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Biofuel production from microalgae: challenges and chances

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Abstract The inherent capability and increased efficiency of microalgae to convert sunlight into solar chemical energy are further enhanced by the higher amount of oils stored in microalgae compared to other land-based plant species. Therefore, the widespread interest in producing biofuels from microalgae has gained considerable interest among leading energy experts and researchers due to the burgeoning global issues stemming from the depletion of fossil fuel reserves, future energy security, increasing greenhouse gas emissions, and the competition for limited resources between food crops and conventional biomass feedstock. This paper aims to present the recent advances in biofuel production from microalgae and the potential benefits of microalgae in the energy and environmental sectors, as well as sustainable development. Besides, bottlenecks and challenges mainly relating to techniques of cultivation and harvesting, as well as downstream processes are completely presented. Promising solutions and novel

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trends for realizing strategies of producing biofuels from microalgae on an industrial and commercial scale are also discussed in detail. Alternatively, the role of microalgae in the circular economy is thoroughly analyzed, indicating that the potential of scaling up current microalgae-based production could benefit from the waste-to-energy strategy with microalgae as a key intermediate. In the future, further research into combining different microalgae biomass pretreatment techniques, separating the microalgae feedstock from the cultured media, developing new species, and optimizing the biofuel production process should be carried out to reduce the prices of microalgae biofuels.

Keywords Microalgae · Biofuel · Bottlenecks · Benefits · Circular economy

Introduction

Along with the extraordinary pace of technological advances, the twenty-first century also faces incredibly difficult global challenges such as overpopulation, climate change, food scarcity, depletion of fossil fuels, etc., (Hoang et al. 2021c; Bhushan et al. 2020; Nayak and Mishra 2016). Indeed, the growing reliance on nonrenewable sources of energy has prompted a growing interest in finding more sustainable solutions to meet the increasing global energy demands; this lesson could be observed more clearly from the shifting progress to clean energy and energy crisis after the COVID19 pandemic (Steffen et al. 2020; Chen et al. 2021). Among the renewable and clean energy sources, solar power presents a vast amount of renewable energy harnessed from the sun; through photosynthesis, plants use sunlight to grow and mature (Udayan et al. 2022; Hariskos and Posten 2014). Therefore, plant biomass-derived carbonaceous materials present a promising and widely available source of feedstock for biofuel production such as microalgae, crop residues, and organic wastes (Hoang et al. 2022; Papathoti et al. 2021; Alami et al. 2021). The conversion of widely available biomass into promising sources of renewable energy such as biofuels has been a popular topic among current scientists and energy researchers (Chen et al. 2022; Aissi et al. 2021; Hoang and Pham 2021). Biofuels are commonly classified into three different categories, including first, second, and third generation. Biofuels produced from food crops are considered first-generation such as bioethanol made from starch. For biofuels that are derived from non-food crops such as lignocellulosic biomass can be grouped into the second-generation category. Last but not least, biofuels derived from microalgae and microbes are considered the third generation category (Sirohi et al. 2022a; SB et al. 2022).

One of the major challenges facing the production of biofuels is the inadequate and inconsistent supply of biomass feedstock (Koyande et al. 2019). Furthermore, the conversion of first-generation biofuels from food crops also raises the controversial food versus fuel debate as they compete for the same amount of arable land and freshwater resources. Recently, different species of microalgae have received much attention for their potential of being a promising feedstock for the production of third-generation biofuels (Mishra et al. 2017). Due to the incredibly fast growth rate and high carbon efficiency, microalgae present major benefits as a substrate for biofuel production. Examining the structure of microalgae, one can find the presence of several bioactive compounds such as lipids, proteins, carbohydrates, and fractions of anti-oxidants (Kusmayadi et al. 2021; Yu et al. 2021a). Besides the potential as a biofuel feedstock, microalgae are packed with a significant amount of nutrients that can be utilized in food

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supplements and animal feed. Microalgae also have a special role in dye products, cosmetics, and the pharmaceutical industry. Furthermore, studies also have found the benefit of microalgae as an effective pollution control agent (Aliyu et al. 2021). Among major industries such as biochemical engineering, food production, and pharmaceutical, there has been an increasing trend in the use of microalgae as a solution to address the burgeoning issues of sustainable production and consumption of food, feed, and fuel, as shown in Fig. 1. Compared to other biofuel alternatives, microalgae-derived biofuels demonstrate a higher potential and practicality (Kazemi Shariat Panahi et al. 2019b). Depending on the species of microalgae, the efficiency of bioethanol can be significantly increased when there is a higher content of carbohydrates (e.g., up to 30% of dry weight) (Kazemi Shariat Panahi et al. 2019a).

Using sunlight via photosynthesis, microalgae are capable of converting nutrients in the water to bioactive compounds that make up the primary cell structure (Verma et al. 2020). As microalgae, they feed off the nutrients (e.g., nitrogen, phosphates, and trace elements) when converting CO_2 into organic compounds with the energy provided from sunlight. Considering this characteristic alone, microalgae present a likely solution for wastewater treatment and CO_2 bioremediation in industrial processes (Cunha et al. 2020), suggesting that microalgae could be a key and critical link in the progress of converting waste to energy. Another significant advantage in the farming of microalgae is that it does not compete for the same resources required for the cultivation of traditional food crops. Furthermore, provided with high concentrations of CO_2, microalgae can grow at a rapid pace while utilizing and offsetting the CO_2 source such as flue gas from thermal power plants (Singh et al. 2019a; Sirohi et al. 2021). Furthermore, the technologies utilized in the production of biofuels from microalgae also have a lower carbon footprint

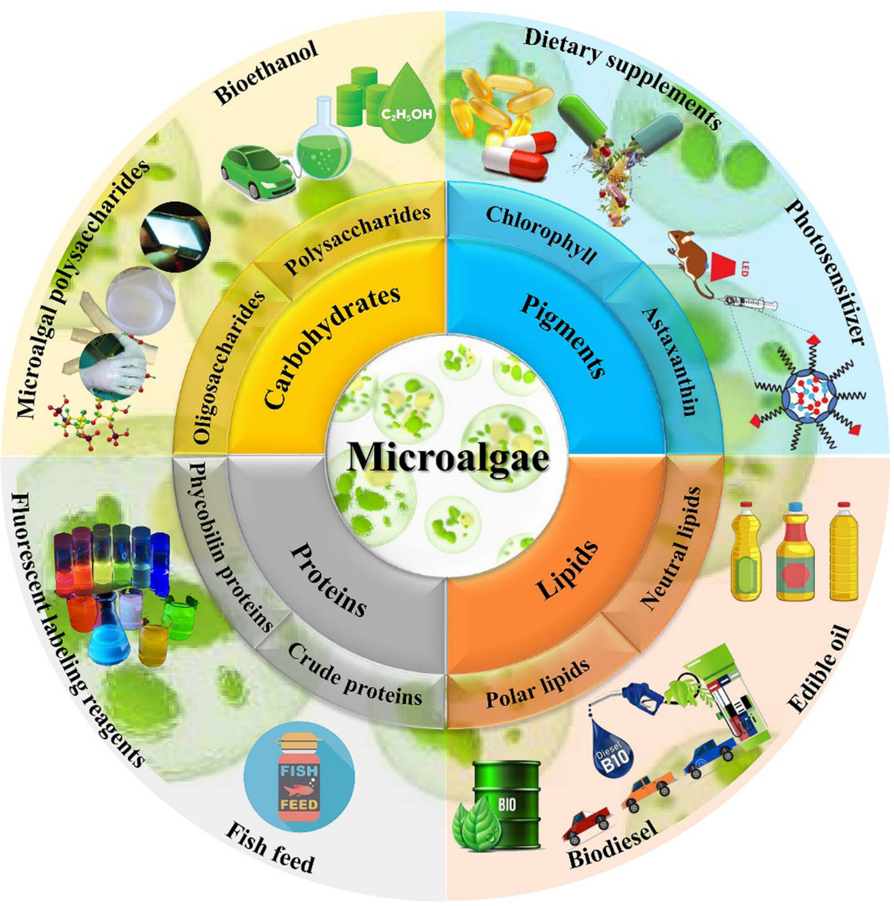

Fig. 1 Potential application of microalgae in various fields

and lower negative impacts on the ecosystem because the equipment used in microalgae-based biofuel technologies has a low-energy consumption level (Bwapwa et al. 2017; Khoo et al. 2020). Due to this reason, promoting the use of microalgae-derived biofuels can yield a positive sustainability effect on the environment since it is considered carbon neutral; thus, there is a significant benefit in pursuing this promising source on the path toward a higher sustainable energy future based on the circular economy and carbon neutrality (Javed et al. 2019).

Despite extensive progress in the development of microalgae technology, significant challenges remain surrounding the techno-economic viability of microalgae-derived biofuels. Among the current obstacles, cultivation techniques need further research to improve the biomass concentration of microalgae grown in suspended culture, as well as find an adequate supply of CO_2 and affordable growth medium for industrial-scale farming (Suparmaniam et al. 2019; MT et al. 2022). Notably, high concentrations of nutrients and different variety of salts are needed in the culture medium to support the growth of microalgae (Baicha et al. 2016). Moreover, increasing cost is a significant issue in the operation of microalgae cultivation systems in suspended cultures (Tan et al. 2015). Upon harvesting, the next step is to decide which conversion method that depends significantly on the conditions of the farmed biomass and the desired biofuel products (Kumar et al. 2020). Overall, there are reasons and gaps to have further studies of the development of biofuels production from microalgae due to several advantages and value-added products. Indeed, this review paper explores existing practices while reviewing the bio-processing of microalgae, also known as bio-refinery of microalgae. This current work also attempts to provide an overview of the current development of third-generation biofuels derived from microalgae. Furthermore, this review paper also addresses the current issues facing the implementation of microalgae bio-refinery and future opportunities for the industry. On the one hand, the paper summarizes the recent development in terms of strategies employed in the production of biofuels from microalgae. On the other hand, it also focuses prospects, opportunities, and challenges on the path toward scaling up microalgae-based biofuel production and improving widespread market penetration. More importantly, this review paper aims to provide insights and inputs in the strategic decisions by various stakeholders such as researchers, legislators, officials, environmental organizations, private sectors in advancing the progress of the biofuel industry overall and improve the viability and cost-effectiveness of the microalgae-based biofuel products specifically.

Microalgae: properties and CO_2 fixation capacity

Compared to various land-dwelling species of flora, microalgae have a significantly higher concentration of light-harvesting pigment known as chlorophyll which results in more effective photosynthesis (Kumar et al. 2021b). Several factors can influence the cellular structure and make-ups of different species of microalgae. Among these, inherent conditions such as species, growth media, environmental factors, and the coexistence of multiple strains within the same culture are examples of factors that can affect the nature and characteristics of microalgae (Kim et al. 2014).

Provided with metabolic versatility, microalgae are capable of being converted into a wide range of value-added products via various processing pathways. For certain species of microalgae with high concentrations of carbohydrates (e.g. 37–55%) such as *Chlorella, Chlamydomonas, Dunaliella, Scenedesmus,* and *Tetraselmis*, the starch-rich components are mainly found in the chloroplasts and cell walls made of cellulose (Dragone et al. 2011). Besides, the lipid contents in microalgae can range between 2 and 77% of the biomass volume for certain species under favorable growth conditions. Lipids in microalgae are grouped based on the end-product from the conversion process, namely biofuel and nutrient supplements (Mimouni et al. 2018; Ho et al. 2020). For the latter category, microalgae present a potentially good source for the manufacturing of omega-3 fatty acids, in which common microalgae species used in the production of omega-3 polyunsaturated fatty acids include *Bacillariophyta, Chlorophyta, Cryptophyta, Haptophyta, Haptophyta, and Rhodophyta* (Ryckebosch et al. 2012, 2014). Last but not least, proteins which are considered an essential product in the biorefinery process of microalgae make up a significant component. Depending on the species, they can range anywhere between 50–70% of the biomass volume in microalgae. Proteins extracted from microalgae have different uses such as in food supplements and animal feeds (e.g., aquaculture and husbandry) (Bertsch et al. 2021). Furthermore, pigments and other bioactive compounds can also be extracted from microalgae. For example, several chemical processes often utilize chlorophylls, phycobilins, and carotenoids that are extracted from several microalgae species such as *Porphyridium cruentum, Synechococcus sp.*, and *Chlorella* (Ummalyma et al. 2020).

