

Review Article

# Exploring the biotechnological applications of *Spirulina maxima*: a comprehensive review

## Explorando as aplicações biotecnológicas da *Spirulina maxima*: uma revisão abrangente

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### Abstract

The *Spirulina maxima* algae is a phototrophic, multicellular, filamentous cyanobacteria of greenish blue tones, without ramifications and is characterized mainly by its helical form, thickness of approximately 3 to 12  $\mu\text{m}$  and length of 500  $\mu\text{m}$ ; its development depends on factors such as temperature, light intensity, pH, aeration speed, carbon dioxide concentration, carbon source, nitrogen source which determine its chemical composition, which is composed of proteins, carbohydrates, lipids, minerals, and vitamins; due to this, it is widely used in industries such as food, pharmaceutical, cosmetics, and energy to obtain different products of great value. This *S. maxima* review addresses morphological characteristics, growth factors, growth methods, and metabolites of biotechnological interest and biotechnological applications for the *S. maxima* microalgae. A brief review of the enzyme production capacity of *S. maxima* and other microalgae is also presented, in addition to mentioning some areas of opportunity to study these and the economic viability of implementing a biorefinery with an integrated approach for the production of biomass and metabolites of biotechnological relevance based on the control of growth variables and the productive and economic efficiency of the process is discussed.

**Keywords:** *Spirulina maxima*, algae development, chemical composition, applications, enzymes production.

### Resumo

A alga *Spirulina maxima* é uma cianobactéria fototrófica, multicelular, filamentosa, de tons azul-esverdeados, sem ramificações, e se caracteriza principalmente por sua forma helicoidal, espessura de aproximadamente 3 a 12  $\mu\text{m}$  e comprimento de 500  $\mu\text{m}$ . Seu desenvolvimento depende de fatores como temperatura, intensidade luminosa, pH, velocidade de aeração, concentração de dióxido de carbono, fontes de carbono e de nitrogênio, que determinam sua composição química, que inclui proteínas, carboidratos, lipídios, minerais e vitaminas. Devido a essa composição, é amplamente utilizada em indústrias alimentícia, farmacêutica, cosmética e energética para obter diferentes produtos de alto valor. Esta revisão de *S. maxima* aborda características morfológicas, fatores de crescimento, métodos de cultivo e metabólitos de interesse biotecnológico, além de suas aplicações biotecnológicas para a microalga *S. maxima*. Uma breve revisão da capacidade de produção de enzimas de *S. maxima* e outras microalgas também é apresentada, além de mencionar algumas áreas de oportunidade para estudá-las e a viabilidade econômica da implementação de uma biorrefinaria com abordagem integrada para a produção de biomassa e metabólitos de relevância biotecnológica com base no controle de variáveis de crescimento e na eficiência produtiva e econômica do processo.

**Palavras-chave:** *Spirulina maxima*, desenvolvimento de algas, composição química, aplicações, produção de enzimas.

## 1. Introduction

Algae are photosynthetic microorganisms that convert natural or artificial light energy into chemical energy. Algae are divided into microalgae, macroalgae, and cyanobacteria (also included as microalgae) and classified as prokaryotes or eukaryotes and unicellular or multicellular. In recent years, the interest in algae use has taken great relevance due to the chemical components in its structure, which are considered of great economic value

and used in pharmaceuticals, food, cosmetics, agriculture, biofuels, and other industries (Coronado-Reyes et al., 2022). Some advantages of growing algae are its growth and reproduction speed because its growth cycle more quickly than other microorganisms in the same conditions (Çelekli et al., 2019). Recent studies have focused on finding the nutritional and environmental conditions that increase the growth and development of biomass and

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increase the content of a particular chemical component (Lafarga et al., 2020).

*Spirulina maxima* is a cyanobacterium (microscopic and prokaryotic) that is the oldest living plant on Earth (approximately 3.6 billion years old) and the first organism with the ability to carry out photosynthesis and generate the oxygen atmosphere for life development. Currently, the main biotechnological microalgae applications are diverse; however, Hernan Cortez Scientist and Conqueror, in 1519 observed that the Aztecs located in Lake Texcoco in Mexico consumed this microalga due to its high protein, vitamins, minerals, and fatty acids content. *Spirulina maxima* is photosynthetic, planktonic, filamentous, multicellular, blue-green color, phototrophic cyanobacterium and helical morphology (Figure 1), capable of forming large populations and grows in high-salinity aquatic ecosystems, such as water bodies saline, alkaline, brackish, and freshwater; It has different uses as a food supplement, livestock feed, and pharmaceutical products, due to its high protein, carbohydrates, vitamins, minerals, fatty acids, pigments, antioxidants content (Rajasekaran et al., 2016; Baleta et al., 2017). The *S. maxima* development depends on several factors, such as temperature, light intensity, pH culture, aeration, carbon dioxide concentration, carbon source, nitrogen source, salts, and inoculum amount; however, the temperature and light intensity have a higher effect on the growth and production of metabolites because these are directly related to the photosynthetic process of this algae (Lafarga et al., 2020; Ragaza et al., 2020).

In recent years, the use of microalgae attributed to its chemical composition consisting mainly of proteins, carbohydrates, fatty acids, antioxidants, minerals, and vitamins has taken on great relevance because these metabolites can have various applications in different areas of the industry, including food, beverages, pharmaceutical, cosmetics, and biofuels; the *S. maxima* cyanobacterium is widely known and used in several industries attributed to its ability to produce metabolites of great biotechnological relevance. Likewise, a little studied area for this microalga is its intra- and extra-cellular enzyme ability production and its applications in the industry (Baleta et al., 2017). Currently, some research reports that *S. maxima* can

produce some enzymes, among which are proteases, lipases, superoxide dismutase (SOD), laccases, and L-asparaginase. However, there is a field that can be studied and explored to determine the production capacity of other types of enzymes from this cyanobacterium (Spier et al., 2020).

The objective of this research was to collect and analyze the information that influences the growth and development of the cyanobacterium *S. maxima* and to review the production and application of enzymes from *S. maxima* since there is little information reported on this topic.

## 2. Morphology and Life Cycle

The *S. maxima* algae is a phototrophic, multicellular filamentous cyanobacterium of greenish blue tones constituted of cells organized in helical trichomes, with a thin external mucilaginous membrane, without branches and absent of heterocyst and characterized by its helicoidal shape (Figure 1), which can be altered by the environmental (temperature), physical and chemical development conditions, likewise its helical shape is maintained in liquid growth means, however, in solid means the filaments take a spiral form which depends mainly on the water content on the surface of the medium, attributed to the hydration or dehydration of oligopeptides which causes the rigidity in the cells (Lukavský and Vonshak, 2000; Can et al., 2017).

The thickness of each filament varies from 3 to 12  $\mu$ m, the diameter of each helix is between 10 to 70  $\mu$ m and the length of the trichomes is approximately 500  $\mu$ m; the variation of these measurements is due to the medium and the growing conditions; the filaments size will depend on the winding degree and its length; which are composed of a cytoplasmic peripheral section, tented by different pigments and another one of the center bodies called nucleoplasm (Alagawany et al., 2021).

The prokaryotic structure of *S. maxima* is constituted by: the capsule of a capsular fiber that covers and protects the filament, a stratified cell wall, chromatoplasm with its photosynthetic system of thylakoids, ribosomes, and DNA fibrils; the stratification of the cell wall or membrane is in four sections which the layers give it higher rigidity in its structure attributed to the presence of  $\beta$ -1,2-glucan and peptidoglycan respectively (Rajasekaran et al., 2016; AlFadhly et al., 2022). This alga contains functional pigments such as chlorophyll, carotenes, and phycobilin, concentrated in the thylakoid system, chloroplasts, and photosystems I and II, and their floating capacity in aqueous media is attributed to the gas contained in the protein vesicles of the intrathyroidal space (Ragaza et al., 2020).

