Thesis for the Degree of Master of Engineering

Effect of Acid Hydrolysis for Volatile Fatty Acids Production from Alginate

by

Huiqing Jin

Department of Chemical Engineering

The Graduate School

Pukyong National University

February 2016

Effect of Acid Hydrolysis for Volatile Fatty Acids Production from Alginate

알지네이트로부터 휘발성 유기산 생산을 위한 산 가수분해의 영향

Advisor: Prof. Hee Chul Woo

by

Huiqing Jin

A thesis submitted in partial fulfillment of the requirements
for the degree of

Master of Engineering

in Department of Chemical Engineering, The Graduate School,
Pukyong National University

February 2016

Effect of Acid Hydrolysis for Volatile Fatty Acids Production from Alginate

A dissertation

by

Huiqing Jin

Approved by:

(Chairman) Jun Heok Lim

(Member) Do Jin Im

(Member) Hee Chul Woo

February 26, 2016

CONTENTS

CONTENTS i

LIST OF TABLES iii

LIST OF FIGURES iv

ABSTRACT vi

I. INTRODUCTION 1

1.1 Generation backgrounds 1

1.2 Research objectives 3

II. LITERATURE REVIEW 4

2.1 Brown algae 4

2.2 Alginate 6

2.3 Biofuels production 10

2.4 Anaerobic fermentation 13

2.5 Pretreatment 15

2.5.1 Dilute acid pretreatment 17

2.5.2 Solid acid catalyst pretreatment 17

III. MATERIALS AND METHODS 20

3.1 Enrichment of VFAs producing microorganism 20

3.2 Pretreatment 21

3.3 Anaerobic fermentation 24

3.4 Analytical methods 26

IV. RESULTS AND DISCUSSION 28

4.1 Molecular distribution 28

4.1.1 Dilute sulfuric acid pretreatment 28

4.1.2 Amberlyst-15 pretreatment 30

4.2 VFAs yield from pretreated alginate 32

4.2.1 Dilute sulfuric acid pretreatment 32

4.2.2 Amberlyst-15 pretreatment 39

V. CONCLUSIONS 46

REFERENCES 49

ACKNOWLEDGEMENT 57

LIST OF TABLES

Table 1 Chemical composition of macroalgae 5

Table 2 Classification of biofuel generations.....11

Table 3 Advantages and disadvantages of pretreatment methods of biomass 16

Table 4 Physical properties of Amberlyst-15 catalyst..... 19

Table 5 Dilute sulfuric acid pretreatment condition..... 22

Table 6 Amberlyst-15 pretreatment condition..... 23

Table 7 Analytical methods..... 27

LIST OF FIGURES

Figure 1 Structural characteristics of alginates: (a) alginate monomers, (b) chain conformation, and (c) block distribution..... 7

Figure 3 Macroalgae conversion process for biofuel production 12

Figure 4 Schematic of the anaerobic digestion process..... 14

Figure 5 Schematic of (a) a batch reactor and (b) biofuels production system 25

Figure 6 Variation molecular weight of alginate using dilute sulfuric acid; (a) H_2SO_4 conc. (b) Reaction temp. 29

Figure 7 Variation molecular weight of alginate using Amberlyst-15; (a) Amberlyst-15 conc. (b) Reaction temp. 31

Figure 8 Effect of different dilute sulfuric acid conc. (w/w) on VFAs production yield; (a) 0%, (b) 0.5%, (c) 1%, (d) 3%, (e) 5%, (f) 10%, (g) Control, at 120℃, 2h..... 33

Figure 9 Effect of different reaction temp. on VFAs production yield using dilute sulfuric acid; (a) 80℃, (b) 100℃, (c) 120℃, (d) 140℃, (e) 160℃, (f) Control, at 3% w/w, 2h..... 34

Figure 10 Effect of dilute sulfuric acid conc.; (a) yield of TVFAs production (b) yield of individual VFAs production at 4th days of anaerobic

fermentation 36

Figure 11 Effect of reaction temp. using dilute sulfuric acid; (a) yield of TVFAs production (b) yield of individual VFAs production at 4th days of anaerobic fermentation 38

Figure 12 Effect of different Amberlyst-15 conc. (w/w) on VFAs production yield; (a) 0%, (b) 1%, (c) 3%, (d) 5%, (e) 10%, (f) Control, at 120℃, 4h 40

Figure 13 Effect of different reaction temp. on VFAs production yield using Amberlyst-15; (a) 60℃, (b) 80℃, (c) 100℃, (d) 120℃, (e) 140℃, (f) Control, at 5% w/w,4h 41

Figure 14 Effect of Amberlyst-15 conc.; (a) yield of TVFAs production (b) yield of individual VFAs production at 4th days of anaerobic fermentation 43

Figure 15 Effect of reaction temp. using Amberlyst-15; (a) yield of TVFAs production (b) yield of individual VFAs production at 4^{th} days of anaerobic fermentation. 45

Effect of Acid Hydrolysis for Volatile Fatty Acids Production from Alginate

Huiqing Jin

Department of Chemical Engineering, The Graduate School,
Pukyong National University

Abstract

Recently brown algae has received considerable attention as a feedstock for biofuel production due to its fast growth rate and high carbon fixation ability. Alginate is the major component of polysaccharide in brown algae, however, it has low solubility in water and low biodegradability due to its chemical structure. Application of various pretreatments for marine macroalgae can make more simple type carbohydrates and enhance the biofuel productivity. Therefore, the objectives of this study are to evaluate the effects of acid pretreatment on volatile fatty acids (VFAs) production from alginate in anaerobic fermentation. The dilute sulfuric acid and Amberlyst-15 were catalysts for the pretreatment of alginate, and different variables (catalyst concentration and reaction temperature) were investigated in order to enhance VFAs production.

Sodium alginate of 10 g/L was pretreated in an autoclave reactor with a working volume of 100 mL. The influence of different catalyst concentrations were varied ranging from 0.5% to 10 % (w/w) at 120℃ for 2 h in dilute sulfuric acid, and from 1% to 10% (w/w) at 120℃ for 4 h in Amberlyst-15. The effect of the reaction temperatures were conducted from 80 to 160℃ at 3% (w/w) for 2 h in dilute sulfuric acid, and from 60 to 140℃ at 5% (w/w) for 4 h in Amberlyst-15. A series of anaerobic fermentation tests were carried out in a glass bottle of 250 mL with working a volume of 200 mL and placed in a shaking incubator of 120 rpm at 35℃.

The molecular weight of alginate for all conditions was significantly reduced by acid pretreatment, and increases of catalyst concentrations and reaction temperatures. The maximum VFAs production yield of 45.4% was obtained with 3%(w/w) at 120℃ for 2 h in dilute sulfuric acid and the yield of 18.1% was obtained with 5%(w/w) at 80℃ for 4 h in Amberlyst-15. These results indicate that acid pretreatment of alginate could be affected by the reduction of molecular weight, and could enhance the VFAs production compared with raw alginate.

I. INTRODUCTION

1.1 Generation backgrounds

Environmental issues, including air pollution and global warming caused by the combustion of fossil fuels, have been increasingly affecting international society and economy. Accordingly, the generation of energy from sources other than fossil fuels is necessary for the reduction of global greenhouse gas emissions. Biofuels are potential alternative energy sources because they offer various benefits related to economics, energy security, and environmental impacts.

Marine macroalgae have recently received considerable attention as feedstock for biofuel production [1]. Because macroalgae such as green-, red-, brown algae have high growth rates, high carbon fixation ability, no land supplement, and easy adaptability to various growing environments from diverse resources, they are significantly being considered as attractive biofuel resources with advantages over terrestrial biomass (i.e., energy crops and lignocellulosic biomass) [2].

In particular, massive brown algae, which are primarily composed of

polysaccharides such as alginate and fucoidan with mannitol and laminaran [3, 4], are being considered as carbon sources of biofuel production. Theoretically, a high yield of organic acids can be generated and used as the intermediated substrate for biofuels production (i.e., biomethane, biohydrogen, biodiesel, and microbial fuel cell etc.). Alginate is the major polysaccharide accounting for up to 40% in brown algae [3]. According to its chemical structure, alginate is not readily hydrolyzed by conventional microorganisms for the biological conversion process [5]. The maximum production of volatile fatty acids (VFAs) from no pretreated alginate was still low, according to a recent report [3]. In this standpoint, further treatment, such as pretreatment, is required to enhance the bioconversion efficiency of alginate in brown algae to biofuel production. To improve the biodegradability of the biomass feedstock, pretreatment has been widely used. Based on the pretreatment methods used for lignocellulosic biomass, several pretreatments have been applied to macroalgae for biofuel pretreatment, including hydrothermal, wet oxidation, ball milling and acid pretreatments [4, 12, 45]. Although various pretreatment methods have been proposed, the suitable pretreatment method should depend on the types of biomass and the biological conversion process being used.

1.2 Research objectives

The acid pretreatment, which has high selectivity and easy availability, among the various pretreatments was considered to have high selectivity for improvement of biodegradability of alginate in this study. The objectives of this research were to evaluate the effect of acid pretreatment on VFAs production from alginate in anaerobic fermentation. The alginate were pretreated with dilute sulfuric acid and Amberlyst-15, and were carried out under different catalyst concentration and reaction temperature on VFAs production.

II. LITERATURE REVIEW

2.1 Brown algae

Macroalgae are macroscopic marine plants or seaweeds which are excellent candidates for biofuel production. Macroalgae with few or zero lignin and low cellulose content, form essential structural components of terrestrial plants. Therefore, it makes macroalgae on average a relatively easy material to digest by microbes in the biofuel production process [8, 9].

Macroalgae are divided into three major groups based on their photosynthetic pigments: brown algae (*Phaeophyceae*), red algae (*Rhodophyta*), green algae (*Chlorophyta*) [12]. The composition of macroalgae are listed in table 1.

Brown algae are an important assemblage of plants that are classified in 19 orders, and 265 genera with more than 1800 species [13]. Brown algae are naturally abundant in nearshore coastal waters with suitable substrate for attachment in low temperatures between 6 and 14℃. Most brown algae contains the pigment fucoxanthin, which is responsible for the distinctive greenish-brown color that gives them their name.

Table 1 Chemical composition of macroalgae [18, 19]

Division	Macroalgae		
	Green algae	Red algae	Brown algae
Species	Green laver, seastaghorn	Laver, alger-alger	Brown-seaweed, kelp
Water [a]	70 – 85%	70 – 80%	79 – 90%
Minerals [b]	10 – 25%	25 – 35%	30 – 50%
Carbohydrates (main compoments)	25 – 50% (Cellulose, Starch)	30 – 60% (Agar, Carrageenan)	30 – 50% (Algiante, Fucoidan)
Proteins [b]	10 – 15%	7 – 15%	7 – 15%
Lipids [b]	1 – 2%	1 – 5%	2 – 5%

a: wet basis b: dry basis

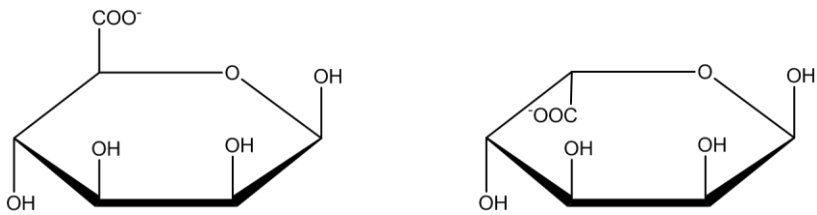
2.2 Alginate

Alginic acid, also called align or alginate, is an anionic polysaccharide distributed widely in the cell walls of brown algae. Its color ranges from white to yellow-brown.

Alginate is now known to be a whole family of linear copolymers containing blocks of (1,4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues. The blocks are composed of consecutive G residues (GGGGGG), consecutive M residues (MMMMMM), and alternating M and G residues (GMGMGM). The structures of alginate monomers, chain conformation and block distribution are illustrated in Figure 1 (a), (b) and (c) respectively.

