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Introductory Chapter: Microalgae Biotechnology. A Brief Introduction

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1. A general overview on microalgal biotechnology

The diversity of beings that make up the biological universe that comprehends the planet is simply dazzling. Not less dazzling than the whole is, with no doubt, each unit in special, even though being able to present itself in some similar aspects morphologically and physiologically to the others. Regardless of the specific pattern of the protein synthesis of each organism, they all perform chemical transformations, however, with due metabolic differences, which stimulate the potential biotechnological interest.

From the multiple metabolic diversities, it has been highlighted under the biotechnological point of view, the microalgae. This terminology comprehends a variety of prokaryotic or eukaryotic organisms, autotrophs and many capable of development themselves heterotrophically. The microalgae have been considered promisors organisms to various biotechnological applications in function of its potential of use to diverse renewable forms of useful substances and sustainable processes.

In morphologic, physiologic and structural characterization terms, the microalgae are organisms beings of extraordinary adaptive capacity susceptible to survive in an environmental diversity. The morphologic term aims to describe the shape, the size and the growth of an organism. The microalgae are highly adaptable not only in the physiologic aspect, but also are carrier of a quite diverse morphology. Mainly with respect to the size of these organisms, the physiologic properties are determinant factors and are directly connected to their cellular structure.

The microalgae term is devoid of taxonomic value. Once they constitute a polyphyletic and highly diversified group of microscopic beings present in aquatic systems, on its great majority photosynthesizes and present a vegetative structure known as thallus, whose cellular differentiation is characteristically small or null [1].

The principal standards to classify these microorganisms are pigmentation, chemical nature of the reserve products, and basis cellular structure. According to Mata et al. [2], under the denomination of microalgae are included organisms with two types of cellular structure: prokaryotic structure, with representatives in the groups *Cyanophyta* and *Prochlorophyta*; eukaryotic structure, with representatives in the groups *Glaucophyta*, *Rhodophyta*, *Ochrophyta*, *Haptophyta*, *Cryptophytes*, *Dinophyta*, *Euglenophyta*, *Chlorarachniophyte* and *Chlorophyta*. However, it stands out under the biotechnological exploitation aspect the groups: cyanobacteria (*Cyanophyta*), Chlorophyceae (*Chlorophyta*) and diatoms (*Ochrophyta*).

The cyanobacteria are classified in the eubacteria kingdom. It has never presented flagella, and by having its cellular organization of the prokaryotic type, it does not possess nucleus or organelles. They present in their structure the chlorophyll and the photosystems I e II, in contrasts to others photosynthetic bacteria, which allow them to realize the photosynthesis in the presence of oxygen. Some species are strictly phototrophic, while others act in, optional mode, being able to grow heterotrophically [3].

On the other hand, the Chlorophyceae present a great variety in the levels of organization, from unicellular, a flagellated microalgae or not, until the morphologically complex thallus. As well as cyanobacteria, the Chlorophyceae can be found in almost all environments, however, about 90% of the total of species (mainly the microscopy forms) occur in fresh water [4].

In relation with diatoms, they have been characterized by a cellular wall denominated frustula, and beyond the chlorophyll a and c, the plastids contain fucoxanthin and other xanthophylls, such as neofucoxanthin, diadinoxanthin and diatoxanthin. The main reserve substance of such organisms is chrysolaminarin, but the cells can also accumulate lipids and in general, are deprived of flagella and almost always heterotrophic. The diatoms inhabit the photic zone of oceans (up to about 200 m deep), seas, lakes, and rivers, presenting benthic and planktonic form [4].

The peculiar properties of each group made these microorganisms beings metabolically differentiated. Although the pigmentary profile of microalgae makes the photosynthesis the principal metabolic model, these organisms stand out by notable versatility to provide the necessary energy to the growth and maintenance, which identify them as a potential source of resources to be exploited biotechnology.

As previously mentioned, the microalgae possess a versatility in relation to the maintenance of their structures, utilizing different energy metabolisms as photosynthesis, respiration, and the nitrogen fixation, its utilization initially depends on the evaluative origin and also of the environment conditions or of the cultivation conditions [5].

