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FACULTY OF SCIENCES AND TECHNOLOGY
BIOLOGICAL SCIENCES DEPARTMENT

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COURSE HANDOUT

1ST YEAR Master Microbiology and Quality Control

Entitled :

Applied and Environmental Microbiology

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FOREWORD

The course of "**Applied and Environmental Microbiology**" is intended for students of the first year Master Microbiology and Quality Control, field of nature and life sciences.

The knowledge required to follow this course is some basic notions of Microbiology, Biotechnology and Biochemistry.

In this course, students will be introduced to the main concepts in microbial ecology and techniques for studying microorganisms in the environment from the digestive tract to the aquatic and soil ecosystems. They will be required to conduct an analysis from the sample taking until decision making. Fermentation technology will allow the student to address the design of a bioreactor and therefore biomass production and of major metabolites. From environmental microbiology, the researchers developed industry applications for the use of micro-organisms in the various areas of interest of Man: food processing, pharmacology agriculture, cosmetology, bioremediation, wastewater treatment... etc. This is what we will develop in this document.

At the end of this course, the student will be able to:

- Understand the methods of studying the ecology of viruses,
- Examine the role of micro-organisms in the biochemistry, and to name and classify microorganisms of interest.
- Understand the current state of knowledge of the material cycle in the environment, bioreactors and biofilms
- Show examples of application of environmental micro-organisms.

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METHODS OF STUDYING MICROORGANISMS

Chapter I: Methods of studying microorganisms

1. Beneficial microorganisms and contamination germs

There are many beneficial microorganisms for humans, used in different areas of life: agriculture, food, pharmacology, cosmetology, environment,... etc. However, excessive use of these tools can lead to a loss of control over their management.

Therefore, it is important to control beneficial microorganisms or contamination germs by physical-chemical methods among others to avoid their transmission.

Furthermore, the germs of contamination are those that are not supposed to be in a so-called environment and are often pathogenic. These microorganisms create ecological balances and can produce toxic substances.

2. Control point targeting

Strict microbiological monitoring of the product manufacturing chain involving the use of beneficial microorganisms is required for the following reasons:

- Manage the necessary amount of germs used and prevent their spread;

- Hygiene diagnosis, control of surfaces in contact with the material;

- Avoid contamination of raw materials and products by pathogenic germs.

3. sterilization practices

In microbiological practice, sterilization is essential to avoid all forms of contamination and work under conditions of complete asepsis. It is defined as a technique of destruction of all microorganisms of any material (glasses, plastic, food, ...etc).

The purpose of sterilization is to ensure that the microorganisms counted or identified are actually from the sample being analyzed. To this end, the following criteria must be met:

- Disinfection of surfaces with appropriate products;

- Hands disinfected with hydro-alcoholic products

- All sample containers shall be sterile.

4. **sampling**

Sampling is the act of taking a certain amount of substances or materials for analysis in a laboratory. Samples can be of different types, from food to water and environment (soil, wood, plants, etc.). Sampling for microbiological analysis is performed in the following situations:

-Verify that the product or process meets quality requirements.

-Temporary assessment, monitoring and verification of the proper application of good hygiene practices in specific manufacturing processes.

-Analytical research to explore or distinguish one or more specific micro-organisms in all areas of scientific research.

5. Methods for the study of microorganisms

The study of microorganisms uses a variety of methods, which can better describe microorganisms based on phenotype and phylogenetic characteristics. The latter begins with sampling, observations and guided experiments.

Indeed, this research begins with sampling, which allows to define microbiological tools in space and time. Then, continue to measure biomass and metabolic activity, and isolate and characterize microorganisms.

All these steps lead to the analysis of the behaviour and function of micro-organisms. However, difficulties can be encountered, which is the non-culturability of most microorganisms in the environment.

5.1. Growth under controlled conditions

Growth is defined as "the orderly increase of all components in an organism, and in microorganisms it leads to an increase in the number of cells. For this reason, many nutrients must be present in the medium. In addition, physical and chemical factors may promote or inhibit this nutrient intake, thereby controlling growth.

5.2. Isolation of pure crops (purification)

The aim of this operation is to obtain different colonies for each different micro-organism. When all the microorganisms that make up a strain are identical, the strain is called pure strain: the colonies obtained by spreading the pure strain must all have the same characteristics.

5.3. Phenotypic characterization

Morphological tests (fresh examination, single and/or double staining): Allows to determine the size, shape, and arrangement of cells.

- **Gram staining** (differential staining) allows to divide the bacteria into two groups: Gram Negative and Gram Positive. This coloration uses a combination of two dyes (Primary Coloring - Purple Crystal Mauve / Secondary Coloring - Safranin Red). The Gram positive wall allows trapping of 1st dye and the Gram negative wall does not allow trapping of 1st dye

- **Malachite green coloration of spores (resistant structures used for survival in adverse conditions).**

Biochemical tests (metabolism)

Physiological tests (respiratory enzyme search: catalase, oxidase, nitrates reductase,. etc.).

5.4. Culture media :

They can be divided into different classes according to their consistency, composition and function.

Based on consistency, they are:

1. **Liquids (broths)**

- Suspension culture (homogeneous or heterogeneous)

- Uniform distribution of nutrients

- Growing large quantities

2. **Solids (agar)**

Solid media are of the same composition as liquid media with a solidifying agent added (example: agar which is a polysaccharide derived from an algae). The agar allows for isolation of distinct colonies and pure cultures.

Depending on the composition, they can be either synthetic, complex or enriched.

5.5. Microscopic observation

The first observation of microbial cells is often done by a (ordinary) clear-field optical microscope, which is equipped with 3 to 4 lenses ranging from *4 magnification up to *100.

This observation allows us to study the morphological appearance of the cells of a microbial species. It includes fresh examination (between slide and slide at G*40) and post-coloration examination (on dried smears fixed without slide at G*100 plus a drop of immersion oil).

The aim is therefore to describe the form, mode of grouping and mobility so visible on examination in its fresh state.

Cytometry methods

The different techniques of:

- ☐ direct light microscopy (the simplest: optical microscope),
- ☐ the phase contrast microscopy (observation of cells without preparation or staining: observation of living cells)
- ☐ the interferential microscope (use of polarized or non-polarized light: observation of transparent or reflective objects),
- ☐ fluorescence microscopy (widely used in microbial ecology: exploits the capacity of certain molecules, fluorochromes or fluorophores: laser), electron microscopy etc...).

The counting of microorganisms by cytometry techniques is most often done by fluorescence microscopy and flow cytometry.

Counting in microbial ecology is important for various applications, including the assessment of the biomass of target microorganisms, especially bacteria.

5.6. Enumeration Methods

The purpose of the count is to determine the concentration of germs contained in the initial sample. For this purpose, different techniques exist, the most used of which are:

1. Measurement of turbidity (optical density suspension haze) by spectrophotometry UV (amount of light that can pass through a sample)

2. Direct counting on the blades (example: Malassez blade)

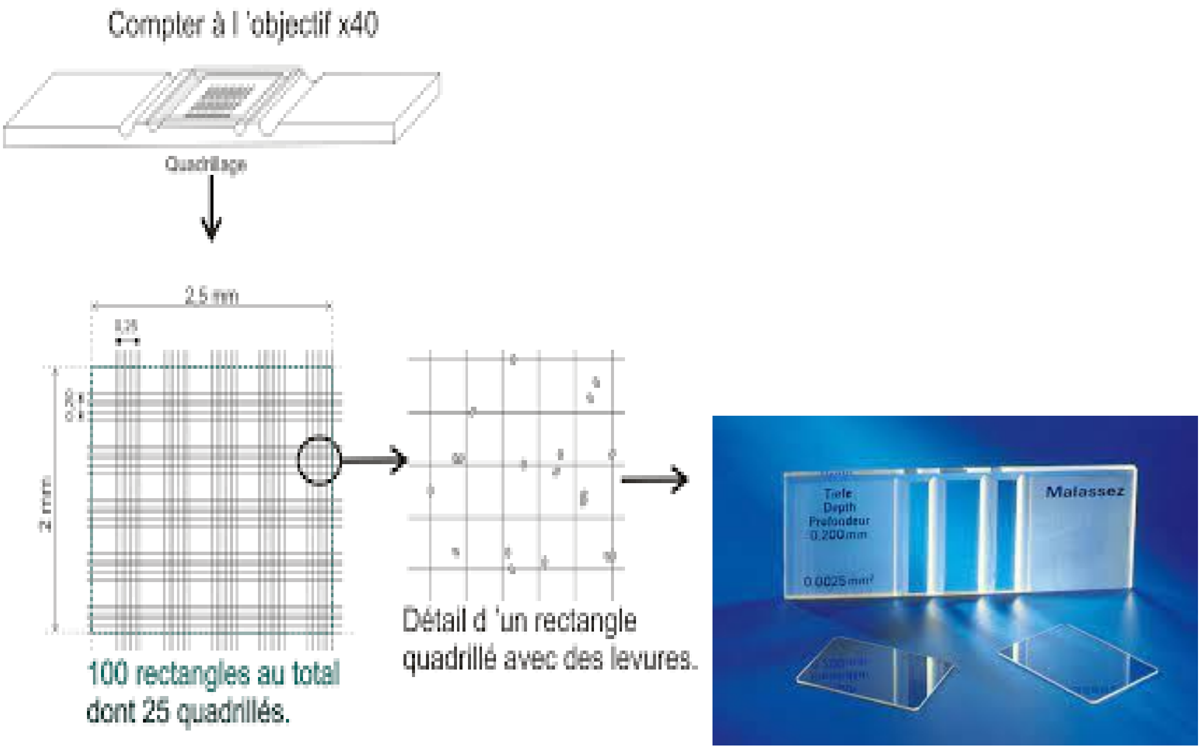


Figure 01: Malassez cell

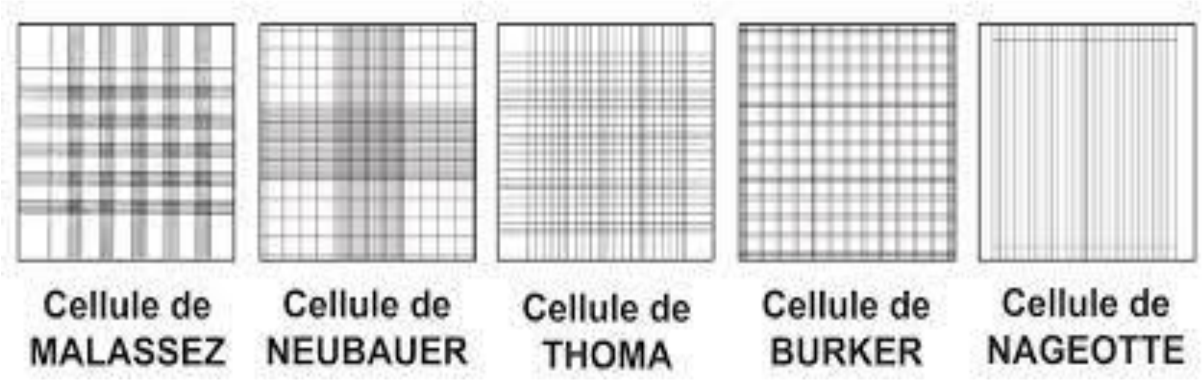


Figure 02: Differents cell counting

3. **Counting of colony forming units (CFU) on a geolocated box**

A viable account is made by diluting the initial sample

Spread samples of the dilutions on an appropriate culture medium

Incubate under appropriate conditions to allow growth

Colonies are formed

Colonies are counted and the initial number of viable cells is determined by function of dilution

4. **Most likely number (MPN): based on probability statistics (Mac Grady table). Liquid medium technique.**

Start with a broth that allows the desired characteristics to be detected (ex: BCPL lactosed broth with bromocresol purple to highlight lactose fermentation in coliforms);

✓ Inoculate different dilutions of the sample to be tested in each of 3 tubes Dilution3 Tubes/Dilution1 ml of each dilution in each tube. After an appropriate incubation period, record POSITIVE TUBES (which have the desired growth and characteristics)

After incubation, the number of tubes that demonstrate the desired characteristics are recorded.

Example of results for coliform search on BCPL media

-Dilutions: Positive tubes: Choose the right suite: 321 and find it in the table of Mc Grady

- Multiply the result by the central dilution factor 150 X 10^2 = 1.5 X 10^4/mL

Since you have 1g in 10mL must multiply by 101.5 X 10^5/g

The different usual methods

The different methods are based on the physico-chemical and biochemical properties of the microorganisms. The usual methods are:

The filter membrane, the most probable number (MPN) method, the isolation method followed by tests (serological, biochemical), immunological techniques, measurement of metabolism, use of bacteriophage, study of changes in pH or redox potential, The search for enzymes or coenzymes.

These methods, which are available to a very large number of laboratories, are based on the study of the morphology and metabolism of microorganisms. These methods consist of observing the fermentation and assimilation of different sugars and carbonaceous substrates, the production of toxins or secondary products, resistance to various antibiotics or natural or synthetic molecules, ...

Using bioluminescence (Lonvaud-Funel and Joyeux 1982) and epifluorescence techniques (Froudière et al. 1990) revealed the existence of Viable Non-cultivable (NVC) populations (Millet 2001).

Regarding the identification of species, phenotypic tests (consumption of certain sugars, respiratory qualities...)

Epifluorescence is a fast, culture-free method that is highly effective for the visualization and counting of viable cells.

1.3. Genetic methods :

- Over the past decade, new methods have been developed that are faster and more molecular-based than traditional methods.

- Today, the laboratory technician can perform these analyses in a matter of hours, whereas the former techniques would have required several days of work by microbiologists.

New methods for detecting microorganisms are based on genetic information, which allows more accurate results than traditional tests, based on biochemical or immunological characteristics, sensitive to environmental conditions

- **Analysis of mitochondrial DNA**

direct extraction of bulk DNA by physical treatments of sonication (sound energy), thermal shocks and purification by chromatography.

- **The different techniques of PCR (Polymerase Chain Reaction)**

Genetic characterization is the most reliable description of a species and individual within the species.

This technique, also known as caryotype, consists of separating the chromosomes of the microorganism under study according to their size by applying alternating cross-sectional and opposite-direction electric fields in an electrophoresis gel. After revealing under ultraviolet light by a fluorescent substance, the obtained profiles allow to highlight differences between strains

ribosomal RNA is a highly conserved molecule in all organisms, so all these techniques rely largely on the properties of this ribosomal molecule and its sequence provides a molecular identity card to identify microorganisms. This discovery has greatly expanded the researchers' view of microbial diversity in almost all ecosystems and significantly broadened the scope of study in a complex environment.

The amplification of sequences of interest is the method of amplifying a specific DNA fragment in order to make it detectable.

VIRAL ECOLOGY

Chapter 2 : Viral ecology

Viruses are important in ecosystems and nutritional networks, especially those that infect prokaryotic and eukaryotic microorganisms.

These acellular microorganisms are heavily involved in large biogeochemical cycles and constitute a major part of the global biomass.

They are ubiquitous in the biosphere and can infect all living things. Thus, they have an impact on the diversity of microbial populations in the ecosystem, the genomic evolution of these populations and major major or indirect biogeochemical cycles(Roux, 2013).

1. Definition

Viruses are infectious microscopic particles that can only replicate themselves by entering cells and using their cellular machinery.

Viruses that infect bacteria are bacteriophages (Chastel, 1992).

2. Historic

The discovery of the virus dates back to the 19th century by Adolf Meyer (1892) on the leaf of tobacco. The German scientist discovered that this disease, which affected tobacco leaves, could spread from one plant to another. Tobacco mosaic virus (VMT) was not identified until 1935 by Wendell Stanley. But it is the work of Dimitri Ivanovsky (1864-1920) that has allowed a first description of the virus.

Later, the microbiologist Martinus Beijerinck (1851-1931) understood that the infectious particle must be much smaller than a bacterium and named it "virus" (Zaitlin, 1998)

However, Robert Koch (1843-1910) and Fredriech Loeffer (1852-1915) were the first authors to identify the virus as a smaller particle than bacteria because it was not retained by Chamberland filters which normally retain bacteria (Mahy, 2005).

On the other hand, Ernest Hanbury Hankin (1865-1939) was the microbiologist who noted the antibacterial power of a virus against Vibrio cholera, but it is the work of Frederick William Twort (1877-1950), which revealed the existence of a virus infecting bacteria, called “bacteriophage” (Roux, 2013*)*.

3. General virus structure

Viruses are infectious particles whose genes (DNA or RNA) are enclosed in a protein envelope: the capsid.

The latter is formed of a set of proteins grouped in subunits called «capsomeres». They are however unable to multiply and grow independently (Roux, 2013).

Despite the morphological description (Figure 1), the genetic analysis was more interesting.

The first virus to be sequenced is actually a bacteriophage, called Enterobacteria phage MS2 (Fiers *et al., 1976).*

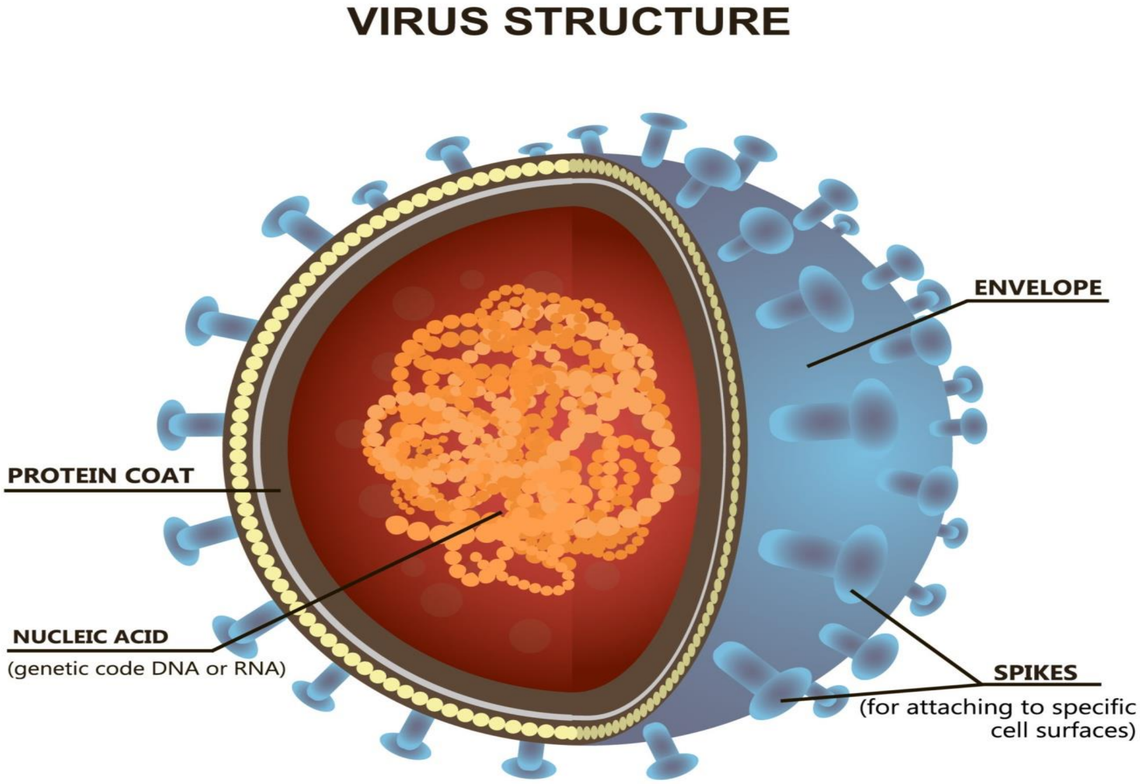


Figure 3 : Virus structure

4. Impact

Viruses are ubiquitous in the biosphere and can infect all living things.

Within ecosystems, viruses impact:

- The quantity and diversity of the microbial population

- The evolution of the genomes of these populations

- The biogeochemical cycles of matter.

The metabolic activity and abundance of microorganisms in ecosystems are affected by viral infections.

5. Aquatic Viral Ecology

The main biological factor limiting virus diversity and abundance appears to be the abundance of susceptible hosts.

Presence of spinous extensions or processes (a polymorphism: ability to present in different forms) observed only in aquatic viruses increases the likelihood of encountering the host, especially in an oligotrophic environment (nutrient-poor environment).

Therefore, it is very likely that all aquatic communities, whether procariotic or eukaryotic, will be infected with viruses in their environment.

From a quantitative point of view, viruses with densities typically between 10^4 and 10^8 are highly dynamic components of aquatic ecosystems, and these viruses are mainly represented by newly produced viral particles (Figure 2).

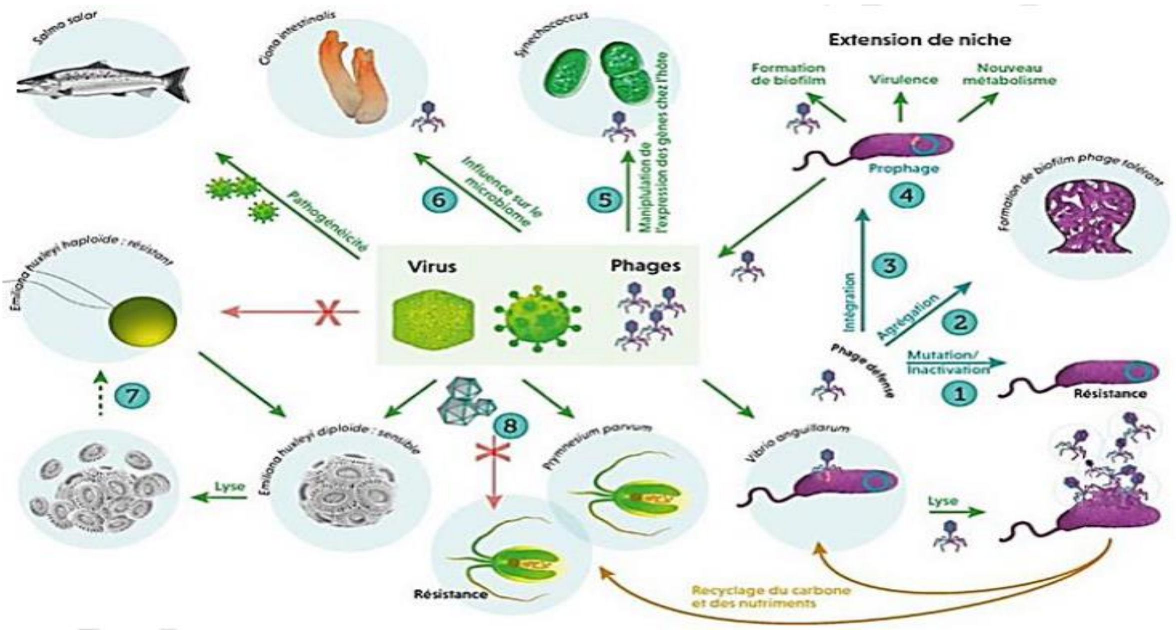


Figure 4: Principles interactions between virus and host in Marin ecosystem (Middelboe and Brussaard, 2017)

Their seasonal dynamics depend on non-biological (temperature, ultraviolet radiation, chemical reagents, etc.) and biological (sensitive hosts, organic charges, etc.) (Amblard *et al., 1998).*

The main factors that may regulate the abundance and production of bacteria in the aquatic environment are temperature, resources, predation and lysis of the virus (Rivkin and Anderson, 1997).