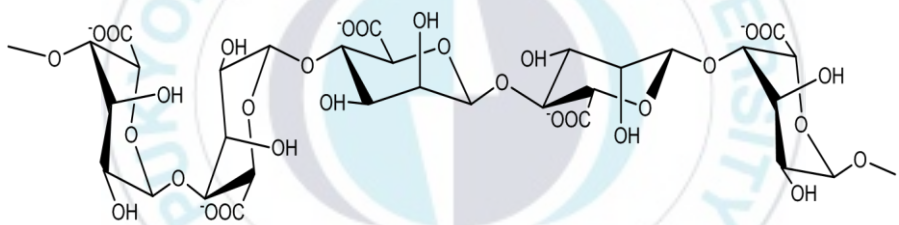
Alginate is presented in the cell walls of brown algae as the calcium, magnesium and sodium salts of alginic acid. The goal of the extraction process is to obtain dry, powdered, sodium alginate. The calcium and magnesium salts do not dissolve in water; the sodium salt does. The alginate industry extraction process is divided into five steps: acidification, alkaline extraction, solid and liquid separation, precipitation and drying [41]. The principle of alginate extraction from seaweed is illustrated in Figure 2.

(a)



β-D-mannuronate (M) **α-L-guluronate (G)**

(b)



G **G** **M** **M** **G**

(c)

Figure 1 Structural characteristics of alginates: (a) alginate monomers, (b) chain conformation, and (c) block distribution [13].

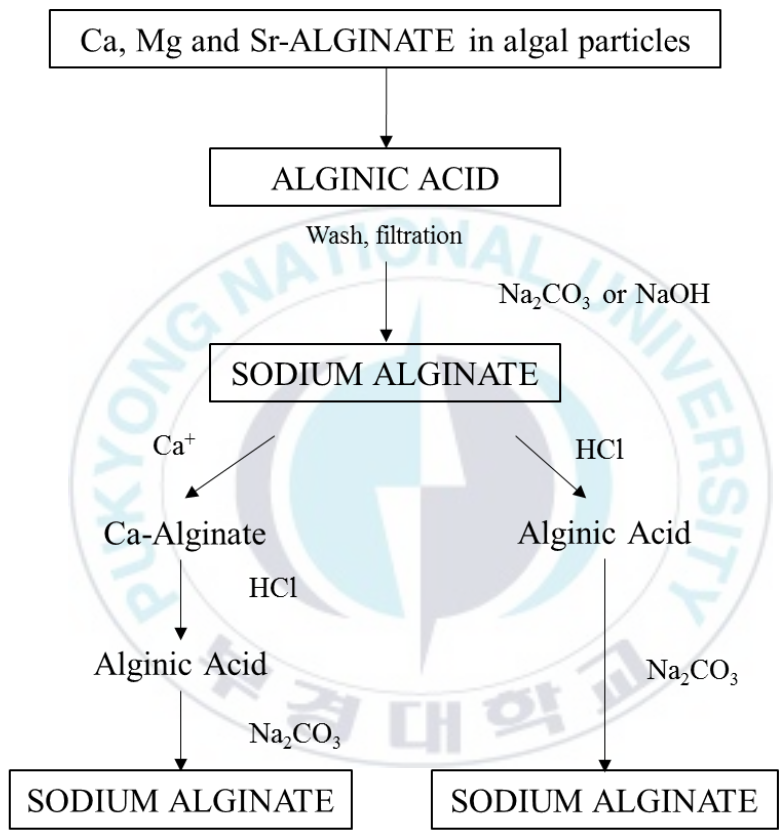


Figure 2 Principle scheme of the alginate extraction from seaweeds.

The uses of alginates are based on three main properties. The first is their ability, when dissolved in water, to thicken the resulting solution. The second is their ability to form gels. The gel forms by chemical reaction, the calcium displaces the sodium from the alginate, holds the long alginate molecules together and a gel is the result. The third property of alginates is the ability to form films of sodium or calcium alginate and fibres of calcium alginates.

2.3 Biofuels production

Biofuels are liquid, gas and solid fuels produced from biomass. Biofuels are divided into three generations in relation to its sources. First generation biofuels are produced directly from food crops by abstracting the oils for use in biodiesel or producing bioethanol through fermentation. Second generation biofuels have been developed to overcome the limitations of first generation biofuels. They are produced from non-food crops such as wood, or organic waste. The third generation of biofuels is based on improvements in the production of biomass. It takes advantage of specially engineered energy crops such as algae as its energy source (Table 2).

The algae can be converted into various types of renewable biofuel including bioethanol, biodiesel, biogas, photobiologically produced biohydrogen, and further processing for bio-oil and syngas production through liquefaction and gasification, respectively. The conversion technologies for utilizing algal biomass to energy sources can be categorized into three different ways, such as biochemical, chemical, and thermochemical conversion, as well as macroalgal biorefinery [11], which has been depicted in Figure 3.

Table 2 Classification of biofuel generations [18]

Generation	1st	2st	3st
Biomass	Sugar-based ethanol, plant oil-based biodiesel (~50% CO2 ↓)	Non-edible biomass (wastes,lignocellulose)- based biofuel (80~90% CO2 ↓)	Improved plants or algae-based biofuel
Process	Bioethanol - Fermentation Biodiesel - Transesterification	Bioprocess - Pretreatment, Saccharification, Fermentation, Algal biotechnology Chemical process - Gasification, Catalysis, FT (BLT)	Bioprocess - Pretreatment, Saccharification, Fermentation, Algal biotechnology Chemical process - Gasification, Catalysis, FT (BLT)
Products	Bioalcohol - Ethanol Biodiesel - FAME	Gasoline - Ethanol - Butanol - Long-chain alcohols - Hydrocarbons Diesel - FT Diesel - Hydrocarbon	Gasoline - Ethanol - Butanol - Long-chain alcohols - Hydrocarbons Diesel - FT Diesel - Algal oil

FT: Fischer-Tropsch, BLT: Biomass-to- liquid, FAME: Fatty Acid Methyl Ester

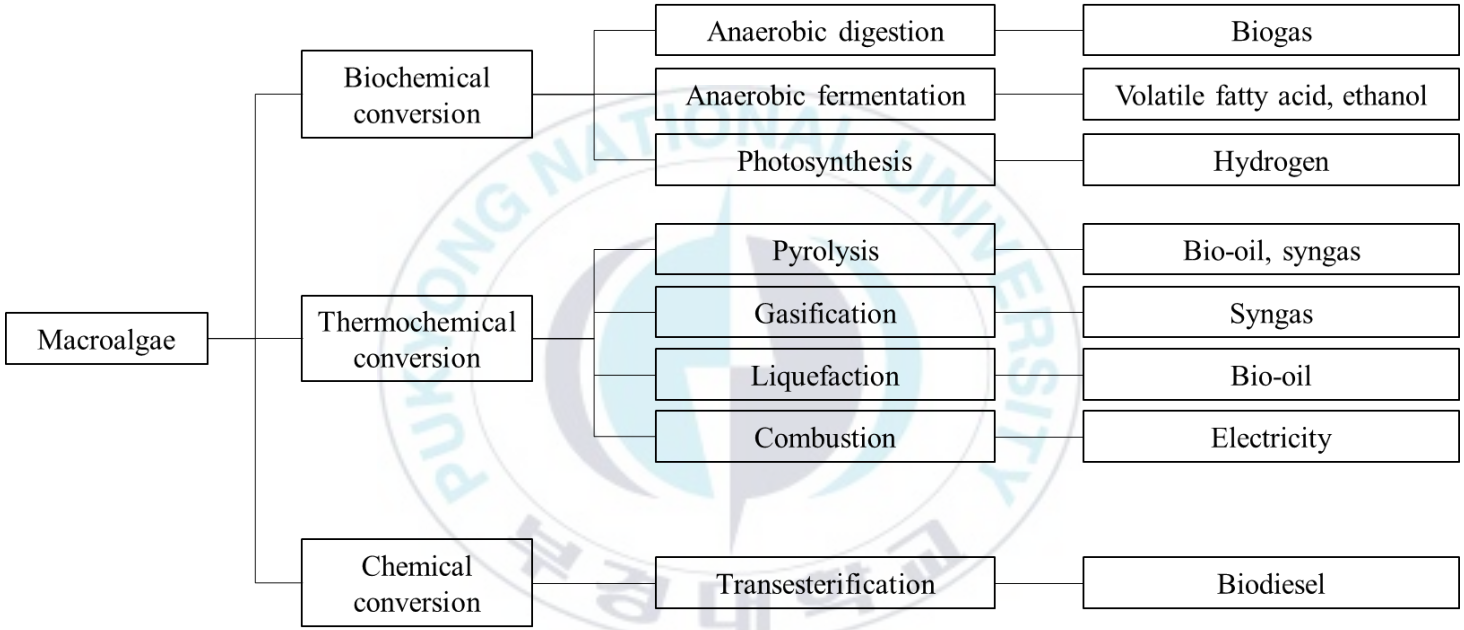


Figure 3 Macroalgae conversion process for biofuel production [11].

2.4 Anaerobic fermentation

Anaerobic fermentation is a method cells use to extract energy from carbohydrates when oxygen or other electron acceptors are not available in the surrounding environment. It is the second stage in the four stages of anaerobic digestion (Figure 4).

In the first stage of hydrolysis, bacteria transform the particulate organic substrate into liquefied monomers and polymers, i.e. proteins, carbohydrates and fats transformed to amino acids, monosaccharides and fatty acids respectively. In the second stage of acidogenesis, acidogenic bacteria transform the products of the first reaction into short chain volatile acids. The products are acetic acid, propionic acid, butyric acid, formic acid, lactic acid, ethanol and methanol, among others. In the third stage of acetogenesis, the rest of the acidogenesis products are transformed by acetogenic bacteria into carbon dioxide, hydrogen and mainly acetic acid.

During the fourth stage of methanogenesis, microorganisms convert the hydrogen and acetic acid formed by the acid formers to methane gas and carbon dioxide.

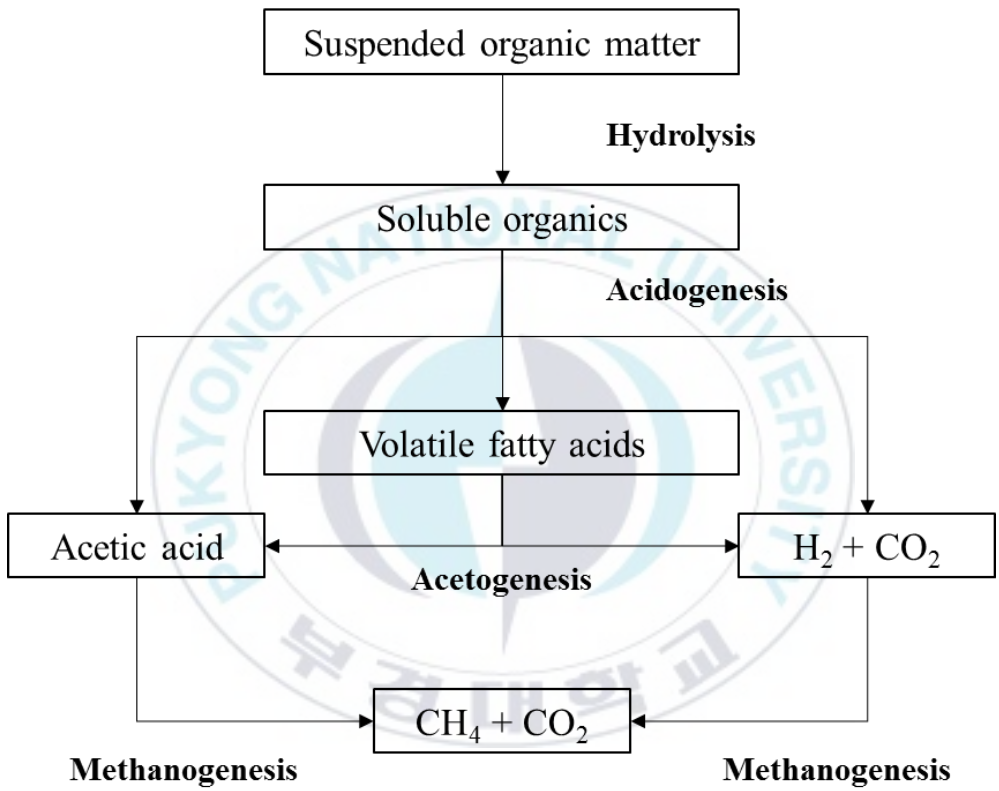


Figure 4 Schematic of the anaerobic digestion process [44].

