Chapter

Microalgae Cultivation for Secondary Metabolite Production

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Abstract

Microalgae including cyanobacteria have been recognized as an excellent source of fine chemicals, renewable fuels, vitamins, and proteins and usually are found in health food stores around the world. However, the accumulation of these compounds generally occurs at end of the exponential growth phase; furthermore, biomass density in cultivation commonly is low. Open cultures have been used for pigment, biofuels, and biomass production, but these types of culture system are not a good choice for the production of fine chemicals, due to contamination problems and the expensive production costs. Closed photobioreactors can be operated in a continuous cultivation providing an increase on biomass density and contamination-free condition and generally working at a maximum growth rate under specific conditions; besides, these systems can recycle the consumed culture medium at least three times before a new enriched medium is supplied, generating a more cost-effective production system. In addition, microalgae metabolism can be manipulated to provoke a specific secondary metabolite accumulation by the addition of organic carbon source or changing light intensity or both. In other words, photobioreactors can operate in continuous mode, with efficient light supply and the supplementation of organic carbon source to produce fine biochemicals such as anticancer, antibacterial, antioxidant, lectins, antiviral compounds, and biofuels.

Keywords: microalgae metabolism, mixotrophy, continuous cultivation, secondary metabolites, fine chemicals, biofuels, pigments

1. Introduction

Industrial reactors for microalgae cultivation have been generally constructed using channels with movement and adapted for a better gas exchange. One of the biggest problems in this culture system is the low density of microalgae cells; they are constructed between 15 and 30 cm deep along the canal, limiting therefore the available light in addition to increasing the potential for contamination. A system proposed to solve the problem of low density and pollution has been found in closed polyethylene pipe systems, having the geometric design of the reactor as its main objective. Some strategies addressing three aspects have been developed to improve cultivation of microalgae in photobioreactors and produce fine chemicals:

(1) the culture medium design—it is necessary to fix the nutrient composition to provide the right source of carbon and energy depending on the microalgae strain and secondary metabolite to be produced; (2) reducing adverse conditions for culture, such as oxygen accumulation, CO_2 efficient supply, and sufficient light distribution. For this purpose, studies on the photobioreactor prototype should be performed; (3) once that photobioreactor prototype works well, critical factor criteria for scale-up bioengineering process should be fixed [1–3].

2. Microalgae metabolism and mixotrophic growth kinetics

Biomass and product productivity are significantly affected by the culture condition; energy and carbon supply impacts directly biomass and product concentration. In effect, different metabolic growth modes for microalgae have been recognized: (a) autotrophy, in which light is the sole source of energy and inorganic carbon is the sole source of carbon; (b) heterotrophy, in which energy and carbon are both obtained exclusively from an organic carbon source, such as glucose, glycerol, and acetate, and growth can proceed without light supply; (c) mixotrophy, in which the photosynthetic microorganisms obtain energy from light and organic carbon sources and carbon is obtained from organic and inorganic carbon sources [4, 5]; and (d) photoheterotrophy, in which carbon can be obtained from organic compound but strictly with a light supply [5]. Chojnacka and Noworyta designed an empirical mathematical model to describe mixotrophic growth; in this model heterotrophic and autotrophic cultures are fractions of mixotrophic growth, but the metabolic interaction of photosynthesis and heterotrophy is important to improve biomass density and consequently secondary metabolite productivity [6].

Light as a source of energy for photosynthetic organisms is the main limiting factor during cultivation process of these organisms. In light intensities above the light saturation point, photosynthesis rate is directly proportional to the incident light supplied. The photosynthetic system of many microalgae becomes saturated to a radiation close to 30% of the total solar irradiance, i.e., between 1700 and 2000 $\mu Em^{-2}\ s^{-1}$. Some species of phytoplankton grow to optimal intensities of 50 $\mu Em^{-2}\ s^{-1}$ and are photoinhibited at around 130 $\mu Em^{-2}\ s^{-1}$. The culture limitation by photoinhibition is the most important problem for commercial cultivation of microalgae. A possible solution is to assume that the heterotrophic metabolism in photosynthetic cells occurs, replacing or supplementing energy and carbon requirements from organic sources. Some studies suggest that mixotrophic, autotrophic, and heterotrophic metabolic activities occur simultaneously during cell growth [7]. The relative contribution of autotrophy to biomass production increases by increasing the light supply coefficient (kJ kg $m^2\ s^{-1}$) or with an increase in the supply of CO_2 and a decrease of organic carbon source supply. For example, at a light supply coefficient of 0.5 at 0.03 and 10% of CO_2 concentration, the ratio of contribution of autotrophy (heterotrophy/autotrophy) to the biomass production was of 98:2 and 70:30, respectively [8]. A respirometric procedure has been proposed to obtain half saturation constant values for several nutrients; it is useful for modeling bioprocess for photosynthetic microorganisms [9]. These methods can be useful to evaluate organic substrates to be used in cultures, in a practical way.

