表1: 結果の見通し

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Optimization of Hydrolysis and Esterification for Biodiesel Production from Wet Microalgae

January 2014

Kenji TAKISAWA
Optimization of Hydrolysis and Esterification for Biodiesel Production from Wet Microalgae

A Dissertation Submitted to
the Graduate School of Life and Environmental Sciences,
the University of Tsukuba
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Agricultural Science
(Doctoral Program in Bioindustrial Sciences)

Kenji TAKISAWA
Contents

Summary ........................................................................................................................................5

Chapter 1 Background ................................................................................................................8

1. Introduction .................................................................................................................................8

2. Microalgae as a biodiesel feedstock .........................................................................................10

   2.1. Microalgal biology .............................................................................................................10

   2.2. Microalgal advantages ......................................................................................................12

3. Biodiesel production from microalgae .....................................................................................13

   3.1. Traditional biodiesel production from microalgae .........................................................13

   3.2. Direct transesterification of microalgal lipid .................................................................14

4. Various direct transesterification methods .............................................................................16

   4.1. Alkaline catalyzed method ............................................................................................16

   4.2. Acid catalyzed method ..................................................................................................17

   4.3. Enzyme catalyzed method ............................................................................................18

   4.4. Supercritical method .....................................................................................................19

   4.5. Microwave- and ultrasound-assisted method ..............................................................20
Chapter 2 Two-step hydrolysis and esterification from wet microalgae

Introduction

Material and Methods

2.1. Materials

2.2. Experimental procedures

2.2.1. Effect of water on transesterification and esterification

2.2.2. Orthogonal experiment

2.2.3. Effect of temperature and volume of sulphuric acid on hydrolysis

2.2.4. Maximum FAME content

2.2.5. Effect of hydrolysis on FAME yield

2.2.6. Effect of methanol volume on esterification of hydrolysates

2.3. FAME and FFA analysis

Results and Discussions

3.1. Effect of water on transesterification and esterification
3.2. Investigation of effective parameters on hydrolysis with orthogonal experiment

.................................................................................................................49

3.3. Effect of temperature and acid on hydrolysis ......................................49

3.4. Esterification of hydrolysates ..........................................................50

3.5. Effect of methanol volume on esterification .....................................52

4. Conclusions .........................................................................................52

5. References ..........................................................................................63

Chapter 3 Simultaneous hydrolysis-esterification from wet microalgae

..........................................................67

1. Introduction .........................................................................................67

2. Material and Methods ..........................................................................70

2.1. Materials ..........................................................................................70

2.2. Experimental procedures ...............................................................71

2.2.1. Maximum FAME content ..........................................................71

2.2.2. SHE with orthogonal experiment ...............................................71

2.2.3. Investigation of effect of water content on SHE .........................72

2.2.4. Effects of various parameters on SHE ........................................73
2.3. FAME and FFA analysis .................................................................73

3. Results and Discussions ...............................................................74

3.1. Effects of various parameters on SHE with orthogonal experiment ........74

3.2. Effect of water content ..............................................................76

3.3. Effect of various parameters .........................................................77

  3.3.1. Effect of temperature ............................................................77

  3.3.2. Effect of methanol volume .....................................................77

  3.3.3. Effect of sulphuric acid volume ............................................78

  3.3.4. Difference of catalysts and alcohols .......................................78

4. Conclusions ..............................................................................80

5. References ..................................................................................91

Chapter 4 Overall conclusions ......................................................95

Acknowledgements .......................................................................97
Summary

It has become obvious that continued dependence on fossil fuel is unsustainable because of global warming by greenhouse gas emission and the future depletion of fossil fuel. Development of renewable energy has attracted much interest for energy sustainability. Biodiesel is a renewable fuel which is produced from oils derived from plants, animals or microbes. It is non-toxic and biodegradable, and has lower emission of greenhouse gas when burned in diesel engine. Various methods such as transesterification, blending, cracking, microemulsification and pyrolysis have been developed to convert oil into biodiesel which is comparable to diesel fuel.

