TOMSK POLYTECHNIC UNIVERSITY

Available online at www.sciencedirect.com

ScienceDirect

Resource-Efficient Technologies 2 (2016) S87–S95

www.elsevier.com/locate/reffit

Research paper

Removal of aqueous benzene in the immobilized batch and continuous packed bed bioreactor by isolated *Bacillus* sp. M1

M.K. Kureel [a], S.R. Geed [a], B.S. Giri [a], A.K. Shukla [b], B.N. Rai [a], R.S. Singh [a,*]

[a] *Department of Chemical Engineering & Technology, Indian Institute of Technology (BHU), Varanasi 221005, India*

[b] *Department of Botany, Institute of Science, Banaras Hindu University, Varanasi 221005, India*

Received 20 June 2016; received in revised form 11 November 2016; accepted 14 November 2016

Available online 20 December 2016

Abstract

Benzene biodegradation was studied in batch and continuous packed bed bioreactors using polyurethane foam (PUF) as packing media by isolated *Bacillus* sp. M1. The values of optimized process parameters were found to be 800×10^6 CFU·mL^{-1}, 400 mg·L^{-1}, 7.0 and 37 °C for inoculum size, substrate concentration, pH and temperature respectively. Continuous packed bed bioreactor (CPBBR) was operated and monitored for 69 days on laboratory scale at various flow rates (10–60 mL·h^{-1}). The steady state removal efficiency was observed more than 90% up to the inlet load of 288 mg·L^{-1}.d^{-1} and elimination capacity was found to be 91.2–266.4 mg·L^{-1}·day^{-1}. Monod growth model was applied for the removal of benzene and values were found to be (K_s: 215.07 mg·L^{-1}; μ_{max}: 0.314 day^{-1}).

Keywords: Biodegradation; Removal efficiency; Benzene; Elimination capacity; Growth kinetic; Batch adsorption

1. Introduction

Benzene is a component of gasoline and aviation fuels and also used extensively as solvents, raw material for synthesis of various organic compounds, plastic, detergents, pesticides, cleaning of laboratory equipment etc. [1]. The following activities and manufacturing processes (other than benzene production or use of benzene as a feedstock) were identified as additional sources of benzene emissions: oil and gas wellheads, petroleum refineries, glycol dehydrators, gasoline marketing, publicly owned treatment works (POTWs), landfills, pulp and paper manufacturing, synthetic graphite manufacturing, carbon black manufacturing, rayon-based carbon manufacturing, aluminum casting, asphalt roofing manufacturing, and use of consumer products and building supplies [1,2]. The annual production of benzene, toluene, and xylenes is 95 million metric tons in the year 2012, while benzene was consumed alone about 40 million metric tons per year [3]. It is frequently found as a contaminant in soil, water, and air from various sources such as emission from industrial units, storage tanks, leakage from pipelines, accidental oil spills, improper waste disposal technique etc. Main source of benzene in groundwater is leakage in underground storage tanks and pipelines [1,4].

Due to its high toxicity, carcinogenicity and mutagenic activity, benzene has been reported as a priority pollutant by several environmental agencies [5,6]. Because of the human health concerns; USEPA set a maximum contaminant level 5 μg·L^{-1} of benzene in drinking water [2]. Exposure to benzene in humans causes severe lung cancer or leukemia, DNA strand breaks and chromosomal damage [7,8]. Due to its toxicity and health hazard to human beings there is an urgent need for the removal of benzene from the contaminated environment.

Several researchers have developed various techniques for the treatment of toxic waste containing BTEX which is generated from industrial processes and bioremediation techniques are one of them which are more promising than physicochemical methods such as extraction, incineration, adsorption, biosorption, catalytic destruction etc. [8–13],.

Bioremediation technique could be more effective because its application often involves manipulating environmental parameters to allow the better growth of the microbial community and ease the rate of biodegradation. The continuous mode of bio-treatment provides several benefits as compared to batch

International Conference on Separation Technologies in Chemical, Biochemical, Petroleum and Environmental Engineering (TECHNOSCAPE 2016).

* Corresponding author. Department of Chemical Engineering& Technology, Indian Institute of Technology (BHU), Varanasi 221005, India.

E-mail address: rssingh.che@itbhu.ac.in (R.S. Singh).

http://dx.doi.org/10.1016/j.reffit.2016.11.010

bio-treatment for pollutant degradation because of their ease of automation and process parameters control resulting in a reduced operational cost, enhanced degradation and eco-friendly [14,15].

Various supporting packing media in bio-filtration of benzene in vapor phase have been used such as granular activated carbon (GAC), sugarcane bagasse, peat, compost, wood charcoal, polyurethane foam etc. [16–22]. In present study, PUF used as packing media for bio-treatment of aqueous benzene PUF offers various advantages as packing media like good strength, durability, high surface area, high moisture retention capacity, light weight etc. Biodegradation efficiencies have been increased in immobilized cells on PUF in terms of sustainability, degradation efficiency and durability as compared to free cells [23]. Immobilized cells also improved cell viability and able to tolerate and treat high concentrations of pollutants for a longer duration [24,25].

Packed Bed Bioreactors (PBBRs) are the most promising and convenient bio-treatment system among the wide range of bio-reactor designs reported with immobilized cells on different packing media [26,27]. A PBR has several advantages such as ease to scaling-up and high-yield operation due to possibility of automation of separation process leading to high degrees of purification, the opportunity of treating a large volume of wastewater continuously by a specified quantity of immobilized cells, and reuse of biomass [14,28].

Various researchers have investigated the degradation of benzene in vapor phase using bacterial communities such as *Pseudomonas* sp., *Rhodococcus* sp, *Klebsiella* sp, and A*lcaligenes xylosoxidans* Y234 in different kinds of bioreactor systems as shown in Table 1. However only few researchers have performed the removal of aqueous phase benzene using bacterial sp. like A*lcaligenes xylosoxidans* Y234, *Pseudomonas putida* MHF 7109, *Pseudomonas putida P. fluorescens* and *P. putida* F1 (ATCC 700007) in hybrid, two-phase partitioning and fibrous bed bioreactor [30,32–35]. In above bioreactor systems have several drawbacks such as a cell over growth, striping difficult to maintain uniform aeration, etc.

To overcome above mentioned problems a novel CPBBR was designed with unique features like provision of multiple ports in order to maintain the uniform air supply and provision of condenser to recycle the benzene vaporized during the bioremediation process.

The objectives of present study were to investigate and compare the performance of batch, PBBR and CPBBR immobilized with *Bacillus* sp. *M1* microbial community under optimum operating conditions. Biodegradation kinetics was also studied and kinetics parameters were calculated using Monod model.

2. Materials and methods

2.1. Enrichment and isolation

The soil samples were collected from the transformer oil contaminated soil nearby DLW Hydel plant Bhikharipur Varanasi, India [25° 26′ N, 82° 92′E and 129 m above the mean sea level]. Benzene utilizing bacteria were isolated from this

Table 1
Literature survey on benzene biodegradation.