Although the contribution of aquatic ecosystems to the release of enteric viral pathogens has been known for decades, The importance of wild virions that educate aquatic communities and food webs has only been highlighted relatively recently.

The evidence of viral infections in both prokaryotic and eukaryotic life domains, as well as in heterotrophic bacteria and protozooplankton, has led marine biologists to question the impact of viroplankton on processes such as

(1) the mortality of micro-organisms,

(2) Protists' nutrition heterotrophic,

(3) the promotion of exchanges of genetic material between populations microbial,

(4) the maintenance of species diversity,

(5) the induction of aggregates plankton, and

(6) the cycle of organic matter in aquatic ecosystems (Amblard *et al., 1984)*

Viruses undoubtedly influence biological processes in aquatic ecosystems to varying degrees, although almost all studies on the ecology of pelagic viruses are conducted during a limited period of the year, Mainly in marine areas and waters located in temperate zones.

This directly affects the calculation of carbon flux and understanding of biological pumps. (series of biological processes leading to transport of carbon from photic zone to seabed)

In fact, carbon dioxide was initially captured from the atmosphere primarily by photosynthetic organisms, including aquatic micro-organisms. Therefore, global warming is expected to be the result of increased greenhouse gases, the most important being carbon dioxide.

6. Viral metagenomes

Metagenomics, or large-scale random sequencing of nucleotide fragments extracted from samples, offers a unique perspective on the viral genome.

Therefore, this kind of recently developed approach highlights the exceptionally rich population of environmental viruses in terms of genes and genotypes(Roux, 2013).

By establishing the Metavir web server, which is the first server dedicated to virion analysis (final form of virus maturation, most often infectious free extracellular form), a new method of analysis adapted to the specificity of the viral genome and the metagenome has been developed.

Today, Metavir offers a set of tools consistent for different virus types. Metavir has more than 300 users and can scan more than 2,000 viruses

The functional potential of the viral genome can be explored by a group of virions. After a rigorous analysis of the potential contamination, it was demonstrated that the viral genome comprises a limited but not negligible set of genes related to cellular metabolism.

Therefore, most viruses undoubtedly act directly on the metabolism of host cells during infection.

As a factor constituting the aquatic viral community, environmental parameters, particularly salinity, are also dominant. The geographical distance between samples seems to have only a minor effect, confirming the remarkable ability of viral capsid to disperse.

However, there seems to be local adaptability in some cases, especially when there is clear competition between host-developed resistance and the ability to infect the virus.

Finally, different families of small single-stranded DNA viruses were characterized by viral meta-analysis. Their apparent simplicity thus reveals a more complex evolutionary mechanism than expected, involving capacities for gene circulation and transfer.

Until now, they were considered the privilege of double-stranded DNA viruses and questioned the acceptable separation between them.

Different groups of viruses are divided according to the nature of their genomes. By allowing studies ranging from the size of communities to specific genotypes, virions are the tool of choice for characterizing viral diversity, understanding the different factors that govern these communities, and thus better understand the location of viruses in the biosphere.

In addition, these studies confirmed that there are close interactions between viruses and cellular organisms. These interactions seem numerous and multiple in nature and consequences, and run through the whole history of organisms.

Therefore, this new knowledge brought by virosomes analysis (vesicle with phospholipid membrane incorporating viral proteins, are able to penetrate into cells) Address some fundamental questions about the origin of major evolutionary innovations or the overall function of ecosystems.

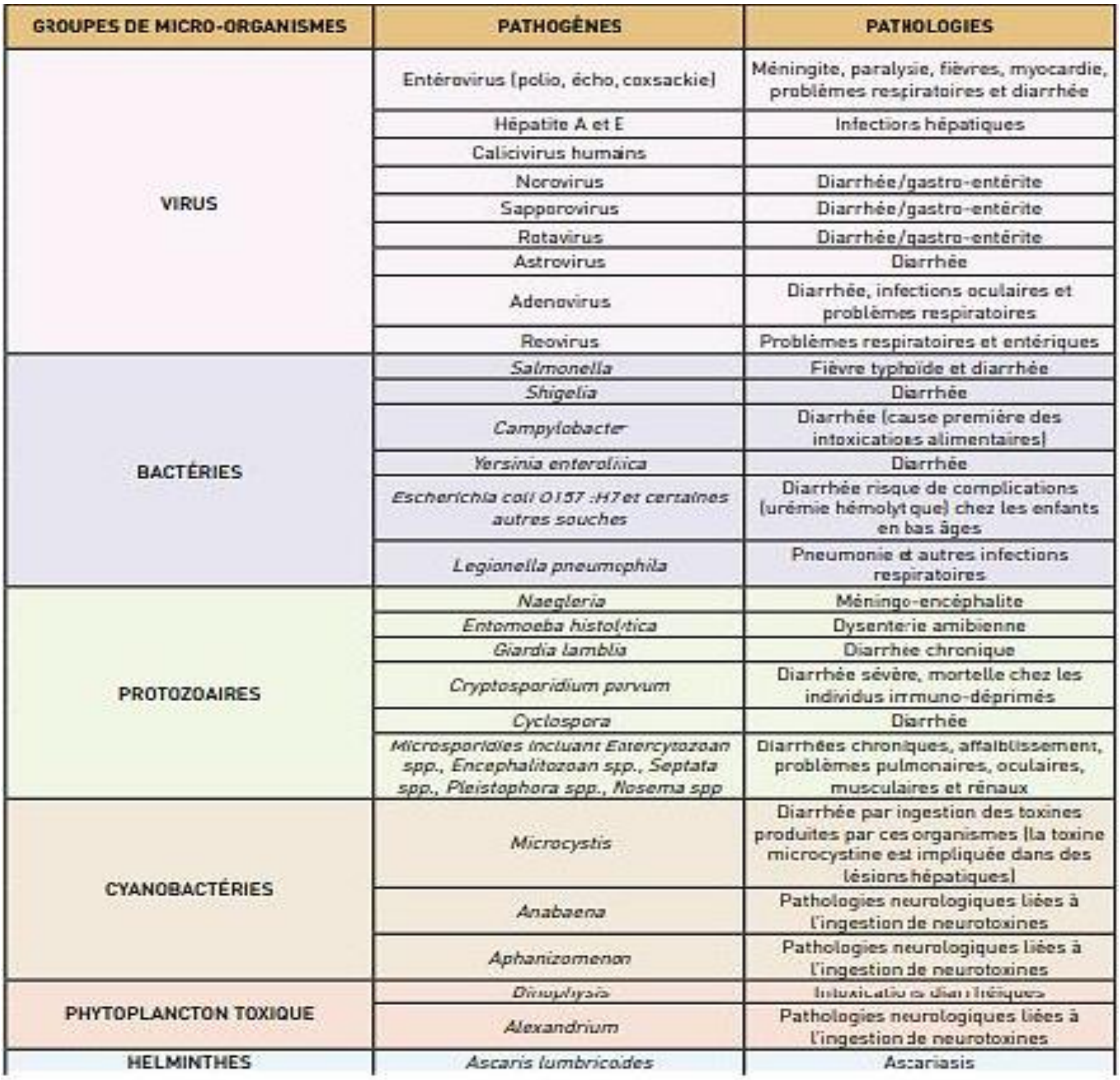
WATER QUALITY AND TREATMENT

Chapter 3 : Water Quality And Treatment

1. Introduction

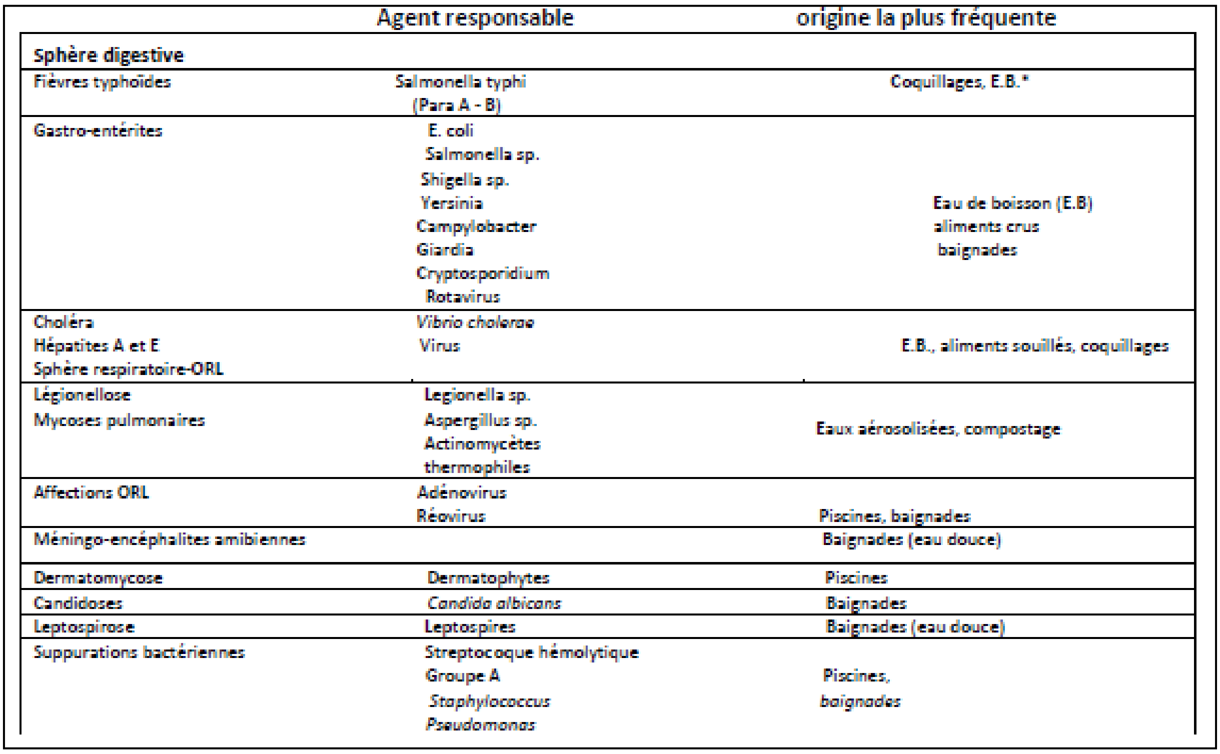
Most of the aquatic microbial community plays an important role in the biogeochemical functions of aquatic ecosystems, but others are not involved, but only transported by water which is an ideal means of transport for pathogenic germs. These come mainly from the digestive tract of humans and animals, it is so-called «fecal» microorganisms, coming from human or animal excrement. Some fecal microorganisms are pathogenic and use water to propagate between hosts causing the waterborne diseases.

Table 1: Groups and genus of pathogenic microorganisms responsible of water borne diseases (Straub and Chandler, 2003)



GROUPES DE MICRO-ORGANISMES	PATHOGÈNES	PATHOLOGIES
VIRUS	Entérovirus (polio, écho, coxsackie)	Méningite, paralysie, fièvres, myocardie, problèmes respiratoires et diarrhée
	Hépatite A et E	Infections hépatiques
	Calicivirus humains	
	Norovirus	Diarrhée/gastro-entérite
	Sapporovirus	Diarrhée/gastro-entérite
	Rotavirus	Diarrhée/gastro-entérite
	Astrovirus	Diarrhée
	Adenovirus	Diarrhée, infections oculaires et problèmes respiratoires
	Reovirus	Problèmes respiratoires et entériques
BACTÉRIES	*Salmonella*	Fièvre typhoïde et diarrhée
	Shigelia	Diarrhée
	Campylobacter	Diarrhée (cause première des intoxications alimentaires)
	Yersinia enterolitica	Diarrhée
	Escherichia coli O157 :H7 et certaines autres souches	Diarrhée risque de complications (urémie hémolytique) chez les enfants en bas âges
	Legionella pneumophila	Pneumonie et autres infections respiratoires
PROTOZOAIRES	*Naegleria*	Méningo-encéphalite
	Entomoeba histolytica	Dysenterie amibienne
	Giardia lamblia	Diarrhée chronique
	Cryptosporidium parvum	Diarrhée sévère, mortelle chez les individus immuno-déprimés
	Cyclospora	Diarrhée
	Microsporidies incluant Enterocytozoan spp., Encephalitozoan spp., Septata spp., Pleistophora spp., Nosema spp	Diarrhées chroniques, affaiblissement, problèmes pulmonaires, oculaires, musculaires et rénaux
CYANOBACTÉRIES	*Microcystis*	Diarrhée par ingestion des toxines produites par ces organismes (la toxine microcystine est impliquée dans des lésions hépatiques)
	Anabaena	Pathologies neurologiques liées à l'ingestion de neurotoxines
	Aphanizomenon	Pathologies neurologiques liées à l'ingestion de neurotoxines
PHYTOPLANCTON TOXIQUE	*Dinophysis*	Intoxications diarrhéiques
	Alexandrium	Pathologies neurologiques liées à l'ingestion de neurotoxines
HELMINTHES	*Ascaris lumbricoides*	Ascariasis

Table 2: Main human water borne infections (Leclerc et coll, 1982)



	Agent responsable	origine la plus fréquente
Sphère digestive		
Fièvres typhoïdes	Salmonella typhi (Para A - B)	Coquillages, E.B.*
Gastro-entérites	E. coli Salmonella sp. Shigella sp. Yersinia Campylobacter Giardia Cryptosporidium Rotavirus	Eau de boisson (E.B) aliments crus baignades
Choléra	*Vibrio cholerae*	
Hépatites A et E	Virus	E.B., aliments souillés, coquillages
Sphère respiratoire-ORL		
Légionellose Mycoses pulmonaires	Legionella sp. Aspergillus sp. Actinomycètes thermophiles	Eaux aérosolisées, compostage
Affections ORL	Adénovirus Réovirus	Piscines, baignades
Méningo-encéphalites amibiennes		Baignades (eau douce)
Dermatomycose	Dermatophytes	Piscines
Candidoses	*Candida albicans*	Baignades
Leptospirose	Leptospires	Baignades (eau douce)
Suppurations bactériennes	Streptocoque hémolytique Groupe A *Staphylococcus* *Pseudomonas*	Piscines, *baignades*

2. Water

Today, due to the increase in water needs, water for food use is sourced from surface water, increasing the risk of microbial pollution and requires complex and expensive treatment. Indeed, wastewater of diverse origin has a microflora characteristic in quantity and quality. For example, a domestic waste water contains approximately 10^7 CFU/ml of fecal bacteria and 10^5 to 10^6 CFU/ml of *Aeromonas*. Hospital waste water is not more polluted urban waste water except for the presence of *Pseudomonas aeruginosa and Staphylococci dorati.*

However, its Salmonella load is three times less. Wastewater treatment systems and microbiological controls on water sources for food use prevent normally any presence of a pathogenic micro-organism in the feed water.

The regulations define drinking water as water that must not contain a number or a concentration of micro-organisms, parasites or any other hazardous substances potential for human health.

The objective of the treatment is to protect consumers from pathogenic microorganisms and

of impurities unpleasant or dangerous for health.

Tap water, an elaborate product, reflects two permanent concerns that are health public, comfort and pleasure to drink.

However, faecal contamination may occur at the source or anywhere in the system water distribution. They may also occur accidentally in the finished product as a result of a treatment and insufficient disinfection. In these conditions consumers will ingest a certain amount of quantity of bacteria, viruses or parasites.

3. Definition of water quality

Microbiological quality is a state of the water characterized by a level of presence of microorganisms (viruses, bacteria, protozoa...) which may cause a greater or lesser health risk.

The microbiological quality classification comprises 4 levels:

Category A, good quality waters: at least 80% of samples give a number of *Escherichia coli* less than or equal to 100 per 100 ml of water and 95% of samples have less than 2,000 per 100 ml. Finally, 90% of the samples collected contain less than 100 fecal streptococci for 100 ml.

Category B, medium quality waters: less than 2,000 E coli per 100 ml in 95% of the samples.

Category C, temporarily polluted waters: the exceedance of 2 000 **E coli** is observed in more than 5%, but in less than one third of the samples. The contaminated site must be subject to immediate measures or medium-term, which will lead to permanent improvements in water quality.

Category D, poor water quality: when the number of Escherichia coli is exceeded in at the use of this water is prohibited.

4. Water Pollution

- Various erosion products cause natural water pollution but it is also caused by acid rain, agricultural fertilizers and microorganisms released with faeces.
- These different types of pollution create a imbalance in the ecosystem (acidification, eutrophication) and affect living beings (destruction of animal species, pollution).

- Pollution is a change in water quality. Domestic water discharges and various human, industrial and agricultural activities are the main sources of pollution of surface waters.

Water is of vital biological and economic importance. The hydrosphere is the foundation of life and ecological balances. Water is both a food, possibly a medicine, a raw material industrial, energy and agricultural, and a means of transport. Its uses are therefore multiple but, as far as the of human health, they are dominated by agriculture and aquaculture, industry and crafts, leisure water, including bathing and, above all, the collective or individual supply of drinking water, usable for food (drinking water, cooking) but also domestic and hygiene.

The degree of water quality required obviously depends on these uses, and particular attention is paid to the quality of water intended for human consumption (EDCH), itself depends on that of the resources in water available. Recall that the bulk of the planet’s resources are represented by ocean waters (97%) which are essential biological reservoirs for human food, but which are very difficult to use.

Sea water is also, after evaporation, the source of water resources continental, underground and surface, very valuable for humanity, but often insufficient here and there, in quantity or quality.

The latter, which puts into question the uses of resources, but also the ecological balances of the environments water, and particularly affected by waste from human activities, very often poorly managed, whether solid waste (household waste, industrial waste), gaseous (air pollution, or liquids (urban, industrial or agricultural waste water, runoff

These waters more or less properly treated, pose risks to the receiving environment, including self-cleaning capabilities natural are limited. This results in ecological alterations and impacts on human uses. Water, which is more or less easy to correct by expensive and complex treatments.

The subsurface or surface water compartment is obviously in contact with the other compartments environmental: soil, air and biosphere. There is a trade-off between these different sectors, depending on the nature of contaminants. In particular, groundwater is more or less protected from contamination of soils, by their depth. Some long-range air pollutants (sulphur oxides and nitrogen, ammonia, in particular) are likely to cause acidification or eutrophication of soils and of water. Water contamination also affects various aquatic organisms directly consumed by human (fish, crustaceans, shellfish) or is indirectly involved in the progressive contamination of the food chain (bio-accumulative persistent organic compounds).

Various forms of pollution affect water resources. “Thermal” pollution is the consequence of the distribution of large quantities of water used for cooling in the aquatic environment, especially in the production of electrical energy by thermal or nuclear power plants. The rise excessive river temperature can change the biological balance of waters with respect to species fish farming and facilitated the development of free amoebas, which are pathogenic to bathers.

Chemical pollution is probably the most common, felt and diverse (organic compounds: Na and chloride, nitrates, phosphate, heavy metals, etc...) that are the cause of real intoxications human.

Finally, microbial and parasitic pollution of water is important. The main source is clearly fecal, due to human and animal excreta, through wastewater more or less well controlled at

technical and sanitary plans. In any case, the primary and secondary treatment of waste water

only partially affect their microbial load and the sludge is highly contaminated except after

Appropriate treatment. Microbial water pollution factors are enteropathogenic bacteria

(*Salmonella, Sigelles, E.coli, vibrions choleric*), viruses (enterovirus type poliovirus, echovirus, virus of hepatitis A, corona and rotavirus) responsible, as appropriate, for gastroenteritis, hepatitis or neuro syndromes meninages; these viruses are generally more persistent in the environment and more resistant to disinfection of bacteria. Parasites are also involved: they are very numerous in tropical countries, to involve a significant water stage in their development cycle, such as Entamoeba coli.

In developed, single-cell parasites such as Giardia lamblia and Cryptosporidium parvum are

pathogens, especially for immunocompromised subjects (weakened immune system); their cysts are also particularly resistant in the environment and to disinfectants.

5. Wastewater treatment

- The treatment plant treats collected wastewater and produces treated water and sewage residues (sludge) that are released into the natural environment. Sludge consists of water, organic matter and minerals
- The presence of harmful substances and pathogenic microorganisms requires water treatment before consumption. There is a difference between treatment to purify water before consumption and treatment to purify wastewater to return water of acceptable quality to the environment.

In theory, wastewater treatment consists of three consecutive treatments:

Primary treatment

Removal of insoluble particles by sieving, addition of alum and other coagulation agents and by other physical techniques

Secondary or biological processing,

Which aims to biodegrade organic matter through various processes, involving microorganisms;

- Bacterial beds
- Activated sludge
- Lagooning

- **Bacterial beds**

- The operation of a bacterial bed consists in making Spray the water to be treated on a specific surface Used as a carrier for microorganism scrubbers which form a film of varying thickness
- The biofilm contains heterotrophic bacteria generally near the surface and autotrophic (nitrifying) bacteria that can proliferate near the bottom. In the upper areas, there is a presence of fungi On the surface green algae Predatory fauna is generally abundant: protozoa, worms, insect larvae, arachnids, snails and slugs.

Figure 5: Operation of a bacterial bed

Sludge activated

- Activated sludge together of aerobic microorganisms maintained in suspension by agitation and/or aeration.
- These microorganisms develop in oxidizing organic matter present in the effluent from the oxygen of the air.

Figure 6: Operation of a Sludge activated

Lagooning

- Purification system with an area basin large and shallow.
- Aerobic and anaerobes are in suspension and/or deposited on the bottom of the work.
- A lagunage may have a aeration artificial stimulating biomass activity.

Figure 7: Lagooning

The third treatment

will ensure ultimate elimination of pollutants and disinfect water:

- Biological removal of inorganic nutrients,
- Chemical removal of inorganic nutrients,
- Elimination/inactivation of viruses,
- Elimination of trace chemicals...

Assessment of organic pollution

- Biochemical oxygen demand (BOD) is the quantity of oxygen expressed in mg/l

consumed under test conditions

(incubation at 20°C, and in the dark) during a time to provide biological the oxidation

of biodegradable organic matters in waste water.

