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أ.د. ايثـــــــــــار كامـــــــل عبـــــــاس

Prof.Dr.Ithar Kamil Abbas

Microbial ecology: an overview

It is the relationship of microorganisms with one another and with their surroundings. It concerns all three of the major Domains of life including Eucarya, Archaea, and Bacteria. Microbes, by their omnipresence, impact the entire biosphere. They are present in virtually all of our planet's environments except the most extreme, from high mountain glaciers to the deepest ocean depths, and from frozen permafrost to superheated hydrothermal vents.

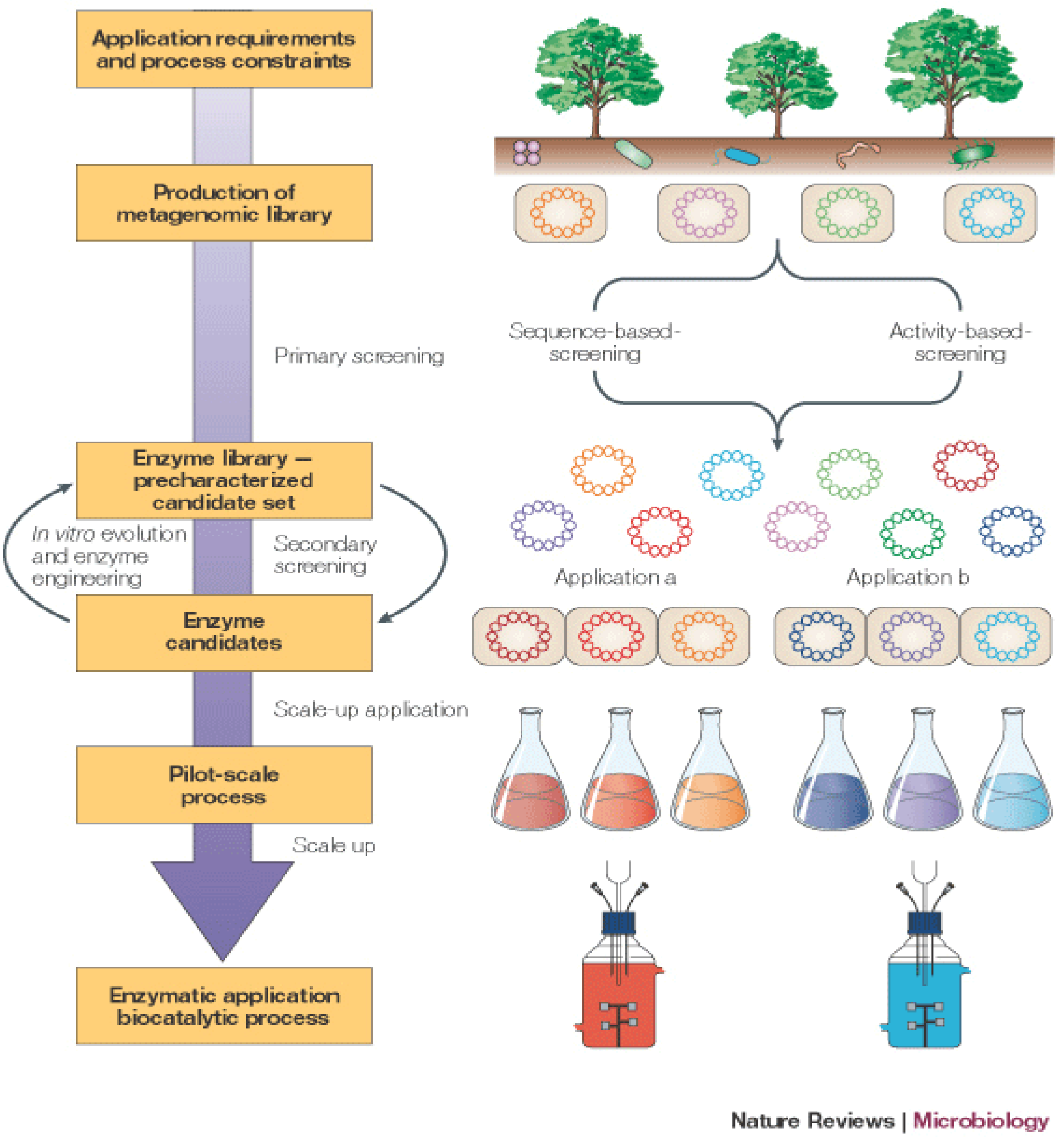
Microbes are the backbone of all ecosystems, even where light cannot penetrate and thus photosynthesis cannot be the basic means to collect energy. In such habitats, chemosynthetic microbes provide energy and carbon to the rest of the ecosystem. Microbes are also decomposers and remineralizers, with the ability to recycle nutrients from other organisms' waste products. They play a critical role in biogeochemical cycles. The nitrogen cycle, the sulfur cycle, the phosphorus cycle and the carbon cycle all depend on microbes in one way or another. For example, nitrogen which makes up 78% of the planet's atmosphere is "indigestible" for most organisms, and the flow of nitrogen into the biosphere depends on a microbial process called fixation. Microbes, especially bacteria, often engage in symbiotic relationships (either positive or negative) with other organisms, and these relationships affect the ecosystem. An example is chloroplasts, which allow eucarya to conduct photosynthesis. Chloroplasts are considered to be endosymbiotic cyanobacteria; these bacteria were among the first to perform photosynthesis, and therefore made and still make major impacts on global climate change.

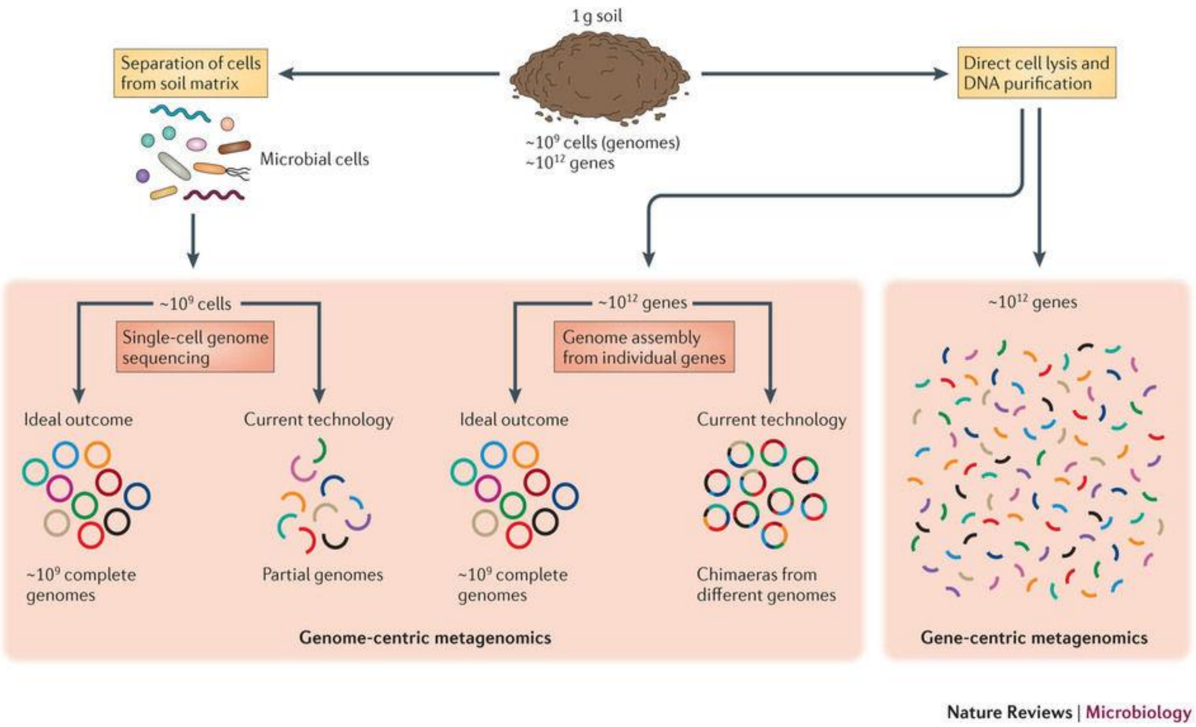
Microbes interact with eukaryotes in a number of ways, ranging from symbiotic or commensal to pathogenic. Plants and animals are home to thousands of bacterial species – some beneficial and some harmful. For example, agricultural practices exert selective pressure on microbial communities that favor microbial communities benefiting a particular crop. Conversely, well-adapted pathogens can destroy plant and animal communities in days or weeks. The adaptations that facilitate interactions of microbes with their eucaryotic hosts range from elaborate signaling processes and structures that provide checks and balances at the population/community level, to elaborate molecular regulatory processes and machines used to interact with the host on a cell-to-cell level.

With modern DNA sequencing techniques, scientists are now examining "metagenomes", or collections of genes that comprise an entire microbial community rather than a single genome.

Metagenomics is the study of genetic material recovered directly from environmental samples. The broad field may also be referred to as environmental genomics, ecogenomics or community genomics. It is A booming area of biotechnology is the industrial use of microorganisms to produce antibiotics, enzymes, and other bioactive compounds. ... The industrial **applications** of **metagenomics** include identification of novel biocatalysts, discovery of new antibiotics, personalized medicine, and bioremediation.

Current projects include agricultural metagenomes as well as projects to reveal species that comprise the "human microbiome". Understanding the biology and ecology of the unseen majority is essential to understanding the health of our own bodies, other animals, plants, ecosystems… and so on.





Knowledge about microbial ecology is directly applied in such fields as agriculture (e.g., food and dairy, plant pathology), industrial and chemical engineering (e.g., industrial biocatalysis), health (e.g., biofilms and pathogenesis), and bioremediation (e.g., pollution elimination). On a broader scale, scientists study microbial ecology in order to understand the role of microbes in the functioning of the biosphere.

Biosphere: is the region of the earth inhabited living organisms including microorganisms. It is consists of:

1- The hydrosphere (earth water supply).
2- Lithosphere (the soil and rocks including earth crust).
3- Atmosphere (the gaseous envelope surrounding earth)

Microbial ecosystem: comprises all microorganisms in a given area together with the surrounding biotic and abiotic factors.

Microbial community: consists of a microbial population of all species living in a given area where the microbial population is the group of individuals belong to the same species.

Microbial habitat: is the location in the environment where microorganisms are found and from which can be isolated.

There are four major microbial habitats related to one another by biotic factors and abiotic (physical and chemical).

1- In the air we breathe (atmospheric habitat).
2- In the water we drink (aquatic habitat).
3- In the soil where our food is grown and cultivated (terrestrial habitat).
4- On and in animals and plants from which our food is produced and in and on human body (biological habitat).

Microorganisms can live in macro habitat such as soil and microhabitat such as air particles, oral cavity, teeth surface, hair root, insect gut …… etc.

Most microorganisms have several habitats in different ecosystmes. For example; *Rhizobium* in terrestrial, aquatic and biological habitats and *Methanobacterium* in aquatic, cow rumen and human made ecosystem (septic tank). However, some microorganisms have just one habitat within a given ecosystem such as *Treponema pallidum* (in human reproductive system), *Mycobacterium tuberculosis* and *M. leprae.*

The ecological niche does not refer to the actual location of the microorganism but related to the function of the species of the population within the community. A niche as a term is used to specify the functional role of microorganism in its habitat. Consequently; microorganisms may have several habitats and only few niches. Three factors determine the microbial function in the habitat:

1- Nutritional needs.
2- Biochemical activity.
3- Tolerance to the environmental conditions.

In natural ecosystem such as cow rumen only cellulolytic bacteria that can degrade cellulose anaerobically and get their energy by fermentation can maintain themselves and flourish in this natural ecosystem. Furthermore, they must be able to tolerate the rumen temperature (35°C) and physical factors or chemical factors and presence of the fatty acid, NH_3 and other harmful products.

At the same time, biological activity of rumen cellulolytic microorganisms plays an important role in food consumer because celluloses are produced only by microorganisms which degrade cellulytic plants and produce simple source of carbon. Alongside, the animals are provided with high amount of N from billions of dead bacterial cells.

Plant residue in the rumen

Rumen microbial community

1- *Ruminococcus flavefaciens*
2- *R. albus*
3- *Bacteroides succinogenes*

4- Cellulolytic fungi and protozoa

Simple sugar (glucose)

A-Source of energy and carbon

Fermentation by **non methanogenic** bacteria

$\downarrow$

formate, succinate, lactate, acetate, ethanol, CO_2, H_2

$CO_2 + H_2 \longrightarrow 2H_2O + CH_4$

Fermentation by **methanogenic** bacteria ex.*Methanobacterium rumenatum*

$\downarrow$

acetate, CO_2, CH_4

B- Billions of dead microbial cells as source of N for animal growth.

C- Biogas for human activity such as electricity and cooking.

D- Fertilizer and soil conditional animal manure and biogas.

Moreover, in natural microbial ecosystem (human body) and cow rumen there are human made ecosystem such as lab fermenter, bioreactor, sewage tanks … etc. in these ecosystems; microbial succession take place.

Microbial succession is the successional changes in microbial population over a period of time as in the following human made ecosystem:

stage	Septic tank	Microbial population
I	Raw sewage (complex organic compounds)	Waste degradation by (aerobic, oxidative

	Lignin, cellulose, fat, protein… etc	population, proteolytic, cellulolytic and lipolytic, microorganisms
II	Product of raw material (Sugar, amino acids, organic acids, other organic compounds)	Facultative anaerobic populations (oxidative and fermentative)
III	Simple organic compounds (nutrients, gases …. etc.)	Anaerobic microbial populations (methane producing bacteria, H_2S producing bacteria)
End products (CH_4, CO_2, H_2… etc) biogas (H_2S, NH_3)		

Therefore, the final product is the biogas and other gases producing under aerobic conditions.

Methane produced by anaerobic *Methanobacterium* and *Methanococcus* from degradation of sewage, organic matter and also by reaction of CO_2 and H_2. Thus from these microbial ecosystems we can get:

1- Energy.
2- Water.
3- Sulfur and other elements.
4- Fertilizers and soil conditions and the environment cleaning up from the sewage.

Prof.Dr.Ithar Kamil Abbas

Bioreactors and fermenters

A bioreactor is a specially designed vessel which is built to support the growth of high concentration of microorganisms. It must be so designed that it is able to provide the optimum environments or conditions that will allow supporting the growth of the microorganisms.

Bioreactors are commonly cylindrical vessels with hemispherical top and/or bottom, ranging in size from some liter to cube meters, and are often made of stainless steel and glass.

The difference between a bioreactor and a typical composting system is that more parameters of the composting process can be measured and controlled in bioreactors.

The sizes of the bioreactor can vary over several orders of magnitudes. The microbial cell (few mm3), shake flask (100-1000 ml), laboratory fermenter (1 – 50 L), pilot scale (0.3 – 10m3) toplant scale (2 – 500 m3) are all examples of bioreactors.

The design and mode of operation of a fermenter mainly depends on the production organism, the optimal operating condition required for target product formation, product value and scale of production. The design also takes into consideration the capital investment and running cost.

- Large volume and low value products like alcoholic beverages need simple fermenter and do not need aseptic condition.
- High value and low volume products require more elaborate system of operation and aseptic condition.

Bioreactors differ from conventional chemical reactors in that they support and control biological entities. As such, bioreactor systems must be designed to provide a higher degree of control over process upsets and contaminations, since the organisms are more sensitive and less stable than Chemicals. Biological organisms, by their nature, will mutate, which may alter the biochemistry of the bioreaction or the physical properties of the organism. Analogous to heterogeneous catalysis, deactivation or mortality occur and promoters or coenzymes influence the kinetics of the bioreaction. Although the majority of fundamental bioreactor engineering and design issues are similar, maintaining the desired biological activity and eliminating or minimizing undesired activities often presents a greater challenge than traditional chemical reactors typically require.

Other key differences between chemical reactors and bioreactors are selectivity and rate.

In bioreactors, higher selectivity — that is, the measure of the system's capability for producing the preferred product (over other outcomes) — is of primary importance. In fact, selectivity is especially important in the production of relatively complex molecules such as antibiotics, steroids, vitamins, proteins and certain sugars and organic acids. Frequently, the activity and desired selectivity occur in a substantially smaller range of conditions than are present in conventional chemical reactors. Further, deactivation of the biomass often poses more severe consequences than a chemical upset.

The Designing of a Bioreactor also has to take into Considerations the Unique Aspects of Biological Processes

A- The concentrations of starting materials (substrates) and products in the reaction mixture are frequently low; both the substrates and the products may inhibit the process. Cell growth, the structure of intracellular enzymes, and product formation depend on the nutritional needs of the cell (salts, oxygen) and on the maintenance of optimum biological conditions (temperature, concentration of reactants, and pH) within narrow limits.

B- Certain substances inhibitors effectors, precursors, metabolic products influence the rate and the mechanism of the reactions and intracellular regulation.

C- Microorganisms can metabolize unconventional or even contaminated raw materials (cellulose, molasses, mineral oil, starch, wastewater, exhaust air, biogenic waste), a process which is frequently carried out in highly viscous, non-Newtonian media.

D- In contrast to isolated enzymes or chemical catalysts, microorganisms adapt the structure and activity of their enzymes to the process conditions, whereby selectivity and productivity can change. Mutations of the microorganisms can occur under sub optimal biological conditions.

E- Microorganisms are frequently sensitive to strong shear stress and to thermal and chemical influences.

F- Reactions generally occur in gas-liquid -solid systems, the liquid phase usually being aqueous.

G- The microbial mass can increase as biochemical conversion progresses. Effects such as growth on the walls, flocculation, or autolysis of microorganisms can occur during the reaction.

