

**SCHOOL OF MATERIALS AND MINERAL RESOURCES ENGINEERING  
UNIVERSITI SAINS MALAYSIA**

**CHARACTERIZATION, DELIGNIFICATION AND HYDROLYSIS OF  
MALAYSIA SWITCHGRASS FOR SUGAR EXTRACTION**

By

**WAI WENG SIN**

**Supervisor: Dr. Norazharuddin Shah Bin Abdullah**

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of the requirement for the degree of Bachelor of Engineering with Honours  
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## DECLARATION

I hereby declare that I have conducted, completed the research work and written the dissertation entitled “**Characterization, Delignification and Hydrolysis of Malaysia Switchgrass for Sugar Extraction**”. I also declare that it has not been previously submitted for the award of any degree or diploma or other similar title of this for any other examining body or University.

Name of Student: Wai Weng Sin

Signature:

Date: 1/6/2017

Witnessed by

Supervisor: Dr. Norazharuddin Shah Bin Abdullah

Signature:

Date: 1/6/2017

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## TABLE OF CONTENTS

<b>Contents</b>	<b>Page</b>
<b>DECLARATION</b>	<b>II</b>
<b>ACKNOWLEDGEMENTS</b>	<b>III</b>
<b>TABLE OF CONTENTS</b>	<b>IV</b>
<b>LIST OF TABLES</b>	<b>VIII</b>
<b>LIST OF FIGURES</b>	<b>IX</b>
<b>LIST OF ABBREVIATIONS</b>	<b>XIV</b>
<b>LIST OF SYMBOLS</b>	<b>XV</b>
<b>ABSTRAK</b>	<b>XVI</b>
<b>ABSTRACT</b>	<b>XVII</b>
<b>CHAPTER 1: INTRODUCTION</b>	<b>1</b>
1.1 Introduction	1
1.2 Problem Statement	2
1.3 Research Objectives	4
1.4 Thesis Outline	5
<b>CHAPTER 2: LITERATURE REVIEW</b>	<b>6</b>
2.1 Introduction	6

2.2 Switchgrass	10
2.3 Lignocellulosic Biomass	11
2.3.1 Cellulose	13
2.3.2 Hemicellulose	14
2.3.3 Lignin	15
2.4 Pre-treatment	16
2.5 Chemical Pre-treatment	21
2.5.1 Acid Pre-treatment	21
2.5.2 Alkaline Pre-treatment	23
2.5.3 Comparison of Acid and Alkaline Pre-treatment	25
<b>CHAPTER 3: MATERIALS AND METHODOLOGY</b>	<b>27</b>
3.1 Introduction	27
3.2 Flowchart	27
3.2.1 Sample Preparation	28
3.2.2 Pre-treatment	30
3.2.3 SEM Characterization	32

3.2.4 Sugar Determination	32
3.3 Chemicals and Materials	33
<b>CHAPTER 4: RESULTS AND DISCUSSION</b>	<b>34</b>
4.1 Introduction	34
4.2 Taxonomy and Identity of the Biomass Sample	35
4.3 Effect of Time Variable and Different Chemicals Use in the Delignification Process	36
4.3.1 Acid Pre-treatment	36
4.3.2 Alkaline Pre-treatment	39
4.3.3 Comparison between Acid and Alkaline Pre-treatment	43
4.4 Concentration of Sodium Hydroxide Reagent	46
4.5 Pulp Density of the Pre-treatment	49
4.6 FESEM Characterization	51
4.6.1 Oven-Dried Sample	51
4.6.2 Acid Treated Switchgrass	52
4.6.3 Alkaline Treated Switchgrass	54

4.6.4 Comparison of Acid and Alkaline Pre-treatment on the Structural Difference	56
4.6.5 Concentration of Alkaline Pre-treatment	59
4.6.6 Pulp Density of Alkaline Pre-treatment	62
4.7 Determination of Sugar Content	65
4.7.1 Concentration of Reagent	65
4.7.2 Volume of Sodium Hydroxide Reagent	66
<b>CHAPTER 5: CONCLUSION</b>	<b>68</b>
5.1 Conclusion	68
5.2 Recommendations	69
<b>REFERENCES</b>	<b>71</b>

## LIST OF TABLES

		<b>Page</b>
Table 2.1	The contents of cellulose, hemicellulose and lignin in different type of lignocellulosic biomass.	12
Table 2.2	Advantages and disadvantages of acid and alkaline pre-treatment of lignocellulosic biomass.	26
Table 2.3	Total sugar or reducing sugar yields of different types of grasses with different types of pre-treatment.	26
Table 4.1	Weight loss of oven-dried switchgrass after acid pretreatment at various residence times	36
Table 4.2	Weight loss of the oven-dried switchgrass after the alkali pre-treatment at various residence time	39
Table 4.3	Weight loss of oven-dried switchgrass with various concentration of alkaline pre-treatment	46
Table 4.4	Weight loss of oven-dried switchgrass with various solid to liquid ratio	49



## LIST OF FIGURES

	<b>Page</b>
Figure 2.1	13
The representation of lignocellulose content in grasses. (a) Grass; (b) Plant cell; (c) Lignocellulose structure in cell wall; (d) Lignin polymer structure; (e) Hemicellulose structure; (f) Cellulose structure	
Figure 2.2	17
Classification of Pre-treatment techniques	
Figure 2.3	18
Graphical representation of the matrix of polymers in which cellulose exists	
Figure 3.1	27
Flow chart of the phases of the project.	
Figure 3.2	28
Flow chart of sample preparation process	
Figure 3.3	29
Switchgrass samples. (A) Washed fresh switchgrass after leaves removal. (B) Oven-dried switchgrass for 48 hours at 60°C. (C) Oven-dried switchgrass for 96 hours at 60°C	
Figure 3.4	30
Oven-dried switchgrass that has been cut into less than 2mm length	
Figure 3.5	30
Pre-treatment process	

