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Biofilter Treatment of Gas Phase β -Caryophyllene at Elevated Temperature

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BIOFILTER TREATMENT OF GAS PHASE β -CARYOPHYLLENE
AT ELEVATED TEMPERATURE

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Civil and Environmental Engineering

by

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ABSTRACT

Industrial wood processing operations generate a variety of gaseous emissions containing volatile organic compounds (VOCs). One of the VOCs emitted from the processing of coniferous softwood tree species is β -caryophyllene, a naturally occurring sesquiterpene of environmental concern. One approach that may be attractive for treating gaseous emissions of β -caryophyllene from wood processing operations is the use of fixed-film processes commonly referred to as biofiltration. Previously, it was established that β -caryophyllene can be successfully treated via biofiltration at temperatures in the mesophilic range. Many gaseous emissions from industrial wood processing operations, however, are higher in temperature than those employed in the initial report on the treatability of β -caryophyllene via biofiltration.

This thesis describes experiments carried out to test the ability of a laboratory-scale biofilter operated at elevated temperature level ($\sim 50^\circ\text{C}$) to remove β -caryophyllene from contaminated air. Results from experiments presented here demonstrate that it is technically feasible to treat β -caryophyllene even at high temperature. Results also demonstrate that with a properly selected microbial enrichment and inoculation strategy, the successful startup of a high temperature biofilter can be achieved over a time interval comparable to that previously reported for biofilters operated in the mesophilic temperature regime. Collectively, the results expand understanding of β -caryophyllene treatment by biofiltration.

CHAPTER 1: INTRODUCTION AND OVERVIEW

1.1 Background

Industrial wood processing operations (e.g., lumber drying and production of composite products such as oriented strand board) generate a variety of gaseous emissions containing volatile organic compounds (VOCs) (Diehl *et al.*, 2000; Granstrom, 2003). One of the VOCs emitted from the processing of coniferous softwood tree species is β -caryophyllene, a naturally occurring sesquiterpene of environmental concern (Hakola *et al.* 2006). One approach that may be attractive for treating gaseous emissions of β -caryophyllene from wood processing operations is the use of fixed-film processes commonly referred to as biofiltration. In a recent study, it was established that β -caryophyllene can be successfully treated via biofiltration at temperatures in the mesophilic range of 31-34 °C (Moe *et al.*, 2013). Many gaseous emissions from industrial wood processing operations, however, are higher in temperature than those employed in the initial report on the treatability of β -caryophyllene via biofiltration.

1.2 Objectives

The overall objective of research described here was to experimentally evaluate the ability of a laboratory-scale biofiltration system to biologically remove β -caryophyllene from contaminated air at an elevated temperature on the order of 50 °C.

1.3 Thesis Organization

Chapter 2 of this thesis contains a brief literature review. Chapter 3 describes initial experiments to assess the ability of a biofilter to remove β -caryophyllene from high temperature contaminated air. Chapter 4 describes additional experiments carried out to test the ability of the biofilter operated at elevated temperature level to remove β -caryophyllene using an alternative enrichment culture and inoculation procedure than was described in Chapter 3. Chapter 5 summarizes the overall conclusions and recommendations for future research. Supplementary data and calculations are provided in appendices.

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CHAPTER 2: LITERATURE REVIEW

This chapter contains a literature review regarding the processes responsible for emission of volatile organic compounds (VOCs) from wood drying and processing operations with an emphasis on terpene emissions. This chapter also presents a summary of previous studies in which biofiltration has been applied for treatment of wood-associated VOC contaminated gas streams, with an emphasis on previous studies of biofilters operated at elevated temperatures.

2.1 Terpene Emissions from the Wood Industry

2.1.1 Overview

Trees are generally divided into two categories: softwood (conifer) and hardwood (broadleaf). On average, softwood species contain a larger amount of potentially volatile extractive materials compared to most broadleaf species (Beakler *et al.*, 2007). Terpenes, which are defined as hydrocarbons built from isoprene (C_5H_8) units, comprise a substantial fraction of the VOCs present in softwood (pine, fir, and spruce). Terpenes are classified according to the number of isoprene units per molecule. In conifers, the terpene groups consist mainly of monoterpenes ($C_{10}H_{16}$), with sesquiterpenes ($C_{15}H_{24}$) and diterpenes ($C_{20}H_{32}$) generally present in smaller amounts (Baumann *et al.*, 1999; Granstrom, 2010).

Terpenes are stored in roots, stumps, bark, branches, twigs, needles and stems. For most pine species, the concentration of terpenes decreases from heartwood to sapwood as well as from root to stem. Ingram reported that the total concentration of terpenes in sapwood, heartwood, and knot tissue from loblolly pine samples was found to be 0.31, 1.51, and 3.31 percent by mass on an oven-dry basis (Ingram *et al.*, 2000). Thompson also suggested knotty lumber samples gave higher emissions of terpenes than lumber samples containing a low proportion of knots (Thompson *et al.*, 2006; Thompson and Ingram, 2006). Kininmonth (1991) reported that terpenes accounted for one-third of the extractive content of radiata pine at ambient temperature. The average concentration of terpenes from 24- to 45-year-old radiata pine trunk achieved 1.8 to 2.3 percent on a dry weight basis (Kininmonth and Whitehouse 1991; Thompson *et al.*, 2006).

Although terpenes are naturally emitted from living trees in low concentration (less than 300 ppm) (van Groenestijn and Liu, 2002), the anthropogenic contribution of terpene emission from wood products industry may be larger (Kleinheinz *et al.*, 1999). Anthropogenic terpene emissions include gaseous terpene releases during logging, chipping, debarking, sawing, drying,

pulping, and the production of boards and pellets (Bagherpour *et al.*, 2005; Diehl *et al.*, 2000; Granstrom, 2010; Rupar-Gadd *et al.*, 2006; van Groenestijn and Liu, 2002). These artificial activities result in comparatively large emissions to air, because terpenes are important components in tree defense mechanisms against mechanical wounds (Stromvall and Petersson, 1993). Anthropogenic emission of terpenes have been reported from many different coniferous tree species including pine (loblolly pine, southern pine, Scots pine, ponderosa pine), fir (Douglas fir, white fir), spruce (Norway spruce, white spruce) and hemlock (Stromvall and Petersson, 1993).

Usually, the emissions of terpenes produced by wood products manufacturing processes account for between 7 and 21 percent of the total VOC emissions (Baumann *et al.*, 1999), but the relative amounts and types of terpenes depend upon the species of wood, the locality, growing conditions (Baumann *et al.*, 1999; Kleinheinz *et al.*, 1999), temperature, and wood moisture content. Granstrom reported that the emission of terpenes per unit mass of oven-dry weight increased with increased drying temperature and decreased wood moisture content. She further reported that sawdust dried in steam dryers emitted 50–70% of the initial monoterpene content when dried to 6–12% moisture content (Granstrom, 2003; Granstrom, 2009).

Baumann *et al.* reported that the types of panels manufactured also had an impact on terpene emissions. For example, a large quantity of terpenes was detected during the production of pressed wood products such as particleboard and oriented strand board (OSB) (Baumann *et al.*, 1999; Langolf and Kleinheinz, 2006); whereas the terpenes were almost absent in emissions from Medium Density Fiberboard (MDF) samples. The differences of MDF, OSB and particleboard manufacturing process may greatly impact the amount of emissions (Baumann *et al.*, 1999).

Air quality is affected by the terpenes both in air and photochemical oxidants, and tropospheric ozone formed in reactions involving terpenes (Granstrom, 2009; Montes *et al.*, 2012). Photochemical oxidants such as hydrogen peroxide, organic peroxide, organic nitrate, and reactive organic aerosols in the presence of nitrogen oxides (NO_x) disturb the photosynthesis of plants causing forest and crop damage, and at sufficiently high concentrations are harmful to humans causing respiratory difficulties and mucous membrane irritation (Bagherpour *et al.*, 2005; Beakler *et al.*, 2007; Granstrom, 2003; Granstrom, 2009; Montes *et al.*, 2012; Thompson and Ingram, 2006).

2.1.2 Emission of Monoterpenes from Wood Industry

Monoterpene are widely distributed in plants but occur in large amounts mainly in conifers. They are identified as the major chemical component in VOC emissions from industrial wood products, such as sawing, pulping and fragrance industries and responsible for much of the characteristic odor of softwoods (Baumann *et al.*, 1999; Diehl *et al.*, 2000; Lavery and Milota, 2000).

Monoterpenes are built from two isoprene units and their general molecular formula is $(C_5H_8)_2$. These compounds may be grouped as bicyclic, monocyclic, and acyclic with one, two, and three double bonds (Lavery and Milota, 2000). The average molecular weight of monoterpenes is approximately 136 g/mole and the boiling points are in the range of 150 °C to 185 °C (Granstrom, 2010; Lavery and Milota, 2000). Fourteen monoterpenes were reported occurring in the oleoresin of conifers. They are α -pinene, β -pinene Δ^3 -carene, camphene, alpha-thujene, limonene, beta-phellandrene, terpinolene, gamma-terpinene, α -phellandrene, α -terpinene, myrcene, tricyclene and p-cymene aromatic. Among them, α -pinene, β -pinene, myrcene, camphene, and limonene received great concern (Stromvall and Petersson, 1993), because they are the chief monoterpenes emitted from lumber drying (Diehl *et al.*, 2000; Stromvall and Petersson, 1993). Δ^3 -carene also received high concern due to its high concentration from pulpwood barking (Stromvall and Petersson, 1993).

According to Granstrom (2003), monoterpene emissions were quantified as comprising 13 to 250 mg/kg oven dried wood from drying spruce sawdust. Edman *et al.* (2003) showed that the geometric mean for monoterpenes emissions was 0.5 mg/m³ within a large lumber mill handling pine, spruce and fir.

Studies also pointed out the monoterpenes in softwood vary with species. According to the report, α -pinene and Δ^3 -carene predominant for Scots pine (*Pinus sylvestris*) (*Picea abies*), and α -pinene followed by β -pinene were the major components in Norway spruce (Stromvall and Petersson, 1993). In addition, Mohseni *et al.* (1998) introduced that α -pinene is of particular interest because the amount of α -pinene released during the pressing process can influence the amount of certain materials being used in the production of man-made board and consequently the cost of the final product.

2.1.3 Emission of Sesquiterpenes from Wood Industry

Sesquiterpenes, terpenes composed of three isoprene units, are naturally emitted from the needles of softwoods, the leaves and fruit of hardwoods, and the bark of many species. According to Granstrom's studies, generally, the sesquiterpene emissions account for about 20% of the monoterpene emissions from Norway spruce sawdust during drying at 140 °C -200 °C (Granstrom, 2009; Granstrom, 2010). Stromvall and Petersson (1993) further reported that the sesquiterpene to monoterpene ratio was 10% in a kraft mill digester blow tank; was 5% at a thermo-mechanical pulp mill pretreatment of chips and sulfite mill seasoning silo; and was 0.5% during spruce debarking, but the types and amounts of sesquiterpene varied with species, surrounding temperature, and wood moisture contents.

The amount of turpentine and sesquiterpenes emissions was compared in some studies. It was reported that sesquiterpenes make up to 0.5 percent of most sulfate turpentines, but up to several percent of turpentines from longleaf pine and about 15% of turpentine from grand fir. Thus, the content of sesquiterpene can be believed varied from 1% to about 20% of turpentine (Granstrom, 2010).

Many kinds of sesquiterpenes have been detected from wood drying in recent years. Granstrom identified isolongifolene, α -gurjunene, α -longipinene, α -copaene and longifolene by condensing the exhaust streams (Granstrom, 2009). Helmig *et al.* (1999) also identified α -humulene, and cyperene as sesquiterpenes emitted from *Pinus taeda* (loblolly pine). Vuorinen *et al.* (2005) detected germacrene-D, (E,E) α -farnesene, α -copaene, and β -bourbonene emitted from clones of *Betula pendula* (silver birch). These terpenes are collected from detached twigs of living birch in July at Finland.

2.2 Biofiltration of Terpenes from Wood Drying and Processing

2.2.1 Overview of Biofiltration

VOCs have been treated by means of various technologies such as absorption, adsorption, scrubbing, and thermal or catalytic oxidation over several decades (Jin *et al.*, 2006). In at least some cases, biofiltration offers a potential alternative to the existing physicochemical techniques for removal of VOCs and hazardous air pollutants (HAPs) (Kleinheinz *et al.*, 1999). In biofiltration, contaminated air is passed through a packed bed containing a solid medium that supports a biofilm. As air moves through the packed bed, contaminant partition into an aqueous or biofilm layer where microorganisms can metabolize the contaminants to innocuous products

such as carbon dioxide, water, and cell mass (Moe and Qi, 2004). It is reported that at least 60 of the 189 HAPs listed in the 1990 Clean Air Act Amendments have been successfully treated using this technology. The HAPs successfully treated in biofilters include ketones, ethers, and alkanes (e.g., acetone, methyl ethyl ketone, methyl isobutyl ketone, methyl propyl ketone, diethyl ether, methanol, n-hexane and n-butyl acetate) as well as aromatic hydrocarbons (e.g., benzene, toluene, xylenes and ethylbenzene) (Miller and Allen, 2004; Miller and Allen, 2005).

The ability of a biofilter to successfully treat VOCs depends to some extent upon the solubility of the compounds in the liquid layer of the biofilm (Mohseni and Allen, 2000). Hence, the hydrophobic characteristics of the terpenes discharged in air emissions can significantly influence their removal capacities in biofilters (Mohseni and Allen, 2000).

2.2.2 Biofiltration of Terpenes

As a widely distributed monoterpene, α -pinene is a relatively hydrophobic compound that has been investigated by several researchers. Mohseni and Allen (2000) showed that α -pinene can be completely removed by biofiltration with elimination capacity reaching 40–45 g/m³bed/h. Langolf et al. (2006) operated a laboratory-scale lava rock-based biofiltration system for the removal of α -pinene, and their system achieved a significantly higher elimination capacity of 100 g/m³bed/h, indicating that preselected microorganisms and lava rock packing medium may be an attractive choice when treating α -pinene. In a study reported by Jin *et al.* (2006), near complete removal of α -pinene was observed up to a load of 100 g/m³bed/h, while 89% removal efficiency was reached at an elimination capacity of 143 g/m³bed/h.

van Groenestijn and Liu (2002) demonstrated that different packing materials that were used to support the growth of fungi affected the removal capacities. In their study, perlite, expanded clay granules, polyurethane foam cubes and compost were compared in fungal biofilter and the volumetric removal capacities of α -pinene were 24, 33, 38 and 24 g/m³bed/h respectively. They found that the use of polyurethane foam cubes was favorable in biofilter because of the high volumetric elimination capacity and low gas pressure drops across the filter bed compared to other media.

