Control of Gaseous Volatile Organic Compounds Using a Sponge-medium Based Rotating Biological Contactor

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By

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CERTIFICATE

This is to certify that **Susant Kumar Padhi** (126104001) has been working under my supervision since July, 2012 as a Ph.D. student. His thesis entitled "**Control of Gaseous Volatile Organic Compounds Using a Sponge-medium Based Rotating Biological Contactor**" is an authentic record of the results obtained from the research work carried out under my supervision in the Civil Engineering Department, Indian Institute of Technology Guwahati, Assam, India. I certify that he has fulfilled all the requirements according to the rules of this institute regarding the investigations embodied in his thesis and this work has not been submitted elsewhere for a degree.

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DECLARATION

I declare that the work embodied in this thesis is the result of investigations carried out by me in the Department of Civil Engineering, Indian Institute of Technology Guwahati India. The sources referred in the creation of this work have been appropriately acknowledged by explicit references or footnotes. Other assistance received has been acknowledged. I have not knowingly copied or used the words or ideas of others without acknowledgement.

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Dedicated

to

my dear

Parents

&

my mentor

Prof. Sharad Gokhale

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by

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ABSTRACT

Volatile organic compounds (VOCs) containing toxic air pollutants mainly benzene, toluene, ethylbenzene and xylene (BTEX) are generated from paint, chemical, and petrochemical industries, and have a harmful impact on human health and the atmosphere. In real scenario, industrial waste gas contains a mixture of VOCs mostly BTEX, the simultaneous treatment of which is challenging. In this research, rotating biological contactor (RBC) has been designed and developed with sponge as a bio-support medium for the control of benzene and BTEX at different combinations. The performance of RBC at different inlet loading rates (ILR), and its effect on elimination capacity (EC) and removal efficiency (RE) have been studied for benzene. After, the treatment of xylene as a single pollutant, a combined toluene and xylene, and BTEX mixture in waste gas streams has been studied.

Prior to the operation of RBC a batch study was carried out to study the biodegradation of benzene and the kinetics involved in the process for modeling. Haldane model was found to be the best. The central composite design (CCD) was used to determine optimum pH and benzene concentration to enhance the benzene biodegradation. The results showed that at the optimum value of pH (7.05) and benzene concentration (332.82 mg/l), the maximum specific growth rate (μ) and degradation rate (r) obtained were found to be 0.05 1/h and 6.01 mg/l h, respectively. The metabolic intermediates produced during benzene biodegradation were determined, which justified the pathway of benzene biodegradation. The *Enterobacter cloacae* SG208 was found to be dominant in the mixed culture responsible for biodegradation of benzene. The optimal pH obtained in the batch study was later used in RBC for the efficient treatment of benzene using a mixed culture at various loading rates.

The results showed that removal at ILR below 8.171 ± 0.162 g/m^3 h was over 92%, and decreased with the further increase in ILR. At higher ILR of 69.375 ± 0.591 g/m^3 h, the maximum EC (EC_{max}) reached to about 45 g/m^3 h with the RE of over 59%, which is higher than the results reported in literatures. The EC of benzene increased with the increase in loading rate, but the RE showed an opposite trend. The production of CO_2, which determines the degree of pollutant degradability, also increased with the increase in EC. Along with benzene, the nutrients (NH_3-N and PO_4-P) from liquid phase also got removed in the RBC.

The screening of enriched cultures was done initially to enhance the performance of RBC for treating xylene, toluene and xylene, and BTEX at various loading rates. The removal efficiency of BTEX was maximum (82%) at the highest ILR of 50.16 ± 2.418 g/m^3 h, higher than toluene and xylene (79%), and xylene (72%). The presence of toxic compounds like xylene enhanced the removal of toluene when treated in mixture. In the BTEX, toluene was found to be highly biodegradable followed by ethylbenzene, benzene and xylene. The higher CO_2 concentration and the RE of VOCs confirmed the better performance of RBC at smaller flow rates. The maximum concentration of CO_2 (4.69 ± 0.25 g/m^3) produced was consistent with the highest overall RE, which was observed during the treatment of BTEX mixture. The RBC also removed nutrients from wastewater and found to have an inhibitory effect with increase in ILR of gaseous VOCs. The stability of RBC has also been investigated, which showed that the supply of nutrient media influences the performance of reactor more. Further, the predominant strain identified in the mixed culture was *Enterobacter cloacae* SP4001, responsible for biodegradation of BTEX. The results of RBC are promising, which might be a better alternative for treatment of gaseous VOCs showing its potential application in industries.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS

Symbol	Description
S	Substrate concentration (mg/l)
μ	The specific growth rate (1/h)
r	Degradation rate (mg/l h)
μ_{max}	The maximum specific growth rate (1/h)
K_s	Half saturation constant (mg/l) or (g/m^3)
K_i	Inhibition constant (mg/l)
D	Composite desirability
d	Individual desirability
k	Number of factors
n_0	Number of replicates
V	Volume of medium (m^3)
V_l	Volume of liquid medium (m^3)
V_d	Volume of sponge medium (m^3)
Q	Gas flow rate (m^3/h)
C	Concentration of VOCs (g/m^3)
C_i	Inlet or influent gas concentration (g/m^3)
Co	Outlet or effluent gas concentration (g/m^3)
t	Time (h)
CO_{2i}	Inlet CO_2 concentration (g/m^3)
CO_{2o}	Outlet CO_2 concentration (g/m^3)
Pco_2	Production of CO_2 or CO_2 production rate **(**g/m^3 h**)**
r_{max}	Maximum biodegradation rate (g/m^3 h)

LIST OF ABBREVIATIONS

Syntax	Description
VOCs	Volatile organic compounds
BTEX	Benzene, toluene, ethylbenzene and xylene
RBC	Rotating biological contactor
PBR	Packed bed bioreactor
MBR	Membrane bioreactor
TBAB	Trickle bed air biofilter
TPPB	Two-phase partitioning airlift bioreactor
ILR	Inlet loading rate ($g/m^3 h$)
EC	Elimination capacity ($g/m^3 h$)
EC_{max}	Maximum EC ($g/m^3 h$)
RE	Removal efficiency (%)
EBCT	Empty bed contact time (min)
HRT	Hydraulic retention time (min)
GC	Gas chromatography
FID	Flame ionization detector
LC-MS	Liquid chromatography mass spectrometer
FESEM	Field emission scanning electron microscope
rpm	Rotation per minute
NTP	Normal temperature and pressure
TCA	Tri-carboxylic acid
MSM	Mineral salt medium
MSA	Mineral salt agar
NH_3-N	Ammonical nitrogen
NO_2-N	Nitrite
NO_3-N	Nitrate
PO_4-P	Phosphorus
DO	Dissolved oxygen
RLR	Reaction-limited region

DLR	Diffusion-limited region
OD_{600}	Optical density or absorbance at 600 nm wavelength
VSS	Volatile suspended solids (mg/l)
CFU	Colony forming unit
RMSE	Root mean square error
CCD	Central composite design
RSM	Response surface methodology
OFAT	One factor at a time
ANOVA	Analysis of variance
AU	Arbitrary unit

CHAPTER 1

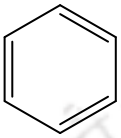
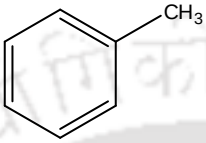
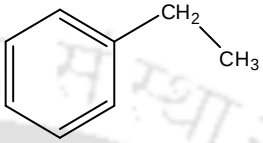
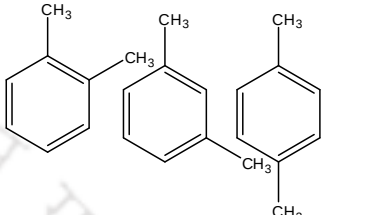
INTRODUCTION

1.1 GENERAL

Volatile organic compounds (VOCs), mainly produced by anthropogenic activities, are prevalent in both outdoor and indoor environments (Rudel and Perovich, 2009). The widely found VOCs are benzene (C_6H_6), toluene (C_7H_8), ethylbenzene (C_8H_{10}) and xylene (C_8H_{10}), commonly known as BTEX. The physico-chemical properties of BTEX are shown in Table 1.1. These BTEX compounds are mostly released from industries such as burning of fossil fuel (41%), chemical industry (22%), coating and surface treatment (18%) in outdoor environments (Álvarez-Hornos et al., 2011). Benzene is a potent carcinogen predominant in indoor environment, released from tobacco smoke, paint and some household products containing petroleum based chemicals (Wolkoff, 1995). Dincer and Muezzinoglu (2008) reported that the wastewater treatment plants and sludge management areas also release VOCs, mostly monoaromatics, i.e. benzene, toluene, and xylene. Similarly, organic influents during waste handling and its biological decomposition produce VOCs (Font et al., 2011). VOCs are also produced from landfills (Kim et al., 2006) and aerobic biological processing of municipal solid waste and composting processes (Pierucci et al., 2005). The industry-originated VOCs represent about 50% of the overall VOCs emissions (Kennes and Veiga, 2013). In china, vehicular exhaust is the major contributor of VOCs, followed by paint industry, and gasoline vapour (Liu and Diamond, 2005). The BTEX compounds participate in atmospheric photochemical reactions and contribute to ozone, which is harmful to the environment (Gallastegui et al., 2017). These compounds are carcinogenic in nature and

classified as hazardous compounds by United States Environmental Protection Agency (USEPA). Exposure to such compounds may cause acute health effects (Singh and Fulekar, 2010).

Table 1.1 The physico-chemical properties of BTEX

Parameters	Benzene	Toluene	Ethylbenzene	Xylene
Formula	 (C_6H_6)	 (C_7H_8)	 (C_8H_{10})	 (20%) (40-60%) (40-60%) (C_8H_{10})
Molar weight (g/mol)	78.11	92.14	106.17	106.16
Density (g/ml)	0.876	0.866	0.867	0.868
Boiling point (^oC)	80.1	110.8	136	138.5
Solubility (mg/l)	1780	500	150	150
Vapor pressure (mmHg)	76	22	7	6.5
Henry's law constant at 25^oC (kPa·m^3/mol)	0.55	0.66	0.84	0.73

The environmental regulations have driven industry and commercial sectors to rely on the widely adopted technologies such as carbon adsorption, incineration, and membrane separation to reduce the environmental damages caused by VOCs (Khan and Ghoshal, 2000). On the other hand, increasing stringent environmental regulations have put heavy cost on treatments and the high volatility property of VOCs makes treatment challenging and

difficult per se (Mudliar et al., 2010). Several conventional physical and chemical methods are commonly applied in industries (Adler, 2001), but face unavoidable problems such as consumption of more raw materials and production of more secondary waste, which damage the environment (Rene et al., 2015). On the other hand, certain biological methods because of several advantages over the physical and chemical methods may become sustainable and next generation techniques for controlling gaseous VOCs (Datta and Philip, 2014). Biodegradation for removal of VOCs in waste gas streams is cheap, easy and attractive. Research is continuing to make biodegradation even cheaper and easier to carry out.

1.2 BIOLOGICAL TREATMENT

In biological treatment (biodegradation), pollutants are converted to CO_2 by microorganisms. Biodegradation technique is efficient in treating pollutants of low concentration, soluble and biodegradable in nature (Van Groenestijn and Hesselink, 1993). This technique is based on the ability of microorganisms to convert VOCs to CO_2, H_2O, inorganic and organic by-products, and new biomass under aerobic condition (Khan and Ghoshal, 2000). The process is expressed by equation 1.1.

$$VOCs + O_2 + microorganisms \longrightarrow CO_2 + H_2O + biomass + byproducts \quad (1.1)$$

The biological treatment methods have several advantages over the physical and chemical methods because biological waste-air treatments are cost effective, simple to operate and also eco-friendly for the removal of VOCs (Delhoménie and Heitz, 2005; Devinny et al., 1998). It requires less energy and raw material, and produce less waste and CO_2. For these reasons, the biodegradation technique is widely accepted in VOCs control by most of the industries (Van Groenestijn and Hesselink, 1993). The biological treatment is performed at ambient temperature, and dose not generates nitrogen oxide and any other waste

streams (Deshusses, 1997). Once the pre-requisite conditions of temperature, humidity and suitable pH range are met, biological waste treatment is tolerant to changes in waste composition, and the process is on-going and effective. The commonly used bioreactors for biodegradation of gaseous VOCs are described in the next section.

1.3 BIOREACTORS FOR WASTE GAS TREATMENT

The biodegradation of gaseous VOCs is accomplished aerobically by different biological reactors such as biofilter (Mathur and Majumder, 2008), biotrickling filter (Cox and Deshusses, 2002), bioscrubber (Lo and Hwang, 2004), membrane bioreactor (Fitch et al., 2003) and suspended bioreactor (Ensley and Kurisko, 1994). The biofilters is widely used for aerobic treatment of VOCs such as benzene (Hassan and Sorial, 2009), toluene (Saravanan et al., 2013), mixture of benzene and toluene (Rene et al., 2015), and BTEX mixture (Rahul et al., 2013) in gas phase. The treatment of VOCs in waste gas streams involves a common mechanism consisting of absorption and biodegradation (Rahul et al., 2012). Though fundamental mechanism for VOCs removal is more or less same in all these bioreactors, there exist a difference in the use of microorganisms, filter materials, and concentration range of pollutants to be treated and so on (Devinny et al., 1998). These techniques have limitations of poor oxygen mass transfer due to restriction of forced aeration and mixing by virtue of the volatility of compounds, which make treatment difficult (Mudliar et al., 2008). In this context, the rotating biological contactor (RBC) is a popular aerobic treatment technique, which overcomes the limitations of conventional bioreactors (Datta and Philip, 2014).

RBC was originally developed for treating wastewaters (Pakshirajan and Kheria, 2012), but nowadays is widely used in industry for the treatment of polluted air. RBC

provides higher oxygen transfer, high surface area and better mixing as compared to other conventional bioreactors (Patwardhan, 2003). Due to rotation, RBC prevents excess biomass accumulation occurring in traditional biofilters (Ravi et al., 2010), and can be operated for a longer period without increasing the pressure drop (Datta and Philip, 2014). Yang et al. (2004) used a bioreactor containing activated sludge with a rotating drum process for treating toluene in waste gas streams. Later, Yang et al. (2008a) developed a multilayer rotating drum biofilter for treating diethyl ether in waste air, which showed high removal efficiency even at higher organic loading rate by utilizing nutrients from the liquid phase. Datta and Philip (2014) used a rotating disc contactor for treating aliphatic ketones in presence of aromatic VOCs such as toluene, ethylbenzene, and o-xylene in gaseous phase from a paint industry. Thus, RBC has emerged as an attractive process and environment friendly alternative for aerobic treatment of gaseous VOCs. To date, a very few literatures are available on the treatment of gaseous VOCs using conventional RBC. However, no work is found on the treatment of benzene and xylene as a single pollutant, a combined toluene and xylene, and BTEX mixture in waste gas streams using RBC.

1.4 RESEARCH AIM AND OBJECTIVES

In this research, a drum based RBC using sponge as a bio-support medium has been designed and developed for the aerobic treatment of BTEX compounds in gas-phase. Sponge-medium based RBC provides high porosity for homogeneous distribution of VOCs and more surface area for uniform development of biofilm. **The main aim of the research has been to control benzene and xylene as a single pollutant, a combined toluene and xylene, and BTEX mixture in waste gas streams using the developed sponge-medium based RBC.**

The research aim has been achieved with the following tasks.

i. Batch study to assess the biodegradation potential of mixed culture at various concentrations of benzene.

ii. Design, installation and start-up of RBC for removal of gaseous benzene using enriched mixed microbial consortium.

iii. Evaluation of the performance of RBC as removal efficiency (RE) and elimination capacity (EC) at various inlet loading rate (ILR) of gaseous benzene.

iv. Screening of enriched cultures for efficient treatment of VOCs.

v. Treatment of xylene, combined toluene and xylene, and mixture of BTEX in waste gas streams at various ILR, and its effect on RE and EC.

vi. The stability of RBC by studying the effect of intermittent operation and shock loading of BTEX.

vii. Identification of potential BTEX degrading microorganism in mixed culture.

viii. Kinetic modeling of the biodegradation of BTEX in RBC.

1.5 NOVELTY STATEMENT

i. Optimization of the biodegradation process of benzene using response surface methodology (RSM).

ii. Use of indigenous mixed culture (collected from petroleum refinery) under normal environmental condition for successful treatment of gaseous VOCs.

iii. Screening of enriched cultures improved the treatment of VOCs.

iv. Successful application of conventional RBC with little modification for removal of gaseous BTEX compounds individually and in mixture is one of its kind.

v. High RE of the modified RBC at higher loading rates of BTEX in waste gas streams.

vi. Identification of *Enterobacter cloacae* SP4001 (KY238115) as a predominant strain in mixed culture responsible for degradation of BTEX is first of its kind.

1.6 ORGANIZATION OF THE THESIS

The thesis has been organized in the following seven chapters.

Chapter 1: Introduction – This chapter gives background of the VOCs, its effect on human health and environment. It highlights the biological methods with advantages over the conventional physico-chemical methods and the potential use of RBC for controlling gaseous VOCs. Further, it describes the main aim of research along with the specific objectives followed by novelty statement, and organisation of the thesis.

Chapter 2: Literature review – This chapter presents the overview of the properties and sources of VOCs, and its effects on human health. It summarizes the drawbacks of various physico-chemical methods for removal of VOCs and provides the advantages of biological techniques over these techniques. Further, various performance and operating parameters are briefly discussed, which influence the biodegradation of VOCs using bioreactors. Subsequently, the chapter includes in-depth critical review on the various biological reactors and its pros and cons to treat gaseous VOCs.

Chapter 3: Materials and methodology – The detailed materials and analytical methods developed and adopted in this study have been described in this chapter. The chapter covers experimental methodologies for biodegradation of benzene in batch reactor and the process optimization using RSM. It also provides the design and installation of sponge-medium based RBC, its operation, acclimatization and biofilm development during the treatment of benzene, xylene, combined toluene and xylene, and mixture of BTEX.

Chapter 4: Kinetic modeling and optimization of benzene biodegradation – This chapter contains the study on the kinetics of benzene biodegradation and modeling of growth kinetics for a mixed microbial culture in the presence of benzene. Further, optimization of pH and benzene concentration has been done using RSM to enhance the biodegradation of benzene. Subsequently, the metabolic intermediates produced during benzene biodegradation have also been analyzed and the predominant benzene degrading microorganism in the mixed culture has been identified.

Chapter 5: Gaseous benzene control using a sponge-medium based RBC – This chapter presents the performance of RBC during the treatment of benzene along with nutrients at ambient temperature and discusses the effect of ILR of benzene on pH, dissolved oxygen, and biomass. Further, the effect of EC on production of CO_2 (Pco_2) has been presented followed by the identification of predominant benzene degrading microorganism and determination of the kinetic constants of benzene.

Chapter 6: Control of BTEX from waste gas streams and performance of RBC – This chapter discusses the screening of enriched cultures for the efficient treatment of xylene as a single pollutant, a combined toluene and xylene, and mixture of BTEX in waste gas streams. The Pco_2, removal of nutrients, and biomass concentration during the treatment has been assessed by varying the operating conditions. The effect of intermittent operation and shock loading has also been investigated to determine the stability of RBC.

Chapter 7: Conclusion and future scope – This chapter summarizes the major conclusions and key findings of the research work, limitations and provides future scope for research.

CHAPTER 2

LITERATURE REVIEW

2.1 GENERAL

The literature has been reviewed on the topics relevant to the objectives of the research. It includes the properties and sources of VOCs, effects of VOCs on environment, and control techniques followed by the advantages of biological methods over the physico-chemical methods. Further, different parameters that influence the treatment of VOCs using biological methods followed by various biological reactors are critically reviewed.

2.2 VOLATILE ORGANIC COMPOUNDS

VOCs are carbon containing organic molecules that also contain hydrogen and oxygen. Some hydrocarbons are VOCs, composed of only carbon and hydrogen atoms but not all VOCs are hydrocarbons (Kennes and Veiga, 2013). VOCs are one of the top five atmospheric pollutants, are also defined as organic pollutants arising from human activities, other than methane, which are capable of producing photochemical oxidants by reaction with nitrogen oxides in the presence of sunlight. VOCs, characterized by low boiling point and high vapor pressure, are prevalent in our atmosphere, and are of major environmental concerns (Singh and Fulekar, 2010). According to the European EC-directive 1999/13/EC, VOCs have a vapour pressure of about 10^{-2} kPa at 20 ^{o}C, with a boiling temperature usually below 250 ^{o}C at normal temperature and pressure (Kennes and Veiga, 2013).

VOCs have wide range of industrial applications, but it also causes environmental pollution (Liu et al., 2010). Among these VOCs, BTEX are toxic and found both in liquid and gaseous form, are the main components in petrochemical effluents and gasoline, and its

exposure is a global environmental problem (Singh and Fulekar, 2010). Cigarette smoke, smokes from wood burning fires, and some household products containing petroleum-based chemicals emit a large amount of VOCs, predominantly benzene in indoors (Cooke, 1991). The outdoor sources are motor vehicle exhausts, burning of fossil fuel, emissions from petrochemical, allied industrial processes and from wastewater treatment plants (Khan and Ghoshal, 2000). The widely found VOCs such as BTEX are mostly released by burning of fossil fuel, chemical industry, coating and surface treatment (Álvarez-Hornos et al., 2011). Kennes and Veiga (2013) reported that major sources of VOCs mostly BTEX are released from industries, which represent about 50% of the overall emission estimates. Higher levels of these VOCs are found in indoors than outdoors in urban areas due to a variety of consumer products used on a daily basis and also due to outdoor pollutants released by the traffic migrating to indoors (Rudel and Perovich, 2009). In china, vehicular exhaust is the major contributor of VOCs, followed by paint industry, and gasoline vapour (Liu and Diamond, 2005).

2.3 EFFECTS OF VOCs ON HUMAN HEALTH AND ENVIRONMENT

The VOCs commonly contain compounds like BTEX are carcinogenic and classified as hazardous compounds by United States Environmental Protection Agency (USEPA) (Singh and Fulekar, 2010). Exposure to VOCs is a global environmental problem due to its several short-term health effects, which include dizziness, eye irritation, and long-term effects, which include asthma, cancer, liver and kidney damages (MDH, 2010). The accumulation of highly toxic VOCs in atmosphere cause severe damage to the environment (Delhoménie and Heitz, 2005). Both liquid and gaseous VOCs at higher concentrations can cause a significant threat owing to their toxic and carcinogenic properties (Rene et al., 2015). The VOCs such as

benzene, toluene, ethyl benzene and xylene (BTEX) are important industrial solvents emitted into the atmosphere in the form of vapor gases during its manufacturing, transportation and disposal (Mathur et al., 2007). These VOCs participate in photochemical reactions to form ozone in the lower atmosphere, which has a significant impact on environment (Gallastegui et al., 2017). Ozone in the troposphere is harmful to human health by causing damage to respiratory system and may also damage the vegetation (Shareefdeen and Singh, 2005).

2.4 CONTROL METHODS OF VOCs

Methods used for control of VOCs are physical, chemical and biological. Several conventional physical methods such as adsorption, absorption are commonly applied in industries, which consume more raw materials and in turn produce a huge amount secondary waste (Datta and Philip, 2014). Similarly, in chemical methods such plasma technology and combustion produces NO_x and greenhouse gases, respectively (Shareefdeen and Singh, 2005). These aspects such as generation of by-products of various controlling methods and its effect on environment are generally ignored. In addition, membrane separation and plasma technology cannot be operated at high temperature and using membrane in biological reactor creates fouling. Table 2.1 summarizes the drawbacks of various physico-chemical methods for removal of VOCs. Biological waste air treatments, on the other hand, are widely accepted, cost effective, require less energy, less raw material and are eco-friendly for treating VOCs (Delhoménie and Heitz, 2005). Fig. 2.1 represents application of various treatment methods depending upon the gas flow rate and concentration (Devinny et al., 1998).

Table 2.1 Summary of physico-chemical VOC removal methods with the drawbacks

Methods	Pollutants[a]	Removal	Remark	References
▪ Physical Dilution	Polluted air and VOCs	–	• Pollutants remain untreated	Corbitt (1990)
Condensation	VOCs	70–85%	• Recovery of condensate difficult, which can offset the operating cost	Khan and Ghoshal (2000)
Adsorption	VOCs	80–90%	• Spent adsorbents produce solid waste and recovery of compound is also expensive	Khan and Ghoshal (2000)
Absorption	VOCs	90–98%	• Waste water released needs further treatment	Khan and Ghoshal (2000)
Membrane separation	Acetone, formaldehyde, and ammonia	95–99%	• Membranes are costly and recovery of solvent may increase the operating cost	Jansen et al. (1994)
Masking	Odors	–	• Application of stronger molecule for neutralization of concentrated VOCs	Planker (1998)
▪ Chemical Combustion	VOCs	95–99%	• Recover of energy is easy but simultaneously produces greenhouse gases	Khan and Ghoshal (2000)
Chemical oxidation	SVOCs	90%	• Expensive and highly unstable in nature	Wei et al. (2012)
Chemical precipitation	Chlorinated VOCs	95–98%	• Regeneration of precipitant is the major problem	Bouwer and McCarty (1982)
Plasma technology	Chlorophenol	99.99%	• Large electrical power consumption and generation of thermal NO_X	Urashima and Chang (2000)

[a]VOCs = Volatile organic compounds
[a]SVOCs = Semi volatile organic compounds

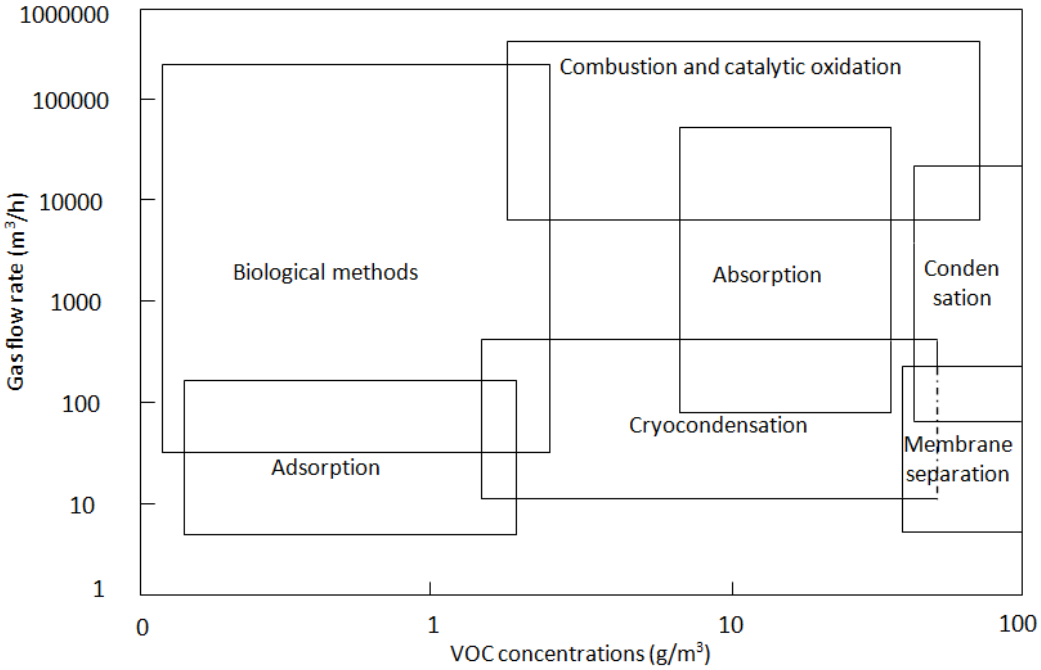


Fig. 2.1 Application of various treatment methods as function of gas flow rate and concentration [From Devinny et al., (1998)]

2.5 BIOLOGICAL METHODS

Biological waste air treatment methods involve the biodegradation of VOCs by microbial enzymes into less toxic compounds, most often produce biomass as end-product (Rene et al., 2015). Biological methods are not only economical but also eco-friendly for treating VOCs at low concentrations and high flow rates (Rene et al., 2010). Hence, the biological methods are widely used and popular for VOCs control, and many industries are also focussing on these methods (Van Groenestijn and Hesselink, 1993). Some biological techniques, because of several advantages over the conventional physical and chemical methods, may become sustainable and next generation techniques for treating gaseous VOCs. The parameters that influence the treatment of VOCs in biological reactors are described in the following sections.

2.5.1 Performance parameters

The parameters that are widely considered for evaluating the performance of biological reactors are shown along with their typical ranges (Table 2.2).

Table 2.2 Performance measure with typical ranges used in biological reactor

Name of parameter	Description	Typical range	Units	References
Empty bed contact time (EBCT)	Measure of gas residence time	15–60	s	Severin et al. (1993)
Inlet loading rate (ILR)	Chemical mass loading rate per unit bed volume	10–160	$g/m^3 h$	Ottengraf and Van Den Oever (1983)
Elimination capacity (EC)	Chemical mass removal rate per unit bed volume	10–160	$g/m^3 h$	Ottengraf and Van Den Oever (1983)
Removal efficiency (RE)	Performance measures	95-99	%	Marsh (1992)

2.5.2 Operating parameters

The operation of bioreactor involves transfer of pollutants from gaseous to the liquid phase, and finally biodegradation of VOCs occurs in liquid phase by the microorganisms (Jansen et al., 1994). The operating parameters that influence the biodegradation process of VOCs in bioreactors are described in the following sections.

2.5.2.1 Transfer of pollutant

The first step is the transfer of pollutants from gaseous to liquid phase. The fundamental assumption of this process is that both phases are at equilibrium as explained by Henry's law. This is expressed by equation 2.1.

$$C_g = H \cdot C_l \quad (2.1)$$

where, C_g is the concentration of pollutant in gaseous phase; H, the Henry's coefficient; and C_l is the concentration of pollutant in liquid phase (Shareefdeen and Singh, 2005).

VOCs having the Henry's coefficient more than 0.01 are volatile but reduce their solubility in water with higher coefficients, which decreases their RE. The Henry's coefficients are in the order: E (0.84) > X (0.73) > T (0.66) > B (0.55).

2.5.2.2 Microorganisms

Several groups of microorganisms including bacteria, fungi, and actinomycetes act as catalysts, which grow on the surface of a solid support and catabolically degrade the VOCs into harmless products (Khan and Ghoshal, 2000). The microorganism is obtained by enriching and acclimatizing the activated sludge (Aizpuru et al., 2001), a specialized inoculum (Grove et al., 2004) and the packing material such as compost (Liu et al., 2002) or enriching the microorganisms present in compost and subsequently used in the bioreactor (Qi and Moe, 2006). Activated sludge from wastewater plants is commonly used because it contains a large variety of microorganisms, which can biodegrade a wide range of VOCs (Wagner et al., 2002). At the same time, some VOCs are not easily biodegradable for which pure-culture-bacterial strain is characterized for its ability to biodegrade target pollutants (Ottengraf et al., 1986). Van Groenestijn et al. (2001) replaced the working consortium from bacteria to fungi due to advantages such as more resistant to acidification and the mycelia of fungi provide a larger surface area facilitating the uptake of hydrophobic volatile compounds, i.e. benzene. Kennes and Veiga (2004) treated benzene in the biofilter in the presence of mixed bacterial and fungal populations with fungi as the dominant species with > 95% RE at low pH of 3. Biodegradation of benzene is very slow, so Mohseni and Allen (2000) treated a

mixture of hydrophobic and hydrophilic contaminated air streams. Several researchers have used different microorganisms for treatment of VOCs. For example, treatment of benzene by *Pseudomonas putida* showed a RE of 97% at lower concentration of 10 mg/m^3, which decreased up to 67% at a concentration of 50 mg/m^3 (Sene et al., 2002). A fungal bioreactor containing *Exophiala lecanii-corni* was used by Woertz et al. (2001) for the treatment of toluene, which showed RE greater than 95% throughout the study at a concentration of 1.886 g/m^3. *Pseudomonas* sp. NBM21 degraded *p*-xylene with a RE higher than 90% for the removal of xylene (Jeong et al., 2006). Natarajan et al. (2017) used mixed microbial culture collected from activated sludge for biodegradation of ethylbenzene and xylene contaminated air at a lower concentration of 1.0 g/m^3, which showed the maximum RE of 89% for ethylbenzene and 78% for xylene. In a biofilter dominated by *Bacillus sphaericus* exhibited more than 99% total BTEX RE at an inlet concentration of 0.681 g/m^3 (Mathur et al., 2007).

