

CHEMICAL MONITORING OF THE BIOREMEDIATION OF ENVIRONMENTS CONTAMINATED BY SPILLS OF OIL AND/OR OIL DERIVATIVES

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Spills of oil, fuel, gasoline and other compounds with low or nil solubility in water are unfortunately frequent news in the times we live in. Recent examples of ecological disasters of this type are the oil tanker Exxon Valdez, which spilled 37,000 tons of oil in Alaska in 1989, the spills during the Gulf War in 1991, which generated particular problems because the Gulf's narrow connection to the Indian Ocean gives it little opportunity to flush out pollutants, the Nakhodka spill of 6200kl of gas oil into Japanese waters in 1997 and, most recently, the ship Prestige, which continues to leak oil off the coast of Galicia.

The best strategies for cleaning up and collecting the oil depend on several factors, such as the type of spill, the characteristics of the site involved and sometimes political considerations. There are several techniques and strategies for controlling spills in marine and aquatic environments. The most commonly used/most suitable for coastal areas and the sea are: i) Physical methods; ii) chemical methods and iii) natural methods.

The conventional methods, such as the physical and chemical techniques, are the first working option for tackling large masses, although it is not possible to recover more than 10-15% of the total spill.

Chemical methods, moreover, involve the input of new substances with a negative environmental impact. Among the natural processes, therefore, bioremediation is one of the most promising methods being considered for the recuperation of oil spill areas. It is a "green", non-intrusive or -disruptive technology. Bioremediation has been defined as the action of adding material to contaminated environments to favour the acceleration of the natural biodegradation process. Its success depends on our capacity to establish and maintain the conditions that enhance the speed of the biodegradation process. Two enhancing methods are possible:

- i)** bioaugmentation and
- ii)** biostimulation.

To ascertain whether or not bioremediation is occurring in a given environment it is necessary to check that both the microbial and chemical population of this environment is being suitably modified. The environmental conditions, in particular the concentration of nutrients and of some spill components, are some of the factors that have to be continually assessed. The following points need to be addressed:

- a)** Analysis of nutrients. In any problem such as an oil spill there is a

high input of carbon into the micro-organisms present in the environment; the nutrients (nitrogen, phosphorous) would limit the effectiveness of the micro-organisms in continuing the bioremediation process. Their concentration is therefore a critical factor in carrying out the most suitable strategy for their augmentation (e.g. a slow-release fertilizer).

b) Chemical analysis of Polycyclic Aromatic Hydrocarbons (PAHs). Aromatic hydrocarbons are toxic for some microorganisms since they dissolve in the cell membrane, but in low concentrations they are easily biodegradable under aerobic conditions.

c) Monitoring of the general environmental conditions. Among the parameters that need to be assessed in monitoring the bioremediation process are dissolved oxygen, pH, salinity and temperature. Available dissolved oxygen is crucial for a rapid recovery of the environment, since the biodegradation of hydrocarbons is an aerobic process. pH is also important, for pH might greatly inhibit the biodegradation process.

The monitoring of the chemical parameters taking part in the bioremediation process calls for quick, robust and reliable analysis methods with the necessary sensitivity and selectivity. The analysis methods selected were:

A) NO_2 : The nitrites are determined using the "Griess Method". This is based on the reaction between sulphanilamide and nitrite to give a diazo compound that then reacts with an aromatic amine, N-(1-Naphthyl)ethylenediammonium chloride, with which it forms a coloured compound showing a maximum ab-

sorption at 540 nm. This method was coupled up with an automatic fluid injection analysis system (FIA system), making the system suitable for routine analysis purposes.

B) NO_3 : The nitrates can also be analysed by the Griess Method if they are first reduced to nitrites. This can be done by using a copperised cadmium column (Cd/Cu), through which the sample is passed so that the nitrates are reduced to nitrites and are then determined together with the latter.

C) $\text{PO}_3\text{-4}$: The phosphates can be determined by means of the "Ascorbic acid method", based on the reduction of the phosphate with molybdate in the acid medium (forming phosphomolybdic acid) and the subsequent reduction thereof with ascorbic acid to molybdenum blue, whose spectrum shows an absorption maximum between 820 and 825 nm.

