

# Effect of Starvation on the Performance and Re-acclimation of Biotrickling Filters for Air Pollution Control

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Biotrickling filters for air pollution control are expected to encounter fluctuating conditions or periods without pollutant supply. In the present study, we investigated the effect of pollutant starvation in bench-scale biotrickling filters treating toluene. The experimental protocol consisted of starving biotrickling filters under various conditions: with or without airflow, with or without liquid recycle, and with or without an alternate carbon source (glucose) supply. The duration of the period without toluene was varied from 2 to 9 days, during time which the biotrickling filters were monitored for biomass content, endogenous and toluene-induced oxygen uptake rates during starvation, and toluene overall elimination capacity after restart. During starvation, all reactors lost their ability to degrade toluene within 5 days, regardless of the mode of starvation. The biomass content significantly decreased during starvation, in particular in those reactors where the recycle liquid was maintained, but this decrease was not critical for future re-acclimation. Glucose addition to starved biotrickling filters had several detrimental effects. It resulted in a faster decrease of the biomass content and slowed the re-acclimation phase. Overall, the results show that the re-acclimation of toluene-degrading biotrickling filters after periods of nonuse is short (10–24 h to re-establish full performance), and they suggest that, in the case of toluene-degrading biotrickling filters, re-acclimation time is largely governed by the induction of key pollutant-degrading enzymes.

## Introduction

Over the past decade, increasingly stringent environmental regulations have opened new possibilities for biological techniques for air pollution control. In many instances, vapor-phase biotreatment has become the alternative of choice to physical and chemical treatment technologies (1, 2). Vapor-phase biotreatment is effective for the control of low concentrations of odors and volatile organic compounds (VOCs) in high flow rates. The most widely utilized bioreactors for air pollution control are biofilters and biotrickling filters. Biofilters work by passing a humidified contaminated airstream through a packed bed usually made of compost and some bulking agents (2). The gaseous pollutants are absorbed in the damp packing material and are then biodegraded by mixed cultures immobilized on the packing.

Biofilters have been widely used for odor abatement and to a lesser extent for VOC control in industry for over several decades (2). Biotrickling filters work in a manner similar to biofilters, except that an aqueous mineral nutrient solution is trickled over the packed bed and that the packing is made of inorganic materials (e.g., plastic rings, lava rocks, structured packing, etc.). Biotrickling filtration is newer than biofiltration, and the number of industrial applications is still limited. Biotrickling filters enable higher pollutant elimination rates to be obtained for a broader range of pollutants (3–6). Efficient removal of, for example, aromatics, some chlorinated hydrocarbons, ethers, and other VOCs had been reported in lab-scale biotrickling filters (3–6). Biotrickling filtration is especially well-suited for the control of relatively poorly biodegradable VOCs or pollutants that release acids upon degradation such as chlorinated and reduced sulfur compounds (3).

Performance of biotrickling filters is usually studied under relatively ideal conditions, i.e., steady-state operation and presence of a single pollutant in laboratory systems. Operation of pilot- or full-scale biotrickling filters in industrial settings may however give rise to operational problems undetected in the laboratory (7). In particular, repeated periods of nonuse was identified as one of the factors that caused a lower pollutant elimination in the field, preventing the establishment of a dense process culture in the reactor (7). Pollutant starvation may be the result of interruptions in the plant operation, weekend recess, holiday breaks, or equipment malfunctions leading to interruptions in the feed of polluted air. Clearly, understanding the phenomena occurring in the absence of pollutant feed and characterization of the recovery of biotrickling performance when resuming normal operation are important aspects for the successful deployment of biotrickling filters, but quantitative data are lacking.

