**Characterization techniques of packing material colonization in gas biofiltration processes**

Yves Andres, Eric Dumont, and Claire Gerente

**Abstract:** Biological air treatment is largely used for the decontamination of large air streams with low pollutant concentrations. As a result of microbial metabolism, the pollutants are degraded into carbon dioxide and water or converted into cell biomass. Three main research domains have emerged within the field of biofiltration: filter bed development, process optimization, and microbiological studies (community, nutrients, ...). The objective of this paper is to focus on the microbial activities in air biofiltration processes. The nutrient contribution and the packing material effects are described. Special attention is paid to the description of techniques for the characterization and identification of the microbial population.

**Key words:** biofiltration, packing material, molecular biology, air treatment.

**Résumé :** Le traitement biologique de l’air est très utilisé pour la décontamination de grands courants d’air faiblement pollués. En raison du métabolisme microbien, les polluants sont dégradés en dioxyde de carbone et en eau ou convertis dans la biomasse des cellules. Trois grands domaines de recherche ont vu le jour en biofiltration : le développement des lits filtrants, l’optimisation des procédés et des études microbiologiques (communauté, nutriments, etc.). L’objectif de cet article est de cibler les activités microbiennes dans les processus de biofiltration de l’air. La contribution des nutriments et les effets du remplissage sont décrits. On prêtera tout particulièrement attention à la description des techniques de caractérisation et d’identification de la population microbienne.

**Mots-clés :** biofiltration, remplissage, biologie moléculaire, traitement de l’air.

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**Introduction**

Air pollution is one of the major problems to be tackled over this century. In recent years, a strong technological base has been developed for air quality control. The driving force behind this development has been public awareness of the need for human health and environmental protection. Consequently, environmental legislation and regulations have been initiated by governments to control air quality. Removing air pollutants from industrial gaseous emissions is achieved by different physical and chemical methods, like the transfer and (or) concentration of compounds in a liquid phase (condensation, absorption) or onto a solid (adsorption) or by the destruction of molecules by thermal and catalytic incineration.

However, more recently new treatment alternatives have been developed involving the biological degradation of pollutants present in the gaseous phase. Thus, these microbial systems can remove a large number of molecules, especially volatile organic compounds (VOCs) and odorous molecules. Such systems present several advantages, among which is their high efficiency and low cost. Moreover, the treatment of polluted air in bioreactors allows the complete degradation or transformation of the contaminants. For the treatment of industrial gaseous emissions, the applications are possible for a concentration of pollutant above 1 g m$^{-3}$ to diluted air (some mg m$^{-3}$ or μg m$^{-3}$). The installation designs cater for airflow ranged from 1000 to 100 000 m$^3$h$^{-1}$ (gas velocity ranged from 50 to 150 m h$^{-1}$) and even more for some specific systems. Typical pollutants concentrations are below 5 g m$^{-3}$.

Biological units commonly used for air management are based on three different technologies: biofilter, trickling filter, and bioscrubber. Recently, two other types of bioreactors have been developed: the membrane bioreactor and the two-phase bioreactor (Le Cloirec et al. 2001). These processes can be distinguished either by the mobility of the microorganisms and the liquid phase (as in the bioscrubber) or only the liquid phase (as in the trickling filter). In biofilters and trickling filters, the microorganisms are fixed on a support or packing material (Ottengraf 1987; Devinny et al. 1999). Waste gas treatment performances of such processes are very interesting when operating parameters, such as waste gas loads for biofiltration and washing solution loads in the case of bioscrubbing and trickling filtering, are well controlled.

The objective of this paper is to focus on the microbial activities in biofiltration processes. The nutrient contribution and the packing material effects are described. Special attention is paid to the description of methods for the determination of the biological population evolutions.
Packing material studies

In biofiltration processes, the gas stream containing the pollution is injected through a bed packed with a solid medium that supports immobilized microorganisms arranged in a biofilm. The pollutant substances transfer from the airflow to the biofilm, where they are degraded by microorganisms, mostly into by-products such as carbon dioxide and water, and they produce biomass (Fig. 1).

In many cases, the gas stream and the water flow used to humidify the biofilm brings all the nutrients required for the development of the microorganisms. The contaminant concentrations in most waste gas streams vary with time due to the inherent nature of the processes that generate them. In other cases the airflow contains only some of the elemental compounds needed for the microorganism growth and makes the biofiltration process not very efficient. So the process could require the addition of nutrients (C, N, P, S, K, Na) or oligo-elements (Ca, Co, Cu, …) to enhance the microbial development. Microorganisms can be initially present in the material (compost, peat, …) or can be inoculated as a consortium (activated sludge, soil extract, …) or as pure culture (Zilli et al. 2001). The bed material inoculation has a direct effect on the length of the start-up period.

