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Combined removal of BTEX in air stream by using mixture of sugar cane bagasse, compost and GAC as biofilter media

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Abstract

Biofiltration of air stream containing mixture of benzene, toluene, ethyl benzene and *o*-xylene (BTEX) has been studied in a lab-scale biofilter packed with a mixture of compost, sugar cane bagasse and granulated activated carbon (GAC) in the ratio 55:30:15 by weight. Microbial acclimation was achieved in 30 days by exposing the system to average BTEX inlet concentration of 0.4194 g m^{-3} at an empty bed residence time (EBRT) of 2.3 min. Biofilter achieved maximum removal efficiency more than 99% of all four compounds for throughout its operation at an EBRT of 2.3 min for an inlet concentration of 0.681 g m^{-3}, which is quite significance than the values reported in the literature. The results indicate that when the influent BTEX loadings were less than 68 g m^{-3} h^{-1} in the biofilter, nearly 100% removal could be achieved. A maximum elimination capacity (EC) of 83.65 g m^{-3} h^{-1} of the biofilter was obtained at inlet BTEX load of 126.5 g m^{-3} h^{-1} in phase IV. Elimination capacities of BTEX increased with the increase in influent VOC loading, but an opposite trend was observed for the removal efficiency. The production of CO_2 in each phase (g m^{-3} h^{-1}) was also observed at steady state (i.e. at maximum removal efficiency). Moreover, the high concentrations of nitrogen in the nutrient solution may adversely affect the microbial activity possibly due to the presence of high salt concentrations. Furthermore, an attempt was also made to isolate the most profusely grown BTEX-degrading strain. A Gram-positive strain had a high BTEX-degrading activity and was identified as *Bacillus sphaericus* by taxonomical analysis, biochemical tests and 16S rDNA gene analysis methods.

Keywords: Biofiltration; BTEX vapour; Compost; Elimination capacity; Sugar cane bagasse; *Bacillus sphaericus*

1. Introduction

A large quantity of volatile organic compounds (VOCs) is released from various industrial plants and processes. These VOCs have already been shown to play an important role in the atmospheric chemistry. VOCs are a major group of pollutants, which have now become a cause of concern worldwide. VOCs are harmful to ecosystem, human health and atmosphere [1–4]. Some VOCs (such as benzene, toluene, ethylbenzene and *o*-xylene (BTEX)) are important industrial solvents that are frequently encountered in industrial operations and contaminated sites. Many BTEX vapours are emitted into the atmosphere during its manufacture, transportation, use and disposal every year. BTEX are volatile aromatic hydrocarbons with toxic properties. Exposure to BTEX can cause neurological, respiratory, genetic and excretory system damage. Use of BTEX has persisted despite toxic properties because of the extent of applications. The Clean Air Act Amendments (CAAA) of 1990, proposed by the United State Environmental Protection Agency (USEPA) places special emphasis on the handling and uses of BTEX compounds which are among the 188 Hazardous Air Pollution (HAPs) linked under the recognized list [5]. Among these compounds benzene is known to be more carcinogenic. It occupies sixth position in the Priority List of Hazardous Substances Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) [6]. Benzene is also classified as hazardous substance in the EPA list of priority pollutants [7], because of its confirmed carcinogenic properties [8–10]. For these reasons, strict regulations and air

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quality standards came into force to drive the researchers to reduce its emissions.

In order to abide by the regulations of the local regulation authorities and governments on the protection of the environment and air quality, industrial plants need to eliminate or reduce the content of toxic chemicals in gaseous effluents prior to their release into the atmosphere. In this context, biofiltration has emerged as an attractive process for the elimination of volatile organic compounds from waste air streams. The biofiltration process has various advantages compared to the conventional processes used in the treatments of gaseous effluents. It is environmental friendly technology appropriate for the treatment of diluted emission. In addition to its low capital and operating cost, biofiltration is convenient for the processing of discontinuous emissions and can be used to eliminate a variety of VOCs simultaneously [11,12].

Although, studies on BTEX biodegradation in biofilter have been conducted over the last 10 years [13–21], results of various studies indicate that the substrate interactions between BTEX compounds often vary with microbial culture and culture conditions. In the recent years, there has been increasing trend towards more efficient utilization of agro-industrial residues, including sugar cane bagasse as packing materials. Further, literature survey reveals that only few researchers have been conducted to investigate biofilter by using sugar cane bagasse as a packing material for treating single or mixed of these four compounds [22–25]. Although, many researchers have reviewed the principles of biological waste air treatment and the advantages over chemical and physical techniques, research is still going on to the biological treatment of waste air for the use of effective new packing media, designs, microbial structure analysis and modelling of VOC removal. Furthermore, attempts to genetically modified bacteria to increase the degradation rate of a single pure organism are being made [26,27]. In the present paper, the biofiltration of BTEX vapours was investigated using a filter material consisting of a mixture of compost as a base material, raw sugar cane bagasse as a supporting material and granulated activated carbon (GAC) in a 55:30:15 (%, w/w) ratio. The main objective of the present work is to investigate the performance of a biofilter in treating a mixture of BTEX loaded air stream under variable loading conditions. Performance of the biofilter was assessed by determining the BTEX removal efficiencies, elimination capacities, CO_2 production, microbial concentration and varying in nutrient concentrations by varying the process parameters and operating conditions. Furthermore, an attempt was also made to isolate a most dominating pure BTEX-degrading strain.

2. Materials and methods

2.1. Biofilter operation

A schematic diagram of the experimental setup for treating the BTEX is shown in Fig. 1. The biofilter consists of Perspex pipe with an internal diameter of 14 cm and effective packing bed height of 60 cm. The biofilter column consisted of two individual sections that were bolted together. Each section was packed with the packing material to a height of 30 cm. A 5 cm plenum was located between two sections to allow for gas sampling and redistribution of the contaminants stream between sections. The volume of the filter bed was 9.2 L. The packing material was supported on the acrylic sieve plate that ensured homogeneous distribution. All experiments were conducted in a temperature controlled chamber at $30 \pm 2\,^{\circ}C$.

