AIR MICROBIOLOGY

1. The air as an environment of microorganisms

Air is an unfavorable environment for microorganisms, in which they cannot grow or divide. It is merely a place which they temporarily occupy and use for movement. Therefore, there are no metabolic connections occurring between different microorganisms in air (such as in soil or water). As a result they form only a random collection of microorganisms, not a microbiocenosis. Microorganisms get into air as a consequence of wind movement, which sweeps them away from various habitats and surroundings (soil, water, waste, plant surfaces, animals and others), or are introduced during the processes of sneezing, coughing, or sewage aeration.

1.1. Why are the air conditions unfavorable for the microorganisms?

There are 3 elementary limiting factors in the air:

● Lack of adequate nutrients,

● Frequent deficit of water, threat of desiccation,

● Solar radiation.

It is obvious that the first factor limits cell growth. As a matter of fact, air, and especially polluted air, contains some organic substances, but they are usually poorly decomposed and there is not enough to be utilized as food. Besides, there are other unfavorable contributing factors.

Microorganisms contained in air are constantly subjected to drying, which definitely stops all processes. Some bacteria are especially sensitive to water deficits which cause bactericidal effects (e.g. gonococci or spirochete which die as soon as they enter the air). Many organisms, however, can successfully cope with water deficits and, although they cannot function properly, their dried up forms survive months and even years (endospores, fungi spores). Solar radiation is also damaging to microorganisms suspended in air as it causes mutation and desiccation (in water and soil the solar radiation is usually very weak or simply does not exist)

2. Adaptation of microorganisms to the air environment

2.1. What types of microorganisms occur in air?

There are 3 main groups of microorganisms that occur in air:

● Viruses

● Bacteria

● Fungi

Bacteria may exist as vegetative or resting form; however fungi occur in the form of spores or fragments of mycelium. Especially in the vegetative season, pollen of anemophilous plants (e.g. grasses and some trees) is abundant in the air. Besides the above, the following can be found in air as well: algae and protozoa cysts and small invertebrates such as worms in forms of eggs or cysts and mites. Besides living microorganisms, their fragments and products, which often exhibit toxic or allergic activities, may also occur in air

2.2. Which microorganisms are best adapted to a prolonged existence in air?

The atmosphere can be occupied for the longest time by those forms which, due to their chemical composition or structure, are resistant to desiccation and solar radiation. They can be subdivided into the following groups:

● Bacterial resting forms,

● Bacterial vegetative forms which produce carotenoidal dyes or special protective layers (capsules, special structure of cell wall),

● Spores of fungi,

● Viruses with envelopes

Resting forms of bacteria

Endospores are the best known resting forms. These structures evolve within cells and are covered by a thick multi-layer casing. Consequently, endospores are unusually resistant to most unfavorable environment conditions and are able to survive virtually endlessly in the conditions provided by the atmospheric air. They are only produced by some bacteria, mainly by *Bacillus* and *Clostridium* genera. Because each cell produces only one endospore, these spore forms cannot be used for reproduction. Another type of resting form is produced by very common soil bacteria, the actinomycetes. Their special vertical, filiform cells, of the so-called air mycelium, undergo fragmentation producing numerous ball-shaped formations. Due to the fact that their production is similar to the formation of fungal, they are also called conidia. Contrary to endospores, the conidia are used for reproduction. There are also other bacterial resting forms, among others, the cysts produced by azotobacters - soil bacteria capable of molecular nitrogen assimilation. The production of carotenoidal dyes ensures cells with solar radiation protection.

Carotenoids, due to the presence of numerous double bonds within a molecule (-C=C-), serve a purpose as antioxidants, because, as strong reducing agents, they are oxidized by free radicals. Consequently, important biological macromolecules are being protected against oxidation (DNA, proteins etc.). Bacteria devoid of these dyes quickly perish due to the photodynamic effect of photooxidation. That explains why the colonies of bacteria, which settle upon open agar plates, are often coloured. The ability to produce carotenoids is possessed especially by cocci and rod-shaped actinomycetes. Rod-shaped actinomycetes, e.g. *Mycobacterium tuberculosis*, besides being resistant to light, also demonstrate significant resistance to drying due to a high content of lipids within their cell wall. High survival rates in air are also a characteristic for the bacteria which possess a capsule, e.g. *Klebsiella* genus, that cause respiratory system illnesses.

Fungal spores

Spores are special reproductive cells used for asexual reproduction. Fungi produce spores in astronomical quantities, for example the giant puffball (*Calvatia gigantea*) produces 20,000,000,000,000 (20 billion!) spores, which get into the air and are dispersed over vast areas. A very common type of spores found in air is that of conidia.

Conidia are a type of spore formed by asexual reproduction. They form in the end-sections of vertical hyphae called conidiophores and are dispersed by wind. The spores of common mould fungi such as *Penicillium* and *Aspergillus* are examples of the above. Spore plants such as ferns, horsetails and lycopods also produce spores. Plant pollen is also a kind of spores.

