# The importance of microalgae biotechnology to the wastewater treatment and biofuels production

# A importância da biotecnologia das microalgas para o tratamento de águas residuais e a produção de biocombustíveis

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**Andreza de Lima Souza**
Public Health and Environment Program, Fundação Oswaldo Cruz (ENSP/FIOCRUZ),
Environmental Engineering Program, Federal University of Rio de Janeiro (PEA/UFRJ)
Avenida Athos da Silveira Ramos, 149, Rio de Janeiro, RJ, Brazil, 21941-909.
E-mail: andreza.lima@poli.ufrj.br

**Marcelle Candido Cordeiro**
M.Sc
Production Engineering Program, Federal University of Rio de Janeiro (UFRJ),
Avenida Aluizio da Silva Gomes, 50, Novo Cavaleiros, Macaé, 27930-560
Production Engineering Program, Federal University of Rio de Janeiro
(PEP/COPPE/UFRJ)
Avenida Horácio Macedo, 2030, Rio de Janeiro, RJ, Brazil, 21941-598
E-mail: marcelle.candido@coppe.ufrj.br.

**Anna Cristina Pinheiro de Lima**
D.Sc
School of Chemistry, Federal University of Rio de Janeiro (EQ/UFRJ)
Avenida Athos da Silveira Ramos, 149, Rio de Janeiro, RJ, Brazil, 21941-909
E-mail: anna.pinheiro.lima@gmail.com

**Carla Patricia Figueiredo Antunes de Souza**
M.Sc
Secretaria Municipal de Saúde do Município do Rio de Janeiro (SMS). Rua Afonso
Cavalcante 455 CASS sala 801, Cidade Nova, Rio de Janeiro
Public Health, Fundação Oswaldo Cruz (ENSP/FIOCRUZ)
Rua Leopoldo Bulhões, 1480, Rio de Janeiro, RJ. Brazil, 21041-210
E-mail: cpatricia.farma@gmail.com

## ABSTRACT

After the Second Industrial Revolution, the use of fossil fuels increased due to growth of cities. The burning of these fuels results in air pollution, which can cause health problems in the most sensitive population, environmental impacts due to $SO_2$, and $CO_2$ emissions in the atmosphere that add to the greenhouse effect. The unbridled consumption of fossil fuels, a non-renewable resource, has stimulated many countries to invest in different types of renewable energies including biofuels. Biofuels can be produced from agricultural crops (first generation); residual biomass from the agricultural sector, which are not fit to the human consumption (second generation); residual microalgae biomass (third generation); and genetically modified microalgae biomass (fourth generation). The microalgae have become an attractive resource for biofuel production because they do not require arable lands for their cultivation and can use wastewater as growth medium, while simultaneously treating this effluent by assimilating the pollutants presents in these waters (nitrogen, phosphorus, and metals). The aim of this work is to analyze the relevance of microalgae applied for wastewater treatment and biofuels production, through an exploratory literature review. Wastewater treatment using microalgae is well documented and presents efficient results for biofuels production; nevertheless the use of microalgae is still in the development phase and the major barrier being economic viability. Additional studies are recommended that explore more microalgae species and focus on genetic engineering to make this biotechnology economically viable.

**Keywords:** Microalgae, Wastewater treatment, Biomass, Biofuel, Biorefineries. Biotechnology.

## RESUMO

Após a Segunda Revolução Industrial, o uso de combustíveis fósseis aumentou devido ao crescimento das cidades. A queima desses combustíveis resulta em poluição do ar, que pode causar problemas de saúde na população mais sensível, impactos ambientais devido ao SO2 e emissões de CO2 na atmosfera que se somam ao efeito estufa. O consumo desenfreado de combustíveis fósseis, um recurso não renovável, tem estimulado muitos países a investir em diferentes tipos de energias renováveis, incluindo os biocombustíveis. Os biocombustíveis podem ser produzidos a partir de cultivos agrícolas (primeira geração); biomassa residual do setor agrícola, que não são adequados ao consumo humano (segunda geração); biomassa residual de microalgas (terceira geração); e biomassa de microalgas geneticamente modificadas (quarta geração). As microalgas se tornaram um recurso atraente para a produção de biocombustíveis, pois não requerem terras aráveis para seu cultivo e podem utilizar as águas residuais como meio de crescimento, ao mesmo tempo em que tratam este efluente assimilando os poluentes presentes nestas águas (nitrogênio, fósforo e metais). O objetivo deste trabalho é analisar a relevância das microalgas aplicadas no tratamento de águas residuárias e na produção de biocombustíveis, através de uma revisão exploratória da literatura. O tratamento de efluentes com microalgas está bem documentado e apresenta resultados eficientes para a produção de biocombustíveis; entretanto, o uso de microalgas ainda está em fase de desenvolvimento e a maior barreira é a viabilidade econômica. São recomendados estudos adicionais que explorem mais espécies de microalgas e se concentrem na engenharia genética para tornar esta biotecnologia economicamente viável.