Given the high concentration of biological molecules contained in the biomass, microalgae present an attractive option to be used as a substrate in energy conversion. The production of specific biofuel relies heavily on the biochemical make-up of the microalgae substrate. For example, various species of microalgae with a higher mass fraction of lipid content provide optimal feedstock for biodiesel production (Brindhadevi et al. 2021). On the other hand, microalgae that have higher carbohydrate content are more suitable for the production of fermentative alcohol (Phwan et al. 2019). Notably, researchers have found ways to modify the biochemical structure of microalgae species with the help of genetic engineering techniques to improve the characteristics of certain strains. Furthermore, the growth rate of selected species of microalgae can be significantly enhanced with the development and application of photobioreactors in the cultivation process (Sirohi et al. 2022b; Ranganathan et al. 2022). Moreover, microalgae species with high concentrations of fatty acids have been regarded as potential sources to supplement conventional petroleum-based fuels and fish oils. Depending on the strains of microalgae, the productivity of biomass and lipid content can vary significantly when they are used as inputs for edible oil production (Shin et al. 2018b). Despite the fact that the investigation into optimal culture conditions to increase the lipid content from microalgae has been conducted, it is unlikely to achieve equally desirable results if the chosen strains are not suitable for the conversion to edible oil (Piligaev et al. 2015). Hence, it is important to consider the microalgae species as it is the major factor affecting the productivity and composition of major components such as fatty acids, carbohydrates, lipids, and proteins (Xue et al. 2021). Figure 2 provides nutrients of different microalgae species for the purpose of biofuel production.

Previous studies have examined different species of microalgae for biofuel production, such as *Chlamydomonas sp.* (Scranton et al. 2015), *Chlorella sp.* (Guccione et al. 2014), *Senedesmus sp., Nannochloropsis sp.*, and a combination of various cultures included both fresh or wastewater strains (Sajjadi et al. 2018). Biological molecules found in microalgae biomass, namely lipid, protein, and carbohydrates, as well as the entire cell structure, can be converted into biofuels through proven techniques. All in all, the development of microalgae as a potential biomass feedstock for third-generation biofuel production presents a significant opportunity and viable solution for achieving a more sustainable energy future.

As reported, microalgae are found to have a higher growth rate, as well as they could fix CO_2 with higher capacity compared to aquatic and agricultural plants and traditional forests. More interestingly, without any evolution beyond the cells, microalgae could adapt to popular conditions of the environment and have a strong growth in the long term (Singh and Ahluwalia 2013). In the growing process, photosynthesis is known as the critical basis in the fixation and storage of CO_2 of microalgae. Many studies indicated that the efficiency of the photosynthesis process for microalgae could reach 10–20% (Li et al. 2008), even microalgae could perform 50% of the photosynthesis process on the Earth (Singh and Singh 2014). However, the level of CO_2 fixation depends much on the types of microalgae and the environmental conditions of cultivation (Ho et al. 2011). For example, *Chlorella* species were found to have strong CO_2 fixation capacity when CO_2 concentration ranged from 5 to 20% (Tang et al. 2011). In another case, *Spirulina* species could fix CO_2 at a high rate value of 37.9% (De Morais and Costa 2007). Besides, it was reported that the use of *N. oculata* for CO_2 fixation in flue gas produced from a coal-fired power plant could offer a high efficiency that around 1/3 of CO_2 could be removed from flue gas (Cheng et al. 2015b). In addition to traditional microalgae, many novel microalgae have been developed to provide excellent capacities in fixing CO_2. In a study by Aghaalipour et al. (2020), they found that *Psammothidium* and *Monoraphidium contortum* species could show a good capacity of CO_2 biofixation at concentration $< 10\%$. In another report based on the experimental results obtained from 20 microalgae species, *Gleocystis ampula* was observed to have the highest growth rate and CO_2 fixation capacity (0.281 g/l.d)

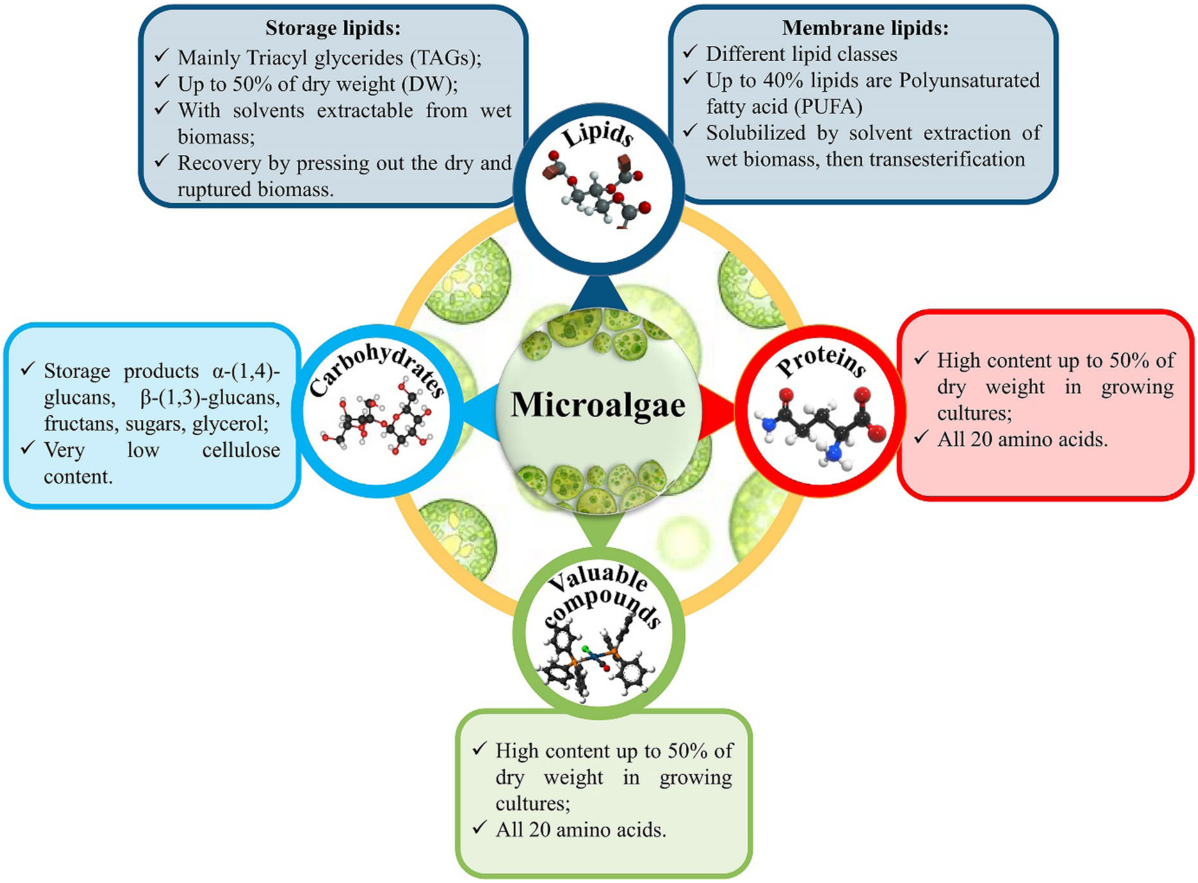

Fig. 2 Nutrients of different microalgae species (Singh and Gu 2010)

(Derakhshandeh et al. 2021). Also, many studies reported that a large number of unfamiliar microalgae could offer excellent capabilities in fixing CO_2 and producing biomass (Song et al. 2020; Ding et al. 2020; Tamil Selvan et al. 2020; Jin et al. 2021; Rodas-Zuluaga et al. 2021; Sung et al. 2021). In general, a critical route from the fixation process of CO_2 by microalgae to biofuel production could be depicted in Fig. 3.

Critical route for biofuel production from microalgae

The existing biorefinery process of microalgae has the potential to yield many high-value products that can be grouped into fuel and non-fuel categories (Chandra et al. 2019), in which common biofuels include bioethanol (or biobutanol), biodiesel, biogas, and bio-hydrogen. In general, Fig. 4 provides examples of advanced methods utilized in the conversion of microalgae biomass to different end-products.

Thermochemical conversion

Thermochemical liquefaction is a technique that can be utilized at low temperature and high-pressure conditions to convert wet microalgae biomass to bio-oil. In this method, the use of a catalyst is optional (Arvindnarayan et al. 2017a). Such an approach benefits from the elimination of the energy-intensive drying process. Through liquefaction, biomolecules such as lipids, carbohydrates, and proteins in microalgae biomass are processed into crude bio-oil. Several factors can affect the efficiency of the process, such as temperature, pressure, reaction time, catalyst, and the natural structure and composition of the microalgal biomass (Hong et al. 2021). Pyrolysis is another

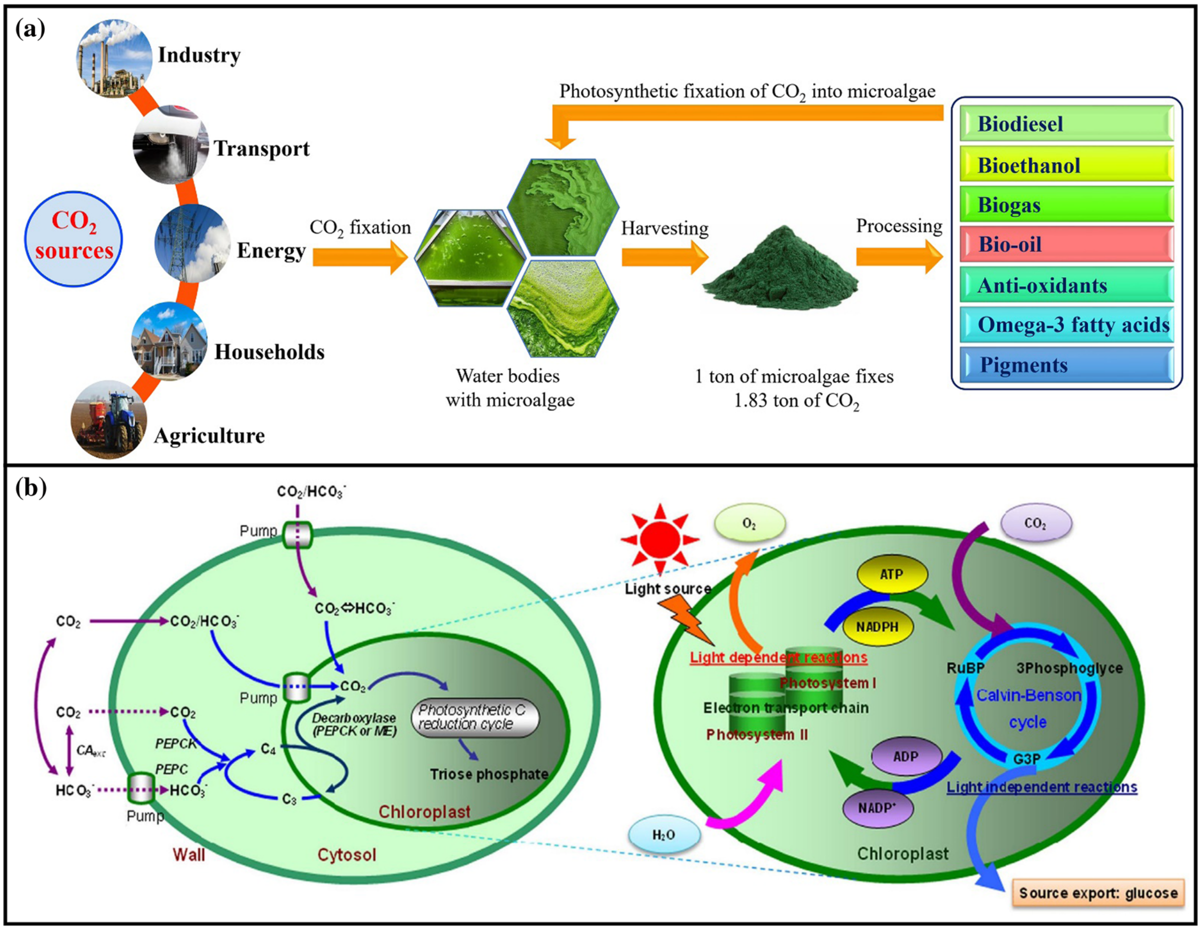

Fig. 3 **a**—Microalgae cultivation from various CO_2 sources and microalgae processing (Zhang and Liu 2021); **b**—Photobiochemical mechanism of fixing CO_2 in microalgae (Zhao and Su 2014) (With permission from Elsevier through LN 5262961242136)

commonly used technique to yield bio-oil, syngas, and charcoal from the combustion of biomass at medium to high temperatures (350–700 °C) without the presence of oxygen (Yang et al. 2019). For fast pyrolysis, the process occurs at a medium temperature range of approximately 500 °C with a really short hot vapor residence time of 1 s. The process has the potential to obtain up to 95,5% bio-oil yield (Venderbosch 2019). In contrast to slow pyrolysis, fast pyrolysis has a lower overall energy consumption due to its fast reaction while yielding a higher amount of bio-oil outputs. Furthermore, bio-oil obtained from the fast pyrolysis process has a lower viscosity compared to its counterpart obtained from slow pyrolysis (Tan et al. 2015). Hence, the fast pyrolysis process is more appropriate for the production of liquid bio-fuel in general and much larger industrial scales specifically (Hoang et al. 2021b).