Figure 3.6	SEM characterization process	32
Figure 4.1	Certification of the biomass	35
Figure 4.2	Weight loss of the sample after the acid pretreatment against the residence time	37
Figure 4.3	Weight loss of alkaline pre-treated sample against the residence time	40
Figure 4.4	Oven-Dried sample, (A) Acid treated oven-dried switchgrass, (B) Oven-dried switchgrass without any treatment, (C) Alkaline treated oven-dried switchgrass	43
Figure 4.5	Close up physical appearance of oven-dried switchgrass. (A) Alkaline treated oven-dried switchgrass, (B) Untreated oven-dried switchgrass, (C) Acid treated oven-dried switchgrass	44
Figure 4.6	Comparison of the weight loss of switchgrass with acid and alkaline pre-treatment for various residence times	45
Figure 4.7	Weight loss of oven-dried switchgrass against the concentration of sodium hydroxide	47
Figure 4.8	Weight loss of oven-dried switchgrass against the pulp density of the mixture	50

Figure 4.10	Comparison of surface structure before and after acid pre-treatment. (A) Acid treated switchgrass, (B) Untreated switchgrass	52
Figure 4.11	Comparison of inner surface structure before and after acid pre-treatment. (A) Acid treated switchgrass, (B) Untreated switchgrass	52
Figure 4.12	Comparison of cross-sectional structure before and after acid pre-treatment. (A) Acid treated switchgrass, (B) Untreated switchgrass	53
Figure 4.13	Comparison of surface structure before and after alkaline pre-treatment. (A) Alkaline treated switchgrass, (B) Untreated switchgrass	54
Figure 4.15	Comparison of inner surface structure before and after alkaline pre-treatment. (A) Alkaline treated switchgrass, (B) Untreated switchgrass	55
Figure 4.16	Comparison of surface structure between acid and alkaline pre-treated switchgrass. (A) Acid treated switchgrass, (B) Alkaline treated switchgrass	57

Figure 4.17	Comparison of inner surface structure between acid and alkaline pre-treated switchgrass. (A) Acid treated switchgrass, (B) Alkaline treated switchgrass	57
Figure 4.18	Comparison of cross-sectional structure between acid and alkaline pre-treated switchgrass. (A) Acid treated switchgrass, (B) Alkaline treated switchgrass	58
Figure 4.19	Surface structure of the different concentration of alkaline treated switchgrass. (A) 2M, (B) 3M, (C) 4M, (D) 5M	59
Figure 4.20	Inner surface structure of the different concentration of alkaline treated switchgrass. (A) 2M, (B) 3M, (C) 4M, (D) 5M	60
Figure 4.21	Cross-sectional surface structure of the different concentration of alkaline treated switchgrass. (A) 2M, (B) 3M, (C) 4M, (D) 5M	61
Figure 4.22	Surface structure of the different pulp density of alkaline treated switchgrass. (A) 0.07g/mL, (B) 0.05g/mL, (C) 0.04g/mL, (D) 0.03g/mL	62
Figure 4.23	Inner surface structure of the different pulp density of alkaline treated switchgrass. (A) 0.07g/mL, (B) 0.05g/mL, (C) 0.04g/mL, (D) 0.03g/mL	63

- Figure 4.24 Cross-sectional surface structure of the different pulp density of 64  
alkaline treated switchgrass. (A) 0.07g/mL, (B) 0.05g/mL, (C)  
0.04g/mL, (D) 0.03g/M
- Figure 4.25 The result of sugar content concentration against the 65  
concentration of the sodium hydroxide reagent.
- Figure 4.26 The concentration of sugar content against the volume of the 66  
sodium hydroxide reagent.

## **LIST OF ABBREVIATIONS**

FESEM      Field Emission Scanning Electron Microscope

GHG        Greenhouse Gas

## LIST OF SYMBOLS

%	Percentage
o	Degree
°C	Degree Celsius
°Cmin <sup>-1</sup>	Degree Celsius per minute
psi	Pounds per square inch
g	Gram
mL	Milliliter
mm	millimeter
M	Molar
g/mL	Gram per milliliter
h	Hours

# **PENCIRIAN, DELIGNIFIKASI DAN HIDROLISIS SWITCHGRASS MALAYSIA UNTUK GULA ESTRAK**

## **ABSTRAK**

Penukaran lignoselulosa ke etanol adalah teknologi berpotensi untuk menambahbaik biobahan-api generasi pertama dan bahan api fosil. Walau bagaimanapun, struktur kompleks biojisim lignoselulosa merupakan cabaran utama kepada penukaran biojisim tersebut. Untuk memperbaiki dan meningkatkan penghadaman enzim switchgrass sebagai biojisim lignoselulosa untuk gula beragi atau gula reduksi dalam proses hidrolisis. Perbandingan antara asid dan alkali pra-rawatan menggunakan asid sulfuric dan nitrium hidroksida bahan mentah biojisim itu akan diselidiki. Pada keadaan kepekatan 1M, 0.1g/mL ketumpatan pulpa, di suhu bilik standard, biojisim itu dipra-rawat masing-masing pada masa satu hingga lima jam. Pra-rawatan tersebut dinilai berdasarkan kehilangan berat jisim sampel yang dirawat. Kedua-dua pra-rawatan turut mencapai kehilangan berat jisim yang bertakung di 4 jam masa rawatan, iaitu 26% dan 26.9% masing-masing. Kemudian, kajian ini telah dijalankan lagi untuk menyelidik keadaan optimum untuk alkali pra-rawatan. Biojisim dirawat untuk 4 jam, 0.1g/mL ketumpatan pulpa, pada suhu bilik dan tekanan yang standard dengan kepekatan yang berbeza dari 1M hingga 5M. Kehilangan berat jisim tertinggi dicatatkan padan 28.5% pada 2M NaOH pra-rawatan. Ketumpatan pulpa mempunyai kesan terhadap pada penurunan berat jisim pada masa rawatan yang tetap dan pada suhu dan tekanan bilik standard. Kajian SEM atas sampel pra-rawat menunjukkan banyak perubahan struktur dalam sampel rawatan alkali dan hanya sedikit atau tiada perubahan fizikal ke atas struktur sampel rawatan asid.