Resistant viruses

Besides cells, the air is also occupied by viruses. Among those that demonstrate the highest resistance are those with enveloped nucleocapsids, such as influenza viruses. Among viruses without enveloped nucleocapsids, enteroviruses demonstrate a relatively high resistance. Besides the previously mentioned resistant forms, the air is also occupied by more sensitive cells and viruses, but their survival is much shorter. It is believed, that among vegetative forms, gram-positive bacteria demonstrate greater resistance than gram-negative bacteria (especially for desiccation), mainly due to the thickness of their cell wall. Viruses are usually more resistant than bacteria.

3. Biological aerosols

3.1. Microorganisms suspended in air as a colloidal system

Microorganisms in air occur in a form of colloidal system or the so-called bio-aerosol. Every colloid is a system where, inside its dispersion medium, particles of dispersed phase occur whose size is halfway between molecules and particles visible with the naked eye. In the case of biological aerosols, it's the air (or other gases) that has the function of the dispersion medium, whereas microorganisms are its dispersed phase. However, it is quite rare to have microbes independently occurring in air. Usually, they are bound with dust particles or liquid droplets (water, saliva etc.), thus the particles of the bioaerosol often exceed microorganisms in size and may occur in two phases:

- Dust phase (e.g. bacterial dust) or
- Droplet phase (e.g. formed as the result of water-vapor condensation or during sneezing).

The dust particles are usually larger than the droplets and they settle faster. The difference in their ability to penetrate the respiratory tract is dependent on the size of the particles; particles of the droplet phase can reach the alveoli, but dust particles are usually retained in the upper respiratory tract. The number of microorganisms associated with one dust particle is greater than in the droplet phase.

3.2. The size of bioaerosols

The average size of bioaerosols ranges from about 0.02 μm to 100 μm. The sizes of certain particles may change under the influence of outside factors (mainly humidity and temperature) or as a result of larger aggregates forming. By using size criterion, the biological aerosol can be subdivided into the following:

- Fine particles (less than 1μm) and
- Coarse particles (more than 1μm)

Fine particles are mainly viruses, endospores and cell fragments. They possess hygroscopic properties and make-up the so-called nucleus of condensation of water vapor. At high humidity water collects around these particles creating a droplet phase. Then, the diameter of the particles increases. Coarse particles consist mainly of bacteria and fungi, usually associated with dust particles or with water droplets.

3.3. Mechanisms protecting lungs against bioaerosols

There are two basic mechanisms which remove aerosols from the inhaled air:

● Mucous-ciliary apparatus,

● Phagocytosis of lung macrophages

Human and animal respiratory tracts are lined with a multi-row epithelium. The tissue is made up of cylindrical cells equipped with cilia and the so-called goblet cells which produce mucus that covers the entire epithelium. The mucus has a high viscosity due to a high content of mucin, as well as bactericidal properties given by **lysozyme** - an enzyme that hydrolyses cell walls of gram-positive bacteria. Both cell types make up a functional unity creating the mucous-ciliary apparatus First, the particles contained in air stick to the mucus, then they are swept along the mucus toward the nasal cavity, next they are either excreted with saliva (e.g. during coughing) or swallowed. This mechanism is rather effective with larger particles of coarse bioaerosol. Fine bioaerosols often escape this trapping and find their way into the pulmonary alveoli. Then, they may get absorbed by the macrophages, which are capable of phagocytosis. Besides the above, there are also other protective mechanisms, e.g. filtration of larger aerosol particles by hair in the nose, the cough reflex, or the inhibitory action of the natural microflora of the respiratory-tracts mucous membrane - called bacterial interference.

However, the efficiency of these mechanisms isn't always sufficient and, especially at high concentrations of bioaerosol and high invasiveness of microorganisms, the respiratory tracts may get invaded causing pathological changes. In addition, the microorganisms which make up the bioaerosol, may find their way into the organism through a food chain (e.g. contaminated surfaces) or through the skin.

Settling of bioaerosols in various parts of the respiratory system is based on the particle size and the strength with which they are inhaled. The same rule governs the non-biological aerosol (dust, smoke, fog). Coarse grain bioaerosol settles mainly in the nasal and throat cavity (especially particles with a diameter larger than 10 µm) and in the bronchial tubes (particles with a diameter 2-10µm). However, the fine-grained bioaerosol reaches even further, all the way to the pulmonary alveoli (particles with a diameter 1µm and less). During forceful inhalations (e.g. hyperventilation, cough) the lungs may be reached by larger particles as well (diameter of 10µm. and more). Particles capable of penetrating the pulmonary alveoli are referred to as the **respirable fraction**. This name refers to all types of particles, not only those of the bioaerosol. It is commonly believed that the respirable fractions consist of particles of diameters less than 10µm.

The contribution of the respirable fraction to the bioaerosol is a measure of its harmfulness as it illustrates the part of the bioaerosol that may penetrate the lungs. That is why methods to determine the size of the respirable fractions are most crucial when determining the level of microbiological air pollution.