Sl.no	Compounds	Bacterial ps.	Packing material	Concentration mg/l	Continuous bioreactor	Removal efficiency/rate of biodegradation	Elimination capacity	Loading rate	References
1	BTEX (air)	Consortia	Granular activated carbon (GAC)	200 mg L^{-1}	Biofilter	≥90%	–	17.6 m^3/m^2	[16]
2	Benzene (air)	Pseudomonas sp.	Sugarcane bagasse	10–50 mg m^{-3}	Biofilter	–	–	6:12 g m^{-3} h^{-1}	[17]
3	BTEX (air)	Fungal	–	0.2–12.6 g m^{-3}	Biofilter	≥95%	73.7 g m^{-3} h^{-1}	338.7 g m^{-3} h^{-1}	[29]
4	BTEX (air)	Mixed culture	Peat	30–70 mg L^{-1}	Biofilter	78–99%	–	–	[18]
5	Benzene (air)	Rhodococcus	Compost	0.01–0.2 g m^{-3}	Biofilter	81–100%	20.1 g m^{-3} h^{-1}	1.2–6.4 g m^{-3} h^{-1}	[19]
6	Benzene (air)	*Klebsiella*sp.	–	20 mg L^{-1}	–	87%	–	–	[30]
7	Benzene (air)	A*lcaligenesxylosoxidans* Y234	–	340 mg L^{-1}	Two-phase partitioning bioreactor	95%	133 g m^{-3} h^{-1}	140 g m^{-3} h^{-1}	[31]
8	Benzene (liquid)	A*lcaligenesxylosoxidans* Y234	–	100 mg L^{-1}	Two-phase partitioning bioreactor	63.8%	–	–	[32]
9	Benzene (liquid)	*Pseudomonas putida* MHF 7109	Cow dung microflora	50–250 mg L^{-1}	Two phase partitioning bioreactor	65%	–	–	[33]
10	Benzene (liquid)	*Pseudomonas putida* F1 (ATCC 700007)	–	4400 mg L^{-1}	Two phase partitioning bioreactor	91.7–183.3 mg/l./h	–	–	[34]

soil using mineral salts medium (MSM). The enrichment of bacteria was done in a MSM media containing ($g \cdot L^{-1}$) of K_2HPO_4, (4.27); KH_2PO4, (3.48); $(NH_4)_2SO_4$, (0.34); $MgSO_4.7H_2O$, (0.46); $FeSO_4$, (0.001); $CaCl_2.2H_2O$, (0.018). Trace elements were in ($mg \cdot L^{-1}$) $CuCl_2.2H_2O$, (0.01); $CoCl_2.6H_2O$, (0.2); $ZnSO_4.7H_2O$, (0.1); $MnCl_2.4H_2O$, (0.03); $Na_2MoO_4.2H_2O$, (0.03); and $NiCl_2.6H_2O$, (0.02) [36]. The pH was adjusted to 7 ± 0.1 For enrichment, 5 g of soil samples were inoculated into 100 mL Erlenmeyer flasks containing 50 ml MSM media and benzene (concentrations ranging from 50 to 1000), then the whole setup was kept in incubator shaker maintained at 37 °C for one week. The maximum growth was observed in flask inoculated with 250 $mg \cdot L^{-1}$ of benzene. Further, this suspension was serially diluted from 10^{-1} to 10^{-9}. From each dilution (10^{-5}–10^{-9}), 100 μL of the sample was taken and inoculated with 100 μL of the culture then transferred to nutrient broth agar plates. The bacterial colonies were observed on the plates and then transferred into the MSM agar plate where benzene was the sole source of carbon. A pure culture of isolates was obtained by repeated plating over benzene coated MSM agar medium. Cell growth was determined by measuring optical density at 600 nm against control by spectrophotometer (Elico SL 210, India). Control was prepared in MSM medium without benzene.

2.2. Molecular characterization of bacterium isolate

Bacterium genomic DNA was extracted using the standard protocols of Sambrook et al. [37], details are described in elsewhere [38]. Genomic DNA was subjected to PCR amplification of 16SrRNA gene with universal primers Bac8F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′) reported by Edwards et al. 1989 and Stackebrandt et al. 1993. DNA amplifications were carried out in a Thermocycler (PCR) (Biorad Laboratories, Inc, Australia). PCR reaction mixture was prepared in a final volume of 50 μL containing 10 mM tris–HCl, 1.5 mM $MgCl_2$, each dNTP at a concentration of 0.2 mM, IU of Taq DNA polymerase, each primer at a concentration of 0.2 mM, and 1 μL of the DNA template. Each cycle consisted of initial denaturation temperature at 94 °C for 2 minutes followed by 30 cycles of amplification program comprising a denaturation step at 94 °C for 50 s, annealing at 48 °C for 30 s and extension at 72 °C for 1.3 minutes and final extension was 72 °C for 6 minutes. Five microliters of the amplified mixture was then analyzed using 1% agarose gel and electrophoresis. The gel was stained with ethidium bromide (EtBr) and visualized on gel doc under UV light. The products of PCR were purified prior to sequencing using PCR purification kit (Axygen, USA) and then sequenced by automated DNA sequencer and analyzer using Big Dye Terminator v3.1 cycle sequencing Kit (DNA sequencer, 3100 DNA analyzer, Applied Biosystems, USA). The sequences obtained first determine the percentage of similar nucleotides to 16S rRNA gene sequences in the GenBank using BLASTN program available in NCBI (National Centre for Biotechnology Information) database. Further, the sequences were aligned manually with published sequences in NCBI database using CLUSTALW multiple sequence alignment program. Algorithm of the neighbor-joining was used. The optimal tree with the sum of branch length equal to 0.02184967 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches .The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree to generate the phylogenetic tree and evolutionary analyses were conducted in MEGA5 [39–41].

16S rRNA gene sequence data of bacterial isolates have been submitted to the GenBank database under accession number KU845306.

2.3. Packing material and adsorption studies

PUF sheet purchased from the local market (Prakash Stationary, Lanka, BHU, India) was cut into cubes of approximately 1 cm^3 size. Pieces were washed by distilled water then ethanol, squeezed and dried in oven at 60 °C for overnight. Further, these pieces were used for packing media in the CPBBR.

Experiments of adsorption studies of benzene were carried out using PUF as adsorbent at various concentrations [42] and results indicated that the PUF had negligible adsorption capacity for benzene.

2.4. Batch and packed bioreactors / experimental setup

Batch experiments were conducted for the optimization of process parameters such as pH, temperature, inoculum size, DO and concentration of pollutants (benzene) at laboratory scale in serum bottles of 100 mL volume. The pH, temperature, DO and inoculum size were varied in a range of 6.0–10, 28–43 °C, 3.4–7.2 and 2.0×10^8–8.0×10^8 CFU mL^{-1} respectively at fixed concentration of 250 mg L^{-1} of benzene. All measurements were made in triplicate to minimize the experimental error. A blank run without inoculum was used as the control and change in benzene concentration with time due to adsorption or transfer to air stream was measured and was found to be insignificant. ANOVA was also applied to study for minimization of experimental error. Optimum values of parameters obtained were used in packed reactors (PBBR and CPBBR) to maximize the percent removal of benzene.

