THE BIOFILTRATION OF INDOOR AIR USING NORTHERN PLANTS

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of

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by

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ABSTRACT

BIOFILTRATION OF INDOOR AIR USING NORTHERN PLANTS

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The role of tropical foliage in the Canada Life Environmental Room (CLER) was evaluated as a means of improving indoor air quality (IAQ) through direct removal of volatile organic compounds (VOCs). There was evidence of VOC sorption to the foliage of eight tropical species, however, leaf concentrations were too small to represent a substantial sink. Since, higher plants are believed to influence VOC removal indirectly by sustaining a diverse microbial population, plant selection need not be restricted to tropical species. A modular biofilter was constructed as a simplified version of the CLER. The plant community consisted of common northern species collected from the wild and held in a stable indoor environment. The biofilter was evaluated as a source of ambient spore, *Legionella pneumophilia* and for its capacity to eliminate indoor VOCs. Spores loads remained stable over the observation period and the system was devoid of the pathogen *L. pneumophilia.* This suggested that the biofilter does not lower IAQ through the production of bioaerosols. In terms of its ability to eliminate VOCs, the biofilter compared favourably with other biofiltration systems. This supports the contention that IAQ could be improved by a community of northern plants configured into a biofilter.

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LIST OF ABBREVIATIONS

2-p	2-pentanone
ANOVA	Analysis of variance
ASHRAE	American Society of Heating, Refrigeration and Air conditioning Engineers
CFU	Colony forming units
C_{GI}	Influent gas concentration (ppbv, μmol m^{-3})
C_{GO}	Effluent gas concentration (ppbv, μmol m^{-3})
CLER	Canada Life Environmental Room
C_R	Removal coefficient
d	Biofilter depth (m)
DCM	Dichloromethane
dwt	Dry weight (g)
EBRT	Empty bed retention time ($m^3_{biofilter}$ s m^{-3}_{air})
ET	Effluent temperature (°C)
fwt	Fresh weight (g)
HPLC	High performance liquid chromatography
HVAC	Heating ventilation and air conditioning (system)
EC	Elimination capacity (μmol m^{-3} h^{-1})
G	Green moss
GC	Gas chromatograph
HVAC	Heating ventilation and air conditioning system
k	Microbial elimination rate (s^{-1})
IAQ	Indoor air quality
OD	Outside diameter (mm)
OSHA	Occupational health and safety association
m_i	Temperature corrected solubility (dimentionless)
MEK	Methylethylketone
MIBK	Methylisobutylketone
NIOSH	National Institute of Occupational Health and Safety
NG	Non green moss
NORCAT	Northern Centre for Advanced Technology
PID	Photo ionization detector
PCE	Tetrachloroethylene
ppbv	Parts per billion by volume
ppm	Parts per million (by mass)
ppmv	Parts per million by volume
psi	Pounds per square inch
SCEBRT	Solubility corrected empty bed retention time ($m^3_{biofilter}$ s m^{-3}_{air})
RBA	Rose bengal agar
RCS	Reuter centrifugal sampler

R_E	Removal efficiency (%)
TCE	Trichloroethylene
TSA	Tryptic soy agar
VOC	Volatile organic compound
WHO	World Health Organization

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CHAPTER 1. GENERAL INTRODUCTION

Indoor Air Quality:

The air quality inside many buildings may be worse than outdoors (Otson & Fellin, 1992) and since North Americans spend in excess of 85% of their time indoors (Jenkins et al., 1992) the quality of indoor air represents a major public health concern. Agents that negatively impact indoor air quality (IAQ) can be broadly placed into many categories; particulates including dust, bioaerosols and asbestos, volatile organic compounds (VOCs), and radon (ASHRAE, 1998). Many of these contaminants may accumulate in buildings when the air exchange rate with 'fresh' outdoor air is restricted. Techniques developed for the biological treatment of industrial waste gas streams may offer an alternative or supplementary means of maintaining IAQ.

In the United States indoor ventilation consumed 10% of the total energy production in 1998 (ASHRAE, 1998). Much of this cost can be attributed to conditioning imported outdoor air in terms of temperature and humidity to create a comfortable indoor climate. In areas where the outdoor climate is uncomfortably hot or cold, the cost of maintaining a comfortable indoor environment is greater. Reducing the quantity of imported air by restricting the building air exchange rate can represent a major cost savings for building operators, particularly in these extreme climates. As a result buildings have been designed to minimize the air exchange with outdoors creating an indoor environment that is increasingly isolated from the outdoor environment.

To a large extent IAQ is governed by the activities of the occupants. For example, building occupants themselves are a source of bioaerosols (Heinemann et al., 1994) and VOCs (Otson & Fellin, 1992). Activities such as cigarette smoking can add over 20 VOCs to the air (Otson & Fellin, 1992). Materials used in the construction of the building may also be a major source of contaminants, releasing VOCs, asbestos and particulates into the air. Low quality air has a substantial impact in the workplace, the Occupational Health and Safety Administration (OSHA, 1999) relate poor IAQ to a loss of productivity, irritation of the eyes, throat and mucous membranes, nausea, asthma, drowsiness and since some of the indoor contaminants are carcinogens even death.

Bioaerosols:

Air quality may be lowered by airborne particles of microbiological origin. Such particles are known as bioaerosols (ASHRAE, 1998). Micro organisms such as bacteria and fungi are ubiquitous both indoors and out but rarely do they present a health concern to the vast majority of the population. Under the right environmental conditions bioaerosols can proliferate indoors to populations that can negatively influence the health of building occupants (Flannigan & Miller, 1994; Maroni et al., 1995) . Health Canada sets safety limits on the number of colony forming units per cubic meter of indoor air (CFU m^{-3}). Fungi concentrations indoors should not exceed 150 CFU m^{-3} and bacterial concentrations should not exceed 500 CFU m^{-3} (Annon., 1995). High bioaerosol concentrations have been associated with building moisture (Singh, 1994) and organic

materials such as soil (Burge et al., 1982). Perhaps the most infamous bioaerosol, *Legionella pneumophilia* is associated with standing water in improperly maintained air conditioning systems (OSHA, 1999).

Bioaerosol exposure may induce several symptoms. Irritation of the respiratory tract and eyes is a mild reaction to bioaerosols. They have also been associated with higher incidence of respiratory tract infections including alveolitis and toxic dust syndrom (Maroni et al., 1995). Bioaerosols have also been linked to allergies and increased incidence of asthma (Husman, 2000). Exposure to *Legionella pneumophilia* can be extremely dangerous for individuals with compromised immune systems, sometimes leading to legionnaires disease which can be lethal (OSHA, 1999).

Volatile organic compounds:

Most of the volatile organic compounds (VOCs) present indoors are derived from indoor sources (Sack, 1992). Almost half of all common household cleaners contain aromatic compounds such as toluene or styrene (Sack, 1992). Varnishes and paints also release aromatic compounds and continue to release VOCs hundreds of hours after their initial application (Sparks, 1991). Glues and lacquers release ketones (Otson & Fellin, 1992) and carpets and cleaning agents release chlorinated compounds such tricholoroethylene or tetrachloroethylene into the air (Sack 1992). As a result of these, and other sources, over 300 VOCs have been detected indoors (Kostiainen, 1995). With inadequate ventilation rates in buildings the frequently the conditions exist where VOCs

may accumulate.

Individual VOCs are rarely present in concentrations greater than a few parts per billion by volume (ppbv) in indoor air (Singh, 1988). Their combined concentration may reach into the 100 ppbv range (Kostiainen, 1995). Although rarely are individual compounds present in sufficient concentration to present a health concern, their combined effect may negatively impact building occupants (Otson & Fellin, 1992). Acute exposure to low concentrations of many VOCs has been associated with irritation of the eyes and respiratory tract (NIOSH, 1998), higher incidences of asthma, eye and respiratory infections and headaches (Maroni et al., 1995). However, the health effects of long term acute exposure to a dilute mixture of many VOCs is not well understood (ASHRAE, 1998).

Industrial Biofiltration:

Biological treatment of industrial waste gas streams has proven to be an economical means of reducing VOC emissions into the environment (Devinny et al., 1999). The biofiltration process contains two essential parts. First contaminants are transferred to the liquid phase by diffusing directly into water, into a biofilm (Ottengraf & van den Oever, 1983) or through a membrane and into the water (Fitch, 2000). Once in solution, VOCs are degraded in a bioreactor. The microbial community in the bioreactor generally oxidize the VOC to CO_2, heat, water, biomass and salts (Devinny et al., 1999; Wani et al., 1997).

Conventional biofiltration relies on three types of bioreactors capable of VOC degradation. They are distinguished by the status of the water phase and the nature of the microbial community. 1) In the case where the water phase is stationary and the microbial community is fixed into a porous medium, the bioreactor can be characterized as a a biofilter. 2) A biotrickling filter contains a fixed microbial community with a flowing water phase. 3)

Bioscrubbers differ from biofilters and biotrickling filters in both the transfer of contaminants to the liquid phase and the nature of the microbial community. In a typical bioscrubber contaminated gas is force through a fine mist, where the mass transfer takes place. Water is collected and moved into a bioreactor where VOCs are degraded by suspended bacteria (Devinny et al., 1999).

In biofilters and biotrickling filters, waste gas is forced through a porous packing material capable of supporting microbial growth (Wani et al., 1997). Packing consists of inert material such as wood chip, peat moss or synthetics (Devinny et al., 1999). The biofilter media must be capable of providing adequate habitat for the microbial population and allowing the mass transfer of gaseous contaminants into the liquid phase (Swanson & Loehr, 1997; Wani et al., 1997). Sustaining a population of microbes capable of VOC degradation is key to an efficient biofilter. As such, environmental parameters such as nutrients, pH, temperature and moisture are controlled to optimize biofilter performance (Wani et al., 1997).

Microorganisms in the biofilter or biotrickling filters are organized in a biofilm or are suspended in the aqueous layer surrounding the biofilter packing material (Devinny et

al., 1999). Often biofilter performance is optimized by inoculating the biofilter with a specific population of microbes known to degrade the target compound (Wani et al., 1997). Inoculation is also used to reduce the acclimation period of the biofilter (Devinny et al., 1999; Swanson & Loehr, 1997). Acclimation of a biofilter to a new compound is marked by a period of increasing biofilter performance until a sustainable peak is reached (Swanson & Loehr, 1997). Wani and coworkers (1997) attribute the acclimation period of a biofilter to the conversion of metabolic pathways and the creation of new enzymes to utilize the new carbon source. The duration of the acclimation period will vary from a few days to a year (Devinny et al., 1999).

Once acclimated, the efficiency of a biofilter will vary with its structural characteristics, including the depth of the biofilter and the flux of air through it (Wani et al., 1997). The flux and depth are typically combined into the empty bed residence time (EBRT). EBRT is the biofilter volume normalized for the volumetric flux through it, ($m^3_{biofilter}$ s m^{-3}_{air}) (Devinny et al., 1999). In industrial biofilters, EBRT typically ranges from a few seconds to a few minutes (Pedersen & Arvin, 1997; Swanson & Loehr, 1997). The greater the contact time between the gas stream and the biofilter, the greater the removal of the contaminant. An increase in EBRT must correspond to an increase in biofilter depth or a decrease in the volumetric flux. As biofilter depth increases performance may be limited by the availability of oxygen, nutrients or low pH (Swanson & Loehr, 1997). Thus, increased depth becomes cost prohibitive and industrial biofilters are generally 1.0 to 1.5 m in depth with contact times ranging from 15 to 60 seconds (Devinny et al., 1999).

Biofilter performance:

VOC removal from waste gas streams can be quantified in many ways. One of the most common means of describing biofilter performance is the removal efficiency (R_E). This is defined as:

$$R_E = \left(1 - \left[\frac{C_{GO}}{C_{GI}}\right]\right) x100\%$$

[1.1]

Where C_{GO} is the effluent concentration (μmol m^{-3} s^{-1}) and C_{GI} is the influent concentration (μmol m^{-3} s^{-1}). R_E is usually expressed as a percent (Devinny et al., 1999). R_E is a useful term for comparing the performance of a single biofilter over time. It is not useful when comparing different biofilters due to differences in construction and operating parameters. To normalize for differences in biofilter depth, air flow and influent concentration between biofilters, removal is often expressed by the term elimination capacity (EC):

$$EC = \frac{[C_{GI} - C_{GO}]}{EBRT} x3600$$

[1.2]

The elimination capacity (EC) (μmol m^{-3} h^{-1}) is the difference between the influent VOC concentration (C_{GI}) (μmol m^{-3}) and the effluent concentration (C_{GO}) (μmol m^{-3}) normalized for EBRT (s).

While the waste gas stream is resident in the biofilter, contaminants must transfer from the gaseous to the liquid phase where they are eliminated by microbial reactions. The removal of a contaminant from the waste gas stream can be limited ,therefore, either by diffusion into the biofilm or by microbial reaction rates (Ottengraf & van den Oever. 1983). Diffusion of contaminants into the biofilm is related through Henry's law and the ideal gas law to the solubility of the contaminant and the temperature of the biofilm (Ottengraf, 1986). Once in the biofilm, the rate of VOC degradation is governed by the interaction between the VOC and the microbial community. Microbial reactions in the biofilm can be described the Michaelis-Menton equation (Ottengraf & van den Oever. 1983). Contaminant removal from the gas stream can be described by the first order equation:

$$\frac{C_{GO}}{C_{GI}} = \exp\left[\frac{-dK}{miVa}\right]$$

[1.3]

(Ottengraf & van den Oever. 1983)

Where C_{GO} and C_{GI} are the effluent and influent concentrations respectively (μmol m^{-3}). Biofilter depth is measured by d (m). The temperature adjusted solubility is measured by mi (dimensionless) which is calculated from Henry's Law and the ideal gas law. K is an estimate of the microbial activity (s^{-1}). Va is the superficial gas velocity ($m\ s^{-1}$). which may be combined with the biofilter depth to calculate EBRT, thus the equation 1.3 could also be written:

$$\frac{C_{GO}}{C_{GI}} = \exp\left[\frac{-EBRTxK}{mi}\right]$$

[1.4]

Where the ratio of EBRT and m_i is the solubility corrected empty bed retention time (SCEBRT).

Indoor air biofilters:

The removal of VOCs from indoor air presents several challenges not faced by industrial biofilters. Concentrations of VOCs indoors are very low compared to industrial concentrations. While, Industrial biofilters deal with gasses in the parts per million or parts per thousand range (Devinny et al., 1999), indoor VOC concentrations are very dilute by comparison typically in the parts per trillion to parts per billion range (Kostiainen, 1995). Secondly, the composition of industrial waste gas is generally known and constant, while the composition of indoor air will vary through time and with the activities in the building (Otson & Fellin, 1992). The goal of an industrial biofilter is to reduce effluent VOC concentrations to comply with environmental regulations. Indoors, biofiltration could be used as a tool to reduce the ventilation rate and maintain IAQ through the removal of VOCs and for the psychological benefits to occupants.

To compensate for these differences an indoor air biofilter sustains a diverse microbial population. The assumption is that a diverse community of microbes will be

better suited to degrade a diverse mixture of VOCs (Binot & Paul, 1989). While some authors have engineered a diverse mixture of microbes (Keuning et al., 1991), others have stimulated microbial diversity with plant-microbe associations (Darlington et al., 2000). In either case, caution must be taken when stimulating microbial growth in an indoor environment. Increased bioaerosol loads in the space may have a negative impact on IAQ despite lower VOC concentrations.

The Canada Life Environmental Room:

The Canada Life Environmental Room (CLER) is a prototype biofilter designed to improve IAQ through the removal of VOCs. It is located in a 160 m^2 board room in the Canada Life Assurance Building (Toronto, Ontario). The CLER is a complex collection of higher plants, mosses and microbes in a recirculating hydroponic system. It is composed of three separate but interconnected sections: a 12 m^2 moss covered wall, a hydroponically grown community of higher plants and a 3500 L aquarium.

Indoor air is drawn through the 12 m^2 wall constructed from a porous backing and covered in moss and a variety of higher plants. Water is constantly trickled down the surface of the wall, which is actually composed of 5 independent panels. Air flow through each of the panels can be controlled separately by adjusting the intake into a dedicated HVAC system. Thus, each panel can be considered a separate biotrickling filter. VOC contaminated air is pulled through the five panels where the contaminants are transferred into the aqueous phase and degraded.

Unlike conventional biotrickling filters, the CLER does not rely on an inert packing material to maintain the microbial population. Instead the microbial degraders are grown on living moss. The use of moss as a packing material may have several advantages over conventional materials. As a living substrate moss has the ability to both regenerate decomposing substrate and form symbiotic associations with microbial communities (Llewellyn, 2000). Furthermore, since it is a photosynthetic organism it may add oxygen to the biofilm, enhancing the degradation of many compounds. While industrial biofilters are typically 1.0 to 1.5 m in depth, CLER contains only a thin layer of moss, 0.025 m thick and retention time is less than one second (Darlington et al., 2000).

Excess water from the moss surface drains into a hydroponic system containing a complex community of higher plants grown on a lava rock base. Contaminants that transfer to the aqueous phase, but are poorly degraded on the surface of the wall, may be degraded in the hydroponic system. In this sense, the system acts as a bioscrubber (Devinny et al., 1999). Likewise, undegraded compounds may also be degraded in the aquarium which connects the wall and the hydroponics as a common irrigation supply.

Results from the CLER indicate that it is possible to improve IAQ with a biofilter, through the removal of VOCs. The VOCs that have been tested at the CLER represent several classes of compounds common to indoor spaces (Otson & Fellin, 1992); these include aromatics (Darlington et al., 2000), ketones (Darlington & Dixon, 1999), aldehydes (Darlington et al., 1998) and chlorinated compounds (Darlington et al., 1998).

The incorporation of large quantities of biomass indoors slightly increase the spore load of the space relative to a reference room in the same building (Darlington et al.,

2000). However, the increase did not raise the spore load above common indoors spore counts reported in the literature (Mouilleseaux & Squinazi, 1994), or above the Health Canada safety guidelines (Annon., 1995). The CLER can be said to improve IAQ through the removal of VOCs without compromising it through excessive production of bioaerosols.

Modular Biofilter:

IAQ is a common problem, and it is desirable to increase the accessibility of this technology. However, construction of a system like the CLER is a major undertaking both in terms of the space required and the cost of renovations. One possible solution is the development of a modular biofilter. A modular biofilter could be constructed off site and installed with minimal disruptions to the occupants and minimal renovation costs.

The CLER included a diverse community of tropical plants many of which were understory, shade tolerant species as some epiphytic species which require very little root space. These species characteristics were subjectively imposed in this prototype biofilter design. The actual role of higher plants in the CLER remains largely unknown and difficult to quantify (Darlington et al., 1998). It has been noted that VOCs can be removed directly by higher plants (Simonich & Hites, 1995; Wolverton et al., 1983) and they have been though to provide ecological infrastructure for microbial communities (Anderson, 1993; Shimp et al., 1993). Therefore, one of the first objectives of this study was to address the role of higher plants in the CLER. This would help to establish a

logical basis for species selection in a proposed modular biofilter which it was hoped could accommodate local (ie northern temperate) species.

Having determined some of the interactions between VOCs and higher plants, further studies were planned on a prototype modular biofilter constructed at the Northern Centre for Advanced Technology (NORCAT). Species selection criteria (both objective and subjective) were applied to create a prototype using north temperate species. The study progressed to include concurrent evaluations of the survivability of various species along with the performance of the ecosystem as a biofilter.

CHAPTER 2: THE ROLE OF HIGHER PLANTS IN AN INDOOR AIR BIOFILTER

Introduction:

The CLER houses over 100 species of higher plant with more than 500 individuals present. While the presence of a large quantity of plant material indoors has an aesthetic appeal, the value of indoor plants as a means of improving IAQ remains poorly studied. In terms of the construction of an indoor air biofilter, the combination of species best suited to VOC removal is not known. Plants may interact with ambient VOCs directly through metabolism (Wolverton et al., 1983) or sorptive processes (Paterson et al., 1995) or they can act indirectly through the stimulation of a diverse microbial community (Anderson, 1993).

Direct removal of VOCs can take the form of sorption or metabolism. Some authors have suggested that higher plants are able to metabolize ambient VOCs. Formaldehyde, for example, is reportedly removed by plants such as the spiderplant (*Chlorophtum elatum var.*) (Giese et al., 1994; Wolverton et al., 1983), although microbial populations in the soil likely play the most important role (Godish & Guindon, 1989). Similarly 25 to 100 part per million concentrations of benzene and hexane respectively were removed from a sealed chamber by the species *Spathuphyllum petite* and *Dracena Janet Craig* (Wood, 2000). The concentrations required to elicit this response were high by indoor air standards (Kostiainen, 1995) and it is not clear that a similar response would be observed at concentrations typical of indoor air.

The most likely route of direct VOC removal is passive sorption, since higher plants have been shown to accumulate VOCs in leaf material (Frank, 1989; Gaggi & Bacci, 1985; Hauk, 1994; Hiatt, 1998; Keymuellen, 1993). Lipophilic VOCs have an affinity for the waxy cuticle layer of many plants (Paterson et al., 1995). Much of the work in this area has focused on the accumulation of chlorinated compounds in the foliar tissue of the genus *pinea* collected outdoors (Frank, 1989; Gaggi & Bacci, 1985; Travis & Hatemer-Frey, 1988). Compounds common to the indoor environment (Singh, 1988) have been poorly represented in the literature. Aromatic compounds are a notable exception since they are common pollutants both indoors (Otson & Fellin, 1992) and out (Keymuellen, 1991). Aromatics include compounds such as: benzene, toluene, ethylbenzene and xylene isomers. Hiatt and coworkers (1998) report the accumulation of aromatic compounds in the leaf tissue of several outdoor species. Their work suggested that terpene producing species have a greater affinity for aromatic VOCs than would be expected by the composition of the cuticle layer. Keymuellen and coworkers (1991) found the aromatic concentration in the leaf tissue of *Pseudotsuga menziesii* was correlated to the atmospheric concentration.

In examining the direct role of foliage as a sink for indoor VOCs, two approaches were taken. The first measured the combined influence of sorption and metabolism on high VOC concentrations (by indoor standards) in sealed bottles. The second focused exclusively on the sorption process at concentrations typical of indoor air.

Material and Methods:

Bottle Experiments:

Leaf samples (2 - 5 g_{fwt}) were cut from 12 species of living plants including: *Nephrolepis exaultata, Clematis jackmanii, Codiaeum varigatum, Deiffenbacia amonena, Nephthytis "White butterfly", Ficus bengaminia, Chrysalidocarpus lutegcens, Polypodium aureusa, Chlorophytum comosum, Aeschynanthus radicans*, and *Yucca aloefolia*. Leaf samples were sealed into glass bottles modified to contain a gas sampling septum. The airspace of each bottle was promptly injected with a concentrated gas mixture designed to yeild 1 part per million by volume (ppbv) of toluene and trichloroethylene (TCE). VOC concentrations in the bottles were measured after the initial injection and again after 12 hours of exposure using an SRI model 310 gas chromatograph equipped with a photoionization detector (PID). Analytes were separated through a 0.53 mm OD MXT-volatiles column at a temperature of 95 °C and a head pressure of 10 psi. Under these conditions the retention times of TCE and toluene were 1.7 and 2.1 minutes respectively. Data for each bottle was converted into a percent relative removal and differences between species treatments, including blanks, were detected with a 1 way ANOVA using SAS version 6.12.

Leaf extractions at the CLER:

The CLER houses approximately 100 species of plant, grown hydroponically on a lava rock substrate. Eight species were selected to represent a large range of plant types present in the system in large enough quantity to allow sampling without impacting the health of the plant. Sampled species included: *Verissa splendens, Hendra helix, Nephrolopis exaltata, Dracena fragrans, Spathyphyllum mona loa, Rhododendron japonica, Aucuba japonica variegata* and *Dieffenbacia manculata.*

The CLER is equipped with a feedback controlled VOC emission system which generated and maintained ambient VOC concentrations in the room (discussed in detail in Chapter 3). Using this system, the ambient concentration of four VOCs, toluene, tetrachloroethylene (PCE), ethylbenzene and ortho-xylene were controlled over a six day period. The first two days the ambient concentrations were maintained at background levels (below 3 parts per billion by volume (ppbv) or 10 $\mu g\ m^{-3}$). The third and fourth day ambient concentrations were increased to 100 ppbv for toluene and PCE, and 60 ppbv for both ethylbenzene and o-xylene. These correspond to concentrations 380, 685, 310 and 310 $\mu g\ m^{-3}$ for each compound respectively. Sampling days five and six ambient concentrations were allowed to return to background levels.

