A COMPARISON OF SUBCRITICAL WATER AND STEAM EXPLOSION PRETREATMENTS FOR ENZYMATIC HYDROLYSIS OF SUGARCANE BAGASSE

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A THESIS SUBMITTED AS A PART OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENGINEERING IN ENERGY TECHNOLOGY AND MANAGEMENT

THE JOINT GRADUATE SCHOOL OF ENERGY AND ENVIRONMENT AT KING MONGKUT'S UNIVERSITY OF TECHNOLOGY THONBURI

2ND SEMESTER 2014

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ABSTRACT

Lignocellulosic biomass is mainly composed of cellulose, hemicellulose, and lignin. To convert lignocellulosic biomass to biofuels/chemicals, the process is usually obtained from (1) a pretreatment step for cellulose crystallinity reduction by the hydrolysis of hemicellulose and delignification, and (2) an enzymatic hydrolysis step of cellulose to produce sugar monomers. Subcritical water and steam explosion are selected as the pretreatment because these pretreatments provide biomass more readily for enzymatic digestion. In sugarcane-producing countries like Thailand, there is an abundant opportunity for the use of sugarcane bagasse. Thus, sugarcane bagasse is selected as the efficient feedstock to produce sugar monomers. The optimum conditions of pretreatment were identified and compared systematically to enhance the enzymatic digestibility of the cellulose fraction for subsequent sugar conversion. In addition, the technical feasibility of process in large scale for biorefinery industry was evaluated.

Central composite design and response surface methodology were used to optimize the subcritical water pretreatment conditions of temperature, time, and biomass/water ratio The optimum conditions of subcritical water were 170.59 °C, 19.31 min, and 1:6.64 bagasse to water ratio which resulted in the reducing sugar yield 297.09 mg/g pretreated (48.76% glucose yield) while the optimal conditions of steam explosion were 200 °C and 5 min with water soaking which led to the highest amount of sugar yield of 353.37 mg/g pretreated (59.12% glucose yield). This indicated that the use of steam explosion pretreatment could be suitable for increasing enzymatic digestibility of sugarcane bagasse. Moreover, the subcritical water pretreatment in 1000 L reactor resulted in the reducing sugars yield of 196.30 mg/g pretreated with the pretreatment conditions at 160 °C for 2 h using 1:12 bagasse to water ratio. The work indicates the potential on scaling up the developed pretreatment process in biorefinery industry.

Keyword: Lignocellulose; Bagasse; Pretreatment; Subcritical water; Steam explosion

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my thesis advisor, Assoc. Prof. Dr. Navadol Laosiripojana, for his guidance and all the support, not only the research methodologies, but also many other methodologies in life. I also thank Dr. Nawin Viriya-empikul for guiding me during my research in Nanomaterials for Energy and Catalysis Laboratory (NEC). He was always available for my questions and gave generously of his time and vast knowledge to discuss together. Moreover, I would like to thanks my other committee members, Dr. Verawat Champreda and Dr. Patrick Rousset for their helpful suggestions and comments during my research, and Assoc. Prof. Dr. Artiwan Shotipruk for being an external examiner.

I would like to acknowledge the financial support for this research by the Joint Graduate School of Energy and Environment, King Mongkut's University of Technology Thonburi, and the National Nanotechnology Center (NANOTEC).

Finally, I gratefully acknowledge my family and my friends for all their support throughout the period of the research.

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CHAPTER 1
INTRODUCTION

1.1 Rationale/Problem Statement

At present, petroleum resources are the major feedstock for the production of commodity chemicals and fuels. Nevertheless, the rapid depletion of these finite resources and the increase in emissions of CO_2 levels are encouraged as a replacement of petroleum with renewable resources such as lignocellulosic biomass, forest biomass, and municipal solid waste. Among various potential large-scale industrial biorefineries, the use of lignocellulose feedstocks is known as one of the most promising approaches particularly in the agricultural-based countries [1, 2]. This concept has been developed from the petroleum-based refinery, which produces multiple products from petroleum. As shown in Fig. 1.1, the objective of biorefinery is to produce multiple products from biomass feedstock using integrated technologies.