- Dose BOD5 is usually used.
- Discharge standards must be less than 20-40 mg/l

Figure 8 : Measuring apparatus for BOD

- Chemical oxygen demand (COD) is anything that can be require oxygen to be oxidized: oxydable mineral salts and most organic compounds.
- The discharge standard should be less than 90-120 mg/l.

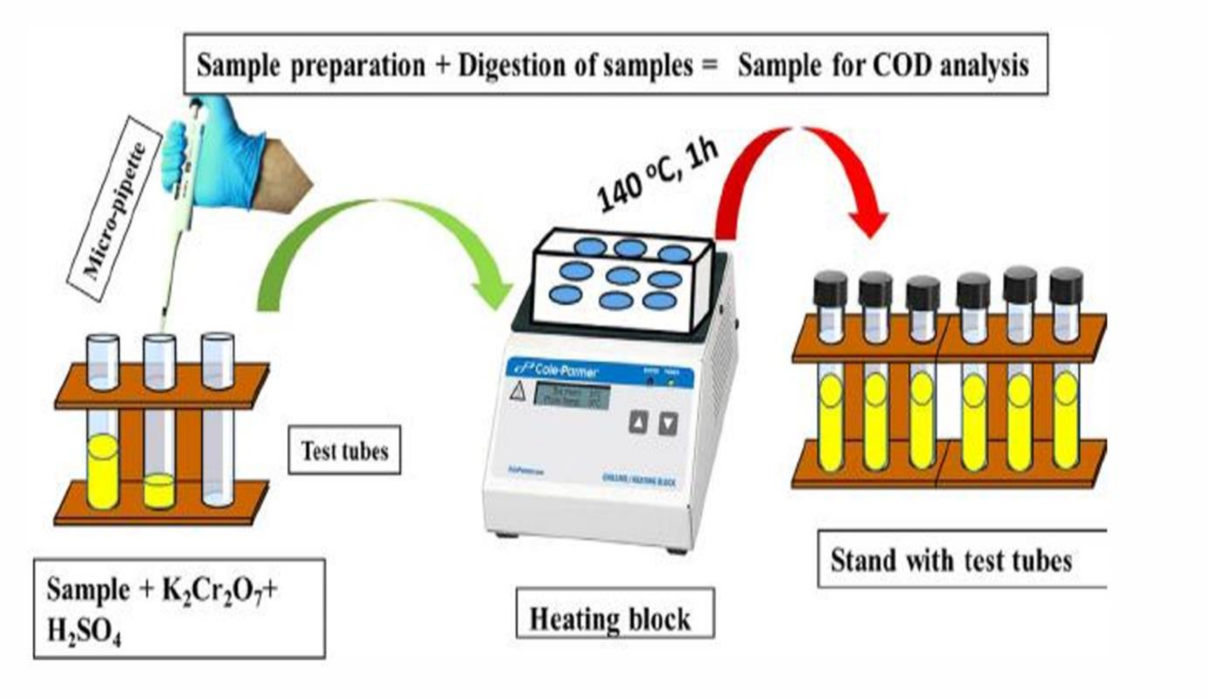


Figure 9 : Measurement method for COD

6. Wastewater treatment plant

- The wastewater treatment plant (WWTP) is an installation for treating domestic or industrial wastewater and rainwater before it is discharged into the natural environment. The objective of treatment is to separate water from undesirable substances in the receiving environment.
- It can use several physical, chemical and biological principles. In most cases, it is the biological process that is involved by the use of bacteria that can degrade organic matter. The treatment plant consists of a series of equipment designed to extract the different pollutants contained in water at different stages.
- Pollutants left in the treatment plant are transformed into sludge (Figure 6). The continuity of equipment is calculated according to the nature of the wastewater collected on the network and the type of pollution to be treated. The capacity of a

sewage treatment plant is measured in population equivalents (E.H). This unit represents the pollution load of the Wastewater generated per capita per day.

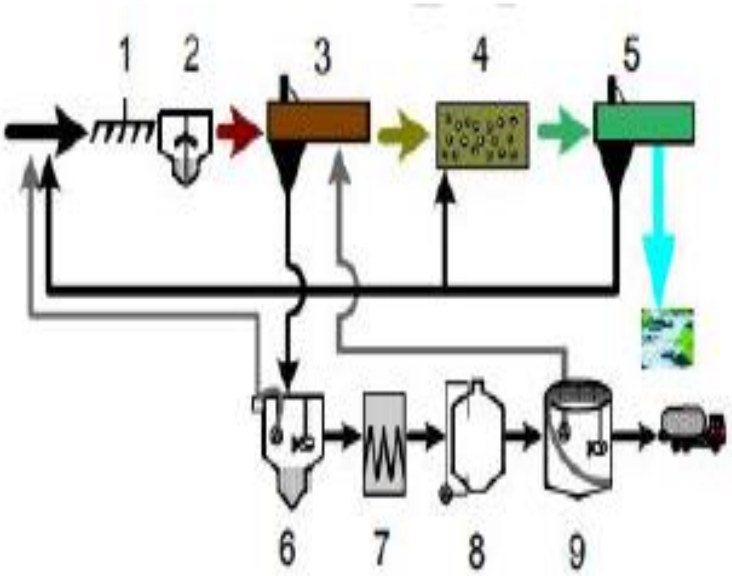


Figure 10 : Simplified diagram of WWTP (Plagellat, 2004)

6.1. Pretreatment

The siding dirty water pass through several grids that retain and remove the largest waste (cotton swab, pieces of paper, plastic, wood...). At the end of this operation, the waters are still grey.

Oil removal: floating oils and fats are recovered on the surface (in a

cylindrical-conical work).

Sand-blasting: heavier sands and gravel settle at the bottom of the same

work and then are sent to the landfill.

6.2. Primary treatment: settling of suspended solids in water.

- In some stations, water can rest for more than two hours in a large basin called the **primary decanter**.

- The water slowly clears its impurities; the fine particles in suspension settle at the bottom of the basin where they are scraped and discharged. This mass of matter forms sludge.
- At this stage, the water is less dirty but still polluted. They are directed to other basins where secondary or biological treatment is carried out.

6.3. Biological treatment

- Among the technologies used for wastewater treatment, the efficiency of biofilm reactors is well known. Then the water enters the aeration tank, also called bioreactor.
- Therefore, the principle of biological treatment is based on the ability of bacteria to degrade harmful organic compounds in water. Once these aerobic bacteria are stimulated by the oxygen flowing through the pond, they feed first on organic pollution and continue to purify the water. These reactors are generally used for water treatment to eliminate pollution from carbon and nitrogen, hydrocarbons, pesticides or medicinal compounds(Picard, 2011).

- Purified microorganisms are heterotrophic bacteria, which are introduced in free form in suspension (treated with activated sludge) or in a fixed form. Noting that the reactor must be fed in continuous or semi-continuous mode because microorganisms feed on organic matter, converting pollutants by adsorption or absorption on bacterial flocs, or by conversion into cytoplasm, or oxidation to CO2 and H2O.
- Biological purification can be performed in aerobic or anaerobic mode, but the aerobic pathway (faster and more complete process) is commonly used (Plagellat, 2004).

In the biofilm membrane reactor used in wastewater treatment plants, the membrane separates two phases, one of which is represented by water or biofilm formation.

This is the case with the use of methanotrophic bacteria, which can be degraded by the cometabolism of chlorinated aromatic hydrocarbons in the presence of oxygen and methane. (Picard, 2011) , as well as denitrifying heterotrophic bacteria (Figure 7), which reduce nitrite or nitrate to gaseous nitrogen.

The water is then transferred to the secondary settling basin. Sludge formed by removing bacterial contamination falls to the bottom of the sedimentation basin where it is concentrated. The sludge is then emptied and used as agricultural fertilizer, otherwise it will be incinerated. They can carry out treatments to dry them (pressing, spinning, etc.).

DIVERSITY OF MICROORGANISMS AND METABOLISM

Chapter 4: Diversity of microorganisms and metabolism

1. Diversity of microorganisms

It is now established that microorganisms dominate the biosphere. The latter, can thrive in extreme environments (cold, hot, salty,...etc). Thus, thanks to their various metabolisms, they maintain among other things the biogeochemical cycles of matter in the environment (carbon, oxygen, nitrogen, phosphorus, sulphur, etc.) and play a major role there.

They are found wherever there is life, whether in aquatic, terrestrial, underground or aerial environments.

In a given ecosystem, microorganisms are often the primary producers, constituting the first link in the food chain, or play the role of decomposers, responsible for the mineralization of organic matter.

In addition to the vital importance of microorganisms in the biosphere, they sometimes have a major impact on bioprospecting for applications in sectors such as food, agriculture, pharmacology, cosmetology and the environment.

1.1. Taxonomic diversity

The taxonomic diversity (diversity and distribution of taxa) of microbial communities is studied separately for the 3 major kingdoms: bacteria, fungi and archaea (Lemmel, 2019, Figure 8).

Microbial diversity is not only the diversity of the number of species that exist, but also the diversity of the characteristics of strains within a species, i.e., the diversity of subspecies (infra-specific). The latter is visible through various metabolic properties, especially when characterizing the genome itself: simple techniques allow to prove this diversity at a very fine scale; they are usually derived from PCR (Balandreau, 2000).

Therefore, the taxonomic diversity of bacteria and archaea is obtained by sequencing a part of the gene coding for 16S ribosomal RNA (16S rDNA), while fungal diversity can be obtained by the 18S rDNA or STI (internal transcribed spacer; that is, a part of the region between 18S)(Bertrand *et al., 2011).*

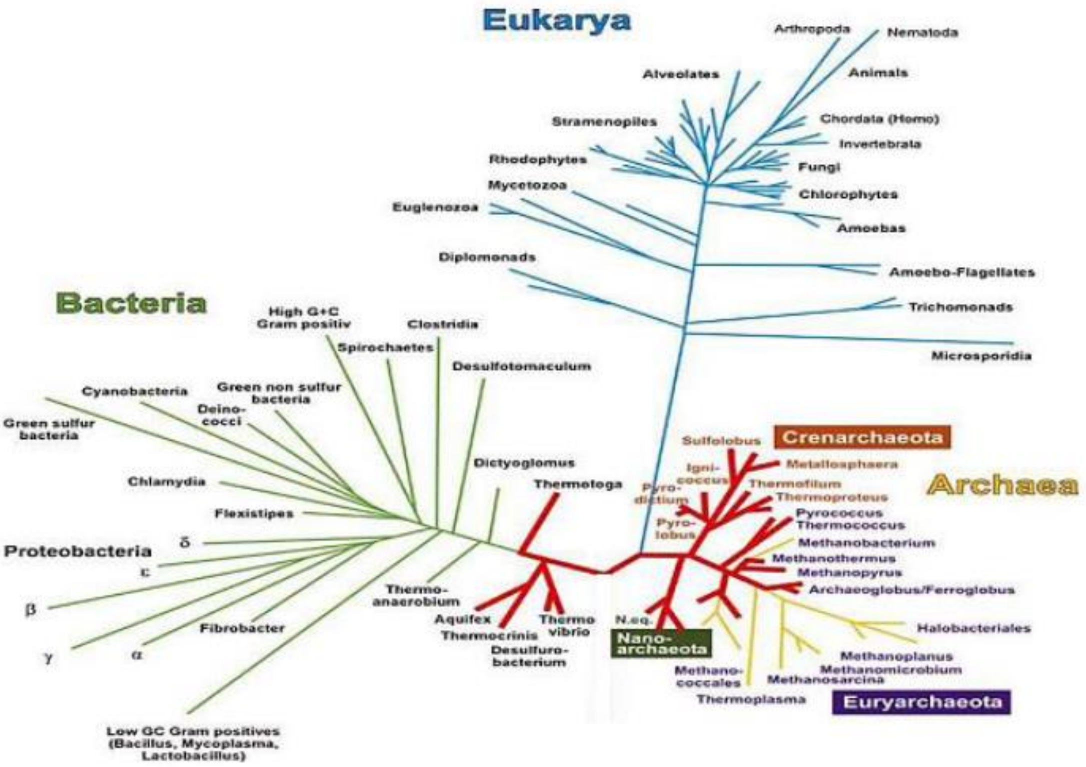


Figure 11 : phylogenic tree of life (Stetter, 2006)

1.2. Functional diversity

The key role of microbial communities in terrestrial ecosystem functions is ensured by their taxonomic diversity, but most importantly their functional diversity.

Traditionally, functional diversity is defined as the diversity of traits, which are the morphological, phenotypic, biochemical, physiological, structural, genomic or behavioural characteristics of organisms (Violle *et al., 2007).*

There are different methods for studying functional diversity, which can be divided into "molecular" (DNA, RNA and protein based) and "microbial activity measurement" methods.

Molecular methods are summarized by the use of metagenomic, metatranscriptomic or metaproteomics, which allows the study of diversity and abundance of genes, transcripts or enzymes that reflect the existence of different functions. The example of real-time quantitative PCR (Figure 9) or microarray will allow to quantify genes or their expression and deduce the presence of functions.

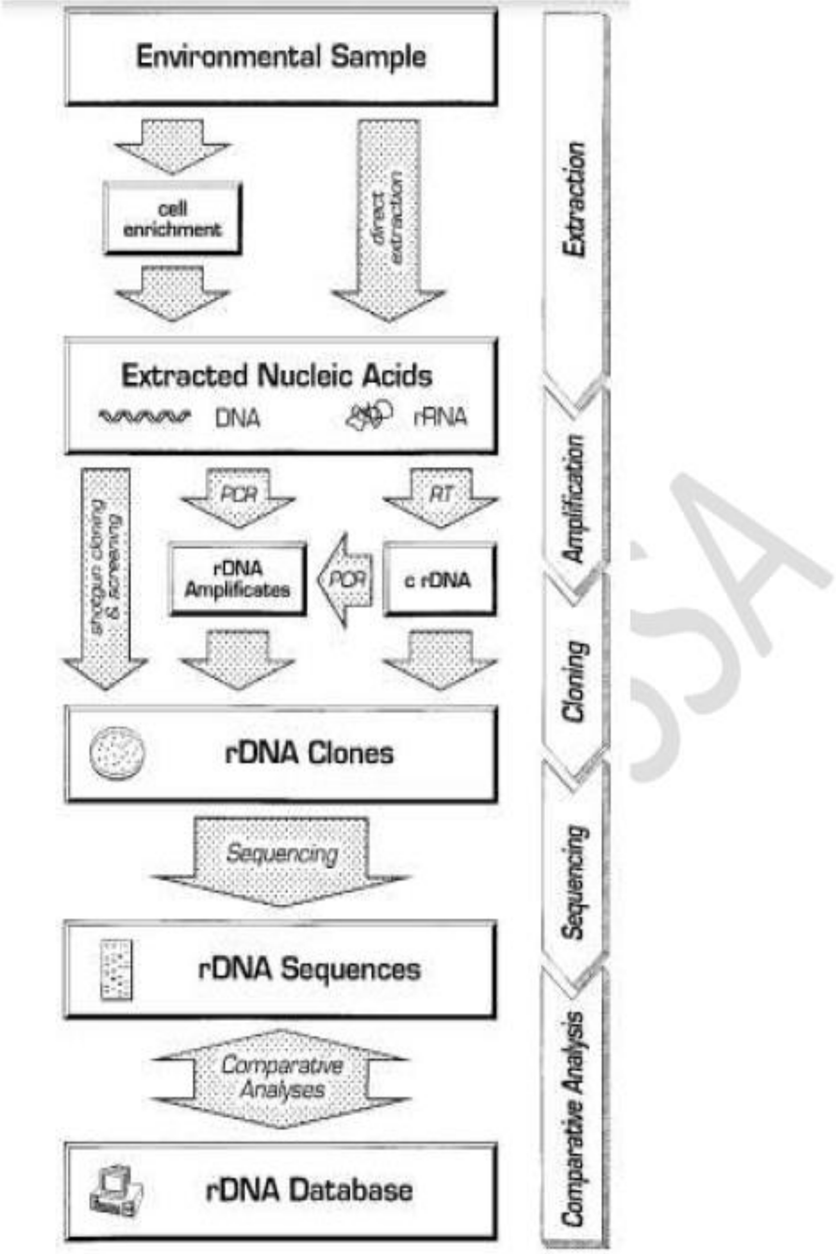
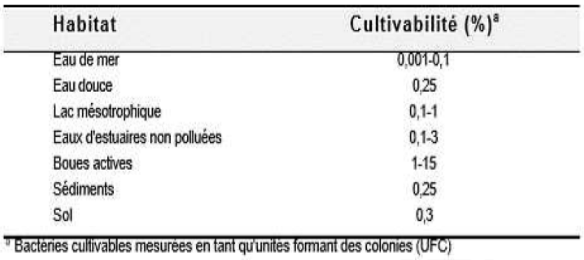


Figure 12 : Differents possibilIties to caracterize a environmental sample (Amann et al., 1995)

1.3.Non-culturability of environmental micro-organisms: an obstacle to the study of microbial diversity

It is almost difficult to grow most microorganisms in the natural environment (Table 3), especially bacteria (Balandreau, 2000).

Table 3 : Pourcentage of cultivable bacteria (Amann et al., 1995)



Habitat	Cultivabilité (%)[a]
Eau de mer	0,001-0,1
Eau douce	0,25
Lac mésotrophique	0,1-1
Eaux d'estuaires non polluées	0,1-3
Boues actives	1-15
Sédiments	0,25
Sol	0,3

[a] Bactéries cultivables mesurées en tant qu'unités formant des colonies (UFC)

This very small percentage of cultivability (table above) can be explained by the following points:

The chosen growth medium cannot be universal, and not all environmental conditions can be reproduced on the same medium, especially for environments with strong physical and chemical gradients. Some cells outside the environment can enter a viable but non-cultivable state. This cellular state corresponds to the adaptation to stress conditions, especially nutritional.

It is accompanied by physiological and structural changes and cannot simply be restored on synthetic supports.

This condition can last from a few days to several years.

Poor people can replace most of the populations that are not well adapted to the growing environment.

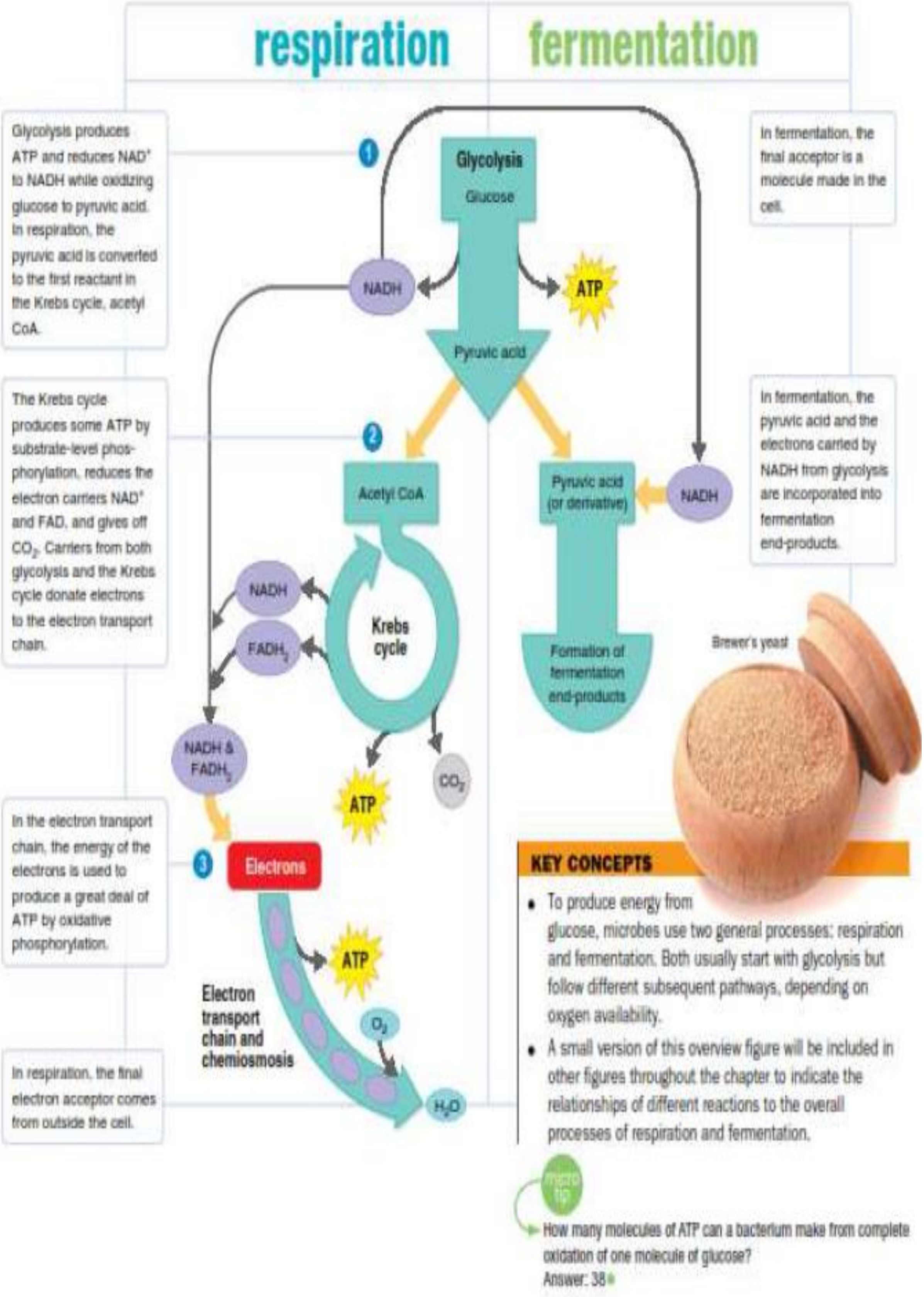
Liquid-rich populations may not grow on solid medium.

The amount of inoculum chosen for enrichment influences the type of cells enriched. Different micro-organisms with similar growth conditions will be difficult to distinguish.

2. Metabolic Diversity

- Metabolism is a set of biochemical reactions that occur in cells.

- It is a process involving processes of degradation (catabolism) and organic synthesis (anabolism).
- Bacteria with a fermentative metabolism can be contrasted with those with a respiratory-type metabolism (Figure 10).
- 1. For bacteria with a fermentative metabolism, the breakdown of glucose is incomplete and results in the formation of various organic compounds (organic acids).
- 2. For bacteria with a respiratory-type metabolism, degradation occurs via the Krebs cycle. The final electron acceptor is oxygen.
- In bacteria, the electron transport system is located in the cytoplasmic membrane.



