THE BIOFILTRATION OF INDOOR AIR II: MICROBIAL LOADING OF THE INDOOR SPACE.

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ABSTRACT

Two ecologically complex indoor air biofilters were tested for their impact on selected airborne spore concentrations. Fungal and bacterial spore concentrations were monitored during the commissioning of a biofilter and found to remain consistent with reference site. While the water path was devoid of the pathogen *Legionella pneumophilia,* irrigation schedules could impact air quality. The air path was not seen to add to the fungal or bacterial concentrations of the ambient air.

INTRODUCTION

Ecologically complex biofiltration systems seek to include a diverse selection of plants and microbes, the assumption being that a diverse system will be better able to assimilate a dilute mixture of volatile organic compounds (1, 2). Indoor plantings, however, have been associated with the production of airborne spores (3) particularly after disturbance by air movement or watering. Since such disturbances are common in indoor air biofilters, it must be established that they are not a source of airborne spores. Production of bioaeosols such as these could reduce indoor air quality, despite the removal of volatiles.

Two ecologically complex systems were tested for their contributions to the ambient airborne spore load of their respective buildings. The Canada Life Environmental Room (CLER) is a prototype biofilter constructed into a 160 m^2 ground floor meeting room in the Canada Life Assurance Company (Toronto, Ontario, Canada). This system contains a 20 m^2 community of tropical species grown hydroponically, a 3.5 m^3 aquarium and a 12 m^2 moss covered wall, which acts as a bioscrubber. Air is circulated through the wall by a dedicated air handling system. Irrigation water for the moss and plant species is taken directly from the aquarium and delivered via low pressure misting lines. The Northern Center for Advanced Technology (NORCAT) (Sudbury, Ontario, Canada) biofiltration system contains a complex community of northern plant species grown hydroponically. The NORCAT biofilter is a stand alone unit able to be retrofit into an indoor space. Like CLER it includes a moss covered wall (4 m^2) designed to act as a bioscrubber. Air is circulated through this wall by a variable speed exhaust fan. These systems contain several design characteristics which may encourage airborne spore production. Circulation of air through the wet biomass creates a stream of moist air, moisture is a major factor in the proliferation of bioaerosols (4). Circulation can also distribute microorganisms or their spores to the occupants of the building. Finally, each system contains between 0.5 m^3 and 3.5 m^3 of water. Stagnant water is believed to be a source of spores indoors (4).

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Three types of bioaerosols were monitored: fungal spores, bacteria and *Legionella pneumophila*. It should be noted that microbial contamination of indoor air is usually not an area of concern (5). Rarely are bioaerosols responsible for serious or life threatening infections. However, among immunocomprimised individuals airborne spores may cause respiratory tract infection, allergic rhinitis, asthma, humidifier fever and hypersensitive pneumonitis (6, 7).

Viable fungal spores Excess mould growth is associated with damp or wet areas (8). The precise relationship between mould growth, sporulation and moisture is complex. While the availability of water or moisture limits the growth of moulds (4, 8), sporulation maybe encouraged at lower relative humidities (9). CLER and NORCAT may encourage fungal activity by providing a water source, however, they may discourage sporulation by increasing the ambient humidity.

A healthy indoor spora is a dilute mixture of species similar to outdoor, (10-12). The indoor environment generally has fewer than 50 colony forming units per cubic meter ($CFU\,m^{-3}$) (11, 13). Health Canada (13) guidelines recommend that no more than 50 CFU m^{-3} of any single species be present in the air stream, excluding *Cladosporium sp.* or *Alternaria sp.* which are extremely common outdoor fungi. A combined total, including all species present, should not exceed 150 CFU m^{-3}.

Viable bacteria Health Canada defines unhealthy bacterial loads as those exceeding 500 CFU m^{-3}(13). Much of the indoor bacterial load can be attributed to building occupants, (14). Nevalainen and coworkers (12) report higher bacterial loads in older buildings and in buildings in more rural settings.

Legionella pneumophila *L. pneumophila* is the causal agent of Legionnaire's disease and Pontiac fever. It is an opportunistic pathogen attacking immunocompromised individuals, less than 5% of the population is at risk (15). There are more than 25 000 cases per year in the US, resulting in more than 4000 deaths (15).

MATERIALS AND METHODS

Four experiments were conducted to measure airborne spore production from ecologically complex biofilters: 1) changes in microbial concentrations during biofilter commissioning, conducted at the NORCAT facility, 2) the low pressure misting system at the CLER was tested as a source of airborne bacteria and fungi, 3) microbes concentrations were measured in the exhaust air stream of the NORCAT system, 4) *Legionella pneumophillia* was sampled at both locations.