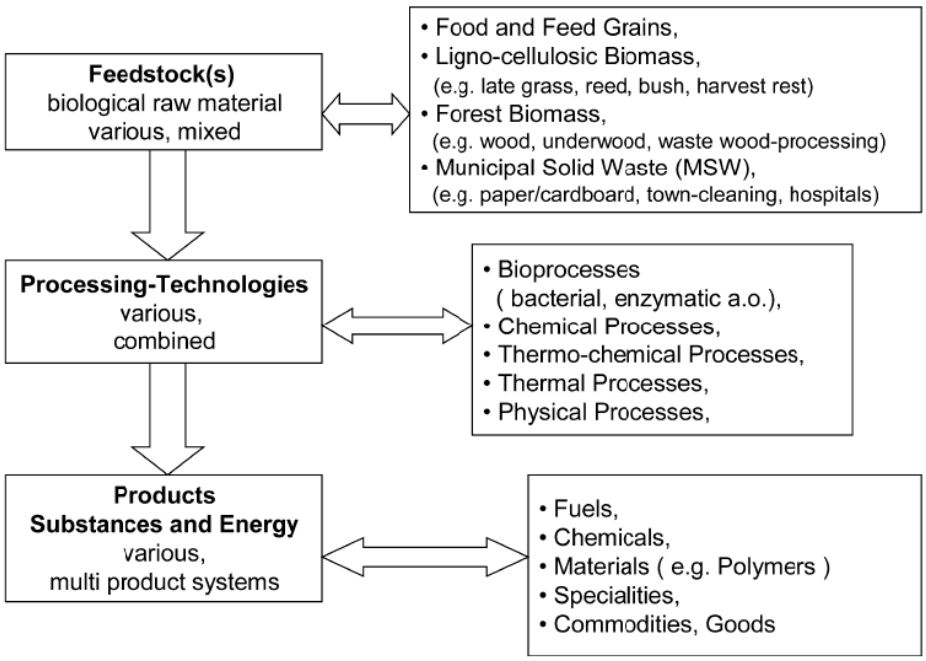


Fig. 1.1 Basic principles of a biorefinery [1]

Lignocellulosic biomass is mainly composed of cellulose (40-50%), hemicellulose (25-30%), and lignin (15-20%). Cellulose, a linear polymer of glucose molecules linked with β-(1-4)-glycosidic bonds is a major structural component of plant cell walls. Hemicellulose is a branched polymer of different 5-carbon sugars (xylose, arabinose) and 6-carbon sugars (glucose, galactose, and mannose) that are linked to cellulose and cross-linked with lignin. Lignin is an aromatic polymer that composed of p-coumaryl alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S) forming a layer of the cell walls. Cellulose, hemicellulose, and lignin form structures in the plant cell wall called macrofibrils as can be seen in Fig. 1.2. To convert lignocellulosic biomass to biofuels/chemicals, it generally requires: (1) a pretreatment step for cellulose crystallinity reduction by the hydrolysis of hemicellulose and delignification (2) an enzymatic hydrolysis step of cellulose to produce sugar monomers [3,4]. It has been known that pretreatment of lignocellulosic biomass enhances the product yield and is therefore of great importance for the efficient biorefinery process.

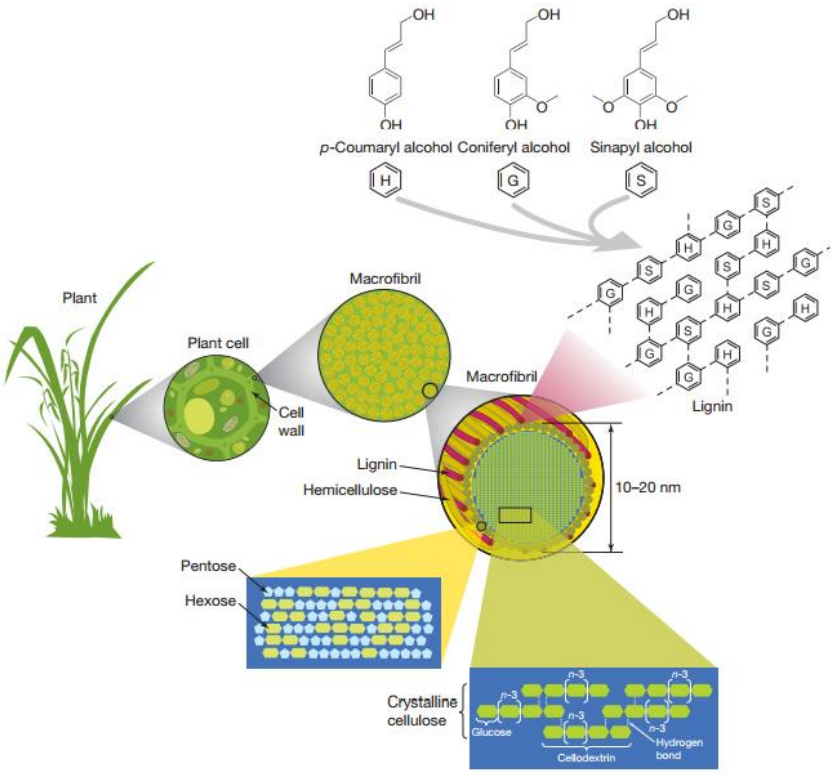


Fig. 1.2 Structure of lignocellulosic biomass [3]

Various pretreatment methods have been developed to change the physical and chemical structures to break down the hemicellulose and lignin shields efficiently.

