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Microalgae

1.1. Definition

Algae are photosynthetic organisms that develop in varied habitats, predominantly in aquatic environments, capable of converting light energy and carbon sources, such as carbon dioxide (CO_2), into "biomass". Depending on their size, they can be classified into two broad categories: "macroalgae" and "microalgae". Macroalgae are multicellular algae of around one centimeter in size which usually grow in ponds of natural fresh water or salt water. Microalgae have a size measured in micrometers and are considered to be single cell algae which grow in suspension, mainly in aqueous solutions [WEN 09].

These microorganisms are considered to be the first producers of oxygen (O_2). Their existence in the oceans dates back to more than three billion years ago. They are responsible for transforming the composition of the atmosphere (CO_2 fixation and O_2 emission) and have allowed the emergence of plant and animal life on Earth. Also referred to as *Phytoplankton*, microalgae represent a food source from the earliest stages of larval life right up to human beings, owing to their specific biochemical composition.

Their adaptation and survival capacities are such that they are able to colonize all types of environments. They are found in thermal waters as well as in ice, in acidic or even hyper saline waters, in caves, in symbiotic relationships with any other type of living organisms, and as parasites, even on humans. They are also able to develop on hard surfaces, such as walls or tree trunks, and even on immersed structures. Certain species can withstand very low or paradoxically extreme temperatures. This faculty of adaptation is the result of their morphological properties as well as their capacity to synthesize different varieties of secondary metabolites.

1.2. Characteristics

Through photosynthesis, these microorganisms synthesize O_2 and primary organic metabolites such as carbohydrates, lipids and proteins. From a cell structure perspective, a microalga has a nucleus, a plasma membrane and contains organelles, essential to its operation, such as chloroplasts, amyloplasts, elaioplasts and mitochondria. It contains three main types of pigments: chlorophylls, carotenoids and phycobiliproteins.

Microalgae take a variety of forms (Figure 1.1): spherical (*Porphyridium*), crescent-shaped (*Closterium*), spiral-shaped (*Arthrospira*), droplet-shaped (*Chlamydomonas*) and even star-shaped (*Staurastrum*).

From a nutritional point of view, microalgae are predominantly photoautotrophic[1] but they can also be heterotrophic or mixotrophic [CHE 11b]. An autotrophic metabolism uses inorganic carbon such as CO_2 or bicarbonate as a carbon source while a heterotrophic metabolism is characterized by a consumption of organic

1 I.e. using light as a source of energy and CO_2 as a source of carbon.

carbon as a carbon source for their development; mixotrophs use both types of carbon sources.

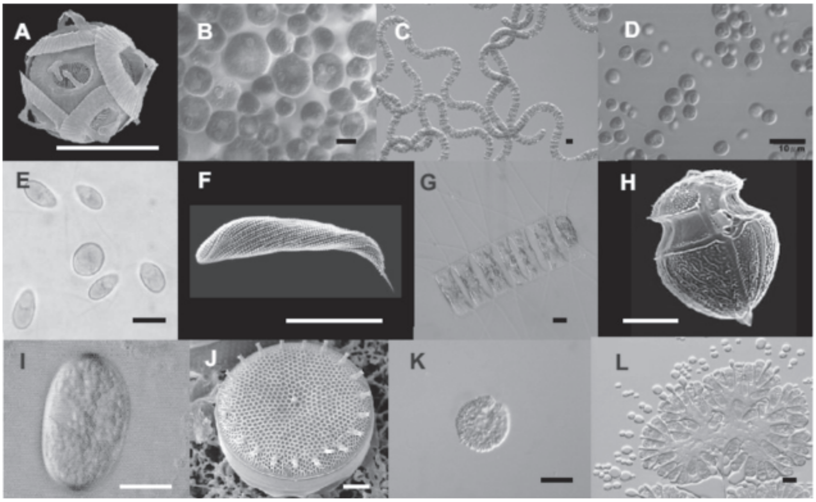

Figure 1.1. *Morphological diversity of microalgae [SUM 09]. a) Gephyrocapsa; b) Haematococcus lacustris; c) Spirulina platensis; d) Chlorella vulgaris; e) Dunaliella tertiolecta; f) Chaetoceros calcitrans; g) Chaetoceros calcitrans; h) Dinophysis acuminate; i) Alexandrium; j) Bacillariophycea; k) Raphidophceae; l) Botryococcus. The length of the line in each figure is equal to 10 µm [SUM 09]*

1.3. Uses of microalgae

Microalgae offer interesting perspectives for applications in diverse areas such as the pharmaceutical industry, agriculture, environment and renewable energy. The main uses are detailed below.

