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AHMED ZABANA RELIZANE UNIVERSITY
FACULTY OF NATURAL AND LIFE SCIENCES
BIOLOGICAL SCIENCES DEPARTMENT

COURSE HANDOUT

3rd YEAR Licence

Microbiology

Intitulé

Environmental Microbiology

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FOREWORD

The course of "Environmental Microbiology" is intended for students of the **third year licence of Microbiology**.

The knowledge required to follow this course is some basic notions of Microbial systematics and Microbial biochemistry.

Environmental microbiology is a dynamic and rapidly evolving field that explores the diverse and intricate roles of microorganisms in the natural world. From the depths of the oceans to the soils beneath our feet, microbes are fundamental drivers of ecological processes, influencing nutrient cycles, climate regulation, and the health of all living organisms. This handout aims to provide a comprehensive overview of the principles, techniques, and applications of environmental microbiology, bridging the gap between fundamental microbial ecology and applied environmental sciences.

Throughout these chapters, the student will discover the remarkable adaptability of microorganisms and their interactions with various environmental factors. Emphasis is placed on cutting-edge research methods, including molecular and genomic tools, that have revolutionized our understanding of microbial communities and their functions.

Whether you are new to microbiology or seeking to deepen your understanding of environmental systems, this course material is structured to support your learning journey with clear explanations, relevant examples, and practical insights. It is our hope that this booklet will inspire curiosity and foster a deeper appreciation of the microbial world that sustains life on our planet.

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CHAPTER 1: INTRODUCTION

CHAPTER 1: INTRODUCTION

Microorganisms are an irreplaceable group of living beings. Ubiquitous in the environment, they are essential to the planet's equilibrium and form a vital link in any ecosystem. The microorganisms are a large and diverse set of microscopic organisms existing as a single cell or as a group. Microorganisms function as **populations** or assemblies of similar organisms (Prescott et *al.*, 2002).

1. AN ECOSYSTEM

An ecosystem is an open system traversed by energy flows and matter cycles, housing a biotic community (biocenosis) made up of living organisms interacting in a given environment, biotope (abiotic community). The biotic community comprises 3 categories of organisms: producers, consumers and decomposers.

2. THE PRESENCE OF MICROORGANISMS IN ECOSYSTEMS

2.1. Diversity of microorganisms in ecosystems :

They form highly diversified populations, well adapted to the living conditions of the microhabitats in which they develop. They are abundant in the soil, in water, in appreciable quantities in the air and particularly abundant on the surface of the skin and mucous membranes of animals and humans, where they find particularly favorable conditions for their development. Microorganisms are a diverse group of organisms summarized in 3 domains (tree of life): prokaryotes (eubacteria), eucaryotes and archaebacteria. Which are of great metabolic diversity, the bacteria in particular that can use practically any substrate as a source of energy and carbon (e.g. from humic acids to plastics).

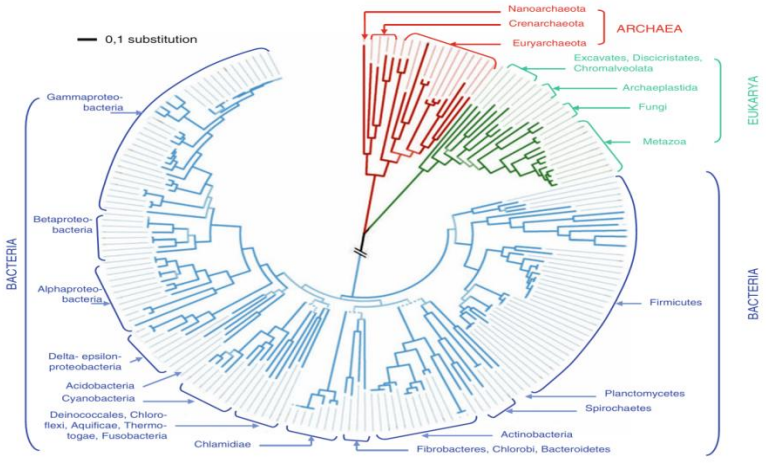

Figure 1 : The tree of life (modified and redrawn from Ciccarelli et *al.* 2006 (Bertrand et *al.* 2014)

2.2. Selection of microorganisms in ecosystems :

Environmental factors lead to the selection of organisms. Microorganisms are able to resist modifications, provided they are not radic*al.*

Adaptation is a word that designates all morphological and physiological characteristics deriving from selection that enables organisms to grow and multiply in a given biotope. This definition implies that any physiological characteristic is an adaptation. By their small size relative to their volume and metabolism associated with their envelopes, prokaryotic cells are in constant interaction with their environment. To follow the evolution of various environmental parameters, such as osmotic pressure, ionic strength, pH, temperature, concentrations of nutrients, or toxic substances, is a necessity for surviv*al.*

Among microorganisms, prokaryotes are those who are most capable of wide adaptability and resistance to changing conditions of the environment. Prokaryotes are practically the only organisms able to resist or even thrive over the extreme conditions of life that exist on our planet. For this purpose, prokaryotic cells have developed adaptive systems that induce **morphological changes** (such as variations in size, changes in envelopes features, productions of protective features such as capsules or spores*, etc.) or **changes in the metabolism** and **physiology** of the cell. These systems are based either on **signal transduction proteins** that are able to perceive changes in environmental parameters and to transmit information to modify other proteins by different **molecular mechanisms**, resulting in metabolic or behavioral adjustments, or on the **regulation of transcription** at **the genetic level** and modifications in the **protein expression** pattern. So, The main regulation systems for molecular adaptation are **transcription, signal transduction, and protein modifications.** Three specialized systems are also: **quorum sensing, phase variation, and antibiosis.** Quorum sensing allows bacteria to trigger some responses when their density is high enough to permit the function to be successful. Phase variation is an adaptive process by which a bacterial subpopulation undergoes frequent, usually reversible phenotypic changes resulting from genetic or epigenetic alterations, allowing rapid modification of the cells physiology. Antibiosis is the ability to synthesize molecules that will impact other taxa and eventually provide a selective advantage to which some microbes respond by resisting to these molecules. Finally, the physiological responses to various environmental parameters such as temperature, oxidants, salinity, acidity, pressure, desiccation, and this translates in different biotopes such as soil, water bodies, sediments, **biofilms, mats**, air, and manmade biotopes.

3. INTERACTIONS BETWEEN MICROORGANISMS

Most biotopes contain many microbial taxa that coexist and interact in many ways, of which few details are known or even considered. These interactions are in most cases not specialized, based mainly on trophic aspects because resources are always limiting, but in some cases there are instances of mutualistic or parasitic relations.

Microbial interaction can be neutral, **Neutralism** corresponds to a situation where two species occupy the same habitat, but not the same niche.

It can be also positive, such as mutualism, cooperation or commensalism, or negative, such as amensalism, parasitism, predation or competition.

3.1. Conflictual Interactions

Martin (2002) has classified into a few categories the known strategies of predation or parasitism: pack predation, epibiotic attachment, direct cytoplasmic invasion, and periplasmic invasion. These strategies constitute a continuum toward more and more specialized forms.

3.1.1. Parasitism

The best-known example of conflictual interaction involving two bacterial taxa concerns *Bdellovibrio bacteriovorus*. This interaction is often described as predation, but corresponds rather to parasitism because it is a lasting interaction, with several cell doublings of Bdellovibrio while inside the periplasm of the target cell. In fact, this bacterium should more accurately be called parasitoid since it leads to the death of the host. The small deltaproteobacterium *B. bacteriovorus* consumes motile cells belonging to many Gram-negative taxa. It starts by entering the periplasm and sealing the pore entrance and then replicates in the peri plasm, yielding daughter cells without flagella that will invade all the cell space. Thereafter, *Bdellovibrio* hydrolyzes cell constituents, forms filamentous cells that become sep tate, lyses the host cell, and releases flagellated offsprings. This way of life and the lack of attack on mammalian cells have even led some to consider the use of Bdellovibrio for the treatment of infections in humans (Stolp and Starr 1963). The genus was divided to yield Bacteriovorax to accommo date marine strains physiologically distinct and thereafter Peredibacter starrii, but all these genera are phylogen etically very close to each other. In

addition, there are many bacteriophages that attack bacteria and act as parasites. Finally, the fungi *Agaricus bisporus* is parasited by *Pseudomonas tolaasi*, forming brown spots on carpophores, i.e., brown blotch disease, and by various other parasitic bacteria and fungi.

3.1.2. Predation

The main microbial predators are protozoa, which regulate bacterial populations in various ecosystems. In soil, however, many bacterial taxa belonging to *Actinobacteria and Proteobacteria* were described as predators of other bacteria and fungi (Zeph and Casida 1986), but few studies have been carried out subsequently, except on the actino bacterium species *Agromyces ramosus* and the proteobacterial genera *Ensifer* (reclassified recently in genus *Sinorhizobium) and Pseudomonas*. Soils and many other biotopes contain bacterivorous organisms belonging to different taxa. This is the case of Caenorhabditis nematodes and Brachionus rotifers. Another type of predation, quite dramatic, involves fungi such as *Hirsutella minnesotensis or Arthrobotrys robusta* that catch and metabolize nematodes. Nematodes are captured using a constrictor ring or adhesive structures such as nets or buttons, after which lytic enzymesattack the nematode. This interaction is used to fight against nematode pests in mushroom farms, intestinal nematodes parasiting sheep (Waller and Larsen 1993), or even phytoparasitic nematodes in soil.

3.1.3. Antibiosis

Amensalism (synonym of antagonism) is an interaction that has a negative effect on one partner but no effect on the other. Amensalism assumes that the two species do not compete significantly with each other. Amensalism is based on a physical or chemical modification of the environment and, in the latter case, often involves the release of toxic compounds. Thus, the production of antibiotics (i.e., **antibiosi**s) may correspond to **interference competition or antagonism**, depending on the nature of the partner affected and on the energy invested in the synthesis of these secondary metabolites.

Antibiosis is generally negative for some of the taxa sharing the same habitat as the antibiotic producer. Therefore, it is often a case of **interference competition,** i.e., an interaction between two species in which one of the two inhibits the development of the other and thus gains greater access to food resources in the biotope. This strategy is widespread in

prokaryotes and eukaryotes and has been extensively studied since Fleming (Fleming 1922), mainly because of its developments in public health. Compounds involved belong to several chemical classes ranging from simple molecules such as aminoglycosides to complex compounds such as macrolides or polypeptides, targeting several cellular functions such as protein synthesis (kanamycin) or RNA synthesis (rifampin). Taxa known to produce antibiotics are bacteria especially soil actinobacteria and fungi, and the types of compound produced and their mechanisms of action. Many discussions have been held to determine if antibiosis was positive for the organism that synthesizes antibiotics, if not we should speak of **antagonism.** It is difficult to determine the cost of antibiotic synthesis, which includes the genetic burden of maintaining dozens of biosynthetic genes, resistance genes, and genes necessary for their transport out of the cell. The presence of an antibiotic is not necessarily detrimental for a given taxon, either because it has acquired genes permitting its degradation, and thus to feed on it, or a mutation has occurred in the genes whose product is the antibiotic target (Birge and Kurland 1969). This kind of phenomenon could in principle occur with any antibiotic. There has been a debate on the relationship between antibiotics and bacteriocins. In contrast to antibiotics, bacteriocins affect bacteria closely related to the producing organism, often belonging to the same species. They are often of a proteinaceous nature.

3.1.4. Competition

Competition is an interaction defined as a simultaneous demand by two or more organisms for a limited environ mental resource, such as a nutrient, water, living space, or light. This is of course the rule in the microbial world where many habitats include several taxa with closely related metabolic capabilities. For example, the addition to the soil of a complex carbon source like deciduous tree litter stimulates many fungi and bacteria capable of metabolizing polymers such as cellulose, hemicelluloses, and pectin. Microbial populations that grow in the soil thus vary according to the carbon sources added (Hery et *al.* 2005). A wastewater treatment plant is another biotope (anthropized), in which is found a rich mixture of carbon molecules inducing fluctuations of the microbial community present (Snaidr et *al.* 1997).

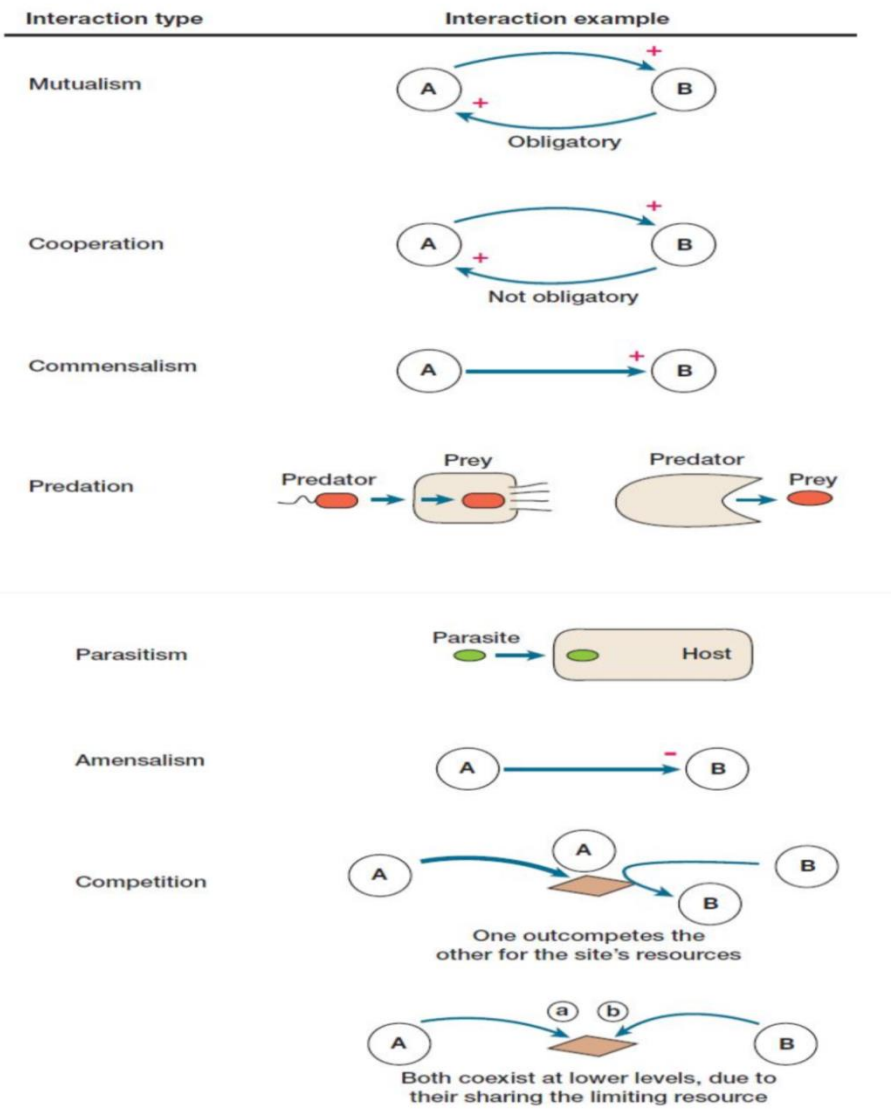

Figure 2 : Microbial interactions (https://microbiologynotes.org/microbial-interactions-in-the-environment/)

3.2. Beneficial Interactions

When comparing various beneficial interactions between microorganisms, it appears that the terms cooperation, mutualism, and syntrophy have been used for conceptually similar phenomena. The term cooperation is sometimes used to designate situations encompassing the other two. Mutual ism is little used to describe microbe–microbe relations, but rather is reserved for relationships between microbes and higher organisms. The term **syntrophy*** would cover just cases where microorganisms have complementary metabo lisms and are in mutualistic situations, each partner providing substrate(s) to the other(s). At one extreme of

the spectrum, there are situations of loose or non-exclusive relationships, based on trophic exchanges thus designated **syntrophic**; at the other extreme are situations of mutualism or symbiosis involving potentially more than trophic aspects of detoxification and entailing toxin synthesis, signaling, etc. It has been argued that the origin of eukaryotic cells was a syntrophy that has evolved into mutualism and finally into an obligatory interaction (Lopez-Garcia and Moreira 1999).

3.2.1. Cometabolism

Cometabolism is a prominent case of beneficial interaction taking place between different microorganisms. It corresponds to cooperation, **syntrophy,** or even symbiosis when metabolisms are different and complementary and when the interaction is sustained. Microorganisms that live in complex habitats such as soils, sediments, or digestive tracts are typically in contact with complex trophic resources they are unable to catabolize alone. For a single organism, cometabolism is the transformation of a compound that cannot serve as sole carbon and energy source (i.e., a non growth substrate), which is made possible by parallel degra dation of a growth substrate. We also speak of cometabolism when several microorganisms must cooperate as a consor tium to synthesize all the enzymes necessary for a catabolic pathway. This is the case of soil isolates individually unable to metabolize polycyclic aromatic compounds, but which are able to do so when they are grown in a consortium (Bouchez et *al.* 1999). It is likely that this consortium includes strains able to compensate for the inhibition caused by a metabolic intermediate by degrading it or when the product of a strain is used as a substrate by a different strain.

3.2.2. Mutualism

The deltaproteobacterium *Syntrophus aciditrophicus* can metabolize various saturated or unsaturated fatty acids, hexanoate and butyrate esters, or benzoate when in coculture with microorganisms capable of metabolizing hydrogen or formate (Mouttaki et *al.* 2007). Anaerobic degradation of saturated fatty acids and aromatic acids in the absence of a terminal electron acceptor necessitates the presence of an organism capable of maintaining a hydrogen partial pressure low enough so that these reactions are thermody namically possible. We therefore find it associated with hydrogenotrophic microorganisms such as *Desulfovibrio* or the archeon *Methanospirillum hungatei*. Mutualism is also found during methan ogenesis. It involves many taxa that perform various steps in the complex pathway that transforms organic

compounds into acetate and eventually into methane. Sometimes the metabolic basis of the association is unknown, as in the case of the two archaea *Nanoarchaeum equitans* (Nanoarchaeota) and *Ignicoccus hospitalis* (*Crenarchaeota)* present in hydrothermal environ ments, which are in close interaction in a relationship described as symbiosis or parasitism (Jahn et *al.* 2008). These two organisms are closely nested into one another and cannot be cultured separately. Another interesting case concerns *Symbiobacterium thermophilum* (Firmicute) (Watsujiet*al.*2006), which can not be cultivated in the absence of a *Bacillus sp*. Both thermophilic bacteria are found in composts. *Bacillus sp*. provides *S. thermophilum* with CO_2 from its respiration, which allows *S.thermophilum* to compensate for the absence of a carbonic anhydrase, an enzyme that allows different processes such as photosynthesis, respiration, pH homeostasis, and ion transport. It also catabolizes indolic compound, which are self-inhibitors to *S. thermophilum*. Some times, diseases of plants and animals are caused by consortia or teams consisting of very different partners. This is the case of seedling blight of rice, caused by the fungus *Rhizopus microsporus*, which contains in its cytoplasm endosymbiotic *Burkholderia* bacteria necessary for production of the viru lence factor rhizotoxin and plant disease (Partida-Martinez and Hertweck 2005). The presence of *Burkholderia* within fungal tissues has been reported in many fungi. Biofilms found in soils, sediments, higher organisms, and man-made environments are complex assemblies that can include many taxa, some more active than others for the synthesis of exopolysaccharides. These polymers, which trap heavy metals or antibiotics, allow the development of a three-dimensional structure and protect all cells present, those synthesizing the polymers as well as the others. Microbial mats include many microbial taxa with complementary physiological properties such as sulfate reduction and sulfate oxidation, photosynthesis, and heterotrophy.

3.2.3. Cooperation

Trophic relations such as the ones above would correspond to cooperation if facultative. One of the main cases of cooperation between microorganisms is quorum sensing. Bacte ria have mechanisms that allow them to control certain physiological functions (such as conjugation) according to cell density, which is useful to ensure success. This quorum sensing mechanism is based on synthesis and perception of signals such as N-acyl homoserine lactone and is sometimes disturbed by higher plants or algae (Teplitski et *al.* 2000; Bauer and Robinson 2002). Thus, it has been shown that gamma-amino butyric acid (GABA) (synthesized by

plants upon wounding or infection by *Agrobacterium*) induces the catabolism of N acyl homoserine lactone and thus may modulate quorum sensing (Chevrot et *al.* 2006). Quorum sensing may also be affected by bacteria that synthesize lactonases, especially *Bacillus thuringiensis and Agrobacterium tumefaciens*, a mechanism that is being evaluated for biological control of various infectious agents.

3.2.4. Commensalism

It is difficult to identify situations of true commensalism between microorganisms, i.e., where a taxon derives benefits from an interaction but the other derives none. This could be the case of bacteria involved in nitrification, a process that occurs in two steps. Chemolithotrophic ammonia-oxidizing archaea and bacteria convert ammonium to nitrite, while nitrite-oxidizing bacteria Nitrobacter and Nitrospira convert nitrite into nitrate. Nitrite oxidizers obviously depend on the provision of nitrite by ammonia oxidizers, while the benefit for the latter is less obvious. However, nitrite is toxic for many taxa, including certain ammonia oxidizers, which would then benefit from nitrite remov*al.* The interaction involved would rather be a cooperation in this case. In many habitats, an element is limiting for bacterial growth and taxa with access to this element will be at the base of a food chain. This is the case of cyanobacteria capable of fixing carbon dioxide through photosynthesis, which are trophic partners of marine bacteria particularly those of the microbial loop. This is also the case for nitrogen-fixing bacteria in the rhizosphere and soils. There are other examples of commensalism, in particular, E. coli that is optionally aerobic, consumes oxygen, and renders the intestinal tract anaerobic and therefore suitable for Bacteroides, a strict anaerobe. Similarly, bacteria involved in milk souring have a fermentative metabolism and release acidic compounds that acidify the medium and provide favorable growth conditions for acid-tolerant lactic acid bacteria. Plants and microorganisms also synthesize and secrete compounds that are toxic to many microorganisms. In the same vein, many toxic compounds from human activity are found in the environment, where they selectively inhibit microbial taxa. Various microorganisms may degrade them, as illustrated with olive wastes metabolized by composting (Zenjari et *al.* 2006) or pentachlorophenol used as a wood treatment that is degraded by *Sphingobium* (Dams et *al.* 2007). These biodegrading microorganisms enable or enhance the growth of others.

3.2.5. Horizontal Gene Transfer Horizontal

Gene transfer may be mutually beneficial and would fall within the framework of coopera tion but is not easy to qualify in terms of ecological interac tion. The best-known case is that of antibiotic treatment in a medium comprising more than one microbial taxon, where initially most bacteria are sensitive to a compound to which they have never been in contact. Mutants resistant to this antibiotic will proliferate. Finally, the DNA fragment con ferring resistance to the antibiotic may be transferred to sensitive cells of the same taxon or belonging to a remote taxon. One of the first described cases of transfer of resis tance genes took place in Birmingham, England, in 1960. A care unit for burn victims found waves of nosocomial infections starting initially with *Klebsiella aerogenes* resistant to carbenicillin and carrying a resistance plasmid (RK2), followed by a second wave of Pseudomonas aeruginosa also carrying a closely related resistance plasmid. It has been shown that this phenomenon involved conjugative plasmid transfer (Ingram et *al.* 1973). Such cases of transfer of antibiotic resistance genes are common nowadays; they threaten our ability to curb infections and are one of the public health problems of greatest concern. Another case of gene transfer following a massive chemical selection pressure was found regarding mercury, a metal present in the bedrock and abundant in many natural or man made habitats such as hydroelectric reservoirs or gold pan ning workshops. It was shown that mer genes carried by conjugative plasmids or transposons and conferring the abil ity to volatilize mercury were transferred between phylogen etically distant bacteria by conjugation (Mindlin et *al.* 2002). A similar phenomenon has also been observed with man made compoundssuch as atrazine, currently the most widely used herbicide in the USA. When the herbicide is added to a field and ends up in the soil, it induces massive transfer to the soil microbiota of a plasmid carrying genes atz and trz for atrazine degradation (Devers et *al.* 2005). atz and trz are often on plasmids, but also on the chromosome near insertion sequences, suggesting that trans position plays an important role in the dispersion of this metabolic competence. Maintaining large plasmids is not neutral in terms of f itness if they do not carry essential genes, which is reflected in the existence of strains having lost their plasmids in the soil, as is the case for many bacteria in particular *Agrobacterium* (Krimi et *al.* 2002).

4. ROLE OF MICROORGANISMS IN THE ENVIRONMENT

We often believe that all microorganisms are harmful to man, but only a few species are pathogenic. The others play a major role in the environment.

- **Production of organic matter:** producing microorganisms can be photolithotrophic (algae, cyanobacteria) or chemolithotrophic (sulfo-oxidizing bacteria, nitrogen-fixing bacteria). In both cases, organic matter is synthesized through a series of complex metabolic reactions, the first of which constitute the Calvin cycle, where the CO_2 drawn from the environment is used to form oses through various specific metabolic pathways, the oses are transformed as required into reserve carbohydrates, lipids, amino acids and then into proteins, nucleic acids...etc
- **Decomposition of organic matter:** decomposing microorganisms are chemoorganotrophs (protozoa, fungi, bacteria). Decomposition is the set of reactions that lead to the mineralization of organic matter. There are two processes involved: putrefaction and fermentation, which are anaerobic degradations of protein and carbohydrate matter respectively. Mineralization also takes place under aerobic conditions through respiration.
- **Contribution to the equilibrium of multicellular organisms.**
- **Intervention in biogeochemical cycles. (***developped more later***).**

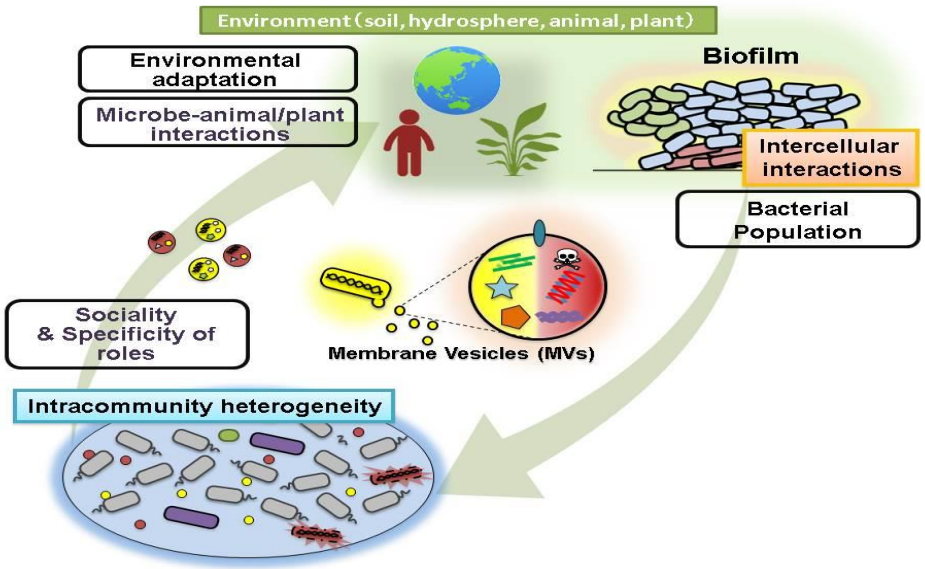

Figure 3 : Microbial ecology and Role of microorganism in ecosystem (https://www.onlinebiologynotes.com)

CHAPTER 2: WATER MICROBIOLOGY

CHAPTER 2: WATER MICROBIOLOGY

1. CHARACTERISTICS OF THE AQUATIC ENVIRONMENT

An important factor in aquatic environments is the movement of materials, whether gaseous, solid or dissolved. The mixing and movement of nutrients, oxygen and wastes that occur in both freshwater and marine environments are factors that control the microbial community. Gases and soluble wastes produced by deep-sea (marine and lacustrine) microorganisms stimulate the activity of other microbial groups. Similar phenomena can occur on a smaller scale: biofilms and microbial mats, where gradients are formed on a scale of a few micrometers. Aquatic environments vary greatly in surface area and volume, from alkaline to extremely acidic. The temperatures at which microorganisms thrive range from -5° to -15°C towards the depths, and up to 113°c in geothermal regions (Prescott et *al.*, 1995).

2. NATURAL WATERS

They include marine and freshwater waters:

2.1. Marine waters

Marine waters have high salinity. They are rich in minerals and gases dissolved. The microorganisms are either suspended or attached to surfaces under marine, or attached to sediments. This diversity includes extremophiles such as barophiles, halophiles and psychrophiles.

2.2. Fresh water

In fresh water, we observe the presence of algae, which often coexist with organic matter and dissolved minerals. Seasonal variations, such as changes in temperature, luminosity and nutrient availability can significantly influence the growth and distribution of algae in these habitats aquatic.

3. SELF-PURIFICATION OF NATURAL WATERS

Self-purification of water involves a complex cooperation between physical and biochemical processes such as: sedimentation, oxidation, exchange of substances volatiles between atmosphere and water, and the release of gaseous products from metabolism in the atmosphere. However, the critical role is played by biological factors. A broad a range of micro-organisms and higher organisms participate in the autopurification. **Bacteria and fungi**

are the most crucial because they are able to mineralize various mineral components. Proteins, simple and complex sugars, fats, cellulose, lignin, wax and others undergo degradation during the autopurification. As a result of mineralization, compounds are created (CO_2, NO^{3-}, SO_4^{2-}, PO_4^{3-}...). With the progression of self-purification, populations of micro-organisms that act in the environment change.

