



Mite growth on fungus under various environmental conditions and its potential application to biofilters

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Abstract. The effects of relative humidity, temperature, pH and vapor-phase toluene concentration on *Tyrophagus putrescentiae* growth on *Cladophialophora* sp. were tested in controlled environmental chambers. It was observed that the mites were able to reproduce readily at relative humidities between 90% and 97% as well as on porous perlite support material pre-soaked in nutrient media of pH 2.5, 4 and 7. Also, the presence of toluene at gas-phase concentrations of 500 to 2000 mg m⁻³ was found to be non-toxic to the mites. The mites, however, were unable to maintain a large population when the temperature was maintained at 14 °C, and overpopulation of the living space led to declines in mite population over time. Overall, it was found to be relatively simple to cultivate mites that may be used for fungal biomass control measures in biofilter applications.

Introduction

Biofiltration is the process of treating a contaminated gas stream in a biologically active bed and has been employed since the early 20th century for the purpose of odor control. In the past two decades, the range of applications for biofiltration systems has broadened, and they are now used to treat waste gas streams polluted with volatile organic compounds and inorganic air toxins (Leson and Winer 1991). These systems have potentially high removal efficiencies for biodegradable contaminants as well as lower investment and operational costs than physical-chemical techniques (van Groenestijn and Hesselink 1993). Although these systems have potential advantages, their use in practice has been limited by their instability (Loy et al. 1997). One problem is that the systems begin to clog with excess biomass after extended periods of operation (Weber and Hartmans 1996; Woertz et al. 2001; Song and Kinney 2000).

Many different biomass control strategies have been employed at the laboratory scale in order to facilitate long-term stability of biofiltration systems. The growth rate of organisms was decreased by limiting the available nutrients (Wübker and

Friedlich 1996; Schönduve et al. 1996) and maintaining high salt concentrations in the nutrient medium (Schönduve et al. 1996). Directionally-switching operation also has been implemented to reduce the clogging rate (Song and Kinney 2000). In this approach, the direction of the inlet feed is periodically reversed to avoid overgrowth in the inlet section of the reactor bed. While these measures succeeded in reducing the rate of biomass clogging, system performance often declined and excess biomass still formed after an extended period of operation. Other approaches for biomass control have included the implementation of mechanical and chemical removal systems. Backwashing of filter material was successful at removing excess biomass, but it required 40% bed expansion (fluidization of the packing material) and two weekly applications (Smith et al. 1996). Chemical rinses also were tested using NaOH, NaClO, and H₂O₂. While biomass was removed during rinsing, the remaining biofilm lost almost all of its biological activity (Cox and Deshusses 1999a).

To overcome the limitations of physical-chemical removal systems, biological control of biomass formation through predator-prey systems has been introduced. Protozoa were studied as potential predators in bacterial systems. Preliminary work showed that the protozoa were tolerant of toluene up to concentrations of 1.33 g m⁻³ (Cox et al. 1999), and they decreased the rate of pressure drop increase in laboratory biofilters treating toluene-contaminated air (Cox and Deshusses 1999b). The predator-prey system also has been applied recently in fungal systems with the use of mites that graze on the fungal mycelium (van Groenestijn et al. 2001; Woertz et al. 2002).

Conditions that favor mite growth in residential households and in food supply stocks have been studied to some extent. No work, however, has been done to determine conditions that promote mite growth in biofilters for the purpose of fungal biomass control. In this study, the relative humidity, pH of nutrient medium, temperature and toluene concentrations in the gas phase were varied to determine which conditions promote the growth of the mite *Tyrophagus putrescentiae* on the filamentous fungus *Cladophialophora* sp. *Cladophialophora* sp. was selected for study because it was successful at degrading volatile organic compounds from polluted gas streams in bench-scale biofilter systems inoculated with *T. putrescentiae*, which was used for biomass control (van Groenestijn et al. 2001; Woertz et al. 2002).