Bioaerosol changes during biofilter commissioning Bioaerosol concentrations were measured during the following phases of commissioning: pre commissioning (dates 210 and 260) addition of the plant material excluding mosses (dates 292 and 302) , addition of mosses (dates 321 and 328) and activation of the air handling system (dates 340 and 348). On each sampling day, seven sites were measured for fungal and bacterial concentrations; four indoor test sites at the NORCAT facility, two outdoor references and 1 off site reference. Sampling data and location were included as variables in the ANOVA (SAS version 8.0).

Airborne spore concentrations were measured with a BIOTEST Reuter centrifugal sampler (Biotest Diagnostics Corporation, Denville, New Jersey). Fungus and bacteria were were collected on rose bengal agar (RBA) and trypic soy agar respectively (TSA) (RWR

Scientific, Ottawa). Samples were incubated for 5-7 days at room temperature (16).
The impact of misting Airborne microorganisms arising from the irrigation system were assessed at CLER on two dates one year apart using a two-stage Anderson sampler. Nutrient agar and Leonian's agar were used to collect bacterial and fungal spores respectively. On each date, four samples were collected, during each of two spray irrigation cycles. Within each cycle, the sampler was operated for 2.5 and 5 minutes during the cycle and 3.5 minutes after the cycle. The plates were incubated for 48 hours at 25 EC, separate counts were made for bacteria and moulds and results were calculated in terms of CFU m^{-3}. Data was analysed as a two factor ANOVA using SAS (Version 6.0).

Microbes in biofilter exhaust air Bacterial and fungal spore loads were measured over seven weekends which were randomly allocated to one of two operational schedules of the NORCAT system. On three of the weekends the biofilter actively circulated air only at night, which is consistent with 'limited operation'. Four weekends the biofilter continuously exchanged air. During 'continuous operation' the ambient air and the exhaust stream were sampled. Data was analysed using pair wise comparisons between operation schedules and between ambient and exhaust. Samples were measured using the same technique as the commissioning experiment.

***Legionella* sampling** Samples were collected from both the NORCAT (8 samples) system and CLER (3 samples) in sterile polypropylene bottles. In addition a sample of moss was collected from each site and included as an additional sample. Samples were analysed by Pathcon Laboratories (Atlanta, Georgia) using a proprietary culture technique.

RESULTS AND DISCUSSION

The viable fungal spore levels observed in the test site ranged from 18 to 58 CFU m^{-3}. This is comparable to other reported values (11, 13). Bacteria ranged from 75 to 535 CFU m^{-3} with half the readings between 100 and 200 CFU m^{-3}. The tests sites did not have significantly higher moulds or bacteria than the reference site (Figure 1 and 2). Comparison of the different stages of commissioning indicated that the biofilter did not significantly impact the quality of air through spores loads (fungal or bacterial).

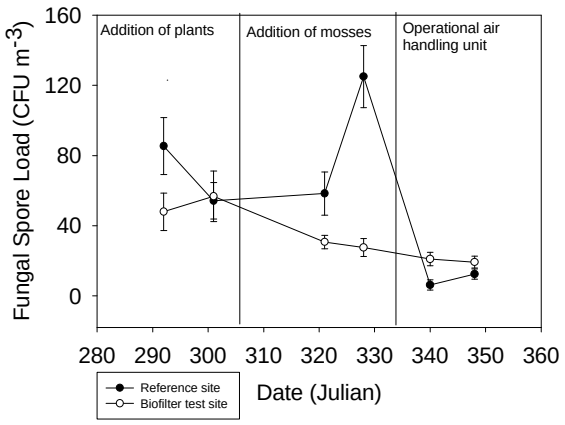

Figure 1 Average fungal concentrations in the test facility and at the reference site throughout biofilter commissioning.

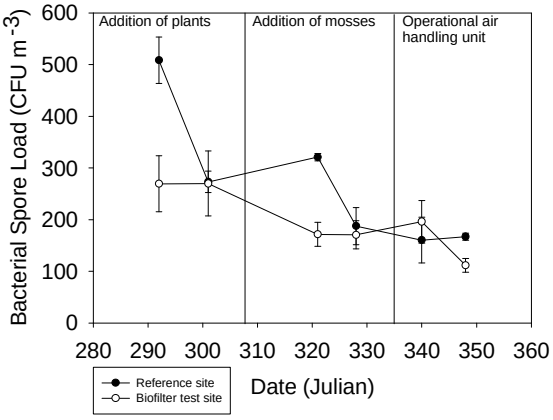

Figure 2 Average bacterial concentration in the test facility and the at the reference site throughout biofilter commissioning.

