

Biodegradation of BTEX in a fungal biofilter: Influence of operational parameters, effect of shock-loads and substrate stratification

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Abstract

The effect of relative humidity (RH: 30% to >95%) of a gas-phase mixture composed of benzene, toluene, ethylbenzene and *para*-, *meta*- and *ortho*-xylenes (BTEX), inlet concentrations ($0.2\text{--}12.6\text{ g m}^{-3}$), and empty bed residence times (EBRTs) (48–144 s) was tested in a fungi-dominant biofilter. A maximum elimination capacity (EC_{\max}) of $244.2\text{ gBTEX m}^{-3}\text{ h}^{-1}$ was achieved at a total inlet loading rate (ILR_T) of $371.2\text{ gBTEX m}^{-3}\text{ h}^{-1}$ (RH: 65%). The transient-state response was tested by increasing the ILR_T , in two steps, from ~ 50 to $850\text{ g m}^{-3}\text{ h}^{-1}$ and from ~ 50 to $320\text{ g m}^{-3}\text{ h}^{-1}$, at a constant EBRT of 41.7 s. Increasing the ILR_T reduced the total BTEX removal efficiency (RE_T) from >97% to 35%, and from >90% to 60% during medium and high shock-load, respectively. When subjected to short (4 d) and long-term (7 d) shut-down periods, the biofilter was able to recover high EC_{\max} of, respectively, 200 and $72\text{ gBTEX m}^{-3}\text{ h}^{-1}$ after resuming operation.

Keywords

Fungi; Relative humidity; pH; Gas to liquid (G/L) ratio; Transient-operations.

1. Introduction

Benzene, toluene, ethylbenzene, *para*-, *meta*-, and *ortho*-xylenes, collectively called BTEX, are important industrial chemicals and are well-known volatile organic compounds (VOCs) of environmental and health concern. Due to improper handling and disposal practices, and the negligence or failure of some process industries to adopt suitable VOC elimination techniques, these compounds can frequently be released into the ambient atmosphere.

Biofilters allow the treatment of low concentrations of contaminants, at high gas-flow rates. In a biofilter, the attached microorganisms convert the gas-phase pollutant(s) into end-products such as carbon dioxide, water, biomass and salts. Recent studies have even successfully evaluated the possibility of converting some volatile pollutants to useful products such as biofuels (Abubackar et al., 2011). The extent to which biological waste-gas purification can occur in a biofilter is affected by several factors: physical and chemical properties of the pollutant(s), degree of biodegradability of the pollutant(s), pollutant loading rate, microbial physiology and ecology, and other suitable environmental conditions such as temperature, relative humidity (RH), and pH (Kennes and Veiga, 2001 and Jorio et al., 2009). Literature reports on the removal of BTEX compounds in fungi-inoculated or fungi-dominant biofilters are sparse, though a few

authors have investigated the removal of BTX or BTEX compounds, as mixtures, in liquid systems using fungi (Oh et al., 1994 and Prenafeta-Boldu et al., 2002). One of the main advantages of favouring the growth of fungi rather than bacteria for the removal of hydrophobic pollutants in biofilters is their ability to degrade these compounds under a broad range of process conditions (Kennes and Veiga, 2004).

Most of the reported studies on the biofiltration of BTEX (B, T, E, *p*-X, *m*-X and *o*-X compounds) were carried out at steady-state. The transient-state behavior of waste-gas treatment systems to sudden variations in operating conditions, during shock-loads or shut-down and re-start operation, has started to receive attention and there are several recent studies that have reported transient behavior with a single pollutant. In field applications, the occurrence of transient conditions, either in the form of an unexpected pollutant shock-load, or complete reactor shut-down, can be either regular or frequent (Seigneur et al., 2004 and Moe and Li, 2005). Such transient operations would lead to instability of the biomass, perturbation between steady-states and would eventually affect the dynamics of pollutant removal and reaction kinetics in the waste-gas treatment system. Substrate starvation is also a type of shock-load where no external substrate is fed to the microbial population, thus depriving them of the essential carbon and energy source. Pollutant starvation can be expected in process industries under the following conditions: overnight or weekend closures, plant maintenance, equipment malfunctioning, and regular change in process operation (Nabatilan et al., 2010). Re-acclimation times after starvation for a biofilter can vary widely depending on the starvation period, pollutant characteristics, microbial activity, packing material, and reactor configuration, among others. A prior knowledge on the re-acclimation times after shut-down of biological waste-gas treatment systems can be important from both design and performance view-point (Qi and Moe, 2006).

In a previous work, Mohammad et al. (2007) investigated gas-phase BTEX removal in a perlite biofilter that reached a maximum elimination capacity (EC_{max}) of $188 \text{ gBTEX m}^{-3} \text{ h}^{-1}$ with 62% removal efficiency (RE), over 212 d of continuous operation. The dominant microorganism in that biofilter was identified as being a fungal *Exophiala* species, a dark gray pigmented dimorphic hyphomycete, which was isolated later from the reactor. In an attempt to strengthen our research understanding with fungal bioreactors, this study was undertaken with the following objectives: (i) to evaluate the performance of the biofilter at different EBRTs (144 and 48 s) at RH values >95%, (ii) to study the biofilter's performance at different RH values of 65%, 45% and 30%, at a constant EBRT, (iii) to identify the effect of maintaining different pH conditions in the filter bed, *i.e.*, 5.9, 7.0 and 4.9, by continuously trickling a constant pH nutrient medium, (iv) to examine the response of the biofilter to high/medium shock-loads of BTEX by increasing their individual concentrations from low to high levels, (v) to examine the recovering capacity of the biofilter after short and long-term shut-down periods, and (vi) to monitor the BTEX stratification profiles along the biofilter height.

2. Methods

2.1. Microorganisms and media composition

The mesophilic biofilter was originally inoculated with a mixed culture taken from refinery sludge (Mohammad et al., 2007). During continuous biofilter operation, at RH >95%, and at an EBRT of 96 s, it was observed that fungi, mainly an *Exophiala* sp.,

became dominant, although some bacteria were also present. A well-defined mineral salt medium having the following chemical composition (per liter in distilled water) was used in all the experiments: 0.5 g K_2HPO_4 , 0.1 g $MgSO_4 \cdot 7H_2O$, 4.5 g KH_2PO_4 , 2 g NH_4Cl , and 2 mL of vitamins and trace minerals solution (Kennes et al., 1996). The pH of this medium was 5.9. The medium was autoclaved at 120 °C for 20 min before adding the filter-sterilized solutions of vitamins and trace minerals. The *Exophiala* sp., isolated from the reactor, was later grown with gas-phase toluene as the sole carbon source and maintained in a sterile atmosphere on 15 g agar L^{-1} , under ambient conditions in a desiccator.

2.2. Biofilter set-up and experiments

The specifications of the perlite biofilter used in this study were the same as described in a previously published work of our research group (Mohammad et al., 2007). Briefly stating, the biofilter was made of glass having an internal diameter of 10 and 70 cm in height. The operating volume of the biofilter was 4 L, and sieved perlite (4–6 mm diameter) was used as the packing material. A perforated Teflon mesh was provided at the bottom to act as a support for the packing material, while another perforated mesh at the top acted as a distributor for gas-phase pollutant flow and mineral medium distribution. Gas sampling ports (0 – inlet, 1 – 20 cm, 2 – 40 cm, and 3 – outlet) sealed with rubber septa were available at equal intervals (20 cm) along the biofilter height.

To generate the BTEX vapors, compressed air was split into two portions, where the major portion of dry air was humidified in a humidification unit placed in a water bath, adjusted to 37 °C or at lower temperatures (20–30 °C), to maintain the desired air's relative humidity. The minor air stream was bubbled through liquid BTEX in a flask to generate the contaminated air stream. The two streams were then mixed in a mixing unit and fed from the top of the reactor in a down-flow mode. In order to maintain adequate nutrients and moisture contents within the filter bed, liquid medium was sprinkled periodically, once every 3 d, from the top of the biofilter, as would usually be done in full-scale systems.

