Oil spills

Mum: Grandma and I watched TV today and heard that a ship accident at sea caused an "oil spill" that seriously damaged the environment. What does it mean, an "oil spill"?

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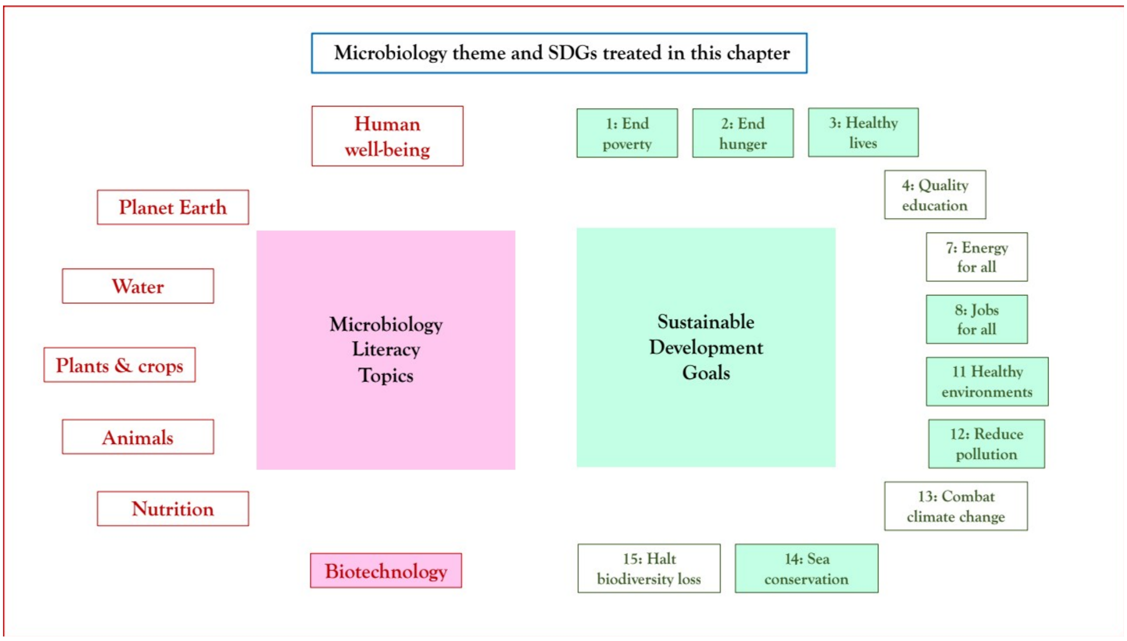
Storyline

We cannot imagine daily life without expenditure of a huge amount of energy that we obtain by consuming the fossil fuels, such as coal, natural gas and, especially, crude oil which is a mixture of liquid petroleum hydrocarbons (LPHs), organic molecules consisting of carbon atoms bonded to each other and to hydrogen atoms. Most, if not all, LPHs are harmful for living beings due to their toxicity, mutagenicity and carcinogenicity (ability to cause cancers). Crude oil and other fossil fuels were formed 50-350 million years ago and are now found in the deep subsurface in various locations worldwide. In order to exploit oil, it must be extracted from its reservoirs and transported around the globe. When either of these operations go wrong, due to various types of accidents, serious contamination of the environment with LPHs can occur. *Oil spills* refer to accidental releases into ocean waters of liquid petroleum hydrocarbons from ships, refineries or oil rigs, and represent one of the most important types of disaster for the marine environment. In addition to killing fish, marine mammals and birds, when an oil slick reaches the shore, it damages wildlife littoral (near shore) habitats and the beaches, thus affecting terrestrial biota and human settlements. Large accidental ocean discharges, for example, the *Amoco Cadiz* and *MT Haven* oil tanker accidents, and the *BP/Deepwater Horizon* discharge, are commercial and environmental catastrophes. Normally, it takes month-long (sometimes year-long) oil cleaning operations to bring the areas around an accident back to normality. For this reason, major efforts have been made to mitigate oil spills with the help of new technology, especially biotechnology, in the attempt to achieve relevant Sustainable Development Goals (SDGs).

The Microbiology and Societal Context

The microbiology: hydrocarbon-degrading marine microbes; bioremediation of contaminated marine environment; biostimulation and bioaugmentation strategies to enhance bio-cleaning efficacy. *Sustainability issues*: oil spills and marine biota and human health; seawater, sediments and coastal pollution; biotechnology to combat oil spills and eliminate pollution.



