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Review Article

BIOREFINERY OF MICROALGAE: THE WORLD'S GREEN GEM FOR THE FUTURE SUSTAINABLE DEVELOPMENT

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ARTICLE HIGLIGHTS

- Integrated biorefinery provides economic and environmental benefits
- Microalgae produce higher lipid content than plants
- Generally, microalgae produce higher carbohydrate contents essential for bioethanol
- Biohydrogen produced by microalgae is a future energy source
- As a biofertilizer, microalgae promotes plant growth

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ABSTRACT

Industrial and household activities leading to many pollutants have been reduced by the presence of microalgae in the phycoremediation. Microalgae transform pollutants into many forms of biorefinery, such as biofuel, biojet, bioethanol, biohydrogen, and biofertilizer. The chemical residue resulted from household and industrial activities has abundant elements (N, P, C) for microalgal cell growth. The contents of a microalgal cell, like lipid and carbohydrates, depend on the nutrition in the medium, the cultivation system, the microorganism-mediator, and the applied technology. *Chlorella vulgaris, Botryococcus braunii, Spirulina platensis, Chlorella* sp., *Chlamydomonas* sp., and *Chlorococcum* sp., are potential lipid-producing microalgae and are applied in biofuel and biojet. The carbohydrate of *Cyanobacteria synechoccus* sp., *Nannochloropsis oculata,* and *Arthospira platensis* is the main content to be utilized in bioethanol. Meanwhile, for the application of biohydrogen, H_2 gas is converted from *Scenedesmus obliquus* fermentation. However, the process of bioethanol and biohydrogen needs bacteria as a mediator of fermentation. *Spirulina* and *Scenedesmus* are examples of microalgae supporting soil fertility as biofertilizers. Extraction of microalgae can increase growth promotors for plants, like amino acids, peptides, and proteins, and also antibacterial and antifungal. Optimizing excellent microalgae content in bioenergy will face several challenges, for example, imbalances of organic waste. However, the phycoremediation of microalgae is a sustainable and futuristic solution to fulfill the need for energy stock.

Keywords: biodiesel, bioethanol, biofertilizer, biojet

INTRODUCTION

The development aspect of a country, such as the economic aspect, tends to increase the consumption of fossil fuels to run factories, households, transportation, as well as to generate electricity and heat. Considering the excessive use of fossil fuels to support human activities, the supply of fossil fuels in the world will not be sufficient, while fossil fuel is also non-renewable. Additionally, the expansion of a country with a war also tends to add to the consumption of fossil fuels. In the future, climate or moderate warming around 2050 will increase energy demand predicted by 25 - 58%. Tropics and southern regions of the United States, Europe, and China will increase energy demand by more than 25% (van Ruijven *et al.* 2019). Although China is an Asian country that used the highest renewables for the production of energy in 2014, coal as a non-renewable source still be used within a quantity of 77%.

The countries in Asia which depend on other non-renewable sources with their quantity are: Indonesia (70% coal), Japan (28% oil), and Malaysia (61% natural gas) (Sharvini *et al.* 2018).

The environmental impact caused by the usage of a non-renewable resource, especially carbon-based oil, has been a spotlight. To produce an eco-friendly profitable energy, microalgae, which is abundant in aquatic systems and other ecosystems, have been observed in many fields. Microalgae have been optimized into many forms, such as biohydrogen, bioethanol, biofuel, biojet, and other forms utilizing microalgae's content, particularly lipid and carbohydrates.

Four algal strains, such as *Chlorella vulgaris*, *Desmodesmus* sp., *Ettlia pseudoalveolaris*, *Scenedesmus obliquus*, have been cultivated within a nitrogen-starving condition (Nzayisenga *et al.* 2020). This treatment triggered more lipid production which is a remarkable biodiesel in the form of triacyglycerols. *Chlorella vulgaris* is also optimized as a bioethanol that its quantity increases if fermentation time expands (Agwa *et al.* 2017). Microalgae-based oil which resulted from *Chlorella* sp. and *Spirulina* sp. was a result of conversion from triglycerides into fatty acid methyl ester and glycerol (Fattah *et al.* 2020). Many species of microalgae have been transformed into green energy. Therefore, this review aimed to describe the advantage of microalgae cultivation to support the application of future green-energy of the world.

REVIEW

Bioprospecting of Microalgae

Microalgae have been widely reported for having the ability to produce various commercial bioproducts, such as bioethanol, biofuel, biodiesel, biohydrogen, biojet, and biofertilizers (Table 1). The relatively high lipid content makes microalgae a candidate for vital bioenergy feedstock in the future. Several strains that produce high amounts of lipid include *Botryococcus*, *Nannochloropsis*, and *Chlorella* sp. Carbohydrate content such as that found in *Arthrospira platensis (*60%) (Markou *et al.* 2013) and *Chlamydomonas* sp. (50%) (Kim *et al.* 2020) is also a critical source of bioethanol. Not only limited to the energy sector, microalgae can also be used as biofertilizers, such as those found in *Schenedesmus obliquus* (Ferreira *et al.* 2019).

Some of the criteria for suitable microalgae strains to be used as candidates for lipid sources to produce biofuels are having a high growth rate, high lipid content, being able to grow in a variety of environmental conditions, including being resistant to contamination, being easily harvested, and being able to be processed using the latest lipid extraction method and potentially developed in many biorefinery activities (Resdi *et al.* 2016).

Two kinds of microalgae cultivation systems are commonly used, namely open pond and photobioreactor. Open pond is a microalgae cultivation system carried out openly (Egbo *et al.* 2018). An open microalgae cultivation system can be applied on a large scale. This system is suitable for wastewater treatment and is sustainable. There are several types of open pond cultivation systems, including shallow lagoons, ponds, inclined systems, circular ponds, mixed ponds, and raceway ponds (Borowitzka & Moheimani 2013).

