Comparative study of the elimination of toluene vapours in twin biotrickling filters using two microorganisms *Bacillus cereus* S1 and S2

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Abstract

BACKGROUND: To investigate the microbial degradation performance of organic pollutants in the atmosphere using a biotrickling filter, two microorganism strains, *Bacillus cereus* S1 and *Bacillus cereus* S2, were selected, identified and inoculated into a twin biotrickling filter for comparison.

RESULTS: Both strains showed good performance towards the degradation of model organic pollutants when gas flow rates ranged from 100 to 600 L h^{-1}. For S1, the total maximum removal efficiency (RE) of toluene was maintained nearly 100% not only at gas flow rates of 100 L h^{-1} corresponding to empty bed residence time (EBRT) 199.44 s, but also at gas flow rates of 200 L h^{-1} (EBRT = 99.72 s) and 300 L h^{-1} (EBRT = 66.48 s). However, S2 had a much lower degradation capability; near 100% removal efficiency was obtained only at the gas flow rate of 100 L h^{-1} although both bacteria belong to the same *Bacillus cereus*. With further increase in gas flow rate, the total REs for both S1 and S2 decreased slightly at first and then dropped sharply to 46% and 35%, respectively, at an EBRT of 33.24 s, corresponding to a gas flow rate of 600 L h^{-1}. Starvation for between 2 and 10 days resulted in the re-acclimation times of both strains ranging between 1.0 and 15.5 h.

CONCLUSION: Strain S1 would be a better choice for inoculation into a biotrickling filter than strain S2, because of its much higher toluene removal capacity and rapid recovery to full performance.

Keywords: air treatment; biodegradation; biotrickling filter; *Bacillus cereus* S1 and S2; toluene

INTRODUCTION

Benzene, toluene, ethyl benzene, and xylenes (BTEX) are a group of typical volatile organic compounds (VOCs). They are common environmental organic pollutants frequently found together at the same contaminated sites, such as anthropic sites, industrial factories, and landfill plants. BTEX are also representatives of hazardous substances commonly used as solvents and intermediates in many manufacturing processes, but are often listed as 'inert'.[1] However, BTEX compounds are far from 'safe' at all concentrations for human and other biota. Benzene, toluene and ethyl benzene are present on the US Environmental Protection Agency Priority Pollutant List. A number of researchers have found that BTEX presented short-term hazards, including potential acute toxicity to aquatic life in the water column, and potential inhalation problems. In addition, BTEX also had chronic effects (i.e. long-term) including changes to the liver and harmful effects on the kidney, heart, lungs, and nervous system. Even immunological, reproductive, fetotoxic, and genotoxic effects were also found to be associated with BTEX compounds.[2–4] Researchers are aware of BTEX for their odour nuisance as well as toxic characteristics, because human beings are frequently exposed to inhalation of airborne BTEX vapour.

So, the removal of BTEX from the atmosphere has received increased attention in recent decades. There are many chemical and physical methods available for removing BTEX from the atmosphere. However, in most cases, these conventional physical–chemical methods, such as vapour scrubbing from the air and adsorption onto activated carbon and other

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adsorbents, are often unsatisfactory for treating low molecular weight VOCs. Organic pollutants may be transferred from the gaseous phase to other phases, and still not be fully destroyed, resulting in the problem of subsequent disposal of the resulting solution and/or solid waste.[5]

In addition to the above-mentioned two technologies, many other psychical–chemical methods, such as photocatalytic oxidation,[6] thermal incineration and catalytic incineration, are efficient abatement technologies for the mineralization of VOCs. But all these technologies are costly compared with biological methods.

Biotreatment processes are environmentally friendly, efficient and cost-effective methods for the mineralization of VOCs, with low energy demand, no need for fossil fuel burning, and are a low temperature treatment. Recently, the biotrickling filter was introduced for the treatment of waste gases. This promising reactor is not only capable of eliminating high concentration pollutants, but also can mineralize a broader range of organic pollutants as well as odour control. Effective removal of various organic pollutants has been reported, for example, nonchlorinated VOCs,[7] polychlorinated VOCs[8] and odorous N- and S-containing compounds[9] using biotrickling filters as bioreactors. Most research work in this field has focused on the selection of packing materials and optimizing process parameters to improve the removal efficiency (RE) of organic pollutants, rather than on microbiological aspects.[10]

However, microorganisms were expected to be the most critical parameter in a bioreactor. In the present work, the degradation efficiencies of toluene with two different strains of gasoline-adapted bacteria, identified as *Bacillus cereus* S1 and *Bacillus cereus* S2, cultivated using the same liquid-microcosm approach were compared in twin biotrickling filter beds. Although these two strains belong to the same family, the same genus and even the same species, the removal efficiencies were different in the two subspecies in terms of BTEX degradation, in this case using toluene as a model VOC. The influence of gas flow rates, variation in inlet toluene concentrations and the effect of starvation mode (without airflow and without liquid recycle) on the toluene degradation performance were also investigated in detail.