The life cycle is a fundamental aspect of the reproduction of *S. maxima*; this process is less complex compared to other cyanobacteria, beginning with the rupture into several parts of a mature trichome (filament) due to the formation of specialized cells called nephridium, which submit a lysis process causing separation, these fragments are composed of groups of 2 to 6 cells known as concrete, from which they generate new trichomes, and during their maturation process they lose nephridium fragments from the original trichome causing cell wall thinning of cells at the ends to take a rounded shape (Grosshagauer et al., 2020); finally, the



**Figure 1.** View of the cyanobacteria *S. maxima* at 40 X magnification.

size of the new trichome increases through binary fission and in parallel the cytoplasm becomes granular, which causes it to acquire its bright bluish-green color and take on its helical shape (Sánchez et al., 2003; Álvarez, 2022).

### 3. Chemical Composition

The *S. maxima* alga has been used as a food source for humans and animals by various ancient civilizations due to the nutritional contribution they observed from its consumption (Gentscheva et al., 2023). Nowadays, this has gained importance worldwide due to its nutritional, antioxidant, and therapeutic effects due to its consumption (Ragaza et al., 2020); from this, in different investigations, the presence of proteins, lipids, carbohydrates, minerals, and pigments has been identified as shown in Figure 2, however, a complete analysis of each biochemical component described below.

#### 3.1. Proteins

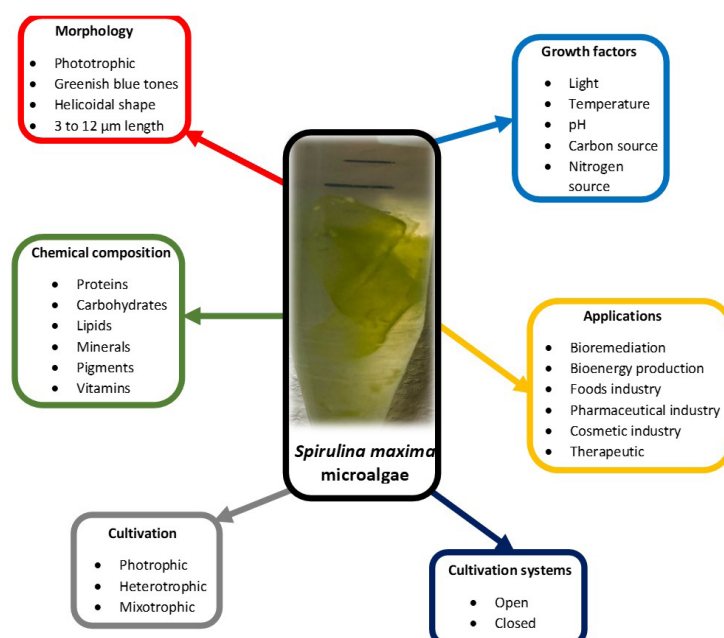
The protein content in *S. maxima* is between 55% to 70% of dry weight, which is higher versus other foods of vegetable origin, which contain around 30% protein; therefore, due to the content and high quality of proteins are considered as food source for human nutrition; however, the protein content in *S. maxima* can vary between 10 and 15% due to factors such as harvest period and light intensity (Lai et al., 2019; Gentscheva et al., 2023). From a qualitative view, the proteins' quality is better because up to 47% of their weight is composed of all essential amino acids, as shown in Table 1 (Tan et al., 2020); the amino acids with the highest proportion in proteins are leucine, valine, and isoleucine with a content of 10.9%, 7.5%, and 6.8%

respectively of total amino acids present; however, there are amino acids with a lower proportion in proteins, that contain sulfur, methionine, and cysteine, which exceed up to 80% the minimum content recommended by the Food and Agriculture Organization (FAO) (Gutiérrez-Salmeán et al., 2015; Lafarga et al., 2021).

Likewise, another aspect that determines the quality of *Spirulina* proteins is their high digestibility attributed to the absence of cellulose in its cell wall, but it has a membrane composed of murein, which favors that up to 90% of the protein can be digestible (Tan et al., 2020). Therefore, the protein obtained from *S. maxima* does not require specific conditions to increase its production, which simplifies the growth of this; however, the exposition at temperatures above 67 °C in a neutral growth medium causes protein denaturation, and when the medium cools form hydrogen bonds in hydrophobic region, which causes the protein gelation (Acquah et al., 2021; Geada et al., 2021).

#### 3.2. Lipids

The lipid content in *S. maxima* is equivalent between 6% and 11% in dry weight constituted of a variety of essential fatty acids as shown in Table 2, among which highlighted linoleic acid (C<sub>18:2</sub>, LA) and γ-linoleic acid (C<sub>18:3</sub>, GLA) to which medicinal and therapeutic properties have been attributed, particularly for GLA it has reported in several researches that it favors the synthesis of prostaglandins and arachidonic acid, in addition to having a higher effect in reducing low-density lipoproteins (cholesterol) compared to LA (Grosshagauer et al., 2020). Among the antioxidant contained in *S. maxima* are polyunsaturated fatty acids; however, the extraction and purification are expensive, so direct consumption of this algae is recommended because it has a better nutritional contribution (Gentscheva et al., 2023).



**Figure 2.** Principal characteristics for cyanobacteria *Spirulina maxima*.

**Table 1.** Amino acid content in *S. maxima* versus animal and vegetable foods.

Contend (g per 100 g de protein)			Vegetables <sup>2</sup>
Amino acids	<i>S. maxima</i> <sup>1</sup>	Meat <sup>2</sup>	
Essential			
Isoleucine	5.71-6.70	1.40-1.60	0.20-0.80
Leucine	9.26-10.17	2.70-3.20	0.30-1.50
Lysine	4.42-4.99	1.80-2.50	0.20-0.60
Methionine	2.05-2.50	0.90	0.10-0.40
Phenylalanine	4.42-5.30	1.20	0.20-1.00
Threonine	4.65-6.20	1.20-1.40	0.20-0.70
Tryptophane	0.08-1.93	0.30	0.10-0.20
Valine	6.08-7.10	1.60-1.90	0.20-0.90
No essential			
Alanine	5.51-9.50	1.50	0.30-1.00
Arginine	7.09-8.0	N.R.	N.R.
Aspartic acid	9.86-11.80	2.50	0.30-1.20
Cysteine	0.57-1.11	0.50	N.R.
Glutamic acid	9.47-13.69	3.50	0.50-1.90
Glycine	1.10-5.70	1.50	N.R.
Histidine	2.20-10.41	N.R.	N.R.
Proline	3.33-4.35	1.50	0.30-0.9
Serine	4.56-5.10	1.50	0.30-0.9
Tyrosine	3.61-5.69	1.00	0.10-0.40

<sup>1</sup>Ragaza et al. (2020);<sup>2</sup>U. S. Department of Agricultura; FoodData Central (USDA, 2023). N.R. = Not Reported

**Table 2.** Fatty acids percentage content in *S. maxima*.

Fatty acids	Nomenclature	Contend (%)
Lauric acid	C <sub>12:0</sub>	0.52 <sup>1</sup>
Myristic acid	C <sub>14:0</sub>	0.23 <sup>2</sup>
Palmitic acid	C <sub>16:0</sub>	46.0 <sup>2</sup>
Palmitoleic acid	C <sub>16:1</sub>	1.26 <sup>2</sup>
Stearic acid	C <sub>18:0</sub>	1.49 <sup>1</sup>
Oleic acid	C <sub>18:1</sub>	5.03 <sup>1</sup>
Linoleic acid	C <sub>18:2</sub>	17.43 <sup>2</sup>
γ-Linoleic acid	C <sub>18:3</sub>	8.87 <sup>2</sup>
Behenic acid	C <sub>22:0</sub>	Trazes-20.01 <sup>2</sup>

<sup>1</sup>Ragaza et al. (2020); <sup>2</sup>Gentscheva et al. (2023).

