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MICROALGAE AS A PROMISING ALTERNATIVE FOR DEVELOPMENT OF BIOREFINERIES: MAIN TECHNOLOGICAL AND ECONOMICAL CHALLENGES

Microalgas como alternativa promissora para o desenvolvimento de biorrefinarias: principais desafios tecnológicos e econômicos

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ABSTRACT

Biorefining is sustainable biomass processing to obtain energy, biofuels and high value products through different technologies and processes for biomass transformation. On the other hand, microalgae have been received great interest as a biofuel feedstock in response to the uprising energy crisis, climate change and depletion of natural sources. However, the development of microalgal biofuels does not satisfy the economic feasibility to reach commercial status. Due to this, different high-value co-products have been produced through the extraction of microalgae fractions to improve the economical profile of this technology, generating in this way the microalgae biorefineries. Examples of these high-value products are pigments, proteins, lipids, carbohydrates, vitamins, and antioxidants, with applications in cosmetics, nutritional and pharmaceuticals industries. To promote the sustainability of this process, an innovative microalgae biorefinery structure is implemented through the generation of multiple products, usually in form of biofuel and other high value products. This review presents the current challenges in the extraction of high value products from microalgae and its integration in the biorefinery. It describes the general characteristics of microalgae, and their potential to be used as a raw material in the biorefinery process.

Keywords: microalgae, bioenergy, bioproducts, biorefineries.

RESUMO

O biorrefinamento é o processamento sustentável de biomassa para a obtenção de energia, biocombustíveis e produtos de alto valor por meio de diferentes tecnologias e processos de transformação de biomassa. Por outro lado, as microalgas têm recebido grande interesse como matéria-prima para biocombustíveis em resposta à crescente crise energética, às mudanças climáticas e ao esgotamento das fontes naturais. No entanto, o desenvolvimento de biocombustíveis microalgais não satisfaz a viabilidade econômica para atingir o status comercial. Por conta disso, diferentes coprodutos de alto valor têm sido produzidos através da extração de frações de microalgas para melhorar o perfil econômico dessa tecnologia, gerando, assim, as biorrefinarias de microalgas. Exemplos desses produtos de alto valor são pigmentos, proteínas, lipídios, carboidratos, vitaminas e antioxidantes, com aplicações nas indústrias cosmética, nutricional e farmacêutica. Para promover a sustentabilidade desse processo, uma estrutura inovadora de biorrefinaria de microalgas é implementada por meio da geração de múltiplos produtos, geralmente na forma de biocombustíveis e outros produtos de alto valor. Esta revisão apresenta os desafios atuais na extração de produtos de alto valor de microalgas e sua integração na biorrefinaria. Descreve as características gerais das microalgas e seu potencial para serem utilizadas como matéria-prima no processo de biorrefinaria.

Palabras-chave: *microalgas, bioenergia, bioprodutos, biorrefinarias.*

INTRODUCTION

In recent years, different conditions such as climate change and the energy crisis have encouraged the society to develop different technologies in order to address these problems. Among the different available alternatives, biorefineries are an option that have been attracted the attention of researchers and the industry. The biorefineries could work such as conventional oil refineries, with the difference that, through sustainable processes, different high-value products are obtained. Biorefineries could use different raw materials for transformation into products, such as corn energy crops, residues from agricultural or livestock industries, among others; However, a raw material that has been shown competitive performances is microalgae (González-Delgado & Kafarov, 2011; Palomeros-Parada; Osseweijer & Posada-Duque, 2016; Chew *et al*., 2017).

Microalgae are photosynthetic microorganisms that could live in freshwater, marine and even in wastewater environments. Also, they have the ability to accumulate compounds (i.e., carotenoids, pigments, fatty acids, vitamins, proteins, among others), that could be commercialized in different industries such as pharmaceuticals, nutrition, energy, etc. (Santos-Ballardo; Valdez-Ortiz & Rossi-Heras, 2016b; Chew *et al*., 2017).

The microalgae use on industrial level is not a novelty, because there are many research on this topic. However, the exploitation of this resource has not been considered optimal, mainly in economic/technical issues; for these reasons, schemes such as biorefineries are proposed to improve the yields of this raw material as much as possible. Nowadays, the microalgae have been positioned as potential candidates for two main products: biofuels and bioactive compounds (Koyande *et al*., 2019 Caguimbal *et al*., 2019; Ambriz-Perez *et al*., 2021).

On biodiesel, microalgae are a possible source because of their high lipid content (with adequate FA profiles) that could be transformed into biofuels. (Martinez-Guerra *et al*., 2018; Gorry; Sánchez & Morales, 2018; Gárate-Osuna, 2020). Regarding to biogas production, different authors reported some microalgae species as good substrates for anaerobic digestion and some of them even can compete with sources that currently are used for this purpose (Fuentes-Grünewald *et al*., 2012; Zamalloa; Boon & Verstraete, 2012; De Vrieze *et al*., 2015; Santos-Ballardo *et al*., 2016a; Merlo *et al*., 2021). Also, bioethanol production is considered feasible for some microalgae species reported with high amount of carbohydrates. Microalgae biomass fermentation for bioethanol production at industrial scales has been investigated and some authors reported as a feasibility option (Harun; Danquah & Forde, 2010; Alfenore & Molina-Jouve, 2016; Farias-Silva & Bertucco, 2016; Martin-Juarez *et al*., 2017).

In terms of bioactive compounds, microalgae biomass has proven to be a promising source of compounds such as pigments, polyunsaturated fatty acids and phenolic compounds. Also, these bioactive compounds have been reported to be related with neuroprotective, anti-inflammatory, antibiotic, antimicrobial, among other properties, which could have potential applications in industries such as pharmaceuticals and nutrition. Furthermore, microalgal pigments (i.e., chlorophylls, carotenoids, and phycobilins), has an important antioxidant activity, and these compounds could be used in nutrition as supplements, showing a positive impact on human health. Also, polyunsaturated fatty acids (PUFA) are other bioproduct obtained of microalgal biomass, PUFA's are considered essential nutritional components, and the consumption of these can prevent health problems, preventing different diseases like mental disorders and cardiovascular problems. Phenolic compounds are other bioproduct obtained of microalgae, these are mostly found in brown microalgae species. These compounds could help to prevent photooxidative processes, which trigger problems such as melanogenesis (Thomas & Kim, 2013; Ummalyma; Sahoo & Pandey, 2020; Oliver *et al*., 2020).

Finally, microalgae biorefineries are a topic that has been studied in recent years, and they are considered a promising alternative to some socio-environmental problems. However, although it is known that it may be technically feasible to obtain different bioproducts in a production line with microalgae biomass, it is important the research and technological development, in order to reach techno-economically feasibility. This review presents the current challenges in the extraction of high value products from microalgae and its integration in biorefineries. It describes the state of the art of the recent literature on microalgae biofuel production, with a strong emphasis on the concept of biorefineries and the derivation of high value products from microalgae. Also, the advantages and disadvantages of the main biofuel conversion processes from microalgae biomass and the possibilities of derivation of high value products are analyzed, in order to present the main challenges facing the development of microalgae biorefineries.

Biorefineries

The biorefinery concept can be compared to the current concept of oil refineries, mainly because it regards to the fractioning of a complex mixture. However, there are two major elements that make them different: 1) the first is the raw material used, because those used in biorefinery are organic matter that not undergone on biodegradation of crude

oil over time; due to this, the possibilities of obtaining more products using biomass as a feedstock are greater; and 2) the second is the application of different existing and emerging technologies in order to obtain bioproducts (González-Delgado & Kafarov, 2011).

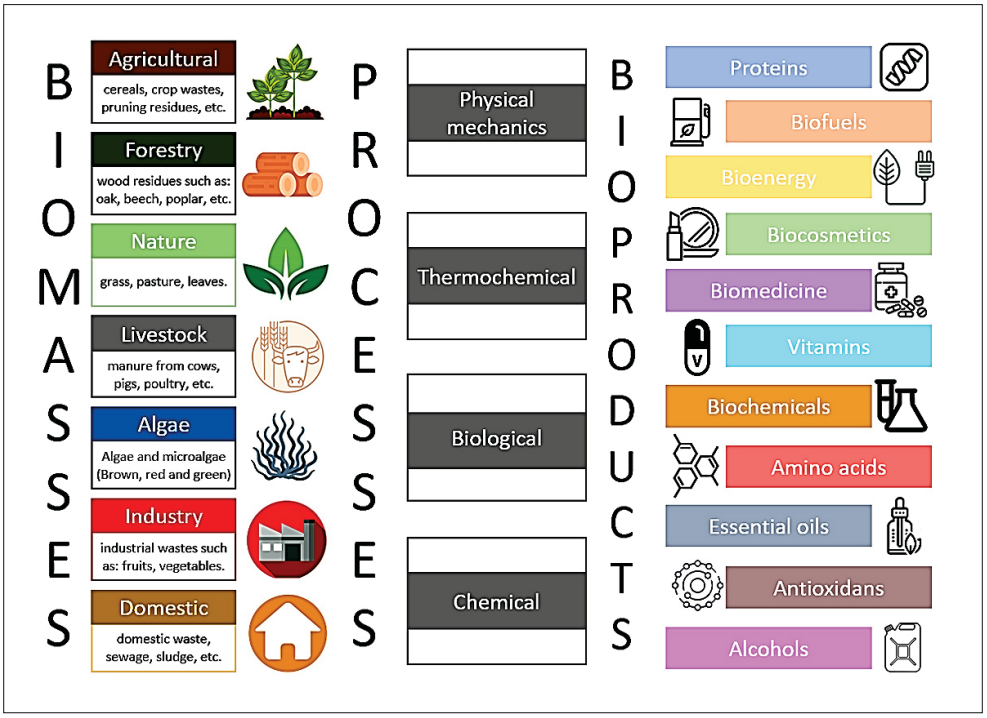
These facilities represent the optimal combination of biological, physical, chemical and thermal, processes for obtaining valuable products. Typically, different technologies are used to produce chemicals, bioenergy and biofuels, food products, biomaterials and other bioproducts. Biorefineries are not an entirely new concept, mainly because the processes are very similar to those used in oil refineries. However, this technology recently have been shown a rapid developing in order to diminish the main environmental problems and energetic issues (Diep *et al*., 2015; Palomeros-Parada; Osseweijer & Posada-Duque, 2016).

Raw materials used in biorefineries

There is a wide variety of organic materials that can be used as feedstocks in a biorefinery, which generally come from five key productive sectors: agricultural, forestry, industrial, aquaculture and domestic sectors (Agencia Provicional de la Energía, 2014).

For biorefineries, the raw material used for bioproducts development could be biomass obtained from different sources. This biomass is transformed (through different processes) in a wide range of products that are finally demanded. Moreover, it can be cultivated for exclusive use in a biorefinery, or could be obtained from industrial sectors, including the residual materials wastes with potential to be transformed into commercial merchandises (Figure 1). For example, the wastes derived from industries like bioenergy, agriculture or livestock farming, can be used to create products such as chemicals, bio-fertilizers, bio-compost, bio-plastics, bioenergy, etc. (Vanthoor-Koopmans, 2013; Chew *et al*., 2017; Behr & Seidensticker, 2020).

Figure 1 – General concept of biorefineries: raw materials, processes and final bioproducts



It is important to highlight that these raw materials are selected under certain criteria, which usually are based on the compounds that accumulate in the organic matter. According to this, plants, and seeds (for example soy, sunflower, coconut palm) are used for their accumulation of lipids and oils, which can be used to create nutrition products or bioenergy. Some waste or energy crops such as potato, sugar beet, corn, wheat, are used as a source of sugars and starch. For example, soy, which is one of the most popular materials under this scheme, is also used to obtain amino acids (proteins) (Behr & Seidensticker, 2020).

Main challenges

Even if the biorefineries development still is a technology with important background, still presents important issues to resolve prior to reach favorable balances at economic, environmental, and technical levels. One of the main topics concerning to the potential use of different biomass sources under this concept, is the development of chain processes that allow an integral use of the feedstocks, if this is achieved the valorization of the biomass could be catapulted in the best possible and integral way (Diep *et al*., 2015; Palomeros-Parada; Osseweijer & Posada-Duque, 2016).

The commercial development of biorefineries has the potential to generate significant advances in the industry, but not before overcoming some challenges. These challenges can be classified into three main categories: technological, commercial and economic (Agencia Provicional de la Energía, 2014).

Initially, the technological challenges are related to the pre-treatments, that many times are required in biomass processing, in order to enhance the viability of the organic matter prior to the extraction of compounds; there are different pre-treatments which can be used such as physical, chemical or biological treatments for biomass transformation; these previous steps are considered important drawbacks for viability of microalgal technology for producing bioproducts. Second, there are commercial challenges, mainly related with the logistics and obtaining of raw materials, because the yields of available microalgal biomass for biorefinery processing is considered very low at these stages. Finally, the economic challenges are focused on 1) the favorable economic-technical balance is only present in some raw materials, of which (energy crops) relatively low yields have been reported in comparison with raw materials with better yields (microorganisms), so it is important to optimize processes and resources to make them economically favorable; and 2) the existence of institutional or governmental support to develop and implement biorefinery projects (Diep *et al*., 2015; Palomeros-Parada; Osseweijer & Posada-Duque, 2016).

One of the feedstocks that are considered potential candidate for their use in biorefineries are the microalgae. Furthermore, there is a huge diversity of species available (green, brown and red microalgae), also they can cumulate large amounts of lipids, pigments, antioxidant compounds, etc., and their properties such as its high growth rates, and their tolerance to stressful conditions (which can be positive for compounds accumulation) have generated interest. Due these advantages, microalgae biomass is considered promising feedstock for use in biorefineries in the future (Santos-Ballardo; Valdez-Ortiz & Rossi-Heras, 2016b).

Microalgae as biorefineries source

Microalgae

Microalgae are photosynthetic microorganisms present in nature, the term *microalgae* refer to those microorganisms that contain chlorophyll "a" and other photosynthetic pigments, and also are able to develop oxygenic photosynthesis. Also, microalgae can grow in saline, fresh and even in wastewater. They have been subject of research because they can present advantageous characteristics for obtaining high value bioproducts, such as bioenergy, biochemicals, biopharmaceuticals, etc. In recent years, these microorganisms have been used in development of biorefineries, because according to several authors, the integral use of microalgal biomass could allow to obtain different value-added products efficiently, such as: biodiesel, biogas, pigments, antioxidant compounds, proteins, fatty acids, polysaccharides, etc. However, there are many microalgal species that, despite their potential, have not been fully exploited, giving rise to new opportunity areas for research (Gómez-Luna, 2007; Chisti, 2007; Santos-Ballardo; Valdez-Ortiz & Rossi-Heras, 2016b; Koyande *et al*., 2019).

Growth conditions on microalgal cultures

The growth and productivity of microalgae is influenced by several culture conditions, some of which are enlisted as follows: pH, temperature, salinity, light, agitation, type of reactor, nutrients and CO_2 availability, among others (Chew *et al*., 2017). These culture conditions can positively or negatively affect the microorganisms growth. Therefore, the knowledge regarding to the effect of these parameters in microalgal growth kinetics is essential to obtain adequate yields of microalgal biomass, as well as for obtaining high quality products from the cultivation of these microorganisms; then, in the first instance it is important to establish optimal growth conditions for microalgal culture, which also will change depending on each species used (Almutairi, 2015). Table I enlisted some important growth conditions and its repercussion into the microalgae cultures.

Table I – Effects of growth conditions of microalgae cultures

Condition	How it affects to microalgae	Reference
Light	Directly influences the overall productivity of microalgae in photoautotrophic cultures, because cultures depend on CO_2 obtained for light as their only carbon source.	Almutairi, 2015
pH	This is related to microalgal productivity and cellular respiration; these factors are closely linked and directly influence each other. This parameter depends on microalgae specie (freshwater or marine microalgae), and it affects the microalgae growth and cumulation of compounds.	Borowitzka, 1982; Park *et al*., 2011; Hernández-Pérez & Labbé, 2014 .
Carbon	At being photosynthetic microorganisms, the carbon obtained from the CO_2 (or an external carbon source) with which they feed, it is used as *food* to generate sugars that help their metabolic activities.	Borowitzka, 1982; Hernández-Pérez & Labbé, 2014
Salinity	This parameter is related to cell turgor, metabolic processes and cellular respiration of microalgae. High levels of salinity can negatively affect microalgae growth.	Velasco *et al*., 2009; Astocondor, 2017
Temperature	Variations in temperature on cultures can lead to changes in the growth rate, lipid content and fatty acid composition of the microorganism. Temperature ranges change in relation to microalgae species.	Arías-Peñaranda *et al*., 2013; Hernández-Pérez & Labbé, 2014
Nitrogen	Nitrogen sources (generally ammonium and nitrate) are inorganic salts essential for cell growth and metabolism, as they are involved in the composition of proteins. The microalgae nitrogen needs can change between species.	Huang & Wen, 2013; Almutairi, 2015

It is important to highlight that microalgae cultures conditions may change depending on the species used, because there is a wide variability among the characteristics reported between the different strains, due to this, each microalga variety has specific and different needs. Furthermore, these culture conditions can be altered in order to accumulate a specific compound, this is possible because subjecting microalgae to stress conditions has shown positive results in compounds accumulation such as fatty acids, pigments, antioxidant compounds, among others (Santos-Ballardo; Valdez-Ortiz & Rossi-Heras, 2016b; Chew *et al*., 2017). In addition to the cultivation conditions, an important factor for optimal microalgae growth is the bioreactor in which the microalgae are grown. There are different types of bioreactors and these are chosen depending on the needs and the products or compounds of interest to be obtained (González-Delgado & Kafarov, 2011).