MATERIALS AND METHODS

Organisms and culture medium

Two microorganism strains were obtained from a wastewater treatment plant of Guangzhou Petrochemical Corporation, and were used to degrade organic pollutants in oily wastewater. The medium used for organisms S1 and S2 was a mineral medium comprising 21.75 g L^{-1} $K_2HPO_4 \cdot H_2O$, 33.40 g L^{-1} $Na_2HPO_4 \cdot 12H_2O$, 8.5 g L^{-1} KH_2PO_4, 40 g L^{-1} NH_4Cl, 22.5 g L^{-1} $MgSO_4$, 36.4 g L^{-1} $CaCl_2$, 0.25 g L^{-1} $FeCl_3$, 0.04 g L^{-1} $MnSO_4 \cdot H_2O$, 0.04 g L^{-1} $ZnSO_4 \cdot H_2O$, 0.04 g L^{-1} $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. All the chemicals were analytical grade reagents (AR), and purchased from Guangzhou Chemical Reagent Co., Inc. (China). The pH value of the mineral medium was adjusted to 5.5 before use. Colonies of S1 were circular, entire, opaque, lipidic, flat, wet, cream-coloured with a diameter of 0.5–3 mm, whereas colonies of S2 were circular, entire, opaque, lipidic, convex, wet, kelly-coloured with a diameter of 1–2 mm. An optical microscope (Leica DMRX, Wetzlar, Germany) was used for the morphological observation of strains S1 and S2. Both bacterial isolates were Gram-positive rods or coccobacilli (S1: ~2 μm; S2: ~4 μm) (Fig. 1(A) and 1(B)). Molecular identification of the S1 and S2 strains were performed according to the method for identification of bacteria using 16S rDNA[11] from the Guangdong Institute of Microbiology, China. The amplified fragment results, based on partial 16S rDNA sequence (863 bp) of S1 and 16S rDNA sequence (869 bp) of S2, indicated that both strain S1 and S2 were closely related to *Bacillus cereus* ATCC BAA-1005 with 100% similarity.

Experimental set-up and standard operation of biotrickling filter

The biological reactor used in the experiment was a custom-made biotrickling filter; a schematic diagram is shown in Fig. 2. It comprises two identical plexiglass columns allowing for parallel or comparison operation of two biotrickling filter beds; the performances of the two columns were considered to be directly comparable. Each biotrickling filter column was constructed of six layers with an internal diameter of 14 cm and 120 cm height, packed with 36 cm of ceramic particles (irregular size, 2–4 mm, specific gravity 1.52 g cm^{-3}, porosity of 55–70%, and with a specific surface area of approximately 3.99 $m^2 g^{-1}$, manufactured by Boshan ceramic factory, Shandong, China). In order to support the filter bed and to ensure homogeneous radial distribution of input gas, a plexiglass mesh was installed at each layer. Sampling ports were located at fixed interval of 15 cm along the height of the columns. The packing materials were inoculated with strains S1 in the left and S2 in the right column of the biotrickling filter, respectively. The air pump, nutrient distributor and peristaltic pump were all controlled by microcomputers. A 150 mL mineral salt medium was trickled every 1.5 h over the bed upper surface to maintain an adequate level of bed filling moisture content and to provide the necessary nutrients for the growth of microorganisms. In order to shorten the start-up phase, optimal nutrient salt and gas–liquid phase toluene were added; the biofilms were completely established after 12 days operation.