2.1 Contribution of autotrophic and heterotrophic metabolisms during the mixotrophic cultivation performance

Prior works [8, 10] conducted a detailed analysis of the heterotrophic and autotrophic modes simultaneously in *Euglena gracilis* and *Spirulina platensis*,

respectively, under strict control of culture conditions; mixotrophic biomass concentration and growth rates resulted in the sum of the biomass or growth rates of heterotrophic and autotrophic cells in growing culture in parallel. The yields produced approximately the same amount of biomass produced in mixotrophic conditions; a mathematical approach can be summarized in Eqs. (1)–(8) [8, 10]. Assuming that mixotrophic growth is derived from cells growing in autotrophic and heterotrophic conditions, it can be described mathematically by defining the contribution of both metabolic growth modes. This can be simplified in Eq. (1):

$$X_M = X_A + X_H \tag{1}$$

Supposing that α is the heterotrophic fraction in mixotrophic growth, X_H (biomass from heterotrophy) may be expressed as follows:

$$X_H = \alpha X_M \text{ with the condition } 0 < \alpha < 1 \tag{2}$$

Combining Eqs. (1) and (2), biomass in mixotrophic (X_M) conditions can be expressed as follows:

$$X_M = \frac{X_A}{1-\alpha} \tag{3}$$

in which X_A is the biomass from autotrophy; the growth rate ratio can be expressed as follows:

$$1 \approx \left[\frac{dX_M/dt}{dX_A/dt + dX_H/dt}\right] \left(g\, d^{-1}\right) \tag{4}$$

The α coefficient is low when the incident light reaches all cells in the photobioreactor, where cell density should be low enough to avoid hiding among cells. Once that cell density increases, heterotrophic fraction increases as well, in the presence of an organic substrate as carbon source. To estimate α at any time, the value of α can be represented by an α_i which can be constant for a period of time, Δt_i.

Then, Eq. (4) can be expressed as follows:

$$\frac{\Delta X_M}{\Delta t_i} = \frac{1}{1-\alpha_i}\frac{\Delta X_A}{\Delta t_i} \tag{5}$$

By replacing in Eq. (5) of autotrophic growth, the equation included incident light:

$$\frac{\Delta X_M}{\Delta t_i} = \frac{1}{1-\alpha_i} K l_0 \tag{6}$$

where $K = \frac{Y_{kJ} A}{V}$.

Integrating Eq. (6) results in

$$\int_{X_{Mi}}^{X_{Mi+1}} \Delta X_M = \frac{K l_o}{1-\alpha_i} \int_{t_i}^{t_{i+1}} \Delta t_i \tag{7}$$

And then, α_i at any time can be calculated:

$$\alpha_i = 1 - \frac{K l_o}{X_{M_{i+1}} - X_{M_i}} (t_{i+1} - t_i) \tag{8}$$

With Eq. (8), α value was 0.02 at the beginning of culture, independent of incident light, to 0.61 after several hours of cultivation. Carbon balance showed inorganic carbon and carbon from glucose were consumed simultaneously; CO_2 produced from respiration was used as carbon source during autotrophic growth [11]. Ogbonna and McHenry observed similar behavior in *Euglena gracilis*. Heterotrophic and autotrophic growth occurred simultaneously and independently [8]; this work defined two fractions instead of one as follows:

$$\frac{dX_M}{dt} = \frac{dX_A}{dt} + \frac{dX_H}{dt} \tag{9}$$

The mixotrophic growth rate dX_M/dt was equal to the sum of the heterotrophic growth rate dX_H/dt and the rate of autotrophic growth dX_A/dt. However, when two metabolic activities interact and when the presence of an organic carbon source affects the autotrophic metabolism or when the light affects the heterotrophic metabolic activity, then the heterotrophic rate in the mathematical description can be modified in accordance with Eq. (10), where dX_M/dt is the total mixotrophic growth rate and β and α are coefficients of the autotrophic and heterotrophic fractions of the total mixotrophic growth rate, respectively:

$$\frac{dX_M}{dt} = \beta\frac{dX_A}{dt} + \alpha\frac{dX_H}{dt} \tag{10}$$

The values of α and β can be calculated on the basis of dX_A/dt autotrophic growth rate and dX_H/dt heterotrophic growth rate, both during mixotrophic culture.