3.3. Minerals

*S. maxima* has minerals in its structure, among which all the essential minerals for human development (as shown in Table 3), so it is considered a rich natural source of minerals (Rajasekaran et al., 2016).

3.4. Vitamins

*S. maxima* contains a variety of vitamins, as shown in Table 4, being β-carotene (Provitamin A) the highest proportion, furthermore to have a higher content compared with other vegetable foods (Rani et al., 2021); likewise, it is rich in vitamins B and E, which are absent or present in minor amounts in vegetable or animal foods; therefore, due its vitamin content and beneficial effects on health

**Table 3.** Mineral content in *S. maxima*.

Minerals	Symbol	Contend (mg per 100 g)
Calcium	Ca	700
Potassium	K	1,400
Magnesium	Mg	400
Sodium	Na	900
Phosphorus	K	800
Copper	Cu	1.2
Iron	Fe	100
Manganese	Mn	5
Zinc	Zn	3
Chromium	Cr	0.28
Selenium	Se	0.04
Boron	B	2.87
Molybdenum	Mo	0.37

Taken and modified from Sharoba (2014) and Anvar and Nowruzi (2021).

generated for its intake, *S. maxima* is considered an excellent source of vitamins (Grosshagauer et al., 2020; Katiyar and Arora, 2020).

3.5. Carbohydrates

The carbohydrate content in *S. maxima* is approximately 13.6%, constituted of glucose, xylose, mannose, galactose, rhamnose and starch that can absorb through the intestinal

**Table 4.** Vitamin content in *S. maxima*.

Vitamins	mg per 100 g <sup>1</sup>
Provitamin A ( $\beta$ -carotene)	140 <sup>2</sup>
Vitamin E	9.86 <sup>1</sup>
Thiamine ( $B_1$ )	3.5 <sup>2</sup>
Riboflavin ( $B_2$ )	4.0 <sup>21</sup>
Niacin ( $B_3$ )	14.0 <sup>2</sup>
Pyridoxine ( $B_6$ )	0.98 <sup>1</sup>
Cobalamin ( $B_{12}$ )	0.16-0.175 <sup>1</sup>
Folic acid	0.01 <sup>2</sup>
Biotin	0.01 <sup>2</sup>
Pantothenic acid	0.1 <sup>2</sup>
Vitamin K	2.2 <sup>2</sup>

<sup>1</sup>Ragaza et al. (2020); <sup>2</sup>Anvar and Nowruzi (2021).**Table 5.** Pigments contained in *S. maxima*.

Pigment	Content (mg 100 g <sup>-1</sup> )
Chlorophyll $\alpha$	1,000 <sup>1</sup>
Phycocyanin	14,000 <sup>1</sup>
Carotenoids	370 <sup>1</sup>
$\beta$ -carotene	55.5 <sup>2</sup>
Echinenone	40.7-48.1 <sup>2</sup>
$\beta$ -cryptoxanthin	22.2-29.6 <sup>2</sup>
3'-hidroxiechinenone	25.9-40.7 <sup>2</sup>
Zeaxanthin	92.5 <sup>2</sup>
Diatoxanthin	18.5 <sup>2</sup>
Canthaxanthin	18.5 <sup>2</sup>
Mixoxanthophyll	48.1-62.9 <sup>2</sup>
Unidentified	11.1-14.8 <sup>2</sup>

<sup>1</sup>Sánchez et al. (2003); <sup>2</sup>Cárdenas-Nieto et al. (2010).

tract due to the absence of cellulose in the cell wall (Zaparoli et al., 2020); the presence of inulin also has been reported, which has high molecular weight, a water-soluble polysaccharide with stimulating activity on the immune system (Ragaza et al., 2020).

### 3.6. Pigments

The pigment content in *S. maxima* (Table 5) is up to 5% of dry weight, as  $\alpha$ -chlorophyll, phycobiliproteins, and carotenoids (as shown in Table 4) (Park et al., 2018). Chlorophyll is a molecule with a greenish tone, characteristic of plants and located in chloroplasts, which allows capturing light energy to carry out photosynthesis (Park et al., 2018); carotenoids are natural pigments, fat-soluble and characterized by tones yellow or orange, synthesized from the exposure of the alga to light sources;  $\beta$ -carotene is a carotenoid contained in high concentrations

in green algae such as *S. maxima*, in recent years it has gained higher relevance due to the positive effects on the health of animals and humans for its consumption (Abreu et al., 2023; Fernandes et al., 2023). Finally, phycobiliproteins are hydrophilic protein structures linked to phycobilins and generated in the photosynthetic apparatus of algae, classified as phycoerythrin, phycocyanin, and allophycocyanin constituted for particles called phycobilisomes; due to their fluorescence in bluish tones, they are used in several industries, although also have antioxidant and antiviral properties, among others (Cardenas-Nieto et al., 2010; Sommella et al., 2018).

## 4. Cultivation Methods and Growth Factors

Plants and algae can perform their metabolic functions under phototrophic conditions through the use of inorganic carbon sources ( $CO_2$ ) and light (natural or artificial) to carry out photosynthesis, and is the most common crop system; however, some disadvantage is the reduction of metabolites generation, biomass growth, due to the self-shading effect due for partial blocking of light passage due biomass density (Markou et al., 2019; Manhaeghe et al., 2020).

The heterotrophic growth of algae is carried out through the use of organic carbon sources (sugars, lipids, acetates) and the absence of light (Mollamohammada et al., 2020); the use of this growth method avoids the limitations associated with the light source and increases the biomass growth and metabolite production significantly (Jin et al., 2021; Li et al., 2022).

Mixotrophic growth consists of the combination of light and carbon (organic and inorganic) sources combination, as the principal energy source in algae photosynthesis, which can carry out growth and generation of metabolites in its light phase (Bhatnagar et al., 2011; Cecchin et al., 2018); however, in the dark phase, there is no energy input from light sources for metabolites synthesis (autotrophic culture), hence having a carbon source, in this phase the alga can obtain energy from its use, and continue with its growth and production of metabolites (heterotrophic culture) (Li et al., 2018; Ray et al., 2022).

The growth of *S. maxima* is affected by environmental factors, which can cause changes in biomass composition and metabolite production (Figure 2). The light is a factor that influences the growth and production of metabolites in *S. maxima*, so the exposure time control (photoperiod) and intensity are essential; however, prolonged exposure can cause that alga to exceed its light capture capacity, causing photoinhibition or photolysis, therefore, various investigations report and recommend between 2,000 kLux to 5,000 kLux for the growth and metabolism of *S. maxima* (Li et al., 2018; Gonzalez Bautista and Laroche, 2021).

The production of metabolites from photosynthesis can be affected by the phenomenon of "self-shading" due to excessive light intensity, in which the cells closest to the light source will be able to carry out their photosynthetic process without problems, but they will cast a shadow for those cells behind, which would cause partial or total inhibition of metabolic functions and growth (Gonzalez Bautista and Laroche, 2021); an option to minimize this



phenomenon is through to induce medium stirring for bubbling during the supply of air or CO<sub>2</sub> in the reactor, in addition to avoiding mechanical stirring with blades since it can damage the cells. Another consideration during algal growth is the light homogeneous distribution in all directions so that a photon light can travel through the system to the furthest point, defined as the reactor depth (Ziganshina et al., 2020; García-López et al., 2020).

Microorganism growth requires that the environmental conditions are appropriate, and temperature is an ambiental important factor for this, particularly since each type of organism has specific temperature intervals in which it can grow and develop optimally (Karemore et al., 2020). The ideal temperature for *S. maxima* is between 30 °C to 35 °C; however, temperatures below 20 °C or above 38 °C reduce their growth; but increase the production of metabolites (proteins, pigments, and lipids) (Soni et al., 2016).