1.3.1. *Nutrition*

Microalgae represent an excellent source of nutrients. They are used for animal feed, as a human food source and in aquaculture. They are used in the manufacture of natural colorants in the food industry. Polysaccharides (hydrosoluble polymers) from microalgae are used in the food industry as gelling agents or thickeners. Glycerol (the molecule involved in the osmoregulatory systems of microalgae) is exploited in the food industry as a sweetener.

1.3.2. *Pharmaceuticals*

Microalgae are an interesting source of bioactive molecules and toxins that have notably been used in the development of new medicines for the treatment of cancerous diseases [PUL 04]. Polysaccharides extracted from microalgae allow the synthesis of antioxidant, antiviral, antitumor and anticoagulants agents. Microalgae are capable of synthesizing vitamins and natural antioxidants.

1.3.3. *Cosmetics*

Several species of microalgae are used industrially in the cosmetics industry [PUL 04, SPO 06], mainly the two species *Arthrospira* and *Chlorella*. Algae extracts with antioxidant properties are used in the manufacture of hair care products, anti-wrinkle products and sun creams. Pigments derived from microalgae are also used for cosmetics.

1.3.4. *Energy*

Algal biomass offers benefits in the production of energy in the form of electricity and/or heat by direct combustion, or in the form of biomethane or biofuels. However, these benefits are only competitive in cases with strong biomass productivity, using simple mechanical harvesting techniques and which present lower production costs than those involved in processes using other types of biomass [CAR 07].

1.3.4.1. *Biomethane production*

Several research projects have confirmed the technical and commercial feasibility of biomethane production from marine biomass, showing great potential [CHY 02]. However, technical obstacles such as the accessibility of the nutrients and high production costs limit the use of microalgae for this application. A way to reduce costs would be, for example, to link the production of methane with the production of secondary high value-added metabolites. Species such as *Gracilaria sp*. and *Macrocystis* are excellent methane-producing organisms.

1.3.4.2. *Biofuel production*

Considering the current global context (increase in the price of oil, depletion of fossil resources, production of greenhouse gases, etc.), it is interesting to consider microalgae as a source of production of different types of biofuel: bio-oil and biodiesel [PAN 11].

Bio-oil from microalgae represents an interesting alternative to liquid biofuels. It is produced by the thermochemical conversion of biomass at high temperatures in the absence of O_2. Two different processes are used: pyrolysis and thermochemical liquefaction. Several studies have been carried out based on the implication of microalgae in bio-oil synthesis [DOT 94, SAW 99, DEM 06]. Areas for improvement for this type of process include the reduction of production costs, the optimization of the culture system, and the improvement of separation and harvesting steps.

The most promising approach involves the production of second and third generation biofuels (ethanol production from lignocellulosic materials and biodiesel production from microalgae). The third generation biofuel addresses the major drawbacks observed in first and second generation biofuels (competing with food production, excessive water consumption and deterioration of soil). Due to certain valuable properties (important biomass productivity, high photosynthetic activity, large lipid storage potential up to 20–50% dry weight), microalgae are 500 to 1,000 times more effective than terrestrial species for biodiesel synthesis.