Kleinheinz *et al.* (1999) used laboratory-scale biofilters packed with aspen wood chips and achieved a maximum volumetric removal capacity of 32 g/m³bed/h. Wood chips have also been used as packing medium because microorganisms from pine wood chips could be used

directly without further enrichment regarding α -pinene removal (Hejazi *et al.*, 2010; Jeong *et al.*, 2006).

Also, some researchers have tried different ways to enhance the removal rate of terpenes in biofilters. Hejazi *et al.* (2010) found that the addition of silicone oil to packing material could increase the mass transfer rate and improve the elimination capacity due to higher absorption capacity occurring when the concentration of hydrophobic pollutants increased during the start-up period.

2.3 Temperature Effects on Biofilter Operation

As mentioned above, multiple researchers have reported successful removal of hydrophilic and hydrophobic chemicals using mesophilic biofilters (Miller and Allen, 2005; Moe and Qi, 2004; van Groenestijn and Liu, 2002). However, the application of biofiltration reported in the literature has largely been limited to the treatment of waste gases at temperatures in the mesophilic range (15 °C to 40 °C). This hinders informed decision making related to the use of this technology for hot waste gases, such as the emissions from wood-related industry drying and pressing operations. Also, the cooling step for hot gases can be costly and may introduce an aqueous waste stream requiring further treatment (Cho *et al.*, 2007; Dhamwichukorn *et al.*, 2001; Jin *et al.*, 2007).

Some research work has been performed recently to explore the potential of high temperature waste gas biotreatment. It has been reported that compared with mesophilic microorganisms, microorganisms that thrived at elevated temperature are more sensitive to temperature increases (Cho *et al.*, 2007). Biological reactions may decrease in rate or stop entirely if the temperature is not suitable for the growth of the microorganisms present. Dhamwichukorn *et al.* (2001) and Jin *et al.* (2007) reported that microorganisms could provide higher degradation rate at 45 °C~55 °C. Thus, the maximum degradation rate for α -pinene in thermophilic biofiltration was higher than that in mesophilic biofiltration. Kong *et al.* operated three biotrickling filters at 40 °C, 55 °C and 70 °C, respectively, and suggested that the acclimation time for microorganisms increased with increasing temperature. They further indicated that there were relatively small changes in removal when temperature varied from 40 °C to 55 °C in the laboratory-scale biofilters treating α -pinene. However, the α -pinene removal significantly decreased when the temperature reached 60°C. When the temperature reached to 65°C, the α -pinene removal was almost zero (Kong *et al.*, 2001).

Montes *et al.* (2010) used silicone oil to enhance the elimination capacity of α -pinene in two laboratory-scale mesophilic and thermophilic biotrickling filters and further reported that higher removal rates for α -pinene were obtained in the thermophilic than in a mesophilic bioreactor. In his study, the elimination capacity for α -pinene at thermophilic and mesophilic biofiltration was 293 g/m³bed/h and 195 g/m³bed/h at similar load. Also, he pointed out removal capacity under overload condition decreased in both mesophilic and thermophilic biofilter, but the performance of the biofilter operated under thermophilic conditions was better than that of the mesophilic system.

Kong *et al.* (2001) further investigated the treatment of a mixture of α -pinene and methanol in a thermophilic biofilter and showed that there were more varieties of α -pinene communities at thermophilic condition than those at mesophilic condition and the species of degraders changed with temperature. Also, the varieties of methanol or α -pinene degraders were more similar to each other at thermophilic conditions (55 °C) than the mesophilic communities (40 °C). Collectively, these results demonstrated that biofilters could be successfully operated at high temperatures for treatment of α -pinene and methanol mixtures. In his study, under thermophilic conditions, the removal rates achieved 60 g/m³bed/h for α -pinene at 55 °C and 100 g/m³bed/h for methanol at 70 °C, respectively.

2.4 β -Caryophyllene

Terpene emissions contribute the formation of photo-oxidants and secondary organic aerosols (SOA) (Granstrom, 2009; Granstrom, 2010). As a sesquiterpenes, β -caryophyllene has the same characteristic with terpene (Li *et al.*, 2011). Thus, it contributes to the depletion of ozone, breaking the natural cycle of NO_x and catalyzing the reaction of smog formation (Bagherpour *et al.*, 2005; Montes *et al.*, 2012). The emission of β -caryophyllene can have adverse effects on both human health and the environment, causing respiratory irritation problems and disturbing the photosynthesis of plants (Beakler *et al.*, 2007; Granstrom, 2009). The formation of SOA by dark ozonolysis of gas-phase β -caryophyllene was quantified by several researchers (Alfarra *et al.*, 2012; Chen *et al.*, 2012; Li *et al.*, 2011).

Generally, sesquiterpenes are more reactive than monoterpenes in the atmosphere due to their low vapor pressure (Granstrom, 2009; Granstrom, 2010) combined with their higher molecular weight (Stromvall and Petersson, 1993). The boiling points of sesquiterpenes are in

the range of 240°C to 270°C. For β -caryophyllene, specifically, the boiling point is around 262 °C (Granstrom, 2009). Physicochemical properties of β -caryophyllene are listed in Table 2-1.

Because experimental data are not available regarding several physicochemical properties of β -caryophyllene, estimation methods were applied using models freely available in the EPI SuiteTM version 4.10 software package (US EPA, 2011, <http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm>) (Moe *et al.*, 2013).

Table 2-1: Physicochemical Properties of β -Caryophyllene (Moe *et al.*, 2013)

Parameter	Value
Molecular formula	C ₁₅ H ₂₄
CAS#	87-44-5
Molar mass (g/mol)	204.36
Density at 20 °C (g/cm ³)	0.902
Boiling Point (°C)	262-264
Log KOW (dimensionless)	6.30 ^a
Water Solubility at 25 °C (mg/L)	0.05 ^b -0.54 ^c
Henry's Law Constant (atm × m ³ /mole) (unitless)	0.69 ^d 28.2 ^d
Vapor Pressure (Pa at 25 °C)	4.16 ^e

^a K_{OW}=octanol-water partition coefficient, estimated using WSK_{OW}Win version 1.67, atom/fragment contribution method.

^b Water solubility estimated using WSK_{OW}Win version 1.41 regression equation.

^c Water solubility estimated using WATERNT version 1.01.

^d Henry's Law constant estimated using the Bond contribution method in HenryWin version 3.20.

^e Vapor pressure estimated as the mean of the Antoine and Modified Grain Method in MPBPWin version 1.43.

The estimated vapor pressure for β -caryophyllene shown in Table S1 is somewhat higher than the value of 1.1 Pa estimated using the alternative approach of (Hoskovec *et al.*, 2005)

Hakola *et al.* (2006) suggested β -caryophyllene appeared to be the dominant sesquiterpene based on testing the seasonal variation of mono-and sesquiterpene emission for live *Pinus sylvestris* (Scots pine) during the midsummer months. Helmig *et al.* (1999) identified β -caryophyllene emitted from *Pinus taeda* (loblolly pine), found the amounts of sesquiterpenes were similar to those of emitted monoterpene in Scots pine. Besides emission from live trees and wood industry operations, it is reported that β -caryophyllene is emitted to the air by various

additional plant species. For example, Arey *et al.* (1995) detected the emissions of β -caryophyllene from *Salvia mellifera* (black sage). Ciccioli *et al.* (1999) found the air emissions of β -caryophyllene from orange orchards in Spain.

Recent research has demonstrated that β -caryophyllene contaminated air can be successfully treated via mesophilic biofiltration (Moe *et al.*, 2013). β -caryophyllene removal efficiency increased from near zero to 96% over a 16-day interval following startup with continuous loading at a contaminant loading rate of 1.2 g-C/m³/hr (with β -caryophyllene loading rate reported in units of grams carbon supplied per m³ bed volume per hour), in a biofilter with an Empty Bed Residence Time (EBRT) of 120 seconds. In subsequent tests with higher loading rates and lower EBCT of 10 seconds, β -caryophyllene removal efficiency higher than 95% was achieved under continuous loading at a rate of as high as 17.9 g-C/m³/hr.

While temperature has been shown to greatly affect biofilter performance, previous research regarding β -caryophyllene treatment in biofilters has been conducted at a single temperature in the mesophilic range (Moe *et al.*, 2013). Due to the high gas temperature range of industrial wood drying and pressing operations, the research reported in this thesis focused on degrading β -caryophyllene at elevated temperature level (50 °C).

2.5 References

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CHAPTER 3: INITIAL EXPERIMENTAL TESTING OF THE HIGH TEMPERATURE BIOFILTER

3.1 Introduction

This chapter describes initial experiments carried out to test the ability of a laboratory-scale biofilter operated at elevated temperature level to remove β -caryophyllene from contaminated air.

3.2 Materials and Methods

3.2.1 Experimental Apparatus

A schematic diagram of the laboratory-scale biofilter apparatus is shown in Figure 3-1. Compressed air passed through a packed column (7.3 cm ID) containing activated carbon (BPL 4 $\times$ 6 mesh GAC, Calgon Carbon, Pittsburgh, PA) to remove unwanted contaminants. A pressure regulator (series R35, Arrow Pneumatics, Broadview, IL) and electronic mass flow controller (Aalborg Inc., Orangeburg, NY) regulated the air flow. Water from a heated recirculating bath (Cole-Parmer, Vernon Hills, IL) was used to heat and humidify the influent air. The humidification column was constructed of clear PVC pipe (7.3 cm ID) and had a total height of 163 cm. The column consisted of two sections divided by a perforated stainless steel support plate that was held in position by a PVC coupling. The bottom section had a height of 50 cm and served as a water storage reservoir. The top section (total height of 113 cm) was packed to a depth of 95 cm with stainless steel 10 mm Interpack media (Jaeger Company, Houston, TX). Air entered the humidification column at a point between the lower water reservoir and the upper packed bed, flowing counter-current to the direction of water flow. Water entered at the top of the humidification column with a flow rate of 1.7 L/min from the water bath set to a temperature of 59 °C.

The biofilter consisted of three water-jacketed sections, each constructed of schedule 80 clear PVC pipe (Harvel Plastics Inc., Easton, PA). The inner diameter of the inner pipe was 7.3 cm, and the inner diameter of the outer pipe was 20.0 cm. A recirculating water bath (Cole-Parmer, Vernon Hills, IL) was used to recirculate heated water (temperature set point 60 °C) in the annular space between the inner and outer pipes at a flow rate of 2.1 L/min. Fiberglass insulation (Thermwell Products Co., Sparks, NV) was affixed to the exterior surface of the biofilter to minimize heat loss. A perforated stainless steel support plate located at the bottom of

each section supported the packing medium. Glass marbles were placed in the bottom of the inlet section to distribute air flow. Compression fittings (1/4" ID, JACO) located at the biofilter inlet and at the outlet of each of the three biofilter sections allowed gas sampling.

Figure 3-1: Schematic diagram of experimental apparatus for the biofilter system.

Gas transfer lines employed in the biofilter system (3/8 in) were wrapped with electrical heating tapes (Cole-Parmer) regulated with Variac controllers (ISE Inc., Cleveland, OH) to maintain the elevated temperature level.

Each of the three biofilter sections contained reticulated polyurethane foam cubes (Honeywell-PAI, Lakewood, CO) as packing medium. The medium, supplied by the vendor in the form of cubes approximately 5.0 cm per side, was cut into cubes approximately 1.25 cm per side prior to use. The physical properties of this packing medium were described previously (Li and Moe, 2005)

β -caryophyllene (TCI America (Portland, OR, catalog No. C0796, >90% purity) was injected into the air flow by a syringe pump (KD Scientific model 1000, Boston, MA, USA) equipped with a 250 μ L glass gas tight syringe (Hamilton, Reno, NV, USA).

3.2.2 Biofilter Inoculation and Operation

3.2.2.1 Initial Inoculation and Operation

A 500 g mass of compost (wet basis) which was derived from citrus peels and other plant materials (residential house in Louisiana) was mixed with 1 L freshly prepared nutrient solution

which consisted of the following constituents added to deionized water: NH_4NO_3 7.5 g/L, KH_2PO_4 6.0 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.6 g/L, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.12 g/L, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 2.03 mg/L, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 1.44 mg/L, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 3.48 mg/L, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 6.06 mg/L, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 1.44 mg/L, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ 0.60 mg/L and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 8.16 mg/L. A 33 mL volume of 1 M/L NaOH was added to adjust the pH from 4.16 to 7.0. The suspension was manually stirred for one minute, passed through a sieve to remove coarse materials, and allowed to quiescently settle for five minutes to separate large particles which visibly accumulated on the bottom of the beaker. A 260 mL volume was transferred to each of three containers along with 1.0 L nutrient solution (composition as above). Thereafter, 39 g polyurethane foam packing material was immersed in each suspension for 80 minutes. During this period, a burette was used to intermittently stir the contents. Each of the three preparations of the packing medium was then transferred to a separate biofilter section, resulting in three sections each with a packed bed depth of 30 cm. That resulted in a total packed bed depth of 90 cm, total packed bed volume of 3.74 L, and total mass of 117 g polyurethane in the biofilter. The total suspended solids (TSS) concentrations before and after immersing the packing medium was measured in triplicate using Standard Method 2540D.

After the biofilter column was assembled, the remaining suspension of compost plus nutrient solution was mixed with an additional 2.0 L freshly prepared nutrient solution (composition as above, 5.78 L total), heated to a temperature of 50 °C, and pH was adjusted to 7.0-7.1 after heating. This was then pumped into the bottom of the biofilter via a peristaltic pump (Masterflex) at a flow rate of 170 mL/min. After filling the column, the suspension was recirculated for a duration of 1 hour (withdrawal at top and reintroduction at the bottom at flow rate 170 mL/min), and then drained from the column at the same flow rate used during filling (170 mL/min).

