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Treatment of Methyl *tert*-Butyl Ether Vapors in Biotrickling Filters. 1. Reactor Startup, Steady-State Performance, and Culture Characteristics

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An aerobic microbial consortium able to biodegrade methyl *tert*-butyl ether (MTBE) was enriched in two waste air biotrickling filters after continuous operation for 6 months. After this acclimation phase, the two laboratory-scale biotrickling filters were able to degrade up to 50 g of MTBE per cubic meter of reactor per hour, a value comparable to other gasoline constituents. Such high performance could be sustained for at least 4−6 weeks. After the acclimation phase, the MTBE degrading biotrickling filters were characterized by their almost full conversion (∼97%) of MTBE to carbon dioxide and the absence of any degradation byproducts in either the gas or the liquid phase. They also exhibited a very high specific degradation activity per amount of biomass (5−10 mg of MTBE per gram dry weight of cell per hour), and a low rate of biomass accumulation. An observed biomass yield of 0.1 g g^{-1} and a specific growth rate of 0.025 day^{-1} were determined for the biotrickling filter process culture. Further data on MTBE mass transfer and on the dynamic behavior of the biotrickling filter are presented in part 2 of this paper. Overall, the results demonstrate that MTBE can be effectively biodegraded under carefully controlled environmental conditions.

Introduction

Methyl *tert*-butyl ether (MTBE) was first introduced in United States in the late 1970s as an octane enhancer in gasoline. The use of MTBE increased rapidly after the 1990 Clean Air Act Amendments required urban areas in nonattainment zones to oxygenate their gasoline in order to reduce tailpipe carbon monoxide and other smog forming emissions. While other oxygenates such as methanol, ethanol, or aliphatic ethers are sporadically used in reformulated gasoline, MTBE has emerged as one of the best oxygenates because of its low cost and blending characteristics. Depending on the season, reformulated gasoline contains 11−15% MTBE by volume. As a result, the production of MTBE has grown rapidly and ranked second among all chemicals in 1993. At the same time, an increasing number of groundwater contaminations with MTBE have been reported (*1*, *2*). While MTBE is thought to be less harmful than other gasoline constituents such as benzene, there is still relatively incomplete knowledge on its health effects (*3*, *4*). Currently, the U.S. Environmental Protection Agency recommends 20−40 ppb as the indicative Health Advisory level for drinking water (*4*). In California, ongoing discussions based on water esthetic criteria (MTBE odor threshold is 45 to 95 ppb and taste threshold is about 135 ppb) target levels as low as 5 or 14 ppb for drinking water. In any event, the challenge will be to find cost effective solutions to prevent contamination of drinking water supplies with the oxygenate while mitigating existing contaminations from leaking underground storage tanks and leaking pipelines.

In the past few years, several studies have been conducted to determine if natural attenuation occurred at MTBE contaminated sites (*5*−*8*). In one of the studies, MTBE mass disappearance was noticed over time, but it could not be firmly demonstrated that in situ biodegradation of MTBE occurred (*5*). Therefore, MTBE is still often considered as a persistent contaminant. Further reasons of concern are that MTBE does not significantly sorb to soil particles and is highly water soluble. Hence MTBE plumes were found to move much more rapidly than usual benzene, toluene, ethyl benzene, and xylene (BTEX) plumes, with in most cases no clear signs of natural attenuation (*2*).

Laboratory experiments were so far unsuccessful in demonstrating MTBE biodegradation under anaerobic conditions (*8*−*11*). While it was first thought that MTBE was aerobically recalcitrant (*12*), recent studies have since shown that MTBE is biodegradable under various aerobic controlled environments (*8*, *13*−*19*). In some cases, complete degradation was observed (*13*, *16*−*19*) while others only reported partial transformation (*8*, *15*). Some studies involved co-metabolism by alkane degrading organisms, possibly involving a Cytochrom P-450 (*16*, *20*, *21*), while in others MTBE was used as the sole carbon and energy source (*14*, *18*, this paper). Two MTBE degrading cultures nitrified ammonia (*8*, *13*). Most cultures were thought to rely on prokaryote activity, but at least one report involved filamentous fungi co-metabolizing MTBE (*20*). The various studies seem to agree on a relatively slow growth rate for the different MTBE degrading cultures, low biomass yields, and sometimes culture instability.

