1.05 |
| **Ultimate Analysis** | | |
| Carbon | 44.13 | 45.54 |
| Hydrogen | 5.807 | 5.347 |
| Nitrogen | 0.139 | 0.450 |
| Sulphur | 0.000 | 0.526 |

Figure 5.1 describes the FTIR spectra of acid treated corn cob (ACC), corn cob immobilized with bacteria before adsorption and corn cob immobilized with bacteria after biodegradation with benzene only, toluene only, o-xylene only and BTX. Acid treated corn cob shows peaks at 3429.8, 2923.77, 1626, 1352, 1039.29 and 570.26 $cm^{-1}$. Pan et al. (2010) also found these peaks in corn cob. There is a presence of peak at 2358 $cm^{-1}$ in all corn cobs having bacteria which is identified as $CO_2$ (Feng et al., 2006). The presence of carbon dioxide is due to the use of conversion of BTX into carbon dioxide by microbes indicating the biodegradation of BTX.

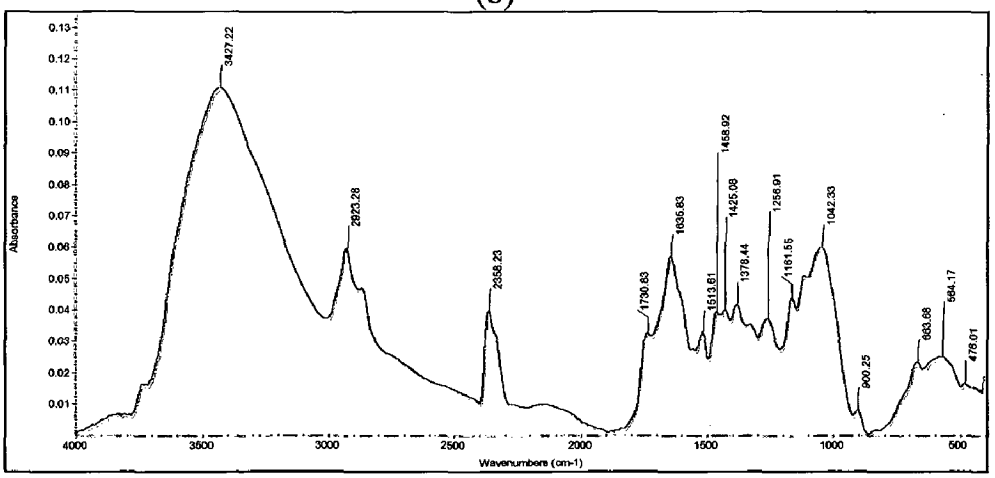
The surface morphologies of corn cob were studied under Scanning Electron Microscope (SEM). The SEM provides crucial information for studying topographic and structural features of corn cob before and after biodegradation. Figure 5.2, 5,3, 5,4, 5.5, 5.6 and 5.7 shows the SEM images of corn cob in various biodegradation studies. We can see from the images that acid treated corn cob contains a large number of pores.


Absorbance
Wavenumbers (cm-1)
3429.28
2923.77
2167.05
1628.95
1362.35
1039.29
570.56

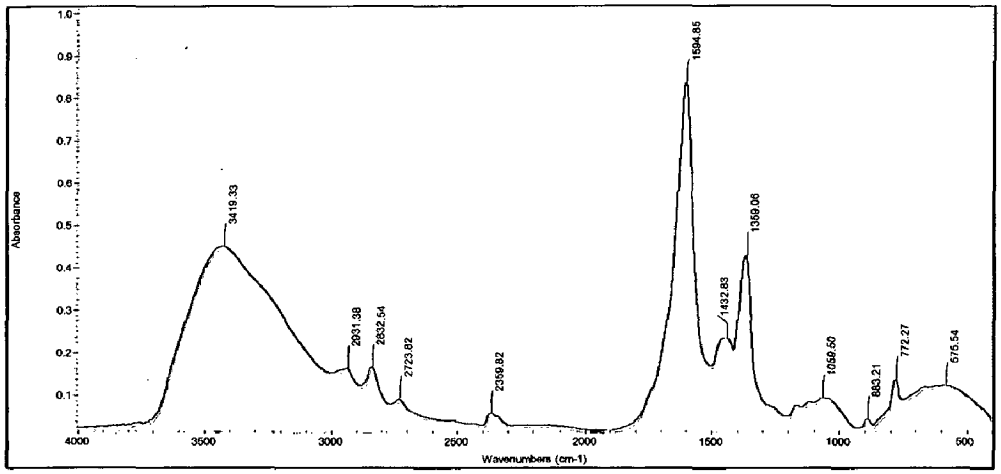
(a)


Absorbance
Wavenumbers (cm-1)
3416.90
2924.92
2358.12
1635.78
1513.92
1458.63
1423.48
1378.73
1256.99
1158.85
1043.28
559.10

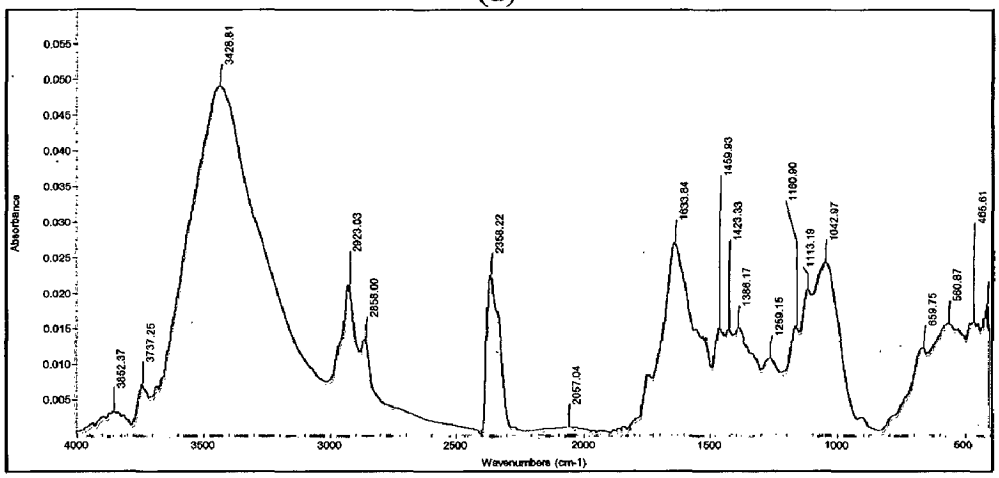
(b)


Absorbance
Wavenumbers (cm-1)
3427.22
2923.28
2358.23
1730.83
1635.83
1513.61
1458.92
1425.09
1378.44
1256.91
1161.55
1042.33
900.25
683.88
584.17
478.01

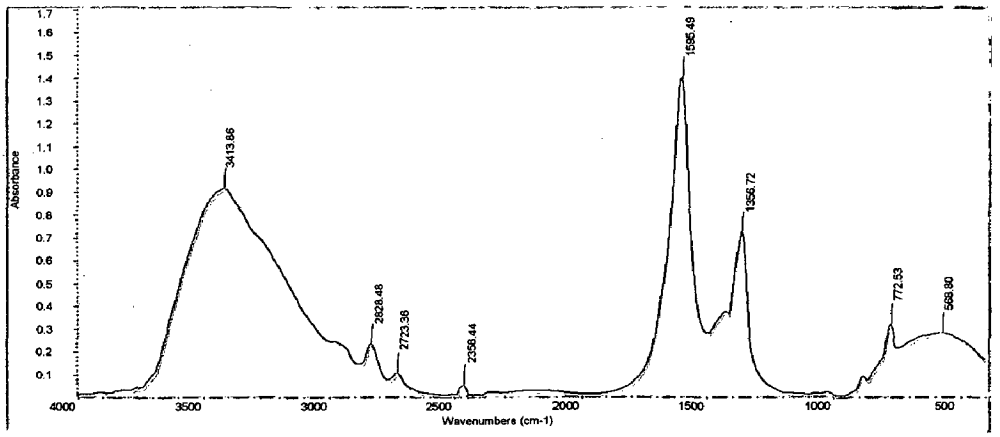
(c)




(d)




(e)




(f)

**Figure 5. 1: FTIR spectra of (a) acid treated corn cob, (b)*A. chroococcum* immobilized on cron cob, (c) after biodegradation of benzene only, (d) after biodegradation of toluene only, (e) after biodegradation of o-xylene only, (f) after biodegradation of BTX**

**Figure 5. 2: SEM image of acid treated corn cob**

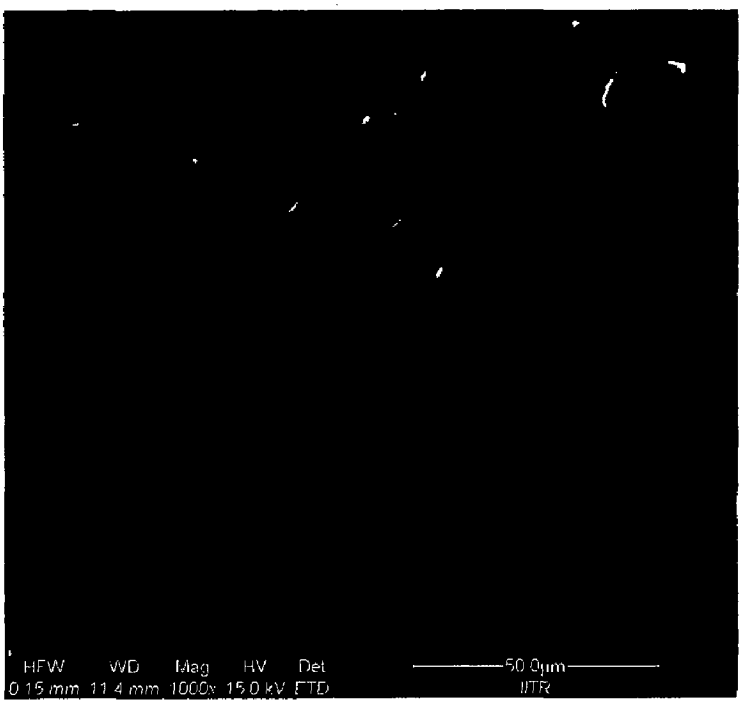



**Figure 5. 3: SEM image of *A.chroococcum* immobilized on acid treated corn cob.**

**Figure 5. 4: SEM image of *A.chroococcum* immobilized on acid treated corncob after biodegradation of benzene only**

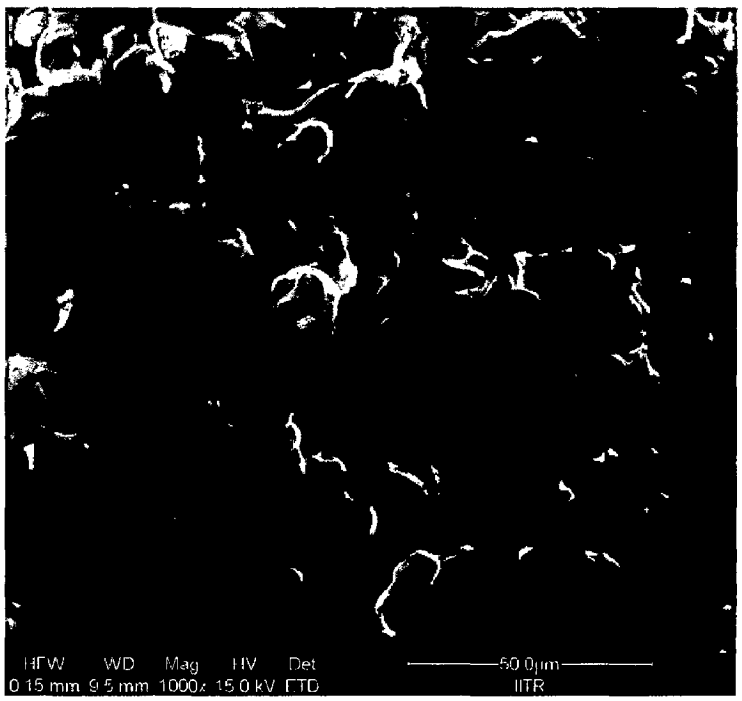



**Figure 5. 5: SEM image of *A.chroococcum* immobilized on acid treated corn cob after biodegradation of toluene only**

**Figure 5. 6: SEM image of *A.chroococcum* immobilized on acid treated corn cob after biodegradation of o-xylene only**

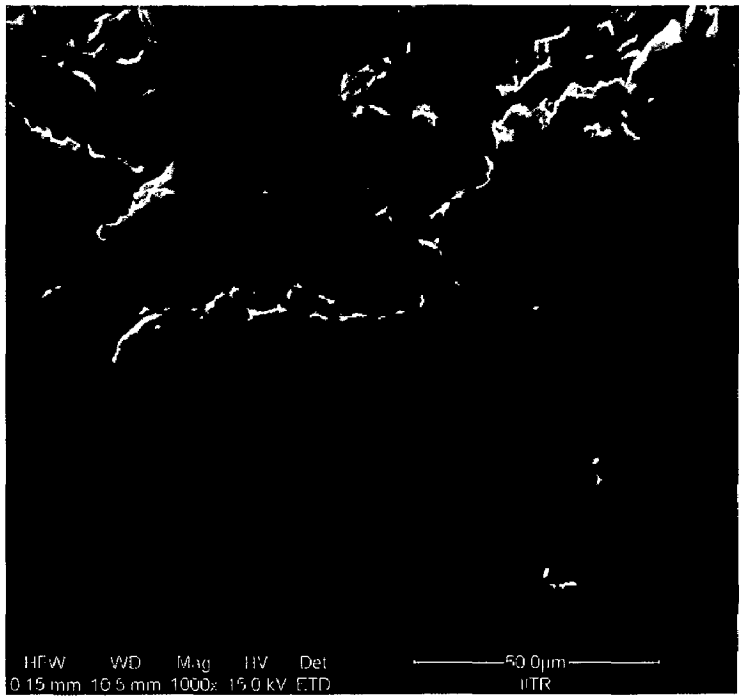



**Figure 5. 7: SEM image of *A.chroococcum* immobilized on acid treated corn cob after simultaneous biodegradation of benzene, toluene and o-xylene (BTX)**

In figure 5.2, we see bacterial population is small amount on the surface of corn cob which is due to immobilization of *A. chroococcum* on corn cob. In figure 5.3, 5,4, 5.5, 5.6 and 5.7, the bacterial population seems to be increased due to increase in their biomass after utilization of benzene, toluene and o-xylene for their growth.

