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Biodegradation kinetics and microbial communities associated with methyl *tert*-butyl ether removal in a biotrickling filter

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Abstract

A microbial culture capable of degrading methyl tertiary butyl ether (MTBE) was enriched in a laboratory-scale biotrickling filter. The reactor was continuously operated in a temperature-controlled condition for more than 100 days. Removal of MTBE vapors from air streams in a biotrickling filter was studied under various operating conditions, including the MTBE loading rate and the type of media in the circulating liquid. The changes in an MTBE-degrading bacterial community were investigated by using polymerase chain reaction (PCR) amplification of bacterial 16S rDNA. A subsequent analysis of the PCR products was performed by a single-strand-conformation polymorphism (SSCP) based on genetic profiles. Our results show that the MTBE removal efficiencies of 98.6–57.3% were observed at the MTBE loading rates of 2.67–50.76 g m^{-3} h^{-1} in the biotrickling filter. The removal efficiency appeared to increase by 27% (from 68 to 95%) in a later phase operation when the nitrogen source of $(NH_4)_2SO_4$ was replaced by $NaNO_3$. Due to the high MTBE loads in the inlet section, MTBE was removed mostly from the inlet section. The parameters estimated by using the Michaelis–Menten equation were 1.607 ± 0.208 ppmv s^{-1} for the maximum removal rate (V_m), and 24.91 ± 0.794 ppmv for the half-saturation constant (K_s). The total number of bands in an electrophoresis gel for various sections in the biotrickling filter using the same nitrogen source was similar, thus indicating similar compositions of species in the biotrickling filter. Nevertheless, communities grown in the presence of $(NH_4)_2SO_4$ and $NaNO_3$ media exhibited a very different pattern, thus revealing a small change in community structure due to the type of media.

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Keywords: Biodegradation; Biotrickling filter; Kinetics; Methyl *tert*-butyl ether (MTBE); Microbial community; Nitrogen source

1. Introduction

Methyl *tert*-butyl ether (MTBE) is a fuel oxygenate added to gasoline to enhance engine efficiency and to reduce the emission of hydrocarbons and carbon monoxide. However, the US Environmental Protection Agency (USEPA) has tentatively classified MTBE as a possible human carcinogen due to reports that have shown MTBE to be a carcinogen in animals [1]. The current guideline limit established by the USEPA is 20–40 μg l^{-1} for MTBE in drinking water [2]. During the past two decades, MTBE has been detected in the soil and groundwater in certain industrial parks in Taiwan. Therefore, the Environmental Protection Administration (EPA) of Taiwan has classified MTBE in the fourth category of toxic chemicals. Due to the apparently detrimental effect of MTBE on health, the EPA is proposing to elevate this substance to the third category of toxicants in the near future. At that time, the regulation of MTBE will be much stricter. Hence, relevant research (such as microbial treatment technology) must be undertaken in advance to reduce the MTBE level in water.

Many studies have reported that physico-chemical treatment technology for MTBE may be costly and possess the potential for by-product formation. Alternatively, biological waste gas treatment is a proven organic compound treatment technology having the advantages of low operating costs and minimum generation of waste by-products in streams [3]. Biofiltration has been successfully applied to control the large air streams contaminated with moderate concentrations of odors and volatile organic compounds (VOCs) from wastewater treatment plants, industrial sources and soil and groundwater remediation operations [4,5]. Among various biofiltration methods, biotrickling filters are more complex and often more expensive to operate. Nevertheless, they often exhibit better performance than other

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technologies, such as biofilters [6]. Typical successful applications for biotrickling filters include the treatment of chlorinated compounds or VOCs [7,8] for which operation parameters, such as inlet concentration of targeted compounds, empty-bed residence time (EBRT) of gas and the recirculating liquid flowrate are crucial.

