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Bacterial dynamics in steady-state biofilters: beyond functional stability

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Keywords

biofiltration; microbial community; dynamics; acclimatization; stability; stratification.

Abstract

The spatial and temporal dynamics of microbial community structure and function were surveyed in duplicated woodchip-biofilters operated under constant conditions for 231 days. The contaminated gaseous stream for treatment was representative of composting emissions, included ammonia, dimethyl disulfide and a mixture of five oxygenated volatile organic compounds. The community structure and diversity were investigated by denaturing gradient gel electrophoresis on 16S rRNA gene fragments. During the first 42 days, microbial acclimatization revealed the influence of operating conditions and contaminant loading on the biofiltration community structure and diversity, as well as the limited impact of inoculum compared to the greater persistence of the endogenous woodchip community. During long-term operation, a high and stable removal efficiency was maintained despite a highly dynamic microbial community, suggesting the probable functional redundancy of the community. Most of the contaminant removal occurred in the first compartment, near the gas inlet, where the microbial diversity was the highest. The stratification of the microbial structures along the filter bed was statistically correlated to the longitudinal distribution of environmental conditions (selective pressure imposed by contaminant concentrations) and function (contaminant elimination capacity), highlighting the central role of the bacterial community. The reproducibility of microbial succession in replicates suggests that the community changes were presumably driven by a deterministic process.

Introduction

Functional stability is one of the main concerns of bio-reactor design, especially when the system is operated under full-scale transient conditions. The investigation of potential links between environmental fluctuations and microbial community structure is one of the most challenging issues in natural and engineered environments (Briones & Raskin, 2003). An essential prerequisite before exploring the transient state is the thorough characterization of system stability under controlled and stable operating conditions, in terms of both functional capacity and microbial community structure. A better understanding of interactions between environmental, functional and microbial components may contribute to

improve system stability by providing diagnostic, control and predictive tools for reactor operation (Briones & Raskin, 2003). The majority of bibliographic data questioning microbial stability in bioreactors refer to wastewater treatment systems, in which the overwhelming uncultivable fraction of microbial diversity could be accessed thanks to the development of molecular tools (Daims *et al.*, 2006). According to the operating specificities of the studied systems, the outcome of microbial dynamics was very disparate and somewhat obscured by the heterogeneity of stability definitions, index calculations and methodological tools available to study microbial communities (Yue *et al.*, 2005). Highly dynamic microbial communities can sustain stable function (Fernandez *et al.*, 1999), whereas in other cases functional

stability is related to stable microbial communities (Genile *et al.*, 2006). Nevertheless, the microbial ecology approach is rarely used in the context of polluted gas biofiltration. Contrary to other engineered ecosystems, the question of discordant structural and functional stability has never been addressed, nor, to our knowledge, the stability in duplicated gas biofilters (BF) using a long-term systematic approach such as the one we present here.

Biofilters are packed-bed reactors used for biological waste gas purification particularly adapted to the treatment of sludge composting emissions (Iranpour *et al.*, 2005). Gaseous contaminants are removed from the gaseous stream by a combination of physicochemical mass transfer and microbial oxidation within the biofilm which develops at the outer surface of the packing material (Aizpuru *et al.*, 2001). Once they are transferred from the gas phase to the biofilm, the contaminants are used as carbon and/or energy source by a complex and diverse microbial community. Biofilters can be considered model ecosystems where inputs and outputs are controlled and monitored. Hence they are relevant tools to approach ecological questions such as the relationship between diversity and functioning. However, many studies suffer from lack of replicates because of the complexity of pilot-scale BF implementation. Moreover, before molecular characterization of microbial communities, a cautious evaluation and optimization must be made of the various methodological steps, such as DNA extraction (Cabrol *et al.*, 2010) or PCR amplification (Von Wintzingerode *et al.*, 1997).

The acclimatization of microbial communities to the bioreactor environment is a critical period which influences the long-term functioning of bioreactors. As such, it has been extensively studied in waste gas and wastewater treatment systems (Tresse *et al.*, 2002). Acclimatization is usually extremely dynamic, as it corresponds to the selection of the most fitted populations emerging from the packing material itself (when organic) as well as from the inoculum. Determining the influence of the origins of the different microbial elements is thus a crucial first step in prospecting the subsequent characterization of spatial and temporal microbial dynamics.

In this context, our objectives were twofold: (i) to determine the origin of the biofiltration community and the factors which drove its acclimatization to the BF, and (ii) to investigate the links between the community structure and the macroscopic function in pilot-scale BF applied in the treatment of a gaseous mixture representative of composting emissions, under long-term stable operating conditions.

For the first objective, our hypothesis was that the structure of the BF community could be influenced by

four factors, namely the endogenous community on the woodchips, the inoculum, the growing conditions within the reactor (e.g. the attachment on an organic carrier, the hydrodynamics of gas and water flows/streams) and the contaminant feeding. We therefore assessed the contribution of each factor, and combination of factors, in shaping community structure during the acclimatization period.

For the second objective, our hypothesis was that, from a spatial point of view, the microbial distribution might reflect the functional stratification which is a characteristic feature of such biofiltration system, whereas, from a temporal point of view, we assumed that the total microbial community could vary independently of the macroscopic parameters.