A photobioreactor is also called a closed system because it is carried out in a closed container made of translucent material (Egbo *et al.* 2018). Several types of photobioreactors are tubular reactors, flat plate reactors, pyramid reactors, fermenter types, and hybrid reactors. Tubular reactors consist of vertical tubular reactors, horizontal tubular reactors, helical tubular reactors, α-shaped reactors, and polyethylene bags and sleeves (Płaczek *et al.* 2017).

Photobioreactors require higher production costs than that of open ponds. However, the water demand in photobioreactor, the risk of contamination, as well as the loss of CO_2 and nutrient is lower compared to that in the open pond. A photobioreactor is made of translucent materials that can be penetrated by sunlight. Photobioreactor productivity is higher compared to that in open ponds. It is easier to regulate culture temperature in a photobioreactor than it is in an open pond. However, the commercialization of open ponds on a large scale has more potential (Egbo *et al.* 2018).

Phycoremediation can be used as a method of environmental recovery. Phycoremediation is safe, environmentally friendly, and relatively easy to perform. In addition, the microalgae biomass produced in this process can be used as a source of biodiesel production (Asiandu & Wahyudi 2021). Bioprospecting of various algal species has resulted in many forms of bioenergy for utilizing the variation of growth-supporting chemical content (Table 1).

Table 1 Prospect of some microalgae species

No.	Algal Species	Bioprospect	Biochemical content or Roles	Ref.
1.	*Arthrospira platensis*	Bioethanol and biodiesel	Carbohydrate content 60%, lipid < 10%, and protein > 20%	(Markou *et al.* 2013)
2.	*Botryococcus braunii*	Biofuel	Lipid content 25 - 75%	(Chisti 2007)
3.	*B. braunii*	Biodiesel	Lipid content 78%	(Nagaraja *et al.* 2014)
4.	*Chlamydomonas* sp.	Biojet	Lipid content 43.34%	(Phang *et al,* 2015; Chen *et al.* 2016)
5.	*Chlamydomonas* sp.	Bioethanol	Carbohydrate content 50.5%, lipid 19%, and protein 24.2%	(Kim *et al.* 2020)
6.	*Chlamydomonas* sp.	Bioethanol	Carbohydrate content 49%	(Qu *et al.* 2020)
7.	*Chlorella* spp.	Biojet	Lipid content 26.72 - 40.38%	(Phang *et al.* 2015; Chen *et al.* 2016)
8.	*Chlorella* sp.	Biofuel	Lipid content 28 - 32%	(Chisti 2007)
9.	*C. minutissima*	Bioethanol	Carbohydrate content 0.125 g/L	(Margarites & Costa 2014)
10.	*C. vulgaris*	Biodiesel	Lipid content > 40%	(Widjaja 2009)
11.	*C. vulgaris*	Biohydrogen	Potentially used in biohydrogen production	(Lakshmikandan & Murugesan 2016).
12.	*C. vulgaris*	Biofertilizer	Increasing N, P, K, Fe, Zn, Mn contents in the soil	(Dineshkumar *et al.* 2020).
13.	*Chlorococcum* sp.	Biojet	Lipid content 39.28%	(Phang *et al.* 2015; Chen *et al.* 2016)
14.	*Crypthecodinium cohnii*	Biofuel	Lipid content 20%	(Chisti 2007)
15.	*Desmodesmus* sp.	Bioethanol	Carbohydrate content 37%	(Qu *et al.* 2020)
16.	*Dunaliella primolecta*	Biofuel	Lipid content 23%	(Chisti 2007)
17.	*Monallanthus salina*	Biofuel	Lipid content > 20%	(Chisti 2007)
18.	*Nannochloropsis* sp.	Biofuel	Lipid content 68%	(Chisti 2007)
19.	*Nannochloropsis* sp.	Biojet	Lipid content 80%	(Bwapwa *et al.* 2018)
20.	*N. gaditana*	Bioethanol	Carbohydrate content 17.7%	(Onay 2018)
21.	*N. oculata*	Bioethanol	Carbohydrate content 252.84 mg/g	(Fetyan *et al.* 2021)
22.	*Parachlorella kessleri*	Bioethanol	Carbohydrate content 40%	(Qu *et al.* 2020)
23.	*Scenedesmus obliquus*	Biofuel	Carbohydrate content 30.2%, lipid 17.9%, and protein 31.4%	(Ferreira *et al.* 2019)

No.	Algal Species	Bioprospect	Biochemical content or Roles	Ref.
24.	*S. obliquus*	Biohydrogen	Potentially used in biohydrogen production	(Ferreira *et al.* 2019)
25.	*S. obliquus*	Biofertilizer	Enhanching the germination of wheat dan barley seeds	(Ferreira *et al.* 2019)
26.	*S. obliquus*	Biofertilizer	Increasing N, P, and K content in soil	(Nayak *et al.* 2019).
27.	*Schizochytrium* sp.	Biofuel	Lipid content 77%	(Chisti 2007)
28.	*Schizocytrium* sp.	Bioethanol	Carbohydrate content 17.3%	(Kim *et al.* 2012)
29.	*Spirullina platensis*	Biofertilizer	Increasing N, P, K, Fe, Zn, Mn contents in the soil	(Dineshkumar *et al.* 2020).
30.	*Synechoccus* sp.	Bioethanol	Carbohydrate content 60%	(Möllers *et al.* 2014)
31.	*Tetraselmis sueica*	Biofuel	Lipid content 15 - 23%	(Chisti 2007)

Biodiesel

Biodiesel from microalgae is considered as having more potential to be developed for overcoming the scarcity of fossil-based energy. When compared to crop-based biodiesel, microalgae are more sustainable for production in the long run. Microalgae biomass obtained from microalgae cultivation activities can be extracted to produce algal oil. Algal oil can be used as a raw material for biodiesel production. Meanwhile, algal oil production produces by-products that can be processed into animal feed and other products. Biomass waste can be processed in an anaerobic digestion system to produce biogas and bioelectricity. Meanwhile, the effluent from this process can be used as a biofertilizer (Chisti 2008).

