An Environmentally Friendly Method for Controlling Biomass in Biotrickling Filters for Air Pollution Control

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ABSTRACT

Biomass accumulation is a major obstacle for long-term, stable operation of biotrickling filters treating high loadings of volatile organic compounds (VOCs). Clogging reduces pollutant removal and increases the pressure drop in biotrickling filters. Several options exist to remove excess biomass or to slow down the accumulation rate, but so far none has succeeded in combining a high VOC removal rate with a low biomass net growth rate. Recently, we observed the invasion of some of our biotrickling filters by small flies. In the best cases, a rapid reduction of the biomass content was observed. The fly larvae rapidly spread throughout the reactor and biomass was rapidly removed from the packing, initially at a rate of 13.1 kg wet weight/m^3.day and increasing up to 70-140 kg/m^3.day. In that case, the wet biomass content in the reactor was reduced from 455 to 28 kg/m^3 reactor in 16 days with 80% of the biomass reduction occurring in 2-4 days. Analysis of the recycle liquid indicated that the major mechanism of biomass removal was detachment of biofilm, although experiments are underway to determine the exact proportion of biofilm detachment and consumption by larvae. We speculate that larval activity loosened the biofilm structure, thus enhancing biofilm detachment by shear-stress from the trickling liquid. Overall, the preliminary results presented herein highlight that the use of fly or other larvae presents a tremendous potential for controlling biomass in biotrickling filters.

INTRODUCTION

Biotrickling filters for waste air treatment have been extensively studied in the laboratory and efficient removal of a wide variety of organic and inorganic compounds from polluted air streams has been reported. However, the industrial deployment of biotrickling filters is still limited in spite of the many promising aspects of the treatment technique.

A major obstacle to implementation of biotrickling filters in industry appears to be the instability of these reactors over the long-term. Due to rapid biomass accumulation in the packed bed, the pressure drop will increase leading to higher operational costs and pollutant removal will decrease. If no remedial action is taken, the reactor will ultimately clog.[3,8,13,17] In the case of a toluene-degrading biotrickling filter with an elimination capacity of 50 g toluene/(m^3 filter bed .

h) with 50% conversion of carbon into biomass and the production of a biofilm with 0.1 kg/L dry matter, 45% carbon on dry weight basis, and a density of 1 kg/L, it would take 82 days of continuous operation to produce a biomass volume as large as the reactor volume. Indeed, rapid clogging of biotrickling filters has been observed.[7,13,14,17] Interestingly, the minimum amount of biomass in the reactor to obtain maximal pollutant-degrading activity is only 16 L/m^3 reactor if one assumes a specific surface area of 200 m^2/m^3 (ref. 15) and an effective biofilm thickness of about 80 μm as estimated in biofilters for waste air treatment.[2,12] This implies that prolonged biotrickling filter operation primarily results in formation of biomass not actively involved in pollutant degradation. This was confirmed by Zuber[19], who demonstrated that no improvement of pollutant removal occurred as biomass accumulated over time in methylene chloride degrading biotrickling filters.

Several options to either prevent excessive biomass production or to remove excess of biomass in biotrickling filters and biofilters have been investigated, including nutrient limitation,[9,10,11,17] addition of growth-limiting concentrations of NaCl,[11,15] discontinuous operation with starvation periods,[8] biomass removal by chemical washing[4,17] by backwashing[13,14] or mechanical removal by stirring of the trickling filter bed.[18] Unfortunately, these methods suffer from various drawbacks. In the case of limitation of bacterial growth, decrease of bacterial activity and hence pollutant elimination capacity is generally observed. Chemical biomass removal suffers from a considerable down-time after treatment, while mechanical biomass removal requires complex reactor design. Finally, full medium fluidization (backwashing) requires expensive equipment and generates large amounts of high BOD wastewater.