$$\beta = \frac{dX_A/dt}{dX_M/dt} \tag{11}$$

$$\alpha = \frac{dX_H/dt}{dX_M/dt} \tag{12}$$

The sum of the values of β and α is 1.0 when the growth proceeds independently and simultaneously; when the sum is more than 1.0, there is an effect of promotion; and when it is <1.0, there is an inhibitory effect. It is clear that when mixotrophic growth occurs, both growth modes, namely, autotrophic and heterotrophic growth, have contribution in the final biomass and metabolite production; Eqs. (1)–(12) may be used to describe mathematically the secondary metabolite formation. An important stoichiometric relationship exists on carbon metabolism, with the pH changes driven by consumption of carbon source. Bicarbonate consumption increases pH, whereas glucose consumption decreases the pH due to CO_2 production, being the reason that pH is kept almost constant during mixotrophic growth, CO_2 consumption by photosynthesis, this balance may reflect the type of predominant metabolism.

3. Photobioreactor systems: open and closed systems

Despite certain variability in the shape of open and closed systems, technical designs for open systems are the type race track, moved by paddles, usually operating at depths of 15–20 cm. At this depth, the growth rate of microalgae can be 15 g m^{-2} d^{-1}, with a lipid content of 25%. Similar designs in terms of operation are the circular ponds, which are commonly found in Asia and Ukraine [3]. The major

disadvantages of open systems are the significant loss of water by evaporation, the loss of CO_2 into the atmosphere, the pollution, and the need for considerable surface for cultivation. Since the 1990s, in certain parameters such as the selection of species with efficient incident light utilization, the path of the incident light through the photobioreactor (PBR), the thickness of the wall, the mixing regime, and release of O_2 via degassing, CO_2 supply, have been focus on several developments [12]. Closed or semi-closed PBRs, based on different design concepts, have been implemented and tested at a pilot level. The latest developments seem to be directed toward tubular or plate-type compact configurations as well as combinations of these major designs in the form of distributing light over an expanded surface [13].

4. Main problems in closed photobioreactors: light supply, temperature, and oxygen accumulation

Microalgae need enough quality and quantity of light supply, and it should be taken into account as a primary critic factor to design proper PBR. Cell density can increase from 10^3 cells ml^{-1} to densities above 10^8 cells ml^{-1}; it produces a reduction of the distance among cells over 250 times, and the cell size can reduce its size 10 times as well. By improving mix capabilities of the PBR, hydrodynamic shearing stress over the cells can be increased; also, it can reduce growth or even cell death at high stress conditions [14]. The temperature has a greater influence on respiration and photorespiration than photosynthesis; when CO_2 or light is limiting for photosynthesis, the influence of temperature is negligible. In contrast, an increase in the temperature will increase significantly the respiration, but flow of carbon through the Calvin cycle increases marginally. In other words, the net efficiency of photosynthesis declines at high temperatures. This effect can worsen in culture suspension by the difference in the solubility of CO_2 and O_2 at high temperatures. Normal temperatures for the growth of microalgae ranged between 25 and 30°C; an increment in the temperature affects the lipid production; at higher temperatures saturated free fatty acids are produced, while low temperatures favor unsaturated free fatty acid formation [15]. High concentration of O_2 can build up in closed PBR; if this happens photosynthesis can be damaged by decreasing microalgae growth, and an improvement in the PBR should be implemented as an effective gas exchange [16].

5. Photobioreactor design and scale-up

The first generation of closed PBR finds limitations over 50–100 L of culture volume; this was not effective for light supply to produce higher biomass density. Several designs of light distribution over the PBR, mainly underwater lamps, optical fiber, and column-shaped photobioreactors, have been used to provide an efficient production system; however, not much success has been obtained [12]. This is the main challenge in the future to find the appropriate scaling criteria for a larger irradiate surface, mass transfer, and coupled steps upstream and downstream processes [17]. The difficulty to scale up PBRs is to establish the inherent relationship among physical parameters involved in the design and the physiology of the microalgae to be cultured. An important design rule is to define quantitatively parameters to describe the interactions between incident light, the light distribution in the PBR, cell growth, and secondary metabolite production.