Bioreactors used for microalgae cultures

One of the most important parameters to take account for microalgae cultivation is the photobioreactor (or reactors), which are mainly divided into two categories: open and closed (Figure 2). First, there are the open cultivation systems, which have the microalgae cultures fully exposed to natural environmental conditions, although these systems can come up with some external sources for enhance the microalgal growth (for example, artificial light to help the microalgae growth or mechanical agitation). Then, the closed cultivation systems, which avoid the contact of the cultures with the environment, these reactors maintain a better control in the conditions determined for the microalgae growth (such as temperature, pH, salinity, etc.). Some examples of these microalgae cultivation systems are in Table II.

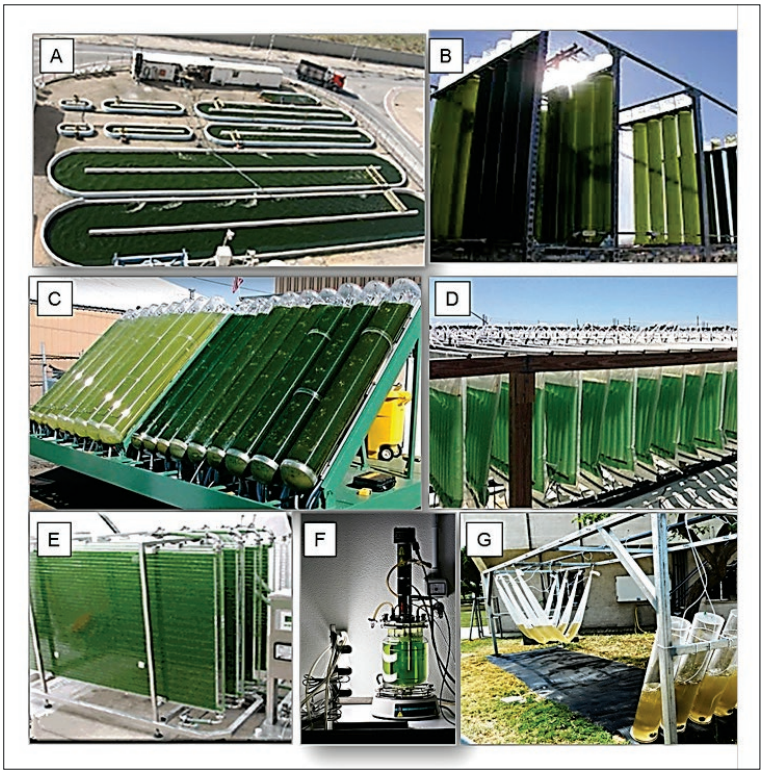


Figure 2 – Main photobioreactors used for microalgae cultures. A) Open ponds (outdoor); B) and C) tubulars photobioreactors airlift (outdoor); D) polyethylene bags (outdoor); E) flat panel systems (indoor); F) tubulars photobioreactors airlift (indoor), and G) Combined system of tubulars photobioreactors and polyethylene bags (outdoor)

Source: adapted from Santos-Ballardo, Valdez-Ortiz and Rossi-Heras (2016b).

Table II – Photobioreactors used in microalgae cultures

Type of system	Reactor	Description
Open	Raceway ponds (Artificial ponds, tanks, thin-layer ponds)	These systems have an overall design. Raceway ponds are the most frequently systems used because are an economical and favorable option to microalgae cultivation. The cost investment is minor compared to closed systems. In these systems the total surfaced used is divided in channels (commonly two to four) where microalgae are recirculated. These systems need mixing mechanisms, for this purpose generally a paddle wheel is added. Also, in some systems the supply of CO_2 and oxygen removal is very important to have an optimal microalgae culture. These systems are adequate in some locations, where conditions are optimal, such as temperature (between 20-30 °C), humidity, pollution, climate, etc.
Closed	Tubular	This system is the most common in closed systems categories, it is also used at industrial scale. The system consists in a group of transparent tubes, usually made of glass or plastic, that has coupled with a pump or airlift technology. The diameter of tubes is commonly of 0.1 m, but this need to be design in relation to O_2 concentration, to prevent oxygen accumulation which is important for optimal system performance. Tubular systems must be settled in an adequate position in order to an adequate sunlight absorption, also large amount of heat could be prejudicious to microalgae cultures.
	Serpentine	This closed system is the oldest one. Similar to tubular photobioreactors, this system consists in straight glassed or plastics tubes connected by U-bends to form a flat loop. This system can be positioned vertically or horizontally. The recirculation of the culture is provided by a pump, and the nutrients are added in an external vessel.
	Manifold	This system is a series of parallel tubes that are connected at the end by two manifolds, one of them is for distribution and the other one is for the culture suspension. Usually coupled with a pump or airlift technology.
	Flat-plate	This bioreactor has a rectangular shape, usually made by a transparent material (plastic or glass) to use solar radiation in an optimal way. The design consists in two parallel panels with a thin-layer of microalgal suspension flowing in between. Also, a gas sparger is usually coupled to this system.

Source: Chisti (2007); Ugwu, Aoyagi and Uchiyama (2008); Weathers *et al*. (2009); González-Fernandez and Muñoz (2017).

Between open and closed systems, there are some advantages and disadvantages. First, the advantages for open systems are the economical price: these facilities are cheaper than closed systems. Usually, raceway ponds are easy to construct and operate, and their operation cost are lower too, usually the cleaning procedures of these systems are easier and the energy consumption are significantly minor to other systems, mainly because they use the direct exposure to sunlight. One of the main advantages, is related to the avoiding of oxygen accumulation into the reactors (which could cause inhibition in closed reactors), mainly because it is directly released to the atmosphere. The main disadvantages are poor conditions control like temperature and pH, because climatological factors are involved, besides, open systems are susceptible to contamination with another microorganism, huge amount of CO_2 can be missed, and important water content can be loss due the evaporation rate, and finally these bioreactors need more space to be constructed in comparison to closed systems (Ugwu; Aoyagi & Uchiyama, 2008; González-Fernandez & Muñoz, 2017).

The closed systems present the following advantages: some authors reported that these bioreactors accumulate more biomass than open systems, allows a better control on the culture conditions, usually, there are compact and easy to operate, low ranges of CO_2 are missed during the operation, and the angle of closed systems can be modified for a better use of solar radiation. However, the disadvantages are a relatively high cost compared to open systems, high energy consumption: the scale up of these systems require

many materials, further, in some system designs the mixture is complicated or insufficient. To conclude, the microalgae cultivation system needs to be chosen based on the microalgae species, how much biomass are needed, which compounds are the objective, and the resources available for the investment (Chisti & Moo-Young, 2003; Lam *et al*., 2019).

Microalgae as biofuels and bioactive compounds source

Based on the great interest to obtain raw materials for bioproducts and biofuels generation with better characteristics in terms of efficiency, economy, and environmental impact; microalgae represents a suitable alternative feedstock for biomass production, mainly because this material presents convenient properties, such as high caloric value, elevated yields, low viscosity, potential to generate high valuable coproducts (such as phytochemicals), also they can be grown in saline, fresh water and even in wastewater, avoiding the competition for arable land and fresh water which could be used for food crops cultivation. Other relevant microalgae properties are their high lipid yield (Table III), which allows the production of liquid biofuels like biodiesel, also its high photosynthetic efficiency that could help to mitigate the climate change in the environment (Caguimbal *et al*., 2019; Ambriz-Perez *et al*., 2021).

In this sense, some authors reports that microalgae cultivation could present the potential to provide an alternative energy source and at the same time improves the environmental situation. Besides, microalgae cultivation systems could represent particularly benefits in urbanized areas, mainly because the microalgal biomass cultures could satisfy the energy and bioproducts demands, while the waste generation and the atmospheric pollution is reduced (Merlo *et al*., 2021).

The perspective of large-scale microalgae production for biofuel applications is a subject of strong interest due to their relatively high lipid, carbohydrate and nutrient contents coupled with a fast growth potential. These properties make them an excellent feedstock for biofuels, as well as a source of different value-added products such as pharmaceutical and nutraceutical merchandises (Singh & Gu, 2010; Santos-Ballardo *et al*., 2016a).

Even if microalgae biomass is at the forefront of alternative energy research due to their substantial potential as a renewable biofuels feedstock; the reality is that microalgae platforms have not reached an adequate industrial-scale bioenergy production due to various technical and economic constraints. The main challenges in microalgae production processes for bioenergy purposes are related to microalgae cultivation, harvesting and downstream processes, and due to this many techno-economic assessments are focused in these topics, besides. Different techno-economic analyses developed, suggest that microalgae production only for biofuel is not economically viable; biorefinery approaches are highly recommended because the valorization of by-products in microalgae production could improve the overall economics of the process (Rodionova *et al*., 2017; Venkata-Subhash *et al*., 2022).

Principal biofuels obtained from microalgae

Concerning to biofuels production, microalgae biomass represents an adequate raw material due to its chemical composition. For example, the microalgal lipids presents similar properties to those obtained from seed oils and shows the potential to be converted

to biodiesel by transesterification (Chisti, 2007). The carbohydrates could be converted to ethanol by alcoholic fermentation. Furthermore, practically all the cellular elements can be converted to bio-methane by anaerobic digestion (Santos-Ballardo *et al*., 2016a). Although past efforts were mainly engaged in the development and handling of microalgae for biodiesel production (Andrade- Nascimento *et al*., 2013; Koutra *et al*., 2020), the utilization of microalgae biomass for other energy forms is drawing increasing attention.

Table III – Lipid content from some microalgae species with biofuel interest

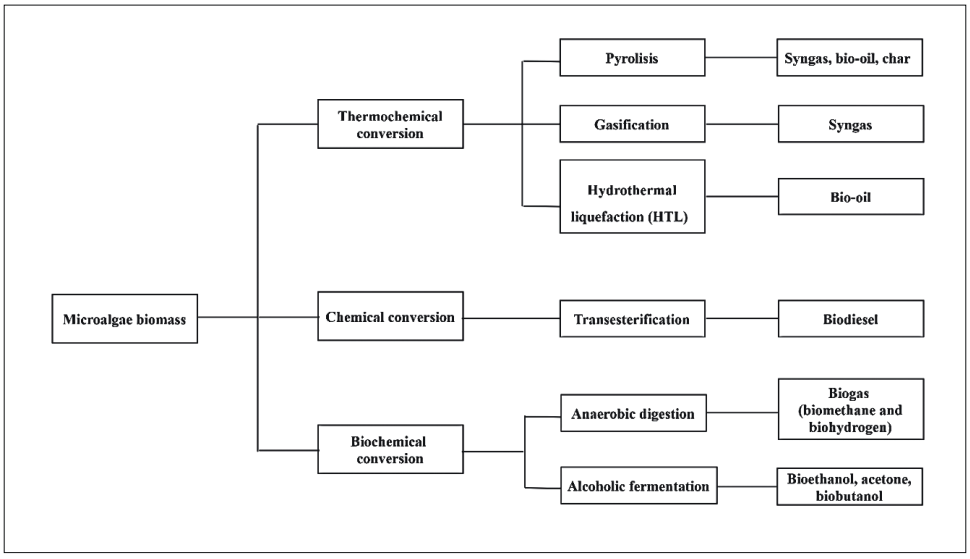
Growth medium basis	Microalgae	Lipids (% dry weight)
Fresh water	*Chlorella emersonii*	63
	Chlorella protothecoides	11-59
	Chlorella pyrenoidosa	27
	Chlorella sorokiana	13-23
	Chlorella sacccharophila	18-54
	Chlorella sp.	19-43
	Chlorella vulgaris	15-58
	Chlorella zofingiensis	51
	Haematococcus pluvialis	35
	Neochloris oleoabundans	26-38
	Scenedesmus dimurphus	31
	Scenedesmus incrassabulus	8-12
	Scenedesmus obliquus	10-43
	Scenedesmus rubescens	27-43
	Scenedesmus sp.	7-53
Seawater	*Chlorella minutissima*	57
	Chlorella sp.	35-52
	Chlorella vulgaris	57
	Dunaliella tertiolecta	24
	Nannochloris sp.	40
	Nannochloropsis oculata	8-54
	Nannochloropsis sp.	24-60
	Tetraselmis suecica	20-54

Source: adapted from Chisti (2007); Mandal and Mallick (2009); Rodolfi *et al.* (2009); Shen *et al.* (2009); Damiani *et al.* (2010); Tan and Lin (2011); Liu *et al.* (2011); Zhen *et al.* (2012); Sforza *et al.* (2012); Arías-Preñadara *et al.* (2013); Aslam *et al.* (2020).

Biofuels from microalgae biomass could be obtained mainly through three routes (Figure 3), such as: thermochemical conversion, biochemical conversion and chemical conversion (Raheem *et al*., 2015, 2018). The biochemical conversion technologies usually use enzymes and/or microorganisms for biomass hydrolysis to obtain fermentable sugars or a mixture of gases (Biogas), these technologies show better performance in specify and environmental impact, but also represents the most expensive way to obtain bioenergy from microalgae (Brennan & Owende, 2010; Santos Ballardo *et al*., 2016a). On the other hand, thermo-chemical conversion technologies use heat and catalysts to obtain intermediate products from microalgae biomass, which can be used directly as energy or could be converted into other biofuels via the previously mentioned routes. It is important to remark that the development of suitable transformation approaches

represents one of the main challenges for economic viability and sustainability of microalgal biofuel production.

Figure 3 – Principal conversion processes for biofuel production through microalgae biomass



Source: adapted from Santos Ballardo *et al*. (2016b); Gorry, Sánchez and Morales (2018); Raheem *et al.* (2018); Djandja *et al.* (2020).

Thermochemical conversion

Thermo-chemical conversion involves the decomposition of organic matter for conversion into fuels, through combination of thermal breakdown (at elevated temperatures and pressures) and chemical reformation of the organic compounds into biofuels, the main processes used are pyrolysis, gasification, hydrothermal liquefaction, torrefaction, and direct combustion; these technologies work at different operating conditions (Table IV) and through these conversion methodologies different biofuels (solid, liquid, and gaseous) could be produced for heat and power generation (Chen *et al*., 2015; Gorry; Sánchez & Morales, 2018).

Table IV – Operational conditions of thermochemical conversion methods

Method	Operational conditions			
	T(°C)	Heating rate (°C s^{-1})	Residence time	Others
Slow pyrolysis	300-700	0.1-1	5-30 min	
Fast pyrolysis	500-800	10-200	0.1-0.3 s	
Flash pyrolysis	850-1000	up to 103	0.5-1 s	
Gasification	700-1000			Controlled O_2 flow
Hydrothermal liquefaction (HTL)	200-400			4-20 MPa
Combustion	800			Air flow

Source: adapted from Brennan and Owende (2010); Naik *et al.* (2010); Raheem *et al*. (2018).

Pyrolysis

Pyrolysis is a combustion process which is developed at high temperatures (350-800 °C) in oxygen absence. It produces fuels products with low-medium calorific power, such as charcoal, gas, and biocrude. The common methods include slow pyrolysis (0.1-1 °C s^{-1}), fast pyrolysis (10-200 °C s^{-1}) and flash pyrolysis (> 1,000 °C s^{-1}), the speed of the process will affect the final composition of the products. For example, slow pyrolysis is easier and less energy demanding process, but usually lower oil yields are obtained, with different compositions in gas and charcoal (Brennan & Owende 2010; Wang *et al*., 2013).

The pyrolysis of microalgae biomass usually is performed between the range of temperatures from 300 to 700 °C, but it could be developed at lower temperatures using a catalyst. The main products are obtained with separation of the oils, followed by condensation of the generated vapors, producing char as a solid residue. The amounts of gas, oil and charcoal obtained from microalgae biomass could present considerably variations (Table V).

Table V – Products distributions obtained from pyrolysis of microalgae biomass

Microalgae specie	Conditions	Production distribution (wt%)				Reference
		Bio-oil	Syngas	Char	Higher Heating Value (MJ kg^{-1})	
Mycrocystis aeruginosa	Fast pyrolysis, 500 °C, 10 °C min^{-1}	24	-	-	29	Maio & Wu, 2004
Chlorella protothecoides	Pyrolysis, fluidized bed, 500 °C, 10 °C min^{-1}, 200 g	18	-	-	30	
Chlorella vulgaris	Outdoor pond, CO_2 enriched, Pyrolysis, 500 °C, 10 °C min^{-1}	33	14	53	5	
Chlorella sp.	Batch Pyrolysis, 450 °C	34	37	9	-	Grierson *et al*., 2009
Dunaliella tertiolecta	Pyrolysis, fluidized bed, 500 °C, 10 °C min^{-1}	24	13	3	-	
Synechococcus	Pyrolysis, 500 °C, 10 °C min^{-1}	38	18	4	-	
Nannochloropsis sp.	Pyrolysis, 400 °C, fixed bed reactor with/without HZSM-5, 10 °C min^{-1} ,1 g	20	25	5	33	Pan *et al*., 2010
Chlorella sp.	Pyrolysis, fixed bed reactor, 450 °C	55	20	0	27	Babich *et al*., 2011
Tetraselmis chuii	IR- pyrolysis, 500 °C, fixed bed, 10 °C min^{-1}, 2.4 g	43	20	7	28	Grierson; Strezov & Shah, 2011
Chlorella protothecoides	Slow pyrolysis, 550 °C, tubular reactor, 450 °C	55	-	-	40	Rizzo *et al*., 2013
Chlorella vulgaris	Closed tubular photobioreactor (PBR), Fast pyrolysis of ethanol extracts, fluidixed bed, 500 °C	53	10	1	57	Wang *et al*., 2013
Nannochloropsis gaditana	Pyrolysis, 600 °C	40	-	-	12.6	Adamczyk & Sajdak, 2018

Some authors mentioned that the microalgae-based pyrolysis oils present higher stability than lignocellulosic oils, but also shows lower calorific values. This product usually contains dissolved water, solids, and nitrogen derivated compounds, which requires catalytic improvement via cracking and hydrogenation to remove them (Raheem *et al*., 2015; Gorry; Sánchez & Morales, 2018; Raheem *et al*., 2018). Concerning to the gaseous products generated during microalgae pyrolysis, mainly consists of CH_4 and CO_2, also some heat exchangers can be used to recover energy from the gaseous product and could be used to dry the microalgae biomass feedstock and/or for heating the pyrolysis chamber, to improve the energy input demands of the overall process (Vardon *et al*., 2014).