During each of the six sampling days, 5 samples were taken from each target species at approximately 16:00 hrs. Each sample contained between 1 and 2 g of fresh, healthy leaf material and were collected into 9 ml environmental sample bottles. Samples were extracted using 1-1.5 ml dichloromethane (DCM) and constantly mixed for five

days, based on the procedure described in Keymuellen and coworkers (1991). Sample bottles were equipped with a teflon/silica septum. This allowed the extraction solvent to be added to the vials without re-exposing the sample to different atmospheric concentrations of the target VOCs. The extract was isolated from the sample vial and filtered using a Cameo 0.22 micron 3N nylon syringe filter. Filtered extract was transferred to sealed extract vials and spiked with 1 μL of a 2000 ppb bromobenzene internal standard solution. The standard solution was included to account for decreased in detector sensitivity between samples. The detection limit of samples increased as the detector lost resolution, as such samples where either the standard or the analyte were below 0.1 mv were considered non detectable.

Samples were analyzed with a chromatographic procedure similar to Keymuellen et al (1991). Analytes were separated through a 0.53 mm OD MXT-volatiles column connected in flash vaporization split/splitless injection mode to an SRI 310 gas chromatograph. The initial injection temperature of 90 °C was maintained for 4 minutes until after the elution of the o-xylene peak. The oven temperature was then ramped at 50 °C min^{-1} to a final temperature of 200 °C and held for five minutes to eliminate any late eluting compounds. Column pressure was isobaric and maintained at 7 psi.

Data was analyzed as a 2 x 2 factorial with 4 levels of VOC and 6 levels of sampling day. Comparisons among significant factors was tested with a Student-Newman-Kuels mulitple comparison test using SAS verison 6.12.

Results and Discussion:

Bottle experiments:

The exposure of leaf tissue to known concentrations of specific VOCs in sealed bottles resulted in barely detectable removal of the VOCs in question. The analysis of variance did not detect differences between treatments, including the blanks (see tables AI.1 and AI.2 in appendix I). This Inferred that leaf tissue had no effect on the concentrations of either TCE or toluene in the septum bottles (Figure 2.1). A disadvantage to the bottle technique was the high degree of variability in both the initial concentrations and the leakage rate of VOCs between bottles. TCE had an average leakage rate of 30.47 % and ranged from 18.35 to 42.71 %, toluene leakage ranged from 27.50 to 48.08 % and averaged 39.64 %. This made meaningful comparisons difficult. Relative removal rates below 48.9 and 57.8 % for TCE and toluene, respectively were not distinguishable from the control bottles, based on a 1 tailed t-test ($\alpha = 0.25$). Thus, bottles treated with plant material require removal rates approximately 18% higher than the control bottles to be detected using this technique.

None of the foliage surveyed removed TCE or toluene with a detectable efficiency. Leaf tissue was removed from the parent plant in order to run the experiment, thus there is some question as to the applicability of these results to living plants. Other authors have investigated the role of foliage in VOC metabolism in living plants (Giese et al., 1994; Wolverton et al., 1983; Wood, 2000) with limited success. In most cases

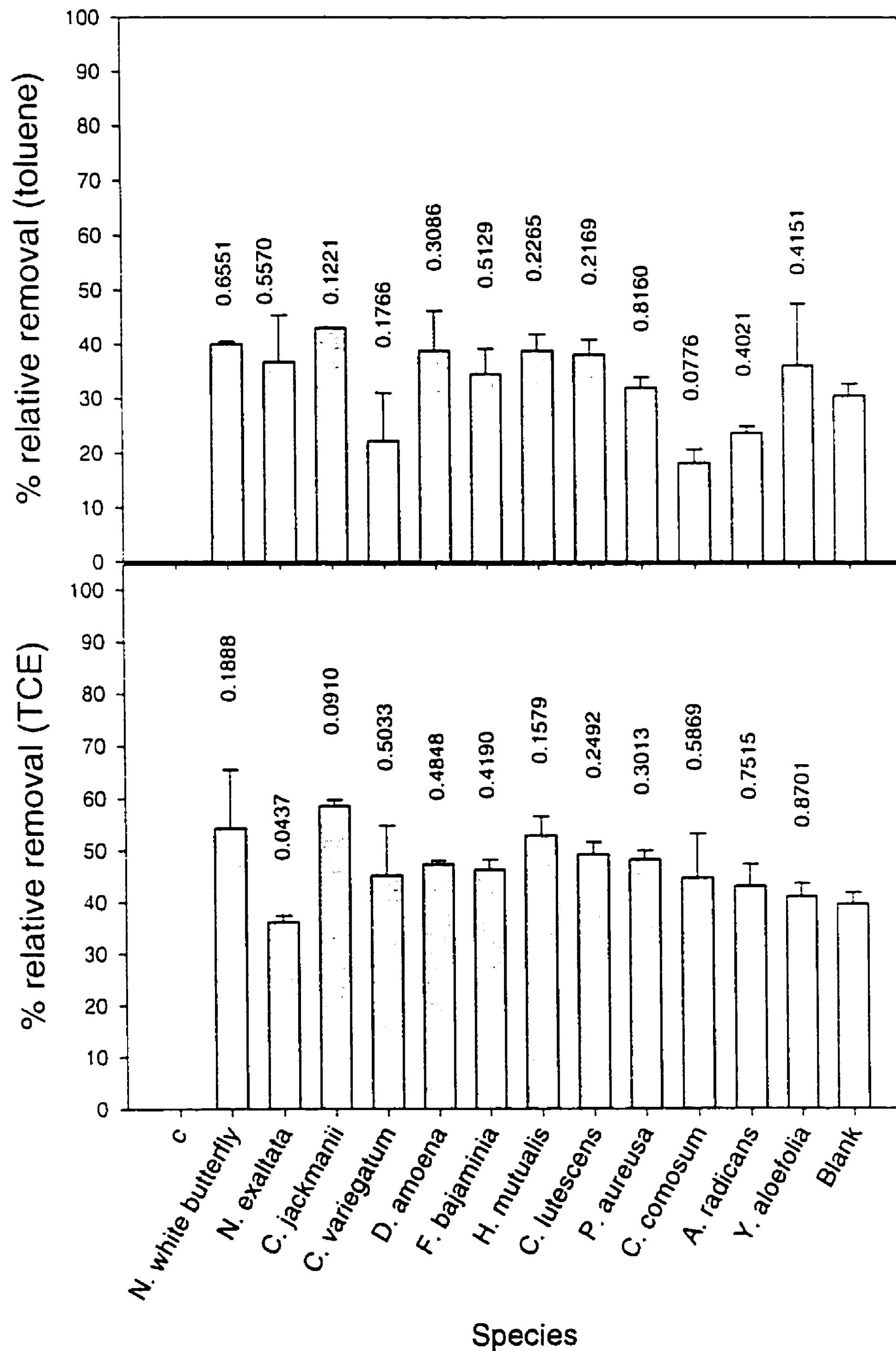


Figure 2.1: Percent relative removal of toluene and trichloroethylene in sealed bottles by the foliage (2-5 g $_{(fwt)}$) of a variety of tropical species. Standard errors are based on 2-5 samples and p-values denote the probability that the treatment differs from the blanks.

metabolism by foliage could not be distinguished from concurrent VOC degradation by microbial communities either on the plant surface or in the soil (Godish & Guindon, 1989; Wood, 2000).

Foliage extraction at the CLER:

Leaf extraction procedures have been successfully used by others to determine the concentration of VOCs in the leaf material of living plants outdoors (Frank, 1989; Gaggi & Bacci, 1985; Keymuellen, 1991; Patterson & D., 1991). Likewise, the procedure was able to detect the presence of all four VOCs in the foliage of all 8 indoor species tested (Table 2.1). *Spathypyllum mona loa* was dropped from the sampling procedure as leaf extractions yielded an extract unsuited to this technique. In the remaining species, variability in the data was greater than expected. Often VOC concentrations ranged over several orders of magnitude. Other authors have also reported large variation in VOC concentrations extracted from leaf tissue (Hiatt, 1998; Keymuellen, 1993). However, the variability reported in this study exceeded both literature reports.

One possible explanation was inadequate mixing of the air in the CLER, particularly since VOC concentrations were very low. The CLER is subjected to less air movement that an outdoor system would encounter, thus it is possible that there were locally concentrated or dilute pockets of VOCs in the space. This might result in differences between the measured room VOC concentration and the concentration affecting the leaf tissue. This point has been made previously (Darlington et al., 1998). A

Species (Days included in average)	Leaf Accumulation ($\mu g\ g^{-1}{}_{fwt}$) (number of samples) ± Standard error			
	Toluene	PCE	Ethyl-benzene	Ortho-Xylene
Verissa splendens (2,3,4,5,6)	1.42 (16) ± 0.54	6.02 (16) ± 2.94	9.10(18) ± 4.93	1.47(15) ± 1.31
Hendra helix (4,5)	2625.28 (6) ±1082.01	344.76 (6) ± 113.28	1583.09 (6) ± 863.26	153.03 (6) ± 65.61
Nephrolopis exaltata (2,3,4,5,6)	0.78 (14) ± 0.28	0.03 (6) ± 0.01	0.20 (13) ± 0.07	0.26 (17) ± 0.064
Dracena fragrans (2,3,4,5)	29.16 (19) ± 17.74	3.02 (8) ± 1.61	9.98 (18) ± 6.08	3.35 (20) ± 2.60
Rhododendron japonica (2,3,4,5)	0.25 (17) ± 0.06	0.20 (15) ± 0.07	0.65 (16) ± 0.14	3668.95 (16) ± 662.76
Aucuba japonica variegata (2,3,4,5)	83.10 (15) ± 58.76	98.03 (10) ± 90.79	106.09 (14) ± 101.25	153.03 (6) ± 65.61
Dieffenbacia manculata (2,4,5,6)	316.38 (12) ± 240.51	71.57 (7) ± 63.98	201.61 (13) ± 183.47	54.34 (13) ± 36.11

Table 2.1: The maximum average accumulation of toluene, tetrachloroethylene, ethylbenzene and ortho-xylene ($\mu g\ g^{-1}{}_{fwt}$) in indoor foliage material. Reported averages, number of samples and standard errors represent the maximum value measured across statistically similar sampling days. Statistical similarity was based on Student-Newman-Kuels muliple comparison tests, statistical analysis is presented in Tables AI.3 to AI.9 in appendix I.

second impact of inadequate mixing in the CLER is the increased diffusive resistance between the atmosphere and the leaf (Nobel, 1999). As a result the extraction procedure may be detecting unmixed pockets of air in the space and not necessarily the sorptive capacity of the leaf material.

A second possibility is the contamination of samples. Due to the nature of the collection site, the lab area was exposed to the same ambient concentrations as the CLER. Great pains were taken to prevent contamination of the extraction solvent with ambient VOCs, and blanks measured before each set of extractions indicated that the solvent was free of contaminants. However, when samples were extracted for five days some may have been contaminated. If this was the case many of the reported values may over estimate of the actual leaf concentration.

Despite variation, the reported values are consistent with the results of other authors reporting on VOC concentrations in leaf tissue collected outdoors. The accumulation of aromatic compounds in leaves of *Rhododendron japonica*, *Nephrolopis exultata* and *Verissa splendens* were similar to the concentrations reported by Keymuellen et al. (1993) in *Pseudotsuga menziesii*. Hiatt and coworkers (1998) report that the leaf concentration of aromatic compounds in several outdoor species ranged 4 order of magnitude from 0.1 to 1070 $\mu g\ g^{-1}$ (dwt). A similar range of leaf concentrations was detected in the leaf tissue of indoor species at the CLER (table 2.1). Notably the VOC accumulations in these studies are expressed as dry weights whereas values here are reported on a fresh weight basis. Patterson and Mackay (1991) suggested that either fresh or dry weights are appropriate and that 3.3 ($\mu g\ g^{-1}_{(fwt)}$ / $\mu g\ g^{-1}_{(dwt)}$) is a reasonable

conversion.

PCE was the most lipophilic of all the compounds tested. It was therefore expected that greater amounts of PCE would accumulate in the leaf tissue (Makay, 1981; Paterson et al., 1995). In most species there was no difference in concentrations of VOCs on a mass basis (see tables AI.3 to AI.9 in appendix I.). Nor could differences be attributed to differences in molecular weight. Molecular weights are 165.85, 106.17, 106.17 and 94.14 for PCE, o-xylene, ethylbenzene and toluene respectively. *Azalea japonica* had a greater affinity for o-xylene than the other aromatic compounds or the lipophilic PCE. *Nephrolopis exaultata* also had less affinity for PCE then the aromatic compounds. No differences were detect between toluene, ethylbenzene and o-xylene in these species.

Changes in leaf concentrations as a result of changes in the ambient concentration were measured in two ways: 1) changes in the average concentration of the detected samples (Figure 2.1) or 2) changes in the number of detectable sample (Figure 2.2). Generally, the aromatic compounds did not exhibit elevated leaf concentrations corresponding with increased ambient concentration on days 3 and 4. In part this may be due to sample contamination. In particular samples collected on day 2 had higher concentrations than samples collected on day 1, even though both were at background VOC concentrations (Figure 2.1, Toluene and O-xylene). As previously discussed the error associated with these results was larger than anticipated and differences between the sampling days may not have detectable against the sampling error. The accumulation of ethylbenzene in *Dracena fragrans* (Figure 2.1 ethylbenzene) is one example of an

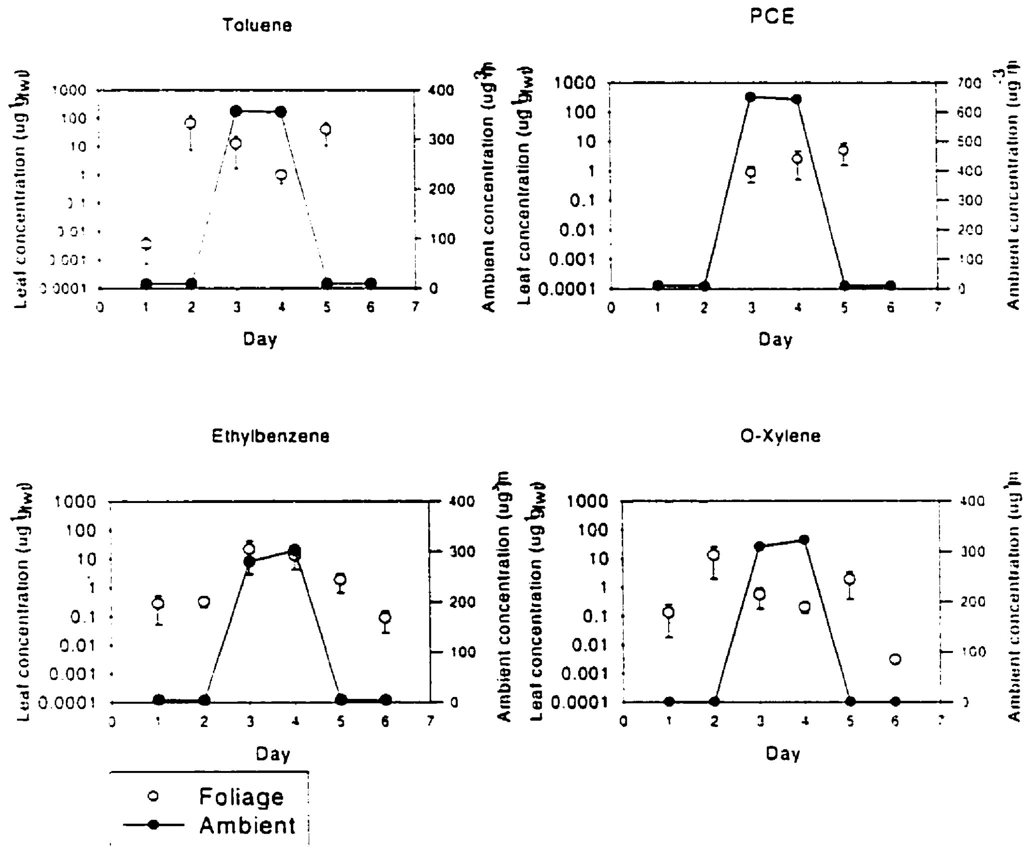


Figure 2.1: Typical accumulation of toluene, tetrachloroethylene, ethylbenzene and o-xylene ($\mu g\ g^{-1}{}_{fwt}$) measured in *Dracena fragrans* at background concentrations ($<10\ \mu g\ m^{-3}$) and at augmented concentrations (380, 685, 310 and 310 $\mu g\ m^{-3}$ for each VOC respectively). Reported averages and errors are based on the detectable samples only. where averages are not reported no detectable samples were measured.

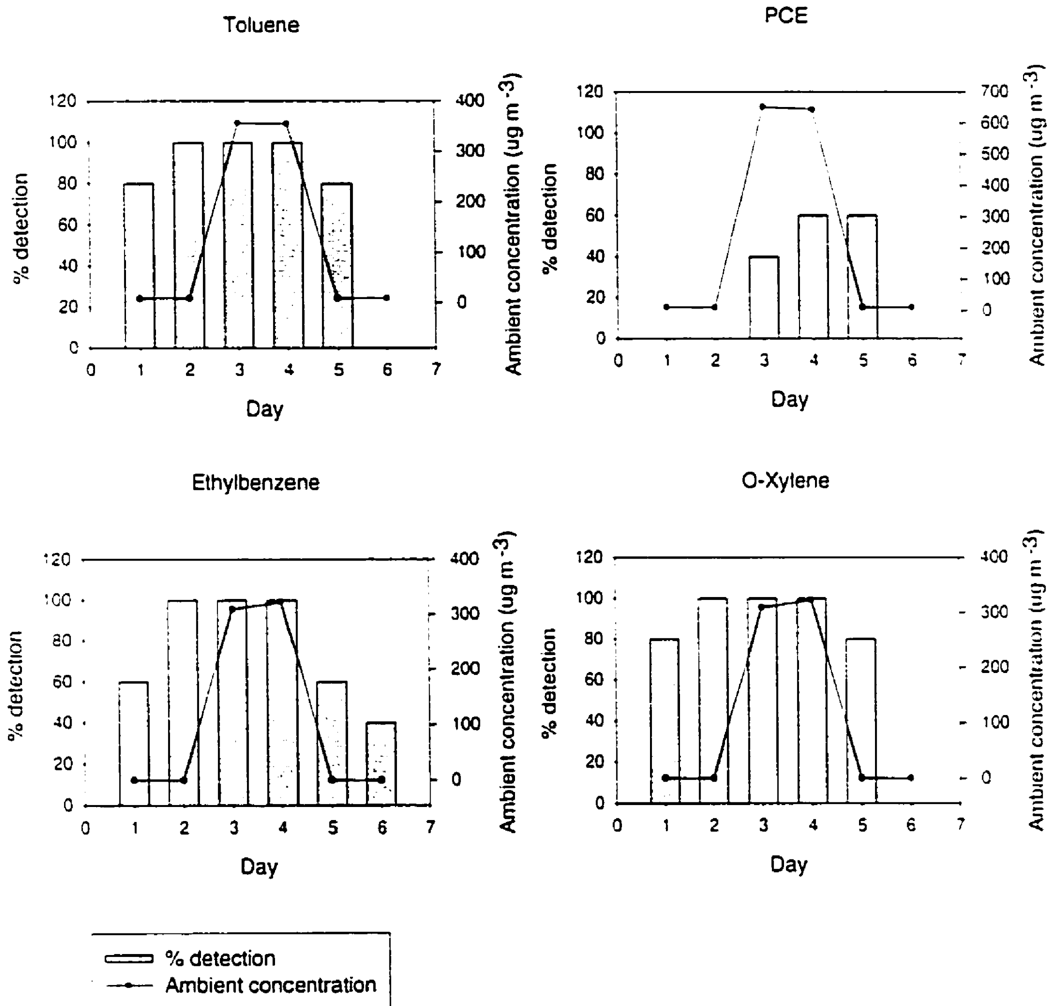


Figure 2.2: The percent of samples where toluene, tetrachloroethylene, ethylbenzene and o-xylene were detected at background (<10 µg m^{-3}) and augmented VOC concentrations (380, 685, 310 and 310 µg m^{-3} for each VOC respectively)

apparent pattern that is not statistically significant.

In some cases PCE became detectable in leaf samples only after exposure to the augmented VOC treatment (100 ppbv or 685 $\mu g\ m^{-3}$) (Figure 2.2). This was the case for *Dracena fragrans*, *Nephrolopis exaultata* and *Hendra helix*. Such a response indicates rapid sorption of PCE to the leaf tissue of some species. Elevated leaf concentrations were also measured on day 5, when the ambient concentrations were returned to background levels.

It is possible that a 48 hour increase in ambient concentration was not long enough to affect a significant change in leaf concentration. Keymuellen and coworkers (1993) found that leaf concentration varied with outdoor concentrations, however, they report a lag of several days between increased ambient and increased leaf concentrations. If this were the case, the leaf air equilibrium established on days 1 and 2 and before the start of the experiment represents the VOC accumulation (Keymuellen, 1993) under typical indoor concentrations (Kostiainen, 1995; Singh, 1988). In terms of a sorptive surface capable of removing VOCs from indoor air the capacity of tropical plants seems limited. VOC concentrations found in leaf tissue in the CLER indicate that there is little storage capacity for VOCs indoors.

While it has been suggested that foliage is a major sink for organic compounds outdoors (Simonich & Hites, 1995), the biomass required indoors to achieve any meaningful removal of VOCs would be enormous. The median toluene concentration . was found in foliage of *Dracena fragrans* which averaged 29.16 $\mu g\ g^{-1}_{(fwt)}$. If this species was to be used to remove a common indoor concentration of toluene from the air, 1 ppbv

or 3.8 $\mu g\ m^{-3}$, in the CLER which has a volume of 640 m^3 (Darlington et al., 2000) over 83 grams of foliage would be required. Furthermore, this rate of sorption can not account for the removal of toluene at the CLER, Darlington and coworkers (Darlington et al., in press) report a mass transfer of 2700.0 μg of toluene per hour through a 0.27 m^2 biofilter 0.02 m deep. To account for the loss of toluene in the space 92.6 g of new leaf material are required each hour, over 2 kilograms each day.

The direct role of plants on the removal of VOCs was minimal. Sorption of VOCs to leaf material had a small impact on the ambient concentration. The bottle experiment did not detect increased rates of VOC removal in bottles with leaf tissue when compared to control bottles. Metabolism of common VOCs by higher plants is not well supported in the literature. Many authors attribute VOC removal to microbial populations associated with higher plants (Godish & Guindon, 1989; Wood, 2000). Thus, higher plants may have the greatest impact on air quality indirectly through the stimulation of rich microbial populations.

Sustained microbial populations may be central to removal of many indoor VOCs (Binot & Paul, 1989; Pritchard et al., 1995). Higher plants are used in the field of phytoremediation to facilitate microbial VOC degradation in soil and water (Anderson, 1993; Shimp et al., 1993). However, interactions between plant and microbial communities in terms of contaminant removal are complex. For a review see Siciliano and Germida (1998).

Microbial populations in the soil are limited by carbon, thus, the community is closely related to the species of plants and the nature of its exudates to the soil (Grayston,

1998). Similarly, microbial population in biofilters may not have adequate substrates to sustain growth under the typically low indoor VOC concentrations (Wani et al., 1997). Thus, the presence of root exudates and decaying material may provide an essential cosubstrates for VOC degradation by microbes (Shimp et al., 1993).

The plant community best suited to this task remains unknown. Tropical foliage species had a limited direct role in VOC removal, although the biofilter as a whole was capable of substantial VOC removal (Darlington et al., in press; Darlington et al., 1998; Darlington & Dixon, 1999). This supports the idea that higher plants act as ecological infrastructure in the biofilter, indirectly improving VOC elimination by sustaining a diverse microbial population. It does not support the idea that tropical species are better suited to this task then other communities of higher plants. It is reasonable to assume that any community capable of supporting microbial diversity will function in the same manner.

Summary and Conclusions:

Extraction of foliar material from the CLER was able to recover VOCs present in the ambient air. The VOC concentrations in the leaf varied with the compound and the species of plant, indicating that some plants have a greater affinity for some VOCs. However, leaf concentrations were too low to have a meaningful impact on the air quality indoors without extremely large quantities of plant material present.

The role of higher plants in an indoor air biofilter should not be limited to the direct impact of VOC removal. Although the sorption of VOCs on the foliage of higher plants is a limited sink for common indoor VOCs plants may play a more important role in the establishment of diverse microbial community. The plants that are best suited to this task are not known.

CHAPTER 3: PLANT SELECTION AND SYSTEM DESIGN.

Introduction:

The biofiltration of indoor air as an alternative or supplementary means of controlling IAQ may be especially applicable in northern climates. As heating costs increase, the economic incentive to restrict ventilation rates also increases. This may allow contaminants to accumulate indoors. Importing tropical species as components of biofilters to these northern sites may be costly, since it would require temperature controlled shipping of the biologicals. Thus, the creation of a tropical ecosystem under these conditions may be cost prohibitive. Also, the characteristics of the higher plant species may not necessarily be critical variables. As an alternative, locally collected or grown species of plants could be substituted for tropical species. Some degree of freedom in plant selection may be available. Previous experiments (Chapter 2) concluded that the direct role of higher plants in VOC removal was small and highly variable and the inclusion of higher plants in indoor bioiflters probably served to enhance the microbial community.

