Available online at www.sciencedirect.com

SciVerse ScienceDirect

Procedia Engineering 42 (2012) 666 – 671

Procedia Engineering

www.elsevier.com/locate/procedia

20th International Congress of Chemical and Process Engineering CHISA 2012
25 – 29 August 2012, Prague, Czech Republic

Evolution of bacterial community in a full-scale biotrickling filter by fluorescence in situ hybridization (FISH)

M. C. Pérez, F. J. Álvarez-Hornos, P. San-Valero, C. Gabaldón and V. Martínez-Soria a*

Department of Chemical Engineering, University of Valencia, Av. De la Universidad s/n, Burjassot 46100, Spain.

Abstract

The performance of a full-scale biotrickling system for the treatment of exhaust gases from two different paint sources at a furniture facility, was investigated applying Fluorescense in situ hybridization (FISH). This technique allowed the detection of major bacteria groups and, therefore, helped in understanding complex microbial communities. The results indicated that *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria*, and *Deltaproteobacteria* were more predominant than *Firmicutes* and *Actiniobacterias*. In addition, a variation in the composition of the bacterial community throughout the time of operation and with the paint source was observed. *Betaproteobacteria* showed similar relative abundance in all analyzed days. However, *Gammaproteobacteri*a, relevant group in the degradation of VOCs, fluctuated with operational changes and the relative abundance of *Alphaproteobacteria* decreased when the composition of pollutants of the emission source was changed.

Keywords: Biotechnologies; biotrickling filter; FISH; volatile organic compounds; waste gas treatment

1. Introduction

Biotrickling filtration could be considered as a suitable and viable technology for controlling the industrial emissions of volatile organic compounds (VOCs) in waste gases. Biotrickling filters (BTFs) use

* Corresponding author. Tel.:+34 963543169; fax:+34 963544898.
E-mail address:Vicente.mtnez-soria@uv.es.

1877-7058 © 2012 Published by Elsevier Ltd.
doi:10.1016/j.proeng.2012.07.459

a specified inert packing material and involve a liquid phase, which trickles through the bed providing nutrients. The biofilm is developed on the packing surface. Removal efficiency in BFTs depends on multiple parameters, being the microbial community a key parameter in the performance of this process. In general, main efforts have been made to improve the feasibility and robustness of the bioprocess, however there are few studies focused on the characterization of the bacterial population in BTFs. For this porpuse, molecular biological techniques are useful tools to analyze microbial community structures during the removal of contaminants in biological processes. Fluorescence in situ hybridization (FISH) is currently a method used for the detection and quantification of the composition of microbial communities in natural and engineered ecosystems. FISH detects nucleic acid sequences by probes with fluorescent dyes that hybridize specifically to its complementary target sequence within the cell [1]. In fact, there are some studies in the field of biofiltration where FISH technique was used to analyze the bacterial community, for example Friedrich applied the FISH technique in a full-scale industrial biofilter resulting that members of the *Betaproteobacteria* followed by *Actinobacteria, Alphaproteobacteria, Cytophaga-Flavobacteria, Firmicutes* and *Gammaproteobacteria* were the most abundant groups and whereas the lower 50 cm of the biofilter proved to be the most active part for the degradation of aldehydes such as 2- and 3-methylbutanal, 2-methylpropanal, and hexanal [2].

In this study, the evolution during more than a year of the bacterial population in an industrial BFT treating two different VOC sources at a furniture facility was analyzed by using FISH.

2. Materials and methods

The full-scale biotrickling unit was supplied by Pure Air Solutions (The Netherlands) following its innovative abatement technology (VOCUS^{TM}) and it has been described by elsewhere [3]. Two different VOC discharge sources were selected, consecutively, as representatives of the diverse paint activities carried out in the industrial facility. In the first focus (A) tested, butyl acetates and xylenes were the major compounds and acetone was the dominant compound in the second focus (B). The plant was operated for more than a year at different air flow rates corresponding to empty bed residence times (EBRT) ranging from 20 to 100 s. During the first seventh months, including start-up period, the VOC removal in the first focus was studied. Afterwards, the BTF was connected to the second source during more than four months. Then, the first source was connected again. At 13th month industrial installation was closured associated to holyday period. During these different periods several biomass samples were taken from the bioreactor to analyze the microbial community.