We reported on several approaches to control the rate of biomass formation in biotrickling filters. One innovative strategy that was investigated is the use of protozoa that prey upon bacteria.[3] Since protozoan predation is coupled with CO_2 and heat generation, overall biomass production per amount of pollutant degraded should be less in the presence of protozoa. Ideally, predation should balance bacterial production in order to obtain a stable biotrickling filter with a constant amount of biomass. More recently, the use of mites has been suggested which appears promising.[1]

In short, our previous study demonstrated that protozoan activity delayed clogging of the biotrickling filter without a reduction of the toluene elimination capacity, but the predation rate was not high enough to fully counterbalance bacterial growth. It was concluded that protozoan predation needed to be stimulated in order to obtain pollutant degradation with zero net-growth. The methods for such stimulation remain to be developed. An alternative solution would be to use other scavengers or predators that are more effective in consuming biomass.

In the present paper, we report on several observations made in laboratory-scale toluene-degrading biotrickling filters in which fly eggs and/or larvae either appeared after some time of operation (probably as a result of introducing eggs with the original microorganism inoculum) or were fly eggs and/or larvae added on purpose to control biotrickling filters. Various levels of biomass reduction were observed as a result of the larvae activity, which suggested interesting possibilities for engineering and controlling the biomass control using fly larvae.

The life cycles of flies may not necessarily be known by chemical and environmental engineers, hence basic background information is warranted . Flies lay eggs that can be highly resistant to

environmental conditions, depending on the species. Eggs of the type of flies discussed herein are typically 0.45 x 0.15 mm in size. They are usually embedded in gelatinous matter and take 3-10 days to hatch. The next stage is called the larva, which is the most active stage as far as consumption of food is concerned and therefore presumably the most interesting as far as biomass control is concerned. Larvae of the type of flies discussed here grow to about 2-10 mm in 5 to 40 days, depending on the specific species and the conditions. They are highly motile and require oxygen. The development of larvae depends on environmental conditions such as availability of food (essentially decayed mass) and temperature. There are several larval instars in the fly life cycle between which the fly grows by shedding the external skeleton. When the larva is mature, it will undergo transformation to the next stage called the pupa. Pupae are typically 7-10 mm long by 1-3 mm diameter; they are usually non motile, and require oxygen. An important aspect is that pupae do not consume any outside food, hence they present little interest for biomass control in biotrickling filter. The pupal stage duration depends on the species and the environmental conditions, primarily temperature, but is roughly 3-10 days long. A mature fly, which is capable of flight, emerges from the pupa, leaving an empty puparium behind and the cycle is complete.

In the present paper, the potential of using of using fly larvae for achieving biomass control in biotrickling filters and future research needs are discussed.

MATERIALS AND METHODS

Biotrickling Filter Equipment and Inoculation Procedure

The results reported herein were obtained from various studies performed in two types of biotrickling filters. The larger reactors were made of clear PVC pipe with an internal diameter of 0.152 m and filled with polypropylene Pall rings with a diameter of 2.5 cm (Flexirings®, Koch Engineering, Wichita, KS). The resulting reactor had a packed bed height of 1 m and a bed volume of 18.5 L. The reactors were operated in a concurrent mode. Toluene inlet concentrations were varied by pumping liquid toluene at different flow rates into the air stream with a metering pump. The trickling liquid (composition per per L: 1 g KH_2PO_4, 1 g K_2HPO_4, 1 g KNO_3. 1 g NaCl, 0.2 g $MgSO_4$, 26 mg $CaCl_2.2H_2O$, 5.2 mg EDTA $Na_4(H_2O)_2$, 1.5 mg $FeCl_2.4H_2O$, 0.12 mg $CoCl_2.6H_2O$, 0.1 mg $MnCl_2.2H_2O$, 0.07 mg $ZnCl_2$, 0.06 mg H_3BO_3, 0.025 mg $NiCl_2.6H_2O$, 0.025 mg $NaMoO_4.2H_2O$, 0.015 mg $CuCl_2.2H_2O$.) was recycled over the filter bed with a centrifugal pump and fresh mineral medium was continuously fed to a vessel at the bottom of the reactor. Excess liquid was drained via an overflow outlet to maintain a constant liquid volume in the vessel. The smaller reactors were of similar construction, except that bed dimensions were 50 cm high by 4 cm ID and that the packing was made of crushed Pall rings, which reduced the size of the packing to about 0.5-1 cm and increased the specific area to about 400 m^2/m^3. The synthetic waste air generation was slightly different, but operating parameters (flows, feed rate, etc.) were prorated to the larger system so that the results could be compared. A schematic of the experimental apparatus is shown in Figure 1 and standard conditions are listed in Table 1. The reactors were inoculated with an undefined mixture of cultures from our collection, with extracts from soil, and with activated sludge from a wastewater treatment plant. It is believed that some of the inocula contained fly eggs which developed over time.

