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Evaluation of Various New Technologies for Valuable Materials Production from Green Macroalgae

Somayeh Daneshvar Hosseini

February 2013

Doctoral Thesis at Osaka Prefecture University
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Somayeh Daneshvar Hosseini

Osaka Prefecture University

February 2013
Chapter 1

Introduction
1. 1. General background of biomass

1. 1. 1. Biomass

Generally biomass is the matter that can be derived directly or indirectly from plant which is utilized as energy or materials in a substantial amount. The resource base includes hundreds of thousands of plant species, terrestrial and aquatic, various agricultural, forestry and industrial residues and process waste, sewage and animal wastes. Many studies have suggested that biomass-derived energy will provide a greater share of the overall energy supply as the price of fossil fuels increase over the next several decades. The use of biomass as a source of energy and bio-chemicals is very attractive, since it can be a zero net CO\(_2\) energy source, and therefore does not contribute to increased greenhouse gas emission [1].

Biomass is quite various and different in its chemical and physical properties, moisture content, mechanical strength, etc.; the conversion technologies to materials and energy are also diversified. Three primary constituents of biomass include cellulose, hemicellulose, and lignin, and can contain other compounds (for example, extractives such as waxes and water insoluble oily compounds). Cellulose is a polymer of glucose, hemicellulose is an oligomer of both C\(_6\)- and C\(_5\)- sugars (mainly glucose and xylose), and lignin is a highly cross-linked polymer. The common molecular formula of cellulose, hemicellulose, and lignin are \([C_6(H_2O)_5]_n\), \([C_5(H_2O)_4]_n\), and \([C_{10}H_{12}O_3]_n\), respectively [2].

Biomass can be used either as materials or energy resources. It is utilized as diversified materials such as food, feed, fiber, feedstock, forest products, fertilizer, fine chemicals, and fuel (8F Use of biomass). Utilization as energy in the form of biofuels occurs on the final stage and biomass is decomposed into carbon dioxide or methane.
and emitted in the air.

With an annual production of biomass up to $1.7-2.0 \times 10^{11}$ tons in the world, only $6 \times 10^9$ tons of it is currently used for food and non-food applications [3]. Estimated amount of annual effectively available biomass (by type) in the USA (California) is shown in Figure 1-1 [4].

Considering the several major subjects including oil depletion, global warming, improving the standards of living, increasing rural economies, energy security, and foreigner currency lead the scientists to develop the cost effective and environmentally friendly conversion technologies and reduce the dependence on fossil fuels [5].

1.1.2. Aquatic plant biomass

Aquatic plant biomass is produced in freshwater and marine environments and has great potential for human uses. Most current aquatic plant biomass includes seed plants and algae.

Alga is photosynthetic organism that grows in many diverse habitats by harvesting energy from the sun to convert water and carbon dioxide into biomass. The easy adaptability to different growing conditions, higher photosynthetic activity, the possibility of growing either in fresh-water or marine-water, and the avoidance of land use make aquatic biomass more interesting than terrestrial biomass [6].

Algae include multi-cellular macroalgae (seaweeds) and unicellular microalgae (phytoplankton). Macroalgae inhabits mostly in seawater; it is classified as Phaeophyta or brown algae, Rhodophyta or red algae, and Chlorophyta or green algae based on the composition of photosynthetic pigments. Generally, the active biomass utilization is made with 220 species of red algae, 88 species of brown algae and 27 species of green
algae in the world [7]. The green macroalgae has evolutionary and biochemical affinity with higher plants.

The distribution of macroalgae is worldwide. They are abundant in coastal environments, primarily in nearshore coastal waters with suitable substrate for attachment. Macroalgae also occurs as floating forms in the open ocean, and floating seaweeds are considered one of the most important components of natural materials on the sea surface [8].

Macroalgae has been reported to contain more than 2,400 natural products of commercial importance to the pharmaceutical, biomedical, and nutraceutical industries [9]. Figure 1-2 shows the various utilizations of macroalgae in different industries [10].

They mainly consist of carbohydrates, polyunsaturated fatty acids (PUFAs), vitamins, minerals, dietary fibers [11, 12], and some of these can be extracted, while others (such as carbohydrates) can be converted to biological building block materials and energy.

Studies of the values of carbohydrate from different species belonging to three families of macroalgae show the concentration of carbohydrate is higher in most of the species of green macroalga [13]. However, this varies and is affected by geographic area, season of the year, and the temperature of the water [14].
Figure 1-1. Amount of annually effectively available biomass in the USA (California) [4].

Figure 1-2. Schematic diagram of utilizations of macroalgae in different industries.
1. 1. 3. Codium fragile

Codium fragile (*C. fragile*) or dead man's fingers is a green macroalgae named for its dark green color and soft, felt-like texture. It has earned the common name dead man's fingers or green sea fingers for its swollen, finger-shaped branches that float in the water, or hang down the sides of rocks and cliffs when the tide is out. Figure 1-3 shows the photo of *C. fragile*. *C. fragile* is a native to the coastal areas of Japan. However it has made its way around the world, growing along the coasts of Southwest Africa, Australia, southern Argentina, Chile, China, Denmark, England (particularly the British Isles), Ireland, Korea, Atlantic and Pacific coasts of North America, Norway, Netherlands, New Zealand, Scotland, and Sweden.

Even though the genus *Codium* is a very common species, it has attracted much attention as a result of its interesting chemical composition [15]. It was found that *C. fragile* contains vitamins, proteins, minerals, unusual fatty acids, sterols, polysaccharides, and especially essential C\textsubscript{16} and C\textsubscript{18} PUFAs [16-18]. However, the composition and level of fatty acids is different for different locations and under different environmental conditions [19]. Table 1-1 shows the selected chemical composition of *C. fragile*.

1. 2. Conversion techniques of biomass

There are many conversion technologies available for changing the quality of biomass (including marine biomass) to match its utilization purposes. They are physical, chemical and biological techniques. Figure 1-4 illustrates typical conversion technologies for variety of biomass. Generally, biomass and particularly marine biomass conversion techniques could be divided into two main groups of conventional
and innovative methods, which explain in detail as follows.

1. 2. 1. Conventional methods

Over the years, conventional methods have been used for extraction and production of useful compounds from algae. Direct solid-solvent and catalytic extraction techniques are examples of the conventional methods which have been used widely for extraction of valuable materials and production of biodiesel fuel from algae [20-22]. Generally organic solvents and particularly hexane have been used to extract the oily compounds. Extraction-transesterification, direct methanolysis and transesterification methods [23, 24] have also been used as conventional methods to produce bio-oil from algae. Most of these methods are based on the choice of solvent with the use of heat and/or agitation [25].

Microalgae have usually been used for conventional extraction treatments due to having higher amounts of lipid compared with macroalgae samples; however, efficient extraction of microalgae lipids is still a challenge. Another shortcoming of the conventional method is using acid base catalysts while their recover is difficult. On the other hand a large volume of wastewater and other solid wastes are produced. Conventional methods are time-consuming and have low selectivity [25]. Extraction of algal oils by conventional methods is both energy- and cost-intensive. The cost of biodiesel fuel production from algae oils through conventional transesterification methods is estimated to be about $23/gal [26]. These disadvantages can be overcome by application of the new environmentally friendly technologies.
Figure 1-3. Typical photograph of macroalgae, i.e. C. fragile.

Table 1-1. Chemical composition of C. fragile.

<table>
<thead>
<tr>
<th>Polysaccharides</th>
<th>Fatty acids</th>
<th>Sterols</th>
<th>Minerals</th>
<th>Others</th>
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<tbody>
<tr>
<td>D-mannose arabinogalactan</td>
<td>Palmitic acid</td>
<td>Fucosterol</td>
<td>Magnesium</td>
<td>Vitamins</td>
</tr>
<tr>
<td></td>
<td>Oleic acid</td>
<td>Isofucosterol</td>
<td>Barium</td>
<td>Proteins</td>
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<tr>
<td></td>
<td>Hexadecatrienoic acid</td>
<td>Cholesterol</td>
<td>Calcium</td>
<td>β-carotene</td>
</tr>
<tr>
<td></td>
<td>Linoleic acid</td>
<td>Sitostrol</td>
<td>Phosphorus</td>
<td>Siphonaxanthine</td>
</tr>
<tr>
<td></td>
<td>Linolenic acid</td>
<td></td>
<td>Sodium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arachidonic acid</td>
<td></td>
<td>Iron</td>
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<td></td>
<td>Behenic acid</td>
<td></td>
<td>Potassium</td>
<td></td>
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<tr>
<td></td>
<td>Myristic acid</td>
<td></td>
<td>Aluminum</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stearic acid</td>
<td></td>
<td>Lithium</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aluminum</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sodium</td>
<td></td>
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</table>
Figure 1-4. Various conversion and pretreatment technologies of biomass resources [5].
1. 2. 2. Innovative methods

There are increasing interest in application of innovative and green methods for conversion and treatment of variety of biomass including marine biomass. The most common techniques include using sub- and supercritical fluids (particularly water and ethanol), thermochemical, and sono-assisted treatment techniques. Each of these techniques has been explained in below.

1. 2. 2. 1. Subcritical water

The subcritical water technique is environmentally friendly which uses water at temperatures ranging between 100 °C and 374 °C under high pressure between 0.1 and 22 MPa to maintain water in the liquid state. Phase diagram of water is shown in Figure 1-5.

At a temperature of 25 ºC and a pressure of 0.1 MPa, water is a polar solvent with a density of 1000 kgm$^{-3}$, a dielectric constant, $\varepsilon$, of 79.73, and an ion production constant, $K_w$, of $1\times10^{-14}$ [27]. However, under high temperature and high pressure conditions, the characteristics of water change. Namely, the hydrogen bonding network between the molecules is weakened by the thermal motion of the molecules, resulting in dissociation of water and a higher concentration of both hydroxide and hydrogen ions in the water [28]. Figure 1-6 shows the scheme of water dissociation [29].

At near critical conditions, the value of the ion production increases considerably from $10^{-14}$ to $10^{11}$ mol$^2$/l$^2$ at about 247 ºC, and decreases sharply at temperatures higher than that temperature. The effect of temperature on ion production of water is shown in Figure 1-7 [30]. Water has maximum ion production at around 250 ºC under saturation vapor pressure. This indicates that subcritical water may possess the
Dielectric constant ($\varepsilon$) expresses the affinity of water, as a reaction media, to reaction materials. When water is heated at temperatures above 100 °C, under sufficient pressure to remain as liquid, its dielectric constant can be changed; changing temperature and pressure can control this value. Figure 1-8 shows the effect of temperature on dielectric constant [32]. For instance, water dielectric constant decreases from 80 (at room temperature) to 27 (at 250 °C) almost equaling to that of ethanol at ambient temperature [33].

These considerable manipulations of physicochemical parameters with pressure and temperature should be important in any application sensitive to the thermodynamic properties of water. These variations offer the possibility of using pressure and temperature to tune the properties of water to optimal values for a given chemical reaction. High temperatures and pressures actually induce a nonpolar solvent behavior of water. As sequence, organic compounds are completely miscible with water.

The subcritical water technique has a wide range of applications, such as extraction and wet oxidation of organic compounds [34]. In addition, this technique can be used for hydrolysis and conversion of biomass and carbohydrates to useful compounds [28, 35-37], treatment of hazardous organics [38], treatment and recovery of waste water [39], and in other applications. During this process, most organic compounds are converted to CO$_2$, N$_2$, and water soluble materials.
Figure 1-5. Phase diagram of water in P-T plane.

Figure 1-6. Scheme of water dissociation.
Figure 1-7. Effect of temperature on ion production constant of water vs. temperature [30].

Figure 1-8. Effect of temperature on dielectric constant of water at 30 MPa [32].
1. 2. 2. Subcritical ethanol

Sub and supercritical alcohols can be classed as a green, are readily available, cost-effective, have a low potential toxicity, are able to dissolve many organic and inorganic compounds, and most of all, can be recycled afterwards by distillation [40]. Since alcohols have low critical pressures ($P_c$) typically between 2.0 - 6.0 MPa, but high critical temperatures ($T_c$), between 200 and 300 °C, they can be used for the chemical degradation of (natural and synthetic) polymeric materials [41]. The critical temperature is usually high due to the abundance of hydrogen-bonding networks present within the solution and relatively higher amount of energy in the form of heat is required for breaking these bonds [42, 43].

In critical conditions, molecules in the substance have high kinetic energy like a gas and high density like a liquid. In addition, dielectric constant of sub and supercritical alcohol is lower than that of liquid. These properties in sub and supercritical media causing homogeneous (one-phase) reactions between sample and alcohol. Comparing with the traditional chemical methods for biomass treatment, this novel technique requires no catalyst and nearly complete conversions can be achieved in a very short time. Therefore, it could successfully overcome most problems in conventional chemical processes.

One of the interested alcohols for method is ethanol. The boiling point and critical point of ethanol has been reported at $T_b= 78.37$ °C, $P_c = 6.14$ MPa, and $T_c= 241$ °C, respectively. Low boiling point, much lower critical point, low dielectric constant of ethanol [44] for dissolve high molecular weight of products, and also low price of it as short-chain alcohol comparing to others, have made it promising solvent for sub and supercritical fluid treatment reactions. It has a wide range of applications,
such as biodiesel synthesis from biomass, non-catalytic transestrification and production of fatty acid methyl ester (FAME) from algae, extraction of cellulose from lignocellulosic and cellulosic biomass, pure fiber production, and chemical recycling processes [45 - 48].

1.2.3. Thermochemical method

The base of thermochemical conversion is pyrolysis process, which include all chemical changes occurring when heat is applied to a material in absence of oxygen. This method can be simply described as Figure 1-9. Thermochemical biomass conversion includes number of roots to produce useful chemicals and energy from the initial biomass feedstock (containing mainly cellulose, hemicellulose and lignin). The main products of marine biomass pyrolysis include water, char, gaseous products and bio-oils. Figure 1-10 shows the composition changing during pyrolysis reaction.

In laboratory scales, for the fundamental studies, thermogravimetric analysis (TGA) is most commonly used of pyrolysis reactions. This method measures the amount and rate of change in the weight of a material as a function of temperature or time in a controlled atmosphere. Figure 1-11-a and b show the typical TGA setup and mechanism of weight change in TGA instrument, respectively. Inside the TGA chamber, ceramic crucibles were used in order to minimize any thermal lag and to optimize heat transfer between thermocouples and crucibles. One microgram balance with platinum surface was placed between crucible and thermocouple with connection to the PC. The instrument will record the mass and the temperature changes in time. The N₂ gas is injected to the chamber to make the condition inert inside the crucible.

There are four different approaches for TGA which are explained as follow.
Heat (500 ~ 700 °C)

\[-(C_6H_{12}O_6)_{m} \rightarrow (H_2+CO+CH_4+ACl_{H_{12}}) + (H_2O+CH_3OH+CH_3COOH+) + C\]

Biomass  Gas  Liquid  Char

**Figure 1-9.** Typical pyrolysis reaction.

**Figure 1-10.** Biomass composition change during pyrolysis [5].
(1) **Constant heating rate technique (Conventional TGA)**

In this TGA approach, a constant heating rate is used throughout the experiment. Heating rates between 0.5 and 50 ºC/minute or less is often required to obtain any resolution enhancement from this approach.

(2) **Constant reaction rate TGA**

Constant reaction rate TGA is a high resolution approach originally developed by Rouquerol [49] and later improved by Paulik & Paulik [50]. In this approach the TGA heating rate is adjusted in order to maintain an operator selected constant rate of weight loss. Constant reaction rate TGA has proved most useful for samples which decompose reversibly.

(3) **Dynamic heating rate TGA (Dynamic high resolution TGA)**

Dynamic heating rate TGA is a high resolution approach (patented by TA Instruments) in which the both heating rate and rate of weight loss continuously vary during decomposition. The heating rate is decreased, however, as the rate of weight loss increases. The result is both enhanced resolution and productivity (generally faster than constant heating rate experiments).

Decomposition kinetics may be obtained from dynamic heating rate TGA experiments using a derivation of the Arrhenius equation [51].
**Figure 1-11-a.** Typical setup of TGA.

**Figure 1-11-b.** Schematic of weight change mechanism in TGA.
(4) Stepwise isothermal TGA

The fourth approach for TGA analysis is the stepwise isothermal analysis [52]. In this TGA technique, the operator defines a maximum heating rate and two weight losses per minute thresholds.

Several advantages of this technique are listed below:

- Sample held isothermal until transition completed.
- Excellent resolution of overlapping transitions.
- Permits careful control of reaction environment.
- Available on all thermal analysis instruments.

This method has also several disadvantages:

- Difficult method development. May require several scans to optimize run conditions
- Inappropriate parameter choices may produce artifacts
- Long run time
- Utility
- Routine analysis of similar samples

The TGA and generally thermochemical treatment reactions, are widely used for the study of composition of multicomponent systems, oxidative stability of materials, estimated lifetime of a product, the effect of reactive or corrosive atmospheres on materials, moisture and volatiles content of materials, and finally for the study of degradation kinetics of organic and inorganic compounds [53, 54].

1. 2. 2. 4. Ultrasonic assisted technique

Ultrasound is acoustic energy at a frequency above the normal upper human hearing limit of 18–20 kHz. When a liquid medium is subjected to high power
ultrasound, cavitation occurs [55, 56]. Cavitation is the production of microbubbles in liquid subjected to a large negative pressure [57]. Due to rectified diffusion, dissolved gas in the liquid becomes free gas in the form of microbubbles [58]. These microbubbles grow during the ultrasound rarefaction cycle, reaching an unstable size, and eventually collapse violently. This could be due to several factors such as collision of the microbubbles, presence of foreign bodies and gradients in the pressure waves [57]. The violent collapse of the microbubble causes the production of localized temperatures and pressures of up to 5000 ºC and 180 MPa, respectively [59]. Figure 1-12 describes the creation of stable cavitation bubbles. Ultrasound propagation in liquid also causes acoustic streaming. This promotes mixing and enhances the homogenous distribution of ultrasonic energy to the liquid [56, 60].

Ultrasonic cavitation is a physical phenomenon whose performance depends upon the frequency, intensity, solvent, temperature, external pressure, bubbled gas, and the direct and indirect ultrasonic application. Figure 1-13 shows the variation in threshold frequency versus intensity for aerated water and air-free water. As can be seen, ten times more power is required to induce cavitation in water at 400 kHz than at 10 kHz. The physical explanation for this lies in the fact that, at very high frequencies, the cycle of compression and decompression caused by the ultrasonic waves becomes so short that the molecules of the liquid cannot be separated to form a void and, thus, cavitation is no longer obtained [61].
Figure 1-12. Creation of stable cavitation bubbles (a) Displacement, (b) transient cavitation, (c) stable cavitation, (d) pressure [5].

Figure 1-13. Variation of intensity of sonication versus the threshold frequency for aerated water (left-hand graph) and air-free water [5].
Ultrasound assisted technology has been applied widely in various biological and chemical processes. The use of ultrasonic in starch hydrolysis has been reported as early as 1933 [62]. Depolymerization of macromolecules, such as starches [63, 64] and reduction of average molecular weight occur as a result of ultrasonication treatment [65]. In these cases, the cavitation breaks the primary backbone bonds resulting in smaller molecules. Ultrasound could be used to enhance particle size reduction, fastest gelatinization, and fractionation of sample into its compounds [66, 67]. It also enhanced the production of ethanol and reduced the use of enzymes during the simultaneous saccharification and fermentation reactions [68, 69].

