Iranian Journal of Chemical Engineering
Vol. 10, No. 2 (Spring), 2013, IAChE

Biofiltration of BTEX in a Mixture Bed of Bagasse and Activated Carbon Using Aspergillus Fungi

B. Mokhtarani[*]*, M. Mafi, M. Keshavarz, H. R. Mortaheb, K. Tabar Heidar*
Chemistry and Chemical Engineering Research Center of Iran, Tehran, Iran

Abstract

Biodegradation of a mixture of benzene, toluene, ethyl benzene, and o-xylene (BTEX) was studied in a two-bed biofilter. The packing material was a mixture of sugarcane bagasse and granulated activated carbon. Aspergillus Brasiliensis and Aspergillus Niger fungi were inoculated in the biofilter, separately. The effects of BTEX concentration, Empty Bed Residence Time (EBRT) and temperature on the biofilter efficiency have been studied. The results indicate that in the biofilter with Aspergillus Brasiliensis fungus, the removal efficiency approaches to 100% for the inlet load of 71.5 $g.m^{-3}h^{-1}$ and EBRT of 2.7 min while total removal efficiency in the biofilter with Aspergillus Niger fungus is obtained for the inlet load of 140.3 $g.m^{-3}h^{-1}$ and EBRT of 1.3 min. The maximum elimination capacity for this biofilter at inlet load of 223.08 $g.m^{-3}h^{-1}$ and EBRT of 0.9 min is 188.95 $g.m^{-3}h^{-1}$.

Keywords: *Biofilter, BTEX, Fungus, Bagasse, Aspergillus Niger, Aspergillus Brasiliensis*

1. Introduction

Benzene, toluene, ethyl benzene, and *o*-xylene (BTEX) are important industrial solvents in various industries such as petrochemical plants, refineries, printing industries, etc. Large quantities of BTEX mixtures are annually released during manufacturing, transportation, utilization, and disposal of these materials. BTEX compounds have toxic properties and are classified as the hazardous substances in the Environmental Protection Agency priority list [1]. Nowadays, emission of BTEX is considered as a major environmental problem. These compounds can cause neurological, respiratory, genetic and excretory system damages [2]. Among these compounds, benzene is known to be more carcinogenic. The emission of these mixtures is often in high flowrates and low pollutant concentrations, which makes their treatment difficult. Biofilteration is an appropriate technique to overcome the treatment difficulties. The method has proven to be effective and economical for treatment of waste gases contaminated by BTEX compounds [3]. Biofilteration is considered as a clean technology with minimum energy

∗ Corresponding author: mokhtaranib@ccerci.ac.ir

requirements and low waste production [4].

The removal of BTEX using biofilter has been investigated by some researchers in recent years [2,4-7]. The early researches were conducted on removal of a single gas (benzene, toluene or xylene) using biofilter [8-14]. However, the removal of BTEX mixtures is more complicated than that of a single gas. Many bacterial strains can biodegrade the BTEX mixtures in the biofilter. Although the BTEX biodegradation has been extensively investigated [2,4,6], fewer researches have been done using fungi. The fungal biofilters show higher elimination capacities for removal of aromatic compounds compared to the bacterial biofilters because of greater resistance of the fungi to the dry and acidic conditions than the bacteria [15]. The humidity and pH are important operational parameters for the biofilters. Oh et al. [16] studied the biodegradation of BTX by the white rot fungus of *Phanerochaete Chrysosporium* in a biofilter. They observed that the biodegradation degree for p-xylene is the highest, while for benzene it is the lowest. Qi et al [17] investigated the ability of five fungal species of *Cladosporium Resinae*, *Cladosporium Sphaerospermum*, *Exophiala Lecanii-corni*, *Mucor Rouxii*, and *Phanerochaete Chrysosporium* for biodegradation of nine volatile compounds (aromatic compounds, organic acids, and ketons) on a ceramic media biofilter. The results showed that *Exophiala Lecanii-corni* and *Cladosporium Sphaerospermum* can biodegrade each of the nine components. The biofilteration of BTEX by *Paecilomyces Variotii* fungus have been studied by Garcia - Pena et al. [7]. They reported that toluene is completely degraded while ethyl benzene, benzene, and xylene are partially metabolized.

In the present work, the biofilteration of BTEX mixture by *Aspergillus Brasiliensis* and *Aspergillus Niger* fungi is investigated. The packing material is a mixture of sugarcane bagasse and granulated activated carbon (GAC). As the characteristics of the packing materials have considerable influence on the biofilter efficiency, sugarcane bagasse, which is an inexpensive residue of sugar industries and has been addressed as a suitable media for biofilteration of aromatic compounds [2-3,18], is used in the present study. The performance of the biofilter was assessed by determining the BTEX removal efficiencies, elimination capacities and microbial concentration.