1.3.4.3. *Biohydrogen production*

Biohydrogen is an effective source of renewable energy and is currently the subject of extensive research and applications. The process of biohydrogen synthesis can take two forms: direct photolysis and indirect photolysis. Direct photolysis is based on the transfer of electrons from water molecules to protons, coupled with a reduction of ferredoxin (protein intervening at the level of the algae photosystem in the transport of electrons and protons) inducing hydrogen synthesis with hydrogenase enzymes [BEN 00]. The indirect method is based on the conversion of starch stored by algae to hydrogen under anaerobic conditions and sulfur limitation [CAR 07]. Several species of microalgae have shown interesting properties in relation to indirect processes, i.e. a large capacity for biohydrogen synthesis under sulfur deprivation conditions. Accordingly, the production of hydrogen from microalgae is a promising niche but requires a better understanding of microalgae metabolism and engineering [BEE 09].

1.3.5. ***Environmental field***

The main environmental applications of microalgae are in wastewater treatment and consumption of CO_2 as a method for reducing greenhouse gas emissions.

1.3.5.1. *Wastewater treatment*

Their capacity to assimilate numerous nutrients necessary for their growth means that microalgae offer an interesting solution for the elimination of these elements; they are also able to fix heavy metals. They thus constitute the main biological element of certain municipal and industrial water treatment systems (mainly tertiary treatment). Due to the assimilation of nitrogen and phosphorus, they contribute in reducing the phenomenon of eutrophication (i.e. degradation) of certain aquatic environments.

In order to reduce the economic costs of these water treatment processes, generated microalgae biomass can be used to produce molecules with high added value, (such as biodiesel, methane, hydrogen etc.). These processes are typically coupled with the elimination of CO_2 in industrial gas emissions, leading to integrated processes.

1.3.5.2. *Agriculture*

Algal biomass constitutes a valuable asset as manure, fertilizer and soil stabilizer in agriculture, and also as a crop accelerator and protector by limiting the proliferation of epiphytes and parasites. Microalgae allow particle adhesion and storage of water in the soil as well as nitrogen fixation. The synthesis of bioactive molecules means that they are likely to influence the growth of terrestrial plants. Microalgae are used, for example, in the production of rice, ensuring nitrogen fixation in tropical and subtropical agriculture. They are also used for surface strengthening in arid regions in order to combat erosion.

1.3.5.3. *Life-support systems*

During space missions, the development of a life-support system for the crew is essential. These systems must fulfill four basic needs: regeneration of a breathable atmosphere (O_2 supply), water recycling, waste treatment and provision of food. In order to respond to these constraints, closed-loop regenerative life support systems use biological systems, such as algae and terrestrial plants. The *Micro-Ecological Life Support System Alternative* (MELISSA) project has been developed by the European Space Agency (ESA) for these purposes [GOD 02]. The microalga *Spirulina* is produced and then dried to be consumed or incorporated into food.

1.3.5.4. *CO_2 sequestration*

The current alarming situation concerning climate change has triggered worldwide awareness. The growing concentration of greenhouse gases (known as "GHG") in the atmosphere has an increasingly important effect on climate change [MAT 95].

Natural absorption no longer compensates for the high production rate of these types of gas; CO_2 having the highest effect, representing more than 68% of total emissions [MAE 95, KON 07, ROM 07]. A dramatic increase in the release of CO_2 in the atmosphere has been observed due to anthropogenic sources. Indeed, released CO_2 was about 7.4 billion tons in 1997, and is estimated to be about 26 billion tons by 2100. There is need to remind the reader of the catastrophic results of climate warming on desertification, increase in the frequency of extreme weather events, disruption of ecosystems and melting of non-polar glaciers resulting in rising sea levels [MOR 97].

In order to reduce the levels of greenhouse gases in the atmosphere, intensive research has focused on the development of new CO_2 reduction techniques (Figure 1.2) [IPC 05]. There are three main types of processes: geological sequestration, chemical processes and bioprocesses [PIR 11].

Geological sequestration relies mainly on the storage of liquid or gaseous CO_2 in geological formations, in the soil [HER 01] or in deep ocean storages [ISR 09]. However, these technologies have many disadvantages such as the possibility of leakage, the contamination of drinking water aquifers, the increase in the acidity of water, the disruption of the marine ecosystem and significant financial costs.