To test these assumptions, we surveyed the spatial and temporal dynamics of BF functioning and microbial community structure during the acclimatization and during long-term steady state in duplicated experimental BF treating composting emissions and maintained under stable conditions. Functional performance and community structure were regularly monitored at different dates and heights by gaseous contaminant removal and denaturing gradient gel electrophoresis (DGGE) profiles, respectively.

Materials and methods

Inlet stream generation

The BF were fed continuously with a synthetic gaseous stream qualitatively and quantitatively representative of sludge composting emissions, whose composition was chosen on the basis of both a literature survey and field measurements in several industrial composting plants treating mixtures of municipal sludge and yard wastes. Taking into account the laboratory feasibility for dynamic generation (in terms of reactions, volatilization and detection constraints), the gaseous mixture was composed of ammonia and six volatile organic compounds (VOCs): butyraldehyde, acetone, methyl ethyl ketone (MEK), methyl butanoate, methyl pentanoate and dimethyl disulfide (DMDS).

To generate the gaseous mixture, the liquid VOCs (> 99% pure; VWR, West Chester, PA) were mixed in isomass proportions, volatilized in a pure air stream via a metering pump (Mikro g/5; Prominent, Heidelberg, Germany) and ammonia was added (purity class 3.8; Messer France S.A.S., Puteaux, France). The gaseous stream was homogenized, further diluted with purified air, adjusted and regulated by mass flow controllers (Thermic mass flow controllers; Brooks Instrument, Hatfield, PA), to reach the desired load.

Inlet stream load

The air flow across the biofilters was set up at 30 L min⁻¹, which corresponds to a superficial gas velocity of 100 m h⁻¹ (representative of full-scale BF operation) and an empty bed retention time (EBRT) of 54 s. Each VOC concentration was on average 4.6 ± 0.5 mg m⁻³, and ammonia concentration was about 20.5 ± 2 mg m⁻³, which corresponds to an organic load of 1.1 ± 0.1 g_C m⁻³ h⁻¹ and a nitrogen load of 1.2 ± 0.1 g_N m⁻³ h⁻¹. The generation system allowed stable and reproducible concentrations to be maintained during the whole 231-day experiment, with relative standard deviations ranging from 10% to 12% on time, depending on compounds.

Pilot-scale biofiltration unit

Biofilters set-up

Two identical polyvinyl chloride (PVC) biofiltration columns BF1 and BF2 (15 cm internal diameter, 230 cm total length) were operated in parallel in downward flow mode, at ambient temperature, for 231 days. Due to the size of the reactors, the complexity of the experimental device and the operation costs, only two replicates could be implemented. At the top, a 30-cm head space was designed to house the humidification system and to homogenize the gas velocities before entering the filter. Each BF was filled with packing material up to a total bed height of 150 cm, corresponding to a packing volume of 26.5 L, divided into five compartments 30 cm in height.

Sampling strategy

Gas-sampling ports were located in the headspaces at the inlet and outlet of each compartment, 0, 30, 60, 90, 120 and 150 cm from the inlet, respectively. This enabled the functional performance of each of the five compartments to be assessed. Due to cost and time reasons, we selected four compartments for biofilm analysis on the basis of functional results. The medium-sampling ports were located at the middle of the four selected compartments (15, 45, 75 and 135 cm from the gas inlet), at the center of the column section.

To evaluate both short- and long-term community dynamics, biomass was sampled with different frequencies. In the first compartment (15 cm from gas inlet), biomass was sampled at days 42, 47, 58, 64, 90, 97, 121, 127, 173 and 231. Biomass was also sampled at deeper BF levels, 45, 75 and 135 cm from gas inlet, with a lower frequency. On the basis of functional results, biomass was not sampled at days 121, 127 and 173 in these lower compartments.

Packing material

The organic carrier was composed of pine bark woodchips screened to a size of 6–16 mm. Its physical, chemical and hydrodynamic properties have been published elsewhere (Cabrol *et al.*, 2009). It was inoculated by recirculation of supernatant from activated sludge collected at a municipal wastewater treatment plant during the first day of operation. The packing material was periodically sprayed by a nutrient solution (15 min every 2 h, corresponding to an inflow of 2.5 ± 1 L day⁻¹) to maintain constant high moisture content and to provide essential nutrients for microbial growth (Khammar *et al.*, 2005). The liquid medium did not provide any carbon or nitrogen, the only available C and N sources being fed by the gas phase.

Functional characterization

Gas phase analysis

At the inlet and outlet of each BF compartment, VOCs were detected daily and quantified on-line with a gas chromatograph (Trace; Thermo Fisher Scientific Inc., Waltham, MA) equipped with a polar capillary column (Nukol Supelco, 30 m, 0.25 mm internal diameter, 0.25 µm film thickness; Sigma-Aldrich, St. Louis, MO) and a flame ionization detector. Ammonia was analyzed daily on-line by a non-dispersive infra-red sensor (MIR 9000; Environnement S.A., Poissy, France) equipped with a heated transfer line.

