Biotechnological cleaners

Bacteria that ‘eat oil’

In today’s society, more than 80% of human activities are closely connected to the production of energy generated by the combustion of oil. The utilisation of this natural fossil resource offers modern man inalienable benefits and comforts. On the other hand, however, there must be the determination and commitment to reduce not only contamination to a minimum but also environmental degradation that hydrocarbons (oil is a mixture of hydrocarbons) can bring about in terrestrial and aquatic ecosystems.
Crude oil is made up of a mixture of substances that present a different molecular structure maintaining the same chemical structure: hydrocarbons, molecules made up of atoms of carbon and hydrogen.
The efforts made to preserve all ecosystems from the input of hydrocarbons are multidisciplinary and involve different sectors of science and industry, and pursue different objectives. For example, activities such as the prevention of accidental and chronic input of hydrocarbons, the implementation of intervention strategies capable of minimising the environmental and economic consequences of contamination, the redevelopment and improvement of severely endangered areas, are necessary.
In this setting, where attention is given to the world we live in, biotechnologies find a natural and practical application in solving environmental problems. The European Federation of Biotechnology (EFB) defines environmental biotechnologies as the combined use of natural and engineering sciences with the objective of utilising living organisms (such as bacteria and plants), cells, parts of organisms and molecules similar to them for a sustainable development. This apparently abstract definition has many practical applications. Research in the field of environmental biotechnologies is focused on achieving objectives that support the realization of energetic and environmental sustainability together with the protection of natural resources, prevention of damage to the environment, solid and liquid waste treatment, gas emission treatment, bioremediation of contaminated areas and development of products and processes that generate less waste and that reduce energetic consumption.

The discovery

The plans for preservation of the territory materialize also include depuration of polluted sea waters and renewal of contaminated sea beds and sediments. The methods utilized make use of innovative technologies. In particular, a state of the art sector of environmental biotechnologies is emerging: bioremediation. This term defines the use of micro-organisms to remove contaminants from aquatic and terrestrial systems (sea and land). The process is based on the metabolic diversity of natural microbe communities. This implies that the techniques that are utilized are based on the natural predisposition of some bacteria to produce enzymes capable of degrading the hydrocarbons that oil is made up of.
The enzymes are proteins, identified on the basis of the function that they carry out; in other words, they are classified according to the substratum (compound) they attack. The function of the enzymes is to behave as catalysts, that is as promoters, making certain reactions easier. These proteins act in such a way that the energy required for a chemical reaction to take place is reduced. Let’s take an example from everyday life: the cleaning of clothes with a detergent – many stains have a natural origin and so the enzymes are present in washing machine detergents: grease stains like oil and fat are easily eliminated by lipase while stains of protein origin (like blood) are washed away more easily if protease is present in the soap.
The micro-organisms utilized in the bioremediation activities are very interesting because they involve hydrocarbonclastic bacteria i.e. bacteria that ‘feed’ on oil using it for their metabolic processes. What’s more, these bacteria have the characteristic that they are native to marine ecosystems (considered as hydrocarbon clearance sites), which implies that that these sea cleaners naturally live in great numbers in waters where oil is present because it is just what they ‘eat’.
From the beginning of the Seventies, various researchers have dedicated an increasing amount of time to studying micro-organisms capable of partially or totally destroying oil and its derivatives that are found accidentally in the sea.

Particularly in the last decade, there have been decisive developments in the field of bioinformatics (a discipline that deals with the study, development and integration of information technology instruments at the service of scientific research in the field of biotechnology), biochemistry and molecular biology. Even microbiology has begun to utilize efficient techniques and methods of research that were unconceivable just a few years ago.

What are these bacteria?

The scientific nomenclature used for bacteria (and also for yeast and moulds) must be interpreted as the surname and name of the micro-organisms; for example, ***Acinetobacte****r* is the surname, that corresponds to the genus to which numerous species of bacteria belong,***venetianus*** is the name of the species discovered in the waters of Venice's lagoon. A curious fact about species of bacteria that belong to the genus *Acinetobacte*r is that they can survive in soap and in disinfectant mixtures; besides, they can even be found on our skin with a probability of up to 25%, but it still is not clear if these bacteria are harmful or inoffensive guests for Man. Let's study in more detail the discovery of these 'environmental friendly' micro-organisms in Italian waters, both in the North and South of the peninsula.

In Venice's lagoon

In 1996, in the waters of Venice's lagoon, a bacterium was isolated and named Acinetobacter venetianus; it was capable of carrying out numerous stages in the process of degrading fuel oil/petroleum. Following this first discovery, many studies have been dedicated to micro-organisms belonging to this part of the sea with the objective of differentiating a microbial community made up of Acinetobacter venetianus and bacterial strains of *Pseudomonas putida*and *Alcaligenes faecalis*.