For experiments involving different pH levels in the filter bed (5.9, 7.0 and 4.9), the nutrient medium, stored in a continuously stirring nutrient hold tank (Vol – 1.5 L), was continuously fed to the biofilter using a peristaltic pump (Watson and Marlow, USA) at a rate of 72 $mL\ min^{-1}$. Both the nutrient medium and the gas-phase pollutants were thus fed co-currently to the reactor in a down-flow mode. The gas to liquid (G/L) ratio was maintained between 43 and 125.6 in these experiments. The pH of the recirculated nutrient medium was maintained constant, at 5.9, 7.0, or 4.9, depending on the experiment, by adding a 2 N NaOH solution to neutralize the acidic metabolites formed during the biodegradation process. The pH was measured by means of a pH electrode (EASYFERM 120, Hamilton) attached to the nutrient collection tank and an on-line pH controller coupled to an electro valve (DO 9765T, Dual 3½ Digit pH redox indicator and regulator, Italy).

The performance of the biofilter was evaluated using the following parameters, defined elsewhere (Kennes et al., 2009a): total BTEX removal efficiency (RE_T , %), total inlet loading rate (ILR_T , $g\ m^{-3}\ h^{-1}$) and the total elimination capacity of the biofilter (EC_T , $g\ m^{-3}\ h^{-1}$). Steady-state biofiltration experiments were carried out by varying the flow rates of the BTEX vapors and humidified air independently to get different initial

concentrations and EBRTs (30–144 s) in the biofilter. The different biofiltration experiments performed in this study are summarized in Table 1, showing the different EBRTs and ILRs tested.

Table 1.

Experimental scheme of biofilter operation for gas-phase BTEX removal.

| Nature of experiment | of EBRT, s | Range of ILR, $\text{g m}^{-3} \text{h}^{-1}$ | of EC, $\text{g m}^{-3} \text{h}^{-1}$ | |
|--|------------|---|---|--|
| <i>1. Effect of relative humidity (RH)</i> | | | | |
| | 144 | 47–315 | 46–249 | |
| RH: >95% | 96 | 8.1–354 | 2.6–214 | |
| | 48 | 37–315 | 22.5–176 | |
| RH: 65% | 96 | 19–371.2 | 14.5–244.2 | |
| RH: 45% | 96 | 25.1–272.6 | 16.8–221.0 | |
| RH: 30% | 96 | 80.4–267 | 54.2–168.8 | |
| <i>2. Effect of medium pH</i> | | | | |
| pH | G/L ratio | EBRT, s | Range of ILR, $\text{g m}^{-3} \text{h}^{-1}$ | Range of EC, $\text{g m}^{-3} \text{h}^{-1}$ |
| 5.9 | 43 | 77.8 | 34.9–460.5 | 16.1–322.2 |
| | 80.2 | 41.7 | 48.8–1051.3 | 37.4–576.4 |
| 7.0 | 83.7 | 45 | 63.9–791.2 | 57.1–638.4 |
| | 125.6 | 30 | 29.1–730.5 | 29–687.8 |
| 4.9 | 83.7 | 45 | 147.5–364.1 | 146.1–357.2 |
| | 125.6 | 30 | 181.2–232.8 | 168.1–208.7 |

During shock-load, shut-down and re-start experiments, the EBRT was maintained constant at 41.7 s, while the air's RH was maintained at >95% during transient-state experiments. The concentrations of individual BTEX were increased from low to high levels, corresponding to ILR_T varying between 48.8 and 853.6 $\text{gBTEX m}^{-3} \text{h}^{-1}$ during shock-load experiments, and between 0 and 257.7 $\text{gBTEX m}^{-3} \text{h}^{-1}$ during shut-down (short-term – 4 d and long-term – 7 d) experiments, according to the details provided in Table 2. Substrate stratification profiles of individual B, T, E, *p*-X, *m*-X and *o*-X compounds were measured along the filter bed height at pH 7.0.

Table 2.

Experimental scheme of biofilter operation for gas-phase BTEX removal under transient-state conditions.

I – During successive shock load tests

| Individual VOCs | B | T | E | <i>p</i> -X | <i>m</i> -X | <i>o</i> -X |
|---|---|----------|-----------|-------------|-------------|-------------|
| Concentration range, g m^{-3} | 0.07–3.92 | 0.14–1.3 | 0.06–1.25 | 0.09–2.3 | 0.08–1.43 | 0.03–1.06 |
| Range of REs, % | 13–95 | 36–100 | 23–92 | 31–100 | 46–100 | 16–100 |
| ILR _{max} , $\text{g m}^{-3} \text{ h}^{-1}$ | 338.7 | 112.5 | 108.3 | 205.2 | 123.4 | 91.7 |
| EC _{max} , $\text{g m}^{-3} \text{ h}^{-1}$ | 73.7 | 71.6 | 63.3 | 105.6 | 61.6 | 20.9 |
| Other parameters | EBRT = 41.7 s, RH: >95%, EC _T = 322.4 $\text{g m}^{-3} \text{ h}^{-1}$ | | | | | |

II – During shut-down and re-start operations

| Individual VOCs | B | T | E | <i>p</i> -X | <i>m</i> -X | <i>o</i> -X |
|---|---|-------|-------|-------------|-------------|-------------|
| Concentration range, g m^{-3} | 0–0.7 | 0–0.5 | 0–0.6 | 0–0.7 | 0–0.4 | 0–0.2 |
| Maximum REs, % | 85.9 | 95.2 | 96.7 | 88.4 | 89.8 | 90.4 |
| ILR _{max} , $\text{g m}^{-3} \text{ h}^{-1}$ | 63.2 | 45.5 | 54.1 | 60.2 | 36.6 | 22.1 |
| EC _{max} , $\text{g m}^{-3} \text{ h}^{-1}$ | 32.5 | 39.8 | 47.9 | 53.2 | 28.3 | 14.5 |
| Other parameters | EBRT = 41.7 s, RH: >95%, EC _T = 203.3 $\text{g m}^{-3} \text{ h}^{-1}$ | | | | | |

Note: RE – removal efficiency; ILR – inlet loading rate; EC – elimination capacity

2.3. Analytical methods

Gas samples collected from the inlet and outlet of the biofilter were analyzed for residual gas phase BTE_{*o,m,p*X} concentrations on a HP 5890 gas chromatograph equipped with a FID, fitted with a 50 m TRACER column and at 250 °C. The flow rates were 30 mL min⁻¹ for H₂ and 300 mL min⁻¹ for air. Helium was used as carrier gas at a flow rate of 2 mL min⁻¹. The initial temperature was 60 °C, followed by heating at a rate of 28 °C min⁻¹–80 °C, then heating at 18 °C min⁻¹ to a final temperature of 96 °C. Two milliliter of gas samples were collected, using gastight syringes (Hamilton, Reno, USA), from sampling ports located at the inlet and outlet of the biofilter. Typical gas elution times in the GC were 7.2, 10.5, 14.6, 15.2, 15.6 and 18.5 min, respectively, for individual BTE_{*o,m,p*X} compounds. CO₂ was analyzed with a HP 5890 gas chromatograph equipped with a TCD. The injection and oven temperatures were 90 and 25 °C, respectively, with the TCD set at 100 °C. A glass U-tube water manometer having an operation range of 0–40 cm was used to periodically monitor the pressure drop across the filter bed height. Moisture content (%) of the filter bed was periodically measured according to the procedure outlined by Mohammad et al. (2007). The relative humidity of the incoming pollutant-air mixture was monitored using a hand held thermo-hygrometer, Model C210 fitted with a flexible sampling probe (G. Lufft Mess- und Regeltechnik, GmbH, Germany).

