Biological waste air treatment in biotrickling filters

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Recent studies in the area of biological waste air treatment in biotrickling filters have addressed fundamental key issues, such as biofilm architecture, microbiology of the process culture and means to control accumulation of biomass. The results from these studies have provided a deeper insight into the fundamental mechanisms involved during biotrickling filtration. In the coming years, these and future advances should allow for the design of better reactor controls and the improvement of pollutant removal in these gas phase bioreactors. Ultimately, this should lead to a more widespread use of biotrickling filters for air pollution control.

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Abbreviations
CAT   computed axial tomography
MTBE   methyl tert-butyl ether
PHS   peat humic substance
TCE   trichloroethylene

Introduction

Biological waste air treatment is an emerging technology which is becoming more popular amongst industries facing increasingly stringent environmental regulation. This technique often offers a cost effective and environmentally friendly alternative to conventional air pollutant control technologies, such as catalytic oxidation or adsorption onto activated carbon. Biological waste air treatment is achieved at ambient temperatures, it does not generate secondary pollutants, such as nitrogen oxides or spent activated carbon, and is positively perceived by the general public. Pollutants are generally converted to carbon dioxide under the action of growing or resting microorganisms, suspended in an aqueous phase or immobilized as biofilms. Air pollution control bioreactors, in particular biofilters, have become in many instances the method of choice for the control of low concentrations of odors, volatile organic compounds, or hazardous air pollutants in large air streams.

Whereas there has been an explosion in the number of papers dealing with biofilters [1], there has not been nearly as many reports on waste air treatment using biotrickling filters. This is unfortunate because these reactors have been shown in several instances to be superior to biofilters when accurate control of the environmental conditions or higher pollutant elimination rates are required. Even so, since the late eighties significant progress has been made in the fundamental understanding of gaseous pollutant elimination in biotrickling filters. In this review, advances in biotrickling filters made in the past year are discussed and placed in an overall perspective for process understanding and process optimization. This review focuses on microbiological aspects of biofilms in waste air biotrickling filters and on recent innovations that will assist biotrickling filters in moving from the lab to the field as a mature technology. Although the past year brought some significant contributions in other aspects of this technology, such as treatment cost evaluation [2], full-scale deployment and effect of support medium [3•,4•,5] or mass transfer considerations [6•,7•], these topics as well as recent advances in waste air biofiltration will not be discussed herein.
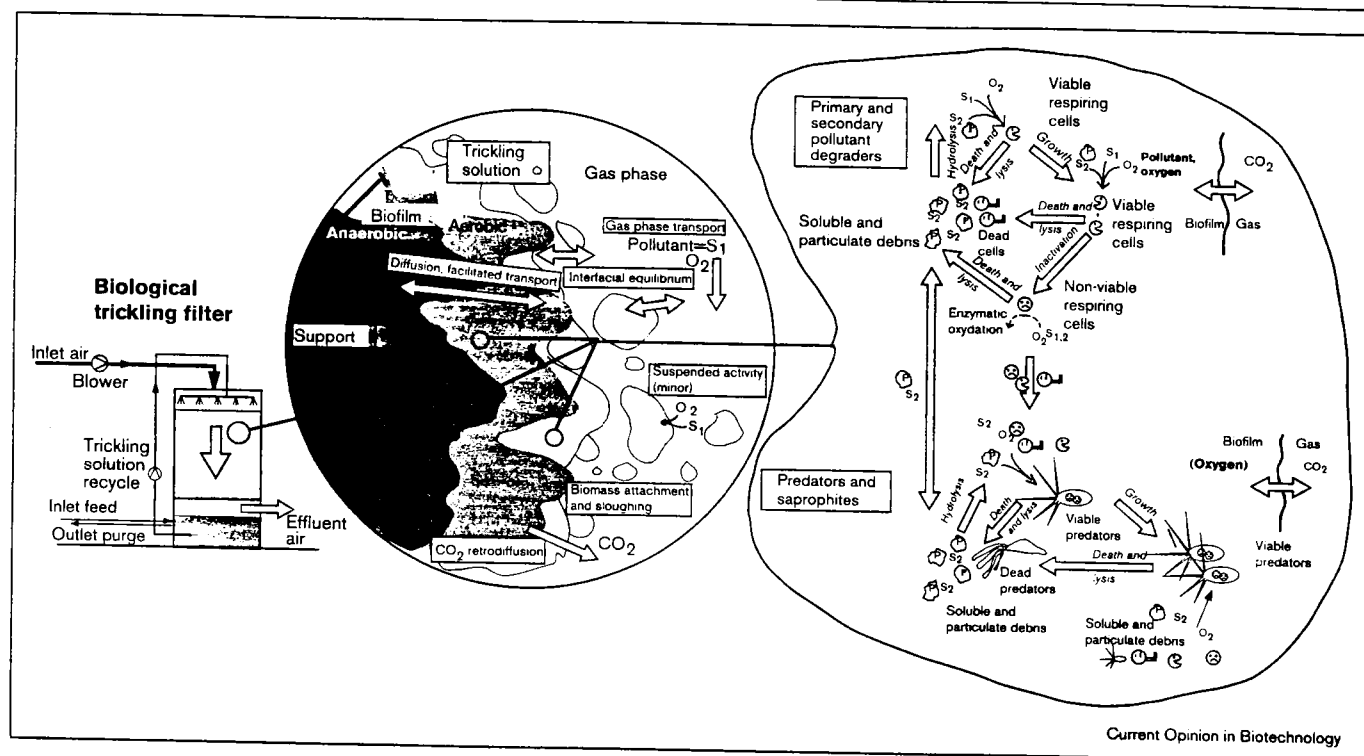
Biotrickling filtration process