H- Continuous bioreactors often exhibit complicated dynamic behavior.

Requirements of Bioreactors

Due to above mentioned demands made by biological systems on their environment, there is no universal bioreactor. However, the general requirements of the bioreactor are as follows:

1-The vessel should be robust and strong enough to withstand the various treatments required such as exposure to high heat, pressure and strong chemicals and washings and cleanings.

2- The vessel should be able to be sterilized and to maintain stringent aseptic conditions over long periods of the actual fermentation process.

3- The vessel should be equipped with stirrers or mixers to ensure mass transfer processes occur efficiently.

4- It should have sensors to monitor and control the fermentation process.

5- It should be provided with inoculation point for aseptic transfer in inoculum.

6- Sampling valve for withdrawing a sample for different tests.

Fermentation technology: -

Fermentation Technology could be defined simply as the study of the fermentation process, techniques and its application. Fermentation should not be seen merely as a process that is entirely focused on the happenings occurring in the fermenter alone! There are many activities that occur upstream leading to the reactions that occur within the bioreactor or fermenter, despite the fermenter is regarded as the heart of the fermentation process.

Fermentation technology is the whole field of study which involves studying, controlling and optimization of the fermentation process right up from upstream activities, mid-stream and downstream or post fermentation activities.

The study of fermentation technology requires essential inputs from various disciplines such as biochemistry, microbiology, genetics, chemical and bioprocess engineering and even a scatter of mathematics and physics.

Fermentation in terms of biochemistry and physiology:-

Fermentation is now defined as a process of energy generation by various organisms especially microorganisms. The fermentation process showed unique characteristics by which it generates energy in the absence of oxygen. The process of energy generation utilizes the use of substrate level phosphorylation (SLP) which do not involved the use of electron transport chain and free oxygen as the terminal electron acceptor.

Engineers definition of fermentation:-

It is only up to recently with the rise of industrial microbiology and biotechnology that the definition of fermentation took a less specific meaning. Fermentation is defined more from the point of view of engineers. They see fermentation as the cultivation of high amount of microorganisms and biotransformation being carried out in special vessels called fermenter or bioreactors.

Their definitions make no attempt to differentiate whether the process is aerobic or anaerobic. Neither are they bothered whether it involves microorganisms or single animal or plant cells.

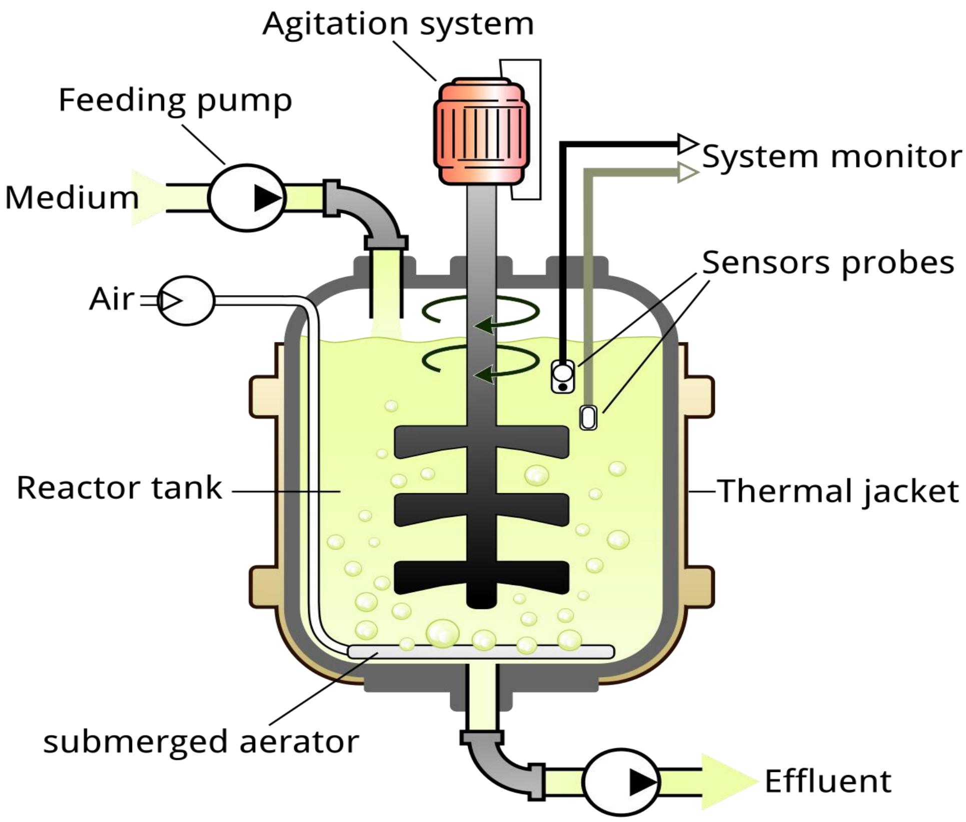
They view bioreactors as a vessel which is designed and built to support high concentration of cells.

What is the different between bioreactor and fermentor?

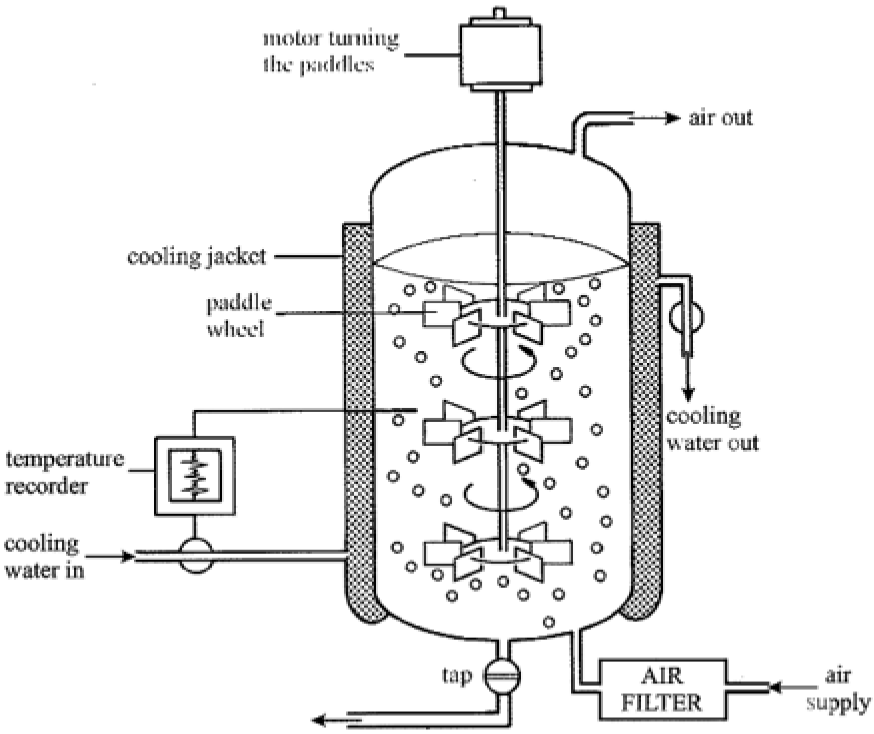
-**T**he systems which are utilized for the prime purpose of enhancing the growth and maintenance of a population of bacterial or fungal cells in a controlled mode are given by the fermentors. In contrast, it is the bioreactor system that is responsible for growth and maintenance of the mammalian and insect cells only.

-There are differences in sterilization process also. While in the case of a fermentor, it has to be sterilized full, a bioreactor is so designed so as to be sterilized empty.

- Bioreactors are large in comparison to fermentors and range in size from a few liters to cubic meters whereas fermentors are typically small having a capacity of up to 2 liters. Bioreactors are cylindrical vessels made of stainless steel.



Bioreactor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Fermentor

Prof.Dr.Ithar Kamil Abbas

Microbial Association-Microbial Interaction

Dr.Ithar Kamil Abbas

Microbial Associations

Many microbial populations interact and establish associations with each other and with higher organisms. Usually the association is nutritional, although other benefits may accrue and the association can become crucial to the survival of one or both partners. In 1879, de Bary coined the term 'symbiosis' to describe any situation where two different organisms live together. Confusingly, some biologists then used the same term specifically to mean the association where both the partners benefited. The term 'symbiosis' will be used in its original non-specific sense in this text. There are many sorts of symbiotic relationship such as mutualism, parasitism, amensalism and competition, predation, protocooperation (synergism) and commensalism between the organisms.

Microbial Associations and Fundamentals of their Interactions:

Table 1 – Impact of symbiotic relationships on organisms.

Relationship	Self	Opponent
Amensalism	Neutral	Harm
Commensalism	Benefit	Neutral
Competition	Harm	Harm
Mutualism	Benefit	Benefit
Parasitism	Benefit	Harm
Predation	Benefit	Harm
Proto-cooperation	Benefit	Benefit

The interactions between the two populations are classified as above according to whether both populations or one of them benefit from the associationship, or one or both populations are negatively affected

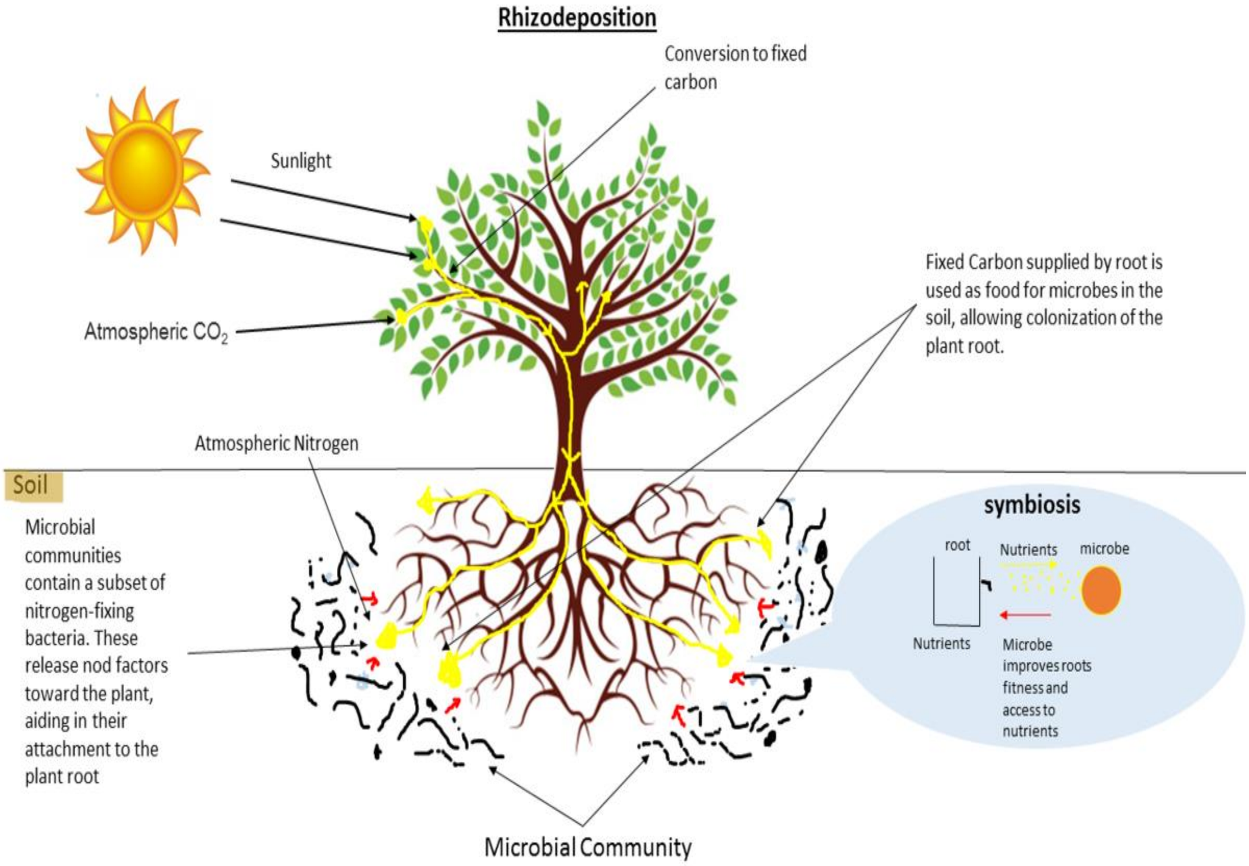
Mutualism and parasitism have been most extensively studied in microbial relationships.

Mutualism

Mutualism describes a relationship in which both associated partners derive some benefit, often a vital one, from their living together. Attempt to summarize the main kinds of mutualistic associations; some of which are trivial and of scientific interest only but others such as Rhizobium legume association, mycorrhizae, coral-microbial association, herbivore-microbial association and lichens are very important, or indispensable, both to the local ecosystem and on a world scale.

Kinds of Mutualistic Associations Involving Microorganisms

Mycorrhizae represent a mutualistic symbiosis between the root system of higher plants and fungal hyphae. Frank, who first noted the existence of such a characteristic association in the roots of Cupulifereae in 1885, coined the term ‘mycorrhiza’. Over the last 20 years, basic works conducted by hundreds of researchers from different countries has shown that this association is fundamental and universally occurring. Among the different symbiotic associations between the soil microorganisms and root of plants, mycorrhizae are the most prevalent as they occur on more than 90% of the vascular plants. However, Kumar and Mahadevan (1984) have studied a large number of mycorrhizal associations are found that they are highly influenced by the toxic substances that, when present, are essentially concentrated in the root of plants. Such substances may be alkaloids, phenolics, terpenoids, tannis, stilbenes, etc.



Advantages

1. The fungus derives nutrients via the root of the plant. Sugars formed in the leaves move down the stem as sucrose. Sucrose itself never accumulates in the fungus; it is converted into isomers such as ‘trehalose’ thus resulting in the low sugar concentration.

2. The fungal hyphae act like a massive root hair system, scavenging minerals from the soil and supplying them to the plant.
3. Because of this associationship the plant partner, in addition to the nutritional benefits, develops drought resistance, tolerance to pH and temperature extremes, and greater resistance to pathogens due to ‘phytoalexins’ released by the fungus

Nitrogen is one of the elements essential to life since it is present in DNA (the blueprint of life) as well as in proteins (cell constituents characteristic of every living being). In gaseous form, nitrogen constitutes 79 % of the atmosphere but most living creatures cannot assimilate it. However, certain bacteria, present in soil, can chemically reduce atmospheric nitrogen (N_2). The bacteria transform nitrogen into ammonia which can be assimilated by plants and which can then circulate up the food chain from plants to herbivores and from herbivores to carnivores. A few legumes and certain shrubs, such as the specked alder, have developed symbiotic relationships with these microorganisms (e.g., *Rhizobium leguminosarum*). They provide them with sugars and carbohydrates and receive, in return, the nitrogen required for their metabolism.

Symbiotic associations of bacteria-plant: Relationship between rhizobia and leguminous plants is very well known and intensely studied symbiosis. It represents an obligate endosymbiosis in which rhizobia of Rhizobium spp. and Bradyrhizobium spp. carry out unique biological nitrogen fixation process inside root nodules of legume host plants. Bacteroids or nodule rhizobia provide fixed nitrogen to fulfill nitrogen requirement of host plant and in turn receives protection, oxygen and photosynthetic nutrients for their growth. Free living nitrogen fixing bacteria like Azospirillum, Frankia and Azotobacter also fix nitrogen but ectosymbiotically with grasses, trees and crop plants respectively. Nitrogen fixing root nodule and free living bacteria are integral living functional components of terrestrial ecosystems. Interaction between aquatic fern, Azolla and cyanobacteria Anabaena spp. is also excellent example of obligatory symbiotic association. Anabaena fix nitrogen into utilizable form for fern and in turn gets protective habitat, oxygen and nutrients formed during Azolla photosynthesis. Different species of indigenous rhizosphere and soil bacteria such as Pseudomonas, Micrococcus, Bacillus, Proteus, Sarcina and Actinomyces also live in association with plant species. They mobilize insoluble phosphate compounds in utilizable form, sequester iron and zinc essential for plant growth and plant host in turn enhance the growth of these bacteria by provision of nutritive root exudates as carbon and energy source. Rhizobacteria protect plant host from deleterious soil microbes and also degrade recalcitrant soil pollutants like hydrocarbons, pesticide residues or heavy metals found near plant rhizosphere. Plant-rhizobacteria association is very beneficial as it forms the basis of phytoremediation and bio fertilization applications.

Herbivore-Microbial Association:

Plants contain about 30% cellulose (dry weight), the large insoluble inert polysaccharide. It would be very much to the advantage of any herbivore to digest the chemical but, however, the only herbivore to possess the appropriate digestive enzyme, cellulose, is snails. All others, from insects to mammals, do not possess this enzyme and they establish mutualistic associationship with cellulose splitting bacteria and protozoa. These microorganisms generally occupy one of the several sites in the gut, the most advanced condition being that in ruminants. Ruminants, such as cow and sheep, have evolved a unique four chambered ‘stomach’ that has helped establish them as extremely successful herbivores. The rumen volume is large compared with size of the mammal (in the cow it is 80-100 L) so that there being a long resistance time for cellulose decomposition. Plant material is chewed, mixed with saliva and passed to rumen. The rumen contains very numerous microorganisms of which about 90% are cellulose secretors. These may be 104 to 105 protozoa. 1010 – 1011 bacteria and 4×10^4 fungi. The contents of the rumen are continually mixed by slow contractions of the wall at 1-2 minute intervals.

