

## **Issues on the optimization of bioreactors of microalgae and cyanobacteria crops for hydrogen and bioproduct productions**

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### **Abstract**

*Optimizing the design and operating parameters for optimum production of hydrogen and other bioproducts is a necessary step to address the rate of production of this energy input. Optimization is basically based on the appropriate choice of microalgae strain along with the available growth conditions. This paper presents a simplified review of the possible monitoring variables for microalgae and cyanobacteria crops. In addition, the design of open pond bioreactors and photobioreactors (PBR) that allow greater control of monitoring and crop parameters were presented. The physicochemical bioproduct characterization, such as fatty acids constituent and gases, is an aspect to be considered. The use of the optimization of the physical-chemical properties for their subsequent processing may improve the production of biofuels and biomass. In addition, the generation of hydrogen in the photosynthetic cycle of bioreactors based on microalgae cultures is presented as a solution to energy demand. And finally, we comment on some findings obtained from Multiphysics computational modeling carried out in PBRs.*

**Keywords:** Microalgae; cyanobacteria; bioproducts; hydrogen; PBR; biofuels.

## **1. Introduction**

The world is facing challenges associated with high demands of energy and rising fossil fuel prices, due to the growing world population and rapid industrialization. Fossil fuels linked to environmental pollution, global warming and are non-renewable. Therefore, it is necessary to expand the processes of generating energy from renewable sources, mainly in sustainable, ecological, renewable, low cost and decentralized methods (AZWAR; HUSSAIN; ABDUL-WAHAB, 2014; MALLICK, 2002).

At present, governments are proactive in securing energy supply and limiting climate change. Many potential fuels were assessed early in this century (CHAUMONT, 1993; SAIFUDDIN; PRIATHARSINI, 2016). Biomass is one of the most promising renewable resources used to produce various types of biofuels, including biodiesel, bioethanol, biogas and biohydrogen. Energy from biomass contributes to a stable supply of energy and to society, maintaining the growth of market activities (SHERIF; BARBIR; VEZIROGLU, 2003). Biomass can be derived, for example, from harvesting specific crops; using raw materials from forestry and other plant residues (CHANG; LIN, 2004). So far, saccharose and starch crops, for example, sugarcane and maize, as well as lignocellulosic materials, such as rice straw, have being used as feedstock for biofuel. Sugars come in various forms, containing about four calories per gram, such as monosaccharides like glucose, fructose and galactose. However, the high cost of hydrolysis lignocellulosic materials is a limiting factor.

In turn, hydrogen is considered a clean and environmentally safe fuel, with renewable resources and a great substitute for fossil fuels. Hydrogen retains high energy density potential, 143 GJ per ton, with many technical, socioeconomic and environmental benefits that guarantee its credibility among all other fuels. Hydrogen is the only recognized fuel that does not produce carbon dioxide as a by-product when it is used in fuel cells to produce electricity (AZWAR; HUSSAIN; ABDUL-WAHAB, 2014; CHANG; LIN, 2004). The largest users of hydrogen are the fertilizer and petroleum industries, at approximately 50% and 37% respectively (CHANG; LIN, 2004).

The production of biohydrogen offers a sustainable alternative and the use of renewable carbon sources can be considered as offsetting the production of carbon dioxide. The hydrogen production process can consume multiple sources of carbon, including organic material from wastewater. Glucose and sucrose are easily degradable and are therefore preferred as model substrates for the hydrogen production. Because of the complexity of the chemical composition and polymeric structure of these carbon sources, the carbon present must be released or transformed to simple sugars. Complex carbon polymers consist of tightly bonded lignin, cellulose and hemicellulose. Cellulose and hemicelluloses may be degraded under the same conditions and add to the cost factor, which is also restrictive (AZWAR; HUSSAIN; ABDUL-WAHAB, 2014; BEHERA; SINGH; ARORA; SHARMA *et al.*, 2015).

Many microorganisms participate in the production of biofuels like hydrogen, but the most accepted are cyanobacteria and green microalgae. These micro-organisms are recently considered as a third-generation raw material, being more effective in converting sunlight into chemical energy and requiring less footprint and less water for cultivation. (KOTAY; DAS, 2007; MANISH; BANERJEE, 2008). In such a case, it is said that the fuel is biohydrogen.

The production of biohydrogen using algae was determined only at the laboratory level, with a consistently

low yield for commercial application. Therefore, optimizing the design and operating parameters for maximum hydrogen production is a necessary step to process the production rate of this energy input. Optimization is primarily based on the microalgae strain with the available growing conditions (MORENO-GARRIDO, 2008). For biofuels to be widely authorized in the energy markets, emphasis should be placed on acclimatization and improvement photosynthetic organisms for the biofuel production (DINCER, 2012). Several physical-chemical pretreatments have been revised to produce hydrogen, biooils and biomass as supplementary nutrients. It requires a step to pierce the algal cell wall with the complex carbohydrate to release simple sugars. Pretreatment methods such as physical (sonication, grinding, and pyrolysis), chemical (acid, alkali, and thermal) and biological (enzymatic) methods are used to break up the algal cell wall, to hydrolyze complex carbohydrates and release fermentable sugars, or merely oil (KAPARAPU; GEDDADA, 2016).

A immobilised cell means that a cell through natural or artificial pathways is prevented from moving independently of the surrounding environment to all parts of the system that are under consideration (BROUERS; SHI; HALL, 1988). Basically, there are six different types of cell immobilization methods. There is covalent coupling, affinity immobilization, adsorption, liquid-liquid emulsion containment, trapping behind a semi-permeable membrane. The use of the immobilization technique contributes to greater resiliency when designing a reactor comparing conventional suspension systems. Additionally, increased cell density and permeability of cell wall, lack of cell washing, and improved system stability are some additional benefits of the cell immobilization technique. Above all, cell trapping in polymeric matrices and self-adhesive cell binding on solid support surfaces are generally more common. The algae cells within its partition are important criteria for a successful trap, while the pores within the gel matrix allow the diffusion of substrates and metabolic products to and from cells (BROUERS; SHI; HALL, 1988). In this case, there is a lot of experience in growing algae in bioreactors. Cell immobilization techniques for the exploration of products expressed by green algae have not yet been studied and could be a reason for the future to improve the processes of extraction of bioproducts from live matrices. In turn, bioreactors are considered a means of producing hydrogen from algae biomass.

The production of bio-hydrogen by micro-organisms has attracted growing worldwide attention, with the potential to be an inexhaustible, inexpensive, and renewable energy source. The development of bioreactors or microalgae immobilizers is a sine qua non condition for large-scale hydrogen production. Bioreactors are closed or open or hybrids systems ranging in size from the small scale (5 to 10 mL) to the larger scale or more than 500,000 L on an industrial scale. Photobioreactors (PBRs) are composed of a series of tubes, tank bags, in which photosynthetic micro-organisms, including algae, are grown and then monitored because light is the critical component for the growth of photosynthetic microorganisms. These bioreactors are PBRs, called continuous wave tank reactor (CSTR), fixed bed bioreactors, membrane bioreactors, multistage bioreactors or hybrid bioreactors.