3.4. Survival and spread of the bioaerosols

Once microorganisms find their way into air, they are influenced by a series of unfavorable conditions, thus a significant number of them perish, mainly as a result of dehydration. The ones that manage to overcome the shock of sudden condition change still continue to be affected by these changes and consequently may die. There are several factors which influence the ability of a bioaerosol to survive in air:

- Particular resistance for a given microorganism (*morphological characteristics),*
- Meteorological conditions (inter alia, air humidity, solar radiation),
- Air pollution,
- The length of time in air.

3.5. Resistance of microorganisms

It is a species dependent feature, which relies on the microorganism's morphology and physiology. Factors responsible for resistance in air include the following:

3.5.1. Relative humidity

The content of water in air is one of the major factors determining the ability to survive. At a very low humidity and high temperature cells face dehydration, whereas high humidity may give cells protection against the solar radiation. Microorganisms react differently to humidity variations in air, but nevertheless most of them prefer high humidity. The morphology and biochemistry of cell-surrounding structures, which may change its conformation depending on the amount of water in air, are crucial. Actually, an exact mechanism of this is not known. Forms of resting spores with thick envelopes (e.g. bacterial endospores) are not particularly susceptible to humidity variations. Gram-negative bacteria and enveloped viruses (e.g. influenza virus, myxo) deal better with low air humidity which is contrary to gram-positive bacteria and non-enveloped viruses (e.g. enteroviruses) that have higher survival rates in high air humidity.

3.5.2. Temperature

Temperature can indirectly affect cells by changing the relative-air humidity (the higher the temperature, the lower the relative humidity) or a direct affect, causing, in some extreme situations, cell dehydration and protein denaturation (high temperatures) or crystallization of water contained within cells (temperatures below 0^{o}C). Therefore, it can be concluded that low (but above 0^{o}C) temperatures are optimal for the bioaerosol (according to some researchers the optimal temperatures are above 15^{o}C).

3.5.3. Solar radiation

Solar radiation has a negative effect on the survival rate of the bioaerosol, both visible as well as ultraviolet (UV) and infrared radiation due to the following factors:

● It causes mutation,

● Leads to the formation of free radicals, which damage important macromolecules.

● Creates a danger of dehydration.

Visible-light rays of about 400-700 nm wavelength, create the so-called photodynamic effect, which produces free radicals within cells, especially compounds such as peroxy and hydroxyl radicals. These radicals demonstrate strong oxidizing activities and may cause damage to crucial macromolecules, e.g. DNA or proteins. UV radiation has a much larger effect on cells than visible light does, especially the rays of 230-275 nm wavelengths. The mechanism of this effect is based on changes to DNA, both directly (e.g. by creating thymine dimer and consequently causing mutation), as well as indirectly, by creating free radicals as in the case of the visible light. In addition, infrared (IR) radiation may have a negative effect upon cells contained in air by heating up and consequently dehydrating them.

4. Air pollution

Pollutants in air, especially hydrocarbons, ozone and nitric oxides, which are activated by solar radiation (especially UV) create various, highly reactive secondary pollutants, commonly described as photochemical oxidizers (among others peroxiacetyl nitrate – PAN and organic peroxides). They are toxic to all living forms including air-suspended microorganisms. In contrast, the non-toxic and non-biological aerosols (dust, fog), disperse and absorb the solar radiation which consequently increases the survival of bioaerosols. It should be noted that the above factors often work simultaneously and are related, e.g. solar radiation increases the temperature, whereas high humidity weakens the radiation.

5. Biological aerosols as a human hazard source

5.1. What types of dangers are connected to the presence of microorganisms in air?

● Infectious diseases (viral, bacterial, fungal and protozoan),

● Allergic diseases,

● Poisoning (exotoxins, endotoxins, mycotoxins).

Bioaerosols may carry microorganisms other than those which evoke respiratory system diseases. The intestinal microorganisms contained in aerosols may, after settling down, get into the digestive system (e.g. by hands) causing various intestinal illnesses.

5.2. Infectious airborne diseases

The mucous membrane of the respiratory system is a specific type of a 'gateway' for most airborne pathogenic microorganisms. Susceptibility to infections is increased by dust and gaseous air-pollution, e.g. SO_2 reacts with water that is present in the respiratory system, creating H_2SO_4, which irritates the layer of mucous. Consequently, in areas of heavy air pollution, especially during smog, there is an increased rate of respiratory diseases. Bioaerosols may, among other things, carry microbes that penetrate organs via the respiratory system. After settling, microbes from the air, may find their way onto the skin or, carried by hands, get into the digestive system (from there, carried by blood, to other systems, e.g. the nervous system). Fungi that cause skin infections, intestinal bacteria that cause digestive system diseases or nervous system attacking enteroviruses are all examples of the above.