Materials and methods

Cultures and medium

Perlite packing medium covered with a biofilm consisting primarily of *Cladophialophora* sp. was obtained from a toluene-degrading bioreactor at TNO Department of Environmental Biotechnology (Apeldoorn, the Netherlands). Mites were obtained from the leachate of a second toluene-degrading bioreactor (TNO, Apeldoorn, The Netherlands), which had been inoculated with *Tyrophagus putrescentiae*.

tiae (Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands). Medium used for soaking the packing samples was prepared as described by Cox et al. (1993). The pH of the medium was adjusted to 2.5, 4, or 7 using either 85% H₃PO₄ or 50% NaOH (Acros, Geel, Belgium).

Henry's coefficient determination

The Henry's coefficient for toluene in dioctyl phthalate (Acros, Geel, Belgium) was determined experimentally by injecting known volumes of toluene into 20 mL glass vials sealed with Teflon-lined septa and containing 10 mL of dioctyl phthalate. The vials equilibrated for four h at 25 °C. Headspace samples (100 mL) were then withdrawn from each vial and injected onto a gas chromatograph equipped with a flame ionization detector (Varion, Model 3800). The peak area was recorded and compared with a linear calibration curve obtained by injecting 100 mL samples of air containing known concentrations of toluene vapor over the concentration range of 0 to 2500 mg m⁻³. Triplicate samples were made for each concentration tested. The Henry's coefficient for toluene in air and dioctyl phthalate was then calculated as 3.69·10⁻³ m³ dioctyl phthalate m⁻³ air.

Environmental chamber studies

Controlled-environment chambers were constructed using 1-L Duran bottles sealed with Teflon-lined screw caps (Schott, Germany). Saturated salt solutions of BaCl₂, KNO₃, or KSO₄ in demineralized water were placed in the bottom of the bottles to maintain constant relative humidities of 90, 94, and 97%, respectively, as described by Cox et al. (1996). A 1.0 g sample of perlite granules (sieved fraction 3.45–4.75 mm average diameter, Pull BV, Rhenen, the Netherlands) covered with a fungal biofilm was added to a 20-mL vial and suspended from the top of the bottle. The fungus served as the sole food source for the mites. Toluene concentrations of 500 or 2000 mg m⁻³ headspace were maintained by adding 1.5 mL or 5.75 mL toluene to 5 mL dioctyl phthalate in a second 20-mL glass vial, also suspended from the top of the bottle. Prior to the addition of mites, some of the perlite samples were soaked in pH-adjusted mineral medium. Mites were added to the perlite by pipetting 0.1 or 0.2 mL (referred to as underpopulated and overpopulated, respectively) of leachate from the mite infested bioreactor directly onto the perlite samples. These two different starting conditions were used to determine if the number of mites per given amount of fungus (i.e., food source) would affect mite growth. The leachate was mixed prior to each addition.

Culture conditions tested in the environmental chambers are provided in Table 1. The different conditions chosen for these experiments were based on the typical range of operating conditions encountered in biofiltration systems. For the mites to be used successfully for fungal biomass control in such applications, the mites must be able to withstand the different environmental conditions that may be experienced. Duplicate chambers were made for each condition tested.

Table 1. Experimental conditions in the controlled-environment chambers.

Culture*	RH (%)	Temperature (°C)	Toluene (mg m ⁻³)	Soaking Medium (pH)
R1	94	25	-**	-
R2	97	25	-	-
R3	90	25	-	-
T1	94	30	-	-
T2	94	14	-	-
V1	94	25	500	-
V2	94	25	2000	-
P1	94	25	-	2.5
P2	94	25	-	4.0
P3	94	25	-	7.0
C1	94	25	-	-

*Cultures R1-3, T1-2, V1-2, and P1-3 were made in duplicate at two different starting conditions, either with 0.1 mL or 0.2 mL of mite inoculum. Culture C1 was made in duplicate, but no mites were added.