## **2. Mechanisms for biofuel production**

Biofuels can be solids, liquids or gases to the extent that they are derived directly from biological sources. The most common solid biofuel is lignified (wood based) cellulose, which can be burned for energy

proposes. Liquid and gaseous biofuels generally require more refining and include bio-ethanol, biodiesel and hydrocarbons for engines, as well as methane from anaerobic digestion. The liquid biofuels mentioned above have significant potential to increase or substitute petroleum fuel for transportation purposes. Currently, ethanol dominates the biofuels market and can be produced through various of methods, primarily heterotrophic fermentation of purified sugars from biomass (CHIARAMONTI, 2007). Biodiesel and other hydrogen-treated biofuels are primarily derived from vegetable oils (lipids) raw materials (VAN GERPEN, 2007).

Lipids used for biofuels have important physiological roles in plants, including energy storage, structural support such as membranes and cell signaling (MURPHY, 2001). Storage lipids differ from structural and signaling lipids in that they are composed primarily of glycerol esters of fatty acids, also known as triacylglycerol (TAG). These lipids are generally stored in a specialized lipid storage compartment, the lipid body. This compartment is found in most oil seed cells and is used to store a variety of TAG molecules, depending on the species (MURPHY, 2001). Vascular plants store large amounts of lipids in the seeds and provide energy for growth during germination. The lipid content and fatty acid composition of oilseeds vary. Environmental changes or human manipulations, such as reproductive or genetic engineering, have been used to alter lipid content and composition (GUSCHINA; HARWOOD, 2007). Although less common, some species such as *Simmondsia chinensis* accumulate stored lipids in the form of waxes, not as TAG. Regardless of the type of final storage, fatty acid biosynthesis again at the plants occurs only in the plastid stroma. While, with the exception of plastid desaturation and some complex lipid biosynthesis, most changes in the fatty acyl residues and TAG synthesis of the acyclic chains are located in the lumen of the endoplasmic reticulum (ER) (GUSCHINA; HARWOOD, 2007). In addition to TAGs, plants also contain membranous lipids. These, unlike TAGs, remain highly preserved in identity and quantity to maintain the normal plant physiology.

Ethanol and biodiesel are mainly derived from plant sources, often food crops, because the established scale of food crops has become a convenient source of biomass required to produce biofuel on a commercially. However, a growing demand for biofuel feedstock has a negative impact on food markets and has led to global controversy over "food versus fuel". Furthermore, land and fresh water for cultivation and long growing-to-harvest periods limit the expansion of the biofuel industries to the amount of arable land. In contrast, unicellular algae require small quantities of land which do not need to be cultivated, have faster growth cycles, have a higher percentage of oil and have been proposed as a better solution to the food and fuel debate. Therefore, particular attention has been paid to algae as the next generation raw material for the biofuel production (CHISTI, 2007). It was proposed that a fuel-only approach for biodiesel production from algae is unlikely given current yields based on economic modeling of production facilities. As a result, attention needs to be paid to genetic manipulation to take advantage of algae's ability to produce high quality fuel, but also potentially to be used as a factory for the production of other value-added products, such as protein therapy (GREENWELL; LAURENS; SHIELDS; LOVITT *et al.*, 2009; WILLIAMS; LAURENS, 2010). In light of this and studies of the selection pressure for photosynthetic efficiency in native environments versus bioreactor environments, it seems that genetic modification is likely to provide the key to unlocking the viability of algal production lines (FLYNN; GREENWELL;

LOVITT; SHIELDS, 2010).

### **3. Biohydrogen production and extraction**

#### **3.1 History**

The ability of unicellular algae to produce H<sub>2</sub> gas under lighting was discovered six decades ago (GAFFRON, 1939; GAFFRON; RUBIN, 1942). The hydrogen production activity in green algae was induced following previous anaerobic incubation of cells placed in the dark (GREENBAUM, 1982; HAPPE; NABER, 1993; ROESSLER; LIEN, 1984; SCHULZ, 1996). A hydrogenase enzyme has been expressed in such incubation and catalyzed, with high specific activity, generating H<sub>2</sub> mediated by light. The reported monomeric form of the enzyme belongs to the Ferrous hydrogenase class (ADAMS, 1990; HAPPE; MOSLER; NABER, 1994; MEYER; GAGNON, 1991; VOORDOUW; STRANG; WILSON, 1989). This functional protein is encoded in the nucleus of unicellular green algae and locates and operates in the chloroplast stroma (HAPPE; MOSLER; NABER, 1994).

#### **3.2 Biochemical mechanism**

The absorption of light by the photosynthetic apparatus is essential for the generation of gaseous hydrogen, because the light energy facilitates the oxidation of water molecules, the release of electrons and protons and the transport of metals from these electrons to ferredoxin. Photosynthetic ferredoxin (PetF) is used as a physiological electron donor for Fe-hydrogenase and thus connects Fe-hydrogenase to the electron transport chain in the green algae chloroplast (TAMAGNINI; LEITÃO; OLIVEIRA; FERREIRA *et al.*, 2007).

#### **3.3 Inhibitory process for oxygen (O<sub>2</sub>)**

Under these conditions, the hydrogenase activity is only transient for a few seconds to minutes, since, in addition to protons and electrons, the light-dependent oxidation of the water molecule involves the release of molecular O<sub>2</sub>. However, oxygen itself is a potent inhibitor of Fe-hydrogenase (GHIRARDI; ZHANG; LEE; FLYNN *et al.*, 2000).

#### **3.4 Questions**

Current technological developments in this field have not yet succeeded in overcoming this mutually exclusive nature of O<sub>2</sub> and H<sub>2</sub> photoproduction reactions. Thus, the physiological meaning and role of Fe-hydrogenase in green algae, which normally grow under photosynthetic aerobic conditions, has been a mystery. Given the sensitivity of Fe-hydrogenase to O<sub>2</sub> and the oxidative conditions in the soil, questions were arise whether hydrogenase is anything more than a relic of a chloroplast's evolutionary past in green algae, producing cellular energy in non-oxygenated environments. It is questionable whether this enzyme and the photosynthesis process can at present be used to produce H<sub>2</sub> gas for commercial purposes (ZHANG; HAPPE, 2001). However, the ability of green algae to generate H<sub>2</sub> gas has been a challenge despite the fundamental and practical importance of the process. A diagram of the cycle in biohydrogen production is shown in Fig. 1.