1.3. The aim of the thesis

The aim of this study is to evaluate and develop an efficient environmentally friendly technique for conversion of \emph{C. fragile} as model of green macroalgae, a low-cost and abundant aquatic biomass, into valuable bio-compounds such as soluble sugars, fatty acids, organic acids, and pure fibers. Furthermore, this study provides sufficient information in order to compare between the effect of different innovative methods on conversion and production yield.

This thesis contains seven chapters. The main focus of each chapter is summarized as follows:

Chapter 1 provides general background of this thesis. In the first part of this chapter, a general introduction about biomass and aquatic biomass, macroalgae, and finally \emph{C. fragile} as model of this group is given, and its composition is presented. In the next part the properties of various innovative conversion techniques related to this thesis are described in detail. The various fields of marine biomass, chemical and material
cycling, and related researches on each method are reviewed.

Chapter 2 dedicates to evaluate the hydrolysis and decomposition of C. fragile as model of green macroalgae under subcritical water conditions in order to obtain value-added materials. Effect of various parameters on the hydrolysis and decomposition reactions is studied. Production of various water-soluble compounds such as organic acids and soluble sugars, and also elemental composition of residual solid and its energy density evaluated.

Chapter 3 deals with treatment and liquefaction of C. fragile under subcritical ethanol conditions. The bio-oil and solid residue composition were investigated in detail. Significant amounts of fatty acid ethyl ester extracted and identified by this method. The fiber content of residual solid and its purity was also investigated.

Chapter 4 describes thermochemical techniques as another innovative method to evaluate the pyrolysis reaction of C. fragile. Furthermore, the kinetics of pyrolysis reactions of C. fragile was studied. The value of activation energies for three pyrolysis stages of C. fragile were obtained with the methods of Flynn, Ozawa, Wall (FOW), Friedman, Kissinger-Akahira-Sunose (KAS), and Coats-Redfern.

Chapter 5 devotes to evaluate the effect of ultrasonic irradiations on C. fragile samples. The efficiency of the method has been evaluated by saccharification yield, changing reaction solvent, bio-oil production, and the purity of extracted fiber. Utilization of ethanol for this method causes to produce the valuable fatty acids ethyl ester. High energy density of residual solids obtained after reaction also makes it as a good alternative resource for energy production via direct combustion.

Chapter 6 simulates the algae conversion pilot plant and estimate the cost and benefit of the process by understanding the mass balance and energy balance.
Chapter 7 summarizes the conclusions of this thesis.

**Nomenclature**

\textit{PUFAs} Polyunsaturated fatty acids
\textit{C. fragile} Codium fragile
\(\varepsilon\) Dielectric constant
\(\{} ^{\circ}C\) Degree Celsius
\(MPa\) Mega pascal
\(K_w\) Water ion production constant
\(P_c\) Critical pressure
\(T_c\) Critical temperature
\(T_b\) Boiling temperature
\textit{FAME} Fatty acid methyl ester
\textit{TGA} Thermogravimetric analysis
\textit{DTG} Derivative thermogravimetric
\textit{wt} Weight

**References**


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Chapter 2

Subcritical water conversion of green macroalgae to value-added materials
2.1. Introduction

The interest in alternative energy sources encourages the scientific community to implement technologies that use not only terrestrial but also aquatic biomass for the production of biofuel and bio-chemicals. One of the main aquatic biomass resources is algae, which are photosynthetic organisms that grow in many diverse habitats by harvesting energy from the sun to convert water and carbon dioxide into biomass. The easy adaptability to different growing conditions, higher photosynthetic activity, the possibility of growing either in fresh-water or sea-water, and the avoidance of land use make aquatic biomass more interesting than terrestrial biomass [1].

Among different type of aquatic biomass, because of the chemical composition of macroalgae [2, 3] they have applied in pharmaceutical, biomedical, and nutraceutical industries [4]. Studies of the values of carbohydrate from different species belonging to three families of macroalgae show the concentration of carbohydrate is higher in most of the species of green macroalgea [5]. However this varies and is affected by geographic area, season of the year, and the temperature of the water [6]. Kumar et al. [5] reported a range from 29.5 to 43.4% of carbohydrate contain in six species of green macroalgae. However Haroon et al. [7] investigated the carbohydrate content of green macroalgae about 54.71±8.17%.

To the best of our knowledge, little information has been made available to date regarding treatment and conversion of macroalgae [8]. Few studies have examined the conversion and decomposition of the carbohydrate fraction of the macroalgae. They have mainly focused on utilization and extraction of specific small molecules such as lutein, β-carotene, and membrane proteins, rather than as raw materials (carbohydrate portions) for ethanol or butanol production [9-14]. In fact, most researchers have
focused on the use of microalgae for the production of biodiesel [1, 8, 15-18]. On the other hand, the available traditional methods are time consuming and use organic solvents [19, 20].

To solve the problems of traditional methods, recently developed innovative and green methods have attracted more attention. The subcritical water technique is an environmentally friendly technique for extractions as well as reactions at temperatures ranging between 100 °C and 374 °C. In fact, tunable physicochemical properties of water in subcritical region, make it environmentally friendly medium for utilization in a variety of chemical processes.

We have used *C. fragile* as a model of green macroalgae which belongs to the *codiales* category. At inner sea of Rinku Park, in Osaka Bay, Japan, the maximum production of macroalgae is about 8.6 tons-wet/day, as calculated by an ecosystem model developed by Nakatani et. al [21]. To date, the application of such a huge amount of macroalgae has been limited to the production of biomethane gas [22].

In the present work, I have taken advantage of water below its critical point to study hydrolysis and decomposition reactions of *C. fragile* as well as production of valuable materials such as soluble sugars.

### 2. 2. Experimental

#### 2. 2. 1. Materials

Fresh *C. fragile* was collected at Rinku Park in Osaka Bay, Japan during May 2010. The average water content of *C. fragile* just after harvesting was found to be 93%. The remaining amount, similar to other marine biomass, is cellulose with some incorporation of hemicelluloses and a wide variety of acidic polysaccharides [23].

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proteins, lipids, chlorophylls, and other organic and inorganic compounds.

As well as water and organic compounds, C. fragile contains considerable amounts of inorganic compounds. To investigate the non-volatile inorganic content of samples, a Yamato Furnace model FM25 was used for combustion under atmospheric conditions. For this purpose approximately 5 g of dried sample was placed in a Yamato ceramics muffle and placed in a preheated furnace with temperatures ranging 150 to 600 °C for three hours to burn and reach a constant weight.

2.2.2. Chemicals

Phenol and sulfuric acid were purchased from Kishida Kagaku (Japan). Other reagents and solvents were purchased from Wako Chemical Industries, Ltd. (Japan).

2.2.3. Procedure

The collected fresh C. fragile was washed and cleaned of foreign debris and towel-dried. It was then divided into 150 g samples in ziplock bags. Finally it was frozen at -40 °C for 2 hours and stored at -10 °C until use.

The sample was ground before subcritical water treatment to attain homogenous slurry. In a typical experiment, a stainless steel tube (SUS316, i.d. 16.5 × 150.4 mm length) with a Swagelok fitting (Swagelok AG) was used as a batch reactor for subcritical water reactions. An accurately weighed amount of 23 g of slurry sample was charged into the reactor. Then it was capped tightly and immersed in a preheated oil bath (Thomas Kagaku Co. Ltd., Celsius M type) at temperatures ranging from 100 to 200 °C or in a preheated salt bath at temperatures ranging from 200 to 240 °C for different reaction times. The reaction time included the heat-up time. The reactor was
shaken both before and during immersion in the salt/oil bath. In fact, the reactor shaking had a marked effect on the rate of heat-up, and could enable it to reach steady state conditions in a very short time. However, this depends on the reactor size and its thickness as well as temperature [24, 25]. After the desired residence time, the reactor was removed from the oil/salt bath and quickly quenched by immersing it into a water bath at room temperature. The reaction pressure was estimated from a steam table [26].

According to the previous literature, most of the biomass sample contained some amount of oily compounds, which can easily dissolve in hexane. Therefore, we tried to extract oily compounds using hexane. All of the reactor contents were poured into a test tube, into which 1 mL hexane was added. After 15 min shaking and centrifuging at 4000 rpm for 15 min, the hexane phase was separated. This extraction step was repeated three times (total hexane volume 3 mL). The hexane-soluble phase was placed at room temperature to evaporate the hexane and obtain a constant weight. During the next step, the aqueous phase and residual solid were isolated. The aqueous phase was increased to 25 mL and filtered with a 0.2 μm filter. Residual solid phase placed in an oven at 60 °C for 48 hours to dry to a constant weight.

2. 2. 4. Analysis

2. 2. 4. 1. Aqueous phase

The amount of TOC in the aqueous phase was determined with an Automatic TOC Analyzer (Toray model TOC-150, Toray Engineering Co., Ltd., Japan). The aqueous phase was appropriately diluted before analysis.

The amount of total soluble sugars in the aqueous phase was determined by a photometric method (phenol-sulfuric acid assay), using D-glucose as a standard curve
Phenol reagent, 5% (v/v) (0.4 mL) was added to appropriately diluted aqueous solutions (0.4 mL). Samples were then mixed with 2 mL of concentrated sulfuric acid using a bottle top dispenser. The solutions were mixed immediately by shaking and allowed to cool to room temperature. After 10 min, the absorbance at 490 nm was determined using a double-beam spectrophotometer (UV-1700 Pharmas Pec-Shimadzu).

To identify products other than sugars in the aqueous phase, liquid-liquid extraction was used. This method is usually used for the extraction of semi-non-polar compounds from water. When extracting compounds that are relatively soluble in water, a salting-out technique is utilized to increase extraction yield. Adding salt to an aqueous phase decreases the solvation power of the solution and the solubility of target compounds and increases the partitioning into the organic solvent. Therefore, 2.0 g NaCl was added to 22 mL aqueous solution, then 4 mL ethyl acetate was added (twice) and shaken for 30 min. Finally, samples were centrifuged at 4000 rpm for 15 min to separate the ethyl acetate phase (total 8 mL) and this was then evaporated to reduce the volume to 1 mL. The ethyl acetate phase was analyzed using a GC-MS analyzer. A Hewlett-Packard HP 6890 gas chromatograph coupled to a quadrupole mass spectrometer HP 5973 equipped with a mass selective detector (MSD) was used for this purpose. A 30 m × 0.25 mm diameter capillary column (Agilent 19091N-133) coated with a 0.25 μm thick film of 5% polyethylene glycol (HP-INNOWax) was used to separate the decomposition products. Helium (99.999%) was used as a carrier gas at a constant flow rate of 1 mL/min. Initially, the oven temperature was held at 35 °C for 10 min and was raised to 300 °C, at a rate of 10 °C/min.

The pH of the aqueous solutions before and after treatment was measured by a
KRK pH meter KP-2Z and a KRK pH/ORP electrode CE-105, Kasahara Chemical Instruments Corp., Japan.

Elemental analysis of the aqueous phase was carried out using an inductively coupled plasma (ICP) atomic emission spectrophotometer (SPS-7800 Plasma spectrometer, Seiko, Japan).

2. 2. 4. 2. Residual solid phase

1.5–2 mg of dried residual solid and also dried initial sample (for comparison) was used to find the carbon, hydrogen, nitrogen, sulfur, and oxygen content. Analysis was carried out with a CHNS (O) analyzer (Perkin-Elmer, model 2400, USA). All samples were analyzed in triplicate.

To identify the inorganic content (ash) of the residual solid after subcritical water treatment, a Yamato Furnace model FM25 and a ceramic muffle were used for combustion under atmospheric conditions.

2. 2.4.3. Hexane soluble phase

Because insufficient amounts of hexane solubles were found in this study, qualitative analyses were not carried out.

2. 3. Results and discussion

2. 3. 1. Specification of initial C. fragile

Figure 2-1 shows the effect of combustion temperature on the residue of the C. fragile for 3 hours of combustion. The sample weight loss increased with temperature up to ~ 64% and leveled off at temperatures above 500 °C. Obviously, the lost and
residual amounts can be attributed to the organic and non-volatile inorganic composition of the sample, respectively. The maximum inorganic composition of the samples was identified as approximately 36% based on dry matter.

The organic composition of *C. fragile* was identified as 39.7% carbon, 6.1% hydrogen, 4.1% nitrogen, and 3.9% sulfur (calculations were normalized based on the organic content of the solid residue) (Table 2-1). These data are consistent with those previously reported for similar macroalgae [23].

2. 3. 2. **Subcritical water treatment**

2. 3. 2. 1. **Effect of residence time**

To determine the optimum residence time for subcritical water treatment of *C. fragile*, the effect of reaction time between 5 and 20 min at 190 °C was studied. Results of TOC analysis of the aqueous phase before and after subcritical water treatment are shown in Figure 2-2. After 5 min reaction at 190 °C, the TOC amount reached 3.7%. However, continuing the reaction time up to 10 min increased this to 8.5% and it then leveled off at longer residence times. These results show that a 10 min residence time is optimal for studying the effect of reaction temperature.
Figure 2-1. Effect of combustion temperature on residue of *C. fragile* under atmospheric conditions.

Figure 2-2. Effect of subcritical water time on TOC yield of *C. fragile* at 190 °C.
Table 2-1. Ultimate analysis of *C. fragile* and its higher heating values for solid samples before and after subcritical water treatment at 10 min residence time. Values were normalized by the organic content of the solid residue (i.e. g element/100 g of organic part of the solid residue).

<table>
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<th>Temperature (°C)</th>
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<th>RSD %</th>
<th>H</th>
<th>RSD %</th>
<th>N</th>
<th>RSD %</th>
<th>S</th>
<th>RSD %</th>
<th>O</th>
<th>RSD %</th>
<th>HHV (MJ kg⁻¹)</th>
<th>Eq. (2-1)</th>
<th>Eq. (2-2)</th>
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<td>±0.5</td>
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2. 3. 2. 2. Effect of temperature

Subcritical water reactions were carried out at temperatures ranging between 100 and 240 °C for 10 min residence time. Figure 2-3 shows a photograph of the *C. fragile* samples before and after treatment at different temperatures. Clearly, with increasing temperature, the color of the slurry phase changed from light green to dark red brown. The color of the solid phase also changed from green to dark brown and its amount decreases with increasing temperature.

Changes in the color of the aqueous phase (Figure 2-3) are mainly due to the hydrolysis and decomposition of chlorophyll in the sample. Obviously, at relatively high temperatures, the amount of solid was reduced by increasing the hydrolysis and decomposition of the sample. The constant amount of solid phase at higher temperatures was attributed to the inorganic composition of the sample.

Figure 2-4 shows the results for the inorganic and organic contents of the residual solid after subcritical water treatment and also total residual solid. At 100 °C the total residual solid amount decreased by ~ 30% but, up to 160 °C, there was little change in the amount of total residual solid. Above 160 °C, the total residual solid declined sharply to ~ 70% at 210 °C and then leveled off. As shown in Figure 2-4, the loss in total solid residue at higher temperatures was attributed mainly to the hydrolysis and decomposition of the organic fraction.

To obtain the un-reacted content as well as inorganic composition of the samples, we examined the ash content (at 600 °C) for each residue after subcritical water treatment (Figure 2-4).
Figure 2-3. Photograph of *C. fragile* before (control) and after subcritical water treatment as a function of temperature for 10 min residence time.
Results (Figure 2-4) show the amount of inorganic content after subcritical water treatment was ~36% and there was little change up to 210 °C. However, this amount decreased at higher temperatures and reached 20% at 240 °C. From the results for total residual solids and the inorganic content of the solid residue, we concluded that at higher temperatures the total solid residue strongly decreased because of hydrolysis of largely residual organic solids. At temperatures higher than 210 °C, the amount of total residual solid leveled off, indicating the existence of inorganic content that cannot decompose or hydrolyze even at higher temperatures.

The amount of hexane-soluble material and the pH of the aqueous solutions as a function of temperature are shown in Figure 2-5. As shown in Figure 2-5, the amounts of hexane-soluble material increased with temperature up to 2% at 210 °C and then leveled off at higher temperatures, showing that this type of algae has less oily compounds than those reported elsewhere [2]. It should be noted that there is a possibility for hydrolysis of oily compounds under subcritical water conditions.

Figure 2-5 also shows the effect of treatment temperature on the pH of the aqueous solution. pH was around 5 at or below 170 °C and then decreased to 4 with increasing temperature up to 210 °C. There was no change in the pH of the sample at higher temperatures. pH change could be attributed to the production of acidic products from decomposition and hydrolysis of C. fragile. With the decreasing pH at higher temperature it is possible to have auto-catalytic reactions, which would accelerate the hydrolysis and decomposition reactions [27, 28].
Figure 2-4. Effect of subcritical water temperature on yield of residual organic and inorganic solid (10 min residence time)

Figure 2-5. Subcritical water temperature course curves for amounts of hexane-soluble material and pH (10 min residence time)
2. 3. 3. Aqueous phase

2. 3. 3. 1. Total organic carbon (TOC)

The TOC in the aqueous phase before and after treatment by subcritical water is shown in Figure 2-6. TOC did not significantly change with temperature up to 140 °C. However, with further increases in temperature, the TOC amount increased and reached a maximum of 14% at 210 °C. The majority of the water soluble organic carbon seems to be derived from decomposition of macromolecules of the algae. Further increases in temperature caused the TOC to decrease to 11% at 240 °C through gasification and carbonization reactions.

2. 3. 3. 2. Total soluble sugars

The cellulosic part of the *C. fragile* could be converted into a wide range of water soluble sugars (such as poly, oligo, and mono saccharides) by hydrolysis under subcritical water conditions. Figure 2-7 shows the amount of total water soluble sugars as a function of temperature at 10 min residence time. The results show that production of the sugars began at 170 °C and reached a maximum amount at 210 °C. By calculation, more than 50% of the solid algae were converted into water soluble sugars. However, at temperatures higher than 210 °C, because of further rehydration and decomposition reactions [29], the amount of total soluble sugars sharply decreased to 7% at 240 °C.

Obviously, water soluble sugars are more attractive for fermentation reactions than solid samples. Therefore, subcritical water treatment could play an important role as a pre-treatment for cellulose-containing biomass samples in fermentation processes.
Figure 2-6. Effect of subcritical water temperature on TOC yield of \textit{C. fragile} for 10 min residence time.

Figure 2-7. Effect of subcritical water temperature on total soluble sugar yield in the aqueous phase obtained from hydrolysis of \textit{C. fragile} at 10 min residence time.
2. 3. 3. 3. Other organic compounds

Several other organic compounds have been identified from decomposition of *C. fragile* under subcritical water conditions. Figure 2-8 shows a typical GC-MS chromatograph of the aqueous phase after treatment at 205 °C for 10 min. Identified decomposition products have also been shown in Figure 2-8, most of which were mainly produced from decomposition of the cellulosic part of *C. fragile*. There are several compounds that we have not yet identified.

In this research work we did not quantify these products. However, from the peak areas in GC-MS chromatographs, it can be estimated that some valuable bio-products could be produced in higher yields. Identified bio-products could be applied in different industries such as nylon, food, fuel, and leather industries [30-33].