Microbial Action in the Rumen of the Ruminants

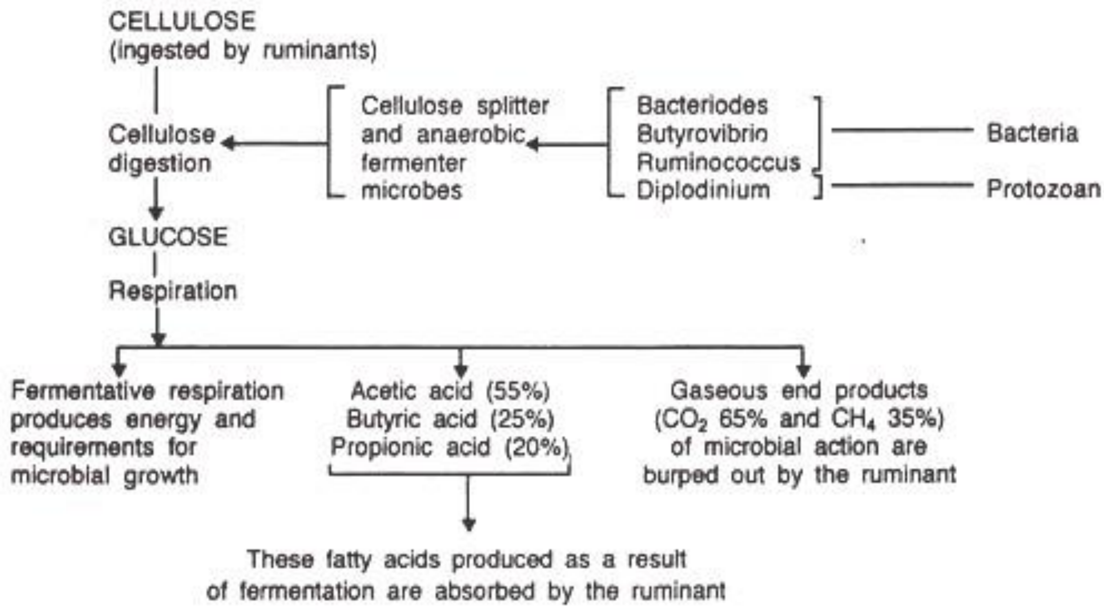


FIG. 33.8. Microbial action in the rumen of the ruminants (*e.g.*, Cow).

Microbes in herbivore-rumen

Though less in number than the bacteria, the protozoa may be 50% of the biomass and they include isotrichia, Diplodinium, Dasytrichia and Epidinium. Generally, these protoza have been assigned a role in increasing bacterial turnover rate by their feeding; they remove about 1% of the bacteria per minute. Some of the protozoa can also degrade cellulose but probably not significantly when compared with the bacteria.Many bacteria have been isolated from the rumen but the main ones are *Rumenococcus flavifaciens*. *R. albus*, *Bacteroids succinogenes*. *B. amylophilus* and *Butyrovibrio*. These and others degrade cellulose and starch and generate, as earlier described, organic acids. Methane is formed by *Methanobacterium ruminnantium*, using hydrogen and carbon dioxide produced by the degradation.The fungi in rumen have only been reported recently; they include some yeasts but more especially there are anaerobic chytrid-like fungi with multiflagellate zoospores which are apparently widely distributed. These fungi may be significant in the digestion of lignocellulose.

Prof.Dr.Ithar Kamil Abbas

Symbiosis of bacteria-animals:

Bacteria are known to be very common endo and ectosymbiotic microorganisms of humans, insects and other animals. All human beings acquire specific bacteria at the birth as normal flora which remain associated throughout the life span. Animals such as cattle also bear specific bacteria as resident microflora. Normal flora bacteria attach and colonize epithelial mucus lining of organs of gastrointestinal, genitourinary and respiratory tract of animals and humans. They represent mutualism as well as commensalism with the host. Normal flora receives protective environment, nutrition and transport from host while as host gets supply of certain B vitamins, development and activation of immune system and protection from pathogenic microbes. Lactobacilli residing in vagina produce lactic acid which protects female genitourinary tract from infectious yeasts like Candida and other pathogenic microbes. Rumen bacteria such as *Propionibacterium spp.* residing in cattle intestine are cellulolytic and actually digest cellulosic food ingested by animal; cattle don't have other means of cellulose utilization and cellulosic content of vegetable matter would otherwise impossible to degrade without aid by rumen bacteria. Cooperative interaction between aphids and bacteria *Buchnera aphidicola* is one of the best example of insect and bacterial symbiosis. Bacteria provide all enzymes necessary for the synthesis of essential amino acids which are lacking in aphid food (plant sap); in return aphids synthesize enzymes required to build up bacterial cell wall. Another example of bacteria-animal symbiosis is of gutless worms of deep hydrothermal vents. They have sulfur oxidizing bacteria as endosymbionts; since they don't have digestive tract, they are completely dependent on nutrition provided by these bacteria. Association of squids, jelly fish and fishes like angler fish and lantern fish with luminescent bacteria is interesting example of symbiotic interaction. Bioluminescence is acquired by luminescent bacteria like Vibrio fischeri which harbor inside the light organs of these animals; it is very useful for location of prey, as path finder in deep sea regions, to attract mate or to warn off or repel the predators.

<u>Association of bacteria-protozoa</u>: Complex interactions among termite, bacteria and protozoan Trichonympha is good example of mutualistic association. Cellulose in the form of plant fiber is the principle food of termite but it is not able to digest without digestive help from Trichonympha. Trichonympha on the other hand relies on bacteria present on its surface for particular lytic enzymes fir complete digestion of cellulosic material. Neither termite nor Trichonympha and associated bacteria live in absence of any of the organism. One more example is relationship between protozoan Mixotricha and bacteria. This protozoan lacks mitochondria but it harbors bacteria that fulfill the vital functions of mitochondria.

Bacteria-algae symbiosis: Obligate relationship is observed between some higher algae and bacteria synthesizing vitamin B12. Some algae cannot produce cobalamin on their own and hence dependent on exogenous supply of cobalamin which is fulfilled by bacteria associated with algal cells. Bacteria in turn derive nutrition from algae produced during photosynthesis. Algae-bacteria associateship is also evident in sewage with high phosphorus concentration and during activated sludge process.

Algae-fungi symbiosis:

The most familiar example of mutualism between microorganisms is that of lichens, which comprise a close association between the cells of a fungus (usually belonging to the Ascomycota) and a photosynthetic alga or cyanobacterium. The fungal partner forms the upper surface of the lichen. The remaining structures below this surface are composed of a network of fungal threads (hyphae) and photosynthetic cells. In most lichens photosynthetic green algae are arranged in a layer (algal layer) just under the outer lichen surface. In some lichens there are additional partners a blue green bacterium. Although many different fungal species may take part in lichens, only a limited number of algae or cyanobacteria do so. Lichens are typically found on exposed hard surfaces such as rocks, tree bark and roofs, and grow very slowly at a rate of a millimetre or two per year. They often occupy particularly harsh environments, from the Polar Regions to the hottest deserts. The photosynthetic, Often unicellular, partner usually exists as a layer of cells scattered among fungal hyphae (figure 6). It fixes carbon dioxide as organic matter, which the heterotrophic fungus absorbs and utilizes this organic matter. The fungal member provides anchorage and supplies inorganic nutrients and water, as well as protecting the alga from excessive exposure to sunlight.

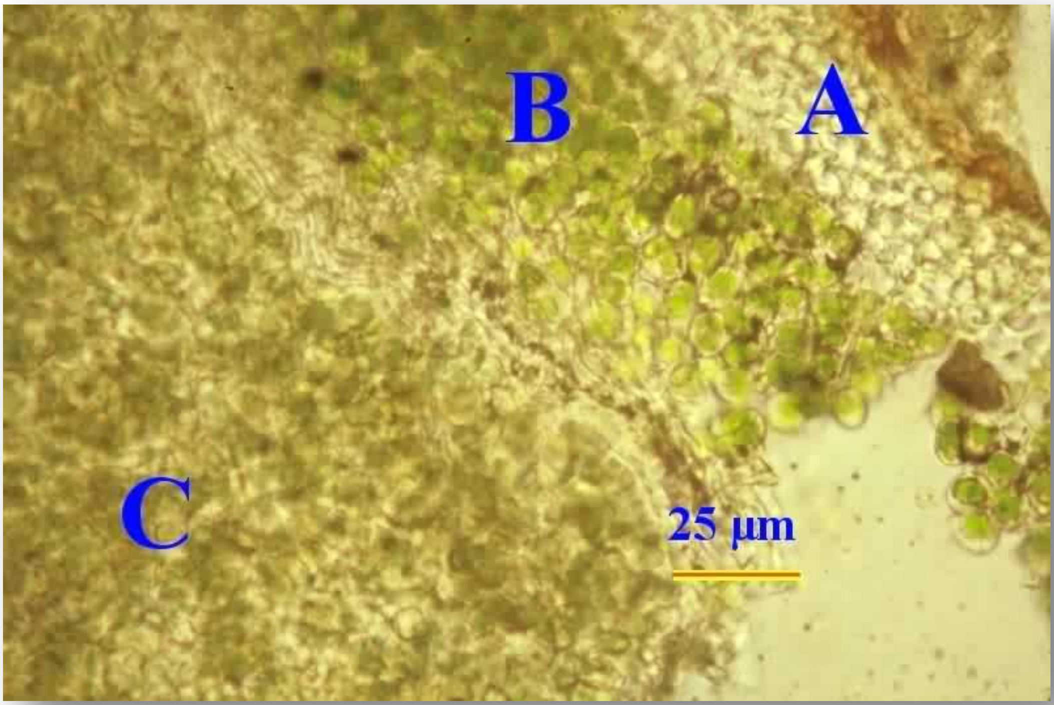


Figure 6: Algae and fungi combine to form lichens. (A) fungal. (B) photosynthetic green algae. (C) blue green bacterium.

Although lichens are tolerant of extremes of temperature and water loss, they have a well-known sensitivity to atmospheric pollutants such as the oxides of nitrogen and sulphur. Their presence in an urban setting is therefore a useful indicator of air quality. Lichens were used for many years as a source of brightly coloured dyes for the textile industry; they are also used in the perfume industry. The dye used in litmus paper is derived from a lichen, belonging to the genus *Roccella*.

It should be stressed that lichens are not just a mixture of fungal and algal cells. They are distinctive structures with properties not possessed by either of their component species. Indeed, the relationship between the two partners of a lichen is so intimate that the composite organisms are given taxonomic status. Many thousands of species of lichen have been identified.

Bacteria-bacteria relationships:

Cyanobacterial blooms, formation of biofilms such as dental plaque, opportunistic infections and involvement of native bacteria or consortia in biodegradation of petrochemicals or pesticides represent classical examples of interactions between different bacteria. Soils and aquatic environments contaminated by oil spills have been found to contain indigenous bacteria like Aeromonas, Pseudomonas, Sphingomonas, Acinetobacter, Brevibacterium, Micrococcus, Arthrobacter and Bacillus actively degrading aromatic and aliphatic components of oil. It was also investigated that rate of degradation was higher when these bacteria worked as consortium than individual strain. Many bacterial species of Pseudomonas, Deinococcus and Actinobacteria have been found to be associated with cyanobacteria forming harmful blooms. Some of these bacteria were also found to degrade algal toxins to receive their nutrient requirements. Bacteria in consortia are able to communicate by **quorum sensing mechanism.**

Bacteria in consortia or biofilms function in coordination and get advantages like protection, nutrition and resistant to antimicrobial agents.

Quorum sensing (QS) : is a process of bacterial cooperative behavior that has an effect on gene regulation. This cell-to-cell communication system involves the production of signaling molecules according to cell density and growth stage. Virulence, the ability to infest a habitat and cause disease, is also governed by such communication signals.

Some of the best-known examples of quorum sensing come from studies of bacteria. Bacteria use quorum sensing to regulate certain phenotype expressions, which in turn, coordinate their behaviors. Some common phenotypes include biofilm formation, virulence factor expression, and motility. Certain bacteria are able to use quorum sensing to regulate bioluminescence, nitrogen fixation and sporulation. The quorum-sensing function is based on the local density of the bacterial population in the immediate environment. It can occur within a single bacterial species, as well as between diverse species. Both Gram-positive and gram-negative bacteria use quorum sensing, but there are some major differences in their mechanisms.

Syntrophy

Syntrophy (Gr. syn=together; trophe-nourishment) is such mutualistic interrelationship between two different microorganisms which together degrade some substances (and conserve energy doing it) that neither could degrade separately. In most cases of syntrophism the nature of a syntrophic reaction involves H_2 gas being produced by one partner and being consumed by the other. Thus, Syntrophy has also been called interspecies hydrogen transfer. Following are some examples of syntrophic associations:

(i) Ethanol fermentation to acetate and eventual production of methane is a good example. Ethanol oxidizing bacterium ferments ethanol producingH2 which is a valuable electron donor for methanogenesis hence used by a methanogen. When both these reactions are summed, the overall reaction is exergonic (i.e., energy releasing). Actually, the oxidation of ethanol to acetate plus H_2 is energetically unfavorable, the reaction becomes favorable when H_2 produced during it is consumed by the methanogens. In this way, both partners thus use the energy released in the coupled reaction of syntrophic association.

Parasitism

Parasitism represents the symbiotic associationship between two living organisms and is of advantage to one of the associates (parasite) but is harmful to the other (host) to a greater or lesser extent. The parasites may be destructive or balanced. The former destroy the host cells in their later stages of development whereas the latter fulfil their demands from the host in such a way that the host cells are not destroyed but continue to live.

Facultative and Obligate Parasites Associations would be easy to describe if organisms always behaved in the same way. Unfortunately, they do not. Many microorganisms, for instance, can survive as both parasites and saprophytes. The fungus Ceratocytstis ulmi, which causes Dutch elm disease, kills the tree and then lives saprophytically on its dead remains. Such an organism which mostly lives as saprophyte but seldom holds the charge of a parasite is referred to as facultative parasite. In contract, downy mildews, powdery mildews, etc. only grow on live protoplasm of the host plant in nature. Such as organism which cannot live elsewhere except on the living protoplasm of its host in nature is called obligate parasite (biotroph). Facultative and obligate parasites often differ in their pathogenic effects, i.e., in their ability to injure the host. Since obligates are restricted to living organisms, their effects on the host are often less severe, although the host may show less vigorous growth. In contrast, facultative parasites which have only recently acquired a host, tend to be more damaging.

Mycoparasitism

When one fungus parasitizes the other, the act is referred to as 'mycoparasitism'. This term has been generally used interchangeably with 'hyperperasitism'. 'direct parasitism' or 'interfungus parasitism'. This incitant is generally called 'mycoparasite' or 'hyperparasite'. Mycoparasitism has been classified into two main groups on the basis of nutritional relationship of parasite with host : necrotrophic and biotrophic.

(a) Necrotrophic Mycoparasitism

The necrotrophic (destructive) parasite makes contact with its host, excretes a toxic substance which kills the host cells and utilizes the nutrients that are released.

(b) Biotrophic Mycoparasitism

The Biotrophic (balanced) parasite is able to obtain its nutrients from the living host cells, a relationships that normally exists in Nature. The Mycoparasitism is of common occurrence and examples can be found among all the groups of fungi from chytrids to higher basidiomycetes .

A three member mycoparasitic associationship has also been reported in which chytridium parasiticum is parasite on Chytridium subercrelatum which, too parasitizes *Rhizidium richmondense*, another chytrid .

The biological control of plant diseases has recently become an area intensive research in view of the hazardous impact of pesticides and other agro-chemical on the ecosystem. Amongst the biological agents, the mycoparasites have attained a significant position. It has been suggested that efforts should be made to investigate the biological control of plant diseases through parasitism and predation. Therefore, the mycologists and plant pathologists are searching for new mycoparasites because the greater number of these the greater would be the chance of exploiting them as agents for biological control. Trichoderma is an important example.

New terms for parasites

The belief that the obligate parasites cannot be grown in laboratory on artificial culture media came at stake when after reports poured in recent years in which there are claims to grow successfully some obligate parasites on culture media. This has prompted the biologists to propose new terminologies in this respect.

Biotroph A parasite which always obtains its food from living tissues regardless of the ease with which it can be cultured in the laboratory on artificial media, is referred to as a biotroph. The term ‘biotroph’ is now being used for obligate parasites because some of them have been successfully cultured in the laboratory during past few decades.

Hemibiotroph

A parasite is called Hemibiotroph if it attacks living in the same way as the biotroph but continues growing and reproducing even after the living tissues are dead. Hemibiotroph, infact represent facultative saprophytes.

Necrotroph

A parasite, when it kills living of the host in advance of penetration during infection and then obtains its food as a saprophyte, is called a nectotroph. Necrotrophs represent the facultative parasites and are also referred to as perthotrophs or perthophytes.