Viral diseases:

After penetrating the respiratory system with inhaled air, particles of viruses reproduce inside the cuticle cells of both the upper and lower respiratory system. After reproduction some of the viruses stay inside the respiratory system causing various ailments (runny nose, colds, bronchitis, pneumonia), whereas others leave the respiratory system to attack other organs (e.g. chickenpox viruses attack the skin). The most noteworthy viruses are:

● Influenza (orthomyxoviruses)

● Influenza, measles, bronchitis, mumps and pneumonia among newborns (paramyxoviruses)

● German measles (similar to paramyxoviruses)

● Colds (rhinoviruses and koronaviruses)

● Cowpox and true pox (pox type viruses)

● Chickenpox (cold sore group of viruses)

● Foot-and-mouth disease (picorna type viruses)

● Meningitis, pleurodynia (enteroviruses)

● Sore throat, pneumonia (adenoviruses)

Bacterial diseases:

Similarly to viruses, some bacteria that find their way to the respiratory system may also cause ailments of other systems. Especially staphylococcus infections assume various clinical forms (bone marrow inflammation, skin necrosis, intestinal inflammation, pneumonia). Often, a susceptible base for development of various bacterial diseases is first prepared by viral diseases, e.g. staphylococcus pneumonia is usually preceded by a flu or mumps. Bacterial airborne diseases include:

● Tuberculosis (*Mycobacterium tuberculosis),*

● Pneumonia (staphylococcus, pneumococci, *Streptococcus pneumoniae,* less frequently chromatobars of *Klebsiella pneumoniae),*

● Angina, scarlet fever, laryngitis (streptococcus),

● Inflammation of upper and lower respiratory system and meningitis (*Haemophilus influenzae),*

● Whooping cough (chromatobars of *Bordetella pertussis*),

● Diphtheria (*Corynebacterium diphtheriae),*

● Legionnaires disease (chromatobars of *Legionella* genus, among others *L. pneumophila),*

● Nocardiosis (oxygen actinomycetes of *Nocardia* genus).

Fungal diseases:

Many potentially pathogenic airborne fungi or the so-called saprophytes live in soil. They usually have an ability to break down keratin (keratinolysis) - difficult to decompose proteins found in horny skin formations, e.g. human or animal hair, feathers, claws. Some of the **keratinolytic fungi,** the so-called **dermatophytes**, cause **mycosis of the outer skin** (dermatosis)**,** as the breakdown of keratin enables them to penetrate the epidermis. Other fungi, after penetrating the respiratory system, cause **deep mycosis** (organ), e.g. attacking lungs. The following are examples of airborne fungi diseases:

● Mycosis (*Microsporum racemosum),*

● Deep mycosis: aspergillosis (*Aspergillus fumigatus),* cryptococcus (*Cryptococcus neoformans*).

Protozoan diseases:

Some protozoa, which are able to produce cysts that are resistant to dehydration and solar radiation, may also infect humans by inhalation. The most common example of the above is: *Pneumocystis carinii* which causes pneumonia. Dangers connected with pathogenic bioaerosols do not concern only human diseases. Other significant diseases are those that attack cultivated plants or farm animals. The following are examples of the above:

● Blight - grain disease caused by *Puccinia graminis*, and

● Aphthous fever - very infectious disease that attacks artiodactylous animals.

5.3. Allergic diseases

Allergy is a changed, hypersensitive reaction of the person or animal to some substances called allergens. Actually, it's an immunologic reaction, in which a needless production of antibodies by B lymphocytes (immunoglobulins) occurs as a hypersensitive response to penetration of antigens (called the **allergen**). Excessively produced immunoglobulins combine with allergens, which cause among other things:

● A release of various compounds (e.g. histamines) from mast cells.

The released compounds induce inflamed reactions in the form of bronchus asthma or hay fever,

● Cause damaged tissue at the place of contact, - allergic pulmonary alveoli inflammation (e.g. the so-called farmer's lung, or mushroom breeder's lung). Many microbes exist as allergens. Besides these, there are other allergenic factors such as anemophilous pollens (e.g. grass, nettle, comose), small arachnids (mites) as well as biological dust (e.g. particles of feathers, hair or droppings). Microorganisms differ in their allergenic influences. The strongest allergens are mold fungi, thermophiles actinomycetes, as well as Gram-negative chromatobars. The strength of allergenic bioaerosols depends not only on the type of microorganisms but also on their concentration.

5.4. Poisoning

Poisoning / intoxication is caused by toxins that are produced by some microorganisms. Endotoxins and mycotoxins are the most significant types of toxins in polluted air.

Endotoxins are the components of Gram-negative bacterial cell walls (A lipid fragment of lipopolysaccharides LPS outer membrane). They demonstrate toxic (and allergenic) effects on mammals. After being inhaled into the lungs, they cause acute inflammation of the lungs.

Mycotoxins are produced by various mold fungi. The most common ones are **aflatoxins** produced by *Aspergillus flavus.* These compounds (there are several types of them) demonstrate strong toxic, mutagenic, carcinogenic, teratogenic (cause malformation in a fetus) actions. Most often they lead to food poisonings, however it has also been indicated, that inhaling dusts which contain aflatoxins may bring about tumours of the liver and the respiratory system.

