International Biodeterioration & Biodegradation 123 (2017) 78–86

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Characterization of bioaerosol emissions from two biofilters during treatment of toluene vapours using epifluorescence microscopy

Saúl Esquivel-Gonzalez [a], Aitor Aizpuru [b], Araceli Patrón-Soberano [c], Sonia Arriaga [a, *]

[a] División de Ciencias Ambientales, Instituto Potosino de Investigación Científica y Tecnológica (IPICYT), San Luis Potosí, Mexico
[b] Universidad del Mar, Puerto Angel, Oaxaca, Mexico
[c] División de Biología Molecular, IPICYT, San Luis Potosí, Mexico

ARTICLE INFO

Article history:
Received 17 April 2017
Received in revised form
5 June 2017
Accepted 6 June 2017
Available online 10 July 2017

Keywords:
Bioaerosol
Epifluorescence microscopy
Fluorochrome
Toluene
Biofilter

ABSTRACT

Emission of bioaerosols from biofilters during the treatment of toluene vapours was studied. A non-culture-dependent technique, known as epifluorescence microscopy (EM), with several fluorochromes was used to characterize and quantify bioaerosols. The bioaerosol emitted concentrations were between 6.4×10^5 and 1.3×10^8 cells m^{-3}_{air} compared with the bioaerosol concentration in ambient air, which was $3.0 \times 10^7 \pm 7 \times 10^6$ cells m^{-3}_{air}. EM allowed for a better estimation of bioaerosol concentrations than culture-dependent techniques. Bioaerosol emission was dependent on the packing material. Perlite was a better packing material in terms of removal efficiency (RE; RE of 60%), with a lower bioaerosol emission (7×10^7 cells m^{-3}_{air}) than Tezontle (RE = 40%; 1.3×10^8 cells m^{-3}_{air}). The main drawback of perlite was acidification of the bed. Bioaerosols in biofilters A and B were composed of Gram-negative bacteria (45% and 40%, respectively), a similar percentage of Gram-positive bacteria (28%) and fungi (27% and 32%, respectively). After the shutdown periods, Gram-positive bacteria were predominant (~60%). The biomass concentrations in leachates were twice those in the air flow and were mainly composed of fungi. Overall, the EM technique is a valuable tool to characterize and quantify bioaerosols in biofilters without under evaluation. This is the first estimation of bioaerosol emissions by biofilters inoculated with a microbial consortium using a noncultivable technique.

1. Introduction

Biofilters are a promising technology for Volatile Organic Compounds (VOCs) abatement and are generally considered to be a cost-effective and environmentally friendly. However, biofilters can generate bioaerosols as a secondary pollution, which has not been well documented, particularly when operated under unsteady conditions, such as fluctuating temperatures, moisture contents, residence times, inlet organic loads and when exposed to shut-down periods (Zilli et al., 2005; Vergara-Fernández et al., 2012a, b; Saucedo-Lucero et al., 2014). Such bioaerosol emissions have detrimental effects on ecosystems and human health, producing toxicity, inflammation, and allergic and asthmatic reactions (Douwes et al., 2003; WHO, 2016). They include all airborne particles of biological origin, such as pollen, viruses, bacteria, bacterial or fungal spores, with an aerodynamic diameter between 0.5 and 100 μm. Until now, the only technique used to quantify bioaerosols emitted in biofilters was based on culture-dependent methods. Previously, six studies have been reported in this field; the first study was carried out by Ottengraf and Konings (1991), in which bioaerosols were collected from six-full scale filter installations using a Millipore all glass impinger. They reported that mainly bacteria were found in the air emission and, to a lesser extent, moulds with concentrations between 10^3 and 10^4 CFU m^{-3}_{air}. Zilli et al. (2005) studied the emission of bioaerosols from biofilters that were used to treat benzene and were packed with two different carriers, such as peat and sieved sugarcane bagasse. These authors reported bioaerosol concentrations of approximately 3.5×10^3 CFU m^{-3}_{air} and indicated that the loading conditions had a strong effect on the bioaerosol emissions. Wang et al. (2009) reported the emission of bioaerosols from a biofilter coupled with a UV lamp for the treatment of chlorobenzene, estimating the bioaerosol concentration to be 1.38×10^3 CFU m^{-3}_{air}. Vergara-Fernández et al. (2012a, b) studied the emission of spores, such as bioaerosols, in a fungal biofilter for the treatment of pentane. The authors used a membrane collector for bioaerosol sampling and

