Performance and Microbial Diversity of a Trickle-Bed Air Biofilter under Interchanging Contaminants

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A trickle-bed air biofilter (TBAB) was evaluated under conditions of interchanging the feed volatile organic compounds (VOCs) in the sequence methyl ethyl ketone (MEK), toluene, methyl isobutyl ketone (MIBK), styrene, and then back to MEK. The obtained performance results revealed that the biofilter provided high removal efficiency within the critical loading of each VOC, which was previously defined in the non-interchanging VOC fed biofilter. The biofilter easily acclimated to the oxygenated compounds (MEK and MIBK), but re-acclimation was delayed for the aromatic compounds (toluene and styrene). Ratios of the molar mass of CO_2 produced per molar mass of VOC removed were investigated. It has been found that the ratios for the aromatic compounds closely resembled the theoretical complete chemical oxidation based ratios while larger differences were encountered with the oxygenated compounds. Denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA genes was used to assess the impact of interchanging VOCs on the bacterial community structure in the biofilter. The results from denaturing gradient gel electrophoresis (DGGE) showed that the structure of the microbial community in the biofilter was different after each interchange of VOCs.

1 Introduction

Biofiltration technology has recently emerged as an efficient and cost-effective technology for the control of volatile organic compounds (VOCs) emission. However, fluctuations in concentration and variation in the waste air composition is the most common situation encountered in the chemical industry, which challenges the application of biofiltration technology.

During the past decade, numerous studies were performed at the bench, pilot, or pilot-field scale reactors to evaluate the effect of inlet concentration on biofiltration performance [1–13]. Target contaminants included hydrocarbons (e.g., benzene, styrene, hexane, toluene, and naphthalene), oxygenated hydrocarbon (e.g., methanol, ethanol, diethyl ether, acetone, and methyl ethyl ketone), chlorinated hydrocarbons (e.g., chlorobenzene and *o*-dichlorobenzene), and sulfur compounds (e.g., hydrogen sulfide). Factors affecting the decontamination efficiency include: nature of the contaminants, packing materials and biofilter configurations, empty bed retention time, volumetric loading rates, nutrient feed flow rates, nutrient solution pH, and flow patterns of air. Jorio et al. [14] and Sorial et al. [15] studied the effect of variation of styrene inlet concentration and gas flow rate on the overall biofilter performance. They found that excess biomass accumulated within the biofilter decreased the overall biofilter performance when the employed inlet concentration exceeded the removal capacity of the biofilter. Deshusses et al. [5] studied the transient behavior of a biofilter under step change of MEK and MIBK concentrations.

They found that the biofilter adapted rapidly (2–5 hours) to the new operating conditions. Cai et al. [3, 4] studied the biofilter behavior in removing oxygenated compounds, and they observed that the biofilter could maintain high removal efficiency when the employed loading rate did not exceed its elimination capacity. They also found that oxygenated compounds favored biomass growth, which would cause channeling in the biofilter and lead to a decrease of biofilter performance. Periodic backwashing operation with media fluidization was necessary for removing the excess biomass in order to maintain a stable high performance. Furthermore, biofilter re-acclimation after backwashing or non-use due to shutdown for factory retooling or equipment repair, or during weekends and holidays is an important factor in biofilter operation. To obtain consistent performance and to control the biofilter more effectively, some researchers have focused on biofilter re-acclimation. Torronen et al. [16] noted that 2–4 hours were required following a two-day period of non-use and that five hours were required following a five-day period of non-use, for removal efficiency to return to previous levels for hexane and phenolic compounds. Togna and Frisch [17] reported styrene re-acclimation periods after two or more days without chemical contact. Standefer and van Lith [18] reported that biofilter removal efficiency returned to previous levels after one hour following a two-day period of non-use and after 5–8 hours following a seven-day period of non-use. Martin and Loehr [19] reported biofilter re-acclimation after restart-up from two intermittent periods. Moe and Qi [20] found out that weekend shut-down periods caused varying effects on removal efficiency for different compounds. Cai et al. [3, 4] and Kim et al. [1, 2] observed that non-use periods did not have noticeable adverse effects on removal efficiency when the employed loading rates did not exceed the elimination capacity of the biofilters and non-use operation could be

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employed as a means of biomass control for these loading rates.