The pH value is an essential parameter for microorganism development, like temperature each one has a specific value for its growth; particularly pH value for microalgae's is approximately 8.0 (Sornchai and Iamtham, 2013; Zapparoli et al., 2020); specifically, the pH value for *S. maxima* is between 9 to 11, and the optimal values are between 9.5 to 10.3; however, variations may occur due to alkalinity and nutrient content, medium temperature, metabolic activity or CO<sub>2</sub> concentration (Ismaiel et al., 2016; Soni et al., 2017).

CO<sub>2</sub> is a source of inorganic carbon used for algae during the light phase of photosynthesis. Its use may depend on factors such as pH, temperature, and light intensity; the recommended concentration of CO<sub>2</sub> for *S. maxima* is between 0.05% to 5.0% v/v; however, concentrations between 0.075 v/v have better results on the production of biomass and metabolites (Mehar et al., 2019; Coronado-Reyes et al., 2022). The production of O<sub>2</sub> carried out from the consumption of CO<sub>2</sub> during photosynthesis, which causes an increase of up to 300% concerning initial content dissolved in the medium, which can cause a reduction of up to 25% in biomass production and the metabolites content, so O<sub>2</sub> concentration must be monitored and controlled during the development of growth kinetics (Acquah et al., 2021; Geada et al., 2021).

Maintaining the nutrient content required for *S. maxima* development is essential, as nitrogen is a macronutrient through which the alga can carry out the biosynthesis of genetic material, proteins, and lipids from nitrates, nitrites, and ammoniacal nitrogen (Rajasekaran et al., 2016), it has also been reported that excess or depletion of nitrogen source can induce an increase in carbohydrates or lipids production respectively (Puspanadan et al., 2018); the phosphorus content is essential in the growth medium and is necessary for the synthesis of nucleic acids and energy molecules such as ATP in cells, the obtaining this nutrient is through adding phosphate salts to the growth medium (Velázquez-Sánchez et al., 2023). Macro and micronutrients are necessary for *S. maxima* growth; the macronutrients (SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup>, Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>) in the medium are essential because they favor structural and metabolic functions; the micronutrients addition (EDTA, B, Mo, Co, Cu) perform catalytic, mediating or regulating functions of enzymatic

reactions and physiological processes (Cardenas-Nieto et al., 2010; Zapparoli et al., 2020); based on *S. maxima* nutritional requirements, some growth media have been standardized, which contain necessary macro and micro nutrients for its growth, among the best-known media is the Zarrouk medium, which has modified the nutrient content to reduce its cost; another alternative for growth media algae is nutrient-rich wastewater; however, a drawback is that the generated algae are not suitable for human and animal consumption (Mehar et al., 2019).

## 5. Cultivation Systems

The open and closed culture systems allow the development and culture of this cyanobacteria (Figure 2); however, for its design is necessary to consider some factors such as culture type, temperature, pH, light, nutrient, and CO<sub>2</sub> requirements; therefore, it is relevant to establish each growth parameter based on cyanobacteria growth (Alfadhly et al., 2022). The open system cultivation system is cheap in its construction, maintenance, and operation; it has higher durability and production capacity versus closed systems; however, some of the disadvantages of this system are the contamination susceptibility by other microorganisms, low CO<sub>2</sub> solubility, and the lack of control over environmental conditions (temperature, evaporation, lighting); therefore this culture method is recommended for those cyanobacteria that grow in highly alkaline or acidic conditions, specific temperature, and particular nutritional requirements (Abreu et al., 2023; Velázquez-Sánchez et al., 2023). The closed cultivation system for this cyanobacteria growth allows have higher advantages over the open system, among which is having greater control over the culture conditions (temperature, lighting, pH, nutrients, agitation), which reduces the possibility of contamination by other microorganisms. Particularly in equipment such as photobioreactors, it is possible to control and optimize biomass growth parameters compared to open systems; one of the main advantages of its use is control of the CO<sub>2</sub> supply and lighting, which favors the growth of biomass and the production of metabolites of interest (Grosshagauer et al., 2020; Abreu et al., 2023).

## 6. Applications

The *S. maxima* applications are diverse; however, the main objective is to achieve biomass development and the production of the specific metabolites, which depends on the nutrient content in the growth medium and the growth factors, some applications of *S. maxima* will be described below (Demain and Sánchez, 2017; Rani et al., 2021).

### 6.1. Feed and nutrition

The use of *S. maxima* as a source of animal feed is another application because it is considered a source of protein for poultry, pigs, and fish; however, in some cases, its use as food causes implications for the quality of the final product, such as in the production of fish and poultry, harming the meat appearance due to the change in meat

coloration; for which reason they must develop research that allows knowing its implications when used as food for the development of animals for human consumption (Altmann and Rosenau, 2022).

In recent years, several foods have increased their nutritional value through microalgae addition attributed to their high content of proteins, antioxidants, vitamins, minerals, and fatty acids; some research indicates that *S. maxima* addition contributes to increasing the nutritional value of different foods. In the baking and biscuit industry, the *S. maxima* addition in several products increases the protein content between 9.43% and 18.11% in a 6% weight/weight ratio (Batista et al., 2019; Haoujar et al., 2022). Likewise, some research mentions that *S. maxima* addition does not represent a risk for its consumption and does not cause changes in the sensory product properties (Guldas et al., 2022). Pasta is another food enriched with *S. maxima* because it improves its nutritional contribution and organoleptic characteristics (Mostolizadeh et al., 2020). The 0.25% weight/weight ratio microalgae addition improves the nutritional content and organoleptic properties. Likewise, with the addition of ratios of up to 5% and 10% weight/weight of *S. maxima* in wheat flour for pasta production, the protein content increases to 10.32% and 14.5% respectively, and the energy contribution increases slightly up to 322.94 kcal g<sup>-1</sup> and 327.60 kcal g<sup>-1</sup> respectively for each addition percentage; enriching foods such as pasta with *S. maxima* can significantly improve the nutritional and organoleptic characteristics of these products (Lemes et al., 2012; Grahl et al., 2018). Dairy products improved with *S. maxima* addition in 1% weight/weight ratio in cheese to improve their physicochemical and organoleptic characteristics (Nakamoto et al., 2023). Likewise, the *S. platensis* addition in ice cream increases the antioxidant activity by up to 39.7% compared to the 32.8% of ice cream without the algae addition, and this product improved the protein content and the organoleptic properties (Malik, 2011). Finally, the *Spirulina* addition to foods can increase the nutrient content, prolong its shelf life, and improve the physicochemical and organoleptic characteristics, without causing adverse effects on human health at the added concentrations.

## 6.2. Therapeutic and health effects

In recent years *S. maxima* have attracted attention due to the effects caused by its consumption on human health; such is the case of the decreased cholesterol levels in blood, attributed to the high g-linoleic acid contained in the seaweed; it also favors the loss of up to 1.4 ± 0.4 Kg after consuming the seaweed for four weeks; likewise, no variations in clinical (blood pressure) or biochemical (hemoglobin, white blood cells, hematocrit) parameters have been reported due to the consumption of *S. maxima* in humans (Anvar and Nowruzi, 2021).

Some reports show that antioxidant content in *S. maxima* can reduce the presence of free radicals that cause cancer; some studies have been carried out in hamsters by the School of Dental Medicine at Harvard University, reported a decrease in oral cancer cells from extracts of β-carotene obtained from *S. maxima* because this component causes the

reduction size of tumors and some cases its disappearance; therefore its consumption can favor the prevention of the appearance of different types of cancer attributed to its content of antioxidants (carotenoids and tocopherols) (Romero et al., 2017; Gentscheva et al., 2023).