To test the applicability of this technology to remote northern locations a modular biofilter was commissioned with species of plants native to northern Ontario, Canada. In the wild, northern plants are subject to large seasonal variations of many factors including: day length, moisture and temperature. Many of these factors are required for normal plant growth and development. Growth of wild species indoors may be limited by their ability to adapt to the stable environmental conditions indoors. To avoid large plant

losses after biofilter commissioning it was necessary to know in advance which species were adaptable to long term indoor survival.

Preliminary Growth Trials:

A preliminary study was conducted before biofilter commissioning to generate a candidate species list. Forty, 18 L storage containers (approximately 600 cm^2 surface area) were used to collected plant specimens from 1 of 4 natural habitats during the week of June 9 1998. Collection sites were located within a 100 km radius of Sudbury Ontario. Sites were selected because they represented a range of habitats that were targeted for logging or mining activities in the near future. This was done to minimize the potential ecological disturbances resulting from the sampling activities.

Sampling strategy and site descriptions:

Sample plots were selected subjectively to maximize the number of species collected. A range of canopy covers representing variable light environments were selected since light was believed to be a limiting factor to survivability indoors. Samples were collected in their native soil to limit transplantation shock and to include species germinating vegetatively or from the resident seed bank in the survey. Four sites were selected to represent a range of ecological habitats and canopy covers:

Site 1: Floating bog. This site contained very little canopy except on the edges where it was bordered by alder thickets in transition into an alder/birch forest. Most canopy in the middle of the site was provided by a sparse population of black spruce, thus, most species collected from this site received full sun light. The root substrate consisted of a thick layer of sphagnum moss. The water table was 20-30 cm below the surface of the peat. Ten samples were collected at this site.

Site 2: Lowland forest. This site was characterized by a thick canopy layer which provided between 50% and 90% cover. The soil at this site consisted of a thick saturated silt layer. The water table at this site was typically within the first 20 cm of the surface, many samples were taken from standing water. Fourteen samples were collected at this site.

Site 3: Stream bed. An open area bordered by a stream and a marsh, with varying degrees of water flow. Water table was extremely high, most species collected were emergent. Light levels were high, with most species receiving full sunlight for the majority of the day. Three samples were collected at this site.

Site 4: Upland forest. The upland forest was characterized by dense canopy cover and a low density of plants in most areas. Soil type varied from peaty soil, to a highly decomposed humus. Most areas were well above the water table. This was a considerably dryer site than the low land forest. Canopy cover ranged from 25 to 80%

cover. Thirteen samples were collected at this site.

Sample Storage:

Plants were initially stored in a damp shaded area within the greenhouses at Cambrian College (Sudbury, Ontario). They were grown in 80 to 100 % shade during the summer season by storing them under benches surrounded with a burlap skirt. To lower the ambient temperature and raise relative humidity a drip line was attached to the top of the burlap. The constantly flowing water maintained a cooler temperature than that of the surrounding greenhouse, which at times exceeded 40 °C. Temperatures under the benches, where the plants were located, did not exceed 30 °C.

Plants were watered regularly, using tap water which had been allowed to dechlorinate by holding it in open storage tanks for at least 24 hours. To account for characteristic water table depths of each habitat, drainage holes were punched into the side of each container.

Plants were moved to an indoor location the last week of September 1999, and kept on a 12/12 light cycle. The light was provided by commercially available flourescent and incandescent bulbs commonly used in indoor spaces. Light levels ranged from 17 to 50 μmol m^{-2} photosynthetically active radiation (PAR). Watering continued regularly throughout the experiment, despite changes in location.

Surviving Species and Characteristics:

Twenty-one species either survived the low light treatment for approximately 10 months, or were able to propagate from the seed bank after sampling. Interestingly, all surviving species were perennial species. Many of the species exhibited signs of nutrient deficiencies towards the end of the observation period. No attempt was made to assess the fitness of individuals as survival was considered the sole criteria for success. Survivability was influenced by collection site (Figure 3.1). Although there was some species overlap between collection sites, each site contributed several unique species. A notable exception was the river bed site which had no surviving species.

In an attempt to generalize successful habitats, the reported environmental preferences were compiled from field guides and native species identification manuals (Chambers, 1996; Legasy, 1995; Newmaster, 1997; Peterson, 1977; Petrides, 1972). Most surviving species were reported to tolerate moist conditions. It was possible that soil moisture was maintained too high. As a result more water tolerant species may have been selected, not on the merit of survival under indoor light conditions, rather on a combination of lighting and watering factors. Nevertheless since the proposed modular biofilter is a hydroponic system, water tolerance would be an asset to species in the final biofilter commissioning.

Three of four sampling sites yielded species that were able to survive indoors for the observation period. The bog collection site yielded the greatest number of surviving species, including all of the surviving woody plants. There was little difference between

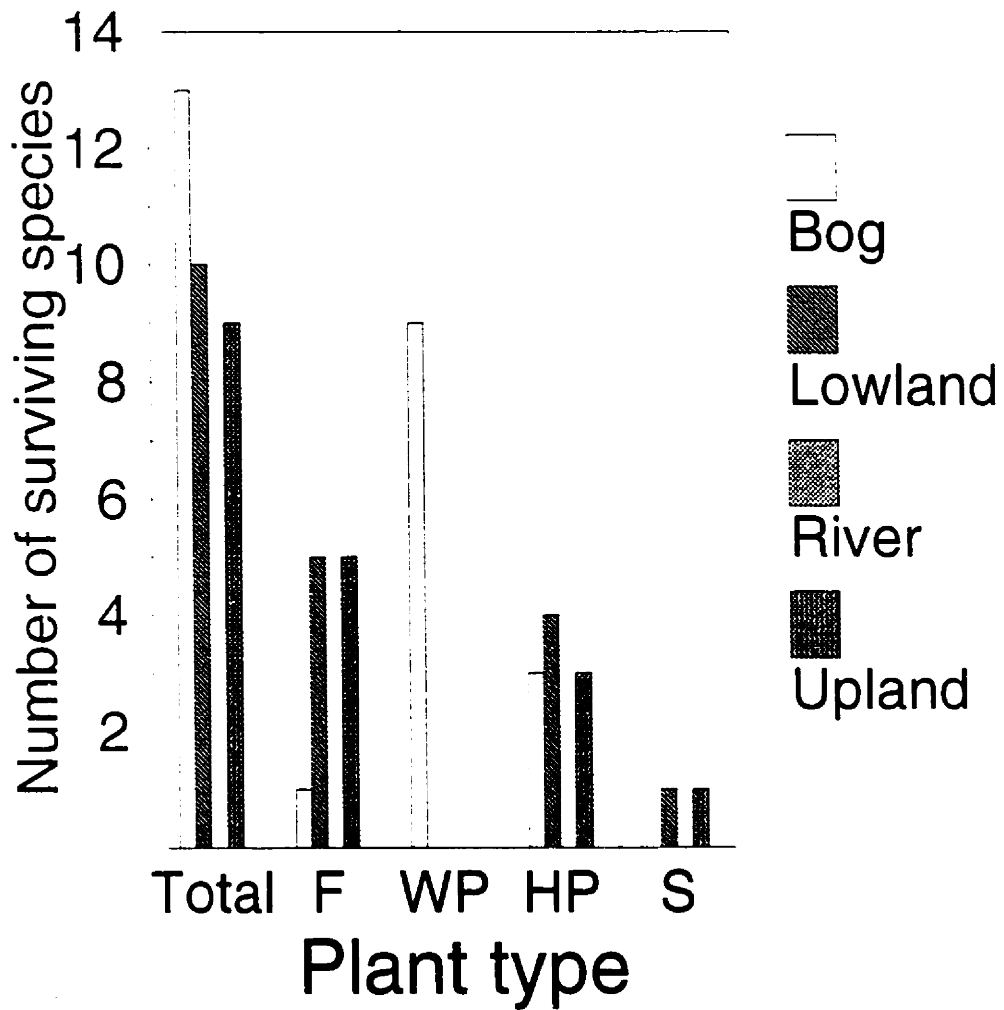


Figure 3.1: Number of species from four habitats surviving a 10 month low light trial. Successful species are organized by plant type: ferns (F), woody plants (WP), herbaceous perennials (HP) and sedges (S).

the upland and lowland forest sites in terms of the number of surviving ferns or herbaceous perennials, however, collections from both sites contained unique species.

Collection Strategy:

In the bog, 65% of surviving species maintained or increased their occurrence in the preliminary trial. There was likely no advantage to emphasizing either transplants or seed bank specimens from this site. Most surviving species grew in bogs, thus collection emphasis in general should be put on the bog site. In the lowland site an equal number of species increased and decreased their occurrence. This suggested a rapid turnover in the population perhaps indicative of an active seed bank or other propagation sources in the soil. Species surviving from the upland site were very stable with 80% of species collected from this site not changing in their occurrence. Transplants should also be emphasized from this location. The riverine site had no surviving species, future collections may attempt to increase survivorship from this site by providing a greater light supply.

Biofilter Commissioning:

The biofilter was located in the foyer of the Northern Centre for Advanced Technology (NORCAT , Sudbury Ontario). The building volume was approximately

20,000 m^3, although the area affected by the biofilter was substantially smaller, approximately 3670 m^3.

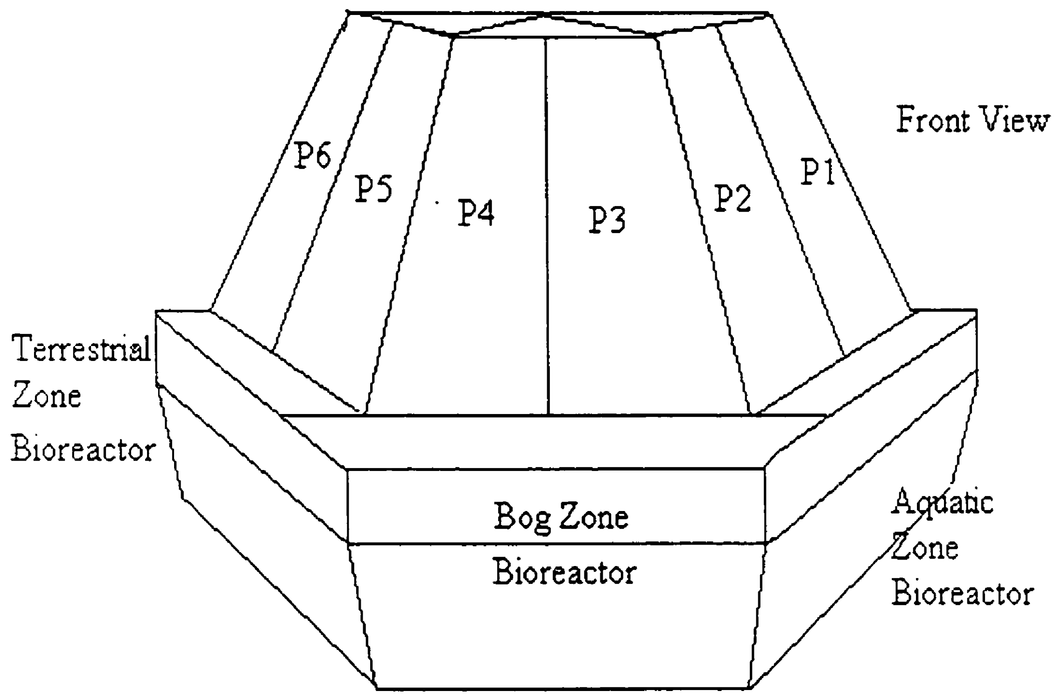
The modular biofilter took the shape of half a hexagon composed of 3, 60° wedges on a 10° incline from vertical. Each wedge contained two biofilter panels divided vertically down the centre, creating a total of 6 individual biofilter panels. The surface area of each panel was 0.67 m^2 (Figure 3.2). Panels were covered with a coconut fibre erosion fabric which acted as a porous backing material for the biologicals on the bioiflter surface.

A continuous catchment basin ran along the base of the biofilter panels. The basin served two purposes; it collected excess water from the irrigation of the biofilter panels and provided habitat for plants to be grown in the system. Microbial communities in the basin may degrade compounds not degraded on the biofilter surface. In this sense, the plant zones also act as a bioreactor and the panels act as bioscrubbers. Contaminants sorbed on the panels but not metabolized could be washed into the basin for further metabolism.

Water flowed through the catchment basin in a recirculating loop. Excess water from the panels was added to the loop at any point, but could only drain to the reservoir through one drain. The basin was divided into three planting zones; an aquatic zone, a bog and a terrestrial zone. Plants specimens were collected and transferred into the respective planting zone on the same day (Julian date 290).

Aquatic zone: This zone contained 0.20 m^3 of open water to a depth of 45 cm held in place by a baffle. Water temperature of this zone was 19.4 ± 0.8 °C (based on daily measurements between January 3rd and June 7th 2000). Circulation of water through this zone relied on the irrigation to panels 1 and 2. Excess water drained into the aquatic zone displacing water over a 45 cm deep baffle designed to prevent the flow of water from the aquatic to the bog zone. The retention time of water in this zone was 1 day.

Plant specimens in the aquatic zone were collected from the stream bed collection site and planted in plastic baskets. Riverine species had low survivorship in the preliminary plant collection. An attempt was made in the biofilter prototype to increase survivorship by increasing the light supply in this zone and in the biofilter in general. However, even with the augmented light supply, species collected from this site had very low survivorship in the biofilter.



Top View

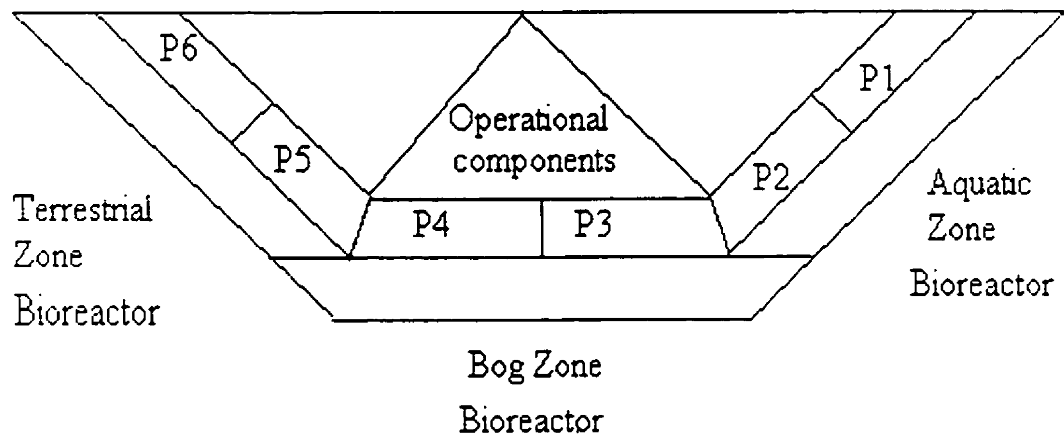


Figure 3.2: Modular biofilter schematic including the location of biofilter panels, bioreactor planting zones and operational components (presented in detail in Figures 3.3 and 3.4).

Bog zone: Excess water from the aquatic zone drained into the bog over the 45 cm deep baffle. The water in the bog zone varied in depth periodically from 0 to 15 cm depth below the bog surface. This varied with the irrigation cycle, with the highest water levels occurring immediately after irrigation (panels were irrigated every 0.5 hours). Bog species (see Table 3.1) were planted in a mixture of rock wool and organic material collected from the bog and supplemented with peat moss.

Inclusion of bog species in the biofilter served two purposes. First preliminary trials suggested that bog species were well suited to indoor conditions. Secondly, the anerobic conditions in natural bogs provide ideal conditions for the degradation of some compounds (Mars et al., 1998; Sun & Wood, 1997). Like the aquatic zone, the bog was designed to act as a bioreactor. In this case offering an anerobic environment degrade compounds. Vertical baffles were installed in the bog to redirect the water flow through the bog and limit short circuiting, thereby increasing the water retention time through this section and improve its potential contaminant removal ability.

Species	Common Name	Biofilter Zone
Kalmia angustifolia	Sheep laurel	Bog
Veronica officinalis	Common speedwell	Terrestrial
Cornus canadensis	Bunchberry	Terrestrial
Oxalis acetisella ssp. Montana	Upright wood sorrel	Wall, Terrestrial
Mitella nuda	Naked mitrewort	Wall, Terrestrial
Coptis trifolia	Goldthread	Wall, Terrestrial
Maianthemum canadense	Canada mayflower	Wall, Terrestrial
Linnaea borealis	Twin flower	Terrestrial
Kalmia polifolia	Bog laurel	Bog
Ledum greonlandicum	Labrador tea	Bog
Chamaedaphne calyclata	Leatherleaf	Bog
Vaccinium oxycoccus	Bog cranberry	Bog
Vaccinium ovalifolium	Oval leafed bilberry	Bog
Acer saccharum	Sugar maple	Terrestrial
Larix laricina	Tamarack	Bog
Picea mariana	Black spruce	Bog
Thuja occidentalis	Eastern white cedar	Terrestrial
Populus balsamifera	Balsam poplar	Terrestrial
Lycopodium annotinum	Interrupted club moss	Terrestrial
Polypodium virginanum	Common polypody	Wall, Terrestrial, Bog
Drypoteris marginalis	Marginal wood fern	Wall, Terrestrial, Bog
Gerum macrophylum	Large leaved avens	Wall, Terrestrial, Bog

Table 3.1: Final species composition and location within the modular biofilter.

Water quality in the bog was measured in a sampling tube installed in the center of the zone. Initially the bog was characterized by a slightly lower pH and low levels of dissolved O_2 when compared with the other planting zones. Over time however, the bog pH increased to the level measured in the other zones (6.79 ± 0.84). A similar phenomenon was observed with dissolved O_2. Although the bog was initially an hypoxic zone (2.5 ppm O_2) over time it became O_2 saturated. However, the degree to which an anerobic or low pH environment was maintained is questionable since pockets of anerobic activity in this zone would not have been detected by the sampling procedure.

Terrestrial zone: Low pH water from the bog was initially drained into a buffer zone present between the bog and terrestrial zones. Bog water flowed over a second baffle and onto a bed of crushed oyster shells. A simple aeration system ensured both mixing and aeration of the water in the bed. Buffered water overflowed into the terrestrial area where the water table was originally maintained from 15-30 cm below the surface. A combination of high salt measurements and relatively neutral pH readings in the bog prompted the removal of the buffering system.

Species transplanted into this zone were collected in the upper soil horizon and consisted of shallow rooted plants. These were placed directly on a rockwool layer of 30 cm deep. Over time compaction of the rockwool raised the water table, so that immediately after an irrigation cycle water was at the surface of the terrestrial zone. Plants from the upland and lowland collection sited were planted in this zone (Table 3.1).

Biofilter surface: Moss was collected at the same time as the higher plants and stored in sealed zip lock bags with 0.25 L of dechlorinated tap water. Samples were stored in a well lit location for approximately 40 to 50 days prior to commissioning.

The surface of the biofilter was commissioned over a period of approximately 10 days (Julian dates 310 to 320). Moss was washed in the biofilter hydroponic water supply to remove any excess detritus or soil particles. It was attached to the wall surface by tucking sections under a 1 cm by 1 cm plastic mesh sewed into the coconut fibre. Although there were several species of moss were present the predominant species was *Eurhynchium riparoides*.

A 2 cm x 2 cm grid constructed of fishing line, was placed over each panel and 50 random quadrats were measured for moss depth and coverage. This provided an estimate of biofilter thickness after 4 and 6 months of growth. Average biofilter thickness varied between panels from 10 to 12 mm, likewise coverage ranged from 61.1 to 85.0 %.

Environmental Systems:

Lighting system:

Light was supplied to the biofilter through 8, 150 watt halogen lamps. Limited natural lighting was available in the foyer of the NORCAT building, thus the supplemental lighting was the main source of lighting. Lamps were located 1 m from the top of the biofilter and approximately 1.8 m from the catchment basin. This system was

limited in its ability to deliver light uniformly across the biofilter surface. Plants on the biofilter panels and in the catchment basin received between 42 and 75 µmol m^{-2} s^{-1} PAR. The best lit sections of the surface were generally in the middle of the panel (0.8 m from the basin) and lower.

Air Path:

The modular biofilter (Figure 3.3) moved air through the biofilter by a dedicated air handling system. The biofilter had a total of six replicated panels which included a 0.67 m^2 coconut fibre surface over a 20 cm deep plenum. Air was pulled through all biofilter panels by a communal 30 cm exhaust fan connected to each plenum by a series of 10 cm ducts. The duct work was attached to each panel in two locations designed to provide a uniform pressure gradient across the surface of each panel. The airflow through each panel was controlled independently by baffles installed in the connecting duct work. A combined air stream was vented vertically (Figure 3.3) into the 3670 m^3 air space at a rate of 0.23 m^3 s^{-1}.

Atmospheric Monitoring and Control:

Flux measurement. Flux through the biofilter surface was calculated from the air velocity (m s^{-1}) in the ducts. Due to non laminar flow, the air velocity in each tube was calculated as the average of 8-12 spot measurements. The reported velocities in panels 1,

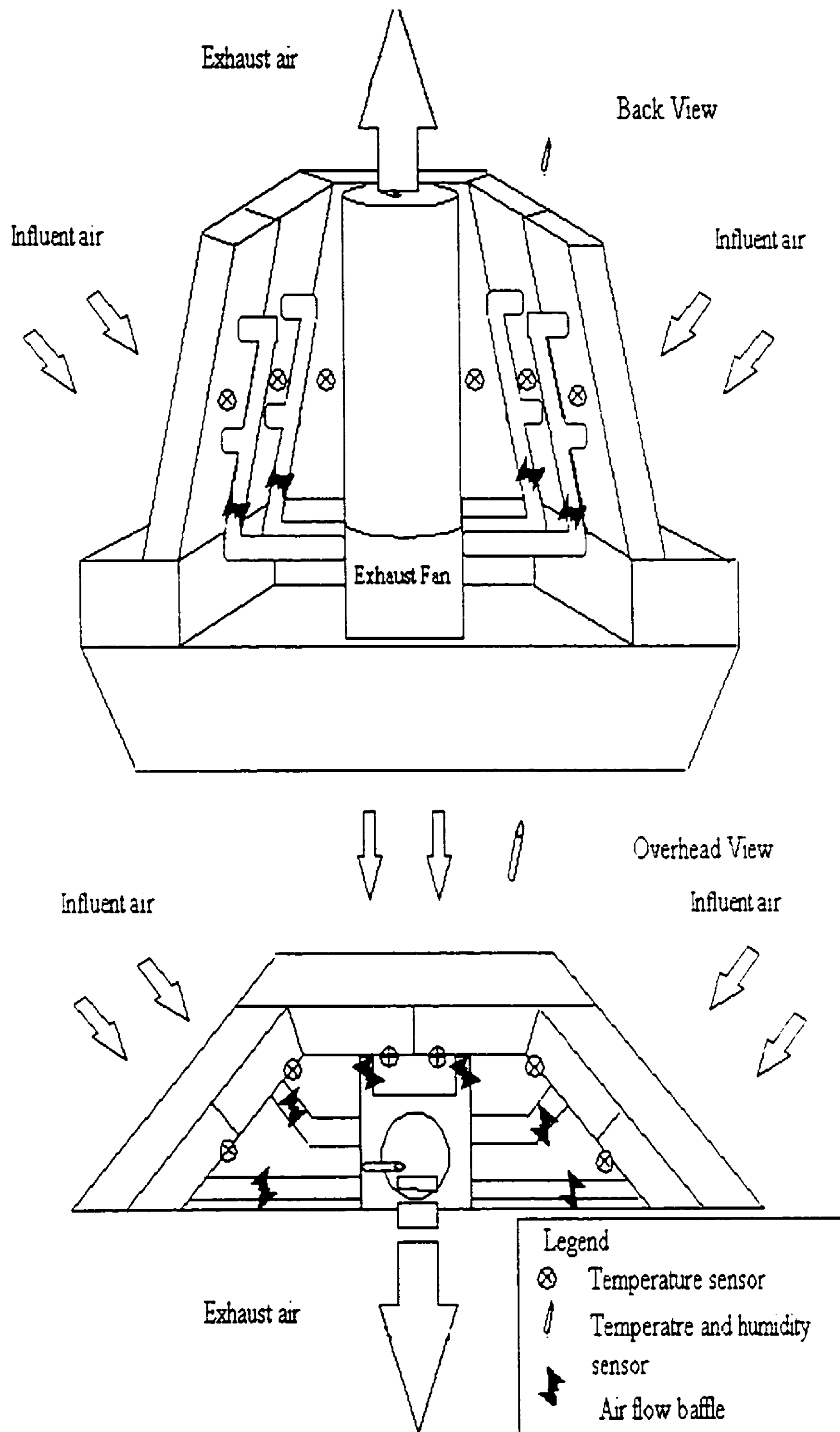


Figure 3.3: The air path schematic for the modular biofilter including location of exhaust fan, temperature and humidity sensors and air flow baffles and air flow direction.