Only a few studies investigated the effect of VOC interchanging on the biofilter performance. Martin and Loehr [19] studied the re-acclimation following changes of contaminant in a biofilter packed with municipal compost. They found that re-acclimation from benzene to toluene was 5.5 hours, 8.1 hours from toluene to benzene, 13 days from benzene to *p*-xylene, and 14 days from *p*-xylene to benzene. It is worthwhile to note that synthetic media used in TBABs are resistant to degradation and hence allow for long-term operation of the biofilter.

With the development of molecular biotechnologies, molecular techniques have been used to study microbial populations and microbial ecology in biofilters, which will optimize biofilter performance [11, 21–32]. Juteau et al. [25] studied the microbial diversity of a compost biofilter treating toluene vapor gas by using serum-bottle assays and mineral agar plates. They observed that only 15 % of the isolated colonies was involved in the toluene degradation. They also observed that *Pseudo-nocardia* and *Rhodococcus* were the dominant species when using 16S rRNA gene-sequence comparison. Acuna et al. [31] studied changing of the amount of microorganisms with respect to acclimation period and operation duration and noted that the amount of toluene degrading microorganisms increased significantly with increased acclimation time. Khammar et al. [32] studied the relationship between the microbial communities and VOC mixture degradation along the peat biofilter's depth by using single-strand conformation polymorphism (SSCP). Eleven VOC mixtures which include oxygenated, aromatic, and halogenated compounds were employed as the target contaminants. They found that the microbial density distribution along the biofilter depth was related to the biodegradation efficiency. However, only one study was reported on how microorganisms responded to the change of VOC employed on a biofilter. Grove et al. [31] assessed the changes in functional diversity of the microbial community in a compost biofilter over time by employing community level physiological profiling (CLPP) with BIOLOG ECO-plates. The biofilter treated the waste airstream contaminated with hexane and later with ethanol. Although no positive conclusion could be drawn due to insufficient samples taken and replication, the CLPP technique was thought to be a reliable tool to assess the variation in the community structure of a biofilter. To the best of our knowledge, no studies have been carried out on TBAB performance behavior on VOC interchange.

The objective of this research was, therefore, to investigate the trickle-bed air biofilter (TBAB) behavior under the interchange of feeding VOCs. The evaluations are focused on the following issues:

(i)   VOC loading rates and elimination capacity,
(ii)   Re-acclimation of the biofilter after interchanging VOCs,
(iii) CO_2 production, and

(iv)   The response of the microbial community to interchanging VOCs.

2 Materials and Methods

The experiments were performed on a lab-scale TBAB under interchange of the feed VOCs in the sequence:

MEK → toluene → MIBK → styrene → MEK

The biofilter continued to be run without reconditioning the media used in pervious runs where MEK was the sole VOC contaminant [3]. The biofilter was constructed of seven cylindrical glass sections with an internal diameter of 76 cm and a total length of 130 cm. The reactor was packed with pelletized diatomaceous earth biological support media to a depth of about 60 cm. The biofilter was run at constant operating temperature of 20 °C. The biofilter was operated in a co-current gas and liquid downward flow mode.

The air flow was set up at the rate of 3.6 L/min for MIBK and MEK with a corresponding empty bed retention time (EBRT) of 0.76 min, at 2.22 L/min for toluene with a corresponding EBRT of 1.23 min, and 1.35 L/min for styrene with a corresponding EBRT of 2.20 min. These conditions were chosen based on our previous studies with each VOC [1–4]. Liquid VOC was injected via a syringe pump and vaporized into the air stream. Each VOC inlet concentration was stepwise increased from 50 ppmv to the maximum allowable inlet concentration, for attaining over 99 % removal, which was determined previously by the authors [1–4]. Buffered nutrient solution was supplied at a rate of 1.5 L/day for both MIBK and MEK, 2.4 L/day for both toluene and styrene, the composition of the nutrient solution was provided in a previous study [7]. In-situ upflow backwash with media fluidization was employed at a rate of 1 hour a week as a strategy for biomass control. The conditions employed during the experimental runs are summarized in Tab. 1.

