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Microbial response and elimination capacity in biofilters subjected to high toluene loadings

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Abstract Elimination capacity (EC) is frequently used as a performance and design criterion for vapor-phase biofilters without further verification of the microbial quantity and activity. This study was conducted to investigate how biofilters respond to high pollutant loadings and ultimately how this affects the EC of the biofilter. Two identical laboratory-scale biofilters were maintained at an initial toluene loading rate of 46 g m^{-3} h^{-1} for a period of 24 days. After the initial biofilm development stage, the loading rates were increased to 91 g m^{-3} h^{-1} and 137 g m^{-3} h^{-1}, respectively. Following a short period of pseudo-steady state, toluene removal efficiencies rapidly declined in both biofilters, with a concurrent decline in both critical and maximum ECs. The decline was mainly due to deterioration in the biodegradation activity of the biofilm and a decline in the toluene-degrading bacterial population within the biofilm phase. The findings imply that high toluene loadings accelerated the deterioration in overall performance due to a rapid accumulation of inactive biomass. As a result, care must be used when relying on EC values for biofilter design and operational purposes, since the values do not appropriately reflect the temporal changes in biodegradation activity and active biomass quantities that can occur in biofilters subjected to high inlet loadings.

Introduction

The removal of a wide variety of odorous and volatile organic compounds (VOCs) from contaminated waste gas streams is a major environmental concern. Vapor-phase biofilters have emerged as an effective alternative among the many conventional abatement technologies available, since they consume comparatively less energy and produce few undesirable byproducts (van Groenestijn and Hesselink 1993). Numerous studies have demonstrated that biofilters can successfully treat a wide range of VOCs; and their application range can be expanded by increasing their VOC-degrading capacity (Kinney et al. 1999; Jang et al. 2004; Song and Kinney 2000; van Groenestijn and Hesselink 1993; van Groenestijn and Kraakman 2004).

The VOC-degrading capacity of biofilters is commonly quantified as a function of pollutant loading by determining the contaminant elimination capacity (EC):

$$\mathrm{EC} = \frac{\left(C_{\mathrm{g,in}} - C_{\mathrm{g,out}}\right) \times Q}{V} \left(\mathrm{g/m^3/h}\right) \quad (1)$$

$C_{\mathrm{g,in}}$ is the inlet VOC concentration in the gas phase (g m^{-3}), $C_{\mathrm{g,out}}$ is the outlet VOC concentration (g m^{-3}), Q is the gas flow rate (m^3 h^{-1}) and V is the biofilter bed volume. EC tests are typically performed by sequentially increasing the inlet pollutant loading stepwise and then calculating the EC corresponding to each pollutant loading. An EC versus pollutant loading curve is used to determine two important indicators, the maximum EC value and the critical EC value. The maximum EC is the point at which the EC curve achieves its highest value, while the critical EC is defined as the point at which the EC curve begins to deviate from the 100% removal line (Deshusses and Johnson 2000). These two parameters are useful not only to evaluate biofilter performance under a given operating condition but also to set design criteria such as biofilter size and empty bed contact time. This design approach is based on the assumption that EC is a stable measure of biofilter performance. However, declines in biofilter performance often occur in biofilters (Kinney et al. 1999; Smith et al. 1996; Weber and Hartmans 1996; Eq. 1).

There are several potential reasons for a decline in biofilter performance, including excess microbial growth and temporal and/or permanent changes in the pollutant-de-

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grading microbial culture. Excess microbial growth in the biofilm phase can cause biofilter operating problems such as clogging and high pressure drops leading to low contaminant removal efficiencies and ultimately system failure (Song and Kinney 2000; Sorial et al. 1995; Weber and Hartmans 1996). Moreover, temporal changes in the relative quantities of microbial components in the biofilm phase can lead to a decline in overall bioreactor performance. Biofilm systems in mixed-culture biofilters are heterogeneous, containing active microbial components that actively degrade pollutants and inactive microbial components that consist of secondary microbial populations and dead cells. As a result, the relative quantity of pollutant-degrading, active biomass can be a critical factor affecting long-term performance and the observed pollutant-degrading activity (Song and Kinney 2001; Juteau et al. 1999).