Extensive research conducted in laboratory and field experiments on biodegradation of VOC and sulfur-containing compounds has shown that these compounds in a biofiltration system can be biodegraded under suitable conditions [9,10]. However, relatively little is known about the structure of a microbial community during aerobic VOC degradation. Furthermore, conventional micro-organism analyses cannot fully explain micro-organism species and the resulting microbial community composition in a biotrickling filter. Capturing microbial community fingerprints therefore requires applying a molecular biological technique. Single-strand-conformation polymorphism (SSCP) genetic profile analysis of 16S rDNA fragments amplified from environmental samples is one of the frequently used methods, being inexpensive and highly sensitive [11,12]. SSCP analysis is based on comparing positions of single-stranded DNA bands on the gel. Each species is usually represented by two single-stranded DNA bands, but it is also possible to have bands from different species migrating to the same position. Some of the bands indicative of the presence of these cultivated strains can be seen in most of the sample profiles, thus suggesting that these strains were indigenous to the study site. In recent years, SSCP has been applied extensively to detect a variety of environmental microbial communities. For instance, Peter et al. [13] used PCR-SSCP (polymerase chain reaction) and demonstrated that compost microbial communities are dominated by a few species with the most favored growth requirements. Lin et al. [14] reported the changes in genetic diversity and spatial distribution of microbial communities in association with the changes in phenol concentration during a bioremediation process. Hence, it is necessary to utilize molecular approaches to provide clues to bacterial types involved in aerobic VOC degradation and determine the relationship between growth and decline among bacterial populations.

In this study, we focused on the microbiological aspects of the process culture and on the biodegradation kinetics for the performance of an MTBE-degrading biotrickling filter. Single-strand-conformation polymorphism was used to detect mixed-culture population changes in the biotrickling filter. Furthermore, physiologically relevant parameters, such as the type of nitrogen source and acceptable inlet loads, were also investigated. With a genetic fingerprinting technique, this research may be useful in understanding how a bioreactor can be stably operated for field applications.

2. Materials and methods

2.1. Experimental set-up

The laboratory-scale biofiltration unit built and used in the experiments reported here is shown schematically in Fig. 1. Its major component was a transparent cylindrical plastic acrylic

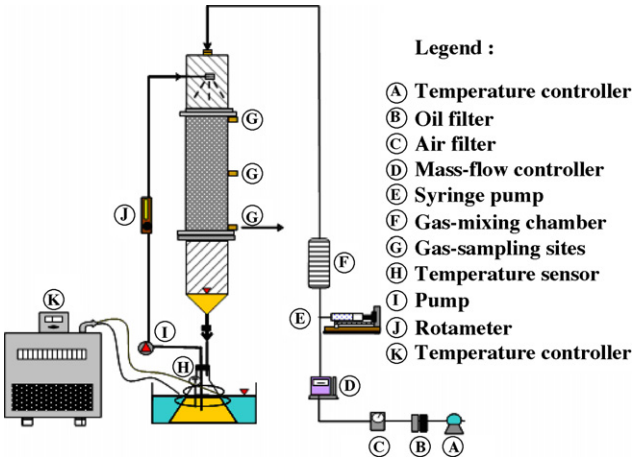

Fig. 1. Schematic diagram of biotrickling filter system.

glass column (10 cm in diameter and 1 m in height). The column was constructed in five sections joined by flanges. Each of the three 20-cm-long center sections were packed with media providing a total bed depth of 60 cm and a total packed-bed volume of approximately 4.71 l. A perforated stainless steel plate located at the bottom of the fourth section supported the packing media. A 20-cm headspace was left at the top for the gas inlet and for the housing of a sprinkler nozzle controlled by a solenoid valve, while a 20-cm bottom space was used as the outlet zone. The circulation liquid was continuously collected in a flask installed in a temperature-controlled water bath at the bottom of the biotrickling filter. Three gas-sampling ports were located between sections, at the inlet, middle and outlet sections, to determine the concentration along the reactor. The trickled-bed was filled with 0.77 kg of 1.6 cm polypropylene Pall rings. The main characteristics of the filtering media included a volumetric weight of 163.8 kg m^{-3}, an initial porosity of 83% and a specific surface area of 360 m^2 m^{-3}. On the surface of the filter media, biofilms of a microbial consortium were initially inoculated with an enriched aerobic microbial culture obtained from a wastewater treatment system in a petroleum refinery plant by using MTBE as one of the carbon sources.