Transesterification is the most common method for the production of biodiesel and consists of a number of consecutive reversible reactions. Triglyceride is converted stepwise to diglyceride, monoglyceride and finally glycerol and a mole of fatty acid methyl ester (FAME) named as biodiesel is liberated at each step. Generally, alcohol and catalyst are needed for transesterification of oil.

Microalgae are unicellular microscopic (2–200 μm) autotrophic organisms which grow by photosynthesis and are the eukaryotic representatives, though the prokaryotic cyanobacteria are often included in algae. Some species contain more than 70% lipid
(dry weight basis). They also grow extremely rapidly under optimal conditions and their growth rates are 100 times faster than terrestrial plants. Oil yield of microalgae containing 70% oil content is 58,700 L/ha year and much higher than other crops (e.g., soybean 446 L/ha year and palm 5950 L/ha year). In addition, microalgal cultivation does not encroach on arable land suitable for food production.

The extraction of lipid from microalgae and their conversion into biodiesel in a single step would be highly valuable as it will bypass the use (and cost) of large quantities of organic solvents. However, such direct transesterification approach has an issue, namely that the existence of water inhibits the reaction. In our study, hydrolysis of lipid to free fatty acid (FFA) from microalgae under high water content was investigated as a pretreatment of direct esterification. Results indicated that the hydrolysis process reduced the inhibition by water in FAME production. Also, FAME obtained by esterification of hydrolysates was increased by 181.7% compared to FAME obtained by direct transesterification under the same amount of water content (80%). Therefore, it was confirmed that hydrolysis process can reduce the negative effect of water on biodiesel production from wet microalgae.

In addition, hydrolysis of wet microalgal lipid to FFA followed by esterification of FFA using acid in one-step process was investigated. The investigation of simultaneous
hydrolysis-esterification (SHE) of wet microalgal lipid was conducted by using L27 orthogonal design and the effects of water content, volume of sulphuric acid, volume of methanol, temperature and time on SHE were examined. As a result, water content was found to be the most effective factor. The effects of various parameters on FAME content and equilibrium relation between FAME and FFA were also examined under water content 80%. Equimolar amounts of sulphuric acid and hydrochloric acid showed similar results. When two-step and simultaneous processes were compared, total reaction time in the two-step process was found to be faster than that seen in the simultaneous process. These methods have great potential in terms of biodiesel production from microalgae since no organic solvents are used, simultaneously reducing the drying cost and lowering the operating cost compared to other traditional methods.
Chapter 1 Background

1. Introduction

Climate change is the most critical global environmental problem. The potential threat of global climate change has increased, and much of this risk has been attributed to greenhouse gas (GHG) emissions by fossil fuel usage. It has become necessary to develop techniques and adopt policies to minimize impacts of global warming which results from the increase in anthropogenic GHG emissions. In 1997, the Kyoto Protocol called for a 5.2% reduction in GHG emissions from 1990 (Wang et al., 2008) and various technologies has been investigated to meet the value. Another problem is the future energy crisis due to depletion of fossil fuels. As shown in Fig. 1-1 (Ahmad et al., 2011), world energy consumption has been gradually increasing. Also, Fig. 1-2 shows energy consumption of each country in 2005 (Saito, 2010). The continuous use of fossil fuels as a primary source of energy is widely recognized to be unsustainable. Therefore, it is absolutely necessary to ensure new energy resources before the world will be confronted with an energy crisis. Currently, various technologies to allow substitution of fossil fuel with renewable energy have been developed.
Biodiesel has been widely known as one of alternative fuel. It can be produced from oils derived from plants, animals or microbes (Graboski and McCormick, 1998), which represents 82% of total biofuel production (Bozbas, 2008). Some methods are currently available and have been adopted for the production of biodiesel fuel. There are four primary ways to produce biodiesel (Table 1-1). Especially, transesterification, which produces the monoalkyl ester of long-chain fatty acid is the most general method.