Changes in the pollutant-degrading activity of biofilters subjected to a high inlet loading have generally not been considered and biofilter performance is characterized at a given point in the operation by the EC without further verification. It has been hypothesized that EC curves determined for moderately loaded biofilters are different from those determined in a biofilter operated at the critical loading rate or higher. In this study, two toluene loading rates, equivalent to the critical EC point and the maximum EC point, were applied to two identical biofilters to determine how continuously high loadings affect the EC of the biofilters over time. In addition, changes in biomass accumulation, toluene-degrading activity and heterotrophic bacterial counts were examined to gain insight into how high toluene loadings affect biofilter performance and the pollutant-degrading microbial population.

Materials and methods

Biofilter configuration and operation

The experimental set-up used in this study was identical to that of a previous study (Song and Kinney 2000; Song and Kinney 2001). The two laboratory-scale biofilters consisted of stainless steel columns (16.2 cm I.D.) packed with porous ceramic pellets (Celite R-635; Celite Co., USA) to an overall packed bed height of 1.0 m. The bacterial consortium used to inoculate the packing material was obtained from a mixed culture grown in another toluene-degrading biofilter in the laboratory. An inoculum solution was cultivated using the consortium prior to biofilter inoculation as described by Song and Kinney (2000).

The filtered compressed air was split into two streams. Pure toluene was injected into one air stream using a syringe pump (model 200; KD Scientific, USA) and the other stream was saturated with a nutrient-laden aerosol generated by a nebulizer (Heart, Westmed, USA). The two air streams were combined and supplied to each biofilter at an empty bed residence time of 1 min. During the first 24 days of operation, the inlet toluene concentrations were maintained at approximately 200 ppm_v (0.76 g m^{-3}) in both biofilters, yielding a toluene loading rate of 46 g m^{-3} h^{-1}. On day 24, the inlet toluene loadings to the biofilters were increased to 91 g m^{-3} h^{-1} and 137 g m^{-3} h^{-1}, respectively.

A fine aerosol generated by the nebulizer was used to supply approximately 120 ml day^{-1} of nutrient solution to each biofilter. The nutrient medium contained 2.72 g l^{-1} KH_2PO_4, 1.42 g l^{-1} Na_2HPO_4, 1.32 g l^{-1} $(NH_4)_2HPO_4$, 10.1 g l^{-1} KNO_3 and trace elements, as described by Ridgway et al. (1990). When the toluene loading was increased in each biofilter, the concentrations of the nitrogen sources (NH_4^+, NO_3^-) were increased proportionally in the nutrient solution to maintain a nearly constant carbon/nitrogen (C/N) supply ratio of 9.8. The other components of the nutrient solution remained unchanged thoughout the biofilter experiments.

Analytical methods

Duplicate gas samples were collected from gas sampling ports using 0.5-mL gas-tight syringes (Hamilton, USA). The grab samples were immediately analyzed using a gas chomatograph (series 6890; Hewlett Packard, USA) equipped with a flame ionization detector and a capillary column (HP-5; Hewlett Packard, USA). EC curves were determined in both biofilters by increasing the inlet concentration stepwise for 2 hours, similar to the procedure used by Deshusses and Johnson (2000). Five to six different loadings were applied and the EC values were calculated for each loading, using the overall toluene removal efficiency observed at each loading. The CO_2 evolution from each section was measured using an infrared CO_2 analyzer (LI-6252; LI-COR, USA).

