

Bioaerosol biodiversity in waste sorting plant: feasibility and uncertainty measurement

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Waste sorting activities are source of occupational bioaerosol exposure that were associated with several health disorders such as rhinitis, asthma and hypersensitivity pneumonitis. So far, the microbial biodiversity data published in this occupational environment were obtained by cultivation of microorganisms on nutrient media prior to identification. Due to the well-known limits of cultural methods, these biodiversity measurements underestimate the real microbial taxon richness. Recent advances in molecular biology provided powerful methods for biodiversity studies such as high-throughput sequencing.

The first aim of the study was to evaluate the suitability of high-throughput sequencing for the assessment of bioaerosol biodiversity in a waste sorting plant (WSP). The second one was to calculate the uncertainty of the global measurement process.

Bioaerosol biodiversity was assessed in a French WSP sorting journal newspapers, papers, cardboards, food packaging and other sorting wastes (4000 tons per month). The WSP employed about 50 workers mainly dedicated to manual sorting in three cabins. In one cabin, three stationary parallel samples were collected by filtration of the air through polycarbonate membrane (0.8 µm) using closed-face cassette (Milipore, France), at 10 L/min and for five hours. An outdoor reference sample was also collected by the same way. After DNA extraction (FastDNA[®] SPIN kit for soil, MP Biomedicals, USA), eukaryotic rDNA 18S and bacterial rDNA 16S were sequenced by an external provider (INRA Transfert, France). Bioinformatic sequences analysis was performed using two pipelines developed by INRA Transfert using Mothur software (Schloss and Westcott, 2009). To evaluate uncertainty of measurement process, the results of bacterial biodiversity were compared and statistical parameters were calculated.

All DNA extracts from samples had suitable concentration for the sequencing (mean: 6ng/µL). Biodiversity was different in the sorting cabin compared with the outdoor reference (figure 1). For bacterial biodiversity, more than 80% of sequences were identified at the phylum rank whereas only 50% of the sequences were identified at the genera rank. Bioaerosols were dominated by four main phyla: *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*. The ten dominant fungal OTU (Operational Taxonomic Unit) in sorting cabin samples represented 95% of eukaryotic sequences with a predominance of *Penicillium* genera

(60%). This agrees with previous findings using the culture method (Breum et al., 1999). In the outdoor reference, *Cladosporium spp.* was the main genera (45%).

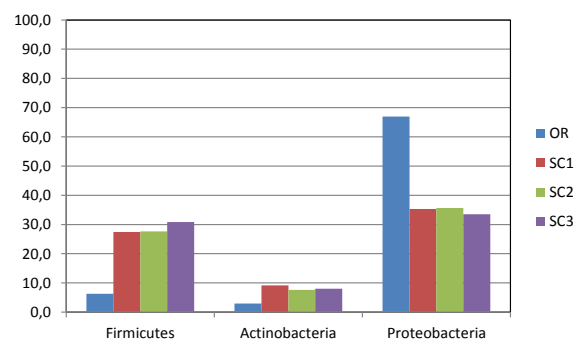


Figure 1. Main bacterial phyla in bioaerosol emitted in a sorting cabin and the outdoor reference. OR : outdoor reference, SC1: sorting cabin sample 1, SC2: sorting cabin sample 2; SC3: sorting cabin sample 3

Uncertainty decreased as the proportion of taxon increased, the more the taxon was present, the less the difference between the three repetitions was important. It would be used to define the taxa which could be studied to provide robust results. The results and the calculation method of uncertainty will be described in the presentation. Shortly, this method allow to demonstrate that *Firmicutes* proportion was four times higher in the sorting cabin than in the reference and that *Actinobacteria* were also predominant in the sorting cabin. *Proteobacteria* were predominant in the reference.

The study shows that high-throughput sequencing is a suitable method for the assessment of bioaerosol biodiversity emitted in WSPs. The calculation of uncertainty allowed discriminating bioaerosols sampled in different situations and gave information about the repeatability of biodiversity measurements. These results provide the first record about biodiversity in bioaerosols from WSPs using high-throughput sequencing and an original method for uncertainty calculation from the subsequent data. The methods will be used to assess parameters that will have an influence in variation of biodiversity in bioaerosols emitted in waste sorting plant.

Breum, N.; Würtz, H.; Midtgaard, U. and Ebbenhøj, N. (1999) *Waste Manage. Res.* **17**, 100-108.

Schloss, P. and Westcott, S. (2009) *Appl. Environ. Microb.* **77**, 3219-3226.