First of all, compressed air was passed through the filtration device to remove moisture, oil and particulate matter. After purification, the filtered air stream was split into two sections: minor and major air stream. For producing BTEX loaded air stream of desired concentration, the minor air stream was passed separately through the glass bottles containing BTEX solution (99% pure) and through the humidifier. The BTEX loaded air streams and humid air were mixed in a glass bottle. Finally, this mixed humidified BTEX loaded air stream were mixed with major stream and then fed to the bottom of the biofilter in up flow mode of operation. Rate of addition of BTEX in the main stream was controlled by regulating the rate of inflow of BTEX bottles. The airflow rates were controlled and measured by a rotameter (JTM, Japsin Industrial Instrumentation, India) for high flow rate (1–10 L min^{-1}) and for low flow rate (1–300 cm^3 min^{-1}). Finally, BTEX concentrations were maintained at the desired value by adjusting the fine brass control valves. Pressure control valve was used for constant airflow to the reactor. The biofilter was operated at various inlet BTEX concentrations and gas flow rates. Samples were collected at a regular intervals of time from the inlet, outlet and as well as from the sampling ports using an airtight syringe and analyzed for residual BTEX.

2.2. Packing material

In the present work biofilter packing media was used as a mixture of compost, sugar cane bagasse and GAC in the ratio of 55:30:15 by weight. The compost used in this study was obtained from the composting facility of Star Paper and Pulp Mills Pvt. Ltd., Saharanpur, India. The compost was stored in a sealed plastic bag at room temperature before its use to maintain its original moisture content. The compost was sieved through 0.8–1 cm screens before used in the biofilter. The physical and chemical compositions of the compost, sugar cane bagasse and GAC used in the biofilter are presented in Table 1. The raw sugar cane bagasse was obtained from local sugar crushing mill. It was sieved through 1–2 cm screens, washed two times with Millipore water, dried in an oven at 80 °C for 2 days, and sterilized at 15 psi for 20 min. Sugar cane bagasse was mixed with a mineral salt solution (nutrient solution) prior to addition to the biofilter unit. The GAC for gas adsorption used in this study was supplied by M/s S.D. Fine Pvt. Ltd. India in the size range of 2–5 mm. After washing the GAC with Millipore water, it was dried in an oven at 105 °C for 72 h. After drying, this was sterilized and stored in a glass bottle until use. After that the compost, raw sugar cane bagasse and GAC were mixed and then fed in the biofilter. Initial conditions were kept as follows: pH 7.0 (adjusted with NaOH or HCl), temperature 30 °C and moisture content (wet weight basis) 56.1%.

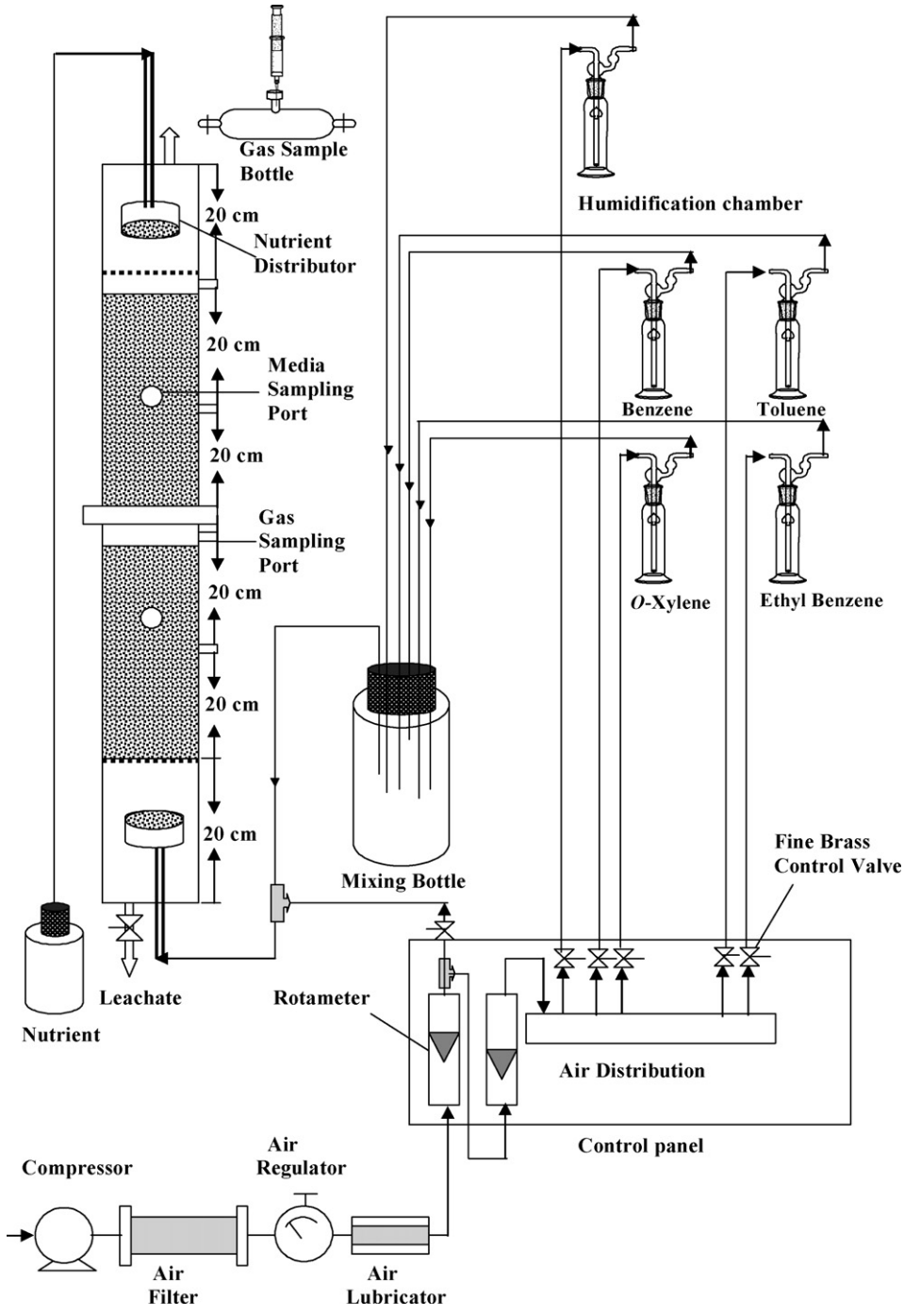

Fig. 1. Schematic diagram of biofilter for treating BTEX waste gases.