2. Materials & methods

2-1. Microorganism

The *Aspergillus Brasiliensis* PTCC (5298) and *Aspergillus Niger* PTCC (5223) fungi were purchased from Persian type culture collection center in Iran. The *Aspergillus Niger* PTCC (5223) is a native strain that is isolated from oil sludge. The *Aspergillus Brasiliensis* PTCC (5298) is equal to ATCC (9642) in American Type Culture Collection (ATCC) and is a preservative strain for the chemicals and fungus resistance testing of the polymers.

2-2. Chemicals

Benzene, toluene, o-xylene and ethyl benzene were purchased from Merck Company. All the chemicals for cultivation of the fungi were also obtained from Merck

Company. The double distillated water was used in all the experiments.

2-3. Cultivation system

The fungi were cultivated in the liquid cultivation media which contained 5 gr peptone and 3 gr meat extract in 1 lit water. In order to increase the adaptation time of the fungus, 25 μlit of the BTEX mixture was added to the cultivation media. The cultivation media were inserted in a Kuehner Lab-Therm shaker incubator for 48 hours at 24^{o}C. The numbers of the fungus colonies were calculated before and after each biofilter experiment. The sterilization of the packing media was performed in an autoclave for 15 min.

2-4. Analytical and monitoring methods

The concentrations of each component in the BTEX mixtures were determined using a Varian CP-3800 gas chromatograph equipped with a packed column (mesh 80-100, 2 m × 2.2 mm) and a flame ionization detector with helium as the carrier gas. The temperatures of injector and detector were 150^{o}C and 200^{o}C, respectively. The initial oven temperature of 55^{o}C was then increased to 80^{o}C for 5 min and finally increased to 105^{o}C at a rate of 2^{o}C per minute. The samples of the BTEX gas mixture were injected into the gas chromatograph with a gas tight 1.0 ml-syringe and the BTEX concentrations were measured by comparison with the standards. The standards for each gas were prepared according to the Lodge's method [19].

The temperature of the biofilter was controlled by a water bath circulator (FP50, Julabo) within ±0.1^{o}C. The temperatures of each bed in the biofilter were determined using a Lutron TM903A multichannel thermometer. The relative humidity of the inlet gas was measured by a humidity meter with ±0.2% accuracy. The Cole-Parmer flow meters were used for measuring the gases flow rates. The pressure drop was determined using a Reed 8230 digital manometer in the range of 0-30 psi and ±0.3% accuracy.

The water content of the bed medium was determined from reduction in mass by drying a known amount of the bed material at 105^{o}C for about 1 day [20]. The pH of the medium was measured according to the standard method [20]. The method for calculating the number of the fungus colonies was based on Sarvanan and Rajamohan [14]. This is the standard method used in microbiology to estimate the number of cells or fungal. Colony-forming unit (CFU) count viable fungal. In this method, a sample of the fungus is mixed with a phosphate buffer solution and diluted 6 times with the order of 0.1. It means that 6 solutions of the fungus and buffer are prepared. Each solution is then poured on an agar plate and incubated for 24 h. The plates with the numbers of colonies between 30-300 are selected and counted.

2-5. Biofilter characteristics

The biofilter set up consists of two glass beds in series. Each bed has 60 cm height, and the internal and external diameters are 5 and 10 cm, respectively. Two 5-cm gaps under each bed are considered for the gas sampling. The biofilter column is equipped with a jacket to control the bed temperature through circulating water. Each section is packed with the packing medium to a height of 50 cm. The packing medium is a mixture of

sugarcane bagasse and GAC with a ratio of 65:35 by mass. The particle size of the sugarcane bagasse and GAC are within 2-5 cm and 0.5-2 mm, respectively. About 200 ml of the nutrient feed is added to the biofilter packing medium every 2 days. The nutrient feed composition contains 0.54; KH_2PO_4, 0.54; K_2HPO_4, 0.5; NH_4NO_3, 0.26; NaCl, 0.025 ($g.Lit^{-1}$); $CaCl_2.2H_2O$, respectively [8].

The gas mixture is prepared by injecting air streams from air pumps into separate vessels of benzene, toluene, ethyl benzene, and o-xylene, and mixing them in a mixing chamber. In order to prevent drying up of the bed by the gas flow, the gas mixture is saturated with an air stream, which is passed through a humidification chamber and added to the gas mixture in the mixing chamber. The input air is passed through a filter to prevent contamination of the biofilter by bacterial species existing in the air. The gas mixture is then entered into the biofilter from the bottom. A schematic diagram of the biofilter is shown in Fig. 1. The moisture content of the packing material is maintained at the desired level by periodically adding nutrient feed from the top of the biofilter. The excess amount of water and nutrient feed are accumulated in the bottom of the biofilter as a leachate, which is periodically circulated to the top by means of a peristaltic pump.