The decrease in BET surface area and pore volume after biodegradation of BTX from 98.2804 to 14.7213 $m^2g^{-1}$ and 0.0495 to 0.0074 $m^3$ $g^{-1}$ respectively can be attributed to utilization of BTX for microbial growth.

**5.2.Individual biodegradation of BTX in batch reactor**

**5.3.1. Effect of pH**

The pH value is a key factor in microbial metabolic processes. It influences the redox potential and enzymatic activity (Jianlong et al., 1997). The pH of the medium was adjusted by 1M NaOH and 1M HCl. A control was also run without microbial cells to measure the loss of different components due to any volatilization. The effect of pH on removal of individual benzene, toluene and o-xylene was studied at 50 mg/L of substrate for pH ranging from 1-12 after allowing the biodegradation for 2 days. Figure 5.8 shows the effect of pH on percentage removal of individual BTX by bacterial cells due to biodegradation. Percentage of initial benzene concentration removed due to biodegradation increased from 17.6% to almost 93.1%. On increasing the pH from 7 to 9, the percentage removal decreased to 67.1%. The removal percentage of original toluene concentration after a period of 2 days increased from 21.2% to 95.1%. On further increase in pH to 9, the percentage removal decreased to 71.8%. It was seen that percentage removal of o-xylene increased from 14.1% to 78.9 % on increasing pH from 4 to 7. On further increase in pH to 9, the removal efficiency decreased to 58.7%. In all cases it was observed that at pH less than 4 or greater than 9, there was no significant removal due to biodegradation. This may be due to the effect of pH on the ionization and therefore binding and interaction of a myriad of molecular processes which in turn affect the metabolic pathway of microorganisms. It could even cause denaturing of proteins which might result in lethal toxicity (Chakraborty et al., 2010). The optimum pH was found to be 7.0.

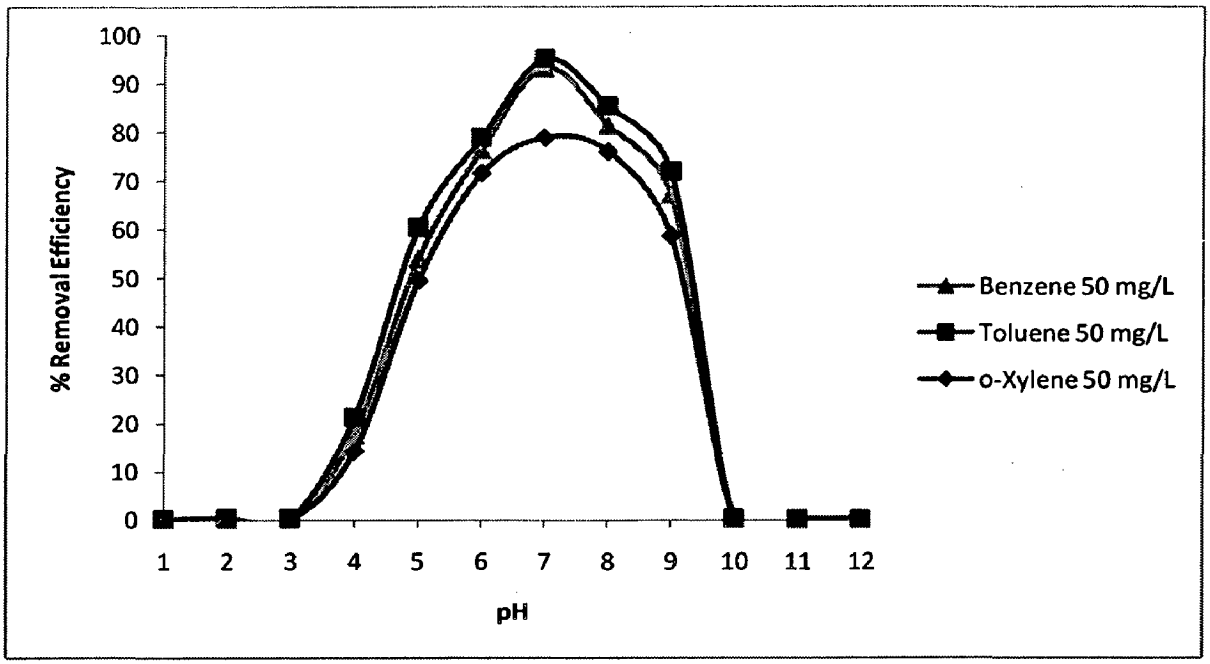



**Figure 5. 8: Effect of pH on the percentage removal efficiency of 50 mg/L of BTX (Temperature =30°C, agitation rate = 150 rpm)**

### 5.3.2. Effect of temperature

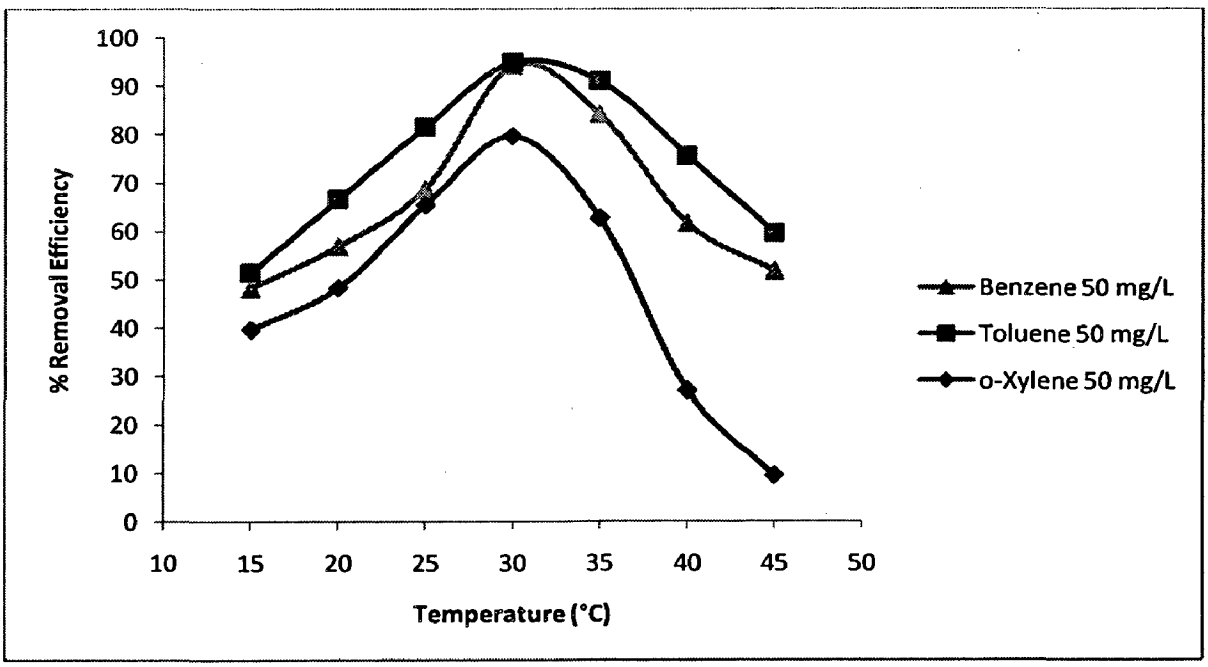



**Figure 5. 9: Effect of temperature on the percentage removal efficiency of 50 mg/L of BTX (pH=7, agitation rate = 150 rpm)**

Figure 5.9 shows the biodegradation of BTX by immobilized cells was carried out at temperatures ranging from 15 °C to 45 °C for an initial concentration of 50 mg/L each of BTX at pH=7. The results reveal that removal efficiency increases on increasing temperature from 15°C to 30°C. The results also seem to follow an exponential trend in this range. On further increasing temperature, the removal efficiency decreases. *A. chroococcum* was able to degrade 94.2%, 94.8% and 79.6% of BTX respectively at 30°C. Therefore *A. chroococcum* can grow from 15°C to 45 °C but maximum removal of BTX occurs at temperature of 30°C. Table 5.2 compares the optimum pH and temperature for optimum biodegradation conditions of different microbes.

**Table 5. 2: Comparison of optimum pH and temperature for optimum biodegradation conditions with other reported works**

| Reference | Microbe | Substrate | Optimum pH | Optimum Temperature (°C) |
|---|---|---|---|---|
| This study | *A. chroococcum* | BTX | 7 | 30 |
| Gurusamy et al., (2007) | *Pseudomonas pictorum* | Phenol | 7 | 30 |
| Jung and Park (2005) | *Rhodococcus pyridinovorans* | Styrene | 7 | 32 |
| Reda and Ashraf (2010) | *B.subtilis*-EPRIS12 | Toluene | 7 | 30 |
| Reda and Ashraf (2010) | *B.laterosporous*–EPRIS41 | Toluene | 7 | 30 |

### 5.3.3. Effect of initial substrate concentration

The effect of initial substrate concentration on the removal of benzene and toluene was studied at 50, 100, 250, 400, 500 mg/L. The effect of initial substrate concentration on the removal of o-xylene was studied at 50, 100, 150, 200 and 250 g/L. Figure 5.10, 5.11, 5.12 shows the percentage of BTX respectively remaining after the biodegradation by *A. chroococcum* with respect to time. Almost 100% biodegradation of benzene was observed in 54, 66, 84 hours when the initial concentration of benzene was 50, 100, 250 mg/L respectively. However only 70.3% and 51.2% degradation was observed in 96 hours when the initial substrate concentration was 400 and 500 mg/L. When the initial concentration of toluene was 50, 100 and 250 mg/L, complete biodegradation was observed in 54, 60 and 78 hours.

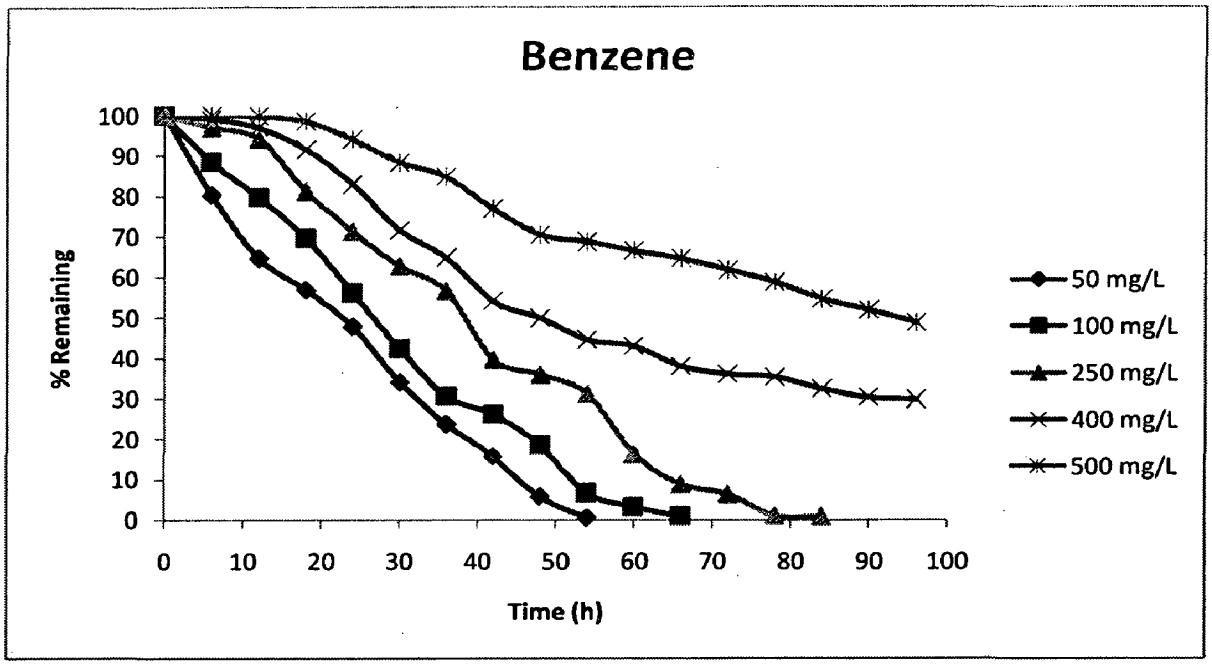



**Figure 5. 10: Effect of initial substrate concentration on removal of Benzene (pH = 7 , Temperature =30°C, agitation rate = 150 rpm)**