A few studies discuss the starvation and restart of biofilters or biotrickling filters for air pollution control (8–15). Most of these studies are concerned with compost-based biofilters. After restarting the reactors after a period of nonuse, a wide variety of responses were reported. This reflects the fact that these studies were conducted with different pollutants, reactor systems, and operating conditions. However, in most cases, re-acclimation after starvation is much faster than the initial start-up phase because of the pre-existence of an acclimated process culture. Only a few limited studies deal with actual measurements of the activity of the process culture during starvation (8, 15). Probably the most interesting observations were made comparing the activity of biofilms in directionally switching (DS) and unidirectional (UD) biofilters treating toluene vapors (16). The biofilm close to the air outlet port of the UD biofilter had a 5-fold lower respiratory activity than that at the inlet port. In the reactor subject to air flow direction change every 3 days, the respiratory activity was uniformly high along the bed length, although portions of the bed were not exposed to toluene for cycles of 3 days. This shows that, in this case, short starvations have minor effects on the process culture. Further detailed investigations on the phenomena occurring during starvation and on the re-acclimation period of biotrickling filters were warranted.

In the present study, we investigated the effect of toluene starvation in 16 parallel bench-scale toluene-degrading biotrickling filters. The experimental protocol considered interrupting the toluene feed for durations ranging from 2 to 9 days under selected conditions (with or without airflow, with or without liquid recycle, and with or without glucose

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TABLE 1. Experimental Matrix for Toluene Starvation Experiments

starvation experiment	toluene supply	air supply	liquid recycle	glucose addition	purpose
A	off	on	on	no	simulate weekend or holiday break with water recycle and air kept on
B	off	off	on	no	simulate blower breakdown
C	off	off	off	no	check potential benefits or electricity savings by shutting blower off during breaks simulate complete shut down check potential benefits or electricity savings by shutting blower and liquid recycle off during breaks
D	off	on	on	yes	check potential benefits of supplying alternate C source during starvation

supply). The effect of the starvation mode on the re-acclimation of the biotrickling filter performance was determined.

## Materials and Methods

**Equipment, Start-Up, and Standard Operation.** All experiments were performed using a custom-made rotating reactor setup allowing for the parallel operation of 20 biotrickling filters (17), of which 16 were used for this experiment. Each biotrickling filter (4.0 cm i.d.) contained a packed bed section of 50 cm height (volume 0.628 L) packed with 0.7–2 cm length crushed polypropylene Pall rings (Koch Engineering, Wichita, KS) and two identical liquid reservoirs of 0.35 L attached to either side of the reactor. The recycle liquid (0.35 L) trickled from the upper reservoir downward through the reactor and was collected in the lower reservoir, usually within about 60 s. The biotrickling filters were periodically rotated back and forth once every 111 s to maintain a semi-continuous trickling of recycle liquid over the packed bed. Details of the experimental setup are described elsewhere, and recent experiments have shown that the performance of rotating biotrickling filters are comparable to those of conventional biotrickling filters (17).

The biotrickling filters were started by adding 340 mL of a mineral medium (18) and 10 mL of toluene-degrading consortium taken from a biotrickling filter (18). All biotrickling filters were operated similarly throughout the entire experiment, except during the starvation phase. At standard operation, toluene-containing air at 2 g m<sup>-3</sup> was passed through the reactor at a flow rate of 50 L h<sup>-1</sup>, corresponding to an empty bed residence time of 45 s and a toluene load of 160 g m<sup>-3</sup> h<sup>-1</sup>. Periodical rotation resulted in an average superficial liquid velocity of 9.0 m h<sup>-1</sup>. As the gas flow was in one direction only, periodical rotation resulted in alternating cocurrent and countercurrent operation. Medium feed to the reactors was with replacement of 100 mL of recycle liquid by fresh mineral medium once a day (average liquid residence time of 3.5 days). Evaporation of water was compensated by addition of about 40 mL of distilled water once every 2–3 days.

**Starvation Experimental Protocol.** After a start-up phase of 8 days at standard operating conditions, the set of 16 biotrickling filters was randomly divided into four groups and assigned to starvation experiments A–D (Table 1). The supply of toluene was then stopped in all biotrickling filters. In experiments A, B, and D, the recycle liquid was removed and replaced by mineral medium after which liquid trickling was continued. Daily removal and addition of recycle liquid was stopped during starvation in experiments A and B. In experiment D, 100 mL of the recycle liquid was replaced daily by mineral medium containing 2 g of glucose L<sup>-1</sup> as an alternate carbon and energy source, which corresponds to about 22% of the actual toluene degraded prior to starvation. The biotrickling filters of experiment C were starved without trickling liquid. For these reactors, the recycle liquids were stored at 4 °C until resuming standard operation. Clean air supply was maintained in experiments A and D, but it was discontinued in experiments B and C.