PBBR was consisted of a cylindrical borosilicate glass column of perimeter [ID: 6 cm; L: 55 cm; working volume: 1000 mL; total volume: 1554 mL] with provision of inlet and outlet at 4 and 44 cm above the bottom. All sampling, inlet and outlet ports were closed by silicon tubing with pinch cork to avoid contamination. Packed reactors were connected by silicon tube (2 mm) for filtered air supply at the bottom of reactor. Recycle of gaseous benzene was done using a condenser (Fig. 1). Flow rate of air was measured and regulated by rotameter (1–5 LPM). PUF pieces soaked in an aqueous solution of benzene of known concentration along with inoculum were filled in PBBR at a height of 25 cm and the air was supplied by an air compressor. Same reactor was operated in the packed and continuous mode. Outlet is blocked in batch mode and a definite amount of benzene (400 mg L^{-1}) was added to the reactor. Same reactor was operated in the continuous mode and called as CPBBR in this paper. Kinetics studies were carried out in batch packed bed bioreactor under optimum conditions.

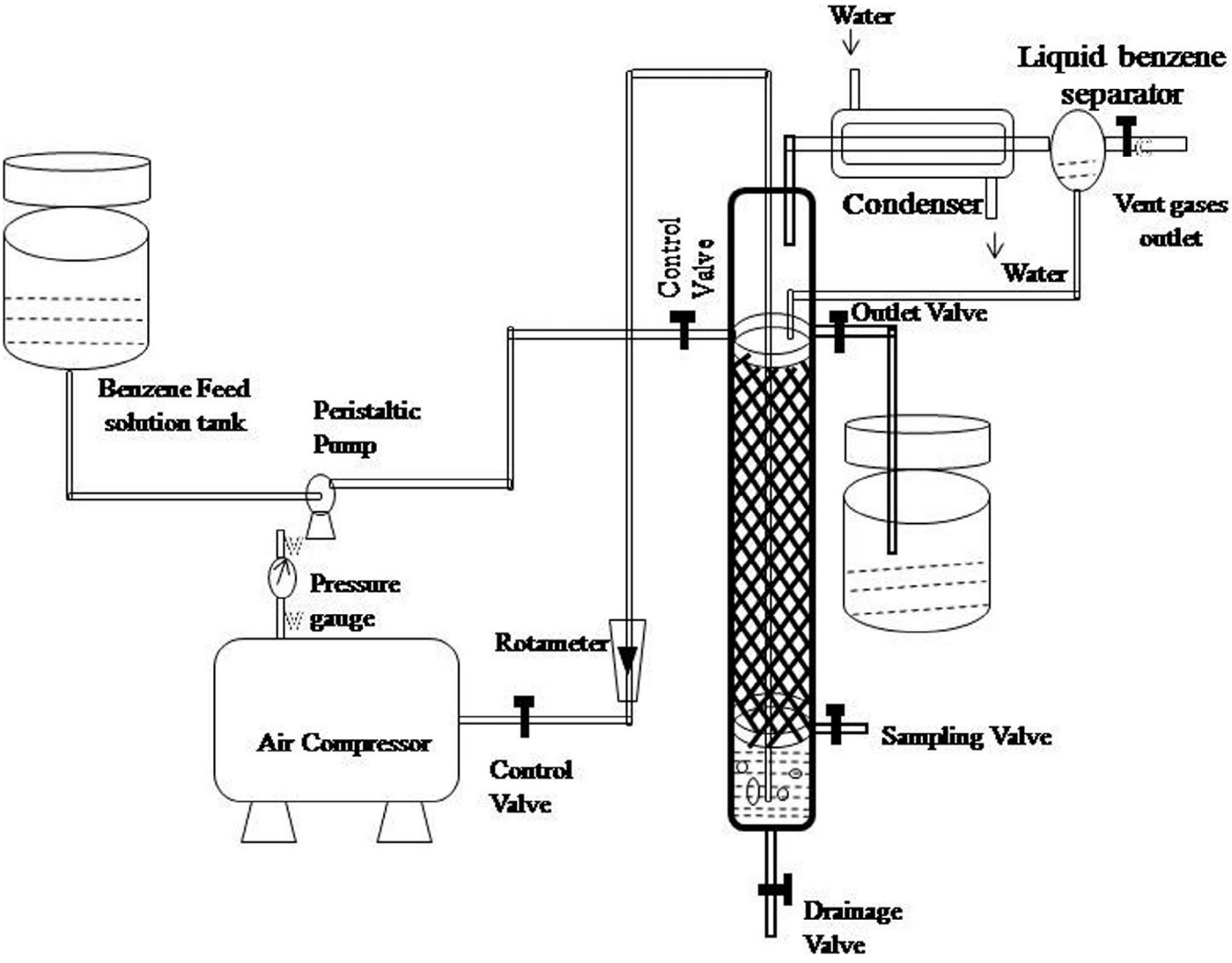

Fig. 1. Flow diagram for bioreactor system (packed/continuous).

2.5. Analysis and performance equations

Benzene in the sample obtained from the reactor was extracted thrice with an equal volume of hexane and subsequently analyzed using a Thermo-Fisher 7610 gas chromatograph (GC) equipped with a flame-ionization detector (FID) and BP-5 capillary column (25 m × 0.32 mm) with nitrogen as carrier gas. The initial temperature was maintained at 60 °C for 60 s and raised to 90 °C at a rate of 8 °C and for 60 s then raised to 160 °C at a rate 8 °C. The injector and detector temperature were kept at 170 °C and 180 °C respectively. Liquid benzene (1 μL) was injected into the GC for analysis using GC syringe.

Performance of the CPBBR under optimized conditions and at various inlet loading rates ($Si.Q/V$ = 10–40 mL·h^{-1}) was calculated in terms of % removal efficiency (RE) and elimination capacity (EC) defined by:

$$\%\ \text{Removal Efficiency}\ (\text{RE}\ \%) = \frac{S_{in} - S_{out}}{S_{in}} \times 100 \quad \text{(I)}$$

$$\text{Elimination Capacity}\ (\text{EC}) = Q\frac{(S_{in} - S_{out})}{V} \quad \text{(II)}$$

$$\text{Inlet Loading Rate}\ (\text{ILR}) = \frac{S_{in}}{V}Q \quad \text{(III)}$$

where S_{in} and S_{out} are the inlet and outlet concentrations of benzene. Q are the volumetric flow rate of feed, V is working volume and ILR is inlet loading rate of the bioreactor.