The vitamin content in *S. maxima* is varied; one of these is Vitamin E (α-tocopherol) which is used as a natural lipid antioxidant because it stops the oxidation reaction of these molecules, so the addition of *S. maxima* to some foods to reduce the oxidation of lipids contained in them; likewise, several studies show that Vitamin E consumption has benefits on human health, such as degenerative diseases prevention, arteriosclerosis, heart disease, cancer, skin, and eye diseases for excessive light exposition; Therefore, the vitamin content in *S. maxima* and its consumption can favor human health in preventing diseases onset (Matos et al., 2017; Bortolini et al., 2022).

Polyunsaturated fatty acids have essential relevance in human health because humans cannot synthesize fatty acids with more than 18 carbons; however, their obtaining can be from another food source such as *S. maxima*, some fatty acids content in this algae as eicosapentaenoic (EPA), docosahexaenoic (DHA), and docosapentaenoic (DPA) may be effective in the treatment and prevention of various diseases such as hypertension, cancer, type 2 diabetes, asthma, arthritis, and kidney and skin disorders, depression, schizophrenia, in addition to being essential for pre and postnatal eye and brain growth and development, among other benefits. Therefore, due to the multiple benefits in human health attributed to its consumption, various food supplements allow the supplied minimum recommended dose of these components (Anvar and Nowruzi, 2021).

Finally, the phycocyanin is a blue protein, soluble in water and non-toxic based on its benefits; several researchers have reported that have antioxidant, nephroprotective, hepatoprotective, anti-inflammatory, anticancer, antimicrobial, and diabetes control properties, among others; due to its beneficial effect on health due to its consumption, its market value ranges from €25.00 mg<sup>-1</sup> when partially purified to €200.00 mg<sup>-1</sup> for high-purity phycocyanin (Belwal et al., 2020; Pez Jaeschke et al., 2021).

## 6.3. Cosmetic products

Recently, different studies have evaluated the addition of *S. maxima* as a principal component in the formulation of cosmetic products for skin care, including sun creams, moisturizers, healing agents, illuminators, and cleansers, to name a few (Ovando et al., 2018; Rodriguez-Concepcion et al., 2018; Ragusa et al., 2021). This cyanobacterium contains a set of components with synergistic action, such as peptides, fatty acids, vitamins, minerals, phycobiliproteins, and antioxidants, which promote improvement in appearance, stimulate the production of proteins (collagen and elastin), repair, hydration, regeneration, protection against UV radiation (Delsin et al., 2015; Nihal et al., 2018; Nowruzi et al., 2020). Finally, the algae addition in cosmetic products favors their efficacy and reduces the probability of skin infections or secondary reactions (Ragusa et al., 2021).

#### 6.4. Bioremediation

Microalgae are microorganisms that can adapt to different growth media, so they can bioremediate industrial or domestic wastewater due to their nutrient content that favors particularly the *S. maxima* growth; however, concentrations higher than 300 mg COD L<sup>-1</sup> inhibit its growth (Borja-Aragón et al., 2017; Garcia-Martinez et al., 2019). The *S. maxima* approximate chemical composition when grown in wastewater is 62.2% protein, 11.3% carbohydrate, 7.0% total lipid, 3.2% chlorophyll, and 6.2% ash; these values are lower than those reported from their growth in mineral medium, attributed to the nitrogen deficit in wastewater causing carbon photo assimilation for protein and chlorophyll production (Han et al., 2021; Rahman et al., 2022). Some reports indicate that *S. maxima* can remove heavy metals, different research reported that *S. maxima* would remove between 80% and 97% of heavy metals content in wastewater; however, this removal capacity is a function of the metal concentration in the wastewater; other investigations report that *S. maxima* also can remove long-chain hydrocarbons, therefore, *S. maxima* is a novel and viable alternative for water bioremediation contaminated with high levels of nutrients (C, N, P, K), hydrocarbons and heavy metals (Wei-Tung et al., 2013; Blanco-Vieites et al., 2022).

#### 6.5. Biofuels

Technological alternatives have been developed that allow contributing to energy demand and reduction of greenhouse emissions, one of these alternatives is anaerobic digestion through which biogas, from organic matter such as microalgae development through wastewater bioremediation, this microalgae is ability to nutrients removal (carbon, nitrogen, phosphorus, potassium, etc.), the biomass generated from this process is used as organic matter in anaerobic digestion due to its chemical composition (Jacuinde et al., 2022); likewise, it has been reported that the biomass of *S. maxima* produced can be used as organic matter in the anaerobic process, from which methane production yields can be reached from 150 Kg CH<sub>4</sub> Kg<sup>-1</sup> o.d.m. (organic dry mass) up to 240 Kg CH<sub>4</sub> Kg<sup>-1</sup> o.d.m, achieving a degradation of up to 70% of the initial dry organic matter (Ramos-Suárez and Carreras, 2014; Rodríguez et al., 2018).

The biodiesel production through *S. maxima* is a viable alternative for its lipid content (Table 1); however, a disadvantage is the high operating costs for the biomass production versus to the production process from petroleum, due that several nutrients required for its growth are expensive; therefore, it is imperative to optimize the factors that have higher influence on its growth, such as nutrients and sources of carbon, nitrogen, and light, to increase the lipid content in the microalgae structure (Gonzalez Bautista and Laroche, 2021; Rahman et al., 2022). Abdo et al. (2016) evaluated the production of biodiesel from *S. maxima* biomass developed in municipal wastewater and reported a lipid content of 7%, which favored biodiesel and glycerol production with 99.3% and 89% of purity respectively, they concluded that biodiesel

production from *S. maxima* developed in wastewater is viable alternative.

### 7. Enzymes Production

The cyanobacterium *S. maxima* is a source of production of various metabolites of biotechnological interest as intracellular and extracellular enzymes, which allow the cell to carry out different catalytic reactions. These enzymes have great biotechnological relevance due to their application in the pharmaceuticals, food, textiles, fuels, and detergents industries (Brasil et al., 2017a). Enzyme production can be expensive since it may require a recovery process with several stages, which considerably increases their cost (Rodrigues et al., 2017). Likewise, some reports indicate that the industrial production of enzymes from *Spirulina* is significantly lower than that of bacteria and fungi, so an alternative to minimize their production costs is to implement biorefinery systems where they can take advantage of the majority of biomolecules contained in this cyanobacterium (Brasil et al., 2017a). Currently, there is not enough information on the production of enzymes from *S. maxima*, so some types of enzymes that microalgae and cyanobacteria can produce, as well as their possible industrial applications, and the economic viability of their large-scale production are described below.

#### 7.1. Amylases

Amylases are a group of hydrolase-type enzymes that are part of the glycohydrolases that carry out the breakdown of carbohydrates such as starch, oligosaccharides, and polysaccharides; amylases are  $\alpha$  and  $\beta$  amylases, isoamylases, glucoamylase, glucosidases are the most applicable, due to their biocatalytic application, stability, high production, due to the amylases represent up to 30% in the enzyme market (Spier et al., 2020). Several microorganisms can produce this type of enzyme; however, recent research has focused on evaluating the enzyme production capacity for some microalgae, and it indicated that microalgae enzymes may have industrial applications such as bioethanol production, paper, drugs, cosmetics, detergents, and in the food industry (for the saccharification and liquefaction of starch, production of beer, infant cereals, flour, and animal feed) (Demain and Sánchez, 2017); likewise, from these studies, it has been reported that the cultivation method (mixotrophic, autotrophic, phototrophic) influences the production of these enzymes (Vingiani et al., 2019). The production of amylases has not been evaluated for cyanobacteria as in *S. maxima*, so there is little or no information about it, making it a new area of exploration due to the biotechnological relevance of these enzymes.