**Indicates that toluene or soaking medium was not used in preparing the cultures.

Enumeration of mites in the leachate and on the perlite

A stereomicroscope (Wild Heerbrugg, Rijswijk, the Netherlands) set at 15x magnification was used to enumerate the number of mites initially present in the leachate inoculum and on the perlite samples after various incubation periods. To determine the initial number of mites in the inoculum, ten 0.1-mL random samples were taken from the leachate and placed on a petri dish. The average number of mites per 0.1-mL sample was 58 ± 18 . To determine the number of mites on the perlite after a given incubation period, the 1-g perlite sample was removed from the controlled-environment chamber, placed in a petri dish, and examined as above. If the number of mites exceeded 500 per sample, the sample was said to have too many mites to count. In these cases, the sample was recorded to have 500 mites/g perlite.

Results

Effect of relative humidity on mite growth

Initially, the lowest relative humidity environment was preferable for mite growth in the overpopulated samples (Figure 1). After three months, however, little difference in the number of living mites per gram of packing was discernible in either the under- or overpopulated samples, and numerous dead mites were observed on the perlite packing.

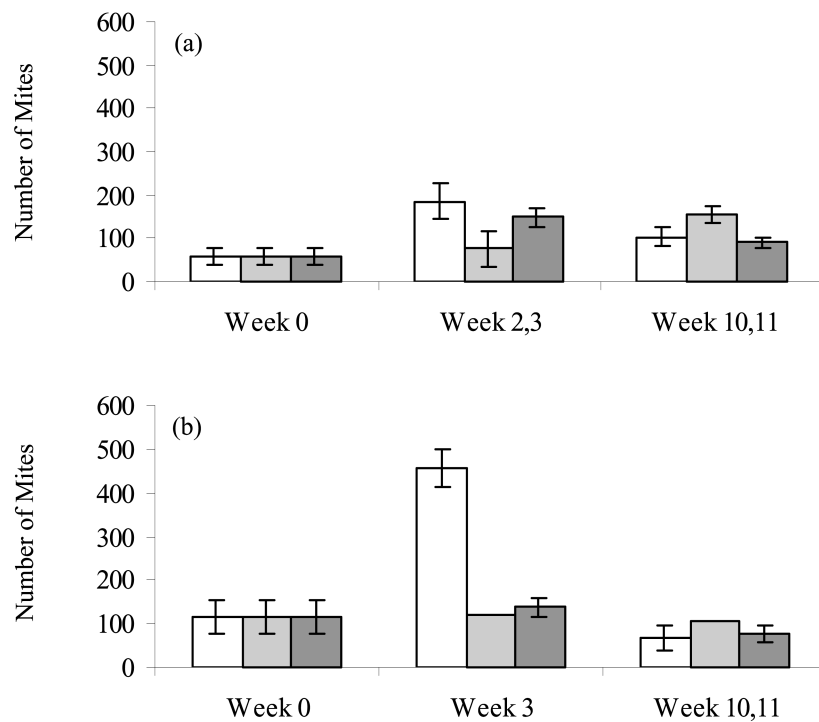


Figure 1. Effect of relative humidity on the growth rate of *Tyrophagus putrescentiae* on *Cladophialophora* sp. under (a) underpopulated conditions and (b) overpopulated conditions: 90 % (white bars), 94% (light grey bars), and 97% (dark grey bars) relative humidities. The samples at 94% RH were counted during weeks 2, 3 and 10.

Effect of soaking medium and pH on mite growth

In the underpopulated samples, rinsing the perlite with an acidic to neutral pH medium prior to inoculation resulted in a higher mite population after the three month incubation period as compared to the samples that were not rinsed (Figure 2). For the overpopulated samples, those rinsed with pH 7 medium initially supported higher mite populations, but after two months, the samples rinsed with acidic media showed higher numbers of mites per gram of perlite. Again, many dead mites were observed on the surface of the packing in these samples after three months of incubation.

