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Microalgae as A Potential Feedstock for Biodiesel Production in The Philippines: A Review

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Abstract

Microalgae are gaining interest as a potential renewable energy resource for biodiesel production due to their high growth rate, photosynthetic efficiency, biomass productivity, and CO_2 fixation rate. In the Philippines, future biodiesel consumption is projected to rise due to the anticipated increase in biodiesel blending mandated by the Philippine Biofuels Law. Massive research efforts were conducted in search for alternative feedstocks in the Philippines. This led to several studies using third-generation feedstock (such as microalgae) for biodiesel production. Previous studies have shown that microalgae can be cultivated in engineered systems and can produce biodiesel with superior quality fuel characteristics, which makes them a promising source of biomass feedstock for biodiesel production. Microalgae is also a sustainable option for feedstock because they occupy less land area compared to other feedstocks and they are not being consumed as food. Microalgal strains documented in the Philippines, such as *Chlorella* sp. and *Chlorolobion* sp., exhibit exceptional lipid productivities and biodiesel properties achieved through experimentation on upstream processes like nutrient limitation and lipid induction. However, only a limited number of studies on microalgae particularly in biodiesel production have been conducted in the Philippines. Thus, the current work highlighted the recent advancements and research on microalgal cultivation for biodiesel production within the Philippine context. Additionally, it provided an in-depth discussion of various factors that affect microalgal growth and lipid induction, microalgal cultivation techniques, and methods for cell disruption.

Keywords: biodiesel, cultivation, lipid induction, microalgae, Philippines

Introduction

Microalgae are a remarkably diversified but specialized class of organisms that have evolved to live in different ecological settings. The enormous diversity and occasionally odd arrangement of cellular lipids, and the capacity of algae to regulate lipid metabolism effectively during stressful conditions, are major factors in their ability to thrive in diverse environmental situations. The lipids may comprise neutral lipids, wax esters, hydrocarbons, and polar lipids, as well as carotenoids, quinones, terpenes, which are prenyl derivatives, and chlorophylls, which are phytylated pyrrole derivatives (Ambika, 2023; Kumari et al., 2022). Microalgae generate fatty acids that can be converted to membrane lipids (glycerol-based), which make up around 5–20% of their dried biomass under favorable culture conditions. Fatty acids are made up of fatty acid derivatives and species with long (C16-C18), medium (C10-C14), and very-long chains ($\geq$C20). Glycosylglycerides are abundant in chloroplasts and make up most of the membrane lipids, along with sizable quantities of phosphoglycerides, which are mostly found in the cytoplasm and several endoplasmic membranes. The primary components of membrane glycerolipids are a variety of polyunsaturated fatty acids that are formed from the “precursor” fatty acids palmitic (16:0) and oleic acids by aerobic desaturation and chain elongation (Hu et al., 2008).

Several species of microalgae change their pathways in lipid biosynthesis to form and accumulate neutral lipids (20–50 percent dry cell weight (DCW), primarily triacylglycerol (TAG), under unfavorable environmental conditions for growth. TAGs are primarily used as energy storage, in contrast to the glycerolipids that are found in membranes, which perform a structural function. However, some data suggest that the TAG biosynthetic pathway in algae may play a more important role in the organism's response to stress. TAGs are produced and then stored in tightly packed lipid bodies in the cytoplasm of the algal cell. However, in some green algae, like *Dunaliella bardawil*, lipid bodies can also form and accumulate in the inter-thylakoid region of the chloroplast. Plastoglobuli are the name for the chloroplastic lipid bodies in the later scenario (Hu et al., 2008). In a process known as transesterification or alcoholysis, these triglycerides can be combined with an alcohol to produce fatty acid methyl or ethyl esters that can be used to make biodiesel.

Another neutral lipid class that can be found in algae is hydrocarbons, which typically make up 5% of the total DCW. Only the colonial green alga *Botryococcus braunii* has been demonstrated to produce significant amounts (up to 80% of DCW) of hydrocarbons (C23-C40) (Pierre et al., 2019), like those observed in petroleum, under unfavorable environmental conditions. As a result, it has been investigated over time as a feedstock for biofuels (Hu et al., 2008). Table 1 lists several microalgae species with their respective oil content and lipid productivity.

Microalgae are used as a cell factory to generate lipids for biofuel because several species are reported to have fast growth rates and produce significant concentrations of TAGs. Algae have the potential to be advantageous as feedstocks for biodiesel because these organisms can: (i) produce and store large amounts of lipids (20-50% of the DCW); (ii) fast growth rate; (iii) grow in brackish and seawater for which competing demand is less; (iv) uses marginal and underutilized lands such as arid, semi-arid, and desert lands for mass cultivation; (v) utilizes growth nutrients (like nitrogen and phosphorus) from wastewater sources that can be used for bioremediation; (vi) capture and utilization of carbon dioxide from fossil fuel-derived power plants lowering emissions of several greenhouse gases; (vii) create by-products with value like biopolymers, animal feed, and proteins; (viii) grow in photobioreactors with higher annual biomass productivity than plants. Some microalgae species that are used in biofuel production, particularly biodiesel, belong to the genus *Acutodesmus, Chlorella, Desmodesmus, Cymbella, Scenedesmus,* and *Chlorococcum* (Figure 1).

The Philippines is expected to increase its biodiesel blending as mandated by the Philippine Biofuels Law (Mojica-Sevilla, 2022). Initially, coconut oil was used as a feedstock for biodiesel production, but due to concerns related to food security and pricing, other feedstocks were studied. Second-generation feedstocks, like *Jatropha curcas*, are more economical than first-generation feedstocks but require a large area of land for plantation, which may compromise the land area intended for food plants. Third-generation feedstocks, like oleaginous microorganisms, specifically microalgae, are a more sustainable option and occupy less land area compared to other feedstocks. Some microalgae such as *Chlorella vulgaris* and *Scenedesmus* sp. can be grown in engineered systems using sewage as the culture medium and coal-fired power stations as the carbon supply. However, since some wild-type species of microalgae lack certain characteristics necessary for large-scale production, it led to the fourth-generation feedstocks, which are genetically modified microorganisms.