2.5 Pretreatment

The purpose of pretreatment is to break down the shield formed by lignin and hemicellulose, to disrupt the crystalline structure and reduce the degree of polymerization of cellulose. Pretreatment techniques have generally been divided into three distinct categories, including physical, chemical, and biological pretreatment.

Physical pretreatment aimed at reducing the particle size and crystallinity of lingo-cellulosic, such as milling processes and high-energy radiation (such as microwave irradiation at different oven's irradiation power), is expensive [40]. Biological pretreatment with lignin-degrading microorganisms is energy-saving but it takes a long time to enrich sufficient lignin-degradation microorganisms [31]. However, chemical pretreatment is simple and quick and may have potential. Chemical pretreatment is the most studied pretreatment technique among all of the pretreatment categories [23]. Table 3 summarises the advantages and disadvantages of pretreatment technologies.

Table 3 Advantages and disadvantages of pretreatment methods of biomass

Pretreatment method	Advantages	Disadvantages
Hydrothermal	-Chemical are not needed -Solubilized hemicellulose	-Works test for feedstocks with low lignin -High temperature and pressure needed
Dilute acid	-High glucose yield -Solubilization hemicellulose -Hydrolysis of hemicellulose -Low residence time	-High cost of acids and for recovery -Needs high temperature -Formation of inhibitor
Alkali	-Utilize low temperature and pressure -Low inhibitor formation -Low sugar loss -Feedstock flexible	-High residence time -Bases can not recovered
Solid acid catalyst	-Environmental friendly -Recyclable -Easily separated from production	-Low stability -Longer reaction duration
Ionic liquid	-Lignin can be recovered -Feedstock flexible -Solvents are not volatile	-Solvents can inhibit fermentation -Cost of solvent

2.5.1 Dilute acid pretreatment

Acid pretreatment was derived from the concentrated acid hydrolysis such as concentrated H_2SO_4 and HCl hydrolysis. Concentrated acid is powerful and effective for cellulose hydrolysis, but it is toxic, corrosive and hazardous and requires a reactor that needs expensive construction material which is resistant to corrosion. Additionally, the concentrated acid must be recovered and recycled after hydrolysis to render the process economically feasible. Alternatively, dilute acid pretreatment has received numerous research interests. It has been successfully developed for the pretreatment of lignocellulosic biomass. Several different acids, including dilute sulfuric acid, dilute nitric acid, dilute hydrochloric acid and peracetic acid, have been reported in the literature.

Dilute acid hydrolysis is performed in two different conditions, namely, high temperature (T >160 ℃) in continuous mode for low solid loading and lower temperature (T≤160 °C) in batch mode for high solid loading.

2.5.2 Solid acid catalyst pretreatment

Solid acid catalyst pretreatment is considered essential for the hydrolysis process, in terms of economical and environmental factors. In order to ensure an efficient process, the solid acid catalysts should be

water-tolerant, have strong acidic sites and surface area. Recently, several studies have reported on the use of various types of solid acid catalysts for the hydrolysis of disaccharides with the aim to ascertain its activity [28].

Amberlyst-15 is one of the powerful solid acid catalysts which is often used in organic synthesis. Amberlyst-15 is mild and highly selective, commercially available, is an excellent strong acid source, is chemically and physically stable, non-toxic, non-corrosive, removable and reusable. Amberlsyt-15 is a brown grey solid which has the following physical properties (Table 4).

Table 4 Physical properties of Amberlyst-15 catalyst

Property	
Ionic form as shipped	hydrogen
Concentration of active sites	≥1.7 eq/L; ≥4.7 eq/kg
Moisture holding capacity	52 to 57% (H+ form)
Shipping weight	770 g/L
Particle size	0.600 to 0.850 mm
Average pore diameter	300Å
Total pore volume	0.40 mL/g

III. MATERIALS AND METHODS

3.1 Enrichment of VFAs producing microorganism

The anaerobic sludge was taken from an anaerobic digester in Su-young waste-water treatment plant, Busan, Korea and then sieved through a 850 μm (20 mesh) for removal of larger particle in anaerobic sludge. It was acid-pretreated using 2 N HCl at 35℃ for 24 h in water bath in order to enhance activity of VFAs producing microorganism and inhibit methanogenesis phase. Inoculum system was continuously operated at 35℃, pH 5.5 and 120 rpm with working volume of 2 L for enrichment of VFAs producing microorganism. The substrate medium contained 20 g/L of glucose as a sole carbon source and inorganic supplements including 5.2 g/L NH_4HCO_3, 120 mg/L K_2HPO_4, 100 mg/L $MgCl_2 \cdot 6H_2O$, 15 mg/L $MnSO_4 \cdot 6H_2O$, 25 mg/L $FeSO_4 \cdot 7H_2O$, 5 mg/L $CuSO_4 \cdot 5H_2O$, 0.5 mg/L $CoCl_2 \cdot 5H_2O$, and 6.7 g/L $NaHCO_3$ [20]. Before the experiments, the glucose-acclimatized sludge was centrifuged at 2500 rpm for 3 min and supernatant part was removed. The rest part was washed out using distilled water to remove remaining substrate and other nutrients. After these procedure was repeated three times and then applied to batch experiment

for microorganism source.

3.2 Pretreatment

The sodium alginate (80-120 mPa·s, Wako Pure Chemical Industries Ltd., Osaka, Japan) of 10 g/L was pretreated in autoclave equipped with Teflon-lined stainless steel with working volume of 100 ml under different catalyst concentrations and reaction temperatures as shown in table 5 and 6. The catalyst concentrations were varied with ranging from 0.5% to 10% (w/w) at 120℃ for 2 h using 1 M H_2SO_4 in dilute sulfuric acid pretreatment, and from 1% to 10% (w/w) at 120℃ for 4 h in Amberlyst-15 pretreatment. The reaction temperatures were operated from 80 to 160℃ at 3% (w/w) for 2 h in dilute sulfuric acid pretreatment, and from 60 to 140℃ at 5% (w/w) for 4 h in Amberlyst-15 pretreatment.

Table 5 Dilute sulfuric acid pretreatment condition

No.	Catalyst conc. (% w/w)	Reaction temp. (℃)	Time (h)
HC1	0		
HC2	0.5		
HC3	1		
HC4	3	120	2
HC5	5		
HC6	10		
HT1		80	
HT2		100	
HT3	3	120	2
HT4		140	
HT5		160	

HC: H_2SO_4 conc., HT: H_2SO_4 temp.

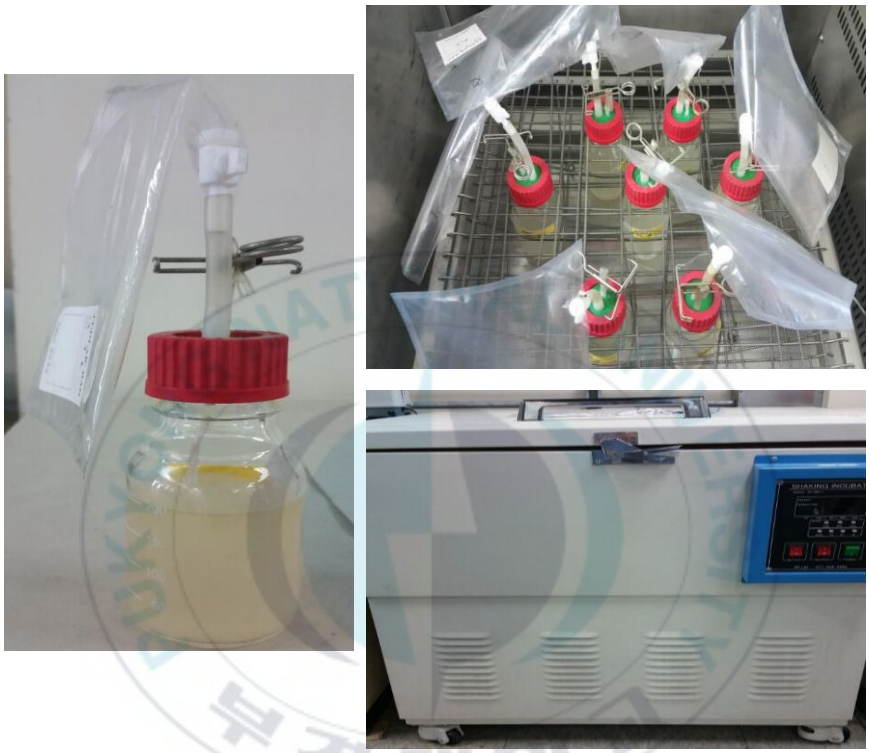
Table 6 Amberlyst-15 pretreatment condition

No.	Catalyst conc. (% w/w)	Reaction temp. (℃)	Time (h)
AC1	0		
AC2	1		
AC3	3	120	4
AC4	5		
AC5	10		
AT1		60	
AT2		80	
AT3	5	100	4
AT4		120	
AT5		140	

AC: Amberlyst-15 conc., AT: Amberlyst-15 temp.

3.3 Anaerobic fermentation

Anaerobic fermentation were conducted in 250 mL glass bottle with 200 mL of working volume. The glucose-acclimated sludge was seeded 5% (v/v) 10 mL of the sludge and nutrient were added into the glass bottle containing 180 mL of pretreated samples. The nutrient composition were 2 g/L NH_4HCO_3, 1 g/L KH_2PO_4, 0.01 g/L $MgSO_4 \cdot 7H_2O$, 0.001 g/L NaCl, 0.001 g/L $NaMoO_4 \cdot 2H_2O$, 0.001 g/L $CaCl_2 \cdot 2H_2O$, 0.0015 g/L $MnSO_4 \cdot 7H_2O$, 0.00388 g/L $FeCl_2 \cdot 4H_2O$ in distilled water. The initial pH was adjusted to 7.6 using 5 N HCl and 5 N NaOH and purged with N_2 gas for 5 min to create anaerobic condition. All experimental glass bottles were incubated at 35℃ in a shaking incubator at 120 rpm to provide better contact between substrate and microorganism.



(a) (b)

Figure 5 Schematic of (a) a batch reactor and (b) biofuels production system.

3.4 Analytical methods

The pretreatment sample was quantified for molecular weight using high performance liquid chromatography HPLC (Utimate 3000, Dionex, USA) which was configured with three types of Waters Ultrahydrogel columns: 120, 500, 1000 in series. Sodium azide solution (0.1 M) was used as a mobile phase at 1 mL/min at 40℃. And measured for total organic carbon (TOC) using TOC analyzer (TOC-VCPH, Shimadzu, Japan).

The fermentation sample was measured for pH values using pH meter, and supernatant extracted from centrifugation at 2500 rpm for 10 min was filtered through a 0.2 μm membrane filter for further analyses. The VFAs (C_2-C_6) were quantified using HPLC (LC-20, Shimadzu, Japan) equipped with an ultraviolet (UV) detector using Aminex HPX-87H column. Every analysis was performed at 65℃ under isocratic condition with 2.5 mM H_2SO_4 as mobile phase. The yield of total volatile fatty acids (TVFAs) (g carbon in TVFAs/g carbon in substrate) was calculated as the carbon amount of TVFAs produced divided by the soluble carbon amount of substrate feeding. The carbohydrate concentration was determined using the phenol-sulfuric acid method. All analytical methods were shown in the Table 7.

$$TVFAs\ yield\ (\%) = \frac{carbon\ amount\ in\ TVFAs\ production\ (g)}{carbon\ amount\ of\ in\ algiante\ (g)}$$

Table 7 Analytical methods

Phase	Parameters	Sampling point	Analytical method
Pretreatment	Molecular weight	After	HPLC (Utimate 3000, Dionex, USA)
	Total organic carbon (TOC)	After	TOC analyzer (TOC-VCPH, Shimadzu, Japan)
Fermentation	pH	Everyday	pH meter (SevenCompact, Mettler Toledo, Korea)
	VFAs*	Everyday	HPLC (LC-20, Shimadzu, Japan)

* acetate, propionate, iso-butyrate, n-butyrate, n-valerate, lactate and formate

IV. RESULTS AND DISCUSSION

4.1 Molecular distribution

4.1.1 Dilute sulfuric acid pretreatment

Figure 6 showed the molecular weight of raw alginate and dilute sulfuric acid pretreated alginate. Clearly, by using the dilute sulfuric acid pretreatment, the molecular weight of alginate decreased as the catalyst concentration and reaction temperature was increased. The reduction of molecular weight is probably by shortening the polymer chain because of glycosidic bond breakage [42]. As shown in figure 6(a), the molecular weight of alginate from 345,902 to 49,622 Da was obtained for catalyst concentration which increased from 0.5% to 10% (w/w). From this result, the molecular weight of alginate was significantly reduced by catalyst concentration compared with catalyst-free hydrothermal pretreatment at 120℃ and raw alginate. Also, a molecular weight of alginate from 579,838 to 2,504 Da was obtained when the reaction temperature increased from 80 to 140℃ (Figure 6(b)).