3. Results and discussion

3.1. Molecular characterization (16s rRNA) of bacterial isolate

Identification of bacteria by molecular characterization is highly sensitive and specific as compared to a biochemical approach to identification. The molecular characterization of the bacterial isolate was carried out using the amplified PCR products of 1.5 kb size confirmed the presence of 16S rRNA

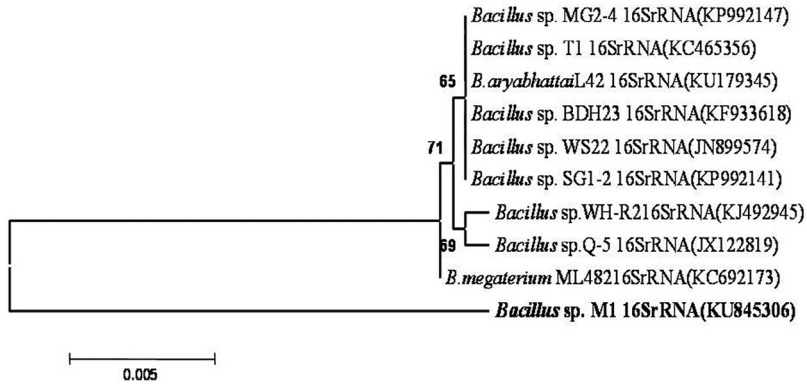

Fig. 2. Phylogenetic tree of isolated bacterial *Bacillus* sp.M1.

gene in the bacterial isolate. The isolate was further confirmed by 16S rRNA sequencing. The bacterial 16S rRNA sequences were aligned with NCBI databases. Sequences of bacterium isolate have shown 99% similarity with *Bacillus* sp. M1 (Fig. 2). The results suggested that the genera of *Bacillus* were found to be predominant and involved in benzene biodegradation. This finding is supported by Liu et al. [43].

3.2. Batch biodegradation

Percentage removal efficiency of benzene was evaluated in the batch experiments by varying the important process parameters namely inoculum level, pH, temperature, DO, and benzene concentration with objective to get the optimum value of these parameters for maximum removal of benzene. Removal increased rapidly as inoculum level increased from 200×10^6 to 800×10^6 CFU·mL^{-1} with an average removal of 93.75% for a period of 25 days (Fig. 3a). The pH range of 6.0–10.0 was used to examine its effect on the removal of benzene as shown in Fig. 3b and optimum pH was found to be 7 at which maximum percent RE of 95% was obtained. RE of benzene was observed by varying the temperature (Fig. 3c) in the range of 28–43 °C and it was observed that RE (%) increased up to 92.3% at 37 °C and then started decreasing

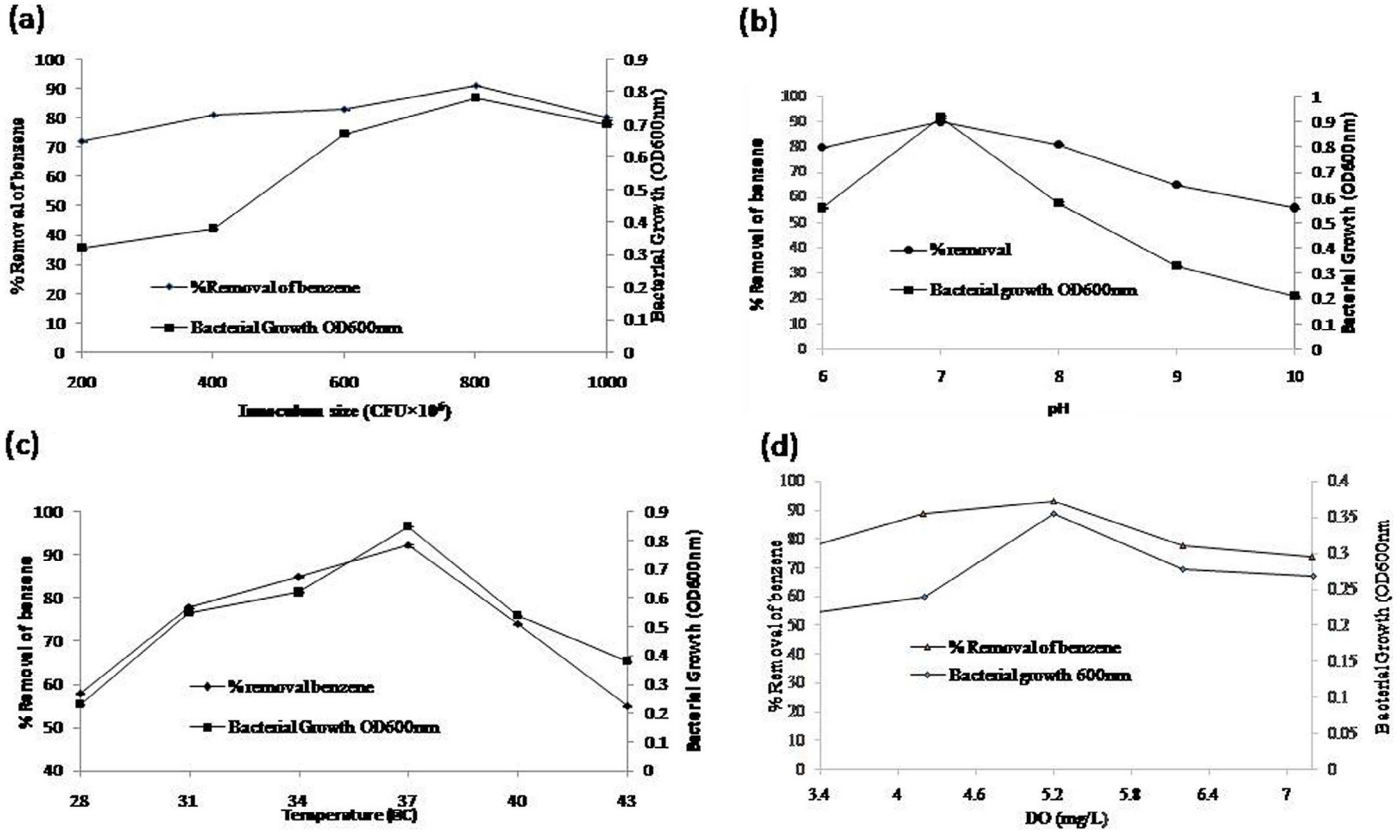

Fig. 3. Study of the optimization of parameter in packed bed reactor (3a, 3b, 3c, 3d) shows the effect of inoculum size, pH, temperature, and OD on % removal of benzene, with respect to bacterial growth.