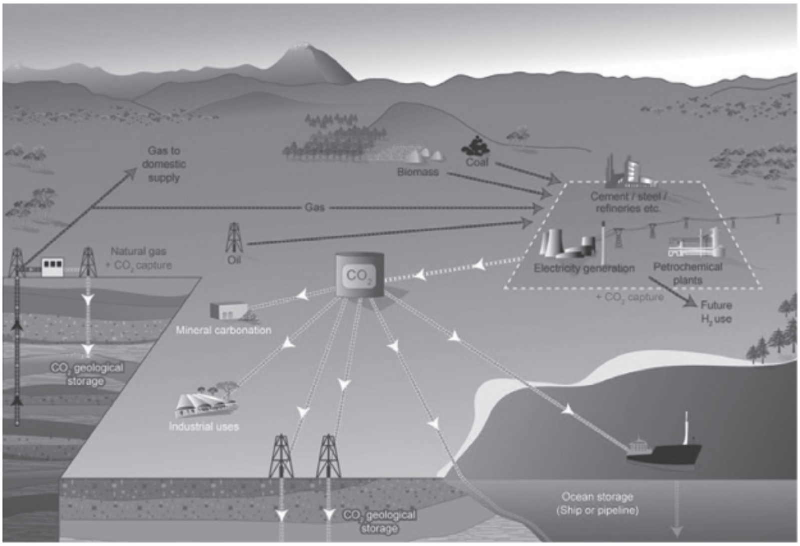

Figure 1.2. *Schematic view of the capture and storage of carbon dioxide [IPC 05]*

Chemical processes include absorption by alkaline solutions [DIA 04], the use of multi-walled carbon nanotubes [SU 09], and adsorption–neutralization on amine enriched carbon [PLA 07]. These methods are expensive and energy-intensive [WAN 08].

The use of biological systems is a very promising alternative solution, relatively efficient, economically feasible and sustainable. These methods are based essentially on photosynthesis, with the transformation of CO_2 into biomass [KON 07, DEM 07]. Two CO_2 sequestration biological pathways exist: one using terrestrial plants and the second photosynthetic microorganisms. In the first method, forests are used to convert CO_2 into cellulosic structures for plants (namely trees) and into humus for soils [ZAM 10]. However, due to limited conversion efficiency, low growth rate, economic and technical disadvantages (possibility of release of stored carbon following a forest fire or damage to trees), numerous studies have focused on the second pathway, which is the implementation of photosynthetic organisms such as microalgae. CO_2 sequestration by microalgae is the main subject of this book and will be discussed in Chapter 2.

1.4. Microalgae cultivation systems

Given the diversity of industrial applications and nutritional and environmental requirements of microalgae culture, the establishment of an efficient cultivation system is a specific and crucial step which depends on the application in question. There are two main categories of cultivation systems: open systems (natural and artificial ponds characterized by a low surface-to-volume ratio) and closed systems (photobioreactors with a high surface-to-volume ratio).

1.4.1. *Open systems*

This type of system is characterized by its technical simplicity, its ease of operation and a relatively long lifespan. These systems essentially involve shallow basins supplied, for example, with wastewater discharged from waste processing factories and fitted with a stirring system formed by rotating structures or impellers. Biomass itself is harvested at the end of the recirculation cycle (Figure 1.3). This type of configuration is known as a *raceway* basin [MOH 05].

Open systems have a wide variety of configurations depending on the size, depth, type of material, agitation system and inclination [TRE 04]. Three main types are used on an industrial scale: lakes and natural ponds (natural ecosystems), circular basins and raceway basins (Figure 1.3).

Figure 1.3. *Variety of open system configurations: a) raceway basin used for the culture of Spirulina platensis in California; b) circular basin with central pivot for the culture of Chlorella in Taiwan; c) non-agitated broad basins used for the culture of Dunaliella salina in Australia*

Natural ecosystems are the most economic mode of culture and the least technically demanding. On an industrial scale, this technique has been used for the culture of the *Dunaliella salina* species [BEN 87]. The main advantage of natural ecosystems is their low installation cost. However, these systems have significant drawbacks, including the limited possibility of controlled cultivation conditions, the inability to control agitation, the poor efficiency of gas–liquid mass transfer and the strong dependency on climatic conditions [BOR 99].