The FISH technique was carried out adapting the procedure described by Amann et al.[4]. The procedure includes the following steps: sampling, fixation of the biofilm, hybridization with the respective probes for detecting the respective target sequences, washing, mounting, visualization and document of results. Biofilm was collected from full-scale biotrickling unit, the samples were homogenized and disaggregated with the Ultra-Turrax (IKA® T18 basic). Then, the samples were fixed to allow the penetration of the fluorescent probes into the cell. Gram negative cells were fixed with 4% paraformaldehyde and Gram positive cells with ethanol. Hybridization consists in annealing of the probe to the target sequence, performed at 46 ºC for 2 h. In this technique, was used a Cy5-labelled EUB_{mix} (general probe) and Cy3-labelled specific probes (Thermo Fisher Scientific, Germany). EUBmix consists of the mix of probes EUB338, EUB338II and EUB338III. Cy5 and Cy3 are fluorescent dyes, that is, fluorochromes with different excitation and maximum emission allowing simultaneous microscopic observation. Table 1 summarizes the oligonucleotide probes used in this study and the percentage of formamide used for each probe. Slides were examined by using a Leica TCS SP confocal laser scanning

microscope (CLSM, Leica Microsystems, Germany). This microscope is equipped with two He-Ne lasers with light emission at 543 and 633 nm, respectively. Five microscopic images were acquired for the negative control and 30 images for the sample with specific and general probes. Each captured image was formed with 2 pictures, one corresponding to EUBmix and other one to specific probe. Finally, specific probes were quantified as a proportion of EUB_{mix} labelled bacteria using image analysis based on the methodology developed by Jubany et al.[5].

Table 1. List of oligonucleotide probes used in this study

Probe	Specificity	Sequence (5'–3')	Formamide (%)
[a]EUB338	*Most Bacteria*	GCTGCCTCCCGTAGGAGT	0 – 50
[a]EUB338-II	*Other Bacteria not detected by EUB338*	GCAGCCACCCGTAGGTGT	0 – 50
[a]EUB338-III	*Other Bacteria not detected by EUB338*	GCTGCCACCCGTAGGTGT	0 – 50
ALF968	*α–Proteobacteria*	GGTAAGGTTCTGCGCGTT	20
BET42A	*β–Proteobacteria*	GCCTTCCCACTTCGTTT	35
-	*Competitor for BET42A*	GCCTTCCCACATCGTTT	35
GAM42A	*γ –Proteobacteria*	GCCTTCCCACATCGTTT	35
-	*Competitor for GAM42A*	GCCTTCCCACTTCGTTT	35
[b]DELTA495a	*Deltaproteobacteria*	AGTTAGCCGGTGCTTCCT	35
[b]DELTA495b	*Deltaproteobacteria*	AGTTAGCCGGCGCTTCCT	35
[b]DELTA495c	*Deltaproteobacteria*	AATTAGCCGGTGCTTCCT	35
-	*Competitor for DELTA495a*	AGTTAGCCGGTGCTTCTT	35
-	*Competitor for DELTA495b*	AGTTAGCCGGCGCTTCKT	35
-	*Competitor for DELTA495c*	AATTAGCCGGTGCTTCTT	35
HGC69A	*Actinobacteria (high G+C gram positive bacteria)*	TATAGTTACCACCGCCGT	25
-	*Competitor for HGC69A*	TATAGTTACGGCCGCCGT	25
[c]LGC354A[b]	*Firmicutes (low G+C gram positive bacteria)*	TGGAAGATTCCCTACTGC	35
[c]LGC354B[b]	*Firmicutes (low G+C gram positive bacteria)*	CGGAAGATTCCCTACTGC	35
[c]LGC354C[b]	*Firmicutes (low G+C gram positive bacteria)*	CCGAAGATTCCCTACTGC	35

a EUB338, EUB338-II, EUB338-III were used in the mixture called EUBmix
b DELTA495a, DELTA495b, DELTAc were used in the mixture called DELTA495mix
c LGC354A, LGC354B, LGC354C were used in the mixture called LGCmix