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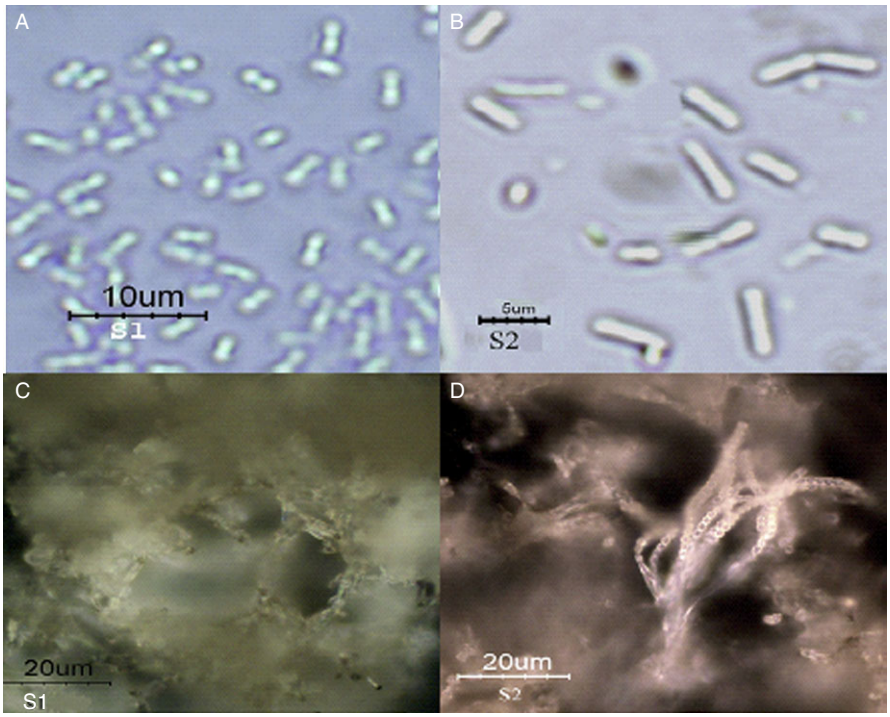

Figure 1. Electron micrograph: (A) *Bacillus cereus* S1 (1000×); (B) *Bacillus cereus* S2 (1000×); (C) structure of *Bacillus cereus* S1 biofilm on the ceramic saddle packed material (500×), and (D) *Bacillus cereus* S2 biofilm on the ceramic saddle packed material (500×).

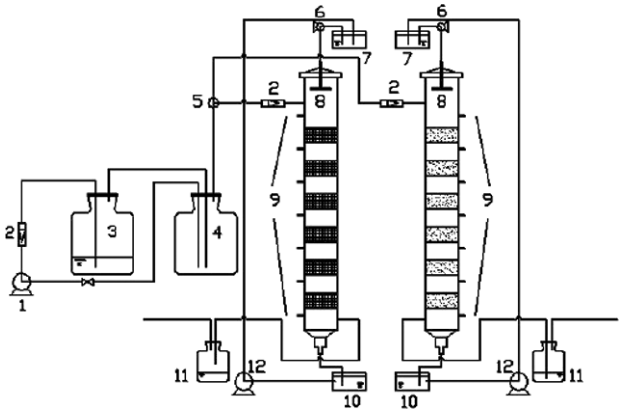

Figure 2. Schematic representation of the experimental biotrickling filter unit: (1) air pump; (2) air flow meter; (3) toluene tank; (4) mixing gas tank; (5) Y-bend; (6) peristaltic pump; (7) medium tank; (8) nutrient distributor; (9) sampling ports; (10) leachate tank; (11) waste gas tank; (12) circulating pump.

Optical microscope photographs were also taken to observe the microbial growth on the ceramic particles. Figure 1(C) and 1(D) show the surface morphologies of S1 and S2 biofilms adhering to the surface of the ceramic particles (×500 magnification). Different shaped microorganism biofilms were formed during the start-up procedure in the two biotrickling filter columns. From Fig. 1(C), it can be seen that the S1 biofilm has an irregular surface, and appears very thick in the porous particle structure. In contrast, many chain-like linear arrangements resembling the organization of fungi are observed on ceramic particle surfaces covered with S2 biofilm.

The start-up procedures were as follows: first, sterile distilled water was passed through the biotrickling filter bed with sterilized ceramic particles installed. After purification, the two air streams were mixed and passed through the packed bed for the degradation experiments. One stream was distributed to sparge the toluene vapour from the liquid bottle; the other stream flowed directly into the mixing bottle to dilute the toluene vapour. The toluene concentrations in the mixed streams were changed by adjusting the gas flow rate of the former stream.

Analytical methods

Air samples were collected at regular time intervals from the inlet, outlet and from the various sampling ports using an airtight syringe. Toluene concentrations were determined using a HP 5890 gas chromatography equipped with a FID detector (Hewlett-packard, USA). A DB-5MS (30 m × 0.25 mm × 0.25 um) capillary column was used for chromatographic separation. The column temperature was programmed to hold at 40 °C for 2 min, increase from 40 °C to 100 °C at 3 °C min^{-1}, followed by an increase to 200 °C at 25 °C min^{-1} (held for 1 min). The carrier gas was ultra-high purity nitrogen at a constant flow rate of 2.2 mL min^{-1}. And a 200 μL gas sample was injected into the column for the concentration determination in the splitless mode.