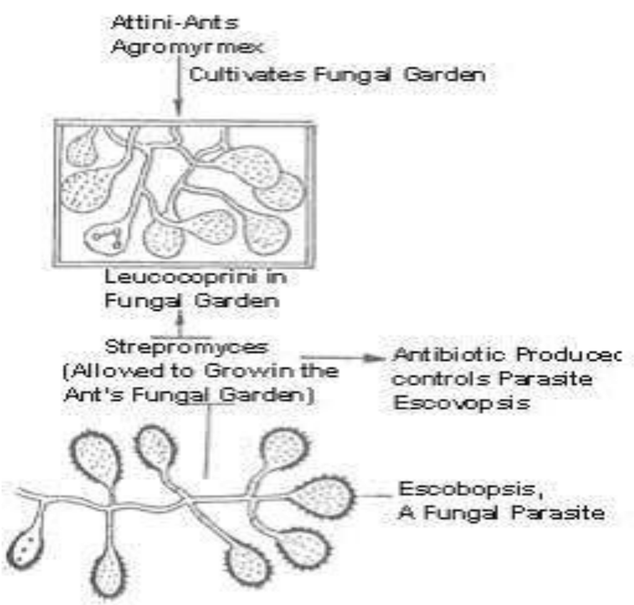
Prof.Dr.Ithar Kamil Abbas

Amensalism

Amensalism : refers to such an interaction in which one microorganism releases a specific compound has a negative effect on another microorganism. That is, the Amensalism is a negative microbe-microbe interaction. Some important examples are:

(i) Antibiotic Production by a microorganism and inhibiting or killing of other microorganism susceptible to that antibiotic is the important example of Amensalism. Concentrations of such antibodies in the bulk of soil or water are certainly small, though there could be a large enough quantity on a micro-habitat scale to give inhibition of nearby microorganisms. The antibiotics reduce the saprophytic survival ability of pathogenic microorganism in soil. The attini ant-fungal mutualistic relationship is promoted by antibiotic producing bacteria (e.g., Streptomyces) that are maintained in the fungal gardens . In this case, streptomyces produces an antibiotic which controls Escovopsis, a persistent parasitic fungus, which can destroy the ant's fungal garden.

(ii) Production of **ammonia** by some microbial population is deleterious to other microbial population. Ammonia is produced during the decomposition of proteins and amino acids. A high concentration of ammonia is inhibitory to nitrate oxidizing populations of Nitrobacter.



A Schematic Diagram Showing the Use of Antibiotic Producing Streptomyces by Attini ants to control the growth of fungal parasite in their fungal Garden.

Competition

In contrast to the positive interactions of mutualism and synergism, competition represents a negative relationship between two populations in which both populations are adversely affected with respect to their survival and growth. In this case, the microbial populations compete for a substance which is in short supply. Competition results in the establishment of dominant microbial population and the exclusion of population of unsuccessful competitors. During decomposition of organic matter the increase in number and activity of microorganisms put heavy demand on limited supply of oxygen, nutrients, space etc. The microbes with weak saprophytic survival ability are unable to compete with other soil saprophytes for these requirements and either perish or become dormant by forming resistant structures.

Predation

Predation typically occurs when one microorganism, the predator, engulfs and digests other microorganisms, the prey, and the former derives nutrition from the latter. In microbial fraternity, however, the distinction between predation and parasitism is not sharp. The interaction between Bdellovibrio bacteria and other small gram-negative bacteria is considered by some as predation but by others as parasitism. Bdellovibrio is apparently quite widespread in aquatic habitats and attacks other bacteria, normally gram-negative ones by boring a hole in the wall, entering the bacterium and causing lysis with the eventual release of many small Vibrio-shaped bacteria. The major microbial predators are the protozoa which may engulf bacteria and more rarely algae and other protozoa. These systems have been used extensively in models and simulations of predator prey-relationship. In the simplest from the protozoan population (e.g., Tetrahymena) is limited by its bacterial food (e.g., Klebsiella) and numbers of both prey and predator show cyclic oscillations. Another such example is of Didinium-paramecium (both protozoa) relationships. Didinium preys on the paramecium until the population of the later becomes extinct. Lacking a food source, the Didinium population also becomes extinct. If a few members of the paramecium population are able to hide and escape predation by the Didinium, then the paramecium population recovers following the extinction of the Didinium. Thus a cyclic oscillation can occur in the population of these two protozoans. Predatory fungi exist and have been considered as possible biocontrol agents for some diseases of plants caused by soil microorganism. Nematodes and protozoa may be trapped by a variety of net-like-hyphae, sticky surface and nooses. The organism is the invaded by hyphae and digested.

Protocooperation synergism

Protocooperation (or synergism), like mutualism, represents an association between two microbial population in which both population benefit from each other, but it differs from the mutualism in that the association is not ‘obligatory’. Both synergistic populations of microbes are able to survive in their natural environment on their own. Protocooperation or synergism allows microbial population to perform metabolic activities such as synthesis of a product which neither population could perform alone.

Following are few examples:

(i) The **Desulfolvibrio** bacteria supply H_2S and CO_2 to Chlorobium bacteria and, in turn, the Chlorobium bacteria make sulphate (SO_4) and organic material available to Desulfovibrio. Thus the mixture of the two bacterial populations produce much more cellular material than either alone.

(ii) Nocardia population metabolizes cyclohexane resulting in degradation products that are used by Pseudomonas population. The Pseudomonas population. The pseudomonas species produce biotin and growth factors that are required for the growth of Nocardia.

(iii) Azotobacter population present in soil fixes atmospheric nitrogen if they have a sufficient source of organic compounds. Other soil bacterial populations such as cellulomones are able to utilize the fixed form of nitrogen and provide the Azotobacter population with needed organic compounds.

Commensalism

Commensalism represents a relationship between two microbial populations in which one is benefit and the other remains unaffected (i.e., neither benefit nor harmed). Thus the commensalism is a unidirectional relationship between two microbial populations, It is quite common, frequently based on physical or chemical modifications of the habitat, and is usually not 'obligatory' for the two population involved. Commensalistic association is often established when one microbial population, during the course of its normal growth metabolism, modifications the habitat in such a way that the other population is benefited.

Following are some examples:

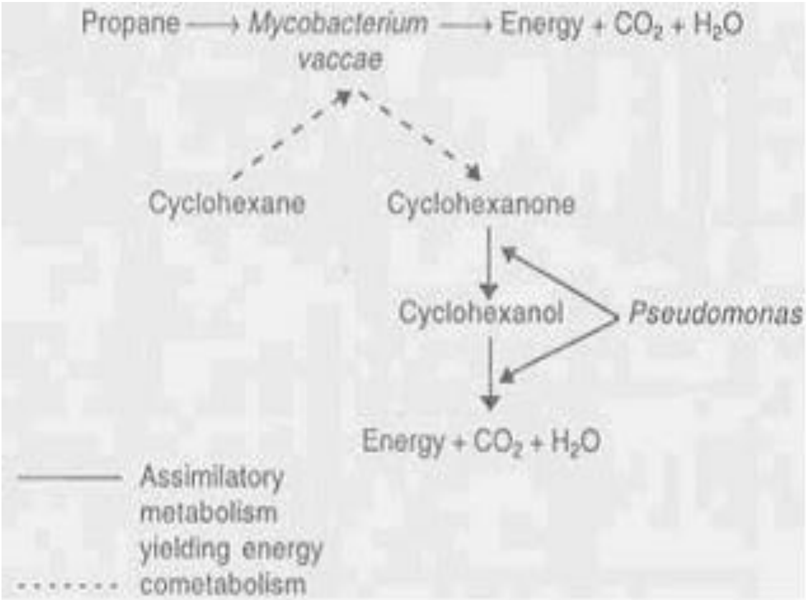
(i) A disease causing microbial population when a lesion on the surface, it creates an entry-passage for other microbial population that otherwise could not enter and grow in the host tissues. For convenience, Mycobacterium leprae, the causative agent of leprosy, open lesions on the body-surface and thus allow other pathogens to establish secondary infections.

(ii) When facultative anaerobes utilize oxygen and lower the oxygen content, they create anaerobic habitat which suits the growth of obligate anaerobes because the latter benefit from the metabolic activities of the facultative anaerobes in such a habitat. On the contrary, the facultative anaerobes remain unaffected. The occurrence of obligate anaerobes within habitats of predominantly aerobic character, such as the oral cavity, is dependent on such commensal relationship.
(iii) Population of Mycobacterium vaccae, while growing on propane cometabolizes (gratuitously oxidizes) cyclohexane to cyclohexanone which is then used by other bacterial population, e.g., Pseudomonas. The latter population is thus benefited since it is unable to oxidise cyclohexane to cyclohexanone. Mycobacterium remains unaffected since it does not assimilate the cyclohexanone.

Cometabolism

Cometabolism is the process in which one organism growing on a particular substrate gratuitously oxidizes (i.e., oxidizes without any motive) a second substrate which is of no use for it. The oxidation products, however, are well used by other organism.

An Example of Commensalism based on cometabolism



Some microbial populations create Commensalistic habitat by detoxifying compounds by immobilization. Leptothrix bacteria deposite manganese on their surface. In this way, they reduce manganese concentration in the habitat thus permitting the growth of other microbial populations. If Leptothrix do not act so, the manganese concentration would be toxic to other microbial population.

Biotechnology

Microbiology makes an important contribution to biotechnology, an area of science that applies microbial genetics to biological processes for the production of useful substances. Microorganisms play a central role in recombinant DNA technology and genetic engineering. Important tools of biotechnology are microbial cells, microbial genes and microbial enzymes.

The genetic information for many biological products and biological processes can be introduced into microbes in order to genetically engineer them to produce a substance or conduct a process. The genes can come from any biological source: human, animal, plant or microbial. This opens the possibility for microbial production of foods, fuels, enzymes, hormones, diagnostic agents, medicines, antibiotics, vaccines, antibodies, natural insecticides and fertilizers, and all sorts of substances useful in our civilization and society. Also, the microbial genes that encode for these substances, most of which are unknown, are a tremendous resource of information for application in medicine, pharmacy, agriculture, food science and biotechnology.

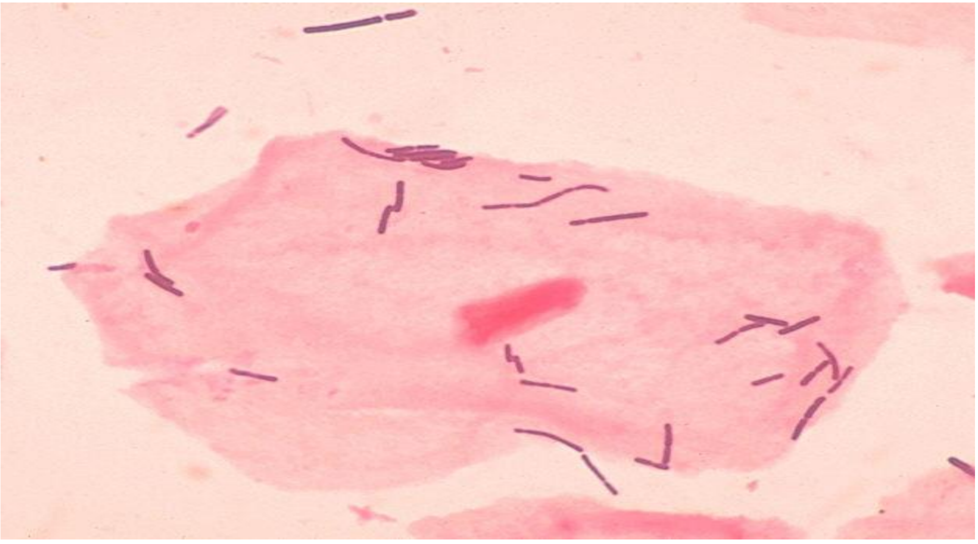
The Impact of Microbes on the Environment and Human Activities

-Beneficial Effects of Microorganisms

Microbes are everywhere in the biosphere, and their presence invariably affects the environment that they are growing in. The effects of microorganisms on their environment can be beneficial or harmful or in apparent with regard to human measure or observation.

The beneficial effects of microbes derive from their metabolic activities in the environment, their associations with plants and animals, and from their use in food production and biotechnological processes.

The microbes that normally live in associations with humans on the various surfaces of the body (called the normal flora), such as *Lactobacillus* and *Bifidobacterium*, are known to protect their hosts from infections, and otherwise promote nutrition and health.



Lactobacillus acidophilus **and a vaginal squamous epithelial cell. CDC. L. acidophilus (informally known as Doderlein's bacillus) colonizes the vagina during child-bearing years. As a lactic acid bacterium, the organism creates a low pH (acidic environment) on the tissues which prevents colonization by potentially harmful yeast and other bacteria.**

Prof.Dr.Ithar Kamil Abbas

Extreme microbial environments

Generally there are many abiotic (physical and chemical) factors selectively influence the survival and proliferation of microorganisms in nature. Microorganisms can tolerate extreme factors and live in several habitats where the environmental conditions are in there extreme level. For examples the icy pool of Antarctica, salt lakes, sea bottom where the pressure is nearly 10 tons/cm^2 others are isolated form hot springs and boilers. Such microorganism referred as to "extremophiles". Although the domain Archaea is known for wide spread in such extreme habitats, extremophiles are present in both Bacteria and Archaea domains.

In general there are ten main groups of extremophiles:

- Thermophiles: microorganisms can thrive at temperature 60-80° C.
- Hyperthermophiles: microorganisms can thrive at temperature 80-121° C.
- Psychrophiles: microorganisms those are capable of growth and reproduction in cold temperatures, ranging from -15 to +10°C.
- Acidophiles: microorganisms that can withstand and even thrive in acidic environments where the pH values are below 3.
- Alkaliphiles: *microorganisms that grow optimally or very well at pH values above 9, often between 10 and 12*.
- Halophiles: microorganisms requiring at least 2M of salt (NaCl) for growth.
- Barophiles: microorganisms live at high pressure.

- ❖ Xerophiles: microorganisms can grow in extremely dry habitats.
- ❖ Osmophiles: microorganisms can grow in high sugar concentrations.
- ❖ Oligotrophs: microorganisms can grow in nutritionally limited habitats.

However, many extremophiles fall under multiple categories for examples microorganisms living inside hot rocks deep under earth's surface are both thermophilic and barophilic, or in deep ocean hot springs. In hot sulfur springs, thermophilic and acidophilic are found at the same time.

Extreme thermophilic microbial community and habitats

Most life as we know it seems to have an upper temperature limit somewhere between 40-50°C. However, several microorganisms live, grow and multiplicate at much higher temperature (55-80 °C). They are called thermophiles to distinguish them from mesophilic microorganisms which grow between 25 and 40°C, psychrophilic microorganisms which grow optimally below 20 °C. Moreover, the extreme thermophile can grow above 80 °C.

The growth of microorganisms at high temperature has posed the question: how do these organisms mange to live in high temperature and we know that ordinary proteins and other cell constituents inactivated or denatured at high temperature?

Researchers, particularly the microbial biochemists, demonstrated that proteins, enzymes and nucleic acids from thermophiles are stable to high temperature do not function well enough at lower temperature.

Thermophilic microorganisms do not constitute a particular genus or genera, thus thermophiles exist among algae, fungi and several bacterial genera.

Hot springs appear the main natural habitat of thermophiles. These springs appeared on the surface; where water is heated by geological processes in the earth's crust. Convection in the water stirs up chemicals like sulphur and methane from surrounding rocks to make this habitat thermo-chemical habitat.

Temperature in hot springs can be as the boiling point of water, although a common range below the boiling temperature. *Thermus aquaticus* isolated from hot springs in yellow stone national park. This bacteria has extensively colonized domestic hot water systems (boilers). Chemolithotrophic sulphur-oxidizing bacteria such as *Sulfolobus acidocaldarius* which capable to grow at 85°C and *Thiobacillus* spp. were isolated from hot sulfurous acid springs where the temperature is 60-70°C and pH value 2-3.

Synechococcus lividus usually with the gliding thermophilic phototrophic bacteria; *Chloroflexus aurantiacus* make a characteristic mat on the surface of hot water.

From the economic viewpoint, thermotrophic and thermophilic fungi play a role in the heating of stored cereals in silo. Thermotrophic *Geotrichum candidum* and *Aspergillus fumigatus* are succeeded, at 50-60°C, by thermophilic *Mucor pusillus* and *Humicola lanuginosa*. At temperature above 65°C, numbers of such fungi diminish and further cereal heating is due to bacteria and actinomycetes growth and exothermic chemical reactions.

Coal waste tips which commonly reach temperatures exceeding 60°C contain thermophiles including unusual acidophilic mycoplasma-like thermophile *Thermoplasma acidophilum* which seems to be restricted to coal tips, probably as a consequence, a requirement for particular growth factors. Generally thermophiles active in the plant residue, manures, humus and any stored self-heating organic residues and materials.

Thermoenzymes extracted from thermophiles are advantageous for industry, because at high temperature most enzymes used to breakdown or denature and so are unable to complete the given reaction. Also the solubility of many reaction components is significantly improved at high temperature. Enzymes from thermophiles include xylanases used for paper bleaching; glycosyl-hydrolases used for food processing and textiles, chitinases for chitin modification in food and health products.

Principles of molecular adaptation of microorganisms to extreme temperature

The main scientific principle of such adaptation is that the essential cell components (membranes, proteins, enzymes, N.A) more heat stable than these of mesophiles.

