Doctoral Thesis

HYDROTHERMAL PRETREATMENT OF MACROALGAE: DETAILED REACTION KINETICS AND MECHANISMS

(大型藻類の水熱前処理: 詳細な反応速度論及び機構)

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ABSTRACT

Hydrothermal process has been identified as a promising technology for pretreatment of wet biomass, particularly marine macroalgae. The key advantage of this technology is that it does not require any chemical solvent but only water as its reaction medium. Hence, drying of macroalgae prior to the hydrothermal process is certainly unnecessary. In fact, the potential of macrolagae as renewable resources has been extensively discussed in numerous studies. Most of the researchers highlighted the importance of pretreatment process of macroalgae in order to enhance the following processes. The remarkable point of macrolagae is that this biomass contains substantial amount of valuable carbohydrates. Thus, recovery and utilization of these carbohydrates are essentially desired. Owing to that, this research was initiated with the main idea is to study the behavior of carbohydrates in macroalgae, specifically kelp prior to its effective utilization.

The research methodology involved in this study consists of two main sections, which are (i) hydrolysis of alginic acid for recovery of its uronic acids, and (ii) hydrothermal treatment process for kinetics study. In the first section, mild concentration of hydrochloric acid (HCl) was employed for the hydrolysis process, which was conducted for about 2.5 h in total, at round 90 °C. The desired uronic acids, mannuronic acid (MA) and guluronic acid (GA) were recovered separately via pH adjustment at 2.85. The undesired impurities were removed through additional

washing and purified uronic acids were dried in desiccator prior to preservation in refrigerator. The later section was conducted by using continuous-flow reactor apparatus. The reaction temperatures ranged between 170 and 250, while residence times varied from 3 to 100 s. For all experiments, pressure was fixed at 25 MPa. Besides the carbohydrates in kelp (GA, MA), several other carbohydrates were also subjected to the hydrothermal treatment, which are glucuronic acid (uronic acid), mannose (aldohexose), and sorbitol (sugar alcohol). The products obtained after the hydrothermal treatment was quantitatively analyzed by using high performance liquid chromatography (HPLC).

The preparation of uronic acid from alginic acid was successfully conducted and the recovery yield was almost 50 %. The key point here is that the desired uronic acids were successfully obtained in a solid form, unlike the other previous studies. Certainly, this methodology employs simple processes to produce MA and GA in easy and quick way. On the other hand, the kinetics study indicated the susceptibility of GA and MA under hydrothermal condition, whose recovery yields were less than 10 % at 170 °C and 15 s of residence time. Similarly, high decomposition rate was observed for another type of uronic acid, namely glucuronic acid. Apparently, lower temperature range is desired for hydrothermal treatment of uronic acids. Perhaps, high reactivity of uronic acids was due to the high electronegativity of their functional group, which is carboxylic group.

The effect of functional group was further evaluated for a sugar family that consists of MA (carboxylic), mannose (aldehyde) and mannitol (hydroxyl). Basically, these sugar compounds have almost similar chemical structure except for the functional group. Among them, MA decomposed faster that the others while mannitol was less susceptible to degradation. Likewise, similar study was conducted

on another sugar family that consists of glucuronic acid (carboxylic), glucose (aldehyde) and sorbitol (hydroxyl). The result showed a good agreement with the previous work, in which glucuronic acid exhibited the highest degradation rate. Unlike mannitol and mannose, different behavior was observed for sorbitol, which decomposed faster than glucose. Owing to that, additional study was conducted in order to investigate the effect of chemical structure on kinetics characteristics of these isomers. Apparently, the hydroxyl configuration in sorbitol caused to higher interaction between water and its carbon atoms, which consequently resulted to higher decomposition rate.

Finally, the kinetics parameters of various carbohydrates in macrolagae were estimated by using Arrhenius equation. Similar activation energies, E_a were obtained for GA, MA, and glucuronic acids, which are 20.6, 28.3, and 22.8 kJ/mol, respectively. However, the pre-exponential factors, A were increased in the order of GA (40.6 s^{-1}) < glucuronic acid (43.4 s^{-1}) < MA (281 s^{-1}). As for sorbitol, the E_a (28.3 kJ/mol) and A (42.9 s^{-1}) are higher than that of mannitol, 26.5 kJ/mol and 3.23 s^{-1}, respectively. Evidently, aldohexoses (mannose and glucose) showed the highest E_a and A than the uronic acids and sugar alcohols.

THESIS STRUCTURE

This thesis comprises of 10 chapters and brief descriptions of each chapter are explained in the following paragraphs.

Chapter 1: Introduction. Briefly, this chapter describes the problems and issues that need to be solved in this study. Besides, explanation on the significance of this study is also provided, specifically its contributions towards the research field.

Chapter 2: Literature review. This chapter provides the significant findings of previous studies. This information is essentially important in order to identify the research gap between the previous and present studies. Consequently, it will determine the originality or novelty of this study. In short, this study focuses on hydrothermal treatment of macroalgae. Thus, the literature review is divided into two main sections, which are (i) discussion about potential of biomass in general, and macroalgae in specific, as renewable resources, (ii) discussion on the available technologies for conversion of biomass, particularly macroalgae.

Chapter 3: Research aim and objectives. The research motivations behind this study are described in this chapter. Based on the motivations, research strategies are planned accordingly in order to achieve the ultimate research aim and objectives.

Chapter 4: Methodology. The experimental setup, procedures and conditions are explained in this chapter. Besides, procedures of various quantitative analyses are also described herein. The details of all chemicals used in this study (experiment and analysis) are provided too. With this information, reproducibility experiment of this study will be possible for future work.

Chapter 5: Recovery of uronic acids through simplified acid hydrolysis. The development of simplified methodology of uronic acids is clearly explained in this chapter. Briefly, the methodology involves 2 main processes (i) hydrolysis of alginic acid by using mild concentration of hydrochloric acid (HCl), and (ii) separation of uronic acids by using pH dependent method. The reliability of the proposed methodology is further evaluated based on quantitative product characteristics. The results of product characteristics are also compared with the previous studies.

Chapter 6: Degradation characteristics of carbohydrates in kelp under hydrothermal condition. The kinetics behavior of the guluronic acid (GA) and mannuronic acid (MA) obtained in Chapter 5 is investigated in this chapter. This study is required prior to the effective utilization of kelp. The experimental work was conducted by using continuous-flow reactor system under subcritical water condition (170-250 °C, 25 MPa, 5-100 s). The decomposition behavior of the uronic acids was

evaluated based on residual ratio of its concentration. Additionally, rate constant was calculated based on first-order reaction and the reactivity was further evaluated based on Arrhenius plot. Finally, reaction parameters of the uronic acids were calculated accordingly.

Chapter 7: Decomposition behavior of mannose, mannitol, and mannuronic acid under hydrothermal condition. The investigation on behavior of kelp compound (MA) is extended to its parents sugar (mannose) and sugar alcohol (mannitol). Similar experimental apparatus and conditions (as in Chapter 6) were employed for these compounds for comparable study. Owing to the different functional groups in each of them, the effect of aldehyde, carboxylic and hydroxyl groups on kinetics characteristics was elucidated. The reactivity was evaluated based on rate constant value and reaction parameters were calculated by using the Arrhenius equation. The finding of this chapter will provides important knowledge on the effect of functional group of sugar compound on kinetics behavior under hydrothermal condition.

Chapter 8: Kinetics behavior of sugar alcohols under hydrothermal condition. The research work of previous chapter is further expanded, in which the effect of chemical configuration of isomers (mannitol and sorbitol) is examined. Likewise, continuous-flow reactor was employed and hydrothermal treatment was conducted under subcritical region. The effect of hydroxyl configuration of these isomers was investigated based on residual yield after the treatment process. Based on the results, the possible reaction mechanism in mannitol and sorbitol was proposed accordingly. Moreover, the results obtained in this study were also compared with

previous studies, whose conditions are significantly different than this study. This information is needed in order to explain the effect of reaction conditions on reactivity of sugar alcohols.

Chapter 9: Hydrothermal treatment of carbohydrates in marine macroalgae. Owing to the significant impact of functional group on kinetics behavior of sugar compounds as described in Chapter 7, similar study was conducted on another family of sugar. In this chapter, glucose (aldehyde group), sorbitol (hydroxyl group), and glucuronic acid (carboxylic group) were used as the reactants. These sugar compounds were chosen to represent the carbohydrates in macroalgae. The reactivity of each sugar was evaluated based on the ratio of unreacted concentration and initial concentration of reactants. The effect of functional group was clearly explained and possible reaction mechanism was also determined. The knowledge obtained in this study could be used as guideline in treating macroalgae under hydrothermal condition.

Chapter 10: Conclusions and recommendation. This chapter summarizes the significant findings obtained throughout this research work. Besides, recommendation for research improvement is also provided for future researchers.

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LIST OF ABBREVIATION

AD	Anaerobic digestion
Alg	Alginic acid
Btu	British thermal unit
C	Carbon atom
Ca	Calcium
$CaCO_3$	Calcium carbonate
cm	Centimeter
CH_4	Methane
CO	Carbon monoxide
CO_2	Carbon dioxide
COOH	Carboxylix
EXP	Exponent
ft	Foot
FC	Fixed carbon
g	gram
GA	Guluronic acid
h	Hour
H	Hydrogen atom
HCl	Hydrochloric acid

$HClO_4$	Perchloric acid
HMF	Hydroxymethylfurfural
HPLC	High performance liquid chromatography
H_2	Hydrogen gas
H_2O	Water
H_2SO_4	Sulfuric acid
H_3O^+	Hydronium ion
ID	Inner diameter
kg	Kilogram
kJ	Kilojoule
K	Potassium
KH_2PO_4	Monopotassium phosphate
K_2HPO_4	Dipotassium phosphate
ln	Natural logarithm
L	Liter
LSE	Least square of error
m	Meter
mg	Milligram
min	Minutes
mm	Millimeter
mmol	Milimole
ms	Millisecond
mL	Milliliter
mM	Milimolar
M	Molar

Man	Mannitol
Mg	Magnesium
MA	Mannuronic acid
MHz	Megahertz
MJ	Megajoule
MPa	Megapascal
MSW	Municipal solid waste
N	Normality
Na	Sodium
NaOH	Sodium hydroxide
Na_2CO_3	Sodium carbonate
NMR	Nuclear magnetic resonance
N_2	Nitrogen gas
OH^-	Hydroxide ion
O_2	Oxygen gas
ppm	Part per million
P	Phosphorus
RID	Refractive index detector
s	Second
SCWG	Supercritical water gasification
SSF	Simultaneous saccharification fermentation
TGA	Thermogravimetric analysis
TOC	Total organic carbon
TRS	Total reducing sugar
VFA	Volatile fatty acid

VM	Volatile matter
$ZnCl_2$	Zinc chloride

LIST OF SYMBOLS

A	Pre-exponential factor
C, C_o	Concentration
c_p	Specific heat capacity
E_a	Activation energy
h	Enthalpy
k	Reaction rate constant
K	Kelvin
m	Mass
n	Mole
Q	Heat
R	Gas constant
t	Time
T	Temperature
W_s	Work shaft
α	Alpha
β	Beta
Δh	Change in enthalpy
Δh_c	Standard heat of combustion
ΔH_{rxn}	Standard heat of reaction

ΔT	Temperature difference
%	Percentage
°C	Degree Celsius

LIST OF PUBLICATIONS

PUBLICATION NO.	TITLE
1	Mohamad, R., Aki, T., Nakashimada, Y., Okamura, Y., Tajima, T., Matsumura, Y., 2016. Decomposition kinetics of uronic acids obtained from kelp under hydrothermal condition. J. Energy Inst. Article in press (available on line, Mar 2016)
2	Mohamad, R., Aki, T., Nakashimada, Y., Okamura, Y., Tajima, T., Matsumura, Y., 2016. Decomposition kinetics of mannose, its sugar alcohol, and its uronic acid under hydrothermal condition. J. Chem. Eng. Japan. 49 (7). Article in press.
3	Mohamad, R., Aki, T., Nakashimada, Y., Okamura, Y., Tajima, T., Matsumura, Y., 2016. Kinetics of sorbitol decomposition under hydrothermal condition. J. Japan. Pet. Inst. 59 (4), 149-154. Accepted for publication.

CHAPTER 1

INTRODUCTION

1.1 Problem statement

The utilization of sustainable and renewable energy is needed for replacement of non-renewable energy like fossil fuels. Typically, many options are available but biomass energy (biofuels) is among the promising alternative energy. Notably, the first generation biofuels are the energy that derived from sugar and starch materials such as sugarcane, corn, soybean and many more. However, the utilization of these kinds of resources is usually created issues related to the human food supply. Owing to this reason, research efforts have been switched to alternative resources that do not have competition issue with human consumption.

In order to overcome these issues, second-generation biofuels has been introduced where lignocellulosic biomass and waste materials have been employed for the energy production instead of using the food crops. For this kind of resources, competition with food supply is not an issue anymore. However, lignocellulosic materials composed of cellulose, hemicellulose, and lignin, which require pretreatment

process prior to its effective utilization. During the pretreatment process, the complex structure of lignocellulosic materials is disrupted and consequently released the desired carbohydrates. Besides, lignocellulosic materials are usually required large cultivation land, which could be another drawback of using this kind of feedstock.

Another alternative resource that has been extensively investigated is marine biomass (macroalgae), which is also known as third-generation biofuels. Since macroalgae could be cultivated in water, they do not require large land area like the lignocellulosic biomass. Moreover, its utilization also does not cause undesired competition with human food supply. Therefore, macroalgae could be one of the most promising resources to substitute the non-renewable resources.

1.1.1 Macroalgae as renewable and sustainable resources

Since the third-generation biofuel has been introduced, extensive discussion on macroalgae was reported in numerous studies. Apparently, significant research efforts have been taken, in which the investigation cover each type of macroalgae, namely red, green, and brown macroalgae. In fact, each of them has different compositions and thus requires distinctive approach of pretreatment. This is mainly because of the carbohydrates in macroalgae are always have dissimilarity characteristics, which consequently affecting their reactivity.

Ulvan is a major carbohydrate in green macroalgae. Chemically, it is sulfated hetero-polysaccharide that contains high amount of rhamnose residue and uronic acids. It fact, this carbohydrate can be used in various application owing to its physico-chemical and biological characteristics. The extraction of ulvan from green macroalgae is usually conducted using a hot water.

In previous studies, it was reported that higher ulvan was extracted by using hot water (Lahaye and Robic, 2007) than by using alkali (Ray and Lahaye, 1995). However, the utilization of sugars released from ulvan is somewhat limited. Perhaps, it was due to the low conversion rate of sugars from *Ulva* species to bioethanol, which has not been over than 20 % (Jiang et al., 2016). In another study, it was reported that lower extraction yield of ulvan was obtained when chelating agent solution was employed (Robic et al., 2009). The researchers also highlighted that lower extraction yield was due to the effect of its strong chemical structures.

On the other hand, red macroalgae contains mainly carrageenan and agar. Indeed, various methods have been developed for the saccharification of these two compounds, including acid hydrolysis and enzymatic hydrolysis. However, these methods have several limitations, which have been highlighted in previous studies. For instance, a group of researchers reported that lower sugar recovery and higher inhibitor concentration were obtained during the hydrolysis process, particularly when batch reactor system was employed (Park et al., 2012). As an alternative, bioconversion technique could be used for the hydrolysis of carbohydrates in red macroalgae. Nevertheless, specific strain is needed in order to obtain higher conversion rate of sugars.

In contrary to green and red macroalgae, less numbers of researches have been conducted on brown macroalgae. Typically, brown macroalgae composes of alginic acid and mannitol as its main carbohydrates. These two valuable compounds have wide range of applications but their utilization is rather limited. This is mainly because of the complex structure of alginic acid itself that makes it difficult to be digested by microorganism.

For that reason, further fractionation of alginic acid is needed where the corresponding monomers will be generated, specifically guluronic acid (GA) and mannuronic acid (MA). The recovery of these uronic acids is expected to enhance the utilization of kelp. However, due to limited numbers of study, the information on these uronic acids is not well documented. Owing to that, it is necessary to investigate the behavior of kelp compound in order to provide the desired information.

1.1.2 Energy extraction from macroalgae

Nowadays, numbers of technology have been developed for the conversion of macroalgae to bioenergy, including thermochemical and biochemical conversion. For example, pyrolysis is employed when the desired products are bio-oils. Typically, this process is carried out at enhanced temperature with the absence of oxygen, which consequently change the chemical structure and physical phase of the initial feedstock. This technology is also suitable for biomass that contains high ash like macroalgae (Ross et al., 2008). Unfortunately, dry feedstock is needed for pyrolysis process, which means that pre-drying is essentially important. Due to that, extra cost is incurred when additional energy is required. Hence, this process is less favorable for macroalgae whose water content was significantly high.

Liquefaction process is another alternative method for energy extraction from biomass. Basically, this process does not require any organic solvent and pre-drying of feedstock unlike the pyrolysis process. However, based on previous study, unfavorable energy recovery was obtained when water content in feedstock is too high, probably exceeded 90 % (Vardon et al., 2012). Therefore, it could be a possible constraint for conversion of macroalgae by using this technology.

Alternatively, hydrothermal treatment process is another potential method for macroalgae. Certainly, this process is suitable for wet biomass particularly macroalgae. This is because of the process itself, which employs water as a solvent and also as reaction medium. Usually, this process is conducted under subcritical or supercritical water conditions, where water remains in liquid phase. The products obtained varying depending on the operating conditions particularly temperature and time. Indeed, numerous studies have been conducted to investigate the potential of this technology for conversion of macroalgae into biofuels and biorefinery products.

1.2 Significance of the study

The findings obtained in this study would be of interest to the industries that utilize macroalgae as their resources, as well to the researchers in this particular field of study. Macroalgae is promising renewable resources that has been extensively investigated as an alternative resource to non-renewable ones. Certainly, many industries have been established in recent years in order to explore the various potentials of macroalgae. This is primarily due to the high carbohydrate contents in macroalgae.

The high demand on macroalgae also justifies the need for effective utilization of the macroalgae. Thus, worldwide researchers have initiated significant efforts in order to explore for any promising methodology. The development of appropriate technology for macrolagae is essentially important to ensure that this resource is utilized efficiently. However, each macrolagae has its own characteristics due to the different compositions. Owing to that, individual study on specific macroalgae is essentially important.

For this research, the experimental works focused specifically on effective utilization of kelp. Prior to its utilization, specific study on its individual compound particularly alginic acid is required. Alginic acid has a complex structure and thus its utilization is somewhat limited especially when microorganism is involved. Due to that, further fractionation of alginic acid into its corresponding uronic acids, namely GA and MA is desirable. However, implementation of specific study of GA and MA is somewhat restricted due to limited or unavailable supply in commercial market.

The limited supply of GA and MA in commercial market is mainly due to the difficulty in recovering these uronic acids as solid products. Furthermore, the preparation process of these compounds is usually involved various processes and time consuming. Owing to that, development of simple and easy method of preparation the

uronic acids is desired. In this research work, the simplified methodology has been proposed, in which the desired uronic acids were successfully obtained as solid products.

Briefly, the proposed methodology was developed based on improvement on previous studies. The preparation of the uronic acids employed in this study is much simpler than the others. Surprisingly, characteristics study on the obtained uronic acids also shows comparable results than the other methodologies. Therefore, this finding demonstrates the reliability of the proposed methodology. With this method of preparation, it is expected that the production of uronic acid could be scaled up at larger scale. Consequently, uronic acids will be also commercially available for further utilization in various industries.

In addition, the availability of uronic acids in the market could also contributed to the industries, which utilizes kelp as its resources. This is because, prior to the effective utilization of kelp, information on its individual compounds is necessary. Indeed, limited numbers of study has been conducted on the individual kelp compound, especially the alginic acid. Since alginic acid would be further fractionated into its uronic acids, kinetics study on GA and MA has more priority than the alginic acid itself. Nevertheless, due to lack of information particularly on the kinetics characteristics, its application has not been widely explored.

In this research, the kinetics characteristics of GA and MA were successfully elucidated under hydrothermal condition, in which kelp will be treated too. The finding of this work would contribute to effective utilization of kelp. By knowing the kinetics behavior of the uronic acids, appropriate pretreatment condition of kelp could be determined. This is therefore could avoid the undesired decomposition of uronic acids. Consequently good recovery yield could be obtained. Without this knowledge,

optimum condition of kelp pretreatment is also difficult to be determined. Perhaps, decomposition of desired products could have happen and make the process less efficient.

Besides kelp, this research also provides information on other carbohydrates in macroalgae in order to obtain the general overview. The main idea is to deliver important knowledge on macrolagae, so that its utilization could be extended for red and green macroalgae too. Hence, industries and researchers whose works are involved with macroalgae could benefits from the finding of this research.

Besides, specific study on various carbohydrates in macroalgae is needed because they are always existed in different forms, such as aldohexose, sugar alcohol and uronic acid. Fundamentally, each of them is differed based on their functional group. For example, aldohexose is a sugar compound with aldehyde group like glucose and mannose. On the other hand, sugar alcohol (mannitol and sorbitol) has hydroxyl as its functional group while uronic acid (GA, MA, and glucuronic acid) has a carboxylic group. These carbohydrates are the sugar compounds that usually observe in all kind of macroalgae (red, green and brown macroalgae).

The different functional groups of those carbohydrates consequently resulted to different kinetics behavior under the same condition. Owing to this reason, this study was conducted where the effect of functional group on kinetics behavior of carbohydrates was investigated. Furthermore, the effect of chemical configuration of carbohydrates (sugar isomers) on kinetics characteristics was also evaluated. It is expected that the findings obtained herewith would have great contribution particularly to the carbohydrate field of study.

CHAPTER 2

LITERATURE REVIEW

2.1 Biomass

Biomass is referred to organic matter that derived from living organism, and is abundantly and readily available on earth. Chemically, biomass is mainly composed of carbon, hydrogen and oxygen. The carbon in biomass is absorbed from the atmosphere as carbon dioxide (CO_2), through photosynthesis of plant materials. At the same time, CO_2 is released to the atmosphere during the combustion of biomass. Thus, it will create a carbon neutral situation, where the amount of CO_2 releases from the biomass consequently returns back to the atmosphere. Moreover, biomass is generally has low ash content, high volatile matter, cheap resource and has many other advantages (Vassilev et al., 2010). Owing to that, significant numbers of research have been conducted by worldwide researchers, on the potential of biomass for various applications.

Besides, the utilization of biomass as renewable energy resources has shown an increasing trend particularly due to the depletion of fossil fuels resources and its environmental impacts. Basically, fossil fuel is also derived from biological materials. Nevertheless, the formation of fossil fuel requires million of years to be produced and thus is considered as non-renewable energy resources. Due to that, when the demand of fossil fuels is getting higher, the world will consequently faces the shortage of fossil fuel supply. Therefore, utilization of alternative resources is extremely desired to fulfill the worldwide energy demand.

Another concern regarding the utilization of fossil fuel is about the environmental issues. This is because, during combustion of fossil fuel, substantial amounts CO_2 gases are released and consequently accumulated in the atmosphere. The high concentration of CO_2 that released to the environment subsequently causes a global warming problem. In order to prevent this problem, biomass has been identified as one of the most promising resources as alternative to fossil fuel.

Generally, biomass can be categorized into two major groups, namely productive biomass (terrestrial and aquatic), and unused biomass (residues and wastes from various industries) (Long et al., 2013). Each of them are however has different compositions and characteristics since they are originated from various resources. Therefore, they are also having different suitability to be used as bioenergy. For example, marine macroalgae (aquatic biomass) are generally having higher moisture content than the terrestrial plants. Thus, this kind of biomass is more favorable for the conversion process that suitable for wet biomass, such as hydrothermal process. Owing to that, different methods are needed for the conversion of biomass, either directly or indirectly used to produce biofuel, in particular.

2.2 Lignocellulosic biomass

Among the many different kinds of biomass, lignocellulosic biomass is one of the favorable resources for biofuel and bio-refineries. Specifically, lignocellulosic biomass refers to plant materials that compose of the combination of cellulose, hemicellulose, and lignin, such as sugarcane bagasse, rice husk, corncob, and others. Cellulose in lignocellulosic biomass exists as a linear polysaccharide while hemicellulose is a polysaccharide with a random structure. Theoretically, the sugar compounds in cellulose and hemicelluose can be further converted to bio-based products through appropriate process. In fact, the potential of lignocellulosic materials for biofuel and biochemical production was successfully evaluated by previous researchers (Cai et al., 2014)(Zheng et al., 2014).

Nevertheless, the existence of lignin consequently resulted to high resistance of lignocellulosic biomass towards the degradation. Basically, lignin consists of aromatic polymer that binding the cellulose and hemicellulose. Therefore, proper pretreatment is essentially important prior to its effective utilization. The effect of pretreatment on the complex structure of lignocellulosic biomass is clearly illustrated in **Fig. 2.1**. During the pretreatment process, complex structure of lignocellulosic biomass is disrupted and subsequently enhances the hydrolysis of both cellulose and hemicellulose. Then, the sugars release from the conversion of cellulose and hemicellulose are further utilized for various applications.

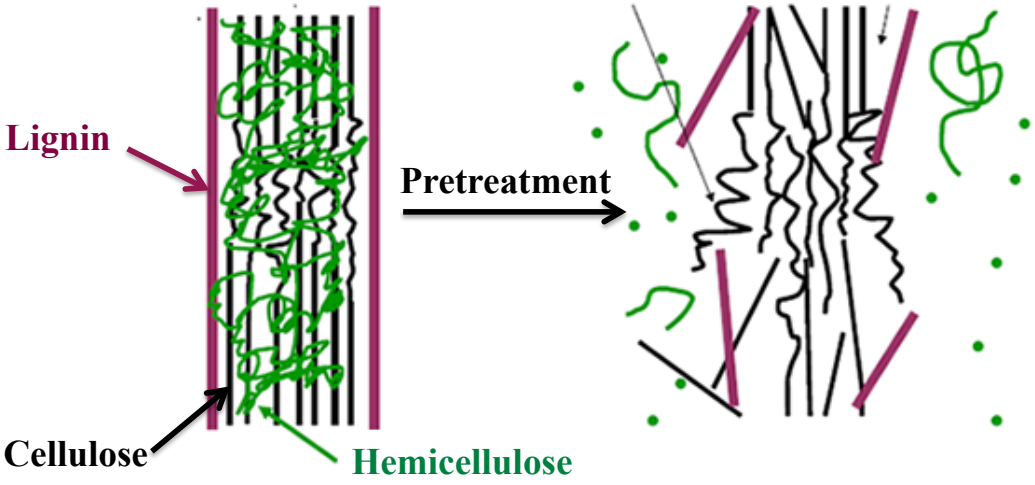


Fig. 2.1 Structure overview of lignocellulosic biomass

In fact, numbers of technology have been developed for the pretreatment process. Nevertheless, due to the complex structure of lignocellulosic biomass, various shortcomings are associated with its utilization. Hence, marine biomass that has simpler structure could be better alternative resources.

2.3 Marine biomass

Marine biomass (macroalgae) is another option of biomass that has great potential for sustainable production of bio-based materials, including biofuels. This is mainly due to its composition that contains high amount of carbohydrates. In fact, numbers of research have been conducted to demonstrate the potential of the carbohydrates in macroalgae to produce valuable products. However, depending on the main composition, different methods were employed for their utilization. In comparison to lignocellulosic biomass, the conversion of macroalgae to bioenergy is

much easier due to the absence of lignin. However, proper pretreatment of macroalgae is still needed for its effective utilization.

Noticeably, the utilization of green macroalgae has been widely explored in various fields. In earlier study, a group of researchers was performed chemical composition analysis on green algae, a species of *Chaetomorpha linum* (Sutour et al., 2015). The researchers reported that the extract of green algae contain high concentration of fatty acids, particularly C16 and C18. Recently, another study was also conducted on utilization of the same species (*Chaetomorpha linum*) specifically for the production of bioethanol and biogas (Yahmed et al., 2016). The proposed concept was successfully demonstrated that both bioethanol and biogas could be obtained from this species through innovative integrated process.