Table 1. Oil content and lipid productivity of some microalgae

Microalga	Oil Content (% Dry Weight)	Lipid Productivity (mg/L/day)	Reference
Botryococcus braunii	33.6	5.5	Lee et al. (2010)
Chlorella pyrenoidosa	11.76	34.8	Rodolfi et al. (2009)
Cylindrotheca fusiformis	17.0	4.78	D'Ippolito et al. (2015)
Dunaliella salina	26.65	9.71	D'Ippolito et al. (2015)
Chlamydomonas sp.	20.92	2.98	D'Ippolito et al. (2015)
Dunaliella tertiolecta	26.65	5.0	D'Ippolito et al. (2015)
Nannochlorosis salina	21.98	7.27	D'Ippolito et al. (2015)
Thalassiosira weissflogii	38.84	8.0	D'Ippolito et al. (2015)
Euglena gracilis	25.0	32.4	Rodolfi et al. (2009)
Chlorella sp.	28.77	151.14	Arguelles & Martinez-Goss (2021)
Phaeodactylum tricornutum	2.09	9.32	D'Ippolito et al. (2015)
Chlorococcum infusionum	21.26	22.08	Arguelles (2021)
Chlorolobion sp.	31.61	227.84	Arguelles & Martinez-Goss (2021)

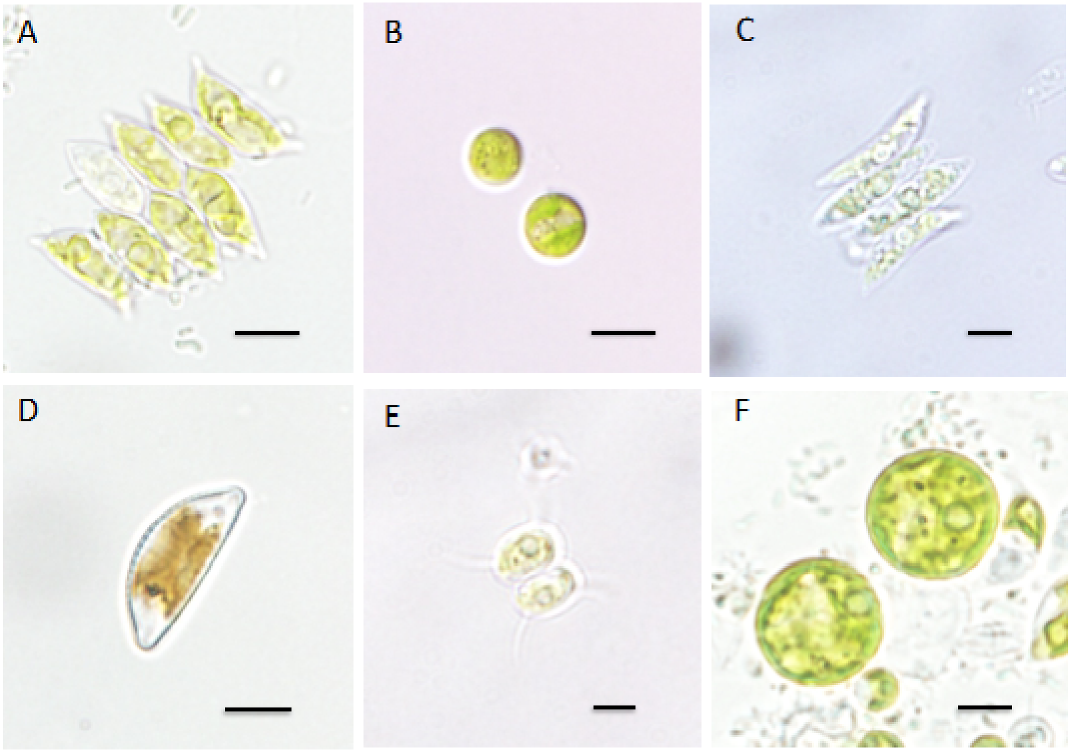

Figure 1. Photomicrographs of some commonly used strains of microalgae for biodiesel production: (A) *Acutodesmus dimorphus* (Turpin) P.M. Tsarenko; (B) *C. vulgaris* Beyerinck [Beijerinck]; (C) *Desmodesmus* sp.; (D) *Cymbella* sp.; (E) *Scenedesmus* sp.; (F) *C. infusionum* (Schrank) Meneghini, All scale bars = 10 ìm.

Through genetic manipulation, the characteristics of microalgae are further enhanced particularly lipid productivity. Finally, due to the need for more biodiesel feedstocks, waste materials were considered, such as used cooking oil and bio-oil from agricultural wastes, which were studied by some institutions and academia in the Philippines.

Despite the numerous papers published on microalgal cultivation, with a focus on biodiesel production, only a limited number of studies have been conducted in the Philippines. Thus, the current work highlighted the recent advancements and research on microalgal cultivation for biodiesel production within the Philippine context. Additionally, it provided an in-depth discussion of various factors that affect microalgal growth and lipid induction, microalgal cultivation techniques, and methods for cell disruption.

Factors Affecting the Growth and Lipid Production of Microalgae

Growth rate, ambient conditions, and life cycle are only a few examples of variables that might impact the biochemical makeup of microalgae. The key factors influencing chemical composition and microalgal growth include light, temperature, the amount of carbon dioxide that is accessible, nutrients, and pH.

Light Intensity

Microalgae, being mostly photoautotrophic, require light for their growth and proliferation. Season and latitude, cloud cover, plant canopy, the vertical position in the photic zone, and water turbidity affect how much light is available for them. Microalgae produced under varying lighting conditions show striking variations in their overall chemical makeup, pigment concentration, and photosynthetic activity. The synthesis of polar lipids is often induced by low light intensity, especially the membrane polar lipids connected to the chloroplast, while high light intensity decreases total polar lipid content while simultaneously increasing the concentration of storage lipids, primarily TAGs (Chin et al., 2023). The intensity of the light can also affect the level of fatty acid saturation. For instance, in *Nannochloropsis* sp., the primary polyunsaturated fatty acid (PUFA) percentage (about 35% of the total fatty acids) held steady despite limited light conditions (Hu et al., 2008). Nonetheless, under light-saturated conditions, it reduced around three times, concurrent with a rise in the amount of saturated and monounsaturated fatty acids. With a few exceptions, it seems from the algal species/strains analyzed that low light favors the production of PUFAs, which are then integrated into membrane structures. However, strong light modifies fatty acid synthesis to increase the production of saturated and mono-unsaturated fatty acids, which are primarily responsible for neutral lipids (Hu et al., 2008).

A study was done on examining the growth of four microalgal varieties (*C. vulgaris, Desmodesmus* sp., *Ettlia pseudoalveolaris, Scenedesmus obliquus*) obtained from the Northern hemisphere, and their potential for generating fatty acids. The study involved cultivating the microalgae under three light intensities of 50, 150, and 300 mE m^{-2} s^{-1}, and determining their biomass and fatty acid output. Results showed that the different strains of microalgae tend to accumulate higher lipid concentrations when cultivated under high light intensities. *Desmodesmus* sp. recorded the highest fatty acid content (6.2%), closely trailed by *S. obliquus* (5.8%), when cultivated under a light intensity of 300 mE m^{-2} s^{-1}. However, *C. vulgaris* and *E. pseudoalveolaris* displayed a decrease in lipid content when cultivated under 300 mE m^{-2} s^{-1} light, despite experiencing an upsurge in biomass, unlike the other microalgae species examined in the experiment (Nzayisenga et al., 2020). Thus, it is possible that distinct microalgal species have unique methods of adaptation in high light intensity which could lead to the accumulation of either higher or lower lipid concentrations (Nzayisenga et al., 2020).

