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Treatment of gaseous alpha-pinene by a combined system containing photo oxidation and aerobic biotrickling filtration

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ABSTRACT

Biofiltration of hydrophobic and/or recalcitrant volatile pollutants is intrinsically limited. In the present study, a combined ultraviolet-biotrickling filter (UV-BTF) was developed to improve the removal of these compounds, and a single BTF as the control was operated under the same conditions. The experimental results showed that the UV-BTF provided higher removal efficiencies than the single BTF at an inlet concentration range of 600–1500 mg m^{-3} under shorter residence times. The maximum elimination capacities (ECs) obtained were 94.2 mg m^{-3} h^{-1} and 44 mg m^{-3} h^{-1} in the combined UV-BTF and single BTF, respectively. The mass ratio of carbon dioxide produced to α-pinene removed in the UV-BTF was approximately 2.74, which was much higher than that of the single BTF (1.99). Polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) analysis indicated that there was more complicated microbial community in the UV-BTF than that in the single BTF. In addition, we investigated the effect of starvation or stagnation on re-acclimation and removal performance from an engineering standpoint. The results showed that the combined UV-BTF could deal with fluctuating conditions or periods without any flow (air or liquid) supply much better than the single BTF.

1. Introduction

Alpha-pinene (α-pinene) is applied in various industries such as pulp and paper industry, forest production, pharmaceutical industry, color printing and paint process etc. [1–3]. Industries that produce or utilize α-pinene usually generate gaseous wastes of this compound with variable concentrations ranging from a few hundreds to thousands of milligrams per cubic meter. Indiscriminate release of α-pinene not only causes health problems to humans, but also promotes the formations of secondary organic aerosols and photochemical smog [4,5]. Therefore, there is still a considerable interest in developing innovative and cost-effective approaches for removal of α-pinene.

Biofiltration of VOCs provides an environmentally friendly and low-cost alternative to other physical and chemical treatment technologies such as incineration, catalytic oxidation, and adsorption [6,7]. Several researchers have demonstrated that a large number of VOCs, like benzene [8], toluene [9] and styrene [10], could be successfully removed by biofiltration. However, α-pinene's hydrophobic property and high toxicity would impede the application of this single purification technology. The reported critical elimination capacities for α-pinene by the bacterial-biofilters were only 3.9–60 g m^{-3} h^{-1} [11,12], which were much lower than those soluble and biodegradable compounds (methanol with 250 g m^{-3} h^{-1} [13] and methyl ethyl ketone with 174 g m^{-3} h^{-1} [14]). Alternatively, some researchers suggested that the advanced oxidation process (AOP) could be utilized as a pretreatment for the subsequent biological processes, since this chemical technology converted some special compounds to several simple intermediates, thus solving the mass transfer limits and the bio-activity inhibition appearing in the biological facilities.

Although AOPs based on the formation of reactive radicals in situ are regarded as very promising methods for the complete removal of the recalcitrant organic pollutants, one major drawback is the relative high operating cost [15,16]. Recently, partial oxidation is preferred by several researchers, owing to its relatively lower cost and the produced biodegradable intermediates. Successes of the combined AOP-activated sludge facilities not only proved the technical possibility in wastewater treatments, but also expanded the types of pollutants treated by the traditional biological facilities [17–20]. Due to the special characteristics of subsequent biofiltration, the ultraviolet (UV) technology is generally chosen as the suitable pretreatment by researchers. UV oxidation

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was demonstrated to convert some gaseous compounds (such as chlorobenzene, toluene, etc.) into soluble and biodegradable intermediates, and the combined systems have been successfully applied in the removal of gaseous hydrophobic and recalcitrant compounds [21–24]. Moussavi and Mohseni [23] found that the combined UV-biofiltration system provided up to 60% additional removal for toluene and *o*-xylene mixtures, and the combined UV-biofiltration system had a much lower pressure drop than the single biofiltration reactor. Den et al. [24] used the combined UV-BTF system for treatment of trichloroethylene and perchloroethylene, and demonstrated that all the photodegradation products were completely removed in the subsequent BTF. Wang et al. [25] also reported the similar results for gaseous chlorobenzene treatment, and suggested that the remaining O_3 generated by UV could control the biomass overgrowth further to achieve longer runs than the single biofilter. For gaseous α-pinene, some researchers only conducted the biodegradability assessments of photodegradation intermediates [26]. Our previous studies showed that the biodegradability of intermediates formed in the photodegradation process was nearly 10 times more than that of the parent compound (α-pinene), and provided a possibility for its removal by the combined system [27,28]. However, the studies on the continuous treatment of α-pinene by the combined system are relatively rare. Integrated studies, which focused on the removal performance and microbial evolution in the combined UV-BTF, are therefore required to better understand the UV-BTF process and to develop feasible methods for treating the hydrophobic and recalcitrant compounds.

In this study, the removal performance and microbial community were investigated using two BTF reactors, one of which possessed a UV reactor as a pretreatment. The comparison of removal performance between the two systems was conducted during the whole operation, and the differences of microorganisms inhabited in the biofilters were analyzed by the denaturing gradient gel electrophoresis (DGGE). Finally, the responses of the single BTF and combined UV-BTF to the abnormal operations (starvation and stagnant) were also evaluated.