Glycolysis produces ATP and reduces NAD^+ to NADH while oxidizing glucose to pyruvic acid. In respiration, the pyruvic acid is converted to the first reactant in the Krebs cycle, acetyl CoA.

The Krebs cycle produces some ATP by substrate-level phosphorylation, reduces the electron carriers NAD^+ and FAD, and gives off CO_2. Carriers from both glycolysis and the Krebs cycle donate electrons to the electron transport chain.

In the electron transport chain, the energy of the electrons is used to produce a great deal of ATP by oxidative phosphorylation.

In respiration, the final electron acceptor comes from outside the cell.

In fermentation, the final acceptor is a molecule made in the cell.

In fermentation, the pyruvic acid and the electrons carried by NADH from glycolysis are incorporated into fermentation end-products.

KEY CONCEPTS

- To produce energy from glucose, microbes use two general processes: respiration and fermentation. Both usually start with glycolysis but follow different subsequent pathways, depending on oxygen availability.
- A small version of this overview figure will be included in other figures throughout the chapter to indicate the relationships of different reactions to the overall processes of respiration and fermentation.

How many molecules of ATP can a bacterium make from complete oxidation of one molecule of glucose?
Answer: 38

Figure 13 : Steps of fermentation and respiration

3. Trophic types

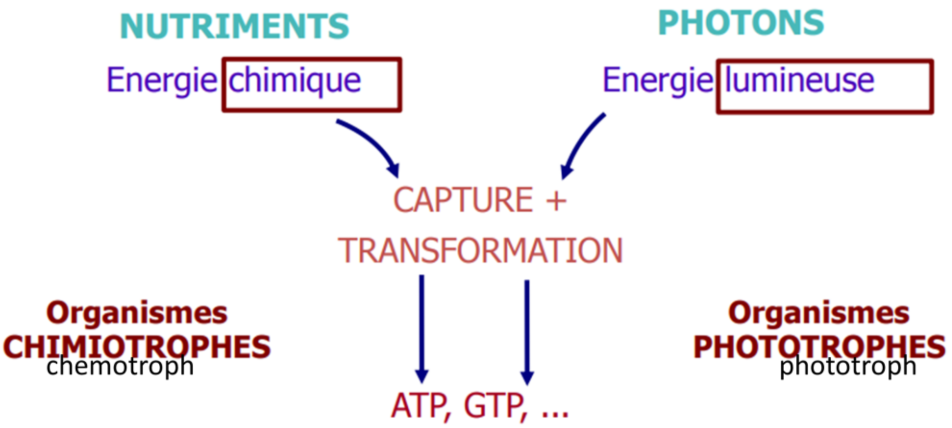
According to the basic and energetic needs of bacteria, different physiological types called trophic types are distinguished the trophic types have a primary interest in bacterial classification

Criteria used to define trophic types

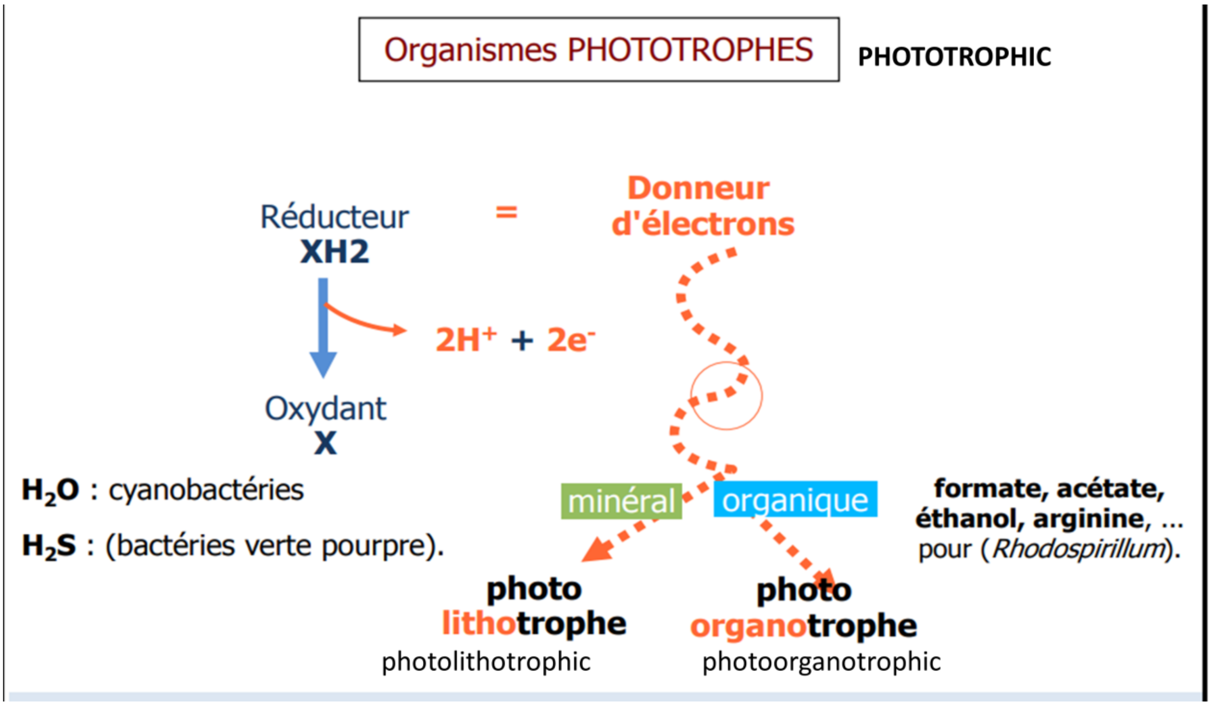
- ✓ Energy source
- ✓ Nature of the electron donor
- ✓ Carbon source
- ✓ Growth factor requirements

CLASSIFICATION BY ENERGY SOURCE

- in the prokaryotes the energy used can be light energy (photons) or chemical energy (energy contained in the covalent bonds between atoms)



CLASSIFICATION BY ENERGY SOURCE AND ELECTRON DONORS



Organismes PHOTOTROPHES

PHOTOTROPHIC

Réducteur **XH2** = **Donneur d'électrons**

$2H^+ + 2e^-$

Oxydant **X**

H_2O : cyanobactéries

H_2S : (bactéries verte pourpre).

minéral

organique

formate, acétate, éthanol, arginine, … pour (*Rhodospirillum*).

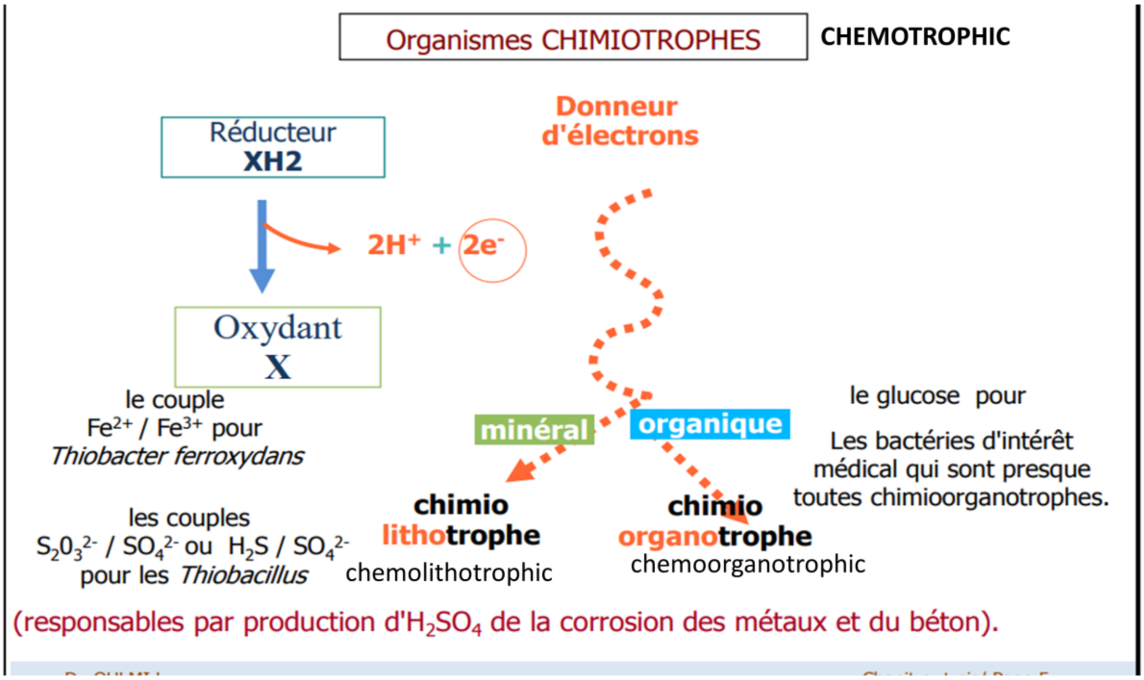
photo lithotrophe

photolithotrophic

photo organotrophe

photoorganotrophic

CLASSIFICATION BY ENERGY SOURCE AND ELECTRON DONORS



Organismes CHIMIOTROPHES

CHEMOTROPHIC

Réducteur **XH2**

Donneur d'électrons

$2H^+ + 2e^-$

Oxydant X

le couple Fe^{2+} / Fe^{3+} pour *Thiobacter ferroxydans*

les couples $S_2O_3^{2-}$ / SO_4^{2-} ou H_2S / SO_4^{2-} pour les *Thiobacillus*

minéral

organique

le glucose pour

Les bactéries d'intérêt médical qui sont presque toutes chimioorganotrophes.

chimio lithotrophe

chemolithotrophic

chimio organotrophe

chemoorganotrophic

(responsables par production d'H_2SO_4 de la corrosion des métaux et du béton).

CLASSIFICATION BY CARBON SOURCE

Depending on the organic (molecule produced only by a living being) or inorganic (carbon dioxide or hydrogen carbonate ions) nature of the carbon source taken from the environment by the bacteria:

CLASSIFICATION BY GROWTH FACTOR REQUIREMENTS

- **Protrophic strain**

able to synthesize the organic compounds essential for its growth .

- **Auxotrophic strain**

unable to synthesize one or more nutrients essential for its growth. bacteria require the presence of these compounds in their environment (amino acids, fatty acids, vitamins and nitrogenous bases)

PLACE OF MICROORGANISMS IN THE LARGE BIOGEOCHEMICAL CYCLES

Chapter 5: Place of micro-organisms in the large biogeochemical cycles

- The structure of biological elements or biological matter pays particular attention to the interaction between the geosphere, atmosphere and biosphere.
- There are major elements: C, H, O, N, S, P, K, Mg and Ca, and trace elements: Fe, Mn, Cu, Co, Ni, V, W, Zn, etc.
-

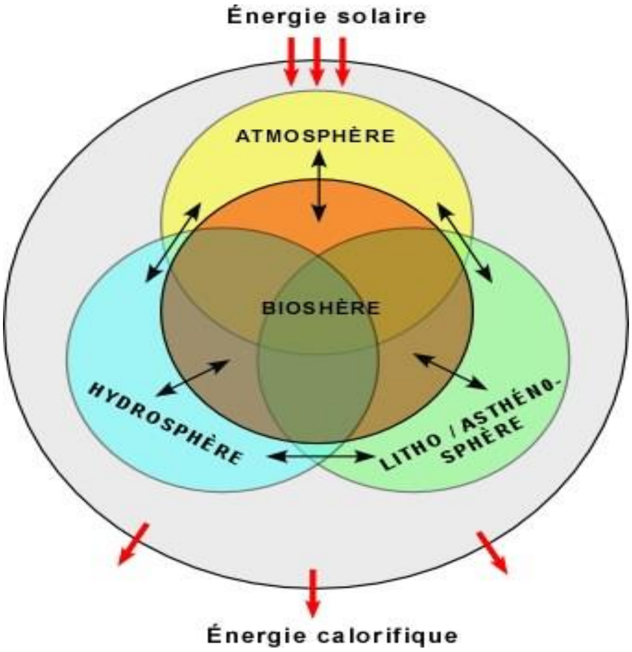


Figure 14 : Structure of biological elements or biological matter

1. Carbon cycle

1.1. Forms of carbon

- In terms of abundance, carbon is considered the fourth chemical element after hydrogen, helium and oxygen.
- Due to volcanic activity, it exists mainly in the mantle as carbonate sedimentary rocks, mainly formed of carbonate and calcium (calcite), and a small amount of mixed carbonate (dolomite) of calcium and magnesium (Gobat et al., 2010).

- the basic element of living matter, and it has two forms:
- **Organic matter: all the molecules that make up living organisms.**
- Inorganic: CO_2 or $CaCO_2$ in the atmosphere, most of the CO_2 and CH_4 produced by respiration and fermentation.
- Photosynthesis is the main input stream of C (CO_2 reservoir in the atmosphere)
- and respiration is the reflux mechanism, so these two chemical reactions are the basis for the recycling of C.
- On the other hand, the purpose of fermentation is "to use non-atmospheric carbon".
- It should also be noted that the industrial carbon flow generated by fossil energy use is much higher than the natural flow.

1.2. Stages of cycle

- The carbon cycle consists of three stages: fixation, mineralization and retention
- 1. Binding is a synthetic step that combines carbon in carbon dioxide into organic molecules.
- 2. In contrast, in the fixation process, mineralization is the stage where the carbon contained in the organic compound returns to the environment as a mineral.
- 3. The new cycle starts again, except that some of the carbon released goes through the retention stage. Carbon is trapped in insoluble compounds and is difficult to degrade or temporarily difficult to degrade.

1.4. Role of microorganisms

- Biological processes are the whole set of microbial reactions, leading to degradation of PAHs and production of secondary metabolites, or even complete mineralization of PAHs into mineral carbon (CO2) (Cerniglia 1992).
- These processes mainly determine the fate of polycyclic aromatic hydrocarbons in soil.
- Degradation can be achieved according to two types of metabolism:
- i) microbial metabolism (or microbial assimilation), using PAHs as a source of carbon or energy to synthesize biomass.
- ii) Co-metabolism, which corresponds to the degradation of PAHs without nutritional and energetic benefits for related organisms but can detoxify the culture medium (Lemmel, 2019).

- Under hypoxic (anoxic) conditions, methane is the bulk of the carbon used by methanogenic bacteria. It will be mineralized as methane, but the effect of methanisation is different. These bacteria effectively promote the spread of methane in the atmosphere, thereby reducing the greenhouse effect.
- According to the carbon assimilation, two groups of microorganisms are considered:
- - Aerobic: decompose organic matter by releasing CO2 (respiration)
- - Anaerobic: decompose organic matter produce CO2 and CH4

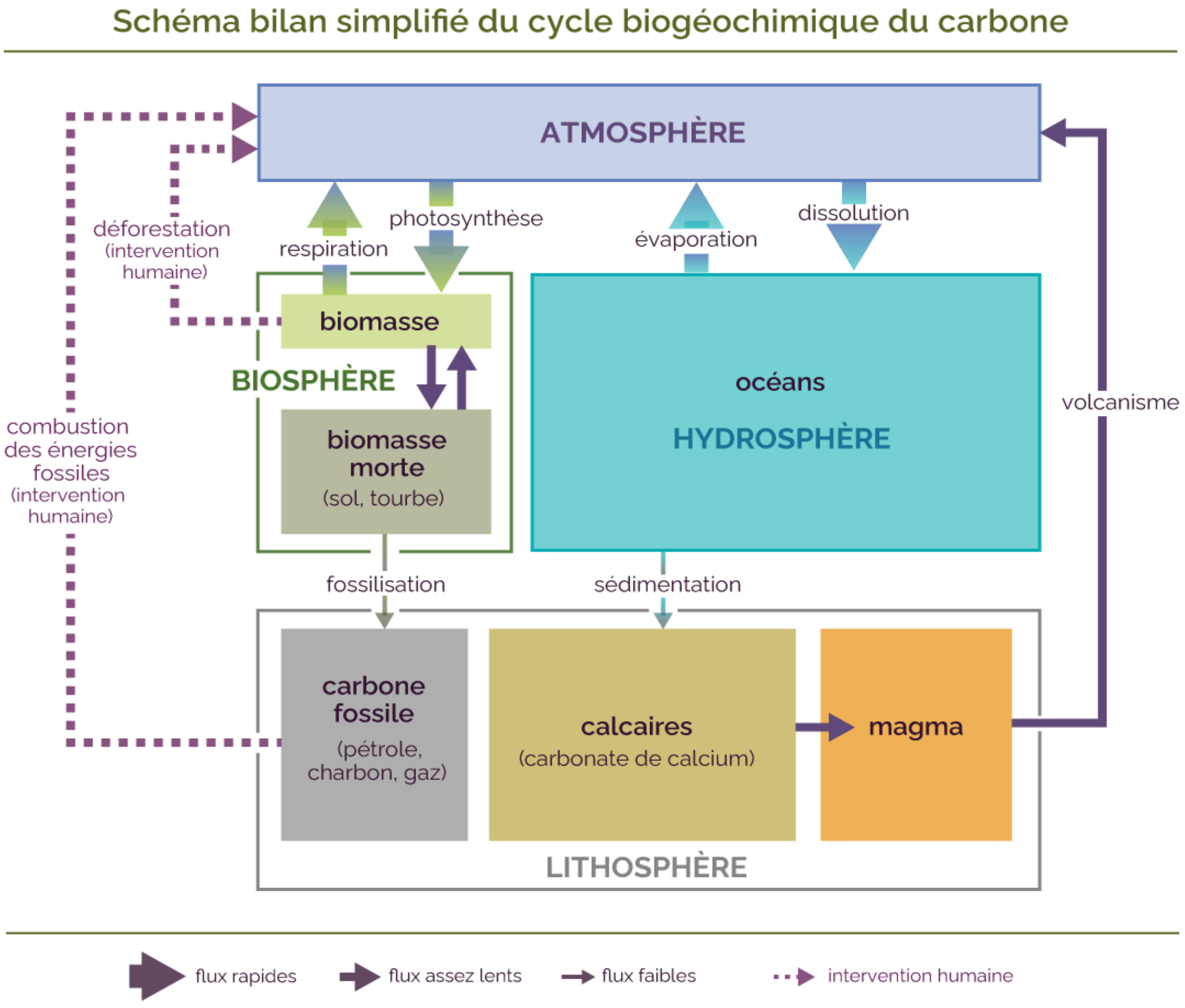


Figure 15 : Biogeochemical cycles

2. Oxygen Cycle

- In the biosphere, most organisms use molecular oxygen as a final electron acceptor during respiration and they are produced in parallel by photosynthesis. In soil, aerobic redox reactions are highly dependent on the presence of oxygen.

2.1.Forms

- In the environment, oxygen exchanges directly with the biosphere and is distributed into five reservoirs (molecular O2, water O2, carbon dioxide O2, minerals O2, carbonates and biomass O2)

2.2.Steps

- Oxygen is highly involved in a wide variety of biological and redox reactions, however, two major metabolites control the flows between reservoirs; namely:
- Oxygen photosynthesis: this type of metabolism is characteristic in the majority part by plants but not only, photosynthetic bacteria (ex: Chlorobium) and cyanobacteria (ex: Pleurocapsa) (phototrophic), hydrogen produced will be used to fix CO2 (autotropy)
- We can therefore see from these two reactions that the oxygen released by phototrophy comes from water while that of CO2 is found in half in biomass and water equally
- Aerobic organotrophic respiration: practically all eukaryotes and a significant fraction of prokaryotes perform this breathing. It includes the inverse reactions of oxygen photosynthesis which maintains a certain stability of the concentration of oxygen in the atmosphere: (Degradation of organic substrates or catabolism)

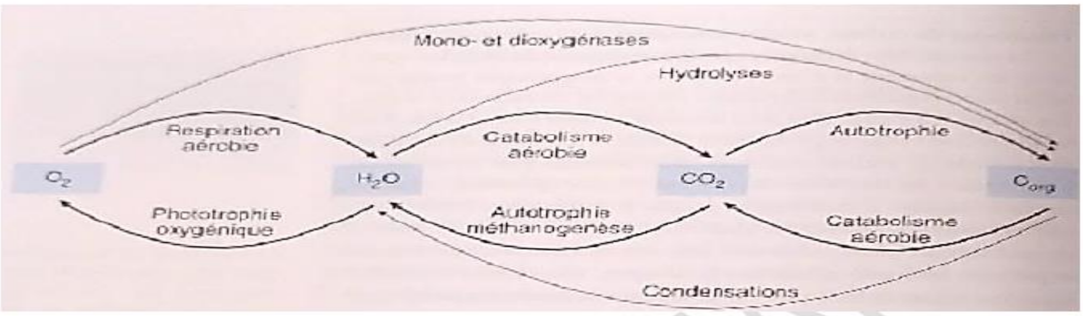


Figure 16 : Oxygen cycle

3. Nitrogen cycle

3.1.Forms

- On the other hand, N represents the 3rd biomass element after C and O in living beings, while organic N is derived mainly from dead biomass.
- Inorganic N is represented by the ions: NH4+, NO2-, NO3-, of which a significant fraction is in the hydrosphere because it is very soluble.

- N ions depend on the presence of oxygen, for example:
- - In an oxidic environment (presence of oxygen), nitrites are transformed into nitrates
- - In an inoxic environment (absence of oxygen), nitrites are transformed into ammonium

3.3.Cycle steps

- The nitrogen cycle is based essentially on:
- **Fixation:** transformation of air's molecular nitrogen into ammonia and ammonium ions. This process can be biological or industrial.
- - Biological fixation in the lithosphere can be done thanks to symbiotic bacteria with legume roots (ex: Rhizobium) while in the aquatic environment it is cyanobacteria that take over(ex : *Trichodesmium)*

☐ Industrial fixation is mainly represented by the manufacture of nitrogen-based products such as chemical fertilizers

☐ Nitrification: transformation of ammonium into nitrites and then into nitrates these first two steps are carried out only by a few rare bacterial species.

- **Nitrates produced may:**
- **Denitrification and return to the atmosphere: transformation of nitrates into molecular nitrogen**
- **Be assimilated and used for the synthesis of nitrogen organic compounds; Assimilation/Mineralization: incorporation of nitrate nitrogen into amino acids and other nitrogen organic compounds**

☐ Be decomposed during ammonification: transformation of organic nitrogen into ammonia nitrogen by heterotrophic microorganisms and denitrification before starting a new cycle.

