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.
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 coprodutos 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.

Palavras-chave: microalgas, bioenergia, bioprodutos, biorrefinarias.
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
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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, biofertilizers, 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](image-url)
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 Provisional 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 CO₂ 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.

| Condition | How it affects to microalgae                                                                 | Reference                           |
|-----------|--------------------------------------------------------------------------------------------|-------------------------------------|
| Light     | Directly influences the overall productivity of microalgae in photoautotrophic cultures, because cultures depend on CO₂ 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 microalgal specie (freshwater or marine microalgae), and it affects the microalga 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 CO₂ (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 microalga 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. | Arias-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).](image-url)
Table II – Photobioreactors used in microalgae cultures

| Type of system | Reactor                        | Description                                                                                                                                 |
|---------------|--------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Open          | Raceway 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 CO₂ 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. |
|               | 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 O₂ 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 prejudicial to microalgae cultures. |
| Closed        | 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 CO₂ 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 CO₂ 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
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.

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

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 saccharophila        | 18-54                 |
|                     | Chlorella sp.                  | 19-43                 |
|                     | Chlorella vulgaris             | 15-58                 |
|                     | Chlorella zofingiensis         | 51                    |
|                     | Haematococcus pluvialis        | 35                    |
|                     | Neochloris oleobundans         | 26-38                 |
|                     | Scenedesmus dimorphus          | 31                    |
|                     | Scenedesmus irgrassabulius     | 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                    |
|                     | Nannochloris oculata           | 8-54                  |
|                     | Nannochloris 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); Aslam et al. (2020).
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**

![Diagram of conversion processes](source)

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                        | 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 O₂ 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 |
|-------------------|------------|-------------------------------|-----------|
| *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, CO₂ 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 with/without HZSM-5, 10 °C min⁻¹ 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, fluidized 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 CH₄ and CO₂; 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 H₂, CO, CO₂ and CH₄. 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 H₂ by employing sorption enhanced reforming (Sanchez-Silva et al., 2013; Raheem et al., 2017).