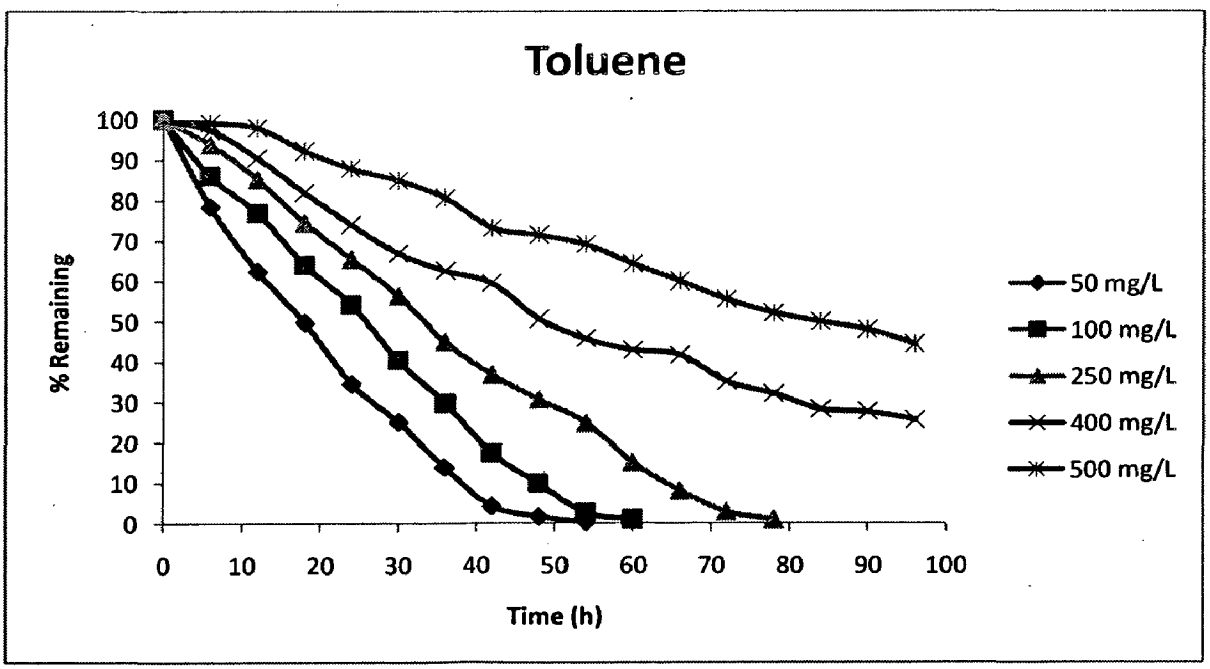



**Figure 5. 11: Effect of initial substrate concentration on removal of Toluene (pH = 7 , Temperature =30°C, agitation rate = 150 rpm)**

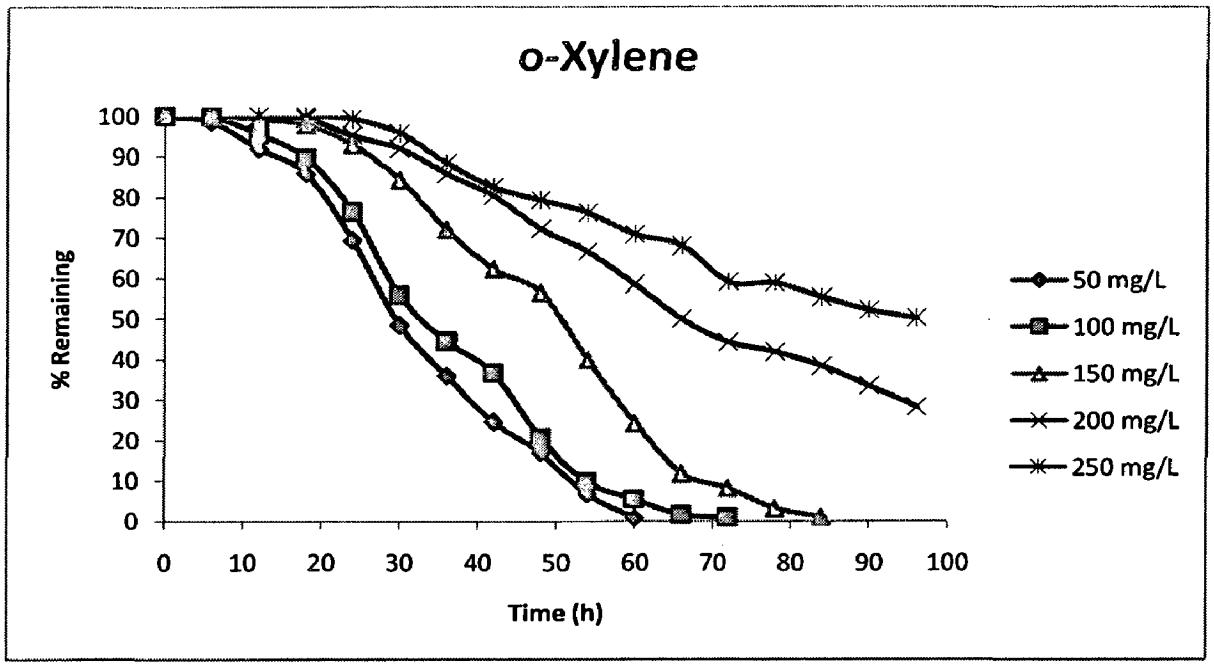



**Figure 5. 12: Effect of initial substrate concentration on removal of o-Xylene (pH = 7 , Temperature =30°C, agitation rate = 150 rpm)**

However in 96 hours, only 25.5% and 44.4% degradation was observed when initial substrate concentration was 400 and 500 mg/L. *A. chroococcum* was able to degrade around 100% of o-xylene at an initial concentration of 50, 100, 150 mg/L in 60, 72 and 84 hours respectively. However it was able to degrade only 72% and 50% for the initial concentration of 200 and 250 mg/L.

The time required for biodegradation was found to increase on increasing initial concentration of substrate. A region of relatively less rate of substrate removal was observed towards the end of substrate consumption curve for high concentrations. The degradation rate was high at low concentrations whereas it was low at high concentrations. It could be due to the deficit of oxygen since no constant supply of oxygen was available as stated by Morgen et al., 1993.

They found oxygen supply found to be a limiting factor in the biodegradation of benzene, toluene, ethyl benzene and xylenes in groundwater (Morgan et al., 1993). Since the experiments were conducted in 500 mL bottle with 100 mL working volume, therefore only a limited amount of air was available for the growth of bacteria.

Another reason could be the fall in pH of the solution over time. Fakhruddin and Quilty (2005) found out that the removal of 2-chlorophenol was inhibited due to significant fall in pH.

A study of change in biomass concentration with time and different initial concentration of benzene, toluene and o-xylene was carried out and results are plotted in Figure 5.13, 5.14 and 5.15 respectively.

The cell growth followed the pattern of a lag phase in which no appreciable change in concentration of biomass was observed. It was followed by a log phase in which biomass concentration increased exponentially. At the end of log phase, the biomass concentration became constant indicating the stationary phase.

When the initial concentration of benzene was increased from 50 mg/L to 500 mg/L, the duration of lag phase increased from 4 hours to 20 hours. The maximum biomass concentration was 24.3, 28.1, 29.4, 18.8 and 13.1 mg/L at 50, 100, 250, 400 and 500 mg/L respectively. *A. chroococcum* was in the lag phase for 2, 4, 6, 10 and 12 hours with the maximum biomass concentration of 26.1, 28.8, 32.3, 20.0 and 10.1 mg/L when the initial concentration for toluene was 50, 100, 250, 400 and 500 mg/L respectively. The duration of lag phase increased from 6 hours to 20 hours on increasing the initial concentration of o-xylene from 50 mg/L to 250 mg/L. The maximum biomass concentration increased on increasing the initial concentration from 50 mg/L to 150 mg/L. On further increment in initial concentration to 250 mg/L, the maximum biomass concentration decreased. A possible explanation that could be given for this behavior is due to self inhibition by substrate (Li et al., 2010).

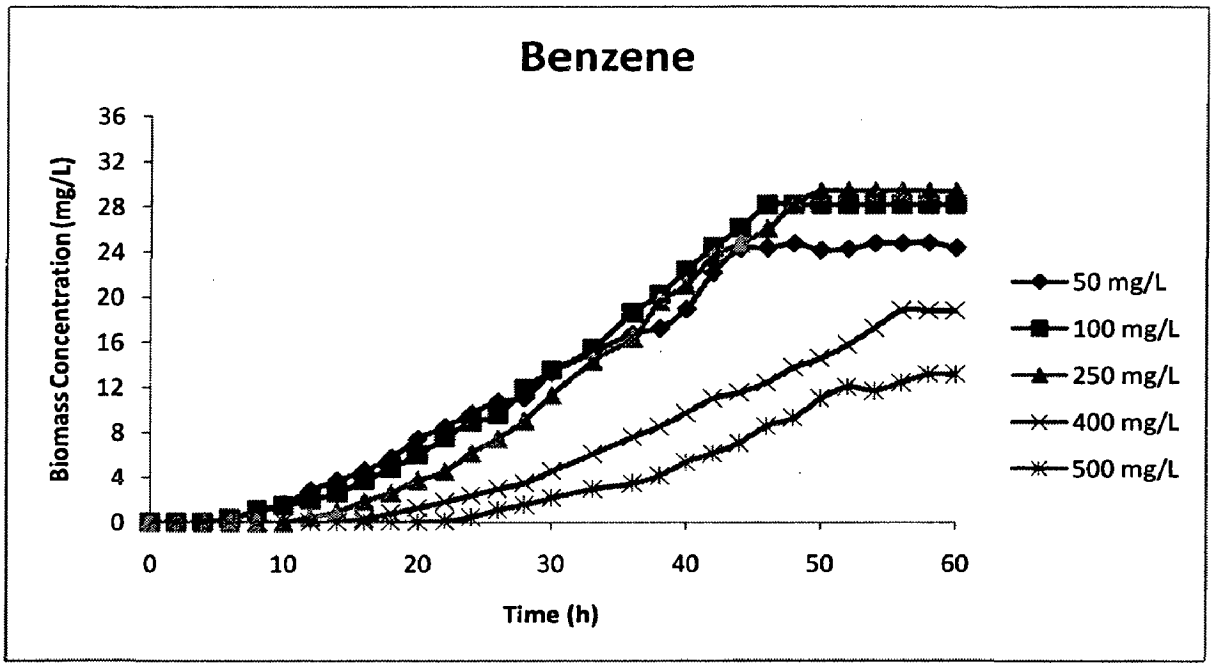



**Figure 5. 13: Effect of initial concentration of Benzene on biomass concentration (pH = 7 , Temperature =30°C, agitation rate = 150 rpm)**

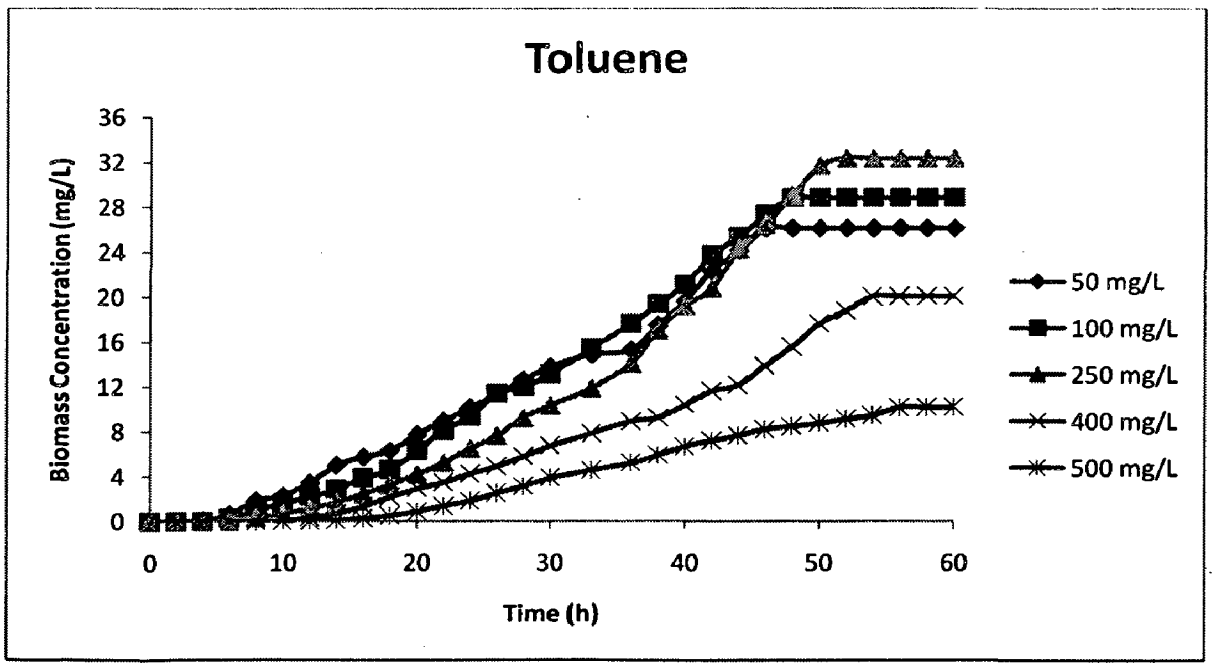



**Figure 5. 14: Effect of initial concentration of Toluene on biomass concentration (pH = 7 , Temperature =30°C, agitation rate = 150 rpm)**