* Corresponding author.
E-mail address: sonia@ipicyt.edu.mx (S. Arriaga).

http://dx.doi.org/10.1016/j.ibiod.2017.06.007
0964-8305/

reported bioaerosol concentrations between 1.8×10^4 CFU m^{-3}_{air} and 4.5×10^3 CFU m^{-3}_{air}. The effect of varying the biofilter operating conditions (inlet load, mineral medium addition periodicity, temperature, moisture content and empty bed retention time) were evaluated in this study. More recently, Saucedo-Lucero et al. (2014) reported the emission of bioaerosols from fungal biofilters that were used to treat hexane. These authors also implemented an advance oxidation technique at the outlet of the biofilter to inactivate bioaerosols. Previous studies indicated that bioaerosol emissions from biofilters are greater than found in ambient air (~10^3 CFU m^{-3}_{air}). However, it is important to better characterize bioaerosol emissions in biofilters in terms of the type of microorganisms, as the level of toxicity among microorganisms varies and biofilters commonly incorporate microbial consortia, including bacteria and fungi. Until now, no study has specifically characterized bioaerosol emissions of biofilters inoculated with such a consortium. Thus, the overall air microbial emissions, such as of bacterial and fungal spores, or even biological cell fragments, should be characterized from biofilters to develop complete technologies, not only for the abatement of VOCs but also to remove the bioaerosol emitted.

Several studies from 1992 until the present have quantified bioaerosols in ambient air, indoors, work places, schools, animal farms and wastewater treatment plants because of the negative health effect of bioaerosols on humans (Sanchez-Monedero et al., 2003; Chen and Li, 2005; Tsai and Macher, 2005; Chi and Li, 2007; Ghosh et al., 2013; Perrino and Marcovecchio, 2016; Szyłak-Szydłowski et al., 2016). Culture-based methods are the main method used to quantify bioaerosols, and to a lesser extent, non-culture methods, such as epifluorescence microscopy (EM), flow cytometry, biochemical or immunological assays and PCR techniques, are used (Lange et al., 1997; Duchaine et al., 1999; Rinsoz et al., 2008; Ghosh et al., 2013, 2015; Perrino and Marcovecchio, 2016). Additionally, the use of culture-based techniques drastically underestimates the concentration of microorganisms in bioaerosol emissions as only approximately 17% of the known fungal spores and approximately 1% of bacteria can be grown in culture media (Bridge and Spooner, 2001; Chi and Li, 2007). Epifluorescence microscopy coupled with fluorochromes is able to characterize bioaerosols (Chi and Li, 2007; Rule et al., 2007; Rinsoz et al., 2008; Perrino and Marcovecchio, 2016). However, few studies have been published on this issue. Chi and Li (2007) used an EM technique to characterize bioaerosols at an ambient air station in Taiwan. They reported bioaerosol concentrations between 100 and 1000 times higher than those obtained by culture-based methods. Rinsoz et al. (2008) sampled and quantified bioaerosols in a waste water treatment plant using EM, classical culture methods and PCR-based methods. They found a high correlation between PCR methods and EM techniques.

On the other hand, several methods for bioaerosol capture have been reported, i.e., liquid impingement is clearly superior to membrane filtration (Griffin et al., 2011; Haig et al., 2016). Therefore, to elucidate the capability and limitations of biofilters for bioaerosol retention and emission, EM techniques are able to adequately quantify bioaerosols. The objective of this study was to evaluate the use of epifluorescence microscopy as a non-culture-based method to quantify and characterize bioaerosols emitted by two biofilters for the treatment of toluene that were packed with a microbial consortium.