On the other hand, red macroalgae compose of mainly agar and carrageenan, whose structural view is shown in **Fig. 2.2**.

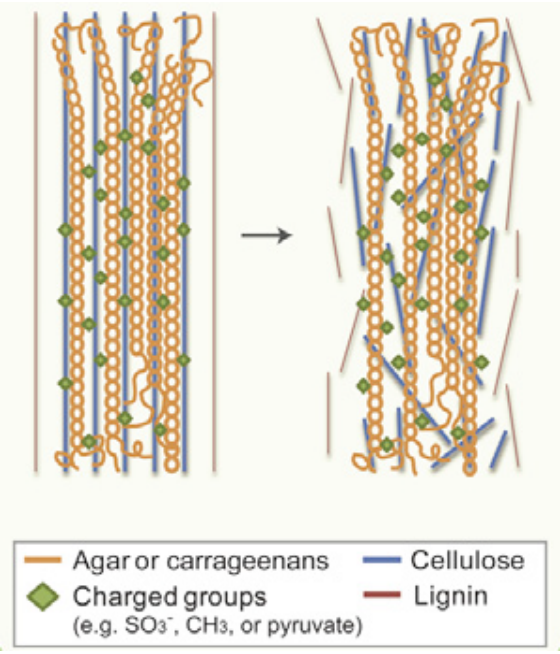


Fig. 2.2 Structure view of red macroalgae

In earlier study, specific research on *Gelidium amansii* was conducted for the production of bioethanol (Kim et al., 2015). The result revealed that substantial amount of ethanol was obtained (about 85 % of product yield).

Besides, a group of researchers was successfully evaluated the potential of brown macroalgae (*Laminaria hyperborea*) for hydrogen and methane gasses production (Cherad et al., 2014). This study reported that the yields of both hydrogen and methane were highly depended on the experimental conditions. Based on recent study, this species contains high amount of alginate (Schiener et al., 2015). Owing to that, various studies were initiated to evaluate the potentials of alginate in brown algae. Specifically, a study was conducted to elucidate the possibility of alginate in brown algae for bio-chemical production (Jeon et al., 2015). The researchers reported that various organic compounds were produced under subcritical water condition.

2.3.1 *Laminaria japonica* (kelp)

In Japan, brown macroalgae of *Laminaria japonica* species also known as kelp, are abundantly available especially in the Honshu and Hokkaido Islands (Radiarta et al., 2011). Notably, this species contains significant amount of carbohydrate (up to 67 wt%), whose compositions are mainly alginic acid and mannitol, as reported in previous study (Xu et al., 2014). Owing to the high carbohydrate contents, significant efforts have been initiated to utilize kelp, especially for biofuels and bio-refinery industries.

In fact, the utilization of kelp as renewable resource was extensively discussed in previous studies. For example, a group of researchers successfully evaluated the potential of *Laminaria japonica* species as precursor to produce the desired bioenergy

and bio-products (Malihan et al., 2012). The recovery of carbohydrates in the macroalgae was conducted using ionic liquid prior to the acid-catalyzed hydrolysis. The results showed that the yield of total reducing sugar (TRS) increased at enhanced temperature.

Similarly, a study was conducted on this species of brown algae for production of reducing sugars (J. N. Park et al., 2012). The researchers investigated the effect of hydrothermal condition and catalyst on the yield of TRS through subcritical water hydrolysis. The highest yield of TRS was obtained at rather low temperature (200 °C) while at higher temperature further decomposition of TRS was possibly occurred. In another study, *Laminaria japonica* was also employed for the production of bioethanol (Lee et al., 2013). In that study, the macroalgae were initially pretreated under hydrothermal condition prior to the enzymatic hydrolysis. The finding revealed that the macroalgae were promising feedstock for bioethanol production.

Besides, biodiesel is another type of biofuel that can be produced from *Laminaria japonica*. The potential of this species as alternative feedstock for biodiesel production was evaluated in previous study (Xu et al., 2014). Initially, mannitol in the macroalgae was recovered and further utilized by microorganism under aerobic condition. As a result, significant amount of lipid was obtained that potentially used as feedstock for biodiesel production. Nonetheless, particular pretreatment is always needed prior to its effective utilization owing to the complex structure of kelp.

2.4 Carbohydrates in macroalgae

Generally, marine macroalgae can be classified into red, green and brown macroalgae (Wei et al., 2013)(Jung et al., 2013). Remarkably, each of them has specific composition of carbohydrates that vary among various species of the same category. For example, some species of brown algae might contain laminarin but the others of the same type might not have. Apparently, different classifications of macroalgae also have different main carbohydrates. For example, red macroalgae contains high amount of agar, carrageenan, and cellulose, whose sugar compositions are mainly glucose and galactose. On the other hand, brown macroalgae composes of mainly alginate and mannitol, as well as laminarin, fucuidan, and cellulose, while green macroalgae contains mainly starch and cellulose.

The lists of all potential carbohydrates in each type of macroalgae are summarized in **Table 2.1**. These carbohydrates can be used in many applications particularly for biofuel and bio-chemicals, as alternative to petroleum-based products. The market distribution for the carbohydrates (carrageenan, agar, and alginate) was reported in previous study, where the major segments include food, pharmaceuticals and biological industries (Bixler and Porse, 2011). Moreover, details discussion of various applications of the carbohydrates in macroalgae were extensively discussed in previous literature (Holdt and Kraan, 2011).

However, in the present study, we focused on the utilization of brown macroalgae, specifically kelp, whose main carbohydrates are alginic acid and mannitol.

Table 2.1 Carbohydrate in marine macroalgae (Jung et al., 2013)

	Green algae	**Red algae**	**Brown algae**
Polysaccharide	Mannan	Carrageenan	Laminarin
	Ulvan	Agar	Mannitol
	Starch	Cellulose	Alginate
	Cellulose	Lignin	Fucuidan
			Cellulose
Monnosaccharide	Glucose	Glucose	Glucose
	Mannose	Galactose	Galactose
	Rhamnose	Agarose	Fucose
	Xylose		Xylose
	Uronic acid		Uronic acid
	Glucuronic acid		Mannuronic acid
			Guluronic acid
			Glucuronic acid

2.4.1 Alginic acid

Alginic acid is polysaccharide in brown algae that has a complex structure. Chemically, alginic acid consists of sequences of uronic acids, namely guluronic acid (GA) and mannuronic acid (MA), whose chemical structure is illustrated in **Fig. 2.3**. Due to its characteristics, alginic acid has lower digestibility by microorganisms, thus requires pretreatment process prior to its effective utilization. Specifically, the pretreatment focuses on hydrolysis of the alginic acid, to produce its corresponding uronic acids. Due to that, various method of hydrolysis of alginic acid has been established, particularly by acid hydrolysis methods (Smidsrod et al., 1963)(Smidsrod et al., 1966)(Haug et al., 1967)(Chandia et al., 2001)(Sánchez-Machado et al., 2004)(Chhatbar et al., 2009).

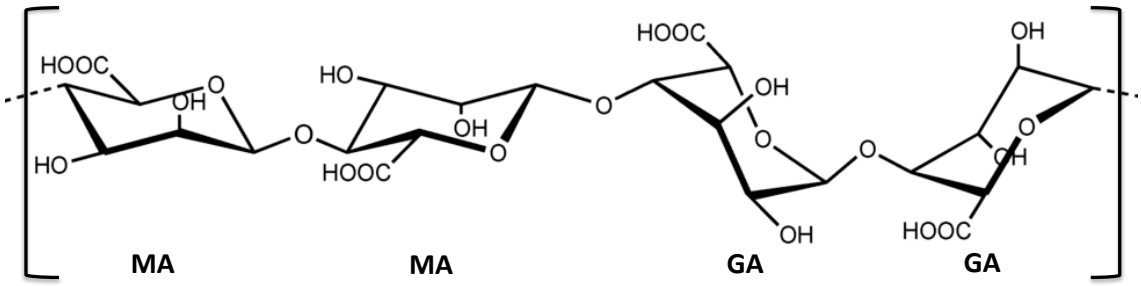


Fig. 2.3 Chemical structure of alginic acid

The comparison of the hydrolysis methods conducted by those researchers is summarized in **Table 2.2**. In short, most of the studies focused on the yield of uronic acids as a mixture solution, rather than the recovery yield as individual uronic acid. Hence, limited information is available on the recovery of GA and MA as individual acid.

Table 2.2 Comparison of hydrolysis methods for alginic acid

No.	Methods	References
1.	The main objective of this study was to determine the degradation behavior of alginate. Briefly, various reducing agents were added into alginate solution, and then were hydrolyzed inside a water bath at constant temperature.	(Smidsrod et al., 1963)
2.	The objective of this study was to determine the ratio of MA and GA in the liquid product of alginate hydrolysis. The researchers used 1 M of oxalic acid and mixed with alginate prior to hydrolysis process. The duration and temperature of hydrolysis were set at 100 °C and between 1 to 10 h,	(Smidsrod et al., 1966)

	respectively. After hydrolysis, calcium carbonate ($CaCO_3$) was added to neutralize the solution. The solid residues were removed while filtrate was evaporated. Finally, ethanol was added into the filtrate and the precipitate was washed and dried.	
3.	The alginate hydrolysis was conducted in 0.3 N of hydrochloric acid (HCl) for 20 min at 100 °C. The liquid phase was then neutralized, evaporated, dialyzed, and freeze-dried. The solid residues were further hydrolyzed for another 20 h, where the remaining solid was then dissolved in alkali. The pH was adjusted at 2.85 and centrifuged to separate the insoluble and soluble fractions. Both fractions were neutralized and dialyzed. Lastly, ethanol was added into both fractions and precipitates formed were washed and dried.	(Haug et al., 1967)
4.	This study employed different types of acid for alginate hydrolysis. a) Sodium alginate was mixed with 80 % of sulfuric acid (H_2SO_4) for 18 h at 0 °C. The mixture was cooled at 0 °C and added with water prior to reflux for 6 h. The uronic acids were separated by ion-exchanged chromatography. b) Sodium alginate was hydrolyzed in 90 % of formic acid for 2 to 6 h at 100 °C. Then, water was added into the hydrolysate and further hydrolyzed for 2 h. The solution was evaporated and remaining residue was dissolved in water, added with triethylamine, and analyzed by HPLC. c) Sodium alginate was hydrolyzed in 3 M HCl for 0.5 h at 100 °C under N_2. The remaining residue was further hydrolyzed in 0.3 M HCl for another 2 h at 100 °C. After	(Chandia et al., 2001)

	centrifuged, the solid residues were dissolved by neutralization, adjusted at 2.85 and centrifuged to separate the solid and liquid fractions. The solid was recovered as it is (GA) while liquid was added with ethanol for the recovery of MA.	
5.	This researcher conducted two methods of hydrolysis. a) Dietary fiber was hydrolyzed in 12 M H_2SO_4 for 0.5 h at 35 °C. Then, ultrapure water was added and further hydrolyzed for 2 h at 100 °C. The solution was passed through ion exchange resin column, where the eluate was collected. Lastly, HPLC analysis was conducted for the eluate that contains mixtures of MA and GA. b) Alginic acid was hydrolyzed in 1 M of oxalic acid for 10 h at 100 °C. Then, pH of the hydrolysate was adjusted at 2.85, where precipitate was collected as GA while filtrate was adjusted at pH 1.5 to collect MA (precipitate).	(Sánchez-Machado et al., 2004)
6.	This study employed microwave for the hydrolysis, where alginate in various concentration of oxalic acid or H_2SO_4 was hydrolyzed between 1 to 5 min. The precipitate was then dissolved by neutralization with sodium carbonate (Na_2CO_3) and adjusted at pH 2.85. Finally, precipitate was collected as GA while filtrate was adjusted at pH 1.0 to collect MA (precipitate).	(Chhatbar et al., 2009)

Besides the acid hydrolysis, hydrothermal technology and microwave-assisted method are the other possible alternatives for alginic acid hydrolysis. Previously, a study was conducted where high temperature of hydrolysis was employed and required large amount of ethanol for the fractionation of the uronic acids (Chandia et al., 2001). Similar study was also conducted but they employed additional processes (dialysis and freeze drying) for the uronic acids separation (Leal et al., 2008). In addition, hydrolysis by microwave irradiation was also conducted by previous researchers, where rapid hydrolysis was achieved (Chhatbar et al., 2009). In short, all of these studies reported on the composition ratio of the uronic acids in hydrolysate.

Theoretically, hydrolysis of alginic acid occurs at the glycosidic bond, between the oxygen atom and hydrogen ion (Xiang et al., 2003). Under hydrothermal condition where water was employed as reaction medium, reaction proceeds and generates its corresponding uronic acids, whose graphical view is shown in **Fig. 2.4**.

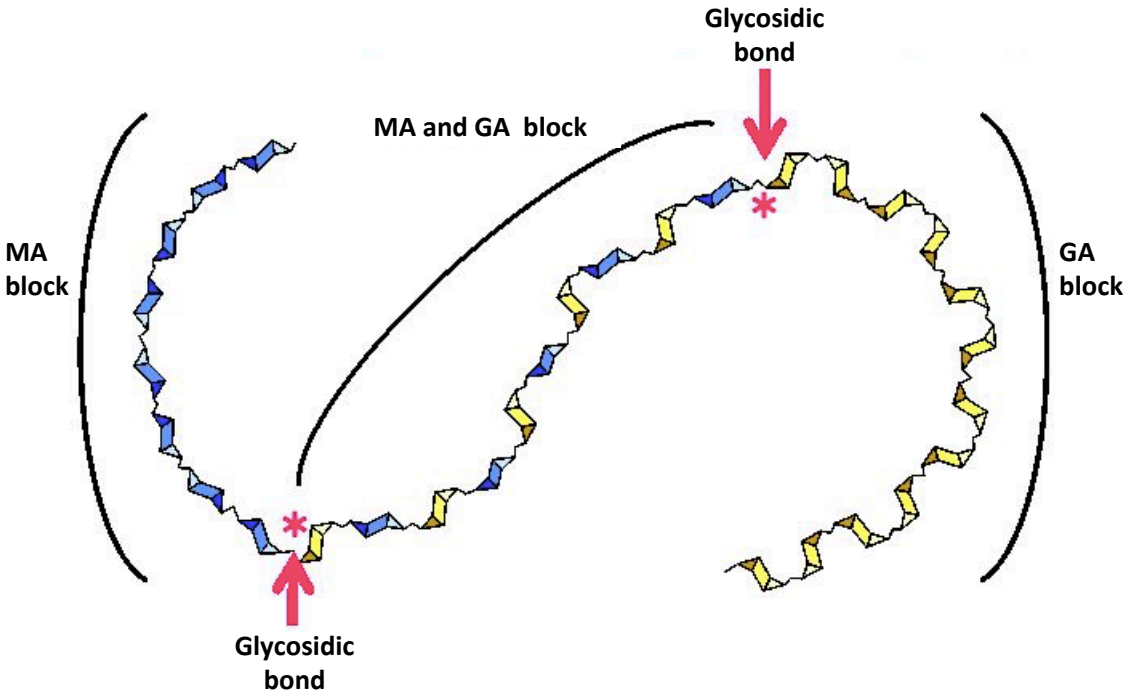


Fig. 2.4 Graphical view of alginic acid hydrolysis

In previous study, a group of researchers reported that the hydrolysis reaction was promoted by the intramolecular catalysis by the carboxyl group (Smidsrod et al., 1963). Further degradation of the uronic acids consequently produces various organic compounds, whose finding was reported in previous study (Aida et al., 2012). The researchers also concluded that alginate was firstly decomposed to its corresponding uronic acids, and consequently the uronic acids decomposed to organic acids.

After the hydrolysis of alginic acid, a homogeneous solution that contained mixture of GA and MA was obtained. Thus, separation of these uronic acids is needed to get the individual acids by changing the pH of the solution. Notably, GA and MA have different acid solubility, where at around pH 2.85, GA was precipitated while MA remained in the solution. However, the effectiveness and reproducibility of the process for separation of individual uronic acid were rather limited and not well reported.

As mentioned earlier, most of the previous studies focused on the composition ratio of MA and GA in the hydrolysate instead of the separation. Therefore, effective separation method is needed for effective utilization of kelp in particular.

2.4.2 Uronic acid

Uronic acid is classified as sugar acid with carboxylic acid (−COOH), as its functional group. Fundamentally, sugar acid is derived from oxidation of hydroxyl group of its terminal carbon, and the name is generally depending on its corresponding parent sugar. For example, the oxidation of glucose consequently produces glucuronic acid as its corresponding sugar acid, whose chemical structures are illustrated in **Fig. 2.5** and **Fig. 2.6**.

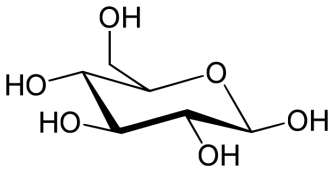


Fig. 2.5 Glucose (before oxidation)

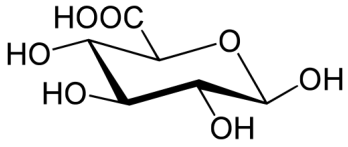


Fig. 2.6 Glucuronic acid (after oxidation of glucose)

The figures clearly show that only the hydroxyl group of carbon 6 (C-6) was oxidized to carboxylic group while the others remained. Usually, the oxidation reaction proceeds with the presence of catalyst. Recently, details reaction mechanism for the oxidation of glucose to glucuronic acid was elucidated where gold nanoparticle catalyst was employed. The finding revealed that high selectivity was achieved where only glucuronic acid was obtained in the absence of base (Wojcieszak et al., 2016).

The other types of uronic acids that were of interest are MA and GA. As mentioned in the previous subsection, these uronic acids exist as the main homopolymers of alginic acid. Hence, hydrolysis of the alginic acid is needed for the recovery of its uronic acids. Furthermore, separation of the uronic acids is also necessary to obtain individual uronic acid prior to their effective utilization. Besides the hydrolysis, both of the uronic acids can be derived from the oxidation of their parent sugars, namely mannose and gulose, respectively. During the oxidation, hydroxyl group of the terminal carbon changed to carboxylic group. The chemical structures of the uronic acids and their corresponding parent sugars are illustrated in **Fig. 2.7** to **Fig. 2.10**.

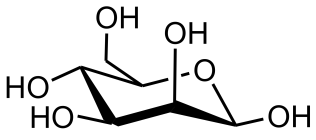
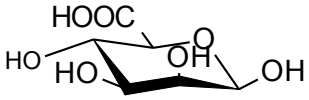


Fig. 2.7 Mannose (before oxidation)



MA

Fig. 2.8 Mannuronic acid (after oxidation of mannose)

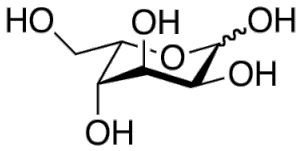
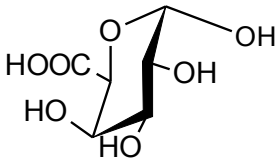


Fig. 2.9 Gulose (before oxidation)



GA

Fig. 2.10 Guluronic acid (after oxidation of gulose)

Uronic acids naturally exist as biopolymers in most plants and animals. For example, D-glucuronic acid is a component of hemicellulose while MA is a component of alginic acid, the main compound of kelp. Since uronic acids are derived from their corresponding hexoses, they naturally exist with six carbon atoms. Besides, they have the properties of monosaccharide, as well as hydroxyl acid. Thus, uronic acids have great potential to be used as renewable resources due to their characteristics.

However, the utilization of uronic acids, particularly GA and MA, has yet to be explored owing to the lack of information on its individual characteristics. Furthermore, these uronic acids are not commercially available in the market, thus their application becomes limited. Hence, recovery of uronic acids from alginic acid of kelp

is essentially important through a simplified procedure so that it will be useful to those involved in the utilization of marine biomass.

2.4.3 Sugar alcohol

Another major compound of kelp is mannitol, a group of sugar alcohol with hydroxyl as its functional group. Chemically, mannitol is derived from reduction reaction of its corresponding aldohexose (mannose), where aldehyde group of mannose is reduced to hydroxyl group. The reduction mechanism of mannose to mannitol is clearly shown in **Figure 2.11**.

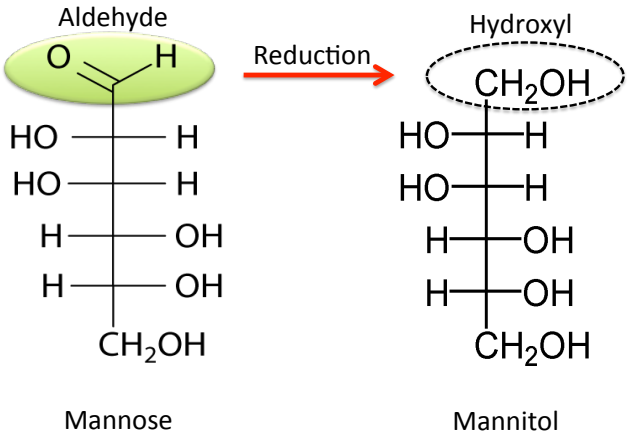


Fig. 2.11 Reduction reaction of mannose

Mannitol is naturally existed in variety of plants including macroalgae, whose composition is significantly high. In previous study, it was reported that kelp contained as much as 31.5 % of mannitol, from 54.5 % of its carbohydrate contents (Jang et al., 2012). Owing to that, recovery of mannitol from plant biomass is needed for its further utilization.

Remarkably, mannitol has broad range of applications particularly in food and pharmaceutical industries. Recently, its application has been broadening to which it was used as feedstock for bioenergy and bio-refineries. In fact, previous researchers successfully evaluated the potential of mannitol in macroalgae for biofuel production (Xia et al., 2015). Specifically, mannitol was employed as substrate for fermentation process to produce hydrogen, ethanol, and volatile fatty acid (VFA). The results indicated that the used of anaerobic fermentative bacteria, which was pretreated prior to the fermentation effectively favored the conversion of mannitol to its desired products. Furthermore, a group of researchers was successfully conducted production of bioethanol from mannitol in kelp (Wang et al., 2013). Surprisingly, the study reported that the bioethanol production was more favorable by using mannitol in macroalgae than that of glucose.

Sorbitol, the isomer of mannitol is another type of sugar alcohol whose chemical structures is only differed on the orientation of hydroxyl group of C2, as shown in **Fig. 2.12**. Similar like mannitol, sorbitol has been widely used as sugar substitute especially in food industry. For example, the potential of sorbitol as alternative sugar in cake was successfully evaluated in previous study. The researchers highlighted that the utilization of sorbitol generally improve the characteristics of cake (Manisha et al., 2012). Recently, new approach was also developed to diversify the utilization of sorbitol as feedstock to produce various ranges of gasoline products (Liu et al., 2016). The finding revealed the importance of controlling the cleavage of C−C and C−O bonds in determining the product yield.

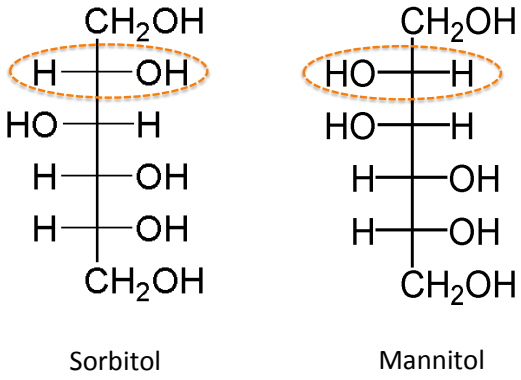


Fig. 2.12 Chemical structures of sorbitol and its isomer

Since sorbitol is the isomer of mannitol, similar reaction characteristics of these sugar alcohol are expected from the experimental results. Thus, general reaction rate could be obtained and applied for the other sugar alcohols. However, in order to demonstrate the similarity or dissimilarity, comparable study on each sugar alcohol is needed. Therefore, this study was initiated to evaluate the comparison on behavior of these sugar alcohol.

2.5 Conversion of macroalgae for bioenergy production

Nowadays, various technologies have been employed for the conversion of biomass to biofuel. Generally, the technologies can be categorized into two groups:

a) Technology with pre-drying

b) Technology without pre-drying

The earlier technology is basically designed for energy extraction from dry feedstock, while the latter is more suitable for energy extraction from wet biomass. As for marine biomass, the technology with pre-drying is less favorable owing to their high moisture content. This is because drying of macroalgae whose water content was significantly high requires great energy consumption. Instead, the technology is more suitable for terrestrial biomass, whose moisture content is generally much lower than marine biomass.

In contrast, production of biofuel particularly via thermochemical and biochemical processes is more favorable for macroalgae. The selection of appropriate process is however depended on several factors including the amount of available feedstock and the types of desired energy (such as gas or liquid fuel). The details of various processes that suitable for conversion of marine macroalgae were discussed in the following subsections.

2.5.1 Thermochemical conversion of macroalgae

Typically, the thermochemical conversion of biomass to biofuel is a process where energy in biomass was extracted through thermal decomposition of its organic compounds. Theoretically, during the heating process with poor oxygen supplied, syngas is generated whose compositions are mainly hydrogen (H_2) and carbon monoxide (CO). On the other hand, in the presence of sufficient air supply, carbon dioxide (CO_2) and water are produced along with heat generation. Both conditions are however known as combustion process.

Technically, combustion is conducted at high temperature around 800 ^{o}C, where the efficiency is greatly depended on the moisture content of its feedstock. A study reported that practically the moisture content of the feedstock should be less than 50 % (McKendry, 2002). Thus, pre-heating of macroalgae whose water content was above than 80 % (McDermid and Stuercke, 2003), is apparently required prior to the combustion process.

Another thermo-chemical process that is widely used for biofuel production from biomass is pyrolysis. In contrast to combustion, this process occurs without air supply and the products vary depending on the reaction temperature and time. For example, slow pyrolysis that operated at lower temperature and longer residence time is generally produced higher char. On the other hand, fast pyrolysis is usually employed to produce higher yield of liquid and gas products.

In fact, a group of researchers revealed that dried macroalgae produced significant amount of bio-oil approximately 65 wt% through fast pyrolysis (Trinh et al., 2013). However, the yields were generally lower than that obtained from lignocellulosic biomass, due to the high ash and mineral contents in macroalgae. Owing to that, pre-treatment of macroalgae is needed for improving the yield as well as the characteristics of the products. Previously, a study was successfully conducted whose finding demonstrated significant improvement on the pyrolysis process and its products after the pretreatment of macroalgae (Choi et al., 2014).

Alternatively, biomass can be converted to the desired biofuel through gasification process where hydrocarbon is converted to syngas through partial oxidation at relatively high temperature. The gases generated can be directly utilized for electricity generation or can be used for others such as for biofuel production.

Gasification was conventionally designed for dry feedstock, similar to combustion and pyrolysis processes.

However, supercritical water gasification (SCWG) was later developed that allowed the utilization of wet biomass, particularly marine macroalgae. Basically, the SCWG is conducted at temperature and pressure above the critical point of water, where liquid and gas are indistinguishable. The graphical view of the phase diagram of water is illustrated in **Fig. 2.13**, in which water acts as en excellent solvent for many substrates under the supercritical region.