☐ Nitro-ammonification: transformation of nitrites into ammonium

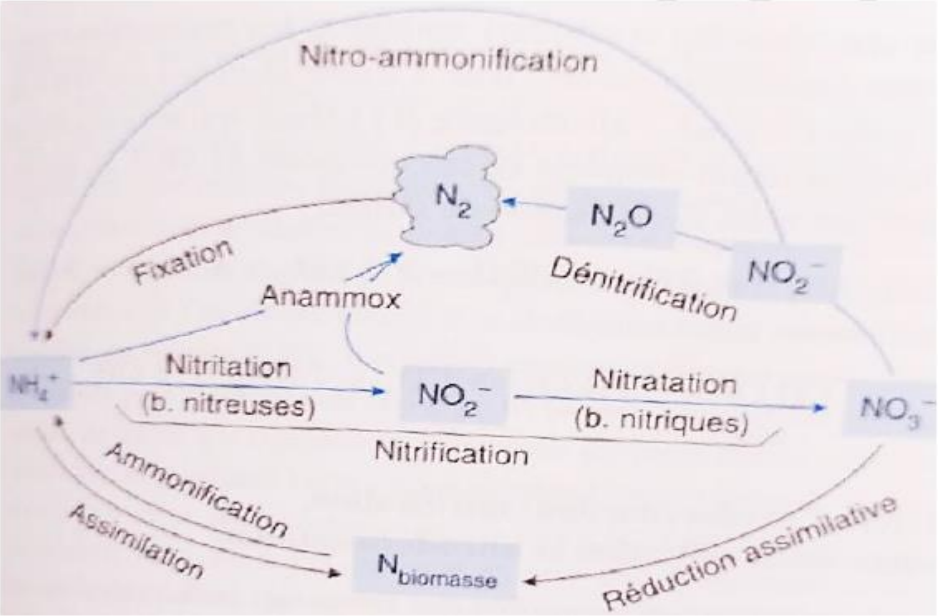


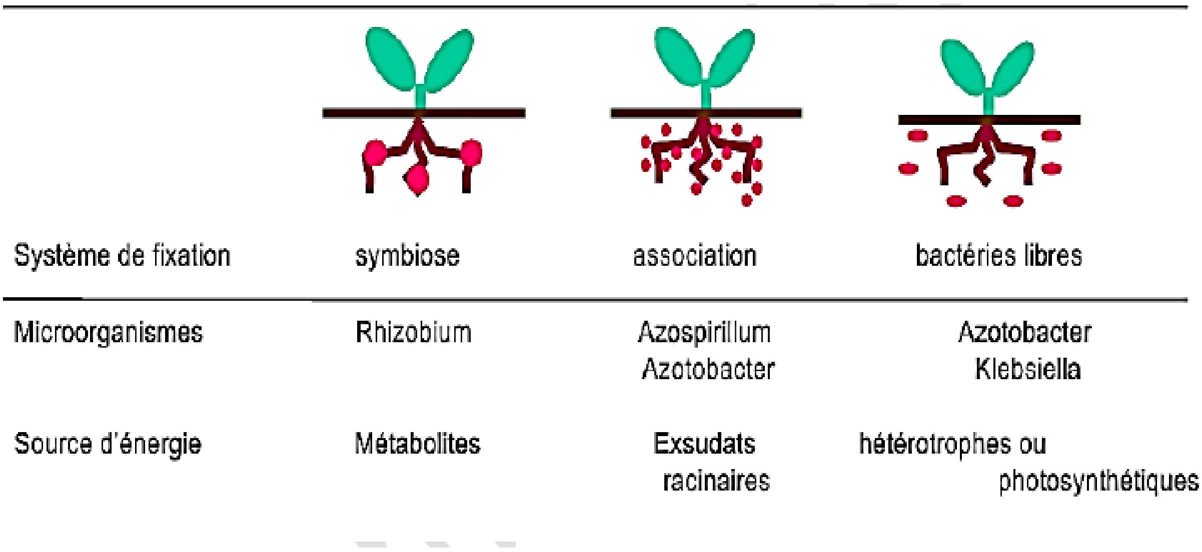
Figure 17 : Nitrogen cycle

- The nitrogen reduction oxidation cycle includes the following reactions:
- Nitrification (oxidation): NH3+ to NO3-, this reaction includes two steps:
- Nitritation (NH3+ to NO2-) ex: Nitrosomonas, Nitrosococcus, Nitrosospira
- Nitratation (NO2-in NO3-) ex: Nitrobacter, Nitrococcus, Nitrospira
- Nitro-ammonification (reduction): NO2-to NH3+

3.5. Nitrogen fixing microorganisms

- There is a large amount of nitrogen in the atmosphere as minerals.
- However, the ability to bind atmospheric nitrogen by the enzyme complex nitrogenase is limited to a few bacteria.
- Legumes can interact symbiotically with rhizobacteria called Rhizobia.. *This interaction involves two processes strictly controlled by plants: bacterial infection and the formation of new organs: nodules, in which atmospheric nitrogen is reduced.*

- *Interestingly, only differentiated bacteria (bacteroids) can fix nitrogen. In free culture, bacteria do not express nitrogenase. Of all the systems linking atmospheric N2, the symbiotic relationship is the most important.*



Système de fixation	symbiose	association	bactéries libres
Microorganismes	Rhizobium	Azospirillum Azotobacter	Azotobacter Klebsiella
Source d'énergie	Métabolites	Exsudats racinaires	hétérotrophes ou photosynthétiques

4. Sulphur cycle

- Sulphur is considered a constituent of living matter and an redox element, as are oxygen, nitrogen and iron (Gobat et al., 2010).

4.1.Forms

- At the level of the biosphere, sulphur exchange is mainly due to two
- mineral deposits:
- 1. Metal sulphide such as: pyrite (FeS_2) which will subsequently undergo oxidation
- 2. Evaporate sulphate such as: gypsum ($CaSO_4$) which will subsequently be reduced
- In biology, sulphur exists in two amino acids: methionine and cysteine, as well as vitamin B (thiamine).
- At soil level, microorganisms transform sulphur into various soluble and gaseous forms, the most important being SO4
- 2: dominant sulphate form in well-drained soils. This is the form available for plants (sulphate ions). It is also easily washable like nitrate.

4.2. cycle steps

- Four main stages summarize the sulphur cycle:
- 1. Sulphate-reduction: this step requires a mineral source of oxidised sulphur to be reduced to hydrogen sulphide (H_2S), pyrrhotin (FeS) or pyrrite (FeS_2)
- 2. Sulfooxidation: this is the reverse step of the previous one that will reproduce the sulphate (SO_4)
- 3. Gypsum precipitation: sulphur is not involved in a redox reaction, it is a dissolution of the sulphate from gypsum ($CaSO_4$).
- 4. Assimilative reduction: plant assimilates sulfate for nutrition and the microbial world uses it as final electron acceptor in respiratory process

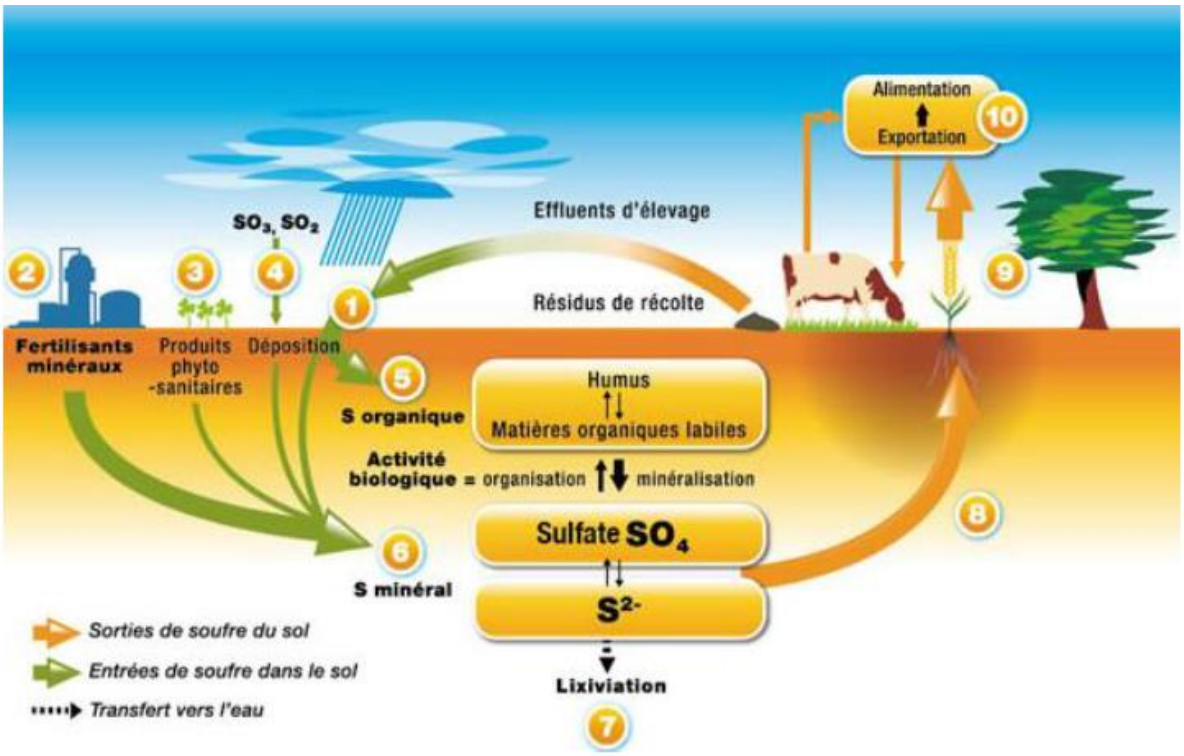


Figure 18 : Sulfur cycle

4.3.Sulphate-reducing bacteria (SRB)

- Sulphur is the electron acceptor of sulfate-reducing bacteria through the redox cycle (from SO_4 to SO_2), such as Desulfovibrio. Note that unlike denitrifying bacteria, BSR is not necessarily anaerobic.
- Sulphate reducing bacteria perform a specific respiration process, leading to the accumulation of a large amount of hydrogen sulfide (Gall, 1975)

5. Phosphorus cycle

- Phosphorus is a minor element of the earth's crust but a major component of biomass, it participates in the composition of multiple components such as nucleic acids, cofactors (NADPH) and lipids.
- Phosphorus is the most important nutrient for plants after nitrogen. It is an important part of all major metabolic processes in plants, such as energy transfer, photosynthesis, signal transduction and respiration (Khan et al., 2010).

6.1.Forms of the phopshore

- Inorganic phosphorus is found in soil, mainly in insoluble mineral complexes such as tricalcium phosphate ($Ca_3(PO_4)_2$), iron phosphate ($FePO_4$) and aluminum phosphate ($AlPO_4$) (Khan et al., 2014) that may appear after repeated application of chemical fertilizers.
- Plants do not have the capacity to absorb these insoluble forms, moreover, only 0.1% of total phosphorus is in soluble form, so it can be used for plant nutrition.
- Therefore, in most agricultural soils, the effective level of phosphorus must be supplemented with chemical phosphate fertilizers. Frequent and reckless application of chemical phosphate fertilizers can interfere with microbial populations, leading to reduced soil fertility, thus reducing crop yields (Gyaneshwar et al., 2002).

6.2. cycle steps

- Phosphorus availability depends on weathering of phosphate rock (Figure 21), so the phosphorous cycle is considered a "slow" cycle (Gobat et al., 2010).

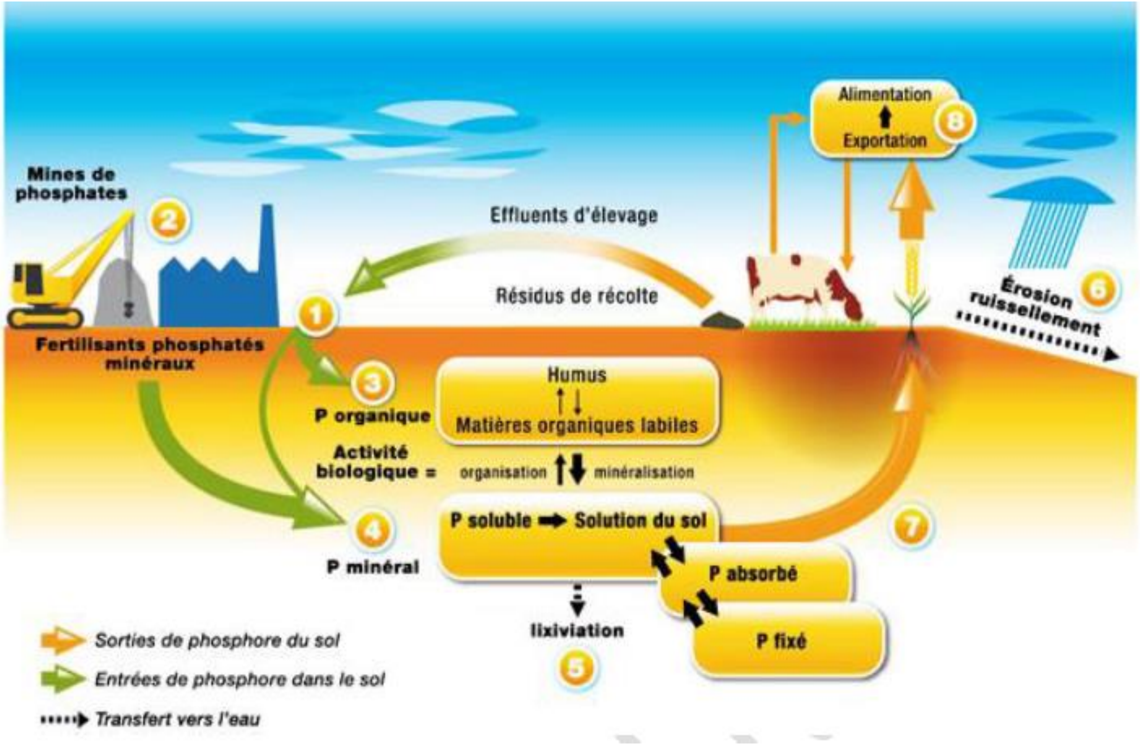


Figure 19 : Phosphurus cycle

6.3. Phyto-availability of phosphorus

- Only 1% of the total phosphorus is supplied to plants in the form of orthophosphate ions (HPO4-, H2PO4-).
- Its form depends on the pH of the medium, in acidic pH: it is associated with iron, aluminum and manganese, and in alkaline pH: calcium salt.
-

6.3.1. Phosphate solubilising bacteria (PSB)

- The ability of some bacteria to convert insoluble phosphate into soluble phosphate (bioavailable for plant) is the best biological treatment method and an environmentally friendly alternative to chemical phosphate fertilizers, Which can improve soil fertility and promote plant growth through solubilization and mineralization processes (Behera et al., 2017).

- Many research efforts have focused on phosphate solubilising bacteria (PSB), which colonize the rhizosphere of many plant species and have proven beneficial effects on plant growth, yield and productivity, and its role in reducing industrial or chemical use (Pande et al., 2017).
- These BSPs can promote plant growth through various mechanisms, such as the release of H+ ions, organic acid production and chelating substances. Therefore, microorganisms can produce low molecular weight organic acids as the main mechanism for phosphate solubilization (Khan et al., 2014; Pande et al., 2017).
-

6.3.2. Mycorrhiza

- Terrestrial vascular plants can establish symbiotic relationships with many microorganisms. In terms of roots, plants can combine with mycorrhizal fungi to form mycorrhizas.
- The main types of mycorrhizal association are: endomicorhize and ectomycorhize
- Mycorrhizal symbiotic enzymes significantly promote plant nutrition, especially the absorption of phosphorus.

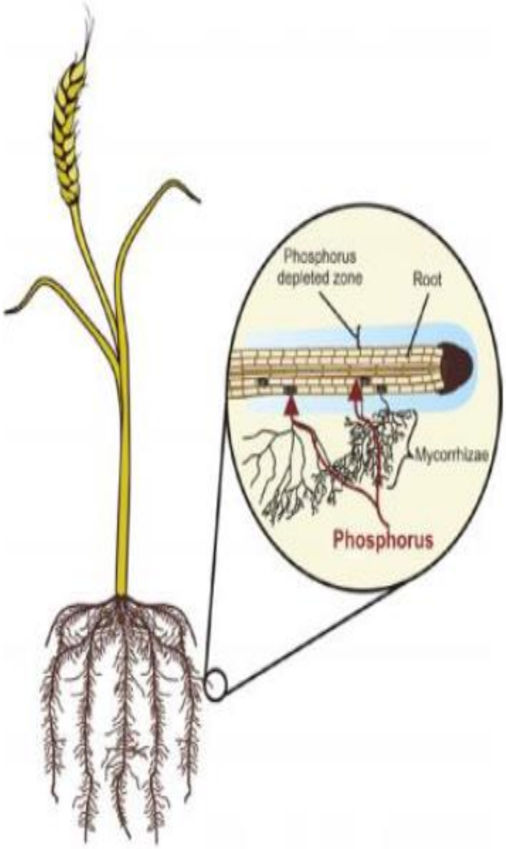


Figure 20 : Formation of mycorhizes

CULTURE AND GROWTH OF MICROORGANISMS

Chapter 6: Culture and growth of micro-organisms

1. Bacterial division

Bacteria reproduce by binary fission: the bacteria grow and then divide into two daughter cells, separated by a partition formed by the cell wall (Figure 25). During the process of division, DNA replicates with other components. Various enzymatic synthesis and degradation systems are involved in cell division.

2. Bacterial growth

Bacterial growth is the orderly growth of all components of bacteria. This results in an increase in the number of bacteria. During the growth process, on the one hand, the nutrients in the environment are depleted, on the other hand, metabolic by-products become toxic for multiplication.

3. Growth curve

- There are five phases, all of which form the growth curve (Figure 26):
- 3.1. Latency phase: the growth rate is zero, which corresponds to the time required for the bacterium to synthesize the enzymes adapted to catalyze the substrates present in its new environment.
- 3.2. Acceleration phase: the growth rate is accelerated.
- 3.3. Exponential phase: the growth rate reaches a maximum and remains constant, this corresponds to a shorter doubling time of the bacteria and the mortality rate is zero.
- 3.4. Stationary phase: the growth rate is equal to the mortality rate. Bacteria synthesize deficiency proteins that make the cell more resistant to toxicity from metabolic by-products;
- 3.5. Phase of decline: growth rate is negative. All nutrients in the medium are depleted (depletion of the culture medium). Therefore, there is accumulation of toxic metabolites. There is a beginning of autolysis of bacteria (cryptic growth), under the action of endogenous proteolytic enzymes.

4. Conditions for growth

4.1. Energy sources

- Bacteria must find in their environment the substances necessary for their energy and cell synthesis. Phototrophic bacteria use light as a source of energy for photosynthesis, from an organic electron source (photoorganotrophic bacterium) or mineral electron source (photolithotrophic bacterium) The chemiotroph bacteria draws its energy from mineral (chemioolithotroph) or organic (chemioorganotrophic) compounds.

4.2. carbon sources

Carbon is one of the most abundant elements in bacteria because it has the constituent character. The simplest carbon dioxide is carbon dioxide (CO2), if it is the only source of C for the bacteria, it is called autotrophic. On the contrary, bacteria called «heterotrophic» use CO2 optionally but can degrade a large amount of hydrocarbon substances (alcohol, acetic acid, lactic acid, polysaccharides, various sugars).

4.3. Sources of nitrogen and sulphur

Bacteria need nitrogen substances to synthesize proteins and nucleic acids. Some bacteria can directly bind molecular nitrogen from the atmosphere, they are called “nitrogen-fixing bacteria”, which play a very important role in soil biofertiliser and plant nutrition. On the other hand, bacteria can incorporate nitrogen compounds by de-amination and transamination reactions. Sulphur is incorporated by bacteria as sulphate or organic sulphur compounds.

4.4. others

Other elements play a role in bacterial metabolism (sodium, potassium, magnesium, chlorine) and in enzymatic reactions (phosphorus, calcium, iron, magnesium, manganese, nickel, selenium, copper, cobalt, vitamins).

5. Environmental factors

Influencing growth The environment of microorganisms can influence their development and proliferation through various factors

5.1.Oxygen

- Depending on their relationship with oxygen, there are several types of bacteria. Strictly aerobic (AS) bacteria can only thrive in the presence of air. Their main source of energy is aerobic respiration. The final molecular oxygen (electron acceptor) is reduced to water (Pseudomonas, Acinetobacter, Neisseria).
- When the partial pressure of oxygen is lower than the partial pressure of air, microaerophilic bacteria (Campylobacter spp., Mycobacterium) multiply better or only this one.
- Optional aero-anaerobic (NAAF) can thrive independently of air.
- Strictly anaerobic (ANS) bacteria can only thrive in the absence of oxygen, which is usually toxic. They have a fermentation metabolism or carry out a respiration whose final acceptor is not oxygen, it is anaerobic breathing.

5.2.Temperature

- Bacteria can be classified according to their optimal growth temperature into four types:
- - Mesophilic bacteria: growth temperature varies between 20 and 40°C, ex: Escherichia coli
- - Thermophilic bacteria: growth temperature varies between 42°C and 70°C, ex: Thermus aquaticus
- - Hyper-thermophilic bacteria: growth temperature is above 80°C, ex: Thermococcus
- - Psychrophilic bacteria: growth temperatures vary between 0 and 10°C, ex: Listeria monocytogenes
- - Psychrotrophic bacteria: growth temperature is close to 0°C with growth optimum close to mesophilic bacteria

5.3.pH

- Depending on whether the pH is neutral, basic or acidic, three classes of bacteria are distinguished:
- - Neutrophil bacteria that grow at pH between 5.5 and 8.5 with an optimum close to 7.
- - Alkalophilous bacteria prefer alkaline or basic pH, ex: Pseudomonas, Vibrio.
- - Acidophilic bacteria that multiply optimally in acidic environments, e.g.: Lactobacillus.

5.4.Osmotic pressure and free water

- Water plays a major role in solubilization and transport of nutrients, it is the seat of metabolic reactions. The activity of water (aw) is inversely proportional to the osmotic pressure of a compound. Thus, it is affected by the presence of more or less salts or sugars dissolved in water.
- - Halophilic bacteria require salt (NaCl) for their growth. This concentration can vary from 1-6% for weakly halophilic up to 15-30% for extreme halophilic bacteria (ex: Halobacterium).
- - Halotolerant bacteria tolerate moderate concentrations of salts but not required for their growth (e.g. Staphylococcus aureus).
- - Osmophilic bacteria require the presence of sugars in the medium for their growth.
- - Osmotolerant bacteria tolerate moderate concentrations of sugars but not required for their growth.
- - Xerophilic bacteria can multiply in the absence of water in their environment

6. Bacterial culture

The microscopic observation of bacteria must be supplemented by other tests on culture medium to be able to characterize and identify them, it is bacterial culture. The latter requires knowledge of the culture media that will be used for the inoculation of the microbial sample to be analyzed but also the interpretation of the culture results.