Membranes and lipids

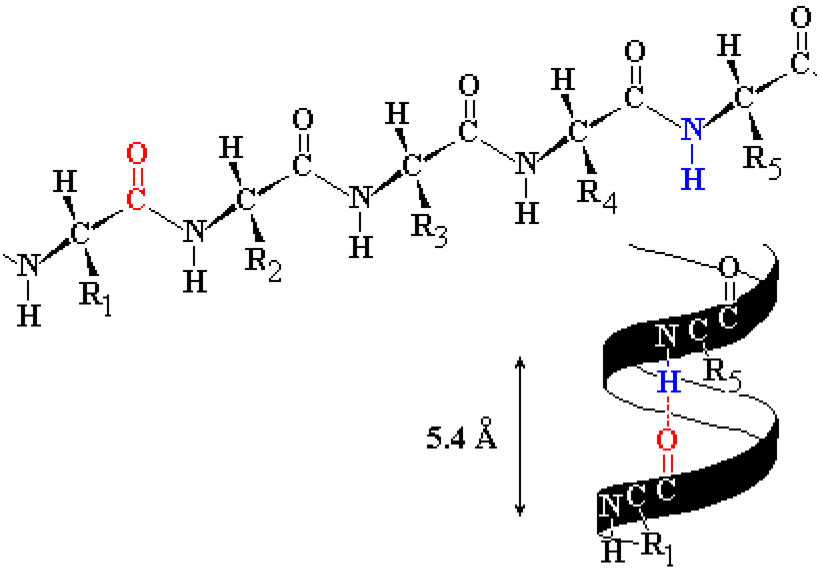
Investigators consider the thermostability of membranes to the melting point of its lipid and the maintenance of membrane stability is an important aspect of life in extreme temperature. It is suggested that domains of gel and liquid must coexist to maintain both structural integrity and the functional capability of a membrane and therefore a larger proportion of high-melting temperature membrane lipid would be predicted in thermophiles, and conversely a larger proportion of membrane lipids fluid at low temperature would be necessary in psychrophiles. Furthermore, bacteria are able to modulate the proportion of the synthesis of such lipids as the growth temperature changes. For example thermophilic *T. aquaticus* in addition to possessing a large proportion of high melting point membrane lipids it shows a decrease in C17 branched fatty acids, with increase in high melting point C16 straight chain fatty acids as the growth temperature increased. Psychrophilic bacteria have large amount of unsaturated low melting point fatty acids. Psychrotrophic mutants of mesophilic *S. typhimurium* show altered fatty acids with fewer saturated fatty acids.

Proteins, Ribosomes and Enzymes

It was stated by many researchers that molecular interactions in the molecules of proteins and enzymes of thermophilic *Geobacillus stearothermophilus* and *T. aquaticus* gave heat stability to these proteins. These molecular interactions increase the hydrophobic ionic bonds and such bonds more stable to high temperature. Also they suggested that protein thermostability depends on amino acids content and sequence. Studying subunits of ribosomes of these bacteria it was stated that the melting point of these subunits are higher than those of the subunits of *E. coli*. These results clearly demonstrated that the thermostability of ribosomes mostly depend on thermostability of ribosomes proteins. Such protein thermostability mostly depends on:

1- Thermophiles both have a larger fraction of their residues in the alpha-helical conformation, and they avoid Proline in their alpha-helices to a greater extent than the mesophiles.

Alpha -helix :is a common secondary structure of protein and is a right hand , coiled or spiral conformation (helix)in which every backbone N-H group donates a hydrogen bond to the backbone C=O group of the amino acid four residues earlier.



2- The presence of amino acids with S-S disulphide bond which give more stability than SH-amino acid, so the increase of cystine (S-S) than cysteine (SH) in protein molecule increased the heat stability.
3- Hydrophobic bonds increase the thermostability of proteins so thermophiles have more amino acids with hydrophobic side chains.
4- Non-protein factors such as Ca^{++}, factor S, etc. increase the thermostability of enzymes of thermophilic bacteria.

Nucleic acids

Comparing nucleic acids of *T. aquaticus* and *T. thermophiles* a true thermophilic lives at 80-85 °C with nucleic acids of *E. coli* it was stated that the main principles of heat-stability of tRNA:

1- Increase in G+C content in relation to A+U content and this gives increase in number of hydrophobic bonds in RNA molecule.
2- High concentration of magnesium ions which protect the nucleic acids against heat denaturation.
3- High percentage of methyl groups linked with tRNA resulted in increase of three folds structure of RNA such structure gives

RNA stability against high temperature as well as against ribonucleases.**(It is type nuclease that catalyzes the degradation of RNA into smaller component.**

Psychrophilic microbial community and habitats

Temperate soils and waters as well as oceans and polar soils yield large numbers of organisms capable of growing at 0-5 °C, but the majority of such isolates seem to be psychrotrophic rather than psychrophilic. These psychrophiles appear to be particularly sensitive to temperature above 20 °C and many are killed by exposure to such temperature for relatively short periods. The stable cold environment such as the cold depths of the oceans and permanently frozen soils harbour the greatest number of such organisms.

Psychrophilic algae such as *Raphidonema nivale* are found in Polar regions with psychrophilic fungi such as *Sclerotina borealis* and are also commonly associated with frozen soils and subterranean caves. Terrestrial psychrophilic bacteria are generally chemoorganotrophic gram negative organisms of the genera *Pseudomonas, Cytophaga* and *Flavobacteruium* although gram positive bacteria such as *Arthrobacter glacialis, Micorococcus cryophilus* and *Bacillus psychrophilus* may be the dominant species in certain subterranean cave. Marine isolates are almost always of the genera *Pseudomonas, Vibrio* and *Spirillum* and the best known example is *Moritella marina* (previously, *Vibrio marinus*) which is widely distributed below the thermocline in the oceans of the world.

In extreme arid environments, microorganisms colonize the microscopic air spaces of porous rocks under a thin rock crust. Such Endolithic microbial communities occur also in the ice free areas of Antarctica and hot deserts. The dry valley of Antarctica is earth's most extreme deserts. Only yeasts appear better adapted to extreme cold desert life than bacteria, filamentous fungi (e.g. the yeasts *Cryptococcus vishniacii* and *Leucosporidium antarcticum*), algae and lichens and these valleys seldom achieve temperature above freezing. Microorganisms that live on ice surface must be able to tolerate wide variation in moisture as the ice freezes and thaws, as well as temperature fluctuation.

Comparing with thermophiles, there are little studies on psychrophiles. Results of these studies mentioned that cell membranes of psychrophiles contain high percent of unsaturated fatty acids such as hexadecanoic, octadecanoic and other fatty acids with short chains and low melting temperature. The enzymes of *M. marina* (hexokinase, malic dehydorgenase, phophofructokinase) loss their activity when incubated at 25-35 °C for one hour. In addition to enzymes and other proteins of these bacteria damaged at 25-35°C.

Acidophiles microbial community

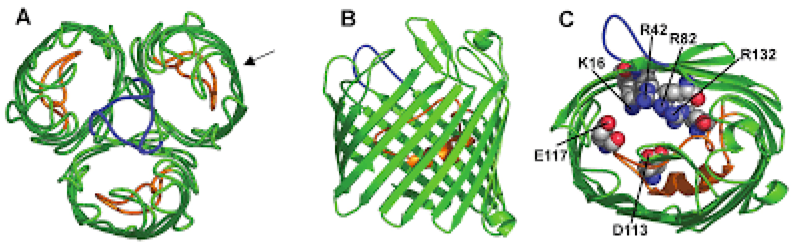
Microbial life in extremely low pH (<3) natural and man-made environments may be considerably diverse. Prokaryotic acidophiles (eubacteria and archaea) have been the focus of much of the research activity in this area, primarily because of the importance of these microorganisms in biotechnology (predominantly the commercial biological processing of metal ores) and in environmental pollution (genesis of "acid mine drainage"); however, obligately acidophilic eukaryotes (fungi, yeasts, algae and protozoa) are also known, and may form stable microbial communities with prokaryotes, particularly in lower temperature (>35°C) environments. Primary production in acidophilic environments is mediated by chemolitho-autotrophic prokaryotes (iron and sulfur oxidizers; *Thiobacillus ferrooxidans*), and may be supplemented by phototrophic acidophiles (predominantly eukaryotic microalgae include *Euglena* spp., *Chlorella* spp., *Chlamydomonas acidophila*, *Ulothrix zonata* and *Klebsormidium fluitans*.) in illuminated sites. The most thermophilic acidophiles are archaea (*Crenarchaeota*) whilst in moderately thermal (40-60°C) acidic environments archaea (*Euryarchaeota*) and bacteria (mostly Gram-positives) may co-exist. Lower temperature (mesophilic) extremely acidic environments tend to be dominated by Gram-negative bacteria, and there is recent evidence that mineral oxidation may be accelerated by acidophilic bacteria at very low (ca. 0°C) environments. Whilst most acidophiles have conventionally been considered to be obligatory aerobic (e.g. *Metallosphaera sedula* and *Sulfurococcus yellowstonii*), there is increasing evidence that many isolates are facultative anaerobes (e.g. *T. ferrooxidans*), and are able to couple the oxidation of organic or inorganic electron donors to the reduction of ferric iron. A variety of interactions have been demonstrated to occur between acidophilic microorganisms, as in other environments; these include competition, predation, mutualism and synergy. Mixed cultures of acidophiles are frequently more robust and efficient (e.g. in oxidising sulfide minerals) than corresponding pure cultures. In view of the continuing expansion of microbial mineral processing (biomining) as a cost-effective and environmentally sensitive method of metal extraction, and the ongoing concern of pollution from abandoned mine sites, acidophilic microbiology will continue to be of considerable research interest well into the new millennium.

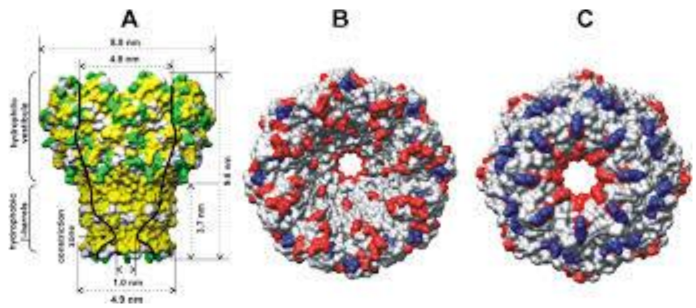
Barophilic microbial community

Three microbial populations found in high pressure environments can be distinguished. Barotolerant microbes are found in the ocean to depth 4 km (400 atm) but grow better at 1 atm (water surface). Barophilic species occur at 5-6 km and grow better at high pressure (500-600 atm) than low. Extreme barophiles live at great depth where the pressure is at least 700 am and will not grow at all at 1 atm. **(1 atm= 1.0132 bar , =10132 bascal)**

As a rule, life is very thin in the open oceans. Some general points are limiting growth of organisms in potential habitats within the oceans. Light penetrates to a depth of 300 meters in the open oceans; therefore, no phototrophs below this depth. Microorganisms living at lower than 1000 m (> 100 atm) must be barotolerant. That is they must be able to withstand the pressure exerted by the water column above. Microorganisms living between 1000-10000 m deep are either barophiles or extreme obligate barophiles that prefer or even require high pressure to grow. Expression of genes from such microorganism may be regulated by environmental pressure. For instance, membrane protein composition is altered in response to increasing pressure. Genes expressed at high pressure, such as the one encoding the porin protein (OmpH) apparently help cells survive at the extreme pressure.

porin protein: is beta barrel protein that cross a cellular membrane and act as a pore through which molecules can diffuse, they act as channels that are specific to different types of molecules.(outer membrane of G- and some G+).





Generally in the surface photic zone (0-300 m) there are abundant biological activity by animals, plants and microbes. In the layer between 300-1000 m still a lot of biological activity, but by the animals and microbes only. At more than 1000 m depth, only a little microbial activity is achieved by barophilic and psychrophilic microorganisms, due to a low nutrient conditions, cold temperature and high pressure.

There are a little investigations about the extremely barophiles because the difficulty in the culturing and identification in the laboratory under high pressure which need a very special techniques and equipments. *Thermus barophilus* is one of the most important barophilic bacteria isolated from deep sea.

Molecular effect of high pressure on bacterial cell

High pressure strongly affects physiology and biochemistry of bacterial cell through:

1- Decrease the binding capacity of enzymes to their substrates so enzymes folded to minimize pressure relate effect.

2- To keep the membrane gel at high pressure cytoplasmic membrane of barophilic bacteria has a higher proportion of unsaturated fatty acids.

3- Pressure can change protein composition. For example (OmpH) protein is synthesized at high pressure but not at low pressures. OmpH is a porin forms channels for diffusion of organic molecules into periplasm. Possibly, pressure sensitive repressor proteins or pressure dependent gene activators turn on/off transcription of specific genes encoding proteins needed for growth at high pressures.

Prof.Dr.Ithar Kamil Abbas

Introduction

Biofilms are complex communities of surface-attached microorganisms, comprised either of a single or multiple species. Over the past few decades, there has been a growing realization that bacteria in most environments are not found in a unicellular, planktonic (free-living) form such as those typically studied in the laboratory, but exist predominantly in multi-cellular surface attached communities called biofilms. Biofilm development is a series of complex but discrete and well-regulated steps (figure 1). The exact molecular mechanisms differ from organism to organism, but the stages of biofilm development are similar across a wide range of micro-organisms. The sequential stages of biofilm development:

(1) Microbial attachment to the surface.

(2) Adhesion, growth, and aggregation of cells into **microcolonies** (figure 2).

(3) Maturation

(4) Dissemination of progeny cells for new colony formation.

Microbial Attachment

Direct contact of the microorganism with the surface is required for attachment and subsequent colonization. The ability of a cell to perform this “initial attachment event” is controlled by both environmental factors, including nutrient levels, temperature, pH, and genetic factors, including the presence of genes encoding motility functions, environmental sensors, adhesins, etc. The mere "touch" of the cell wall with the biomaterial alters the microorganism's phenotypic expression to begin production of a sticky adhesin that attaches the cell to the surface.

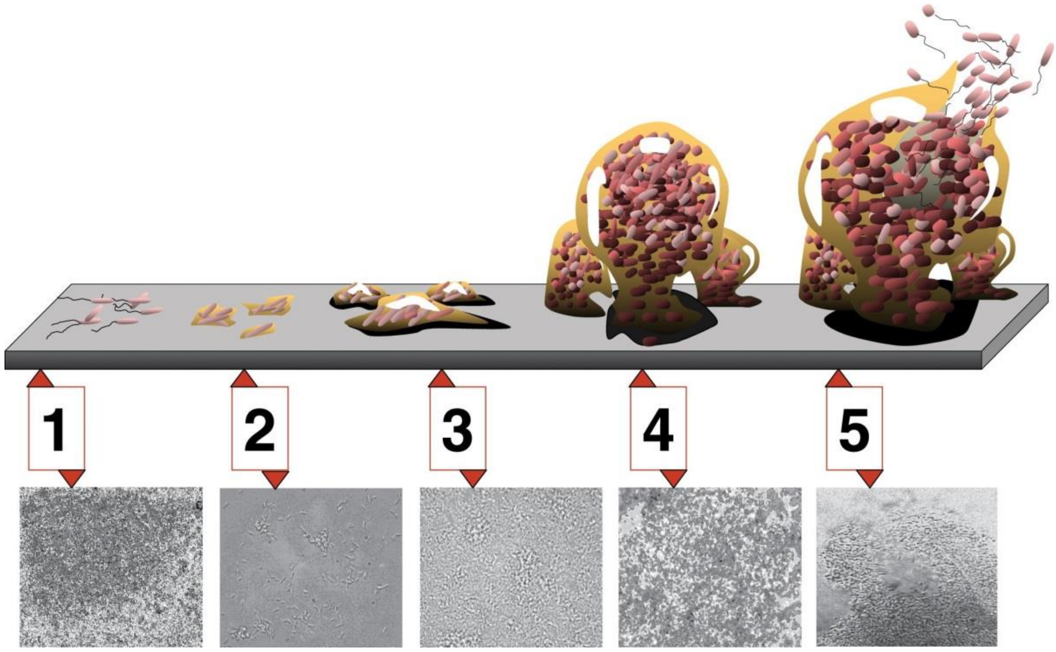


Figure 1. Schematic diagram of biofilm formation and growth

The matrix of host products serves as scaffolding for the simultaneously developing biofilm and provides receptor-binding sites for newly arriving bacteria.

Adhesion and Microcolony Formation

The attachment of a small number of bacterial cells is all that is needed to initiate biofilm formation anywhere along the system. Within a few seconds, the progression of phenotypic changes in the bacteria remarkably alters protein expression to further produce species-specific adhesions that irreversibly anchor the cell to the surface. Type IV pili are involved in a type of surface-associated motility called **twitching**, and this twitching might be required for the aggregation of cells into microcolonies.

Within as few as 12 minutes, the adherent cells **upregulate** genes that direct production of accumulation proteins and polysaccharides, which firmly attach the cells to the substratum and to each other as they undergo exponential binary division.

After initial attachment, the cells begin to grow and spread as a **monolayer** on the surface. As the cells continue to divide, the daughter cells spread outward and upward from the attachment point to form cell clusters. The production of **exopolysaccharides** (EPS) or "slime" embeds the aggregating cells to form **microcolonies**. Typically, the microcolonies are composed of 10% to 25% cells and 75% to 90% EPS matrix, with a consistency similar to a viscous polymer hydrogel (figure 2).

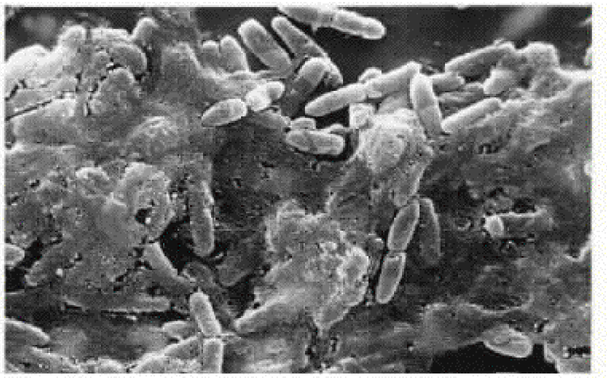


Figure 2. Electron micrograph of interior surface of a vascular catheter removed from a patient showing growth of a bacterial biofilm of *P. aeruginosa*.

The continued formation of the biofilm community evolves according to the **biochemical** and **hydrodynamic** conditions, as well as the **availability** of nutrients in the immediate environment. The structural organization is mainly influenced by hormone-like regulatory signals produced by the biofilm cells themselves in reaction to growth conditions. This interactive network of signals allows for communication among the cells, not only controlling colony formation but also regulating growth rate, species interactions, toxin production, and invasive properties. The cell clusters are structurally and metabolically heterogeneous, and both aerobic and anaerobic processes occur simultaneously in different parts of the multicellular community.

