ISOLATION AND IDENTIFICATION OF MICROBIAL POPULATIONS FROM AN ODOUR TREATING BIOFILTER

V. S. Bessa[1], S. C. Moura[1], I. I. R. Baptista[2], R. Ferreira Jorge[2] and P. M. L. Castro[1]

[1]CBQF/Escola Superior de Biotecnologia – Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal
vsbessa@esb.ucp.pt; plcastro@esb.ucp.pt
[2]WeDoTech, Lda., Edifício CiDEB, Rua Dr. António Bernardino de Almeida s/n, 4200072 Porto, Portugal.

ABSTRACT

Complex odour emissions are normally associated to the operation of wastewater treatment plants, composting facilities and agro-industry activities. The most common contaminants are hydrogen sulfide (H_2S), organic reduced sulfur compounds (e.g.,CH_3SH), and volatile organic compounds (VOCs). These compounds can be treated using biological air treatment systems, such as biofilters. Five different types of material, including pieces of wood and polymeric material, were collected from different locations of a biofilter treating odours at an organic waste treatment plant and subjected to microbiological characterization. The different materials showed high heterogeneity of microbial colonization, being the diversity higher in a heather based material. After random amplification of polymorphic DNA (RAPD) analysis, a total of 22 different isolates were identified by 16S rRNA sequencing analysis. Ten isolates demonstrated capacity to grow on solid sulphur oxidizing medium. Their capacity to oxidize sulphur compounds in liquid medium is being further studied.

MATERIAL AND METHODS

- ✓ **Sampling** Five different types of material (classified from A to D), including pieces of wood and polymeric material that composed the biofilter mix were collected from different locations of a biofilter treating odours at an organic waste treatment plant, in Leiria, Portugal (Figure 1)
- ✓ **Microbial counts** Colony forming units (CFUs) were determined based on the surface-plate counting procedure (in Nutrient Agar (NA)). The original samples were then prepared to be stored at -80ºC with 30% glycerol.
- ✓ **Bacterial isolation** Different bacterial colonies were isolated based on size, morphology and pigmentation, from NA plates using a streak-plate procedure. Pure bacterial isolates were stored at -80ºC with 30% glycerol.
- ✓ **DNA Sequencing Analysis** After 16S rRNA extraction, a Random Amplified Polymorphic DNA (RAPD) using OPA3 primer was performed. The isolates amplification was carried out with the universal primers f27 and r1492 (Lane, 1991) under standard polimerase chain reaction (PCR) conditions (Rainey et al., 1996).
- ✓ **Growing in Sulphur Oxidizing Medium** Each different isolate was plated in Sulphur Oxidizing Medium (SOM). With those that grew, batch liquid cultures were set up. Cultures were incubated on an orbital shaker (150 rpm) at 25ºC. Optical density (λ600nm) was monitored for 20days.

Figure 1.. Different materials collected in the biofilter (A and B: pieces of wood; C and D: heather based material; D and E: polystyrene).

REFERENCES

- ✓ Lane, D.J. (1991) 16S/23S rRNA sequencing. In: Stackebrandt E & Goodfellow M, John Wiley and Sons Ltd. (Ed). Nucleic acid techniques in acterialsystematics. West Sussex, England.
- ✓ Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R.M. and Stackebrandt, E. (1996) The genus Nocardiopsis represents a phylogenetically coherenttaxon and a distinct actinomycete lineage: proposal of Nocardiopsaceae fam. nov.. International Journal of Systematic Bacteriology 46:1088-1092.

ACKNOWLEDGEMENTS

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RESULTS AND DISCUSSION

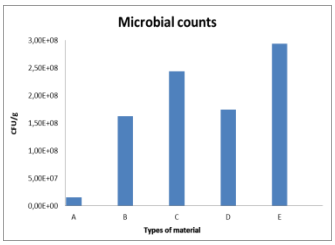

Figure 2: Microbial counts obtained from the five different materials within the biofilter.

- ✓ CFU/g ranged from 10^7 to 10^8 from each different sample analyzed;
- ✓ Different materials showed high heterogeneity of microbial colonization;

Higher diversity in heather based material (B)

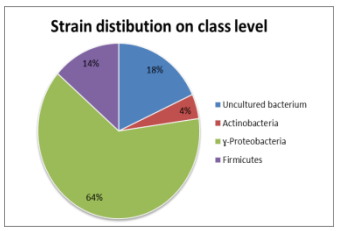

Figure 3: Proportions of taxonomic groups represented by the isolates.

- ✓ A total of 22 different isolates were analysed and sequenced;
- ✓ In all the materials, the dominant population belonged to the γ-proteobacteria genera

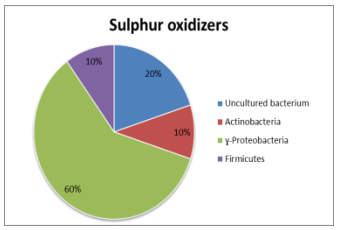

Figure 4: Proportions of taxonomic groups represented by the isolates able to oxidize sulphur compounds.

- ✓ Ten isolates were able to grow in SOM;
- ✓ The dominant population (60%) was γ-proteobacteria genera.

The identified γ-Proteobacteria isolates are commonly found in contaminated sources.

Table 1: List of the isolates able to grow in solid SOM and their closest relatives identified by 16S rRNA

Isolate	Phylogenetic affiliation	Closest relatives		
		Identification (NCBI/BLAST accession number)	Similarity (%)	Origin
A1	Firmicutes	Paenibacillus illinoisensis strain IB-1087 (FN422001)	98	Garden Soil
A2	γ-proteobacteria	Pseudomonas fluorescens strain E10 (HQ420253)	99	Pear
A3	γ-proteobacteria	Pseudomonas poae strain NBB19 16S (HQ256531)	100	Lake
A5	γ-proteobacteria	Pseudomonas reactans strain PB-St3 (GU459213)	100	sugarcane plant
B9	γ-proteobacteria	Pseudomonas sp. TAD054 (FJ225205)	99	rhizosphere soil
B11	Actinobacteria	Curtobacterium sp. VM11 (DQ238838)	98	PAH contaminated soil
D1	γ-proteobacteria	Pseudomonas sp. PcFRB120 gene (AB569969)	100	plant rhizosphere
D3	Uncultured bacterium	Uncultured bacterium clone Luq_GN470_006 (HQ445717)	100	deep saprolite and saprock
E4	Uncultured bacterium	Uncultured bacterium (FN814110)	100	phyllosphere of lettuce
E5	γ-proteobacteria	Pseudomonas syringae strain 100-p8_H12 (GU068645)	100	maple sap

CONCLUSIONS

- ✓ The five different types of material collected showed high microbial diversity;
- ✓ The dominant population belonged to γ-proteobacteria genera;
- ✓ Between the sampled materials, no significant differences were found in microbial population;
- ✓ Ten of the isolates showed capacity to oxidize sulphur compounds.

FURTHER WORK

- ✓ Assess the ability of the 10 isolates that grew on solid medium to grow in liquid medium supplied with a H_2S gas source.