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Biomass accumulation-influencing factors in microalgae farms

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ABSTRACT: Due to the emergence of large microalgae farms and increased competition in this sector, the search for higher productivity is common. One way to achieve this goal in microalgae production is to optimize the factors that influence their growth during the cultivation stage to increase the accumulation of bio-compounds of interest. In this stage, the factors that most influence are: nutrition, gas diffusion, light intensity and quality and, finally, stirring, which directly affects all other factors. Thus, a review and an evaluation of the influence and importance of stirring were performed in the present study. The nutrients that most influence biomass accumulation are carbon, nitrogen and phosphorus, but their proportion is directly related to the proposed objective for microalgae. In the diffusion of gases, it is essential to supply adequate CO₂ for the growth of microalgae, and flue gases can be used. Also, it is necessary to ensure proper removal of photosynthetic O₂, which could inhibit microalgae metabolism and slow their growth rate. It is important to provide the appropriate light intensity for photosynthesis, but excess may cause photoinhibition in cultivation. Stirring is of paramount importance to ensure nutrient distribution in the medium, gas diffusion (incorporation of CO₂ and removal of O₂) and adequate exposure of microalgae to light, reducing the effects of photoinhibition and self-shading.

Key words: nutrition, stirring, lighting, productivity

Fatores que influenciam o acúmulo de biomassa em fazendas de microalgas

RESUMO: Com o surgimento de grandes fazendas de cultivo de microalgas e aumento da competição nesse setor, é comum a busca por maior produtividade. Uma maneira de alcançar esse objetivo na produção de microalgas é otimizar os fatores que influenciam o crescimento das mesmas, durante a etapa de cultivo, para aumentar o acúmulo de biocompostos de interesse. Nesta etapa, os fatores que mais influenciam são: nutrição, difusão de gases, intensidade e qualidade luminosa e, por último, agitação, que afeta diretamente todos os demais fatores. Desta forma, foi realizada no presente estudo uma revisão e também uma avaliação da influência e importância da agitação. Os nutrientes que mais influenciam o acúmulo de biomassa são o carbono, nitrogênio e fósforo, porém, a proporção dos mesmos está diretamente relacionada ao objetivo proposto para a microalga. Na difusão de gases, é imprescindível o fornecimento de CO₂ adequado para o crescimento das microalgas, podendo-se utilizar gases de queima. Além disso, deve-se garantir a remoção adequada de O₂ produzido pela fotossíntese, que poderia inibir o metabolismo da microalga e diminuir sua taxa de crescimento. É importante fornecer a intensidade luminosa adequada para a fotossíntese, porém, o excesso pode provocar fotoinibição no cultivo. A agitação é de suma importância para garantir a distribuição dos nutrientes no meio, a difusão de gases (incorporação de CO₂ e remoção de O₂) e adequada exposição das microalgas à luz, reduzindo os efeitos de fotoinibição e de auto sombreamento.

Palavras-chave: nutrição, agitação, iluminação, produtividade



INTRODUCTION

Microalgae are photosynthetic microorganisms with high growth rate, capable of accumulating large amounts of proteins, lipids and carbohydrates at levels that are comparable or superior to those of the main grains produced in the world (Lum et al., 2013). Their use by mankind is old, and the first reports date back to 2000 years ago, when Chinese peoples used the cyanobacterial strains *Nostoc*, *Arthrospira* (spirulina) and *Aphanizomenon* in their food (Potvin & Zhang, 2010). Years later, in 1524, the Franciscan missionary friar Toribio de Benavente reported that the Aztecs, who lived in the valley of Mexico, collected a cyanobacterial biomass (*Arthrospira*) from the surface of some lakes to eat (Milledge, 2011). However, large-scale open microalgae cultivation began to be studied only after World War II, mainly in the United States, Japan and Germany (Spolaore et al., 2006).