The packing material represents an important part of a biofilter as microorganisms grow on its surface. Focusing on their characteristics, packing materials can be described with five main properties (Perry and Green 1997), which will strongly influence the system efficiency and its costs:

- Particle size, bed porosity, and specific surface area allows the biomass attachment. Bed porosity ranges usually from 0.5 to 0.9 (Devvinny et al. 1999) and the specific surface area from 300 to 1000 m².m⁻³ (Perry and Green 1997).
- Nutritive capacities: the microbial metabolism must be complete with the essential nutrients that are not contained in the waste stream.
- Strong mechanical resistance and low bulk density will ensure better hydrodynamic properties avoiding bed packing down.
- Significant water retention capacities: the bed humidity rate ranges between 40% and 60% (Morales et al. 2003)
- High buffer capacities will avoid high pH fluctuations while microorganisms produce acid or basic metabolism compounds.

In addition, low cost and ease of supply is required for industrial facilities. Figure 2 summarizes the packing material properties needed to have a good biofilm development and the hydrodynamic and economic criteria.

For process optimization purposes, many authors investigated various packing or carrier materials to provide the nutrients or to improve the hydrodynamics, with, in both cases, the economical criteria in view. Table 1 gives some examples of material that has been studied in air biofiltration. As can be seen from this not exhaustive table, a large diversity of organic or inorganic compounds can be used as packing material in biofilters. For better hydrodynamic behaviour, organic material can be mixed with a solid one to increase the bed porosity. In the case of an inorganic carrier, nutrients have to be provided by a specific solution. With economy in mind, many agriculture or industrial by-products and wastes are being investigated as new biofiltration bed material. An interesting alternative is to synthesize a reactive packing material, which is added directly into the bed where it slowly dissolves and releases the essential nutrients (Gaudin et al. 2008). One general conclusion for a good packing material choice is the one that allows better hydrodynamic properties, provides microbial development, and which is cheaper to use.

Some authors have studied the effect of the addition of nitrogen or phosphorus sources on the biofilter efficiency even for organic beds (Nikiema et al. 2005).

Microbial development investigations

Various ways can be followed to describe the microbial life, colonization, and the population evolution on the bedding material involved in biofiltration processes. Figure 3 presents different approaches, which could be set up for the study of the microbial population immobilized on the biofilter media.

Two main survey methods can be described: (i) conservative approaches by using direct microscopic observation or microbial growing methods; (ii) destructive approaches with cell macromolecule extraction followed by analysis using molecular biology tools, enzymatic activity measurement, or protein, phospholipids, or polysaccharides analysis. For this latter step, a cell extraction from the carrier material followed by a lysis and macromolecules purification is needed.

From the last two decades, many nucleic acid methods have been developed to characterize the genetic fingerprint of microbial populations found in various environments. These techniques are much used in the biological treatment research to understand the microbial population evolution and diversity related to the processes efficiency.

Commonly used microbiological methods

One way of surveying microorganisms populations in biofilm is by the direct observation of the biofiltration bed material. For this purpose, a scanning electronic microscope can be used (Acuña et al. 2002; Qi and Moe 2006). Qi and Moe (2006) have observed that for biofilter treating paint solvent, a pH decrease enhances the domination of fungi in the microbial population. But this approach leads to limited results and in many cases the observation has to be completed with plate count (Qi and Moe 2006) or polymer extraction and measurement (Gaudin et al. 2008). In the latter study, the protein content increase was correlated to the microbial colonization of the packing material due to the pollutant biodegradation.

Webster et al. (1997) have proposed following the microbial ecosystem evolution by phospholipid fatty acid (PLFA) determination in a compost and granular activated carbon biofilter used to remove VOCs and low H₂S concentration. The PLFA assays were used to infer microbial density, metabolic stress status, and community structure.

A second way of investigation is the method involving microbial growth. Despite the fact that the complex microbial environment in a biofilter makes microbial enumeration by traditional plate counting methods difficult (and often less than 10% of the total viable population is counted) and imprecise, this approach is used. However, it is possible to
characterize the main viable microorganisms present on the packing material. Moreover, the microbial identification of the isolated viable microorganisms can be done through conventional biochemical methods like the Apilab identification system (Andrés et al. 2006; Pineda et al. 2004). For example, Langolf and Kleinheinz (2006) have measured a microbial density from $10^6$ to $10^7$ colony forming units per g of lava rock from a biofilter treating alpha pinene and they identify *Bacillus* and *Rhodococcus* as predominant genera.