RESULTS AND DISCUSSION

Effects of the gas flow rates

The effect of gas flow rates on the removal efficiencies of the biotrickling filter filled with ceramic particles having S1 or S2 biofilms were investigated at a fixed toluene inlet concentration of about 2.0 mg L^{-1}. The

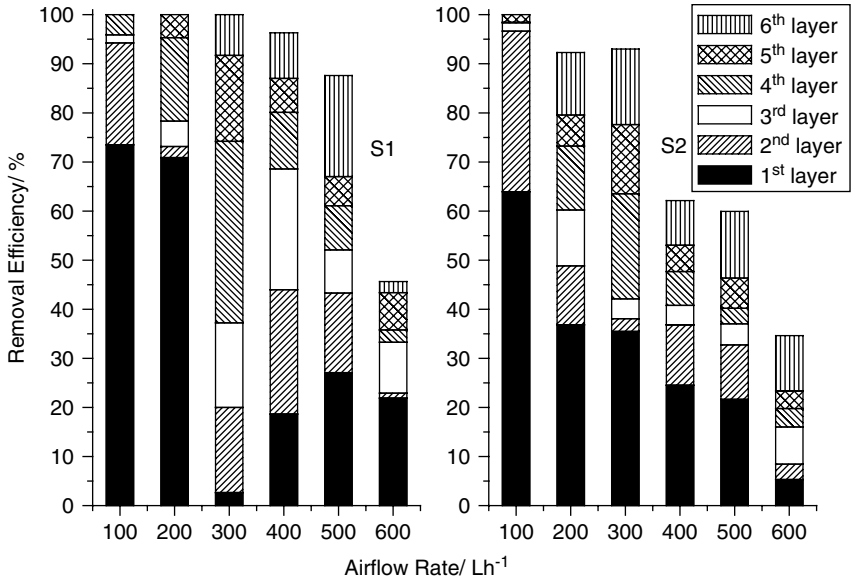

Figure 3. Removal efficiency comparison of biotrickling filter filled with ceramic particles having *Bacillus cereus* S1 or *Bacillus cereus* S2 biofilm at different gas flow rates: initial concentration (S1) = initial concentration (S2) = 2.0 mg L^{-1}.

gas flow rates were varied between 100 and 600 L h^{-1}. Figure 3 shows the influence of gas flow rates on the RE of toluene in the biotrickling filter columns. Overall, higher REs were obtained at lower gas flow rates, while higher gas flow rates led to lower removal rates for both S1 and S2 strains. However, S1 and S2 had different degradation trends. For the strain S2, the maximum total RE of 100% was achieved only at a gas flow rate of 100 L h^{-1}, which corresponds to an empty bed residence time (EBRT) of 199.44 s (Table 1). In this period, 97% of the toluene was degraded by the first layer (64%) and second layer (33%) of the biotrickling filter added together. In other words, the first and second layers play an important role in removing toluene. Eventually, after the toluene had passed through the fifth layer, 100% of the pollutant was removed. With further increase in gas flow rate and decrease in EBRT, REs decreased slightly until a gas flow rate of 400 L h^{-1}. Finally, total RE dropped sharply to 35% at an EBRT of 33.24 s. At this stage, the removal rate of each layer also changed accordingly, but the removal rate of first layer showed the same trend as the total removal rate. The total elimination capacity (EC) trend for strain S2 were almost the same as the REs, that is, lower EBRT results in both lower RE and EC (Table 1). For EBRT falling from 199.44 to 39.89 s, EC decreased slowly from 152.02 to 101.52 g m^{-3} h^{-1}; then fell abruptly to 55.77 g m^{-3} h^{-1} at EBRT = 33.24 s.

In contrast to S2, the strain S1 had a much higher RE at the same treatment conditions. Total toluene RE_{max} was maintained at 100% not only at an EBRT of 199.44 s (100 L h^{-1}), but also at EBRTs of 99.72 s (200 L h^{-1}) and 66.48 s (300 L h^{-1}). It is also worth pointing out that the degradation trends in the bioreactors were different at gas flow rates of 100, 200 and 300 L h^{-1}, respectively. That is to say, 100% removal rates were obtained after toluene passed the fourth, fifth and sixth layers at gas flow rates 100, 200 and 300 L h^{-1}, respectively. For further increases in gas flow rates (400 and 500 L h^{-1}), there was a slightly drop in removal rates (RE = 97% at 400 L h^{-1} and RE = 88% at 500 L h^{-1}). When gas flow rate was increased from 500 to 600 L h^{-1}, the RE dropped significantly, from 88% down to 46%. This is because the reaction efficiencies were mainly controlled by mass transfer of toluene from the air to the biofilm and gas phase boundary layer, which were in turn