From the use of the carbon source, we can differentiate two basic types of nutrition from microorganisms: autotrophic or heterotrophic. Between the autotrophic exist the photosynthetics,

whose obtain energy for the metabolism of light, and the heterotrophic, whose obtain energy to the metabolism originated from inorganic compounds or of ions and the nitrogen fixation [5]. The photosynthetic cultivation involves the utilization of light as the only energy source, which is converted in chemical energy by the photosynthesis process.

On this cultivation conditions, there is a direct relation between the photosynthetic activity and the microorganism's growth, since the light is the substrate and its intensity influences in the specific rate of growth, being capable to be identified or not by the photo-inhibition [6]. The reactions of light capture on upper plants and eukaryotic algae occur in the internal membranes of chloroplasts, in thylakoids and in the plasma membrane ramification, where are found the photosynthetic pigments (chlorophylls, carotenoids, and phycobiliproteins) and the enzymes necessary for the use of light and conversion of carbon dioxide. The pigments are located in highly organized structures called photosystem I (PSI), or reaction center (P700), and photosystem II (PSII) or reaction center (P680), whose are interconnected through an electron transport chain. The photosystems are enzymatic complexes capable of utilize the light as a reducing element, producing the driving force for the transport of electrons. On prokaryotic microalgae, these structures are in the thylakoids. When the light is absorbed, a series of oxidation reactions are started [7, 8].

In the presence of organic molecules as sugars, organic acids and acetate, some microalgae and cyanobacteria are capable of using the heterotrophic metabolism in the dark. Thus, the metabolism consists in the substitution of atmospheric CO₂ of the photosynthetic cultures by exogenous carbon sources, which makes it possible the increase of the concentration of biomass and of the productivity. Furthermore, the microalgae are capable of metabolize different forms of nitrogen compounds to endure the growth and the cellular manutention. Nitrogenous organic sources, such as urea and amino acids, have access to the interior of the cell through active transport. Some amino acids have been used as source of carbon and nitrogen to support the microalgal growth in the dark, but the most widespread form as a source of nitrogen is urea, which is hydrolyzed in NH₃ and CO₂, being able the two generated compounds to being utilized to the cellular growth [9].

Beyond the mentioned metabolisms, it is common to observe another metabolic process, the mixotrophic. This one is equivalent to the autotrophic and the heterotrophic, where the organic compounds and CO₂ are necessary to the growth, and where they are operated simultaneously the respiratory and the photosynthetic system, although the realization of photosynthesis is its principal energy source. However, the mixotrophic organisms assimilate organic compounds as a carbon source, while using inorganic compounds as electrons donors [10].

Particularly, in relation to photosynthetic cultivation, this presents a unique demand in industrial biotechnology, which is the contribution of light energy to the cells. This feature substantially modifies the bioreactor configuring for the microalgal cultivation. In this sense, the choice of the ideal photobioreactor is a crucial factor to the good performance of any microalgae culture system, since this is one function of the environment conditions and of the cultivation. Without taking into consideration any economic aspect, the photobioreactor must present some basic design requirements: efficient supply of light energy and CO₂; controlled temperature; suitable mixing system; availability of nutrients; facility in the control of reaction conditions; and facility in the scale-up [11].

Both the quantity and quality of the light source affect the cellular growth rate. When the intensity of the light is low, the growth rate is proportional to the light intensity. However, when the light intensity is much higher than the value of the saturation constant, it occurs the photoinhibition of the growth, which generally is caused by the reversible damage to the photosynthetic apparatus. The natural or artificial illumination can be utilized in function of the required characteristics in the cultivation system [6, 11].

In relation to the nutrition, despite of the different between the species, to a good growth, the culture medium must provide all the macronutrients and micronutrients demanded. In the group of the macronutrients are C, N, O, H and P, whose are considered as essential, and also Ca Mg, S, and K. In case of the macronutrients it has the Fe, Mn, Cu, Mo and Co, some species also need low concentrations of vitamins [12].