2. Materials and methods

2.1. Experimental set-up

Fig. 1 shows a schematic diagram of the combined UV-BTF system and the control BTF system. The spiral photoreactor (custom made) was made of quartz glass with an effective volume of 2.25 L, in the center of which were installed special low-pressure mercury vapor lamps (36 W, Electric Light Sources Research Institute, Beijing, China), emitting 184.9 nm wavelength (0.206 mW cm^{-2} from 1 m) and producing ozone on line through the photolysis of oxygen. Since 184.9 nm wavelength could penetrate through the quartz, α-pinene absorbed the UV light directly and was decomposed by the combinations of photolysis and photooxidation (ozone, hydroxyl radical, etc.). More detailed description about the UV reactor and the optimal photodegradation condition for the subsequent BTF can be found in our previous study [27,28]. Considering the light intensity decay, the lights were changed twice during the whole operation, on day 55 and day 110, respectively. The light intensity was considered to be equal for the continuous operation of 55 d, since the intensity attenuation could be less than 20% within 84 d according to the manufacture.

The 2 biofilters were made of Plexiglas and each consisted of 2 equal sections of 12 cm in diameter and 55 cm in height. The ether-based polyurethane foams (PU-foam) were utilized as the biofilm-carriers (the mean size, the stacking density and the porosity were 14–18 mm, 155 kg m^{-3} and 90.8%, respectively), and the effective volume of the packing materials in each section was 4.86 L. Ports were placed along the biofilter column and used for gas sampling, filter material sampling and temperature/pressure-drop monitoring.

α-pinene contaminated stream was generated by passing air through a sparger containing α-pinene (purity 97%, J&K Chemical Company, China) and then mixed with the other flow of the ambient air (purified by activated carbon to remove other background VOCs) in the gas-mixer. Different ratios of these streams, which were controlled by the mass flow meters and the humidifier, determined the inlet concentration of α-pinene and the relative humidity of the mix stream.

2.2. Microorganisms inoculation

The microorganisms for inoculation were made up of the acclimated active sludge and the prepared α-pinene degraders suspension (volume ratio = 6:1). The active sludge was originated from a full-scale wastewater treatment plant (Hangzhou, China) and acclimated by α-pinene as the carbon source to form a stable microbial consortium for nearly one month. The previously isolated α-pinene degraders, *Pseudomonas veronii* PT (Genebank no.: FJ169494) [29] and *Pseudomonas fluorescens* ZW (Genebank no.: GU357489) [30], were large-scale cultured in a 4 L jacketed tubular reactor controlled by an automatic control system equipped with a feedback loop (monitoring the DO, temperature and pH). 2.3 mL pure α-pinene liquid was added to the mineral medium, which dispersed in the form of droplet-like (its water solubility was 2.49 mg L^{-1}) and was utilized by these strains as the carbon source. After 48 h culture, the biomass increased from the initial value of 10.9 mg L^{-1} to the final value of 223 mg L^{-1}. These cells were harvested by centrifugation at 9000 × *g*, 4 °C for 10 min and resuspended in 0.05 mol L^{-1} phosphate buffer solution (pH 7.2) to obtain a final biomass concentration of 510 mg cell L^{-1}.

The obtained inoculation was re-circulated through the packed PU foams using a peristaltic pump at a rate of 100 mL min^{-1} for 24 h in order to allow the biomass attaching to the support material. During the whole operation, the biofilters were equipped with a sprayer for feeding a circulating mineral medium at a constant flow rate of 180 mL min^{-1}, supplying the nutrient for the microbial growth. 500 mL of recycled liquid was replaced with the fresh mineral medium every 3 d (average liquid residence time of 9 d).

2.3. Experimental procedure

Both the combined UV-BTF and single BTF were operated for more than 5 months under the desired operational conditions which are summarized in Table 1. α-Pinene stream with a relative humidity of 35–40% was firstly introduced into the photoreactor. Through photodegradation of 18 s, the remaining α-pinene together with more soluble and biodegradable intermediates [28] entered into the subsequent BTF. The expected residence time (18 s) was achieved by changing the reactor volumes through the photoreactors in series. Experiments were carried out by varying the flow rates of streams to obtain different initial concentrations and total empty bed residence times (EBRT), and the performance comparisons were processed by the calculated removal efficiency (RE), the elimination capacity (EC) and the production of CO_2 (P_{CO_2}).

2.4. Analytical methods

2.4.1. VOC and CO_2 analysis

α-Pinene concentration was analyzed on-line by an Agilent 6890N gas chromatograph (GC) with a flame ionization detector (FID). The gas samples were collected using a six-way valve with a gas sampling loop and then transferred into a silica HP-Innowax