Self-purification of water = degradation of organic matter (decomposition by micro-organisms) + removal of mineral forms from nitrogen (nitrification and denitrification) + elimination of microorganisms (amensalism, predation, autolysis...).

The most commonly encountered scrubbers are *Pseudomonas, Acinetobacter and Flavobacterium, Aeromonas and the Enterobactériaceae. Clostridium and Desulfovibrio* also intervene in the purification. **The algae**, by photosynthesis, ensures oxygenation of the environment. Aerobic fermentation or oxidation of organic matter by microorganisms lead to the formation of mineral matter. In the absence of oxygen, fermentation releases methane and often harmful sulphides for the ecosystem.

Finally, total nitrogen and sulphur mineralization is carried out using chemolithotrophs. Nitrogen in its forms: nitrate (NO3-), nitrite (NO2-) ammonium (NH4+) is removed by: Nitrification: During nitrification, ammonium is transformed into nitrite and then nitrate into aerobic conditions, under the effect of microorganisms: *Nitrosomonas and Nitrobacter*. Denitrification: Denitrification takes place under anaerobic conditions. The breakdown of ammonium directly into N_2 can be achieved by bacteria Anammox.

4. NATURAL WATER MICROBIOLOGY

4.1. Marine waters microorganisms

Phytoplankton: diatoms, Xantophyceae, dinoflagellates, flagellates.

Zooplankton: dominated by protozoa: foraminifera, radiolarians...

The most common bacteria are cyanobacteria such as *Oscilatoria* (e.g.: Red Sea). Gram - bacteria are generally found because they have an outer membrane containing exo-enzymes used to degrade organic matter in the external environment, so they don't need to synthesize enzymes and therefore don't lose energy. Example of Gram -*: Flavobacterium, Pseudomonas, Aeromonas, Vibrio, Acinetobacter*. Gram + example: *Bacillus, Clostridium (in spore form) and Staphylococcus*

4.2. Freshwater microorganisms

Water is a medium that allows the survival and development of a wide variety of organisms (Table 1). Microbial diversity depends on the nutrients available, and their various centrations (ranging from extremely low levels to very high), transitions from aerobic to anaerobic zones, and the mixing of oxidants and of reducing agents in this dynamic environment. Furthermore, the penetration of light into many anaerobic zones creates environments favorable to certain types of photosynthetic microorganisms.

Cold, clear ground water may contain just a few hundred bacteria per milliliter, whereas surface water (river, lake, etc.) loaded with sediment and organic matter may contain several thousand.

The main source of organic matter in illuminated surface waters is activity photosynthetic, mainly due to phytoplankton. **Synechococcus** is a common genus in plankton; it can reach densities of 10^4 to 10^5 cells per millilitre, at the surface of the oceans. *Picocyanobacteria* (very small cyanobacteria) can represent 20 to 80% of the total phytoplankton biomass on which predators depend.

Table 1: Surface water microorganisms

Microorganisms		Genus
Bacteria	Photolithotroph	*Chlorobium, Chromobacterium*
	Chemolithotroph	Bactéries fixatrices d'azote : *Azotobacter, Aerobacter* Bactéries nitrifiantes : *Nitrozomonas, Nitrobacter* Bactéries méthanogènes : *Methanobacterium, Methanococcus*
	chemoorganotroph	*Flavobacterium, Sarcina, Serratia, Pseudomonas, Micrococcus, Bacillus, Clostridium, Nocardia, Escherichia coli, Proteus vulgaris, Streptococcus faecalis, Enterobacterb aerogenes, Salmonella typhi, Vibrio*...etc
Algae		*Euglena, Chromulina, Navicula, Nautococcus*...etc
Fungi		*Cladosporium,* levure...etc
Protozoa		*Vorticella, Stentor, Paramecium*

5. FOODWEB AND MICROBIAL LOOP

In pelagic zones of lakes, carbon and energy circulates within a complex web of interactions between the organisms and their biotopes, and between organisms themselves. The organic carbon (primary production) is synthetized from mineral carbon (CO_2) by photosynthesis performed by macrophytes, phytoplankton and at a lesser extent by photo- and chemosynthetic bacteria. Some heterotrophic bacterial species are also able to incorporate CO_2 by anaplerotic reactions. Then, the phytoplankton is ingested by herbivorous zooplankton (predation) which itself consumed by planktono phagous fishes. Finally, these planktono phagous fishes are prey for carnivorous fish species. Metabolites released by living organisms and those resulting in the post mortem degradation of phytoplankton produces DOM that initiates the functioning of a trophic network named **"microbial loop"** (Amblard et *al.* 1998).

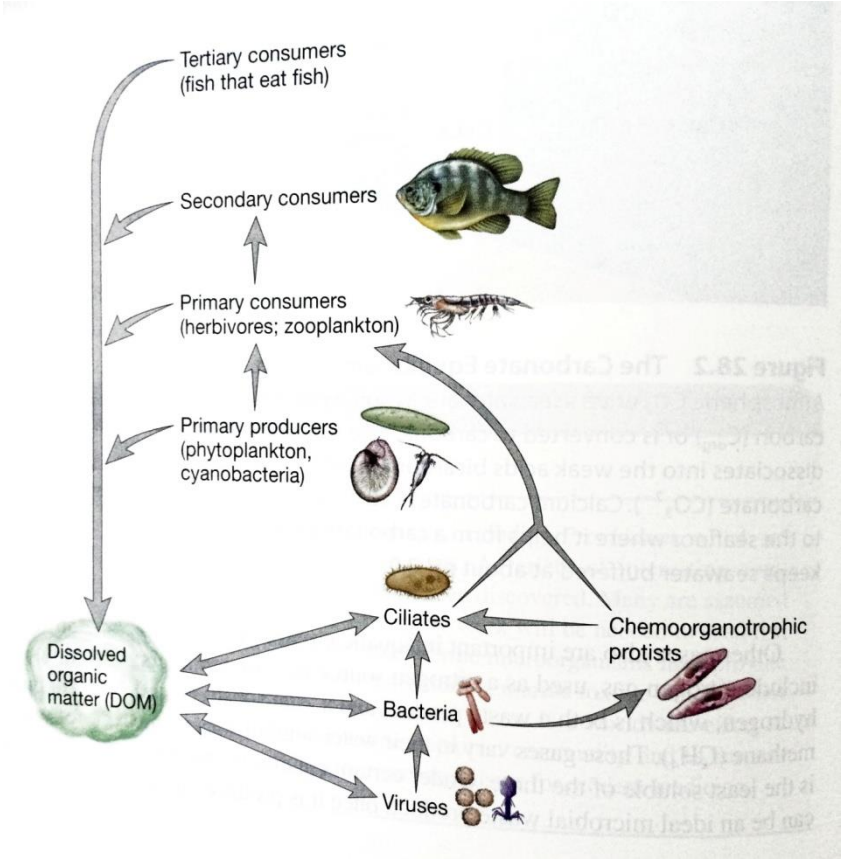

Figure 4 : Microbial loop (https://www.ritubiology.com/2016/07/01/aquatic-microbiology-iii-food-chains-food-webs/microbial-loop/)

Then, these products are degraded and metabolized by heterotroph prokaryotes that are abundant in pelagic zones of lakes and by pico-cyanobacteria and pico-eukaryotes. Then, these microorganisms are ingested by the zooplankton; their major predators are the uni cellular mixotrophic- and heterotrophic eukaryotes (ciliates, Dinobiontes, "amibs," etc.). Other unicellular eukaryotes serve also as prey to Ciliates which are then consumed by the metazoan of the zooplankton. The detritic allochthonous OM, particular or dissolved, is also degraded by the microorganisms. This transfer of matter and energy accumulated in prokaryotes, via the bacterivorous organisms, ensures a better recycling of the organic matter.

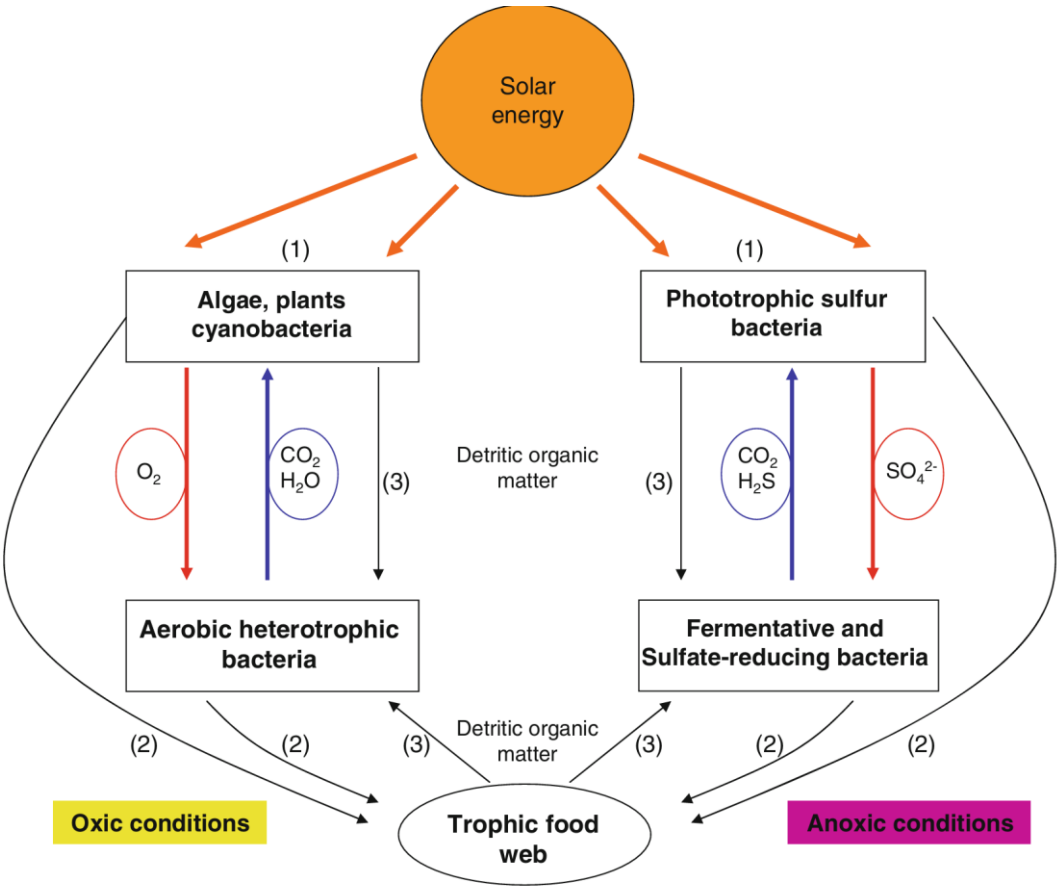

Figure 5 : Comparison of the primary production (oxygenic photosynthesis due to algae, macrophytes, and cyanobacteria) and para primary production (anoxygenic phototrophic bacteria) in aquatic ecosystems and their contribution to food webs. 1 photosynthetic production, 2 organic matter incorporation in food webs, 3 organic matter degradation

6. DRINKING WATER TREATMENT

When water is obtained from intact reservoirs fed by a clear mountain stream or from deep wells, it requires a minimum of treatment to make it potable. Many cities, however, obtain their water from polluted sources, such as rivers that have received municipal and industrial waste upstream. Water treatment is not intended to produce sterile water that is free of pathogenic microbes (Tortora et *al.*, 2010).

Water purification can involve a variety of steps, depending on the type of impurities in the raw water source.

Water purification can involve a variety of steps, depending on the type of impurities in the raw water source. For example, if the raw water contains large quantities of iron and manganese, which will often precipitate when the water is exposed to air, it may be necessary to aerate the water and use other methods to remove these ions early in the purification sequence. Typically, municipal water is purified by a process consisting of the following stages. :

6.1. Coagulation

Coagulation is often the first step in a water treatment process. When particles are slow to settle or are non settling, chemicals (coagulants) with a positive charge such as aluminum are added to the water. These react with the unwanted particles to form larger particles, called floc. The larger size and weight of the flocs then causes them to settle rapidly.

6.2. Flocculation

Flocculation follows coagulation. When chemicals (coagulants) are added to the water, gentle mixing occurs to form larger particles called flocs. Additional chemicals such as alum (potassium aluminum sulfate) and lime, may be added during this step to aid in forming the flocs. Both coagulation and flocculation are effective at removing fine, suspended particles that attract and hold bacteria and viruses to their surface. These first two steps can remove up to 99.9% of the bacteria and 99% of the viruses from water supplies. They also remove some of the organic matter that gathers as water travels across the landscape, from raindrop to river. However, certain taste and odor problems may remain.

6.3. Sedimentation

As flocs are formed, the larger size and weight of the flocs cause the large particles to settle rapidly. These particles settle because they are heavier than the water. And some particles will spontaneously settle out from standing water. This is the sedimentation process.

6.4. Filtration: After flocculation, the water is further purified by passing through a filtration unit. Sand filters, which rely on the physical trapping of fine particles and flocs, are usually used for this purpose. This filtration removes up to 99% of remaining bacteria (Prescott et *al.*, 2010). Some protozoa, cysts and oocysts are only removed from the water by this filtration. Most micro-organisms are trapped by adsorption on the surface of sand particles. These systems can be complemented with activated carbon filters. Carbon removes not only fine particles, but also dissolved organic chemical pollutants. This treatment system can remove viruses (which are more difficult to eliminate than bacteria and protozoa) with an efficiency of around 99.5%. Low-pressure membrane filtration is also used, with pore openings as small as 0.2 μm (Tortora et *al.*, 2010).

6.5. Disinfection: After filtration, the water is treated with a disinfectant. This stage generally involves chlorination or ozonation, the latter becoming increasingly popular. When chlorination is used, and given that organic matter neutralizes chlorine, constant attention must be paid to maintaining effective chlorine levels. The chlorine dose must be large enough to leave free chlorine residual at a concentration of 0.2 to 2.0 mg/liter. However, the creation of disinfection by-products (DBPs) is a cause for concern, such as trihalomethanes (THMs), which form when chlorine reacts with organic matter. Some of these compounds may be carcinogenic. Ozone treatment is popular because it leaves no taste or odour. Because it has little residual effect, ozone is usually used as a primary disinfection treatment, followed by chlorination. The use of UV light is also a complement or alternative to chemical disinfection (Prescott et *al.*, 2002, Tortora et *al.*, 2010).

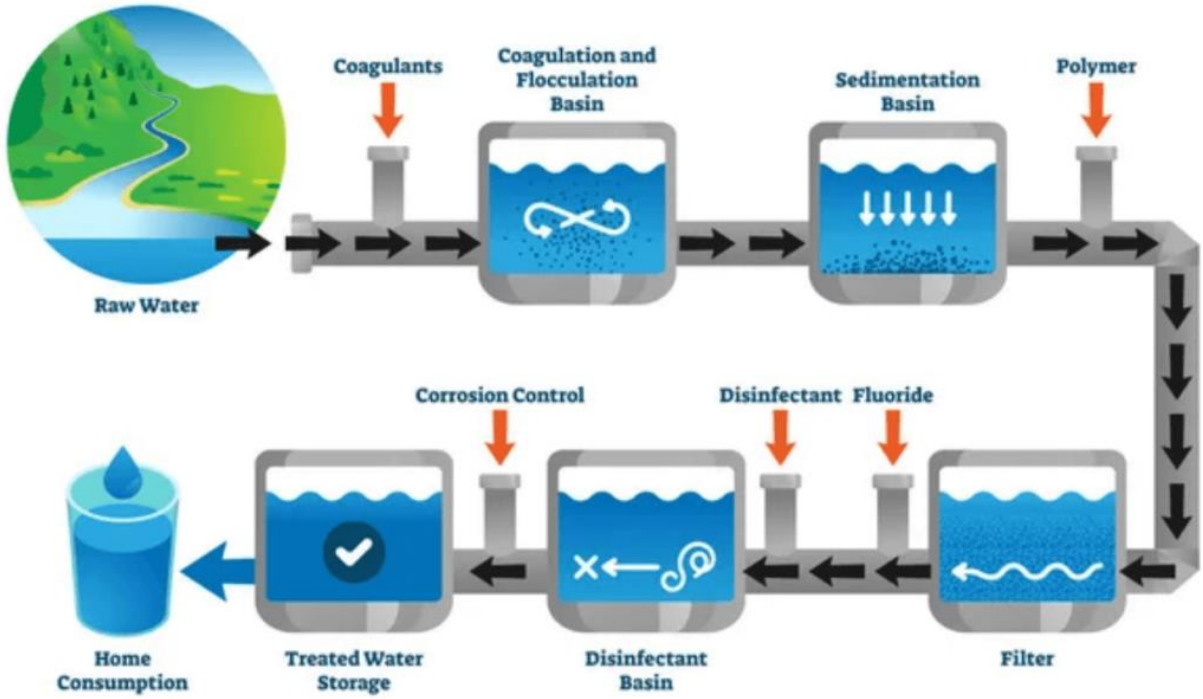

Figure 6 : Water purification plant (https://spartanwatertreatment.com/drinking-water-treatment-overview/)

7. QUALITATIVE WATER ANALYSIS

Historically, most of our concerns about water purity have been associated with disease transmission. Consequently, tests have been developed to determine the safety of water (Tortora et *al.*, 2010).

Health monitoring and the detection of pathogenic micro-organisms are an important part of microbiology. A wide range of viral, bacterial and protozoan diseases result from contamination of water by human faeces. Although many of these pathogens can be detected directly, environmental microbiologists use specific organisms as indicators of pathogen contamination of water (Prescott et *al.*, 2002).

Coliforms, of which Escherichia coli and Enterobacter aerogenes are members of the Enterobacteriaceae family, account for around 10% of intestinal microorganisms in humans and animals. These bacteria are widely used as indicator microorganisms, losing their viability in freshwater at a slower rate than most major intestinal pathogens. When such enteric indicator bacteria are not detectable in a specific volume (100 ml) of water, the water is considered potable [from the Latin potabilis: fit to drink], for human consumption (Prescott et *al.*, 2002, Tortora et *al.*, 2010).

7.1. Fermentation test

Coliforms are defined as gram-negative facultative anaerobic non-sporulating rods, fermenting lactose with gas formation within 48 hours at 35 ° C. The original coliform test used to meet this definition involves a presumptive, a confirmatory and a final demonstrative test. The presumptive stage is performed using tubes inoculated with three different volumes of sample to give an estimate of the most probable number (MPN) of coliforms in the water. The whole process requires at least 4 days for transfers and incubations. Unfortunately, coliforms comprise a wide range of bacteria whose primary source may not be the intestinal tract. To overcome this difficulty, tests have been developed to detect the presence of fecal coliforms. These are coliforms from the intestines of warm-blooded animals, which can thrive at the more restrictive temperature of 44.5°C.

To test for the presence of coliforms and fecal coliforms, a series of simple, specific and more effective tests have been developed to recover stressed coliforms. These include the membrane filtration technique, the coliform presence-absence (PA) test and the Colilert test for detection of coliforms and E. coli by a defined substrate (Prescott et *al.*, 2002).

7.2. Membrane filtration technique. The membrane filtration process is a more direct method for determining the presence and enumeration of coliforms, to assess the microbiological characteristics of water. The water sample is passed through a membrane filter of specified porosity (0.45μm). The filter, bearing the trapped bacteria on its surface, is transferred to a solid support of suitable medium. Use of the appropriate medium enables rapid detection of total coliforms (Endo), fecal coliforms (bile salt medium (m-FC agar) containing blue aniline dye), and fecal streptococci (azide-containing medium (KF agar) with TTC (triphenyltetrazolium chloride) by their characteristic colonies. This method is suitable for waters with low turbidity (which may clog the filter), and containing relatively few bacteria (non-coliforms) that would mask the results (Prescott et *al.*, 2002, Tortora et *al.*, 2010).

7.3. P/A tests and ONPG and MUG tests

More simplified tests for detecting coliforms and fecal coliforms are currently available. The presence-absence test (PA test) can be used for coliforms, in which a water sample (100 ml) is incubated in a single culture flask with a triple broth concentrate containing lactose, tryptose lauryl broth, and purple bromocresol as an indicator. The PA test is based on the assumption of total absence of coliforms in 100 ml of drinking water. A positive test results in the

production of acid (a yellow color) and constitutes a presumptive positive test requiring confirmation (Prescott et *al.*, 2002).

A more recent and convenient method for detecting coliforms, specifically the fecal coliform E. coli, has been developed by the Colilert defined substrate test. A 100 ml water sample is added to a specific medium containing o-nitrophenyl-ß-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-ß-D-glucuronide (MUG) as the only nutrients. Coliforms produce ß-galactosidase, which acts on ONPG, releasing o-nitrophenol and giving a yellow color within 24 hours at 35°C, indicating their presence in the sample. E. coli is the only coliform that almost always produces ß-glucuronidase. To verify this, the medium is observed under UV light. When E. coli is present, a fluorescent product is observed. If the test is negative for the presence of coliforms, the water is considered fit for human consumption.

These simple tests can detect the presence or absence of coliforms or E.coli, and can be combined with the multiple tube method to enumerate them. It can also be applied to solid media, such as the membrane filtration process, where colonies fluoresce under UV light (Prescott et *al.*, 2002, Tortora et *al.*, 2010).

Other indicator organisms. Fecal enterococci are increasingly used as an indicator of fecal contamination in brackish and marine water. In salt water, these bacteria die at a slower rate than fecal coliforms, providing a more reliable indicator of possible recent pollution (Prescott et *al.*, 2010). An even bigger problem is that some pathogens, particularly viruses and protozoan oocysts, are more resistant than coliforms.

An even bigger problem is that some pathogens, particularly viruses and protozoan oocysts, are more resistant than coliforms to chemical disinfection. Thanks to the use of sophisticated virus detection methods, it has been shown that chemically disinfected water samples free of coliforms are still often contaminated with enteric viruses. *Giardia lamblia cysts and Cryptosporidium* oocysts are so resistant to chlorination that their complete elimination by this method is probably impossible, and mechanical methods such as filtration are necessary. As a general rule, viruses are more resistant to chlorination than *E.coli, and Cryptosporidium and Giardia* cysts are 100 times more resistant than viruses (Tortora et *al.*, 2010).

7.4. Molecular techniques

Molecular amplification techniques using real-time PCR are also used to detect viral and coliform contamination of water (Weinert et *al.*, 2010).

8. WATER POLLUTION PHENOMENA

8.1. Eutrophication

It is a natural or anthropogenic phenomenon that corresponds to an excessive increase of the amount of organic matter in aquatic ecosystems, including lakes, lakes and ponds, rivers and estuaries. It is a slow process that occurs as follows:

- Release of organic matter
- Excessive intake of nitrates, phosphates and ammoniums
- Excessive growth of algae and aquatic plants
- Reduced transparency
- Accumulation of organic matter
- Decomposition of organic matter by aerobic bacteria while consuming oxygen
- Decreased level of dissolved oxygen in depth
- Dominance of harmful anaerobic bacteria producing methane and sulphide of hydrogen
- The ecosystem becomes anoxic which leads to the death of fish, invertebrates and aquatic plants: disappearance of the biocenosis (irreversible change)

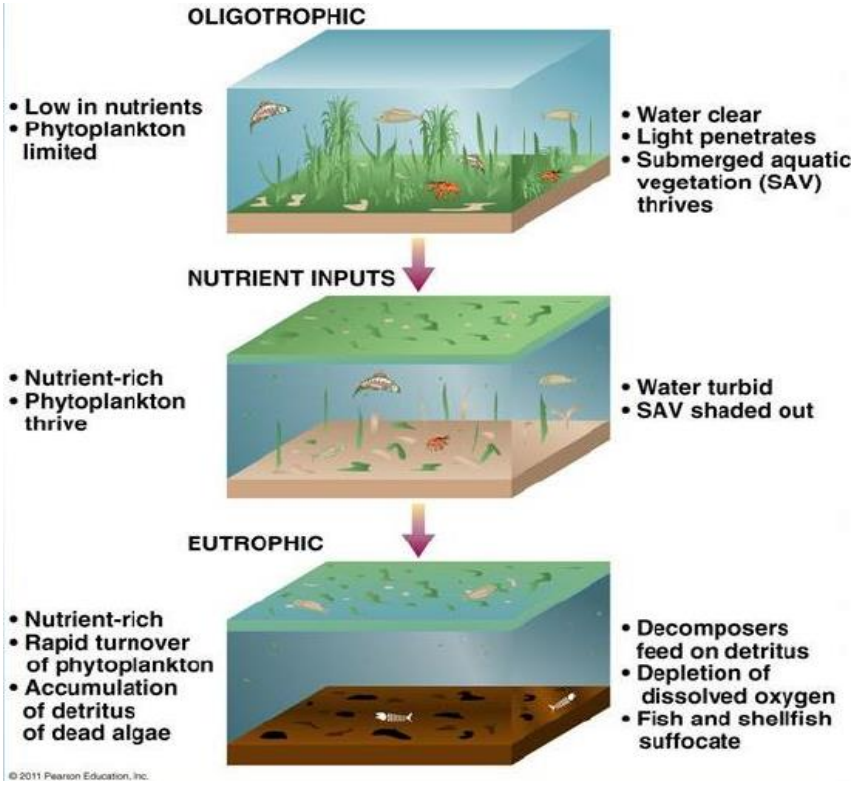

Figure 7 : Eutrophication of aquatic ecosystem (https://oneclass.com/class-notes/ca/u-of-manitoba/envr/envr-1000/2594503-envr-1000-lecture-3.en.html)

Eutrophication can be caused by natural sources, such as nutrient inputs by rivers and streams, or by anthropogenic sources such as water discharges tion, agricultural fertilizers and industrial activities.

8.2. Bacterial biofilm

Biofilm A biofilm is defined as an agglomeration of microorganisms adhering to the surface of a hard substrate or which develops at an interface between two phases (e.g., liquid-solid, liquid-liquid, liquid-gas, solid gas). Biofilm formation is a natural phenomenon and biofilms are ubiquitous. They will occur on many occasions, when a non-sterile liquid enters into contact with a solid surface; many of the prokaryotes occurring in the liquid will progressively adhere to the surface. During the adhesion, many of these prokaryotes will express their capacities to synthesize and excrete biopolymers. As a result, bacteria in biofilms are surrounded by these polymers, which often confer protection and enhance their adherence to the surface. These biopolymers are also known as extracellular polymeric substances (EPS), which mainly comprise polysaccharides (polymers of sugar molecules) and proteins (**Bertrand et *al.*, 2015).**

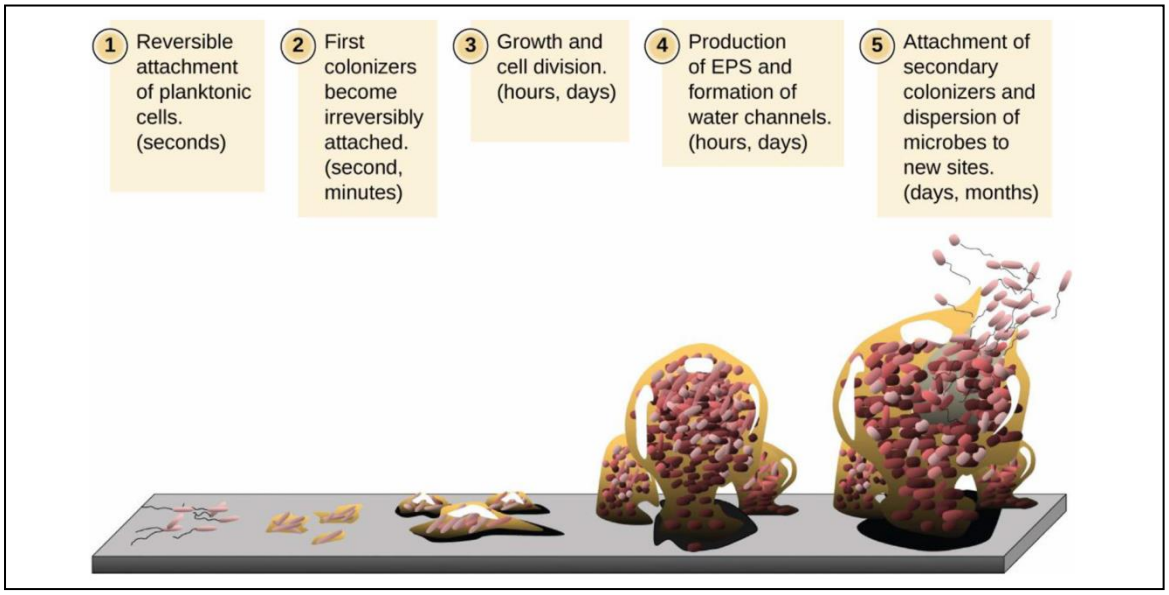

Figure 8 : Description of various stages involved in the develpment of a biofilm. 1. Bacterial adhesion to the surface, 2. Cell-to-cell adhesion, 3. Attached cell monolayer, 4. Maturation of a biofilm and formation of exopolymeric substances and 5. Detachment. (Tshikantwa et *al.*, 2018)

a) Initial attachment

Free (plankton) bacteria attach themselves reversibly to a surface, often by means of electrostatic and hydrophobic forces. This stage is still fragile and bacteria can easily break off.

b) Irreversible attachment

The bacteria begin to secrete adhesive substances such as extracellular polysaccharides (EPS), which allow a more stable and irreversible fixation to the surface.

c) Formation of microcolonies

Attached bacteria proliferate and form microcolonies. The production of EPS increases, forming a matrix that encapsulates bacterial cells and provides protection against external conditions.

d) Maturation of biofilm

Microcolonies develop into complex three-dimensional structures. Water channels are formed inside the biofilm, allowing the diffusion of nutrients, oxygen and waste.

e) Dispersion

In this last step, some bacteria leave the mature biofilm to colonize new surfaces. This process can be triggered by environmental changes or chemical signals, allowing bacterial populations to spread and survive.