6.1.Types of culture media The culture medium is a growth support for microorganisms, it must contain nutrients and energy necessary for their multiplication. There are different types of culture media depending on their composition and use.

- Synthetic medium: its composition is known exactly in quantitative and qualitative manner.

- Complex medium: its composition is partially known because it contains raw materials more or less complex such as: serum, blood, peptone, liver, meat, soy and casein.

- Enriched medium: it is a nutrient-rich medium that allows the growth of demanding sprouts. It is therefore added to suspensions rich in organic molecules (blood, serum, globular extract,...etc).

- Selective medium: it is a medium that will promote the growth of desired microorganisms cultivated despite other undesirable microorganisms. It therefore contains inhibitory and stimulatory molecules.

- Differential medium: it is a medium that facilitates the distinction between colonies of the microorganisms targeted and colonies of other germs present on the same medium. Most media contain elements to show biochemical traits.

Enrichment media: it is a medium used to accelerate the growth of a demanding or slow-growing germ.

CONTROL OF MICROORGANISMS

Chapter 7: The control of microorganisms.

Microorganisms compared to humans are beneficial and necessary. Many are also undesirable

(food spoilage). So you have to be able to destroy them or at least be able to inhibit their

development. It is the control of microorganisms.

The means of controlling development are very numerous and can be classified into three categories (depending on the nature): - - -

physical agents (widely used, for example heat, radiation)

chemical agents: toxic (very active against microorganisms) but will exercise their activity

on human or animal cells. So they cannot be used therapeutically.

Selective toxicity, opposes microbial multiplication, without acting on the host cells. These are used in therapy.

1. Control of microorganisms by physical and chemical agents (General)

When antimicrobial treatment is applied, a certain effect is sought which may be the destruction of the population, the reduction of the population or inhibition of population growth.

Sterilization: This is the process by which an object or habitat is destroyed or removed from “all” the living cells, viable spores and viruses. A sterile object is considered to be completely free of

of these agents to viable state. When a chemical agent allows sterilization it is called a sterilizing agent.

Disinfection: It is the destruction, inhibition or elimination (consists of filtration) of microorganisms potentially pathogenic. Disinfectants are chemical agents usually used on

Inanimate objects. A disinfectant does not necessarily sterilize an object, because it can still leave Viable spores and some micro-organisms.

Decontamination: It is closely associated with disinfection. Through this process, the microbial population is reduced to levels considered safe by public health standards. For example, the decontamination agents to clean dishes in restaurants.

Antisepsis: It is not always necessary to control microorganisms on objects. You also need

control on living tissue. Antisepsis is the prevention of infection by the use of antiseptic, agents chemicals applied to the tissue in order to destroy or inhibit the development of the pathogen. They should not destroy the host tissue too much, antiseptics are generally less toxic than disinfectants.

Antimicrobial: depending on its effect, a specific suffix can be used. The suffix "cide" means that it has an action destructive.

Example: germicidal agent, an agent that will destroy pathogenic microorganisms, not necessarily the spores.

When a agent destroyed the spores on will say that he is sporicide.

Some agents have a specific action on a type of micro-organism, it will be indicated in prefix (example: fungicides).

Suffix «static» growth inhibition, these agents are less powerful (example: bacteriostatic effect).

The action of these agents is reversible, if they are eliminated the microorganisms can resume their growth.

Antimicrobial agents are often sought for action on pathogens, but also on non-microbial

pathogens. In a very numerous situation what is sought is the reduction of the population

total microbial

2. Microbial lethality kinetics

The destruction of a microbial population is neither instantaneous nor complete. Death is usually

exponential. That is, a fraction of the population dies at a constant time interval.

Microorganisms are considered dead when they are unable to reproduce under conditions that usually support their growth and reproduction

6.2.1. Conditions affecting antimicrobial activity

Population size: It takes longer to destroy a large population than a small one.

Population composition

The effectiveness of an agent varies with the type of organism treated because microorganisms vary greatly

in sensitivity.

Concentration or intensity of an antimicrobial agent. Usually, a higher concentration

It is faster (but this relationship is not linear).

Duration of exposure: Long-term exposure plus organisms killed

Temperature: An increase in temperature increases its activity.

Local environment: Several factors (pH and organic matter concentration) can

also influence efficiency.

6.2.2. The use of physical methods in control - - - -

Heat

Low temperatures

Filtration

Radiation

Heat

Wet heat: Easily kills different types of microorganisms (viruses, bacteria, fungi). Degrades

nucleic acids, denatures proteins, and breaks membranes.

Dry heat: Less effective, requires higher temperatures and longer exposure times.

Oxidation of cellular constituents and denaturation of proteins.

Low temperatures

Freezing: Inhibits microbial reproduction due to lack of liquid water. Some micro Organisms are killed by the rupture of membranes due to the formation of ice crystals.

Refrigeration: Reduces bacterial growth and reproduction.

Filtration: Used to reduce microbial population in heat-sensitive solutions and sometimes to sterilize solutions. Also used to reduce the microbial population of air.

Filtration of liquids:

Thick filters: Made of fibrous or granular materials fixed in a thick layer containing Small diameter twisted channels. Microbial cells are eliminated by physical trapping and also by adsorption to the filter surface.

Filter membranes: Porous membranes with defined pore diameters that eliminate Microorganisms mainly by physical trapping.

Air filtration: - - -

Surgical masks.

Cotton plugs on culture bottles.

Laminar flow biological safety hoods using HEPA (High-Efficiency Particulate Air filters).

Radiation:

Ultraviolet (UV) radiation: Is very lethal but does not penetrate glass, film or dust, water and other substances.

Ionizing radiation: penetrates objects deep down. Destroys bacterial endospores but not always viruses. Used for sterilization of antibiotics, hormones, sutures, single-use plastic food and items.

6.2.2. The use of chemical methods in control

Alcohols: Bactericides, Fungicides, but not sporicides. Some lipid-containing viruses are also destroyed. Denature proteins and dissolve membrane lipids.

Halogens – chlorine: - -

Oxidizes the cellular constituents. Important for disinfection of water distribution and swimming pools. It is also used in the dairy and food industries, as well as

Personal use disinfectant.

Kills bacteria and fungi, but not spores.   Can react with matter

organic and thus create carcinogenic compounds.

Aldehydes: Highly reactive molecules. They are sporicides and can be used as chemical disinfectants. They can be combined with nucleic acids and proteins for inactivate.

Sterilizing gases: Used to sterilize heat-sensitive objects. Germicides and sporicides. Se combine with proteins to inactivate them.

BIOREACTOR TECHNOLOGIES

Chapter 8: Bioreactor technologies

1. Bioreactor definition

The bioreactor is a large-scale production system of “target products” that uses microorganisms as real plants to produce metabolites or degradation substrates (Figure 28), a biochemical reaction occurs (Figure 27).

The term bioreactor includes fermentation tanks (old term) and cell incubators (biotechnical equipment used for bacterial culture, which can maintain a constant environment for cell culture).

A bioreactor, also called a fermenter or propagator, is a device in which one multiplies

micro-organisms (yeasts, bacteria, microscopic fungi, algae, animal and plant cells)

for biomass production (ecology), or for the production of a metabolite or bioconversion

of a molecule of interest.

In the 1800s, Pasteur, Kutzing, Schwann, and Cagniard-Latour demonstrated that fermentation was caused by yeasts, which are living organisms (Hochfeld 2006). The term "fermentation" is

both aerobic and anaerobic metabolism. It is a process that multiplies the biomass of

living microorganisms, and possibly to use its metabolism.

Unlike the simpler systems used to grow micro-organisms, such as vials, the

bioreactor allows to control the culture conditions (temperature, pH, aeration, etc.), and therefore it

It allows for more reliable information to be collected.

Laboratory models range from 0.1 to 15 litres. The models used for testing are

industrialization (called "pilots") range from 20 to 1,000 litres, while those intended for industrial production

may exceed 1,000 m3 (ethanol production case). Models of disposable bioreactors exist on the market since 1995, used mainly for volumes ranging from millilitres to a few hundred litres.

In tissue engineering, the term bioreactor can refer to a system that allows for tissue culture. Purpose is not to produce metabolites but a complete tissue composed of cells and the matrix extracellular.

2. The principle

A fermenter or bioreactor or propagator, is an apparatus in which microorganisms are multiplied

(yeasts, bacteria, microscopic fungi, algae, animal and plant cells) for the production of

biomass, or for the production of a metabolite or the bioconversion of a molecule of interest.

The bioreactor allows to control the conditions of culture (temperature, pH, aeration, etc.), and therefore it

It allows for more reliable information to be collected.

A fermenter is usually built on the model of a bioreactor, but without an aeration system.

In the field of biotechnology, the term fermenter is sometimes used without any distinction by

compared to the bioreactor. It allows differentiation of the type of culture (bacteria, yeast for fermenter and

animal cells for bioreactor).

The first industrial fermentation processes were extrapolated versions of these domestic recipes.

To meet the growing needs, new dimensions have developed from

the ability of microorganisms to produce in significant quantities, primary or secondary metabolites

of industrial interest, covering most of the major sectors of economic activity: chemistry, pharmacy,

energy, food, agriculture and the environment.

Fermentation reactions is a process that involves microorganisms that develop in

consuming a portion of a reagent called substrate and transforming the other into various product. The process of

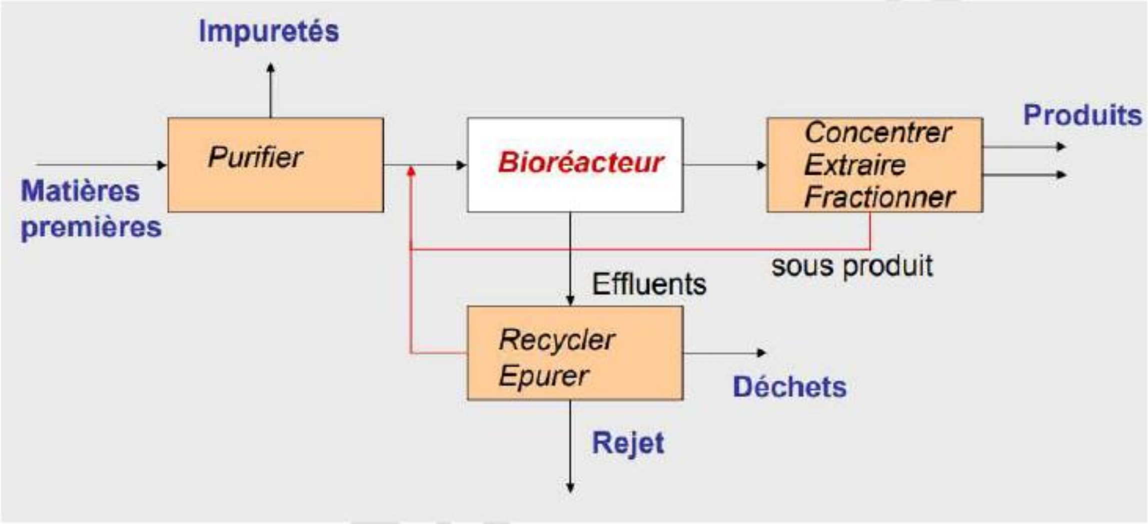
preparation and processing is implemented, whether it be laboratory size (1 to 15 liters), at

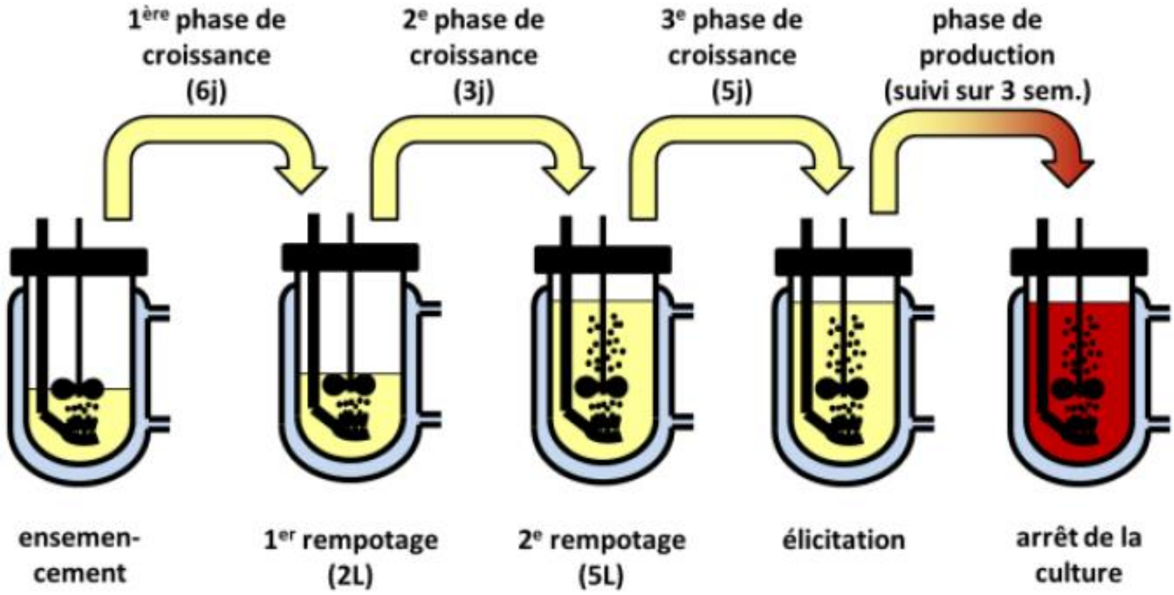
the pilot scale (1 to 5 m3) or industrial scale (up to 300 sometimes 500 m3), is called Fermenters. Their mode of

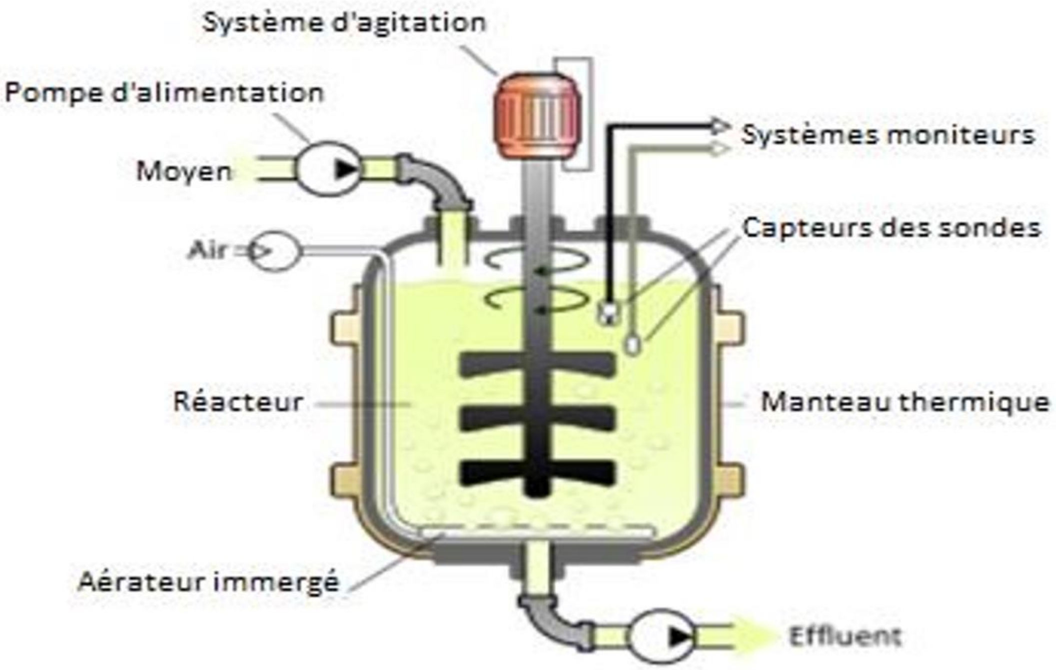
operation, is either discontinuous (batch), semi continuous (fed- batch) or continuous.

3. Operation of a bioreactor

- The bioreactor vessel must contain a suitable culture medium for seeding selected live cells (Figure 30).
- After a first phase of growth (a few days), a first repotting is carried out. When the biomass is sufficiently concentrated again, a second medium is added to ensure that the storage tank reaches its maximum capacity.
- The cells will then multiply during a third and final phase of growth at the end of which the suspension is selected.







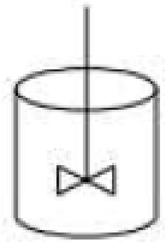
Fig…… : Bioréacteur

Figure 21 : Experimentation in bioreactor (Chastang, 2014)

4. Types of reactors

4.1.batch or closed reactor

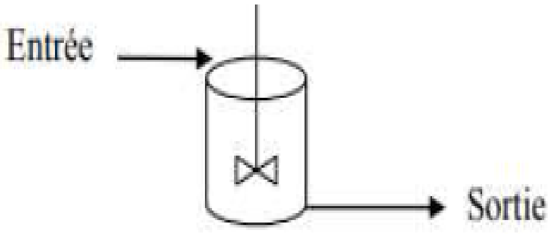
These are reactors that have neither input (not powered) nor output (no draw)



Réacteur Agité

4.2. CONTINUOUS OR OPEN REACTOR

- These reactors have an inlet (powered) and an outlet (drawn). There are two types of reactor in this category:
- Perfectly stirred or mixed reactor and unstirred reactor (no mixture) or piston reactor.
- Reactors with an inlet or outlet are called semi-closed reactors or fedbatch, and generally are classified as closed reactors.



Réacteur Continu Parfaitement Agité (RCPA)

Réacteur Piston (RP)

5. MAJOR FAMILIES OF MEMBRANE BIOREACTORS

- **TWO MAIN FAMILIES OF MEMBRANE BIOREACTORS CAN BE DISTINGUISHED:**

5.1.EXTERNAL MEMBRANE BIOREACTORS

The membranes are placed in a housing. The housing can be mounted in series and/or parallel. These are tubular or flat membranes, organic or mineral so-called internal skin (filtration is carried out from the inside of the membrane to the outside).

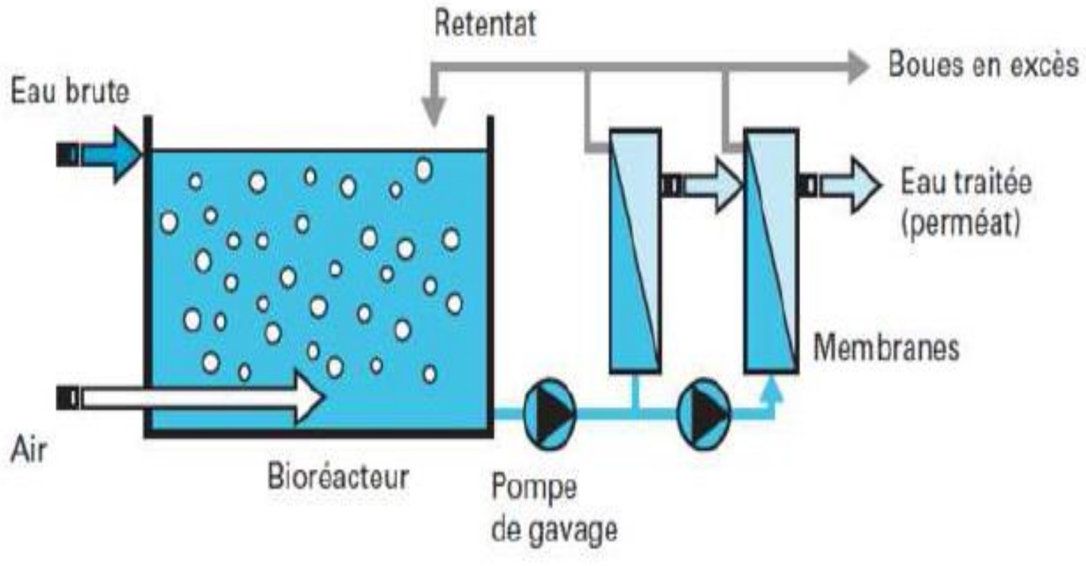


Figure 22: EXTERNAL MEMBRANE BIOREACTORS

5.2. Submerged membrane bioreactors

The membranes are placed directly in the activated sludge. These are hollow or flat membranes, organic fibres called outer skin (filtration is carried out from the outside of the membrane to the inside). Filtration is ensured by hydrostatic pressure or vacuum. The control of the accumulation of materials on the surface of the membranes is ensured by a dedicated aeration complemented by automatic cleaning phases (backwashing, filtration stop).

Some aeration may be deducted from the biomass's oxygenation requirements. When the membranes are placed in a dedicated tank, recirculation of this tank to the aeration tank is necessary. If the membranes are placed directly in the aeration tank, the need for recirculation depends on the configuration (channel, integral mix, piston).

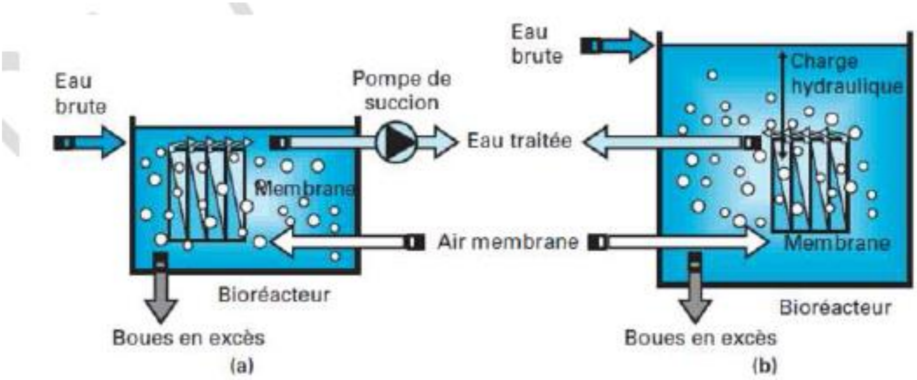


Figure 23 : Submerged membrane bioreactors

BIOFILM

Chapter 9: Biofilm

In most natural environments, the association with surfaces in structures called biofilms is the main way of life for microorganisms. Surface bonding is an effective way to walk in a supportive micro-environment instead of being swept away by the current. Biofilms exist in almost all systems where microorganisms exist.

1. Historic

For more than 150 years, whenever microbiologists have used direct methods to examine the natural populations of these organisms that grow in real ecosystems, the idea that bacteria grow preferentially on the surface has emerged.

The dutch scientist antonie van leuwenhoek (1632-1723) used his primitive but effective microscope to describe the aggregates of «animacules» which he scraped from the surface of human teeth.

About two centuries later, claude zobell (1904-1989) examined natural marine populations under the direct microscope and concluded that these bacteria were attracted to surfaces sometimes attached, forming sessile populations.

