



# Thermophilic biofiltration of methanol and $\alpha$ -pinene

S Dhamwichukorn<sup>1</sup>, GT Kleinheinz<sup>2,3</sup> and ST Bagley<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, Michigan Technological University, Houghton, MI 49931-1552; <sup>2</sup>School of Forestry and Wood Products, Michigan Technological University, Houghton, MI 49931-1295, USA

**Biofiltration systems utilizing thermophilic (55°C) bacteria were constructed and tested for the removal of methanol and  $\alpha$ -pinene — two important volatile organic compounds (VOCs) in the forest products industry. Thermophilic bacterial mixtures that can degrade both methanol and  $\alpha$ -pinene were obtained via enrichment techniques. Two bench-scale thermophilic biofiltration systems (1085 and 1824 cm<sup>3</sup>) were used to examine compound removals at different residence times, with influent concentrations of 110 ppmv methanol and 15 ppmv  $\alpha$ -pinene. At a residence time of 10.85 min, the smaller system had removal efficiencies of >98% for methanol, but only 23% for  $\alpha$ -pinene. The larger system was operated with the same parameters to evaluate residence time and surfactant effects on compound removals. At a residence time of 18.24 min, both methanol and  $\alpha$ -pinene removal rates were  $\geq$ 95%. However,  $\alpha$ -pinene removal dropped to 26% at a residence time of 6.08 min; methanol removal was not affected. Subsequent addition of a surfactant mixture increased  $\alpha$ -pinene removal to 94% at the shortest residence time. No residual  $\alpha$ -pinene was detected with the support medium Celite R-635, indicating that the surfactant may increase mass transfer of  $\alpha$ -pinene. *Journal of Industrial Microbiology & Biotechnology* (2001) 26, 127–133.**

**Keywords:** thermophilic; biofiltration; biodegradation;  $\alpha$ -pinene; methanol; VOCs

## Introduction

Biofiltration systems utilizing mesophilic microorganisms and operating typically at around 20–25°C have been used successfully for degrading volatile organic compounds (VOCs) such as methane, gasoline, toluene, trichloroethylene, ethyl benzene, chlorobenzene, and terpenes [6,13,14,20]. However, drawbacks can exist in certain applications. For example, in various industries, the contaminated hot air stream effluent (50–60°C) must be cooled before entering the system for treatment [5]. This cooling process could be costly and time-consuming. A biofiltration system that operates at temperatures typical of such industrial effluents could theoretically eliminate the cooling step and thus reduce processing time and cost. Using thermophilic, instead of mesophilic, microorganisms may also provide the added advantage of higher degradation rates and thus, higher removal rates [5]. Previous biofiltration studies with thermophiles have demonstrated this. For example, a recent study showed a greater removal rate for ethyl acetate in a biofiltration system at 45–50°C compared to mesophilic systems (30–37°C) [4]. Another study showed that ethanol can be degraded by thermophilic fungi with a high removal rate by forcing the hot gases containing ethanol (at about 60°C) through the biofiltration unit using porous ceramic particles as a support medium [27]. Biofiltration of toluene at 48–50°C, utilizing an active compost of maple leaves and alfalfa as a support medium, has also been demonstrated [15].

Because VOCs are released from the forest product industry at thermophilic temperatures (50–60°C), the use of thermophilic biofiltration systems may be particularly advantageous over

mesophilic biofiltration systems. The VOCs released by the forest products industry also vary widely in physical, chemical, and biological properties, as well as in concentrations. Simulating an emission stream in the laboratory containing five to seven major compounds emitted from the manufacturing industries is difficult because of the wide variation in compound properties. Therefore, in this feasibility study, methanol and  $\alpha$ -pinene were selected as representative VOCs because of their very different physical and chemical properties, their predominance among discharges, their high rate of discharge, and their environmental importance [28]. Methanol has high aqueous solubility, a low boiling point (64.7°C), and a low octanol–water partition coefficient. Methanol is among the top (over 49%) Toxic Release Inventory compounds and, as an air pollutant, its discharge must be regulated [26]. In contrast,  $\alpha$ -pinene has low solubility in water (2.5 ppm at 23°C [16]), a high boiling point (154°C), and a high octanol–water partition coefficient.  $\alpha$ -Pinene is one of the major VOCs emitted by the forest products industry and, as a monoterpene (the main pollutants emitted by manufacturing industries, especially the forest products industry [26]), it can react in the atmosphere to form free radicals and deplete the ozone [3].

Previous studies have shown that both methanol and  $\alpha$ -pinene can be degraded in liquid culture under both aerobic [1,7,12,16,22,24] and anaerobic conditions [8,9]. Under aerobic conditions, both mesophilic [12,16,24] and thermophilic [1,7,22] temperatures have been used. While several researchers have investigated the performance of mesophilic biofiltration for methanol [24] and for  $\alpha$ -pinene [12,17,18] removal, no studies have been reported on thermophilic removal of these compounds.