Compressed air was passed through oil and air filters to remove oil, particulate matter and microbes. After purification, the air to the biotrickling filter was mass-flow controlled. MTBE was continuously injected by a syringe pump (Kd Scientific, model 100) into the influent air stream, where it vaporized and entered the gas-mixed chamber for further mixing. The flow rates of both the gas and solvent streams were controlled by using previously calibrated flow-meters to obtain the desired gas flow rate and contaminant concentration at the biotrickling filter entrance. A mineral solution was continuously recirculated over the trickled-bed by using a sprinkler nozzle controlled by a solenoid valve and centrifugal pump. The liquid feed contained all of the necessary macronutrients, micronutrients and buffers. The biotrickling filter system was operated in a co-current manner with the air and liquid flows directed downward. Throughout the experiments, the recirculating liquid flow rate, gas empty-bed residence time and bioreactor temperature were generally maintained at 400 ml min^{-1}, 85 s and 30–32 °C, respectively.

2.2. Analytical methods

To examine the performance of the biotrickling filter, the inlet and outlet MTBE concentrations were measured. Air samples were withdrawn from various locations on the column in a 5-ml gastight syringe equipped with Luer-lok valves and directed to a gas chromatograph (GC) equipped with a flame ionization detector (FID) (Shimadzu 14B). The MTBE inlet mass loading rate L ($g\,m^{-3}\,h^{-1}$), the biotrickling filter elimination capacity (EC) ($g\,m^{-3}\,h^{-1}$) and the biotrickling filter removal efficiency (RE) (%) were determined by using the relationships between the influent and effluent gas phase concentrations, the gas flow rate and the biotrickling filter material volume [6].

2.3. Kinetic analysis

The kinetic parameters of V_m and K_s were determined by assuming a plug air flow in the biotrickling column and by using the Michaelis–Menten equation, as shown in Eqs. (1) and (2) [15]. The experimental values of the removal rate (R_e) were determined by using Eq. (1) with six-set experimental data at each inlet/outlet concentration level for a given EBRT. The five-level inlet MTBE concentrations were 10, 50, 100, 200 and 300 ppmv. Subsequently, the initial estimated values of V_m and K_s were substituted into Eq. (2) to obtain a predicted removal rate (R_p). A pair of V_m and K_s was determined by a trial-and-error method and by minimizing the residual sum of square deviations for removal rates (R_e and R_p) between the experimental and predicted values calculated with the equations to determine the reasonable kinetic parameters. All of the computations were performed by using a solver function in the spreadsheet program Microsoft Office Excel 2003.

$$R_e = \frac{C_{in} - C_{out}}{t_{EBRT}} \quad (1)$$

$$R_p = \frac{V_m \times (C_{in} - C_{out})/\ln(C_{in}/C_{out})}{K_s + (C_{in} - C_{out})/\ln(C_{in}/C_{out})} \quad (2)$$

where R_e and R_p represent the experimental and predicted removal rates ($ppmv\,s^{-1}$), V_m represents the maximum removal rate ($ppmv\,s^{-1}$), K_s the saturation constant (ppmv), C_{in} and C_{out} the respective inlet and exit MTBE concentrations in the gas phase (ppmv) and t_{EBRT} is the gas empty-bed residence time (min).

2.4. DNA extraction

Genomic DNA for polymerase chain reactions (PCR) was extracted from mixed-culture cells by using genomic DNA purification kits (Bilight, GGP 100/200) in accordance with the manufacturer's instructions. Approximately 100–200 mg of biomass was harvested in duplicate at various stages of the biotrickling filter operation and used immediately for DNA extraction. The template DNA typically ranged from 100 to 200 ng μl^{-1}. To obtain the best fingerprint in an electrophoresis gel, the concentration of the template DNA solution was adjusted to 50 ng μl^{-1}, after which the final solution was stored at 4 °C for use as the PCR template.