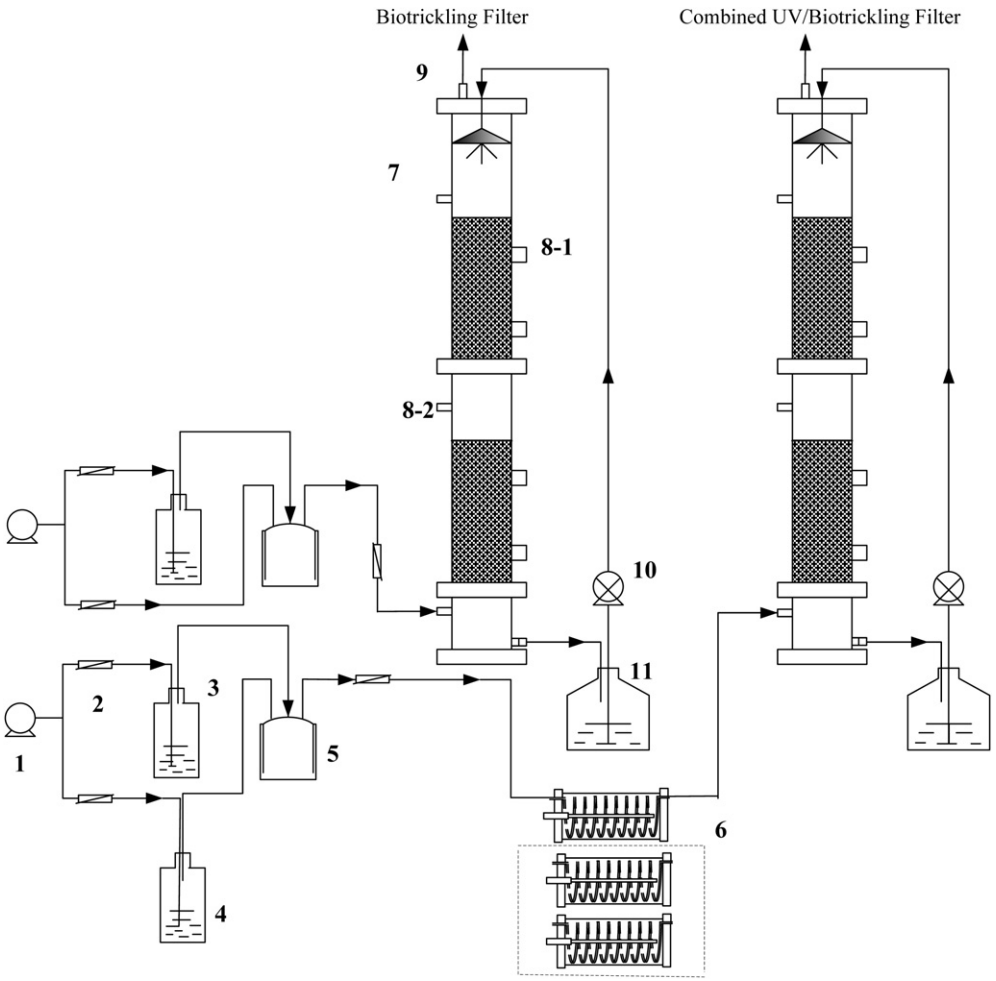

Fig. 1. Experimental setup for the combined UV-BTF and the single BTF: (1) air pump, (2) flow meter, (3) α-pinene sparger, (4) deionized water, (5) mixing chamber, (6) photoreactor, (7) BTF, (8-1) carrier sampling port, (8-2) gas sampling port, (9) exhaust port, (10) peristaltic pump, and (11) Nutrient solution.

capillary column (30 m × 0.32 mm × 0.5 μm, J&W Scientific, USA). The operating conditions of GC were as follows: injector: 250 °C, oven: 140 °C for 3.5 min and detector: 300 °C.

The sampling method of CO_2 was similar to that of α-pinene, and the analysis was conducted on an Agilent 6890 GC equipped with a thermal conductivity detector (TCD) and a silica HP-Plot-Q capillary column (30 m × 0.32 mm × 20 μm, J&W Scientific, USA). The operating conditions were as follows: injector: 90 °C, oven: 40 °C for 4 min and detector: 100 °C.

2.4.2. Biofilm morphology analysis

Scanning electron microscopy (SEM) was used to visualize the morphology of biofilms in the biofilters. The samples were gently washed with 0.1 M phosphate buffer (pH = 7) and fixed with 2.5% glutaraldehyde and 1% osmic acid for 12 and 2 h, respectively. The fixed samples were dehydrated by successive passages of 30, 50, 70, 85 and 95% ethanol solutions and absolute ethyl alcohol. The dehydrated samples were dried with a CO_2 Critical Point Dryer, and were observed by the scanning electron microscopy (Model XL30 ESEM, Philips Eindhoven, The Netherlands) after their spraying treatments.

2.4.3. 16S rRNA gene PCR and denaturing gradient gel electrophoresis

DNA was extracted from the biofilms using a Fast DNA SPIN Kit for environmental samples (V2.2, Shennengbocai, Shanghai, China) according to the protocol described by the manufacturer. The primer set 341F (containing a GC clamp CGCCGGGGGC GCGCCCCGGGCGGGGCGGGGGCACGGGGGG) and 534R (*Escherichia coli* numbering system) described by Muyzer et al. [31] were used to amplify the V3 region of bacterial 16S rRNA gene sequences. The polymerase chain reaction (PCR) cycling was performed in a PTC-200 DNA Engine Thermal Cycle (BioRad, Hercules, CA, USA), and the denaturing gradient gel electrophoresis (DGGE) was performed using a D-Code 16 cm × 16 cm acrylamide gel system (BioRad, Hercules, CA, USA) according to our previous study [8].

3. Results and discussion

3.1. The comparison of 90-day operation between the 2 systems

3.1.1. Start-up

α-Pinene inlet concentrations together with the RE for every day at each EBRT are illustrated in Fig. 2. For the single BTF, the removal of α-pinene increased gradually after 6 d, indicating that the biofilm began to form. The overall RE reached 90% and was stable at this level on day 13. When the inlet concentrations increased to 850 and 1000 mg m^{-3} on day 14 and day 21, the nearly stable RE was achieved around 80% and 66.75% after a short adaptation period, and the maximum ECs were respectively 26.72 and 25.51 g m^{-3} h^{-1}. When the identical inlet stream was introduced into the combined UV-BTF, the performance was much better than

Table 1
Summary of the experimental operating conditions.

Phase		I			II			III			
Period (d)		1–11	12–17	18–28	31–50	51–70	71–90	91–110	111–165		
		Start-up			Stable operation			UVRT effects[a]	No α-pinene supply	No liquid recycle	No flows supply
EBRT (s)	BTF	97	97	97	58	41	35	26–62	35	35	35
	UV-BTF	18 + 78	18 + 78	18 + 78	18 + 39	18 + 23	18 + 17	(9–45) + 17	18 + 17	18 + 17	18 + 17
Concentration (mg m^{-3})		600	1000	1200	600–1500	600–1500	600–1500	1500	1500	1500	1500

[a] Effects of UV residence time on microbial community.

that of the single BTF under the same total EBRTs and the maximum EC arrived at 39 g m^{-3} h^{-1} on day 14. These results indicated that the combined system performed satisfactorily after a start-up period of only 16 d, which shortened a third of the one relative to the single BTF.