Commercial application and the emergence of the first microalgae farms began in mid-1960 but, until approximately the year 2000, the production and utilization of these microorganisms were limited to human and animal nutrition. Currently, large companies, such as Cyanotech Co. and DIC Co., are producing microalgae in large farms, not only for use as a dietary supplement, but also as a means of production of several high value-added bio-compounds, such as β -carotene, astaxanthin and polyunsaturated fatty acids - PUFAs (Maeda et al., 2018). Combined with the increased interest in biofuel production, microalgae production jumped from 1000 t year⁻¹ in 1999 to 19000 t year⁻¹ in 2016. It is estimated that in 2024 the annual production of microalgae will reach 27500 t year⁻¹, with a market value of U\$ 1.1 billion (Dmytryk et al., 2018).

With the growth in microalgae market, emergence of new companies and increased competition in the sector, it is common to search for higher productivity and, consequently, reduction in production costs. One way to achieve this goal in microalgae production is to optimize the factors that influence their growth, during the cultivation stage, in order to increase the accumulation of bio-compounds of interest (Chew et al., 2017). In this stage, the most important factors are: nutrition, diffusion of gases, light intensity and quality and, lastly, stirring, which directly influences all other factors (Voloshin et al., 2016).

Thus, a review and also an evaluation of the influence and importance of stirring were carried out in the present study.

NUTRIENTS

The ideal culture medium for microalgae must contain in its composition inorganic elements such as nitrogen (N), phosphorus (P), iron (Fe), among others, which may vary according to the cultivated species. The minimum nutritional requirements needed for their growth can be determined using the approximate molecular formula $\text{CO}_{0.48}\text{H}_{1.83}\text{N}_{0.11}\text{P}_{0.01}$. From the analysis of the molecular formula of biomass, it is concluded that about 50% of the biomass is composed of carbon (C) (Chisti, 2007).

Nitrogen is the second most abundant element in microalgal biomass and its concentration can range from 1 to

14% in dry mass. It can be absorbed in the inorganic forms NO_3^- , NO_2^- , NO, NH_4^+ and, in some cases, as N_2 or in the organic form, through urea or amino acids (Perez-Garcia et al., 2011). Reduction of nitrogen concentration in the cultivation allows lipids and carbohydrates to be synthesized preferentially (Yang et al., 2011).

Phosphorus concentration in the dry biomass of microalgae can range from 0.05 to 3.3% (Sacristán de Alva et al., 2018). This nutrient is considered highly important, as it can be limiting, affecting the biomass productivity of several microalgae species if it is absent or present at low concentrations in the medium (Matouke et al., 2018). In natural environments, as well as in wastewater, phosphorus is present in various forms, such as orthophosphate, polyphosphate, pyrophosphate and metaphosphate (Markou et al., 2014). In addition, there are various types of agricultural fertilizers that can be used to supplement microalgae cultivation with phosphorus, such as phosphates and superphosphates, produced from rock phosphate.

Microalgae can accumulate intracellular reserves of phosphorus, such as polyphosphate granules. This reserve can be used when phosphate is exhausted in the medium, a behavior known as luxury uptake or accumulation (Powell et al., 2009). This ability is interesting when the objective is to remove phosphorus from wastewater; however, in cultures in which synthetic fertilizers are used, luxury uptake should be avoided so that it is possible to maximize the biomass production per mass of nutrients added (Markou et al., 2014).

For the adequate growth of microalgae, the medium should contain other nutrients (micronutrients), besides the macronutrients already mentioned (nitrogen, phosphorus and potassium). The essential micronutrients are Mg, S, Ca, Na, Cl, Fe, Zn, Cu, Mo, Mn, B and Co, with emphasis on magnesium, sulfur and iron (Mg, S and Fe, respectively). Both wastewater and seawater are good sources of most of these micronutrients (Markou & Georgakakis, 2011). It is also possible to use fertilizers and salts as sources of these micronutrients (Markou et al., 2014).

CARBON DIOXIDE (CO₂)

Microalgae are able to grow in autotrophic systems, using light and carbon dioxide, in heterotrophic systems, where organic compounds are used as a source of energy and carbon, or also in mixotrophic systems, where the light source and the organic substrate are simultaneously used as a source of energy, in addition to CO₂ and organic substrate as sources of carbon (Chojnacka & Facundo-Joaquin, 2004).

