Bioprocess Biosyst Eng (2010) 33:219–226
DOI 10.1007/s00449-009-0315-z

ORIGINAL PAPER

A bioactive foamed emulsion reactor for the treatment of benzene-contaminated air stream

Farshid Ghorbani Shahna · Faride Golbabaei · Javad Hamedi · Hossein Mahjub · Hossein Reza Darabi · Seyed Jamaleddin Shahtaheri

Received: 16 December 2008 / Accepted: 18 March 2009 / Published online: 7 May 2009
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Abstract An adapted bioactive foamed emulsion bioreactor for the treatment of benzene vapor has been developed. In this reactor, bed clogging was resolved by bioactive foam as a substitute of packing bed for interfacial contact of liquid to gaseous phase. The pollutant solubility has been increased using biocompatible organic phase in liquid phase and this reactor can be applied for higher inlet benzene concentration. Experimental results showed a benzene elimination capacity (EC) of 220 g m^{-3} h^{-1} with removal efficiency (RE) of 85% for benzene inlet concentration of 1–1.2 g m^{-3} at 15 s gas residence time in bioreactor. Assessment of benzene concentration in liquid phase showed that a significant amount of transferred benzene mass has been biodegraded. By optimizing the operational parameters of bioreactor, continuous operation of bioreactor with high EC and RE was demonstrated. With respect to the results, this reactor has the potential to be applied instead of biofilter and biotrickling filters.

Keywords Bioactive foamed reactor · Benzene · Air pollution control · Biodegradation

F. Ghorbani Shahna · F. Golbabaei (✉) · S. J. Shahtaheri
Department of Occupational Health, School of Public Health, Institute of Public Health Research, Tehran University of Medical Science, Enghelab Street, 16 Azar Ave, PB 14155-6446, Tehran, Iran
e-mail: fgolbabaei@tums.ac.ir

J. Hamedi
Division of Microbiology, Department of Biology, Faculty of Science, University of Tehran, Enghelab Street, Tehran, Iran

H. Mahjub
Department of Biostatics and Epidemiology, School of Public Health, Centre of Health Research, Hamadan University of Medical Science, Mahdieh Street, Shahid Fahmide Blv, PB 4171, Hamadan, Iran

H. R. Darabi
Chemistry and Chemical Engineering Institute of Iran, Km 17 Tehran-Karaj Freeway, Pajoohesh Blv, PB 14335-186, Tehran, Iran

Introduction

Volatile organic compounds (VOCs) are of special significant air pollutants. They readily volatilize to the atmosphere and can be distributed over large regions thus leading to a population-wide exposure to these chemicals. Because of their relatively high vapor pressure, they are found predominantly in the atmosphere, causing different environmental problems, such as stratospheric ozone depletion, ground-level ozone formation, global green house effect, and toxic health effects on humans, animals, plants and ecosystems [1].

Volatile organic compounds are considered to be the most hazardous of air pollutants emitted by industrial facilities. Among VOCs, monochromatic hydrocarbons such as benzene, toluene and xylene (BTX) are the toxic substances. Releasing these pollutants into the ambient air may lead to adverse effects on air quality and threat public health and welfare [2].

Biological treatment is a novel established technology for the control of air pollution among the air pollution control methods and since it is effective for the control of odors and volatile organic compounds, especially in high flow rates, low concentrations cases, it can be an alternative for physical and chemical treatment techniques [1, 3, 4].

The most widely used bioreactors for air pollution control are biofilters and biotrickling filters but both have

major limitations [5–7]. Due to the low activity of the attached cells to packing bed, biofilters typically have low pollutant elimination capacities, while biotrickling filters often exhibit higher performance than biofilters, but often it can be clogged from excessive biomass growth on the bed, which results in high pressure drop and process instability [8–10]. Excess biomass clogs the reactor, which requires costly remedial or preventive actions [5].

To overcome these inherent problems, two techniques have been introduced in the recent years. These techniques include: (1) Two phase partitioning bioreactor (TPPB), which is based on the use of water-based immiscible and biocompatible organic phase that is allowed to float on the surface on a cell containing aqueous phase [4, 10–12] and (2) Foamed emulsion bioreactor (FEBR) that consists of an emulsion of highly active pollutant-degrading microorganisms and a water-immiscible organic phase, which is made into a foam with the air being treated [3, 5, 13]. The FEBR is similar to TPPB, but the amount of organic phase is low and it uses a biocompatible surfactant for foam production. To attain high volumetric pollutant removal rates, the FEBR relies on a high-density culture of actively growing organisms. At the same time, bed clogging and associated pressure drop problems are resolved by using a moving foam rather than an immobilized culture growing on a bed [5].