Table 1. Removal efficiency at different gas flow rate as a function of EBRT

		Bacillus cereus S1		*Bacillus cereus* S2	
Gas flow rate (L h^{-1})	EBRT (s)	Removal efficiency (%)	Total elimination capacity (g m^{-3} h^{-1})	Removal efficiency (%)	Total elimination capacity (g m^{-3} h^{-1})
100	199.44	100	115.09	100	152.02
200	99.72	100	112.09	92	141.67
300	66.48	100	113.07	93	135.85
400	49.86	96	113.69	62	103.50
500	39.89	88	104.78	60	101.52
600	33.24	46	52.49	35	55.77

Initial concentration (S1) = Initial concentration (S2) = 2.0 mg L^{-1}.

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controlled by the residence time. Thus, the lower the gas flow rate, the longer the gas contact time between toluene and microorganisms. There is then adequate time for microbes to completely biodegrade toluene after entering the biofilm; thus lower gas flow rate results in higher REs. In the case of high gas flow rates, RE was very low due to the shorter residence time, and thus the toluene was unable to make sufficient contact between gas and biofilm for degradation. This conforms to the viewpoint that gas flow rate is a significant limiting parameter in biotrickling filtration.

In summary, when gas flow rates were no higher than 300 L h^{-1}, there was a high, steady RE for both S1 and S2 strains. Thus, in all further experiments, 300 L h^{-1} was chosen as the optimum gas flow rate for the degradation of toluene. It is also worthwhile pointing out that strain S1 had a lower EC and higher RE than strain S2 at the same treatment conditions. Overall, strain S1 had a steadier removal capacity and less fluctuation than strain S2.

Effects of variation of inlet toluene concentrations

Keeping the flow rate fixed at 300 L h^{-1} and EBRT fixed at 66.48 s, the toluene concentration was increased from 0.72 to 10.00 mg L^{-1} for strain S1, and from 0.49 to 5.72 mg L^{-1} for strain S2. The response of the biotrickling filter was determined by regularly analysing the outlet gas concentrations of each layer. After adjusting toluene concentrations, the system was allowed to stabilize for 24 h before again changing the concentration. The ECs of S1 and S2 at standard operating conditions in each layer and the total ECs at different concentrations are presented in Fig. 4. From the figure, it can be seen that toluene concentration plays an important role in the RE of the biotrickling reactor. The total ECs of outlet gas in both biotrickling filter columns initially increased rapidly to a maximum value and then fell. For the strain S1, total RE approached 99% (Table 2) at an inlet toluene concentration of 0.72 mg L^{-1}, but the total EC was only about 35.63 g $m^{-3} \cdot h^{-1}$. As toluene concentration was further increase to 4.75 mg L^{-1}, the ECs increased dramatically to 223.82 g m^{-3} h^{-1}. It is also worth pointing out that the total RE did not show much decrease during this period. Total EC increased slightly when toluene concentration was increased from 4.75 to 5.81 mg L^{-1} (EC = 233.73 g $m^{-3} \cdot h^{-1}$), while total RE fell sharply from 94% to 80%. Then a steep increase in total EC was observed; a maximum EC of 300.47 g $m^{-3} \cdot h^{-1}$ was reached, and a slightly lowered RE (75%) with further increase in toluene inlet concentration to 8.19 mg L^{-1}. This total EC value greatly exceeded the maximum values reported for toluene degradation in biotrickling filters as summarized by Cox[12] and by Iranpour.[13] The maximum toluene inlet concentrations were 2.6 to 52.6 times greater than the toluene inlet concentrations typically reported in other bacterial systems. The EC values obtained were much higher than the maximum toluene ECs obtained using a new design of reactor (285 g $m^{-3} \cdot h^{-1}$)[14] and a fungi bioreactor (270 g $m^{-3} \cdot h^{-1}$).[15] When the inlet concentration was set to 10.00 mg L^{-1}, both lower total EC (276.23 g $m^{-3} \cdot h^{-1}$) and lower total RE (58%) were obtained. The results suggest that the biotrickling filter performance will decrease even further if toluene inlet concentrations are further increased.