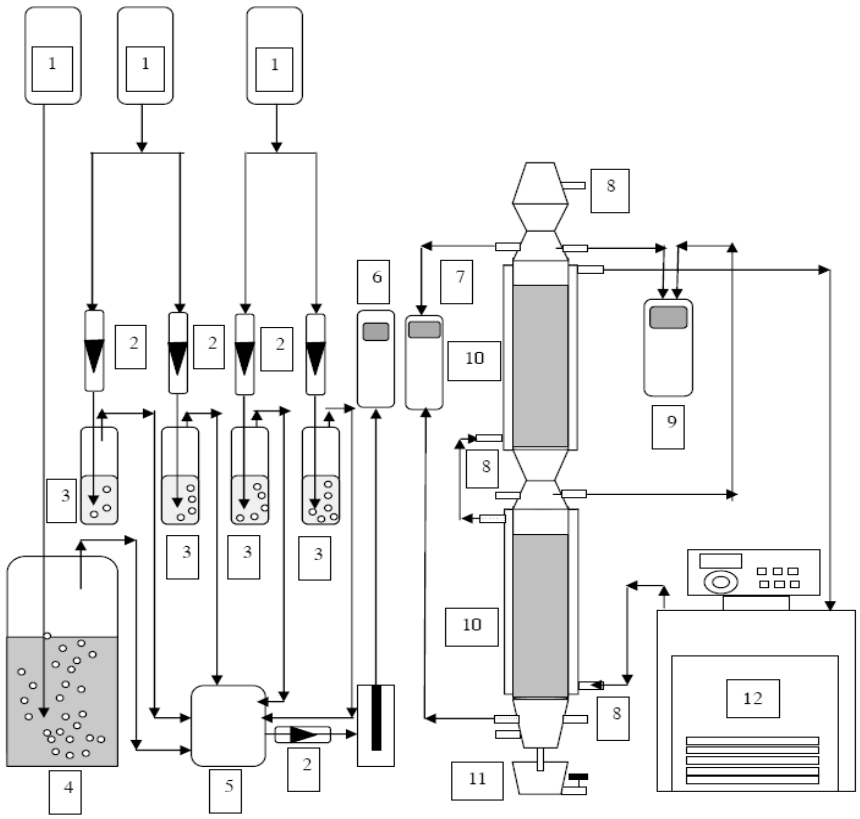

Figure 1. a schematic diagram of the studied biofilter, 1, air compressor; 2, flow meter; 3, BTEX storage vessel; 4, humidification system; 5, mixing chamber; 6, humidity meter; 7, manometer; 8, sampling port; 9, thermometer; 10, biofilter bed; 11, leachate chamber; 12, water bath.

The experiments were performed in different phases. For the strain of *Aspergillus Brasiliensis* PTCC (5298), the biofilter efficiency was studied in 3 phases with different empty bed residence time (EBRT) and inlet load. For the strain of *Aspergillus Niger* PTCC (5223), the experiments were done in 4 phases.

3. Results & discussion

The experiments were carried out with two strains of the fungi. In order to show that the biodegradation of the BTEX is due to the existence of the *Aspergillus* Fungi rather than the physical adsorption on the activated carbon, an experiment was performed without the fungi. The result indicated that the system was saturated with BTEX after a short time (about 4-6 hours) i.e., the inlet and outlet concentrations of BTEX were the same. Also, as the biofilter bed and the air input were sterilized during the experiments, the contamination probability is near to zero.

In the first group of the experiments, the strain of *Aspergillus Brasiliensis* PTCC (5298) was inoculated in the biofilter. The humidity and pH of the packing media was 55% and 7.4, respectively. The effects of BTEX inlet concentration and empty bed residence time (EBRT) on the biofilter efficiency were studied. The experiments were performed in 3 phases where operational parameters of each phase are reported in Table 1.

The variations of removal efficiency in the biofilter for each component through the 3 phases are shown in Fig. 2, in which, the removal efficiency is defined as:

$$RE = (C_i - C_0)/C_i \times 100 \quad (1)$$

C_i and C_0 are the inlet and outlet concentrations of BTEX compounds, respectively.

As the figure shows, the removal efficiency of the biofilter for each component in phase A is about 100%. In this phase, the inlet load is about 17.6 ($g.m^{-3}.h^{-1}$).

In phase B, the inlet concentrations and the inlet load of BTEX are increased and the EBRT is reduced to 2.7 min. The analysis results show that the fungus degrades the BTEX mixture and the removal efficiency reaches to 100%.