A microbial community

In the laboratory the micro-organisms of different species are studied in individual bacterial cultures. In practice, some test tubes and Petri dishes (round plastic transparent containers suitable for the growth of bacteria, yeast and mould colonies) are filled with a suitable specifically selected soil and with appropriate dilutions for each species that forms the community. Then, at fixed time intervals, the growth of mixed bacterial cultures (formed by all the different species that live in the microbial community) is monitored.

The objective is that of studying the dynamics of the microbes in a microcosm (an environment recreated and simplified in the laboratory that has the essential characteristics of a bigger natural system) that contains the hydrocarbon that should be metabolized.

This is the first step undertaken by researchers to understand the way in which bacterial cells stick to the particles of fuel oil or petroleum and also to be able to get to know the complicated mechanism of interactions that take place between the various micro-organisms that share the same habitat. If we want to go into further detail regarding the daily life of the '**sea cleaners'**, microbiology studies are not sufficient and it is necessary to resort to molecular biology because the bacterial cultures are treated with various molecular analysis techniques to be able to study the DNA image. In this way some DNA traits of the microbes are cloned and their dimensions and precise position are quantified.

To better understand the different jobs of these biologists, let us imagine a bacterial cell as a small village that is being flown over by a glider: if the pilot is a microbiologist he will fly at an altitude of 200m and will take a photograph of all the residential area and its surroundings, if the pilot is a molecular biologist he will descend to an altitude of 20m and in his photograph the streets, the roofs and windows of the houses will be clearly visible and some shop sign boards will be legible. Both the photographs are equally necessary to understand the name of the village and how life goes on in and out of it.

Along the Sicilian coasts

The Institute for Coastal Marine Environment - Messina section (Istituto per l'Ambiente Marino Costiero sezione di Messina IAMC – ME) is devoted to the study of hydrocarbonclastic bacteria and their geographic distribution, to increasing the knowledge of their genetic characteristics, to understanding how they work and their potential use in the processes of naturally decreasing the marine environments contaminated by oil. This Italian research institute coordinates different national and international projects and their possible utilization in environmental remediation operations. In this context, numerous areas of the Sicilian coast are monitored with the objective of analysing the distribution of the different taxonomic groups, i.e. the attribution of a nomenclature that allows the classification of the oil-eating bacteria by means of microbiology and molecular biology techniques, as in the case of the bacteria discovered in the sea waters around Venice. Even these 'cleaners' were given a surname and name, enabling us to know them better. In 2003, among the different bacteria that were isolated, some new genera were described: Alcanivorax whose genetic outfit has been totally sequenced (i.e. all the traits of its DNA are known), Oleiphilus, Thalassolituus and Oleispira. All the bacterial species belonging to these four genera are formed by constrained marine micro-organisms capable of surviving only in sea water or by organisms defined as ubiquitous because they can be found in oceanic environments particularly where oil is present. A detail about the life of bacteria belonging to the genus Oleispira is that they are psychrophile - this means that their ideal growth temperature is below 15°C, in particular, they live well at a temperature between 2 and 4°C.

Research in Antarctica

For a couple of years now, also in Antarctica researchers have been carrying out studies on bacteria capable of reducing hydrocarbon pollution in the sea. In February 2005 we read the news about a three-year expedition of Italian researchers whose intent is to increase the knowledge regarding Antarctic bacteria and cyanobacteria so as to understand their biodiversity and discover new mixtures with possible applications in biotechnology. During the first two years the researchers will carry out some sampling and some preliminary treatments of the samples collected in Antarctica at the 'Mario Zucchelli' Station. During the third year of the project, work on the collected and treated material will take place in Italy, in the competent laboratories. Sampling is the process of selecting specimens such as water columns, surface water, sediment and ice cores, with living organisms (in particular, bacteria and sponges).

But why so much interest for Antarctic marine organisms?

Oceans represent more than 70% of the Earth, every drop of sea water is animated by micro-organisms, the majority of which Man is not familiar with: it is estimated that 9 out of 10 species of bacteria are unknown! Bacteria that live in cold seas are very promising because they have adapted to constantly low temperatures, therefore to grow and survive they have had to optimize their characteristics. Furthermore because of their capacity to adapt in extreme environments, these bacteria have not been found in other ecosystems yet and therefore belong to unknown genera and new species. The study of the mechanisms with which they deal with the 'job of eating oil' is of great interest from a biotechnological point of view to understand how they are made and what kind of enzymes and compounds they produce to degrade oil and its derivatives. In fact these bacteria are very efficient in climatic conditions that normally produce a slowing down of the metabolism. Very simply, biotechnologies try to utilise the perfect instruments that have always been known in the bacterial micro-world.