Biotrickling filters are biological scrubbers in which a polluted air stream is passed through a packed bed on which a mixed culture of pollutant-degrading organisms is naturally immobilized. The packed bed is generally made of an inert material, such as random or structured plastic packing or, less often, polyurethane foam [4•] or lava rocks [3•]. It provides the necessary surface for biofilm attachment and for gas–liquid contact. As described in Figure 1, the elimination of a gaseous pollutant in a biotrickling filter is the result of a complex combination of different physico-chemical and biological phenomena. The following discussion seeks to identify the different steps likely to occur during treatment, so that reviewed papers can be placed in a general perspective for their contribution to the understanding of the process.

While the contaminated air is forced through the packed bed in either a downflow or upflow motion, an aqueous phase is recycled over the packing to provide moisture and mineral nutrients to the immobilized mixed culture of pollutant-degrading microorganisms. The fact that the trickling liquid is recycled suggests that co-current flow (air in a downflow motion) is preferred over counter-current flow (upflow air stream). In the upflow configuration, air might pick up pollutant from concentrated liquid just before leaving the bioreactor, which would reduce the removal efficiency of the system. Experiments have failed, however, to show a significant difference between upflow or downflow [8,9].

As contaminated air moves through the packed bed, pollutant vapors and oxygen can transfer either to the trickling water or directly to the biofilm [7•]. Adsorption onto the support is usually minimal in biotrickling filters because of the inertness of the support. As the trickling water moves down the bioreactor, it will contact the biofilm and provide a means to control the process

Figure 1

Biotrickling filtration principle, fate of pollutants during the process, and simplified biology of the process culture. O_2, oxygen; P, particulate matter and cell debris; S_1, primary substrates (pollutants); S_2, secondary substrates.

culture conditions, such as pH, mineral nutrient and salt concentrations, conductivity, and so on. Inside the biofilm, biodegradation is mediated by mixed cultures of bacteria and fungi thriving in a complex ecosystem, including secondary pollutant degraders and predators, such as protozoa and other higher organisms (Figure 1). The kinetics of pollutant elimination in the biofilm are influenced by both environmental conditions and mass transfer of essential substrates. The system is continuously supplied with essential mineral nutrients, such as nitrogen, phosphorus, potassium, and trace elements in the recycle liquid. Mineral nutrient availability, in particular nitrogen and to a lesser extent phosphorus, is often restricted, however, to either reduce biomass formation or to minimize treatment costs. Hence, it is most likely that predators such as protozoa, nematodes and higher organisms frequently observed in trickling filters play an important role in recycling key nutrients (Figure 1). Pollutant biodegradation may be accompanied by the formation of end products, such as chloride or sulfate, and/or partially oxidized metabolites, such as carboxylic acids. These may be inhibitory to the process culture and are best purged with the liquid drain along with small amounts of biomass. Usually, less than 10% of the carbon-pollutant entering the system leaves via the purge [10].

