Geophysical Research Abstracts
Vol. 14, EGU2012-1640, 2012
EGU General Assembly 2012

A cell extraction method for oily sediments

M. Lappé and J. Kallmeyer
Institute of Earth and Environmental Science, University of Potsdam, Potsdam, Germany (lappe@geo.uni-potsdam.de)

Hydrocarbons can be found in many different habitats and represent an important carbon source for microbes. As fossil fuels, they are an important economical resource and, through natural seepage or accidental release, they can be major pollutants. Oil sands from Alberta, Canada, and samples from the seafloor of the Gulf of Mexico represent typical examples of either natural or anthropogenically affected oily sediments.
DNA-specific stains and molecular probes bind to hydrocarbons, causing massive background fluorescence and thereby massively hampering cell enumeration. The cell extraction procedure of Kallmeyer et al. (2008) separates the cells from the sediment matrix, producing a sediment free cell extract that can then be used for subsequent staining and cell enumeration under a fluorescence microscope. In principle, this technique can also be used to separate cells from oily sediments, but it was not originally optimized for this application and does not provide satisfactory results.
Here we present a modified extraction method in which the hydrocarbons are removed prior to cell extraction by a solvent treatment. Due to the reduced background fluorescence the microscopic image becomes clearer, making cell identification and enumeration much easier. Consequently, the resulting cell counts from oily samples treated according to our new protocol were significantly higher than those treated according to Kallmeyer et al. (2008).
We tested different amounts of a variety of solvents for their ability to remove hydrocarbons and found that n-hexane and – in samples containing more biodegraded oils – methanol, delivered the best results. Because solvents also tend to lyse cells, it was important to find the optimum solvent to sample ratio, at which the positive effect of hydrocarbon extraction overcomes the negative effect of cell lysis. A volumetric ratio of 1:2 to 1:5 between a formalin-fixed sediment slurry and solvent delivered highest cell counts. Extraction efficiency was around 30 to 50% and was checked on both oily samples spiked with known amounts of E.coli cells and oil-free samples amended with non-biodegraded and biodegraded oil. The method provided reproducible results on samples containing very different kinds of oils with regard to their degree of biodegradation. For strongly biodegraded oils, like those from the Alberta oil sands, methanol turned out to be the most appropriate solvent. For less biodegraded oils, like those from sediments from the Gulf of Mexico, n-hexane delivered best results. The relative amount of polar groups increases with an increasing level of biodegradation. Therefore polar solvents like methanol are better suited to dissolve biodegraded oils than less polar solvents like n-hexane.
Our new method only provides a minimum estimate of cell abundance, as some cells are either lysed by the solvent treatment or remain attached to mineral grains and therefore do not end up in the cell extract but remain in the sediment pellet which will not be used for further microbiological analysis.