Another alternative is a bioactive foam reactor (BFR), operating with a surfactant bubble solution containing pollutant-degrading microorganisms [14]. In this reactor, organic phase has not been used. The past studies demonstrate that organic phase has a key role in absorption of VOCs, especially at high concentrations. The function of organic phase was to adsorb/desorb high concentration of pollutants. In comparison to biofilter and biotrickling filter, the BFRs have a high elimination capacity (EC) for large interfacial area between the gas and fine foam, but in the high concentration (generally higher than 300 mg m^{-3}) removal efficiency (RE) in BFRs is reduced [3, 6].

Toluene as a representative of VOCs and oleic alcohol as an organic phase have been used in past studies [3, 5, 6]. The benzene has lower solubility (and bioavailability) in aqueous phase in comparison to toluene and therefore, it is biodegraded lower than toluene in the same condition. Benzene is a very hazardous pollutant that can produce very adverse health effects, e.g. leukemia and lymphomas (blood cancer), while the adverse effects of toluene is light. The purpose of this study was designing of the two-phase foam bioreactor for biodegrading benzene-laden air stream and studying other organic phases, surfactants and variables for achieving optimum operation conditions for bioreactor. The model estimation of bioreactor behavior to operation parameter was other goal of this study. In addition to this determination of the maximum EC, RE and optimum percentage of organic phase, oxygen content, mode of operation, residence time and associated parameters have been other goals of this study. Finally, the statistical model of bioreactor behavior to operation parameter was estimated.

Materials and methods

Microorganism and mineral nutrients

The soil of oil and petrol storage tanks at a storage and distribution complex near Hamadan city situated at the west of Iran was sampled based on the assumption that it contains benzene-degrading microorganisms. First, the soil samples at the sterile condition were scrubbed with sterile NaCl serum and the produced dark solution was poured in a glass vial. After 6 h the clear supernatant of samples was poured in the 1 L flask and mineral nutrient was added to it with 3:1 (vol) ratio. The nutrient medium consisted of: 1.2 g L^{-1} KH_2PO_4, 1.2 g L^{-1} K_2HPO_4, 0.2 g L^{-1} $MgSO_4$, 1 g L^{-1} NaCl, 1 g L^{-1} KNO_3 and trace element that comprised: 26 mg L^{-1} $CaCl_2{\cdot}2H_2O$, 5.5 mg L^{-1} EDTA $Na_4(H_2O)_2$, 1.3 mg L^{-1} $FeCl_3{\cdot}4H_2O$, 0.12 mg L^{-1} $CoCl_2{\cdot}6H_2O$, 0.1 g L^{-1} $MnCl_2{\cdot}2H_2O$, 0.07 mg L^{-1} $ZnCl_2$, 0.06 mg L^{-1} H_3BO_3, 0.025 mg L^{-1} $NiCl_2{\cdot}6H_2O$, 0.025 mg L^{-1} $NaMoO_4{\cdot}2H2O$, 0.015 mg L^{-1} CuCl2·2H2O.

The benzene-degrading microorganisms were extracted and enriched in two steps. First, 60 mL of the above mentioned solution was poured in 300 mL bottles fitted with a butyl rubber septum. The 240 mL of headspace guaranteed sufficient air for aerobic degradation. Thus, 100 mg L^{-1} of benzene was added to any bottle as the sole carbon and energy source. All the flasks were incubated at 25–30 °C temperature on a rotary shaker at 150 rpm in a dark environment. The ability of samples for degrading benzene in head space and solution analysis was evaluated by gas chromatography (UNICAM 4600) with FID. A similar flask, which contained 1% of NaCN as a blank sample has been prepared for control of a benzene loss from volatilization and diffusion from septum. After consumption of benzene, samples were washed with sterile air and again the cycle was started. In each cycle, dissolved oxygen (DO) was measured with HACH DO meter (model sesion6, USA). Optical density was monitored with UV–Vis spectrophotometer (UV-1700 Shimadzu pharmaspec, Japan) at 600 nm as index of cell growth. After several cycles, the enriched centrifuged solution and fresh nutrient mineral solution were added for using in bioreactor. In the second step, the mixed culture was grown by bubbling 0.8–1.2 g m^{-3} benzene-laden air through nutrient medium in a 3 L flask and concentrated by centrifugation before each experiment.

The minimum concentration of biomass for introducing to bioreactor was 10 mg L^{-1}. The biomass concentration of these inoculums was measured by overnight drying of aliquots at 70 °C.

The various organic phases (including *n*-hexadecene, oleic alcohol and 1-octadecene) and surfactants were studied for application at reactor and the best of them was selected.