Between 1935 and 1978, microbiologists at the forsyth dental center, including ron gibbons and van hoot, observed the microbial biofilms that make up the plaque and form macroscopic scales on the tooth surface.

Additionally, ralph mitchell and kevin marshall (1964) studied the early stages of bacterial biofilm formation in pure culture and distinguished reversible and subsequent adsorption of bacteria to the surface as the first stage of biofilm formation (costerton, 1999).

2. Définition

Biofilms are defined as complex microbial communities, characterized by cells that attach to the matrix and to each other via a matrix of self-produced polymer extracellular substances (SPE)

Clusters of microbial cells may belong to the same species and therefore be homogeneous in nature or different species and therefore heterogeneous in nature.

The adhesion surface can be biotic (mucosa, skin, plant...etc) or abiotic (wood, water,..etc.). Otherwise defined by Declerck (2010) as complex and natural assemblages of microorganisms that involve a multitude of trophic interactions.

The microorganisms in biofilms live in an autogenic matrix of hydrated extracellular polymer substances (eps), which forms their immediate environment.

Eps can be of different kinds, mainly polysaccharides, proteins, nucleic acids and lipids.

These substances provide the mechanical stability of the biofilm, promote its adhesion to the surface and form a cohesive three-dimensional polymer network that interconnects and temporarily immobilizes the cells in the biofilm.

In addition, the biofilm matrix acts as an external digestive system by bringing extracellular enzymes closer to cells, allowing them to metabolize colloidal and dissolved solid biopolymers (flemming and wingender, 2010).

3. Structure

Biofilm structure will be affected by substrate loading on the biofilm surface, hydrodynamic conditions (including shear stress on biofilm and type of organism or physiological group) (van loosdrecht et al., 1995).

Water accounts for 97% of the mass of most biofilms (sutherland, 2001). The cells in the biofilm are surrounded by extracellular polymers (eps), which constitute the immediate environment of these cells. Some eps, especially those that form capsules, are more closely related to the cell surface with other eps.

The morphology of the resulting biofilm may be smooth and flat, rough, fluffy or filamentous, and the porosity of the biofilm may also vary, with large mushroom-shaped colonies surrounded by voids filled with water (table 5).

All these forms allow cells to temporarily attach biofilm, and to the micro-combination of mixed species and coexist for a long time. This provides highly diverse habitat in a small area and promotes biodiversity (flemming and wingender 2010).

Biofilms are aggregates separated by an interstitial fluid, which transports nutrients to waste produced by cells and bacteria. These aggregates contain bacterial cells, which are

divided into an external polymer matrix composed primarily of polysaccharides and proteins (de beer et al., 1994; stewart, 2003).

In fact, extracellular polysaccharides form the main part of the eps matrix (wingender et al., 2001). In a true ecosystem, wild bacterial strains are covered with an extracellular polysaccharide (eps) layer, through which long fimbriae exist to form an effective part of the cell surface (costerton 1999).

However, in hybrid biofilms, the presence of eps-producing substances may lead to the incorporation of other substances that do not synthesize matrix polymers (sutherland 2001).

Table 4: Functions of extracellular polymeric substances in bacterial biofilms (Flemming and Wingender, 2010).

Table 1 | **Functions of extracellular polymeric substances in bacterial biofilms**

Function	Relevance for biofilms	EPS components involved
Adhesion	Allows the initial steps in the colonization of abiotic and biotic surfaces by planktonic cells, and the long-term attachment of whole biofilms to surfaces	Polysaccharides, proteins, DNA and amphiphilic molecules
Aggregation of bacterial cells	Enables bridging between cells, the temporary immobilization of bacterial populations, the development of high cell densities and cell–cell recognition	Polysaccharides, proteins and DNA
Cohesion of biofilms	Forms a hydrated polymer network (the biofilm matrix), mediating the mechanical stability of biofilms (often in conjunction with multivalent cations) and, through the EPS structure (capsule, slime or sheath), determining biofilm architecture, as well as allowing cell–cell communication	Neutral and charged polysaccharides, proteins (such as amyloids and lectins), and DNA
Retention of water	Maintains a highly hydrated microenvironment around biofilm organisms, leading to their tolerance of dessication in water-deficient environments	Hydrophilic polysaccharides and, possibly, proteins
Protective barrier	Confers resistance to nonspecific and specific host defences during infection, and confers tolerance to various antimicrobial agents (for example, disinfectants and antibiotics), as well as protecting cyanobacterial nitrogenase from the harmful effects of oxygen and protecting against some grazing protoza	Polysaccharides and proteins
Sorption of organic compounds	Allows the accumulation of nutrients from the environment and the sorption of xenobiotics (thus contributing to environmental detoxification)	Charged or hydrophobic polysaccharides and proteins
Sorption of inorganic ions	Promotes polysaccharide gel formation, ion exchange, mineral formation and the accumulation of toxic metal ions (thus contributing to environmental detoxification)	Charged polysaccharides and proteins, including inorganic substituents such as phosphate and sulphate
Enzymatic activity	Enables the digestion of exogenous macromolecules for nutrient acquisition and the degradation of structural EPS, allowing the release of cells from biofilms	Proteins
Nutrient source	Provides a source of carbon-, nitrogen- and phosphorus-containing compounds for utilization by the biofilm community	Potentially all EPS components
Exchange of genetic information	Faciliates horizontal gene transfer between biofilm cells	DNA
Electron donor or acceptor	Permits redox activity in the biofilm matrix	Proteins (for example, those forming pili and nanowires) and, possibly, humic substances
Export of cell components	Releases cellular material as a result of metabolic turnover	Membrane vesicles containing nucleic acids, enzymes, lipopolysaccharides and phospholipids
Sink for excess energy	Stores excess carbon under unbalanced carbon to nitrogen ratios	Polysaccharides
Binding of enzymes	Results in the accumulation, retention and stabilization of enzymes through their interaction with polysaccharides	Polysaccharides and enzymes

EPS, extracellular polymeric substances.

4. Properties

The role of the matrix in conferring ecological benefits to all cells of the biofilm, especially those furthest from the surface

The biofilm competition simulation shows that polymer producers have a strong evolutionary advantage over non-producers, perhaps because the polymer brings the daughter cells of polymer producers closer to an oxygen-rich environment (Xavier et al., 2007).

Biofilm has several characteristic properties, namely:

The micro-organisms forming the biofilms will undergo phenotypic changes, corresponding to behavioural changes;

The formed matrix provides a physical protective barrier against external conditions and pathogens. The SPE matrix has the ability to act as an anion exchanger, which can physically prevent some antibacterial agents from entering the biofilm (Kokare et al., 2009).

The metabolic activity of micro-organisms is low, because they are less exposed to danger and overall contact with the external environment;

Microorganisms must exchange genes, so they must acquire new characteristics. One of the advantages of living biofilms is their ability to acquire genetic elements that can be transmitted at a faster rate. There are numerous reports of increased binding rates of bacterial biofilms (Angles et al., 1993; Hausner and Wuertz. 1999). This indicates that evolution by horizontal transfer of genetic material can occur rapidly in biofilms, This makes it an ideal environment for the emergence of new pathogens by acquiring factors of antibiotic resistance and virulence and environmental viability (Watnick and Kolter, 2000).

The adhesion substrate may be degraded or mineralized by the microorganisms that make up the biofilm.

5. Formation

There are generally three different stages in the biofilm life cycle of bacteria: (i) binding of bacteria to substrate, (ii) maturation of biofilm, and (iii) separation and release of biofilm. Then spread in the environment (Donlan, 2002).

The formation of bacterial biofilm must begin with a small amount of bacterial cells adhering to the surface, but in order to make the bacteria react with the surface. Indeed, planktonic bacterial cells release protons and signaling molecules when they pass through the fluid (Costerton 1999).

The bacteria can then form a transient association with the surface and/or other microorganisms previously attached to the surface. This brief connection has allowed him to find a place to live. When a bacterium forms a stable connection as a member of a small colony, it chooses the neighbor where it will live.

Finally, buildings rise with the formation of three-dimensional biofilms. Sometimes bacteria associated with biofilms are removed from the biofilm matrix. Photomicrographs of these stages of the biofilm formed by a single bacterial species are shown in Figure 34.

Although these microphotographs are static views of the various stages of biofilm formation, biofilms are not a mass of fixed cells (Watnick and Kolter, 2000).

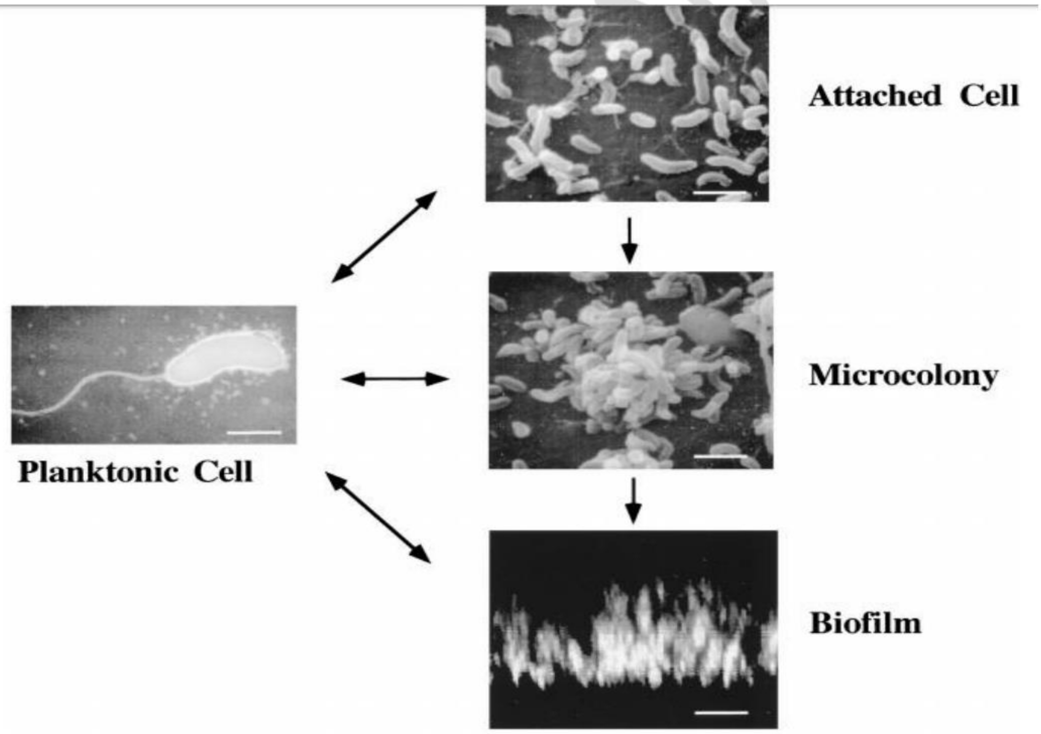


Figure 24 : Microscopic Study Of Formation Steps Of Biofilm By Vibrio Cholerae (Watnick And Kolter, 2000)

In summary, for biofilm to form, five simple steps must occur (Figure 35):

1) Adhesion of the first batch of colonizing agents to the substrate (reversible reaction). As a result, bacterial physiology has developed into a "biofilm" phenotype, characterized by the overproduction of foreign polymers that “bind” the bacterial complex (Jouenne 2008).

2) Attachment to other individuals by gathering to form micro-colonies (irreversible reaction). The colonization phase involves a more or less dense and rapid accumulation by cell division of adherent bacteria or continuous recruitment of bacteria (Picard, 2011). It has recently been shown that signal transduction between cells (quorum sensing) plays a role in cell attachment (Kokare et al., 2009).

3) Primary maturation corresponding to the formation of several microcolonies. The maturity stage of biofilms is characterized by an increased size structure through cell proliferation and synthesis of extracellular polysaccharides (Picard, 2011).

4) Secondary maturation which corresponds to the final aspect of the biofilm

5) Detachment when nutrient deficiency or aging of biofilm caused by insufficient nutrition or non-biological factors (such as stress or biological) cause cells to fall out or disperse. When conditions in the biofilm change, these interactions can determine which cells survive and which die (Watnick and Kolter, 2000).

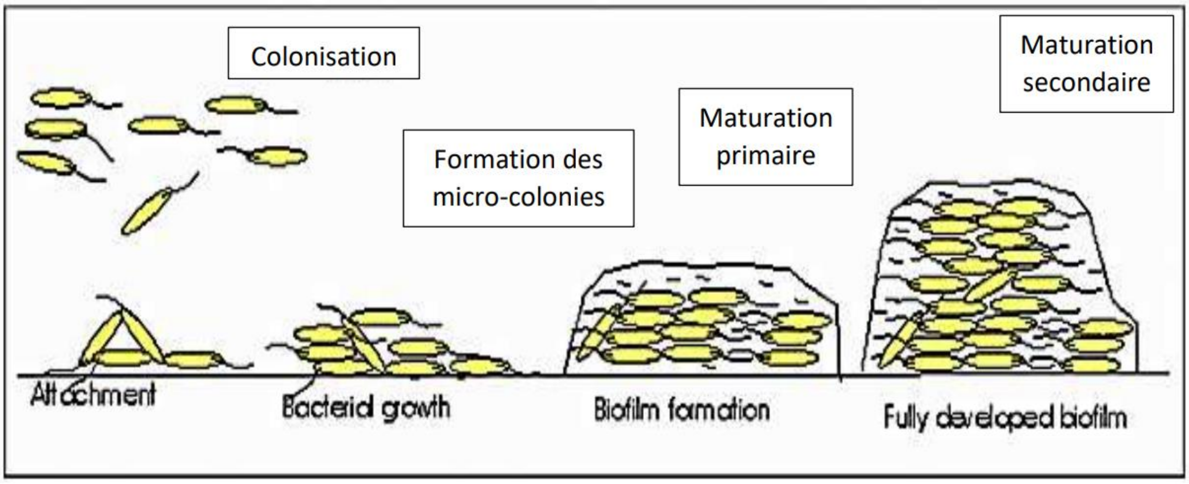


Figure 25 : Schema of biofilm formation (Kokare et al., 2009)

6. Applications

6.1. Treatment and contamination of water

The biofilm would play a double role in water. In the pipes, biofilm formation can infect water potability despite the presence of disinfectant due to their inefficiency or resistance developed by germs. In contrast, heterogeneous biofilms are used voluntarily in municipal sewage sludge, which allows the organic matter of the suspension to be digested and thus contributes to the purification (biological treatment).

6.2. Bio-indicator of pollution:

The biofilm in surface water is mainly composed of heterologous microorganisms. In contaminated conditions, fecal bacteria may interact with these biofilms, suggesting that they can act as reservoirs for pathogenic bacteria in contaminated rivers (Balzer et al., 2010). On the other hand, Rocher and colleagues (2003) studied the structure of sewer biofilms and the content and distribution of aliphatic and aromatic hydrocarbons in the biofilms of the Marais watershed (Paris, France). They found that carbon in biofilms and other sediments The hydrogen compounds are different, so the biofilm can be used as an indicator of contamination by aliphatic hydrocarbons in the organic layer.

6.3. Pathogenesis:

The biofilms on clinical devices may be composed of Gram-positive and Gram-negative bacteria, which can have various origins: skin of patients or medical personnel, tap water or other environmental sources. In addition, the role of biofilms in implant infections has been established in many human systems. For example, the bacterial genera Staphylococcus and Streptococcus can form biofilms on the tissues of the heart valves causing pathologies (Kokare et al., 2009).

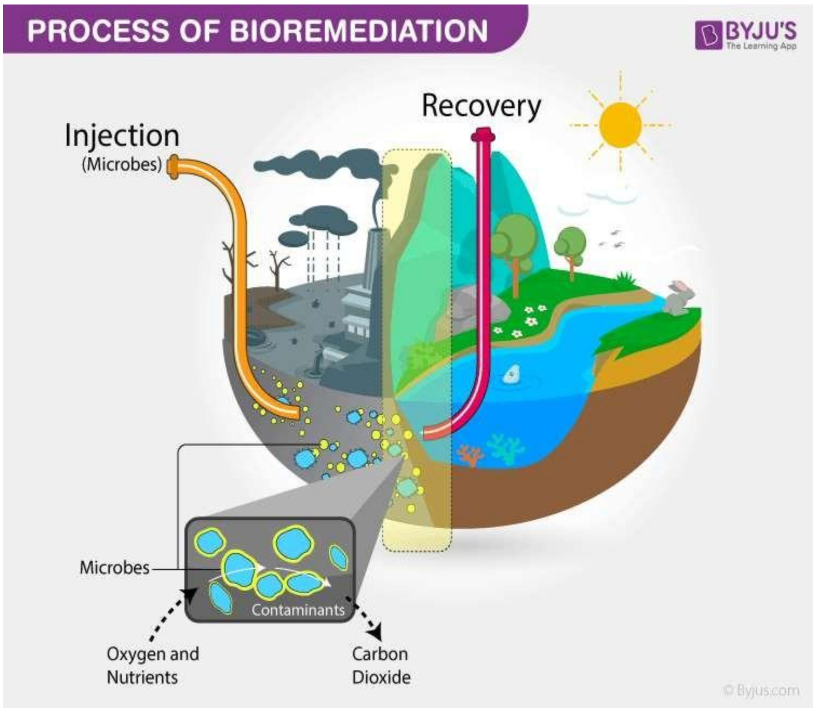
BIOREMEDIATION

Chapter 10 : Bioremediation

1. Definition

Bioremediation that exploits the properties of certain living organisms to extract pollutants and restore contaminated environments.

Bioremediation most often involves plants (phytoremediation) or microorganisms, or both together. Indeed, microbial stimulation in the rhizosphere can increase plant performance in depollution.



2. Principle of bioremediation

The process of bioremediation involves activating the natural ability of many organisms, most often microscopic, to degrade pollutants into inert compounds such as water and carbon dioxide.

- These organisms may be indigenous (already present in the polluted area), or exogenous (added to the environment), or they may be taken from the contaminated site, cultivated in the laboratory and then reintroduced into the soil (bioaugmentation).

▸ Bioremediation is generally carried out in an aerobic condition, however the application of anaerobic bioremediation systems allows for the degradation of a number of recalcitrant molecules.

3. Factors to be optimized and maintained throughout the bioremediation process

- The concentration and bioavailability of the pollutant in environmental conditions: if it is too high, it can be harmful to the same microorganisms that have the ability to biotransform them.
- Humidity: Water availability is essential for living organisms, as well as for the enzymatic activity of acellular biological catalysts. In general, a relative humidity of 12 to 25% should be maintained in soils undergoing biorestauration.
- Temperature: it must be within the range that allows the survival of the applied organisms and/ or the required enzymatic activity.
- Bioavailable nutrients: essential for the growth and multiplication of microorganisms of interest. In particular, carbon, phosphorus and nitrogen must be controlled, as well as some essential minerals.
- The acidity or alkalinity of the aqueous medium or pH (measurement of H + ions in the medium).

The availability of oxygen: in most bioremediation techniques, aerobic microorganisms are used (for example in composting, bio-cells and «landfarming»), and aeration of the substrate is necessary. However, anaerobic microorganisms can be used in bioremediation processes under highly controlled laboratory conditions (using bioreactors).

4. Microorganisms used in bioremediation

▸ Microorganisms are subdivided into the following groups (Vidal, 2001): - In the presence of oxygen (aerobic), examples : Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus and Mycobacterium. These microbes have often been reported as degrading pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as their sole source of carbon and energy. - In the absence of oxygen (anaerobic), example: Phanaerochaete chrysosporium is a fungus that has the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants.

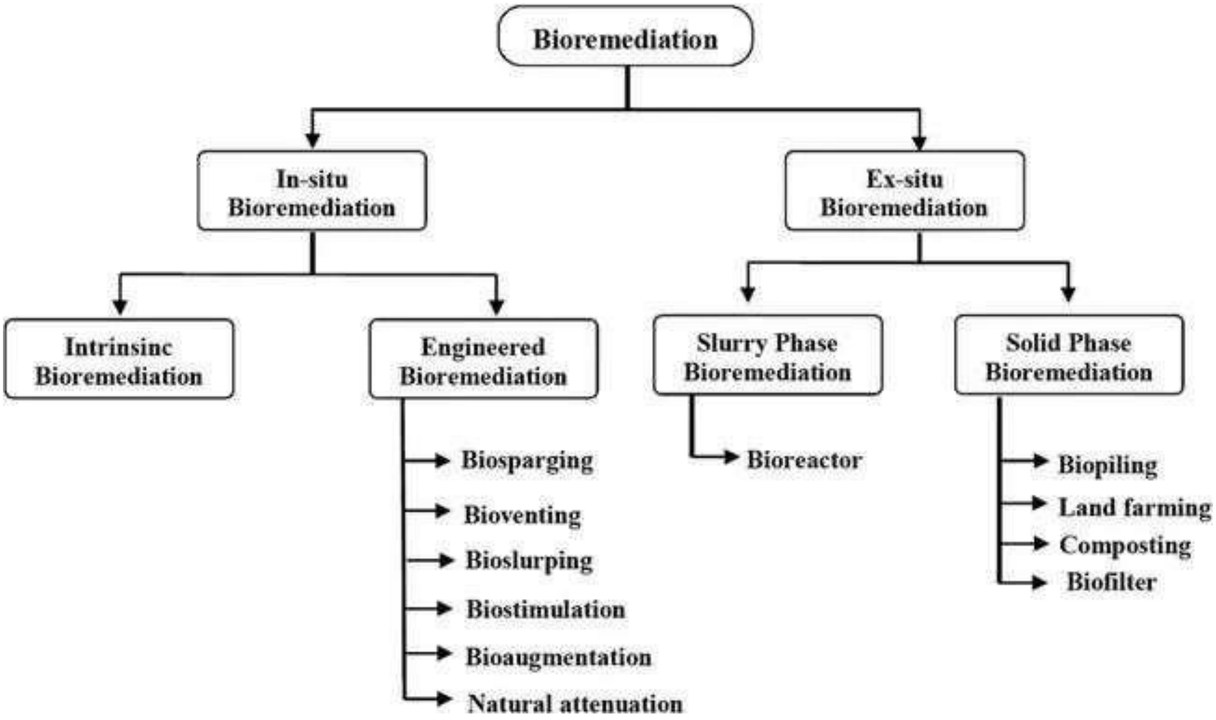
- Anaerobic bacteria are less common than aerobes.

▸ However, they are of great interest in the bioremediation of polychlorinated polyphenyls, trichloroethylene and 1,2 dichloroethane. In all cases, the operation involves not only checking the availability of depolluters but also the constant adjustment of their efficiency conditions: quantity and type of nutrients, oxygen concentration, pH, temperature and salinity.