The carbon source most utilized in photosynthetic cultivations is the carbon dioxide, which can be in its normal or dissociate form (HCO_3^-) in the cultivation medium. The ideal concentration of CO_2 in the medium still is not well elucidate and it varies according to the specie of microalgae, however, generally are used concentrations between 3 and 15%. Considering the low solubility of CO_2 in liquids, there must be an efficient transference of CO_2 to the medium, in order to raise the volumetric mass transfer coefficients (K_La) to guarantee a suitable cellular growth [13].

The control of temperature also is indispensable in order to assurance the stability of the culture. In general, the ideal temperature of the cultivation occurs in the mesophyll region ($25\text{--}35^\circ\text{C}$), although some thermophilic strains resist to temperatures in the range of 60° . The majority of the cultivation systems assume the variation of temperature as a result of environment variation, though the use of heating mantle, serpentines, and external heat exchangers can be installed for the control of the temperature of microalgae bioreactors [12, 13].

Finally, agitation is a necessary operation in the cultivation of these microorganisms, since it ensures the spatial uniformity of reaction vessels, favoring the exposure of cells to light, the heat transfer, and the thermal stratification, as well as improving gas exchange. A suitable mixture minimizes yet the formation of cellular aggregates that increase the global inefficiency of the bioreactor. Although fundamental to the suitable development of the process, the operation of the mixture is related to hydrodynamic stresses associated to the cellular shear, which damages and inhibit the microalgal growth. The microalgae bioreactors are normally equipped with pneumatic aeration systems and mechanical agitation, or yet with a combination between these systems [14].

The cultivation of microalgae in large scale have started before the middle of the twentieth century, since then it has already been reported a wide range of cultivation systems. The differentiation of these cultivation systems depends principally of the cost, the type of product desired, the source of nutrients and the CO_2 capture. The culture systems are generally classified according with its conditions of project as open or closed systems [15].

Traditionally, the open systems have been widely used to the cultivation in large scale, due to its simplicity and low cost. These systems of cultivation present two principal configurations: circular and raceway ponds, that consist in a shallow tank (20–30 cm deep) of circular or oval

geometry, equipped with mechanical agitation systems, which expose the culture medium to the air by bubbling. Unfortunately, these photobioreactors allow only a limited control of the operation conditions. Besides that, the productivity is low, due the low absorption of light in the tank bottom and the major probability of contamination. Other limitations of these type of cultivation include a major necessity of space of land to the cultivation, losses by evaporation, high temperatures and, consequently, low efficiency of mass transfer [16].

An alternative to the open photobioreactors are the closed systems, which enable a great variety of configuration and significantly increase the performance of the cultivation. Three main configurations dominate the arrangements of closed photobioreactors are the tubular systems, the flat plates and the vertical columns. These systems are characterized by high photosynthetic efficiencies associated to a greater precision and control of the operational variables, lower risk of contamination and minimization of water losses by evaporation. Although, are severely limited by capital costs and scale-up [15, 17].

Futhermore, microalgae are important bioresources that have a wide range of biotechnological applications. The metabolic characteristics of the microalgae make these microorganisms an important source of resources to be explored. Associated with photosynthetic metabolism, the respiration and nitrogen fixation constitute important metabolic routes, passable of being biotechnologically explored for diverse purposes [18, 19].

The utilization of microalgae for the treatment of wastewater is particularly attractive, due to its abilities in assimilating nutrients as organic matter, NO_3^- , PO_4^{3-} , NH_4^+ , CO_2 and heavy metals [20]. The biological treatment of wastewaters occurs in heterotrophic bioreactors, where the organic matter and the inorganic nutrients are simultaneously converted in biomass in the absence of light. These processes are considered a cheap alternative to the conventional forms of treatment of secondary and tertiary effluents [21]. On the other hand, the photosynthetic cultivations of microalgae demonstrate to be one of the mitigation technologies of CO_2 most promisor, since that these microorganisms present high photosynthetic rates when compared to other upper plants, besides having a high resistance to high concentrations of carbon dioxide [8].