# **CHARATERIZATION, DELIGNIFICATION AND HYDROLYSIS OF SWITCHGRASS FOR SUGAR EXTRACTION**

## **ABSTRACT**

Lignocellulose-to-ethanol conversion is a promising technology to supplement the first generation biofuels and fossil fuels. However, the recalcitrant structure of the lignocellulosic biomass is a major challenge to the efficient conversion of the biomass. To improve and increase the enzymatic digestibility of switchgrass as a lignocellulosic biomass for fermentable sugar or reducing sugar production in hydrolysis, comparison of acid and alkaline pre-treatment using sulphuric acid and sodium hydroxide of the biomass feedstock was investigated. At the condition of 1M concentration, 0.1g/mL pulp density, at standard room and temperature, the biomass was pre-treated respectively at 1, 2, 3, 4 and 5 hours of residence time. Pre-treatment was evaluated based of the weight loss of the treated samples. Both of the pre-treatment achieved the stagnant weight loss at the 4 hours residence time, which were 26% and 26.9% respectively. Then, the research was carried out further to investigate the optimum conditions for the alkaline pre-treatment. The biomass was treated at 4 hours residence time, 0.1g/mL, at standard room temperature and pressure with different concentration varying from 1 to 5M. The highest weight loss was recorded at 28.5% at the 2M NaOH pre-treatment. Pulp density had limited effect on the weight loss at constant residence time and concentration at room temperature and pressure. SEM studies on the pre-treated samples showed a great deal of structural change in the NaOH pre-treated samples and a little or no physical changes on the acid pre-treated samples.

# CHAPTER 1

## INTRODUCTION

### 1.1 Introduction

Lignocellulosic biomass has become a resource which is renewable and it can be converted to chemicals and fuels. Its utilisation for the fuel production to produce energy has high potential in helping to reduce the emissions of CO<sub>2</sub> that could eventually contribute to prevent global warming [1]. The recent research and development efforts have put more focus into a two steps bioconversion process that involve : 1) liberation of the fermentable sugars from the lignocellulose and, 2) conversion of sugars into chemicals or fuel by hydrolysis or fermentation [2]. The Lignocellulosic biomass to serve as a renewable feedstock has a great potential but has not been realized due to the focus on the petroleum-based feedstocks in 1920s and the difficulty to depolymerize the lignocellulose into its component monomeric sugars [3].

There are three major components in the lignocellulosic materials which are cellulose, hemicellulose and lignin. Cellulose and hemicellulose are structural carbohydrates in the plant tissues. Fermentable sugars can be produced from the depolymerisation of cellulose and hemicellulose through enzymatic hydrolysis. On the other hand, lignin is a complex aromatic polymer that serves as a barrier, surrounding the carbohydrates fraction by forming a crust, to minimize the accessibility of the carbohydrates to hydrolytic enzymes [4].

Therefore, delignification process would highly improve the enzymatic hydrolysis to digest the lignocellulose. Research and development efforts have been put in vigorously in pursuing the pre-treatment of lignocellulosic feedstocks [5]. There are three major categories of delignification process which are physical, chemical and biological pre-treatment. In some cases, where the only option available is physical pre-treatment because the energy required is higher than the theoretical energy content available in the biomass. This method requires high amount of cost and is not likely to be used in the full scale commercial process [6]. Biological pre-treatment involves the use of microorganisms to degrade the lignin and hemicellulose while leaving the cellulose intact [7-9]. Biological pre-treatment involves mild conditions and cheap. However, the disadvantages are the low rate of hydrolysis and require long treatment time [10]. There are still some current efforts to the biological pre-treatment. Research efforts are put in on the combination of biological pre-treatment with the other pre-treatments and in developing novel microorganisms to increase the rate of hydrolysis. There are a lot of methods involve in the chemical pre-treatment. The use of sodium hydroxide as alkaline pre-treatment and sulphuric acid as acid pre-treatment have been extensively studied and show promise [5].

## **1.2 Problem Statement**

Various types of wild grasses in Malaysia are left unutilized. These grasses are abundant and have no industrial use. This may cause problem to the social community and the household. For example, snake and insects attack. Therefore, I am looking into the opportunity and possibility to turn these grasses to some industrial usage. The

possibility to derive sugar from the grass would be one of the main concern of this project.

Various researches have been done to focus on only one of the various chemical pre-treatment. The efforts on the comparison between the chemical pre-treatment methods have seldom been done. There are different strengths and weaknesses of the different type of chemical pre-treatment. Besides that, pre-treatment to remove the lignin has seldom been on the spot light to be focused solely. Various researches have been putting in efforts combining the pre-treatment together with the hydrolysis process to increase the yield of sugars. There are too many factors that could potentially affect the yield of sugars. Therefore, breaking down the whole process into smaller part for analysis can be more accurate.

Acid can be a delignification agent and also play a role in the hydrolysis process to act as a catalyst in the enzymatic hydrolysis. This may affect the yield of the sugars with the double roles of the acid. Therefore, the process has to be separated to be able to analyze on the efficiency of the acid to remove the lignin on the lignocellulose structure. Switchgrass is one of the abundant grasses that grow easily in Malaysia without a lot of efforts to be put on it. Although there are already some companies in United States of America commercially processing it to produce renewable energy but there is no effort so far on the research on the biomass cellulose in the South East Asia region.

There also not much effort on the energy-transfer of the delignification process without disturbing the structure of the biomass with the physical process. Characterization of the biomass after the pre-treatment to analyze on the change of

structure of the biomass has seldom been focused as those researches would continue with the enzymatic hydrolysis process with the pre-treated sample. Various researched have focused on characterizing the content of the biomass rather than the structure change of the biomass.

### **1.3 Research Objectives**

- To characterize physically on the oven-dried switchgrass before and after the pre-treatment.
- To study the efficiency of the alkaline and acid pre-treatment on removing the lignin
- To study the possibility of deriving sugar content from the switchgrass

## **1.4 Thesis Outline**

Chapter one discusses on the introduction of dissertation including background of sugar extraction using non-consumable plant, problem statement, objectives of this work and the scope of this project.

Then, chapter two discusses on the overview survey of literature on the switchgrass for sugar extraction, chemicals used for pretreatment and their effects and importance of the chemical pretreatment in reference to widely relevant to the previous works or related to this study.

Next, in the chapter three discusses about the raw materials being used in this project and the methodology of this project. The flow sheets and steps of experiment are stated in this chapter. The Characterization methods used in this project also stated in this chapter.

After that, chapter four discusses the results and findings of the experimental works that have been done and analyze the efficiency of acid and alkaline pre-treatment on the switchgrass.

