Essay: PROJECT ON MICROBIAL DEGRADATION OF ORGANIC COMPOUNDS; IMPLICATIONS FOR THE BIOSPHERE

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1.0. Introduction/Background to the Study

Accumulation of organic pollutants can cause serious problems in various environmental compartments due to their toxicity, persistence and bioaccumulation. Depending on the environmental compartment in which organic compounds are present (e.g. soil, benthic sediments, surface and ground waters), they can undergo slow changes resulting from biodegradation, a major degradation process.

Biodegradation is a multi-step process, which is taking place in the presence of a number of microorganisms that often act synergistically. The range and rate of biodegradation processes depend on several factors such as the composition and activity of bacterial flora, the properties and "age" of a pollutant, the content of nutritive ingredients and physio-chemical properties of the medium in which the reactions occur.

In 1990 Joseph and Rita concluded that the biodegradation of petroleum and other hydrocarbons in the environment is a complex process, who's quantitative and qualitative aspects depend on the nature and amount of the oil or hydrocarbons present, the ambient and seasonal environmental conditions, and the composition of the autochthonous microbial community.

Microbial degradation of oil has been shown to occur by attack on aliphatic or light aromatic fractions of the oil, with high-molecular-weight aromatics, resins, and asphaltenes considered to be recalcitrant or exhibiting only very low rates of biodegradation, although some studies have reported their removal at high rates under optimal conditions. In aquatic ecosystems, dispersion and emulsification of oil in oil slicks appear to be prerequisites for rapid biodegradation; large masses of mousse, tar balls, or high concentrations of oil in quiescent environments tend to persist because of low surface areas available for microbial activity. Petroleum spilled on or applied to soil is largely adsorbed to particulate matter, decreasing its toxicity but possibly also contributing to its persistence. Biodegradation rates generally increase with increasing temperature; ecosystems exposed to extremely low temperatures degrade hydrocarbons very slowly. The microbial degradation of petroleum in aquatic environments is limited primarily by nutrients such as nitrogen and phosphorus; salinity and pressure may be important in estuarine and deep-sea regions, respectively. Oxygen, nutrient concentrations, moisture, and pH are predominant factors in determining biodegradation rates in soil. Hydrocarbon degradation by microbial communities depends on the composition of the community and its adaptive response to the presence of hydrocarbons. Bacteria and fungi are the key agents of degradation, with bacteria assuming the dominant role in marine ecosystems and fungi becoming more important in freshwater and terrestrial environments.

Adapted communities, i.e., those which have been previously exposed to hydrocarbons, exhibit higher biodegradation rates than communities with no history of hydrocarbon contamination. The mechanisms of adaptation include both selective enrichment and genetic changes, resulting in a net increase in the number of hydrocarbon utilizing organisms and in the pool of hydrocarbon-catabolizing genes within the community. The association of such genes with plasmid DNA may also lead to an increased frequency of plasmid-bearing microorganisms. Seeding of petroleum-contaminated water or soils with hydrocarbon utilizing bacteria has met with some success, particularly in situations in which chemostats or fermenters have been used to control conditions and reduce competition from indigenous microflora. Probably the most significant recent developments in hydrocarbon biodegradation ecology involve the demonstration of anaerobic metabolism of aromatic hydrocarbons and application of DNA probes to the analysis of genetic adaptation of microbial communities upon exposure to aromatic hydrocarbons. The expansion of the DNA probe method to the detection of genes encoding the catabolism of other classes of hydrocarbons, as well as to the detection of specific RNAs, should revolutionize the study of the microbial degradation of hydrocarbons in the environment and remove many of the biases and limitations imposed by conventional culture techniques. Recombinant DNA technology may also allow the construction of bacterial and fungal strains exhibiting improved capability for hydrocarbon metabolism and suitability as seed organisms for the elimination of hydrocarbon pollutants, but field studies must await the resolution of the issue of release of genetically engineered microorganisms into the environment.

However, the scope of microbial degradation of organic compounds could be widened with its implications on the biosphere- either positive or negative as the case may be.