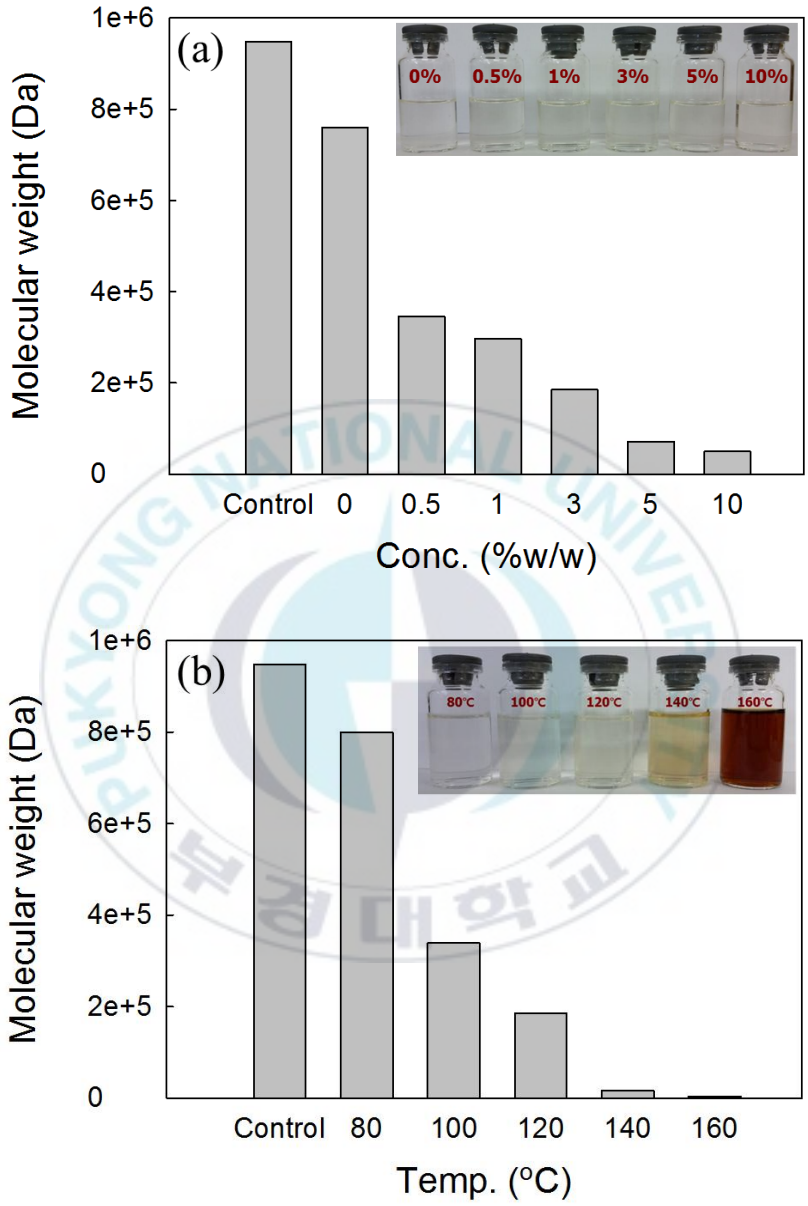


Figure 6 Variation molecular weight of alginate using dilute sulfuric acid; (a) H_2SO_4 conc. (b) Reaction temp.

4.1.2 Amberlyst-15 pretreatment

The molecular weight of alginate pretreated under various catalyst concentrations and reaction temperatures by Amberlyst-15 was shown in Figure 7(a) and (b), respectively. Compared to the alginate without pretreatment (727,919 Da) and catalyst-free hydrothermal pretreatment (158,587 Da), the molecular weight of alginate was remarkably reduced to below 120,000 Da even though lower Amberlyst-15 concentration was applied which indicates that the addition of the catalyst enhanced the depolymerization of the alginate. The increasing thermal energy gives rise to a decrease of relative dielectric permittivity [43], which catalysis the hydrolysis of alginate more effectively. Furthermore, the molecular weight of alginate with reaction temperature of 60, 80, 100, 120, 140℃ was reduced from 808,650 to 596,854, 156,913, 24,318 and 3,950 Da, respectively. Thus, the molecular weight significantly reduced with increasing reaction temperature compared with the untreated alginate of 889,701 Da. Previously, it was shown that the depolymerization of alginate was clearly accelerated by reaction temperature [43], which is in agreement with our results. As a consequence, increasing the Ambelyst-15 concentrations and reaction temperatures were efficient for the depolymerization of alginate.

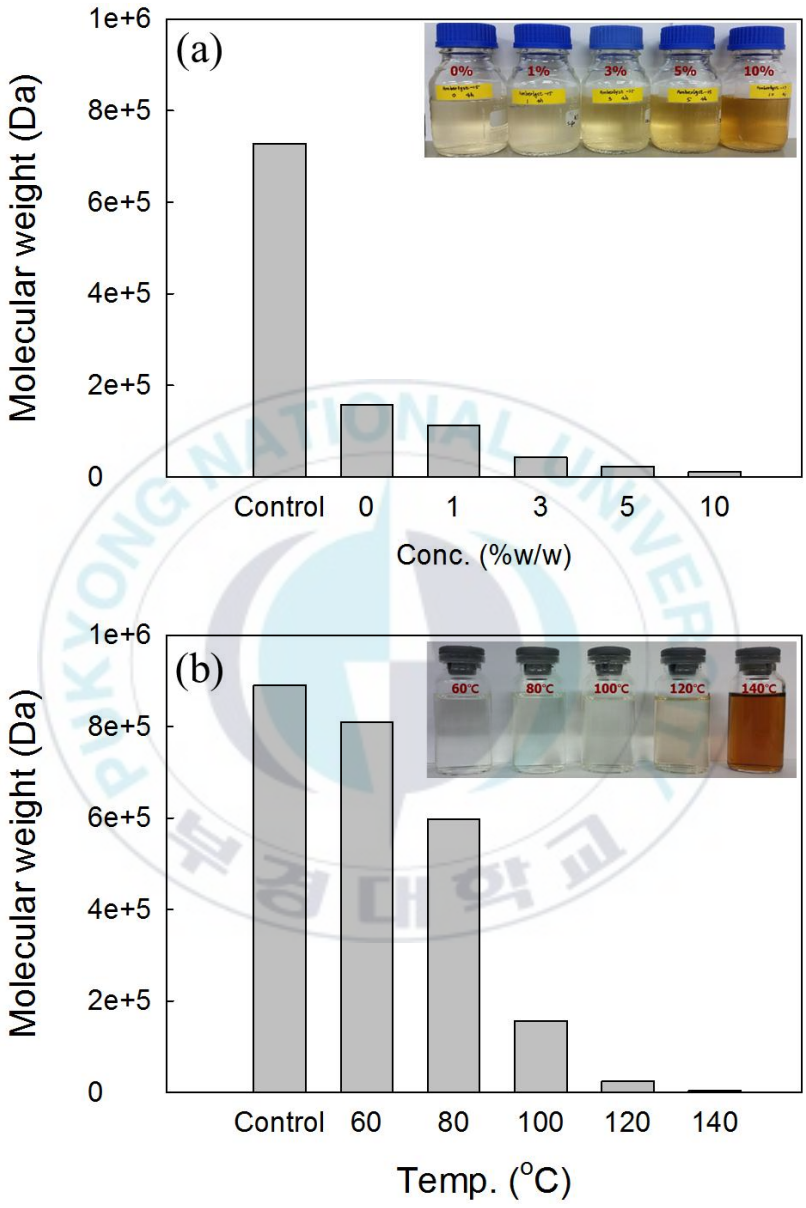


Figure 7 Variation molecular weight of alginate using Amberlyst-15; (a) Amberlyst-15 conc. (b) Reaction temp.

4.2 VFAs yield from pretreated alginate

4.2.1 Dilute sulfuric acid pretreatment

In this study, acetic, propionic, iso-butyric, n-butyric, and others (n-valeric, latic and formic acid) were measured at different catalyst concentrations and reaction temperatures. As shown in figure 8 and figure 9, acetic acid was the most prevalent VFAs, which was probably due to its stability compared to other VFAs [36]. High acetic acid was promising since acetic acid is the main feedstock for biomethane production via anaerobic fermentation [37]. After 8 days of fermentation, acetic acid slowly decreased, it was well-known that acetic acid could be degraded directly by methanogens [39]. Propionic, iso-butyric, n-butyric were also detected but in smaller percentages (<10.3%). There were several possible reasons for the different components of VFAs, such as the sludge quality, sludge components, pretreatment methods, and degradation conditions.

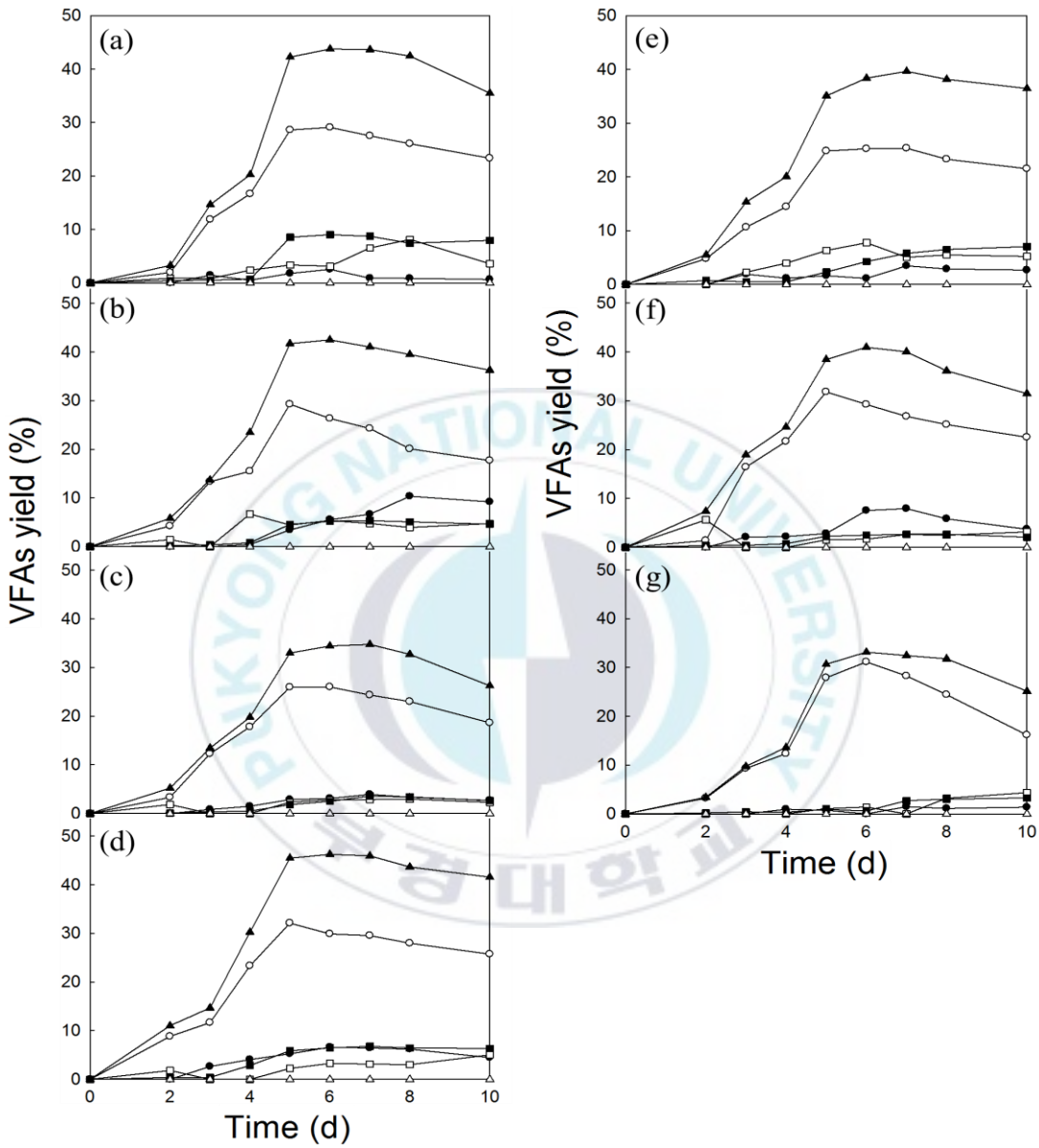


Figure 8 Effect of different dilute sulfuric acid conc. (w/w) on VFAs production yield; (a) 0%, (b) 0.5%, (c) 1%, (d) 3%, (e) 5%, (f) 10%, (g) Control, at 120℃, 2h; acetate (○), propionate (●), iso-butyrate (□), butyrate (■), others (△), and total VFAs (▲).