In phase C, the EBRT is reduced to 1.8 min and the removal efficiency decreases to 40%. The reduction of the removal efficiency may be attributed to the fungus population. The inlet load for this phase is much higher than the biodegrading capacity of the fungus. For this reason, the average inlet load is reduced to 35.8($g.m^{-3}.h^{-1}$) but the removal efficiency increased up to 70%.

Table 1. Operation parameters in biofilter using *Aspergillus Brasiliensis* fungus.

phase	BTEX Inlet Concentration (ppm)				Average Inlet Load ($g.m^{-3}.h^{-1}$)	EBRT (min)	T (^oC)	Time (day)
	B	T	E	X				
A	65.33	58.91	60.80	64.60	17.60	3.5	24	18
B	156.71	149.82	144.63	142.84	55.04	2.7	24	35
C	65.66	65.53	61.47	65.18	35.82	1.8	24	14

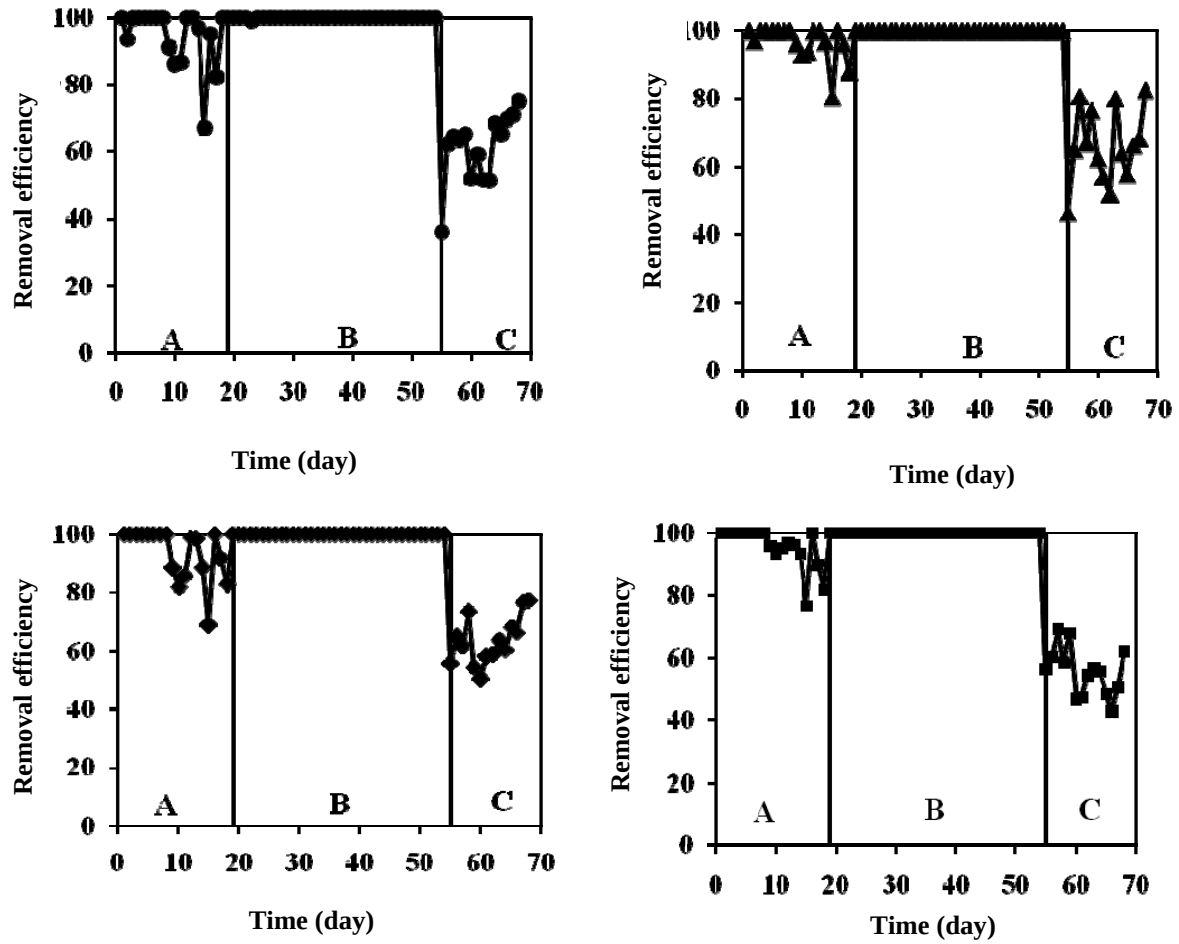

Figure 2. Variations of removal efficiency with time in different phases for *Aspergillus Brasiliensis* fungus, (●) benzene, (▲)toluene, (♦) ethyl benzene, (■) o-xylene, The operational conditions are according to Table 1.