Organic Compounds

Organic compounds are any compounds that contain carbon (with a few exceptions). All other compounds are referred to as inorganic compounds. In almost all organic compounds, carbon atoms are bonded to hydrogen atoms or other elements that are near carbon in the periodic table, especially nitrogen, oxygen, sulphur, phosphorus, and the halogens. Other elements, including metals and non-metals, may also be present.

The carbon in organic compounds forms four bonds, which enables it to form complex, branched-chain structures, ring structures, and even cage-like structures.

To recognize a compound as organic, look for an indication of the presence of carbon in its name, chemical formula, or diagram. However, there are a few exceptions to this rule.

Certain compounds that contain carbon are classified as inorganic carbon compounds. These include any compounds that contain carbonates, (i.e., $CaCO_3$); carbides, (i.e., SiC); and oxides (i.e., CO_2, CO). What are some common organic compounds?

Two common organic compounds are hydrocarbons and alcohols.

i. Hydrocarbons

A hydrocarbon is an organic compound that contains only the elements carbon and hydrogen. The simplest of all organic compounds is the hydrocarbon molecule called methane (CH_4) which consists of a carbon atom bonded to four hydrogen atoms. Other hydrocarbons are formed by linking two or more carbons together to make a chain.

ii. Alcohols:

An alcohol is a kind of organic compound that contains C, H, and O in a specific structure.

Microbial Degradation of Organic Compounds

Biological decomposition of persistent organic pollutants by microorganisms is one of the most important and effective ways to remove these compounds from the environment. Usually, the biodegradation of organic compounds is a multi-step process, which is taking place in the presence of a number of microorganisms that often act synergistically. The biodegradation rate in real aquatic environment depends on characteristics of the aquatic system, the presence of particulate matter, concentration of inorganic and organic nutrients, temperature, oxygen concentration, redox potential and adaptation of the microbial population. In soil, the range and rate of biodegradation processes depend on several factors such as soil temperature, soil moisture content, the composition and activity of bacterial flora, the properties and “age” of a pollutant and the content of nutritive ingredients.

Microorganisms, in comparison to other organisms, have a particular predisposition to adapt to novel environmental conditions and the ability to utilize compounds that are not the products of their own metabolism, as substrates needed for energy production and structure building. In general, microorganisms can be divided into autotrophic that use carbon dioxide as a carbon source, and heterotrophic that obtain carbon from decomposition of organic matter and man-made organic substances. The processes in the organic compounds take place due to direct contact with the microbial cellular enzymes. One molecule of enzyme can catalyze decomposition of millions of organic molecules per minute.

The reactions mediated by microorganisms are, to a large degree, similar to those occurring in higher organisms. Therefore, the aromatic compounds undergo epoxidation and hydroxylation, the aliphatic ones are oxidized and degraded through β–oxidation pathway, and the nitro-organic derivatives are metabolized with the use of nitroreductases. Microorganisms can also mediate the processes that the higher organisms are not capable of, e.g. decomposition of aromatic ring or dehalogenation.

In general, there is a relatively large variety of microorganisms in the natural environment; a higher diversity has been observed in the microbial biocenoces associated with sediments than in ground waters originating in formations of large pore size and inhabited by mobile microorganisms. Microorganisms residing in sediments are sedentary, permanently bound and living in pores of small diameter.

The characteristic morphological types are gram-negative rods, among others, aerobic rods from the genera Pseudomonas, Flavobacterium, Azotobacter and Rhizobium, non-obligatory anaerobic species Aerobacter aerogenses, and anaerobic species from the genus Desulfovibrio.

From among the gram-positive bacteria, typical for this environment is the genus Arthrobacter, as well as bacterial spores from the aerobic genus Bacillus and anaerobic genus Clostridium.