5. What are the different types of bioremediation?.

There are several types of bioremediation, including natural mitigation, bioaugmentation, biostimulation and phytoremediation

The concept of bioremediation includes biodegradation, which refers to the decomposition and detoxification of contaminants by microorganisms and plants. This natural process can be improved by a variety of methods:



I. Types of bioremediation in situ

- These methods consist of treating the polluted substances at the source of pollution.
- It requires no excavation and minimal or no disturbance of the ground during construction.
- There are two types of in situ bioremediation: intrinsic and artificial.

A. Intrinsic bioremediation

- Intrinsic bioremediation, also known as natural reduction, is an in situ method of bioremediation involving passive remediation of polluted environments without the need for external force (human intervention).
- This procedure involves the promotion of the native or indigenous microbial population.
- Use of aerobic and anaerobic microbial mechanisms to biodegrade contaminants, including those that are resistant.
- Due to the lack of external force, the procedure is less expensive than other in situ techniques.

B. ARTIFICIAL BIOREMEDIATION

1. Bioaugmentation

- Bioaugmentation refers to the introduction of specialized microorganisms, whether naturally occurring or genetically engineered, into polluted soil or water for their remarkable ability to decompose or detoxify a specific contaminant or group of contaminants.
- To speed up the remediation process, microorganisms with potential for using or detoxifying pollutants are usually identified, grown in the laboratory and then delivered to contaminated sites.

- Dehalococcoids sp. , which dechlorines trichloroethylene (TCE) into ethene, has the potential to be used as a bioaugmentation agent in the rehabilitation of groundwater contaminated with TCE.
- Bioremediation of BTEX-contaminated soils is effective when inoculated with microbial populations capable of metabolising benzene, toluene, ethylbenzene and xylene (BTEX).
- Bioaugmentation has been effective with groups of microorganisms from the same or different taxonomic groups (e.g., microbial mats and bacteria-microalgae/cyanobacteria assemblages).

2. Biostimulation

- This technology involves introducing cultures of micro-organisms to the surface of the contaminated environment with the objective of increasing the biodegradation of organic contaminants.
- Generally, microorganisms are selected on the basis of their ability to Degrade organic compounds in the site to be cleaned. The culture may include one or more species of microorganimes. Nutrients are usually added to the solution containing the microorganisms. This suspension of the micro-organism is brought to the surface of the soil under natural conditions or injected into the contaminated site under pressure.

This technology is widely used to decontaminate sites containing Hydrocarbons: The selected microorganisms are bacteria with a high capacity to digest these hydrocarbons.

3. Bioventing (bioventilation) :

- The most common in situ treatment, it involves transporting air and nutrients to contaminated soil through wells to stimulate the natural metabolism of bacteria. In other words, biological ventilation uses a low airflow and provides only the amount of oxygen needed for biodegradation while minimizing volatilisation and release of contaminants into the atmosphere (Vidal, 2001

- Applicability
- Soils contaminated with petroleum hydrocarbons, non-chlorinated solvents, pesticides, wood preservatives and other organic contaminants have been successfully remediated by bioventilation.

4.Biosparging

- is the injection of pressurized air below the water table level to increase the oxygen concentration in the water table and increase the rate of biodegradation of pollutants by bacteria found in nature. This process increases mixing in the saturation zone, thus enhancing contact between soil and groundwater (Vidal 2001).

5. Bioaspiration (bioslurring)

- This method combines vacuum assisted pumping, extraction of the vapours from the soil and Bio-ventilation to clean soil and groundwater through indirect oxygenation and promotion of the biodegradation of pollutants.
- This technology is intended for the recovery of capillary products, light liquids in non-aqueous phase (LNAPL), unsaturated and saturated zones during depollution.
- This method is used to decontaminate soil contaminated with pollutants
- volatile and semi-volatile organic.
- The approach uses a "slurp" that spreads in the free product layer and sucks them
- liquids from this layer.
- As they rise, the pumping machine brings the LNAPs to the surface where they are isolated from air and water. In this method, soil moisture limits air permeability and decreases oxygen transfer rate, thus decreasing microbial activity.
- Although this method is not suitable for restoring low permeability soils, it is a cost-effective process because it uses less groundwater and reduces storage, treatment and disposal costs.

6 . Monitored natural mitigation

- Monitored natural mitigation (MNA) involves dependence on processes
- natural to obtain the cleaning of pollutants.
- The consideration of NMM for remediation of contaminated aquifers and groundwater systems generally requires modelling and evaluation of the rates of degradation of contaminants, exposure pathways, Impacts on sensitive receptors and prediction of contaminant concentrations downstream of the migrating contaminant plume.
- In general, the relevance of the NMA is assessed on a case-by-case basis. The evaluation of NMA is not an easy task; it requires multidisciplinary expertise in microbiology, chemistry, hydrogeology and geochemistry, among others.
- Applicability
- The MNA has been effective, especially for hydrocarbons. Generally, fuel and halogen-containing volatile organic compounds are examined for IEM.

7. Phytoremediation

- Phytoremediation is a bioremediation process that uses plants to remove, transfer, stabilize or destroy contaminants in soil and groundwater. Different mechanisms are involved in phytoremediation, including:
- Biodegradation of the rhizosphere: Plants release natural substances through their roots, providing nutrients to soil microorganisms, which improves biological degradation of contaminants.
- Phyto-stabilization: Chemical compounds produced by plants immobilize contaminants, preventing their
- movement and reducing their toxicity.
- Phyto-accumulation (phytoextraction): The roots of plants absorb contaminants, as well as other nutrients and water, which accumulate in shoots and leaves. This method is mainly used for metal-containing contaminants.

- Hydroponic stream treatment systems (rhizofiltration): Plants are grown in greenhouses with their roots in the water, allowing plants to absorb contaminants from the water. Saturated roots are harvested and eliminated.
- Phyto-volatilization: Plants absorb water containing organic contaminants and release the contaminants into the air through their leaves.
- Phyto-degradation: Plants metabolize and destroy contaminants in their tissues.
- Hydraulic control: The trees correct indirectly by controlling the movement of groundwater. The dense root mass of trees absorbs large amounts of water, helping to regulate the groundwater and limit the spread of contaminants.

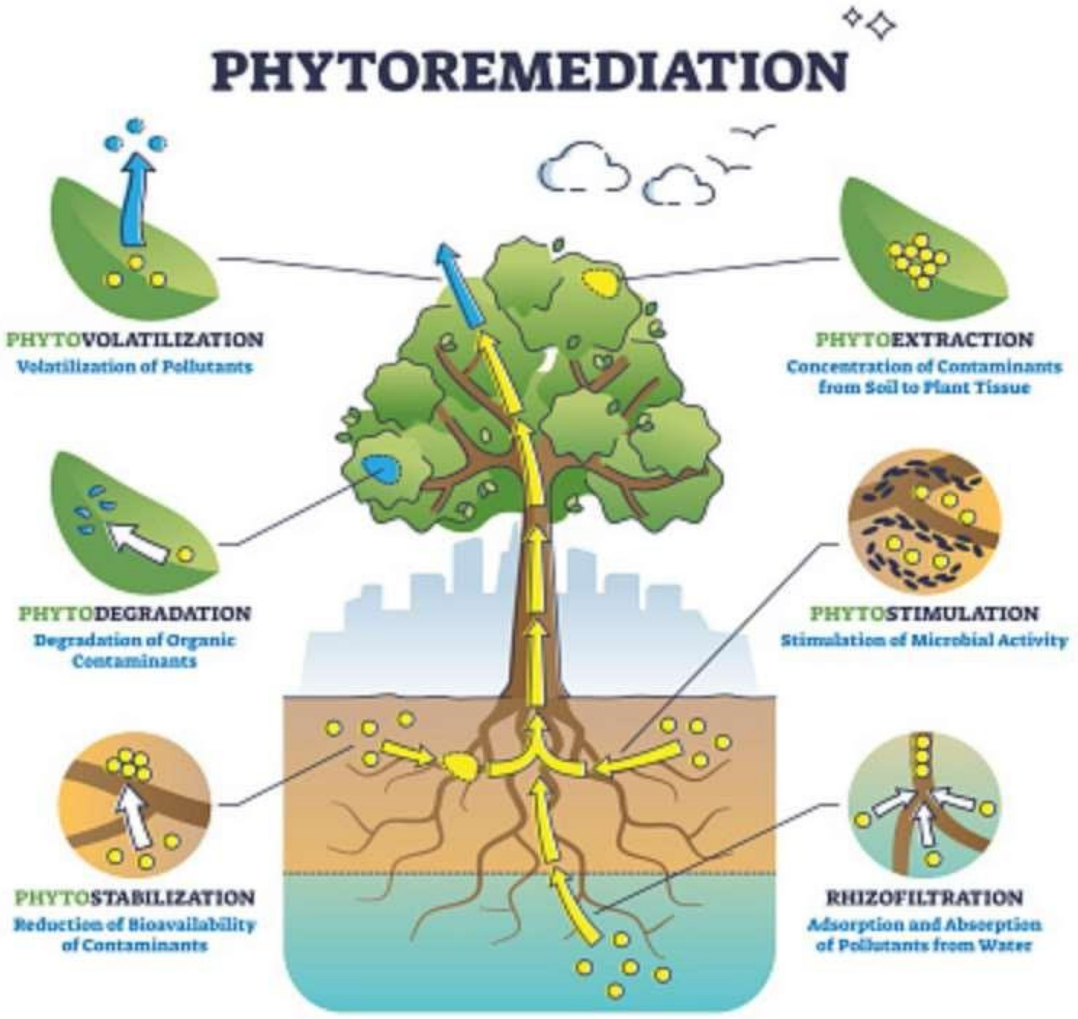


Figure 26 : Phytoremediation

Influence of rhizospheric microflora on phytoremediation

- Rhizosphere microbiota components can improve potential
- plant mediator by direct or indirect action (Glick et al., 1998).
- The indirect aspect occurs when microorganisms stimulate resistance of plants face
- to plant pathogens and helps the plant stay healthy by attacking plant pathogens.
- The direct aspect is synthesized by the biofertilising power of microorganisms in the soil.
- In summary, the role of microorganisms in phytoremediation is very important and can be
- expressed in the following points:

- Detoxification of certain pollutants to better enable their assimilation by the plant;

- The increase of catabolic activities between soil and plant to promote growth of the latter;

- The stabilization of pollutants in the rhizosphere to increase their phytoavailability.

- ☐ Heavy metals and radionuclides are generally removed by extraction, transformation and sequestration.
- ☐ Organic pollutants such as hydrocarbons and chlorinated chemicals are generally eliminated by degradation, rhizoremediation, stabilization and volatilization; However, mineralization is possible when certain plants such as willow and alfalfa are used.

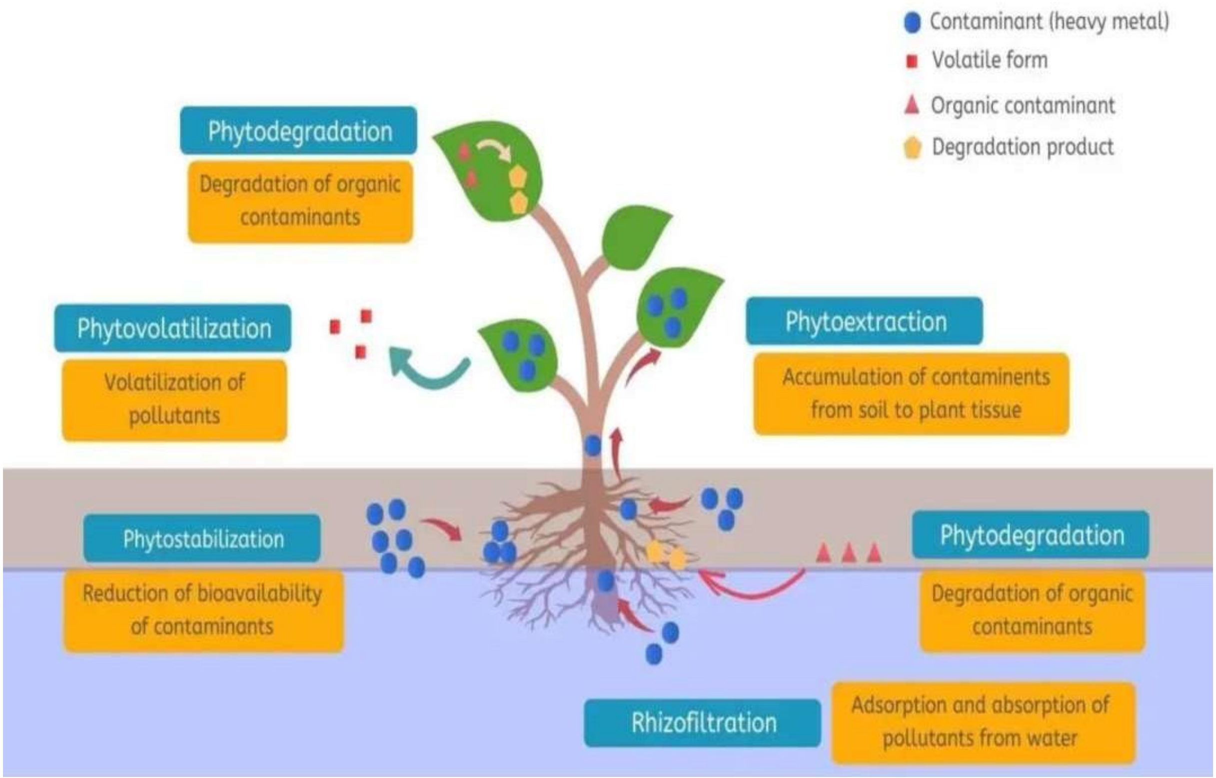


Figure 27 : Influence of rhizospheric microflora on phytoremediation

II. Biorestauration ex situ

- Ex-situ bioremediation approaches involve the removal of pollutants from contaminated sites and their transfer to a new site for treatment.

There are two types of ex-situ bioremediation

A. Solid phase treatment

- Solid phase bioremediation is an ex-situ process that involves Excavation and piling of contaminated soil.
- It also includes municipal, industrial and household waste as well as organic wastes such as leaves, animal manure and agricultural waste.
- The pipes are used to transport bacterial growth throughout the pious.
- Ventilation and microbial respiration require air to flow through the pipes.

- Compared to liquid phase procedures, solid phase systems require a large area and require more time to be cleaned.
- Solid phase treatment methods include biopiles, Windrows, land cultivation and composting, among others.

1. Biopiles

- Biopiles are a treatment technology in which excavated soils are combined with soil improvers and placed in above-ground enclosures equipped with an aeration system and leachate collection system.
- Biodegradation is frequently used to clean up petroleum hydrocarbons in excavated soils.
- To reduce the possibility of contaminants entering uncontaminated ground water or soil, the treatment area is usually covered with an impermeable membrane. Various fertilizer and supplement formulas are used to increase microbial activity in biopiles.
- The piles of earth can reach a height of up to 6 meters, although the optimal height is 2-3 meters. As a general rule, a vacuum or positive pressure air distribution system is built under the ground and maintained. The biopiles are covered with plastic film to reduce runoff, evaporation and volatilization, which can also result in increased solar heating.
- If there are volatile organic compounds (VOCs) in the soil, it may be necessary to clean the air out of the ground before it is released into the sky. The operation of biopiles can take from a few weeks to several months.
- **Applicability**
- Biopile treatment has been effective for combustible hydrocarbons and non-halogenated VOCs. This method has also been used to treat halogenated VOCs and pesticides; however, the success rate will vary and some chemicals may be unworkable.

2. Compostage

- Composting (windrows) is a controlled biological process in which contaminated soil excavated is mixed with filler agents and organic amendments (wood chips, hay, manure, green waste, etc.) in a proportion appropriate to provide the right balance of carbon and nitrogen required. for thermophilic microbial activity.
- Under aerobic and anaerobic conditions, microbial activities convert organic pollutants (such as polycyclic aromatic hydrocarbons (PAHs) and 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT)) into harmless stable compounds.
- During the composting process, heat produced by indigenous microorganisms during the decomposition of organic matter will cause a thermophilic phase (55 to 65 degrees Celsius) essential for the transformation of hazardous pollutants.
- Maintaining adequate oxygenation (through windrowing rotation), moisture content (through irrigation) and temperature can increase degradation efficiency.
- The different composting designs include (1) aerated static compost heaps, in which compost heaps are aerated by blowers or vacuum pumps, (2) mechanical agitated tank composting, in which the compost is placed in a reactor vessel and mixed and aerated, and (3) in windrows, a more cost-effective method in which the compost is placed in long heaps called windrows and periodically mixed with mobile equipment.
- Applicability
- By using the composting process, biodegradable pollutants in soils and sediments can be removed. From pilot and large scale operations have demonstrated that aerobic and thermophilic composting can reduce the explosive content of such as trinitrotoluene (TNT), RDX, HMX and ammonium picrate at tolerable levels.
- This method is also suitable for PAHs and DDT residues.

Aeration and humidity are essential to maintain the conditions for a good fermentation. Composting can be done at home or collectively through industrial processes.

Figure 28: Compostage

3. Land agriculture

- Land agriculture is a large-scale bioremediation technique in which contaminated soil excavated is deposited on beds with a predetermined depth and aerated by turning or periodic tillage (about 4 to 12" tillage depth).
- During this procedure, soil parameters such as moisture content, aeration, pH and soil improvers such as soil fillers, fertilizers etc. are manipulated to achieve the highest possible rate of degradation of pollutants.
- This mechanism allows aerobic microbial digestion through the availability of oxygen, nutrients and moisture.
- **Applicability**
- The cultivation of land has been effective in treating petroleum hydrocarbons. The chlorinated and nitrated substances decompose slowly.
- Diesel fuel, fiouls, oil sludge, wood preservation waste, and insecticides are also successfully treated.

4. BIOFILTRATION

This method consists of the use of a bioreactor to treat gaseous emissions .

The principle consists in using microorganisms to degrade pollutants contained in the air to be treated : the aqueous phase (contaminated air) is put into contact with an aqueous phase in which the microbial population develops, also known as biomass.

- In a biofiltration unit, the air to be purified (decontaminate) first passes through a filter and a humidifier in order to remove particles (dust, fats) present in the gas and bring the humidity level to 100%. The air is then fed into a reactor (vessel) containing a packing of highly porous materials (very moisture-hungry). On the surface of the particles that make up the lining is a biofilm which corresponds to a water film containing microorganisms (bacteria and fungi) whose function is to degrade pollutants in the air to be treated.
- This technology is for example used to treat air pollutant by

xylene or nitrogen compounds

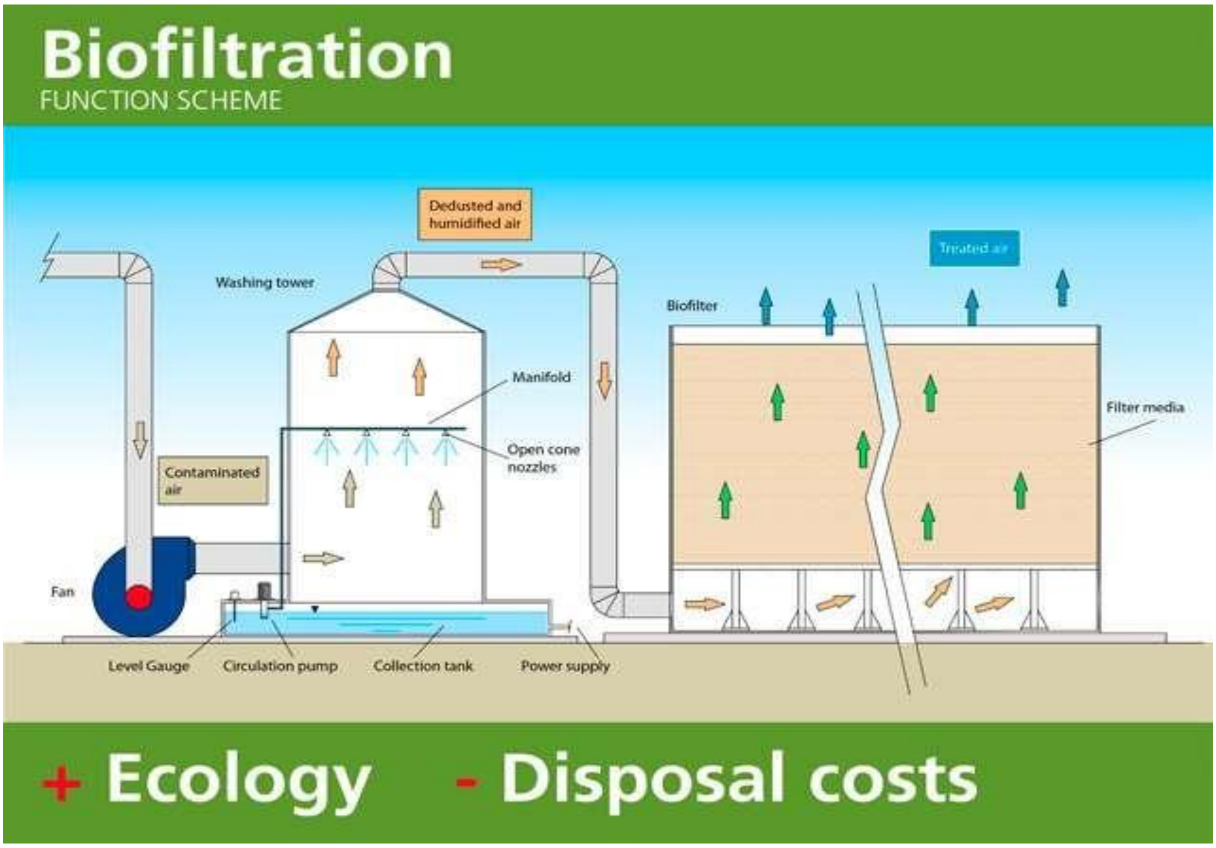


Figure 29 : Biofiltration

B. Sludge phase bioremediation

Sludge bioremediation is a somewhat faster treatment method than others.

In the bioreactor, contaminated soil is mixed with water, nutrients and oxygen to produce the optimal environment for micro-organisms to break down soil pollutants.

This procedure involves separating rocks and debris from contaminated soil.

The additional water concentration depends on the amount of contaminants, the rate of biodegradation and soil physico-chemical parameters.

Using vacuum filters, pressure filters and centrifuges, the soil is extracted and dehydrated according to this procedure.

- **Applicability**

Sludge phase bioreactors have been shown to be effective in soil and sediment remediation contaminated with petroleum hydrocarbons, explosives, solvents, pesticides and other contaminants.

When treating diverse and impermeable soils, as well as when faster treatments are required, bioreactors are preferable to in situ biological methods.

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