Bioreactor setup

The bioreactor consists of an air compressor, an oxygen tank, dehumidifier and activated carbon beds, dynamic system for generation of benzene vapors, foam column, cell reservoir and defoamer (Fig. 1). The compressed air was dehumidified in a ceramic bed and then the conditioned air passed through a column filled with activated carbon to remove VOCs. For this reason, dried treated air was mixed to oxygen. This bioreactor has high oxygen and pollutant mass transfer rates due to large interfacial area between gas and liquid of fine foam and a high portioning of pollutants into the organic phase. Thus, for achieving a high elimination capacity and removal efficiency of pollutants, the oxygen in air is insufficient and needs additive oxygen. After mixing of air and oxygen, it is introduced to dynamic generation system of benzene air stream. Introduced air is divided to two parts. A slow stream of air was bubbled through impinger (glass vial) containing pure benzene (Merck Co, Germany) and the second stream was bypassed. Thus, two streams are mixed together again and are metered with flow meter. By adjusting the flow rates of two streams, the various concentrations for benzene were achievable. In this method, vapor concentration was maintained stable and the fluctuation of operation being variable (e.g. room temperature or solvent volume) the effect was very low. It has not used of bypassed air in the some of other studies [3–5, 10, 12, 14]. Vapor concentration was related to operation parameters strongly and need to continue monitoring of these parameters without bypassed air.

The volume of pure benzene in the impinger and temperature of room in the study period is better to be stable. To remove the air-borne microorganisms, the contaminated air to benzene was passed through 0.22 μm pore size sterile bacteriological filter. For all the experiments described herein, the heat resistant parts were autoclaved and ethanol vapor has been rinsed up to bioreactor for several hours for sterilization. The sterilization of inlet air and all parts of bioreactor prevented interferences of other microorganisms on benzene-degraded microorganism's performance.

The benzene air stream enters to foam column. The plexiglass foam column (4 cm diameter, 48 cm high and 0.6 L volume) had a fine air sparger at the bottom of column. The introduced benzene-contaminated air stream passed through the sparger while emulsion consisting of nutrient medium, the active culture and the *n*-hexadecene 6% (vol) as organic phase (Merck Co) and 0.2% (vol) Triton X-100 as biocompatible surfactant (Merck Co) was introduced at the bottom of bioreactor by a peristaltic pump (SR25-S300 thomas Co, USA).

Fine foams were generated and rised up the column. After rising the reactor, the foams left the column through a side port and were defoamed in a defoamer with continuous spraying the emulsion from cell reservoir. The defoamer was a 1 L volume glass flask. The sprayed and defoamed liquid was returned to reservoir (a 1 L flask) and recycled to foam column. The liquid in cell reservoir was continuously stirred to be introduced to column as a homogenous emulsion. Total volume of emulsion in this bioreactor was 0.6 L.