After microalgae biomass undergoes either the pyrolysis or hydrothermal liquefaction (HTL) process, the dark brown liquid output is determined as bio-oil. The main difference between these two methods is that the former can only occur under dry conditions (i.e., less the 5% of moisture contained in the biomass feedstock) while HTL can be conducted using any type of microalgae biomass (Toro-Trochez et al. 2019). Within these methods, depolymerization of organic compounds takes place without the presence of oxygen (Sun et al. 2020). The microalgae biomass feedstock is first broken down into smaller molecules through various thermophysical processes such as dehydration, dehydrogenation, deoxygenation, and decarboxylation. One of the most significant factors driving the difference in the yield and quality of bio-crude is microalgae biomass constituents. According to Arvindnarayan et al. (2017b), there is a high potential in obtaining high energy content from the pyrolysis of *Chlorella* species of microalgae. Furthermore, bio-oil obtained from microalgae biomass can be easily blended with conventional transportation fuel without the need for major modifications to existing engines. According to the experiment reported by Jena and Das (2011), the pyrolysis of *Spirulina platensis* at 350 °C yielded 39.7, 23.8, and 19.2wt.% of biochar, bio-oil, and gases, respectively. According to Shakya et al. (2017), an experiment was conducted when subjecting nine species of microalgae to hydrothermal liquefaction at 280 °C and 320 °C in operating temperature and 0.7 MPa of pressure. The study confirmed the highest yield of bio-crude output from the conversion of lipid-rich *Nannochloropsis* at 320 °C. Furthermore, a series of investigations by Zhu et al. (2016) utilized microalgae biomass of two different species, namely *Scenedesmus* and *Spirulina* to produce bio-oil. The authors compared the results from the application of hydrothermal liquefaction (300 °C and 10–12 MPa) and the pyrolysis method (heating to 450 °C at a rate of 50 °C/min) to the above microalgae strains. As a result, both approaches yielded comparable results in terms of bio-oil output. Notably, the experiments obtained 24–45% of bio-oil with a high heating value (35–37 MJ/kg). Hence, the outputs of the pyrolysis of microalgae biomass depend on the species and the pyrolysis conditions. Moreover, the researchers also confirmed the advantage of hydrothermal liquefaction over the pyrolysis method in terms of energy consumption ratio. When the moisture content of wet biomass exceeded 80%, the ECR values of hydrothermal liquefaction and pyrolysis were measured in the range of 0.44–0.63 and 0.92–1.24, respectively. Recently, researchers have made significant progress on the development of microalgae biomass pyrolysis to convert microalgae into biofuels. For example, one study has successfully obtained nearly 54.4% (wt./wt.) yield of bio-oil from microwave-enhanced pyrolysis subjected to a CO_2 environment when using freshwater microalgae blooms naturally occurring in lakes as the main feedstock (Zhang et al. 2016). In another instance, the hydropyrolysis of the native microalgae consortium was observed to yield up to 31% of bio-oil under 200 °C operating temperature (Choudhary et al. 2017). Recent studies have explored the use of heterogeneous catalysts to enhance the conversion efficiency in the hydrothermal liquefaction of microalgae (Xu et al. 2019a; Kohansal et al. 2019). In their study, Saber et al. (2016) conducted low-temperature hydrothermal liquefaction using Nannochloropsis as the primary biomass feedstock. The experiments were conducted using three different catalysts, including nano-Ni/SiO_2, synthesized zeolite, and Na_2CO_3, under three different temperatures (210, 230, and 250 °C). Among these catalysts, nano-Ni/SiO_2 yielded the highest amount of bio-oil (30 wt.%), followed by Na_2CO_3 (24.2 wt.%) and zeolite (24 wt.%). In another comparable work, the hydrothermal liquefaction at 300 °C of Nannochlopsis in the presence of Ni supported TiO_2 obtained a 48.23% yield of bio-crude (Wang et al. 2018).

Under low-oxygen conditions, biomass can be heated at relatively high temperatures (800–1000 °C) to yield combustible gas mixtures in a process known as gasification (Clark and Deswarte 2014). The output of syngas typically contains several common gases such as CO, H_2, CH_4, and CO_2 (Hoang et al. 2021a). Although syngas has a low energy density (4–6 MJ/m^3), it is highly suitable for domestic heating and cooking by combustion in gas engines or gas turbines (McKendry 2002). In a study by Zhu et al. (2016), they conducted an experiment utilizing both wood biomass and microalgae with a 9:1 ratio in a co-gasification process. Compared to only using wood biomass, the co-gasification of microalgae and wood yielded higher H_2, CO, and CH_4 by 3–20%, 6 -31%, and 9–20%, respectively. Likewise, Raheem et al. (2015) performed the gasification of Chlorella biomass at 950 °C yielding H_2, CO, and CH_4 up to 2.9, 22.8, and 10.1 wt.% of biomass, respectively. In a study by Liu et al. (2017), they found that the gasification process exhibited an overall 81.6% efficiency with a combustion gas yield of 1.05 Nm^3/kg. The authors also confirmed the factors influencing the cold gas efficiency, such as steam and moisture content in the feedstocks, as well as the mixture of the microalgae substrate to a lesser degree. More importantly, the most significant factor is the moisture content in the gasification and biomass feedstock that affects the composition of the syngas output (Azadi et al. 2014). A study conducted by Diaz-Rey et al. (2015) also has shown the potential to obtain hydrogen-rich precursors with measured HHV up to 25 MJ Nm via gasification

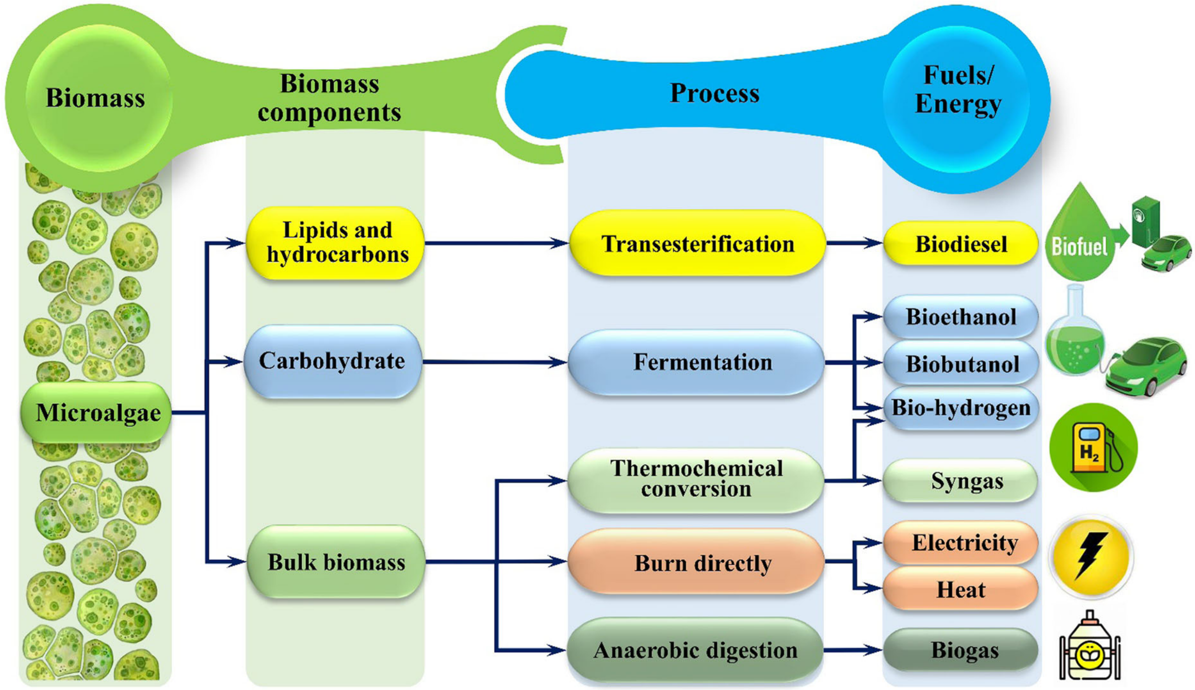

Fig. 4 Different routes for biofuels/bioenergy production from microalgae (Abo et al. 2019)

of microalgae. Such a process still faces major obstacles in dealing with the high concentration of nitrogen and minerals found in microalgae feedstock. One way to reduce the ash content in the output has been suggested by Liu et al. (2019b) that could eliminate the amount of ash by 67.6% when acid washing the microalgae feedstock with HCL. Other studies also pointed out that the elevated concentration of potassium in microalga ash could enhance gas yield, while the presence of calcium could activate carbon capture and deamination (Liu et al. 2020a; López-González et al. 2014). Table 1 provides a summary of different studies on thermochemical conversion of microalgae into biofuels.

Biochemical conversion

Anaerobic digestion is considered to be a popular biochemical conversion of biomass into biofuels. Biogas, which makes up mainly of CH_4 and CO_2 along with a small amount of H_2S, is the output of the anaerobic digestion process in which microbes break down and convert the organic biomass of microalgae. Typically, a constant temperature is maintained throughout the anaerobic digestion process to ensure the optimal condition for the growth of the microbes. The temperature range can be classified as mesophilic (30–42 °C) and thermophilic (43–55 °C). Most anaerobic digesters are constructed based on the characteristics of the former operating conditions, given their prevalence and stability in the treatment of different types of biomass (Gonzalez-Fernandez and Muñoz 2017). Commonly, there are three main stages of anaerobic digestion, including hydrolysis, fermentation, and methanogenesis. Initially, simple sugars are obtained from the hydrolysis of complex polysaccharides in biomass. Then, the fermentation process takes place to transform sugars into alcohols, acetic acid, volatile fatty acids, and traces of H_2 and CO_2. The gas mixture is further transformed into CH_4 (60–70%) and CO_2 (30–40%) in the presence of methanogens (Cantrell et al. 2008). The measured energy content of the biogas product is 20–40% of the lower heating value of the biomass feedstock. Brennan and Owende (Brennan and Owende 2010) have observed the suitability of wet biomass containing 80–90% of moisture as feedstock in an anaerobic digestion process. Several studies have attempted to use microalgae as the primary feedstock for the production of bio-methane via anaerobic digestion. Nevertheless,

Table 1 Thermochemical conversion methods of microalgae to bio-oil/syngas/hydrogen

Microalgae types	Thermochemical method	Temperature, °C	Biofuel types	Yield	Heating value	Reference
Acutodesmus obliquus	Supercritical water gasification	690	Hydrogen	13,944 kg/h	–	Nurdiawati et al. (2019)
Chlorella vulgaris	Gasification	800	Hydrogen	81.6%	–	Liu et al. (2017)
Scenedesmus sp.	Gasification	650	Hydrogen	81.6%		Gholkar et al. (2021)
Saccharina latissima	Gasification	500	Hydrogen	11.0 mol/kg	27.6 MJ/m^3	Onwudili et al. (2013)
Scen. Almeriensis	Gasification	600	Hydrogen	–	25 MJ/m^3	Díaz-Rey et al. (2015)
Chlorella vulgaris	Gasification	850	Hydrogen	–	–	Liu et al. (2019a)
Phaeodactylum tricornutum	Hydrothermal gasification	420	Hydrogen	3.9 mol/kg	–	Bagnoud-Velásquez et al. (2014)
Galdieria sulphuraria	Hydrothermal gasification	500	Hydrogen	45.7%mol	–	Ibrahim et al. (2020)
Spirulina	Hydrothermal gasification	290	Bio-oil	46.6 wt%	33.6 MJ/kg	Liu et al. (2021)
Cyanobacteria	Pyrolysis	550	Bio-oil	41.1 wt%	–	Sotoudehniakarani et al. (2019)
Sargassum tenerrimum	Liquefaction	260–300	Bio-oil	33.0 wt%		Biswas et al. (2020)
Chlorella pyrenoidosa	Liquefaction	290	Bio-oil	78.9 wt%	–	Jin et al. (2014)
Scenedesmus obliquus	Liquefaction	243–320	Bio-oil	39.6 wt%	–	Arun et al. (2020a)
Scenedesmus abundans	Liquefaction	220–320	Bio-oil	43.4 wt%		Sundar Rajan et al. (2019)
Scenedesmus obliquus BR003	Hydrothermal liquefaction	450	Bio-oil	16.75 wt%	36.05 MJ/kg	Rocha et al. (2020)
S. obliquus SAG276-10	Pyrolysis	600	Bio-oil	54.4 wt%	–	Hu et al. (2020)
Nannochloropsis sp.	Hydrothermal liquefaction	260	Bio-oil	29.8 wt%	37 MJ/kg	Li et al. (2014)
Nannochloropsis sp.	Hydrothermal liquefaction	300–400	Bio-oil	59 wt%	39 MJ/kg	Xu and Savage (2017)
Chlamydomanas debaryana	Pyrolysis	800	Bio-oil	12.8 wt%	–	Aramkitphotha et al. (2019)
U. prolifera	Pyrolysis	–	Bio-oil	41.3 wt%	39.04 MJ/kg	Ma et al. (2020)
Saccharina japonica	Pyrolysis	–	Bio-oil	39.05 wt%	27.19 MJ/kg	Ly et al. (2019)
Dunaliella Sp	Hydrothermal liquefaction	350	Bio-oil	11.81 wt%	–	Shahi et al. (2020)
Scenedesmus almeriensis	Microwave-induced pyrolysis	800	Syngas	93.8 vol%	–	Beneroso et al. (2013)
Spirulina, chlorella	Microwave-induced pyrolysis	700	Syngas	73.3 vol%	–	Hong et al. (2017)
Nannochloropsis sp.	Hydrothermal gasification	500	Syngas	16.4 mmol/kg	21.1 MJ/m^3	Brown et al. (2010)
Chlorella vulgaris	Pyrolysis	600	Syngas	43.7 vol%	–	Yuan et al. (2015)
S. japonica	Pyrolysis	350	Syngas	22 vol%	–	Ly et al. (2016)
Chlorella sp.	Microwave-induced pyrolysis	550	Syngas	16 vol%	–	Borges et al. (2014)