2.3. Chemicals and mineral medium

The nutrient solution (basal salts medium (BSM)) with about 10 mL min^{-1} was continuously sprayed two times in a day for 30 min on the top of the packing media through the nutrient distribution system. The BSM solution used for the continuous tests had the following composition per liter of water: K_2HPO_4, 0.91 g; $Na_2HPO_4{\cdot}2H_2O$, 2.39 g; KNO_3, 2.96 g; $(NH_4)_2SO_4$, 1.97 g; $FeSO_4{\cdot}7H_2O$, 0.2 g; $MgSO_4{\cdot}7H_2O$, 2.0 g; $MnSO_4{\cdot}7H_2O$, 0.88 mg; $Na_2MoO_4{\cdot}2H_2O$, 1 mg; $CaCl_2$, 3 mg; $ZnSO_4{\cdot}7H_2O$, 0.04 mg; $CoCl_2{\cdot}6H_2O$, 0.04 mg. All the chemicals used were AR grade with more than 99% purity.

2.4. Analytical methods

Concentrations of BTEX gas were analyzed by using a Netel India Limited (model-MICHRO 9100) gas chromatograph equipped with a capillary column type HP5 (30 m × 0.249 mm × 0.25 μm film thickness, temperature limits: −60 to 325 °C) and with a flame ionization detector. The

Table 1
Characteristics of the bagasse, compost and GAC used in the biofilter

Parameters	Bagasse	Compost	GAC
Physical compositions			
Particle diameter (cm)	1–2	0.8–1	0.2–0.5
pH	6.24	7.65	7.12
Moisture content (%) (at field capacity)	48.3	69.2	39.7
Specific gravity	–[a]	1.592	–
Wet filled weight (g), w_1	180	330	90
Dry filled weight (g), $w_2 = w_1/(1 + \text{moisture})$	121.3	195	64.4
BET surface area (m^2/g)	4.146	–	939.058
Total pore volume of pores (cm^3/g)	0.0041	–	0.47
Average pore diameter (Å)	39.429	–	20.007
Maximum pore volume (cm^3/g)	0.009	–	0.47
Median pore diameter (Å)	4887.11	–	10.178
Chemical compositions (%)			
C	43.22	7.585	78.8
H	6.097	3.584	1.977
N	0.006	0.065	0.012
S	0.81	0.334	0
O*	43.95	63.63	14.59
Ash	5.91	24.8	4.62

O* = 100 − (C + H + N + S) − Ash.
[a] Not determined.

injector, oven and detector temperature were maintained at 210, 60, and 230 °C, respectively. The hydrogen gas was used as the fuel and nitrogen was used as the carrier gas at a flow rate of 20 mL min^{-1}. CO_2 concentration was determined by using the same GC equipped with a Porapack Q column (2 m length, 1/8 in. ID, 80/100 mesh) and thermal conductivity detector (TCD). The calibration curve was prepared by injecting known amounts of the BTEX into a sealed bottle equipped with a Teflon septum according to the standard procedure [28]. The injected amount of BTEX was allowed to evaporate in the air space within the bottle at experimental temperature (30 °C). For the calibration, air samples are drawn from the bottle by a 1 mL gas tight syringe (Hamilton–Bonaduz–Schweiz) and analyzed by gas chromatograph. The air samples were drawn from the various sampling ports by using a gas tight syringe and analyzed.

The pH values of nutrient solution and leachate were measured by a digital pH meter. C, H, N and S analysis of compost sugar cane bagasse and GAC were carried out by Elemental Analyzer System, GmbH, model Vario-EL V3.00. Scanning electron micrographs of the sugar cane bagasse, at the beginning and after 5 months of operation were carried out by using a scanning electron microscope (SEM) (Model LEO435VP, LEO Electron Microscopy Ltd., England). For SEM, the microorganisms were fixed with 2% glutaraldehyde aqueous solution for 1 h at less than 20 °C, washed with phosphate buffer (pH 7.0) and then dehydrated with ethyl alcohol, dried and given a metal coating with gold. The particle size analysis was done using standard sieves. The specific surface area and the pore diameter of the bagasse and GAC were measured by nitrogen adsorption isotherm using an ASAP 2010 V2.00 C Micromeritics instrument and by Brunauer–Emmett–Teller (BET) method by using the software of Micromeritics. Nitrogen was used as cold bath (77.12 K).

2.5. Isolation of BTEX-degrading pure culture

The un-identified microbial culture was obtained from a biofilter which had been operated for 6 months to remove mixture of BTEX. One gram of sample was taken with sterile steel forceps from the sampling port provided at the top and bottom of the biofilter. The samples were replaced with fresh compost (1 g) each time. The withdrawn samples were mixed with 10 mL of sterilized deionized water (Milli-Q Millipore 18.2 MΩ cm^{-1} resistivity) and then shaken in a vortex shaker for 10 min and then converted to desired concentration through serial dilution technique. Diluted sample was then allowed to stand for 10 min. One milliliter of this sample was serially diluted up to 10^{-10} in sterile buffer (phosphate buffer, pH 7.0). Serially diluted samples were then spread aseptically on the solid nutrient agar plate and the pour plates were incubated at 30 °C for 24 h. Several different colonies were obtained after incubation. They were plated on solid nutrient agar plate until reaching complete purification. By this type of streaking 10 isolates were found to have grown profusely.

After obtaining of 10 pure isolated colonies, they were checked for its ability to grow in 20 mL of basal salts medium (BSM) with 50 μL of BTEX by inoculating them into separate serum bottles. In order to understand degradation ability, the experiments were conducted in duplicating using 20 mL BSM volume in 125 mL serum bottles. The loopfuls of single colony from each plate were inoculated in serum bottles separately. The serum bottles were shaken at 120 rpm and 30 °C for 48 h. Subsequently, 1 mL of culture of each bottles were transferred to 20 mL fresh BSM media and 100 μL of BTEX for another 48 h incubation. After this, these cultures were streaked from serum bottles on solid nutrient agar plate. Colonies obtained were reinoculated into 20 mL MSB with 100 μL BTEX to confirm the utilization of BTEX. The serum bottles were incubated and monitored.