Moreover, some studies investigated the effects of light sources and light wavelength instead of light intensity. A study investigated the impact of light quality on the growth and accumulation of high-value substances in *Chlorella* sp. HQ cultivated in coastal saline-alkali leachate. Results showed that the algal density under blue light was significantly higher than other light groups. The highest lipid content and TAGs content were obtained under red light and blue-white mixed light, respectively (Liu et al., 2021). In the Philippines, Carpio et al. (2015) determined the effects of light sources such as Compact Fluorescent Lamp (CFL) and Light Emitting Diode (LED) and light wavelength (white, red, and blue) on the growth and lipid content of *C. vulgaris* Beijerinck. The study reported that the dry biomass concentration and lipid content of *C. vulgaris* was the highest under white light, followed by red and blue light, for both CFL and LED light sources (Carpio et al., 2015).

Temperature

Temperature is one of the important factors influencing all forms of life. All microorganisms have a characteristic optimal growth temperature at which they exhibit the highest growth and reproduction rates (Arguelles, 2021). While most of algae are mesophilic, some are thermophilic while some others are cryophilic. The impact of temperature was evaluated on the fatty acid composition of four tropical microalgal species such as two cryptomonads (*Rhodomonas* sp. and *Cryptomonas* sp.), diatom *Chaetoceros* sp., and prymnesiophyte (unidentified strain - NT19) and in an industrially important microalgal strain, *Isochrysis* sp. (used as the control). The results showed that when cells were cultivated at 25°C, the diatom *Chaetoceros* gave the highest concentration of lipid (16.8% dry weight). But NT19, *Isochrysis* sp., *Cryptomonas* sp., and *Rhodomonas* sp. when cultivated at temperatures between 27 and 30°C, had significantly larger levels of lipids at 15.5, 12.7, 21.4, and 21.7 percent dry weight, respectively (Renaud et al., 2002).

A study done by Can et al. (2021) examined the influence of varying temperatures on both biomass and lipid content of *C. vulgaris*. The findings revealed that as the temperature increased, there was an increase in algal biomass, although the effect on lipid production was not as noticeable as on algal growth. Nonetheless, in all groups, the highest lipid production was recorded at 30°C, compared to other temperatures (Can et al., 2021). In addition, Parichehreh et al. (2021) investigated the effects of several physicochemical factors on the growth and lipid accumulation of *Chlorella* sp. and temperature is one of the factors considered. Results showed that along with light intensity, temperature substantially impacted both biomass production and lipid biosynthesis in contrast to the chemical parameters. Similar to the previous study, as per the experimental findings, increasing the temperature of the algal culture from 20° to 32°C resulted in a moderate increment in the microalgae's biomass density but had the most significant beneficial impact on the biomass productivity (Parichehreh et al., 2021).

Carbon Dioxide and Other Carbon Sources

Microalgae utilize carbon dioxide (CO_2) as a source of carbon for the synthesis of lipids and other chemical molecules. Elevated concentrations of CO_2 in microalgal cultivation can enhance the growth of the organism since it is being utilized in the process of photosynthesis. Microalgae are highly efficient photosynthetic organisms that can consume large amounts of CO_2 and grow rapidly. They can double their biomass every 4-6 hours and are capable of thriving in diverse environmental conditions. Microalgae have high oil content, up to 80% in some species, which make them suitable for converting CO_2 into fuels (Alami et al., 2021a). A study was conducted on two species of microalgae, *Isochrysis galbana* and *Nannochloropsis*, which have high biomass and oil content ranging from 7.0% to 40.0% and 22.7% to 52.0%, respectively (Alami et al., 2021a). Results of the study showed that *I. galbana* CCMM5001 and *Nannochloropsis* sp.

CCMM7001 have the potential to serve as a renewable feedstock for biodiesel production and to decrease CO_2 emissions. The optimal condition for culturing these two marine microalgae, which are rich in oil, is a 10% concentration of CO_2. Under this condition, the two strains of microalgae can accumulate up to 490.00±4.60 (fluorescence value of a single cell is 10–6) neutral lipids in a single cell, with triglycerides being the most abundant component (Wang et al., 2018).

However, excess CO_2 in the culture medium is transformed into carbonic acid. This compound causes acidification of the culture medium causing a drastic effect on the growth and metabolism of the microalgae (Alishah Aratboni et al., 2019). A study conducted by Ying et al. (2014) showed that changes in pH of the culture medium caused by varying concentrations of carbon dioxide damaged some of the important enzymes that are involved in the process of photosynthesis. Thus, it is crucial to always consider the optimum pH levels among various industrially important microalgal species during mass cultivation to maximize biomass lipid production in the algal cells.

Other studies used other carbon sources instead of CO_2 only. In a study conducted on *Nannochloropsis* sp., carbonates derived from flue gas were supplemented on the cultivation of biomass, and it was observed that the use of soluble carbonates as a carbon source for growth was found to be slightly more effective than using CO_2 gas alone. The 20% carbonates solution yielded the maximum dry biomass of 0.55 g, while the maximum dry biomass yield of 0.44 g was achieved using 15% dissolved CO_2 gas (Alami et al., 2021b). Moreover, a recent study showed that *Chlorella* sp. PG96 exhibited a preference for bicarbonate over CO_2 as a source for lipid biosynthesis. The findings of the study revealed that adding 4,000 mg/L of sodium bicarbonate to the culture medium increased lipid accumulation in the algal cells. A higher concentration of bicarbonate can increase the CO_2 fixation rate by promoting the synthesis of carbohydrates through the Calvin cycle, which in turn enhances lipid production through the Kennedy pathway (Parichehreh et al., 2021).

Nutritional Requirements

Like other chlorophyllous plants, algae require the following elements for growth: nitrogen (N), phosphorous (P), silicon (Si), iron (Fe), magnesium (Mg), and sulfur (S) (Barua & Munir, 2021), as well as minute amounts of zinc (Zn), manganese (Mn), cobalt (Co), and copper (Cu). Among the nutrients considered, nitrogen has the greatest impact on algae’s lipid metabolism. Previous studies documented that several algal taxa could produce high concentration of industrially important lipids (TAGs), in nitrogen-starved culture conditions (Alishah Aratboni et al., 2019). A study explored the impact of different stress factors, including nitrogen starvation, on the production of lipids in four species of unicellular green algae belonging to genus *Scenedesmus*, *Ankistrodesmus*, and *Chlorococcum*. Results showed that there was a sustained rise in the accumulation of neutral lipids (TAGs) in the cells of *Ankistrodesmus* and *Scenedesmus* microalgae for up to 40 hours of incubation, which was followed by a considerable decrease in lipids after 72 hours, under nitrogen-starved conditions. In *Chlorococcum* cultures, the maximum lipid biosynthesis (59.7%) occurred after 60 hours of incubation with nitrogen starvation, leading to a nearly threefold increase in lipid accumulation compared to the original culture (22.1%) (Saatovich et al., 2021). Similarly, efficient lipid production was observed on nitrogen-starved conditions for other species of microalgae such as *C. vulgaris*. A reduction in nitrogen concentration resulted in a decrease in biomass and an increase in lipid content. The maximum lipid amount of 20.80% dry weight (DW) was achieved from the culture grown at 30 °C in a medium with no sodium nitrate (nitrogen source) (Can et al., 2021). In the Philippines, Arguelles et al. (2019; 2018) cultivated *Chlorella* sp., *Desmodesmus* sp., and *Chlorolobion* sp. under nitrogen-limited growth conditions, and had a 20-35% increase in lipid yield and productivity.