3. Results and discussion

The changes of the microbial population associated to different operational conditions were monitored by the FISH technique. In addition, FISH analysis of the inoculum (coming from a municipal WWTP) was also carried out. Figure 1 shows the relative abundance of the general groups of bacteria in the full-scale biotrickling filter; in particular, *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Actinobacteria* (high G+C Gram-positive bacteria) and *Firmicutes* (Low G+C gram-positive bacteria) are expressed as percentage of EUB338mix stained cells.

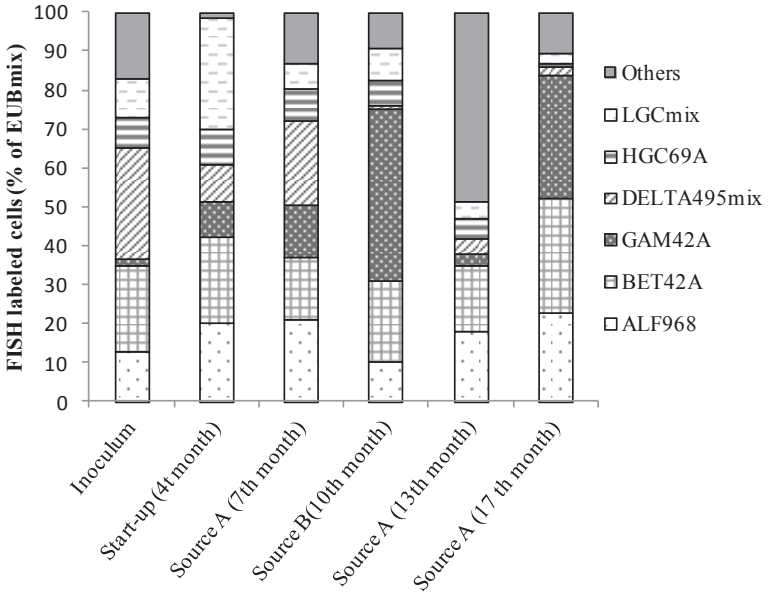

Fig. 1. Time-dependent changes in bacterial community composition using domain to class-specific FISH probes

It can be observed a variation in the composition of the bacterial community throughout the time of operation and with the source selected as well. In first place, a high relative abundance of bacteria was identified, with an average of 80%. This fact indicates that despite the complexity of the samples analyzed, the characterization of the microbial community was successful and, therefore, the selection of probes used in this study was suitable. The complexity of the samples is due to the high diversity of bacterial presenting into the biofilm caused by the complex mixture of volatile organic compounds hardly degradable in the emission source. In addition, changes in the bacterial community in comparison with the initial inoculum community were observed, depending on time but especially on the different VOC emission composition associated with the paint-solvent products used in each spray booth. Figure 1 shows as *Betaproteobacteria* is maintained at similar rates in all analyzed days with values ranging from 16% to 30%. The relative abundance of *Alphaproteobacteria* decreased when source B was connected, previous value of abundance, around 20%, was achieved when source A was selected again. *Gammaproteobacteri*a, relevant group in the degradation of VOCs, fluctuate with operational changes and with composition of pollutants of the emission source. For example, at 4th month, the abundance relative of *Gammaproteobacteria* was 10%, at 10th month 44% and at 17th month 31%.

Therefore, the change in the paint formulation did not only affect the pilot unit's performance but also affected the microbial population composition. In this sense, a sudden change of the relative abundance of *Gammaproteobacteria* and *Deltaproteobacteria* between the 7th and 10th month associated to the variation in the emission composition was observed. *Gammaproteobacteria* varied from 13% to 45% and *Deltaproteobacteria* from 22% to 6% when the source was changed. Finally, the shift in the population at 13th month could be related to the starvation period associated to holidays closure in the industrial installation. In 17th month of operation, after the holiday period a recovery of the bacteria population was observed. Both at 10th as at 17th month, a high relative abundance of *Gammaproteobacteria* was

identified, 44% at 10th month and 31% at 17th month. These percentages can be explained by ability of species belonging to *Gammaproteobacteria* group to degrade VOCs [5]. Figure 2 shows a high relative quantity of *Gammaproteobacteria* at 10th month respecting general probe EUB338mix. In the last place, in the full-scale biotrickling filter has been more abundant Gram negatives bacteria (*Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria)* compared to Gram positives bacteria (*Actinobacteria* and *Firmicutes*) in all days.