2, 5, and 6 was the average of 12 spot measurements. Measurements were taken at 1.5, 4.0, 6.5 and 9.0 cm depths across 3 cross sectional axes through the duct. Reported velocities in panels 3 and 4 were average values measured at the same depths across duct 2 axes. Velocity was measured with a Velocicalc air velocity meter (model 8345, TSI Inc., Shoreview MN.).

Temperature and humidity monitoring. Temperature was measured in the exhaust airstream of each panel using an Omega RTD temperature transmitter (model TX94, Omega Engineering Inc., Stanford CT). Temperatures and relative humidities were measured in the room and in the 30 cm exhaust vent with Omega SS RH probes (model HX94c, Omega Engineering Inc., Stanford CT.) (Figure 3.4). Temperature and humidity data was automatically logged every two minutes using a National Instruments Analogue Input Card. Reported data consisted of hourly averages of the raw data.

In line gas chromatography. Gas samples were drawn through 1/8" copper tubing from 7 locations (the ambient air and the exhaust of each of the 6 panels). Exhaust measurements were taken between each of the plenums and the exhaust fan. Selection of the gas sampling site was automated through a multiplexer and controlled through the gas chromatograph (GC). Upon receiving signal from the GC, the multiplexer activated the appropriate solenoid and air was drawn from the specific sampling location to a 10 position gas sampling valve with a 1 ml sample loop (Valco Instruments Inc. Houston TX). In the 'Load' position, the loop was open and thoroughly flushed with sample air

for several minutes. In the 'Inject' position, the loop was closed and a 1 ml sample was isolated and injected into the GC for analysis.

VOC concentrations were analyzed using either an SRI model 310 GC or an SRI model 8600a GC. In either case, analytes were separated through a 0.53 mm OD, 30 m RESTEK MXT - volatiles column and detected using a photoionization detector (PID) set on high gain. The column temperature was isothermic and varied from 45 to 90 °C (depending on analysis). The column head pressure was isobaric at 10 psi.

Ambient VOC control. Ambient concentrations of VOCs were controlled through a feedback system, linking the in-line GC (described above) and a custom VOC emission system. The emission system was programable with up to 4 diurnal VOC profiles. In a typical case, the target VOC concentration started at 0 ppbv at 16:00 ramped up to 60 ppbv at 24:00 and back to 0 ppbv by 08:00. The influent concentration of each VOC was measured by the GC, the information was transmitted to the emitter system through an RS232 connection. Upon receiving the influent concentration, the emitter system compared it to the target concentration. If the ambient concentration was less than the target concentration the emitter system added solvent saturated air to the exhaust stream of the bifilter. Solvent saturated air was generated by pulsing air through a midget impinger containing pure solvent. The quantity of solvent added to the space was controlled by controlling the pulse duration (a function of the difference between the actual and target concentrations). Each solvent had a separate dedicated air pump and impinger system allowing the concentration of each VOC to be controlled separately. If

the ambient concentration was greater than the target concentration no solvent was added to the ambient air, allowing the concentration to slowly decrease through biofiltration and leakage from the building. This exposure technique has been described in detail elsewhere (Darlington & Dixon, submitted).

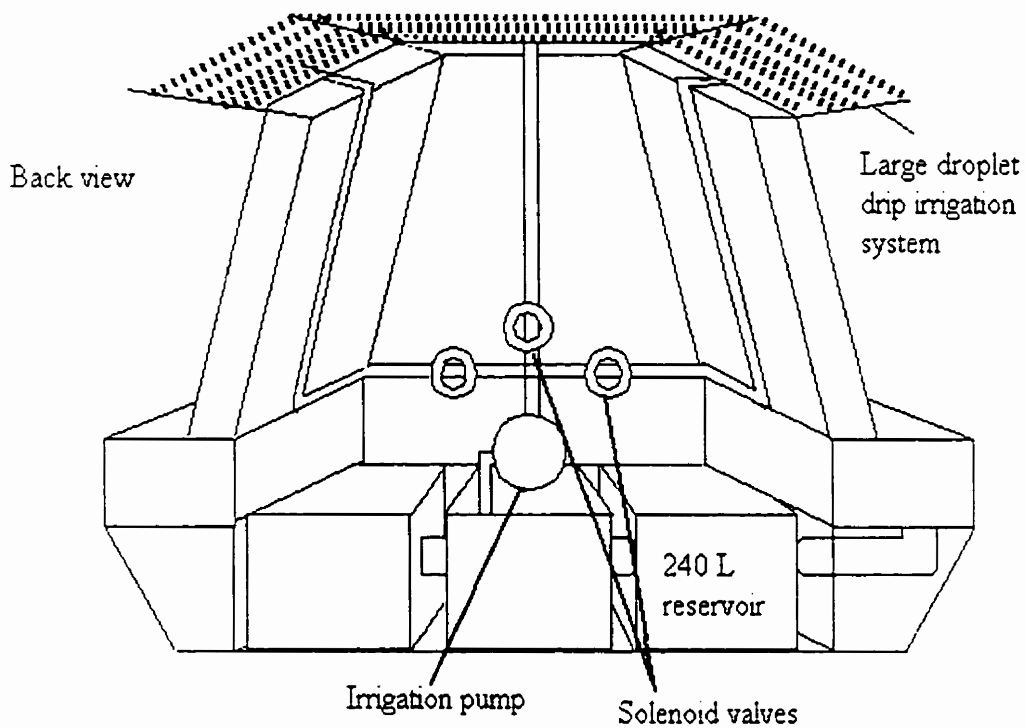
Septum bottle calibration technique: The GC was calibrated against gaseous mixtures of the target compounds. Calibration mixtures were made in glass bottles modified to contain a septum. A concentrated mixture was created by injecting 1.0 μL of pure solvent into the 1 L bottle. To avoid needle discrimination air was drawn into the syringe before and after the pure solvent so that the precise volume of solvent could be recorded. All target solvents were added to the mixture at the same time and 5 minutes was given to allow the solvents to fully evaporate in the bottle. A stock solution was created by injecting 10 ml of the concentrated mix into a 1 L bottle, reducing the concentration by a factor of 100x. Calibration curves were generated by measuring the concentration of a series of bottles diluted from the stock bottle. To avoid cross contamination of samples, the 10 ml glass syringe was thoroughly flushed with carbon filtered room air between all gas transfers.

Water Monitoring and Control:

Watering schedule and delivery. The biofilter surface was watered with a custom large droplet drip system (Figure 3.4). This system proved to be limited in its ability to deliver water uniformly across the biofilter surface. To compensate, some areas were over watered to ensure that the majority of the surface received minimum water. The watering schedule was adjusted so that all biofilter panels were still wet to touch immediately prior to the beginning of the next irrigation cycle. Each section was watered for approximately 45 seconds every 30 minutes.

Watering cycles were automatically controlled through custom software and a data acquisition card (National Instruments Corp., Toronto, ON) which, through a series of relays, controlled power to a diaphragm pump (Pump Systems Inc., Dickinson, ND) and three solenoid valves (SV-303 1/4" brass Omega-flo 2 way general purpose solenoid valves, Omega Engineering Laval, QUE). At the beginning of each watering cycle, the computer would activate the relays to provide power to the pump and sequentially open the solenoid valves to water each of the biofilter wedges.

Irrigation water was pumped from the 240 L reservoir to the biofilter surface. The reservoir consisted of three 80 L containers connected through ABS tubing. Water was pumped from the central container through the large droplet drip irrigation system: consisting of a series of 5 1.25 cm ID chlorinated polyvinylchloride (CPVC) tubes located above the biofilter surface (Figure 3.5). Water was forced through 0.8 mm holes drilled at



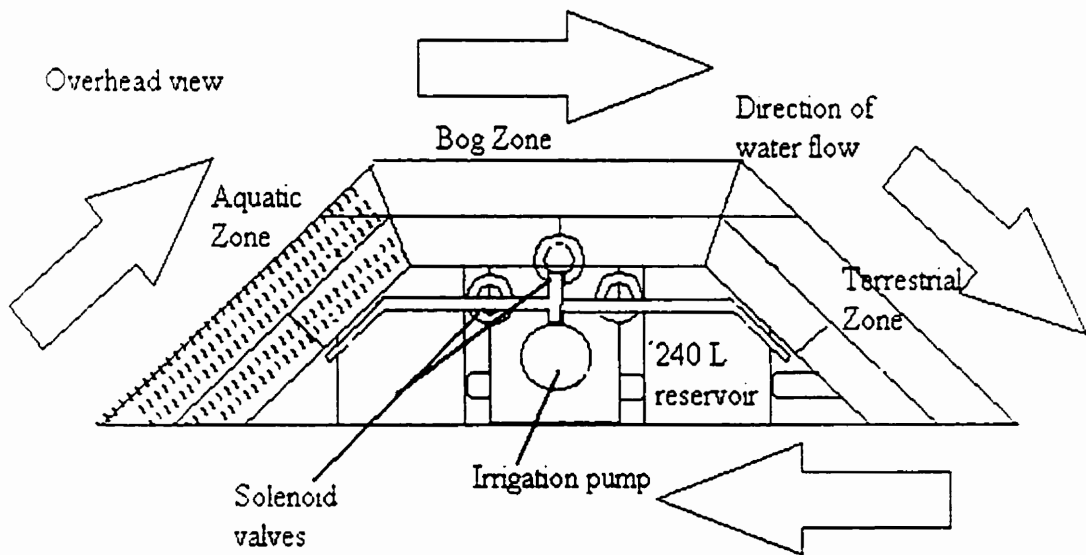


Figure 3.4: The water path schematic of the modular biofilter including the location of the irrigation pump, solenoid valves, large droplet drip irrigation system and water reservoir and flow direction.

2.5 cm intervals along the length of the tubes.

Water lost from the system through the combined processes of evaporation and transpiration averaged of 69.2 ± 2.1 L day^{-1}. An additional 18.8 ± 1.7 L day^{-1} of water was required to maintain water quality in terms of electrical conductivity (EC) and pH. Therefore the total water requirements of the system averaged 88 L day^{-1}.

Water quality monitoring. Parameters such as pH and EC were measured daily (Figure 3.5) using hand held pH and EC metres. Measurements were recorded from the reservoir, aquatic, bog and terrestrial zones in the catchment basin.

It was possible through the monitoring exercise to maintain the water quality parameters within an acceptable range. The average pH of the system was 6.79 ± 0.84. Average conductivity was 354.63 ± 41.57 µS cm^{-1}. Instrumental failure between julian date 95 and 135 allowed the EC to climb to above 450 µS cm^{-1}. However, several consecutive days of system flushing was able to return EC to an acceptable level (Figure 3.5).

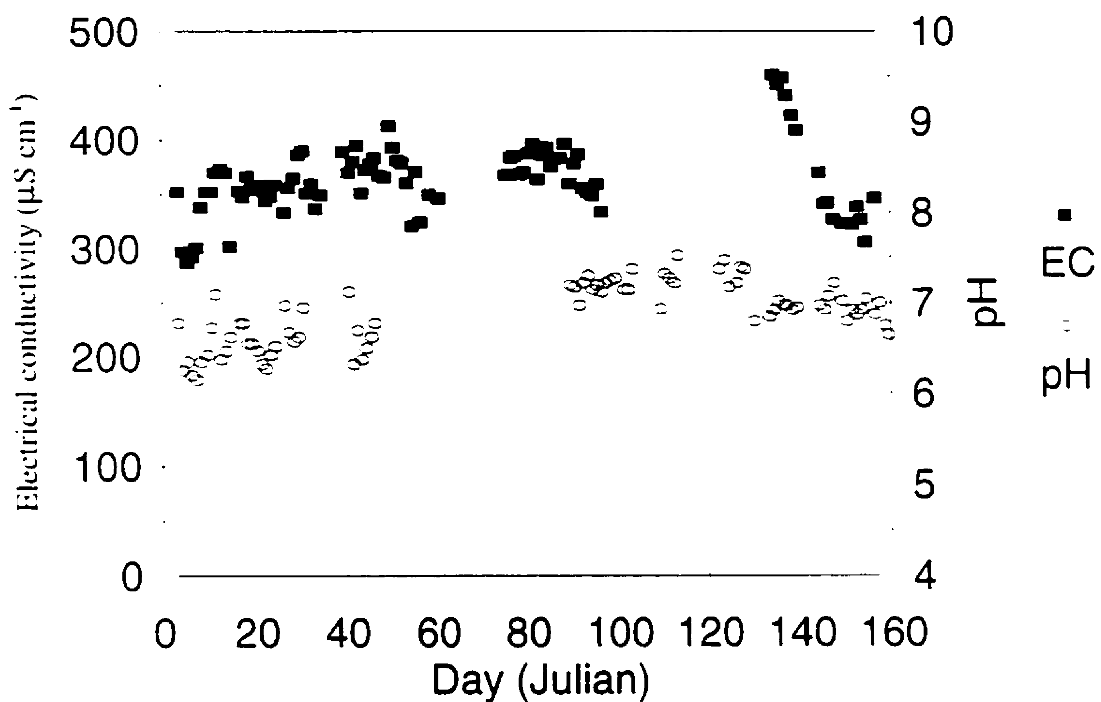


Figure 3.5: pH and EC (µS cm^{-1}) measurements during 160 days of biofilter operation. Each data point is the average of four samples, one taken in the aquatic, bog and terrestrial bioreactor zones and the reservoir.

Nutrient Balance: Water samples were collected on a daily basis from the reservoir prior to the addition of water. Water was filtered through a 0.2 micron filter into a 25 ml sample bottle and frozen until analysis. Water samples were analyzed for nutritional anions and cations with a Dionex high pressure liquid chromatograph (HPLC) . Cations were separated through a DX-120 column at a pressure of 1450 psi and a flow of 2 ml min^{-1}. Anions were separated through a DX-500 column at a pressure of 1226 psi and a flow of 1 ml min^{-1}.

The modular biofilter tended to maintain higher concentrations of nutrients than a typical marsh but less than a riverine marsh (Richardson, 1978). Nutrients were generally stable over the 140 day monitoring period (Figure 3.6). Notably phosphorous and nitrogen are almost absent from the system, suggesting that the addition of some nutrients may benefit the health of the system. The addition of nitrogen, phosphorous and potassium is common in industrial biofilters to maintain high removal rates (Devinny et al., 1999).

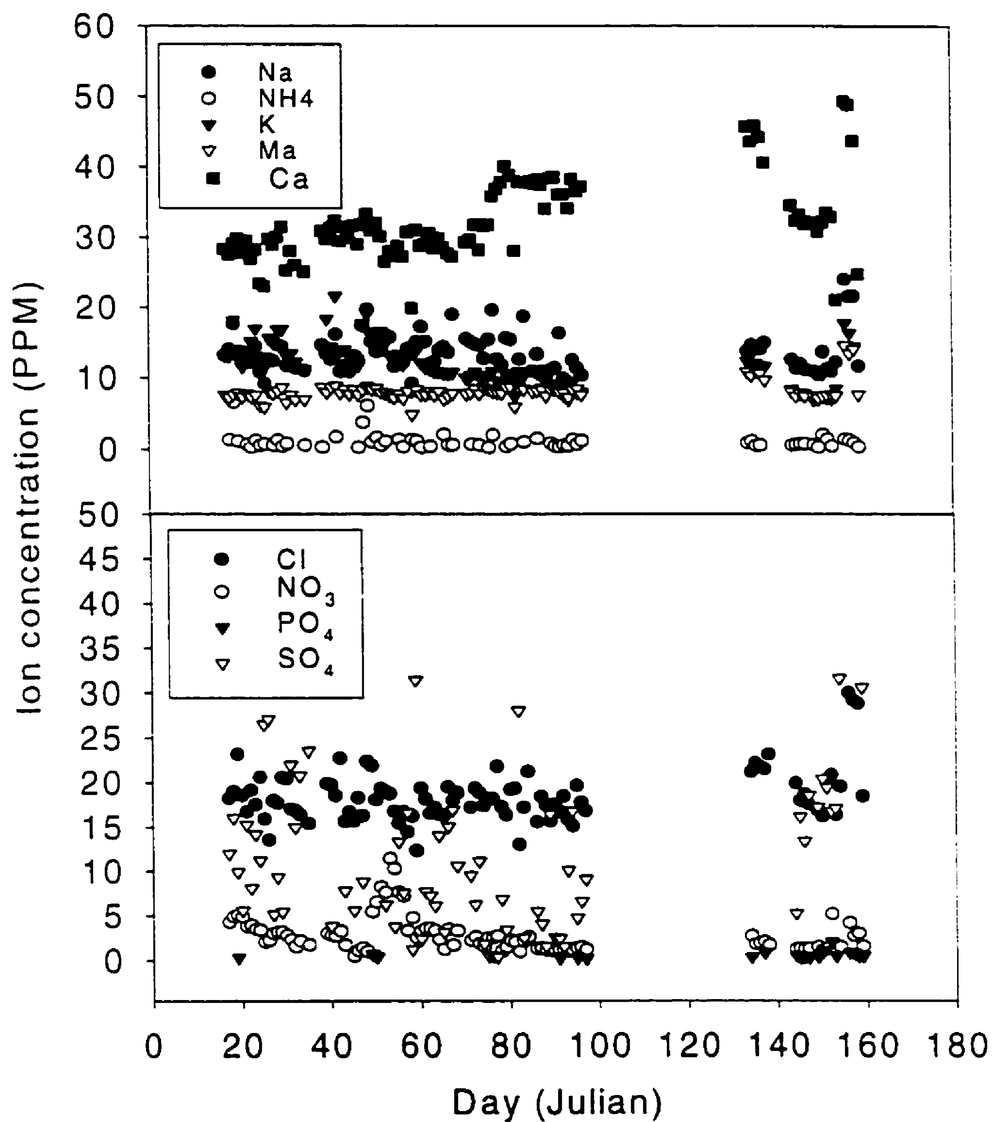


Figure 3.6: Anion and cation nutrient concentrations in parts per million (ppm) measured in the biofilter reservoir over 160 days of biofilter operation.

Conclusions:

An ecosystem based on northern plant species was constructed as part of an indoor air biofilter. A preliminary collection of plants was observed over a 10 month period to determine which species were suited to indoor survival. Three of four collection sites yielded species able to adapt to indoor conditions for the observation period. Increased lighting was installed in the final biofilter construction to encourage the survival of riverine species. However, this attempt was not successful.

Once commissioned, the system contained 6 biofilter panels covered in locally collected moss species. Higher plants were grown hydroponically in a bioreator. It was possible to control environmental factors such as lighting, electrical conductivity, pH and moisture to create an adequate environment for plant survival. The plants received low levels of supplemental lighting throughout the experiment. Nutrients in the water were low but comparable to other natural and constructed systems.

CHAPTER 4: IMPACT OF AN INDOOR AIR BIOFILTER ON AIRBORNE SPORE CONCENTRATIONS.

Introduction:

The modular biofilter included a diverse selection of northern plants, mosses and their associated microbes, the assumption being that a diverse system would be better able to assimilate a dilute mixture of volatile organic compounds (VOCs) (Anderson, 1993; Shimp et al., 1993). Indoor plantings, however, have been associated with the production of bioaerosols such as airborne spores (Burge et al., 1982) particularly after disturbance by air movement or watering. Since such disturbances are common in indoor air biofilters and since many of the species included in the modular system are not typically grown indoors, it must be established that they are not a source of airborne spores. Production of bioaerosols such as these could reduce indoor air quality (IAQ), despite the removal of volatile organic compounds (VOCs).

Although, rarely are bioaerosols responsible for serious or life threatening infections, among immunocomprimised individuals bioaerosols may cause respiratory tract infection, allergic rhinitis, asthma, humidifier fever and hypersensitive pneumonitis (ASHRAE, 1998; Dales, 1991). Allergic response to bioareosols is far more common, affecting between 6% and 15% of the population (Miller, 1992). Three categories of bioaerosols were considered in this study; viable fungal spores, viable bacteria and *Legionella pneumophila.*

Viable Fungal Spores:

Fungi are common indoor contaminants that have been linked to several health problems, including, asthma, sick building syndrome and allergies (Flannigan, 1994; Norback, 1994). The availability of moisture is a limiting factor for the growth of most

moulds indoors (Miller, 1992; Singh, 1994). As a result mould growth tends to reach unhealthy levels in the presence of damp or wet areas. A Swedish survey (Norback, 1994) found that 50% of buildings with excessive mould growth also had water damage.

Despite this, the relationship between mould growth, sporulation and moisture is complex. Often "mouldy dwellings", with excessive fungal growth, do not have higher airborne fungal spore loads than "healthy dwellings" (Nevalainen et al., 1994). Increased relative humidity significantly reduced the airborne concentration of fungal spores for three common indoor genera (Pasanen, 1991). However, Foarde and coworkers (1994) tested the viability of moulds under different relative humidity regimes, and found that there is a minimum humidity required to sustain mould colonies. While water is a limiting growth requirement for moulds (Norback, 1994; Singh, 1994), sporulation is encouraged at lower relative humidities (Pasanen, 1991). The modular biofilter both contained standing water which would contribute to mould production and added water vapour to the space which would discourage sporulation.

There is substantial variability in the number of fungal colonies measured indoors. Mouilleseaux and coworkers (1994) reported the indoor fungal counts to range from a few colony forming units to 700 colony forming units per cubic metre (CFU m^{-3}). However, the bulk of the buildings surveyed had fewer than 50 CFU m^{-3}. The three year average of Canadian federal buildings reported fungal bioaerosol loads of 40 CFU m^{-3} (Annon., 1995). Darlington and coworkers (2000) reported an increase in the viable spore load in the air at the Canada Life Environmental Room, they report a mean value of 115 CFU m^{-3}.

A healthy indoor spora generally has a mixture of species similar to outdoors, with reduced numbers (Flannigan, 1994; Mouilleseaux & Squinazi, 1994; Nevalainen et al., 1994). As such, there is evidence of a seasonal variation in indoor spora (Reponen, 1992). Health Canada (Annon., 1995) guidelines recommends that no more than 50 CFU

m^{-3} of any single species be present in the air stream, excluding *Cladosporium sp.* or *Alternaria sp.* that are extremely common outdoor fungi. A combined total, of all species present, should not exceed 150 CFU m^{-3} in all seasons excluding summer. Fungal loads of 500 CFU m^{-3} are acceptable during the summer if the species present are of the genus *Cladosporium* or other tree and leaf fungi.

Viable Bacteria:

The comfort of building occupants may be reduced by bacteria. As such they are a major factor in the quality of indoor air (Flannigan, 1994). The most publicized bacterial agent is *Legionella pneumophila*, this will be discussed separately in this study. For other bacteria the ACGIH committee on bioaerosols (Morey, 1986) defines colony counts in excess of 500 CFU m^{-3} as "high". Bacterial loads associated with the CLER were between 27 and 146 CFU m^{-3} (Darlington et al., 2000).

Like fungal spores, bacterial levels are thought to be influenced in part by the outdoor concentrations. Nevalainen and coworkers (1994) report higher bacterial loads in buildings located in more rural settings. However, indoor sources such as improperly maintained HVAC systems (Reponen, 1992) and building occupants (Heinemann et al., 1994) are believed to have the greatest impact on IAQ. The diverse nature of the modular biofilter may support a greater number of bacterial species than a typical HVAC system, thus, it should be evaluated as a potential source of bacterial bioaerosols.

Legionella pneumophila:

Legionella pneumophila quickly became a public health concern following outbreaks of Legionnaire's disease and Pontiac fever in 1973 (OSHA, 1999). Most

strains of this bacteria proliferate in cooling towers and improperly maintained ventilation systems (OSHA, 1999). Although *L. pneumophilia* is an airborne pathogen, it proliferates in standing water. Thus, water samples are the most sensitive method for the detection of this pathogen (OSHA, 1999).

Fatal infections can result from exposure to the species *L. pneumophila*, which is specifically associated with Legionnaire's disease and Pontiac fever. The Occupational Safety and Health Association (OSHA) estimates there are 25 000 cases per year in the US, resulting in more than 4000 deaths. *L. pneumophila* is an opportunistic pathogen attacking a small portion of the population ($< 5\%$) who are immunocompromised (OSHA, 1999). Because of the potentially extreme health problems associated with the genus *Legionella*, it was necessary to ensure that it is not present in unsafe levels in the biofilter. Concentrations below 1 CFU ml^{-1} are considered safe, concentrations above 10 CFU ml^{-1} merit remedial action (OSHA, 1999).