3. Results and discussion

3.1. Performance at different EBRTs

Steady-state experiments at three different EBRTs, *i.e.*, 144, 96 and 48 s, were performed by maintaining the RH at >95%, and by increasing the concentration of individual BTEX compounds gradually from low to high values (Table 1). The total BTEX concentration (C_{inBTEX}) was thus increased from 1.9 to 12.6, 0.2 to 9.4, and 0.5 to 4.2 g m⁻³ at EBRTs of 144, 96, and 48 s, respectively. During this study, the total BTEX inlet loading rate (ILR_T) varied from 8.1 to 354 g m⁻³ h⁻¹. At an EBRT of 144 s, for BTEX concentrations below 9 g m⁻³, more than 85% total BTEX removal (RE_T) was noticed ($ILR_T < 230$ g m⁻³ h⁻¹). However, for loads exceeding 300 gBTEX m⁻³ h⁻¹, the performance of the biofilter gradually levelled off and a maximum total elimination capacity (EC_T) of 200 g m⁻³ h⁻¹ (occasionally 250 g m⁻³ h⁻¹) was reached (Fig. 1a). During biofilter operation at an EBRT of 48 s, the ILR_T were varied from 37 to 315 gBTEX m⁻³ h⁻¹. It was observed that, for low BTEX concentrations the RE was close to 100%. For inlet concentrations close to 2 g m⁻³ (ILR_T of 150 gBTEX m⁻³ h⁻¹), the RE_T were only about 68%. A further increase in ILR_T during this phase, decreased the BTEX removal significantly, with about 50% removal at an ILR_T close to 300 gBTEX m⁻³ h⁻¹ (Fig. 1a). This decrease in RE_T could be attributed to insufficient contact time between the gas-phase pollutant and the biofilm. Substrate toxicity, competitive inhibition, and the formation of toxic intermediates by non-specific enzymes may also be responsible for the poor RE_T at high concentrations of BTEX (Bielefeldt and Stensel, 1999). More details concerning the biofilter operation at an EBRT of 96 s can be found in Mohammad et al. (2007). The results are summarized in Fig. 1a, showing that the biofilter performance at 96 s was something intermediate between the performances at 48 and 144 s. Fig. 1a shows a near linear relation between ILR_T and EC_T , at different EBRTs up to an inlet load of at least 140 gBTEX m⁻³ h⁻¹. However, for higher ILR_T tested at the three EBRTs, the EC_T increased at a slower rate, becoming nearly constant at inlet loads beyond 200 gBTEX m⁻³ h⁻¹. This clearly shows the existence of two different operating regimes, as previously described in the literature (Jorio et al., 2009 and Kennes and Veiga, 2001), *i.e.* the diffusion limitation regime (DLR) and the reaction limitation regime (RLR): (i) under DLR, for $ILR_T < 140$ gBTEX m⁻³ h⁻¹, the EC_T increased linearly with an increase in ILR_T because the gas-biofilm interface is relatively small and complete mineralization of BTEX occurred in the fungi-dominant biofilm, and (ii) under RLR, when the ILR was >140 gBTEX m⁻³ h⁻¹, the EC_T remained almost constant and decreased slightly depending on the EBRT. In RLR, the biofilm is fully active and the RE_T of BTEX is controlled only by the rate of biodegradation. The critical ILR_T to achieve >90% RE_T ($ILR_{T,critical}$), at EBRT of 144, 96, and 48 s, were found to be around 225, 191, and 148 gBTEX m⁻³ h⁻¹, respectively. The results corroborate some of the literature findings, as the critical load in biological waste-gas treatment systems have shown to decrease with an increase in the gas-flow rate, *i.e.*, decreasing EBRTs (Kennes and Veiga, 2001). The maximum EC_T was 249 g m⁻³ h⁻¹, achieved at an ILR_T of 285 gBTEX m⁻³ h⁻¹ with 85% RE_T , when experiments were performed at an EBRT of 144 s.

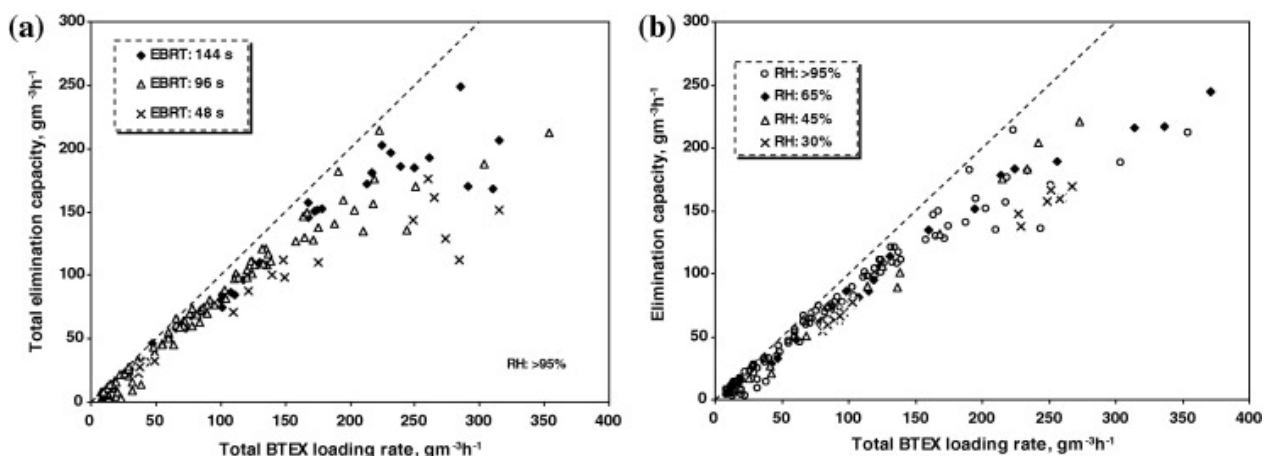


Fig. 1.

Effect of total ILR_T on the EC_T of the biofilter at different operating conditions: (a) EBRTs, and (b) RH.

3.2. Performance at different RH

In order to understand the influence of the relative humidity (RH, %) of the incoming BTEX-polluted air on the pollutant removal characteristics in the biofilter, experiments were performed at three different RH values of 65, 45, and 30%, at a constant EBRT of 96 s. Maintaining the EBRT at 96 s allowed a comparison of the biofilter performance with the results obtained in another previous study at a RH of >95%. The concentrations of individual BTEX were varied as follows: B = 0.01–1.2 g m⁻³, T = 0.2–4 g m⁻³, E = 0.06–2.6 g m⁻³, *p*-X = 0.05–1.9 g m⁻³, *m*-X = 0.04–3 g m⁻³, and *o*-X = 0.03–2.1 g m⁻³. The ILR_T was varied from 19 to 371.2 gBTEX m⁻³ h⁻¹ during the 90 d experimental study. Fig. 1b compares the effect of ILR_T on the EC_T of the biofilter at different RH, including the ones previously obtained at >95% RH. It is evident from Fig. 1b that the total BTEX elimination capacity (EC_T) increased linearly with an increase in the ILR_T , up to ~200 gBTEX m⁻³ h⁻¹, and after that, an increase in the ILR_T slowed down the rate of increase in EC_T values. The EC_T stabilized between 135 and 244 g m⁻³ h⁻¹ for $ILR_T > 210$ gBTEX m⁻³ h⁻¹. Thus, for $ILR_T < 210$ gBTEX m⁻³ h⁻¹ the RE_T was maintained consistently at >80%, irrespective of the RH conditions. However, the biofilter did not exhibit any marked difference in the critical ILR_T values, contrary to what was observed earlier for changes in EBRTs, despite the variation in RH from very low (30%) to normal operational values of a conventional biofilter (>95%). This can be explained by the fact that some mineral medium was occasionally fed, twice a week, to the biofilter, as would also be done for full-scale systems. This allows maintaining the water content of the filter bed sufficiently high, avoiding any marked effect of the waste-gas relative humidity.