9. ORIGIN AND CONTENT OF WASTEWATER

Usually there are two main categories of wastewater (also called effluents):

(**i) Urban wastewater (or domestic)** resulting from house holdor commercial activities to gether with **rainwater (stormwater)**. It contains grease, soap, and detergents; suspended and organic or mineral dissolved solids; and a very large diversity of microorganisms. Household wastewater consists of greywaters and waters from toilets, baths, and sinks.

(ii) Industrial wastewater discharged by factories. Unlike domestic wastewater, they are characterized by their wide diversity according to theuse of water during the industrial process.

UWW is characterized by different parameters, the most important of which are suspended solids (VSS) and the weight in oxidizable matters, mainly organic.

Oxidizable matters in waters are characterised by two quantitative indices:

- Chemical Oxygen Demand (COD), which corresponds to the amount of oxygen necessary to the chemical oxida tion of the totality of the reduced organic and mineral compounds
- Biochemical Oxygen Demand (BOD_5), which corres ponds to the amount of oxygen consumed by organisms in 5 days to oxidize the reduced organic and mineral compounds
- Other parameters are taken into account: nitrogenous matters expressed in total nitrogen (TN) corresponding to the sum of organic and ammonium nitrogen ($N\text{-}NH4^+$) and phosphorus matters expressed in total phosphorus (TP) and/or in phosphorus ($P\text{-}PO_4^3$). It is note worthy that 95 % of urban wastewater consist of biodegrad able matter. The remaining part discharged in environment is then submitted to the action of natural biodegradation processes.

10. WASTEWATER TREATMENT

Because of the discharge diversity, treatments (and pretreatments) are carried out through physicochemical and biological processes. The wastewater is submitted to the physico-chemical treatments before the microbiological treatment. The different treatment processes depend on the origin of the pollution, on its chemical composition (organic or mineral), and on its soluble colloidal or particulate form. Various processes have been implemented and take into account the UWW volume to treat, the economical constraints linked to the functioning of the WWTP, and the quality of water required which depends on the discharge area.

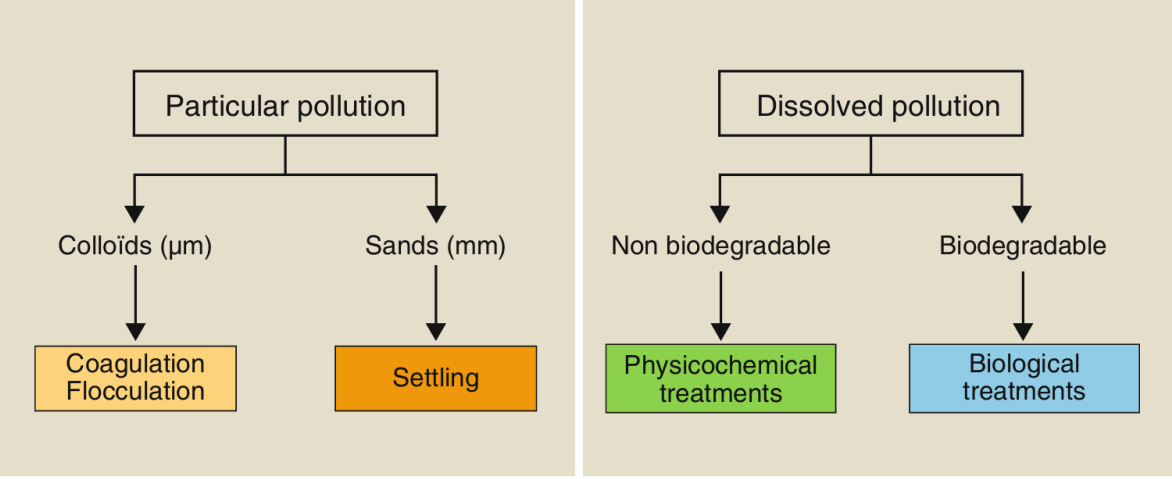

Figure 9 : General outline of the various methods used in a wastewater treatment plant

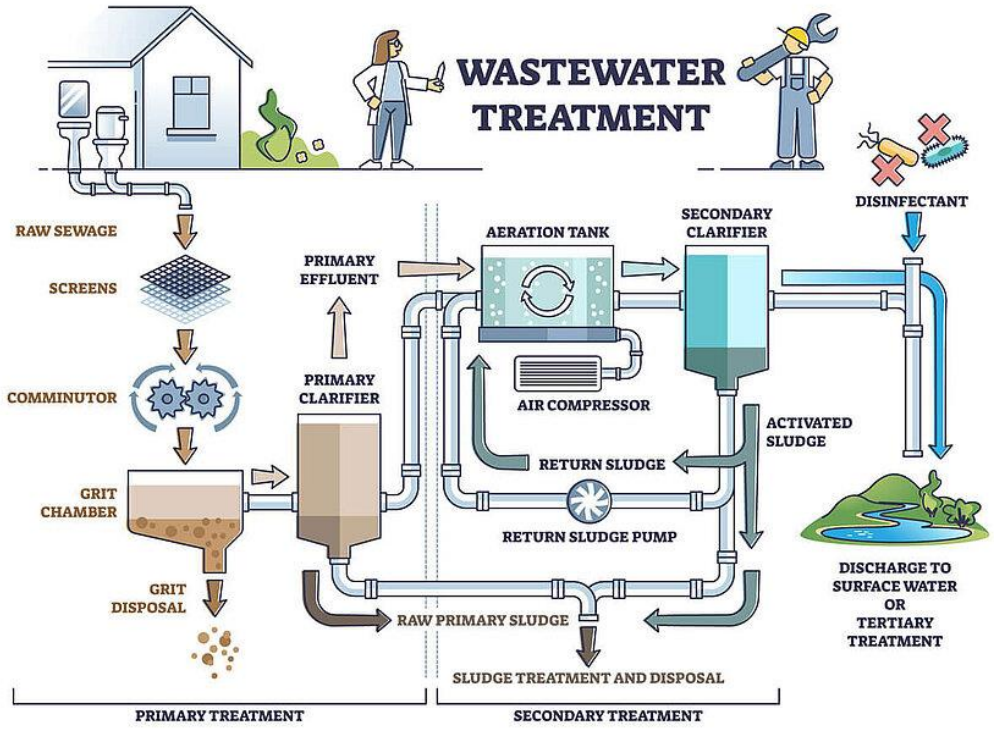

Figure 10 : Wastewater treatment plant (https://www.elementar.com/en/blog/efficient-wastewater-treatment-and-the-role-of-toc-in-process-control)

10.1. Pretreatments

Pretreatments enable the treatment plant to operate properly, but they generate some operational constraints linked to the refusal recovery, sanitation, and maintenance. The WWTP are usually equipped with the following preliminary treatments:

• **Screening, Sieving**: separation by size

• **Grit removal, Degreasing, Settling**: separation by density or density ratio to water.

These pretreatments enable the elimination of the coarse matters, which are liable to damage mechanical parts and/or hamper the efficiency of the subsequent steps such as settling or aeration of the biological tank. From Coarse to Fine Screening The purpose of screening when water passes between the grids or through the holes is to keep any matter bigger than the cross. So, it protects mechanical equipments and prevents pipe clogging. The separation limit is based on the more or less fine meshes of the grids, which can be straight or curved: (i) Coarse screening or prescreening for the mechanical retention of the heaviest waste (papers,

textile fibers, plastics, pieces of wood, etc.): width > 40 mm (ii) Average screening for the mechanical screening of all the little compounds (plastics, etc.): width > 10 mm (iii) Fine screening: width < 10 mm.

Periodically, a rake or comb moves up along the grid to get rid of the waste stuck and then spills it into a receptacle placed behind the grid. It is then compacted, put in dumpsters, and conveyed in a final waste storage center, and it will not be treated by sludge process. After grit removal, water must be raised from a low level (wastewater gravity collection in the WWTP low point) to a high level (+10 m) to allow a gravity flow in the WWTP (run-of-river). No pumping equipment will be used in the WWTP. This raise must not chop oil slicks; hence, Archimedean screws are used. Grit Removal Degreasing Water will be submitted to a degritting and a degreasing phase. The purpose is to eliminate the heaviest Volatile Suspended Solids (VSS) by settling and grease residues by f lotation. To recover grease residues and sand, a surface and bottom scraper placed on a bridge crane pushes the compounds towards the end. Air is transferred in an air diffuser to accelerate the elimination of greases. Thus, the created bubbles (d ¼ 1 mm) will bring greases to the surface. The more important the incoming flow is, the higher the rate of climb will be. The recovering of sand in wastewater avoids the following: **(i)** Operating problems of installations linked to the sedimentation of sands in aeration basins, in clarifiers, and in digesters; (**ii**) Problems of excessive wears of stirrers as well as pipe clogging or intermediate structures.

Sand settling is a grain settling with a constant fall velocity (the size, form, and density of the solid particle do not vary). After this step, a fine screening is proposed (1 cm) to eliminate the little VSS and particularly packing of fibers. Sand and fibers are evacuated towards final waste storage centers. At this processing stage, the fine VSS are still present and leave them settling by gravity would be too long compared with the flow coming into the plant. This quantity of VSS represents about 10 % of the incoming VSS. To increase the settling velocity, VSS will be artificially thickened (coagulation–flocculation) because these fine particles can be assimilated to resin grains exchanging generally negatively charged ions.

Figure 11 : Example of screening. (a) Grid screening; (b): grid and comb for elimination of accumulated pollution on the grid (Photographs courtesy of Societe des Eaux de Marseille)

Figure 12 : Screw lift (treatment plant of the town of Vitrolles, France) (Photo: courtesy of Socie´te´ des Eaux de Marseille)

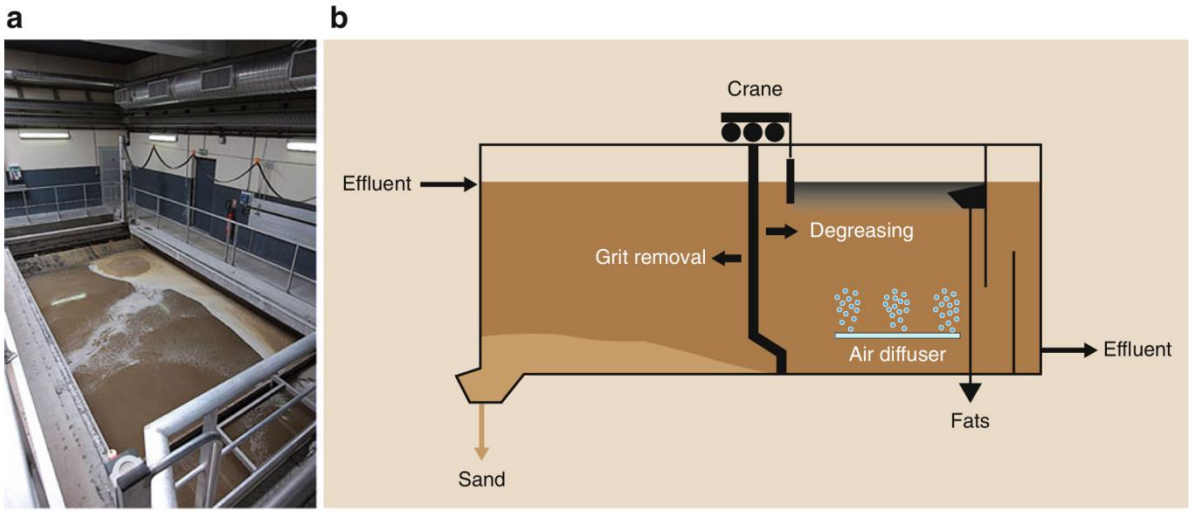

Figure 13 : Basin grit and oil removal with crane to scrape the surface and eliminate oils and deposited sands. (a) Photograph of a disposal (Photo: courtesy of Socie´te´ des Eaux de Marseille). (b) Longitudinal section of a basin

10.2. Primary Treatments or Physico chemical Treatments

Coagulation–Flocculation

The particles involved by these treatments are colloids. Their origin varies: soil erosion, dissolution of mineral substances, decomposition of organic matters, etc. These particles have a very small diameter (less than 1 μm) and are particularly responsible for the color and the turbidity of the surface water. The decantation velocities of these particles are very slow: coagulation–flocculation is therefore necessary. The main purpose of coagulation is the destabili zation of the suspended particles, to make easy their agglom eration. Flocculation facilitates contact between destabilized particles. They begin to agglomerate to form a floc that will be eliminated by settling. However, as colloids are negatively charged, they repel one another and make any agglomeration impossible. If repulsions are eliminated, particles will agglomerate, grow, and settle quickly. The addition of high oxidation state cations such as Fe^{3+} and Al^{3+} avoids the repulsion of electric charges. So, in the WWTP, charges will be brought by a small quantity of charged coagulants ($FeCl_3$, $Al_2(SO_4)_3$). During this process, phosphates precipitate in the form of insoluble salts.

Settling

Settling is the step after coagulation–flocculation. It is car ried out in various types of settlers: horizontal, vertical, or lamellar settlers (Degremont, 2008). Since inlet of the plant, the water has generally been submitted to these pretreatments in confined premises and at low pressure to prevent the diffusion of unpleasant odors in the atmosphere. These odors will generally be treated with a chlorinated solution and soda. Flocculation, coagulation, and settling are also part of the elimination process of microorganisms, particularly of enteric origin, among which some of them are pathogenic.

10.3. Secondary Treatments

Secondary treatments have biological treatments with or with out oxygen and a settling step in the activated sludge process. Among biological treatments, a distinction is made between extensive and intensive biological processes.

a) Extensive biological processes

Artificial marshes or lagoons use aquatic plants (floating, emergent or submerged) and their associated microorganisms to treat liquid household waste, industrial effluents or mine drainage water.

Lagooning uses the purifying capacity of shallow water bodies. Wastewater is sent to a series of basins. Oxygen is supplied by exchanges with the atmosphere. Organic pollution is degraded by bacteria present in the water. This treatment method eliminates 80 to 90% of BOD and 20 to 30% of nitrogen, and contributes to a significant reduction in germs. It does, however, have the disadvantage of requiring large surface areas.

Extensive Water Treatments in Lagoons and Planted Beds

Wastewater treatment has tobe objective to reach water quality criteria in terms of suspended solids, organic matter, andnutrients before its release into the environment. Many systems use biological activities, which, inwastewater treatmentplants, are optimized to achieve maximum efficiency. These biological activitiesmay alsobe used in extensive treatment processes suchas lagoon treatment plants o rmacrophytic treatment plants.These treatments are less efficient in terms of performance (degradation rate, residence time) but not in the quality of the treated water. In both cases, the role of microorganisms is essential. The lagoon treatment process is a

natural alternative to other treatment systems for the purification of urban sewage and industrial effluents (e.g., food, paper). In lagoon treatment systems, raw sewage will stay in several basins (usually three), shallow (maximum 1.5m) and of great area. The biological activities of aerobic and anaerobic degradation, as well as primary production and grazing by zooplankton, allow purification in the successive lagoons.There si dence time in this case is very long (50–80 days), which makes these system shaving large buffer capacities with respect to changes in load and effluent flow. As in the large majority of treatment systems, the first steps include a screening for there moval of large size solids, a grit chamber to eliminate sand and other solid deposits, and oil/water separation that removes fat that may disrupt the treatment system. The pre-treated effluent is then discharged into the first and largest basin, which provides aerobic (in the water) and anaerobic (sludge and sediment) bacterial degradation of most of the organic load. The main anaerobic metabolisms involved are fermentations, nitrates respiration (including denitrification), and methanogenesis. If the lagoon treatment contains brack ish water (coastal environments), sulfate reducting activ ity can cause problems related to the production of smelly and toxic sulfide. These mineralization activities produce large quantities of mineral salts (NH^{4+}, NO_2, PO_4^3) and gas (N_2, CH_4, CO_2, H_2S). The second, shallower pond, allows primary production by microalgae that will use the nutrients originating from organic matter degra dation in the first pond. Due to the presence of oxygen arising by oxygenic photosynthesis, degradation proceeds through aerobic metabolism. The third pond allows the development of primarily herbivorous zooplankton (protozoans and metazoans), which will reduce the microalgae load. There are variations to this lagoon treatment process: (i) Aerated lagoons replace oxygen production by microalgae by forced ventilation. Only two ponds are then required (degradation and sedimentation), thus reducing the surface. This system generates costs due to forced aeration.

(ii) Anaerobic lagoons require deeper ponds (more than 4 m) and high organic load for the establishment of anoxic conditions. For proper operation, the temper ature should be high (at least 25 C). Epuration yields through lagoon treatment are good, and most important, it allows excellent treat ment of pathogenic microorganisms due to residence times that are long enough to permit competition between microorganisms (indigenous microorganisms out-competing nonnative microorganisms). Pathogen elimination is also due to the action of light radiation (UV). This explains why lagoon treatment systems are sometimes used as tertiary treatment after activated sludge or extended aeration. This process has very low operational costs and excellent integration into the landscape. The investment depends on the size of the

basins, which, in turn depend on the volumes of effluent to be treated. Thus, for big cities, the area required (10–20 m^2 per capita equivalent) is often a limiting factor. In lagoon treatment processes, primary production is driven by microalgae. It can also be driven by macrophytes, but the use of the latter takes place in the context of planted beds. These processes use macrophyte plants (reeds, iris) and rhizosphere microorganisms for treatment. The roots of plants provide oxygen and simple organic molecules that promote bacterial aerobic growth. Depending on the distance to the roots of the plants, degradation will be aerobic or anaerobic. Planted beds are of ten coupled with sand filters (vertical or horizontal) for individual effluent treatments. The planted beds, in addition to good overall degradation, accumulate large quantities of metals and thus ensure their effective decontamination. Obviously, the biomass has no value and in this case should be incinerated. The metals contained in the ash can be recovered by bioleaching.

Figure 14 : Aerial view of the lagoon treatment plant of Meze (Herault, France) showing successive ponds used for wastewater purification. Pools 1, 2, and 3 correspond to those set up in 1980 during the installation of the station and using a classical lagoon treatment process. Pools 4 (two) correspond to tertiary treatments installed in 1996. The pools 5, installed in 1997, permit pretreatment (flocculation of raw material) under anaerobic conditions (deep ponds) with, in addition, aeration of the surface waters to reduce odors. Finally, in 2002, four high load material ponds (pools 6) have been installed to allow a more efficient aerobic treatment (Photograph courtesy from CCNPT, Lagunage de Meze)

b) Intensive biological processes

These encompass a whole range of techniques that use bacterial cultures to “consume” pollutants.

Aerobic Biological Treatments

Treatment of urban and industrial effluents mainly requires a biological process either under aerobic or anoxic, even anaerobic conditions (terminology used in process engi neering will be specified later). Processes vary according to the type of culture either free or fixed. In the first case, microorganisms are maintained in suspension in the liquid treated (activated sludge). In the second case, bacterial cultures are fixed on a solid support (pozzolan, gravel, Raschig rings) where they develop to form a biofilm, which will eliminate pollution. In processes of fixed cultures, microorganisms constitute bacteria beds* or develop on disks or other granular beds. These processes are not detailed here because they are less and less attractive due to their excessive dimensioning to obtain a suitable purification rate. The activated sludge process consists in a suspended biomass aeration tank where air is injected in order to provide oxygen. This is the most widespread process in the world to treat wastewater. This results from the composition of urban wastewater constituted at 95 % of biodegradable matters. The purpose of wastewater treatment plants is to degrade a maximum of organic matters, the remaining part is sub mitted to the action of the natural processes of biodegra dation. For example, in France, this process represents about 60 % of the wastewater treatment plants and 80 % of the treatment capacity.

In the aeration tank, pollutants in waters are next to living microorganisms which can metabolize them partly or totally according to their metabolic capacity. Free microorganisms are very few, most of them are agglomerated in a mucilage composed of exopolymers they synthesize. They form biomasses or bacterial flocs. Zoogloea ramigera, one of the microorganisms in the f locs, is associated to a great number of other bacterial species. To avoid the settling of flocs, activated sludge must be stirred. By malfunctions of the plant, an excessive amount of filamentous bacteria (*Thiothrix, Leucothrix, actinobacteria*, etc.) usually present in activated sludge may hamper the floc settling in the secondary settler; this dysfunction is called bulking sludge. Eukaryotic and heterotrophic microorganisms (flagellates, ciliates free or fixed on the flocs, amoeba, etc.) and metazoa (rotifers, nematodes, etc.) living in the activated sludge feed on free bacteria, the biggest also catch small eukaryotic microorganisms; some (amoeba, nematodes) can ingest the floc biomass. Sludge

eukaryotes take part to the elimination of pathogens and facilitate the development of flocs, whose exopolymers bring a better protection of prokaryotes against environmental factors. Based on the observation of this microfauna, which is very sensitive to the variations of in situ conditions, it can be verified if the ecosystem works well and how are its perturbations. For example, an important quantity of rotifers shows a good stability of the purifying biomass and good water purification; in contrast, a lot of small flagellate or amoeba communities are the sign of recent sludge, sudden high load, a bad aeration, and a poor quality of purified water (Degre´mont 2008).

Figure 15 : Basin for biological treatment of wastewater with surface aerator (Photo: courtesy of Socie´te´ des Eaux de Marseille

Aerobic degradations are obtained by an adapted biomass in constant and sufficient concentration in the aerated tank. The biomass adsorbs and eliminates a more or less important part of pollution according to the detention time in the tank and to the organic load received by the treatment plant. The dissolved pollution is partly transformed into sludge by

bac terial assimilation. Another part of the biodegradable organic matter is mineralized and releases as carbon dioxide, water, sulfate ions, ammonium ions, etc. In the same tank, in pres ence of oxygen, ammonium ions resulting from degradation of organic matter as well as those brought by the effluent are oxidized to nitrate ions by two communities of aerobic, chemolithotrophic, and autotrophic microorganisms. Ammo nium oxidizing prokaryotes achieve the conversion from NH_4^+ to NO_2 and nitrite oxidizing prokaryotes achieve the conversion from NO_2 to NO_3. Most of the wastewater treatment plants eliminate properly carbon compounds, whereas nitrogen and phosphorus compounds are processed only in the most performing plants. Twomajorparameters must bedetermined in the activated sludge process; dimensioning and aeration of the tanks:

- Dimensioning of tanks For an optimum functioning, the structure will depend on dimensioning parameters, which are primarily mass and volume load, hydraulic detention time*, and sludge detention time called “sludge age”. Load represents the quantity of pollution measured in COD or in BOD_5 supplied every day to the treatment plant and in relation either to the volume of the aeration tank (volume load/flow) or to the quantity of measured bio mass: Volatile Suspended Solids (VSS) or mass load (ml), which represents the biomass active part.

$$\text{Mass load } (C_{\text{m}}) = \frac{\text{kg of biodegradable injected per day } (\text{BOD}_5)}{\text{quantity of biomass in the system } (\text{VSS})}$$

Mass load is expressed in kg of BOD5 to be treated per kg of VSS per day.

$$\text{Volume load } (C_{\text{v}}) = \frac{\text{kg of biodegradable injected per day } (\text{BOD}_5)}{\text{reactor volume } (\text{m}^3)}$$

The volume load is expressed in kg of BOD5 to process/ m^3 of reactor/day. This notion of mass load is important because it influences: **(i)** The purification efficiency of an activated sludge: The low mass load corresponds to high purification rates and high mass load to lower

rates**. (ii)** The excessive production of biological sludge: For a low load, endogenous respiration is more important thanforahighloadbecauseofthesubstraterestriction. **(iii)** The requirements for oxygen in relation to the eliminated pollution: The importance of endogenous respiration with a weak load leads to consumption of oxygen in relation to eliminated pollution more than those obtained with high load. Low mass load also increases the age of sludge, which means an increase in a chemolithotrophic and autotrophic community and the possibility to obtain better stabilized sludge and better flocs. The advantages of a low mass load are obvious but have

- **Aeration of activated sludge**

The biological aeration tank is the core of the wastewater treatment plant since the suspended aerobic microbial culture degrades the pollutant load of wastewater in presence of dissolved oxygen. This oxygen is brought by an aeration device whose functioning amounts to 50–70 % of the total energetic cost of the wastewater plant, which corresponds to one-third of the total opera tional cost approximately. With such costs, optimizing oxygen transfer or the energetic control of wastewater treatment processes has become a major challenge. The efficiency of aerobic bacteria in the wastewater treatment strongly depends on the oxygen supply and consequently on its transfer from the gaseous phase towards the liquid phase, which must not be a restrictive step of the process operation. Whatever the medium may be studied, an increase in the viscosity of the liquid phase or of the suspension involves a decrease of the oxygenation capacity of the medium; thus, it generates degradation in the performances of the aerobic biological systems.

Biological Nitrogen and Phosphorus Removal

The nitrates constituted during the aerobic process in the activated sludge tank are removed during denitrification by heterotrophic and chemolithoautrophic autotrophic bacteria responsible for anammox by recycling waters of the aeration tank in an anoxic tank (no free oxygen but presence of bonded oxygen, particularly under the form of NO3) upstream of the aerobic reactor. The organic or mineral molecules in wastewater will bring by oxidation the energy necessary to the reduction of nitrates to nitrogen. In this type of process, the reduction of nitrates can be incomplete and stopped to the stage N2O (greenhouse gas) which is released to the atmosphere. In these conditions, a tertiary treatment is necessary. As far as phosphorus

removal is concerned, the phosphate removal of the effluent is achieved in an anaerobic tank (absence of free and bonded oxygen, in particular under the form of NO_3) upstream of the anoxic tank and immediately after the primary treatment. The operators of wastewater treatment used to describe the different tanks of a WWTP as the expression of aerated tank (aerobic) to indicate oxygenated environ ments (presence of free oxygen) and the expression of anoxic tank to indicate environments without free oxygen but containing combined oxygen (particularly under the form of nitrate). The expression of anaerobic tank is only for environments without free or bonded oxygen (i.e., nitrate). For biologists, these expressions have different meanings. Indeed, oxic (or aerated) and anoxic, respectively, chara cterize biotops in which oxygen is present or absent; bonded oxygen is not taken into account in this definition. Besides, aerobic and anaerobic, respectively, characterize organisms (or biological processes), which require the presence or absence of molecular oxygen to live. The principle of phosphorus removal is the following one: in anaerobic conditions (in anaerobic tanks), various bacterial communities, probably most of them belonging to Betaproteobacteria, use their polyphosphates to synthetize from acetate or other volatile organic acids intracellular polyhydroxyalkanoates (PHA), releasing phosphate in the tank. These bacteria under aerobic conditions reconstitute stocks of cellular polyphosphates more important than the previous ones. They will be eliminated with the cells during the secondary settling (Seviour et *al.* 2003). Frequently in the WWTP, the aerobic activated sludge treatment is combined in the same tank to anoxic treatments (denitrification) and anaerobic treatments (phosphorus removal). The activated sludge tank is compartmentalized. Each compartment is intended for a specific treatment. Such devices can be observed, for example, at the WWTP of Gre´asque (France), where the central annular zone of the tank is only for the anaerobic process and the peripheral zone for aerobic and anoxic processes. Another example is represented by the WWTP of Skaekinge (Denmark), whose tank is divided into three annular compartments. The central compartment is devoted to phos phorus removal, the most peripheral one to aerobic process, and in the intermediate one to denitrification (Degre´mont 2008). Alternation in the aerobic tank of aerated and non aerated periods (sequential aeration) enables the combina tion of aerobic treatment and denitrification in the same PHA ATP Poly - P tank. Wastewater professionals use the expression "biological treatement by referring to the whole processes that they define as aerobic, anoxic, and anaerobic wastewater are submitted to.