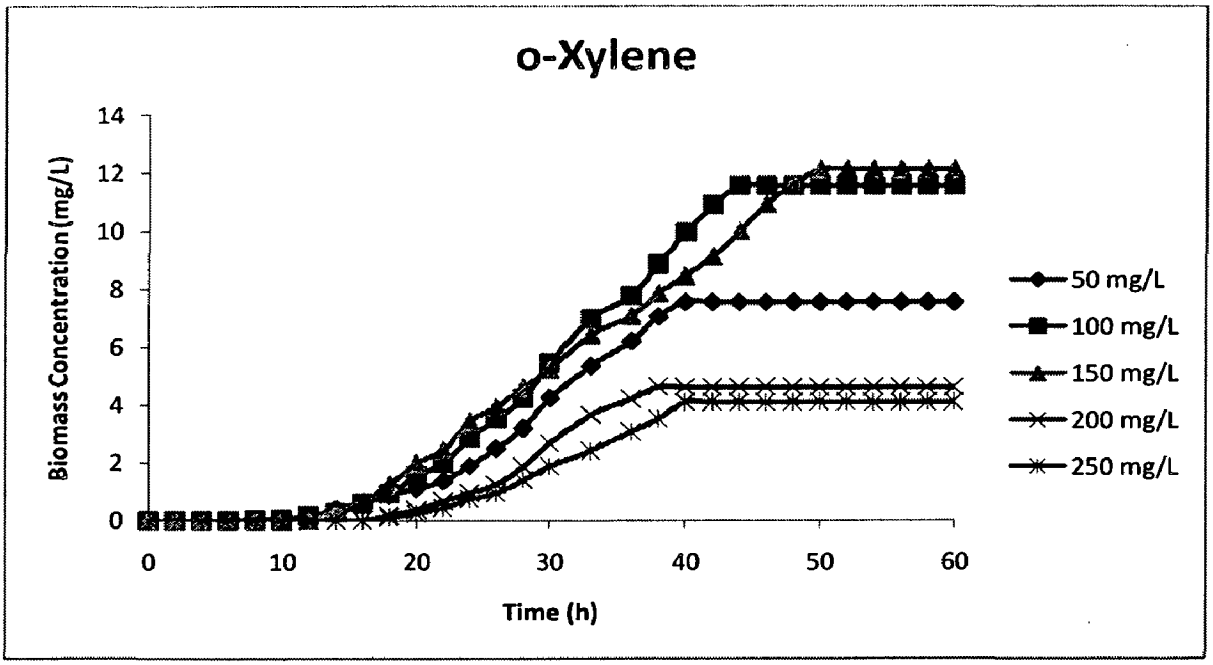



**Figure 5. 15: Effect of initial concentration of o-Xylene on biomass concentration (pH = 7 , Temperature =30°C, agitation rate = 150 rpm)**

### 5.3.Microbial growth kinetics

The specific growth rate in exponential phase was calculated using the following equation (Abuhamed et al., 2004).

$$\mu = \frac{\ln(X_2 / X_1)}{(t_2 - t_1)} \qquad (1)$$

Here $X_1$ and $X_2$ represents the biomass concentration at the start and end of the log phase respectively. $t_1$ and $t_2$ represents time of start and end of log phase respectively. The experimental maximum value of specific growth rate was observed at initial concentration of 50 mg/L of substrate in each case i.e 0.261, 0.246 and 0.255 for BTX respectively. The specific growth rate was observed to decrease with an increase in initial concentration in each case. Amir et al. (2009) also observed the decrease in specific growth rate of consortium of bacterial culture used for biodegradation of o-xylene on increasing initial concentration of o-xylene. The Haldane-Andrews model was used to describe the specific growth rate for a single substrate as given by following equation (Kim et al., 2005):

$$\mu = \mu_{max} \frac{S}{K_s + S + \frac{S^2}{K_i}} \qquad (2)$$

where $\mu_{max}$ represents maximum specific cell growth rate (1/h), S is the substrate concentration (mg/L), $K_S$ is the saturation constant (mg/L) and $K_i$ is the self inhibition constant. The Andrew-Haldane model was fitted to the experimental data and was found to have high correlation (Figure 4.5).

From the graph it has been observed that maximum specific growth rate occurred at initial concentration of 90, 120 and 20 mg/L for BTX respectively. Table 5.2 compares the kinetic parameters obtained in this work with other works on biodegradation of BTX. *A. chroococcum* was found to have higher maximum specific growth rate than *P. Putida* and *P. aeruginosa*. A higher $K_S$ value (38 mg/L) was found in case of toluene indicating that *A. chroococcum* can grow better at higher concentrations of toluene than benzene and o-xylene (Kim et al., 2005). Trigueros et al. (2010) also found that maximum specific growth for toluene was highest and lowest for o-xylene which is in accordance with the results obtained from this study.

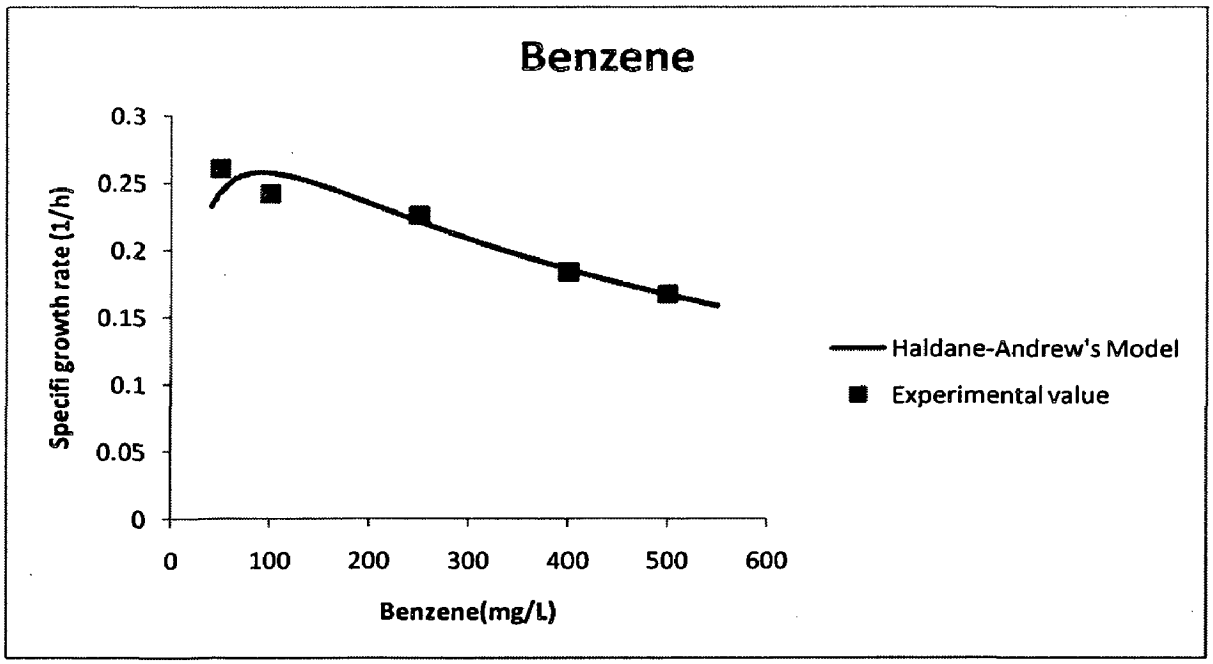



**Figure 5. 16: Variation of specific growth rate with initial concentration of benzene(pH = 7 , Temperature =30°C, agitation rate = 150 rpm)**

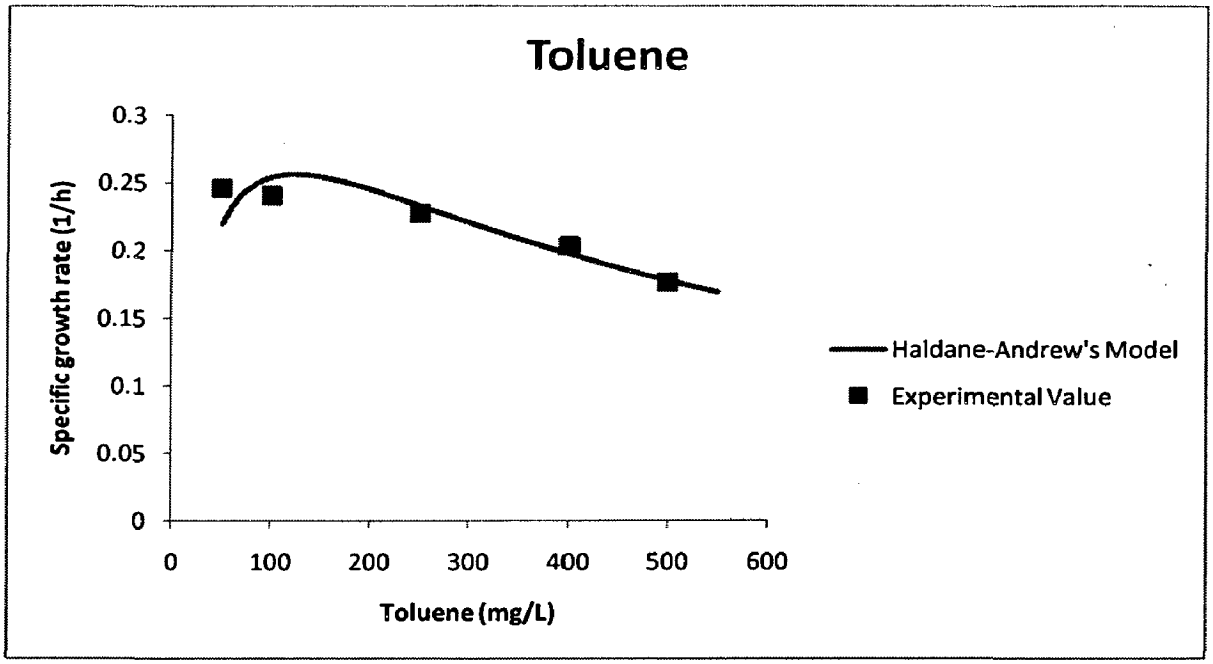



**Figure 5. 17: Variation of specific growth rate with initial concentration of toluene (pH = 7 , Temperature =30°C, agitation rate = 150 rpm)**

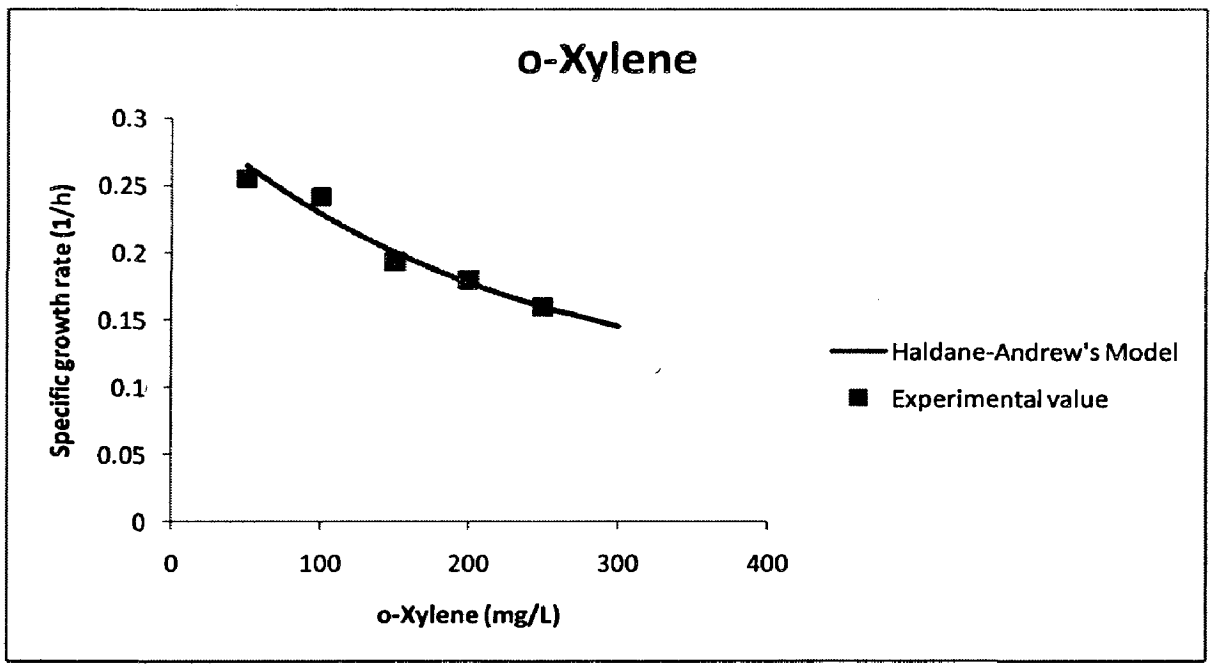



**Figure 5. 18: Variation of specific growth rate with initial concentration of o-Xylene (pH = 7 , Temperature =30°C, agitation rate = 150 rpm)**

. The high value of $K_i$ indicates that the inhibition effect can be observed only in a high concentration range (Abuhamed et al., 2004). A higher $K_i$ value also indicates that the culture is less sensitive to substrate inhibition.