Functional performance

Based on the contaminant concentrations (C) at the different sampling levels, the functional performance was defined as removal efficiency (RE), calculated as follows, for the compound *i*, at biofilter height *h*:

$$RE_{h,i} = \frac{C_{i,\text{inlet}} - C_{i,h}}{C_{i,\text{inlet}}}$$

Microbial community investigation

DNA recovery

DNA was recovered from the biofilm on the woodchips, according to a previously optimized protocol (Cabrol *et al.*, 2010). Briefly, cells were dislodged from the carrier by vigorous shaking in physiological buffer, and DNA was extracted using a commercial extraction kit (Fast-DNA SPIN Kit for Soil; MP Biomedicals, Irvine, CA). DNA was quantified by absorbance at 260 nm (Biophotometer; Eppendorf, Hamburg, Germany).

Polymerase chain reaction

The V3 region of the eubacterial 16S rRNA gene was PCR amplified using a previously described protocol (Cabrol *et al.*, 2010). PCR products were quantified by absorbance at 535 nm after PicoGreen staining (Quant-iT ds DNA HS reagent; Invitrogen, Carlsbad, CA).

Denaturing gradient gel electrophoresis

DGGE was performed with Ingeny phor U-2 system (Goes, The Netherlands) according to the protocol of Muyzer *et al.* (1993), with a denaturing gradient ranging from 43% to 63% as previously described (Cabrol *et al.*, 2010), and 500 ng per sample was loaded onto the gel. Gel images were analyzed with BIONUMERICS software (Applied Maths, Ghent, Belgium).

Numerical analysis

The cumulative proportion of relative band intensity (ranked in decreasing order) was plotted against the cumulative proportion of species richness (relative band number), which is known as the Lorenz–Pareto curve. From this representation, the evenness degree of the community was assessed by the Gini coefficient (normalized area between the Lorenz curve and the diagonal line describing a perfect distribution equity) and y intercept at 0.2, $y_{0.2}$ (contribution to abundance of the first 20%-ranked species) (Marzorati *et al.*, 2008; Wittebolle *et al.*, 2008) (see Supporting Information).

The diversity was measured by the Shannon index H' , which took into account both the number of DGGE bands and their relative intensity:

$$H' = - \sum_i^n p_i \log(p_i)$$

where p_i is the relative abundance of the i^{th} band of the profile.

After exclusion of the rarest bands (i.e. < 3% intensity in all samples) the initial data matrix (relative intensities according to position) were standardized and square root-transformed to downweight the influence of more abundant species masking shifts among less abundant species (Crouzet *et al.*, 2010). The pairwise similarity index $S_{i,j}$ between community profiles i and j was calculated by the Bray–Curtis coefficient:

$$S_{i,j} = 100 \cdot \left(1 - \frac{\sum_{k=1}^N |p_{i,k} - p_{j,k}|}{p_{i,k} + p_{j,k}} \right)$$

where $p_{i,k}$ is the relative abundance of the k^{th} band in the profile i , and $p_{j,k}$ is the relative abundance of the k^{th} band

in the profile j , in the transformed matrix. The dissimilarity matrix (given by $100 - S_{ij}$) was used to perform non-metric multidimensional scaling (nMDS) and hierarchical clustering with UPGMA linking, using MATLAB[®] software (The Mathworks, Natick, MA). One-way ANOSIM (analysis of similarity) was carried out with PRIMER software (PRIMER-E Ltd, Plymouth, UK) to examine the statistical significance of the effects of space and time on the bacterial community structure (significance data reported as P -values). We tested the correlation between the bacterial community structure and two environmental variables: contaminant inlet concentration (IC) and contaminant elimination capacity (EC). First, this correlation was visualized by a ‘bubble’ representation on an MDS map, by allocating to each sample position a symbol whose size was proportional to the value of the environmental variable considered (IC or EC), calculated at the corresponding date and height. Then, Spearman correlation coefficient and the associated P significance level were computed with PRIMER software (permutation test with 999 permutations) between (i) the rank dissimilarity matrix from the genetic structure (Bray–Curtis coefficient) and (ii) the rank dissimilarity matrix generated from each of the environmental variables (using Euclidean distance on log-transformed data) (Clarke & Ainsworth, 1993).

Methodological validation

The reproducibility of the whole methodology, from the sampling step to the DGGE pattern analysis, was evaluated on a BF woodchip sample which was further divided into eight replicated subsamples, each independently processed. The community structure was $93\% \pm 2\%$ similar (Bray–Curtis) between all pairwise comparisons of the eight replicates, thus validating the methodology. Statistical treatment of multivariate data by nMDS with the Bray–Curtis similarity index is recommended as that most adapted to abundance data (Clarke & Ainsworth, 1993). When we tested a different similarity index (based on presence/absence data) and different ordination methods (PCA, clustering, nMDS) for the statistical treatment, the global grouping of samples was conserved, indicating the robustness of our statistical treatment (data not shown).

Acclimatization study

The acclimatization was defined as the period necessary to reach and maintain high and stable performance for several weeks. High functional efficiency was obtained as soon as 10–26 days (see Results) and we waited until day 42 to study the acclimatization of the microbial community, in order to ascertain the functional steady-state at the time of first biomass sampling.

Initial communities

The endogenous microbial community on the packing material was analyzed on duplicated woodchip samples W1 and W2 before inoculation. The inoculating community produced by the sludge inoculum was analyzed on duplicated sludge samples S1 and S2.