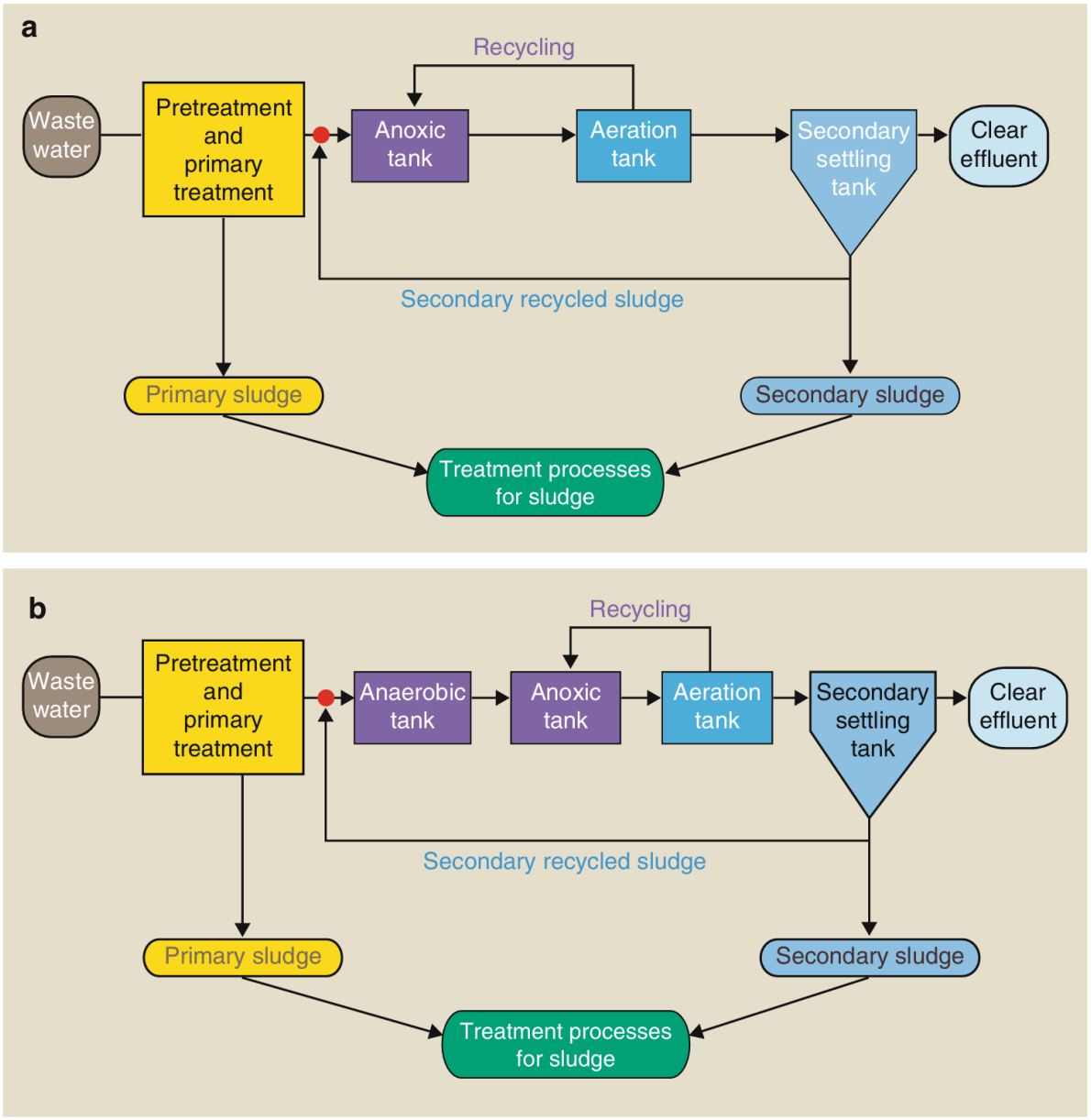

Figure 16 : Removal of nitrates and phosphates by microbiological processes. **(a) Removing of nitrates.** The basin is called anoxic because it does not contain free dioxygen. The dioxygen may be present in the combined form of nitrate. **(b) Removing of nitrates and phosphates**. The diagram shows the method of the STEP Phoredox (Modified and redrawn from Degre´mont 2008). There are several other processes which differ by the number of reactors and their respective positions as well as the mode of effluent recirculation and secondary sludge (Degre´mont 2008). Drawing: M.-J. Bodiou

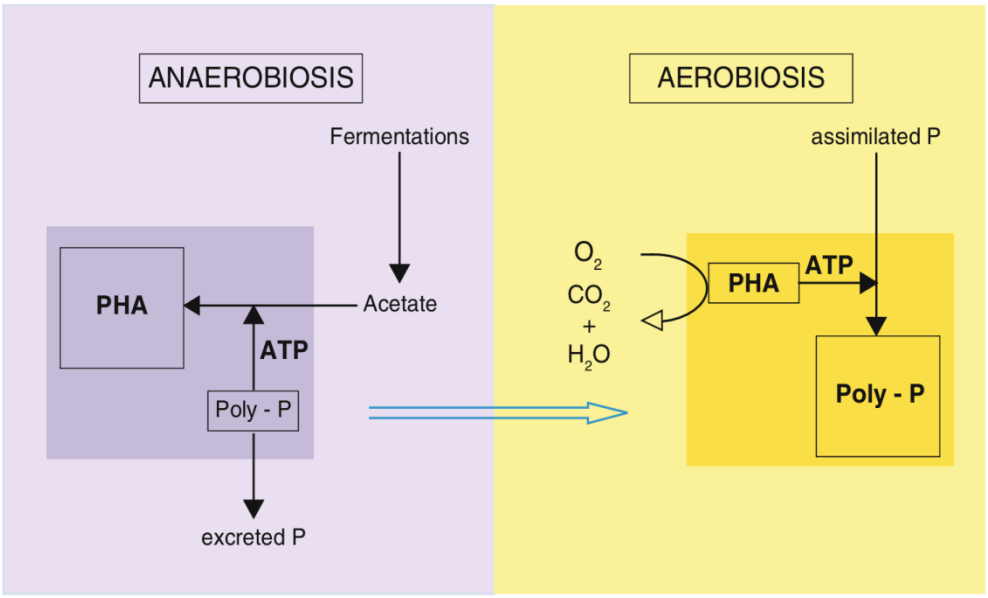

Figure 17 : Mechanism of bacterial phosphorus remov*al.* P phosphate, PHA polyhydroxy alkanoate. In anaerobic conditions, the synthesis of PHA transforms cellular storages of polyphosphate; these last are reconstituted massively in aerobiosis, via an aerobic metabolism. Drawing: M.-J. Bodiou

Figure 18 : Aerobic treatment tank. 1 Anaerobic zone, 2 aerobic and anoxic zone

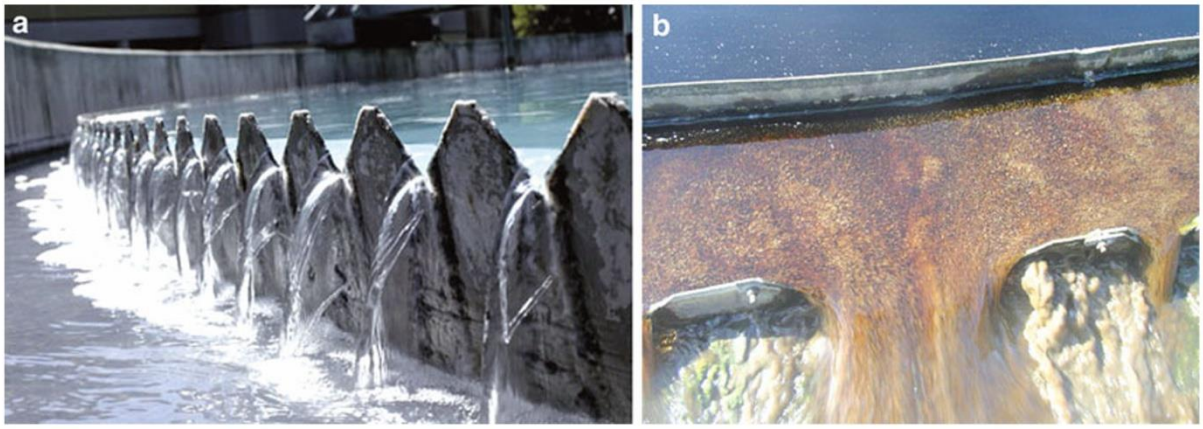

Figure 19 : Secondary clarifier. Overflow of the decanter: (a) effective settling (the effluent is clear), (b): insufficient settling, the effluent still contains suspended materials (presence of activated sludge) (Photographs Benoit Marrot and Philippe Moulin, courtesy of Socie´te´ des Eaux de Marseille)

Secondary Settling in Aerobic Activated Sludge

Treatments Another stage to separate microorganisms and the treated liquid is necessary to prevent any discharge of suspended solids in the natural environment. Flocs are therefore separated from the treated water by a secondary settling. Apart of the settled sludge is recycled in order to enable the reseeding of the biological aerated tank (aeration tank) and thus to maintain a biomass concentration enough to purify the effluent. The other part, namely, the excessive sludge, is periodically extracted to be treated in composting centers, for example. In practice, activated sludge process remains limited by a lack of flexibility towards the variations of the composition of the pollutant loads and the biomass concentration; it is expressed by significant secondary settling problems if the settling is more than 4 $g \cdot L^{-1}$. Besides, because of the increase in the production capacity of the industrial sites and of stricter discharge standards, biological wastewater treatment plants are generally undersized and cannot meet the specification required by the legislator. These drawbacks have been a tremendous challenge for the scientific commu nity and the wastewater professionals. This challenge has been solved by the coupling of biological processes (treat ment in bioreactor) and physical processes (processes with porous membranes) with biomass recycling which led to the membrane bioreactor (MBR).

Anaerobic Biological Treatments

The purification of effluents can also be completely carried out in anaerobic conditions (in the biological sense) in anaerobic reactors. During the degradation, the organic matter is transformed into gas (carbon dioxide, methane). Compared with aerobic purification, anaerobic purification is slower and generates a less important biomass production, since the involved microorganisms have a metabolism which releases very little energy (cf. Sects. 3.3.2 and 3.3.3). This treatment mode is generally used to purify agricultural or industrial wastewater and to stabilize primary sludge (sludge methanization).

All aerobic processes produce an excess microbial mass or holding sludge, containing numerous refractory organic by-products. Sludge from aerobic treatment is often reprocessed by anaerobic digestion, together with the settled matter from primary treatment.

Anaerobic digestion is a natural biological process that degrades organic matter in the absence of oxygen, transforming it into simpler elements, divided between a gaseous phase, the combustible biogas, and a solid-liquid mixture, the fertilizing digestate. Anaerobic digestion or methanization is in fact a means of producing renewable energy, biogas which can be used to replace fossil fuels, and digestate which can be used as an organic soil improver.

The process can be broken down into 4 main stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis.

(1) **Hydrolysis** breaks down organic macromolecules (proteins, lipids, etc.) into monomers (simple sugars, amino acids, fatty acids, etc.).

(2) **Acidogenesis** ferments the resulting monomers: acidogenic bacteria break down the monomers in organic matter into acids and alcohol.

(3) **Acetogenesis** converts the resulting molecules into precursor molecules for methanogenesis: acetic acid, hydrogen and CO2, which are then used by the methanogenic bacteria.

(4) Methanogenesis: this produces biogas, composed of methane CH4 (60%), CO2 (40%) and digestate.

10.4. Tertiary Treatments: Nitrogen and Phosphorus Removal by Other Processes; Complementary Refining Treatments

If after secondary treatments nitrogen and phosphorus concentrations do not meet standards specified by legisla tion, tertiary physicochemical treatments are implemented. Nitrogen removal under the form of nitrogen can be obtained after alkalinization and chlorination, whereas phos phates can be removed by precipitation with salts of iron, aluminum, or calcium. Other more specific tertiary treatments (non-exhaustive list) contribute to refine the quality of the discharged water: (i) Activated carbon filtration to remove organic matters, which are resistant to biological treatments (ii) Reduction of the load of pathogen agents by coagulation–flocculation (iii) Ultrafiltration, nanofiltration, and reverse osmosis.

10.5. An Innovative Process: Membrane BioReactors (MBR) Clear effluent

In the MBR system, the bioreactor and the membranes, tubular or plane, have each a specific function: (i) The organic degradation of the dissolved organic pollution is achieved in the bioreactor with adapted bacteria. (ii) The separation of particulate pollution is obtained via membranes, which constitute a physical barrier for suspended solids (VSS) that cannot get through. Membrane bioreactors are innovative systems. Their development has been undeniable since the beginning of the twenty-first century (Stephensons 2000; van der Roest et *al*. 2002; Cornel and Krause 2008). The related scientific studies have been developing exponentially, and conse quently this treatment process has been implemented more and more worldwide. The expansion of membrane bioreactors is due mostly to limitations of the secondary settler in the classical treatment and to the evolution of effluents to be treated. Because of effluents are more or less polluted, containing various and new types of pollutants such as xenobiotics, with varying loads, it is necessary to apply adaptable, flexible, and highly efficient purifica tion processes in order to meet the more and more strict discharge standards. The two sets of membrane reactors (submerged or external membranes) meet these require ments for urban as well as industrial effluents (Figs. 16.14 and 16.15a, b). Unlike the conventional biological systems, MBR enables the purification improvement with a higher biomass concentration in the biological tanks (about 10–15 g·L 1 wet weight). Besides, they produce less sludge, are more com pact, and provide a total retention of suspended solids. A further development of MBR mainly depends on the control of the phenomena which limit the performances of the pro cess. In the first place, the membrane clogging is impossible to

eliminate totally; therefore, it should be controlled with one of the following solutions: (i) Preventive: air is blown at the surface of the membrane to avoid clogging. (ii) Curative: optimization of cycles and wash solutions. The oxygen supply to microorganisms in biological tanks is among the limiting phenomena. However, the clear advantages of membrane bioreactors largely compensate for these restricting phenomena. Nowadays, MBR are being optimized, and their future is very promising in a wide range of application fields, such as Food and Drink Industries, petrochemical industries, landfills, or urban wastewater. It is still possible to argue about the more important cost of the MBR system compared with the conventional one.

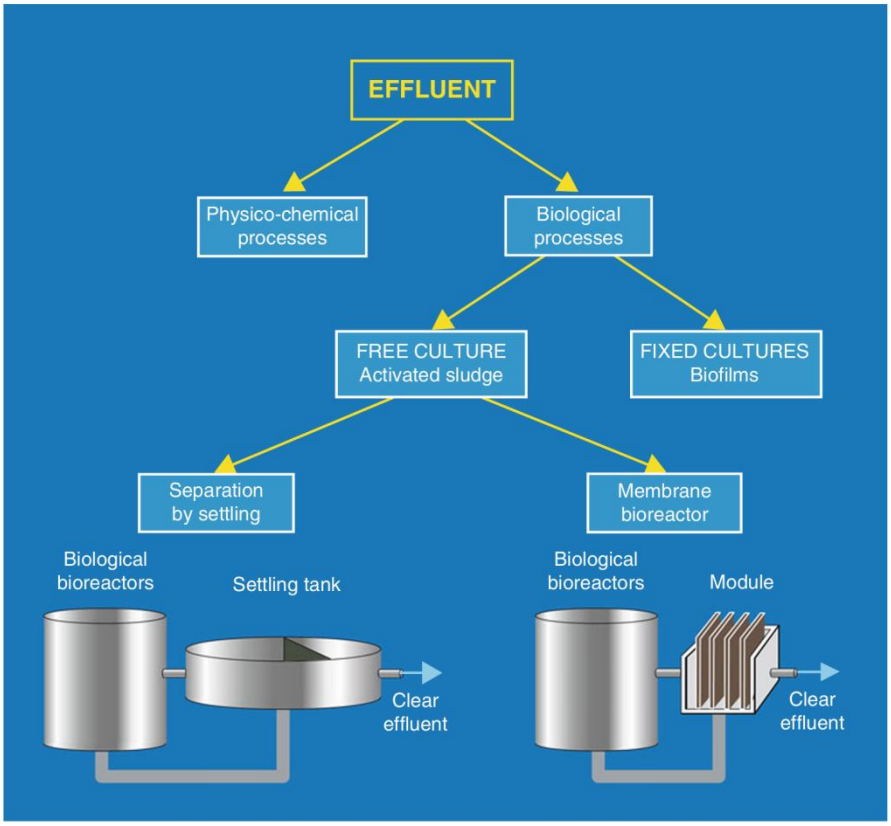

Figure 20 : Place of membrane bioreactors in the two disposal of wastewater treatment. The physicochemical processes correspond to the primary treatment; in biological processes, primary treatment is supplemented with secondary treatment. Drawing: M.-J. Bodiou

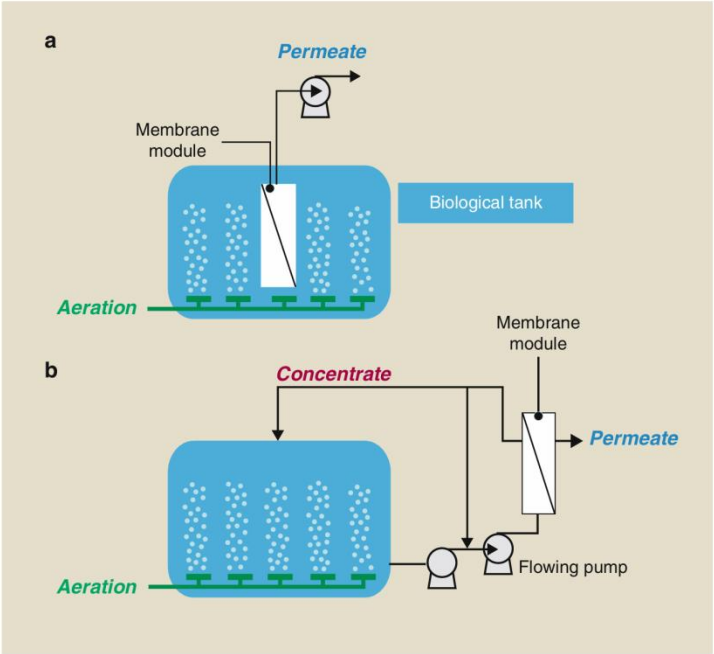

Figure 21 : Drawings illustrating the devices with immersed (a) and external (b) membranes in a treatment with membrane bioreactors. Drawing: M.-J. Bodiou

CHAPTER 3: SOIL MICROBIOLOGY

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Soil is formed by the transformation of a parent rock under the influence of physical, physico-chemical and biological factors. Soil can be defined as the pedosphere, the intersection of the lithosphere, hydrosphere, biosphere and atmosphere, modified over time and possibly by human action.

1. SOIL COMPONENTS:

Soil is made up of mineral particles of varying size, shape and composition, living organisms, organic matter in various stages of decomposition, gases, water and dissolved mineral elements. These components are not, of course, homogeneously mixed, and different levels of organization can be distinguished in a soil, ranging from colloids (clay-humus complex = association between clay particles and organic matter) to soil horizons and macro-environments such as the rhizosphere.

The formation of clay-humus complexes and aggregates is the main structural feature of most soils. Clay-humus complexes are formed when microflora and plant roots produce filaments and mucilage that combine with clay particles. Soil structure then develops, when physical forces (desiccation, swelling-reduction of volume, alternating freezing and thawing, animal action....) induce the formation of aggregates. Aggregation is one of the main factors controlling soil microbial activity and the soil organic matter cycle.

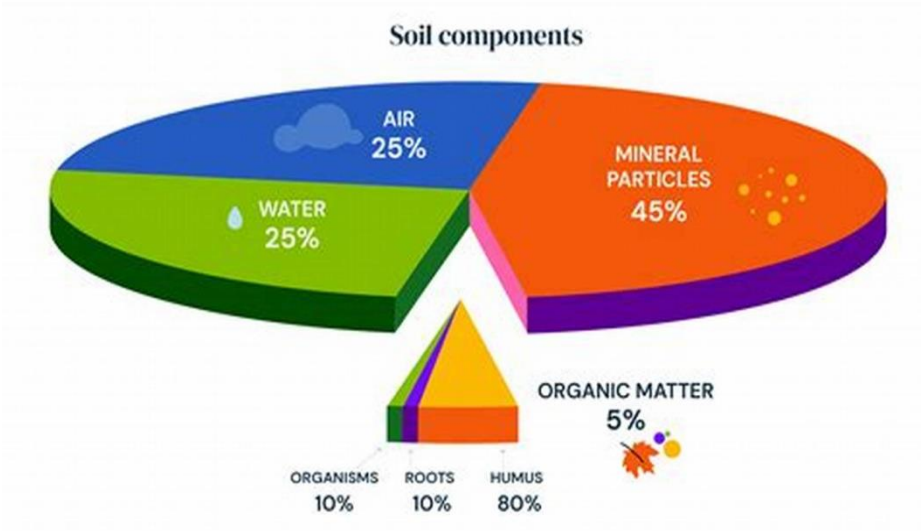

Figure 22: soil components (https://wmswcd.org/conservation-priorities/healthy-soil/)

Mineral components

Mineral components result from alteration of the parent rock and exogenous inputs resulting from erosion. Mineral particles are classified according to their size, and are traditionally divided into the following categories:

Table 2: size of different mineral particles of soil

Category	Size range (mm)
Gravel	>2.0
Coarse sand	0.2 – 2.0
Fine sand	0.02 – 0.2
Silt	0.002 – 0.02
Clay	<0.002

https://www.researchgate.net/figure/Classification-of-particles-according-to-size-by-IUSS-and-USDA-systems_tbl1_336641743

The relative content of sands, silts and clays determines the texture of soils and their classification into textural groups (e.g. clayey sand, sandy clay loam, etc.).

Texture partly determines the level of soil aggregation and influences biological activity.

The content and nature of clays in the soil are important characteristics that condition the pore size of the soil and its ability to retain water. The clay fraction has the greatest influence on soil microorganisms, due to the large surface areas it covers and the absorption phenomena that develop. One gram of clay (particles < 2µm) can contain more than 1010 particles, corresponding to a total surface area of around 8 x 10^6 cm^2. The clay fraction is particularly rich in mineral elements, containing silica, oxygen and aluminum, as well as the following elements: Fe, Mg, K, Ca, Na.

Water and air

Water in aggregates influences the activity of microorganisms in aggregates in two main ways:

- the space available for growth increases with water content, which is therefore a positive factor,

- oxygen diffusion decreases with water content, the diffusion coefficient being 1000 times lower in water than in air, so increasing water content is a negative factor.

These two opposing effects mean that there is generally an optimum water content for microbial activity in a soil, although this value obviously depends on texture and structure. For example, nitrifying or sulfooxidizing activity is highest at water contents corresponding to pF 2.5, i.e. a boundary value between capillary and gravity water.

In flooded soils, where the activity of certain groups of anaerobic bacteria is high (sulfate-reducers, nitrogen-fixers, methanogens, etc.), this activity increases with water content, reaching an optimum at saturation.

Organic matter

Soil contains various types of organic matter, which can be grouped into four main classes. The quantity and nature of organic matter present in the soil largely determine its characteristics and condition its fertility. It is important for ecologists and agronomists to distinguish between organic matter that is specific to the soil, i.e. humus in the strict sense, and foreign, incompletely or untransformed substances that are simply mixed with it.

The preponderance of non-humified fractions in a soil often indicates reduced biological activity, leading to the build-up of an unused stock of nutrients and the appearance of aggressive mobile substances that degrade the soil.

The dominance of humified fractions often corresponds to soils with good structure, intense microbiological activity and balanced nutrient cycles.

It contributes to soil aggregation and buffers nutrients. However, there are also inert humus and mobile humic substances. The distinction between humified and non-humified organic matter is therefore not absolutely clear-cut in ecological terms.

Humic substances are mainly short-chain polyphenols, polymerized with phosphorus or sulfur compounds. Based on chemical extraction methods using alkaline solvents, three main types of humic fractions are traditionally distinguished:

- Fulvic acids: extractable and remaining dissolved or dispersed after acidification.

- Humic acids, extractable but precipitating at pH 1.

- Humin, a non-extractable residue.

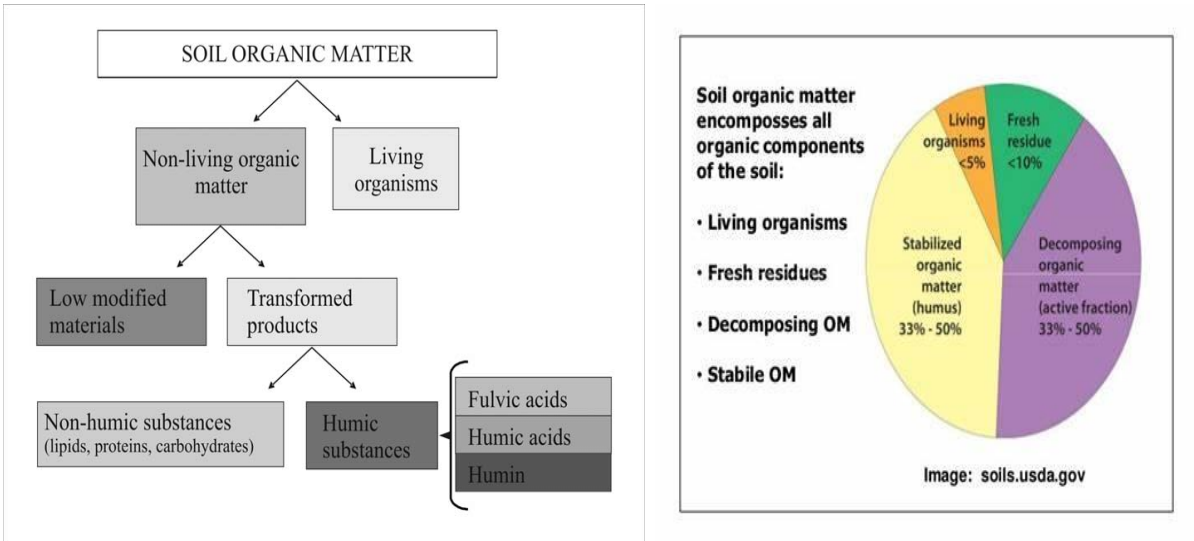

Figure 23: Major classes of soil organic matter (https://www.researchgate.net/figure/Principal-components-of-soil-organic-matter-Adapted-from-FAO-2005_fig19_293132330)

2. MAJOR GROUPS OF SOIL MICROORGANISMS

Soil microorganisms are represented by a number of metazoans, protozoans, microscopic algae, fungi, bacteria, actinomycetes, cyanobacteria and viruses.

Soil flora (micro flora) e.g. Bacteria, fungi, Actinomycetes, Algae and Soil fauna (micro fauna) animal like eg. Protozoa, Nematodes, earthworms, moles, ants, rodents. Relative proportion / percentage of various soil microorganisms are: Bacteria-aerobic (70%), anaerobic (13 %), Actinomycetes (13%), Fungi /molds (03 %) and others (Algae Protozoa viruses) 0.2-0.8%. Soil organisms play keyrole in the nutrient transformations.

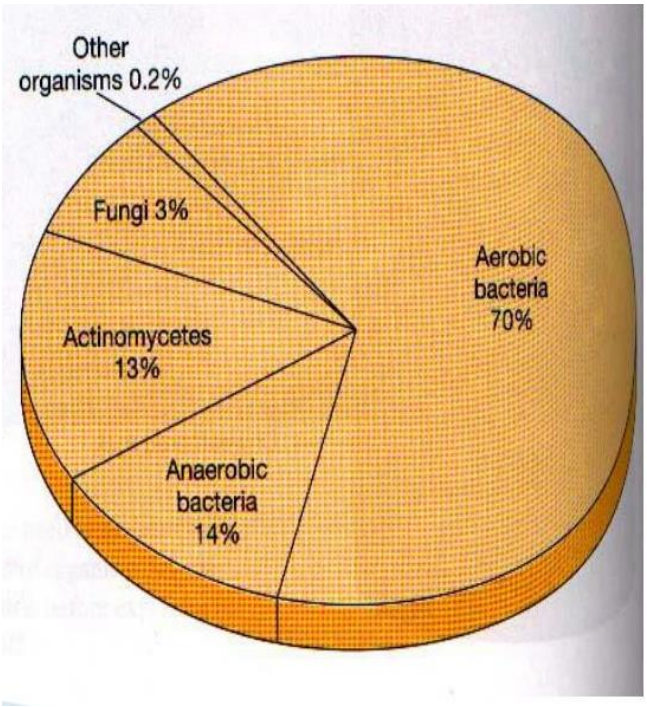

Figure 24: Proportion of different soil microorganisms
https://www.slideserve.com/turi/mikrobiologi-tanah

- Bacteria are generally the most numerically important group in soil. In waterlogged or heavily compacted soils, the number of aerobic bacteria is reduced, whereas the microaerophilic and finally the anaerobic bacteria will increase. most bacteria prefer nutrient-rich soils of neutral or slightly alkaline pH and a close C/N-ratio
- Fungi, in general, tend to dominate over bacteria and actinomycetes in acidic soils as they can tolerate a wider range of pH levels. and a wide C/N-ratio, they dominate in raw humus and moder and mull soils. Most fungi are aerobic except for yeasts,
- Actinomycetes in the soil are mesophilic organisms that are sensitive to acidity/low pH (optimum pH range 6.5-8.0) and waterlogged soil conditions.
- The inhabitation of soil by protozoa depends on the structure and texture of the soil. Mastigophorans (flagellates) tend to dominate in drier soils, while ciliophorans (ciliates) are abundant in moist soil.
- Cyanobacteria are phototrophic bacteria that are important in soils where light and water are available.

Table 3 : soil microorganism with example (https://www.brainkart.com/article/Soil-Microorganisms_35270/)

Soil Microorganisms	Examples
Bacteria	*Agrobacterium* *Bacillus* *Clostridium* *Pseudomonas*
Actinomycetes	*Actinomyces* *Nocardia* *Streptomyces*
Fungi	*Aspergillus* *Fusarium* *Alternaria* *Cladosporum*
Soil algae	*Anabaena* *Oscillatoria* *Nostoc*
Protozoa	*Colpoda* *Nematodes* *Pleurotricha* *Heteromita*
Bacteriophages	T4 Bacteriophages

In exposed soils, the dominant groups of microorganisms in terms of microbial biomass are mainly bacteria, actinobacteria, and fungi. These microorganisms play a crucial role in the decomposition of organic matter and the release of essential nutrients for plants.

In submerged soils, such as rice paddies, cyanobacteria and anaerobic bacteria (which do not need oxygen to survive) are often predominant. These microorganisms are adapted to low-oxygen conditions and contribute to processes such as nitrogen fixation and the decomposition of organic matter in aquatic environments.