Effect of temperature on mite growth

The mites were tolerant of temperatures up to 30 °C, but did not reproduce readily at 14 °C (Figure 3). In both the underpopulated and overpopulated samples, a rapid decline in the number of mites per gram packing was observed after one month in the samples maintained at 14 °C. After three months, there were many dead mites

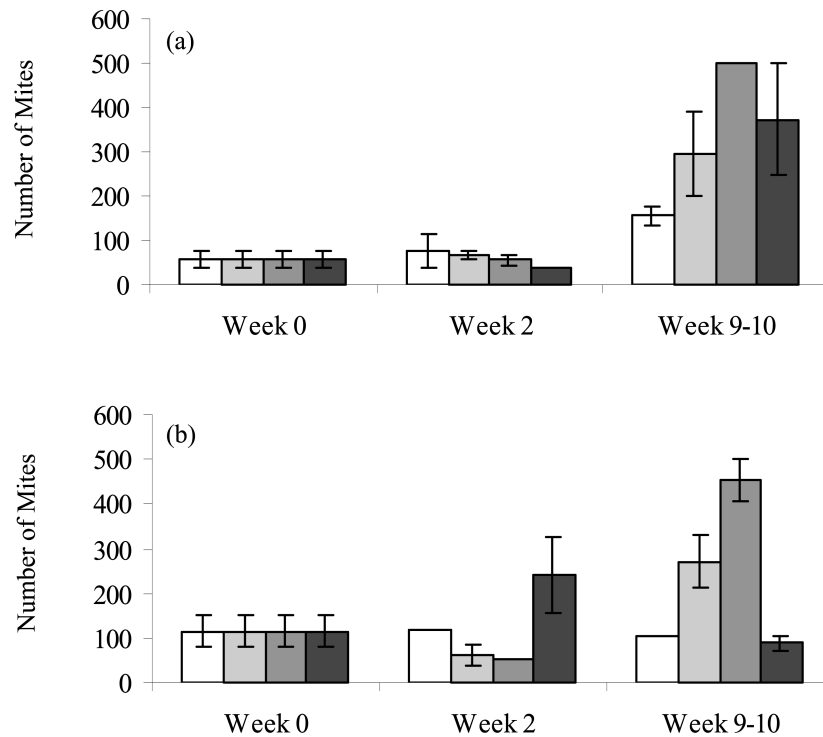


Figure 2. Effect of soaking medium pH on the growth rate of *Tyrophagus putrescentiae* on *Cladophora* sp. under (a) underpopulated conditions and (b) overpopulated conditions: pH 2.5 (light grey bars), pH 4 (medium grey bars), pH 7 (dark grey bars) and no soaking medium applied (white bars). The samples with no rinsing were counted during weeks 2, 3 and 10.

on the samples kept at 30 °C; however, almost no dead mites were observed on the 14 °C samples, most likely because the decomposition of the initial population was expedited by the trampling of the surviving mites in the confined space.

Effect of toluene on mite growth

In both the under- and overpopulated samples, the presence of moderate to high toluene concentrations in the headspace of the chambers had no toxic effect, and the mites were capable of rapid reproduction during the first six weeks of incubation (Figure 4). After three months, the number of mites per gram of perlite decreased significantly in all samples exposed to toluene vapor, and many dead mites were observed.