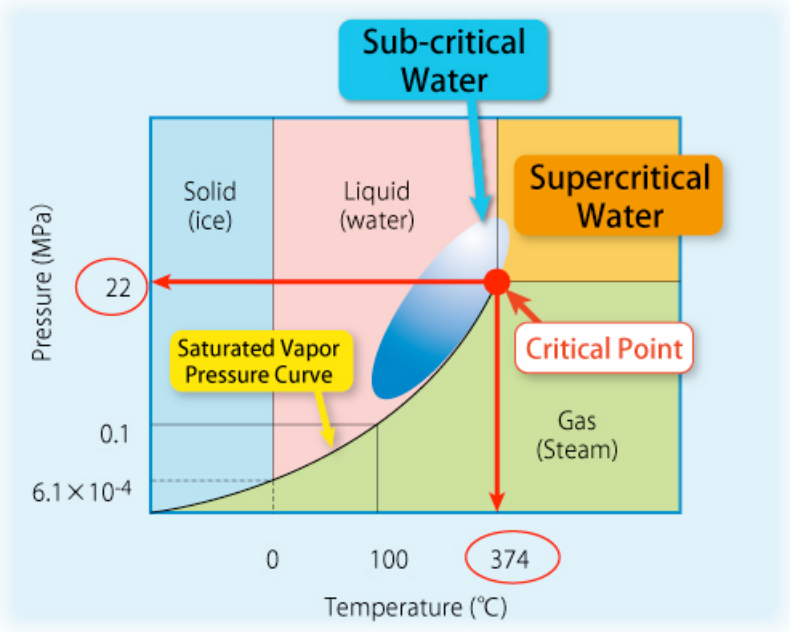


Fig. 2.13 Phase diagram of water

The potential of SCWG of macroalgae was later evaluated by group of researchers where the results demonstrated significant amount of methane and hydrogen were produced (Cherad et al., 2014). Besides, the results were in accordance with another study that revealed great potential of SCWG in producing methane and

hydrogen gasses (Schumacher et al., 2011). Since the process itself requires water as the reaction medium, pretreatment of macroalgae (drying) is certainly unnecessary unlike the other thermo-chemical processes. However, pre-treatment is needed for removal of minerals in macroalgae to avoid the formation of excessive char.

Liquefaction is also another option under thermochemical technology for conversion of macroalgae in particular, into liquid biofuel. In comparison to pyrolysis, this process requires lower temperature but higher pressure and suitable for wet biomass. During the liquefaction, the biomass decomposition occurs under sub-critical condition with the addition of catalyst and consequently produces smaller molecular compounds. The detail reaction mechanism of liquefaction was extensively discussed by previous researcher (Demirbaş, 2000). Besides, the production of bio-oil from macroalgae was evaluated in previous study (Zhou et al., 2010). In short, the researchers were successfully obtained bio-oils that composed of various compounds. However, further improvement was needed for the liquefaction process due the lower product yield.

2.5.2 Biochemical conversion of macroalgae

The conversion of marine macroalgae into biofuel is generally more effective by using biochemical process, whose technologies have been well established. However, each of the technology is employed depending on the desired biofuels, such as biogas, bioethanol or biodiesel. In fact, numerous studies have been conducted on the utilization of macroalgae as promising feedstock for biogas production via anaerobic digestion (AD) (Nkemka and Murto, 2010)(Hinks et al., 2013)(Fan et al., 2015).

Chemically, biogas composes of mainly methane (CH_4) and CO_2 gasses that are generated from decomposition of organic matters in the macroalgae. During the process, sequence of reactions occurs namely hydrolysis, fermentation and methanogenesis. Briefly, carbohydrate in macroalgae is hydrolyzed into soluble sugars and consequently converted by fermentative bacteria to biogas as the final product. In fact, it was reported in previous study that biogas has about 650-750 Btu/ft^3 of heating value. Owing to that, it can be further utilized in various ways, such as for heating, generating electricity, and also as biofuel for transportation purposes. Therefore, utilization of biomass specifically macroalgae for biofuel production is apparently a promising option than using the fossil fuel.

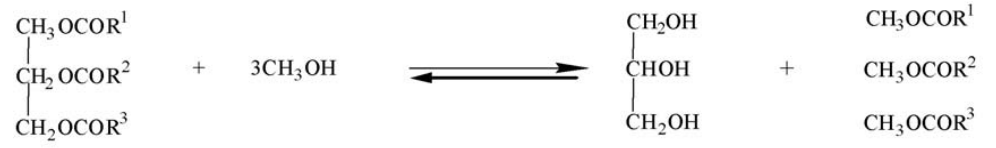
Bioethanol is another type of biofuel that can be obtained from macroalgae via alcoholic fermentation. Fundamentally, the fermentation involves the used of enzymes and microorganism, where the carbohydrates in macroalgae is converted to bioethanol. For example, in the case of fermentation where *Saccharomycess cerevisiae* was employed, the sugar in macroalgae was converted to bioethanol, as represented in Eq. 2.1.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \qquad (2.1)$$

In comparison to lignocellulosic biomass, macroalgae is generally has insignificant amount of lignin, and thus easier to be hydrolyzed by microorganism. In fact, numbers of researches were conducted to evaluate the potential of marine macroalgae as the promising resources for bioethanol (Borines et al., 2013)(Kim et al., 2011)(Trivedi et al., 2013).

Specifically, a group of researchers was successfully demonstrated the potential of macroalgae as a feedstock for the bioethanol production. The researchers highlighted that significant amount of bioethanol was produced by continuous reactor than that of batch reactor (J. H. Park et al., 2012). Nevertheless, pretreatment of macroalgae is essentially important to obtain higher yield of bioethanol. This is because macroalgae are often contained significant amount of mineral that could be the drawback for fermentation process.

Another kind of biofuel that can be obtained from conversion of macroalgae is biodiesel. Generally, biodiesel is produced through a reaction known as transesterification, where triglyceride is reacted with alcohol to form biodiesel. During the process, glycerol is also produced as by-product. For instance, when triglyceride is reacted with methanol using homogeneous catalyst, methyl ester (biodiesel) and glycerol are generated as represented by Eq. 2.2.



Triglyceride Methanol Glycerol Methyl ester (biodiesel) (2.2)

Various methods have been developed for the transesterification that employed either acid or base catalyst in a homogeneous or heterogeneous condition. Besides, recent study also demonstrated the possibility of using waste catalyst from steel industry in the production of biodiesel (Khan et al., 2016).

In addition, the potential of macroalgae as carbon source for transesterification was successfully reported in previous work whose finding showed about 90 % of product yield was obtained at optimum condition (Suganya et al., 2013). Specifically for *Laminaria japonica* species, it was highlighted in previous study that this macroalgae favored the lipid production, a feedstock for biodiesel (Xu et al., 2014). Nonetheless, the efficiency of biodiesel production is greatly depended on the pretreatment of macroalgae prior to the transesterification reaction.

2.6 Pretreatment of marine macroalgae

Notably, marine macroalgae are one of the promising resources with broad ranges of applications, particularly for bioenergy production. In fact, many researchers have put significant efforts to demonstrate the potential of macroalgae as sustainable resources. However, the used of macroalgae often requires pretreatment process prior to their utilization. The discussions on the importance of pretreatment of macroalgae have been well documented by numerous studies. Besides, several methods have been developed for the pretreatment process, with the aim for improving the subsequent processes such as fermentation and transesterification.

2.6.1 Hydrothermal pretreatment technology

This technology has been widely implemented for pretreatment of biomass, either for lignocellulosic biomass or marine biomass. This is mainly due to economic point of view, in which the process is generally considered as cost effective. Particularly, the process itself employs water as the solvent that apparently cheaper than

any other chemical solvent, hence considered as environmental friendly process. Besides, since it does not involve any harsh chemical, the equipment has less corrosion problem.

Fundamentally, the hydrothermal process is conducted at high temperature and pressure, where water as the reaction medium remains in liquid state. At this condition, the hydrogen bond in biomass starts weakening consequently promotes the autoionization of water into hydronium ions (H_3O^+) and hydroxide ions (OH^-) (Ruiz et al., 2013).

The important parameters of this technology that might affect the product yield including reaction temperature, residence time, and solid to liquid ratio. In fact, most studies reported higher degradation of biomass was obtained at higher temperature and longer residence time. Under extreme condition, probably undesired reaction occurs where the desired product is further decomposed. Thus, appropriate operating condition is needed for the recovery of target product. Owing to that, kinetics study is essentially important to avoid unnecessary reaction.

Practically, hydrothermal technology is appropriate method for treating macroalgae that generally contains high amount of water. This is because, the process itself employs water as the reaction medium, and thus does not requires drying process for the wet feedstock. Besides, under hydrothermal condition, water acts as an excellent solvent and consequently favors the conversion of wet biomass into biofuels. Moreover, the applications of marine macroalgae have been extended to many other industries including food, pharmaceuticals, cosmetic as well as bio-chemicals.

Chemically, during the hydrothermal pretreatment where high temperature and pressure are employed, reaction between macroalgae and water is preceded. Consequently, decomposition reaction of macroalgae occurs and varieties of products are generated depending on the operating conditions. Owing to the importance of pretreatment process, the effects of hydrothermal pretreatment on decomposition behavior of macroalgae have been extensively discussed.

Theoretically, under the subcritical water condition, sugar compounds in macroalgae are released and consequently favored the subsequent process like fermentation. Previously, a study was conducted where macroalgae were pretreated under hydrothermal condition in a batch type reactor. The results showed significant effect of experimental conditions on the product yield, specifically reaction temperature, time, solid to liquid ratio, and severity factor. Additionally, the product yield was significantly improved when pretreated macroalgae were subjected to enzymatic hydrolysis (Kim et al., 2014).

In another study, macroalgae were pretreated in batch reactor that contained water as the reaction medium, at high temperature and pressure prior to simultaneous saccharification fermentation (SSF) for bioethanol production. Positive results were obtained when pretreated macroalgae produced higher ethanol than the untreated ones (Schultz-Jensen et al., 2013).

2.6.2 Chemical pretreatment of macroalgae

Typically, hydrothermal pretreatment technology was developed using only water as the reaction medium. However, the technology has been further explored to enhance its effectiveness. In earlier study, a group of researchers was successfully

implemented hydrothermal pretreatment of *Laminaria japonica* under acidic condition for bioethanol production. The results showed that enzymatic digestibility was relatively higher for treated macroalgae than that without pretreatment (Lee et al., 2013). Perhaps, the addition of low concentration acid had successfully promoted the hydrolysis of the macroalgae under hydrothermal condition. Consequently, the released sugar from the treated macroalgae favored the subsequent process.

Furthermore, the effectiveness of the hydrothermal treatment with dilute acid concentration was also reported in another study. The researchers found that the treated macroalgae under acidic condition apparently released higher sugars than the control sample. Consequently, the sugars were utilized by microorganism during the fermentation process to produce bioethanol (Borines et al., 2013). Besides, the effect of thermo-acidic condition on pretreatment of macroalgae for methane production was clearly explained in recent study (Barbot et al., 2015).

In addition, alkaline pretreatment is another alternative for chemical pretreatment of macroalgae. This method is usually employed for pretreatment of lignocellulosic biomass. Practically, the process was comparatively simpler than other pretreatment methods. Previously, a study was conducted to evaluate the effect of alkaline pretreatment of macrolagae on volatile fatty acid production (Pham et al., 2013). The results showed the dependence between reaction time and product yield. Furthermore, the alkaline pretreatment is more favorable to brown macroalgae than the red and green algae. Perhaps, the compositions of macroalgae greatly affect the effectiveness of the alkaline pretreatment.

2.6.3 Biological pretreatment of macroalgae

Another potential approach for pretreatment of macroalgae involves the biological activity of microorganism. Specifically for brown algae that compose of high alginic acid, biological treatment is one of the best options for hydrolysis. The application of this technology for production of volatile fatty acid (VFA) was reported in previous study (Pham et al., 2013). Briefly, the macroalgae employed in that study was first subjected to fermentation by microorganism, and then followed by VFA fermentation. The finding showed the yield of VFA was apparently higher for macroalgae that were biologically treated.

Nevertheless, the efficiency of biological treatment is greatly depended on the types of microorganism because its ability differs depending on the composition of macroalgae. Another research group was also conducted biological pretreatment of marine algae via enzymatic hydrolysis (Trivedi et al., 2013). In that study, the total reducing sugar obtained after the enzymatic hydrolysis showed increasing trend at longer reaction time, which was further utilized for bioethanol production.

2.7 Effective utilization of macroalgae (kelp)

Notably, marine biomass contains significant amounts of carbohydrate that can be recovered and utilized in diverse ways. In order to ensure that these resources are being utilized efficiently, quantitative analysis is necessary. For this purpose, analyses on mass and energy balances are particularly important. Prior to that, the information on overall process flow for kelp utilization is needed with appropriate system analysis.

Typically, the main objective of any system is to maximize the product yields and at the same time to minimize the cost, especially the energy requirement. Unfortunately, the information on analysis of overall process flow of kelp utilization is somewhat limited.

The carbohydrates obtained from pretreated kelp could be utilized in multiple ways. For example, they can be used as feedstock for the production of high value added oils. In fact, our research group was successfully produced value added lipid from kelp (Arafiles et al., 2014). The results showed higher conversion of carbohydrate in kelp to fructose (approximately 83 %) that was further utilized for value added lipid production. Furthermore, the waste generated from the oil fermentation process also could be supplied to methane fermentation. However, the information on mass and energy balances was not reported in this study.

In addition, the carbohydrates in kelp could potentially use as feedstock for methane fermentation. Typically, this process consists of three steps, namely acidogenesis, acetogenesis, and methanogenesis, where methane can be produced through single-stage or two-stage process. In the previous study conducted by our research group, a single-stage process was employed for the production of methane from kelp (Miura et al., 2015). The finding showed the methane production from kelp was improved via fed-batch cultivation without dilution of salinity. Nonetheless, the evaluation on mass and energy balance of the methane production was not available. Actually, this kind of information is certainly desired to provide guidelines to other researchers. Due to the lack of study reported about it, this topic could be an interesting point of discussion.

Basically, wastes are generated from anaerobic fermentation of biogas production as well from oil fermentation process. Thus, proper waste treatment is needed prior to disposal. Besides that, the treatment process could be designed for mineral recovery. This is because, kelp contain significant amount of minerals that will remain through out the system. Nevertheless, the effectiveness of the overall kelp utilization is greatly depended on the overall mass and energy balance of the system.

CHAPTER 3

RESEARCH AIM AND OBJECTIVES

3.1 Research motivation

The utilization of renewable energy as an alternative to fossil fuel energy has attracted great attention from worldwide researchers. This is due to the worldwide energy crisis that caused by fossil fuel depletion as well as environmental issues. Fundamentally, fossil fuels are formed through natural processes, where temperature and pressure in the earth crust play an important role. Furthermore, they require million of years to be formed before the fuels can be used for various applications.

Owing to that, sustainable supply of fossil fuels has becoming more crucial. Additionally, the world energy demands has also shown an increasing trend and hence resulted to imbalance between energy supply and demand. Therefore it is essentially important to reduce the dependency on fossil fuels.

On the other hand, climate change issue has generated significance awareness among the energy consumers. Nowadays, the average world temperature has shown an increasing trend. This is mainly due to the greenhouse effects that causes by the presence of excessive carbon dioxide (CO_2) gas in the atmosphere. Basically, the CO_2 gas is generated when fossil fuel is consumed for energy production. Besides that, other problems are also anticipated from utilization of fossil fuels including the rising of sea level, changing of precipitation, as well as expansion of dessert particularly in subtropic areas.

In order to overcome these issues, utilization of renewable energy is the best alternative. Basically, renewable energy is the energy that derived from natural resources. Among the many different kinds of natural resources, biomass is one of the most abundant resources that available on the earth. Thus, biomass energy could be a promising alternative energy. Indeed, numerous studies have been conducted on the utilization of various types of biomass for wide range of applications, including for biofuels production. Among them, marine biomass (macroalgae) has started obtaining extensive interest from researchers of various fields. This is mainly due to several factors, particularly because of its composition that contains significant amounts of carbohydrate. Hence, recovery of these carbohydrates from macrolagae is a great opportunity to be discovered.

Specifically, *Laminaria japonica* or kelp is a brown macroalgae that abundantly available in Japan. Recently, its utilization as renewable resource has been extensively discussed in many studies. Specifically, it is due to the high amount of alginic acid and mannitol in kelp. These sugar compounds have been widely used in various applications, such as foods, pharmaceuticals, and cosmetics industries. In recent

studies, the potentials of these sugars for biofuel and biorefineries productions have also been explored.

Nevertheless, proper pretreatment is needed prior to the recovery of these valuable carbohydrates from macroalgae. Appropriate methodology will consequently determine its recovery yield. In order to determine the best pretreatment condition of macroalgae, kinetics study of its individual compound is required. This is to ensure that good recovery yield is obtained after the pretreatment process. Currently, many technologies have been developed specifically for pretreatment of macroalgae. Among them, hydrothermal process has been identified as potential method of pretreatment for wet biomass like macroalgae. This is because of the process itself that requires water as reaction medium. Unfortunately, specific studies on the individual carbohydrate in macroalgae under hydrothermal condition is limited and not well reported. Owing to that, this research was initiated where hydrothermal treatment process was employed through out this study.

For the case of kelp, the information regarding kinetics characteristics of its carbohydrates (alginic acid and mannitol) under hydrothermal condition is somewhat limited. Partly, it is due to the unavailability of alginic acid in commercial market. Besides, it is not easy for microorganism to digest the alginic acid due to its complex structure. Thus, further fractionation of alginic acid is needed to produce its corresponding uronic acids, namely guluronic acid (GA) and mannuronic acid (MA). Unfortunately, the kinetics behavior of GA and MA is yet to be reported. Hence, it is necessary to study the behavior of these uronic acids under hydrothermal condition prior to the kelp pretreatment. For that purpose, preparation of individual uronic acid (solid form) is needed.

Previously, several methodologies have been developed for hydrolysis of alginic acid, whose procedures involved several processes and rather complicated. Also, most of them focused on the ratio of GA and MA in hydrolysate. Only few studies had discussed on the recovery of the uronic acids as solid products but yet the product characteristics were not well reported. Owing to this reason, development of simplify methodology of uronic acid preparation is necessary prior to the kinetics study.

Besides kelp, a study on other carbohydrates in macroalgae is also within the scope of this study. Generally, macrolagae contain various kinds of carbohydrates, whose composition differs among different species. Besides, the carbohydrates are always existed in different types of sugar such as aldohexoses, sugar alcohols, and uronic acids. Since all of these sugars have different functional groups, their reactivity also might be different under the same pretreatment condition. Therefore, a study on the effect of functional group on kinetic characteristics of sugar is an interesting subject of discussion. Additionally, these sugar compounds can be isomerized under certain condition. Owing to this possibility, the effect of chemical structures of isomers is also another important point to be investigated.

By considering all of these issues, hydrothermal treatment of carbohydrates in macroalgae is needed to obtain guidelines for recovery their valuable carbohydrates. The title of this study is “Hydrothermal pretreatment of macroalgae: Detailed reaction kinetics and mechanisms”.

3.2 Research aim and objectives

The aim of this study is obtain guidelines for hydrothermal pretreatment of macroalgae for recovery of their valuable carbohydrates. In order to achieve this goal, the specific objectives of this study are as follows:

1. To develop simple methodology for recovery of guluronic acid (GA) and mannuronic acid (MA) from alginic acid that originally derived from kelp.
2. To elucidate the decomposition behavior of GA and MA under hydrothermal condition.
3. To determine the effect of functional groups on the kinetics characteristics of various sugars under hydrothermal condition.
4. To investigate the effect of chemical structures on the kinetics behavior of sugars (isomers) under hydrothermal condition.

CHAPTER 4

METHODOLOGY

4.1 Introduction

The experimental procedures employed in this study were clearly described in this chapter. The details descriptions were provided with appropriate illustration of experimental apparatus and experimental conditions. Besides, explanation on quantitative product analyses was also provided, including their analysis conditions.

In this study, the experimental procedures were divided into two (2) main stages, which are (i) hydrolysis and (ii) hydrothermal processes. The first stage was employed at the beginning of this study mainly for the separation of uronic acids from alginic acid. The later process was employed for the kinetics study of various carbohydrates in macroalgae under hydrothermal condition.

4.2 Experimental setup and procedures

4.2.1 Hydrolysis of alginic acid

The experimental procedure began with partial hydrolysis of alginic acid using hydrochloric acid (HCl). Firstly, 1 % (w/v) slurry of alginic acid was treated with 3 mol/dm^3 HCl; an amount equal to approximately 3 % of the total volume was added. The hydrolysis was conducted in a hot water bath at 90 °C for 30 min. Afterward, the solid phase was recovered by vacuum filtration and further hydrolyzed with 0.3 mol/dm^3 HCl for 2 h at the same temperature. Then, the solid phase was collected and dissolved in 1 mol/dm^3 sodium hydroxide (NaOH). As a result, a homogeneous solution containing the desired uronic acids was obtained. The amounts of 0.3 mol/dm^3 HCl and 1 mol/dm^3 NaOH required were equal to approximately 100 and 50 % of the initial volume of the alginic acid solution, respectively. The experimental apparatus used for hydrolysis process is shown in **Fig. 4.1**.

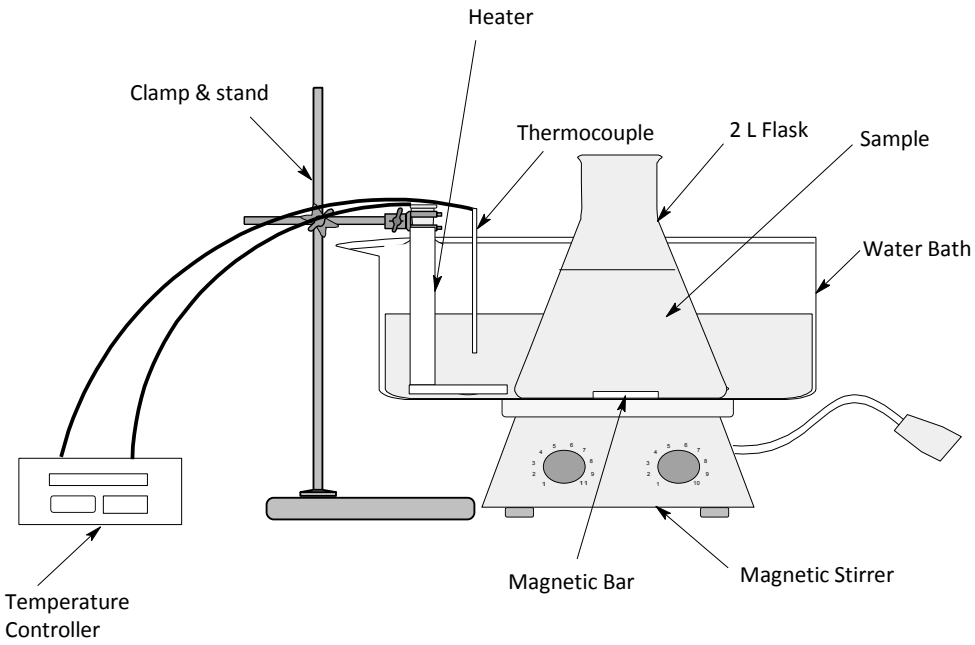


Fig. 4.1 Hydrolysis of alginic acids

Subsequently, the pH of the mixture solution of uronic acids was adjusted to 2.85 for the recovery of individual acids using 1 mol/dm^3 HCl. Owing to the difference in acid solubility, GA was collected as an insoluble solid product, while MA remained in solution in the filtrate. Then, an equal amount of ethanol was added to the filtrate for the recovery of desired MA as a solid product. The experimental flow is illustrated in **Fig. 4.2**.

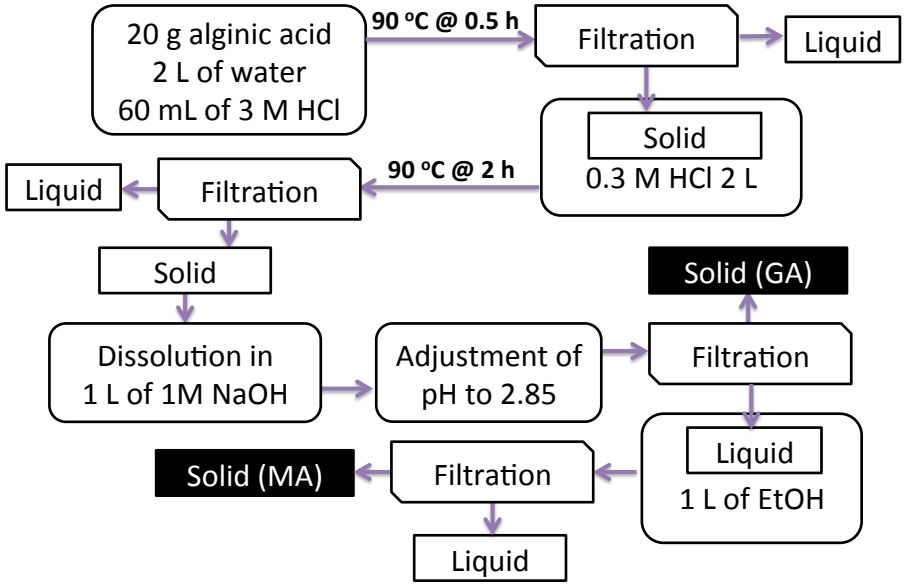


Fig. 4.2 Preparation of uronic acids

Additionally, both GA and MA were washed thoroughly with distilled water to remove the impurities, particularly sodium salt. The wet uronic acids were dried in desiccator instead of drying oven to avoid further degradation. The dried uronic acids were kept in a closed container prior to the preservation in a refrigerator, and product analysis.

4.2.2 Hydrothermal treatment of carbohydrates in macroalgae

The experimental apparatus employed in this study is similar with previous work conducted by our laboratory members (Yong and Matsumura, 2012). The schematic diagram of the experimental procedure is shown in **Fig. 4.3**.

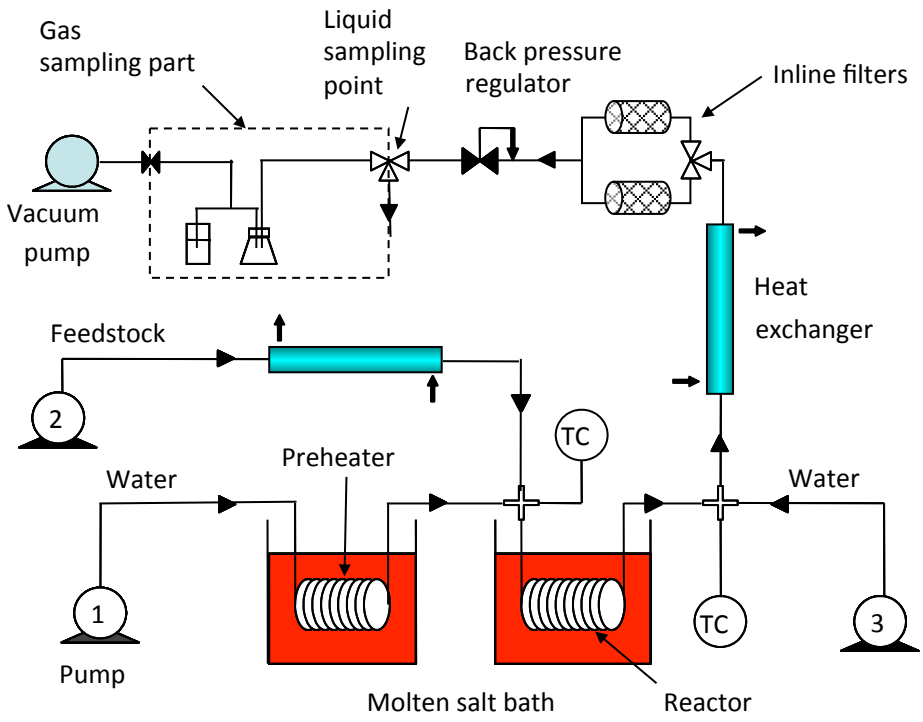


Fig. 4.3 Continuous flow reactor system

Briefly, a continuous flow reactor (stainless steel) was employed for the hydrothermal treatment of carbohydrates. The reactor length was changed according to the desired residence times, while the inner diameter was fixed at 1 mm. The feedstock was supplied at a ratio of 1 to 4 of the pre-heated hot water that was pumped from a different line and consequently mixed at the entrance of the reactor. The concentrations of feedstock in the reactor was 1 wt%. The reaction occurred inside the reactor at the desired temperature and pressure. After certain residence time, the hot liquid effluent

from reactor was mixed with cool water and further cooled in the heat exchanger. The cooled product stream was later depressurized by back-pressure regulator. Finally, liquid product was collected at specified point for further analysis.