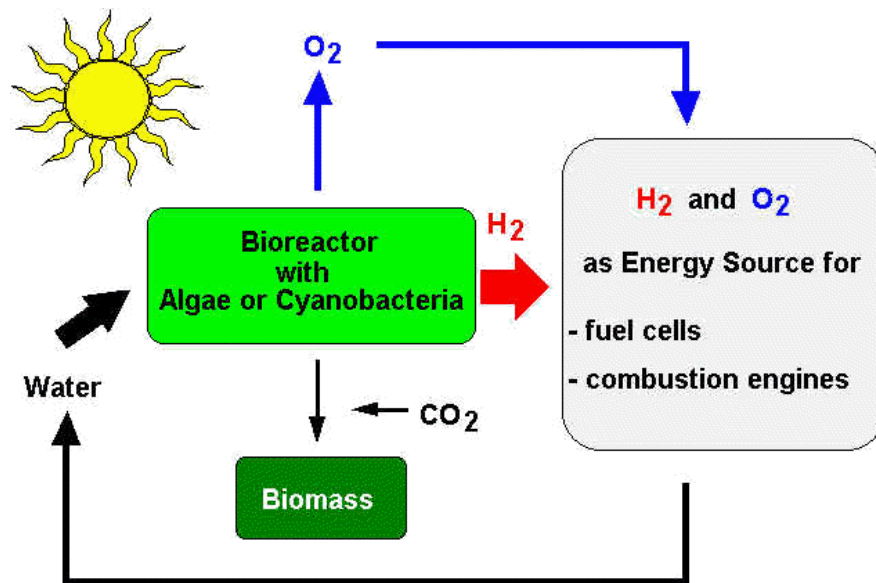


Figure 1. Hydrogen production cycle in bioreactors.

#### 4. Microalgae Production

Growing microalgae as a raw material for biofuels can be the solution for burning fossil fuels. This sounds like a viable resource for biodiesel and biogas. Some microalgae have up to 70% lipid in their structure and are capable of producing more than 30 times the amount of oil (per year and per unit soil area) compared to oilseed crops (CHISTI, 2007). This is because the fact that they have the doubling of biomass in a very short time, the use of a smaller physical space, the ability of being grown in areas not suitable for agriculture and the lower generation of waste (LOURENÇO, 2006). Algae constitute a highly diverse group of microorganisms. Thus, microalgae can be found in virtually all aquatic systems, including places with a wide range of physicalchemical development parameters. The biodiversity of these organisms represents an important technological feature, allowing the cultivation of various genera and species in a wide range of operational (THAJUDDIN; SUBRAMANIAN, 2005; XU; MIAO; WU, 2006). Another benefit is that microalgae use CO<sub>2</sub> as a carbon source to grow, being able to capture carbon dioxide from emissions from power plants or any other CO<sub>2</sub>-emitting process. Microalgae may be grown in closed photobioreactors (PBRs) as shown in Fig. 2, or in open systems (open ponds, swimming pools or small lakes), as shown in Fig. 3 (a) and (b). Since racing ponds are less productive and require a large amount of land, PBRs appear to be better candidates for industrial production of microalgae biofuels in the future.



Figure 2. Compact conduits-array Photobioreactor (PBR). Source: Núcleo de Pesquisa e Desenvolvimento em Energia Autossustentável NPDEAS - UFPR (2016)

A number of research groups have already recognized the demand for algae and biofuel. The Center for Research and Development of Self-Sustainable Energy (NPDEAS/UFPR) selected high-fat microalgae strains, improved the culture medium, and investigated the use of degraded water for culture and reuse of the culture medium. New compact geometries for driven PBRs and growth kinetics analysis on the pilot and industrial scale (RIBEIRO; MARIANO; VARGAS, 2016). The design and mathematical modeling of the PBR is very important for the development of a microalgal biomass oil extraction process and the biodiesel production from microalgae oil.



(a)



(b)

Figure 3. Hybrid open ponds. (a) Open ponds for controlling microorganism and nutrients (b) closed system type swimming pool.

The various research approaches required to make any process possible with microalgae, the need for a cropping system with high productivity per occupied area, low cost of installation and operation, stands out. To develop such a system, it is necessary to understand the parameters of the microalgae growing process and the systems that have already been used.

Factors such as temperature, sunlight, pH and the nutrient composition of the growing medium directly influence the cellular composition of the microalgae. Where these condition can be controlled by PBR engineering and architecture, the microalgae biomass can be increased. The pH and nutrient composition in medium can be controlled by devices installed in the PBR, but solar radiation and room temperature are variables that depend on the location of the system.

## **5. Some relevant control factors in monitoring microalgae crops**

### **5.1 Temperature**

Optimal temperature plays an important role in the growth of microalgae. An increase in temperature can lead an increase in biomass to a certain level, where growth can be inhibited due to inactivation of proteins by heat shock (MAHBOOB; RAUF; ASHRAF; SULTANA *et al.*, 2012). Studies carried out on biomass production at temperatures of 30, 40 and 50 °C, and found that, for high growth, the optimal temperature for the microalgae *Chlorella vulgaris* is 30°C (CHINNASAMY; RAMAKRISHNAN; BHATNAGAR; DAS, 2009). Kerby and Stewart (1988) reported that the ideal temperature for rapid growth, especially for *Chlorella* species, should be between 20 and 32°C (KERBY; STEWART, 1988).

However, there is the possibility of preadaptation of crops to temperature values outside the range considered ideal. Isolation of species tolerant to high temperatures (40 - 60 °C) has been considered an important criterion in the selection of the microorganism because it would allow the direct injection of carbon dioxide from thermal processes (ONO; CUELLO, 2007).

Microalgae cultures in the PBRs, at excessive or reduced temperatures, show a decrease in growth. However, it is possible to try to control the temperature in the values defined as ideal for a given crop by installing systems that use heat exchange to reach the ideal temperature. While these systems increase microalgal productivity, their main disadvantage is the high cost and energy expenditure. It is possible to determine the effect of temperature on the specific growth rate of microalgae by keeping all other variables constant. Indeed, the growth rate reaches a maximum at a specific temperature.