Legionella pneumophila is native to fresh water lakes and streams. In its natural habitat, it accounts for less than one percent of the bacterial population (Fliermans, 1981). Competition and antagonism from other natural biota may keep it from reaching epidemic levels in its natural habitat and make it difficult to isolate (Fliermans, 1981). It exists in a wide range of environmental conditions. Although growth temperatures ranging for 5.7 to 63 °C have been reported (Fliermans, 1981), the optimum temperature is reported to be between 35 and 46 °C (OSHA, 1999). Acceptable pH ranges from 5.5 to 8.1, dissolved O_2 0.3 to 9.6 ppm and electrical conductivity from 18 to 106 $\mu S\,cm^{-2}$ (Fliermans, 1981). It is also reported to thrive with algal growths (OSHA, 1999). *Legionella pneumophila* is incapable of multiplying in cold flowing water, but it may persists in decaying matter in river sediment (Stout, 1985).

Materials and Methods:

Bioaerosol Measurement:

Bioaerosol measurements were taken using a BIOTEST Reuter centrifugal sampler (RCS). RCS is an impact sampler that generates a high speed air current and forces colony forming units (CFUs) in the air stream to impact on agar strips. The sampler was programmed to operate for 4 minutes and sampled 0.16 m^3 of air per sample. CFUs were cultured on selective media, fungus was grown on rose bengal agar (RBA) (RWR Scientific, Ottawa, ON) and bacteria was cultured on trypic soy agar (TSA) (RWR Scientific, Ottawa, ON). Both TSA and RBA strips were incubated for 5-7 days at room temperature (20 to 25°C) as per NIOSH guidelines (NIOSH, 1998).

Impact of Plant Material and Ventilation on Ambient Spore Counts:

Viable spore and bacterial colony counts were determined prior to commissioning, during the commissioning phases and post commissioning (during biofilter operation). Prior to the commissioning of the biofilter, spore loads were measured during two periods in July and September of 1999. Bacterial and fungal measurements were taken over several days in both sampling periods. Sampling dates included in the analysis were Julian days 208, 210, 212, at the end of July and 258, 260, 262, 264 during September. The commissioning phases of the biofilter were further divided into the following stages: the addition of plant material, without moss (sampled on Julian days 292 and 302), the subsequent addition of moss material (sampled on Julian days 321 and 328). The fully commissioned biofilter with air circulation through the moss was sampled on Julian days 340 and 348. On each sampling day, three fungal and bacterial measurements were taken

from each of seven sampling sites: four within the test facility (within 5 to 20 metres from the biofilter), one from the reference site in the adjacent building, and two outdoor measurements. Once fully operational, samples were also taken from the effluent stream of the biofilter.

The number of colony forming units was counted using a hand held colony counter (Fisher Scientific). Colony numbers were converted to colony forming units per cubic metre (CFU m^{-3}) by dividing the count by the volume of air sampled. Data was analysed as a two factor (sampling site and sampling date) anova using the GLM procedure in SAS (Version 8). The reference site was compared to the test sites using a one-tailed t-test (α =0.05). Since only one reference site was considered the concentration of spores in this location (based on 3 measurements) was compared to the pooled average concentrations (based on 3 measurements at each of 4 locations) and variance of the spores in the test facility.

Operational Schedule and Effluent Bioaerosols:

The impact of biofilter operation on ambient bioaerosols was studied over the course of seven weekends following the commissioning of the biofilter. Weekends were chosen because ambient spore loads on weekends are less influenced by building traffic and ventilation. Bacterial and fungal bioaerosol loads were measured once Friday afternoons, twice on Saturday and Sunday and once Monday mornings. Weekends were randomly allocated to one of two operational schedules. The biofilter actively circulated air only at night for three weekends, which is consistent with 'limited operation'. On weekend allocated to limited operation three fungal and three bacterial samples were measured from the ambient air (5 m from the biofilter). The biofilter was run continuously for the remaining four weekends. This was consistent with 'extended

operation', during this operational schedule three fungal and bacterial measurements were taken both in the foyer (5 m from the biofilter) and directly in the biofilter effluent. Data was analysed using the proc glm procedure in SAS (Version 8). Differences were detected using paired t-tests between operational schedules and between ambient and effluent bioaerosol concentrations.

Legionella Sampling:

Water samples were collected in sterile polypropylene bottles. Four samples were collected from the biofilter one from each of; the reservoir, aquatic, terrestrial and bog zones. In addition, a sample of moss was rinsed in 250 ml of reverse osmosis water. the rinse was included as a fifth sample. Samples were shipped to Pathcon Laboratories (Atlanta, Georgia) for analysis and quantification.

Results and Discussion:

Impact of Plant Material and Ventilation on Ambient Spore loads:

The average fungal spore concentration in the test facility was 56 ± 11 CFU m^{-3} in the period prior to commissioning. During and after the commissioning phases the average concentrations in the test facility ranged from 18 to 58 CFU m^{-3} (Figure 4.1). The reference site ranged from 6 to 125 CFU m^{-3} over the same period. Although site was a significant factor, the reference site was not significantly different from three of the 4 test sites (see Tables AII.1 and AII.2 in Appendix II). Furthermore, t-test measuring the difference between the pooled test site and the reference site indicated that the test site did not exceed the fungal loads of the reference on any sampling days (see Table AII.3 in

appendix II).

Four genera of fungi were found in the facility (Table 4.1). *Cladosporium sp.* is a common genus of outdoors fungus (Annon., 1995). It is excluded from the guidelines because of its prevalence. *Aspergillus sp.* and *Penicillium sp.* are genera which include several common species of fungi. Other genera identified were *Ulocladium* and *Epicoccum*.

The modular biofilter did not significantly increase the ambient fungal load beyond that of a normal building either during the phases of commissioning or during biofilter operation. Mouilleseaux and coworkers (1994) reported indoor fungal concentrations to range from a few units to 700 CFU m^{-3}, however, buildings typically had fewer than 50 CFU m^{-3}. Darlington et al. (2000) reported a mean value of 115 CFU m^{-3}, in a similar biofiltration system.

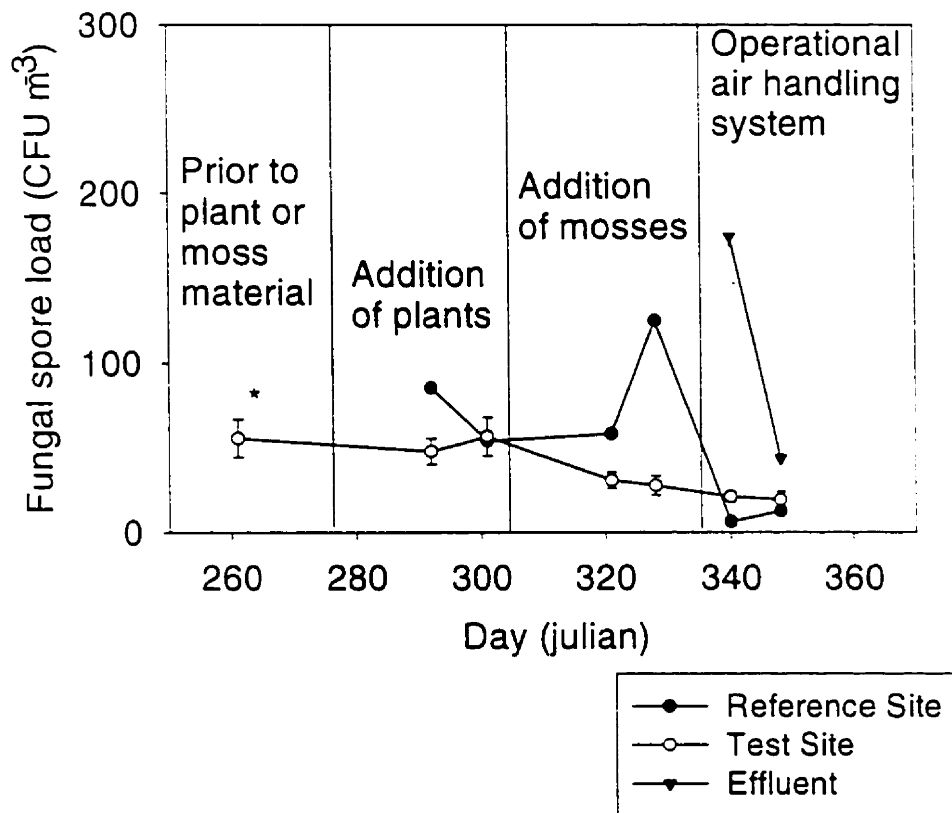


Figure 4.1: Average ambient fungal spore load (CFU m^{-3}) sampled prior to and during the addition of plants and mosses and with a fully operational biofilter. Samples were taken at the test site (4 sites, measured 3 times each), the reference site (measured 3 times) and in the biofilter effluent (measured 3 times) when operational. Note: * for display purposes data collected prior to commissioning was averaged and plotted on day 264. Data consists of 7 measurements taken between day 208 and 264.

Genera	Foyer	Lobby	Hall 1	Hall 2	Average of Detectable Sites.
Aspergillus sp.	nd	7	nd	nd	7
Cladosporium sp.	13	56	181	38	72 ± 32.3
Ulocladium sp.	7	19	25	13	16 ± 3.35
Epicoccum sp.	nd	nd	nd	13	13
Penicilium sp.	nd	19	nd	19	19

Table 4.1: Frequency of fungal genera (CFU m^{-3}) in the 4 test locations within the NORCAT test facility.

In general bacterial loads exceeded fungal loads in the airspace (Figure 4.2). Unlike the analysis of the fungal spore loads, the analysis of variance for the bacterial concentrations revealed that there was a significant interaction between sampling site and date (see Tables AII.4 and AII.5 in Appendix II). Comparisons between the test and reference sites indicated that the test site did not have significantly higher bacterial loads than the reference site (see Table AII.6 in Appendix II). A possible explanation is locally higher pockets of bacteria caused by the influx of outdoor air or building occupants. Sampling sites close to entrances (Hall 1 and Foyer 1) seemed to be occasionally affected by higher outdoor concentrations. These are also high traffic areas and contamination of samples can not be ruled out as a possible explanation.

Prior to commissioning the bacterial load was measured as 322 ± 40 CFU m^{-3} in the test facility. During the commissioning phases and operation of the biofilter, the bacterial spores ranged from 75 to 535 CFU m^{-3} with almost half the readings between 100 and 200 CFU m^{-3}. Darlington and coworkers (Darlington et al., 2000) reported values of between 27 and 146 CFU m^{-3} at the CLER. There was a general decrease in the bacterial load over the course of the experiment, likely the result of a seasonal effect. Increased bacterial bioaerosol loads did not correspond to changes in the commissioning of the biofilter (see Figure 4.2).

Health Canada defines colony counts in excess of 500 CFU m^{-3} as "high". However, Reponen and coworkers. (1992) propose an upper bacterial limit of 5000 CFU m^{-3} for subarctic dwellings. Thus, there is considerable variation in the acceptable concentration of bacteria indoors. As with fungi, bacterial counts are thought to be influenced by outdoor concentrations (Nevalainen et al., 1994; Reponen, 1992). Within this analysis, however; the addition of the outdoor concentration did not improve the model.

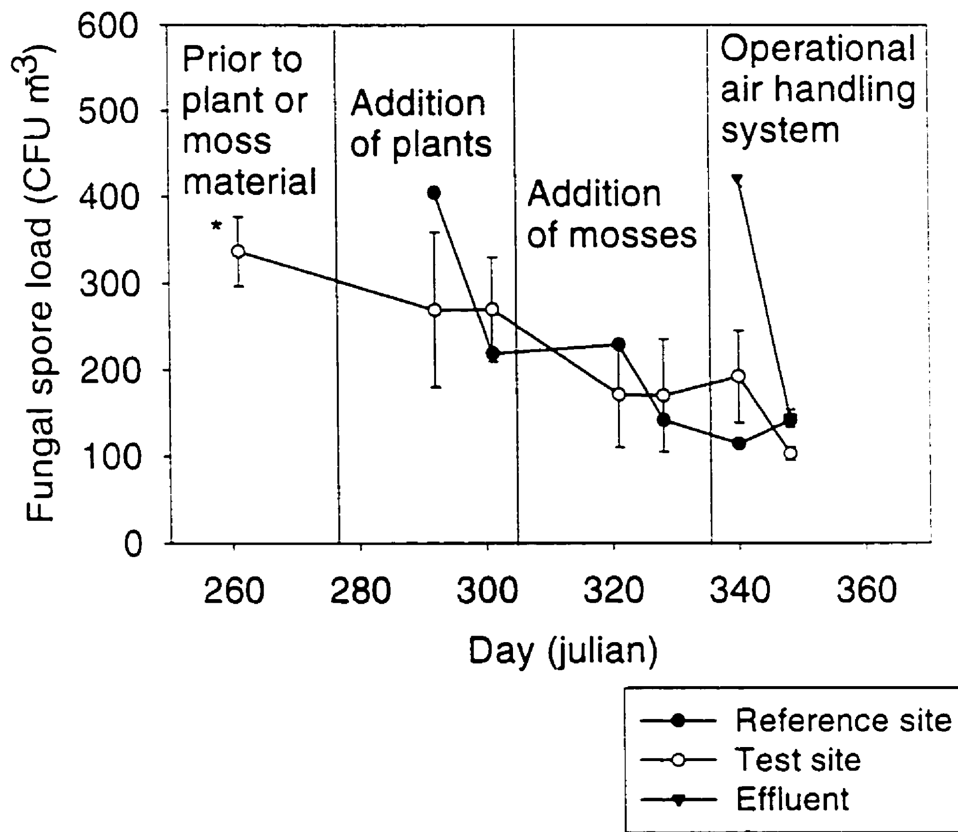


Figure 4.2: Average ambient bacterial spore load (CFU m^{-3}) prior to and during biofilter commissioning with plants and mosses and with a fully operational biofilter. Samples were collected in the test facility (4 sites sampled 3 times each), at the reference facility (measured 3 times) and in the biofilter effluent (measured 3 times) when operational. Note: * for display purposes the average of all data collected prior to commissioning is presented as a single point on day 264. Data for this period consisted of 7 samples taken between day 208 and 264.

Operational Schedule and Effluent Bioaerosols.

Higher bacterial and fungal spore concentrations in the exhaust stream of the biofilter on day 340 prompted further investigation into the relationship between ambient and effluent bioaerosol loads. It should be noted that although concentrations on this date were high compared to other measurements taken in the test site, they were not high compared to workplace safety guidelines (Annon., 1995). Furthermore, the higher exhaust stream readings on day 340 did not have a measurable impact on the ambient air (Figure 4.1 and 4.2).

If the biofilter acts as a source of spores the 'extended operation' of the biofilter would be expected to have a greater impact on spore counts than its 'limited operation'. However, no significant differences in fungal or bacterial loads could be detected between the operational schedules (Figures 4.3 and 4.4). Furthermore, during continuous operation there was no significant difference between ambient and exhaust measurements (Figures 4.3 and 4.4). If the biofilter was a source of spores, the exhaust stream would contain a higher concentrations than the ambient air.

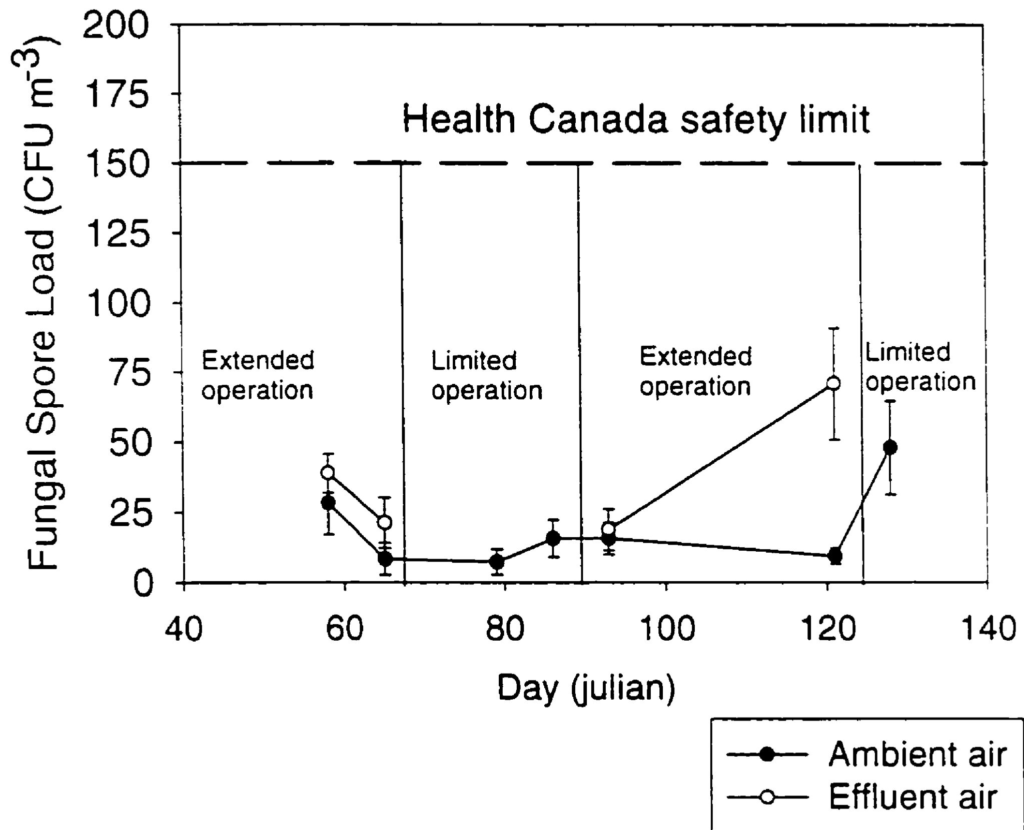


Figure 4.3: Ambient and exhaust fungal spore concentrations (CFU m^{-3}) collected during 'extended' and 'limited' biofilter operation. Exhaust measurements collected during extended operation only. All values are compared to the Health Canada safety guideline of 150 CFU m^{-3}.

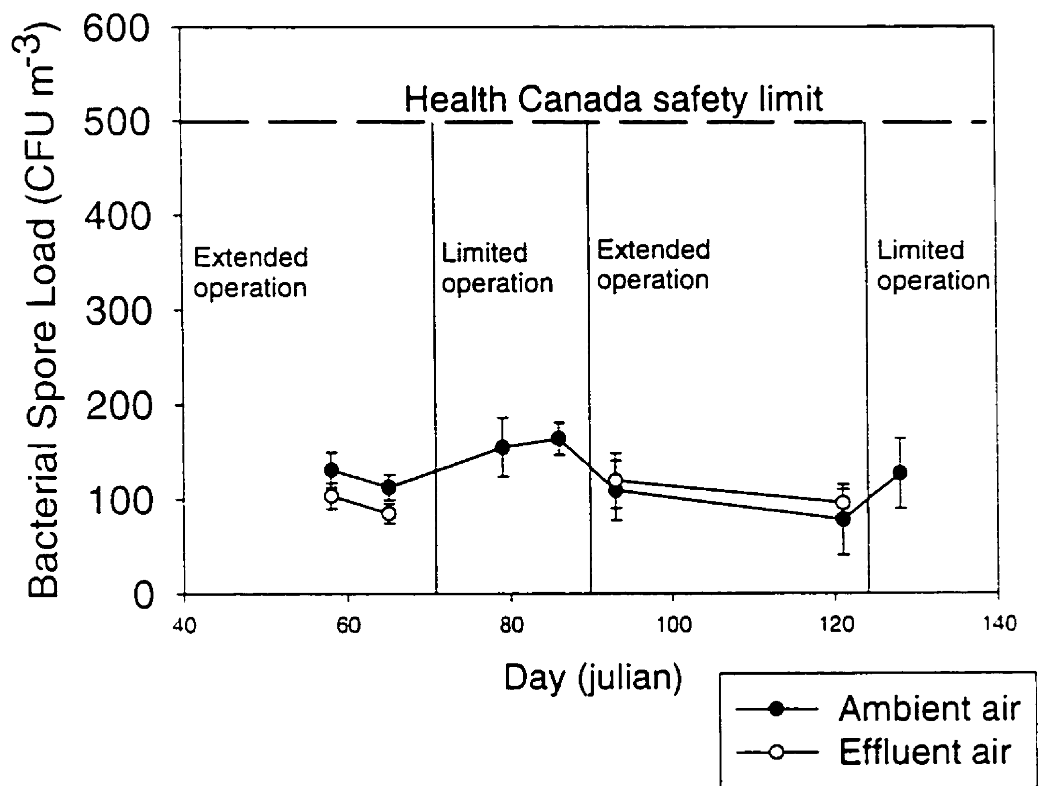


Figure 4.4: Ambient and exhaust bacterial spore concentrations (CFU m^{-3}) collected during 'extended' and 'limited' biofilter operation. Exhaust measurements collected during extended operation only. All values are compared to the Health Canada guideline of 500 CFU m^{-3}.

However, the high exhaust concentrations of mould and bacteria on day 340 (Figures 4.1 and 4.2) indicated that the biofilter may have acted as a source of bioareosols when the ventilation system was started for the first time. This impact was sort lived since further measurements of the exhaust stream did not show significantly increased concentrations of spores (Figures 4.3 and 4.4). Furthermore, neither the ambient or the exhaust measurements exceeded the Health Canada safety guidelines in any sample (Annon., 1995).

Legionella:

Legionella pneumophila was not detected in this system under the present operating conditions (Table 4.2). The environmental parameters such as water temperature and salinity, likely limited the growth of this pathogen. *Legionella sp.* is reported to grow in tap water between 25 and 42 °C, with an optimum growth temperature of 37 °C (Morey, 1986), although, it has been reported in water as cold as 6°C (Fliermans, 1981). The modular system maintained water temperatures below 20 °C, with an average temperature of 19.4 ± 0.8 °C . Higher temperatures give *Legionella sp.* a competitive advantage allowing it to proliferate to unsafe population levels (Stout, 1985). Furthermore, the salt content of the modular biofilter was maintained between 250 and 400 $\mu S\ cm^{-2}$. The reported electrical conductivity range of the bacterium is between 18 and 106 $\mu S\ cm^{-2}$ (Fliermans, 1981). Should the environment change in favour of the proliferation of *Legionella sp.*, it is possible that it could survive perhaps even reaching epidemic levels, continued monitoring is recommended.

Planting zone	**CFU ml^{-1}**
Aquatic zone	n/d
Bog Zone	n/d
Reservoir	n/d
Terrestrial Zone	n/d
Moss	n/d

Table 4.2: Occurrence of *Legionella pneumophila* in the biofilter planting zones. Bacterial concentrations marked by n/d were not detectable.

Summary and Conclusions:

A series of experiments were conducted to determine the impact of an indoor air biofilter on the indoor bioaerosol load. Bioaerosols were broadly placed into three categories: viable fungal spores, bacterial spores and *Legionella pneumophila.* Neither the viable fungal or the bacterial counts were significantly higher than the reference site. Analysis of air from the exhaust stream indicates no elevation in the bacteria or fungal spore loads, nor did it exceed safety recommendation. Similarly, third party analysis of *Legionella pneumophila* revealed no detectable colony forming units in any of the supplied water samples. The inclusion of a modular biofilter in an office space had no negative impacts on the air quality through the production of bioaerosols.

CHAPTER 5: REMOVAL OF METHYLETHYLKETONE, TOLUENE AND TRICHLOROETHYLENE WITH AN INDOOR AIR BIOFILTER.

Introduction:

Research activities at the Canada Life Environmental Room (CLER) have demonstrated that an indoor air biofilter containing tropical plants was capable of removing common indoor volatile organic compounds (VOCs) thus improving indoor air quality (IAQ) (Darlington et al., 2000; Darlington et al., in press; Darlington et al., 1998; Darlington & Dixon, 1999). Previous experiments have indicated that an ecosystem containing northern species of higher plants could be established indoors as part of an indoor air biofilter (Chapter 3). There was no indication that the modular biofilter negatively impacted IAQ through the production of airborne spores (Chapter 4). However, it remains unknown whether an indoor biofilter containing northern species of higher plants is able to improve IAQ through the elimination of VOCs.

It has been previously discussed that the role of higher plants in an indoor air biofilter served mainly to enhance the microbial diversity of the system (Chapter 2). To test the efficacy of a northern community of higher plants, this study focused on three compounds which have been reported to be removed from biofiltration systems at different rates. Methylethylketone (MEK) is an indoor pollutant that has many sources including paints, varnishes and adhesives (Otson & Fellin, 1992; Singh, 1988). It has been reported to be highly biodegradable in conventional biofiltration systems (Agathos et al., 1997; Deshusses et al., 1995; Deshusses et al., 1996; Wani et al., 1997). Toluene

represents a class of compounds collectively known as BTEX compounds, these include benzene, toluene, ethylbenzene and xylenes. BTEX, particularly toluene, have been well studied in conventional biofiltration systems (Ottengraf & van den Oever, 1983; Pedersen & Arvin, 1997). Toluene removal was also reported in an indoor air bioiflter, Darlington and coworkers (1998) report a 50% removal efficiency of toluene at the CLER. Microbial communities adapt quickly to metabolize MEK, the acclimation period of biofilters to MEK has been reported to take approximately 4 days (Deshusses et al., 1996). Acclimation of biofilter to toluene is reported to take 5 to 6 days (Bibeau et al., 1997).