The overall objective of this study was to evaluate the feasibility of using a thermophilic biofiltration system for removal of some important VOCs released by the forest products industries. The first specific objective was to recover and characterize thermophilic (55°C) microorganisms that were capable of utilizing methanol and  $\alpha$ -pinene as their sole carbon and energy sources. The second

Correspondence: Dr ST Bagley, Department of Biological Sciences, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931-1295, USA  
<sup>3</sup>Current address: Department of Biology and Microbiology, University of Wisconsin Oshkosh, Oshkosh, WI 54901, USA.

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objective was to construct and test a bench-scale biofiltration system utilizing the recovered thermophilic microorganisms for the removal of methanol and  $\alpha$ -pinene.

## Materials and methods

### Sources of chemicals

All chemicals were analytical grade.  $\alpha$ -Pinene and Triton X-100 were purchased from Aldrich Chemical (Milwaukee, WI); Bradford reagent, Brij35, and Brij58 were purchased from Sigma Chemical (St. Louis, MO), and methanol and other chemicals were purchased from Fisher Scientific (Itasca, IL).

### Isolation and characterization of thermophilic bacteria

Samples were obtained from thermophilic composting piles and sludge-amended soil at a pulp-and-paper mill in Thailand. Enrichment cultures were prepared by adding soil samples to two flasks with Bushnell–Haas minimal medium (BHM; Difco Laboratories, Detroit, MI). One flask was supplemented with 0.5% v/v ( $\sim 3955$  mg/l) methanol, the other with 0.5% v/v ( $\sim 4290$  mg/l)  $\alpha$ -pinene (the high levels of the substrates were added to prevent resource limitation for bacterial growth). The culture flasks were incubated in a gyratory shaker at 200 rpm and 55°C. The contents were transferred 10 times until the microbial cell densities reached approximately  $10^7$ – $10^9$  cells/ml. Increases in microbial populations were monitored using direct observation with differential interference contrast and epifluorescence microscopy (with acridine orange) techniques. The liquid cultures were maintained by weekly transfers and were used later as inocula for the biofiltration systems.

Bacteria were recovered from both enrichments by streaking onto R2A Agar (Difco) with incubation at 55°C. Morphological characteristics on R2A agar, such as size, color, and form, as well as Gram staining and microscopic observations, were used to distinguish between different types of bacterial isolates. At the start of the study, morphological characteristics on Tryptic Soy Agar (Difco) were also examined; use of this medium was discontinued as characteristics appeared too similar for all recovered colonies.

### Methanol and $\alpha$ -pinene degradation studies

Two inocula for the degradation studies were prepared by growing cells, as described above, for 3 days in BHM — one with methanol and the other with  $\alpha$ -pinene. Each bacterial mixture (for each substrate) was centrifuged at  $15,300\times g$  for 15 min; the cell pellets were washed twice using BHM. Three 125-ml EPA-grade VOC bottles with Teflon-butyl septa (Fisher Scientific) were set up (one control and two samples) for each sampling time for each substrate. For each VOC control bottle, 25 ml of BHM was added along with 30  $\mu$ l of methanol ( $\sim 950$  ppm) or 2  $\mu$ l of  $\alpha$ -pinene ( $\sim 70$  ppm), but no cells were added. For the test bottles, 23 ml of BHM was added with 2 ml of inoculum in addition to the same amount of methanol or  $\alpha$ -pinene as the control bottles. The initial protein concentrations were between 21 and 23  $\mu$ g/ml. The bottles were incubated in the shaker at 55°C at 200 rpm. This procedure was conducted twice.

For methanol degradation studies, liquid samples for methanol determination were removed through the septum of each bottle with a gas-tight syringe and stored immediately in borosilicate glass autosampler vials at 4°C. The remaining liquid was used for protein

assays to determine bacterial growth, using Bradford reagent [2]. Methanol analyses were performed within 24 h on an HP 5890 gas chromatograph (GC; Hewlett Packard, Palo Alto, CA) with a flame ionization detector (FID).

For the  $\alpha$ -pinene degradation studies, 5 ml of liquid was removed at each sampling time for protein assays. The 20 ml remaining in the bottle was extracted using liquid–liquid extraction techniques with hexane as the organic solvent. The final  $\alpha$ -pinene volume was calculated following extraction. The samples were stored in borosilicate glass autosampler vials (Hewlett-Packard) at 4°C, until analyzed using the GC-FID (within 24 h). The  $\alpha$ -pinene content in the organic phase (after hexane addition) was considered to represent the total  $\alpha$ -pinene content.

### Thermophilic biofiltration system set-up and operation

A general schematic of the thermophilic biofiltration system is shown in Figure 1. A humidified air stream containing methanol and  $\alpha$ -pinene was introduced into the biofiltration unit through the bottom *via* two in-line syringe pumps (KD Scientific, Boston, MA). Air concentrations of approximately 110 ppmv methanol and 15 ppmv  $\alpha$ -pinene were chosen for this study based on industrial site work [19] which indicated that these concentrations were fairly typical at a forest products facility such as an oriented strand board mill.