This, together with the poor definition of the microbial mechanisms involved during MTBE biodegradation, has, up to date, hampered the development of viable bioremediation processes for MTBE. Unfortunately, MTBE has quite different physicochemical properties than its aromatic co-pollutants, making it more expensive to treat MTBE wastes with conventional techniques (*22*, *23*). In many remediation cases, such as air sparging, soil vapor extraction, air stripping, or wastewater treatment operations, large air streams contaminated with MTBE are generated that require further treatment. This poses a serious economic challenge. Adsorption of MTBE vapors requires disposal of spent adsorbent while catalytic oxidation generates nitrogen oxides and requires additional fuel. Other emerging technologies such as membrane processes or advanced oxidation process are not yet ready to be deployed in the field. Alternatively, biological waste air treatment has proven to be an extremely cost effective and environmentally friendly technology for the control of large air streams contaminated with moderate concentrations of volatile organic compounds (*24*, *25*). The most promising bioreactors for air pollution control are biofilters and biotrickling filters.

Biofilters work by passing a humid stream of contaminated air through a damp packing material—usually compost with bulking agents—on which pollutant degrading bacteria are

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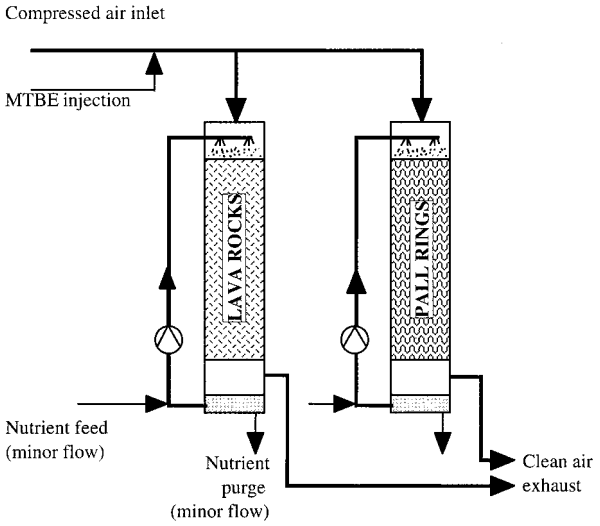

FIGURE 1. Schematic diagram of the experimental setup.

naturally immobilized (*24*, *26*). Biotrickling filtration is a variation of biofiltration where an inert support is used and a scrubbing solution is continuously or intermittently recycled over the packing to provide the process culture with the necessary moisture, nutrients, and optimal conditions (*27*). While biotrickling filters are more complex and often more expensive to operate than biofilters, they often exhibit higher performance than biofilters. This is because they allow a better control of environmental conditions and because they rely on growing organisms rather than on resting organisms as in the case of biofilters. Typical successful applications for biotrickling filters include the treatment of chlorinated volatile (*28*, *29*) or reduced sulfur compounds (*30*, *31*) for which a good control of the pH and a continuous purging of degradation metabolites (chloride and sulfate, respectively) are essential. Other successful applications include the treatment of volatile organic compounds (VOCs) and hazardous air pollutants (HAPs). Because biotrickling filters allow for strict control of process culture conditions, they offer promise for the treatment of MTBE, for which biodegradation is difficult and may result in the accumulation of partially oxidized products such as formaldehyde or *tert*-butyl alcohol (TBA) or in pH changes due to nitrification. Recent biofiltration work at a wastewater treatment plant found that it took more than a year before biodegradation of MTBE began. The performance was typically in the range of 6−8 g m^{-3} h^{-1} with 95−100% removal (*18*). Hence the motivation for the present study was to develop a more efficient and sustainable process for MTBE vapor control. This study reports on the enrichment of a MTBE degrading consortium capable of using MTBE as the sole carbon and energy source and on the deployment of the consortium in two laboratory-scale biotrickling filters. Aspects pertaining to the MTBE mass transfer in biotrickling filters and to the dynamic behavior of MTBE treatment are presented in part 2 of this paper (*32*).

Materials and Methods

Biotrickling Filter Setup and Operating Conditions. Two parallel laboratory-scale biotrickling filters were constructed and served both culture enrichment and to investigate the performance of MTBE removal from synthetic waste gas. A schematic of the experimental apparatus is shown in Figure 1. Each trickling filter consisted of clear PVC pipe with a packed bed height of 0.5 m (total reactor height: 1.5 m, internal diameter: 0.153 m, bed volume: 9 L). Reactor 1 was filled with 8.81 kg of wet lava rock (1−3 cm diameter, initial bed porosity of 50%), and reactor 2 was filled with 0.94 kg of 2.5 cm polypropylene Pall rings (Flexirings, Koch Engineering, Wichita, KS). The Pall ring bed had an initial porosity of 90% and a specific surface area of 206 m^2 m^{-3}. A mass flow controller (Series 200F, Porter Instrument Company, Inc., Hatfield, PA) was used to provide a metered flow of synthetic waste air to both reactors. Liquid MTBE (purity greater than 99.9%, Fisher Scientific, Pittsburgh, PA) was directly injected into the air stream using a metering pump (QVG50-RH00STY, Fluid Metering, Inc., Syosset, NY). The resulting synthetic waste gas was introduced at the top of the column (co-current flow). The reactor temperature was maintained between 19 and 21 °C.