Transesterification consists of a number of consecutive reversible reactions (Freedman et al., 1986). Triglyceride (TAG) is commonly reacted with methanol and is converted stepwise to diglyceride, monoglyceride and finally glycerol as shown in equation (1) and (2). A mole of fatty acid methyl ester (FAME) is liberated at each step.

\[
\begin{align*}
\text{Triglyceride} + \text{Methanol} & \leftrightarrow \text{Diglyceride} + \text{Fatty acid methyl ester} \\
\text{Diglyceride} + \text{Methanol} & \leftrightarrow \text{Monoglyceride} + \text{Fatty acid methyl ester} \\
\text{Monoglyceride} + \text{Methanol} & \leftrightarrow \text{Glycerol} + \text{Fatty acid methyl ester}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2\text{COOR}_1 + \text{CH}_3\text{OH} & \leftrightarrow \text{CH}_3\text{COOR}_1 + \text{CH}_2\text{OH} \\
\text{CH}_2\text{COOR}_2 + \text{CH}_3\text{OH} & \leftrightarrow \text{CH}_3\text{COOR}_2 + \text{CH}_3\text{OH} \\
\text{CH}_2\text{COOR}_3 + \text{CH}_3\text{OH} & \leftrightarrow \text{CH}_3\text{COOR}_3 + \text{CH}_2\text{OH}
(\text{Triglyceride}) & \leftrightarrow (\text{Methanol}) (\text{Fatty acid methyl ester}) (\text{Glycerol})
\end{align*}
\]
There are some reasons chosen as an attractive renewable energy. Biodiesel is biodegradable and less toxic. Also, it can be used in existing diesel engines with little or no modification (Demirbas, 2002). Furthermore, 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 cultivation area of crops for biodiesel production is growing with the increased biodiesel production. Increasing demand for biodiesel crop cultivation may result in the destruction of natural habitats. Additionally, the increment of the use of vegetable oil for biodiesel production accelerates the competition with food production. Therefore, new alternative feedstock is eagerly anticipated to meet the biodiesel production demand.

2. Microalgae as a biodiesel feedstock

2.1. Microalgal biology

Microalgae are prokaryotic or eukaryotic thallophytes, have no sterile covering of cells around the reproductive cells and have chlorophyll $a$ as photosynthetic pigment (Lee, 1980). Microalgae are present in a wide range of environmental conditions and it is estimated that more than 50,000 species exist (Richmond, 2004). Prokaryotic cells (cyanobacteria, blue-green algae) lack membrane-bound organelles and are more similar
to bacteria rather than algae. On the other hand, eukaryotic cells have these organelles that control the functions of the cell (Brennan and Owende, 2010). Eukaryotes are categorized into a variety of classes mainly defined 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 either be autotrophic or heterotrophic. Some algae are mixotrophic (Lee, 1980). The biosynthesis route of TAG in microalgae may consist of the following three steps: (a) the formation of acetyl coenzyme A (acetyl-coA) in the cytoplasm; (b) the elongation and desaturation of the carbon chain of fatty acid; and (c) the biosynthesis of TAG (Huang et al., 2010). In general, \( \text{L-\(\alpha\)-phosphoglycerol and acetyl-coA} \) are two major elements required for the biosynthesis of TAG. Microalgae form and accumulate more TAG, which is the main lipid, under stress conditions. Lipid accumulation begins when microalgae exhaust nitrogen from the medium. Also, the excess supply of carbon is assimilated by the cells and is converted into TAG. Synthesized lipid was stored within their cells which can no longer divide (Meng et al., 2009). TAG does not perform a structural role but serve as a carbon energy source.
2.2. Microalgal advantages