The biofilter performance can be evaluated from removal efficiency in terms of elimination capacity (*EC*) versus inlet loading rate (*IL*) by the following equations:

$$EC = Q(C_i - C_0)/V \quad (2)$$

$$IL = QC_i/V \quad (3)$$

where *Q* and *V* are the gas flow rate and bed volume, respectively. The elimination capacity reflects the capacity of the biofilter to remove the pollutants [2]. The BTEX elimination capacities for various loading rates are plotted in Fig. 3, in which experimental data are represented by the points and 100% removal is indicated by the dotted line. As the figure shows, the elimination capacity of the BTEX increases with the increase of inlet load. The maximum elimination capacity at inlet load is 71.5 $g.m^{-3}h^{-1}$ in phase B with EBRT of 2.7 min. When the EBRT is decreased to 1.8 min, the elimination capacity is reduced to 30%.

The pressure drop is an important factor in operation of a biofilter. It determines the power of blowers to pass the gas through the

the biofilter. In this research, the pressure drop was monitored during the operational period. Fig. 4 shows the variation of pressure drop in the biofilter. The packing media of the biofilter is removed in the case of high pressure drop (more than 70 mmHg), which is mainly due to decrease in the bed porosity [21]. The bed is then repacked. As the figure shows, the pressure drop was below 40 mmHg during the whole operation time.

In order to assess the degradation of the BTEX mixture by *Aspergillus Brasiliensis*, the number of *Aspergillus Brasiliensis* colonies are determined in the first and the last operation days. The results show that the number of the fungal colonies is increased from 3×10^8 to 1.04×10^{10} cfu/gr.

In the second group of experiments, the strain of *Aspergillus Niger* PTCC (5223), which is isolated from oil sludge, was inoculated in the biofilter. The experiments were performed in 4 phases where the operational parameters are reported in Table 2.

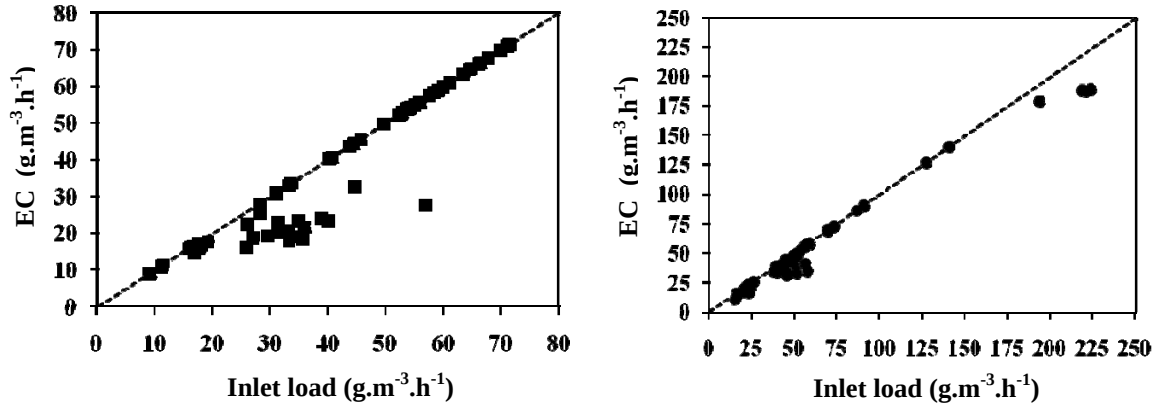

Figure 3. Influence of BTEX loading rate on elimination capacity for (■) *Aspergillus brasiliensis* and (●) *Aspergillus Niger* fungi.

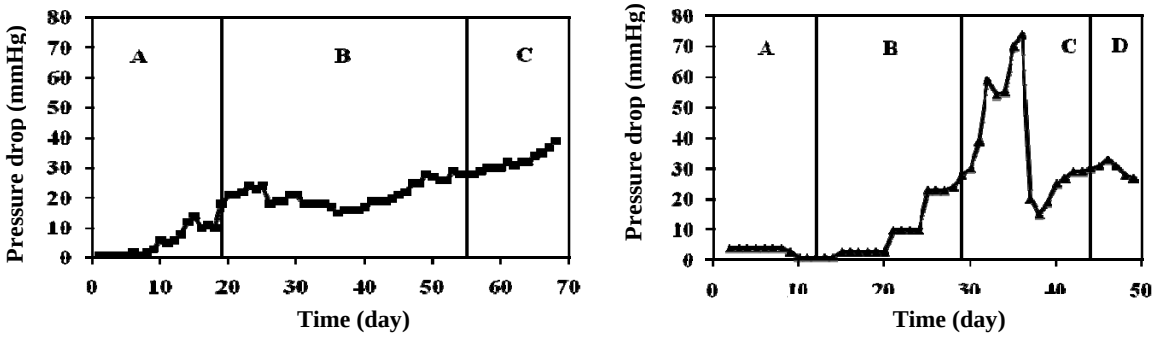

Figure 4. Variation of pressure drop in biofilter with time in different phases for (■) *Aspergillus brasiliensis* and (▲) *Aspergillus Niger* fungi.

