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Performance of a biotrickling filter in the removal of waste gases containing low concentrations of mixed VOCs from a paint and coating plant

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Abstract The performance of a field-scale biotrickling filter (BTF) in the removal of waste gases containing low concentrations of mixed volatile organic compounds was evaluated. Results showed that acetone and methyl ethyl ketone (MEK) were more easily removed than toluene and styrene. The removal efficiency for acetone and MEK reached over 99% on days 28 and 25 of the operation, whereas those for toluene and styrene were 80 and 63% on day 38. The maximum individual elimination capacities for styrene, toluene, acetone, and MEK were 10.2, 2.7, 4.7, and 8.4 g/m^3 h, respectively. These values were achieved at inlet loading rates of 13.9, 3.3, 4.8, and 8.5 g/m^3 h, respectively, at an empty bed retention time of 14 s. the removal efficiency for styrene and toluene rapidly increased from 67% and 83% to 86% and over 99%, respectively, when the concentration of ammonia nitrogen ($N–NH_4^+$) and phosphates (P) in the nutrients increased from 350 to 840 mg/l and 76 to 186 mg/l. When the BTF was restarted after a four-day shutdown, the removal efficiency for toluene was restored to over 99% within a week. However, that for styrene was not restored to its previous level before the shutdown. No noticeable adverse effect on acetone and MEK removal was observed. Denaturing gradient gel electrophoresis results for the bacterial community in the BTF during VOC removal showed that proteobacterial phylum was dominant, and the changes of nutrient concentration and shutdown periods may have played a role in the community structure differences.

Keywords Biotrickling filter · Volatile organic compounds · Bacterial community · Denaturing gradient gel electrophoresis

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Introduction

Paints and coatings are widely used for automobiles, architectural structures, household appliances, and ships. Paint and coating yields in China have rapidly increased in the past years. Large volumes of waste gases containing volatile organic compounds (VOCs) are released into the atmosphere during the

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manufacture of these materials, which impose acute and chronic effects on workers and nearby residents. Several technologies have been developed for the removal of VOCs from gas streams; among these technologies, biofiltration has emerged as an efficient and cost-effective air pollution control technology. Biofiltration is also an environment-friendly technology because it is based on the ability of microorganisms to convert a large variety of VOCs into harmless oxidation products (e.g., water and carbon dioxide) (Malhautier et al. 2005).

The biofiltration system applied in industries is usually operated under unsteady states or transient loading conditions results from shutdown, intermittent running or load shocking, which are the most commonly encountered problems in the field. Contaminant concentration frequently varies throughout the manufacturing process. The inlet loading of the biofiltration system sometimes increases many times over, passing on load shock to the system and resulting in contaminant breakthrough (Rene et al. 2009). Discontinuous emissions of waste gases are also common in industrial processes because of overnight, weekend shutdowns and scheduled maintenance activities; these are additional challenges confronting the application of the biofiltration system. The microbial activity and microbial ecosystem may be affected during idle periods because of the lack of carbon or biofilm dehydration. Therefore, evaluating the effect of shutdown periods on biofiltration system performance is important. Although several reports demonstrating the performance of biofiltration systems under unsteady states have been published (Kim et al. 2008; Sempere et al. 2008), experimental testing on such conditions remains very limited. More information about biofiltration performance under field conditions is especially needed to all assessment of the combined effects of shutdown, shock loading, and other variations when treating waste gases containing VOC mixtures.

Additionally, industrial waste gases generally contain multiple contaminants, individual VOCs are rarely found alone. Thus, study of biofilters treating VOC mixtures is of practical significance. Results of degradation studies on VOC mixtures showed that the removal of one component may be affected by other components in the mixture (Reardon et al. 2002; Shim et al. 2006; Lee and Cho 2009; Chan and Lai 2010; Chan and Lin 2010); such interaction involves enhancement, inhibition, and co-metabolism (Littlejohns and Daugulis 2008). Few studies have focused on the biological removal of paint and coating VOCs and only a few pilot/full-scale biofilters have been reported. Mathur and Majumder (2008) described a coal biofilter that treats simulated paint VOC mixtures that contain methyl ethyl ketone (MEK), toluene, *n*-butyl acetate, and *o*-xylene (MTBX). A nearly 100% removal was achieved at influent loadings of less than 120 g/m^3 h. Martínez-Soria et al. (2009) successfully applied a pilot-scale biotrickling filter (BTF) filled with polypropylene rings in treating VOC emissions from a furniture manufacturing facility.