The first biofiltration system (System 1) consisted of a 4.5 cm-diameter and 60 cm-long borosilicate glass column (1085 cm<sup>3</sup> empty bed volume) surrounded with a water jacket that maintained temperatures at approximately 55°C by using a heating/recirculating pump (HAAKE, Karlsruhe, Germany). A residence time of 100 ml/min (coefficient of variation, CV, of 6.8%) was used throughout this portion of the study. Celite R-635 (Celite, Lompoc, CA), a diatomaceous earth/ceramic material, was used as the biofiltration bed medium or solid support as it has been shown to be effective in other biofiltration studies [11,25] and was not expected to be affected by the high temperatures. Water was added at approximately weekly intervals (in 100-ml increments) to maintain moisture in the bed. The system was checked frequently to assure that there were no leaks. Air for on-line gas chromatography analysis was withdrawn from both the influent and effluent air streams using an in-line multiport gas sampling valve (Valco, Houston, TX). Gas samples were drawn directly from the sampling valve and analyzed *via* an on-line GC (HP 6890). The sample transfer line temperature was maintained at 55°C. A 10-min equilibration period was programmed into the sampling sequence to insure that all sample lines had been flushed after each sample was taken.

The bacterial mixtures (obtained from the enrichments) were grown on either methanol or  $\alpha$ -pinene as described above for 7 days on a gyratory shaker at 55°C. The contents of the methanol or  $\alpha$ -pinene flask were centrifuged at  $15,300\times g$  for 10 min, washed with 0.85% saline, recentrifuged, and then resuspended in 0.85% saline for a 10-fold concentration. The cells from both the methanol and  $\alpha$ -pinene flasks were then combined in a 1:1 ratio. Cells were added to System 1 every 7 days until the recovered cell densities appeared to have been stabilized (day 105). A 10-ml liquid sample was taken from the biofiltration system on a weekly basis to conduct a direct count (DC; using a Petroff–Hausser counting chamber and cells stained with methylene blue) and a viable count (VC; using R2A agar). No additional bacteria were added to this first system after day 105, although a nutrient solution (BHM