2.5.2.3 Filter materials

Filter materials used in filter beds provides support to microbial growth. The most important characteristics of filter material are high surface area, low pressure drop, high porosity for homogeneous distribution of gas, good water retention capacity, neutral pH, less maintenance and total organic matter should be more than 55% (Oh and Choi, 2000). Peat, soil, compost, and wood chips are often used as filter materials. Compost is widely used, which offers varied microbial ecosystem, good water holding capacity, good permeability of air and also contains large amount of nutrients, but it become compact leading to increase in pressure drop (Alexander, 1999). Some bed structures, for example, lime stone shows pH buffering capacity (Smet et al., 1996) and activated carbon possesses adsorbing capacity (Abumaizar et al., 1998). Further, a few studies have reported the use of other filter materials, such as

polyurethane foam to remove toluene, with over 90% of removal efficiency (Yang et al., 2004) and perlite to remove toluene with 99% efficiency (Woertz et al., 2002). Similarly, sugarcane bagasse is used to remove benzene with almost 100% efficiency initially, which later decreased (Christen et al., 2002) and Mudliar et al. (2008) used rotating rope to remove pyridine with efficiency of more than 85%.

2.5.2.4 Air flow rates

The air flow rates significantly influences the performance of biodegradation (Elmrini et al., 2004). The degradation of VOCs depends on both the flow rate and concentration. Higher air flow rate decreases the contact time, which leads to incomplete biodegradation of VOCs (Christen et al., 2002). Moreover, at higher flow rate, the water in the filter bed is stripped, which tends to desiccate the filter material of bioreactor (Detchanamurthy and Gostomski, 2012). To enhance the degradation of VOCs, the EBCT should be greater, which is the case of low-flow rates. The EBCT varies from 15 second to several minutes, in most of the successfully operated bioreactors during VOC degradation. The low flow rates provide longer EBCT, which increases the RE of VOC (Martin et al., 2002).

2.5.2.5 Pollutant concentrations

The concentration of VOCs to be treated is important for an effective operation. Slightly soluble VOCs at low concentrations and soluble compounds at higher concentrations are absorbed into the biofilm causing biodegradation (Delhoménie and Heitz, 2005). The oxygenated hydrocarbons are readily biodegradable than the linear alkanes, followed by the aromatic hydrocarbons and chlorinated VOCs (Delhoménie and Heitz, 2005). The inlet VOC concentrations higher than a threshold value may lead to substrate inhibition and may tend to inhibit microbial growth and activity (Tang et al., 1996). Moreover, the higher inlet VOC

concentrations lead to insufficient availability of oxygen (Ottengraf, 1987). It is reported that 0.113 g/m^3 of toluene achieves removal efficiency up to 99%, but it decreases up to 82% when the inlet concentration is doubled (Detchanamurthy and Gostomski, 2012). Delhoménie and Heitz (2005) reported that generally inlet VOC concentrations do not exceed 5 g/m^3 in biofilters, which tends to inhibit its performance.

2.5.2.6 Nutrients

Microorganisms obtain their carbon and energy by utilizing the VOCs present in the inlet air stream along with nitrogen, phosphorous, minerals and trace elements for successful operation (Morales et al., 1998). Filter materials like compost, peat, and soil contain nutrients for growth of microorganisms but in case of synthetic filter materials like polyurethane foam, nutrient is provided from outside for better removal efficiency (Weckhuysen et al., 1993). The constant supply of nutrients, irrespective of the filtering material, such as KNO_3, KH_2PO_4, $(NH_4)_2SO_4$, $CaCl_2$, $MgSO_4$, $FeSO_4$, Na_2MoO_4, NH_4HCO_3, Vitamins B_1, etc. in form of aqueous solution are essential (Wu et al., 1999).

2.5.2.7 Oxygen requirement

Aerobic biodegradation of VOCs requires oxygen, which is generally supplied with the pollutant stream. A minimum of 100 mole of oxygen is required per each mole of gaseous pollutant in aerobic biodegradation process (Williams and Miller, 1992). In polluted air, oxygen is usually a limiting factor. Supply of additional oxygen using a blower increases the dissolved oxygen (DO) concentration in bioreactor, which enhances the performance. The commonly used biofilters or packed bed bioreactors (PBR) have a limitation of poor oxygen mass transfer (Mudliar et al., 2008). The increase in concentration of VOCs increases the loading rates but reduces DO concentration significantly. Thus, the performance of PBR

decreases due to poor transfer of DO to the microorganisms (Sahoo et al., 2013). Therefore, in this context, RBC has emerged as an attractive bioreactor, which provides higher oxygen transfer for aerobic biodegradation of VOCs (Mudliar et al., 2010).

2.5.2.8 pH

Most microorganisms survive and grow in a specific pH range but the optimum pH range for biodegradation is 7−8, which supports the growth of bacteria and actinomycetes (Mudliar et al., 2010). Maximum degradation of BTEX is observed at a pH between 7.0 and 8.0 (Lu et al., 2002; Lee et al., 2002). The effect of pH on degradation of alkyl benzene between 3.5 and 7.0 is studied, and which revealed that degradation of alkyl benzene increases with pH (Veiga et al., 1999). Hassan and Sorial (2010) utilized acidic pH to encourage the growth of fungi for the treatment of benzene vapours. The VOCs containing hetero atoms (S, Cl, N) turn into acidic products, which reduce the pH of the medium, affecting the EC (Christen et al., 2002). To maintain the neutral pH, buffer materials i.e. limestone (Smet et al., 1996), NaOH (Zilli et al., 2000), $NaHCO_3$ (Tang et al., 1996) are added.

2.5.2.9 Temperature

The range of mesophilic temperature 25–35 ^{o}C, is suitable for activities of aerobic microorganisms and strongly influences the activities of microorganism (Marsh, 1992). VOCs with a temperature over 40 ^{o}C are cooled by diluting with ambient air before the gas enters into the bioreactor. In the same way, when the temperature is below 10 ^{o}C, heating of the gas stream is required (Shareefdeen and Singh, 2005). For example Park et al. (2002) found the maximum toluene degradation rate at a range of 30–35 ^{o}C, which is also used by Lee et al. (2002) for degradation of BTEX. Some researchers have also studied the feasibility of toluene degradation under thermophilic conditions at a temperature of range 50–60 ^{o}C

(Matteau and Ramsay, 1999). Similarly, the elimination of methanol and α-pinene by thermophilic bacterial consortium at a temperature of 55ºC is studied by Allen et al. (2000) and Dhamwichukom et al. (2001), respectively. Therefore, the activities of microorganisms increase with the rise in temperature but, the solubility of VOCs in water and the sorption capacity of filter solids decreases. This impedes the partitioning out of the gaseous phase at higher temperatures (Leson and Winer, 1991).

2.5.3 Metabolic processes and degradation pathways of BTEX

The VOCs, oxidized by catabolic pathway, become a source of energy and carbon for microbial cell growth (Jindrova et al., 2002). The degradation of VOCs is dominated by oxidation-reduction reactions, which involves external transfer of electrons catalysed by microorganisms (Yu et al., 2001a). The BTEX compounds are relatively water-soluble and therefore they are often degraded by indigenous microbial population (Jindrova et al., 2002). The degradation of BTEX occurs by several aerobic-metabolic-pathways usually by two enzymes catalyzed reaction (mono-oxygenases and di-oxygenases) into substituting catechol (Zhang et al., 2013). Benzene and ethylbenzene are degraded to catechol and 3-ethylcatechol, respectively, while toluene and m-xylene give 3-methylcatechol as an intermediate product (Jindrova et al., 2002). These catechols further undergo cleavage to produce succinate, fumarate, pyruvate, and acetyl-CoA, which are entered into tri-carboxylic acid cycle (TCA cycle), producing CO_2 and water as by-products (Fig. 2.2).

Different compounds follow different metabolic pathways but the interaction among the compounds occurs when their metabolic pathway of degradation is same (Du Plessis et al., 2001). The presence of ethyl acetate reduces the removal of toluene, is reported by Liu et al. (2002) and oxygenated compounds are removed more efficiently as compared to aromatic

compounds, as reported by Khammar et al. (2005). The competitive inhibition between benzene and toluene, and its inhibition by the p-xylene co-metabolism is reported by Oh et al. (1994). A number of studies also proved that inhibition is attributed to the accumulation of intermediate metabolites in the medium (Yu et al., 2001a).

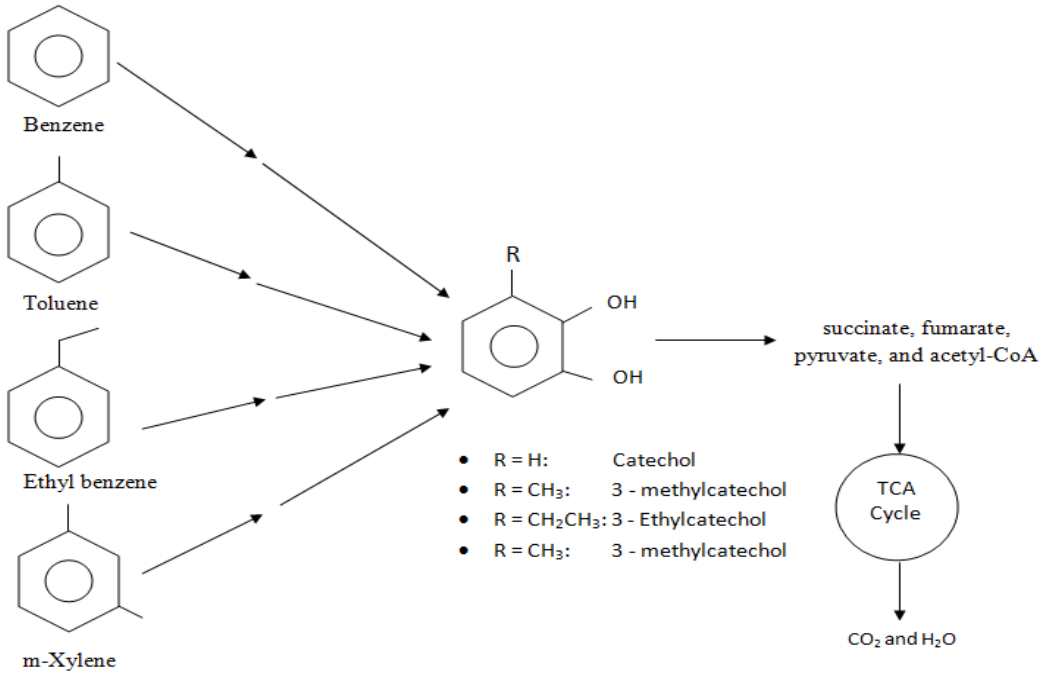


Fig. 2.2 Metabolic degradation pathway of BTEX

2.6 BIOLOGICAL REACTORS

There are a number of biological reactors which can accomplish biodegradation of VOCs, such as biofilter, biotrickling filter, bioscrubber, membrane bioreactor, suspended bioreactor, and rotating biological contactor. Though fundamental mechanism for VOC removal is more or less same in all these systems, there exist a difference in the use of microorganisms, filter material, and concentration range of pollutants to be treated. Table 2.3 summarizes the characteristics, advantages and disadvantages of the various biological reactors for waste gas treatment containing VOCs.

Table 2.3 Characteristics, advantages and disadvantages of the various biological reactors

Type of bioreactors	Characteristics	Advantage	Disadvantage	Reference
• Biofilters	• Immobilized microorganism • Immobile water phase	• Low operating cost • High gas liquid surface area	• Clogging of the medium • Large area requirement	Mudliar et al. (2010)
• Biotrickling filters	• Immobilized microorganism • Nutrient solution recirculated	• Better retention for slow growing microorganism • Low pressure drop	• More complex to construct and operate. • Disposal of excess sludge	Mudliar et al. (2010)
• Bioscrubbers	• Suspended biomass consisting of two units i.e. scrubber and a bioreactor	• No clogging problem • Better control of nutrient	• Slowest growing microorganism wash out • Disposal of excess sludge	Shareefdeen and Singh (2005)
• Membrane bioreactor	• Microorganisms in two forms – fixed film and suspended growth cultures	• No gas phase clogging • Suitable for low water solubility compounds	• High operating and capital cost • Clogging of the liquid channel due to excess biomass	Kumar et al. (2008)
• Suspended bioreactor	• Suspended biomass consisting of single unit	• Absence of plugging and easier biomass control • No drying of medium	• High operating and capital cost • High mass loading rate decreases the performance	Neal and Loehr (2000)
• Rotating biological contactor	• Attached and suspended growth of biomass • Both aerobic and anaerobic degradation occurs	• Provides higher oxygen transfer, better mixing and high surface area • Lower operating cost • Enable better contact of the air with biofilm	• Not suitable for highly concentrated pollutants • Difficult to scale up • Slow process startup	Patwardhan (2003); Cortez et al. (2008)

2.6.1 Biofilter

The biofilter is a fixed-bed bioreactor in which polluted air flows through the filter bed and biological oxidation of VOCs occurs after it is diffused into the biofilm. The operation of biofilter involves a series of steps like transfer from gaseous phase to the water phase, adsorption onto medium, absorption into the biofilm and finally biological oxidation gives rise to CO_2, H_2O and other mineral products (Devinny et al., 1998; Kennes and Thalasso, 1998). The performance and operating parameters of a biofilter as well as other bioreactors are almost similar described in the section 2.51 and 2.5.2.

In Europe, most of the chemical industries use biofilters for control of VOCs, odours, organic and inorganic air pollutants that are toxic to humans (Leson and Winer, 1991). Air containing toluene vapour treated in the biofilter inoculated with *Pseudomonas putida* (MTCC 102) is described by Singh et al. (2010). Compost based biofilters for the treatment of toluene is operated at 0.108−0.15 m^3/h gas flow rates having an inlet concentration of 0.5−13 g/m^3 for a period of 102 days. REs in these cases are observed in the range of 48−100% and ECs of 26−180 g/m^3 h (Znad et al., 2007).

2.6.2 Biotrickling filter

The biotrickling filter consists of a fixed bed through which a gas stream containing VOCs passes and the nutrient solution is recirculated continuously through the bed. In biotrickling filter, the adapted bacterial monocultures or mixed cultures immobilize on granular materials, such as synthetic polymers or activated carbon (Weber and Hartmans, 1996). The nutrient solution provides moisture, nutrient, and pH control to the biofilm and allows the removal of inhibiting products (Shareefdeen and Singh, 2005). The filtering materials facilitate easy flow of the gaseous VOCs and liquid through the bed, which promote the development of the

microflora. As filtering materials are synthetic and inert in nature, biotrickling filters need inoculation of activated sludge (Lu et al., 2000). Due to the permanent trickling mechanism, the VOCs is first transferred to liquid medium before undergoing biodegradation, and thus it is more adapted for the elimination of water soluble VOCs (Mudliar et al., 2010). It is not suitable for those VOCs which are relatively less soluble in water e.g. toluene (Kirchner et al., 1991) and whose inlet concentration is above 50 g/m^3, which limits the oxygen-diffusion (Kozliak et al., 2000). Therefore, the biotrickling filters remove VOCs of low concentrations efficiently (Kirchner et al., 1996). In a typical biotrickling filter, the inlet concentration of a VOC is generally less than 0.5 g/m^3 (Delhoménie and Heitz, 2005). There is a major drawback of accumulation of biomass in the filter bed, which can be overcome by physical, chemical and biological methods. The physical or mechanical treatments include bed back washing (Smith et al., 1996) and bed stirring (Laurenzis et al., 1998), whereas the chemical treatments include limiting the amount of nutrient and water, as well as the use of disinfectant to dissociate the attachment between biomass and bed (Cox and Deshusses, 1999a). The biological treatments include the use of biomass predators such as protozoa (Cox and Deshusses, 1999b). Amongst, the back washing is efficient and effective method (Cai et al., 2004).

Cox and Deshusses (1999b) used a bio-trickling filter with two protozoan species to control biomass for the treatment of toluene vapours. The method yielded the EC of 32.2 g/m^3 h at an inlet concentration of 1.025 g/m^3. Recently, several chemical scrubbers are converted into bio-trickling filters (Kraakman, 2003). In spite of that, biotrickling filters are preferred less due to more operating cost and restriction of solubility of VOCs.

2.6.3 Bioscrubber

The bioscrubber is a combination of absorption column and a bioreactor which contains activated sludge (Delhoménie and Heitz, 2005). In bioscrubbers, gas stream containing VOCs is brought in contact with water in a spraying tower with inert packing, which results in absorption of toxic compounds in water phase and release of clean air from the top of the column, whereas contaminated water is entered into the bioreactor portion for treatment. After the treatment, the waste solution is recycled in the absorption column, and the sediment-biomass is recycled in the bioreactor either partly or whole (Mudliar et al., 2010). The addition of nutrient, temperature, and pH can be easily controlled for microbial growth and its activity (Van Groenestijn and Hesselink, 1993). This type of reactors are less popular for treatments of VOCs since most of the VOCs are volatile and less water soluble but the recent advances and process designs indicate a growing interest in its applications (Le Cloirec et al., 2001). To eliminate less soluble compounds, Mortgat (2001) suggested the addition of emulsifying agents (silicon oil, phthalate) in the liquid phase, which favour the better mass transfer. Most of the bioscrubbers use activated sludge from wastewater treatment plant as inoculum (Ottengraf, 1987), but in some cases, specific degrading strain can also be inoculated.

An airlift bioreactor is also a type of bioscrubber in which for biodegradation two distinct zones are made by a draft tube into an inner gassed region called riser and an outer ungassed region called down-comer (Ward, 1989). The gaseous VOCs streams enter the reactor through sparger located at the bottom of the riser. Airlift bioreactors are extensively used in water treatments. Its application in gas treatment is developed by Yu et al. (2001b). Lo and Hwang (2004) used an airlift bioreactor for toluene biodegradation and achieved the

RE in the range of 50–90%. The RE decreases with the increase in VOC flow rate and loading rate in the airlift bioreactors (Vergara-Fernandez et al., 2008). The use of airlift bioreactor for gaseous VOCs treatment is still in its infancy and therefore a focus on large scale treatment is needed. The airlift bioreactor is a new bioscrubber having effect of both absorption and biodegradation described by Edwards and Nirmalakhandan (1999) and successful used for treatment of BTEX compounds in gas phase.

The performance of the treatment of VOCs can be enhanced with some modifications in conventional bioscrubbers. For example, in two-liquid phase bioscrubber, addition of an organic solvent to the liquid phase enhances the degradation of hydrophobic compounds (Déziel et al., 1999). The addition of 10–30% water immiscible solvent to the liquid phase enhances the absorption of hydrophobic compounds such as alkane, benzene, styrene, phenol, and naphthalene (Mudliar et al., 2010). The water immiscible solvents such as silicon oil, paraffin oil, dibutyl phthalate, and pristine are frequently used in two-liquid phase bio-scrubbers, amongst, silicon oil is the best (Yeom and Daugulis, 2001). However, in two stage bio-scrubbers, the first stage contains autotrophic organisms for oxidation of inorganic compounds and the second stage contains heterotrophic organisms for removal of VOCs (Le Cloirec et al., 2001). Despite all these developments, the use of bioscrubbers to treat water soluble VOCs are limited.

2.6.4 Membrane bioreactor

In membrane bioreactors (MBRs), the gaseous VOCs are transferred at the interface of gas and liquid through a membrane, where they are degraded by biofilm attached to the other side of the membrane (Kumar et al., 2008). In general, the VOCs which provides, carbon source and oxygen are supplied to the biomass from the gaseous phase, whereas nutrients are

supplied through the liquid phase and membrane is used as the interface between the phases (Reij et al., 1998). Microorganisms are in two forms – fixed film cultures and suspended growth cultures. The most important characteristics of the MBR over other bioreactors are the selective permeation of pollutants and its promising application for removal of poorly water soluble VOCs. The diffusion of contaminants across the membrane interface is driven by the concentration difference between the gas phase and the biofilm (Reij et al., 1997). Excess biomass accumulation in the spiral wound membrane decreases the reactor performance and increases the pressure drop (Freitas Santos et al., 1995). The clogging of hollow fibre membrane bioreactor is studied by higher liquid velocity. It is reported that the performance gradually decreases after a period of 3−6 months as the biofilms are prone to aging (Reij and Hartmans, 1996). The advantage of membrane bioreactors over biofilters is the optimum humidification of biomass and removal of degradation products due to the presence of discrete water phase, by which biomass remains active throughout the process (Mudliar et al., 2010).

The membrane bioreactors are best suited for removals of hydrophobic pollutants since they provide a larger gas-liquid interface and have excellent mass transfer properties (Reij et al., 1995). In the treatment of trichloroethene (TCE) in a hollow-fibre membrane biofilter having both aerobic and anaerobic zones, anaerobic zone causes partial dechlorination of TCE, which degrades further in the aerobic zone of the biofilm (Parvatiyar et al., 1996). The hollow-fibre-polyporous-polypropylene membrane bioreactor remove up to 98% of benzene (Fitch et al., 2003), and 97 % of toluene (Ergas and McGrath, 1997). Bauerle et al. (1986) reported the use of capillary non-porous polydimethylsiloxane membrane for removal of xylene using activated sludge as an inoculum with a RE of 96%.

2.6.5 Suspended bioreactor

In suspended bioreactors, the gas stream of VOCs is injected in the bulk of the liquid containing suspended microorganisms (Shareefdeen and Singh, 2005). This produces biomass, which can be removed by gravity sedimentation and the settled biomass can be recycled so that a desired mixed-liquor-suspended-solids concentration can be maintained in the bioreactor (Neal and Loehr, 2000). In these bioreactors, the easier biomass and nutrient control over immobilised systems are the potential advantages (Bielefeldt and Stensel, 1998). The VOC degradation in a biofilter occurs due to microorganisms residing in a bioflm, whereas it is in case of suspended bioreactors occurs due to suspended microorganisms in an agitated mixture (Neal and Loehr, 2000).

These bioreactors are effective for treating gases containing VOCs having mass loadings in the range of about 5–30 g/m^3 h. The co-metabolic degradation of trichloroethylene with phenol and toluene by suspended cell bioreactor is described by Ensley and Kurisko (1994). Neal and Loehr (2000) also reported that the biofilters and suspended bioreactors achieve similar RE of VOCs up to about 96–99.7%. The RE of VOCs as high as up to 99% is observed at an organic loading of 1.6 $x10^2$ g/m^3 h in suspended bioreactor (Carvalho et al., 2009).

2.6.6 Rotating biological contactor

The most popular immobilized growth based reactor is RBC, developed initially for wastewater treatment (Pakshirajan and Kheria, 2012; Sarayu and Sandhya, 2012), but nowadays is widely used for the treatment of gaseous pollutants. RBC consists of a set of discs connected to a horizontal bar that supports the biofilm is rotated at 1-10 rpm (Antonie, 1976). The discs, which is also called as supporting medium in RBC, are partially submerged

in the nutrient solution and partially exposed to the air enabling better contact between the gas and liquid (Datta and Philip, 2014). The rotation of the discs, which exerts shear-force on the biomass, strips the excess biomass, thus prevent the clogging of media (Patwardhan, 2003). The performance of RBC depends on the rotational speed, organic loading rate, hydraulic retention time (HRT), supporting media, biofilm characteristics, and DO levels to operate the bioreactor at optimum condition (Cortez et al., 2008). Increasing the organic loading rate reduces the retention time of the substrate within the reactor, which results in the decrease of RE and vice versa (Najafpour et al., 2006). Shorter HRT results in the lower RE, whereas longer HRT is not economically feasible (Hanhan et al., 2005).

RBC media provides surface area for attachment of the biofilm (Cortez et al., 2008). Some studies have reported the use of biosupport media such as polyurethane foam, rotating rope, modified RBC discs by attaching netlon sheets (Radwan and Ramanujam, 1997), and plastic discs attached with a layer of polyurethane foam to enhance the adhesion of microorganisms in RBC (Guimarães et al., 2005). Biofilm is a living microbial system obtained by enriching and acclimatizing the activated sludge, or specialized inoculums which is used in RBC. To avoid clogging due to excess biofilm growth in the supporting media, different mechanisms such as variable rotation speed and reverse shaft rotation are followed. RBC has a compact design so it requires relatively small land for installation and the biodegradation occurs in a closed system, which overcomes the generation of odors (Cortez et al., 2008). It has low energy consumption because of less speed of rotation and the supporting media are lighter in weight so that they offer little resistance to rotation, low operating and maintenance cost as compared to conventional extended aeration systems (Greaves, 1990).

Boumansour and Vasel (1998) examined the relationship between diffusion from the gas phase to the liquid phase and rotational speed in a disc based RBC. According to Sassi et al. (1996), the most important operating parameters are substrate loading, volumetric flow rate, shaft speed, liquid density, and diameter of the disc. Further, Von Rohr and Ruediger (2001) suggested for feeding of air tangentially to the disc or through perforations in a hollow shaft to improve the contact between the gas and liquid. Yang et al. (2008b) recently designed and developed a rotating drum biofilter for better distribution of VOCs, oxygen, nutrients and biomass using polyurethane foam as a bio-support medium. Typical operational parameters, which influence the performance of RBC, are shown in Table 2.4.

The commonly used fixed bed bioreactors have a limitation of poor oxygen mass transfer for aerobic biodegradation of gaseous VOCs (Mudliar et al., 2008). Due to continuous rotation, RBC provides higher oxygen transfer, better mixing and prevents excess biomass accumulation, making RBC a promising and a next generation technique for controlling gaseous VOCs (Datta and Philip, 2014). However, a thorough understanding of biodegradation kinetics of VOCs is required prior to the design and development of RBC. The effect of concentration and flow rate of gaseous VOCs has significant impacts on the performance of RBC. Hence, it is important to optimize these parameters so that RBC can be operated in stable conditions for longer periods during the treatment of gaseous VOCs.

Table 2.4 Typical operational parameters which influence the performance of RBC

Types of RBC	Pollutant	EBCT	Rotation speed	Organic loading rate	Removal	Remark	Referenes
RBC	Mixture of ketones and toluene, ethylbenzene, and o-xylene	30–45 s	24 rpm	108–672 g/m^3 h	88.1–100%	• Overall RE is dropped with the increase in loading rate	Datta and Philip (2014)
Multilayer rotating drum biofilter	Diethyl ether	30 s	1 rpm	32.1–256 g/m^3 h	43–99%	• The RE decreases with the increase in VOC loading rate	Yang et al. (2008a)
Rotating drum biofilter	Hexane	30–120 s	–	2.95 kg/m^3 h	31.1–57.0% for multilayer RDB and 29.5–50.0% for hybrid RDB	• The RE increases with the increase in EBCT. Hexane shows low RE due to its poor water solubility and high Henry's law constant	Yang et al. (2008b)
Hybrid rotating drum biofilter	Toluene	38 s	1 rpm	0.505–2.02 kg/m^3 day	74.1–99.8%	• An increase in the organic loading rate decreases the overall RE of the reactor	Yang et al., (2004)

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 GENERAL

This chapter describes in detail the analytical and experimental methodologies to achieve the objectives of the research. It covers experimental methodologies for biodegradation of benzene in batch reactor and the process optimization using RSM. It also provides the design and installation of sponge-medium based RBC, its operation, acclimatization and biofilm development during the treatment of VOCs.

3.2 MATERIALS

3.2.1 Instrumentation

Various instruments used in the present study, their use and model specification are listed in Table 3.1.

3.2.2 Glassware and chemicals

All glass wares used in this study were purchased form Borosil, India. The glass wares were cleaned using chromic acid solution followed by washing with tap water, then distilled water, and finally dried at 105 ^{o}C for an hour, before being used for experiment.

Analytical grade (99.5-99.8% purity) chemicals of benzene, toluene, ethylbenzene, and xylene obtained from Hi-media, India were used for this study. Analytical grade minerals such as $(NH_4)_2SO_4$, K_2HPO_4, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, $CaCl_2$, $ZnSO_4 \cdot 7H_2O$, $MnSO_4$, H_3BO_3, $NiCl_2$, $COCl_2$, $(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$, $FeCl_3$, and $CuSO_4 \cdot 5H_2O$ purchased from Hi-media, India, were used to prepare the mineral salt medium (MSM).

Table 3.1 Instruments and their specifications used for the present study

Instruments	Parameters measured/Application	Model specification
• Gas chromatography	• Benzene	• CIC, GC Dhruva, India
• VOC analyzer	• BTEX	• Ion science, PhoCheck TIGER, UK
• Liquid chromatography mass spectrometer	• Metabolic intermediates during benzene degradation	• LC-MS, Model-Q-Tof Premier, USA
• Field emission scanning electron microscope	• Biofilm morphology on the surface of sponge media	• Zeiss, Model-Sigma,
• CO_2 analyzer	• CO_2	• TSI, IAQM-7545, USA
• UV-visible spectrophotometer	• Biomass concentration in terms of cell growth	• Cary 50, Varian, USA
• pH meter	• pH	• Systronics, model-361, India
• Dissolved oxygen meter	• Dissolved oxygen (DO)	• Eutech, CyberScan-DO 110, Singapore
• Muffle furnace	• MLVSS or VSS	• Multispan, India
• Hot air oven	• MLSS	• ICT, India
• BOD incubator	• Incubation of microbial culture	• ICT, India
• Shaking incubator	• For growth kinetic study	• Labtech, LSI-1005R, India
• Centrifuge	• To separate biomass from solution	• REMI CM-8 PLUS, India
• Water purification system	• To provide ultra-pure water	• MERCK MILLIPORE, India
• Autoclave	• Sterilization	• REICO, India
• Magnetic stirrer	• Uniform mixing of solution	• Tarsons, SPINT-5020
• Air compressor	• To provide air stream to RBC	• ROCKER-320, Tarsons
• Geared motor with speed controller	• To rotate the shaft of RBC with a uniform speed	• LINIX-LYC13220S, China
• Light microscope	• Morphology of microorganism	• Nikon Eclipse-E200, India
• Weighing balance	• Wight of chemicals	• KERN-ABJ 120-4NM
• Horizontal laminar airflow	• Maintenance of microbial culture in aseptic manner	• Clean air system, CAH-1800, India
• Peristaltic pump	• Feeding of nutrient medium into RBC	• Miclins, PP-20-EX, India

3.3 LABORATORY ANALYSIS

3.3.1 VOCs and CO_2

Benzene concentration in the liquid samples were analyzed by gas chromatography (Model – GC Dhruva, Chromatography and Instruments Company, Vadodara, India), equipped with a capillary column BPX 70 (30 m x 0.32 mm x 0.25 µm) and with a flame ionization detector (FID). The injector, oven and detector temperature were maintained at 210, 60, and 230 ºC, respectively (Mathur et al., 2007). The hydrogen gas was used as the fuel and nitrogen gas was used as the carrier at a flow rate of 30 ml/min. The known amount of BTEX compounds were injected in n-hexane, kept in a GC vial and sealed with Teflon septum as per the standard method (Lodge, 1991). The samples were further injected in GC-FID for analysis and the peaks obtained for respective compounds. Basing on the peak area calibration curves were prepared for BTEX as shown in Appendix-I and were used to analyze the unknown concentrations. The liquid samples from the batch experiments were collected at discrete time intervals, centrifuged at 10,000 rpm for 10 min to separate cell mass from the supernatant, and then residual benzene was extracted with n-hexane for analysis in gas chromatography (GC). Similarly, during the screening of enriched culture, the concentrations of BTEX in the liquid sample were analyzed in GC and the concentration of the samples were determined from the calibration curves as shown in Appendix-I. To determine metabolites during benzene biodegradation, the sample was analyzed in liquid chromatography mass spectrometer (LC-MS, Model: Q-Tof Premier, USA).