**Palavras-chave:** Microalgas, Tratamento de águas residuais, Biomassa, Biocombustível, Biorrefinarias, Biotecnologia.

## 1 INTRODUCTION

Since the First Industrial Revolution, the fossil fuels have become the foremost component of the world's energy mix. However, their use generates some impasses, especially the ability of the planet to handle the growing energy demand of contemporary societies. Due to runaway consumption, this fuel will become scarce in the future, making renewable energy sources more attractive (DEMIRBAS, 2009; MATA; MARTINS; CAETANO, 2010; UTAMA et al., 2014). Several countries are already seeking alternatives and investing in other technologies, such as solar, thermal, photovoltaic, hydroelectric, geothermal, wind, and biofuels (CORAM; KATZNER, 2018).

Biofuels are fuels of biological origin derived from biomass which did not fossilize. Depending on their origin, they have different classifications such as biodiesel, bioethanol, biogas, biomethane (DEMIRBAS, 2009).

First generation biofuels (1G) are produced from agricultural crops that could produce foods such as sugarcane, in Brazil, and the corn, in USA. According to the reports by the Food and Agriculture Organization (FAO), one of the biggest problems of the first generation biofuels is that they compromise the availability of food commodities (AHMAD et al., 2011; DAROCH; GENG; WANG, 2013; VALDIVIA et al., 2016). Second generation biofuels (2G) are obtained from residual agricultural biomass, which are found in high quantities and are not appropriate for human consumption (AHMAD et al., 2011; CARDONA ALZATE; SOLARTE TORO; PEÑA, 2018; DAROCH; GENG; WANG, 2013). Third generation biofuels (3G) are produced from residual microalgal biomass (AHMAD et al., 2011; DAROCH; GENG; WANG, 2013; FARIED et al., 2017). The fourth generation (4G) has the same origin as the third; however; it is focuses on studies of metabolic engineering (DUTTA; DAVEREY; LIN, 2014; LÜ; SHEAHAN; FU, 2011; SHUBA; KIFLE, 2018).

In this context, microalgae have emerged as a potential sustainable biomass resource (AMARO; GUEDES; MALCATA, 2011). Moreover, these microorganisms can treat wastewater by eliminating nitrogen, phosphorus, and metals, which are pollutants present in these waters (CAI; PARK; LI, 2013).

The use of microalgae biotechnology to obtain biofuels is relevant because the microbial biomass composition is formed mostly of organic macromolecules, such as protein, carbohydrates, lipids, the latter one found in rate values of 7–23% (RIZWAN et al., 2018; ZHU, 2015a). Microalgae are resistant to temperature changes, luminosity, and pH (CHISTI, 2007a). They are able to perform photosynthesis, which consists of a natural physicochemical process, where the microalgae capture the $CO_2$ present in the atmosphere or in the industrial environment and converts solar energy into chemical energy in the form of biomass (PRIYADARSHANI; SAHU; RATH, 2012).

The use of microalgae instead of terrestrial plants to produce biofuels, tend to be more cost-effective, since they have a much higher oil yield than and require much less land space (HATTAB; GHALY, 2015; KROUMOV et al., 2016; WIJFFELS; BARBOSA, 2010).

The aim of this study was to report the relevance of microalgae biotechnology, in a context of biorefineries. This exploratory literature review focused on the applications of microalgae in wastewater treatment and the limiting factors of studies related to microalgae biotechnology in Brazil.

## 2 MICROALGAE

Microalgae are photosynthetic microorganisms capable of surviving in marine and freshwater environments. They can be prokaryotes, for example cyanobacteria (*Cyanophyceae*), or eukaryotes, such as green algae (*Chlorophyceae*), gold algae (*Chrysophyceae*), and diatoms (*Bacillariophyceae*) (HATTAB; GHALY, 2015).