Phosphorus is necessary for the growth of microalgae in various cellular functions, including energy transmission and nucleic acid production. Phosphorus frequently becomes one of the most significant growth-limiting variables in algal culture since the production of protein in cells requires a significant amount of this element (Saklani et al., 2023). Aside from that, phosphorus also affects the lipid production of some species of microalgae. When phosphorus is limited, *Monodus subterraneus*, *Phaeodactylum tricornutum*, *Chaetoceros* sp., *I. galbana*, and *Pavlova lutheri*, all had higher lipid contents, primarily TAG. However, *Nannochloris atomus* and *Tetraselmis* sp., have lower lipid contents. Increased phosphorus deprivation of the marine algal species studied was shown to increase the relative content of 18:1 and 16:0 as well as a decrease in the relative contents of 18:4w3, 20:5w3, and 22:6w3. (Hu et al., 2008). Moreover, the growth of certain diatom species is impacted by the quantity of phosphorus present in the culture medium. In a recent study, the appropriate level of phosphorus required for the diatom *Amphora copulata* was optimized. The study concluded that the optimal concentration of phosphate for this diatom is 6.5 mg/L, under which *A. copulata* was effective in accumulating lipids (Govindan et al., 2021). Nonetheless, some species like *C. vulgaris* did not show any significant changes in lipid production under phosphorus-deficient conditions (Parichehreh et al., 2021).

pH

The hydrogen ion concentration's negative logarithm is used to define pH. The pH of a medium influences the solubility of minerals and carbon dioxide in the culture and either directly or indirectly affects the metabolism of algae, including its ability to carry out photosynthesis (Ratomski & Hawrot-Paw, 2021). Enzymatic activities can be influenced by pH, and similarly, microalgal metabolism can be affected by pH fluctuations. Altering the external pH of the medium results in the formation of a new pH gradient between the cell and the medium, leading to a shift in equilibrium between the interior and exterior of the cell. This change has the potential to induce modification of the intracellular pH (Filali et al., 2021). Aside from that, the pH of the culture medium also influences the lipid synthesis of certain species of microalgae. A study showed that the biomass and lipid productivity of microalgae *Chlorella zofingiensis* improved when the pH of the medium was regulated. The culture with pH regulation showed significantly elevated lipid content, with lipid productivity of 37.48 mg/L-day, which was 6.7 times greater than the productivity of the culture with no pH regulation (Zhu et al., 2014). A more recent study revealed that lipid induction of microalgae can be influenced by pH. The research findings demonstrate that after a six-hour incubation at pH 4.0, both *Chlorococcum macrostigmatum* UT4 and *Scenedesmus armatus* UT39 showed a 1.3-fold increase in lipid content. The highest lipid accumulation (63.7%) was observed in *C. macrostigmatum* UT4 after 12-18 hours of incubation, but further incubation up to 24 hours resulted in a slight decrease in lipid content. When incubated in nutrient media at pH 9.0, lower lipid accumulation was observed compared to pH 4.0, particularly in *C. macrostigmatum* UT4 where lipid accumulation was two times lower during incubation from 12-18 hours (Saatovich et al., 2021).

Microalgal Lipid Induction

Microalgal growth manipulation is effective in producing target groups of lipids and free fatty acid methyl esters (FAMEs) via optimization of some of the important chemical components of the culture medium. Such manipulation triggers microalgae to develop fatty acids and important lipids that can be harnessed in several industrial applications. Previous studies showed that limitations in the concentrations of nitrogen and phosphorus (in the culture medium), high salt concentration, high light intensity, and temperature can cause oleaginous microalgae to produce 20 to 80% triacylglycerols from the harvested dried algal biomass (Arguelles, 2021; Shokravi et al., 2020).

When microalgae are exposed to stress conditions (like nutritional deficiency, and high temperature), the cell division rate always gradually decreases. However, certain algal species can maintain active fatty acid production in these circumstances if there is enough CO_2 and light for photosynthesis (Sharma et al., 2012). Microalgal cells redirect the synthesis and deposit fatty acids in the form of TAGs when microalgal growth slows down and new membrane components are not needed. TAG synthesis could act as a defense mechanism in these circumstances. Under typical growth conditions, after ATP and NADPH generated by photosynthesis are utilized for biomass production, ADP and NADP+ are recycled and become available again as acceptor molecules in photosynthesis (Sharma et al., 2012). The pool of NADP+, the main electron acceptor, can also be depleted when cell growth is hindered by a shortage of resources. Since photosynthesis is mostly regulated by the amount of light present and cannot be entirely stopped, this could put the cell in a perilous scenario and harm many cell components. Increased fatty acid production (which is then stored in TAGs) replenishes the pool of NADP+ under stress growth conditions since NADPH is used in FA biosynthesis (Sharma et al., 2012).

Microalgal Lipid Productivity and FAME Profiles

The suitable characteristics needed in algal species for optimum biodiesel production are high oil content and high lipid productivity. However, literature highlights a primary economic challenge concerning algae species, as they exhibit conflicting characteristics: some show high biomass production but low lipid content, while others have high lipid content but low biomass production (Chen et al., 2017; Ghosh et al., 2016). Lipid content and biomass production both rely on lipid productivity, making it a crucial factor of particular significance in large-scale microalgal lipid production processes. (Sibi et al., 2016). Traditional methods to enhance lipid productivity involve manipulating culture conditions, using chemical additives and applying stress (Khoo et al., 2023). In the Philippines, Arguelles and Martinez-Goss (2021) reported strains of microalgae that have highly comparable lipid productivities to other microalgal species, as presented in Table 1. These values of lipid productivity were obtained by exposing the microalgae to stress such as nitrogen-limited conditions. Usually, these stress-inducing techniques often have a negative impact on algal growth, leading to reduced lipid productivity (Shin et al., 2018), but the reported lipid productivities of these microalgae demonstrate their potential as feedstocks for biodiesel production despite being exposed to stress and without undergoing further genetic manipulation. Nonetheless, engineering approaches are gaining significant attention due to the genetic and biochemical diversity of microalgae, as evidenced in the literature (Hess et al., 2018). These approaches involve modifying the genetic and metabolic properties of microalgae, offering promising avenues to address the challenges associated with achieving higher lipid productivity in microalgal-based biodiesel production.