Bacterial loads were significantly influenced by the irrigation cycle, increasing approximately three fold during the cycle. The increase in fungal spore loads was found to be not significant. The elevated bacterial loads during the irrigation cycles could be attributed to two factors: the disturbance of the soil (3) or the atomization of aquarium water used for irrigation. Airborne spore counts were higher than mean levels reported for other commercial indoor spaces, but were well within the reported ranges (11). The room housing the biofilter had a slightly higher average spore count than the reference room. Airborne bacteria counts were dependant on the sampling year ($Pr > F = 0.06$). Subsequent to the initial bacterial loads of 53 CFU m^{-3}, changes in the maintenance of CLER declined the bacterial load to 27 CFU m^{-3}. These results are discussed in detail elsewhere (17).

Table 1 Ambient airborne spore counts at CLER affected by irrigation and year. Samples collected during irrigation with water from the recirculating aquarium (1) or were collected after the cycle (0).

During Irrigation Cycle	Year	Mould (CFU m^{-3})	Bacteria (CFU m^{-3})
0	1995	107	53
0	1996	76	27
1	1995	179	146
1	1996	98	62

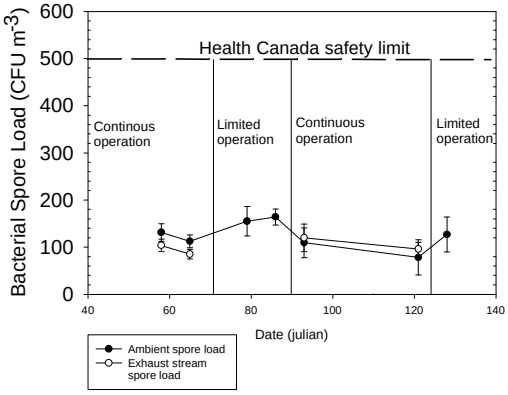

Figure 3 Ambient bacterial concentrations collected during continuous and limited biofilter operation. Exhaust measurements collected during continuous operation.

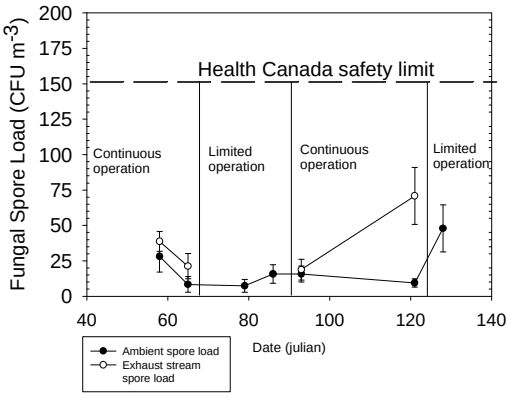

Figure 4 Ambient fungal concentrations collected during continuous and limited biofilter operation. Exhaust measurements collected during continuous operation.

On only one occasion was there a significant difference between ambient and exhaust measurements (Figure 4). Should the biofilter be a source of spores, the exhaust stream would contain a higher concentrations than the ambient air. Furthermore, ambient microbial counts did not increase with the duration of biofilter operation. Should the biofilter act as a source of

bioaerosols, the 'continuous operation' which exchanges a third more air than 'limited operation' should have generated a higher ambient spore loads (Figure 3 and 4).

Legionella pneumophila was undetectable (less than 1 CFU ml^{-1}) in either biofilter. The current operating conditions likely limit its growth. *Legionella sp*. is reported to grow in tap water between 25 and 42 EC (7). Both biofilters were maintained at water temperatures below 20 EC. In nature *Legionella sp.* would account for less than 1% of the natural bacteria population due to competition and predation. The ecologically diverse nature of these system suggests similar processes might exist here. Finally, the salt content of the biofilters are at or above the tolerance range of the bacterium. The NORCAT system was maintained between 250 and 400 µS cm^{-2} , CLER is maintained at 70 to 150 µS cm^{-2}. The reported electrical conductivity range of the bacterium is between 18 and 106 µS cm^{-2}(18).

SUMMARY AND CONCLUSIONS

Tests were conducted on the impact of an ecologically complex biofilters on the concentration of selected bioaerosols indoors. Neither viable fungal spore nor bacteria concentrations showed a significant increase over the reference site. Results of the misting experiment indicated that increased bacterial concentrations were associated with the watering system. Although not at unsafe concentrations modification of the irrigation and maintenance schedules reduced bacterial and fungal concentrations. Measurements from the exhaust stream did not show an increase relative to the ambient concentrations, indicating that the air path is not a source of bioaerosols. *Legionella pneumophila* was not found in any water samples. This technology does not lower ambient air quality through the production of bacterial or fungal spores or through the production of the pathogen *Legionella pneumophilia.*

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