**Table 5. 3 : Comparison for kinetic parameters of Haldane- Andrews' model**

| | | Benzene | | | Toluene | | | o-Xylene | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $\mu_{max}$ ($h^{-1}$) | $K_s$ (mg/L) | $K_i$ (mg/L) | $\mu_{max}$ ($h^{-1}$) | $K_s$ (mg/L) | $K_i$ (mg/L) | $\mu_{max}$ ($h^{-1}$) | $K_s$ (mg/L) | $K_i$ (mg/L) |
| *A. chroococcum* | This work | 0.368 | 19.482 | 427.687 | 0.41457 | 38.015 | 398.430 | 0.328 | 1.505 | 238.63 |
| *P. putida F1* | Abuhamed et al. (2003) | 0.62 | 1.65 | 180 | 0.61 | 6.47 | 88 | - | - | - |
| *P.putida* | Readron et al. (2000) | 0.73 | 0.12 | - | 0.86 | 13.8 | - | - | - | - |
| *P. aeruginosa* | Lin et al. (2007) | 0.194 | 8.349 | 191.89 | 0.0064 | 9.851 | 48.48 | 0.006 | 1.427 | 153.55 |
| Bacterial culture | Kelly et al. (1996) | 0.049 | 0.004 | - | 0.046 | 0.003 | - | 0.383 | 0.125 | - |
| *P.putida F1* | Trigueros et al. (2010) | 0.22 | 0.92 | 150 | 0.46 | 45 | - | 0.19 | 2.55 | 5 |

## 5.4.Simultaneous removal of BTX in batch reactor

### 5.4.1. Effect of pH

The pH value is a key factor in microbial metabolic processes. It influences the redox potential and enzymatic activity (Jianlong et al., 1997). The pH of the medium was adjusted by 1M NaOH and 1M HCl. A control was also run without microbial cells to measure the loss of different components due to any volatilization. The effect of pH on percentage removal of 20 mg/L each of benzene, toluene and o-xylene for pH ranging from 1-12 after allowing the biodegradation for 2 days was studied. Figure 5.19 shows the effect of pH on percentage removal of combined BTX by bacterial cells due to biodegradation. A similar trend was observed in the combined BTX biodegradation as in individual biodegradation. Almost negligible removal was observed at pH less

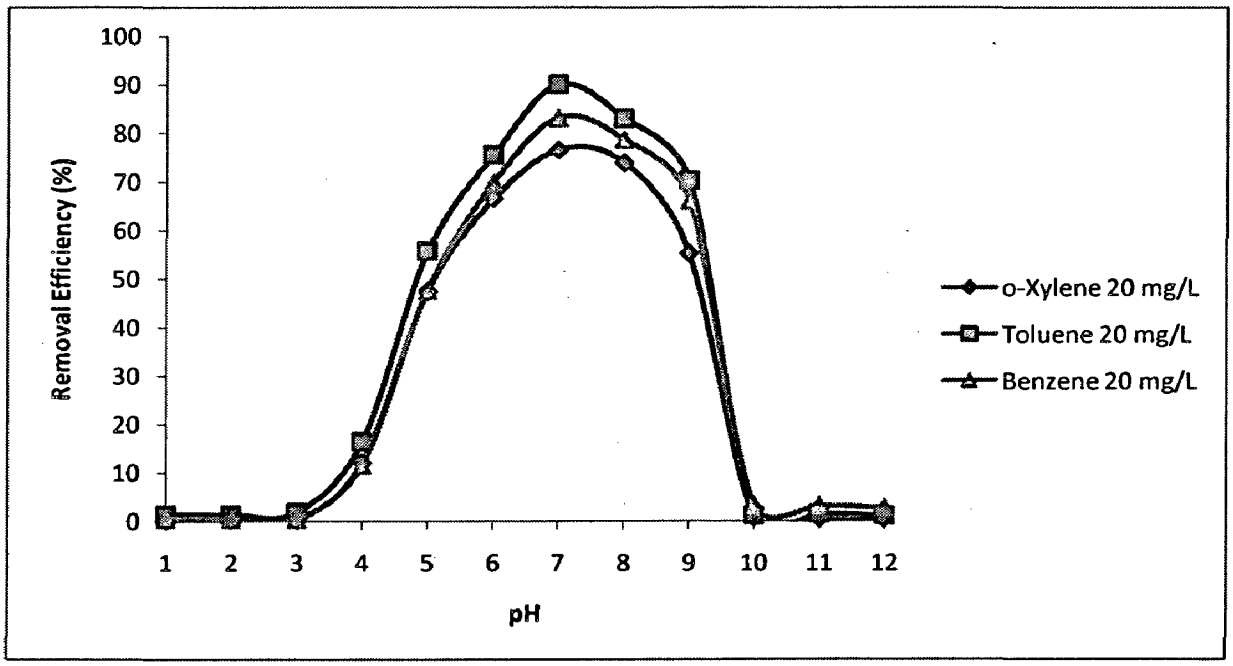



**Figure 5. 19 : Effect of pH on the percentage removal efficiency of simultaneous removal of 20 mg/L each of BTX (Temperature =30°C, agitation rate = 150 rpm)**

than 4 and greater than 9. This may be due to the effect of pH on the ionization and therefore binding and interaction of a myriad of molecular processes which in turn affect the metabolic pathway. It could even cause denaturing of proteins which might result in lethal toxicity (Chakraborty et al., 2010). The removal of benzene, toluene and o-xylene was inhibited by the presence of other substrate. The maximum removal was observed when pH was 7 in all cases. The

maximum removal of benzene, toluene and o-xylene was found to be 85.3%, 93.4% and 72.9 % respectively.

### 5.4.2. Effect of temperature

Simultaneous biodegradation of BTX by immobilized cells was carried out at temperatures ranging from 15 °C to 45 °C for an initial concentration of 20 mg/L each of BTX at pH=7. The results are shown in figure 5.20. The results follow the same trend as observed in individual biodegradation of BTX. The results reveal that removal efficiency increases on increasing the temperature from 15°C to 30°C.

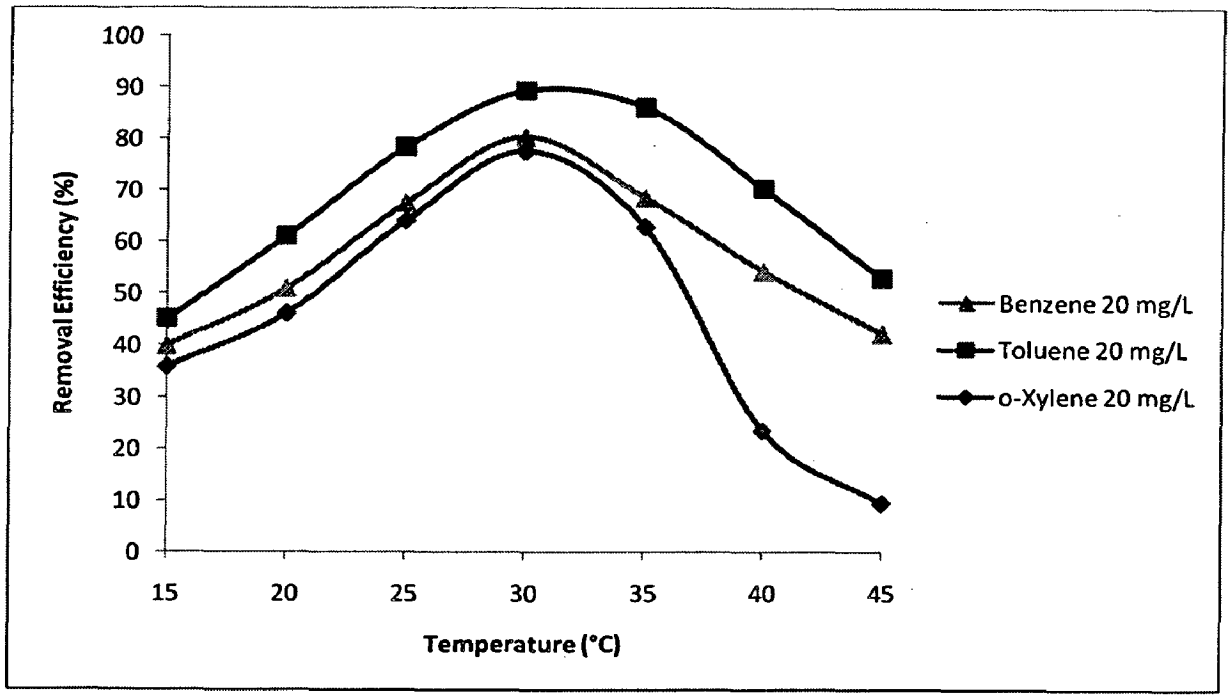



**Figure 5. 20: Effect of temperature on the percentage removal efficiency of simultaneous removal from 20 mg/L each of BTX (pH=7, agitation rate = 150 rpm)**

The results also seem to follow an exponential trend in this range. On further increasing temperature, the removal efficiency decreases. *A. chroococcum* was able to degrade 80.4%, 89.3% and 77.6% of BTX respectively at 30°C. Therefore *A. chroococcum* can grow from 15°C to 45 °C but maximum removal of BTX occurs at temperature of 30°C. Gurusamy et al. (2007) also found maximum degradation rate for phenol at 30 °C using *Pseudomonas pictorum.* Reda and Ashraf (2010) also reported 30 °C as the optimum temperature for toluene biodegradation by both *B.subtilis*-EPRIS12 and *B.laterosporous* – EPRIS41.

## 5.5.Biotrickling Filtration

### 5.5.1. Startup of bioreactor

The biotrickling filter was sterilized by passing steam from autoclave at 15 psi for 30 min to avoid any type of contaminations. Sterilized corn cob was kept in a one day old culture for 24 hours. This sterilized corn cob was used as packing for biotrickling filter. After addition of sterilized corn cob, nutrient media was circulated to top of the biotrickling filter at rate of 14 mL $min^{-1}$. 500 mL of recycled nutrient media was replaced with fresh nutrient media once a day. Experiments were conducted at 30°C. The pH of the medium was monitored and maintained at 7.0 after the addition of fresh nutrient media.

The performance evaluation of biotrickling filter under different inlet loading rate (LR) and empty bed residence time (EBRT) was studied for 128 days. The flow rate of air and inlet concentration was varied to provide different EBRT and LR. The operating conditions of biotrickling filter are provided in Table 5.3.

**Table 5. 4: Operating conditions of biotrickling filter**

| Phase | Operating Days | Flow Rate (L/min) | Average Pollutant Concentration (g $m^{-3}$) | | | Average Loading Rate (g $m^{-3}$ $h^{-1}$) | EBRT (min) |
|---|---|---|---|---|---|---|---|
| | | | Benzene | Toluene | o-Xylene | | |
| 1 | 24 | 1 | 0.262 | 0.254 | 0.255 | 24.034 | 1.925 |
| 2 | 10 | 2 | 0.510 | 0.507 | 0.509 | 95.164 | 0.963 |
| 3 | 18 | 3 | 0.792 | 0.782 | 0.790 | 220.951 | 0.642 |
| 4 | 29 | 4 | 1.141 | 1.148 | 1.139 | 427.381 | 0.481 |
| 5 | 47 | 5 | 1.315 | 1.313 | 1.312 | 614.152 | 0.385 |

Each phase was operated till a pseudo-steady state was reached i.e, when the removal efficiency was within ±2% for 3 days. The performance evaluation of the biotrickling filter was calculated using the following equations (Mathur et al., 2007).

Inlet Loading Rate ($gm^{-3}h^{-1}$), $LR = \frac{QC_i}{V} \times 60$

Elimination Capacity ($gm^{-3}h^{-1}$), $EC = \frac{Q(C_i - C_0)}{V} \times 60$

Removal Efficiency, $RE(\%) = \frac{(C_i - C_0)}{C_i} \times 100$

where Q is air flow rate (L $min^{-1}$), V is volume of bed (L), $C_i$ and $C_o$ are inlet and outlet gas concentrations (g $m^{-3}$).