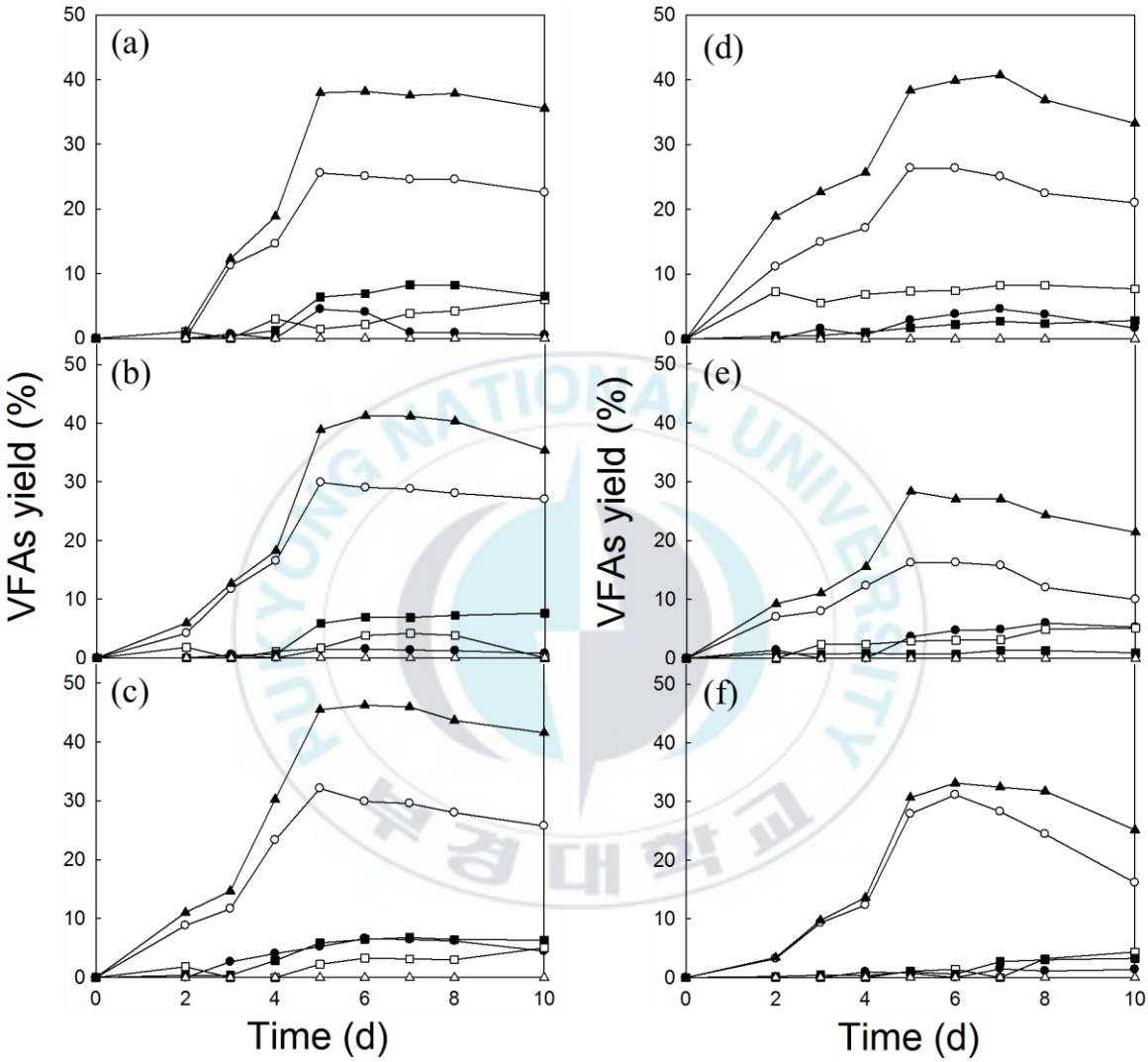


Figure 9 Effect of different reaction temp. on VFAs production yield using dilute sulfuric acid; (a) 80℃, (b) 100℃, (c) 120℃, (d) 140℃, (e) 160℃, (f) Control, at 3% w/w, 2h; acetate (○), propionate (●), iso-butyrate (□), butyrate (■), others (△), and total VFAs (▲).

The TVFAs yielded by different dilute acid concentrations in anaerobic fermentation were shown in Figure 10(a). The maximum TVFAs yield was achieved 45.4% with 3% (w/w) of catalyst concentration at 120℃ for 2 h after 5 days, which was 1.5 times higher than the control set (untreated alginate) of 30.7%. It was mainly due to a large number of soluble minor molecular compounds contained in the pretreated alginate and these soluble compounds could be easily utilized [30]. However, the VFAs yield decreased when the concentration of catalyst was increasing beyond 3% (w/w). This is because higher acid concentration might degrade the sugar compounds and produce more by-product inhibitors such as 5-HMF [35]. Figure 10(b) was the yield of individual VFAs in the TVFAs at the 4th day of fermentation. The TVFAs yield ranging from 19.8% to 30.2% was higher than control set (13.6%), and acetic acid was the major component, accounting for over 90.6% of TVFAs yield.

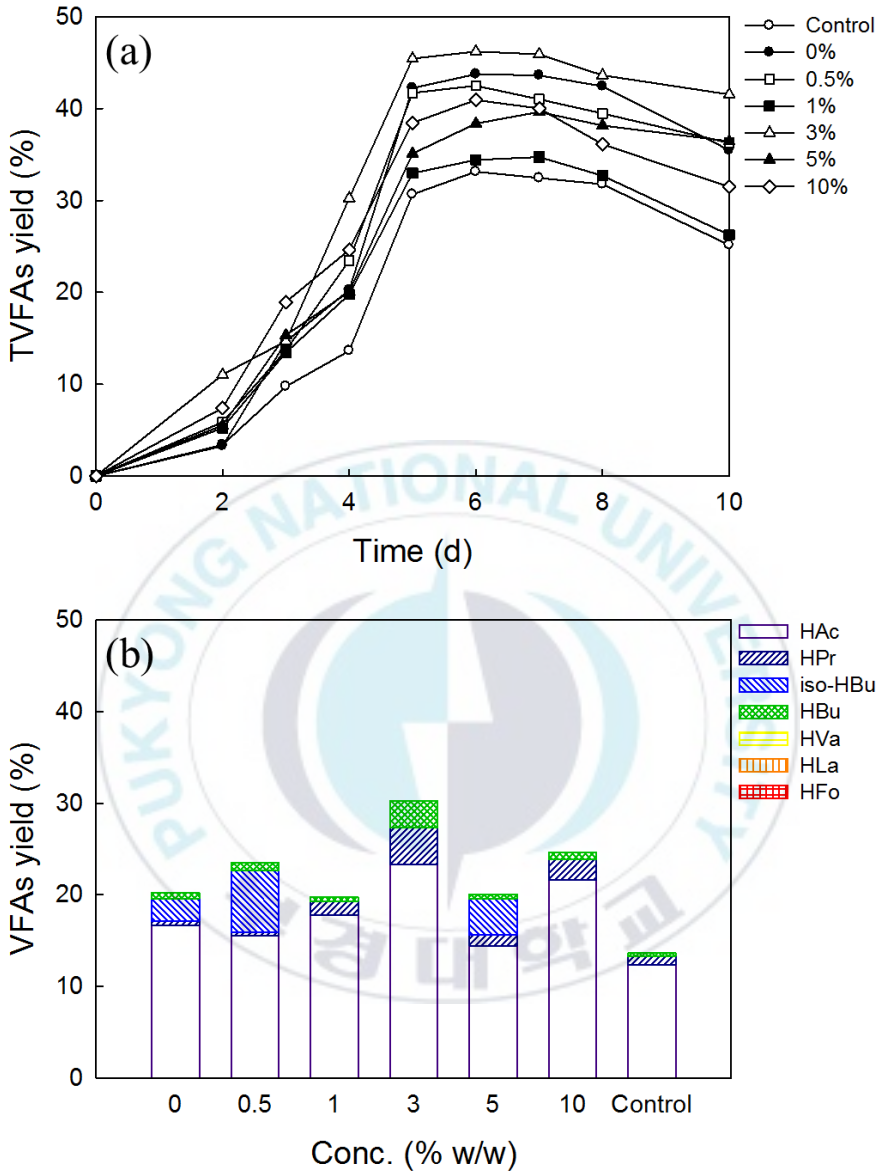


Figure 10 Effect of dilute sulfuric acid conc.; (a) yield of TVFAs production (b) yield of individual VFAs production at 4th days of anaerobic fermentation.

Figure 11(a) showed the effect of reaction temperatures on TVFAs yield in the dilute sulfuric acid pretreatment. The highest TVFAs yield of 45.4% was obtained at 120℃, which was markedly higher than control set of 30.7%. Mostly, after maximum degrees of TVFAs yield was reached on the 5th day, TVFAs yield stayed constant or decreased slightly. A recent study showed that the highest increase of methane production from cassava residues compared to untreated samples was 56.96% and was achieved at 158℃ with an additional 3% (w/w) H_2SO_4 [30]. It can be seen, that the optimum condition was similar with our research. The yield of individual VFAs production on the 4th day was shown in figure 11(b), VFAs yield with ranging from 13.6% to 30.2%. The dominating fermentation products for all conditions were acetic, propionic, iso-butyric and butyric acid.