### **5.2 pH**

The ideal pH range for most algae species is 5 to 9. However, it found that, at pH 5, the productivity of the microalgae *Chlorella vulgaris* increases considerably. After 9 days, the concentration at pH 5 was  $9.5 \times 10^7$  cell/mL, at pH 7 it was  $4.5 \times 10^7$  cell/mL, and  $1.5 \times 10^7$  cell/mL at pH 9, under the following conditions: initial density of  $100 \times 10^4$  cell/mL, temperature of 24 °C, photoperiod of 24:0 (light/dark) and brightness  $97 \mu\text{mol} \cdot \text{photon} / \text{m}^2 \cdot \text{s}$ . According to Becker (2004), pH influences the solubility of CO<sub>2</sub> and minerals, interfering directly or indirectly in the metabolism of algae (BECKER, 2007). According to Esteves (1998), in aqueous media, inorganic carbon can be in the form of CO<sub>2</sub>, H<sub>2</sub>CO<sub>3</sub>, HCO<sub>3</sub><sup>-</sup> (bicarbonate) or CO<sub>3</sub><sup>-</sup> (carbonate) and their proportions depend on the pH. At high (basic) pHs, the proportions of HCO<sub>3</sub><sup>-</sup> or CO<sub>3</sub><sup>-</sup> increase. Thus, the availability of CO<sub>2</sub> increases in a culture with acid pH, since it is the carbon source used by algae. The gradual increase in pH in the culture medium is an indicative of microalgae growth (ESTEVEZ, 1998). The increase in pH occurs because the biological cellular activity, which produces a reduction in the dissolved inorganic carbon by the consumption needed for cell growth, forcing a shift of



the carbonate-bicarbonate balance in the buffer system (BERENGUEL; RODRIGUEZ; ACIÉN; GARCIA, 2004). Due to the low solubility of CO<sub>2</sub> in aqueous solutions, it is necessary to supply it throughout the process (KLASSON; ACKERSON; CLAUSEN; GADDY, 1991). Another study assesses the effect of the hydrogen ion concentration on the growth of *Chlorella vulgaris* between pH 3.0 and 11.5 and showed increased growth in the pH range 5.5 to 8.0 (MAYO; NOIKE, 1994).

### **5.3 Oxygen concentration [O<sub>2</sub>]**

Another factor to consider, according to Oswald (1988), is the concentration of O<sub>2</sub> in the microalgae culture medium. High levels of dissolved O<sub>2</sub> may generate photo-oxidative damage in cells with a parallel reduction in growth efficiency (OSWALD, 1988). However, since oxygen is a product of photosynthetic metabolism, its formation and solubilization in PBRs are indicative of high rates of inorganic carbon consumption (MUÑOZ; KÖLLNER; GUIEYSSE; MATTIASSON, 2004).

### **5.4 Stirring and flow dynamics**

Stirring is an important variable for providing more homogeneous exposure to light; increase nutrient availability, so that in constant agitation, no deposition to avoid microalgae sedimentation which would lead to excessive exposure of the upper layers and underexposure of the lower layers (COUTTEAU; SORGELOOS, 1992; MONTEIRO; LUCHESE; ABSHER, 2010).

### **5.5 Radiative transfer at light-dark cycle**

Light is the most important factor that influences the growth of photosynthetic organisms, representing the main source of energy (SOLETTO; BINAGHI; LODI; CARVALHO *et al.*, 2005). However, exposure to high photosynthetic photon flux density (PPFD) can increase the production of harmful reactive oxygen species, such as H<sub>2</sub>O<sub>2</sub>, damaging the cellular structure, or inducing the phenomenon of photoinhibition, that is, the reduction of microalgae photosynthesis capability causing bleaching and death (MEHLITZ, 2009; MÜLLER; LI; NIYOGI, 2001; SOLETTO; BINAGHI; LODI; CARVALHO *et al.*, 2005). Soletto *et al.* evaluated the luminous intensity in the growth of microalgae, using different densities of photosynthetic photon flux in *Spirulina platensis* based on a reactor with a volume of 4 L. The authors obtained maximum photosynthetic efficiency with 125 μmol·photon·m<sup>-2</sup>·s<sup>-1</sup> (SOLETTO; BINAGHI; LODI; CARVALHO *et al.*, 2005). The photoinhibition phenomenon for low PPFD and excess CO<sub>2</sub> inhibited growth. Redaelli *et al.* (2011) also studied the influence of light intensity (2200, 10000, 17000, 24500 lux) on carbon bio fixation in very short *Chlorella*, using a 2.2 L by PBR, and observed that the best results were obtained for intensity 17000 lux, reaching a biomass of 0.38 g/L and a specific growth rate of 0.61 day<sup>-1</sup> (REDAELLI; KOCHER; DIERINGS; JARENKOW *et al.*, 2011).

### **5.5 Influence of UV and oxidative stress**

Oxidative stress is defined as the increase in the production of species in excess of cellular antioxidant defenses (MOSKAUG; CARLSEN; MYHRSTAD; BLOMHOFF, 2005). The consequence of oxidative stress can cause damage to lipids, proteins and DNA, with the development of pathologies and subsequent aging (FINKEL; HOLBROOK, 2000). Vegetables synthesize bioactive compounds with antioxidant

characteristics, containing multiple phytochemical structures, the majority fraction of which are polyphenols (SCALBERT; WILLIAMSON, 2000), tannins, lignans and flavonoids (KUSKOSKI; ASUERO; GARCÍA-PARILLA; TRONCOSO *et al.*, 2004). Many polyphenols have antioxidant features (for example, reducing agents) and can react directly with reactive species, forming less reactive products (MOSKAUG; CARLSEN; MYHRSTAD; BLOMHOFF, 2005). Plants produce these phytochemicals as a defense mechanism for several factors that can cause stress. Relevant factors are ultraviolet radiation (UV) (CANTOS; GARCÍA-VIGUERA; DE PASCUAL-TERESA; TOMÁS-BARBERÁN, 2000; DUVAL; SHETTY; THOMAS, 1999; HERNANDO; MALANGA; FERREYRA, 2005; JANKNEGHT; DE GRAAFF; VAN DE POLL; VISSER *et al.*, 2009; MALANGA; CALMANOVICI; PUNTARULO, 1997; ZUDAIRE; ROY, 2001) and temperature variations (WONG; CHU; MARCHANT; PHANG, 2007). Many organisms produce different substances capable of absorbing ultraviolet light (COCKELL; KNOWLAND, 1999; ROZEMA; BJÖRN; BORNMAN; GABERŠČIK *et al.*, 2002). Among these chemical compounds there are the so-called "screens", such as MAAs (mycosporin-type amino acids), produced by many algae and some cyanobacteria (BJÖRN; PAPAGEORGIOU; BLANKENSHIP, 2009). Studies in vascular plants have shown that RUV-B (280-315 nm) stimulates the production pathways of some metabolites, including polyphenols and flavonols that have proven to be UV radiation absorber compounds, minimizing damage to the normal physiological functions of their tissues (REUBER; BORNMAN; WEISSENBOCK, 1996). In addition, there are studies that show the presence of photoprotective compounds against UV radiation in algae (GARCIA - PICHEL, 1994; GÓMEZ; PÉREZ-RODRÍGUEZ; VIÑEGLA; FIGUEROA *et al.*, 1998; HOYER; KARSTEN; SAWALL; WIENCKE, 2001).