The gaseous samples released from RBC were analyzed to determine the benzene concentration using GC-FID with similar method mentioned above. A calibration curve was prepared by injecting a known amount of benzene in methanol at different concentrations

into a sealed bottle with Teflon septum and was analyzed by GC. The calibration curve of benzene using methanol as a solvent shown in Appendix-I was used to determine concentrations of sample. The gaseous samples containing benzene were collected at inlet and outlet ports of RBC daily by scrubbing in methanol (10 ml methanol for 1 min) and concentrations were analyzed by GC as described by Pandey et al. (2007). The concentrations reported are the average of two replicates (n = 2). However, during the treatment of xylene, combined toluene and xylene, and BTEX mixture, the gas phase concentrations were measured daily at inlet and outlet ports of RBC by VOC analyzer having PID detector (Ion science, UK) (Gandu et al. 2013).

The CO_2 concentrations were measured by an automatic CO_2 analyzer (TSI, IAQM-7545, USA) at air inlet and outlet ports of the RBC.

3.3.2 Dissolved oxygen and pH

The dissolved oxygen (DO) concentration of effluent liquid was measured daily using DO meter (Eutech, CyberScan DO 110). The pH of effluent liquid was measured using digital pH meter (Systronics, model-361, India), which was calibrated periodically using standard buffer solutions.

3.3.3 Nutrients

The $(NH_4)_2SO_4$, and K_2HPO_4 and KH_2PO_4 were continuously supplied to RBC in the form of nutrient media or MSM as the sources of nitrogen and phosphorus, respectively. The concentrations of ammonical nitrogen (NH_3-N), nitrite (NO_2-N), nitrate (NO_3-N), and phosphorus (PO_4-P) of effluent liquid were determined at steady state. The effluent liquid sample was centrifuged at 10,000 rpm for 5 min, and the supernatant was used for the analysis. The analyses of these nutrients were carried out as per the standard methods

(APHA, 2005). For example, NH_3-N was determined by phenate method, NO_2-N by N-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrocholoride) colorimetric method, NO_3-N by ultraviolet spectrometric screening method, and PO_4-P was determined by the stannous chloride method.

3.3.4 Cell density

The cell density or cell growth was determined by measuring its absorbance at 600 nm wavelength (OD_{600}) using a UV-visible spectrophotometer (Cary 50, Varian, United States). The OD_{600} was then expressed as dry cell weight (MLSS) by a calibration curve plotted between OD_{600} and the dry weight of biomass (Sahoo et al., 2014), is shown in Appendix-II. One unit of OD_{600} is equal to 525 mg/l of dry cell weight.

3.3.5 Biomass concentration and cell viability

The biomass concentration as volatile suspended solids (VSS) in liquid effluent was determined in accordance with the standard methods (APHA 2005).

The colony forming unit (CFU) of the biofilm grown on the sponge medium was analyzed by collecting biomass from both ends and middle of RBC, mixed together in equal proportions (w/w). The predetermined homogenized mixed sample was suspended in sterile water and serially diluted for microbial colony counting by pour-plate method. Nutrient agar was used for the total microbial count, while the mineral salt agar (MSA) supplemented with a desired quantity of benzene along with agar 1.5% (w/v) was used for the determination of specific benzene degraders (specific count) (Mudliar et al. 2008). The results of the total microbial count and specific count were expressed as CFU/g of the biofilm weight. The specific count expressed as CFU/ml was also analyzed in liquid effluent throughout the operation to ensure the increase in numbers of benzene degraders.

3.3.6 Isolation and characterization of predominant VOCs degrading strain

The 1 ml of enriched-mixed microbial culture was inoculated in 100 ml of MSM with an optimum pH and benzene concentration. The flask was then incubated at 30 ºC and 150 rpm for a period of 48 h and the grown culture was serially diluted and cultured on MSA plates containing benzene. Plates were incubated at 30 ºC for 48 h, after which the colonies grown were individually selected and streaked in MSA plates at various increasing concentrations of benzene. The bacterial colonies that profusely developed at higher concentration of benzene were selected and re-streaked on nutrient agar for identification. Singh and Fulekar (2010) used the same technique for the isolation of potential benzene degrading microorganism from cow dung. The isolated predominant benzene degrading colonies from the mixed culture were gram stained and analyzed for biochemical and physiological characterization using KB003 identification kit for *Enterobacteriaceae* (Himedia, India). It was found that the isolates belong to the member of same genus. Further, the molecular characterization was performed to re-identify the strain (Mathur et al., 2007). The genomic DNA was extracted from the isolate and the fragment of 16S rDNA gene was amplified with universal bacterial primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) using polymerase chain reaction (Sriprapat and Thiravetyan, 2016). The 16S rDNA sequencing was performed by Xcelris Labs Ltd., Ahmedabad, India. The 16S rDNA gene sequence was used to accomplish BLAST alignment in the GenBank database. First fifteen sequences were selected on the basis of maximum identity score and aligned using CLUSTAL W software, and phylogenetic tree was prepared with the molecular evolutionary genetic analysis package (MEGA 5) based on the study of Gandu et al. (2013).

The above methodology was followed for the isolation and characterization of the predominant strain responsible for biodegradation of benzene after the batch kinetic study is explained in detailed by Singh and Fulekar, (2010). After the successful operation of RBC for treatment of benzene and the mixture of BTEX in waste gas streams, the same technique was used for isolation and identification of predominant benzene and BTEX degrading strains in mixed culture responsible for biodegradation.

3.3.7 Microscopic analysis

Light microscope (Nikon Eclipse-E200, India) was used to determine the cell morphology of isolated strain. Whereas, the biofilm morphology grown on the bio-support medium (sponge) was analyzed by field emission scanning electron microscope (FESEM, Model: Sigma, Zeiss). The small fragments of sponge containing the biofilm were collected on 0^{th} day and 138^{th} day. The microorganisms in the sponge medium were fixed with 2% glutaraldehyde, washed with phosphate buffer (pH 7.0) and then dehydrated with ethyl alcohol, dried and mounted on aluminum stubs for gold coating. Then, the image of sponge medium before (0^{th} day) and after (138^{th} day) were scanned in FESEM to determine the micrograph.

3.4 BATCH STUDY FOR BIODEGRADATION OF BENZENE

3.4.1 Collection and isolation of mixed microbial culture

The activated sludge was collected from a wastewater treatment plant of petrochemical industry located in Guwahati, India. The sludge was initially allowed to settle for a few hours, and then 10 ml of settled sludge was mixed with 100 ml of saline water (1%). After shaking for 30 min, the sludge was again allowed to settle for 1 min to remove the inorganic solids. Further, supernatant was centrifuged in a centrifuge (Remi, India) at 10,000 rpm for 3

min to obtain the microbial cells. This technique is used by Datta and Philip (2012) to isolate the mixed microbial culture from activated sludge. The microbial culture was then enriched in MSM containing 1 g/l of glucose by varying benzene concentration in the range of 25–600 mg/l under the agitation of 150 rpm at 30 ^{o}C for a period of 16 days.

3.4.2 Biodegradation of benzene

A batch study was carried out to determine the biodegradation kinetics of benzene in a MSM at a pH of 6.7 ± 0.05 by varying the benzene concentrations from 25 to 600 mg/l. Erlenmeyer flasks of 250 ml capacity containing 100 ml of MSM with Teflon liner screw cap and a sampling port on the bottom were used. The enriched mixed culture was grown overnight and inoculated in the flasks to obtain an initial OD_{600} from 0.11–0.15. The flasks were kept on shaking incubator (LSI-1005R, India) at 30 ^{o}C with 150 rpm. For each concentrations of benzene (25, 50, 100, 200, 300, 400, 500, 600 mg/l), batch experiments were performed in duplicate along with abiotic control. The flasks were removed at an interval of 6 h time span up to 12 h, and then at every 12 h for a period up to 72 h. A sample volume of 2 ml was collected from the sampling port of flasks using the gas-tight syringe to estimate cell growth and its corresponding benzene biodegradation. In control flasks, benzene concentrations were decreased by 10–16% at the end of each batch study for eight different concentrations, and the degradation rates were estimated after correcting with the control experiments.

3.4.3 Growth kinetic models

Several growth kinetic models are used to estimate the biokinetic parameters in the biodegradation experiments. These models are substrate inhibition models such as Haldane (Mathur and Majumder, 2010), Aiba (Kim et al., 2005), Monod inhibition (Priya and Philip, 2013) and noninhibition model such as Monod growth (Kim et al., 2005). The kinetic data

generated from the batch study were used to estimate the biokinetic parameters such as maximum specific growth rate (μ_{max}), half saturation constant (K_s), and inhibition constant (K_i) by a nonlinear regression method using Microsoft Excel solver program (Krithika and Philip, 2016).

The growth of microorganism by utilising benzene as a single substrate was represented by Monod model (Saravanan et al., 2008) as given by equation 3.1.

$$\mu = \frac{\mu_{max} \cdot S}{K_s + S} \tag{3.1}$$

where, μ is the specific growth rate (1/h), μ_{max} the maximum specific growth rate (1/h), K_s the half saturation constant (mg/l), and S is the substrate concentration (mg/l).

However, the Monod model does not satisfactorily explain the inhibition of substrate on microbial growth. Therefore, the growth kinetics of the culture due to substrate inhibition can be represented by the substrate inhibitory models such as Haldane, Monod inhibition, and Aiba. The equation 3.2 shows the Haldane model (Saravanan et al., 2011).

$$\mu = \frac{\mu_{max} \cdot S}{K_S + S + \frac{S^2}{K_i}} \tag{3.2}$$

where, K_i is the inhibition constant (mg/l). The Halden model is based on the effect of substrate on growth of the culture (Kumar et al., 2005). For inhibitory type growth kinetics, the Monod model has been modified with the additional kinetic parameter, K_i, which is referred to the Monod inhibition model (Datta and Philip, 2012) given in equation 3.3.

$$\mu = \frac{\mu_{max} \cdot S \cdot K_i}{(K_S + S)(K_i + S)} \tag{3.3}$$

Another model of inhibitory kinetics was proposed by Aiba (Sahoo et al., 2011) to represent microbial growth kinetics, as given by equation 3.4.

$$\mu = \frac{\mu_{max} \cdot S \cdot \left[exp\left(\frac{-S}{K_i}\right)\right]}{(S+K_S)} \quad (3.4)$$

3.4.4 Experimental design for process optimization

Central composite design (CCD) based on response surface methodology (RSM) was employed for determining optimum pH and benzene concentration to enhance the specific growth rate and degradation rate of the culture. Therefore, two factors (k = 2), pH (X_1) and benzene concentration (X_2) were chosen as the variables of the function at low value (-1), central (0) and high (+1). Table 3.2 summarizes the levels and variables involved in the design strategy. The pH and concentration range were selected based on the previous literature. The total numbers of experimental runs were formulated as

$$2^k + 2k + n_0 \quad (3.5)$$

where, k is the number of factors, and n_0 is the number of replicates at the center point (Sahoo et al., 2010).

Thus, a total of 13 experiments in duplicate were carried out. For optimization, batch studies were conducted and samples were collected at regular intervals from each experimental run to analyze cell growth and benzene concentrations as described earlier in section 3.4.2. Control experiments were also carried out simultaneously to assess the abiotic losses. The regression analysis of the experimental data was studied using MINITAB 16.

Table 3.2 Experimental ranges and levels of independent variables

Variables	Symbol	Coded levels		
		Low (-1)	Center (0)	High (+1)
pH	X_1	6	7	8
Benzene concentration	X_2	25	312.5	600

3.5 RBC FOR BIODEGRADATION OF VOCs

3.5.1 Design and installation

The RBC was built of a closed chamber with a rotating contactor consisting of perforated drum supported by a sponge medium. The chamber was made of 6 mm perspex sheet of length of 410 mm, width 400 mm and height 400 mm with a working volume of 0.0656 m^3 (65.6 L). Fig. 3.1 shows the schematic of the laboratory scale RBC with important components.

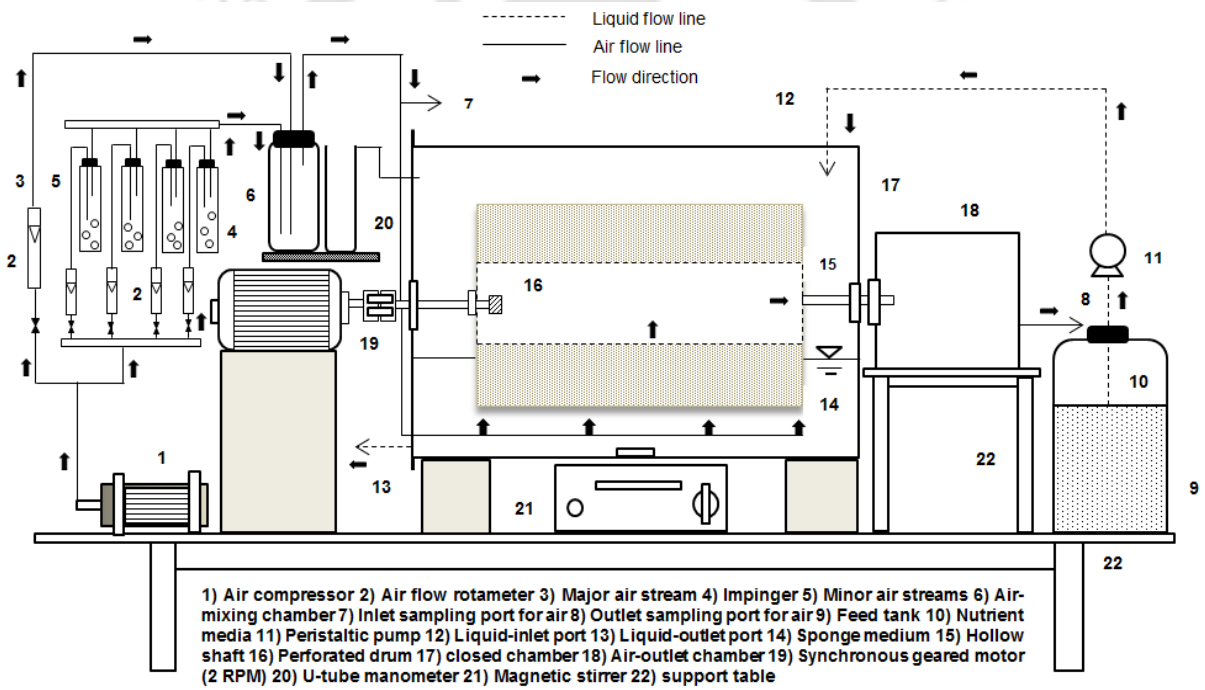


Fig. 3.1 Schematic of RBC for the control of VOCs

For maintenance and cleaning, one side of the chamber was bolted so that it could be opened while the rest of the parts were sealed by placing butyl rubber gaskets in between them to ensure the chamber airtight. The sponge medium of volume 0.008 m^3 was mounted concentrically over the drum to support the biofilm growth. The drum was closed by disks at both ends, and was placed horizontally in the center of the chamber with the help of stainless steel hollow shaft. The shaft at its one side was coupled with a geared motor having a speed

controller and the other side was connected to the air outlet chamber. The shaft rotates the drum at 2 rpm along with the supporting medium during operation. The inlet and outlet ports were provided for feeding and discharge of MSM by a peristaltic pump. Similarly, inlet and outlet ports were provided for sampling of gaseous VOCs. Fig. 3.2 shows the picture of the RBC after design and complete installation before starting the operation.

Fig. 3.2 The RBC after design and complete installation in picture

3.5.2 Operation

The MSM was continuously supplied to the RBC at a flow rate of 5.1 l/d and discharged with the same flow rate at the outlet. The submergence of sponge medium in the nutrient solution was consistently about 26% to initiate the growth of biofilm. The air stream was divided into minor and major air streams to connect to the mixing chamber and to the impingers, respectively. The waste gas containing VOCs was generated from the impingers at normal temperature (27 ± 3 ºC) and pressure, connected to the mixing chamber. Rotameters were used to control the major and minor air streams for maintaining a constant flow rate and a desired empty bed contact time (EBCT). The waste gas enters the reactor through the air inlet

port and is released at the bottom of RBC through air diffusers. The gaseous VOCs are absorbed partly into the nutrient medium, causing the suspended cell biodegradation (SCB). The unabsorbed VOCs pass through the supporting medium, where the biodegradation occurring by the biofilm grown, and finally exit through the hollow shaft into the outlet chamber. The gaseous samples from the air inlet and outlet ports were collected every 24 h at different loading rates to determine the RE. The biomass was mixed uniformly in the liquid medium of RBC by a magnetic stirrer and a U-tube manometer (in cm of H_2O) was attached to the RBC to measure the pressure drop. Fig. 3.3 describes the operation procedure of RBC.

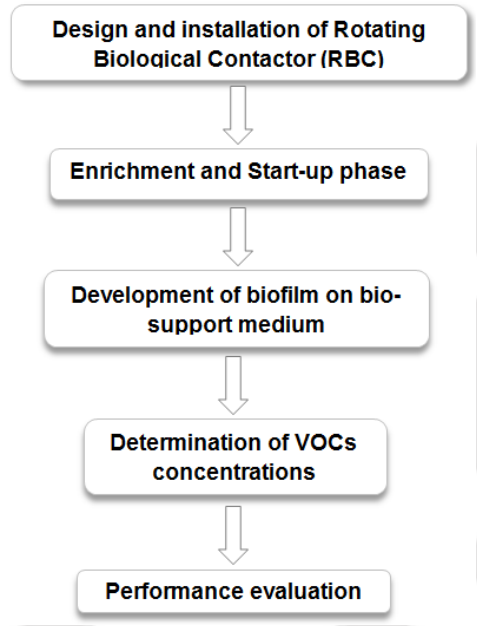


Fig. 3.3 Operational procedure of RBC

3.5.3 Seeding and medium

The source of a mixed biomass was the activated sludge, obtained from the wastewater treatment plant of the Indian Oil Corporation, Guwahati, which was used as an inoculum in RBC for the treatment of VOCs in the waste gas streams. Table 3.1 shows the characteristics of the activated sludge with the presence of macro and micro nutrients such as carbon, nitrogen, phosphorus and sulfate. The MSM was prepared with the constituent (g/l of water):

K_2HPO_4 (0.8), KH_2PO_4 (0.4), $(NH_4)_2SO_4$ (1), $MgSO_4 \cdot 7H_2O$ (0.5), $CaCl_2$ (0.125), $FeSO_4$ (0.01), $NaHCO_3$ (0.048), H_3BO_3 (0.0225), $ZnSO_4 \cdot 7H_2O$ (0.06), $NiCl_2$ (0.018), $CuSO_4 \cdot 5H_2O$ (0.0025), $MnSO_4$ (0.0375), and $COCl_2$ (0.014). These chemicals were of the analytical grades of 99.5–99.8% purity and the pH of the MSM was 6.95 ± 0.05.

Table 3.3 Characteristics of the activated sludge used in RBC

Parameters	Units	Quantity[a]
pH	–	6.97 ± 0.03
DO	mg/l	2.9 ± 0.15
Temperature	^oC	26 ± 0.5
Mixed liquor suspended solids	mg/l	7162 ± 20
Volatile suspended solids	mg/l	5476 ± 16
Biological oxygen demand	mg/l	120 ± 5
Chemical oxygen demand	mg/l	7272 ± 31
Total Kjeldahl nitrogen	mg/l	630 ± 7.5
Total phosphate	mg/l	1.734 ± 0.027
Total sulphate	mg/l	941.197 ± 10

[a] The values represent the mean and standard deviation of two replicates

3.5.4 Acclimatization and development of biofilm

The RBC was initially filled with 15 l of MSM and 2 l of activated sludge. When the RBC was rotated at 2 rpm, the biomass gradually adhered onto the supporting medium, initiating the biofilm formation. The VOCs concentration was gradually increased, together with 1 g/l glucose as cometabolite, to acclimatize and achieve optimum cell growth. During the start-up phase, the RBC was operated in a closed loop with respect to biomass to maximize the adherence to the support medium and to prevent biomass loss (Kennes and Veiga, 2001). To ensure increase in the number of microorganisms, VSS of the liquid phase, total microbial count, specific plate count was analyzed in the start-up phase.

3.5.5 Performance evaluation

The performance of the RBC was evaluated by RE (%) and EC (g/m^3 h) for different ILR (g/m^3 h) of gaseous VOCs at different EBCT (min), estimated by the following equations:

Inlet loading rate, $ILR = \left(\frac{Q \cdot C_i}{V}\right)$ (3.6)

Elimination capacity, $EC = \frac{Q(C_i - C_o)}{V}$ (3.7)

Empty bed contact time, $EBCT = \frac{V}{Q}$ (3.8)

Removal efficiency, $RE(\%) = \left(\frac{C_i - C_o}{C_i}\right) \times 100$ (3.9)

Overall removal efficiency, $RE(\%) = \left(\frac{\sum C_i - \sum C_o}{\sum C_i}\right) \times 100$ (3.10)

CO_2 production rate, $P_{CO_2} = \frac{Q\left(CO_{2_o} - CO_{2_i}\right)}{V}$ (3.11)

where, Q is the gas flow rate (m^3/h), V the total medium volume in RBC (m^3), and C_i and C_o are the inlet and outlet concentrations (g/m^3) of VOC, respectively. The CO_2 concentration (g/m^3) at inlet and outlet of RBC are designated as CO_{2i} and CO_{2o}, respectively.

3.5.6 Kinetic models for biodegradation of VOCs

The kinetic coefficients describe the kinetic behavior of RBC are determined from the steady state outlet concentrations for various inlet concentrations of VOCs using a modified Monod equation. Mathur et al. (2006) used the same method to determine the kinetic coefficients in the biofilter. The assumptions made for RBC are as follows: (1) Oxygen limitation is not present in the reactor hence the system is aerobic and (2) At steady state the growth rate of

microorganism is in equilibrium with its decay rate. Hence, the kinetic coefficients were considered to be constant over the period of time (Rahul et al., 2012).

The kinetic coefficients were determined using the plug flow model without dispersion at steady state using the equation 3.12.

$$\frac{dC}{dt} = -Q\frac{dC}{dv} + r \tag{3.12}$$

where, C is concentration of VOC (g/m^3), Q the gas flow rate (m^3/h), t the time (h), V the medium volume (m^3), and r is the rate of reaction defined by equation 3.13.

$$r = \frac{r_{max}C}{K_s+C} \tag{3.13}$$

where, r_{max} is the maximum biodegradation rate per unit of medium volume of RBC (g/m^3 h) and Ks is the half saturation constant (g/m^3).

At steady sate, the accumulation term dC/dt equals zero. Integrating equation (3.12) under the given conditions, $C = C_i$ at $V = 0$ (at the inlet) and $C = C_o$ at $V = V_l + V_d$ (at the outlet), where V_l is the liquid medium volume and V_d is the sponge medium volume in RBC**.** Solving equations (3.12) and (3.13), resulting equation 3.14.

$$\frac{\frac{v_l+v_d}{Q}}{C_i - C_o} = \frac{K_s}{r_{max}}\frac{1}{C_{ln}} + \frac{1}{r_{max}} \tag{3.14}$$

The equation (3.14) can further be simplified and represented as equation 3.15

$$\frac{V/Q}{C_i - C_o} = \frac{K_s}{r_{max}}\frac{1}{C_{ln}} + \frac{1}{r_{max}} \tag{3.15}$$

where, V is the total medium volume (V_l is the liquid medium volume and V_d is the sponge medium volume, m^3), C_i and C_o the influent and effluent concentrations (g/m^3) of VOC, respectively, and $C_{\ln}$ is the log mean concentration $[(C_i - C_o)/\ln(C_i/C_o)]$.

CHAPTER 4

KINETIC MODELING AND OPTIMIZATION OF BENZENE BIODEGRADATION

4.1 GENERAL

In biodegradation process, microorganisms plays a vital role in metabolizing aromatic hydrocarbons for their growth and development are an effective way of treating hazardous wastes (Ojumu et al., 2005). Benzene is one of major VOCs produced from natural and anthropogenic activities and also causes environmental pollution (Liu et al., 2010). Several microorganisms having ability to degrade aromatic hydrocarbons such as benzene and its derivatives are isolated and identified. *Pseudomonas* sp. is reported to be the most common and efficient strain (Ferhan et al., 2002). The biodegradation of benzene is investigated by many researchers using pure culture (Reardon et al., 2000; Zhang et al., 2013), coculture (Shim and Yang, 1999), mixed microbial culture (Maliyekkal et al., 2004), and activated sludge (Lodaya et al., 1991). The effect of benzene, toluene, and xylene concentration on the specific growth rate and degradation rate of mixed microbial culture is studied by Maliyekkal et al. (2004). The Monod and Haldane kinetic models can predict accurately the biodegradation of toxic compounds like benzene and toluene (Priya and Philip, 2013). Kim et al. (2005) investigated the kinetics of benzene biodegradation and estimated the biokinetic constants like maximum specific growth rate (μ_{max}), half saturation constant (K_s), and inhibition constant (K_i) using *Pseudomonas* species at neutral medium pH.

The microbial cell growth varies with a different external source of carbon unless acclimatized to a specific substrate. Some studies have shown that even after acclimatization, microorganisms exhibit inhibition due to high toxicity of substrate (D'adamo et al., 1984).

The growth of microorganisms is inhibited when substrate concentration is high and leads to starvation when it is low (Singh and Fulekar, 2010). Therefore, knowing optimum concentration at which maximum biodegradation will occur is extremely important. A few studies have applied one-factor-at-a-time (OFAT) method for process optimization to improve biodegradation (Zhao et al., 2016). The method, however, takes more time and also is inefficient in determining the interactions among variables and prediction of optimum environment (Zu et al., 2013). There exists a method based on response surface methodology (RSM), such as central composite design (CCD), overcomes the above mentioned drawbacks (Yao et al., 2009). The same method is used by Sahoo et al. (2010) for optimizing the media components and to enhance 4-chlorophenol biodegradation and the specific growth rate of the culture.

In this research, the batch kinetic study has been performed in shake flasks over a concentration ranging from 25 to 600 mg/l to determine the specific growth rate and degradation rate using a mixed microbial culture. The experimental data has been used to determine the biokinetic constants such as μ_{max}, K_s, and K_i using Haldane, Monod inhibition and Aiba substrate inhibition models, and noninhibition model of Monod. Further, CCD followed by RSM has been employed to optimize the process variables to enhance the specific growth rate and degradation rate of the culture. At the optimum combination, the metabolic intermediates produced during benzene biodegradation have also been analyzed. An attempt has been made to isolate the predominant benzene degrading microorganism from the mixed culture and identify using morphological, biochemical, physiological, and 16S rDNA techniques (Singh and Fulekar, 2010).

4.2 KINETICS OF BENZENE BIODEGRADATION

Fig. 4.1(a) shows the effect of initial benzene concentration on cell growth (OD_{600}). No lag phase has been observed during the growth, indicating no sudden inhibition of benzene. This might be because the mixed culture was enriched with high concentration of benzene (Kim et al., 2005). The cell growth was less at low concentration up to 50 mg/l due to starvation and maximum at 300 mg/l concentration. It decreased with further increase in the benzene concentration, indicating the inhibition. Observing the trend in the figure, it can be concluded that mixed culture at higher concentration of benzene takes a long time to reach the stationary phase, which may be attributed to its toxicity, especially beyond 300 mg/l (Hamed et al., 2003). Further, the effects of benzene concentration on the specific growth rate and the degradation rate of the culture were studied. The specific growth rate (μ) has been estimated from the cell growth versus time curves for each benzene concentration using equation (4.1).

$$\mu = \frac{\boldsymbol{ln}\,(X_2/\boldsymbol{X_1})}{(\boldsymbol{t_2}-\boldsymbol{t_1})} \tag{4.1}$$

where, X_2, X_1 are the dry cell weights at maximum and initial growth time t_2 and t_1, respectively, during the logarithmic phase. In the growth curves (Fig. 4.1(a)), it was observed that at benzene concentrations of 25–300 mg/l, the time to reach the end of logarithmic phase was 48 h, while beyond that it was 60 h up to 500 mg/l and 72 h for 600 mg/l. The degradation rate (r) has also been determined for each concentration from the slope of the biodegradation curve as shown in Fig. 4.1(b).

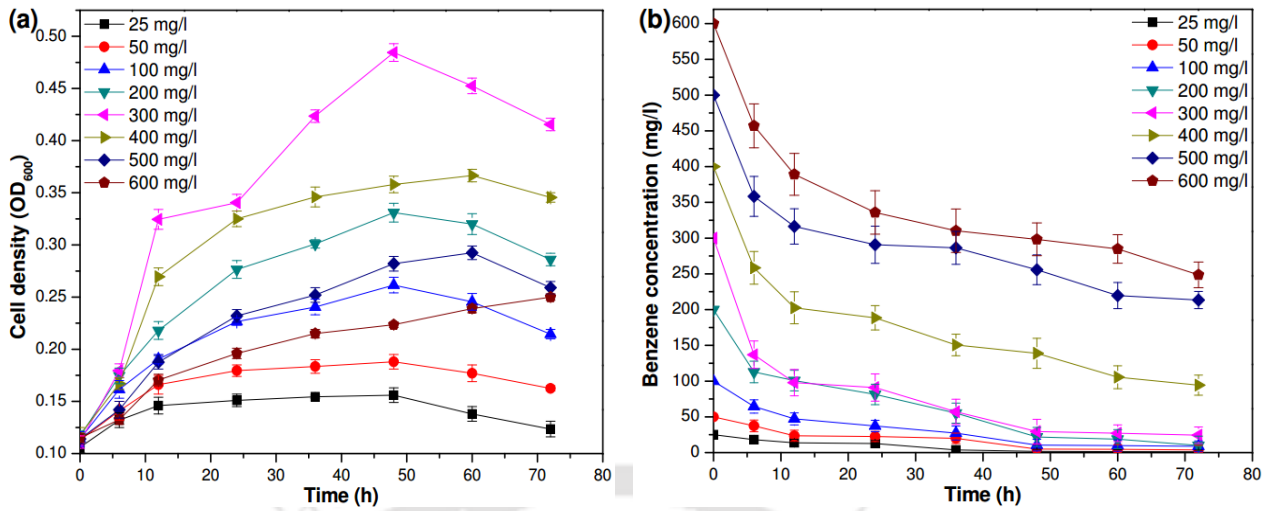


Fig. 4.1 Effect of benzene concentration on (a) cell growth and (b) biodegradation

Both specific growth rate and degradation rate increased with increase in benzene concentration up to 300 mg/l, and thereafter decreased due to inhibition. The maximum specific growth rate of 0.045 ± 0.001 1/h and degradation rate of 5.536 ± 0.089 mg/l h were observed at 300 mg/l of benzene concentration. Shim and Yang (1999) reported the inhibition of cell growth occurring at a concentration of 135 mg/l benzene, in which both specific growth rate and degradation rate are less as compared to the present study.

4.3 MODELING OF GROWTH KINETICS

It has been observed that the specific growth rate increases with the increase in benzene concentration, but decreases beyond 300 mg/l which indicates the substrate inhibition (Fig. 4.2). This process has been modeled using various kinetic models to determine the biokinetic constants such as μ_{max}, K_s, and K_i. These kinetic models are shown in Table 4.1, which were solved by nonlinear regression method (Krithika and Philip, 2016). Fig. 4.2 presents the comparison of the experimental and modeled specific growth rate. The Halden modeled values matched well with the observed values. Table 4.2 shows the biokinetic constants obtained by the models and comparison with the literature values.