In order to demonstrate the biofilm formations in both BTFs, the packing materials with attached biomass were collected after 25 d and subjected to SEM analysis (figures not shown). Through the comparison of SEM photos, the apparent microbial colony morphology in the combined UV-BTF was richer than those in the single BTF. Although the photolysis of air would produce ozone, the previous study indicated that the O_3 content detected in the outlet of the photoreactor was below 0.04 mg m^{-3} when the residence time was controlled at 27 s [27], which implied that most produced ozone reacted with α-pinene. Meanwhile, the circulating spray liquid in BTF could also absorb the remaining ozone in the stream (its solubility in water was 1372 mg L^{-1} at 1 atm and 0 °C, 13 times more than that of oxygen), further avoiding the inhibition to the subsequent bacteria.

Some photodegradation intermediates such as aldehydes, ketones, and volatile fatty acids, which were detected in our previous study [28], were directly introduced into the subsequent BTF. The biodegradable components not only accelerated the development of the stable microbial consortium but also improved the mass transfer of the parent compound. Tsang et al. [32] indicated that the volatile fatty acids were easily biodegraded by microorganisms and the period for biofilm formation was shorter than that of hydrophobic and recalcitrant compound (α-pinene, BTEX, etc.). Mohseni and Allen [33] also reported that the RE for α-pinene was nearly 100% on day 18 in the presence of gaseous methanol, which was much higher than that of the single α-pinene. It was proposed that the presences of biodegradable compounds would promote the start-up of the subsequent BTF.

3.1.2. Steady-state

Following the successful start-up, the performance of the systems was investigated at different EBRTs (58, 41 and 35 s) and different inlet concentrations (600–1500 mg m^{-3}), both of which were known to affect the REs significantly. As shown from Fig. 2 during the stable operation of nearly 2 months, the REs of the single BTF were between 25% and 76%, much lower than those of the combined UV-BTF (57–98%) under the same conditions. At concentrations below 650 mg m^{-3} and an EBRT of 58 s, 76% of the incoming α-pinene vapor was removed by the single BTF. With a higher inlet concentration and a shorter EBRT applied, the performance of the single BTF was worsening. In the later operation, with the increase of inlet load (IL) to 130 g m^{-3} h^{-1} at an inlet concentration of 1500 mg m^{-3} and an EBRT of 41 s, the RE dropped to about 25%. Note that with this increase in α-pinene loading, the performance of the combined system initially dropped less and ultimately recovered better than the single BTF. The comparisons of the performance revealed that that the combined UV-BTF was more suitable for the purification of higher inlet concentration at a short EBRT from an engineering standpoint.

Since high-concentration VOCs fed into the bioreactor would inhibit the bacterial growth, the biofiltration was generally regarded as a suitable treatment for the low-concentration VOCs removal. Rene et al. [34] and Zilli et al. [35] reported that high-concentration VOCs would inhibit biological activity strongly, and thus had a negative effect on the removal performance. In the combined UV-BTF system, high-concentration VOCs could be converted to relatively low-concentration VOCs by the photodegradation process, thereby reducing the inhibition to the subsequent microorganisms. Our previous study indicated that UV photodegradation could remove about 15% of α-pinene at the inlet concentration varied from 600 mg m^{-3} to 1500 mg m^{-3} [27]. The introduction of

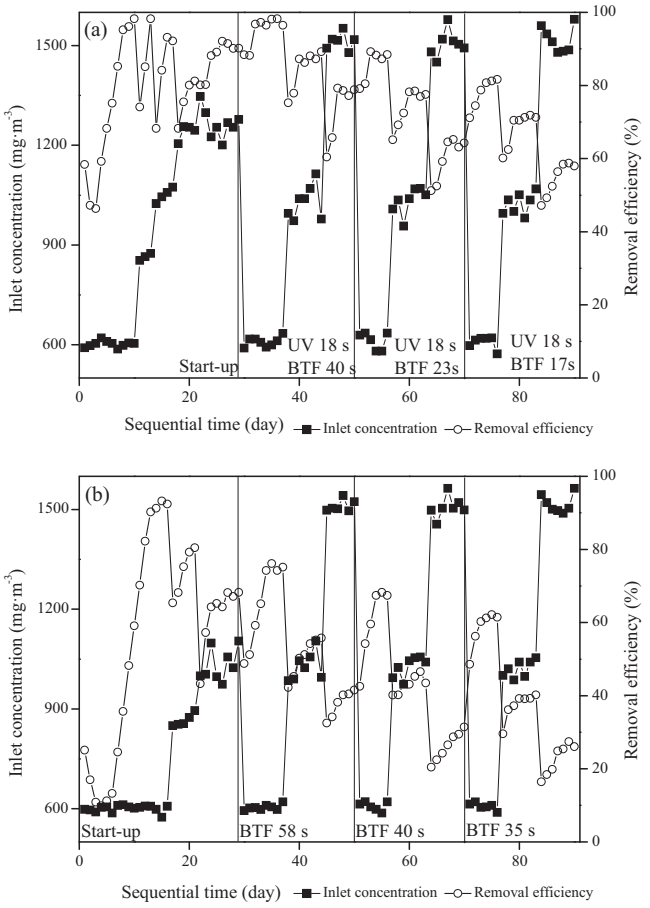

Fig. 2. Performance of the combined UV-BTF (a) and single BTF (b) during the start-up and stable operational periods.