To encourage the use of microalgae, it is necessary to implement a step-by-step system at different levels. The first step is the bioprospecting for selecting the most

promising strain to produce a specific secondary metabolite and is the interaction of various disciplines, such as the analytical chemistry, biochemistry, molecular biology, and microbiology. The second step is the development of the culture medium, applicable to the largest volume. The third step is the strategy to analyze the scaling-up; biochemical or bioprocess engineers play an important role at this point. Strain and medium selection is carried out at flask level; the type of metabolism for the desired metabolite production, namely, mixotrophic, heterotrophic, or autotrophic growth, is also defined in this step. Operation parameters are fixed at small PBR scale; once the critic factors are overcome, PBR is ready to apply a scale-up procedure, from pilot to industrial production [18]. At the same time, recovery and purification steps should be performed. The last step of scale-up process should be a feasibility economic and technological analysis, in which production costs are obtained [19]. Quinn et al. constructed and validated a scalable growth model with species-specific variables, such as light and temperature; it can be used with PBR dimensions to accurate growth modeling for life cycle analysis.

6. Energy efficiency received by microalgae in photobioreactors

Many aspects should be considered to obtain high concentration of biomass and secondary metabolites. Microalgae need energy from light to drive photosynthesis and growth. However, many of these organisms are able to use organic compounds as a source of chemical energy from respiratory mechanism. Although the terms of mixotrophy, autotrophy, photoheterotrophy, and heterotrophy are not well-defined, the influence of organic carbon energy and incident light energy can be quantitatively described in terms of biomass and secondary metabolite production.

Assuming that autotrophy growth occurred in cells absorbing incident light on the irradiate surface of the reactor, growth depends on the specific energy yield (Y_{kJA}), in other words, the amount of energy required to produce an amount of biomass; it can be defined by the following equation for continuous cultures:

$$Y_{kJA} = \frac{DX_A V}{I_O A} \tag{13}$$

Moreover, microalgae growing in mixotrophic mode and energy from carbon source can be included in Eq. (13), as follows:

$$Y_{kJM} = \frac{DX_M V}{I_o A + \Delta H_S D V (S_o - S)} \tag{14}$$

Yield equations can be achieved in continuous cultivation, where D is the dilution rate (h^{-1}). Energy efficiency in batch and continuous cultures for *Spirulina* was calculated in values of 5.0×10^{-3} g biomass kJ^{-1} [11] and 2.4–4.8×10^{-3} g biomass kJ^{-1} [20]. There are two different points of view concerning energy efficiency which should be mentioned: one recently, a photocolor spirometer has been used for direct measurements of photosynthesis (calorimetry) and oxygen evolution at different light intensities [21], and the other one uses a photobioreactor to measure the overall light and carbon energy necessary to produce biomass and secondary metabolites [5]. The first can be useful to provide a potential energetic yield measurement because it considers the metabolic energy flows in the cells; the second provides data necessary for bioengineering purposes, specifically for the photobioreactor design and scaling-up procedures to follow.

Mixotrophic growth can be described according to **Figure 1**. On the left-hand side, in the photosynthetic growth by the consumption of CO_2 from culture medium, in the presence of light, biomass is produced, and oxygen is produced as well. On the other side, biomass is also produced but from organic carbon source consumption. The photosynthesis and respiration rates depend on several factors, such as microalgae species, O_2 and CO_2 availability, light supply, organic carbon source availability, temperature, pH, etc., but the main factor is the ability of microalgae to use O_2 and CO_2 at the same time [5].

The balance for energetic yield ($Y_{X/kJ}$) can be described as follows: (i) autotrophic growth; total energy comes from incident light, and the biomass is formed from inorganic carbon:

$$\Delta ATP_T = \Delta ATP_{hv} \tag{15}$$

$$\frac{\Delta X_A}{kJ_{hv}} \tag{16}$$

Heterotrophic growth, energy, and biomass are provided by an organic carbon source:

$$\Delta ATP_T = \Delta ATP_{Glu} \tag{17}$$

$$\frac{\Delta X_H}{kJ_{Glu}} \tag{18}$$

Then, in mixotrophic growth, energy is supplied by both incident light and chemical energy from the organic carbon source and biomass from both inorganic and organic carbon sources.