Concerning the removal of individual BTE_{*p,m,o*}-X compounds, in the concentration range tested, T, E, and *m,o*-X showed >70% REs, compared to B and *p*-X whose REs were <55% at all the tested RH conditions. The maximum EC_T of the biofilter, at a RH of 65%, was 244.2 g m⁻³ h⁻¹, which is comparable to the maximum EC_T value achieved in this study at an EBRT of 144 s with >95% RH (Table 1). It is often recommended in the literature to pre-humidify the waste-gas to be treated in a biofilter, up to values exceeding 95% RH. However, the present results show that this is not necessarily a prerequisite if water is sprinkled twice a week on top of the filter bed. The critical load

would remain unchanged, although the EC will level off somewhat faster at the lowest RH reaching a lower EC_{max} than at higher RH. The reactors performance should not drop as long as the water content of the biofilter remains above 40%. In case of fungal biofilters, this value could even be lower.

3.3. Performance at different pH

The effect of filter bed pH was studied by continuously recirculating the nutrient medium at the desired pH (5.9, 7.0, and 4.9) over the filter bed at a trickling rate of 72 mL min^{-1} . At pH 5.9, two G/L ratios were studied, *i.e.*, 43 and 80.2, while the EBRTs were maintained at either 77.8 or 41.7 s, respectively. Experiments at pH 7.0 and 4.9 were carried out for 33 and 24 d, at G/L ratios of 83.7 and 125.6, respectively (Table 1). Although the biofilter operation now resembles that of a conventional biotrickling filter, by doing so the desired pH levels could continuously be maintained constant within the filter bed. Failure to do so would eventually lead to possible fluctuations of the filter bed's pH affecting the main goal of this experiment. Fig. 2a–f shows the 6 phases of biofilter operation at different pH conditions and the respective inlet concentrations and RE profiles of individual BTE p,m,o -X compounds, while Fig. 2g shows the total BTEX concentrations and the corresponding RE $_T$ profiles. The concentrations of BTE p,m,o -X during the first 20 d operation were as follow: B = 0.2–3.7 g m^{-3} , T = 0.08–1.1 g m^{-3} , E = 0.06–3.15 g m^{-3} , p -X = 0.07–1.9 g m^{-3} , m -X = 0.08–2.9 g m^{-3} , o -X = 0.04–1.3 g m^{-3} . From Fig. 2a–g it can be seen that, during the first 20 d of operation at pH 5.9 and at a G/L ratio of 43 (EBRT – 77.8 s), the removal of individual BTEX compounds varied widely. The values were as low as <20% in the case of B and p,o -X, while the removal of other VOCs (TE m -X) occasionally reached >80%. The RE $_T$ also fluctuated between ~20% and 70% depending on the removal of individual compounds in the mixture (Fig. 2g). The fluctuating removal profiles during the initial 20 d of operation can be attributed to the operating conditions of the biofilter, *i.e.*, with a continuous trickling phase in order to maintain the filter bed pH constant, and the requirement of a re-acclimation phase in this mode of operation, or pollutant mass transfer limitations due to high BTEX concentrations. Between days 21 and 54 (pH: 5.9, EBRT: 41.7 s) the concentrations of individual BTEX compounds were increased further in order to envisage the maximum performance of the biofilter. The total BTEX concentrations were thus varied from 0.56 to 12.1 g m^{-3} , corresponding to ILR $_T$ ranging from 48.8 to 1051.3 $\text{gBTEX m}^{-3} \text{ h}^{-1}$; and a maximum RE $_T$ of 77% was noticed when the ILR $_T$ were <80 $\text{gBTEX m}^{-3} \text{ h}^{-1}$. At this stage the biofilter experienced clogging problems due to excess biomass growth on the perlite, leading to flooding conditions near the inlet of the biofilter (top-section). The biofilter was stopped for ~2 h to drain the excess nutrient medium, and the packing was removed from the biofilter. 500 mL of fresh perlite was added to the original packing with biomass, and then re-introduced into the biofilter. After a brief shut-down period (8 h), the biofilter was started with an operating bed volume of 4.5 L. The concentrations of individual BTEX were brought down to values <2.5 g m^{-3} , corresponding to total BTEX concentrations ranging from 0.2 to 9.8 g m^{-3} , during the next phase of operation that was performed at a pH of 7.0. At an EBRT of 45 s (G/L ratio: 83.7) in this phase (days 55–74), the RE of individual VOCs, as well as the RE $_T$ increased significantly, despite subjecting the biofilter to ILR $_T$ as high as 791.2 $\text{gBTEX m}^{-3} \text{ h}^{-1}$. The removal of individual VOCs, such as BTE, was >80%, although the removal of xylene isomers dropped occasionally to values <70% depending on their concentrations in the mixture. During days 75–88 (EBRT: 30 s), for inlet BTEX concentrations varying between 0.2 and 6.08 g m^{-3} , the

RE_T were maintained consistently at $>90\%$. Regarding experiments at pH 4.9 (days 89–111), the concentration of individual BTEX were maintained at $<1.2 \text{ g m}^{-3}$, corresponding to ILR_T ranging from 147.5 to 364.1 $\text{gBTEX m}^{-3} \text{ h}^{-1}$ at an EBRT of 45 s, and from 181.2 to 232.8 $\text{gBTEX m}^{-3} \text{ h}^{-1}$ at an EBRT of 30 s. In this concentration range, all VOCs were removed with more than 80% except benzene. Thus, for total BTEX concentrations $<4.5 \text{ g m}^{-3}$, the RE_T in this phase, at pH: 4.9, ranged from 88 to 100%.