An aqueous mineral medium liquid phase was continuously recirculated over the packed beds using centrifugal pumps (Dayton Electric Mfg. Co., Chicago, IL) at a volumetric flow rate of 0.15 m^3 h^{-1}. Fresh mineral medium (in g L^{-1}: K_2HPO_4, 2; KH_2PO_4, 1; NH_4Cl, 0.75; $MgSO_4$, 0.5; $CaCl_2$, 0.018; and 1 mL L^{-1} trace element solution (*33*)) was continuously fed by a peristaltic pump at an average flow rate of 50 mL h^{-1}. All chemicals were of high-purity grade (>99.9%, Fisher Scientific, Pittsburgh, PA). At selected times, peat humic substances (PHS) (Biogene, Prodex, Acron, OH) were added to the medium at a concentration of 0.25 mg L^{-1}. A constant liquid volume of 3 L was maintained at the bottom of the reactors by an overflow outlet, and the dynamic holdup in each biotrickling filter was constant at approximately 0.8 L. Apart from their packing material, the operation of the two parallel biotrickling filters was identical. Under these conditions, absorption and/or diffusion of MTBE through PVC pipe was found to be negligible. In both cases, the pressure drop across the beds remained below 5 cm of water gauge. An overview of the experimental plan is reported in Table 1. Throughout the experiments, the MTBE inlet concentration was generally maintained between 0.65 and 0.85 g m^{-3}.

Definitions and Performance Reporting. The performance of the systems was expressed as elimination capacity (EC) or removal efficiency (RE), defined in eqs 1 and 2, as a function of the inlet and outlet gas concentrations (C_g), the air flow rate (Q), and the packed bed volume (V). The elimination capacity represents the amount of pollutant degraded per unit of trickling filter bed volume and time; it is often reported as a function of the pollutant loading L (eq 3).

$$\mathrm{EC} = \frac{(C_{g,in} - C_{g,out})Q}{V} \qquad (\mathrm{g\ m^{-3}\ h^{-1}}) \qquad (1)$$

$$\mathrm{RE} = \frac{(C_{g,in} - C_{g,out})100}{C_{g,in}} \qquad (\%) \qquad (2)$$

$$\mathrm{L} = \frac{C_{g,in}Q}{V} \qquad (\mathrm{g\ m^{-3}\ h^{-1}}) \qquad (3)$$

MTBE and Other Analyses. *Liquid Samples Analysis.* A cell-free aqueous sample (1 μL) was automatically injected into a Hewlett-Packard model 6890 GC fitted with a HP-FFAP column (50 m × 320 μm × 0.5 μm). The injection was by pulsed splitless injection mode. The oven temperature was held at 80 °C for 1 min and was ramped to 120 °C (50 °C min^{-1}) while the pressure remained constant (initial gas flow through the column: 1.2 mL min^{-1}). The detection was with a flame ionization detector (FID). MTBE and *tert*-butyl alcohol (TBA) were quantified using standards of known concentrations. The detection limit for MTBE was about 2 mg L^{-1}. Formaldehyde was analyzed with the chromotropic acid method as described by Levaggi and Feldstein (*34*).

Gas Samples Analysis. Grab samples from either inlet or outlet streams were automatically injected into a Hewlett-Packard 5890 Series II gas chromatograph by a 10-port gas sampling valve. Simultaneous analysis of CO_2 and MTBE or

TABLE 1. Overview of the Experimental Schedule

day	characteristic operation	EBRT[a] (s)
−180 to 0	reactor startup, various attempts to enhance performance, no or minimal MTBE removal, liquid feed as fill and draw (50% of the scrubbing liquid is replaced every day to every other day)	90
0 to 26	reinoculation on day 0	90
26	scrubbing liquid feed is switched to continuous (50 mL h^{-1} per reactor)	90
33	PHS addition in the medium	90
38	reactor 2 is re-inoculated with liquid from reactor 1	90
63	volumetric load increase	54
69	transient behavior studies (see part 2 (*32*)): inlet concentration is increased from 0.66 to 0.82 g m^{-3}	54
70	transient behavior studies (see part 2 (*32*)): EBRT is reduced from 54 to 39 s	39
74	reactor 1 is stopped	N/A
98	reactor 2 is used for liquid biodegradation studies (unpublished results)	N/A

[a] EBRT: empty bed retention time = gas flow rate/bed volume.

other volatiles was achieved with a packed column (80/100, 8 ft × 1/4 in., Supelco, Bellefonte, PA) and a thermal conductivity detector, and with a capillary column (Supelcowax 10, 30 m × 0.53 mm × 1 μm) and a FID detector, respectively. At standard operating conditions, influent and effluent streams were analyzed in triplicate once per day.