Using microbial growth, community level physiological profiling (CLPP) with BIOLOG EcoPlates™ can be realized to describe changes in the functional diversity of the microbrial community for various packing materials and pollutants. This method involves the inoculation of plates containing 96
Table 1. Example of packing material used in biofiltration studies.

<table>
<thead>
<tr>
<th>Material</th>
<th>Pollutant</th>
<th>pH</th>
<th>Specific surface area (m²g⁻¹)</th>
<th>Chemical composition C/H/N (%)</th>
<th>Elimination capacity (g m⁻³h⁻¹)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granular high mineralized peat (soil)</td>
<td>Ethylbezene</td>
<td>6.2</td>
<td>1.3</td>
<td>17.0/1.8/0.8</td>
<td>45 (IL 55)</td>
<td>Alvarez-Hornos et al. 2008</td>
</tr>
<tr>
<td>Fibrous peat</td>
<td>Ethylbezene</td>
<td>4.8</td>
<td>13.4</td>
<td>48.5/5.8/0.6</td>
<td>120 (IL 135)</td>
<td>Alvarez-Hornos et al. 2008</td>
</tr>
<tr>
<td>Perlite (volcanic rock)</td>
<td>Alpha-pinene</td>
<td></td>
<td></td>
<td></td>
<td>24 (IL 69)</td>
<td>van Groenestijn and Liu. 2002</td>
</tr>
<tr>
<td>Expended clay</td>
<td>Alpha-pinene</td>
<td></td>
<td></td>
<td></td>
<td>33 (IL 90)</td>
<td>van Groenestijn and Liu. 2002</td>
</tr>
<tr>
<td>Polyurethane foam cubes</td>
<td>Alpha-pinene</td>
<td></td>
<td></td>
<td></td>
<td>38 (IL 96)</td>
<td>van Groenestijn and Liu. 2002</td>
</tr>
<tr>
<td>Compost (household vegetable, fruit, garden wastes)</td>
<td>Alpha-pinene</td>
<td></td>
<td></td>
<td></td>
<td>24 (IL 64)</td>
<td>van Groenestijn and Liu. 2002</td>
</tr>
<tr>
<td>Horse manure</td>
<td>H₂S</td>
<td>4.3</td>
<td></td>
<td>53.2/5.4/0.5</td>
<td>1 (IL 79)</td>
<td>Barona et al. 2004</td>
</tr>
<tr>
<td>Sludge</td>
<td>H₂S</td>
<td>7.2</td>
<td></td>
<td>28.4/4.8/4.3</td>
<td>5 (IL 79)</td>
<td>Barona et al. 2004</td>
</tr>
<tr>
<td>Soil and algae</td>
<td>H₂S</td>
<td>6.9</td>
<td></td>
<td>21.1/2.7/3.1</td>
<td>10 (IL 79)</td>
<td>Barona et al. 2004</td>
</tr>
<tr>
<td>Pig manure and sawdust</td>
<td>H₂S</td>
<td>6.5</td>
<td></td>
<td>20/2.0/0.6</td>
<td>46 (IL 79)</td>
<td>Barona et al. 2004</td>
</tr>
<tr>
<td>Coconut fiber</td>
<td>Toluene</td>
<td></td>
<td>0.75</td>
<td>47.3/5.7/0.5</td>
<td>89.9 (IL 110)</td>
<td>Maestre et al. 2007</td>
</tr>
<tr>
<td>Compost</td>
<td>Toluene</td>
<td></td>
<td>5.12</td>
<td>28.7/3.3/2.9</td>
<td>95 (IL 110)</td>
<td>Maestre et al. 2007</td>
</tr>
<tr>
<td>Peat</td>
<td>Toluene</td>
<td></td>
<td>1.21</td>
<td>31.6/3.4/1.2</td>
<td>71.6 (IL 110)</td>
<td>Maestre et al. 2007</td>
</tr>
<tr>
<td>Pine leaves</td>
<td>Toluene</td>
<td></td>
<td>0.23</td>
<td>46.4/5.3/0.6</td>
<td>85 (IL 110)</td>
<td>Maestre et al. 2007</td>
</tr>
<tr>
<td>Bagasse–compost–GAC (55:30:15)</td>
<td>BTEX</td>
<td>6.2</td>
<td>4.14</td>
<td>43.2/6.1/0.006</td>
<td>83.65 (IL 126.5)</td>
<td>Mathur et al. 2007</td>
</tr>
<tr>
<td>Inorganic bed</td>
<td>CH₄</td>
<td></td>
<td></td>
<td></td>
<td>29.2 (IL 68)</td>
<td>Nikiema et al. 2005</td>
</tr>
<tr>
<td>Mature compost bed</td>
<td>CH₄</td>
<td></td>
<td></td>
<td></td>
<td>12.5 (IL 68)</td>
<td>Nikiema et al. 2005</td>
</tr>
<tr>
<td>Coir pith fiber</td>
<td>Toluene</td>
<td>4.4–5.7</td>
<td></td>
<td></td>
<td>96.75 (IL 120.72)</td>
<td>Krishnakumar et al. 2007</td>
</tr>
<tr>
<td>Pine bark</td>
<td>H₂S</td>
<td>4.5</td>
<td></td>
<td>53.7/0.3/5.5</td>
<td>6.35 (IL 9.13)</td>
<td>Dumont et al. 2008</td>
</tr>
<tr>
<td>Peat–glass bed (4:1 v/v)</td>
<td>Styrene</td>
<td></td>
<td></td>
<td></td>
<td>63 (IL 400)</td>
<td>Zilli et al. 2001</td>
</tr>
<tr>
<td>Peat–glass bed (4:1 v/v)</td>
<td>Toluene</td>
<td></td>
<td></td>
<td></td>
<td>242 (IL 1000)</td>
<td>Zilli et al. 2001</td>
</tr>
<tr>
<td>Perlite</td>
<td>TEX</td>
<td>72</td>
<td></td>
<td></td>
<td></td>
<td>Kennes and Veiga 2004</td>
</tr>
</tbody>
</table>