D) HYDROCARBONS: These compounds are usually analysed by chromatography, either gas chromatography (GS) or High Performance Liquid Chromatography (HPLC). These techniques are limited, however, not only due to their high cost but also due to the thermal ability of some PAHs (GC) and also the fact that there is normally a need for a previous derivatization of the analytes (HPLC). This means that these techniques are not totally suitable for the sort of routine analysis to be carried out here. Recent research work (Digambara Patra, A.K. Mishra, Talanta 55 (2001) 143-153) has shown that more or less complex mixtures of PAHs can be

resolved and quantified by synchronous fluorimetric methods, based on measuring the intensity both of the mixture (sample) and of standard solutions of the compounds to be determined, in the optimum measuring conditions for each one. This gives us an equation system of n equations with n unknowns, enabling us to find the coefficients that, multiplied by the concentration of the standard solution, give the concentration of the compound in the mixture (sample).

The advantages of this method are its simplicity and low cost, the fact that no previous treatment of the sample is necessary (previous separation of the components) and the reduction in the number of readings to be made. Nonetheless, to ensure that the values obtained by this method are reliable, a determination is also made of the total hydrocarbons. This is done by means of a liquid-liquid extraction with dichloromethane and subsequent measuring of the fluorescence of the extract recomposed in hexane using Chrysene as the standard, present at a constant proportion in all crude oil. This method is mainly based on the one described by Keizer and Gordon in *J. Fish. Res. Bd. Canada*, 30: 1039, 1973 and is recommended in "Timothy R. Parsons, Yoshiaki Maita, Capol M. Lalli: A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press (1984)"

E) Other Parameters

i. O_2 : This nutrient is measured in situ by using a commercial system, the oxygen selective electrode or Clarke electrode, which is calibrated at the

moment of making the measurement. The equipment also takes into consideration the correction of salinity and temperature.

ii. pH. This parameter is measured in the laboratory using a glass electrode calibrated between pH 7 and pH 9.

iii. Conductivity. This parameter is also measured in situ using a portable conductivity meter. The salinity of the water can then be calculated from the conductivity value and the water temperature (also given by the conductivity meter).

Sampling

The analysis is going to be carried out in the laboratory, so a sample of the sediment, sand and water needs to be collected in a suitable way, using special containers and at low temperature.

The samples both of soil and water were taken in accordance with the rules of EPA (US Environment Protection Agency). The areas are contaminated in a patchy way so it is difficult to make a representative sample. In this work a stratified sample was made on 5 m² of beach, dividing the sampling area into a number of sectors or quadrants and taking independent samples in each sector in accordance with the proportionality rule (more samples in more contaminated zones), with a sampling frequency of 1-2 samples a week. The independent samples are brought together in a single sample; this has the advantage of greater accuracy (a high number of samplings per sample). The depth is also important in these samples. Crude oil rarely penetrates to a depth of more

than 3 cm in sediment so the samples were taken with this depth.

The sample taking site selected was the beach called Playa de Rodiles in Villaviciosa (Asturias). Previous visits had found signs of fuel oil being washed up on this beach and there is also an almost permanent contingent of workers mopping the oil sludge washed ashore.

Previous Treatment and Analysis of the Samples

The water samples are given no previous treatment apart from filtering. The soil samples do need previous treatment, however, in this case a liquid-solid extraction. The literature on the subject mentions a great deal of solvents for extracting the sample analytes needed. Of all these methods the most suitable turned out to be:

* Milli-Q water, 25 g. of soil per 50 ml of water, agitated for one hour. The plant available phosphorous is extracted and this is perhaps what is of most interest to us here.

The samples were analysed using the aforementioned methods. Up to now

the levels of nitrates, nitrites and phosphates have never topped the legal levels laid down in Royal Decree 140/2003 of 7 February 2003, regulating the levels of contaminants in human drinking water, and they fall within the limits considered to be normal in marine environments.

To sum up, this work has optimised the routine analysis methods necessary for the analytical control of the most important parameters involved in the bioremediation of oil spill scenarios (except for the individual PAH identification and quantification method by synchronous fluorescence, which proved to suffer serious interference). The objectives laid down at the start of this research work were therefore met. This opens up the possibility of ongoing, routine, rapid and reliable monitoring of the recuperation capacity of the Asturian coast and also of the levels of existing nutrients. This will enable us to ascertain which bioremediation stimulation methods will be the most suitable. ■