The performance of a biofiltration reactor depends substantially on the robustness of the microbial community. Understanding the microbial community is essential in optimizing the process parameters of the bioreactor. However, literature survey revealed that only a few studies have been carried out on analyzing microbial community structures in a VOC mixture-removing bioreactor. Some authors have focused on the changes and diversity in the community structure of bioreactors involving only a single VOC (Grove et al. 2007; Wang et al. 2009; Goncalves and Govind 2009). Khammar et al. (2005) investigated the spatial structure of microbial communities residing in a peat biofilter treating a complex mixture of VOCs by single-strand conformation polymorphism. They demonstrated that the distribution of the biodegradation activities of VOC is correlated with the spatialization of microbial density and diversity. Cai et al. (2008) used denaturing gradient gel electrophoresis (DGGE) to assess the effect of interchanging the VOCs in the bacterial community structure in the biofilters. The authors employed two aromatic compounds (toluene and styrene) and two oxygenated compounds (MEK and methyl isobutyl ketone (MIBK) as the target contaminants. They found that the microbial community structure in the biofilter changed after each VOC interchange, but the microbial species in the VOC mixture-removing biofilters did not show significant difference.

In the present study, a field-scale BTF was designed and applied in the treatment of waste gas emissions from a paint and coating manufacturing plant. The first objective of the study was to evaluate the performance of the BTF in treating the VOC mixture-loaded air stream from the paint and coating

manufacturing process under unsteady-state operations. The effect of a shutdown and nutrients on BTF performance was comprehensively investigated. Furthermore, the response of the bacterial community composition in the BTF to shutdowns and changes in nutrient was also examined by polymerase chain reaction (PCR)-DGGE technique.

Materials and methods

BTF and operations

A field-scale BTF with size of 1.5 × 1.5 × 1.8 m was made of stainless steel. It was installed in a paint and coating plant, and operated under ambient temperature (15–37°C). Pall rings (Zhen Da Co. Ltd., China), with an average size of 16 × 12 × 3 mm, were used as filter materials. The bulk density, porosity, and specific surface area of the pall rings was 690 kg/m^3, 71%, and 378 m^2/m^3, respectively. A perforated sieve plate was placed at the bottom of the filter bed to support the packing materials. The effective packed volume of the filter bed was 1.58 m^3. A sampling port in the column wall for collecting the packing materials was located 0.3 m to the bottom of the packed BTF bed. VOC-polluted air was extracted by a fan from a workshop at a flow rate of 405 m^3/h, passed through a humidifier to increase moisture content, and subsequently delivered downward into the BTF. The calculated EBRT was 14 s.

The nutrient solution stored in a tank located at the bottom of the filter was recycled using a peristaltic pump at 2 l/min for 5 min every hour, and was completely renewed every week. The peristaltic pump was connected to a spray nozzle located at the top of the BTF to spray the solution uniformly onto the packing materials. The solution volume was periodically maintained at 600 l through the addition of tap water. The base nutrient solution (1×) contained 15 mg/l KH_2PO_4, 140 mg/l Na_2HPO_4, 10 mg/l $MgSO_4{\cdot}7H_2O$, 10 mg/l $CaCl_2{\cdot}2H_2O$, 6 mg/l $FeSO_4{\cdot}7H_2O$, and 450 mg/l NH_4Cl. The concentrations of ammonia nitrogen (NH_4^+–N) and phosphates (P) in the nutrient solution was 140 and 31 mg/l, respectively. The nutrient concentration was increased following the procedure described in Table 1 for the consideration of the increase in VOC mixture-inlet loading at the last phase of the experiment. The bed drop, ambient temperature, and pH of the nutrient solution were also monitored.

Table 1 The nutrient using procedure

Operating time (d)	1–30	31–57	58–65	66–80
The concentration of the nutrient	2×	2.5×	6×	8×

The activated sludge used to inoculate the BTF was drawn from the on-site wastewater treatment station of the company. To develop biofilms on the surface of packing materials, 150 l of the activated sludge plus 450 l of nutrient solution was continuously recycled for two days during which only fresh air was supplied to the BTF before the startup.