In addition, microalgae must possess the right kind of fatty acid methyl esters (FAMEs) composition in order to produce high-quality biodiesel. C16 and C18 FAMEs are two of the most observed fatty acids generated by microalgae and are recognized as the most common FAMEs important in biodiesel production (Sathya et al., 2012). The carbon chain length of both the saturated and unsaturated fatty acids in crude oil extracts generally affects the properties of biodiesel such as cetane number, oxidative stability, and cold-flow properties (Mussharraf et al., 2012). A suitable fatty acid composition should consist of a combination of MUFAs and short-chain SFAs to meet the biodiesel standards (Shokravi et al., 2022). The most common FAMEs used for biodiesel consist of C14–C18 fatty acids such as palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3w3) (Knothe, 2008; Andrew et al., 2022]. In addition, high amount of myristic acid (C14:0), palmitic acid (C16:0), and palmitoleic acid (C16:1) in a microalgal lipid will help in meeting the required specifications of biodiesel standard (Knothe, 2008; Andrew et al., 2022). Lipids rich in oleic acid (C18:1) were documented to have good fuel balance, including oxidative stability, ignition quality, viscosity, combustion heat, cold filter plugging point, and lubricity (Pugliese et al., 2020; Andrew et al., 2022). Thus, the list of FAMEs listed above are referred to as desirable fatty acids and are widely utilized as reference fatty acids used in the assessment of lipid quality for biodiesel production. In general, biodiesel production prefers crude oil extracts that are rich in saturated fatty acids (SAFAs) and monounsaturated fatty acids (MUFAs) since these fatty acids are crucial in increasing the superior oxidative stability and energy yield of biodiesel. However, oils containing MUFAs are prone to solidification at low temperatures, while oils rich in PUFAs have very good cold-flow properties but are very vulnerable to oxidation. Such characteristics of these unsaturated fatty acids cause adverse effects on fuel combustion and conservation (Mussharraf et al., 2012; Arguelles et al., 2018). PUFAs are long-chain unsaturated fatty acids that are commonly found in plants, fungi, animals, and microalgae. These are fatty acids that are commercially important since they have positive effects on the human body. The presence of a high concentration of polyunsaturated FAME in the microalgal lipid profile causes a negative effect on the oxidative stability of the biodiesel since these fatty acids are prone to free radical attack (Mandotra et al., 2014). On the other hand, long-chain saturated and monounsaturated FAMEs are important for biodiesel production since they improve the oxidative stability of the fuel with no effect on the cold flow properties of biodiesel (Mandotra et al., 2014).

Microalgal Cultivation

Microalgae have the capacity for rapid growth. They are a suitable choice for usage as a raw material for industry because of their high photosynthetic efficiency and ability to store high concentrations of bioproducts inside of their cells (Randrianarison & Ashraf, 2017). Microalgae will not be competing for resources because their growth requires less fertile soil, freshwater, herbicides, and pesticides than other feedstocks do. (Khan et al., 2018). In addition, wastewater can be utilized to grow microalgae, which aid in the bioremediation of wastewater, including residential sewage water and palm oil milling effluents (Selmani et al., 2013). Moreover, growing microalgae can reduce atmospheric carbon dioxide through photosynthesis, making a significant contribution to the fight against the greenhouse effect and global warming (Tan et al., 2020). Microalgae cultivation still faces difficulties due to the low production of biomass when grown in a liquid medium. Their development rate is accelerated using technologies like open ponds and photobioreactors to make up for these drawbacks (Tan et al., 2020).

Open Pond

One of the earliest and most basic methods of mass microalgae culture is open pond cultivation. Open ponds are frequently used in the industry because of their inexpensive operation and maintenance costs, as well as their much lower cost during construction. However, despite the wide cultivation area, microalgae grown in natural water have a significantly lower cell concentration, necessitating the use of a very effective harvesting technique. Another problem with open pond cultivation systems is the potential for bacterial and protozoan contamination, which renders the products ineffective and toxic (Tan et al., 2020).

The raceway pond is one of the most widely used open pond designs for the biomass production of microalgae. To ensure that nutrients are distributed equally and to prevent sedimentation of the microalgae biomass, it is made up of a series of closed-loop channels that are about 30 cm deep and paddlewheels (Tan et al., 2020). Raceway ponds have been regarded as one of the best open pond cultivation designs currently accessible due to their energy efficiency. In the Philippines, two pilot-scale open raceway pond systems were constructed to observe the biomass productivity of *C. vulgaris* Beijerinck in an open setting. The first was a raceway with an oval shape and a central island; the second was a multi-stage raceway pond (MSRP) with numerous portions divided by baffles. The resulting biomass productivity is on par with or even better than that of other commercial open-pond farming methods utilizing *C. vulgaris* (Matanguihan et al., 2020).

Photobioreactor

A photobioreactor is a type of bioreactor used to cultivate phototrophs like microalgae in a closed system that prevents direct material exchange between the culture and its surroundings. A photobioreactor can circumvent several issues that open pond culture design frequently encounters (Tan et al., 2020). They are more space-efficient due to their smaller size, and they provide a closed and tightly controlled environment for the culture's growth, resulting in a contamination-free, single-strain microalgae culture (Posten, 2009). In the Philippines, *C. vulgaris* Beijerinck was grown in a 21-L externally lighted vertical-column draft-tube airlift photobioreactor and the study reported that the photobioreactor is appropriate for cultivation of *C. vulgaris* on a large scale and can be kept running continuously for at least 30 days with strict flowrate monitoring (Santiago et al., 2013).

Cell Disruption Methods

Microalgae have rigid cell walls that affect the contact between the extractant and lipids. On average, the tensile strength of their cell walls is approximately 9.5 MPa, which is three times stronger than the cell walls of plants (Bharte & Desai, 2021). Some microalgae species have tough cell walls that serve as a deterrent to the utilization of microalgae for industrial purposes (Alhattab et al., 2019; Lari et al., 2019). Algaenan, also known as sporopollenin, is a hydrocarbonaceous, nonhydrolyzable biopolymer that is found in the tough outer layer of various microalgae species (Alhattab et al., 2019) The inclusion of algaenan in the microalgal cell wall lessens the microalga's vulnerability to disruption processes such mechanical, enzymatic, and chemical hydrolysis (Burczyk et al., 2014). Some examples of the microalgal species that contain algaenan in their cell walls are the Chlorophyceae and Trebouxiophyceae species of the Chlorophyta (Ye et al., 2023).

Aside from the algaenan, the presence of cellulose also influences the rigidity of microalgal cell walls since its purpose is to support the structure of the cell. *C. zofingiensis* is an example of a microalga with cellulose in cell walls that comprises 70% of the total dry cell wall weight (Alhattab et al., 2019) Moreover, cell walls of microalgae like *Tetraselmis suecica* and *Tetraselmis striata*, contain complex sugars (Alhattab et al., 2019) which interfere to the extraction of intracellular products. Hence, selecting an appropriate cell disruption method is necessary to ensure efficient lipid extraction. There are various cell disruption methods used in microalgae, and they can be categorized as mechanical or non-mechanical.

Mechanical Methods

Mechanical methods provide an effective strategy for breaking the cell walls of microalgae because they are less dependent on the species of microalgae being processed and are also less prone to contaminate the recovered lipid product. However, they are energy-intensive, and some generate heat that damages the end products (Kumar et al., 2015). Some examples of mechanical methods include high-pressure press, homogenizers, bead mills, and ultrasonic extraction.