2.5. Polymerase chain reaction

The microbial communities were analyzed by using the PCR-SSCP method as described by Lee et al. [11] and Schwieger and Tebbe [12], with modifications. The V3 region of the 16S rDNA, corresponding to the nucleotide positions 334–514 of the *Escherichia coli* sequence, was amplified with the primers EUB1 (5′-CAGACTCCTACGGGAGGCAGCAG-3′) and UNIV2 (5′-GTATTACCGCGGCTGCTGGCAC-3′). The PCR program included an initial denaturation at 94 °C for 5 min, and 30 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, followed by a final extension of 72 °C for 5 min. The PCR products of 200 bp were verified by gel electrophoresis on 1.2% agarose gels and stored at 4 °C for further use.

2.6. SSCP gel electrophoresis

A Hoefer SE260 vertical gel electrophoresis apparatus was used for SSCP analysis. The electrophoresis was conducted in 8% polyacrylamide gel for 45 min at a constant voltage of 300 V. The gel temperature was maintained at 4 °C by using a refrigerated circulating water bath. The DNA samples were mixed with equal volumes of a denaturing solution (95% formamide, 10 mM NaOH, 0.02% bromophenol blue, 0.02% xylene cyanol and 20 mM EDTA) heated to 95 °C for 5 min and snap-freezed on ice before loading. After electrophoresis, the gels were visualized by using the silver-stain method, sandwiched between two pieces of mylar membrane and dried. The relative positions of the DNA bands in the SSCP gels were analyzed by using LabWork software.

3. Results and discussion

3.1. Nitrogen source effect

Fig. 2 shows the removal efficiency and pH of the biotrickling filter operating continuously for 80 days with $(NH_4)_2SO_4$ and $NaNO_3$ as a nitrogen source. The efficiency of MTBE removal in this phase (days 60–65) can be maintained at near 80% for both nitrogen sources. However, the removal efficiency decreased sharply from near 80 to about 56% for the next 15 days (i.e., days 66–80) with $(NH_4)_2SO_4$ as a nitrogen source. During the decrease in the removal efficiency, it was found that the pH decreased gradually (pH 5.3, reduced to about 4.3) with the increasing number of days of operation, while using $(NH_4)_2SO_4$ as a nitrogen source. In contrast, the pH tended to remain neutral while the efficiency of MTBE removal was maintained at around 95% when $NaNO_3$ was used as a nitrogen source during days 66–80. The increase in MTBE removal is believed to be mostly due to the neutral environment, which provides a better condition for increasing the biomass in the biotrickling filter. A comparison of the two nitrogen sources used indicated that the efficiency of MTBE removal was similar in the earlier phase operation

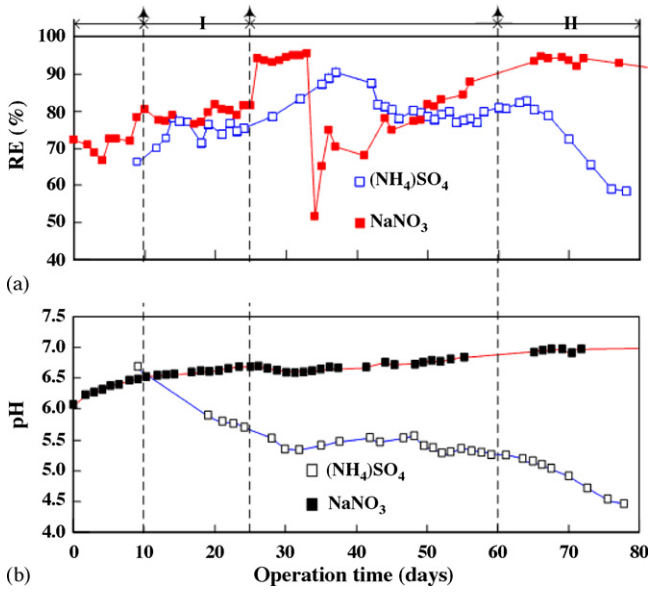

Fig. 2. Removal efficiencies (a) and pH (b) of biotrickling filter operated continuously for 80 days, with $(NH_4)_2SO_4$ and $NaNO_3$ as a nitrogen source.