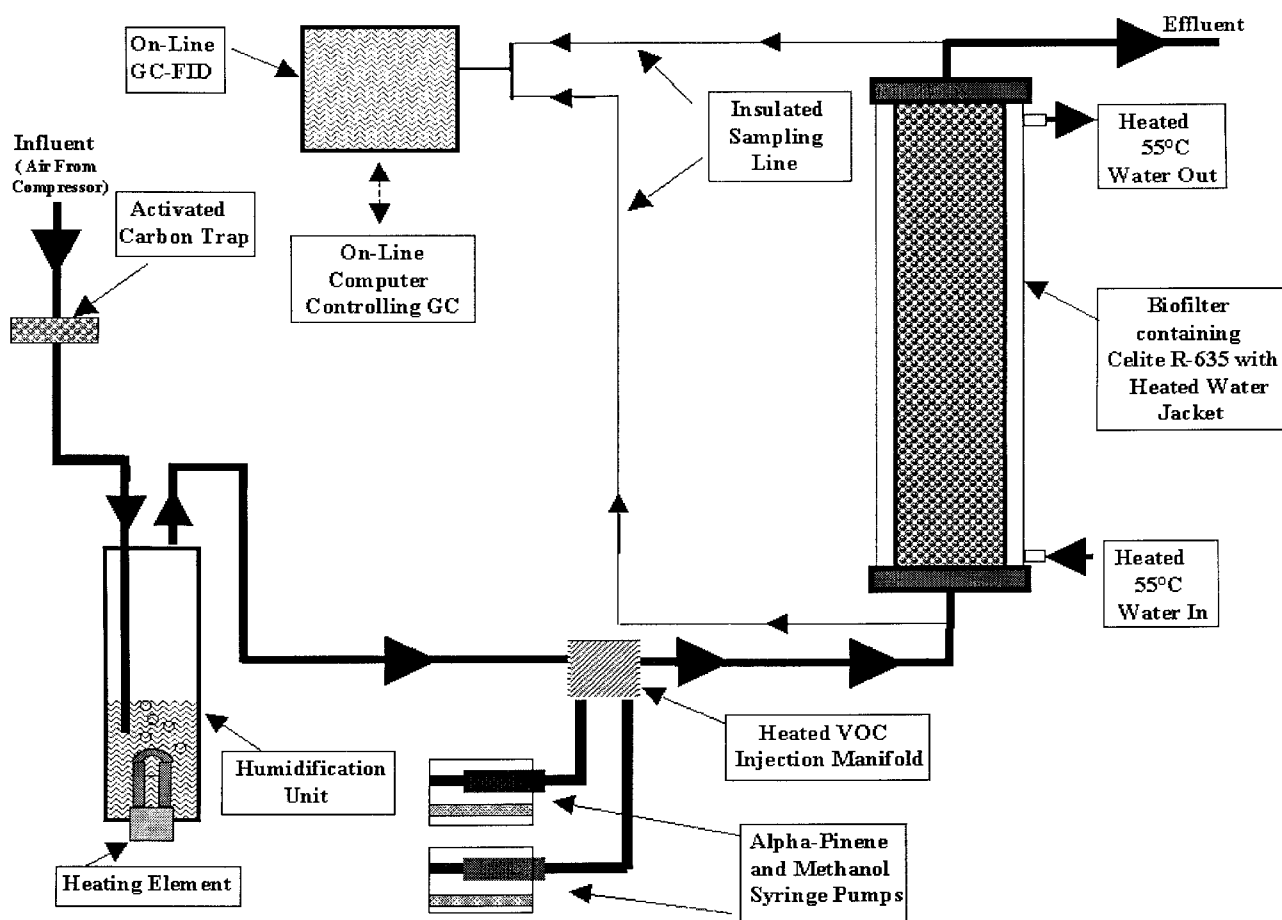


Figure 1 Schematic of the thermophilic biofiltration system.

without methanol or  $\alpha$ -pinene) was periodically added to the column instead of water when more liquid was needed.

In order to determine the effect of residence time on removal of the test compounds ( $\alpha$ -pinene, in particular), a second biofiltration system (System 2) was set up with the same parameters except that the column size was  $4.5 \times 120 \text{ cm}^2$  ( $1824 \text{ cm}^3$ , empty bed volume), i.e., 68% larger than the first system. The flow rate in System 2 was initially set at 100 ml/min (days 1–115) and was later raised to 300 ml/min (days 115–250). Beginning on day 200, 100 ml of a non-ionic surfactant mixture composed of Triton X-100 (0.05% w/v), Brij35 (0.1% w/v), and Brij58 (0.05% w/v) in distilled water solution was added weekly to study the effects of the surfactants on  $\alpha$ -pinene removal (by increasing  $\alpha$ -pinene's solubility). These surfactants are considered non-toxic and resistant to biodegradation [21]. Because of differences in solubility and other properties, each surfactant distributes in different concentrations in each of the physical conditions [23], such as solid and liquid phases, within the biofiltration system. Using a mixture of surfactants ensured that some surfactants were distributed everywhere within the system, enhancing the  $\alpha$ -pinene solubility throughout the system. In preliminary studies,  $\alpha$ -pinene solubility at 55°C increased from <2 ppm to over 200 ppm with all of the surfactants (data not presented). Compound monitoring, analysis techniques, and bacterial inocula were the same as for the first system, except that cell suspensions were added in 200-ml volumes every 10–14 days.

### Support medium analyses

Operation of System 1 was stopped after 174 days. The support medium was separated into top, middle, and bottom sections and each section was analyzed for levels of methanol,  $\alpha$ -pinene, and bacteria. Approximately 5 g (wet weight) of Celite from each section was placed into each of two 40-ml amber glass bottles (Quorpak, Bridgeville, PA). For methanol analyses, 10 ml of distilled water was added to a vial. For  $\alpha$ -pinene analyses, 15 ml of hexane was added for each vial. All vials were then placed on a tumbler for 30 min. Sufficient water or hexane was removed to completely fill an autosampler vial, which was stored at 4°C before the analysis using the GC techniques described above. Celite moisture content was determined by weighing approximately 1 g of Celite from each section before and after heating in an oven at 106°C for 18 h.

For bacteriological analyses, 30 ml of 0.85% NaCl was added to 30 g of Celite from each section. Each mixture was sonicated for 1 min to remove attached cells using a Branson Model 450 Sonifier (Branson Ultrasonics, Danbury, CT) with a constant duty cycle set at its lowest power level. The cell suspension was filtered through Whatman no. 1 filter paper and the filtrate was used for microbial enumeration by both DC and VC techniques as described above. Analyses were also conducted on the liquid remaining in the bottom of this unit.

The support medium of System 2 was also analyzed for levels of methanol,  $\alpha$ -pinene, and bacteria as described for System 1. In

addition,  $\alpha$ -pinene extractions were also conducted using 10 ml of distilled water instead of 15 ml of hexane to determine if any  $\alpha$ -pinene was retained in the surfactant- $\alpha$ -pinene complexes that could dissolve in water.

## Results

### Recovery and initial characterization of thermophilic bacteria

Microscopic examination of the liquid cultures indicated that there was approximately a 10-fold difference in observed (DC,  $\sim 10^7$ – $10^9$  cells/ml) versus recovered (VC,  $\sim 10^6$ – $10^8$  CFU/ml) bacterial levels throughout the course of this study. Three distinct bacterial colony types were recovered from methanol enrichment on R2A agar; all three were able to degrade methanol in pure culture (data not presented). Two distinct bacterial colony types were recovered from  $\alpha$ -pinene enrichment, both of which appeared morphologically and microscopically similar to the two dominant colony types from the methanol enrichment. These two types were also able to degrade both  $\alpha$ -pinene and methanol in pure culture (data not presented). Both Gram-positive and Gram-variable bacteria were found. The Gram-positive isolate produced endospores under aerobic conditions, as is typical for *Bacillus* sp.