For each set of four biotrickling filters, starvation was continued for 2, 3, 5, or 9 days, after time which standard operation was resumed. The effect of starvation was determined by comparing both the toluene elimination capacity of the biotrickling filter and the biological activity of the immobilized biomass before and after starvation.

**Analytical Methods.** Toluene concentrations in inlet and outlet air were determined in triplicate by direct injection of 0.5-mL grab gaseous samples into a model 8860 flame ionization detector (SRI Instruments, Las Vegas, NV). Typical standard deviation between the measurements was about 5%. The amount of wet immobilized biomass in the biotrickling filters was determined (±1 g) by regularly weighing the reactors after draining the recycle liquid for at least 30 min. For representative sampling, any viscous recycle liquid trapped inside the packed bed was first removed by gentle flushing with 1 L of distilled water.

Measurement of biological activities was done by oxygen uptake rate (OUR) determinations. Portions of the packing with immobilized biomass were transferred to buffer containing 1 g of KH<sub>2</sub>PO<sub>4</sub> and K<sub>2</sub>HPO<sub>4</sub> each L<sup>-1</sup> in a 40-mL EPA vial, and the biomass was suspended by vortexing the vial for 20 s. OUR experiments were done at room temperature in a 2.7-mL custom-made vessel fitted with a YSI oxygen probe and meter (Yellow Springs, OH). Samples were first saturated with air and monitored for endogenous respiration rate for 2–5 min depending on the activity. The toluene-induced OUR was determined after addition of a concentrated toluene solution to reach a final concentration of 0.19 mM in the vessel, and the OUR was corrected for the endogenous respiration. OUR experiments were done in duplicate, and rates were correlated to the protein content of each sample. Protein content was determined using the Biorad DC Protein Assay (Hercules, CA) after boiling the samples in 1 M NaOH for 10 min.

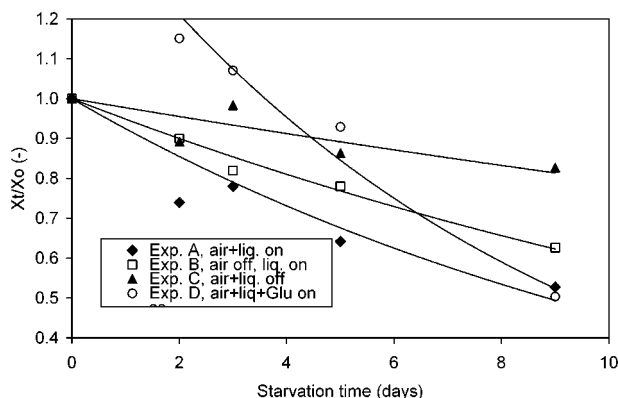
## Results

**Performance before Starvation.** After inoculation of the biotrickling filters, mineral medium was added on a daily basis for 8 days to sustain a rapid microbial growth and to allow for the full development of toluene degradation activity before starting the starvation experiments. The steady-state characteristics of the biotrickling filters at the end of the acclimation phase are presented in Table 2. Previous experiments with the same setup and similar conditions had shown a rapid start-up, and the reactors reached steady state after 3–4 days after inoculation (17). Here, a rapid accumulation of immobilized biomass was observed with on average about 160 kg wet biomass per m<sup>3</sup> reactor formed in 8 days. At this high biomass content, a few biotrickling filters exhibited an increase in the dynamic holdup in the countercurrent mode of operation. This did not influence toluene removal as shown by a paired *T*-test of each reactor performance at cocurrent and countercurrent, which revealed that toluene removal was not statistically different at the 99% level. Over the 20 biotrickling filters, toluene removal was on average slightly lower during countercurrent operation, probably because of lower pollutant mass transfer rates (17). The average toluene elimination capacities during cocurrent and countercurrent