Some numbers concerning oil rig and very large crude carrier (VLCC) oil spills

• On March 16, 1978, the VLCC oil tanker *Amoco Cadiz* broke in half and sunk off the coast of Brittany (France), letting its cargo of 246,000 metric tons of light crude oil spill into the waters of the English Channel. The slick spread quickly, covering an area of 1440 square miles. 76 beaches suffered oil spill effects, and more marine life was killed than by any other oil spill previously recorded.
• In June 1979, a collapse of the oil well *Ixtoc 1*, operated by Mexico's state company Pemex in the Gulf of Mexico, resulted in the discharge of approximately 480,000 cubic meters of oil. The oil slick resulting from the blowout measured around 1100 square miles and affected Rancho Nuevo, a region of the Mexican coastline housing prominent nesting sites for Kemp's Ridley sea turtles.
• On April 11, 1991, MT Haven, carrying about 144,000 tons of crude oil, exploded off the coast of Genoa, Italy. As a result of the accident, 30-40,000 metric tons of oil poured into the sea, while the residual 80,000 metric tons partially burned during the fire or went down in the sunk tanker. For the next 12 years, the Mediterranean coast of Italy and France, especially around Genoa and southern France, was polluted.
• In April 2010, a spill from a seafloor oil gusher led to the explosion of the British Petroleum oil rig, *Deepwater Horizon*. The oil well blowout discharged approximately 500,000 metric tonnes of oil and at least 250,000 metric tonnes of natural gas into the deep water of the Gulf of Mexico. The accident is considered to be the largest oil spill in the petroleum industry's history, and also made extensive damage to the marine environment. According to the Centre for Biological Diversity, the oil spill killed over 82,000 birds, 25,900 marine mammals, 6,000 sea turtles, and tens of thousands of fish, among others. It had a major impact on regional seafood industries and cost BP in excess of $ 60b (https://espis.boem.gov/final%20reports/5518.PDF).

Oil spills: the Microbiology

1. ***The ocean contains significant numbers of diverse naturally-occurring (autochthonous) microbes that make a meal of crude oil***. While only large accidental ocean discharges that result in oil spills receive most of the attention, smaller and chronic incidents like pipeline and tanker leaks are a regular occurrence. In all cases, as a result of human activity, oil is introduced into the sea at a global scale, leading to the widespread distribution of LPHs in the marine environment. Luckily for us, the ability to degrade petroleum hydrocarbons, i.e. converting them to biomass and CO_2, is a metabolic feature found relatively frequently among marine microorganisms, which are designated hydrocarbonoclastic or oil-eating bacteria (see ***Abo*** in the **MicroDefenders Portrait Gallery**). Typically, these microorganisms comprise less then 0.1% of the marine microbiota in pristine ecosystems, but "bloom" to become prevalent (up to 90%) in oil-polluted ecosystems. It is hard to overestimate their contribution to prosperity of life on our planet, since without their 'invisible' metabolic activities our oceans would turn into muddy lifeless water bodies covered with a thick film of crude oil.

Since crude oil is a complex mixture of different hydrocarbons, the complete degradation of LPHs can be achieved only though the collective action of associated but independent microorganisms, specialized to degrade different LPH fractions. Aliphatic hydrocarbons or alkanes varying in sizes and structures can constitute up to 50% of crude oil, depending on the oil source, and are typically converted to primary alcohols by oxidation of the terminal carbon, before entering general metabolism via the ß-oxidation pathway. Polyaromatic (cyclical) hydrocarbons are amongst the most important components of crude oil, and their molecular stability, hydrophobicity and low water solubility are some of the main factors contributing to their persistence in the marine environment. Nevertheless, these hydrocarbons serve as a meal for different marine microbes which initiate degradation by the incorporation of both oxygen atoms of an O_2 molecule into the substrate by aromatic ring-hydroxylating dioxygenases. A second type of dioxygenase then opens the hydroxylated aromatic ring, which then enters central metabolism. In both cases, metabolism of the LPHs converts them to microbial biomass, CO_2 and water.