Figure 1. Schematic of the experimental apparatus.

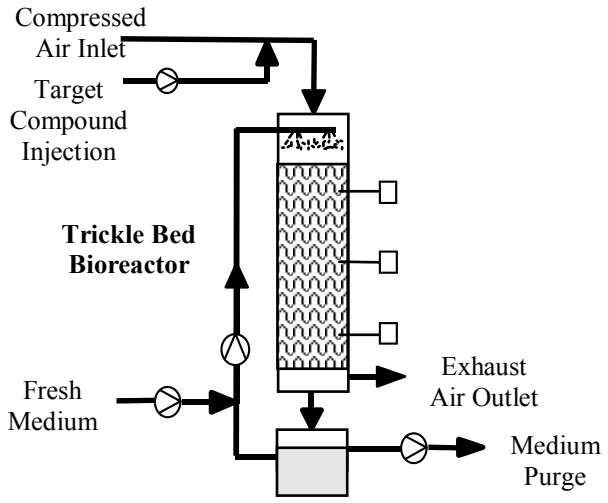


Table 1. Standard operating conditions (larger reactors)

Operating Parameter	Range
Toluene Concentration (g/m^3)	0.12 – 3.0
Toluene Load Rate (g/m^3h)	6 – 240
Gas Empty Bed Residence Time (min)	0.5 – 2.0
Superficial Velocity of Recirculating Nutrient Medium (m/hr)	1.0 - 8.2
Gas Superficial Velocity (m/min)	0.5 – 2.0
Liquid Space Velocity (hr^{-1})	1 - 8
Fresh Nutrient Medium Feed Rate (l/day)	5.7 – 11.4
Air & Liquid Temperature (^oC)	22 – 25
Recirculating Nutrient Medium Volume (liter)	9

Analytical Methods

Toluene concentration in inlet and outlet streams was measured on-line by a stand alone SRI FID detector connected to a data logger. Influent and effluent streams were analyzed daily. The recycle liquid was analyzed for pH, suspended biomass activity by measuring toluene induced

oxygen uptake rate (OUR), optical density (at 600 nm) using standard methods or methods reported previously.[3-6] For determination of the amount of wet biomass in the reactor, the liquid circulation was stopped and the liquid was allowed to drain from the reactor for 30 minutes. The reactor was weighed with a precision of 5 g using a model 7300 scale from Pennsylvania Scale Company (Leola, PA). The amount of wet biomass in the reactor was calculated as the increase of reactor weight as compared to the weight of the dry and clean reactor including the packing on day 0. Pressure drop measurements over the filter bed was determined with a water U-tube during standard operation.

RESULTS AND DISCUSSION

Association of Fly Larvae and Steady Biomass Content (Zero Net Growth)

After a two year study two toluene degrading biotrickling filters during which various aspects of operation were investigated, it was noticed that one biotrickling filter reactor suddenly achieved a constant weight and that the other reactor lost biomass and then reached a zero net-growth situation. There was no detrimental effect on the pollutant elimination and very steady operation was reached. At the same time, fly larvae were noticed in the biofilm, while adult flies were present in the headspace of these reactors. The small adult flies (2-4 mm) were identified as the scatopsid fly *Coboldia fuscipes* which is often associated with decaying fruits. The larvae did not appear to be very motile in contrast to observations reported in the next section. A review of all operating conditions did not reveal any change in reactor operation, hence it was hypothesized that the presence of the larvae was the most possible cause for the zero net growth. Possible mechanisms of biomass removal included predation of biofilm material by growing larvae, and detachment of biofilm patches under the mechanical action of the larvae. More research was warranted.