Kim, Koo and Lee (2014) reported the pyrolysis of *Scenedesmus* sp. biomass in a fluidized bed reactor. Microalgae showed convenient bio-oil yields. Microalgae bio-oil was characterized by similar carbon and hydrogen contents and showed higher H/C and O/C molar ratios compared to other oleaginous materials. The pyrolytic oils from microalgae contained more oxygen and nitrogen and less sulfur than petroleum and palm oils. Also, the microalgae bio-oil present high concentrations of aliphatic compounds, fatty acid alkyl ester, alcohols, and nitriles. Which means that microalgae biomass processes by pyrolysis showed potentials for alternative feedstock for green diesel, commodity, and valuable chemicals.

Adamczyk and Sajdak (2018) studied the pyrolysis performance of marine microalgae biomass, *Nannochloropsis gaditana*, using three different temperatures (400, 500, 600 °C). The results indicate that the bio-oil obtained from of *N. gaditana* pyrolysis under 600 °C showed the highest heating value (12.6 MJ/kg) and the highest efficiency (38-40%). Within the liquids products were identified some alkanes and alkenes. Also, in 500 °C pyrolysis conditions, the gaseous products exhibited the highest concentrations of methane. These properties of the bio-oil and its gaseous products demonstrated that *N. gaditana* can be used as a renewable energy resource and chemical feedstock. Additionally, the biochar from all processes contained almost 70% ash, which shows the potential for being used as a fertilizer, because it does not contain any heavy metals.

Pyrolysis is one of the most studied conversion technologies for microalgae biomass, this technology has the potential to process microalgae into biofuels and fine chemicals. However, due to a negative energy balance issue (caused for an obligated microalgae drying process prior the pyrolysis), this process has been questioned for its application viability. To solve this problem, innovative solutions, such as drying devices powered by renewable energies, new pyrolysis process and equipment with a high energy efficiency, have been studied. Furthermore, compared to pyrolytic products from cellulosic biomass, the microalgal bio-oils showed less oxygen content, more hydrocarbons, higher gross heating values; also, some light olefins, alkanes, and fuel gases can be obtained with variations of the operation parameters; also, microalgal biochar is considered as a good soil amendment (Yang *et al*., 2019).

Gasification

Among the thermochemical technologies available, the biomass gasification is considered a promising process mainly because it shows the best cost/efficiency ratio for biomass to bioenergy conversion (De Lasa *et al*., 2011). Gasification is considered an adaptable chemical technology that allows a wide range of organic feedstocks. The process

involves partial oxidation with controlled amounts of air, oxygen, or steam at different temperatures (700-1000 °C), producing gases mixtures named syngas, which is mainly composed of H_2, CO, CO_2 and CH_4. Syngas is considered as a biofuel with low calorific value (between 4-6 MJ m^{-3}) and it could be used as a fuel for heating, gas engines and turbines for electricity generation (Raheem *et al*., 2018). Furthermore, the gasification can also be reorganized to obtain other products such as liquid hydrocarbon fuels using Fischer-Tropsch synthesis (for example methanol, gasoline, and diesel fuels), also hydrogen production is available using water-gas-shift (WGS) reaction. The WGS reaction can be optimized to produce syngas enriched with H_2, by employing sorption enhanced reforming (Sanchez-Silva *et al*., 2013; Raheem *et al*., 2017).

For microalgae gasification, the biomass usually is heated under low O_2 concentration, and/or using natural air, and sometimes a mixture of both could be used; the goal of the process is to obtain an incomplete combustion of the biomass generating syngas (main product), where the yield could improve depending on the combustion conditions (Sikarwar *et al*., 2017). Table VI shows an overview of microalgae gasification.

Duman, Uddin and Yanik (2014) reported the gasification of residues from *Nannochloropsis oculata,* using a fixed bed reactor with water steam, the range of temperatures used was 600-850 °C. The results showed the formation of syngas composed mainly of H_2 (50%), CO_2 (35%), while CH_4 and CO were found in smaller proportions (10 and 6%, respectively), also, the carbon conversion was 70%.

Adnan and Hossain (2018) developed a new integrated CO_2 gasification process for *Nannochloropsis oculata* biomass. The performance of the process was evaluated by determining producer gas compositions, gasification system efficiency (GSE), and cold gas efficiency (CGE). The process optimization was performed by varying CO_2 to carbon ratio (CO_2/C) at different pressures, steam to carbon ratio and equivalence ratios (ERs). It is showed that the introduction of CO_2 to the gasification process has a positive effect on the CO concentration, a negative effect on the H_2 concentration, and minimum effects on CGE and GSE. The gasification of the microalgae shows the best performance in terms of H_2/CO ratio and CGE, compared to the gasification of other biomasses. The microalgae biomass demonstrated several valuable characteristics for future bioenergy research in relation to high syngas production.

Hydrothermal liquefaction (HTL)

Usually, the microalgae biomass slurries harvested after cultivation presents elevated moisture content *(*70-80% approximately); this represent one of the foremost difficulties in the handling of microalgal biomass, mainly because high energy inputs are necessary for pumping, dewatering and drying processing of the biomass (Santos Ballardo *et al*., 2016a). Furthermore, the microalgae hydrothermal liquefaction (HTL) occurs in a temperature range between 300-400 °C and pressures between 40-200 bar. One of the main advantages of this technology is that the process occurs in water, avoiding all the energy demands related with dewatering procedures (Chiaramonti *et al*., 2017). The product distributions range typically between 10-73% of biocrude, 8-20% of gas and 0.2-0.5% of char (Brand *et al*., 2014).

Table VI – Overview of the results from gasification of microalgal biomass

Microalgae wet biomass	Conditions	Syngas composition (vol. %)	Observations	Reference
Chlorella vulgaris	350 °C, Electric furnace	CO_2 (44-49), CH_4 (16-38), H_2 (10-35)	Nitrogen in the algal biomass was converted to NH_3 during gasification	Minowa & Sawayama, 1999
Chlorella vulgaris	700 °C, 15 min, Ru/TiO_2	CO_2 (26), CH_4 (25), CO (22), H_2 (1)	Higher gas/H_2 yields with higher T, lower feed load and longer residence time	Chakinala *et al*., 2009
Saccharina latissima	450 °C, 30 min, NaOH, Ni, batch reactor	CO_2 (50-51), H_2 (25-69), CH_4 (12-29), CO (2-4)	Higher H_2 yield compared to *Chlorella vulgaris* and *Spirulina platensis*	Stucki *et al*., 2009
Tetraselmis sp.	850 °C, co-gasification of 10% microalgae with 90% coal in a fixed bed reactor	CO_2 (13), CO (12), H_2 (9), CH_4 (2)	Lower H_2/CO_2 and Higher CO yields with increasing T. Showed limitations due to ash accumulation	Alghurabie *et al*., 2013
Nannochloropsis sp.	450 °C, Ru/C, KOH, NaOH, Pd/C. Minibatch reactor	H_2 (48), CO_2 (36), CH_4 (15), CO (1)	Ru/C was most efficient catalyst for H_2 enrichment in gas mixture	Guan *et al*., 2013
Nannochloropsis sp.	700-1000 °C, 1-10 bar, 10000 °C min-1, Fixed bed.	85 % total gas yield	Highest Net Energy Balance (NEB) of 0.71 MJ MJ^{-1} for gasification to gas and lowest for pyrolysis to bio-oil (0.57 MJ Mj^{-1})	Khoo *et al*., 2013
Chlorella vulgaris	Quartz capillary reactor 450 °C, 30 min. Batch reactor	CO_2 (35-45), CH_4 (17), H_2 (18-57), CO (1-5)	In presence of NaOH/Ni higher H_2 yield + lower phenol/tar yields	Onwudili *et al*., 2013
Nannochloropsis gaditana	850 °C, TGA	H_2 (45), CO (33), CO_2 (12), CH_4 (4),	Prolounged oxidation stage due to increased O_2 content. Higher H_2 production by steam concentration.	Sanchez-Silva *et al*., 2013
Spirulina platensis	800 °C, co- gasification with wood. Fixed bed reactor	CO (40), CO_2 (25), H_2 (19), CH_4 (8)	CO/CO_2 concentrations increase with increased algae co-feeding, whereas H_2 and CH_4 first decrease and then increase gradually. Limitations due to high ash content.	Yang *et al*., 2013
Spirulina platensis	800-1000 °C. Fixed bed reactor	H_2 (34-48), CO_2 (31-37), CO (10- 18), CH_4 (9-11)	Gas composition depends on temperature. Highest theoretical yield of 0.64 g MeOH from 1 g biomass at 1000 °C.	Yang *et al*., 2013
Spirulina platensis	450 °C, 30 min, NaOH, Ni	CO_2 (36-38), H_2 (21-60), CH_4 (21- 26), CO (4)	NaOH/Ni led to significant increase in H_2 production and reduction in phenols/tars.	Duman; Uddin & Yanik, 2014
Nannochloropsis oculata	850 °C, 15 min, Fe_2O_3-CeO_2 Fixed bed reactor	H_2 (50), CO_2 (35), CH_4 (10), CO (6)	Gas yield depends on algae characteristics, process parameters and catalyst loading. Catalytic activity increased tar degradation + H_2 production.	Duman; Uddin & Yanik, 2014
Spirulina platensis	> 400 °C, Ru/ZrO2; Ru/C	CO_2 (38-77), CH_4 (2-52), H_2 (6-29)	Hydrothermal process. Complete gasification in presence of Ru	Rizwan; Lee & Gani, 2015

Besides, the primary aim of HTL is the production of low molecular weight bio-oil. Also, aqueous phase is a secondary important product obtained in this process. Moreover, it is reported that during HTL of microalgal biomass, nearly to 25-40% of the carbon and around 50% of the nitrogen contained in the feedstock are transferred into aqueous phase as dissolved carbon dioxide and nitrogen-containing compounds, respectively (Djandja *et al*., 2020).

During HTL some compounds are removed from the biomass, others are separated into oligomers and monomers, after that, the production of small fragments is realized by transformations such as cleavage, dehydration, decarboxylation and deamination, these fragments could be rearranged to form new compounds by condensation and cyclization. For example, the carbohydrates could be transformed to aromatics compounds, proteins can be rearranged to piperidine, pyrrole and amide compounds while triglycerides (TAG) could be hydrolyzed to fatty acids (Raza, 2014). Among other benefits, HTL shows the opportunity to develop the recovery of phosphorous salts and the conversion of the nitrogen to ammonium to recycle the nutrients for the microalgae cultures which represent a lower cost in this part of the process (Gorry; Sánchez & Morales, 2018). Table VII resumes some research of the HTL on microalgal biomass.

Table VII – Summary of hydrothermal liquefaction (HTL) of microalgae biomass

Microalgae specie	Chemical composition (wt %)			Conditions		Bio-oil (wt %)	Higher Heating value (MJ kg^{-1})	Reference
	Carbohydrates	Proteins	Lipids	T (°C)	Time (min)			
Golenkinia sp.	27	45	17	350	60	30	37.1	Yang *et al*., 2004
Nannochloropsis sp.	12	52	28	350	60	43	39	Brown *et al*., 2010
Chlorella vulgaris	9	55	25	350	60	36	35.1	Biller & Ross, 2011
Porphyridium cruentum	40	43	8	350	60	27	37.5	
Desmodesmus sp.	20	44	14	375	5	49	35.4	Garcia-Alba *et al*., 2011
Spirulina platensis	31	49	11	350	60	40	35.2	Jena & Das, 2011
Chlorella vulgaris	9	55	25	300	60	47	37.5	
Chlorogloeopsis fritschii	44	5	7	300	60	39	32	
Nannochloropsis oculata	8	57	32	350	60	35	34.5	Biller *et al*., 2012
Spirulina platensis	20	65	5	300	60	36	36.1	
Scenedesmus dimorphus	16	43	18	350	60	25	33.6	
Chlorella sp.	13	10	14	300	90	66	34.2	
Nannochloropsis sp.	22	53	14	300	90	36	37.1	Barreiro *et al*., 2013
Phaeodactylum tricornutum	-	38	22	375	5	54	35.9	
Tetraselmis sp.	22	58	14	350	5	65	35	Eboib *et al*., 2014
Nannochloropsis oceanica	24	36	30	350	60	40	39.5	Yoo *et al*., 2015
Bacillariophyta sp.	27	30	8	325	60	18	36.5	Huang *et al*., 2016
Cyanobacteria sp.	35	35	1	325	45	21	33.9	
Tetraselmis sp.	27	52	11	350	60	31	35.5	Yan *et al*., 2019

Furthermore, the microalgal HTL products yield depends on different operational parameters, for example the temperature, reaction time, catalyst, and co-solvent. Variations on these parameters, and on the microalgal chemical composition, are traduced into significant differences in the HTL bio-oil yields; for example, the reports showed values from 9 to 65% for *Spirulina* and *Botryococcus barunii*, respectively, and values up to 97% for *Dunaliella tertiolecta* (Fortier *et al*., 2014; Gorry; Sánchez & Morales, 2018; Djandja *et al*., 2020)

Li *et al*. (2014), reported a comparison of the HTL oil yields using two microalgae species: *Nannochloropsis sp.* (as a low-lipid and high-protein biomass) and *Chlorella sp.* (high-lipid and low-protein microalgae), the results showed a production of 55 and 83% bio-oil, respectively, which means that the oil content of the feedstock present a high influence in the reaction yield. Finally, the final product in both cases represents an energy content of 25 MJ kg^{-1}.

Yan *et al*. (2019) reported HTL of *Tetraselmis* sp. wet biomass under high temperature (280-350 °C) and pressure (5-21 MPa), in this process the associated water in the wet biomass was used as the reaction medium. The results showed that the conversion of *Tetraselmis* sp. was promoted by higher reaction temperature. The bio-oil yield increased from 26.3 ± 1.6% to 31.0 ± 2.1% as the temperature was increased from 275 °C to 350 °C. Also, the addition of 10% isopropyl alcohol as co-solvent promoted a 14.5 ± 4.9% increase in bio-oil yield and increased the gas production.

Finally, although there are many scientific works concerning to thermochemical conversion of microalgae biomass, the commercial implementation of biofuel production from microalgae using these conversion processes is still at an early stage, also economical, and energetic positive balances have still to be demonstrated (Raheem *et al*., 2018).

One of the key challenges is the harvesting and drying of microalgae biomass, due to its high energy consumption. However, this is not an issue for hydrothermal liquefaction, which is a "wet-route" process, due to this at the present stage, HTL appears a more interesting technology, however, this process is capital intensive, due to the high pressures required. T gasification and combustion conversion processes are also potential technologies for biofuel production, these processes require high temperatures, which will result in greater energy use (Aliyu; Lee & Harvey, 2021).

It is important to remark that additionally to the use of whole microalgae feedstocks, thermo-chemical technologies also represent an important valorization route for co-products in biofuel production systems. One example is the valorization of defatted residue remaining after conversion to biodiesel via transesterification. This residual algae biomass is mainly comprised of proteins, carbohydrates, and unutilized lipids, which can be utilized to produce gaseous and liquid biofuels and biochar, depending on the experimental conditions used (Pradhan *et al*., 2017).

Chemical conversion

Transesterification

Biodiesel consist of a mixture of acylated fatty acids (FA), obtained via transesterification of triacylglycerides (TAGs) and/or esterification of FA with alcohols. Microalgae biodiesel is generally produced through the extraction and further transesterification of algal oil. Furthermore, this biofuel is compatible with fossil diesel and

possess similar characteristics such as cetane number, higher heating value (HHV), flash point, and kinematic viscosity (Azadi *et al*., 2014; Gorry; Sánchez & Morales, 2018).

Transesterification is the reaction of TAGs with alcohol (usually methanol), in the presence of a catalyst, which produces glycerol and fatty acid methyl esters (FAME or biodiesel) derived from TAGs. The complete biomass conversion depends on lipid profile, oil impurities, catalyst nature, temperature, and time (Chisti, 2007).

Usually, biodiesel is produced from some oil crops (such as soy, sunflower, and palm), through transesterification. This biofuel is considered a non-toxic and biodegradable alternative fuel; in addition, it offers similar performance to petroleum diesel in engines, while reducing emissions of sulfur and other particles characteristic of fossil diesel (Brenan & Owende, 2013).