$$\Delta ATP_T = \Delta ATP_{hv} + \Delta ATP_{Glu} \tag{19}$$

$$Y_{M_{kJ}} = \frac{\Delta X_M}{kJ_{hv} + kJ_{Glu}} \tag{20}$$

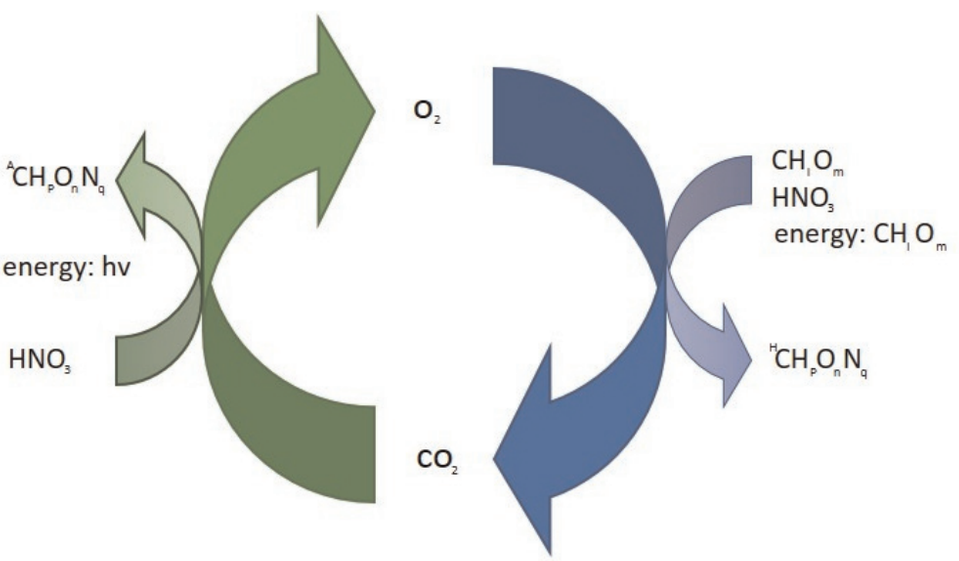

Figure 1.
Drawing describing the interaction of heterotrophy and autotrophy during mixotrophic growth. A, biomass from autotrophy; H, biomass from heterotrophy, modified from [5].

7. Secondary metabolite production

In the mixotrophic growth mode, certain molecules are accumulated, and there is a need to elucidate which metabolite is able to accumulate under specific growth conditions, but in general mixotrophic growth, it seems to be an efficient way for secondary metabolite accumulation [15, 22].

7.1 Fine chemicals

Several high valuable products have been described to be produced by photosynthetic microorganisms: antitumor agent from *Amphidinium* sp., food supplements from *Dunaliella* and *Isochrysis galbana*, antioxidants from *Phaeodactylum tricornutum*, and elastase inhibitor from *Oscillatoria agardhii* [17]. Algae biomass can accumulate or produce (i) bioenergy-based products, such as ethanol, methanol, biodiesel, biohydrogen, biogas, and long-chain hydrocarbons; (ii) staple food and vitamins such as yellow-white proteins, β-carotene, and phycobiliproteins, such as phycocyanin; (iii) polyunsaturated fatty acids, such as linolenic acid and arachidonic acid, that is, omega-3 fatty acids [23, 24]; (iv) base compounds for cosmetic industry and plant growth regulators; and (v) compounds with anticancer, antimicrobial, and antiviral activities.

Spirulina platensis showed higher antioxidant activity than other microalgae tested; *Nostoc muscorum* and *Oscillatoria* sp., moreover, have an important increment of phycobiliproteins by increasing nitrogen to the culture medium. It produces an important increment of the antioxidant activity in aqueous extracts of these microalgae. These extracts exhibit anticancer activity as well; in the extracts phenolic compounds, terpenoids, and alkaloids have been detected which can be responsible for several biomedical activities [24]. Water extracts of *S. platensis* have shown vulvovaginal antifungal activity on *Candida* and antifungal activity on several strains of *Candida* sp.; this can be the basis for therapeutic treatments, where secondary effects seem to be absent [25].

7.2 Pigments

Dietary supplements have been produced from biomass of microalgae; they include pigments and colorants from *Haematococcus pluvialis*, *Chlorella* sp., *Dunaliella*, red algae, cyanobacteria, and *S. platensis* [23]. A profile of natural pigments in dietary supplements of *Spirulina* including 51 pigments has been found in commercial products [26]. Pre-column reaction with DPPH radical followed by fast UHPLC-PDA separation revealed different classes of pigments grouped among carotenes, xanthophylls, and chlorophylls. Diadinoxanthin, alloxanthin, cantha-xanthin, diatoxanthin, zeaxanthin, and echinenone were found in powder and tablets as minor components, in addition to β-carotene as a major component of dietary supplements [26]. Astaxanthin from *H. pluvialis*, c-phycocyanin from *Limnothrix* sp., and phycoerythrin from *Phormidium* have been produced [27–29], respectively.