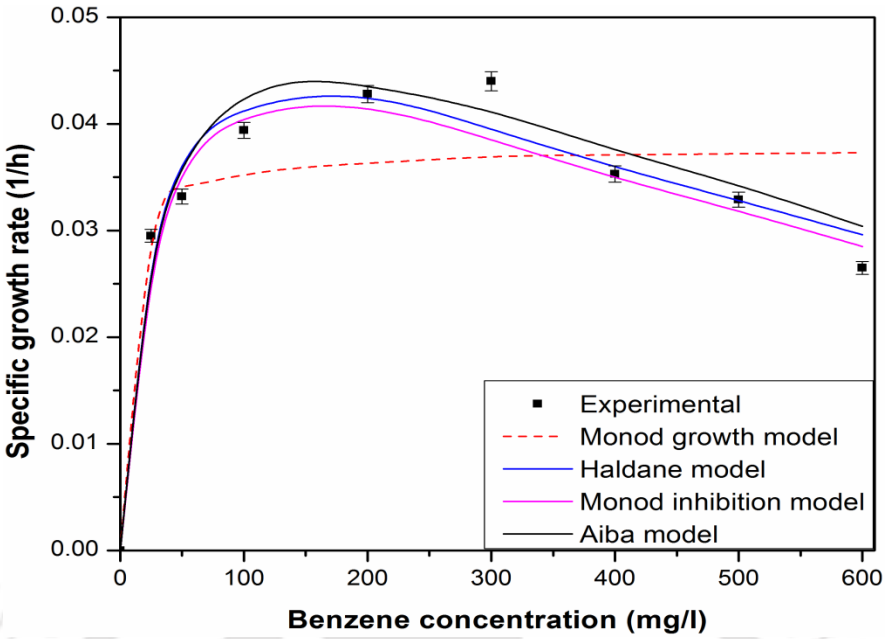


Fig. 4.2 Experimental and modeled specific growth rate of the mixed culture at various benzene concentrations

The results strengthened the fact that the Halden model is the best, which described the biodegradation kinetics of benzene with much lower root mean square error (RMSE), i.e. 0.0033. The K_i was 488 mg/l, which was much higher than the value of 100 mg/l found by Kang et al. (2016), indicating the mixed culture was less susceptible to inhibition (Mathur and Majumder, 2010). The reason could be the acclimatization at high benzene concentration. The results of this study, except the K_s value obtained by Haldane model, matched well with the results reported by Mathur and Majumder (2010). The *Ks* value was slightly lower, which is indicating that the minimum substrate concentration at which the microorganism can grow. The low μ_{max} and high K_i value of this study may be due to the higher range of benzene concentration used in the batch kinetic process. The results of the Monod inhibition model and the Haldane model were almost similar, while the Aiba model predictions were slightly higher than the Haldane model, which compared with the previous studies in Table 4.2. The Monod growth model showed a substantial difference in the K_s

value with the others. This may be because the Monod growth model is a noninhibition model, and the others are inhibition models.

Table 4.1 Different kinetic models for estimation of biokinetic parameters

Model	Mathematical form	References
Monod growth model	$\mu = \frac{\mu_{max} \cdot S}{K_s + S}$	Saravanan et al. (2008)
Haldane model	$\mu = \frac{\mu_{max} \cdot S}{K_S + S + \frac{S^2}{K_i}}$	Saravanan et al. (2011)
Monod inhibition model	$\mu = \frac{\mu_{max} \cdot S \cdot K_i}{(K_s + S)(K_i + S)}$	Datta and Philip (2012)
Aiba model	$\mu = \frac{\mu_{max} \cdot S\left[exp\left(\frac{-S}{K_i}\right)\right]}{(S + K_s)}$	Sahoo et al. (2011)

μ = specific growth rate (1/h), μ_{max} = maximum specific growth rate (1/h), K_s = half saturation constant (mg/l), S = substrate concentration (mg/l), K_i = inhibition constant (mg/l)

4.4 OPTIMIZATION OF PROCESS PARAMETERS USING RSM

The optimization of process parameters to enhance benzene biodegradation was carried out by the design of experiment as a function of factors. Two factors or parameters such as pH (X_1) and benzene concentration (X_2) were chosen as the variables at five coded levels *(-α, -1, 0, +1, +α*). As per the CCD matrix, 13 experiments were performed in duplicate and the specific growth rate and degradation rate were determined as described in section 4.2. Table 4.3 shows the levels and variables with their experimental and predicted responses of specific growth rate (Y_1) and degradation rate (Y_2). The obtained experimental results were fitted to the quadratic polynomial equation (Eq. 4.2).

Table 4.2 The biokinetic parameters of various models and their comparison with the literature values for benzene biodegradation

Micro-organism	Monod growth model				Haldane model				Monod inhibition model				Aiba model			
	μ_{max} (1/h)	K_s (mg/l)	K_i	RMSE	μ_{max} (1/h)	K_s (mg/l)	K_i (mg/l)	RMSE	μ_{max} (1/h)	K_s (mg/l)	K_i (mg/l)	RMSE	μ_{max} (1/h)	K_s (mg/l)	K_i (mg/l)	RMSE
Mixed culture (present study)	0.038	4.9	–	0.0062	0.069	40.9	488	0.0033	0.076	45	443	0.0036	0.070	48	725	0.004
	0.14[a]	4.5[a]	–	–	0.29[b]	15[b]	100[b]	–	0.03[d]	54[d]	230[d]	–	0.3[a]	30[a]	240[a]	–
	–	–	–	–	0.16[c]	71[c]	340[c]	–	–	–	–	–	–	–	–	–

[a]Kim et al. (2005)
[b]Kang et al. (2016)
[c]Mathur and Majumder (2010)
[d]Priya and Philip (2013)

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i \cdot X_i + \sum_{i=1}^{k} \beta_{ii} \cdot X_i^2 + \sum_i \sum_j \beta_{ij} \cdot X_i \cdot X_j \quad (4.2)$$

where, Y is the predicted response variable, β_0 the intercept coefficient, k the number of factors, X_i and X_j the independent variables, β_i the liner coefficient, β_{ii} the quadratic coefficient, and β_{ij} is the interaction coefficient.

Table 4.3 Central composite design matrix showing the experimental and predicted responses of specific growth rate and degradation rate of the mixed culture

Run order	pH (X_1)	Concentration (X_2, mg/l)	Specific growth rate (Y_1, 1/h)		Degradation rate (Y_2, mg/l h)	
			Experimental	Predicted	Experimental	Predicted
1	6.00 (-α)	312.50 (0)	0.0308	0.0312	2.576	2.568
2	8.00 (+α)	312.50 (0)	0.0348	0.0348	3.206	3.035
3	7.00 (0)	600.00 (+α)	0.0284	0.0270	4.310	4.076
4	7.00 (0)	312.50 (0)	0.0485	0.0474	5.636	5.743
5	7.00 (0)	312.50 (0)	0.0476	0.0474	5.595	5.743
6	7.71 (+1)	109.21 (-1)	0.0345	0.0333	1.515	1.560
7	7.71 (+1)	515.79 (+1)	0.0307	0.0317	3.641	3.890
8	7.00 (0)	312.50 (0)	0.0470	0.0474	5.870	5.743
9	6.29 (-1)	109.21 (-1)	0.0340	0.0326	1.144	1.073
10	7.00 (0)	312.50 (0)	0.0460	0.0474	5.715	5.743
11	7.00 (0)	312.50 (0)	0.0480	0.0474	5.901	5.743
12	6.29 (-1)	515.79 (+1)	0.0265	0.0273	3.582	3.716
13	7.00 (0)	25.00 (-α)	0.0301	0.0319	0.504	0.559

4.4.1 Analysis of variance and RSM model

The significance of the coefficients for specific growth rate and degradation rate was determined by *t*-test and the results are presented in Table 4.4a. A large *t*-value with a desired *p*-value indicates that the corresponding coefficient is significant (Su et al., 2011). Therefore, from *t*-test results, the pH and the concentration have individually a considerable effect on the specific growth rate and the degradation rate (*p*-value < 0.05), but no interaction effect

has been observed, as confirmed by the insignificant *p*-value. Table 4.4b shows the results of the analysis of variance (ANOVA). Basically, a higher *F*-value associated with a small *p*-value indicates the respective coefficient is significant (Tanyildizi et al., 2005). The *p*-values for the linear and square terms of the regression models for both specific growth rate and degradation rate were found to be < 0.05, indicating the results have been significant. However, the interaction terms were insignificant (*p*-value > 0.05) for both the regression models. The lack of fit (*F*-value = 3.70 and *p*-value = 0.119 for specific growth rate, and *F*-value = 3.08 and *p*-value = 0.153 for degradation rate) is insignificant or in other words models were significant. A good correlation (R^2 = 0.9839 and 0.9944 for specific growth rate and degradation rate, respectively) also indicated the good agreement the responses predicted by the model with the experimental values as shown in Fig. 4.3. Based on these results, the regression models for both specific growth rate and degradation rate are demonstrated by equations (4.3) and (4.4), respectively.

$$Y_1 = 0.0474 + 0.0012X_1 - 0.0017X_2 - 0.0072X_1^2 - 0.0089X_2^2 + 0.0009X_1X_2 \quad (4.3)$$

$$Y_2 = 5.743 + 0.165X_1 + 1.243X_2 - 1.470X_1^2 - 1.712X_2^2 - 0.078X_1X_2 \quad (4.4)$$

where, Y_1 is the specific growth rate, Y_2 the degradation rate, X_1 the pH, and X_2 is the concentration of benzene.

Table 4.4a Estimated regression coefficients for specific growth rate and degradation rate

Term	Specific growth rate (Y_1, 1/h)			Degradation rate (Y_2, mg/l h)		
	Coefficient	*t*	*p*	Coefficient	*t*	*p*
Constant	0.0470	74.855	0.000	5.7434	68.058	0.000
pH (X_1)	0.0012	2.585	0.036	0.1651	2.475	0.043
Concentration (X_2)	-0.0017	-3.421	0.011	1.2433	18.636	0.000
pH × pH (X_1^2)	-0.0072	-13.422	0.000	-1.4708	-20.558	0.000
Concentration × Concentration (X_2^2)	-0.0089	-16.727	0.000	-1.7128	-23.941	0.000
pH × Concentration ($X_1 \times X_2$)	0.0009	1.306	0.233[a]	-0.0780	-0.827	0.436[a]

[a]Insignificant values

Table 4.4b The analysis of variance of specific growth rate and degradation rate

	Specific growth rate[a] (1/h)					Degradation rate[b] (mg/l h)				
Source	DF	SS (x10^{-3})	MS (x10^{-3})	*F*	*P*	DF	SS	MS (x10^{-3})	*F*	*P*
Regression	5	0.860	0.172	85.68	0.000	5	44.0299	8.8060	247.30	0.000
Linear	2	0.037	0.018	9.19	0.011	2	12.5847	6.2924	176.71	0.000
Square	2	0.819	0.410	204.17	0.000	2	31.4208	15.7104	441.20	0.000
Interaction	1	0.003	0.003	1.71	0.233[c]	1	0.0243	0.0243	0.68	0.436[c]
Residual (Error)	7	0.014	0.002			7	0.2493	0.0356		
Lack of fit	3	0.010	0.003	3.70	0.119	3	0.1740	0.0580	3.08	0.153
Pure error	4	0.004	0.001			4	0.0752	0.0188		
Total	12	0.874				12	44.2791			

DF = degree of freedom, SS = sum of squares, MS = mean square

[a]R^2 = 98.39, R^2 (adj) = 97.24

[b]R^2 = 99.44, R^2 (adj) = 99.03

[c]Insignificant value

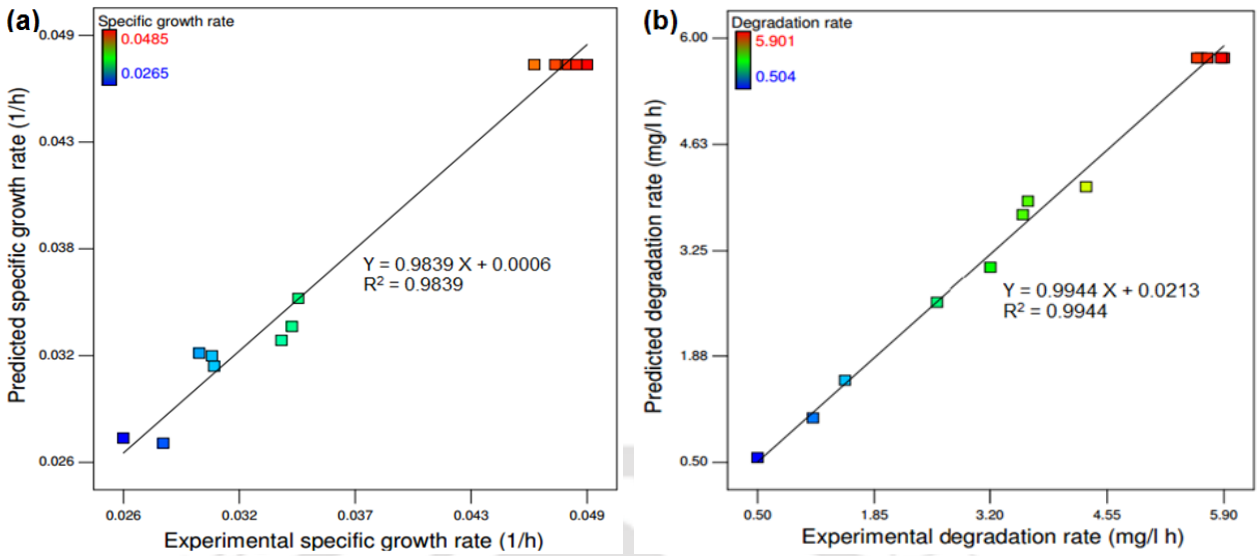


Fig. 4.3 Correlation between the experimental and predicted (a) specific growth rate and (b) degradation rate

4.4.2 Effect of variables on responses

The individual and interaction effects of pH and benzene concentration on both responses specific growth rate and degradation rate have been illustrated with the surface and contour plots. The surface plots in Fig. 4.4 depict that both specific growth rate and degradation rate increases with increasing pH and reach to peak at pH of about 7. The optimal pH of 7 is also being reported by Lee et al. (2002) for benzene biodegradation. Increase of benzene concentration made benzene readily available to microorganisms, as a result both specific growth rate and degradation rate increased. Beyond 300 mg/l, responses decreased indicating inhibition caused by benzene toxicity. The inhibition effect of benzene at concentration beyond 158 mg/l is observed by Monero et al. (2003). As observed in Fig 4.4, the lower and higher levels of both variables results in lower specific growth rate and degradation rate. The response surface contours identify the interactions between variables to predict maximum response. The elliptical contours indicate better interaction between the variables, whereas

circular contours indicate insignificant interaction (Zhao et al., 2016). The contour plots (Fig. 4.4) were circular indicating the less significance of the interaction effect between pH and the benzene concentration, which was also confirmed in statistical analysis (Table 4.4a). Similar non-significant interaction between pH and atrazine concentration are reported during atrazine degradation by Zhao et al. (2016).

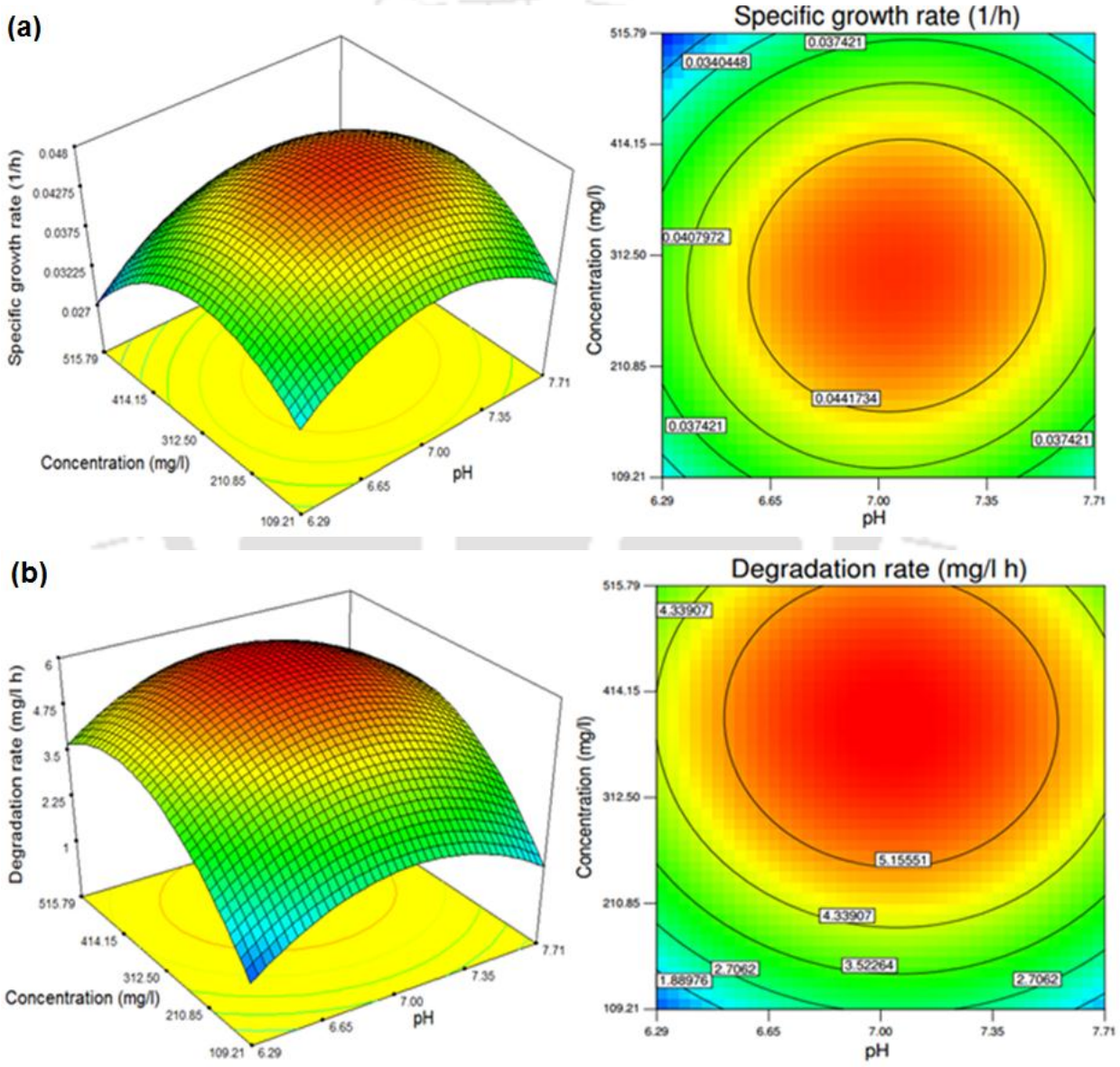


Fig. 4.4 Surface and contour plots of (a) specific growth rate and (b) degradation rate for the interaction between pH and benzene concentration

4.4.3 Response optimization and model validation

Response optimizer of MINITAB 16 (trial version), which takes the desirability function into account, was used to find optimum pH and benzene concentration for achieving maximum response. The individual desirability (*d*) and composite desirability (*D*) for both responses such as specific growth rate and degradation rate was near to 1, shown in Fig. 4.5, which signifies that the function linearly increases to achieve the target values (Sahoo et al., 2010). At an optimum pH of 7.05 and benzene concentration of 332.82 mg/l, the predicted maximum specific growth rate (μ) and degradation rate (r) were 0.047 1/h and 5.854 mg/l h, respectively (Fig. 4.5).

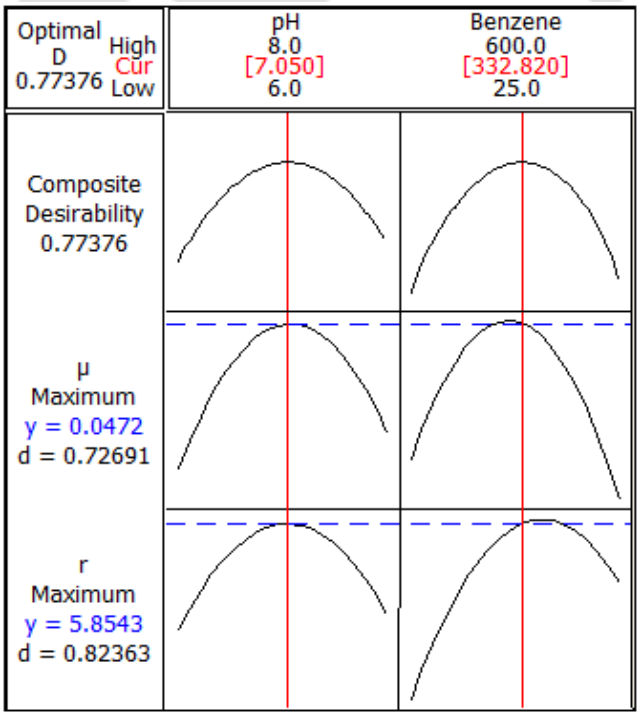


Fig. 4.5 Response surface optimization of pH and benzene concentration for achieving maximum specific growth rate (μ) and degrdation rate (r)

Further, duplicate experiments were performed at the optimum values. The specific growth rate and degradation rate were found to be 0.050 ± 0.002 1/h and 6.011 ± 0.103 mg/l

h, respectively. These values have been in good agreement with the predicted values, thus validated the models. Moreover, after optimization the specific growth rate and degradation rate were improved, from 0.045 ± 0.001 to 0.050 ± 0.002 1/h and 5.536 ± 0.089 to 6.011 ± 0.103mg/l h, respectively. Mathur and Majumder (2010) reported inhibition of benzene at concentration of 148 mg/l with the degradation rate of 3.9 mg/l h. Some other recent studies, for example, Tsai et al. (2013) found benzene inhibition at 52.63 mg/l and Kang et al. (2016) at 100 mg/l. The values observed in this study have been high, i.e. degradation of benzene concentration of up to 332.820 mg/l without inhibition. Therefore, the process optimization carried out in this study not only improved the biodegradation but also overcame the inhibition effect at higher concentration.

4.5 INTERMEDIATES OF BENZENE BIODEGRDATION

In order to determine the metabolic intermediates during benzene biodegradation, the sample was collected from the optimized biodegradation experiment after 48 h incubation for analysis in LC-MS. The result of LC-MS showed the presence of catechol (m/z = 109.22), cis-1,2-dihydrobenzene-1,2-diol (m/z = 112.11), and 2-hydroxymuconate semialdehyde (m/z = 142.06) as metabolites, which confirms biodegradation of benzene by indigenous mixed microbial culture as shown in Fig. 4.6.

Dioxygenase is the main enzyme involved in aerobic metabolic pathway to oxidize the aromatic ring of benzene by incorporating two oxygen atoms to form 2-hydroxy-substituted compounds (Zhang et al., 2013). A putative pathway for benzene biodegradation by mixed microbial culture was proposed (Fig. 4.7) based on the LC-MS analysis and the previous study by Singh and Fulekar (2010). This pathway presented the catalytic oxidation of benzene into cis-1,2-dihydrobenzene-1,2-diol, then to catechol, which is further converted

to 2-hydroxymuconate semialdehyde to substrates of the citric acid cycle (TCA cycle) to produce CO_2 and water (Jindrova et al., 2002). The catechol is identified to be the major intermediate of aerobic benzene biodegradation, mineralizes to carbon and electron, which supports the growth of biomass (Yu et al., 2001a). The formation and accumulation of intermediates during benzene biodegradation has important implication for the treatment of benzene in waste streams. These intermediates produced during biodegradation are toxic to microorganisms and the successful treatment of benzene is not effected until the intermediates are removed (Yu et al., 2001a). The metabolic intermediates produced during the benzene biodegradation, in this study, verified the benzene biodegradation pathway established by Jindrova et al. (2002).

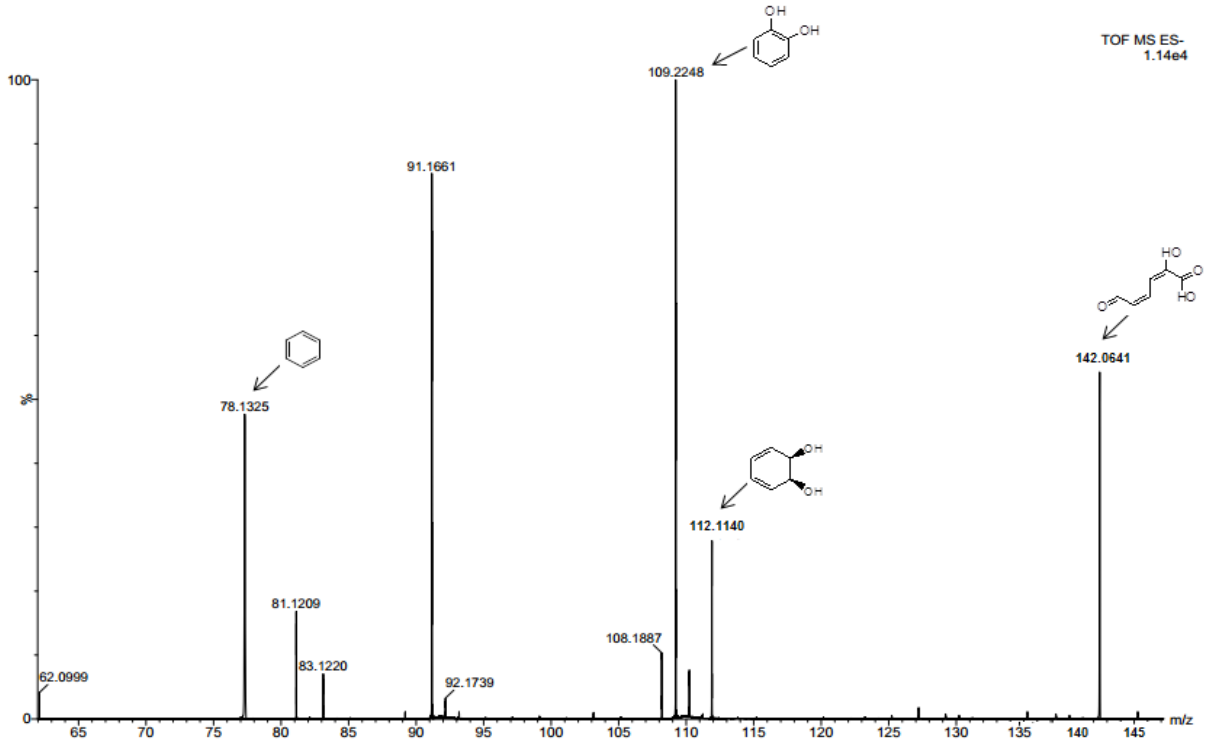


Fig. 4.6 Mass spectrum of benzene (m/z = 78.13), catechol (m/z = 109.22), cis-1,2-dihydrobenzene-1,2-diol (m/z = 112.11), and 2-hydroxymuconate semialdehyde (m/z = 142.06)

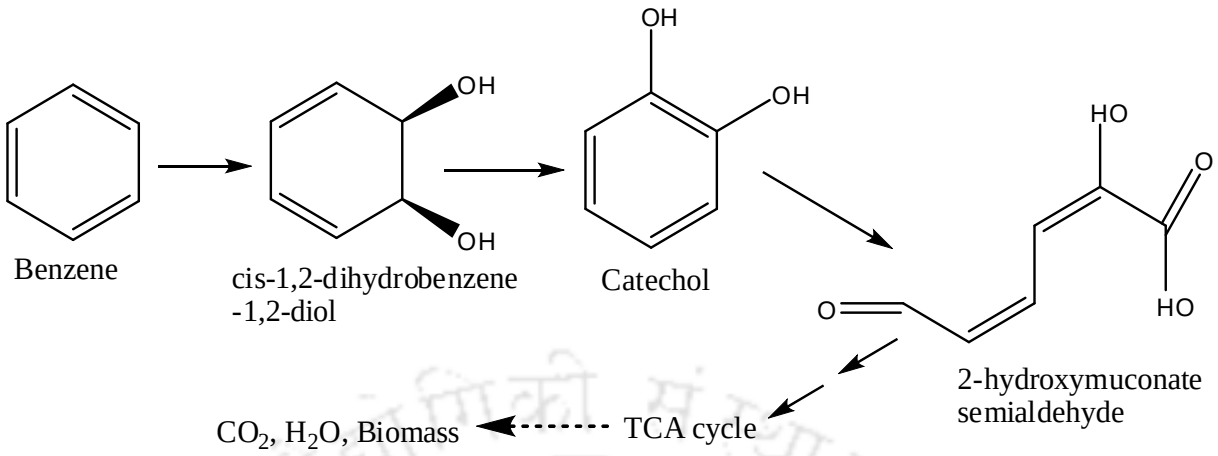


Fig. 4.7 A proposed pathway of benzene biodegradation

4.6 IDENTIFICATION OF THE PREDOMINANT BENZENE DEGRADING STRAIN

Morphological, biochemical and physiological characterizations were carried out to identify the predominant benzene degrading isolates in the mixed culture, shown in Table 4.5. The microscopic analysis revealed that the isolates were gram negative, and rod shaped. It showed positive for Voges Proskauer's, citrate and fermentation of glucose, and negative for methyl red test. It also exhibited negative for oxidase and positive for catalase activity test. These isolates showed a common characterization results belonging to facultative-anaerobic group, tentatively identified as *Enterobacter* species, predominantly present in the mixed culture. For further confirmation, the strain was analyzed using 16S rDNA sequencing method (Mathur et al., 2007). The 16S rDNA sequence determined for the isolate was compared with the previously available sequences of related microorganism in GenBank (Gandu et al., 2013). The sequence of the identified culture was similar (99%) to *Enterobacter cloacae* strain M-5 based on nucleotide homology and phylogenetic analysis (Fig. 4.8).

Table 4.5 Morphological, biochemical and physiological characterizations

Characteristics	Results[a]
Gram staining	−
Cell form	Rod
ONPG	+
Lysine utilization	−
Ornithine utilization	+
Urease	V
Phenylalanine deamination (TDA)	−
Nitrate reduction	+
H_2S production	−
Citrate utilization	+
Voges Proskauer's	+
Methyl red	−
Indole	−
Malonate utilization	V
Esculin hydrolysis	V
Arsbinose	+
Xylose	+
Adonitol	−
Rhamnose	+
Cellobiose	+
Melibiose	+
Saccharose	+
Raffinose	+
Trehalose	+
Glucose	+
Lactose	+
Oxidase	−
Catalase	+

[a]V = 11–89% positive, + = positive (more than 90%), − = negative (more than 90%)

The isolated strain was designated as *Enterobacter cloacae* SG208 and the sequence data submitted to NCBI GenBank under accession number KU297784.1. The *E. cloacae* SG208, identified as a predominant microorganism in the mixed culture in this study, is the major strain responsible for degradation of benzene. A few studies have reported *E. cloacae* for biodegradation of 2,4,6-trinitrotoluene (French et al., 1998) and 2-chlorobenzoic acid (Khleifat et al., 2015). This study demonstrated that the strain predominantly present in mixed culture can degrade benzene to a significantly high level of concentration with a high degradation rate. The results obtained in this study are the first of its kind.