There are many advantages to using microalgae as a biodiesel feedstock. The most important point is their higher growth rates and oil productivity compared to conventional crops (Minowa et al., 1995). Generally, microalgae have oil levels in the range of 20 to 50% by weight of dry biomass (Table 1-2), but higher productivities can be also reached (Mata et al., 2010). Microalgae commonly double their biomass within 24 h, and algal cells divide as frequently as 3.5 h during the exponential growth phase (Chisti, 2007). Table 1-3 shows comparison of some sources of biodiesel. Oil yield is 58,700 L ha\(^{-1}\) year\(^{-1}\) for microalgae containing 30% oil by weight, compared with 636 L ha\(^{-1}\) year\(^{-1}\) for soybean and 5366 L ha\(^{-1}\) year\(^{-1}\) for palm (Mata et al., 2010). If microalgae contain 70% oil by weight, oil of 136,900 L ha\(^{-1}\) year\(^{-1}\) can be produced. In terms of the cultivation area, microalgae are clearly advantageous because of their higher biomass productivity and oil yield. Also, microalgae do not compete for land with crops used for food production, 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 the 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% or more (Salih, 2011 and Zhaoa et al., 2011). Microalgae can also be used to treat wastewater. Microalgae have been shown to be efficient in nitrogen and phosphorus removal (Mallick, 2002), and the combination of microalgal cultivation with wastewater treatment can generate the 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 such as ethanol and methane by fermentation, and can be supplied as livestock feed and fertilizer. 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 & Cysewski, 2000); or silica derived from diatom cell walls (Gordon et al., 2009).

3. Biodiesel production from microalgae

3.1. Traditional biodiesel production from microalgae

Fig. 1-3 shows flow diagram of biodiesel production from microalgae. In the cultivation step, it is important to consider different factors which influence algal growth: light, temperature, nutrient concentration, CO₂, pH, salinity, contamination and
so on. Cultivated microalgae are harvested and concentrated by sedimentation, centrifugation, flocculation or membrane filtration. Though centrifugation is often employed, the process energy is high. The development of more cost-viable and energy-efficient method is needed. After drying and cell disruption of harvested biomass are implemented as appropriate, it is subject to oil extraction step. There are three common methods to extract oil from microalgae: (1) expeller/press, (2) solvent extraction and (3) supercritical fluid extraction. Currently, the most popular extraction method is Soxhlet extraction using hexane as a solvent. Extracted oil is converted to biodiesel through transesterification. Finally, crude biodiesel become a biodiesel product after purification.

3.2. Direct transesterification of microalgal lipid

Currently, the most common biodiesel production method from microalgae is the extraction of lipid using organic solvents (e.g., hexanes, chloroform, and methanol), followed by the FAME generation from extracted lipid by transesterification. This results from the traditional method using terrestrial feedstock such as soybean or rapeseed. However, the extraction of oil from microalgal cells is prevented by their rigid cell walls. Mechanically crushing algal biomass to extract oil is not easy to be
performed using the existing crushing equipments. Also, life cycle analysis conducted on the process of biodiesel production from microalgae indicates 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 extraction of lipid from microalgae and its conversion into biodiesel in a single step would be highly valuable as it will bypass the use (and cost) of large quantities of organic solvent and the distillation cost to recovery solvent (Fig. 1-3). Alcohol can be simultaneously used for the extraction of oil and as acyl acceptor for transesterification. Such direct transesterification can simplify the fuel conversion process, potentially reducing the overall process cost, hence reducing the final fuel product costs as well. Direct transesterification has been used as an analytical technique to determine the fatty acid composition of lipid containing tissue (Lepage and Roy, 1984, Park and Goins, 1994, and Rodríguez-Ruiz et al., 1998). Also, it has been reported that direct method can result in greater FAME yield than the extraction followed by transesterification approach (Lepage and Roy, 1984, Siler-Marinkovic and Tomasevic, 1998, Lewis et al., 2000). Direct approach has been shown to be effective in making biodiesel from both pure (Johnson and Wen, 2009 and Vicente et al., 2009) and mixed cultures of microorganisms (Dufreche et al., 2007 and Mondala et al., 2009).
addition, this method confirmed that TAG, free fatty acid (FFA) and phospholipid all contributed to the formation of FAME (Wahlen et al., 2011). Furthermore, process wastes and pollution could also be reduced by this method (Haas et al., 2007).

4. Various direct transesterification methods

Various catalysts are used in order to perform direct transesterification of microalgal lipid. Advantages and disadvantages of the main types of catalysts used for transesterification are listed in Table 1-4. In addition to them, new methods such as supercritical method and microwave-assisted transesterification have also been investigated. They are described in detail below.