Analytical methods

The biofiltration was run under intermittent conditions because of the discontinuous manufacturing schedule, with contaminants supplied only 8 h a day and 5 days a week. Using 1L Tedlar gas bags, inlet and outlet gas samples were collected every 2 or 3 days a week during the manufacturing hours of the factory (Monday to Friday, 9:00 to 9:30 a.m.). However, to comprehensively assess the effect of the shutdown and nutrients on BTF performance in removing each VOC, the inlet and outlet gas samples were collected daily from days 58 to 80. The VOC concentration was measured using a voyager portable digital gas chromatograph (Photovac, Inc., USA), which consists of a built-in three-column configuration and dual electron capture and photo ionization detectors. Styrene and toluene were determined using blank fused silica and a Supelcowax10TM column, respectively, while acetone and MEK were determined using a Quadrex 007-1 column. Nitrogen was used as carrier gas. The column temperature was isotheral at 60°C. The detection limits for the aforementioned compounds were below 0.03 ppm. The ammonia nitrogen of the nutrient solution was determined using Nessler's reagent colorimetric method (according to the Chinese national standard method GB7479-87). pH was monitored periodically using a pH meter (SevenMulti S40). The removal efficiency (RE) and elimination capacity (EC) of each component were calculated as follows:

$$RE(\%) = \left(\frac{C_{in} - C_{out}}{C_{in}}\right) \times 100$$

$$EC(g/m^3 \cdot h) = (C_{in} - C_{out}) \times \frac{F}{V}$$

where C_{in} is the inlet concentration (g/m^3), C_{out} is the outlet concentration (g/m^3), F is the gas flow rate (m^3/h), and V is the packed volume (m^3).

Bacterial community analysis

Packing materials (10 g, wet weight) were collected from the BTF for analysis, mixed with 50 ml phosphate buffer (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO4, and 1.4 mM KH_2PO4; pH 7.3), and vortexed for 30 min. The packing materials were discarded after detachment, and the liquid phase containing the biofilm was centrifuged at 8,000 rpm for 10 min. The pellet was resuspended in 2.7 ml DNA extraction buffer (100 mM Tris, pH 8.0; 100 mM EDTA, pH 8.0; 1.5 M NaCl; and 1% CTAB) and 20 µl proteinase K (10 mg/ml) in a 5 ml centrifuge tube. The mixture was shaken at 225 rpm for 30 min at 37°C, after which 300 µl 20% SDS was added. The samples were then incubated at 65°C for 60 min and gently shaken once every 20 min. The lysates were centrifuged at 6000×*g* for 10 min followed by chloroform extraction, as described by Yin and Xu (2009). The bacterial V6 regions were PCR-amplified using primers FP968GC and R1401 (Kimura et al. 2003). PCR reactions were carried out on 50 µl reaction volumes containing 1.5 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphate, 1 µM each of the primers, 0.1 mg/ml analytical grade bovine serum albumin, and 2.5 U DNA polymerase using a Mastercycler gradient thermocycler (Eppendorf, Tiangen, Beijing). The cycling parameters were 94°C for 5 min, followed by 30 cycles of 94°C for 40 s, 55°C for 30 s, and 72°C for 40 s, with a final extension at 72°C for 5 min. The PCR product was electrophoresed in 1.0% (w/v) agarose gel and visualized after staining with 0.5 µg/ml GoldView (Applygen Technologies Inc., Beijing).

DGGE

DGGE analysis was carried out using a D-Code Universal Mutation Detection System (Bio-Rad Laboratories Inc.). The PCR products were loaded onto 10% polyacrylamide gel (10% (w/v), 37.5:1 acrylamide/biacrylamide) with a linear denaturant gradient increasing from 30 to 60% (100% denaturant defined as 7 M urea plus 40% formamide). DGGE was performed using 50 µl of PCR product in 1× TAE buffer (40 mM Tris, 20 mM acetate, 1 mM EDTA pH 7.4) at 60°C and 80 V for 12 h. The resulting gel was stained with Goldview and visualized with ImageQuant 350 (GE Healthcare, USA) and digitalized image was analyzed using Quantity one version 4.4 software (Bio-Rad, USA), background was subtracted using rolling disk with the diameter of 50. Relative band densities were calculated by the software.

The discriminable bands were excised, re-amplified with the above-mentioned primers, and sequenced after cloning into pMD 18-T vector (Shanghai Sangon Company, China) to obtain insight into the dynamic changes that occur in the composition of the BTF bacterial community under different operating conditions. The nucleotide–nucleotide Basic Local Alignment Search Tool (BLASTn) was used to search for nucleotide sequence similarities in the National Center for Biotechnology Information website, and the sequence in the database most similar to each clone was used as reference.