3. BIOTIC INTERACTIONS OF MICROORGANISMS ASSOCIATED WITH PLANTS

3.1. Parasitism

Most parasitic diseases of plants are caused by fungi or oomycetes with the remainder due to bacteria or viruses (Agrios 1997).

The majority of phytopathogenic **fungi and oomycetes** affect the aerial parts of plants, causing diseases such as downy mildew (caused by oomycetes *Phytophthora parasitica* on

tomato or *Plasmopara viticola* on vines), powdery mildew (*Erysiphe graminis* on herbaceous or *Uncinula necator* on vines), corn smut (*Ustilago maydis* on corn), rust (*Puccinia spp*. on cereals and *Uromyces spp*. on Fabaceae), or rot (*Botrytis cinerea* on grapevine) (Agrios 1997; Heitman 2011). In some cases, the disease symptoms are subtle, like ergot (*Claviceps purpurea*) or head blight of cereals (*Fusarium spp*.) because the phytoparasite produces mycotoxins such as lysergic acid [*C. purpurea* (Lorenz et *al.* 2007)]; trichothecenes, fumonisins, and zearalenone [*Fusarium spp.* (Osborne and Stein 2007)]; and ochratoxins and aflatoxins [(*Penicillium spp. and Aspergillus spp*. (Berthiller et *al.* 2013)].

Damping-off is generally caused by oomycetes such as *Aphanomyces cochlioides* on sugar beet and more particularly *Pythium spp.* on most crops, but fungi such as *Rhizoctonia solani* can also cause damage in vegetables or forest nurseries.

Many **foliar bacteria** are phytopathogenic, such as *Erwinia amylovora* (fire blight pathogen of pear and apple), *Xanthomonas campestris* (which causes stalk lesions and necrosis), or *Pseudomonas syringae* (causing cankers in many fruit trees or brown necrotic spots on the glumes of wheat) (Agrios 1997). Part of P. syringae epiphytes produce IceC membrane protein, which has an ice nucleation activity and promotes frost damage on strawberry (Lindow and Brandl 2003). Some are soil-borne phytopathogenic bacteria and are responsible for vascular disease (Ralstonia solanacearum), soft rot (Pectobacterium carotovorum), or gall (*Agrobacterium tumefaciens, Streptomyces scabies*). Certain *Agrobacterium tumefaciens* carry the Ti plasmid that confers them the ability to transfer genes to the plant (Krimi et *al.* 2002; Tzfira and Citovsky 2006).

Some **protozoa** including Phytomonas are phytopathogens. These trypanosomes are responsible for heart-rot disease of coco nut palms, and they can be transmitted back and forth from host plants to insects.

Finally, some **viruses** such as poxvirus cause significant damages, but most of the time, they need vectors to infect their host plants. For instance, parasitic nematodes (e.g., *Xiphinema spp*.) serve as vectors of viruses such as the grapevine fanleaf virus (GFLV). Moreover, the wound caused by stylet penetration of the parasitic nematodes may also facilitate the plant infection by fungal (Verticillium), oomycete (Phytophthora), or bacterial (Clavibacter michiganense) pathogens. Besides nematodes, the main virus vectors are Hemiptera insects,

but some viruses can also be transmitted to the plant by fungi (Olpidium spp.) or protists (Polymyxa betae) or between plants through parasitic plants such as dodder (Agrios 1997).

3.2. Symbiosis

Certain bacteria and fungi form a mutualistic symbiosis with plants (Cardon and Whitbeck 2007). Those between bacteria and plants consist mainly of **nitrogen-fixing symbioses** involving the floating fern *Azolla* (with the cyanobacterium Anabaena), phylogenetically diverse plants called **actinorhizal** (with the actinobacterium *Frankia*), and Fabaceae (with selected alphaproteobacteria, such as *Rhizobium*, and selected betaproteobacteria) (Mathesius 2003; Huguet et *al.*2005).

The Rhizobium–Fabaceae dialogue involves molecular signals produced by the plant (including flavonoids) and leads to bacterial synthesis of a lipo-chito-oligosaccharide termed Nod factor, which is a signal inducing root nodulation (Mathesius, 2003).

In the case of Frankia and of photosynthetic *Bradyrhizobium*, the bacterial signal involved is different, and indeed their genomes are devoid of canonical nod genes (Giraud et *al.* 2007; Normand et *al.* 2007).

The Fabaceae nodule is formed by proliferation of cells from the cortex and the pericycle. The bacteria usually enter the plant through root hairs, which are deformed during the interaction, and find themselves in an infection thread of plant origin. This thread enables proliferation and delivery of bacteria to plant cells in the nodule. Once endocytosed by nodule plant cells, the bacteria differentiate into bacteroids but remain separated from the cytoplasm of the plant cell by the peribacteroid membrane.

In *Medicago truncatula*, this transformation into bacteroids is triggered by nodule-specific cysteine-rich peptides resembling antimicrobials from the plant innate immune response (Vande Velde et *al.* 2010). The peribacteroid membrane and the bacteroid(s) it contains constitute the symbiosome. The nitrogen fixed by the bacteroids will serve as nitrogen source for the plant, which in return provides a source of carbon and energy (25–35 ATP necessary for each N2 reduced) in the form of dicarboxylic acids (mainly malate). The nitrogenase is sensitive to oxygen but is protected by low oxygen diffusion within the nodule. The oxygen

supply to the bacteroid is provided by leghemoglobin, a carrier of both bacterial (heme) and plant origin (globin). The leghemoglobin allows a high flux of oxygen without the latter reaching a concentration deleterious for the nitrogenase. The nitrogen fixed by the bacteroid is transferred to the plant in the form of ammonia and more rarely alanine. It will be transported in the phloem sap as amides (for temperate Fabaceae, with indeterminate nodules) or ureides (for tropical Fabaceae, with determinate nodules). In the bacterial partner, key genes (over fifty) involved in symbiosis are generally clustered as islands of genes, often plasmid-borne. They may be transferred horizontally, which probably explains their presence in both alphaproteobacteria and betaproteobacteria (Moulin et *al.* 2001).

The **mycorrhizal symbioses** implicate plant roots **(**Mathesius, 2003; Bailly et *al.*, 2007). The fungus provides the plant with minerals taken from the soil (phosphorus and to a lesser extent nitrogen), whereas the plant provides carbon substrates derived from photosynthesis (Brundrett 2009). The vast majority of plants are mycorrhized in nature, the few plant species not forming mycorrhizae belong mainly to the family Chenopodiaceae (beets) and Brassicaceae (rape seed, Arabidopsis). There are several types of mycorrhizae, particularly ectomycorrhizae, arbuscular endomycorrhizae, and ectendomycorrhizae (Mukerji et*al.*2000; Brundrett 2009). Ectomycorrhizal fungi (Ascomycetes and Basidiomycetes) develop extensively at root tips, forming fungal sheaths over short roots, which is very characteristic and easily visible to the naked eye (Brundrett 2009). The mycelium grows between root cortical cells but does not penetrate living cells, thus forming an intercellular network called the Hartig net, which is involved in nutrient exchanges between the two partners. The functioning of ectomycorrhizae is under the metabolic and genetic control of both partners (Bailly et *al.* 2007). Arbuscular endomycorrhizal fungi (*Glomeromycota* division) do not develop a mantle around the root (Brundrett 2009). The endomycorrhizae are widespread and affect about 90 % of plant species. They are found mainly in herbaceous plants and in some woody species. The fungus penetrates through plant cell walls and develops arbuscules and vesicles in cortical cells. Ectendomycorrhizae are intermediate between ectomycorrhizae and endomycorrhizae; an external mantle may be produced, but the fungus penetrates into root cells, as coils (arbutoï¨d mycorrhizae; among *Ericaceae*) or very short hyphae (monotropoid mycorrhizae; among *Pyrolaceae*). The establishment of mycorrhizal symbiosis shows common features with that of bacterial nitrogen-fixing symbioses (Mathesius 2003). Before

contact, the mycorrhizal fungus stimulates the production of fine roots to increase the contact sites, secreting hormones including the auxin indole-3-acetic acid (IAA).

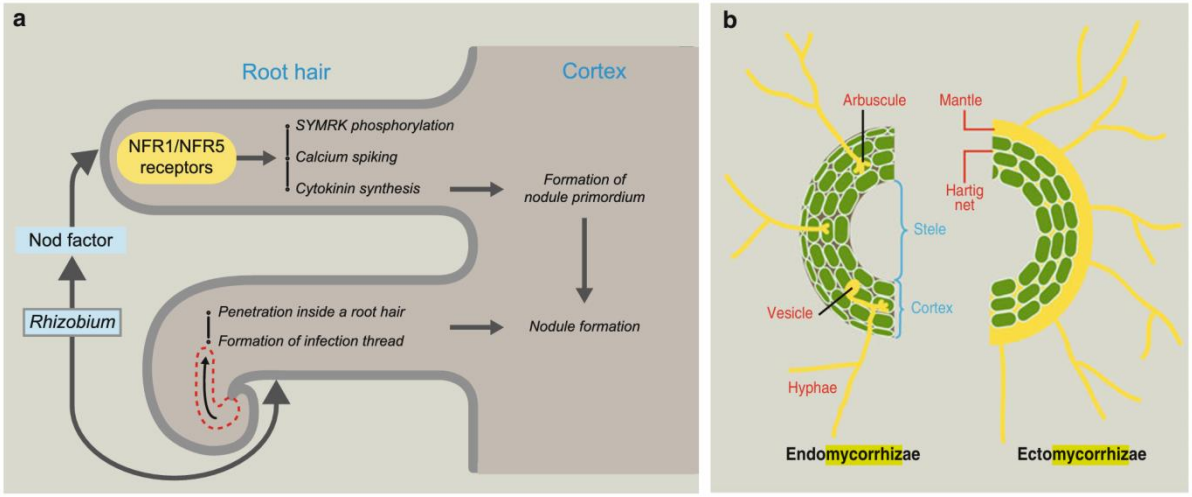

Figure 25 : Symbiotic interactions between microorganisms and plants. (a) Root nodulation process. (From Oldroyd 2007). (b) Structural comparison of endomycorrhizae and ectomycorrhizae (Drawing: M.-J. Bodiou)

After contact, the fungus enters the root and must overcome defense mechanisms. It switches from a saprophytic to a biotrophic phase, which recalls the case of biotrophic parasites. Signaling mechanisms between partners in the establishment of a nitrogen-fixing symbiosis or a mycorrhizal symbiosis involve common symbiosis genes (SYM) in the plant. The structure of the Myc factors, i.e., sulfated and non-sulfated lipo-chito-oligosaccharides consisting of four substituted N acetylglucosamines, is very close to that of the Nod factors, which reinforces the view that the two types of symbioses are functionally very close (Maillet et *al.* 2011). In terms of evolution, the bacterial symbioses (relatively young; about 65 million years) and the ectomycorrhizal symbiosis (also recent; about 180 million years) probably recruited SYM genes already involved in the arbuscular mycorrhizal symbiosis, which is older (probably 400 million years) (Simon et *al.* 1993).

3.3. Cooperation

Several bacteria and fungi actively cooperate (syn. associative symbiosis) with the plant. In the case of **bacteria**, this ability is mainly found in plant growth-promoting rhizobacteria

(PGPR). PGPR are documented mainly in *Proteobacteria* and *Firmicutes* and to a lesser extent in *Actinobacteria*. Growth stimulation usually results from a combination of direct and indirect phytobeneficial effects (Dobbelaere et *al.* 2001). Direct phytobeneficial effects may entail improved mineral nutrition of the plant, e.g., via free-living nitrogen fixation or phosphate solubili zation (Dobbelaere et *al.* 2003) and/or improved water uptake through rhizosphere soil structuration by bacterial exopolysaccharides (Amellal et *al.* 1998) or aquaporin stimulation (Groppa et *al.* 2012). Interference with plant hormonal metabolism may also be involved, via bacterial production of phytohormones (auxins and cytokinins) and/ or bacterial deamination of 1-aminocyclopropane-1-carboxylate (ACC), the ethylene precursor in the plant (Dobbelaere et *al.* 2003). The auxin indole-3-acetic produced by PGPR helps getting around plant defense mechanisms, thereby facilitating PGPR colonization of the plant (Remans et *al.* 2006). Finally, phytobeneficial effects also include triggering of systemic resistance in the plant, in particular, induced systemic resistance (ISR) pathways relying on jasmonate and ethylene (van Loon et *al.* 2006).

Indirect phytobeneficial effects of PGPR entail inhibition of parasitic bacteria, oomycetes, fungi, nematodes, and even parasitic plants (e.g., Striga), mainly through competition or antagonism (Chapon et *al.* 2002; Raaijmakers et *al.* 2009). Competition can take place for macronutrients (such as organic carbon), micronutrients (such as soluble ferric iron via high-affinity siderophores), and/or infection sites. Antag onism (syn. amensalism) may involve extracellular lytic enzymes (e.g., cellulases, chitinases, proteases), which act on the cell wall of pathogenic microorganisms, their virulence factors (such as fusaric acid from *Fusarium oxysporum*, degraded by some *Burkholderia*), or their intercellular signals (such as N-acyl homoserine lactone in Pectobacterium carotovorum) (Raaijmakers et *al.* 2009). Antagonism may also involve type III secretion system effectors with the ability to reduce the virulence of certain pathogens (Rezzonico et *al.* 2005). Finally, antagonism can rely on production of antimicrobial secondary metabolites (antibiosis), such as 2,4 diacetylphloroglucinol, and a single antagonistic strain often produces several of these antimicrobial metabolites (Raaijmakers et *al.* 2009). Some pathogens can defend them selves by suppressing the production of these metabolites (case of 2,4-diacetylphloroglucinol, via fusaric acid from Fusarium) or the expression of genes involved in root colo nization by the antagonistic bacterium (Fedi et *al.* 1997; Raaijmakers et *al.* 2009). The metabolites released by PGPR are important for their interactions with the plant and plant pathogens, and many of them play

multiple roles. Indeed, 2,4-diacetylphloroglucinol (enabling antagonism toward phytoparasites) and some siderophores such as pyoverdine (involved in iron competi tion) can also induce plant resistance (Raaijmakers et *al.* 2009). Phenylacetic acid, synthesized by *Azospirillum brasilense* Sp245 and others, is both an auxinic phytohormone and an antimicrobial compound. Therefore, certain PGPR are multifunctional in that they can act both on plant (via induction of resistance and ACC deamination) and phytopathogens (antagonism or competition).

Fungi in cooperation with the plant remain poorly documented compared with PGPR. Their direct phytobeneficial effects include solubilization of mineral nutrients (*Trichoderma and Gliocladium*) and the induction of systemic resistance in plants (*Trichoderma, Gliocladium, and nonpathogenic F. oxysporum*) (Harman et *al.* 2004). However, indirect phytobeneficial mechanisms are more important. Hyperparasitism toward different phytoparasitic oomycetes and fungi is one of the modes of action of *Trichoderma*. The fungus coils around its target, e.g., R. solani, and secretes lytic enzymes (chitinases and cellulases) that alter phytoparasite cell wall. The degradation products will allow chemotropism toward the phytoparasite. Physical contact between fungi will trigger external colonization and then penetration of the phytoparasite by Trichoderma. Trichoderma and Gliocladium may also antagonize phytoparasites through the production of antimicrobial secondary compound (Harman et *al.* 2004). Finally, competition for trophic resources can be a major mode of action, as in nonpathogenic *F. oxysporum* (Alabouvette et *al.* 2006).

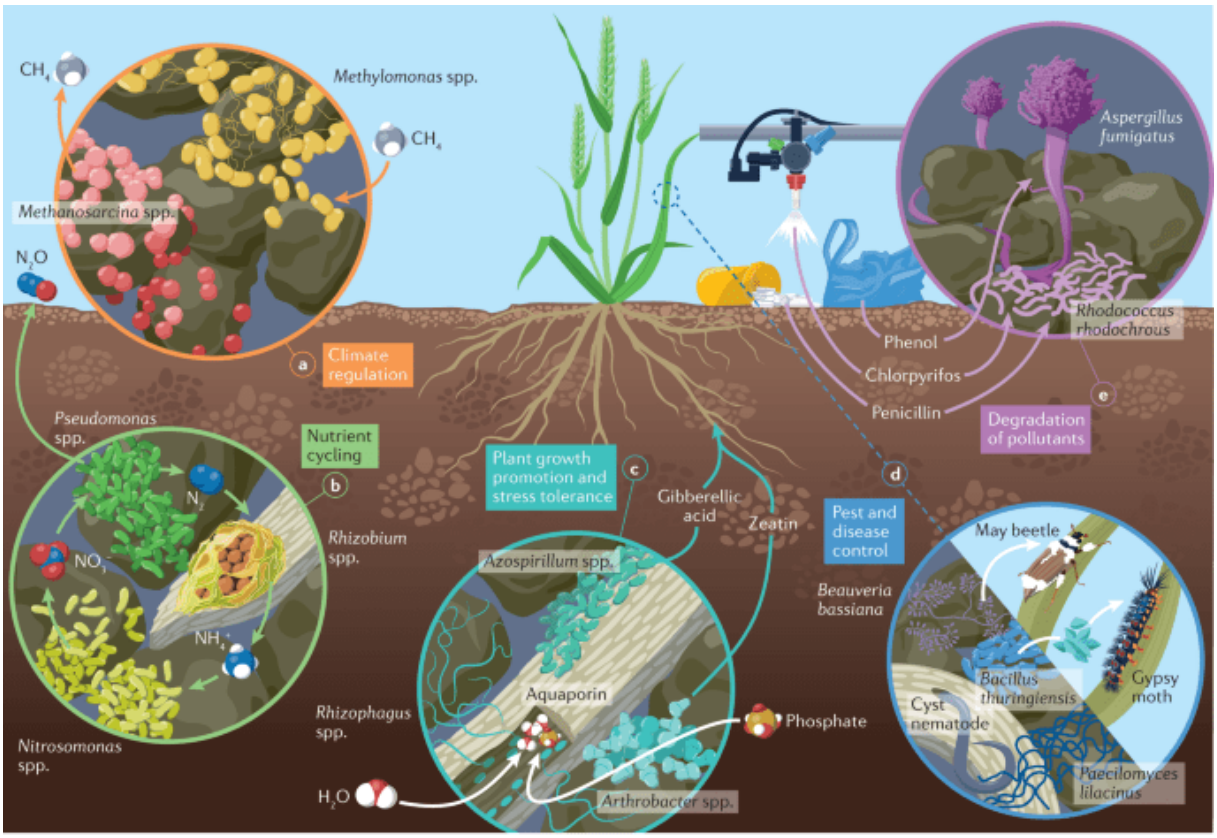

Figure 26 : soil microbiome **(https://usys.ethz.ch/en/news-events/news/archive/2022/11/soil-microbiomes.html)**

4. ROLE OF MICROORGANISMS IN THE BIOGEOCHEMICAL CYCLES OF C, N AND S

In order to grow, to reproduce and to maintain their structural and functional integrity, living organisms select a limited number of chemical compounds in their surrounding environment to build organic biomolecules that form the basic units of cells. When dying, the cells release their organic constituents back to the environment as simple minerals, primarily as the result of the activity of microorganisms. Any biological element thus undergoes a continuous cycle, called “biogeochemical cycle”, in which it passes alternately from a mineral, non-living status to a status of living matter. The oxidation state of organic elements such as carbon, nitrogen, or sulfur forms can also be modified without being incorporated into living organisms because these elements can be used as electron donors or acceptors

14.1.1 A Biogeochemical Cycle Includes Three Key Steps

1. Mineral elements (MI) are fixed by autotrophic organisms, qualified as primary or paraprimary producers. These organisms use light energy (oxygenic and anoxygenic photosynthesis) or chemical energy (aer obic and anaerobic chemolithotrophy) to fix CO_2 and build their organic biomolecules. In terrestrial environments, primary producers are mainly vascular plants; the contribution of unicellular.

algal in soils being of minor importance. This contrasts with aquatic environments where phytoplanktonic microorganisms play the major role; large marine algae (i.e., seaweed) only develop on coastal areas. Chemolithotrophic and photolithotrophic bacteria are mainly autotrophic. When biomass is produced from carbon and electron donors known from a metabolism pathway, the production is then qualified as paraprimary production. This is the case of photosynthetic purple and green bacteria that con sume sulfide produced by sulfate-reducing bacteria. Paraprimary production also applies to the amount of biomass produced by methanogenic archaea that consume hydrogen produced by fermentative bacteria. The term of primary production when applied to bacte ria (prokaryotes) relates only to microorganisms that typically use light as energy source and electron donors of geochemical origin (H_2, H_2S, NH_3, Fe^{2+}). This is for example the case of purple or green bacteria that per form anoxigenic photosynthesis by oxidizing sulfide from ground via volcanism;

2. The organic matter synthesized by autotrophs flows through food webs and is used by heterotro phic consumers that release carbon dioxide gas and pro duce organic waste or minerals;

3. At the death of organisms, organic matter (MO) is transformed into mineral form (MI) by the action of aerobic (aerobic respiration; Fig. 27, pathway 3) and anaerobic (fermentation and anaerobic respiration; Fig. 27, pathway 4) heterotrophic microorganisms known as the main decomposers, because they recycle elements from all trophic levels of the food chains. It is estimated that 90 % of the CO_2 produced in the biosphere results from the activity of bacteria and fungi. The microbial decomposers can, in turn, serve as food for higher trophic organisms (feeding of bacteria, worms, filter feeders). The mineral elements released by decomposers will be reused later. They can also come from abiotic sources such as erosion of rocks or human exploitation of mineral deposits (Fig. 27, pathway 5).

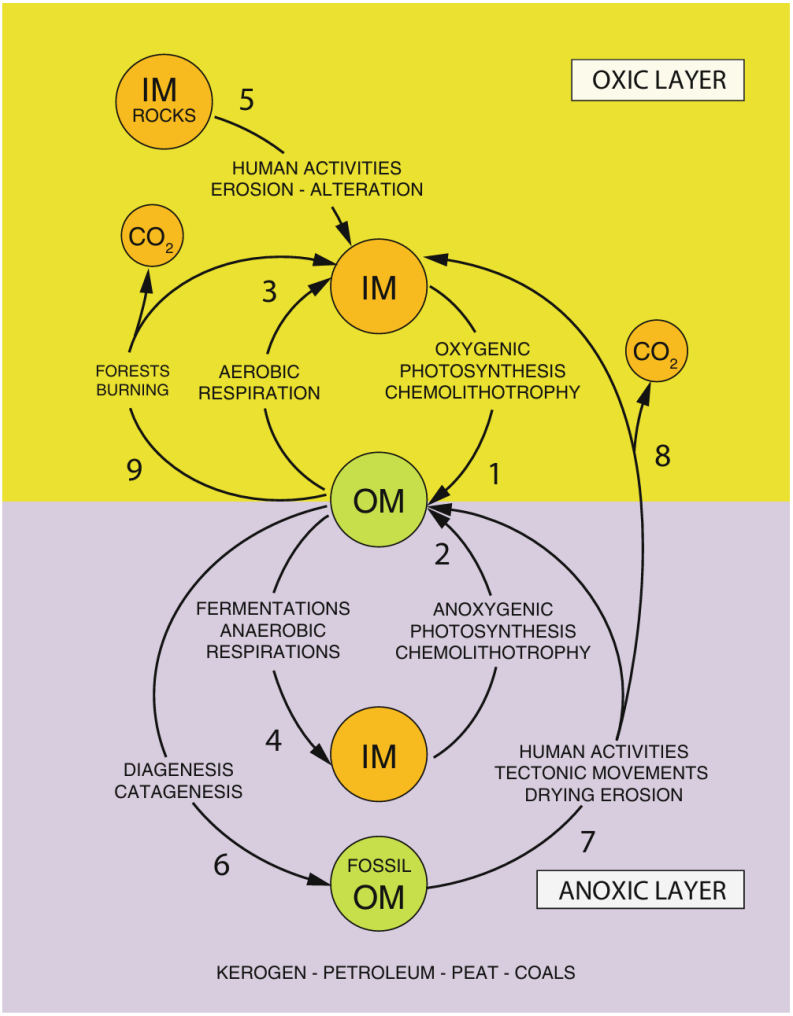

Figure 27 : Transformation of organic and mineral matter in ecosystems. IM Inorganic Matter, OM Organic Matter, 1,2 Organic matter production from mineral compounds in oxic (1) and anoxic (2) conditions, 3,4 Organic matter mineralizationin oxic (3) and anoxic (4) conditions, 5 Rock degradation and dissolution of mineral elements, 6 Storage and trapping of organic matter in soils and anoxic sediments (fossilization), 7,8 Release of organic matter from fossil stocks in anoxic (7) and oxic (8) conditions, 9 Mineralization of organic matter by burning. The study of the degradation of organic matter can be company at different levels; it can be analyzed from a global approach (content of carbon, nitrogen, etc..) through the analysis of a class of compounds (proteins, carbohydrates, lipids, humic acids, etc.) till a precise analysis of the compounds at the molecular level (fatty acids, sterols, quinones, hopanes, saturated hydrocarbons, and aromatic pesticides, etc.). The terms “oxic” and “anoxic” characterize environments containing or not containing free oxygen (dioxygen). Aerobic microorganisms are living in oxic environment and anaerobic microorganisms in anoxic environments (Drawing: M.-J. Bodiou)

14.2 The Carbon Cycle

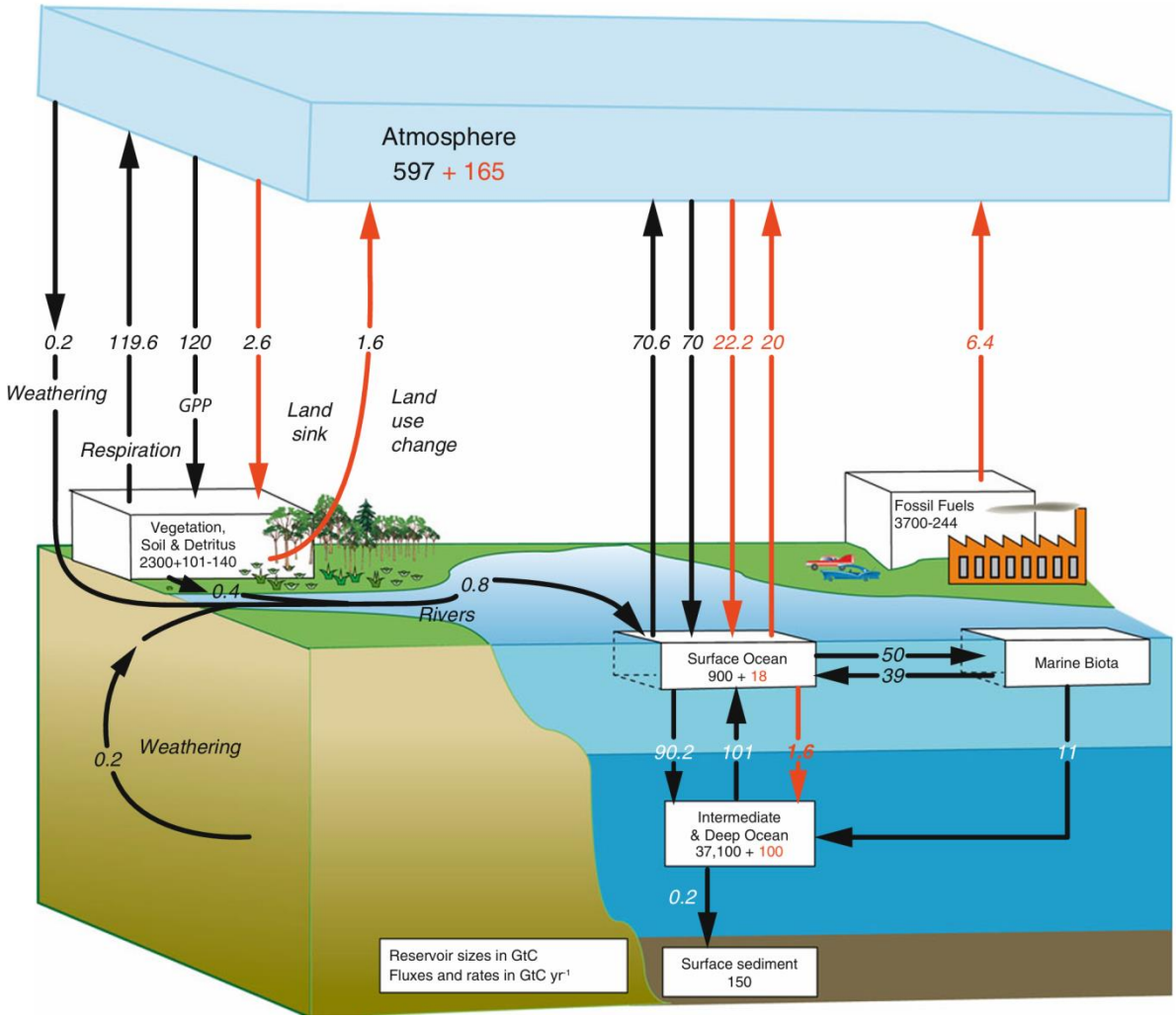

Figure 28 : Global carbon cycle and fluxes between different compartments of the Earth

The CO2 content of the atmosphere (0.0368 % or 368 ppm) is very low compared with that of nitrogen (78 %) and oxygen (21 %). The size of this reservoir is about 762 Pg C (1 Pg ¼ 1 petagram carbon ¼ 1015 g). The atmosphere also contains methane (CH4: ~1.7 ppm) and carbon monox ide (CO: 0.1 ppm) as trace gases. Carbon is present in both organic and inorganic forms in continental and oceanic reservoirs. Oceanic inorganic carbon is involved in the car bon cycle at a short time scale (years to a few hundred years), whereas the continental inorganic carbon is involved at a longer time scale (millions of years). The continental reservoir of biomass is about 2,261 Pg C, which is divided into 550 Pg C for vegetation and 1,711 Pg C for soils and detritus. The terrestrial vegetation thus contains less carbon than the atmosphere (550 versus 762 Pg C), while the first meter of soil contains 2–3 times more

carbon (1,500–2,000 Pg C) than the atmosphere. Half of these two sub-reservoirs of organic carbon is in dense forests then, by decreasing order of importance, in open forests, grasslands, tundra, wetlands, and agricultural ecosystems as well. Almost all terrestrial living carbon is in plants; animals (including the Human species) only represent 0.1 % of this carbon. The oceans contain 37,000 Pg of inorganic carbon and 1,000 Pg of organic carbon, i.e., 50 times more than the atmosphere and 70 times more than the terrestrial vegetation. The vast majority of carbon is found in the mesopelagic and bathypelagic ocean. The sediment contains 6,000 Pg C, with a very slow renewal ("turnover") time. The chemistry of inorganic carbon in seawater is more complex than that of oxygen or that of CO2 in the

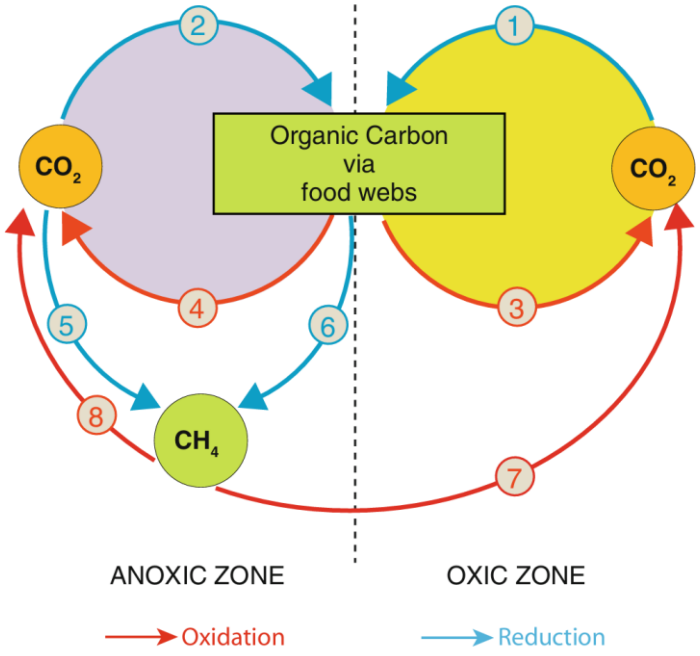

Figure 29 : Role of microorganisms in the carbon cycle.