4.1. Alkaline catalyzed method

There are few researches of direct transesterification using homogenous alkaline catalyst (Xu and Mi, 2011, Velasquez-Orta et al., 2012 and Velasquez-Orta et al., 2013). Many microalgae have high FFA contents. FFA reacts with alkaline catalyst to form soap, consumes the catalyst, and results in the low tranesterification reaction as shown in equation (3) (Al-Zuhair, 2007).
\[
R - \text{COOH} + \text{KOH} \rightarrow R - \text{COOK} + \text{H}_2\text{O}
\]

(Free fatty acid) (Potassium hydroxide) (Soap) (Water) \hfill (3)

Therefore, alkaline catalyst is usually not recommended for the direct tranesterification from microalgae. However, if microalgae have low FFA content, alkaline catalyst is the most suitable because transesterification proceeds faster than with acid catalyst. Alkaline catalysts are also less corrosive to equipment than acid catalysts (Freedman, 1986). Moreover, alkaline catalyst has a higher tolerance for water than acid catalyst (Kusdiana and Saka, 2004). Also, presumably as the sole paper of direct transesterification using heterogenous catalyst, Li et al. (2011) reported a maximum conversion of 28% on the direct transesterification of dried \textit{Nannochloropsis} sp. by reacting microalgae with 10% of an alkaline heterogeneous catalyst (Mg-Zr) in the volume ratio of methanol to dichloromethane 2: 1 at 65 °C for 4 h.

4.2. Acid catalyzed method

Direct transesterification from microalgae has been mainly conducted using homogenous acid catalyst because of their high FFA content. Acid catalyst can convert FFA into FAME as opposed to alkaline catalyst as shown in equation (4) (Ehimen et al.,
2010).  

\[ R - \text{COOH} + \text{CH}_3\text{OH} \rightarrow \text{CH}_3 - \text{COOR} + \text{H}_2\text{O} \]  
(Free fatty acid) (Methanol) (Fatty acid methyl ester) (Water)  

(4)

In fact, when Nagle and Lemke (1990) examined the effect of acid and alkaline catalysts for the conversion of microalgae oil, acid catalyst resulted in higher FAME yield than alkaline catalyst under the same reaction conditions. However, direct transesterification with acid catalyst is weak in the water existence. FAME yield was reduced with an increment in water content for direct transesterification using Chlorella biomass (Ehimen et al., 2010). FAME yield of 81.7 % was observed for the biomass with 0.7% water content, while only the yield of 19.5 % was for the biomass with 73 % water content.

4.3. Enzyme catalyzed method

As far as I know, the report of Tran et al (2013) is only direct method using enzyme catalyst. In the work, the direct conversion of the lipid in Chlorella vulgaris ESP-31 into FAME was performed using immobilized Burkholderia lipase as the catalyst after the microalgal biomass (water content of 86–91% and oil content 14–63%) was
pretreated by sonication to disrupt the cell walls. Lipase produced by an isolated strain \textit{Burkholderia} sp. C20 was immobilized on hybrid nanomaterials (Fe$_3$O$_4$–SiO$_2$) grafted to a long chain alkyl group as supporters (Liu et al., 2012 and Tran et al., 2012). The immobilized lipase worked well with wet microalgal biomass. In this method, it is important that the microalgal biomass has a high lipid content to achieve over 90% biodiesel conversion with a lower biocatalyst loading and better lipase recycle efficiency.

4.4. Supercritical method

The catalyst free method has been recently developed by employing supercritical methanol (Demirbas, 2009). This process is conducted at high reaction temperature and pressure (Kusdiana and Saka, 2004). Supercritical methanol can form a single phase with lipids, in contrast to the two phases at the normal conditions. This can be achieved because the decrease in the dielectric constant of methanol at supercritical state. In such supercritical method, the reaction was achieved in a very short time within 2–4 min, and FFA and TAG can be simultaneously esterified and transesterified. Also, supercritical method has a possibility of an alternative process to reduce cost associated with drying microalgae as the reaction is not inhibited under high water content. When this method
was using wet algal biomass containing about 90% water, 90% of FAME yield was reached (Patil et al., 2011a). Two-step process was also reported (Levine et al., 2010); in the first step, wet microalgal biomass reacts to hydrolyze intracellular lipid to FFA under subcritical water condition, and in the second step, the wet FFA rich solid are subjected to supercritical direct transesterification. However, as it now stands, this method may be disadvantageous due to the adverse process economics as well as safety concerns (Marchetti and Errazu, 2008).