Generally, FAME can be produced using crops with high oil concentrations, also, is able the production using waste streams with high FA contents (for example: cooking oils). However, some authors reports that high oil yields could be obtained from microalgae with minimum land usage (Table VIII**).** The reports of the high lipid content in microalgae generates some expectation for the potential use of this material as source for biodiesel production (Chisti, 2007; Gouveia, 2011; Santos-Ballardo; Valdez-Ortiz & Rossi-Heras, 2016b).

Table VIII – Comparison of microalgae with other vegetable feedstocks used for biodiesel production

Raw material	Oil content (% dry weight)	Oil yield (L ha year^{-1})	Area required for cultivation (m^2 year Kg Biodiesel^{-1})	Biodiesel productivity (Kg ha year^{-1})
Maize (*Zea mays* L.)	44	172	66	152
Hemp (*Cannabis sativa* L.)	33	363	31	321
Soy (*Glicine max* L.)	18	636	18	562
Jatropha (*Jatropha curcas* L.)	28	741	15	656
Camelina (*Camelina sativa* L.)	42	915	12	809
Canola (*Brassica napus* L.)	41	974	12	862
Sunflower (*Helianthus annus* L.)	40	1070	11	946
Castor bean (*Ricinus communis*)	48	1307	9	1156
Palm (*Elaeis guineensis*)	36	5366	2	4747
Microalgae (Low oil content)	30	58700	0.2	51927
Microalgae (Medium oil content)	50	97800	0.1	86515
Microalgae (High oil content)	70	126900	0.1	121104

Source: adapted from Chisti (2007); Gouveia (2011); Santos-Ballardo, Valdez-Ortiz and Rossi-Heras (2016b).

TAGs feedstocks with chain length between C15 and C22 and low unsaturation level are most suitable for biodiesel production. Some investigations reports that some microalgae species shows FAs profiles that presents an adequate chain length for biodiesel production, however, also shows high unsaturation levels, which can be an obstacle, mainly because it could negatively affect oxidative stability, heat of combustion and cetane number of the final product (Williams & Laurens, 2010). Apart from neutral TAGs, microalgae also

contain polar lipids like phospholipids and glycolipids, which can affect the production and quality of the biodiesel. Regarding to this, some authors report that these kinds of lipids could be processed to biodiesel using alternative methods such as microwave-assisted acid transesterification in supercritical methanol (Wahlen; Willis & Seefeldt, 2011; Liu *et al.*, 2011).

Biodiesel production from microalgae usually is developed with acid or base catalysts in homogeneous phase, which represents a two-step method (oil extraction using solvents followed by transesterification), resulting in high water consumption and energy input. Among these methods the acid-catalyzed transesterifications are less sensitive to the presence free FAs and water and consequently mitigate saponification and emulsification risks, enhancing the product recovery. However, the acid catalysts show these advantages, at this time they are not selected for commercial purposes, mainly due their lower activity compared to alkaline catalysts, also requires higher temperatures and longer reaction times (Martinez-Guerra *et al*., 2018; Gorry; Sánchez & Morales, 2018).

Due to the energetic/economical disadvantages of these transesterification processes, some researchers have been developed alternatives methods for microalgal biodiesel production; for example, the *in situ* transesterification, that is a single-step model, which avoids the expensive steps of dewatering/drying of microalgal biomass. For example, the supercritical extraction process of lipids can be coupled with a transesterification reaction to enable a one-pot approach. Supercritical methanol or ethanol is employed as both the oil-extraction medium and the transesterification reagent. Finally, the use of biocatalysts such as lipases for developing the TAGs transesterification offers an environmentally attractive option to the conventional processes, reduces the energy-input, and facilitates the removal of glycerol (Guldhe *et al*., 2016; Deshmukh; Kumar & Bala, 2019). Table X shows some research for microalgal biodiesel production through transesterification in homogeneous phase (using different process conditions and catalyst types).

Although, the lipidic microalgae shows high potential for biodiesel production, there are some constrains such as the high microalgal biomass production cost, scalability, limited growth rates and the need of applying environmental stress to enhance lipid production are the key bottlenecks for industrial scale biodiesel production from microalgae (Lü; Sheahan & Fu, 2011).

Recent technoeconomic studies has shown that the reduction of the microalgal biodiesel production costs to a competitive level which promotes the commercialization is extremely challenging. There is necessary the development of future research for improvements in all the steps of the down-stream processing of this product (Deshmukh; Kumar & Bala, 2019; Aliyu; Lee & Harvey, 2021)

Biochemical conversion

Bioethanol

Bioethanol production from microalgae is considered a feasible technological development, mainly because some species can reach 50% of their dry weight (DW) in carbohydrates (Table IX), which can then be hydrolyzed and fermented with high yields (Farias-Silva & Bertucco, 2016).

Some authors have reported three possible routes for microalgae bioethanol production. The first one is considered as the traditional process in which the biomass are

subjected to pretreatment steps, hydrolysis, and alcoholic fermentation. The second route is the use of metabolic pathways in dark conditions, redirecting the photosynthesis to produce alcohols (ethanol), hydrogen and some acids. The third route is through the photofermentation process, which is less viable in nature (Markou *et al.*, 2013).

Table IX – Carbohydrates present in different microalgae species

Microalgae	Total carbohydrate content (% dry weight)
Chlamydomonas reindhartii	17
Chlorella pyrenoidosa	26
Chlorella sp.	19
Chlorella vulgaris	12-17
Chlorococcum sp.	32.5
Dunaliella bioculata	4
Dunaliella salina	32
Euglena gracilis	14-18
Isochrysis galbana	7.7-13.6
Isochrysis sp.	5.2-16.4
Mychonastes afer	28.4
Nannochloropsis oculata	8
Porphyridium cruentum	40
Prymnesium parvum	25-33
Scenedesmus abundans	41
Scenedesmus dimorphus	21-52
Scenedesmus obliquus	15-51.8
Spirogyra sp.	33-64
Spirulina platensis	8-20
Spirulina máxima	13-16
Synechoccus sp.	15
Tetraselmis sp.	24
Tetraselmis suecica	15-50

Source: adapted from Martin-Juarez *et al.* (2017).

The traditional bioethanol production (by hydrolysis and fermentation) from microalgae biomass usually consist in 3 main stages: 1) recovery of fermentable starch stored in the algae cells (which means the breakdown of cell structure through pretreatments), 2) starch hydrolysis using different techniques such as thermic, mechanic, acid, alkaline and enzymatic treatments, and 3) alcoholic fermentation of the released sugars to ethanol using yeast strains (Vitovà *et al.*, 2015; Farias-Silva & Bertucco, 2019).

Finally, separation and purification of ethanol is necessary, and usually is realized by distillation-rectification dehydration of the initial diluted alcohol product (10-15% ethanol). The purified ethanol (with around 95%) is then extracted and condensed (McKendry, 2002). Table X shows an overview of bioethanol production from microalgal biomass using hydrolysis and alcoholic fermentation.

Some authors have recommended the valorization of the residual biomass (for example in thermochemical processes, or for anaerobic digestion) mainly because the biomass cultivation represents around 55-80% of the final alcohol market price (Lam & Lee, 2012).

Table X – Overview of bioethanol production from microalgal biomass using hydrolysis and alcoholic fermentation

Microalgae	Biomass Concentration (g L^{-1} dry cell weight)	Productivity (g ethanol L day^{-1})	Type and Conditions of Hydrolysis	Yield of hydrolysis (%)	Yield of fermentation (%)	Reference
Chlamydomonas reindhartii	50	7	Enzymatic (αamylase and glucoamylases)	94	60	Choi; Nguyen & Sim, 2010
Chlamydomonas faciata Ettl 437	100	14.4	Enzymatic (glutase)	80	69	Asada *et al*., 2012
Scenedesmus obliquus	20-500	-	Acid (Sulfuric acid 3 N, 30 min and 120 °C)	71-96	-	Miranda; passarinho & Gouveia, 2012
Antrosphira platensis	12-13	-	Acid (sulfuric and nitric acid 0.5 N and 100 °C)	80	56	Markou *et al*., 2013
Chlorella vulgaris FSP-E	10-80	-	Acid (Sulfuric acid 0.36 N, 20 min and 121 °C)	95	90	Ho *et al*., 2013
*Dunaliella tertiolecta**	50	8-9	Chemic-enzymatic (amyloglucosidase and after HCl 0.5 N, 15 min, and 121 °C)	80	82	Lee *et al*., 2013
Synechococcus PCC 7002	100	30	Enzymatic (lyzozyme and α glucannases)	80	86	Mollers *et al*., 2014
Chlorella vulgaris	10	0.58	Enzymatic (pectinases)	45-70	89	Kim *et al*., 2014
*Scenedesmus bijugatus**	20	-	Acid (Sulfuric acid 0.36-1.08 N, 45 min and 130 °C)	84	70	Ashokkumar *et al*., 2015
Chlorella sp. KR-1*	50	12-14	Acid (HCl 0.3 N, 15 min and 121 °C) and Enzymatic (pectinases)	98 (acid) and 76 (enzymatic)	80	Lee *et al*., 2015

* Residual biomass after lipids extraction.

In regard to the alcoholic fermentation process of microalgal biomass, is well known at industrial levels, to obtain higher yields, it is necessary an extensive screening of microalgae strains with high carbohydrate content and/or induce the accumulation of intracellular starch using culture variations. It is important to remark that the polysaccharides on the microalgal cell walls are not easily fermentable for bioethanol production by microorganisms due to this, sometimes is necessary pretreatment methods focused on polysaccharides degradation, among the available technologies, the acids pre-treatments have been proposed as the best option compared to other methods, mainly in terms of cost-effectiveness and low energy consumption (Harun; Danquah & Forde, 2010; Alfenore & Molina-Jouve, 2016).

Furthermore, the alcoholic fermentation of microalgae biomass presents lower energy consumption, and a much simpler process in comparison with the biodiesel production system. In addition, the CO_2 produced as a by-product during the fermentation

process can be recycled as carbon source for microalgae cultivation, thus reducing greenhouse gas emissions as well. Moreover, the hydrolysis/fermentation process presents the highest rate biomass conversion, due to the well-known high efficiency of enzymes and yeasts used for biomass conversion into products. The main drawbacks of this route are the multistep processes required, which represents high energy demands, also the use of enzymes and yeasts, which signifies a considerable proportion of the overall process costs (Santos-Ballardo *et al*., 2016a; Farias-Silva & Bertucco, 2019).

Some authors report the link of the biodiesel production with generation of bioethanol; Dragone *et al.* (2010) developed alcoholic fermentations from *Chlorococum* sp. residues (obtained from lipid extraction for biodiesel), obtaining bioethanol concentrations of up to 3.83 g L^{-1}, from 10 g L^{-1} residual biomass.

Furthermore, the dark fermentation of microalgae consists of biohydrogen production by the microalgae themselves, through the consumption of intracellular starch obtaining at the same time bioethanol. Fermentative and hydrolytic microorganisms hydrolyze complex organic polymers into monomers, which are subsequently converted into a mixture of organic acids of low molecular weight and alcohols, mainly acetic acid and ethanol (Ueno; Kurano & Miyachi, 1998).

Different microalgae species are able of ethanol production through the cell wall by using some intracellular process in the absence of light, the main species include: *Chlamydomonas reinhardtii, Chlamydomonas moewusii, Chlorella vulgaris, Oscillatoria limnetica, Oscillatoria limosa, Gleocapsa alpícola, Cyanothece* sp., *Chlorococcum littorale, Spirulina* sp. and *Synechococcus* sp (Santos-Ballardo *et al*., 2016a; Farias-Silva & Bertucco, 2019).

However, dark fermentation is an inefficient process in terms of hydrogen productivity, because approximately 80-90% of the initial chemical oxygen demand (COD) remains in the form of acids and alcohols after the process. Even under optimal operating conditions, typical yields are reported between 1 and 2 mol H_2 per mol of glucose. The ethanol production is enhanced by the accumulation of carbohydrates in the microalgae cells through photosynthesis, and then the microalgae are forced to synthesize ethanol through fermentative metabolism, directly using their carbohydrate and lipid reserves when switching to dark conditions. Nevertheless, it can be concluded that microalgae dark fermentation is not yet an efficient process for bioethanol production (Abo-hashesh *et al*., 2011; Farias-Silva & Bertucco, 2016).

Furthermore, as in other technologies for biofuel production using microalgae biomass, the suitable of dark fermentation application depends on its insertion into an integrated scheme (which means the use of the residuals of the process). The final by-product of this process is a mixture of volatile fatty acids and solvents, depending on the operational conditions and the microorganisms present (Gorry; Sánchez & Morales, 2018).

Biogas

Biogas is mainly composed of CH_4 and CO_2, with traces of other gases such as H_2S. This biofuel is produced by the anaerobic digestion (AD) of organic matter. Currently, the AD is widely recognized as a mature and profitable process for obtaining renewable primary-energy. The energy content of the biogas is determined mostly by its methane (CH_4) content, which has a higher heating value (HHV) of 39.3 MJ m^{-3}. Also, the residual effluents from the AD could be used as a fertilizer or nutrient additive for cattle (González-Fernández *et al*., 2012).

The anaerobic digestion of microalgae biomass has been the subject of extensive research and the main conclusion is that some species of microalgae can be good substrates for anaerobic digestion, obtaining biogas with high methane contents and with potential to replace the biomass of some higher plants used frequently. However, the microalgae biogas potential is clearly dependent on the species properties used as feedstock and should be studied separately. Also, other biomass properties and operational parameters have to be analyzed, for example: temperature, pH, volatile solids amount, carbon/nitrogen ratio (C/N), substrate bacterial contact, hydraulic retention time and feeding rate, among others (Santos-Ballardo *et al*., 2016a).

It is important to remark that the properties of the consortiums of microorganisms responsible for the degradation of the microalgal organic matter are one of the main factors that affect the biogas production. Due to this, it is very hard to estimate the behavior of these microorganisms in the interactions with different microalgae biomass used. Because of this, an adequate selection and standardization of different inoculum that will be used for degradation of microalgae biomass has to be performed prior the AD trials (De Vrieze *et al*., 2015). An overview of biogas yields from various microalgae is available in Table XI.

Table XI – Results of different studies of anaerobic digestion from microalgal biomass

Microalgae species	Reactor	Temperature (ºC)	HRT (days)	Methane production (LCH_4gVS^{-1})	Biogas % CH_4	References
Chlorella and *Scenedesmus*	Batch	35-50	30	0.17-0.32	62-64	Golueke; Oswald & Gotaas, 1957
Tetraselmis suecica	CSTR[a]	35	14	0.31	72-74	Asinari *et al*., 1982
Spirulina	Semi-continous	30	33	0.26	68-72	Samson & LeDuy, 1983
Chlorella vulgaris	Batch	28-31	64	0.31-0.35	68-75	Sanchez-Hernandez & Travieso-Cordoba, 1993
Chlorella sp. and *Scenedesmus* sp.	CSTR[a]	35	10	0.09-0.136	69	Yen & Brune, 2007
Arthrospita platensis	Batch	38	32	0.29	61	Mussgnug *et al*., 2010
Chlamydomonas reinhardtii	Batch	38	32	0.39	66	Mussgnug *et al*., 2010
Chlorella Kessleri	Batch	38	32	0.22	65	Mussgnug *et al*., 2010
Dunaliella salina	Batch	38	32	0.32	64	Mussgnug *et al*., 2010
Euglena gracilis	Batch	38	32	0.32	67	Mussgnug *et al*., 2010
Scenedesmus obliquus	Batch	38	32	0.18	62	Mussgnug *et al*., 2010
Chlorella and *Scenedesmus*	Batch	35	40	0.16	70	Gonzáles-Fernández *et al*., 2011
Chlorella vulgaris	CSTR[a]	35	28	0.24	NS	Ras *et al*., 2011
Scenedesmus obliquus	FTR[b]	33	2.2	0.296	74.3	Zamalloa; Boon & Verstraete, 2012
Scenedesmus obliquus	FTR[b]	54	2.2	0.462	77.1	Zamalloa; Boon & Verstraete, 2012

(continuation Table XI)

Microalgae species	Reactor	Temperature (ºC)	HRT (days)	Methane production (LCH_4gVS^{-1})	Biogas % CH_4	References
Phaeodactylum tricornutum	FTR[b]	33	2.1	0.6	75.1	Zamalloa; Boon & Verstraete, 2012
Phaeodactylum tricornutum	FTR[b]	54	2.3	0.628	78.6	Zamalloa; Boon & Verstraete, 2012
Nannochloropsis salina	Semi-continous	37	40	0.13	64-68	Park & Li, 2012
Chlorella vulgaris	Batch	35	30	0.138[c]	69.9	Mendez *et al*., 2013
Neochloris oleoabundans	Batch	35	34-50	0.308	NS	Frigon *et al*., 2013
Chlorella sorokiniana	Batch	35	34-50	0.283	NS	Frigon *et al*., 2013
Micratinium sp.	Batch	35	34-50	0.36	NS	Frigon *et al*., 2013
Botrycoccus braunii	Batch	35	34-50	0.37	NS	Frigon *et al*., 2013
Isochrysis spp.	Batch	35	34-50	0.408	NS	Frigon *et al*., 2013
Chlorella sp.	Batch	35	90-95	0.34	74	Bohutskyi; Betenbaugh & Bouwer, 2014
Nannochloropsis sp.	Batch	35	90-95	0.36	72	Bohutskyi; Betenbaugh & Bouwer, 2014
Thalassiosira weissflogii	Batch	35	90-95	0.38	74	Bohutskyi; Betenbaugh & Bouwer, 2014
Tetraselmis sp.	Batch	35	90-95	0.42	79	Bohutskyi; Betenbaugh & Bouwer, 2014
Pavlova_cf sp.	Batch	35	90-95	0.51	73	Bohutskyi; Betenbaugh & Bouwer, 2014
Nannochloropsis oculata	Batch	37	12	0.279	73.9	Marsolek *et al*., 2014
Chlorella vulgaris	Batch	35	29	0.142-0.148[c]	67.5	Mendez *et al*., 2014
Scenedesmus	Batch	37	32-40	0.14	79.1	Ramos-Suárez & Carreras, 2014
Tetraselmis suecica	Batch	37	30	0.31	59.6	Santos-Ballardo *et al*., 2015
Tetraselmis suecica	Batch	37	30	0.173	73.2	Santos-Ballardo *et al*., 2015
Tetraselmis suecica	Batch	37	30	0.133	68.1	Santos-Ballardo *et al*., 2015

[a] CSTR: completely stirred tank reactor.