(I: days 10–25). However, the removal efficiency appears to have increased by 27% (from approximately 68 to 95%) in the later phase operation (II: days 60–80) when $NaNO_3$ was used as the nitrogen source. It appears that $NaNO_3$ can provide better performance and ensure a longer operational life for the biotrickling filter. This is consistent with previous observations that nitrate is used preferentially over sulfate [16]. Moreover, the relatively low biodegradation capability in the ammonium conditions is believed to be the result of the decrease in pH possibly caused by nitrifying activity. Nitrifying bacteria oxidize ammonium to nitrite and then nitrate, leading to acid production. The two reactions are carried out by different micro-organisms but the processes are closely coupled and normally there is no accumulation of nitrite [17].

The overall performance of the biotrickling filter, with $NaNO_3$ as a nitrogen source for a 100-day period, is shown in Fig. 3. The biotrickling filter was exercised at MTBE inlet concentrations (10 and 100 ppmv), a loading rate of 15.12 g m^{-3} h^{-1}, an EBRT of 85 s and a liquid flow rate of 400 ml min^{-1}. In the first 8 days of operation in Phase I, a removal efficiency averaging 65% was observed for a 100-ppmv

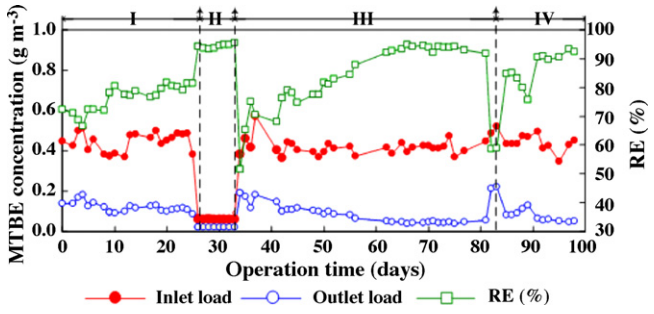

Fig. 3. Overall performance of biotrickling filter, with $NaNO_3$ as a nitrogen source for a 100-day period.

inlet load. After an adaptation period of 8 days, a consistent removal efficiency of 80% was obtained for the next 17 days (days 9–25). In Phase II (days 26–33), the removal efficiencies increased to an average of 94.3% for an MTBE inlet concentration of 10 ppmv. In Phase III (days 34–83), the removal efficiencies decreased to approximately 51.6% initially, then gradually increased to as high as 95% for an MTBE inlet concentration of 100 ppmv. In Phase IV (days 84–100), the removal efficiencies varied between 60 and 94% for a 100-ppmv load and operation temperatures of 15–38 °C. Therefore, these results indicate that $NaNO_3$ is a suitable nitrogen source for use in a biotrickling filter. Moreover, it appears that the biotrickling filter can be operated consistently during shock loading and temperature variations.

3.2. MTBE load effect and concentration profiles

To determine the removal characteristics throughout the unit, the biotrickling filter was subjected to a range of load conditions. Fig. 4 shows the variation in EC and RE with MTBE loading rates. As can be seen in figure, the EC increased with the amount of the influent load. It was found that the removal efficiency