Table 2
Biological characteristics of highly BTEX-degrading isolated strains *Bacillus sphaericus* from an active biofilter

Biochemical and culture conditions	
Gram straining	+
Kligler Iron Agar Slant	Butt-yellow Slant-red
Catalase	+
Oxidase	+
Indole	−
Methyl red	+
Voges-Proskauer	+
Citrate	−
H_2S production	−
Glucose	+
Maltose	+
Sucrose	−
Lactose	−
Xylose	−
Urease	+
Morphology under microscope	
Cell type (shape)	Rods
Color	Yellowish white
Size	0.5–0.6 μm × 1.6–2.8 μm
Surface	Smooth
Arrangement	Coherent cluster
Density	Opaque
Elevation	Convex
Motility	Positive
Physiological characteristics	
pH	
3	+
5	+
6	++
7	+++
8	++
9	+
11	+
Conditions	30 °C, aerobic
RPM	125

2.6. Identification of bacteria

The identification procedure of isolated bacteria was performed according to Buchanan and Gibbons (1974) 'Bergey's Manual' [29]. The morphological, physiological and biochemical tests of only the most profusely grown strain are listed in Table 2. Based on the tests, with reference to the Bergey's manual, the selected most profusely grown BTEX-degrading strain was tentatively identified as genera *Bacillus*. The selected strain was then also re-identified in Institute of Microbial Technology (IMTECH), Chandigarh, India, as *Bacillus sphaericus* and characterized in terms of its morphology and biochemistry.

2.7. Amplification and sequencing of 16S rRNA gene (rDNA)

Genomic DNA was isolated using standard bacterial procedures [30]. The following primers were used for PCR amplification of the 16S ribosomal DNA: 63f (5′-AGGCCTAACACATGCAAG TC-3′), 1387r (5′-GGGCGGAGTGTACAAGGC-3′) [31]. The PCR mixtures (50 μL) contained 25 pmol of each primer, 200 μM of each of deoxynucleoside triphosphate, PCR buffer (Promega, Madison, WI, USA), 0.5 U of Taq DNA polymerase (Promega, Madison, WI, USA) and 10 ng of DNA per μL. The thermocycling conditions consisted of a denaturation step at 94 °C for 1 min, 28 amplification cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min and a final polymerization for 4 min with a MJ Research Thermalcycler (MJ Research, PTC-100, USA) [32]. PCR products were visualized on 1.0% agarose gels and the products were excised with Montage DNA gel extraction kit (Millipore Corporation Bedford, MA, USA) following the manufacturer's instructions. The PCR products were cloned in a pGEM-T vector (Promega, Madison, WI, USA) by following the manufacturer's instruction. Several clones containing the insert were isolated. Both strands of the 16S sequences were sequenced by the dideoxynucleotide method [33]. Nucleotide sequence similarities were determined using BLAST (National Center for Biotechnology Information databases).

2.8. Performance evaluation of biofilter

The performance of the biofilter was evaluated in terms of the removal efficiency (%) and the elimination capacity (EC) ($g\,m^{-3}\,h^{-1}$) of the filter bed, which were estimated by the following equations:

$$\text{inlet loading rate, LR} = \frac{QC_i}{V} \quad (1)$$

$$\text{elimination capacity, EC} = \frac{Q(C_i - C_o)}{V} \quad (2)$$

$$\text{removal efficiency, RE}\,(\%) = \frac{C_i - C_o}{C_i} \times 100 \quad (3)$$

where C_i and C_o is the inlet and outlet BTEX concentration.

Performance of biofilter was investigated with respect to the loading rate (LR) of BTEX in $g\,m^{-3}\,h^{-1}$. To do that, loading rate was expressed in terms of the combination of two parameters: BTEX concentration ($g\,m^{-3}$) and flow rate ($L\,min^{-1}$). These two parameters were actually varied in the reactor to study the performance of the reactor to maintain the precalculated loading rate.

3. Results and discussion

3.1. Biofilter performance

The biofiltration of air stream containing BTEX was carried out over 140 days at various operating conditions in an up flow mode compost based biofilter. Reactor had been operated in the five phases (Table 3). Various flow rate and concentration were maintained so that the corresponding loading rate could be maintained and regulated in the reactor to study the performance and shock study (step input) of the reactor. Fig. 2 shows the removal efficiencies of BTEX as a function of operating time. The each run was operated till pseudo steady state is

Table 3
Operating conditions of each phase for the compost based biofilter experiments for BTEX

Phase	Operating period (days)	Flow rate (L min^{-1})	Pollution concentration (g m^{-3})				Average loading (g BTEX m^{-3} h^{-1})	EBRT (min)
			B	T	E	X		
I	0–30	4	0.092–0.1207	0.082–0.106	0.0934–0.122	0.091–0.1207	10.94	2.3
II	31–40	4	0.322–0.357	0.23–0.258	0.23–0.259	0.209–0.238	27.43	2.3
III	41–65	6	0.4554–0.508	0.369–0.404	0.41–0.445	0.419–0.445	68.00	1.53
IV	66–96	6	0.75–1.308	0.567–0.946	0.128–0.829	0.393–0.732	117.07	1.53
V	97–141	8	1.229–1.413	–[a]	–	–	68.86	1.15

[a] Not applicable.

achieved. Pseudo steady state was presumed when the changes in the BTEX removal efficiency were within 5% for three successive days. The removal efficiencies in each run were gradually increased, reached stable and rapidly decreased after the sudden change in empty bed residence time (EBRT) or influent concentration.

Phase I lasted from days 1 to 31. Flow rate of the gases mixture and average concentration of BTEX were maintained at 4 L min^{-1} and at 0.149, 0.0946, 0.108 and 0.108 g m^{-3}, respectively, so that the average loading rate 10.94 g m^{-3} h^{-1} can be applied to the reactor. Corresponding EBRT was 2.3 min. Gradual increase in removal efficiency was observed (Fig. 2) more than 99.5% after 30 days of operation for all four compounds. The results showed that the removal efficiency of toluene was the most favorable than others. Time required for achieving more than 99% removals for toluene is 25 days (Fig. 2). At the end of first phase, more than 99.5% removal of all four compounds was achieved at the steady-state condition. These results are consistent with the reported acclimation periods from several weeks to several months [34].