Table 1 continued

Microalgae types	Thermochemical method	Temperature, °C	Biofuel types	Yield	Heating value	Reference
Scenedesmus sp.	Flash volatilization	600	Syngas	16 vol%	–	Gholkar et al. (2019)
Porphyra	Gasification	450	Syngas	87 vol%		Adnan et al. (2020)

the process achieves very low yields due to the inability of bacteria to break down the outer cell structure of microalgae. Furthermore, the presence of free ammonia due to the low carbon to nitrogen ratio further inhibits the formation of methane (Chandra et al. 2019). In a study by Zamalloa et al. (2011), they confirmed relatively lower increases in methane output from the digestion of *Scenedemus obliquus* and *Phaeodactylum tricornutum* under mesophilic (0.296 L CH_4/g) and thermophilic (0.462 L CH_4/g). According to Trivedi et al. (2015), higher biomass yields are observed in microalgae biomass compared to biomass obtained from terrestrial plants (e.g., Jatropha curcas). However, the latter has a lower feedstock cost which improves its economic viability.

To convert microalgae to bio-alcohol, major biomass constituents, such as sugars, starch, and cellulose, are first degraded in the presence of yeast or bacteria. Further distillation is often required to achieve higher concentrations of diluted alcohol (Alves et al. 2020). Carbohydrates are mainly found in starch reserves in cell bodies as well as cellulose/polysaccharides in the cell walls. Jönsson et al. (2016) have observed a high degree of difficulty in fermenting carbohydrates found in biomass in the presence of microbes. Hence, it is necessary to break down these carbohydrates into monomeric sugars via hydrolysis using either chemical or biological agents (enzymes) before further processes (Lin et al. 2019). Nevertheless, enzymatic hydrolysis is less favorable due to the high costs associated with enzyme procurement and the required pre-treatment of microalgal biomass (Tan et al. 2015).

As part of the alcoholic fermentation process, the monomeric sugars derived from initial treatments of biomass are further processed into the desired outputs. De Farias Silva and Bertucco (de Farias Silva and Bertucco 2016) have observed the conversion of sugars found in Saccharomyces and Zymomonas into bioethanol under an oxygen-free environment. Furthermore, the fermentation process can occur in two different routes in which hydrolysis and fermentation can take place separately or simultaneously in the same reaction chamber. According to Brennan and Owende (Brennan and Owende 2010), the conversion of *Chlorella vulgaris* which contains 37% of starch per dry cell weight results in nearly 65% of ethanol output. In another study, Choi et al. (2010) were able to produce 0.235 g of bioethanol from each gram of *C. reinhardtii* biomass via the separated hydrolysis and fermentation method. Compared to other conventional fermentation methods, the use of anaerobic fermentation to convert microalgae biomass into bioethanol is considered a relatively more simple process. In one study, Daroch et al. (2013) investigated the use of genetically modified species of microalgae (e.g., *Chlamydomonas perigranulata*) that revealed positive results in terms of bioethanol production due to the presence of self-ferment carbohydrates. To improve the yield of ethanol, starch-rich microalgae such as Chlorella vulgaris is one of the most popular strains with the potential to achieve up to 65% conversion efficiency, and a high bioethanol yield can be obtained from dark fermentation of microalgae at 30 °C (Javed et al. 2019).

To produce biobutanol, the conventional method typically involves the conversion of sugars into a combination of acetone, butanol, and ethanol (ABE) (Veza et al. 2021). Theoretically, under a controlled fermentation process, the optimal ABE yield is 0.41 g per gram of sugar which is slightly lower than 0:5 g of bioethanol per gram of sugar. A mixture of CO_2 and H_2 gas is also obtained as by-products in the process. The common product ratio of these components is 3:6:1, respectively. Hence, butanol accounts for the largest portion of the outputs (Bellido et al. 2014). According to Chen et al. (2015), there is lower productivity in the production of biobutanol due to the strong resistance of degraded substances resulting from the initial steps of the fermentation process.

Furthermore, there is only a single species of microalgae (i.e., *Clostridium spp.*) that can be converted to biobutanol leading to the lower efficiency of the conversion process. Given the fact that *Clostridium spp.* is saccharolytic, the conversion process of starch-rich microalgae into biobutanol is relatively easy as that of bioethanol. In another instance, Ellis et al. (2012) utilized biomass obtained from microalgae grown in wastewater with *Clostridium saccharoperbutylacetonicum N1,4* in an ABE fermentation process. In this experiment, microalgae that were pretreated with acid and basic chemicals resulted in 2.74 g/L of total ABE from the fermentation process. On the other hand, a much higher yield of 9.74 g ABE/L when enzymatic hydrolysis (xylanase and cellulases) was applied. As reported, ABE biofuel has more beneficial physicochemical properties than those of fossil fuel, indicating that it could be used as the potential biofuel for engine applications (Veza et al. 2019, 2020).

Biohydrogen is produced biologically as a result of the metabolic reactions of microbes. Renewable biohydrogen presents a more attractive option compared to other biofuel productions. Regarded as a potential alternative fuel, biohydrogen is regarded to be cleaner and more sustainable while having a relatively high energy content (142 MJ/kg). Biohydrogen can be produced via different biological techniques, including photo-fermentation, dark fermentation, and electro-bio-hydrogenation. There are major pros and cons associated with each of these methods in terms of feasibility, sustainability, and energy efficiency (Kadier et al. 2018; Sivagurunathan et al. 2017). Microalgae have recently gained the interest of researchers as the potential third-generation biomass feedstock for the production of renewable biohydrogen. Studies have reported the use of different species of microalgae in the generation of biohydrogen, such as *Scenedesmus, Chlorella, Synechocystis, Anabaena, Nostoc,* and *Tetraspora harbor hydrogenase* (Eroglu and Melis 2011; Anwar et al. 2019). Naturally, some microalgae strains can use sunlight and water as the source of electrons and energy respectively to produce photo-biological biohydrogen. Moreover, hydrogen and carbon dioxide can be obtained when subjecting microalgae biomass as the organic substrate in the photo-fermentation process with the help of photosynthetic micro-organisms (Arun et al. 2020b). In another study, a 200 ml/L.h hydrogen production rate was observed when using *Clostridium butyricum* in the dark fermentation of *C.vulgaris* biomass which contains a high level of carbohydrate, in which biohydrogen also accounted for 66% of the total volume of biogas produced in the above process (Liu et al. 2013). In addition, biological hydrogen is also a potential power source for electricity produced from fuel cells (Ban et al. 2019; Eroglu and Melis 2016; He et al. 2017). In general, biofuel yield achieved from the biochemical conversion of microalgae is summarized in Table 2.

Chemical conversion

Microalgae-derived bio-oils inherently have a higher viscosity compared to diesel oils, it is thus necessary to perform a transesterification process on the microalgae oils to lower their overall viscosity before applying to engines (Akubude et al. 2019). To improve the efficiency of biodiesel from microalgae, the direct transesterification method is deemed more favorable. Notably, alcohol acts as both the reactant and solvent in a transesterification process. Due to its affordability and easy access, it is common to use methanol in this process over other types of alcohols (e.g., methanol, ethanol, propanol, butanol, and amyl alcohol). Given that alcohol is hardly soluble in different kinds of oils, the presence of a catalyst is also important due to its enhancing the solubility of alcohol. The direct application of lipid contained in wet microalgae biomass is another cost-effective strategy to reduce the cost associated with drying and extracting moisture content from the biomass feedstock. Therefore, researchers have explored the development of advanced wet lipid extraction techniques (Lakshmikandan et al. 2020). Moreover, to penetrate and break down the cell wall of microalgae within the lipid extraction process, researchers have applied different pretreatment techniques to the biomass feedstock, such as high-pressure homogenization, ultrasound sonication, microwave irradiation (Howlader and French 2020), osmotic shock, liquid hot water, Triton-X-100 application, shake mill (Ramola et al. 2019). According to Singh et al. (2019b), solvothermal methods were applied to obtain lipids from Spirulina in a microwave environment with a capacity of 750 W at 60 °C for 30 min. Furthermore, mixing an equal amount of hexane and methanol has been proven to be effective in large-scale operations (Shin et al. 2018a). The application of ionic

Table 2 Biochemical conversion of microalgae to bio-hydrogen/bio-methane/bio-ethanol/bio-butanol

Microalgae types	Biochemical method	Condition reactions	Biofuel types	Yield	Reference
Microcystis wesenbergii/Microcystis aeruginosaare	Anaerobic sludge with butyrate-acetate	37 °C	Bio-hydrogen	31.42 ml/g	Xu et al. (2019b)
Scenedesmus sp./Monoraphidium.sp	Thermophilic inoculum with acetate-butyrate	37 °C; pH = 5.5		0.72 ml/g	Kumar et al. (2016)
Chlorella sp./Scendesmus sp.	Anaerobic sludge with butyrate-acetate	35 °C; pH = 7		45 ml/g	Sivagurunathan et al. (2018)
Arthrospira platenis/Laminaria digitata	Fermentation with butyrate-acetate	35 °C at pH = 6		85 ml/g	Xia et al. (2016)
Chlorella sp. TISTR 8411 with Molasses	Anaerobic sludge	55 °C		90.12 ml/g	Jehlee et al. (2019)
Scenedesmus obliquus	Fermentation with Clostridium butyricum	58 °C		116.3 ml/g	Ortigueira et al. (2015)
Chlorella sp.	Photo-fermentation	30 °C		48%vol	Liu et al. (2020b)
Chlorella sp	Dark-fermentation	pH = 6		47.2 ml/g	Phanduang et al. (2019)
Scenedesmus obliquus	Enterobacter aerogenes	30 °C		56.8 ml/g	Batista et al. (2015)
C. vulgaris	Heat-treated anaerobic sludge	60 °C		135 ml/g	Wieczorek et al. (2014)
Phaeodactylum tricornutum	Anaerobic digestion after being pretreated by ultrasound	–	Bio-methane	284–287 ml/g	Caporgno et al. (2016)
Chlorella vulgaris	Anaerobic digestion	40 °C; pH = 6.5		411 ml/g	Kavitha et al. (2019)
Chlorella vulgaris	Anaerobic digestion after being pretreated by enzyme	–		86.6–200.8 ml/day	Mahdy et al. (2016a)
Chlorella vulgaris/Scenedesmus sp.	Anaerobic digestion after being pretreated by enzyme	–		170–250 ml/g	Mahdy et al. (2016b)
Scenedesmus sp./Chlorella sp.	Anaerobic digestion	35 °C		237 ml/g	Olsson et al. (2018)
Scenedesmus quadricauda	Anaerobic digestion	35 °C		222 ml/g	Garoma and Nguyen (2016)
Chlorella sp.	Anaerobic digestion	35 °C		547 ml/g	Lu and Zhang (2016)
Chlorella sorokiniana/Scenedesmus sp	Anaerobic digestion	37 °C		560 ml/g	Wágner et al. (2016)
Prosopis juliflora	Simultaneous saccharification and fermentation	72 h	Bio-ethanol	21.45 g/l	Sivarathnakumar et al. (2019)
Spirulina platensis	Fermentation	20 h		4.2 g/L	Rempel et al. (2019)
Closteriopsis acicularis	Fermentation	–		14.9 g/L	Bibi et al. (2021)
Tetraselmis sp	Fermentation	50 h		15.7 g/l	Constantino et al. (2021)
C. vulgaris ESP-31	Hydrolysate	–		0.0761 g/g	Yu et al. (2020)