For microalgae gasification, the biomass usually is heated under low O₂ 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 H₂ (50%), CO₂ (35%), while CH₄ 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 CO₂ 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 CO₂ to carbon ratio (CO₂/C) at different pressures, steam to carbon ratio and equivalence ratios (ERs). It is showed that the introduction of CO₂ to the gasification process has a positive effect on the CO concentration, a negative effect on the H₂ concentration, and minimum effects on CGE and GSE. The gasification of the microalgae shows the best performance in terms of H₂/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 | CO₂ (44-49), CH₄ (16-38), H₂ (10-35) | Nitrogen in the algal biomass was converted to NH₃ during gasification | Minowa & Sawayama, 1999 |
| *Chlorella vulgaris*   | 700 °C, 15 min, Ru/TiO₂ | CO₂ (26), CH₄ (25), CO (22), H₂ (1) | Higher gas/H₂ 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 | CO₂ (50-51), H₂ (25-69), CH₄ (12-29), CO (2-4) | Higher H₂ 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 | CO₂ (13), CO (12), H₂ (9), CH₄ (2) | Lower H₂/CO₂ 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 | H₂ (48), CO₂ (36), CH₄ (15), CO (1) | Ru/C was most efficient catalyst for H₂ 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 | CO₂ (35-45), CH₄ (17), H₂ (18-57), CO (1-5) | In presence of NaOH/Ni higher H₂ yield + lower phenol/tar yields | Onwudili et al., 2013 |
| *Nannochloropsis gaditana* | 850 °C, TGA | H₂ (45), CO (33), CO₂ (12), CH₄ (4), | Prolonged oxidation stage due to increased O₂ content. Higher H₂ production by steam concentration. | Sanchez-Silva et al., 2013 |
| *Spirulina platensis*  | 800 °C, co- gasification with wood. Fixed bed reactor | CO₂ (40), CO₁ (25), H₂ (19), CH₄ (8) | CO/CO₂ concentrations increase with increased algae co-feeding, whereas H₂ and CH₄ first decrease and then increase gradually. Limitations due to high ash content. | Yang et al., 2013 |
| *Spirulina platensis*  | 800-1000 °C. Fixed bed reactor | H₂ (34-48), CO₂ (31-37), CO (10-18), CH₄ (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 | CO₂ (36-38), H₂ (21-60), CH₄ (21-26), CO (4) | NaOH/Ni led to significant increase in H₂ production and reduction in phenols/tars. | Duman; Uddin & Yanik, 2014 |
| *Nannochloropsis oculata* | 850 °C, 15 min, Fe₂O₃, CeO₂. Fixed bed reactor | H₂ (50), CO₂ (35), CH₄ (10), CO (6) | Gas yield depends on algae characteristics, process parameters and catalyst loading. Catalytic activity increased tar degradation + H₂ production. | Duman; Uddin & Yanik, 2014 |
| *Spirulina platensis*  | > 400 °C, Ru/ZrO₂; Ru/C | CO₂ (38-77), CH₄ (2-52), H₂ (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                  | Carbohydrates (wt %) | Proteins (wt %) | Lipids (wt %) | T (°C) | Time (min) | Bio-oil (wt %) | Higher Heating value (MJ kg⁻¹) | Reference               |
|-----------------------------------|----------------------|-----------------|--------------|--------|------------|---------------|-------------------------------|-------------------------|
| 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                          | Garcia-Alba et al., 2011|
| Desmodesmus sp.                  | 20                   | 44              | 14           | 375    | 5          | 49             | 35.4                          | Jena & Das, 2011        |
| Spirulina platensis              | 31                   | 49              | 11           | 350    | 60         | 40             | 35.2                          |                         |
| Chlorella vulgaris                | 9                    | 55              | 25           | 300    | 60         | 47             | 37.5                          |                         |
| Chlorogloeoepsis fritschii       | 44                   | 5               | 7            | 300    | 60         | 39             | 32                            |                         |
| Nannochloropsis oculata          | 8                    | 57              | 32           | 350    | 60         | 35             | 34.5                          |                         |
| Spirulina platensis              | 20                   | 65              | 5            | 300    | 60         | 36             | 36.1                          | Biller et al., 2012     |
| 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   |
| Phaeodactyulum 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. 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...
MICROALGAE AS A PROMISING ALTERNATIVE FOR DEVELOPMENT OF BIOREFINERIES: MAIN TECHNOLOGICAL AND ECONOMICAL CHALLENGES

...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 (m² 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 (Martínez-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 reinhardtii   | 17                                        |
| Chlorella pyrenoidosa       | 26                                        |
| Chlorella sp.               | 19                                        |
| Chlorella vulgaris          | 12-17                                     |
| Chlorococcum sp.           | 32.5                                      |
| Dunaliella biculata        | 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                                     |
| Synechococcus 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⁻¹ day⁻¹) | Type and Conditions of Hydrolysis | Yield of hydrolysis (%) | Yield of fermentation (%) | Reference                  |
|--------------------------|---------------------------------------------|-----------------------------------|----------------------------------|--------------------------|--------------------------|---------------------------|
| Chlamydomonas reinhardtii| 50                                         | 7                                 | Enzymatic (α-amylase and glucoamylases) | 94                       | 60                       | Choi; Nguyen & Sim, 2010   |
| Chlamydomonas faciata    | 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 |
| Antrospira 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 glucanases) | 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 CO₂ 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 H₂ 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 CH₄ and CO₂, with traces of other gases such as H₂S. 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 (CH₄) 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 microalgal 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 microalgal 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 consortia 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 microalgal biomass used. Because of this, an adequate selection and standardization of different inoculum that will be used for degradation of microalgal 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 (°C) | HRT (days) | Methane production (LCH₄/gVS⁻¹) | Biogas % CH₄ | References |
|--------------------|---------|------------------|-----------|-------------------------------|--------------|------------|
| Chlorella and Scenedesmus | Batch | 35-50 | 30 | 0.17-0.32 | 62-64 | Golueke; Oswald & Gotaas, 1957 |
| Tetraselmis suecica | CSTRᵃ | 35 | 14 | 0.31 | 72-74 | Asinari et al., 1982 |
| Spirulina | Semi-continuous | 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. | CSTRᵃ | 35 | 10 | 0.09-0.136 | 69 | Yen & Brune, 2007 |
| Arthospita 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 | González-Fernández et al., 2011 |
| Chlorella vulgaris | CSTRᵃ | 35 | 28 | 0.24 | NS | Ras et al., 2011 |
| Scenedesmus obliquus | FTRᵇ | 33 | 2.2 | 0.296 | 74.3 | Zamalloa; Boon & Verstraete, 2012 |
| Scenedesmus obliquus | FTRᵇ | 54 | 2.2 | 0.462 | 77.1 | Zamalloa; Boon & Verstraete, 2012 |
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**Table XI**