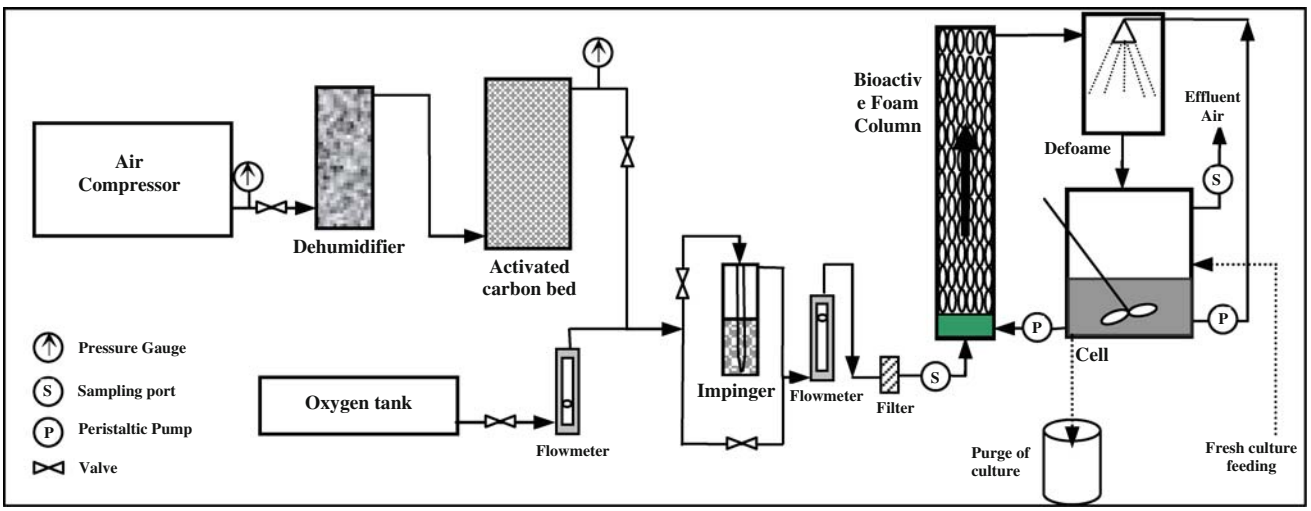

Fig. 1 Schematic of the bioactive foamed emulsion reactor applied in this study

The continuous operation of bioreactor was guaranteed by replacing 10–20% (vol) of culture medium described above except the potassium nitrate, that was increased fourfold to 4 g L^{-1}. The fresh culture had the similar percentage of organic phase (6% *n*-hexadecene) and surfactant (0.2% Triton X-100).

The effect of organic phase amount and oxygen content on the RE and EC of bioreactor at various conditions have been studied. In addition, determination of the optimum conditions of bioreactor operations including the residence time, the oxygen content, concentration of organic phase etc., RE and EC were evaluated for different conditions. After the determination of the optimum conditions, the performance of the bioreactor at the operation mode for continuously 1 week was studied.

Analytical methods

To determine removal efficiency and elimination capacity of bioreactor, the gaseous samples were collected from the two sampling ports placed at before foam column and after cell reservoir at outlet of bioreactor. Samples were directly injected into gas chromatograph (UNICAM 4600, UK) with flame ionizing detector (FID) and glass column 1.5 m×4 mm id packed with 10% SE 30 on chromosorb W-AW-DMCS 100-120. The concentration of CO_2 at inlet and outlet of bioreactor was measured with Testo model 535 CO_2 meter (Hotek technologies Inc, USA). By determining the difference between inlet and outlet concentrations of CO_2, the carbon content of CO_2 was calculated and by dividing it with carbon content of benzene biodegraded (C-CO_2/C-benzene biodegraded), the percentage of C-mineralized was determined.

For measuring benzene concentration in liquid phase, 30–50 mL of purge culture was filtered by 0.22 μm pore size sterile bacteriological filter for the removal of particulates and microorganisms. Then, the filtered liquid was centrifuged at 3,500 rpm for 10 min for separating organic and aliquots phases. 10 μl of organic phase was immediately injected to GC/FID and concentration of benzene was calculated by comparing the produced peak area with standard curve.

Results and discussion

n-Hexadecene was selected as organic phase for its biocompatibility and high partition coefficient for benzene [12, 15, 16]. The oleic alcohol as best organic phase was selected by Kan and Deshusses [3, 5] for toluene, while the results of this study showed that *n*-hexadecane has a better performance in comparison to oleic alcohol and 1-octadecene to adsorb/desorb benzene. Whereas the silicone DC in some of previous similar studies [3, 5, 13] was selected as biocompatible surfactant, but in this study, the Triton X-100 as an alternative showed good performance. This surfactant is biocompatible and has a good foaming ability [6, 17]. The optimum concentration of organic phase was determined based on RE and EC of bioreactor. Results in Fig. 2 demonstrate that RE and EC of bioreactor have been increased relatively fast with increasing organic phase concentration up to 4%. From 4 to 6%, the increase of RE and EC had been slow. The higher than of 6%, RE and EC was remained stable therefore, the 6% (v/v) of *n*-hexadecane was determined as optimum in comparison to 3–5% (v/v) oleic alcohol for toluene [3, 5]. The maximum EC of the 250 g m^{-3} h^{-1} was achieved at 93% removal efficiency. Without organic phase the RE was reduced to 68% and EC to 163.68 g m^{-3} h^{-1}. According to the principle of TPPB [10], this reduction for high concentration of benzene was more. As long as the organic phase content was higher than 7% (vol), because of oily state of *n*-hexadecene, the foam at column was slope and broken down, thus the use of higher concentration of that was impossible.

The effect of gas residence time (or linear velocity) on EC and RE was studied. At residence time less than 10 s, the foam was instable and could not rise up the column. In the range of 10–15 s, the RE of bioreactor was increased significantly. In the range of 15–60 s, increase in the RE was insignificant but EC was reduced. The trend of RE and EC changes with respect to residence time is shown in Fig. 3.