In this sense, the microalgae present versality to associate the treatment processes of wastes with the parallel production of inputs. The main biomolecules of commercial interest are the intracellular substances (pigments, fatty acids, proteins, and carbohydrates), the extracellular substances (carbohydrates and volatile compounds) [22–24].

The crescent interest in the natural and organic production has pressed the development environmentally correct technologies to a sustainable agriculture. The reduction in the fertility of the soils, the low efficiency in the use of chemical fertilizers, the increase of the environment pollution, and the decline of the productivity of important of agricultural crops are associated to the need to develop and implement biofertilization techniques that promote a reduction in the use of chemical fertilization in parallel to the increase of the efficiency of the use of these nutrients. In this way, the use of microalgae, preferentially the cyanobacteria, whether of free-living or symbiotically associated to other organisms, is considered an alternative in potential to supply the practices of organic agriculture. Independent of the metabolic pathway adopted in the cultivation, photosynthetic or heterotrophic, the microalgal biomass present mineral

substantial composition, that can reach 25% of the biomass dry weight. Furthermore, the presence of mineral elements of commercial importance as nitrogen (N), phosphorus (P) and potassium (K) potentiate the use of these in biosolids in the formulation of organic fertilizers [25].

In additional, when processed through chemical or biological reactions, the microalgae can provide different types of renewable biofuels that are called of biofuels of third generation. These include biodiesel, biohydrogen and bioethanol. The biodiesel is a mixture of alkyl esters and fatty acids obtained by transesterification. The transesterification is a reaction of multiple phases, where triglycerides are converted into diglycerides, subsequently these are converted into monoglycerides, which are then converted into esters (biodiesel) and glycerol (co-product) [2]. The bioethanol is obtained by biochemical processes through the fermentation of sugars (cellulose, xylose, galactose, arabinose, glucose and mannose) of the biomass and subsequent hydrolysis of the starch and cellulose content by a thermochemical process. On the other hand, the biohydrogen can be produced through two enzymatic pathways: direct photolysis or indirect photolysis [23, 26].

The commercial exploration in large scale of the microalgal intracellular content has started in the decade of 1950, motivated by the elevated content of proteins of biomass to utilization as an alternative food resource [27]. Since then, it has opened a wide range of passable products to be explored. Between them are found the pigments, classified in carotenoids, phycobiliproteins and chlorophylls that are responsible for the colors yellow/orange, red/blue and green, respectively [24]. The cyanobacteria in particular synthesize high levels of phycobiliproteins, with percentages that reach up to 8% of its dry weight. These pigments have been used as non-radioactive fluorescence markers when covalently bound to antibodies, biotin, lecithin and hormones. Beyond these applications, the phycobiliproteins present important antioxidant and anti-inflammatory activity. In function of the stability of molecules, the phycocyanin is used in the formulation of cosmetics as perfumes and makeup to the eyes. The carotenoids are other important class of pigments abundantly found in microalgae. It is well known to the pro-vitamin A activity of β -carotene and in its effects on the vision and in the immune system. Beyond this, the antioxidant activity of the carotenoids is associated to the prevention of cancer, atherosclerosis, degenerative diseases, and aging [28]. In consequence of these properties, innumerable carotenoids have been approved by the regulatory agencies in diverse countries as natural dyes of food and feed, with special emphasis to the astaxanthin produced by the chlorophyceae *Haematococcus pluvialis*, which represents the largest natural source of this carotenoid. This pigment has been extensively utilized in the feeding of salmon and trout as a coloring agent. Finally, it must be considered some keto-carotenoids and glycosylated carotenoids found exclusively in microalgal cells such as myxoxanthophyll, equinenone and canthaxanthin [24].

Some proteins, peptides and amino acids present biological functions associated to nutritional benefits and the human health. Thus, as the majority of species of microalgae present contents above 50% of protein in dry weight, these biopolymers can be used as nutraceuticals or included in formulation of functional foods. Beyond of the hypolipidemic and hypoglycemic properties, the ingestion of unicellular proteins is associated to the reduction of the cholesterol and the triglyceride levels. Finally, some proteins of microalgal origin

are associated to the stimulation of the production of the hormone cholecystokinin that regulates the appetite suppression and therefore, has been considered in the formulation of functional foods against the obesity.