Table 2 continued

Microalgae types	Biochemical method	Condition reactions	Biofuel types	Yield	Reference
Spirulina	Simultaneous saccharification and fermentation	72 h		73 g/l	Luiza Astolfi et al. (2020)
Chlamydomonas sp. QWY37	Fermentation	37 °C, 96 h	61 g/L	Qu et al. (2020)	
Hydrodictyon reticulatum	Fermentation	30 °C, 48 h	93.5%	Min et al. (2021)	
Chlorella	Simultaneous saccharification and fermentation	32 °C, 84 h	0.28 g/g	Phwan et al. (2019)	
Microcystis sp.	Fermentation	30 °C, 43.6 h	18.57 g/L	El-Mekkawi et al. (2019)	
Clostridium acetobutylicum	Fermentation	37 °C, 30–40 h	Bio-butanol	13.8 g/l	Tsai et al. (2020)
C. zofingiensis	Fermentation with *C. acetobutylicum*	–		4.2 g/l	Onay (2020)
Chlorella vulgaris JSC-6	Fermentation with *C. acetobutylicum*	37 °C		13.1 g/l	Wang et al. (2016)
Nannochloropsis sp	Fermentation with *C. acetobutylicum* B1787 after being pretreated by acid	–		10.9 g/l	Efremenko et al. (2012)
C. sorokiniana	Fermentation with *C. acetobutylicum* B1787 after being pretreated by H_2SO_4 and NaOH	–		3.86 g/l	Cheng et al. (2015a)
Chlorella sp.	Fermentation with *C. acetobutylicum*	37 °C		6.23 g/l	Onay (2018)
Chlamydomonas reinhardtii	Fermentation	37 °C, 20 h		12.67 g/l	Figueroa-Torres et al. (2020)
Chlorella vulgaris JSC-6	Fermentation with acidogenesis phase after being pretreated by H_2SO_4 and NaOH	–		0.58 mol/mol	Wang et al. (2016)

Table 3 Chemical conversion of microalgae to biodiesel

Microalgae types	Condition reactions	Biodiesel yield, %	Reference
Chlorella sp.	Ratio of methanol/oil = 83; Ratio of chloroform/oil = 30; H_2SO_4 concentration = 0.08 mol/l; Ultrasonic power = 137 W, reaction time = 100 min	81.2	Karimi (2017)
Chlorella	Methanol/oil molar ratio = 12:1; Temperature = 40 °C, reaction time = 12 h;	> 97	Wu et al. (2017)
Nannochloropsis gaditana	4 kg biomass in 16 L of water/36.4 L hexane/36.4 L methanol/ 18.2 L H_2SO_4 98%; Reaction time = 2 h before vacuum distillation refining	85.5	Torres et al. (2017)
Microalgae	Ethanol/oil molar ratio = 4.5:1; Temperature = 30 °C, reaction time = 13 h;	98	Gumbytė et al. (2018)
Nannochloropsis gaditana	1.5 ml methanol/0.1 ml chloroform/0.3 ml HCl; Heated at 95 °C; Reaction time = 2 h;	90	Kim et al. (2015)
S. obliquus	Methanol/oil molar = 12:1 with WO3/ZrO2 as catalyst; Temperature = 100 °C;	94.58	Guldhe et al. (2017)
Microalgae	Ethanol/oil molar ratio = 3:1; Temperature = 30 °C; Reaction time = 26 h;	96.9	Makareviciene et al. (2017)
Nannochloropsis gaditana	H_2SO_4 concentration = 4.06 M and 6.67 ml (ethyl acetate; Heated at 113.6 °C; Reaction time = 2 h;	97.8	Park et al. (2017)
Microalgae	Methanol/oil molar = 3.2:10 with CaOAl2O3 as catalyst; Temperature = 50 °C;	88.89	Narula et al. (2017)
Isochrysis galbana	Methanol/oil molar = 12:1; Temperature = 65 °C	99.7	Silitonga et al. (2017)
Anabaena PCC 7120	Methanol/oil molar = 18:1 with Ba_2TiO_4 as catalyst; Temperature = 65 °C; Reaction time = 3 h;	98.41	Singh et al. (2019c)
Acutodesmus obliquus	Methanol/oil molar = 3:1 with immobilized *Candida rugosa* lipase; Temperature = 50 °C;	95.36	Guldhe et al. (2019)
Spirulina platensis	Methanol/oil molar = 12:1 with β-Sr_2SiO_4 as catalyst; Temperature = 65 °C; Reaction time = 104 min;	97.88	Singh et al. (2020)
Chlorella vulgaris/Chlorella protothecoides	[C_{16}mim][NTf_2]/[Bmim][Cl])as catalyst; Temperature = 60 °C; Reaction time = 5 h;	100	Lozano et al. (2020)
Chlorella vulgaris	Tetrabutylphosphonium formate as catalyst	98	Malekghasemi et al. (2021)
Chlorella vulgaris	*In-situ* transesterification; Temperature = 60 °C; Reaction time = 25–35 h;	> 90	Nguyen et al. (2021)

liquids can also improve the efficiency of lipid extraction from microalgae.

Presently, microalgae are gradually gaining a favorable assessment from experts as the potential sources of biodiesel production, considering the rapid growth rates and lipid composition (50–70%) (Satputaley et al. 2017). Compared to petroleum-based diesel with 46 MJ/kg caloric values, microalgae-derived biodiesel has a comparable energy content ranging between 39 and 41 MJ/kg (Goh et al. 2019). Relatively high conversion efficiency (70–75 wt%) can be achieved in the production of biodiesel via hexane or supercritical CO_2 extraction method. According to Umdu et al. (2009), a very high conversion efficiency rate of 97.5% was obtained in the transesterification of *Nannochloropsis oculate* with the support of CaO and Al_2O_3 catalysts at 50 °C. Furthermore, the addition of Zn, Ti, and Al-based catalysts in the transesterification of green microalgae was detected to yield a 90.2% conversion efficiency under 350–400 °C and 2500 psi operating conditions (McNeff et al. 2008). In another example, the transesterification of *C. protothecoides* along with 75% of lipase (Candida sp.) in a methanol-containing medium at 38 °C resulted in the conversion efficiency of 98.15% after 12 h (Cheng et al. 2009). According to Cheng et al. (2020), a reduction in biodiesel yield from lipids extracted with chloroform and n-hexane by as much as 41% and 65%, respectively. On the other hand, the same authors observed up to a 9% increase in FAME yield from lipids resulting from the transesterification process in the presence of TEPDA. Furthermore, the application of n-hexane/formic acid has been shown to improve the biodiesel yield compared to chloroform/methanol extraction enhanced by ultrasound, microwave, hydrothermal, and dilute nitric acid pretreatments. Particularly, Xia et al. (2020) observed an increase of biodiesel yield in the range of 79–99% by applying the mixture of n-hexane/formic acid at the ratio of 9:2 by volume at 80 °C for 2 h. Recently, the in-situ transesterification method has been gaining traction as an alternative for the conversion of biodiesel from microalgae due to the overall lower production cost resulting from the elimination of expensive biomass drying and lipid extraction steps (Ghosh et al. 2017; Ma et al. 2019; Mandik et al. 2020).

Currently, there is a significant amount of interest among the scientific research community in the generation of biodiesel from microalgae due to several advantages, such as the rapid growth rate and enhanced lipid accumulation of microalgae. Furthermore, microalgae-based biodiesel also exhibits higher energy content (i.e., up to 34% increase) compared to conventional ethanol (Moshood et al. 2021). Despite its potential, further research is needed to examine the practicality of scaling up biodiesel production via the wet lipid extraction method. The alkaline-catalyzed process is among the most prevalent and well-developed methods. However, this method is not without its setbacks associated with the high energy requirement, the challenge in glycerin and catalyst post-process extraction. Considering the negative impacts from the use of alkaline-based catalysts, researchers have proposed alternative biodiesel production methods without the need for a catalyst known as the supercritical fluid method (Ortiz-Martínez et al. 2019). This new process utilizes a single reactor to convert microalgae biomass into biodiesel under operating temperatures between 250 °C and 350°. Compared to traditional transesterification and lipid extraction pathways supported by co-solvent, the supercritical method requires a significantly lower amount of energy inputs (Dickinson et al. 2017).

Potential benefits of microalgae-based biofuels

The rapid rate of anthropogenic-related environmental degradation in the latter part of the twentieth century has resulted in unprecedented destruction of natural habitats and depletion of natural resources compared to all previous parts of human history (Zaimes 2016). Increasingly stringent regulations are putting pressure on the current trend of global consumption of fossil-based energy sources. Furthermore, producers are also facing the challenge of designing products with lower environmental footprints and incorporating more sustainable components into the manufacturing process. Diverse factors are driving sustainable development progress, namely human, cultural, political, and socio-economic issues (Xu and Chen 2020). These adverse and irreversible consequences are apparent on a global scale that not only affects ecosystems on earth but also human inhabitants and their livelihoods on this planet. Considering this multifaceted sustainability perspective, advances in the fields of science and engineering could potentially offer powerful solutions to address issues related to the satisfaction of current demands without compromising the needs of future generations. Specifically with chemical production, incorporating sustainability elements are of utmost importance in enhancing the current manufacturing processes. Compared to conventional fossil fuels, biomass-derived biofuels present a more attractive alternative that is considerably cleaner and more sustainable (Cai et al. 2019).

Among various sources of biomass feedstock, microalgae are becoming a more popular choice in the production of third-generation biofuels. Microalgae serves as a promising feedstock in the production of biofuels and other value-added byproducts. Cultivated in aquatic environments, microalgae, which comprise either single-cell or multi-cellular photosynthetic microorganisms, are capable of using water and solar energy to fix atmospheric CO_2 to chemical energy into the forms of lipids, carbohydrates, and proteins contained in the structure of the microalgae biomass. Similar to other plant species, microalgae are major sources of carbon dioxide sequestration that accounts for up to 40% of global CO_2 (Sydney et al. 2019; Kholssi et al. 2021). As shown in Fig. 5, autotrophic microalgae absorb sunlight during photosynthesis to convert carbon dioxide into chemical energy. As a result, biomass and oxygen are the outputs of this natural process. To produce each ton of microalgae biomass, nearly two tons of CO_2 along with 0.1 tN, 0.010 tP, and 0.015 tK are needed. Besides, up to two tons of O_2 are emitted in the process. Microalgal biomass production has several advantages in terms of wastewater treatment and biofuel production. Several families of microalgae are suitable for the above purposes, including *Spirulina (Arthrospira), Chlorella, Dunaliella,* and *Haematococcus* (Draaisma et al. 2013).

To ensure the optimal growth of autotrophic microalgae, it is important to ensure plenty amount of sunlight and CO_2. Previous studies have concluded that 8–10 mol of photons are needed to obtain one mole of carbohydrates. On the other hand, the generation of each kilo of biomass necessitates nearly 2 kg of input CO_2 (Kumar et al. 2011). The sustainability of microalgae production is supported by the use of natural sunlight and atmospheric CO_2 (or from flue gas) (Vo Hoang Nhat et al. 2018). Because of their capability to fix atmospheric CO_2 via the photosynthesis process, microalgae farming can help to reduce the current carbon stock in the atmosphere (Acién et al. 2012). In their experiment, Chiu et al. (2008) investigated the conversion efficiency of CO_2 fixation in the farming of Chlorella sp. in a photobioreactor. As a result, the authors observed various rates of CO_2 reduction and removal efficiency given different concentrations of CO_2. Specifically, the following

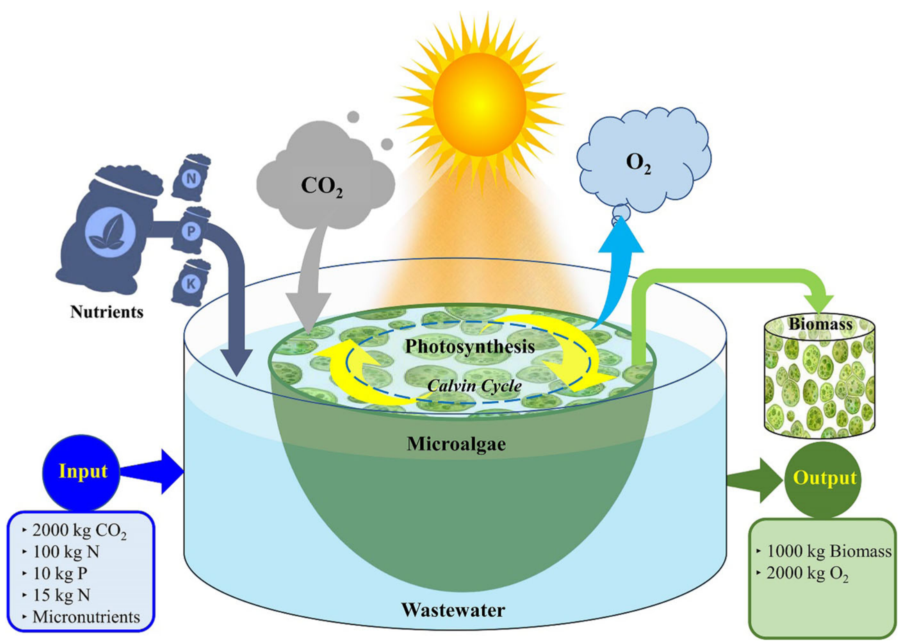

Fig. 5 The relationship between inputs and outputs in microalgae production (Fernández et al. 2021)

rate of reduction (g/h) and removal efficiency (%) are provided with the following CO_2 concentrations at 2% (0.261 g/h and 58%), 5% (0.316 g/h and 27%), 10% (0.466 g/h and 20%) and 15% (0.573 g/h and 16%). Besides, earlier studies have shown that increasing the level of CO_2 concentration from 5 to 15% could enhance the lipid composition and productivity in microalgae biomass (Jiang et al. 2011). In another study, the cultivation of Spirulina sp. and Scenedesmus obliquus was carried out in a three-stage serial tubular photobioreactor. The daily amounts of CO_2 fixed in the process were recorded at 0.413 and 0.260 g/L for the above microalgae strains, respectively (Chisti 2007). In general, the capability of microalgae in removing atmospheric CO_2 via photosynthesis can further be enhanced upon scaling up the existing microalgae-based biofuel production (Table 3).