### 5.5.2. Removal Efficiency

Figure 5.21, 5.22, 5.23 and 5.24 shows the removal efficiency of benzene, toluene, o-xylene and total VOC respectively as function of day. In each phase, it was found that removal efficiency first increased and reached stability and after that decreased rapidly on increasing loading rate. Phase 1 was operated at an average VOC Loading rate of 24 g $m^{-3}$ $h^{-1}$. The flow rate of air was maintained at 1 L $min^{-1}$ so that an EBRT of 1.925 min could be applied to biotrickling filter. Only 44, 49 and 34% removal was obtained on its first day of operation. It took 17, 15 and 18 days to reach the removal efficiency of greater than 90% for benzene, toluene and o-xylene respectively. It took further 7, 9, 6 days to achieve pseudo steady state. The maximum removal efficiency observed for benzene, toluene and o-xylene was more than 99% in every case in Phase 1. An acclimation period of one month has been reported for a bench scale biofilter treating benzene, toluene, ethyl benzene and o-xylene (BTEX) polluted air (Mohammad et al., 2007). A period of 30 days was required for a biofilter exposed to BTEX mixtures in air stream (Mathur et al., 2007). The results are in consistency with the reported acclimation periods from several weeks to several months (Juneson et al., 2001). Acclimation period can be reduced to as low as 12 days by inoculating the biotrickling filter media with the adapted microbial aggregates (Shareefdeen and Baltzic, 1994; Acuna et al., 1999, Jorio et al., 2003).

On day 25, the air flow rate was increased to 2 L min-$^1$ so that an EBRT of 58 s could be applied to the system. The average inlet concentration of benzene, toluene and o-xylene in Phase 2 was 0.510, 0.50 and 0.50 $gm^{-3}$ respectively. The inlet loading rate was quadrupled to 95.164 g $m^{-3}$ $h^{-1}$. Due to sudden increase in loading rate, the removal efficiency decreased to 79.7 %, 83.6% and 68% from 99.7%, 99.8% and 99.5% for benzene, toluene and o-xylene respectively. It took 10 days to recover from this shock loading. The maximum removal observed in this phase which lasted for 10 days was 99.6%, 99.7% and 98.9% for benzene, toluene and o-xylene respectively.

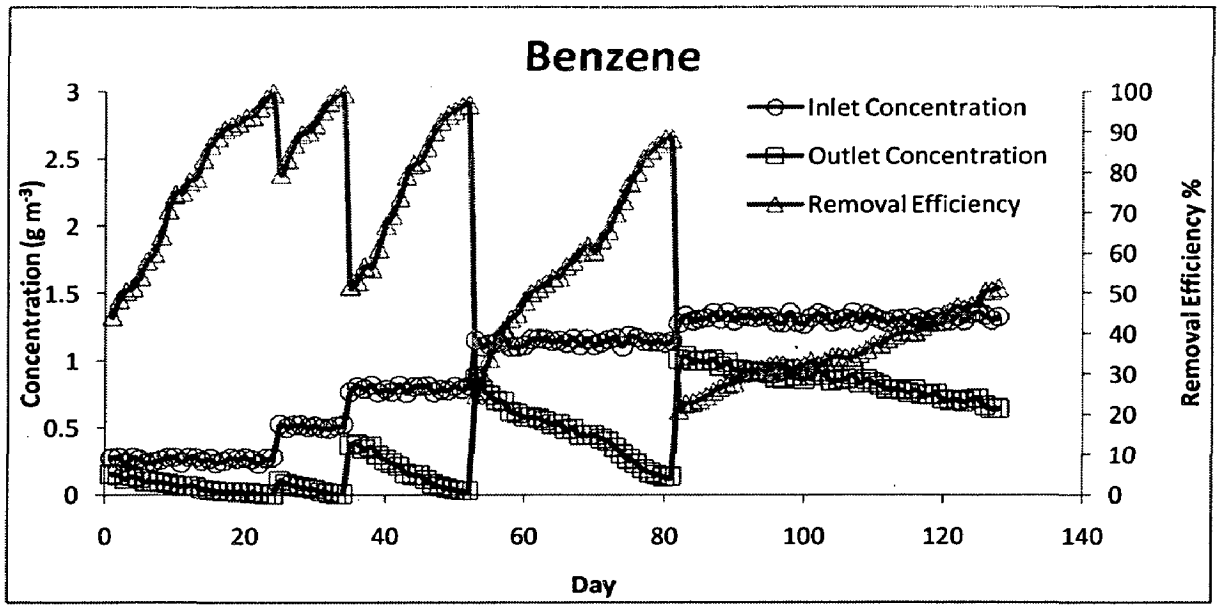



**Figure 5. 21: Profile of benzene removal under different loading conditions in biotrickling filter**

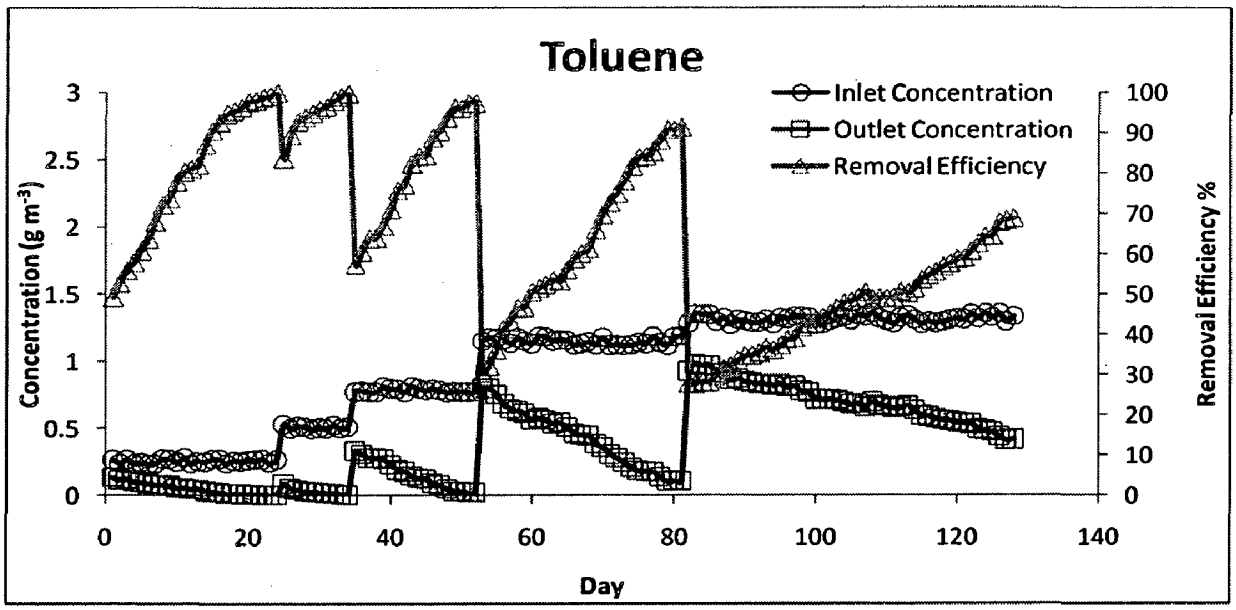



**Figure 5. 22 : Profile of toluene removal under different loading conditions in biotrickling filter**

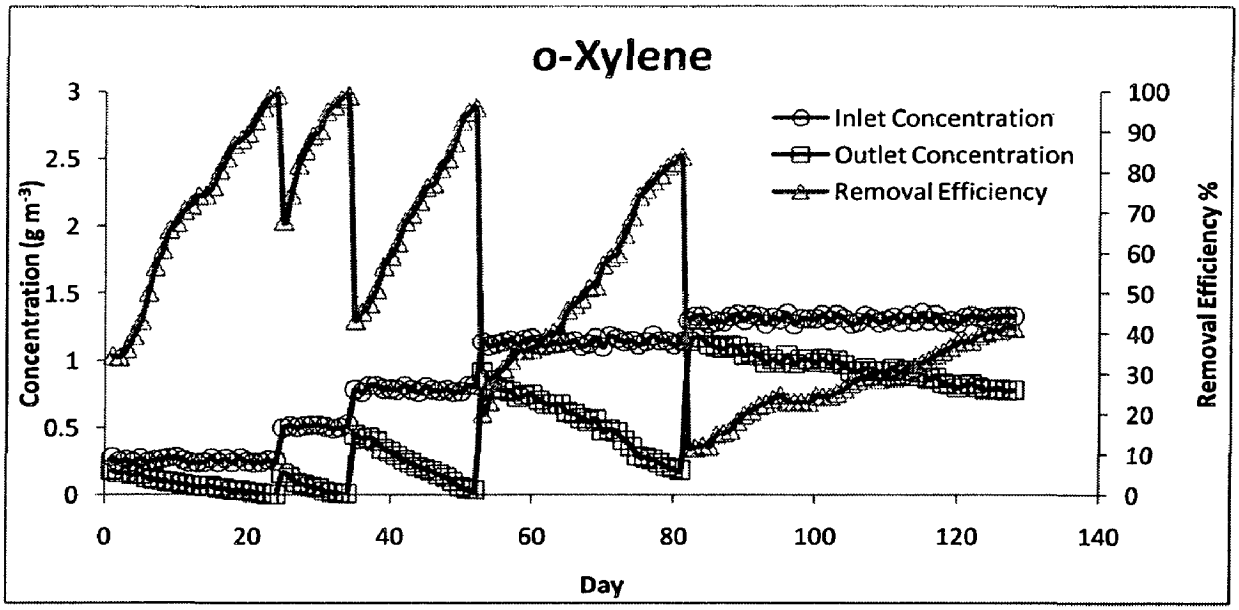



**Figure 5. 23 : Profile of o-xylene removal under different loading conditions in biotrickling filter**

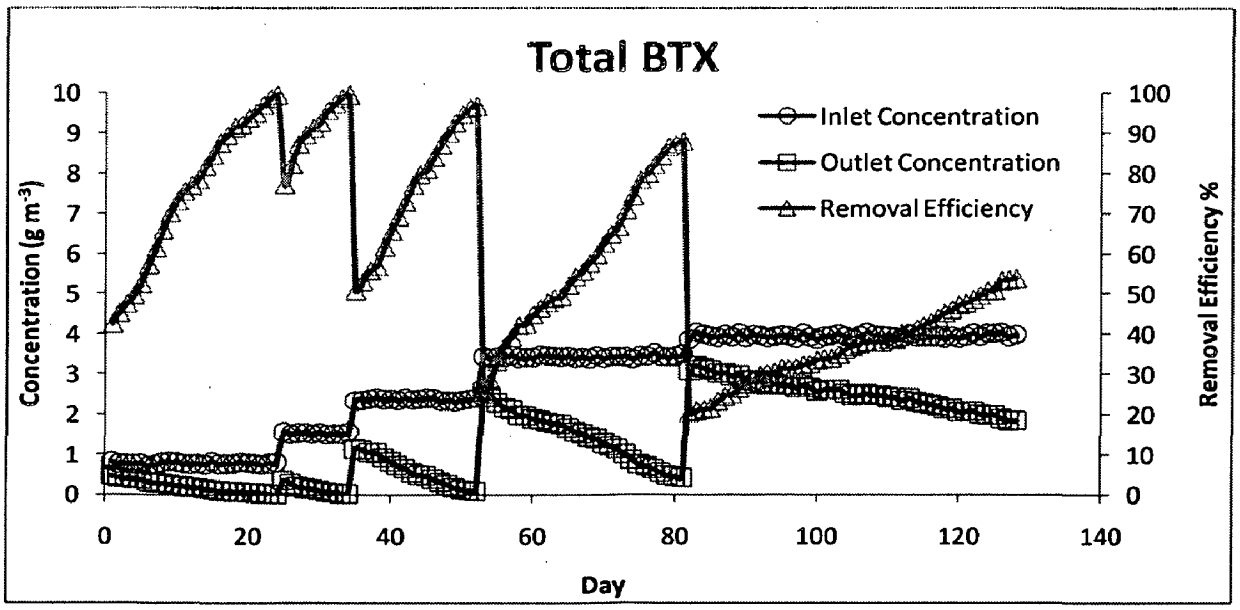



**Figure 5. 24: Profile of total VOC removal under different loading conditions in biotrickling filter**

For Phase 3, the air flow rate was increased to 3 L $min^{-1}$ so that and EBRT of 39 s could be applied to the system. The average inlet concentration of benzene, toluene and o-xylene in this phase was 0.79, 0.78 and 0.79 $gm^{-3}$ respectively. The inlet loading rate was increased more than two times to 220 g $m^{-3}$ $h^{-1}$ . The removal efficiency decreased approximately to 52%, 57% and 53 on Day 35 due to this shock loading. To recover from this sudden increase in inlet loading rate, it took 18 days to reach pseudo-steady state where the maximum removal was 96.8%, 97.4% and 96% for benzene, toluene and o-xylene respectively. In this case too, toluene had highest removal efficiency followed by benzene and o-xylene.

To apply an EBRT of 29 s, the air flow rate was increased to 4 L $min^{-1}$ at the start of Phase 4 on Day 53. The inlet BTX loading rate was doubled to 427 g $m^{-3}$ $h^{-1}$ due to increase in average inlet concentration of benzene, toluene and o-xylene to 1.14, 1.15 and 1.14 $gm^{-3}$. This increase in loading rate caused the removal efficiency of benzene, toluene and o-xylene to decrease to 25%, 30% and 20% respectively. The removal efficiency of benzene, toluene and o-xylene gradually increased to 88%, 91% and 83 % respectively after 29 days.