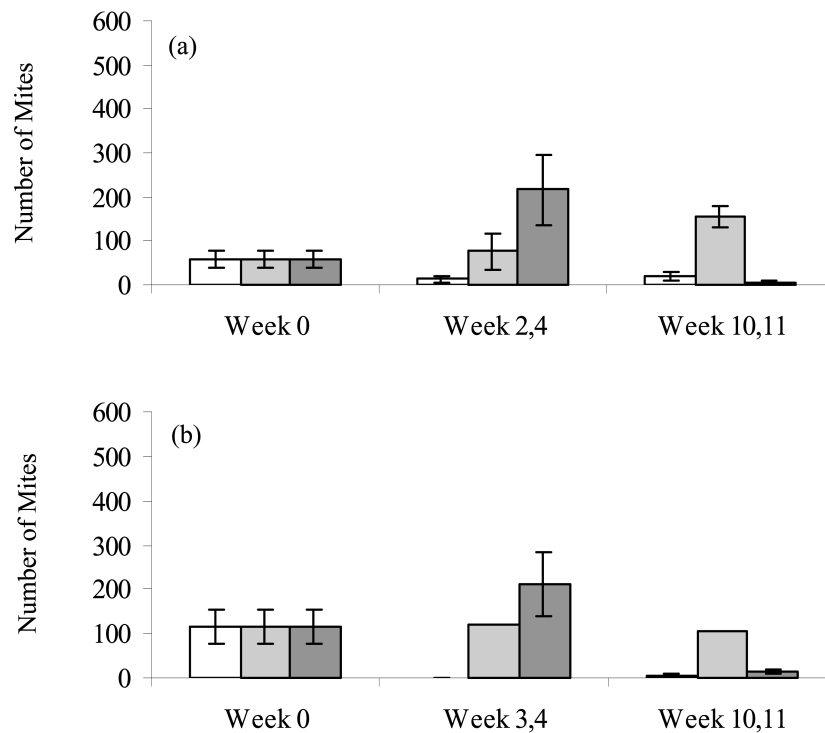


Figure 3. Effect of temperature on the growth rate of *Tyrophagus putrescentiae* on *Cladophialophora* sp. under (a) underpopulated conditions and (b) overpopulated conditions: 14 °C (white bars), 25 °C (light grey bars) and 30 °C (dark grey bars). The samples at 25 °C were counted during weeks 2, 3 and 10.

Fungal and mite population changes

Upon inspection of the perlite samples over time, it was observed that the amount of fungal biomass present on the packing surface decreased as the mite population increased. For example, on pieces of packing where a large population of mites (>20) was counted, the packing would have large sections of exposed, bare surface. On pieces of packing with few mites, on the other hand, an even coating of fungal biomass was observed over the entire surface of the packing. In no samples, however, was all the fungal biomass initially provided entirely degraded by the mites. The mites were often found in clusters on only a few of the perlite pellets, creating a food shortage on that single pellet. The effect of overcrowding was likely due to the fact that when the eggs hatched, all mites stayed in the same area and caused local overcrowding.

In order to minimize disturbances to the environmental chambers as well as due to the large amount of time it took to count the mites in each sample, only three data points were collected for every condition tested. Although general trends were observed in the mite growth, some discrepancies are discernible. For example, there

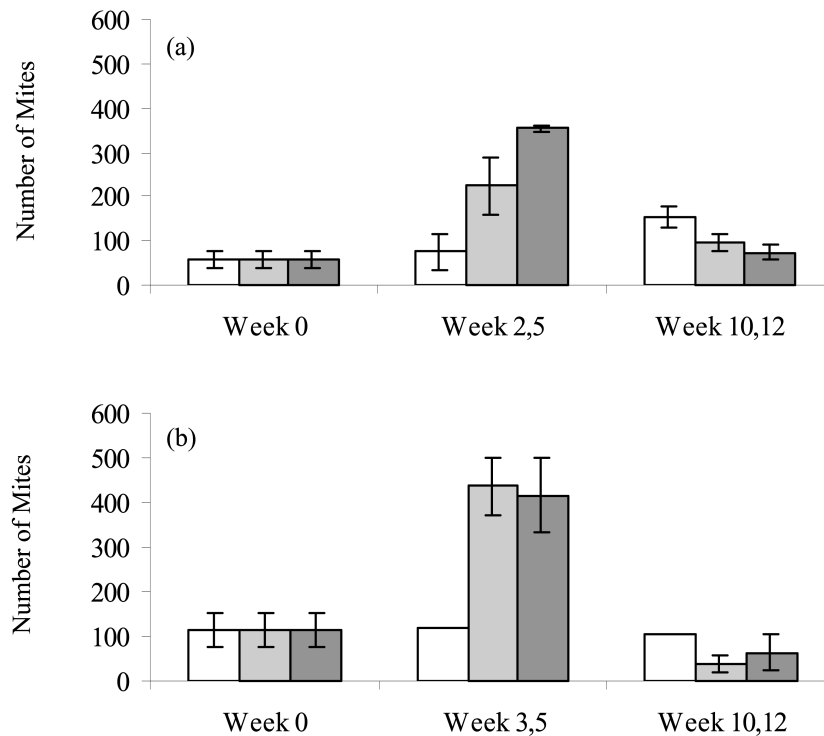


Figure 4. Effect of vapor-phase toluene concentration on the growth rate of *Tyrophagus putrescentiae* on *Cladophialophora* sp. under (a) underpopulated conditions and (b) overpopulated conditions: 0 mg m⁻³ (white bars), 500 mg m⁻³ (light grey bars), and 2000 mg m⁻³ (dark grey bars) of toluene in the vapor-phase. The samples with no toluene in the headspace were counted during weeks 2, 3 and 10.