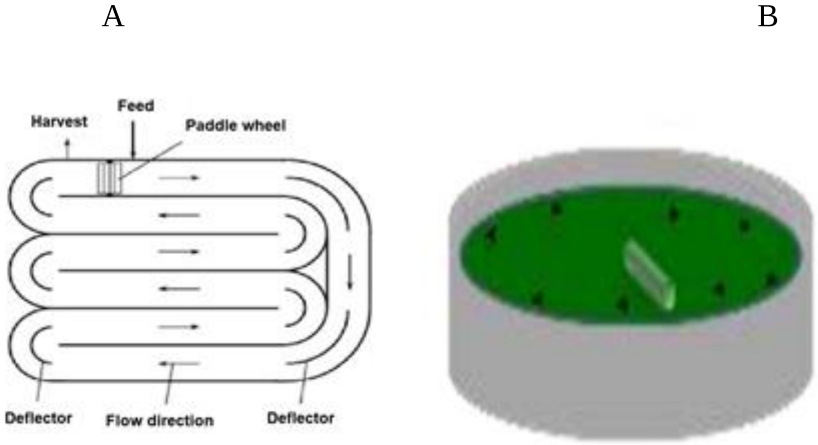
Microalgae are rich at three main chemicals compounds: proteins (6–52%), carbohydrates (5–23%), and lipids (7–23%) (RIZWAN et al., 2018; RIZWAN; LEE; GANI, 2015; ZHU, 2015b). From a cultivation method, which uses microalgae, several with high value-added products are used in animal feed, pharmaceutical and medicinal, cosmetic, and biochemistry products (MORENO-GARCIA et al., 2017; SUGANYA et al., 2016). To produce biofuels, the microalgae need to contain or produce high quantity of lipids, which range from 1 to 70% (CHISTI, 2007a; ROSENBERG et al., 2008). *Chlorella vulgaris* and *Dunaliella* sp. can have more than 50% lipid content, but with different productivity (MATA; MARTINS; CAETANO, 2010). In addition, their versatile nature permits growth in many wastewater treatment systems, to remove pollutants present in these waters (ABINANDAN; SHANTHAKUMAR, 2015). In combination, biofuels are also important products produced using microalgae (CAVALCANTI et al., 2021; JANKOWSKA; SAHU; OLESKOWICZ-POPIEL, 2017).

### 2.1 CULTIVATION

The use of microalgae begun in 1960s, in Japan, when C*hlorella* sp. was targeted. However, the motivation for use of these microorganisms as energy began in the 1970s, when the world suffered the first oil crisis. Since then, alternative energies are seen as beneficial due to the concern about oil prices and environmental issues. Following World War II, the United States, Germany, Japan, and England developed crop methods and engineering methodologies for greater quantities of microalgae and on a large scale (FARIED et al., 2017). For cultivation, three options of bioreactors are available: Open ponds, photobioreactors (CHISTI, 2007b), and biofilm algae (KIRAN; KUMAR; DESHMUKH, 2014; MANTZOROU; VERVERIDIS, 2019).

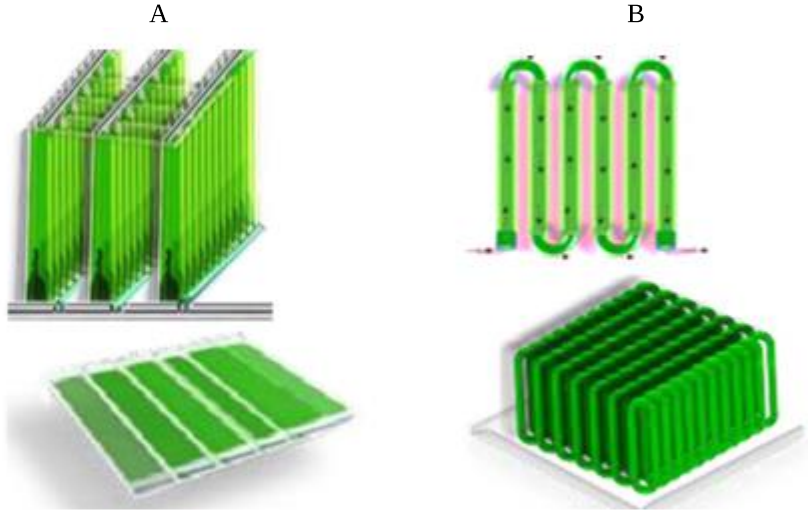
Open ponds are open systems which include natural ponds using circular, raceway, and inclined systems (Fig. 1).

**Figure 1** – Layouts of open ponds: (A) raceway and (B) Circular pond. Adapted from (CHISTI, 2007a; RASTOGI et al., 2017).