Immediately following this initial inoculation procedure, air flow to the biofilter was started with a flow rate of 2.24 L/min, and β -caryophyllene injection began with a flow rate of 10 $\mu\text{L/hr}$. The corresponding empty bed contact time (EBCT) for the biofilter was 100 seconds, with the EBCT calculated on the basis of dry air flow rate at standard temperature and pressure. The EBCT calculated for the biofilter accounting for water evaporation and temperature of 50 °C was 81 seconds (see Appendix A1). The start of β -caryophyllene contaminated air was designated as time zero. (Time zero = 8:10 p.m. on March. 18, 2013). Time was measured in days from the start of pollutant loading.

At weekly intervals, gas flow was temporarily halted, and a 6.0 L volume of nutrient solution with composition as described above was filled and drained from the column. NaOH was added to adjust the pH to 7.0.

Both influent and effluent pollutant concentration were measured in terms of carbon concentration using a model 600 HFID hydrocarbon analyzer. Measurements were collected at one minute intervals for a minimum of 1 hr/day from day 1 to day 63. From day 64 (May 20, 2013) to day 81 (June 6, 2013), the syringe pump used for injecting β -caryophyllene was turned off (on account of a malfunctioning fume hood that caused no capacity to provide exhaust of the effluent gas). Influent and effluent concentration data were not collected during this period (days 64-81).

3.2.2.2 Re-inoculation and Operation

After 81 days of operation (June 7, 2013), two different kind of soil (Clegg's Nursery, Inc., Baton Rouge, LA) were used to grow inoculum. Type I soil contained 100% forest product compost, and type II soil was comprised of a mixture of 60% composted forest products and 40% sand and perlite. Neither of the soils was subjected to secondary heat treatment or other disinfection following composting.

The 8 L of nutrient solution with all constituents added at one sixth of the concentration described in Section 3.2.2.1 was used to develop the biofilter inoculum. The nutrient was pre-heated to 50 °C and its pH was adjusted to 7.0 before using.

A 500 g mass of type I and II soil (wet basis) was added to 4 L freshly prepared nutrient solution (composition as above) respectively, and then the slurry was manually stirred for one minute. After passing through a sieve to remove coarse materials, the slurry was allowed to quiescently settle for five minutes to separate sand which visibly accumulated on the bottom of the flask. A 3 L volume of supernatant from each flask was decanted then mixed together. After adjusting its pH to 7.0, the resulting 6 L suspension was used to re-inoculate the biofilter. The inoculums was introduced into the biofilter by a peristaltic pump (Masterflex) at a flow rate of 170 mL/min. After filling the column, the suspension drained from the column at the same flow rate used during filling (170 mL/min). The drainage from the biofilter was evenly separated into two sparged-gas bioreactors (Appendix B and C), resulting in 2.6 L suspension in each bioreactor. 400 ml deionized (DI) water was added to each bioreactor to supply an initial 3 L liquid level.

In an attempt to improve biofilter performance, after 14 days cultivation of the two sparged-gas bioreactors (June 21, 2013), the 3 L volume of the inoculum in one of the two bioreactors was mixed with 3 L freshly prepared nutrient solution (pre-heated to a temperature of 50 °C) and pH of the resulted suspension was adjusted to 7.0. Then, the resulting suspension was re-introduced to the biofilter, filling the column and then draining from the column by a peristaltic pump (Masterflex) at a flow rate of 170 mL/min.

At weekly intervals, nutrients were added to the biofilter. The concentrations of constituents in the nutrient solution supplied to the biofilter on a weekly basis were changed to different levels over time (Table 3-1).

Table 3-1: Summary of Biofilter Operating Conditions

Period	Times of operation (days)	Nutrient concentration	Inoculum / Notes
1	0-63 (63 days)	1	Inoculated with suspension of residential compost (at t=0).
2	64-81 (18 days)	1	No β -caryophyllene loading (due to fume hood malfunction)
3A	82-125 (44 days)	$\frac{1}{6}$	Inoculated with suspension of composted forest products on day 81. Inoculated with enrichment culture (see Appendix B) on day 95.
3B	126-158 (33 days)	$\frac{1}{2}$	The HFID was temporarily unavailable on account of a broken gas sampling pump during days 130-157
3C	159-172 (14 days)	$\frac{1}{2}^*$	

Nutrient concentration is expressed relative to the concentration used to develop the biofilter inoculum. $1\times$ concentration refers to nutrient solution composition identical to that described in Section 3.2.2.1. $1/6\times$ and $1/2\times$ concentration refers to nutrient solution with all constituents added at one sixth times and one half times the concentration described in Section 3.2.2.1, respectively. $1/2^*$ concentration stands for nutrient solution with all constituents added at half of the concentration except the ammonium nitrate which was added at the same concentration as described in Section 3.2.2.1.

From day 82 to day 125 (Period 3A), a 6.0 L volume of nutrient solution with composition as described above (one sixth of the concentration described in Section 3.2.2.1) was filled and drained from the column at weekly intervals. In the following 33 days (Period 3B, from day 126 to day 158), on weekly basis, a 6.0 L volume of nutrient solution with all constituents added at one half the concentration described in Section 3.2.2.1. From day 159 to day 172 (Period 3C), the constituents of nutrient solution which was used to do weekly addition was changed to one half of

the original concentration except for ammonium nitrate which was added at the same concentration as described in Section 3.2.2.1. For all of the periods of operation described in this Chapter, the nutrient solutions were pre-heated to 50 °C and their pH was adjusted to 7.0 before use.

All of the periods of operation described in this Chapter were conducted in the same condition (β -caryophyllene injection rate of 10 μ L/hr and air supplied continuously at a flow rate of 2.24 L/min. The corresponding EBCT for the biofilter calculated accounting for water evaporation and temperature of 50 °C was 81 seconds).

Both influent and effluent pollutant concentration were measured in terms of carbon concentration using a model 600 HFID hydrocarbon analyzer. Measurements were collected at one minute intervals for a minimum of 1 hr/day except during the time periods of days 64-81 (when β -caryophyllene loading was temporarily discontinued on account of a fume hood malfunction) and days 130-157 (when the HFID was temporarily unavailable on account of a broken gas sampling pump).

3.2.3 Analytical Procedures

At daily intervals, to compensate for evaporative losses of water in the humidification column, deionized (DI) water was added to each water bath. Both influent and effluent pollutant concentrations were measured in terms of carbon concentration using a model 600 HFID hydrocarbon analyzer (California Analytical, Orange, CA). Measurements were collected at one minute intervals for a minimum of 1 hr/day. HFID calibration was calibrated on daily basis using certified calibration standard (Air Liquide, Houston, TX). The pH of the drainage was measured by briefly opening a valve located at the bottom of the column (1 min in every two hrs), and then measuring the pH of the drained liquid using a model 290A pH meter with temperature compensation (Orion Research, Boston, MA, USA).

Influent, effluent and monitoring port gas temperatures were measured using an electronic temperature probe (Oakton Temp Lab, China). Weekly relative humidity measurements were conducted using a traceable digital hygrometer/thermometer (Fisher Scientific, Pittsburgh, PA).

3.3 Results

The suspended solids concentrations of the suspension measured before and after immersing the packing material during the inoculation conducted immediately prior to startup

were 1970 mg/L and 1650 mg/L, respectively, indicating that roughly 1.2 g of the compost suspended solids was retained on the packing medium during the Period 1 inoculation procedure.

Data regarding the overall performance for all of the periods of operation described in this Chapter is presented in Figure 3-2. The average influent and effluent pollutant concentration is depicted in Figure 3-2(a). Each data point represents the average of concentrations measured at one minute intervals over a duration of at least one hour, and error bars represent standard deviation. Red arrows denoted days when the biofilter system was inoculated. Green arrows denoted the days when concentration of nutrients were changed. The purple arrow denotes both re-inoculating and nutrients concentration changing.

Influent, effluent, and intermediate gas monitoring port temperatures are shown in Figure 3-2(b). At packed bed depths of 0 cm (inlet), 30 cm, 60 cm, and 90 cm (outlet), the average temperatures were 50.4, 50, 51.24, and 51.56, respectively, all close to target temperature of 50 °C.

Data points depicted in Figure 3-2(c) are the pH of drainage. As shown, pH fluctuated between 4 and 6.3 for the first 64 days. A cyclic pattern was observed on a weekly basis, with pH initially decreasing on days immediately after nutrient solution and then an increase during the last four days prior to the next nutrient addition. After resumption of β -caryophyllene loading following the period when it was temporarily stopped (from day 65 to day 81), the pH had smaller fluctuations, within the range of 5.7 to 6.9, again with cyclic variation on a weekly basis.

During Period 1 (the first 63 days), the influent and effluent concentrations were 84.8 ± 30.1 and 83.0 ± 3.3 ppm C (mean $\pm$ standard deviation), respectively, indicating very little or no β -caryophyllene degradation during this Period. At day 64 (12:45 p.m. on May 20, 2013), β -caryophyllene loading was temporarily stopped on account of a fume hood malfunction. Thus, no effluent and influent concentration data were collected during Period 2.

Following resumption of pollutant loading on day 82, a lower concentration nutrient solution was employed (addition at one sixth of the initial concentration) and the biofilter was re-inoculated with a mixture of composted forest products (Period 3A). The influent concentration was 78.8 ± 30.6 ppm C, essentially the same as the previous influent concentration measurement, and the effluent concentration was 75.7 ± 6.87 ppm C which is quite similar to the average effluent concentration in Period 1. Although the average effluent concentration did not vary much for the first two weeks, after re-inoculating the biofilter with the enrichment culture

Figure 3-2: (a) The average influent and effluent pollutant concentration. Green arrows denoted the days when concentration of nutrients were changed. Red arrows showed the system was inoculated. Purple arrow represented both re-inoculating and nutrients concentration changing; (b) Gas temperatures measured at various packed bed depths within the biofilter; (c) The pH of drainage collected at the bottom of the biofilter column.

cultivated in the sparged-gas bioreactor on day 95, the effluent pollutant concentration exhibited a consistent pattern on a weekly basis. As shown in Figure 3-3, the mean effluent concentration decreased to 64.9 ± 1.98 ppm C, one day after the weekly nutrient addition, and then gradually increased to 75.49 ± 1.63 , near the average influent concentration, on the day just before the next nutrient filling.

Figure 3-3: The average effluent concentration as a function of time since nutrient addition from day 96 to day 124 in Period 3A

From day 125 to day 157 (Period 3B), all constituents in the nutrient solution supplied to the system on a weekly basis were added at one half of the concentration employed in Period 1 (three times the concentrations employed during Period 3A. Over the first five days of operation (i.e., days 125-130), the effluent concentration was consistently lower than the influent, showing a promising trend for biodegradation (Figure 3-2a). On day 130 (June 26, 2013), however, the HFID pump broke and no pollutant concentration data was collected from day 130 to day 157.

From day 158 (Period 3C), during the weekly nutrient additions, the system received one half nutrient concentration (relative to the nutrient solution employed during Period 1) except for ammonium nitrate which was added at a concentration identical to that described for Period 1. The average influent and effluent in this Period were 77.53 ± 27.20 and 57.50 ± 10.40 ppm C, respectively. Compared with Period 3A, the average influent concentration in these two periods was nearly the same; however, the average effluent concentration in this period was appreciably lower. Also, it can be seen the effluent concentration decreased immediately after nutrient was

added, and it reached the local minimum concentration 43.81 ± 2.04 ppm C on the third day (shown in Figure 3-2 and Figure 3-4).

Figure 3-4: Effluent concentration as a function of time since nutrient addition with data plotted as average of two weeks of operation during Period 3C.

The mean daily loading rates, removal efficiency and elimination capacities for Period 3C is depicted on Figure 3-5. The mean daily loading rates were varied around 1.40 ± 0.46 g C/m³/hr (corresponding to 1.59 ± 0.52 g β -caryophyllene/m³/hr), whereas the removal efficiency and elimination capacity fluctuant over weekly intervals. The overall removal efficiency was $26.72 \pm 8.47\%$, and the highest removal efficiency were $41.56 \pm 8.30\%$ and $42.02 \pm 9.78\%$ on the third day after nutrient addition. The pollutant elimination capacity was 0.37 ± 0.18 g C/m³/hr, corresponding to an elimination capacity of 0.42 ± 0.20 g β -caryophyllene/m³/hr.

Figure 3-5: (a) Loading rates and removal efficiency and (b) elimination capacities during Period 3C.

3.4 Discussion and Conclusions

In Period 3A, the mean effluent concentration decreased following the weekly fill-and-drain nutrient additions and then increased to near the average influent concentration just before the next nutrient addition. Three plausible reasons for the temporary increase in biofilter performance (i.e., decrease in effluent concentration) following the weekly nutrient addition procedures followed by a subsequent decrease in performance (i.e., increase in effluent concentration) are: (1). temporary alleviation of a bed moisture limitation brought about by wetting (and subsequent drying due to incomplete humidification) (Qi *et al.*, 2005); (2). temporary alleviation of a nutrient limitation followed by microbial consumption of available nutrients (Moe *et al.*, 2013); (3). washout of accumulated intermediate products harmful to microbes. The weekly relative humidity measurements for all of the monitoring ports was 100% during all periods of operation, indicating that drying of the packing medium was likely not the reason for diminished treatment performance. The increased availability of nutrients is regarded a likely cause. After increasing the nutrients concentration in Period 3C, the effluent concentration was appreciably lower than that in Period 3A, suggesting that the system was affected by a nutrient limitation. The accumulation and washout of inhibitory intermediate products; however, cannot be excluded based on the data collected.

According to Kong *et al.* (2001)'s result, an acclimation period of 42 days was required for a biotrickling filter treating high temperature (55°C) α -pinene contaminated air following

inoculation with with a mixed microbial culture that was treating bleach kraft mill effluent. Similarly, Montes et al. (2010) reported an acclimation period of around one month for a biotrickling filter operated at 55°C and EBCT of 60 seconds to degrade α -pinene following inoculation using pre-cultivated leachate. In Period 1 operation reported in this thesis chapter for treatment of β -caryophyllene, however, the effluent concentration was consistently close to the influent concentration throughout the initial 64 days of operation. Performance improved over time following reinoculation of microbial populations from multiple sources, but the increase in performance was gradual and never reached the levels reported in the only literature available for biofiltration of β -caryophyllene (Moe *et al.*, 2013).

In the mesophilic temperature regime reported by Moe et al. (2013), a startup period of only about three weeks was required to remove >90% of the β -caryophyllene at a loading rate comparable to that employed in the high-temperature system reported here. Differences in the performance of the system reported here may have resulted from a combination of factors including the differences in operating temperature and inoculum sources. Nevertheless, the fact that performance of the biofilter did eventually improve demonstrates that degradation of β -caryophyllene in a high temperature (e.g., 50°C) biofilter is possible, albeit with lower elimination capacity than reported previously for biofilters in the mesophilic operating range.