Presently, it has been estimated to require a significantly large sum of money to startup a carbon credit economy. For example, if it was assumed a price tag of US$100 per credit of carbon, close to one trillion dollars would be needed (Bird et al. 2011). Furthermore, the annual emissions of carbon from fossil fuel sources are estimated to be around 8 Gt which also equals 8 billion carbon credits each year (Bird et al. 2011). Hence, fuels that do not contribute to the carbon emissions stock in the atmosphere can be considered carbon negative. In this sense, biofuels can be seen as more advantageous than conventional fossil fuels due to their negative carbon association. On the one hand, it would still require the consumption of fossil fuels in the cultivation of microalgae. On the other hand, the microalgae biomass itself used as the feedstock for biofuel production is considered carbon negative. In a separate study, the application of 15 g of *A. fragilissima* biomass was observed to produce up to 34.1% and 29.5% of carbon dioxide from the HTL and pyrolysis process, respectively (Arun et al. 2020c). As the CO_2 obtained from the valorization process of plant-based biomass is fed back into the natural carbon cycle, microalgae biomass can be deemed carbon–neutral due to the prior photosynthesis process during its growth. Thus, the consumption of microalgae biomass can lead to a negative flow of CO_2 as it is taken out of the natural cycle. On the other hand, if we compare to the CO_2 emitted from the process of burning coal will increase the carbon stock in the atmosphere unless it can be subsequently captured and sequestered (Arun et al. 2020b). To ensure that microalgae-based biofuels are carbon negative, the cultivation methods should prioritize the reduction of carbon positive sources of energy. Furthermore, microalgae are capable of withstanding the presence of NOx and SOx in flue gas as long as the amount of SOx does not go above 400 ppm (De Bhowmick et al. 2019). Considering this dilemma, microalgae present an attractive biomass feedstock for a biofuel future (Fernández et al. 2021).

As mentioned above, common biofuels obtained from microalgae include bioethanol, biodiesel, bio-oil, biomethane, bio-hydrogen, and bio-butanol that could be used as alternative fuels for vehicle and transportation means with lower carbon emissions (Karthikeyan et al. 2018; Rajak et al. 2019; Hoang et al. 2020). Moreover, the sustainability of biofuel production from microalgae on an industrial scale was also confirmed (Hossain et al. 2019). Several benefits of this approach to produce biofuels include a relatively simple adaptation of natural habitat in the cultivation of microalgae without the need of competing with precious arable lands for farming of food and cash crops. Furthermore, microalgae also demonstrate the capability to achieve high photosynthesis efficiency and high lipid content. Notably, the integrated process of generating different types of biofuel, including biodiesel, bioethanol, and biogas, from a single feedstock has been shown to significantly improve the conversion efficiency of the microalgae biomass. Hence, it could lead to the improvement in the economic efficiency of the overall microalgae biorefinery process. Current progress made in the area of genetic and metabolic engineering promises to further enhance the cost-effectiveness and environmental sustainability of biofuel production from microalgae biomass. Indeed, Carbohydrate-rich microalgae such *as Zymomonas mobilis, Saccharomyces cerevisiae., Chlorella sp., Dunaliella sp., Spirogyra sp.,* and *Scenedesmus sp.* present as promising biomass feedstock for the generation of biofuels (Özçimen and Inan 2015; Özçimen et al. 2020; Culaba et al. 2020). In addition, the potential of certain strains of microalgae (e.g., *Chlorella protothecoides*) could hold up to 55% of lipid in their biomass structure under a nitrogen-deprived cultivation environment, facilitating biodiesel production. (Xu et al. 2006). Hence, the development of microalgae biorefinery processing is integral to the realization of a zero-emission and sustainable circular economic model that can utilize wastewater-cultivated microalgae as a source of renewable bio-energy and bio-fuel (Serrà et al. 2020). Furthermore, experts believe that biomass can be the single renewable source of carbon-based fuel that is capable of supplying future energy demand. Due to the characteristics and composition of different microalgae species, it is possible to determine the desired product outputs as part of the conversion process. Within an integrated process of sustainable microalgae biofuel production, several stages are highly dependent and interconnected, requiring careful planning and monitoring of environmental, operational, and socio-economic conditions as well as externalities (De Bhowmick et al. 2019).

Taking sustainable development into account, the shifting of arable land and valuable resources such as freshwater from growing food to energy crops further drives the intense food versus fuel debate. Progress made in the current production of third-generation biofuels from microalgae presents a promising solution to the food versus fuel dilemma. Strong drivers for the transition to microalgae-biofuels are the positive impact on greenhouse gas reduction and saving of valuable arable land and freshwater resource. Indeed, the global consumption of freshwater in farming worldwide is estimated to be around 2700 km^3 and is projected to rise close to 4000 km^3 by the year 2050 (Rao et al. 2000). The significant requirements of freshwater inputs in growing energy crops face considerable pushback from the wider public considering the lack of safe drinking water in impoverished communities worldwide. Another adverse impact of farming energy crops is the potential abuse of fertilizers and pesticides that would cause surface water pollution leading to eutrophication and ecological contamination (Deknock et al. 2019). In contrast to energy crops, the outstanding advantage of microalgae is the ability to be cultivated on marginal lands, wastewater, and seawaters that are unsuitable for conventional food and energy crops. Besides, the conversion of microalgae into biofuels can also gain additional economic benefits from the generation of value-added byproducts as part of the process.

To sustain the growth of microalgae, nutrients are needed besides sunlight. Due to this reason, options to utilize wastewater or seawater that are unsuitable for drinking and farming of food crops could further improve the overall sustainability to improve the carbon footprints of microalgae-based biofuel production. Provided that wastewater has a considerably higher level of individual amino acids, microalgae can be supported in such a medium. Cultivation of microalgae can benefit from using nutrients present in wastewater such as common contaminants (e.g., NH_4^+, NO_3^-, and PO_4^{3-}) (Ahmed et al. 2021; Vargas-Estrada et al. 2021). The growth rate of microalgae is less influenced by the changes in seasonal weather during the year, ensuring an uninterrupted growth cycle. Moreover, microalgae can be cultivated in wastewater, and microalgae farming has a lower environmental footprint because it does not consume freshwater (Lu et al. 2020). Studies have confirmed the capability of microalgae in lowering both the chemical oxygen demand and biochemical oxygen demand in wastewater. Common species that have been demonstrated to be effective in treating wastewater include *Scendesmus sp.*, *Chlorella sp.*, and *Chlamydomonas reinhardtii* (Wang et al. 2010; Kong et al. 2010; Park et al. 2011). Depending on the microalgae species, the extraction rate of metal ions (e.g., aluminum, calcium, ferum, mangan, and magnesium) can fluctuate between 50 and 99% (Wang et al. 2010; Woertz et al. 2009). However, Mathews (Mathews 2008) noted the optimal thresholds of nickel (1.0 ppm) and vanadium (0.1 ppm) contained in the flue gas have on the overall productivity of microalgae. Considering all these factors, Singh et al. (2011a) have concluded that microalgae are the leading candidate among existing energy crops having an energy and biofuel yield measured at 793–4457 GJ/ha and 24,355–136,886 L/ha, respectively (Rao et al. 2000). Furthermore, the current economics of biofuel production from microalgae can be enhanced by utilizing unconventional sources of nutrients such as fermented liquid (biogas liquor), animal manure, and other agricultural residues (Markou and Georgakakis 2011; Samorì et al. 2013). Beyond the positive economic benefits of microalgae cultivation using wastewater, this method also enhances the overall sustainability of the entire microalgae-based biofuel supply chain. Recently conducted Life Cycle Assessment (LCA) studies on the use of wastewater for microalgae-biofuel production have also highlighted the improved sustainability attribute. Compared with convention fuel crops such as corn, grass, and canola, the LCA of microalgae grown using wastewater resulted in improving sustainability and lowering the ecological footprints and subsequent biofuel production (Clarens et al. 2010). In another study, Yang et al. (2011) concluded that the cultivation of microalgae using wastewater could save up to 90% of freshwater. Besides, it could also eliminate the need for up to 94% of nitrogen consumption and close to 100% requirements of other common nutrients such as S, K, and Mg. If these strategies are effectively utilized, it could avoid the use of a large amount of freshwater and fertilizer while enabling the sequestration of atmospheric CO_2. Grown in wastewater, microalgae can break down inorganic and organic matters via physical and biological pathways offering an innovative approach to wastewater treatment (Yin et al. 2020). Therefore, to realize the double advantages of microalgae on top of wastewater treatment, significant progress must be made in the current technology to ensure the downstream processing of microalgae can produce biofuel and other valuable biological compounds (Ubando et al. 2021). Overall, the practice of microalgae farming is considered much more sustainable compared to conventional agriculture due to the nonrequirement of arable land and freshwater.

Microalgae-based biofuel production presents several positive socioeconomic and sustainability benefits. Indeed, the value-added byproducts obtained from processing microalgae can make up for the lower capital cost of the overall production. Among these include mineralized carbon and high-value biological compounds. Either one or both of these byproducts can be obtained depending on the selection of microalgae species as the biomass feedstock. In terms of bioactive compounds, they can range from fine chemicals to compounds produced in large quantities such as fats, polyunsaturated fatty acids, oil, natural dyes, sugars, pigments, antioxidants, etc. (Sharma et al. 2019; Tang et al. 2020). These compounds can yield high economic value due to their diverse commercial uses. Hence, microalgae have the potential to contribute to significant breakthroughs in different sectors within biotechnology, such as biofuel production, nutrition, and food supplements, cosmetics, aquaculture, pharmaceuticals, and pollution control (Ranganathan et al. 2022; Suganya et al. 2016). Among the key advantages, the development of this sector can be an important economic driver for both urban and rural communities while contributing toward improving livelihoods and advancing social sustainability (Pachapur et al. 2020). In these instances, local biofuel industries can be supported by various public and private funding that would benefit the local economy of rural communities (El Semary 2020). The continued progress made in the development of microalgae-based biofuel offers potential employment opportunities for those that already have relevant skills in the conventional oil and fossil fuel industry on their transition to a more sustainable sector (Correa et al. 2019). As existing biofuels continue to face increasing obstacles associated with surging food prices and capital constraints, its prospect remains in question, which begs the need for a more sustainable alternative.

The consumption of microalgae-biofuel can contribute to reducing the amount of CO_2 emitted into the atmosphere, which is an important advantage to the current exploitation of fossil fuels. Continued research and development of various species of microalgae that are suitable for biofuel production can significantly contribute toward lowering the carbon footprints and more sustainable energy production and consumption in the future. Future advances in technology associated with microalgae farming and harvesting have the potential to lower fossil fuel consumption and overall carbon footprints. All in all, the development of microalgae cultivation and microalgae biomass feedstock for biofuel production have significant advantages to the socio-economic and long-term sustainability of communities around the world.