4.5. Microwave- and ultrasound-assisted method

Microwave radiation is a non-ionizing radiation that influences molecular motions. A molecule with a dipole moment is sensitive to external electric fields. In microwave-assisted transesterification, methanol absorbs microwave radiation, redirecting its dipole quickly. This enables the destruction of the methanol-lipid interface (Patil et al., 2011b). The microwave transfers energy in an electromagnetic form and the oscillating microwave field tends to move continuously to polar ends of molecules or ions (Azcan and Danisman, 2008). Consequently, collisions between the moving molecules produce heat (Marra et al., 2010) and it can shorten reaction time (Lidstrom et al., 2001). Ultrasonic technology is also an effective method to enhance
mass transfer rate between immiscible phases (Pan et al., 2002). This high frequency sound wave compresses and stretches the molecular spacing of media in which it passes through, and these molecules remain continuously vibrating with the formation of fine micro-bubbles or micro-cavities (Ji et al., 2006 and Lam et al., 2010). Generally, homogenous 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. There are mainly two approaches which produce FAME from lipids such as TAG and FFA; one is the organic solvent–extraction of lipid from microalgae followed by transesterification of the lipid extracts, and the other is direct transesterification from microalgal biomass. The former has the disadvantage of the increased cost caused by using organic solvent. On the other hand, the latter considerably can reduce the process energy because it produces biodiesel without the need for organic solvent. In addition, other research showed that direct transesterification could convert phospholipid into FAME as well as TAG and FFA. Therefore, direct transesterification should be considered as a promising biodiesel production process from microalgae. There are many approaches of direct
transesterification as mentioned above. It is considered that the important keys to commercialization are the process energy. For the future, further researches are desired in order to develop the cost-effective technology.
Table 1-1. Different methods of biodiesel production (Leung et al., 2010)

<table>
<thead>
<tr>
<th>Methods</th>
<th>Definition</th>
<th>Advantage</th>
<th>Disadvantage</th>
<th>Problems of using in engines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct use and blending</td>
<td>Direct use as diesel fuel or blend with diesel fuel</td>
<td>Liquid nature-portability</td>
<td>Higher viscosity</td>
<td>Coking and trumpet formation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Heat content (~80% of diesel fuel)</td>
<td>Lower volatility</td>
<td>Carbon deposits</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Readily available; renewability</td>
<td>Reactivity of unsaturated hydrocarbon chains</td>
<td>Oil ring sticking; thickening and gelling of the lubricating oil</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower cetane number</td>
<td>Irregular injector needle sticking; incomplete combustion</td>
</tr>
<tr>
<td>Micro-emulsions</td>
<td>A colloidal equilibrium dispersion of optically isotropic fluid microstructures with dimensions generally in the 1–150 nm range formed spontaneously from two immiscible liquids and one or more ionic or non-ionic amphiphiles</td>
<td>Better spray patterns during combustion</td>
<td>Lower fuel viscosities</td>
<td>Heavy carbon deposits; increase lubrication oil viscosity</td>
</tr>
<tr>
<td>Thermal cracking (pyrolysis)</td>
<td>The conversion of long-chain and saturated substance (biomass basis) to biodiesel by means of heat</td>
<td>Chemically similar to petroleum-derived gasoline and diesel fuel</td>
<td>Energy intensive and hence higher cost</td>
<td>–</td>
</tr>
<tr>
<td>Transesterification</td>
<td>The reaction of a fat or oil with an alcohol in the presence of catalyst to form esters and glycerol</td>
<td>Renewability; higher cetane number; lower emissions; higher combustion efficiency</td>
<td>Disposal of by-product (glycerol and waste water)</td>
<td>–</td>
</tr>
</tbody>
</table>
Table 1-2. Lipid content and productivities of different microalgae species (Mata et al., 2010).