The third target compound was trichloroethylene (TCE). Chlorinated compounds, such as, TCE are typically degraded anaerobically (Devinny et al., 1999). Non specific oxygenases produced in toluene or phenol metabolism have been reported to aerobically degrade TCE, however, such reactions are generally self limiting due to the toxic nature of the chemical products (Mars et al., 1998; Sun & Wood, 1997). The modular biofilter includes a bioreactor planted with locally collected bog species designed to create an anaerobic environment where compounds such as TCE can be degraded (discussed in Chapter 3).

The objectives of this study were threefold. The first objective was to determine whether the target compounds could be removed with this system. Second, the time required for the acclimation of the biofilter to these compounds will be monitored. The acclimation of a biofilter is associated with a period of increasing performance. Thus, the removal efficiency was monitored during the preliminary exposure period and quantified using isolated moss samples to detect acclimation. The final objective was to measure the

impact of flux on the operation of the biofilter by measuring the removal and elimination of the target compounds under different flux treatments.

Materials and Methods:

Preliminary Exposure:

Prior to exposing the biofilter to elevated concentrations of MEK, toluene, and TCE 40 moss samples (1-2 g fresh weight) were removed from the biofilter panels. Moss samples were divided into two treatments. 'Green' healthy moss was collected from the surface layer of the biofilter with as much detritus as possible removed. The non-green treatment was collected from below the surface layer with as much green moss as possible removed.

All moss samples were sealed in 1 L glass bottles (Bernardin Ltd. Toronto, ON) modified to contain a gas sampling septum. Bottles were injected with 10 ml of a gas mixture containing 2062.5 μmol m^{-3} of each of the target compounds and mixed thoroughly to create a homogeneous mixture of 20.6 μmol m^{-3} MEK, toluene and TCE in the bottle. Samples were drawn from the bottle with a 10 ml glass syringe (Hamilton Co., Reno, NV) and loaded into a gas sampling port (Valco Instrument Co., Houston, TX) where 1 ml of gas was automatically injected into the gas chromatograph (GC). Gas was analyzed using an SRI model 310 GC, analytes were separated through a 0.53 mm OD, 30 m RESTEK MXT - volatiles column and detected using a photoionization detector (PID)

set on high gain. The column temperature was 95 °C with a head pressure of 10 psi. Under these conditions the retention times were 1.3, 1.7, and 2.1 minutes for MEK, TCE and toluene respectively. An internal standard was necessary to account for bottle leakage. Thus, TCE was included since it has been shown it to be recalcitrant on this time scale in similar experiments (Llewellyn, 2000).

Gas samples were drawn from the bottles immediately after treatment, and after 5, 20, 40, 60, 120, 240, 360 and 480 minutes or until the target compound became undetectable. Prior to removing gas samples, the air contents of the bottles were thoroughly mixed by pumping the syringe 5 times. Between samples the syringe and the sampling port were flushed with carbon filtered room air to prevent cross contamination. VOC degradation in the bottles was calculated based on a logarithmic decay curve. The parameter used in analysis was the decay constant, K (s^{-1}), which was normalized for the moss dry weight ($g_{(dwt)}$).

During the preliminary exposure period, the modular biofilter was exposed to the target compounds on a nightly basis. This period lasted for approximately 14 days. The air flux through each biofilter panel was set to a air velocity of approximately 0.05 m s^{-1}. The ambient VOC concentrations were increased from 0 at 16:00 to 2.48 μmol m^{-3} by 24:00 and back to 0 by 08:00. VOC control and monitoring is discussed in Chapter 3.

Moss sampling was repeated after the 14 day preliminary exposure period. Data was analyzed as a two factor anova with two levels of exposure and two types of moss. Differences between treatment combinations were detected using pair-wise comparisons (SAS version 6.12).

Biofilter Flux and VOC Removal:

Four biofilter panels were randomly set to one of three air velocities approximately, 0.10, 0.06 or 0.02 m s^{-1}, for five days each. The VOC profile remained consistent with the preliminary exposure period. The removal coefficient (C_R) was calculated as the slope of the effluent regressed onto the influent VOC concentrations. C_R was calculated on a nightly basis for each panel by VOC combination and used as the input parameter to SAS (release 6.12). Data was analyzed as a completely randomized block design using the panels as the blocking factor. Removal was related to the empty bed retention time (EBRT), and the effluent temperature (ET), using the glm procedure of SAS (release 6.12).

Results and Discussion:

Preliminary Exposure:

The removal coefficients (C_R) of MEK and toluene decreased over the course of the preliminary exposure period (Figure 5.1). Such a response indicates acclimation. Acclimation has been associated with changes to the microbial population either in adaptation of metabolic pathways to utilize the new food source (Swanson & Loehr, 1997) or increased numbers (Deshusses et al., 1996). Though sparsely removed in the initial nights of exposure (R_E <0.05), the removal efficiency increased to 25% by day 6

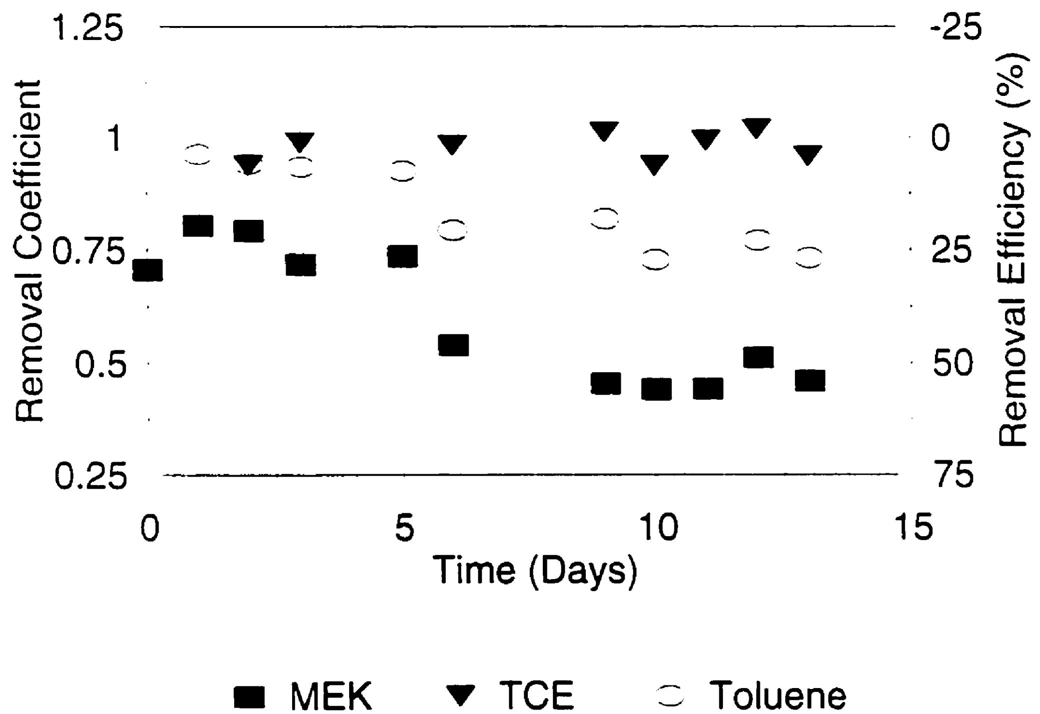


Figure 5.1: Acclimation of a typical biofilter panels to repeated nightly exposure of methylethylketone, toluene and trichloroethylene over a 14 day preliminary exposure period. Data is presented from panel 1.

and maintained that level of removal for the duration of the preliminary exposure. This is typical of reported toluene acclimation periods (Bibeau et al., 1997; Llewellyn, 2000). The biofilter showed signs of acclimation to MEK by day 6, which was slightly slower than reported by other authors (Deshusses et al., 1996; Llewellyn, 2000). MEK removal reached 55% removal and remained constant there for the duration of the preliminary exposure. No signs of acclimation were observed in the removal of TCE, which remained near 0% removal for the entire trial.

Acclimation of the biofilter was also measured through the decay rate of toluene and MEK in sealed bottles by moss samples isolated from the wall. Prior to exposure the decay of toluene did not differ from zero in either the green or non-green treatments (Figure 5.2). After the preliminary exposure period there was a significant removal of toluene in both treatments. No difference was detected between moss collected from the surface and moss collected below the surface. Based on this, uniform toluene removal was expected throughout the biofilter depth. The increased decay rate of toluene in sealed septum bottles (Figure 5.2) corresponded with the data collected from the biofilter (Figure 5.1) in the preliminary exposure period.

The decay rate of MEK did not differ significantly between the green and non-green treatments sampled before exposure ($P = 0.2164$) (Figure 5.2), inferring that removal of MEK was initially uniform throughout the depth of the biofilter. Post exposure, there was a significant difference between the green and non-green treatments ($P = 0.0139$). This indicates stratification of the microbial community occurred in the moss with exposure to MEK. Despite the divergence of the green and non-green

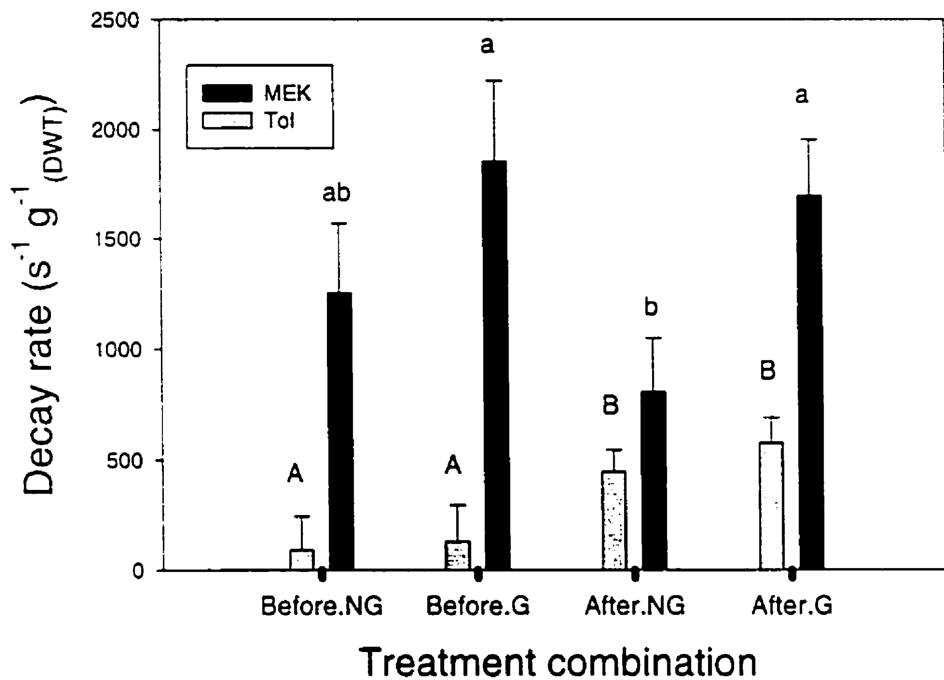


Figure 5.2: Logarithmic decay of an initial concentrations of 20.6 μmol m^{-3} methylethylketone and toluene by green (G) and non-green (NG) moss samples taken from the modular biofilter before and after exposure to the target compounds. Means with the same letter are not significantly different based on pair-wise t-tests (uppercase for toluene and lowercase for MEK).

samples over the exposure period, there was no difference in the decay rates over the preliminary exposure period (ie. moss samples taken before exposure did not decay MEK faster than moss samples taken after exposure ($P = 0.3145$)).

During the same period the removal of MEK measured through the biofilter increased from 25 to more than 50% (Figure 5.1). Increased removal through the biofilter did not correspond to increases in the decay rates measured in the septum bottles. Indicating that there may be limitations in relating results of this technique to the removal in the biofilter itself. MEK was consumed quickly by the moss samples in the bottles, limiting the number of measurements used to calculate the decay. Thus, greater sampling, particularly in the initial half hour of exposure in the bottles, may greatly increase the sensitivity of this technique. This was supported by the decay rates of toluene in the bottles. Slower toluene removal in the sealed bottles allowed a greater number of measurements to be included in the slope estimate. This reduced the variance and allowed greater separation of treatments.

Biofilter Flux and VOC Removal:

Empty bed retention time (EBRT) in industrial biofilters typically ranges from a few seconds to a few minutes (Pedersen & Arvin, 1997; Swanson & Loehr, 1997). Due to higher air velocities and thinner biofilter, retention through the modular biofilter was considerably less ranging from 0.1 to 0.5 seconds. Retention time is a key factor in the complete breakdown of organic compounds in biofilters (Wani et al., 1997). Ottengraf

(1986) indicated that EBRT, along with temperature, were proportional to the natural log of the removal coefficient (Equation 1.3). However, the analysis of data collected in this experiment revealed that temperature was a not a significant factor in removal of MEK or toluene (see Tables AIII.1 and AIII.5 in Appendix III). This was not surprising since the 3 to 4 °C temperature range was very small and the experiment was not designed to isolate a temperature effect. The removal coefficients of MEK and toluene were fitted to the model: $\ln C_R = A_{panel} + B(EBRT)$ (see Appendix II for ANOVA). MEK and toluene removal correlated well with the EBRT with r^2 of 0.75 and 0.82 respectively (Figure 5.3).

The removal of MEK and toluene through the biofilter increased with increasing EBRT. MEK was removed most effectively typically ranging from 42% removal at the shortest EBRT to 74% removal at the longest retention time(Figure 5.3). At a similar range of flux, Darlington and coworkers (2000) report removal efficiencies of between 30 and 60% for the removal of toluene. These are almost identical to the removal efficiencies measured through panel 3. Other panels showed slightly less toluene removal. Panel 4, in particular, showed poor toluene removal (Figure 5.3).

TCE was not removed through the biofilter panels (Figure 5.3). The interactions of TCE in the environment are complex. It is known to be degraded under anaerobic conditions but there is little evidence that it can be degraded aerobically. Although some strains of bacteria are capable of aerobic cometabolism of TCE in the presence of toluene or phenols (Mars et al., 1998), no such metabolism could be detected.

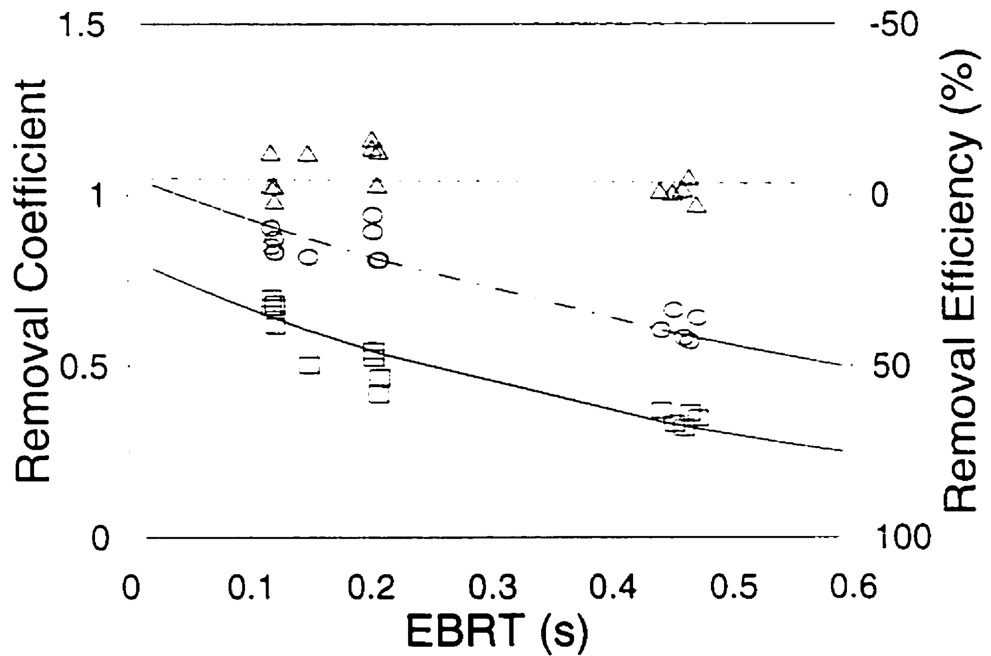


Figure 5.3: The removal coefficient and removal efficiency of methylethylketone, toluene and trichloroethylene versus the empty bed retention time of a typical panel.

The elimination capacities were calculated using equation 1.2, based upon the removal coefficient and EBRT. Since equation 1.2 requires a constant influent concentration a theoretical influent concentration of 1.24 μmol m^{-3} applied to the calculation. This concentration represented the midpoint of the exposure range. The elimination capacity of MEK through the biofilter panels ranged from 2.58 x 10^{4} to 1.98 x 10^{4} μmol m^{-3} h^{-1} (Table 5.1). This was lower than the reported elimination capacities for MEK typically measured at much higher influent concentrations and much longer EBRTs. In conventional biofilters, elimination capacities are based on higher influent concentrations, generally ranging from 69.35 x 10^4 to 416.09 x 10^4 μmol m^{-3} h^{-1}, and longer retention times ranging from tens of seconds to minutes (Deshusses et al., 1995; Devinny et al., 1999). The modular system was characterized by low influent concentrations, 1.24 μmol m^{-3} h^{-1}, and short retention times, 0.1 to 0.5 seconds. The elimination of MEK increased at shorter retention times indicating that the greatest absolute removal of MEK corresponded with the fastest air velocities.

The toluene elimination capacity was calculated the same way, it ranged from 3.36 x 10^3 to 1.43 x 10^4 μmol m^{-3} h^{-1} (Table 5.1). Ottengraf and van den Oever (1983) report approximately 21.71 x 10^4 μmol m^{-3} h^{-1} elimination capacity for toluene at high influent concentrations from 32.56 x 10^4 to 195.35 x 10^4 μmol m^{-3} h^{-1} and retention times between 4 and 71 seconds. Concentrations used in this study ranged from 0 to 2.71 μmol m^{-3}, very low by industrial standards. Similar to the results reported for MEK, three of the biofilter panels showed an increased elimination at faster retention time. Panel 4, however, was characterized by fairly consistent low removal at all retention times (Table

5.1). This panel showed a slight decrease in elimination at short retention times.

To account for differences in the panels, removal was normalized for EBRT and solubility of VOCs using the first order equation. When the EBRT is normalized for solubility (equation 1.4), K measures the slope between solubility corrected empty bed retention time (SCEBRT) and the natural log of the removal coefficient (lnC_R) (Ottengraf & van den Oever, 1983). There were significant differences between panels when the data was fit to the model $lnC_R = A_{panel} + K(SCEBRT_{panel})$. Analysis of variance is presented in tables AIII.7 to AIII.12 in Appendix III.

For the removal of MEK, all panels had similar slopes of $K = 0.02188\ s^{-1}$, however, panel 2 had a higher intercept value. This was observed by the lower MEK elimination through panel 2 (Table 5.2), and may indicate breakthrough. Panel 2 also had the shallowest biofilter depth of the panels and substantially less coverage than 2 other panels. Similarly, all panels had the same slope in the removal of toluene, $K = 0.2555\ s^{-1}$, this value is very close to reported values (Darlington et al., in press). Toluene removal through panels 2 and 3 were greater than the other panels, likewise they had a lower intercept value.

	EBRT	MEK	MEK	Toluene	Toluene
Panel		3 (Highest)	2(Lowest)	3(Highest)	4(Lowest)
Parameter					
C_R	0.1	0.58	0.68	0.70	0.93
R_E (%)	0.1	42.0	32.3	29.7	7.09
EC (μmol m^{-3} h^{-1})	0.1	2.58 x 10^4	1.98 x 10^4	1.42 x 10^4	0.34 x 10^4
C_R	0.5	0.26	0.30	0.42	0.56
R_E (%)	0.5	74.0	69.6	57.6	44.0
EC (μmol m^{-3} h^{-1})	0.5	0.92 x 10^4	0.86 x 10^4	0.55 x 10^4	0.42 x 10^4

Table 5.1: Removal coefficients, removal efficiencies and elimination capacities of methylethylketone and toluene through the most and least effective panels at a theoretical influent concentration of 1.24 μmol m^{-3}.

Summary and Conclusions:

Moss samples isolated from the wall prior to exposure significantly reduced ambient MEK concentrations in septum jars. No significant difference could be detected in MEK degradation between exposure periods by isolated moss samples even though the biofilter as a whole exhibited increased removal efficiency. This suggested that the septum jar technique or procedure was limited in its ability to reflect the removal of contaminants through the biofilter. Qualitatively though, this technique may prove useful in detecting the initial presence or absence of an active VOC degrading population of microbes.

The elimination of the target VOCs was lower than values reported for industrial biofilters but similar to other indoor air biofilters. The latter is a more appropriate comparison since, differences in the inlet concentrations and the retention times between indoor air and industrial waste gas may make such a comparison unrealistic. The removal rate of toluene compares favourably with rates reported from the CLER, confirming that a northern community provided suitable ecological infrastructure for VOC removal. The greatest removal rates of MEK and toluene were observed at the longest retention times. However, gains made by increasing the contact between the biofilter and the contaminant stream were offset by reduced flow rates. The greatest elimination of MEK and toluene was observed at shortest retention times.

CHAPTER 6: ELIMINATION OF KETONES WITH AN INDOOR AIR BIOFILTER.

Introduction:

Previous experiments have indicated that an indoor air biofilter containing northern species of higher plants and mosses was able to remove some VOCs from the ambient air (Chapter 5), without reducing air quality through the production of spores (Chapter 4). It has been proposed that a diverse community of microbes would be capable of degrading low concentrations of ketones (Binot & Paul, 1989). Ketones make up a class of compounds common to the indoor environment (Otson & Fellin, 1992) and to industrial processes (Otson & Fellin, 1992; WHO, 1998; WHO, 1990). There are several sources of ketones indoors, they are a common product of incomplete combustion processes such as wood burning or cigarette smoke and are also emitted from building occupants (Otson & Fellin, 1992). Ketones are highly degradable compounds in conventional biofiltration systems (Deshusses et al., 1996). The concentration of ketones in an indoor environment (Kostiainen, 1995) is several orders of magnitude less than the ketone concentrations treated in industrial biofilters (Deshusses et al., 1996; Hwang, 1997). Thus, ketones are an ideal class of compounds for elimination through the modular biofilter.

This study focussed on the removal of three ketones through the modular biofilter. Acetone is a very common VOC indoors (Otson & Fellin, 1992). It is used as a solvent in industrial processes, volatilises off of building materials, is used as a drying agent in paint, varnishes and some cleaning agents and is even exhaled in the breath of building

occupants (WHO, 1998). Darlington and co workers (1999) report successful removal of acetone through the CLER where the removal efficiency ranged from 45% to 80%. Methylisobutylketone (MIBK) is a common indoor contaminant (Kostiainen, 1995; Otson & Fellin, 1992). It is an additive to foods and makes up a minor component of paints and lacquers (WHO, 1990). MIBK is also produced during the incomplete combustion of fossil fuels which often infiltrate the indoor space. It has been studied in industrial biofilters where the elimination capacity was 2.99 x 10^5 μmol m^{-3} h^{-1} at influent load of 3.99 x 10^5 μmol m^{-3} h^{-1} retention times between 90 and 180 seconds. Elimination is reduced to 8.08 x 10^4 μmol m^{-3} h^{-1} in the presence of equal concentrations of methylethylketone (MEK) (Deshusses et al., 1995) (Deshusses et al., 1996). 2-pentanone (2p) is a common, although less studied, compound which is persistent indoors (Kostiainen, 1995). Chemically, it is similar to acetone with one chain lengthened by 2 carbons. It is also similar to MIBK; lacking a carbon side chain.

The objectives of this study were three fold. The first objective was to test whether the modular biofilter system was able to remove a mixture of ketones from the ambient air space. Removal can be measured two ways, by measuring the decay of VOCs in sealed septum bottles and by measuring the influent and effluent concentrations of VOCs as they move through the biofilter. The second objective was to measure the impact of the air flow and, thus, empty bed retention time (EBRT) on removal coefficient for each of the three ketones. Finally to use the first order relationship (equation 1.3) to measure and compare the elimination rates between compounds and between panels by normalizing panels for the substrate concentration in the biofilters aqueous phase.

Materials and Methods:

VOC removal in sealed septum bottles:

The analysis of moss samples followed the procedure outlined in Chapter 5, with the following changes. The column temperature was set at 45 °C , the head pressure remained constant at 10 psi. Under these conditions the retention times were 1.2, 3.4, 4.7 and 7.8 minutes for acetone, 2-pentanone, MIBK and PCE respectively. PCE replaced TCE as the internal standard in this analysis, since, it did not co-elude with other analytes. Gas samples were drawn from the bottles immediately after treatment, and again after 10, 20, 40, 60, 120, 240, 360 and 480 minutes or until the target compounds became non detectable. Unlike the procedure outlined in Chapter 5, 10 samples of 'green' and 'non-green' moss were taken before and after a initial exposure period.