In autotrophic growth, microalgae perform oxygenic photosynthesis and fix carbon dioxide (Yang et al., 2000). From the fixed carbon, part is used for cell maintenance and for growth, while another part is stored in several forms, varying according to the different species of microalgae. In addition, the amount of carbon stored is dependent on environmental conditions (Durán et al., 2018).

Microalgae require from 1.8 to 2.0 kg of CO₂ to produce 1 kg of biomass (Chisti, 2007). Considering this stoichiometric ratio, the amount of CO₂ present in the air (0.03%) is not

enough to provide the necessary gas pressure in the cultures to promote high productivity. Thus, to increase photosynthetic efficiency in their growth, microalgae cultures should be supplemented with carbon, either in the form of salts, such as bicarbonate, or by injection of CO₂-rich air (Cho et al., 2011; Park et al., 2011). A study conducted by Dúran et al. (2018) demonstrated that, using air injection (600 mL min⁻¹) in a photobioreactor, microalgae showed optimal growth with up to 20% (volume per volume) of CO₂ present in the injected air (maximum value tested by the authors), not differing much from the control with 2.0% of CO₂, known as the optimal value for microalgae growth. This creates the possibility of using CO₂ from industrial burning, a process that generates on average 5.0% (volume per volume) of CO₂, and this concentration may reach up to 20%, depending on the technology and type of fuel used (Ge et al., 2011). This use combines a low-cost source of carbon for microalgae with the reduction of CO₂ emissions to the atmosphere.

The supply of CO₂ to microalgae cultures allows increasing biomass productivity, but the reduction of pH, resulting from the increase of CO₂ availability in the aqueous phase, can inhibit the growth of some species of these microorganisms (Pires et al., 2012).

pH AND SALINITY

Hydrogen potential (pH) has great relevance in microalgal cultures, as it determines the solubility of minerals and carbon dioxide in the medium, besides affecting the microalgae themselves (Qiu et al., 2017). Several factors may influence the pH of the culture medium, such as composition and buffering capacity, amount of dissolved carbon dioxide, temperature and metabolic activity of the cells (Singh & Dhar, 2011).

Different species of microalgae have different levels of tolerance to the pH of the culture medium, which may affect the growth rate, but the most common pH values for microalgae culture vary from 6 to 8 (Zhu, 2015; Rai & Gupta, 2017). Wu et al. (2016) studied the effect of pH on the growth of *Scenedesmus* sp. LX1. These authors varied the pH from 5 to 11 and observed that, for pH values of 7, 9 and 11, there was no significant difference of growth in the cultures. However, at pH 5 there was a significant limitation in the growth of the microalgae used, as illustrated in Figure 1.

The use of buffers reduces pH variation in the cultures; however, for large-scale systems, the use of buffers increases the cost of production, which makes it unfeasible. One way to regulate pH variation is through the aeration of the cultures by pumping atmospheric air (0.03% of CO₂) or CO₂-enriched air, because carbon dioxide, when dissolved, reduces the pH of the medium (Valdés et al., 2012).

During microalgae cultivation, salinity is another factor that requires attention, because it tends to increase due to the intense evaporation that occurs in cultures in open tanks, increasing their concentration in the medium. Some species of microalgae are very restricted in terms of salinity, especially those found in freshwater environments. In general, microalgae can be divided as to their tolerance to

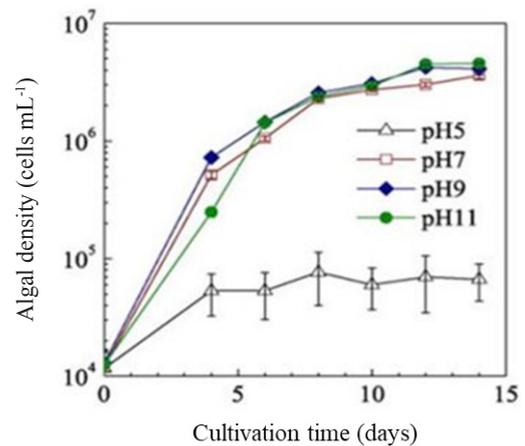


Figure 1. Effect of pH on biomass production in culture of *Scenedesmus* sp. LX1 (Wu et al., 2016)

salinity as follows: oligohaline, when they can develop only in water with low salinity (maximum salinity between 0.5 and 5 g kg⁻¹); mesohaline, when they develop in environments of moderately saline water, with salinity between 5 and 18 g kg⁻¹, and polyhaline, when they can develop in highly saline water, with salinity between 18 and 30 g kg⁻¹ (Venkata Mohan & Devi, 2014).