The air flow rate was increased to 5 L $min^{-1}$ so that an EBRT of 23 s could be applied to bioreactor in Phase 5. The inlet concentration of benzene, toluene and o-xylene was increased to 1.31 g $m^{-3}$ which increased the inlet loading rate to 614 $gm^{-3}h^{-1}$. Due to sudden increase in inlet loading rate, the removal efficiency of benzene, toluene and o-xylene decreased to 21, 28 and 12% respectively. The removal efficiency took 47 days to reach pseudo-steady state.

### 5.5.3. Elimination Capacity

Elimination capacity is an important parameter for the evaluation of biotrickling filter performance as it provides capacity of biotrickling filter to remove pollutants. It is defined as the amount of pollutants degraded per unit of reactor volume and time for the various loading rates. It provides a direct comparison between different biotrickling filter systems. Figure 5.25, 5.26, 5.27 and 5.28 shows the changes in elimination capacity with respect to inlet loading rate for benzene, toluene, o-xylene and total BTX respectively. The experimental points are plotted with the help of symbol whereas the continuous line shows the theoretical 100% removal efficiency line.

It was observed that in all cases almost 100% removal could be obtained till Phase 4 i.e when the inlet loading rate was less than 150 $gm^{-3}h^{-1}$ for each pollutant. On further increment in loading rate, no marginal increase in elimination capacity was observed.

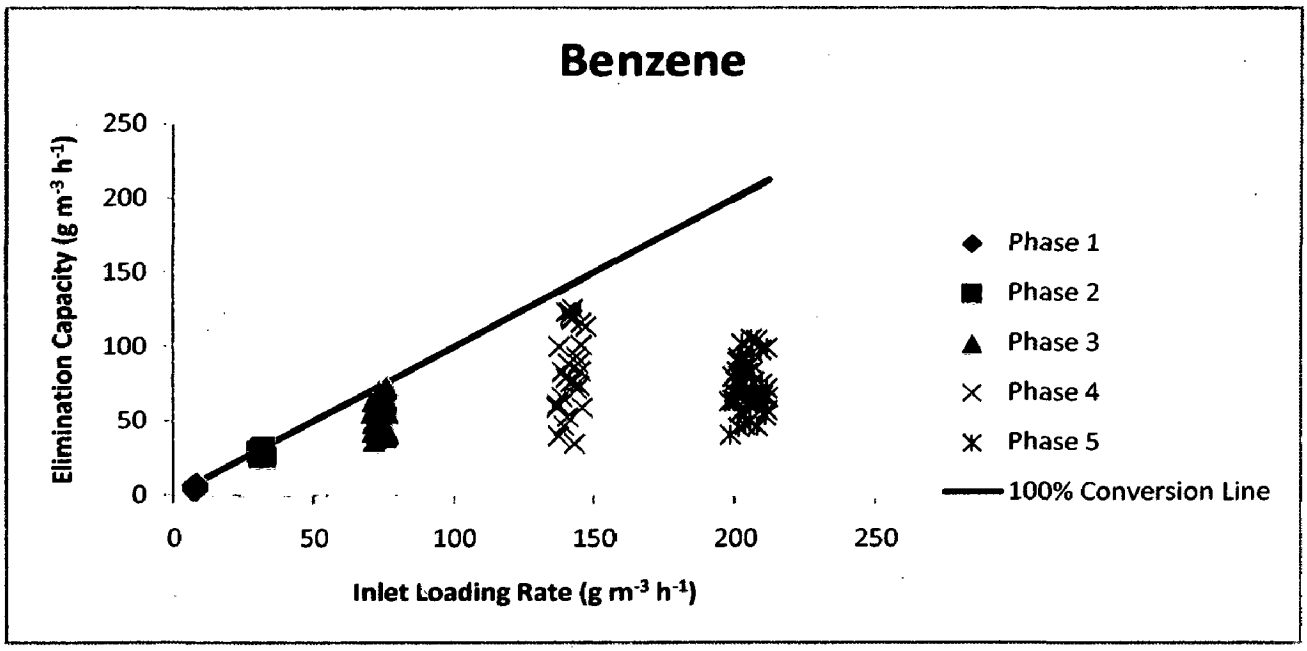



**Figure 5. 25: Elimination Capacity of benzene for different inlet loading rate**

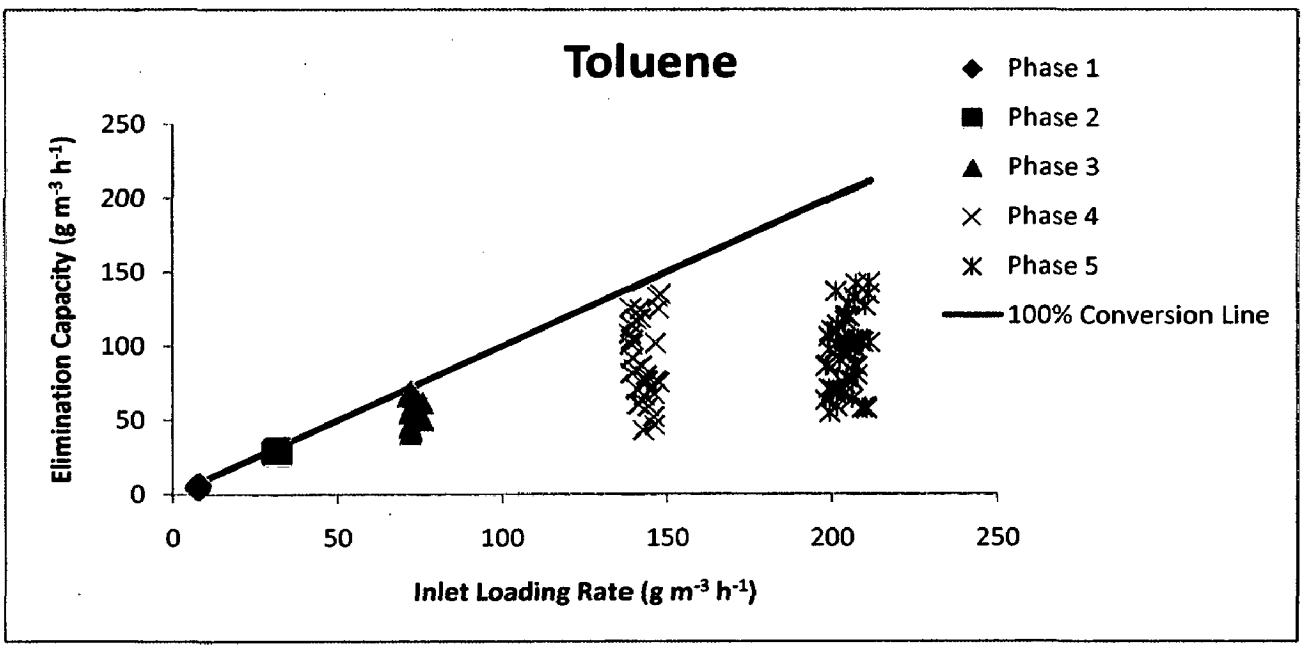



**Figure 5. 26: Elimination Capacity of toluene for different inlet loading rate**

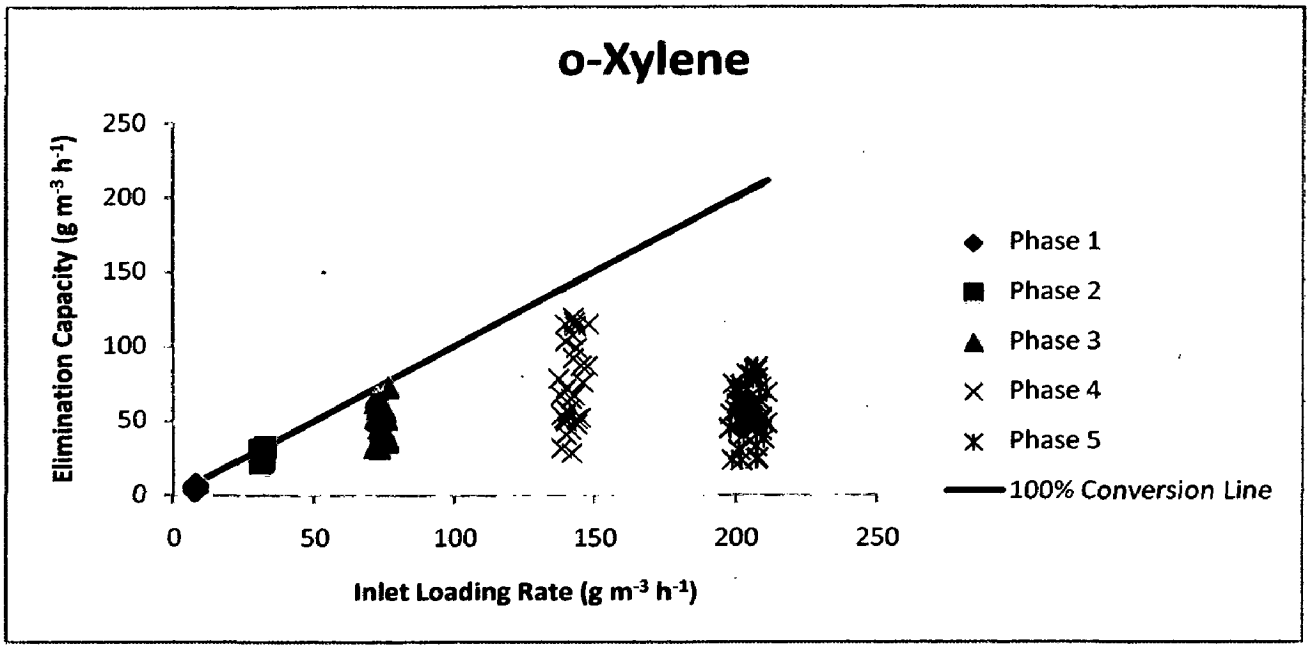



**Figure 5. 27: Elimination Capacity of o-Xylene for different inlet loading rate**

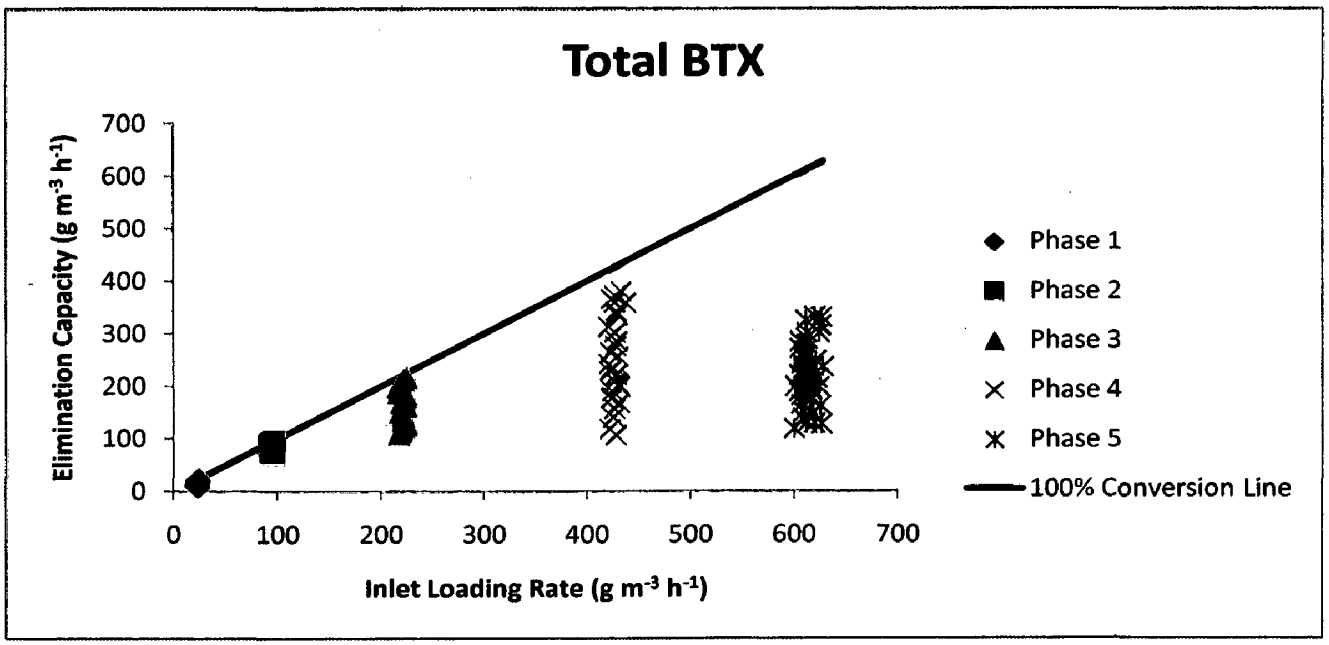



**Figure 5. 28: Elimination Capacity of total btx for different inlet loading rate**

The maximum elimination capacity observed was 126.26, 143.22, 119.59 g $m^{-3}h^{-1}$ for benzene, toluene and o-xylene respectively when their loading rate was 142.49, 211.355 and 142.63 g $m^{-3}h^{-1}$respectively. The overall maximum elimination capacity was 381.19 g $m^{-3}h^{-1}$ at

loading rate of 433.61 g $m^{-3}h^{-1}$.The reduction in elimination capacity on increasing the loading rate from Phase 4 to Phase 5 incases of benzene and o-xylene can be due to poor removal of benzene and o-xylene in Phase 5. Jeong et al. (2009) found maximum elimination capacity to be 220 g $m^{-3}h^{-1}$ for biofilter packed with biosol containing *Pseudomonas* sp. NBM21 and *Rhodococcus* sp. BTO61 used for treatment of ethyl benzene and xylene isomers. Kwon and Cho (2009) found maximum elimination capacity for biofilter treating BTEX to be 86 and 67 g $m^{-3}h^{-1}$ incase of cork and granular activated carbon packing respectively. Moussavi and Mohseni (2008) found the maximum elimination capacity to be 642 g $m^{-3}h^{-1}$ for phenol biodegradation in biotrickling filter packed with polyurethane cubes. Garci-Pena et al. (2008) found maximum elimination capacity to be 110 g $m^{-3}h^{-1}$ for biofilter containing *Paecilomyces variotii* immobilized on vermiculite for treatment of BTEX laden air.