1 Organic carbon production (biomass) from CO2 reduction (autotrophy) Under oxic conditions: chemolithotrophic bacteria and archaea, photosynthetic micro-eukaryotes, cyanobacteria,

2 Organic carbon production from CO2 reduction (autotrophy) under anoxic conditions: chemolithotropic bacteria and archaea, anoxygenic photolithotrophic bacteria,

3,4 Use of organic carbon as energy source and oxidation to CO2 (mineralization) under oxic conditions (3, aerobic respiration) and anoxic conditions (4, anaerobic respiration),

5 Methane production (methanogenesis) by methanogenic archaea from CO2 reduction (CO2 respiration),

6 Methane production by methanogenic archaea from reduction of C1 organic compouds (methanol, formic acid, etc.), C2 (acetate) or C3 (trimethylamine),

7,8 methane oxidation to CO2 (methanotrophy) by methylotrophic bacteria under oxic conditions (7) or by methanotrophic archaea under anoxic conditions (8); Blue arrows represent reduction processes; red arrows are oxidation processes (Drawing: M.-J. Bodiou)

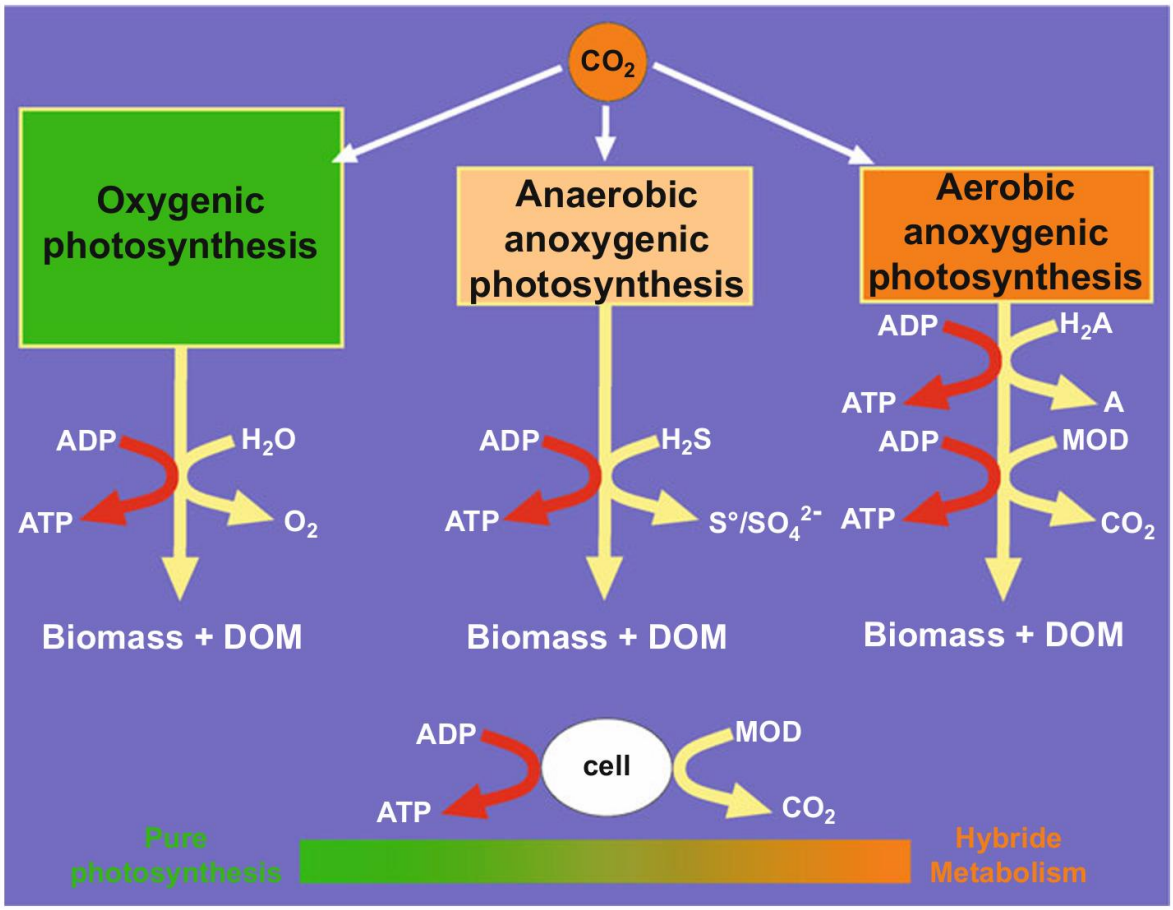

Figure 30 : Relationship between illumination, dissolved organic matter (DOM), and the biological production processes. Schematic representation of the three main mechanisms of photosynthetic production that exist in the water column. Although there is no quantitative assessment of the importance of these processes in marine environment, oxygenic photosynthesis (water photolysis with released dioxygen) is known as the dominant process. H2A electron donor (Modified and redrawn from Karl 2002)

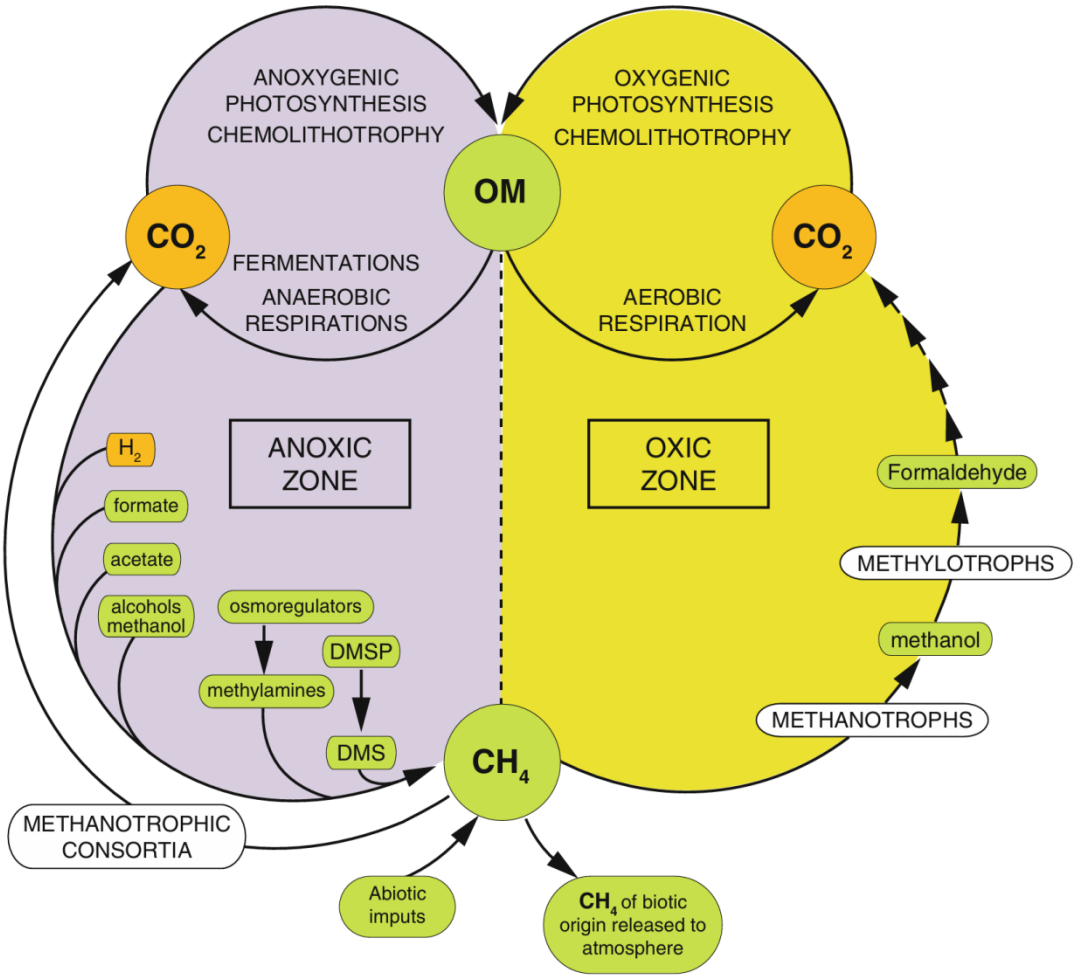

Figure 31 : Production and transformation of methane in the carbon cycle. Methane production from CO2 (CO2 respiration) or simple organic compounds by methanogenic archaea; methane oxidation to CO2 by methanotrophic and methylotrophic bacteria under oxic conditions, or by a bacterial consortium under anoxic conditions (Drawing: M.-J. Bodiou)

14.3 Nitrogen Cycle

Nitrogen is one of the major nutrients used by plants and animals. Combined nitrogen refers to all forms of nitrogen other than dinitrogen (N2). Organic nitrogen (Norg) is nitrogen which

is bound to carbon to form complex molecules. In living beings, organic nitrogen is present mainly in the form of proteins (nitrogen constitutes 18 % of proteins), nucleic acids (DNA, RNA), in certain polysaccharides such as chitin (shell of arthropods) or peptidoglycan (membrane wall of bacteria). Nitrogen is present in various reservoirs on Earth. Inputs and outputs from the differ ent reservoirs are due to physical, chemical, and biological reactions. The nitrogen cycle is complex and consists of multiple redox reactions in which nitrogen passes through many states of oxidation,

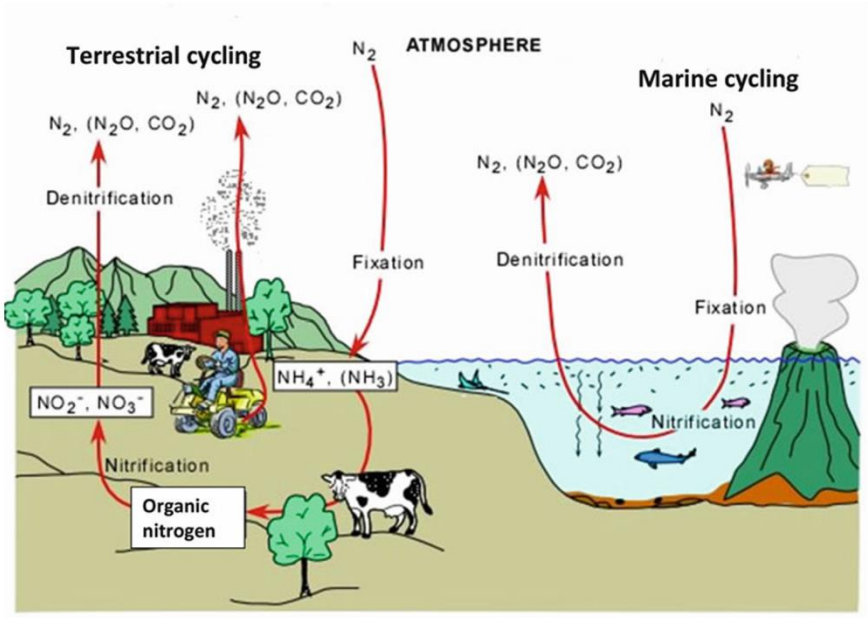

Figure 32 : Global nitrogen cycle and fluxes between different compartments of the Earth (Modified from Plane`te Terre, Bourque et Dansereau, Laval University Que´bec)

The inter-conversion between the different nitrogen forms represents the biogeochemical cycle of nitrogen which is mainly made of biological processes in which microorganisms play a predominant role. The nitrogen biogeochemical cycle comprises a series of redox reactions for transforming nitrogen compound. The nitrogen cycle is of particular interest since it will regulate the amount of nitrogen available for the food chain. Nitrogen is assimilated as ammonium or nitrate; ammonium is derived from the mineralization of organic matter or from the reduction of nitrate. Dinitrogen is relatively inert and may be directly used only by some microorganisms in a process called nitro gen fixation (Fig. 33, **pathway 3**) that converts inorganic nitrogen into biologically available substrates: ammonium ($NH4^+$) and its conjugate acid, ammonia (NH_3). This is the main mechanism for the introduction of nitrogen into the biosphere. Ammonium and ammonia can also be converted to nitrate (NO_3) and nitrite (NO_2)

during the nitrification (Fig. 33, **pathway 4**), an aerobic process which is performed by specialized microorganisms. During denitrifi cation (Fig. 33, **pathway 5),** the nitrate is transformed into gaseous compound: nitric oxide (NO), nitrous oxide (N_2O), and finally dinitrogen (N_2) which is quickly released back to the atmosphere. It is important to note that different pro cesses can lead to the same product (Herbert, 1999).

Although denitrification is often considered as the sole pro cess of dissimilatory reduction of nitrate (using nitrate as an electron acceptor), the dissimilatory nitrate reduction to ammonium (DRNA, Fig. 33, **pathway 6**), also called ammonification of nitrate, can be the dominant process in some ecosystems. This process presents a major ecological interest because, unlike denitrification which corresponds to a net loss of nitrogen in the ecosystem, DRNA ended by ammonium production which is then biologically available for the food web. However, the production of ammonium via DRNA is lower compared with ammonification.

Anammox is a biological process where energy is produced, in which nitrite and ammonium are converted directly into dinitrogen, ammonium being the electron donor and nitrite the electron acceptor (Mulder et *al.,* 1995) (Fig. 33, **pathway 7**) For a long time, denitrification has been considered as the only process leading to the production of dinitrogen and responsible of the net loss of nitrogen from the ecosystems. Another process producing dinitrogen called anammox (acro nym for “anaerobic ammonium oxidation ” was discovered in wastewater treatment systems. This process allows the anaerobic conversion of ammonium and nitrite into molecular nitrogen.

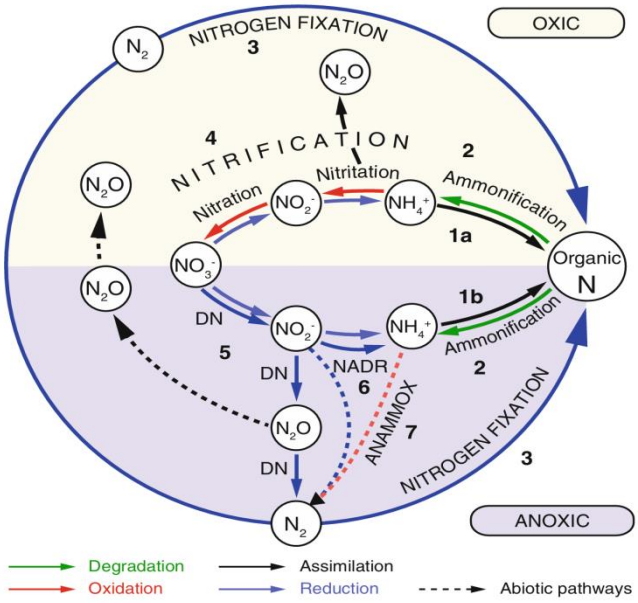

Figure 33 : Nitrogen cycle. 1a ammonium assimilation,1b nitrate assimilation, 2 Ammonification: mineralization, 3 fixation, 4 nitrification, 5 denitrification, 6 DRNA dissimilative reduction of nitrate to ammonium, 7 anammox: anaerobic ammonium oxidation (Drawing: M.-J.Bodiou)

14.4 Sulfur Cycle

Sulfur is an abundant element and exists under different solid, liquid, soluble, or gaseous forms. Unlike nitrogen, sulfur is very scarce in the atmosphere where it is present in both oxidized (SO_2) and reduced (H_2S, organo-sulfur compounds such as dimethyl sulfide or DMS) forms. Sulfur compounds in the atmosphere have a natural or anthropogenic (combustion of fossil materials) origin. In the atmosphere, sulfur is oxidized to sulfur dioxide, and to sulfate, which can cause acid rain phenomena due to the formation of sulfuric acid (H_2SO_4). In addition, sulfate is an extremely hygroscopic compound that promotes cloud for mation and thus participates in the reverberation of a fraction of solar radiations

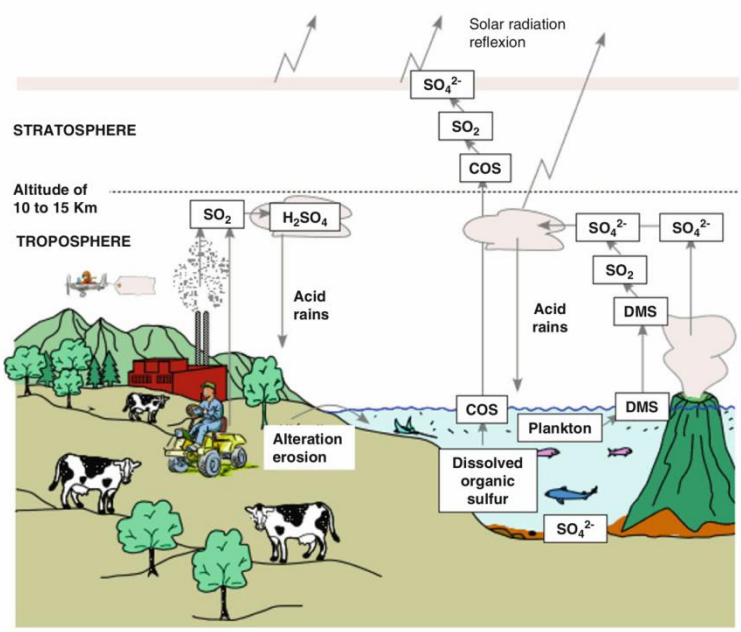

Figure 34 : Global sulfur cycle and fluxes between the different earth main compartments. DMS dimethyl sulfide, COS carbon oxysulfide (Redrawn from Pierre Andre Bourque and Pauline Dansereau, University Laval, Que´bec, Canada)

in Nature, sulfur undergoes a series of chemical or biological transformations occurring in oxic or anoxic conditions. All of these redox reactions constitute the biogeochemical cycle of sulfur in which biological processes, primarily those from prokaryotes, are predominant.

This cycle includes the **sulfur assimilation** (**Fig. 35, pathway 1**) by living organisms, either directly as sulfide or indirectly as sulfate. In the latter case, an **assimilatory sulfate-reduction** step is indeed necessary, in order to incorporate sulfur into organic matter.

The mineralization of organic matter associated with the death of living organisms results in the release of inorganic sulfur, mainly in the form of sulfide, a phenomenon called sulfhydrization (**Fig. 35, pathway 2**).

Another process contributing to the production of reduced sulfur is grouped under the terms of dissimilatory sulfate-reduction or sulfate respiration (**Fig. 35, pathway 3**). During this process, all the oxidized forms of sulfur such as sulfate (SO_4^{-2}), thiosulfate ($S_2O_3^{-2}$), sulfite (SO_3^{-2}), or elemental sulfur (S^0) may be used as electron acceptors by prokaryotes (bacteria and Archaea) and reduced to sulfide (S^{-2}). This activity most often occurs in reduced and anoxic environments.

The sulfur oxidation processes (Fig. 35, **pathway 4)** will use reduced or partially oxidized sulfur compounds as electron donors. Sulfur oxidation involves photolithotrophic anoxygenic metabolisms in anoxic conditions, and chemolithotrophic metabolisms in oxic and anoxic conditions. Although sulfate, sulfur, and sulfide are the three major reservoirs of sulfur, intermediate compounds such as thiosulfate ($S_2O_3^{-2}$), have a very important role in the functioning of the biogeochemical cycle of sulfur. The thiosufate used as electron donor or acceptor generates, in the sulfur cycle, a phenomenon called the "thiosulfate shunt" (Jørgensen 1990). This compound can actually either be oxidized to sulfate (SO_4^{-2}), dismutated to sulfate (SO_4^{-2}) and sulfide (HS) (disproportionation of thiosulfate), or be reduced to sulfide.

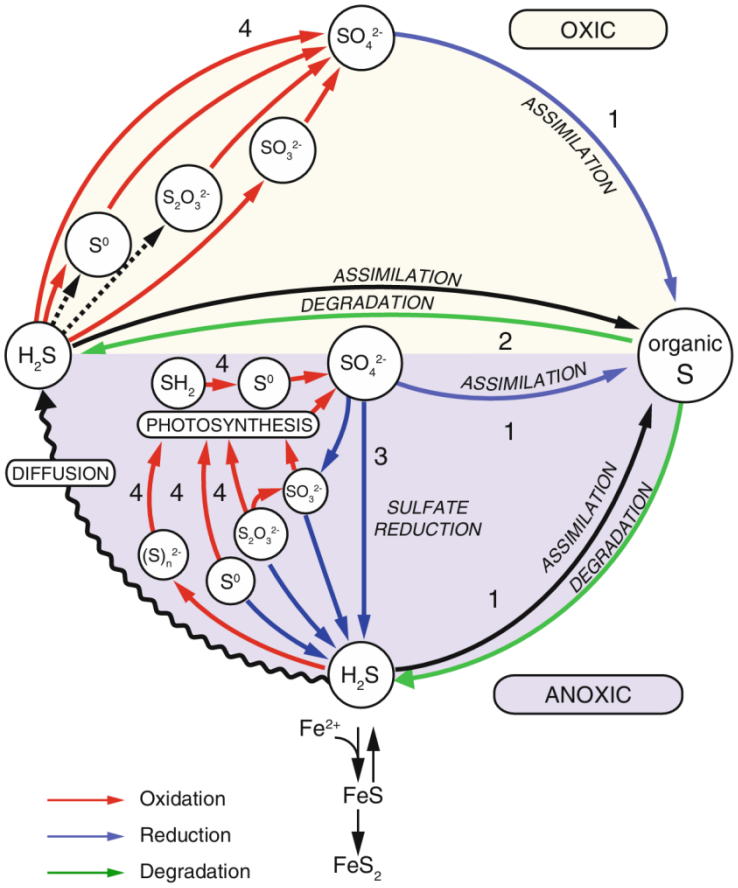

Figure 35 : Biogeochemical sulfur cycle under oxic and anoxic conditions, showing the main biological transformations (Drawing: M.-J. Bodiou)

CHAPTER 4: MICROBIOLOGY OF THE DIGESTIVE TRACT

CHAPTER 4: MICROBIOLOGY OF THE DIGESTIVE TRACT

1. MICROBIOLOGY OF THE HUMAN DIGESTIVE TRACT

The intestinal microflora is in constant interaction with food and the human organism, forming a complex ecosystem. Indispensable for the proper functioning of the body, intestinal microflora should be better understood thanks to new exploration techniques.

In humans, the intestinal microflora contains around 100,000 billion bacteria belonging to over 400 different species. This represents a considerable biomass, whose activity is reflected in the in vivo production of enzymes and metabolites. The study of intestinal microflora cannot be separated from its environmental context, i.e. the host and the food. Together, they form an integrated ecosystem with multiple interrelationships. Any change in one or other of its components is likely to upset the balance and functioning of the whole.

1. TOPOGRAPHICAL DISTRIBUTION OF DIGESTIVE FLORA

The distribution of flora varies according to the segments of the digestive tract. It depends on the oxygen content of the environment, secretions from the upper digestive tract, available nutrients and the speed of transit (rapid from mouth to cecum, slower thereafter). Overall, there is an increasing oral-aboral gradient:

- in the stomach, due to a low pH 2, the flora is virtually non-existent (below 10^3 CFU/g) / (CFU = Colony Forming Units).

- in the small intestine, pH 4-5, there is both quantitative variation (duodenum 10^3-104 CFU/g, jejunum 10^4-10^6 CFU/g, ileum 10^6-10^8 CFU/g) and qualitative variation: aerobic bacteria are gradually replaced by strict anaerobic bacteria. There are few bacteria in the small intestine, where they play virtually no role,

- in the colon, pH 7, transit slows down considerably, leading to stasis and a significant increase in the bacterial population (from 10^9 to 10^{11} CFU/g). The colon is a veritable fermentation chamber, the site of numerous biotransformations of foods not assimilated in the small intestine. The colon is the only zone permanently colonized: the essentially anaerobic microbial flora is dense and active, producing numerous metabolites locally.

The bacteria present in the digestive tract are xenobiotics which, as they are not recognized by the host, should be rejected. However, this enormous bacterial mass is tolerated and is even responsible for non-specific stimulation of the immune system. This cannot be explained by stasis alone: it is likely that bacterial strains have the capacity to adhere either to mucins or to colonic cells via specific (adhesins) or non-specific (ionic bonds, hydrogen bonds) systems.

2. CLASSIC COMPOSITION OF HUMAN INTESTINAL FLORA

A normal flora can be "simplistically" defined as all the species constantly present in the ecosystem and capable of multiplying under the environmental conditions of the digestive tract. But published results vary widely, depending on sampling methods, microbiological methods, the presence of bacteria of dietary origin, intestinal physiology and environmental context. We are far from knowing all the species and their different types, and therefore have a good grasp of the variations in flora induced by changes in diet, exogenous bacteria, antibiotic substances, etc.

The bacteria usually present in the small intestine belong to the genera :

Lactobacillus, Streptococcus, and a few species of the Enterobacteriaceae family at low concentrations up to the ileum, where they appear dominated by Gram-negative anaerobic species belonging to the Bacteroides genus.

In the colon, 4 types of flora can be distinguished:

- **Dominant flora** ($N>10^9$ CFU/g) exclusively anaerobic: Bacteroides, Eubacterium, Bifidobacterium, Peptostreptococcus, Ruminococcus, Clostridium, Propionibacterium,
- **Sub-dominant flora** ($10^6>N>10^8$ CFU/g): various species of the Enterobacteriaceae family (especially E.coli) and the genera Streptococcus, Enterococcus, Lactobacillus, Fusobacterium, Desulfovibrio, Methanobrevibacter,
- **Residual flora** ($N<10^6$ CFU/g): bacteria in transit or repressed by resident flora,
- **Fecal flora**: easily accessible for analysis, it contains many dead species and is not representative of the different ecological niches of the digestive microbial ecosystem. Analysis of the faecal flora gives only a very limited view of the ecosystem, but allows pathogenic or potentially pathogenic strains to be found in the host.

3. ESTABLISHMENT OF INTESTINAL FLORA

At birth, the digestive tract is normally sterile, but it is rapidly colonized by bacteria from the direct environment (particularly the mother's flora), reaching a population of between 10^9 and 10^{11} CFU/g after 48 h in the colon. A complex phenomenon, still poorly understood and relatively specific to each animal species, colonization occurs differently depending on whether the child is born vaginally or by Caesarean section. In infants, the microbial flora shows great variability: predominance of *Bifidobacterium* in the majority of breast-fed children, or predominance of *Lactobacillus* in formula-fed children. In children aged 1 to 4, in parallel with dietary diversification, a gradual change in flora is observed, tending towards that of adults and referred to as “normal flora”.