Bottlenecks, challenges, and prospects in biofuel production progress from microalgae

Bottlenecks and challenges

Over the past decade, there has been a growing interest in the development of third-generation biofuels from microalgae biomass. Research projects on different aspects of microalgae cultivation and biofuel production have been conducted among private enterprises, academics, government agencies, and public research institutions. Nevertheless, it still requires a significant amount of time before reaching widespread commercial adoption of microalgae-based biofuels as the cost of third-generation biofuels is still much higher than conventional alternatives (Chowdhury and Loganathan 2019). Based on recent estimates, the cost of microalgae bio-oil fluctuates in the range of US $300–2600. Another study also has confirmed that the cost of microalgae-derived oil is twice as much as that of petroleum oil. According to Chisti et al. (2007), a liter of microalgae oil is estimated to be around $2.80, not including the cost of distribution, marketing, sale, and other taxes. Among the current literature on microalgae-based biofuel production, there have been a number of Techno-Economic Analysis (TEA) studies examining the practicality of different technological approaches (Vo Hoang Nhat et al. 2018). Current projections of biofuel production cost are either obtained from past research or small-scale experimental studies. More than often, the modeling of these cost estimates has not been supported and validated by actual data, while several input assumptions still need to be taken into account, such as growth rate, nutrient requirement, lipid productivity, and energy consumption (Chia et al. 2018). There are more studies on the cultivation of microalgae in open raceway ponds relative to the photobioreactor method, which costs between 2 to 2.5 times more than the former process (Barry et al. 2016). Based on current cost estimates, prices of microalgae-based biofuel would fluctuate in the range of $0.44/L and $8.76/L (Richardson et al. 2012; CARD 2018). Given these price calculations, microalgae oil is not cost-competitive against other convention fuel options. Hence, it is necessary to drive down the cost of microalgae biofuels through process optimization and advancement in cultivation methods. In addition, further systematic optimization of both production and conversion processes has the potential to drive down these costs in the near future (Chowdhury et al. 2019).

Even though a wide range of research studies have been conducted on biofuel production from microalgae over the last several decades, in reality, only a small number of pilot projects have been tested for real-world applications, while commercialization has yet to achieve any meaningful results (Su et al. 2017). Currently, there has not been an actual case study of continuous microalgae-based biofuel production on an industrial scale. Indeed, several obstacles still face the scaling up of production and product commercialization associated with microalgae-based biofuels, such as high operation and capital investment cost. Up to date, there have been several investigations into microalgae cultivation using an open system and photobioreactors. In one example, Davis et al. (2011) has conducted an overall economic analysis to identify potential cost-saving strategies. In the open system, the authors obtained $8.52/gal for 25 g/m^2.day, which yielded a cost of $9.84/gal of biodiesel. On the other hand, the estimated cost for the photobioreactor approach was recorded at $18.10/gal for 1.25 kg/m^3.day resulting in the biodiesel cost of $20.53/gal (Davis et al. 2011). Hence, it is time-consuming to find sufficient solutions to resolve potential technical challenges concerning the development of species suitable for sustainable biofuel production within a plausible timeframe. Among these concerns, the structure of the microbial community in microalgae cultures raises the question of the conversion efficiency of biofuel production (Zabed et al. 2020). The main issue facing the cultivation of microalgae is the propagation of biomass in the raceway pond and photobioreactor. Additional pretreatment steps and an effective yeast fermentation process are necessary for the conversion of microalgae-based bioethanol. Further research into the cultivation of engineer-modified species of microalgae with fast-growing properties is still needed to advance existing bioethanol production. In several case studies, the use of automation technology has been cited to significantly improve microalgae yield and biofuel productivity. Furthermore, only selected strains of microalgae containing a high concentration of fatty acids or lipids are deemed suitable for biodiesel production. Hence, it significantly affects the overall production costs (Branco-Vieira et al. 2020). Because of the varying environmental conditions and regeneration of microalgae after successive subcultures, the quantity and quality of lipid that can be obtained can differ significantly. Furthermore, the biggest issues with microalgae biofuel production are the relatively low biomass yield and low level of volatile solid compounds in biomass. In the case of cultivating microalgae by wastewater, several recent studies have presented innovative solutions to improve microalgae biorefinery processes by combining bioenergy production and wastewater treatment (De Bhowmick et al. 2019; Chandra et al. 2019). In this approach, wastewater streams can be utilized as the nutrient source in the cultivation of microalgae (Hemalatha et al. 2019a, 2019b). However, there are still several challenges facing the cultivation of microalgae given the different characteristics of various effluent streams such as origin, pretreatment processes, and nutrient contents resulting in uncontrolled variables in the propagation of microalgae biomass. Even though microalgae can process common nutrients present in wastewater streams, there are still certain nutrients and inhibitors found in wastewater that negatively affect microalgae growth. Furthermore, incompatible carbon to nitrogen ratio could reduce the optimal growth rate leading to lower biomass productivity (Li et al. 2020). Provided the increase in contaminants in microalgae biomass, the additional downstream processing steps would increase the overall production cost. These issues are further tied to other factors such as fertilizer costs, harvesting, moisture extraction, and biomass concentration.

Despite the major advantages offered by the consumption of bioenergy converted from microalgae, the widespread deployment of this clean and sustainable source of energy still faces significant obstacles, such as identifying suitable strains of microalgae, input resource requirement, biomass harvesting, seasonal variability in feedstock supply, and appropriate conversion method. Among these obstacles, the low rate of sunlight distribution negatively affects the growth and biomass yield of microalgae culture. Due to the small size of microalgae cells, the low biomass concentration further raises the cost of microalgae biomass harvest. High moisture content in biomass also poses another major challenge that would require additional energy-intensive drying stages. Compared to traditional farming, the cultivation of microalgae requires significantly higher capital investment in facility construction and maintenance to ensure the proper and suitable growing conditions for microalgae. Concerning the obstacles facing biomass cultivation and the application of lipid and protein extraction methods to produce biofuels, there have been proposals on the development of biorefinery processing that could eliminate the cultivation and harvesting steps within the existing production cycle. In one instance, the investigation into lipid-extracted biomass obtained only a third of the amount of biofuel output (Quinn et al. 2014). Currently, there are several stages involved in the conversion of microalgae-extracted lipids to biodiesel. On top of that, additional use of other chemicals is also necessary, as well as extra refining steps are also taken to ensure the quality of biodiesel output is suitable for operating engines (Stengel and Connan 2015). In another example, dark fermentation for biohydrogen production is a more complex process that requires the removal of CO_2 prior to the start of the treatment. This method also yields a lower hydrogen output. To achieve a higher energy density, further processing of the gas output is necessary, including drying and compressing to less than a few thousand PSI. However, the gas compression method is energy-intensive, and such expensed energy cannot be recovered. Besides, many other issues concerning bio-hydrogen production have to mention the fluctuation of production yield that is caused by the metabolic variation among hydrogen-emitting organisms (Kumar et al. 2021a). Besides, the challenge of reducing furfural and 5-hydroxymethylfurfural involved in the pre-processing stage has not yet been sufficiently addressed. The requirement to remove these unwanted toxic contaminants necessitates the use of specific adsorbent materials (Sudhakar et al. 2016).

Important socioeconomic advantages, such as energy security and independence from fossil energy sources, could be realized from the continued development of advanced biofuels production (Gasparatos et al. 2013). There have been instances in which biofuels have received significant funding from public and private sources in several countries, indicating evidence among the supportive regulatory framework and the role of policies in advancing the biofuel industries (Sun et al. 2019). Hence, there are strong reasons to identify the lack of supportive policies and effective funding mechanisms as major obstacles in maintaining the momentum of the biofuel sectors. Furthermore, the inadequate funding also has slowed the transition away from capital-intensive first-generation towards second and third-generation biofuels (Mathimani and Mallick 2018). This is a major obstacle that hinders the progress toward long-term energy security and long-term sustainability of the society as long as the reliance on non-renewable, carbon-emitting fossil fuels persists (Balsalobre-Lorente et al. 2018).

Considering the positive benefits offered by this sustainable fuel resource, microalgae are believed to act as an important source of sustainable and carbon–neutral biomass feedstock. Several methods can be used to convert microalgae into third-generation biofuels, including widely used techniques such as transesterification, pyrolysis, anaerobic digestion, hydro-treatment, and fermentation (Adeniyi et al. 2018; Pourkarimi et al. 2019; Solé-Bundó et al. 2019). However, the current slow pace of development has hindered the progress made in advancing the cost-effectiveness of biofuel production from microalgae biomass. Future successes in promoting wider commercial adoption of microalgae-based biofuel production will depend on the effective solutions to these existing challenges (Wang and Yin 2018).

Prospects and perspectives

Compared to current fossil fuel prices, the production cost of microalgae-based biodiesel is significantly higher than conventional fuel options. One of the main factors driving up the production cost of advanced biofuels is the high capital requirement associated with nutrients (e.g., nitrate and potassium) and freshwater supply for the cultivation of microalgae. Studies have shown that these expenses can account for between 20 and 30% of the total production cost of microalgae-based biodiesel (Clarens et al. 2010; Chen et al. 2011). Therefore, the cultivation of microalgae plays an integral part in the biofuel supply chain and biofuel cost. Conventional farming also runs the risk of runoff from fertilizers and pesticides, leading to increased surface water contamination and eutrophication of lakes and streams. These environmental issues should be at the forefront of future development of microalgae-based fuel to avoid undermining the environmental and social sustainability aspects of the overall production. Among current research efforts into this sector, a credible foundation has been established based on a thorough analysis of technological feasibility and production cost optimization that could serve as the initial steps toward more advanced research and development. Particularly, the operation cost should take into account the cost of land, production infrastructure, harvesting equipment, downstream processing, fixed and variable operational expenses (Beal et al. 2012; Daroch et al. 2013; Javed et al. 2019). Continued research should focus on lowering these costs. A potential strategy to reduce this cost is to cultivate microalgae cultures in wastewater that has the potential to be more cost-effective and sustainable on a large production scale (Ahmed et al. 2021; Hena et al. 2021). With the use of wastewater as the growing medium, microalgae can utilize the nutrients available in the waste effluents. Hence, such a process can produce microalgae biomass while treating the wastewater at the same time (Yu et al. 2021b). Furthermore, a higher level of biomass and lipid productivity can be obtained in the case of inorganic wastewater via mixotrophic cultivation. Indeed, carbon is the main source of nutrients that drives the growth and lipid production of microalgae. The use of organic and inorganic carbon can lead to different overall cultivation costs. According to Suali et al. (2012), an innovative approach has been proposed on the use of sweet sorghum as a carbon source for farming microalgae. It is estimated to cost approximately from \$0.027 to \$0.48 in terms of sweet sorghum cultivation (Gao et al. 2010; Singh and Ahluwalia 2013). Studies have reported the lipid production of up to 73% of microalgae biomass when supplying 25–50 g/L of sweet sorghum in the cultivation of microalgae. Another cheap and highly accessible source of carbon is flue gases (Suali and Sarbatly 2012). Considering the fact that microalgae can reach a 10% threshold of CO_2 fixation, continued development to improve these positive characteristics will contribute positively to driving down the cultivation cost and lowering global greenhouse gas emissions (Rawat et al. 2013). In their studies, Mu et al. (2014) investigated different factors involving the production of biofuels from wastewater-grown microalgae, including (1)—open pond versus photobioreactor-based cultivation methods; (2)—multiple biomass conversion techniques; (3)—nutrient supply (Mu et al. 2014). The authors confirmed significant advantages from using wastewater over freshwater in the cultivation of microalgae biomass for biofuel production. Hence, applying the wastewater microalgae cultivation method can potentially lower the production cost of biofuels up to nearly 50% improving their cost-competitiveness with conventional petroleum-based alternatives. Nevertheless, variables such as the nutrient profile of wastewater streams and subsequent biomass processing stages still have a significant influence on the overall efficiency of the production. Due to this reason, the potential to effectively scale up current operations further relies on the accessibility and adequate supply of suitable wastewater streams. Currently, there is still significant room for continued improvements made to the existing production technologies before feasible commercialization.

