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Overview of Biodiesel Production from Microalgae

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Biodiesel is a renewable fuel that is produced from oils derived from plants, animals, or microbes. It is nontoxic and biodegradable. Various methods such as transesterification, blending, cracking, microemulsification, and pyrolysis have been developed to convert oils from biological sources into biodiesel that is comparable to diesel fuel. Transesterification, by a number of consecutive reversible reactions, is the most common method: triglycerides are converted stepwise to diglycerides, monoglycerides, and finally glycerol, with the liberation of fatty acid methyl ester defined as biodiesel at each step. Generally, an alcohol and a catalyst are needed for transesterification of triglycerides.

Microalgae are microscopic ($2−200 \mu$m), autotrophic organisms that can grow by photosynthesis. Most are eukaryotes, but the prokaryotic cyanobacteria are often included. Some microalgae contain more than 70% (w/w, dry basis) lipids. Microalgae grow extremely rapidly under optimal conditions and can grow 100 times faster than terrestrial plants. The oil yield from microalgae containing 30% oil content is 58700 L ha^{-1} $year^{-1}$, which is much higher than that from other crops (e.g., 636 L ha^{-1} $year^{-1}$ for soybean and 5366 L ha^{-1} $year^{-1}$ for oil palm). Importantly, microalgal cultivation need not encroach on arable land suitable for food production.

Currently, there are several areas of research on transesterification of microalgal oil. The conversion efficiency of biodiesel production via transesterification depends on microalgal characteristics, amount and type of alcohol used, catalyst, operating temperature, and reaction time. This study reviews the technologies that generate biodiesel from microalgae by transesterification. The relative performances of alkaline, acidic, and enzymatic catalysts are evaluated. New techniques used in biodiesel production, e.g., methods using microwaves and supercritical solutions, are discussed.

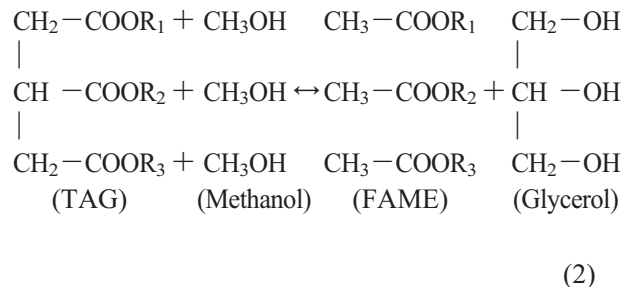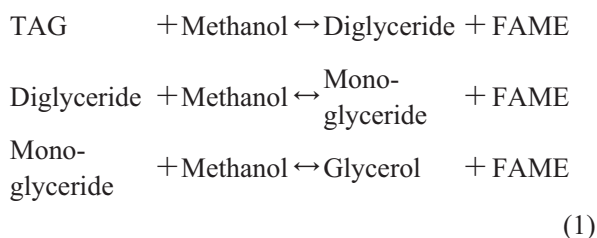**Key words**: microalgae, biodiesel, transesterification, catalyst

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1. Introduction

Climate change is the most critical global environmental problem. The potential threat of global climate change has increased, and much of the risk has been attributed to greenhouse gas (GHG) emissions by fossil fuel usage (Wuebbles *et al*., 2001). It has become necessary to develop techniques and to adopt policies to minimize impacts of global warming that result from the increase in anthropogenic GHG emissions. In 1997, the Kyoto Protocol called for a 5.2% reduction in GHG emissions from 1990 levels (Wang *et al*., 2008), and various technologies have been investigated to meet this goal. Another problem is a future energy crisis due to depletion of fossil fuels. The continuous use of fossil fuels as a primary source of energy is widely recognized to be unsustainable (Khan *et al*., 2009). Therefore, it is absolutely necessary to ensure new energy resources before the world is confronted with an energy crisis.