Denaturing gradient gel electrophoresis (DGGE) of the 16S ribosomal DNA genes of the microbial community was performed to determine changes in the most abundant microbial populations during the operation of the reactor. For DGGE analysis, genomic DNA was extracted from each sample using the Ultraclean soil DNA extraction kit (Mo Bio Laboratories, Inc.) according to the manufacturer's instructions. The DNA extracts were used as a template material for the polymerase chain reaction (PCR). PCR was performed in 50 μL reaction volume using a reaction mixture of 1X PCR buffer, 200 μM of each deoxynucleoside triphosphate, 2.0 mM $MgCl_2$, 0.025 U of *Taq* DNA polymerase/μL (Qiagen), and 0.5 μM of each primer. The primers were used to amplify the variable V3 region of the 16S rDNA gene. The primers were 341–358f (containing a GC clamp): '-CGCCCGCCGCGCGCGGCGGGCGGGGCGG-GGGCACGGGGGGCCTACG GGAGGCAGCAG-3' and 518–534R: 5'-ATTACCGCGGCTGCTGG-3'   (manufactured by the University of Cincinnati DNA Core lab). These primers were used to amplify conserved regions of the 16S

Table 1. Experimental conditions for this study.

	I	II	III	IV	V
Operational periods [d]	1–20	21–131	132–158	159–185	186–192
Target VOC	MEK	Toluene	MIBK	Styrene	MEK
Inlet concentration [ppmv]	400	50–250	50–150	50–200	400
Loading rate [kg COD/(m³d)]					
Range	5.63	1.14–5.72	1.09–3.26	0.48–1.90	5.63
Critical load*	5.63	3.52	3.26	1.90	5.63
EBRT [min]	0.76	0.76, 1.23	0.76	2.02	0.76

* Critical load represents maximum allowable load to attaining over 99 % removal, which was determined previously by the authors [1–4].

rRNA genes in a wide range of bacterial species [33]. Amplification of DNA was performed in a GeneAmp PCR system 2400 (Perkin Elmer) by using the following program: an initial denaturing step at 94 °C for 5 min, followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min and 30 s, and final extension at 72 °C for 10 min. PCR products were verified visually (5 μL) using 1 % agarose gel electrophoresis in 1X TBE and SYBR Green I staining (Molecular Probes).

DGGE was performed using a Bio-Rad D-Code system following published procedures [33]. The PCR products were loaded onto 8 % [w/V] polyacrylamide gels containing a denaturing gradient between 15 and 55 % (100 % denaturant defined as 7 M urea plus 40 % vol/vol formamide). Electrophoresis was performed for 20 hours in 0.5 X TAE (20 mM Tris, 10 mM acetic acid, 0.5 mM EDTA, pH 8.3) at 35 volts and 60 °C. After electrophoresis, the gels were stained for 30 minutes in 1 X TAE using SYBR Green I staining (Molecular Probes). The stained gel was photographed using the Bio-Rad Gel Doc 2000.

Gas phase samples for VOC analysis were taken with gas-tight syringe and were measured by using a GC equipped with a FID. A GC equipped with a thermal conductivity detector was used for determining the CO_2 concentration in the effluent gas. Liquid phase samples were analyzed for nitrate and volatile suspended solid (VSS) concentration, total carbon, and inorganic carbon.

3 Results and Discussion

3.1 TBAB Performance Overview

The biofilter performance with respect to VOC removal is shown in Fig. 1. The feed VOCs were interchanged in the sequence:

MEK (stage I) → toluene (stage II) → MIBK (stage III) → styrene (stage IV) → MEK (stage V)