2. 3. 3. 4. Inorganic compositions

Some of the inorganic compounds present in the sample could be extracted and dissolved in water. Therefore, the inorganic elements of the liquid phase before and after subcritical water treatment were evaluated to determine the inorganic composition. Results are presented in Figure 2-9. Nine main elements were identified: Ca, Mg, Na, K, P, Al, Ba, B, and Si. The results demonstrate that even without treatment by only crushing the sample, some part of the inorganic compounds could be released to water. However, with treatment under subcritical water conditions, relatively high amounts of the inorganic compounds could be extracted into water because of decomposition of cell walls and macromolecular structures. The amount of inorganic elements in the aqueous phase after treatment at 190 °C was approximately 1.2 times greater than in untreated samples.
Figure 2-8. Typical GC-MS chromatograph of the aqueous phase of *C. fragile* after treatment by subcritical water at 205 °C for 10 min residence time.

Figure 2-9. Comparison of inorganic composition in the aqueous phase before and after treatment of *C. fragile* under subcritical water conditions (190 °C for 10 min).
2. 3. 4. Solid residue phase

2. 3. 4. 1. Elemental analysis

Residual solids after treatment as well as initial C. fragile were subjected to ultimate analysis. The results of the analysis are shown in Table 2-1. The values in Table 2-1 were normalized based on the organic content of the solid residue (i.e. g element/100 g of the organic part of the solid residue).

Table 2-1 shows that wt% of carbon did not vary significantly with increasing temperature up to 150 °C. However, at higher temperatures this increased sharply and reached approximately 60.3 wt% at 240 °C. This means that the amount of fixed carbon after treatment by subcritical water increases with temperature. Fixed carbon, by definition, is the carbon content of a sample obtained from oxidation and combustion of the cellulosic part of a dried sample. The increase in fixed carbon with temperature increases the energy density and consequently increases the heating value of the residual solid after treatment.

On the other hand, the hydrogen wt% decreased with increasing subcritical water temperature from 6.1 to 3.5 wt%. The results showed that the nitrogen wt% of C. fragile varied only slightly between 4.1–7.1 wt% and increased with temperature. Variation of sulfur content was between 3.9–1.3 wt% and essentially decreased with increasing subcritical water temperature. Results showed that the oxygen content of the solid residue after subcritical water treatment decreased from 46.3 to 29.7 wt%. The change in the composition of the sample after treatment was due to hydrolysis and decomposition reactions of the C. fragile.

2. 3. 4. 2. Higher heating value (HHVs) of solid residue
The HHVs of biomass can be either measured experimentally by bomb calorimeter [34] or calculated from the ultimate and/or proximate analyses. A number of formulae have been proposed for calculating HHVs of biomass based on proximate analysis [35-37], amongst which Goutal’s formula is the oldest and best known [38].

Meanwhile, there are various formulae for estimating the HHVs of different biomass sources, using their ultimate analysis data. In this study, I used data obtained from the CHNS analyzer (ultimate analysis data) for estimating its HHVs with the aid of three main formulae:

1) The HHVs calculated based on the formula proposed by Demirbas [34]:

\[
\text{HHV (MJ kg}^{-1}\text{)} = \{33.5[C] + 142.3[H] - 15.4[O] - 14.5[N]\} \times 10^{-2} \quad (2-1)
\]

This was derived by using the oxidation heats of C and H and the reduction heat of O, assuming that the effect of the N content of a biomass fuel on its HHV was negative.

2) Another calculation is based on Dulong’s formula [39] which defines HHVs as a function of the C, H, O, and S contents of the sample:

\[
\text{HHV (MJ kg}^{-1}\text{)} = \{337 \times C + 1428 (H - O/8) + 95 \times S\} \times 10^{-3} \quad (2-2)
\]

3) Finally, HHVs were calculated from the C, H, O, N, and S contents of the sample using Boie’s formula [40]:

\[
\text{HHV (MJ kg}^{-1}\text{)} = \{151.2 \times C + 499.77 \times H + 45.0 \times S - 47.7 \times O + 27.0 \times N\} \times 2.326 \times 10^{-3} \quad (2-3)
\]

The results are listed in Table 2-1.

By using these formulae, it was generally realized that the HHVs of the initial sample were relatively low. Treatment of the samples under subcritical water conditions (up to approximately 180 °C) showed no change in the HHVs of the solid phases or, rather, declined somewhat for unknown reasons. At temperatures higher than 180 °C,
the HHVs of the residual solid increased with increasing subcritical water temperature. The results show that there is good agreement between these three formulae for my sample.

Comparison between these formulae, i.e. Demirbas formula (2-1), Dulong formula (2-2), and Boie formula (2-3) indicates that latter predicts somewhat higher HHVs for *C. fragile* even for the initial sample. However, there is excellent correlation between formulae 2-1 and 2-2.

In fact, the HHV for the algae is relatively high due to its high carbon content. After subcritical water treatment this amount increases relatively with temperature, because of the increasing carbon content of the solid residue, which causes a higher energy density. This shows that even solid residue after treatment can be a good resource as an alternative fuel and can be used for energy production.

### 2. 4. Conclusions

The results presented in this chapter show that subcritical water technology is a green process with potential for treatment of marine biomass. The hydrolysis reactions were effectively carried out without using organic solvents, acid, base, or enzymes. The majority of the solid cellulosic macromolecules of the biomass were hydrolyzed and solubilized into the aqueous phase. Obviously, water soluble sugars are more attractive for fermentation reactions than solid samples. Apart from soluble sugars, several other compounds were also produced from decomposition of the algae, which can be classified as building block materials.

The amount of oil extracted was low but it is possible that the oily compounds may hydrolyze to water-soluble compounds.
Treatment of the algae under subcritical water conditions shows promising results not only for solubilization of solid biomass but also conversion of solids into higher energy values. Based on ultimate analysis results, water insoluble residual solids after treatment had a high carbon content, which caused an increased energy density in the solid, along with increased higher heating values. This shows that the solid residue can be a very good alternative energy resource to algae itself.

In this research work I used *C. fragile* as a model for marine biomass. The hydrolysis of other marine biomass is possible under subcritical water conditions. Finally, this technique was carried out in a batch type reactor and it may be possible to use continuous plug flow reactors for marine biomass samples.

**Nomenclature**

*C. fragile*  *Codium fragile*

*TOC*  Total organic carbon

*HHV*  Higher heating value

**References**


[38] M. Goutal, Compt. Rend. 135, 477, 1902.


Chapter 3

Liquefaction of green macroalgae in subcritical ethanol
3. 1. Introduction

Many efforts have been made to liquefy and convert marine biomass to fuels and bio-chemicals using conventional and innovative technologies such as solvent extraction, pyrolysis, hydrothermal liquefaction, sub and supercritical fluid technologies [1-6]. The application of conventional methods to produce biodiesel from microalgae is by lipid extraction with solvent and then transesterification of lipids with methanol/ethanol. This process requires a microalga of high lipid content, but the efficient extraction of algal lipids is still a challenge. Based on available reports, among the innovative methods, special characteristics of subcritical ethanol (such as low critical point and dielectric constant [7]) have made it promising solvent for the liquefaction of marine biomass.

As mentioned in chapter 1, extraction of oily compound of C. fragile after treatment by subcritical water technique is difficult due to the intensive hydrolysis level by this method. On the other hand, huge amount of wastewaters produce after reaction. Thus far, there are very few studies about the treatment of macroalgae using alcohols (under their critical points) as solvents to produce valuable bio compounds.

In the present study, to solve these problems we take the advantages of subcritical ethanol technique in a batch reactor to liquefy the C. fragile and produce bio-oil compound in higher yield. The ease of recovery and recycling of ethanol waste after reaction is another advantage of this method.

Effects of temperature and time on the liquefaction yields were studied. The bio-oil composition and solid residues were analyzed in detail using elemental analysis, Fourier transform infrared (FTIR) spectroscopy, gas chromatography–mass spectrometry (GC–MS).
3. 2. Materials and methods

3. 2. 1. Materials

Phenol and sulfuric acid were purchased from Kishida Kagaku (Japan). Other reagents and solvents were purchased from Wako Chemical Industries, Ltd. (Japan).

Fresh *C. fragile* was collected at Rinku Park in Osaka Bay, Japan during 2011. The average water content of *C. fragile* just after harvesting was found to be 93%. The remaining amount, similar to other marine biomass, is cellulose with some incorporation of hemicelluloses and a wide variety of acidic polysaccharides [8], proteins, lipids, chlorophylls, and other organic and inorganic compounds.

3. 2. 2. Procedure

The collected fresh *C. fragile* was washed and cleaned of foreign debris and towel-dried. It was then complete dried using Freeze-drying method (TAITEC freeze dryer VD-16).

Figure 3-1 depicts the details of the procedure for treatment and separating liquefaction products. Briefly, in a typical experiment, a stainless steel tube (SUS316, i.d. 16.5 × 150.4 mm length) with a Swagelok fitting (Swagelok AG) was used as a batch reactor for subcritical ethanol reactions. An accurately weighed amount of about 2 g of freeze dried sample and 18 ml of pure ethanol were charged into the reactor. Then it was capped tightly and immersed in a preheated oil bath (Thomas Kagaku Co. Ltd., Celsius M type) at temperatures ranging from 100 to 205 °C for different reaction times ranging 2 to 15 min. The reaction time included the heat-up time. After the desired residence time, the reactor was quickly cooled down to room temperature by immersing it into a cold water bath. The reaction pressure was estimated from a steam table [9].
All of the reactor contents were poured into a test tube. After 15 min shaking and centrifuging at 4000 rpm for 15 min, the ethanol phase was separated. This extraction step was repeated three times (total ethanol phase volume 25 mL). A portion of the ethanol-soluble phase was placed at room temperature to evaporate and obtain ethanol-soluble yield. Another portion of this phase was used for identification of bio-oil composition. In the next step, the residual solids were washed three times with pure water to final volume of 25 mL to release water-soluble compound out, and filtered with a 0.2 μm filter. Residual solid phase placed in an oven at 60 °C for 48 h to dry to a constant weight.

3. 2. 3. Analysis

The amount of TOC in the aqueous phase was determined with an Automatic TOC Analyzer (Toray model TOC-150, Toray Engineering Co., Ltd., Japan). The aqueous phase was appropriately diluted before analysis.

The amount of total soluble sugars in the aqueous phase was determined by a photometric method (phenol-sulfuric acid assay), using D-glucose as a standard curve [3]. This analysis procedure has been reported in my previous report [5]. Double-beam spectrophotometer (UV-1700 Pharmas Pec- Shimadzu) was used for this purpose.

Concentration of organic acids in the aqueous and ethanol phases were determined by HPLC, using a pump (Shimadzu LC-20AD, Shimadzu Co., Japan) with ODS (STR ODS-II, Shinwa Chemical Ind. Ltd., 10L × 4.6 mm I.D.), guard column (SCR-102H, 6.0 × 50 mm) and ion-exclusion chromatography columns (Shim-pack SCR-102H, 8 mm × 300 mm, Shimadzu Co., Japan) in series and their detection affected using post-column pH-buffered electroconductivity detection (Shimadzu...
COD-10A VP, Shimadzu Co., Japan). The mobile phase was 5 mM p-toluensulfonic acid solution at a flow rate of 0.8 mL/min. Mixtures of 5 mM p-toluensulfonic, 20 mM Bis-Tris and 100 μM EDTA were used as post-column reagents, all at flow rates of 0.8 mL/min. The thermostat (CTO-20AC) temperature was kept at 45 ºC.

A Hewlett-Packard HP 6890 gas chromatograph coupled to a quadrupole mass spectrometer HP 5973 equipped with a mass selective detector (MSD) was used for identification of bio-oil composition. A 30 m × 0.25 mm diameter capillary column (Agilent 19091N-133) coated with a 0.5 μm thick film of 5% phenyl methyl siloxane (HP-5 Trace Analysis) was used to separate the decomposition products. Helium (99.999%) was used as a carrier gas at a constant flow rate of 1 mL/min. Initially, the oven temperature was held at 50 °C for 2 min and was raised to 290 °C, at a rate of 7 °C/min.

The element composition of the dried residual solid and also dried initial sample (for comparison) was analyzed by CHN analyzer (YANACO Co., Ltd., MT-6). The higher heating value was calculated for each sample using the equation (see equation 3-1) [10]. These calculations are based on the carbon, hydrogen, and nitrogen content.

\[
HHV \text{ (MJ. Kg}^{-1}\text{)} = [5.22C^2 - 319C - 1647H + 38.6C \times H + 133N + 21028] \times 10^{-3} \quad (3-1)
\]

FTIR spectroscopic analysis of initial dry sample, residual samples after subcritical ethanol treatment, and pure cellulose was performed by JASCO FTIR-6100 over a range of 600 - 4000 cm\(^{-1}\). All measurements were carried out by means of KBr plates.
Figure 3-1. General procedure of subcritical ethanol treatment and separation of products.
3. 3. Results and discussion

3. 3. 1. Evaluation of reaction conditions

Reactions were carried out at temperatures ranging between 100 and 205 °C for 2, 5, 10, and 15 min residence times in order to determine the optimum reaction conditions in the production of solid residual, ethanol soluble, and water soluble materials. As shown in Figure 3-2-a, at 2 min residence time, increasing the reaction temperature had not significant effect on the yield of water and ethanol solubles, and residual solid. However, with increasing the reaction time to 5 min and in continue 10 and 15 min (Figure 3-2-b ~ d) the amount of residual solid decreases from 61 % at ambient temperature to minimum amount of 17 % at 200 °C at 15 min (Figure 3-2-d).

For ethanol solubles, as shown in Figure 3-2-b, after 5 min reaction time maximum amount of 11% was obtained. However, at longer reaction times (10 and 15 min) the ethanol soluble yield significantly increased and reached to maximum amount of 32% at 200 °C (see Figure 3-2-c and d).

For water solubles, with temperature raising up to 205 °C at 5 min residence time, solubility of *C. fragile* in water increased gradually from 35 to 57% (Figure 3-2-b). Results indicated that reaction time has not significant effect on the solubility of *C. fragile* in water phase.
Figure 3-2. Effect of subcritical ethanol treatment time and temperature on the yield of water soluble, ethanol soluble, and remained solid.
3.3.2. Total organic carbon (TOC)

The TOC results of the water phase before and after treatment by subcritical ethanol are shown in Figure 3-3. At 2 min residence time, TOC yield did not significantly change with temperature increasing. However, at 5 min reaction time its amount gradually started increasing from 160 °C and reach to maximum amount of 7% at 205 °C. At 10 min residence time, the TOC amount start increasing at 160 °C and reached a maximum of 10% at 190 °C. The results of 15 min reaction time show the maximum of 9% at 180 °C. Further increases in temperature at 10 and 15 min reaction time, caused the TOC to decrease to 4% at 205 and 200 °C, respectively, through the gasification and carbonization reactions. TOC amount in subcritical ethanol treatment was relatively low owing to not completely hydrolysis of cellulosic part of the algae.

3.3.3. Total soluble sugars

Figure 3-4 shows the amount of total water soluble sugars as a function of temperature at different residence times. Temperature does not have significant effect on production of sugars at 2 min reaction time. The results show that production of the sugars began at longer reaction times at around 160 °C. The maximum amount was obtained 18%, 40%, and 41% for 5, 10, and 15 min residence time at 200, 190, and 180 °C, respectively. However, at higher temperatures, because of further decomposition reactions [1], the amount of total soluble sugars sharply declined.

Obviously, using subcritical ethanol will result less soluble sugars compared to subcritical water reactions, since later one could hydrolyzed macromolecules of the cellulose more effective than former one. Water soluble sugars are more attractive for fermentation reactions than solid samples. However the average of produced soluble
sugars under subcritical ethanol conditions is lower than those produced under subcritical water conditions [4].

3. 3. 4. Total organic acids

Organic acids can be produced by decomposition and hydrolysis of macromolecules of biomass, carbohydrates and amino acids [11]. In this work levulinic, pyroglutamic, formic, and acetic acids were identified and quantified in ethanolic phase after subcritical ethanol treatment. On the other hand, water phase which obtained by washing solid residue after liquefaction reaction, shows D-malic, levulinic, pyroglutamic, butyric, and phosphoric acids. The results of total organic acids in both ethanol and water phase at different temperature and reaction time was shown in Table 3-1. As shown, with temperature raising the amount of total organic acids increased with time, and at 10 and 15 min reaction times their amounts reach to maximum at 180 ºC and then declined. This reduction could be attributed to the decomposition of organic acids at higher temperature and production of gaseous products.
Figure 3-3. Effect of subcritical ethanol temperature on the yield of TOC in water phase at different reaction time.

Figure 3-4. Effect of subcritical ethanol temperature on the yield of water soluble sugars at different reaction time.
Table 3-1. Organic acids yield as function of subcritical ethanol temperature at 2, 5, 10, and 15 min residence times.

<table>
<thead>
<tr>
<th>Temperature (ºC)</th>
<th>2 min</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.036</td>
<td>0.036</td>
<td>0.036</td>
<td>0.036</td>
</tr>
<tr>
<td>100</td>
<td>N.D.</td>
<td>0.032</td>
<td>0.037</td>
<td>N.D.</td>
</tr>
<tr>
<td>120</td>
<td>N.D.</td>
<td>0.031</td>
<td>0.042</td>
<td>N.D.</td>
</tr>
<tr>
<td>160</td>
<td>0.038</td>
<td>0.052</td>
<td>0.077</td>
<td>0.073</td>
</tr>
<tr>
<td>180</td>
<td>0.048</td>
<td>0.084</td>
<td>0.088</td>
<td>0.085</td>
</tr>
<tr>
<td>190</td>
<td>N.D.</td>
<td>0.078</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>200</td>
<td>0.054</td>
<td>0.074</td>
<td>0.061</td>
<td>0.059</td>
</tr>
<tr>
<td>205</td>
<td>N.D.</td>
<td>0.088</td>
<td>0.049</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

*Organic acids in ethanol phase + Organic acids in water phase*
3. 3. 5. Production of bio-oils

Bio-oils produced by liquefaction and extraction from the macroalgae in ethanol are a dark brown liquid with good flow at room temperature. The bio-oils compounds were analyzed by GC-MS. Figure 3-5 shows the typical total ion chromatographs along with comparison of bio-oil contents in untreated and treated samples. Table 3-2 listed the typical identified products obtained in ethanol at 200 °C after 15 min reaction time. The relative contents of compounds identified take up about 40.3 % of the total area for each chromatograph. It can be seen that bio-oils obtained in alcohols are mainly composed of fatty acid esters, N-containing-compounds, carbohydrates, hydrocarbons, and fatty alcohols/ketones. The fatty acid esters (C\textsubscript{4}–C\textsubscript{24}), that is, none N-containing ethyl esters, are the most abundant compounds in the bio-oils, and among these compounds, hexadecanoic acid ethyl ester (RT = 28.4 min, in Table 3-2), has a contents for 5.8 % of the total area. Also, some other esters, such as pentanoic acid, 4-oxo-, ethyl ester (RT = 11.6 min, in Table 3-2) were identified, which are possibly generated from the reactions of organic acids with alcohols. The identification of furfural diethylacetal (RT = 11.9 min, in Table 3-2), is believed to derive from the decomposition of the polysaccharides and cellulose. Also, some hydrocarbons, such as heptadecane (RT = 24.0 min, in Table 3-2) were identified in bio-oils. In addition, several fatty alcohols or fatty ketones were also found in bio-oils.