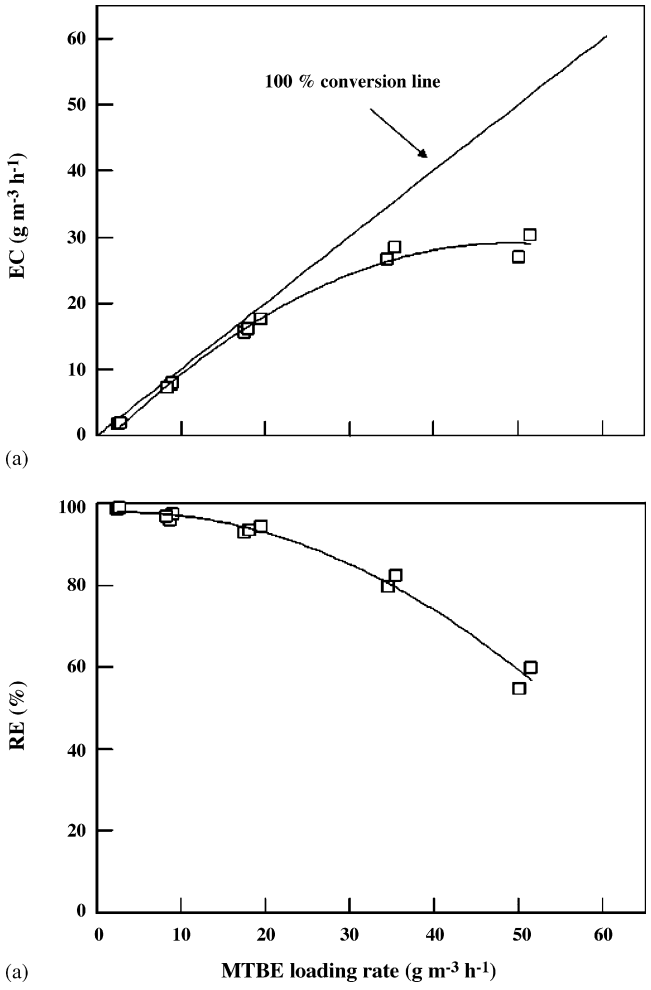

Fig. 4. Elimination capacity (a) and removal efficiencies vs. MTBE loading rates (b).

was maintained at 80% for an inlet load of 35.03 g m^{-3} h^{-1} (230 ppmv or 0.83 g m^{-3}) but declined at higher loads. It appears that growth of micro-organisms is inhibited at higher MBTE loading rates. Under the test conditions (MTBE loading rates at 2.67–50.76 g m^{-3} h^{-1}, temperature of recirculation liquid at 32 °C, rate of recirculation liquid at 400 ml min^{-1} and air empty-bed residence time of 85 s), the MTBE removal efficiencies ranged from 98.6 to 57.3%. The elimination capacity and the removal efficiency kept an approximately linear relationship when the inlet concentration was less than 0.83 g m^{-3} (i.e., $L < 35.03$ g m^{-3} h^{-1}) and the removal efficiency was greater than 80%. In this case, the reaction system became mass-transfer-limited, and the EC value was proportional to the MTBE loads. However, the system was changed to reaction-limited when the inlet concentration was larger than 0.83 g m^{-3} (i.e., $L > 35.03$ g m^{-3} h^{-1}) and the removal efficiency declined gradually. After the influent concentration was further increased to 1.21 g m^{-3} (i.e., an MTBE loading rate at 50.76 g m^{-3} h^{-1}), the EC was not further enhance, as indicated in Fig. 4. Hence, the highest observed range of the EC during this experiment is believed to be in the range of 30–35 g m^{-3} h^{-1}. This elimination capacity seems unremarkable, because in literature a higher maximum EC of 50 g m^{-3} h^{-1} for MTBE can be found for a biotrickling filter [6]. However, the maximum EC of this biotrickling filter is approximately four-fold higher than that of a compost biofilter for treating MTBE [18]. We believe that our results can be attributed to the higher moisture content applied in this biotrickling filter, the capability for MTBE control of which makes it an appealing application in the treatment of high-solubility contaminants, such as MTBE. A close examination of the data presented in Fig. 4 reveals that high REs were obtained up to loading rates of nearly 15–20 g m^{-3} h^{-1}. Therefore, this result can be used to design full-scale biotrickling filters for high MTBE removal efficiencies.

To provide further insight into the operation of the biotrickling filter unit, a comparison of the contribution of two biotrickling filter sections (0–30 and 30–60 cm) to the overall performance was accomplished. Fig. 5 shows the concentration profiles of the two sections in the axial direction of the biotrickling filter for various inlet MTBE loading rates. The biotrickling filter was effective in removal of MTBE in the upper section but performed poorly in the lower section, thereby demonstrating that most biological reactions occurred in the top section of the system. Obviously, a preponderance of the pollutants was eliminated in the upper section.