2. ***Bioremediation of oil contaminated marine environment***. Clean-up and recovery from an oil spill is a very complex and difficult operation and depends upon many factors, including the type of oil spilled, the temperature of the water, the location of the spill, and the types of shorelines and beaches (if any) involved. Elimination of all treatable negative consequences of oil spills may take weeks, months or even years. Human operations aimed to combat accidental oil discharge typically start with physical clean-ups, involve various technologies, among which the controlled burning, oil skimming, and use of chemical dispersants are most frequently applied. Controlled burning can significantly reduce the amount of discharged oil in seawater, but can cause significant air pollution. Skimming is a very efficient operation to collect floating discharged oil by deploying large floating sorbents (called *booms*) as barriers that round up and reduce the dispersal of an oil slick, and then removing the contained oil by skimming. Unfortunately, "corralling" and skimming require calm waters at all phases of the process, which is rare on the open sea.

Application of a chemical dispersant allows rapid dissipation of an oil slick by dispersing the oil in the form of micro-droplets throughout the water column. While this is a very efficient operation to remove discharged oil from the sea surface, it does not resolve the problem of intoxication of marine biota dwelling underwater and in the sediment.

To clean up or remediate contaminated marine environment, dispersed oil must be eliminated. It is exactly this process that is named bioremediation and that primarily refers to the capabilities of marine hydrocarbonoclastic bacteria to clean up and restore polluted habitats. Functioning as a part of a biological network composed of diverse microbial community members, they are responsible for initial transformation of toxic LPHs into harmless intermediates, which finally end up with production of microbial biomass, CO_2 and water.

3. ***Biostimulation strategies to enhance the bioremediation efficacy***. Understanding microbial community functioning is essential to performance management during remediation of a polluted marine environment, in particular for identifying and alleviating rate limiting parameters. A number of factors can influence the response of hydrocarbonoclastic bacteria in the event of a spill, and also their efficacy in degrading hydrocarbons. Low temperature of deep and polar ocean ecosystems (usually 2-4°C), low levels of nutrients (nitrogen and phosphorus) in seawater, and low bioavailability of LPHs (especially at low temperatures), are among the most important factors, which negatively affect the bioremediation performance of an oil-contaminated marine environment. Although we cannot increase the temperature of the sea at a contaminated site, we can nevertheless stimulate the hydrocarbonoclastic bacteria to do their job better and faster. This may be achieved by application of N- and P-containing fertilizers, to alleviate rate limiting nutrient concentrations, and dispersants, to increase rate limiting LPH bioavailability.