Several studies have been carried out in the following macro and microalgae: *Chlamydomona nivalis* strain (DUVAL; SHETTY; THOMAS, 1999), *C. augustae*, *Naviculla uncertain* (WONG; CHU; MARCHANT; PHANG, 2007), *Chlorella sp.* (VIMALABAI; KULANDAIVELU, 2002; WONG; CHU; MARCHANT; PHANG, 2007), *Isochrysis galbana* (VIMALABAI; KULANDAIVELU, 2002), *Ascophyllum nodosum* (PAVIA; CERVIN; LINDGREN; ÅBERG, 1997) and *Ulva fasciata* (SHIU; LEE, 2005), among others, to assess resistance and the effect of exposure to UV radiation. There is evidence that organisms such as microalgae may have adaptive responses to exposure to UV radiation, increasing levels of polyphenols as photoprotectors (DUVAL; SHETTY; THOMAS, 1999). This process can be used as a very useful mechanism for the production of antioxidants of natural origin, the selection of more adapted plant organisms and their exposure to controlled UV radiation to increase the production of phytocomposites (polyphenols) through the photoprotective response of these algae. The importance of microalgae in aquatic ecosystems in primary production and in trophic plots is to understand how sensitive these organism are to UV-B (radiation UV-B). This research evaluated the adaptation strategy to the effect of ultraviolet B radiation on marine microalgae *Chlorella sp.* through the capacity of production of polyphenols and total antioxidant.

## 6. Physical-chemistry techniques for characterizing bioproducts and crop medium

The production of substances within the life cycles of microalgae crops makes easier to understand and adjust certain control factors to produce fatty acids, esters and other substances that form the basis of lipids

and biofuels. The balance of the biophysical parameters for crop optimization is analyzed with the aid of the capacity factors in the production of these raw materials. The definition of the capacity factors requires an understanding of the physicochemical properties of the culture media and the strains used. Chemical analysis techniques such as gas chromatography, magnetic resonance imaging, Fourier transform infrared spectroscopy, and other thermal and rheological analysis techniques, expanded the comprehension on the bioproduct compositions of a limited number of strains of cyanobacteria. The photosynthetic efficiency to produce monounsaturated fatty acids (double bond at the carbon chain) is related to a high morphological complexity that are identified in the raw materials of lipids present in crops. The feature of unsaturation in the fatty acid and the basis of the lipid production were identified in a complementary way with FT-IR as well as the hydrogen coupling factors at the carbon positions of the chain studied by  $^1\text{H-NMR}$  technique. Other rheological studies carried out showed viscosity parameters of non-Newtonian fluids susceptible to a strain rate like the base lipids of biodiesel such as beef tallow. Studies in thermal stability based on thermogravimetric analysis (TGA) found degradation of lipid materials, presented a maximum mass loss of 380 and 497 °C. The following table summarizes some physicochemical features of the fatty acids made in three strains of cyanobacteria (RÓS; DA; SILVA; SILVA-STENICO *et al.*, 2013).

In other thermal characterization studies based on differential scanning calorimetry (DSC) established the degree of randomness of residues by analyzing crystallization and fusion in microalgae ethylic biodiesel (BATISTA; LUCCHESI; CARARETO; COSTA *et al.*, 2018). Those studies are a base of knowledge in the characterization of the bioproducts of algae crops.

Table 1. Physicochemical properties of lipids at three cyanobacteria for biodiesel production

Analysis Technique	M. aeruginosa NPCD-1	Trichormus sp. CENA77	Synechococcus sp. PCC7942
Long chain saturated factor (gas chromatography)	5.7	5.0	5.2
Viscosity (Rheology) [cP]	52.7	59.1	62.3
Set points by maximum mass lose (TGA analyzer) [°C]	200 - 321	321 - 442	210 - 560
Formation ethyl esters ( $^1\text{H-NMR}$ ) 4 – 4.2 ppm	100%	50%	94%

## 7. Modeling computational for PBRs optimal design

The production of microalgae in pond models and in PBRs requires an in-depth analysis of other variables associated with the light transfer, both on close or open systems in a controlled matter. Furthermore, the understanding about hydrodynamic parameters in which the culture medium are mixed with the captured gases in the  $\text{CO}_2$  fixation process as an integral part of the photochemistry developed by the various microalgae strains.

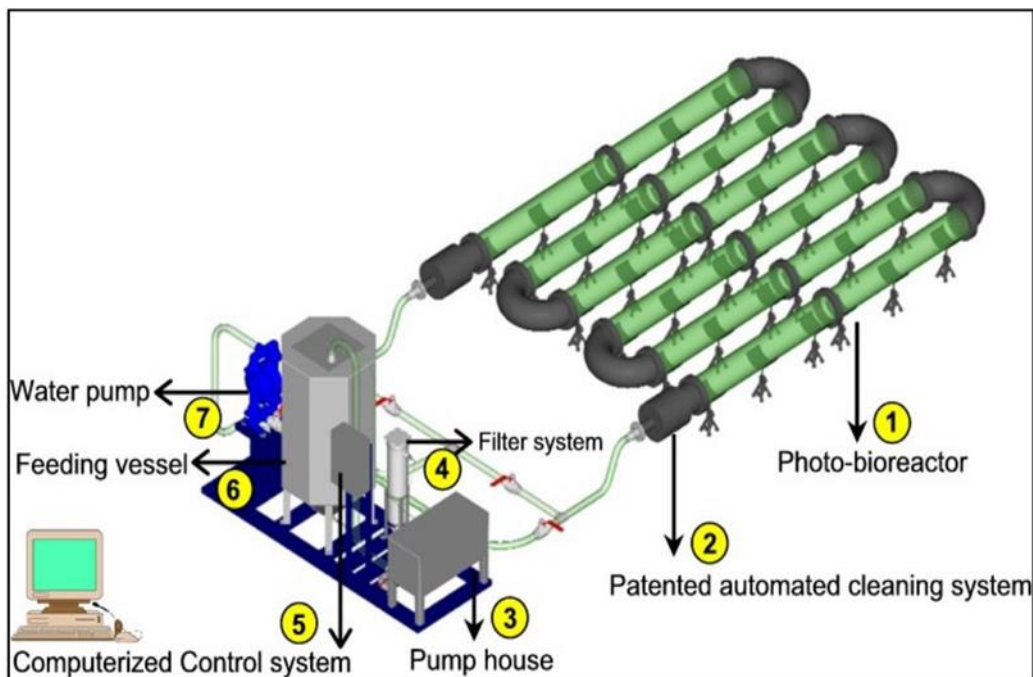


Figure 5. Compact conduits-array Photobioreactor System Design. Source: Algaelink 2007.