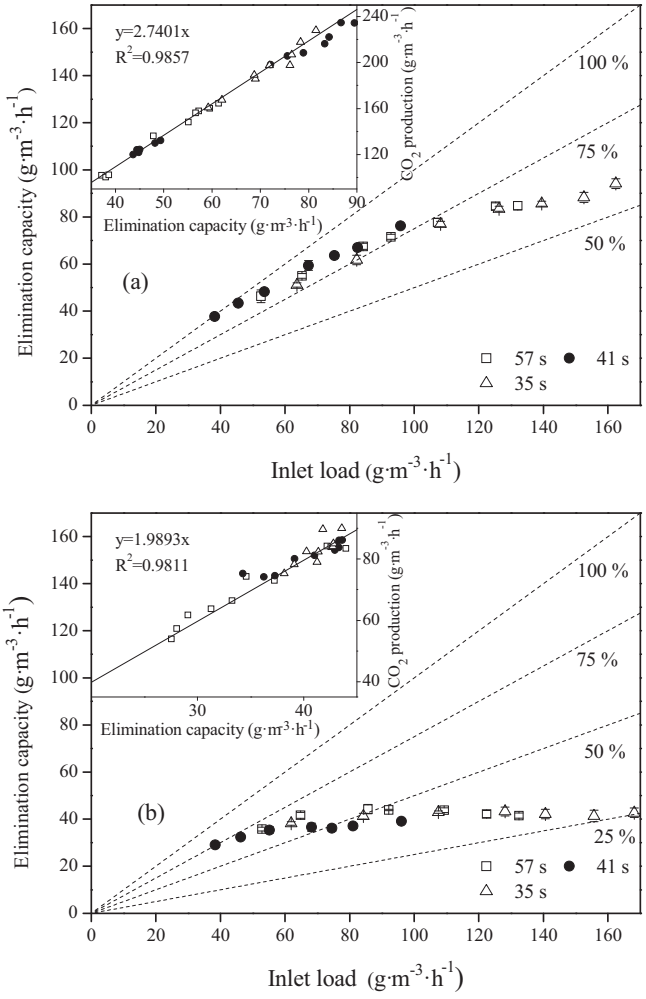

Fig. 3. Elimination capacities and CO_2 productions of the combined UV-BTF (a) and single BTF (b) at different inlet loads.

relatively lower α-pinene together with some biodegradable intermediates into the BTF resulted in a better performance, not only reducing the inhibition but also promoting the activity restoration of some microorganisms. Wang et al. [25] indicated that the UV pretreatment could reduce the inhibitory effects to the bacterial in the subsequent BF for the removal of high-concentration chorobenzene.

3.2. ECs and P_{CO_2} under different ILs

The degree of pollutant degrading capacity for a treatment system is commonly quantified as a function of pollutant loading by determining the EC values. It is evident from Fig. 3 that the relationship between IL and EC was linear until a critical value, after which the EC approached to a maximum value asymptotically. Though fluctuations were found in the ECs, a relationship between EC and IL was observed in both BTFs. In the single BTF, the maximum ECs of 37.2, 42.2 and 44 g m^{-3} h^{-1} were observed at EBRTs of 51, 41 and 35 s, respectively. From Fig. 3a, it can be seen that 75% RE could be maintained up to an IL of about 40 g m^{-3} h^{-1} at EBRT of 51 s, while it dropped to nearly 25% at the EBRT of 35 s under the IL of 150 g m^{-3} h^{-1}. In the combined UV-BTF, the maximum ECs of 66.1, 84.5 and 94.2 g m^{-3} h^{-1} were achieved at the same EBRTs (Fig. 3b). A nearly complete RE could be maintained up to an IL of 40 g m^{-3} h^{-1} at EBRT of 51 s, while it dropped to 60% with a much higher IL applied. Excluding the EC induced by the photodegradation process, the ECs of the subsequent BTF increased 31.2%, 20.6% and 15.2% respectively at each designed EBRT. These results suggested that the UV pretreatment had a positive effect on the subsequent biodegradation, and the combined removal was higher than the sum of the removal induced by the single UV plus the single BTF.

In any biological treatment process, some biodegradable VOCs are easily degraded to carbon dioxide, water and biomass aerobically without any second pollution generated [36]. Hence, it is important to monitor the profile of CO_2 in the inlet and outlet gaseous stream to acquire valuable information on the mineralization extent of the pollutant. It was observed that there was a linear relationship between P_{CO_2} and the observed ECs (Fig. 2). For complete chemical oxidation of α-pinene to CO_2 and H_2O, the ratio of CO_2 produced to the parent compound removed should be 3.24 on a mass-basis. The slopes of lines fitted by the experimental results were approximately 1.99 and 2.74 for the single BTF and the combined UV-BTF (Fig. 3), respectively, indicating that the two systems could mineralize 61.4% and 84.6% of the incoming α-pinene vapor under all the test conditions. Results from the comparisons of the EC and P_{CO_2} indicated that UV pretreatment contributed more to the conversion of α-pinene, while the subsequent BTF accounted for over 50% of the entire CO_2 production (Table 2). Raymond et al. [37] reported that some simple organic compounds could be easily mineralized by the microorganisms. Jin et al. [38] also showed that over 80% of methanol could be mineralized by the biofiltration, completely. Therefore, through the photodegradation, most α-pinene were converted to some simpler intermediates, which would provide a possibility for their complete mineralization by the subsequent BTF. The difference between CO_2

Table 2
Contribution percents of each unit to the removal and mineralization.