Negative controls

During the first 42 days, two supplementary biofilters, NC1 and NC2, were added as negative controls. They were run under the same operating conditions as the biofilters (in terms of inoculum, hydrodynamics and nutrients) but fed with pure air without contaminants.

Community analysis

On day 42, woodchips were sampled from NC1 and NC2 at two different levels (10% and 50% of bed height) and from BF1 and BF2 at three different levels (20%, 40% and 60% of bed height). For the four sample types, DNA extraction, PCR amplification and DGGE analysis were performed as presented earlier, except that the denaturing gradient was broader (30–60%) to adapt to the higher diversity in sludge and initial woodchip samples.

Results

Biofiltration efficiency

The removal profiles being similar for the five oxygenated compounds (acetone, MEK, butyraldehyde, methyl butanoate and methyl pentanoate), they will be referred to as the ‘oxygenated’ family without distinction of the individual compounds.

The efficiency profile during start-up was similar for both duplicates. In the most active section, i.e. in the first 30 cm of bed height, a high and stable efficiency level was reached after 5–10 days for ammonia and oxygenated compounds, and after 26 days for DMDS removal.

After day 26, the long-term efficiency was slightly different in the two duplicates. BF2 maintained stable maximal performance during the whole experiment (Table 1), with 99% removal of oxygenated compounds within the first 30 cm of filter, and 90% removal of DMDS after 60 cm of filter. Ammonia removal first required two filter sections and then improved to the same level as oxygenated compounds. BF1 experienced two different stable periods. Until day 85, BF1 was less efficient than BF2, as 60 and 90 cm of filter bed were required to reach nearly-complete removal of, respectively, oxygenated compounds and DMDS (Table 1, period I). Nevertheless, after day 89, most of the degradation activity migrated towards the first section, with high and stable removal efficiencies in the first 30 cm until the end of the experiment, similar to those of BF2 for all compounds (Table 1, period II).

Acclimatization of the microbial community

The communities of initial sludge (S) and woodchips (W) were compared with the communities of negative controls (NC) and biofilters (BF) at day 42 (i.e. after 2 weeks of functional steady-state) by univariate and multivariate analysis of DGGE patterns. The duplicated samples exhibited good reproducibility (see SD in Table 2).

Only the dominant species are addressed by DGGE. Bearing this in mind, the species richness and Shannon diversity index were found to be statistically similar in the inoculum sludge, the initial woodchips and the NC samples, but were significantly lower in the BF samples (Table 2), revealing a selection towards a reduced number

Table 1. RE of oxygenated compounds (acetone, MEK, butyraldehyde, methyl butanoate and methyl pentanoate), DMDS and ammonia at four depth levels in biofilters BF1 and BF2. RE values were measured daily and averaged by stable periods: Period I (until day 85), Period II (from day 89 until the end) or the whole experiment. Standard deviations are indicated in brackets

Pilot – distance from gas inlet	% RE (SD)					
	5 oxygenated VOCs		DMDS		Ammonia	
	Period I	Period II	Period I	Period II	Period I	Period II
BF1 – 30 cm	76 (12)	98 (2)	37 (13)	66 (10)	52 (10)	90 (7)
BF1 – 60 cm	90 (5)	100	63 (11)	88 (15)	80 (10)	98 (2)
BF1 – 90 cm	100	–	94 (8)	94 (8)	100	100
BF1 – 150 cm	–	–	95 (6)	95 (6)	–	–
	Whole period		Whole period		Period I	Period II
BF2 – 30 cm	99 (3)		68 (10)		64 (14)	95 (6)
BF2 – 60 cm	100		90 (14)		97 (4)	97 (4)
BF2 – 90 cm	–		91 (10)		99 (1)	99 (1)
BF2 – 150 cm	–		96 (6)		100	100

Table 2. Species richness (number of bands) and Shannon diversity index, in sludge inoculum (S), initial packing material (W), NC and biofilter samples (BF). The values for S and W were averaged between duplicates. The values for BF and NC were averaged between duplicates and sampling levels (20%, 40% and 60% of bed height from gas inlet for BF; 10% and 50% for NC). Standard deviations are given between brackets. The second part of the table represents the *P*-values obtained by Student *t*-tests when comparing the Shannon diversity index between pairwise samples. The same statistical trends were obtained for richness values (data not shown)

	S (<i>n</i> = 2)	W (<i>n</i> = 2)	NC (<i>n</i> = 2*2)	BF (<i>n</i> = 2*3)
Species richness	31 (0)	29 (1)	23.8 (4.5)	16.5 (3.3)
Shannon diversity index H	4.6 (0.1)	4.5 (0)	4.1 (0.3)	3.7 (0.3)
Statistical analysis for H (Student test <i>P</i> -values)				
S (<i>n</i> = 2)	–			
W (<i>n</i> = 2)	0.14	–		
NC (<i>n</i> = 2*2)	0.11*	0.25*	0.54 [†]	
BF (<i>n</i> = 2*3)	0.0032*	0.0082*	0.041*	> 0.81 [†]

Significant differences at 5% risk are given in bold type.

*For these pairwise comparisons, the diversity at the different heights was averaged for NC and BF.