Strain S2 showed almost the same trend as S1: total EC increased with increasing toluene concentration, and toluene degradation reached a peak at an inlet toluene concentration of 4.72 mg L^{-1}, with a maximum EC of 228.87 g $m^{-3} \cdot h^{-1}$ (RE = 97%)

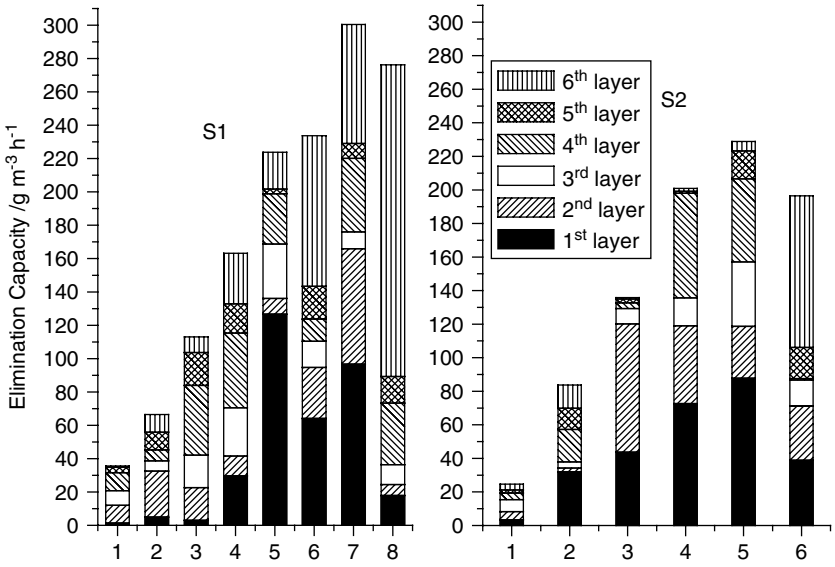

Figure 4. Elimination capacities comparison of biotrickling filter filled with ceramic particles having *Bacillus cereus* S1 and *Bacillus cereus* S2 biofilm at different initial concentrations: gas flow rate (S2) = gas flow rate (S1) = 300 L h^{-1}. S1: 1: 0.72 mg L^{-1}; 2: 1.35 mg L^{-1}; 3: 2.28 mg L^{-1}; 4: 3.34 mg L^{-1}; 5: 4.75 mg L^{-1}; 6: 5.81 mg L^{-1}; 7: 8.19 mg L^{-1}; 8:10.00 mg L^{-1}. S2: 1: 0.49 mg L^{-1}; 2: 1.80 mg L^{-1}; 3: 2.72 mg L^{-1}; 4: 4.14 mg L^{-1}; 5: 4.72 mg L^{-1}; 6: 5.72 mg L^{-1}.

Table 2. Removal efficiency of S1 and S2 at different initial concentrations of toluene

Bacillus cereus S1			*Bacillus cereus* S2		
Concentration (mg L^{-1})	Total removal efficiency (%)	Total elimination capacity (g m^{-3} h^{-1})	Concentration (mg L^{-1})	Total removal efficiency (%)	Total elimination capacity (g m^{-3} h^{-1})
0.72	99	35.63	0.49	100	24.70
1.35	99	66.44	1.80	93	83.76
2.28	100	113.07	2.72	100	135.83
3.34	98	163.20	4.14	97	200.91
4.75	94	223.82	4.72	97	228.87
5.81	80	233.73	5.72	67	196.51
8.19	75	300.47			
10.00	58	276.23			
Average	88	176.57		92	145.10

Gas flow rate (S1) = Gas flow rate (S2) = 300L h^{-1}.

(Table 2; Fig. 4). A possible explanation is that as concentration increased, a higher concentration of contaminant occurred in the biofilm, resulting in faster specific biodegradation rates and higher ECs. A slight decrease in toluene EC (196.51 g m^{-3} h^{-1}) and dramatic drop in total RE (67%) were observed when the inlet toluene concentration was increased to 5.72 mg L^{-1}. Clearly, total toluene ECs and REs will decrease with further increase in toluene concentration for strain S2. The main difference between S1 and S2 is that the strain S2 has much lower EC and RE values than strain S1 at the approximate equivalent inlet concentration. But it is worthwhile mentioning that at this point, the EC of S2 reached a maximum, while EC of S1 was still increasing. In other words, the performance of the biotrickling filter seeded with strain S1 was much steadier, (with an average toluene EC of 176.57 g m^{-3} h^{-1} at all toluene concentrations tested) than that of S2 (average toluene EC of 145.10 g $m^{-3} \cdot h^{-1}$) at an EBRT of 66.48 s. Although maximum toluene inlet concentration (5.72 mg L^{-1}) and EC (228.87 g m^{-3} h^{-1}) for S2 were slightly lower than those of S1, the total maximum toluene EC was 1.8 to 12.7 times greater and the inlet concentration was 1.5 to 30.1 times higher than those reported for other bacterial systems.[12] Also, from Fig. 4, it is worth mentioning that the sixth layer microbes play a much more important role in the removal of toluene when the toluene concentration is near maximum for both S1 (10.00 mg L^{-1}) and S2 (5.72 mg L^{-1}) strains.