Wet Biomass Accumulation. To determine the amount of wet biomass in each reactor, the recycle medium was allowed to drain for 10 min and the reactor was then weighed with a precision of 5 g using a model 7300 scale (Pennsylvania Scale Company, Leola, PA). The amount of wet biomass in the reactor was calculated as the increase of reactor weight as compared to the weight of the wet reactor including the packing on day 0. Dry biofilm weight was found to be 5.38% ± 0.18% of the wet weight by weighing wet and oven dried (90 °C) samples of biofilm.

Inoculum Origin and Culture Conditions. Groundwater samples and aquifer material from two long-term MTBE contaminated sites were mixed and introduced in both biotrickling filters for enrichment using MTBE as selective pressure. The first sample was from the Borden Aquifer (gift of M. Schirmer, University of Waterloo, Ontario, Canada) where chloride, BTEX, and MTBE were injected in 1988. After eight years, extensive analyses showed that only 3% of the initial MTBE mass could be accounted for (Schirmer et al., 1998). The second sample (soil and contaminated water up to 2.6 ppm MTBE, gift of E. D. Reynolds, The Reynolds Group, Tustin, CA) was from a leaking underground storage tank site in Southern California. No further tests were performed to determine if one or the other sample contained the successful MTBE degrading consortium.

Results and Discussion

Culture Enrichment in the Biotrickling Filter and Culture Characteristics. After inoculation of both biotrickling filters with the mixture of contaminated soil and groundwater, various attempts were made to shorten the startup phase. Methanol was fed to one system (Pall ring biotrickling filter) to promote biomass growth. This proved unsuccessful, and methanol feed was discontinued. It took about 6 months of continuous operation before a slight elimination of MTBE (about 1 g m^{-3} h^{-1}) could be noticed in the reactors. Since the MTBE removal was less than 5% of the incoming feed, biodegradation was confirmed by draining the scrubbing solution out of the reactors and monitoring the decrease of MTBE over time, after the reactors were closed. MTBE was initially present in the gas phase at a concentration of 0.65 g m^{-3} and was rapidly depleted (Figure 2). Its depletion coincided with the increase of an unknown metabolite (reported as methanol equivalent in Figure 2). While the retention time of the peak of the metabolite on the gas chromatograph coincided with the peak of methanol or this of *tert*-butyl alcohol, a rapid calculation based on gas−liquid

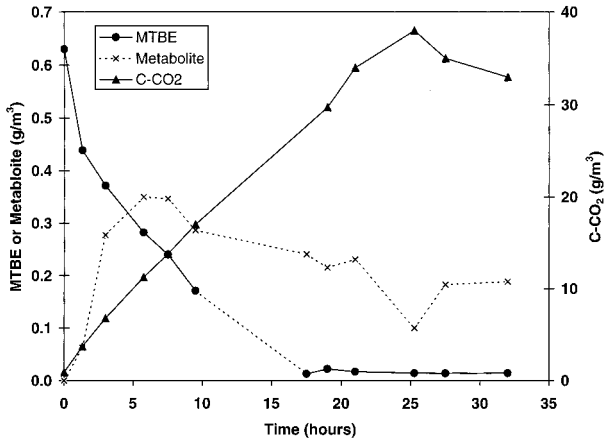

FIGURE 2. Batch mode biodegradation of MTBE: production of carbon dioxide and of an unidentified metabolite (reported as methanol equivalent) in a closed biotrickling filter (Pall ring biotrickling filter) after drainage of the recycle fluid.

equilibrium showed that the concentrations measured in the gas phase where too high to be any of these two compounds. No further attempts were made to identify the metabolite, since its production ceased as the performance of the reactor improved. It is probable that the metabolite was rapidly biodegraded as the process culture matured over time. During the experiment, carbon dioxide concentration increased, but far more than the theoretical value calculated based on the degradation of MTBE. This indicates that a significant amount of secondary substrates was available in the biofilm. On the basis of the slope of MTBE depletion, an approximate elimination capacity of 0.2−1 g m^{-3} h^{-1} can be estimated. This is a very low value, but the process culture was not yet fully developed. Even so, it is interesting to notice that the MTBE decrease follows almost a straight line even at low concentrations, indicating that the observed kinetic is probably close to zero order until very low concentrations. This is a definite advantage for a gas phase bioreactor.