High Pressure Press. Pressing entails applying intense pressure on the algal biomass in order to rupture the cell walls and release the contents. Algae are mechanically compressed while maintaining their intracellular contents, which can subsequently be pushed out. Different press configurations, such as screw, expeller, or piston, are made to accommodate the various physical characteristics of various strains of algae (Show et al., 2015). Nonetheless, a study utilized two pieces of glass in compressing microalgae treated with biodiesel and methanol at a force of 360 N for 20 minutes. The two pieces of glass were turned 360 degrees in both clockwise and counterclockwise directions twice each minute while being pressed. After that, all components were reassembled for methanol washing. Microalgal lipids from the subsequent methanol phase were recovered for transesterification (Huang et al., 2017).

Homogenizers. Homogenizers based on high-pressure liquid shear cell disruption techniques have been effective in large-scale applications (Show et al., 2015). Due to its scalability, ability to run continuously,

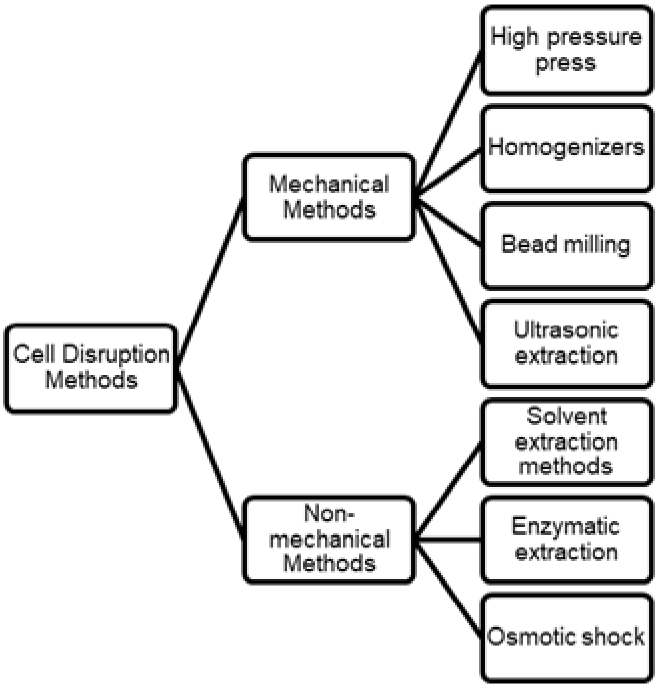

Figure 2. Some cell disruption methods for microalgae.

and capacity to treat wet biomass, high-pressure homogenization has been frequently used in microalgae cell disruption operations (Ekpeni et al., 2015). Although most microalgae have shown resistance to mechanical cell disruption, it has been demonstrated that processing wet microalgae concentrate with up to 25% (w/w) solids as a predecessor to lipid recovery using high-pressure homogenization is highly effective and does not consume a significant amount of energy (Lee et al., 2012). A study evaluated the rupturability of three species of microalgae such as *Chlorella* sp., *T. suecica*, and *Nannochloropsis* sp. using high-pressure homogenization and compared quantitatively to the yeast *Saccharomyces cerevisiae*. *Nannochloropsis* sp. was the most significantly challenging to disrupt, while *T. suecica* was the most prone to rupture followed by *Chlorella* sp. and *S. cerevisiae*. These findings enable the microalgae under investigation to be evaluated in terms of their sensitivity to rupture, adding a further criterion to strain selection and process design.

Bead milling. Bead mills have a vertical or horizontal cylindrical compartment with a central shaft that is motorized and supports a group of discs or another agitating component. A significant number of tiny, fast-moving steel or glass beads are placed throughout the compartment to give the necessary degree of charge and high-velocity spinning cell-breaking action (Show et al., 2015). In a study, lipids, carbohydrates, and proteins from *C. vulgaris* were successfully fractionated using a combination of bead milling and enzymatic hydrolysis. A horizontal 75 mL bead mill filled to 65% with 0.4 mm Y_2O_3 stabilized ZrO_2 beads was utilized to break apart the microalgal wall. A recovery yield of 75%, 31%, and 40%, respectively, for lipids, carbohydrates, and proteins was obtained using only bead milling. However, the recovery yield significantly increased when paired with enzymatic hydrolysis in cells (Alavijeh et al., 2020). Hence, using bead milling solely for cell disruption of microalgal cells is less efficient than combining it with other cell disruption methods.

Ultrasonic extraction. Utilizing a reactor to generate ultrasonic waves that cause cavitation bubbles in a solvent substance is the principle behind ultrasonic-assisted extraction. When these bubbles burst adjacent to algal cell walls, shock waves, and liquid jets are created, breaking the cell wall and releasing the contents of the broken cell into the solvent. A study compared the effectiveness of various lipid extraction methods on two species of microalgae, such as *Chlorella* sp. and *Spirulina* sp. The best method to extract lipids from both types of microalgae was found to be ultrasonication, which produced oil yields of 6.6% for *Spirulina* sp. whereas *Chlorella* sp. has 9.4%. Additionally, both types of microalgae successfully underwent transesterification of ultrasonic-assisted extraction samples, demonstrating their viability as a renewable energy source (Rizwanul Fattah et al., 2020).

Non-Mechanical Methods

The next sections address non-mechanical cell disruption techniques for extracting algal oil, including the hexane solvent method, supercritical fluid extraction, enzymatic extraction, and osmotic shock.

Solvent Extraction Methods. The extraction of oil and its conversion to methyl esters is of primary importance in the biofuel industry. Some of the well-known methods of microalgal oil extraction that are non-mechanical are the hexane solvent method and supercritical fluid extraction. Other solvents can also be used in extracting oil, but it is important to note that the solvents should not dissolve in water, be simple to get, have a low boiling point, and be reusable as their primary requirements (Show et al., 2015). Some of the solvents that can be alternatives to hexane are chloroform, acetone, benzene, and cyclohexane (Show et al., 2015). Lipid extracts of *C. vulgaris* Beijerinck were subjected to various extraction methods in the Philippines, and the study revealed that the fatty acid composition was influenced by the extraction method used. Sacaranlao et al. (2019) reported that the solvent system 2:1 (v/v) chloroform:methanol resulted in the highest lipid yield and extracted a wider range of fatty acids compared to hexane, which produced a lower yield of mainly medium-chain fatty acids suitable for biodiesel production. The use of more polar solvents was shown to extract a broader variety of fatty acid types in contrast to less polar solvents (Saracanlao et al., 2019).

Supercritical fluid extraction. Supercritical fluid extraction makes use of liquefied CO_2 to extract oil from microalgae. Under pressure and heat, carbon dioxide liquefies to the point that it possesses both liquid and gaseous characteristics. The solvent used to extract the oil is then this liquefied fluid. The supercritical fluid extraction method is very efficient that it can extract almost 100% of the total oil content of dried biomass. However, it is expensive and requires special equipment.

Enzymatic Extraction. Enzymatic extraction uses enzymes in hydrolyzing microalgal cells to release the oil into a suitable solvent media. The employment of enzymes, either alone or in conjunction with a physical disruption technique like ultrasound, has the potential to speed up and increase the yield of extractions (Mercer & Armenta, 2011). Although it is expensive, it can be combined with other disruption techniques for species that are more resilient to disruption (Show et al., 2015).