Biotrickling filter re-acclimation

The performance of biotrickling filters is usually studied under relatively ideal conditions, such as steady-state operation in laboratory systems. However, the operation of pilot scale biotrickling filters in industrial settings may give rise to operational problems undetected in the laboratory.[16] In particular, repeated periods of non-use were identified as one of the factors that caused lower pollutant elimination in the field. So, it is very meaningful to investigate the recovery of the biotrickling filter after a certain starvation period. That is, the bacteria were starved of toluene for a short period (i.e. no carbon source for the microorganisms) and mineral medium, to simulate complete shutdown and to test the recovery capacity. For each of the biotrickling filter columns, the standard operation (toluene-containing air at a concentration of about 2 mg L^{-1} was passed through the biotrickling filter bed at a flow rate of 300 L h^{-1}, corresponding to an EBRT of 66.48 s) was resumed after starvation for 2, 4, 6 and 10 days. The effect of starvation was determined by comparing the REs of toluene before and after the different starvation intervals.

Figure 5 demonstrates the re-acclimation profiles of toluene REs after resuming at the standard operating conditions for several starvation intervals. It was observed clearly that, for the strain S2, after 2, 4, 6 and 10 days of starvation there was a sudden decline in RE each time, which could be due to the death of microorganisms and lysis during starvation.[17] This recovered quickly, with recovery times of 3 h (RE = 91%), 4.5 h (RE = 75%), 9.5 h (RE = 75%) and 15.5 h (RE = 83%) after 2, 4, 6 and 10 days starvation, respectively. It should be noted that, the maximum REs of S2 each time were much higher than 65%, which was the RE before the starvation period. The same fact was also observed by Du *et al.*[18], who found that proper starvation can improve the REs of microorganisms. This may be interpreted as the long-term operations of a biotrickling filter will often lead to excess biomass formation and clogging of the biotrickling filter bed when nutrient loadings and pollutant concentrations are high. A decrease in the amount of biomass during starvation will increase the overall lifespan of the biotrickling filter,[19] and thus, maintain or even improve the EC of organic pollutants in the biotrickling filter.

From Fig. 5 it is also found that the REs fluctuated widely and there was no apparent correlation with elapsed time during each re-acclimation period after starvation. Two probable explanations for the observed phenomena may exist. One is that a significant decrease in microbial population may occur during starvation due to biomass death and lysis;[17] the other reason is that the microbes may lose degrading

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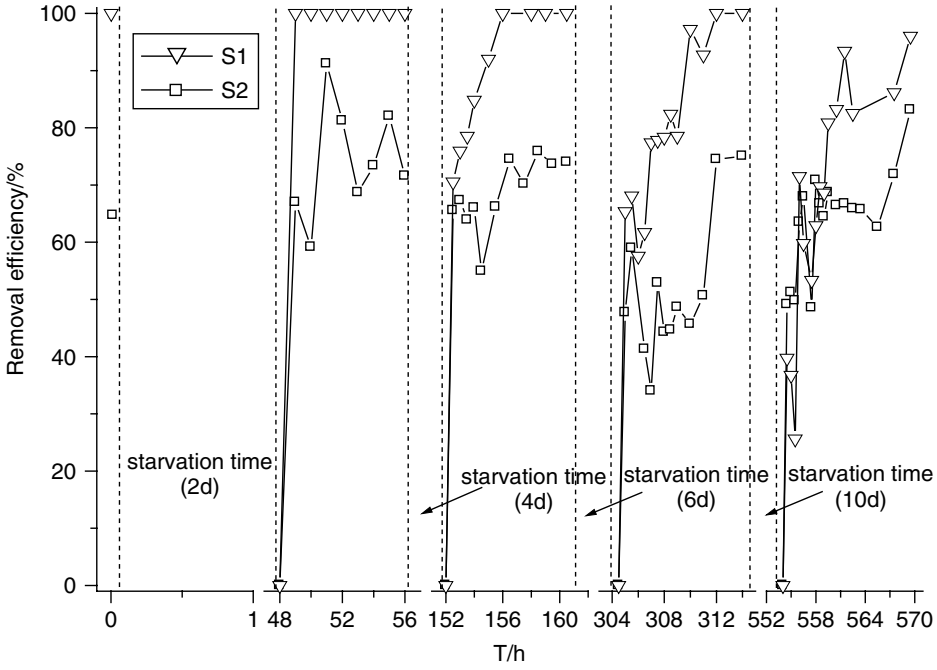