Subsequently, a biofilm sample was taken from the reactors and evaluated for its ability to degrade MTBE in liquid batch cultures. High MTBE degradation rates (up to 2 ppm h^{-1}) were obtained after three 1-week-long subcultures with 100 ppm MTBE. While the culture had a very low biomass yield coefficient, MTBE degradation was not subject to substrate inhibition at concentrations as high as 500 ppm. The same consortium was also capable to degrade TBA at a similar rate. The MTBE degrading culture consisted of at least six Gram positive and negative bacteria, bacilli and cocci, fungi, protozoa, and rotifers. Similarly to other MTBE degrading cultures (*35*), this consortium formed dense aggregates when grown on MTBE in liquid cultures. Further characterization of the consortium is underway. After three

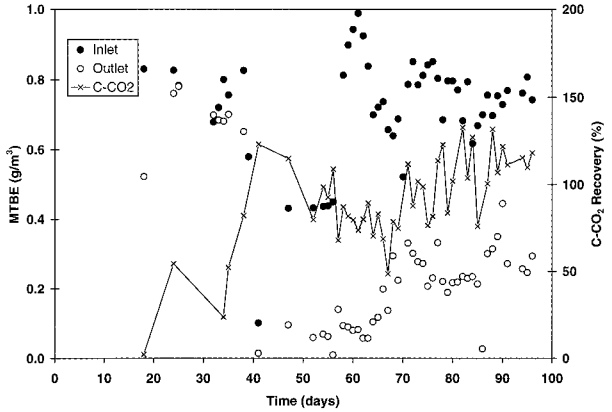

FIGURE 3. MTBE inlet and outlet gas phase concentrations and recovery of the degraded MTBE as carbon dioxide for reactor 2 over the duration of the experiment. Note that time zero corresponds to the re-inoculation of the biotrickling filters with the competent liquid mixed culture.

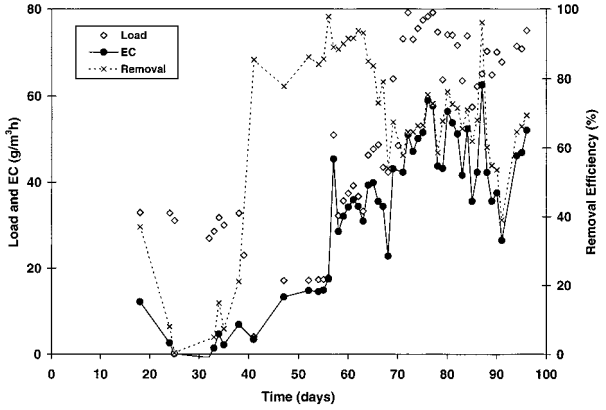

FIGURE 4. MTBE loading, elimination capacity, and percentage removal over time for reactor 2 (Pall rings packing).

subcultures in shaken flasks, the resulting consortium was re-inoculated in the trickling filters.

It was not surprising to find that obtaining a competent aerobic microbial consortium for MTBE degradation required a relatively long acclimation and/or growth period. From the most current research available, only a few microbial cultures capable of completely degrading MTBE have been isolated. Interestingly, conventional shake flask enrichment techniques seem to fail so far with MTBE. Most MTBE degrading consortia have been obtained in biofilters or other types of bioreactors (this study, *18*, *36*). This suggests that attached growth might be a key parameter to obtain an active MTBE degrading consortium as it provides a favorable environment for slow growing microorganisms. This may be because close cell proximity promotes genetic exchanges between species which are required for the establishment of proper genotype(s) (*37*) or because it stimulates metabolic complementation between the biofilm community members. In liquid culture, metabolic complementation between community members is indeed well documented for various biochemical process of environmental concerns such as nitrifying organisms (*38*, *39*), methanogens (*40*), and various xenobiotics degraders (*37*, *41*, *42*). For biofilms, such metabolic interdependency between community members has been investigated for dental plaque communities and bacterial mediated corrosion communities, but surprisingly little attention has been given to biofilter catabolic flora. Our experience tends to show that, in addition to phenotype and/or genotype changes, the structural integrity of the aggregate (in liquid culture) or of the biofilm (in the trickling filters) may be an important requirement for the MTBE catabolic activity to be expressed within the consortium.

Biotrickling Filter Startup. Figure 3 shows the inlet and outlet concentrations of MTBE and carbon dioxide production for biotrickling filter 2; loading, elimination capacity, and percentage removal are reported in Figure 4. Biotrickling filter 1 data were very similar and are not shown. At the beginning of the experiment, a number of measures were taken in order to possibly improve MTBE elimination. Continuous medium supply started on day 26. Starting on day 33, 0.25 mg L^{-1} peat humic substances (PHS) were added to the scrubbing solution of both biotrickling filters. The removal efficiency started to increase after 25 days for the lava rocks biotrickling filter and after 35 days for the reactor equipped with polypropylene Pall rings. Performance reached a relatively steady value at about 95% removal after 52 and 40 days for the lava rocks and Pall ring reactors, respectively. The slight differences in startup observed between the two biotrickling filters are most probably due to the differences in surface properties of the support materials. Clearly expanded lava rocks offer many small pores easily colonized by microorganisms, whereas the polypropylene surface is not very favorable for bacterial attachment.