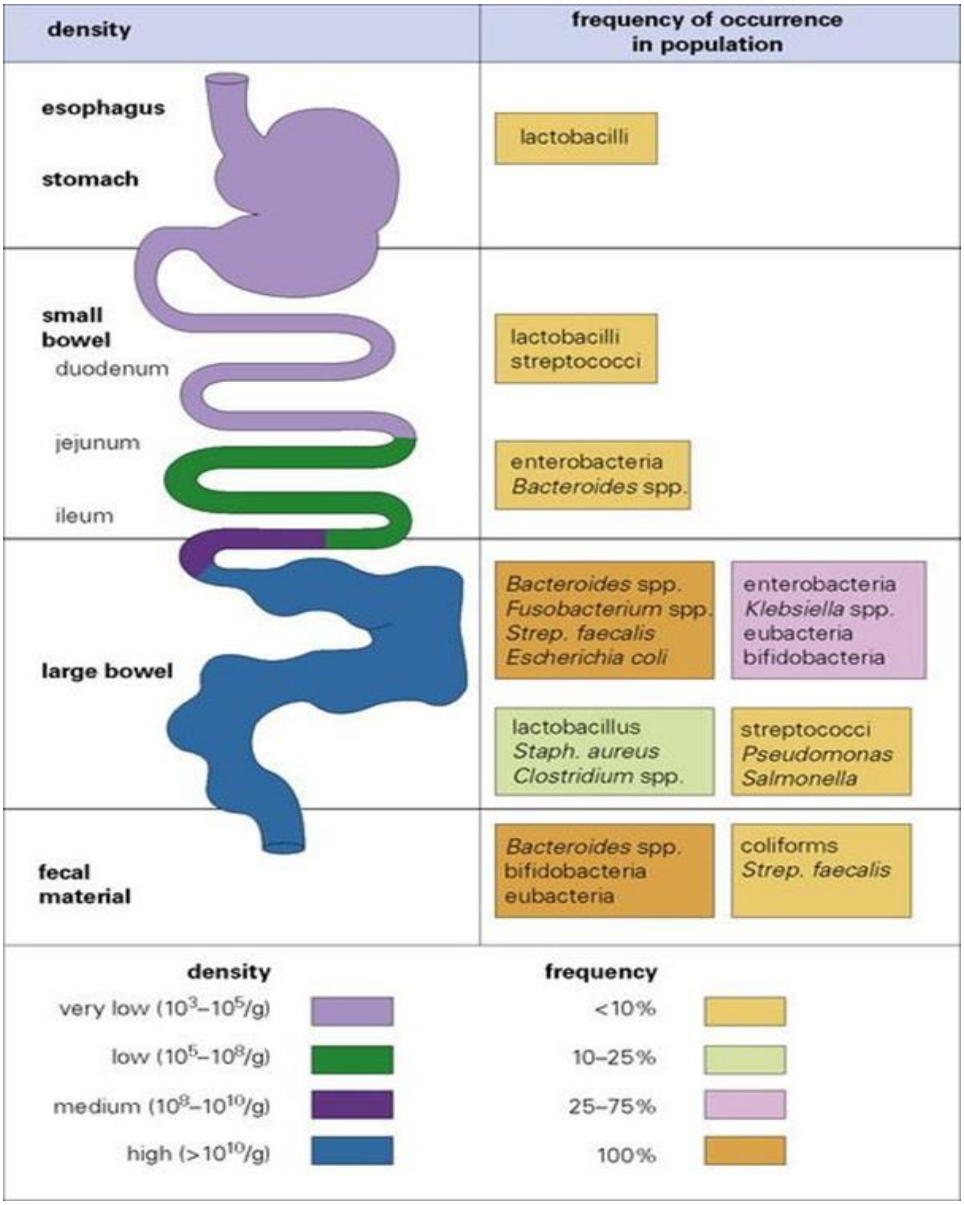

Figure 36: Simplified diagram describing the compartments of the human digestive tract and their microflora (https://schoolbag.info/biology/microbiology/9.html)

4. MAIN FACTORS MODIFYING THE HUMAN INTESTINAL MICROBIOTA:

a) Food:

The composition of the diet (rich in fiber, proteins, fats, sugars, etc.) directly influences the diversity and abundance of intestinal microorganisms.

Diets high in fiber promote beneficial fermentation bacteria, while diets high in fat and sugars can disrupt the microbial balance.

b) Antibiotics and Drugs:

Antibiotics can reduce microbial diversity and eliminate some beneficial species, allowing pathogens to develop.

Other drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs), may also affect the microbiota.

c) Age:

The microbiota evolves throughout life, from birth to old age. Infants, adults and the elderly have distinct microbial compositions.

d) Mode of Birth:

Babies born by vaginal route acquire their microbiota mainly from their mother, while those born by caesarean section have a different microbiota initially.

e) Breastfeeding:

Breast milk provides specific oligosaccharides that feed certain beneficial bacteria, such as bifidobacteria.

f) Environment:

Exposure to various environmental micro-organisms, such as those found in soils, pets and living places, influences the composition of the microbiota.

g) Genetic factors:

Genetic predispositions may play a role in microbial diversity and susceptibility to microbial imbalances.

h) Stress and Lifestyle:

Stress, exercise and other aspects of lifestyle can influence the gut microbiota. For example, chronic stress can cause microbial imbalances.

5. ROLES OF THE HUMAN INTESTINAL FLORA

a/ Digestive effects

Anatomical and histological changes related to the presence of microflora are highlighted by comparing what happens in conventional animals compared with axenic (germ-free) animals. It is thus found that:

- The absence of flora causes a slowdown in intestinal transit and a dilatation of the cecum (effect on motor skills),
- The rate of cell renewal and mitotic index are significantly reduced in axenic animals (effect on trophication).

b/ Nutritional effects

• beneficial to the host

- production of short-chain fatty acids that decrease hepatic cholesterol synthesis; one of them, butyric acid, is the main source of energy for the colic mucosa,
- degradation of unabsorbed carbohydrates (starch, pectin, glycoproteins) leading to the production of host-assimilable organic acids (acetate, propionate, butyrate) and gas (CO_2,H_2)
- hydrolysis of unabsorbed food lipids thanks to bacterial lipases and conjugation of primary bile acids, essential for a good absorption of fats,
- breakdown of certain proteins and amino acids (tryptophan), allowing the recovery of nitrogen,
- vitamin intake: some optional anaerobic bacteria (E.coli, E.aerogenes) are able to synthesize in vitro a wide range of vitamins (biotin, riboflavin, pantothenic acid, pyridoxine and vitamin K). Strict anaerobic bacteria (C.butyricum, Veillonella sp.) are able to synthesize vitamin B12, of great use for local bacterial growth. There is no precise data on the use of these vitamins by the host, especially by humans.

• **Adverse to host:**

- ❖ carbohydrate metabolism: the ß-glucuronidase activities release from the ß-glucuronides of carcinogenic aglycones,
- ❖ nitrogen metabolism: the degradation by the microflora of nitrates and secondary amines leads to the production of carcinogenic nitrosamines,
- ❖ metabolism of xenobiotics: possibility of drug inactivation (inactivation of digoxin by Eubacterium lentum) or production of toxic metabolites. Thus the bacterial myrosinases, able to hydrolyze glucosinolates of cruciferous (cabbages, brussels sprouts, turnips...) can be responsible for diarrhea. Similarly, after large and prolonged consumption of cabbages, the metabolites derived from 5-vinyl-oxazolidine-2-thione (goitrine) are responsible for a significant decrease in the uptake of iodine by the thyroid.

c/ Protection against infection

It is first exercised by the barrier effect exerted by the resident flora vis-à-vis exogenous bacteria ("resistance to colonization"), by total elimination of the exogenous strain (drastic effect), or by maintaining the exogenous strain in sub-dominance (permissive effect). The mechanisms explaining these phenomena, which are poorly known, are closely related to the dominant strict anaerobic strains of resident flora.

The digestive flora also stimulates local immunity, as shown by comparisons of the immune status of conventional and axenic animals.

Both effects may, under certain conditions, be increased by some **probiotic** yeasts or bacterial strains (lactic acid bacteria) in transit.

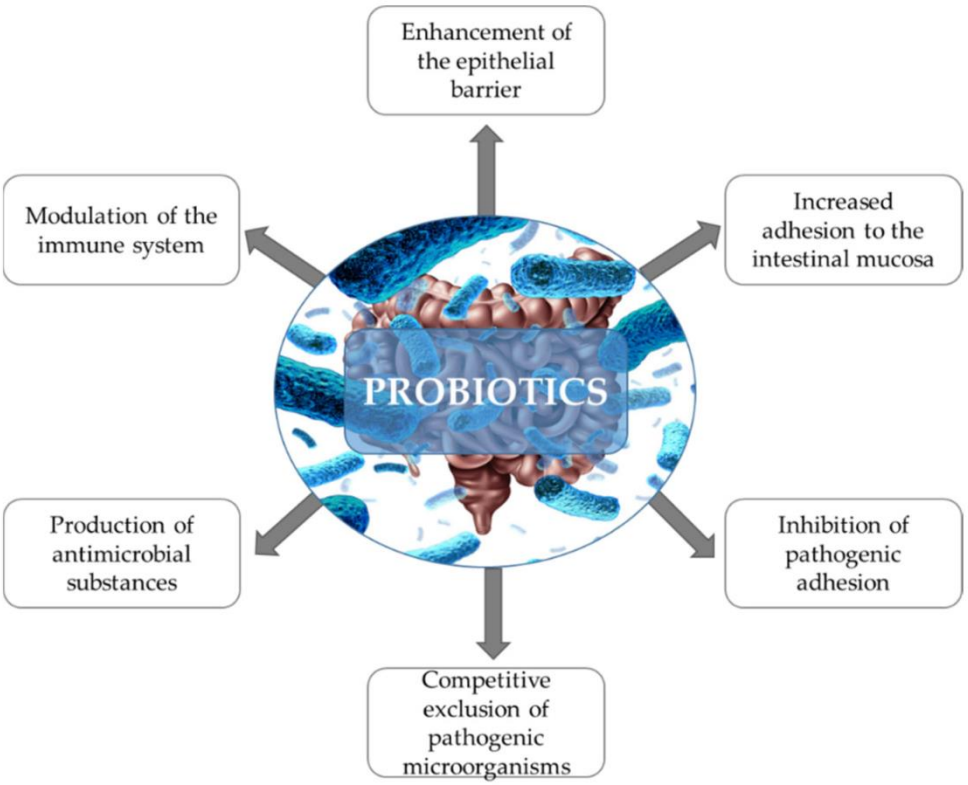

Figure 37 : Major mechanisms of probiotic activity in the human body **(Udayakumar et *al.*, 2022)**

2. MICROBIOLOGY OF THE RUMINANTS DIGESTIVE TRACT

The digestive function of ruminants is characterized by the existence of a micropopulation, in the pre-stomachs, especially in the rumen. This micropopulation is characterized by its extreme diversity: there are a large number of protozoa, fungi and bacteria, the latter population constituting the rumen flora. This ruminal population, characteristic of ruminants, develops during the early stages of the young's life.

1/ ANATOMY OF THE DIGESTIVE TRACT

The digestive tract of sheep is similar to that of other ruminants, it consists of three unequal parts: stomach, small intestine, large intestine.

1-1/ Stomach:

This is the digestive portion between the esophagus and the intestine. It occupies 3/4 of the abdominal cavity. It consists of four compartments: the rumen (panse), the network (reticulum), the leaflet (omasum), the caillette (abomasum) which is considered as the true stomach. The volume and weight of the stomach varies with the level of ingestion, ration composition and feeding behaviour.

1-1-1/ Panse or rumen:

It occupies the left part of the abdomen. It is a bulky bag representing 85 to 90% of the volume of the stomach and 70 to 75% of the total volume of the digestive tract.

The rumen wall is made up of a muscular tunic which constitutes the bulk of its mass. The contractions of these muscles ensure that food is continuously stirred. The rumen is lined with a mucosa ensuring absorption of soluble nutrients. The different pockets of the rumen communicate through a wad of two protrusions which is the oesophageal drop.

1-1-2/ Network or reticule:

It is placed in front of the belly, against the diaphragm. Its inner wall is lined with honeycomb-like alveoli covered with horny papillae. These cells increase the surface area of contact with food. They play a major role in circulation and sorting, allowing only sufficiently fragmented food particles to pass into the leaflet, with other particles retained in the rumen where they undergo rumination and microbial degradation. This is the reason why rumen and leaflet are considered to be a single organ called rumen reticulum.

1-1-3/ Leaflet or omasum:

It is a roughly spherical reservoir, larger than the network. Its inner wall is lined with many mucous membranes. Like the leaves of a book, hence its name. These lamellae, deposited in parallel with the passage of food, ensure the filtration of food particles and the absorption of water and minerals from the digestive contents, before their arrival in the clot.

1-1-4/ Caillette or abomasum:

It is elongated, folded in spiral ridges. The luminal epithelium consists of secretory cells that produce mucus, hydrochloric acid and pepsin (pH: 2-3). It ends in the pylorus, which connects it to the duodenum.

1-2/ Intestines:

1-2-1/ Small intestine:

It is divided into the duodenum, jejunum and ileum. Its mucosa is rich in villi which form a surface for absorption and secretion. Its development depends on the diet and species.

1-2-2/ Large intestine:

It is formed by an elongated reservoir: the cecum (0.75m), the colon (9m) which winds in a spiral and an ovoid pocket ending at the anus which is the rectum.

2/ THE PHYSIOLOGY OF THE DIGESTIVE TRACT

2-1/ Physico-chemical parameters:

2-1-1/ pH:

It plays an important role in regulating microbial activity. It is practically stable (6-7). But a rapid fermentation can lower the pH to less than 5, which is favorable for the growth of microorganisms that mainly produce propionate and lactate. The abundant and continuous salivation assures to the contents of the rumen a buffering power, by the supply of a large quantity of ions bicarbonate and phosphate.

2-1-2/ Temperature:

It is generally higher than that of the body: 39°- 40.5°C. However, it can reach 41°C during the great fermentation.

2-1-3/ Redox potential:

The rumen is a highly anaerobic ecosystem, with an average oxidoreduction potential of -350mv. The area near the epithelium is very vascularized. A microbial population is optionally attached to it and aerobically contributes to the elimination of traces of oxygen, thus maintaining the ecosystem in anaerobic conditions.

2-1-4/ Osmotic pressure:

It is identical to blood under normal feeding conditions. After absorption of water, the osmotic pressure decreases. But given the permeability of the rumen wall, it reaches equilibrium after ten hours. The osmotic pressure of saliva is lower than that of blood. Its arrival continues in the rumen, little affects the pressure of the medium.

2-1-5/ Gas phase:

Its average composition is:

Co2 - - - - - - - - - - - - - - - 60-65% CH4 - - - - - - - - - - - - - - - 25-30%

N2 - - - - - - - - - - - - - - - 6-9% O2 - - - - - - - - - - - - - - - 0,3-0,6%

H2 - - - - - - - - - - - - - - - 0,1-0,3% H2S - - - - - - - - - - - - - - - 0,01%

2-1-6/ Water phase:

2-1-6-1/ Volatile fatty acids:

They are present at a concentration of about 0.1N in the rumen. They are in balance with their formation and absorption through the rumen wall and their passage to the intestine with the digesta.nThe volatile acids guys (AGV) have the following composition: acetic acid (70%), propionic (20%), butyric (8-9%), methyl-butyric acid and isobutyric acid, valeric and isovaleric acid (1-2%). They are an important source of energy for the animal, especially that produced during the fermentation of cellulose.

2-1-6-2/ Organic acids

They are found in very small quantities because they are quickly absorbed and metabolized.

2-2/ Rumination or meric mastication:

It is the act by which food is brought back from the rumen into the oral cavity to be subjected to a second mastication making the particles finer and a second salivation before returning to

the belly to be fermented there. Indeed, rumination facilitates the action of microbial fermentation and digestion of all food compounds. Once the mechanical grinding is complete, the food is in the gut where it will undergo a second digestion.

2-3/ Digestion:

Digestion involves physical phenomena (grinding, transit,...) and phenomena due to digestive secretions or the action of the microbial population developed in the digestive tract, which allows the animal both to use plant cellulose and to ensure its nitrogen nutrition by degrading simple and synthesize B vitamins and vitamin K. In ruminants, The main feature is that food is subjected to microbial actions in the pre-stomachs before undergoing the action of enzymes from the digestive tract. These phenomena mainly concern the anterior part of the digestive tract (reticulo-rumen) where the physico-chemical conditions are favourable to the action of micro-organisms.

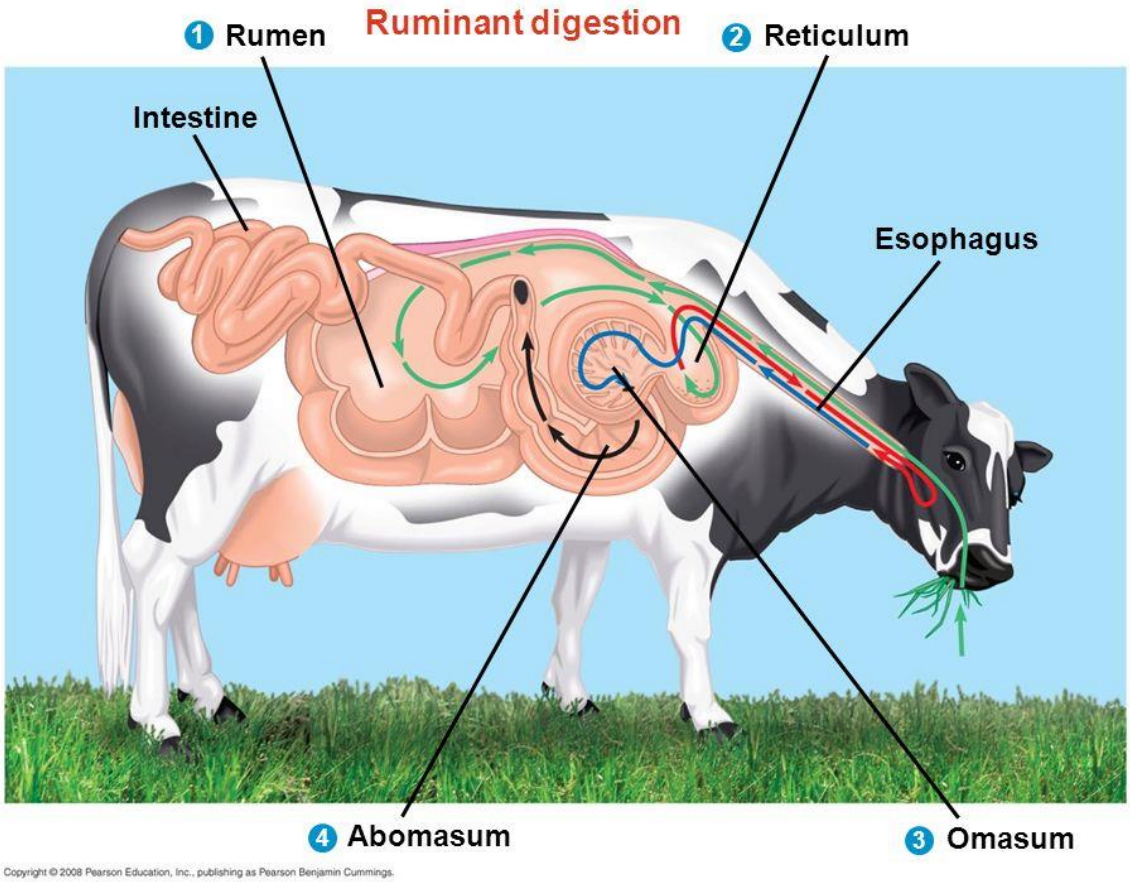

Figure 38 : ruminant digestion

(https://bot1320.nicerweb.com/Locked/media/ch05/ruminant.html)

3/ THE MICROFLORA OF THE DIGESTIVE TRACT:

1- Bacteria:

The ruminal flora is characterized by its extreme diversity, the number of bacterial species colonizing the rumen being important, and presenting varied enzymatic activities. The rumen of an adult contains about 1010 bacterial cells/ml, bacteria alone represent about 50% of the microbial biomass. It is composed mainly of non-sporulated strict anaerobic bacteria.

Among the main bacterial species: Cellulolytics (Bacteroides succinogenes, Ruminococcus albus, Ruminococcus flavefaciens, Butyrivibrio fibrisolvens), Hemicellulolytics (Bacteroides ruminicola, Butyrivibrio fibrisolvens), Amylolytics (Bacteroides amylophilus, Streptococcus bovis (optional aerobic), Bacteroides ruminicola).

Bacteria colonize the digestive tract of ruminants rapidly. From the first day, the first bacteria settle: Escherichia coli and Streptococci, while cellulolytic bacteria appear on the 4th day in 75% of young ruminants. These can be grouped according to the type of substrate attacked in the rumen. The substrates fermented by ruminal bacterial species are multiple, so they can be found in different ecological niches (degradation of cellulose, starch, proteins, lipids, use of ammonia, organic acids, methane production etc.)

2- Protozoa:

Protozoa are cellular eukaryotic organisms. There are 02 types in the rumen: flagellates and ciliates. Ciliates represent about half of the microbial biomass and their concentration varies from 104 to 106 cells/ml, it is distributed between solid particles and liquid phase.

The majority of protozoa found in the rumen belong to the branch of the ciliates, and represented by two groups, both of the subclass of the Trichostomatiae. The «holotriches» belong to the order of the Vestibuliferida, and the «entodiniomorphes» to the order of the Entodiniomorphidae, under the order of the Entodiniomorphines, and family of the Ophryoscolecidés.

Among the Entodiniomorphidés, there are a large number of genera: the genera Entodinium (a genus difficult to classify on the basis of morphological aspect), Eodinium (whose type species is Eodinium lobatum), Diplodinium, Eremoplastron, Eudiplodinium,Ostracodinium, Polyplastron, Diploplastron, Metadinium, Epidinium, Enoploplastron, Ophryoscolex, Epiplastron, Elytroplastron.

Concernant les Holotriches, les genres rencontrés dans le rumen sont majoritairement Isotricha et Dasytricha, ainsi que, en moindre nombre, les genres Oligoisotricha, Microcoetus, Buetschliidae, Parabundleia, Polymorphella, Blepharoconus et Paraisotricidae)

The type of food ration strongly conditions populations of protozoa. And are very sensitive to non-nutrition and can disappear in 2-3 days of diet.

Entodiniomorphs digest cell walls and chloroplasts, with cellulolytic enzymes found in all protozoa of this order. Nevertheless, the presence of cellulases of bacterial origin does not allow to bring the unambiguous expected of a hair rather than bacterial origin. Larger protozoa can also degrade hemicellulose. On the other hand, protozoa play an important role in starch hydrolysis by ingesting starch granules and soluble sugars, thus reducing the accessibility of these substrates to amylolytic bacteria.

Interactions with other microorganisms are numerous: protozoa ingest endogenous and exogenous bacteria as a source of proteins for their cell synthesis. Predation increases the concentration of ammonia and phosphate and also the bacterial concentration and its efficiency because there are more usable nutrients. The protozoa are not essential for digestion but their presence improves digestibility, standardizes fermentation between meals.

3- Fungi:

The rumen mushrooms were discovered only late. The fungal population is estimated at 10^3 and 10^5 cells/ml or about 10% of the microbial biomass. Zoospores attach to particles of already damaged plants. Rhizoid penetrating into tissues by proteolysis. The fungi found in the rumen are strict anaerobes, which is quite exceptional in the group of fungi, do not have mitochondria, no cytochromes and only ensure the fermentation of cellulosic tissues. Three species are described: Neocallimastix frontalis, Piromonas communis and Sphaeromonas communis.

The proteolytic activity is ensured by metallopores, they hydrolyze the extensin of the walls. They contain a lot of amino acids, the content of adenine and thymine is important, and as such, the proteins of mushrooms are highly digestible. Fungi produce a significant amount of H2 and are therefore associated, in metabolic reactions, with methanogenic bacteria, dihydrogen-consuming bacteria. Cellulolytic bacteria decrease fungal activity. Removal of fungi decreases digestibility and increases the propionate ratio.

4- Viruses:

125 morphological types of bacteriophages have been observed in the rumen. Their role among the microbial population is not well known. Although they lyse Streptococcus bovis and Bifidobactéruim thermophilus in vitro. Viruses are an important component of the ruminal environment and likely play roles in the ecology of the rumen; however, their activation mechanisms in the rumen remain unclear and their interactions with other components of the microbiome, diet, and physicochemical properties of the ruminal environment and subsequent effects on the health and production of livestock animals.

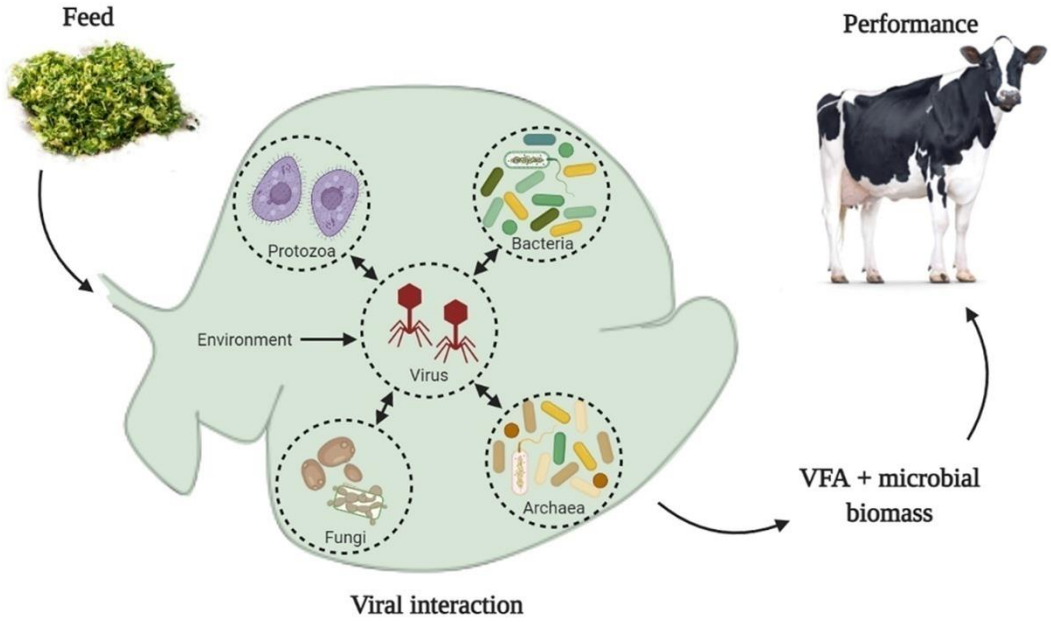

Figure 39 : interaction between all groups of microorganisms in the ruminal environment. Lobo & Faciola (2021).

CHAPTER 5: CONTAMINATION AND HYGIENE OF PREMISES

CHAPTER 5: CONTAMINATION AND HYGIENE OF PREMISES

1. SOURCES OF MICROBIAL CONTAMINATION: AIR, WATER, PERSONNEL, SURFACE AND MATERIAL, RAW MATERIALS.

1.1. Air:

Air microorganisms are generally carried by media of varying size: dust (10 to 100µm), droplets and micro-droplets emitted from the human respiratory tract or by aerosolization (reduction of spores, ... to make them small enough to be inhaled) (10 to 1,000µm) and condensation nuclei from the evaporation of droplets (2 to 5µm). The smallest particles, in the order of a micron, persist in the atmosphere for a long time because their theoretical speed of fall is slow (about 1 metre in 8 hours); they can be kept in suspension and diffuse at a distance from their emission point; They can penetrate deeply into the respiratory tract and reach the pulmonary alveoli.

The air microflora consists of a base flora and an accidental flora.

a) Saprophytic flora:

This basic flora is made up of the germs of the environment, composed of some bacteria and spores of microscopic fungi. It is consistently found in samples from a standard culture medium.

The bacteria regularly found in air samples are Gram-positive bacteria, usually the most resistant in the outdoor environment: *Micrococcus* or sporulated bacilli such as *Bacillus*.

The mycelial flora is very diversified and more abundant in summer and autumn (*Penicillium, Aspergillus, Mucor, Alternaria, Botrytis*). Yeasts belonging to the genera *Rhodotorula, Pichia, Kloeckera* can also be encountered.

b) Accidental flora of human, animal or hydro-telluric origin:

This flora comes to supercontaminate the base flora. Human or animal microorganisms can be divided into two groups:

- those whose life span will be limited in the outdoor environment and which will rarely be found in samples (viruses and fragile bacteria);

- those able to survive for a more or less prolonged time in the outdoor environment and which will be indicators of fecal contamination (*E. coli, Enterococcus...),* rhinopharyngeal or cutaneous (*Staphylococcus, Neisseria, Corynebacterium*).

Micro-organisms of hydro-telluric origin (bacteria such as *Pseudomonas, Flavobacterium, Acinetobacrer,* microscopic fungi and their spores) have a life independent of human presence. They proliferate with sometimes small amounts of nutrients (*Pseudomonas*).

1. 2. Water:

Water can be a powerful vector of contamination because it is often rich in micro-organisms. Water used in product processing or cleaning may cause contamination. But it is the waste water that is the major risk: they become the sites of development of microorganisms from other agents.