[b] flow-through reactors.

[c] LCH_4gCOD^{-1}

Source: adapted from Santos-Ballardo *et al*. (2016a).

The relationship between the amount of carbon and nitrogen present in organic materials is expressed as the C:N ratio. This parameter plays a crucial role for an effective and stable AD process. The optimal C:N ratio for biogas production ranges between 15:1 and 30:1. When the C:N ratio of the feedstock material is higher than 30:1, means that the nitrogen content may be insufficient to fulfill the protein demands of the anaerobic microbial consortium, causing a fast nitrogen consumption in the reactors, resulting in a rapid diminished of CH_4 production (Zeshan & Visvanathan, 2012).

Contrastingly, lower C:N ratio causes nutritional imbalance and lower biogas production, this imbalance leads to nitrogen build-up and release in the form of ammonia (NH_3) during digestion causing the bacterial inhibition (Hidaka *et al*., 2014; Kwietniewska & Tys, 2014).

Some difficulties have been identified with AD of microalgae biomass (for whole microalgae and lipid extracted biomass), mainly due to a general low C:N ratio present in microalgae species, which can vary between 3.1 and 14.87 (Sialve; Bernet & Bernard, 2009; Zhao *et al*., 2014; Santos-Ballardo *et al*., 2015). To overcome the problems with low C:N ratios, several researchers have investigated co-digestion, where microalgae biomass has been co-digested with other waste streams or biomass (such as wastepaper or glycerol) to increase the C:N ratio (González-Fernández; Molinuevo-Salces & García-Gonzalez, 2011; Santos-Ballardo *et al*., 2015).

For example, Ramos-Suárez, Martínez and Carreras (2014) reported the anaerobic co-digestion of biomass from microalgae *Scenedesmus* and cladodes from *Opuntia maxima* cladodes, obtaining stable AD even at high load rates of organic matter, resulting in reduction of ammonia inhibition and high methane yield, and reported increases in methane yield in the range of 63.9 and 66.4%. In addition, Santos-Ballardo *et al*. (2015) reported the methane potential from residual microalgal biomass from *Tetraselmis suecica*, obtaining methane production improvement of 252% for the co-digestion with glycerol compared with the microalgae biomass alone.

Other main issue for the anaerobic digestion of microalgae is the resistance of the cell wall of these microorganisms. The endurance of the microalgae cell walls is related to the presence of hardly biodegradable polymers which obstructs an efficient microalgae degradation, showing a great impact on the anaerobic digestion performance for some microalgae biomasses. The efficiency of the hydrolytic bacterial is strongly affected by the cell wall structure and composition of microalgae (Kwietniewska & Tys 2014; Ward; Lewis & Green, 2014; Santos-Ballardo *et al*., 2016a).

Different authors reported that is necessary the disruption of the cell wall (trough a pretreatment step before the anaerobic digestion) for enhance the availability of the organic matter for the bacterial inoculum. Several methods of algal biomass pre-treatment show the potential to increase the organic matter biodegradability, enhance the production rates, and improve the CH_4 yields, these pre-treatments techniques can be classified as thermal, mechanical, chemical, and biological (González-Fernández *et al*., 2012; Passos *et al*., 2014; Ward; Lewis & Green, 2014; Santos-Ballardo *et al*., 2016a).

Passos *et al*. (2013) pretreated microalgal biomass for biogas production using microwave process. Using optimized conditions, the maximum yield was achieved reporting 307 mL of biogas per volatile solid added (mL biogas g^{-1}VS), compared to 172 mL biogas g^{-1}VS obtained without any pretreatment. They concluded that microwave irradiation enhanced the disintegration and digestibility of microalgae and the main parameter influencing the solubilization was the specific energy applied to the biomass.

Furthermore, Alzate *et al*. (2012) realized a comparison between thermal, ultrasound and biological pre-treatments, using biomass from mixtures of microalgal species. The results showed that the biological pre-treatment generates an untraceable enhancement on CH_4 yield for all the microalgae tested; meanwhile, thermal pretreatment (170 °C and 6.4 bars) was the best operational method for all the biomass analyzed, achieving methane production increments between 46 and 62%. Due to this, they

proposed the thermal pre-treatment as the most effective option for enhancing the methane yield on microalgae biomass.

Finally, concerning to the production of biofuels from microalgae, even if some authors presents an optimistic outlook regarding the sustainability of these products in outdoor conditions and in their contribution to energy security, other authors comment that it is not clear yet if the microalgal biofuels have the real potential to replace/complement the current fossil fuel consumption and contribute to energy security, especially at a large scale (Fuentes-Grünewald *et al*., 2012; Merlo *et al*., 2021).

Some authors have shown several doubts about the economic and the energetic feasibility of this technology, mainly because it could be considered as very expensive compared to other energy sources, also usually requires high energy inputs; Due to this, there is no clear scenario about the future evolution of the microalgae biofuel market and the real potential of commercialization (Itoiz *et al*., 2012). Due to this, the microalgae potential for obtaining different products (with better economical balances) have been explored recently.

Microalgae to bioactive compounds

Microalgae have been widely recognized as good source of a huge variety of natural products, with diverse application in several sectors, including energy, nutrition, pharmaceutics, and cosmetics. The activity of the bioactive ingredients extracted from microalgal cells has been studied by several authors. It is important to remark that in the context of integrated biorefinery approach, these bioactive compounds could represent an important enhance on the economic aspects of microalgal technology, due to their high market values (Bule *et al*., 2018; Koutra *et al*., 2020).

Microalgae are enriched with different types of active compounds, such as phycobiliproteins, fatty acids, vitamins, fatty acids, antioxidants, and pigments; with huge biotechnological and industrial interest due their nutritional properties and the potential application in many pharmaceutical industries, mainly due to its neuroprotective, antibiotic, and anti-inflammatory properties (Begum *et al*., 2016; Ummalyma; Sahoo & Pandey, 2020).

Pigments

Microalgal pigments, including chlorophylls, carotenoids, and phycobilins, plays an important role on the photosynthesis, light harnessing, and to maintain the correct function and integrity of microalgal cells. Besides, these pigments show a high antioxidant activity, they have the potential to be used as natural colorants, nutritional supplements, and ingredients of cosmetics products; furthermore, within the most well-known pigments includes chlorophylls (a and b), astaxanthin, -carotene, lutein, violaxanthin, and fucoxanthin (Table XII). The production and accumulation in the microalgal cells depend on several factors, including the species used and variations in the culture conditions (Da Silva-Ferreira & Sant'Anna, 2017; Koutra *et al*., 2020).

Furthermore, increases in cellular chlorophyll concentrations are usually observed under low light intensity conditions, mainly because microalgae shade adaptation. This reduction in chlorophyll concentration has been reported under micronutrient and/or

nutrient depletion (mainly nitrogen and phosphorus). The accumulation of other photosynthetic pigments such as carotenoids, could be triggered by stressful environmental conditions (including high temperatures, osmotic stress and increments in the light intensity (Markou & Nerantzis, 2013; Aslam *et al*., 2020).

Table XII – Summary of pigments extraction from microalgae biomass

Microalgae	Product	Extraction method	Extraction efficiency	Observations	Reference
Haematococcus pluviales	Astaxantin	Physical disruption and solvent extraction	35% (35.1 mg g^{-1})	Grinding cell wall disruption	Jaime *et al*., 2010
Chlorella pyrenoidosa	Chlorophyll	Solvent extraction	2.9% (11.4 mg g^{-1})	Organic solvent is required	Bai *et al*., 2011
Isochrysis galbana	Chlorophyll	Solvent extraction	5.60%	Organic solvent is required	Bai *et al*., 2011
Anabaena NCCU-9	Zeaxanthin	Repeated freezing and thawing	128.8 mg g^{-1}	Optimization of culture conditions	Hemlata; Bano & Fatma, 2011
Chlorella saccharophila	Zeaxanthin	Ultrasonication and cell disruption	72.2% (11.3 mg g^{-1})	Improved extraction method	Singh *et al*., 2013
Chlorella saccharophila	β-carotene	Ultrasonication and cell disruption	37.3% (5.1 mg g^{-1})	Improved extraction method	Singh *et al*., 2013
Spirulina platensis	Phycocyanin	Photobioreactor with CO_2 fixation	92.2 mg g^{-1}	Enchanced by engineering strategies	Chen *et al.,* 2013
Haematococcus pluviales	Astaxantin	Solvent extraction	46 mg l^{-1}	Highest yield obtained with 6% CO_2	Cheng *et al*., 2016
Haematococcus pluviales	Astaxantin	Cell disruption and solvent extraction	32.5 pg $cell^{-1}$	-	Kim *et al*., 2016
Phaeodactylum tricornutum	Fucoxanthin	Variations in cell culture	2.3 mg L d^{-1}	-	McClure *et al*., 2018

Examples of carotenoids extract obtained from microalgae are the production of natural astaxanthin obtained from *Haematococcus pluvialis* biomass; as well the β-carotene production from *Dunaliella* salina; both products were evaluated to determine antitumor activity, suggesting their high health protective role. The carotenoids extracted from *Nannochloropsis oleoabundans*, composed mainly by violaxanthin, lutein, and monoesters, were tested against colon cancer cells showing positive results, the antitumor activity mainly was correlated with the monoester carotenoids (Castro-Puyana *et al*., 2017; El-Baz *et al*., 2018).

Other products with high commercial importance are the phycobiliproteins, which are water-soluble pigments present in the family of red algae (*rhodophytes, cryptomonads, glaucocystophytes*) and in some cyanobacteria. Some phycobiliproteins consist of phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC) constituents, and they have been used as natural dyes and as nutraceutical compounds in different biotechnological applications (Parmar *et al*., 2011; Koutra *et al*., 2020).

Phycoerythrin is naturally responsible for red coloration in red algae whereas blue pigment such as phycocyanin is mainly present in cyanobacteria. The main uses of these compounds are in immunofluorescence techniques (usually linking these pigments to protein markers); due to this, the phycobiliproteins have the highest market values within

all the microalgal-derived products. Moreover, their use as chemical tags, the phycobiliproteins are also utilized in cosmetics and as food colorants because of their high coloration effects (Arad & Yaron, 1992; Ummalyma; Sahoo & Pandey, 2020).

Other pigment with high interest is the fucoxanthin, which could be obtained from brown microalgae cultures. This pigment is recognized for its high antioxidant activity, and some authors have been reported a contribution to prevention of diseases correlated with oxidative stress; also, antitumor, antidiabetic activity, and chemoprevention have been attributed to fucoxanthin consumption (Mikami & Hosokawa, 2013). Regarding to this, McClure *et al*. (2018) reported a productivity of 2.3 mg L d^{-1} of fucoxanthin, using variations on the culture conditions of *Phaeodactylum tricornutum*, this represents an significant source of this important pigment.

Polyunsaturated fatty acids

Polyunsaturated fatty acids (PUFA) are widely recognized as essential nutritional components that assist in prevention of different health problems. The increments in PUFAs demands have motivated the search of alternative sources of eicosapentaenoic acid (EPA) and docosahexae-noic acid (DHA). Furthermore, microalgae have great potential for producing PUFAs, due to this, some advances on microalgal technology have been focused on the production of PUFAs as a sustainable source (Wang *et al*., 2015).

PUFAs, including EPA, DHA, -linolenic, and arachidonic acid, represent important target products from microalgal biomass, as alternatives to fish oil. Microalgae cultures for PUFA production must be developed under strict control, mainly because the fatty acid profiles show high variability and is widely affected by different factors such as: the microalgae species used, the environmental conditions such as temperature, agitation, light, and carbon supply, as well as by the growth phase of the culture (Borowitzka, 2013; Boelen *et al*., 2017).

Some authors have been reported that the PUFAs consumption presents several health benefits against different affections (cardiovascular diseases, cognitive decline, and mental disorders), due to this, the market value of these products is considered high (Shahidi & Ambigaipalan, 2018).

Furthermore, Oliver *et al*. (2020) reported that *Phaeodactylum tricornutum* is one of the two diatoms whose genomes have been completely sequenced, which allows to a high develop in metabolic engineering of high EPA producing strains. Based on its rapid growth, high lipid content and omega-3 PUFA concentrations, this microalga exhibits a large commercial potential. Also, Tanakaa *et al*. (2017) reported EPA productivity up to 136 mg L d^{-1}, under optimized photoautotrophic conditions of the marine diatom *Fistulifera solaris,* suggesting its potential use for large-scale EPA production.

Two main families of microalgae could be considered as the main omega-3 PUFA producers: *Thraustochytriacea* and *Crypthecodiniacea* family. Within these, the *Schyzochitrium*, *Ulkenia* and *Crypthecodinium* species present the higher amount of omega-3 PUFA accumulation, especially DHA (Gupta; Barrow & Puri, 2012).

Phenolic compounds

Different phenolic compounds with antioxidant properties are found in marine microalgae. These compounds are present as constituents of various brown microalgal groups such as *Sargassaceae*, *Fucaceae*, and *Alariaceae* (Ummalyma; Sahoo & Pandey, 2020).

Terpenoids, phlorotannins, phenolic pigments, and bromophenols are the major classes of phenols found in marine microalgal resources. Within these, phlorotannins are the most important group concerning to the market price and usually are used in the cosmetics industry. The main function of the phlorotannin phenolic compound in the cells, is to provide shield against photooxidative stress induced by UV-*b* radiation and show inhibitory effects on melanogenesis. Usually, these bioactive compounds are extracted from brown algae like *Ecklonia cava*, *Fucus vesiculosus*, and *Ascophyllum nodosum* (Thomas & Kim, 2013; Oliver *et al*., 2020). Also, the antioxidant activity of microalgae can be attributed to several phenolic compounds (gallic, cinnamic, salicylic, caffeic, ferulic, and *p*-coumaric acids) that have been identified in different microalgal species *(*Aslam *et al*., 2020).

Furthermore, depending on the extraction solvent used to obtain microalgal extracts (for example acetone, methanol, ethanol, chloroform, water or hexane, among others) different compounds could be extracted, including phenols, flavonoids, tannins, fatty acids, and pigments. On the other hand, some vitamins (including A, B, C), minerals (such as Ca, Mg, K), and chemical compounds (including phenols and sterols), represent interesting valorization options for microalgal biomass (Luo; Su & Zhang, 2015).

Microalgal biorefineries

As it was explained above, microalgae have attracted increasing attention over the past decades, because they are considered as potential feedstock for a wide variety of products, which could include products with low until extremely high prices (Koutra *et al*., 2020).

Between the alternative uses of biomass, biofuels are a highly promising option with numerous advantages in terms of CO_2 mitigation and renewable energy production. However, several technical improvements are needed before to reach the industrial production and commercialization of microalgal biofuels. Some authors have been concluded that microalgal biofuels production alone is not sustainable; thus, biorefinery approaches is highly recommended to make integral use of microalgal biomass, use all the valuable fractions that are available, and enhance the economic/energetic balance on this technology (Aslam *et al*., 2020; Ummalyma; Sahoo & Pandey, 2020).

It is important to remark that this strategy not only contributes to the economic viability of the process, but also, allows the production of highly valuable resources from microalgae; these products range from nutrients and food supplements, fine organic chemicals with different applications, along with energetic products (biofuels) such as biodiesel, bioethanol and biomethane (Zhu, 2014; Santos-Ballardo *et al*., 2016a).

Different research has been dedicated to find microalgae species which presents compounds profiles that allows the development of biorefinery concept; usually, these approaches start through primary processes which delivery the products with the greatest commercial interest or those that can be exploited more widely, subsequently the potential for secondary products are analyzed (Chew *et al*., 2017). Some works on microalgae biorefineries are shown in Table XIII.

One option to develop the biorefinery concept is combining biofuel production (biodiesel or biohydrogen) prior to anaerobic digestion (from residual biomass) to improve methane yield (González-Fernández *et al*., 2012). Regarding to this, Bohutskyi *et al*. (2015) studied the methane potential from lipid-extracted algal residues from *Auxenochlorella*

protothecoides reaching up to 0.25 liters of methane per gram of volatile sold added (L CH_4 gVS^{-1}) and increasing the energetic yield from algal biomass by more than 30%. Additionally, the authors proved that the recycling of the anaerobic digestion effluent for biomass cultures reduced the cost of the supplied nutrients by up to 45%, enhancing with this the process sustainability especially for scaled-up processes. Another work showed that the anaerobic digestion of *Tetraselmis suecica* residual biomass (obtained after oil extraction), reached a final methane production of 0.175 L CH_4 g VS^{-1}, obtaining approximately an increment of 103 % of methane yield compared with the untreated biomass (0.086 L CH_4 g VS^{-1}) (Santos-Ballardo *et al*., 2015).