**TABLE 2. Performance of the Biotrickling Filters at the End of the Growth Phase (Day 8), i.e., Just before the Starvation Experiment**

parameter	average	SD	no. of filters analyzed
toluene inlet ( $\text{g m}^{-3}$ )	1.979	0.008	2 <sup>a</sup>
toluene outlet, cocurrent ( $\text{g m}^{-3}$ )	1.17	0.12	16
toluene outlet, countercurrent ( $\text{g m}^{-3}$ )	1.29	0.12	16
gas flow ( $\text{m}^3 \text{h}^{-1}$ )	0.050	0.0063	16
average toluene elimination capacity ( $\text{g m}^{-3} \text{h}^{-1}$ )	58.8	7.0	16
wet biomass ( $\text{kg (m of reactor)}^{-3}$ )	160	24.0	16
endogenous OUR ( $\mu\text{g of O}_2 \text{(mg of protein)}^{-1} \text{min}^{-1}$ )	4.36	0.60	5
toluene-induced OUR ( $\mu\text{g of O}_2 \text{(mg of protein)}^{-1} \text{min}^{-1}$ )	3.75	1.76	5

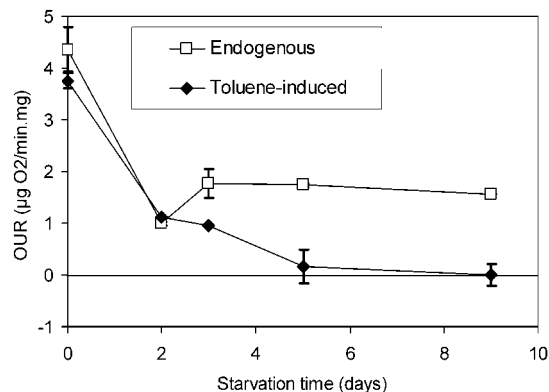
<sup>a</sup> All reactor inlets share a common line.



**FIGURE 1. Decrease of biomass amount in the reactors vs starvation time. Solid lines show fit (forced through point 1 at time zero, except for experiment D) with first-order biomass decay kinetics of  $0.078 \text{ day}^{-1}$  for experiment A ( $r = 0.94$ ),  $0.053 \text{ day}^{-1}$  for experiment B ( $r = 0.99$ ),  $0.023 \text{ day}^{-1}$  for experiment C ( $r = 0.83$ ), and  $0.11 \text{ day}^{-1}$  for experiment D ( $r = 0.98$ , origin not included).**

operation was  $63 \pm 10$  and  $54 \pm 7 \text{ g of toluene m}^{-3} \text{ h}^{-1}$ , respectively, which is not statistically different. This is slightly lower than the  $79 \text{ g m}^{-3} \text{ h}^{-1}$  found previously under relatively similar conditions (17). A possible explanation for the difference between the two experiments may be that relatively high biomass contents at the end of the acclimation phase caused a lower performance. Also, nutrient loadings in the previously published experiments were higher. Still, the low standard deviation reported in Table 2 indicates that the performance of the 16 biotrickling filters prior to starting the starvation experiments was relatively uniform and comparable.

**Effect of Starvation on the Biomass Content.** As shown in Figure 1, the decrease of the amount of wet immobilized biomass in the biotrickling filters was a first-order function with respect to time for the various types of starvation examined. The best fit was for experiments B ( $r = 0.99$ ). Decrease of biomass may be due to biomass death and lysis, endogenous respiration of the process culture, secondary processes such as predation by higher organisms, and/or shear by the liquid recycling. Biomass decrease was the lowest in starvation experiment C, which differed from the other experiments by the absence of liquid recycling. This indicates that biomass detachment due to liquid shear stress is an important contributing factor to the overall biomass loss in the other experiments with liquid recycling. However, it should be stressed that the experiments presented herein were conducted at a relatively high liquid trickling velocity, which probably resulted in a high shear. Lower trickling rates should generally result in less biomass detachment, although



**FIGURE 2. Effect of starvation on the endogenous and toluene-induced oxygen consumption rate of immobilized biomass. Experiment A: starvation with supply of toluene-free air and liquid recycling on is shown.**

detachment also depends on the mechanical properties of the biofilm. These are known to vary significantly depending on the biofilm composition (19). Passing pollutant-free air during nonuse while maintaining liquid recycling had no marked effect (experiment A vs experiment B), indicating that oxygen availability is not a crucial factor in biomass decay. Addition of glucose as an alternate carbon and energy source (experiment D) resulted in an initial increase of the biomass content, but extended toluene starvation with regular glucose addition caused an unexpected loss of biomass at a rate faster than observed in all other experiments. This would suggest a stimulation of biomass decay in the presence of glucose, but the mechanisms are unclear.