The experimental results indicated that ethanol under subcritical conditions has exhibited outstanding transport characteristics, which can facilitate reactant mixing to promote the liquification and conversion of algal material [12]. According to the GC–MS analysis results of bio-oils and some other studies, a series of complicated reactions occurred during the conversion process, which includes the decomposition and
repolymerization of the components of macroalgae (e.g., lipids, carbohydrates, and proteins). Studies on the noncatalytic transesterification of model compounds in subcritical ethanol [13-15] such as free fatty acids, as well as some bonded fatty acids (triglycerides), have revealed their different reaction pathways. Because the alcohol has involved in the reactions, a large number of transesterification and esterification reactions could exist in the conversion process during the liquefaction of macroalgae C. fragile in alcohols. Typically, the long-chain fatty acid esters are generated from lipids (triglycerides) by transesterification, and the esterification of some free fatty acids with alcohols can also form fatty acid esters. Another point of interest is that the organic acids from the decomposition of carbohydrates can undergo esterification and substitution reactions to form their corresponding esters and derivatives. In addition, the further reaction of several decomposition products of proteins with alcohols causes to form N-containing esters. The analysis results indicate the possibility of having the lipids and oily compounds from carbohydrates and proteins parts of algae as well.
Figure 3-5. Typical total ion chromatographs of (a) Untreated (control) and (b) treated *C. fragile* at 200 °C for 15 min.
Table 3-2. Identified products from *C. fragile* treatment under subcritical ethanol at 200 °C in 15 min.

<table>
<thead>
<tr>
<th>No.</th>
<th>RT (min)</th>
<th>Identified compound name</th>
<th>Peak Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.1</td>
<td>Hexanoic acid ethyl ester</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>11.6</td>
<td>Pentanoic acid, 4-oxo-, ethyl ester</td>
<td>4.2</td>
</tr>
<tr>
<td>3</td>
<td>11.9</td>
<td>Furfural diethyl acetal</td>
<td>3.1</td>
</tr>
<tr>
<td>4</td>
<td>12.5</td>
<td>3-(Methylthio) propanoic acid ethyl ester</td>
<td>1.8</td>
</tr>
<tr>
<td>5</td>
<td>24.0</td>
<td>Heptadecane</td>
<td>1.3</td>
</tr>
<tr>
<td>6</td>
<td>24.9</td>
<td>Tetradecanoic acid</td>
<td>1.1</td>
</tr>
<tr>
<td>7</td>
<td>25.5</td>
<td>Tetradecanoic acid, ethyl ester (ethyl myristate)</td>
<td>0.3</td>
</tr>
<tr>
<td>8</td>
<td>26.8</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
<td>1.4</td>
</tr>
<tr>
<td>9</td>
<td>27.9</td>
<td>n-Hexadecanoic acid</td>
<td>9.1</td>
</tr>
<tr>
<td>10</td>
<td>28.0</td>
<td>Ethyl 9-hexadecenoate</td>
<td>1.9</td>
</tr>
<tr>
<td>11</td>
<td>28.3</td>
<td>Hexanoic acid, anhydride (hexanoic anhydrate)</td>
<td>1.3</td>
</tr>
<tr>
<td>12</td>
<td>28.4</td>
<td>Hexadecanoic acid, ethyl ester (ethyl palmitate)</td>
<td>5.8</td>
</tr>
<tr>
<td>13</td>
<td>30.7</td>
<td>Linoleic acid ethyl ester</td>
<td>0.5</td>
</tr>
<tr>
<td>14</td>
<td>30.8</td>
<td>Ethyl Oleate</td>
<td>3.1</td>
</tr>
<tr>
<td>15</td>
<td>32.7</td>
<td>5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)-</td>
<td>1.7</td>
</tr>
<tr>
<td>16</td>
<td>32.8</td>
<td>5,8,11,14,17-Eicosapentanoic acid, methyl ester, (all-Z)- (EPA)</td>
<td>1.0</td>
</tr>
<tr>
<td>17</td>
<td>34.0</td>
<td>9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester</td>
<td>0.5</td>
</tr>
<tr>
<td>18</td>
<td>35.2</td>
<td>(Z)14-Tricosenyl formate</td>
<td>0.4</td>
</tr>
<tr>
<td>19</td>
<td>35.9</td>
<td>Heptadecanoic acid, ethyl ester</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>40.3</td>
</tr>
</tbody>
</table>
3. 3. 6. Ultimate analysis and energy density of residual solid

Ultimate analysis of residual solid obtained after 5 and 10 min treatment of *C. fragile* in ethanol at various temperatures are shown in Table 3-3. The HHVs of sample calculated based on equation 3-1. It can be seen that the carbon, hydrogen, and nitrogen contents have greatly increased in comparison to that of the untreated sample. The carbon contents of treated sample at 5 min reaction time are 31 ~ 35 wt% and the HHVs of residual solids are around 14-15 MJ/kg, while the carbon contents and HHVs of 10 min treated sample were higher at the same reaction temperature. However, results show high oxygen (19-31 wt%) and nitrogen contents (3-7 wt%) of treated sample than that of algae feedstock (8.4 and 2.1 wt%, respectively). By treatment of *C. fragile*, carbon contents (fixed carbon) increase and consequently the energy density and the heating value of the residual solid after treatment increased somewhat. However, since these reactions have done with no hydrolysis reactions, it seems that the structure of cellulose has not significantly changes, which cause to obtain almost similar fixed carbon and HHVs at different reaction conditions. The carbon content of residual solid by using this method is much lower than those obtained from hydrothermal treatment of *C. fragile* [4]. It is indicated that subcritical in alcohol medium displays better a solvent function than that of water [12], and can enhance the liquefying of algae and promote the extraction of bio-oil as well as obtaining pure cellulose which will be discussed in the next section.
Table 3-3. Ultimate analysis of *C. fragile* and its higher heating values for solid samples before and after liquefaction in ethanol at 5 and 10 min residence time.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Temperature ( ºC)</th>
<th>Elemental compositions (wt%)</th>
<th>H/C</th>
<th>HHV (MJ/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
<td>N</td>
</tr>
<tr>
<td>untreated</td>
<td>25</td>
<td>25.5</td>
<td>4.6</td>
<td>2.1</td>
</tr>
<tr>
<td>100</td>
<td>33.7</td>
<td>5.7</td>
<td>3.5</td>
<td>27.5</td>
</tr>
<tr>
<td>120</td>
<td>32.1</td>
<td>5.6</td>
<td>3.9</td>
<td>24.5</td>
</tr>
<tr>
<td>160</td>
<td>33.1</td>
<td>5.9</td>
<td>3.1</td>
<td>29.5</td>
</tr>
<tr>
<td>180</td>
<td>31.4</td>
<td>5.3</td>
<td>4.0</td>
<td>19.5</td>
</tr>
<tr>
<td>190</td>
<td>33.8</td>
<td>5.7</td>
<td>4.5</td>
<td>25.4</td>
</tr>
<tr>
<td>200</td>
<td>34.6</td>
<td>5.9</td>
<td>5.0</td>
<td>26.7</td>
</tr>
<tr>
<td>205</td>
<td>34.2</td>
<td>5.7</td>
<td>4.5</td>
<td>25.6</td>
</tr>
</tbody>
</table>

5

|            |                   | C   | H   | N   | O   |       |     |
| 100        | 35.3              | 6.0 | 3.9 | 30.6| 2.0 | 15.1  |
| 120        | 34.8              | 6.0 | 4.3 | 30.1| 2.1 | 15.1  |
| 160        | 34.9              | 5.9 | 4.3 | 28.9| 2.0 | 15.1  |
| 180        | 35.8              | 6.0 | 5.2 | 28.7| 2.0 | 15.4  |
| 190        | 37.2              | 5.9 | 7.3 | 24.3| 1.9 | 16.1  |
| 200        | 32.5              | 5.9 | 6.0 | 24.0| 2.2 | 14.7  |
| 205        | 25.5              | 4.1 | 4.7 |     | 1.9 | 14.2  |

10
3. 3. 7. Evaluation of remained solid using FTIR

Refereeing to the previous results, since subcritical ethanol did not hydrolyzed completely the macromolecules of the algae; it was expected to obtain the pure cellulose after treatment and after extraction of all extractives from the algae. FTIR technique was used to compare the initial algae, treated algae under subcritical ethanol, and pure cellulose peaks.

Results are shown in Figure 3-6. FTIR peak of the treated sample is very similar to the peak of the pure cellulose. On the other words, subcritical ethanol could successfully extract all extractives from the algae and result almost very pure cellulose with less hydrolyzed amount. This pure cellulose has vast pharmaceutical and industrial applications.
Figure 3-6. FTIR spectra of pure cellulose, initial (control) dried *C. fragile*, and residual solid after 10 min treatment of *C. fragile* in ethanol at 205 °C.
3. 4. Conclusions

The results of this chapter showed complete liquification of valuable materials with less hydrolysis reaction of cellulosic part of the algae. Very valuable bio-oils produced from C. fragile treatment in ethanol are composed of fatty acid (C$_4$–C$_{24}$) ethyl esters, N-containing compounds, sugars, fatty alcohols/ketones, and hydrocarbons. These products could industrially use in bio-fuel production, methane fermentation, pharmaceutical products, and so on.

Treatment of the algae under subcritical ethanol conditions show promising results not only for solubilization of solid biomass and/or production of fatty acids, but also obtain of very pure residual solid similar to those of pure cellulose, with vast industrial applications. Since residual solid also shows high energy density, it can also be a very good alternative energy resource as well. From engineering point of view, for industrialization of a process it is important to explore the production of biofuels or synthesis of value-added chemicals via one step reaction, which may help to develop a new path for the use of the macroalgae in the industrial scales.

Finally, subcritical ethanol treatment is a green and zero emission process in which ethanol waste after reaction could be easily purified and recycles back to the reaction system.

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hydrolysis in subcritical and supercritical water,” Journal of Supercritical Fluids, 13, 261-268, 1998.


Chapter 4

Pyrolytic behavior and kinetic studies of selected green macroalgae
4. 1. Introduction

Thermochemical conversion techniques are one of the advanced methods for conversion of biomass feedstock (containing mainly cellulose, hemicellulose and lignin) into useful chemicals and energy. Pyrolysis is one of the industrially sound processes among the thermochemical conversions techniques which has been commonly used to convert the different kinds of biomass into bio-oil, bio-char, water, and gaseous products [1] in the absence of oxygen. For the fundamental studies of pyrolysis behavior of biomass, thermogravimetric analysis (TGA) is most commonly used in laboratory scales. TGA measures the amount and rate of change in the weight of a material as a function of temperature or time in a controlled atmosphere. The TGA is used not only for study decomposition kinetics of organic and inorganic compounds, but also widely used for the study of composition of multicomponent systems, oxidative stability of materials, estimated lifetime of a products, the effect of reactive or corrosive atmospheres on materials, moisture and volatiles content of materials [2, 3]. Traditionally, non-isothermal and constant heating rate of TGA have been used to obtain kinetic information with the constant heating rate method developed by Flynn and Wall [4]. The pyrolysis characteristics of terrestrial biomass have been widely investigated, and the corresponding kinetics has been established [5]. However, there are few studies on marine biomass especially macroalgae [6].

The present study has determined the pyrolysis behavior of *C. fragile* by application of TGA and DTG techniques in order to evaluate the kinetics of pyrolysis of *C. fragile*. In fact, prior to scale up a pyrolysis system, it is necessary to evaluate the influence of different parameters such as pyrolysis temperature, particle size, and heating rate, and so on. Value of the activation energies were also estimated in various
combustion rates and investigated in detail.

4. 2. Materials and methods

4. 2. 1. Materials

Fresh macroalgae (C. fragile) was collected at Rinkou Park in Osaka, Japan on 2011. The average water content of C. fragile just after harvesting was found to be 93%. Obviously their compositions can vary from batch to batch and by harvesting season [7]. However the remaining solid amount, similar to other marine biomass, is cellulose with some incorporation of hemicelluloses and a wide variety of acidic polysaccharides [8] proteins, lipids, chlorophylls, and other organic and inorganic compounds.

4. 2. 2. Procedure

The sample was washed, cleaned of foreign debris, and towel-dried. It was then complete dried using freeze-drying method (TAITEC freeze dryer VD-16). All the experiments have been performed with initial dry mass close to 20 mg. In fact by freeze drying the moisture content of sample can be reduced to levels acceptable for thermal processing [9]. The dried sample was ground in a laboratory mill MF 10.1 from IKA Works, Inc. USA. It was sieved into six different particle sizes of the sample ranging between <75 and >1400 μm. Proximate experiments were carried out using a thermogravimetric analyzer (Seiko Exstar 6000 TG/DTA 6300, Seiko Instruments Inc.). Ceramic crucibles were used in order to minimize any thermal lag and to optimize heat transfer between thermocouples and crucibles. The instrument will record the mass and the temperature changes in time (Figure 4-1-a and b). The experiments were performed in an atmospheric pressure and under pure nitrogen gas (99.999%) with flow rate of 20
ml/min. It was performed in non-isothermal condition from room temperature to 700 °C.

Pyrolysis was carried out by vary the heating rates from 5 to 50 °C/min. The weight losses occurring in correspondence to temperature rises were continuously recorded with a computer working in coordination with the instrument. Effect of particle size (ranging between <75 and >1400 μm) and initial sample weight (ranging between 5-25 mg dry sample) have been studied. Most of the results are presented in TG plot which is plot between weight reduction and temperature. Alternative presentations of results are given as the derivative of the TG or rate of weight reduction against temperature. In this study, all experiments were replicated twice.

4. 2. 3. Techniques for studying reaction kinetics

Figure 4-2 shows a general flow chart of kinetic studies for both homogeneous and solid state reactions along with mathematical methods and solutions. Generally these techniques can be grouped into two categories: isothermal and non-isothermal techniques. For solid-state reactions, for example, in a TG analysis, there are several ways to obtain kinetic data and the most popular methods are isothermal and non-isothermal methods. Figure 4-2 also shows some different types of computational methods that are used in determining kinetic parameters using isothermal and non-isothermal methods. Many mathematical methods have been developed to evaluate these techniques; model-fitting and model-free methods are the most common two groups of methods used to analyze either isotherm or non-isothermal reaction kinetics, which will be discussed later in this section.
Figure 4-1-a. Typical setup of TGA.

Figure 4-1-b. Schematic of weigh change mechanism in TGA.
Figure 4-2. Major techniques for studying reaction kinetics.
4.2.4. Kinetic parameters estimation

In this study to estimate kinetic parameters, the solid state non-isothermal (isoconversional) method was used. This method is applied for the description of more complex processes where lots of chemical reactions are running simultaneously; however, their mechanisms are not exactly known [10]. It also has been widely utilized when describing decomposition of biomass [11, 12].

Under non-isothermal conditions in which a sample is heated at constant rate [13], mass loss data from the thermogravimetric analysis can be recalculated into conversion which is defined as follows:

\[ \alpha_T = \frac{(m_{w_0} - m_{wT})}{(m_{w_0} - m_{w\infty})} \]  

(4-1)

where \( m_{w_0} \) is the initial mass of sample, \( m_{wT} \) is the mass at temperature \( T \), and \( m_{w\infty} \) is final mass of sample at the end of reaction. This method employs a heating rate (\( \beta \)), usually linear, to raise the temperature. A linear heating program follows:

\[ T = T_0 + \beta \times t. \]  

(4-2)

Here \( \beta \) is the heating rate (°C/min) and \( T \) is temperature (°C) at time \( t \) (min). The following relationship can be defined for non-isothermal solid-state reactions:

\[ \frac{d\alpha_T}{dT} = \frac{d\alpha_T}{dt} \times \frac{dt}{dT}, \]  

(4-3)

and

\[ \frac{d(\alpha_T)}{dt} = A e^{\frac{E_a}{RT}} f(\alpha_T). \]  

(4-4)

Under non-isothermal conditions the explicit temperature dependence of the rate equation is given by substituting equation (4-4) into equation (4-3):

\[ \frac{d\alpha_T}{dT} = A/\beta \times e^{-\frac{E_a}{RT}} \times f(\alpha_T). \]  

(4-5)

Equation (4-5) represents the differential form of the non-isothermal rate law.
Rearranging (4-5) gives:

\[
\frac{d\alpha_r}{f(\alpha_r)} = \frac{A}{\beta} \exp \left( \frac{-E_a}{RT} \right) \times dT.
\]  

(4-6)

Integrating the differential non-isothermal rate law, equation (4-6) produces the integral form of the non-isothermal rate law:

\[
\int_{0}^{\alpha_r} \frac{d\alpha_r}{f(\alpha_r)} = \frac{A}{\beta} \int_{0}^{T} \exp \left( \frac{-E_a}{RT} \right) \times dT,
\]  

(4-7)

hence, letting

\[
g(\alpha_r) = \int_{0}^{\alpha_r} \frac{d\alpha_r}{f(\alpha_r)} ,
\]  

(4-8)

then

\[
g(\alpha_r) = \frac{A}{\beta} \int_{0}^{T} \exp \left( \frac{-E_a}{RT} \right) dT.
\]  

(4-9)

This integral is called the "temperature integral" equation.

Assume that:

\[x = \frac{E_a}{RT},\]

(4-10)

then

\[
g(\alpha_r) = \frac{AE_a}{\beta R} \int_{0}^{\infty} \exp(-x) x^{-2} dx.
\]  

(4-11)

Equation (4-11) can be written as [13]:

\[
g(\alpha_r) = \frac{AE_a}{\beta R} p(x),
\]  

(4-12)

where:

\[
p(x) = \int_{0}^{\infty} \frac{\exp(-x)}{x^2} dx.
\]  

(4-13)

Equation (4-12) is the general equation of non-isothermal reaction rate suggested first time by Doyle [14, 15].

The integral in the right-hand side of equation (4-12) (i.e. \(p(x)\)) has no analytic
solution, but has many approximations. Thus, several approximated equations have been proposed in literature. Some of these approximations lead to a linear relation between the logarithm of $g(\alpha)$ and a predetermined function of $T$, in such a way that the activation energy can be determined from the slope of the plot of $\ln g(\alpha)$ versus the predetermined $T$ function. Hereafter, the most common approximations used in the analytical solution of the “temperature integral” equation will be discussed. In non-isothermal kinetics, the Flynn-Wall-Ozawa (FWO) [16, 4], Kissinger-Akahira-Sunose (KAS) [17-19], Friedman (FR) [20] and Coats-Redfern [21, 22] methods are the most popular representative of the isoconversional methods which used in this study.

4. 3. Results and discussion

4. 3. 1. Characteristics of the thermal degradation process on C. fragile

Figure 4-3 illustrates the typical TG profile of C. fragile dehydration, primary devolatilisation, consequently char formation, and finally char oxidation by proximate analysis. Holding freeze dried sample at temperatures below 110 °C for 20 min cause to lose weight near 6.5%. This weight loss corresponds to the moisture and cell water content of the sample.

The second weight reduction with temperature rising up to 700 °C is attributed to devolatilisation and pyrolysis of mainly natural organic macromolecules. All the volatiles are evolved until 700 °C, and only the char remain. In this step, the yield of the volatile material is around 58% of the initial weight of sample. The char content can be found from the oxidation of the produced char by switching the carrier gas from nitrogen to air at 700 °C. In this step, the char is oxidized into carbon dioxide and
carbon monoxide as well as other gaseous products. The weight differences, before and after switching the gas, is used to determine the fixed carbon, which is about 10.5% of the weight sample. The remaining residue represents the ash content (25%). The ash production of marine biomass is generally higher than grass biomass and lignocellulosic biomass, which is in good coincidence with the fact that marine biomass contains higher salinity [6, 9].