Stage I was the last 20 days of a previous MEK biofilter study [1–4] at an inlet concentration of 400 ppmv with a corresponding loading rate of 5.63 kg COD/(m³d) and 0.76 min EBRT. At stage II, the contaminant was switched to toluene and the EBRT was initially maintained at 0.76 min EBRT. The biofilter got re-acclimated to the 99 % removal for 50 ppmv and 100 ppmv inlet concentration, but failed to get re-acclimated for the 250 ppmv even after 31 days of operation. Furthermore, the removal efficiency decreased to 80.4 % just prior to the next backwashing. An inlet concentration of 250 ppmv with a corresponding loading rate of 5.72 kg COD/(m³d) exceeded the elimination capacity limit of 3.52 kg COD/(m³d) (see Tab. 1) and the 0.76 min EBRT was thought to be not long
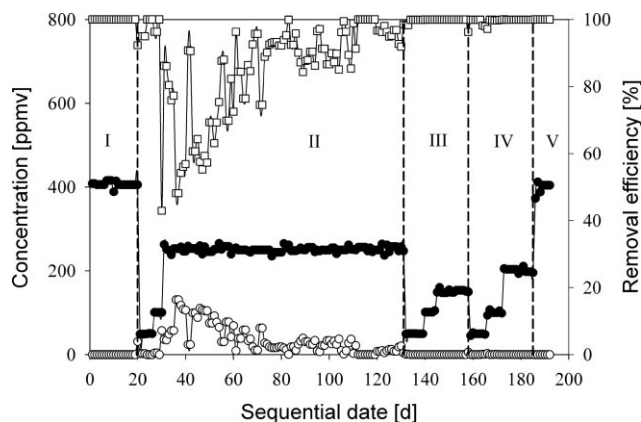

Figure 1. Biofilter performance with respect to sequential days. (□ Removal efficiency [%]; ● Inlet concentration, [ppmv]; ○ Outlet concentration, [ppmv]. I – MEK, II – Toluene, III – MIBK, IV – Styrene, and V – MEK).

enough for obtaining a consistent high performance (> 99 %). Thus on day 62 after backwashing, the EBRT for toluene was increased to 1.23 min while maintaining the inlet concentration at 250 ppmv, which provided a critical loading rate of 3.52 kg COD/(m³d). The overall removal efficiency increased to 92 % just prior to the next backwashing. On day 69, after backwashing was conducted, the removal efficiency increased to 95.7 % on the next day, but decreased to 74.5 % on day 72. On day 76, after backwashing was conducted, the removal efficiency recovered to 88.9 % in 300 min. The removal level was maintained at 92–93 % during this cycle. On day 84, after backwashing was conducted, the removal efficiency recovered to 99.3 % in 300 min, but decreased to 84.3 % just prior to the next backwashing. Twelve backwashing cycles were conducted at this loading rate. Although the overall removal efficiency increased gradually to 99 % after backwashing, this removal level could not be maintained for the whole cycle. The removal efficiency was ranging from 92 % to 99 % during the last two backwashing cycles. The performance in the

twelve backwashing cycles for a loading rate of 3.52 kg COD/(m³d) showed that the biofilter reached its maximum removal capacity under the current operation conditions.

On day 132, stage III was started. After backwashing was conducted, the target VOC was changed to MIBK with an initial concentration of 50 ppmv at 0.76 min EBRT. The biofilter obtained consistent 99 % removal efficiency up to 150 ppmv inlet concentration corresponding to the critical loading rate of 3.26 kg COD/(m³d). On day 159, after backwashing, the VOC was switched to styrene (stage IV) at 50 ppmv initial inlet concentration and 2.02 min EBRT. The biofilter re-acclimated to the 99 % removal level after one day and maintained this consistent removal level up to the critical loading rate of 1.90 kg COD/(m³d). However, it is worth mentioning that the re-acclimation was delayed with the increase of the employed loading rates. At stage V, the target VOC was switched back to MEK with 400 ppmv inlet concentration (5.63 kg COD/m³d) at 0.76 min EBRT. The biofilter obtained consistent over 99 % removal efficiency at this loading rate.