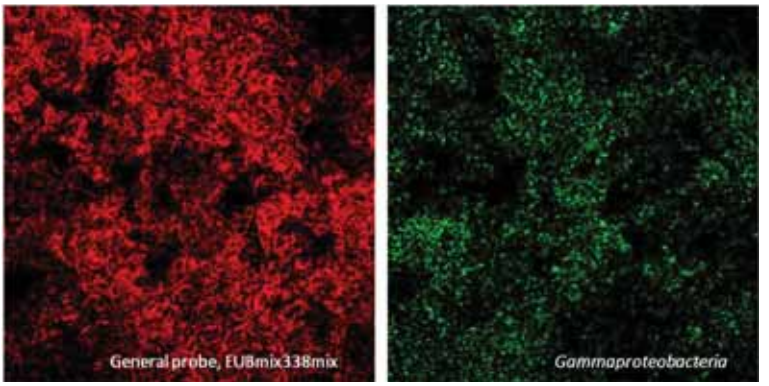

Fig. 2. *Gammaproteobacteria* at 10th month of operation. (a) General probe; (b) specific probe

4. Conclusions

In the present work, the FISH technique allowed the detection of the microbial populations existing in the full-scale biotrickling filter in the coating sector. Operational conditions were changed over the study period and therefore it reflects an evolution in the bacterial community in the process. A high abundance relative of bacteria was identified, an average of 80% and identifying reaching 98%. FISH analysis indicates that the microbial population composition depends on the solvent-paint applied in the booths, so the differences between source A and B were evident. The results obtained showed higher relative abundance of Gram negatives bacteria and relevant changes of *Gammaproteobacteria,* important microorganisms in the degradation of organic solvents.

Finally, it would be interesting to deepen into the microbiology of this process to identify the predominant bacteria species, in order to study the role which play in the VOC removal and in the robustness and effectiveness of the process.

Acknowledgements

The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under *REA* grant agreement nº 284949. Financial support from Ministerio de Ciencia e Innovación (Project CTM2010-15031/TECNO) and Generalitat Valenciana (ACOMP/2012/209), Spain, is also acknowledged. M.C.Pérez has a FPU grant from Ministerio de Educación, Cultura y Deporte, Spain.

References

[1] Moter A, Göbel UB. Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. *J Microbiol.Methods* 2007;**41(2)**:85-112.

[2] Friedrich U, Van Langenhove H, Altendorf K, Lipski A. Microbial community and physicochemical analysis of an industrial waste gas biofilter and design of 16S rRNA-targeting oligonucleotide probes (vol 5, pg 183, 2003). *Environ Microbiol* 2003;**5(5)**:439.

[3] Lafita C, Penya-Roya JM, Gabaldón C, Martínez-Soria V. Full-scale biotrickling filtration of volatile organic compounds from air emission of wooden coating activities. *J Chem Tech Biotech*, in press.

[4] Amann R, Fuchs BM, Behrens S. The identification of microorganisms by fluorescence in situ hybridisation. *Curr Opin Biotechnol* 2000;**12(3)**:231-236.

[5] Jubany I, Lafuente J, Carrera J, Baeza JA. Automated thresholding method (ATM) for biomass fraction determination using FISH and confocal microscopy. *J Chem Techn Biotech* 2009;**84(8)**:1140-1145.

[6] Mosqueda G, Ramos-González M, Ramos JL. Toluene metabolism by the solvent-tolerant Pseudomonas putida DOT-T1 strain, and its role in solvent impermeabilization. *Gene* 1999;**232(1)**:69-76.