[†]Pairwise comparisons between the different height combinations.

of species under contaminant load. As depicted by the Gini coefficient and γ -intercept at 0.2 calculated from Pareto–Lorenz representations (Supporting Information), the degree of evenness of species distribution was conserved in all samples, even after 42 days' exposure to contaminants, indicating that the species loss affected both dominant and minority species (within the community fraction detected by DGGE). The communities were characterized by a rather balanced distribution of species, where the 20% most fitting species dominated at elevated abundance (representing 45% of total band intensity), whereas the remaining 80% species were still available at low abundance.

Apart from the richness and diversity, there was a clear group effect between S, W, NC and BF in terms of species composition and relative abundances, as illustrated by clustering (Fig. 1) and statistically confirmed by ANOSIM (*P*-value 0.1%). The NC samples and the BF samples displayed two homogeneous community structures, with

63% \pm 6% and 63% \pm 8% similarity, respectively. The sludge inoculum constituted the most distinct group, with only 29% \pm 7% similarity with NC and 24% \pm 4% similarity with BF group. The woodchip endogenous community had only a 40% \pm 4% and 30% \pm 5% similarity with NC and BF communities, respectively. After 42 days, there was only a 38% \pm 5% similarity between communities fed with (BF) and without (NC) contaminants (Fig. 1).

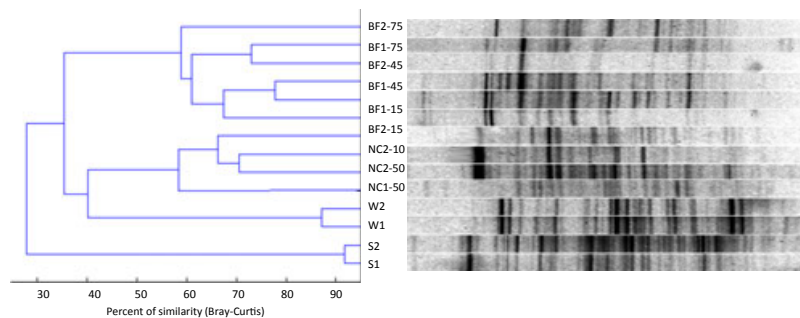
Microbial diversity in biofilters

The effects of time and height – and their interaction – on microbial diversity (Shannon index) were tested by a two-way ANOVA, using BF1 and BF2 as replicates. There was no significant effect of sampling time on biodiversity at any stage (*P* = 0.003). However, there was a significant height effect (Student *t*-test *P* = 0.006) due to a higher diversity in the inlet compartment (0–30 cm, richness 40 \pm 7 bands, H' (Shannon index) 4.99 \pm 0.02), compared to the lower compartments, where the diversity was statistically homogeneous (30–150 cm, richness 27 \pm 7 bands, H' 4.37 \pm 0.07). The estimation of the Shannon index encompasses dual information about microbial richness, on the one hand, and community evenness, on the other hand. All the observations made for the Shannon index were also statistically verified for richness. However, the evenness (Gini coefficient and $\gamma_{0.2}$ -intercept) was not influenced by time or height effects, as verified by ANOVA with BF1 and BF2 as duplicates (Supporting Information). So it can be assumed that the height effect detected on microbial diversity mostly affected the microbial richness rather than the community evenness. The BF effect, assessed by considering the different sampling times as replicates, was not significant: the microbial diversity and its stratification were constant and reproducible in duplicated BF.

Temporal dynamics of the inlet microbial community

The structures of the communities from the 15-cm samples were analyzed by UPGMA clustering and nMDS

Fig. 1. UPGMA clustering of DGGE patterns based on Bray–Curtis similarities. S1, S2: Duplicated samples from inoculum sludge. W1, W2: Duplicated samples from initial woodchips. NC1, NC2: Samples from duplicated negative controls at day 42. BF1, BF2: Samples from duplicated biofilters at day 42. The number next to the pilot name indicates the sampling depth in cm from the gas inlet.



(Supporting Information), with both giving similar trends. Both BF communities stayed in relative proximity to each other until day 173 and the ANOSIM confirmed there was no significant difference between replicate structures ($P \gg 0.05$). The samples clustered more according to time than to pilot identity.

Within each BF community, there was a strong time effect confirmed by ANOSIM ($P = 0.001$). The microbial community at 15 cm was dynamic during the whole experiment, without convergence towards a climax community. The microbial structure shifts led to new structures, still similar between BF1 and BF2 (Supporting Information). The dissimilarity between successive sampling dates was plotted against time according to the moving window approach (Fig. 2) and ranged from 19% to 42% ($30\% \pm 8\%$ on average in BF1 and $29\% \pm 7\%$ in BF2). The dissimilarity level was in the same range between near sampling dates (1-week interval) and between more distant dates (more than 8-week interval). The magnitude of temporal changes did not depend on the time elapsed between two consecutive sampling dates. The divergence between consecutive dates tended to be accentuated slightly with time, up to the end of the experiment in BF2 and up to day 173 in BF1 (Fig. 2).