Biodiesel is a widely known alternative fuel. It can be produced from oils derived from plants, animals, or microbes (Graboski and McCormick, 1998), and currently represents 82% of total biofuel production (Bozbas, 2008). Biodiesel is usually defined as the monoalkyl esters of long-chain fatty acids derived from transesterification of renewable feedstocks (Meher *et al.*, 2006). Transesterification consists of a number of consecutive reversible reactions (Freedman *et al.*, 1986). Triglycerides (or triacylglycerols: TAGs) are commonly reacted with methanol and are converted stepwise to diglycerides, monoglycerides, and finally glycerol, as shown in equations 1 and 2. From 1 mole of starting TAG, 1 mole of fatty acid methyl ester (FAME) is liberated at each step.

$$TAG + Methanol \leftrightarrow Diglyceride + FAME$$

$$Diglyceride + Methanol \leftrightarrow Mono\text{-}glyceride + FAME$$

$$Mono\text{-}glyceride + Methanol \leftrightarrow Glycerol + FAME$$

$$(1)$$

$$
\begin{array}{l}
CH_2-COOR_1 + CH_3OH \quad CH_3-COOR_1 \quad CH_2-OH \\
| \\
CH\ -COOR_2 + CH_3OH \leftrightarrow CH_3-COOR_2 + CH\ -OH \\
| \\
CH_2-COOR_3 + CH_3OH \quad CH_3-COOR_3 \quad CH_2-OH \\
\ (TAG) \qquad (Methanol) \qquad (FAME) \qquad (Glycerol)
\end{array}
$$

$$(2)$$

There are several reasons why biodiesel is an attractive renewable energy: it is biodegradable and has low toxicity; it can be used in existing diesel engines with little or no modification (Demirbas, 2002); and it can be blended in any ratio with traditional petroleum-based diesel fuel (Peterson, 1991). On the other hand, there are some drawbacks in the current biodiesel production technology: the area of crops for biodiesel production is growing, potentially resulting in the destruction of natural habitats and competing with food production (Janaun and Elis, 2010). Therefore, new feedstocks are needed to meet demand.

2. Microalgae as a biodiesel feedstock

2.1 Microalgal diversity

Microalgae are defined as prokaryotic or eukaryotic thallophytes that lack a sterile covering of cells around the reproductive cells and have chlorophyll *a* as a photosynthetic pigment (Lee, 1980). Microalgae are present in a wide range of environments, and it is estimated that more than 50000 species exist (Richmond, 2004). Prokaryotic microalgae (cyanobacteria, blue-green algae) lack membrane-bound organelles and are more similar to bacteria than to algae. In contrast, the cells of eukaryotic microalgae have organelles that control the functions of the cell (Brennan and Owende, 2010). Eukaryotic microalgae are categorized into a variety of classes defined mainly by their pigmentation, life cycle, and basic cellular structure (Khan *et al.*, 2009). The most important classes include green algae, red algae, and diatoms. Algae can be autotrophic or heterotrophic, and some are mixotrophic (Lee, 1980).

2.2 TAG biosynthesis in microalgae

Microalgae synthesize and store TAGs, which are their main lipids, under stress conditions, such as when their nitrogen supply is exhausted, and when an excess supply of carbon is assimilated. They store the TAGs as a carbon energy reserve within cells that can no longer divide (Meng *et al.*, 2009). The route of TAG biosynthesiss consists of three steps: (a) the conversion of acetyl-CoA to malonyl-CoA; (b) the elongation and desaturation of the carbon chain of fatty acids; and (c) the biosynthesis of TAG (Huang *et al.*, 2010).

The conversion of acetyl-CoA and CO_2 into malonyl-CoA occurs has two steps and is catalyzed by an enzyme complex (Hu *et al.*, 2008). First, CO_2 is transferred to nitrogen by the biotin carboxylase prosthetic group of acetyl-CoA carboxylase. In the next step, the activated CO_2 is transferred from biotin to acetyl-CoA to form malonyl-CoA, which is catalyzed by carboxyltransferase (Ohlrogge and Browse, 1995). The fatty acid elongation condenses malonyl-CoA molecules and acetyl-CoA; after several repeated reaction steps, the ACP-thioesterase cleaves the acyl chain and liberates the fatty acid (Courchesne *et al.*, 2009). The biosynthesis of TAG is performed by the condensation of glycerol-3-phosphate with acyl-CoA to form lysophosphatidic acid, which is catalyzed by glycerol-3-phosphate acyltransferase. After this reaction, phosphatidic acid, diacylglycerol and TAG are synthesized by a series of catalytic reactions.