EBRT(s)	Parameters	Contribution percents (%)				
		Combined			Sole	
		UV	Down[a]	Up[b]	Down	Up
57	EC	47.30	20.01	32.69	68.24	31.76
	P_{CO_2}	14.88	63.84	21.28	43.28	56.72
41	EC	35.93	27.55	36.52	62.14	37.86
	P_{CO_2}	10.45	55.52	34.03	45.21	54.79
35	EC	34.68	32.66	32.66	58.34	41.66
	P_{CO_2}	9.73	51.45	38.82	48.24	51.76

[a] The down section of the BTF.
[b] The up section of the BTF.

productions could also provide an evidence that the mineralization role was much strong in the UV-BTF than that in the single BTF.

3.3. Microbial analysis

Changes in the microbial community structure present were revealed by 16S rRNA PCR-DGGE of microbial samples taken from the single BTF and the combined UV-BTF in different operational phases (Fig. 4). Obviously, both of the microbial community structures tended to become stable with the operation, and there were no significant variations. With the introduction of gaseous photodegradation intermediates and α-pinene into the subsequent BTF together, the microbial structure tended to become different from the one in the single BTF. In the combined UV-BTF, it was speculated that several strains, which appeared and gradually became the predominant species with the operation, might be the degraders of carbonyl intermediates. Therefore, the numbers of discriminable bands in Fig. 4 were 27 and 17 respectively for the combined UV-BTF and the single BTF on day 90. It seemed that there was more complicated microbial community in the combined system than that in the single one.

To better understand the differences in microorganisms among samples, some brighter bands were excised and sequenced (Table 3). The most dominant strains in all the bacterial communities were α-pinene degraders (P. PT and P. ZW, Band B/C for single BTF and Band M/N for combined UV-BTF in the DGGE images), which were inoculated in the biofilters during the start-up period and existed throughout the whole operation. Sequence analysis and BLAST searchers revealed that there were six other bacteria (corresponding to Band F, H, I, J, K and O, respectively), which were classified as the members of four different groups, inhabiting only in the combined UV-BTF. We found that many of them were closely related to the bacteria that had the biodegradability for carbonyl compounds (data shown in Table 3) [39–43]. These sequenced results indicated that the photodegradation intermediates mainly consisting of organic acids (which made the surrounding turning into weak acid of pH value at 6.1–6.2) and aldehydes provided good conditions for these bacterial growths. The Shannon index of general diversity H [44] was also calculated to evaluate the microbial diversity, and they were 0.79 and 1.39 respectively for the samples taken from the single BTF and the combined UV-BTF. Owing to microbial diversity and more stable community present in the subsequent BTF, the photodegradation products and remaining α-pinene could be completely removed by the biofiltration, thus producing a higher RE and a larger P_{CO_2} observed from this study.

3.4. Responses of BTF to UV residence time and the non-use periods

3.4.1. Effect of UV residence time on the performances of the BTF

Our previous study showed that the photodegradation residence time affected the types and amounts of the intermediates, and resulted in different biodegradability [28]. The responses of the subsequent BTF to the various photodegradation reaction times (9, 18 and 45 s) are investigated under the same α-pinene concentration of 1500 mg m^{-3} (Fig. 5a). When a shorter UV residence time (such as 9 s) was applied, the RE and mineralization rate produced by photodegradation only were 16% and 11.2%, respectively. Most intermediates were hydrocarbons, the properties of which were similar to that of α-pinene, and the performances of the subsequent BTF were not improved much more. But with the UV residence time extended to 45 s, about 75% of α-pinene was removed by the photodegradation, and the organic acids accounted for a large proportion in the formed intermediates, making the sub-surroundings of the biofilter more weak acid. Since some α-pinene degraders grow well in the neutral condition (about pH 7) [29,30], an acidic environment would inhibit their bioactivities and thus had a negative effect on the biological removal performance. In order to investigate the UV residence times on the microbial community, changes in microbial community structure of the biofilm samples on day 90, 95, 104 and 110 were revealed by 16S rRNA PCR-DGGE (Fig. 5b). Box 1 represented the α-pinene degraders, Box 2 was the organic acid degraders, and Boxes 3–4 was the carbonyl (aldehyde or ketones) degraders. The bands belonging to the carbonyl and organic acids tended to become faint on day 95, for that some carbonyl or acid intermediates were not formed under a relative short photodegradation residence time. With the extended residence time, these types of intermediates appeared again in the outlet from the UV reactor, and thus Bands F and H–J gradually appeared in the DGGE images. With a residence time extended to 45 s on day 110, the microorganism named as *Acidovorax caeni*, which could biodegrade organic acids, gradually became the predominant strain among the inhabitants, and the intensity of Band K was brighter than those of Band F and H–J. These results indicated that the types and amounts of intermediates would have a significant effect on the microbial evolution.

3.4.2. BTF responses to the non-use periods

Performance of biotrickling filters is usually studied under relatively ideal situations, such as steady-state operation, well-supply operation, etc. However, operational problems undetected in the laboratory may burst out when the pilot- or full-scale biofilters were operated in industrial settings. Therefore, in this study, both the single BTF and the combined UV-BTF were operated under the mimic practical shutdown from day 111 to 165, in order to compare their responses and re-acclimations to the starvations (no VOC supply, no liquid supply and no flows supply). For each BTF, starvation was continued for 7 d, after time which the standard operation was resumed. The effects of starvation are determined by comparing the REs before and after different operations, and the re-acclimation profiles are shown in Fig. 6. It could be seen that, for the Starvation I, a relatively high RE for the combined UV-BTF appeared soon after a re-start of 2 d, which was shorter than that of the single BTF (the RE arrived to 26% after 7 d). A probable explanation for the different responses was that the biofilm rapidly switched from a highly active metabolism (growth) to a lower metabolism (endogenous respiration) when the carbon source supply was discontinued [45]. Determination of the oxygen consumption rate (OUR) by the biofilms collected before and after starvation were conducted, and their values respectively decreased from 0.89 mg to 0.34 mg of O_2 (mg of dry cell weight)$^{-1}$ h^{-1} and 1.16 to 0.78 mg of O_2 (mg of dry cell weight)$^{-1}$ h^{-1}. Such results indicated the bacterial inhabited in