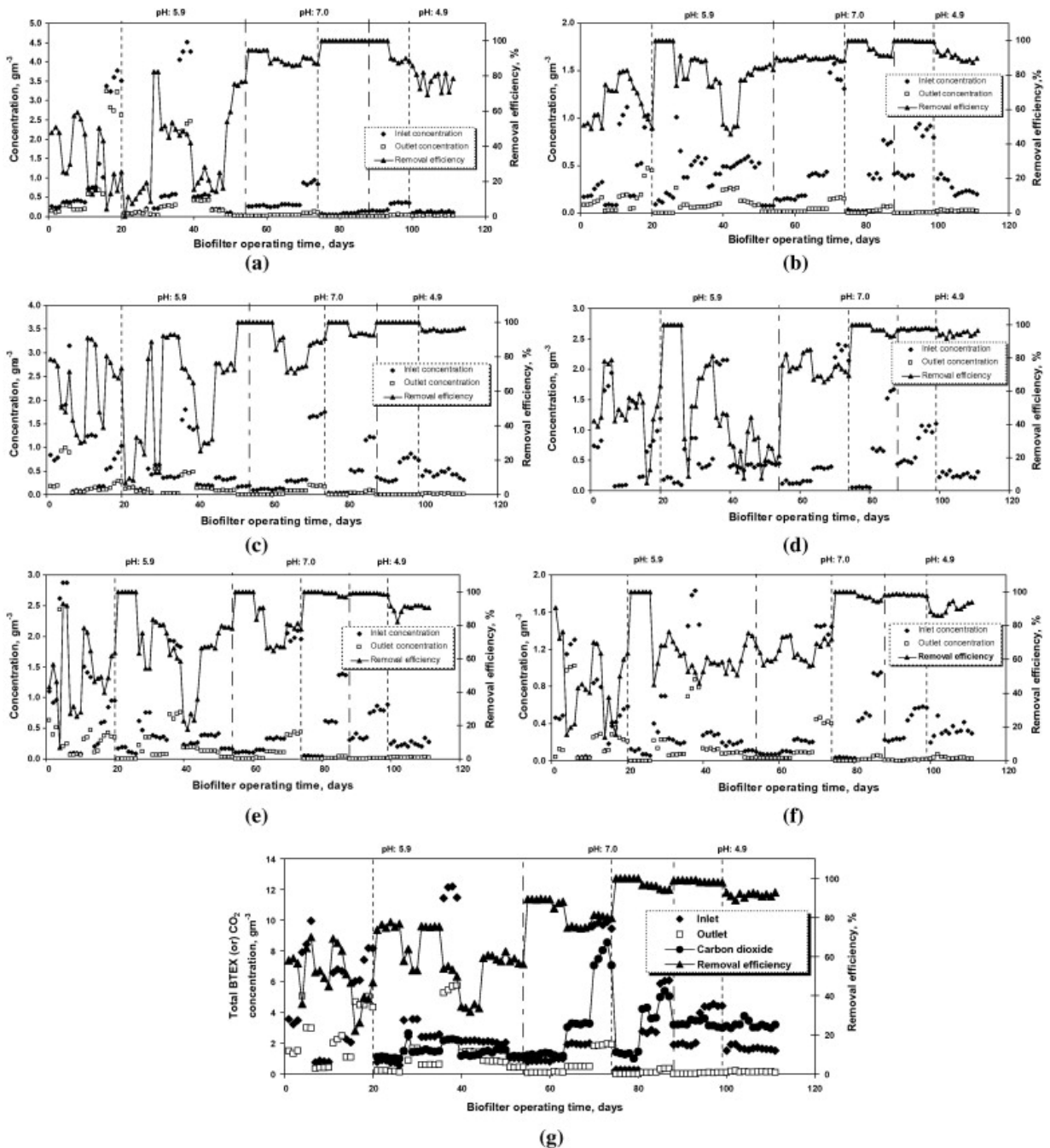


Fig. 2.

Individual BTEX concentration and removal profiles at different pH conditions; (a) benzene, (b) toluene, (c) ethylbenzene, (d) *p*-xylene, (e) *m*-xylene, (f) *o*-xylene, and (g) total BTEX concentrations and RE_T profiles.

Fig. 3a–c illustrates the effect of ILR of individual BTEX on the EC, while Fig. 3d compares the effect of total BTEX loading rates (ILR_T) on the EC_T of the biofilter, at different pHs. As evident from these figures, still higher EC could have been achieved for the individual VOCs at pH 7.0 and 4.9 because near linear relationships between ILR and EC were noticed over all the range of tested ILRs. Among the BTEX compounds, ethylbenzene and *m*-xylene were removed better, reaching EC_{max} of, respectively, 113 and 121 g m⁻³ h⁻¹ at pH 5.9, and 135 and 187 g m⁻³ h⁻¹ at pH 4.9. The removal of benzene or xylenes has nearly always been reported to be less efficient by either bacteria or fungi in comparison with other benzene-pollutants (Prenafeta-Boldu et al., 2002 and García-Peña et al., 2008). Regarding total BTEX loading to the biofilter, for ILR_T <400 gBTEX m⁻³ h⁻¹, a near linear relationship was noticed for the EC_T profiles at pH 7.0 and 4.9, reaching a maximum EC_T of 687 g m⁻³ h⁻¹ at an ILR_T of 730.5 gBTEX m⁻³ h⁻¹. The maximum EC_T observed in this study is higher than most of the EC_{max} values reported in the literature for the biofiltration of mixture of VOCs (<10–340 g m⁻³ h⁻¹). Table 3 gives an overview of the sources of inoculum, the EBRTs tested, and the EC_{max} achieved during the biotreatment of BTEX, TEX, TX, TXT, and MTBX mixtures in biofilters.

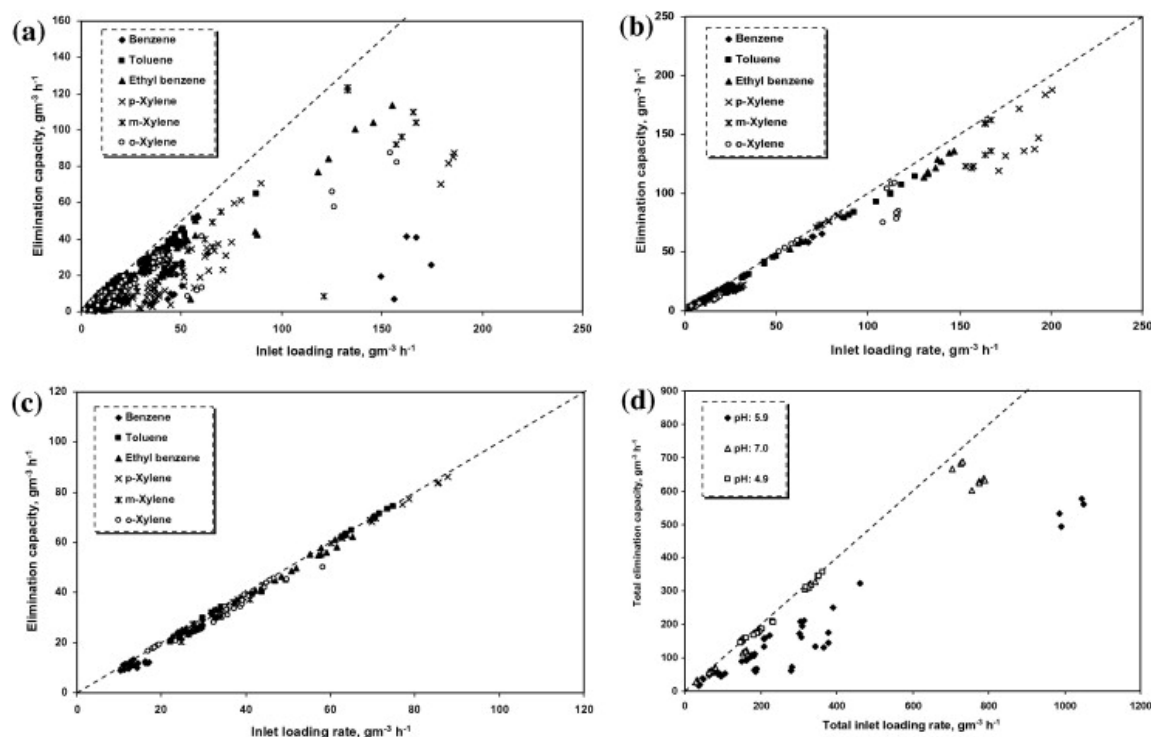


Fig. 3.

Effect of ILR on the EC of the biofilter for individual BTEX compounds at different pH conditions; (a) pH: 5.9, (b) pH: 7.0, (c) pH: 4.9, and (d) effect of ILR_T on the EC_T at different pH conditions.

Table 3.

Literature information on BTEX removal in biofilters and typical maximum elimination capacity (EC_{max}) values reported.

| Pollutants | Inoculum | EBRT (or) air velocity | Packing material | EC_{max} ($g\ m^{-3}h^{-1}$) | Reference |
|------------|--|-------------------------------------|---|----------------------------------|----------------------------|
| BTEX | <i>Paecilomyces variotii</i> ¹ | 1.7 min | Vermiculite | 110 ^{1*} | García-Peña et al. (2008) |
| BTX | Isolated bacterial strains | 4.4 min | Dry peat moss | 40–45 ^{2*} | Choi and Oh (2002) |
| BTX | <i>Pseudomonas putida</i> strain PPO1 ³ | 40 s ^{3*} | Dry peat, perlite | 40–50 | Oh and Bartha, (1997) |
| BTEX | Enriched consortia ⁴ | 0.5–3 min | Mature composted pine bark | NA ^{4*} | Strauss et al. (2004) |
| TX | Mixed microbial population ⁵ | 0.03 m ³ h ⁻¹ | Peat, bark and wood | ~17 ^{5*} | Marek et al. (1999) |
| BTX | Activated sludge ⁶ | SV: 50 h ⁻¹ | Cubic polyurethane foam | 340 ^{6*} | Shim et al. (2006) |
| TXT | Microbes present in compost ⁷ | 65 s | Compost, organic binder | 110 ^{7*} | Delhoménie et al. (2003) |
| BTX | Activated sludge ⁸ | 60 s | 5 different types of packing ^{8*} | 260 | Ortiz et al. (2003) |
| TEX | Activated sludge ⁹ | 16–120 s | Peat | 85 ^{9*} | Gabaldon et al. (2006) |
| MTBX | Activated sludge ¹⁰ | 42–106 s | Coal | 184 ^{10*} | Mathur and Majumder (2008) |
| BTEX | Mixed culture ¹¹ | 1.2–2.3 min | Compost, baggase, GAC | 83.6 ^{11*} | Mathur et al. (2007) |
| BTo-X | Enriched microbial consortium ¹² | 30–90 s | Wheat bran, redwood powder and diatomaceous earth | 97.7 ^{12*} | Chen et al. (2010) |
| BTX | <i>Phanerochaete chrysosporium</i> ¹³ | AV: 0.13–0.53 mh ⁻¹ | Glass beads or hydroballs | <10 ^{13*} | Oh et al. (1998) |
| BTEX | Mixed culture ¹⁴ | 90 s | Cork, GAC | Cork-86 GAC-67 ^{14*} | Kwon and Cho (2009) |
| Tp-X | Non-defined mixed culture | 180 s | Small stones | 40 ¹⁵ | Gallastegui et al. (2011) |
| TX | Mixture of bacteria ¹⁶ | 78, 102 s | Conditioned peat | 115 ^{16*} | Jorio et al. (1998) |