In fact, pyrolysis of *C. fragile* involving thermal devolatilisation consists of a very complex set of reactions. The reactions can be represented as the sum of thermal devolatilisation reactions of the individual components of oily compounds, cellulose, hemicellulose, and so on.

Figure 4-4 shows typical derivative thermogravimetric (DTG) curve of *C. fragile* for heating rate of 50 °C/min. This curve indicates that there are three main zones in the pyrolytic process of *C. fragile*. As it is clear from Figure 4-4, the sample reveals large differences in degradation behavior during zone II of the DTG curve. It can be seen that there are three peaks apparent in this zone. The area under the peak represents the weight loss during the reaction. As the temperature increased, the rate of the devolatilisation process also increases.

For heating rate of 50 °C/min, the first zone occurs as the temperature increase from 40 °C to 142 °C, while the second zone occurs as the temperature increase from 142 °C to 450 °C. Zone III starts from 450 °C and ends at 700 °C.

However, these critical temperatures can be varied by heating rate [23]. The corresponding temperatures to each zones and stages as function of heating rates are tabulated in Table 4-1. These data was used in this study to distinguish the different zones of each process and their respective reaction kinetics.
Generally, during pyrolysis, the moisture is removed initially at a temperature below 110 ºC in the zone I [24]. Above 110 ºC in zone II, the chemical bonds of macromolecules break to release the volatile compounds. Prior to decomposition of macromolecules, oily compounds degrade and evaporate form the sample (zone II, stage I). At relatively higher temperatures, it has been reported [25] that hemicelluloses degrade faster than cellulose. Above 250 ºC in stage II, the celluloses may start to break and release more volatiles till up end of the zone II [26]. In zone III, the degradation rate is slow corresponding to the degradation of other less volatile compounds. In fact zone III is the carbonization step and mass loss in this zone can be attributed to char decomposition as well as inorganic ash decomposition and volatilization [6, 9].

4. 3. 2. Effect of particle size on pyrolysis of *C. fragile*

The influence of the particle size was investigated for six different particle sizes of the sample ranging between <75 and >1400 μm. Figure 4-5 shows only three TGA curves for comparison. As shown in this figure, particle size does not have a significant effect on the TG profile of the *C. fragile* pyrolysis. In contrast, as the particle size increased from 75 to 1400 μm, the char yield decrease somewhat (see Figure 4-5 and Table 4-2). This may be due to the amount of heat transfers from different particle sizes. The samples consisting of larger particle may have had higher rate of heat transfer. As a result, during the second zone, larger amounts of sample are decomposed and less amounts of char remained at the end.

On the other hand, Table 4-2 shows that the ash content decreased somewhat. This may be caused by the effect of heat transfer and diffusion in the bulk of the sample. Heat transfer inside the bulk with larger particles is more efficient in comparison with
the bulk with smaller particles, which produces smaller amounts of ash. Similar results were reported by other research groups [27-29]. In addition, this may be due to the inorganic components separated from the lignocellulosic structure during size reduction of the sample, and tend to accumulate in smaller size fractions [30].

4.3.3. Effect of initial weight on pyrolysis of *C. fragile*

The effect of pyrolysis reaction was studied on initial weight of the *C. fragile* (Table 4-3). Due to the change of initial amount, the TG curves indicate that the change in pyrolysis behavior was observed only in the third zone (curves are not shown here). However, initial weight did not have significant effect on the first and the second zones of the pyrolysis of the *C. fragile*. It was clear that the char yield increased as the initial weight increase. It seems that the inert gas would encounter a higher diffusion resistance inside the bed by increasing the initial weight. Since the bed sample was getting higher inside the crucible, the heat transfer also would be affected adversely, and thus more fixed carbon (bio char) was produced (see Table 4-3).
Figure 4-3. TG profile for proximate analysis of *C. fragile* for the particle size of 150–250 μm, 20 mg of initial sample, and heating rate of 50 °C/min.

Figure 4-4. Typical DTG curve of *C. fragile* at heating rate of 50 °C /min including characteristic temperature zones and stages.
Table 4-1. Temperatures of initial, peak, and final weight loss of *C. fragile* sample obtained from DTG curves.

<table>
<thead>
<tr>
<th>Heating rate ($\beta$, °C/min)</th>
<th>Zone I</th>
<th>Zone II</th>
<th>Zone III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_i$</td>
<td>$T_f$</td>
<td>$T_{max}$</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>85</td>
<td>56</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>90</td>
<td>57</td>
</tr>
<tr>
<td>20</td>
<td>40</td>
<td>101</td>
<td>61</td>
</tr>
<tr>
<td>50</td>
<td>40</td>
<td>142</td>
<td>73</td>
</tr>
</tbody>
</table>

Figure 4-5. TG curve of *C. fragile* at typical different particle sizes with heating rate of 50 °C/min and initial sample weight of 20 mg.
Table 4-2. Char percentage for different values of particle size of the sample for *C. fragile* pyrolysis with initial weight 20 mg and heating rate 50 °C/min.

<table>
<thead>
<tr>
<th>Average particle size (μm)</th>
<th>Char (%)</th>
<th>RSD (%)</th>
<th>Ash content (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 75</td>
<td>11.1</td>
<td>1.7</td>
<td>33.0</td>
<td>1.7</td>
</tr>
<tr>
<td>75 ~ 150</td>
<td>10.4</td>
<td>2.1</td>
<td>31.0</td>
<td>0.7</td>
</tr>
<tr>
<td>150 ~ 250</td>
<td>10.4</td>
<td>0.7</td>
<td>24.7</td>
<td>0.4</td>
</tr>
<tr>
<td>250 ~ 600</td>
<td>10.3</td>
<td>1.2</td>
<td>25.0</td>
<td>0.2</td>
</tr>
<tr>
<td>600 ~ 850</td>
<td>9.8</td>
<td>1.8</td>
<td>24.9</td>
<td>0.9</td>
</tr>
<tr>
<td>850 ~ 1000</td>
<td>9.9</td>
<td>2.3</td>
<td>24.6</td>
<td>0.8</td>
</tr>
<tr>
<td>1000 ~ 1400</td>
<td>9.5</td>
<td>1.8</td>
<td>24.5</td>
<td>0.3</td>
</tr>
<tr>
<td>≥ 1400</td>
<td>8.9</td>
<td>2.1</td>
<td>24.5</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>10.0</strong></td>
<td>-</td>
<td><strong>26.5</strong></td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4-3. Effect of initial amount of the sample on the bio char production at heating rate of 50 °C/min and particle size of 150 ~ 250μm.

<table>
<thead>
<tr>
<th>Initial sample weight (mg)</th>
<th>Char (%)</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>9.3</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>9.7</td>
<td>0.8</td>
</tr>
<tr>
<td>15</td>
<td>10.2</td>
<td>1.2</td>
</tr>
<tr>
<td>20</td>
<td>10.4</td>
<td>0.7</td>
</tr>
<tr>
<td>25</td>
<td>10.4</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>10.0</strong></td>
<td>-</td>
</tr>
</tbody>
</table>
4. 3. 4. Effect of heating rate on pyrolysis of *C. fragile*

Effect of different heating rates has also been studied for the pyrolysis of *C. fragile* sample. Figure 4-6-a shows the DTG curve and also Figure 4-6-b and 4-6-c show the TG curves of conversion rates at different heating rates from 5 to 50 ºC/min as function of pyrolysis temperature and time, respectively.

As shown in Figure 4-6 (a-c), there is a shift in conversion lines caused by various heating rates. At higher heating rates, individual conversions are reached at higher temperatures. In other word at higher heating rates, higher temperatures are required to achieve the same conversion level [10, 31].

The maximums of the decomposition rate are also slightly shifted towards higher temperatures. This fact can be due to a consequence of heat and mass transfer limitations. It means that temperature in the furnace space can be a little higher as the temperature of particle, and the rate of devolatilization is higher than the release of volatilities. Because of the heat transfer limitation, temperature gradients may exist in the particle. Temperature in the core of a particle can be a bit lower than temperature on the surface, and different devolatilization processes or releasing rates can occur. This is the reason why it is necessary to have a small particle, homogenous sample and heat transfer surface between the sample and the crucible as large as possible [10].

At higher heating rate, the devolatilisation process occurs sooner due to the increase the heat transfer between the crucible and the sample (Figure 4-6-c). Faster heating rates cause the primary devolatilisation to complete rapidly, because the temperature for secondary devolatilisation has been reached rapidly. Since the faster heating rate lead to less efficient heat transfer, the devolatilisation rate increases faster than that at lower heating rates, thus the peak of the devolatilisation rate shifts.
Figure 4-6-a. Pyrolysis DTG curves of *C. fragile* at different heating rates as function of temperature with particle size of 150 ~ 250 $\mu m$ and initial sample weight of 20 mg.

Figure 4-6-b. Pyrolysis TGA curves of *C. fragile* at different heating rates as function of temperature with particle size of 150 ~ 250 $\mu m$ and initial sample weight of 20 mg.
Figure 4-6-c. Pyrolysis TGA curves of *C. fragile* at different heating rates as function of pyrolysis time with particle size of 150 ~ 250 μm and initial sample weight of 20 mg.

Table 4-4. Char yield for different values of heating rate in pyrolysis of *C. fragile* with average particle size 150 ~ 250μm and initial weight 20mg.

<table>
<thead>
<tr>
<th>Heating rate $\beta$ (°C/min)</th>
<th>Char %</th>
<th>RSD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>8.7</td>
<td>1.3</td>
</tr>
<tr>
<td>10</td>
<td>9.3</td>
<td>0.7</td>
</tr>
<tr>
<td>20</td>
<td>9.5</td>
<td>0.2</td>
</tr>
<tr>
<td>50</td>
<td>10.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Meanwhile, the ash yield increases with the heating rate increase. At the lower heating rate, the heat transfer between the crucible and the sample are more efficient. This exists in a proper drying and devolatilisation during the first and second zones, respectively. As a result, char yield increases with the heating rates increase, as shown in Table 4-4.

4. 3. 5. Kinetic analysis of the pyrolysis process for C. fragile

For the kinetic analysis we assume that:

1. The pyrolysis process was assumed to take place in three zones (see Figure 4-4). Since zone II is the main step of pyrolysis process, therefore, the three stages of C. fragile pyrolysis in the zone II was only chosen for kinetic studies. The temperatures selected to distinguish between stages of the zone II are shown in Table 1.

2. The moisture and adsorbed water content released up to 110 ºC was excluded from pyrolysis data.

3. All the pyrolysis reactions assumed to be irreversible ones [32].

4. Since it is impossible to identify all the compounds in the pyrolysis of C. fragile, the model is based on only weight loss.

5. Simple solid-state pyrolysis reaction model was assumed for C. fragile sample. To estimate kinetic parameters, the isoconversional methods were used.

6. Among the several approximated analytical solution methods, the most common methods were selected to calculate the activation energy of the pyrolysis of C. fragile.

7. The effects of the internal mass transport as a significant resistance inside the
particles during pyrolysis are not considered in this study.

The experimental data were processed in order to obtain activation energies [10]. Predefined conversions were in the range from 0.05 to 0.7. From the set of data at different heating rates (5, 10, 20, and 50 °C/min), the isoconversional lines for predefined conversion were calculated. Because at higher temperatures no significant changes occurred in conversion, the isoconversional lines were not very precise. Different heating rates gave different Arrhenius plots; therefore a series of activation energy ($E_a$) values could be determined from the slopes of the each isoconversional straight lines at conversion degrees ($\alpha$). The pre-exponential factor could be obtained from the intercept of the isoconversional line.

4. 3. 5. 1. Flynn, Ozawa, and Wall (FOW) method

Flynn, Ozawa, and Wall (FOW) method [4, 16] is one of the non-isothermal methods which use to determine the energy of activation ($E_a$) at constant several conversion degrees ($\alpha$). This method starts with equation (4-6). Therefore integration will be as:

$$
\int_0^\alpha \frac{d\alpha_T}{f(\alpha_T)} = \frac{A}{\beta} \int_0^T e^{E_a/R\cdot T} dT.
$$ (4-14)

Integration and rearranging of equation (4-14) gives:

$$
\log \beta = \log(A) - 2.315 - 0.4567 \frac{E_a}{RT}
$$ (4-15)

For $\alpha$ constant, $\ln(\beta)$ versus $1/T$ obtained at several heating rates yields a straight line whose slope allows evaluation of the apparent activation energy [31].

Figure 4-7 shows the Arrhenius plots of $\ln(\beta)$ versus $1/T$ at constant conversions but different heating rates. The activation energies ($E_a$) determined from the slope of each
line along with line equations and $R$-square values calculated by FOW method for each $\alpha$ are listed in Table 4-5. $R$-squared values show good fitting of the straight lines obtained by FOW method. Therefore, it can be concluded that activation energies calculated by FOW methods are valid [23].

4. 3. 5. 2. The method of Friedman

This method [20] is one of the earliest isoconversional methods, which according to the non-isothermal rate law equation (4-5); rearranging gives:

$$ \beta \frac{d\alpha_t}{dT} = Ae^{-\frac{E_a}{RT}} f(\alpha_t). $$

(4-16)

Then by taking logarithm of both sides of equation (4-16), the following equation is obtained:

$$ \ln(\beta \frac{d\alpha}{dt}) = \ln(A_{\alpha} f(\alpha)) - \frac{E_a}{RT}. $$

(4-17)

Hence, a plot of $\ln(\beta d\alpha/dT)$ versus $1/T$ at each conversion degree ($\alpha_T$) gives activation energy $E_a$ from the slope of the plot. The constructed plots are shown in Figure 4-8. The activation energies ($E_a$) along with line equations and $R$-square values calculated by Friedman method for each $\alpha$ are listed in Table 4-6.
Figure 4-7. FOW plots of *C. fragile* at different conversion fractions.

Table 4-5. Activation energies calculated by FOW method.

<table>
<thead>
<tr>
<th>( \alpha_f )</th>
<th>Curve fit equation</th>
<th>( R )-Squared value</th>
<th>( E_a ) (kJmol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>( y = -16.1x + 36.909 )</td>
<td>0.86</td>
<td>293.9</td>
</tr>
<tr>
<td>0.1</td>
<td>( y = -10.9x + 24.313 )</td>
<td>0.96</td>
<td>198.5</td>
</tr>
<tr>
<td>0.2</td>
<td>( y = -12.4x + 26.39 )</td>
<td>0.96</td>
<td>225.7</td>
</tr>
<tr>
<td>0.3</td>
<td>( y = -15.3x + 30.854 )</td>
<td>0.95</td>
<td>278.6</td>
</tr>
<tr>
<td>0.4</td>
<td>( y = -17.9x + 34.143 )</td>
<td>0.94</td>
<td>325.9</td>
</tr>
<tr>
<td>0.5</td>
<td>( y = -20.2x + 36.44 )</td>
<td>0.91</td>
<td>368.5</td>
</tr>
<tr>
<td>0.6</td>
<td>( y = -25.2x + 42.737 )</td>
<td>0.80</td>
<td>458.9</td>
</tr>
<tr>
<td>0.7</td>
<td>( y = -22.9x + 37.483 )</td>
<td>0.98</td>
<td>417.3</td>
</tr>
</tbody>
</table>
Figure 4-8. Friedman plots of *C. fragile* at different conversion fractions.

Table 4-6. Activation energies calculated by Friedman method.

<table>
<thead>
<tr>
<th>$\alpha_T$</th>
<th>Curve fit equation</th>
<th>$R$-Squared value</th>
<th>$E_a$ (kJmol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>$y = -28.0x + 56.232$</td>
<td>0.99</td>
<td>233.1</td>
</tr>
<tr>
<td>0.1</td>
<td>$y = -17.2x + 37.495$</td>
<td>0.94</td>
<td>142.9</td>
</tr>
<tr>
<td>0.2</td>
<td>$y = -26.2x + 50.464$</td>
<td>0.96</td>
<td>218.0</td>
</tr>
<tr>
<td>0.3</td>
<td>$y = -28.2x + 52.217$</td>
<td>0.98</td>
<td>234.6</td>
</tr>
<tr>
<td>0.4</td>
<td>$y = -31.8x + 55.83$</td>
<td>0.98</td>
<td>264.5</td>
</tr>
<tr>
<td>0.5</td>
<td>$y = -32.2x + 53.65$</td>
<td>0.97</td>
<td>267.5</td>
</tr>
<tr>
<td>0.6</td>
<td>$y = -33.1x + 51.743$</td>
<td>0.98</td>
<td>275.3</td>
</tr>
<tr>
<td>0.7</td>
<td>$y = -39.7x + 58.797$</td>
<td>0.97</td>
<td>329.9</td>
</tr>
<tr>
<td>0.8</td>
<td>$y = -36.2x + 48.732$</td>
<td>0.99</td>
<td>301.1</td>
</tr>
</tbody>
</table>
4. 3. 5. 3. Kissinger-Akahira-Sunose (KAS) method

The Kissinger-Akahira-Sunose (KAS) method [18, 19] is based on the following equation,

\[
\ln \left( \frac{\beta}{T_a^2} \right) = \ln \left( \frac{AR}{E_a g(\alpha)} \right) - \frac{E_a}{RT} .
\]

(4-18)

The activation energies \((E_a)\) can be determined from the linear plots of the \(\ln \beta/T^2\) versus \(1/T\) temperature corresponding to each conversion degree (Figure 4-9). Same as the other methods the activation energies can be determined without a precise knowledge of the reaction mechanism. The activation energies \((E_a)\) along with line equations and \(R\)-square values calculated by KAS method for each \(\alpha\) are listed in Table 4-7.

4. 3. 5. 4. The method of Coast-Redfern

This method [21, 22] is a non-isothermal model free method and uses the integral form of the non-isothermal rate law (Equation 4-5). Assuming \(f(\alpha_T) = (1-\alpha_T)^n\), the following equation can be derived from the fundamental equation:

\[
g(\alpha_T) = \frac{d\alpha}{dT} = \frac{A}{\beta} \int_{T_0}^{T} e^{-E_a/RT} dT ,
\]

(4-19)

where \(n\) is the order of the reaction. The integration for \(n=1\) gives:

\[
\log \left[ \frac{-\log(1-\alpha)}{T^2} \right] = \log \frac{AR}{\beta E_a} \left[ 1 - \frac{2RT}{E_a} \right] - \frac{E_a}{2.3RT} ,
\]

(4-20)

and for \(n \neq 1\)

\[
\log \left[ \frac{-\log(1-\alpha)^{(1-n)}}{T^2(1-n)} \right] = \log \frac{AR}{\beta E_a} \left[ 1 - \frac{2RT}{E_a} \right] - \frac{E_a}{2.3RT} .
\]

(4-21)

The left-hand side of equation (4-21) versus \(1/T\) was plotted, and the slope of these lines gave the \(E_a\) values. In the present study, the orders 0.5, 1, and 1.5 were
plotted for equation (4-21) and the best correlation coefficients were obtained for \( n = 1 \). The straight line plots for \( C. \text{fragile} \) are given in Figures 4-10 (a-d), and the calculated activation energies are cited in Table 4-8 (and for comparison in Figure 4-11).