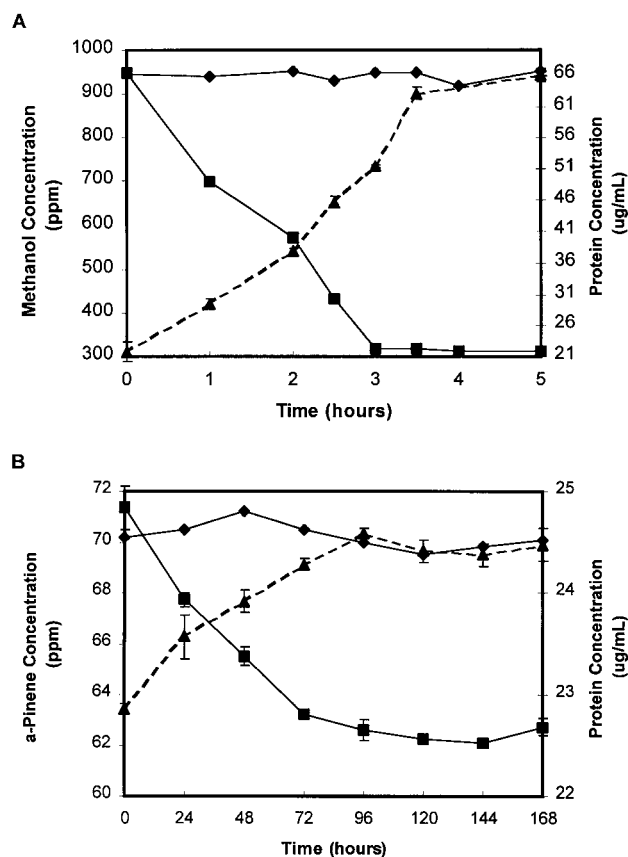
Although the bacterial types recovered on R2A agar were consistently present in all samples from the biofiltration systems, additional viable bacteria that could not be recovered using the VC techniques were also present. The DC levels from the enrichments (and later from the biofiltration systems) continuously increased in parallel with the VC levels, indicating that at least some of the additional cells detected using the DC technique were, in fact, viable. For this reason, inocula for the biofiltration systems were prepared by transferring samples from the liquid cultures (originally derived from the enrichments) rather than using only the recovered bacterial types. The VC data were also used to monitor any change in levels and types of the recovered bacterial populations.

### Methanol and $\alpha$ -pinene degradation studies

The results for methanol and  $\alpha$ -pinene degradation by the bacterial mixtures along with changes in bacterial protein levels are shown in Figure 2A and B, respectively. Methanol biodegradation was detected after a 12-h lag period (not represented in Figure 2A), followed by a 3-h period of rapid degradation. Maximum degradation rates were determined from the period showing the highest compound degradation [16], i.e., for the methanol culture during the first hour after the lag phase (251 mg/l/h). Overall, 65% of the methanol was removed by this thermophilic bacterial mixture at a growth rate of 0.92/h. Growth likely slowed (even with 300 ppm methanol remaining) due to oxygen limitation in these small bottles. In contrast to methanol, the second mixture of bacteria grew slowly, but consistently, on  $\alpha$ -pinene at 55°C ( $k$  of 0.08/h) after a 24-h lag phase. The maximum  $\alpha$ -pinene degradation rate occurred during the 24-h period after the lag phase (0.15 mg/l/h).

### Operation of thermophilic biofiltration System 1

Summary data for System 1 are shown in Table 1. The first 20 days of operation were considered to be an equilibration period. The average influent concentrations of methanol and  $\alpha$ -pinene were



**Figure 2** Bacterial growth and degradation of methanol (A) and  $\alpha$ -pinene (B) in liquid culture at 55°C, as measured by removal of methanol or  $\alpha$ -pinene (■) and increases in protein concentrations (▲). (◆) (◇) Represents control methanol or  $\alpha$ -pinene levels with no cells. Data presented as mean  $\pm$  SD of four replicates.

112 and 17 ppmv, respectively. The average removal of methanol from this system was consistently very high (over 97%) with relatively low variability.  $\alpha$ -Pinene removal averaged only 23% with high variability. The limited aqueous solubility of compounds such as  $\alpha$ -pinene appears to have caused this low removal [10].