6. Basic sources of bioaerosol emission

There are two basic **sources of bioaerosol**:

- Natural,
- Artificial

Natural sources are mainly soil and water, from which microorganisms are being lifted up by the movement of air, and from organisms such as fungi, that produce gigantic amounts of spores that are dispersed by the wind. Therefore, there are always a given number of microorganisms in the air, as a natural background. It is estimated, that the air is considered to be clean, if the concentration of bacteria and fungi cells does not exceed 1000/m3 and 3000/m3 respectively. This latter statement is only true when the concentration of microorganisms consists of saprophytic organisms, not pathogenic organisms. If the concentration of microorganisms in the air exceeds the above values, or contains microorganisms dangerous to humans, then such air is considered to be **microbiologically polluted.**

From the hygienic point of view, **living sources of bioaerosols** related to human activity, are more important than the natural sources. The emissions from these sources are dangerous due to the following two reasons:

- They may distribute pathogenic microorganisms,
- They often cause a high increase of microorganisms in the air, significantly exceeding the natural background.

The emission sources of biological aerosols can have a localized character (e.g. aeration tank) or a surface character (e.g. sewage-irrigated field).

The most important sources of bioaerosol emission are:

- Agriculture and farming-food industry,
- Sewage treatment plants,
- Waste management.

Module Two

THE ROLE OF MICROBES IN PROSPECTING, RECOVERY AND DEGRADATION OF PETROLEUM PRODUCTS

Microorganisms have tremendous capacity to metabolize various compounds. They can also quickly adapt to environments where they evolve new metabolic abilities to grow. Thus, the metabolic capacity of microorganisms is even larger when their fast evolving and adaptive nature is considered. Microbes can degrade selected compounds (chloronitrobenzenes, parathion and nitrophenols, carbazole and dibenzothiones). Especially environmentally important Gram-positive bacteria.

1. **Bioremediation**:

Bioremediation is the use of biological systems for the reduction of pollution from air or from aquatic or terrestrial system; it also involves extracting a microbe from the environment and exposing it to a target contaminant so as to lessen the toxic component. Thus, the goal of bioremediation is the employment of biosystems such as microbes, higher organisms like plants (phytoremediation) and animals to reduce the potential toxicity of chemical contaminants in the environment by degrading, transforming, and immobilizing these undesirable compounds.

Biodegradation is the use of living organisms to enzymatically and otherwise attack numerous organic chemicals and break them down to lesser toxic chemical species. Biotechnologists and bioengineers classify pollutants with respect to the ease of degradation and types of processes that are responsible for this degradation, sometimes referred to as treatability. Biodegradation with microorganisms is the most frequently occurring bioremediation option. Microorganisms can break down most compounds for their growth and/or energy needs. These biodegradation processes may or may not need air. In some cases, metabolic pathways which organisms normally use for growth and energy supply may also be used to break down pollutant molecules. In these cases, known as cometabolisms, the microorganism does not benefit directly. Researchers have taken advantage of this phenomenon and used it for bioremediation purposes.

A complete biodegradation results in detoxification by mineralising pollutants to carbon dioxide (CO_2), water (H_2O), and harmless inorganic salts. Incomplete biodegradation (i.e., mineralization) will produce compounds that are usually simpler (e.g., cleared rings, removal of halogens), but with physical and chemical characteristics different from the parent compound. In addition, side reactions can produce compounds with varying levels of toxicity and mobility in the environment.

Biodegradation may occur spontaneously, in which case the expressions “intrinsic bioremediation” or “natural attenuation” are often used. In many cases the natural circumstances may not be favourable enough for natural attenuation to take place due to inadequate nutrients, oxygen, or suitable bacteria. Such situations may be improved by supplying one or more of the missing/inadequate environmental factors. There are millions of indigenous species of microbes living at any given time within many soil environments. The bioengineer simply needs to create an environment where those microbes are able to use a particular compound as their energy source.

Life Chemical Dynamics (Biochemodynamics) of Bioremediation.

Bioremediation success depends on the following:

(1) The growth and survival of microbial populations; and

(2) The ability of these organisms to come into contact with the substances that needs to be degraded into less toxic compounds

(3) Sufficient numbers of microorganisms to make bioremediation successful;

