

Assessment of the Biological Contribution to Monitored Natural Recovery of Anthropized Freshwater Sediments

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Background/Objectives. Aquatic ecosystems are suffering from different anthropogenic pressures due to contaminants released by discharges from domestic and industrial wastewaters. Among the organic pollutants, petroleum hydrocarbons are of major concern because of their wide persistence and toxicity. Monitored natural recovery (MNR) of historically contaminated sediments is a viable and cost-effective remediation strategy that relies on natural physical, chemical, and biological processes to isolate, degrade or reduce the toxicity of contaminants. For a proper implementation of MNR the characterization of the ability of autochthonous microbial communities to biodegrade petroleum hydrocarbons is necessary.

The studied sediments come from two sites of a freshwater course with a history of oil pollution and whose recovery began 20 years ago. Four sediments cores were extracted of each site using a hammer piston tool. In a previous study, water samples were analyzed, showing significant difference in the dissolved oxygen between the two sites, while hydrocarbons were not detected. Likewise, surface sediment horizons showed the lowest or non-detectable hydrocarbon concentrations. By contrast, deeper horizons were contaminated with aliphatic and aromatic hydrocarbons. At cultivable level, the counts of anaerobic heterotrophic bacteria were one to two orders of magnitude higher than aerobic heterotrophic bacteria.

The aim of the present work was to assess the hydrocarbon degrading capacity of autochthonous microbial communities through the prediction of relative abundance of functional genes encoding enzymes for hydrocarbon degradation pathways by an *in silico* metagenomics approach.

Approach/Activities. The *Bacteria* and *Archaea* diversity were investigated by sequencing V4 region of 16S rRNA genes by Illumina. The sequencing data obtained were used to perform the taxonomic assignment (Qiime 1.9.1) and *in silico* predictions of metagenomic functions with PICRUSt software.

Results/Lessons Learned. Sequencing results showed that surface sediments were more diverse than deeper sediments (5.7-6.1 and 3.3-4.5 Shannon index, respectively) and relative abundance of *Bacteria* ($94 \pm 0.5\%$) overcame *Archaeas* ($5.5 \pm 5.4\%$) in all cores.

Bacteria belonging to the classes *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Clostridia* and *Bacilli* were predominant; whereas the *Methanomicrobia* class was predominant among *Archaeas*.

As indicated by the functional prediction, independently of physicochemical characteristics of the sites, the fatty acid metabolism, toluene, nitrotoluene, aminobenzoate, benzoate, limonene, naphthalene and polycyclic aromatic hydrocarbon degradation pathways were present in all horizons with the benzoate pathway being the most enriched.

The prediction also indicated that the abundance of alkylsuccinate synthase (EC: 2.3.1.54) sequences were 15-fold higher than alkane-1-monooxygenase (EC: 1.14.15.3) sequences, suggesting the predominance of alkane anaerobic degradation.

Only the aerobic PAH degrading genes, naphthalene 1,2-dioxygenase (EC:1.14.12.12) and PAH dioxygenase (EC:1.13.11.-) were founded with PICRUSt prediction.

The predictive metagenomics approach provides clear evidence of the hydrocarbon degradation potential of the autochthonous microbial community and their possible contribution to MNR.