It should be noted that a decrease in the biomass amount during starvation is not a concern when the biomass content is high. Long-term operation of biotrickling filters will often result in excess biomass formation and clogging of the filter bed when nutrient loadings and pollutant concentrations are high (18). Hence, periods of starvation associated with a decrease of biomass will increase the overall lifespan of the biotrickling filter as reported by Hekmat et al. (8). On the other hand, starvation during the early start-up may delay the buildup of a critical mass of pollutant degrading biofilm and ultimately result in suboptimum performance, especially in applications with slow net biomass growth (7). In the present experiments, the lowest wet biomass content observed after starvation was  $67 \text{ kg m}^{-3}$  reactor (experiment D after 9 days of starvation). For our packing, with a specific surface area of approximately  $485 \text{ m}^2 \text{ m}^{-3}$ , this corresponds to an average biofilm thickness of  $137 \mu\text{m}$ . A usual rule of thumb for the effective biofilm thickness is about  $50\text{--}100 \mu\text{m}$  (20–22). Thus one can conclude that the present starvation experiments did not cause biomass contents to drop to levels where biodegradation rates would be limited by the amount of biomass. This means that any loss of toluene removal after starvation is related to the activity of the remaining biomass, rather than to limiting amounts of biomass. This is further demonstrated below by determining the toluene degradation activity of the biotrickling filter process culture.

**Effect of Starvation on Biological Activity.** Pollutant elimination capacities of biofilters and biotrickling filters have been correlated with the respiratory activity of the immobilized biomass (23, 24). Determination of the oxygen consumption rate in batch experiments therefore allows for rapid, off-line assessment of the effect of starvation on the activity of the biotrickling filter process culture. Figure 2 shows the endogenous and toluene-induced oxygen uptake rates of the immobilized biomass versus starvation time for experiment A. Similar trends were observed for the other experiments. In all cases, the endogenous respiration activity dropped by about 60% within 2 days and remained relatively



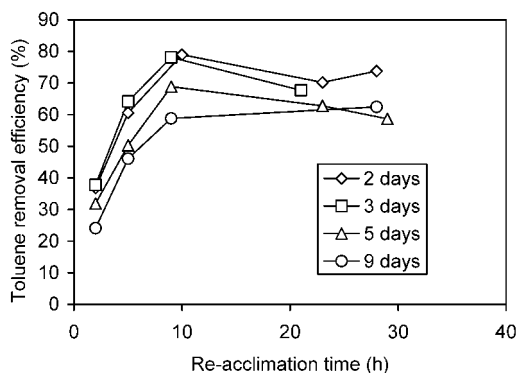


FIGURE 3. Re-acclimation of biotrickling filters after 2–9 days of nonoperation. Experiment A: starvation with liquid recycle on and supply of toluene-free air is reported. The legend shows the starvation duration.

constant thereafter. Before starvation, the endogenous OUR was  $4.36 \mu\text{g of O}_2 \text{ (mg of protein)}^{-1} \text{ min}^{-1}$  in all reactors. It decreased to values ranging from 1.51 (experiment A) to 2.38 (experiment D)  $\mu\text{g of O}_2 \text{ (mg of protein)}^{-1} \text{ min}^{-1}$  as the average values of 4 determinations over 2–9 days of starvation. A similar trend was made by Arcangeli and Arvin (25) for the starvation of a mixed culture grown on toluene in a biofilm reactor. A probable explanation for the observed transient decrease of endogenous respiration followed by an essentially constant value is that the process culture rapidly switches from a highly active metabolism (growth) to a lower metabolism (maintenance) when the toluene supply is discontinued. After all rapidly biodegradable materials are consumed, a pseudo-steady-state is observed. During this phase, biodegradable materials and hydrolysis products serve as substrates for primary and secondary degraders and for predators.