Biodegradation of Organic compounds in Soil and Benthic Sediments

Soil is a mixture of organic, mineral, gaseous and liquid components inhabited by numerous microorganisms. Organic matter in soil consists of the remnants of decomposing plants and humic substances. The mineral components of soil are particles of weathering rocks, silt and hydrated oxides of Aluminium. Organic compounds are decomposed by microorganisms that live in soil; however, the rate of decomposition depends on the properties of an organic substance, microbial genotypes, pH, nutritive properties of soil, soil temperature, soil moisture content and soil adsorption characteristics. Enzymes cause microbial decomposition of substances, and it generally occurs after an initial latency period during which the microorganisms adapt to novel substratum. In many cases, the reactions taking place in soil are similar to those occurring in sediments, although usually only the surface layer of sediment contains oxygen. Most of the sediment constitutes the anaerobic compartment.

Polycyclic aromatic hydrocarbons get transferred into the environment as a result of the incomplete combustion of organic substances at high temperature. The ability of microorganisms to degrade polycyclic aromatic hydrocarbons depends on the number of aromatic rings in a given compound. Microorganisms can decompose compounds with 2 to 4 benzene rings and, at the same time, use the process as a carbon source. A larger number of rings, i.e. 5 to 6, give the compound higher resistance to microbial “attacks”.

Biodegradation of Organic Compounds in Ground- and Surface Waters

Phenoxyacetic acids are herbicides widely used in agriculture. These compounds are selective because grains are resistant to their herbicidal properties while leafy plants display sensitivity. Herbicides get into waters by means of diffuse run-offs from agricultural fields.

Degradation in the Present and Absent of Oxygen

The microbial degradation of hydrocarbons in the presence of oxygen has been known for many years. In all studied cases, molecular oxygen is used as highly reactive co-substrate in initial reactions catalyzed by enzymes termed oxygenases.

Due to this essential role of molecular oxygen doubts about the feasibility of anaerobic degradation of hydrocarbons have long prevailed. However, about 15 years ago reports on the isolation of anaerobic bacteria that could completely oxidize toluene to CO2 were published. In the meantime a variety of denitrifying, sulphate and iron reducing and phototrophic bacteria were isolated that could also anaerobically degrade toluene and other alkylbenzenes. In addition, the anaerobic oxidation of n-alkanes was demonstrated under conditions of denitrification, sulphate-reduction and methanogens.

Biodegradation pathway of Xenobiotic compound

In biodegradation processes, depending on the oxidation state of the pollutant, compounds can be either electron donors or electron acceptors. In the bacterial respiration, oxygen is the most common electron acceptor. In aerobic biodegradation of aromatic compounds, oxygen plays an important dual role: (1) act as an electron acceptor for the aromatic pollutants and (2) with the help of oxygenation reactions activate the substrate. The aerobic degradation of aromatic compounds has been widely studied; some polluted environments are often anoxious such as aquifers, aquatic sediments, and submerged soils, requiring alternative electron acceptors such as nitrate, Fe (III), and sulfate (Chakraborty and Coates, 2004; Wilson and Bouwer, 1997; Bouwer and Zehnder, 1993; Cao et al., 2009).

Biodegradation Pathway

Some of the xenobiotics like petroleum hydrocarbons, chlorinated aliphatics, benzene, toluene, phenol, naphthalene, fluorine, pyrene, chloroanilines, pentachlorophenol and dichlorobenzenes are rapidly and potentially degraded by the aerobic degradation process. Many bacterial consortia capable to grow on these chemicals they are producing enzymes which degrade toxic compounds to non-toxic compounds.

The degradation process can be divided into (1) aerobic and (2) anaerobic degradation

Aerobic biodegradation:

Xenobiotic compound + O_2 CO_2 + H_2O + biomass + residue(s)

Anaerobic biodegradation:

Xenobiotic compound CO_2 + CH_4 + H_2O + biomass + residue(s)

In the process of aerobic degradation, carbon dioxide is produced. If there is no oxygen, an anaerobic degradation process occurs and methane is produced instead of carbon dioxide. The conversion of biodegradable materials to gases like carbon dioxide, methane, and nitrogen compounds, this process is called mineralization. Mineralization process is completed, when all the biodegradable biomass is consumed and all the carbon is converted into carbon dioxide (Kyrikou et al., 2007). Alkanes consisting long carbon chains and straight structures considered to be more prone to aerobic biodegradation.