LIGHT INTENSITY AND QUALITY

The optimal condition for microalgae growth will depend on light intensity, wavelength and photoperiod to which the cells are exposed (Iasimone et al., 2018). Light intensity is directly related to the photochemical phase of photosynthesis, when light absorption occurs through the chlorophyll molecules, synthesis of adenosine triphosphate (ATP) and photolysis of water. In general terms, photosynthesis can be defined as the process in which light energy allows the synthesis of carbohydrates and oxygen from carbon dioxide and water (Corrêa et al., 2017).

Light intensity is one of the pillars of microalgae cultivation and requires special attention. The amount of light received by the cultured cells is directly related to the carbon that will be fixed, influencing the growth rate of the cultures (Fontoura et al., 2017). The light source can be either artificial or natural (solar), the latter being the most economically viable due to its availability. Nevertheless, in high value-added cultures, artificial light can be employed because it allows the precise control of photosynthesis and photoperiod, besides allowing the control of the light spectrum in microalgae production, which can be transformed in gains of quality and productivity (Schulze et al., 2014).

Photosynthesis in microalgae increases as light intensity increases, until it reaches a maximum rate at the saturation point (Figure 2) (Rai & Gupta, 2017). Above the saturation point, the excess light leads to a phenomenon called photoinhibition, defined by alteration and eventual inactivation of photosystem II (PSII), affecting electron transport in the chain of reduction reactions from NADP⁺ to NADPH. Photoinhibition and photosynthesis can be classified as moderate or intense, which determines whether this inhibition is dynamic or chronic (Nikolaou et al., 2016).

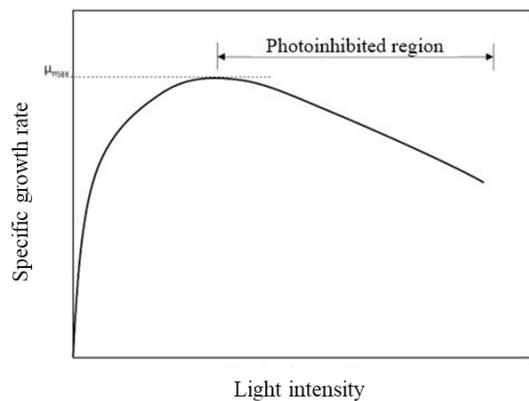


Figure 2. Effect of light intensity on the specific growth rate of microalgae (Chisti, 2007)

The depth that the light can reach in the culture does not depend on the intensity. In other words, even if light intensity is sufficiently high to cause photosynthesis inhibition, the light may not reach shaded cells, affecting the efficiency in biomass production. Hence, for complementing the natural light, or even for cultures under artificial light, it is recommended to place LEDs inside the medium to improve the delivery and distribution of photons (Lee & Palsson, 1995).

The photosynthetically active radiation, useful for microalgae, is located within the range from 400 to 700 nm of the light irradiance, corresponding to 50% of the solar radiation and intensity from 800 to 1,000 W m⁻² (Zhang et al., 2015). This is corroborated by the results of Fu et al. (2013), who demonstrated that *Dunaliella salina* obtained the highest production of biomass and carotenoids (β -carotene and lutein) with the combined use of 75% of red light (wavelength around 700 nm) and 25% of blue light (wavelength around 400 nm), compared to just the red light.

In microalgae culture tanks, there are light/dark (L/D) cycles induced by the stirring system of the medium. These cycles have been related to the increase in the photosynthetic conversion and biomass productivity in microalgae (Takache et al., 2015). These authors found that the efficiency of photosynthesis in *Chlamydomonas reinhardtii* was improved when L/D cycles of less than 20 s were applied, with increase in the growth rate of up to 40%, depending on the conditions of the L/D cycle.