4.3 Experimental conditions

The hydrothermal treatment of various carbohydrates was conducted under subcritical water condition. The summaries of experimental conditions for Chapter 6 to 9 are shown in **Table 4.1** to **4.4**, respectively.

Table 4.1 Experimental conditions for Chapter 6 (Decomposition kinetics of uronic acids obtained from kelp under hydrothermal condition)

Parameters	**Experimental conditions**
Feedstock	Mannuronic acid (MA)
	Guluronic acid (GA)
Types of reactor	Continuous flow (stainless steel)
Reactor diameter	1 mm (ID)
Reactor length	0.2 to 6 m
Feedstock concentration	1 wt%
Feedstock to water ratio	1 : 4
Reaction temperature	170 to 250 °C
Residence time	5 to 100 s
Operating pressure	25 MPa

Table 4.2 Experimental conditions for Chapter 7 (Decomposition kinetics of mannose, its sugar alcohol, and its uronic acid under hydrothermal condition)

Parameters	**Experimental conditions**
Feedstock	MA, mannose
Types of reactor	Continuous flow (stainless steel)
Reactor diameter	1 mm (ID)
Reactor length	0.2 to 0.9 m
Feedstock concentration	1 wt%
Feedstock to water ratio	1 : 4
Reaction temperature	170 to 250 °C
Residence time	3 to 20 s
Operating pressure	25 MPa

Table 4.3 Experimental conditions for Chapter 8 (Kinetics of sorbitol decomposition under hydrothermal condition)

Parameters	**Experimental conditions**
Feedstock	Sorbitol
Types of reactor	Continuous flow (stainless steel)
Reactor diameter	1 mm (ID)
Reactor length	0.9 m
Feedstock concentration	1 wt%
Feedstock to water ratio	1 : 4
Reaction temperature	170 to 250 °C
Residence time	15 to 20 s
Operating pressure	25 MPa

Table 4.4 Experimental conditions for Chapter 9 (Kinetics characteristic of carbohydrates in marine macroalgae under hydrothermal condition)

Parameters	**Experimental conditions**
Feedstock	Sorbitol, glucuronic acid
Types of reactor	Continuous flow (stainless steel)
Reactor diameter	1 mm (ID)
Reactor length	0.9 m
Feedstock concentration	1 wt%
Feedstock to water ratio	1 : 4
Reaction temperature	170 to 250 °C
Residence time	15 s
Operating pressure	25 MPa

4.4 Product analysis

4.4.1 Moisture content

The moisture content of the uronic acids obtained after the hydrolysis of alginic acid was measured in the following procedure. Approximately 1 g of sample was weighed and put in a drying oven at 80 °C until consistent weight was obtained (about 5 h). The difference between the initial and final weights of the uronic acids was used as the amount of water contained in the samples as represented in Eq. 4.1.

$$\text{Moisture content [\%]} = \frac{\text{Initial weight - Final weight [g]}}{\text{Initial weight [g]}} \text{ x } 100 \qquad (4.1)$$

Consequently, recovery yield was calculated based on the dry weight of uronic acid obtained per initial dry weight of alginic acid used in the hydrolysis, as represented in Eq. 4.2.

$$\text{Yield [-]} = \frac{\text{Product dry weight, g}}{\text{Feedstock dry weight, g}} \quad (4.2)$$

4.4.2 Karl-Fischer titration

The trace amounts of water in dried uronic acids were measured by using Karl-Fischer titrator that operated based on conventional titration method. Firstly, the device was switched on and selection of method was done accordingly. Prior to that, about 10 mg of sample was dissolved in 1 mL of dried ethanol. When the device was ready, sufficient amount of sample was introduced into the reaction vessel that contained methanol as the solvent. The actual value of sample weight was entered in the program prior to start the analysis. In this analysis, water content was measured directly by the instrument. The results of water content were expressed in term of percentage.

4.4.3 High performance liquid chromatography (HPLC) analysis

The quantitative analysis using HPLC (Shimadzu Corp.) was conducted for the uronic acids obtained after the alginic acid hydrolysis as well as for the liquid products obtained after the hydrothermal treatment process. Three (3) different kinds of column were employed for the HPLC analysis for quantification of various compounds. The column KS-802 from Showa Denko K. K. was employed for quantification of sugar compounds by using refractive index detector (RID 10-A, Shimadzu Corp.). The eluent

used was deionized water whose flow rate was set at 0.7 cm^3/min while the oven temperature was fixed at 60 °C.

The other column was SCR 102 HG (Shimadzu Corp.) was employed for quantification of organic acids that generated during the hydrothermal treatment of carbohydrates. For this analysis, 5 mmol/dm^3 of perchloric acid ($HClO_4$) was prepared as the eluent. Similarly, the eluent flow rate was set at 0.7 cm^3/min and a refractive index detector (RID 10-A, Shimadzu Corp.) was used, but the oven temperature was fixed at 40 °C.

The quantitative analysis of ring compounds (5-hydroxymethylfurfural (HMF), furfural) was conducted using DE 413L column (Showa Denko K. K.) at 40 °C of oven temperature. The eluent was prepared at 1:1 ratio of 5 mmol/dm^3 $HClO_4$ and acetonitrile, whose flow rate was 0.7 cm^3/min. Unlike the others, UV-vis detector (SPD 10-A, Shimadzu Corp.) was used for the analysis. The analysis conditions for each column are summarized in **Table 4.5**.

Table 4.5 Analysis condition of HPLC

	KS-802	**SCR 102 HG**	**DE 413L**
Detector	RID-10A	RID-10A	SPD-10A
Eluent	Deionized water	5 mM $HClO_4$	5 mM $HClO_4$: Acetonitrile
Eluent flow rate	0.7 cm^3/min	0.7 cm^3/min	0.7 cm^3/min
Oven temperature	60 °C	40 °C	40 °C

The analysis started with appropriate setup of column and its detector. Then, machines LC-10AD and SIL-20A were purged with respective eluent. After finish purging, the computer program was set up where analysis condition was inserted accordingly. Then, the computer program was connected to the machines. When the oven temperature reached the target value, eluent flow rate was increased gradually until the desired flow rate. Afterwards, RID flow was turned on and was turned off after 40 min. The RID-10A detector was then balanced until zero value appeared on the display screen. Next, schedule analysis was performed where total numbers of sample and injection as well as sample details were inserted in the program. When the system was ready, auto-sampler was rinsed prior to start the analysis.

The decomposition of feedstock concentration was calculated based on the residual ratio, which was defined as the ratio of unreacted feedstock concentration after hydrothermal treatment (C) to initial feedstock concentration (C_o), as represented by Eq. 4.3.

$$\text{Yield [-]} = \frac{\text{Unreacted feedstock [mol/dm}^3\text{]}}{\text{Initial feedstock [mol/dm}^3\text{]}} \tag{4.3}$$

4.4.4 Thermogravimetric analysis (TGA)

The TGA was conducted on dried samples of uronic acid for determination of fixed carbon (FC), volatile matter (VM), ash content, and moisture content. At first, appropriate gas line (air or nitrogen) was connected to the TGA machine. After turning on the machine, gas flow rate was set at the desired value. Then, balance was set to zero prior to weigh the sample of approximately 5 to 10 mg. Afterwards, the computer program was set up where the analysis conditions were entered accordingly.

The analysis was conducted at 950 °C of furnace temperature, 40 °C/min of heating rate, under air and nitrogen environment that flowed at 50 mL/min. When the system was ready, the analysis was started accordingly. The data obtained after the TGA was analyzed accordingly to obtain the desired information.

4.4.5 Total organic carbon (TOC) analysis

The carbon recovery yield was calculated based on the TOC analysis results. At first, carrier gas was supplied to the TOC analyzer. Then, the machine and computer were turned on accordingly. After that, the TOC program was open for the startup procedure, which required about 2 hours for system stabilization. After the system was stabilized, samples were put in the analysis rack, while the sample information was entered to the program and finally the TOC analysis was started immediately.

Theoretically, the amount of carbon in feedstock is equal to carbon in product. The carbon yield was calculated based on the ratio between carbons in product to carbon in feedstock, as represented in Eq. 4.4.

$$\text{Carbon yield [-]} = \frac{\text{Total carbon in product [mg/L]}}{\text{Total carbon in feedstock [mg/L]}} \quad (4.4)$$

4.4.6 1H-Nuclear Magnetic Resonance (NMR)

The 1H-NMR analysis was conducted using 500 MHz NMR equipment (Varian, 400MR). Approximately 15 mg of sample was dissolved in 0.6 cm^3 of deuterium oxide (D_2O) containing 0.1 % acetonitrile prior to the analysis. Maleic acid was introduced as internal standard.

4.5 Materials

Alginic acid that is originally produced from kelp was purchased from Sigma-Aldrich as a mixed polymer of mannuronic acid (MA) and guluronic acid (GA). The other raw materials are mannose, sorbitol, and glucuronic acid were also purchased from the same supplier, Sigma Aldrich. The chemicals used for the alginic acid hydrolysis namely HCl and NaOH, were both supplied by Nacalai Tesque.

As for the product analysis, various analytical grade chemicals were employed. The acetonitrile and $HClO_4$ were used as the HPLC eluent and were purchased from Sigma Aldrich. Several standards were also used for the HPLC analysis including formic acid, glyceraldehyde, 5-HMF, furfural, and glycolaldehyde were all obtained from Nacalai Tesque. As for NMR analysis, the analysis chemicals include deuterium oxide (D_2O) and maleic acid were supplied by Sigma Aldrich. The methanol used for Karl-Fischer analysis was purchased from Nacalai Tesque.

CHAPTER 5

Recovery of uronic acids through simplified acid hydrolysis

5.1 Introduction

The objective of this chapter was to recover guluronic acid (GA) and mannuronic acid (MA) from alginic acid that originated from kelp. The simplified methodology was proposed where individual uronic acid was recovered as solid product. Several methodologies have been developed for the alginic acid hydrolysis but most of them focused on the hydrolysis itself instead of uronic acids recovery. Thus, information on individual product recovery is rather limited and has not been widely reported.

Due to that, the utilization of uronic acids as renewable resources has yet to be explored. Since uronic acids have great potential to be used as renewable resources, development of simplified recovery methodology is apparently necessary. The proposed methodology should be simple and quick so that it is useful for those involved in the utilization of marine macroalgae specifically kelp.

5.2 Experimental procedures

Two-stage hydrolysis was employed to produce mixtures of uronic acids (GA and MA) that were further separated using a pH-dependent method. Firstly, 1 % (w/v) of alginic acid slurry was hydrolyzed with 3 M hydrochloric acid (HCl) for 30 min. Afterwards, the precipitate was recovered and further hydrolyzed in 0.3 M HCl for another 2 h. The hydrolysis was conducted in a hot water bath at about 90 °C for specified time.

The remaining solids after hydrolysis was then dissolved in 1 M sodium hydroxide (NaOH) and adjusted to pH 2.85 for recovery of insoluble fraction (GA) and filtrate containing soluble MA. The solid MA was recovered by addition of ethanol into the filtrate. Finally, both GA and MA were thoroughly washed with deionized water and ethanol, respectively. The recovered GA and MA were then subjected to several analyses:

a) Moisture content

b) Karl-Fischer

c) Thermogravimetric analysis (TGA)

d) High performance liquid chromatography (HPLC)

e) 1H-Nuclear magnetic resonance (NMR).

5.3 Results and discussion

The uronic acids obtained from the proposed methodology were characterized by several analyses. The characteristic information was needed to ensure that the method employed was successfully obtained the desired uronic acid.

5.3.1 Moisture content analysis

The wet uronic acids obtained after washing procedure were subjected to moisture content analysis to determine the recovery yield. The measurement was repeated twice and the results are shown in **Table 5.1**.

Table 5.1 Moisture content of wet uronic acids

Moisture content [%]					
GA			MA		
Ex150608	Ex150626	Average	Ex150608	Ex150626	Average
80.90	81.75	81.30	78.94	79.11	79.03

The product yield was calculated based on the ratio between weight of uronic acids (excluding the amount of moisture) and the dry weight of feedstock (alginic acid), The yields for GA and MA were about 26 wt% and 22 wt%, respectively, which the total recovery yield was around 48 wt%. Different yields between the uronic acids probably due to the their original composition in kelp.

The results in Table 5.1 showed significant amounts of water content in both uronic acids. Thus, further drying is required to obtain a dried product. However, proper precaution is essential to avoid undesired degradation of the uronic acids. Owing to that, the wet uronic acids were dried in desiccator that filled with molecular

sieve, for certain period of time until consistent dry weight was obtained. The moisture content of the dried uronic acid was later determined by using Karl-Fischer titration device.

5.3.2 Karl-Fischer titration

Basically, Karl-Fischer device operated based on conventional titration method. The device employed methanol as the reaction medium (solvent) because of its rapid reaction and ability to dissolve many samples. Principally, during the titration, water was reacted with iodine and the end point of titration was reached after water was completely consumed. The trace amounts of water in the solid uronic acids after drying in desiccator were summarized in **Table 5.2**.

Table 5.2 Moisture content of dried uronic acids

Moisture content [%]					
GA			MA		
Ex150706	Ex150827	Average	Ex150706	Ex150827	Average
0.47	0.97	0.72	0.82	0.81	0.82

Apparently, the moisture content decreased significantly after the drying process, where less than 1 % of water remained in the dried uronic acids. The results indicated the effectiveness of the drying method employed in this study, which was conducted at room temperature instead of higher temperature. Thus, the product degradation could also be minimized or prevented.

The dried GA and MA obtained at the end of drying process are shown in **Fig. 5.1** and **5.2**, respectively. Apparently, the uronic acids were somewhat differed on the outlook, where GA was slightly yellowish while MA was more whitish.

Fig. 5.1 Dried GA

Fig. 5.2 Dried MA

5.3.3 Thermogravimetric analysis (TGA)

The dried uronic acids were subjected to TGA, which mainly used for determination of fixed carbon (FC), volatile matter (VM), and ash content. Since the uronic acids were obtained from alginic acid, similar analysis was also conducted on alginic acid for comparison. The summary of TGA results is shown in **Table 5.3**. The results revealed that the uronic acids obtained in this study contain low amount of ash. In fact, the results for GA and MA were somewhat comparable with alginic acid.

Table 5.3 Summary of TGA results

Sample	Analysis code	FC [mg/mg-dry]	VM [mg/mg-dry]	Ash [mg/mg-dry]	Total
MA	tg160314a-N_2 tg160315a-air	0.2721	0.6244	0.1035	1.0
GA	tg160314b-N_2 tg160315b-air	0.2220	0.7147	0.0633	1.0
Alginic acid	tg160128c-N_2 tg160202a-air	0.2216	0.7378	0.0407	1.0

The TGA profiles for alginic acid, MA and GA are shown in **Fig. 5.3(a)** and **(b)** to **Fig. 5.5(a)** and **(b)**, respectively, under different environments.

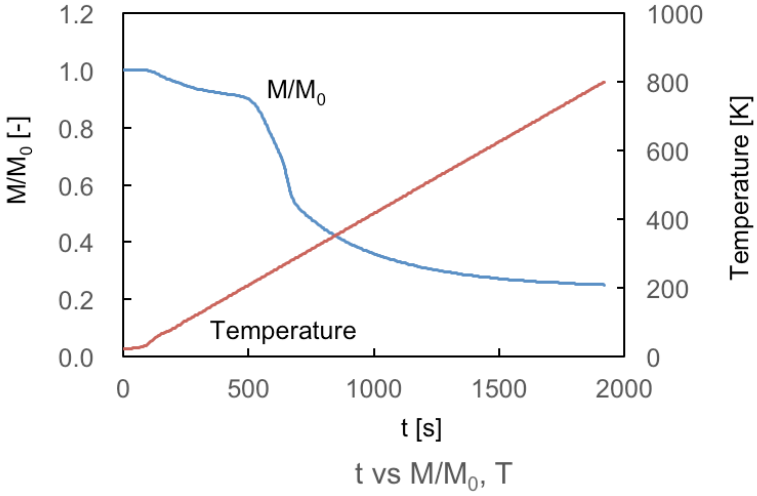


Fig. 5.3(a) Alginic acid – N_2

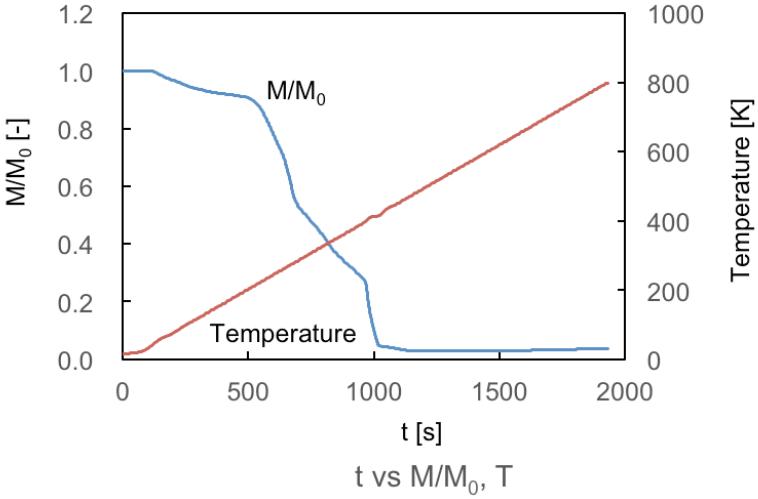


Fig. 5.3(b) Alginic acid – air

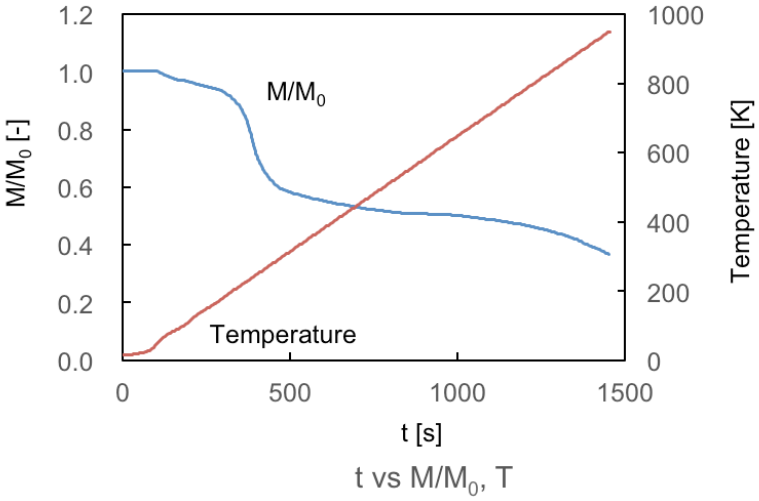


Fig. 5.4(a) Mannuronic acid – N_2

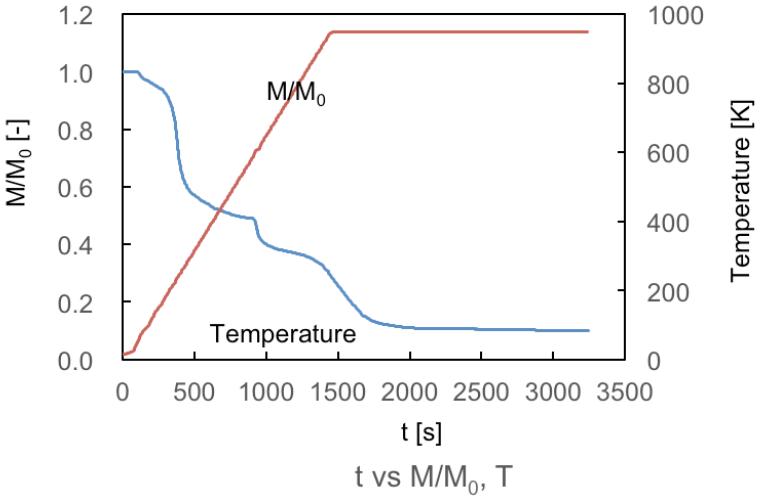


Fig. 5.4(b) Mannuronic acid – air

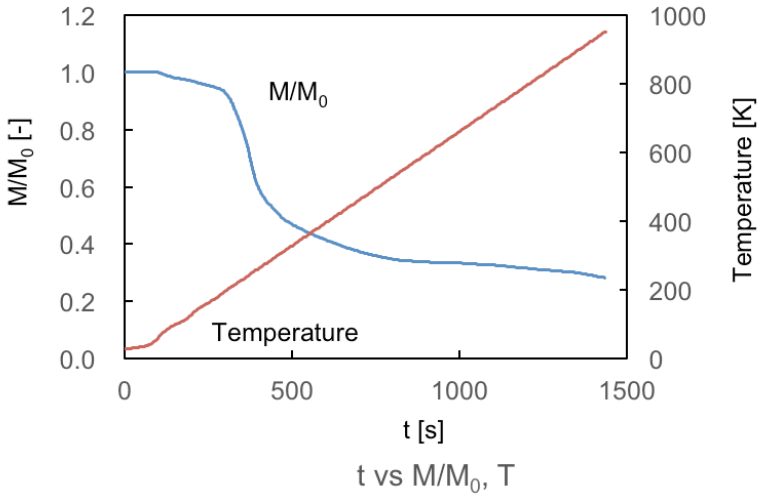


Fig. 5.5(a) Guluronic acid – N_2

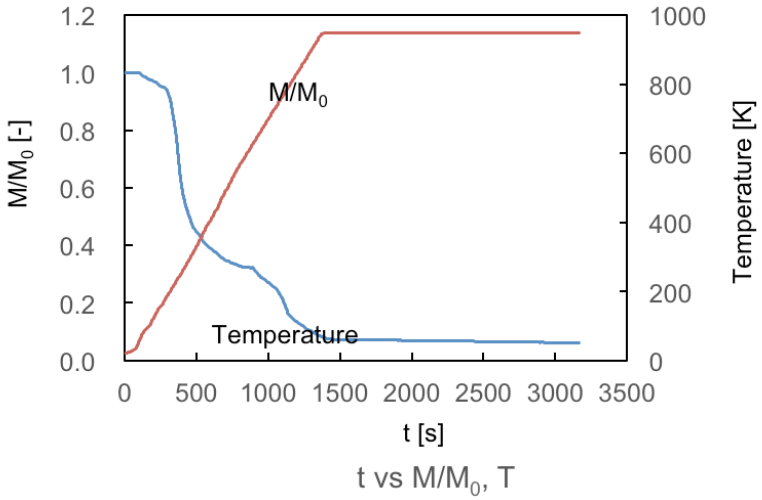


Fig. 5.5(b) Guluronic acid - air

The first stage associates with a small weight loss due to dehydration, which accounts for the moisture content. The second stage represents the main volatilization reaction, where most of the sample weight was lost as volatile matter while the remaining mass corresponds to the char content of the samples. When air was introduced to the environment, oxidation process occurred and thus the remaining weight after oxidation was mainly ashes.

5.3.4 High performance liquid chromatography (HPLC)

The HPLC analysis was performed in order to determine the retention time of the uronic acids. Based on the HPLC charts, the respective peaks of GA and MA were observed at approximately 10.8 and 12.1 min. Apparently, the retention times of uronic acids are dissimilar with any other organic compounds, whose retention times are shown in **Table 5.4**.

Table 5.4 List of organic acids

Acid	Retention time [min]
Formic acid	13.1
Acetic acid	15.3
Lactic acid	11.3, 13.2
Glycolic acid	11.5
Succinic acid	13.3
Malic acid	12.2
Dihydroxyacetone	18.6, 26.3
Butyric acid	17.6

These results indicated the uronic acids would not be interfered by decomposed products that might be generated during the alginic acid hydrolysis. Since only single peak was observed for each uronic acid, its purity in the range of HPLC was confirmed. Furthermore, it is also confirmed that the obtained uronic acids are monomers because if oligomers are included, the peak should not be single.

Theoretically, the separation of these uronic acids using HPLC depends on the type of column. In this analysis, a sugar column, Shodex KS-802, was employed, whose separation mode is a combination of size separation and ligand exchange. This separation uses the interaction between the positive charges of metal cations and the negative charges of hydroxyl groups (OH^-) on saccharides. The configuration of OH^- will influence the strength of interactions with the metal cations. Since GA and MA have dissimilar configurations, they have different retention times. Other researchers have also detected the shorter retention time for GA than for MA using mixture solutions containing these acids; the retention time was between 9 and 11 min (Sánchez-Machado et al., 2004).

5.3.5 1H-Nuclear magnetic resonance (NMR)

In addition to the HPLC analysis, 1H-NMR analysis was also conducted to detect trace contaminant if any, and to be sure that the products are really uronic acids. **Fig. 5.6** and **5.7** show the 1H-NMR spectra of MA and GA, respectively. Each spectrum shows five significant peaks, whose assignments are comparable with those from a previous study in which only an alginate sample was analyzed instead of individual uronic acids (Tako et al., 2000). Furthermore, the peaks agreed with those from another study that employed a very long hydrolysis time: approximately four times longer than ours (Steginsky et al., 1992).