Aerobic degradation pathway of alkane degradation is the oxidation of the terminal methyl group into a carboxylic acid through an alcohol intermediate, and after all completes mineralization through β-oxidation. The aerobic degradation process of aromatic compound involves their oxidation by molecular oxygen; after oxidation steps intermediates are outcome, then it enters into central metabolic pathways, including the Krebs cycle and β-oxidation. During aerobic respiration microorganisms use oxygen to hydroxylate the benzene ring, resulting in the subsequent fission of the ring. Enzymes are involved in these processes are mono- and di-oxygenase enzymes, incorporate one or two atoms of oxygen, respectively, into the ring.

Anaerobic biodegradation pathway

Some pollutants are not mineralized by aerobic degradation process; they are highly recalcitrant due to increase in halogenations. Substitution of halogen, nitro and sulfo groups on the aromatic ring is increase the electrophilicity of the molecule. These xenobionts resist the electrophilic attack by oxygenases in aerobic degradation. Some recalcitrant that are persisting under aerobic condition are polychlorinated biphenyls (PCBs), chlorinated dioxins and some pesticides like DDT. It is necessary to overcome the high persistence of halogenated xenobiotics from the biosphere, for achieving these, reductive attacks by anaerobic bacteria is of great value. Anaerobic bacteria performed reductive dehalogenation either by a gratuitous reaction or a new type of anaerobic respiration, this process reduces the degree of chlorination and makes the product more accessible for mineralization by aerobic bacteria. During anaerobic degradation reductive dehalogenation is the first step of degradation of PCBs (Poly chlorinated biphenyl), dehalogenation done under anaerobic conditions where organic substrates act as electron donors. PCBs accept electrons to allow the anaerobic bacteria to transfer electrons to these compounds. Anaerobic bacteria are capable to degrade xenobiotics that are present in various anaerobic habitats like water laden soils, reticuloruminal contents, inter alia sediments, gastrointestinal contents, sludge digesters, feedlot wastes, groundwater, and landfill sites. The major groups of anaerobic bacteria that are capable of degrading xenobiotic compounds – Acidovorax, Bordetella, Pseudomonas, Sphingomonas, Variovorax, Veillonella alkalescens, Desulfovibrio spp., Desulfuromonas michiganensis, and Desulfitobacterium halogenans, D. oleovorans, G. metallireducens, D. Acetonicum.

Factors Affecting HC Biodegradation

Several environmental factors influence biodegradation of petroleum HC such as temperature, pH, nutrient and oxygen availability, salinity, pressure, and light. Bioavailability of contaminant is also an important aspect of biodegradation. Presence of HC degrading populations of microbes at sufficiently high levels is a prerequisite for an effective bioremediation. Occurrence and abundance of microorganisms in environment depend on availability and diversity of carbon sources. Surface soil has high organic matter but subsurface and deep layers has lower organic content. Because of a low amount of organic matter organisms in these regions are often dormant. Various physicochemical factors affecting HC degradation are described below (Maier, 2000).

• Oxygen availability: HC degradation takes place both in presence and absence of oxygen. However aerobic conditions are more favourable as oxygenases are the primary enzymes needed for degradation to occur. Oxygenases function in presence of oxygen, so degradation rates are higher in aerobic conditions as compared to those under anaerobic conditions.

• Nutrient availability: The process of biodegradation can be enhanced by addition of essential nutrients such as nitrogen and phosphorous. In case of petroleum oil spills where nitrogen shortages can be acute; carbon, nitrogen and phosphorous are added in the ratio of approximately.

Biosphere

Biosphere includes all living communities on earth, from the profusion of life in the tropical rain forests to the photosynthetic phytoplankton in the world's oceans. In a very general sense, the distribution of life on earth reflects variations in the world's environments, principally in temperature and the availability of water. Figure 29.1 is a satellite image of North and South America, collected over eight years, the colors keyed to the relative abundance of chlorophyll, a good indicator of rich biological communities. Phytoplankton and algae produce the dark red zones in the oceans and along the seacoasts. Green and dark green areas on land are dense forests, while orange areas like the deserts of western South America are largely barren of life.