A number of experiments were conducted to quantify the number of larvae in the reactors and firmly link the presence of the larvae to the low/zero overall biomass yield that was observed. One complicating factor for proving the cause and effect is the complex life cycle of flies: eggs -> larvae -> pupae -> adults which is affected by numerous factors. In one experiment, larvae were taken out of the reactor and were fed biofilms. The results (in terms of biofilm mass change with time) were not conclusive. Other experiments tried to eradicate the flies using a potent organophosphate insecticide that was applied to one bioreactor while monitoring the reactor weight, but again the results were inconclusive. Unfortunately, the complexity of the systems and our inability to cultivate the fly prevented us from firmly showing that a cause and effect relationship existed between the presence of the fly larvae and the low/zero biomass yield.

Observations of Larvae Associated with Significant Biomass Reduction

Case 1

In another biotrickling filtration experiment, a first observation of larvae occurred after 100 days of operation, at which time the reactor contained a significant amount of biofilm biomass. The larvae appeared first at the top of the bed and gradually colonized the rest of the bed underneath. Most of the larvae had a length of about 2–3 mm and a diameter of about 1 mm. They were highly motile. Interestingly, the biofilm biomass at the top of reactor was gradually removed from the top of the bed, leaving a clean section of packing on top of the bed. A clear demarcation

was visible (Figure 3). This behavior continued for 12 days during which the net biomass reduction rate was about constant at 13.1 kg/m^3 day. The removal of toluene was unchanged during this phase and ranged from 50-70 % and elimination capacities ranging from 30-42 g/m^{3}h. The unchanged performance was most probably due to the excess toluene removal capacity in the rest of the reactor. The color of the recycle liquid was not significantly different from this during standard operation, before the larvae appeared (light yellow OD = 0.016 at 600 nm with no or very little suspended biomass). The number of larvae gradually increased and some larvae, pupae and adult flies were found in the recycle reservoir. The pupae were yellow with black stripes. Although no detailed investigations were conducted, pupae kept in a beaker with some liquid and biofilm typically transformed into adult flies within 3-5 days.

Figure 2. Weight of the biomass in the biotrickling filter over time (day 0 is time of initial startup).