These ponds are simple projects, are cheaper to build, and have high production capacity (CARLSSON; VAN BEILEN; MÖLLER; CLAYTON, 2007). Photobioreactors are closed systems used to cultivate one microalgae specie for long periods without contaminations and are known as tubular and flat plate (Fig. 2).

**Figure 2 –** Representations of photobioreactors (a) flat plate (b) tubular. From: (RASTOGI et al., 2017).



The first has a combination of transparent tubes made of glass, plexiglass, or polycarbonate (KIRAN; KUMAR; DESHMUKH, 2014). The closed system is better because the temperature is regulated (PATEL et al., 2017). **Table 1** presents the greatest advantages and disadvantages of these systems.

**Table 1:** Advantages and disadvantages of microalgal systems.

| Croping system | Advantages | Disadvantages | Reference |
|---|---|---|---|
| Open pond | • Lower operation costs;<br>• Simple design. | • Requires extensive lands;<br>• More risk of contamination. | (CARLSSON; VAN BEILEN; MÖLLER; CLAYTON, 2007; COLLING KLEIN; BONOMI; MACIEL FILHO, 2018a; WOLKERS H, BARBOSA M, KLEINEGRIS DM, BOSMA R, 2011). |
| Photobioreator | • No contamination;<br>• Temperature, pH, and light intensity can be controlled. | • Higher operating cost than the open system;<br>• Low biomass production. | (BERNER; HEIMANN; SHEEHAN, 2014; KUMAR et al., 2015; RASTOGI et al., 2017; SEVDA et al., 2017). |
| Algae biofilm | • Produces more concentrated biomass than closed photobioreactors. | • Not effective to control the micralgae species and temperature. | (WOLKERS H, BARBOSA M, KLEINEGRIS DM, BOSMA R, 2011). |

Algae biofilm include algae, cyanobacterial, and heterotrophic bacteria, which live in symbiosis (SCHNURR; ALLEN, 2015) and are embedded in extracellular polymeric substances (EPSs) that grow on a solid surface (MANTZOROU et al., 2018). The biofilm allows development, growth, and fixation of microalgae on the desired solid surface for long periods (ZENG et al., 2015)..

### 2.1.2 Harvesting

The methods to harvest the microalgae are the flocculation, gravity sedimentation, and centrifugation as well as manual techniques (BRENNAN; OWENDE, 2010; GARBOWSKI et al., 2017). The microalgae specie, cell density, and cultivation conditions should be taken into consideration (CARLSSON; VAN BEILEN; MÖLLER; CLAYTON, 2007). In wastewater treatment systems, which use unit operations, setting secondary tank, sedimentation, filtration, clarification secondary and water, appropriate and affordable techniques are important to collect the biomass (ABINANDAN; SHANTHAKUMAR, 2015).

### 2.1.3 Microalgae and wastewater treatment

Microalgae can remove nitrogen and phosphorus in polluted waters. Due to their versatile nature, they can grow while simultaneously removing these pollutants

(ABINANDAN; SHANTHAKUMAR, 2015). Oliveira et al. (2017) isolated microalgae *Asterarcys quadricellulare* in a wastewater treatment. The microalgae were cultivate at a temperature of 24.2 ± 1.2 °C under mixotrophic conditions under 12 h light/dark intervals in 2-L photobioreactors. The researchers obtained absorbed of significant pollutant quantities (not specified) causing a remediation of this water. Unfortunately, the authors did not specified which photobioreactor was used in the research, or from which effluent they removed the microalgae for future analyses.

Conventional systems are not economic. Biological wastewater treatment systems can remove organic matter, preventing nitrogen and phosphorus concentrations in sewage from being discharged into water bodies, which otherwise could result in eutrophication (HAANDEL et al., 2009; ZHI et al., 2016). Eutrophication is the enrichment of the aquatic environment with nutrients, causing the growth of aquatic organisms and plants that can reach levels which interfere with desirable uses of the water body (HAANDEL et al., 2009).