#### 7.2. Peroxidases

Peroxidases are enzymes that carry out the oxidation of inorganic compounds through peroxide hydrogen, classified as peroxidases of animal and plant origin and used as biosensors for wastewater treatment or in paper production, and can degrade pollutants, pesticides, and



dyes (Spier et al., 2020). The higher activity of microalgae peroxidases is achieved in acidic conditions and is resistant to high temperatures, promoting its catalytic activity (Demain and Sánchez, 2017; Vingiani et al., 2019). Some reports indicate that different microalgae and diatoms have been identified with the capacity to produce peroxidases; however, their function in the metabolic pathway in which they carry out their function has only been studied (Cirulis et al., 2013). Currently, there are no reports on peroxidase production through *Spirulina*, so it is a new field of study due to so it is a new field of study to explore due to their biotechnological relevance.

### 7.3. Proteases

Proteases are a group of enzymes that cause the breaking of peptide bonds in peptides or proteins, and their applications span food, pharmaceutical, and detergent formulation industries (Murphy et al., 2000; Brasil et al., 2017b). These enzymes have a relevant metabolic function in microalgae since they allow for maintaining control in the senescence stage of the organelles in cyanobacteria. The enzymatic activity of proteases increases when algae are in stress conditions due to the limitation of light, nutrients, or apoptosis (Spier et al., 2020). They are classified into endo and exo peptidases depending on the site of action, exopeptidases cut at the C-terminal end (carboxypeptidases) or at the N-terminal end (aminopeptidases) of a substrate and endopeptidases carry out the cuts in the internal of the substrate. Likewise, some reports indicate that the majority of proteases produced by algae require  $\text{Ca}^{+2}$  as a cofactor to carry out their catalytic activity, and the production capacity of these enzymes is mainly related to the availability of the nitrogen source and its nature. Therefore, it is necessary to develop new studies with different nitrogen sources to induce the catalytic activity of these enzymes in microalgae (Brasil et al., 2017b; Vingiani et al., 2019). Yada et al., (2005) obtained a protease from *S. platensis* and reported that the enzyme isolated has an approximate size of 81 kDa and does not require calcium to maintain its catalytic activity and carries out selective lysis reactions of phycobiliproteins. On the other hand, Elleuch et al., (2021) characterized an extracellular protease produced from *Spirulina platensis*, indicating that the enzyme activity increased by the presence of  $\beta$ -mercaptoethanol, 5,5-dithio-bis-(2-nitrobenzoic acid) and its inhibited by metal ions  $\text{Hg}^{2+}$  and  $\text{Zn}^{2+}$ ; they reported an enzymatic activity of 159.79 U mL<sup>-1</sup> and its production can increase by using the Zarrouk growth medium with the addition of 0.625 g L<sup>-1</sup> of NaCl and K<sub>2</sub>HPO<sub>4</sub> respectively at an initial pH of 9.5; finally, they suggest that it may be an enzyme with wide application in the food industry due to its high activity at a neutral pH and low production cost since it is recovered in the residual growth medium after obtaining the biomass.

### 7.4. Lipases

Lipases with enzymes known as triacylglycerol hydrolases carry out the hydrolysis of long-chain triacylglycerols into simpler fatty acids and glycerol. Likewise, catalyzes aminolysis, esterification,

interesterification, and transesterification reactions in a restricted aqueous medium. These enzymes have relevance in the pharmaceuticals, foods, beverages, and detergents industries, for they have higher application and biotechnological importance (Ali et al., 2023). Currently, studies on lipase production and characterization from microalgae are few concerning microorganisms such as bacteria and fungi. However, some studies have found that some lipases from microalgae are similar to known lipases, particularly those produced by fungi (AlFadhly et al., 2022). The extracellular lipases obtained from microalgae have higher resistance to high temperatures (40 to 70 °C) and a wide pH range (5 to 11), from which there is a need to develop new studies that evaluate the effect of growth factors on production and their characterization due to the biotechnological relevance they have in industry (Spier et al., 2020). Demir and Tükel (2010) evaluated the enzymatic capacity of a crude enzyme extract, reporting an enzymatic activity of 0.23 U mL<sup>-1</sup> and a specific enzymatic activity of 0.12 U protein<sup>-1</sup>; they subsequently isolated and characterized a lipase by purifying the crude extract approximately 375 times, reporting a molecular weight of 45 kDa, isoelectric point of 5.9 and a specific enzymatic activity of 45 U mg Protein<sup>-1</sup>.

### 7.5. Phytases

Phytases (myo-inositol-hexakis-phosphate-phosphohydrolase) are enzymes that catalyze the hydrolysis of the anti-nutritional factor phytic acid and its salts, increasing the availability of phosphorus and minerals; this acid is the main form of phosphorus (80%) in seeds, grains, and cereals (Joudaki et al., 2023). This type of enzymes has relevance in animal and human nutrition because they are widely used in the food industry for the production of cereal-based foods, to increase the availability of nutrients that promote health improvement for its consumption. The phytases are in three types depending on the position where they dephosphorylate phytic acid: 3-phytases, 6-phytases, and 5-phytases, and they are in the intracellular fraction of animals, plants, and microorganisms, among which are microalgae (Gessler et al., 2021). Currently, there is little information on algae capable of producing this enzyme, so there is a field of study due to the biotechnological relevance of this enzyme. However, there is not enough available information to demonstrate the production of phytases from cyanobacteria, particularly *Spirulina*, which is a new area of study to understand their capacity and viability for phytase production.

### 7.6. Superoxide dismutase (SOD)

The enzyme superoxide dismutase is a metalloenzyme that can contain the metal ions of iron (Fe SOD), manganese (Mn SOD), nickel (Ni SOD), copper or zinc (Cu/Zn SOD) and iron or zinc (Fe/Zn SOD) which all of these They are considered isoforms of SOD, and they also can convert superoxide radicals into oxygen and hydrogen peroxide, protecting cells and tissues from oxidative damage; In microalgae, this enzyme protects cells against oxidative stress induced by environmental conditions (exposure to

chemical or biological agents, heavy metals and radiation). SOD with catalase and peroxidase enzymes constitute an enzymatic defense group against reactive oxygen species induced by environmental stress (Brasil et al., 2017a; Rosa et al., 2021). This enzyme is present in prokaryotic and eukaryotic cells; however, there is currently little information on SOD activity in microalgae. Some of the applications of SOD are its therapeutic and prophylactic application in humans, preserving biological material such as organs for transplant, and contributing to the preservation of non-perishable foods, among other applications (Cirulis et al., 2013; Spier et al., 2020; Kumar et al., 2022). Gunes et al., (2015) evaluated and compared the activity of SOD in the cyanobacterias *S. nidulans*, *S. platensis*, and *Pseudanabeana sp.*, concerning protein production, reported an enzymatic activity of 8.0 U mL<sup>-1</sup>, 0.27 mg mL<sup>-1</sup> total protein and specific enzymatic activity of 30.0 U mg protein<sup>-1</sup> for *S. platensis*. Similarly, Morelli and Scarano (2004), evaluated the SOD activity by adding Cu to a growth medium for the microalga *Phaeodactylum tricornutum*. They reported that the addition of copper at a concentration of 10 µMol increased SOD activity by up to 40% after 48 h of exposure, which helps reduce the oxidative damage generated by the presence of copper. Odenthal et al. (2024) carried out an extensive bibliographic review in which they mention that the microalga *C. sorokiniana* subjected to conditions of absence of a nitrogen source causes an increase in the enzymatic activity of SOD and CAT; however, they mention that the addition of plant hormones to the growth medium further increased the activity of these enzymes up to 49.76 ± 4.72 and 81.41 ± 7.87 U mg<sup>-1</sup> Protein for CAT and SOD, respectively; likewise, mentioned that when the microalga *Tetrademus dimorphus* subjected to the absence of nitrogen, an increase in the catalytic activity of SOD of up to 3,857.92 ± 1,052.67 U mg<sup>-1</sup> was observed, which on the third day decreased to 1,273.73 ± 155.25 U mg<sup>-1</sup> and the activity recorded for CAT was up to 54.07 ± 2.79 U × 10<sup>3</sup> U mg<sup>-1</sup> on the third day. Ismaiel et al. (2016) evaluated the influence of pH on the enzymatic activity of SOD, CAD, and peroxidase (POD) for *S. platensis* and reported that a growth pH of 7.5 and 11.0 favored an enzymatic activity for SOD up to 11.09 ± 0.45 U mg<sup>-1</sup> Protein; likewise, a growth pH of 7.5 promoted the increase in the enzymatic activity for CAD and POD reaching values of up to 4.11 ± 0.05 U mg<sup>-1</sup> and 30.50 ± 0.05 U mg<sup>-1</sup>, concluding that the pH in the growth medium has a significant effect on the enzymatic activity for these antioxidant enzymes.