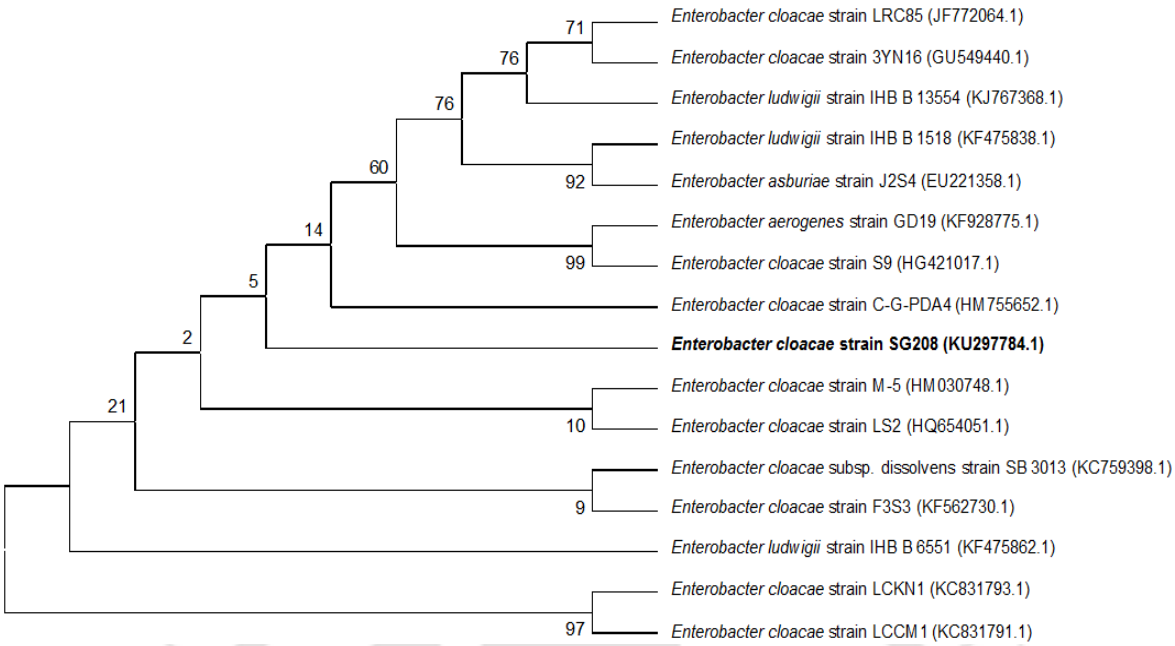


Fig. 4.8 Phylogenetic tree of *E. cloacae* SG208 on the basis of 16S rDNA sequencing

4.7 CONCLUSION

A batch study was performed to estimate the biokinetic constants using various kinetic models. The results of Haldane model matched well with the experimental results. The optimization using RSM enhanced the specific growth rate and degradation rate of the mixed microbial culture at higher concentration of benzene, which is reported to be inhibitory in the literature. The results of the study on the intermediates of benzene degradation validated the established pathway of benzene biodegradation. *E. cloacae* SG208 has been found to be the predominant organism in the enriched mixed culture responsible for degrading benzene.

CHAPTER 5

GASEOUS BENZNE CONTOL USING A SPONGE-MEDIUM BASED RBC

5.1 GENERAL

BTEX are widely found VOCs, released in large quantity from industries. Benzene is more carcinogenic and classified as a hazardous compound by United States Environmental Protection Agency (USEPA) (Singh and Fulekar, 2010). The application of biodegradation techniques for industrial VOCs treatment has become popular because they overcome the drawbacks of physico-chemical methods, are economical and environment friendly (Rene et al., 2015). Several bioreactors such as biofilter, biotrickling filter, and bioscrubber are used earlier for continuous treatment of benzene (which involves a common mechanism consisting of absorption and biodegradation) (Datta and Philip, 2014). The commonly used biofilters or packed bed bioreactors (PBR) are suitable for treating VOCs of low concentration but have a limitation of poor oxygen mass transfer (Mudliar et al., 2008). With the increase in concentration of VOCs the corresponding loading rate also increases for which DO concentration significantly reduces. As a result the performance decreases due to poor transfer of DO to the microorganisms (Sahoo et al., 2013).

RBC is one of such novel bioreactors, which overcomes some of the drawbacks and limitations of the conventional bioreactors and offers scope for modification in its design, materials and operating parameters to provide higher removal efficiency (Datta and Philip, 2014). The activated sludge from a wastewater treatment plants contains a wide variety of organisms, which biodegrade a range of pollutants (Wagner et al., 2002). A mixed microbial consortium from activated sludge is used in RBC to biodegrade VOCs such as

dichloromethane by Ravi et al. (2015). Literature is scarce on the use of RBC for gaseous VOCs control (Vinage and Von Rohr, 2003). Therefore, the research carried out in this study for the treatment of gaseous benzene using RBC is the first of its kind.

The optimal conditions obtained in the batch study (Chapter 4) were used in a lab scale RBC using mixed culture for the efficient treatment of benzene in waste gas streams at various loading rates. In this study, the RBC designed unlike the existing RBC, uses open-pore reticulated polyurethane sponge mounted over a perforated drum to support the uniform growth of biofilm layer. The main goal has been to investigate its performance for treating gaseous benzene at different ILR, study its effect on EC and RE. Further, the effect of Pco_2 on EC, and removal of the nutrients (NH_3-N and PO_4-P) has also been examined in RBC to prove its potential in removing gaseous benzene and nutrients simultaneously.

The work involved the design and installation and operation of RBC, measurement and analysis of gaseous benzene, CO_2, biomass, and nutrients, and then performance evaluation of the RBC followed by the isolation of benzene degrading microorganisms and determination of the kinetic constants.

5.2 OPERATION OF RBC AND DEVELOPEMNT OF BIOFILM

The RBC after design was installed successfully for the desired operation of treating benzene from a waste gas stream. During the start-up phase, the RBC was operated in a closed loop with respect to biomass and the benzene concentration was gradually increased from 0.081 g/m^3 to 1.925 g/m^3, along with 1 g/l glucose to acclimatize and achieve optimum cell growth within RBC. To ensure increase in the number of benzene degrading microorganisms, total microbial count, specific plate count, and VSS of the liquid phase in start-up period was analyzed. After 30 days, a thin biofilm layer was observed on sponge medium (Fig. 5.1) and

the RBC was available for continuous removal of benzene in waste gas streams at different loading rates.

Fig. 5.1 Biofilm developed on RBC at 30th day

5.3 PERFORMANCE OF RBC

5.3.1 Removal of benzene

The biodegradation of waste-gas-streams containing benzene was carried out at various operating conditions over 138 days. The RBC was operated in nine phases, shown in Table 5.1. Each phase was operated till the pseudo-steady state reached. The removal efficiencies in each phase increased gradually, reaching a steady state, and thereafter decreased rapidly with the sudden change in inlet concentration and flow rate. The benzene absorbed partly in liquid phase, resulting in a suspended cell biodegradation, and the unabsorbed benzene was degraded by the biofilm developed in the rotating drum portion of RBC.

Table 5.1 Operating conditions of each phase of RBC for treating benzene

Phases	Time (days)	Inlet concentration (g/m^3)	Flow rate (m^3/h)	ILR[a] (g/m^3 h)	EC[a] (g/m^3 h)	EBCT (min)
Start-up	0–32	0.081–1.925	0.121	–	–	12.44
I	33–45	0.483–0.565	0.121	2.534 ± 0.113	2.258 ± 0.111	12.44
II	46–59	0.719–0.785	0.181	5.409 ± 0.128	4.603 ± 0.245	8.28
III	60–72	0.819–0.872	0.242	8.171 ± 0.162	7.057 ± 0.364	6.20
IV	73–86	1.189–1.280	0.302	14.779 ± 0.398	12.184 ± 0.450	4.96
V	87–99	1.439–1.571	0.363	21.949 ± 0.574	17.534 ± 1.149	4.13
VI	100–112	1.982–2.052	0.424	34.164 ± 0.338	25.415 ± 1.470	3.54
VII	113–125	2.629–2.918	0.484	53.224 ± 1.692	37.807 ± 2.325	3.10
VIII	126–138	3.145–3.239	0.545	69.375 ± 0.591	40.793 ± 4.025	2.75

[a] The values represent the mean and standard deviation in that phase

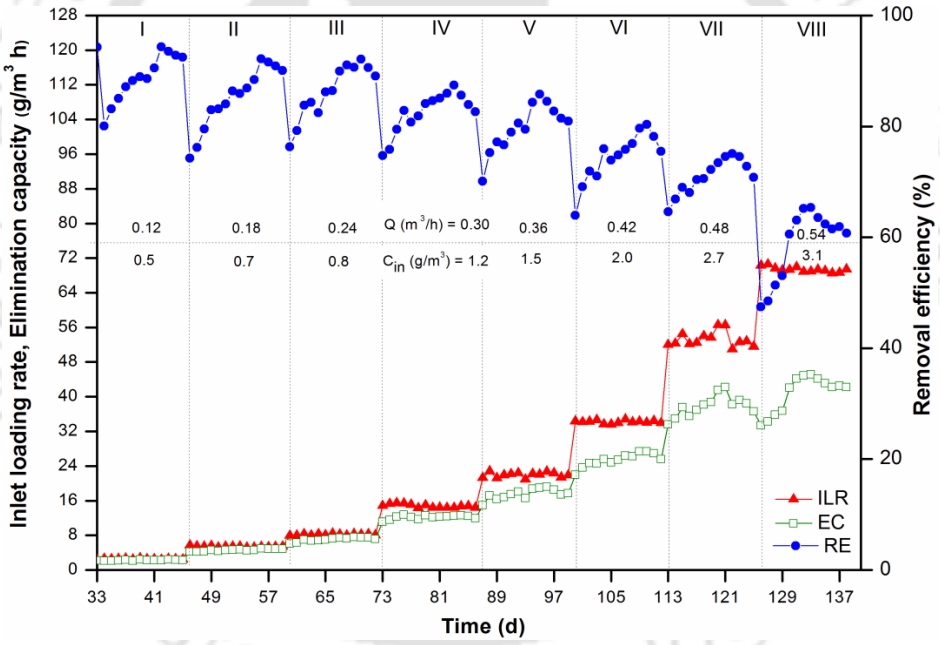


Fig. 5.2 Performance of RBC at various loading rate of benzene

The combinations of different flow rates and concentrations were studied and at different loading rates the performance of RBC was evaluated, shown in Fig. 5.2. The start-up phase lasted for about 32 days followed by the phase I from day 33 to 45 at EBCT of 12.44 min. In phase I, the flow rate was 0.121 m^3/h, at which the average loading rate applied was 2.534 ± 0.113 g/m^3 h**.** On 33rd day, the removal efficiency was 95%, which might be

attributed to the benzene absorbed into the nutrient solution and also diffused into the sponge medium. Similar findings are reported by Yang et al. (2004) for the removal of gaseous VOCs using hybrid rotating drum biofilter. The efficiency was decreased to 80% on day 34, which later increased and reached to 94%, with the maximum on day 42. In phase II, loading rate was increased to 5.409 ± 0.128 g/m^3 h. In this phase, the flow rate of air stream and EBCT was maintained at 0.181 m^3/h and 8.28 min, respectively. With the sudden increase in the loading rate, the efficiency decreased from about 93% to 74% initially, and regained gradually and reached 92% at steady state. The phase III lasted for about 13 days. In this, the flow rate was 0.242 m^3/h (with EBCT 6.20 min), which increased the loading rate to 8.171 ± 0.162 g/m^3 h. Initially removal efficiency was decreased from 90% to 76% and then recovered up to 92% at steady state. The phases IV, V, VI, and VII lasted for 14, 13, 13, 13 days, respectively. In these phases, responses were similar to that of phases I to III. In phase VIII, the flow rate was increased to 0.545 m^3/h, which increased the average loading rate to 69.375 ± 0.591 g/m^3 h. Initially, the removal efficiency dropped to 47%, which may be attributed to the sudden higher loading of benzene, which later recovered and reached only up to 65% due to decrease in biomass concentration.

Further, the overall performance of RBC was studied by establishing the relationship between ILR and RE using a regression analysis. The relationship between them has been represented by RE = 89.5–0.379(ILR). For which, the *t*-value was 163 and *p*-value was 0.00 (<0.05) indicating that the relationship between RE and ILR was significant. The analysis of variance (ANOVA) with a larger *F*-value, smaller *p*-value (<0.05), and high value of R^2 indicates the significance of the regression model (Sahoo et al. 2010). These parameters, i.e. *F*-value = 523, *p*-value = 0.00, and R^2 = 0.85, justified the results were significant.

Kim (2003) reported the maximum benzene removal efficiency of 98% at inlet concentration of 0.7 g/m^3 in a biofilter. Hassan and Sorial (2010) found it above 75% at concentration below 2.5 g/m^3 at acidic pH by encouraging the growth of fungi in a biofilter. However, their earlier study reported it above 80% for the concentration below 2.6 g/m^3 by the same biofilter (Hassan and Sorial, 2009). It was in the present study found to be over 59% at a concentration of 3.184 ± 0.027 g/m^3, higher than the value reported earlier. Table 5.2 shows the comparison of benzene removal efficiencies of previous studies, mostly carried out on biofilters with the present study using RBC. It has been observed that at low concentration, different bioreactors showed similar removal efficiencies, but at higher concentration, RBC showed a better performance. Similar findings are also reported by Ravi et al. (2013).

The performance was also evaluated in terms of EC for various loading rates, which is defined as the amount of benzene degraded per unit of medium volume and time. The EC is shown in Fig. 5.3 as a function of ILR. The EC increased with the increase in influent benzene loading, but the removal efficiency was decreased. From Fig. 5.3, it was observed that when the ILR was less than 8.171 ± 0.162 g/m^3 h, nearly 100% removal was achieved. The region below 8.171 ± 0.162 g/m^3 h in Fig. 5.3 corresponds to diffusion-limited region (DLR), in which benzene concentration might be insufficient to activate the biomass and the region beyond it corresponds to reaction-limited region (RLR), in which biomass was insufficient to degrade the benzene. The maximum EC (EC_{max}) was 45.090 g/m^3 h at an average benzene ILR of 69.375 ± 0.591 g/m^3 h in phase VIII, which is way higher as compared to the results obtained by Lu et al. (2002) and Kim (2003), i.e. 34 g/m^3 h and 20 g/m^3 h, respectively, indicating the better performance of RBC over biofilters.

Table 5.2 Comparison of various reactors for removal of gaseous benzene

Type	Pollutant	Supporting medium	Concentration (g/m^3)	Loading (g/m^3 h)	Removal efficiency (%)	Microbial culture	References
RBC	Benzene	Sponge	<3.18	<69.38	>59.0	Activated sludge	Present study
Biofilter	Benzene and monochloro-benzene[b]	Compost and woodchips	–	2.00	97 ± 6%	*Acinetobacter calcoaceticus*	Pandey et al. (2010)
Biofilter	Benzene	Sugarcane bagasse	0.10	6.10	63.0	*Pseudomonas* sp. NCIMB 9688	Sene et al. (2002)
Biofilter	Benzene	Powdered compost	0.20	24.80	81.0	Compost	Zilli et al. (2005)
Biofilter	Benzene	Granular activated carbon	0.70	23.30	75-98.0	Activated sludge	Kim (2003)
Biofilter	Benzene and Toluene[b]	Compost	0.12−0.95	–	13.8−72.7	Mixed microbial consortium	Rene et al. (2015)
TBAB[a]	Benzene	Pellets	<2.55	<76.80	>75.0	Activated sludge	Hassan and Sorial (2010)
TBAB[a]	Benzene	Pellets	<2.60	<76.80	>80.0	Activated sludge	Hassan and Sorial (2009)

[a] Trickle bed air biofilter
[b] Benzene as part of a mixture.

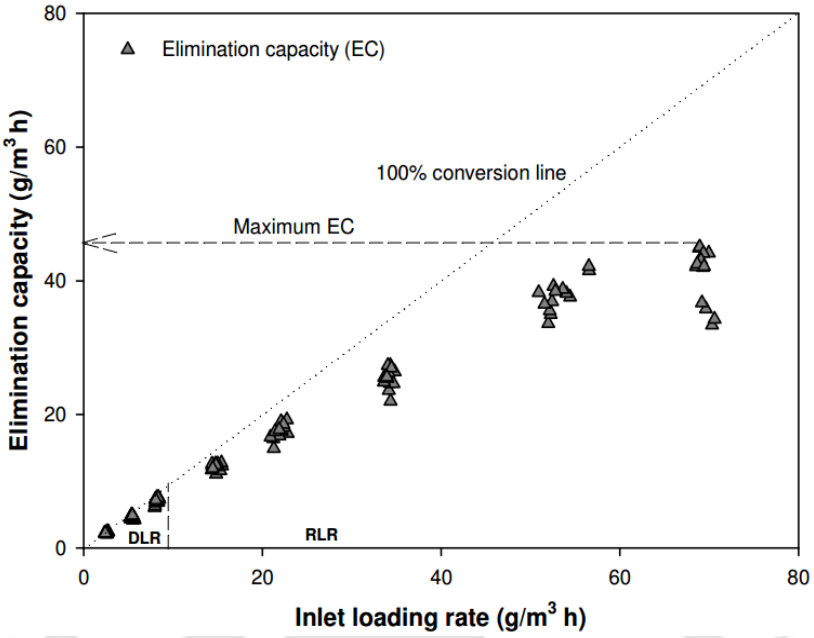


Fig. 5.3 EC as a function of ILR in RBC

5.3.2 Combined removal of benzene and nutrients

Benzene itself is a source of carbon for microorganisms, additionally, the nutrients like nitrogen and phosphorous are essential for optimal operation of RBC. The ammonium ion concentration as a source of nitrogen in the MSM at the inlet was found to be 212 mg/l during the study. The ammonium, nitrate, and phosphorus ion concentration in the effluent of RBC were measured at steady state. The nitrification process was found to be inhibited with the increasing ILR, represented by a higher residual ammonium ion concentration of 26.037 ± 0.227 mg/l in effluent at a loading rate of 69.375 ± 0.591 g/m^3 h in phase VIII. At the loading rate of 2.534 ± 0.113 g/m^3 h, removal of ammonium was 97%, which decreased to 88% at a higher loading rate of 69.375 ± 0.591 g/m^3 h. The removal of ammonium ion concentration increased the nitrate ion concentration at lower ILR, but at higher ILR the nitrification process was found to be inhibited (Fig. 5.4). Similar findings are reported by Datta and Philip (2014) in a study wherein heterotrophs fed on organic carbon grow faster

than the nitrifiers, thus, nitrification inhibits at higher organic loading rate. However, nitrite ion was found to be very less throughout the period (<0.027 mg/l). The phosphorus ion concentration was 233 mg/l during the process. Its removal was 67% at lower ILR of 2.534 ± 0.113 g/m^3 h**,** which gradually decreased to 44% at higher ILR of 69.375 ± 0.591 g/m^3 h, lesser as compared to ammonical-nitrogen removal. Fig. 5.4 shows the effect of ILR on removal of NH_4-N, PO_4-P, and NO_3-N formation. The results in the removal of NH_4-N and PO_4-P are promising and indicate that RBC can be effective in controlling gaseous benzene as well as simultaneously removing nutrients in liquid phase.

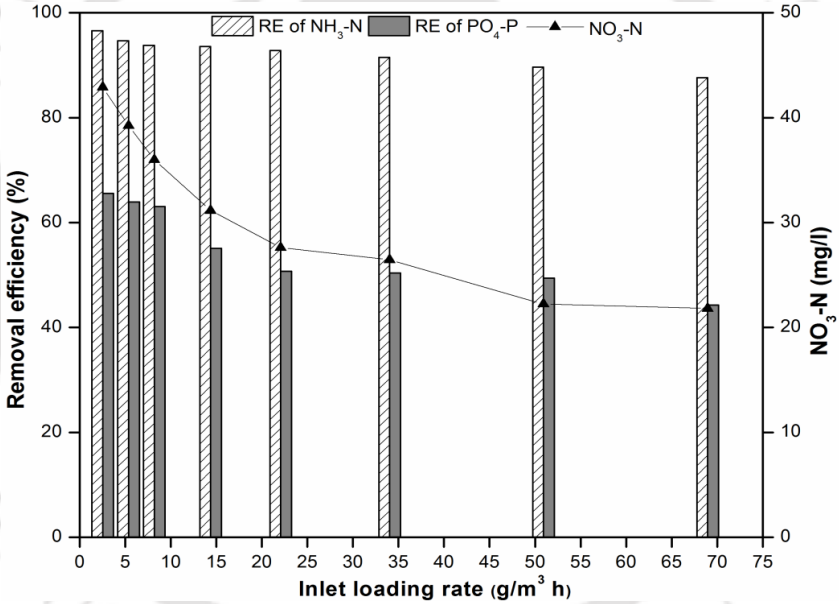


Fig. 5.4 Effect of inlet loading rate on removal of NH_3-N, PO_4-P, and NO_3-N formation

5.3.3 Effect of ILR on DO and pH

As a consequence of increase in flow rate in the subsequent phases, the DO concentration reduced significantly, due to higher ILR of gaseous benzene. However, it remained above 1.8 mg/l at all the loadings and operating conditions in different phases, as shown in Fig. 5.5. Mudliar et al. (2008) have also found similar results during biodegradation of pyridine using

novel rotating rope bioreactor (RRB), i.e. above 2.4 mg/l, indicating better oxygen mass transfer than that is observed in conventional packed bed bioreactors.

The pH of the inlet MSM was 6.95 ± 0.05 in which sodium bicarbonate was used as a buffer to prevent the appreciable change of pH in RBC. The pH was slightly reduced during biodegradation and was found to be between 6.95–6.29. It was nearly 6.35 ± 0.05 at higher ILR of 69.375 ± 0.591 g/m^3 h in phase VIII (Fig. 5.5). This might be due to increase in the production of CO_2, which resulted into carbonic acid and the formation of acidic intermediates during biodegradation in the liquid phase (Singh et al., 2017).

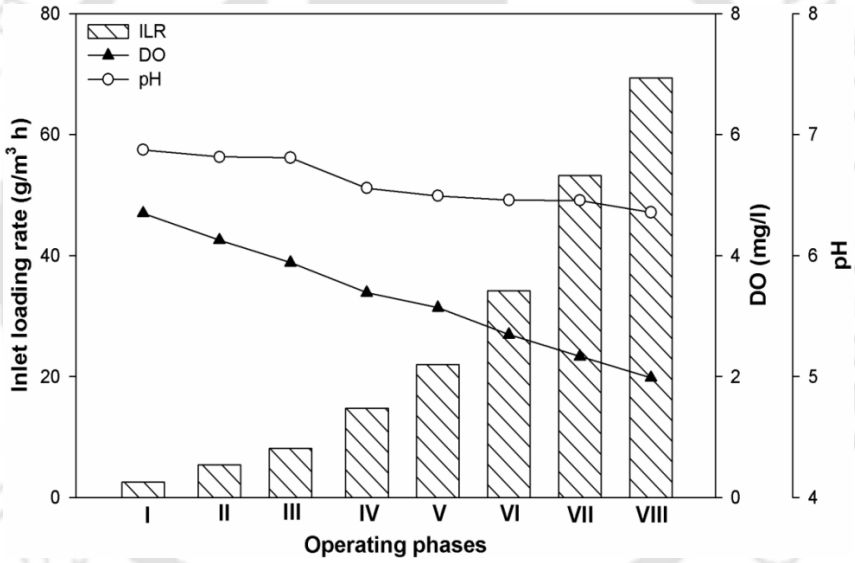


Fig. 5.5 Effect of inlet loading rate on DO and pH

5.4 PRODUCTION OF CARBON DIOXIDE

Production of carbon dioxide in biodegradation indicates the degree of pollutant degradability, since pollutants are finally biodegraded to water and carbon dioxide. The biodegradation is often assessed by means of carbon dioxide production rate, which is always not easy to get through mass balance, as CO_2 may also be generated through endogenous respiration (Kennes and Veiga, 2013). On the other hand, autotrophic bacteria may also use

CO_2 as a source of carbon. The CO_2 production rate was calculated by equation 3.11. The Pco_2 in each phase presented in Fig. 5.6 along with the EC for benzene. The increase or decrease in the production of CO_2 varied with the increase or decrease in the EC. The linear equation obtained between production of CO_2 to the EC of benzene was $Pco_2 = 1.47EC$. The ratio of the production of CO_2 to benzene consumed (EC) was 1.47. With the increase in benzene concentration, both ILR and EC were gradually increased, which led to the production of more CO_2 in the effluent stream. Similar correlation equation, i.e. $Pco_2 = 2.933EC$, is reported by Mathur et al. (2007) for removal of BTEX using biofilter.

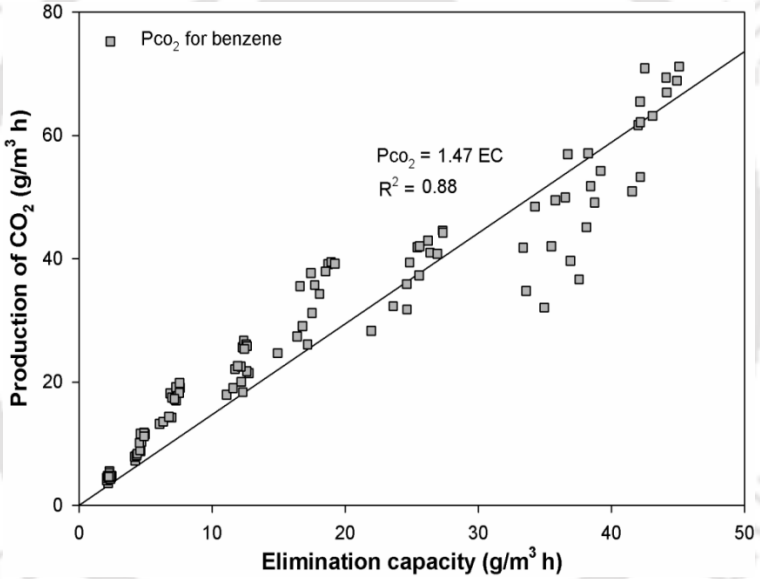


Fig. 5.6 Production of CO_2 as a function of EC

5.5 FESEM CHARACTERIZATION OF SPONGE MEDIUM

To ensure the microbial growth on support medium (sponge), a comparison of the field emission scanning electron microscopic image of sponge medium before (on zero day) and after experiment (138 day) was carried out. Compared to initial image of sponge medium, biofilm was clearly observed on the pore as well as surface of sponge medium after 138 days of operation (Fig. 5.7).

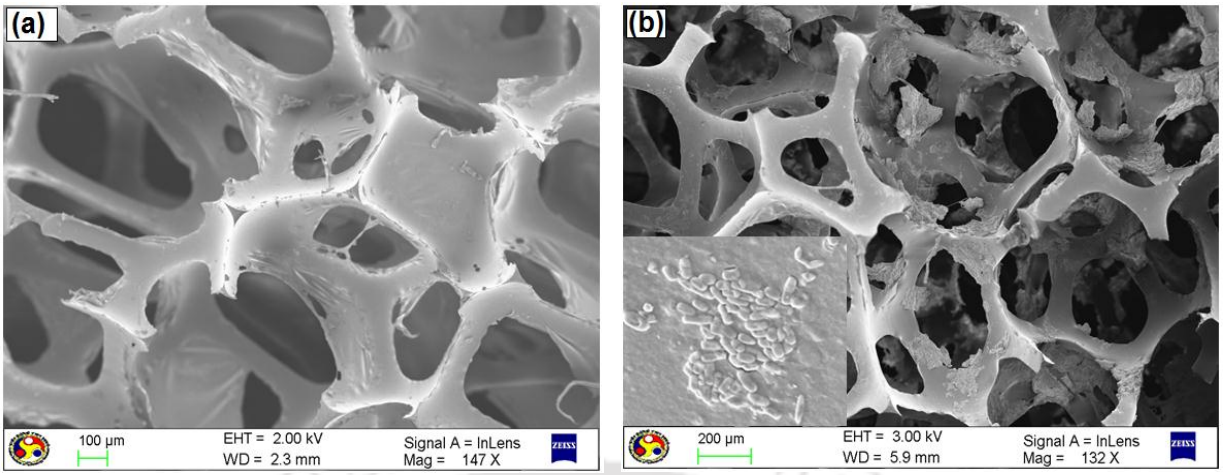


Fig. 5.7 The FESEM image of sponge-medium (a) at 0^{th} day and (b) 138^{th} days of operation

5.6 BIOMASS CONCENTRATION AND MICROBIAL COUNT

The VSS in the liquid effluent of RBC was gradually increased during the operation at various ILR of benzene shown in Table 5.3. The VSS was much higher at the end of the study might be due to sloughing of biomass from sponge medium. Yang et al. (2004) reported increases in the amount of biomass on the supporting sponge medium, may have produced a higher biomass concentration in the effluent liquid.

The specific count and total microbial count for RBC biofilm were $(27 \pm 3) \times 10^6$ and $(57 \pm 5) \times 10^6$ CFU/g, respectively at phase I at ILR of 2.534 ± 0.113 g/m^3 h, which gradually increased to $(106 \pm 7) \times 10^6$ and $(156 \pm 12) \times 10^6$ CFU/g, respectively up to the end of the phase (Table 5.3). The microbial count exponentially increased up to phase IV and later became stable. It was slightly lesser in phase VIII as compared to phase VII due to the sudden high loading of benzene. These counts were also done for liquid medium, found to be lesser as compared with the RBC biofilm, i.e. $(15 \pm 3) \times 10^5$ and $(37 \pm 4) \times 10^5$ CFU/ml, respectively at ILR of 2.534 ± 0.113 g/m^3 h**,** which gradually increased to $(107 \pm 9) \times 10^5$ and $(145 \pm 10) \times 10^5$ CFU/ml respectively after 138 days (Table 5.3).

Table 5.3 Biomass concentration and microbial count (total and specific) at various loading rates of benzene in RBC

Phases	Benzene loading rate (g/m^3 h)	VSS in effluent liquid[a] (mg/l)	Benzene degraders on biofilm[a] (CFU/g)	Total microbial count on biofilm[a] (CFU/g)	Benzene degraders in effluent liquid[a] (CFU/ml)	Total microbial count in effluent liquid[a] (CFU/ml)
I	2.534 ± 0.113	216 ± 13	$(27 \pm 3) \times 10^6$	$(57 \pm 5) \times 10^6$	$(15 \pm 3) \times 10^5$	$(37 \pm 4) \times 10^5$
II	5.409 ± 0.128	265 ± 18	$(46 \pm 5) \times 10^6$	$(81 \pm 4) \times 10^6$	$(35 \pm 5) \times 10^5$	$(54 \pm 6) \times 10^5$
III	8.171 ± 0.162	384 ± 29	$(66 \pm 4) \times 10^6$	$(109 \pm 7) \times 10^6$	$(64 \pm 6) \times 10^5$	$(93 \pm 7) \times 10^5$
IV	14.779 ± 0.398	423 ± 25	$(88 \pm 6) \times 10^6$	$(122 \pm 6) \times 10^6$	$(88 \pm 4) \times 10^5$	$(116 \pm 5) \times 10^5$
V	21.949 ± 0.574	487 ± 31	$(96 \pm 5) \times 10^6$	$(139 \pm 9) \times 10^6$	$(95 \pm 5) \times 10^5$	$(125 \pm 9) \times 10^5$
VI	34.164 ± 0.338	545 ± 36	$(103 \pm 8) \times 10^6$	$(153 \pm 11) \times 10^6$	$(103 \pm 9) \times 10^5$	$(136 \pm 8) \times 10^5$
VII	53.224 ± 1.692	595 ± 35	$(108 \pm 6) \times 10^6$	$(166 \pm 14) \times 10^6$	$(113 \pm 7) \times 10^5$	$(148 \pm 11) \times 10^5$
VIII	69.375 ± 0.591	475 ± 30	$(106 \pm 7) \times 10^6$	$(156 \pm 12) \times 10^6$	$(107 \pm 9) \times 10^5$	$(145 \pm 10) \times 10^5$

[a] The values represent the mean and standard deviation in that phase

5.7 CHARACTERIZATION AND IDENTIFICATION OF BENZENE DEGRADERS

Morphological, physiological and biochemical characterizations of isolates were performed to identify the benzene degraders and the results are given in Table 5.4.