Results and discussion

BTF startup

An experiment was conducted in a paint and coating manufacturing plant to evaluate the performance of the BTF in purifying waste gases containing VOC mixtures in low concentrations. Styrene, toluene, acetone, and MEK were the primary pollutants detected in the waste gases. The average inlet concentrations and standard deviations for styrene, toluene, acetone, and MEK within 80 days after startup were 17.9 ± 13.5, 2.0 ± 2.2, 5.1 ± 4.7, and 19.5 ± 66.9 mg/m^3, respectively. A high standard deviation means that the concentration of each contaminant had considerably fluctuated over the entire experiment periods. BTF performance during the startup period is depicted in Fig. 1. As shown in Fig. 1a, b, the removal efficiencies for acetone and MEK gradually increased with increasing durations and were over 99% on days 28 and 25 of the

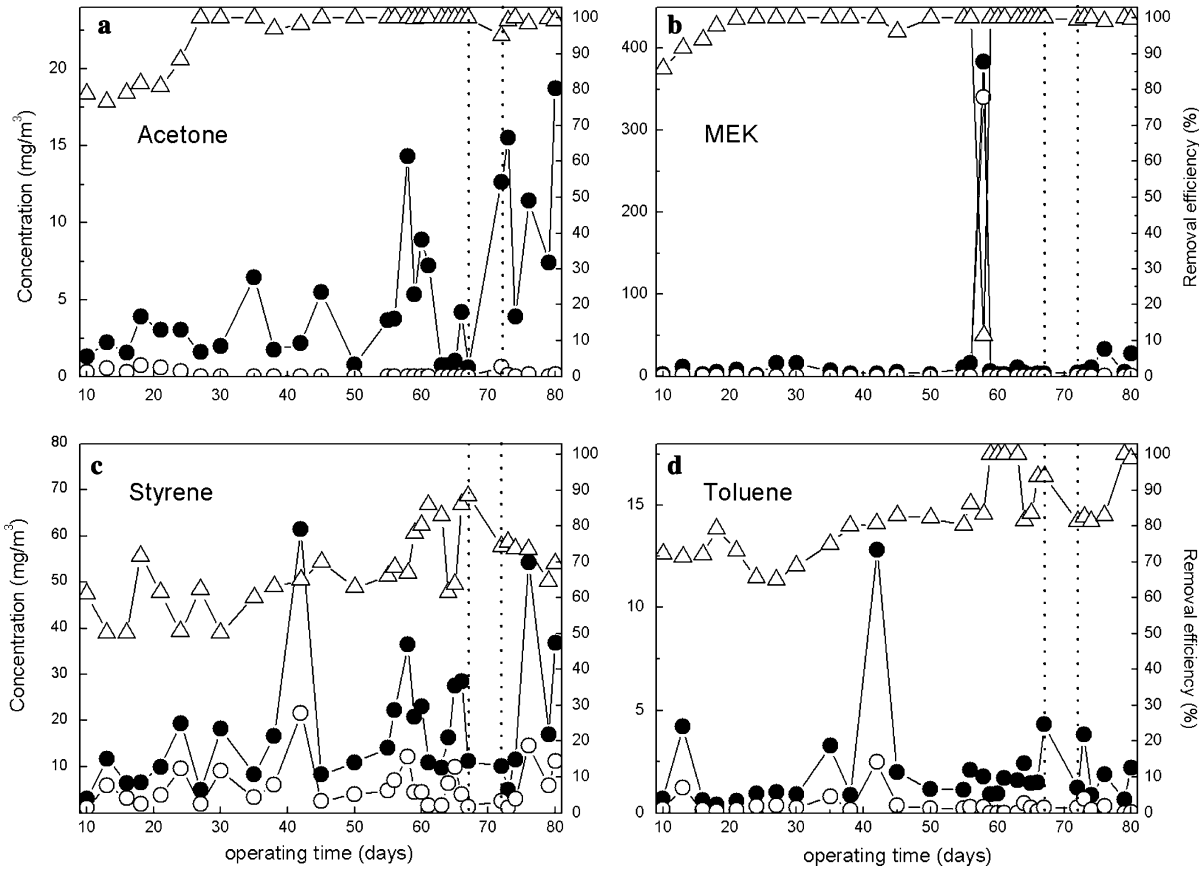

Fig. 1 Performance of BTF in the removal of **a** acetone, **b** MEK, **c** styrene, and **d** toluene (*open triangle* removal efficiency, *open circle* outlet concentration, *filled circle* inlet concentration.) (the *dotted line* indicates the shutdown period)

operation, respectively; the efficiencies remained constant during nearly the entirety of the experimental periods except on day 58, during which the MEK removal efficiency dramatically dropped because of the sudden increase in MEK inlet concentration.