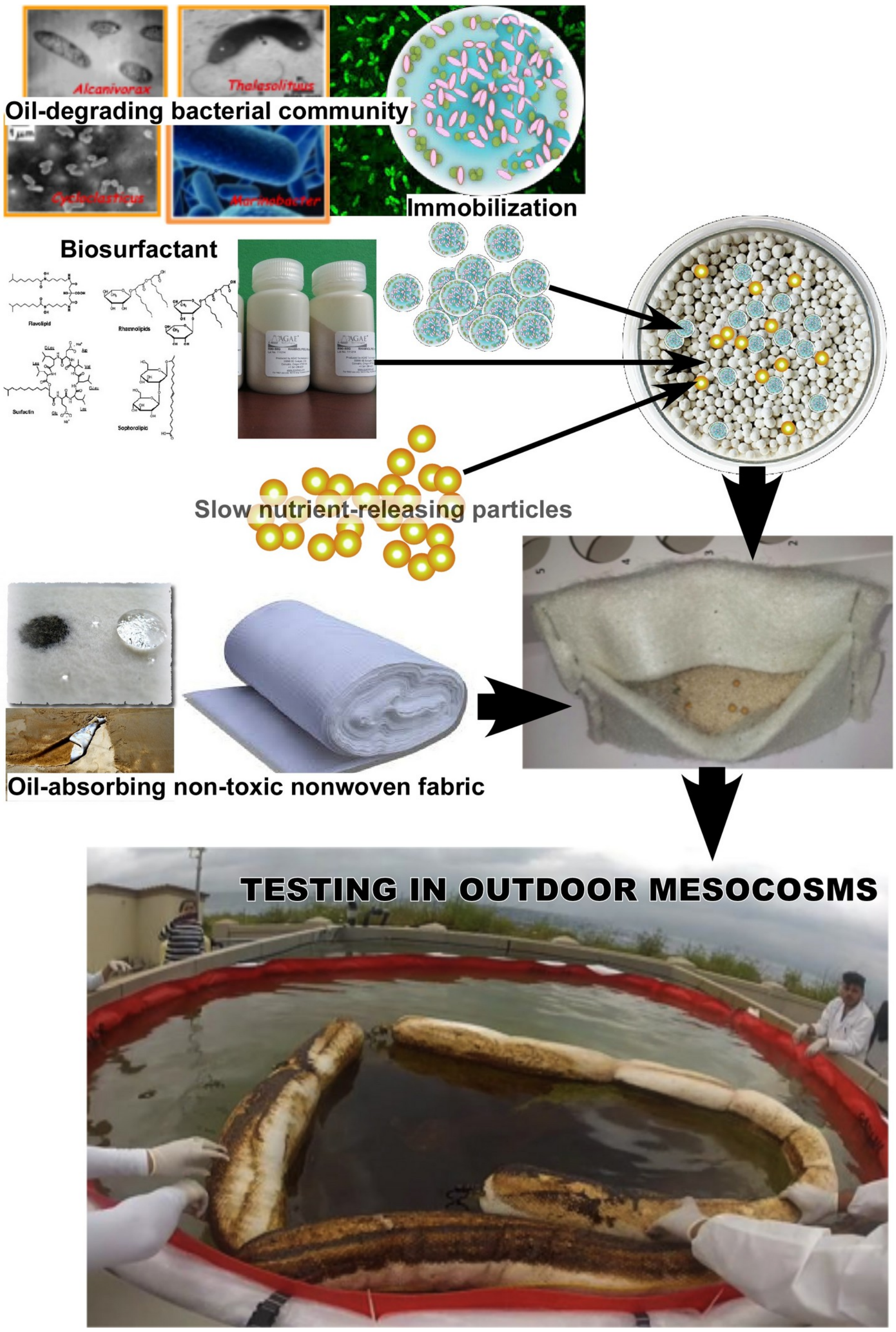
In an effort to achieve high oil degradation efficiency during the biostimulation, researchers have investigated many fertiliser recipes and compositions to optimize the process. The best results were obtained through application of various slow nitrogen- and phosphorous-releasing hydrophobic matrices. These dual capacities are very important since, after distribution of the fertiliser over the oil spill by planes (aerial spraying) or by spraying from the ships directly, such composite particles will concentrate at the oil slick, due to hydrophobic-hydrophobic interactions (oil is also hydrophobic), analogous to the mutual attraction of magnetic materials, instead of sinking or dispersing and becoming diluted to effectively zero concentration in the water. Slow release of nutrients from the particles, in turn, determines long-term effectiveness of biostimulation by delivering required nutrients to hydrocarbon-degrading microbes (which are also gather at the oil slick) over a long period of time.

As stated above, dispersant use exposes aquatic life to dispersed oil. But small droplets are more readily biodegraded by hydrocarbonoclastic bacteria. Therefore, there is an important requirement: the dispersant should be minimally toxic. While many chemical dispersants exhibit significant toxicity, many microbes fortunately produce surface-active compounds, called bioemulsifiers and biosurfactants, that act as dispersants. Application of these bio-based substances as oil dispersants is a very attractive approach in biostimulation of oil-eating marine microbes and is being developed in many biotechnological laboratories to enhance the bioremediation efficacy of oil-contaminated sites.

4. ***Bioaugmentation strategies to enhance the bioremediation efficacy***. Although biostimulation of oil-eating microbes is a very promising scenario to combat oil spills, the time it takes for oil-eating microbes to multiply to maximal concentrations mediating maximal degradation rates is significant. And the cleaner, more pristine, the water before the spill, the lower the concentration of existing hydrocarbonoclastic bacteria, and the longer the period during which bacterial numbers are rate limiting for biodegradation. During this period of multiplication from low pre-spill bacterial concentrations to maximal post-spill concentrations, the oil is effecting serious ecological damage. Bioaugmentation is the process of addition of metabolically active oil-eating microbes in significant amounts to alleviate initial microbial biomass rate limitation and immediately attain high rates of bioremediation. Ideally, indigenous, i.e. varieties of oil-eating microbes originally present in the contaminated location, should be implemented to boost the oil degradation. Fortunately for us, many marine hydrocarbonoclastic bacteria are truly cosmopolitan, i.e. widely distributed in the world's oceans, and their utilization in bioaugmentation scenarios does not create concerns about invasive new species.