Table 5.4 The morphological, physiological and biochemical characteristics of benzene degraders

Characteristics	Results[a]	Results[a]
Gram staining	−	−
Color	White	Purple
Cell form	Rod	Rod
Motility	+	+
ONPG	+	+
Lysine utilization	−	+
Ornithine utilization	+	+
Urease	V	−
Phenylalanine deamination (TDA)	−	−
Nitrate reduction	+	−
H_2S production	−	−
Citrate utilization	+	−
Voges Proskauer's	+	−
Methyl red	−	−
Indole	−	−
Malonate utilization	V	−
Esculin hydrolysis	V	+
Arsbinose	+	−
Xylose	+	−
Adonitol	−	−
Rhamnose	+	−
Cellobiose	+	−
Melibiose	+	−
Saccharose	+	−
Raffinose	+	−
Trehalose	+	−
Glucose	+	−
Lactose	+	−
Oxidase	−	+
Catalase	+	+
Identification	*Enterobacter* sp.	*Pseudomonas* sp.

[a]V = 11–89% positive, + = positive (more than 90%), − = negative (more than 90%)

Four profusely developed strains grown in presence of benzene were isolated. Out of which three showed common characteristics and the isolates were gram-negative rod, with positive for catalase, citrate, nitrate reduction, Voges Proskauer's, and glucose fermentation. It showed negative for methyl red and oxidase tests. Based on the tests, the isolates were facultative-anaerobic bacterium, with reference to Bergey's manual (Buchanan and Gibbons, 1974) the strains were tentatively identified as genera *Enterobacter*. The remained one culture was gram-negative rod, with positive for citrate, catalase, and oxidase tests. Whereas, negative for Voges Proskauer's, methyl red, and indole tests. These characteristics tentatively identified the isolate as genus *Pseudomonas*. *Enterobacter* sp. was found to be the predominant benzene degrader strain and the most efficient isolate that survived at a higher concentration of benzene. The *Enterobacter cloacae* is used by Arulazhagan et al. (2010) for the biodegradation of polycyclic aromatic hydrocarbons.

5.8 KINETICS OF BENZENE BIODEGRADATION

In the present study, the kinetic parameters are determined from steady state outlet concentrations for various inlet concentrations of benzene using modified Monod equation (Eq. 5.1). The mathematical model is described in detailed in section 3.5.6.

$$\frac{V/Q}{C_i - C_o} = \left(\frac{K_S}{r_{max}} \cdot \frac{1}{C_{ln}}\right) + \frac{1}{r_{max}} \qquad (5.1)$$

where, C_{ln} is the log mean concentration $[(C_i - C_o)/\ln(C_i/C_o)]$. $[(V/Q)/(C_i - C_o)]$ plotted against $(1/C_{ln})$, and the slope (K_s/r_{max}) and intercept $(1/r_{max})$ were determined. From the Fig. 5.8, the r_{max} and K_s were calculated as 30.769 g/m^3 h and 2.224 g/m^3, respectively, with a correlation coefficient (R^2) of 0.94. The value of r_{max}/K_s obtained for benzene is higher than the value reported in the literature for the same compound (Mathur et al., 2012).

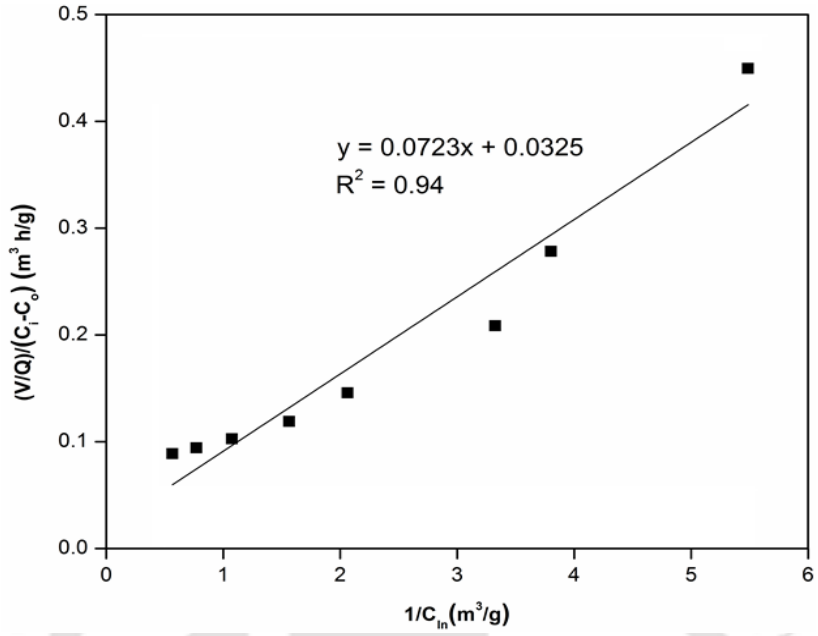


Fig. 5.8 Determination of kinetic parameters of benzene

5.9 CONCLUSION

The RBC designed in this study with a sponge medium supported on a perforated rotating drum has been found to be effective in the removal of gaseous benzene with efficiency up to about 95%. At even high benzene loading rate of 69.375 ± 0.591 g/m^3 h, the removal has been over 59%, which is higher in comparison with the results reported in literatures. The biomass changed considerably during the operation, but was found to be much higher in the later phases due to sloughing, which provided stable performance. The biomass was evenly distributed on the sponge without a problem of diffusion of nutrients into the biofilm as happens in biofilters. *Enterobacter* sp. was found to be the predominant strain responsible for the biodegradation of gaseous benzene. Thus, the RBC provides dual benefits of treating gaseous benzene as well as nutrients from wastewater simultaneously using industrial sludge as an inoculumn, which shows its potential usefulness for industrial applications.

CHAPTER 6

CONTROL OF BTEX FROM WASTE GAS STREAMS AND PERFORMANCE OF RBC

6.1 GENERAL

The VOCs such as BTEX are emitted to the air on a large scale by industrial and manufacturing operations (Qi and Moe, 2006). Biological waste gas treatment of VOCs depends on microorganisms, which have ability to oxidize and degrade these compounds into carbon dioxide, water, and biomass as end products (Cox and Deshusses, 2002; Rene et al., 2012), with useful secondary products used as biofuels (Abubackar et al., 2011). The research, presented in the previous chapter, has demonstrated the biological treatment of single pollutant (benzene) from a gas stream using mixed culture. In real scenario, gas contains a mixture of volatile organics mostly BTEX. Degrading all simultaneously might pose challenges in the operation and the performance of RBC due to complex interaction between these compounds and with microorganisms. A few studies have reported this concern. For example, the mixture of pollutant increases the complexity of the system might be due to microbial and substrate interactions (Rahul et al., 2013). The interaction effects between pollutants may reduce the removal efficiencies of the target pollutants reported by Gallastegui et al. (2011). The changes in flow rate and concentration of VOCs are detrimental for the microbial culture, which reduces the performance of the system (Rahul et al., 2013).

Since, the RBC developed in this research has shown good performance as observed in treating benzene, it has been further used to treat a mixture of VOCs. A few studies have also reported the use of different types of RBCs for treating various VOCs. Rotating drum

biofilter is used successfully to treat toluene (Yang et al., 2004), and diethyl ether (Yang et al., 2008a) in waste gas streams. Later, Datta and Philip (2014) used rotating disc contactor for treating mixture of VOCs from waste gas streams. Literature studies thus reveals that RBC is used for treatment of single pollutant and very limited researches for mixture of pollutants. However, no work is found for treatment of BTEX in waste gas using RBC.

First, the screening of acclimatized cultures was done to understand the effect of single a pollutant enriched culture for the efficient treatment of VOCs. After screening, the activated sludge was inoculated in RBC and acclimatized with single pollutant, which showed better degradation efficiency. Before starting the treatment of mixture of BTEX by enriched culture, xylene as a single pollutant, and then combined toluene and xylene has been treated. The performance of RBC at various ILR of VOCs as single and combine has been investigated, and its effect on RE and EC has been studied. The Pco_2, removal of nutrients, and biomass concentration during treatment has also been assessed by varying the operating conditions. The effect of intermittent operation and shock loading were also investigated to study the stability of RBC.

6.2 BATCH STUDIES FOR SCREENING OF CULTURE

The mixed microbial culture was isolated and enriched with the target pollutants as described in detail in section 3.4.1. Depending on the source of carbon, five different enriched cultures were prepared by acclimatizing benzene, toluene, ethylbenzene and xylene as a sole source of carbon in the concentration range of 100–600 mg/l, and equal mixture of BTEX at concentration from 25 to 150 mg/l. Screening of five enriched cultures were conducted in batch shake flasks to assess the biodegradation efficiency in presence of equal concentration of BTEX mixture up to 150 mg/l as shown in Fig. 6.1. Among the five, xylene enriched

culture showed highest degrading potential for BTEX mixture, followed by toluene, BTEX, ethylbenzene and benzene enriched culture over a period of 36 h and hence was selected. The xylene enriched culture exhibited maximum biodegradation for BTEX concentrations of 400 mg/l. Single-pollutant-enriched culture is effective for the treatment of a mixture of VOCs as reported by other researchers (Shim and Yang, 1999; Datta and Philip, 2012). Therefore, the activated sludge was inoculated in the RBC and acclimatized with xylene, which was used for subsequent treatment of xylene, combined toluene and xylene, and a mixture of BTEX.

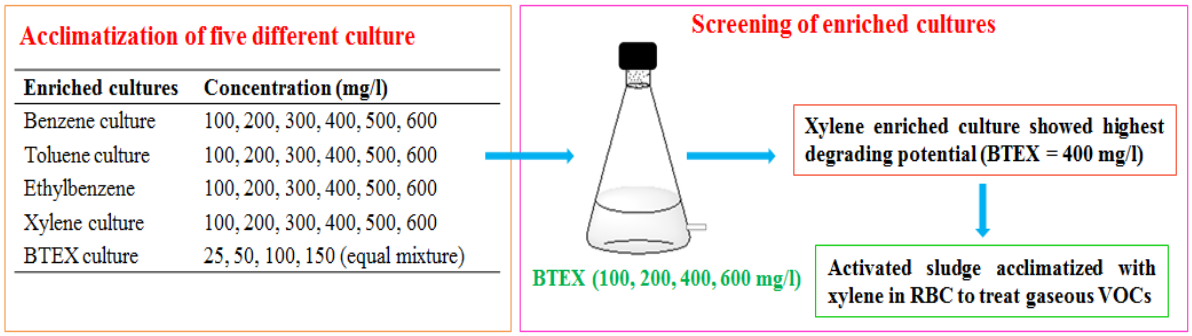


Fig. 6.1 Screening of enriched culture

6.3 START-UP AND ACCLIMATIZATION PHASE OF RBC

Prior to the start-up of RBC, the lowest gaseous xylene concentration of 0.409 ± 0.023 g/m^3 was supplied to absorb in the liquid as well as bio-support medium. When the outlet concentration of xylene equaled the inlet concentration, then 2 l of activated sludge as a source of biomass was added into the 15 l of nutrient media in the RBC. The xylene concentration was increased from 0.424 ± 0.033 to 1.428 ± 0.064 g/m^3, additionally 1 g/l of glucose was supplied for acclimatization and optimum biomass growth within RBC. Due to rotation of RBC, the biomass immobilized onto the bio-support medium and initiation of biofilm was occurred. During this phase, the RBC was operated in batch mode to increase the attachment of biomass onto the bio-support medium and to prevent the loss of biomass

(Kennes and Veiga, 2001). The xylene removal was lower than 40% in the beginning, which subsequently improved. After 18 days of operation, the removal efficiency reached to about 67% and a thin biofilm was also developed on bio-support medium, indicating the biomass was enriched with xylene. Then, the RBC was continuously operated for treatment of xylene, combination of toluene and xylene, and BTEX mixture in waste gas streams.

6.4 TREATMENT OF XYLENE

The removal of xylene in the RBC investigated over a period of 74 days (I-IX phases) at various operating conditions has been presented in Table 6.1. In each phase, removal efficiency gradually increased, attained steady state and then decreased with the sudden change in flow rate. Treatment of xylene from gas streams was carried out at various flow rates (0.12–0.30 m^3/h) and at various concentrations ranging from 0.463 ± 0.011 to 3.613 ± 0.051 g/m^3. The phases I to III were operated at a concentration of 0.459 ± 0.025 g/m^3 and at the flow rate in the range of 0.12–0.30 m^3/h for which the corresponding ILR was obtained from 2.222 ± 0.052 to 5.533 ± 0.158 g/m^3 h. Initially, the RE was 74% in phase I at ILR of 2.222 ± 0.052 g/m^3 h and it reached up to 92% on day 27 (Fig. 6.2). In phase II, with increase in flow rate from 0.12 to 0.24 m^3/h, the EBCT reduced from 12.4 to 6.2 min, which decreased the RE to 72% and recovered gradually up to 90%. Subsequently, in phase III, with increase in flow rate the ILR increased to 5.533 ± 0.158 g/m^3 h, which reduced the removal efficiency to 87% at steady state. The concentration was increased further from 0.459 ± 0.025 to 1.409 ± 0.045 g/m^3 in phase IV and was maintained up to phase VI with three different flow rates (0.12, 0.24, and 0.30 g/m^3). The loading rate obtained from 6.728 ± 0.167 to 16.939 ± 0.210 g/m^3 h was applied in these phases. It is evident that with increase in ILR the RE gradually decreased up to 83% at the end of phase VI. The concentration was increased further by 2.5

times to 3.610 ± 0.059 g/m^3 and the flow rates in the range of 0.12–0.30 m^3/h were applied in phase VII–IX, which achieved a maximum loading rate of 43.739 g/m^3 h. Similar responses had been noticed in these phases to that of phases I to VI, but the removal efficiency dropped significantly to 61% at a higher loading of 43.361 ± 0.351 g/m^3 h in phase IX and then recovery of RE was observed maximum up to 72%.

Table 6.1 Operating condition for each phase of RBC for treating xylene

Phases	Time (days)	Inlet concentration (g/m^3)	Flow rate (m^3/h)	ILR[a] (g/m^3 h)	EC[a] (g/m^3 h)	EBCT (min)
Start-up	0–18	0.424–1.428	0.12	–	–	12.4
I	19–27	0.443–0.483	0.12	2.222 ± 0.052	1.900 ± 0.151	12.4
II	28–35	0.431–0.486	0.24	4.347 ± 0.165	3.638 ± 0.327	6.2
III	36–42	0.436–0.479	0.30	5.533 ± 0.158	4.452 ± 0.424	4.9
IV	43–50	1.364–1.434	0.12	6.728 ± 0.167	5.310 ± 0.404	12.4
V	51–59	1.377–1.446	0.24	13.571 ± 0.192	10.805 ± 0.689	6.2
VI	60–66	1.393–1.427	0.30	16.939 ± 0.210	13.447 ± 0.799	4.9
VII	67–75	3.585–3.626	0.12	17.333 ± 0.170	12.684 ± 0.809	12.4
VIII	76–83	3.576–3.630	0.24	34.614 ± 0.261	24.741 ± 1.447	6.2
IX	84–92	3.588–3.645	0.30	43.361 ± 0.351	30.022 ± 1.547	4.9

[a]The values represent the mean and their standard deviation in that phase

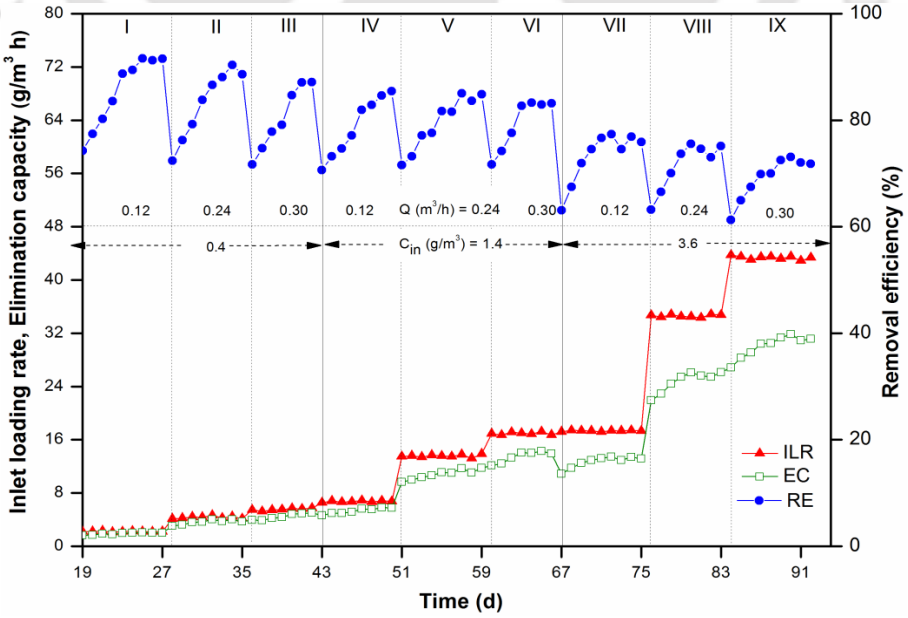


Fig. 6.2 Performance of RBC treating xylene

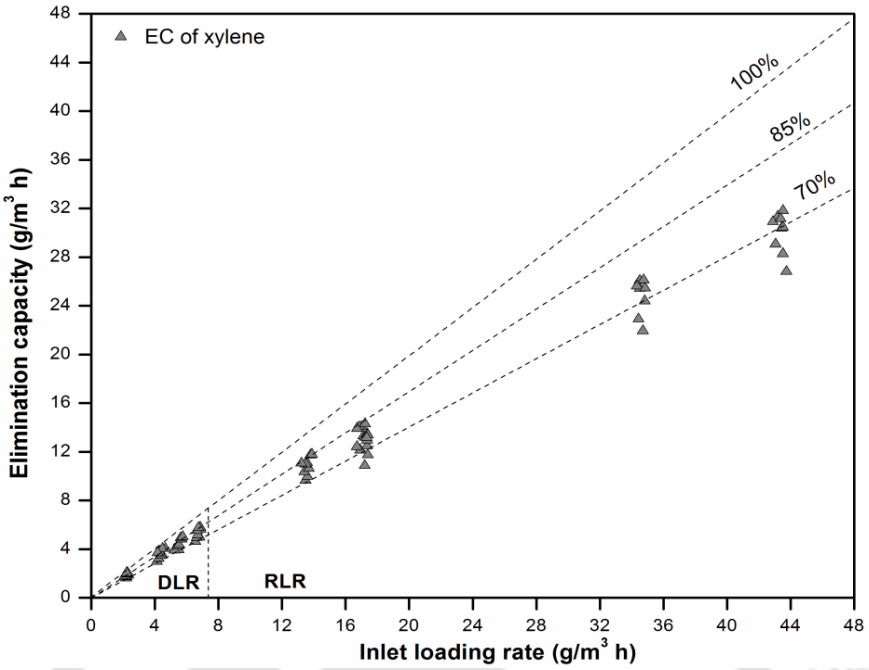


Fig. 6.3 Influence of ILR of xylene on the EC

The RBC performance was assessed in term of EC at various loading rates is shown in Fig. 6.3. The 45º line means 100% conversion, similarly, the other two line show 85% and 70% conversion. The EC was different at different phases due to change in loading rate and removal rate. At lower loading rates up to 6.728 ± 0.167 g/m^3 h, the conversion was almost 100%, which belongs to DLR. As the loading rate increased up to 16.939 ± 0.210 g/m^3 h, the EC decreased nearly 85%. The decline in xylene removal with further increase in loading rate (beyond 6.728 ± 0.167 g/m^3 h) was observed, which indicated the RLR. In phase IX, at a higher loading rate of 43.361 ± 0.351 g/m^3 h, the EC which was slightly less initially, slowly recovered up to 70%. It can be thus concluded that with increase in loading rate, EC increases but the RE shows opposite trend. The EC_{max} of 31.822 g/m^3 h was observed for xylene at a loading rate of 43.531 g/m^3 h in this study, which is significantly higher as compared to the EC_{max} of 26.5 g/m^3 h reported by Gallastegui et al. (2011).

6.5 COMBINED TREATMENT OF TOLUENE AND XYLENE

The combined treatment of toluene and xylene was carried out in RBC from day 93 to 161 at equal concentrations of both compounds to achieve equal loading rates (Table 6.2). In phase I–III, the toluene and xylene concentrations were maintained at about 0.241 ± 0.028 g/m^3 and 0.233 ± 0.021 g/m^3, respectively, at flow rates of 0.12–0.30 m^3/h to achieve a total loading rate up to 5.814 ± 0.344 g/m^3 h. The RE of toluene was about 86%, whereas that of xylene was above 97% at steady state in the end of phase III (Fig. 6.4a, b). In phase IV, with increase in toluene and xylene concentrations to 0.673 ± 0.043 g/m^3 and 0.661 ± 0.034 g/m^3, respectively, the total loading rate increased to 6.402 ± 0.235 g/m^3 h (Fig. 6.4a-c). Initially, the removal of toluene reduced to 83% and then recovered up to 95%. However, at the same time the RE of xylene was slightly less i.e. 91% at steady state. With the further increase in total loading rate to 12.469 ± 0.449 g/m^3 h in phase V, the toluene RE dropped to 81% initially, later recovered to 93%. However, for xylene a maximum removal of 88% was observed in the same phase. The RE in phase VI decreased subsequently to 90% and 85% for toluene and xylene, respectively, at a total loading rate of 15.447 ± 0.525 g/m^3 h. The responses in phase VII–IX were almost similar to those in phases I–VI. The RE has progressively decreased with increase in total ILRs for both compounds. At the total loading rate of 42.016 ± 0.666 g/m^3 h in phase IX, the maximum RE of toluene and xylene was 83% and 74% (Fig. 6.4a-c), respectively. Overall, toluene removal was more effective than xylene. At high toluene concentration, the biodegradation of xylene was inhibited because of the strong inclination of microbial culture to toluene, which outcompetes the removal of xylene (Prenafeta-Boldú et al. (2002). On the contrary, Gallastegui et al. (2011) reported that the presence of xylene might increase the RE of toluene during the treatment of a mixture.

Table 6.2 Operating condition for each phase of RBC for treating toluene and xylene

Phases	Time (days)	Inlet concentration (g/m^3)		Flow rate (m^3/h)	ILR[a] (g/m^3 h)	EC[a] (g/m^3 h)	EBCT (min)
		Toluene	Xylene				
I	93–100	0.198–0.253	0.201–0.255	0.12	2.212 ± 0.075	2.058 ± 0.176	12.4
II	101–107	0.215–0.280	0.190–0.250	0.24	4.592 ± 0.252	4.301 ± 0.416	6.2
III	108–114	0.199–0.275	0.220–0.270	0.30	5.814 ± 0.344	5.426 ± 0.427	4.9
IV	115–122	0.598–0.729	0.630–0.709	0.12	6.402 ± 0.235	5.702 ± 0.282	12.4
V	123–130	0.607–0.697	0.598–0.707	0.24	12.469 ± 0.449	10.800 ± 0.510	6.2
VI	131–137	0.626–0.705	0.591–0.671	0.30	15.447 ± 0.525	12.922 ± 0.384	4.9
VII	138–146	1.691–1.846	1.667–1.843	0.12	16.915 ± 0.396	13.525 ± 0.776	12.4
VIII	147–153	1.665–1.813	1.694–1.784	0.24	33.610 ± 0.655	26.730 ± 1.346	6.2
IX	154–161	1.685–1.831	1.657–1.764	0.30	42.016 ± 0.666	31.486 ± 2.171	4.9

[a] The values represent the mean and their standard deviation in that phase

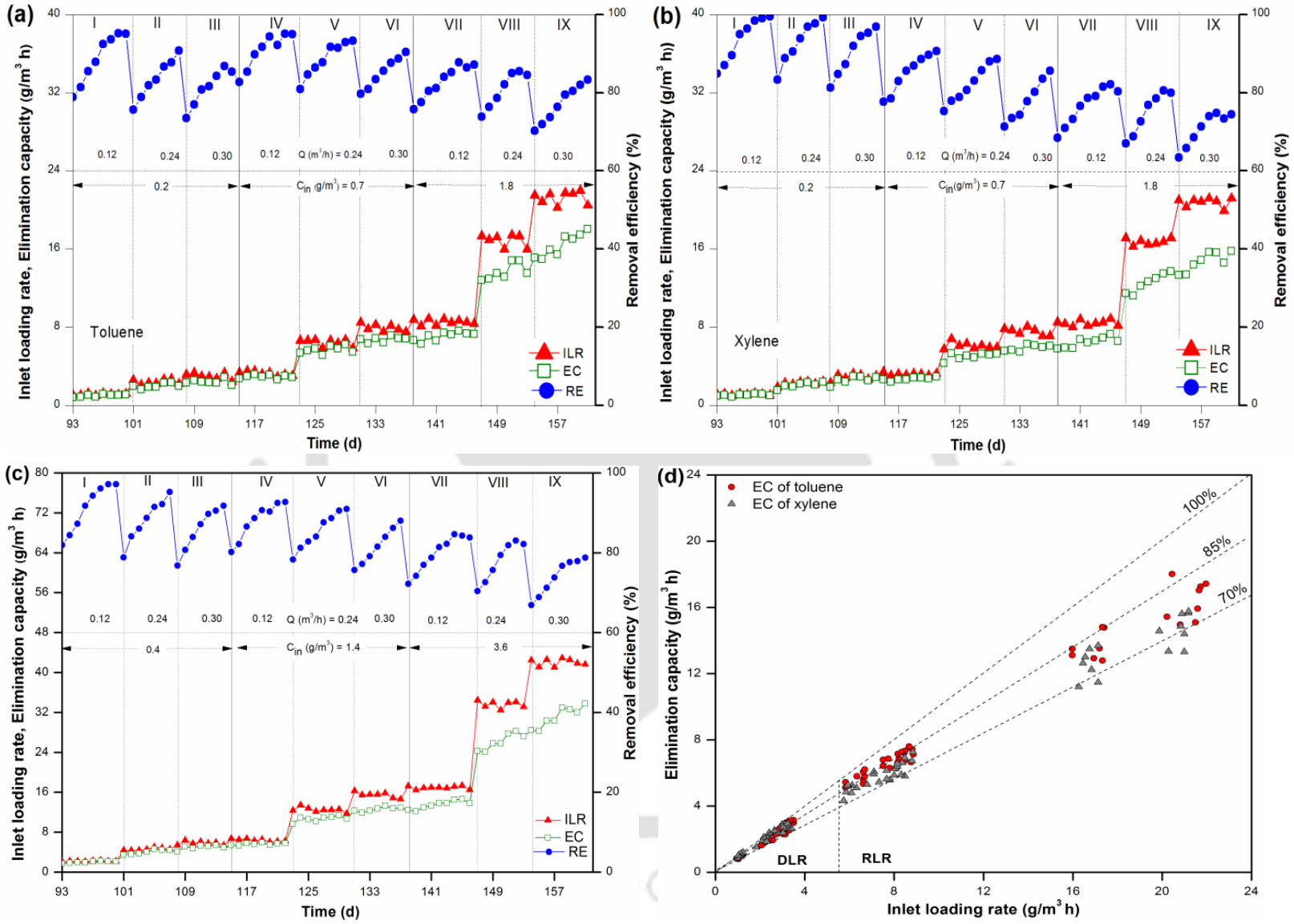


Fig. 6.4 Performance of RBC treating toluene and xylene mixture (a) toluene removal (b) xylene removal (c) overall removal and (d) Effect of ILR on EC

The performance of RBC has been quantified in terms of EC of toluene and xylene as a function of loading rates. The relationship of ECs with the corresponding ILRs for toluene and xylene is shown in Fig. 6.4d. The RBC was operated at a toluene loading rate between 1.106 ± 0.093 and 21.240 ± 0.610 g/m^3 h, and xylene loading rate between 1.119 ± 0.080 and 20.777 ± 0.431 g/m^3 h. The EC was nearly 100% for toluene at loading rate up to 6.402 ± 0.347 g/m^3 h (Fig. 6.4d). Similarly, for xylene it was lower i.e. 3.171 ± 0.116 g/m^3 h. The 100% conversion line indicated that the RBC reached to its maximum EC under this loading rate (Singh et al., 2010). It has been observed that toluene is highly biodegradable and its EC is more than 70% throughout the process, xylene is comparatively less biodegradable. The decline in EC of xylene was observed up to 64% at the starting of phase IX, which, with increase in loading rate gradually recovered only up to 74%. The toluene removal was up to 83% in the same phase higher than that of xylene. This result justifies that toluene is more susceptible to biodegradation than xylene, which is also reported in some studies (Strauss et al., 2004; Kim et al., 2009). The overall EC for both toluene and xylene increased with the increase in loading rate, but the RE concurrently decreased (Gallastegui et al., 2017).

For the mixture of toluene and xylene, the overall EC_{max} of 33.781 g/m^3 h was obtained at the total inlet load of 41.631 g/m^3 h in phase IX, which is slightly higher than the EC_{max} of 31.822 g/m^3 h obtained for xylene as a single pollutant as reported in section 6.4. For the mixture, The EC_{max} for toluene was 18.023 g/m^3 h, higher as compared to that of xylene with EC_{max} of 15.758 g/m^3 h. The higher molecular weight and the steric hindrance due to the second methyl group in xylene might cause the reduction of EC of xylene in the presence of toluene (Jorio et al., 1998).

6.6 TREATMENT OF BTEX MIXTURE

The RBC was operated from day 162 to day 231 for treating the mixture by varying BTEX concentration in the range of 0.103–1.124, 0.110–1.308, 0.105–1.228, and 0.104–1.157 g/m^3, respectively, at three different flow rates of 0.12, 0.24, 0.30 m^3/h to achieve a total loading rate from 2.602 ± 0.217 to 50.160 ± 2.418 g/m^3 h (Table 6.3). The starting concentrations of BTEX in inlet air were 0.128 ± 0.026, 0.135 ± 0.015, 0.139 ± 0.018, and 0.141 ± 0.023 g/m^3, respectively, with a total loading rate of 2.602 ± 0.217 g/m^3 h (Fig. 6.5a-d). Initially, the removal of xylene and toluene were 85% and 91%, respectively, and the removal of benzene and ethyl benzene were relatively low i.e. 69% and 72%, respectively. Gradually the removal of BTEX reached up to 89%, 98%, 91%, and 95% on the day 169. Progressively the ILRs of BTEX were increased and the RBC was operated with nine different loading rates. In phase IX, the overall RE was dropped to 68% initially at total ILR of 50.160 ± 2.418 g/m^3 h and recovered up to 82% (Fig. 6.6a). Maximum total ILR of BTEX mixture applied to the RBC was 54.504 g/m^3 h, which showed the overall RE of 81%. The removal of toluene and ethylbenzene was above 70%, while that of benzene and xylene was less, around 63% throughout the operation process. Biodegradation of BTEX in a fungal biofilter reported similar observations (Rene et al., 2012). The maximum RE was obtained for toluene throughout the process and it was found to be the highly biodegradable compound among the BTEX (Hu et al., 2007). However, Rene et al. (2005) reported the presence of toluene might inhibit the removal of other compounds. It was observed that the overall RE was decreased either due to increase in concentration or with increase in flow rate, which reduces EBCT as shown in Fig. 6.6a. The results indicated that concentration of BTEX significantly influences the RE as compared to the flow rate.