Biofilm samples were collected periodically from the eight sampling ports located along each biofilter column. For the chemical oxygen demand (COD) and protein analyses, samples were prepared by adding an appropriate amount of packing material to 10 ml of deionized water. The samples were homogenized using an orbital vibrator for 5 min and a sonicator for 1 min. The COD was measured using a COD vial (Hach, USA) according to a closed-reflux colorimetric method (APHA et al. 1992). The total biomass protein concentration was quantified using a protein assay kit (Sigma, USA), based on the modified Lowry method and using bovine serum albumin as standards. For the in vitro toluene-degrading activity and enumeration analyses, biofilm samples were collected by adding an appropriate amount of packing material to 10 ml of saline solution (Song and Kinney 2000) and then homogenized in an orbital vibrator for 1 min. The in vitro toluene-degrading activity was measured by determining the initial toluene biodegradation rates, using an analytical procedure developed by Yee et al. (1998). The activity was normalized by protein content to obtain the specific activity, expressed as $\mu g_{\text{toluene}}\ mg^{-1}_{\text{protein}}\ h^{-1}$. Bacterial populations were enumerated by the spread plate method and expressed as colony-forming units (CFU), as described previously (Song and Kinney 2001). R2A media (Difco, USA) was used to count total heterotrophic bacteria, while agar plates made with hydrocarbon minimal medium [1.34 g l^{-1} KH_2PO_4, 1.42 g l^{-1} $NaHPO_4$, 0.5 g l^{-1} KNO_3, 2.38 g l^{-1} $(NH_4)_2SO_4$,

8.0 g l^{-1} granular agar, and trace elements] were used in a sealed chamber containing toluene vapor for the enumeration of toluene-degrading bacteria only.

Daily carbon accumulation in the biofilm phase was determined by subtracting the carbon exiting the bioreactor system via CO_2 evolution and the liquid leachate from the carbon input to the biofilm phase from toluene degradation (Song and Kinney 2000):

$$\begin{aligned}&\text{Cumulative Carbon (g}-\text{c)}\\&=\sum_{\text{day}}\Big[\text{Toluene degration (g}-\text{C)}\\&\quad-\text{CO}_2\text{ evolution (g}-\text{C)}\\&\quad-\text{Carbon in the leachate (g}-\text{C)}\Big]\end{aligned}\tag{2}$$

The differences in toluene and CO_2 concentrations in the inlet and the outlet gas steams were converted into equivalent carbon mass units, while the carbon lost in the leachate was determined by COD measurements.

Results

Biofilter performance

Initially, both biofilters were operated for 24 days at a toluene loading rate of 46 g m^{-3} h^{-1} to establish a stable biofilm. During this initial period, both biofilters achieved high toluene removal efficiencies of greater than 99.9% (Fig. 1). On days 21–23 in both biofilters, EC tests were performed to determine the critical EC point and the maximum EC (Fig. 2). At a loading of 91 g m^{-3} h^{-1}, the EC line began to deviate from the 100% removal line, defining it as the critical EC point (Deshusses and Johnson 2000). In addition, the maximum EC was determined to be 120 g m^{-3} h^{-1} at a toluene loading rate of 143 g m^{-3} h^{-1}. On day 24, the inlet toluene loadings were increased to 91 g m^{-3} h^{-1} in one biofilter (referred to as "BioR-Critical") and 137 g m^{-3} h^{-1} in the second biofilter (referred to as "BioR-Max"). BioR-Critical was operated for an additional 21 days at the elevated, critical EC point (overall 45 days) and BioR-Max was maintained for an additional 23 days at the maximum EC point (overall 47 days).