Table 1. Lipid contents and productivities of different microalgae species (Mata *et al.,* 2010).

Marine and freshwater microalgae species	Lipid content (% dry weight biomass)
Ankistrodesmus sp.	24.0–31.0
Botryococcus braunii	25.0–75.0
Chaetoceros muelleri	33.6
Chlorella emersonii	25.0–63.0
Chlorella protothecoides	14.6–57.8
Chlorella sorokiniana	19.0–22.0
Chlorella vulgaris	5.0–58.0
Chlorella sp.	10.0–48.0
Chlorella pyrenoidosa	2.0
Chlorella	18.0–57.0
Chlorococcum sp.	19.3
Crypthecodinium cohnii	20.0–51.1
Dunaliella salina	6.0–25.0
Dunaliella primolecta	23.1
Dunaliella tertiolecta	16.7–71.0
Dunaliella sp.	17.5–67.0
Ellipsoidion sp.	27.4
Euglena gracilis	14.0–20.0
Haematococcus pluvialis	25.0
Isochrysis galbana	7.0–40.0
Isochrysis sp.	7.1–33
Monodus subterraneus	16.0
Monallanthus salina	20.0–22.0
Nannochloris sp.	20.0–56.0
Nannochloropsis oculata.	22.7–29.7
Nannochloropsis sp.	12.0–53.0
Neochloris oleoabundans	29.0–65.0
Nitzschia sp.	16.0–47.0
Oocystis pusilla	10.5
Pavlova salina	30.9
Pavlova lutheri	35.5
Phaeodactylum tricornutum	18.0–57.0
Scenedesmus obliquus	11.0–55.0
Scenedesmus quadricauda	1.9–18.4
Scenedesmus sp.	19.6–21.1
Skeletonema sp.	13.3–31.8
Skeletonema costatum	13.5–51.3
Spirulina platensis	4.0–16.6
Spirulina maxima	4.0–9.0
Thalassiosira pseudonana	20.6
Tetraselmis suecica	8.5–23.0
Tetraselmis sp.	12.6–14.7

Table 2. Comparison of microalgae with other biodiesel feedstocks (Mata *et al.*, 2010).

| Plant source	Oil content (% dry weight biomass)	Oil yield (L oil/ha year)	Land use (m^2 year/kg biodiesel)
Corn	44	172	66
Hemp	33	363	31
Soybean	18	636	18
Jatropha	28	741	15
Camelina	42	915	12
Canola/Rapeseed	41	974	12
Sunflower	40	1070	11
Castor	48	1307	9
Palm oil	36	5366	2
Microalgae (low oil content)	30	58,700	0.2
Microalgae (medium oil content)	50	97,800	0.1
Microalgae (high oil content)	70	136,900	0.1

2.3 Microalgal advantages

Microalgae offer many advantages as a biodiesel feedstock. The most important one is their higher growth rates and oil productivity than conventional crops (Minowa *et al.*, 1995). Generally, microalgae have oil levels in the range of 20% to 50% (w/w, dry basis) (Table 1), but higher levels can be reached (Mata *et al.*, 2010). Microalgae commonly double their biomass within 24 h, and cells divide as frequently as every 3.5 h during the exponential growth phase (Chisti, 2007). Oil yield is 58700 L ha^{-1} year^{-1} for microalgae containing 30% oil, compared with 636 L ha^{-1} year^{-1} for soybean and 5366 L ha^{-1} year^{-1} for oil palm (Table 2; Mata *et al.*, 2010). If microalgae contain 70%, 136 900 L oil ha^{-1} year^{-1} can be produced (Mata *et al.*, 2010). In terms of cultivation area, microalgae are clearly advantageous over land plants because of their higher biomass productivity and oil yield, and they do not compete for land with crops used for food, fodder, and other products (Huang *et al.*, 2010). Microalgae can be grown in a number of environments that are unsuitable for growing other crops, such as fresh, brackish, or salt water or non-arable lands (Patil *et al.*, 2008). CO_2, which is essential to autotrophic cultivation of microalgae, can be provided by industrial facilities such as power plants and boilers, where the CO_2 concentration in emitted gases may reach 15% (v/v) (Salih, 2011; Zhao *et al.*, 2011). Microalgae can also be used to treat wastewater, removing nitrogen and phosphorus efficiently (Mallick, 2002) and generating