The second system is based on a mode of culture in artificial circular basins. It is widely used in wastewater treatment processes [BOR 05]. This configuration is composed of a circular basin fitted with a mechanical agitation system with a central pivot. It presents significant limitations, mainly due to the inefficiency of the agitation system in large basins, high energy consumption and high installation costs.

The last open culture system is the raceway type. It uses a configuration with individual units or with a series of multiple loops. The medium in these basins can be homogenized by various techniques: impellers, propellers, airlift systems and pumps. On an industrial scale, this type of configuration is used for the culture of species such as

Chlorella sp., *Spirulina platensis*, *Haematococcus sp.* and *D. salina* [BOR 05]. However, this configuration has disadvantages such as the settling of algae in the basins [MOH 05] and a significant cultivation surface.

Other systems, such as basins with inclined configurations, have been developed to increase the surface area-to-volume ratio and therefore productivity [RIC 99]. This technology has been widely used in the Czech Republic for the culture of *Chlorella sp.*, *S. platensis* as well as *Scenedesmus sp.* [MOH 05]. It has technical and economic limitations due to the high costs of pumping operations, maintenance and installation [TRE 04].

In conclusion, open systems present many disadvantages, including a very low volumetric productivity compared to that which is theoretically expected, the difficulty of controlling operating conditions, the potential risks of contamination by other organisms, significant installation surfaces and high harvesting costs. In addition, a low yield of CO_2 consumption in these culture systems may be due to the difficulty of controlling cultivation settings [CHI 07, SHE 09].

1.4.2. *Closed systems: "photobioreactors"*

Closed systems or *photobioreactors* allow better control of external factors in the bioprocess than open systems. Microalgae are exposed to a light source through a transparent material. These systems may be optimized for the chosen application and in accordance with the biochemical properties of the cultivated species [ALC 10].

Photobioreactors can take varying forms (plane, tubular, horizontal, inclined, vertical or spiral) (Figure 1.4); they may use different agitation systems (airlift system or pumps, etc.) and be constructed using different types of materials (glass or plastic, rigid or flexible). The design of the photobioreactor relies on the optimization of the availability of light energy to microorganisms and on agitation efficiency. Two main configurations are found, depending on the geometry used.

1.4.2.1. *Tubular photobioreactors*

These are composed of one or more transparent tubes which may be arranged horizontally, vertically or in spirals. The following technologies are used:

– *Stirred tank reactor* (*Carboy* or *Bag*) [CHI 01]: this type of system is typically used for the manufacture and maintenance of inocula in crops on a very large scale in the field of aquaculture. It is composed of a tank equipped with an agitation system with a shaft driven by a motor or with magnetic bars. It is easy to implement and maintain, but has a low surface-to-volume ratio.

– *Bubble column reactor*: this is a cylinder in which agitation is produced through dispersion of the gaseous phase in the form of small bubbles. The surface-to-volume ratio is relatively high, its cost is low and the thermal and mass transfer rates satisfactory.

– *Airlift reactor*: this is a cylinder with two interconnected zones, one known as a *riser*, where the gaseous mixture is diffused, and one known as a *downcomer* in which there is no gas supply. The liquid phase is set in motion naturally, by the difference in the weight of water columns between the ventilated and non-ventilated areas. Stirring is carried out by bubbling gas. This type of configuration has disadvantages related to implementation and a low residence time.

– *Tubular photobioreactor*: this type of configuration uses long transparent glass or plastic tubes. It can be vertical, horizontal, spiral or even inclined.

1.4.2.2. *Flat-plate photobioreactors*

These are made of two transparent rectangular panels between which a thin layer of culture circulates [KUM 11]. This configuration is used with different geometries – vertical, horizontal and tilted. The benefits of these photobioreactors include a large surface-to-volume ratio, a low light path and a good quality of gas–liquid mass transfer. Their limitations relate to the increase in production costs following the recarbonation of cultures, mainly due to the increase of pH, to the phenomenon of clogging and to the difficulty of installation design.