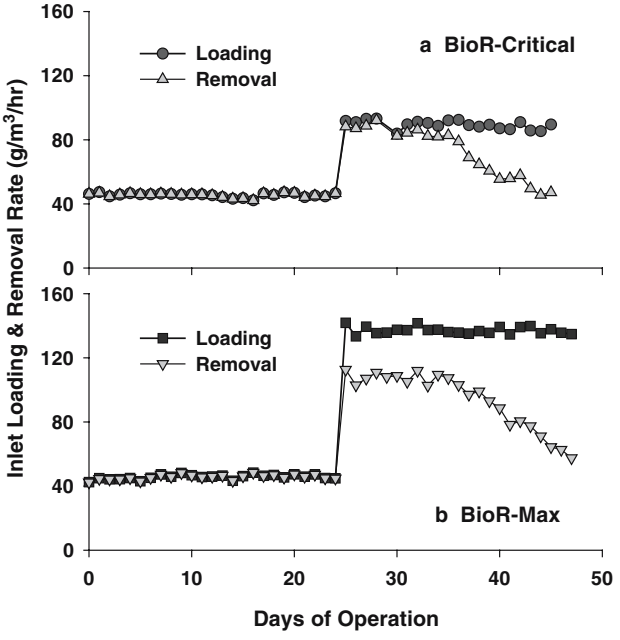

Fig. 1 Inlet toluene loading and removal rates in the biofilters: **a** BioR-Critical, **b** BioR-Max

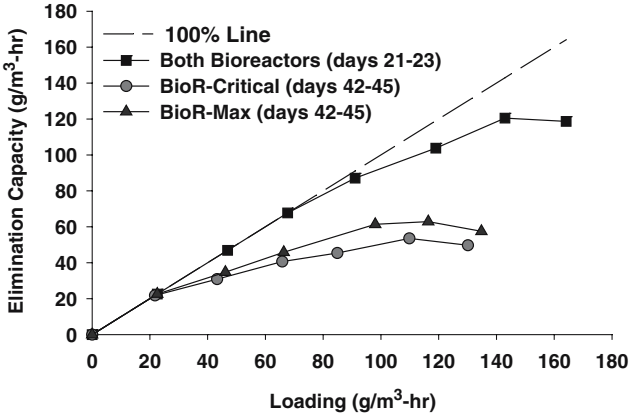

Fig. 2 Toluene EC curves determined in the biofilters

A drop in the overall toluene removal efficiency followed the loading increase in each biofilter (see Fig. 1). In BioR-Critical, the removal efficiencies were maintained at a pseudo-steady-state level of 96% for approximately 10 days. However, on day 35, the removal efficiency started to decrease rapidly. By day 45 when BioR-Critical operation was terminated, the overall removal efficiency had declined to 46%. In addition, the critical EC and maximum EC were reevaluated in the BioR-Critical biofilter on days 42–45. As shown in Fig. 2, the critical EC and maximum EC substantially decreased to 22 g m^{-3} h^{-1} and 54 g m^{-3} h^{-1}, respectively.

At the increased loading in BioR-Max (137 g m^{-3} h^{-1}), a 12-day pseudo-steady-state period was observed with an overall removal rate of approximately 78% (Fig. 2). A rapid decline in overall efficiency was also observed in BioR-Max and the decline in biofilter performance progressed at a rate similar to that in BioR-Critical. The critical EC and maximum EC determined on days 42–45 in BioR-Max were 23 g m^{-3} h^{-1} and 63 g m^{-3} h^{-1}, respectively, which were similar to those of BioR-Critical (see Fig. 2).

Even though a short period of pseudo-steady-state was observed in each biofilter after the increase in toluene loading, the decline in activity began almost immediately after the load increase, as evident in the toluene profiles along the column. In each case, the daily toluene profile (i.e., a toluene degradation pattern along the length of the biofilter) shifted from an exponential removal curve to a linear profile (data not shown), although the overall toluene removal efficiencies remained relatively unchanged during the pseudo-steady-state period.