Some efficient solvents for lipid extraction in microalgae are petroleum ether, n-hexane, and chloroform. The percentage ratio of solvents with high yield conversion values is 75% ethanol and petroleum ether, 75% ethanol and n-hexane, and 75% ethanol and chloroform. The combination produced a yield of about 85%. This solvent can be used to carry out direct transesterification of *Chlorella* spp. biomass (Zhang *et al.* 2015).

Microalgae have many advantages over higher plants in the production of biofuels. Microalgae are photosynthetic and absorb CO_2 from the air. It is one strategy to reduce greenhouse pollutants in the atmosphere. Microalgae have a rapid growth rate and biomass can be produced in a short time. Microalgae require a smaller land area than higher plants. Also, microalgae can be harvested easily (Widjaja 2009).

One candidate for biodiesel production is *Chlorella vulgaris*. This strain is capable of accumulating lipid in relatively high amounts. The treatment in the form of N-depletion initiates lipid accumulation in the microalgae to produce high lipid accumulation at the end of incubation. Lipid accumulation is more than 40% in cultures that experienced N-depletion on day 27. Cultivation on day 20 produces a biomass of 0.86 mg/L, with a lipid percentage of 29.53%, and a lipid productivity of 12.77 mg/L/day. The accumulation of lipid can be higher when implementing vacuuming at low temperatures in the extraction process (Widjaja 2009).

Moreover, compared to other plants in lipid production, microalgae have a higher lipid conversion value. Lipid conversion value of microalgae with low lipid content reaches 58.700 L/ha/year (Fig. 1). Meanwhile, palm oil only reaches a conversion of 5.366 L/ha/year. This amount is much lower than that of microalgae. It means that palm oil production is not optimal as a future energy source. This is also related to lower productivity with large cultivation area (Rahman *et al.* 2019; Rajvanshi & Sharma 2012). Meanwhile, some strains have already been reported as oleaginous microalgae, as presented in Figure 2, which accumulates high amounts of lipid, such as *Botryococcus brauni* (> 75%), *Schizotrium* sp. (> 75%), *Nannochloropis* sp. (> 60%), to widely

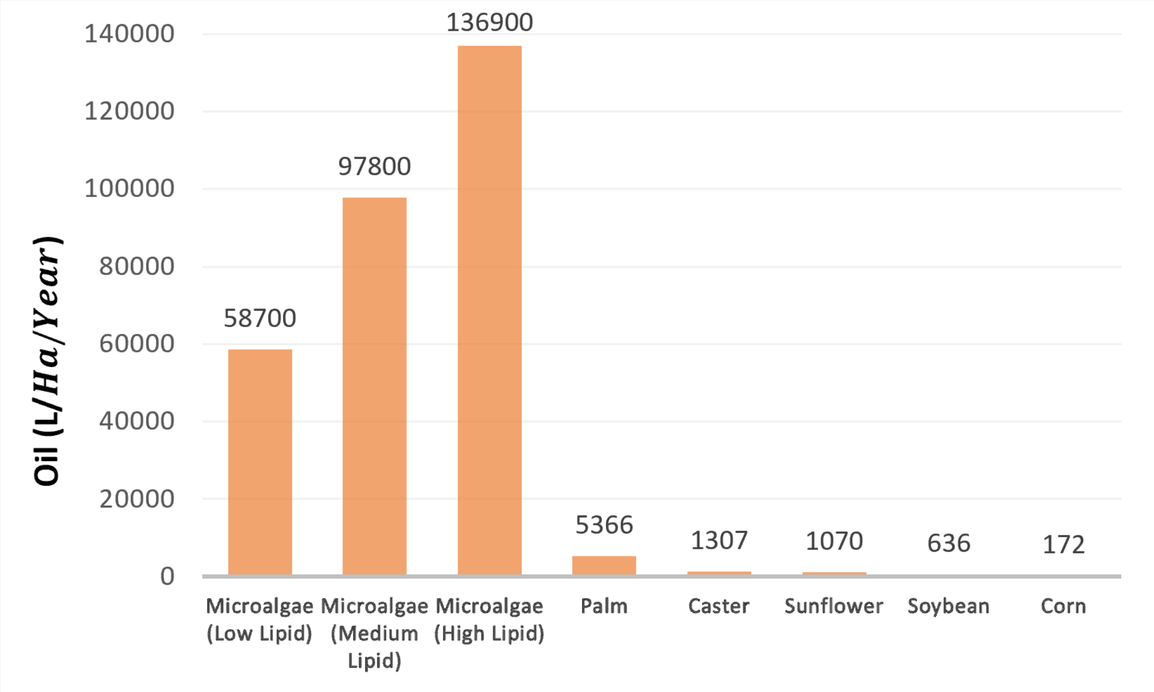

Figure 1 Oil yield comparison between microalgae and other biomass sources (Rahman *et al.* 2019; Rajvanshi & Sharma 2012)