The ambient VOC profile during the exposure period remained consistent with Chapter 5 with the exception of acetone which increased from 0 at 16:00 to a maximum of 4.95 μmol m^{-3} at 24:00 and returned to 0 by 8:00.

VOC removal through the biofilters:

Generally, the analysis of ketone removal followed the procedure outlines in Chapter 5 with the following changes: the VOC profile in the flux experiment was identical to the preliminary exposure period and six panels were used in the ketone

analysis instead of four.

Results and Discussion:

VOC removal in sealed septum bottles:

Bottle experiments were subjected to a 2 x 2 factorial analysis with exposure (before versus after acclimation) as one factor and moss type (green versus non-greeen) as the second. All treatment combinations showed some ability to remove all three ketones (Figure 6.1) suggesting that there was an inherent ability to remove ketones in this system. The 'before' exposure decay rates were similar between green and soiled treatments. This indicated a relatively uniform microbial community through the biofilter depth.

Increased removal rates on the biofilter surface 'after' acclimation indicated greater microbial activity in this region. Moss sampled from below the surface did not show the same increase (Figure 6.1). Swanson and Loehr (1997) suggested that removal gradients through the biofilter may indicate oxygen or nutrient gradients. While this may be true in industrial biofilters which are 1 - 1.5 m deep, the biofilter panels varied in depth from 11 to 15 mm and were regularly irrigated with oxygen saturated water. Thus, it is unlikely that differences in ketone removal can be attributed to nutrient or oxygen deficiencies at greater depths. Population differences may exist between the surface and

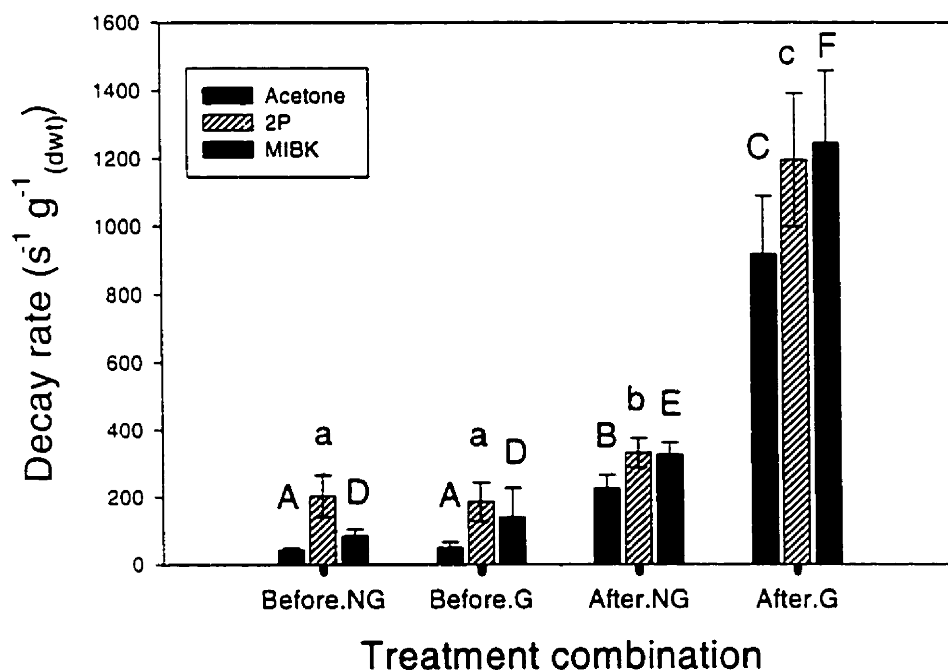


Figure 6.1: Logarithmic decay rate of acetone, 2-pentanone and methylisobutylketone by green and non-green moss samples taken from the modular biofilter before and after exposure to the target compounds. Means with the same letter are not significantly different. Acetone is designated upper-case A, B and C. 2P is designated lowercase a, b and c. MIBK is designated upper-case D, E and F.

subsurface of the biofilter. Microbes more able to assimilate ketones will thrive where ketone concentrations are greatest, on the biofilter surface.

VOC Removal through the Biofilter Panels:

Empirically, the data fit well to the first order kinetic model (Ottengraf & van den Oever, 1983) that predicts a log-linear relationship between the removal coefficient (C_R) and the retention time (EBRT) (equation 1.3). The removal coefficients for all three target compounds were fit to the model, $\ln C_R = A_{Panel} + B(EBRT) + C(ET)$. Where the EBRT is the empty bed retention time ($m^3_{biofilter}\ s\ m^{-3}_{air}$) and ET is the effluent temperature (°C) (See Tables AIV.1 to AIV.6 in Appendix IV for statistical analysis). Unlike previous experiments (Chapter 5) ketone removal was found to vary significantly with the effluent temperature.

The removal coefficient (C_R) for all three ketones decreased with increasing EBRTs (Figure 6.2). This was expected since increased retention time provided greater contact between the airstream and the biofilm. 2-P was removed most efficiently from the airstream varying between 44.3 and 78.1 % removal depending on retention time (Figure 6.2). The removal of MIBK varied from 19.2 to 61.5% over this range of retention times. Acetone removal was most sensitive to EBRT (Figure 6.2) 28.9 % of the acetone concentration was removed at short retention times, 79.3 % at long retention times.

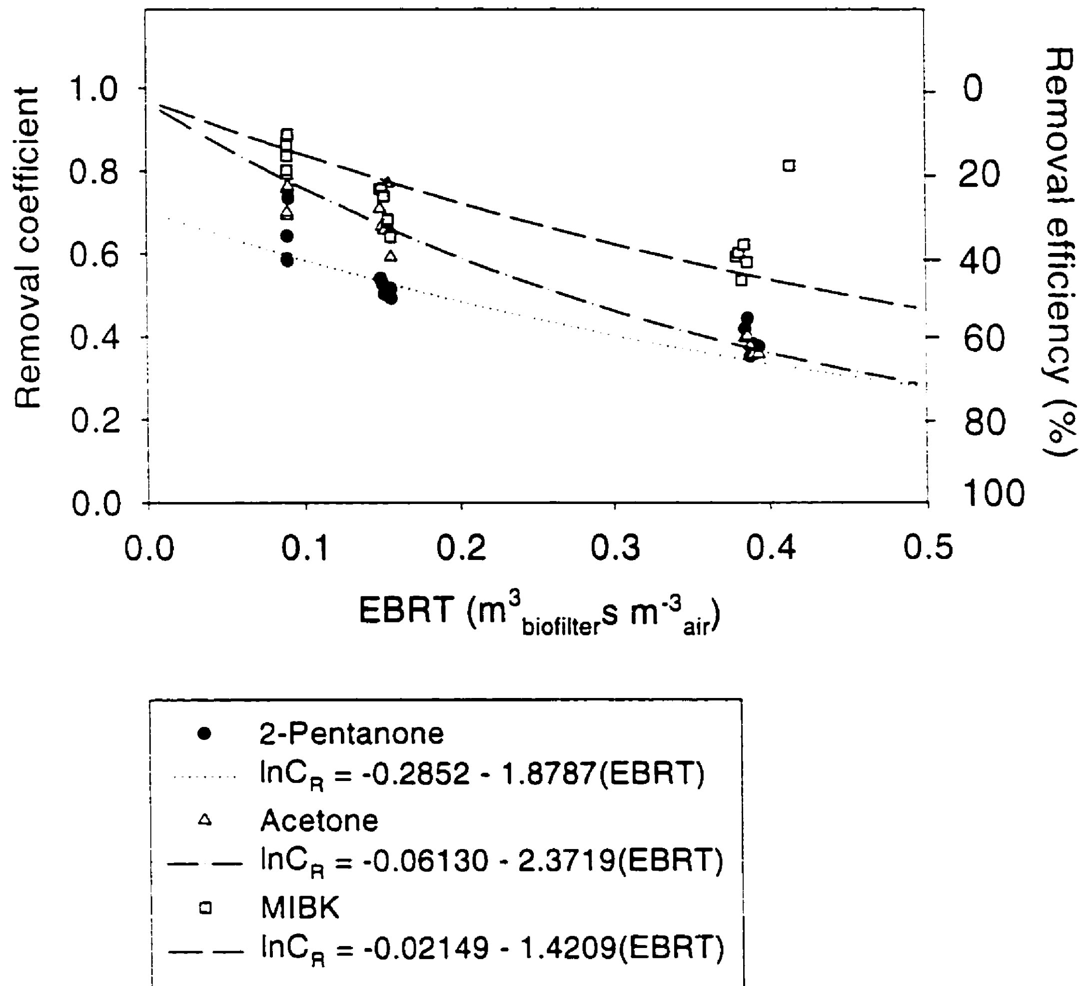


Figure 6.2: Typical removal coefficients and efficiency of 2-pentanone, acetone and methylisobutylketone versus empty bed retention time at an average effluent temperature of 18.6 °C. Data presented from panel 2.

The elimination capacity normalizes the removal coefficient for the volumetric flux and the influent VOC concentration of the biofilter (equation 1.2). Since removal coefficients were calculated as slopes, the midpoint of the VOC exposure range was used as the influent in this calculation. Similar to MEK and toluene (Chapter 5) the elimination capacities of the ketones were greatest at the shortest retention times (Table 6.1).

The elimination of MIBK through the modular biofilter ranged from 0.55×10^4 to 0.69×10^4 $\mu mol\ m^{-3}\ h^{-1}$. Deshusses and coworkers (1996) report an MIBK elimination capacity of 8.08×10^4 $\mu mol\ m^{-3} h^{-1}$ when a mixture of MIBK and MEK is passed through a biofilter. For a pure stream of MIBK, they report an elimination capacity as high as 29.94×10^4 $\mu mol\ m^{-3} h^{-1}$ (Deshusses et al., 1995). There are large differences between the influent concentrations in this experiment (0.99 $\mu mol\ m^{-3}$) and the values reported by Deshusses and coworkers (1996) (0.77×10^4 $\mu mol\ m^{-3}$). It is not known whether a realistic comparison can be made between two biofilters operating under such vastly different conditions. Differences the elimination of MIBK through this system are within an order of magnitude of reported values despite a 3 order of magnitude difference in influent concentrations.

Acetone has been thoroughly studied in both industrial biofilters (Hwang, 1997) and in an indoor air biofilter (Darlington & Dixon, 1999). The CLER had an elimination capacity between 15.49×10^4 and 24.10×10^4 $\mu mol\ m^{-3} h^{-1}$ where influent concentrations ranged from 4.48 to 22.38 $\mu mol\ m^{-3}$ (Darlington & Dixon, 1999). These are similar to the results reported here. Hwang and coworkers(1997) report elimination capacities

Parameter	EBRT	2-Pentanone	MIBK	Acetone
C_R	0.1	0.557	0.808	0.711
R_E (%)	0.1	44.27	19.2	28.9
EC(µmol $m^{-3} h^{-1}$)	0.1	2.22 x 10^4	0.69 x 10^4	2.86 x 10^4
C_R	0.4	0.219	0.385	0.207
R_E (%)	0.4	78.1	61.45	79.3
EC (µmol $m^{-3} h^{-1}$)	0.4	0.98 x 10^4	0.55 x 10^4	0.20 x 10^4

Table 6.1: Typical removal coefficients, removal efficiencies and elimination capacities of 2-pentanone, MIBK and acetone at a theoretical influent concentration of 1.24 µmol m^{-3} and a theoretical temperature of 18.6 °C.

equal to the loading rate for loads less than 137.69 μmol m^{-3} h^{-1}. This suggests, full removal of acetone at low concentrations. Both the data presented here and the results of others (Darlington & Dixon, 1999) dispute their results.

There was no correlation between effluent temperature and EBRT ($r^2 < 0.05$) (Figure 6.3). The biofilter temperature varied little over this range of flux. Likewise the solubility of VOCs would have varied little. The first order kinetic model (Ottengraf & van den Oever, 1983) (equation 1.3) assumes that removal is diffusion limited because of low influent concentrations. If this assumption is valid, the microbial activity (K) should remain constant over this range of EBRT. In a purely diffusion limited process $\ln C_R$ is proportional to the EBRT and the solubility (Equation 1.4). The microbial rate constant (K) ,presented in Figure 6.4, represents the slope of the relationship between $\ln C_R$ and the solubility corrected empty bed retention time (SCEBRT) (Devinny et al., 1999; Ottengraf, 1986). Despite the increased possibility of breakthrough at higher fluxes, there was a good correlation between $\ln C_R$ and the SCEBRT over the examined range of air flows. The r^2 of 2-pentanone, MIBK and acetone were 0.65, 0.73 and 0.87 respectively (see Tables AIV.7 to AIV.12 in Appendix IV for analysis of variance).

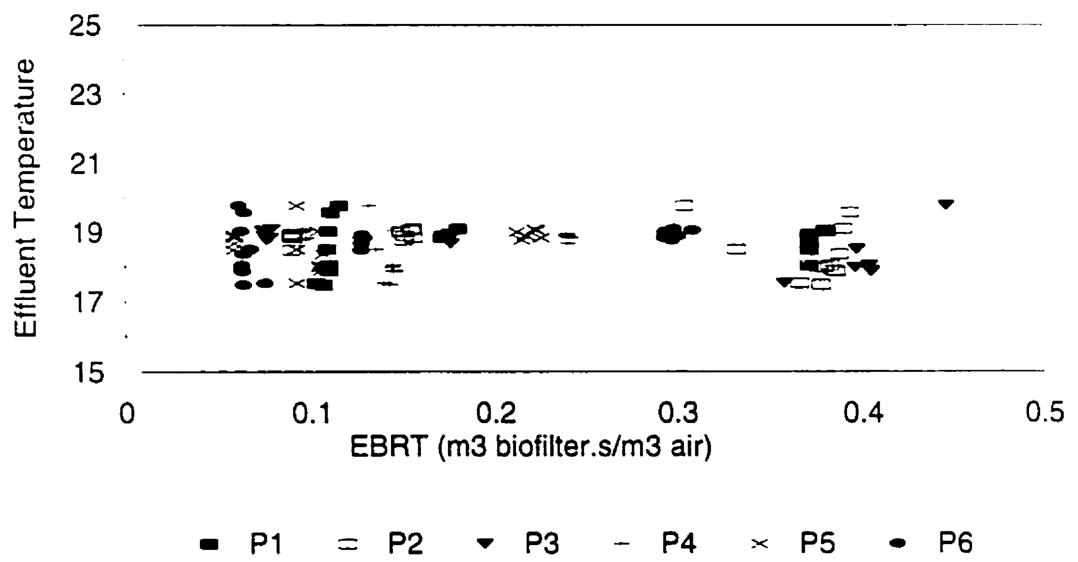


Figure 6.3: Effluent temperature versus empty bed retention time for the different panels.

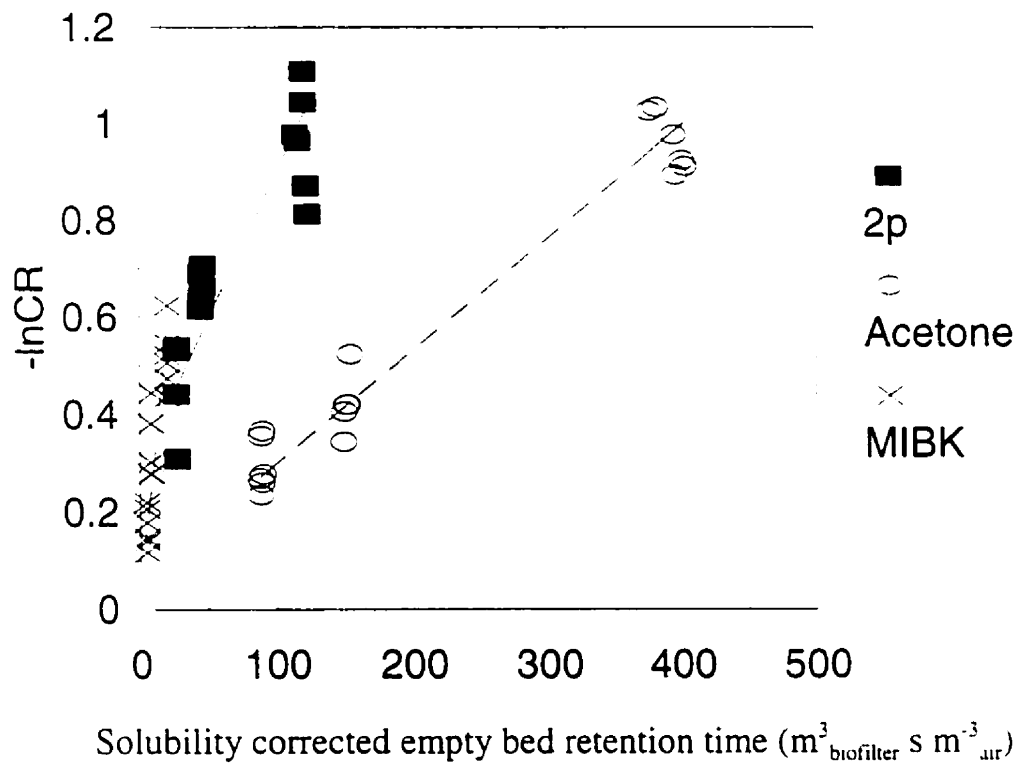


Figure 6.4: The negative natural logarithm of the removal coefficients of 2P, acetone and MIBK, expressed as a function of the solubility corrected empty bed retention time ($m^3_{biofilter}$ s m^{-3}_{air}). Microbial activity rates are for panel 2 calculated as the slopes of the presented lines.

The elimination parameters for each panel by VOC treatment combination were subjected to analysis of variance. Significant differences were detected with pair-wise comparisons, and an experiment wise error term. The microbial activity of 2-P did not differ between panels, 0.00718 $\pm$ 0.00128 s^{-1}. The microbial activity of acetone averaged 0.00272 s^{-1} $\pm$ 0.000305. There were significant differences between the slopes. panel 1 was the highest with an activity of 0.00383 s^{-1}, panel 4 was the lowest with a microbial activity of 0.00196 s^{-1}. Significant differences were also found between panels in the removal of MIBK which averaged 0.0311 s^{-1} $\pm$ 0.00576, the microbial activity on this compound ranged from 0.0520 s^{-1} (panel 5) to 0.0238 (panel 6). The microbial rates reported here are very similar to the previously reported value of 0.0218 s^{-1} for the microbial activity on MEK (Chapter 5).

Differences in microbial activities between panels may reflect differences in moss coverage and biofilter depth or differences in the degrader communities between panels. While some of these variables are accounted for by the SCEBRT term. it does not account for their impact on breakthrough in the system. Incomplete biofilter coverage leads to channelization of the waste gas stream. In a thin biofilter, gas channels may form allowing gas to pass through the biofilter without coming into contact with the biofilm. Some differences in the microbial activities between panels may be the result of breakthrough. However, if breakthrough were the only factor differentiating panels. a biofilter panel with a high degree of breakthrough for one compounds would have a similar breakthrough for all compounds. The data presented does not support this. The fact that the ranking of microbial activities depends on the compound suggests some

variety in the degradation community.

Summary and Conclusions:

A complex community of northern temperate and boreal species designed to act as a biofilter was able to remove three common ketones present at concentrations that ranged from 0 to 4.95 μmol m^{-3}. The ketone removal could be described by a log-linear relationship between the removal coefficient, the empty bed retention time and the effluent temperature. The relationship varied between ketones. The removal of 2-P was the highest followed by acetone and then MIBK. All three target compounds were eliminated from the air stream at rates comparable to other indoor air biofilters and industrial biofilters. The elimination capacities ranged from approximately 0.2 x 10^4 to 2.86 x 10^4 μmol m^{-3} h^{-1}. Elimination of VOCs was greatest at the shortest retention times for all three compounds. When removal was normalized for the differences in EBRT and solubility, MIBK was found to be eliminated in the biofilm most effectively, followed by 2-P and acetone. Variation between panels and between compounds inferred that there was some variety between the biofilter panels that could not be accounted for by the SCEBRT or by breakthrough. Such variety inferred that a diverse community of microbes was present in the system.

CHAPTER 7: GENERAL CONCLUSIONS.

A modular biofilter was constructed as a supplementary or alternative means of controlling indoor air quality (IAQ). Tropical plants have been used in other systems as a means of removing volatile organic compounds (VOCs) directly from the air through sorptive or metabolic processes or indirectly by supporting a diverse microbial community. Examination of the role of higher plants at the Canada Life Environmental Room (CLER) found toluene, tetrachloroethylene, ethylbenzene and o-xylene accumulated in foliage, however, concentrations were too low to have a meaningful impact on IAQ. It was concluded that plants in an indoor biofilter serve mainly to enhance the microbial diversity in the system.

A potential application of this technology may be in areas where tropical species are difficult or costly to obtain. Therefore, based on the results from the CLER, it was decided that the modular biofilter would be commissioned with northern species of higher plants. The successful incorporation of northern species into an indoor space was not a certainty. However, preliminary survivability trials indicated that some species were adaptable to the indoor environment and a stable community of northern plants was established indoors.

The community was evaluated for its negative impact on IAQ through the production of airborne spores and the pathogen *Legionella pneumophilia*. The results of this series of experiments show no signs that the modular system added to the bacterial or fungal spore load of the test facility. The airborne pathogen *Legionella pneumophilia*

was tested separately, no colonies were detected. On going monitoring of the biofilter for bioaerosols and *legionella sp.* is recommended, since, environmental conditions may change to encourage the proliferation of these agents.

The modular system compared favourably with other biofiltration systems in the removal of VOCs from the air. Ketones were easily removed in the system. The decay rate of ketones in sealed bottles indicated that the majority of the degradation occurred on the biofilter surface. The first order kinetic model suggests that contaminant removal decreased exponentially with biofilter depth. Decay rates measured in isolated moss samples support this hypothesis. They also lend credence to the design of the biofilter, which emphasizes surface area as opposed to biofilter depth. Improvements to biofilter design should include a greater surface area.

Toluene represents a class of moderately biodegradable compounds that have been well studied in other biofiltration systems. Toluene removal suggests that the modular biofilter would be capable of removing a wide variety of similar compounds.

All target compounds were removed with the exception of trichloroethylene (TCE). TCE represents a class of compounds that is notoriously difficult to biodegrade aerobically. Although the bog zone was included to provide an anaerobic water path through the system, its success was questionable. Thus, technical challenges remain in the elimination of common persistent compounds such as chlorinated compounds. Despite this, a community of northern plants installed into a modular biofilter was able to improve IAQ through the removal of VOCs without compromising it through the production of airborne spores or pathogens.

APPENDIX I: STATISTICAL ANALYSIS RESULTS FOR CHAPTER 2

Table AI.1: Analysis of variance for the decay of 1 part per million by volume of toluene in sealed bottles. Treatments included 11 species of tropical plants and blanks as controls.

Source	df	Type III SS	MS	F	Pr < I T I
Species	12	2880.07	240.01	1.5	0.3552
Error	36	7527.69	209.10		
Total	48	10407.75			

Table AI.2: Analysis of variance for the decay of 1 part per million by volume trichloroethylene in sealed bottles. Treatments included 11 species of tropical plants and blanks as controls.

Source	df	Type III SS	MS	F	Pr < I T I
Species	12	1987.57	165.63	1.46	0.1848
Error	36	4082.29	113.40		
Total	48	6069.87			

Table AI.3a: Analysis of variance for the accumulation of toluene, trichloroethylene, ethylbenzene and ortho-xylene in the leaf tissue of *Rhododendron japonica*. The analytical model was log (leaf concentration) = A*VOC +B*Sampling day + C*(VOC x Sampling day).

Source	df	Type III SS	MS	F	Pr < I T I
VOC	3	183.11	61.04	57.79	<0.0001
Sampling Day	5	72.87	14.57	13.80	<0.0001
VOC x Sampling Day	13	26.15	2.01	1.90	0.0484
Error	58	61.26	1.06		
Total	79	393.42			

Table A I.3b: Student-Newman-Kuels multiple comparison test of statistical difference between the VOC treatment analysed in Table AI.3a.

SNK-Grouping	Mean	N	VOC
A	-5.4481	23	o-xylene
B	-8.6270	21	ethylbenzene
B C	-9.1136	19	toluene
C	-9.6255	17	tetrachloroethylene

Table AI.3c: Student-Newman-Kuels multiple comparison test of statistical difference between sampling day treatment analysed in Table AI.3a.