According to a study, a combined application of cellulase, pectinase, and xylanase can boost lipid extraction yields from *Scenedesmus* microalgae by 96.4% when compared to untreated algal biomass. At 45 °C and pH 4.4, 190 min of mixed enzymatic treatment resulted in a lipid recovery of 86.4%. The enzymatic breakdown of microalgal cell wall components is responsible for the increase in lipid extraction efficiency (Zhang et al., 2018). Although enzymatic treatment is an effective substitute for microalgal lipid extraction, a thorough cost-benefit analysis is still required to determine its practical application potential (Zhang et al., 2018). Fortunately, a novel approach was initially used to generate microalgal biodiesel from wet *Chlorella* biomass using a two-step enzymatic process (hydrolysis and transesterification) mediated by cellulase and liquid lipase TL. Cellulase achieved the highest hydrolysis efficiency, according to the results, when hydrolysis circumstances were ideal. Liquid lipase TL was a potential contender in the transesterification of the pretreated wet microalgal biomass to yield economically viable biodiesel. Overall, this two-step enzymatic process proved a cost-effective, environmentally friendly way to make renewable commercial biodiesel from wet microalgal biomass (He et al., 2018).

Osmotic shock. Osmotic shock, on the other hand, uses high concentrations of a solute or other additive (such as salt, substrates, neutral polymers like polyethylene glycol, or dextran) to put stress on the cells, causing them to rupture and release the internal components (Show et al., 2015). Osmotic treatment was tested as a method to extract lipids from two lipid-rich microalgal species, *Chaetoceros muelleri* and *Dunaliella salina*, without the use of solvents. According to the results, *C. muelleri* is a good species for a combined system that produces biodiesel and biogas, and up to 72% of the lipids in *C. muelleri* were removed. Based on this species, a sustainable microalgae-based refinery might be possible using the technique suggested in cell disruption (González-González et al., 2019). A successive study using *C. muelleri* also resulted in 72% lipids released during osmotic shock pre-treatment (González-González et al., 2021). Nevertheless, due to its expensive cost, osmotic shock has not gained widespread use in the industry (Mercer & Armenta, 2011).

Current Scenario of Biodiesel Production in the Philippines

Biodiesel consumption in the Philippines is expected to continue its upward trajectory in the future, driven by the proposed increase of biodiesel blending as stipulated by the Philippine Biofuels Law (Mojica-Sevilla, 2022). To comply with the requirements of the law, a one percent biodiesel blend (B1) was implemented in 2007 and followed by a two percent biodiesel blend (B2) in 2009. The biodiesel blend in the present is still B2, although it is projected to increase to B5 in the future. The Philippines Biodiesel Association (TBPA) advocates for a gradual increase from B2 to B5, and the Department of Energy (DOE) awaits the

Table 2. Comparison of some cell disruption methods: cost and energy efficiency (Kumar et al., 2015).

Method	Efficiency Rating	Cost Involved	Energy Requirement	Remarks
High-Pressure Press	Low to moderate	High cost	Intensive	Heat production and potential compound damage
Bead milling	Moderate	Cost-effective	Intensive	Challenging to scale up
Ultrasonic extraction	High	High particularly in capital and maintenance costs	Intensive	Poor quality product as a result of process damage
Hexane solvent method	Moderate	High cost	Intensive	Regulation concerns, environmental, health, and fire risks
Supercritical Fluid Extraction	High	High cost	Intensive	Environmental and safety issues
Osmotic Shock	Moderate to high	Low -cost method	Less Energy	Requires longer treatment time (not less than 48 h)

endorsement of the National Biofuels Board (NBB) to increase the blend mandate (Mojica-Sevilla, 2022).

The increasing demand for biofuels is always accompanied by a surge in the demand for feedstock. The Philippines initially used coconut oil as feedstock for biodiesel production. However, the sustainability of feedstock remains a challenge for the industry due to concerns related to food security and pricing. Given such a situation, massive efforts in searching for other feedstocks were conducted. This led to more studies on the woody plant *J. curcas,* a second-generation feedstock for biodiesel. Compared to first-generation feedstocks, *J. curcas* is a more economical source of oil for biodiesel production and it does not compete with food supply. *J. curcas* does not produce edible oil and it may grow in low-fertility soil with little maintenance (Escobar et al., 2008; Ewunie et al., 2021). Further studies were also conducted for its process development from 2005 to 2009 in the Philippines and funded by the Department of Science and Technology (DOST). However, many of the studies were shelved by DOST because of the lacking economic feasibility (Mojica-Sevilla, 2021). Moreover, it will require a large area of land for plantation which could be challenging particularly in urban areas. Although second-generation feedstocks do not compete with the demand for food supply, it competes in another aspect which is the land area for plantation (Ma & Liu, 2019). It is still highly debatable that such a practice may ultimately compromise land areas that are intended for plants that are consumed as food. Hence, researchers are challenged to seek other feedstocks that are more sustainable than the first- and second-generation feedstocks, which led to studies on oleaginous microorganisms such as bacteria, yeast, microalgae, and fungi.

Oleaginous microorganisms are the third-generation feedstocks for biodiesel. In the Philippines, most of the studies for third-generation feedstocks were related mostly to microalgae. Compared to other sources, microalgae may be grown in engineered systems using sewage as the culture medium and coal-fired power stations as the carbon supply since they have demonstrated considerable potential as feedstock for biodiesel (Ma & Liu, 2019). It also occupies less land area compared to other feedstocks and some species are consumed as food, so it is more sustainable. Some of the species that are mostly studied are *C. vulgaris* and *Scenedesmus* sp. Matanguihan et al. (2020) designed, fabricated, and evaluated the performance of open raceway ponds for the biomass production of *C. vulgaris* Beijerinck, and results showed that the biomass productivity is comparable to commercial raceway ponds for *C. vulgaris*. Arguelles et al. (2019; 2018) reported two strains of high lipid and protein-producing epilithic microalga, which both belong to the genera *Desmodesmus* sp. These species are suitable candidate feedstock for biodiesel and its protein-rich biomass can be used as animal feed. Another study evaluated two green microalgae species such as *Chlorolobion* sp. and *Chlorella* sp. that have the potential as feedstock for the production of biodiesel with good fuel quality (Arguelles & Martinez-Goss, 2021). Mean oil contents of 31.61% and 28.77% and lipid productivity of 227.84 and 151.14 mg/L/day were quantified, respectively (Arguelles & Martinez-Goss, 2021). The properties of biodiesel observed for *Chlorella* sp. were good cetane number (68.79), low kinematic viscosity (2.78 mm^2 s^{-1}), oxidation stability (10.44 h), and low density (0.88 g cm^{-3}). Likewise, *Chlorolobion* sp. has low density (0.89 g cm^{-3}), oxidation stability (8.93 h), low kinematic viscosity (2.79 mm^2 s^{-1}), and cetane number (65.17). Nitrogen starvation increased the lipid yield, but it decreased the biomass production of the two green microalgae. Finally, a study assessed the use of *C. infusionum* (EAU-10) as a potential feedstock for the production of biodiesel. By limiting the nitrogen source, high oil content of 21.26% was produced by the microalgae (Arguelles, 2021). However, most of the experiments for microalgae were done at the laboratory stage and there is a need to conduct more studies at the industrial level.