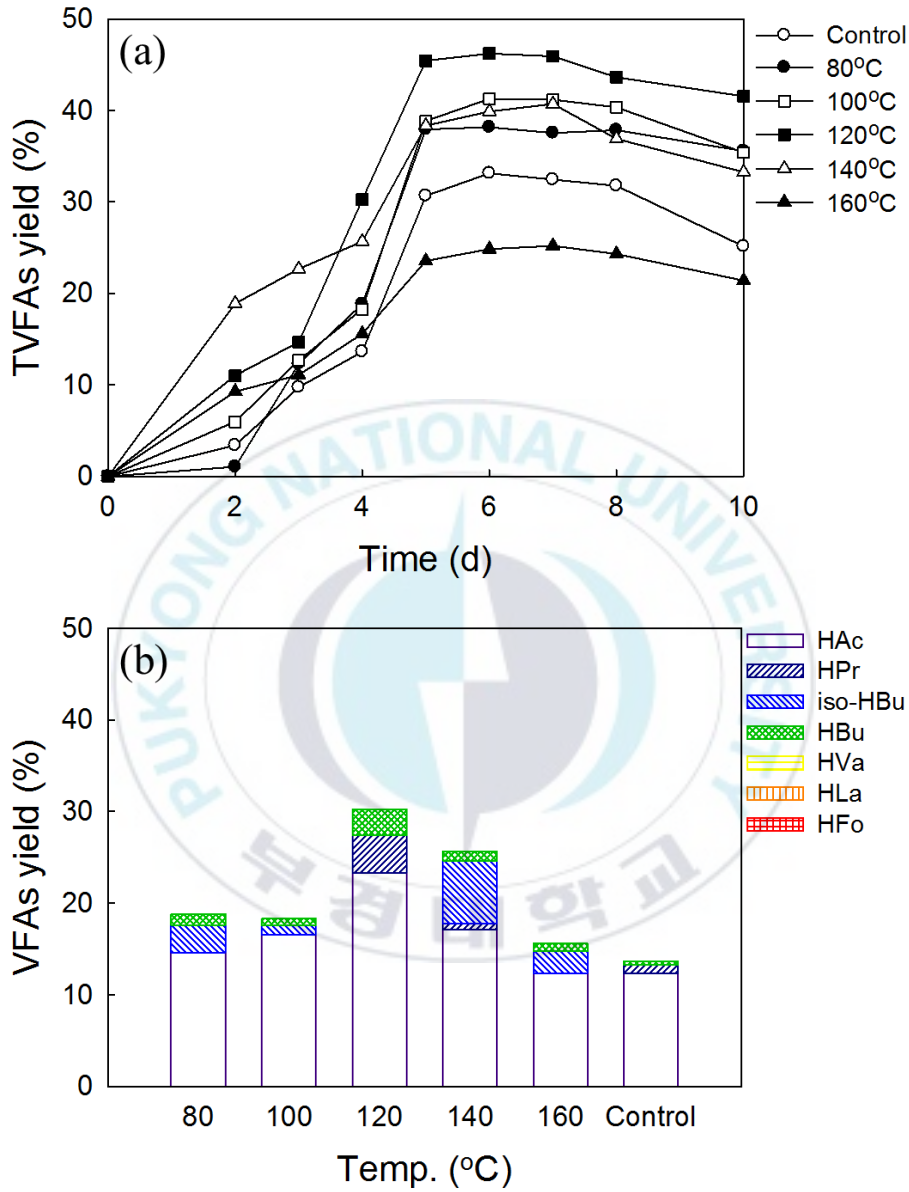


Figure 11 Effect of reaction temp. using dilute sulfuric acid; (a) yield of TVFAs production (b) yield of individual VFAs production at 4th days of anaerobic fermentation.

4.2.2 Amberlyst-15 pretreatment

Figure 12 and 13 showed that, acetic acid was the main constituent with 59% - 89% of the total VFAs. This observation is in line with previous reports that acetic acid was the dominant VFA during anaerobic fermentation of microalgal biomass [38]. Although valeric acid was also detected, its percentage in the total VFAs was less than 1%. And the percentage of formic acid increased up to 16% of total VFAs, but then decreased to zero during fermentation. One possible reason for this, as previously mentioned, is that the formic acid could not be substantially accumulated at a high temperature [37].

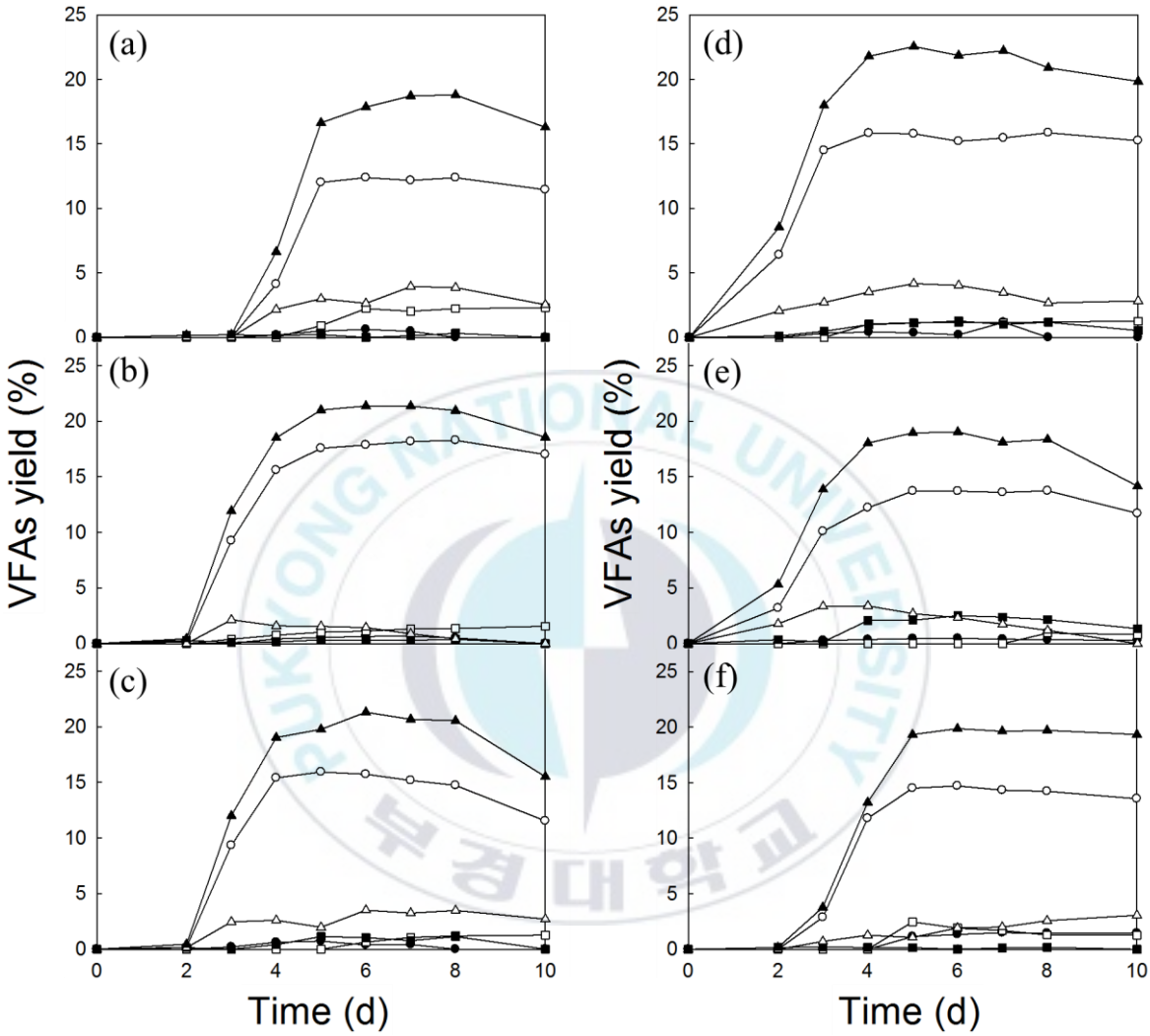


Figure 12 Effect of different Amberlyst-15 conc. (w/w) on VFAs production yield; (a) 0%, (b) 1%, (c) 3%, (d) 5%, (e) 10%, (f) Control, at 120℃, 4h; acetate (○), propionate (●), iso-butyrate (□), butyrate (■), others (△), and total VFAs (▲).

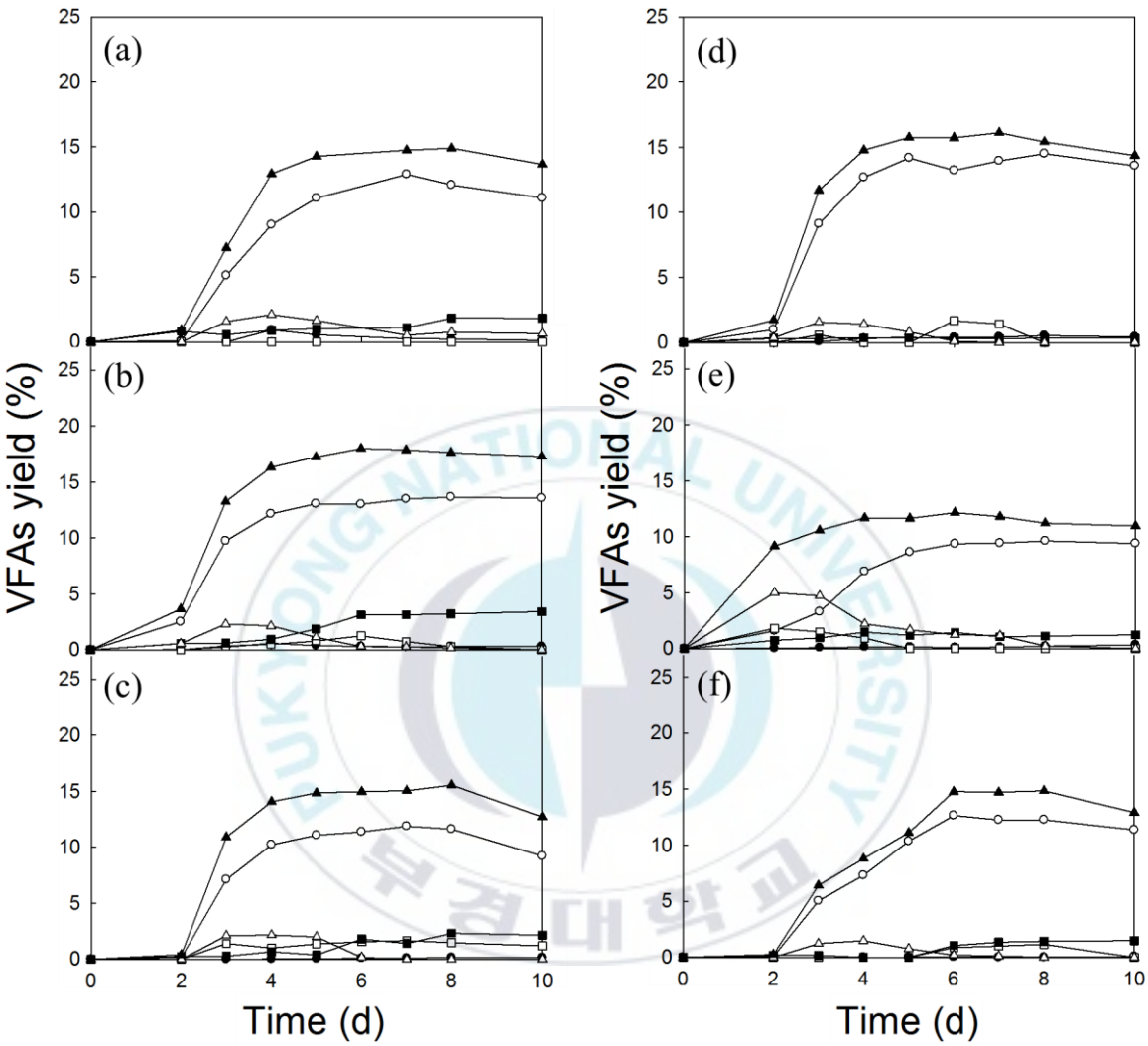


Figure 13 Effect of different reaction temp. on VFAs production yield using Amberlyst-15; (a) 60℃, (b) 80℃, (c) 100℃, (d) 120℃, (e) 140℃, (f) Control, at 5% w/w,4h; acetate (○), propionate (●), iso-butyrate (□), butyrate (■), others (△), and total VFAs (▲).

The effect of Amberlyst-15 concentration on TVFAs yield of alginate was shown in Figure 14(a). As shown in the figure, a higher concentration of Amberlyst-15 accelerates the hydrolysis and anaerobic fermentation and improved the VFAs production rate, compared with 0% (catalyst-free) and control, which can be explained due to the increase in the total number of available active catalytic sites for the reaction [27]. The highest TVFAs yield of 23% was obtained at catalyst concentration of 5% (w/w) on the 4th day. However, the VFAs decreased by over 5% (w/w) of Amberlyst-15. It showed that the alginate pretreated with a high concentration of Amberlyst-15 may the toxicity for microorganism growth and VFAs production. Figure 14(b) showed that, on the 4th day, TVFAs were produced ranging from 13% to 23%, and the dominant product was acetic acid in the range of 62% - 89% of TVFAs in each condition. Even though, a similar range of acetic acid was obtained at the concentration of 1%, 3%, 5% (w/w), higher iso-butyric and butyric acid accumulations were obtained at 5% (w/w). It also showed that the catalyst-free hydrothermal alginate has the lowest performance. This result indicated that Amberlyst-15 has the most significant effect on VFAs production.