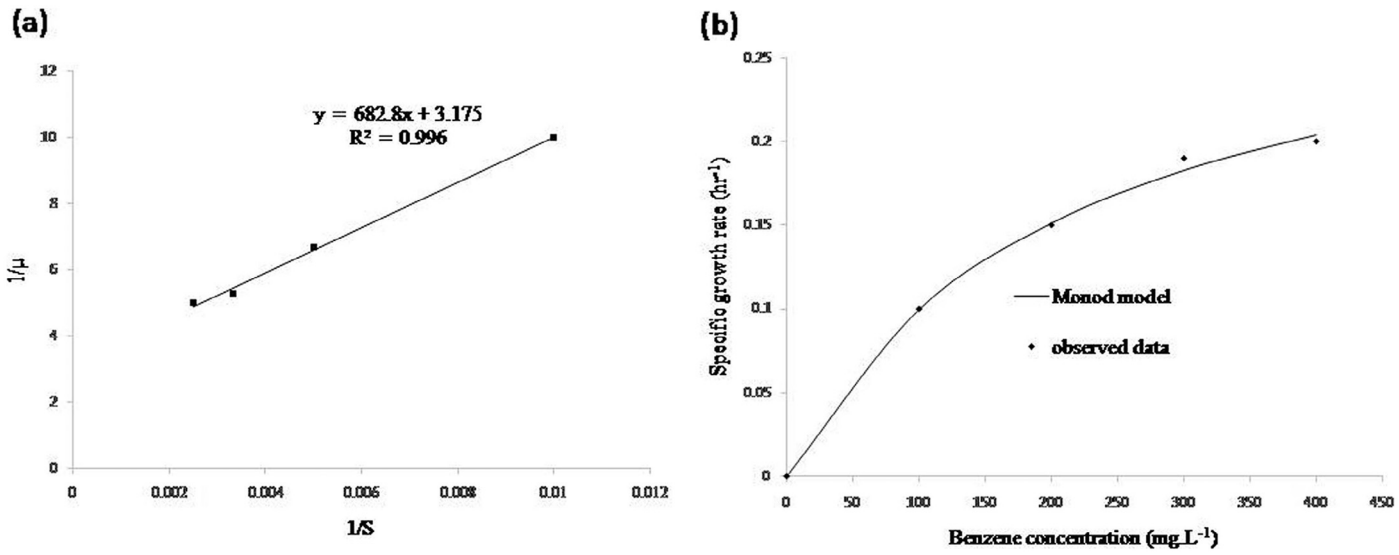

Fig. 4. (a) Monod model plotted 1/μ vs 1/S; 4. (b) Monod model fitted with observed and experimental data.

(Fig. 3c). The results obtained in the present study are similar to the results of Das et al. [44]. With increase in DO in the range of 3.4–7.2 mg·L^{-1}, RE (%) increased rapidly to 93.1% at DO level of 5.2 mg·L^{-1} (Fig. 3d) and then start decreasing [45]. The optimum value of inoculum level, pH, temperature, DO, and benzene concentration were found to be 8×10^8 CFU mL^{-1}, 7.0, 37.0 °C, 5.2 mg L^{-1} and 250 mg L^{-1} respectively and used in subsequent experiments for PBBR and CPBBR.

3.3. Kinetics of biodegradation in packed bed batch study

Monod kinetic model (Monod 1949) was used to investigate the kinetics of microbial growth and utilization of benzene as given below:

$$\mu = \frac{1}{x}\frac{dx}{dt} = \frac{\mu_{max} S}{K_s + S} \quad \text{(IV)}$$

where μ is specific growth rate (h^{-1}), μ_{max} is maximum specific growth rate (h^{-1}), K_s is half-saturation constant (mg L^{-1}), X, S, and t are microbial cell, initial substrate concentrations (mg L^{-1}), and time, respectively. For a given initial microbial cell X_o, the microbial cell concentration X at time t can be given by

$$\mu = \log \frac{\left(\frac{X_2}{X_1}\right)}{(t_2 - t_1)} \quad \text{(V)}$$

Monod model fitted to the experimental data for growth kinetics is shown in Fig. 4a and 4b. The estimated growth kinetic parameters (μmax and Ks) obtained from Monod growth model were found to be K_s = 215.07 mg·L^{-1}, μ_{max} = 0.314 day^{-1} which is better than earlier reported studies [10,46].

3.4. Biodegradation of benzene in PBBR and CPBBR

PBBR was operated for the concentration range of 100–500 mg·L^{-1} to observe the effect of initial concentration on the percent removal under optimized conditions obtained from batch experiments. Removal of benzene was increased and observed around 90% at concentration of 400 mg·L^{-1} and then started decreasing with further increase in concentration which may be due to several possibilities such as different types of inhibition effects.

Further the performance of PBBR was studied in the continuous mode (CPBBR) under optimum condition obtained from batch experiments (except temperature) by varying the flow rate (10–60 mL/h) and benzene concentration in the same range as it was in PBBR (Fig. 5). Initially, CPBBR was acclimated with microbial culture for 21 days using glucose as a sole carbon source. During the experiments, ambient temperature was changed in the narrow range of 35–38 °C. Hence, there was no temperature control was applied. Initially, the bioreactor was operated at low flow rate of 10 mL·h^{-1} to establish the proper bacterial growth and steady-state condition. The steady state was achieved on the 28th day of operation which was evident from almost constant removal of benzene (95%). On the 32th day, the flow rate was increased to 20 mL·h^{-1}. A sharp dip in RE was observed on the 33th day after that RE again improved and attained the value of more than 93% during 36th–38th days. On the 39th and 46th days, the flow rate was increased to 30 and 40 mL·h^{-1}, respectively and the bioreactor again behave similarly i.e. a sharp decrease and followed by resumption in the performance and corresponding stabilized RE were found to be 92.5 and 88.75% for flow rates of 30 and 40 mL·h^{-1} respectively. Similarly corresponding to flow rates of 50 and 60 mL·h^{-1} the stabilized removal efficiencies were found to be 81.5% and 78% respectively. Beyond 40 mL·h^{-1}, the decrease in RE was more sharp which indicates the possibility of change in controlling mechanism in the bioreactor [20–22]. Hassan et al. [47] reported the benzene loading up to 34.1 g m^{-3} h^{-1} and removal efficiency consistently over 98% was achieved while at loading of 76.8 g m^{-3} h^{-1} removal efficiency was observed above 80% and 6.9 g m^{-3} h^{-1} EC [10,29,31,42–47].