Other operating parameters were held essentially constant thoughout the entire experiment. For instance, the pressure drop across both biofilter columns was found to be less than 0.1 inch of water (25 Pa) thoughout the experimental period. In addition, the moisture content of the packing materials was maintained in a range 45–60% (w/w), which is generally within the range required to achieve full biological activity (Gostomski et al. 1997). The continuous supply of nutrient medium via the aerosol delivery system could have had an inhibitory effect on the microorganisms by increasing the salinity within the biofilm phase (Holden et al. 1997). However, another study using the same biofilter and nutrient supply system showed that greater than 99% toluene removal efficiencies could be maintained over 78 days of operation at a toluene loading of 46 g m^{-3} h^{-1} (Song and Kinney 2001). Therefore, the rapid drop in toluene removal efficiency within the relatively short period of biofilter operation was more likely due to a limitation in biodegradation activity, rather than other environmental factors in the biofilters.

Carbon balance and biomass accumulation

Biomass accumulation was monitored by completing the carbon balances for both biofilters (Diks et al. 1994; Song and Kinney 2000). Solid lines in Fig. 3a, b represent the cumulative carbon in the biofilters, calculated using Eq. 2. A carbon balance in the biofilm phase was also completed by converting the measured COD values for biomass samples to an equivalent carbon mass unit (bars in Fig. 3). Assuming 100% oxidation and a zero oxidation state for the organic matters measured in the COD tests, the COD measurements can be converted to g-carbon (C) units using a conversion factor of 0.36 g-C for 1.0 g-COD. Theoretically, the cumulative carbon quantities calculated using Eq. 2 should be equivalent to the carbon accumulation in the biofilm phase as determined by the COD measurements. In each biofilter, the total biomass accumulation as calculated by Eq. 2 and that determined via the COD measurements (lines, bars in Fig. 3) were relatively consistent, indicating that the carbon closure was experimentally valid over the biofilter operating period.

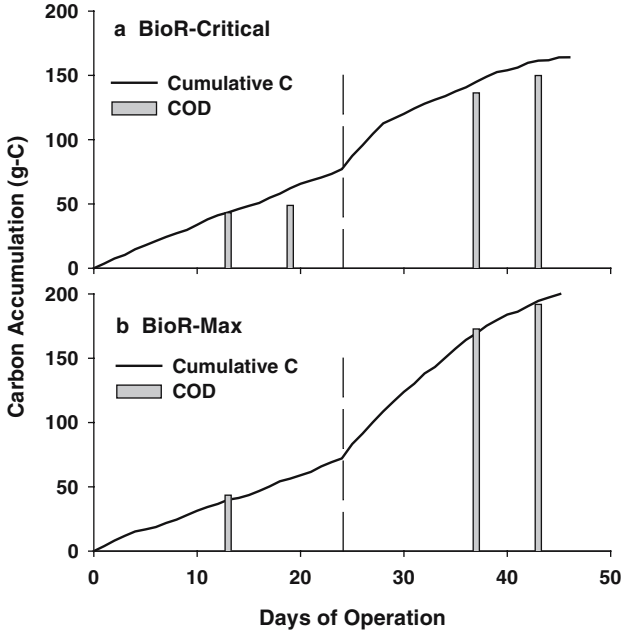

Fig. 3 Total carbon accumulated in the biofilm phase within each biofilter: **a** BioR-Critical, **b** BioR-Max. *Solid lines* were calculated using Eq. 2 and represent cumulative carbon. *Dotted lines* are used to indicate the day when the higher toluene loadings were applied. *Bars* represent the carbon accumulation in the biofilm phase in terms of COD

These cumulative carbon profiles show that biomass in both biofilters continuously accumulated during the entire operational period. During the initial 24-day period at the toluene loading rate of 46 g m^{-3} h^{-1}, both biofilters accumulated biomass at an identical rate (3.2 g-C day^{-1}) and the yield coefficient was calculated to be 0.15 g-$C_{biomass}$/g-$C_{toluene}$. More rapid carbon accumulation occurred immediately after the loading increase in BioR-Critical. Similarly in BioR-Max, substantial increases in the rate of biomass accumulation (5.8 g-C day^{-1}) and the yield coefficient (0.18 g-$C_{biomass}$/g-$C_{toluene}$) were observed after the loading increase.