Lastly, chapter five discusses on the conclusion and the recommendation for future study.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Introduction

The incessant increase in world energy consumption and concerns over the deteriorating, increasingly competitive fossil fuels have resulted in an urgent search for alternative energy sources that can power the world the sustainable way. Besides that, long term environmental concerns have also resulted in a great amount of effort putting into the research in the past couple of decades on renewable sources of energy to replace fossil fuels. Burning fossil fuels such as coal, oil and natural gas releases CO<sub>2</sub>, which is a major source that contributes to the global warming [11].

In the United States of America, with only 4.5% of the world's population, they are responsible for about 25% of the global energy consumption and 25% of global CO<sub>2</sub> emissions [11]. The global fossil fuel reserves were expected to be depleted by the next 40-50 years due to the rapid increase in the consumption rate of this non-renewable fuels. There for it is mandate for search of an alternative and eco-friendly energy source [12]. The conversion of abundant lignocellulosic biomass to biofuels presents a viable option for improving energy security and reducing greenhouse gas emissions [13].

Comparing to the fossil fuels which came from the organism that grew millions of years ago, the biofuel were produced from the plants grown today. They were cleaner-burning comparing to the fossil fuels, and the shorter time frame needed to grow the

plants. The fuels made from them did not emit CO<sub>2</sub> to the atmosphere when the fuels were burnt. There had been reports said that the cellulosic ethanol and ethanol produced from other biomass resources had potential to cut the greenhouse gas emissions by 86% [14]. In a research work that had been done by Ryan et al., it stated that for every 1000L of bioethanol from lignocellulosic biomass, there were approximately 2.6Mg carbon dioxide gas emission was saved [15]. Therefore, biofuels derived from the biomass sources can be an alternate source of energy in the future. There is a main reason which is the fact that the bioethanol produces nearly twice as much energy as it consumes, make its potential as a sustainable biofuel. Thus, it can be utilized as an alternative to the fossil fuel in the commercial scale [16].

On the economic side of view, the average price of gasoline in year 2005 was 2.56 dollars per gallon, which was 0.67 dollar higher than the average price of gasoline in year 2004 [11]. Yet, in June 2008, the price of the gasoline in the United States of America had reached 4.10 dollar per gallon. Novozymes, an enzyme production company in the United States, estimated (2007 values) that the cost of enzymes to depolymerize cellulose and hemicellulose to sugars for fermentation would be about 40-100 times higher than the cost of enzymes for starch hydrolysis to glucose on a per gallon ethanol basis [17]. Besides that, major cellulose producers estimate (2010 values) the cost of fungal celluloses to be about 0.5 dollar per gallon of cellulosic of the total cost of ethanol produced [18].

The economy founded on biomass instead of fossil fuels represented a significant shift in socio-economic, agricultural, energy and technical systems. This type of



economy met many of the requirements for sustainability from environmental, social and economic perspectives [19]. There are several potential benefits transiting to a bio-based economy. Benefits such as reduction in greenhouse gas (GHG) emissions, decrease in dependence on fossil fuel resources and improved food security [20]. In the recent years, there are a lot of policies and incentives given and implemented across the world to encourage the production and consumption of renewable energies, such as biofuels. The Europe biofuel directive and the American Policy Energy Act both aim to increase the consumption of ethanol by 2022 [21, 22].

In the last decade the use of biofuels across the world had been increased steadily. In 2000, there are approximately 18.2 billion liters of ethanol were consumed in North America compared to 83 billion liters in 2012, this had accounted for approximately 83% of the total ethanol produced globally [23]. Europe still remained as the largest producer and consumer of biodiesel, which amounted for 42% of total biodiesel production [24]. Several market challenges still remained now despite the increase in global production of biofuels. The existing market challenges such as the sustainability concern, environmental impact and economic value of the fuel compared to the other transportation fuels for example biomethane [24]. Therefore, as a result of that, financial incentives were given such as tax reliefs, subsidies and feedstock establishment grants had been implemented to help stimulate research, production and consumption of liquid biofuel in Europe [25].

The production of biofuels currently relied a lot on ethanol and diesel from starch and sugars, which was known as the first generation biofuels. However, there had been

a lot of debate and argument surrounding the sustainability and the use of food crops for fuel. This had led to the increase in the food prices and raised concerns for food crisis [26]. For instance, corn grain was the predominant feedstocks for conventional ethanol fuel production in United States. In year 2008, the U.S. ethanol industry produced a record of 9 billion gallons of corn-based ethanol, which was a 38% of increment compared to the previous year [27]. However corn-based ethanol production had affected and diverted corn away from food markets, thus inevitably resulting in food-fuel competition [28]. Besides that, there are studies showed that the first generation biofuel can have a negative impact to the environment, which was increasing the GHG emissions [26].

Because of this, efforts had been put in and the second generation of biofuels produced from lignocellulosic biomass, for example, municipal wastes, grasses, waste paper and energy crops, had been identified as an alternative as the food crops were not used as feedstocks. These lignocellulosic biomasses were more sustainable and have a positive environmental impact as the feedstocks grown are carbon neutral [26]. However, the second generation biofuels were not yet been produced commercially, but a considerable number of pilot and demonstration plants had been announced or set up in recent years, with the effort of research activities mostly taking place in North America, Europe and a few emerging countries such as Brazil, China, India and Thailand [29]. The main challenges that were faced for the second generation biofuels was high initial investment costs as well as the higher costs of end product compared to the fossil fuels or some of the first generation biofuels. There were companies reported that they will commercialize the production of second generation biofuels within the

coming years, with the help of incentives, subsidies and support such as new blending mandates from the government [29].

## **2.2 Switchgrass**

Switchgrass (*Panicum Maximum*) was a perennial native warm season grass species. It was considered as a potential lignocellulosic feedstock for ethanol production because of its excellent growth in various soil and climate conditions and it had low requirements for agricultural inputs for production [30]. In Malaysia, this type of grass can be easily found at the side of the riverbank. It did not require much effort to grow this plant. Furthermore, this plant can grow independently in both the dry season and rainy season in Malaysia. Therefore, this plant can grow throughout the year with minimal problem. According to the research done by Schmer et al., with an annual biomass yield reaching up to 11.1 Mg/ha, switchgrass was capable of producing 5.4 times more renewable energy than nonrenewable energy consumed. On top of that, the greenhouse gas (GHG) emissions from switchgrass-based ethanol were 94% less than those from the gasoline [31].