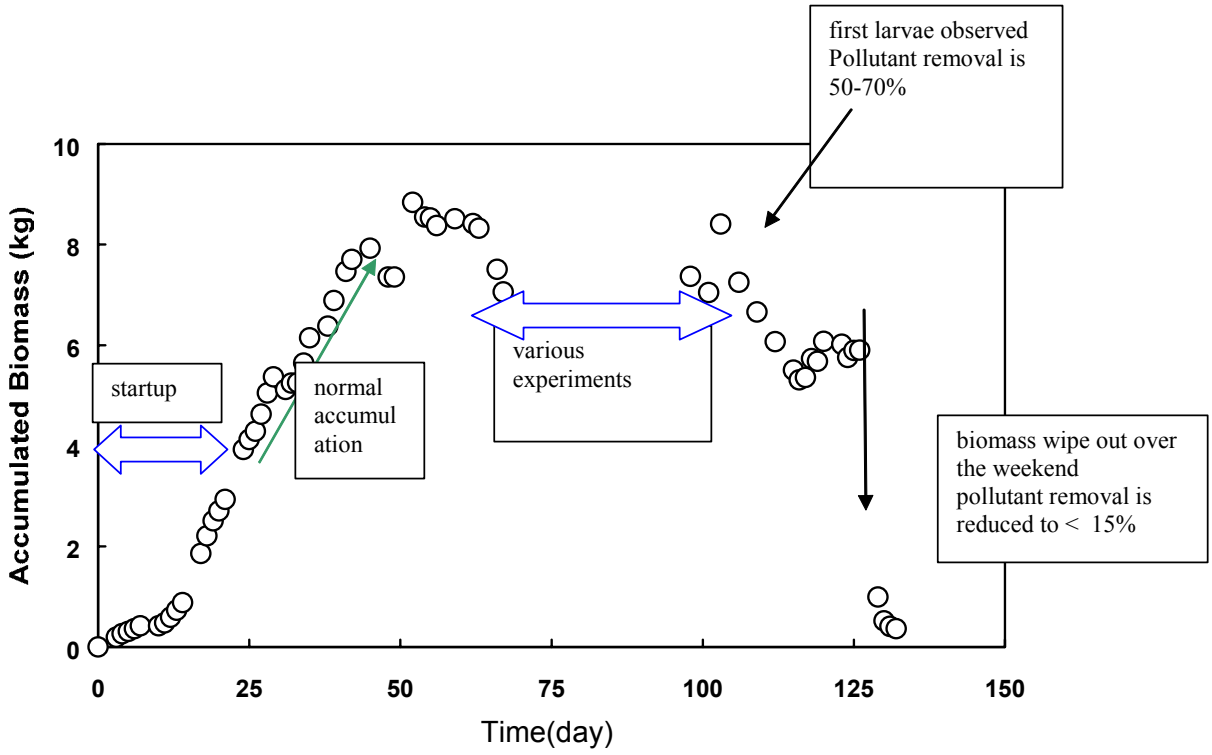
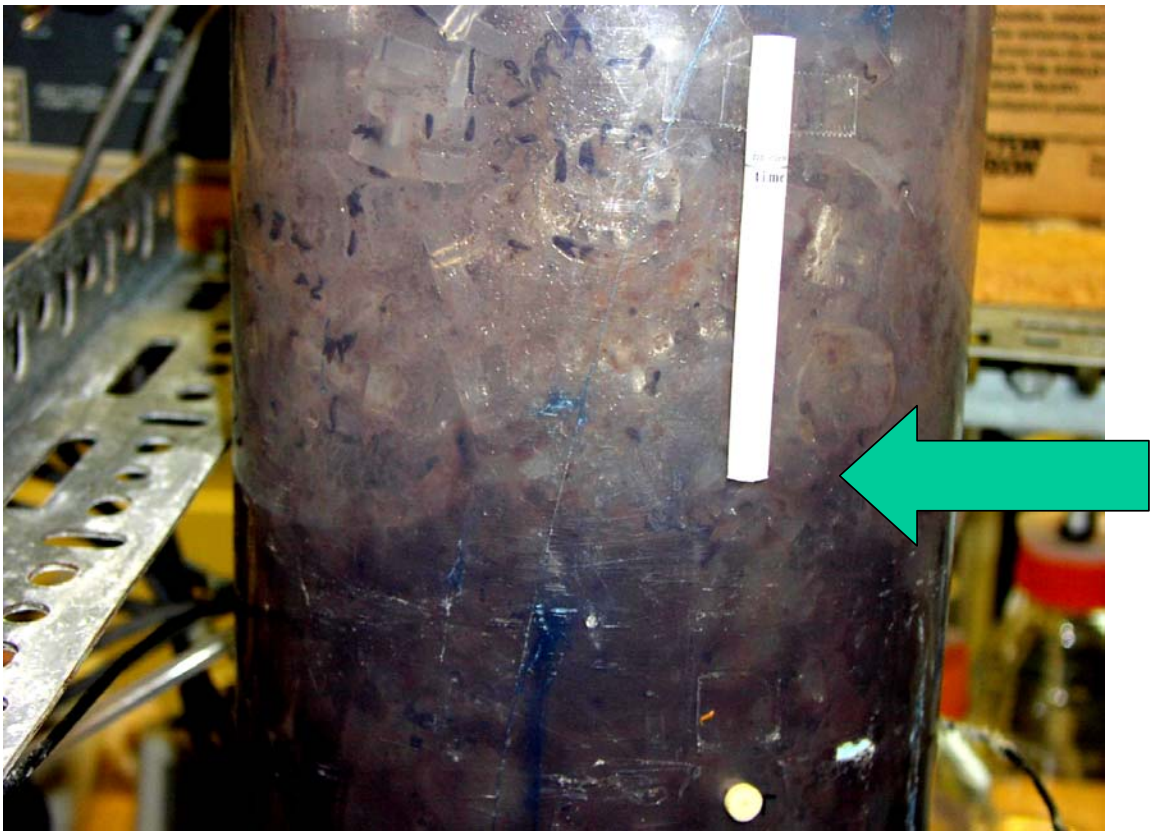