### 4.3.5.5. Comparison of activation energies from different methods

Table 4-9 listed the activation energies calculated by different methods for each conversion fraction as well as each stage. The average activation energies are also calculated for each stage. The average values of \( E_a \) in the range of 0.05 to 0.1 (stage I) are 73.4, 67.0, 188.0, and 218.7 kJ/mol, those in the range of 0.2 to 0.6 (stage II) are 42.1, 141.8, 251.9, and 331.5 kJ/mol, and those in the range of 0.7 to 0.8 (stage III) are 12.6, 171.5, 315.4, and 417.3 kJ/mol, which obtained for each stage by Coast-Redfren, KAS, Friedman, and FOW methods, respectively.

The activation energies calculated by FOW and Friedman methods for each three stages are higher than those calculated by KAS and Coats-Redfern methods, it can be said that the results are fairly compatible with each other [33]. The values of the apparent activation energies obtained by Coast-Redfren method are lower than those of FWO, Fredmean, and KAS methods [13].

The data show that energy of activation dependent of conversion (see Table 4-9) and the apparent activation energies sharply increase with increase in the degree of conversion for FOW, Friedman, and KAS methods.
Figure 4-9. KAS plots of *C. fragile* at different conversion fraction.

Table 4-7. Activation energies calculated by KAS method.

<table>
<thead>
<tr>
<th>αᵣ</th>
<th>Curve fit Equation</th>
<th>R-Squared value</th>
<th>$E_a$ (kJmol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>$y = -9.9x + 9.8623$</td>
<td>0.96</td>
<td>82.3</td>
</tr>
<tr>
<td>0.1</td>
<td>$y = -6.2x + 4.4282$</td>
<td>0.95</td>
<td>51.7</td>
</tr>
<tr>
<td>0.2</td>
<td>$y = -11.3x + 11.843$</td>
<td>0.95</td>
<td>94.3</td>
</tr>
<tr>
<td>0.3</td>
<td>$y = -14.2x + 16.235$</td>
<td>0.94</td>
<td>118.1</td>
</tr>
<tr>
<td>0.4</td>
<td>$y = -16.7x + 19.433$</td>
<td>0.93</td>
<td>139.3</td>
</tr>
<tr>
<td>0.5</td>
<td>$y = -19.0x + 21.625$</td>
<td>0.9</td>
<td>158.2</td>
</tr>
<tr>
<td>0.6</td>
<td>$y = -23.9x + 27.827$</td>
<td>0.78</td>
<td>199.0</td>
</tr>
<tr>
<td>0.7</td>
<td>$y = -20.9x + 20.766$</td>
<td>0.86</td>
<td>173.455</td>
</tr>
<tr>
<td>0.8</td>
<td>$y = -20.4x + 18.042$</td>
<td>0.99</td>
<td>169.7</td>
</tr>
</tbody>
</table>
Figure 4-10-a. Coats-Redfern plots of *C. fragile* (with average particle size $150 \sim 250 \mu m$, heating rate $5 \text{ min}^{-1}$) for stages I, II, and III.

Figure 4-10-b. Coats-Redfern plots of *C. fragile* (with average particle size $150 \sim 250 \mu m$, heating rate $10 \text{ min}^{-1}$) for stages I, II, and III.
Figure 4-10-c. Coats-Redfern plots of *C. fragile* (with average particle size 150 ~ 250 μm, heating rate 20 min⁻¹) for stages I, II, and III.

Figure 4-10-d. Coats-Redfern plots of *C. fragile* (with average particle size 150 ~ 250 μm, heating rate 50 min⁻¹) for stages I, II, and III.
Table 4-8. Activation energies calculated using Coats-Redfern method for each stage as function of heating rate.

<table>
<thead>
<tr>
<th>Heating rate</th>
<th>Stage</th>
<th>Curve fit equation</th>
<th>$R$-Squared value</th>
<th>$E_a$ (kJmol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_1$ 5 °C/min</td>
<td>I</td>
<td>$y = -3.2x + 0.7771$</td>
<td>0.95</td>
<td>61.6</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>$y = -1.7x - 3.0866$</td>
<td>0.96</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>$y = -0.6x - 5.0234$</td>
<td>0.97</td>
<td>11.6</td>
</tr>
<tr>
<td>$\beta_2$ 10 °C/min</td>
<td>I</td>
<td>$y = -3.1x + 0.2596$</td>
<td>0.90</td>
<td>59.7</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>$y = -2.3x - 2.0583$</td>
<td>0.91</td>
<td>43.3</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>$y = -0.7x - 4.7433$</td>
<td>0.95</td>
<td>14.9</td>
</tr>
<tr>
<td>$\beta_3$ 20 °C/min</td>
<td>I</td>
<td>$y = -4.5x + 3.3629$</td>
<td>0.9</td>
<td>86.8</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>$y = -2.1x - 2.3732$</td>
<td>0.91</td>
<td>41.2</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>$y = -0.5x - 5.185$</td>
<td>0.91</td>
<td>10.0</td>
</tr>
<tr>
<td>$\beta_4$ 50 °C/min</td>
<td>I</td>
<td>$y = -4.5x + 2.3154$</td>
<td>0.94</td>
<td>86.2</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>$y = -2.7x + 1.6937$</td>
<td>0.91</td>
<td>51.3</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>$y = -0.7x - 4.9175$</td>
<td>0.97</td>
<td>13.9</td>
</tr>
</tbody>
</table>

Figure 4-11. Activation energies calculated from Coats-Redfern method for each stage.
Table 4-9. Activation energies corresponding to the decomposition of *C. fragile* for each conversion and each stage; calculated based on four methods: FOW, Friedman, KAS, and Coast-Redfren.

<table>
<thead>
<tr>
<th>Stage</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>FOW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{a(0)}$</td>
<td>293.9</td>
<td>198.5</td>
<td>225.7</td>
</tr>
<tr>
<td>$E_{a\text{ (ave)}}$</td>
<td>218.7</td>
<td></td>
<td>331.5</td>
</tr>
<tr>
<td>Friedman</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{a(0)}$</td>
<td>233.1</td>
<td>142.9</td>
<td>218.0</td>
</tr>
<tr>
<td>$E_{a\text{ (ave)}}$</td>
<td>188.0</td>
<td></td>
<td>251.9</td>
</tr>
<tr>
<td>KAS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{a(0)}$</td>
<td>82.3</td>
<td>51.7</td>
<td>94.3</td>
</tr>
<tr>
<td>$E_{a\text{ (ave)}}$</td>
<td>67.0</td>
<td></td>
<td>141.8</td>
</tr>
<tr>
<td>Coast-Redfren</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$E_{a(0)}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$E_{a\text{ (ave)}}$</td>
<td>73.4</td>
<td>42.1</td>
<td></td>
</tr>
</tbody>
</table>

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4.4. Conclusion

In the present study the thermal decomposition of *C. fragile* along with its kinetics was investigated in detail. I have realized that pyrolysis of macroalgae can be influenced by the pyrolysis temperature, particle size, and heating rate. Therefore, it is necessary to evaluate all these parameters for any type of marine biomass prior to scale up the pyrolysis system. I assume that the activation energy is a function of conversion. In fact, the value of the activation energy can give an idea about the optimum reaction conditions in process chemistry, it gives an idea about the thermal stability and the expected lifetime of a compound to be kept at a certain temperature or it provides information in quality research [34]. This study comprised the various kinetic models proposed in literature for *C. fragile* decomposition. The thermodynamic data estimated from the proposed models were in good agreement with the experimental values.

Nomenclature

*C. fragile*  *Codium fragile*

*FOW*  Flynn, Ozawa, Wall

*KAS*  Kissinger-Akahira-Sunose

*TGA*  Thermo Gravimetric Analysis

*DTG*  Derivative Thermo Gravimetric

*TG*  Thermo Gravimetric

*(α)*  Conversion degrees

*(β)*  Heating rate

*E<sub>a</sub>*  Activation Energy (KJ/mol)

*A*  Frequency factor (s<sup>-1</sup>)
\( R \)  Universal gas constant (\( R = 8.314 \text{ J/(mol} \cdot \text{K}) \))

\( T \)  Temperature (°C)

\( f(\alpha) \)  Functional relationship between decomposed solid reactant and the reaction rate.

\( m_0 \)  Initial mass of sample (mg)

\( m_\infty \)  final mass of sample (mg)

References


Chapter 5

Ultrasonic assisted treatment of selected green macroalgae
5. 1. Introduction

The mechanical and chemical effects of ultrasound are believed to accelerate the extraction of organic compounds from plant materials [1]. The possible benefits of ultrasound in extraction are mass transfer intensification, cell disruption, improve penetration, and capillary effects [2].

The use of ultrasonic in starch hydrolysis has been reported as early as 1933 by Szent-Gyorgyi [3]. Depolymerization of macropolymers, such as starches [4, 5] and reduction of average molecular weight, occurs as a result of ultrasonication treatment [6]. In these cases, the cavitation breaks the primary back-bone bonds resulting in smaller molecules. Ultrasound could be used to enhance particle size reduction, fastest gelatinization, and fractionation of sample into its compounds [7, 8].

It also enhances the production of ethanol and reduces the use of enzymes during the simultaneous saccharification and fermentation reactions [9, 10]. These studies employed a bench-scale batch ultrasonic system to pretreat the samples to enhance sugar release during saccharification.

Conversion and production of valuable materials from green macroalgae as a model of marine biomass can be performed using the number of traditional methods or more innovative methods such as sub- and supercritical fluids and pyrolysis methods, which have been explained in the previous chapters in details. In this chapter, in order to evaluate the better conversion and treatment method, the effect of ultrasonic irradiations on C. fragile was investigated. The efficiency of the method has been evaluated by saccharification yield, changing reaction solvent, bio-oil production, and the purity of extracted fiber.

In this study, I assumed that the most probable mechanism for ultrasonic
enhancement of extraction was the intensification of mass transfer and easier access of the solvent to C. fragile cell. The collapse of cavitation bubbles near the sample cell walls was expected to produce the cell disruption together with a good penetration of the solvent into the cells, through the ultrasonic jet.

5. 2. Experimental

5. 2. 1. Materials

Fresh C. fragile was collected at Rinkou Park in Osaka, Japan during 2011. Phenol and sulfuric acid were purchased from Kishida Kagaku (Japan). Ethanol, D-glucose, and potassium bromide were purchased from Wako Chemical Industries, Ltd. (Japan). Microcrystalline cellulose powder was purchased from Sigma-Aldrich (USA).

5. 2. 2. Methods

The ultrasonic system was a bench scale Brason Sonifier 450 with 20 kHz horn type homogenator and maximum output amplitude of 10 μm_{pp} (peak-to-peak). Rosette type glass vessel developed by Maeda [11] was used in order to promote propulsion of the sample together and better exposure of sample. Four external loops continuously delivered the algal material under vibrating tip of the horn. In this way we managed to avoid the agglomeration effect without employing an external propelling device. Figure 5-1 shows the schematic of experimental set up for ultrasonic system.

In this study the effects of two kinds of solvent, i.e. pure water and ethanol, have been investigated separately at the same sonication conditions to evaluate and compare the optimum reaction conditions. The collected fresh C. fragile was washed and cleaned foreign debris and towel-dried. It was then dried in freeze dryer and kept in
freezer for further experiments. An accurately weighed amount of about 1 g of freeze
dried sample dispersed in 80 ml of solvent (ethanol or pure water) in a rosette glass
vessel which take place in water cooling bath (Figure 5-1). Samples treated at three
different amplitude levels, 3, 6, and 10 μm_{pp} for 1 to 20 min reaction time. Reaction was
done by keeping the temperature at 22 ºC using water cooling bath. After desired
reaction time, samples transferred to suitable test tube and centrifuged for 20 min at
4,000 rpm, then aqueous phase and residual phase isolated. Residual solid in test tube
washed with solvent again, shaked, and centrifuged 20 min at 4,000 rpm. This
extraction step was repeated three times (total aqueous volume 100 mL). Water soluble
phase was filtered with a 0.2 μm filter. Ethanol soluble phase was separated from
residual solids. Residual solid phase was placed in an oven at 60 ºC for 48 h to dry to a
constant weight. Figure 5-2 shows the procedure diagram of sonication of C. fragile.
Figure 5-1. Schematic of experimental set up for ultrasonic system.
Figure 5-2. Schematic diagram of experimental and extraction procedure of sonication.
5. 2. 3. Analysis

The amount of total soluble sugars in the aqueous phase was determined by a photometric method (phenol-sulfuric acid assay), using D-glucose as a standard curve [12, 13]. Double-beam spectrophotometer (UV-1700 Pharmas Pec- Shimadzu) was used for this purpose.

In order to identify the ethanol soluble materials, particularly bio-oil composition before and after sonication reaction, a Hewlett-Packard HP 6890 gas chromatograph coupled to a quadrupole mass spectrometer HP 5973 equipped with a mass selective detector (MSD) was used. A 30 m × 0.25 mm diameter capillary column (Agilent 19091N-133) coated with a 0.5 μm thick film of 5% phenyl methyl siloxane (HP-5 Trace Analysis) was used to separate the decomposition products. Helium (99.999%) was used as a carrier gas at a constant flow rate of 1 mL/min. Initially, the oven temperature was held at 50 °C for 2 min and was raised to 290 °C, at a rate of 7 °C/min.

The element composition of the dried residual solid and also dried initial sample (for comparison) was analyzed by CHN analyzer (YANACO Co., Ltd., MT-6). The higher heating value was calculated for each sample using the equation (5-1) given by Tushar [14]. The calculation is based on the carbon, hydrogen, and nitrogen content.

\[
HHV \ (MJ. \ Kg^{-1}) = [5.22C^2 - 319C - 1647H + 38.6C \times H + 133N + 21 \ 028] \times 10^{-3} \quad (5-1)
\]

FTIR spectroscopic analysis of initial dry sample, residual samples after ultrasonic assisted treatment, and pure cellulose (for comparison) was performed by JASCO FTIR-6100 over a range of 600–4,000 cm\(^{-1}\). All measurements were carried out by means of pure and dried KBr plates.
5. 3. Results and discussion

5. 3. 1. Effect of sonication on sugar yield

The sugar yields of untreated and treated *C. fragile* at different reaction times by application of ultrasonic are shown in Figure 5-3. Analysis of untreated (control) sample shows approximately 4.3% of total soluble sugars in aqueous phase. For treated samples, the amount of sugars released from decomposition of *C. fragile* is proportional to both ultrasonic power level (amplitude) and the reaction time.

Sonication at low level powers (i. e. amplitude 3) causes to release approximately 8% sugar after 1 min; however, increasing the sonication time does not have significant effect on sugar yield and it increases slightly to 11 % up to 20 min treatment.

Medium level of sonication powers (i. e. amplitude 6) seems to be caused the more cell disruption and saccharification reaction in water medium, and consequently increases the releasing of soluble sugar gradually up to 14% in 20 min. Furthermore, with increasing power of the sonication (i. e. amplitude 10), the saccahrification reaction increases and it is obtained satisfaction amount of soluble sugars after 20 min residence time.

These results clearly approved that it could be obtained higher yields of the soluble sugars from cell disruption of the *C. fragile* samples with increasing of sonication power. The highest sonication power was selected as optimum conditions for further studies. Furthermore, reaction time had less impact on the saccharification reactions compared to the sonication power.

5. 3. 2. Effect of solvent on solubility of *C. fragile*
Figure 5-4 shows the effect of solvent type (pure water or ethanol) in sonication times ranging between 1 to 20 min on the dissolution and decomposition of *C. fragile* at selected output amplitude $10 \, \mu m_{pp}$. Results show the solubility of untreated sample in water and ethanol are 9.4% and 6.3%, respectively. After 1 min sonication, the solubility in water medium dramatically increases and reaches to 48.8%; however, with increasing residence time, solubility of the sample does not have conspicuous increase. The maximum solubility which obtained by sonication of *C. fragile* in water is 59% after 10 min reaction time. At longer residence times this amount decreases somewhat and reaches to 54.5 %.

Results of sonication of *C. fragile* in ethanolic media show that after 1 min reaction, the solubility reaches to 11.6%. This amount gradually increased with increasing the residence time and reached to maximum amount of 15% after 20 min residence time.

Higher dissolution amount of *C. fragile* in water medium clearly proves that sonication solvent has great effect on the reaction efficiency. Still there are several other environmentally friendly solvents which could be examined on the sonication treatment of *C. fragile*. 

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Figure 5-3. Effect of sonication of *C. fragile* in water medium on the yield of soluble sugars as a function of power and time.

Figure 5-4. Comparison of solubility of *C. fragile* in different solvent at output amplitude 10 μm_{pp}. 
5. 3. 3. Identified decomposition products in ethanol phase

The composition of ethanolic phase extracted before and after sonication of C. fragile was analyzed by GC-MS. The amount of the decomposition products was small owing to less dissolution of C. fragile in ethanol medium during sonication reaction (see Figure 5-4). Table 5-1 shows the identified products in ethanolic phase after 20 min sonication by output amplitude 10 $\mu m_{pp}$. The relative contents of identified compounds take up about 37.96 % of the total peak area. As result very high promising amount of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol) is identified and quantified at RT of 26.8 min with 28.71% of peak area. Heptadecane as a hydrocarbon (RT of 24.05 min) was also identified in this phase with 5% of total peak area. Around 4% of total peak area has concerned to 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)- (EPA), which it is a polyunsaturated fatty acid and metabolically active. There are several other peaks which could not be identified in this study.

5. 3. 4. Effect of sonication treatment on the residual solids

Effect of sonication treatment on residual solids has been investigated at different reaction times ranging from 1 to 20 min in two different reaction media (pure water and ethanol). Results of sonication in pure water and ethanol are shown in Figure 5-5. The results prove that the amount of solid residue in water medium is much less than ethanol medium. After only 1 min reaction in water medium, remained solid decreases from 90.6% to 51.2%. However, increasing the sonication time does not have a significant effect on remained solids, and its amount reaches to 45.4% up to 20 min reaction time. Sonication of C. fragile in ethanolic medium causes to decrease the solid residue up to 85.1% after 20 min reaction time.
5. 3. 5. Ultimate analysis and energy density evaluation of the residual solids

In order to evaluate the variation of elemental compositions of solid residue of *C. fragile* at different conditions and its relative energy density, ultimate analysis of solid residues have been studied. The results are shown in Table 5-2. It can be seen that the carbon, hydrogen, and nitrogen contents of sonicated samples at water medium are higher in comparison to that of the ethanolic medium sonication. Carbon, hydrogen, nitrogen, and higher heating values of samples at water medium are about of 37, 6, 2.5 wt%, and 15 MJ/kg, respectively. These amounts for sonicated samples in ethanol medium were 25, 4, 2 wt%, and 13 MJ/kg, respectively.

As shown in Table 5-2, sonication times do not have significant effect on variation on CHN compositions and HHVs of samples after sonication. It means sonication power is not strong enough to completely decompose the cellulosic structure of *C. fragile*, and even in water medium hydrolysis reactions have not taken place to increase the carbon content and consequently the energy density and the heating value of the residual solid. It causes to obtain almost similar fixed carbon and HHVs at different reaction times. The large difference in wt% of elemental compositions and HHVs could be found by changing the sonication medium from pure water to ethanol, which could be attributed to ease of solubility and dissociation of *C. fragile* in water medium rather than ethanol.
Table 5-1. Main identified products from sonication of *C. fragile* in ethanol phase at power of 10 amplitudes in 20 min.