Production of pigments is affected by the amount of light supplied, and in combination with mixotrophic growth mode, phycocyanin, chlorophyll-a, and carotenoid concentrations, increased as light intensity increased, the concentration increased at least 30% in *S. platensis* [4, 11]. Production of chlorophylls and carotenoids increases 1.5 fold in *Chlorella vulgaris* in stirred tank photobioreactor [4]. Carotenoid accumulation and composition seem to be induced by light intensity, nitrogen starving, and salt stress. Higher light and salt stresses active synergistically

carotenogenesis, mechanism such as esterification of astaxanthin and adonixanthin in *Scenedesmus* sp. [30].

Red marine microalga has proven their ability to produce pigments and hydrocolloids, due to their diversity, and perhaps produce a diversity of high valuable compounds. Fine chemicals are used as cosmetics, nutraceutics, and therapeutic agents; some are used in the food industry, diagnostic, biomedical research, and biosensor. Carbon source such as glucose, sucrose, glycerol, or acetate in the culture medium can help for accumulation of β-carotene and zeaxanthin by red microalga [31].

7.3 Antioxidants

Microalgae produce a wide range of antioxidants, some of them involved in the scavenging machinery of photosynthesis, respiration, and oxidative protection mechanisms. Pigments (carotenoids, chlorophylls, phycobiliproteins) play an important role in the photosynthetic mechanism in tocopherols including α, δ, and γ tocopherols, and pigments are accumulated as secondary metabolites at different amounts. Tocopherols have been found in *Nannochloropsis oculata*, *Tetraselmis suecica*, *Spirulina maxima*, *Chaetoceros* sp., *Synechococcus*, and *Porphyridium cruentum*. These compounds are formed depending several factors, such as microalga specie, growth phase, nutrient availability, light supply, and oxygen concentration, but also their production is affected by the processes of extraction and purification [32–34].

7.4 Biofuels

Lipid metabolism can be induced by a nitrogen-limiting condition; nitrogen obtained from amino acid catabolism is assimilated via the glutamate-glutamine pathway; then, it is stored as an amino acid. The excess of carbon obtained from photosynthesis or glycolysis is redistributed into carbon-containing compounds. Carbon enters lipid metabolism via gamma-aminobutyrate pathway, glycolysis, and the tricarboxylic acid cycle [35]; malonyl-CoA is formed via acetyl-CoA from respiration; then, lipogenesis proceeds [15]. Supplementing microalgae cultures with an organic carbon source increases the productivity of biomass, lipid, and carbohydrates, enhancing the production of biodiesel, ethanol, starch, and polyunsaturated fatty acids. However, organic carbon source addition has limitations, for example, the cost and the bacterial contamination during cultivation. Progress on biorefineries has been focused on mixotrophic cultivation to enhance either secondary metabolite accumulation or fine chemicals [36]. Triacylglycerol content in *Neochloris oleoabundans*, *Dunaliella* sp., and *Botryococcus braunii* is more abundant when glycerol was used as organic carbon source than with autotrophic cultures. Profile of free fatty acids is also different. Saturated free fatty acids increase significantly in the presence of glycerol, but unsaturated free fatty acids decrease in general [37]. Biomass productivity and also the lipid productivity increased with the addition of acetate, glucose, and glycerol; although lipid content is smaller than other cultures, light supply also affected the content of lipids [38]. In contrast, lipid concentration in *Chlorella protothecoides* was as high as 55%, four times those obtained in autotrophic growing cells. Microalgae metabolic pathways for lipid accumulation are influenced by nitrogen-limiting conditions and carbon metabolism, where distribution pathways contribute to lipid biosynthesis [39, 40]. Biomass is considered as a renewable fuel source and does not affect the overall balance of CO_2 in the atmosphere. Algal biofuel production coupled to a biomass power plant waste can serve as a cost-effective process to enhance microalgae biomass and

biofuel productivity by sequestration of the CO_2 produced in the power plant [35]. Productivity of biodiesel from oily plant crops, in terms of produced oil by surface production, varies from 27.57 to 972 L per ha, whereas that from microalgae cultivation is 7688–23,067 L per ha [23].

Biofuels derived from algal biomass depend on algal species: for biodiesel, *Cladophora fracta*, *C. prototheco*ides, and *B. braunii*; for biohydrogen, *C. prototheco*ides, *S. platensis*, and *Chlamydomonas reinhardtii*; for bioethanol *Palmaria*, *Porphyra*, *Ascophyllum*, *Ulva lactuca*, *Tetraselmis* sp., and *Chlorococum* sp.; and for biogas *C. reinhardtii*, *Chlorella kessleri*, and *Spirogyra neglecta* [23, 41–44]. To produce biodiesel from microalgae, it is very important to select strains with oil content over 50% to improve biodiesel yield. With respect to oil content, microalgae can be divided into low, medium, and high oil content strains [45].