In contrast to the endogenous respiration, toluene-induced oxygen consumption rates of the immobilized biomass decreased exponentially with time and approached zero in 5 days (Figure 2). No major differences were observed between the four experiments, and an overall first-order decay of toluene oxidation activity of  $0.414 \text{ day}^{-1}$  ( $r = 0.88$ ) was calculated from the pooled data. The fact that the rate of decrease of toluene oxidation activity was identical in all experiments indicates that the absence of toluene was the determining factor rather than the absence of liquid recycle and/or supply of pollutant-free air. Decreasing activity due to the absence of an inducing substrate is a well-known phenomenon. Pure cultures of toluene-induced *Pseudomonas putida* completely lost degrading activity in 300 h in the absence of toluene (26), whereas Roch and Alexander (27) reported a half-life of toluene oxidation activity of as low as 2 h for a toluene-deprived, unidentified bacterium isolated from forest soil. In the experiments of Jenkins and Heald (26), the addition of ethanol as a noninducing substrate caused a 2-fold increase in the rate of decrease of toluene oxidation over time. This was attributed to stimulation of energy-limited turnover of the toluene degrading enzymes by ethanol. In a sense, experiment D with addition of glucose was similar to this of Jenkins and Head (26); however, no such stimulatory effect on the loss of toluene oxidation was observed. An important difference though between Jenkins and Head and our experiments is that they worked with planktonic cells, while the present work was performed with biofilms. Hence, it is possible that deep portions of the biofilm in the biotrickling filter remained unexposed to glucose because of diffusion limitations. The fact that glucose is rapidly degradable and that the actual glucose loading was lower than the toluene loading probably increased this effect.

**Re-acclimation Studies.** Figure 3 shows the re-acclimation profiles of toluene removal after resuming standard operation

after 2–9 days of starvation in experiment A (no toluene, liquid and air kept on during starvation). Re-acclimation profiles after restarting biotrickling filters from starvation experiments B and C were similar to those of Figure 3 and are not shown. Re-acclimation from experiment D took about twice as long and is discussed further in the paper. For experiments A–C, although the toluene oxidation activity of the biomass was completely lost within 5 days (Figure 2), re-acclimation with complete recovery of the toluene elimination capacity required about 10 h irrespective of the duration of the starvation. Comparable values have been reported for restarting biotrickling filters degrading polyalkylated benzenes (8) and biofilters degrading styrene (9, 10), hexane and phenols (11), and unidentified pollutants (12).

An interesting result, in addition to the short re-acclimation, is the observation that several biotrickling filters had a higher performance shortly after the restart than before the starvation phase. One example is in experiment A where some of the reactors exhibited elimination capacities as high as  $100\text{--}120 \text{ g m}^{-3} \text{ h}^{-1}$  for up to 2 days as compared to about  $60 \text{ g m}^{-3} \text{ h}^{-1}$  prior to the starvation. One possible explanation for this is that, during starvation, biomass death and lysis releases nutrients that are then utilized by the primary degraders after resuming toluene supply. Another possible explanation is that during starvation the reactors lost 10–50% of their biomass (Figure 1). As discussed by Alonso et al. (28), this should result in an increase in gas–biofilm interfacial area, hence possibly higher performance. Further research would be needed to determine the extent of both phenomena in the biotrickling filters.

Comparison of the different starvation experiments demonstrated that the presence or the absence of the gas flow and/or the liquid recycling did not greatly affect the time required for full re-acclimation. This is different from observations made with compost biofilters where air-off starvation and longer starvation induced a longer re-acclimation (13). While the exact causes for the differences are not known, the reactor systems were sufficiently different (biofilter vs biotrickling filter) to be cautious with direct comparisons. Still, an important implication of our results for industrial biotrickling filter operation is that significant energy savings can be made by shutting the blower and the recycle liquid pump off during down time without adverse effects as far as the recovery of full pollutant elimination is concerned. However, before implementation, one should carefully consider potential problems such as the generation of odors or corrosion resulting from anaerobic conditions in the biofilm.