Figure 3 emphasizes the fact that an unusually long acclimation phase was needed until some MTBE degradation activity could be noticed. This was despite vigorous inoculation with competent microorganisms, a procedure which had proven to shorten acclimation down to a few hours to a few days in other biofiltration applications (*24*). Biotrickling filters are, in general, more difficult to start than biofilters because the process culture needs to attach to a carrier, form a biofilm to overcome wash-out, and is subject to shear stress. At first, a parallel can be drawn between the 4-week-long acclimation phase of a biotrickling filter for nitrobenzene removal (*43*) and the experiments reported herein. However, for nitrobenzene, the reason for the slow start might have been that biodegradation released potentially toxic metabolic byproducts such as nitrite, which inhibited the process culture. In the case of MTBE, no metabolites were detected after reinoculation on day 0, which would have caused a similar inhibitory effect. It is more likely that the real causes of slow startup were the difficulty to establish a thriving consortium as discussed in the previous section, the slow growth rate, and the low biomass yield of the process culture. As a matter of fact, biotrickling filters degrading other pollutants that are better growth substrates can be subject to complete clogging by excess biomass in a matter of weeks (*44*). For comparison, the present biotrickling filters only accumulated 300−600 g of wet biomass (less than 10% of the reactor volume) within the first 60 days of the experiment. This would form a biofilm of about 160−320 μm thick on the packing, which is close to the optimum thickness of 200−300 μm we estimate for a biotrickling filter. The slow rate biomass accumulation is consistent with the slow growth rate of the MTBE degraders and the high degree of MTBE mineralization observed in the biotrickling filter (see section on carbon balance and Table 2), which was typically 20−40% higher than that for toluene (*45*).

Further research to improve the startup of biosystems for the treatment of MTBE is warranted. In this context, it is interesting to note that the peat humic substances appeared to have had a beneficial effect on the performance of the systems (see day 33 onward in Figure 4). Peat humic substances were originally developed as plant growth stimulant (*46*) but have also been used as stimulant for bacterial activity in various aqueous systems (*47*). The mechanisms by which PHS stimulate biological activity are not well understood, but the concentration at which PHS have an effect rule out any cometabolic process. A possible

TABLE 2. Comparison of Key Metabolic and Kinetic Parameters of MTBE and Toluene Degrading Biotrickling Filters

parameter	biotrickling filter 1[a] MTBE	biotrickling filter 2[a] MTBE	biotrickling filter degrading toluene[b]
period examined (days)	71 to 74 (4 days)	72 to 88 (16 days)	22 to 34 (12 days)
average load (g m^{-3} h^{-1})	76.5 ± 2.4	71.9 ± 6.5	70.7
average elimination capacity (g m^{-3} h^{-1})	41.7 ± 3.9	50.0 ± 7.2	32.5
average C-CO_2 recovery (%)	97 ± 17	98 ± 16	51.6
wet biomass at start (g)[c]	1050	650	4535[d]
wet biomass at end (g)[c]	1120	970	7835[d]
biomass production rate (g_{dw} m^{-3} h^{-1})[c]	4.4 ± 1.1	5.0 ± 1.2	22.3
biomass yield (g_{dw} g^{-1}_{VOC})	0.105	0.0996	0.69
specific activity (g_{VOC} g^{-1}_{dw} h^{-1})	(6.4 ± 0.5) 10^{-3}	(10 ± 2) 10^{-3}	(2.7 ± 0.8) 10^{-3}

[a] This study. [b] Compiled from Cox and Deshusses (*45*). [c] Conversion: dry biomass weight (dw) = wet biomass × 0.0538. For the toluene biotrickling filter, the conversion factor is 0.046 (*45*). [d] Value for a 23.6 L biotrickling filter.