### 5.5.4. Carbon Dioxide Production

Microbes aerobically degraded organic pollutants into water and carbon dioxide and use it as an essential carbon source for the microbial growth. Therefore the measurement of production of carbon dioxide provides valuable information about the degree of pollutants degradability. Figure 5.29 shows the carbon dioxide production rate versus loading rate and elimination capacity.

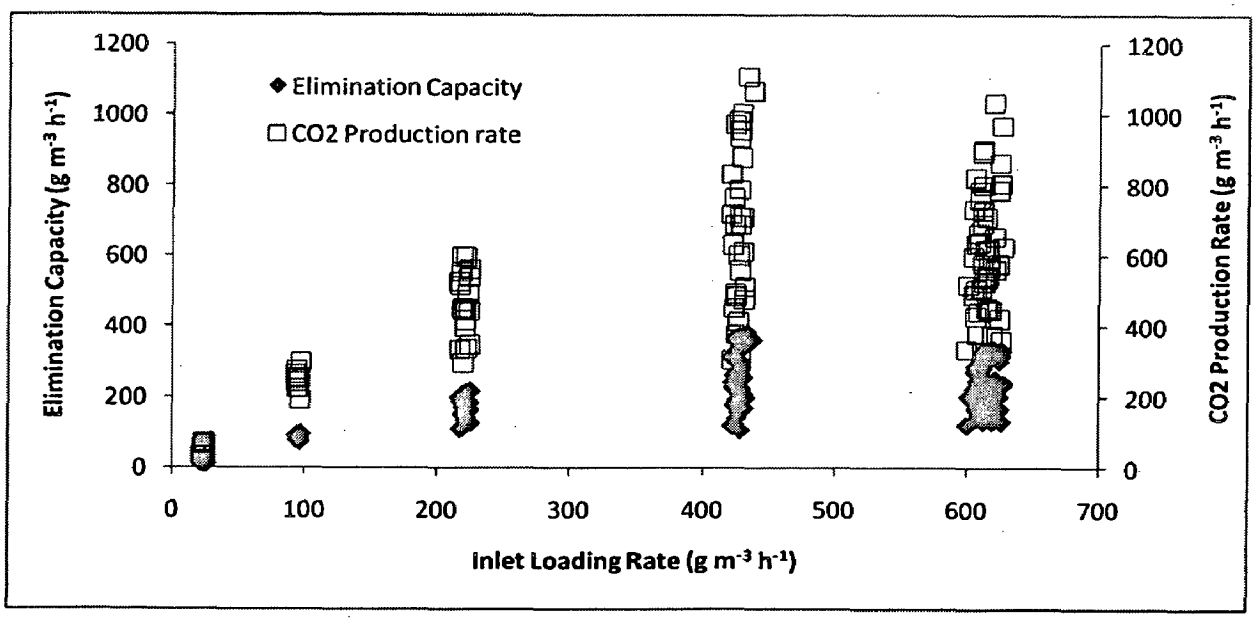



**Figure 5. 29: Carbon dioxide production rate versus loading rate and elimination capacity**

There was increase in production of carbon dioxide with increase in elimination capacity and loading rate. At high inlet loading rate, there was more elimination of pollutants which meant that more carbon dioxide needs to be produced for greater microbial activity. The following equations represent the microbial degradation of benzene, toluene and o-xylene into carbon dioxide and water.

$$C_6H_6 + 7.5O_2 \rightarrow 6CO_2 + 3H_2O$$

$$C_7H_8 + 9O_2 \rightarrow 7CO_2 + 4H_2O$$

$$C_8H_{10} + 10.5O_2 \rightarrow 8CO_2 + 5H_2O$$

Theoretically, 3.384, 3.347 and 3.320 mass of carbon dioxide will be produced for each mass of benzene, toluene and xylene consumed respectively. A plot of EC v/s $CO_2$ production rate is shown in Figure 5.30. From the figure 5.30, mean experimental data lies around the liney=2.754 x indicating a ration of 2.754 between carbon dioxide production rate and elimination capacity.

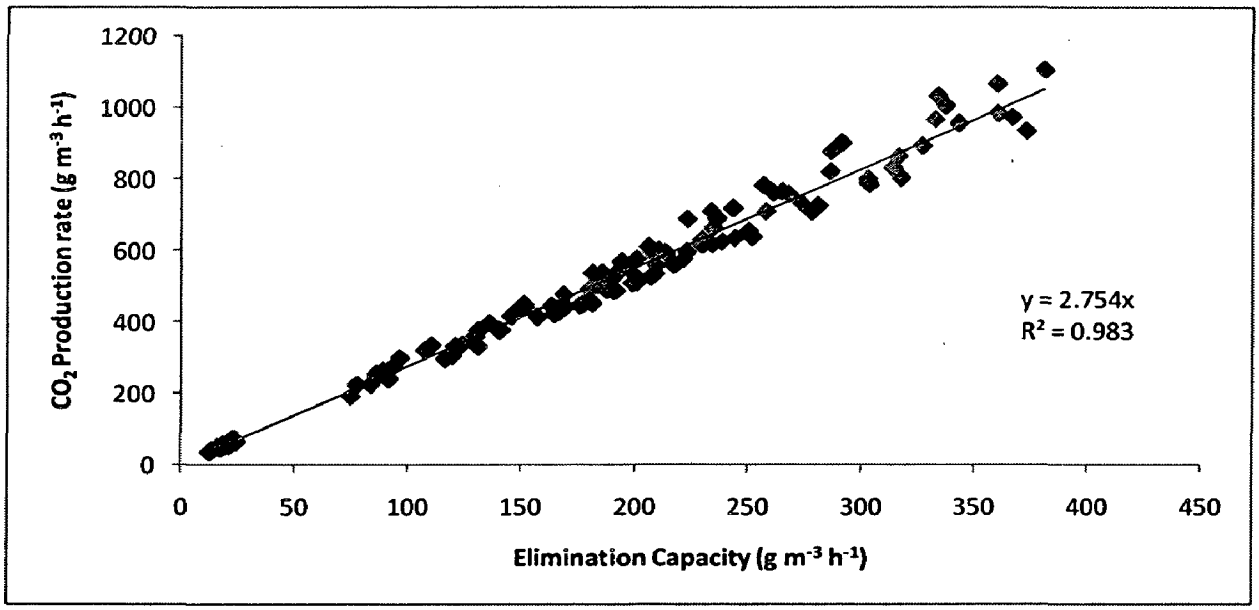



**Figure 5. 30: $CO_2$ production rate as a function of elimination capacity**

Incase of complete oxidation this ratio would have been around 3.3 indicating that a fraction of consumed organic carbon from BTX is used for microbial growth according to following metabolism

BTX+ Oxygen → Carbon Dioxide + Water + Cellular material

The observed deficit in production of carbon dioxide is due to two reasons. First reason is a fraction of carbon is used for microbial growth while the second reason is the accumulation of carbon dioxide in biofilm as $HCO_3^-$, $H_2CO_3^-$, $CO_3^{2-}$, causing a deficit of carbon dioxide in the gas phase.

### 5.5.5. Pressure Drop

Pressure drop across the bed is an important parameter in biotrickling filtration as it determines the amount of energy needed by the compressor or blower to force the VOC contaminated gas stream through the bed. Increment in energy requirement increases the capital costs and therefore pressure drop across the bed should not be too high (Abumaizar et al., 1998). The pressure drop was measured at the end of a phase. The pressure drop has been plotted against EBRT and phase no. in Figure 5.31. There was significant increase in pressure drop when EBRT was decreased. This was due to the fact that pressure has liner relationship with superficial gas flow rate.

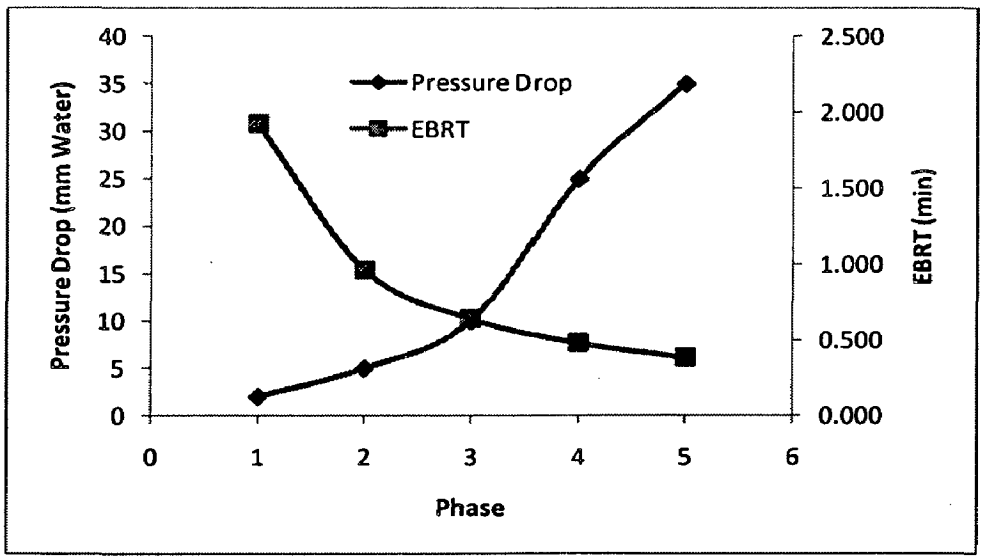



**Figure 5. 31: Pressure drop across bed at the end of a phase**

# CHAPTER 6

# CONCLUSION AND RECOMMENDATIONS

## 6.1.Conclusions

Biological treatment is an attractive alternative for low concentration gas streams because of its low energy consumption, relatively moderate operating costs and minimal by-products generation. Studies were conducted for biodegradation of benzene, toluene and o-xylene in batch reactor and biotrickling filter by use of *Azotobacter chroococcum*. Following conclusion were obtained from the above studies:

- FTIR spectra, SEM and BET surface area analysis confirmed the biodegradation of BTX by *Azotobacter chroococcum* immobilized on cob.
- Optimum pH and temperature was found to be 7 and 30 °C in all cases of degradation by *Azotobacter chroococcum* immobilized on corn cob.
- Almost 100% biodegradation of benzene was observed in 54, 66, 84 hours when the initial concentration of benzene was 50, 100, 250 mg/L respectively When the initial concentration of toluene was 50, 100 and 250 mg/L, complete biodegradation was observed in 54, 60 and 78 hours. *A. chroococcum* was able to degrade around 100% of o-xylene at an initial concentration of 50, 100, 150 g/L in 60, 72 and 84 hours respectively
- *A. chroococcum* was found to have higher maximum specific growth rate than *P. Putida* and *P. aeruginosa*.
- The biotrickling filter was found to degrade toluene better than benzene and o-xylene. Around 99% removal efficiency was achieved at an EBRT of 58 s and inlet concentration at 1.56 g $m^{-3}$.
- The maximum elimination capacity observed was 126.26, 143.22, 119.59 g $m^{-3}h^{-1}$ for benzene, toluene and o-xylene respectively when their loading rate was 142.49, 211.355 and 142.63 g $m^{-3}$ $h^{-1}$respectively.
- A ratio of 2.754 between carbon dioxide production rate and elimination capacity was observed.
- Maximum pressure drop of 35 mm was observed at an EBRT of 0.5 min.

## 6.2.Recommendations

On the basis of present studies, the following recommendations are made:-

- Biofiltration /biotrickling filtration study should be done on real industrial waste gases.
- Studies should be done to find new VOC degrading microorganisms.
- Studies should be done to find the affect of micro nutrients for treatment of mixture of VOCs.
- Kinetic modeling of the bioreactor should be investigated.

# CHAPTER 7

# PUBLICATIONS

- "Biodegradation of o-xylene by *Azotobacter chroococcum*" *International Journal of Advanced Biotechnology and Research* (2012) 3:502-508.
- "Biofiltration of Volatile Organic Compounds (VOCs) – An Overview", *Research Journal of Chemical Sciences* (2011) 1:83-92.
- "Biodegradation of BTX contaminated air by *Azotobacter chroococcum* in biotrickling filter" *Bioresource Technology* **(Under Review)**
- "Determination of mass transfer coefficients for mixture of compost, sugarcane bagasse and granular activated carbon as packing media in biofilter", *Bioremediation Journal* **(Second Revision Submitted)**

# CHAPTER 8

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