Note: MTBX: methyl ethyl ketone (MEK), toluene, *n*-butyl acetate, and *o*-xylene; TXT: toluene, xylene and trimethylbenzene, TEX: toluene, ethylbenzene and xylene; GAC: granular activated carbon; SV: space velocity; EBRT: empty bed residence time; AV: air velocity; RE: removal efficiency; EC_{max}: maximum elimination capacity; NA: not available.

Superscripts: Significant details and results, as reported by the authors. 1 – This culture was previously adapted to toluene in batch systems; 1* – EC_{max} of T: 70 g C m⁻³ h⁻¹, EB: 40 g C m⁻³ h⁻¹, B: 10 g C m⁻³ h⁻¹; 2 – Source: wastewater treatment plant; 2*- EC of B, T, *o*-X, and *m,p*-X were 10.8, 14.2, 1.8, and 15.4 g m⁻³ h⁻¹, respectively; 3 – Isolated from an industrial sludge that was enriched on equal parts of B,T, *p*-X; 3* – In order to increase the RE_T from 50% to 90%, the authors suggested an EBRT of 80 s; 4 – Previously acclimated to toluene in a biofilter for over 4-months at an ILR of 32 g m⁻³ h⁻¹; 4* – Total BTEX load: 18.1 g m⁻³ h⁻¹. The RE followed the order: E > B > *o*-X > *m*-X > *p*-X; 5 – Adaptation of soil micro-biota to BTEX; 5* – Local EC monitored in the middle layer of the filter bed; 6 – BTX degradable culture; 6* – The EC_{max} for B, T, and X were 200, 238, and 400 g m⁻³ h⁻¹; 7 – Total culturable, the toluene-specific and the nitrifying bacterial counts were measured; 7* – Biodegradability followed the order: T > X > B; 8 – Adapted to toluene and then to BTX vapours; 8* – Peat, activated carbon, pine tree bark, Rashig glass rings and vermiculite; 9 – Activated sludge was acclimated for 2 months with TEX mixture in a reactor; 9* – Individual EC_{max} of T, E, and X were 90, 100, and 65 g m⁻³ h⁻¹; 10 – Acclimated in nutrient medium containing 50 µL of each of MTBX; 10* – Maximum removal rates of MTBX were: 0.085, 0.033, 0.16, and 0.024 g m⁻³ h⁻¹; 11 – Microorganisms from previously operated biofilter, and identified as *B. Sphaericus*; 11* – Nearly 100% BTEX was removed at ILR_T < 68 g m⁻³ h⁻¹; 12 – This consortium was grown on BTo-X compounds; 12* – EC_{max} at EBRTs of 90, 60, 45, and 30 s were 55, 73, 81 and 97 g m⁻³ h⁻¹; 13 – *P. chrysosporium* was able to mineralize all BTX components simultaneously; 13* – EC_{max} for B, T, and X were 2.75, 0.77, and 0.24 g m⁻³ h⁻¹; 14 – Microorganisms from public sewage was grown in toluene-saturated environment; 14* – Degradation of benzene and xylene were poor compared to toluene; 15 – EC_{max} for *p*-X and T were 26.5, and 40.3 g m⁻³ h⁻¹. The presence of *p*-X had an enhancing effect on the toluene removal efficiency; 16 – Identified as *Pseudomonas putida*, *Rhodococcus* sp., and *Arthrobacter paraffineus*; 16* – EC_{max} for T and X were 165, and 66 g m⁻³ h⁻¹.

3.4. Carbon dioxide profiles in the biofilter

The variation of CO₂ production, PCO₂, as a function of the EC_T under different experimental conditions, *i.e.*, at different RH and pH, was calculated. It was observed that the PCO₂ values increased with an increase in the EC_T of the biofilter, irrespective of the operational condition, and maximum PCO₂ values reached 680 gCO₂ m⁻³ h⁻¹ for the highest ILR_T tested in the biofilter (>700 gBTEX m⁻³ h⁻¹). The experimental data also reveal that the variation was nearly linear in all cases with a carbon dioxide yield (PCO₂/EC_T) by weight of 0.98 (RH experiments) and 0.91 (pH experiments), respectively. This confirmed the mineralization of the individual BTEX compounds to CO₂, water, and other biodegradation end-products. The carbon dioxide yield observed in this study is less than the expected carbon dioxide yield (~3.3) resulting from the

stoichiometric equations for the complete mineralization of BTEX, when omitting biomass formation (Chen et al., 2010 and Gallastegui et al., 2011). The discrepancies between theoretical and observed CO₂ profiles are not unusual during continuous biofilter operation, especially when the biofilter is subjected to high loads of VOC mixtures. Some of the CO₂ produced could have accumulated in the liquid-phase in the form of HCO₃⁻, H₂CO₃ and CO₃²⁻ or mineral acids could have formed as biodegradation end-products. However, to obtain the theoretical CO₂ yield of ~3.3 for BTEX oxidation, the formulae of biomass is not considered. Thus, for instance, by taking into account the formulae of biomass as C₅H₇NO₂ (Kennes and Veiga, 2001) and ammonium chloride (NH₄Cl) as the nitrogen source in the mineral medium, the stoichiometric equation for complete mineralization of toluene can be written as follows:



Similar stoichiometric equations can be derived for other VOCs used in this work.