PBRs, like presented on Fig.5, are the most suitable design to measure and monitor the mass transfer, and the amount of gases in the culture medium, and the wall surface deposition of microalgae that limit the penetration and the scattering of light throughout the system. The close and compact PBR model, depicted on Fig 5, developed over a decade ago shows the parts of the microalgae cultures process in a general fashion. Many determining factors in the growth and life cycle of microalgae cultures have been modeling computationally based on these revolutionary designs. A computational model was developed at ANSYS FLUENT whose PBR held a cylindrical tube with an internal radius of 7.5 cm and 40 cm height, the light emitted at solid angles by irradiating light sources with wavelength among 400 and 700 nm. The simulation results were able to establish relationships among the intensity of incident radiation the radial tube distance, the number of cells in the column, the scattering effect of the rays, the size of the gas bubbles, and the air-mass flow rates (WHEATON; KRISHNAMOORTHY, 2012). The findings were the greater efficiency in radiative transfer for bubbles between 10 and 100 micrometers. Many other characteristics can be analyzed in these controlled bioreactor designs (NAUHA; ALOPAEUS, 2013).

## 8. Conclusion

The problems that the world is currently facing regarding climate change and energy demand have forced governments to strengthen policies regarding alternative energy resources, clean, sustainable, and renewable energy sources. Microalgae cultivation has been positioned as a strategy that builds on these new challenges and becomes an important solution in the transition to the gradual reduction in dependence on fossil fuels.

Despite the importance and interest that this energy resource has reached, its production at an industrial level is still very low and therefore requires great efforts to channel studies that lead to optimizing and improving its efficiency with a view to raising its energy capacity factor and the production of feedstock

as the basis of industrial activity. The monitoring of physicochemical variables as well as the characterization of substances harvested in crops will allow the necessary readjustment to make government and private investors in this energy sector much more attractive. The development of new PBRs based on new designs will allow optimizing the culture media of microalgae and cyanobacteria that are part of the spectrum of almost 100 species among the more than 10 thousand that have not yet been investigated. Without a doubt, photosynthetic efficiency based on photochemical and radiative processes should continue to be the target of many studies for the selection of new strains of natural or genetically manipulated origin. Computational modeling using Multiphysics software code offers great advantage for controlled farming systems, and great strides have been made in understanding ideas about hydrodynamics, as well as radiation and mass transfer in a predictable fashion.

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## 7. References

- ADAMS, M. W. The structure and mechanism of iron-hydrogenases. **Biochimica et Biophysica Acta (BBA)-Bioenergetics**, 1020, n. 2, p. 115-145, 1990.
- AZWAR, M.; HUSSAIN, M.; ABDUL-WAHAB, A. Development of biohydrogen production by photobiological, fermentation and electrochemical processes: a review. **Renewable and Sustainable Energy Reviews**, 31, p. 158-173, 2014.
- BATISTA, F.; LUCCHESI, K.; CARARETO, N.; COSTA, M. *et al.* Properties of microalgae oil from the species *Chlorella protothecoides* and its ethylic biodiesel. **Brazilian Journal of Chemical Engineering**, 35, n. 4, p. 1383-1394, 2018.
- BECKER, E. W. Micro-algae as a source of protein. **Biotechnology advances**, 25, n. 2, p. 207-210, 2007.
- BEHERA, S.; SINGH, R.; ARORA, R.; SHARMA, N. K. *et al.* Scope of algae as third generation biofuels. **Frontiers in bioengineering and biotechnology**, 2, p. 90, 2015.
- BERENGUEL, M.; RODRIGUEZ, F.; ACIÉN, F.; GARCIA, J. Model predictive control of pH in tubular photobioreactors. **Journal of Process Control**, 14, n. 4, p. 377-387, 2004.
- BJÖRN, L. O.; PAPAGEORGIOU, G. C.; BLANKENSHIP, R. E. A viewpoint: why chlorophyll a? **Photosynthesis research**, 99, n. 2, p. 85-98, 2009.
- BROUERS, M.; SHI, D.; HALL, D. [70] Immobilization methods for cyanobacteria in solid matrices. *In: Methods in Enzymology*: Elsevier, 1988. v. 167, p. 629-636.
- CANTOS, E.; GARCÍA-VIGUERA, C.; DE PASCUAL-TERESA, S.; TOMÁS-BARBERÁN, F. A. Effect of postharvest ultraviolet irradiation on resveratrol and other phenolics of cv. Napoleon table grapes. **Journal of Agricultural and Food Chemistry**, 48, n. 10, p. 4606-4612, 2000.
- CHANG, F.-Y.; LIN, C.-Y. Biohydrogen production using an up-flow anaerobic sludge blanket reactor. **International Journal of Hydrogen Energy**, 29, n. 1, p. 33-39, 2004.
- CHAUMONT, D. Biotechnology of algal biomass production: a review of systems for outdoor mass culture.

**Journal of Applied Phycology**, 5, n. 6, p. 593-604, 1993.

- CHIARAMONTI, D. Bioethanol: role and production technologies. *In: Improvement of crop plants for industrial end uses*: Springer, 2007. p. 209-251.
- CHINNASAMY, S.; RAMAKRISHNAN, B.; BHATNAGAR, A.; DAS, K. C. Biomass production potential of a wastewater alga *Chlorella vulgaris* ARC 1 under elevated levels of CO<sub>2</sub> and temperature. **International journal of molecular sciences**, 10, n. 2, p. 518-532, 2009.
- CHISTI, Y. Biodiesel from microalgae. **Biotechnology advances**, 25, n. 3, p. 294-306, 2007.
- COCKELL, C. S.; KNOWLAND, J. Ultraviolet radiation screening compounds. **Biological Reviews**, 74, n. 3, p. 311-345, 1999.
- COUTTEAU, P.; SORGELOOS, P. The use of algal substitutes and the requirement for live algae and their replacement by artificial diets in the hatchery and nursery rearing of bivalve molluscs: an international survey. **Journal of Shellfish Research**, 11, p. 467-476, 1992.
- DINCER, I. Green methods for hydrogen production. **International journal of hydrogen energy**, 37, n. 2, p. 1954-1971, 2012.
- DUVAL, B.; SHETTY, K.; THOMAS, W. H. Phenolic compounds and antioxidant properties in the snow alga *Chlamydomonas nivalis* after exposure to UV light. **Journal of Applied Phycology**, 11, n. 6, p. 559, 1999.
- ESTEVEZ, F. D. A. Fundamentos de limnología. Ed. **Interciencia. Brasil**, p. 122-124, 1998.
- FINKEL, T.; HOLBROOK, N. J. Oxidants, oxidative stress and the biology of ageing. **nature**, 408, n. 6809, p. 239, 2000.
- FLYNN, K. J.; GREENWELL, H. C.; LOVITT, R. W.; SHIELDS, R. J. Selection for fitness at the individual or population levels: modelling effects of genetic modifications in microalgae on productivity and environmental safety. **Journal of theoretical biology**, 263, n. 3, p. 269-280, 2010.
- GAFFRON, H. Reduction of CO<sub>2</sub> with molecular H<sub>2</sub> in green plants. **Nature**, 143, p. 204-205, 1939.
- GAFFRON, H.; RUBIN, J. Fermentative and photochemical production of hydrogen in algae. **The Journal of General Physiology**, 26, n. 2, p. 219-240, 1942.
- GARCIA - PICHEL, F. A model for internal self - shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. **Limnology and Oceanography**, 39, n. 7, p. 1704-1717, 1994.
- GHIRARDI, M. L.; ZHANG, L.; LEE, J. W.; FLYNN, T. *et al.* Microalgae: a green source of renewable H<sub>2</sub>. **Trends in biotechnology**, 18, n. 12, p. 506-511, 2000.
- GREENBAUM, E. Photosynthetic hydrogen and oxygen production: kinetic studies. **Science**, 215, n. 4530, p. 291-293, 1982.
- GREENWELL, H. C.; LAURENS, L.; SHIELDS, R.; LOVITT, R. *et al.* Placing microalgae on the biofuels priority list: a review of the technological challenges. **Journal of the royal society interface**, 7, n. 46, p. 703-726, 2009.
- GUSCHINA, I. A.; HARWOOD, J. L. Complex lipid biosynthesis and its manipulation in plants. *In: Improvement of crop plants for industrial end uses*: Springer, 2007. p. 253-279.
- GÓMEZ, I.; PÉREZ-RODRÍGUEZ, E.; VIÑEGLA, B.; FIGUEROA, F. L. *et al.* Effects of solar radiation