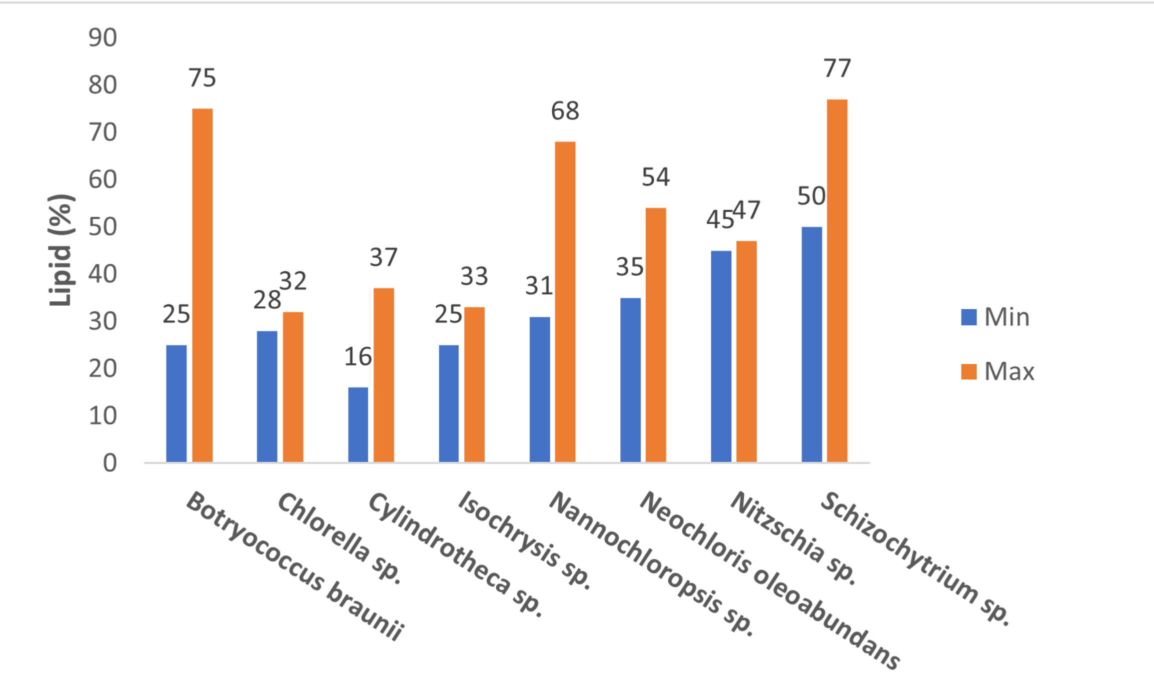

Figure 2 Lipid content in some microalgae

Note: Modified from Christi (2007).

cultivated strain, such as *Chlorella* sp. (> 30%) (Chisti 2007).

Several technologies can be used in the production of microalgae-based biofuels, including biochemical conversion, transesterification, thermochemical conversion, and direct combustion. Biochemical conversion is a biofuel production method that involves microorganism fermentation. Transesterification is a biofuel production technique using the reaction between alcohol and microalgal lipid. Thermochemical conversion is a method that uses high temperature to convert biomass into alcohol, hydrocarbon fuels, and other chemical substances. The process includes gasification, pyrolysis, and liquefaction (Culaba *et al.* 2020). Direct combustion is used to produce electrical energy using microalgae biomass residue.

The microalgae biomass is converted through the use of heat which will produce electrical energy.

Some microalgae accumulate lipid in a high percentage which is essential in biofuel production (Fig. 2). One of the strains that contains high lipid is *Botryococcus braunii* with lipid content of 25 - 75%. Additionally, lipid accumulation in *Nannochloropsis* sp. peaked at 68%. Lipid content in *Schizochytrium* sp. is up to 77%. Other lipid-accumulating microalgae are *Chlorella* sp., *Crypthecodinium cohnii*, *Dunaliella primolecta*, *Monallanthus salina*, and *Tetraselmis sueica* with lipid content of 28 - 32%, 20%, 23%, > 20%, and 15 - 23%, respectively (Chisti 2007).

Botryococcus braunii cultivated in a photobioreactor is able to reach a biomass of 4.1 g/L. Lipid accumulation in the microalgae is higher with increasing incubation time. The highest lipid accumulation reached 78%. At 42 days of incubation, the lipid extract obtained from 5 g of dry biomass was 3.8 g or 78% (Nagaraja *et al.* 2014). Some *B. braunii* isolates can be found in Miyagi, Fukui, and Kochi, Japan, at pH 6.7 - 8.0. Meanwhile, several isolates from Indonesia, namely Palangka Raya, Pundu, Bunto, Muara Teweh, and Tenggarong are found at pH 6.0 - 7.7 (Kawamura *et al.* 2020).

There are differences in fatty acid composition in microalgae and palm oil. Palm oil contains methyl stearate, methyl oleate, methyl palmitate, and methyl myristate as much as 5.73%, 55.43%, 37.37%, and 1.46%, respectively. Meanwhile, the contents of methyl stearate, methyl oleate, methyl palmitate, and methyl myristate in algal oil (*Spirulina* sp.) are 6.04%, 49.20%, 44.76%, and trace (Pradana *et al.* 2020). Moreover, lipid of *Spirulina platensis* contains C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1. The FFA value of this lipid is relatively high, reaching 18.7%. The use of 80% alcohol in the microalgae lipid transesterification process produces a biodiesel yield of 84.7%.

The biodiesel quality test produced on microalgae-based biodiesel has a viscosity of 4.8 mm^2/s, density of 886 kg/m^3, flash point at 172 °C, cloud point at 5 °C, pour point at -1 °C, and cetane number 60.73. Considering other biodiesel, the fatty acid composition of the biodiesel is dominated by a palmitic acid of 48.35%, while the content of other fatty acids, namely mystic acid, linolenic, linoleic, palmitoleic, oleic, stearic, and lauric is 20.9%, 7.84%, 5.37%, 2.66%, 2.41%, 2.02%, and 0.7%, respectively. From this analysis, biodiesel produced by *S. platensis* lipid is very appropriate to be used as a biodiesel source (El-Shimi *et al.* 2013).

Biojet

Some potential microalgae strains in biojet production are found in Malaysia, including *Chlorella* sp., *Chlamydomonas* sp., and *Chlorococcum* sp. Lipid content in *Chlorella* spp. peaks at 26.72 - 40.38%, the lipid content of *Chlamydomonas* sp. is 43.34%, and in *Chlorococcum* sp. is 39.28%. The productivity of lipid production in *Chlorella* spp. is 12.71 - 30.57 (mg/L/day), *Chlamydomonas* sp. at 14.03%, and *Chlorococcum* sp. at 22.55%.

To produce 1 kg of biojet through HEFA (Hydroprocessed Esters and Fatty Acids), 4.14 - 6.25 kg of *Chlorella* spp. biomass, 3.86 kg of *Chlamydomonas* sp. biomass, and 4.26 kg of *Chlorococcum* sp. biomass are needed (Phang *et al.* 2015; Chen *et al.* 2016). The production of biojet through HEFA can be applied by converting microalgal oil extracted from its biomass through hydrotreatment (Lundquist *et al.* 2010; Chen *et al.* 2016).