Switchgrass can also be combusted directly or co-fired with the coal to lower the emissions associated with the burning of the particular fuel. However, for this to be practical or to be commercialized, the current coal or co-fired applications had to be retrofitted. Switchgrass was not the only or possibly even the best biomass species for cellulosic ethanol production, but it did possess some ecological characteristics that make it a very good candidate. There were several positive qualities that switchgrass could offer:

- Pest and disease resistance
- High yield of cellulose
- Low fertility needs
- Cultivars that are locally adapted and relatively available
- Excellent wildlife habitat
- Carbon sequestration in its extensive and very deep root system
- Tolerance of poor soils and wide variations of soil pH
- Drought and flood tolerance
- Efficient water use in grassland ecosystem.

But many other grasses may possess the same characteristics as well [Switchgrass/Lee] Switchgrass could regenerate itself after it had been cut for harvest. It requires little fertilizers and pesticides to flourish. Other than that, it helps trap CO<sub>2</sub> in the ground [32, 33].

In the future, this fast growing plant can be targeted as the potential energy crops not only because of its high productivity per hectare but also due to its abundance, availability and utilization of the whole plant which increase the percentage of the total cellulose and hemicellulose content and relatively less lignin content [34].

### **2.3 Lignocellulosic Biomass**

There were different types of lignocellulosic biomasses such as wood based, non-wood biomasses that include agricultural residues, sugar cane bagasse, switchgrass, cotton fiber and etcetera. Among these different types of lignocellulosic biomasses, switchgrass which was consider as the short rotation crops had high yields of up to

40Mg/ha/year as compared to corn feedstocks which had only yields of up to 7Mg/ha/year [35].

The composition of the lignocellulosic material was made up of mainly three different types of polymers, namely cellulose, hemicellulose and lignin, which three of them were associated with each other [36].

Table 2.1: The contents of cellulose, hemicellulose and lignin in different type of lignocellulosic biomass [8]

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Nut shells	25–30	25–30	30–40
Corn cobs	45	35	15
Grasses	25–40	35–50	10–30
Paper	85–99	0	0–15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15–20	80–85	0
Cotton seed hairs	80–95	5–20	0
Newspaper	40–55	25–40	18–30
Waste papers from chemical pulps	60–70	10–20	5–10
Primary wastewater solids	8–15	NA <sup>b</sup>	24–29
Swine waste	6.0	28	NA <sup>b</sup>
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12.0

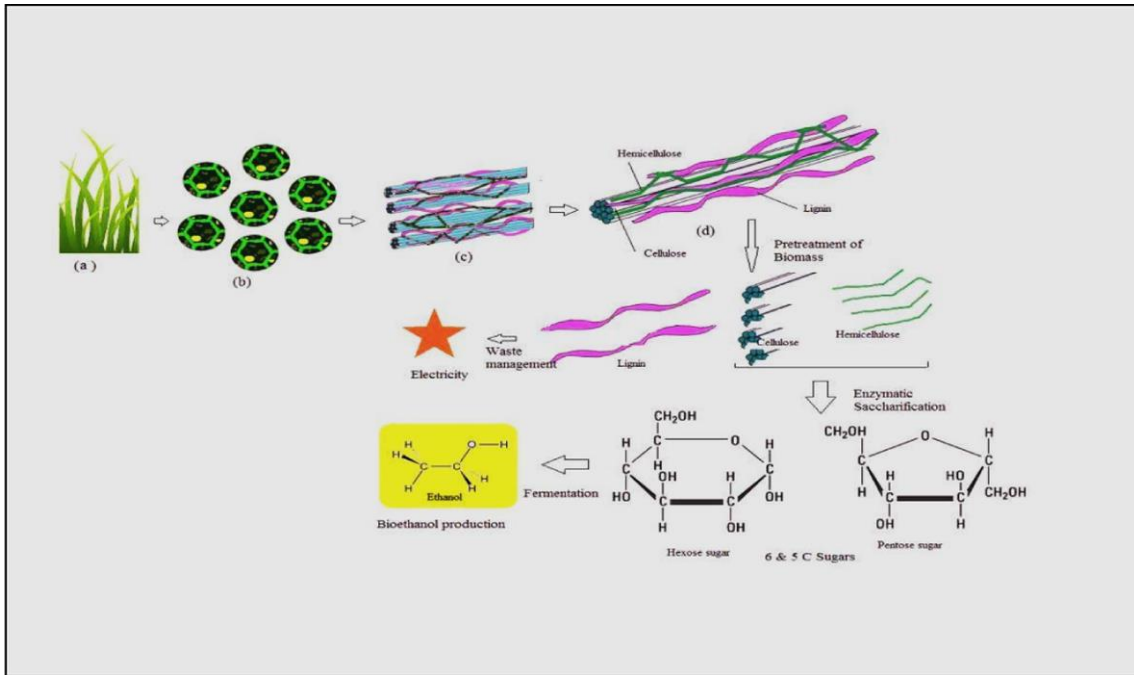


Figure 2.1: The representation of lignocellulose content in grasses. (a) Grass; (b) Plant cell; (c) Lignocellulose structure in cell wall; (d) Lignin polymer structure; (e) Hemicellulose structure; (f) Cellulose structure. [53]

### 2.3.1 Cellulose

Cellulose was a structure that exist of D-glucose subunits, linked by  $\beta$ -1,4 glycosidic bonds [36].

The cellulose in a plant was consisted of parts with a crystalline organized structure, and parts with a, not well-organized, amorphous structure. The cellulose strains were tangled and bundled together, forming a so called fibrils or cellulose bundles. There cellulose fibrils were mostly independent and weakly bonded together through hydrogen bonding [37].

### 2.3.2 Hemicellulose

Hemicellulose was a more complex carbohydrate structure that consisted of different polymers like pentoses (examples: xylose and arabinose), hexoses (examples: mannose, glucose and galactose), and sugar acids. The dominant component of hemicellulose from the hardwood and agricultural plants, like grasses and straw, was xylan, and if for softwood, it was glucomannan [36, 38].

Hemicellulose had a lower molecular weight compared to cellulose, and branches with short lateral chains that consist of different sugars, which were the polymers that could be hydrolyzed relatively easier [36]. Hemicellulose served as a connection connecting the lignin and the cellulose fibers and gave the three polymers cellulose-hemicellulose-lignin a network and enhanced the rigidity of the network [37].