### 7.7. Carbonic anhydrase

Carbonic anhydrase is a zinc-requiring metalloenzyme that catalyzes carbonic acid formation from water and carbon dioxide. In photosynthetic microorganisms, its function is the fixation of inorganic carbon when the availability of CO<sub>2</sub> is less than 3% v v<sup>-1</sup>; therefore, when CO<sub>2</sub> concentration increases, the enzymatic activity decreases, and when the availability of CO<sub>2</sub> is little, enzymatic activity increases considerably (Talekar et al., 2022). Various studies have evaluated the catalytic activity of this enzyme in microalgae such as *Chlorella sp.*,

*Chlamydomonas reinhardtii*, *Phaeodactylum tricornutum*, *Pleurochrysis wallei*, *Dunaliella salina* *Dunaliella parva*, and *Chlorella sorokiniana* (Brasil et al., 2017b; Spiera et al., 2020); however, there is little information on the production of this enzyme in cyanobacteria like *Spirulina*, so it is a new field of study to be studied.

### 7.8. Laccases

Laccases are classified as oxidases and contain multiple copper atoms that carry out phenolics and non-phenolic compounds oxidation, which are extracellular monomeric glycoproteins found in plants, insects, liques, and microorganisms. This glycoprotein captures O<sub>2</sub> from the air, which it uses as an electron acceptor to oxidize various compounds and produce water, oxidizing complex polymers into phenolic compounds (Khatami et al., 2022); they are considered green catalysts and could use in the food and paper industry and to degrade xenobiotics, and phenolic compounds, and dyes in industrial effluents (Spiera et al., 2020). Afreen et al. (2017) isolated and characterized a laccase from *S. platensis*, said the enzyme had a size of 66 kDa, optimal temperature between 3 °C to 50 °C, and maintained its stability and activity up to alkaline pH values. (up to 8.0); also report that said enzyme can degrade up to 96% of the anthraquinone blue dye; however, there are no reports on its catalytic capacity. Subsequently, Ellatif et al. (2021) evaluated textile dye degradation capacity using different microalgae including *S. platensis*, and quantified the enzymatic activity for laccases and lignin peroxidase; they found that this alga can degrade up to 91% brazilwood dye, 87% crystal violet dye, 66.1% malachite green dye, 50% naphthol green B dye, and 36% orange G dye, all at concentration of 0.2 g L<sup>-1</sup> of dye; likewise, they reported an enzymatic activity for laccases and lignin peroxidases of 580 U mL<sup>-1</sup> and 250 U mL<sup>-1</sup> for brazilwood dye, 550 U mL<sup>-1</sup> and 290 U mL<sup>-1</sup> for crystal violet dye, 370 U mL<sup>-1</sup> and 250 U mL<sup>-1</sup> for malachite green, 340 U mL<sup>-1</sup> and 210 U mL<sup>-1</sup> for naphthol green B and 200 and 270 for organic G dye respectively for each enzyme; this demonstrates that *S. platensis* can produce enzymes for the degradation of dyes and its possible use on an industrial scale. Finally, Sami et al. (2024) evaluated the estrone degradation capacity using the laccase enzyme from the *Spirulina CPCC-695* strain. Initially, the extract was partially purified in four stages with a purity of around 45% and reported a specific activity of 204.14 U mg<sup>-1</sup> Protein, with a weight of 80 kDa; they also indicate that the maximum enzymatic activity is at a pH of 3.0 and a temperature of 40 °C; finally, they mention that for this enzyme the kinetic constants (K<sub>m</sub>, V<sub>max</sub>) were 0.58 mM ABTS<sup>•</sup> and 480.54 mM ABTS<sup>•</sup> min<sup>-1</sup>, reaching an estrone degradation efficiency of around 91%, which is considered an alternative for the bioremediation of estrone.

### 7.9. L-asparaginase

L-asparaginase is an intracellular enzyme produced by plants, mammals, and microorganisms (bacteria, fungi, and algae) that carries out the L-asparagine hydrolysis into L-aspartic acid and ammonia, from which it has application in the food industry to prevent the formation of acrylamide when foods are processed at high temperatures and in the

pharmaceutical industry to treat diseases such as cancer and leukemia (Castro et al., 2021). Different researches indicate that microalgae are a viable alternative for L-asparaginase production because it is considered a safe source of production of this enzyme. Several studies on L-asparaginase report that *S. platensis* induced by a stress condition (high nitrogen, and salinity level, and addition of  $\text{FeSO}_4(7\text{H}_2\text{O})$  increases its production of L-asparaginase up to 0.275 U, with a specific enzymatic activity of 0.166 U  $\text{mL}^{-1}$  in cytoplasmic extracts (Brasil et al., 2017b; Spiera et al., 2020). El Baky and El Baroty (2016) produced, purified, and characterized L-asparaginase from the cyanobacterium *S. maxima* at concentration of 5 g  $\text{L}^{-1}$   $\text{NaNO}_3$  in the growth medium increases the enzymatic activity of L-asparaginase up to 898 IU and specific enzymatic activity of 2.21 IU  $\text{mg protein}^{-1}$ ; likewise, they report that the temperature and pH of maximum activity were 37 °C and 8.5 respectively. Microalgae are a viable alternative due to their capacity to produce enzymes with biotechnological applications; however, this area has been little explored compared to other microorganisms (El-Baky and El-Baroty, 2016). Specifically, *S. maxima* cyanobacterium is a potential enzyme production source that may be of biotechnological interest, so it is necessary to develop research to evaluate the production capacity of the different types of enzymes, physicochemical characterization, and establish the conditions that promote its production, so there is a study field to explore for this cyanobacterium.

#### 7.10. Catechol oxygenase

Catechol oxygenase enzymes participate in the degradation of halogenated aromatic compounds, which gives them a relevant role in the bioremediation of these contaminants. These enzymes contain iron in the active site, necessary to carry out the enzymatic activity, and the intradiol enzymes contain Fe III, and the extradiol enzymes contain Fe II. The oxidation state of iron each one different in the selectivity of the catechol groups (Setlhare et al., 2018). From that Tomar and Jajoo (2021), evaluated the potential of the microalga *Chlorella vulgaris* for the bioremediation of fluoranthene (FLT), and reported that from an optical density  $\text{OD}_{680}=0.10$  reduced up to 58% of FLT in 72 h and up to 97% in 168 h, they determined that the enzyme that carried out said degradation was catechol 2,3 dioxygenase which increased its content up to 2 times in the culture that contained 5  $\mu\text{M}$  of FLT and up to 2.4 times at a concentration of 25  $\mu\text{M}$  of FLT, so it could be a biotechnological application for the bioremediation of waters with the presence of FLT, it is worth mentioning that the authors mention that there are no reports on other microalgae that carry out such degradation, so it is a study gap that can contribute to environmental biotechnological implementation for other strains of microalgae particularly on *S. maxima*.