Some methods for converting microalgae biomass into biojet fuel are hydrothermal liquefaction; biomass gasification and Fischer-Tropsch, comprising pyrolysis; cracking, reforming, and upgrading; deoxygenation and decarboxylation, transesterification, and fractional distillation (Bwapwa *et al.* 2018). Hydroprocessing requires relatively low production costs, suitable for large-scale production.

Nannochloropsis sp. is one of the future candidates for biojet production. This strain grows well at temperatures of 15 - 25 °C. Lipid accumulation in the microalgae increased by 80% after being treated in the form of nutrient starvation for 3 days. The bio-oil extracted from the microalgae can then be processed through pyrolysis to produce the next oil fraction in the form of biojet. The characteristics of the biojet fuel produced from this microalgae are a heating value of 44 MJ/kg, freezing point at -30 °C, flash point at 68 °C, density at 15 °C which is 1.38 g/mL with total sulfur of 0.27 wt% (Bwapwa *et al.* 2018).

Bioethanol

Microalgae can also be utilized in producing bioethanol due to the high accumulation of carbohydrates as a result of their photosynthesis. As an example, Cyanobacteria *Synechoccus* sp. biomass contains 60% carbohydrates. Their carbohydrate compounds can be hydrolyzed and then fermented using *Saccharomyces cerevisiae* involving some enzymes to convert carbohydrates into ethanol. The ethanol conversion value reaches 0.22 g per one gram of dried biomass or 30 g/L. Furthermore, the sugar of carbohydrates in *Synechoccus* sp. includes glucose (60%), as well as galactose, xylose, arabinose, and mannose with concentrations of less than 5% (Möllers *et al.* 2014).

Chlamydomonas sp. can also be used in bioethanol production with an average yield reaching 0.22 g/g residual biomass. Microalgal biomass growth can be initiated by cultivating the microalgae with 80 μm photon/m^2/s light. The percentage of carbohydrates in the microalgae cells was 50.5%, with 24.2% protein and 19% lipid. The main composition of the fatty acids that make up the microalgae lipid included polyunsaturated fatty acids (PUFAs) and Monounsaturated Fatty Acids (MUFAs) dominated by C16:4 with a percentage of 25.6%, followed by C18:3 (19.4%), C18:2 (5.9%), C18:1 (7.8%), and other fatty acids. The high carbohydrate content in microalgae is a promising source of bioethanol production (Kim *et al.* 2020).

Arthrospira platensis also contains a high carbohydrate percentage which is nearly 60%. Meanwhile, the protein content is more than 20%, and lipid content is less than 10%. The yield of bioethanol produced from *Arthrospira platensis* biomass reaches 16.27% using 0.5 N H_2SO_4, with a bioethanol yield of 16% g/g of dried biomass (Markou *et al.* 2013).

Also, *Nannochloropsis oculata* contains carbohydrates of 252.84 mg/g dried biomass. The yield of carbohydrates produced from the mixotrophic cultivation is 268.53 mg/g, while from the phototrophic cultivation the carbohydrates produced is 177.73 mg/g of dried biomass. The reducing sugar content is 232.39 mg/g at a concentration of 3% H_2SO_4 with a saccharification percentage of 92.2%. The sugar is dominated by galactose, fucose, rhamnose, rabinose, xylose, and mannose (Fetyan *et al.* 2021).

Nannochloropsis gaditana can be cultivated using municipal wastewater. The yield of bioethanol produced in municipal wastewater is 30%, equal to 94.3 mg/g dried biomass with an accumulation of carbohydrates of 17.7%. The biomass produced on the 14th day of incubation is 2.33 g/L (Onay 2018). Meanwhile, to optimize the production of biomass and carbohydrates in microalgae like *Arthrospira platensis*, optimum K_2HPO_4 and $NaHCO_3$ concentrations are required, because the accumulation of lipid in the microalgae is influenced by the concentration of the two kinds of nutrients in the medium (Tourang *et al.* 2019).

The integration of bioremediation and microalgae-based biofuel production can also be carried out in bioethanol production. Microalgae *Parachlorella kessleri*, *Desmodesmus* sp., and *Chlamydomonas* sp. isolated from pig farms can remediate swine wastewater. The biomass of each microalgae cultivated in a mixotrophic culture during an incubation period of 8 days is 5 g/L, 3.1 g/L, and 6.3 g/L, respectively. The carbohydrate content in each isolate is 40%, 37%, and 49%. Meanwhile, the percentage of COD reduction in each isolate reaches 47%, 38%, and 47%. The enzymes involved in the production of bioethanol are glucoamylase, α-amylase, β-glucosidase, endoglucanase, and cellobiohydrolase. Meanwhile, in *Chlamydomonas* sp. the maximum ethanol concentration obtained was 61 g/L (Qu *et al.* 2020). Another potential isolate in bioethanol production is *Schizocytrium* sp. with a yield of 11.8 g/L (Kim *et al.* 2012), *Chlorella minutissima* with carbohydrates of 0.125 g/L (Margarites & Costa 2014).

Biohydrogen

Microalgae are also pivotal in biohydrogen production. One of many species, *Scenedesmus obliquus* produces high-purity biohydrogen through fermentation of about 67.1 mL H_2/g VS and 167.8 mL H_2/mL FM. The microalgae can be cultivated using brewery wastewater to minimize the production cost as well as remediate the environment (Ferreira *et al.* 2019). The production of biohydrogen can also be carried out using *Chlorella* sp. through the process of anaerobic biomass metabolism without the presence of sulfur. Microalgae cells are known of their capability in carrying out anaerobic metabolic processes by using carbon sources in their cells. However, the accumulation of hydrogen increases with the addition of glucose in the medium in the anaerobic phase (Song *et al.* 2011).