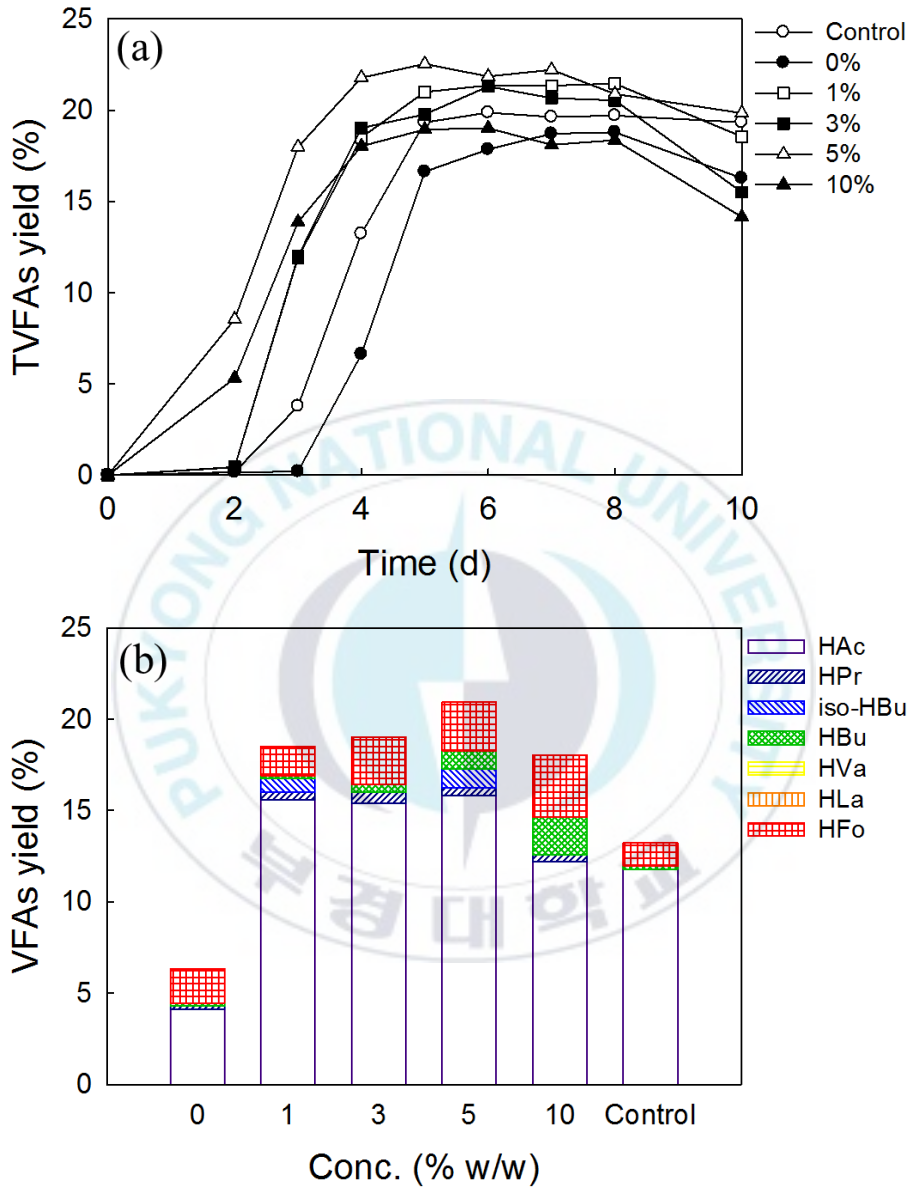


Figure 14 Effect of Amberlyst-15 conc.; (a) yield of TVFAs production (b) yield of individual VFAs production at 4th days of anaerobic fermentation.

Figure 15(a) shows the effect of reaction temperatures on TVFAs production. At 80℃ with 5% (w/w) for 4 h, the highest VFAs yield of 18% was obtained. It can be seen that on the 2nd day for fermentation, at 140℃, the VFAs yield surpassed those obtained at a lower temperature. However, after the 2nd day, instead of increasing, the VFAs stabilized at a yield of 11%. This result indicates that high temperature can cause a negative effect on the VFAs production due to the formation of an undesirable by-product [29]. In addition, Amberlyst-15 may also be deactivated at a higher temperature due to the low thermal stability of the ion-exchange catalyst. Furthermore, a high temperature could also cause the reduction of the catalyst surface area as well as the number of H^+ active sites [28]. On the 4th day of fermentation, after pretreatment, the amount of produced VFAs was much greater than for the untreated alginate (figure 15(b)) and acetic acid was the major component in all conditions, ranging from 59% to 83% of TVFAs. From these results, it could be concluded that the yield of TVFAs production was influenced by reaction temperatures and optimum conditions for VFAs production of alginate of 10 g/L were: 5% (w/w) of Amberlyst-15 at 80℃ for 4 h.

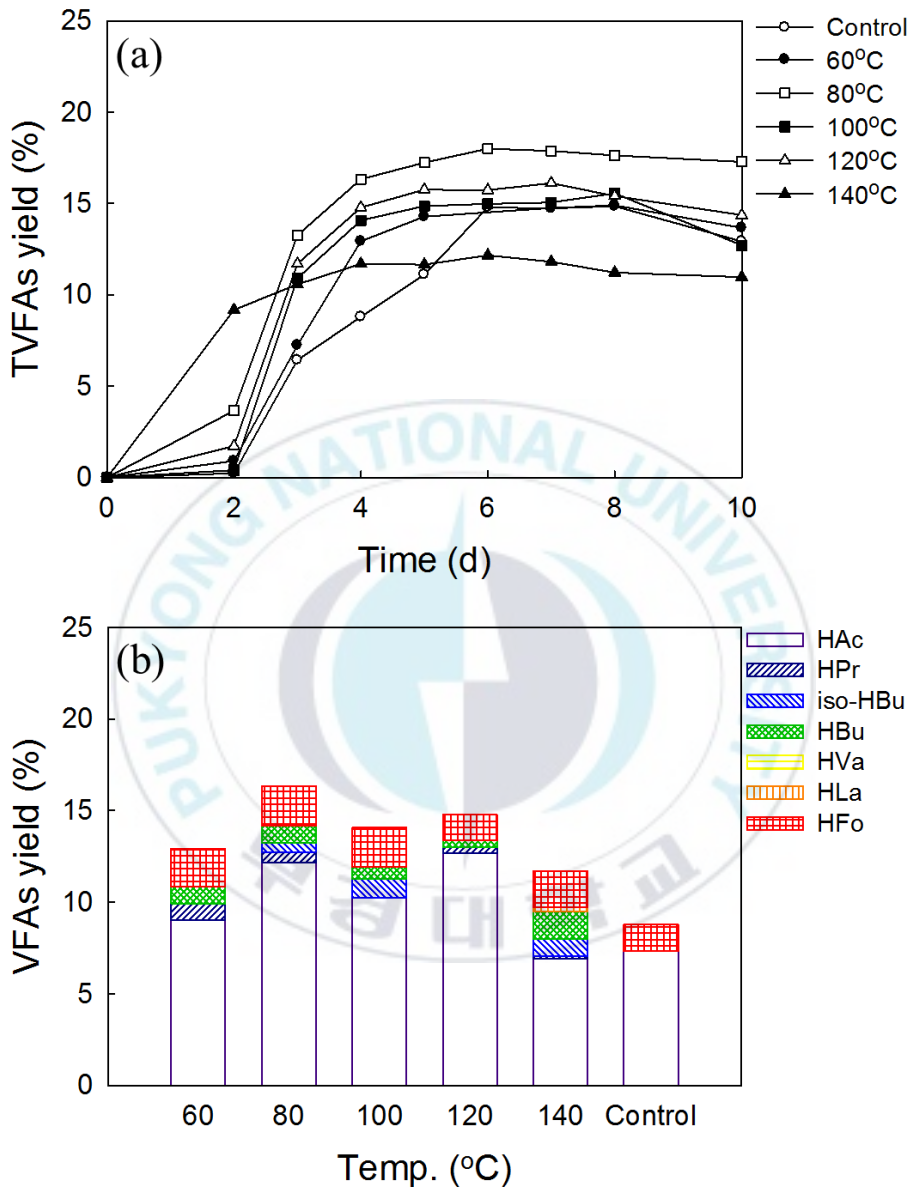


Figure 15 Effect of reaction temp. using Amberlyst-15; (a) yield of TVFAs production (b) yield of individual VFAs production at 4th days of anaerobic fermentation.

V. CONCLUSIONS

In this study, VFAs were produced from alginate pretreated with dilute sulfuric acid and Amberlyst-15. The pretreatment varied with different catalyst concentrations and reaction temperatures in order to enhance the depolymerization of alginate and VFAs production.

From the results, alginate was effectively depolymerized with increasing catalyst concentrations and temperature in the dilute sulfuric acid pretreatment. In the Amberlyst-15 pretreatment, depolymerization of alginate was more significantly affected by the reaction temperature than the catalyst concentration. The anaerobic fermentation of alginate is possible even without any pretreatment. However, acid pretreatments were beneficial for VFAs production from alginate. The maximum VFAs production yield of 45.4% from alginate of 10g/L was obtained 3% (w/w) at 120℃ for 2h in dilute sulfuric acid. And in the Amberlyst-15 pretreatment, the highest yield of 18.1% obtained was 5% (w/w) at 80℃ for 4h. Dilute sulfuric acid pretreatment was found to be more effective pretreatment for improving yields of VFAs.

알지네이트로부터 휘발성 유기산 생산을 위한 산 가수분해의 영향

김혜경

부경대학교 대학원 화학공학과

요약

알긴산염은 갈조류 내 탄수화물의 주요성분이며 이론적으로 다양한 형태의 바이오에너지원으로 활용가능하지만, 구조적인 특징으로 인해 생물학적 분해가 어렵다고 알려져있다. 바이오연료의 효율적인 생산을 위해서는 전처리 과정이 필수적이며, 이러한 전처리에는 물리적 및 화학적, 생물학적인 방법이 있다. 본 연구에서는 알지네이트로부터 휘발성 유기산 생산 효율을 높이기 위하여 산 전처리 방법을 적용하였다. 산 전처리를 위해 균일계 산 촉매인 황산 (dilute sulfuric acid)과 불균일계 산 촉매인 Amberlyst-15를 사용하였으며, 이 때 촉매의 농도 및 반응 온도 변수 실험에 대한 알지네이트의 분해 및 유기산 생산 수율에 미치는 영향을 평가하였다.

황산 전처리의 촉매 농도 변수 실험은 0.1%-10% (w/w)의 범위에서 120℃, 2시간동안 반응하였고, 반응온도 변수 실험은 80℃-160℃에서 3% (w/w) 황산, 2시간동안 반응하였다. Amberlyst-15 전처리의 촉매농도 변수 실험은 1%-10% (w/w)에서 120℃, 4시간

동안 반응하였고 반응온도 변수 실험은 60°C-140°C의 범위에서 5% (w/w) Amberlyst-15, 4시간동안 반응하였다.

황산 및 Amberlyst-15의 산 촉매를 사용하여 알지네이트를 전처리한 결과, 촉매농도 및 온도가 높아짐에 따라 알지네이트의 분자량이 낮아지는 것으로 나타났다. 순수 알지네이트의 평균 분자량은 758,276 Da인 반면에, 황산 농도를 1%에서 10% (w/w) 증가시킨 결과, 분자량은 276,992 Da에서 24,030 Da로 감소하였으며, 반응 온도 160°C에서 전처리한 분자량은 2,504 Da로 낮아졌다. Amberlyst-15의 산 촉매를 사용한 전처리에서는 촉매 농도 1%에서 120.000 Da이하로 감소하였고, 반응 온도가 140°C까지 높아짐에 따라 3,950 Da로 감소하였다. 또한 알지네이트의 산 전처리가 유기산 생산 수율에 미치는 영향을 확인하였다. 혐기성 발효 온도 35°C 에서 최대 유기산 생산 수율을 확인한 결과, 황산 전처리의 경우는 촉매 농도 3%(w/w), 전처리 온도 120°C인 조건에서 유기산 수율이 45.4%로 나타났다. Amberlyst-15 전처리의 경우 촉매 농도 5%(w/w), 전처리 온도 80°C에서 유기산 수율 18.1%를 얻었다. 이러한 결과는 알지네이트의 산 전처리에 의해 분자량 감소 및 휘발성 유기산 생산 효율에 효과적인 것으로 확인하였다.