Closed systems offer many advantages. They allow us to control the hydrodynamic conditions of culture, reduce the risk of contamination by undesirable microorganisms, decrease water consumption, offer a large surface-to-volume ratio, a high biomass productivity and CO_2 fixation capacity. However, their main limitations are: the high costs of building and operation, difficulty in scaling up and the formation of biofilms on the walls of the photobioreactor reducing light availability. Accordingly, several studies have attempted to overcome the technical limitations of closed systems with the aim of reducing the light path [JAN 02], and of obtaining a more economical system for temperature control [CAR 01].

1.5. Factors affecting algae cultivation

The effectiveness of microalgae cultivation depends on a number of environmental, physical and biological parameters, related to the intrinsic characteristics of the algal species, and to the geometry of the production system. These parameters not only affect the photosynthetic activity and productivity in biomass, but also the physiological and metabolic behavior of microalgae.

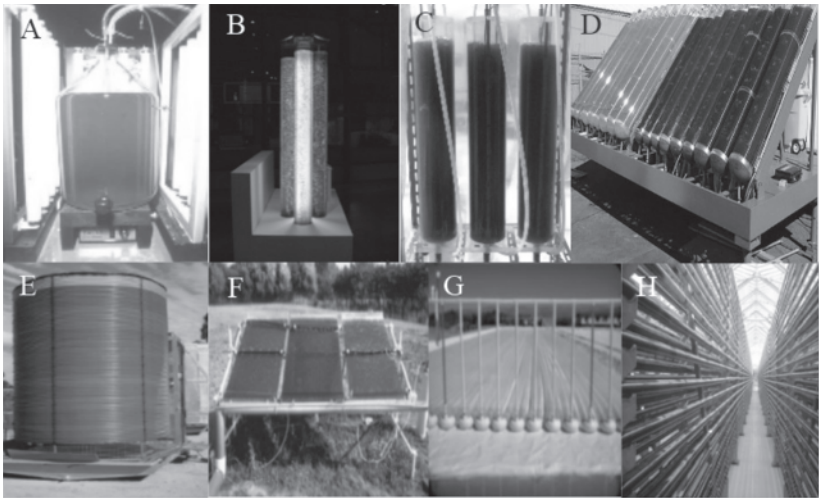

Figure 1.4. *Variety of photobioreactor configurations: a) stirred tank photobioreactor; b) 5 L bubble column photobioreactor based on work by the Plymouth Marine Laboratory , United Kingdom; c) airlift photobioreactor designed by Algain Energy; d) inclined photobioreactor based on the studies conducted by the US Geological Survey agency or "USGS" in the south–west USA; e) 1,000 L tubular-helical photobioreactor of the "BIOCOIL" type at the University of Murdoch, East Australia; f) inclined-plate photobioreactor; g) commercial horizontal photobioreactor under construction in the desert of California at the junction of Death Valley; h) tubular photobioreactor, controlled environment agriculture and energy*

The most important parameters are light, temperature, pH, salinity, nutrient concentrations, and the presence of toxic elements [KUM 10]. These are detailed below. Other parameters related to the hydrodynamic behavior of the reactor (for example residence time, rate of mass transfer, degree of homogeneity of the medium) can play a role on the availability of nutrients and light energy [KUM 10].

1.5.1. *Light*

Light is essential for the metabolism of photosynthetic microalgae. The supply of light energy can occur naturally through solar energy or artificially using fluorescent tubes which emit light at a specific wavelength.

Algae growth depends on the availability and efficient use of light energy. An inadequate light supply is likely to limit productivity and growth even if the other parameters are at optimal values. Depending on the degree of illumination, microalgae can be in the phase of respiration (in case of absence of light), in limitation (insufficient inflow of light), in saturation (with a maximum photosynthetic yield) or in inhibition (loss of photosynthetic activity due to excessive light intensity).

Light is therefore a very important parameter in microalgae cultivation and is regarded as a substrate when modeling the culture (detailed in section 3.2.3).