New applications

Whereas conventional compost or soil bed biofilters are limited to the elimination of odorous compounds and non-chlorinated volatile organic compounds, a wider range of pollutants can potentially be treated in biotrickling filters. This is because environmental conditions can be better controlled in the latter reactors and potentially toxic dead-end metabolites can be purged out of the system via the free liquid. Also, laboratory biotrickling filters offer the opportunity to work with monocultures, possibly of genetically engineered microorganisms.

Oh and Bartha [11] reported the first elimination of nitrobenzene vapors in a laboratory-scale biotrickling filter. They used a stable microbial consortium enriched from sewage sludge immobilized on perlite. During the four week startup, the inlet nitrobenzene concentrations had to be kept relatively low ($<80\,mg\,m^{-3}$) to avoid poisoning of the process culture. Thereafter, high and sustained nitrobenzene elimination was observed: typically 80–90% degradation for inlet concentrations ranging from 100–300 $mg\,m^{-3}$ and an empty bed gas contact time of 21 seconds. This corresponds to an elimination capacity of 50 $g\,m^{-3}\,h^{-1}$, a high value that could lead to an economically viable process. A nitrogen balance showed

that 98% of the nitrobenzene nitrogen was converted into ammonia while a small amount of nitrite was produced.

Two other compounds of general interest were newly reported to be biodegraded in laboratory biotrickling filters: di-ethyl ether, a compound not widely studied in gas phase bioreactors [12]; and the gasoline additive methyl tert-butyl ether (MTBE) [13••]. Whereas Eweis *et al.* [14•] demonstrated for the first time that low concentrations of MTBE could be eliminated in biofilters, Fortin and Deshusses [13••] achieved removal efficiencies of 75% for inlet concentrations of $0.8\,g\,m^{-3}$ with an empty bed residence time of less than a minute in biotrickling filters. This corresponds to an elimination capacity of $50\,g\,m^{-3}\,h^{-1}$, an extremely high value for a compound which biodegradation *in-situ* still remains a challenge. Higher removal percentages were obtained at lower loadings. Fortin and Deshusses reactors were originally inoculated with various samples of aquifer material and soil contaminated with MTBE. Interestingly, MTBE removal was significant only after addition of traces of a peat humic substance (PHS) extract to the recycle liquid. As biomass accumulated in the reactors, the benefits of the PHS were no longer significant. While several reports exist on biostimulation using PHS in wastewater treatment, the exact mechanisms involved in biostimulation using PHS are yet to be elucidated [15].

Also noteworthy is a study by Sun and Wood [16••], who immobilized a pure culture of *Burkholderia cepacia* $PR1_{23}$ (TOM_{23C}) constitutively expressing toluene *ortho*-monooxygenase to cometabolize the biodegradation of trichloroethylene (TCE) vapors in a biotrickling filter. Aerobic biodegradation of TCE only occurs through cometabolism, and a growth substrate (usually toluene, methane, propane, phenol, or ammonia) is required to induce the expression of the appropriate TCE-degrading enzyme. *B. cepacia* $PR1_{23}$, however, expresses toluene *ortho*-monooxygenase constitutively, which circumvents the problem of competitive inhibition of TCE oxidation by the usual inducers during the growth phase. Sun and Wood [16••] used a glucose solution as a carbon and energy source and observed TCE eliminations up to 200 times higher than previously reported. As observed in other bioreactors for TCE aerobic cometabolism, however, rapid inactivation of the TCE-degrading enzyme by TCE breakdown products (TCE epoxide) remained a problem, and TCE degradation in an industrial biotrickling filter in the near future is not very probable.