7.5 Proteins

Soluble proteins have been used as nutritional supplements and personal care products or insoluble proteins for animal feeds [36]. Protein production has been reported in *S. platensis* using beet vinasse-supplemented culture media, in tubular photobioreactor biomass, which reached to 6.5 g L^{-1} and 168 mg L^{-1} d^{-1} of protein productivity. Continuous cultivation was also suitable for protein production from *S. platensis* using a medium supplemented with beet vinasse [46].

Incorporation of carbon from an organic carbon source, the type of carbon source, the amount supplemented to the culture, and the specie of microalgae are important for lipid accumulation in the cells of microalgae. The content of protein mostly increases by the addition of an organic carbon source, but lipid content decreases, although productivity of biomass, protein, and lipids increases substantially in the presence of organic carbon source [38].

Figure 2 represents the secondary metabolite production along with biomass that should be included in balance equations. The main components are carbon, hydrogen, oxygen, and nitrogen; in other words, a secondary metabolite can be a fraction of the total biomass, and it can be defined as ΘΔX, where is the fraction corresponding to the secondary metabolite produced, for chlorophyll accumulation, and it depends on the availability of carbon and nitrogen sources [40].

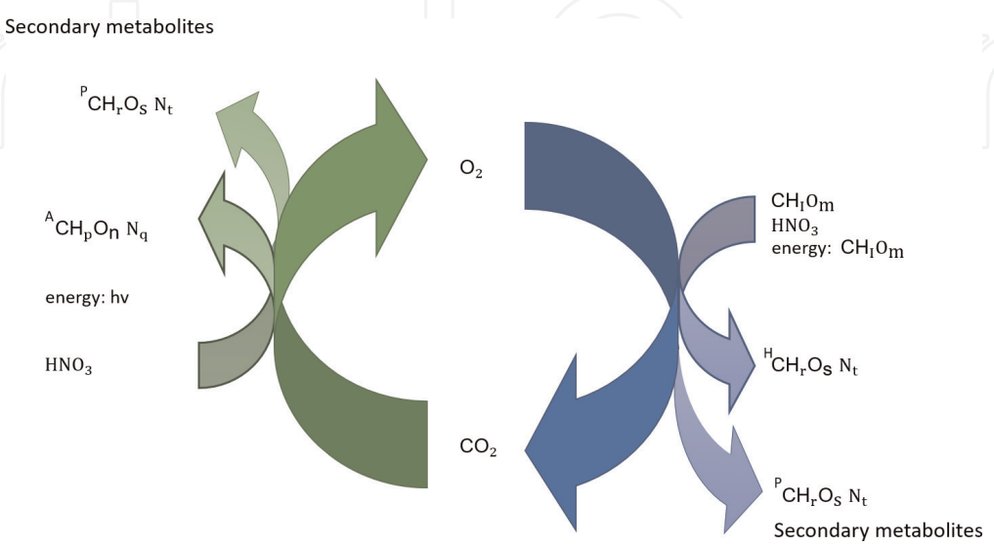

Figure 2.
Drawing describing the production of secondary metabolites under mixotrophy. P, metabolite from autotrophy and heterotrophy; A, biomass from autotrophy; H, biomass from heterotrophy. Modified from Ref. [5].

Mixotrophy is coupled with three metabolic mechanisms, glycolysis, Calvin-Benson-Bassham, and the tricarboxylic acid cycle, where ATP is formed in the tricarboxylic acid cycle helping to drive electron flux on the light reactions of the photosynthesis to generate NADH, which is needed in the tricarboxylic acid cycle. These mechanisms are focal point to perform metabolic engineering, which open new routes to enhance the synthesis of fine chemicals by microalgae [47].

8. Concluding remarks

In the past, microalgae cultures were used as components of aquaculture feeds and human food supplements. Recently, new alternatives have been opened for the production of fine chemicals and biofuels. However, production costs have been a concern; several efforts have been made to reduce processing costs to construct a profitable process. In this context, Allen et al. propose an integration of biology, ecology, and engineering topics for a sustainable biofuel and bioproduct production from microalgae [48].

The potential markets of value-added products from microalgae are nutraceuticals for human applications and nutraceutical with applications for animal and fish feed, bulk chemicals, and biofuels, with commercial costs of 100 €/kg biomass, 5–20 €/kg biomass, <5 €/kg biomass, and <0.4 €/kg biomass, with a volume market of 60 million, 3–4 billion, >50 billion, and >1 trillion €, respectively [49].