Cellular density typically increases to a steady state within 1-2 weeks, depending on the species and local environmental conditions. Expanded growth evolves into complex 3-D structures of **tower- and mushroom-shaped** cell clusters. Adjoining microcolonies are connected by **water channels** that serve as a primitive circulatory system for delivery of nutrients and removal of wastes.

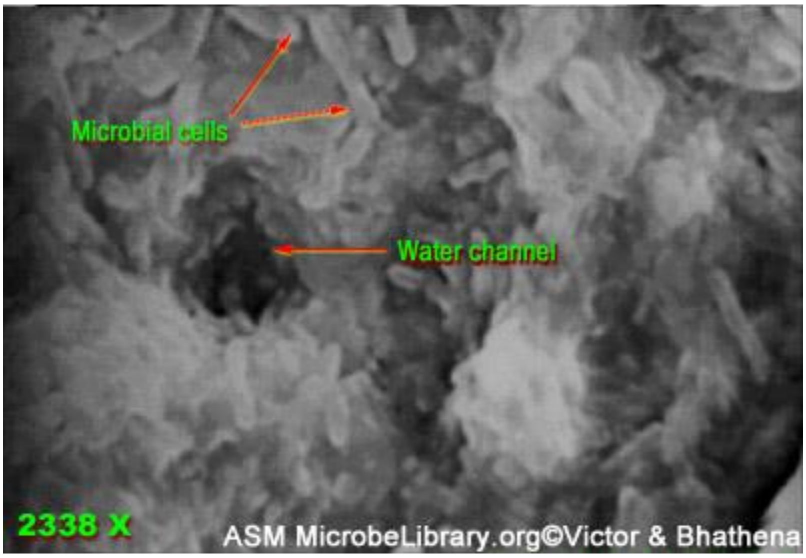


Figure 3: Water channels in a biofilm

The thickness of the biofilm is variable (13-60 µm) and uneven, as determined by the balance between growth of the biofilm and detachment of cells. Depending on the initial number of attached organisms, the multilayered cell clusters develop as patchy networks or form a contiguous layer over the surface of the catheter.

The dimension of biofilms in vivo is only on the order of tens of micrometers, but they contain thousands of bacteria in a very compact space.

Dispersal and Dissemination of Biofilm Cells

The formation of biofilm is a universal strategy for microbial survival. In order to colonize new surfaces and to prevent density-mediated starvation within the mature biofilm, the cells must detach and disseminate. However, those released in clumps retain antibiotic resistance and may embolize at a distant anatomic site to develop **metastatic** infections such as endocarditis or osteomyelitis. Dispersal is accomplished by shedding, detachment, or shearing:

1. **Shedding**: occurs when daughter cells from actively growing bacteria in the upper regions of the microcolonies are released from the cell clusters. Due to increased cell density a programmed set of events (cell-cell signaling) within the biofilm leading to a local hydrolysis of the extracellular polysaccharide matrix (alginase in the case of *P. aeruginosa*), and conversion of a subpopulation of cells into motile planktonic cells, which leave the biofilm.
2. **Shearing**: biofilms are exposed to variable flow rates and shear forces. When the shear force of the infusion exceeds the tensile strength of the viscous biofilm, fragments break away.
3. **Detachment**: Clumps or fragments of detached biofilm may contain thousands of cells, but they leave behind an adherent layer of cells on the surface to regenerate the biofilm.

Beneficial biofilms

Water and wastewater treatment

One of the best examples of successful, beneficial application biofilms to solve a huge problem is in the cleaning of wastewater. A system that uses the proper microorganisms (in the form of a biofilm) to process wastewater and sewage: if the contaminated water was passed through such a biofilm, perhaps the microorganisms in the biofilm would remove the harmful organic material from the water by decomposition (figure 4). In order to prevent the microorganisms in the biofilm to get into the filtered water, or for chunks of biofilm to detach from the colony and make it through the system. Ideally, the biofilm stays attached to the filtration system and can be cleaned when the system is flushed.

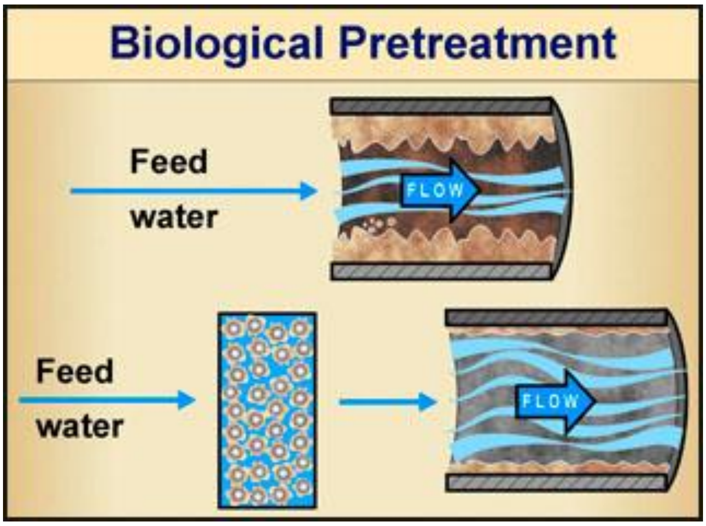


Figure 4: Biological treatment using bacterial biofilm

Interestingly, drinking water and wastewater that have been processed with a biofilm system in a treatment plant are more "biologically stable" than water filtered by other types of treatment systems. This just means that there is likely to be less microorganism contamination in water that has passed through a biofilm-based filter than there is in water that has passed through some alternative treatment system. This implies that biofilm treated water typically has lower disinfectant demand (e.g., use of chlorine) and disinfection by-product formation (e.g., that unsavory taste and smell of chlorine) potential than water treated in other ways if the water prior to treatment is high in the kind of nutrients the biofilm craves (which in this case is organic carbon).

Remediation of contaminated soil and groundwater

One of the less obvious beneficial applications of biofilms is in cleaning up oil and gasoline spills. Certain bacteria will degrade oil and gasoline. Bioremediation using biofilms has emerged as a technology of choice for cleaning up groundwater and soil at many sites contaminated with hazardous wastes. It is done by introducing a biofilm into the contaminated ground and providing the necessary environment below the surface of the ground to encourage the biofilm to clean up the polluted area (illustrated in figure 5).

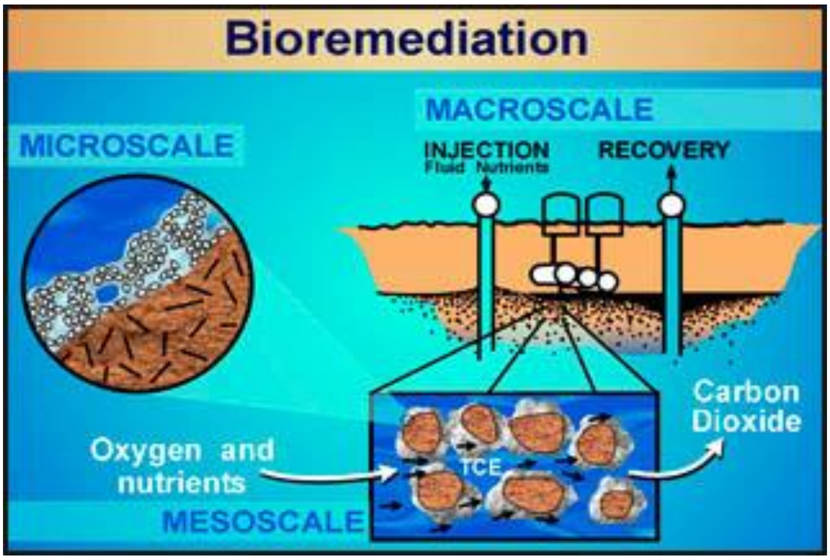


Figure 5: Bioremediation by biofilm

Microbial leaching

Billions of bacteria are employed in the mining industry, extracting gold, silver, cobalt and other metals. How is a biofilm engineered to accomplish this task? A specific bacteria with a particular affinity to the ore must be found. This idea has led to the most common biofilm supported leaching process, called "heap leaching." Low grade ore is placed in a "heap," and sprayed with a mildly acidified water solution that encourages the growth of particular bacteria that degrades the ore, releasing water soluble cupric ion (copper) that can then be recovered from the water. Currently approximately 10 to 20 percent of copper mined in the United States is extracted from low grade ore with the assistance of biofilms. And mining companies are making a considerable investment to extend this process to the extraction of other precious metals.

Nuisance effects of the Microbial Biofilms

Microbial biofilms are considered to be nuisance because these badly affect everything from human health to important industrial products. Reduction of sulphur to SO_2 by anaerobic, and oxidation of metals by aerobic microbial population of the biofilms, respectively, results in plugging and corrosion of the water supply pipes.

The formation of the microbial biofilms on wetted surfaces of the water supply pipes and tubes increases the fluid frictional resistance, reduces the cross sectional area for flow, enhances the roughness of the surface and viscoelasticity of the fluids.

A release of detached biofilms in water distribution system and from cooling towers not only lowers the quality of the drinking water but also increases the incidence of pathogen dissemination in the atmosphere.

Dental plaques and various other animal and human diseases of lungs, intestinal and urinary tracts are the result of the development of the microbial biofilms within the tissues of the organs. Moreover, the development of the microbial biofilms reduces the effectiveness of the antibiotics against the pathogens.

Prof.Dr.Ithar kamil Abbas

Bioremediation

Much concern has concentrated on the visible effects of pollution, but the hidden effects are also of great importance. The necessity to decontaminate polluted sites is recognized, both socially and politically, because of the increasing importance placed on environmental protection and human health. As the number of sites and levels of contamination rise, so does the need to develop effective and affordable methods for decontamination.

What is Bioremediation?

Bioremediation can be defined as ***any process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition.***

Bioremediation is a combination of two words - bio, short for biological, and remediation, which means to remedy. The use of plants to clean up the environment, known as **phytoremediation**, is also considered a type of bioremediation.

Bioremediation provides a technique for cleaning up pollution by enhancing the same biodegradation processes that occur in nature. Depending on the site and its contaminants, bioremediation may be safer and less expensive than alternative solutions such as incineration or land filling of the contaminated materials. It also has the advantage of treating the contamination in place so that large quantities of soil, sediment or water do not have to be dug up or pumped out of the ground for treatment.

For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate.

Like other technologies, bioremediation has its limitations. Some contaminants, such as chlorinated organic or high aromatic hydrocarbons, are resistant to microbial attack. They are degraded either slowly or not at all, hence it is not easy to predict the rates of clean-up for a bioremediation exercise; there are no rules to predict if a contaminant can be degraded. Bioremediation techniques are typically more economical than traditional methods such as incineration, and some pollutants can be treated on site, thus reducing exposure risks for clean-up personnel, or potentially wider exposure as a result of transportation accidents. Since bioremediation is based on natural attenuation the public considers it more acceptable than other technologies.

Applications

Naturally occurring bioremediation and phytoremediation have been used for centuries. For example, desalination of agricultural land by phytoextraction has a long tradition.

Bioremediation techniques have been successfully used to remediate soils, sludge's, and ground water contaminated with petroleum hydrocarbons, solvents, pesticides, wood preservatives, and other organic chemicals. Bench- and pilot-scale studies have demonstrated the effectiveness of anaerobic microbial degradation of nitro toluene in soils contaminated with munitions wastes.

What is the pilot plant?

A **pilot plant** is a small industrial system which is operated to generate information about the behavior of the system for use in design of larger facilities. **Pilot plant** is a relative term in the sense that plants are typically smaller than full-scale production plants, but are built in a range of sizes.(Air sparge /soil vapor extraction, high vacuum ,Dual phase extraction, groundwater pump and treat…etc.)

Bioremediation is especially effective for remediating low level residual contamination in conjunction with source removal.

The contaminant groups treated most often are polycyclic aromatic hydrocarbons (PAHs), non-halogenated semivolatile organic compounds (SVOCs) (not including PAHs), and BTEX (an acronym that stands for Benzene, Toluene, Ethylbenzene, and Xylenes).

Because the two contaminant groups most commonly treated using bioremediation are SVOCs and PAHs, it may be difficult to treat them using technologies that rely on volatility. In addition, bioremediation treatment often does not require heating; nevertheless, relatively inexpensive inputs, such as nutrients, and usually does not generate residuals requiring additional treatment or disposal. Also, when conducted in situ, it does not require excavation of contaminated media. Compared with other technologies, such as thermal desorption and incineration (which require excavation and heating), thermally enhanced recovery (which requires heating), chemical treatment (which may require relatively expensive chemical reagents), and in situ soil flushing (which may require further management of the flushing water), bioremediation may have a cost advantage in the treatment of nonhalogenated SVOCs.

While bioremediation, nor any other remediation technology, cannot degrade inorganic contaminants, it can be used to change the valence state of inorganics and cause adsorption, immobilization onto soil particulates, precipitation, uptake, accumulation, and concentration of inorganics in micro or macroorganisms. These techniques, while still largely experimental, show considerable promise of stabilizing or removing inorganics from soil.

Factors of Bioremediation

The control and optimization of bioremediation processes is a complex system of many factors. These factors include: the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population; the environment factors (type of soil, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients).

Limitations

Factors that may limit the applicability and effectiveness of the process include:

1. Cleanup goals may not be attained if the soil matrix prohibits contaminant-microorganism contact.
2. The circulation of water-based solutions through the soil may increase contaminant mobility and necessitate treatment of underlying ground water.
3. Preferential colonization by microbes may occur causing clogging of nutrient and water injection wells.

The above factors can be controlled with proper attention to good engineering practice.

Types of bioremediation

The main types of bioremediation are as follows:

1- <u>Biostimulation</u>

Nutrients and oxygen - in a liquid or gas form - are added to contaminated water or soil to encourage the growth and activity of bacteria already existing in the soil or water (indigenous microorganisms).

The nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants. All of them will need nitrogen, phosphorous, and carbon.

Carbon is the most basic element of living forms and is needed in greater quantities than other elements. In addition to hydrogen, oxygen, and nitrogen it constitutes about 95% of the weight of cells.

Phosphorous and sulfur contribute with 70% of the remainders. The nutritional requirement of carbon to nitrogen ratio is 10:1, and carbon to phosphorous is 30:1.

The disappearance of contaminants is monitored to ensure that remediation occurs.

2- Bioaugmentation

Microorganisms that can clean up a particular contaminant are added to the contaminated soil or water. Bioaugmentation is more commonly and successfully used on contaminants removed from the original site, such as in municipal wastewater treatment facilities. To date, this method has not been very successful when done at the site of the contamination because it is difficult to control site conditions for the optimal growth of the microorganisms added.

3- Intrinsic Bioremediation

Also known as natural attenuation, this type of bioremediation occurs naturally in contaminated soil or water. This natural bioremediation is the work of microorganisms and is seen in petroleum contamination sites, such as old gas stations with leaky underground oil tanks.

Environmental requirements

Microbial growth and activity are readily affected by pH, temperature, and moisture. Although microorganisms have been also isolated in extreme conditions, most of them grow optimally over a narrow range, so that it is important to achieve optimal conditions.

If the soil has too much acid it is possible to rinse the pH by adding lime. Temperature affects biochemical reactions rates, and the rates of many of them double for each 10 °C rise in temperature.

Above a certain temperature, however, the cells die. Plastic covering can be used to enhance solar warming in late spring, summer, and autumn. Available water is essential for all the living organisms, and irrigation is needed to achieve the optimal moisture level.

The amount of available oxygen will determine whether the system is aerobic or anaerobic.

Hydrocarbons are readily degraded under aerobic conditions, whereas chlorurate compounds are degraded only in anaerobic ones. To increase the oxygen amount in the soil it is possible to till or sparge air. In some cases, hydrogen peroxide or magnesium peroxide can be introduced in the environment.

Soil structure controls the effective delivery of air, water, and nutrients. To improve soil structure, materials such as gypsum or organic matter can be applied. Low soil permeability can impede movement of water, nutrients, and oxygen; hence, soils with low permeability may not be appropriate for *in situ* clean-up techniques.

Bioremediation Strategies

Different techniques are employed depending on the degree of saturation and aeration of an area. *In situ* techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance.

Ex situ techniques are those that are applied to soil and groundwater at the site which has been removed from the site via excavation (soil) or pumping (water).

***In situ* bioremediation**

These techniques are generally the most desirable options due to lower cost and less disturbance since they provide the treatment in place avoiding excavation and transport of contaminants. *In situ* treatment is limited by the depth of the soil that can be effectively treated. In many soils effective oxygen diffusion for desirable rates of bioremediation extend to a range of only a few centimeters to about 30 cm into the soil, although depths of 60 cm and greater have been effectively treated in some cases.

The most important land treatments are:

Bioventing is the most common *in situ* treatment and involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface.

In situ biodegradation involves supplying oxygen and nutrients by circulating aqueous solutions through contaminated soils to stimulate naturally occurring bacteria to degrade organic contaminants.

It can be used for soil and groundwater. Generally, this technique includes conditions such as the infiltration of water-containing nutrients and oxygen or other electron acceptors for groundwater treatment.

Biosparging. Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the system.

Bioaugmentation. Bioremediation frequently involves the addition of microorganisms indigenous or exogenous to the contaminated sites. Two factors limit the use of added microbial cultures in a land treatment unit: 1) nonindigenous cultures rarely compete well enough with an indigenous population to develop and sustain useful population levels and
2) most soils with long-term exposure to biodegradable waste have indigenous microorganisms that are effective degrades if the land treatment unit is well managed.