The behavior of the BTF for the other two aromatic compounds differed from that for the two oxygenated compounds. Figure 1c, d show that the BTF exhibited relatively poorer performance and took a longer time to acclimate for toluene and styrene compared with the other two oxygenated compounds. On day 38 of the operation, the removal efficiencies for toluene and styrene were only 80 and 63%, respectively. The removal efficiency for toluene continued to improve slowly, finally reaching an efficiency of over 99% on day 59. The removal efficiency for styrene reached 86% on day 61 and subsequently dropped to 62%, a decrease that can be attributed to the depletion of nutrients (discussed below). The acclimation periods needed for styrene and toluene were longer than those for acetone and MEK. The presence of acetone and MEK may have suppressed the microbial activity that degrades styrene, resulting in poorer styrene removal.

The first step to biofiltration is the transfer of pollutants from air to water or biofilm (Ottengraf and Van Den Oever 1983). Hydrophilic compounds are more easily and rapidly removed in biofiltration systems than are hydrophobic compounds, and the removal of the latter can be suppressed by the former (Ikemoto et al. 2006). A similar behavior was reported by Mohseni and Allen (2000), who observed that the presence of methanol in the system significantly decreased the removal rate for α-pinene. In another biofilter simultaneously treating ethyl acetate and toluene, the presence of ethyl acetate in the system also significantly reduced toluene removal (Liu et al. 2002). In addition, the running model of

the bioreactor influenced the acclimation time. Moe et al. showed that a biofilter subjected to intermittent loading conditions took a longer time to reach high performance than did one subjected to continuous loading conditions (Moe and Qi 2005; Qi and Moe 2006).

BTF performance was also evaluated in terms of the EC of each contaminant at various loading rates. The maximum individual EC values of styrene, toluene, acetone, and MEK were 10.2, 2.7, 4.7, and 8.4 g/m^3 h, respectively. These were achieved at inlet loading rates of 13.9, 3.3, 4.8, and 8.5 g/m^3 h, respectively, and at an EBRT of 14 s. Comparing the EC values with those reported by other authors is difficult because of the different compositions of the VOC mixtures and the limited number of studies on the subject matter. Mathur et al. (2008) studied the removal of paint VOC MTBX mixture using a coal biofilter. A maximum EC of 185 g/m^3 h was obtained at an MTBX load of 278 g/m^3 h, with an EBRT of 42.4 s. Cai et al. (2008) observed that a biofilter integrated with two bed sorption units can maintain a consistent 99% removal efficiency in treating VOC mixtures of toluene, styrene, MEK, and MIBK when the inlet load rate does not exceed 34 g/m^3 h. Most of the studies on biofiltration were conducted at laboratory scale. However, as referred by Sempere et al. (2010), industrial emissions present variable and discontinuous concentrations that hinder the performance of field-scale BTFs. They studied the removal of air emissions from painting and wood finishing in a pilot BTF, and the average EC during the operating hours of the factory for typical days ranged from 32.8 to 12.7 g/m^3 h as the EBRT increased from 10 to 35 s. But the corresponding removal efficiency increased only from 49 to 70%.

The pressure drop across the filter bed was kept very low (<20 pa/m) during the experiments, which indicated that the bioreactor had no clogging problems and the biomass accumulation was very slow under the tested conditions. These results can be attributed to the low inlet VOC concentrations and the discontinuous running model of the bioreactor, which resulted in fewer microorganisms produced in the bioreactor. Thus, additional methods for controlling biomass growth under these conditions were unnecessary. Similar results were drawn by Martínez-Soria et al. (2009), who observed that the pressure drop in a BTF was kept at less than 15 pa/m when the BTF was supplied low inlet VOC feeding.

Effect of shutdowns on BTF performance

Throughout the BTF operation, uncontaminated air and nutrient solution were supplied during the idle periods of the bioreactor on nights and weekends. From days 68 to 71, the biofiltration filter was shut down for four days because of a holiday, during which no air and nutrient solution were supplied to the bioreactor. Figure 1 shows that the shutdown periods caused varying effects on the removal efficiency for the different contaminants. When the BTF restarted after a four-day shutdown, the removal efficiencies for toluene and styrene significantly decreased from 94 and 88% to 81 and 74%, respectively. The removal efficiency of toluene was restored to more than 99% within a week, whereas the styrene removal efficiency was not restored to its previous level and ranged from 65 to 75% at days 72 to 80. A potential explanation for this phenomenon is that the shutdown periods may have negatively affected the styrene-degrading microorganisms. The results may be further confirmed using PCR-DGGE (see below), which revealed that some changes in the bacterial community profile occurred after the reactor restarted. Conversely, the removal of acetone and MEK was only slightly influenced by the four-day shutdown. The removal efficiencies for acetone and MEK were quickly restored to 100% within 2 days after the bioreactor was restarted. These results are similar to those obtained from a fungal biofilter, in which the shutdown had no noticeable adverse effect on the removal of *n*-butyl acetate; the removal of MEK and methyl propyl ketone was adversely affected for a few hours, and toluene removal was adversely affected for a few days (Moe and Qi 2004).