Re-acclimation after nonoperation was always faster than the 3–4 days required for the initial acclimation of identical biotrickling filters [Cox and Deshusses (17) and this study]. This and the fact that full activity was recovered with only minor reactor mass increase (not shown) suggest that re-acclimation does not require the buildup of significant amounts of new toluene-degrading biomass. Instead, it is likely that re-acclimation consists merely of the induction of the toluene degradation pathway. Interestingly, re-acclimation of the biotrickling filters starved in the presence of glucose required roughly twice as long as biotrickling filters starved without glucose (Figure 4). We do not have a good explanation for this phenomenon. Although glucose in the recycle liquid was not determined, residual glucose in the trickling liquid probably only suppressed the induction of toluene degradation for the first few hours after restart until glucose was certainly completely consumed. Data in Figure 4 indicate a more prolonged effect, although some may be due to intrinsic variability between the performances of individual reactors. While, further research would be needed to identify the mechanisms of inhibition, the results suggest that there are no benefits of adding an alternate carbon source during starvation, if the particular carbon source added is not

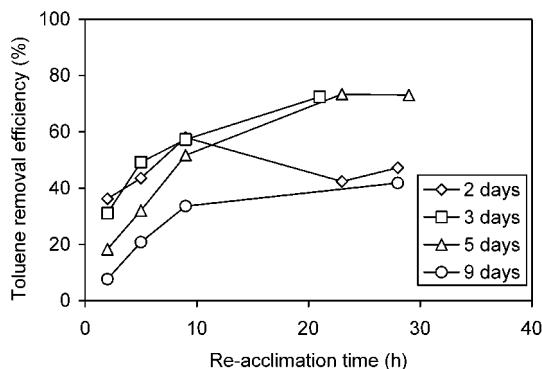


FIGURE 4. Re-acclimation of biotrickling filters after 2–9 days of nonoperation: Experiment D, i.e., starvation with liquid recycle amended with glucose is reported. The legend shows the starvation duration.

inducing the enzymes responsible for the biodegradation of the key pollutants.

Overall, the results of the present study demonstrate that re-acclimation of biotrickling filters after starvation is not a major problem for easily biodegradable pollutants such as toluene. This is particularly true when high concentrations of pollutant are treated. The results of this study further suggest that key enzyme induction is the most important condition for the rapid restart of biotrickling filters. These overall conclusions can reasonably be generalized for the treatment of all easily biodegradable compounds in biotrickling filters. However, they may not necessarily apply to the treatment of low concentrations or the treatment of compounds that are difficult to degrade. For the treatment of compounds that are difficult to degrade such as MTBE, nitro-, or chlorobenzenes, efficient biodegradation usually requires long start-up phases (4–6). Hence, re-acclimation after an extended starvation may be slow. On the other hand, in the case of the treatment of low concentrations of easily degradable compounds, one can reasonably assume that re-acclimation after a single starvation event will probably not be a problem and will follow the patterns described in this paper. However, repeated starvations at low concentrations may result in a net decrease of the biomass in the biotrickling filter over time. In this case, re-acclimation will most probably resemble the initial startup.

In light of this discussion, one can imagine two strategies to improve the performance of reactors subject to frequent pollutant starvations. The first one is the artificial addition of the inducing pollutant to the reactor during nonoperation. This will maintain the process culture in an active state. Such additions can be done via the inlet air or the recycle liquid depending on the physical form of the substance. Another possibility to supply small concentrations of pollutants during down time would be to install a small adsorption unit (e.g., granular activated carbon or zeolites) upstream of the biotrickling filter. As discussed by several authors (29–31), the adsorber will slowly release pollutants when the process air contamination is low. Such a setup could possibly reduce the size of the required biotrickling filter by equalizing the load over time. The second approach that is proposed would be to inoculate the biotrickling filter with mutant microorganisms that constitutively express the target pollutant degradation pathway. In the case of toluene, such microorganisms are available (32), although their competitiveness in complex and open systems such as biotrickling filters remains to be proven.

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