In phase II, loading rate has been increased by nearly three times from 10.94 to 27.42 g m^{-3} h^{-1}. Here average BTEX concentration was maintained at 0.337, 0.244, 0.223, 0.224 g m^{-3}, respectively. In this phase, the flow rate of main stream and EBRT was kept constant at 4 L min^{-1} and 2.3 min. With the sudden increase in the loading rate to the reactor removal efficiency of BTEX decreased approximately from 100% to 61%, 71%, 71% and 52% initially, but later on it recovered gradually from this shock loading to 97% at steady state. Due to this shock load there was a fall in the removal efficiency, it took 3 days to recover. Phase II was lasted for almost 10 days.

In phase III, initially loading rate has been increased further by two times: from 27.42 to 68 g m^{-3} h^{-1}. Flow rate of gas mixture was maintained at 6 L min^{-1} (EBRT 1.53 min). In this phase also same response similar to phase II had been observed once again. There were initial high sudden decrease in removal efficiency from 97% to less than 93% and then there was the recovery in the removal efficiency of BTEX up to 93% for all of the compounds.

In the phase IV, the gas flow rate entering the biofilter remain unchanged at 6 L min^{-1} same as the previous phase, but the concentrations of BTEX were changed on daily basis and moisture content in the bed was also not properly maintained. The loading rate was increased from 68 to 117 g m^{-3} h^{-1}. This is done to

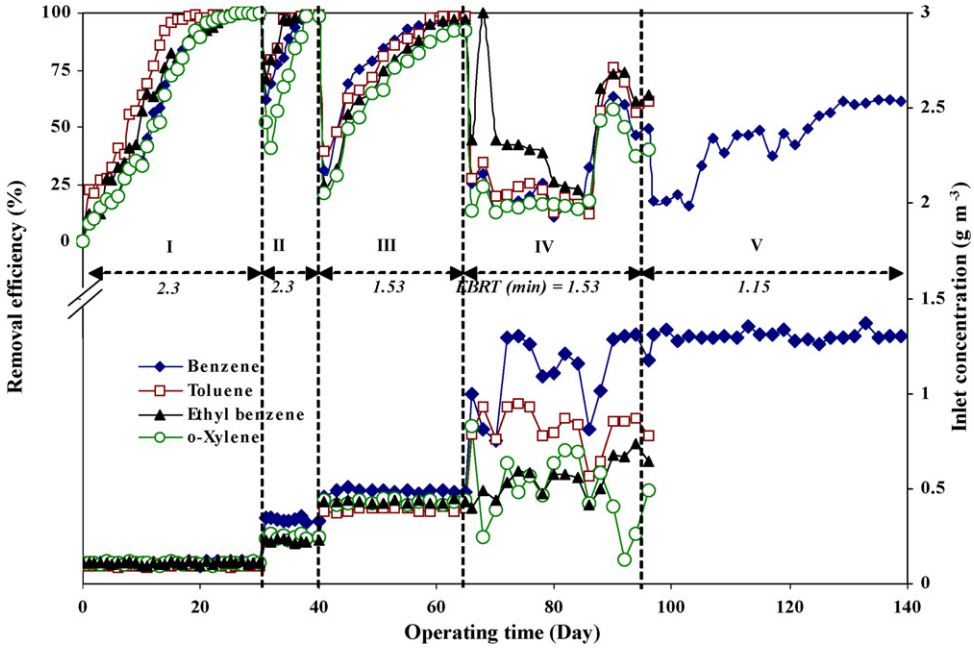

Fig. 2. Overall performance of compost based biofilter in the removal of benzene, toluene, ethyl benzene and *o*-xylene with time.

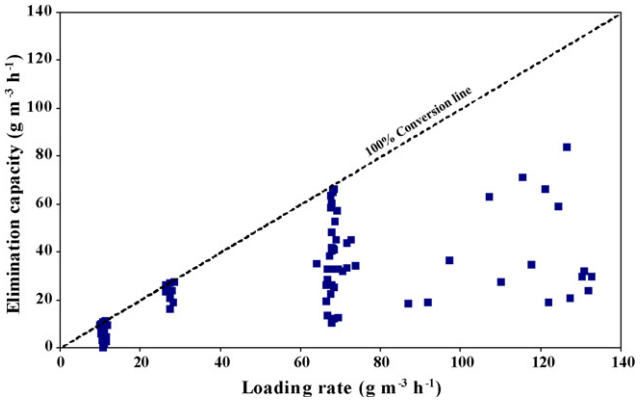

Fig. 3. Influence of BTEX loading rate on the elimination capacity of the biofilter.

observe the changes in the performance of the biofilter, when the concentrations of BTEX are quickly changed. During this phase it was observed that the removal efficiency was also changed in initial days. But after 20 days, the removal efficiencies with more than 63% of all the four compounds were stable. In initial stage of this phase the removal efficiencies were poor. This could be due to the insufficient moisture content and substrate inhibition at the sudden change in BTEX.

Phase V lasted from days 97 to 141 for 44 days with only benzene at average concentration of 1.319 g m^{-3}. Although the flow rate increased from 6 to 8 L min^{-1}, the loading rate decreased from 117 g m^{-3} h^{-1} to nearer to the same as in the phase III, i.e. at 68.8 g m^{-3} h^{-1}. Though initial high sudden decrease in removal efficiency was observed, later on there was the recovery in the removal efficiency up to 61%. In this phase the removal efficiency was gradually increased but not reached greater than 63%. This could be due to the biomass concentration in the biofilter decreased after the sudden change in the flow rate and concentrations of BTEX in phase IV and maintaining of improper moisture since the biomass concentration is also an important factor for biofilter performance.