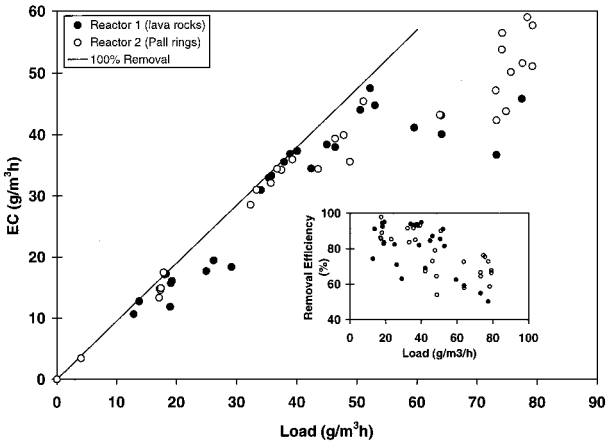

FIGURE 5. MTBE elimination capacity vs MTBE loading; the inset shows removal percentage as a function of the loading.

explanation is that humic acids form complexes with MTBE or any other growth limiting substrate and improve their assimilation. Further experiments with PHS revealed that while humic substances had a pronounced effect at the reactor startup, they only had a marginal effect once an effective biofilm had been established (results not shown), and effective MTBE removal could be obtained without PHS. This suggests that the main effect of PHS was to help in the initial colonization of the packing by competent cultures, rather than changing the intrinsic kinetics of MTBE biodegradation. Experiments performed in shake flasks confirmed that PHS stimulated the growth of the consortium (not shown, Fortin and Deshusses, unpublished results). Clearly, the use of PHS for the biostimulation of environmental bioprocesses requires further investigation.

Steady-State Performance. During the experiment, MTBE loadings were increased several times (see Table 1) by keeping the inlet concentrations constant and increasing the air flow rate, i.e., decreasing the empty bed retention time (EBRT). As reported in Figure 4 this load increase induced a significant breakthrough of MTBE after day 63. Eventually, at high loadings the reactors reached their maximum elimination capacity (Figure 5). In this regime, the process is kinetically limited and zero order biodegradation kinetics exists throughout the entire height of the biotrickling filter. For both biotrickling filters, a sustained maximum elimination capacity of 42−50 g m^{-3} h^{-1} was obtained. In general, reactor 2 had elimination capacities slightly higher than reactor 1, but both performed remarkably well in the light of the fact that MTBE was considered recalcitrant until recently and that MTBE maximum elimination capacity was found to be close to that of other VOCs. Cox and Deshusses (*45*) reported a maximum of 70 to 85 g m^{-3} h^{-1} for toluene in a very similar system, Mpanias and Baltzis (*48*) found that the maximum elimination capacity for mono-chlorobenzene was about 60 g m^{-3} h^{-1}, Oh and Bartha (*43*) reported 50 g m^{-3} h^{-1} for nitrobenzene. The present performance is also much higher than that reported by Eweiss et al. (*18*, *35*) in a biofilter which reached 8 g m^{-3} h^{-1} for an EBRT of 1 min. This is very encouraging, since such performance would allow a viable full-scale process to be operated. Further examination of the data presented in Figure 5 reveals high removal percentages are obtained up to loadings of approximately 15−20 g m^{-3} h^{-1}. This would be the target loading if a reactor would be designed for high removal percentages, while the 50 g m^{-3} h^{-1} value would be used to design system for high MTBE removal rate. Still, further research may be needed before effective full-scale biotrickling filters can be properly designed and operated. While Figure 5 is a good representation of the performance for a wide range of pollutant loadings and medium MTBE concentrations, one should remember that the loading can be varied by changing either the empty bed retention time or the inlet pollutant concentration. At this time, it is unclear how the biotrickling filters would perform at very low MTBE concentrations and high flow rates, but at similar MTBE loadings. These conditions may be encountered for the treatment of waste air generated by air strippers. Ultimately, the reduction of the performance will depend on the half-saturation rate constant of MTBE biodegradation. If the K_s value is low compared to the concentrations treated, performance will remain similar to those reported in Figure 5, if concentrations are lowered and loading kept constant. On the other hand, if K_s is comparable to the treated concentrations, performance will be reduced proportionally as reported previously for toluene (*49*). Also, while the present studies involved MTBE only, a number of phenomena can occur if other compounds are simultaneously treated with MTBE, as it would probably occur in a practical applications. The rate of MTBE biodegradation could be increased by cometabolism as in other liquid phase biodegradation studies (*16*, *20*, *21*), or it could be subject to cross inhibition as observed for ketones in biofilters (*50*). Clearly, further definition of the kinetics parameters governing the biodegradation of MTBE is warranted.

It is interesting to find no real significant differences between the performance of the two biotrickling filters (maximum elimination capacities of 42 ± 4 and 50 ± 7 g m^{-3} h^{-1} for reactors 1 and 2, respectively) although the packings were quite different. Kirchner et al. (*51*) studied the effect of various support materials for the treatment of propionaldehyde by *Pseudomonas fluorescens* in biotrickling filters. For low cell density or slow biodegradation rate, i.e., in the kinetically limited regime, different performances were found for different supports, but they found no significantly different performances between the packings when mass transfer was the rate-limiting step. In the present case, since the reactors were clearly limited by the biological reaction (see part 2),

the similar performance between the two reactors indicates that both reactors had approximately the same amount of active biomass.