It was also clearly demonstrated in this study that the decline in the toluene removal rate corresponded to a decline in the rate of biomass accumulation at the elevated toluene loadings (Fig. 2). During the final phase of BioR-Critical operation when the biofilter performance declined rapidly, the biomass yield coefficient also decreased to less than 0.07 g-$C_{biomass}$/g-$C_{toluene}$. Similarly, at the end of BioR-Max operation, the yield coefficient also dropped to less than 0.09 g-$C_{biomass}$/g-$C_{toluene}$, which was substantially lower than that determined during the initial start-up period.

Toluene degrader fraction and microbial activity

Table 1 summarizes the total heterotrophic and toluene-degrading bacterial counts determined in each biofilter immediately after inoculation and during each toluene loading condition. The toluene-degrading fraction of the total plate-countable bacteria in the biofilm phase is also presented. In both biofilters, the number of heterotrophic bacteria increased with time, but the toluene-degrading fraction of the total population decreased rapidly when the biofilters were subjected to elevated toluene loadings. At the end of these experiments, less than 20% of the total heterotrophic bacterial population in either biofilter consisted of toluene-degrading bacteria, indicating that the non-toluene-degrading population became dominant in the biofilm phase. In our previous study conducted at a constant toluene loading of 46 g m^{-3} h^{-1} (Song and Kinney 2000), a gradual decrease in the toluene-degrading fraction was also observed with increasing toluene exposure time. However, in this previous study the toluene-degrading fraction still constituted 43% of the total bacteria population, even after 43 days of biofilter operation.

Changes in biofilter performance were also examined by determining the in vitro toluene-degrading activity of bio-

Table 1 Bacterial counts and in vitro toluene-degrading activity in the biofilters

	BioR-Critical				BioR-Max			
	Total heterotrophic counts[a] (CFU g_{pellet}^{-1})	Toluene-degrading counts[a] (CFU g_{pellet}^{-1})	Toluene-degrading fraction (%)	In vitro toluene-degrading activity[a] (μg mg^{-1} h^{-1})	Total heterotrophic counts[a] (CFU g_{pellet}^{-1})	Toluene-degrading counts[a] (CFU g_{pellet}^{-1})	Toluene-degrading fraction (%)	In vitro toluene-degrading activity[a] (μg mg^{-1} h^{-1})
Immediately after inoculation	0.19×10^{10} ($\pm0.02\times10^{10}$)	0.17×10^{10} ($\pm0.01\times10^{10}$)	89.1	–	0.09×10^{10} ($\pm0.01\times10^{10}$)	0.07×10^{10} ($\pm0.01\times10^{10}$)	82.9	–
Initial loading[b]	0.59×10^{10} ($\pm0.06\times10^{10}$)	0.40×10^{10} ($\pm0.04\times10^{10}$)	67.4	31.9 (±9.8)	1.08×10^{10} ($\pm0.24\times10^{10}$)	0.69×10^{10} ($\pm0.13\times10^{10}$)	63.1	28.5 (± 4.4)
Elevated loading[c]	2.13×10^{10} ($\pm1.10\times10^{10}$)	0.35×10^{10} ($\pm0.12\times10^{10}$)	15.9	6.0 (±2.5)	1.82×10^{10} ($\pm0.86\times10^{10}$)	0.36×10^{10} ($\pm0.17\times10^{10}$)	20.3	8.3 (±2.5)

[a]Each data point represents an average value obtained from eight different biofilm samples collected from sampling ports located across each biofilter column as described by Song and Kinney (2000)
[b]Performed on days 13, 14, 16 and 17 at the initial loading conditions
[c]Performed on days 37, 38, 42 and 43 at the elevated loading conditions

film samples collected from the biofilter (Table 1). During the initial moderate loading period (days 0–23), high toluene-degrading activity was maintained in both biofilters. After the loading increase in BioR-Critical, a rapid activity drop was observed. The highest toluene loading in BioR-Max also resulted in a substantial activity decline. These changes in the in vitro microbial activity are consistent with the decline in overall toluene removal efficiency. Nevertheless, the quantity of total biomass continued to increase in each biofilter with time, even though the toluene-degrading activity of the biomass continued to decline.