(4) The microbial environment must be habitable for the microbes to thrive. Sometimes, concentrations of compounds can be so high that the environment is toxic to microbial populations. Therefore, the bioengineer must either use a method other than bioremediation or modify the environment (e.g., dilution, change of pH, pumped Oxygen, adding organic matter, etc.) to make it habitable. An important modification is the removal of non-aqueous-phase liquids (NAPLs) since the microbes' biofilm and other mechanisms usually work best when the microbe is attached to a particle; thus, most of the NAPLs need to be removed, by vapor extraction. Thus, low permeability soils, like clays, are difficult to treat, since liquids (water, solutes, and nutrients) are difficult to pump through these systems. Usually bioremediation works best in soils that are relatively sandy, allowing mobility and greater likelihood of contact between the microbes and the contaminant. Therefore, an understanding of the environmental conditions sets the stage for problem formulation (i.e., identification of the factors at work and the resulting threats to health and environmental quality) and risk management (i.e., what the various options available to address these factors are and how difficult it will be to overcome obstacles or to enhance those factors; that make remediation successful). In other words, bioremediation is a process of optimization by selecting options among a number of biological, chemical and physical factors these include correctly matching the degrading microbes to conditions, understanding and controlling the movement of the contaminant (microbial food) so as to come into contact with microbes, and characterizing the abiotic conditions controlling both of these factors. Optimization can vary among options, such as artificially adding microbial populations known to break down the compounds of concern. Only a few species can break down certain organic compounds. Two major limiting factors of any biodegradation process are toxicity to the microbial population and inherent biodegradability of the compound. Numerous bioremediation projects include in situ (field treatment) and ex situ (sample/laboratory treatment) waste treatment using biosystems

Many microorganisms can adapt their catabolic machinery to utilize certain environmental pollutants as growth substrates, thereby bioremediating the environment. Some microorganisms in carrying out their normal metabolic function may fortuitously degrade certain pollutants as well. This process termed cometabolism obviously requires adequate growth substrates. Diazotrophs, such as *Azotobacter vinelandii*, beyond their ability to fix atmospheric nitrogen also have the capacity, in some case, to cometabolise petroleum hydrocarbons.

A case study:

A research was carried out on bioremediation involving two bacteria, a hydrocarbonoclastic and diazotrophic bacteria. The hydrocarbonoclastic was tentatively identified as *Pseudomonas* sp. and designated as NS50C10 by the Department of Microbiology, University of Nigeria, Nsukka. The diazotrophic bacteria was *Azotobacter vinelandii*, which was isolated from previously crude oil contaminated soil. This study describes the mineral media and procedure for isolation and multiplication of the bacteria to the required cell density. Crude oil spill was simulated by thoroughly mixing 50, 100, and 150 mg fractions of crude oil with 100 g batches of a composite soil sample in beakers. The soil samples were taken from a depth of 0–50 cm from the Zoological garden, University of Nigeria, Nsukka. The mixing was conducted using a horizontal arm shaker adjusted to a speed of 120 rpm for 30 minutes. The contaminated soil samples, in beakers, were inoculated with optimal combinations (cell density) of NS50C10 and *A. vinelandii*. Water was added to the crude oil contaminated soil samples (both inoculated and those not inoculated to a saturation point but not in excess), and then the samples were left to stand undisturbed for seven days. NS50C10 was applied first, followed by *A. vinelandii*, 12 hours later. At the seventh day of soil treatment, 20 sorghum grains (previously soaked overnight in distilled water) were planted in each soil sample followed by irrigation to aid germination. Seven days after the planting of the sorghum grains, the soil from each beaker was carefully removed. The number of germinated seed per batch of soil sample was noted, the length of radicules was measured, and the mean length was taken from each batch. The results of this experiment showed that *Pseudomonas* sp. grew well on agar plates containing a thin film of crude oil as the only carbon source, while *A. vinelandii* did not. However, cell-free extract of *Azotobacter vinelandii* fixed atmospheric nitrogen as ammonium ion (NH_4^+) under appropriate condition. The specific growth rate values in contaminated soil samples inoculated with both normal NS50C10 and *A. vinelandii* (consortium) were highest in all cases. By adding an aerobic, free living diazotroph *A. vinelandii* with the *Pseudomonas sp.* (NS50C10), an improvement on bioremediation of soil over that of the pure NS50C10 alone was achieved to the order of 51.96 to 82.55%. This innovative application that uses the synergetic action of several microorganisms to clean up oil-polluted soil has potential application for the bioremediation of oil contaminated soil in the Niger delta region.

The method described above is the biotechnological application known as *bioaugmentation* which is the addition of selected organisms to contaminated soils (sites) in order to supplement the indigenous microbial population and speed up degradation. This bioremediation method by the authors has been applied in bioremediation especially in Niger delta areas of Nigeria. The authors also serve in the capacity of industrial consultants in the specialized field of crude oil pollution clean-up procedures using this specific biotool (bioremediation).

Biofiltration: This is a pollution control technique employing the use of living material to capture and biologically degrade process pollutants. Common uses of biofiltration processes are for processing waste water, capturing harmful chemicals or silt from surface runoff, and microbiotic oxidation of contaminants in air. In multimedia-multiphase bioremediation, waste streams containing volatile organic compounds (VOCs) may be treated with combinations of phases, that is, solid media, gas, and liquid flow in complete biological systems. These systems are classified as three basic types: biofilters, biotrickling filters, and bioscrubbers. Biofilms of microorganisms (bacteria and fungi) are grown on porous media in biofilters and biotrickling systems.