Table 2. Operational parameters in biofilter using *Aspergillus Niger* fungus.

Phase	BTEX Inlet Concentration (ppm)				Average Inlet Load ($g.m^{-3}.h^{-1}$)	EBRT (min)	*T* (°C)	Time (day)
	B	T	E	X				
A	71.91	49.76	55.62	53.24	21.16	2.7	24	10
B	95.94	85.99	79.56	81.67	47.43	1.8	24	17
C	82.69	59.12	67.17	66.39	50.79	1.3	24	7
C	95.23	76.25	74.54	80.50	60.14	1.3	30	8
D	169.05	157.40	178.28	162.52	186.95	0.9	30	6

The variations of removal efficiency in the biofilter for each component through the 4 phases are shown in Fig. 5. In phase A of the experiments, the average inlet load is 21.16 $g.m^{-3}.h^{-1}$, and the removal efficiency after 3 days from start up increases to more than 90%. The reduction in removal efficiency in the 8^{th} day is due to the lack of the nutrient feed in the biofilter. Adding the nutrient feed into the biofilter increases the removal efficiency. This behavior is observed for toluene, ethyl benzene and o-xylene. In phase B, the BTEX inlet load is increased and the EBRT is reduced to 1.8 min. The removal efficiency increases to 90% after 3 days when the fungus has adapted well in the biofilter. Reducing EBRT to 1.3 leads to 50% reduction in the removal efficiency. The main reason for reduction in the removal efficiency in this phase is attributed to increase in the pressure drop (as the pressure drop was increased to 75 mmHg the packing media had to be replaced), and also to increase in the inlet load. In order to increase the activity of the fungi in the biofilter, the temperature was increased to $30^{o}C$ in the middle of phase C. It was then observed that the removal efficiency had reached 100%. In the final phase, when the EBRT was reduced to 0.9 min, the removal efficiency was decreased to 70%. The average inlet load is 186.9 $g.m^{-3}.h^{-1}$, which is larger than that for the biofilter with *Aspergillus Brasiliensis.* This behavior is identical for benzene, toluene, ethyl benzene, and o-xylene.

The BTEX elimination capacities for various loading rates are plotted in Fig. 3. As the figure shows, the maximum elimination capacity of the biofilter is 188.95 $g.m^{-3}h^{-1}$ at the inlet load of 223.08 $g.m^{-3}h^{-1}$ in phase D with EBRT of 0.9 min. The removal efficiency approaches to 100% at the inlet load of 140.3 $g.m^{-3}h^{-1}$.

The variation of pressure drop in the biofilter using *Aspergillus Niger* is shown in Fig. 4. The pressure drop variation is not significant in phases A and B but it increases after the 20^{th} day of the operation so that in the 37^{th} day of the operation, the packing media had to be replaced.

The numbers of the *Aspergillus Niger* colonies are determined in the first and the last operation days. The results show that the number of fungal colonies is increased from 7×10^{8} to 1.3×10^{10} cfu/gr, that shows the fungus could grow and degrade the BTEX mixture in the biofilter.

Comparison of the results of this research

and those by Mathur et al. [2] and Garcia-Pena et al. [7], is reported in Table 3. As the table shows, the elimination capacity for the strain of *Aspergillus Niger* is higher than that of the others.