STIRRING AND DIFFUSION OF GASES

Efficient stirring of the culture medium is important for obtaining high concentrations of cells. Stirring has the function of keeping cells in suspension, eliminating thermal stratification, distributing nutrients and making gas exchanges more efficient. Stirring can reduce the degree of self-shading and reduce the probability of photoinhibition, distributing the light evenly among all microalgae (Zhu, 2015). In addition, stirring is also responsible for allowing the capture of CO₂ from the atmosphere and facilitating the transfer of biosynthesized O₂ from the liquid phase to the gaseous phase, which stimulates photosynthesis in microalgae culture (Doucha et al., 2009; Zhu et al., 2014; Wang & Lan, 2018).

The relationship of stirring with lighting becomes more evident when the culture has high cellular concentration

because, under this condition, the light is blocked by the cells of the superficial region and light intensity decreases sharply with culture depth (Ogbonna & Tanaka, 1998). In a study conducted by Sánchez et al. (2013), it was possible to note that in raceway culture systems stirred by paddles, the daily growth of *Isochrysis galbana* microalgae culture was double when compared to the system without stirring (8.8×10^5 and 4.0×10^5 cells mL⁻¹ d⁻¹). This shows the great importance of stirring the culture medium in the industrial processes of microalgae production.

The growth rate of photosynthesizing microorganisms increases with the increment of turbulence promoted by stirring, but above an optimum level of turbulence, this growth decreases sharply due to damage caused to the cells (Doucha et al., 2009; Wang & Lan, 2018). Many photosynthesizing microorganisms have a fragile cell wall, some are mobile or filamentous and can be susceptible to physical stress. Thus, it is desirable that the stirring is performed with the minimum possible hydrodynamic stress (Ogbonna & Tanaka, 1998; Ación et al., 2017).

Culture stirring can be classified into two main groups: stirring by air injection and mechanical stirring. In tanks stirred by air injection there is gas-liquid or gas-liquid-solid contact, where the content is pneumatically stirred by an upward current of pressurized air. In mechanical stirring, motors provide the energy required for mechanical elements to transfer this power to the medium, in the form of displacement of the liquid (Singh & Dhar, 2011).

The most used method of stirring for industrial production of microalgae, in open system, is the mechanical stirring by paddles ("raceway"). These paddles promote circulation and formation of vortices in the area near the mixing paddle. This mechanism can reduce the effect of shading among the microalgae that are located in deeper regions of the tank, turning them to regions close to the tank surface (Chisti, 2007).

CULTURE STRUCTURES

Microalgae production, depending on the species, environmental conditions, culture structure used and the relationship among the factors, can achieve a high lipid concentration.

Microalgae cultivation can be performed in open or closed systems, and both have advantages and limitations. In the open cultivation system, raceway-type, more employed nowadays due to its economic viability and ease of flow, the limiting factors are the temperature and seasonal variations of solar radiation. The advantages of these systems are low costs of installation and operation (Borowitzka, 2003).

Another important point of the biotechnological process of growing microalgae is the use of environmental conditions that stimulate the synthesis of the product that is to be obtained. These processes can be divided in several ways, highlighting the classification regarding the conduction of the culture, which can be discontinuous (batch), fed-batch, semi-continuous and continuous (Dias et al., 2015).

Thus, it is strictly necessary that the factors influencing microalgae growth would be aligned and optimized to achieve high productivity, because in order for microalgae to

be effectively used in the production of biofuels, the process should be as productive and low-cost as possible.

CONCLUSIONS

1. Large-scale production of microalgae is an emerging technology, and technological studies and development are needed to make it even more competitive with the existing systems.

2. The metabolic variability of microalgae is an important factor to be considered, and it is necessary to conduct studies to determine the behavior of the strain under different growth conditions. From these results it is possible to design the future production system.

3. It is important to define the composition of the culture medium, along with the other variables: CO₂, pH, light intensity and stirring, in order to determine the conditions which lead to maximum concentrations of the bio-compounds of interest.

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