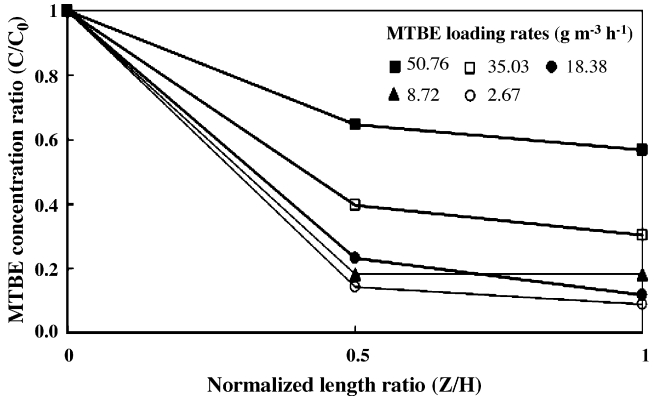

Fig. 5. Dimensionless MTBE concentration profiles across depth of biotrickling filter.

These removal variations in the incremental biotrickling filter length are believed to be due primarily to sufficient organic load, moisture content and nutrients in the upper sections of the biotrickling filter, resulting in an elevated population of micro-organisms. However, the lower removals observed in the bottom sections of the biotrickling filter may be partially attributed to the low carbon source, moisture content and nutrients in the biotrickling filter that could have a negative effect on the microbial activity. Fig. 5 also shows that the reduction of the MTBE concentration diminished as the loading rate decreased in the upper section. However, this concentration-reduction pattern was not observed in the lower section of the biotrickling filter.

3.3. Microbial community structure

Due to the significant difference in removal efficiency in a biotrickling filter when using $(NH_4)_2SO_4$ and $NaNO_3$ as a nitrogen source, a PCR-SSCP technique was employed to explore the changes in the microbial community during biodegradation in the biofilm. To evaluate the changes and to link them with a simultaneously diminishing substrate concentration in the microcosms, two biofilm samples were taken from the filter at different operation environments after 60 days. The first sample was taken from a biotrickling filter using $(NH_4)_2SO_4$ as a nitrogen source, having a removal efficiency of 76.4% and a pH of 4.5. The second sample was taken from a filter using $NaNO_3$ as a nitrogen source, having a removal efficiency of 93.5% and a pH of 7.

Fig. 6 shows that, after a 60-day operation period, different medium types resulted in different microbial community structures. The lanes designated L1, L3 and L5 in Fig. 6 represent communities developed in $(NH_4)_2SO_4$ medium cultures, the samples being taken from the upper, middle and lower sections of the biotrickling filter, respectively. Similarly, the lanes designated L2, L4 and L6 in Fig. 6 represent communities developed in $NaNO_3$ medium cultures. The total number of bands in these profiles for various sections in the biotrickling filter using the same nitrogen source is similar, thus indicating compositions of similar species in the biotrickling filter. However, communities grown in the presence of $(NH_4)_2SO_4$ and $NaNO_3$ media exhibited a different pattern, thus revealing a small change in community structure due to the media. Because the competition capability of MTBE-degrading species for nutrients declined for the biotrickling filter using $(NH_4)_2SO_4$ as a nitrogen source, the removal efficiency decreased as a complicated band appeared in SSCP gel. The substrate degradation rate has been known to affect community structure [19]. Our results show that the MTBE was degraded at higher rates when using $NaNO_3$ media than with $(NH_4)_2SO_4$ (Fig. 2(a)). MTBE with high degradation rates frequently generate communities with simple structures, probably by favoring the growth of a few dominant species, as exemplified in Fig. 6 when using $NaNO_3$ media.