SNK-Grouping	Mean	N	Sampling Day
A	-5.2718	5	6
B	-7.4814	18	3
B	-7.5966	14	5
B	-7.9556	16	4
B	-8.4189	16	2
C	-10.3543	11	1

Table AI.4a: Analysis of variance for the sorption of toluene, trichloroethylene, ethylbenzene and ortho-xylene in the leaf tissue of *Nephrolopis exultata*. The analytical model was log (leaf concentration) = A*VOC +B*Sampling day + C*(VOC x Sampling day).

Source	df	Type III SS	MS	F	Pr < I T I
VOC	3	12.14	8.83	1.82	0.1564
Sampling Day	5	128.67	25.74	10.94	<0.0001
VOC x Sampling Day	15	32.02	2.13	0.91	0.5612
Error	50	117.61	2.35		
Total	73	305.16			

Table AI.4b: Student-Newman-Kuels multiple comparison test of statistical difference between sampling day treatment analysed in Table AI.4.

SNK-Grouping	Mean	N	Sampling Day
A	-7.2570	7	6
A	-7.8861	13	3
A B	-8.8847	6	5
A B	-9.0201	15	4
A B	-9.4908	14	2
B	-11.3199	13	1

Table AI.5a: Analysis of variance for the sorption of toluene, trichloroethylene, ethylbenzene and ortho-xylene in the leaf tissue of *Dieffenbacia manculata*. The analytical model was log (leaf concentration) = A*VOC +B*Sampling day + C*(VOC x Sampling day).

Source	df	Type III SS	MS	F	Pr < I T I
VOC	3	12.14	4.04	1.72	0.1749
Sampling Day	5	218.68	25.74	10.94	<0.0001
VOC x Sampling Day	15	32.03	2.13	0.91	0.5612
Error	58	117.61	2.35		
Total	79	305.16			

Table:AI.5b Student-Newman-Kuels multiple comparison test of statistical difference between sampling day treatment analysed in Table AI.5a.

SNK-Grouping	Mean	N	Sampling Day
A	-5.5330	11	3
A B	-6.8852	15	4
A B	-6.9298	7	5
B	-7.3263	16	2
B	-7.7035	9	6
C	-9.9344	16	1

Table AI.6a: Analysis of variance for the sorption of toluene, trichloroethylene, ethylbenzene and ortho-xylene in the leaf tissue of *Dracena fragrans*. The analytical model was log (leaf concentration) = A*VOC +B*Sampling day + C*(VOC x Sampling day).

Source	df	Type III SS	MS	F	Pr < I T I
VOC	3	3.29	1.10	0.50	0.6856
Sampling Day	5	88.63	17.73	8.03	<0.0001
VOC x Sampling Day	11	9.83	0.89	0.40	0.9485
Error	58	128.08	2.21		
Total	77	234.86			

Table AI.6b: Student-Newman-Kuels multiple comparison test of statistical difference between sampling day treatment analysed in Table AI.6a.

SNK-Grouping	Mean	N	Sampling Day
A	-8.1324	15	5
A B	-8.4456	14	2
A B	-8.6395	17	3
A B	-8.6520	18	4
B C	-10.1571	3	6
C	-11.5034	11	1

Table AI.7a: Analysis of variance for the sorption of toluene, trichloroethylene, ethylbenzene and ortho-xylene in the leaf tissue of *Hendra helix*. The analytical model was log (leaf concentration) = A*VOC +B*Sampling day + C*(VOC x Sampling day).

Source	df	Type III SS	MS	F	Pr < I T I
VOC	3	4.69	1.56	0.85	0.4740
Sampling Day	5	107.82	21.56	11.69	<0.0001
VOC x Sampling Day	13	3.55	0.27	0.15	0.9997
Error	51	94.17	1.84		
Total	72	241.47			

Table AI.7b: Student-Newman-Kuels multiple comparison test of statistical difference between sampling day treatment analysed in Table AI.7a.

SNK-Grouping	Mean	N	Sampling Day
A	-5.0880	20	4
A B	-5.7327	10	5
B C	-6.7729	13	3
B C	-6.9760	9	2
C D	-8.0518	5	6
D	-8.7039	16	1

Table AI.8a: Analysis of variance for the sorption of toluene, trichloroethylene, ethylbenzene and ortho-xylene in the leaf tissue of *Aucuba japonica variegata*. The analytical model was log (leaf concentration) = A*VOC +B*Sampling day + C*(VOC x Sampling day).

Source	df	Type III SS	MS	F	Pr < | T |
VOC	3	5.64	1.88	0.98	0.4120
Sampling Day	4	138.99	34.75	18.11	<0.0001
VOC x Sampling Day	12	7.41	0.618	0.32	0.9816
Error	46	88.27	1.92		
Total	65	242.36			

Table AI.8b: Student-Newman-Kuels multiple comparison test of statistical difference between sampling day treatments analysed in Table AI.8a.

SNK-Grouping	Mean	N	Sampling Day
A	-7.6765	6	5
A	-7.7428	16	2
A	-8.6444	17	4
A	-8.9540	11	3
A	-8.9540	16	1

Table AI.9a: Analysis of variance for the sorption of toluene, trichloroethylene, ethylbenzene and ortho-xylene in the leaf tissue of *Verissa splendens*. The analytical model was log (leaf concentration) = A*VOC +B*Sampling day + C*(VOC x Sampling day).

Source	df	Type III SS	MS	F	Pr < I T I
VOC	3	13.30	4.43	3.50	0.0206
Sampling Day	5	132.98	26.60	21.01	<0.0001
VOC x Sampling Day	14	5.35	0.38	0.30	0.9918
Error	61	77.23	1.27		
Total	83	231.98			

Table AI.9b: Student-Newman-Kuels multiple comparison test of statistical difference between VOC treatment analysed in Table AI.9a.

SNK-Grouping	Mean	N	VOC
A	-8.7720	23	ethylbenzene
A	-9.1064	22	toluene
A B	-9.3581	20	tetrachloroethylene
B	-10.0512	19	o-xylene

Table AI.9c: Student-Newman-Kuels multiple comparison test of statistical difference between sampling day treatments analysed in Table AI.9a.

SNK-Grouping	Mean	N	Sampling Day
A	-8.0982	18	3
A	-8.3897	16	4
A	-8.6001	10	5
A	-9.0437	3	6
A	-9.3976	18	2
B	-11.4706	19	1

APPENDIX II: BIOAEROSOL ANALYSIS OF VARIANCE OUTPUTS.

Table AII.1: Analysis of variance for the viable fungal spore load during biofilter commissioning.
Spore load = A + B*(Site) +C*(Date)+D*(Site*Date)+E*(Outside concentration).

Source	df	Type III SS	MS	F	Pr > f
Model	30	56181.064	1872.702	2.18	0.0058
Error	56	48027.808	857.639		
Site	4	12292.075	3073.019	3.58	0.0144
Date	6	17333.851	2888.975	3.37	0.0066
Site*Date	19	24558.907	1292.574	1.51	0.1188
Outside	1	279.484	279.484	0.33	0.5704
Total	86	104208.87			

Table AII.2: Multiple comparisons of the site factor on the viable spore count (CFU m^{-3}) during biofilter commissioning.

Treatment by site	Mean	Least Significant Difference	Student-Newman-Kuelhs
College	52.94	A	A
Foyer 1	44.49	A	A
Foyer 2	40.97	A	AB
Hall 1	37.5	AB	AB
Hall 2	17.71	B	B

Table AII.3: Students t-values comparing the pooled test facility fungal spore loads to the reference site spore loads. Significance is based on a two tailed test at a significance level of 0.5. The critical t-value is 2.132.

Sampling date	Pooled test facility load	Reference facility load	t- value
292	47.91 ± 18.00	85.42 ± 16.22	-2.0831
301	56.77 ± 26.43	54.17 ± 10.34	0.0985
322	30.73 ± 10.94	58.33 ± 12.27	-2.5229
328	27.60 ± 13.22	125.0 ± 17.67	-7.3688
340	20.31 ± 6.45	6.25 ± 4.50	2.1804
348	22.40 ± 11.07	12.5 ± 1.70	0.08936

Table AII.3: Analysis of variance for the bacterial spore load during biofilter commissioning :
Spore load = A + B*(Site) +C*(Date)+D*(Site*Date)+E*(Outside concentration).

Source	df	Type III SS	MS	F	Pr > f
Model	30	1364608.811	45486.96	3.87	<0.0001
Error	62	728954.697	11757.334		
Site	4	352461.9021	88115.4755	7.49	<0.0001
Date	6	414443.0718	69073.8453	5.87	<0.0001
Date x Site	19	532140.6679	28007.4036	2.38	0.0054
Outside	1	1396.8652	1396.8652	0.12	0.7315
Total	92	2093563.50			

Table AII.4: Multiple Comparison of the site factor on the bacterial bioaerosol load (CFU m^{-3})during biofilter commissioning.

Treatment-Site	Mean	Least Significant Difference	Student-Newman-Kuelhs
Hall 1	306.62	A	A
College	251.19	AB	AB
Foyer 2	214.58	ABC	ABC
Foyer 1	163.16	BC	BC
Hall 2	111.81	C	C

Table AII.3: Students t-values comparing the pooled test facility bacterial spore loads to the reference site spore loads. Significance is based on a two tailed test at a significance level of 0.5. The critical t-value is 2.132.

Sampling date	Pooled test facility load	Reference facility load	t- value
292	269.27 ± 89.24	404.17 ± 44.71	-0.6545
301	269.79 ± 60.15	218.75 ± 20.69	0.3674
322	171.35 ± 26.43	229.17 ± 7.41	-0.9472
328	170.31 ± 28.21	141.67 ± 35.84	0.4398
340	192.19 ± 53.21	114.58 ± 37.31	0.6315
348	103.13 ± 7.74	141.67 ± 9.00	-2.1554

APPENDIX III: REGRESSION OUTPUTS FOR METHYLETHYLKETONE, TOLUENE AND TRICHOROETHYLENE FLUX EXPERIMENTS.

Table AIII.1: Analysis of variance for the removal of methylethylketone. The analytical model was $C_R = A_{panel} + B*(EBRT) + C(ET)$.

Source	df	Type III SS	MS	F	Pr > I T I
Panel	3	0.0544	0.01812	3.21	0.0299
EBRT	1	0.9247	0.9247	164.04	0.0001
ET	1	0.0087	0.0087	1.55	0.2183
Error	54	0.3044	0.0056		
Total	59	1.2951			

Table AIII.2: Regression output for the removal of methylethylketone through all panels. Parameters are reported for the model $C_R = A_{panel} + B*(EBRT)$.

Parameter	Estimate	Standard Error	t value	Pr > I T I
A_{panel1}	0.5043	-0.09	0.9309	0.0286
A_{panel2}	0.5397	1.08	0.2868	0.0278
A_{panel3}	0.4528	-1.96	0.0552	0.0276
A_{panel4}	0.5068	2.88	0.0057	0.1759
EBRT	-0.9155	1.25	0.2183	0.0121

Table AIII.3: Analysis of variance for the removal of trichloroethylene. The analytical model was $C_R = A_{panel} + B*(EBRT) + C(ET)$.

Source	df	Type III SS	MS	F	Pr > I T I
Panel	3	0.0324	0.0108	8.28	0.0001
EBRT	1	0.0016	0.0016	1.24	0.2707
ET	1	0.0423	0.0423	32.49	0.0001
Error	54	0.0703	0.0013		
Total	59	0.1415			

Table AIII.4: Regression output for the removal of trichloroethylene through all panels. Parameters are reported for the model $C_R = A_{panel} + B*(ET)$.

Parameter	Estimate	Standard Error	t value	Pr > I T I
A_{panel1}	1.5764	2.06	0.0444	0.0138
A_{panel2}	1.5253	-1.71	0.0938	0.0134
A_{panel3}	1.5152	-2.51	0.0150	0.0133
A_{panel4}	1.5481	18.31	0.0001	0.0845
ET	-0.0330	-5.70	0.0001	0.0058

Table AIII.5: Analysis of variance for the removal of toluene. The analytical model was $C_R = A_{panel} + B*(EBRT) + C(ET)$.

Source	df	Type III SS	MS	F	Pr > I T I
Panel	3	0.2656	0.0885	24.20	0.0001
EBRT	1	0.7401	0.7401	202.24	0.0001
ET	1	0.0094	0.0094	2.57	0.1145
Error	54	0.1976	0.00366		
Total	59	1.2103			

Table AIII.6: Regression output for the removal of toluene through all panels. Parameters are reported for the model $C_R = A_{panel} + B*(ET)$.

Parameter	Estimate	Standard Error	t value	Pr > I T I
A_{panel1}	1.1724	-1.44	0.1547	0.231
A_{panel2}	1.1028	-4.60	0.0001	0.0224
A_{panel3}	1.0322	-7.81	0.0001	0.0222
A_{panel4}	1.2057	8.51	0.0001	0.1417
EBRT	-0.8191	-14.22	0.0001	0.0576

Table AIII.7: Analysis of variance for the regression of the natural log of the removal coefficient of methylethylketone onto the solubility corrected empty bed retention time. The input model $Ln(C_R) = A_{panel} + B*(SCEBRT)$.

Source	df	Type III SS	MS	F	Pr > F
Panel	3	0.2014	0.0671	2.96	0.0400
SCEBRT	1	4.1849	4.1849	184.72	0.0001
Error	55	1.2460	0.0227		
Total	59	5.6154			

Table AIII.8: Regression outputs for the correlation of the natural log of the removal coefficient of methylethylketone onto the solubility corrected empty bed retention time. The input model $Ln(C_R) = A_{panel} + B*(SCEBRT)$.

Parameter	Estimate	T for Ho: Parameter = 0	Pr > | T |	Standard Error
A_{panel1}	0.2565	0.05	0.9594	0.0550
A_{panel2}	0.1965	-1.04	0.3026	0.0550
A_{panel3}	0.3574	1.88	0.0648	0.0550
A_{panel4}	0.2537	4.87	0.001	0.0521
SCEBRT	0.0023	13.59	0.0001	0.0002

Table AIII.9: Analysis of variance for the regression of the natural log of the removal coefficient of tricholoethylene onto the solubility corrected empty bed retention time. The input model $Ln(C_R) = A_{panel} + B*(SCEBRT)$.

Source	df	Type III SS	MS	F	Pr > F
Panel	3	0.0206	0.0069	3.59	0.0192
SCEBRT	1	0.0036	0.0036	1.88	0.1758
Error	55	0.1051	0.0019		
Total	59	0.1284			

Table AIII.10: Regression outputs for the correlation of the natural log of the removal coefficient of trichloroethylene onto the solubility corrected empty bed retention time. The input model $Ln(C_R) = A_{panel} + B*(SCEBRT)$.

Parameter	Estimate	T for Ho: Parameter = 0	Pr > I T I	Standard Error
A_{panel1}	-0.0691	-0.36	0.7221	0.0160
A_{panel2}	-0.325	1.93	0.583	0.0160
A_{panel3}	-0.0943	2.30	0.0255	0.0160
A_{panel4}	-0.0634	-4.19	0.0001	0.0151
SCEBRT	0.0001	1.37	0.1758	0.0001

Table AIII.11: Analysis of variance for the regression of the natural log of the removal coefficient of toluene onto the solubility corrected empty bed retention time. The input model $Ln(C_R) = A_{panel} + B*(SCEBRT)$.

Source	df	Type III SS	MS	F	Pr > F
Panel	3	0.5753	0.1918	18.30	0.0001
SCEBRT	1	1.7979	1.7979	171.55	0.0001
Error	55	0.5764	0.0105		
Total	59	2.8301			

Table AIII.12: Regression outputs for the correlation of the natural log of the removal coefficient of toluene onto the solubility corrected empty bed retention time. The input model $Ln(C_R) = A_{panel} + B*(SCEBRT)$.

Parameter	Estimate	T for Ho: Parameter = 0	Pr > I T I	Standard Error
A_{panel1}	0.0294	13.10	0.001	0.0374
A_{panel2}	0.1111	1.89	0.0644	0.0374
A_{panel3}	0.2225	4.07	0.0002	0.0374
A_{panel4}	-0.04116	7.04	0.2506	0.0354
SCEBRT	0.0024	-1.16	0.001	0.0035

APPENDIX IV. REGRESSION OUTPUTS FOR 2-PENTANONE, ACETONE AND METHYLISOBUTYLKETONE FLUX EXPERIMENTS.

Table AIV.1: Analysis of variance output for the removal of 2-pentanone. Parameters are reported for the model $C_R = A_{panel} + B*(EBRT) + C*(ET)$.

Source	df	Type III SS	MS	F	Pr > F
Panel	5	0.41476	0.08295	3.51	0.0063
EBRT	1	3.40296	3.40296	144.08	0.0001
ET	1	0.08724	0.08724	3.69	0.0581
Error	82	1.93673	0.02362		
Total	89	5.77775			

Table AIV.2: Regression output for the removal of acetone. Parameters are reported for the model $C_R = A_{panel} + B*(EBRT) + C*(ET)$.

Parameter	Estimate	T for Ho: Parameter = 0	Pr > I T I	Standard Error
A_{panel1}	1.2015	-0.06	0.9511	0.0573
A_{panel2}	1.0874	-2.18	0.0323	0.0540
A_{panel3}	1.0624	-2.54	0.0131	0.0563
A_{panel4}	1.0544	-2.68	0.0089	0.0563
A_{panel5}	1.0339	-3.03	0.0033	0.0565
A_{panel6}	1.2051	1.66	0.10 14	0.7274
EBRT	-1.8683	-12.00	0.0001	0.1556
ET	-0.0743	-1.92	0.0581	0.0386

Table AIV.3: Analysis of variance output for the removal of acetone. Parameters are reported for the model $C_R = A_{panel} + B*(EBRT) + C*(ET)$.

Source	df	Type III SS	MS	F	Pr > F
Panel	5	0.80532	0.16106	12.53	0.0001
EBRT	1	6.45785	6.45785	502.23	0.0001
ET	1	0.056298	0.056298	4.38	0.0392
Error	90	1.15726	0.012858		
Total	97	8.42100			

Table AIV.4: Regression output for the removal of acetone. Parameters are reported for the model $C_R = A_{panel} + B*(EBRT) + C*(ET)$.

Parameter	Estimate	T for Ho: Parameter = 0	Pr > I T I	Standard Error
A_{panel1}	1.0176	4.11	0.0001	0.0409
A_{panel2}	0.9521	2.55	0.0123	0.0402
A_{panel3}	0.7823	-1.63	0.1068	0.0412
A_{panel4}	0.7515	-2.48	0.0151	0.0395
A_{panel5}	0.8520	0.06	0.9506	0.0402
A_{panel6}	0.8495	1.78	0.0780	0.4765
EBRT	-2.4712	-22.41	0.0001	0.1103
ET	-0.05304	-2.09	0.0392	0.0253

Table AIV.5: Analysis of variance output for the removal of methylisobutylketone. Parameters are reported for the model $C_R = A_{panel} + B*(EBRT) + C*(ET)$.

Source	df	Type III SS	MS	F	Pr > F
Panel	5	0.3343	0.06686	5.57	0.0002
EBRT	1	2.1514	2.1514	179.30	0.0001
ET	1	0.0698	0.0698	5.82	0.0180
Error	84	1.0079	1.0079		
Total	91	3.6439			

Table AIV.6: Regression output for the removal of methylisobutylketone. Parameters are reported for the model $C_R = A_{panel} + B*(EBRT) + C*(ET)$.

Parameter	Estimate	T for Ho: Parameter = 0	Pr > I T I	Standard Error
A_{panel1}	1.0176	4.03	0.0456	0.0425
A_{panel2}	1.0346	2.12	0.0365	0.0393
A_{panel3}	1.0319	-1.91	0.0601	0.0402
A_{panel4}	0.8716	0.68	0.5004	0.0400
A_{panel5}	0.9050	-1.10	0.2764	0.0395
A_{panel6}	0.9483	2.13	0.0358	0.4446
EBRT	-1.4810	-13.39	0.0001	0.1106
ET	-0.05710	-2.41	0.0180	0.0237

Table AIV.7: Analysis of variance for the regression of the natural log of the removal coefficient of 2-pentanone onto the solubility corrected empty bed retention time. The input model $Ln(C_R) = A_{panel} + B*(SCEBRT) + C*(Panel \times SCEBRT)$.

Source	df	Type III SS	MS	F	Pr > F
Panel	5	0.2711	0.0542	2.19	0.0639
SCEBRT	1	2.7815	2.7815	112.52	0.0001
SCEBRT x Panel	5	0.2391	0.0478	1.93	0.0987
Error	74	1.8292	1.8292		
Total	85	5.3636			

Table AIV.8: Regression outputs for the correlation of the natural log of the removal coefficient of 2-pentanone onto the solubility corrected empty bed retention time. The input model $Ln(C_R) = A_{panel} + B*(SCEBRT)$.

Parameter	Estimate	T for Ho: Parameter = 0	Pr > |T|	Standard Error
A_{panel1}	-0.1972	-0.53	0.5944	0.1285
A_{panel2}	-0.3790	-2.25	0.0271	0.1111
A_{panel3}	-0.4264	-2.80	0.0065	0.1064
A_{panel4}	-0.2483	-0.85	0.4002	0.1415
A_{panel5}	-0.1975	-0.58	0.5655	0.1194
A_{panel6}	-0.1286	-1.65	0.1024	0.0777
SCEBRT	-0.0072	-5.60	0.0001	0.0013

Table AIV.9: Analysis of variance for the regression of the natural log of the removal coefficient of acetone onto the solubility corrected empty bed retention time. The input model $Ln(C_R) = A_{panel} + B*(SCEBRT) + C*(Panel \times SCEBRT)$.

Source	df	Type III SS	MS	F	Pr > F
Panel	5	0.1379	0.0275	2.20	0.0629
SCEBRT	1	5.0906	5.0906	405.58	0.0001
SCEBRT x Panel	5	0.3035	0.0607	4.84	0.0007
Error	78	0.9790	1.8292		
Total	89	7.6190			

Table AIV.10: Regression outputs for the correlation of the natural log of the removal coefficient of acetone onto the solubility corrected empty bed retention time. The input model $Ln(C_R) = A_{panel} + B*(SCEBRT)_{panel}$.

Parameter	Estimate	T for Ho: Parameter = 0	Pr > I T I	Standard Error
A_{panel1}	-0.09715	-0.02	0.9880	0.1285
A_{panel2}	-0.1145	-0.18	0.8555	0.1111
A_{panel3}	-0.2381	-1.87	0.0657	0.1064
A_{panel4}	-0.0470	0.54	0.5927	0.1415
A_{panel5}	0.0165	1.45	0.1521	0.1194
A_{panel6}	-0.0984	-1.65	0.1024	0.0777
$SCEBRT_{panel1}$	-0.0020	2.21	0.0302	0.0004
$SCEBRT_{panel2}$	-0.0021	1.64	0.1058	0.0004
$SCEBRT_{panel3}$	-0.0023	1.26	0.2106	0.0003
$SCEBRT_{panel4}$	-0.0037	-1.57	0.1205	0.0005
$SCEBRT_{panel5}$	-0.00383	-1.90	0.0607	0.0005
$SCEBRT_{panel6}$	-0.00279	-9.15	0.0001	0.0003

Table AIV.11: Analysis of variance for the regression of the natural log of the removal coefficient of methylisobutylketone onto the solubility corrected empty bed retention time. The input model $Ln(C_R) = A_{panel} + B*(SCEBRT) + C*(Panel \times SCEBRT)$.

Source	df	Type III SS	MS	F	Pr > F
Panel	5	0.1197	0.0239	1.91	0.1022
SCEBRT	1	1.8238	1.8238	145.51	0.0001
SCEBRT x Panel	5	0.1511	0.0302	2.41	0.0436
Error	78	0.9776	0.0125		
Total	89	3.6959			

Table AIV.12: Regression outputs for the correlation of the natural log of the removal coefficient of methylisobutylketone onto the solubility corrected empty bed retention time. The input model $Ln(C_R) = A_{panel} + B*(SCEBRT)_{panel}$.

Parameter	Estimate	T for Ho: Parameter = 0	Pr > |T|	Standard Error
A_{panel1}	-0.9949	-0.02	0.9880	0.0760
A_{panel2}	-0.0596	-0.18	0.8555	0.0933
A_{panel3}	-0.2013	-1.87	0.0657	0.0770
A_{panel4}	0.0128	0.54	0.5927	0.0969
A_{panel5}	0.0058	1.45	0.1521	0.0818
A_{panel6}	-0.1461	-1.65	0.1024	0.0549
$SCEBRT_{panel1}$	-0.0241	-0.04	0.9642	0.0072
$SCEBRT_{panel2}$	-0.0352	-0.14	0.8879	0.0081
$SCEBRT_{panel3}$	-0.0267	-0.42	0.6747	0.0070
$SCEBRT_{panel4}$	-0.04358	-1.40	0.1664	0.0110
$SCEBRT_{panel5}$	-0.0521	-2.82	0.0055	0.0099
$SCEBRT_{panel6}$	-0.0238	-4.14	0.0001	0.0058

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