3.5 References

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CHAPTER 4: OPERATION AND PERFORMANCE OF A BIOFILTER INOCULATED WITH BIOMASS PRE-GROWN ON POLYURETHANE FOAM SUPPORT MEDIUM

4.1 Introduction

This chapter describes additional experiments carried out to test the performance of a laboratory-scale biofilter to remove β -caryophyllene from high temperature ($\sim 50^\circ\text{C}$) contaminated air using an alternative enrichment culture and inoculation procedure than was described in Chapter 3.

4.2 Materials and Methods

4.2.1 Experimental Apparatus

A schematic diagram of the laboratory-scale biofilter apparatus is shown in Figure 4-1. Compressed air passed through a packed column (7.2 cm ID) containing activated carbon (BPL 4 $\times$ 6 mesh GAC, Calgon Carbon, Pittsburgh, PA) to remove unwanted contaminants. A pressure regulator (series R35, Arrow Pneumatics, Broadview, IL) and electronic mass flow controller (Aalborg Inc., Orangeburg, NY) regulated the air flow. Water from a heated recirculating bath (Cole-Parmer, Vernon Hills, IL) was used to heat and humidify the influent air. The humidification column was constructed of clear PVC pipe (7.2 cm ID) and had a total height of 163 cm. The column consisted of two sections divided by a perforated stainless steel support plate that was held in position by a PVC coupling. The bottom section had a height of 50 cm and served as a water storage reservoir. The top section (total height of 113 cm) was packed to a depth of 95 cm with stainless steel 10 mm Interpack media (Jaeger Company, Houston, TX). Air entered the humidification column at a point between the lower water reservoir and the upper packed bed, flowing counter-current to the direction of water flow. Water entered at the top of the humidification column with a flow rate of 1.7 L/min from the water bath set to a temperature of 59°C .

The biofilter consisted of three water-jacketed sections, each constructed of schedule 80 clear PVC pipe (Harvel Plastics Inc., Easton, PA). The inner diameter of the inner pipe was 7.3 cm, and the inner diameter of the outer pipe was 20.0 cm. A recirculating water bath (Cole-Parmer, Vernon Hills, IL) was used to recirculate heated water (temperature set point 60°C) in the annular space between the inner and outer pipes at a flow rate of 2.1 L/min. Fiberglass

insulation (Thermwell Products Co., Sparks, NV) was affixed to the exterior surface of the biofilter to minimize heat loss. A perforated stainless steel support plate located at the bottom of each section supported the packing medium. Glass marbles were placed in the bottom of the inlet section to distribute air flow. Compression fittings (1/4" ID, JACO) located at the biofilter inlet and at the outlet of each of the three biofilter sections allowed gas sampling.

Figure 4-1: Schematic diagram of experimental apparatus for the biofilter system.

Gas transfer lines employed in the biofilter system (3/8 in) were wrapped with electrical heating tapes (Cole-Parmer) regulated with Variac controllers (ISE Inc., Cleveland, OH) to maintain the elevated temperature level.

Each of the three biofilter sections contained reticulated polyurethane foam cubes (Honeywell-PAI, Lakewood, CO) as packing medium. The medium, supplied by the vendor in the form of cubes approximately 5.0 cm per side, was cut into cubes approximately 1.25 cm per side prior to use. The physical properties of this packing medium were described previously (Li and Moe, 2005). β -caryophyllene (TCI America (Portland, OR, catalog No. C0796, >90% purity) was injected into the air flow by a syringe pump (KD Scientific model 1000, Boston, MA, USA) equipped with a 250 μ L glass gas tight syringe (Hamilton, Reno, NV, USA).

4.2.2 Biofilter Inoculation and Operation

The biofilter was inoculated by taking polyurethane foam cubes that were incubated in an aerobic sparged gas reactor containing an enrichment culture that was initially inoculated with

composted forest products to allow attached growth of β -caryophyllene degrading microorganisms (see Appendix C for details). The 900 mL (12.9 g dry basis) packing medium which was cultivated in the sparged-gas bioreactor was equally divided into three parts, and each part was mixed with 34.7 g virgin reticulated polyurethane foam cubes. The resulting packing mixture was then filled into each biofilter section to a packed bed depth of 30 cm. This resulted in a total packed bed depth of 90 cm, total packed bed volume of 3.74 L, and total mass of 117 g packing medium (dry basis).

After the biofilter was assembled and the system reached the target temperature of 50 °C (approximately 1 hour), the biofilter was filled with nutrient solution using a peristaltic pump (Masterflex) at a flowrate of 170 mL/min. After filling the column, the nutrient solution was drained from the column at the same flow rate used during filling (170 mL/min). The nutrient solution contained the following constituents: NH_4NO_3 7.5 g/L, KH_2PO_4 6.00 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.60 g/L, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.12 g/L, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 2.04 mg/L, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 1.44 mg/L, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 3.48 mg/L, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 6.06 mg/L, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 1.44 mg/L, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ 0.60 mg/L and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 8.16 mg/L. The pH of the nutrient solution was adjusted to pH 7.0 by addition of 1.0 M NaOH after heating to 50 °C prior to addition to the biofilter. This nutrient solution was the same composition as that employed for development of the enrichment culture (see Appendix C) but with all constituents added at six times the concentration employed for development of the enrichment culture (see Appendix C).

Immediately following the nutrient addition, air flow to the biofilter was started with a flow rate of 2.24 L/min, and β -caryophyllene injection began with a flow rate of 10 $\mu\text{L}/\text{hr}$. The air volumetric flow rate was measured and regulated prior to heating, humidification, and β -caryophyllene injection and is reported here in units of standard liters per minute of dry air, with standard conditions corresponding to $T=70^\circ\text{F}$ (21.1 °C), $P=14.69$ psi (absolute). The corresponding empty bed contact time (EBCT) for the biofilter immediately following startup was 100 seconds, with the EBCT calculated on the basis of dry air flowrate at standard temperature and pressure. The empty bed contact time (EBCT) for the biofilter accounting for water evaporation and temperature of 50 °C was 81 seconds (see Appendix A3). The start of β -caryophyllene contaminated air supply was designated as time zero. (Time zero = 3:30 p.m. on Sep. 13, 2013). Time was measured in days from the start of pollutant loading.

Deionized make-up water was added to the humidification column water reservoir and biofilter water bath on a daily basis to compensate for evaporation loss. At daily intervals, water accumulated in the bottom of the biofilter column was drained (1 min in every two hrs) by briefly opening a valve located at the bottom of the column. At weekly intervals, nutrients were added to the biofilter by temporarily halting gas flow, filling the column with freshly prepared nutrient solution (pre-heated to a temperature of 50 °C and pH adjusted to 7.0-7.1 after heating), and then draining from the column before restoring normal operation.

After 35 days of operation (Oct. 16, 2013), the influent gas flow rate remained the same as before (2.24 L/min, EBCT 89 seconds); however, the concentrations of all constituents in the nutrient solution supplied during the weekly fill-and-drain nutrient addition procedure were increased to twice that of the initial days of Period 1a. After the nutrient addition on day 50, the gas flow rate and β -caryophyllene injection rate were increased to twice their initial values. The start of the higher loading rate is hereafter referred to as Period 2 operation. Starting on day 56 (Nov, 5, 2013), the nutrient addition approach was changed. On a daily basis, 250 mL nutrient solution (pre-heated to a temperature of 50 °C and pH adjusted to 7.0-7.1 after heating) was sprayed at the top of the uppermost packed bed section of the biofilter column on a daily basis, and the interval of the 6 L fill-and-drain of nutrient solution was adjusted to be every two weeks (as opposed to one week during the prior periods). The biofilter operation conditions is listed in Table 4-1.

Table 4-1: Biofilter Operating Conditions

Period ID	Days	Gas flow rate (slpm) ^a	Empty bed contact time (seconds) ^b	Nutrient concentration ^c
1a	0-35	2.24	100	1×
1b	36-49	2.24	100	2×
2	50-84	4.48	50	2×

^a Gas flow rate prior to heating, humidification, and β -caryophyllene injection, reported here in units of standard liters per minute of dry air [with standard conditions corresponding to at T=21.1 °C, P= 14.69 psi (absolute)].

^b Calculated on the basis of dry air flow at standard temperature and pressure noted in footnote a.

^c Nutrient concentration is expressed relative to the concentration employed at startup. 1× refers to nutrient solution with all constituents added at the same concentrations as used at startup. 2× indicates nutrient solution with all constituents added at twice the concentration employed at startup.

4.2.3 Analytical Procedures

Both influent and effluent pollutant concentrations were measured in terms of carbon concentration using a model 600 HFID hydrocarbon analyzer (California Analytical, Orange, CA). Measurements were collected at one minute intervals for a minimum of 1 hr/day. The HFID was calibrated on daily basis using certified calibration standard (Air Liquide, Houston, TX). Pollutant removal profiles as a function of biofilter height were determined by measuring pollutant concentrations from intermediate sampling ports at regular (~weekly) time intervals.

Gas temperatures were measured using an electronic temperature probe (Oakton Temp Lab, China). Relative humidity was measured using a traceable digital hygrometer/thermometer (Fisher Scientific, Pittsburgh, PA). The pH was measured using a model 290A pH meter (Orion Research, Boston, MA, USA) with temperature compensation. Headloss was measured using a water manometer (Dwyer Instruments, Michigan, IN).

4.3 Results

4.3.1 Summary of Overall Biofilter Performance Following Inoculation

As summarized in Table 4-2, during Period 1a (days 1-35), the average influent and effluent pollutant concentration were 83.0 ± 35.6 ppm C and 37.3 ± 27.5 ppm C, respectively (mean $\pm$ standard deviation, parts per million by volume as carbon), corresponding to an average loading rate of 1.90 ± 0.80 g β -caryophyllene/m³/hr (grams β -caryophyllene per m³ packed bed volume per hour). After increasing the concentration of nutrients, in Period 1b (days 36-49), the average influent and effluent pollutant concentrations were 79.9 ± 27.4 ppm C and 10.11 ± 6.38 ppm C, respectively. (mean $\pm$ standard deviation, parts per million by volume as carbon), corresponding to an average loading rate of 1.83 ± 0.62 g β -caryophyllene/m³/hr (grams β -caryophyllene per m³ packed bed volume per hour).

During Period 2 (days 50-84), the average influent and effluent pollutant concentration were 68.8 ± 21.4 ppm C and 10.3 ± 9.6 ppm C, respectively (mean $\pm$ standard deviation, parts per million by volume as carbon), corresponding to an average loading rate of 3.15 ± 0.91 g β -caryophyllene/m³/hr (grams β -caryophyllene per m³ packed bed volume per hour).

The overall performance is shown in Figure 4-2. Data points depict the average influent and effluent concentration measured at one minute intervals for a minimum of 1 hr/day. Error bars represent one standard deviation.

Table 4-2: Summary of Biofilter Loading Conditions for Each Period

Period ID	Influent concentration (ppm C) (mean±standard deviation)	Loading rate (g C/m ³ /hr)	Loading rate (g BC/m ³ /hr)
1a	83.0±35.6	1.67±0.71	1.90±0.80
1b	79.9±27.4	1.61±0.54	1.83±0.62
2	68.8±21.4	2.77±0.80	3.15±0.91

4.3.2 Biofilter Performance during Period 1 Operation

Figure 4-3 depicts influent and effluent pollutant concentrations during Period 1 operation (days 1-49). The average influent concentration considering all of Period 1 operation (i.e., both Period 1a and 1b) was 82.1 ± 33.5 ppm C (mean±standard deviation), corresponding to 93.2 ± 38.0 ppm β -caryophyllene (mean±standard deviation). Although the average daily influent was relatively consistent, on a short term basis (i.e., within an hour long monitoring interval) there was considerable fluctuation, hence a large standard deviation. The variation in influent concentration can likely be attributed to discharge of β -caryophyllene droplets from the syringe needle into the glass injection port followed by droplet spreading and evaporation leading to short-term intervals of increasing and decreasing influent gas-phase concentrations (as opposed to consistent evaporation from the end of the injection needle without droplet formation).

During the first day following startup, the effluent concentration increased over time, consistent with initial removal due to adsorption of β -caryophyllene to the polyurethane packing medium as was observed in abiotic testing under identical loading conditions (see Appendix A). The breakthrough from the first two days of Period 1 is shown separately in Figure 4-4. Data points presented the mean concentration for one hour time increments and the error bars represent standard deviation. As shown, the effluent concentration started to increase after 5 hours continuous loading. Because the adsorption capacity of the polyurethane foam was relatively small (see Appendix A), the adsorption capacity was exhausted relatively quickly, and the influent and effluent concentrations were essentially identical within two days. The effluent reached 95% of the average influent concentration after 29 hours of operation and 98% of the average influent concentration after 35 hours of operation.

Figure 4-2: The average influent and effluent pollutant concentration. Red arrows indicated the weekly nutrient addition. Green arrow denoted the days when concentration of nutrients were changed in along with weekly nutrient addition. Purple arrow represented the starting of spraying 250 mL nutrient solution at the top of the biofilter column.

Figure 4-3: The average influent and effluent concentration during Period 1 (from $t=0$ to day 49). Red arrows indicated the weekly nutrient addition. Green arrow denoted the days when concentration of nutrients were changed in along with weekly nutrient addition.

Figure 4-4: The average biofilter effluent pollutant concentration during the first two days of Period 1 operation.

Beginning roughly six days after the start of operation, the effluent pollutant concentration began to consistently decrease, reaching a local minimum of 45.1 ± 1.8 ppm C on day 14. From days 15 to 35, at weekly intervals, the effluent pollutant concentration decreased following the weekly nutrient addition (indicated by red arrows), reached a local minimum

within 4 days, and then steadily increasing until the next nutrient addition day (Figure 4-5). After receiving the higher nutrient solution on day 36 (all constituents added at twice the concentration of Period 1a, twelve times the concentration employed for development of the enrichment culture (see Appendix C), as denoted by green arrow), the effluent concentration kept at a low level (around 8 ppm C) before nutrient addition. After day 37, the effluent pollutant concentration remained stable for the remainder of Period 1 operation.