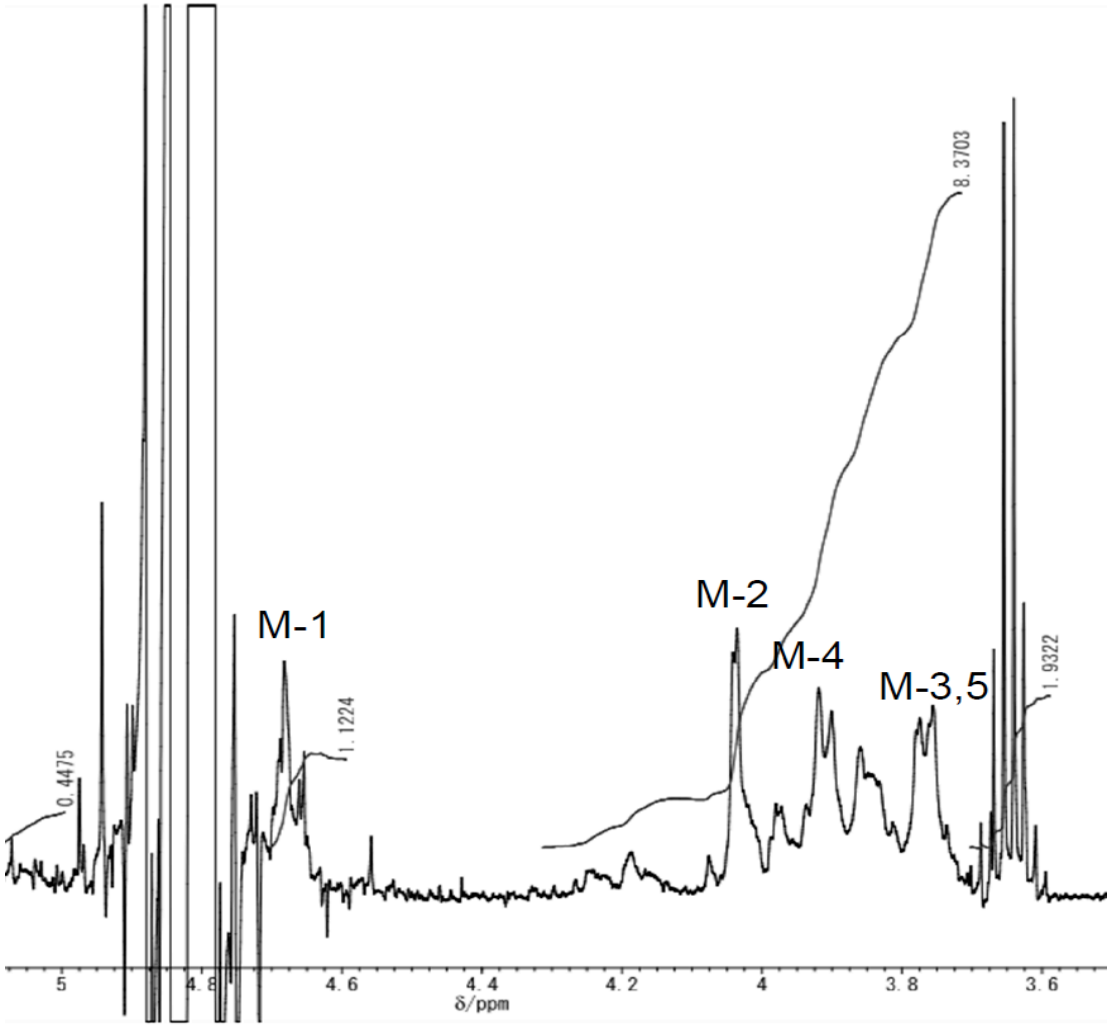


Fig. 5.6 1H-NMR spectrum of MA

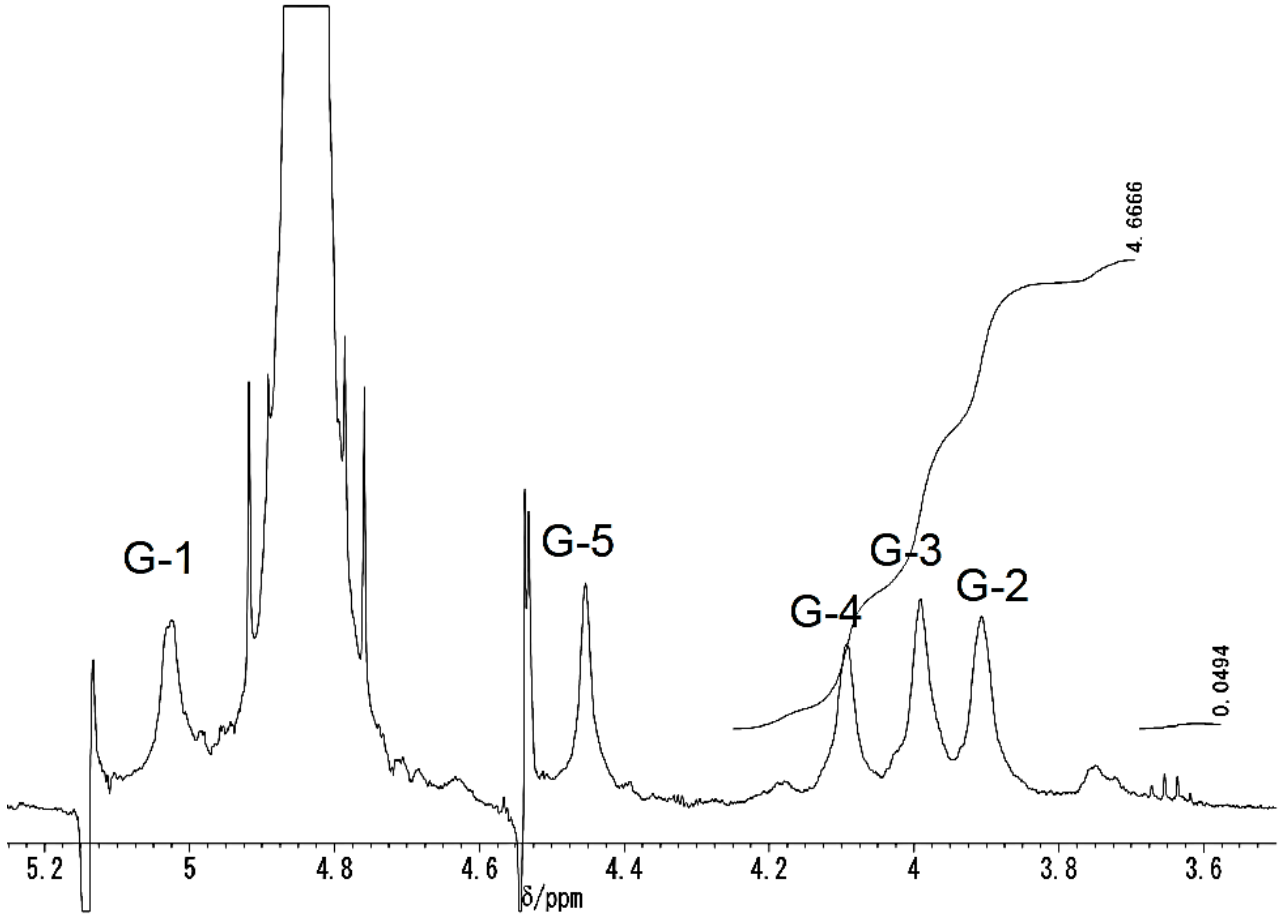


Fig. 5.7 1H-NMR spectrum of GA

Notably, the strong signal for residual D_2O protons and remaining water in the sample appeared at approximately 4.8 ppm in both spectra shown in Fig. 5.6 and 5.7. The residue of ethanol, which was used in uronic acid recovery, was also observed at approximately 3.65 ppm in the 1H-NMR spectrum of MA. However, it was by trace amount so that it could not be detected by HPLC. The chemical shifts of each uronic acid were expressed in parts per million (ppm), as shown in **Table 5.5**.

Table 5.5 Chemical shifts of 1H-NMR spectrum [ppm]

	H-1	**H-2**	**H-3**	**H-4**	**H-5**
MA	4.68	4.04	3.78	3.93	3.75
GA	5.03	3.90	4.00	4.10	4.45

5.4 Evaluation on the proposed methodology

Compared to various previous methodologies to hydrolyze alginic acid shown in Table 2.2, the methodology implemented in this study is much simpler. Basically, the hydrolysis was carried out at approximately 90 °C in the air instead of 100 °C under nitrogen. Only a hot water bath and magnetic stirrer were employed for the hydrolysis. Thus, this method required a lower temperature and eliminated the need for a nitrogen supply. Furthermore, the amount of ethanol used in the uronic acid recovery was significantly lower than that used in a previous study; for every unit volume of filtrate, the previous method required five units of ethanol, while the method in this study required equal volumes of ethanol and filtrate.

Even though a lower quantity of ethanol was used in this work, HPLC and NMR analyses showed that the recovery of uronic acids displayed good reproducibility and purity. Moreover, recovery of the final product only required vacuum filtration before preservation in the refrigerator for storage purposes. These findings provide essential support for the procedure developed here as a reliable method of uronic acid isolation.

5.5 Conclusion

The simplified methodology was proposed for the recovery of GA and MA from alginic acid. The methodology employed acid hydrolysis, precipitation by pH adjustment and ethanol addition, and filtration. The moisture content of the recovered uronic acids was about 79–81 %. The yield obtained for MA was higher than that for GA with a maximum total product recovery of approximately 48 %. The ash content was comparable between the uronic acids and alginic acid. Furthermore, the purity of samples was confirmed by 1H-NMR analysis. The retention time of GA was 10.8 min while that of MA was 12.1 min. Recovery of both acids as solid products was successfully conducted without byproduct formation. This methodology uses simple technique only and allows pure MA and GA monomer easily and quickly.

CHAPTER 6

Degradation characteristics of carbohydrates in kelp under hydrothermal condition

6.1 Introduction

This research was conducted with the aim to study the behavior of kelp compounds under hydrothermal condition. Recently, kelp has attained significant attention among worldwide researchers owing to its substantial amounts of carbohydrate that has wide range of application. Indeed, numbers of study were conducted to evaluate the potential of kelp for biofuel production (Hinks et al., 2013)(Horn et al., 2000)(Lee et al., 2013). A group of researchers was also successfully evaluated the thermochemical characteristic of kelp as a biofuel (Ross et al., 2008). Besides biofuel, its nutritional values were reported in previous study in which the composition of amino acids, fatty acids and dietary fiber of kelp were determined (Dawczynski et al., 2007).

Prior to effective utilization of kelp, proper pretreatment is essentially required. Notably, kelp composes of mainly alginic acid, which is not easily hydrolyzed or digested by microorganism. Owing to that, numbers of technology have been developed for the pretreatment of kelp. However, among various technologies, hydrothermal treatment has been identified as promising method for kelp pretreatment. This process is one of the most favorable methods for wet biomass because the process itself employs water as the reaction medium. Basically, during the hydrothermal pretreatment at high temperature and pressure, kelp structure is disrupted, which consequently enhance the hydrolysis of its compounds. However, further degradation of the desired products should be avoided by determining a suitable condition of kelp pretreatment. Thus, the temperature limitation of kelp compounds under hydrothermal condition is desirable.

The potential of hydrothermal process for production of reducing sugar from kelp was reported in previous study (Park et al., 2012). The researchers highlighted the effect of experimental conditions (temperature and time) on the decomposition behavior of sugar compounds in kelp. Furthermore, production of organic acids from kelp compound (alginic acid) under hydrothermal condition was also reported in another study (Aida et al., 2012). Similarly, the finding revealed significant effects of reaction temperature and time on the decomposition behavior alginic acid.

Remarkably, the behavior of alginic acid has been well documented in previous studies. For example, the decomposition behavior of alginic acid was elucidated by a group of researchers whose results revealed the effect of intramolecular catalysis of carboxyl group (Smidsrod et al., 1966). Moreover, the degradation characteristic of alginic acid was also significantly influenced by the presence of phenolic compounds (Moen et al., 1997)(Smidsrod et al., 1963).

Additionally, numbers of researches were conducted to elucidate the effect of hydrolysis condition on the structure of alginic acid (Zhang et al., 2006)(Lu et al., 2015). Nevertheless, the information on behavior of its individual uronic acids, mannuronic acid (MA) and guluronic acid (GA) is somewhat limited. Certainly, this information is needed prior to determining the appropriate pretreatment conditions of kelp.

For that purpose, this research was initiated to elucidate the kinetic behavior of GA and MA under hydrothermal conditions, where continuous flow reactor was employed. The decomposition characteristics of the uronic acids were evaluated at various reaction temperatures and residence times under subcritical water condition. The liquid product obtained after the hydrothermal treatment was quantitatively analyzed with high performance liquid chromatography (HPLC). The effect of temperature on the concentration of the uronic acids was evaluated based on the residual ratio between the remaining concentration in liquid product and the initial concentration.

The temperature dependence of reaction rate constant was evaluated based on Arrhenius equation where reaction parameters, which are activation energy, E_a and pre-exponential factor, A were calculated. In short, the objective of this research is to provide the guidelines for recovery of uronic acids during hydrothermal pretreatment of kelp. Consequently, kelp utilization can be further explored as sustainable and renewable resources.

6.2 Experimental procedures

Prior to the hydrothermal process, feedstock was prepared in advance following the procedures described in previous chapter (Chapter 5). Afterwards, kinetics study was conducted where the feedstock was subjected to hydrothermal process under subcritical water condition.

6.2.1 Feedstock preparation

Briefly, uronic acids were prepared through two-step hydrolysis of alginic acid. At first, 1 wt% of alginic acid was hydrolyzed with addition of 3 mol/dm^3 hydrochloric acid (HCl) whose volume was approximately 3% of total volume, at about 90 °C for 30 min. Then, the solid residues were recovered and further hydrolyzed in 0.3 mol/dm^3 HCl for another 2 h. Afterwards, the remaining precipitate was collected and dissolved in 1 mol/dm^3 sodium hydroxide (NaOH) in which a mixture of GA and MA was obtained.

Owing to different pH solubility, both uronic acids were separated by pH adjustment at pH 2.85 where GA was recovered as insoluble product while MA remained soluble in the solution. The solid MA was recovered by addition of ethanol into the solution. The wet uronic acids were further washed in order to remove the undesired residues or contaminants, which might remain on the surface of the uronic acids. Upon completion, the washed uronic acids were dried in desiccator at room temperature until consistent dry weight was obtained. The dried samples were then preserved in refrigerator prior to hydrothermal process.

6.2.2 Hydrothermal treatment of uronic acids

The hydrothermal process was conducted using continuous flow reactor, whose details of experimental apparatus were described in previous study (Yong and Matsumura, 2012). Briefly, feedstock and pre-heated hot water were pumped from separate lines and mixed just before the entrance of reactor. After certain residence times, hot liquid effluent from the reactor was mixed with cool water and further cooled in heat exchanger prior to depressurize by back pressure regulator. The liquid product was then collected at specified sampling point.

6.2.3 Experimental conditions

The hydrothermal treatment of the uronic acids was conducted following the experimental conditions summarized in **Table 6.1**.

Table 6.1 Experimental conditions

Parameters	**Experimental conditions**
Feedstock	Mannuronic acid (MA)
	Guluronic acid (GA)
Types of reactor	Continuous flow (stainless steel)
Reactor diameter	1 mm (ID)
Reactor length	0.2 to 6 m
Feedstock concentration	1 wt%
Feedstock to water ratio	1 : 4
Reaction temperature	170 to 250 °C
Residence time	5 to 100 s
Operating pressure	25 MPa

6.2.4 Product analysis

The liquid product obtained after the hydrothermal treatment of uronic acids was analyzed by HPLC (Shimadzu Corp.). A column Shodex KS-802 was employed for quantification of reactant concentration in the liquid effluent. The analysis was conducted at 60 °C of oven temperature, using deionized water as eluent at a flow rate of 0.5 cm^3/min. The effect of temperature on decomposition behavior of uronic acid was evaluated based on residual ratio between the unreacted reactant concentration and initial reactant concentration.

6.3 Results and discussion

The kinetics behavior of uronic acids under hydrothermal condition was evaluated at various temperatures, and the reaction rate constant at each temperature was calculated accordingly. The temperature dependence of reaction rate constant was then evaluated following the Arrhenius equation.

6.3.1 Effect of temperature on decomposition behavior of uronic acids

The remaining concentration of reactant in liquid product was quantified by using HPLC. At each reaction temperature, the yield percentage was calculated as presented in **Fig. 6.1**.

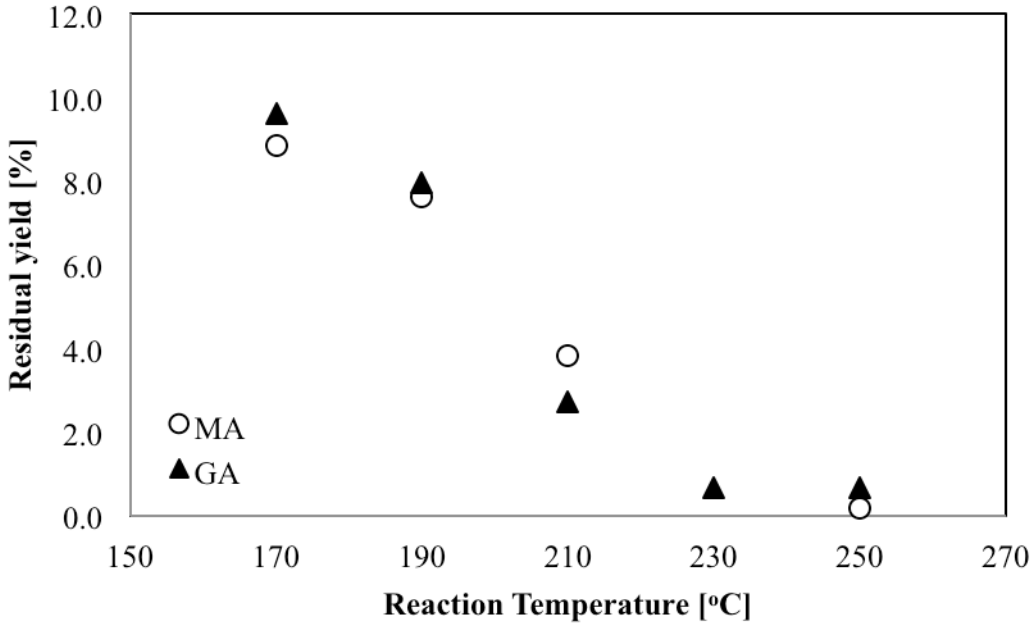


Fig. 6.1 Effect of temperature on residual yield of uronic acids at 15 s of residence time

The data plotted in Fig. 6.1 showed comparable yields between MA and GA for each reaction temperature. This result indicated that both uronic acids have similar reactivity. Furthermore, since the reaction occurred in a continuous flow system whose concentration changes with time, exponential decrease of the concentration with residence time was expected. Thus, the decomposition reaction was assumed following a first-order reaction.

The kinetics behavior of these uronic acids was compared with others uronic acids whose results were reported in previous study (Usuki et al., 2008). The researchers highlighted that the uronic acids (galacturonic acid and glucuronic acid) employed in their study were not followed first-order reaction. This is therefore demonstrated that different uronic acids have dissimilar reactivity. Perhaps, further study is needed for various uronic acids but it is beyond the scope of this study. The ultimate objective of this work is to determine the kinetics behavior of GA and MA in

which decomposition reaction is prevented. Therefore, first order reaction should be appropriate for elucidating the degradation characteristics of the uronic acids.

Apparently, the concentration of both uronic acids showed a decreasing trend towards the enhanced temperatures. Thus, these results indicated that faster reaction occurs at higher temperatures and consequently results to lower concentration of remaining feedstock. Previously, a study was conducted where decomposition of sodium alginate under hydrothermal condition was elucidated (Matsushima and Minoshima, 2005). The results demonstrated the effect of reaction temperature on the selective hydrolysis of glycosidic bonds. However, they conducted the experiment at a very short residence time (88 ms) than that employed in this present work (15 s). Perhaps, at longer residence times, most of the uronic acids are decomposed. In fact, the temperature limitation of the uronic acids is needed for determining the pretreatment condition of kelp.

A first-order reaction was employed for the calculation of reaction rate constant of GA and MA as shown in **Table 6.2**. Besides, the results were compared with glucose whose decomposition characteristics under hydrothermal condition were well reported in previous studies. Among them, a detailed study was conducted on decomposition of glucose, whose results demonstrated the significant effect of experimental condition on reaction mechanism (Matsumura et al., 2006).

Similarly, the researchers also observed faster degradation rate of glucose at higher temperature range. Nevertheless, the decomposition rate of glucose was much slower than GA and MA at the same temperature range (170–250 °C). Obviously, GA and MA are more susceptible to degradation than glucose of six carbons. In fact, the susceptibility of other uronic acids than pentose (five carbons) was also reported in previous study (Usuki et al., 2008).

Table 6.2 Comparison of reaction rate constant

T [^{o}C]	Rate constant, k [s^{-1}]		
	MA	GA	Glucose (Matsumura et al., 2006)
170	1.62E-01	1.56E-01	
175			8.52E-05
190	1.72E-01	1.69E-01	
200			5.36E-04
210	2.18E-01	2.41E-01	
225			2.21E-03
230		3.36E-01	
250	4.29E-01	3.34E-01	4.98E-03
300			2.34E-01
350			5.04E-01
400			4.92E+00

6.3.2 Determination of reaction rate parameters of uronic acids

The Arrhenius equation was employed in order to evaluate the dependency between temperature and reaction rate constants, as shown in Eq. 6.1.

$$k = A\exp\left(\frac{-E_a}{RT}\right) \qquad (6.1)$$

where k, A, E_a, R, and T represent the reaction rate constant, pre-exponential factor, activation energy, gas constant, and absolute temperature, respectively.

Figure 6.2 shows the Arrhenius plots of the uronic acids, in which similar trends were observed for GA and MA. This is expected since both of them are only differed on their hydroxyl configuration. Besides, the Arhenius plots of glucuronic acid and galacturonic acid were also shown in Fig. 6.2, whose decomposition rates were calculated based on previous data (Usuki et al., 2008). Remarkably, GA and MA decomposed much faster than the glucuronic acid and galacturonic acid while glucose degraded slower than the uronic acids.

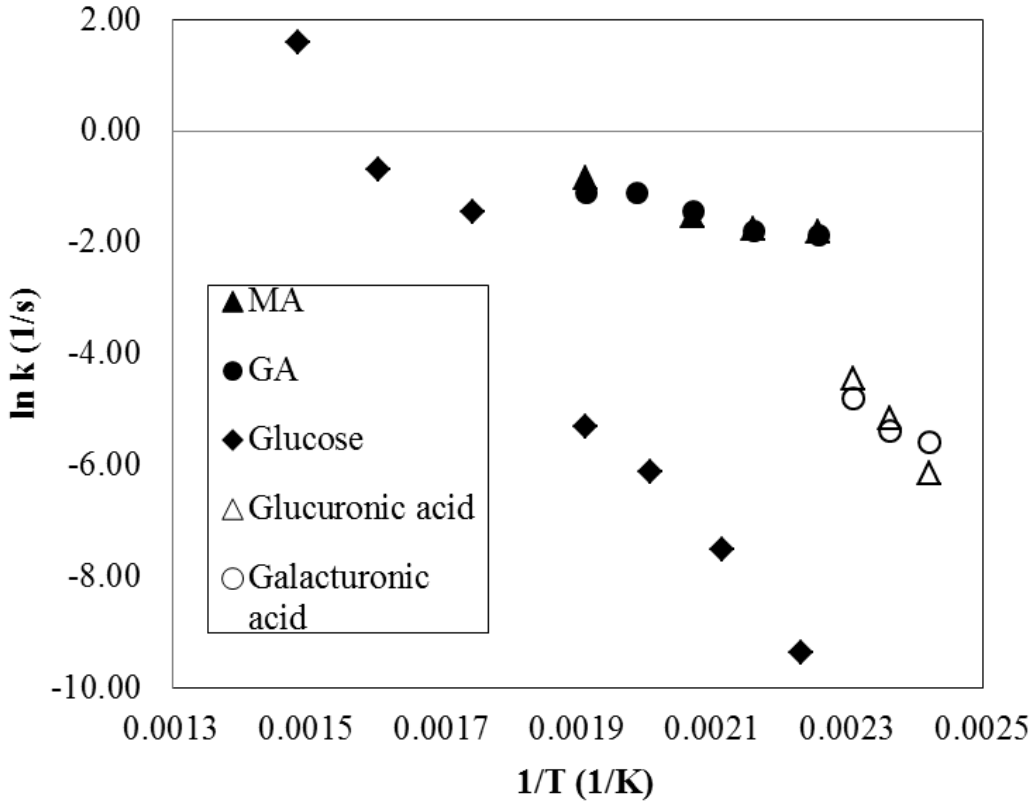


Fig. 6.2 Comparison of Arrhenius plot

Apparently, uronic acids showed higher reactivity than that of glucose. Probably, the decomposition reaction of uronic acids is favored by the presence of carboxylic group, which is the only different between them. The high electronegativity of the carboxylic group resulted to weaker bond between C-3 and C-4 and consequently favored the cleavage of the bonds. Nevertheless, the higher reactivity of GA and MA than galacturonic acid and glucuronic acid is remained unclear. Perhaps, experiment on these uronic acids by using the same apparatus that was used for GA and MA should be conducted in the future study.

Based on the Arrhenius plots, reaction parameters (E_a and A) of GA and MA were calculated together with their statistical value of 95 % reliability as shown in **Table 6.3**. The data in Table 6.3 showed that activation energies of the uronic acids are somewhat similar, but significantly smaller that glucose. On the other hand, pre-exponential factors of the uronic acids are also greatly different from glucose.

Table 6.3 Reaction parameters of GA, MA, and glucose

	Pre-exponential factor, A [s^{-1}]		Activation energy, E_a [kJ/mol]	
	Least square	95 % reliability	Least square	95 % reliability
MA	2.82E+02	(2.90±0.98)E+02	28.3	28.3±0.9
GA	4.06E+01	(3.90±0.44)E+01	20.6	20.8±0.5
Glucose	3.44E+12	(21.27±17.3)E+12	152.6	147.0±4.1

6.3.3 Uronic acids decomposition in batch reactor

The hydrothermal treatment of uronic acids in a continuous-flow reactor resulted to low recovery yields, indicating their susceptibility to degradation. Owing to that, a comparison study on behavior of the uronic acid in batch reactor system is seemed necessary. For that purpose, recovery yields of the uronic acids were calculated based on the actual temperature profile of batch reactor as illustrated in **Fig. 6.3**.

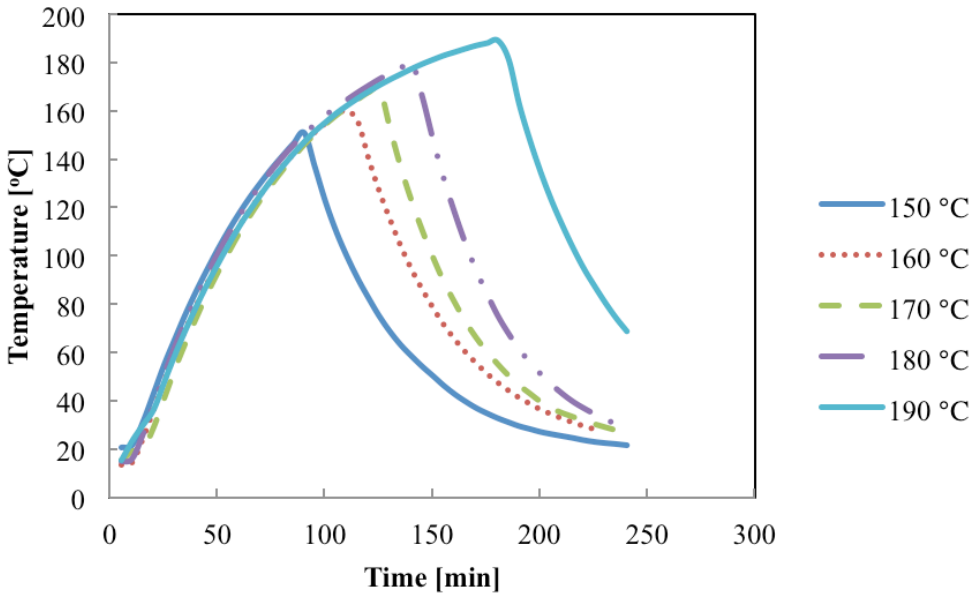


Fig. 6.3 Actual temperature profile of batch reactor

The temperature profile in Fig. 6.3 for that particular batch reactor was obtained from actual experimental procedure of kelp pretreatment. In this case, desired temperatures employed in the calculation were slightly lower than that employed for continuous reactor, which are between 150–190 °C. The numerical integration of reaction rate equation was performed to calculate the amount of uronic acids that decomposed from the initial concentration following the temperature range.

The decomposition yields of the uronic acids obtained from the integration calculation are shown in **Table 6.4**. The calculated data showed the yield obtained from batch reactor is considerably lower than that obtained from continuous reactor system. This is therefore indicated that continuous-flow reactor is more superior to batch reactor for the case of uronic acids recovery from kelp. Perhaps, low recovery yields in batch system is due to slow heating and cooling that associated with the reactor.

Table 6.4 Recovery yields of uronic acids in batch reactor system

Temperature [^{o}C]	Yield [mol/mol]	
	MA	GA
150	1.60E-03	1.14E-05
160	2.06E-04	7.39E-07
170	4.84E-06	5.30E-09
180	3.47E-09	3.55E-13
190	7.47E-14	1.98E-19

6.4 Conclusion

The hydrothermal treatment of mannuronic acid (MA) and guluronic acid (GA) that originated from alginic acid was successfully conducted. A first-order reaction was assumed for elucidating the decomposition behavior of the uronic acids under hydrothermal condition. Based on Arrhenius equation, the reaction parameters were calculated accordingly. Remarkably, the activation energy (E_a) of MA was somewhat higher than that of GA, which are 28.3 and 20.6 kJ/mol, respectively. Similarly, the pre-exponential factor (A) of MA (282 s^{-1}) was also higher than GA (40.6 s^{-1}).

In short, recovery of uronic acids from kelp should be conducted at rather low temperature to avoid further degradation. Besides, continuous-flow system is more favorable for kelp pretreatment than that of batch reactor system.

CHAPTER 7

Decomposition behavior of mannose, mannitol, and mannuronic acid under hydrothermal condition

7.1 Introduction

Among the many renewable energy resources, biomass is easily and readily available on the earth. Remarkably, the utilization of biomass as renewable energy resources has shown an increasing trend owing to its sustainable supply and environmental friendly. In fact the sustainability of biomass resources for energy utilization was extensively discussed by a group of researchers (Sadamichi et al., 2012). The researchers highlighted about biomass sustainability from various aspects, including environmental, economic and social points of view.