3.2 Biofilter Response after Change of Contaminants

Effluent samples were collected at prescheduled time intervals to evaluate the biofilter response subsequent to the interchange of VOCs. Fig. 2 shows the effluent response corresponding to each VOC after interchanging. Re-acclimation period was considered to have been achieved when 99 % of the original biofilter performance was attained. Fig. 2 illustrates that for the oxygenated compounds (MIBK and MEK), the biofilter got re-acclimated in 300 and 30 min, respectively. The quick re-acclimation of MEK as compared to MIBK is explained later under bacterial community analysis. In the case of the aromatic compounds (toluene and styrene), the biofilter got re-acclimated in 4 and 2 days, respectively. This indicates that the metabolism of aromatic compounds is much more complicated as compared to that of oxygenated compounds. Aerobic biodegra-
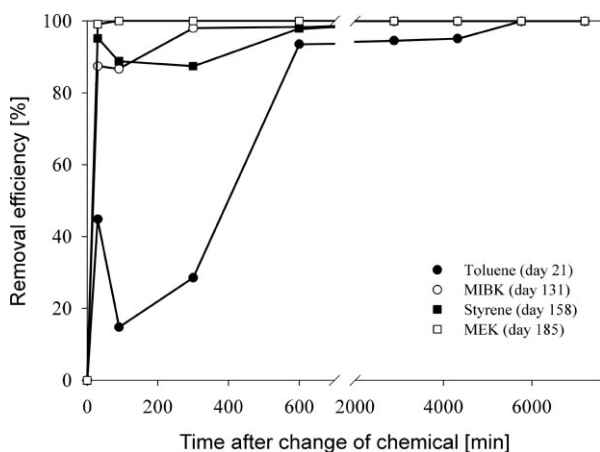
dation of an aromatic compound involves two steps: activation of the ring involving the incorporation of molecular oxygen into the ring, and ring cleavage involving the cleavage of the bond between carbon atoms of the two hydroxyl groups [34].

Furthermore, it is speculated that the mass transfer difference between the oxygenated compounds and aromatic compounds plays an important role in the re-acclimation behavior. For aromatic compounds, the dimensionless Henry's law constants for styrene and toluene are 0.109 and 0.280, respectively [35]. In the case of the oxygenated compounds, MEK and MIBK, the dimensionless Henry's constants are 0.00194 and 0.00062, respectively [35]. Hence, it is speculated that the microorganisms in the biofilter will have more available carbon in the case of oxygenated compounds because of their low Henry's law constants and will favor their growth and will eventually allow re-acclimation response of the biofilter to be quicker as compared to the aromatic compounds.

3.3 Production of CO_2 in the Biofilter with Change of Contaminants

The change of VOC removal is accompanied with a change in the production of CO_2 because of VOC degradation by microorganisms. For a more quantitative analysis of these results, the CO_2 production rate is plotted against the biofilter VOC removal rate in Fig. 3. The experimental average ratio of CO_2 production to VOC removal was equal to 2.02, 5.23, 3.98, 7.85, and 2.77 for stage I, II, III, IV, and V, respectively. The theoretical stoichiometric ratios in the case of complete chemical oxidation of the corresponding VOCs are 4, 7, 6, 8, and 4 for stage I, II, III, IV, and V, respectively. The discrepancy between these experimental values and theoretical values is typical during the process of biodegradation of the VOCs, since some of the removed carbon is converted into biomass for microbial growth. Nonetheless, the relatively small difference between the complete chemical
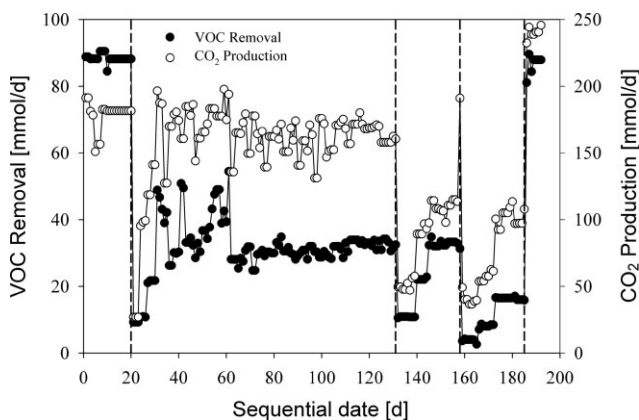

Figure 2. Effluent response corresponding to interchange of feeding VOCs.

Figure 3. VOC removal and CO_2 production with time. (I – MEK, II – Toluene, III – MIBK, IV – Styrene, and V – MEK).

oxidation based ratio and the experimental ratio for toluene (stage II) and styrene (stage IV) indicates that these aromatic compounds were eliminated by aerobic biodegradation.