Note: BTEX: benzene, toluene, ethyl benzene, O-xylene; IL: inlet load; GAC, granular activated carbon; TEX, toluene, ethyl benzene, O-xylene.

UP20°: CH₃N₂O, H₃PO₄, CaCO₃ (C/N/P molar ration 100/5/1).
wells, 95 being impregnated with separated organic carbon compounds. Determination of CLPP is based on sole carbon substrate utilization profiles and was first demonstrated by Garland and Mills (1991) to distinguish between different communities of heterotrophic soil bacteria. BIOLOG use was described for compost biofilter treating hexane and methanol (Grove et al. 2004; Weber et al. 2007), coir pith-based filters for toluene removal (Krishnakumar et al. 2007), or various compost gas phase bioreactors for trichloroethylene biofiltration (Sukesan and Watwood 1997). Many authors agree that BIOLOG EcoPlates™ provide a simple and rapid way for assessing changes in the microbial community structure of biofiltration processes. The technique finds application in monitoring the health of the microbial population and it might be expected that significant changes in the community structure precede a decrease of the removal efficiency of the operation unit. Moreover, this approach can also give some information about the microbial community in response to various stresses.

For the purpose of determining the microbial kinetic parameter necessary to biofilter modelling and design, Dehoménie et al. (2008) proposed a method based on the direct use of packing material, colonized by microorganisms from a steady-state biofilter, which is directly inserted into the thermostated batch reactors. The test consists in following the evolution of substrates (toluene or methane) and carbon dioxide mass change versus time. The experimental data are described as Monod and Haldane kinetic models.

**Nucleic acid-based methods**

As the previous approach gives only partial data, nucleic acid-based methods can be used to get more information. Indeed, with the development of polymerase chain reaction (PCR) and DNA sequencing technology, it is possible to amplify target DNA sequences and to use it in microbial community characterization. The comparison between the quantification of culturing bacteria and the estimation of the total bacterial population with nucleic acids tools was studied by Nehme et al. (2008) for the bioaerol diversity in swine buildings. One explanation of the difference can be attributed to the fact that for culturable bacterial counts the main culture conditions used are mesophilic and aerobic, and with a metagenomic approach the authors show the presence in the air samples of aero-tolerant or anaerobic bacteria. Figure 4 gives a general description of nucleic acid-based methods. In this approach there are two key factors: the DNA extraction and the primer selection, which is not the same for bacteria, fungi, or archaea DNA amplification. For microbial identification the response will also depend on the gene clone libraries’ content. Moreover, the PCR amplification step has to be optimized to produce enough replicon to allow a significant characterization.

In comparison to the time-consuming method based on isolation techniques, fluorescence in situ hybridization (FISH) facilitates the identification procedure. The microbial community characterization is realized with oligonucleotide probes targeting domain, class, and subclass specific regions of the 16S, 18S, and 23S rRNAs. From results presented by Friedrich et al. (1999) it can be expected that waste gas loading will be an important factor in determining the microbial communities of biofilters. Apart from waste gas composition, the filter material itself may also have an impact on the microbial community. The bacterial population from a biofilter packed with granular activated carbon used for biodegradation of volatile sulphur compounds was characterized using the FISH technique (Ho et al. 2008). The main observation revealed that inoculated *Pseudomonas* sp. remained the predominant microbial community member (56%–70%) after 415 d of evaluation.

Denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA genes can be used to assess the microbial diversity and richness in biofilters. In this technique, the total DNA is first extracted from the samples and purified. Subsequently, the purified DNA is amplified by PCR and finally the DNA strands are separated using DGGE. Using DGGE, Cai et al. (2008) observed that interchanging volatile organic compound (VOC) conditions in a filter treating VOC mixture has an impact on the bacterial community structure. These authors have shown that the microbial community...
was different after each interchange of VOCs. With the same method, Sercu et al. (2005) have observed that the microbial community of the biofilm present in a biotrickling filter treating H₂S and dimethyl sulphide presented a low diversity and was stable during process running. Moreover, in the same study, the planktonic microbial community showed very little resemblance to the biofilm community and was more variable over time, although its diversity was comparable to that of the biofilm community. Shim et al. (2006) have observed that the microbial community structure was composed of two large groups, which could be distinguished based on the presence or absence of methyl tert-butyl ether in a biofilter used for benzene, toluene, and xylene removal. Moreover, many of the dominant bacteria in the biofilm were closely related to bacteria isolated from aromatic hydrocarbon-contaminated sites and (or) oil wastewater.

In addition, Khammar et al. (2005) have shown that a link between the spatial structure of microbial communities and the biodegradation of a mixture of 11 VOCs in a peat biofilter could exist. Oxygenated compounds were removed at the top of the filter and aromatics at the bottom. Comparison of single-strand conformation polymorphism patterns clearly showed a shift in community structure, which is function of depth inside biofilters. Steele et al. (2005) have used automated ribosomal RNA intergenic spacer analysis (ARISA) to study the structure of three microbial communities through acclimation to ethanol removal in liquid culture and in two biofilters, respectively, packed with sand and lava rock. Diversity indices have shown a high diversity for biofilter with inconsistent ethanol biodegradation and low diversity in successful process with ethanol removal efficiency close to 80%. Borin et al. (2006) have followed the microbial succession in a compost-packed biofilter treating benzene by plate counts, ribosomal RNA intergenic spacer analysis and DGGE. Their main conclusions are that the number of cultivable aerobic heterotrophic bacteria and species diversity increased with benzene availability. Moreover, comparison of DGGE and ARISA profiles of the total community and isolated strains showed that a complex bacterial population evolution occurred in the biofilter in response to the increasing benzene concentration and that the cultivable bacteria played a major role in pollutant degradation.

Finally, it is also possible to detect microorganisms with specific metabolic ability via the targeting of the catabolic gene in the microbial community. For example, Sakano and Kerkhof (1998) have followed the microbial community structure change in ammonia biofilters using ammonia monooxygenase (amoA) genes. Tell et al. (2007) have used the detection of aromatic catabolic gene (xylene monooxygenase) expression to compare various organic solid media for VOC biofiltration.

The combination of isolation and nucleic acid-based techniques leads to microbial identification by comparison of 16S rRNA sequences. Nikiema et al. (2005) describe the presence of *Methylocystis parvus* in methane biofiltration. Roy et al. (2003) have identified *Pseudomonas putida* as...
the numerical predominant toluene-degrading strain in their biofilter and Mathur et al. (2007) have isolated a Gram-positive bacteria with a high BTEX-degrading activity identified as Bacillus sphaericus.

**Conclusion**

Despite its central role in biofiltration, the ecology of the microbial community in biofilters is not sufficiently studied. The microbial development is strongly related to the nature, the quality, and the ability of packing material to provide nutrients and good life conditions for microorganisms. The classical microbiological techniques give some information for bioprocess optimization, but not enough data to understand the microbial ecology. Nucleic acid-based assessments of bacterial diversity have to be carefully analyzed due to the fact that PCR tools could easily amplify contaminant DNA resulting in biased information. However, these techniques allow the possibility of producing numerous probes allowing microbial identification and microbial population diversity analysis, and also community changes in relation to process modification. All these results indicate that community fingerprints show promising responses, which could be helpful for industrial scale biological treatment process.

In future studies, comparison between biomass activity and biomass quantification by PCR tools can provide information about the microbial potential and the real activity. If a difference is observed, this indicates that there was room to optimize the pollutant removal (Gilbert et al. 2008). Finally, gene expression analysis and (or) population diversity and dynamic could be approached by DNA microarray.

**References**