<table>
<thead>
<tr>
<th>No.</th>
<th>RT (min)</th>
<th>Identified compound names</th>
<th>Peak area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24.05</td>
<td>Heptadecane</td>
<td>5.23</td>
</tr>
<tr>
<td>2</td>
<td>26.82</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol)</td>
<td>28.71</td>
</tr>
<tr>
<td>3</td>
<td>32.86</td>
<td>5,8,11,14,17-Eicosapentaenoic acid methyl ester (all-Z)- (EPA)</td>
<td>4.02</td>
</tr>
</tbody>
</table>

Figure 5-5. Effect of sonication time and solvent on remained solids of *C. fragile*. 

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Table 5-2. Ultimate analysis and higher heating values of *C. fragile* before and after sonication in water and ethanolic media at different reaction time.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Sonication time (min)</th>
<th>Elemental compositions (wt%)</th>
<th>HHV (MJ/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td>Pure Water</td>
<td>Control</td>
<td>37.35</td>
<td>6.05</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>37.54</td>
<td>5.91</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>37.52</td>
<td>6.33</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>36.34</td>
<td>6.33</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>37.27</td>
<td>6.24</td>
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<td>15</td>
<td>37.33</td>
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<td></td>
<td>20</td>
<td>37.27</td>
<td>6.21</td>
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<tr>
<td>Ethanol</td>
<td>Control</td>
<td>25.34</td>
<td>4.13</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>24.71</td>
<td>3.88</td>
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<tr>
<td></td>
<td>3</td>
<td>24.98</td>
<td>3.89</td>
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<td></td>
<td>5</td>
<td>25.34</td>
<td>4.11</td>
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<tr>
<td></td>
<td>10</td>
<td>25.67</td>
<td>4.33</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>25.37</td>
<td>4.52</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>25.65</td>
<td>4.76</td>
</tr>
</tbody>
</table>
5. 3. 6. Structural studies of the residual solids

FTIR spectra of residual solids before and after sonication at different reaction times have been studied in the frequency range of 4,000 – 600 cm\(^{-1}\) in transmission mode. Figures 5-6 and 5-7 show the typical FTIR spectra of pure cellulose (for comparison), untreated and treated samples by sonication at 10 and 20 min in ethanolic and water media, respectively. The broad band in the 3,600-3,100 cm\(^{-1}\) region, which is due to the OH-stretching vibration, gives considerable information concerning the hydrogen bonds [15]. The peaks characteristic of hydrogen bonds from the spectra of amorphous cellulosic structure of untreated and treated \textit{C. fragile} samples show lower intensity and small shifting of peaks to higher wavenumber values in comparison with the initial cellulosic samples. It can be correlated with the scission of the intra- and intermolecular hydrogen bonds.

The presence of amorphous cellulosic structure in samples can be further confirmed by the shift of the band from 2,900 cm\(^{-1}\), corresponding to the C–H stretching vibration, to higher wavenumber values and by the strong decrease in the intensity of this band. The adsorption bands from the 1,500-899 cm\(^{-1}\) region are strongly reduced in intensity, or even absent. In addition, the FTIR absorption band at 1,430 cm\(^{-1}\), assigned to a symmetric CH\(_2\) bending vibration, decreases. This band is also known as the crystallinity band, indicating that a decrease in its intensity reflects reduction in the degree of crystallinity of the samples.

Since the main structure of \textit{C. fragile} is cellulose especially in cell walls; and also, sonication in water or ethanolic medium does not hydrolyze the samples completely, it is expected to obtain the structure as same as pure cellulose from residual solids after treatment. As shown in Figures 5-6 and 5-7, shorter time of sonication
change the structure of residual solid somewhat. However, increasing the sonication time up to 20 min causes to increase the adsorption band in each region which has resulted to increase the similarity of treated samples with pure cellulose fibers.

As main and interesting result, by sonication of *C. fragile* it is possible to extract valuable materials along with production of very pure cellulosic fibers. Recently, there are increasing interests for pure cellulosic fibers; for example alginate fibers. Alginate fibers are resistance to fire and electromagnetic wave, and they can be used in various industries due to their mentioned specifications and also high durability and low weight.
Figure 5-6. FTIR spectra of pure cellulose, initial *C. fragile*, residual solid of untreated sample, and treated samples by sonication for 10 and 20 min in ethanolic media.

Figure 5-7. FTIR spectra of pure cellulose, initial *C. fragile*, residual solid of untreated sample, and treated samples by sonication for 10 and 20 min in water media.
5.4. Conclusion

The ultrasonic assisted treatment of *C. fragile* results in disruption of cell walls and release of intercellular materials. Improvement in sugar release into water extracts is proportional to the sonication amplitude and reaction time somewhat. The total sugar release is improved by as much as 20% with respect to untreated sample. Temperature control and heat generation during the sonication do not affect the sugar release, indicating the cell rupture caused by ultrasound to be the main mechanism for better separation and product yield. On the other hand, compared to water medium reactions, by utilization of this method in ethanolic medium, it can be produced significant amount of fatty acids ethyl esters.

High energy density of the residual solids after sonication reactions make them as a very good alternative source for energy production. On the other hand, base on the results of FTIR analysis, high purity and very similar characteristics of these residual solid to the pure cellulosic fibers, and also high amount of remained residual solids in comparison with the other innovative methods, make it very good technique for bio-fiber production.

Finally because of production of a very value added compounds as well as simplicity of such a green process, the proposed method could be easily scaled up to pilot and industrial plants without any technical problems.
Reference


Chapter 6

Process simulation and pilot plants design for treatment of selected green macroalgae
6. 1. Introduction

Since approximately 50% of global biomass is thought to be generated in a marine environment [1], marine biomass has great potential as a feed stock for future biofuel and bio-chemicals generation. In addition, the issues arising with increasing the proportion of land use for biomass crops and the “food versus fuels” debate are not applicable to marine biomass [2].

For instance, the macroalgae industry has an estimated total annual value of US$ 5.5–6 billion, produced from 7.5–8 million tons of naturally growing and cultivated macroalgae harvested worldwide. The main use is as food products for human consumption, which generate approximately US$ 5 billion per year, with the remainder generated from extracted hydrocolloids, fertilizers and animal feed additives [3]. Such a huge amount of marine biomass motivated researchers to find industrially-sound green techniques for treatment; and consequently these techniques must capable to be scaled up from bench scale to pilot and industrial scales.

In fact, for industrialization of a process, it is impossible to explore all the important features and variables via experimental investigations alone. Numerical simulation is an invaluable and powerful tool for the analysis, design, and economic evaluation of the individual process units, and for comparing and optimizing various process alternatives. Numerical simulations naturally cannot replace experimental studies, but are more of a tool used in the planning and evaluation of the experiments [4]. The large plant design is initially based on a traditional approach (production process flow charts analysis and plant layout study), and it is then supported by a simulation model mainly used to investigate the system behavior in different operative scenarios. The plant simulation model recreates the entire production process (based on different
The focus of the research work presented in this chapter is the design and economic feasibility studies of four pilot plants (based on the different evaluated laboratory techniques) devoted to produce different types of value-added products from decomposition of the selected green macroalgae. The aim of this chapter is to simulate a pilot plant with capacity of 10 kg/h. Mass balance and energy balance of such a simulated pilot plants including cost studies were investigated in details.

6.2. Process simulation using *Aspen Plus*® software

Mathematical modeling and simulation studies are powerful tools to predict the performance of new processes. With the development of computer technology, computer simulation has become an important tool in the design of particularly chemical pilot and industrial plants. Among several computer simulation softwares, *Aspen Plus* is a comprehensive chemical process modeling system, used by the world’s leading chemical and specialty chemical organizations, and related industries to design and improve their process plants. *Aspen Plus* allows plant engineers to view simulation results and plant data side by side within the process model. It uses the underlying physical relationships (e.g., material and energy balances, thermodynamic equilibrium, rate equations) to predict process performance (e.g., stream properties, operating conditions, and equipment sizes). *Aspen Plus* is one of the most popular process simulation software programs used industrially and academically.

In this research work, *Aspen Plus*® version 2006.5 software (Aspen Technology Inc. Cambridge, Massachusetts, USA) was used for process simulations. Based on the experimental data obtained from previous chapters (i.e. sub and
supercritical water, subcritical ethanol, pyrolysis, and ultrasonic assisted experiments), I attempted to simulate pilot plants for the macroalgae treatment. My model includes the steps of drying, gasification or liquefaction, syngas, bio-oil production, and fermentation reactions. The model is set in Aspen Plus®.

6. 2. 1. Materials

The models of the macroalgae treatment plants are implemented on Aspen Plus® simulation tool with capacity of 10 kg/h. For simplicity, in all techniques I assumed the simulated plants to have single continuous tubular reactors. Macroalgae and char components are defined as non-conventional components based on their ultimate analysis including C, H, O, N, S and ash elements, and proximate analysis. The plants are generally (depended on the method) composed of a drying unit, screw pump, main tubular reactor, a centrifuge, and separator. Overviews of the whole integrated processes are provided in Figures 6-1 to 6-4.

I set a simple specifications for the units used in the simulation of each plants. For example, the drying unit is composed of a rotary dryer, and macroalgae was dried with air at 100 °C. Screw pumps were selected to operate either at normal or higher pressures. All equipment was made of stainless steel. Table 6-1 shows the main unit operations used in the simulation processes of the plants.
Table 6-1. Major unit operations along with general specifications used in the simulation process

<table>
<thead>
<tr>
<th>Unit Operation</th>
<th>Operation Pressure (MPa)</th>
<th>Operating Temperature (°C)</th>
<th>Utilization Pilot Plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screw pump</td>
<td>Up to 10</td>
<td>Up to 300 °C</td>
<td>All pilot plants</td>
</tr>
<tr>
<td>Tubular reactors</td>
<td></td>
<td></td>
<td>Sonication pilot plant</td>
</tr>
<tr>
<td>Sonication apparatus</td>
<td></td>
<td></td>
<td>Subcritical water/Subcritical ethanol/Pyrolysis/Sonication</td>
</tr>
<tr>
<td>Centrifuge</td>
<td></td>
<td></td>
<td>Subcritical water/Pyrolysis/Sonication</td>
</tr>
<tr>
<td>Drier</td>
<td></td>
<td></td>
<td>Sonication pilot plant</td>
</tr>
<tr>
<td>Evaporator</td>
<td></td>
<td></td>
<td>Subcritical water/Pyrolysis/Sonication</td>
</tr>
<tr>
<td>Separator tank</td>
<td></td>
<td></td>
<td>Subcritical water/Pyrolysis/Sonication</td>
</tr>
<tr>
<td>Fermentation tank</td>
<td></td>
<td></td>
<td>Subcritical water/Pyrolysis/Sonication</td>
</tr>
<tr>
<td>Water gas shift reactor</td>
<td></td>
<td></td>
<td>Subcritical water/Pyrolysis/Sonication</td>
</tr>
</tbody>
</table>
6. 2. 2. Process modeling

(1) Subcritical water pilot plant

Figure 6-1 shows the process unit of macroalgae conversion. As shown, high pressure screw pump feed fresh macroalgae (as is) through the system, and increase the pressure to maintain the water as liquid. Inside tubular subcritical reactor, decomposition, hydrolysis, and or extraction reactions take places (at optimum temperature, 210 ºC). Whole products, then move out to a centrifuge in order to separate the phases. Residual solid enters the drier set at 60-70 ºC to completely dry and store in storage tank. Solid phase which consists of mainly pure cellulose can be used in several industries, such as pharmaceutical, polymer industries, as well as direct combustion processes.

In the liquid phase, water insoluble organic phase must be separated, which is mainly consists of bio-oil. This product is a relatively expensive and has vast industrial applications. Remained aqueous phase has very high TOC amounts which can be used in fermentation processes to produce biogas or bio-ethanol. Another alternative application of liquid phase is utilization of water gas shift reactions in order to produce hydrogen gas. The simulation process as well as material and energy balances are based on methane fermentation reactions of the liquid phase, and has not been not investigated other possibilities in this research work.
Figure 6-1. Overview of the modeled macroalgae subcritical water treatment plant.
(2) Subcritical ethanol pilot plant

Figure 6-2 shows the process unit of macroalgae conversion plant using ethanol instead of water in the system. The difference between this process and subcritical water process is only initial feedstock. In the case of subcritical water, fresh macroalgae with approximately 93% of water directly subjected to the unit; however, for subcritical ethanol process, it is necessary to dry the sample prior to operation.

Firstly dried macroalgae passes through the high pressure screw pump to mix with ethanol. The stream then enters to the tubular continuous subcritical ethanol reactor. In the reactor, decomposition and transesterification of sample takes place at around 190 ºC and 10 MPa. The stream thereafter transfers to the centrifuge to separate ethanol and solid phases. Ethanol stream enters to the ethanol recovery evaporator unit at 90 ºC temperature. The purpose of this unit operation was not only to separate ethanol, but also to recycle it back to the system. High ethanol recycle is desirable in a transesterification reaction process because it reduces feed costs. Contrary to similar simulations, evaporation is chosen in the procedure instead of a distillation column [6]. This is because only a simple separation is needed due to ethanol’s low boiling point compared to the other components. After evaporating the ethanol, bio-oil collects and stores as biofuel materials. The solid washes with pure water in solid washing unit. Then the stream enters the mixer to enhance the extraction of water soluble materials from residual solid. After separation of the phases by centrifuge, residual solid enter the drier unit set at 60-70 ºC to completely dry and store in storage tank as pure cellulose fibers. They can be also directly combusted in the boiler to provide heat and power for the process. Definitely, as I will see later, the main disadvantage of this process is drying of macroalgae which will be increased the operation cost somewhat.
Figure 6-2: Overview of the modeled macroalgae subcritical ethanol treatment plant.

**Marine biomass**

**Dried**

- **Marine biomass**
- **Dried**

- **High Pressure Screw Pump**
- **Continuous Subcritical Ethanol Reactor**
- **Evaporator**
- **Centrifuge**
- **Heater**
- **Vacuum Drying**

- **FEED-IN**
- **IN**
- **OUT**
- **ETOH-OUT**
- **LIQ-OUT**
- **SLID-OUT**
- **MIX-IN**
- **WATER-IN**
- **Pure water**
- **Liq-Oil**
- **BIO-OIL Products**
- **Water Soluble Products, including soluble sugars**
- **Pure Cellulose Fibers**
(3) Pyrolysis pilot plant

As described in Chapter 4, pyrolysis is a powerful technique to obtain bio-oil as well as bio-char from a variety of biomass compounds. There are several industrial scale plants all over the world working on bio-oil production. In this chapter, however, I simulate a pilot plant for macroalgae conversion into bio-oil, bio-gas, and bio-char. Figure 6-3 describes the process unit for pyrolysis system. Dried feedstock must be utilized. Pyrolysis process takes place up to 400 °C, and as shown in Figure 6-3, carries out in the absence of oxygen gas (i.e. nitrogen atmosphere). Gaseous and liquid products, after cooling down, can be collected separately using as separator unit. Bio-char is the remained solid from pyrolysis reaction, which nourishes soils, protects water quality, provides market value to biomass waste, creates clean energy, reduces greenhouse gas (GHG) emissions, and sequesters CO$_2$ for thousands of years [6].
Figure 6-3. Overview of the modeled macroalgae pyrolysis treatment plant.
(4) Ultrasonic assisted pilot plant

Ultrasonic assisted macroalgae treatment is quite new method. To the best of my knowledge, there is no academic or pilot plant treatment reports for macroalgae treatment using this method yet. I aimed to use the experimental data obtained from the bench scale experiments to simulate a pilot plant. A general process flow diagram of such a pilot plant is shown in Figure 6-4. The process is similar to previous ones except the reactor which here is a sonication apparatus. There is lack of technical and specification data necessary for simulation of a pilot scale sonication apparatus, therefore we roughly estimate the energy balance for the system, as will be shown in the next section. The main product of sonication process is pure cellulosic fiber which is very valuable intermediate by-product for industrial applications. From hydrolysis of cellulose macromolecules, soluble sugars can also be obtained in aqueous phase which is a very attractive for fermentation processes.
Figure 6-4. Overview of the modeled ultrasonic assisted macroalgae treatment plant.
6. 2. 3. Economic assessment and feasibility studies

Since each process is capable of producing bio-oil, pure cellulosic fiber, and other valuable materials at the acceptable level, it is of interest to conduct an economic assessment to determine process viability, and determine if any one process is advantageous over the others. The cost and feasibility of each pilot plant is calculated based on the data obtained from experimental analysis as well as simulation process using Aspen Plus software. It worth to note that, for accurate feasibility studies of any pilot plant, several other technical and thermodynamic data is needed, which was not possible to obtain them in this research work. Hence, I do not estimate the purification process cost, plant construction expenses, utility cost, and labors costs and just do a roughly calculation to estimate the general benefits of each proposed process. The results of estimation of operation cost, value of obtained products, composition of bio-oils and their value are summarized in Tables 6-2(a-c) to 6-5(a-c) for subcritical water, subcritical ethanol, pyrolysis, and ultrasonic assisted processes, respectively.

Estimated energy and material cost of 10 kg/h subcritical water pilot plant (without considering the manufacturing and utility cost) is around 16 ¥/h. On the other hand the final products from this system such as bio-oil, cellulosic fiber, methane gas, and carbon dioxide, showed the higher value of about 655 ¥/h. Definitely, it must be added the cost of separation and purification of these products and along with changing the products yield after purification process as well.

Subcritical ethanol pilot plant shows slightly higher cost (29 ¥/h) comparing to subcritical water pilot plant, which can be attributed to the pre-drying cost of wet sample. Because of completely recovery of ethanol in this process, its cost has no effect on the total expenses. Due to the transesterification reactions, very high valuable
products obtained such as fatty acid ethyl ester, which cause to total high benefit of about 4100 ¥/h.

Simulation results of pyrolysis pilot plant evaluate cost of 12 ¥/h for the process unit. Because of high cellulose content of the sample, final products of this process are mostly bio-oil and bio-char, and mixture of gaseous product (syngas) with roughly value 42 ¥/h.

Simulation of 10 kg/h ultrasonic assisted pilot plant shows cost of about 10 ¥/h, which is obviously cheaper than the other mentioned simulated plants. The estimation of produced compound by this process shows the value of about 335 ¥/h; owing to production of higher amounts of very valuable bio-oils.

Obviously, each process has its much advantages and disadvantages. In comparison, the subcritical ethanol system has more benefit than the others. However, still several other experiments are needed to understand the kinetics and thermodynamics of the decomposition reactions of the macroalgae in order to entirely evaluate simulation of the processes.
Table 6-2 (a). Total operation cost of subcritical water pilot plant with capacity of 10 kg/h.

<table>
<thead>
<tr>
<th>Plant process cost</th>
<th>Estimated energy/material consumption</th>
<th>Unit Price ¥/h</th>
<th>Total Price ¥/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine biomass material</td>
<td>10 kg/h (wet)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Screw pump</td>
<td>0.075 kW/h</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Subcritical reactor heating</td>
<td>0.25 kW/h</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>0.375 kW/h</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Separator</td>
<td>0.015 kW/h</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Drier</td>
<td>0.054 kW/h</td>
<td>1.08</td>
<td>1.08</td>
</tr>
<tr>
<td>Fermentation reactor</td>
<td>0.006 kW/h</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Mixer of fermentation</td>
<td>0.006 kW/h</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Total Process Cost/h</strong></td>
<td>-</td>
<td><strong>16.12</strong></td>
<td><strong>16.12</strong></td>
</tr>
</tbody>
</table>

Table 6-2 (b). Value of the decomposition products obtained under subcritical water pilot plant.