Figure 4-5: Effluent concentration as a function of time since nutrient addition with data plotted as average of three weeks of operation from day 15 to day 35.

Figure 4-6 (a) depicts the average loading rates and pollutant removal efficiencies during Period 1. As shown in the figure, the mean loading rate during Period 1a and Period 1b were 1.67 ± 0.71 g C/m³/hr and 1.61 ± 0.54 g C/m³/hr, respectively. The removal efficiency decreased at the very first beginning because of the adsorption of packing medium, and then it followed an overall trend of increasing over the following 10 days. On day 18, the average removal efficiency was 81.9%. On a weekly basis, the removal efficiency increased following nutrient addition and reached the peak value of the week in the following 4 days (reaching local maxima of 91.7%, 93.2%, 93.0%, and 90.0% on days 21, 31, 38, and 45, respectively). Removal efficiency then decreased until the next nutrient addition.

Figure 4-6 (b) shows the biofilter's elimination capacity during Period 1. It can be seen that the elimination capacity depicted the pattern as same as removal efficiency. On day 18, the elimination capacity was 1.30 ± 0.74 g C/m³/hr, corresponding to an elimination capacity of 1.48 ± 0.83 g β -caryophyllene/m³/hr. The average elimination capacity reached 1.41 ± 0.58 g

C/m³/hr in Period 1b, corresponding to an elimination capacity of 1.44 ± 0.72 g β -caryophyllene/m³/hr.

Figure 4-6: (a) Loading rates and removal efficiency and (b) elimination capacity for Period 1 biofilter operation.

Pollutant concentration profiles measured along the biofilter height are shown in Figure 4-7. At the beginning of Period 1 (i.e., during the first three weeks of operation), slower pollutant removal was observed in the first column section followed by more rapid pollutant removal throughout the last two sections. Over time, the pollutant removal in the first biofilter section

increased, eventually surpassing that of the downstream sections. On days 38 and 45 (the sixth and seventh weeks, respectively), the first section removed 67.6% and 66.8%, respectively, of the contaminant loading from the polluted air. The “local” elimination capacity calculated based only the flow of pollutants entering and exiting the first biofilter section on these days were 3.32 ± 1.52 g C/m³/hr and 3.11 ± 1.08 g C/m³/hr (at corresponding “local” loading rates of 4.92 ± 1.55 g C/m³/hr and 4.66 ± 1.10 g C/m³/hr, respectively). In terms of β -caryophyllene (as opposed to carbon), the “local” elimination capacity calculated based only the flow of pollutants entering and exiting the first biofilter section on days 38 and 45 were 3.77 ± 1.73 g β -caryophyllene /m³/hr and 3.54 ± 1.36 g β -caryophyllene /m³/hr, respectively (at corresponding “local” loading rates of 5.58 ± 1.75 g β -caryophyllene /m³/hr and 5.30 ± 1.39 g β -caryophyllene /m³/hr, respectively).

Figure 4-7: VOC concentration profiles measured on day 3, 10, 17, 24, 31, 38 and 45 during Period 1 biofilter operation.

4.3.3 Biofilter Performance during Period 2 Operation

After 49 days of operation, the dry gas flow rate was increased from 2.24 to 4.48 L/min, decreasing the EBCT accounting for water evaporation and temperature of 50 °C from 81 to 40.5 seconds (see Appendix A3). The average influent concentration decreased from 82.1 ± 33.5 ppm C in Period 1 to 68.8 ± 21.4 ppm C in Period 2. The average effluent concentration was 10.3 ± 9.6 ppm C in the 35 days of Period 2 operation. As shown in Figure 4-8, in Period 2, the effluent concentration decreased to 9.04 ± 0.52 ppm C immediately after nutrient addition (indicated by

red arrows). Then, the effluent concentration increased for the remainder of the week, possibly because of a nutrient limitation. Starting on day 56 (as denoted by green arrow), a 250 mL volume of nutrient solution was sprayed at the top of the uppermost packed bed section of the biofilter column on a daily basis. The effluent concentration gradually decreased from 36.4 ± 5.5 ppm C to 8.00 ± 0.71 ppm C over the following two weeks. After the biweekly fill-and-drain nutrient addition procedure on day 70, the effluent concentration decreased to 7.04 ± 0.74 ppm C within two days.

The pollutant loading rates and the pollutant removal efficiency for Period 2 operation are shown in Figure 4-9. The mean loading rate increased from 1.65 ± 0.66 C/m³/hr in Period 1 to 2.77 ± 0.80 C/m³/hr in Period 2. As shown in the figure, the pollutant removal efficiency varied a lot (46%-90%) in Period 2. The mean removal efficiency considering all of Period 2 was 77.0%.

Elimination capacity followed the same pattern as removal efficiency. The mean elimination capacity in this Period was 2.12 ± 0.72 g C/m³/hr, corresponding to an elimination capacity of 2.40 ± 0.82 g β -caryophyllene/m³/hr.

Figure 4-8: The average influent and effluent concentration during Period 2 (day 50 to day 84). Red arrows indicated the nutrient fill and drain. Purple arrow represented the starting of spraying 250 mL nutrient solution at the top of the biofilter column.

Figure 4-9: (a) Loading rates and removal efficiency and (b) elimination capacities for Period 2.

Figure 4-10 depicts the pollutant concentration profiles measured along the height of the biofilter at different times in Period 2. On day 52, two days after the most recent 6 L nutrient solution fill-and-drain (on day 50), β -caryophyllene was removed quicker in 30 to 90 packed bed height than in 0 to 30 packed bed height. On day 59, two days after the most recent 6 L nutrient solution fill-and-drain (on day 57) but with daily spraying of 250 mL nutrient solution, the pollutant removal in the 30 to 90 cm packed bed heights was somewhat lower, with roughly linear removal throughout the bed height. With the daily spraying for the following two weeks, the pollutant removal profile was roughly linear with pollutant removal throughout the entire column height on day 70. On day 71, the biofilter system received another fill-and-drain nutrient

addition, then the pollutant profile shifted, with somewhat more rapid pollutant removal in the first section followed by slower pollutant elimination up to the outlet height. The maximum “local” elimination capacity (calculated based only the flow of pollutants entering and exiting a single 30 cm biofilter section) observed during Period 2 operation was 4.35 ± 1.40 g C/m³/hr (at corresponding “local” loading rate of 7.96 ± 2.21 g C/m³/hr) on day 77 in the first section from the inlet. The corresponding elimination capacity in terms of β -caryophyllene (rather than carbon) on day 77 was 4.94 ± 1.59 g β -caryophyllene/m³/hr (at a corresponding “local” loading rate of 9.04 ± 2.51 g β -caryophyllene/m³/hr).

Figure 4-10: VOC concentration profiles measured during on day 52, 59, 66, 70, 73, 77 and 81 during Period 2 biofilter operation.

4.3.4 pH and Temperature

The pH measured in leachate collected at the bottom of the biofilter column is shown in Figure 4-11. In Period 1, the pH ranged from 6.84 to 5.77. The pH value dropped throughout the entire first week. However, from the second week, at weekly intervals, pH value increased following the nutrient addition, and then it decreased (albeit with some minor fluctuations) until the next nutrient addition).

The pH varied between 6.02 and 6.74 in Period 2. As shown in Figure 4-11, the pH value dropped after 3 days of operation in Period 2, indicating the pH varied along with nutrient limitation. From day 56 onward, when nutrients were added on a daily basis, pH was roughly constant at pH 6.6.

Figure 4-11: The pH of drainage for Period 1 (day 1 to day 49) and Period 2 (day 50 to day 84)

Influent, effluent, and intermediate gas monitoring port temperatures were measured on a weekly basis. Gas temperatures measured at various packed bed depths within the biofilter during Period 1 and Period 2 are shown in Figure 4-12. It can be seen that temperature in all of the biofilter sections was close to target temperature level 50 °C. At packed bed depths of 0 cm (inlet), 30 cm, 60 cm, and 90 cm (outlet), the average temperatures were 48.0, 49.9, 51.0, and 51.9 °C, respectively.

Figure 4-12: Gas temperatures measured at various packed bed depths within the biofilter during all of the periods described in this chapter.

4.4 Discussion

Previous research demonstrated that gas-phase β -caryophyllene can be successfully treated using biofilters operated in the mesophilic range (Moe *et al.*, 2013). The results presented in this thesis chapter further demonstrate that β -caryophyllene can be successfully removed from biofilters operated at higher temperature levels (e.g., 50 °C). This expands the temperature range over which biofilters are known biodegrade sesquiterpenes.

Compared with previous work on biofiltration of β -caryophyllene in a system operated in the mesophilic temperature range (Moe *et al.*, 2013), the biofilter performance reported in this chapter operated at a temperature of approximately 50 °C appeared to be somewhat less stable and more sensitive nutrient supply. A possible reason for that is that at high temperature, the pKa value for ammonia is lower (Garcia and Angenent, 2009; Zhang *et al.*, 1994) and the Henry's Law constant is higher (Muck and Steenhuis, 1982; Rong *et al.*, 2011), reflecting greater potential for abiotic stripping of ammonia from the nutrient solution present in the packed bed to the gas phase at high temperatures.

Greater transfer of ammonia from the aqueous phase to the gas phase in the higher temperature system (50 °C) than that in a mesophilic condition (30 °C) may have resulted in the lack of a readily bioavailable supply of nutrients. Further research, however, is required to fully elucidate the role that nutrient limitation may (or may not) have played in the observed performance. It is also possible that the microbes able to biodegrade β -caryophyllene at high temperature may have differing nutritional requirements and growth kinetics than those able to biodegrade β -caryophyllene at lower temperature. Regardless, after increasing the concentration of the nutrient solution on day 36 (all constituents added at two times the concentration employed for the initial 35 days), the removal efficiency increased and remained at a relatively high level for the remainder of Period 1 operation. This suggests that biofilter performance may have been adversely affected by nutrient limitation during the first 35 days of operation, and it demonstrates that relatively high β -caryophyllene removal efficiency can be achieved in high temperature biofilters. Nutrient depletion over time may occur because of microbial consumption as well as ammonia transferring to the air phase.

In the experiments described in this Chapter, the inoculation procedure employed polyurethane foam packing medium which was pre-cultivated at 50 °C in a sparged-gas bioreactor. In contrast to the biofilter inoculated with compost without pre-cultivated treatment

(Chapter 3), the removal efficiency and elimination capacities were obviously higher. These results demonstrate that the startup of high temperature β -caryophyllene degrading biofilters can be successfully achieved over time frames comparable to biofilters operated at lower temperatures. The inoculation strategy reported here, pre-cultivation of the polyurethane cube packing medium in a bioreactor used to develop an enrichment culture, may prove useful in future experimental studies and/or full-scale systems.

Similar results were obtained previously by Prado *et al.* (2005). In Prado's experiment (treating contaminants comprised of α -pinene), in order to check the effect of the inoculum on the performance of the biofilter, four inoculums with different biomass concentrations and biomass adaptations were used to inoculate biofilters. All of these biofilters were packed with lava rock and operated with an EBCT of 48 seconds at a temperature of 25 °C. The results suggested that different inoculum dramatically affected the start-up time and the performance of the biofilter. Adapted inoculum was a useful way to shorten the start-up time (Prado *et al.*, 2005).

Except the inoculum, acclimation period is affected by temperature. Kong (2001) investigated the removal of α -pinene in three parallel biofilters operated at three different temperatures of 40 °C, 55 °C and 70 °C. All three biofilters were packed with inert 180 mm NORPAC polypropylene packing and were inoculated with a mixture of a microbial culture that was treating bleach kraft mill effluent (BKME) in a sequencing batch reactor (SBR) at 55 °C and degrading α -pinene in a bench-scale biofilter packed with wood-chip media at 40 °C. Their results showed that α -pinene removal was not achieved in a biofilter operated at 70 °C. For biofilters operated at 40 °C and 55 °C, acclimation time was about 10 days and 42 days, respectively. Montes (2010) also evaluated the performance α -pinene degradation in both mesophilic and thermophilic biotrickling filters inoculated with leachate, containing predominantly *Ophiostoma stenoceras* (a fungus). Different start-up periods were observed in both biotrickling filters in spite of using enriched inocula from the same source. For biotrickling filters operated at 30 °C, the acclimation period was 10 days. For biotrickling filters operated at 45 °C, the acclimation period was around 1 month.

Luvsanjamba further compared the acclimation period for the biotrickling filter operated at 22 °C and the biotrickling filter operated at 52 °C. He used high density polyethylene KMB carrier rings as packing medium to degrade dimethyl sulfide in biotrickling filter operated at 22 °C and 52 °C. Highly enriched sludge from a membrane bioreactor treating landfill leachate

was used as inoculum for these two filters. Although similar loads were used in both filters, the acclimation period was much higher in the case of the thermophilic biotrickling filters, around 25 days to reach removal efficiency exceeding 80% at EBCT of 100 seconds, and shorter in the case of mesophilic system, around 17 days to reach removal efficiency exceeding 98% at the same EBCT (Luvsanjamba *et al.*, 2008). He also employed the same inoculum to remove mixture of isobutyraldehyde and 2-pentanone from high temperature inlet air (52 °C) and from ambient temperature inlet air (22 °C). The result showed that a start-up period of 16 days and 26 days was required in 22 °C and 52 °C biotrickling filter, respectively (Luvsanjamba *et al.*, 2007). All of these results indicated the time for acclimation increased with increasing temperature, with shorter start-up periods at room temperature than under thermophilic conditions.

4.5 References

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CHAPTER 5: OVERALL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

5.1 Overall Conclusions

The laboratory-scale studies reported in this thesis demonstrated that β -caryophyllene can be successfully removed from contaminated air through the use of a biofilter operated at high temperature ($\sim 50\text{ }^{\circ}\text{C}$). This expands our knowledge that the biodegradation of sesquiterpenes at high temperature is possible.

In research described in chapter 3, after inoculating a biofilter with residential compost, the effluent concentration was quite close to the influent concentration even after 64 days of operation. This suggests that β -caryophyllene degrading microbes able to grow at elevated temperature either are not ubiquitous or that they grow slowly. Following inoculation with composted forest products and an enrichment culture derived from composted forest products as well as increases in nutrient concentrations supplied to the system, the biofilter eventually removed a portion of the influent β -caryophyllene after long-term operation. After long-term operation, the biofilter exhibited a consistent pattern on a weekly basis, with effluent pollutant concentration initially decreasing after weekly nutrient additions followed by a roughly linear increase until the next nutrient addition. Such a pattern indicates that the system may have been affected by a nutrient limitation. Similar results were reported by (Moe *et al.*, 2013), who reported that the performance of a β -caryophyllene degrading biofilter operating within a mesophilic temperature regime improved by increasing the concentration of nutrients.