| Microalgae species               | Reactor     | Temperature (°C) | HRT (days) | Methane production (LCH₄gVS⁻¹) | Biogas % CH₄ | References                        |
|----------------------------------|-------------|------------------|------------|-------------------------------|--------------|-----------------------------------|
| Phaeodactylum tricornutum        | FTRb        | 33               | 2.1        | 0.6                           | 75.1         | Zamalloa; Boon & Verstraete, 2012 |
| Phaeodactylum tricornutum        | FTRb        | 54               | 2.3        | 0.628                         | 78.6         | Zamalloa; Boon & Verstraete, 2012 |
| Nannochloropsis salina           | Semi-continuous | 37             | 40         | 0.13                          | 64-68        | Park & Li, 2012                   |
| Chlorella vulgaris               | Batch       | 35               | 30         | 0.138c                        | 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               |
| Micractinium sp.                 | Batch       | 35               | 34-50      | 0.36                          | NS           | Frigon et al., 2013               |
| Botryococcus 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         | 0.142-0.148c                  | 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     |

* CSTR: completely stirred tank reactor.

b flow-through reactors.

c LCH₄gCOD⁻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 CH₄ 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 (NH₃) 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 CH₄ 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⁻¹VS), compared to 172 mL biogas g⁻¹VS 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 CH₄ 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 pluvialis* | Astaxanthin  | Physical disruption and solvent extraction | 35% (35.1 mg g⁻¹)    | Grinding cell wall disruption                    | Jaime et al., 2010     |
| *Chlorella pyrenoidesa* | 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 CO₂ fixation        | 92.2 mg g⁻¹           | Enchanced by engineering strategies              | Chen et al., 2013      |
| *Haematococcus pluvialis* | Astaxanthin  | Solvent extraction                       | 46 mg L⁻¹             | Highest yield obtained with 6% CO₂               | Cheng et al., 2016     |
| *Haematococcus pluvialis* | Astaxanthin  | 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 phyco-
biliproteins 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 docosahexaenoic 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 Cryptothecodiaceae family. Within these, the Schyzochitrium,
Ulkenia and Cryptothecidium 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-\textit{b} radiation and show inhibitory effects on melanogenesis. Usually, these bioactive compounds are extracted from brown algae like \textit{Ecklonia cava}, \textit{Fucus vesiculosus}, and \textit{Ascophyllum nodosum} (Thomas & Kim, 2013; Oliver \textit{et al.}, 2020). Also, the antioxidant activity of microalgae can be attributed to several phenolic compounds (gallic, cinnamic, salicylic, caffeic, ferulic, and \textit{p}-coumaric acids) that have been identified in different microalgal species (Aslam \textit{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 \textit{et al.}, 2020).

Between the alternative uses of biomass, biofuels are a highly promising option with numerous advantages in terms of \textit{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 \textit{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 \textit{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 \textit{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 \textit{et al.}, 2012). Regarding to this, Bohutskyi \textit{et al.} (2015) studied the methane potential from lipid-extracted algal residues from \textit{Auxenochlorella}
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protothecoides reaching up to 0.25 liters of methane per gram of volatile solid added (L CH$_4$ g VS$^{-1}$) 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, microalgal 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 CH₄ 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 hand, 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 an important opportunity area and further developed is 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 CO₂ 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.

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