an environmental benefit. Furthermore, microalgae produce valuable co-products or by-products such as biopolymers, proteins, carbohydrates, and residual biomass. They can be used as energy sources through the generation of ethanol and methane by fermentation, and can be supplied as livestock feed and fertilizer owing to their high N:P ratio. They can also produce a variety of chemical products such as pharmaceuticals and platform chemicals; highly unsaturated fatty acids such as docosahexaenoic acid (Molina Grima *et al.*, 2003); proteins and carbohydrates, which can be used as gross nutrients (Knuckey *et al.*, 2006); specific compounds such as pigments (Lorenz and Cysewski, 2000); and silica derived from diatom cell walls (Gordon *et al.*, 2009).

3. Biodiesel production from microalgae

3.1 Traditional biodiesel production from microalgae

Biodiesel production from microalgae requires several steps (Fig. 1). In the cultivation step, it is important to consider different factors that influence algal growth: light, temperature, nutrient concentration, CO_2 supply, pH, salinity, contamination, and so on. Harvested microalgae are concentrated by sedimentation, centrifugation, flocculation, or membrane filtration. Although centrifugation is often used, energy consumption is high. The developments of more cost-viable and energy-efficient methods are needed. After the harvested biomass is dried, cells are disrupted and the oil is extracted. Three common methods are
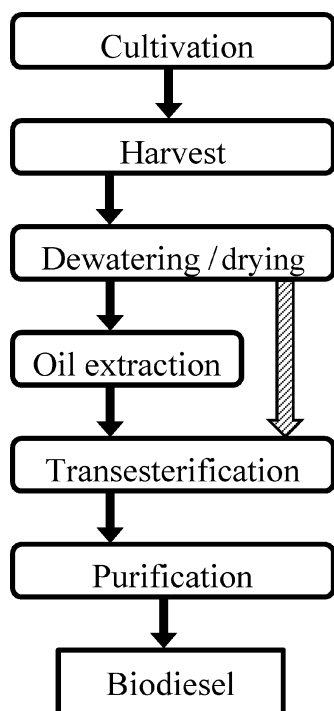
Fig. 1. Steps for biodiesel production from micro-algae. In some procedures, the oil extraction step before transesterification is bypassed (hatched arrow).

used to extract oil from microalgae: expeller/press, solvent extraction, and supercritical fluid extraction. Currently, the most popular extraction method is Soxhlet extraction using hexanes as a solvent. The extracted oil is converted to biodiesel through trans-esterification, and the crude biodiesel is purified into the final biodiesel product.

3.2 Direct transesterification of microalgal lipids

Currently, the most common method for the production of biodiesel from microalgae involves the extraction of lipids using organic solvents (e.g., hexanes, chloroform, methanol), followed by trans-esterification to generate FAME. This is the traditional method used with terrestrial feedstock such as soybean or rapeseed. However, the rigid cell walls of microalgae prevent the efficient extraction of oil: mechanical crushing of algal biomass to extract oil is not easily performed using existing crushing equipment. Also, life cycle analysis showed that 90% of the process energy is consumed by oil extraction, indicating that any improvement in lipid extraction will have a significant impact on the economics of the process (Lardon et al., 2009). Therefore, the extrac-

tion of lipids from microalgae and their conversion to biodiesel in a single step would be highly valuable, as it would bypass the use (and cost) of large quantities of organic solvents and the distillation cost of solvent recovery (Fig. 1).