Based on the DC and VC results of the liquid from System 1, relatively high levels of bacteria were present throughout most of the operating period. There was a consistent difference of  $10^1$ – $10^3$  cells/ml between the DC and VC levels. The initial population levels in the liquid at the bottom of the system were around  $10^3$  and  $10^5$  cells/ml (VC and DC, respectively). After 60 days of operation, the cell densities increased to about  $10^7$ – $10^{10}$  cells/ml (VC and DC, respectively). The levels remained at about  $2.2 \times 10^{10}$  (CV of 0.62%) and  $4.4 \times 10^7$  (CV of 1.2%) for the DC and VC values, respectively, for the remainder of the operating period, even though no new cells were added after day 105. There was also no change in compound removal rates during this time period, indicating that a stable population of organisms had been established.

Operation of System 1 was stopped as  $\alpha$ -pinene removal did not improve with operating time. The Celite from this system had a relatively low moisture content (average of about 28%). No residual methanol was recovered from the Celite obtained from any portion of the column (all values less than the detection limit of 0.1 ppm). It is possible that some methanol might have been lost from

**Table 1** Methanol and  $\alpha$ -pinene removal by thermophilic biofiltration Systems 1 and 2<sup>a</sup>

Parameter	System <sup>b</sup>	Flow rate (ml/min)	Methanol	$\alpha$ -Pinene
Influent concentration (ppmv; mean/CV, %)	1	100	112 (27)	17 (9.0)
	2	100/300	110 (16)	14 (6.9)
Average removal (%; mean/CV, %)	1	100	97 (1.2)	23 (21)
	2	100	95 (3.4)	96 (14)
	2	300	90 (5.8)	26 (5.1)
	2	300+surfactant	97 (0.9)	95 (5.8)

<sup>a</sup>Based on day 20 through the end of the study, as days 1–20 were considered to be an equilibration period. The mean values are calculated from the daily values (e.g., as presented in Figures 3 and 4 for methanol and  $\alpha$ -pinene, respectively, in System 2).

<sup>b</sup>System 1 had a volume of 1085 cm<sup>3</sup> and an empty bed retention time of 10.85 min; System 2 had a volume of 1824 cm<sup>3</sup> and an empty bed retention time of 18.24 min (100 ml/min) and 6.08 min (300 ml/min).

the Celite during its removal from the system, although little methanol was expected to be recovered due to rapid growth of the test bacteria with this substrate (Figure 2A) and the very high removal levels (Table 1). Residual  $\alpha$ -pinene (6–18  $\mu$ g/g dry weight Celite) was detected in samples from all sections of the system — totaling only about 6 mg for the entire system.

The levels of bacteria were highest at the bottom section of System 1, indicating that more growth and degradation occurred in the portions at the influent of the carbon source ( $\alpha$ -pinene and methanol) and at the site of liquid collection. DC levels ranged from  $2.7 \times 10^9$  cells/ml at the bottom section to  $2.6 \times 10^7$  cells/ml in the top section. The highest DC levels were found in liquid from the bottom section ( $2.6 \times 10^{10}$  cells/ml). There continued to be a consistent difference between the VC and DC values, although the differences were typically less on the support media (10- to 100-fold) than in the liquid (1000-fold).

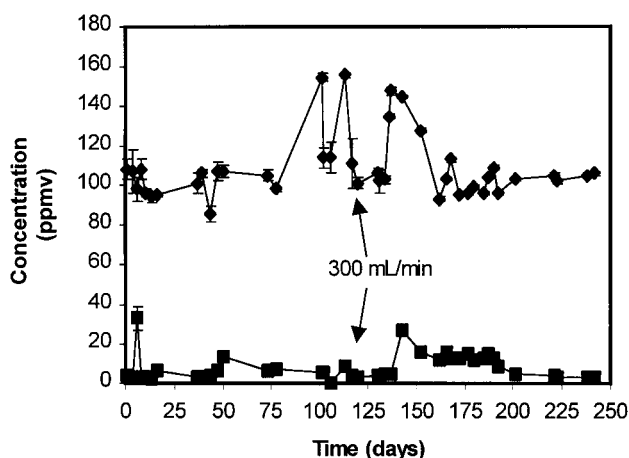
### Operation of thermophilic biofiltration System 2

The influent and effluent levels (ppmv) from System 2 are presented in Figures 3 and 4, respectively, and summary data are presented in Table 1. As with System 1, the first 20 days of operation were considered to be an equilibration period. The empty bed retention time of System 2 was 18.24 min at 100 ml/min and

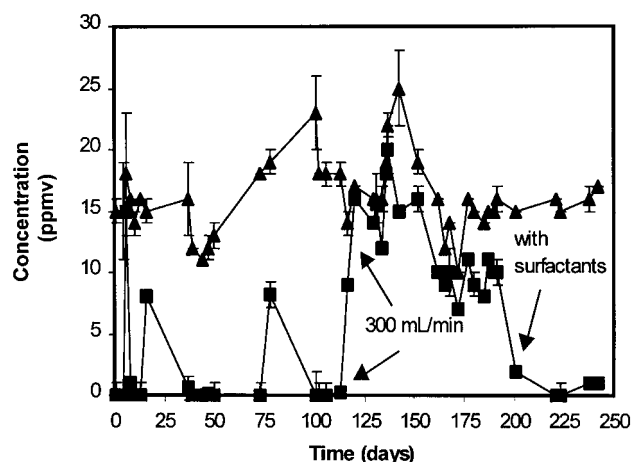
6.08 min at 300 ml/min, compared to 10.85 min for System 1 (at 100 ml/min). These longer residence times, compared to conventional residence times (25–60 s [15]), should have provided ample opportunity for bacteria to degrade the compounds. The overall performance of the second system in removing methanol and  $\alpha$ -pinene is described as elimination capacity (EC) versus loading (influent levels), both as grams per cubic meter per hour, in Figure 5A and B, respectively.