was a large difference in the maximum number of mites observed in the under- and overpopulated samples maintained at 90% relative humidity (Figure 1). The reason for such discrepancies was most likely due to not counting the samples more often. The underpopulated sample, for example, may have reached a higher maximum population, but this may have occurred between the second and third data points.

To obtain an estimate of the overall mite growth during the three-month experiment, all of the data collected for the underpopulated samples was plotted over time (Figure 5). The trend suggests that the mites reproduced at a steady rate for the first eight to ten weeks of incubation and then began to die off rapidly.

Discussion

From the experiments conducted at different relative humidities (Figure 1), it was observed that the mites initially grew best at the lowest relative humidity tested (90%). These results correspond to what others have observed in studies conducted to determine conditions that favor storage mite growth, including *T. putrescentiae*.

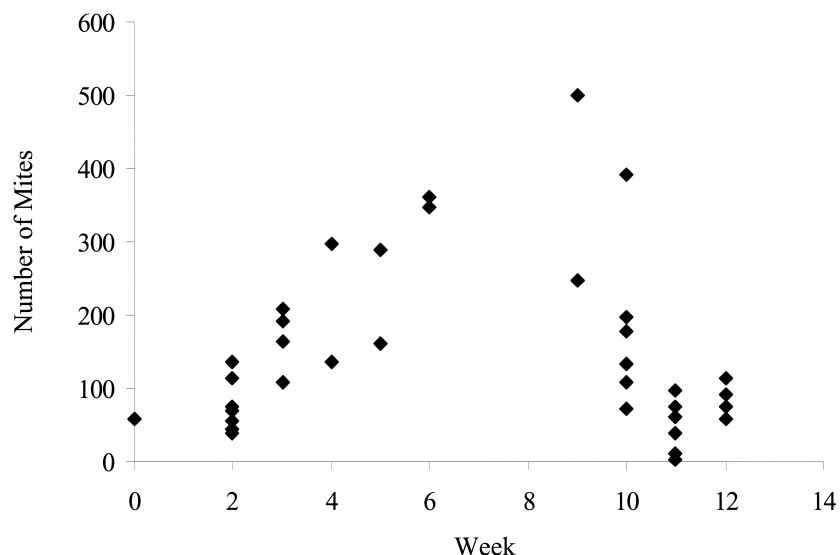


Figure 5. Reproduction of mites in the underpopulated samples independent of growth conditions.

In those studies, it was observed that many storage mites, as well as *T. putrescentiae* grow well at relative humidities between 65 and 90% (Rivard 1961; Ree and Lee 1997; Hodgson 1976; Parkinson 1990). Also, in experiments to determine how to minimize mite growth in residential houses, it has been noted that relative humidities in the range of 35%–100% promote mite infestation (Colloff 1991). In order to eliminate *T. putrescentiae* infestation, Hodgson (1976) proposed extended exposure periods to a dry atmosphere. In biofilter applications, the relative humidity of the inlet gas stream is often adjusted to near 100% using a prehumidification system. This is done in order to prevent stripping of the water from the packing material. If mites were to be used in biofilters, the extent of prehumidification may need to be adjusted to allow for sufficient growth of the mites. In biofilters currently operating in our laboratory, it has been observed that the mites tend to migrate to the top of the bed, where the relative humidity is the lowest (personal observation). Also, since fungal biofilters are tolerant of lower moisture contents and water activities (Cox et al. 1996), lowering the inlet relative humidity should not have a significant effect on the overall performance of the reactor, as long as sufficient care is taken that such excessive drying does not occur.