In the periods described in Chapter 4, both pre-cultivated polyurethane cubes and higher concentration of nutrient solution was employed, β -caryophyllene biodegradation was observed much more quickly (within six days following startup). In addition, the removal efficiency and elimination capacities were obviously higher than was observed for the biofilter inoculated with residential compost without pre-cultivation (i.e., the system reported in chapter 3). This demonstrates that the use of a sparged-gas bioreactor to cultivate an enrichment culture combined with pre-inoculation of polyurethane cubes can greatly shorten the startup of β -caryophyllene degrading biofilters operated at elevated temperature (around $50\text{ }^{\circ}\text{C}$). This result is consistent with Prado *et al.*'s conclusion that different inocula can dramatically affected the start-up time and the performance of biofilters (Prado *et al.*, 2005). Although the increase in performance was never reached the levels reported in the only literature available for

biofiltration of β -caryophyllene (Moe *et al.*, 2013), removal efficiencies in excess of 90 % were observed at empty bed contact times (EBCTs) 100s and pollutant loading rate 1.61 g C/(m³ h) (grams pollutant measured as carbon per cubic meter packed bed volume per hour) and at empty bed contact times (EBCTs) 50s and pollutant loading rate 2.77 g C/(m³ h), respectively.

5.2 Recommendations for Future Research

The research reported in this thesis examined the removal of β -caryophyllene under continuous loading, however, in many real world applications, contaminant concentrations may vary with time, and pollutant loading can be interrupted over various time intervals ranging from minutes to days or even weeks (e.g., during weekends or holiday shutdowns). Thus, more work investigating the performance of biofilters treating terpenes under discontinuous loading conditions should be done.

In addition, β -caryophyllene is not the only pollutant present in hot waste gases emitted from industry. A multitude of other pollutants are released during the industrial processing of tobacco, food, pharmaceutical and rubber (i.e., curing process) and from rendering process, chemical manufacturing, waste treatment (i.e., composting) and combustion of gasoline (Cho *et al.*, 2007; Luvsanjamba *et al.*, 2007). At this point in time, however, only a relatively limited number of pollutants have been studied for treatment in biofilters operated at high temperature. These include ethanol vapors (Cox *et al.*, 2001), α -pinene (Dhamwichukorn *et al.*, 2001), BTEX (benzene, toluene, ethylbenzene, and xylene) (Cho *et al.*, 2007; Mohammad *et al.*, 2007), sulfur-containing malodorous gases (Datta *et al.*, 2007; Ryu *et al.*, 2009), methyl tert-butyl ether (MTBE) (Moussavi *et al.*, 2009), isobutyraldehyde, 2-Pentanone (Luvsanjamba *et al.*, 2007), that have been successfully treated by biofilters operated at high temperature. Further experiments for treating other kinds of harmful hot gas waste by high temperature biofiltration is needed. Also, the contaminant constituents present in industrial off-gases are almost always a mixture. Treatment of complex mixtures emitted by industries is of great practical concern for implementation of full-scale biofilter operations.

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APPENDIX A: ABIOTIC ADSORPTION CAPACITY TEST

As the initial step in preparing to test the ability of a biofilter to treat gas-phase β -caryophyllene at the elevated temperature level (around 50 °C), experiments were conducted to measure the abiotic sorption capability of the packing medium employed in subsequent biotic experiments.

A.1 Abiotic Adsorption Test

After the biofilter column was initially assembled, 39 g (dry basis) previously cut polyurethane foam packing medium was evenly filled to a depth of 30 cm in each of the three biofilter sections, resulting in a total packed bed depth of 90 cm, total packed bed volume of 3.74L, and total mass of 117 g packing medium in the biofilter. 6L distilled water was pre-heated to 50 °C to maintain a high temperature condition. Then the heated water was introduced into the bottom of the biofilter via a peristaltic pump (Masterflex) at a flowrate of 170 mL/min. After filling to saturate the packing medium, water drained from the column at the same flow rate used in filling.

The experiment was conducted with β -caryophyllene injection rate of 10 μ L/hr and air supplied rate of 2.24 L/min. The biofilter was subjected to a continuous loading condition. Pollutant concentration exiting the system was measured as a function of time using a total hydrocarbon analyzer as described in section 4.2.3.

A.2 Results

Figure A-1 provided the experimental data collected from the effluent of biofilter for polyurethane adsorption tests. It can be seen that 95% pollutant breakthrough occurred after 16.58 hrs. Complete breakthrough was achieved after 18.92 hrs of operation (effluent concentrations varied <5%).

During the absorption process, the gas phase concentration of β -caryophyllene progressively decreased and was transferred to the water phase and finally to polyurethane. After 6 hours, polyurethane lost the ability to absorb all the amount of pollutant and then a portion of β -caryophyllene could be measured from the effluent port of the biofilter.

Mass balance calculations indicate that the pollutant mass entering and exiting the biofilter column differed by 0.05564 g C (The adsorption calculation is attached in Appendix A.

Assuming that all of the pollutants measured as Carbon were only comprised of β -caryophyllene (0.88 g C / per g β -caryophyllene based on the formula $C_{15}H_{24}$), the pollutant mass accumulating in the biofilter column was calculated to be 0.06317 g β -caryophyllene. The corresponding mass of β -caryophyllene adsorbed per unit mass of polyurethane foam was calculated to be 0.540 mg/g.

β -caryophyllene was also removed from biofiltration system operated at lower temperature level of 30 °C. In contrast, 95% pollutant breakthrough occurred after five days of continuous loading and complete breakthrough was achieved after 5.833 days of operation (effluent concentrations varied <5%) (Moe *et al.*, 2013). Apparently, it takes shorter time for β -caryophyllene to achieve complete breakthrough in this high temperature biofiltration system. Also, in the previous biofiltration system, the pollutant mass entering and exiting the biofilter column differed by 0.939 g C and the pollutant mass accumulating in the biofilter column was calculated to be 1.06 g β -caryophyllene (Moe *et al.*, 2013). It can be seen these values are obvious larger than the biofilter operated at high temperature.

Figure A-1: Experimental measurements of the effluent VOC concentration during the abiotic adsorption test with the polyurethane foam packing medium.

After the first 18.9 hrs, the effluent pollutant concentration was around 82 ppm C, corresponding to an average loading rate of 1.88 g β -caryophyllene/m³/hr (grams β -caryophyllene per m³ packed bed volume per hour).

A.3 Related Calculation

$$\text{Biofilter Packed Bed Volume } V = \frac{\pi d^2 h}{4}$$

Where:

V = volume (cm³)

d = diameter=7.275 cm (based on manufacturer's specification of average ID =2.864 in, see <http://www.clearpvcpipe.com/pdf/pipeclear.pdf>)

h=height = 90 cm total (measured)

$$V = \frac{\pi d^2 h}{4} = \frac{\pi (7.257 \text{ cm})^2 (90 \text{ cm})}{4} = 3741 \text{ cm}^3 = 3.74 \text{ L}$$

Calculated the corresponding empty bed contact time (EBCT) for the biofilter immediately following startup

$$EBRT = \frac{V}{Q} = \frac{3.74 \text{ L}}{2.24 \text{ L/min}} = 1.67 \text{ min} = 100 \text{ s}$$

A.3.1 Calculated Actual Gas Flow Rate during Period 1

Gas flow rate measured prior to humidification, heating and β -caryophyllene addition during Period 1 was 2.24 SLPM (standard liters per minute) with standard conditions corresponding to T=75 °F (=21.1 °C =297.0K), P=14.69 psi (absolute) (=0.9996 atm) (Aalborg Instruments).

The gas flow rate in units of moles per minute calculated using the Ideal Gas Law would be:

$$n = \frac{PV}{RT} = \frac{(0.9996 \text{ atm})(2.24 \text{ L})}{\left(0.082057 \frac{\text{atm} \cdot \text{L}}{\text{mol} \cdot \text{K}}\right)(297 \text{ K})} = 9.188 \times 10^{-2} \frac{\text{mol}}{\text{min}}$$

The volumetric flow rate of β -caryophyllene injected into the gas flow was 10 μL / hour. The corresponding molar flow rate would be:

$$\frac{\text{moles}}{\text{min}} = 10 \frac{\mu\text{L}}{\text{hr}} \times \frac{1 \text{ mL}}{1000 \mu\text{L}} \times \frac{0.902 \text{ g}}{1 \text{ mL}} \times \frac{1 \text{ mol}}{204.36 \text{ g}} = 4.4 \times 10^{-5} \frac{\text{mol}}{\text{hr}} = 7.36 \times 10^{-7} \frac{\text{mol}}{\text{min}}$$

The saturation mixing ratio (mass of water per mass dry air) at T=50 °C and P=1.0 atm is 85.84 g/kg (http://www.srh.noaa.gov/epz/?n=wxcalc_mixing_ratio)

Assuming influent dry air and air molar mass of 28.964 g/mol (de Nevers, 2000), the

molar flow rate of water be:

$$9.188 \times 10^{-2} \frac{\text{mol}_{\text{air}}}{\text{min}} \times \frac{28.964 \text{g}_{\text{air}}}{1 \text{mol}_{\text{air}}} \times \frac{1 \text{kg}_{\text{air}}}{1000 \text{g}_{\text{air}}} \times \frac{85.84 \text{g}_{\text{H}_2\text{O}}}{1 \text{kg}_{\text{air}}} \times \frac{1 \text{mol}_{\text{H}_2\text{O}}}{18.015 \text{g}_{\text{H}_2\text{O}}} = 1.268 \times 10^{-2} \frac{\text{mol}}{\text{min}}$$

The total molar flow rate would be:

$$\text{Total} = (9.188 \times 10^{-2}) \frac{\text{mol}}{\text{min}} \text{air} + (1.268 \times 10^{-2}) \frac{\text{mol}}{\text{min}} \text{H}_2\text{O} + (7.36 \times 10^{-7}) \frac{\text{mol}}{\text{min}} \beta\text{-caryophyllene} = 0.1046 \frac{\text{mol}}{\text{min}}$$

Converting to a volumetric flow rate at T=50 °C and P=1 atm, we get:

$$Q = \frac{nRT}{P} = \frac{\left(0.1046 \frac{\text{mol}}{\text{min}}\right) \left(0.082057 \frac{\text{atm} \cdot \text{L}}{\text{mol} \cdot \text{K}}\right) (323 \text{K})}{(1.0 \text{atm})} = 2.77 \frac{\text{L}}{\text{min}}$$

Calculating the EBRT based on the humid gas flow during Period 1:

$$\text{EBRT} = \frac{V}{Q} = \frac{3.74 \text{L}}{\left(2.77 \frac{\text{L}}{\text{min}}\right)} = 1.35 \text{min} = 81 \text{sec}$$

A.3.2 Calculated Actual Gas Flow Rate during Period 2

The gas flow rate in units of moles per minute calculated using the Ideal Gas Law would be:

$$n = \frac{PV}{RT} = \frac{(0.9996 \text{atm})(4.48 \text{L})}{\left(0.082057 \frac{\text{atm} \cdot \text{L}}{\text{mol} \cdot \text{K}}\right) (297 \text{K})} = 1.838 \times 10^{-1} \frac{\text{mol}}{\text{min}}$$

The volumetric flow rate of β -caryophyllene injected into the gas flow was 20 μL / hour.

The corresponding molar flow rate would be:

$$\frac{\text{moles}}{\text{min}} = 20 \frac{\mu\text{L}}{\text{hr}} \times \frac{1 \text{mL}}{1000 \mu\text{L}} \times \frac{0.902 \text{g}}{1 \text{mL}} \times \frac{1 \text{mol}}{204.36 \text{g}} = 4.4 \times 10^{-5} \frac{\text{mol}}{\text{hr}} = 1.47 \times 10^{-6} \frac{\text{mol}}{\text{min}}$$

The saturation mixing ratio (mass of water per mass dry air) at T=50 °C and P=1.0 atm is 85.84 g/kg.

Assuming influent dry air and air molar mass of 28.964 g/mol (de Nevers, 2000), the molar flow rate of water be:

$$1.838 \times 10^{-2} \frac{\text{mol}_{\text{air}}}{\text{min}} \times \frac{28.964 \text{g}_{\text{air}}}{1 \text{mol}_{\text{air}}} \times \frac{1 \text{kg}_{\text{air}}}{1000 \text{g}_{\text{air}}} \times \frac{85.84 \text{g}_{\text{H}_2\text{O}}}{1 \text{kg}_{\text{air}}} \times \frac{1 \text{mol}_{\text{H}_2\text{O}}}{18.015 \text{g}_{\text{H}_2\text{O}}} = 2.536 \times 10^{-2} \frac{\text{mol}}{\text{min}}$$

The total molar flow rate would be:

$$\text{Total} = (1.838 \times 10^{-1}) \frac{\text{mol}}{\text{min}} \text{air} + (2.536 \times 10^{-2}) \frac{\text{mol}}{\text{min}} \text{H}_2\text{O} + (1.47 \times 10^{-6}) \frac{\text{mol}}{\text{min}} \beta\text{-caryophyllene} = 0.2092 \frac{\text{mol}}{\text{min}}$$

Converting to a volumetric flow rate at T=50 °C and P=1 atm, we get:

$$Q = \frac{nRT}{P} = \frac{\left(0.2092 \frac{\text{mol}}{\text{min}}\right) \left(0.082057 \frac{\text{atm} \cdot \text{L}}{\text{mol} \cdot \text{K}}\right) (323\text{K})}{(1.0\text{atm})} = 5.54 \frac{\text{L}}{\text{min}}$$

Calculating the EBRT based on the humid gas flow during Period 1:

$$EBRT = \frac{V}{Q} = \frac{3.74\text{L}}{\left(5.54 \frac{\text{L}}{\text{min}}\right)} = 0.675 \text{ min} = 40.5 \text{ sec}$$

Averaged Influent concentration in Period 1: C=82.10 ppm C:

$$\begin{aligned} \frac{\mu\text{g}}{\text{m}^3} &= \text{ppmc} \times MW \times \frac{1000P}{RT} \\ &= 82.10 \times 12 \times \frac{1000 \times 1}{0.08206 \times 323} = 37170 \frac{\mu\text{g}}{\text{m}^3} = 37.17 \frac{\text{mgC}}{\text{m}^3} \end{aligned}$$