In addition, most wild-type microalgae species lack the essential traits required for large-scale production, including but not limited to rapid growth rate, high lipid content, robustness, adaptability to varying environmental conditions, capability to thrive in cost-effective media, favorable cell flocculation properties, ease of downstream processing, and straightforward genetic manipulations (Carino & Vital, 2023). Hence, research has been done in genetically modifying microalgal species to achieve those characteristics leading to the fourth-generation biodiesel feedstocks. Among the extensively explored techniques for improving these microalgae strains are random mutagenesis, adaptive laboratory evolution (ALE), and genetic engineering (Carino & Vital, 2023). Random mutagenesis, particularly UV mutagenesis, emerges as a preferred method for microalgae breeding due to its inherent flexibility and ease of manipulation. Notably, it does not necessitate detailed genetic information about the target organism, making it a versatile and practical option for enhancing microalgae strains in biodiesel production (Arora & Philippidis, 2021; Carino & Vital, 2023). In the context of the Philippines, Carino & Vital (2023) utilized UV-C mutagenesis to improve the productivity of *C. vulgaris*. They successfully generated and isolated two mutants that show promising potential for future commercial production, particularly within the Philippines.

Furthermore, waste materials like used cooking oil and bio-oil obtained from agricultural residues were also taken into consideration as potential feedstocks for biodiesel production. In December 2019, the DOST Industrial Technology Development Institute (ITDI) completed a study titled “Characterization/Performance Testing of the Biodiesel/Diesel Blends from Combined Feedstock of Various Vegetable and Used Cooking Oils.” DOST-ITDI developed optimized processes to produce biodiesel from refined palm oil, used cooking oil, and rubber seed oil. Four different combinations of biodiesel were tested, and the resulting methyl esters could be used as a fuel additive in petroleum diesel when blended with coconut methyl ester, according to the recommended blending ratio for binary and tertiary blends (Mojica-Sevilla, 2022). Aside from that, the DOST-Philippine Council for Industry, Energy, and Emerging Technology Research and Development (PCIEERD), in collaboration with other institutions in the Philippines, conducted research on advanced biofuels such as bio-oil production from agricultural waste. The study utilized a prefabricated reactor to conduct pyrolysis on corn stover, which yielded

Table 3. Summary of advancements and research in microalgal cultivation for biodiesel production in the Philippines

Microalgal Species	Highlights and Significant Findings	Reference
C. vulgaris	Biomass production of *C. vulgaris* using photobioreactor	Santiago et al. (2013)
C. vulgaris	Effective light source and light wavelength for the cultivation of *C. vulgaris*	Carpio et al. (2015)
C. vulgaris	Biomass production of *C. vulgaris* using open raceway pond	Matanguihan et al. (2020)
C. vulgaris	Influence of extraction methods on fatty acid composition and optimization of methods for lipid extracts of *C. vulgaris*	Saracanlao et al. (2019)
Desmodesmus sp.	Cultivation of a high lipid and protein-producing microalgae (Desmodesmus sp.) for biodiesel production	Arguelles et al. (2018)
Desmodesmus sp.	Cultivation of a high lipid and protein-producing epilithic microalga that is suitable feedstock for biodiesel	Arguelles et al. (2019)
Chlorella sp. *and Chlorolobion* sp.	Superior biodiesel properties of algal oil produced by the two microalgal strains	Arguelles & Martinez-Goss (2021)
C. infusionum	Nitrogen starvation-induced lipid accumulation	Arguelles (2021)
C. vulgaris	UV-C mutagenesis was employed to improve *C. vulgaris* for biodiesel production	Carino & Vital (2023)

improved bio-oil and char yields when the temperature, time, and catalyst were optimized. The study recommends further investigation, such as equipment scaling for bio-oil production from other agricultural waste sources, exploring higher pyrolysis temperatures, and evaluating the cost-effectiveness of producing bio-oil and char through economic analyses (Mojica-Sevilla, 2022).

At present, the Philippines continues to depend on coconut oil as the primary source of biodiesel production. To respond to the need for increasing biodiesel blend, the DOE remains committed to finding promising feedstock for biodiesel, and microalgae are still being considered. Based on the comprehensive information provided above, ranging from research conducted on upstream processes to downstream processes for biodiesel production using microalgae as feedstock, achieving a more sustainable source of energy appears not only feasible but also promising. Reported strains of microalgae such as *Chlorella* sp. and *Chlorolobion* sp. have superior lipid productivities and biodiesel properties that were achieved through a series of experiments on upstream processes such as nutrient limitation and lipid induction. The factors that are affecting microalgal biomass production, lipid content, and lipid productivity are now well-studied and optimized as evidenced by the available studies on *Chlorella* sp. and other microalgae, offering more opportunities to enhance their potential for biodiesel production. Aside from that, the possibility of large-scale production is substantiated by studies investigating cell disruption methods and the cultivation of microalgae using photobioreactors and open pond systems that are well-suited to the environmental conditions of the Philippines. Hence, integrating these extensively investigated upstream and downstream processes and suitable cultivation systems will constitute a remarkable advancement towards establishing a sustainable and efficient biodiesel production process, utilizing microalgae as a highly promising renewable energy source.

Conclusions

Microalgae are excellent feedstock for the production of biodiesel. These microorganisms are capable of efficient carbon sequestration, exhibit high growth rates, and can be cultivated in marine and/or freshwater medium. In the Philippines, the current scenario for biodiesel consumption is still expected to increase in the future due to the proposed increase of biodiesel blending as mandated by the Philippine Biofuels Law, which led to massive research efforts conducted in search for alternative feedstocks ranging from the first-generation to fourth-generation feedstocks. Microalgae showed greater potential as feedstock for biodiesel as compared to other sources. Reported microalgal strains in the Philippines, like *Chlorella* sp. and *Chlorolobion* sp., demonstrate superior lipid productivities and biodiesel properties achieved through experiments on upstream processes such as nutrient limitation and lipid induction. Factors affecting microalgal biomass production, lipid content, and lipid productivity are well-researched and optimized, offering opportunities for enhanced biodiesel production. Large-scale production feasibility is supported by studies on cell disruption methods and microalgal cultivation using photobioreactors and open pond systems, well-suited to the Philippines' environmental conditions. Integrating these optimized processes represents a significant advancement towards a sustainable and efficient biodiesel production process, as highlighted by the research conducted in the Philippines utilizing microalgae as feedstock. Nevertheless, future investigations targeting an in-depth study on strain improvement using molecular means and evaluation of biodiesel quality (and efficiency) are recommended to further enhance lipid production in microalgae for biodiesel production.

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Supplementary Materials

Supplementary materials is not available for this article.

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