5. ***Integrated biotechnological solutions for combating marine oil spills.*** Oil spill biotechnology is a rapidly developing, exciting topic. For example, as can be seen in a visit to the website of a recently completed European Project, "Kill•Spill" (2012-2016), a self-regenerating *bio-boom* oil sorbent with enhanced absorption and bioremediation capabilities is being developed. As mentioned above, the most common equipment used for controlling and combatting modest and small-scale oil spills is the application of large floating sorbents in forms of sausages, called *oil booms*. Until now, after saturation with oil, the boom sorbents must be replaced with new material, which engenders operational and waste treatments costs. But what can happen if the boom sausages are filled with petroleum-degrading microbial consortia? To proceed even further and more advanced, what can happen if the boom sausages are filled with (i) petroleum-degrading microbial consortia, plus (ii) slow-releasing nutrients, plus (iii) bio-based dispersant? In the presence of absorbed oil, the last two additives will activate the biomass of oil-eating microbes that will thus regenerate the sorbent and increase its lifetime. This product is

referred to as the Bio-Boom.



The preparation and testing of the Bio-Boom. In the presence of the efficient sorbent, the oil contaminant is absorbed and slowly degraded by the hydrocarbon-degrading community placed within the Bio-Boom.

Relevance to Sustainable Development Goals and Grand Challenges (https://sdgs.un.org/2030agenda)

Small-, medium- and, especially, large-scale oil spills occur during oil extraction, transportation and processing. They significantly pollute marine environments and damage marine and coastal biota. Prevention of these negative effects of oils spills through the action of hydrocarbonoclastic, or oil-eating natural microbial populations, relates to several SDGs including:

- **Goal 1. End poverty**. Fishing is an important source of employment and income in coastal communities of low-income countries. Oil spills that poison local fish stocks, and prevent or diminish the harvesting of finfish and shellfish, may also increase poverty in such communities. The acceleration of a return to normality, though bioremediation, reduces the impact of oil spills on poverty.
- **Goal 2. End hunger.** Fishing is an important source of food in coastal communities of low-income countries. As indicated for Goal 1, bioremediation can reduce shortages on finfish and shellfish sources of food.
- **Goal 3. Healthy lives.** Hunger favours the eating of contaminated food, including oil-contaminated seafood, which can have significant negative health consequences. As indicated for Goal 1, bioremediation can reduce the time taken to restore healthy seafood.
- **Goal 8. Promote full employment.** See Goal 1.
- **Goal 11. Make cities and human settlements inclusive, safe, resilient and sustainable.** Coastal settlements are often in regions of natural heritage that are vulnerable to marine disasters. During the accident on Deepwater Horizon oil platform, discharged oil affected more than 450 miles of the Gulf of Mexico coastline, considerably damaging the coastal ecosystem, and negatively impacting coastal communities. Normally, many years are needed for an ecosystem to recover fully from an oil spill, so scientists have been looking into ways to expedite the clean-up of affected areas. There are many positives that can be taken from production of oil-eating microorganisms and their subsequent application by spreading over contaminated areas. Accelerated and facilitated LPHs degradation would help ecosystems to quickly recover from an oil spill disaster turning it into healthy environment much faster.
- **Goal 12. Eliminate pollution.** The accidental entry of crude oil into marine environment leads to changes in natural microbial communities, decreasing diversity but, fortunately for us, with some microorganisms positively responding to and playing vital role in the degradation of LPHs. Without these tiny creatures, the discharged oil is likely to accumulate producing a thick global ocean surface oil slick, affecting the algal photosynthesis, oxygenation of seawater and intoxicating marine animals and planktonic and benthic microbiota.
- **Goal 14. Sea conservation.** Both chronic and accidental oil spills cause drastic effects on all levels of marine planktonic and benthic life: they damage the health of aquatic mammals, birds and fishes, affect coastal flora and fauna and shore vegetation. Discharge of crude oil provokes significant shifts in the marine microbial communities by decreasing their diversity, but at the same time initiating a bloom in the abundance of hydrocarbonoclastic bacteria. These microorganisms play pivotal role in the biodegradation and ultimate fate of the oil, thus remediating polluted habitats. The need to minimize environmental damage makes it worth developing effective and easy-to-apply oil slick combating methods and technologies, including usage of oil-eating microorganisms. Comprehensive study and understanding of their physiology is necessary for further use in cleansing technologies and for the conservation of the clean sea.