As indicated in Table 1, the removal of methanol in System 2 at 100 ml/min (CV of 5.1%) was approximately 95%, similar to that of System 1. In contrast,  $\alpha$ -pinene removal increased to 96% with only moderate variability. When the residence time was increased to 300 ml/min (CV of 4.9%), methanol removal remained similar at 95%, but  $\alpha$ -pinene removal dropped to 26%. This indicates that residence time, and consequently retention time, is a major influence in the removal of  $\alpha$ -pinene in this thermophilic biofiltration system.

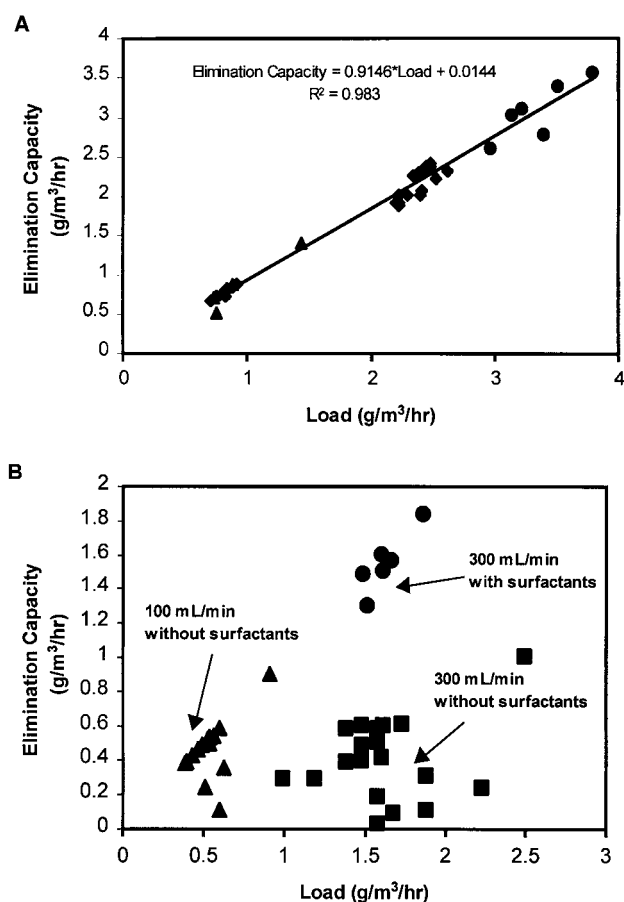
Because the low solubility of  $\alpha$ -pinene appears to have contributed to its poor removal by mass transfer limit, a surfactant mixture which would aid the transfer was added from day 200 until operation was stopped on day 250. Figure 4 shows that  $\alpha$ -pinene removal rates increased to 95% after the surfactants were added (Table 1). Methanol removal also increased (up to 97%). This indicates that the problem of low  $\alpha$ -pinene solubility or



**Figure 3** Methanol influent ( $\blacklozenge$ ) and effluent ( $\blacksquare$ ) concentrations for thermophilic biofiltration System 2. (Flow rate of 100 ml/min from days 1 to 115 and 300 ml/min flow rate from days 115 to 250. The surfactant mixture was added from days 200 to 250. Data presented as means  $\pm$  SD of five samples.)



**Figure 4**  $\alpha$ -Pinene influent ( $\blacktriangle$ ) and effluent ( $\blacksquare$ ) concentrations for thermophilic biofiltration System 2. (Flow rate of 100 ml/min from days 1 to 115 and 300 ml/min flow rate from days 115 to 250. The surfactant mixture was added from days 200 to 250. Data presented as means  $\pm$  SD of five samples.)



**Figure 5** Methanol (A) and  $\alpha$ -pinene (B) loading and elimination capacities in thermophilic biofiltration system 2 at flow rates of 100 ml/min ( $\sigma$ ), 300 ml/min without surfactant ( $\nu$ ), and 300 ml/min with surfactants ( $\bullet$ ).

bioavailability can be alleviated by surfactant addition and that the added bacteria have the ability to degrade  $\alpha$ -pinene once it is more accessible. The initial bacterial numbers in the liquid (at the bottom of the column) were about  $10^4$  and  $10^6$  cells/ml for the VC and DC, respectively. After 40 days of operation, the DC values stabilized at  $6.2 \times 10^9$  cells/ml (CV of 1.1%) throughout the rest of the study. After 80 days of operation, the VC values maintained at  $1.2 \times 10^6$  CFU/ml (CV of 1.1%).