Biomining: Bacteria leaching is now used throughout the world as an additional technique for extracting metals from ores. Metals which can be extracted in this way include copper, uranium, cobalt, lead, nickel, and gold. Biomining is a generic term that describes the processing of metal-containing ores and concentrates of metal containing ores using microbiological technology. Biomining has application as an alternative to more traditional physical-chemical methods of mineral processing. Commercial practices of biomining can be broadly categorized in two, namely, mineral biooxidation and bioleaching. Both processes use naturally occurring microorganisms to extract metals from sulphide bearing minerals. Minerals biooxidation refers to the process when it is applied to enhance the extraction of gold and silver, whereas bioleaching usually refers to the extraction of base metals, such as Zinc, Copper, and Nickel.

Collectively, minerals biooxidation and bioleaching are commercially proven, biohydrometallurgical or biomining processes that are economic alternatives to smelting, roasting, and pressure oxidation to treat base and precious metals associated with sulphide minerals. Metals are essential physical components of the ecosystem, whose biologically available concentrations depend primarily on geological and biological processes. Elevated levels of metals at specific sites can create a significant environmental and health problem when the release of metals through geological processes of decomposition and anthropogenic processes far exceeds that of natural processes of metal cycling. Metal contamination of both aqueous and terrestrial environments is of great concern, due to the toxicity and persistence of metals in the ecosystem and their threat to animal and human health. Bacteria play an important role in the geochemical cycle of metals in the environment, and their capabilities and mechanisms in transforming toxic metals are of significant interest in the environmental remediation of contaminated sites.

Microorganisms colonize and shape the Earth in many ways, and their ability to adsorb and transform metals can shade light on solving pollution problems and proposing solutions in the clean-up of contaminated site.

Extraction Role of Microbes in Biomining: Although many undiscovered microbial communities are involved in biomining, some of the popular and discovered bacteria responsible are: *Leptospirillum ferrooxidans*, *Acidithiobacillus thiooxidans*, and *Acidithiobacillus ferrooxidans*. There is a good understanding of the exact role of microbes in biomining, thanks to today's sophisticated instrumentation that can examine materials at the atomic level. Given the fact that many microbes float freely in the solution around the minerals, many microbes attach to the mineral particles forming a biofilm. The microbes, whether they are freely floating or whether they are in the biofilm, continuously devour their food sources—iron (chemically represented as Fe^{2+}) and sulphur. The product of the microbial conversion of iron is "ferric iron," chemically represented as "Fe^{3+}". Ferric iron is a powerful oxidizing agent, corroding metal sulphide minerals (e.g., pyrite arsenopyrite, chalcocite, and sphalerite) and degrading them into dissolved melts, such as copper, zinc, and more iron, the latter being the food source for the microbes. The sulphide portion of the mineral is converted by the microbes to sulphuric acid. Uranium occurs in oxidation states ranging from U (III) to U (VI), with the most stable species, U (VI) and U (IV), existing in the environment. U (VI) is predominant in the oxic surface waters, and $UO2^{2+}$ (uranyl) always forms stable, soluble complexes with ligands such as carbonate, phosphate, and humic substances. In natural waters the solubility of U (VI) usually increases several orders of magnitude at higher pH values, due to complexation with carbonate or bicarbonate. By contrast, U (IV) is commonly found in the anoxic conditions and is present primarily as an insoluble uranite (UO2). Therefore, reduction of the soluble uranyl to the insoluble uranite seems to be an effective means to immobilize uranium in the anoxic environment to decrease the potential release of the mobile species.

Microbial Gold Mining: In some precious metal deposits, gold occurs as micrometer-sized particles that are occluded, or locked, within sulphide minerals, principally pyrite (an iron sulphide mineral) and arsenopyrite (an arsenic containing iron sulphide mineral). To effectively recover the precious metals, the sulfides must be degraded (oxidized) to expose the precious metals. Once the sulphides are sufficiently degraded to expose the gold and silver, a dilute solution of cyanide is used to dissolve the precious metals. If the occluded gold and silver are not exposed by breaking down the sulphide minerals, the cyanide cannot help in the release of the metals and recovery will be low. The ferric iron that is produced by the microorganism is the chemical agent that breaks down (oxidizes) the sulfide mineral. The microorganisms can be thought of as the manufacturing facility for producing the ferric iron. Microorganisms in the ore are destroyed by lime. Cyanide leaching can be accomplished in another heap or the oxidized and lime-conditioned ore can be ground and cyanide leached in a mill. The residue slurry is rinsed with fresh water, neutralized with lime, subjected to solid/liquid separation, and the solid residue is cyanide leaching to extract the gold. Gold recoveries are in the 95–98% range.

Advantages of biomining using organisms include the following.

(1) Biomining microorganisms do not need to be genetically modified; they are used in their naturally occurring form.

(2) Unlike humans, animals, and plants, microorganisms reproduce by doubling; that is, when there is abundant food (iron and sulfur) for biomining microbes and optimal conditions (sufficient oxygen, carbon dioxide and a sulfuric acid environment), a microbe will simply divide. Thus, in heap of minerals biooxidation for pretreating gold ores, there are about one million microbes per gram of ore.