Light is thus an essential parameter affecting cell growth. Lighting devices can be natural, and directly use solar energy, or artificial through the use of fluorescent tubes around the reactor. Light sources immersed in the culture (LED or fiber optics) are also applied. This kind of device's design must account for various parameters (optical path, optical depth that characterizes the medium's degree of transparency and ratio of illuminated surface per culture volume). Light availability varies according to the depth and to the cellular density of the culture medium. Several studies have examined the distribution of light within the reactor and the design for effective CO_2 sequestration, as well as biomass production [MOR 00]. The photobioreactor geometry must therefore be optimized to ensure optimum light available to microalgae.

1.5.2. *Temperature*

The growth rate of microalgae depends on the temperature. This parameter allows regulation of the physiological and morphological cellular responses of the microorganism [KUM 10]. Temperature can cause changes in the cellular structure, and particularly in its volume (a temperature higher than the optimum value induces an increase in the cell volume). Microalgae generally tolerate a temperature range between 15 and 26°C with an optimal cellular concentration around 23°C. Temperature range for growth is species dependent. It is also worth noting that the increase in temperature affects the metabolism of CO_2 fixation by microalgae [KUM 11]. However, considering the perspective of using microalgae to sequester CO_2 from industrial activities, flue gases are generally at high temperatures (for example from cement kilns), and can induce an increase in the temperature of the culture medium. It is then necessary either to choose microalgae which tolerate high temperatures [TAL 13], or to cool the hot gases before injecting them into the culture medium [WAN 08, LAM 11].

1.5.3. *pH*

This parameter essentially depends on the concentration of CO_2 dissolved in the culture medium, governed by chemical balances between the different forms of carbon in water (CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}). A high supply of CO_2 can cause acidification of the environment, and this is likely to inhibit the growth of microalgae. Similarly, the presence of sulfur monoxide (SO), a toxic element, causes significant acidification of the environment and thereby limits growth. Accordingly, pH control in cultures is necessary in order to promote the growth of species with specific environmental requirements [KUM 11]. In general, the algae growth is the best with a pH close to neutrality. However, certain species tolerate extreme values.

1.5.4. *Nutrients*

1.5.4.1. *Carbon*

The carbon source of an algal culture in the condition of autotrophy is present in the liquid phase in the form of various species in balance: CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}. These balances are governed by the equations of dissociation of water and the carbonate–bicarbonate mixture presented below at a temperature of 25°C:

$$H_2O \leftrightarrow H^+ + OH^- \qquad pK_w = 14 \qquad [1.1]$$

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \qquad pK_1 = 6.38 \qquad [1.2]$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \qquad pK_2 = 10.37 \qquad [1.3]$$

These different forms of carbon give the Total Inorganic Carbon (TIC), the concentration of which is given by the following expression, where the brackets denote the volumetric concentrations (the concentration of $H_2CO_3^-$ is neglected):

$$[TIC] = [CO_2] + [HCO_3^-] + [CO_3^{2-}] \qquad [1.4]$$

During photosynthesis, the consumption of CO_2 causes a gradual increase in pH; in the presence of significant concentrations of dissolved CO_2, the pH drops resulting in a lower consumption of CO_2. This may be due to the reduction of the activity of enzyme RuBisCO, responsible for CO_2 fixation [SOB 00]. Algae species can demonstrate a preference for one of the forms of carbon (CO_2 or HCO_3^-) depending on the biological mechanism of concentration of CO_2. Generally, microalgae prefer CO_2 as a source of inorganic carbon [CAR 06].

1.5.4.2. *Nitrogen*

Nitrogen is an essential nutrient for algae growth. Nitrogen content in microalgae is around 7% of the algal dry matter. The nitrogen source of the culture may be organic (urea) or inorganic (nitrate NO_3^-, ammonium NH_4^+) [ALC 10]. Nitrogen is involved in the major metabolic pathways of microalgae. Therefore, an increase in the concentration of nitrogen, up to a certain limit value, leads to greater cellular and protein productivity and more significant chlorophyll synthesis. A deficiency of this element induces a significant accumulation of lipid reserves (polyunsaturated fatty acids) [CHE 11a] and a limitation of photosynthetic and cellular activities [ALC 10].