3.5. Performance under shock-load conditions

After the completion of steady-state experiments at different EBRTs, the next step was to evaluate the biofilter's performance under shock-loading conditions (Table 2). As seen in Fig. 4a–c, prior to the first shock-load, the total inlet BTEX concentrations were held constant at 0.5 g m⁻³ for 2 days in order to achieve high BTEX removals (≥97% RE_T). After that, the inlet concentrations of individual B, T, E, *p*-X, *m*-X and *o*-X were increased from 0.07 to 3.92, 0.14 to 1.3, 0.06 to 1.25, 0.09 to 2.3, 0.08 to 1.43, and 0.03 to 1.06 g m⁻³, respectively, corresponding to a total BTEX concentration of 9.8 g m⁻³ on the 3rd day. Thus, by increasing the concentrations of individual VOCs, the ILR_T also increased from about ~50 to 850 gBTEX m⁻³ h⁻¹ (Fig. 4c). The response of the biofilter was fast, as seen from the sudden drop in RE_T from 90% to 35%. However, an increase in the ILR_T from 50 to 850 g m⁻³ h⁻¹ also exhibited a marked increase in the EC_T of the biofilter, from 44 to 322 g m⁻³ h⁻¹. During this shock-load, the RE of individual B, T, E, *p*-X, *m*-X and *o*-X decreased to REs <20% for benzene and *o*-xylene, ~50% for ethylbenzene and *m*-xylene, and ~45% for both toluene and *p*-xylene, respectively. This high shock-load was applied to the biofilter for the next 4 d, and when pre-shock conditions were restored, the RE_T of the biofilter also increased to >90%, thus stabilizing the EC_T of the biofilter at 46 g m⁻³ h⁻¹ at an ILR_T of 50 g m⁻³ h⁻¹. When a second, lower, shock-load of 320 gBTEX m⁻³ h⁻¹ was applied on the 11th day, the total BTEX removal (RE_T) dropped only by 30%. During this shock-loading step, the removal of individual VOCs were as follows: B-28%, T-63%, E-78%, *p*-X-45%, *m*-X-76%, and *o*-X-44%. Anew, original removal performances were also re-established quickly in the biofilter when the ILR_T was lowered. This shows the sensitivity of the biofilter to changes in loading rate patterns. An increase in the ILR_T from 50 to 320 g m⁻³ h⁻¹ also increased the EC_T of the biofilter from 45 to 199 gBTEX m⁻³ h⁻¹. The maximum EC_T achieved under such high successive shock-loads in the biofilter was 322 g m⁻³ h⁻¹ at the highest overload tested, with 38% BTEX removal (Fig. 4c). The EC_{max} of individual B, T, E, *p*-X, *m*-X and *o*-X compounds were as follows: 73.7, 71.6, 63.3, 105.6, 61.6, and 20.9 g m⁻³ h⁻¹, respectively. The biofilter performance during successive shock-loads was limited by the biological reaction, a problem often encountered in biological waste-gas treatment systems treating hydrophobic VOCs like BTEX at high concentrations (Kennes and Veiga,

2001 and Kennes et al., 2009b). During large transient operations, either the mass transfer capacity or the reaction capacity of the initial sections of the bed is exceeded and contaminants move into the next sections where the microbial populations and reaction capacities are low and contaminant breakthrough may occur (Wright et al., 2005).

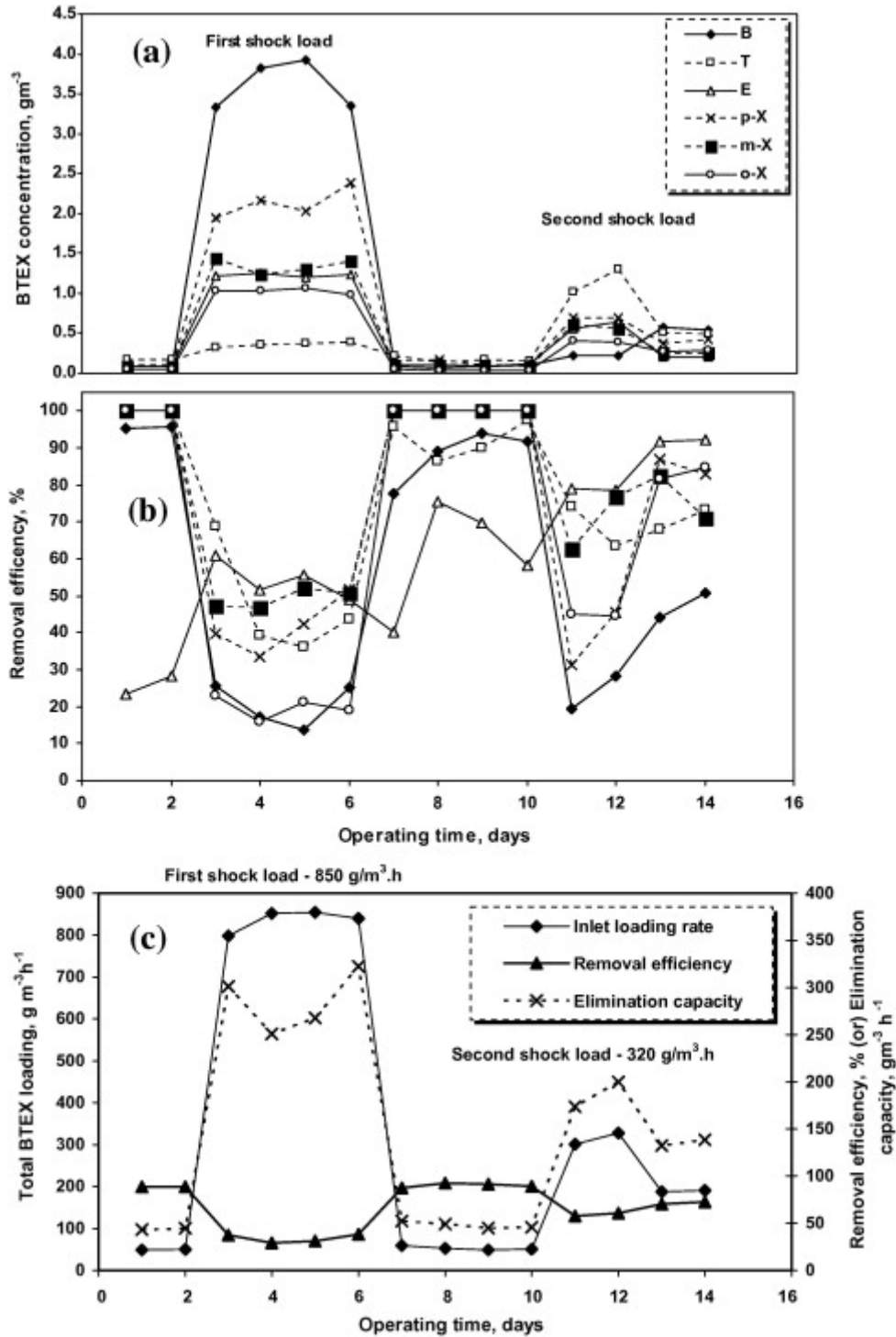
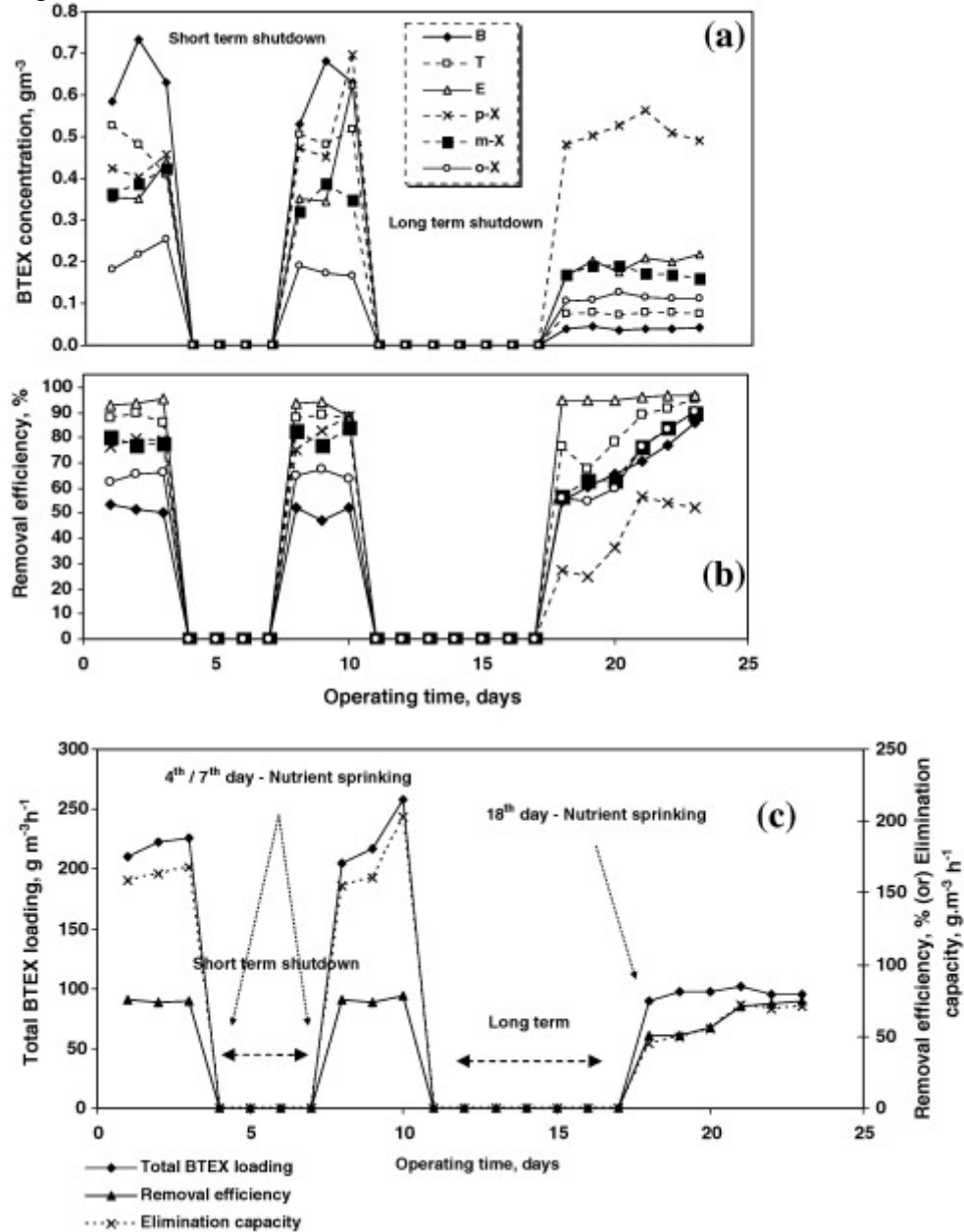