Table XIII – Microalgal Biorefineries attempts

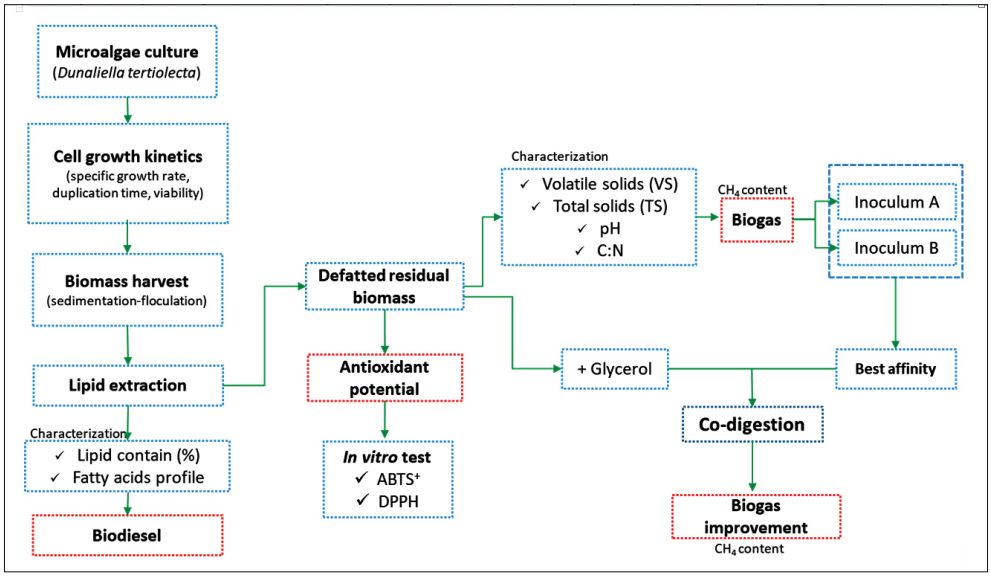
Species	Processes	Bioproducts	Potential	Reference
C. reinhartii, *C. kessleri*, *Spirulina* sp., *A. platensis*, *S. obliquus*, y *D. salina*	Biogas production from microalgal biomass (separately)	Biogas	Use as a biorefinery and improvement in methane yield compared to conventional substrates	Mussgnug *et al*., 2010
Tetraselmis suecica	Lipid extraction, the residual biomass used for biomethane production, as well the co-digestion with the residual glycerol	Main products: Fatty acids (glycerol as byproduct) Secondary products: Biomethane	The residual biomass has potential for biomethane production, and the by-product obtained from the extraction of fatty acids (glycerol) improved the methanogenic potential through a co-digestion	Santos-Ballardo *et al*., 2015
Chlorella sorokiniana	Bioremediation and biofuels	Biofuel production potential	When microalgae grown in wastewater, heavy metals were removed, and biomass showed adequate characteristics for biofuel production	Guldhe *et al*., 2017
Dunaliella tertiolecta	Fast pyrolysis to obtain bio-oil and char from primary processes	Main products: β-carotene, phytosterols, fatty acids Secondary products: Biofuels and fertilizer	Potential use for the residual biomass (obtained after the extraction of the main products), to produce biofuels (bio-oil) and char	Francavilla *et al*., 2018
Phaeodactylum tricornutum	Protein, carbohydrates and lipids extraction	Biofuels (biodiesel, bioethanol) and bioproducts (Proteins and carbohydrates)	Under the proposed scheme, microalgae biomass can be exploited to obtain large quantities of bioproducts	Bronco-Vieria *et al*., 2020

Biofuel production is not the only option for microalgal biorefineries development. For example, Francavilla *et al*. (2018) analyzed the potential of *Dunaliella tertiolecta* biomass under a biorefinery concept, reporting the valorization of residual biomass obtained after the production of chemical compounds (with high commercial value) such as β-carotene, phytosterols and fatty acids. The microalgal residual biomass was used in a rapid pyrolysis process for bio-oil and char production, which can be used as biofuels and fertilizers, respectively. They concluded that *Dunaliella tertiolecta* has the versatility to produce large amounts of different biocomposites and bioenergetics.

Furthermore, Gárate-Osuna (2020) analyzed the potential of *Dunaliella tertiolecta* to obtain bio-products under a biorefinery scheme; the author studied the viability of the intracellular lipids for biodiesel production; besides the residual defatted biomass was analyzed as substrate for biogas generation, and the antioxidant potential (of different

extracts) from the defatted residual biomass was studied (Figure 4). The results are enlisted as follows: the accumulation of intracellular lipids was 15.69 ± 2.94%, in addition, according to the profile of fatty acids observed in the lipidic fraction, the obtained oil presents adequate characteristics for biodiesel production. Regarding to the biogas potential of the residual biomass, accumulated methane values up to 0.202 ± 0.006 L CH_4 gSV^{-1} were obtained. Finally, the antioxidant capacity of residual biomass extracts was determined by ABTS and DPPH, where values of up to 18.70 ± 0.85 micromoles of Trolox equivalent per gram of residual biomass on a dry basis (µM TE g BR^{-1}) were obtained. This work shows the viability to produce a chain of several products with different properties and market values.

Figure 4 – Scheme for the study developed by Gárate-Osuna (2020), for biorefinery potential determination from *Dunaliella tertiolecta* biomass



Challenges and future prospect

Recently, microalgae technology has gained scientific and commercial attention, mainly because several strains of microalgae could synthesize and cumulate larger amounts of high value compound such as proteins, lipids, pigments, vitamins, PUFAs, antioxidants, among others.

However, several challenges still remain without solutions, principally in the aspects of the compound recovery process (for example: scalability of the extraction methods, energy demands and viability of scalability of certain processing methods). Moreover, for the conventional solvent extraction, is hard to find and appropriate solvent which provide high yield of products and be environmentally friendly. Due to this, emerging technologies such as ultrafiltration and microfiltration have been introduced for the extraction of microalgae products. Furthermore, the microalgae cultivation should follow the regulations set by the Food and Drugs Administration (FDA) agency in order to ensure the microalgae products safety for human consumption.

On the other and, the microalgae biorefinery concepts for production of biofuels and other valuable products usually presents low yields, mainly because several steps are

required to obtain specific purity level on the products desired, due to this, a strategic process integration must be used to reduce the number of purification steps.

Also, research in characterization, extraction and valorization of the protein present in residual biomass is a0n important opportunity area and further developed id required.

Different authors pointed that there are important issues to resolve, mainly related to a relatively small market for different products, possible losses caused by product degradation, outdoor culture growth conditions and their effects on the yields of biomass and bioactive compounds, as well as long term stability studies of the microalgal products. These studies are important in compounds like pigments which can be easily degrade due to temperature, light, and other microorganisms.

The cultivation of algae near to strategic points like power plants could work as bio-sequestration strategy for CO_2 mitigation and at the same time realize develop nutrient recycling and environmental remediation representing a prominent research field.

Besides, metabolic engineering of microalgae cells is a promising tool to develop algal biochemical factories with commercial interest.

Finally, comprehensive economic and environmental studies must be conducted regarding the production viability of high-value compounds from microalgae, in addition to that, the life cycle analysis of several high-value compounds must be carried out to evaluate the sustainability of the processes. In conclusion, more efforts should be performed to reduce product loss and minimize energy costs, and at the same time is important to reach environmentally friendly large scale downstream processing for the high value compounds extraction from microalgae.

CONCLUSION

The biorefinery concept has been identified as the most promising way for the creation of a biomass-based industry. It has advantages over linear processes of biomass transformation using waste materials, and the increase in bioproducts obtainable per unit of area and the expectation to revitalize rural areas, which is why it should be taken into consideration when it comes to looking for a use for raw materials.

Incorporating this concept in biofuel production chains is considered the only way to reach a viability in this technology, this only through the valuation of the by-products generated. Additionally, microalgae show high potential for use as a feedstock in biorefinery processes, because they contain different metabolites of interest and recently have been used to obtain different products (with different range of market prices) in linear production chains. In addition, the use of microalgae for biofuel production have been raised over the past few years, due to this, several methods have been developed and improved, in order to obtain microalgal lipids, which sometimes represents the half of the way for microalgae-based biorefinery setting. Finally, it is important the improvement in technology and standardization of the viability of these kinds of technologies, but without a doubt, in the future the microalgal biorefineries will play an important role in the world economy.

REFERENCES

Abo-hashesh, M.; Wang, R. & Hallenbeck, P.C. Metabolic engineering in dark fermentative hydrogen production: theory and pratice. *Bioresource Technology 2011*, v. 102, p. 8414-8422, 2011.

Adamczyk, M. & Sajdak, M. Pyrolysis behaviours of microalgae *Nannochloropsis gaditana*. *Waste Biomass Valor*, v. 9, p. 2221-2235, 2018. https://doi.org/10.1007/s12649-017-9996-8.

Adnan, M.A & Hossain, M.M. Gasification performance of various microalgae biomass – A thermodynamic study by considering tar formation using Aspen plus. *Energy Conversion Management*, v. 165, p. 783-793, 2018.

Agencia Provicional de la Energía. *Biorrefinerías: una oportunidad de negocio para las zonas rurales e industrias.* Diputación de Ávila: Zink Soluciones Creativas, 2014.

Alghurabie, I.K.; Hasan, B.O.; Jackson, B.; Kosminski, A. & Ashman, P.J. Fluidized bed gasification of Kingston coal and marine microalgae in a spouted bed reactor. *Chem. Eng. Res. Des.*, v. 91, n. 9, p. 1614-1624, 2013.

Alfenore, A. & Molina-Jouve, C. Current status and future prospects of conversion of lignocellulosic resources to biofuels using yeasts and bacteria. *Process Biochemistry*, v. 51, n. 11, p. 1747-1756, 2016.

Aliyu, A.; Lee, J.G.M. & Harvey, A.P. Microalgae for biofuels: a review of thermochemical conversion process and associated opportunities and challenges. *Bioresource Technology Reports*, v. 15, p. 100694, 2021.

Almutairi, A. Producton of biofuels from the green alga Tetraselmis. *Producton of biofuels from the green alga Tetraselmis*. Sheffield, UK: Thesis, July 2015.

Alzate, M.E.; Muñoz, R.; Rogalla, F.; Fdz-Polanco, F. & Pérez-Elvira, S.I. Biochemical methane potential of microalgae: influence of substrate to inoculum ratio, biomass concentration and pretreatment. *Bioresource Technology*, v. 123, p. 488-494, 2012.

Ambriz-Perez, D.L.; Orozco-Guillen, E.E.; Galán-Hernandez, N.D.; Luna-Avelar, K.D.; Valdez-Ortiz, A. & Santos-Ballardo, D.U. Accurate method for rapid biomass quantification based on specific absorbance of microalgae species with biofuel importance. *Letters in applied Microbiology*, 2021. DOI: 10.1111/lam.13519.

Andrade-Nascimento, I.; Izabel-Marques, S.S.; Dominguez-Cabanelas, I.T.; Andrade-Pereira, S.; Druzian, J.I.; Oliveira de Souza, C.; Vital-Vich, D.; Correia de Carvalho, G. & Andrade-Nascimento, M. Screening microalgae strains for biodiesel production: lipid productivity and estimation of fuel quality based on fatty acids profile as selective criteria. *Bioenergy Resource*, v. 6, n. 1, p. 1-13, 2013.

Arad, S.M. & Yaron, A. Natural pigments from red microalgae for use in foods and cosmetics. *Trends Food Sci. Technol.*, v. 3, p. 92-97, 1992.

Arías-Peñaranda, M.T. *et al.* Biodiesel production from microalgae: cultivation parameters that affect lipid production. *Acta Biológica Colombiana,* v. 18, n. 1, p. 43-68, sept.-nov. 2013.

Asada, C.; Doi, K.; Sasaki, C. & Nakamura, Y. Efficient extraction of starch from microalgae using ultrasonic homogenizer and its conversion into ethanol by simultaneous saccharification and fermentation. *Natural Resources*, v. 3, p. 175- 179, 2012.

Ashokkumar, V.; Salam, Z.; Tiwari, O.N.; Chinnasamy, S.; Mohammed, S. & Ani, F.N. An integrated approach for biodiesel and bioethanol production from *Scenedesmus bijugatus* cultivated in a vertical tubular photobioreactor. *Energy Conversion and Management*, v. 101, p. 778-786, 2015.

Asinari, C.M.A.D.S.; Legros, A.; Naveau, H.P. & Nyns, E.J. Biomethanation of the marine algae Tetraselmis. *Int. J. Sustain. Energy*, v. 1, p. 263-272, 1982.

Aslam, A.; Fazal, T.; Uzzaman, Q.; Shan, A.; Rehman, F.; Iqbal, J.; Rashid, N. & Rehamn, M.S.U. Biorefinery of microalgae for nonfuel products, p. 197-210, *in* Yousuf A. (ed.). *Microalgae cultivation for biofuels production*. Academic Publish Elsevier, 2020. DOI: 978-0-12-817536-1.

Azadi, P.; Brownbridge, G.; Mosbach, S.; Smallbone, A.; Bhave, A.; Inderwildi, O. & Kraft, M. The carbon footprint and non-renewable energy demand of algae-derived biodiesel. *Appl. Energy*, v. 113, p. 1632-1644, 2014.

Astocondor, M.M.-H.-T.-D. Crecimiento poblacional y productividad de la microalga nativa Chlorella peruviana bajo diferentes salinidades. *Revista de Investigación Vet. Perú*, p. 976-986, 2017.

Bai, M.; Cheng, C.; Wan, H. & Lin, Y. Microalgal pigments potential as byproducts in lipid production. *J. Taiwan Inst. Chem. Eng.*, v. 42, p. 783-786, 2011.

Babich, I.V.; Van der Hulst, M.; Lefferts, L.; Moulijn, J.A.; O'Connor, P. & Seshan, K. Catalytic pyrolysis of microalgae to high-quality liquid bio-fuels. *Biomass Bioenergy*, v. 35, n. 7, p. 3199-3207, 2011.

Begum, H.; Yusoff, F.M.D.; Banerjee, S.; Khatoon, H. & Shariff, M. Availability and utilization of pigments from microalgae. *Crit. Rev. Food Sci. Nutr.*, v. 56, p. 2209-2222, 2016.

Behr, A. & Seidensticker, T. *Refined raw materials! En In: Chemistry of Renewables.* Berlin, Heidelberg: Springer, 2020. https://doi.org/10.1007/978-3-662-61430-3_20.

Boelen, P.; van Mastrigt, A.; van de Bovenkamp, H.H.; Heeres, H.J. & Buma, A.G.J. Growth phase significantly decreases the DHA-to-EPA ratio in marine microalgae. *Aquacult. Int.*, v. 25, p. 577-587, 2017.

Bohutskyi, P.; Betenbaugh, M.J. & Bouwer, E.J. The effects of alternative pretreatment strategies on anaerobic digestion and methane production from different algal strains. *Bioresour Technol.*, v. 155, p. 366-372, 2014.

Bohutskyi, P.; Ketter, B.; Chow, S.; Adams, K.J.; Betenbaugh, M.J.; Allnutt, F.C. & Bouwer, E.J. Anaerobic digestion of lipid-extracted *Auxenochlorella protothecoides* biomass for methane generation and nutrient recovery. *Bioresour Technol.*, v. 183, p. 229-239, 2015.

Borowitzka, M. Mechanisms in algal calcification. *Prog. Phycol.*, p. 137-177, 1982.

Borowitzka, M.A. High-value products from microalgae and their development and commercialization. *J. Appl. Phycol.*, v. 25, p. 743-756, 2013.

Brand, S.; Hardi, F.; Kim, J. & Suh, D.J. Effect of heating rate on biomass liquefaction: differences between subcritical water and supercritical ethanol. *Energy*, v. 68, p. 420-427, 2014.

Brennan, L. & Owende, P. Biofuels from microalgae-a review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sustain. Energy Rev.*, v. 14, n. 2, p. 557-577, 2010.

Bronco-Viera, M.; San Martin, S.; Agurto, C.; Freitas, M.A.V.; Martins, A.A.; Mata, T.M. & Caetano, N.S. Biotechnological potential of *Phaeodactylum tricornutum* for biorefinery process. *Fuel.*, v. 268, p. 117357, 2020.

Bule, M.H.; Ahmed, I.; Maqbool, F.; Bilal, M. & Iqbal, H.M.N. Microalgae as a source of high-value bioactive compounds. *Front. Biosci.*, v. 10, p. 197-216, 2018.

Caguimbal, N.A.L.E.; Devanadera, M.K.P.; Bennet, R.M.; Arafiles, K.H.V.; Watanabe, K.; Aki, T. & Dedeles, G.R. Growth and fatty acid profiles of Halophytophthoravesicula and Salispinaspinosa from Philippine mangrove leaves. *Lett Appl Microbiol.*, v. 69, p. 221-228, 2019.

Castro-Puyana, M.; Pérez-Sánchez, A.; Valdés, A.; Ibrahim, O.H.M.; Suarez-Álvarez, S.; Ferragut, J.A.; Micol, V.; Cifuentes, A.; Ibáñez, E. & García-Cañas, V. Pressurized liquid extraction of *Neochloris oleoabundans* for the recovery of bioactive carotenoids with antiproliferative activity against human colon cancer cells. *Food Res. Int.*, v. 99, p. 1048-1055, 2017.