Potential Implications for Decisions

1. Individual

Currently, our daily lives are based on consumption of enormous amount of fossil fuels such as coal, natural gas and, especially, crude oil that are used to produce energy and various types of chemicals and materials. Development of alternative, environmentally friendly sources of energy and green chemistry processes is more compelling but challenging. However, we must abandon this fossil fuel-dependent lifestyle before it's too late. To achieve this, humanity needs a cohort of young scientists and entrepreneurs that will create and implement these innovative principles and methods. A key individual decision is therefore: should I personally become part of the global effort to find and implement environmentally-friendly alternatives to fossil fuels?

2. Community policies

a. Setting up alternative types of energy production (hybrid wind and solar electric systems; tidal energy, etc.).

b. Setting up the biological systems to produce biogas and biofuel using local organic wastes and livestock management by-products.

c. Oil refineries and harbours for oil tankers should be located very remotely from the cities and national wildlife parks. Decreased consumption will decrease the risk of pollution due to minimizing the amount of extracted and transported crude oil.

3. National policies

a. Creating effective incentives and disincentives, such as taxation, to reduce use of fossil fuels and increase safety relating to their extraction, transportation and use, and tightening norms and fines for violators that cause oil pollution.

b. Setting up increasingly sophisticated observation systems (including satellite communication systems) to remotely detect and to monitor the genesis and spread of oil spills at all scales.

c. Setting up increasingly efficient and instantly-responding oil spill combat teams providing emergency services around the world.

d. Investment in scientific research to find efficient and reliable biotechnologies to enhance and accelerate cleaning-up facilities of oil-impacted marine habitats.

e. Investment in industrial implementation of these innovative biotechnologies.

Pupil Participation

1. Class Discussion

- ***a.*** How many alternative/green types of energy production can you name?
- ***b.*** How many chemical products made of crude oil can you name?
- ***c.*** What do you think will happen if oil spill occurs near of your home/city?
- ***d.*** Would you like to be as a volunteer to help the wild life impacted by oil spill?
- ***e.*** Discuss the principle of a rate-limiting parameter. Do you think the addition of something essential to a process that is not rate-limiting would increase process activity? Argue your response using a nitrogen fertiliser and a biosurfactant, as parameters influencing oil degradation

2. Pupil stakeholder awareness

- ***a.*** How we can reduce our daily dependence on fossil fuel consumption
- ***b.*** Would you like to be an environmental scientist or engineer resolving the problem of global pollution of our planet?
- ***c.*** Can you think of any way we can change the fact that modern humanity is completely dependent on crude oil and other fossil fuels? If so, when do you think this will happen?

3. ***Exercises***
    - ***a.*** How would you design a small-scale bio-boom barrier in the area with permanently leaking contaminants (around docks and pilings) or in environmental resources area, beaches and off-shore aquaculture hatcheries to protect them from modest and low-scale oil spills? What would go inside of bio-boom first?
    - ***b.*** How can you reduce the usage of fossil fuel consumption in your own city? Which form of alternative/green energy production systems, particularly adapted to your urban environment, would you install?
    - ***c.*** Which human activities consume most crude oil? How can we minimize this consumption?

4. ***Excursions***
    - ***a.*** Visiting an oil refinery, particularly the wastewater treatment plants installed there, to understand the role of hydrocarbonoclastic (oil-eating) microorganisms in oil degradation.
    - ***b.*** Visiting petroleum industries and companies providing services to them to know how their activities are designed to prevent accidental discharge of crude oil into environment.

The Evidence Base, Further Reading and Teaching Aids

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Glossary

Asphaltenes are molecular substances that are found in crude oil, along with resins, aromatic and saturated hydrocarbons. Asphaltenes consist primarily of carbon, hydrogen, nitrogen, oxygen and sulfur with the C:H ratio of approximately 1:1.2, depending on the asphaltene source. The molecular structure of asphaltenes is difficult to determine because the molecules tend to stick together in solution. These materials are extremely complex mixtures containing hundreds or even thousands of individual chemical species. Asphaltenes do not have a specific chemical formula: individual molecules can vary in the number of atoms contained in the structure, and the average chemical formula can depend on the source. Asphaltenes in the form of bitumen products from oil refineries are used as paving materials on roads, shingles for roofs, and waterproof coatings on building foundations.