Fig. 4. Response of fungi-dominant biofilter to successive shock-loads: (a) individual BTEX concentration profiles, (b) BTEX removal efficiency profiles, and (c) total BTEX removal and elimination capacity profiles as a function of total BTEX loading rate.

3.6. Performance under shut-down and re-start operations

Fig. 5a–c shows the individual BTEX concentrations, RE profiles, ILR_T and EC_T profiles in the biofilter during short- and long-term shut-downs. In order to prevent the microorganisms in the biofilter from experiencing any substrate inhibition effects, the individual maximum BTEX concentrations tested during these experiments ($<0.72 \text{ g m}^{-3}$) were much lower than the concentration levels of BTEX tested during shock-load experiments (Table 2). During short-term shut-downs, BTEX was not fed to the biofilter, thus depriving the biomass of the essential carbon source; and during long-term shut-downs neither the pollutant nor air was passed through the system, and nutrients were added only during the re-start phase. Under such conditions, the microorganisms could survive in the bioreactor for a limited period of time by scavenging the remaining organic matter and nutrients adsorbed onto the packing and converting them into useful energy and cellular material. Such temporary starvation would place the biomass under stress and decrease their enzyme activities. Prior to the first shut-down period, the ILR of each individual BTEX compounds was $<50 \text{ g m}^{-3} \text{ h}^{-1}$, and the corresponding REs were 50, 85, 95, 79, 77 and 66% for B, T, E, *p*-X, *m*-X and *o*-X, respectively, at a total BTEX concentration of 2.9 g m^{-3} . From Fig. 5c, during the re-start phase (day 8), it is evident that the biofilter was able to maintain its RE after a short-term shut-down reaching a maximum EC_T of $200 \text{ g m}^{-3} \text{ h}^{-1}$ with about 75% RE_T . However, after a long-term shut-down, even when the total inlet BTEX concentration was reduced to 1 g m^{-3} in comparison to the previous value of 2.9 g m^{-3} , the RE of the biofilter dropped by almost 25%. During continuous operation at the same inlet concentration after re-start, the biofilter was able to recover and reach again $\sim 75\%$ RE_T in about 4 d. As described earlier, re-acclimation times during shut-down and re-start have shown to vary depending on the process conditions and microbial activities. Elmrini et al. (2001) reported that a re-acclimation period of 8 d was needed for a biofilter handling toluene vapors to recover its original state, after a prolonged shut-down period of 8 months. In this study, although the removal of benzene and ethylbenzene remained unaffected during the second re-start phase, the RE of T, *p*-X, *m*-X and *o*-X dropped from 85% to 76%, 79% to 27%, 77% to 56%, and 66% to 56%, respectively (Fig. 5b). During extended shut-down periods, the nutrients in the biofilter would have maintained the activity of the biomass and stability of the biofilter. During this experimental phase, the EC_{max} of individual B, T, E, *p*-X, *m*-X and *o*-X compounds were as follows: 32.5, 39.8, 47.9, 53.2, 28.3, and $14.5 \text{ g m}^{-3} \text{ h}^{-1}$, respectively.

Fig. 5.



Response of fungi-dominant biofilter to shut-down and re-start operations: (a) individual BTEX concentration profiles, (b) BTEX removal efficiency profiles, and (c) total BTEX removal and elimination capacity profiles as a function of total BTEX loading rate.

3.7. Substrate stratification in the biofilter during steady-state operations

Substrate stratification profiles were measured under the following conditions: pH = 7.0, EBRT = 45 s, B = 0.08 g m⁻³, T = 0.4 g m⁻³, E = 1 g m⁻³, p-X = 1.3 g m⁻³, m-X = 1.2 g m⁻³ and o-X = 0.9 g m⁻³, respectively, corresponding to an ILR_T of 394 gBTEX m⁻³ h⁻¹ (Fig. 6). T, E, m-X and o-X were removed better (~50%) in the lower section of the filter bed, while B and p-X were removed only in the upper section of the filter bed. The REs of T, E, m-X and o-X were higher than 75%, while the removal of B and p-X was less than 40%. The differences in the removal profiles could

be due to the different microbial species developing in the biofilter during its long-term operation, their specific activities, and the metabolic pathways utilized by the dominant strains involved in BTEX degradation. Apparently, changes in experimental conditions (EBRT, concentrations of BTEX, RH, pH, and transient-state studies) could affect the composition of the process culture and both the rate of mass accumulation and the rate of pollutant degradation (Heitzer et al., 1991). The results from this study showed the versatility of the fungi-dominant biofilter to handle both neutral and mildly acidic pH conditions, reaching high EC_T and RE_T under long-term steady and transient-state operations.

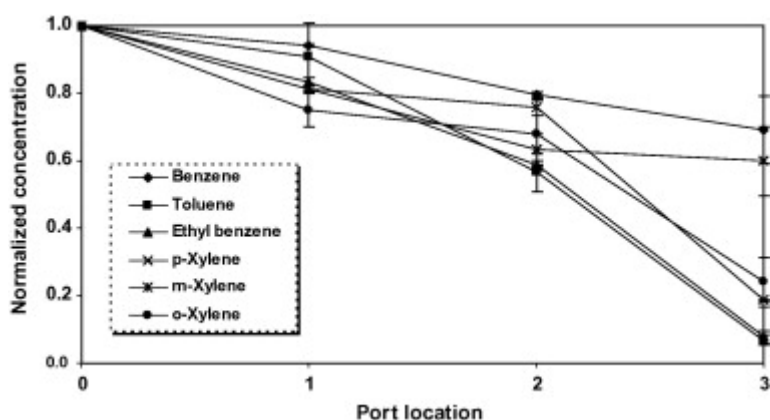


Fig. 6.

Substrate stratification profiles in the biofilter.

4. Conclusions

The critical ILR_T to achieve $>90\%$ RE_T was found to drop at lower EBRTs. The results showed that it is not a pre-requisite to humidify the incoming waste-gas to $>95\%$ RH, if water is sprinkled twice a week on top of the filter bed. When the biofilter was operated at a constant pH (7.0/4.9), for $ILR_T < 400 \text{ gBTEX m}^{-3} \text{ h}^{-1}$, a near-linear relationship was noticed for EC_T profiles. The biofilter was sensitive to successive shock-loads, and could easily recover its performance after intermittent short and long-term shut-down operations, and such transient-state conditions did not affect all different BTEX compounds in a similar way.

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