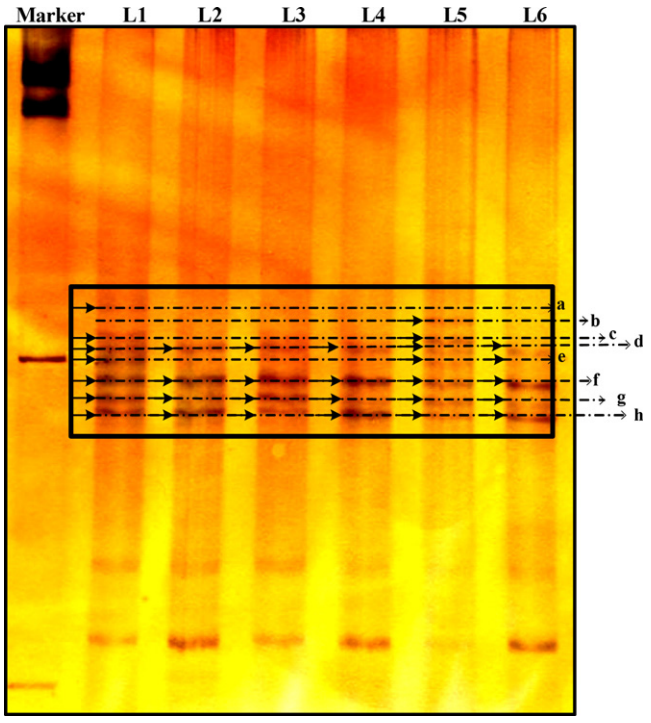

Fig. 6. SSCP bacterial community profiles obtained from 16S rDNA PCR amplicons during continuous tests for influence of ammonium sulfate and sodium nitrate in circulation solution. L1: $(NH_4)_2SO_4$ medium, upper filter; L3: $(NH_4)_2SO_4$, middle filter; L5: $(NH_4)_2SO_4$, lower filter; L2: $NaNO_3$, upper filter; L4: $NaNO_3$, middle filter; L6: $NaNO_3$, lower filter.

3.4. Kinetic parameters

As summarized in Table 1, the two sets of parameters estimated by using Eqs. (1) and (2) were 1.607 ± 0.208 ppmv s^{-1} for the maximum removal rate (V_m) and 24.91 ± 0.794 ppmv for the half-saturation constant (K_s). A relatively low level of K_s was found, thus indicating a high MTBE affinity in the biotrickling filter and thereby leading to a high removal rate. The low variation in K_s in various MTBE loads indicated that the biotrickling filter was operated in a low range of loads, resulting in an insignificant effect of the load on K_s. Conversely, the maximum removal rates were influenced by the MTBE load, with an increase in V_m by increasing the load. With the kinetic data of V_m and K_s, the reaction rate (R) in Eq. (2) was determined for the given MTBE inlet and outlet loads. Obviously, the reaction rate increased with the increase in loads between 1.73 and 23.74 g m^{-3} h^{-1} (10–200 ppmv). Beyond 200 ppmv, the increase rate declined, which was attributed to the high MTBE load. When given the different seeding organisms and packing materials in the biotrickling filters, the performance characteristics for various biotrickling filters can be examined by using these kinetic parameters.

Table 1
Kinetic parameters for biotrickling filter

Inlet concentration (ppmv)	Carbon loading rate (g C m^{-3} h^{-1})	V_m (ppmv s^{-1})	K_s (ppmv)
10	1.73	1.383	25.43
50	5.98	1.459	24.89
100	12.85	1.554	24.82
200	23.74	1.887	23.67
300	34.55	1.752	25.75
Mean		1.607	24.91
Standard deviation		0.208	0.794

4. Conclusions

The results demonstrate that a biotrickling filter appears to be very effective for MTBE removal under carefully controlled conditions. The pH of the solution tended to remain neutral, and the MTBE removal efficiency was maintained at around 95% when using $NaNO_3$ as a nitrogen source. This study clearly reveals that cultures using sodium nitrate as a medium can degrade toxic compounds easily. MTBE removal at the bottom section of the biotrickling filter was consistently lower than for the top section, probably due to an insufficient micro-organism growth with a low load and nutrients in the bottom section. It was observed that the relatively low K_s indicated a high MTBE affinity in the biotrickling filter and consequently led to a high MTBE removal rate. However, the low variation in K_s in various MTBE loads indicated that the biotrickling filter was operated in the low range of MTBE loads, thus resulting in an insignificant effect of the MTBE load on K_s. The maximum removal rates were influenced by the MTBE load, with an increase in V_m by increasing the load. MTBE with higher degradation rates frequently appears to generate communities with simpler structures, probably by favoring the growth of a few dominant species. However, further investigation is needed for a better understanding of the link between the composition of a microbial community and the efficiency of the reactor; moreover, the dynamics of the bacterial population may provide superior information to ensure the success of the biotrickling filter system.

Acknowledgments

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