Averaged mass loading rate of carbon:

$$\begin{aligned} 37.17 \frac{\text{mgC}}{\text{m}^3 \text{ gas}} \times 2.77 \frac{\text{Lgas}}{\text{min}} \times 60 \frac{\text{min}}{\text{hr}} \times \frac{1\text{m}^3}{1000\text{L}} &= 6.178 \frac{\text{mgC}}{\text{hr}} = 6.178 \times 10^{-3} \frac{\text{gC}}{\text{hr}} \\ \frac{6.178 \times 10^{-3} \frac{\text{gC}}{\text{hr}}}{3.74\text{L} \times \frac{1\text{m}^3}{1000\text{L}}} &= 1.65 \frac{\text{gC}}{\text{m}^3 \text{ hr}} \end{aligned}$$

Average mass loading rate of β -caryophyllene

$$1.65 \frac{\text{gC}}{\text{m}^3 \text{ hr}} \times \frac{204.36\text{gBC}}{180\text{gC}} = 1.88 \frac{\text{gBC}}{\text{m}^3 \text{ hr}}$$

Effluent averaged concentration: C=43.03 ppm C:

$$\begin{aligned} \frac{\mu\text{g}}{\text{m}^3} &= \text{ppmc} \times MW \times \frac{1000P}{RT} \\ &= 43.03 \times 12 \times \frac{1000 \times 1}{0.08206 \times 323} = 19481 \frac{\mu\text{g}}{\text{m}^3} = 19.48 \frac{\text{mgC}}{\text{m}^3} \end{aligned}$$

Averaged mass of carbon exiting the biofilter system:

$$19.48 \frac{\text{mgC}}{\text{m}^3 \text{ gas}} \times 2.77 \frac{\text{Lgas}}{\text{min}} \times 60 \frac{\text{min}}{\text{hr}} \times \frac{1\text{m}^3}{1000\text{L}} = 3.238 \frac{\text{mgC}}{\text{hr}} = 3.238 \times 10^{-3} \frac{\text{gC}}{\text{hr}}$$

It took 18.92 hours for clear polyurethane to reach the breakthrough concentration-82.45

ppm C

$$6.178 \times 10^{-3} \frac{gC}{hr} \times 18.92 hr = 0.1169 g$$

$$3.238 \times 10^{-3} \frac{gC}{hr} \times 18.92 hr = 0.06126 g$$

The pollutant mass entering and exiting the biofilter column differed by

$$0.1169 g - 0.06126 g = 0.05564 gC$$

The pollutant mass accumulating in the biofilter column

$$0.05564 gC \times \frac{204.36 gBC}{180 gC} = 0.06317 gBC$$

The weight of polyurethane

$$3 \times 39 = 117 g$$

The corresponding mass of β -caryophyllene adsorbed per unit mass of polyurethane foam

$$\frac{63.17 mg}{117 g} = 0.540 \frac{mg}{g}$$

A.4 References

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APPENDIX B: ENRICHMENT CULTURE 1 (USED TO RE-INOCULATE BIOFILTER DESCRIBED IN CHAPTER 3)

B.1 Introduction

This appendix describes the procedure used to cultivate an enrichment culture that was used to re-inoculate the biofilter described in Chapter 3 of this thesis. The experimental apparatus, operation, and results from the enrichment culture experiments are presented.

B.2 Material and Methods

B.2.1 Chemicals

β -caryophyllene purchased from TCI America (Portland, OR, CAS No.87-44-5) was used as target pollutant.

B.2.2 Apparatus Setup

As shown in Figure B-1, a 4.0 L glass kettle reactor (Pyrex, Acton, MA) was employed to develop the enrichment culture. Electrical heating tapes (Cole-Parmer) were affixed to the exterior surface of the reactor to control temperature at a target level of 50 °C. Compressed air flowed through a pressure regulator (series R35, Arrow Pneumatics, Broadview, IL) and a rotameter (Gilmont Instruments, Swedesboro, NJ) at a flow rate of 1.0 L/min. Then air passed through a glass tube with airtight compression seals, and a port where a Thermogreen LB-1 half-hole septum (Supelco, Bellefonte, PA) was inserted to allow a glass gas-tight syringe (Hamilton, Reno, NV, USA) to deliver β -caryophyllene. The injection rate of delivering β -caryophyllene was set on a syringe pump (KD Scientific model 1000, Boston, MA, USA). The β -caryophyllene containing air passed through an aeration stone submerged in the sparged gas bioreactor.

Figure B-1: Schematic diagram of the gas-sparged bioreactor used for cultivating β -caryophyllene degrading enrichment culture (Hu, 2012).

B.2.3 Bioreactor Operation

Two different kinds of soil (Clegg's Nursery Inc., Baton Rouge, LA) were used to inoculate the sparged gas reactor used to grow the inoculum eventually used in the biofilter experiment (described in Chapter 3). Type I soil contained 100% forest product compost and type II soil was comprised of 60% forest compost mixed with 40% sand and perlite after the decomposing process. Both of these soils were without pre-heating and disinfection treatment.

The 8 L of nutrient solution with the concentration of the following constituents was used to develop the biofilter inoculum: NH_4NO_3 1.25 g/L, KH_2PO_4 1.00 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.10 g/L, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.02 g/L, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.34 mg/L, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.24 mg/L, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.58 mg/L, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 1.01 mg/L, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.24 mg/L, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ 0.10 mg/L and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 1.36 mg/L.

A 500 g mass each of type I and II soil (wet basis) were added to 4 L nutrient solution (composition as above) respectively, and then the slurry was manually stirred for one minute. After passing through a sieve to remove coarse materials, the slurry was allowed to quiescently settle for five minutes to separate sand which visibly accumulated on the bottom of the flask. A 3 L volume of the supernatant from each flask was then mixed together. After adjusting pH to 7.0 with NaOH, the resulting total of 6 L suspension was inoculated to biofilter by a peristaltic pump (Masterflex) at a flow rate of 170 mL/min. After filling the column, the suspension drained from the column at the same flow rate. The drainage from biofilter was collected, and 2.6 L

suspension plus 400 mL deionized (DI) water was added to the bioreactor to supply 3 L liquid level. Gas flow was turned on to deliver clean air at the rate of 1.0 L/min. 0.33 mL β -caryophyllene was injected to bioreactor using syringe pump by the rate of 0.06 mL/hr every single day. After 5.5 hours injection, the syringe pump stayed on, but nothing was injected during the rest of day. The amount of injected β -caryophyllene was 1 mL per 3-day interval to keep the balancing of β -caryophyllene injection and evaporation rate.

On a daily basis, liquid temperature was measured with electronic temperature monitor (Oakton Temp lab, China) by submerging the probe into the liquid in bioreactor. Deionized (DI) water was added to compensate for evaporative losses of water in the sparged gas reactor. The biomass on lid and on surface inner wall above aqueous phase was also washed into suspended solution by this amount of DI water. Two 10 mL aliquots of the liquid suspension were removed for measuring the concentration of Total Suspended Solids (TSS) using Standard Method 2540D. Another 80 mL liquid mixture was used for measuring pH with a model 290A pH meter (Orion Research Inc., Boston, MA, USA). pH was adjusted to 7.0 by addition of NaOH on a daily basis. Then 100 mL of nutrient solution (composition described as above) was added to maintain a liquid volume of 3.0 L. Replacement of 100 mL/day with a working liquid volume of 3 L corresponds to a hydraulic residence time of 30 days.

After 14 days cultivation (June 21, 2013), the inoculum in the bioreactor was used to re-inoculate the biofilter described in Chapter 3 of this thesis. The 3 L volume of the inoculum in bioreactor was mixed with 3 L freshly prepared nutrient solution and pH of the resulted suspension was adjusted to 7.0. Then, the resulted 6 L suspension was re-introduced to biofilter, filled the column and then drained by gravity using a peristaltic pump (Masterflex) at a flow rate of 170 mL/min. Then the container with the resulted suspension was shaken, so that sediment at the bottom is well-mixed. Take 2.7 L the resulted suspension and pour it into the previous bioreactor. 300 mL activated sludge which was derived from aerobic tank in Port Allen Wastewater Treatment Plant was also added into the bioreactor, resulting in a total of 3.0 L volume.

As described before, β -caryophyllene delivering rate and gas flow rate were 0.06 mL/hr and 1.0 L/min, respectively. The amount of injected β -caryophyllene was 1 mL per 3-day intervals.

B.2.4 Biodegradation Test

Temperature plays an important and often decisive role in the distribution of organisms on the earth. Mesophilic microorganisms are those whose optimal growth temperature is about 30-40 °C. Thermophilic microbes exhibit optimal growth rates at high temperatures (>50 °C). Thermotolerant microbes are those that are able to survive at high temperatures but which exhibit maximum growth rates at lower temperatures. Adaptation to life at high temperatures requires that microbes possess enzymes that are thermally stable (Houbraken, 2012).

In an effort to define whether the microbial populations are thrives and prefers in high temperatures, serum bottle tests were designed and it configured as shown in Figure B-2. Eighteen serum bottles were put in there temperature level- room temperature (around 21 °C), 30 °C and 50 °C. There were two treatments for six bottles at each temperature level and one treatment was replicate three times: 1 with β -caryophyllene, 2 without β -caryophyllene.

Figure B-2: Serum bottles used for β -caryophyllene biodegradation test.

Each 168 mL glass serum bottle contain 9.5 mL nutrient solution (composition as used in B.2.3), 0.5 mL inoculum and 5 five cubes of packing medium approximately 1.25 cm per side. The inoculum was comprised of an aliquot of the aqueous-phase removed from the sparged gas bioreactor used to inoculate the biofilter described in Chapter 3 (mixed with type I soil (100% forest product compost), type III soil (60% forest compost and 40% sand and perlite after decompose process) after 14 days of cultivation. The inoculum for the serum bottles was removed from the sparged gas reactor before adding the activated sludge derived from Port Allen.

For the treatments with β -caryophyllene, 2 mL β -caryophyllene was dispensed into the upright glass tubes inside the serum bottles. At the time of inoculating the serum bottles (June 22, 2013), the TSS concentrations for sparged gas reactors was 595 mg/L.

The serum bottles were then sealed with butyl rubber stoppers and aluminum crimp caps and stored at a controlled temperature in the dark. Because carbon dioxide is produced with microbial activity, accumulates in the headspace and reduces the concentration of oxygen, two BD Precision Glide needles were used to puncture the butyl rubber stoppers simultaneously to release carbon dioxide, inject oxygen from air and re-equilibrate to atmospheric pressure every three days. Biomass accumulation was determined visual observation.

B.3 Results and Discussion

B.3.1 Temperature

The measured temperatures for the liquid culture in the bioreactor are shown in Figure B-3. As shown, the liquid temperature in the reactor was relatively stable throughout the 130 days of cultivation. Temperature ranged from 47 to 52 °C, averaging 49.51 ± 1.14 °C. Thus, the measured temperature was close to the target temperature of 50 °C.

Figure B-3: The measured temperature in bioreactor used to inoculate the biofilter described in Chapter 3.

B.3.2 pH

The measured pH in the sparged-gas bioreactor is shown in Figure B-4. The pH was around 6.9 for the first 46 day and then stabilized at near 6.7 for the following 84 days. The

decrease in pH on day 47 may be indicative of the start of nitrification, a process that produces acid that may have lowered the pH; however, ammonium, nitrite, and nitrate concentrations were not measured, so no definitive conclusions can be drawn regarding whether that was the cause.

Figure B-4: The measured pH in the bioreactor used to inoculate the biofilter described in Chapter 3.

B.3.3 Total Suspended Solids (TSS)

The measured Total Suspended Solids (TSS) concentrations are shown in Figure B-5. TSS concentrations were measured every two days and each point shown represents the average of duplicate measurements. As shown, TSS concentrations decreased over time. The maximum TSS concentration observed, 825 mg/L at a time 16 days after startup, corresponds to the day when 300 mL high concentration activated sludge (TSS concentration was 8320 mg/L) derived from an aerobic municipal wastewater treatment plant (Port Allen, LA) was added (denoted by red arrow). Although TSS values varied widely (825-105 mg/L), it was clear from both TSS concentration measurements and visual observations that suspended particulate matter were constantly available throughout the study period.

Figure B-6 shows an image of bioreactor after 76 days of cultivation. The turbidity of the liquid was progressively small by visual observation, indicating the quantity of biomass was decreasing over time in aqueous phase. At the same time, the quantity of biomass growing above the water line (on the inside walls and lids) increased over time. It can be seen in Figure B-6, biomass was visually observed on the glass side wall and lid of bioreactor.

Figure B-5: The measured TSS concentration in bioreactor used to inoculate the biofilter described in Chapter 3. The arrow denotes the addition of activated sludge.

Figure B-6: Photograph of the sparged-gas bioreactor used to inoculate the biofilter described in Chapter 3. The photograph was taken 76 days after the start of cultivation.

The orange-colored biomass was observed on the lid and inside wall above the water level right after the startup. Immediately following the activated sludge addition on day 14, the amount

of orange-colored biomass on the inside wall and lid sharply increased. After 54 days of cultivation (July 30, 2013), green-colored biomass was also visually observed to grow on the lid of bioreactor.

Foaming was observed immediately after the startup at bioreactor. Within the period of cultivation, the quantity of foaming decreased. As we can see in Figure B-6, there was small amount of foaming after 76 days of cultivation.

B.3.4 Serum Bottle Tests

For the bottles supplied with β -caryophyllene, biomass was visually observed on the glass side walls after 18 days of incubation at 30 °C. Six days later, biomass could be seen on the side wall of the bottle cultivated at room temperature. Visual observation revealed accumulation of biomass on the side wall of the serum bottle over time. After 70 days of cultivation at 30 °C, orange colored and white colored biomass attached polyurethane can be clearly seen (See Figure C-7-4). However, only a little bit orange colored biomass was seen by visual observation for bottles incubation at 50 °C after 70 days of cultivation (picture was not shown), indicating that the microorganisms may be thermotolerant rather than thermophilic. According to Cooney and Emerson (1964), thermophilic indicates that microbial populations grow most quickly at high temperatures (at or above 50 °C). Thermotolerant means that the organisms are able to survive high temperatures (at or above 50 °C), but doesn't necessarily mean that the organism grow well at that condition. Additionally, visual observations revealed the liquid being more turbid for the bottles cultivated at 30 °C than those cultivated at room temperature, suggesting that 30 °C is the most favorable temperature among the three temperature levels tested.