The biofilter performance was also evaluated in terms of the elimination capacity (EC) of BTEX for the various loading rates, which is defined as the amount of BTEX degraded per unit of reactor volume and time for the various loading rates. The EC, which reflects the capacity of the biofilter to remove the pollutants, is plotted in Fig. 3 as a function of inlet BTEX load. Square symbols represent the experimental data of BTEX while a dotted line indicates the 100% removal. Significant variation of the EC in various phases was observed due to the change in influent concentration and removal rate. The elimination capacities of benzene, toluene, ethyl benzene and *o*-xylene increased with the increase in influent VOC loading, but an opposite trend was observed for the removal efficiency. From Fig. 3, it is clear that when the influent BTEX loadings were less than 68 g m^{-3} h^{-1}, nearly 100% removal could be achieved. The maximum elimination capacity of the biofilter was 83.65 g m^{-3} h^{-1} at inlet BTEX load of 126.5 g m^{-3} h^{-1} in phase IV. During phase III, when the biofilter was operated at average load of 68 g m^{-3} h^{-1}, the maximum EC was achieved as 66.07 g m^{-3} h^{-1}. But the EC was achieved only 44.9 g m^{-3} h^{-1} in phase V, when the biofilter was operated nearly at the same average load of 68.86 g m^{-3} h^{-1}. This could be due to the poor removal in the phase V, because the EC is maximum at maximum removal efficiency. The literature reveals that the most of the biofilters for treatment of mixture of VOCs (paint solvents) are operated at EBRTs in the range from 40 s to 2 min with loading rates in the range from 6 to 40 g m^{-3} h^{-1} [35,36]. Zilli et al. [25] observed that the maximum elimination capacities of benzene removal in biofilters inoculated with *Pseudomonas* sp. NCIMB 9688 and packed with raw and sieved sugarcane bagasse and with peat were 3.2, 6.4 and 26 g m^{-3} h^{-1} at 6.1, 12 and 31 g m^{-3} h^{-1} loading rates, resulting in 52%, 53% and 84% removals, respectively. Sene et al. [23] studied removal of benzene vapour from gaseous streams in two identically sized lab-scale biofiltration columns and observed a maximum elimination capacities obtained at an inlet load of 6.12 g m^{-3} h^{-1} were 3.50 and 3.80 g m^{-3} h^{-1} with raw and ground sugarcane bagasse, respectively. Christen et al. [22] studied the degradation of ethanol in bioreactor inoculated with *Candida utilis* (*C. utilis*) and packed with sugar cane bagasse. At a higher aeration rate (ethanol load of 153.8 g m^{-3} h^{-1}), the biofilter displayed an average removal efficiency of 70% and an elimination capacity of 107.7 g m^{-3} h^{-1}.

3.2. Production of carbon dioxide

In biofiltration process the production of CO_2 (P_{CO_2}) is an important parameter to evaluate the degree of pollutant degradability, because pollutants are finally biodegraded to water and carbon dioxide and utilized as carbon source to form biomass for microbial growth. In Fig. 4 the elimination capacities of biofilter are presented along with the production of CO_2. In general, increase/decrease in the elimination capacity is accompanied with an increase/decrease in the production of CO_2. The production of CO_2 in each phase (g m^{-3} h^{-1}) presented in Fig. 4 was obtained at steady state (i.e. at maximum removal efficiency). The linear equation obtained from experimental data was formulated as $P_{CO_2} = 2.9335EC + 8.0139$. When the value of EC was zero (i.e. no carbon source was introduced into the biofilter), the P_{CO_2} value was 8.0139 g m^{-3} h^{-1}. This P_{CO_2} produced was likely due to endogenous respiration of microorganism pop-

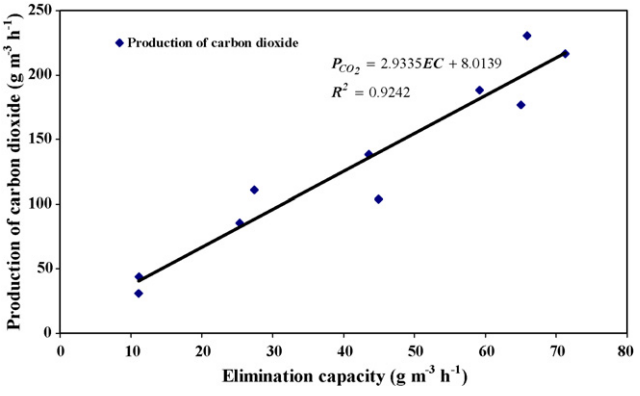

Fig. 4. Variation of production of carbon dioxide with elimination capacity for BTEX.

ulations that did not utilize BTEX as carbon source and the desorption of CO_2 generated by BTEX biodegradation from the packing media. The ratio of the production of CO_2 to BTEX consumed (EC) was approximately 2.9335.

3.3. Effects of nutrients

Microorganisms require nutrients such as nitrogen, phosphorus, potassium, sulphur and trace metals in addition to a carbon source to form new cell material. Among nutrient elements, nitrogen typically makes up 12–13% of dry cell mass and phosphorus makes up 2–3% of the dry cell mass [37]. Hence, nitrogen has been the crucial limiting factor for a long operation for biofilter if adequate nutrients are not available. Generally, available nitrogen utilized by microorganism populations is present in inorganic forms such as ammonia and nitrate. Several studies have been conducted to investigate the effect of nutrients on the performance of biofilters treating single pollutant [38–41]. Further, literature survey reveals that only few researchers have been conducted to assess how nutrient levels affect the degradation of VOC mixtures. Holubar et al. [42] investigated the effect of nitrogen limitation on both a young and a mature steady-state biofilm in a trickle-bed filter treating toluene and *n*-heptane. They demonstrated that while biofilm growth responded strongly to the amount of nitrogen available, the hydrocarbon degradation efficiency reached a maximum of 60% and could not be increased by further addition of nitrogen. Fig. 5a shows the effects of nitrogen limitation on benzene and *o*-xylene removal in the biofilter for 31 days by removing all the nitrogen-containing components from the nutrient feed. During the first 7 days the benzene and *o*-xylene removal increased right after the nitrogen source was eliminated from the nutrient feed. It is suspected that the high salt concentration in the nutrient solution may have inhibited the activity of the aromatic degrading microorganisms. When the nitrogen-containing components were removed from the nutrient solution, the ionic strength of the