Thus, microalga system is viable because nitrogen and phosphorus feed microalgae improving their growth while removing these elements from wastewater treatments (RAZZAK et al., 2013; ZENG et al., 2015). Studies involving *Chlorella* sp., *Scenedesmus* sp., and *Chlamydomonas* sp. concluded that they can efficiently remove those pollutants (XIAO et al., 2012). Another study reported efficient $CO_2$ absorption using *Chlorella* sp. and *Chlorococcum* sp. According to the researchers, the concentration of $CO_2$ increased up to 5% and the effective removal of dissolved organic nutrients in wastewater (YADAV; DASH; SEN, 2019). A pathway to reduce the damages would be integrating conventional systems with a microalgae pond to remove heavy metals and toxic compounds (ABINANDAN; SHANTHAKUMAR, 2015; MATAMOROS et al., 2015). Although, many results have proven the ability of microalgae to eliminate pollutants, the synergistic and antagonistic effects could consider the possibility of combination between pollutants (DELRUE et al., 2016). Through microalgae, quantity of biomass can be obtained and then be used to produce biofuels, as well as carbon sequestering that are emitted through direct and indirect oxidation of organic carbon in wastewater treatment plants, minimizing greenhouse gases emission (IASIMONE et al., 2017).

According to (SHCHEKOTYKHIN; JANNACH; SCHMITZ, 2015), microalgae are considered a sustainable technology, given their beneficial action in wastewater. The advantages resulting from the cultivation of microalgae in these waters are:

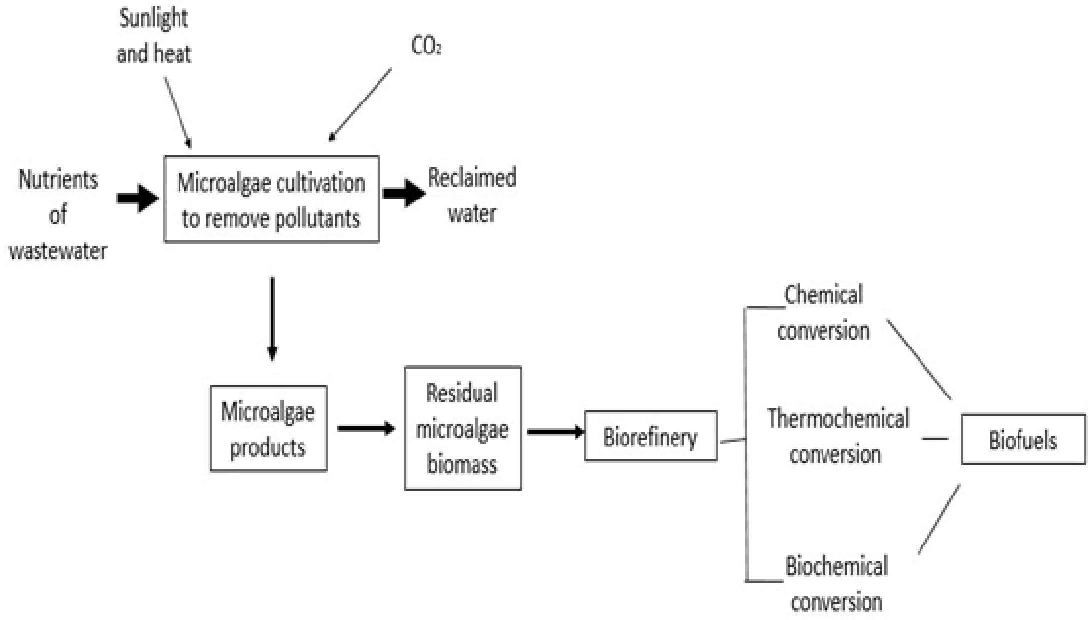
- low treatment cost;
- low energy demand;
- low sludge production;
- generation of biomass for the biofuel production.

Gonçalves et al. (2017) describe the relevance of studies on wastewater treatment using microalgae.  Due to this in the medium/long term, the use of chemical fertilizers will be impractical, especially with respect to the production of biofuels. The authors also emphasize that most publications compare source of nutrients from chemical fertilizers with the application of wastewater for microalgal cultivation. Studies involving metabolic engineering with other factors can be used to enhance the development of microalgae consortia.

## 2.2 BIOREFINERY AND BIOFUEL PRODUCTION FROM MICROALGAE BIOTECHNOLOGY

Biofuels from microalgae is considered environmentally friendly and sustainable. To obtain biofuels, a specie of microalgae needs to produce a certain quantity of oil and consider the cell structure, cell form, and the oil composition (LUTZU et al., 2016). The production of biofuels occur in  microalgal species with high levels of lipids, and their synthesis of mainly nonpolar triglycerides (which are the best substrates to produce biodiesel) can be useful to change the cultivation conditions (TRINIDAD et al., 2013). This production can occur through three types of routes: thermochemical, chemical, and biochemical (ZHU et al., 2014). In the first case, the biomass is tested at different temperatures. The goal is to have products with different attributes. Therefore, the thermochemical process can produce synthetic gas, bio-oil through liquefaction, a mixture of products through pyrolysis, and electricity production through direct combustion. The chemical process can obtain lipids through extraction. The appropriate solvent must be economic, non-polar, volatile, etc. (SHEVCHENKO; SHEVCHENKO, 2001). The biochemical process uses biologic activity of some organisms to produce specific fuels (BRENNAN; OWENDE, 2010). Anaerobic digestion can produce biogas, bio-hydrogen and others fuels (DEMIRBAS, 2011). Figure 3 shows the diagram of a microalgae biorefinery.