Table 6.3. Operating condition for each phase of RBC for treating BTEX mixture

Phases	Time (days)	Inlet concentration (g/m^3)				Flow rate (m^3/h)	ILR[a] (g/m^3 h)	EC[a] (g/m^3 h)	EBCT (min)
		Benzene	Toluene	Ethyl benzene	Xylene				
I	162–169	0.106–0.179	0.117–0.164	0.112–0.169	0.111–0.172	0.12	2.602 ± 0.217	2.306 ± 0.282	12.4
II	170–176	0.103–0.159	0.127–0.145	0.105–0.180	0.104–0.133	0.24	4.869 ± 0.240	4.327 ± 0.305	6.2
III	177–183	0.115–0.164	0.110–0.169	0.113–0.171	0.122–0.169	0.30	6.577 ± 0.564	5.700 ± 0.646	4.9
IV	184–192	0.245–0.366	0.279–0.384	0.293–0.354	0.294–0.379	0.12	6.217 ± 0.318	5.379 ± 0.304	12.4
V	193–200	0.293–0.385	0.277–0.396	0.290–0.352	0.285–0.346	0.24	12.469 ± 0.275	10.629 ± 0.493	6.2
VI	201–207	0.277–0.348	0.280–0.349	0.285–0.386	0.280–0.356	0.30	15.411 ± 0.705	12.996 ± 0.819	4.9
VII	208–216	0.831–1.131	0.938–1.535	0.911–1.080	0.890–1.157	0.12	20.073 ± 1.054	16.257 ± 1.639	12.4
VIII	217–223	0.822–1.206	0.954–1.256	0.845–1.064	0.902–1.098	0.24	39.888 ± 2.149	31.233 ± 3.307	6.2
IX	224–231	0.911–1.124	1.003–1.308	0.885–1.228	0.810–1.157	0.30	50.160 ± 2.418	38.223 ± 3.298	4.9
Intermittent operation									
X	232–238	0.968–1.254	1.163–1.342	1.014–1.159	0.912–1.293	0.30	48.766 ± 1.981	36.287 ± 4.083	4.9
XI	239–245	1.021–1.245	1.110–1.401	0.963–1.291	0.954–1.188	0.30	49.375 ± 2.219	31.772 ± 4.826	4.9
Shock loading									
XII	246–250	1.736–1.991	1.647–2.048	1.712–1.969	1.890–2.017	0.30	91.540 ± 3.190	51.969 ± 5.254	4.9
XIII	251–255	2.105–2.301	2.223–2.437	2.091–2.285	2.045–2.366	0.30	111.232 ± 4.226	54.593 ± 6.974	4.9

[a] The values represent the mean and their standard deviation in that phase

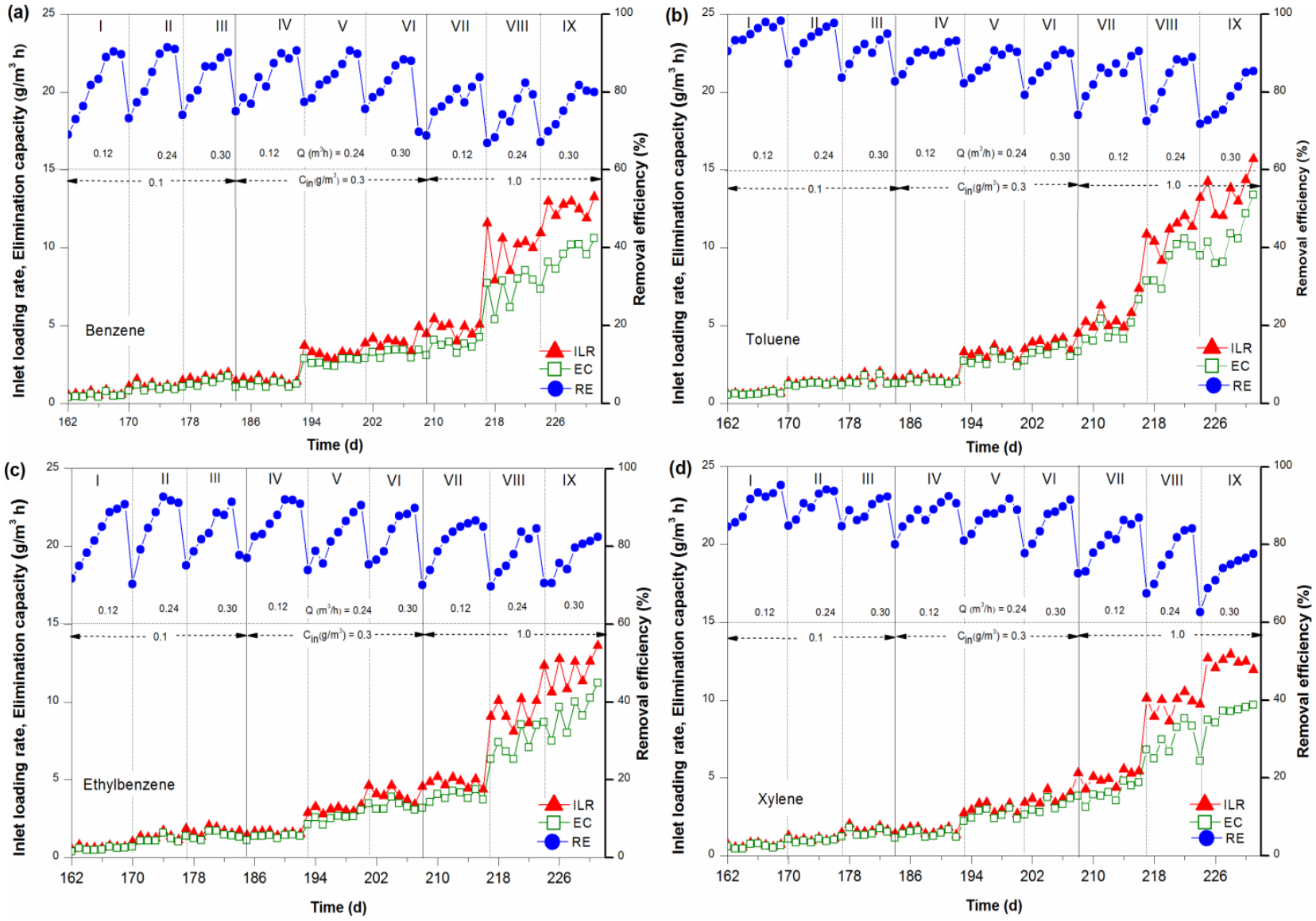


Fig. 6.5 Performance of RBC treating mixture of BTEX (a) benzene removal (b) toluene removal (c) ethylbenzene removal and (d) xylene removal

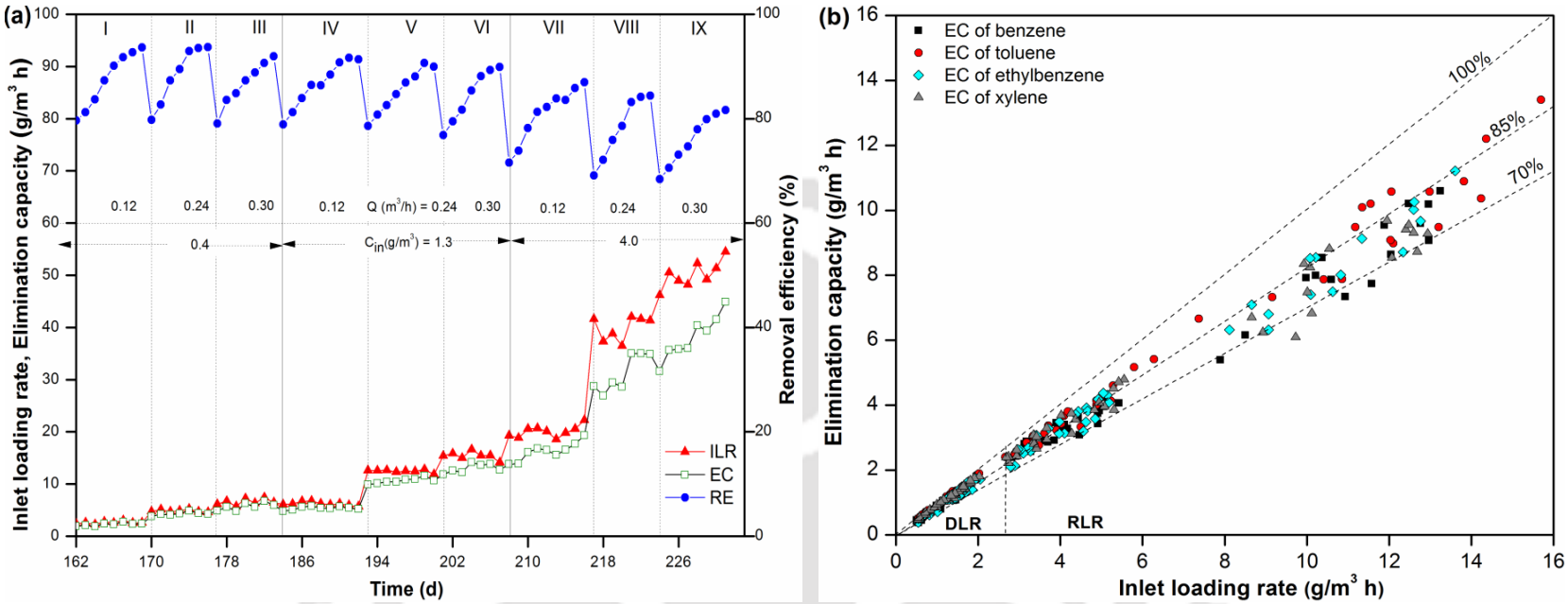


Fig. 6.6 RBC treating BTEX mixture (a) overall removal and (b) effect of ILR on EC

The EC was nearly 100% initially at lower loading rate. However, with further increase in loading rate the total EC deviated from 100% conversion line. It increased linearly with increase in loading rate up to 12.469 ± 0.275 g/m^3 h, corresponds to DLR, beyond this it increased at slower rate, corresponds to RLR (Fig. 6.6b). The order of EC_{max} decreased as follows: toluene (13.403 g/m^3 h) > ethylbenzene (11.217 g/m^3 h) > benzene (10.596 g/m^3 h) > xylene (9.681 g/m^3 h). The findings of this study are in agreement with those of García-Peña et al. (2008). This may be due to the enrichment of culture with a high toxic compound like xylene, which can efficiently degrade the less toxic compounds like toluene and ethylbenzene in BTEX mixture (Maliyekkal et al., 2004). Natarajan et al. (2017) reported that the microbial culture has strong inclination to toluene and ethylbenzene as compared to benzene and xylene due to the higher toxicity of these compounds. The EC_{max} obtained for BTEX in this study is higher than the value reported by Rahul et al. (2013).

In comparison to the previous studies on BTEX treatment using various bioreactors, the RBC in this research performed better at steady state due to higher oxygen mass transfer (Table 6.4). However, the EBCT in this system (4.9–12.4 min) is slightly higher than the previous report of Rene et al., (2012) for the treatment of BTEX by various bioreactors (0.5–4.4 min). Littlejohns and Daugulis (2009) reported a steady state EC of 18.7 g/m^3 h during the BTEX treatment in a two-phase partitioning airlift bioreactor (TPPB), and an EC_{max} of 20.24 g/m^3 h was observed in a biofilter by Rahul et al. (2013). Lu et al. (2000) used trickle-bed air biofilter (TBAB) for removal of BTEX and obtained an EC_{max} of 34.3 g/m^3 h at an ILR of 42.9 g/m^3 h. In a recent study, Akmirza et al. (2017) investigated the anoxic biotrickling filtration of BTEX and found the average EC of 4 g/m^3 h with a steady state overall RE of 58%. In the present research, the EC_{max} of BTEX was reached to 44.8 g/m^3 h, at

an average ILR of 50.16 g/m^3 h, which is higher than most of the EC_{max} values reported in the literature (Table 6.4). Rene et al. (2012) used a fungal biofilter for treating BTEX under transient-state condition, which resulted in a significant drop in the RE at high BTEX load. Similar finding is also reported by Mohammad et al. (2017) in a thermophilic biofilter dominated by fungi, where the transient-state condition reduced the RE of BTEX up to 40%.

6.7 STABILITY OF RBC

The stability of RBC was investigated during the intermittent operations and shock loading of BTEX in order to represent the actual scenario. During the intermittent operations, the RBC was shut-down twice for 2 days and after restart the performance was evaluated. In first shut-down phase both the supply of BTEX mixture in waste gas and the rotation of RBC were stopped for 2 days. After the shut-down phase, on day 234 with a total BTEX loading rate of 47.221 g/m^3 h, the overall RE observed to be decreased by 18%. However, within 4 days of operation the RE quickly recovered and reached up to 84% on day 238 (Fig. 6.7). Ravi et al. (2015) reported the necessity of re-acclimatization phase for microorganisms in the RBC after a period of shut-down to regain its stability. During the second shut-down phase only the supply of nutrient media was terminated while retaining the supply of gaseous VOCs and RBC rotation unchanged for 2 days (239–240 day). The overall RE decreased by 35% on day 241 due to significant drop in pH from 6.82 to 5.41. The nutrient supply was resumed and the performance of RBC gradually improved and the RE reached up to 76% during 242–245 day of operation at a total loading rate of 49.375 ± 2.219 g/m^3 h (Fig. 6.7). The results from this study confirmed that the supply of nutrient media influences the performance of reactor more than the supply of gaseous VOCs and rotation of RBC, which is consistent with the result of Datta and Philip (2014).

Table 6.4 Performance of various bioreactors for removal of BTEX from waste gas streams

Type of bioreactor	Pollutant	Supporting medium	Concentration (g/m^3)	ILR (g/m^3 h)	EC (g/m^3 h)	RE (%)	Microbial culture	References
RBC	BTEX	Sponge	<4.20	50.16	<44.8	>76.0	Activated sludge	Present study
Biofilter	BTEX	Corn-cob	<0.39	<20.39	<20.24	~100	*Bacillus sphaericus*	Rahul et al. (2013)
TPPB[a]	BTEX	Silicon oil	0.8	20	18.7	>76	*Pseudomonas sp.*	Littlejohns and Daugulis (2009)
TBAB[b]	BTEX	Pellet	<0.8	<21.0	<21.0	>96.0	Activated sludge	Sorial et al. (1997)
TBAB[b]	BTEX	Coal particles	<2.6	<42.9	<34.3	80.0	Activated sludge	Lu et al. (2000)
Anoxic biotrickling filter[c]	BTEX	Kaldnes rings	~2.9	5.7	4	58	Activated sludge	Akmirza et al. (2017)
Biofilter	BTEX	Sugarcane bagasse, compost and GAC	1.30	68.86	44.9	63.0	Compost	Mathur et al. (2007)
Biofilter	BTEX	Vermiculite	–	250	70	20–30	*Paecilomyces variotii*	García-Peña et al. (2008)
Biofilter[d]	BTEX	Perlite	<12.6	<371	<244	>50	*Exophiala sp.*	Rene et al. 2012
Biofilter[e]	BTEX	Perlite	<11	880	360	40	*Exophiala sp.*	Mohammad et al. (2017)

[a] Two-phase partitioning airlift bioreactor
[b] Trickle bed air biofilter
[c] Biodegrdation of BTEX in anoxic biotrickling filter using nitrate as the electron acceptor
[d] Biodegradation of BTEX in a fungal biofilter under transient-state condition
[e] Treating high BTEX load under transient-state operation at thermophilic temperature (~50 ºC)

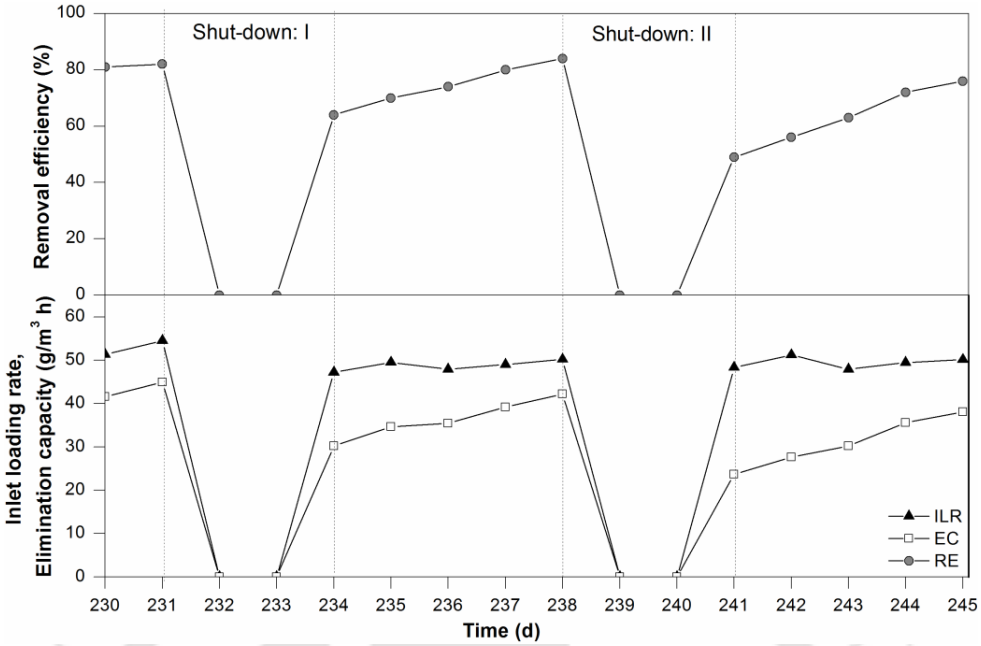


Fig. 6.7 Effect of intermittent operation on performance of RBC

Further, the performance of RBC was also evaluated for two different shock loads, in which a sudden variation in concentration of VOCs was occurred to investigate the robustness of RBC. In first phase of shock loading, at a flow rate of 0.30 m^3/h the concentration of BTEX was almost doubled to 7.667 ± 0.320 g/m^3 to achieve a loading rate of 91.540 ± 3.190 g/m^3 h (Table 6.3). Initially, the overall RE was dropped to 51% but improved further up to 64% and reached the steady state within 4 days of operation (Fig. 6.8). Later, when the concentration of BTEX increased to 9.104 ± 0.392 g/m^3 at a fixed flow rate of 0.30 m^3/h, which in turn increased the total loading rate to 111.232 ± 4.226 g/m^3 h. In this phase, it could be noticed that the overall RE of BTEX reduced quickly to 40% due to the sudden shock load and reached a steady state removal of 53% on day 255. Successive shock loading of BTEX reduced the overall performance of RBC due to limited biological activity and reaction, generally observed in biological waste gas treatment systems (Mohammad et al., 2017).

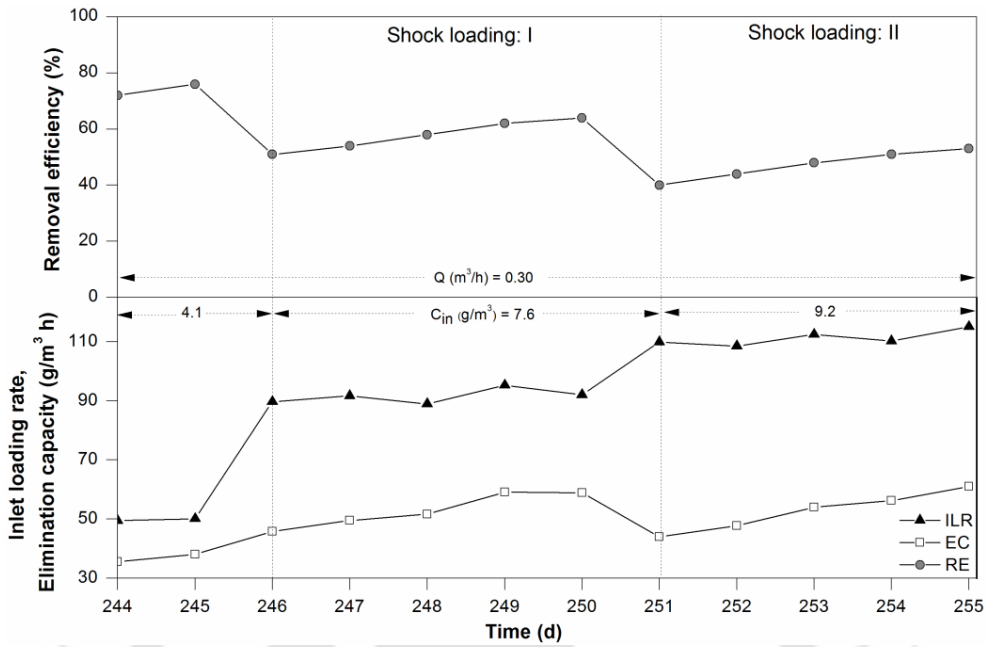


Fig. 6.8 Effect of shock loading on performance of RBC

6.8 EFFECT OF ILR ON DO, pH, AND REMOVAL OF NUTRIENTS

DO is the main limiting factor for optimum and stable operation of RBC during the treatment of xylene, combined toluene and xylene, and BTEX mixture. The xylene was treated with three different flow rates (0.12, 0.24, and 0.30 g/m^3) at a concentration of 0.459 ± 0.025 g/m^3 in phase I to III. Initially, at a lower flow rate of 0.12 g/m^3 the DO was observed to be 4.58 ± 0.13 mg/l, which increased with increase in flow rate and reached to 5.65 ± 0.12 mg/l. However, in subsequent phases xylene concentration was increased and the same three different flow rates were maintained, which increased the corresponding loading rate due to which the DO significantly reduced. At the end of phase IX, the DO was observed to be 3.05 ± 0.11 mg/l, resulting into a poor performance of RBC due to less amount of DO available for microorganisms (Sahoo et al., 2013). Similarly, the DO decreased from 5.0 ± 0.07 to 1.95 ± 0.12 mg/l for toluene and xylene mixture, and from 4.50 ± 0.08 to 2.80 ± 0.08 mg/l for BTEX mixture with increase in loading rates.

The pH of inlet nutrient medium containing sodium bicarbonate as a buffering agent was maintained at 6.95 ± 0.05, supplied continuously to RBC. The pH of effluent leachate was measured daily throughout the process. It reduced from 6.94 ± 0.10 to 6.49 ± 0.07 during the treatment of xylene and from 6.85 ± 0.06 to 6.37 ± 0.06 during the treatment of toluene and xylene mixture with increase in loading rates might be due to formation of acidic intermediates and CO_2 during the biodegradation of VOCs in the liquid phase (Saravanan and Rajamohan, 2009). The decrease was marginal up to 6.29 ± 0.12 during the treatment of BTEX mixture, which was as a result of more acidification due to simultaneous biodegradation of four compounds (Gallastegui et al., 2017).

The nutrients like nitrogen and phosphorus are essential in addition to the VOCs, which serve as source of carbon for microorganisms for efficient operation of RBC. In nutrient media, NH_3-N concentration of 212 mg/l, and PO_4-P concentration of 233 mg/l were constantly supplied till the last phase. In aerobic biodegradation process, nitrification occurs by autotrophic microorganisms, which converts ammonium ion into nitrate (Datta and Philip, 2014). At a lower xylene loading rate of 2.222 ± 0.052 g/m^3 h, the nitrate concentration of 70.881 ± 0.045 mg/l was observed with ammonia removal efficiency of 98% in the effluent at steady state. However, at a higher xylene loading rate of 43.361 ± 0.351 g/m^3 h, the removal of ammonia was reduced up to 83%. For the combined toluene and xylene at a higher loading rate of 42.016 ± 0.666 g/m^3 h the removal of ammonia was 90% and for BTEX mixture at a loading rate of 50.160 ± 2.418 g/m^3 h, it was 87%. This result signifies that with increase in inlet loading of xylene, combined toluene and xylene, and BTEX mixture, nitrification is inhibited, which increases the concentration of residual ammonium ion in effluent. Increasing loading rate stimulates the growth of heterotrophic

microorganisms which feed on organic carbon than the nitrifying microorganisms, thus nitrification was inhibited (Gomez et al., 2000). The removal of phosphorus was low as compared to ammonia, which decreased from 68% to 51% during the treatment of xylene, from 62% to 54% during the combined treatment of toluene and xylene, and from 63% to 52% during the treatment of BTEX mixture with increase in loading rate. Mathur et al. (2007) reported in a study that nitrogen is an essential nutrient, which is utilized more by microorganisms as compared to phosphorous for their growth and development.

6.9 CARBON DIOXIDE PRODUCTION

The regular production of CO_2 during the process indicates that the biodegradation of VOCs is taking place. Microorganisms use VOCs as a source of carbon and degrade in aerobic condition to carbon dioxide and water. A positive gradient of CO_2 was observed at the outlet indicating the biodegradation of VOCs in the RBC. The outlet CO_2 concentration is a function of inlet xylene, toluene and xylene, and mixture of BTEX concentrations for various flow rates of gas is presented in Fig. 6.9a-c. At the lower gas flow rate of 0.12 m^3/h, the outlet CO_2 concentration was initially 1.927 ± 0.170 g/m^3, which increased with increase in xylene concentration to the maximum of 3.859 ± 0.267 g/m^3 and decreased to 3.391 ± 0.196 and 3.167 ± 0.139 g/m^3 at the higher flow rates of 0.24 and 0.30 m^3/h, respectively, as shown in Fig. 6.9a. The results showed that the CO_2 concentration was higher at lower flow rates, which confirms the better performance of RBC at smaller flow rates (Jorio et al., 2000). Similarly, for toluene and xylene mixture, the maximum CO_2 concentration was 4.155 ± 0.262 g/m^3 (Fig 6.9b), whereas for BTEX mixture it was 4.688 ± 0.251 g/m^3 (Fig 6.9c) at the lower flow rate of 0.12 m^3/h. The maximum concentration of CO_2 produced was consistent with the highest overall RE, which was observed during the treatment of BTEX mixture.

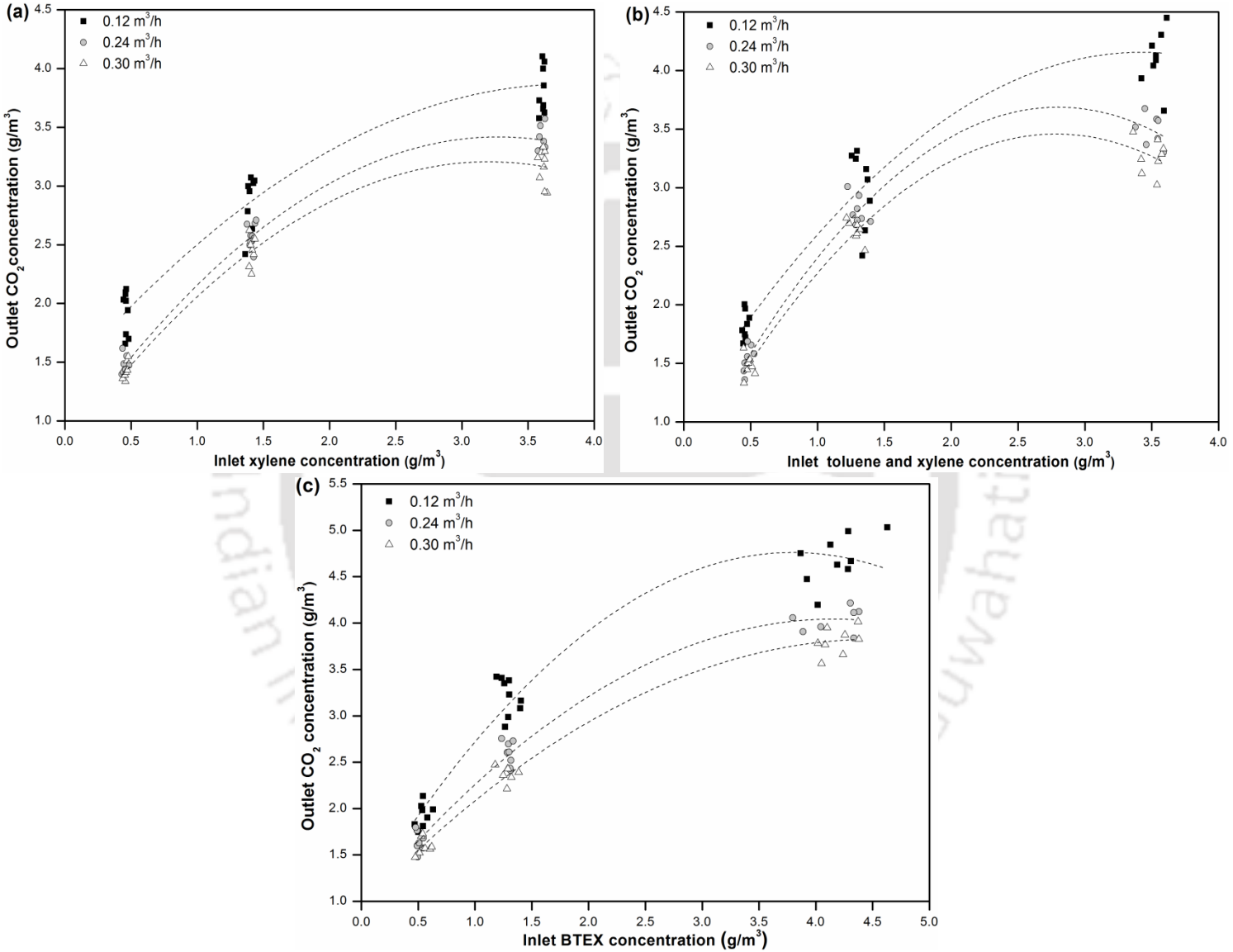


Fig. 6.9 Outlet CO_2 concentration for various gas flow rates of (a) xylene (b) mixture of toluene and xylene and (c) BTEX mixture

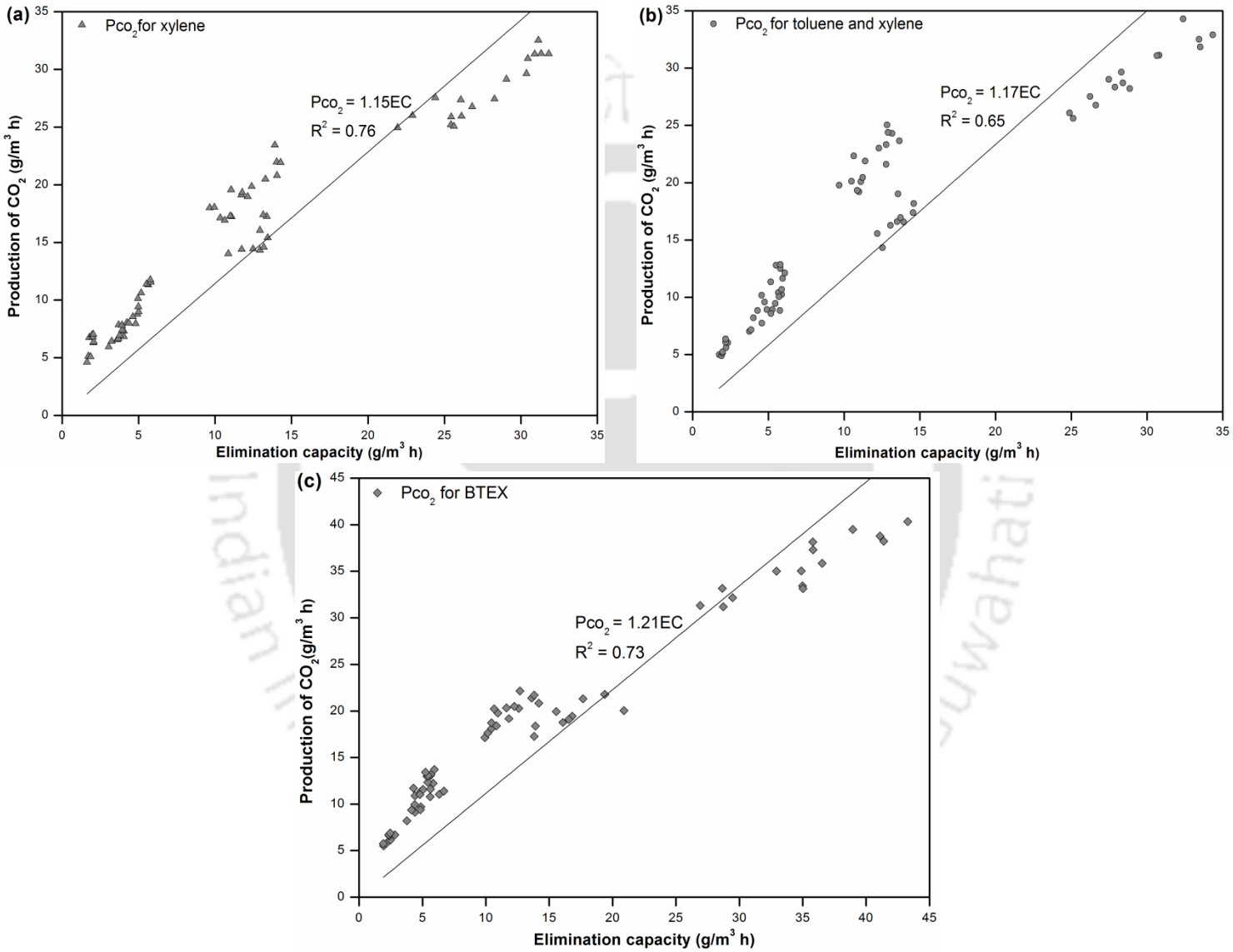


Fig. 6.10 Production of CO_2 with EC for (a) xylene (b) mixture of toluene and xylene and (c) BTEX mixture

Pco_2 is an important parameter to determine the performance of RBC. With the increase in concentration of VOC, CO_2 concentration was increased but reduced at higher flow rates (Saravanan and Rajamohan, 2009). The Pco_2 as a function of EC for various operating conditions for xylene, toluene and xylene, and BTEX mixture is shown in Fig. 6.10a-c. Experimental results show that the Pco_2 versus EC is nearly linear. The relation between Pco_2 and their corresponding EC was described by the linear regression equation, $Pco_2 = 1.15EC$ for xylene (Fig. 6.10a), $Pco_2 = 1.17EC$ for toluene and xylene (Fig. 6.10b), and $Pco_2 = 1.21EC$ for BTEX mixture (Fig. 6.10c). This demonstrates that the CO_2 yield (Pco_2/EC) was maximum (1.21) for BTEX mixture, which revealed that the treatment of more than one pollutant together might enhance the biodegradation, results into more CO_2 production (García-Peña et al., 2008). The deficit in CO_2 yield in this study than the expected (theoretical CO_2 yield = ~3.3) may be due to biodegradation of VOCs in the liquid phase, accumulation of CO_2 in bio-support medium and in biofilm as CO_3^{2-}, HCO_3^- and H_2CO_3 (Saravanan and Rajamohan, 2009). The values of CO_2 yield obtained during the treatment of xylene, toluene and xylene, and BTEX mixture are consistent with other reported results of 0.98 to 1.82 (Wu et al., 2006; Gallastegui et al., 2017; Rene et al., 2012).