Ex situ **bioremediation**

These techniques involve the excavation or removal of contaminated soil from ground.

Landfarming is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous biodegradative microorganisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of superficial 10–35 cm of soil. Since landfarming has the potential to reduce monitoring and maintenance costs, as well as clean-up liabilities, it has received much attention as a disposal alternative.

Composting is a technique that involves combining contaminated soil with nonhazardous organic amendants such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevated temperature characteristic of composting.

Biopiles : are a hybrid of landfarming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of landfarming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic and anaerobic microorganisms.

Bioreactors. Slurry reactors or aqueous reactors are used for *ex situ* treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the processing of contaminated solid material (soil, sediment, sludge) or water through an engineered containment system. A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas) mixing condition to increase the bioremediation rate of soil bound and water-soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous microorganisms) capable of degrading target contaminants. In general, the rate and extent

of biodegradation are greater in a bioreactor system than *in situ* or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable. Despite the advantages of reactor systems, there are some disadvantages. The contaminated soil requires pretreatment (e.g., excavation) or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction (e.g., vacuum extraction) before being placed in a bioreactor.

Microbial population for bioremediation process

Microorganisms can be isolated from almost any environmental conditions. Microbes will adapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream. The main requirements are an energy source and a carbon source. Because of the adaptability of microbes and other biological systems, these can be used to degrade or remediate environmental hazards.

We can subdivide these microorganisms into the following groups:

<u>Aerobic</u>

Examples of aerobic bacteria recognized for their degradative abilities are *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*. These microbes have often been reported to degrade pesticides and *hydrocarbons*, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy.

<u>Anaerobic</u>

Anaerobic bacteria are not as frequently used as aerobic bacteria. There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene **(TCE),** and chloroform.

<u>Ligninolytic fungi</u>

Fungi such as the white rot fungus Phanaerochaete chrysosporium have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corn cobs.

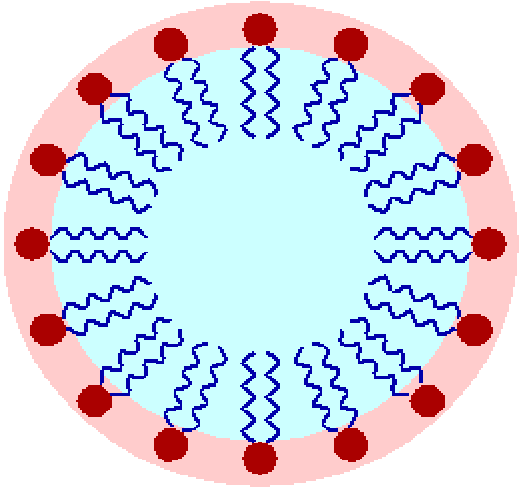
Methylotrophs

Aerobic bacteria that grow utilizing methane for carbon and energy. The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatics trichloroethylene and 1,2-dichloroethane. For degradation it is necessary that bacteria and the contaminants be in contact. This is not easily achieved, as neither the microbes nor contaminants are uniformly spread in the soil. Some bacteria are mobile and exhibit a chemotactic response, sensing the contaminant and moving toward it. Other microbes such as fungi grow in a filamentous form toward the contaminant. It is possible to enhance the mobilization of the contaminant utilizing some *surfactants such as sodium dodecyl sulphate (SDS).

***Surfactants**: are compounds that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants.

Example:

oil in aqueous suspension, such as might occur in an emulsion of oil in water. In this example the surfactant molecules' oil-soluble tails project into the oil, while the water-soluble ends remain in contact with the water phase



Biosorption

Is a technique that used for removal of heavy metals from waste water by the passive binding to living or non-living microorganisms or other biomaterials.

Is a physiochemical process that occurs naturally in certain biomass which allows it to passively concentrate and bind contaminants onto its cellular structure.

Biosorption is an innovation technology that employs inactive and dead biomass for the removal and recovery of metals from aqueous solutions .

Biosorption is therefore independent of the metabolic activities of the organism and occurs due to interactions between solute and external components constituting the cellular structure of the organism.

Usually , **the biosorbent** , unlike mono functional ion exchange resins, contains variety of functional sites including : carboxyl , imidazole, sulphydryl amino , phosphate , sulphate , thioether , phenol, carbonyl, amide and hydroxyl moieties.

The environmental uses of biosorption represents of :

Pollution interacts naturally with biological systems. It is currently uncontrolled, seeping into any biological entity within the range of exposure. The most problematic contaminants include heavy metals, pesticides and other organic compounds which can be toxic to wildlife and humans in small concentration. There are existing methods for remediation, but they are expensive or ineffective. However, an extensive body of research has found that a wide variety of commonly discarded waste including eggshells, bones, peat, fungi, seaweed, yeast and carrot peels can efficiently remove toxic heavy metal ions from contaminated water. Ions from metals like mercury can react in the environment to form harmful compounds like methyl mercury, a compound known to be toxic in humans. In addition, adsorbing biomass, or biosorbents, can also remove other harmful metals like:arsenic, lead, cadmium, cobalt, chromium and uranium. Biosorption may be used as an environmentally friendly filtering technique. There is no doubt that the world could benefit from more rigorous filtering of harmful pollutants created by industrial processes and all-around human activities.

Primary idea of biosorption ensured , using biomass as a tool in environmental cleanup has been around since the early 1900s when Arden and Lockett discovered certain types of living bacteria cultures were capable of recovering nitrogen and phosphorus from raw sewage when it was mixed in an aeration tank. This discovery became known as the activated sludge process which is structured around the concept of **bioaccumulation** and is still widely used in wastewater treatment plants today. It wasn't until the late 1970s when scientists noticed the sequestering characteristic in dead biomass which resulted in a shift in research from bioaccumulation to biosorption.

What is the different between bioabsorption and bioaccumulation :

Biosorption is a metabolically passive process, meaning it does not require energy, and the amount of contaminants a sorbent can remove is dependent on kinetic equilibrium and the composition of the sorbents cellular surface. Contaminants are adsorbed onto the cellular structure.

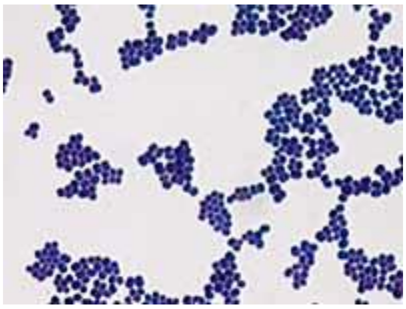
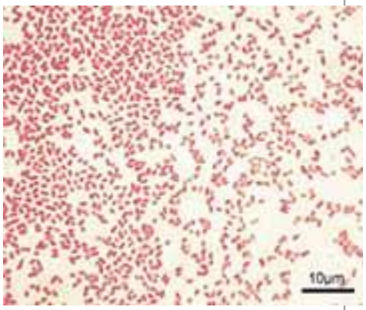
Bioaccumulation is an active metabolic process driven by energy from a living organism and requires respiration.

Both bioaccumulation and biosorption occur naturally in all living organisms .In terms of environmental remediation, biosorption is preferable to bioaccumulation because it occurs at a faster rate and can produce higher concentrations. Since metals are bound onto the cellular surface, biosorption is a reversible process whereas bioaccumulation is only partially reversible.

Biosorbents primarily fall into the following categories **bacteria , fungi, algae , industrial wastes , agricultural waste , natural residues, and other biomaterials .**among the most promising types of biosorbents studied are **algal biomass** have been reported to have high metal binding capacities due to their containing of functional groups on the cell wall. These functional groups are carboxyl , hydroxyl and sulphate , which can act as binding sites for metals.

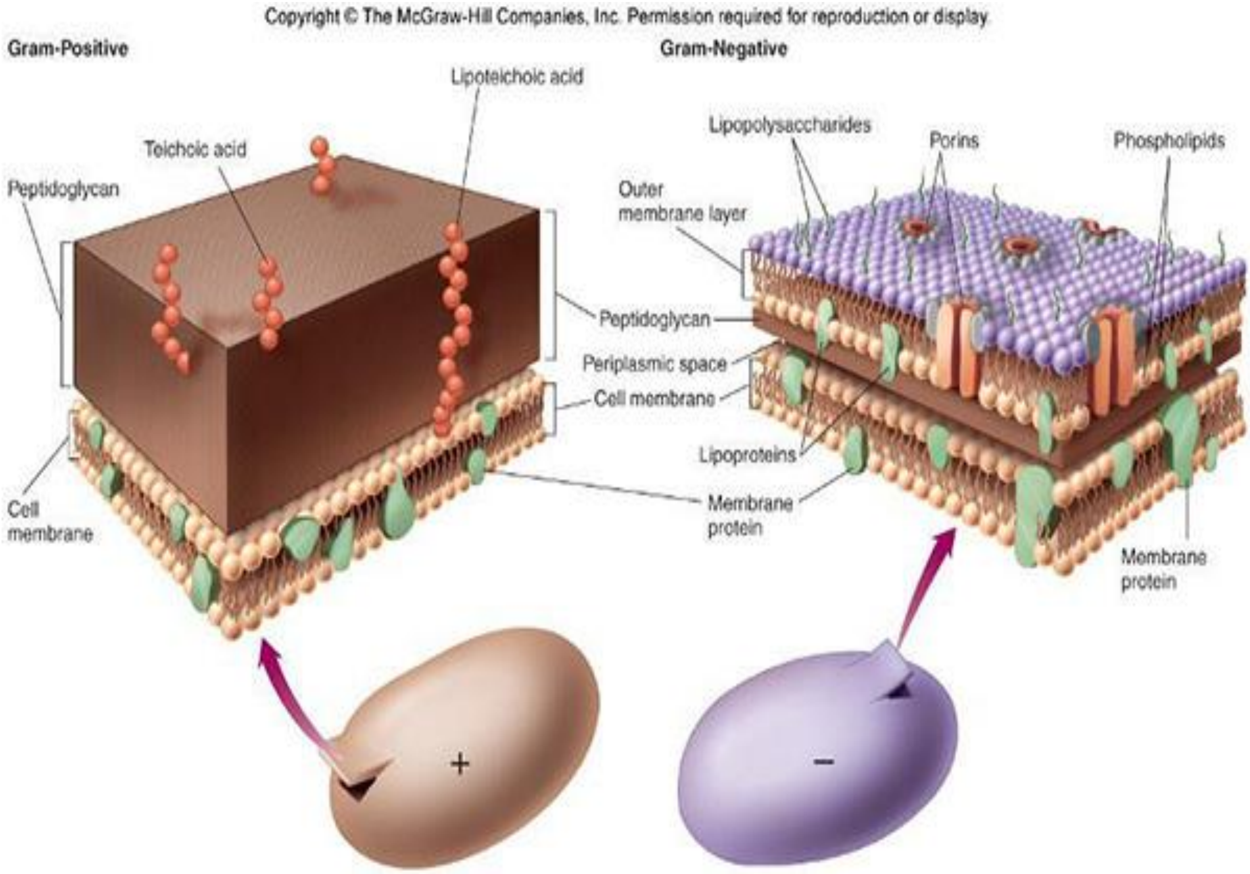
Bacteria as a bioabsorbent : the biosorption is independent of metabolism , it may be the most significant proportion of total uptake.

***Differences between Gram Positive and Gram Negative Bacteria*:**

S.N.	Characteristics	Gram Positive	Gram Negative
1	**Gram Reaction**	 Retain crystal violet dye and stain blue or purple	 Can be decolorized to accept counterstain (safranin) and stain pink or red
2	**Cell Wall**	Cell Wall is 20-30 nm thick.	Cell Wall is 8-12 nm thick.

3	**Cell Wall**	The wall is Smooth.	The wall is wavy.
4	**Peptidoglycan Layer**	Thick (multilayered)	Thin (single-layered)
5	**Teichoic Acids**	Present in many	Absent
6	**Periplasmic Space**	Absent	Present
7	**Outer Membrane**	Absent	Present
8	**Porins**	Absent	Occurs in Outer Membrane
9	**Lipopolysaccharide (LPS) Content**	Virtually None	High
10	**Lipid and Lipoprotein Content**	Low (acid-fast bacteria have lipids linked to peptidoglycan)	High (because of presence of outer membrane)
11	**Mesosomes**	Quite Prominent	Less Prominent
12	**Flagellar Structure**	2 rings in basal body	4 rings in basal body
13	**Toxin Produced**	Exotoxins	Endotoxins or Exotoxins
14	**Resistance to Physical Disruption**	High	Low
15	**Cell Wall Disruption by Lysozyme**	High	Low (requires pretreatment to destabilize outer membrane)
16	**Susceptibility to Penicillin and**	High	

	Sulfonamide		Low
17	**Susceptibility to Streptomycin, Chloramphenicol and Tetracycline**	Low	High
18	**Inhibition by Basic Dyes**	High	Low
19	**Susceptibility to Anionic Detergents**	High	Low
20	**Resistance to Sodium Azide**	High	Low
21	**Resistance to Drying**	High	Low



Comprised of thick peptidoglycan about 90% of cell wall, imbedded in cell wall teichoic acid some of lipid linked to form lipoteichoic. pepetidoglycan, teichoic acid lipoteichoic acid ,teichuronic acid give the gram positive bacterial wall positive charge., but the cell wall of negative bacteria is thinner composed of 10-20% peptidoglycan and it's have outer membrane composed of phospholipid and lipopolysaccharides. Peptidoglycan, phospholipid and lipopolysaccharides give gram negative bacteria cell wall negative charge.

The ionic functional groups(carboxyl , hydroxyle, amine and SH) in gram positive bacteria and phosphate in gram negative bacteria responsible for the metal binding capability of cell wall.(ex: Carboxyl group of Streptomyces bind copper).

Three mechanisms account for the sequestration of the metallic ions by the microbial cell wall: adsorption, micro precipitation and nucleation.

Metal ion sorption is dependent not only on the type, number and accessibility of the functional sites but also the mesh diameter of the cell wall framework.

Sorption studies with dead cells indicate that most of the interaction occurs on or within the outer cell membrane. It has been reported that, for *E. Coli*, the phosphoryl groups in the lipopolysaccharides are mainly responsible for the high divalent ion capacity, however, other studies seem to indicate that metals accumulate along the peptidoglycan layer in the cell membrane.

Algae

Metabolism- independent accumulation of metals is often rapid and usually completes in 5-10 min. In common with other microbial groups, many potential binding sites occur in algal cell walls, which include polysaccharides , cellulose , uronic acid and proteins.

The main constituents of the cell walls of algae are carbohydrates. They may be divided into materials soluble in boiling water such as mucilaginous or pectic substances and constituents that are insoluble in water. There are two fractions within the cell wall: a fibrillar part that gives the wall its strength and an amorphous part in which the fibrils are embedded. The fibrillar fraction of the brown algal wall consists of cellulose and the structural component is probably stiffened by insoluble alginate.The amorphous embedding matrix is composed of glycoproteins.

Trace metals have a vital role to play as constituents of enzymatic systems in biological processes. Interactions of metallic species with proteins and polysaccharides found constituting the cellular structure of algae have been proposed. Amino-carboxyl groups from polysaccharides as well as nitrogen and oxygen of the peptide bond have counter ions which could be available for replacement by metal ions. Heavy metals have been found to show strong affinity for protein. Metal sorption was found to be reversible with bound metal released by different counter ions. Strontium was able to displace zinc in the alga *Vaucheria* even though the zinc was bound to the cell walls suggesting that electrostatic attraction plays an important role in the metal uptake process. The strength of' sequestering of various divalent ions to *Vaucheria* cell walls was proportional to formation of ions or stability constants, suggesting that covalent bonding is also important in the uptake process.

Examples:

Copper sorption by algae: Copper is one of the most common of the industrial metals. Absorption of excess copper by man results in "Wilson's disease" in which excess copper is deposited in the brain, skin, liver, pancreas and myocardium. Copper is a nutrient necessary for algal growth but at high concentrations, it finds use as an algaecide.

In *Nitzschia palea*, the major portion of the copper was found in the cell wall and slime envelope which constituted 6% of the total carbon of the cell. Polysaccharides extracted from *Chlorella stigmatophora* were shown to bind copper in solution. Various algal species within the same genus, although producing polysaccharides did not however show similar metal sorption characteristics. Free carboxylic groups (contributed by uronic acids) and their relatively homogenous distribution in the polysaccharide of C. stigmatophora were found to play a major role in metal sequestration.

Lead sorption by algae : Lead has been found to have a high affinity to algal cells and polysaccharides of algae. Prolonged exposure to lead in the environment resulted in entrapment of lead ions within the cell wall. Eventually, the lead diffused through the cell wall to the plasma and eventually to the cytoplasm. In laboratory experiments, algae specimen *Cladophora sp*. packed in columns removed 85% of lead from the feed solution at near neutral pH. Regeneration of the lead laden column was achieved by washing the algal biomass with small quantities of (0.01-0.1)M EDTA in 0. 1M sodium acetate at pH7.5. During lead sorption, release of calcium, magnesium and potassium along with small amounts of sodium were observed, indicating a cation exchange type phenomenon was responsible for metal sorption. Some researchers screened 45 algal biomass samples (native and cross-linked) for the uptake of lead. Lead uptake was found to be highest among the Phaeophyta (order of algae). Among the Phaeophyta, lead biosorption decreased as follows: *Fucus*>*Ascophyllum*>*Sargassum*>*Padina*. These trends in lead uptake for the various species are to be viewed with caution since no material pretreatment (e. g. washing with dilute HCl) was carried out.