<table>
<thead>
<tr>
<th>Product name</th>
<th>Production amount kg/h</th>
<th>Product value ¥/h</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure cellulose fiber</td>
<td>0.20</td>
<td>201</td>
<td>Ref [7]</td>
</tr>
<tr>
<td>Total soluble sugars</td>
<td>0.34</td>
<td>-</td>
<td>intermediate product</td>
</tr>
<tr>
<td>Inorganic elements</td>
<td>0.15</td>
<td>1.43</td>
<td>-</td>
</tr>
<tr>
<td>Bio-oil*</td>
<td>0.01</td>
<td>449.57</td>
<td>Ref [8]</td>
</tr>
<tr>
<td>Methane gas</td>
<td>0.08</td>
<td>2.33</td>
<td>28 m³ = 660 ¥</td>
</tr>
<tr>
<td>Carbone dioxide</td>
<td>0.14</td>
<td>0.2</td>
<td>1 tone = 1580 ¥</td>
</tr>
<tr>
<td>Unknown compounds (based on TOC results)</td>
<td>0.12</td>
<td>0.1</td>
<td>roughly estimation</td>
</tr>
<tr>
<td><strong>Total products (value)/out of 0.7 kg dry feed</strong></td>
<td>0.7</td>
<td><strong>654.64</strong></td>
<td>¥/h</td>
</tr>
</tbody>
</table>

Table 6-2 (c). Composition/value of bio-oil obtained under subcritical water pilot plant.

<table>
<thead>
<tr>
<th>*Bio-oil individual composition</th>
<th>Production amount kg/h</th>
<th>Unit Price ¥/kg</th>
<th>Total Price ¥/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid (10% of total bio-oil)</td>
<td>0.0014</td>
<td>22000</td>
<td>29.7</td>
</tr>
<tr>
<td>Archidonate Acid (2% of total bio-oil)</td>
<td>0.0003</td>
<td>1300000</td>
<td>351</td>
</tr>
<tr>
<td>Hexanoic acid (60% of total bio-oil)</td>
<td>0.0081</td>
<td>8500</td>
<td>68.85</td>
</tr>
<tr>
<td>3,7,11,15, tetramethyl 2 hexadecane 1-ol (10% of total bio-oil)</td>
<td>0.0014</td>
<td>(1) 1000000</td>
<td>-</td>
</tr>
<tr>
<td>Unknown bio-oil (18% of total bio oil)</td>
<td>0.0024</td>
<td>10</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>0.0135</td>
<td></td>
<td><strong>449.57</strong></td>
</tr>
</tbody>
</table>

(1) The presented price is based on pure materials and has not considered in the estimation of bio-oil total price.
Table 6-3 (a). Total operation cost of subcritical ethanol pilot plant with capacity of 10 kg/h.

<table>
<thead>
<tr>
<th>Plant process cost</th>
<th>Estimated energy/material consumption</th>
<th>Unit Price ¥/h</th>
<th>Total Price ¥/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-drying of wet marine biomass</td>
<td>0.25 kW/h</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Marine biomass material</td>
<td>10 kg/h (wet)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Screw pump</td>
<td>0.056 kW/h</td>
<td>1.12</td>
<td>1.12</td>
</tr>
<tr>
<td>Subcritical reactor heating</td>
<td>0.131 kW/h</td>
<td>2.62</td>
<td>2.62</td>
</tr>
<tr>
<td>Centrifuge ×2</td>
<td>0.437 kW/h</td>
<td>8.75</td>
<td>17.5</td>
</tr>
<tr>
<td>Evaporator</td>
<td>0.062 kW/h</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Drier</td>
<td>0.054 kW/h</td>
<td>1.07</td>
<td>1.07</td>
</tr>
<tr>
<td>Solid wash tank</td>
<td>0.01 kW/h</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Total Process Cost/h</strong></td>
<td></td>
<td>-</td>
<td>20.52</td>
</tr>
</tbody>
</table>

Table 6-3 (b). Value of the decomposition products obtained under subcritical ethanol pilot plant.

<table>
<thead>
<tr>
<th>Product name</th>
<th>Production amount kg/h</th>
<th>Product value ¥/h</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure cellulose fiber</td>
<td>0.23</td>
<td>232</td>
<td>Ref [7]</td>
</tr>
<tr>
<td>Total soluble sugars</td>
<td>0.12</td>
<td>-</td>
<td>intermediate product</td>
</tr>
<tr>
<td>Bio-oil*</td>
<td>0.12</td>
<td>3894.33</td>
<td>Ref [8]</td>
</tr>
<tr>
<td>Methane gas</td>
<td>0.07</td>
<td>2.6</td>
<td>28 m³ = 660 ¥</td>
</tr>
<tr>
<td>Carbone dioxide</td>
<td>0.14</td>
<td>0.22</td>
<td>1 tone=1580 ¥</td>
</tr>
<tr>
<td>Unknown compounds (based on TOC results)</td>
<td>0.13</td>
<td>0.1</td>
<td>roughly estimation</td>
</tr>
<tr>
<td><strong>Total products (value)/out of 0.7 kg dry feed</strong></td>
<td>0.7</td>
<td>4129.26</td>
<td>¥/h</td>
</tr>
</tbody>
</table>

Table 6-3 (c). Composition/value of bio-oil obtained under subcritical ethanol pilot plant.

<table>
<thead>
<tr>
<th>*Bio-oil individual composition</th>
<th>Production amount kg/h</th>
<th>Unit Price ¥/kg</th>
<th>Total Price ¥/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid (10 % of total bio-oil)</td>
<td>0.012</td>
<td>22000</td>
<td>264</td>
</tr>
<tr>
<td>Archidonate Acid (2% of total bio-oil)</td>
<td>0.002</td>
<td>1300000</td>
<td>3120</td>
</tr>
<tr>
<td>Hexanoic acid (50% of total bio-oil)</td>
<td>0.06</td>
<td>8500</td>
<td>510</td>
</tr>
<tr>
<td>3,7,11,15, tetramethyl 2 hexadecane 1-ol (10% of total bio-oil)</td>
<td>0.012</td>
<td>(1)1000000</td>
<td>-</td>
</tr>
<tr>
<td>Unknown bio-oil (28% of total bio oil)</td>
<td>0.034</td>
<td>10</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>0.12</td>
<td></td>
<td>3894.33</td>
</tr>
</tbody>
</table>

(1) The presented price is based on pure materials and has not considered in the estimation of bio-oil total price.
Table 6-4 (a). Total operation cost of pyrolysis pilot plant with capacity of 10 kg/h.

<table>
<thead>
<tr>
<th>Plant process cost</th>
<th>Estimated energy/material consumption</th>
<th>Unit price ¥/h</th>
<th>Total price ¥/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine biomass material</td>
<td>10 kg/h (wet)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Pre-drying of wet marine biomass</td>
<td>0.25 kW/h</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Screw pump</td>
<td>0.075 kW/h</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Pyrolysis reactor heating</td>
<td>0.187 kW/h</td>
<td>3.75</td>
<td>3.75</td>
</tr>
<tr>
<td>Crusher</td>
<td>0.037 kW/h</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Heat exchanger + circulation pump</td>
<td>0.012 kW/h</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Nitrogen gas</td>
<td>2.5 L/h</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Total Process Cost/h</strong></td>
<td><strong>-</strong></td>
<td><strong>11.35</strong></td>
<td><strong>11.8</strong></td>
</tr>
</tbody>
</table>

Table 6-4 (b). Value of the decomposition products obtained by pyrolysis pilot plant.

<table>
<thead>
<tr>
<th>Product name</th>
<th>Production amount kg/h</th>
<th>Product value ¥/h</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-oil*</td>
<td>0.21</td>
<td>42</td>
<td>1kg~200 ¥</td>
</tr>
<tr>
<td>Bio-char</td>
<td>0.29</td>
<td>0.13</td>
<td>1kg ~4 ¥ Ref [9]</td>
</tr>
<tr>
<td>Mixture of gaseous products</td>
<td>0.08</td>
<td>0.07</td>
<td>1 tone = 960 ¥</td>
</tr>
<tr>
<td>Unknown compounds (based on TOC results)</td>
<td>0.12</td>
<td>0.12</td>
<td>roughly estimation</td>
</tr>
<tr>
<td><strong>Total products (value)/out of 0.7 kg dry feed</strong></td>
<td><strong>0.7</strong></td>
<td><strong>42.32</strong></td>
<td>¥/h</td>
</tr>
</tbody>
</table>
### Table 6-5 (a). Total operation cost of ultrasonic assisted pilot plant with capacity of 10 kg/h.

<table>
<thead>
<tr>
<th>Plant process cost</th>
<th>Estimated energy/material consumption</th>
<th>Unit price ¥/h</th>
<th>Total price ¥/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine biomass material</td>
<td>10 kg/h (wet)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Screw pump</td>
<td>0.025 kW/h</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Sonication apparatus</td>
<td>0.375 kW/h</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>0.025 kW/h</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Separator</td>
<td>0.015 kW/h</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Drier</td>
<td>0.054 kW/h</td>
<td>1.08</td>
<td>1.08</td>
</tr>
<tr>
<td><strong>Total Process Cost/h</strong></td>
<td></td>
<td>10.38</td>
<td><strong>10.38</strong></td>
</tr>
</tbody>
</table>

### Table 6-5 (b). Value of the decomposition products obtained by ultrasonic assisted pilot plant.

<table>
<thead>
<tr>
<th>Product name</th>
<th>Production amount kg/h</th>
<th>Product value ¥/h</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure cellulose fiber</td>
<td>0.32</td>
<td>319.8</td>
<td>Ref [7]</td>
</tr>
<tr>
<td>Total soluble sugars</td>
<td>0.13</td>
<td>-</td>
<td>intermediate product</td>
</tr>
<tr>
<td>Bio-oil*</td>
<td>0.003</td>
<td>13.45</td>
<td>Ref [8]</td>
</tr>
<tr>
<td>Methane gas</td>
<td>0.043</td>
<td>1.32</td>
<td>28 m³ = 660 ¥</td>
</tr>
<tr>
<td>Carbone Dioxide</td>
<td>0.079</td>
<td>0.11</td>
<td>1 tone=1580 ¥</td>
</tr>
<tr>
<td>Unknown compounds (based on TOC results)</td>
<td>0.255</td>
<td>0.1</td>
<td>roughly estimation</td>
</tr>
<tr>
<td><strong>Total products (Value)/out of 0.7 kg dry feed</strong></td>
<td><strong>0.7</strong></td>
<td><strong>334.78</strong></td>
<td>¥/h</td>
</tr>
</tbody>
</table>

### Table 6-5 (c). Composition/value of bio-oil obtained under ultrasonic assisted pilot plant.

<table>
<thead>
<tr>
<th>*Bio-oil individual composition</th>
<th>Production amount kg/h</th>
<th>Unit price ¥/kg</th>
<th>Total price ¥/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA (1% of total bio-oil)</td>
<td>0.000034</td>
<td>140000</td>
<td>4.76</td>
</tr>
<tr>
<td>Hexanoic acid (30% of total bio-oil)</td>
<td>0.00102</td>
<td>8500</td>
<td>8.67</td>
</tr>
<tr>
<td>3,7,11,15, tetramethyl 2 hexadecane 1-ol (7% of total bio-oil)</td>
<td>0.000238</td>
<td>(1)1000000</td>
<td>-</td>
</tr>
<tr>
<td>Unknown bio-oil (62% of total bio oil)</td>
<td>0.002108</td>
<td>10</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>0.0034</td>
<td></td>
<td><strong>13.45</strong></td>
</tr>
</tbody>
</table>

(1) The presented price is based on pure materials and has not considered in the estimation of bio-oil total price.
6. 3. Conclusions

My research work provides models of macroalgae conversion units with the capacity of 10 kg/h for the production of bio-oil, pure cellulosic fiber, and bio-char as well as gaseous products. The model is implemented on Aspen Plus®. The simulation results are validated with experimental measurements at laboratory scales. The computer simulation proves that it is possible to successfully scale up the data obtained from bench scale to larger ones.

Subsequently, an economic assessment using mass and energy balances revealed the cost and benefit of each process individually. Clearly it is shown that all proposed innovative techniques have extremely high benefit in even large scales. Simulation results highlight the strong impact of the bio-oil production step in the overall performance of the conversion plants. Finally, it worth to mention that for more precise simulation of such pilot plants there are several other thermodynamic parameters which must be considered and evaluated.

Reference


Chapter 7

General conclusions
In this thesis I have investigated application of green technologies for marine biomass conversion in order to reduce environmental impacts of traditional methods as well as making the conversion process commercially viable. Among several environmentally acceptable techniques, the most efficient and innovative ones were selected for the study. Subcritical water, subcritical ethanol, pyrolysis, and ultrasonic assisted are the techniques which have been utilized and evaluated in this research work. To the best of my knowledge, some of these techniques have been utilized for the first time on marine biomass conversion studies. A kind of green macroalgae called *C. fragile* was selected as a typical model for the marine biomass.

The first objective of this study was to evaluate and then improve the efficiency of each technique in order to obtain valuable compounds such as soluble sugars, oily compounds, organic acids, and pure cellulosic fibers. Wherever it was possible, kinetics of the conversion and decomposition reactions have been studied. The second objective was to provide the sufficient information in order to have better comparison between the efficiency of different techniques on conversion yield and production of valuable materials. The third objective was to scale up the processes from bench to pilot plant scales using experimental data obtained from each technique. Cost estimation and economical assessment for the simulated pilot plants have also been investigated. The major results of the thesis are summarized as follows:

Chapter one provides general background of this thesis. In the first part of this chapter, a general introduction about marine biomass, macroalgae, and particularly *C. fragile* as a typical model of this group were given, and its composition was presented. In the next part, the properties of various conventional and innovative techniques related to this thesis were described in detail. The various fields of marine biomass, chemical and
material cycling, system design and economical assessment, and the other related researches on each proposed method as well as previous literature were reviewed.

In Chapter two, the hydrolysis and decomposition of *C. fragile* under pure subcritical water conditions has been proposed in order to obtain value-added materials. Effect of reaction conditions such as temperature and time on the hydrolysis reactions was studied. As an interesting and main finding, it was observed that hydrolysis and decomposition reactions were effectively carried out without utilization of any organic solvent, acid, base, and/or enzyme. Production of various water-soluble compounds such as organic acids, soluble sugars, several other valuable compounds such as furfural and 5-hydroxymethylfurfural were identified and quantified. As conclusion, I obtained very promising results on utilization of subcritical water technique on either solubilization of almost completely solid type marine biomass, or even improving the energy density of the solid marine biomass without solubilization, depended on the reaction conditions.

In Chapter three, instead of water, ethanol under its critical conditions was used in order to evaluate its effect on the decomposition and formation of value-added compounds. As an interesting and main finding, it could successfully converted the oily part of the macroalgae directly into biodiesel fuel compounds, without using any chemical compounds such as potassium hydroxide solution which are using usually in the biodiesel production procedure. Due to this one step liquefaction and transestrification reactions in the ethanolic medium, higher values and amounts of very expensive bio-oil compounds such as hexanoic acid ethyl ester, n-hexadecanoic acid, ethyl myristate, ethyl oleate, ethyl palmitate, EPA, heptadecanoic acid ethyl ester, and etc. could be produced. It was also possible to extract cellulose fibers with an extremely higher purity which have high demand in pharmaceutical, bio-polymer and bio-plastic industries. Compared to subcritical water technique, the
advantage of this method is the ease of purification and recycling back of ethanol to the reaction system without producing any waste materials and solution.

In Chapter four, pyrolysis technique was used for conversion of the C. fragile. In order to understand the kinetic reaction of decomposition of C. fragile and its activation energy, pyrolytic behavior of C. fragile was studied using thermogravimetric analysis (TGA) method. In fact, this method is usually necessary to evaluate prior to scale up the pyrolysis reactions. To estimate kinetic parameters, the solid state non-isothermal (isoconversional) method was used. This method is applied for the description of more complex processes (such as decomposition of C. fragile) where lots of chemical reactions are running simultaneously, while their mechanisms are not exactly known. This study comprised the various kinetic models proposed in literature for C. fragile decomposition. The thermodynamic data estimated from the proposed models were in good agreement with the experimental values.

In Chapter five, the effect of ultrasonic irradiations as a more innovative technique on treatment of C. fragile was developed. The efficiency of the method was evaluated by analyzing of decomposition products such as saccharides, oils, as well as produced pure cellulosic fibers. I realized that sonication media has great influence on the reaction efficiency, the product types could be varying by choosing different media. Another interesting finding was production of very valuable fatty acids ethyl esters such as EPA and phytol in ethanolic medium. Remained solid had a very pure structure similar to those of cellulose. On the other hand, increasing energy density of residual solid after treatment makes them also as a good alternative resource for energy production via direct combustion process.

In Chapter six, based on the experimental data obtained from previous chapters,
four large pilot plants for macroalgae conversions were simulated using Aspen Plus® software with capacity of 10 kg/h. Subsequently, economic assessments using mass and energy balances (without considering the purification process cost, construction cost, utility, labor cost, and etc.) revealed the cost and benefit of each scaled-up system individually. The obtained products from each treatment technique represented absolutely high value of profit for each process. Specifically, the simulation results highlighted the strong impact of the bio-oil production step in the overall performance of the treatment plants. As final conclusion, it was shown that all proposed innovative treatment methods have extremely high benefit in large scale units. Owing to the industrial importance of marine biomass, there will always be a scope to improve the productivity of the process, therefore, for more precise simulation of such large scale plants, still, there are several other thermodynamic parameters which must be considered and evaluated in future research works.
<table>
<thead>
<tr>
<th>No.</th>
<th>Title of the article</th>
<th>Author(s)</th>
<th>Journal’s Name, Vol., Pages, and Year</th>
<th>Corresponding chapter</th>
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<tr>
<td>2</td>
<td>Conversion of Macroalgae into the Value added Compounds</td>
<td>S. Daneshvar, F. Salak, K. Otsuka</td>
<td><em>Proceeding of Japan Institute of Energy, pp. 84-85, (Osaka, Japan, 2011).</em></td>
<td>Chapter 2</td>
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<td>5</td>
<td>Macrolgae Pyrolysis and Its Devolatilisation Kinetics</td>
<td>S. Daneshvar, F. Salak, K. Otsuka</td>
<td><em>Proceeding of The 3rd International Conference on Chemistry &amp; Chemical Engineering (ICCCE 2012), Vol. 38, pp. 77-81, (Jeju Island, South Korea, 2012).</em></td>
<td>Chapter 4</td>
</tr>
<tr>
<td>6</td>
<td>Proximate Analysis of Green Algae</td>
<td>S. Daneshvar, F. Salak, K. Otsuka</td>
<td><em>Proceeding of 7th International Chemical Engineering Congress &amp; Exhibition (7th IChEC &amp; E), Total 7 pages, (Kish Island, Iran, 2011).</em></td>
<td>Chapter 4</td>
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