Biofilm architecture

Pollutant elimination is the result of many, interdependent processes that simultaneously take place inside the biofilm (see Figure 1). To date, little information exists about biofilm architecture in biotrickling filters. Previous work with scanning confocal laser microscopy discussed the existence of cell-free channels extending from the biofilm–liquid interface to the substratum and their

possible role in enhancing pollutant and oxygen mass transfer [17]. A new and promising development is the use of computed axial tomography (CAT) X-ray scanning to characterize the biofilm macro architecture in biotrickling filters [18••]. CAT scans of a toluene-degrading biotrickling filter containing a large amount of biomass (70% of reactor volume) immobilized on polypropylene Pall rings showed a heterogeneous distribution of biomass with large areas completely filled with biomass whereas other sections of the reactor were covered by <1 mm thick biofilms. Further image analysis revealed the presence of air/water channels ranging in size from $<5–380\,mm^2$, with smaller channels ($0–60\,mm^2$) contributing to more than 80% of the interfacial area. Biomass accumulation in this biotrickling filter resulted in a decrease of the biofilm-specific surface area from $220\,m^2\,m^{-3}$ (surface area of the clean packing) to $101\,m^2\,m^{-3}$. An even more drastic decrease was expected as the reactor was nearly clogged by biomass, but analysis of the scans on a sub-millimeter scale indicated a rough biofilm surface, which significantly increased the biofilm-specific surface area. In the future, further application of high resolution X-ray and possibly CAT scanning techniques could contribute to a better understanding of the architecture of biofilms as advanced image processing allows three dimensional structures of biofilms to be resolved. Such progress could lead to a better understanding of pollutant mass transfer in biotrickling filters and ultimately to a better design of materials for process culture support.

Population dynamics

Although biotrickling filter performance clearly depends on the type of microorganisms present, few studies deal with population dynamics. This is regrettable as biotrickling filters are open systems and if specialized strains are inoculated they will have to compete with others from the outside environment. Pollutant degradation in biotrickling filters is usually attributed to bacteria; however, Weber and Hartmans [19] found that fungi may play an important role. They inoculated two biotrickling filters for toluene removal with different inocula, and observed a predominant development of fungi in one biotrickling filter and bacteria in the other, although operational conditions were identical. Interestingly, under nutrient-limited conditions, the biotrickling filter containing predominantly fungi showed a much higher toluene removal capacity (27 g carbon $m^{-3}\,h^{-1}$ versus 13 g carbon $m^{-3}\,h^{-1}$ for the one with bacteria). The use of fungi in waste air biotrickling filters remains a relatively unexplored area. In the future, it may show promise for the biotreatment of recalcitrant volatile compounds, especially in the light of the wide substrate range of some of the peroxidase enzymes secreted by lignolytic fungi [20].

Classical microbiological techniques such as plate counting are only appropriate for the detection of culturable cells [21], which may constitute only a minority of the biotrickling filter culture. The use of genetic probes allows

one to quantify the contribution of selected individual species in the overall pollutant elimination. In a 1996 study, Møller et al. [17] found a homogeneous distribution of Pseudomonas putida throughout the biofilm of a biotrickling filter using 16S rRNA probes in combination with scanning confocal laser microscopy. This species contributed for 65% of the toluene elimination capacity of the biotrickling filter, although constituting only 4% of the total population. In subsequent investigations with a similar experimental set-up, Pedersen et al. [22••] found that during the biotrickling filter startup, the relative abundance of P. putida in the biofilm decreased from about 40% one day after inoculation, to a constant value of 10% after twelve days of operation. At this time, toluene degradation by P. putida was only 11% of the overall toluene elimination capacity, although it showed the highest toluene degradation rate in suspended batch cultures out of the four toluene-degrading species isolated from the bioreactor [22••]. The authors attributed the observed differences to the difference of nutrient availability, as the 1996 [17] and the 1997 [22••] experiments were performed at different nutrient loadings. Another plausible explanation is that toluene elimination in the 1997 study [22••] was achieved by species other than P. putida, which exhibited lower maximum toluene elimination rates but were better adapted to thrive under nutrient-poor conditions. In a more general sense, these findings emphasize that for the selection of specialized species in biotrickling filters, one should not only consider the maximum pollutant degradation rate, but also the tolerance to suboptimal conditions and the competitiveness in complex biological ecosystems.