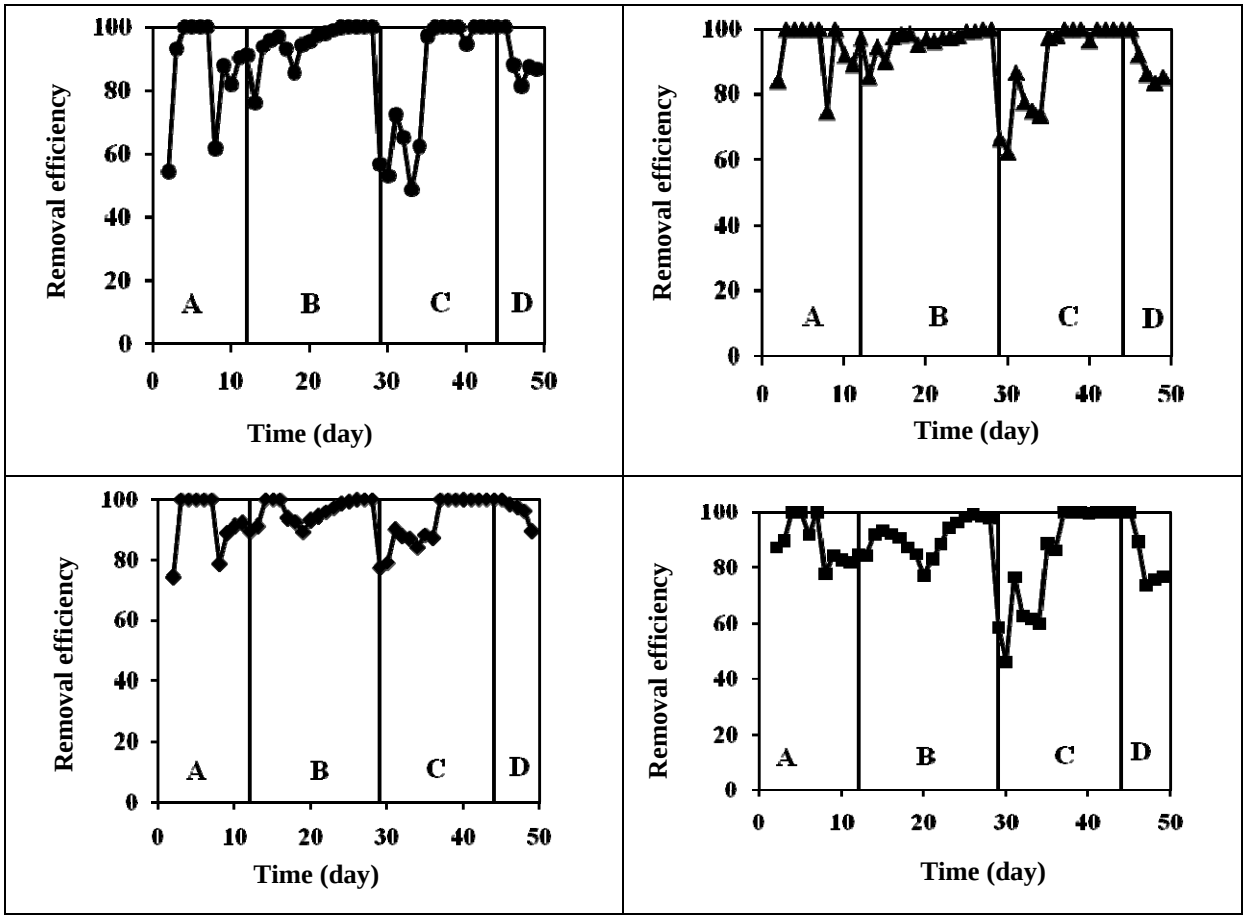

Figure 5. Variations of removal efficiency with time in different phases for *Aspergillus Niger*, (●) benzene, (▲) toluene, (♦) ethyl benzene, (■) o-xylene. The operational conditions are according to Table 2.

Table 3. Comparison of the elimination capacities of biofilter in the present research with those in literature for biodegradation of BTEX.

Name of Component	Microorganism	Biofilter Bed	EC ($g.m^{-3}.h^{-1}$)	Reference
BTEX	Microorganism in compost	Bagass and compost	84	[2]
BTEX	Fungus *Paecilomyces variotii*	Activated carbon and vermiculite	110	[7]
BTEX	*Aspergillus Niger*	Bagass and activated carbon	140	This work
BTEX	*Aspergillus Brasiliensis*	Bagass and Activated carbon	71	This work

4. Conclusions

Biofilteration of BTEX mixture was performed using a two-bed laboratory scale biofilter. The packing media was a mixture of sugarcane bagasse and GAC. Two strains of *Aspergillus Brasiliensis* and *Aspergillus Niger* fungi were inoculated in the biofilter in two separate steps. The effects of EBRT, BTEX inlet concentration, and temperature on the biofilter efficiency were studied. The biofilter efficiency is reduced by reducing EBRT. Increase of the temperature stimulates the activity of the fungus and increases the biofilter efficiency. According to the experimental data, for the biofilter with *Aspergillus Brasiliensis,* the removal efficiency reaches to 100% for the inlet BTEX load of up to 71.5 $g.m^{-3}h^{-1}$ at EBRT of 2.7 min. However, in biofilter with *Aspergillus Niger*, the removal efficiency reaches to 100% for the inlet load of 140.3 $g.m^{-3}h^{-1}$ with the EBRT of 1.3 min. The maximum elimination capacity is 188.95 $g.m^{-3}h^{-1}$ for the inlet load of 223.08 $g.m^{-3}h^{-1}$ with EBRT of 0.9 min. The results of this research imply that the biofilter with *Aspergillus Niger* fungus is more efficient than that with *Aspergillus Brasiliensis*. Moreover, as the strain of *Aspergillus Niger* is isolated from oil sludge, it is completely compatible for biodegradation of hydrocarbon and aromatic mixtures.