Figure 3. Picture of the top of the bed. The contrast has been changed to emphasize the demarcation (green arrow) between the clean section of the bed and the undisturbed section of the bed. In the clean sections, some larvae and pupae can be seen.



An exact quantification of the number of larvae in the biotrickling filter was not possible, but in light of the simultaneous observation of the larval bloom and the biomass wipe-out, there was no longer any doubt that the biomass reduction was caused by the presence of the larvae.

After observing biomass decreasing rapidly for several days, attachment of biomass was apparent at the top of the bed, which is consistent with the fact that biomass usually grows faster at the gas inlet port. Next, a pseudo steady-state was reached where biomass weight in the reactor was constant. This phase lasted 8 days. At that time, the pressure drop over the bed was 1 cm of water column which is about 1 cm lower than prior to the development of the larvae in the reactor.

Case 2

Three weeks after the first appearance of larvae (day 124, see Figure 2), a larval boom was observed. Unfortunately, most relevant events took place over a weekend, and detailed observation of the biomass decrease associated with the larval outbreak was missed. Qualitatively, it seemed that the larvae were of a different type (black, length of 3 to 5 mm, diameter of 0.5 mm) although identification of the flies did not point to different species. The flies were tentatively identified as *Telmatoscopus albipunctatus*, a psychodid fly species often associated with decaying material, drain, and wastewater treatment activities (Figure 4).

Figure 4. Picture of the pupae (left) and flies (right)

Over the weekend event, the entire content of the biotrickling filter was wiped out as indicated by the drastic weight decrease on day 124-128 in Figure 2. Large amounts of foam were observed overflowing from the recycle reservoir, and some recycle liquid was spilled and lost. The recycle liquid turned to a very dark brown solution (Figure 5), slightly viscous because of the large amount of suspended matter. The suspended solids in recycling medium on the first sampling after the event was 0.6025 % dry weight basis or about 12% based on a wet weight basis (95% water content) which is much higher than anything we previously observed in our biotrickling filters. Details listed in Table 2 show the typical decrease resulting from washing-out the suspended solid after day 124-128, while soluble material remained low. Microscopic observation of the recycle liquid indicated a heavy presence of protozoa and rotifers together with larvae. Cell debris were visible. The high concentration of solids in the recycle liquid indicates that the major mechanism of biomass removal was detachment of biofilm. Interestingly, compared to earlier experiments where chemicals, in particular bleach, were used for removing biomass from clogged biotrickling filter, removal of biomass from the packing by the larvae was extremely effective. The packing appearance in the reactor was clean, with very large number of mature larvae (5-10 mm long), while chemical treatment usually was relatively inefficient in removing biomass from the inside of the rings.[4]

Figure 5. The recycle liquid before (left) and after the larval bloom (right).

Table 2. Characteristics of the recycle liquid.

Day (time after 2nd bloom)	Suspended solids (ppm)	$TOC_{soluble}$(ppm)	RE_{Tol} (%)*
Before 2nd boom	0	42	50 - 70
4th	6025	82	15
5th	4850	99	17
11th	1590	82	10
12th	1160	74	9
13th	1080	70	9
14th	860	69	9
15th	970	44	9
19th	280	33	3
20th	210	29	3

* Toluene loading rate = 60g/m^3 hr.