The modeling of bioreactor behavior to operational parameters was a novel idea in comparison to similar studies [3, 5]. The model simulation of bioreactor EC in relation to inlet concentration of benzene at various residence times is illustrated in Fig. 4. Based on estimated

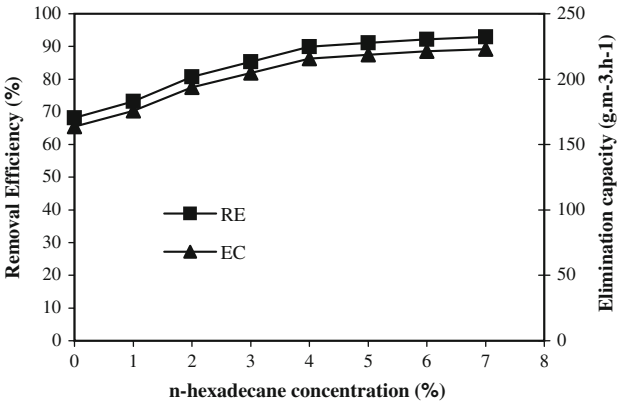

Fig. 2 Effect of organic phase concentration on the bioreactor elimination capacity and removal efficiency for benzene. Experimental condition was: 1 g m^{-3} of inlet benzene concentration, 15 s of residence time, 10 g L^{-1} of biomass concentration

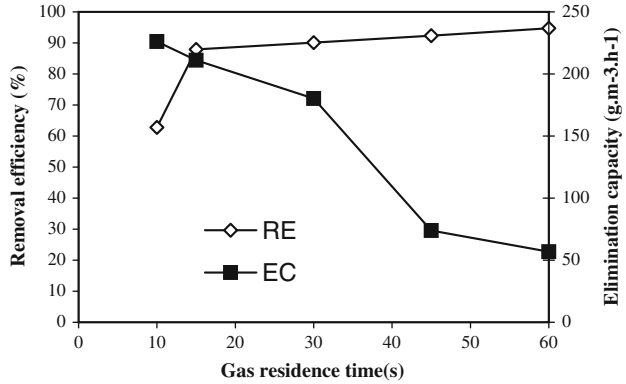

Fig. 3 Effect of gas retention time on the bioreactor elimination capacity and removal efficiency for benzene. Experimental condition was: 1 g m^{-3} of inlet benzene concentration, oxygen content of 40%, and 10 g L^{-1} of biomass concentration

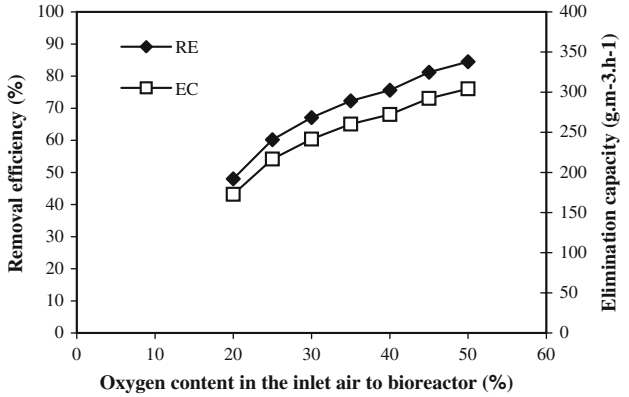

Fig. 5 Effect of oxygen content in the inlet air to bioreactor on the removal efficiency and elimination capacity. Experimental conditions: 1.5 g m^{-3} of inlet benzene concentration, 15 s of residence time, 15 g L^{-1} of biomass concentration

models, relation of EC to benzene loaded concentration was linear for residence time of 30, 45 and 60 s, but was quadratic for the 15 s residence time. The model predicted for residence time of 15 s indicated that the elimination capacity remained stable with increasing benzene concentration of higher then 1,500 mg m^{-3}. This was primarily due to mass transfer limitation of bioreactor.

For best biodegradation of benzene, especially at high inlet concentration and for high concentration of biomass, high oxygen diffusion of gas phase to aliquots phase is necessary. The 21% oxygen content in inlet air was insufficient for complete biodegradation of high concentration of benzene especially in concentrations higher than of 1 g L^{-1} based on stochiometric calculation. Thus additional oxygen was needed. This additional oxygen can be supplied using an oxidant agent like hydrogen peroxide. But this agent may be toxic for microorganisms. Addition of pure oxygen to inlet air to bioreactor is the second resolving method. The relation of oxygen content at inlet gas stream of bioreactor with RE and EC had been shown in Fig. 5. Note that the biomass concentration in nutrient media can affect this relation so that by increasing the biomass, the needed oxygen for biodegradation should be increased, while our experiments showed that RE and EC of reactor has been changed significantly for culture density more than 15 g L^{-1}. Unnecessary high concentration of biomass (>15 g L^{-1}) produced another problem. High biomass density reduced the foam stability and the foam exploded sooner. Our experiments demonstrated that the 5–15 g L^{-1} biomass concentration was appropriate for continuous operation of bioreactor and for this range; the oxygen amount of 40% at inlet gas stream with benzene

