## Biodegradation of ethyl acetate and toluene mixtures by a peat biofilter: respirometry monitoring and dynamics of living and dead bacterial cells

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ABSTRACT. To investigate the microbial degradation of ethyl acetate and toluene mixtures in biofiltration, three laboratory-scale reactors, inoculated with a two-month conditioned culture from activated sludge, were operated for a continuous period of 6 months. Biofilters were packed with a fibrous peat that has been previously shown as an adequate biofilter material for TEX removal. Each reactor was downflow fed with air contaminated with ethyl acetate, toluene or a 1:1 mixture of ethly acetate and toluene, respectively, at a constant EBRT of 90 s. Inlet concentration was progresively increased from 0.1 to 4.0 g m<sup>-3</sup>. The maximum elimination capacity found for toluene as a sole contaminant was 90 g m<sup>-3</sup> h<sup>-1</sup>, but the presence of ethyl acetate decreased the toluene degradation with a maximum elimination capacity of 35 g m<sup>-3</sup> h<sup>-1</sup>. From gas concentration profiles, a stratification in the substrate consumption was observed: nearly no toluene biodegradation ocurred until ethyl acetate, the easily biodegradable compound, was metabolised. This study has focused on two main objectives: first, to implement an easy and low-cost technique for monitoring the biofilter performance based on carbon dioxide production; second, to determine the microbial dynamics of living and dead cells by using two acid-nucleic staining (Syto 9 and propidium iodide) and enumeration by direct epifluorescence microscopy. Results are of particular relevance for better understanding the operational and ecological aspects of the biofiltration, and will also be useful for mathematical modelling. Biofilters fed with the individual VOC were used to determine the experimental mass ratio of carbon dioxide produced to the compound removed. Values of 3.13 g CO<sub>2</sub>/g-C and  $3.03 \text{ g } \text{CO}_2/\text{g-C}$  for ethyl acetate and toluene, respectively, were found, resulting in overall yield coefficients of 0.15 g of dry biomass produced per g of ethyl acetate consumed and 0.30 g of dry biomass produced per g of toluene consumed. By using these calibrated coefficients, the carbon dioxide production in the two-component biofilter was succesfully simulated, showing that microbial metabolism for degrading ethyl acetate or toluene is independent on the simultaneous presence of both compounds. Colonization dymanics were monitored in four sections of the biofilters. Similar bacterial concentrations were found for the three biofilters, with values ranging between  $1.2 \times 10^9$  -  $2.6 \times 10^{10}$  cells per gram of dry peat for total bacteria, and  $1.1 \times 10^9$  -  $2.1 \times 10^{10}$  cells per gram of dry peat for living bacteria. In the first stages of the operation a greater bacterial density in the top zones of biofilters was observed, and a progressive colonisation of the lower zones of biofilters was achieved with operation. The high loads applied in the last stages of the operation showed a loss of bacterial density in the upper zones of biofilters, along with an increase in the dead cells percentages up to 40%.