6.10 BIOMASS CONCENTRATION

The biomass concentration expressed as VSS (APHA, 2005) increased considerably from one phase to the other in the effluent liquid of RBC throughout the operations. It indicates the utilization of VOCs efficiently in subsequent phases. The VSS concentration in the liquid phase found to be much higher (3537 ± 36 mg/l) at the end of the operation. This might be due to the removal of biomass from the bio-support medium, which provided long-term and stable performance of RBC (Fig. 6.11). Yang et al. (2004) reported increases in the amount

of biomass on the bio-support medium, may have produced a higher biomass concentration in the effluent liquid.

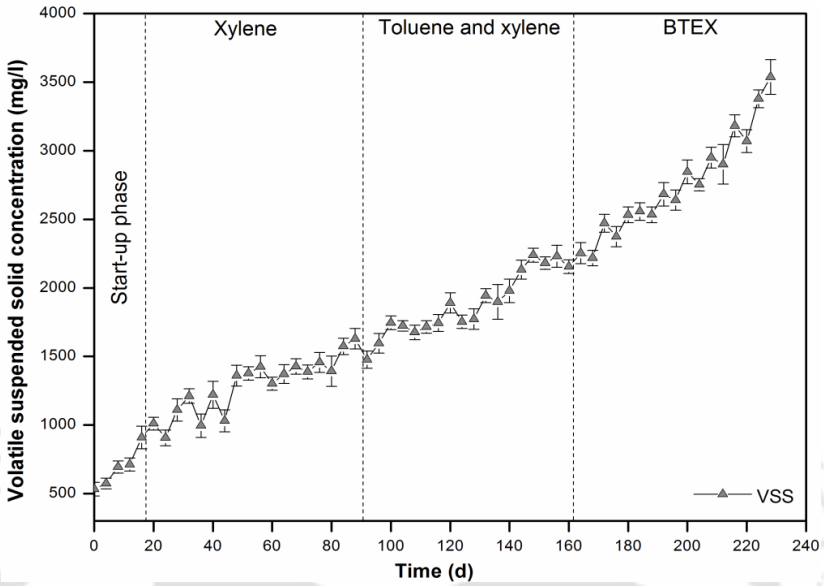


Fig. 6.11 Biomass concentration (VSS) in the liquid phase of RBC

6. 11 IDENTIFICATION OF BTEX DEGRADING MICROORGANISMS

After successful operation of RBC for 231 days of treating xylene, combined toluene and xylene, and BTEX mixture, an attempt was made for isolation and identification of the predominant BTEX degrading microorganisms in mixed culture. The isolation and identification of predominant BTEX degraders by biochemical and molecular characterizations is described in detail in section 3.3.6. The results of biochemical characterization were similar as described in the section 5.7, which showed that the mixed culture contains *Enterobacter* species, became dominant responsible for BTEX biodegradation, although *Pseudomonas* species was also present. The predominant *Enterobacter* species was further analyzed by molecular characterization and its 16S rDNA sequence was compared with the previously available sequences with those in GenBank. The sequence of the identified strain had 99% similarity with *Enterobacter cloacae*, subsp.

Dissolvens, strain TN2002013 based on the phylogenetic analysis (Fig. 6.12). The isolated culture was designated as *Enterobacter cloacae* strain SP4001 and the sequence data submitted to NCBI GenBank under accession number KY238115. Wang et al. (2015) reported the biodegradation of p-xylene by *Enterobacter* species in biotrickling filter.

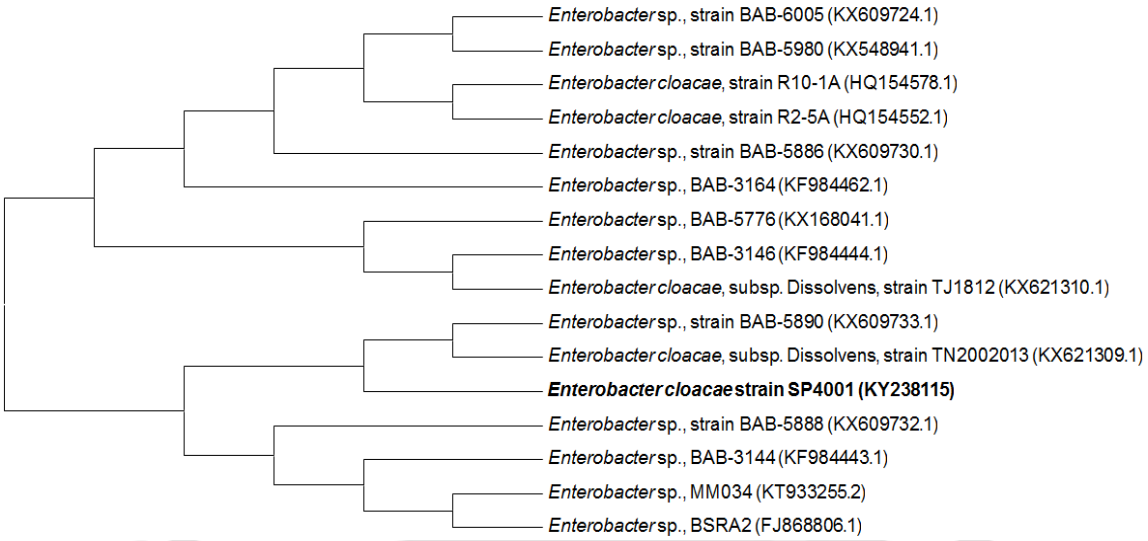


Fig. 6.12 Phylogenetic tree of *E. cloacae* SP4001 on the basis of 16S rDNA sequencing

6.12 ANALYSIS OF BIOKINETIC PARAMETERS

The biokinetic parameters describe the kinetic behaviour of RBC, are obtained from the steady state outlet concentrations for various inlet concentrations of xylene, combined toluene and xylene, and a mixture of BTEX. During the treatment of mixture of compounds the characteristics such as solubility, volatility, and biodegradability of one compound vastly differ from other (Mathur and Majumder, 2008). Therefore, various mechanisms may be responsible for their efficient biodegradation. The kinetics of the system was best represented by the modified Monod model as shown in Eq. (6.1) and the kinetic parameters were determined. The equation 6.1 describes the mathematical model for determining the biokinetc parameters. The mathematical model is described in detailed section 3.5.6.

$$\frac{V/Q}{C_i - C_o} = \frac{K_s}{r_{max}} \frac{1}{C_{ln}} + \frac{1}{r_{max}} \quad (6.1)$$

The kinetic constants r_{max} and K_s can be obtained by a best fit straight line plot of $(1/C_{ln})$ versus $[(V/Q)/(C_i - C_o)]$ using Eq. (6.1) for xylene, toluene and xylene, and BTEX are shown in Figs. 6.13a, 6.13b, and 6.14, respectively. During the treatment of xylene, the r_{max} and K_s were estimated as 37.878 g/m^3 h and 2.041 g/m^3, respectively. During the combined treatment, the r_{max} values of 20.408 and 16.207 g/m^3 h, and K_s values of 0.90 and 0.632 g/m^3 were calculated for toluene and xylene, respectively. However, for BTEX mixture, the r_{max} values were 12.722, 16.077, 14.471 and 11.806 g/m^3 h, and K_s values were calculated as 0.681, 0.734, 0.751 and 0.636 g/m^3, respectively. The values of r_{max}/K_s obtained in this study are higher than the values reported in the literature (Mathur and Majumder, 2008; Rahul et al., 2012), which indicate that the RBC is more efficient as compared to the other bioreactors.

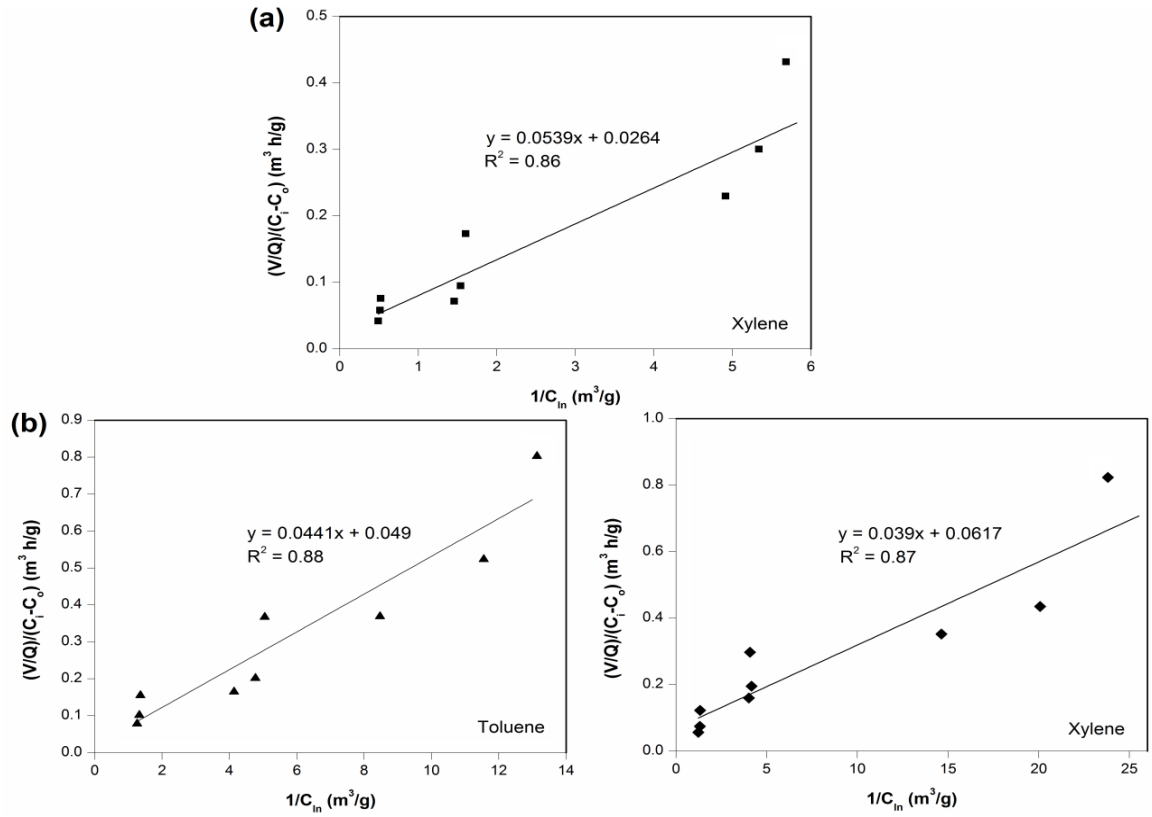


Fig. 6.13 Determination of kinetic parameters of (a) xylene and (b) toluene and xylene

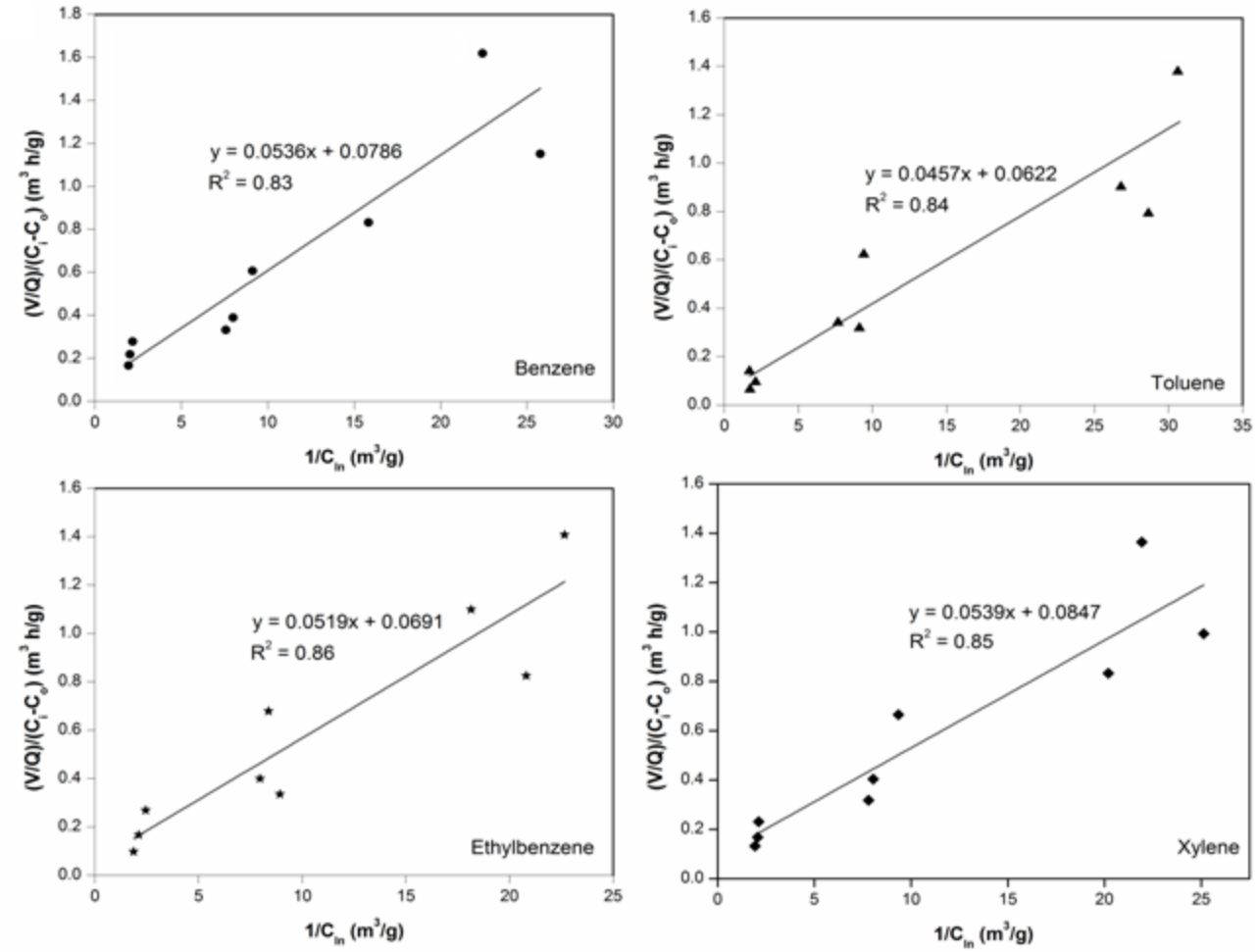


Fig. 6.14 Determination of kinetic parameters of BTEX

6.13 CONCLUSION

Screening of enriched cultures and acclimatization of RBC with xylene reduced the time period to reach the steady state and enhanced the treatment of VOCs. The RE of xylene was found to be up to 72% at the highest ILR of 43.361 ± 0.351 g/m^3 h. The presence of toxic compounds like xylene had an enhancing effect on toluene removal when treated in mixture, as it improved the overall RE up to 79% at the loading rate of 42.016 ± 0.666 g/m^3 h. The toluene removal was more effective than xylene and at high toluene concentration the biodegradation of xylene was inhibited. In the BTEX mixture, the order of removal was toluene > ethylbenzene > benzene > xylene. The overall RE of BTEX was up to 82% at the highest loading rate of 50.160 ± 2.418 g/m^3 h, which was higher than the RE of xylene and that of combined toluene and xylene. The gradual increase in loading rate of VOCs had inhibitory effect on DO and the removal of nutrients. The higher CO_2 concentration and RE of VOCs confirmed the better performance of RBC at smaller flow rates. *Enterobacter cloacae* strain SP4001 is identified as a predominant strain in mixed culture responsible for biodegradation of BTEX compounds. The RBC is effective in removing xylene, toluene and xylene, and BTEX mixture in waste gas streams along with nutrients in wastewater, which shows its dual application and potential use for treating industrial waste streams.

CHAPTER 7

CONCLUSION AND FUTURE SCOPE

7.1 GENERAL

This chapter summarizes the major conclusions and key findings of the research work, limitations and provides future scope for the research.

7.2 MAJOR CONCLUSIONS

- Optimization of pH and concentration prior to RBC operation for a specific VOC is helpful.
- The sponge-medium RBC removed benzene with higher efficiency.
- Screening of enriched cultures enhanced the treatment of VOCs.
- The sponge-medium RBC removed gaseous BTEX with higher efficiency even at higher loading rates than obtained by various conventional techniques by several researchers.
- The sponge-medium provided high surface area for development of biofilm, high porosity for homogeneous distribution of gas and low pressure drop, which provided improved and stable performance of RBC.
- *Enterobacter cloacae* SP4001 was identified as a predominant strain in mixed culture responsible for degradation of BTEX.
- The RBC provided dual benefits of treating gaseous VOCs as well as nutrients from wastewater using industrial sludge as an inoculumn, which shows its potential application in industries.

7.3 KEY FINDINGS

Chapter IV:

- The results of the batch study performed on benzene biodegradation by indigenous mixed microbial culture revealed the increase of cell growth with increase in benzene concentration and inhibition beyond 300 mg/l.
- The kinetics involved in the process was modeled reasonably well with the Haldane model.
- The RSM enhanced the benzene biodegradation by optimizing the pH and benzene concentration.
- The intermediates produced during benzene biodegradation confirmed the established pathway of benzene biodegradation.
- *E. cloacae* SG208 (KU297784.1) was found to be the predominant organism in the enriched mixed culture responsible for degrading benzene.

Chapter V:

- The RBC with a sponge-medium supported on a perforated rotating drum was found to be effective in controlling gaseous benzene up to about 95%.
- Even at higher loading rate (69.375 ± 0.591 g/m^3 h) the RBC performed well, providing RE of over 59%, which has been higher in comparison with the results reported in the literature.
- The performance of the RBC was stable as the biomass changed considerably during the operation and was much higher at the later phase due to sloughing.
- The *Enterobacter* sp. was identified to be the predominant strain biodegrading

gaseous benzene.

- The flow rate beyond 0.30 m^3/h decreased the RE up to about 80% due to less EBCT. Therefore, lower flow rates of 0.12, 0.24 and 0.30 m^3/h were used in subsequent study of RBC at various concentrations of BTEX.

Chapter VI:

- The screening of enriched cultures and acclimatization with xylene improved the treatment efficiency of xylene, combined toluene and xylene and BTEX mixture, and reduced the time period to reach the steady state.
- Even at higher loading rate (43.361 ± 0.351 g/m^3 h) the RBC was effective in removing gaseous xylene at efficiency up to 72%.
- During the combined treatment of gaseous toluene and xylene, the overall removal efficiency reached up to 79% at steady state with a higher loading rate (42.016 ± 0.666 g/m^3 h). In this study, the toluene removal was more effective than xylene and at higher loading rate of toluene the removal of xylene was inhibited.
- The treatment of BTEX mixture showed toluene more biodegradable, followed by ethylbenzene, benzene and xylene. The overall removal efficiency of BTEX mixture reached up to 82% even at the loading rate of 50.160 ± 2.418 g/m^3 h, which was higher as compared to the removal of xylene and combined toluene and xylene.
- Intermittent operation of RBC confirmed that the supply of nutrient media influences the performance of reactor more.
- The inhibitory effect was found to be more on dissolved oxygen, pH and removal of nutrients with increasing loading rate of VOCs.
- The higher outlet CO_2 concentration and the RE of VOCs confirmed the better

performance of RBC at smaller flow rates due to more EBCT.

- *E. cloacae* SP4001 (KY238115) was found to be the predominant strain in the mixed culture responsible for degradation of BTEX compounds.

7.4 LIMITATIONS

- Shaft bearings and mechanical units required frequent maintenance due to continuous rotation, which may increase the operational and maintenance cost.
- The initial cost of installation of RBC is more as compared to the other bioreactor. Moreover, it takes longer time for start-up process.
- At higher concentrations, the performance of RBC decreases.
- The loss of VOCs in effluent liquid is neglected due to the relatively less solubility.

7.5 FUTURE SCOPE

- The optimization of operation of RBC using RSM might have a better prospective for treatment of VOCs, which may consume less time and able to determine the interactive effect of variables on removal of VOCs.
- UV photo-oxidation can be studied as a pre-treatment process to improve the RE at higher concentration by RBC.
- The use of fungus or pure culture strain, specific to different VOCs, which may give higher RE as compared to bacterial systems, may be studied.
- It may be possible to determine the percentage of removal of VOCs in liquid medium and in sponge-medium separately.
- The detailed degradation mechanism and pathway of the BTEX compounds, and mass balance during biodegradation can also be studied.

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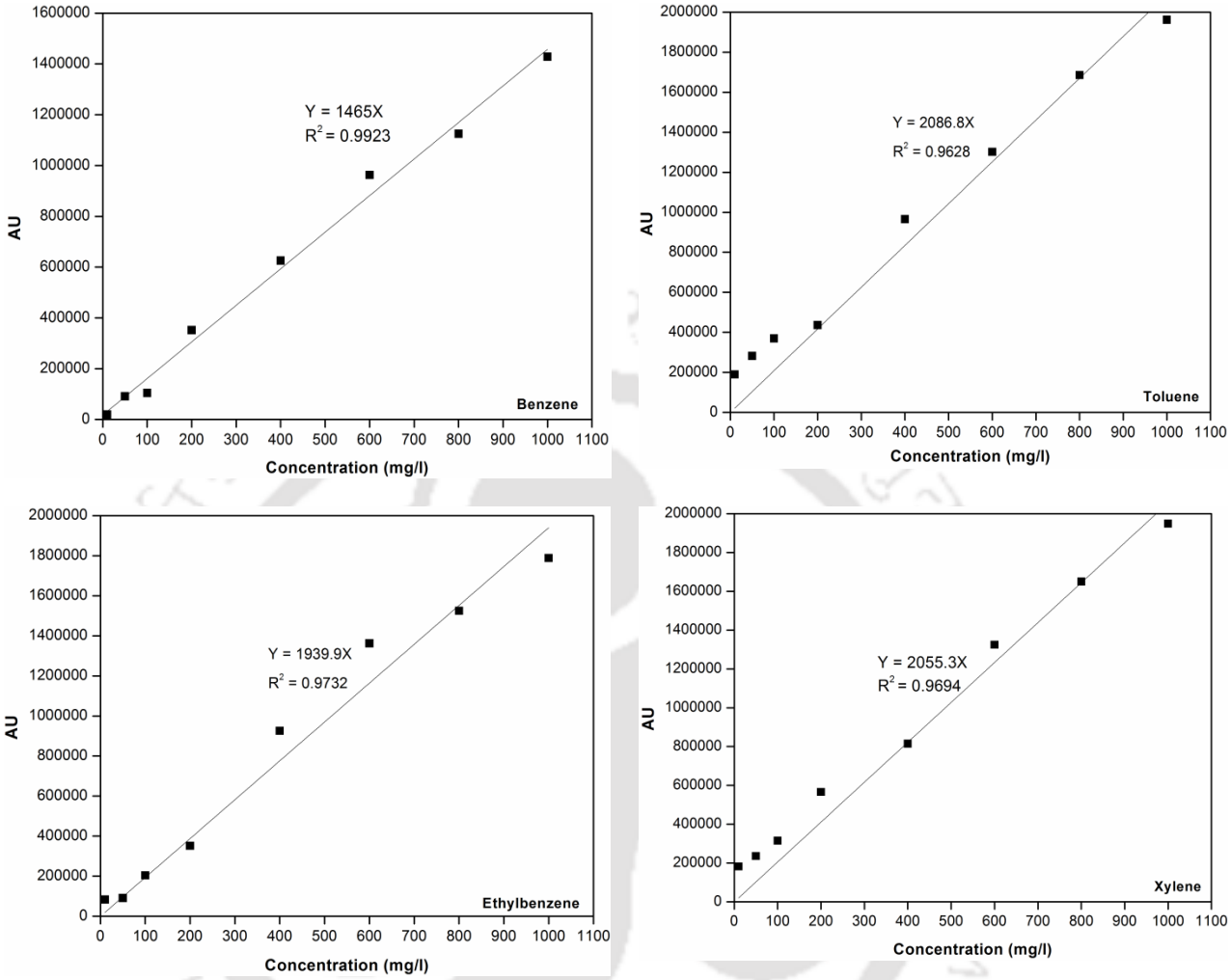
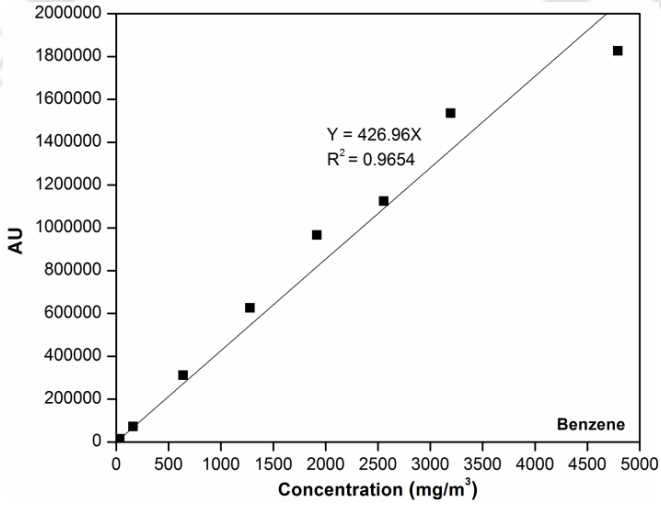
APPENDIX-I

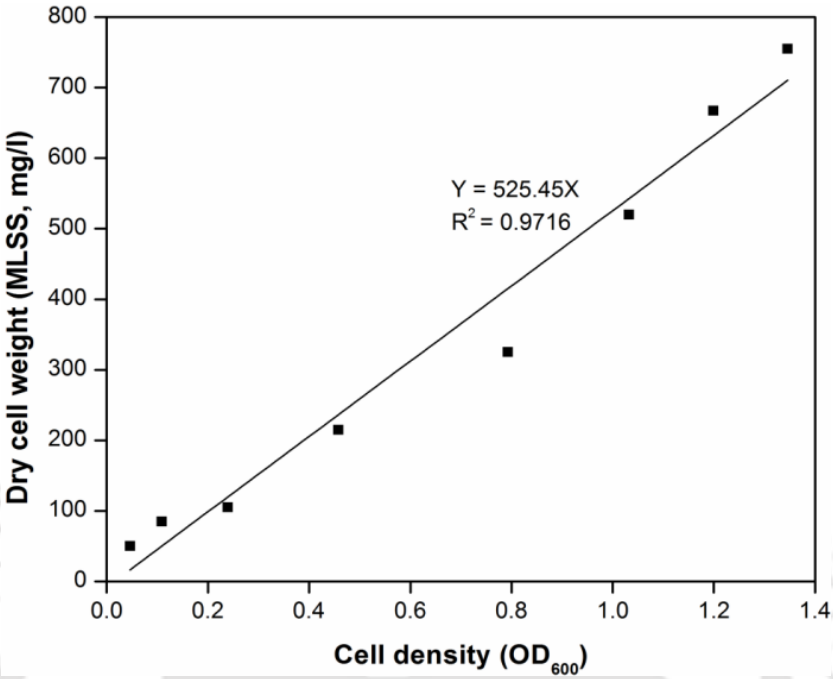


Calibration curve obtained for BTEX using n-hexane as a solvent



Calibration curve obtained for benzene using methanol as a solvent

APPENDIX-II



OD_{600} versus dry weight of biomass

PUBLICATIONS FROM THE RESEARCH

Refereed International Journals:

I. Published

1. Padhi, S.K., Gokhale, S. 2014. Biological oxidation of gaseous VOCs–rotating biological contactor a promising and eco-friendly technique. *Journal of Environmental Chemical Engineering* 2, 2085–2102.

2. Padhi, S.K., Gokhale, S., 2016. Benzene control from waste gas streams with a sponge-medium based rotating biological contactor. *International Biodeterioration & Biodegradation* 109, 93–103.

3. Padhi, S.K., Gokhale, S., 2017. Benzene biodegradation by indigenous mixed microbial culture: Kinetic modeling and process optimization. *International Biodeterioration & Biodegradation* 119, 511–519

4. Padhi, S. K., & Gokhale, S. (2017). Treatment of gaseous volatile organic compounds using a rotating biological filter. *Bioresource Technology* 244, 270–280.

Conference:

1. Padhi, S.K. and Gokhale, S., 2015. Biodegradation of an indigenous microorganism in a batch reactor. *Bio Tech 2015., Int. Conf. on Advances in Biotechnology,* 13-15 March 2015, IIT Kanpur.
2. Padhi, S.K. and Gokhale, S., 2015. Optimization and modelling of growth kinetics during benzene biodegradation in batch reactors. *Int. Conf. on New Horizons in Biotechnology,* 22-25 November 2015, Trivandrum, India.
3. Padhi, S.K. and Gokhale, S., 2015. A hybrid rotating biological filters to control VOC from waste gas streams. *CHEMCON 2015,* 27-30 December 2015, IIT Guwahati, India.
4. Padhi, S.K. and Gokhale, S., 2016. Performance of rotating biological filter on removal of xylene from waste gas streams. *RECYCLE 2016,* 1-2 April 2016, IIT Guwahati, India. (**Best oral presentation award**)
5. Padhi, S.K. and Gokhale, S. (2016). Performance of rotating biological filter treating mixture of xylene and toluene from waste gas streams. *Asia-Pacific conference on biotechnology for waste conversion*, 6-8 December 2016, **Hong Kong Baptist University, Hong Kong**.