The fact shows that low-conversion efficiency and large input resource requirements (e.g., land, freshwater) hinder the potential of scaling up biofuel production from conventional sources of biomass. Current advances and potential breakthrough discoveries in genomic research of microalgae hold the key to increasing the outlook of biofuel production from cellulosic biomass (Brar et al. 2021). By employing mutagenesis or transgenesis technique, the cellular structure and mechanics of an organism can be targeted and altered based on the desired output (Gan and Maggs 2017; Spicer and Molnar 2018). With advances made in this innovative research front, new strains with enhanced lipid productivity and concentration can be developed through molecular breeding. Besides, there are also ongoing efforts in discovering new species of microalgae that offer a higher capability for biofuel production, although these newer strains are yet ready for cultivation and production on a commercial scale. Selected species with demonstrated resistance against potential harm caused by viruses, fungi, and microzooplanktonic grazers are suitable for cultivation in wastewater. Besides, advanced genetic engineering methods can be explored in enhancing the photosynthetic efficiency in microalgae domestication. There has been a great deal of research interest looking into the potential of increasing the lipid content through altering the metabolic activities of microalgae and down-regulate phosphoenolpyruvate carboxylase to inhibit the breakdown of free fatty acids in microalgae (Wang et al. 2017; Aratboni et al. 2019). Besides altering the genetic makeup of selected microalgae species, the application of metabolic engineering techniques promises to improve the biofuel conversion efficiency. For example, the use of the carbon partitioning method, along with increasing the inhibition light threshold, has been observed to significantly enhance the growth and lipid productivity of microalgae (Huang et al. 2020; Bamary and Einali 2021). According to Zaslavskaia et al. (2001), photoautotrophic diatom *Phaeodactylum tricornutum* was detected to sustained growth in complete lack of sunlight after being interserted with glucose transporter genes from *Chlorella*. However, researchers are cautioned by the high environmental sensitivity of the genetically modified organism when exposed to external factors that could result in unpredictable metabolic pathways, in which environmental stress can prompt metabolic changes in selected species of microalgae that could imbue the biomass with additional metabolite (Singh et al. 2011b). As reported, fifty thousand different species of microalgae have been currently identified and sampled that offers a wealth of potential resources and knowledge base for continued research in postgenomic technologies concerning microalgae cultivation for biofuel production (Elisabeth et al. 2021). Continued developments in this research direction promise advancement in several different aspects related to the cultivation of microalgae including hydrocarbon productivity and storage in microalgae biomass, fast growth rate, improved metabolic activities, and enhanced nutrient conversion efficiency via photosynthesis (Khan et al. 2017). Future research should focus on examining the effectiveness of cultivating genetically modified strains of microalgae in an actual culture environment along with advanced methods of metabolic modifications of microalgae. Furthermore, further research is still required to improve the potential to scale up existing operations and successfully implement industrial modules in the cultivation of relevant species of microalgae. Besides, there are also significant obstacles facing the harvesting of microalgae biomass. Currently, the commonly used technique, namely self-flocculation or bio-flocculation, has demonstrated its effectiveness while being fairly affordable (Li et al. 2021; Ray et al. 2021). Given its maturity in the practice of wastewater treatment, its application in microalgae cultivation has yet to be fully explored. Potential areas of research can be of interest when examining the self-flocculation ability of different microalgae species. Furthermore, the self-flocculation process can be significantly improved upon the enhancement of mixed culture cultivation and modifying existing environmental factors affecting the growth of microalgae, these interesting observations should be further examined.

One of the major goals of the transition toward a bio-based circular economy is to ensure equal and fair access to the energy supply of all people while promoting the long-term sustainability of the planet. Compared to other conventional first and second-generation biofuels, microalgae-based biofuels have a certain important edge in terms of ethical quality because the cultivation of microalgae does not compete for valuable arable land and freshwater resource that can be used for growing food crops. However, the production of biofuels from microalgae still faces moral dilemmas similar to other types of fuels to a certain extent. Thus, in the future, one should take into account several elements such as considering human rights, justice, solidarity, sustainability, and stewardship in attempting to formulate an ethical argument in supporting or discounting the development of new energy sources or specifically microalgae-based biofuels in this case (Oncel 2013; Zhu et al. 2014; Lima 2021). Indeed, the development of an integrated biorefinery is an effective strategy in lowering the cost of microalgae-based biofuel production because the integrated system allows for more efficient operations of the different stages, including breaking down of microalgae biomass, isolation and extraction of bioactive components, and conversion to biofuels. Moreover, improvements in pretreatment processes of integrated biorefinery such as harvesting and drying biomass could result in far greater energy and cost savings. The integrated biorefinery can exploit the active bio components of microalgae besides lipid such as carbohydrates and proteins that can be converted into value-added byproducts, showing that the increased economics gained from utilizing high value-added by-products from the microalgae-based conversion process can potentially help to offer a solution to address labor and income inequality issues while building a stronger resilience in local communities (Atabani et al. 2021; Banu et al. 2020; Goswami et al. 2021). As part of the integrated biorefinery system, the economic value of biomass utilization and conversion is maximized to yield different products such as biofuels, chemicals, and feeds (Stiles et al. 2018; Reno et al. 2020; Morseletto 2020). Nevertheless, the biorefinery process yielding biofuels and chemicals does necessitate the need for large-scale operations to meet the current market demand for these biomaterials. Hence, the biorefinery process should be constructed based on the industrial perspective to ensure the integration of different liquid biofuels output as part of the large-scale operation. To save on operational costs, all major components of microalgae biomass should be taken into account in producing various types of liquid fuel due to their different properties. Recent studies have shown the successive production of different liquid biofuels, namely biodiesel, bioethanol, and bio-oil, from microalgae biomass (Fernández-Acero et al. 2019; Devadas et al. 2021; Bolognesi et al. 2021). It was found that the extraction of lipid from microalgae serves as an important ingredient in the conversion of biodiesel and bio-oil. Upon the extraction of lipids from microalgae biomass, the residues can be subjected to saccharification and bioethanol fermentation processes which take advantage of the available polysaccharides. To maximize the economic values of microalgae biomass, the residual biomass can subsequently be put through pyrolysis to yield bio-oils (Lee et al. 2015). These studies have demonstrated the successful capability of yielding multiple liquid biofuel products from the integrated microalgae biorefinery process. By integrating co-production and decarbonization of electricity as part of the entire process, the obtained biofuel is more comparable to the conventional counterpart in terms of financial feasibility and long-term sustainability (Adeniyi et al. 2018). Additional cost savings can be realized from the ability to procure input resources (e.g., CO_2, nutrients, water) from less expensive sources (Reis and Gouveia 2016). However, in the future, new extraction techniques should be further investigated to offer a more efficient way of removing lipid from microalgae biomass, even in the absence of lignin. Besides, advanced techniques should be applied to allow for the extraction to occur under high-moisture conditions can eliminate the need for a high energy-intensive water extraction process. On a global scale, a smooth transition under cooperation and coordination among various stakeholders will ensure the gradual maturation of the sector to avoid any potential market instability. To reduce the chance of market monopolization, R&D results should be made available to the public while the practice of fair trade should be incorporate to uphold the industry's ethical standards (Tait et al. 2011; Oncel 2013). Moreover, further investments for R&D studies are needed to improve the output yield while also lowering the overall production cost (Fernández et al. 2021). In general, applying microalgae biomass as a feedstock in the biorefinery process has the potential to achieve added benefits from various value-added by-products.

The integration of advanced microalgae biorefinery with wastewater treatment also has positive effects on greenhouse gas reduction through effective sequestration of carbon dioxide. Several studies have also proposed the use of mixed-biomass feedstocks, such as plant-based waste, food discard, agricultural residues, sewage effluents, to sustain the continuous production of the biorefinery (Brilman et al. 2017; Chen et al. 2014; Wang et al. 2019). Besides, the presence of auto-sedimentation traits in selected species of microalgae can eliminate the need for using flocculants and other energy-intensive harvesting techniques. This will improve the recyclability of the used effluents in microalgae cultivation and biofuel production as well as enhance the yield of the biofuel output of the microalgae biomass feedstock. In addition, the economic feasibility of microalgae-based biofuel production cannot be realized without the integration of other available technologies. In the future, the application of microalgae as the anodic feedstock for the case of microbial fuel cells should be further investigated and exploited. Particularly, both the extracted oil content and the carbohydrates and protein-containing in residual microalgae biomass can serve as the anode in the fuel cell system. Particularly, both the extracted oil content and the carbohydrates and protein-containing in residual microalgae biomass can serve as the anode in the fuel cell system (Ndayisenga et al. 2018; Mekuto et al. 2020). According to a close-circuit microbial fuel cell system developed by Kakarla and Min (Kakarla and Min 2019), the CO_2 produced from the residual microalgae biomass at the anode is transferred to the cathode chamber where the CO_2 can be fixed by the fresh microalgae. In this instance, the microalgae grown in the cathode chamber can be propagated by fixing on the supplied CO_2. However, issues related to the practical application and commercialization should be examined through TEA and LCA, despite TEA and LCA can only be conducted on pilot-scale demonstration projects on the use of mixed biomass feedstock in an integrated closed-loop biorefinery with a multi-product recovery scheme (Mishra et al. 2019), resulting in limiting the potential assessment and prediction of issues facing larger scale commercial production. Above the feedstock supply challenge, other obstacles facing large-scale conversion of biomass to biofuels have to mention the ability to recover waste after each stage of the process as well as the impact of biomass harvesting methods on wastewater effluents valorization. To address these challenges, an innovative solution to overcome these current obstacles has been proposed toward improving energy efficiency and sustainability of the microalgae biorefinery process, as shown in Fig. 6. This new approach also emphasizes the capability of reducing both liquid and solid waste streams through recycling and reusing materials as part of the circular economy. Furthermore, the integration of advanced microalgae biorefinery with wastewater treatment also has positive effects on greenhouse gas reduction through effective sequestration of carbon dioxide.

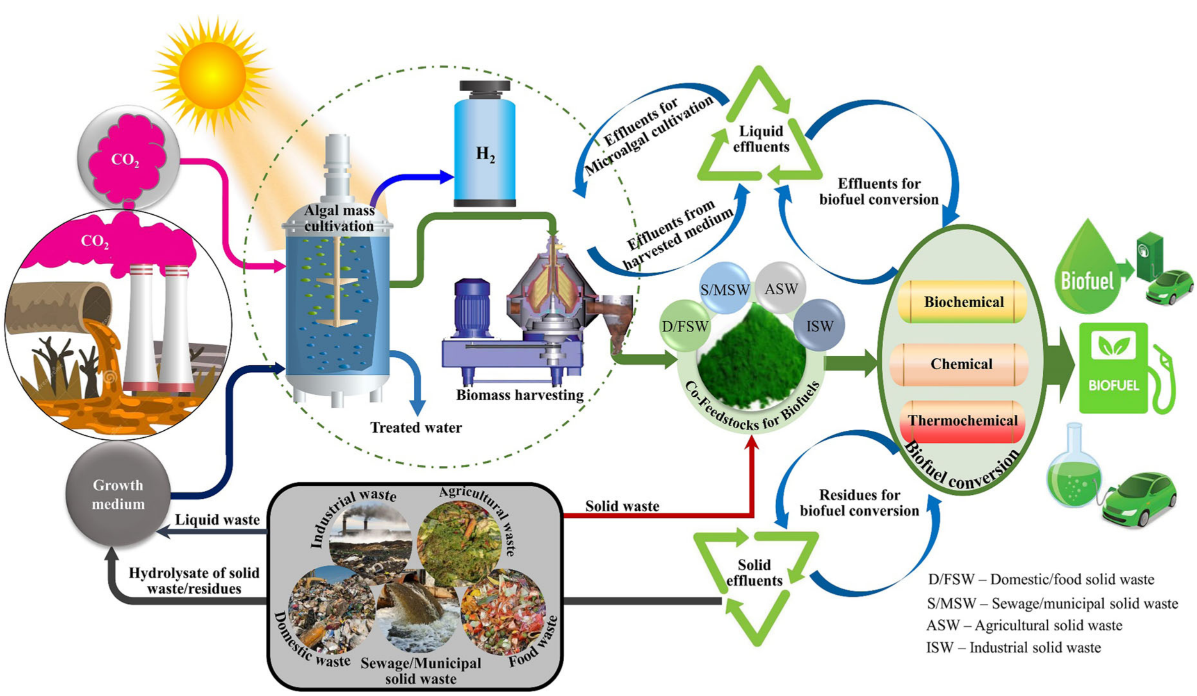

Fig. 6 Sustainable approach based on circular bio-economy for bioenergy production from microalgae aiming to reduce CO_2 emissions and waste

Conclusions

In this paper, the latest update on the current landscape of microalgae-based biofuel research, including the benefits and existing challenges for the multitude of biofuels that can be derived from microalgae such as biodiesel, bioethanol, biohydrogen, biomethane, was comprehensively analyzed. Regarding the benefits of microalgae biofuels, it was indicated that the high ability to fix CO2 in the atmosphere, a significant reduction of environmental pollution as using wastewater as a nutrient in microalgae cultivation, and contribution to sustainable development are considered as the main advantages of microalgae biofuels. However, it was found that the biggest obstacle remains given the costly energy requirements in the cultivation, harvesting, and pre-processing treatment of microalgae feedstock, resulting in a further increase in the overall processing cost. Therefore, possible future research directions should investigate the application of recombinant gene technology to enhance the metabolic pathways aiming to result in the creation of biofuel-grade chemicals. Furthermore, interdisciplinary research should be carried out aiming to improve the productivity of biofuel production. Last but not least, the integration of microalgae biorefinery processing and the economic practicality of microalgae-based biofuel production should be further explored in the context of sustainable development and circular economy.

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