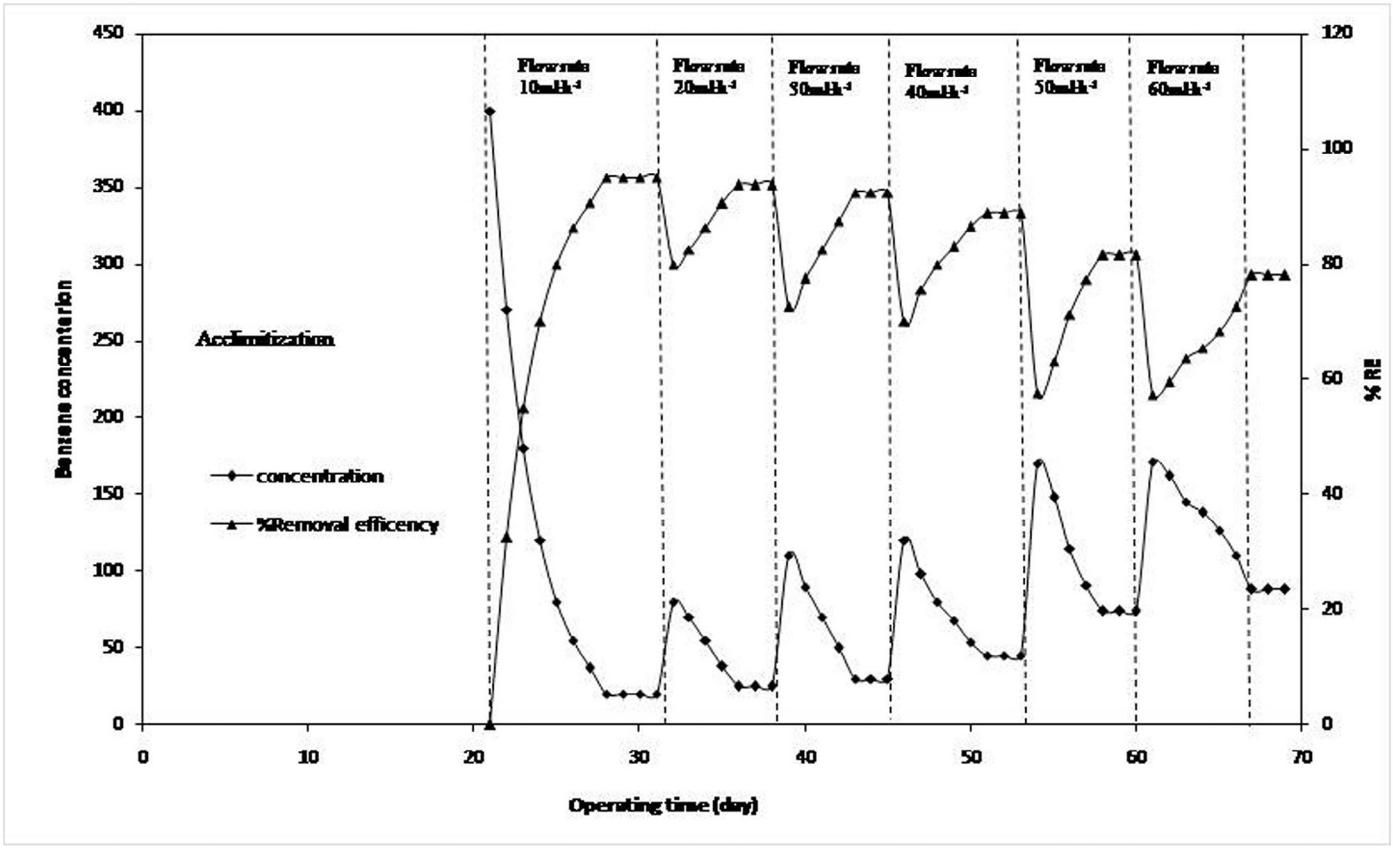

Fig. 5. Bioreactor performance with change in feed flow rate of inlet benzene concentration.

Fig. 6 shows the variation of the elimination capacity and removal efficiency with respect to the inlet loading rate of benzene. The curve of removal efficiency-inlet loading shows two distinct zones of mass transfer and bio-reaction. For the inlet loading rate ranging from 96 to 576 mg.L^{-1}·d^{-1}, the RE is approximately constant and more than 90% up to the loading rate of 400 mg·L^{-1}·d^{-1} thereafter started slightly decreasing continuously. EC increased linearly with inlet loading rate of benzene and attained maximum value of 449.2 mg·L^{-1}·d^{-1} at the loading of 576 mg·L^{-1}·d^{-1}. However, the EC increases continuously with increasing loading of benzene which is evident from slightly change in slope of EC vs Inlet Loading curve (Fig. 6). This observation also supports the change in controlling mechanism during the biodegradation process in the reactor [20–22]. Lu et al. 2002 [48] reported the maximum EC for benzene which was found to be 34 mg·L^{-1}·d^{-1} and another study of Sene et al. 2002 [49] have reported RE and EC of 63%, 3.8 mg·L^{-1}·h^{-1}, respectively at LR of 6.1 mg·L^{-1}·h^{-1} in bio-filter [11,50–52]. At higher concentrations of benzene, substrate inhibition may result in the poor removal efficiency [36].

At low loading rates, the diffusional flux through the biofilm will also be low and so the pollutant will be consumed within the biofilm without reaching the innermost layer of it. The biofilm will remain deficient in the substrate and the biodegradation capability of microbes will not be fully utilized. Under this situation whole biodegradation process is regulated by mass transfer limitations and RE become almost constant with increase in inlet loading rate of pollutant. Other possible reason could be the interaction between growth limiting substrate and bacterial population attached to the surface of PUF for benzene degradation [53].

At higher loading rates, the diffusional flux is high and so the pollutant is easily reaching to innermost layers of biofilm. Now sufficient substrate in form of pollutant is available to microbes and consummation of pollutant by biodegradation control the whole process (bio-reaction controlling zone). Under this situation the RE start decreasing with inlet loading rate of pollutant. In the present study at loading rate of 384 mg·L^{-1}·d^{-1} the process is changing from mass-transfer to bio-reaction controlling (Fig. 6). It is always desirable to operate bioreactors in the bio-reaction controlling zone with acceptable level of RE. For practical operations, the inlet loading rate at which mechanism in the bioreactor changes from mass transfer to bio-reaction controlling and the point of intersection of removal efficiency and elimination capacity curves (Fig. 6) may be taken as an approximate estimate of the operating concentration range of the bioreactor. ANOVA analysis found that $p < 0.05$ and the average value of concentration and removal was 89.14 mg·L^{-1} and 77.14% respectively, standard error (3) and deviation was 17.32 for removal.

4. Conclusions

Efficacy of *Bacillus* sp. M1 supported on PUF for biodegradation of benzene in batch and CPBBR have been demonstrated