The solubility of the different hemicellulose compounds was in the descending order as the list below: mannose, xylose, glucose, arabinose, and galactose. When the temperature increased, the solubility of the hemicellulose compound would be increased as well. However, the solubility of higher molecular polymers could not be predicted, because of the unknown melting points of the polymers [39]. The hemicellulose compounds started to dissolve into the water around 180°C under neutral conditions according to the research work done by Bobleter [40]. However, in the research work done by Garrote et al., the hemicellulose started to become soluble at the temperature around 150°C [41]. The solubility of the lignocellulose components did not only depend on the temperature, it also depended on the other aspects for example, moisture content and pH [36].

The xylan content inside the hemicellulose can be extracted quite well in an acid or alkaline environment, while glucan can only hardly be extracted in an acid environment. It needed stronger alkaline environment than xylan in order for it to be extracted [36, 42, 43]. Therefore, apparently xylan was the part that can be extracted easily.

In comparison with the cellulose and lignin, hemicelluloses were the part that were most thermal-chemically sensitive [44]. During the thermal chemical pre-treatment, the side groups of the hemicellulose were the first to react, then followed by the hemicellulose backbone [45].

### **2.3.3 Lignin**

Lignin was ranked the third most abundant polymers structure in the plant structure, came after cellulose and hemicellulose. It was present in the cellular wall. It was an amorphous heteropolymer consisting of three different phenylpropane units (*p*-coumaryl, coniferyl and sinapyl alcohol) that were held together by different kind of linkages. [46]. The main purpose of lignin was to give the plant structural support, impermeability to liquid especially water and resistance against microbial attack and oxidative stress because the cellulose and hemicellulose are reducing in nature. The amorphous heteropolymer was also non-water soluble and optically inactive. All these conditions increased the difficulty for the degradation of the lignin to happen [36].

Besides that, lignin was also similar to hemicellulose which normally started to dissolve into water around 180°C under neutral conditions [40]. The solubility of the



lignin in acid, neutral or alkaline environments depended on the precursor (*p*-coumaryl, coniferyl, sinapyl alcohol or combinations of them) of the lignin [47].

Lignin was one of the main factors causing the recalcitrance of biomass [48]. Lignin prevented enzymatic degradation of lignocellulose in two ways. First, it acted as the primary obstacle for the cellulase access to the cellulose or the hemicelluloses. Second, hydrophobic interaction between lignin and enzyme molecules causes unproductive binding of enzyme to the lignin, thus it reduced the availability of the enzyme for the cellulose hydrolysis [49, 50].

## **2.4 Pre-treatment**

There were three essential ways to convert the lignocellulosic biomass to ethanol which were the pre-treatment of the recalcitrant feedstock, hydrolysis of the cellulose or hemicellulose matrix and fermentation of the cellulose to ethanol [51]. The conversion of lignocelluloses to ethanol is more challenging compare to corn-to-ethanol process because of the complex structure of the plant cellular wall as described in the previous section. Besides the general saccharification and fermentation, an extra step of pre-treatment was needed to break down the lignin and make the cellulose matrix more susceptible to cellulolytic enzymes during the hydrolysis process [52].

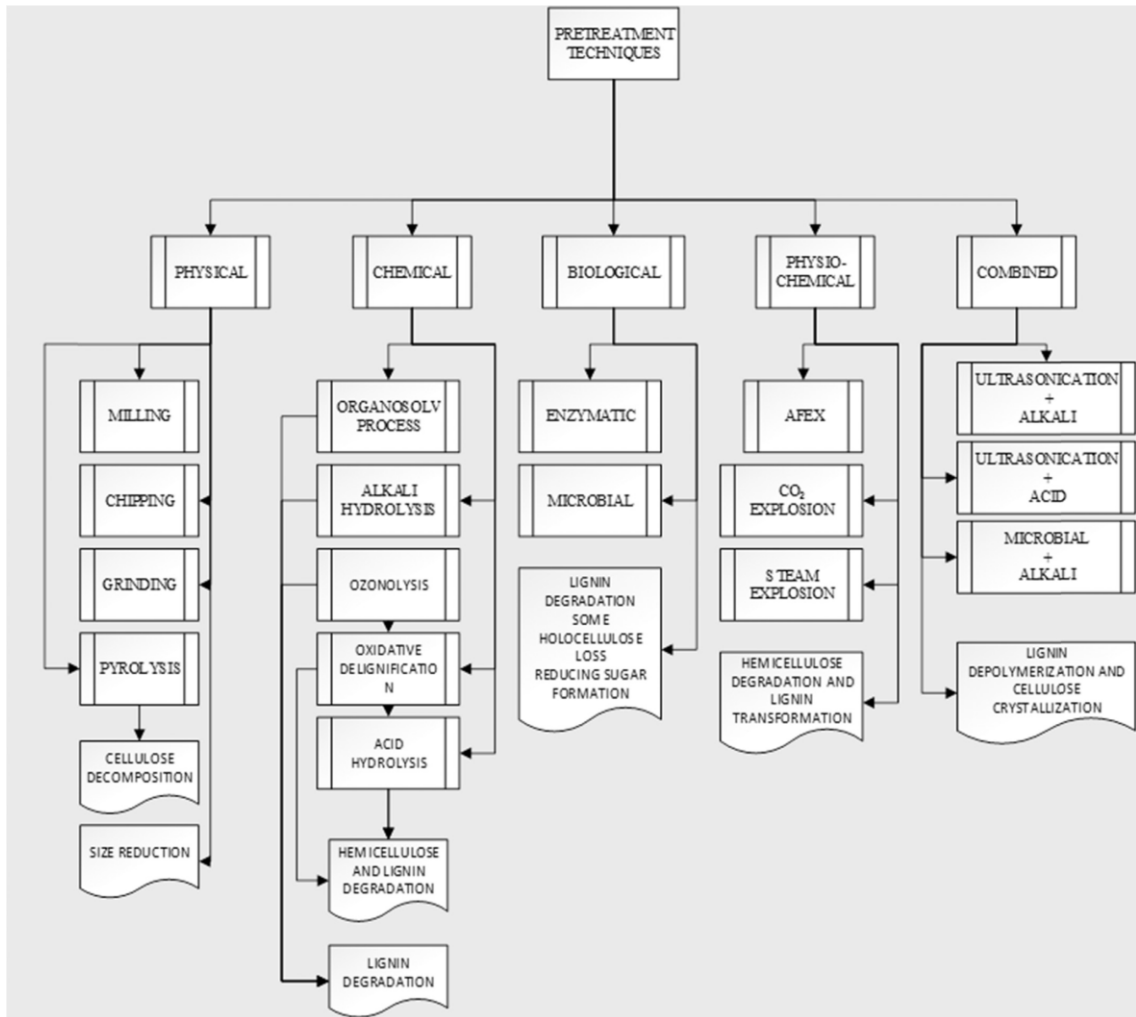


Figure 2.2: Classification of Pre-treatment techniques [53]