Implications of Microbial degradation for the Biosphere

Plastic pollution in the marine environment is the cause of several hazardous and ecologically damaging effects. Plastic debris poses a direct threat to wildlife, with many and varied species documented as being negatively impacted by plastic items. The main dangers associated with plastic objects for most species surround entanglement in and ingestion of said items. Juvenile animals in particular often become entangled in plastic debris, which can result in serious injury as the animal grows, not to mention restriction of movement, preventing animals from properly feeding and, in the case of mammals, breathing. A wide variety of species have been reported to be negatively impacted by plastic debris: marine birds, sea turtles, cetaceans, fur seals, sharks and filter feeders are just some of those documented. Marine birds are particularly susceptible to ingestion of plastic objects that they mistake for food. Plastic ingested by these animals persists in the digestive system and can lead to decreased feeding stimuli, gastrointestinal blockage, decreased secretion of gastric enzymes and decreased levels of steroid hormones, leading to reproduction problems.

Plastic particles in the ocean have been shown to contain quite high levels of organic pollutants.

Toxic chemicals, such as polychlorinated biphenyls (PCBs), nonylphenol (NP), organic pesticides, such as dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs) and bisphenol A (BPA) have been consistently found throughout oceanic plastic debris . The presence of these compounds further increases the risks associated with ingestion of plastic debris by wildlife, and additionally, many of these compounds can undergo significant biomagnification and may potentially pose a direct risk to human health.

However, metabolic diversity of bacteria makes them a useful resource for remediation of pollution in the environment. Bacteria have been utilised in the clean-up of oil spills, PCBs and heavy metals, such as arsenic, mercury, cadmium and lead . There are sufficient examples to suggest there are few if any substances that cannot be utilised at least in part by microbes for metabolic activities.

Biodegradation is an attractive alternative to current practices for waste disposal, as it is generally a cheaper process, potentially much more efficient and does not produce secondary pollutants, such as those associated with incineration and landfill. In some cases, it may even be possible to obtain useful end products with economic benefit from bacterial metabolism of pollutants, for example, ethanol for use in biofuels.

Some other positive implications include;

i. Mineralization

This is a process by which organic matter is decomposed to released simpler, inorganic compounds (e.g. CO_2, NH_4^+, CH_4, N_2, e.t.c.)

ii. Removal or transformation of toxic organic compounds, oil in the sea, whether from natural seepage or spillage undergoes weathering and loses its lighter fractions so that it sinks; emulsification can also take place.

Short Comings of Microbial Degradation

Clean-up activities or microbial degradation could have ecological consequences, for instance;

i. Degradation of DDT to DDE which result from the dehalogenation of DDT, is still of environmental concern.

ii. Degradation of TCE, a widely used solvent to vinylchloride if degraded under anoxic conditions could synthesis a dangerous carcinogen (venylchloride)

$Cl_2=CHCL$ $ClHC=CH_2$

iii. Methane which is one of the end products of mineralization is more than 20 times as effective as CO_2 at trapping heat in the atmosphere (global warming)

iv. Many of organic compounds that are added into the environments are chiral. However microorganisms can degrade only one isomer of a substance; the other isomer will remain in the environment.

v. Studies have shown that microbial communities in different environments will degrade different enertiomers. Changes in nutrient supplies and environmental conditions can alter the patterns of chiral form degradation.

Conclusion

The pollution of the environment by organic compounds has a consequence on the biosphere. Such organic compounds are used in sizeable areas and are even applied to soil surfaces inform of pesticides or fertilizers, and eventually accumulate beneath the ground surface, reaching rivers and seas. The natural microbiota is continuously exposed to chemicals therefore, it is no surprise that these microorganisms, that inhabit in polluted environments, are armed with resistance by catabolic processes to remove the toxic compounds. Biological degradation by organisms (fungi, bacteria, viruses, protozoa) can efficiently remove organic pollutants from the biosphere, especially organochlorines, organophosphates, polythene and carbamates used in agriculture.

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