The re-acclimation time after shutdown varied depending on the VOC tested, packing materials, shutdown periods. Several studies reported that the pollutant removal or the structure of the microbial community was only slightly influenced during the idle phase, and recovered to the original level when waste gases were reintroduced into the bioreactor. Ho et al. (2008) reported that a biofilter, which inoculated *Arthrobacter* sp. and was packed with granular activated carbon, lost only 1.9 and 8.3% removal capacities for TMA and NH_3, respectively, after a

10-day shutdown. These losses were attributed to the slight drop in the packing materials into which TMA and NH_3 were absorbed and which were temporarily supplied to the microbes during shutdown. However, the supply of air and moisture during the shutdown period is necessary for minimizing the re-acclimation period and is essential to maintaining microbial activity (Jang et al. 2006a). Lee et al. (2009) reported that only one day was required for re-adaptation after a two-week shutdown even though no air or nutrients were supplied to the biofilter during the shutdown period. This short re-adaptation period in their work was assumed related to the higher water-holding capacity of the packing materials (i.e., polyurethane). Dorado et al. (2010) also confirmed that this type of packing material has a relatively large water-holding capacity compared with other inorganic support media. However, in the present study, the BTF was installed outside the paint manufacturing plant and easily suffered from environmental conditions. After a four-day shutdown, during which no nutrient and water were supplied, the BTF may have lost part of its capability to degrade the less easily biodegradable compounds of the feed mixture. This diminished capability can be attributed to the dehydration of the biofilm, causing an adverse effect on the microorganisms degrading styrene, a phenomenon similar to that reported by Sempere et al. (2008). Thus, supplying water during shutdown periods is necessary, especially when the biofiltration system is applied in the field.

Effect of the nutrient concentration on the performance of the BTF

The BTF designed in the experiment was packed with inert inorganic materials, thereby requiring the periodic supply of suitable nutrients to support microorganism growth and maintain BTF performance. The samples were periodically collected from the nutrient solution storage tank, and the concentration of ammonia nitrogen in the solution was detected to comprehensively evaluate the relationship between the nutrients and BTF performance. Figure 2 illustrates the relationship between ammonia nitrogen concentration, styrene removal efficiency, and pH. The results showed that nutrient concentration had a significant effect on BTF performance, especially on styrene removal. The removal efficiency for styrene fluctuated below 70% when the concentration of ammonia nitrogen in the nutrients was lower than 350 mg/l. However, when the concentration of ammonia nitrogen in the nutrients increased from 350 to 840 mg/l on day 58, the removal efficiency for styrene rapidly increased from 67% on day 58 to 86% on day 61, and subsequently decreased to 64% on day 65. These results are attributed to the depletion of the nutrients, resulting in a decrease in ammonia nitrogen concentration from 840 to 252 $N–NH_4^+$ mg/l. When the concentration of ammonia nitrogen increased to 1120 $N–NH_4^+$ mg/l on day 66, the removal efficiency for styrene was restored and reached 88% on day 67. The higher amount of nutrients effectively stimulated the microbial degradation rate. The response of toluene removal to the nutrients was similar to that of styrene (Fig. 1b). The removal efficiencies for acetone and MEK were not influenced when the nutrients were depleted. Thus, toluene and styrene, the relatively less biodegradable compounds, were more easily influenced by nutrient concentration than the more biodegradable compounds acetone and MEK. The addition interval of nutrients should not exceed five days. When the nutrients were exhausted, the cells of the microorganisms underwent endogenous respiration, and the substrate was used simply to maintain cell viability (Acuña et al. 2002). The pH of the nutrient solution decreased with nutrient depletion. This pH drop may have been caused by nitrification, NH_4^+ is transformed to NO_3^- and