Discussion

A rapid decline in biofilter performance and toluene-degrading activity was observed in both biofilters when they were operated at high toluene loading rates representing the critical EC point and the maximum EC. The decline in biofilter performance resulted in a concurrent decline in the critical EC and maximum EC. The EC values, which are commonly used as key indicators of biofilter performance, were not constant over time at loading rates greater than or equal to the critical EC point. In contrast, both critical and maximum ECs determined on day 33 in our previous study (Song and Kinney 2001) were almost identical to those determined on day 56 using the same biofilter at a continuous toluene load below the critical point of 46 g m^{-3} h^{-1}. This finding shows that EC values vary substantially with time in biofilters operated at elevated loading conditions. As a consequence, great care must be taken when using EC data, since the EC curve is generated under ideal conditions with the basic assumption that EC is a stable measure of biofilter performance over an extended period of operation. Furthermore, when interpreting EC values, it should be taken into account that ECs fundamentally depend on the quantity of active biomass under a given set of operating conditions.

Continuous accumulation of biomass in the biofilm phase is one possible reason for the decline in biofilter performance and EC. Our previous study (Song and Kinney 2000), using the same biofilter system operating at a constant toluene loading of 46 g m^{-3} h^{-1}, showed that biomass clogging and eventual system failure occurred when the cumulative carbon total exceeded 250 g-C after 75 days of continuous operation. However, the decline in biofilter performance in the current study was not likely due to complete biomass clogging. Rather, the decrease in specific surface area due to biomass accumulation lowered the mass transfer flux of toluene from the gas phase to the biofilm phase. A numerical model which uses a cellular automaton approach to incorporate biomass accumulation with time (Song and Kinney 2002) predicts that increases in biofilm thickness lead to a rapid decrease in specific surface area and eventually overall removal efficiency. Another interesting finding in the model simulation is that toluene profiles along the column shift from exponential curves to linear profiles as biofilter performance deteriorates, a prediction which is consistent with the experimental findings in this study.

Along with the effect of biomass accumulation, an intrinsic decline in pollutant-degrading activity also contributes to the deteriorating performance of biofilters subjected to high loading rates. The declines in removal efficiency in both BioR-Critical and BioR-Max at high toluene loadings clearly imply that a higher toluene loading initiated the deterioration in microbial activity. Leddy et al. (1995) demonstrated that non-toluene-degrading variants appeared in a wild-type cell culture of *Pseudomonas putida* as a result of plasmid instability. They also showed that plasmid instability (i.e., plasmid deletions and mutations) was mediated by metabolic byproducts in the toluene degradation pathway, such as benzyl alcohol. Mirpuri et al. (1997) also demonstrated that the number of non-toluene-degrading variants increased with toluene exposure period, yielding a gradual decline in biodegradation activity in a bioreactor. These

results imply that exposure to high toluene concentrations over long periods of operation makes toluene-degrading bacteria either less prolific than other non-toluene-degrading bacteria or causes them to lose their ability to degrade toluene either temporarily or permanently. Overall, high toluene loadings accelerate the deterioration in biofilter performance due to a rapid accumulation of non-toluene-degrading biomass.

Since continuous exposure to toluene results in a decline in toluene-degrading activity, the highest toluene loading rate at the maximum EC point was expected to accelerate the decline. However, the rates of the activity decline were similar in both biofilters operated at the critical EC and the maximum EC. These experimental results imply that the decline in the toluene-degrading activity progresses with time at a similar rate if the toluene loading is increased above the critical EC point. This study also clearly demonstrates that biofilters treating toluene need to be operated at inlet loading rates below a threshold value in order to maintain stable biofilter operation over an extended period.

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