In the experiments conducted to determine the effect of pH on mite growth, the samples that were rinsed with acidic pH medium prior to inoculation supported higher mite populations after three months than those samples that were not rinsed (Figure 2). The acidic to neutral pH media rinses provided additional moisture and nutrients allowing for the possible degradation of storage materials by the fungus, thus providing an additional carbon source for the mites. The initial drop in mite population in most of the pH-adjusted samples was most likely due to the large amount of water initially present on the surface of the packing. After the packing

no longer had standing water on its surface, the mite population in most samples increased. The ability of the mites to grow on the rinsed packing, regardless of the pH of the rinsing medium, indicates that the use of periodic rinsing of biofilters for the purpose of nutrient and moisture addition should not harm the mite population, as long as large pools of water are not present for extended periods. Also, if the mites are reproducing too rapidly, the pH of the medium may be decreased to slow the rate of growth. It has been previously shown that fungi isolated from biofilters are able to grow well at pH values between 3 and 7 (Cox 1995; Woertz and Kinney 2000), therefore, adjusting the pH of the medium should not greatly affect contaminant removal by the fungus.

Although the mites were able to withstand a large range of other environmental factors, they showed little ability to reproduce and sustain a large population at low temperatures (Figure 3). Although *T. putrescentiae* has been shown to live a normal life cycle at 11 °C, typically fewer eggs are laid and it takes longer for these eggs to develop at this temperature (Barker 1967). The slow development of *T. putrescentiae* at low temperatures would therefore account for the low population observed on the samples stored at 14 °C in this study. The inability of the mites to tolerate low temperatures may hinder their use in biofilters in cold climates or during the winter, and periodic mite addition may be necessary to ensure a stable mite population in such environments. This apparent disadvantage, however, may also help to control mite growth in other biofilters. The use of a cold water rinse or cooled inlet gas stream may slow the reproduction rate of the mites in the event that the biofilter becomes overpopulated with these predators, which potentially may destroy most of the active fungal biofilm. Cooling the gas stream, however, may be economically impractical. The ability of the mites to grow well at temperatures up to 30° is promising, since most biofilters operate in a temperature range below 30 °C to maintain an active biofilm. More extreme temperatures should be tested to determine the fate of mites in such situations.

In addition to tolerating different environmental conditions, it was observed that toluene was not toxic to the mites at concentrations typically found in off-gas streams (Figure 4). In fact, mite growth was observed to be higher in the samples in which toluene was present than those samples that had no toluene in the vapor phase. This also may have been a result of the fungus growing after being put into the chamber, thus increasing the food supply for the mites. Regardless, these results suggest that when designing a mite/fungal biofilter system, the loading rate may not have to be lowered to avoid toxicity effects on the secondary population as suggested for protozoan/bacterial systems (Cox et al. 1999).

Although most of the conditions tested did not have a negative effect on the mites' ability to reproduce using *Cladophialophora* sp. as their food source, overpopulation of the living space appeared to hinder growth after an extended period (Figure 5). In almost all samples, after a two to three month period, large numbers of dead mites were observed on the packing and a large decrease in the mite population was recorded. The sudden drop in mite population was most likely a result of overcrowding and the accumulation of waste by-products. In the experiments conducted, no new food supply was introduced into the systems nor were the

chambers cleaned of accumulating waste. The lack of ample food and the build up of potentially toxic waste likely impeded growth of the mites after a critical population had been established. It is presumed that such problems would not be as critical in operating biofilters, since the biomass (i.e., food source) is continually increasing and most systems are periodically rinsed with nutrient medium (García-Peña et al. 2001; Woertz et al. 2001). Also, the constant flow of air through the system would remove toxic, gaseous by-products from the bioreactor. Although more rigorous counting is required to quantify the mite growth curves, this study indicates the relative tolerance of mites to different environmental conditions and supports the use of mites for biomass control in biofiltration systems, as suggested by preliminary bench-scale studies (van Groenestijn et al. 2001; Woertz et al. 2002).

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