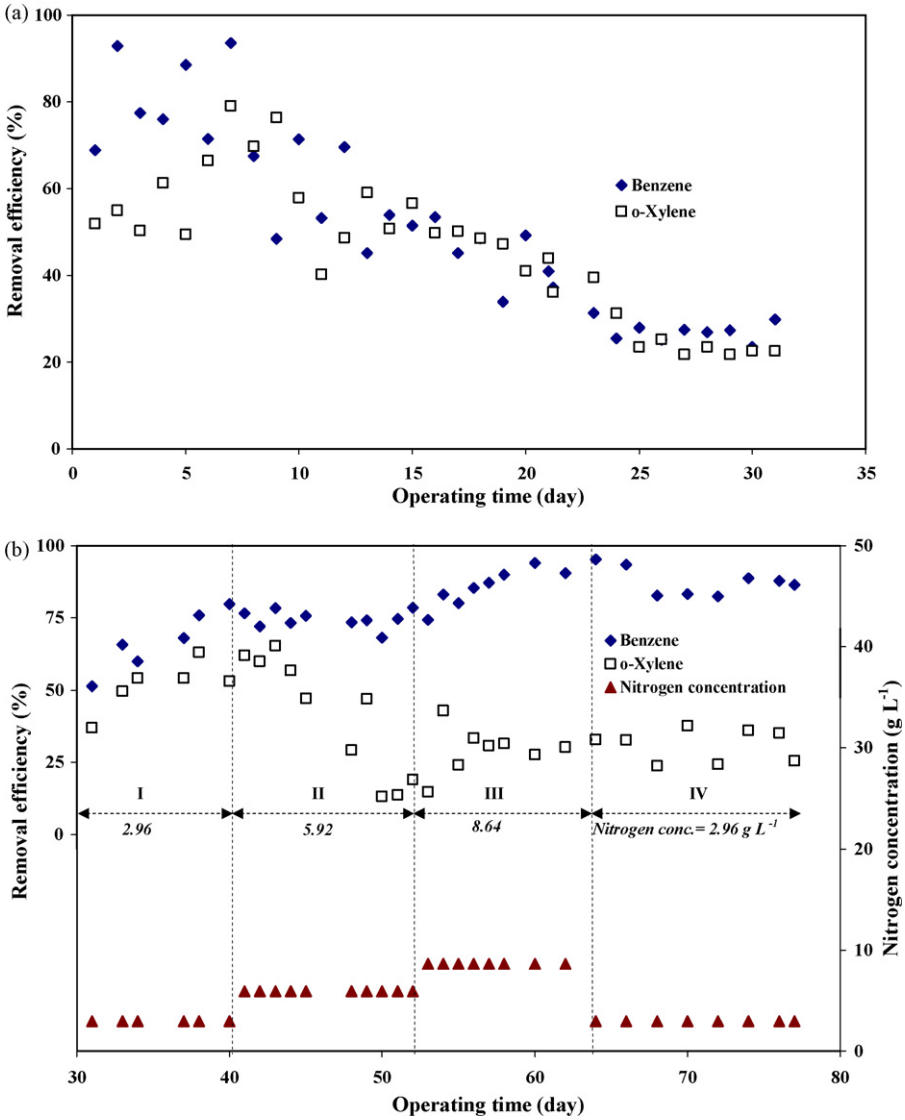

Fig. 5. Effect of nitrogen limitation on benzene and *o*-xylene removal in the biofilter: (a) when nitrogen totally removed from nutrient solution and (b) when different nitrogen level applied in nutrient solution.

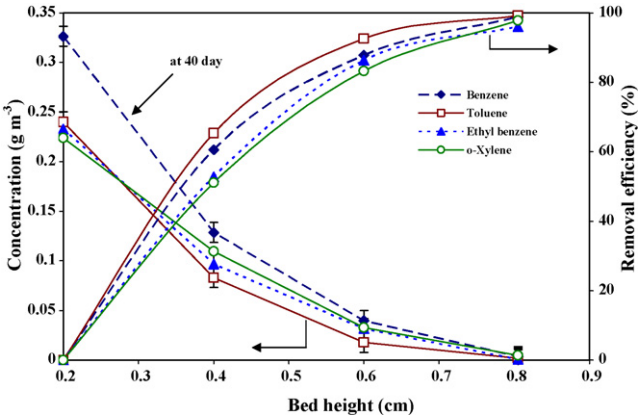

Fig. 6. Inlet concentration and removal efficiency profile along biofilter bed height.

solution was greatly reduced which may have been the reason for the initial improvement in the aromatic hydrocarbon degrading capacity of the biofilters. However, as the nitrogen content in the column became severely depleted, the benzene and *o*-xylene removals gradually decreased. After 28 days operation the removal efficiency (20%) of the benzene and *o*-xylene was constant. It can be presumed that the nitrogen concentration was almost completely depleted. This shows that the nitrogen limitation would be the limiting nutrient ultimately if no addition source were introduced into the biofilter.

Fig. 5b describes the variation of nitrogen along the operation time in compost based biofilter. From the above figure, it has been observed that the benzene removal efficiency increased with the increase in nitrogen addition but *o*-xylene removal increased with the nitrogen up to a point. This result suggests that the high concentrations of nitrogen in the nutrient solution may adversely affect microbial activity possibly due to the presence of high salt concentrations.

3.4. Effects of bed height

One of the aims of this experiment was to determine the local BTEX concentration along the biofilter bed height. For this purpose the sampling ports were provided at the various locations along the bed length. The biofilter was operated in up flow mode. Therefore, highest local concentration of BTEX was at the bottom of the bed and lowest local concentration of BTEX was at the top of the bed. It is important to note that the localized concentration profile provides the valuable information on the degradation profile. BTEX removal profiles within the biofilter bed at the end of phase II on day 40 are shown in Fig. 6. Here BTEX concentration was maintained at 0.326, 0.241, 0.232, 0.224 g m^{-3}, respectively, and EBRT was kept constant at 2.3 min. Results indicate that the removal efficiency was more in bottom part than in middle and upper part of the biofilter. This was due to the fact that more carbon sources, moisture contents and nutrients were present in the bottom section of biofilter, which caused a higher metabolic reaction and thus led to faster biodegradable rate. The moisture content of compost biofilter was varied in the range of 45–70% though the moisture content in the biofilter throughout the study was maintained at around 60%. The optimum temperature range of compost based biofilter is varied in the range of 40–60% [14]. Nearly 65% of toluene and benzene were removed in the first 25 cm of bed depth from lower section, while ethyl benzene and *o*-xylene were removed around only 52%. Fig. 6 shows that the removal of ethyl benzene and *o*-xylene penetrated more slowly into the entire column depth. All four BTEX compounds were removed by 90% up to the second section of biofilter while rest of the 10% was removed in the upper part. The BTEX removal profiles along the height of the biofilter were similar to those previously reported for BTEX and paint VOCs degrading biofilters [21,43].