Additionally, hydrogen gas can also be produced through fermentation which utilizes bacteria and microalgae biomass. *Scenedesmus obliquus* biomass can be fermented by *Enterobacter aerogenes* and

Clostridium butyricum to produce energy in the form of hydrogen gas which can be converted into electrical energy. Dry biomass fermentation of *Scenedesmus obliquus* using *Clostridium butyricum* produces a biohydrogen yield of 113.1 mL H_2/g VS algae. Meanwhile, the yield of biohydrogen produced through the fermentation of *E. aerogenes* is 57.6 mL H_2/g VS algae. In addition, fermentation can also be carried out using wet biomass with lower resulting. However, the production costs and time by using wet biomass are more prospective (Batista *et al.* 2014).

Biohydrogen production using *Chlorella vulgaris* can also be enriched by using *Valoniopsis pachynema* extract as a nutrition source for the microalgae. The extract is added to the *C. vulgaris* growth medium to form hydrogen gas through the metabolism of the microalgal cells. The maximum biohydrogen produced from the culture was 0.002 g/hour/L (Lakshmikandan & Murugesan 2016).

Biofertilizer

Microalgae biomass or waste can also be used in the production of biofertilizers. Biomass contains various essential nutrients that can be utilized by plants to enhance their growth. The use of microalgae-based biofertilizers is potentially developed considering the massive use of chemical fertilizers that are harmful to the environment. The use of fertilizer itself is getting higher due to the increasing growth of the human population in the world. To meet agricultural needs, agricultural activities as well as the demands for fertilizers will be higher in the future (Dineshkumar *et al.* 2019).

Extracts of blue-green algae are reported to be able to increase several chemical properties and the resistance of a plant to pathogens. The microalgae extract enhances the growth promoters, amino acids, peptides, and proteins, and even triggers antibacterial and antifungal properties of these plants (Dineshkumar *et al.* 2019). Microalgae also enhance the nutrient richness of the soil. Microalgae increase the carbon and nitrogen content and balance the pH and electrical conductivity of the soil. Some species of blue-green algae are important in the process of nitrogen fixation, for example, *Anabaena*, *Nostoc*, *Aulosira*, and *Tolypothrix* (Karthikeyan *et al.* 2007; Dineshkumar *et al.* 2019).

Spirullina and *Chlorella* are two microalgal strains essential in enriching the soil to increase the yield of agricultural activities without causing side effects like common chemical fertilizers. Moreover, microalgae are also considered potential candidates for producing biopesticides, such as *Chlorella*, *Dunaliella* sp., *Coscinodiscus* sp., *Tetraselmis* sp.,and *Spirullina* sp. (Dineshkumar *et al.* 2019).

Scenedesmus obliquus cultivated using brewery wastewater is capable of producing various kinds of essential chemical products. It is also able to reduce the content of NH_3, total nitrogen, P-PO_4^{3-}, and COD in the waste with concentrations of 81%, 88.2%, 29.7%, and 70.8% reduction, respectively. Based on chemical properties analysis, the microalgae cells incubated using brewery wastewater until the stationary phase contain 31.4% protein, 30.2% carbohydrates, 17.9% lipid, and 4.4% FAMEs. The fatty acid composition of the microalgae is dominated by C16:0 of 23.03% (Ferreira *et al.* 2019).

Furthermore, *Scenedesmus obliquus* biomass cultivated using brewery wastewater increases the germination rate of wheat and barley seeds. Germinated seeds fed with *S. obliquus* biomass has longer roots, higher in size, and better color saturation. It triggers growth promoters in the seeds of the two samples provided with the microalgae biomass. Therefore, the microalgae can be considered as growth initiators of the plant (Ferreira *et al.* 2019).

The lipid-free residue of *Scenedesmus* sp. is also able to enhance the availability of N, P, and K in the soil. The provision of biomass residue can be used as a slow-release organic matter in the soil (Nayak *et al.* 2019).

C. vulgaris and *S. platensis* are also reported to be able to enrich the soil to support the onion growth rate. The soil compounds administered with *C. vulgaris* biomass contain N, P, K, Fe, Zn, and Mn with concentrations of 18, 14.22, 58.82, 3.46, 1.03, and 2.01 mg/kg, respectively. Meanwhile, the content of N, P, K, Fe, Zn, and Mn in soil administered with *S. platensis* biomass are 18.2, 14.24, 57.86, 3.42, 1.13, 2.06 mg/kg, respectively. The nutrient value estimated after the onion harvesting on the tested soil is shown to be higher than the control. After planting for 100 days, the height of the onions treated with *C. vulgaris* becomes more than 45 cm and almost 50 cm for soil treated with *S. platensis*. The administration of the two microalgae biofertilizers is also reported to increase chlorophyll and carotenoid contents in onions, as well as an increase in the content of total solute sugars, amino acids, and total phenols (Dineshkumar *et al.* 2020). In addition, the use of cow dung added with *S. platensis* or *C. vulgaris* also increases growth, yields, chemical components, and minerals in corn (Dineshkumar *et al.* 2017).

Utilizing microalgae for wastewater remediation and alternative energy source for sustainable environment

Microalgae cultivation can be integrated with environmental bioremediation processes. Various kinds of organic waste resulting from industrial activities can be used as nutrition sources in the cultivation of microalgae. Also, wastewater can be used as a water supply in microalgal biomass cultivation.

The water used in the microalgae cultivation can be reused in the next cultivation cycles to reduce the dependence on clean water. The cultivated microalgae biomass using various kinds of organic waste is the feedstock of some biorefinery activities, such as the production of energy, bulk chemicals, and feed. Meanwhile, microalgal wastewater can be applied as a soil enhancer to fertilize agricultural land safely (Al-Jabri *et al.* 2021).

Organic wastes containing various nutrient compounds, such as nitrogen, phosphorus, and carbon, will be absorbed by microalgal cells to carry out their metabolic processes. Additionally, CO_2 gas produced from industrial activities and diesel fuel can be absorbed by their cell and used as an essential material in photosynthesis. Other microorganisms, such as bacteria, can be integrated with single-strain microalgal biomass, in which the bacteria will break down complex chemicals in organic waste that will then be used as a nutrient source for microalgae cells (Al-Jabri *et al.* 2021).