1.5.4.3. *Phosphorus*

Phosphorus is involved in several metabolic pathways and cellular regulations [CHE 11a, DRO 73]. It accounts for approximately 1% of the dry algal matter. Microalgae are able to use the inorganic forms of phosphorus as well as its organic forms, via hydrolysis with the help of an enzyme of the phosphatase family [ALC 10].

Phosphorous deficiency influences the photosynthetic activity mainly at the level of the enzyme RuBisCO, the accumulation of lipid reserves [WAN 08] and the biomass productivity. Phosphorus may form precipitates with metal ions and therefore has to be supplied in excess into the medium.

1.5.4.4. *Microelements*

Several organic and inorganic microelements are necessary for the growth of microalgae, including sulfur, iron, magnesium, potassium and sodium; the same applies to micronutrients such as copper, manganese, zinc, cobalt, molybdenum, etc.

A deficiency in one of these microelements may lead to reduced algae growth. A deficiency in sulfur induces an inhibition of protein synthesis and photosynthetic activity of microalgae. Iron deficiency causes cellular metabolic changes with a density and cell size decrease and an inhibition of protein and lipid synthesis. This element also acts as a catalyst in the synthesis of chlorophyll. Magnesium is essential to nitrogenase activity in the cellular metabolism of microalgae. A copper deficiency is likely to affect the photosynthetic mechanism of microalgae. A deficiency in molybdenum may influence the metabolic process of nitrogen assimilation at cellular level.

1.5.5. *Medium salinity*

A change of salinity in the medium induces osmotic and ionic stresses which can lead to the formation of precipitates, to an increase in the lipid content of algae, to a higher concentration in carotenoids in some species of *Dunaliella* [BOR 90] and to an inhibition of growth [GOM 03]. Furthermore, an increase in salinity may inhibit photosynthetic activity [LU 99].

1.5.6. *Agitation*

The homogeneity of the culture medium represents a fundamental point in ensuring optimal biomass productivity and high CO_2 fixation capacity. This parameter must therefore be taken into account during the design of the photobioreactor. Low agitation leads to difficulties in accessing nutrients and light to cells, to biomass settling phenomena and to the creation of “stagnant“ zones, within which culture conditions are unfavorable; it can lead to cellular mortality through toxic product accumulation. However, excessive agitation leads to shearing of algae cells which causes physiological and metabolic deficiencies as well as structural damage [SUL 03]. Consequently, the choice of a suitable agitation system must involve a compromise between these two types of constraint. The agitation systems most commonly used are [KUM 10]:

– Pumping: this allows a good mixing efficiency to be obtained but with low gas–liquid mass transfer rates;

– Mechanical agitation: this allows mixing efficiency and a good gas–liquid mass transfer rate to be obtained, but can induce hydrodynamic stresses;

– Agitation by gas injection: this provides the best result in terms of agitation and gas–liquid mass transfer rate, while reducing hydrodynamic stress. However, it can damage microalgae especially in the case of high concentrations.

1.5.7. *Gas–liquid mass transfer*

A good quality of gas–liquid mass transfer provides the maximum amounts of available CO_2 and regulation of pH through CO_2 dissolution in the liquid [KUM 10]. The effectiveness of gas–liquid transfer is measured by determining the volumetric mass transfer coefficient noted $k_L a$; this parameter multiplied by a CO_2 concentration gradient gives the quantity of CO_2 transferred by time and liquid volume. This transfer depends on many parameters, mainly on the diffusivity of CO_2 in both phases, the flow of gas and hydrodynamics.

1.6. Conclusion

Microalgae have several applications. The main examples of use of microalgae have been presented in this chapter. The culture of these microorganisms in photobioreactors allows better control of the operating conditions (pH, temperature, light, nutrient supply, agitation, etc.). Because the growth of microorganisms is very sensitive to these different parameters, it is therefore important to maintain them at optimal values in order to maximize the biomass productivity of the bioprocess. Photobioreactors are preferred since they allow a better control of the culture and its conditions.

Microalgae culture modeling and control in photobioreactors will be detailed in the following chapters, more particularly in the case of microalgae culture for CO_2 biofixation.