Human contamination occurs either through consumption of drinking water or food contaminated by water, or during a bath or contact with waters for recreational use (the cutaneous-muscular route), but the respiratory route (*Legionella*) or even the digestive route (*Yersinia, Listeria*) are increasingly found.

Pathogenic microorganisms include species of human or animal feces most frequently encountered belong to the genera Shigella, responsible for dysentery (*S. dysenteriae*), Salmonella, responsible for water diseases, typhoid and paratyphoid (*S. typhi and S. parathyphi*), the germ (*Vibrio cholerae*), is also widely present in the usual microflora of waters and soils. Parasites (*Cryptosporidium, Giardia*); Yeasts (*Candida albicans*); and Viruses (enteroviruses, hepatitis A) also.

1.3. Personnel:

Personnel present in production areas may be the source of contamination: through direct contact (with the skin), or indirect (hair, scales, hairs....). While it is easy enough to prevent this contamination (hat, glove...), it presents a great health risk (disease vector).

1.4. Surface and Material:

Surfaces of production areas in industrial environments (floor, wall, ceiling...) and equipment (machine, transport medium...) can be covered with micro-organisms which then form a biofilm that is fairly resistant to cleaning. This biofilm is often from contamination from air or water. Surfaces are contaminated either by contact, or by sedimentation of microorganisms

present in the air. In hospital settings, the most contaminated surfaces are: floors, bed barriers, tables, patient clothing, pillows and mattresses.

However, not all bacterial species isolated from these surfaces are necessarily pathogenic but the flora resulting from human activity such as bacteria of the cutaneous flora or mucous flowers and some bacterial species of water flowers natural bacteria such as Pseudomonas aeruginosa may be responsible for nosocomial infections.

1. 5. Raw material:

Depending on the transformation processes applied to the raw material, the microbial flora initially present on it may be found in the final product, with various consequences depending on the nature of the microorganisms present. The raw material must be contaminated due to several sources:

- **Aerial flora:** is composed of a basic flora (cells and spores) and an accidental flora related to a mechanical disturbance that may be human.
- **Water and soil**: may contain:
  - ✓ Bacteria: *Achromobacter, Enterobacter, Bacillus, Micrococcus*
  - ✓ Yeasts: *Aspergillus, Rhizopus, Penicilliun*
  - ✓ Molds: *Saccharomyces, Torula.*
- **Personnel:** personnel must be contaminated on the effect of several sources:
  - ✓ Surfaces in production and work areas and equipment that can be covered with microorganisms, which form a biofilm, are fairly resistant to cleaning.
  - ✓ The wrong manipulations during work.
  - ✓ Raw materials initially contaminated.
  - ✓ Ambient air.

2. MAIN CONTAMINATIONS AND HYGIENE RULES: HOSPITAL SETTINGS, INDUSTRIAL ENVIRONMENTS

2.1. Hospital settings

The term hospital environment usually includes air, water, surfaces (floors, walls, furniture, equipment), linen, food, medical devices, waste.

The hospital environment is heavily contaminated with micro-organisms of human origin or specifically environment*al.* This contamination varies qualitatively and quantitatively over

time, from one institution to another and within the same institution, depending on services, patients, care and techniques. The microorganisms present in the hospital environment are extremely varied (bacteria, yeasts, filamentous fungi, viruses and parasites) and can belong to both opportunistic species and species usually pathogenic for humans.

a) Bacteria:

Two types of bacteria can be found in the patient environment:

- bacteria of human origin (skin, mucous membranes), including multidrug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus*, extended-spectrum beta-lactamase-producing *Enterobacteriaceae*, and vancomycin-resistant *Enterococcus*.
- bacteria of environmental origin, some of which have frequent natural resistance to antibiotics, particularly Gram-negative bacilli such as *Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia, Burkholderia cepacia, Legionella pneumophila*, or atypical mycobacteria.

b) Fungi:

Among the other microorganisms involved in nosocomial infections, yeasts and especially environmental filamentous fungi (*Aspergillus spp*.) are very well adapted to survival and multiplication in the environment.

c) Viruses:

Viruses can also contaminate the environment, most often from the human reservoir constituted by patients and hospital staff. Certain viruses responsible for nosocomial infections in pediatrics, such as respiratory syncytial virus or rotavirus, survive for varying lengths of time in the environment. Rotaviruses, for example, can survive for several days on hands and for one to 10 days or more on dry, non-porous surfaces in a low-humidity environment (< 50%), compared to 6 hours for respiratory syncytial virus.

d) Parasites:

Infectious forms of certain parasites are eliminated in very large quantities in nature from parasitized hosts. This is particularly the case for *Cryptosporidium parvum*, amoeba cysts, *Giardia intestinalis*, and other parasites such as *Cyclospora* and microsporidia. In addition, free amoebas present in water systems are likely to harbor and promote the survival and

multiplication of *Legionella spp*. The viability of these parasites in the external environment is prolonged, and the means of detection and prevention remain limited.

Current techniques, including molecular biology, have made it possible to identify environmental sources of nosocomial infections:

- risk of airborne transmission (*Aspergillus* or other filamentous fungi) and infection in immunocompromised patients during outdoor work;
- risk of airborne transmission from an aqueous reservoir via humidifiers, nebulizers (*Acinetobacter spp., Pseudomonas aeruginosa, Legionella pneumophila*), hot water systems, or air treatment systems for *Legionella pneumophila*;
- contact transmission of *Mycobacterium xenopi, Pseudomonas aeruginosa*, or hepatitis C virus from medical devices;
- Contact transmission of Pseudomonas aeruginosa or other resistant bacteria (*Serratia marcescens, Burkholderia cepacia, Stenotrophomonas maltophilia*, etc.) from contaminated antiseptics.

2.1.1. Risk areas in hospitals

Hospital premises are classified according to infectious risk into four types of areas:

- **Minimal risk areas**: administrative areas, corridors, offices, etc.
- **Medium risk areas**: long-stay wards, maternity wards, psychiatric wards, etc.
- **High-risk areas**: rooms where immunocompromised patients, cancer patients, or patients with cirrhosis are located.
- **Very high-risk areas**: rooms where patients have low immune defenses due to their illness, such as burn units, transplant units, operating rooms, and neonatal units.

Nosocomial infections: An infection is considered “nosocomial” when it is acquired in a healthcare facility and appears within 48 hours of admission. For surgical site infections, infections occurring within 30 days of surgery are considered nosocomi*al.*

2.1.2. Hospital hygiene

This takes into account all clinical, microbiological, and epidemiological aspects of infections, as well as the organization of care, maintenance of hospital equipment, environmental management, and staff protection. It is an indicator of quality of care and safety.

a) Hand washing: The goal is to prevent hand-borne transmission and eliminate transient flora.

b) Wearing gloves: This is necessary during any contact with biological fluids (blood, urine, etc.) in order to prevent the risk of infection and protect healthcare personnel.

c) Professional attire: This must be changed daily and whenever it becomes soiled.

d) Isolation: Isolation measures aim to establish barriers to the transmission of microorganisms:

- from one patient to another;
- from a patient to a caregiver;
- from a caregiver to a patient;
- from the environment to the patient.

e) Waste disposal: To prevent the risk of infection, hospital waste must be disposed of acording to certain procedures.

f) Antiseptics

Antisepsis: A temporary procedure that allows living tissue, within its tolerance limits, to eliminate or kill microorganisms and/or inactivate viruses.

An antiseptic: A product or process used for antisepsis under defined conditions. Antiseptics are used only on living tissue. They are medications.

A **good antiseptic** must be soluble in water or alcohol, stable over time, have a broad spectrum of activity, be incapable of inducing resistance, and have no side effects. Antiseptics are bacteriostatic/cidal and/or virucidal and/or fungicid*al.*

g) Disinfectants

Disinfection is a temporary operation that eliminates microorganisms and/or inactivates viruses carried by inert media (unlike antiseptics). It is only used on decontaminated and

rinsed equipment. Decontamination is a temporary process that eliminates microorganisms. It is only used on contaminated equipment.

H) Sources and vehicles of infection

Hospital infections originate from:

1) The patient's own flora: **autoinfection;**

2) The flora of another patient: **cross-infection**. The germ in question is then transmitted:

a) by direct contact from one patient to another (saliva droplets, patient's hands);

b) through the air (dust from textiles carrying the flora of a patient);

c) by staff : who directly collect the germs on their hands or gown and transmit them to another patient, or who cultivate the germs on their own airway and intestinal mucous membranes;

d) through objects :

- contaminated by the patient themselves: accommodation equipment (sanitary facilities, blankets), medical equipment;
- contaminated by the hands of hospital staff in the hospital (kitchen, laundry room, treatment room, etc.);
- contaminated by visitors;
- contaminated by sick staff or carriers of germs;
- contaminated by contaminated food or water.

Airborne transmission can occur under the following conditions:

a) by inhalation of saliva droplets projected from person to person : childhood diseases, pulmonary tuberculosis

b) by inhaling water droplets aerosolized into the air by a cold humidifier.

c) by inhaling infected water droplets (e.g., pyocyanic).

d) by inhaling humidified air contaminated with *Legionella* during humidification in air conditioning systems.

e) by inhaling droplets of hot water (< 5 µ) from showers and sinks (*Legionella*).

2.2. Industrial environments

Human activities are often the source of industrial pollution. Industries release polluting fumes, air, and water, as well as waste that is harmful to the environment and health. Industrial pollution has very serious effects on human health. There are several types of pollution:

2.2.1. Air pollution

Industrial pollution is caused by the presence of toxic particles in the air; it is caused by industrial fumes (refineries, car exhaust fumes -CO2-), fertilizers or pesticides, methane, and ozone. These pollutants increase the risk of disease in humans:

- respiratory diseases: asthma, bronchiolitis, tonsillitis, respiratory failure, and allergies;
- cardiovascular diseases: myocardial infarction, angina pectoris, and strokes ;
- risks to human reproduction;
- cancers;
- skin diseases.

2.2.2. Soil pollution

Soil pollution is caused by chemical fertilizers or pesticides that are spread on agricultural land to improve yields. Their use causes:

- food poisoning;
- cancers;
- endocrine (gland) disruption.

2.2.3. Water pollution

Water pollution is linked to soil pollution because water seeps into the ground and contaminates groundwater. The health risks are:

- allergenic effects (causing allergies): rhinitis, conjunctivitis, asthma
- viruses and microbes that make water undrinkable and cause many diseases.

2.2.4. Classification of premises into sensitivity zones

Food processing plant premises are classified into sensitivity zones according to the operations carried out on the product. The different levels are determined either by the number of germs per m3 or by the degree of dustiness. Statistically, it is accepted that 10,000 particles of 0.5µm correspond to 1 germ. Classification into inert, sensitive, or ultra-sensitive zones corresponds to the assessment of biocontamination risks.

a. Inert zone (low or medium risk zone: level 1)

In general, the product is not in contact with the air (except for cooking areas). The air distributed in this type of zone will, if necessary, be at a controlled temperature and humidity.
The following cases apply

- areas for receiving/storing raw materials (packaged products or in bins) at low temperature;
- areas for receiving/storing dry raw materials (spices, etc.) with humidity classification;
- cooking areas;
- areas for packaging/boxing pre-packaged products.

b. Sensitive area (high-risk area: levels 2 and 3)

In sensitive areas, the dust class is 350000 and 3500000.

• ***Risk level 2***

- ✓ Dust control

  3500000 particles of 0.5 µm/m^3 → less than 350 germs/m^3;

- ✓ Cleaning and disinfection

  ≤ 2 microorganisms/cm^2;

• ***Risk level 3***

- ✓ Dust control

  350,000 particles of 0.5 µm/m^3 → less than 35 germs/m^3;

- ✓ Cleaning and disinfection

  ≤ 0-2 microorganisms/cm^2.

The cases are as follows

- ❖ slicing, cutting, processing of products (e.g. meat);
- ❖ preconditioning (which may also be classified as an ultra-sensitive area depending on the type of product in question).

c. Ultra-sensitive area (very high risk area: level 4)

Area in which the product is made highly susceptible to biocontamination as a result of the operations performed on it or human handling. In this type of microbiologically controlled area, the rules are strict and costly:

- air purification;

- class 3,500 dust control, i.e.:

3,500 particles of 0.5 µm/m^3 → less than 0.35 germs/m^3;

- cleaning and disinfection less than 0.2 microorganisms/cm^2;

- control of contamination by incoming and outgoing items;

- control of staff access;

- appropriate clothing;

- importance of staff training.

-Laminar flow hoods are a less expensive solution but can only be used when processing operations can be carried out on a work surface.

The cases are as follows

- ❖ grinding areas (minced beef, etc.);
- ❖ cooling areas before preconditioning (the product must be cooled to a temperature ≤ 10°C in less than 2 hours);
- ❖ assembly/preconditioning areas (ready meals, etc.).

2.2.5. Hygiene of surfaces and equipment

a. Cleaning and disinfection

Surfaces, containers, and equipment that come into contact with food products must be kept clean.

Three types of operations contribute to maintaining good surface hygiene:

- cleaning, which aims to remove visible dirt: this generally consists of organic substances from raw materials or the product being manufactured. This is physical cleanliness;
- disinfection, which can be carried out at the same time as cleaning, but is more effective when performed after thorough cleaning and rinsing of surfaces. This is microbiological cleanliness;
- Rinsing is intended to remove all traces of previously used products, without of course introducing new dirt or microorganisms. This is chemical cleanliness. Poor rinsing can result in the presence of undesirable residues of detergents and disinfectants in food.

Depending on the risk areas, a cleaning and disinfection procedure should be defined:

- **low-risk area**: simple cleaning can be carried out;

- **medium-risk area**: a three-step procedure is applied, i.e., pre-washing, cleaning-disinfection, and final rinsing;

- **high-risk area**: a five-step procedure is used, i.e., pre-washing, cleaning, rinsing, disinfection, and final rinsing;

- **very high-risk area**: these are microbiologically controlled rooms that also require a five-step procedure.

b. Staff hygiene control

Some industries are highly automated, but others involve personnel in many operations during food manufacturing (handling, inspection, etc.). The following points should be taken into account when assessing potential hazards related to staff hygiene: health status; cleanliness (particularly hand hygiene); clothing.

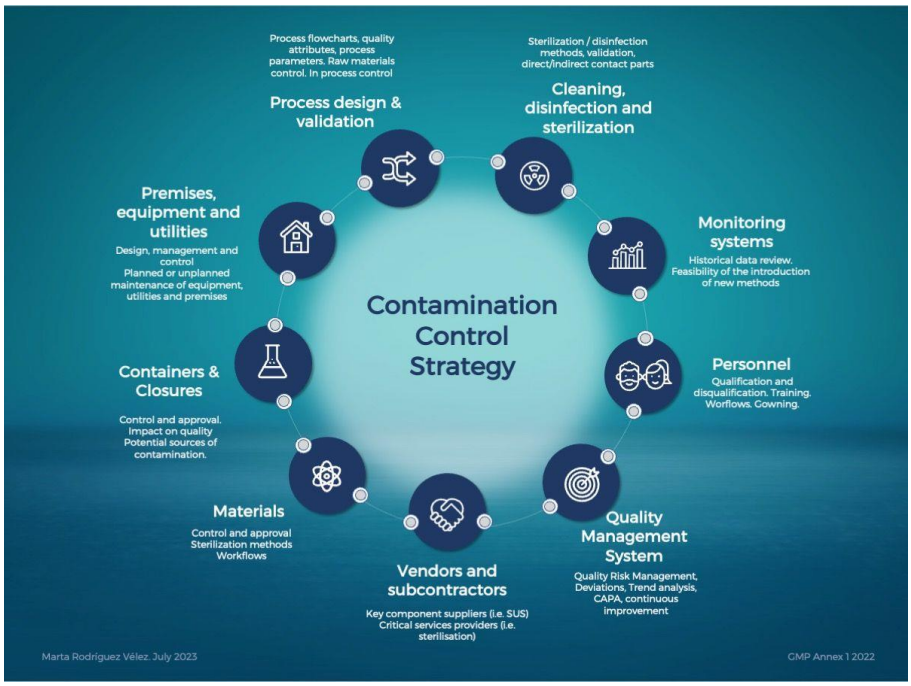

Figure 40: Contamination control strategy

c. Cleanliness

- **Cleanliness of hair (beard and moustache)**

Even when well maintained, hair, beards, and moustaches harbor microorganisms, mainly bacteria.

Therefore, in sensitive or ultra-sensitive areas, hair must be properly covered with a head covering.

- **Hand hygiene**

It is often through “dirty” hands, i.e., hands colonized by potentially pathogenic transient flora, that contamination is transferred to food. Hands are home to many microorganisms, which are generally found in greater numbers on the fingertips, particularly under the nails, and between the fingers. Rings and bracelets should not be worn, as they are a source of microbial nests.

Hand washing must be done according to an established method and frequency. It must be done systematically before any action on clean equipment and after any contaminating action, particularly after using the toilet, in order to prevent the risk of food poisoning. Simple washing removes dirt and skin flakes and reduces transient flora by 30 to 40%. Antiseptic or hygienic washing reduces skin flora by 80%. It acts on the flora on the surface and to a lesser

extent on germs in the deeper layers of the skin. If gloves are worn, they must be used properly.

- **Work clothing**

Clothing contaminated with organic matter through contact with food during production becomes an excellent medium for the development of microorganisms and can cause secondary contamination; it must be changed at regular intervals, washed, and disinfected between uses.

There must be changing rooms where appropriate clothing can be put on, i.e., clothing offering a higher or lower level of protection depending on the type of area and the level of risk of product contamination:

- inert area: normal clothing;
- sensitive area: lab coat, cap, and gloves;
- ultra-sensitive area: lab coat, cap, shoe covers, gloves, and mask.

d. Main types of chemical agents

a. Oxygenated oxidizing agents: Hydrogen peroxide H_2O_2 (or oxygenated water) is an effective antiseptic at 3% in aqueous solution and is used in dental hygiene. Ozone (O_3) is a product that can be used for water disinfection.

b. Chlorine and derivatives: Chlorine gas and its derivatives are the most commonly used antiseptics for treating drinking water and swimming pools, disinfecting premises, etc.

c. Other halogens (iodine and derivatives): Iodine is not very soluble in water, but it dissolves easily in alcohol or aqueous solutions of potassium or sodium iodide, which are used to disinfect superficial wounds.

d. Heavy metals and salts: certain metals have a microbicidal effect even at low concentrations due to the interactions they can have with cellular proteins. The most commonly used are silver, mercury, copper, and zinc salts.

e. Alcohols: Ethanol has a good microbicidal effect at dilutions of 50% to 70%. It is inactive on spore-forming organisms. It is used as a skin disinfectant, but its action is superficial: it denatures proteins. Methanol is less active and more harmful.

f. Phenols, cresols, other phenolic and aromatic compounds: These are used as microbicidal agents and are not particularly active on spore-forming organisms.

g. Dyes: Their antiseptic power varies. They are used for local or general purposes (disinfection of wounds, urinary disinfectant). The main ones are: methylene blue, malachite green, brilliant green, gentian violet. Some act by altering the membrane, others complex with nucleic acids.

h. Soaps and detergents: Soaps have antiseptic properties that vary depending on the species.

i. Acids, anhydrides, aldehydes: Acids have an indirect antimicrobial action due to their pH effect.

REFERENCES

REFERENCES

Altier G. (2005). Genetic and environmental control of Salmonella invasion. J Microbiol 43:82–92

Amellal N., Burtin G., Bartoli F., Heulin T. (1998). Colonization of wheat roots by an exopolysaccharide-producing Pantoea agglomerans strain and its effect on rhizosphere soil aggregation. Appl Environ Microbiol 64:3740–3747

Atlas R.M., Bartha R. (1981). Microbial ecology: fundamentals and applications. Addison-Wesley, Reading

Arlie J.P., Ballerini D., Nativel F., (1984). Les procédés modernes de fabrication de l'éthanol de fermentation, Revue de l'Institut Français du Pétrole, 39 (6), p.781.

Auge R.M. (2004). Arbuscular mycorrhizae and soil/plant water relations. Can J Soil Sci 84:373–381

Bacci M. Jr., Anversa M.M., Pagnocca F.C. (1995). Cellulose degradation by Leucocoprinus gongylophorus, the fungus cultured by the leafcutting ant Atta sexdens rubropilosa. Anton Leeuw Int J G 67:385–386

Ba¨ckhed F., Ding H., Wang T., Hooper LV., Koh GY., Nagy A., Semenkovich CF, Gordon JI (2004). The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA101:15718–15723

Ba¨ckhed F, Ley RE, Sonnenburg JL, Petreson DA, Gordon JI. (2005). Host-bacterial mutualism in the human intestine. Science 307:1915–1920

Bacchin, P. (2006). Cours de biotechnologies et bioprocédés. Procédés de séparation et membranes. Université Paul Sébatier. Toulouse. France.

Bailly J, Debaud JC, Verner MC, Plassard C, Chalot M, Marmeisse R, Fraissinet-Tachet L. (2007). How does a symbiotic fungus modulate expression of its hostplant nitrite reductase? New Phytol 175:155–165

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Balcewicz-Sablinska MK, Keane J, Kornfeld H, Remold HG. (1998). Pathogenic Mycobacterium tuberculosis evades apoptosis of host macrophages by release of TNFR2, resulting in inactivation of TNF-alpha. J Immunol 161:2636–2641

Bandi C., Trees A.J., Brattig N.W. (2001). Wolbachia in filarial nematodes: evolutionary aspects and implications for pathogenesis and treatment of filarial diseases. Vet Parasitol 98:215–238

Bannerman D.D., Goldblum S.E. (1997). Endotoxin induces endothelial barrier dysfunction through protein tyrosine phosphorylation. Am J Physiol 273:217–226

Baratin M. et al (2005). Natural killer cell and macrophage cooperation in MyD88-dependent innate responses to Plasmodium falciparum. Proc Natl Acad Sci U S A 102:14747–14752

Barbault R. (1997). Ecologie Ge´ne´rale. Structure et fonctionnement de la biosphe`re, 4th edn. Masson, Paris, 281 p

Barre´-Sinoussi F et al (1983.) Isolation of a T-lymphotropic retrovirus from a patient at risk for Acquired Immune Deficiency Syndrome (AIDS). Science 4599:868–871

Bauer WD., Robinson JB. (2002). Disruption of bacterial quorum sensing by other organisms. Curr Opin Biotechnol 13:234–237

Baumann P., Moran N.A,. Beaumann L. (2000). Bacteriocyte- associated endosymbionts of insects. In: Dworkin M (ed) The prokaryotes. Springer, NewYork, pp 1–67

Beattie GA., Lindow SE. (1999). Bacterial colonization of leaves: aspectrum of strategies. Phytopathology 89:353–359

Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, PiluzzaG, Bullitta S, Glick BR (2005). Cadmium-tolerant plant growth promoting bacteria associated with the roots of Indian mustard (Brassica juncea L. Czern.). Soil Biol Biochem 37:241–250

Bennett AE, Bever JD (2007). Mycorrhizal species differentially alter plant growth and response to herbivory. Ecology 88:210–218

Bentley SD et al (2002). Complete genome sequence of the model actinomycete Streptomyces coelicolor A3(2). Nature 417:141–147

Bertaux J, Schmid M, Prevost-Boure NC, Churin JL, Hartmann A., Garbaye J., Frey-Klett P. (2003). In situ identification of intracellular bacteria related to Paenibacillus spp. in the mycelium of theectomycorrhizal fungus Laccaria bicolor S238N. Appl EnvironMicrobiol 69:4243–4248

Berthiller F et al (2013). Masked mycotoxins: a review. Mol Nutr Food Res 57:165–186

Bertrand J.-C. · Pierre Caumette Philippe Lebaron · Robert Matheron Philippe Normand · Télesphore Sime-Ngando et *al.* (eds.) (2015). Environmental Microbiology: Fundamentals and Applications: Microbial Ecology, DOI 10.1007/978-94-017-9118-2_9, Springer Science+Business Media Dordrecht 2015

Birge EA, Kurland CG (1969) Altered ribosomal protein in streptomycin-dependent Escherichia coli. Science 166:1282–1284

Bjorklof K, Nurmiaho-Lassila EL, Klinger N, Haahtela K, Romantschuk M (2000). Colonization strategies and conjugal gene transfer of inoculated Pseudomonas syringae on the leaf surface. J Appl Microbiol 89:423–432

Blanc C, Sy M, Djigal D, Brauman A, Normand P, Villenave C (2006). Nutrition on bacteria by bacterial-feeding nematodes and consequences on the structure of soul bacterial community. Eur J Soil Biol 42:S70–S78

Bohrerova Z, Stralkova R, Podesvova J, Bohrer G, Pokorny E (2004). The relationship between redox potential and nitrification under different sequences of crop rotations. Soil Till Res 77:25–33

Bonkowski M (2004). Protozoa and plant growth: the microbial loop in soil revisited. New Phytol 162:617–631

Bouchez M, Blanchet D, Bardin V, Haeseler F, Vandecasteele JP (1999). Efficiency of defined strains and of soil consortia in thebiodegradation of polycyclic aromatic hydrocarbon (PAH) mixtures. Biodegradation 10:429–435

Ciccarelli FD, Doerks T, von Mering C, Creevey CJ, Snel B, Bork P (2006). Toward automatic reconstruction of a highly resolved tree of Life. Science 311:1283–1286

Donlan RM. (2002). Biofilms : Microbial lifes on surfaces, Emerg Infect Dis. 8 : 881-890

Dudkowski, A. (2000). L'épandage agricole des boues de stations d'épuration d'eaux usées urbaines. Courrier de l'environnement de l'INRA n°41, 134-135p

Feng G., Zhang X., Li X., Tian C., Tang C. & Rengel Z. (2002). Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. Mycorrhiza, 12: 185-190.

Goswami, M., Chakraborty, P., Mukherjee, K., Mitra, G., Bhattacharyya, P., Dey, S., & Tribedi, P. (2018). Bioaugmentation and biostimulation: a potential strategy for environmental remediation. J Microbiol Exp, 6(5), 223-231.

Kosaric N., Vardar-Sukan F., (2000). Economic and Energy Aspects of Ethanol Fermentation, The Biotechnology of Ethanol-Classical and Future Applications,

Leung M, (2010). Bioremediation: techniques for cleaning up a mess, Journal of Biotechnology, vol. 2, pp. 18-22, 2004.

Lobo R.R. and Faciola A.P., (2021). Ruminal Phages – A Review. *Front. Microbiol.* 12:763416. doi: 10.3389/fmicb.2021.763416

Martin MO (2002). Predatory prokaryotes: an emerging research oppor tunity. J Mol Microbiol Biotechnol 4:467–477

Ogier J.C., Ballerini D., Leygue J.P., Rigal L., Pourquié J., Production d'éthanol à partir de biomasse lignocellulosique, Oil and Gas Science and Technology, Revue de l'IFP, 1999, 54 (1), p.67.

Pédro, G. (2007). Cycles biogéochimiques et écosystèmes continentaux. EDP sciences.

Philippe Normand, Pierre Caumette, Philippe Goulas, Petar Pujic, and Florence Wisniewski-Dye (2015). Chapter 9 : Adaptations of Prokaryotes to Their Biotopes and to Physicochemical Conditions in Natural or Anthropized Environments, in : Bertrand J.-C. · Pierre Caumette Philippe Lebaron · Robert Matheron Philippe Normand · Télesphore Sime-Ngando et *al.* (eds.), 2015. Environmental Microbiology: Fundamentals and Applications: Microbial Ecology 293-347.

Roux S., (2013). Diversité, évolution et écologie virale : des communautés aux génotypes. Analyse bioinformatique de métagénomes viraux. Thèse de doctorat. Université Blaise Pascal

Udayakumar, S.; Rasika, D.M.D.; Priyashantha, H.; Vidanarachchi, J.K.; Ranadheera, C.S., *(*2022). Probiotics and Beneficial Microorganisms in Biopreservation of Plant-Based Foods and Beverages. *Appl. Sci.*, *12*, 11737. https://doi.org/10.3390/app122211737

Xu J, Bjursell MK, Himrod J, Deng S, Carmichael LK, Chianq HC, Hooper LV, Gordon JI (2003) A genomic view of the human Bacteroides thetaiotaomicron symbiosis. Science 299:2074–2076

Yamamoto H, Fujikura K, Hiraishi A, Kato K, Mayi Y (2002) Phylogenetic characterization and biomass estimation of bacterial endosymbionts associated with invertebrates dwelling in chemosynthetic communities of hydrothermal vents and cold seeps. Mar Ecol Prog Ser 245:61–67

Zenjari B, El Hajjouji H, Ait Baddi G, Bailly JR, Revel JC, Nejmeddine A, Hafidi M (2006) Eliminating toxic compounds by composting olive mill wastewater-straw mixtures. J Hazard Mater 138:433–437

Zeph LR, Casida LE (1986) Gram-negative versus Gram-positive (Actinomycete) nonobligate bacterial predators of bacteria in soil. Appl Environ Microbiol 52:819–823

Zhanq Y, Gao W, Meng Q (2007) Fermentation of plant cell walls by ruminal bacteria, protozoa and fungi and their interaction with fibre particle. Arch Anim Nutr 61:114–125

Zychlinsky A, Sansonetti PJ (1997) Perspectives series: host/pathogen interactions. Apoptosis in bacterial pathogenesis. J Clin Invest 100:493–495.