The Celite from System 2 had a slightly higher moisture content (about 30.5%) than the Celite from System 1. Hexane was used to extract  $\alpha$ -pinene at the start of the System 2 support analysis, but no residual  $\alpha$ -pinene was detected in any of the hexane-extracted samples. Distilled water was then used instead of hexane, but no residual  $\alpha$ -pinene or methanol was recovered from any Celite sample (the detection limit of the GC-FID was 0.01 ppm). The surfactants were also distributed in all portions of the system (based on observation of surfactant foam in all sections/samples of the support media). Surfactant presence likely resulted in more effective access of bacteria to the  $\alpha$ -pinene as no residual  $\alpha$ -pinene was detected from any sample.

Based on DC tests, the levels of bacteria detected in different sections of System 2, as well as in the liquid, were similar to those found with System 1. The differences between DC and VC were typically less on the support media (only 10-fold) than in the

liquid (100-fold) and both of these differences were less than those observed for System 1. As the recovered types did not change throughout the study (from those presented in the original inocula), the results indicate that these types were able to grow better under the operating conditions found in System 2. As with System 1, the DC and VC levels were higher at the bottom (influent) section of the system than on top.

## Discussion

Methanol was readily degraded by the mixture of thermophilic bacteria in the liquid degradation studies and in both of the biofiltration systems, consistently achieving at least 95% removal of methanol in the waste stream having levels representative of those found in some forest products industries. The growth and maximum degradation rates reported in this study are greater than those reported for mesophilic studies (at 27°C), which showed growth rates of only 0.16/h [24]. This consortium also had a growth rate that was greater than other thermotolerant methanol-degrading bacteria (studied at 50–55°C) [1,7]. Methanol is very soluble in water, allowing the bacteria sufficient opportunity to utilize it as a carbon and energy source. The linear relationship between methanol loading and removal indicates that a maximum overall elimination capacity was not reached; thus, loading rates for this system could be greater than those obtained during this study. It is expected that these high removal rates would be exhibited with larger systems having shorter residence times (and higher loading levels).

A mixture of thermophilic  $\alpha$ -pinene-degrading bacteria was also recovered and found to utilize  $\alpha$ -pinene as its sole source of carbon and energy, but at relatively low removal rates. The total amount of  $\alpha$ -pinene consumed in the liquid degradation studies appears to be greater than that reported for mesophilic studies at 23°C, but the overall thermophilic degradation rates are lower [16]. Limited  $\alpha$ -pinene degradation was also detected by *Bacillus pallidus* at 60°C in liquid culture [22]. Unlike methanol,  $\alpha$ -pinene is barely miscible in water, even at room temperature, which may account for the low degradation and growth rates observed at 55°C.

The results show that many factors limited  $\alpha$ -pinene removal in the thermophilic biofiltration systems.  $\alpha$ -Pinene entering these systems needs to be available to the bacteria if degradation is to occur. Mass transfer seems to be the major limitation for  $\alpha$ -pinene degradation or removal. The physical and chemical properties, such as polarity and molecular structure, of  $\alpha$ -pinene affect its solubility [28]. Likewise, temperatures within the thermophilic range can decrease the solubility of many VOCs [5]. The main effect of high temperature in the biofiltration systems seems to be the ineffective mass transfer of  $\alpha$ -pinene from air to the bacteria both within the liquid culture and in the biofilm on the Celite solid support media. Shorter residence times combined with high temperature may result in most of the  $\alpha$ -pinene passing through the system untreated, causing a low elimination capacity (Figure 5B) when compared with other studies [12,17,18], which used higher moisture-containing support media and much lower temperatures.

Increased  $\alpha$ -pinene removal, along with consistently high methanol removal, was found with the larger biofiltration System 2 at a residence time of 18.24 min, providing an indication that this type of thermophilic treatment system may provide a viable alternative to other types of treatment. In addition, this type of system has the possibility of removing compounds with very

different properties simultaneously. However, the solubility or mass transfer limitation of  $\alpha$ -pinene removal needs to be resolved. Surfactant addition to increase  $\alpha$ -pinene mass transfer is a possible option to increase  $\alpha$ -pinene removal at shorter residence times (Figure 5B). Also, it is possible that systems with higher water amount (such as trickling bed biofilters) and different support media, particularly ones with higher moisture contents (60–70%) [12,18], could facilitate greater mass transfer of  $\alpha$ -pinene and thus, its removal. This effect is likely because water and moisture can help the partitioning of VOCs from air to the liquid phase [13]. Overall, the results show the feasibility of applying thermophilic biofiltration systems in the forest products industry for removal of compounds as varied as methanol and  $\alpha$ -pinene.

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