The main equation to count the efficiency of biosorption processes by microorganism(removal percentage) is :

$$\%\text{removal} = \frac{C_{\text{i}} - C_{\text{f}}}{C_{\text{i}}} \times 100$$

Where :$C_{i=}$ the initial concentration, C_f =the final concentration

Summary of various metal sorption results of algal biomass.

Metal	Species	Metal uptake mmol g -I	Reference	Comments
Copper	*Ascophyllum. nodosum*	1.2	Leusch et al.	Fresh water
Cadmium	*Sargassum fluitans*	0.81	Leusch et al.	

Lead	*Ecklonia radiata*	1.36	Matheickal et al.	
Nickel	*Sargassum fluitans*	0.78	Leusch et al	
Zinc	*Sargassum natans*	0.4-0.75	Holan et a1.	
Chromium	*Sargassum fluitans*	0.77	Kratochvil et al.	
Cobalt	*Ascophyllum nodosum*	1.7	Kuyucak et al.	

Fungi and yeasts :

Metabolism-independent binding of metal ions to fungal and yeast cell wall is usually rapid and large amounts may be bound. The biosorption capacity of dead biomass maybe greater , equivalent to, or less than that of living cells and this may depend on the killing process used. As in other microbes , a variety of ligands may be involved including carboxyl, amino, phosphate , hydroxyl and sulphydryl groups. In certain fungi , and especially yeasts , greater amount of metals may be taken up by transport than by biosorption. However , for many filamentous fungi, it appears that general biosorption account for major portion of metal uptake.

Fungi possess a true cell wall which, as in the case of bacteria, is rigid. The chemical and structural characteristics of the fungal cell wall is rather different from those of bacterial cells. Various polysaccharides are the primary constituents of the fungal cell wall (up to 90%) and are often complexed with proteins, lipids and other substances (e. g. pigments). The fungal cell wall has a microfibrillar structure and consists of several layers; (1) an outer layer of glucans, mannans or galactans and (2) an inner microfibrillar layer which is made of parallel chains of chitin, in some cases these may be replaced by cellulose chains, or in certain yeast, of non-cellulosic glucan. Chitin is a polymer of N-acetyl-D-glucosamine; cellulose consists of chains of D-glucopyranose, mostly linked by ß-1,4-glucosidic bonds. These chains are strengthened by proteins, lipids and polysaccharides. Some researchers suggest that phosphodiester and carboxyl groups present within the fungal cell wall confer the electrical surface potential.

Competition between divalent metal cations reduces the capacity for particular metal species. Some researchers found that the uptake capacity of *Rhizopus arrhizus* for cadmium decreased by as much as 70% in the presence of UO 2+. No competitive effects were observed with sodium. Carboxylate and phosphate functional groups were thought to be responsible for the uptake. Others suggested that chitin in the cell wall plays an important role in the uptake of uranium and thorium. Competition in the presence of iron (Fe^{2+}) strongly affected the sorption isotherms for uranium, reducing the maximum capacity (0.76 mmol g^{-1}) by 78% in the presence of 1.8 M Fe^{2+} at pH4 and ambient temperature.

Agriculture wastes :

In this case , a variety of by-products are incorporated , e.g. wool, olive cake ,sawdust , pine needles, almond and coal. Either protein – based animal fiber or cellulose-based plants fibers bear many amino, carboxylic or hydroxyl functional groups, which play a major role in metal binding.

Mechanism of bacterial biosorption:

Transport across cell membrane :

Heavy metal transport across membrane by the same mechanism used to transport essential ions such as potassium , magnesium and sodium and that may confused by the presence of heavy metals of the same charge and ionic radius with essential ions and this mechanism is not associated with metabolic activity.

Physical adsorption :

This is takes place with the help of Van dervals force . That the metal biosorption take place through electrostatic interaction between the metal ions in solutions and cell wall of microbial cells.

Ion exchange : Cell wall of biomass contain monovalent and bivalent ions exchange with metal ions , for example: the alginates of marine algae occur as salts of K^{+}, Na^{+}, Ca^{2+} , and Mg^{2+}.these ions exchange with ions such as CO^{2+},Cd^{2+} and Zn^{2+} resulting uptake of heavy metals.

Precipitation

A variety of precipitation and minerals have been found associated with bacterial surfaces further it may be dependent on cells metabolism if the microorganism produce compounds that favour the precipitation , or it may not be dependent on cells metabolism if it occurs after chemicals interaction between the metal and cell wall surface.

Complexation

Metal uptake by complex formation of cell surface by producing proteins called metallothionein which induced by metal ions and these bind metal ions . The complexing or chelating agents solubilize metal ions. The chelating compounds may be organic acids (citric, oxalic, tartaric) or complex compounds such as sidrophores,(ion –carrier , are small , high affinity iron chelating compounds secreted by microorganism such as bacteria, fungi . they are amongst the strongest soluble Fe^{3+} binding agent known).

Biosorption experimental procedures:

The experimental procedure consists of the following steps: Preparation of the bisorbent , Characterization of biosorbent , Preparation of the stock solutions and Analysis of metal ion concentration .

1- **Batch studies**: have to be used to evaluate the required fundamental such as biosorbent efficiency , optimum experimental conditions, biosorption rate and possibility of biomass regeneration.

Factors influence bacterial batch biosorption:

pH

Increasing the pH increase the negative charge on cell surface until all the functional groups are deprotonated which favors the electrochemical attraction and adsorption of cations and anion with cells with increasing positive charge due to lower pH values. Metals ions in solution undergo hydrolysis as the pH increase .

Temperature :

Temperature affect biosorption with the range 20-35^{o}C , higher temperature enhance sorption due to increase surface activity and kinetic energy solute. Physical damage may occur for the biosorbent at higher temperature and that lead to reduce biosorption capacity of the microorganism.

Biosorbent concentration:

Lower biosorbent concentration increase the amount of solute biosorbed due to increase surface area of biosorbent in turn increasing the binding sites. At high sorbent concentration with insufficient solute to completely cover the binding sites resulting in low solute uptake.

The initial solute concentration the sites available for sorption become fewer compared with the solute present and that reduce biosorption.

Agitation rate : When increasing the agitation rate, the diffusion rate of solute become higher due to enhanced turbulence and decrease in thickness of the liquid boundary layer which enhance the solute diffuse through boundary layer.

2-Continuous biosorption :

It is prefer in industrial application , different columns are used like packed bed columns (are a hollow tubes , pipes or other vessels that filled with a packing material. It can use as a chemical reactor) have been established as effective, economically. Other column like continuous stirred tank reactors are useful but its costs used when biosorbent is powder. This method require high flow rate to keep the biosorbent particles in suspension. Various parameters affect the packed bed biosorption, including the length of the sorption zone , uptake, removal efficiency and the slope of the break through curve.

Desorption:

If the biosorption process were to be used as an alternative to the wastewater treatment scheme, the regeneration of the biosorbent may be crucially important for keeping the process costs down and in opening the possibility of recovering the metals extracted from the liquid phase. For this purpose it is desirable to desorb the sorbed metals and to regenerate the biosorbent material for another cycle of application. The desorption process should:

1. Yield the metals in a concentrated form.
2. Restore the biosorbent to close to the original condition for effective reuse with undiminished metal uptake.

3. No physical changes or damage to the biosorbent.

While the regeneration of the biosorbent may be accomplished by washing the metal- laden biosorbent with an appropriate solution, the type and strength of this solution would depend on the extent of binding of the deposited metal. Dilute solutions of mineral acids like hydrochloric acid, sulphuric acid, acetic acid and nitric acid can be used for metal desorption from the biomass. Salt solutions, alkalies, deionised distilled water and buffer solutions were employed as well.

Prof.Dr.Ithar Kamil Abbas

What is biodegradation?

Biodegradation is the decay or breakdown of materials that occurs when microorganisms use an organic substance as a source of carbon and energy. For example, sewage flows to the wastewater treatment plant where many of the organic compounds are broken down; some compounds are simply biotransformed (changed), others are completely mineralized. These biodegradation processes are essential to recycle wastes so that the elements in them can be used again. Recalcitrant materials, which are hard to break down, may enter the environment as contaminants.

Biodegradation is a microbial process that occurs when all of the nutrients and physical conditions involved are suitable for growth. Temperature is an important variable; keeping a substance frozen can prevent biodegradation. Most biodegradation occurs at temperatures between 10 and 35°C. Water is essential for biodegradation. To prevent the biodegradation of cereal grains in storage, they must be kept dry. Foods such as bread or fruit will support the growth of mold if the moisture level is high enough. The microorganisms need energy plus carbon, nitrogen, oxygen, phosphorus, sulfur, calcium, magnesium, and several metals to grow and reproduce. The oxidation of organic substances to carbon dioxide and water is an exothermic (heat-releasing) process. For each mole of oxygen used as electron acceptor (oxidant), about 104 kilocalories (435 kJ) of energy is potentially available. All organisms make use of only part of this energy. The rest is lost as heat. This can be seen in composting when the compost becomes hot. Biodegradation can occur under aerobic conditions where oxygen is the electron acceptor and under anaerobic conditions where nitrate, sulfate, or another compound is the electron acceptor.

Bacteria and fungi, including yeasts and molds, are the microorganisms responsible for biodegradation. Environmental managers want to use biodegradation when it is needed and prevent it when preservation is important. Chemicals are commonly used to treat wood in buildings and other structures to prevent biodegradation. Wooden posts and pilings are treated with creosote or copper compounds to prevent rotting. Compounds that inhibit biodegradation are often added to automobile antifreeze solutions, aircraft deicer formulations, and other products to preserve the original qualities of the product. These products and chemicals can enter the environment and become contaminants. The inhibitors have a negative effect when the product becomes a waste and is to be biodegraded. For example, biodegradation of aircraft deicer formulations in airport runoff is often inhibited because of the benzotriazoles that are present to preserve the formulation.

Degradation of Petroleum

All natural organic compounds are degradable. If any organic compound produced in the ecosphere were inherently resistant to recycling, huge deposits of this material would have accumulated throughout the geological ages. Substantial organic deposits, such as fossil fuels, accumulate only under conditions adverse to biodegradation. Petroleum is, in one sense, a natural product, resulting from the anaerobic conversion of biomass under high temperature and pressure. Most of its components are subject to biodegradation, but at relatively slow rates. Petroleum hydrocarbons can be divided into four classes: saturates, aromatics, asphaltenes (phenols, fatty acids, ketones, esters, porphyrins), and resins (pyridines, quinolines, carbazoles, sulfoxides, amides).

The fate of most petroleum substances in the marine environment is ultimately defined by their transformation and degradation due to microbial activity. About a hundred known species of bacteria and fungi are able to use oil components to sustain their growth and metabolism. In pristine areas, their proportions usually do not exceed 0.1-1.0% of the total abundance of heterotrophic bacterial communities. In areas polluted by oil, however, this portion increases to 1-10%.

Evidence for the effectiveness of oil bioremediation should include:

1. Faster disappearance of oil in treated areas than in untreated areas.
2. A demonstration that biodegradation was the main reason for the increased rate of oil disappearance.

To obtain such evidence, one has to be careful in selecting proper oil analysis procedures as well as in interpreting the data.

Biochemical processes of oil degradation with microorganism participation include several types of enzyme reactions based on oxygenases, dehydrogenases, and hydrolases. These cause aromatic and aliphatic hydrooxidation, oxidative deamination, hydrolysis, and other biochemical transformations of the original oil substances and the intermediate products of their degradation.

Hydrocarbons differ in their susceptibility to microbial attack and generally degrade in the following order of decreasing susceptibility:

n-alkanes > branched alkanes > low-molecular weight aromatics > cyclic alkanes.

It is difficult to examine biodegradation kinetics and relative rates, without basic knowledge of the various microbial species and specific environmental conditions.

Petroleum-based products are the major source of energy for industry and daily life. Petroleum is also the raw material for many chemical products, such as plastics, paints, and cosmetics. The transport of petroleum across the world is frequent and the amounts of petroleum stocks in developed countries are enormous. Consequently, the potential for oil spills is significant. The volume of spills usually exceeds the inherent remediation capacity for any given environment, resulting in a significant ecological impact. Type specimens of bacterial strains used in bioremediation exist in various repositories (e.g., ATCC, DSM, etc.), or are commercially available and are usually covered by patent rights. Each of these strains may yield dramatic results *in vitro* for specific target compounds. However, the overall success of such strains in treating a wide range of contaminants in situ remains limited. The reintroduction of indigenous microorganisms isolated from a contaminated site after culturing seems to be a highly effective bioremediation method, especially when microbial growth is supplemented by oxygen and fertilizers. Thus, bioremediation is normally achieved by stimulating the indigenous microbiota (naturally occurring microorganisms). Stimulation is achieved by the addition of growth substrates, nutrients, terminal electron acceptor, electron donors, or some combination therein, resulting in an increase in contaminant biodegradation and biotransformation. This notion was confirmed in the large scale operation for bioremediation after the oil spill from the Exxon Valdez in Alaska, with the addition of nitrogen and phosphorus fertilizers.

Microbial mats have a potential to immobilize and probably also degrade pollutants. This capacity of microbial mats may partly decrease the damaging impact of pollutant spills on coastal ecosystems. Development and survival of microbial mats after oil spills was reported in coastal areas of Kuwait after the Gulf War.

Microorganisms involved in the laboratory experiment

Identification of the key organisms that play a role in pollutant degradation processes is relevant to the development of optimal in situ bioremediation strategies. Indeed, efforts have been made to characterize bacterial communities and their responses to pollutants, to isolate potential petroleum degraders, and to identify the functional genes involved in particular degradation processes.

Cyanobacterial mats developing in oil-contaminated sabkhas in the African coasts of the Gulf of Suez and in the pristine Solar Lake, Sinai were collected for laboratory studies. Both mat samples showed efficient degradation of crude oil in the light, followed by an intense bloom of Phormidium spp. and Oscillatoria spp. The major controlling factor for biodegradation is the hydrophilic/hydrophobic interface between the brines and the benthic bacterial mat. It was observed that oil droplets attached to a cluster of the cyanobacterial species of Phormidium, while many heterotrophs migrated to the oil/water interface. As the degradation process takes place at the oil/ water interface, emulsification is the first step in the process. Indeed, cyanobacterial polysaccharides play a major role in the emulsification of the oil, actually breaking the oil into small droplets that are subsequently attacked by the heterotrophs. Initial experiments with axenic cyanobacterial cultures did not show oil degradation. Recent reports have demonstrated that photosynthetic microorganisms, particularly cyanobacteria, may play a direct or indirect role in the metabolism and degradation of hydrocarbons. Cyanobacteria such as Anabaena cyiindrica, P. faveolarum, Oscillatoria sp. strain JCM, and Agmenellum quadruplicatum can degrade different aromatic compounds. Cyanobacteria are present in association with oil-degrading bacteria and prevent them from being washed out, by immobilizing them in their extracellular polysaccharide. In addition, cyanobacteria also supply these bacteria with oxygen and fixed nitrogen. This indirect role of cyanobacteria can be important to the overall success of the biodegradation process.

When model mixture of petroleum contained aliphatic and aromatic compounds exposed to benthic cyanobacteria*; Oscillatoria* sp. and *Phormidium* sp., only the decalin (an aromatic compound) was missed. In the case of the planktonic microorganisms under anaerobic conditions, *Desulfovibrio oxyclinae* biodegraded mostly the aromatic compounds, whereas *Marinobacter* sp. under oxic conditions biodegraded the normal hydrocarbon faster than the aromatics. Strains of sulfate-reducing bacterium and aerobic heterotrophs were capable of degrading model compounds of both aliphatic and aromatic hydrocarbons. These experiments indicated that the aromatic compounds were more water-soluble and hence available to the *D. oxyclinae* bacteria whereas, in the *Marinobacter* sp. experiment, it is possible under these conditions that the bacteria released extracellular bioemulsifiers, which made the normal hydrocarbon more available to biodegradation.

Factors affecting the microbial degradation of petroleum

The degree and rates of hydrocarbon biodegradation depend upon:

1. The structure of hydrocarbon molecules:

The paraffin compounds (alkanes) biodegrade faster than aromatic and naphthenic substances. With increasing complexity of molecular structure (increasing the number of carbon atoms and degree of chain branching) as well as with increasing molecular weight, the rate of microbial decomposition usually decreases.

2. The physical state of the oil, including the degree of its dispersion.
3. The most important environmental factors that influence hydrocarbon biodegradation include:
    a) Temperature.
    b) Concentration of nutrients and oxygen.
    c) Species composition.
    d) Abundance of oil-degrading microorganisms.
4. The rate of biodegradation depends on both physicochemical and biological variables:

It has been coined the term "bioavailability of hydrophobic organic compounds", which is a function of phase-solubility and solution-transport processes. The ability of hydrophobic organic compounds to be solubilized and transported into the immediate vicinity of bacterial cells capable of metabolizing them is potentially the rate-limiting step in bioremediation. Degradation of hydrocarbons in the presence of synthetic surfactants is a delicate issue. Generally, the toxicity of surfactants increases with their hydrophobicity. The use of surfactants of biological origin solves the toxicity problem.

The polymeric biosurfactant Alasan is a high molecular weight bioemulsifier complex of anionic polysaccharides and proteins produced by Acinetobacter radioresistens KA53.

The effect of Alasan was tested on the solubilization and biodegradation of polyaromatic hydrocarbons (PAHs). The presence of Alasan increased significantly the rate of [14C]-phenanthrene mineralization by *Sphingomonas paucimobilis* EPA505. This result suggests that Alasan solubilized PAHs by a physical interaction, probably hydrophilic.

These complex and interconnected factors influencing biodegradation and the variability of oil composition make interpreting and comparing available data about the rates and scale of oil biodegradation in the marine environment extremely difficult.