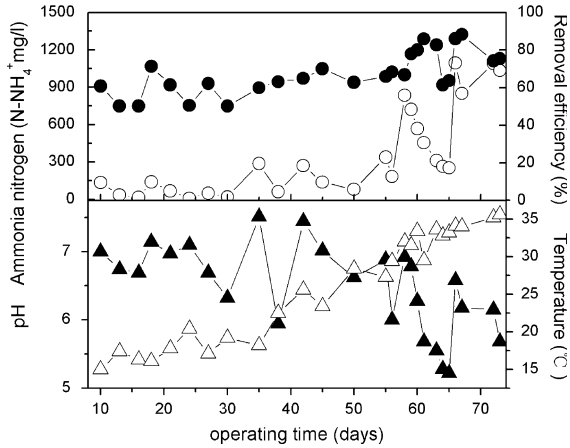

Fig. 2 Relationship among the concentration of ammonia–nitrogen, styrene removal, and pH (*filled circle* styrene removal efficiency, *open circle* concentration of ammonia nitrogen, *filled triangle* pH, *open triangle* temperature)

alkalinity is consumed. The accumulation of NO_3^- resulted in the increase in denitrifying bacteria (see Section 3.4). As shown in Fig. 2, the ambient temperature recorded at every gas sampling varied from 15 to 37°C, and no obvious direct effect of temperature on bioreactor performance was observed, indicating that the biofiltration technique can be applied in a broad range of temperatures. Thus, the removal efficiency was mainly influenced by the nutrients, pH, and constituents of the VOC mixture. The nutrients are required for the maintenance of microbial activity and the consequent removal of the pollutants. A gradual decrease in the removal capacity can be observed under a limited nutrient environment (Son et al. 2005). Acuña et al. (2002) also reported that using a peat biofilter packing material amended with high nutrient concentration can yield toluene consumption rates higher than those obtained with lower nutrient concentrations.

Microbiological analyses

DGGE of bacterial 16S rDNA was performed to analyze the samples that were periodically collected from days 0 to 80 to gain insight into the dynamic changes that occur in the composition of the BTF bacterial community under unsteady-state conditions. Duplicate PCR yielded nearly indistinguishable DGGE results (results not shown).

The activated sludge drawn from the on-site wastewater treatment station of the paint company was used as inoculums for the BTF. Figure 3 shows the DGGE profiles of the BTF bacterial community during the VOC removal process. The band pattern of the sample collected during the reactor startup was considerably different from those obtained on subsequent days. Three intense bands (4, 5, and 7) observed on day 0 did not appear in subsequent sampling days. By contrast, bands 2, 3, 6, and 8, which were not observed on day 0, were consistently present in most of the samples collected after acclimation. These results indicated that the activated sludge from the on-site wastewater treatment station may not be suitable as initial inoculums because of the lack of aromatic compound-degrading microorganisms. It may have been one of the important reasons for the two-month acclimation period of the reactor for styrene and toluene. Band 1 was observed in all the samples but has a lower intensity. Band 9

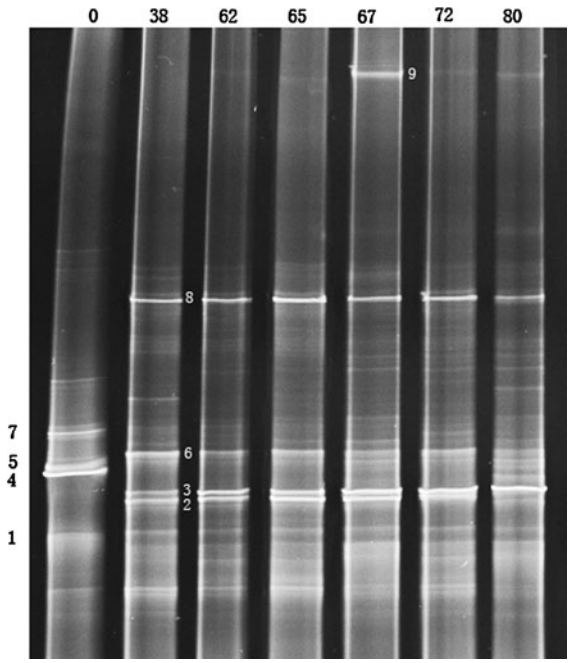

Fig. 3 DGGE profiles of the bacterial community in the BTF

appeared on day 67 under high nutrient concentration. Several bands with low intensities between bands 6 and 8 also began to appear on day 67 probably because the addition of a higher concentration nutrient on day 66 enhanced the activity of the VOC-degrading microbes, resulting in the accumulation of metabolic products and stimulation of heterotrophic microorganisms.