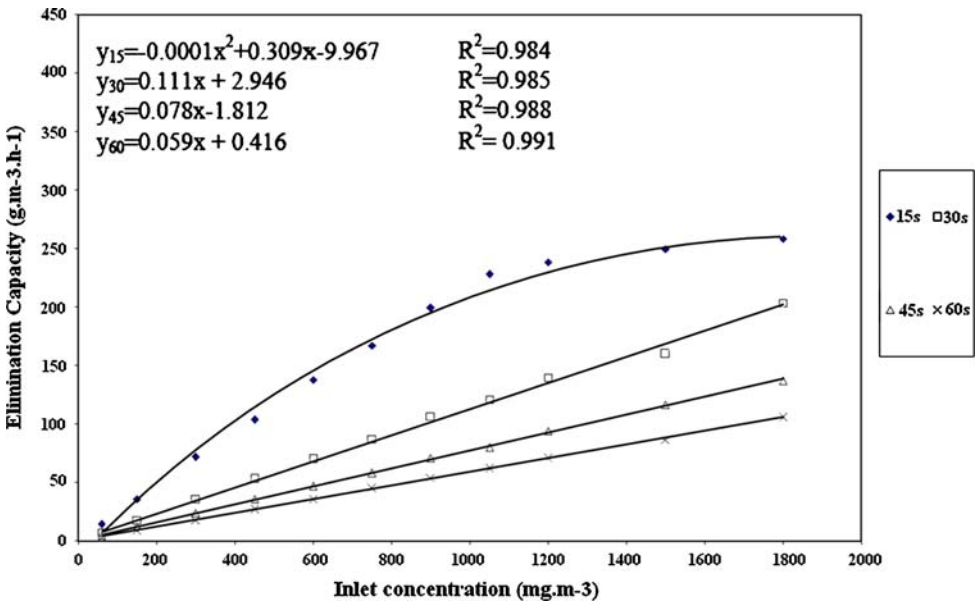

Fig. 4 Changes of elimination capacity and model prediction of bioreactor for various inlet concentration and gas residence time. Experimental condition: 10 g L^{-1} of biomass concentration and the oxygen content in inlet air of 40%

concentration of 1 g m^{-3} was suitable. With 21% oxygen in air, 48% of RE for benzene concentration below 1 g m^{-3} was achieved. The EC of bioreactor at pure oxygen injection condition was increased to 386 g m^{-3} h^{-1}.

The predicted model to elimination capacity in relation to oxygen content for various inlet loading of benzene was indicated in Fig. 6. The elimination capacity of reactor was not increased significantly for inlet loading higher than that of 225 mg m^{-3} h^{-1} without oxygen addition to inlet air based on estimated model. This means that the elimination capacity of bioreactor is related to oxygen content strongly so that for inlet loading of 450 mg m^{-3} h^{-1}, the EC was 199.16 and 321.28 mg m^{-3} h^{-1} for 20% (no oxygen supply) and 60% oxygen content in inlet air, respectively. The effect of oxygen content on EC was more significant with increasing inlet load of benzene to bioreactor.

The performance of bioreactor was monitored for continuous operation for 1 week (Fig. 7). Benzene inlet concentration was about 1–1.2 g m^{-3}. Removal efficiency in preliminary hours was relatively low (about 76%) due to acclimation phase. After 24 h, the removal efficiency increased to 87%. The average RE and EC for continuous operation of bioreactor were 85.44% and 220 g m^{-3} h^{-1}, respectively. The EC of this bioreactor for benzene has been higher than of biofilter and biotrickling filter. The maximum EC for peat-packed biofilter by Mario Zilli et al. [18], polyurethane biofilter by Won et al. [19], activated carbon biofilter by Kim [20] and by Li et al. [21] were reported as 26, 100, 20 and 120 g m^{-3} h^{-1}, respectively. Chungsying et al. [22] determined benzene EC of 64 g m^{-3} h^{-1} for a trickle bed biofilter. In TPPB, EC of 141 ± 12 g m^{-3} h^{-1} have been reported by Nielsen et al. [23]. In the Nielson study, the content of organic phase was 33% which was more than 6% in this study. But the biomass concentration in their work was less than 10 g L^{-1} while here it is 10–15 g L^{-1}. In comparison to previous studies, this bioreactor has higher EC and the ability for biodegrading higher concentration of benzene. Continuous exchanging of nutrient mineral and the need for additive oxygen in high inlet concentration of benzene (>1 g m^{-3}) are the two limitations of this bioreactor. Based on our findings, 71–81% of C-benzene degraded at continuous

