ADVANTAGES OF COUPLING THE FINGERPRINTING AND BIOCHEMICAL TECHNIQUES IN CONTAMINATION ANALYSIS

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Introduction

Thousands of chemicals with different physicochemical properties and toxicity are used in our society and many of them can be detected in environmental (e.g., water, sediment, soil, air and biota) samples. The contaminant class of concern of this presentation is represented by petroleum and its refinery products. Oil spills can affect ecosystems and human health due to the content of toxic and mutagenic compounds such as benzene that is recognized carcinogen. The studies of pollution due to these contaminants can be performed by the employ of fingerprinting methodologies, that is based on a standard technique applied for environmental chemical analysis; e.g., chromatography – mass spectrometry (GC-MS).

The two different fingerprinting techniques are based on the study of the mixture pollutant (compositional fingerprinting) or on the analysis of the isotopic composition of atoms such as carbon, chlorine, hydrogen and sulfur (isotopic fingerprinting) to get answers such as the characterization of the type of pollutant and the identification of the source of pollution. Specifically, the compositional fingerprinting is applied in studies of pollution due to the products of oil processing, while the isotopic fingerprinting can be used also in case studies concerning halogenated hydrocarbons, such as chlorinated. The compositional fingerprinting will be more deeply treated in this presentation, on the other a short summary is given for the isotopic fingerprinting methodology.

This second technique is based on the existence of stable isotopes in almost all the elements present in our solar system. The stable isotope do not decay, but chemical, physical and biological processes will select one isotope over other by some small amount. Moreover, isotopes of a chemical element differ only in the number of neutrons in their nuclei. In this way there will be a concentration of one or another isotope either in a reactant or in a product. When used together with chemical composition, stable isotope ratios of total hydrocarbons or hydrocarbon fractions can offer a powerful fingerprinting tool. Isotope ratios are useful because they are altered by chemical, physical and biological processes and they differ from sample to sample. For example, biodegradation of a pollutant can be monitored by the analysis of the isotopic ratios $^{13}C/^{12}C$ because the weaker ^{12}C-X bonds are broken more easily then the ^{13}C-X bonds. This ratio and the relative delta are measured and interpreted as following:

$$\frac{13C}{12C} \quad \frac{Number\ of\ 13\ C\ atoms\ in\ sample}{Number\ of\ 12\ C\ atoms\ in\ sample}$$

(1.1)

$$Delta\ or\ \delta 13C\ ppt = \left[\frac{\frac{13\,C}{12C}sample - \frac{13\,C}{12\,C}std}{\frac{13\,C}{12\,C}std}\right] * 1000$$

(1.2)

In nature the approximate ratios for the major stable isotopes present in the environment are:

D / H	1/6500
$^{13}C / ^{12}C$	1/90
$^{15}N / ^{14}N$	1/250
$^{18}O / ^{16}O$	1/500
$^{34}S / ^{32}S$	1/22.5

The technique of compositional fingerprinting allows the identification of the source of potential contamination between different possible sources by the analysis of the composition of a mixture pollutant that can provide useful information about the origin of the contamination and processes; such information may be obtained either by evaluating the presence and abundance of individual compounds that the pattern of distribution of hydrocarbon constituents within a Total Ion Chromatogram (TIC) obtained with a GC/MS. The GC / MS is a laboratory technique widely used for the characterization and environmental monitoring, in use for several years. The combined use of the two instruments allows a rapid separation of complex mixtures and identification of compounds that compose them. Gas chromatograph (GC) separates the samples into the individual components, which are in turn introduced into the mass spectrometer (MS) where the molecules are fragmented into ions constituents. The ions are then separated from the mass and tracked by an electron multiplier. The result of the mass spectrometer is characteristic for each individual compound and can be used to identify each component of the extracted sample through a comparison with spectra already catalogued in a separate library.