Spatial distribution of microbial communities along the biofilter height

All the microbial structures originating from the first compartment (15 cm) clustered together, clearly more tightly and further apart from lower stages, in BF1 as in BF2 (Fig. 3a). This height effect was confirmed by ANOSIM ($P = 0.001$): the pairwise comparisons between all

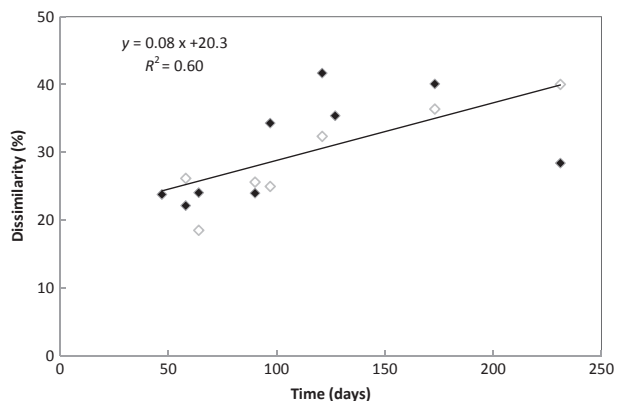


Fig. 2. Moving window analysis based on DGGE patterns for BF1 (◆) and BF2 (◇). Each point represents the dissimilarity between communities sampled at consecutive dates at the inlet stage (15 cm), based on the Bray–Curtis index. The dotted line represents the dissimilarity between duplicates (BF1 and BF2) along the time course. The solid line corresponds to the linear regression obtained from BF1–BF2 means.

compartments revealed that the community structure in the first compartment (15 cm) was the most significantly different from all others (mean R statistic 0.65), whereas intermediate- and low-compartment communities (45–135 cm) were not statistically different (R statistics $\ll 0.5$).

Between 45 and 135 cm the samples were not discriminated by the filter height but clustered more or less by sampling date (47- and 58-day samples separated from 64 to 97 days in BF1; 42- to 64-day samples separated from 64 to 231 days in BF2), indicating the temporal succession. Globally, in both replicates, the inlet community (15 cm) exhibited less temporal dynamics than the lower stages communities, where the samples were more scattered and divergent (Fig. 3b). Moreover, the reproducibility of community structures between replicates was significantly higher ($P = 0.0002$) in the inlet stage ($74\% \pm 6\%$ similarity) than down the filters ($58\% \pm 9\%$ similarity).

As clearly visualized by the bubble plots on nMDS representation, the segregation of inlet community structures according to time and height was correlated to two macroscopic parameters calculated at the corresponding date and height: (i) the selective pressure imposed by contaminant IC and (ii) the global contaminant EC. The bubble plot is only shown for the global contaminant EC (Fig. 3b) but the same trend was observed for contaminant IC (Supporting Information). This ‘visual’ correlation was further confirmed by the Spearman permutation test: in BF1, P -values were 0.003 and 0.05 for the correlations of genetic structure with IC and EC, respectively; in BF2, P -values were 0.005 and 0.03 for the correlations of genetic structure with IC and EC, respectively.

Discussion

In the present study, we used DGGE to survey the spatial and temporal dynamics of the biofiltration community. Several studies showed that the DGGE detection limit (impairing the detection of minority species), the multiple operon sequences (resulting in multiple bands for a single species) and the co-migration (resulting in an artifactual unique band for several different species) (Muyzer *et al.*, 1993; Muyzer & Smalla, 1998) prevent DGGE from correctly estimating the absolute diversity. Nevertheless, DGGE is a good tool to compare microbial communities between several samples and thus its use complies with our objective, which was to characterize globally the spatial and temporal dynamics of community structure, and not to identify particular species or to access absolute diversity.

Acclimatization study

The operating conditions imposed in NC (e.g. continuous air stream, biofilm-mode development, inoculation) were

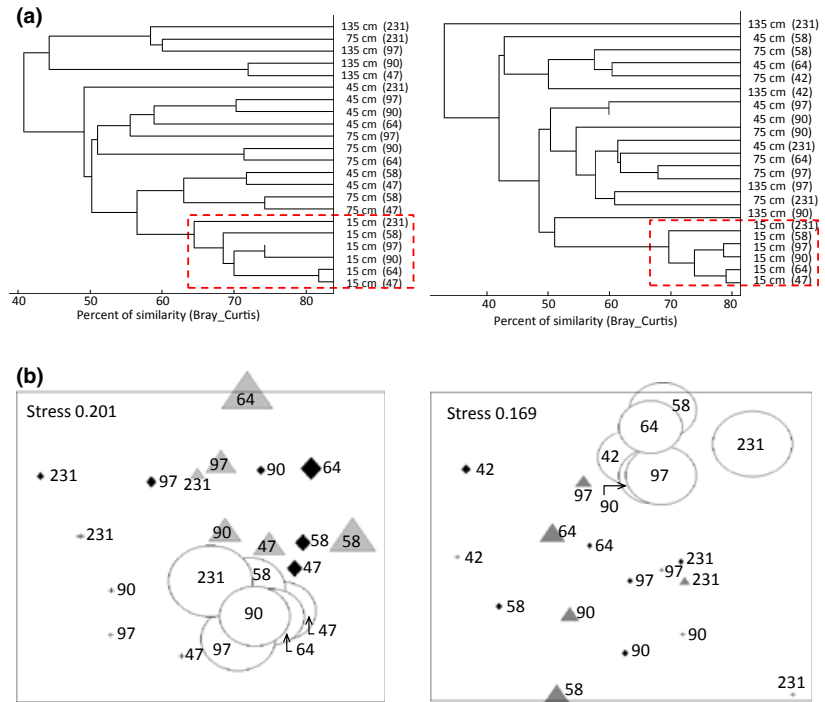


Fig. 3. Spatial and temporal patterns of microbial succession in BF1 (left) and BF2 (right), based on DGGE analysis. (a) UPGMA clustering based on Bray–Curtis similarities, according to biofilter depth (in cm from gas inlet) and sampling day (between brackets); the x-axis represents the similarity. (b) nMDS ordination of DGGE patterns at 15 cm (◆), 45 cm (◇), 75 cm (+) and 135 cm (▲) from the gas inlet. The symbol size is proportional to the global contaminant elimination capacity calculated at the corresponding date and height, according to a bubble representation.