Biomass removal rate ranged from 70-140 kg_{ww}/m^3.day which is significantly larger than the 13.1 kg wet weight/m^3.day observed during the larval invasion that had occurred days earlier in the same reactor. The reasons for the differences are unknown, but could be related to the fact that the density of the larvae may have been higher during the second invasion, or possibly to the fact that the larvae appeared to be different (although the fly identification suggested the organisms were identical). Overall, the wet biomass content in the reactor was reduced from 455 to 28 kg/m^3 reactor in 16 days with 80% of the biomass reduction occurring in the last 2-4 days. The pressure drop fell to close to 0 mm water column.

As indicated on Figure 2 and Table 2, the rapid loss of biomass from the reactor correlated with a drop in toluene removal efficiency. Toluene removal remained low (10-20%) for an extended period, most likely because of the lack of biomass in the biotrickling filter. No attempts were made to control the fly/larvae in a concern for keeping larvae and adults alive for future breeding. Means to control adult flies and larvae would be needed if this biomass control method was to be deployed at an industrial level. Several fly control agents exist, but were not investigated.

Subsequent to this experiment, flies were collected and various attempts were made to multiply *T. albipunctatus* using standard rearing techniques. Unusual difficulties were encountered as it seemed that the flies did not readily want to lay eggs. Providing flies a small amount of cotton partially submerged in an organic infusion enhanced oviposition by the flies; although some individuals attached eggs to side of petri dishes. Successful culture conditions included trickling filters using the biofilter nutrient medium and corn syrup as a carbon source, an infusion of steer manure, or a 3:1 combination (by weight) of pulverized rodent chow and brewer's yeast. The development time from egg hatching until adult emergence, at room temperature (23°C) and a food abundance of 0.0025 g ml^{-1} of the rodent chow-yeast diet provided on day 1 after hatching, was 16 ± 1 days (mean ± SD; N = 8). At lower food levels and high densities of fly larvae, development time through the larval stages is prolonged considerably (e.g., 3-5 times longer).

Case 3. Inoculation of Larvae to a Biotrickling Filter: Controlled Conditions Experiment

After the successful cultivation of *T. albipunctatus*, a small toluene-degrading biotrickling filter (4 cm ID, 50 bed height) which had been operated under conditions promoting biofilm growth was inoculated with about 40-60 young larvae (1-3 mm). The biotrickling filter was subject to extended monitoring (toluene in/out, pressure drop, OD recycle liquid, CO_2 production) so that greater insight as to the mechanisms and specific rate of biomass removal could be determined. Larvae were observed visually over time, and were found to colonize the entire reactor within 5 days. Unfortunately, 7 days after adding the larvae, the inlet air line was inadvertently disconnected, and the reactor did not receive any air for 48 hours. Under these conditions, the bioreactor turned anaerobic, and all larvae eventually died. In contrast to the larvae, the biofilm quickly recovered and effective toluene removal was observed within 24 h of resuming the air flow. The experiment was stopped and postponed until a later date, when new larvae would become available.

CONCLUDING REMARKS

The use of dipterous larvae presents a tremendous potential for controlling biomass in biotrickling filters, especially in light of the high costs of other biomass control methods.[4,5] We believe that the most promising approach is to periodically add fly eggs or larvae and allow a major fraction of the biofilm mass to be removed in a short time. This should prove easier than trying to achieve a zero net growth. Biomass growth would be allowed between biomass removal events. Note that fly/larvae control could be required to avoid long restart periods. The susceptibility of the fly to short periods with low oxygen concentration suggest that asphyxiation could be an effective non-pesticide means of eliminating the flies from the biotrickling filter.

While this study is preliminary, it highlights the fact that a multidisciplinary approach is needed to further develop the method. At this time, it seems that research needs include finding the most effective larvae (in terms of robustness and specific rate of biomass removal), elucidating the mechanisms of biomass removal (consumption and/or predation), and finding effective means for controlling larvae in the reactor and controlling the possible nuisance of fly emissions from the reactor.

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