Alcohol can be used simultaneously for the extraction of oil and as an acyl acceptor for transesterification. Such direct transesterification can simplify the conversion process, potentially reducing the overall cost and hence the final fuel product cost. Direct transesterification has been used to determine the fatty acid composition of lipid-containing tissues (Lepage and Roy, 1984; Park and Goins, 1994; Rodríguez-Ruiz et al., 1998), and it can result in greater FAME yield than extraction followed by transesterification (Lepage and Roy, 1984; Siler-Marinkovic and Tomasevic, 1998; Lewis et al., 2000). The direct approach has been shown to be effective in making biodiesel from both pure (Johnson and Wen, 2009; Vicente et al., 2009) and mixed cultures of microorganisms (Dufreche et al., 2007; Mondala et al., 2009). This method confirmed that TAGs, free fatty acids (FFAs), and phospholipids all contributed to the formation of FAME (Wahlen et al., 2011). Furthermore, it could reduce process wastes and pollution (Haas et al., 2007).

4.   Direct transesterification methods

4.1   Alkaline catalysis

There are few studies of direct transesterification using homogeneous alkaline catalysts (Xu and Mi, 2011; Velasquez-Orta et al., 2012; Velasquez-Orta et al., 2013). Many microalgae have a high FFA content. FFA reacts with an alkaline catalyst to form soap, which consumes the catalyst and results in a low transesterification yield, as shown in equation 3 (Al-Zuhair, 2007):
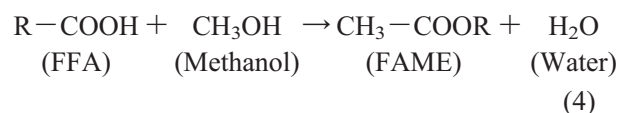
$$R-COOH + \quad KOH \quad \rightarrow R-COOK + \quad H_2O$$
(FFA)   (Potassium   (Soap)   (Water)
hydroxide)

$$(3)$$
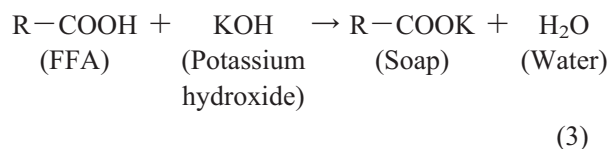
Therefore, alkaline catalysts are usually not recommended for direct transesterification from micro-algae. However, if the microalgae have a low FFA content, alkaline catalysis is more suitable than acidic catalysis because it proceeds faster. Alkaline catalysts are also less corrosive to equipment than acidic catalysts (Freedman et al., 1986). Furthermore, alka-

line catalysis has a higher tolerance for water than acidic catalysis (Kusdiana and Saka, 2004). Moreover, the sole study of direct transesterification using a heterogeneous catalyst reported a maximum conversion of 28% for transesterification of dried *Nannochloropsis* sp. by reacting the microalgae with 10% (w/w) alkaline heterogeneous catalyst (Mg-Zr) in methanol: dichloromethane (2:1, v/v) at 65℃ for 4 h (Li *et al*., 2011).

4.2 Acidic catalysis

Because of the often high FFA content of microalgae, direct transesterification has been conducted mainly via homogeneous acidic catalysis. Acidic catalysts can convert FFAs into FAME, as opposed to soap formation by alkaline catalysts, as shown in equation 4 (Ehimen *et al*., 2010):

$$R-COOH + CH_3OH \rightarrow CH_3-COOR + H_2O$$
$$\text{(FFA)} \quad \text{(Methanol)} \quad \text{(FAME)} \quad \text{(Water)}$$
$$(4)$$

In fact, when Nagle and Lemke (1990) compared the efficacies of acidic catalyst and alkaline catalyst, acidic catalysis resulted in a higher FAME yield than alkaline catalysis under the same reaction conditions. However, direct transesterification with acidic catalysts is weakened by the presence of water: the FAME yield from the direct transesterification of *Chlorella* biomass was reduced from 81.7% at a 0.7% (w/w, wet basis) water content to only 19.5% at a 73% water content (Ehimen *et al*., 2010).