not sufficient to reduce the initial woodchip diversity (Table 2). Nevertheless, despite the absence of contaminants, they induced substantial modifications of the endogenous community structure (Fig. 1), similar to previous works (Pineda *et al.*, 2004; Sei *et al.*, 2004). The NC community was mostly influenced by the endogenous woodchip community.

By contrast, the continuous 42-day contaminant feeding led to a selection of the most fitted species in BF, suggesting community specialization, competitive exclusion, toxic effect of the contaminants and/or reduction of resource variety (Table 2). The diversity decrease during acclimatization is often reported (Stoffels *et al.*, 1998; Steele *et al.*, 2005; Sercu *et al.*, 2006; Ding *et al.*, 2008). In the present case, the BF community was mostly influenced by the contaminant loading, whereas the inoculation had only a limited impact compared to the endogenous community (Fig. 1). As previously reported (Stoffels *et al.*, 1998; Dollhopf *et al.*, 2001; Tresse *et al.*, 2002), the inoculum structure may not be as decisive as the operating conditions in shaping the community which subsequently develops in the biofilters, provided that the inoculation and/or the organic packing material bring enough diversity to allow the emergence of adapted species. The dynamics during start-up and divergence from the inoculum have been reported frequently (Steele *et al.*, 2005; Borin *et al.*, 2006; Sercu *et al.*, 2006), even when the inoculum had

previously been acclimatized to the contaminants (Stoffels *et al.*, 1998; Tresse *et al.*, 2002; Falk *et al.*, 2009). This dynamics during start-up can be attributed to drastic changes between inoculum and BF environmental conditions (Kaewpipat & Grady, 2002), mainly in terms of growth conditions (attached vs. planktonic biomass) and substrate availability (Tresse *et al.*, 2002; Miura *et al.*, 2007; Falk *et al.*, 2009).

Temporal dynamics in the active section

We focused on the first compartment (0–30 cm), where most of the degradation activity occurred (Table 1).

Functional stability

Both BF maintained a high and stable removal performance (Table 1). However, the relatively lower efficiency in BF1 during the first period could be linked to difficulties in maintaining a high and stable moisture content in the inlet compartment. During this period, the moisture content within the first section of BF1 was $51\% \pm 17\%$ (w/w), whereas it was $67\% \pm 5\%$ (w/w) at the same level in BF2, probably because of differences in packing organization and channeling. The crucial importance of moisture content on transfer efficiency and microbial activity has already been shown (Auria *et al.*, 1998; Sun *et al.*, 2002).

High and stable microbial diversity

The BF maintained a stable extent of diversity (richness, evenness and Shannon index) throughout the experiment, in accordance with most reports for wastewater treatment systems (Nadarajah *et al.*, 2007; Falk *et al.*, 2009; Ayarza *et al.*, 2010). Although the DGGE is not an exhaustive technique, the crowded profiles suggest a high level of diversity. First, this high diversity – even in the compartments without gaseous contaminants – may result from the proliferation of saprophytes using products from cell lysis, the extracellular polymeric substances (EPS) matrix or packing material (Stoffels *et al.*, 1998; Borin *et al.*, 2006). In some BF, saprophytes dominate (Juteau *et al.*, 1999; Song & Kinney, 2000; Hekmat *et al.*, 2004), whereas the active primary-degraders can represent only 4% of the community (Møller *et al.*, 1996). Secondly, the high diversity can be interpreted as the expression of species coexistence through niche differentiation (Fernandez *et al.*, 1999; Siripong & Rittmann, 2007). Apart from the variety of microhabitats found in biofilms (Lydmark *et al.*, 2007), the coexistence of different species may be favored by functional complementarity, resource partitioning or positive interactions (Massol-Deyá *et al.*, 1997; Loreau *et al.*, 2001).

Dynamic microbial community structure

The present work revealed that a stable community was not necessary to achieve and maintain high and stable functionality. The extent of microbial dynamics was much larger than the magnitude of functional oscillations (Fig. 2, Table 1). The structural shifts of population intensified at the end of the study, which was still the most functionally stable period. Since the key study of Fernandez *et al.* (1999), and despite the variety of definitions of stability, index calculations and methodological tools to study microbial communities, numerous works attested that stable function could be sustained by highly dynamic communities (Friedrich *et al.*, 2003; Stamper *et al.*, 2003; Graham *et al.*, 2007; Miura *et al.*, 2007; Nadarajah *et al.*, 2007; Wittebolle *et al.*, 2008; Ayarza *et al.*, 2010).