On the other hand, the relatively big discrepancy between these two ratios for MEK (stage I and V) and MIBK (stage III) confirms that the oxygenated compounds favored biomass growth and accumulation in the biofilter and a possibility of denitrification may have occurred [36]. This behavior could be also supported by the nitrate nitrogen utilization for the four interchanged VOC substrates which was 0.0265 g N/g COD for MEK, 0.0258 g N/g COD for MIBK, 0.0090 g N/g COD for toluene, and 0.0103 g N/g COD for styrene. Furthermore, the small discrepancies between these experimental values and theoretical values for toluene and styrene could also be caused by a low net energy yield from the degradation of aromatic toluene and styrene [37]. In contrast, the degradation of oxygenated MEK and MIBK could result in higher net energy yields which allows for the higher production of biomass.

3.4 Analysis of the Bacterial Community

DGGE was used to examine the impact of interchanging VOCs on the bacterial community structure in the TBAB. Samples for DGGE analysis were collected once a week from the backwashing solution of the TBAB. It is worthwhile to note that backwashing was conducted while the biofilter was offline with 50 % bed expansion, i.e., full media fluidization. Hence, samples collected were representative for the whole media depth.

Fig. 4 shows the DGGE banding patterns of the 16S rDNA fragments. The DGGE banding patterns were for samples from the last backwashing cycle before switching to the next VOC. Preliminary analysis of DGGE banding patterns revealed dramatic changes in the structure of the bacterial community from stage I to stage II after interchanging VOC from MEK to toluene. Many new bands appeared at stage II, and some bands that appeared at stage I disappeared or became faint. Moreover, some bands that appeared intense in one treatment became faint after VOC interchange and vice versa (see arrows in Fig. 4).
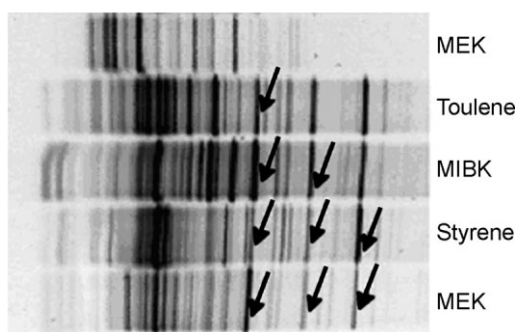

Figure 4. DGGE banding patterns.

This could indicate that the relative abundance among the different microbial species has changed. It is interesting to note that despite the difference in the bacterial community structure as revealed by the banding patterns in Fig. 4, the removal efficiency after re-acclimation was 99 % in the TBAB. Furthermore, it is noticed that the microbial diversity for MEK (stage I) was still available when MEK (stage V) was switched back. It is speculated that this microbial retention could be the possible reason for quick re-acclimation to MEK as compared to MIBK (see Fig. 2).

It is also noticed from Fig. 4 that the banding patterns difference from stage II to stage V were much less than the difference between stage I and II, however, the relative concentration of each microbial community was different. Future analysis of DGGE banding patterns from different times after the VOC has been interchanged will be conducted and representative patterns are expected to show the gradual organic compound adapting process. Different statistical tools such as cluster analysis, principle component analysis (PCA), and diversity indices will be employed on treating these patterns in order to have a better understanding of the impact of interchanging VOCs on the bacterial community structure and diversity in the TBAB.

4 Conclusions

The results obtained in this study supported the following conclusions:

1. The biofilter easily acclimated to oxygenated compounds (MEK and MIBK), while the biofilter re-acclimation was delayed for a period over 2 days for the aromatic compounds (styrene and toluene).
2. Once the biofilter got re-acclimated to the new contaminant, high and consistent performance could be maintained provided the critical loading rates are not exceeded.
3. The destructed aromatic compounds (toluene and styrene) were eliminated exclusively by aerobic biodegradation, however, the destructed oxygenated compounds (MEK and MIBK) were eliminated by aerobic biodegradation and possible denitrification.
4. The results from DGGE showed that the microbial community structure in the biofilter was different after each interchange of VOCs.

Acknowledgements

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