Figure 5. Re-acclimation period after starvation: initial concentration (S1) = initial concentration (S2) = 2.0 mg · L^{-1}; gas flow rate (S1) = gas flow rate (S2) = 300 L h^{-1}.

activity during the period of absence of toluene.[20] Thus the removal capacity of live microorganisms is reduced, and some of them cannot re-acclimatize to the inlet toluene instantly after starvation. Another reason could concern the liquid film thickness and the biofilm surface morphology. That is to say, periodically trickled mineral salt medium may result in different thicknesses of liquid film and biofilm. A thinner liquid film can shorten the diffusion distance of toluene, and capture by bacteria of toluene molecules is easier and more rapid with a thin film. The capability of the biotrickling filter to purify toluene in the waste gas is thus intensified, and vice versa,[8] because most of the degradation occurs in the biofilm, although in selected cases, significant activity can occur in the recycle liquid.[21]

For strain S1, the degradation capacity is much higher than that of strain S2. After 2 days starvation, no apparent acclimation period was observed. Only 1 h of recovery time needed for total toluene RE to recover to 100%. Then, after 4 days and 6 days starvation, the recovery trends were very similar to the pattern for S2. That is, there was a sudden fall in toluene REs and subsequently a quick recovery. The recovery times were 4 h (RE = 100%) and 7.5 h (RE = 100%) after 4 days and 6 days starvation, respectively. And finally, a recovery time of 15.5 h was obtained after 10 days starvation, and maximum RE remains very high, up to 96%. It is worthwhile pointing out that the REs of S1 are all higher than the maximum REs of S2 for any starvation period.

From this experiment, it was found that the re-acclimation times for both strains to reach their full capacities were much shorter than other researcher have found: 10–24 h recovery time for 2–9 days interruption,[19] 3 days for an 8 day interruption,[17] and 5 h for a 2 to 3 day interruption[22], to re-establish full performance. In this current work, the re-acclimation times to reach full capacity were very short, ranging between 1 and 15.5 h for S1, and between 3 and 15.5 h for S2. Weekend shutdown periods (2 days) had no noticeable adverse effect on the REs of toluene, and both strains recovered very quickly. This illustrates that the biotrickling filter could be used effectively to treat discontinuously generated toluene emitted from systems with weekend shutdowns. However, a longer recovery time than that for weekend shutdown will be required when a longer starvation occurs, for example, 10 days shutdown. Thus, we can safely conclude that *Bacillus cereus* S1 not only has a much higher toluene removal capacity, but also can recover its full removal capacity more swiftly than *Bacillus cereus* S2 in these packed biotrickling filter experiments.

CONCLUSIONS

Results showed that the REs of toluene in the biotrickling filter strongly depended on both EBRTs and microorganisms. At the same treatment conditions, with decreasing EBRTs (from 199.44 to 33.24 s), the REs of both *Bacillus cereus* S1 and *Bacillus cereus* S2 decreased gradually. Overall, the column seeded with S1 showed better performance than that seeded with S2. The RE fell from 100% to 46% for S1, and from 100% to 35% for S2, respectively. S1 reached an RE of 100% at EBRTs ranging from 66.48 to 199.44 s, while for S2, 100% toluene RE was obtained only at an EBRT of 199.44 s. The performance of the two strains for the degradation of toluene at different concentrations also varied dramatically. Strain S1

always exhibited better ECs and REs than those for S2. At a gas flow rate of 300 L h^{-1} (EBRT = 66.48 s), the maximum total ECs of S1 and S2 were 300.47 and 228.87 g m^{-3} h^{-1} at inlet concentrations of 8.19 and 4.72 mg L^{-1}, respectively. The biotrickling filter was found to be capable of withstanding different periods of starvation (2–10 days) with rapid recovery times (1–15.5 h) to full performance after starvation. Longer periods of idle phase required longer periods of re-acclimation. Overall, strain S1 is a better choice for the removal of toluene in the biotrickling filter than strain S2, because of its higher removal capacity and rapid recovery to full capacity after different starvation intervals.

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