From the laboratory to the sea

Michail Yakimov, the researcher at IAMC – ME who coordinates the European project Comode (Communities of marine micro-organisms for oil degradation) can virtually answer this fundamental question, quoting his declarations 2003, regarding the new bacteria genera discovered in the waters off the Sicilian coast (Alcanivorax, Oleiphilus, Thalassolituus and Oleispira): "We are in the presence of truly highly specialized biological machinery and as such they cannot but have a high affinity towards hydrocarbons, utilising them as a means of sustenance. The result is, in fact, the degradation of recalcitrant compounds (oil) with the production of autochthonous biomass, water and gas. Our experiments have demonstrated that in the presence of nutrients, hydrocarbonclastic bacteria respond promptly to the input of oil, becoming, in 10-15 days, the predominant bacterial population." In fact, in the last years, this research unit has carried out experiments of bioaugmentation (i.e. increase in the number of oil-eating microbes indigenous to an area of the sea) which have been implemented by adding suitable densities of hydrocarbonclastic bacteria to the natural environment, in polluted areas of the sea. This has allowed the scientists to observe the possible acceleration of the natural processes of biodegradation of oil on an average scale.

Before the research began experiments directly in the sea, a series of tests were started in the laboratory. These experiments were carried out on a small scale in microcosms that demonstrate how, in the presence of suitable nutrients, the hydrocarbonclastic bacteria promptly degrade the oil introduced into the environment, proliferating rapidly and reaching a share of about 90% of the total bacterial population. The researchers utilized compounds that contain nitrogen and phosphorous as suitable nutrients because they promote the development and the activities of these bacteria. The quantity of these elements that was added was instrumental to speed up the degradation of oil, that injects a greater quantity of carbon into the environment respect to normal conditions.
These experiments were the occasion to study in more detail how these bacteria degrade oil by means of particular water-soluble compounds that they themselves produce: biosurfactants. Biosurfactants emulsify oil into drops so that the bacterial cell can absorb them more easily. The final products of oil degradation by the agency of hydrocarbonclastic bacteria are carbon dioxide molecules, water and new biomass. In fact, on finding food available in their environment, the bacterial colonies proliferate very rapidly.
The experiments carried out on small scale were only appetizers for oil-eating bacteria since researches then continued on a medium scale. The capabilities of hydrocarbonclastic bacteria were also studied in more capacious containers, the mesocosms. The latter are tanks filled with sea water and sediments, collected off the coast in sea waters that have been contaminated with oil. Within them sea currents are simulated with appropriate machinery. Some parameters are monitored with the help of a sensor that registers the temperature, the pH and the quantity of oxygen present in the water. These studies have allowed us to understand how bioremediation techniques could be accomplished directly in the sea.

Bioremediation

Both the Earth and some parts of the sea can be polluted by a high concentration of contaminants of organic origin derived from natural or industrial processes.
One of the most up-to-date techniques is bioremediation. This technique is also considered sustainable because its application is considered at a low risk of secondary effects on terrestrial and marine ecosystems.
It involves the use of micro-organisms or plants to clean up an area which contains unwelcome compounds.
Bioremediation is based on the use of micro-organisms and plants that are specifically identified in the laboratory because they are capable of degrading a particular pollutant in particular conditions with maximum efficiency.
The practical realization of this technique is planned in successive phases:

- the pollution situation is studied, pinpointing the kind of pollutant, evaluating its concentration in the environment and its dangerousness for the ecosystem in which it is found. It is necessary to evaluate the extension of the polluted area and assess if the latter can modify its dimensions in time;
- to understand with which species of bacteria the pollution is to be attacked in the best and most convenient way;
- to isolate the specific strain of the micro-organism or of the microbial community necessary to reach the objective;
- to increase the number of bacterial colonies in appropriate quantities providing them nutrients;
- to inoculate, that is, physically introduce bacteria in the polluted area and to monitor ‘how much they eat’ and at what speed, and to control the products that are formed as a result of the degradation processes of the pollutant.

In the case in which the pollutant is oil, in other words, a mixture of hydrocarbons, the biodegradation process can take place by means of an aerobic or anaerobic metabolic mechanism. In bioremediation activities, the aerobic process in which the biodegradation of oil can take place by means of bacteria, has often proved to be more significant because the speed at which oil is eaten is greater. When the amount of oxygen decreases to such a point that it is insufficient for the bacteria to feed on, then we say that oxygen has become the limiting factor of the reaction and the speed of the biodegradation processes decreases till it comes to a standstill.

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