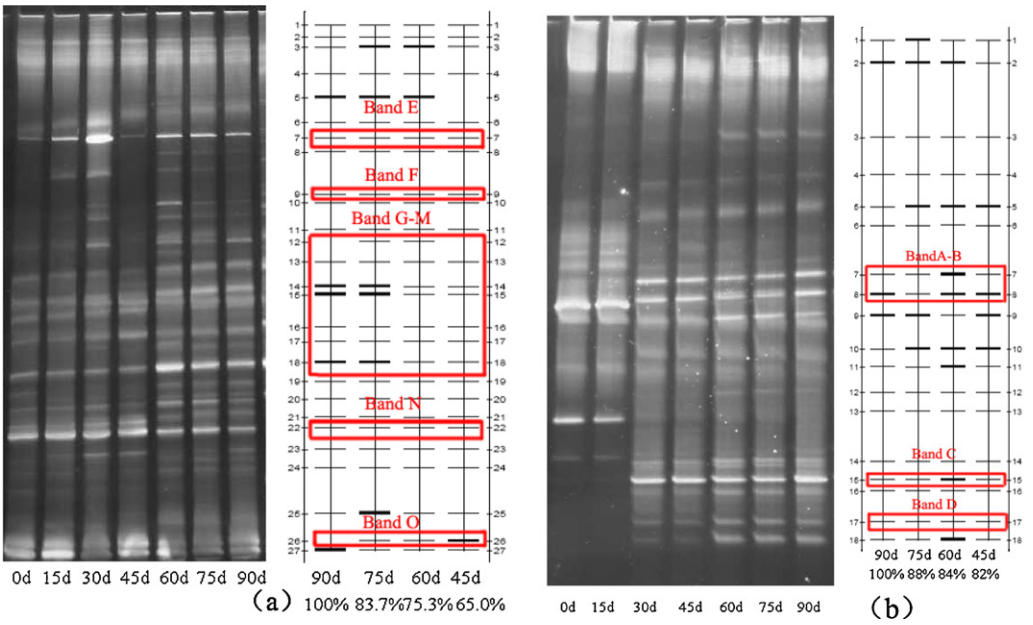

Fig. 4. DGGE profiles of the bacterial communities in the combined UV-BTF (a) and single BTF (b) during the whole operation.

Table 3
Nucleotide sequence similarity and the biodegradation characteristics of bacteria.

Band	Phylogeny	Closest relatives (accession nos.)	Similarity (%)	Biodegradability by Ref.
A[a]	Actinobacteria	*Mycobacterium terrae* (M29568)	96	–
B	γ-Proteobacteria	*Pseudomonas veronii* (AF064460)	100	α-Pinene [29]
C	γ-Proteobacteria	*Pseudomonas fluorescens* (AJ308308)	100	α-Pinene [30]
D	β-Proteobacteria	*Comamonadaceae bacterium* (EF018197)	97	–
E	γ-Proteobacteria	*Pseudomonas grimontii*(AF268029)	98	–
F	β-Proteobacteria	*Ralstonia pickettii*(AY741342)	95	Toluene etc. and metabolites [42,43]
G	γ-Proteobacteria	*Pseudomonas frederiksbergensis*(AJ249382)	100	–
H	α-Proteobacteria	*Sphingomonas paucimobilis* (AM237364)	97	Aldehyde [40]
I	γ-Proteobacteria	*Pseudomonas stutzeri*(AJ308315)	94	Aldehyde [41]
J	γ-Proteobacteria	*Pseudomonas fragi*(AF094733)	100	Aldehyde [41]
K	β-Proteobacteria	*Acidovorax caeni* (AM084006)	98	Organic acids [39]
L	Actinobacteria	*Mycobacterium terrae* (M29568)	97	–
M	γ-Proteobacteria	*Pseudomonas veronii* (AF064460)	98	α-Pinene [29]
N	γ-Proteobacteria	*Pseudomonas fluorescens* (AJ308308)	100	α-Pinene [30]
O	β-Proteobacteria	*Alcaligenes faecalis* (D88008)	99	Toluene etc. and metabolites [42,43]

[a] The bands are designated as shown in Fig. 4.