The biomass conversion can be done through various methods to produce certain bioenergy products. The lipid contained in biomass can be converted into biomethane, or directly go through the transesterification reaction to produce biodiesel. Through hydrothermal liquefaction, the biomass can be turned into bio-crude oil. Sugar or carbohydrate-based contents can also be processed by fermentation to produce bioethanol. Moreover, bulk chemical products that can be transformed from microalgal biomass are glycerols, and polysaccharides, to Poly-β-Hydroxyalkanoate (PHA) bioplastics.

In feed industries, microalgal biomass is pivotal in producing feed, livestock feed, and poultry feed. Also, the cultivation water can be utilized as a biofertilizer in agricultural activities. Therefore, the cultivation and biorefinery system of microalgae is categorized as a green and zero-pollutant industry (Al-Jabri *et al.* 2021).

However, the challenges that must be solved in integrating microalgal biomass production using wastewater are optimizing the organic waste bioremediation, optimizing the growth of microalgal biomass, minimizing production costs, and optimizing the harvesting process (Al-Jabri *et al.* 2021).

Moreover, phycoremediation of organic wastewater using microalgae can be hampered by inconsistencies or imbalances in the organic content of the waste that can affect the growth rate of the microalgal strain. Microalgae cultivation using organic waste is also susceptible to contamination, such as antagonistic bacteria and fungi. Open pond systems are also vulnerable to the presence of predators that will compete with the microalgal strains and to environmental changes. The differences in pH, temperatures, and light intensity affect microalgal growth. Meanwhile, the use of a photobioreactor in cultivating microalgae on a large scale is considered ineffective due to the high production costs (Ahmad *et al.* 2021).

Although microalgae-based biorefinery faces various challenges, it remains promising considering the multiple benefits, such as environmental services. Microalgae are the largest CO_2 absorbents, much higher than C3 and C4 plants. The high rate of CO_2 fixation into various primary and secondary metabolites, such as carbohydrates, lipid, proteins, and pigments, in microalgae is another advantage (Mondal *et al.* 2017). Microalgae fixes around 50 gigatons of CO_2 every year (Ashour *et al.* 2024; Sommer *et al.* 2002). Microalgae culture with a volume of 1,000 ha can absorb 2,100,000 tonnes of CO_2/year (Ashour *et al.* 2024; Kadam 1997). Therefore, microalgae are essential in reducing greenhouse gases, mitigating climate change, and remediating wastewater (Gonepalli & Oroszi 2024).

Moreover, microalgae cultivation can be integrated with industrial CO_2 as they need CO_2 in their photosynthesis process (Santoso *et al.* 2023). For example, the microalgae biorefinery in Caltagirone, Italy, uses a vertically-stacked horizontal photobioreactor with a volume of

40.4 m^3 capable of producing 1,200 kg of dried biomass per year. With this large volume, cultured microalgae (*Chlorella vulgaris*) can absorb carbon dioxide of 153 kg CO_2, eq/kg DW (Gurreri *et al.* 2024).

Another environmental service is phycoremediation, wastewater remediation using microalgae. The use of wastewater as alternative nutrients for microalgae reduces production costs due to the procurement of expensive media. From an environmental perspective, remediation carried out by microalgae on wastewater is an effective, environmentally friendly strategy for overcoming the accumulation of wastewater in the environment (Velásquez-Orta *et al.* 2024). For example, microalgae-based industries that utilize Palm Oil Mill Effluent (POME) are predicted to have a sustainability index covering environmental, social, economic, and technological values of 67.30%, 82.17%, 70.99%, and 73.67%, respectively, with an average sustainability index of 73.53% (Santoso *et al.* 2023). Microalgae can be used to remediate palm oil mill effluent (POME) to rid the environment of this waste pollution. The POME remediation process may involve several microalgae strains that are capable of producing relatively high lipid, such as *Botryococcus braunii*, *Nannochloropsis* sp., and *Chlamydomonas* sp. The resulting lipid can be utilized as a source of biofuel production (Resdi *et al.* 2016).

Microalgae are also known as potential absorbents for various heavy metals found in wastewater. Large-scale microalgae cultivation also allows the absorption of heavy metals in water bodies/reservoirs in higher volumes. They possess a biosorption mechanism including physical adsorption, ion exchange, complexation, and precipitation. The absorbed heavy metals can be processed through reduction, biotransformation, oxidation, and bioprecipitation. The use of microalgae as a biosorbent is a safer and more economical method compared to physical or chemical methods (Rinanti *et al.* 2021).

On the other hand, there are some harvesting techniques to optimize the process of harvesting microalgae biomass, centrifugation, filtration, flocculation, and floatation (Ahmad *et al.* 2021). Centrifugation and membrane microfiltration are more optimum than harvesting biomass by coagulation. Biomass harvesting of *Chlorella* sp. by centrifugation and microfiltration membranes yielded a biomass of 0.1174 ± 0.0308 g and 0.1145 ± 0.0268 g. Both harvesting methods are known to have the same biomass optimization. The biomass produced by the coagulation method is lower than the two previous methods. In addition, the total lipid produced from the two previous methods is almost the same. Of the two methods, the harvesting process in membrane microfiltration method is considered more promising. This method requires lower production costs with relatively high lipid contents. Moreover, the method can also be used in harvesting low biomass quantities (Ahmad *et al.* 2014).

In the coagulation method, the low biomass and lipid productivity happen due to the coagulation process entrapping the cells caused by the differences in charge between microalgae cells and coagulants. However, when viewed from the percentage of water removal, the centrifugation method removed water by 100% compared to the percentage of water removal in the membrane microfiltration method which is only 90%, while the water removal in the coagulation method is 95% (Ahmad *et al.* 2014). On the other hand, the harvesting method using cell immobilization using a matrix is also considered an effective harvesting method with a high percentage of lipid production. Cell immobilization increases lipid yield and fatty acid composition in the microalgae (Rushan *et al.* 2019).