Chakinala, A.G.; Brilman, D.W.; van Swaaij, W.P. & Kersten, S.R. Catalytic and non-catalytic supercritical water gasification of microalgae and glycerol. *Ind. Eng. Chem. Res.*, v. 49, n. 3, p. 1113-1122, 2009.

Chiaramonti, D.; Prussi, M.; Buffi, M.; Rizzo, A.M. & Pari, L. Review and experimental study on pyrolysis and hydrothermal liquefaction of microalgae for biofuel production. *Appl. Energy*, v. 185, p. 963-972, 2017.

Chen, C.Y.; Kao, P.C.; Tsai, C.J.; Lee, D.J. & Chang, J.S. Engineering strategies for simultaneous enhancement of C-phycocyanin production and CO2 fixation with Spirulina platensis. *Bioresour. Technol.*, v. 145, p. 307-312, 2013.

Chen, W.H.; Lin, B.J.; Huang, M.Y. & Chang, J.S. Thermochemical conversion of microalgal biomass into biofuels: a review. *Bioresource Technology*, v. 184, p. 314-327, 2015. ISSN 0960-8524.

Cheng, J.; Li, K.; Yang, Z.; Zhou, J. & Cen, K. Enhancing the growth rate and astaxanthin yield of Haematococcus pluvialis by nuclear irradiation and high concentration of carbon dioxide stress. *Bioresour. Technol.*, v. 204, p. 49-54, 2016.

Chew, K.W.; Yap, J.Y.; Show, P.L.; Suan, N.H.; Juan, J.C.; Ling, T.C. & Chang, J.-S. Microalgae biorefinery: high value products perspectives. *Bioresource Technology*, p. 1-43, 2017.

Chisti, Y. Biodiesel from microalgae. *Biotechnology Advances*, p. 294-306, 2007.

Chisti, Y. & Moo-Young, M. Bioreactors. *Encyclopedia of Physical Science and Technology*, 2003.

Choi, S.P.; Nguyen, M.T. & Sim, S.J. Enzymatic pretreatment of *Chlamydomonas reinhardtii* biomass for ethanol production. *Bioresource Technology.*, v. 101, p. 5330-5336, 2010.

Da Silva-Ferreira, V. & Sant'Anna, C. Impact of culture conditions on the chlorophyll content of microalgae for biotechnological applications. *World J. Microbiol. Biotechnol.*, v. 33, p. 247-271, 2017. DOI: 10.1016/b0-12-227410-5/00067-3.

Damiani, M.C.; Popovich, C.A.; Constenla, D. & Leonardini, P.I. Lipid analysis in Haematococcus pluvialis to assess its potential use as a biodiesel feedstock. *Bioresour Technol.*, p. 3801-3807, 2010.

De Lasa, H.; Salaices, E.; Mazumder, J. & Lucky, R. Catalytic steam gasification of biomass: catalysts, thermodymanics and kinetics. *Chem. Rev.*, v. 111, p. 5404-5433, 2011. DOI: 10.1021/cr200024w.

Deshmukh, S.; Kumar, R. & Bala, K. Microalgae biodiesel: A review on oil extraction, fatty acid composition, properties and effect on engine performance and emissions. *Fuel Processing Technology*, v. 191, p. 232-247, 2019.

De Vrieze, J.; Raport, L.; Willems, B.; Verbrugge, S.; Volcke, E.; Meers, E.; Angenent, L.T. & Boon, N. Inoculum selection influences the biochemical methane potential of agro-industrial substrates. *Microbial. Biotechnol.*, v. 8, n. 5, p. 776-786, 2015.

Diep, N.Q.; Sakanishi, K.; Nakagoshi, N.; Fujimoto, S.; Minowa, T. & Tran, X.D. Biorefinery: Concepts, current status, and development trends. *International Journal of Biomass and Renewables*, p. 1-8, 2015.

Djandja, O.S.; Wang, Z.; Chen, L.; Qin, L.; Wang, F.; Xu, Y. & Duan, P. Progress in hydrothermal liquefaction of algal biomass and hydrothermal upgrading of the subsequent crude bio-oil: a mini review. *Energy Fuels.*, v. 34, n. 10, p. 11723-11751, 2020. https://doi.org/10.1021/acs.energyfuels.0c01973.

Duman, G.; Uddin, M.A. & Yanik, J. Hydrogen production from algal biomass via steam gasification. *Bioresour. Technol.*, v. 166, p. 24-30, 2014. DOI: 10.1016/j.biortech.2014.04.096.

El-Baz, F.K.; Hussein, R.A.; Mahmoud, K. & Abdo, S.M. Cytotoxic activity of carotenoid rich fractions from Haematococcus pluvialis and Dunaliella salina microalgae and the identification of the phytoconstituents using LCDAD/ ESI-MS. *Phytother Res.*, v. 32, p. 298-304, 2018.

Farias-Silva, C.E. & Bertucco, A. Bioethanol from microalgae and cyanobacteria: A review and technological outlook. *Process Biochemistry*, v. 15, n. 11, p. 1833-1842, 2016.

Farias-Silva, C.E. & Bertucco, A. Bioethanol from biomass: a promising approach in biorefinery. *Braz. Aarch. Biol. Technol.*, v. 62, 2019. https://doi.org/10.1590/1678-4324-2019160816.

Fortier, M.O.P.; Roberts, G.W.; Stagg-Williams, S.M. & Sturm, B.S. Life cycle assessment of bio-jet fuel from hydrothermal liquefaction of microalgae. *Appl. Energy*, v. 122, p. 73-82, 2014.

Francavilla, M.; Kamaterou, P.; Intini, S.; Monteleone, M. & Zabaniotou, A. Cascading microalgae biorefinery: fast pyrolisis of Dunaliella tertiolecta lipid extracted-residue. *Algal Research*, v. 184-193, 2018.

Frigon, J.C.; Matteu-Lebrun, F.; Abdou, R.H.; McGinn, P.J.; O'Leary, S.J.B. & Guiot, S.R. Screening microalgae strains for their productivity in methane following anaerobic digestion. *Appl. Energy*, v. 108, p. 100-107, 2013.

Fuentes-Grünewald, C.; Garcés, E.; Alacid, E.; Sampedro, N.; Rossi, S. & Camp, J. Improvement of lipid production in the marine strains *Alexandrium minutum* and *Heterosigma akashiwo* by utilizing abiotic parameters. *J. Ind. Microbiol. Biotechnol.*, v. 39, p. 207-216, 2012.

Gárate-Osuna, A.J. Análisis del potencial de la microalga *Dunaliella tertiolecta* para la obtención de bioproductos bajo el concepto de biorrefinería. Thesis of Master in Applied Sciences, Universidad Politécnica de Sinaloa, 2020.

Golueke, C.G.; Oswald, W.J. & Gotaas, H.B. Anaerobic digestion of algae. *Appl. Microbiol.*, v. 5, p. 47-55, 1957.

Gómez-Luna, L. Microalgas: aspectos ecológicos y biotecnológicos. *Revista Cunama de Química*, p. 3-20, 2007.

González-Delgado, A.D. & Kafarov, V. Microalgae based biorefinery: issues to consider. *CT&F - Ciencia,Tecnología y Futuro*, v. 4, n. 4, p. 5-22, 2011.

González-Fernandez, C. & Muñoz, R. *Microalgae-based biofuels and bioproducts.* United Kingdom: Woodhead Publishing, 2017.

González-Fernández, C.; Molinuevo-Salces, B. & García-González, M.C. Evaluation of anaerobic codigestion of microalgal biomass and swine manure via response surface methodology. *Appl. Energy*, v. 88, p. 3448-3453, 2011.

González-Fernández, C.; Sialve, B.; Bernet, N. & Steyer, J.P. Impact of microalgae characteristics on their conversion to biofuel. Part II: focus on biomethane production. *Biofuels Bioprod. Biorefining*, v. 6, p. 205-218, 2012.

Grierson, S.; Strezov, V.; Ellem, G.; Mcgregor, R. & Herbertson, J. Thermal characterisation of microalgae under slow pyrolysis conditions. *J. Anal. Appl. Pyrolysis*, v. 85, n. 1, p. 118-123, 2009.

Grierson, S.; Strezov, V. & Shah, P. Properties of oil and char derived from slow pyrolysis of *Tetraselmis chui*. *Bioresour. Technol.*, v. 102, n. 17, p. 8232-8240, 2011.

Gorry, P.L.; Sánchez, L. & Morales, M. Microalgae biorefineries for energy and coproduct production, *in* Jacob-Lopes, E.; Queiroz-Zepka, L. & Queiroz, M.I. Energy from microalgae. *Springer Green Energy and Technology*, 2018 https://doi.org/10.1007/978-3-319-69093-3_5

Gouveia, L. *Microalgae as a feedstock for biofuels*. London, New York: Springer Heidelberg Dordretch, p. 2-20, 2011.

Guan, Q.; Wei, C.; Ning, P. & Gu, J. Catalytic gasification of algae *Nannochloropsis sp.* in sub/supercritical water. *Procedia Environ. Sci.*, v. 18, p. 844-848, 2013.

Guldhe, A.; Singh, P.; Kumari, S.; Rawat, I.; Permaul, K. & Bux, F. Biodiesel synthesis from microalgae using immobilized *Aspergillus niger* whole cell lipase biocatalyst. *Renewable Energy*, v. 85, p. 1002-1010, 2016.

Guldhe, A.; Ansari, F.; Singh, P. & Bux, F. Heterotrophic cultivation of microalgae using aquaculture wastewater: a biorefinery concept for biomass production and nutrient remedation. *Ecological Engineering*, p. 47-53, 2017.

Gupta, A.; Barrow, C.J. & Puri, M. Omega-3 biotechnology: thraustochytrids as a novel source of omega-3 oils. *Biotechnol. Adv.*, v. 30, p. 1733-1745, 2012.

Harun, R.; Danquah, M.K. & Forde, G.M. Microalgal biomass as a fermentation feedstock for bioethanol production. *J. Chem. Technol. Biotechnol.*, v. 85, n. 2, p. 199-203, 2010.

Hemlata, P.G.; Bano, F. & Fatma, T. Studies on Anabaena sp NCCU-9 with special reference to phycocyanin. *J. Algal Biomass Util.*, v. 2, n. 1, p. 30-51, 2011.

Hernández-Pérez, A. & Labbé, J. Microalgas, cultivos y beneficios. *Revista de Biología Marina y Oceanografía,* v. 49, n. 2, p. 157-173, ago. 2014.

Ho, S.; Huang, S.; Chen, C.; Hasunuma, T.; Kondo, A. & Chang, J. Bioethanol production using carbohydrate-rich microalgae biomass as feedstock. *Bioresource Technology*, v. 135, p. 191-198, 2013.

Hidaka, T.; Inoue, K.; Suzuki, Y. & Tsumori, J. Growth and anaerobic digestion characteristics of microalgae cultivated using various types of sewage. *Bioresour. Technol.*, v. 170, p. 83-89, 2014.

Huang, X. & Wen, W. Effects of nitrogen supplementation of the culture medium of the growth, total lipid content and fatty acid profiles of three microalgae (Tetraselmis subcordiformis, Nannochloropsis oculata and PAvlova wiridis). *Appl. Phycol.*, p. 129-137, 2013.

Huang, Y.; Chen, Y.; Xie, J.; Liu, H.; Yin, X. & Wu, C. Bio-oil production from hydrothermal liquefaction of high-protein high-ash microalgae including wild *Cyanobacteria* sp. and cultivated *Bacillariophyta* sp. *Fuel*, v. 183, p. 9-19, 2016.

Itoiz, E.S.; Fuentes-Grünewald, C.; Gasol, C.M.; Garcés, E.; Alacid, E.; Rossi, S. & Rieradevall, J. Energy balance and environmental impact analysis of marine microalgal biomass production for biodiesel generation in a photobioreactor pilot plant. *Biomass Bioenergy*, v. 39, p. 324-335, 2012.

Jaime, L.; Rodríguez-Meizoso, I.; Cifuentes, A.; Santoyo, S.; Suarez, S.; Ibáñez, E. & Señorans, F.J. Pressurized liquids as an alternative process to antioxidant carotenoids' extraction from Haematococcus pluvialis microalgae. *LWT Food Sci. Technol.*, v. 43, p. 105-112, 2010.

Kim, K.H.; Choi, I.S.; Kim, H.M.; Wi, S.G. & Bae, H. Bioethanol production from the nutrient stress-induced microalga *Chlorella vulgaris* by enzymatic hydrolysis and immobilized yeast fermentation. *Bioresource Technology*, v. 153, p. 47-54, 2014.

Kim, S.W.; Koo, B.S. & Lee, D.H. A comparative study of bio-oils from pyrolisis of microalgae and oil seed waste in a fluidized bed. *Bioresource Technology*, v. 162, p. 96-102, 2014.

Kim, D.Y.; Vijayan, D.; Praveenkumar, R.; Han, J.I.; Lee, K.; Park, J.Y.; Chang, W.S.; Lee, J.S. & Oh, Y.K. Cell-wall disruption and lipid/astaxanthin extraction from microalgae: Chlorella and Haematococcus. *Bioresource Technology*, v. 199, p. 300- 310, 2016.

Khoo, H.H.; Koh, C.Y.; Shaik, M.S. & Sharratt, P.N. Bioenergy co-products derived from microalgae biomass via thermochemical conversion-life cycle energy balances and CO2 emissions. *Bioresource Technology*, v. 143, p. 298-307, 2013.

Koutra, E.; Tsafrakidou, P.; Sakarika, M. & Kornaros, M. Microalgal biorefinery, p. 163-185, *in* Yousuf, A. (ed.). *Microalgae cultivation for biofuels production.* Academic Publish Elsevier, 2020. DOI: 978-0-12-817536-1.

Koyande, A.K; Show, P.L.; Guo, R.; Tang, B.; Ogino, C. & Chang, J.S. Bio-processing of algal bio-refinery: a review on current advances and future perspectives. *Bioengineered*, v. 10, n. 2, p. 574-592, 2019. https://doi.org/10.1080/21655979.2019.1679697.

Kwietniewska, E. & Tys, J. Process characteristic, inhibition factors and methane yields of anaerobic digestion process, with particular focus on microalgal biomass fermentation. Renew. Sust. Energy Rev., v. 34, p. 491-500, 2014.

Lam, M.K. & Lee, K.T. Microalgae biofuels: a critical review of issues, problems, and the way forward. *Biotechnol. Adv.*, v. 30, p. 673-690, 2012.

Lam, M.; Loy, A.C.; Yusup, S. & Lee, K. Biohydrogen Production From Algae. *Biohydrogen*, p. 219-245, 2019. DOI: 10.1016/b978-0-444-64203-5.00009-5.

Lee, O.K.; Kim, A.L.; Seong, D.H.; Lee, C.G.; Jung, Y.T.; Lee, J.W. & Lee, E.Y. Chemoenzymatic saccharification and bioethanol fermentation of lipid-extracted residual biomass of the microalga, *Dulaliella tetriolecta*. *Bioresource Technology*, v. 132, p. 197-201, 2013.

Lee, O.K.; Oh, Y. & Lee, E.Y. Bioethanol production from carbohydrate-enriched residual biomass obtained after lipid extraction of *Chlorella* sp. KR-1. *Bioresource Technology*, v. 196, p. 22-27, 2015.

Liu, J.; Huang, J.; Sun, Z.; Zhong, Y.; Jiang, Y. & Chen, F. Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic *Chlorella zofingiensis*: assessment of algal oils for biodiesel production. *Bioresource Technology*, v. 102, n. 1, p. 106-110, 2011.

Luo, X.; Su, P. & Zhang, W. Advances in microalgae-derived phytosterols for functional food and pharmaceutical applications. *Mar. Drugs*, v. 13, p. 4231-4254, 2015.

Lü, J.; Sheahan, C. & Fu, P. Metabolic engineering of algae for fourth generation biofuels production. *Energy Environ. Sci.*, v. 4, p. 2451-2466, 2011.

Mandal, S. & Mallick, M. Microalga *Scenedesmus obliquus* as a potential source for biodiesel production. *Appl. Microbiol. Biotechnol.*, p. 281-291, 2009.

Marsolek, M.D.; Kendall, E.; Thompson, P.L. & Shuman, T.R. Thermal pretreatment of algae for anaerobic digestion. *Bioresource Technology*, v. 151, p. 373-377, 2014.

McKendry, P. Energy production from biomass (part 2): conversion technologies. *Bioresource Technology*, v. 83, n. 1, p. 47-54, 2002.

Markou, G.; Angelidaki, I.; Nerantzis, E. & Georgakakis, D. Bioethanol production by carbohydrate-enriched biomass of Antrospira (Spirulina) platensis. *Energies*, v. 6, p. 3937-3950, 2013.

Markou, G. & Nerantzis, E. Microalgae for high value compounds and biofuels production: a review with focus on cultivation under stress conditions. *Biotechnol. Adv.*, v. 31, p. 1532-1542, 2013.

Martinez-Guerra, E.; Howlader, M.S.; Shields-Menard, S.; French, W.T. & Gude, V.G. Optimization of wet microalgal FAME production from Nannochloropsis sp. under the synergistic microwave and ultrasound effect. *Int. J. Energy Res.*, v. 42, p. 1934-1949, 2018. https://doi.org/10.1002/er.3989.