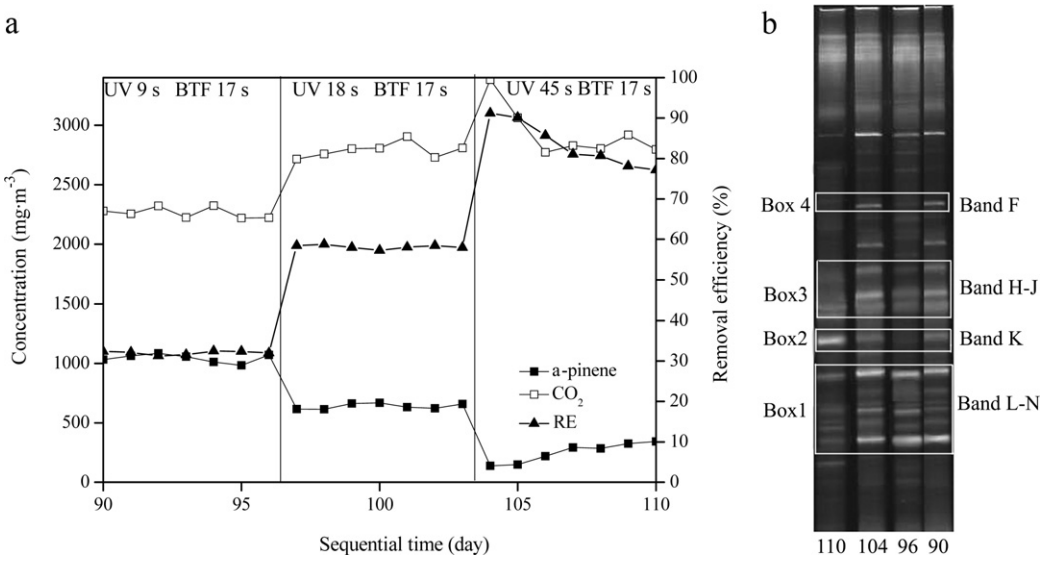

Fig. 5. Effects of UV reaction times on the removal performances (a) and bacterial communities (b) of the subsequent BTF.

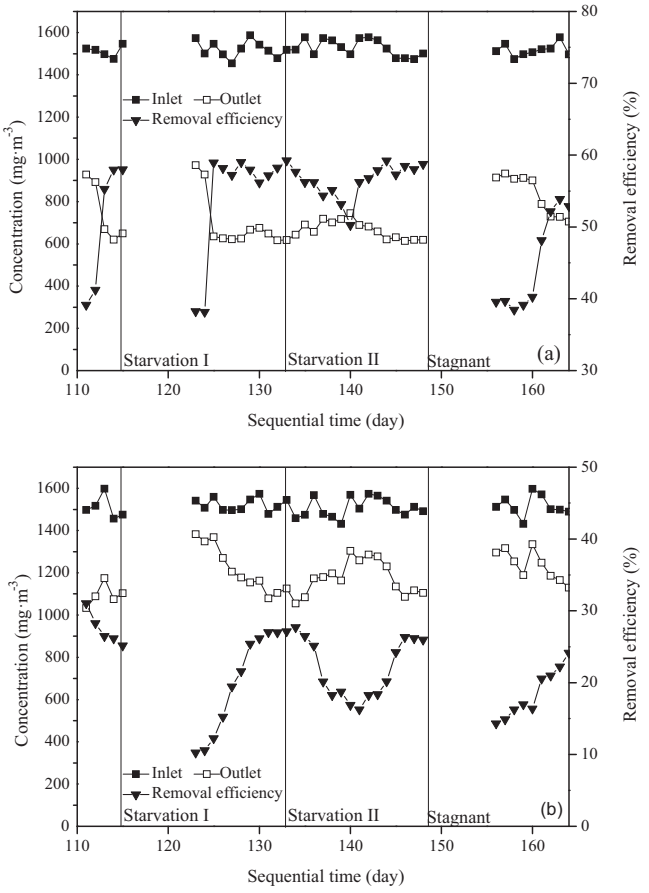

Fig. 6. Re-acclimations after the starvations for the combined UV-BTF (a) and single BTF (b).

the subsequent BTF went into the endogenous respiration later and their activity did not lose much more, owing to the water-soluble photodegradation products accumulating previously could serve as the carbon source when the carbon source did not supply. Although the specific types of products remaining in the liquid were failed to be analyzed, the differences of CO_2 concentrations in the outlets between the single and the combined BTF ($-242\,mg\,m^{-3}$) could also indirectly explain the above phenomenon. Cox and Deshusses [46] indicated that the re-acclimation of BTF after starvation was not a major problem for easily biodegradable pollutants, and the re-startup period was usually shorter than those for recalcitrant compounds. Therefore, the introduction of α-pinene and its photodegradation intermediates in the biofilter would make the lower activities restore soon, and the removal capability increased to the normal level after the standard operation. In Starvation II, α-pinene was introduced in both reactors but no nutrient liquid was supplied. Both of the performances were gradually worsening (the REs were respectively 18.74% and 53.17% 7 d after the stop of nutrient supply), but the combined UV-BTF re-acclimated soon after the nutrient liquid provided again as compared to the single one. Without any liquid or carbon supplies in Starvation III, an anaerobic environment would form in the biofilters [47], which affected the microbial structure and the subsequent re-acclimation. Although the performance of combined BTF did not recover to the normal level, the RE could be 55%, much higher than that of the single one (below 25%).

Comparison of the different starvation experiments demonstrated that the presence or the absence of the gas flow or the liquid recycling did not greatly affect the re-acclimation time for the combined UV-BTF. Therefore, an important implication of our results for industrial BTF operation was that the additions of some biodegradable compounds (e.g., some photodegradation products) during the non-operation and early re-start phase could shorten the re-acclimation period, and this method would maintain the biofilm in an active state [46].

4. Conclusions

This article focused on the comparisons of α-pinene removal by the single BTF and combined UV-BTF systems. Results showed that the REs and ECs of the combined system were higher than those of the single one, especially for the treatment of high-concentration VOCs ($600–1500\,mg\,m^{-3}$). PCR–DGGE analysis provided valuable information on the effects of photodegradation and corresponding residence time on the microbial structure in the subsequent BTF. In addition, comparison of the different starvation experiments demonstrated that the combined UV-BTF required a shorter re-acclimation after non-operation than the single BTF, which was important for actual application of BTF from an engineering standpoint. The combined UV-BTF described in this study could provide a better removal capability for hydrophobic and recalcitrant air pollutants.

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