4.3 Enzyme catalysis

To our knowledge, the only report of a direct method using enzyme catalysis is that of Tran *et al*. (2013). After *Chlorella vulgaris* ESP-31 biomass (water content 86%−91% [w/w, wet basis], oil content 14%−63% [w/w, dry basis]) was sonicated to disrupt the cell walls, the lipids were directly converted into FAME by using immobilized *Burkholderia* sp. C20 lipase as the catalyst. The lipase was immobilized on a hybrid nanomaterial ($Fe_3O_4-SiO_2$) grafted to a long-chain alkyl group as a supporter (Liu *et al*., 2012; Tran *et al*., 2012). The immobilized lipase worked well with wet microalgal biomass. With this method, it is important that the microalgal biomass have a high lipid content to achieve ＞90% biodiesel conversion with a low biocatalyst loading and high lipase recycling efficiency.

4.4 Supercritical solvent method

A catalyst-free method has recently been developed using supercritical methanol (Demirbas, 2009). This process is conducted at a high reaction temperature and pressure (Kusdiana and Saka, 2004). Supercritical methanol can form a single phase with lipids, in contrast to two phases under ambient pressures and temperatures. This can be achieved because the dielectric constant of methanol is decreased in the supercritical state. In the supercritical method, the reaction is achieved within very short times of 2−4 min, and FFA and TAG can be simultaneously esterified and transesterified. This method also has the possibility of reducing costs associated with drying microalgae because the reaction is not inhibited by high water content. When this method was used with wet algal biomass containing about 90% water content (w/w, wet basis), 90% FAME yield was reached (Patil *et al*., 2011b). A two-step process was also reported (Levine *et al*., 2010): in the first step, wet microalgal biomass was reacted to hydrolyze intracellular lipids to FFA under subcritical conditions, and in the second step, the wet FFA-rich solids were subjected to supercritical direct transesterification. However, as it now stands, supercritical solvent methods may be unsuitable owing to poor process economics and safety concerns (Marchetti and Errazu, 2008).

4.5 Microwave- and ultrasound-assisted methods

Microwave radiation influences the motions of molecules with a dipole moment. In microwave-assisted transesterification, methanol absorbs microwave radiation, rapidly reorienting its dipole. This enables the destruction of the methanol–lipid interface (Patil *et al*., 2011a). The oscillating microwave field tends to move continuously toward the polar ends of molecules or ions (Azcan and Danisman, 2008). Consequently, collisions between moving molecules produce heat (Marra *et al*., 2010) that can shorten the reaction time (Lidström *et al*., 2001). Ultrasonic technology is also an effective method to enhance mass transfer between immiscible phases (Pan *et al*., 2002). The ultra-high-frequency sound waves compress and stretch the molecular spacing of the medium through which they pass, and the molecules remain continuously vibrating with the formation of fine micro-bubbles or micro-cavities (Ji *et al*., 2006; Lam *et al*., 2010). Generally, homogeneous alkaline catalysts have been used in these approaches.

5. Conclusions

Biodiesel production from microalgal lipids holds great potential for a new energy industry because some microalgae have high productivity of biomass and oil. Two main approaches have been used to produce FAME from lipids: organic solvent extraction of lipids from microalgae followed by transesterification of the lipid extracts, and direct transesterification from microalgal biomass. The former has the disadvantage of the increased cost caused by the use of organic solvent. In contrast, the latter can reduce the process energy considerably, because it produces biodiesel without the need for organic solvent. In addition, other research has shown that direct transesterification can convert phospholipids, as well as TAGs and FFAs, into FAME. Therefore, direct transesterification, via many approaches, offers promise for biodiesel production from microalgae. The key considerations to commercialization are the process energy and the effect of water. Further research is required to develop a cost-effective technology that is less affected by the presence of water.

6. Acknowledgments

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