- on photosynthesis, UV-absorbing compounds and enzyme activities of the green alga *Dasycladus vermicularis* from southern Spain. **Journal of Photochemistry and Photobiology B: Biology**, 47, n. 1, p. 46-57, 1998.
- HAPPE, T.; MOSLER, B.; NABER, J. D. Induction, localization and metal content of hydrogenase in the green alga *Chlamydomonas reinhardtii*. **European Journal of Biochemistry**, 222, n. 3, p. 769-774, 1994.
- HAPPE, T.; NABER, J. D. Isolation, characterization and N - terminal amino acid sequence of hydrogenase from the green alga *Chlamydomonas reinhardtii*. **European Journal of Biochemistry**, 214, n. 2, p. 475-481, 1993.
- HERNANDO, M.; MALANGA, G.; FERREYRA, G. Oxidative stress and antioxidant defenses due UV radiation in sub-antarctic marine phytoplankton. **Sci. Mar**, 69, n. Suppl 2, p. 287-295, 2005.
- HOYER, K.; KARSTEN, U.; SAWALL, T.; WIENCKE, C. Photoprotective substances in Antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages. **Marine Ecology Progress Series**, 211, p. 117-129, 2001.
- JANKNEGHT, P. J.; DE GRAAFF, C. M.; VAN DE POLL, W. H.; VISSER, R. J. *et al.* Antioxidative responses of two marine microalgae during acclimation to static and fluctuating natural UV radiation. **Photochemistry and photobiology**, 85, n. 6, p. 1336-1345, 2009.
- KAPARAPU, J.; GEDDADA, M. N. R. Applications of immobilized algae. **Journal of Algal Biomass Utilization**, 7, p. 122-128, 2016.
- KERBY, N.; STEWART, W. The biotechnology of microalgae and cyanobacteria. **Biochemistry of the algae and cyanobacteria**, p. 326-327, 1988.
- KLASSON, K.; ACKERSON, M.; CLAUSEN, E.; GADDY, J. Bioreactor design for synthesis gas fermentations. **Fuel**, 70, n. 5, p. 605-614, 1991.
- KOTAY, S. M.; DAS, D. Microbial hydrogen production with *Bacillus coagulans* IIT-BT S1 isolated from anaerobic sewage sludge. **Bioresour Technol**, 98, n. 6, p. 1183-1190, Apr 2007.
- KUSKOSKI, E. M.; ASUERO, A. G.; GARCÍA-PARILLA, M. C.; TRONCOSO, A. M. *et al.* Actividad antioxidante de pigmentos antocianicos. **Food Science and Technology**, 24, n. 4, p. 691-693, 2004.
- LOURENÇO, S. O. **Cultivo de microalgas marinhas: princípios e aplicações**. RiMa São Carlos, 2006.
- MAHBOOB, S.; RAUF, A.; ASHRAF, M.; SULTANA, T. *et al.* High-density growth and crude protein productivity of a thermotolerant *Chlorella vulgaris*: production kinetics and thermodynamics. **Aquaculture international**, 20, n. 3, p. 455-466, 2012.
- MALANGA, G.; CALMANOVICI, G.; PUNTARULO, S. Oxidative damage to chloroplasts from *Chlorella vulgaris* exposed to ultraviolet - B radiation. **Physiologia Plantarum**, 101, n. 3, p. 455-462, 1997.
- MALLICK, N. Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. **biometals**, 15, n. 4, p. 377-390, 2002.
- MANISH, S.; BANERJEE, R. Comparison of biohydrogen production processes. **International Journal of Hydrogen Energy**, 33, n. 1, p. 279-286, 2008.