At first, structural oscillations can be explained by uncontrolled and undetected fluctuations of environmental conditions. Moreover, in the absence of disturbance, the complex network of microbial interactions creates the conditions for non-equilibrium dynamics (Fernandez *et al.*, 1999; Curtis *et al.*, 2003; Carrero-Colón *et al.*, 2006; Vandermeer, 2006; Graham *et al.*, 2007). The absence of correlation between RE and total community structure is also attributed to functional redundancy, with different species able to carry out the same function (Fernandez

et al., 1999; Briones & Raskin, 2003; Franklin & Mills, 2006; Ayarza *et al.*, 2010), especially in highly diverse ecosystems carrying out unspecialized function (Massol-Deyá *et al.*, 1997; Gentile *et al.*, 2006; Wittebolle *et al.*, 2009). The reproducibility of microbial succession in BF1 and BF2 duplicates tends to suggest that, in our BF, microbial dynamics was driven by deterministic rather than stochastic processes. On the basis of the DGGE profiles, the population dynamics can only be asserted for the major populations detected by this technique. However, due to the interaction network within the community, high dynamics is also assumed for the minor species.

Microbial and functional stratification along the biofilter height

Both biofilters exhibited the same stratification pattern of EC, with better and faster elimination of ammonia and oxygenated compounds compared to DMDS (Table 1). The sequential degradation may be due to (i) transfer limitation of poorly soluble sulfur compounds (Sheridan *et al.*, 2002; Chen *et al.*, 2008) or (ii) biodegradability limitation, competition and inhibition of hydrophobic compound metabolism by preferential hydrophilic substrates (Deshusses *et al.*, 1999; Aizpuru *et al.*, 2001; Khammar *et al.*, 2005).

From a microbial point of view, we highlighted the significantly higher diversity of the community near the gas inlet, suggesting that the constant availability of various resources provided conditions for the development of a more diverse microflora. The diversity–function debate being highly polemic (Naeem *et al.*, 2004), we cannot infer from this result a causal relation between high diversity and high RE. We also evidenced the stratification of the microbial structures along the filter bed (Fig. 3), statistically correlated to the longitudinal distribution of environmental conditions (contaminant concentrations) and function (EC). This highlights the central role of the bacterial community, whose composition was selected by the contaminant load, and which in turn influenced the macroscopic performance. Several works in waste gas and wastewater treatment reactors emphasized not only the higher diversity at the inlet sections (Calvo-Bado *et al.*, 2003; Rowan *et al.*, 2003; Roy *et al.*, 2009) but also the markedly different community structures in the biofilters in relation to macroscopic parameters (Li & Moe, 2004; Khammar *et al.*, 2005; Ding *et al.*, 2008; Roy *et al.*, 2009).

Eventually, as others have done for wastewater treatment reactors (Sercu *et al.*, 2006; Lozada *et al.*, 2007; Van der Gast *et al.*, 2008), we highlighted the stabilizing effect of the selective pressure imposed by constant contaminant loading in the upper stage, which tends to reduce

temporal and inter-replicate divergence. Another source of variability in the lower stages may be the fluctuations of the liquid phase percolating through the filter, whose composition and concentrations (resulting from the transfer and partial degradation of primary contaminants within the first stage) could not be measured in this study.

Conclusions

The pragmatic processing of DGGE profiles carried out in this work provides relevant information indicating some partial coupling between community structure and function in gas biofilters. The community which colonized the BF is completely different from the initial communities (endogenous and inoculum communities) but we can assume that the initial high diversity may have been a determinant for the selection of the most fitted community. By assessing the spatial and temporal variability of the community, we highlighted that the selective pressure imposed in the biofilters was sufficient to strongly influence the community diversity, composition and dynamics. This work suggested that a stable function could be maintained, notwithstanding constant community changes probably driven by deterministic process. The flexible community created by functional redundancy may act as insurance in case of perturbation: increasing the chance for resistant species to emerge from a pool of species with different characteristics, which may improve the system stability under transient conditions (Fernandez *et al.*, 2000; Loreau *et al.*, 2001). This intrinsic dynamic feature under stable operating conditions will have to be taken into consideration when assessing the impact of transient conditions on biofiltration ecosystems to avoid misinterpretations. Targeting the total community was a first approach to assessing the community dynamics: further research will not only target the total bacterial community (which encompasses inactive and dormant cells, as well as saprophytes) but also focus on active and functional populations actually involved in contaminant degradation, in order to indicate more significant correlations between structure and function of the ecosystem.

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Fig. S1. Evolution of microbial community structure with time, in the first compartment (15 cm from gas inlet) of biofilters BF1 (♦) and BF2 (◇).

Fig. S2. Evolution of microbial community structure with time, in the first compartment (15 cm from gas inlet) of biofilters BF1 and BF2, visualized by UPGMA clustering based on Bray–Curtis similarities.

Fig. S3. Spatial and temporal patterns of microbial succession in BF1 (left) and BF2 (right), based on DGGE analysis, visualized by nMDS ordination of DGGE patterns at 15 cm (♦), 45 cm (○), 75 cm (+) and 135 cm () from the gas inlet.

Fig. S4. Representation of the microbial community evenness thanks to the Lorenz–Pareto curves, during acclimatization (a) and in BF1 at the inlet stage according to time (b).