<table>
<thead>
<tr>
<th>Marine and freshwater microalgae species</th>
<th>Lipid content (% dry weight biomass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankistrodesmus sp.</td>
<td>24.0–31.0</td>
</tr>
<tr>
<td>Botryococcus braunii</td>
<td>25.0–75.0</td>
</tr>
<tr>
<td>Chaetoceros muelleri</td>
<td>33.6</td>
</tr>
<tr>
<td>Chlorella emersonii</td>
<td>25.0–63.0</td>
</tr>
<tr>
<td>Chlorella protothecoides</td>
<td>14.6–57.8</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>5.0–58.0</td>
</tr>
<tr>
<td>Chlorella sp.</td>
<td>10.0–48.0</td>
</tr>
<tr>
<td>Chlorococcum sp.</td>
<td>19.3</td>
</tr>
<tr>
<td>Cryptophyllum cohnii</td>
<td>20.0–51.1</td>
</tr>
<tr>
<td>Dunaliella salina</td>
<td>6.0–25.0</td>
</tr>
<tr>
<td>Dunaliella primolecta</td>
<td>23.1</td>
</tr>
<tr>
<td>Dunaliella tertiolecta</td>
<td>16.7–71.0</td>
</tr>
<tr>
<td>Dunaliella sp.</td>
<td>17.5–67.0</td>
</tr>
<tr>
<td>Ellipsoidion sp.</td>
<td>27.4</td>
</tr>
<tr>
<td>Euglena gracilis</td>
<td>14.0–20.0</td>
</tr>
<tr>
<td>Haematococcus pluvialis</td>
<td>25.0</td>
</tr>
<tr>
<td>Monallanthus salina</td>
<td>20.0–22.0</td>
</tr>
<tr>
<td>Nannochloris sp.</td>
<td>20.0–56.0</td>
</tr>
<tr>
<td>Nannochloropsis oculata</td>
<td>22.7–29.7</td>
</tr>
<tr>
<td>Nannochloropsis sp.</td>
<td>12.0–53.0</td>
</tr>
<tr>
<td>Neochloris oleoabundans</td>
<td>29.0–65.0</td>
</tr>
<tr>
<td>Nitzschia sp.</td>
<td>16.0–47.0</td>
</tr>
<tr>
<td>Oocystis pusilla</td>
<td>10.5</td>
</tr>
<tr>
<td>Pavlova lutheri</td>
<td>35.5</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>18.0–57.0</td>
</tr>
<tr>
<td>Scenedesmus obliquus</td>
<td>11.0–55.0</td>
</tr>
<tr>
<td>Scenedesmus quadricauda</td>
<td>1.9–18.4</td>
</tr>
<tr>
<td>Scenedesmus sp.</td>
<td>19.6–21.1</td>
</tr>
<tr>
<td>Skeletonema costatum</td>
<td>13.5–51.3</td>
</tr>
<tr>
<td>Spirulina platensis</td>
<td>4.0–16.6</td>
</tr>
<tr>
<td>Thalassiosira pseudonana</td>
<td>20.6</td>
</tr>
<tr>
<td>Tetraselmis suecica</td>
<td>8.5–23.0</td>
</tr>
</tbody>
</table>
Table 1-3. Comparison of microalgae with other biodiesel feedstocks (Mata et al., 2010).

