BIOAEROSOL PRODUCTION FROM INDOOR AIR BIOFILTERS

J Mallany[1]*, A Darlington[2], and M Dixon[1]

[1]Controlled Envrionmental Systems Research Group, Department of Plant Agriculture, University of Guelph, Guelph, On, Canada, N1G 2W1
[2]Air Quality Solutions Ltd., 55 Callander Dr., Guelph, On, Canada, N1E 4H6

ABSTRACT
Several indoor air biofilters containing higher plants, mosses and microbes have been incorporated into functional offices, where they are a supplemental means of controlling indoor air quality through the removal of volatile organic compounds. In theory a rich microbial community indoors may in fact lower air quality through the production of microbial agents such as spores or aerial bacteria. Questions have arisen regarding the impact of an indoor air biofilter on ambient spore concentrations including the pathogen *Legionella pneumophilia*. This study presents empirical evidence which indicates that indoor air biofilters do not increase the spore load of "treated" air under stable conditions and creates only minor increases when subjected to disturbance events.

INDEX TERMS
Indoor air quality, Biofiltration, *Legionella*, bioaerosols, Spores

INTRODUCTION
Indoor air biofilters containing higher plants, mosses and microbes have been successfully used to remediate some indoor air quality (IAQ) issues (Darlington and Dat and Dixon 2001, Darlington *et al* 2000 and Darlington and Dixon and Pilger 1998). In these systems, air from an occupied space (the influent) is drawn through wet biomass allowing airborne pollutants, such as volatile organic compounds, to be sorbed and broken down by the biological community. The "treated" air (the effluent) is then returned to the space, diluting the concentration of airborne contaminants.

There are several characteristics of these systems which may encourage airborne spore production. The biofilters contain recirculating water over porous surfaces and may add humidity to the ambient air. Moisture is a major factor in the proliferation of bioaerosols (Singh *et al* 1994). Airborne spores may be further encouraged by the presence of dead plant matter, which provides a carbon source for microbial growth. Furthermore, the circulation of air through the biofilters can potentially distribute microorganisms or their spores throughout the airspace of the building. Experiments were conducted to better define how the operation of the system influenced IAQ through the production of airborne spores.

MATERIALS AND METHODS
The Biofilters:
Two biofilters were tested. The Canada Life Environmental Room (CLER) is the larger of the systems, it was constructed as a component of a large meeting room and features a dedicated HVAC system. This system has been discussed in detail elsewhere (Darlington et al

* Contact author email: jmallany@uoguelph.ca

1996, Darlington and Dixon and Dat 1998). The second biofilter was a smaller modular unit designed as a retro-fit into existing spaces. Both biofilters consisted of a complex community of plants, mosses and microbes which could be separated into three interconnected systems: a porous wall planted with mosses and small plants ($10m^2$ and $4m^2$, CLER and module respectively), a hydroponically grown community of higher plants and an aquarium. The wall functioned as a biofilter by pulling air from the building (the influent) through the biological community. As air moved through the wall, chemical contaminants were removed from the airstream and treated air (the effluent) was returned to the influent air mass (Figure 1). The hydroponic region served two purposes; it collected excess water from the irrigation of the biofilter panels and provided habitat for plants in the system. The CLER contained a larger volume of water (3.5 vs. $0.5m^3$) and a larger planting area (20 m^2 compared to $0.25m^2$) than the modular unit.

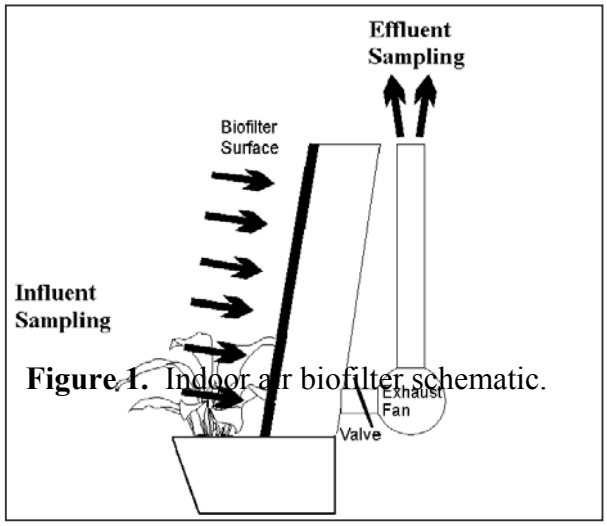

Figure 1. Indoor air biofilter schematic.

Microbial communities in the basin may degrade compounds not degraded on the biofilter surface. In this sense, the plantings also acted as a bioreactor and the moss covered wall acted as bioscrubbers. Microbial growth was not deliberately discouraged on either the moss covered wall or in the hydroponic region, nor was the system inoculated with any specific microbial populations.