Microorganisms play a crucial role in the removal of pollutants from waste gases, and inoculation with proper microbial combination is necessary for better performance of biofiltration systems. Jang et al. (2006b) reported that a biofilter using different combinations of bacterial cultures showed enhanced styrene removal efficiency compared with those using a single culture. Hernández et al. (2010) confirmed that a biofilter inoculated with a pre-adapted microbial population achieved higher nitrification rates and VOC removal efficiencies than did a biofilter inoculated with sludge from a municipal wastewater treatment plant.

Nine discernable bands were excised and used for sequence analysis to better understand the dynamic changes in the bacterial community during biofiltration. The closest relative, nucleotide sequence similarity, phylogeny, and relative abundance of the

Table 2 Nucleotide sequence similarity and relative abundance of sequenced DGGE bands

Band[a]	Closest relatives	Phylogeny	Sources	similarity	Accession	Relative abundance of DGGE bands					
						0[b]	38	62	65	67	72	80
1	Unidentified bacterium	*β*-Proteobacteria	Effluent	99%	AY157117	1.5%	6.6%	4.3%	4.5%	3.5%	4.8%	3.0%
2	*Xanthomonadales* bacterium	*γ*-Proteobacteria	Effluent	99%	EU403632	0%	8.9%	14.7%	16.0%	11.7%	5.6%	2.3%
3	Unidentified bacterium	*γ*-Proteobacteria	Enrichment cultures	99%	AB426189	0%	7.8%	18.8%	12.9%	15.6%	18.7%	25.8%
4	Unidentified bacterium	*γ*-Proteobacteria	Biofilter	93%	AJ318161	11.2%	0%	0%	0%	0%	0%	0%
5	Unidentified bacterium	*β*-proteobacteria	Wastewater	96%	AB504647	4.6%	0%	0%	0%	0%	0%	0%
6	Unidentified bacterium	*α*-Proteobacteria	wastewater	95%	DQ988316	0%	19.1%	12.4%	11.2%	6.0%	12.6%	4.3%
7	Unidentified bacterium	*β*-proteobacteria	Activated sludge	98%	AF502222	32.4%	0%	0%	0%	0%	0%	0%
8	*Pandoraea* sp. JB1	*β*-proteobacteria	Soils	99%	DQ167022	0.0%	14.6%	17.9%	18.8%	13.4%	17.8%	12.9%
9	Denitrifying bacterium	*δ*-proteobacteria	River sediment	96%	FJ802270	0%	0%	0%	1.3%	13.8%	1.4%	2.6%

[a] The bands are designated as shown in Fig. 3

[b] The numbers indicate different operating periods

DGGE bands are listed in Table 2. These bands were individually identified as different members of the phylum Proteobacteria by comparison with the GenBank database. Three bands (1, 5, 7, and 8) were grouped with the *β*-proteobacteria class, another three bands (2, 3, and 4) were clustered within the *γ*-proteobacteria class, while the band 6 belongs to *α*-proteobacteria class. These results suggest that the Proteobacteria class was dominant in the BTF. On the basis of the analysis of the DGGE profile using the Quantity One software, we determined that the intensity of bands 2 and 3 were lower on day 38 (8.9 and 7.8%, respectively), but began to increase on day 62 (16.0 and 12.9%, respectively). This result may be attributed to the stimulation of VOC-degrading microbial growth caused by the higher nutrient concentration. However, the relative intensity of band 2 was reduced to 5.6% on day 72, which corresponds to the re-startup of the bioreactor after a four-day shutdown. The shutdown may have negatively affected some VOC-degrading microbes. Band 2 may be associated with the styrene-degrading microorganisms because the removal efficiency for styrene was not restored to its previous level and fluctuated from 63–78% after re-startup.

Sequence analysis revealed that that the most closely related sequences in the GenBank database were almost all clones from polluted environments or waste treatment systems. The source of the closest relatives to the sequenced bands was listed in Table 2.

Conclusion

This study evaluated the removal performance of a BTF in the treatment of waste gases containing mixed VOCs during the startup period. The BTF was successfully started up under unsteady-state conditions, such as fluctuations in the VOC concentration and an intermittent running model, but required a longer time to acclimate to toluene and styrene than to acetone and MEK. The nutrient concentration and shutdown significantly affected the removal of aromatic compounds. High nutrient concentrations improved BTF performance in the removal of styrene and toluene. Further investigation on the bacterial community shift during the startup was conducted. Proteobacterial phylum was the dominant microbe in

the BTF, and initial inoculums played an important role in the enhancement of BTF removal capacity. The change of nutrient concentration and shutdown periods may have caused a shift in the composition of the bacterial community, and led to the deterioration of BTF performance in treating hydrophobic compounds.

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