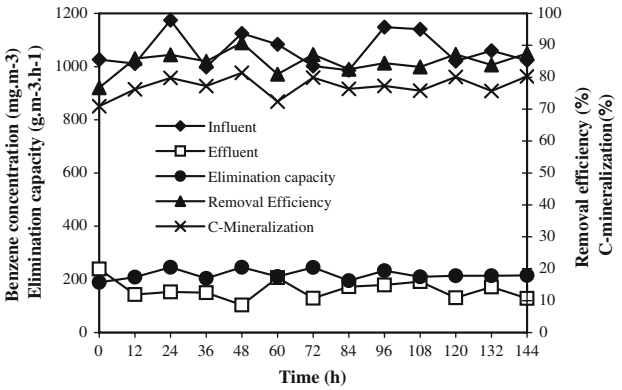

Fig. 7 Continuous operation of bioreactor. Operation conditions: 6% *n*-hexadecane as organic phase, 0.2% Triton X-100 as surfactant, residence time of 15 s, biomass concentration of 10–15 g L^{-1}, The oxygen content in inlet air of 40%. C-mineralization is the percent of C-CO_2/C-benzene degraded

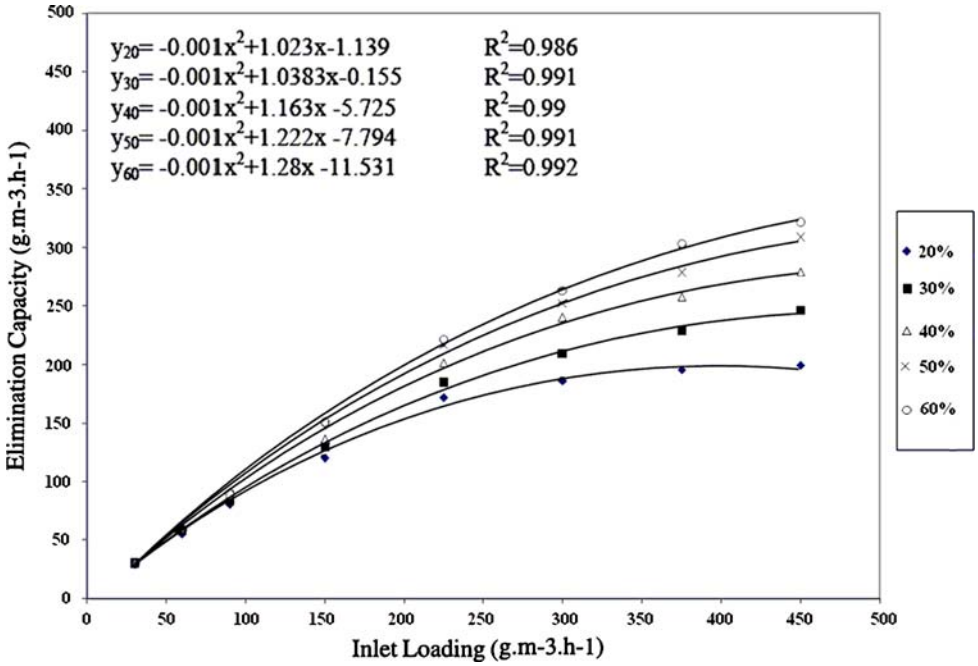

Fig. 6 Changes of elimination capacity and model prediction of bioreactor for various oxygen content in inlet air. Experimental condition: 10 g L^{-1} of biomass concentration and the gas residence time of 15 s

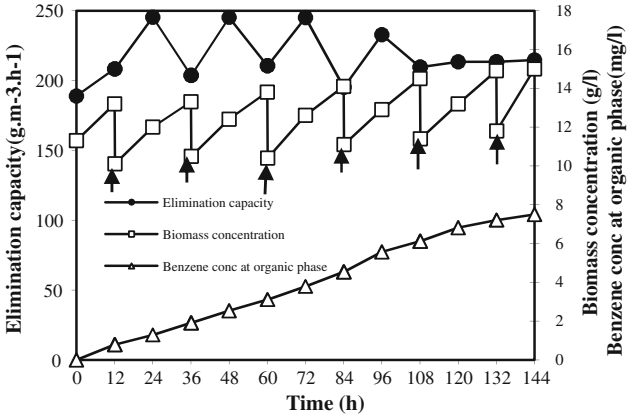

Fig. 8 Changes of the elimination capacity, biomass concentration and benzene concentration in the liquid phase in continuous operation of bioreactor. The *arrow* indicates feeding of nutrient medium to bioreactor. Condition is similar to Fig. 5

operation of bioreactor was served to as C-CO_2 and residual has been utilized for cell production.