Microbes in biofilter exhaust air:

Bacterial and fungal spore concentrations were measured with a BIOTEST Reuter centrifugal sampler (Biotest Diagnostics Corporation, Denville, NJ). The sampler was operated for a four minute period and sampled 0.16 m3 of air per sample. Fungi and bacteria were collected on rose bengal agar (RBA) and trypic soy agar respectively (TSA) (RWR Scientific, Ottawa, ON). Samples were incubated for 5-7 days at room temperature (NIOSH 1998). Samples were collected in the air entering the modular biofilter (the influent) and as it exited the biofilter (the effluent). Influent concentrations were compared to the effluent concentrations using a one factor ANOVA.

Biofilter activation:

Following a period of biofilter inactivity fungal and bacterial spores were collected in the CLER airspace and an indoor reference site outside of the immediate influence of the biofilter. Three reference and airspace samples were taken before the biofilter air handling system was activated. With the initiation of the air handling system biologically filtered air is returned to the airspace in a recirculating manner. Monitoring continued four hours following the biofilter reactivation. All samples were measured using the RCS sampling procedure described above.

Biofilters under dynamic conditions:

Bacterial and fungal spore loads in the space impacted by the modular biofilter were measured over seven treatment periods of 64 hours, allocated to one of two operational schedules. On three of the periods, the biofilter actively circulated air for 16 hours per day, which is consistent with 'limited operation'. The remaining four treatment periods the biofilter exchanged air 24 hours per day, which is consistent with 'extended operation'.

Fungal and bacterial spore concentrations were measured in the building air during the 1st, 16th, 24th, 40th, 48th and 64th hours of operation. Sampling times were arranged to be consistent between treatments and so that measurments were always taken when the biofilter was actiev. Samples were collected using the RCS procedure described above.

Legionella sampling:

Water samples were collected from the modular biofilter after one and eight months of operation and from the CLER biofilter after five and seven years of operations. All samples were collected in sterile polypropylene bottles. In addition samples of moss from the biofilters surfaces were included from each site. Samples were analysed by Pathcon Laboratories (Atlanta, Georgia) using a proprietary culture technique.

RESULTS AND DISCUSSION

Microbes in biofilter exhaust air:

During the commissioning of the biofilter both fungal and bacterial spores were observed to remain well below safety guidelines. Health Canada (1995) recommends that no more than 150 CFU m^{-3} of fungal spores should be present in the air stream. Reponen *et al* (1992) recommends a limit of 5000 CFU m^{-3} bacterial spores for a similar climate and time of year. Although there was no increase in the ambient spore loads near the biofilter during the commissioning period, a single measurement in the effluent stream of the biofilter taken during the initial startup revealed spore levels of 175 (fungal) and 423 CFU m^{-3} (bacterial). A second measurement taken 1 week later showed a greatly reduced concentration in the effluent stream (44 and 144 CFU m^{-3} for fungi and bacteria respectively).

Table 1: Average fungal and bacterial spore load and standard errors measured in the influent and effluent of the modular biofilter. P values represent the probability that no difference exists between sampling locations based on a 1 factor ANOVA for each spore type.

	Fungal spore load (CFU m^{-3})	**Bacterial spore load (CFU m^{-3})**
Influent air	17.6± 5.3	112.9 ± 10.97
Effluent air	23.4 ± 5.0	103.9 ± 12.6
P-value	0.0187	0.7781

To further investigate the impact of the biofilter on the air mass, spore measurements were taken during 64 hour periods of low building traffic and occupancy. Using this approach the impact of building occupants and of outdoor air could be greatly reduced. No increase in the fungal spore load was detected over this period despite an increase ($P > 0.05$) in the exhaust air (Table 1.). Over the same period the bacterial spores generally decreased, which supports the hypothesis that occupants are the major source of bacterial spores indoors (Heinemann and Beguin and Nolard. 1994). No increase in the bacterial spore load was observed in the effluent stream (Table 1).

Biofilters under dynamic conditions:

The release of spores from the biofilter may be sensitive to changes in the airflow, rather than air flow itself. Evaluating the modular biofilter under two operational schedules tested this hypothesis. When under 'continuous operation' (24 hours per day) the airflow through the system remained constant. During 'staggered operation' (16 hours per day) the airflow was

stopped for 8 hours resulting in a "start-up burst" of airflow each day. This treatment did not effect the fungal spore contribution to the space (Table 2). It is likely that air flow through the biofilter was insufficient to disturb spores present in the biofilter. Air velocities of 1.0 ms-1 may be required to release the spores of several common indoor genera into the airstream (Pasanen et al 1991), and the air flow through the biofilter was controlled at a maximum velocity of 0.1 m s-1. Bacterial spore loads measured during 'staggered operation' were slightly elevated (P = 0.077) compared to those sampled during 'continuous operation'.

Table 2: Average ambient fungal and bacterial spore load (CFU m^{-3}) and standard errors measured during continuous (24 hours per day) and staggered (16 hours per day) operation of the biofilter fan. P values represent the probability that no difference existed between operational schedules based on a pairwise t-test for each spore type.