For the bottles without β -caryophyllene, no biomass was seen by visual observation. The observation of biomass growth in bottles supplied with β -caryophyllene but not in bottles without β -caryophyllene suggests that microbes have the ability to utilize β -caryophyllene as their carbon resources.

B.4 References

- Cooney D, G Emerson, R (1964). Thermophilic fungi An account of their biology, activities, and classification. W. H. Freeman, California, 188 pages.
- Houbraken JS, H. Frisvad, J. C. Rasamsonia, a new genus comprising thermotolerant and thermophilic Talaromyces and Geosmithia species Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology (2012) 101: 403-421

APPENDIX C: ENRICHMENT CULTURE 2 (USED TO INOCULATE BIOFILTER DESCRIBED IN CHAPTER 4)

C.1 Introduction

This appendix describes the procedure used to cultivate an enrichment culture that was used to inoculate the biofilter described in Chapter 4 of this thesis. This Appendix describes the experimental apparatus, operation, and results from the enrichment culture experiments.

C.2 Material and Methods

C.2.1 Chemicals

β -caryophyllene purchased from TCI America (Portland, OR, CAS No.87-44-5) was used as target pollutant in the bioreactor experiment.

C.2.2 Apparatus Setup

As shown in Figure C-1, a 4.0 L glass kettle reactor (Pyrex, Acton, MA) was employed for the enrichment culture. Electrical heating tapes (Cole-Parmer) were affixed to the exterior surface of the reactor to control temperature at a target level of 50 °C. Compressed air flowed through a pressure regulator (series R35, Arrow Pneumatics, Broadview, IL) and rotameter (Gilmont Instruments, Swedesboro, NJ) at a flow rate of 1.0 L/min. Then, air passed through a glass tube with airtight compression seals, and a port where a Thermogreen LB-1 half-hole septum (Supelco, Bellefonte, PA) was inserted to allow a glass gas-tight syringe (Hamilton, Reno, NV, USA) to deliver β -caryophyllene. The injection rate of delivering β -caryophyllene was set on a syringe pump (KD Scientific model 1000, Boston, MA, USA). After β -caryophyllene and compressed air mixed, the mixture passed through an aeration stone submerged in the bioreactor.

Figure C-1: Schematic diagram of the gas-sparged bioreactor used for cultivating β -caryophyllene degrading enrichment culture (Hu, 2012).

C.2.3 Bioreactor Operation

Two different kinds of soil (CLEGG's, Baton Rouge, LA) were used to inoculate the sparged gas reactor used to grow the inoculum eventually used in the biofilter experiment (described in Chapter 4). Type I soil contained 100% forest product compost and type II soil was mixed with 60% forest compost and 40% sand and perlite after de-composing process. Both of these soils were without pre-heating and disinfection treatment.

The 8 L of nutrient solution with the concentration of the following constituents was used to develop the biofilter inoculum: NH_4NO_3 1.25 g/L, KH_2PO_4 1.00 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.10 g/L, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.02 g/L, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.34 mg/L, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.24 mg/L, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.58 mg/L, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 1.01 mg/L, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.24 mg/L, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ 0.10 mg/L and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 1.36 mg/L.

A 500 g mass each of type I and II soil (wet basis) were added to 4 L nutrient solution (composition as above) respectively, and then the slurry was manually stirred for one minute. After passing through a sieve to remove coarse materials, the slurry was allowed to quiescently settle for five minutes to separate sand which visibly accumulated on the bottom of the flask. A 3 L volume of supernatant from each flask was then mixed together. After adjusting pH to 7.0 with Sodium Hydroxide, the resulting total of 6L suspension was inoculated to biofilter by a peristaltic pump (Masterflex) at a flow rate of 170 mL/min. After filling the column, the suspension drained from the column at the same flow rate. The drainage from biofilter was

collected, and 2.6 L suspension plus 400 mL deionized (DI) water was added to the bioreactor to supply 3 L liquid level.

In order to define the effect of nitrification in cultivation, 1.5 mg allylthiourea (ATU) was added to the bioreactor (St. Louis, MO, CAS No. 109-57-9). It is reported that 0.5 mg/L ATU could efficiently inhibit bacteria of the genus *Nitrosomonas* activity and secures a complete blockage of the first stage ($\text{NH}_4^+ \rightarrow \text{NO}_2^-$) of nitrification during the incubation period (Young, 1973). In addition, 1.2 L polyurethane foam (approximately 1.25 cm per side) was also immersed in this bioreactor.

Gas flow was turned on to deliver clean air at the rate of 1.0 L/min. 0.33 mL β -caryophyllene was injected to bioreactor using syringe pump by the rate of 0.06 mL/hr each day. After 5.5 hours injection, syringe pump stayed on, but nothing was injected during the rest of day. The amount of injected β -caryophyllene was 1 mL per 3-day intervals for keeping the balancing of β -caryophyllene injection and evaporation rate. On a daily basis, liquid temperature was measured with electronic temperature monitor (Oakton Temp lab, China) by submerging the probe into the liquid in bioreactor. Deionized (DI) water was added to compensate for evaporative losses of water in the sparged gas reactor. The biomass on lid and on surface inner wall above aqueous phase was also washed into suspended solution by this amount of DI water. Two 10 ml aliquots of the liquid suspension were removed for measuring the concentration of Total Suspended Solids (TSS) using Standard Method 2540D. Another 80 ml liquid mixture was used for measuring pH with a model 290A pH meter (Orion Research Inc., Boston, MA, USA). pH was adjusted to 7.0 by addition of NaOH on a daily basis. Then, 100 ml of nutrient solution (composition described as above) was added to maintain a liquid volume of 3.0 L. Replacement of 100 mL/day with a working liquid volume of 3 L corresponds to a hydraulic residence time of 30 days.

After 97 days of operation (Sep 13, 2013), the 900 ml packing medium was taken out from the bioreactor and used to inoculate the biofilter described in Chapter 4 of this thesis.

Following the removal of polyurethane foam packing medium for use in the biofilter experiment described in Chapter 4, the sparged gas was continued to be operated as described before, with β -caryophyllene delivering rate and gas flow rate were remained at 0.06mL/hr and 1.0 L/min, respectively. The amount of injected β -caryophyllene was 1 mL per 3-day intervals.

C.2.4 Biodegradation Test

Temperature plays an important and often decisive role in the distribution of organisms on the earth. Mesophilic microorganisms are those whose optimal growth temperature is about 30-40 °C. Thermophilic microbes exhibit optimal growth rates at high temperatures (>50 °C). Thermotolerant microbes are those that are able to survive at high temperatures but which exhibit maximum growth rates at lower temperatures. Adaptation to life at high temperatures requires that microbes possess enzymes that are thermally stable (Houbraken, 2012).

In an effort to define whether the microbial populations are thrives and prefers in high temperatures, serum bottle tests were designed and it configured as shown in Figure C-2. Eighteen serum bottles were put in there temperature level- room temperature (around 21 °C), 30 °C and 50 °C. There were two treatments for six bottles at each temperature level and one treatment were replicate three times: 1 with β -caryophyllene and nitrification inhibitor; 2 with nitrification inhibitor, no β -caryophyllene

Figure C-2: Serum bottles used for β -caryophyllene biodegradation test.

Each 168ml glass serum bottle contain 9.5 mL nutrient solution (composition as used in C.2.3), 0.5 mL inoculum and 5 five cubes of packing medium approximately 1.25 cm per side. The inoculum was comprised of an aliquot of the aqueous-phase removed from sparged gas bioreactor used to inoculate the biofilter described in Chapter 4 (mixed with type I soil (100% forest product compost), type III soil (60% forest compost and 40% sand and perlite after decompose process) and nitrogen inhibitor after 14 days of cultivation.

For the treatment with nitrification inhibitor, allylthiourea (ATU) was added to eliminate the effects of producing nitrite bacteria. The concentration for ATU is about 0.5 mg/L in the sample. For the treatments with β -caryophyllene, 2 mL β -caryophyllene was dispensed into the upright glass tubes inside the serum bottles. At the time of inoculating the serum bottles (June 22, 2013), the TSS concentrations for the sparged gas reactor was 180 mg/L.

The serum bottles were then sealed with butyl rubber stoppers and aluminum crimp caps and stored at a controlled temperature in the dark. Because carbon dioxide is produced with microbial activity, accumulates in the headspace and reduces the concentration of oxygen, two BD Precision Glide needles were used to puncture the butyl rubber stoppers simultaneously to release carbon dioxide, inject oxygen from air and re-equilibrate to atmospheric pressure every three days. Biomass accumulation was determined visual observation.

C.3 Results and Discussion

C.3.1 Temperature

The measured temperature in the bioreactor which was used to inoculate the biofilter described in Chapter 4 was shown on Figure C-3. As shown, the liquid temperature in the reactor was relatively stable throughout the 130 days of cultivation. Temperature ranged from 47 - 53 °C, averaging at 49.95 ± 1.03 °C. Thus, the measured temperature was close to the target temperature (50 °C).

Figure C-3: The measured temperature in the bioreactor used to inoculate the biofilter described in Chapter 4.

C.3.2 pH

The pH measured in the sparged-gas bioreactor is shown in Figure C-4. The pH fluctuated at 6.86 during the first 90 days and then it started falling down from day 91. The decreasing pH indicated the starting of nitrification process. On day 97 (Sep 11, 2013), all of the packing mediums in the bioreactor was taken out, following pH value dramatic went up to 6.84 (denoted by red arrow). After that, the trend of pH value was depressed and went back to 6.75 during the following 15 days of cultivation. Compared with Figure B-4, it takes longer time to bring the pH in this bioreactor down than it takes in bioreactor described in Appendix B, possibly indicating nitrogen inhibitor is effectively slow down the nitrification process.

Figure C-4: The measured pH in the bioreactor used to inoculate the biofilter described in Chapter 4. The arrow denotes the day when all of the packing mediums was taken out from bioreactor.

C.3.3 Total Suspended Solids (TSS)

The measured Total Suspended Solids (TSS) concentrations are shown in Figure C-5. TSS concentrations were measured every two days and each point shown represents the average of duplicate measurements. As shown, TSS value kept decreasing during the entire cultivation. The adsorbing packing medium combined with nitrogen inhibitor made TSS concentration in this bioreactor apparently lower than it in the bioreactor described in Appendix B. On day 97 (Sep 11, 2013), all of the packing mediums in the bioreactor was taken out. TSS value went up (as shown by red arrow) because the microorganisms cannot attach on the packing medium anymore. Although TSS values varied widely (543–10 mg/L in the bioreactor), it was clear that suspended particulate matter were constantly available throughout the study period.

Figure C-5: The measured TSS concentration in bioreactor used to inoculate the biofilter described in Chapter 4. The arrow denotes the day when all of the packing mediums was taken out from bioreactor.

Figure C-6 was the image of bioreactor after 76 days of cultivation. The turbidity of the liquid was progressively small by visual observation, indicating the quantity of biomass was decreasing over time in aqueous phase. At the same time, the quantity of biomass growing above the water line (on the inside walls and lids) increased over time. Compared with the bioreactor described in Appendix B, less biomass was visually observed on the glass side wall and lid of this bioreactor.

The orange-colored biomass was started to show on the inside wall and lid of the bioreactor after 39 days of cultivation (July 15, 2013) and the biomass was dramatic increased after 97 days of cultivation (Sep 11, 2013) on account of taking out all of the packing mediums combined with starting ammonia oxidization. Also, only a small amount of foam was observed 7 days after startup and it disappeared in the following 10 days of operation.

Figure C-6: Photograph of the sparged-gas bioreactor used to cultivate the inoculum for the biofilter described in Chapter 4. Photograph taken 76 days after the start of cultivation.

C.3.4 Serum Bottle Tests

For the bottles supplied with β -caryophyllene, biomass was visually observed on the glass side walls after 18 days of incubation at 30 °C. Six days later, biomass could be seen on the side wall of the bottle cultivated at room temperature. Visual observation revealed accumulation of biomass on the side wall of the serum bottle over time. After 70 days of cultivation at 30 °C, orange colored and white colored biomass attached polyurethane can be clearly seen (Figure C-7-3 and Figure C-7-4). However, only a little bit gel like biomass has been seen by visual observation for bottles incubation at 50 °C after 70 days of cultivation (Figure C-7-1), indicating that the microorganisms may be thermotolerant rather than thermophilic. According to Cooney and Emerson (1964), thermophilic indicates that microbial populations grow most quickly at high temperatures (at or above 50 °C). Thermotolerant means that the organisms are able to survive high temperatures (at or above 50 °C), but doesn't necessarily mean that the organism grow well at that condition. Additionally, visual observations revealed the liquid being more turbid for the bottles cultivated at 30 °C than those cultivated at room temperature, suggesting that 30 °C is the most favorable temperature among the three temperature levels tested.

For the bottles without β -caryophyllene, no biomass was seen by visual observation (Figure C-7-2). The observation of biomass growth in bottles supplied with β -caryophyllene but not in bottles without β -caryophyllene suggests that microbes have the ability to utilize β -caryophyllene as their carbon resources.

Compared with the bottle without inhibitor described in Appendix B, less biomass was seen on the side wall and polyurethane (Figure C-7-3 and Figure C-7-4), showing that nitrogen inhibitor efficiently secures the blockage of nitrification process during the incubation period.

Figure C-7: Picture of polyurethane placed in serum bottle after 70 days of cultivation. Treatment with β -caryophyllene and nitrification inhibitor at 50 °C (1); treatment with nitrification inhibitor, no β -caryophyllene at 50 °C (2); treatment with β -caryophyllene and nitrification inhibitor at 30 °C (3); treatment with β -caryophyllene, no nitrification inhibitor at 30 °C (4).

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THE VITA

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She began her Master of Science degree study in the Fall of 2009 in the School of Renewable Natural Resources at Louisiana State University under the direction of Dr. Qinglin Wu. During this period, she studied properties of nano-cellulose crystals. After that, she transferred to Civil Engineering to work with Dr. Moe as a master student. The exploration in the direction of high temperature biofilter made her feel novel and excited. Yiying Yue is expected to graduate with a Master of Science degree in Civil Engineering in May 2014.