This technique is mainly useful in study cases of petroleum (or its refined products) pollution. Oil is a complex mixture of up to tens of thousands of hydrocarbons (alkanes, alkenes, polycyclic aromatic compounds – PAH- etc..) formed from a wide variety of organic material chemically converted under the action of different geological conditions for a long period of time. The crude oil initially contains carbon and hydrogen as well as small amounts of sulphides, oxygen, nitrogen and metals such as nickel, vanadium and iron. For commercial purposes, crude oil undergoes a refining process. The three main petroleum products are light distillates (gasoline), middle distillates (kerosene and diesel) and heavy distillates (heavy oils and lubricants). Both the conditions of origin and the industrial process immediately gives the final product a "fingerprint" (or "mark" feature that appears in the form of a TIC chromatogram. Petroleum products are important pollutants because are used as fuels in cars (gasoline and diesel), aircrafts and ships, for heating and electricity generation, as lubricants in machinery and for the production of chemicals and plastics. Figures 1 and 2 show the chromatograms of three of the main products of the processing of petroleum; gasoline, kerosene and diesel fuels.
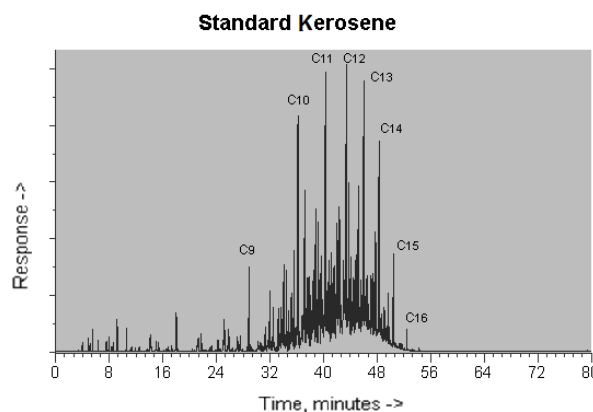

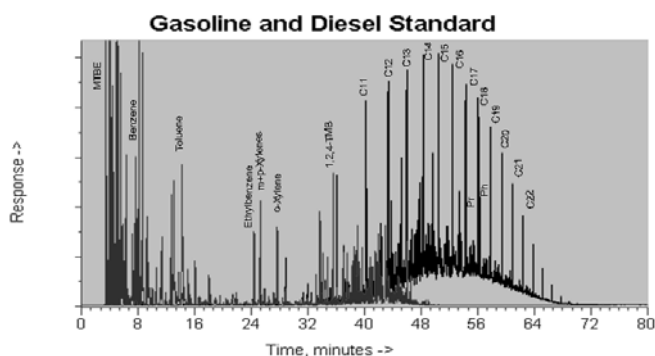**Fig. 1 – Chromatogram of a kerosene standard**

Fig. 2 – Chromatograms of gasoline and diesel standards

Once in the environment spilled crude oil or petroleum products are subject to a series of physical (e.g., evaporation, emulsification, natural dispersion, dissolution and sorption), chemical (e.g., photodegradation) and biological (e.g., microbial degradation) processes that have, as a consequence, the alteration of the composition of the products or mixtures of products thus of their fingerprints. Particular interest in identifying the mixture pollutant and the source of the pollution are the biological processes. In particular, the *in situ* bioremediation, or biological process in which microorganisms metabolize organic pollutants in inorganic material such as carbon dioxide methane, water and inorganic salts. This type of remediation can occur naturally but is not ubiquitous, and not in all environmental conditions.

Compounds are more or less susceptible to biodegradation due to their chemical structures, for this reason it is possible to define a sequence in biodegradation of the different compounds as following : n-alkanes>> monocyclic alkanes >> alkylbenzenes >> isoprenoids >> alkylnaphthalenes >> bicycloalkanes >> steranes >> terpanes.

Table 1 illustrates changes in gasoline and diesel fuels composition during increasing level of biodegradation.

Tab. 1 – Changes in gasoline and diesel fuels composition during increasing level of biodegradation (from Alimi et al. 2003). – *for a clearful comprehension see at the end of text*

fuel type			degradation level	chemical composition	
Bunker C fuel	Diesel	Gasoline	1	abundant n-alkanes	increasing level of biodegradation
			2	light-end n-alkanes removed	
			3	middle range n-alkanes, olefins, benzene and toluene removed	
			4	more than 90% of n-alkanes removed	
			5	alkylcyclohexanes and alkylbenzens removed; isoprenoids and C0-naphtalene reduced	
			6	isoprenoids, C1-napthalenes, benzothiophene and alkylbenzothiophenes removed, C2-naphtalenes selectively reduced	
			7	phenantrenes, dibenzothiophenes and other polynuclear aromatic hydrocarbons reduced	
			8	tricyclic terpanes enriched, regular steranes selectively removed C31-to C35-homohopanes reduced	
			9	tricyclic terpanes, diasteranes and aromatic steranes abundant	
			10	aromatic steranes and demethylated hopanes* predominant	

* only present under special conditions
Change in gasoline, diesel fuel, and Bunker C composition during biodegration

There are also several biodegradation ratios used to measure the degree of biodegradation. Two of these ratios are represented by n-C17/pristine and n-C18/phytane, where n-alkanes (n-C17 and n-C18) are correlated to the isoprenoids pristine and phytane. This because as the fuel contaminant is biodegraded, bacteria preferentially consume the n–alkanes cited leading to an enrichment of pristine and phytane. In fact, the isoprenoids are less susceptible to biodegradation than the n-alkanes, as shown in Tab. 1.