	Ambient fungal spore load (CFU m^{-3})	**Ambient bacterial spore load (CFU m^{-3})**
Continuous operation	15.3 ± 4.1	107.1 ± 14.3
Staggered operation	23.6 ± 7.6	149.0 ± 18.0
P-value	0.3299	0.0777

Biofilter reactivation after inactivity:

There was a temporary increase in the fungal spore load of the CLER following reactivation of the biofilter (Figure 2a.). This increase was short lived and did not represent a safety concern. The bacterial bioaerosols collected were generally below the reference readings. A decline in the reference site in this period was likely a result of decreased building occupancy during the monitoring period (Figure 2b). These results suggest that for the measured bacterial population, biofilter activity had a smaller impact on IAQ than building activities.

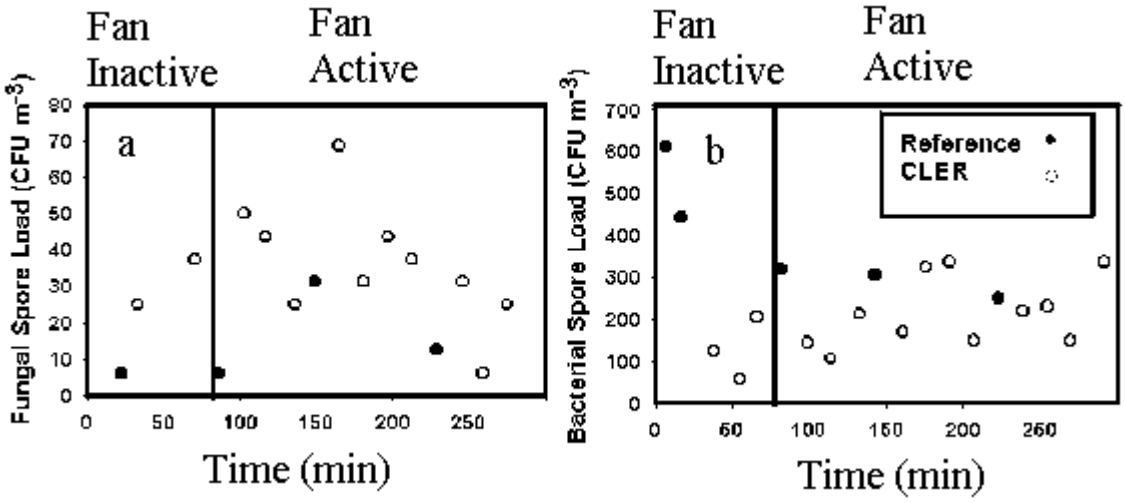

Figure 2: Fungal (a) and bacterial (b) spore loads (CFU m^{-3}) following biofilter fan reactivation

Legionella sampling:
Legionella pneumophila is the causal agent of Legionnaire's disease and Pontiac fever. It is an airborne pathogen, known to proliferate in standing water such as improperly maintained HVAC systems. Exposure of immuno-compromised individuals to *Legionella sp.* may result in fatal infection (OSHA 1999). Two indoor air biofilters were tested for the presence of *Legionella sp.* The watering system of the modular biofilter was sampled during its first and eighth month of operation; the watering system of the CLER biofilter was sampled during its fifth year of operation. All samples contained undetectable concentrations (less than 1 CFU ml^{-1}) of the pathogen.

These results suggest that indoor biofilters containing complex communities of plants and microbes do not support the proliferation of *Legionella sp*. There may be several reasons for this. In its natural habitat, *Legionella sp.* accounts for less than 1% of the natural bacteria population due to competition and predation. The ecologically diverse nature of these systems suggests similar processes might have existed here. Finally, the salt content of the biofilters was at or above the tolerance range of the bacterium. The modular biofilter was maintained between 250 and 400 $\mu S\ cm^{-2}$, and the CLER was maintained at 70 to 150 $\mu S\ cm^{-2}$. The reported electrical conductivity range of the bacterium is between 18 and 106 $\mu S\ cm^{-2}$ (Fliermans *et al* 1981). The temperature of both biofilters is maintained below 20 C; well below the reported optimum of the pathogen (ASHRAE 1998).

SUMMARY AND CONCLUSIONS

Tests were conducted to assess the impact of incorporating an indoor air biofilter containing higher plants, mosses and microbes into an operational office environment. Increased bioaerosol measurements were only observed in the effected airspace after a major disturbance to the system such as the shortterm increase observed during the initial start-up, or after extended periods of no air flow. No observations conducted during major disturbance events indicated bioareosol concentrations that were a concern to public health or safety. The minor disturbances inherent with normal bioifilter operation, including changes in airflow rates produced only minor increases in the biofilter exhaust and no observable effects in the surrounding space. The system was also tested for the pathogen *Legionella pneumophila,* which was not detectable in any samples. These results indicate that normal operation of this technology in an indoor environment does not compromise IAQ through the production of bacterial or fungal spores or through the production of the pathogen *Legionella pneumophilia.*

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