2. Material and methods

2.1. Inoculum and mineral salt medium (MSM)

Activated sludge from a waste water treatment plant in San Luis Potosí, Mexico, was used as the inoculum. MSM was composed of ($g\,L^{-1}$): $(NH_4)_2SO_4$, 0.5; KH_2PO_4, 0.7; K_2HPO_4, 0.7; $MgSO_4$-$7H_2O$, 0.3. The MSM was reconstituted with 200 μL of a trace element solution of ($g.L^{-1}$): FeSO4·7H2O, 0.015; MnSO4·H2O, 0.012; ZnSO4·7H2O, 0.013; CuSO4·7H2O, 0.0023; CoCl2·6H2O, 0.0015; and H3BO3, 0.0015 (Saucedo-Lucero et al., 2014). The pH was adjusted to 7 with a NaOH solution (1.0 N). MSM was added every day or every other day according to several stages of operation, as described in Table 1.

2.2. Biofilter set-up and operation

Two cylindrical glass biofilters with an effective volume of 2.2 L were used; each biofilter was divided in two identical modules (1.1 L) of 0.097 m in diameter and 0.450 m in height. One biofilter was packed with Tezontle particles (The Home Depot, México) with a diameter of 8 mm (BA) and the other with Perlite (Multiperl, ®Grupo Perlita de la Laguna, S. A. de C. V.) with an average diameter of 3.3 mm (BB). Each reactor was provided with longitudinal gas and biomass sampling ports. Both biofilters were operated in down flow mode for 238 days. An inlet load of 50 ± 5 g m^{-3} h^{-1} of toluene and an empty bed residence time of 72 s were used. Mineral medium was sprayed at the top of the biofilter with a flow rate of 10.6 mL min^{-1} for a period of 20 min. Several stages of operation were attained while varying the periodicity of MSM addition and applying shutdown periods according to Table 1. Shutdown periods were applied and consistent in stopping the toluene feed; then, only air was added during this periods. Packing materials were well characterized in terms of their density, pH, bed void fraction and water retention capacity according to the methodologies previously reported by Arriaga and Revah (2009).

2.3. Bioaerosol sampling and analysis

Bioaerosol collection was performed by liquid impingement. A glass impactor, Impinger AGI-30 (Ace Glass, Inc., Vineland, USA), with an outlet that was connected to a vacuum pump (Thomas Scientific, No. DOA-P704A-AA), was operated at a flow rate of 12.5 L min^{-1} for 30 min. The vacuum flow of the pump was fixed with a valved acrylic rotameter (Coleparmer, International). The inlet of the impinger consisted of the outlet flow of the biofilter (2.5 L min^{-1}_{air}) and an ambient air line at 10 L min^{-1} provided with a 1.0 μm hydrophilic glass fibre filter (Merck Millipore), which eliminated the ambient bioaerosol. The outlet flow of the rotameter was checked with an infrared calibrator (Defender 510, Bios International). Sterile impingers were loaded with 20 mL of a filtered PBS solution prior to sampling. Bioaerosol was collected from the exit and entrance (control) of the biofilter air stream in triplicate (see Fig. 1). After collection, the samples were centrifuged two times at 13,300 rpm for 30 min, and the final volume of the sample

Table 1
Experimental stages of operation from biofilters treating toluene vapors.

Stages	Lapse time of operation, d		BA and BB	
			IL ($g\,m^{-3}h^{-1}$)	MSM periodicity
S1	0–60		50	Every other day
S2	60–161			Every day
S3	SP1-24 h	162–163	0	
		163–174	50	
	SP2-48 h	174–176	0	
		176–232	50	
	SP3-72 h	233–235	0	
		235–238	50	

S1, S2, S3: Stages of operation; SP1, SP2, SP3: Shutdown periods of 24, 48 and 72 h; BA: Biofilter packed with Tezontle; BB: Biofilter packed with Perlite; IL: Inlet Load; MSM: Mineral salt solution.