**Figure 3 –** Diagram of a biorefinery Adapted from: (JANKOWSKA; SAHU; OLESKOWICZ-POPIEL, 2017; NAIK et al., 2010).



### 2.2.1 Economic viability

The use of microalgae just to obtain biofuel is not economical yet compared with fossil fuel (BRASIL; SILVA; SIQUEIRA, 2017; XIN et al., 2016). The amount of water and nutrients, such as carbon, nitrogen and phosphorus for development (CHEN; ZHAO; QI, 2015) and the maintenance with the cultivation techniques to obtain biomass algae is high and the low biomass concentration directly influences the cost (MEDIPALLY et al., 2015; ZHU; KETOLA, 2012). In a critical review Rogers et al. (2014) figured that the operation costs was approximately 134 million dollars per year. The authors concluded that alternatives and solutions exist for these problems, but developments are still needed for economic and sustainable resolutions.

Currently, biofuel production using microalgae species is not profitable (MOBIN; ALAM, 2017), but it could be lucrative if the high-value coproducts, such as β-caroten, astaxanthin, acid docosahenoic, acid eicosapentaenoic, pigments bioactive in-functional, colorant naturals, polysaccharides, antioxidants, algae extracts, among others, are also produced.

Some alternatives indicate that the biorefinery idea is good, if other products are produce from biomass, beyond the biodiesel. These coproducts would be a way to make biofuel production from microalgae more economically attractive (CHISTI, 2007b; PATEL et al., 2017). The use of waste for microalgae cultivation and multilevel processing for the extraction of total energy from the mix can reduce the cultivation costs and thus reduce the associated biofuel prices (CHEN et al., 2018). More investment in genetic and metabolic engineering is aimed at increase lipid production (CHISTI, 2007a; GONÇALVES; PIRES; SIMÕES, 2017; MEDIPALLY et al., 2015; SHIN et al., 2016).

### 2.2.2 Microalgae biotechnology and limiting factors of microalgae biotechnology in Brazil

Brazil begun research about biofuel in 1970s, during the oil crisis. The National Alcohol Fuel Program (ProÁlcool) was founded that stimulated the production and commerce of alternative fuels to substitute the fossil fuel (HALL et al., 2009; LAGO et al., 2012). Currently, Brazil is considered the largest producer of biofuels. Along with the United States, it lead the bioethanol commerce (CAPAZ et al., 2020; LAMERS et al., 2011).

Brazil has a lot of sun during most of the year and a rich flora with 40.989 species that can facilitate biofuel production. Petrobras and Embrapa have led project for production of biodiesel from marine microalgae (BRASIL; SILVA; SIQUEIRA, 2017). In addition, microalgae cultivation could help to improve sanitization rates and expand the biofuel production in Brazil.

The challenges reported are more confidence to invest in biorefinery through incentives in laboratorial scales to enhance the usefulness of microalgae biomass. Recent developments in microalgae biotechnology are overcoming challenges. Brazil has many research centers; however, it needs to create public policy that stimulate the creation of enterprises, which want to invest in microalgae biotechnology (ANDRADE; TELLES; LEITE CASTRO, 2020; BRASIL; SILVA; SIQUEIRA, 2017; COLLING KLEIN; BONOMI; MACIEL FILHO, 2018b; RIZWAN et al., 2018).

## 3 CONCLUSION

Biofuel production is an alternative to confront energy demand. The microalgae biomass production development through processes and technologies allow harvest of these organisms for biofuels production. They can be produced in marginal lands, using salty, brackish, or residual waters.

Using wastewater to grow microalgae has many benefits, such as nitrogen and phosphorus removal from wastewaters and reduced eutrophication of hydric bodies. These nutrients can be efficiently recovered and recycled to produce microalgal biomass, which can be used to produce biofuels.

More research needs to be conducted as many microalgae species have not been explored. These studies are important to prove the genetic engineering application as a way to decode microalgae genes, gaining more economic viability in comparison with fossil fuels.

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