The pre-treatment was considered to be the most expensive stage of the bioconversion process accounting for nearly 0.30 dollar/gallon of ethanol produced which was also equal to 20% of the total production cost [51, 54]. There were a lot of research and development efforts had been undertaken for different conversion processes with the aim to reduce the processing costs to be able to compete with the first generation applications [29]. Therefore, the pre-treatment process had become the main focus of the conversion studies [51, 55, 56].

Pre-treatment can partially remove the lignin and hemicellulose, thus reduced the cellulose crystallinity and increased the porosity of the biomass [57]. Pre-treatment was considered to be achieved by breaking down the lignin seal and hemicellulose sheathing over cellulose and by disrupting the crystalline structure of the cellulose [52].

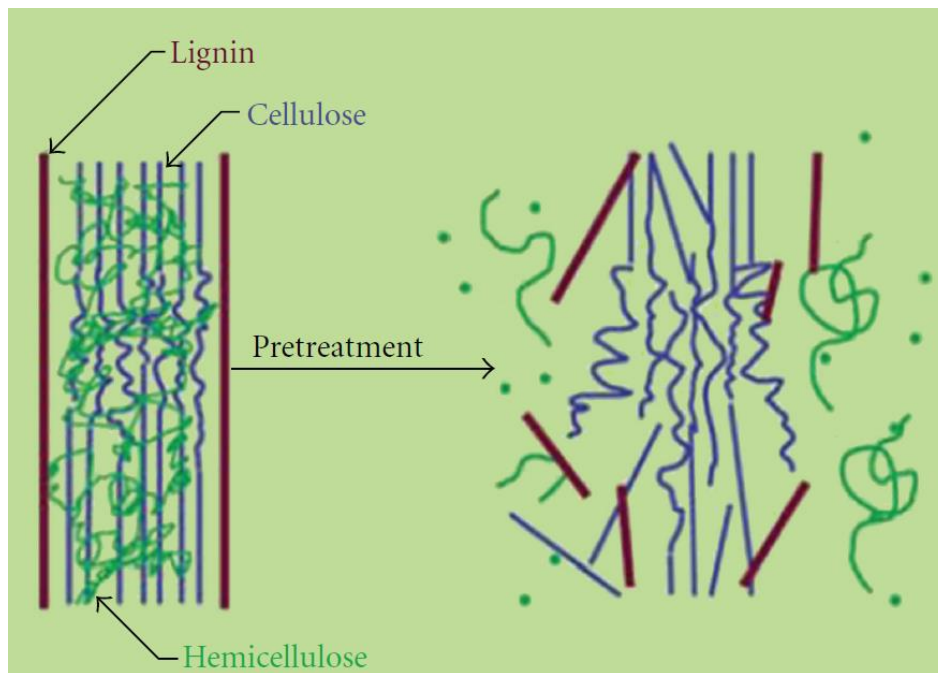


Figure 2.3: Graphical representation of the matrix of polymers in which cellulose exists. Pre-treatment of the biomass by different methods liberated the cellulose by removing the hemicellulose and lignin from the matrix before hydrolysis [54, 58]

Lignocelluloses that consisted of cellulose, hemicellulose, lignin, extractives and several inorganic materials were needed to be recalcitrant [48, 59, 60, 61]. Considering the dimer cellobiose as the basic unit, cellulose could be considered as an isotactic (identical spatial arrangement of the side chains) polymer of cellobiose. The cellulose chains were packed into microfibrils which were stabilized by hydrogen bonds as shown

in Figure 2.3 [54]. The hemicelluloses and amorphous polymers of different sugars as well as other polymers such as pectin linked up these fibrils and were covered by lignin [62]. If the enzymatic hydrolysis of biomass is to proceed in typical processes, the crystalline structure of the cellulose needed to be disrupted to increase the accessible area to the cellulose and the lignin and the hemicellulose separated from the cellulose, so that the enzyme treatment later would be more effective (Figure 2.3).

There were several goals that the pre-treatment aimed to achieve. Numerous pre-treatment strategies had been developed to increase the yield of fermentable sugars and to enhance the reactivity of cellulose. The typical goals of pre-treatment were listed below:

- Production of highly digestible solids that enhances sugar yields during the enzymatic hydrolysis
- Minimize the degradation of sugars (mainly pentoses) including those that derived from the hemicellulose
- Reduce the formation of inhibitors for subsequent fermentation steps
- Recovery of lignin to be converted into valuable co-products
- Increase the cost efficiency by operating in reactors of moderate size and by minimizing heat and power supply.

All these goals were equally important and needed to be achieved in order to be more competitive compared to the first generation biofuels and the fossil fuels [6].

Pre-treatment technologies were classified into four main categories which were physical pre-treatment, chemical pre-treatment physicochemical pre-treatment and biological pre-treatment.

Physical pre-treatment involved breakdown of biomass size and crystallinity by cutting, milling and grinding. It would improve hydrolysis results due to the reduction in crystallinity hence improved mass transfer characteristics from reduction in particle size [6]. The size reduction by physical pre-treatment decreased the heat and mass transfer limitations that occurred due to large particle size of the biomass and increased the bulk density, thus it allowed the pre-treatment of more concentrated feedstock [55, 63]. Physical pre-treatment was an expensive method and likely will not be used in a full-scale process [6].

Biological pre-treatment was a pre-treatment that involved the use of microorganisms mainly fungi to degrade lignin and hemicellulose but leave the cellulose intact [7, 8, 9]. During biological pre-treatment, the lignin degradation occurred through the action of lignin degrading enzymes secreted by the fungi. Biological pre-treatments involved only mild conditions and were of low cost. Despite that, it is seldom used because of the low rates of hydrolysis and long pre-treatment times required compared to other pre-treatment technologies [10]. But there were still efforts in biological pre-treatments which were to combine this technology with other pre-treatments and in developing novel microorganisms for rapid hydrolysis [7, 9].