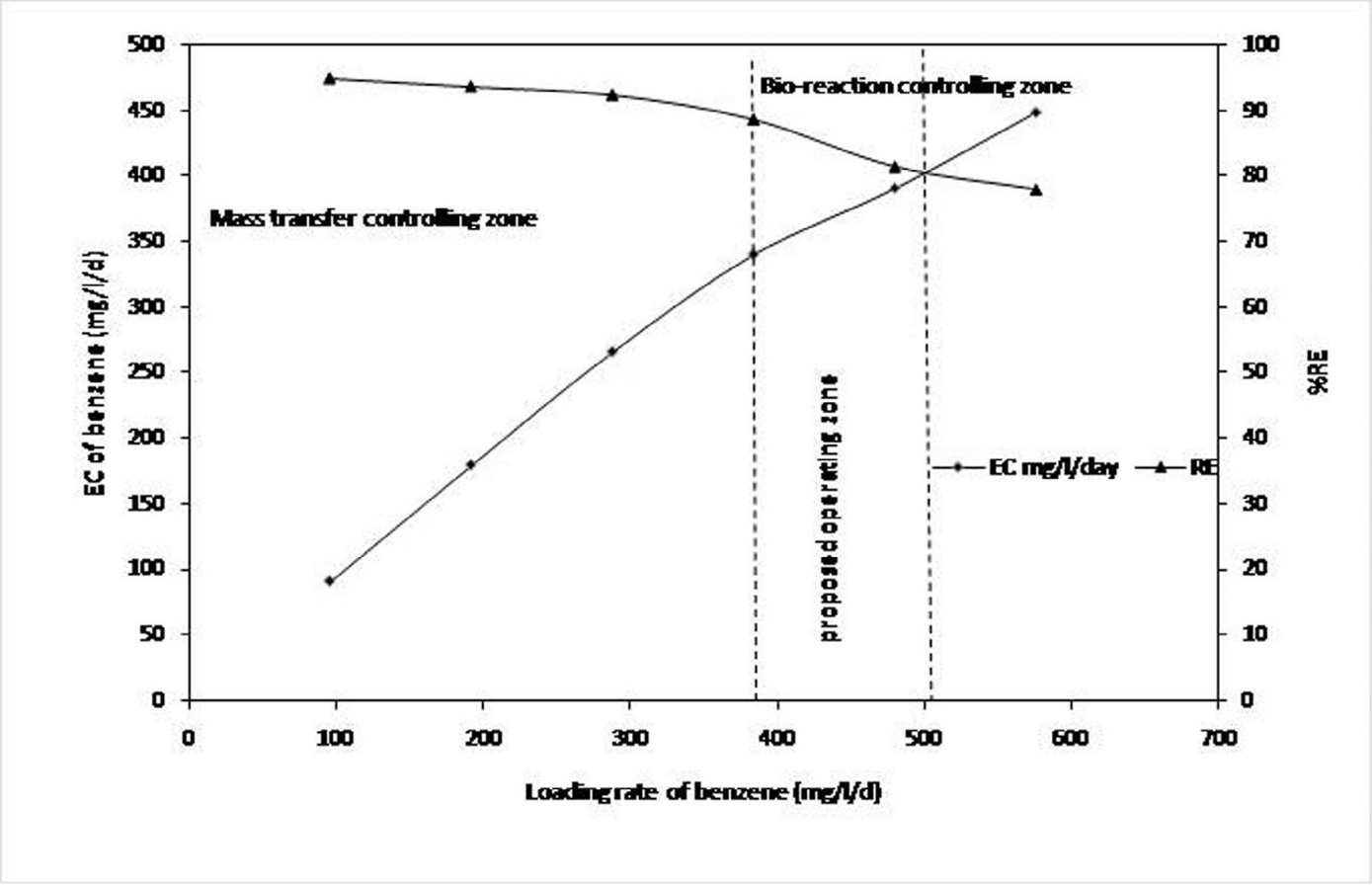

Fig. 6. Effects of inlet benzene load on the removal efficiency and elimination capacity.

under optimum condition. CPBBR have shown potential to remove more than 90% of benzene up to the inlet loading of 288 $mg \cdot L^{-1} \cdot d^{-1}$. The operational stability and longevity of PUF immobilized cells were found to be high. *Bacillus* sp. M1 is capable of degrading benzene in batch and PUF packed reactor. Growth kinetic model of Monod was fitted well for biodegradation of benzene.

Acknowledgement

The author (MKK) gratefully acknowledge Ministry of Human Resource Development, India (MHRD, India) for providing financial support and the Indian institute of technology (BHU) Varanasi for providing lab to conduct research work.

References

[1] R. Chakraborty, J.D. Coates, Hydroxylation and two crucial steps of anaerobic benzene degradation by Dechloromonas Strain RCB, Appl. Environ. Microbiol. 71 (2005) 427–5432.
[2] USEPA, United States Environmental Protection Agency, National primary drinking water regulations, EPA. 816-F, 09-004, 2009.
[3] M. Bender, Global Aromatics Supply-Today and Tomorrow DGMK Conference, October, Dresden, Germany, 9–11, 2013.
[4] K. Taleb, J. Markovski, Z. Velickovic, J. Rusmirovic, M. Rancic, V. Pavlovic, et al., Arsenic removal by magnetite loaded amino modified nano/microcellulose adsorbent. Effect of functionalization and media size, Arbian J. Chem. (2016).
[5] EPA, Priority Pollutants, Code of Federal Regulations, Title 40, Part 423, Appendix A, USA, Chapter 1, 1996.
[6] ATSDR, Priority list of hazardous substances, Agency for Toxic Substances and Disease Registry, 2005.
[7] S.V. Rana, Y. Verma, Biochemical toxicity of benzene, J. Environ. Biol. 26 (2005) 157–168.
[8] Y. Takahata, Rapid intrinsic biodegradation of benzene, toluene, and xylenes at the boundary of gasoline-contaminated plume under natural attenuation, Appl. Microbial. Biotechnol. 73 (2006) 713–722.
[9] M.G. Pillai, P. Simha, A. Gugalia, Recovering urea from human urine by biosorption onto microwave activated carbonized coconut shells: equilibrium, kinetics, optimization and field studies, J. Environ. Chem. Eng. 2 (1) (2014) 46–55.
[10] P. Simha, M. Mathew, M. Ganesapillai, Empirical modeling of drying kinetics and microwave assisted extraction of bioactive compounds from Adathodavasica and Cymbopogoncitratus, Alex. Eng. J. 55 (1) (2016) 141–150.
[11] M. Ganesapillai, P. Simha, The rationale for alternative fertilization: equilibrium isotherm, kinetics and mass transfer analysis for urea-nitrogen adsorption from cow urine, Res. Efficient Technol. 1 (2) (2015) 90–97.
[12] P. Simha, A. Yadav, D. Pinjari, A.B. Pandit, On the behaviour, mechanistic modelling and interaction of biochar and crop fertilizers in aqueous solutions, Res. Efficient Technol. 2 (3) (2016) 133–142.
[13] P. Simha, P. Banwasi, M. Mathew, M. Ganesapillai, Adsorptive resource recovery from human urine: system design, parametric considerations and response surface optimization, Procedia Eng. 148 (2016) 779–786.
[14] O. Tepe, A.Y. Dursun, Combined effects of external mass transfer and biodegradation rates on removal of phenol by immobilized Ralstoniaeutropha in a packed bed reactor, J. Hazard. Mater. 151 (2008) 9–16.