3.5. Microscopic observations

The scanning electron micrograph (SEM) can provide information about the microbial community on the biofilter media. The biomass of individual particle can be mapped. From such precision, important factor such as filter media coverage, thickness and activity can be determined for modelling. The SEM of microbial growth on various types of media before and after experiment has already been shown by some researchers [44,45]. A comparison of the scanning electron microscopic images of sugar cane bagasse before and after experiment is shown in Fig. 7a and b. Compared to the initial sugar cane bagasse, a biofilm on the surface of the sugar cane bagasse was observed clearly after 140 days of operation. An even growth of microbial community on the surface of the pore of the bagasse is

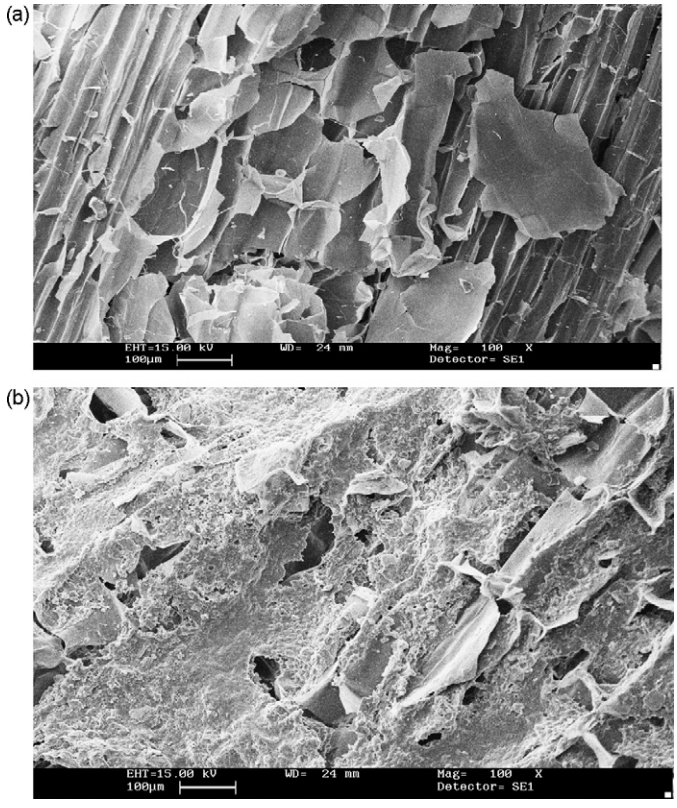

Fig. 7. The biofilm morphology of microorganisms on the surface of sugar cane bagasse by SEM: (a) at the beginning and (b) after 140 days operation (100×).

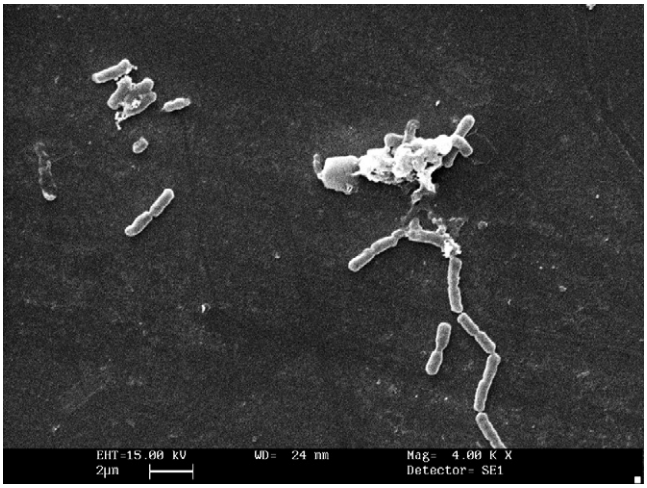

Fig. 8. Scanning electron micrograph of profusely grown BTEX-degrading isolates.

clearly visible. Initially, the degree of acclimatized depends upon the adaptive capacity of the microorganism in the sugar cane bagasse, substrate concentration and its availability and on other necessary environmental conditions. Several groups of microorganisms are involved in the degradation of air pollutants in biofilters including bacteria, actinomycetes and fungi [46].

3.6. Characterization and identification of the pure culture

The most efficient isolates were selected. Microscopic analysis of the isolated BTEX-degrading strain, designated as *B. sphaericus,* shows that this organism is a Gram-positive (Table 2 and Fig. 8).

The 16S rDNA sequences, determined for this isolates were compared to previously published 16S rRNA gene sequences for the Gram-positive bacteria and related microorganisms. Comparison of the 16S rRNA sequences of isolates with those in the GenBank, EMBL and DDBJ databases revealed consistently high similarities (more than 95% similarities) with species of *B. sphaericus.* After taxonomical identification and 16S rDNA analysis, isolate was identified as *B. sphaericus*.

4. Conclusions

The performance of biofilter under various BTEX loading was investigated in this study in a biofilter containing mixture of sugar cane bagasse, compost and GAC as the packing media for the treatment of air streams contaminated with the BTEX compounds. The following specific conclusions are drawn from the results presented in this study:

1. After inoculation, microbial acclimation needed approximately 30 days and simultaneously degradation of BTEX was also observed. The rapid attainment of more than 99.5% removal efficiency in an initial phase indicates that the inoculation procedure used in this study can provide rapid startup and for the good growth of microbial population for the degradation of the mixture of BTEX.
2. The compost based biofilter achieved maximum removal efficiency greater than 99% of all four compounds throughout its operation at an EBRT of 2.3 min for an inlet concentration of 0.681 g m^{-3} which is considerably better than the reported values in the literature. Nearly 100% removal could be achieved when the influent BTEX loadings were less than 68 g m^{-3} h^{-1} in the biofilter. The maximum elimination capacity of the biofilter was 83.65 g m^{-3} h^{-1} at the inlet BTEX load of 126.5 g m^{-3} h^{-1} in phase IV.
3. The production of carbon dioxide is an important parameter to evaluate the degree of pollutant degradability. Increase in the CO_2 production with elimination capacity increases the removal of BTEX could mainly due to the growth of microorganisms. This shows the good performance of the biofilter with the microbial activity.
4. The high concentrations of nitrogen in the nutrient solution may adversely affect microbial activity possibly due to the presence of high salt concentrations.
5. The pure strain, *B. sphaericus*, isolated in this study, has higher potential for capable of degrading mixture of BTEX. This isolate grew in a pH range from 3.0 to 11.0 with an optimum range of 6.0–8.0. Moreover, *B. sphaericus* has potential for the use in biofilter for the remediation of BTEX contaminated environments.

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