It should be emphasized that good performance could only be obtained after a proper density of a competent process culture was established. In this case, the minimum required amount was about 15−20 kg (wet weight) per cubic meter of bed which is at the lower end of what is usually found for toluene (Cox and Deshusses, unpublished results). With common VOCs, building up this baseline biomass is usually fairly rapid, but with MTBE it is much slower because of the slow growth rate and the low biomass yield coefficient typical for MTBE degrading cultures. This low growth may be perceived as a drawback, but the biggest obstacle for industrial deployment of biotrickling filters is their rapid clogging by growing biomass when degrading substances with high yield coefficients. The present report of sustained MTBE elimination for over 2 months with no noticeable accumulation of biomass shows that clogging will not occur if MTBE is the dominant pollutant treated in the biotrickling filter.

Biomass Yield, Carbon Balance, and MTBE Metabolism. To date, little data exist on the mineralization of MTBE to carbon dioxide and possible formation of degradation metabolites. Several authors report that the first step in the MTBE biodegradation pathway is the cleavage of the ether bond leading to the formation of TBA and formaldehyde (*14*, *16*, *20*). Hence both liquid and gaseous samples were carefully analyzed for the presence metabolites such as TBA, formaldehyde, or other byproducts, but none were found. These results are consistent with the high degree of mineralization of MTBE that was observed (Figure 3 and Table 2) and also with shake flask experiments showing no formation of TBA during MTBE biodegradation. We also observed that the mixed culture was able to completely biodegrade TBA at a rate similar to that of MTBE (Fortin, Deshusses, unpublished), which may explain why TBA was never observed in the biotrickling filters.

Carbon dioxide and MTBE were analyzed simultaneously to assess the degree of mineralization. The ratio of the production of carbon-CO_2 to the carbon-MTBE degraded is shown as a function of time in Figure 3. Typically, the carbon recovery ratio ranged between 80 and 120%, which indicates that most of the MTBE was used to satisfy the energy requirement of the process culture rather than for growth. It also demonstrates that the MTBE degraded is converted to harmless carbon dioxide.

During active growth of the biofilm, monitoring the biomass weight in the reactors allows one to estimate the apparent specific growth rate of the process culture assuming Monod kinetics. The observed specific growth rates, μ, were equal to 0.023 day^{-1} and 0.026 day^{-1} for reactors 1 and 2, respectively. As a comparison, Salanitro (*52*) found a specific growth rate of 0.05 day^{-1} for his MTBE degrading mixed culture. When the reactors had reached a steady state with respect to MTBE performance (after day 30−35 for reactor 1 and after day 45−50 for reactor 2), it was experimentally determined that only about 20 g of wet biomass accumulated every day in each biotrickling filter. Using these data and the elimination capacity enables the calculation of an overall yield coefficient specific for the process culture in the biotrickling filter (Table 2). The biomass yield obtained range from 0.09 to 0.12 $g_{dw}\ g^{-1}_{MTBE}$ which is consistent with other MTBE studies (*14*, *53*) but much lower than the usual values of 0.4−0.8 $g_{dw}\ g^{-1}_{substrate}$ observed for other VOCs. With a similar experimental setup but degrading toluene, the biomass production rate was found to be approximately 5 times higher than with MTBE (Table 2). It should, however, be emphasized that the biomass yield presented here is valid for the consortium in the biotrickling filter, and it includes endogenous respiration and predation. As mentioned earlier, such a low biomass yield coefficient is a drawback for system startup, but it is an advantage for the long run, as the biotrickling filter will not be subject to clogging by overgrowing biomass.

On the basis of the numbers of Table 2, a specific activity defined as the mass of MTBE degraded per gram of dry biomass in the biotrickling filter per hour can be calculated. This value was 5.5×10^{-3} and 11×10^{-3} $g_{MTBE}\ g^{-1}_{dw}\ h^{-1}$ for reactor 1 and 2, respectively. The difference between the two trickling filters is due to the higher biomass content in biotrickling filter 1 but a relatively similar performance to reactor 2. Still, the specific activities obtained are significantly higher than those found for toluene in similar systems (Table 2). This emphasizes that (1) competent MTBE degrading culture is extremely active in turning over MTBE into carbon dioxide, mostly because of the difficulty in obtaining sufficient energy and growth out of MTBE, and (2) in the toluene degrading biotrickling filter, a good fraction of the biomass was inactive. Still a number of questions pertaining to the biodegradation of MTBE and to the proper management of MTBE degrading cultures for remediation purposes remain unanswered. But overall, the results show very promising perspectives for future field implementation of biotrickling filters and for the development of effective strategies for MTBE bioremediation.

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