Specifically, marine macroalgae is one of the most abundant resources of biomass. Indeed, many researchers have been focusing on the utilization of marine biomass as alternative resource for renewable energy production. This is because of the composition of macroalgae itself, which contain high amount of carbohydrates that have wide range of applications. Particularly, numbers of study have been conducted on the

utilization of macroalgae for biofuel production (Adams et al., 2011)(Hinks et al., 2013)(Xia et al., 2015).

Laminaria japonica or kelp is a species of brown macroalgae that abundantly available, specifically in Japan. Chemically, kelp contains high amount of carbohydrate that composes of mainly alginic acid and mannitol. Indeed, the potential of kelp has been extensively discussed by a group of researchers that reported its application in diverse areas of biorefinery (Jung et al., 2013). The researchers highlighted great potential of kelp for production of various biomaterials and biofuel.

Besides kelp, specific study on kelp compounds is also of interest. For example, the potential of mannitol as a feedstock for biodiesel production was successfully reported in previous study (Xu et al., 2014). The results obtained from that study demonstrated that mannitol is favorable feedstock for lipid production. On the other hand, the application of alginic acid is rather complicated owing to its complex structure. Thus, hydrolysis of alginic acid is needed prior to its effective utilization where the desired uronic acids are obtained, specifically mannuronic acid (MA).

Clearly, mannitol and MA are sugar compounds that can be obtained from kelp. Besides, both of them can also be derived from the same aldohexose, namely mannose. Theoretically, reduction of mannose will produce mannitol while oxidation of mannose will produce MA. Previously, a group of researchers was successfully employed a reducing agent (sodium borohydrate) for production of mannitol (Hricovíniová, 2011). During that process, the functional group of mannose was changed from aldehyde to hydrolxyl group.

In another study, the synthesis of MA from mannose was investigated, in which the aldehyde of mannose was oxidized to carboxylic group (Niemann and Link, 1933). Owing to the dissimilarity of functional groups between the parent sugar and its corresponding sugar alcohol and uronic acid, comparison of kinetics behavior among them is an interesting point of discussion. Thus, specific study on each of the sugar compounds is essentially important, particularly during pretreatment process. For that purpose, similar experimental conditions of pretreatment process are needed for comparable results.

Remarkably, pretreatment of kelp is necessary for the recovery of its carbohydrates (mannitol and alginic acid). The objective of the pretreatment process is mainly to convert the carbohydrates in macroalgae into valuable products. Nowadays, numbers of technologies have been developed for pretreatment of macroalgae, whose water content is significantly high. One of the most promising technologies for macroalgae is hydrothermal treatment process. In this process, water is used as the reaction medium and thus drying process of feedstock is certainly unnecessary. Besides, this process is also environmental friendly because it does not employ any other chemical as its reaction medium. In fact, the recovery of kelp compound is facilitated by the excellent properties of water at high temperature and pressure, which consequently disrupts the kelp structure.

The effect of hydrothermal process on kelp pretreatment was investigated in previous study (Matsumoto et al., 2014). The researchers employed a batch reactor system to elucidate the behavior of organic compounds in kelp under hydrothermal condition. Besides, numbers of studies were also conducted hydrothermal pretreatment of kelp for various applications. For example, a study was conducted on the production of various reducing sugars from kelp pretreatment (Park et al., 2012). The main

findings of that research revealed the effects of reaction temperature and catalyst on the product yield. In another study, a group of researchers was successfully evaluated the potential of kelp compound for organic acids production (Aida et al., 2012).

Nevertheless, a particular study on the individual kelp compound is rather limited. This information is essentially important to avoid unnecessary degradation of carbohydrates in kelp. Consequently, effective utilization of kelp as renewable resources could be achieved. Therefore, the objective of this study is to elucidate the individual behavior of MA (that derived from alginic acid) and mannitol under hydrothermal condition.

Notably, mannitol and MA that originated from kelp shared the same parent sugar, namely mannose, and they are only differed on their functional groups. Thus, a study on the effect of functional group is an interesting point to be discussed. This is because, during pretreatment of biomass, its sugar compounds are always changed to related sugar alcohol and uronic acid, which consequently affect its reactivity.

7.2 Experimental procedures

Mannuronic acid (MA) was prepared according to the experimental procedures described in subsection 5.2, while the other feedstock (mannitol and mannose) were purchased from Sigma Aldrich. The decomposition behavior of these sugar compounds was evaluated under hydrothermal condition.

7.2.1 Preparation of MA

A two-step hydrolysis was employed for preparation of MA from alginic acid. The first hydrolysis was performed where 1 wt% of alginic acid was mixed with 3 mol/dm^3 hydrochloric acid (HCl) and heated in water bath at 90 °C for 0.5 h. The remaining solid was further hydrolyzed in 0.3 mol/dm^3 HCl for another 2 h. The solid remained after second hydrolysis was then dissolved in 1 mol/dm^3 sodium hydroxide (NaOH).

Due to different acid solubility, MA was remained in liquid solution at pH 2.85. In order to obtain a solid MA, ethanol was added into the solution. Additional ethanol was added to wash out undesired impurities on MA. The drying process of wet MA was conducted at room temperature using a desiccator that filled with molecular sieves. The dried MA was kept in close container and preserved in refrigerator prior to hydrothermal process.

7.2.2 Hydrothermal treatment of sugar compounds

Details description of experimental apparatus employed in this study were clearly explained in the earlier work conducted by our research group (Yong and Matsumura, 2012). Rapid heating of feedstock was obtained when pre-heated hot water was mixed with feedstock at the entrance of reactor. The reaction started immediately for specified residence time and quickly stopped when the hot effluent mixed with cool water. Heat exchanger was employed for further cooling before the effluent being depressurized by back-pressure regulator and collected for analysis.

7.2.3 Experimental conditions

The experimental conditions employed in this study are summarized in **Table 7.1**. For this chapter, hydrothermal experiment was conducted on MA and mannose, while the data for mannitol was obtained from our previous work.

Table 7.1 Experimental conditions for Chapter 7 (Decomposition kinetics of mannose, its sugar alcohol, and its uronic acid under hydrothermal condition)

Parameters	**Experimental conditions**
Feedstock	MA, mannitol, mannose
Types of reactor	Continuous flow (stainless steel)
Reactor diameter	1 mm (ID)
Reactor length	0.2 to 0.9 m
Feedstock concentration	1 wt%
Feedstock to water ratio	1 : 4
Reaction temperature	170 to 250 °C
Residence time	3 to 20 s
Operating pressure	25 MPa

7.2.4 Quantitative analysis of liquid products

High-performance liquid chromatography (HPLC) instrument was employed for quantitative product analysis. The concentration of reactant in feedstock and liquid product was quantified using a sugar column (KS-802) and a refractive index detector (RID-10A). The analysis was conducted using deionized water (eluent) whose flow rate was set at 0.7 cm^3/min at 60°C of oven temperature.

Additional HPLC analysis was performed for quantification of decomposed products that produced during the hydrothermal process. Specifically, SCR-102 HG and DE 413L columns were employed for quantification of organic acids and ring compounds, respectively. For both columns, similar oven temperature (40 °C) and eluent flow rate (0.7 cm^3/min) were employed. However, different eluents were used, which are 5 mmol/dm^3 of perchloric acid ($HClO_4$) for SCR-102 HG and 5 mmol/dm^3 of $HClO_4$ mixed with acetonitrile (1:1) for DE 413L. In addition, different detectors namely refractive index and UV-vis were employed for SCR-102HG and DE 413L columns, respectively. The decomposition yields were calculated based on the ratio between reactant concentration in product and in feedstock.

7.3 Results and discussion

The effect of hydrothermal process on decomposition behavior of sugar compounds was evaluated under subcritical water condition. The corresponding reaction rate constant was calculated for each reaction temperature. The Arrhenius equation was employed to evaluate the dependency of reaction rate constant on reaction temperature.

7.3.1 Decomposition behavior of mannose (aldohexose)

A study on decomposition characteristics of mannose was conducted under subcritical water condition. The effect of reaction temperature on the behavior of mannose was evaluated based on the residual ratio between unreacted reactant concentration and initial reactant concentration as shown in **Fig. 7.1**.

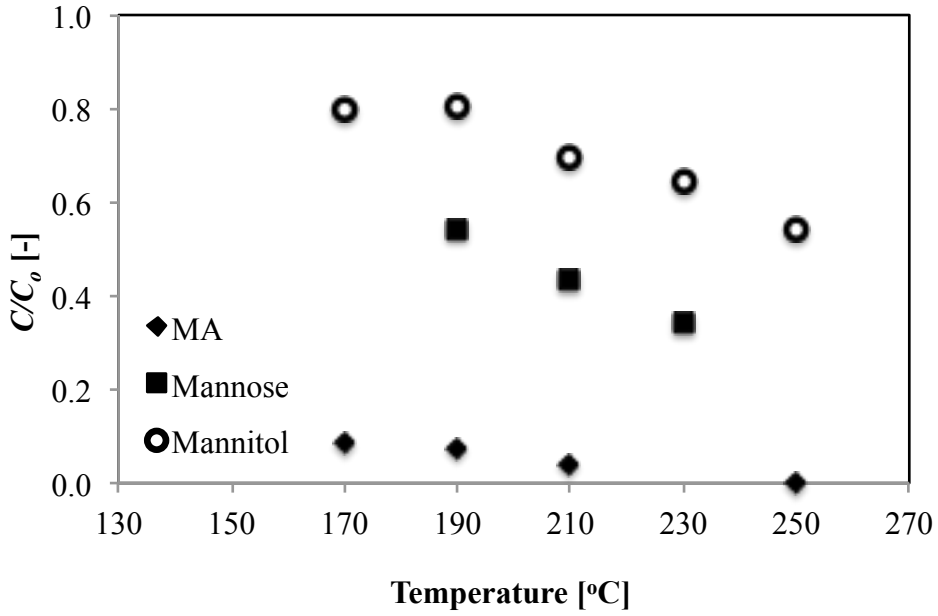


Fig. 7.1 Effect of temperature on decomposition yields of mannose (residence time = 5 s), MA (residence time = 15 s) and mannitol (residence time = 80 s). *C*: concentration of remaining reactant; C_o: initial concentration of reactant)

The decomposition yield of mannose was obtained from two sets of experiment. Clearly, the data plotted in Fig. 7.1 showed that higher temperatures resulted to lower decomposition yield. The yield of mannose was dropped from about 55 % to 34 % when the reaction temperature increased from 190 °C to 230 °C, indicating higher reactivity at enhanced temperature. Thus, reaction at high temperature range should be avoided for hydrothermal treatment of mannose in order to prevent any unnecessary reaction.

In addition, the decomposition yield of mannose was also evaluated at different residence time, as shown in **Fig. 7.2**.

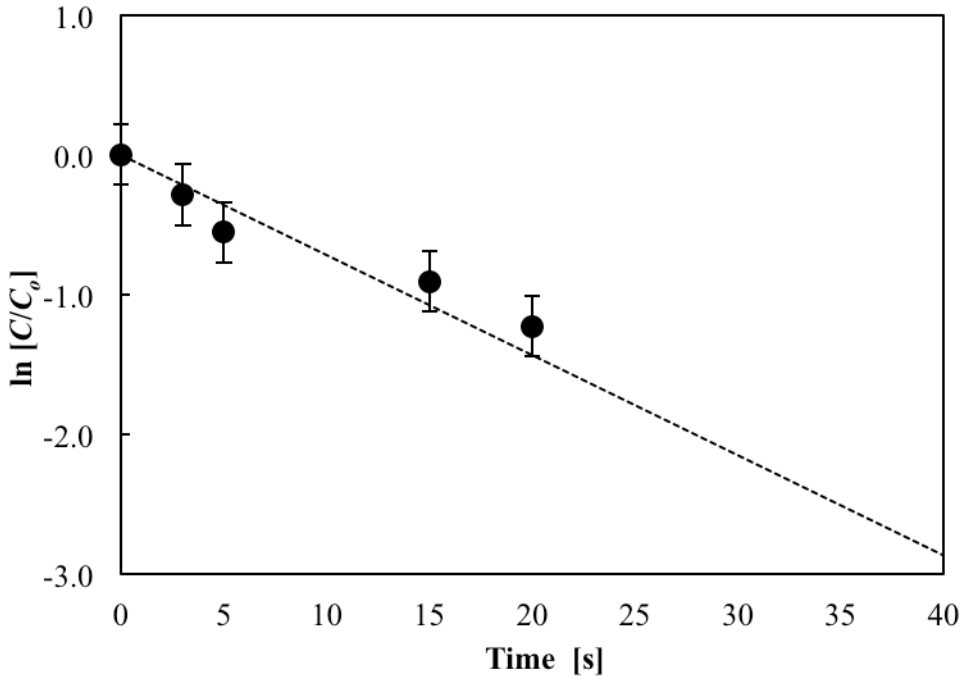


Fig. 7.2 Effect of residence time on remaining concentration of mannose under isothermal condition (190 °C)

Apparently, the concentration of mannose decreased exponentially as the residence time increased. However, this is the case for shorter residence times only. Perhaps, further investigation is needed at longer residence time but it is beyond the scope of this study. The data obtained in the present work showed that the decomposition reaction of mannose obeyed a first-order reaction.

In fact, the behavior of mannose was evaluated by numbers of researchers. However, the experimental conditions employed in previous studies were somewhat different from the ones used in this study. For instance, kinetics behavior of mannose under subcritical condition was successfully evaluated, in which aqueous ethanol was used as reaction medium (Gao et al., 2015). The results showed that mannose produced high amount of fructose, whose concentration increased at the beginning but started to decrease at prolonged residence time. Surprisingly, fructose was not observed in the

present study when water was used as the reaction medium. Probably, the reaction medium significantly affects the reaction mechanism of mannose under subcritical condition.

In addition, another study was also conducted by a group of researchers whose results showed the formation of fructose from mannose under subcritical water condition (Usuki et al., 2007). Similarly, the concentration of fructose was initially increased but it was decreased afterwards possibly due to further degradation. Nevertheless, fructose was not observed in the liquid product of the present study. Probably, fructose produced from mannose was undergone dehydration process, which consequently produced 5-hydroxymethylfurfural (HMF). However, the reason of different findings even though similar reaction medium was employed is remained unclear. Perhaps, different experimental set-up resulted to dissimilar finding between them.

7.3.2 Analysis of decomposed products of mannose

Indeed, mannose reacted faster at high temperature ranges and consequently produced various compounds. Clearly, the results obtained in this study showed an increasing trend of 5-HMF formation as the temperature increased. The amounts of 5-HMF generated at 190, 210, and 230°C were about 2.52, 6.28, and 38.8 mmol/m^3, respectively. Chemically, when dehydration of fructose occurs, 5-HMF will be generated. This fact was reported in previous study whose results revealed the formation of 5-HMF as the major product obtained from hexoses decomposition reaction (Khajavi et al., 2005).

Perhaps, mannose used in this experiment was first isomerized to fructose, and consequently dehydrated to 5-HMF. Thus, the absence of fructose in liquid product could possibly due to higher reactivity of fructose changed to 5-HMF than its formation through isomerization of mannose.

Another major compound observed in the liquid product of mannose decomposition is glycolaldehyde. Likewise, increasing trend was also observed for glycolaldehyde, whose concentration at 190, 210, and 230°C was 0, 96, and 931 $mmol/m^3$, respectively. Furthermore, its concentration was also significantly higher than the 5-HMF. Glycolaldehyde could be produced through a reaction, namely retro-aldol condensation. Theoretically, the reaction proceeds when the bond between alpha (α) and beta (β) carbons of mannose was cleaved and consequently produced other compounds with smaller molecular weight including glycolaldehyde.

It was reported in previous study that glucose was first decomposed to glycolaldehyde and erythrose and later decomposed to other smaller compounds (Kabyemela et al., 1999). Since mannose is also a group of aldohexoses like glucose, perhaps erythrose should be observed in the liquid product of mannose decomposition. However, in the present study, erythrose in liquid product was remained unidentified due to limitation of analysis. In future, further investigation is needed for quantification of other compounds in liquid products, including erythrose.

The comparison between glycolaldehyde and 5-HMF obtained in this study is clearly shown in **Fig. 7.3**.

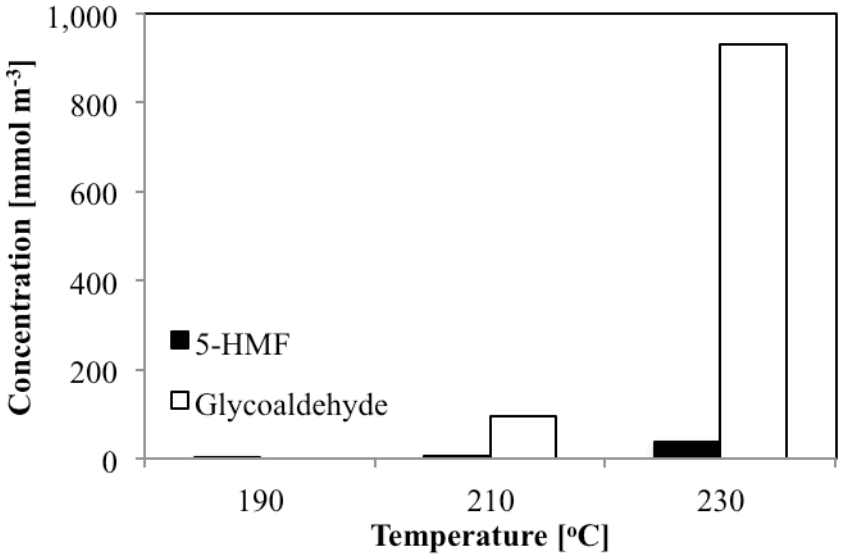


Fig. 7.3 Decomposition products of mannose under hydrothermal condition

7.3.3 Decomposition behavior of mannuronic acid (uronic acid)

Uronic acid is another group of sugars that of interest in this study. Notably, mannuronic acid (MA) is the corresponding uronic acid of mannose. They are of sugar family that differed on the functional groups. Chemically, oxidation of mannose leads to the formation of MA, where the hydroxyl group of terminal carbon of mannose changed to carboxylic group. Owing to this dissimilarity, the effect of functional group on their behavior under hydrothermal condition is an interesting subject of discussion. Unlike mannose, the characteristic of MA under hydrothermal condition is not well studied. Thus, this research was initiated to provide the desired information on decomposition behavior of MA under subcritical water condition. The behavior of MA at different reaction temperatures was evaluated based on the concentration of unreacted MA found in liquid product, as shown in Fig. 7.1.

The data plotted in Fig. 7.1 shows the reactivity of MA at temperature ranged from 170 to 250 °C, which apparently the reaction rate increased at higher temperatures. This is the fact of reaction rates that are generally less favorable at lower range of temperatures. The results obtained in this study revealed the susceptibility of MA in comparison to its parent sugar, mannose. Obviously, at the same reaction condition, MA decomposed much faster than mannose. Perhaps, functional group could be the main reason for such a different reactivity.

Fundamentally, the carboxylic group in MA has high electronegativity effect. Thus, during hydrothermal process, oxygen atoms of the carboxylic group attract electron and consequently resulted to cleavage of C-C bond. The susceptibility of different kinds of uronic acid (glucuronic acid and galacturonic acid) was also investigated in previous study (Usuki et al., 2008). The researchers reported that the uronic acids of 6 carbons are more susceptible to degradation than that of pentoses of 5 carbons.

Based on the results obtained in the present work, perhaps the reaction temperature employed for hydrothermal process was too severe for MA. At the lowest reaction temperature i.e. 170 °C, almost 90 % of MA was already decomposed. For that reason, treatment condition should be carefully decided when recovery of MA is desired.

7.3.4 Decomposition behavior of mannitol (sugar alcohol)

Mannitol is another family member of mannose and MA, whose chemical structure is somewhat similar except for their functional groups. Basically, this sugar alcohol could be derived from the reduction mechanism, where aldehyde of mannose

changed to hydroxyl group. Likewise, different reactivity on the decomposition behavior of these sugar compounds was also expected. However, information on the effect of functional group on behavior of these sugars under hydrothermal condition has not been reported yet.

Owing to that, this study was initiated with the aim to provide clear overview on the different behavior between sugar alcohol (mannitol), and its corresponding parent sugar (mannose) and uronic acid (MA). For that purpose, the experimental works were conducted under the same reaction conditions. The effect of hydrothermal process on remaining concentration of mannitol was calculated and plotted in Fig. 7.1. Remarkably, the amount of unreacted mannitol was greatly higher than the others, under the same reaction temperatures. This is therefore revealed that mannitol is less susceptible to degradation at temperature ranges employed in this study.

Possibly, another reaction occurs during the hydrothermal treatment of mannitol. Instead of cleavage of C-C bond, dehydration could be potential reaction that occurred during that process. According to previous report, intramolecular dehydration reaction was favored when mannitol was subjected to hydrothermal treatment at high temperature. Subsequently, the reaction produced several dehydration products, including 2,5-Anhydromannitol and 1,4-Anhydromannitol as the major products (Yamaguchi et al., 2014). The researchers reported that at 250 °C, approximately 36 h of residence time was needed to achieve about 90 % of sorbitol conversion. This result demonstrated the high resistance of mannitol under hydrothermal condition.

In fact, mannitol is one of the main carbohydrates that can be recovered from kelp. Thus, the recovery could be conducted at rather high temperatures due to its great resistance to degradation. However, MA is also another major carbohydrate in kelp that was easily degraded at high temperature. By knowing the temperature tolerance of both

mannitol and MA, appropriate pretreatment condition of kelp could be decided to prevent undesired reaction.

7.3.5 Determination of reaction rate parameters

The decomposition reaction of mannose, MA, and mannitol was determined to be a first-order reaction. This is based on the results obtained, where concentration of unreacted reactants was decreased exponentially at shorter residence time. The reaction rate constant (k) was calculated for each reaction temperature as shown in **Table 7.2**.

Table 7.2 Summary of reaction rate constants

Temperature [^{o}C]	Rate constant, k [s^{-1}]		
	Mannose	MA	Mannitol
170		1.62×10^{-1}	2.79×10^{-3}
190	7.04×10^{-2}	1.72×10^{-1}	2.73×10^{-3}
210	1.68×10^{-1}	2.18×10^{-1}	4.60×10^{-3}
230	2.16×10^{-1}		5.58×10^{-3}
250		4.29×10^{-1}	7.78×10^{-3}

Apparently, the reaction rate constants increase in the order of mannitol, mannose and MA. Different reaction rates among them could be possibly due to the effect of functional groups, whose reactivity was also affected by the water properties during the hydrothermal process. In fact, water shows different properties at low and high temperature ranges, which consequently affects the reaction mechanism. This

finding was reported in previous study that observed the effect of temperature on decomposition behavior of sugars (Matsumura et al., 2006).

The temperature dependence of reaction rate constant was evaluated based on the Arrhenius equation, as shown in Eq. 7.1.

$$k = A\exp\left(\frac{-E_a}{RT}\right) \qquad (7.1)$$

where k, A, E_a, R, and T denote the reaction rate constant, pre-exponential factor, activation energy, gas constant, and absolute temperature, respectively. The differences of Arrhenius plot between mannose, MA and mannitol were illustrated in **Fig. 7.4**.

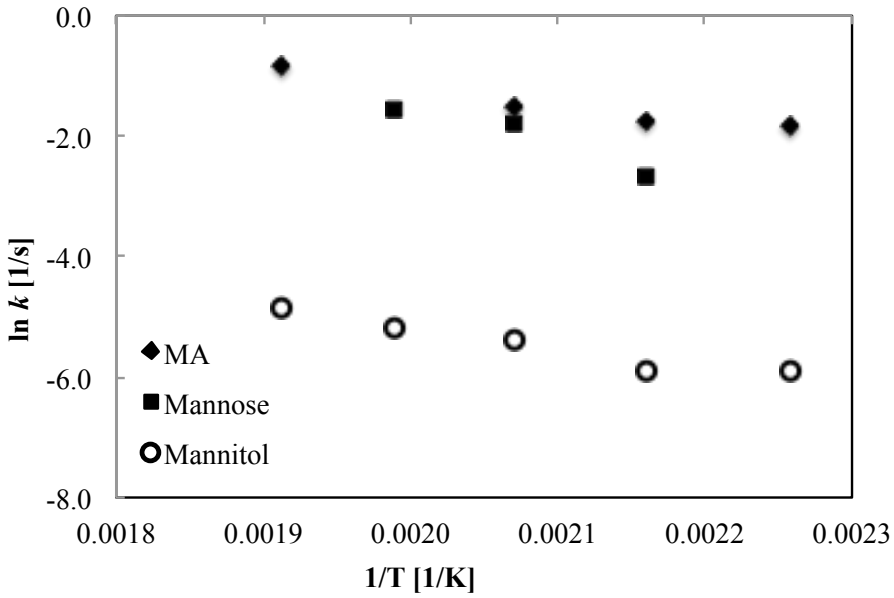


Fig. 7.4 Arrhenius plot of mannose, MA and mannitol

Evidently, the decomposition rate of MA was the highest among the sugar compounds. Besides, mannose was also showed a similar behavior like MA, whose rate of decomposition was somewhat slower than MA. However, mannitol showed a great different on its reactivity among the others, under the same experimental condition. This is therefore indicated the importance of functional group on the reaction rate of sugar compounds. Perhaps, the reaction is more favorable for compounds with carboxylic and aldehyde groups than the ones with hydroxyl group.

For further evaluation, pre-exponential factor (A) and activation energy (E_a) were calculated for each sugar compounds as shown in **Table 7.3**.

Table 7.3 Summary of reaction parameters

	Pre-exponential factor [s^{-1}]		Activation energy [kJ/mol]	
	Least square	95 % Reliability	Least square	95 % Reliability
Mannose	9.60E+03	(9.35±2.33)E+03	44.6	44.8±0.98
MA	2.82E+02	(2.90±0.70)E+02	28.3	28.3±0.88
Mannitol	6.19E+00	(6.38±1.51)E+00	29.1	29.1±0.89

7.4 Conclusion

The degradation characteristic of sugar family that consists of aldohexose (mannose), uronic acid (MA) and sugar alcohol (mannitol) was successfully evaluated under hydrothermal condition. Apparently, different reactivity was observed in which, the reaction rate increases in the order of mannitol, mannose, and MA. Perhaps, the presence of different functional groups on each of them significantly affects their reactivity.

The high electronegativity of carboxylic group in MA resulted to high degradation rate, while hydroxyl group in mannitol is more resistant under experimental conditions employed in this study.

The decomposed products of mannose were generated through different mechanisms. The 5-HMF was produced when mannose was isomerized to fructose, and subsequently fructose was dehydrated to produce 5-HMF. On the other hand, retro-aldol condensation was also occurred during hydrothermal treatment of mannose and consequently produced smaller molecular weight compounds including glycolaldehyde.

CHAPTER 8

Kinetics behavior of sugar alcohols under hydrothermal condition

8.1 Introduction

The discussion on potential of biomass as renewable energy resources has been reported in numerous studies. Particularly, the impact of biomass utilization on several aspects including environmental, economical, and social was extensively discussed by a group of researchers (Sadamichi et al., 2012). Nowadays, there are many types of biomass, in which their utilization have been widely explored. Among them, the potential of macroalgae has attracted significant attention of worldwide researchers.

Basically, macroalgae is a multicellular plant that exhibits similar characteristics like terrestrial plants. They contains considerable amount of carbohydrates that can be utilized in wide range of applications. For example, the potential of carbohydrate in macroalgae as a feedstock for bioethanol production was successfully demonstrated in previous study (Kim et al., 2011). The researchers employed feasible methodology, which involved acid hydrolysis and enzymatic treatment to produce the desired product.

Another study demonstrated the possibility of using macroalgae for biogas production through anaerobic fermentation (Fan et al., 2015). This research focused on the different reaction environment where macroalgae was subjected to seawater system. Surprisingly, comparable product yield was obtained between the seawater and freshwater systems.