[15] R. Sheeja, T. Murugesan, Mass transfer studies on the biodegradation of phenols in up-flow packed bed reactors, J. Hazard. Mater. 89 (2002) 287–301.
[16] A.J. Riyad, K. Walter, H.E. Smith, Biofiltration of BTEX contaminated air streams using compost-activated carbon filter media, J. Hazard. Mater. 60 (1998) 111–126.
[17] L. Sene, A. Converti, M.G.A. Felipe, M. Zilli, Sugarcane bagasse as alternative packing material for biofiltration of benzene polluted gaseous streams a preliminary study, Bioresour. Technol. 83 (2002) 153–157.
[18] A. Mallakin, O.P. Ward, Degradation of BTEX compounds in liquid media and in peat Biofilters, J. Industrial. Microbiol. 16 (1996) 309–318.
[19] B. Sara, M. Massimo, B. Lorenzo, Z. Mario, C. Hanene, H. Abdennaceur, et al., Microbial succession in a compost-packed biofilter treating benzene-contaminated air, Biodegradation 17 (2006) 181–191.
[20] A.K. Shukla, V. Pranjali, R.S. Singh, S.N. Upadhyay, S.K. Dubey, Biofiltration of trichloroethylene using diazotrophic bacterial community, Bioresour. Technol. 101 (2010) 2126–2133.
[21] K. Singh, R.S. Singh, B.N. Rai, S.N. Upadhyay, Biofiltration of toluene using wood charcoal as the biofilter media, Bioresour. Technol. 101 (2010) 3947–3951.
[22] R.S. Singh, B.N. Rai, S.N. Upadhyay, Removal of toluene vapor from air stream using a biofilter packed with polyurethane foam, Proc. Safety Environ. Protect. 88 (2010) 366–371.
[23] H. Ehrhardt, H. Rehm, Semicontinuous and continuous degradation of phenol by Pseudomonas *putida* P8 adsorbed on activated carbon, Appl. Microbiol. Biotechnol. 30 (1989) 312–317.
[24] N.K. Patil, Y. Veeranagouda, M. Vijaykumar, S.A. Nayak, T. Karegoudar, Enhanced and potential degradation of o-phthalate by *Bacillus* sp. immobilized cells in alginate and polyurethane, Int. Biodeter. Biodegrad. 57 (2006) 82–87.
[25] J. Ha, C.R. Engler, J.R. Wild, Biodegradation of coumaphos, chlorferon, and diethylthiophosphate using bacteria immobilized in Ca-alginate gel beads, Bioresour. Technol. 100 (2009) 1138–1142.
[26] A. Livingston, H. Chase, Development of a phenol degrading fluidized bed bioreactor for constant biomass holdup, Chem. Eng. J. 45 (1991) B57–B66.
[27] B.E. Quail, G.A. Hill, A packed-column bioreactor for phenol degradation model and experimental verification, J. Chem. Technol. Biotechnol. 52 (1991) 545–557.
[28] Z. Aksu, G. Bülbül, Investigation of the combined effects of external mass transfer and biodegradation rates on phenol removal using immobilized P. putida in a packed-bed column reactor, Enzyme Microb. Technol. 22 (1998) 397–403.
[29] E.R. Rene, B.T. Mohammad, M.C. Veiga, C. Kennes, Biodegradation of BTEX in a fungal biofilter influence of operational parameters, effect of shock-loads and substrate stratification, Bioresour. Technol. 116 (2012) 204–213.
[30] Y.H. Sung, Y.J. Young, Removal of benzene in a hybrid bioreactor, Proc. Biochem. 34 (1999) 281–288.
[31] C.T. Davidson, A.J. Daugulis, Addressing biofilter limitations: A two-phase partitioning bioreactor process for the treatment of benzene and toluene contaminated gas streams, Biodegradation 14 (6) (2003) 415–421.
[32] Y. Sung-Ho, D.J. Andrew, Benzene degradation in a two-phase partitioning bioreactor by *Alcaligenes xylosoxidans*Y234, Proc. Biochem. 36 (2001) 765–772.
[33] D. Singh, M.H. Fulekar, Benzene bioremediation using cow dung microflora in two phase partitioning bioreactor, J. Hazard. Mater. 175 (2010) 336–343.
[34] H.A. Tareq, B. Emine, L.M. Ülkü, L.M. Tanju, The biodegradation of benzene, toluene and phenol in a two-phase system, Biochem. Eng. J. 19 (2004) 137–146.
[35] I. Garcia-Pena, I. Ortiz, Biofiltration of BTEX by the fungus Paecilomycesvariotii, Int. Biodeter. Biodegrad. 62 (4) (2008) 442–447.
[36] S.L. Tsai, C.W. Lin, C.H. Wu, C.M. Shen, Kinetics of xenobiotic biodegradation by the *Pseudomonas* sp. YATO411 strain in suspension and cell-immobilized beads, J. Taiwan Inst. Chem. Eng. 44 (2013) 303–309.
[37] J. Sambrook, R.W. David, Molecular Cloning a Laboratory Manual, 3rd ed., Coldspring-Harbour Laboratory Press, UK, 2001.
[38] A.K. Shukla, P. Vishwakarma, S.N. Upadhyay, A.K. Tripathi, H.C. Prasana, S.K. Dubey, Biodegradation of trichloroethylene (TCE) by methanotrophic community, Bioresour. Technol. 100 (2009) 2469–2474.
[39] K. Tamura, M. Nei, S. Kumar, Prospects for inferring very large phylogenies by using the neighbor-joining method, Proc. Natl. Acad. Sci. U.S.A. 101 (30) (2004) 11030–11035.
[40] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4 (4) (1987) 406–425.
[41] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, Mol. Biol. Evol. 28 (10) (2011) 2731–2739.
[42] R.S. Singh, B.N. Rai, S.N. Upadhyay, Removal of toluene vapour from air stream using a biofilter packed with polyurethane foam, Pro. Saf. Environ. Protect. 88 (2010) 366–371.
[43] J.H. Liu, J.P. Maity, J.S. Jean, C.Y. Chen, C.C. Chen, S.Y. Ho, Biodegradation of benzene by pure and mixed cultures of *Bacillus* sp, World. J. Microbiol. Biotech. 26 (2010) 1557–1567.
[44] R. Das, P. Dey, P. Choudhary, K.K. Sufia, Benzene utilization as growth substrate by a newly isolated *Aerococcus* sp. strain BPD-6 indigenous to petroleum hydrocarbon contaminated oily sludge, Int. J. Sci. Eng. Res. 5 (10) (2014).
[45] S.V. Mohan, S. Shailaja, M. Ramakrishna, K.B. Reddy, P.N. Sharma, Proc. Biochem. 41 (2006) 644–652.
[46] D.J. Kim, J.W. Choi, N.C. Choi, B. Mahendran, C.E. Lee, Modeling of growth kinetics for *Pseudomonas* spp. during benzene degradation, Appl. Microbiol. Biotechnol. 69 (2005) 456–462.
[47] A.A. Hassan, G. Sorial, Biological treatment of benzene in a controlled trickle bed air biofilter, Chemosphere 75 (10) (2009) 1315–1321.
[48] C. Lu, M.R. Lin, C. Chu, Effects of pH, moisture, and flow pattern on tricklebed air biofilter performance for BTEX removal, Adv. Environ. Res. 6 (2002) 99–106.
[49] L. Sene, A. Converti, M.G.A. Felipe, Sugarcane bagasse as alternative packing material for biofiltration of benzene polluted gaseous streams: a preliminary study, Bioresour. Technol. 83 (2002) 153–157.
[50] J.P. Vander Hoek, L.G.C.M. Urlings, C.M. Grobben, Biological removal of polycyclic aromatic hydrocarbons, benzene, toluene, ethyl benzene, xylene and phenolic compounds from heavily contaminated ground water and soil, Environ. Technol. 10 (2) (1989) 185–194.
[51] J.L.R. Pantoja Filho, L.T. Sader, M.H.R.Z. Damianovic, E. Foresti, E.L. Silva, Performance evaluation of packing materials in the removal of hydrogen sulphide in gas-phase biofilters Polyurethane foam, sugarcane bagasse, and coconut fibre, Chem. Eng. J. 158 (3) (2010) 441–450.
[52] M. Ganesapillai, P. Simha, K. Desai, Y. Sharma, T. Ahmed, Simultaneous resource recovery and ammonia volatilization minimization in animal husbandry and agriculture, Res. Efficient Technol. 2 (1) (2016) 1–10.
[53] S.F. Thornton, K. Tobin, J.W. Smith, Comparison of constant and transient-source zones on simulated contaminant plume evolution in groundwater: implications for hydrogeological risk assessment, Groundw. Monitor. Remed. 33 (3) (2013) 78–91.