McClure, D.D.; Luiz, A.; Gerber, B.; Barton, G.W. & Kavanagh, J.M. An investigation into the effect of culture conditions on fucoxanthin production using the marine microalgae Phaeodactylum tricornutum. *Algal Res.*, v. 29, p. 41-48, 2018.

Mendez, L.; Mahdy, A.; Timmers, R.A.; Ballesteros, M. & Gonzáles-Fernández, C. Enhancing methane production of Chlorella vulgaris via thermochemical pretreatments. *Bioresource Technology*, v. 149, p. 136-141, 2013.

Mendez, L.; Mahdy, A.; Demuez, M.; Ballesteros, M. & González-Fernández, C. Effect of high pressure termal pretreatment on Chlorella vulgaris biomass: organic matter solubilization and biochemical methane potential. *Fuel*, v. 117, p. 674-679, 2014.

Merlo, S.; Durany, X.G.; Tonon, A.P. & Rossi, S. Marine microalgae contribution to sustainable development. *Water 2021*, v. 13, 1373, 2021. https://doi.org/ 10.3390/ w13101373.

Mikami, K. & Hosokawa, M. Biosynthetic pathway and health benefits of fucoxanthin, an algae-specific xanthophyll in Brown seaweeds. *Int. J. Mol. Sci.*, v. 14, p. 13763-13781, 2013.

Minowa, T. & Sawayama, S. A novel microalgal system for energy production with nitrogen cycling. *Fuel*, v. 78, n. 10, p. 1213-1215, 1999.

Miranda, J.R.; Passarinho, P.C. & Gouveia, L. Pre-treatment optimization of *Scenedesmus obliquus* microalga for bioethanol production. *Bioresource Technolgy*, v. 104, p. 342-348, 2012.

Mollers, K.B.; Cannella, D.; Jorgensen, H. & Frigaard, N. Cyanobacterial biomass as carbohydrate and nutriente feedstock for bioethanol production by yeast fermentation. *Biotechnology for Biofuels*, v. 7, p. 1-11, 2014.

Mussgnug, J.H.; Klassen, V.; Schluter, A. & Kruse, O. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. *J. Biotechnol.*, v. 150, p. 51-56, 2010.

Naik, S.N.; Goud, V.V.; Rout, P.K. & Dalai, A.K. Production of first and second generation biofuels: a comprehensive review. *Renew. Sustain. Energy Rev.*, v. 14, p. 578-597, 2010.

Oliver, L.; Dietrich, T.; Marañon, I.; Villaran, M.C. & Barrio, R.J. Producing omega-3 polyunsaturated fatty acids: a review of sustainable sources and future trends for the EPA and DHA market. *Resources*, v. 9, p. 148, 2020. doi:10.3390/resources9120148.

Onwudili, J.A.; Lea-Langton, A.R.; Ross, A.B. & Williams, P.T. Catalytic hydrothermal gasification of algae for hydrogen production: composition of reaction products and potential for nutrient recycling. *Bioresource Technology*, v. 127, p. 72-80, 2013.

Palomeros-Parada, M.; Osseweijer, P. & Posada-Duque, J. Sustainable biorefineries, an analysis of practices for incorporating sustainability in biorefinery design. Industrial Crops and Products, p. 1-9, 2016.

Pan, P.; Hu, C.; Yang, W.; Li, Y.; Dong, L.; Zhu, L.; Tong, D.; Qing, R. & Fan, Y. The direct pyrolysis and catalytic pyrolysis of *Nannochloropsis sp.* residue for renewable bio-oils. *Bioresource Technology*, v. 101, n. 12, p. 4593-4599, 2010.

Park, J. *et al*. Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology,* v. 102, p. 35-42, 2011.

Park, S. & Li, Y. Evaluation of methane production and macronutrient degradation in the anaerobic co-digestion of algae biomass residue and lipid waste. *Bioresource Technology*, v. 111, p. 42-48, 2012.

Parmar, A.; Singh, N.K.; Kaushal, A.; Sonawala, S. & Madamwar, D. Purification, characterization and comparison of phycoerythrins from three different marine cyanobacterial cultures. *Bioresource Technology*, v. 102, n. 2, p. 1795-1802, 2011.

Passos, F.; Solé, M.; García, J. & Ferrer, I. Biogas production from microalgae grown in wastewater: effect of microwave pretreatment. *Appl. Energy*, v. 108, p. 168-175, 2013.

Passos, F.; Uggetti, E.; Carrere, H. & Ferrer, I. Pretreatment of microalgae to improve biogas production: a review. *Biores. Technol.*, v. 172, p. 403-412, 2014.

Pradhan, R.R.; Pradhan, R.R.; Das, S.; Dubey, B. & Dutta, A. Bioenergy combined with carbon capture potential by microalgae at flue gas-based carbon sequestration plant of NALCO as accelerated carbon sink, p. 231-244, *in* Goel, M. & Sudhakar, M. (ed.). *Carbon utilization*. Singapore: Springer Singapore, 2017.

Raheem, A.; Azlina, W.W.; Yap, Y.T.; Danquah, M.K. & Harun, R. Thermochemical conversion of microalgal biomass for biofuel production. *Renew. Sustain. Energy Rev.*, v. 49, p. 990-999, 2015.

Raheem, A.; Dupont, V.; Channa, A.Q.; Zhao, X.; Vuppaladadiyam, A.K.; Taufiq-Yap, Y.H.; Zhao, M. & Harun, R. Parametric characterization of air gasification of *Chlorella vulgaris* biomass. *Energy Fuels*, v. 31, n. 3, p. 2959-2969, 2017.

Raheem, A.; Prinsen, P.; Vuppaladadiyam, A.K.; Zhao, M. & Luque, R. A review on sustainable microalgae-based biofuel and bioenergy production: recent developments. *Journal of Cleaner Production*, v. 181, p. 42-59, 2018.

Ramos-Suárez, J.L. & Carreras, N. Use of microalgae residues for biogas production. *Chem. Eng. J.*, v. 242, p. 86-95, 2014.

Ramos-Suárez, J.L.; Martínez, A. & Carreras, N. Optimization of the digestion process of Scenedesmus sp. and Opuntia maxima for biogas production. *Energy Convers Manag.*, v. 88, p. 1263-1270, 2014.

Ras, M.; Lardon, L.; Sialve, B.; Bernet, N. & Steyer, J.P. Experimental study on a coupled process of production and anaerobic digestion of Chlorella vulgaris. *Biores Technol.*, v. 102, p. 200-206, 2011.

Raza, H. *Aspen simulation of hydrothermal liquefaction process for the conversion of algae to renewable fuels and chemicals*. Beaumont, TX, USA: Lamar University, 2014.

Rizwan, M.; Lee, J.H. & Gani, R. Optimal design of microalgae-based biorefinery: economics, opportunities and challenges. *Appl. Energy*, v. 150, p. 69-79, 2015.

Rizzo, A.M.; Prussi, M.; Bettucci, L.; Libelli, I.M. & Chiaramonti, D. Characterization of microalga *Chlorella* as a fuel and its thermogravimetric behavior. *Appl. Energy*, v. 102, p. 24-31, 2013.

Rodionova, M.V.; Poudyal, R.S.; Tiwari, R.A.; Voloshin, R.A.; Zharmukhamedov, S.K.; Nam, H.G.; Zayadan, B.K.; Bruce, B.D.; Hou, H.J.M. & Allakhverdiev, S.I. Biofuel production: challenges and opportunities. *International Journal of Hydrogen Production Energy*, v. 42, n. 12, p. 8450-8461, 2017.

Rodolfi, L.; Zittelli, G.C.; Bassi, N.; Padovani, G.; Biondi, N.; Bonini, G. & Tredici, M.R. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering*, v. 102, p. 100-112, 2009.

Samson, R. & LeDuy, A. Improved performance of anaerobic digestion of Spirulina maxima algal biomass addition of carbon-rich wastes. *Biotechnol. Lett*, v. 5, p. 677-682, 1983.

Sánchez-Hernández, E.P. & Travieso-Córdoba, L. Anaerobic digestion of Chlorella vulgaris for energy production. *Resour. Conserv. Recy.*, v. 9, p. 127-132, 1993.

Sanchez-Silva, L.; López-González, D.; Garcia-Minguillan, A.M. & Valverde, J.L. Pyrolysis, combustion, and gasification characteristics of *Nannochloropsis gaditana* microalgae. *Bioresour. Technol.*, v. 130, p. 321-331, 2013.

Santos-Ballardo, D.U.; Font-Segura, X.; Sánchez-Ferrer, A.; Barrena, R.; Rossi, S. & Valdez-Ortiz, A. Valorization of biodiesel production wastes: anaerobic digestion of residual *Tetraselmis suecica* biomass and co-digestion with glycerol. *Waste Manag. Res.*, v. 33, p. 250-257, 2015.

Santos-Ballardo, D.U.; Rossi, S.; Reyes-Moreno, C. & Valdez-Ortiz, A. Microalgae potential as a biogas source: current status, restrains and future trends. *Reviews in Environmental Science and Bio/technology*, v. 15, p. 243-264, 2016a.

Santos-Ballardo, D.U.; Valdez-Ortiz, Á. & Rossi-Heras, S. *Energía verde a partir de microalgas: biogás como estrategia para una biorefinería sustentable. Antecedentes, retos y fundamentos.* Saarbrü ken, Deutschland, Alemania: Editorial Académica, 2016b.

Sforza, E.; Ciapriani, R.; Morosinotto,T.; Bertucco, A. &Giacometti. Excess CO_2 supply inhibits mixotrophic growth of *Chlorella protothecoides* and *Nannochloropsis salina*. *Bioresource Technology*, p. 523-529, 2012.

Shahidi, F. & Ambigaipalan, P. Omega-3 polyunsaturated fatty acids and their health benefits. *Annu. Rev. Food Sci. Technol.*, v. 9, p. 345-381, 2018.

Shen, Y.; Pei, Z.; Yuan, W. & Mao, E. Effect of nitrogen and extraction method on algae lipid yield. *Int. J. Agric. Biol. Eng.*, p. 51-57, 2009.

Sialve, B.; Bernet, N. & Bernard, O. Anaerobic digestion of microalgae as necessary step to make microalgae biodiesel sustainable. *Biotechnol. Adv.*, v. 24, p. 409-416, 2009.

Sikarwar, V.S.; ZHao, M.; Fennell, P.S.; Shah, N. & Anthony, E.J. Progress in biofuel production from gasification. *Prog. Energy Combust. Sci.*, v. 61, p. 189-248, 2017. DOI: 10.1016/j.pecs.2017.04.001.

Singh, J. & Gu, S. Commercialization potential of microalgae for biofuels production. *Renew Sust. Energy Rev.*, v. 14, p. 2596-2610, 2010.

Singh, D.; Puri, M.; Wilkens, S.; Mathur, A.S.; Tuli, D.K. & Barrow, C.J. Characterization of a new zeaxanthin producing strain of Chlorella saccharophila isolated from New Zealand marine waters. *Bioresource Technology*, v. 143, p. 308-314, 2013.

Stucki, S.; Vogel, F.; Ludwig, C.; Haiduc, A.G. & Brandenberger, M. Catalytic gasification of algae in supercritical water for biofuel production and carbon capture. *Energy Environ. Sci.*, v. 2, n. 5, p. 535- 541, 2009.

Tanakaa, T.; Yabuuchia, T.; Maedaa, Y.; Nojimaa, D.; Matsumotob, M. & Yoshino, T. Production of eicosapentaenoic acid by high cell density cultivation of the marine oleaginous diatom *Fistulifera solaris*. *Bioresource Technology*, v. 245, p. 567-572, 2017.

Tan, Y. & Lin, J. Biomass production and fatty acid profile of a *Scenedesmus rubescenslike* microalga. *Bioresource Technology*, p. 10131-10135, 2011.

Thomas, N.V. & Kim, S.K. Beneficial effects of marine algal compounds in cosmeceuticals. *Mar. Drugs*, v. 11, n. 1, p. 146-164, 2013.

Ueno, Y.; Kurano, N. & Miyachi, S. Ethanol production by dark fermentation in the marina green alga, *Chlorococcum littorale*. *Journal of Fermentation and Bioengineering*, v. 86, n. 1, p. 38-43, 1998.

Ugwu, C.; Aoyagi, H. & Uchiyama, H. Photobioreactors for mass cultivation of algae. *Bioresource Technology,* v. 99, n. 10, 4021e4028, 2008.

Ummalyma, S.B.; Sahoo, D. & Pandey, A. Microalgal biorefineries for industrial products, p. 187-196, *in* Yousuf A. (ed.). *Microalgae cultivation for biofuels production*. Academic Publish Elsevier, 2020. DOI: 978-0-12-817536-1.

Vanthoor-Koopmans, M.; Wijiffels, R.H.; Barbosa, M.J. & Eppink, M.H.M. Biorefinery of microalgae for food and fuel. *Bioresource Technology*, v. 135, n. 1, p. 142-149, 2013.

Vardon, D.R.; Sharma, B.K.; Jaramillo, H.; Kim, D.; Choe, J.K.; Ciesielski, P.N. & Strathmann, T.J. Hydrothermal catalytic processing of saturated and unsaturated fatty acids to hydrocarbons with glycerol for in situ hydrogen production. *Green Chem.*, v. 16, n. 3, p. 1507-1520, 2014.

Velasco *et al.* Effect of the ligth intensity, temperature and salinity on the growth of Isochrysis galbana (T-ISO). *Revista Intrópica*, p. 92-99, 2009.

Venkata-Subhash, G.; Meghna Rajvanshi, G.; Raja Krishna K.; Uma Shankar S.; Venkatesh P.; Sridharan G. & Santanu D. Challenges in microalgal biofuel production: a perspective on techno economic feasibility under biorefinery stratagem. *Bioresource Technology*, v. 343, 126155, 2022.

Vitovà, M.; Bisova, K.; Kawano, S. & Zachleder, V. Accumulation of energy reserves in algae: from cell cycles to biotechnological applications. *Biotechnology Advances*, v. 33, n. 6, p. 1204-1218, 2015.

Wahlen, B.D.; Willis, R.M. & Seefeldt, L.C. Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. *Bioresource Technology*, v. 102, n. 3, p. 2724-2730, 2011.

Wang, K.; Brown, R.C.; Homsy, S.; Martinez, L. & Sidhu, S.S. Fast pyrolysis of microalgae remnants in a fluidized bed reactor for bio-oil and biochar production. *Bioresource Technology*, v. 127, p. 494-499, 2013.

Wang, J.; Wang, X.D.; Zhao, X.Y.; Liu, X.; Dong, T. & Wu, F.A. From microalgae oil to produce novel structured triacylglycerols enriched with unsaturated fatty acids. *Bioresource Technology*, v. 184, p. 405-414, 2015.

Ward, A.J.; Lewis, D.M. & Green, F.B. Anaerobic digestion of algae biomass: a review. *Algal Res.*, v. 5, p. 204-214, 2014.

Williams, P.J.L.B. & Laurens, L.M. Microalgae as biodiesel biomass feedstocks: review analysis of the biochemistry, energetics economics. *Energy Environ. Sci.*, v. 3, n. 5, p. 554-590, 2010.

Yan, H.; Hoekman, S.K.; Cui, Z.; Jena, U. & Das, P. Hydrotermal liquefaction of marine microalgae using co-solvents. *Algal Research.*, v. 38, p. 101421, 2019.

Yang, C.; Li, R.; Zhang, B.; Qiu, Q.; Wang, B.; Yang, H.; Ding, Y. & Wang, C. Pyrolisis of microalgae: a critical review. *Fuel Processing Technology*, v. 186, p. 53-72, 2019.

Yang, K.C.; Wu, K.T.; Hsieh, M.H.; Hsu, H.T.; Chen, C.S. & Chen, H.W. Co-gasification of woody biomass and microalgae in a fluidized bed. *J. Taiwan Inst. Chem. Eng.*, v. 44, n. 6, p. 1027-1033, 2013.

Yen, H.W. & Brune, D.E. Anaerobic co-digestion of algal sludge and waste paper to produce methane. *Bioresource Technology*, v. 98, p. 130-134, 2007.

Zamalloa, C.; Boon, N. & Verstraete, W. Anaerobic digestibility of Scenedesmus obliquus and Phaeodactylum tricornutum under mesophilic and thermophilic conditions. *Appl Energy*, v. 92, p. 733-738, 2012.

Zhao, B.; Ma, J.; Zhao, Q.; Laurens, L.; Jarvis, E.; Chen, S. & Frear, C. Efficient anaerobic digestion of whole microalgae and lipid extracted microalgae residues for methane residues for methane energy production. *Bioresource Technology*, v. 161, p. 423-430, 2014.

Zhen, Y.; Chi, Z.; Lucker, B. & Chen, S. Two-stage heterotrophic and phototrophic culture strategy for algal biomass and lipid production. *Bioresource Technology*, p. 484-488, 2012.

Zhu, L. The combined production of ethanol and biogas from microalgal residuals to sustain microalgal biodiesel: a theoretical evaluation. *Biofuels Bioprod. Biorefining*, v. 8, p. 7-15, 2014.

Zeshan, O.P.K. & Visvanathan, C. Effect of C/N ratio and ammonia-N accumulation in a pilot-scale thermophilic dry anaerobic digester. *Bioresource Technology*, v. 113, p. 294-302, 2012.