REFERENCES

[1] Kyung A Jung, Seong-Rin Lim, Yoori Kim, Jong Moon Park, "Potentials of macroalgae as feedstocks for biorefinery", *Bioresource Technology*, 135, 182-190 (2015).

[2] Na Wei, Josh Quarterman, Yong-Su Jin, "Marine macroalgae: an untapped resource for producing fuels and chemicals", *Trends Biotechnol.*, 335, 308-313 (2013).

[3] Jiyun Seon, Taeho Lee, Seong Chan Lee, Hong Duc Pham, Hee Chul Woo, Minkyung Song, "Bacterial community structure in maximum volatile fatty acids production from alginate in acidogenesis" *Bioresource Technology*, 157, 22-27 (2014).

[4] Hong Duc Pham, Jiyun Seon, Seong Chan Lee, Minkyung Song, Hee-Chul Woo, "Maximization of volatile fatty acids production from alginate in acidogenesis", *Bioresource Technology*, 148, 601-604 (2013).

[5] Thi Nhan Pham, Youngsoon Um, Hyon Hee Yoon, "Pretreatment of macroalgae for volatile fatty acid production", *bioresource Technology*, 146, 754-757 (2013).

[6] Rojan P. John and G.S. Anisha, "Macroalgae and their potential for biofuel", *Plant Sciences Reviews*, 6, 1-15 (2011).

[7] Adam D Hughes, Maeve S Kelly, Kenneth D Black, Michele S Stanley, "Biogas from Macroalgae : is it time to revisit the idea?", *Biotechnology for Biofuels*, 5, 86 (2012).

[8] Seung Gon Wi, Hyun Joo Kim, Shobana Arumugam Mahadevan, Duck-Joo Yang, Hyeun-Jong Bae, "The potential value of the seaweed Ceylon moss (*Gelidiumamansii*) as an alternative bioenergy source", *Bioresource Technology*, 100, 6658-6660 (2009).

[9] Alberto Vergara-Fernandez, Gisela Vargas, Nelson Alarcon, Antonio Velasco, "Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system", *Biomass and Bioenergy*, 32, 338-344 (2008).

[10] Louise A. Lewis and Richard M. McCourt, "Green algae and the origin of land plants", American Journal of Botany, 91, 1535-1556 (2004).

[11] Shuvashish Behera, Richa Singh, Richa Arora, Nilesh Kumar Sharma, Madhulika Shukla and Sachin Kumar, "Scope of algae as third generation biofuels" *Bioengineering and Biotechnology*, 2, 90 (2014).

[12] Ji ye Lee, Pan Li, Jieun Lee, Hyun Jin Ryu, Kyeong Keun Oh "Ethanol production from *Saccharina japonica* using an optimized extremely low acid pretreatment followed by simultaneous saccharification and fermentation", *Bioresource Technology*, 127, 119-125 (2013).

[13] TA Davis, B Volesky, A Mucci, "A review of the biochemistry of heavy metal biosorption by brown algae", *Water Research*, 37, 4311-4330 (2003).

[14] Minkyung Song, Hong Duc Pham, Jiyun Seon, Hee Chul Woo, “Marine brown algae: A conundrum answer for sustainable biofuels production”, *Renewable and Sustainable Energy Reviews*, 50, 782-792 (2015).

[15] W.A.J.P. Wijesinghe, You-Jin Jeon, “Biological activities and potential industurial application of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweeds: A review”, *Carbohydrate Polymers*, 88, 13-20 (2012).

[16] Michel G, Tonon T, Scornet D, Cock JM, Kloareg B, “The cell wall polysaccharide metabolism of the brown alga *Ectocarpus siliculosus*. Insights into the evolution of extracellular matrix polysaccharides in Eukaryotes”, *New Phytologist*, 188, 82-97 (2010).

[17] Vivekanand Vivekanand, Vincent G. H. Eijsink, Svein J. Horn, “Biogas production from the brown seaweed *Saccharina latissima*: thermal pretreatment and codigestion with wheat straw”, *Applied Phycology*, 24, 1295-1301 (2012).

[18] Ho Nam Chang, Nag-Jong Kim, Jongwon Kang, and Chang Moon Jeong, “Biomass-derived volatile fatty acid platform for fuels and chemicals”, *Biotechnology and Bioprocess Engineering*, 15, 1-10 (2010).

[19] Park Jae-ll, Woo Hee-Chul, Lee Jae-Hwa, “Production of Bio-energy from Marine Algae: Status and Perspectives”, *Korean Chemical Engineering Research*, 46, 833-844 (2008).

[20] Myoung-Joo Lee, Ji-Hyeon Song, Sun-Jin Hwang, “Effect of acid pretreatment on bio-hydrogen production and microbial communities during dark fermentation”, Bioresource Technology, 100, 1491-1493 (2009).

[21] Kuen Yong Lee and David J. Mooney “Alginate: properties and biomedical applications”, Prog Polym Sci., 37, 106-126 (2012).

[22] Jung, K. A., Lim, S.R., .Kim, Y., Park, J. M. “Potentials of macroalgae as feedstocks for Biorefinery”, *Bioresour. Technol*. 135, 182–190 (2013).

[23] Yi Zheng, Zhongli Pan, Ruihong Zhang “Overview of biomass pretreatment for cellulosic ethanol production”, *Int Agric & Biol Eng*, 2, 51-68 (2009).

[24] Grohmann K, Torget R, Himmel M. “Dilute acid pretreatment of biomass at high solid concentrations” *Biotechnol Bioeng Symp*, 17, 135 − 151 (1986).

[25] Parveen Kumar, Diane M. Barett, Michael J. Dewiche, and Pieter Stroeve “Methods of pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production”, *Ind. Eng. Chem. Res.*, 48, 3713-3729 (2009).

[26] Fayyaz AliShah,Qaisar Mahmood, Naim Rashid, Arshid Pervez, Iftikhar Ahmad Raja, Mohammad MaroofShah “Co-digestion, pretreatment and digester design for enhanced methanogenesis”, *Renewable and Sustainable Energy Reviews*, 42, 627-642(2015).

[27] Inn Shi Tan, Man Kee Lam, Keat Teong Lee “Hydrolysis of macroalgae using heterogeneous catalyst for bioethanol production”, *Carbohydrate Polymers*, 94, 561-566 (2013).

[28] Razif Harun, Michael K. Danquah “Influence of acid pretreatment on microalgal biomass for bioethanol production”, *Process Biochemistry*, 46, 304-309 (2011).

[29] Florian Monlau, Eric Latrille, Aline Carvalho Da Costa, Jean-Philippe Steyer, Helene Carrere, “Ehancement of methane production from sunflower oil cakes by dilute acid pretreatment”, *Applied Energy*, 102, 1105-1113 (2013).

[30] Qinghua Zhang, Lei Tang, Jianhua Zhang, Zhonggui Mao, Li Jiang, “Optimization of thermal-dilute sulfuric acid pretreatment for enhancement of methane production from cassava residues”, Bioresource Technology, 102, 3958-3965 (2011).

[31] S. Xie, J.P. Frost, P.G. Lawlor, G. Wu, X. Zhan, “Effects of thermo-chemical pretreatment of grass silage on methane production by anaerobic digestion”, Bioresource Technology, 102, 8748-8755 (2011).

[32] T.V. Fernandes, G.J. Klaasse Bos, G.Zeeman, J.P.M. Sanders, J.B. van Lier, “Effect of thermo-chemical pretreatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass”, *Bioresource Technology*, 100, 2574-2579 (2009).

[33] Maria Dyah Nur Meinita, Bintang Marhaeni, Tjahjo Winanto, Dwi Setyaningsih, Yong-Ki Hong, "Catalytic efficiency of sulfuric and hydrochloric acids for the hydrolysis of *Gelidium latifolium* (Gelidiales, Rhodophyta) in bioethanol production", Journal of Industrial and Engineering Chemistry, 27, 108-114 (2015).

[34] G. Jard, C. Dumas, J.P. Delgenes, H. Marfaing, B. Sialve, "Effect of thermochemical pretreatment on the solubilization and anaerobic biodegradability of the red macroalga *Palmaria palmata*", 79, 253-258 (2013).

[35] Jeong-Hoon Park, Ji-Yeon Hong, Hyun Chul Jang, Seung Geun Oh, Sang-Hyoun Kim, Jeong-Jun Yoon, Yong Jin Kim, "Use of *Gelidium amansii* as a promising resource for bioethanol: A practical approach for continuous dilute-acid hydrolysis and fermentation", *Bioresource Technology*, 108, 83-88 (2012).

[36] Armando T. Quitain, Muhammad Faisal, Kilyoon Kang, Hiroyuki Daimon, Koichi Fujie, "Low-molecular-weight carboxylic acids produced from hydrothermal treatment of organic wastes", *Journal of Hazardous Materials*, B93, 209-220 (2002).

[37] Zhangbing Zhu, Zhidan Liu, Yuanhui Zhang, Baoming Li, Haifeng Lu, Na Duan, Buchun Si,Ruixia Shen, Jianwen Lu, "Recovery of reducing sugars and volatile fatty acids from cornstalk at different hydrothermal treatment severity", *Bioresource Technology* (2015).

[38] Arumuganainar Suresh, Charles Seo, Ho Nam Chang, Yeu-Chun Kim, "Improved volatile fatty acid and biomethane production from lipid removed microalgal residue (LRlAR) through pretreatment", *Bioresource Technology*, 149, 590-594 (2013).

[39] Shin, S.G., Lee, S., Lee, C., Hwang, K., Hwang, S., "Qualitative and quantitative assessment of microbial community in batch anaerobic digestion of secondary sludge", *Bioresource Technology*, 101, 9461–9470 (2010).

[40] Alvira, P., Tomas-Pejo, M., Ballesteros, M., Negro, M.J., "Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolisis", *Bioresource Technology*, 101, 4851-4861 (2010).

[41] Peggy Vauchel, Raymond Kaas, Abdellah Arhaliass, Regis Baron and Jack Legrand, "A New Process for Extracting Alginates from *Laminaria* digitata: Reactive Extrusion", *Food and Bioprocess Technology*, 1, 297-300 (2008).

[42] Zahra Habibi Kelishomi, Bahram Goliaei, Hossein Mahdavi, Alireza Nikoofar, Mahmood Rahimi, Ali Akbar Moosavi Movahedi, Fatemeh Mamashli, Bahareh Bigdeli, "Antioxidant activity of low molecular weight alginate produced by thermal treatment", *Food Chemistry*, S0308-8146 (2015).

[43] Wonjin Jeon, Chunghyeon Ban, Geonu Park, Tae-Kying Yu, Jeong-Yong Suh, Hee Chul Woo, Do Heui Kim, “Catalytic hydrothermal conversion of macroalgae-derived alginate: effect of pH on production of furfural and valuable organic acids under subcritical water conditions”, *Journal of Molecular Catalysis A: Chemical*, 399, 106-113 (2015).

[44] Lise Appels, Jan Baeyens, Jan Degreve, Raf Dewil, “Principles and Potential of the anaerobic digestion of waste-activated sludge”, *Progress in Energy and Combustion Science*, 34, 755-781 (2008).

ACKNOWLEDGEMENT

First of foremost, I would like to express my sincere gratitude to my supervisor, Professor Hee Chul Woo, for his valuable advice, patient guidance and support during the process of my studying in Korea. He has been providing a large amount of knowledge, the direction and skill of research. His continuous supports helped and encouraged me to carry on my study properly and accomplish this thesis scientifically.

I would like to thank the appraiser committee members of my thesis, professor Jun Heok Lim and professor Do Jin Im. Thank you for letting my defense be an enjoyable moment, and for their brilliant and helpful comments and suggestions.

I warmly appreciate that all members of laboratory, Jiyun Seon, Seong Chan Lee, Jae Hyung Choi, Yong Beom Park, Wei Zhang, Kwon Hyun Joo, Hyeonwoo Oh and Jino Chirappattu John for their cooperation and for all the kindness and friendship. This lab gave me a comfortable environment as well as invaluable help during the time spent working with them.

Finally, showing utmost gratitude to my family. Words cannot express how grateful I am to my father, my mother, and all my close

relatives for their love, encouragement and support for me not only in the thesis time but also I the whole of my life. And lastly, I offer my regards and blessings to all my friends who supported me in any respect during completion of the project. Thanks for their unwavering encouragement throughout my graduation.