Biomass and benzene concentration in the liquid phase were measured each 12 h (Fig. 8). Each 24 h 60–100 mL of reactor solution was exchanged with fresh nutrient. This exchanging helped a high performance operation of bioreactor [3, 13]. The results of benzene measurement in the liquid phase of bioreactor showed that the microorganisms in liquid phase were very active and a significant part of introduced benzene was biodegraded. In comparison to content of entered benzene to bioreactor, 7.5 mg L^{-1} of benzene concentration in the liquid phase is very low after 1 week operation of reactor. The amount of dissolved oxygen at this period was in the range of 83.2–90.1%, which was sufficient for aerobic condition for cell activity.

Finally, the behavior of bioreactor was studied for various loads and concentration at specific condition. The results of Fig. 9 demonstrate that the benzene removal and elimination capacity of the bioreactor has a specific trend to 1 g m^{-3} (240 g m^{-3} h^{-1}). In the inlet concentration higher than 1 g/m^3, the efficiency of bioreactor has not increased significantly. Thus, the inlet concentration of 1 g m^{-3} as basic level or experiment level was selected. In addition, the estimated statistical model for removal efficiency and elimination capacity of bioreactor was determined for optimum condition.

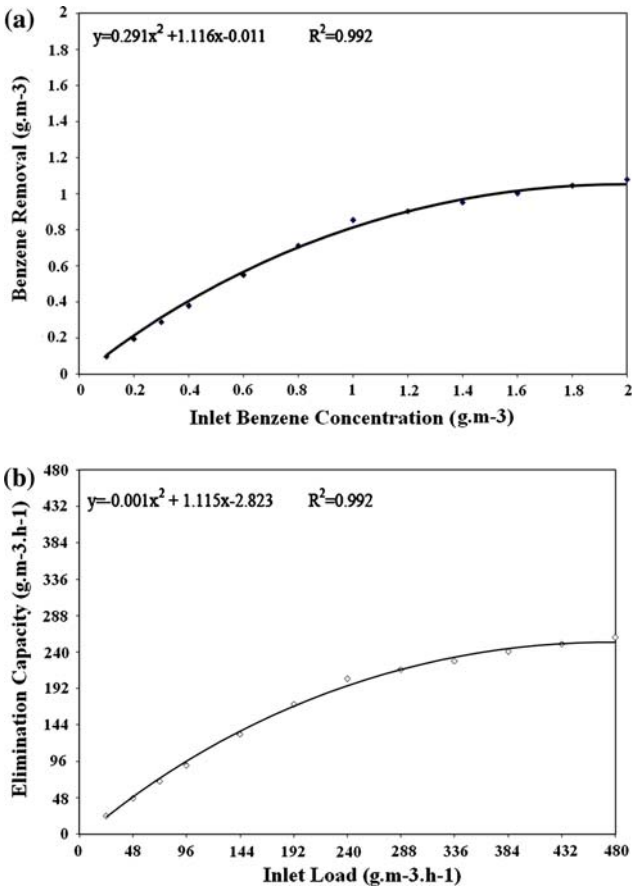

Fig. 9 Trend of removal efficiency and elimination capacity of bioreactor for various inlet concentrations (**a**) and loads (**b**). Experimental conditions: residence time of 15 s and 10 g L^{-1} of biomass concentration. Oxygen content was 40%

Conclusion

The ability of bioactive foamed emulsion bioreactor for the treatment of benzene has been confirmed successfully. The reactor potential for short and long period was demonstrated. In the previous studies, only toluene was utilized as model contaminant at foam reactor while in this research, benzene was evaluated as more hazardous pollutant in relation to toluene. In the tested bioreactor herein, the mass transfer of benzene from the gas to the liquid phase was fast due to the increased interfacial surface area of the surfactant foams. The benzene solubility was increased by organic phase. The optimum residence time (15 s) of this bioreactor was less than biofilters and biotrickling filters. This low residence time has increased the EC of bioreactor. The results showed that this reactor was flexible for high concentration of pollutant, especially by adding a second immiscible phase to the culture medium. The effect of various operation parameters (oxygen content, residence time, organic phase concentration and culture density) of bioreactor was investigated on the removal efficiency and elimination capacity of bioreactor and optimum conditions for continuous operation of bioreactor was determined. The 220 g m^{-3} h^{-1} as the average EC for continuous operation of this reactor was two to ten times higher than that of biofilter and biotrickling. In addition the difficulty due to bed clogging and low efficiency for high concentration of pollutants was eliminated. At higher inlet benzene

concentration and higher culture density, oxygen limitation occurred. By providing additive oxygen and periodical exchanging of nutrient medium, these restrictions could be resolved. Overall, with respect to the results of this and similar studies, the potential of bioactive foamed emulsion reactor was indicated as alternative of classic bioreactors in the future.

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