Laminaria japonica, also known as Japanese kelp is the most common brown macroalgae in Japan. Its utilization as renewable resources has been widely explored owing to the considerable amount of sugar alcohol, namely mannitol. Fundamentally, mannitol can be derived through reduction reaction of aldehyde group of mannose. In fact, the reduction of mannose that led to the formation of mannitol was reported in previous study (Hricovíniová, 2011). The researchers were successfully developed a practical methodology for mannitol production, whose yield was considerably high. Instead of chemical synthesis, mannitol can also be recovered from kelp. However, pretreatment of kelp is necessary prior to the recovery process.

Since macroalgae contain high water content, hydrothermal process could be the promising method of pretreatment. Basically, this process employs water as an excellent solvent and also as a reaction medium under high temperature and pressure conditions. During hydrothermal treatment of kelp, its structure was disrupted and consequently released the desired carbohydrates (mannitol). Nevertheless, appropriate condition is needed for pretreatment of kelp to prevent undesired decomposition of the carbohydrates.

Owing to that, specific study was conducted to investigate the decomposition behavior of mannitol under hydrothermal condition prior to the pretreatment of kelp (Matsumoto et al., 2015). The researchers reported that mannitol degraded faster than glucose, which indicating the needs for fast reaction at somewhat lower range of

temperature. Furthermore, that study demonstrated that mannitol decomposition followed a first order reaction and the temperature dependence of reaction rate constant was represented by Arrhenius equation.

However, information on such reaction is rather limited specifically for other kinds of sugar alcohol. Thus, it is difficult to determine whether general reaction rate is existed for all sugar alcohols. For that reason, a study on the isomer of mannitol could be the best option for comparing the reaction behavior of sugar alcohols. Basically, these isomers are only differed on the orientation of hydroxyl group at C-2, while the other hydroxyl groups are identical between them. Owing to that, it is expected that both of them show similar decomposition behavior under hydrothermal condition. If they are exhibiting comparable behaviors, perhaps the reaction rate obtained for mannitol can be applied for the other sugar alcohols too.

Sorbitol has been widely used as sugar substitute, particularly in food industry. Thus, information regarding its reaction characteristics is also well reported in previous studies. Particularly, decomposition mechanism of sorbitol under non-isothermal condition was successfully elucidated by a group of researchers (Birta et al., 2008). They reported that the decomposition of sorbitol proceeded when dehydration reaction occurred and subsequently produced isosorbide as its decomposition product.

On the other hand, the behavior of sorbitol under isothermal condition was also investigated in several studies. Surprisingly, sorbitol decomposition under isothermal condition showed similar reaction mechanism like the non-isothermal ones. This fact was reported in previous work, in which sorbitol dehydration was investigated at subcritical water condition (Yamaguchi et al., 2011). The researchers highlighted that high temperature resulted to high conversion of sorbitol, whose reaction proceeded via dehydration mechanism. Similar study was also conducted on sorbitol with the addition

of catalyst. In that study, it was reported that dehydration reaction was the main reaction for conversion of sorbitol.

Nevertheless, those researches were only focused on the dehydration products of sorbitol, where the reaction was occurred at high temperature and trace amount of water was employed as reaction medium. Instead, our research objective is to determine the appropriate condition for hydrothermal treatment of macroalgae for recovery of sugar alcohol. This information is needed in order to prevent undesired decomposition of sugar alcohol during pretreatment of macroalgae. Therefore, kinetics behavior of sorbitol at relatively lower temperature range and under higher amount of water is more desirable. For the purpose of conducting comparable study between sorbitol and mannitol, similar experimental conditions are necessary. Since the behavior of mannitol was successfully evaluated earlier, similar experiment is needed for sorbitol too. Thus, this study was initiated with the aim to investigate the decomposition behavior of sorbitol for comparison with its isomer, mannitol.

Briefly, the experimental work was conducted using a continuous flow reactor whose temperature varied between 170 and 250 °C at 25 MPa. The decomposition reaction was evaluated based on the effect of temperature and residence time. The corresponding reaction rate constant at each temperature was later calculated and its dependency on temperature was evaluated by Arrhenius equation. Consequently, reaction parameters of sorbitol was calculated and compared with the ones of mannitol.

8.2 Experimental procedures

The decomposition kinetics of sorbitol was evaluated using a commercial sorbitol, purchased from Sigma Aldrich. The experimental condition used in this study is similar with the ones employed in previous study, to obtain comparable results (Matsumoto et al., 2015).

8.2.1 Hydrothermal treatment of sorbitol

The hydrothermal treatment of sorbitol was conducted in a continuous flow reactor system whose experimental apparatus was described by previous researchers (Yong and Matsumura, 2012). The reactor used is a stainless steel type with 1 mm of inner diameter. Initially, water was pumped into preheater to achieve the target temperature. From another line, feedstock was pumped at a ratio of 1 to 4 of the preheated hot water. Both feedstock and hot water were mixed at the reactor inlet and reaction occurred at certain residence times. The liquid effluent from reactor was then mixed with cool water prior to further cooling in heat exchanger. The cooled product stream was then depressurized by back-pressure regulator and liquid product was collected for further analysis.

8.2.2 Experimental conditions

The kinetic study on decomposition of sorbitol was conducted according to the experimental conditions summarized in **Table 8.1**.

Table 8.1 Experimental conditions for kinetics of sorbitol decomposition under hydrothermal condition

Parameters	**Experimental conditions**
Feedstock	Sorbitol
Types of reactor	Continuous flow (stainless steel)
Reactor diameter	1 mm (ID)
Reactor length	0.9 m
Feedstock concentration	1 wt%
Feedstock to water ratio	1 : 4
Reaction temperature	170 to 250 °C
Residence time	15 to 20 s
Operating pressure	25 MPa

8.2.3 HPLC analysis of liquid products

The quantitative analysis of liquid product obtained after the hydrothermal treatment of sorbitol was conducted using high-performance liquid chromatography (HPLC) from Shimadzu, Japan. A specific column (Shodex KS-802) was employed for quantification of sorbitol concentration in the liquid effluent. The analysis was performed using deionized water (eluent) whose flow rate was 0.7 cm^3 min^{-1} while oven temperature was set 60 °C. Then, a residual ratio between the concentration of unreacted sorbitol in liquid product (C) and initial concentration of sorbitol in feedstock (C_o) was calculated to evaluate its decomposition behavior.

8.3 Results and discussion

The effect of hydrothermal treatment on sorbitol decomposition behavior was evaluated at various temperature and residence time. The yield was calculated based on residual ratio between the concentration of unreacted sorbitol and its initial concentration in feedstock. The reactivity of sorbitol was determined based on the reaction rate constant obtained at each reaction temperature. Furthermore, the reaction parameters were calculated to evaluate the temperature dependence of reaction rate constant, where Arrhenius equation was employed. The data obtained in this study was then compared with mannitol, whose data was reported in previous study (Matsumoto et al., 2015).

8.3.1 Effect of residence time on sorbitol concentration

The effect of residence time on decomposition behavior of sorbitol was investigated at isothermal condition (210 °C). The residual yield of sorbitol at different residence time was calculated accordingly and plotted in **Fig. 8.1**. Apparently, the data plotted in Fig 8.1 shows linear relationship between the residual ratio and residence time. This is therefore indicated that the decomposition of sorbitol under hydrothermal condition is a first-order reaction. In addition, error bar was also included in the figure, whose error calculation was based on measurement error. In fact, this finding shows a good agreement with other studies, whose experimental condition was completely different with ours (Yamaguchi et al., 2011)(Li et al., 2013). Even though the experiments were conducted under different conditions, they still exhibited a first-order kinetics, particularly at short residence time.

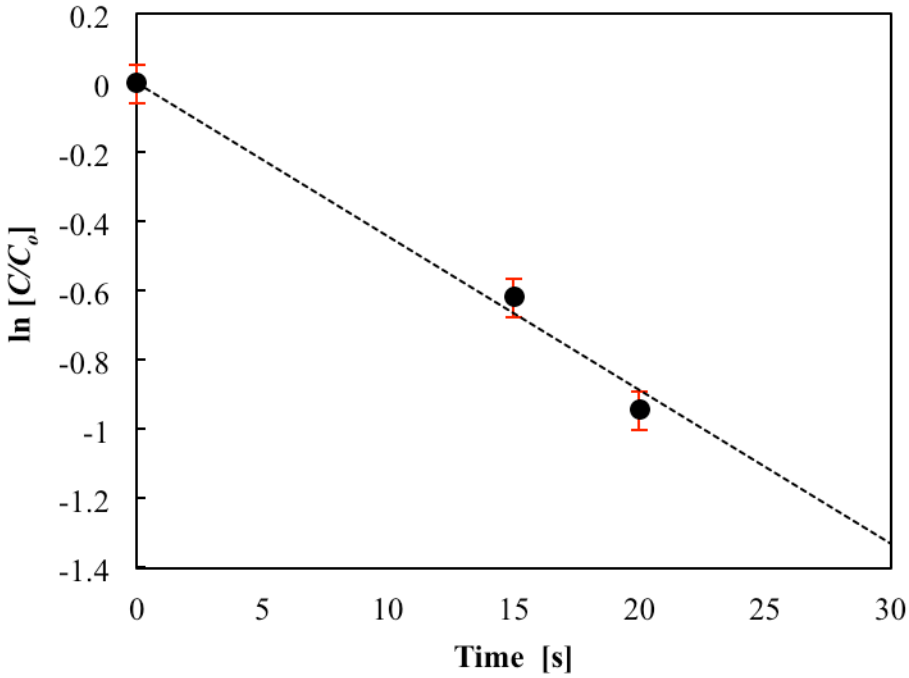


Fig. 8.1 Effect of residence time on sorbitol decomposition at 210 °C

As mentioned earlier, dehydration is the main reaction that occurred under trace amount of water. The same reaction could be occurred in the case of high amount of water condition. However, when more water was employed, perhaps the reaction between sorbitol and water is more favorable than the dehydration reaction. As for the dehydration reaction, the decomposition of sorbitol occurred when there is sufficient temperature dependent energy. This is therefore accounting for the first-order reaction.

On the other hand, reaction between sorbitol and water could possibly be a second-order reaction in total. This is based on assumption that each of sorbitol and water exhibit first-order reaction. Perhaps, when different concentration of sorbitol was employed, the concentration of water was also affected accordingly. Consequently, first-order reaction might not be obtained. Nevertheless, it this study, the reaction is pseudo-first order owing to the presence of excessive water. As a result, the reaction appear to be a first-order reaction.

8.3.2 Effect of temperature on sorbitol concentration

The hydrothermal treatment of sorbitol was conducted at various reaction temperatures. The residual yields of sorbitol were calculated for each temperature as illustrated in **Fig. 8.2**. The data in Fig. 8.2 clearly shows that the residual ratio of sorbitol decreased at enhanced temperature. It was expected because at higher temperature, more energy is generated and consequently improves the reaction rates. Therefore, the reaction is expected to follow the Arrhenius law.

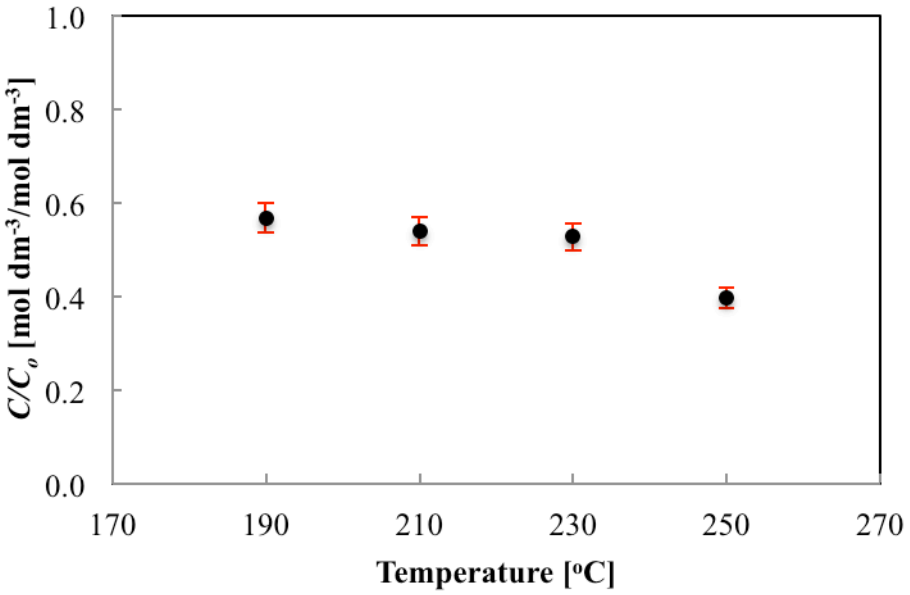


Fig. 8.2 Effect of temperature on sorbitol decomposition at 15 s residence times

Nevertheless, the reaction rate constant of sorbitol obtained in this study was considerably higher than that obtained in previous study (Yamaguchi et al., 2011). Possibly, different experimental setup and conditions employed among them resulted to different findings.

In another study where specific catalyst was employed to improve the reaction, approximately 60 min was required for complete conversion of sorbitol at 220 °C. In contrast, only about 120 s was needed for complete conversion of sorbitol employed in our experiment. Supposedly, faster reaction is expected for reaction with the presence of catalyst, but the results obtained in this study show the opposite. It seems like different reaction mechanism could be occurred during hydrothermal treatment of sorbitol under high amount of water. Probably, reaction between sorbitol and water is occurred rather than dehydration reaction, as reported in the previous works.

8.3.3 Reaction rate constants of sorbitol

Table 8.2 showed the decomposition rate constant (k) of sorbitol, which was calculated based on first-order reaction. Likewise, the same order of reaction was determined when the data obtained in previous study was fitted by using least square error (LSE) method (Yamaguchi et al., 2011). Their data is also shown in Table 8.2 for comparison purposes. As expected, the rate constants of sorbitol showed an increasing trend towards the higher temperatures. However, sorbitol in the present work reacted much faster than those reported in the previous study (for example at 250 °C). This should not be happening if the same reaction mechanism occurs in both studies. Perhaps, the experimental conditions other than temperature greatly affected the mechanism, which resulted to different reactivity.

Possibly, water availability during the reaction could be the main reason that contributed to different findings. For example, when inadequate water is supplied to the system, reaction that involves water will also become limited. In contrary, if sufficient amount of water is available for reaction, different mechanism might be preceded. In both cases, dissimilar reactivity could be expected.

Table 8.2 Summary of reaction rate constants

T [^{o}C]	Rate constant, k [s^{-1}]			
	Sorbitol	Sorbitol (Yamaguchi et al., 2011)	Mannitol (Matsumoto et al., 2015)	Mannitol (Yamaguchi et al., 2014)
170			2.79E-03	
190	3.78E-02		2.73E-03	
210	4.13E-02		4.60E-03	
230	4.27E-02		5.58E-03	
250	6.15E-02	4.20E-05	7.78E-03	1.67E-05
275		1.84E-04		9.49E-05
287		3.23E-04		1.81E-04
300		5.35E-04		3.53E-04

The Arrhenius law was employed to evaluate the temperature dependence of the reaction rate constant, whose equation is shown in Eq. 8.1.

$$k = A\exp\left(\frac{-E_a}{RT}\right) \qquad (8.1)$$

Here, the k, A, E_a, R, and T are the reaction rate constant, pre-exponential factor, activation energy, gas constant, and absolute temperature, respectively. As mentioned earlier, decomposition rates of sorbitol obtained in this study were significantly higher than that obtained in the earlier study. The differences between them are clearly plotted in **Fig. 8.3**.

Obviously, different reaction mechanisms might have happen when sorbitol was subjected to hydrothermal treatment at dissimilar reaction conditions. Particularly, dehydration reaction of sorbitol was reported in previous work, when limited amount of water was employed (Yamaguchi et al., 2011). Similarly, the same reaction was

examined when sorbitol was reacted in the presence of catalyst (Li et al., 2013). In both studies, they observed the presence of isosorbide in liquid product, as the main product of sorbitol dehydration reaction.

Generally, when 1 mol dm^{-3} of sorbitol concentration was employed, about 50 molecules of water were distributed to 1 molecule of sorbitol. With this amount of water molecules, only about 2 to 3 layers of water will be formed around the sorbitol molecule. This is because of the sorbitol itself, which is considered as a big molecule with 6 carbons, 6 oxygen and 14 hydrogen. As in the case when catalyst was added (for example 70 wt% of $ZnCl_2$), the movement of water molecules in the bulk phase is rather restricted. Due to that reason, the reaction between water and sorbitol was also retarded. Instead, this reaction is favored when excessive amount of water was employed as the reaction medium, as in the case for kelp pretreatment.

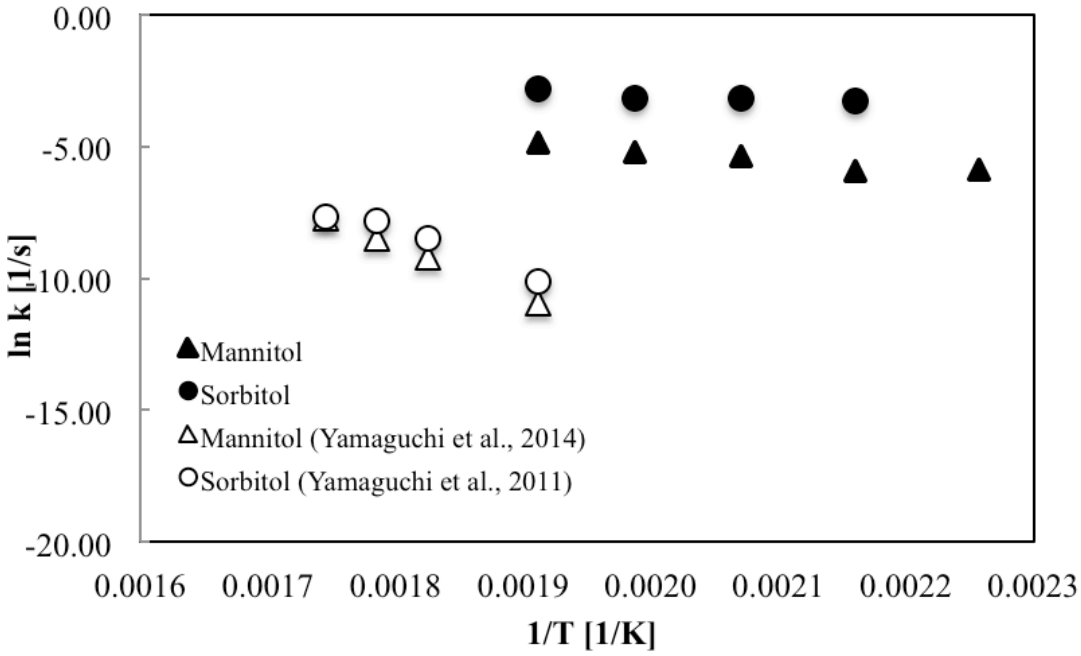


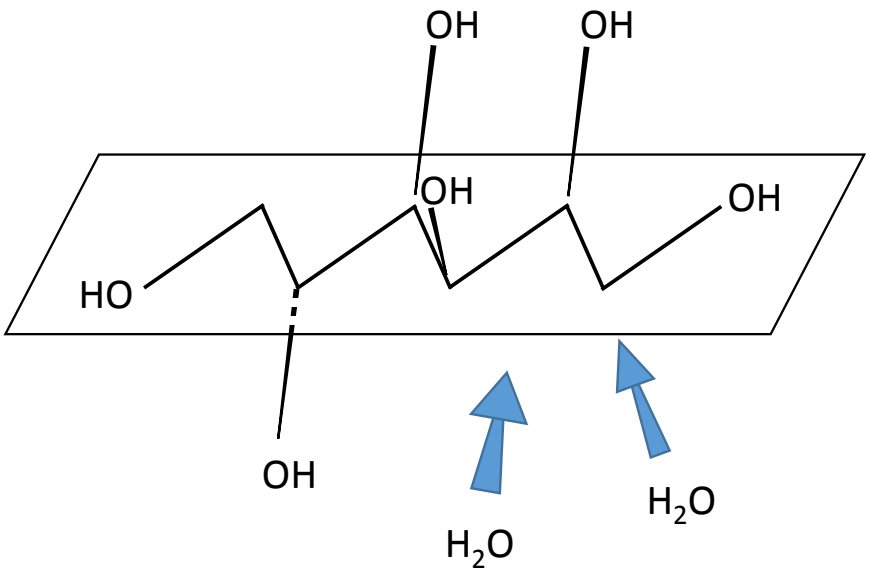
Fig. 8.3 Arrhenius plot

8.3.4 Comparison on kinetics characteristics between sorbitol and mannitol

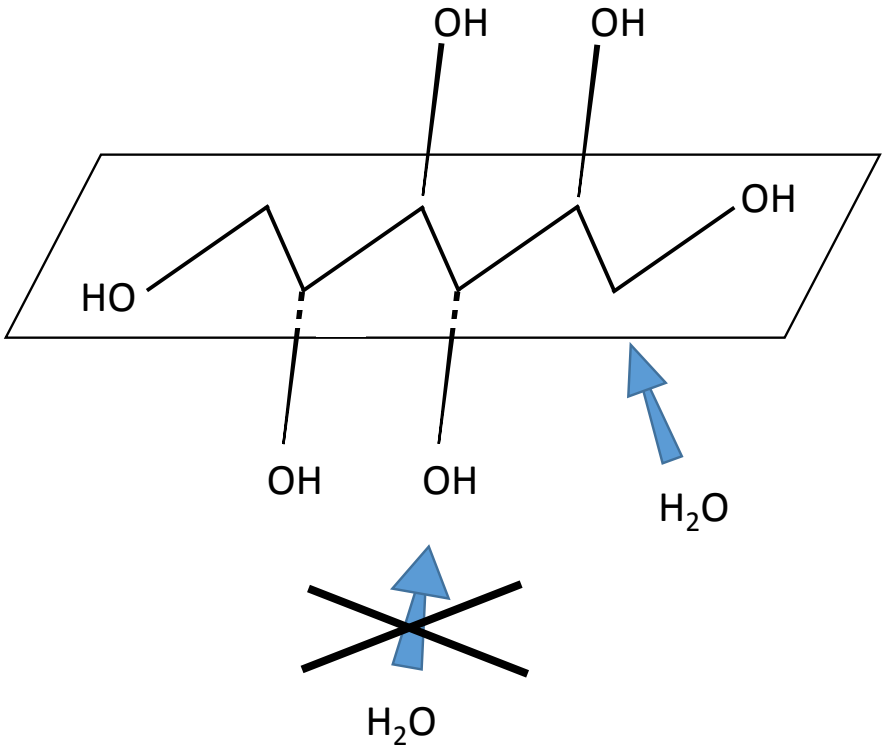
In this study, the behavior of mannitol was compared with its isomer, sorbitol. This information is needed in order to investigate the possibility whether general decomposition rate is existed for sugar alcohols. For better overview, Arhenius plot of mannitol is also shown in Fig. 8.3. The data was obtained from our research group, who employed large amount of water during the hydrothermal treatment process. Apparently, decomposition rate of sorbitol was slightly higher than mannitol under the same experimental condition. These results indicated that general rate constant of sugar alcohols is not existed. Therefore, individual study on each of sugar alcohol is required to determine its reaction rate constant.

Previously, a group of researchers had successfully investigated the behavior of mannitol. However, they employed a highly concentrated mannitol (limited amount of water) in order for dehydration reaction to be achieved. The data obtained in that study was fitted accordingly using LSE analysis to determine the reaction rate constant as shown in Table 8.2. The temperature dependence of the rate constant was further evaluated based on Arrhenius plot as shown in Fig. 8.3. The figure showed that different reactivity was obtained for mannitol under limited and excess amount of water. Specifically, faster reaction was obtained when mannitol was treated under high amount water.

In each case (limited and excess amount of water), the reaction rate of sorbitol was found higher than mannitol. Particularly, great different was observed when they were treated by large amount of water. The main difference between these isomers is only on their hydroxyl orientation. Possibly, different reactivity among them might be due to this dissimilarity. The effect of the hydroxyl orientation is illustrated in **Fig. 8.4**.



(a) sorbitol



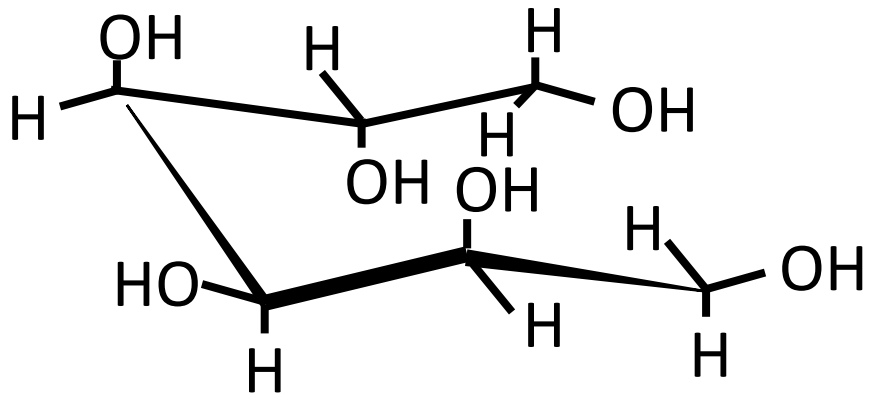
(b) mannitol

Fig. 8.4 Effect of chemical configuration on reaction mechanism in sorbitol and mannitol

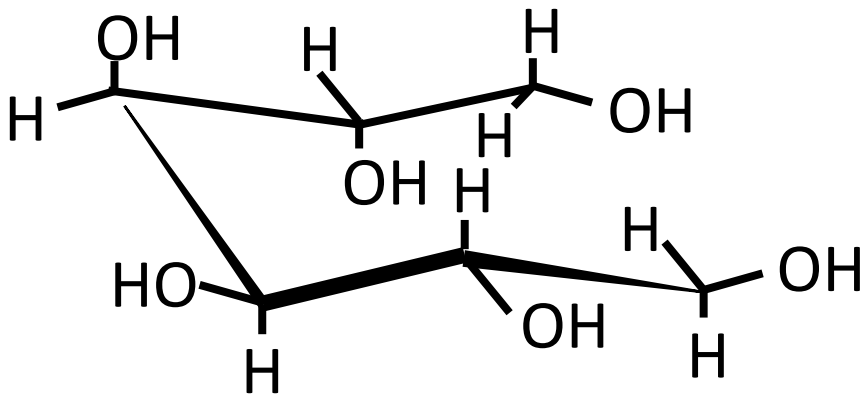
Figure 8.4 explained the possible reaction mechanism that might have occurs in sorbitol and mannitol under high amount of water. Clearly, the same orientation of hydroxyl groups on C3 to C5 was observed for sorbitol. This is therefore resulted to a condition where carbon atoms of sorbitol are easily attacked by water molecules. However, this is not the case for mannitol whose hydroxyl group on C5 prevents attack from water. Subsequently, the reactivity of mannitol becomes slower than that of sorbitol.

On the other hand, when limited water was employed for treatment of sorbitol and mannitol, dehydration reaction is favored through interaction between the hydroxyl groups. In previous study, it was reported that dehydration of sorbitol produced 1,4-anhydrosorbitol and 2,5-anhydrosobitol as the main products (Yamaguchi et al., 2011). Similarly, 1,4-anhydromannitol and 2,5-anhydromannitol was obtained as the main product of mannitol dehydration (Yamaguchi et al., 2014). Evidently, these products were obtained through interaction between the hydroxyl groups of C1 and C4 as well as C2 and C5.

The interaction between these atoms was further evaluated using a chair confirmation as illustrated in **Fig. 8.5**. Clearly, axial conformation was observed for the hydroxyl groups in C2 and C5 of sorbitol. In contrast, equatorial confirmation was observed for hydroxyl group in C2 of mannitol that creates bigger distance between the hydroxyls. As a result, dehydration reaction of mannitol is retarded. Although orientation of hydroxyl group significantly affects the reactions, each of them has different inhibition mechanism. Therefore, the ratio of rate constant between sorbitol and mannitol differs depending on the amount of water available for the reaction to proceed.