- MAYO, A. W.; NOIKE, T. Effect of glucose loading on the growth behavior of *Chlorella vulgaris* and heterotrophic bacteria in mixed culture. **Water research**, 28, n. 5, p. 1001-1008, 1994.
- MEHLITZ, T. H. Temperature influence and heat management requirements of microalgae cultivation in photobioreactors. 2009.
- MEYER, J.; GAGNON, J. Primary structure of hydrogenase from *Clostridium pasteurianum*. **Biochemistry**, 30, n. 40, p. 9697-9704, 1991.
- MONTEIRO, M. P. D. C.; LUCHESE, R. H.; ABSHER, T. M. Effect of three different types of culture conditions on *Spirulina maxima* growth. **Brazilian Archives of Biology and Technology**, 53, n. 2, p. 369-373, 2010.
- MORENO-GARRIDO, I. Microalgae immobilization: current techniques and uses. **Bioresource technology**, 99, n. 10, p. 3949-3964, 2008.
- MOSKAUG, J. Ø.; CARLSEN, H.; MYHRSTAD, M. C.; BLOMHOFF, R. Polyphenols and glutathione synthesis regulation. **The American journal of clinical nutrition**, 81, n. 1, p. 277S-283S, 2005.
- MURPHY, D. J. The biogenesis and functions of lipid bodies in animals, plants and microorganisms. **Progress in lipid research**, 40, n. 5, p. 325-438, 2001.
- MUÑOZ, R.; KÖLLNER, C.; GUIEYSSE, B.; MATTIASSON, B. Photosynthetically oxygenated salicylate biodegradation in a continuous stirred tank photobioreactor. **Biotechnology and bioengineering**, 87, n. 6, p. 797-803, 2004.
- MÜLLER, P.; LI, X.-P.; NIYOGI, K. K. Non-photochemical quenching. A response to excess light energy. **Plant physiology**, 125, n. 4, p. 1558-1566, 2001.
- NAUHA, E. K.; ALOPAEUS, V. Modeling method for combining fluid dynamics and algal growth in a bubble column photobioreactor. **Chemical engineering journal**, 229, p. 559-568, 2013.
- ONO, E.; CUELLO, J. L. Carbon dioxide mitigation using thermophilic cyanobacteria. **Biosystems engineering**, 96, n. 1, p. 129-134, 2007.
- OSWALD, W. J. Micro-algae and wastewater treatment. **Microalgal biotechnology**, p. 305-328, 1988.
- PAVIA, H.; CERVIN, G.; LINDGREN, A.; ÅBERG, P. Effects of UV-B radiation and simulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. **Marine Ecology Progress Series**, 157, p. 139-146, 1997.
- REDAELLI, C.; KOICHEM, L. H.; DIERINGS, T.; JARENKOW, A. *et al.* Influência da intensidade da luz sobre a biofixação de carbono em *Chlorella minutissima*. **Universidade Federal do rio Grande do Sul. Porto Alegre**, 2011.
- REUBER, S.; BORNMAN, J.; WEISSENBOCK, G. A flavonoid mutant of barley (*Hordeum vulgare* L.) exhibits increased sensitivity to UV - B radiation in the primary leaf. **Plant, Cell & Environment**, 19, n. 5, p. 593-601, 1996.
- RIBEIRO, R. L.; MARIANO, A. B.; VARGAS, J. V. Software para Simulação de Crescimento de Microalgas em Fotobiorreatores Tubulares. **Proceeding Series of the Brazilian Society of Computational and Applied Mathematics**, 4, n. 1, 2016.
- ROESSLER, P. G.; LIEN, S. Activation and de novo synthesis of hydrogenase in *Chlamydomonas*. **Plant physiology**, 76, n. 4, p. 1086-1089, 1984.
- ROZEMA, J.; BJÖRN, L. O.; BORNMAN, J.; GABERŠČIK, A. *et al.* The role of UV-B radiation in



- aquatic and terrestrial ecosystems—an experimental and functional analysis of the evolution of UV-absorbing compounds. **Journal of Photochemistry and Photobiology B: Biology**, 66, n. 1, p. 2-12, 2002.
- RÓS, P.; DA, C.; SILVA, C. S.; SILVA-STENICO, M. E. *et al.* Assessment of chemical and physico-chemical properties of cyanobacterial lipids for biodiesel production. **Marine drugs**, 11, n. 7, p. 2365-2381, 2013.
- SAIFUDDIN, N.; PRIATHARSINI, P. Developments in bio-hydrogen production from algae: a review. **Res J Appl Sci Eng Technol**, 12, n. 9, p. 968-982, 2016.
- SCALBERT, A.; WILLIAMSON, G. Dietary intake and bioavailability of polyphenols. **The Journal of nutrition**, 130, n. 8, p. 2073S-2085S, 2000.
- SCHULZ, R. Hydrogenases and hydrogen production in eukaryotic organisms and cyanobacteria. **Journal of marine biotechnology**, 4, n. 1, p. 16-22, 1996.
- SHERIF, S. A.; BARBIR, F.; VEZIROGLU, T. N. Principles of hydrogen energy production, storage and utilization. 2003.
- SHIU, C.-T.; LEE, T.-M. Ultraviolet-B-induced oxidative stress and responses of the ascorbate–glutathione cycle in a marine macroalga *Ulva fasciata*. **Journal of Experimental Botany**, 56, n. 421, p. 2851-2865, 2005.
- SOLETTI, D.; BINAGHI, L.; LODI, A.; CARVALHO, J. *et al.* Batch and fed-batch cultivations of *Spirulina platensis* using ammonium sulphate and urea as nitrogen sources. **Aquaculture**, 243, n. 1-4, p. 217-224, 2005.
- TAMAGNINI, P.; LEITÃO, E.; OLIVEIRA, P.; FERREIRA, D. *et al.* Cyanobacterial hydrogenases: diversity, regulation and applications. **FEMS microbiology reviews**, 31, n. 6, p. 692-720, 2007.
- THAJUDDIN, N.; SUBRAMANIAN, G. Cyanobacterial biodiversity and potential applications in biotechnology. **Current science**, p. 47-57, 2005.
- VAN GERPEN, J. Biodiesel production. *In: Improvement of Crop Plants for Industrial End Uses*: Springer, 2007. p. 281-289.
- VIMALABAI, C.; KULANDAIVELU, G. Effects of prolonged UV-B enhanced fluorescent radiation on some marine microalgae. **Biologia plantarum**, 45, n. 3, p. 389-394, 2002.
- VOORDOUW, G.; STRANG, J. D.; WILSON, F. R. Organization of the genes encoding [Fe] hydrogenase in *Desulfovibrio vulgaris* subsp. *oxamicus* Monticello. **Journal of Bacteriology**, 171, n. 7, p. 3881-3889, 1989.
- WHEATON, Z. C.; KRISHNAMOORTHY, G. Modeling radiative transfer in photobioreactors for algal growth. **Computers and electronics in agriculture**, 87, p. 64-73, 2012.
- WILLIAMS, P. J. L. B.; LAURENS, L. M. Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics. **Energy & Environmental Science**, 3, n. 5, p. 554-590, 2010.
- WONG, C.; CHU, W.; MARCHANT, H.; PHANG, S. Comparing the response of Antarctic, tropical and temperate microalgae to ultraviolet radiation (UVR) stress. **Journal of Applied Phycology**, 19, n. 6, p. 689-699, 2007.
- XU, H.; MIAO, X.; WU, Q. High quality biodiesel production from a microalga *Chlorella protothecoides*

by heterotrophic growth in fermenters. **Journal of biotechnology**, 126, n. 4, p. 499-507, 2006.  
ZHANG, L.; HAPPE, T. Biochemical and morphological characterization of sulfur-deprived and hydrogen-producing *Chlamydomonas reinhardtii* (green algae). **Science Access**, 3, n. 1, 2001.  
ZUDAIRE, L.; ROY, S. Photoprotection and long-term acclimation to UV radiation in the marine diatom *Thalassiosira weissflogii*. **Journal of Photochemistry and Photobiology B: Biology**, 62, n. 1-2, p. 26-34, 2001.

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