#### 7.11. Desaturases and elongases

Desaturases are enzymes capable of carrying out fatty acid dehydrogenation reactions, converting a single bond between two carbons (C-C) into a double bond (C=C) in the acyl of a fatty acid stereospecifically (Cerone and

Smith, 2022). Elongases are enzymes that carry out the elongation of long-chain fatty acids higher than 20 carbons; they are essential in several cellular processes as lipid metabolism and membrane structure (Wang et al., 2023). Some microalgae contain this type of enzyme to carry out the production of long-chain fatty acids, such as DHA or its intermediates. In particular, Pereira et al. (2004) used the microalgae *Pavlova* and *Isochrysis*, which produce high amounts of  $\omega$ 3-PUFA (polyunsaturated fatty acids), EPA (eicosapentaenoic acid, 20:5 n-3) and DHA (docosahexaenoic acid, 22:6 n-3), due to this they carried out the identification of the genes involved in the conversion of EPA to DHA. They reported the identification of a gene called *pavELO* that encodes an elongase enzyme in *Pavlova* carries out the catalytic reaction of EPA to  $\omega$ 3-DPA, said enzyme had high specificity to n-6 and n-3  $\text{C}_{20}$ -PUFA substrates; they identified a gene called *IgD4* in *Isochrysis* of a desaturase that converts  $\omega$ 3-DPA into DHA; finally, when carrying out a co-expression study in yeast of the *pavELO* and *IgD40* genes, reported that both genes are capable of functioning together to convert EPA into DHA. Currently, there are no reports on identifying this type of enzyme in *Spirulina* strains, which is a new study field due to the biotechnological relevance that this type of enzyme could have.

#### 7.12. Oxidoreductases

Oxidoreductase enzymes are biological catalysts that carry out redox reactions since they exchange electrons between donor and acceptor molecules; this type of enzyme catalyze a wide variety of chemical reactions and is classified into oxidases, dehydrogenases, hydroxylases, oxygenases, peroxidases, and reductases. Most of these types of enzymes depend on the nicotinamide cofactor (NAD and NADPH) and are characterized by having several redox centers to carry out catalytic reactions; currently, due to their high specificity, efficiency, and biodegradability, they are used in the textile, medical, food and chemical synthesis industries (Younus, 2019). A recent study by Dagsuyu and Yanardag (2023) purified and characterized the enzyme thioredoxin reductase from commercial *S. platensis* tablets, found that after a 415-step purification process of the crude extract obtained a yield of 19% and quantified a specific activity of up to 0.764 U  $\text{mg}^{-1}$  Protein, also determined that the ions  $\text{Se}^{4+}$ , sodium cefazolin, teicoplanin, and tobramycin increase enzymatic activity; and ions such as  $\text{Ag}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ni}^{2+}$ , some vitamins ( $\text{B}_3$ ,  $\text{B}_6$ , C and, U) and medications (metformin, diclofenac, acyclovir) have inhibitory effects on the enzyme; later, in another study in which carried out a two-stage purification process by affinity chromatography, achieved a yield of 9.7% and a specific activity of 5.77 U  $\text{mg}^{-1}$  Protein, they also determined that the molecular weight of the subunit was 45 kDa and indicated that a pH of 7 and a temperature of 40 °C are the optimal parameters for higher enzymatic activity and determined that the values of the Michaelis constant ( $K_m$ ) and maximum velocity ( $V_{\text{max}}$ ) for NADPH were 5  $\mu\text{M}$  and 0.0033 u  $\text{mL}^{-1}$  respectively (Dagsuyu and Yanardag, 2024).



The use of *Spirulina* has several applications in various areas, attributed to the effects of its components due to its consumption or application; however, despite this, news researchers are currently continuing with the aim of increasing the biomass production and its metabolites based on the search for environmental and nutritional conditions, together with the implementation of improvements in bioreactors and its instrumentation; however, research must develop where that secondary intra and extracellular components as enzymes of biotechnological relevance are quantified, recovered and used to have an integral use of this cyanobacterium.

## 8. Economic Viability of a *S. maxima* Biorefinery

*S. maxima* is one of the most biotechnologically relevant microalgae due to its content of proteins, lipids, antioxidants, vitamins, and minerals, has higher economic and commercial importance attributed to the diversity of applications in different industries; due to this, *S. maxima* covers approximately 30% of the microalgae biomass produced worldwide (Kern et al., 2017); however, for its production on an industrial scale from refineries where the main objective is the production of biomass to obtain various bioproducts of economic and commercial interest, these being an alternative to have better use of the microalgae fractions considering aspects considering environmental, economic and social points (Posada et al., 2016; Brasil et al., 2017a). In industrial-scale biorefineries, aspects must be considered to maintain an integral process for biomass production (cultivation and harvesting) and subsequent stages for the specific bioproducts production; the implementation of an integrated process allows the resource optimization as water, nutritional, and energy; therefore, from this, we can reach a more sustainable production process. The efficient use and exploitation of water as an essential resource in biorefineries is of great importance, as well as obtaining macro and micronutrients from waste sources that contribute to supplying the majority of nutrients required for the development of microalgae. It will contribute to maintaining a sustainable process by minimizing the environmental effects and reducing costs for the acquisition and nutrient supply required for biomass development (Xin et al., 2016; Jacuinde et al., 2022).

One aspect to consider for biomass obtaining is the culture medium and appropriate growth conditions for microalgae growth (pH, temperature, aeration, luminosity), so it is necessary to study technological alternatives that maintain the operation of the microalgae, the facilities at the lowest operating and maintenance costs. Building a bioreactor for growing and harvesting biomass can result in a high initial cost; however, if designed correctly and requires little maintenance, costs will decrease at this stage (Costa et al., 2019). Biomass harvesting is a critical stage because it can represent up to 30% of the cost of the process. The stage objective is to eliminate the higher amount of water until obtaining biomass with a weight content between 10% and 25% of solids. Among the most used methods for harvesting, they include mechanical

processes (sedimentation, filtration, centrifugation, and flotation), chemical (coagulation and flocculation), and biological processes (auto and bio flocculation). In the harvesting stage, it is necessary to consider the microalgae characteristics (size and cell density) to maintain the biomass quality for the desired product (Jesus et al., 2018; Costa et al., 2019).

The biorefinery implementation for *S. maxima* has different expenses, among which the acquisition of land and the costs for construction of the plant stand out; for the production of biomass, the culture medium (nutrients), process control, maintenance and consumption of water and electrical energy; for the cultivation stage, must be considered the process equipment, water and electricity consumption, operating personnel, maintenance, gas consumption, and quality laboratory supplies costs; cost reduction is achieved through the correct process equipment maintenance and the technologies implementation for efficient use of energy and water, in addition to the implementation of technological alternatives that enable the electrical energy production (Posada et al., 2016; Costa et al., 2019). The biorefinery economic viability for *S. maxima* microalgae can be viable through the appropriate design of the plant stages, particularly considering the reduction of operating costs and the optimization and automation of the process stages.

## 9. Conclusions

*S. maxima* is a microalga used worldwide for its adaptability, accelerated growth, chemical composition, and intracellular and extracellular metabolites. Based on this, several investigations focus on obtaining the higher biomass production and metabolites of biotechnological interest in food, cosmetic, pharmacological, and energetic industries. Likewise, for a microalgae biorefinery to be economically viable, must be considered as an integral process between the biomass production and metabolites purification of biotechnological interest without compromising the process efficiency and the economy. The enzyme production from *S. maxima* is a little-studied area in which some reports indicate that this microalga can produce some enzymes with biotechnological interest; however, there are some types of enzymes about which there are no reports, so it is necessary to know, evaluate and optimize the growth factors that induce the other enzyme types production from *S. maxima* because the microalgae enzymes have higher chemical stability and thermal resistance. Due to this, there is a large study field on the extracellular enzyme production capacity of *S. maxima*; the work team is evaluating the growth factors' effect on *S. maxima* on extracellular enzymatic activity.

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