Biofilm activity

Biokinetic parameters are mostly determined in liquid suspensions, an environment quite different from the biofilm: this raises the question of whether data obtained from liquid suspensions can be extrapolated to the biofilm. Mirpuri et al. [23•] compared the biodegradation kinetics of toluene degradation of suspended P. putida 54G cells and of biofilm cells grown on ceramic Raschig rings in a biotrickling filter. The specific toluene degradation activity of biofilm samples, expressed per amount of protein, was three to five times lower than the specific activity of freely suspended cells and decreased both with time of exposure to toluene in the biotrickling filter and increases in the toluene gas phase concentration. When expressed per number of toluene-culturable cells, however, the specific activity was the same for free suspended and biofilm-grown cells. Apparently, long-term exposure of biofilm cells to toluene caused inactivation of a major part of the biofilm, thus causing a lower specific activity when expressed per amount of protein [23•]. It should be emphasized that Mirpuri et al. [23•] determined the activity of biofilm-grown cells by suspending the biofilm in medium and measuring toluene uptake, whereas Møller et al. [17] and Pedersen et al. [22••] determined the activity in situ by correlating the rRNA content to cellular activity.

This is not necessarily directly comparable. The latter authors estimated that the toluene degradative activity of P. putida biofilm cells in situ was about 20% [22••] to 57% [17] of that of suspended cells at optimal conditions. Identification of the factors causing cells in biofilms to be less active than in suspension is needed to optimize the pollutant degradation rate in biotrickling filters.

Experiments with pure cultures of P. putida 54G in flat plate vapor phase bioreactors provided more insight into the toxic effect of toluene on the biofilm [24,25••]. Long-term exposure to increasing concentrations of toluene resulted in an increase in the fraction of respiring cells that could not grow on toluene [25••]. Comparison of oxygen profiles in the biofilm measured with a microsensor and microscopic examination of cryosections of the biofilm indicated that inactive cells had accumulated at the biofilm–liquid interface, whereas active toluene-oxidizing cells were mainly present in deeper parts of the biofilm, that is, near the substratum [24,25••]. This contradicts the generally well accepted concept of the presence of a thin layer of active cells at the biofilm–liquid interface separated from the substratum by an anaerobic, inactive layer as shown in Figure 1. Villaverde et al. [25••] postulate that additional mass transfer resistance in the outer, inactive biofilm layer may protect the active cells close to the substratum from the toxic effects of high concentrations of toluene. It was further observed that high toluene concentrations resulted in an increase of the non-toluene-associated oxygen consumption in the biofilm, up to 97% of the total respiration rate, possibly a result of cryptic growth on leakage and lysis products from injured cells [26]. These results indicate the importance of secondary processes (i.e. processes not directly related to degradation of the primary pollutant) even in a single-species biofilm.

Biomass control in biotrickling filters

In order to maximize the volumetric pollutant elimination capacity, a continuous supply of mineral nutrients is required to sustain an actively growing process culture. Consequently, without a means to control biomass accumulation, the amount of immobilized biomass will increase until the biotrickling filter ultimately gets clogged. This is the greatest challenge facing the deployment of biotrickling filters in the field. For a toluene-degrading biotrickling filter, Cox et al. [10] found wet biomass accumulation rates ranging from $3.1-9.8\,kg\,m^{-3}_{reactor}\,day^{-1}$ while degrading on average $20-40\,g\,toluene\,m^{-3}_{reactor}\,day^{-1}$. At this regime, marked reactor instabilities (decrease in toluene elimination and increase in pressure drop) occurred after 3–5 months of operation [10]. The major cause for declining pollutant elimination capacities is believed to be the reduction of the biofilm-specific surface area with increases of biomass content [27•]. As a long-term (years) stable performance is required, biomass control in biotrickling filters has gained much attention over the past two years. Current biomass control strategies either aim at reduction

of the biomass accumulation rate or at the removal of excess biomass. Recent advances are discussed below.

Reduction of the biomass accumulation rate

Addition of growth-inhibiting concentrations of sodium chloride [28,29] and limitation of potassium [30] or nitrogen [19,29] have been shown to reduce the accumulation of biomass in biotrickling filters. This was associated, however, with a reduction of the volumetric pollutant elimination capacity, hence, larger biotrickling filters would be required to treat the same waste air stream.