Beyond the cholesterol, some microalgae species produce unconventional sterols such as brassicasterol, campesterol, stigmasterol, and sitosterol. In function of the high levels of sterols, these species have been used in the formulation of rations for the growth of juveniles, especially oysters [29]. In addition, the solar blocking compounds derived from microalgae have emerged as an alternative to the synthetic molecules and/or molecules of botanical origin. The compounds with photoprotective action include two main classes: the amino acids of mycosporine type (MAAs), and the scytonemin. These compounds present high blocking efficiency, photostability and low toxicity. The MAAs are compounds derived from imino-carbonyl of the cyclohexenone chromophore from mycosporins, that possess a conjugated nitrogenous substituent ring (an amino acid or an aminoalcohol). These compounds are soluble in water and present UVB action. They are found in both eukaryotic and prokaryotic microalgae. The scytonemin is an alkaloid indole, liposoluble, found exclusively in cyanobacteria. Not included in any of the previous chemical groups, compounds such as ciguatoxin, karatungiol, okadaic acid, and gamma-aminobutyric acid have been identified in microalgal extracts. Some marine dinoflagellates, belonging to the Dinophyta division, synthesize ciguatoxin and okadaic acid that present antifungal action. The okadaic acid has also been associated to the promotion of the secretion of the nerve growth factor (NGF). The karatungiol is another antibiotic molecule produced by marine dinoflagellates with antifungal and antiprotozoal activity. Finally, the gamma-aminobutyric acid is an amino acid that has a stimulating and regulating action of the brain development. It is associated to the neuronal excitability and the muscle tone.

Another class of compounds with positive effect in the human health are the fatty acids. Inside this group, to the majority of species, the polyunsaturated fatty acids, of the families $\omega 3$ and $\omega 6$, correspond to the largest fraction, being capable to get to 60% of the total lipids. On the other hand, microalgae also are classified as a good source of mineral salts such as phosphorus, iron, manganese, copper, zinc, magnesium and calcium. This composition makes the biomass a passable source of being utilized as food supplement in aquiculture and also as fertilizing. Carbohydrates can also be produced intracellularly. They are found in the form of starch or simple sugars such as arabinose, xylose, mannose, galactose and glucose, as well as less common sugars such as rhamnose, fucose and uronic acids [30].

In addition, microalgae present the capacity to accumulate extracellular polymeric substances (EPS) on the surface of the cell as a form of protection for them. EPS's are heterogeneous matrices of polysaccharide polymers, proteins, nucleic acids and phospholipids. The microalgal exopolymer have multiple industrial applications. In this sense, they can be applied in the food industry as thickeners and gelling agents. In the pharmaceutical industry they can be used as a hydrophilic matrix for controlled release of medicines, in the development of bacterial vaccines and to increase non-specific immunity. In addition, some EPS's possess biosurfactant characteristics and are being used in bioremediation of waters and soils [31].

Thus, based on issues above summarized, it's possible to conclude that the diversity of important applications of microalgae in innumerable technological routes of production makes these microorganisms become biocatalysts with a wide potential of agricultural and mainly industrial exploration. Regardless of these potentialities, the competition with consolidated technological routes based, for example, on non-renewable fossil inputs, often makes economically unfeasible in the present scenario the microalgae-based processes and products. Therefore, new industrial approaches have been proposed and implemented in order to effectively enable the technical and economic success of these technologies. The integration and intensification of processes associated to the concept of biorefinery have been considered as the main engineering strategies that will enable a large commercial exploration of the microalgae-based processes. These new technological routes are orientated to the effective use of the industrial resources based on equipment, materials and processing techniques. These three approaches of engineering of process will allow in mid-term the consolidation of microalgae-based processes as effective enhancers of the industrial sustainability, balancing the vectors of the environment, economy and the society.

The chapters presented in this book are intended to help provide a deeper understanding and insight into promises and challenges for microalgal biotechnology to be a substantial contributor to future of food, feed, chemicals, pharmaceuticals, energy, and fertilizer supplies.

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