References

[1] USEPA, Priority Pollutants, Code of Federal Regulations, Title 40, (Chapter 1, Part 423, Appendix A), (1996).

[2] Mathur, A. K., Majumder, C. B. and Chatterjee, S., "Combined removal of BTEX in air stream by using mixture of sugar cane bagasse, compost and GAC as biofilter media", J. Hazard. Mater. 148, 64, (2007).

[3] Sene, L., Converti, A., Felipe, M. G. A. and Zilli, M., "Sugarcane bagasse as alternative packing material for biofiltration of benzene polluted gaseous streams: A preliminary study", Bioresour. Technol., 83, 153, (2002).

[4] Torkian, A., Dehghanzadeh, R. and Hakimjavadi, M., "Biodegradation of aromatic hydrocarbons in a compost biofilter", J. Chem. Technol. Biotechnol., 78, 795, (2003).

[5] Abumaizar, R. J., Kocher, W. and Smith, E. H., "Biofiltration of BTEX contaminated air streams using compost-activated carbon filter media", J. Hazard. Mater., 60, 111, (1998).

[6] Oritz, I., Revah, S. and Auria, R., "Effect of packing material on the biofilteration of benzene, toluene and xylene vapors", Environ. Technol., 24, 265, (2003).

[7] Garcia-Pena, I., Ortiz, I., Hernandez, S. and Revah, S., "Biofiltration of BTEX by the fungus Paecilomyces variotii", Int. Biodetr. & Biodegr., 62, 442, (2008).

[8] Auria, R., Frere, G., Morales, M., Acun, M. E. and Revah, S., "Influence of mixing and water addition on the removal rate of toluene vapors in a Biofilter", Biotech. & Bioeng., 68, 448, (2000).

[9] Ibeau, L., Kiared, K. and Brzeznski, R., "Treatment of air polluted with xylense using a biofilter reactor", Water, Air and Soil Pollution, 118, 377, (2000).

[10] Delhoménie, M. C., Bibeau, L., Gendron, J., Brezezinski, R. and Heitz, M., "A study of clogging in a biofilter treating toluene vapors", Chem. Eng. J., 94, 211, (2003).

[11] Delhomenie, M. C., Bibeau, L., Bredin, N., Roy, S., Brousseau, S., Kugelmass, J. L., Brezezinski, R. and Heitz, M., "Biofiltration of air contaminated with toluene on a compost-based bed", Adv. Environ. Res., 6, 239, (2002).

[12] Namkoong, W., Park, J. S. and VanderGheynst, J. S., "Biofiltration of gasoline vapor by compost media", Environ. Poll., 121, 181, (2003).

[13] Jeong, E., Hirai, M. and Shoda, M., "Removal of o-xylene using biofilter inoculated with Rhodococcus sp. BTO62", J. Hazard. Mater., 152, 140, (2008).

[14] Saravanan, V. and Rajamohan, N., "Treatment of xylene polluted air using press mud-based biofilter", J. Hazard. Mater., 162, 981, (2009).

[15] Maestre, J. P., Gamisans, X., Gabriel, D. and Lafuente, J., "Fungal biofilters for toluene biofiltration: Evaluation of the performance with four packing materials under different operating conditions", J. Chemosphere, 67, 684, (2007).

[16] Oh, Y. S., Choi, S. C. and Kim, Y. K., "Degradation of gaseous BTX by biofilteration with Phanerochaete chrysosporium", J. Microbiol., 36, 34, (1998).

[17] Qi, B., Moe, W. M. and Kinney, K. A., "Biodegradation of volatile organic compounds by five fungal species", Appl. Microbiol. Biotechnol., 58, 684, (2002).

[18] Mathur, A. K. and Majumder, C. B., "Biofilteration of benzene emission: Biofilter response to variation in the pollutants inlet concentration and gas flow rate", J. Scientific & Industrial Res., 67, 243, (2008).

[19] Lodge, J. P., Method of air sampling and analysis, Lewis Publishing, New York, (1989).

[20] APHA, Standard method for the examination of water and wastewater, 18th ed., American Public Health Association, Washington, D. C., (1998).

[21] Singh, R., Agnihotri, S. S. and Upadhyay, S. N., "Removal of toluene vapor using agro-waste as biofilter media", Bioresour. Technol., 97, 2296, (2006).