(3) High altitudes have no effect on the biomining microorganisms. However, additional air must be supplied to give the organisms an optimal performance.

(4) Biomining using microorganisms does not produce dangerous waste products. Base metals, for example, zinc and copper are recycled and neutralized with lime/limestone.

(5) The biomining microbes cannot escape from the heap or bioreactor to cause environmental problems. These microbes exist in the environment only where conditions are suitable (i.e., sources of iron and sulfur are oxidized, air and a sulfuric acid environment).

Biomining as a biotool has not been explored in Nigeria. Though Nigeria has many solid minerals in different states of the country, some of the minerals are tin (found in Plateau, Nassarawa, Kaduna, Bauchi and Gombe States), gold (found in Oyo, Osun and Ondo states), copper (Edo and Benue States), tantalite (Gombe, Plateau, Kaduna, and Nasarawa States), and uranium (Bauchi State) among others. The procedures, equipments, nonawareness/interest by government as well as competition with the physicochemical methods of extraction of these minerals are the greatest limitation in the exploitation and usage of this biotool in mining of minerals from their ores. Providing information to Nigeria scientists, ministries and government agencies is the solution to this limitation. Thus, the essence of the suggestion here is to create awareness in this regard.

Biomonitoring: In a broad sense, biological monitoring involve any component that makes use of living organisms, whole or part as well as biological systems to detect any harmful, toxic, or deleterious change in the environment. There are various components employed in biomonitoring of contaminants in the environment. They include biomarkers (biological markers), biosensors, and many others. Biomonitoring or biological monitoring is a promising, reliable means of quantifying the negative effect of an environmental contaminant.

Biological Markers- A biomarker is an organism or part of it, which is used in soliciting the possible harmful effect of a pollutant on the environment or the biota. Biological markers (biomarkers) are measurement in any biological specimen that will elucidate the relationship between exposure and effect such that adverse effects could be prevented. The use of chlorophyll production in *Zea mays* to estimate deleterious effect of crude oil contaminants on soils
is a typical plant biomarker of crude oil pollution [11].When

a contaminant interacts with an organism, substances like enzymes are generated as a response. Thus, measuring such substances in fluids and tissue can provide an indication or “marker” of contaminant exposure and biological effects resulting from the exposure. The term biomarker includes any such measurement that indicates an interaction between an environmental hazard and biological system [39]. It should be instituted whenever a waste discharge has a possible significant harm on the receiving ecosystem. It is preferred to chemical monitoring because the latter does not take into account factors of biological significance such as combined effects of the contaminants on DNA, protein, or membrane. Onwurah et al. [37] stated that some of the advantages of biomonitoring include the provision of natural integrating functions in dynamic media such as water and air, possible bioaccumulation of pollutant from 103 to 106 over the ambient value, and/or providing early warning signal to the human population over an impending danger due to a toxic substance. Microorganisms can be used as an indicator organism for toxicity assay or in risk assessment. Tests performed with bacteria are considered to be most reproducible, sensitive, simple, economic, and rapid [40] (Table 3).

10.4. Biosensor. A biosensor is an analytical device consisting of a biocatalyst (enzyme, cell, or tissue) and a transducer, which can convert a biological or biochemical signal or response into a quantifiable electrical signal [46]. A biosensor could be divided into two component analytical devices comprising of a biological recognition element that outputs a measurable signal to an interfaced transducer [24]. Biorecognition typically relies on enzymes, whole cells, antibodies, or nucleic acids, whereas signal transduction exploits electrochemical (amperometric, chronoamperometric, potentiometric, field-effect transistors, conductometric, capacitative), optical (absorbance, reflectance, luminescence, chemiluminescence, bioluminescence, fluorescence, refractive index, light scattering), piezoelectric (mass sensitive quartz crystalmicrobalance),magnetic, or thermal (thermistor, pyroelectric) interfaces [24]. The biocatalyst component of most biosensors is immobilized on to a membrane or within a gel, such that the biocatalyst is held in intimate contact with the transducer and may be reused. Biosensors are already of major commercial importance, and their significance is likely to increase as the technology develops [46]. Biosensors are still emerging biotechnology for the future in environmental biomonitoring since they have specific limitations. Biosensors on a general sense are often employed for continuous monitoring of environmental contamination or as bioremediation process monitoring and biocontrol tools to provide informational data on what contaminants are present, where they are located, and a very sensitive and accurate evaluation of their concentrations in terms of bioavailability. Ripp et al. [24] explained that bioavailability measurements are central to environmental monitoring as well as risk assessment because they indicate the biological effect of the chemical, whether toxic, cytotoxic, genotoxic,mutagenic, carcinogenic, or endocrine disrupting, rather than mere chemical presence as is achieved with analytical instruments. As the name suggests they are biological instruments that detest and signal the presence of harmful contaminants in the environment. There are different types based on the biological components on which their sensitivities are based (Figure 4). Some of them, though not exhaustive are the following.