Several studies have found that the factors that most influence or limit the application of *in situ* bioremediation process in groundwater are:

1. Source and concentration of the contaminant;
2. Composition and chemical structure of the contaminant → PAHs, PCBs, heavy metals, some chlorinated solvents, etc. are not biodegradable. On the opposite, the biodegradation leads to the formation of more toxic compounds;
3. Solubility, transport, adsorption, dispersion and volatility of contaminants – BIOAVAILABILITY;
4. Identification, determination and monitoring of contaminants;
5. Composition chemical, physical and microbiological of the groundwater;
6. Chemical composition and mechanisms of soil contaminated site;
7. Hydrology and hydrogeology of the contaminated site;
8. Environmental conditions, presence of electron acceptors and sources of nutrients;
9. Biodegradation of the contaminants and the presence of microbial populations able to degrade them.

From this overview it is clear the mutual influence between the chemical aspect, that is determined by the analysis of the

compositional fingerprinting, and the microbiological characterization of the site from which the sample came from. Moreover, each site has unique characteristics, and then official criteria to evaluate the effectiveness or the ineffectiveness of the bioremediation have not been defined. Considering all these aspects, it was decided to prepare, within a Doctorate Project, a laboratory study with the goal to define a survey instrument of monitoring and characterization built by coupling the compositional and the microbial fingerprinting.

The microbial fingerprinting is a study of the microbial population of the polluted site, that allows to its characterization. Depending on the mixture pollutant present, there are several bacterial strains and the composition of that community will vary in relation with the present conditions or in relation to the contaminants biodegradation occurring.

Through an analysis of biological called T-RFLP (Terminal Restriction Fragment Length Polymorphism) is obtained a chromatogram similar to what is obtained for chemical analysis in GC-MS (as shown in Figure 3). Each peak corresponds to a bacterial strain, as well as each peak in the chromatogram corresponds to a chemical compound of the mixture pollutant.
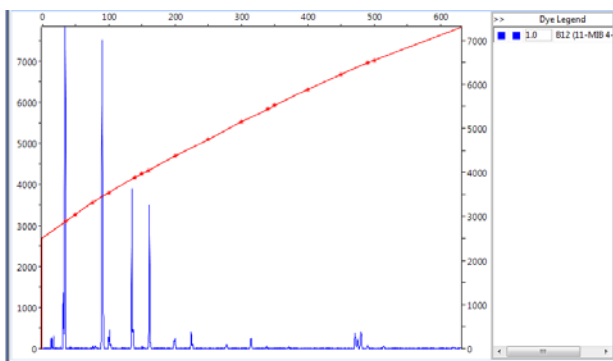

Fig. 4 - Chromatogram of the microbial population present in a sample analyzed

Design experiment:
This topic is the subject of a Doctoral in Environmental Enginnering at Politecnico di Milano. The objective of the study is the determination of an integrated survey instrument by the coupling of the compositional and the microbial fingerprinting through the determination of the physical-chemical parameters that most affect the action of microorganisms in the process of in situ bioremediation. To do this, the study has focused on the observation of some parameters already present in the list (granulometry of the soil, organic matter presence and concentration of the contaminant) and of two new parameters (presence of mixtures of contamination and salinity).

The different environmental conditions were reproduced through microcosms prepared in serum bottles sealed. The soil and the groundwater contained in these serum bottles have been sampled at for different times.

Parameters analyzed:
- Particle size → 2 levels: 50-50% gravel-sandy and sandy silt 50-50%
- Salinity → 2 levels: 0.45 g / L and 4 g /
- Mixtures → 3 levels: 100% diesel fuel, gasoline and 100% 50-50% gasoline and diesel Organic matter available → 2 levels: 10 mg and 100 mg of turf
- Concentrations mixtures → 2 levels: 100 ppm and 1000 ppm
- Analysis time → 4 levels: T0 = 0 days, T1= 10 days , T2 = 70 days and T3 = 140 days

Analysis of results: in progress

In conclusion, compositional fingerprinting is a useful tool for a specific site investigation and fuel contaminant identification because it gives detailed composition information of spilled oil or refining products both in soil and groundwater samples, tacking account of the wide variables that can interfere with the pollutants structure, such as all the weathering processes. These data helps also in the identification of the source contamination. The combination of its results with results coming from microbial fingerprinting should give data more accurate

and complete, leading to an effective survey instrument of monitoring and characterization.

<u>REFERENCES</u>

Alimi, H., Ertel, T. And Schug, B. (2003) Fingerprinting of hydrocarbon fuels contaminants: literature review. Environmental Forensics, 4:25-38.