## **2.5 Chemical Pre-treatment**

In order to increase the elimination of lignin and hemicelluloses, decreased the crystallinity index (CrI) and the degree of polymerization (DP) of cellulose, chemical and physico-chemical pre-treatment methods had evolved as attractive methods [64]. Chemical pre-treatment was used mainly for internal degradation of lignin and hemicelluloses by breaking down the internal lignin and hemicellulose bonds. The hemicellulose and lignin bonds mainly referred to the chemical bonds between the lignin, galactose and arabinose residues on the side chains of hemicellulose molecules [65].

### **2.5.1 Acid Pre-treatment**

Acid pre-treatment involved the use of concentrated and diluted acids to break the rigid and complex structure of the lignocellulosic materials. The most commonly used acid was dilute sulphuric acid ( $\text{H}_2\text{SO}_4$ ), which had been commercially used to pre-treat wide variety of biomass types, for example switchgrass [64, 66], corn stover [67, 68], spruce (softwood) [69], and poplar [70, 71]. Dilute sulphuric acid had traditionally been used to manufacture furfural by hydrolysing the hemicellulose to simple sugars such as xylose, which later continued to convert into furfural [72]. However, other acids have also been studied, such as hydrochloric acid (HCl) [73], phosphoric acid ( $\text{H}_3\text{PO}_4$ ) [74, 75] and nitric acid ( $\text{HNO}_3$ ) [76]. Acid pre-treatment had the ability to remove hemicellulose and had been used to fractionating the components of lignocellulosic biomass [74].

Recent research articles had reviewed the development of acid pre-treatment of biomass over the years and highlighted the advantages and disadvantages and the optimum conditions of operation [77, 78, 79]. Hemicellulose and lignin are solubilized with minimal degradation [80]. However, the samples had to be washed extensively or a detoxification step is required to remove the acid before fermentation step [66, 68, 69]. Besides that, the corrosive nature and toxicity of the acids would need an adequate material for the reactor to withstand the required experimental conditions and corrosiveness of the acids. On top of that, another bottom neck was the production of fermentation inhibitors like furfural and hydroxymethyl furfural (HMF) which would reduce the effectiveness of the pre-treatment method and further processes [66,70].

In a research work done by Ying Yang et al., bioethanol production potential of three oven-dried switchgrass germplasms containing 26.65-29.28% glucan, 17.92-19.37% xylan, and 17.74-19.23% lignin was investigated. The evaluation of the effect of three acid concentrations (0.5, 1.0, and 1.5% w/v) with the residence times (30, 45, and 60 minutes) on composition of all germplasms indicated significant hemicellulose solubilisation relying greatly on the pre-treatment intensity. There was no apparent delignification observed during pre-treatment. The results of the weight loss of the oven-dried switchgrass were discussed in the chapter four with comparison to the result of this project.

The optimum conditions for the acid pre-treatment depended highly on the targeted sugars and the purpose of the pre-treatment. The optimal conditions for obtaining the maximum sugar yield varied depending on whether the goal was to obtain

maximum yield after the pre-treatment or after the enzymatic hydrolysis of the pre-treated solids or if the goal was to obtain maximum yield after both steps [80].

### **2.5.2 Alkaline Pre-treatment**

Alkaline pre-treatment involved the use of bases, such as sodium, potassium, calcium, and ammonium hydroxide for the pre-treatment of lignocellulosic biomass. The importance of alkaline pre-treatment in grasses was due to the fact that, alkaline pre-treatment that offered solubilisation of more than 50% of grass lignin by destruction of alkaline-labile ester linkage and also solubilizes high free phenolic content which increased the lignin solubility [79].

The mechanism of alkaline hydrolysis was to be saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components for example lignin and other hemicellulose. With the removal of the crosslinks, the porosity of the lignocellulosic materials increased [65]. The used of the alkaline caused degradation of ester and glycosidic side chains resulting in structural alteration of lignin, cellulose swelling, partial decrystallization of cellulose [29, 30, 35]. It would cause partial solvation of hemicellulose as well [42, 44].

Sodium hydroxide (NaOH) had been the most studied alkaline for many years, it had been shown to disrupt the lignin structure of the biomass, increased the accessibility of enzymes to cellulose and hemicellulose [29, 30, 31]. Dilute NaOH treatment of lignocellulosic materials caused a lot of effects which were swelling, led to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in



crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of lignin structure [48, 55].

Sun et al. had studied the effectiveness of different alkaline solution by analysing the delignification and dissolution of hemicellulose in wheat straw. They found that the optimal condition was using 1.5% sodium hydroxide for 144 hours at 20°C which resulted in 60% release of lignin and 80% release of hemicellulose [37]. Kingsley C. et al. had done a research on two different pre-treatment methods for switchgrass. Aqueous sodium hydroxide (0.5-1.0% w/v, 90°C, 1h), dilute sulphuric acid, H<sub>2</sub>SO<sub>4</sub> (0.5-6.0% v/v, 121°C, 1h) were the method used in the study to determine the digestibility of switchgrass during enzymatic hydrolysis. Switchgrass pre-treated with 0.5% w/v sodium hydroxide generally produced glucose in higher concentrations than sulphuric acid. Besides that, the SEM study on the pre-treated samples showed a great deal of pore formation in NaOH pre-treated samples and a little or no physical changes on the acid pre-treated samples. Furthermore, lignin analysis as well showed a considerable decrease in lignin content in the NaOH pre-treated samples and only a slight decrease in lignin content for the acid pre-treated samples [81].

Jiele Xu et al. had done a research work to improve the enzymatic digestibility of switchgrass by investigative the sodium hydroxide pre-treatment of the biomass feedstock. Jiele Xu et al. had carried out research at 121, 50, 21°C, raw switchgrass biomass at a solid to liquid ratio of 0.1g/mL was pre-treated, respectively for 0.25-1, 1-48, and 1-96 h at different NaOH concentrations which were 0.5, 1.0, 2.0% w/v. At the best condition of pre-treatment (50°C, 12h and 1.0% NaOH), the yield of total sugars