(a) sorbitol



(b) mannitol

Fig. 8.5 Chair conformation of sorbitol (axial) and mannitol (equatorial)

8.3.5 Determination of reaction rate parameters

The kinetics behavior of sorbitol and mannitol was further evaluated based on the reaction parameters, specifically activation energy (E_a) and pre-exponential factor (A). The calculated value of E_a and A are shown in **Table 8.3**.

Table 8.3 Reaction parameters of sorbitol and mannitol

	Pre-exponential factor, A [s^{-1}]		Activation energy, E_a [kJ/mol]	
	Least square	95 % reliability	Least square	95 % reliability
Sorbitol	4.29E+01	(4.65±1.53)E+01	28.3	28.1±1.26
Mannitol	3.23E+00	(3.18±1.07)E+00	26.5	26.5±1.35

Besides, the reaction parameters were also statistically evaluated at 95 % reliability as shown in the same table. The results showed that activation energy of sorbitol is slightly higher than its isomer, mannitol but not that much. However, pre-exponential factor values indicated that sorbitol decomposed faster than mannitol. Indeed, this finding shows good agreement with the proposed reaction mechanism illustrated in Fig. 8.4. Slower reaction rate of mannitol was due to the limited access of water to the carbon atoms. In short, although similar activation energies were obtained as a result of similar transition states, pre-exponential is greatly affected by the stearic issues.

8.4 Conclusion

The kinetics characteristic of sorbitol under hydrothermal condition was successfully investigated. The experimental work was conducted under subcritical water condition (between 170 and 250 °C) where continuous flow reactor was used. A first-order equation was employed to express the reaction rate, which is also having good agreement with Arrhenius law. Apparently, the reaction rate constant obtained in this study was significantly higher than the previous works. Perhaps, the difference was due to availability of water during the reaction.

In addition, different reactivity between sorbitol and its isomer, mannitol was likely due to their hydroxyl group configurations. Certainly, sorbitol decomposed faster than mannitol because of the high accessibility of water molecules on the carbon atoms of sorbitol. This was the case when excessive water was available for reaction. In another condition when less amount of water was employed, different reaction mechanism was expected. In short, decomposition was favored in large amount of water condition while dehydration was likely occurred when only trace amount of water was employed. Finally, the reaction parameters of sorbitol were calculated to be 28.3 kJ mol^{-1} (activation energy) and 42.9 s^{-1} (pre-exponential factor).

CHAPTER 9

Hydrothermal treatment of carbohydrates in marine macroalgae

9.1 Introduction

Marine biomass has wide range of applications due to its composition that contains significant amount of carbohydrates. Indeed, its utilization as a feedstock for bioenergy production has been successfully investigated in numerous studies. For example, it was reported in previous study that macroalgae is a potential resource for production of various products including bioethanol (Baghel et al., 2016). The researchers also proposed new methodology that fully utilized the macroalgae. In addition, another study also focused on production of bioethanol (Lee et al., 2013). They reported that pretreatment condition of macroalgae greatly affects the glucose production.

Generally, marine macroalgae can be classified into three (3) groups, namely red, green and brown macroalgae. Each of them contains different compositions of carbohydrate, whose amount is also varied among species in a same group. Previously, a study was conducted by a group of researchers on various species of green macrolagae

(McDermid and Stuercke, 2003). In that study, it was reported that the carbohydrate contents of various species of green macroalgae was between 4 to 40 wt%.

In another study, a proximate composition analysis revealed that red macrolagae (*Gelidium amansii*) contains as much as 71 wt% of carbohydrate (Jang et al., 2012). The researchers also reported that about 62 % of sugar conversion was obtained from the carbohydrates. Furthermore, it was also reported in the same study that brown macroalgae (*Laminaria japonica*) contains approximately 55 wt% of carbohydrates, whose conversion was about 32 %. Owing to the high carbohydrate content in macroalgae, significant numbers of study have been conducted. Most of them are focusing on effective utilization of the carbohydrates in various applications.

Basically, carbohydrates in macroalgae exist in the forms of polysaccharides and monosaccharide, as reported in previous study (Jung et al., 2013). Obviously, glucose was found in all types of macroalgae (red, green and brown algae). This sugar compound is a group of aldohexose with 6 carbons and aldehyde as its functional group. Notably, sugar compounds can be changed to another form depending on types of reaction.

For example, glucose can be converted to its corresponding uronic acid, namely glucuronic acid. This uronic acid is obtained when hydroxyl group on C-6 of glucose oxidize to carboxylic group. Perhaps, glucose in macroalgae is also changed to the uronic acid under certain condition. Hence, the presence of glucuronic acid in macroalgae is well expected. In fact, it was reported that glucuronic acid was observed mostly in green and brown macroalgae (Jung et al., 2013).

Another possible reaction that might be occurred for glucose is a reduction reaction. During this reaction, aldehyde group of glucose is reduced to hydroxyl group, which consequently generate sorbitol, its corresponding sugar alcohol. Indeed, red macroalge showed great potential in producing sorbitol as been reported in previous study (Karsten et al., 1996).

Briefly, glucose, glucuronic acid, and sorbitol are considered as one (1) family, in which each of them differs on their functional group. Owing to that, different characteristics are expected for these sugar compounds under the same hydrothermal condition. Typically, they can be produced via chemical synthesis process. However, recovery of these sugars from biomass is also possible. Nowadays, numbers of technology have been developed for the recovery of carbohydrates from biomass, particularly for macroalgae. This kind of biomass needs proper pretreatment due to its high water content. Thus, hydrothermal pretreatment is the most favorable process for macroalgae because the process itself requires water as reaction medium.

Nevertheless, macroalgae is usually composes of various types of sugars, whose reactivity might be different among each other under the same treatment condition. This is due to the presence of different functional groups in different type of sugars. Therefore, the main objective of this study is to elucidate the effect of functional groups on behavior of sugars during hydrothermal process.

9.2 Experimental procedures

In this study, all model compounds of the carbohydrates in marine macroalgae (glucuronic acid and sorbitol) were purchased from Sigma-Aldrich and were of analytical grade chemicals. The kinetics study was performed for the specified

carbohydrates under hydrothermal condition by using a continuous reactor system. The effect of experimental condition on sugar behavior was elucidated accordingly using appropriate reaction order and Arrhenius law.

9.2.1 Hydrothermal treatment of sugar compounds

The detail description of experimental apparatus employed in this study was explained in previous work conducted by our research group (Yong and Matsumura, 2012). The hydrothermal treatment of sugar compounds was conducted using continuous flow reactor (stainless steel), whose inner diameter was 1 mm. The reaction temperature was changed from 190 to 250 °C, at 25 MPa of operating pressure, under subcritical water condition.

Briefly, the experiment started when water was pumped into preheater to achieve desired temperature. From another line, feedstock was pumped at a ratio of 1:4 of feedstock to hot water, and both were mixed at the entrance of reactor. After certain residence time, hot effluent was mixed with cool water and was further cooled in heat exchanger. Finally, liquid product was collected at sampling point after being depressurized by back-pressure regulator. The liquid product was kept in closed container and properly preserved in refrigerator prior to HPLC analysis. This step is needed to prevent undesired product degradation upon storage.

9.2.2 Experimental conditions

The hydrothermal treatment of carbohydrates in macroalgae was conducted based on experimental conditions shown in **Table 9.1**. In this study, glucose was not used as a feedstock. However, the data used in this study was obtained from previous work, which was conducted by our research group.

Table 9.1 Summary of experimental conditions

Parameters	**Experimental conditions**
Feedstock	Sorbitol, glucuronic acid
Types of reactor	Continuous flow (stainless steel)
Reactor diameter	1 mm (ID)
Reactor length	0.9 m
Feedstock concentration	1 wt%
Feedstock to water ratio	1 : 4
Reaction temperature	170 to 250 °C
Residence time	15 s
Operating pressure	25 MPa

9.2.3 Quantitative analysis of liquid products

The liquid product obtained after hydrothermal treatment process was subjected to quantitative analysis, specifically high performance liquid chromatography (HPLC). The analysis was conducted in order to determine the concentration of reactant in feedstock and in the liquid effluent. Furthermore, the analysis was needed to analyze the decomposition products after the hydrothermal process. For that purpose, HPLC analysis was conducted by using three different columns separately. Each column was employed for quantification of different groups of compound.

The first column is Shodex KS-802, which was used to quantify sugar compounds. The analysis was performed at 60 °C of oven temperature by using deionized water as the eluent and at a flow rate of 0.7 cm^3/min. The second column is SCR 102HG, which was used particularly for determination of organic acids. For this column, oven temperature was set at 40 °C. The analysis was carried out using 5 mmol/L of perchloric acid ($HClO_4$) as eluent, at 0.7 cm^3/min of flow rate. For both columns, refractive index detector (RID) was employed. Lastly, DE 413L column was used for compounds with ring structure like furfural and 5-hydroxymethylfurfural (HMF). The analysis was conducted using mixture of $HClO_4$ and acetonitrile at a ratio of 1:1 as eluent. The flow rate of eluent was 0.7 cm^3/min while oven temperature was 40 °C.

The concentration of each compound was calculated based on standard calibration curves, which were prepared beforehand. Based on that, decomposition rate of reactant and recovery yield of decomposition products was determined accordingly.

9.3 Results and discussion

This study focused on the kinetics characteristics of various sugars with different functional groups. For this purpose, a sugar family that consists of aldohexose (glucose), sugar alcohol (sorbitol), uronic acid (glucuronic acid), was employed. Different behavior was expected for these sugar compounds owing to the different functional groups presence in each of them. The decomposition rate of these sugars was evaluated at different temperatures and rate constant was calculated by using the appropriate reaction order. Furthermore, the temperature dependence of rate constant was determined the Arrhenius law.

9.3.1 Kinetics behavior of sugars at various temperatures

The effect of reaction temperature on the residual concentration of glucose, sorbitol and glucuronic acid was plotted in **Fig. 9.1**.

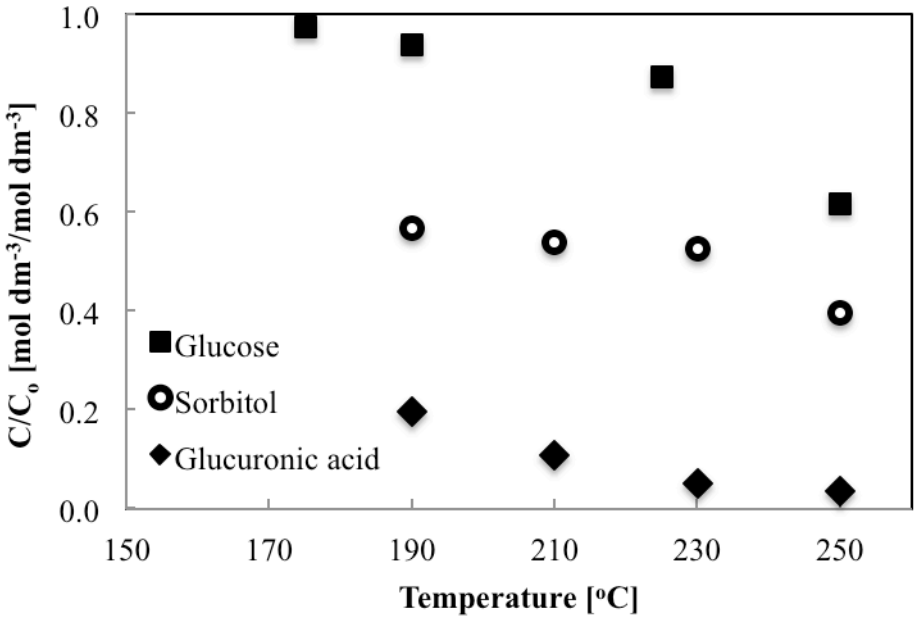


Fig. 9.1. Effect of temperature on yield of glucuronic acid, sorbitol, and glucose in a continuous flow reactor

The data for glucose were obtained from previous study (Matsumura et al., 2006). Figure 9.1 shows that temperature has significant effect on the decomposition of all the sugar compounds regardless of their functional groups. However, the degree of susceptibility to degradation among them was apparently different. Particularly, the yields of glucuronic acid were greatly affected at the temperature range employed in this study, while glucose was less affected among the others. These results indicated that different types of sugar compounds have different ranges of temperature tolerance.

Since these sugar compounds are only differed on their functional groups, different reactivity among them could be possibly due to this dissimilarity.

Specifically, glucuronic acid with carboxyl group as its functional group, could not withstand even at 190 °C, where almost 80 % were decomposed at this temperature. This finding is in accordance with previous study, which revealed the high decomposition rate of this uronic acid at lower temperature ranged between 170 to 200 °C (Wang et al., 2010). Similarly, a group of researchers also observed a rapid degradation of glucuronic acid at temperature ranged from 140 to 160 °C (Usuki et al., 2008). Since the reaction temperature employed in the present study is much higher than the previous studies (190 – 250 °C), lower yield of glucuronic acid in the liquid product after hydrothermal process is well expected.

In comparison with other kinds of uronic acid, similar trend of high reactivity was also observed for galacturonic acid (Wang et al., 2009), as well as for mannuronic acid and guluronic acid (Mohamad et al., 2016), indicating the susceptibility of uronic acid in general, towards degradation under subcritical water condition. The susceptibility of uronic acid towards degradation under hydrothermal condition is probably due to the induction factor created by the presence of carboxyl group at C-6. In fact, this carbon has high electronegativity, and thus resulted to a weaker bond between C-3 and C-4. Consequently, cleavage of the bond is favored, producing simpler structure of organic compounds such as formic acid, glyceraldehyde as well as 5-HMF and furfural.

In contrast, glucose with the aldehyde group showed higher temperature tolerance than glucuronic acid and sorbitol. For example, at the same temperature of 190 °C, only about 6 % of glucose were decomposed after the hydrothermal process, while approximately 40 % of sorbitol still remained in the liquid product. However, the

yield of glucose was then decreased significantly at higher temperature of 250 °C. In fact, this finding is in good agreement with previous study that observed faster degradation of glucose at higher temperature range (Khajavi et al., 2005).

Nevertheless, the yield of glucose was much higher compared to its sugar alcohol and uronic acid, indicating its higher resistance to degradation under hydrothermal condition. The high resistance of glucose towards degradation could possibly due to the effect of isomerization of glucose during the hydrothermal process. Perhaps, during the isomerization, a strong double bond between the C-1 of the aldehyde group and C-2 was formed, which consequently prevent the cleavage of the bond. Therefore, significant amount of energy is needed for breaking down the bond.

Sorbitol can be derived through reduction of glucose, where the aldehyde group of glucose changes to hydroxyl group. Unlike glucose, sorbitol has the hydroxyl group as its functional group at C-1, which basically has a weaker bond than the double bond of glucose. Due to that, the reactivity of sorbitol was found higher than glucose, under the same reaction temperature, as shown in Fig. 9.1. However, the decomposition rate of sorbitol is much lower than the glucuronic acid. In fact, a study conducted by previous researchers also observed lower reactivity of sorbitol during hydrothermal process (Yamaguchi et al., 2011). This phenomenon could possibly explain that the reaction between water and hydroxyl group of sorbitol is less favored than the reaction between water and carboxylic groups of uronic acid, under hydrothermal condition. Besides the reaction between water and the hydroxyl group of sorbitol, dehydration reaction was also occurred during the hydrothermal treatment process, particularly under limited amount of water condition (Li et al., 2013).

9.3.2 Decomposition products analysis

The analysis of decomposition products after hydrothermal treatment was conducted for glucuronic acid, owing to its high reactivity. The quantitative analysis was carried out using HPLC equipped with different types of column. The presence of organic acids (formic acid and glyceraldehyde) and ring compounds (furfural and 5-HMF) were observed on the HPLC charts. The result is in accordance with previous study that was also found the existence of several organic compounds in the liquid product, after hydrothermal treatment of uronic acid (Aida et al., 2012). Nevertheless, different types of organic compounds were obtained in this present study because of different uronic acid was employed. The amount of the organic compounds was calculated and summarized in **Table 9.2**.

Table 9.2 Yield of decomposition products of glucuronic acid

Products	Concentration [mmol/dm^3]			
	190 °C	210 °C	230 °C	250 °C
Formic acid	3.60	5.17	4.98	4.21
Glyceraldehyde	3.20	2.30	0.62	0.54
5-HMF	0	0.11	0.39	0.39
Furfural	0.07	0.17	0.51	0.44

Glyceraldehyde can be derived from retro-aldol reaction of either glucose and fructose (Weenen, 1998). Perhaps, it can also be formed through retro-aldol reaction of glucuronic acid, since this uronic acid has almost similar structure with glucose. Due to the high electronegativity of the glucuronic acid, glyceraldehyde was easily produced, where cleavage of the bond occurred between C3 and C4.

However, the yield of glyceraldehyde shows a decreasing trend at elevated temperature, indicating that the reaction is more favorable at lower temperatures. Probably, formic acid was produced after the retro-aldo reaction, where the remaining part of glucuronic acid was cut between C4 and C5, and consequently produced glycolic acid too. Nevertheless, the presence of glycolic acid was not detected in the liquid product, possibly due to the limitation of the HPLC column employed in this study. Initially, the yield of formic acid shows an increasing trend but later it was decreased slightly at temperature above than 210 °C.

Based on previous study, 5-HMF is the main product of decomposition of hexoses, where dehydration is the key reaction (Khajavi et al., 2005). Owing to the similarity between glucuronic acid and glucose, 5-HMF could be produced through the dehydration reaction of glucuronic acid. Unlike glucose, glucuronic acid has the carboxylic group, which further reduced to form the 5-HMF. The results show that the concentration of 5-HMF in the liquid product was increased at higher temperature. Besides, we also observed significant amount of furfural, which the concentration was much higher than 5-HMF.

Usually, furfural is produced from the dehydration of pentoses. However, the formation of furfural from 6-carbon compounds is also possible. In case of glucuronic acid, the decarboxylation reaction is necessary to produce furfural. Perhaps, during the hydrothermal reaction at high temperature and pressure, decarboxylation of carboxylic group of glucuronic acid was occurred. Consequently, it will produce furfural through the dehydration reaction. The concentration of furfural obtained in the liquid product was the highest at temperature of 230 °C but then it was decreased slightly.

9.3.3 Reaction rate parameter of various sugars

The decomposition of sorbitol and glucuronic acid was determined to be a first-order reaction. The reaction rate constant (k) of each compound were calculated and compared with glucose, as summarized in **Table 9.3**. The results clearly show that the reactivity of these sugar compounds under the same experimental condition was increased in the order of glucose followed by sorbitol and glucuronic acid.

Table 9.3 Reaction rate constants

T [°C]	Rate constant, k [s^{-1}]		
	Sorbitol	Glucuronic acid	Glucose (Matsumura et al., 2006)
175			8.52E-05
190	3.78E-02	1.08E-01	
200			5.36E-04
210	4.13E-02	1.49E-01	
225			2.21E-03
230	4.27E-02	1.99E-01	
250	6.15E-02	2.20E-01	4.98E-03

In order to evaluate the temperature dependence of the reaction rate constants, Arrhenius equation was employed as represented in Eq. 9.1.

$$k = A\exp\left(\frac{-E_a}{RT}\right) \qquad (9.1)$$

where *k, A,* E_a, *R*, and *T* are the reaction rate constant, pre-exponential factor, activation energy, gas constant, and absolute temperature, respectively.

The comparison of the Arrhenius plots is shown in **Fig. 9.2**. Apparently, different reactivity was observed among glucose, and its corresponding uronic acid (glucuronic acid) and sugar alcohol (sorbitol). The presence of different functional groups in these sugar compounds could possibly be the main factor that might affect their susceptibility to degradation. Perhaps, the experimental condition employed in this study was too severe for the uronic acid that has a carboxylic group. Thus, hydrothermal pretreatment of biomass particularly macroalgae containing high amount of uronic acids should be conducted at rather lower temperature range to avoid further degradation.

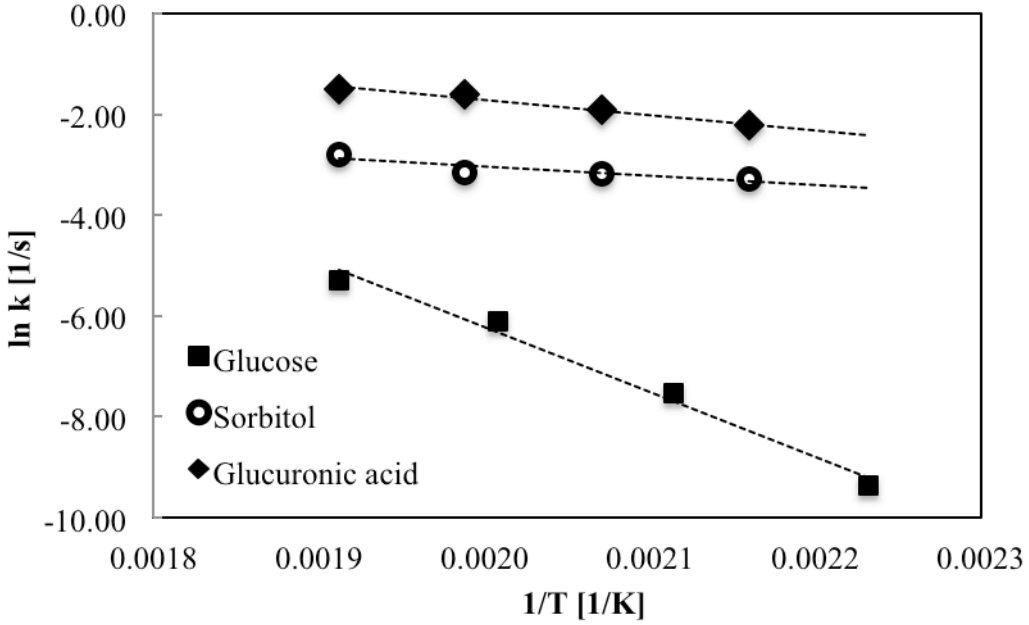


Fig. 9.2 Comparison of Arrhenius plots

Furthermore, the reaction parameters of (activation energy and pre-exponential factor) of each compound were also calculated and summarized in **Table 9.4**. Statistical treatment on the parameters was also conducted at 95 % reliability as shown in the same table.

Table 9.4 Reaction rate parameters

	Pre-exponential factor, A [s^{-1}]		Activation energy, E_a [kJ/mol]	
	Least square	95 % reliability	Least square	95 % reliability
Sorbitol	4.29E+01	(4.65±1.53)E+01	28.3	28.1±1.3
Glucuronic acid	4.34E+01	(4.31±0.41)E+01	22.8	22.8±0.4
Glucose	3.44E+12	(21.17±17.3)E+12	152.6	147±4.1

9.4 Conclusion

The behavior of sugar compounds under hydrothermal condition was greatly affected by the reactivity of their functional groups. The high electronegativity of carboxylic group in glucuronic acid subsequently resulted to higher degradation rate. In contrast, the effect of isomerization between C-1 (aldehyde group) and C-2 of glucose resulted to the formation of strong double bond, which relatively less susceptible to degradation. In fact, the reactivity of sorbitol with hydroxyl group is lower than glucuronic acid but higher than glucose. Due to the different reactivity of these sugar compounds, proper pretreatment condition of macroalgae is needed to avoid unnecessary decomposition.

CHAPTER 10

CONCLUSIONS AND RECOMMENDATION

10.1 Conclusions

The preparation of guluronic acid (GA) and mannuronic acid (MA) from alginic acid was successfully conducted by using the simplified methodology proposed in Chapter 5 of this thesis. Briefly, the hydrolysis of alginic acid was performed in mild acid concentration while separation of the desired uronic acid was carried out by pH adjustment method. Principally, with the proposed methodology, individual uronic acid was successfully recovered whose product characterization was also comparable with the ones obtained from different methods. Besides, the formation of byproduct was not observed indicating a good recovery process. Even though the recovery yield was rather low than expected, 48 % of yield is still a reasonable value. The most important point is the individual GA and MA was easily and quickly obtained by using simple processes only.

The kinetics characteristic of the GA and MA that were obtained in Chapter 5 was successfully evaluated under hydrothermal condition. The decomposition behavior of these uronic acids was determined to be a first-order and obeyed the Arrhenius law. The reaction parameters (activation energy, E_a and pre-exponential factor, A) were calculated accordingly whose results showed that MA has higher E_a and A (28.3 kJ/mol and 282 s^{-1}, respectively) than that of GA (20.6 kJ/mol and 40.6 s^{-1}, respectively). The comparison of types of reactor also revealed the superiority of continuous reactor than batch type reactor.

The decomposition behavior of MA was then compared with other sugars of its family, namely mannose (aldohexose) and mannitol (sugar alcohol). Basically, they have almost similar chemical structures except for the functional groups. Owing to that, different kinetics behaviors were observed under the same hydrothermal condition, whose reactivity increases in the following sequence: mannitol < mannose < MA. The highest reactivity of MA was possibly due to the presence of its carboxylic group that has high electronegativity. During the hydrothermal process, two main reaction mechanisms were observed, which are dehydration reaction and retro-aldol condensation.

The effect of chemical structures on kinectics characteristics of sugar alcohol was also evaluated for mannitol (kelp compound) and its isomer, sorbitol. For comparable study, similar experimental conditions were employed for these isomers. In this study, first-order reaction and Arrhenius law were used to explain the kinetics behavior of the sugar alcohols. Apparently, higher decomposition rate was observed for sorbitol than mannitol, which likely due to its hydroxyl configuration that allows more interaction between water and its carbon atoms. Nevertheless, the reaction rates of sorbitol and mannitol were significantly higher than that obtained in previous studies.

Perhaps, dissimilar reaction mechanisms were involved between the present and previous studies due to the different amounts of water availability.

Besides the kelp compounds, behavior of other carbohydrates that are usually presence in macroalgae was also elucidated in this research work. Again, kinetics characteristic was investigated for sugars that have almost similar structure but different functional groups, namely glucose (aldehyde), glucuronic acid (carboxylic), and sorbitol (sugar alcohol). The result obtained was in a good agreement with Chapter 7 that was also observed higher reactivity of uronic acid than the others, which probably due to the high electronegativity of its functional group. Perhaps, slower decomposition rate of glucose was due to its isomerization, which consequently formed a strong double bond.

10.2 Recommendation

Despite the successful finding obtained in the entire study, there are several areas of improvement that could be considered for better outcomes. The recommendations for future work are as follows:

1) In chapter 5, it was reported that the recovery yield of uronic acids was rather low than the expectation. Thus, extra attention could be given during downstream processing (i.e. conventional filtration), which was expected to be the main reason for product losses.

2) Besides that, drying process of uronic acids could be conducted by using freeze-drying method instead of drying in desiccator, in order to accelerate the drying time.

3) During the hydrothermal treatment of uronic acids whose results were discussed in Chapter 6, the concentration of unreacted uronic acid in liquid product was significantly low. Perhaps, the temperatures (170 to 250 °C) employed in this study were too severe for the uronic acids. Thus, it would be better to conduct additional experiments at lower range of temperatures around 100 to 200 °C in order to have better overview on the kinetics behavior.

4) Two kinds of reaction mechanism were identified during hydrothermal treatment in Chapter 7, which consequently produced 5-HMF and glycolaldehyde. Perhaps, further analysis could be conducted on the decomposition products to identify other possible products too. When more compounds are identified, possible reaction pathway could be proposed.

5) In chapter 8, the recovery yields of sorbitol and mannitol were significantly higher than that obtained in previous study. It was assumed that different reaction mechanisms were involved between these studies. In order to ensure that the assumption is correct, higher concentration of feedstock (similar like previous studies) should be employed in future study.

6) The results obtained in chapters 7 and 9 revealed the susceptibility of uronic acids than the aldohexoses and sugar alcohols. Perhaps, comparison with other uronic acids could be conducted under the same experimental condition so as to obtain general overview on behavior of uronic acids under hydrothermal condition.

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