Bioaugmentation or biological augmentation is the addition to contaminated sites of prokaryotic (archaeal and/or bacterial) cultures, possessing high metabolic (degradation) potential, required to speed up the rate of biological decomposition of a contaminant. Organisms that originate from contaminated areas may already be able to break down waste, but perhaps inefficiently and slowly. Bioaugmentation is a type of bioremediation in which it requires studying the indigenous varieties present in the location to determine if biostimulation is possible.

Bioremediation broadly refers to any process wherein a biological system (typically archaea, bacteria, microalgae, fungi, and plants), living or dead, is employed for removing environmental pollutants from air, water, soil, flue gasses, industrial effluents etc., in natural or artificial settings. The natural ability of organisms to adsorb, accumulate, and degrade common and emerging pollutants has attracted the use of biological resources in treatment of contaminated environment

Biostimulation involves the modification of the environment to stimulate existing archaea and/or bacteria capable of bioremediation. This can be done by addition of various forms of rate limiting nutrients or fertilizers and electron acceptors, such as phosphorous, nitrogen and oxygen. Alternatively, remediation of hydrocarbon contaminants in anaerobic environments may be stimulated by adding electron donors (organic substrates), thus allowing indigenous microorganisms to use these contaminants as electron acceptors.

Chemical dispersants. A chemical dispersant is a mixture of surface-active compounds (see below) or emulsifiers and solvents (substance that dissolves a solute, resulting in a solution) that help break oil into small droplets following an oil spill. Small droplets are easier to disperse throughout a water volume, and small droplets may be more readily biodegraded by oil-eating microorganisms in the water.

Fertilisers. A fertiliser is any material of natural or synthetic origin that is applied to soil or to water in form of various substances used by organisms to survive, grow, and reproduce. For most modern agricultural and environmental practices, fertilization focuses on three main macro nutrients: nitrogen (N), phosphorus (P) and potassium (K) with occasional addition of various supplements for micronutrients.

Fossil fuel is a hydrocarbon-containing material formed naturally in the earth's crust during the anaerobic decomposition of buried dead (plants and animals) that is extracted and burned as a fuel. The conversion from these materials to high-carbon fossil fuels typically require a geological process of millions of years. Main fossil fuels are coal, crude oil and natural gas. Fossil fuels may be burned to provide heat for use directly (such as for cooking or heating), to power engines (such as internal combustion engines in motor vehicles), or to generate electricity.

Hydrocarbonoclastic bacteria, also known as *hydrocarbon degrading bacteria*, *oil degrading bacteria* or *HCB*, are a heterogeneous group of prokaryotes which can degrade and utilize various hydrocarbon compounds, such paraffins and (poly)aromatics, as the principal source of carbon and energy. Despite being present in most of environments around the world, several of these specialized bacteria live in the sea and have been isolated from polluted seawater.

Metalloids. A metalloid is a type of chemical element which has a preponderance of properties in between, or that are a mixture of, those of metals and nonmetals. There is no standard definition of a metalloid and no complete agreement on which elements are metalloids. The six commonly recognized metalloids are boron (B), silicon (Si), germanium (Ge), arsenic (As), antimony (Sb) and tellurium (Te). Five elements are less frequently so classified: carbon (C), aluminium (Al), selenium (Se), polonium (Po) and astatine (At). Despite the lack of specificity, the term remains in use in the literature of chemistry.

β-Oxidation pathway. In biochemistry, ß-oxidation is the catabolic process by which fatty acids molecules are broken down to generate acetyl-CoA, which enters in the central metabolic and respiratory pathways, such the citric acid cycle, to generate energy. It is named as such because the beta (second, following the Greek alphabetical order) carbon of the fatty acid undergoes oxidation to a carbonyl (-COOH) group.

Pristine ecosystem. An ecosystem in its original, unspoiled conditions.

Resins. Most crude oil and plant resins are composed of terpenes, a class of unsaturated hydrocarbons with the formula $(C_5H_8)_n$. Comprising more than 30,000 compounds, these compounds are further classified by the number of carbons: monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), as examples.

Surface-active compounds or surfactants. The word "surfactant" is a blend of ***surf**ace-**act**ive **a**ge**nt***, coined since 1950s. Surfactants are compounds that decrease the surface or interfacial tension between two liquids, between a gas and a liquid, or between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers or dispersants.