The overall biomass accumulation rate not only depends on the growth of primary degraders, but also on secondary processes (Figure 1). Diks et al. [31] showed the existence of a biological equilibrium in a dichloromethane-degrading biotrickling filter where secondary processes, presumably endogenous respiration, cryptic growth and predation of bacteria by higher organisms, caused a near complete mineralization of dichloromethane. The pollutant elimination capacity in the case of Diks et al. [31] was, however, less than $10 \, g \, carbon \, m^{-3} \, h^{-1}$, and equilibrium may not have been reached at higher pollutant loadings. Stimulation of secondary processes may be necessary to ensure a zero net biomass growth at higher pollutant degradation rates. We investigated [32•] the use of protozoa that prey upon bacteria to reduce biomass formation in high-performance toluene-degrading biotrickling filters. Addition of protozoa resulted in a slightly higher toluene elimination capacity and a lower rate of biomass accumulation due to an increase of carbon mineralization. Clogging of the biotrickling filter amended with protozoa was nevertheless observed, but at a much later stage than in the control biotrickling filter. It was concluded that selection and stimulation of protozoa specialized in grazing of biofilms was required to improve the efficiency of biomass control [32•].

Chemical and mechanical removal of biomass

Periodical removal of excess biomass is an alternative to balancing the growth rate for high performance biotrickling filters. Smith et al. [33] and Sorial et al. [34] investigated backwashing (upflow) of the bed with water. Efficient biomass removal required fluidization of the packing material, thus causing a bed expansion of 40%. So far, backwashing seems to be limited to those biotrickling filters containing a packing that can be fluidized. It is yet unclear how the relatively high frequency of backwashing (about twice a week, Sorial et al. [34]) and the larger reactor volume to allow for bed expansion will affect the economics of the process after scaled-up to industrial biotrickling filters. Weber and Hartmans [19] reported chemical washing of biotrickling filters. They used a 0.1 M NaOH solution and obtained a stable biotrickling filter with a constant biomass content by washing once every two weeks. An advantage of chemical washing over backwashing is that much lower liquid flow rates can be

applied. Loss of microbial activity due to the chemical is probable, however, and thus the recovery time after chemical washing becomes a critical issue. When using a 0.1 M NaOH washing solution, activity of the biotrickling filter was fully restored within one day [19]. An alternative to washing techniques is mechanical removal of biomass by periodically stirring the trickle-bed [30,35•]. While the idea is appealing, the practical feasibility of this proposal remains to be demonstrated.

Regardless of the proposed biomass control method, further discussion should address the issue of minimizing operation and maintenance costs and the issue of disposing of possibly large amounts of excess biomass before the deployment of the proposed strategies in an industrial setup.

Conclusions

Recent research in the field of biotrickling filtration for air pollution control has focused on various aspects pertaining to the microbiology of pollutant degrading microorganisms in biofilms, kinetics of pollutant uptake, and means to control biomass accumulation. Significant progress has been made in these areas. Nevertheless, additional information on the fundamental principles underlying biotrickling filtration is needed. Key questions to be addressed are concerned with the complex ecology of biofilms. In particular, studies are needed to understand the overall role of secondary processes (i.e. those processes not directly associated with the elimination of the primary pollutant) and how these can be controlled in practice. In the future, the ability to control the ecology of biofilms in biotrickling filters may enable optimal balancing of the net growth of biomass, so that reactor stability can be ensured over several years. Additional research is needed to better define the kinetic relationships for pollutant biodegradation. Particularly relevant for future implementation of biotrickling filters in the field is to develop an understanding of the biodegradation of mixtures of pollutants, to define the role of oxygen and of ancillary nutrients on the rate of biodegradation and on the biomass yield, and to determine the influences of various stresses, such as changing conditions and mass transfer limitations.

Overall, the review of recent research in biotrickling filtration emphasized that in order to progress in the understanding of the process, more studies involving in situ analysis are required. Clearly, this will involve a more extended application of the modern tools of biotechnology. This should enable the establishment of baseline information presently missing for rational reactor design and optimum process operation. This, together with applied research using pilot or technical-scale biotrickling filters, showing the economic viability of biotrickling filtration for a number of applications, should enable optimum technology transfer from the laboratory into the field in the coming years.

Acknowledgements

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