Another method is floatation, a method of harvesting microalgal biomass by utilizing gas bubbles to separate the microalgae biomass from particles or other components. This process is also known as the reverse sedimentation process. This method is usually combined with the coagulation method early in the harvesting process (Barros *et al.* 2015; Kucmanová & Gerulová 2019). The advantages of the floatation method are that the method can be applied on a large scale, can be done in a short time, and requires only a small space. However, the floatation method usually requires a chemical coagulant and is not suitable for marine microalgae strains (Barros *et al.* 2015; Singh & Patidar 2018; Kucmanová & Gerulová 2019).

Centrifugation is a quick harvesting method. However, it requires relatively high production costs and high energy consumption. Also, the speed of centrifugation causes cell damage and reduces valuable cell compounds (Cheirsilp *et al.* 2019). Meanwhile, this method is still effective, fast, high harvesting efficiency, and can be carried out on almost all types of microalgae, and is precise on a laboratory scale or small scale (Abdelaziz *et al.* 2013; Barros *et al.* 2015; Singh & Patidar 2018).

Furthermore, flocculation is a method of harvesting microalgal biomass that utilizes the charge difference between the microalgal cell surface and the added flocculant. The flocculants usually used are organic or inorganic (Abdelaziz *et al.* 2013). Meanwhile, the harvesting method with sedimentation is considered unfavorable. This is because the sedimentation capacity of microalgal cells is relatively low, produces lower biomass concentrations, and requires a high volume of microalgal biomass (Branyikova *et al.* 2018).

Meanwhile, filtration is a harvesting method that passes the microalgae culture through a filter membrane to accumulate the biomass on the membrane (Barros *et al.* 2015; Kucmanová & Gerulová 2019). This highly effective method can be used in almost all microalgal strains. However, fouling can be a challenge as it increases production costs, membrane replacement, and pumping costs. Therefore, the membrane must be cleaned regularly (Barros *et al.* 2015; Singh & Patidar 2018; Kucmanová & Gerulová 2019). Several species of microalgae can be harvested by using a filtration membrane, such as *C.pyrenoidosa*, *Nannochloropsis* sp., *Chlorella zofingiensis*, and *Dunaliela salina* (Castro-Muñoz & García-Depraect 2021).

The transition from laboratory-scale microalgae cultivation to mass-scale for fulfilling the demand has various challenges. Scaling up to an industrial scale requires a complex system and high costs. Generally, some critical factors in scaling up a microalgae-based biorefinery are the desired goal or end product, the type of species cultivated, culture (open or closed), water source, nutrient source, light, temperature, mixing system, process monitoring, contamination control, harvesting strategy and technique, to environmental impact analysis. In a mass-scale cultivation, the culture must be adjusted to the available space, cost, external factors, carrying capacity, risk of contamination, and supporting tools. Generally, open ponds have cheaper operating costs than closed bioreactors, but the risk of contamination is much higher. Industrial-scale microalgae cultivation also requires large amounts of water (Novovesk *et al.* 2023)

The production cost of a microalgae-based biorefinery using a closed system is still relatively high. The cost required to produce 1 kg of dried biomass using tubular photobioreactors and bubble columns using artificial lights is around EUR 290 - 329 and EUR 587 - 573, respectively (Oostlander *et al.* 2020). One example is a biorefinery based on *Nannochloropsis* sp. as an aquaculture feed developed in Central Germany using PBR for 30 years. The net present value (NPV) of the biorefinery reached EUR 4.5 million after 30 years, or equal to a return on investment (ROI) of 1.87%. The production costs are dominated by PBR procurement, maintenance, and labor costs. However, with the increasing demand and selling price of aquaculture feed, it has been reported that microalgae reduces production costs by 44% to 60%. The operating cost of the microalgae-based biorefinery is dominated by staff costs (40.2%), mechanical equipment (16.3%), electrical equipment (11.9%), administration (9.5%), CO_2 (8.1%), and other costs. Meanwhile, investment costs are dominated by the glass for PBR (28%), dryer (23%), buildings (20%), mixer (6%), and centrifuge (3%) (Schade & Meier 2021). On the other hand, the production cost for an open pond integrated with wastewater is estimated to reach AUD 1.7 per kg dried weight as the use of wastewater reduces production costs by 16% (Alavianghavanini *et al.* 2024).

Therefore, to overcome some challenges in scaling up microalgae culture, it is necessary to optimize biomass production through culture engineering and genetic engineering. It is also necessary to optimize the process, including the use of liquid waste containing lots of nutrients, the use of industrial CO_2, adjusting the culture system (open/closed), optimizing harvesting methods, and increasing energy efficiency. To realize this, sustainable research and development are needed in ensuring the long-term prosperity of this biorefinery. The selling price of microalgae biomass is indeed relatively high and prospective. For example, the market value of *Arthrospira* (*Spirulina*) is predicted to reach USD 2 billion in 2026, with Europe as the main market (70%), followed by Asia-Pacific (14%), North America (10%), Middle East and Africa (5%), and Latin America (1%) (Show 2022).

CONCLUSION

Due to their rapid growth rate, high metabolite content, and ability to grow in a variety of contaminants, microalgae are considered as the prospected abundant green gem that can be utilized in many biorefinery activities. Microalgal metabolites including lipid, proteins, and carbohydrates are prosperous materials in the bioindustry. Those metabolites can be converted into many kinds of products in the sectors of energy, pharmaceuticals, feed, and biofertilizers to biojet fuel. Many technologies, like hydroprocessing, are affordable and recommended choices to produce biojet fuel using microalgal biomass on a large scale. Bioethanol is one of the main final products in the conversion of microalgal carbohydrates by fermenting the metabolite by bacteria. In agroindustry, microalgae's nutritional compounds can be utilized as a biofertilizer to enrich the soil to support plant growth. To minimize the production cost, the cultivation system can be integrated with phycoremediation by using some wastewater as microalgal cell media. The huge potency of microalgae urges the optimization of the cultivation and transformation. Key strategies include utilizing waste effectively, choosing suitable cultivation methods, controlling contamination through biological agents, and making use of sunlight and industrial CO_2. It is also crucial to improve microalgal strains for optimizing outcomes.

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