High value-added products such as antiviral, anticancer, and antioxidants are target products to be obtained from microalgae, since it is an alternative process that can be continuously cultivated of axenic cultures in a closed photobioreactor adapted with a special light source of irradiation, such as fiber-optic or halogen lamps. In this case, biomass increases as long as microalgae receive light and the broth hydrodynamic allows enough movement to reach the illuminated surface (see **Table 1**), in continuous cultivation. Once the light limitation occurred and due to the effect of washing out, biomass starts to decrease to a new dilution rate. When an organic carbon source has a positive effect on the growth, continuous cultivation can be used as well, to produce an increment in biomass density (**Table 1**) and secondary metabolite formation as well, producing an increment of biomass and in the metabolites. Productivity also has a substantial increment at same light intensity and same dilution rate (D, h^{-1}). Productivity and biomass concentration have been obtained in semicontinuous cultivation with a biomass of 5.31 g L^{-1} and productivity of 1.32 g L^{-1} d^{-1} [50]. Therefore, semicontinuous cultivation seems to be a good strategy as well.

Secondary metabolite production can be effectively improved, by three advantages,(i) using a continuous process (up- and downstream processes),

I_o (J cm^{-2} h^{-1})	ΔX_A (g L^{-1})	$\Delta X_A D$ (g L^{-1} h^{-1} × 10^{-3})	ΔX_M (g L^{-1})	$\Delta X_M D$ (g L^{-1} h^{-1} × 10^{-3})
3.22	0.050	1.74	0.079	2.76
5.85	0.092	3.21	0.136	4.75
11.11	0.175	6.11	0.241	8.41
18.98	0.301	10.81	0.405	14.13

Modified from Ref. [51].

Table 1.
Biomass concentration and productivity in continuous culture, in autotrophic and mixotrophic conditions (D = 0.03 h^{-1}).

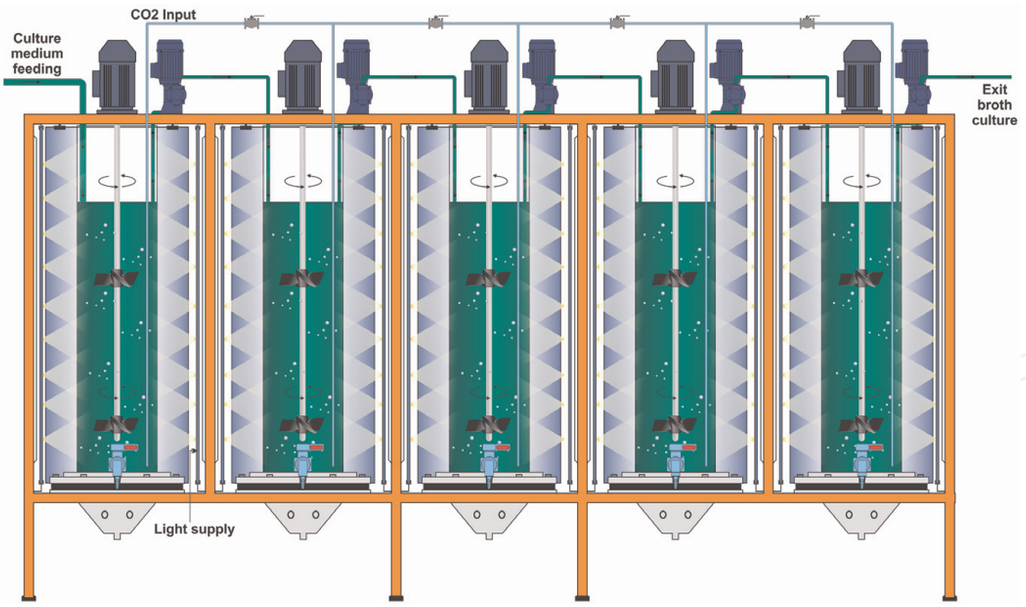

Figure 3.
Schematic representation of a series of photobioreactors to operate in continuous cultivation to produce fine chemicals.

(ii) implementing mixotrophic cultivation, and (iii) recycling broth medium at least three times (**Figure 3**).

Acknowledgements

The authors thank Mr. Mauricio Ramos for the drawing of figures and the Instituto Politécnico Nacional for funding this work.

Conflict of interest

The authors declare that there is no known conflict of interest associated with this publication.

Notes/thanks/other declarations

Thanks to Lada Bozic for helping in an efficient communication with IntechOpen.

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