<table>
<thead>
<tr>
<th>Plant source</th>
<th>Oil content (% dry weight biomass)</th>
<th>Oil yield (L oil/ha year)</th>
<th>Land use (m²/year/kg biodiesel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>44</td>
<td>172</td>
<td>66</td>
</tr>
<tr>
<td>Hemp</td>
<td>33</td>
<td>363</td>
<td>31</td>
</tr>
<tr>
<td>Soybean</td>
<td>18</td>
<td>636</td>
<td>18</td>
</tr>
<tr>
<td>Jatropha</td>
<td>28</td>
<td>741</td>
<td>15</td>
</tr>
<tr>
<td>Camelina</td>
<td>42</td>
<td>915</td>
<td>12</td>
</tr>
<tr>
<td>Canola/Rapeseed</td>
<td>41</td>
<td>974</td>
<td>12</td>
</tr>
<tr>
<td>Sunflower</td>
<td>40</td>
<td>1070</td>
<td>11</td>
</tr>
<tr>
<td>Castor</td>
<td>48</td>
<td>1307</td>
<td>9</td>
</tr>
<tr>
<td>Palm oil</td>
<td>36</td>
<td>5366</td>
<td>2</td>
</tr>
<tr>
<td>Microalgae (low oil content)</td>
<td>30</td>
<td>58,700</td>
<td>0.2</td>
</tr>
<tr>
<td>Microalgae (medium oil content)</td>
<td>50</td>
<td>97,800</td>
<td>0.1</td>
</tr>
<tr>
<td>Microalgae (high oil content)</td>
<td>70</td>
<td>136,900</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Table 1-4. Advantages and disadvantages of the main types of catalysts used for transesterification (Atadashi et al., 2013)

<table>
<thead>
<tr>
<th>Type of catalyst</th>
<th>Example</th>
<th>Merits</th>
<th>Demerits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneous catalyst</td>
<td>Alkaline catalysts: NaOH, KOH, CH₂ONa, CH₃OK</td>
<td>Less corrosive, high reaction rate</td>
<td>Formation of saponified product, emulsion formation, high water and energy consumption, huge, high wastewater discharges, high purification cost. Feedstocks are limited to 0.5 wt FFAs, not recycle</td>
</tr>
<tr>
<td>Acid catalysts:</td>
<td>H₂SO₄</td>
<td>Zero soap formation, the catalyst can be used to catalyze both esterification and transesterification simultaneously</td>
<td>More waste as a result of neutralization, recycling difficulty, high purification cost, energy consuming, low reaction rates</td>
</tr>
<tr>
<td>Heterogeneous catalysts</td>
<td>Solid alkaline catalysts and solid acid catalyst: MgO, CaO, ZnO KOH/NaY, CaO/MgO, Al₂O₃–SnO, KOH/K₂CO₃, Al₂O₃–ZnO, Ca(NO₃)₂/Al₂O₃, CaO/Al₂O₃, KOH/Al₂O₃, Al₂O₃/KI, Sr(NO₃)₂/ZnO, ZrO₂/SO₄²⁻–TiO₂/SO₄²⁻, ETS-10 zeolite, zeolite HY, and zeolite X</td>
<td>Environmentally friendly, easily recycle, less discharges, less separation difficulty, high purity glycerol, lower cost of separation, insignificant leaching of CaO/Al₂O₃</td>
<td>Leaching effects, catalysts preparation is complicated and expense relatively slow rates</td>
</tr>
<tr>
<td>Enzymes catalysts:</td>
<td>Candida antarctica B lipase, Rhizomucor mehei lipase, candida rugosa Pseudonas cepacia, M. mehei (Lypozyyme), M. mehei (Lypozyyme IM60), Aspergillus niger, P. fluorescens, R. Oryzae</td>
<td>Zero saponification products, nonpolluting, easily separable, lesser separation cost, high purity glycerol and biodiesel products, environmentally benign</td>
<td>Catalysts inhibition by water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Simple glycerol recovery</td>
<td>High cost of enzymes</td>
</tr>
</tbody>
</table>
Fig. 1-1. World marketed energy consumption (Ahmad et al., 2011).
Fig. 1-2. Energy consumption of each country (Saito, 2010).
Fig. 1-3. Common biodiesel production procedure from microalgae.
6. References


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Chapter 2 Two-step hydrolysis and esterification from wet microalgae
Chapter 3 Simultaneous hydrolysis-esterification from wet microalgae
Chapter 4 Overall conclusions
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