Pretreatment using pressurized hot water (subcritical method) is an attractive approach for the processing of lignocellulosic biomass due to its advantages on environmental friendliness, chemical handling, operational costs, and potential on hemicelluloses dissolution. Hydrothermal pretreatment where fiber is heated in water at 150-230 °C, is considered as an attractive method due to a cost-effective, simple operation and properties of equipment corrosion (chemical handling). During hydrothermal pretreatment, water molecules break down the long chain of hemicellulose [5-6]. Steam explosion is another common method for pretreating lignocellulosic materials. Moreover, both subcritical water and steam explosion require little or no chemicals because their particular solvent is water, which is environmentally-friendly and cost-effective.

Table 1.1 Global sugarcane productions of main sugarcane-producing countries

Country	Sugarcane production (tonnes/yr)	%
Brazil	386,232,000	28.60
India	290,000,000	21.48
China	93,900,000	6.95
Thailand	74,071,952	5.49
Pakistan	52,055,800	3.86
Mexico	45,126,500	3.34
Columbia	36,600,000	2.71
Australia	36,012,000	2.67
Cuba	34,700,000	2.57
USA	31,178,130	2.31
Philippines	25,835,000	1.91
Other	244,581,738	18.11
TOTAL	1,350,293,120	100

As seen in Table 1, sugarcane is mostly grown in Brazil, India, China, Thailand, Pakistan, Mexico, Columbia, Australia, Cuba, the USA, and the Philippines. The volume in these countries reaches as high as 82% of total global sugarcane production. [7] In

sugarcane-producing countries, such as Thailand, there is an abundant opportunity for the use of sugarcane bagasse.

In this work, sugarcane bagasse is selected as an efficient feedstock to produce sugar monomers. Subcritical water and steam explosion pretreatment will be performed under several operating conditions in order to increase biomass digestibility prior to the enzymatic hydrolysis. The optimum pretreatment conditions will be determined based on experimental design studies.

1.2 Literature Review

As described above, the main objective of this research is to study the subcritical water and steam explosion pretreatment of sugarcane bagasse. The work aims to study and identify the optimum conditions to increase the enzymatic digestibility of the cellulose fraction for subsequent sugar conversion. Until now, several pretreatment technologies and enzymatic hydrolysis have been widely developed. Details of these previous research studies are presented below:

1.2.1 Pretreatment

Lignocellulosic biomass is mainly composed of three components, including cellulose, hemicellulose, and lignin. Pretreatment step is one of the most important processes in the biofuel production. Pretreatment method refers to the solubilization and separation of components in biomass. It makes the remaining solid biomass more accessible for further chemical or biological treatment. Each pretreatment has different impacts on compositional and structural features of biomass. Several pretreatment technologies have been developed during the last decades. Those methods are usually classified into physical, biological, chemical, and physicochemical pretreatments. The effective pretreatment technologies can be defined as below:

- Enhance the formation of sugars
- Minimize sugar degradation and formation of inhibitory products
- Decrease the degree of polymerization and crystallinity index
- Disrupt lignin-carbohydrate linkages to lignin and hemicelluloses
- Increase the porosity of biomass materials

A.) Biological Pretreatments

The biological pretreatment typically uses microorganisms, including brown, white, and soft rot fungi, to degrade hemicellulose and lignin, but slightly for cellulose since it is more resistant to the fungi compared to other components. Normally, white rot fungi which is selective to lignin degradation over cellulose can be successfully applied in microbial pretreatments. Several white rot fungi such as *Phanerochaete chrysosporium*, *Ceriporia lacerata*, *Cyathus stercolerus*, *Ceriporiopsis subvermispora*, *Pycnoporus cinnarbarinus*, and *Pleurotus ostreaus* have been examined on different lignocellulosic biomass showing high delignification efficiency [8]. Advantages of biological pretreatment are low capital cost, low energy requirement, no chemicals required, mild environmental condition, and no inhibitory compound formation.

Ray *et al.* [9] reported that brown fungal pretreatment significantly improved the enzymatic hydrolysis of Pinusradiata sapwood. Six different fungi, including *C. puteana* (brown rot), *P. placenta* (brown rot), *T. versicolor* (white rot), *C. globosum* (soft rot), *T. viride* (mould), and *Mucor sp.* (mould) were examined in this biological pretreatment. *C. puteana* (brown rot) gave the highest yield of glucose as shown in Fig. 1.3. The brown rot pretreatments under mild conditions gave cellulose-to-glucan yields in the order of 70% biomass (Pine sapwood comprises ~ 44-47% glucose) after enzymatic saccharification, representing the good potential in lignocellulosic biomass pretreatment.

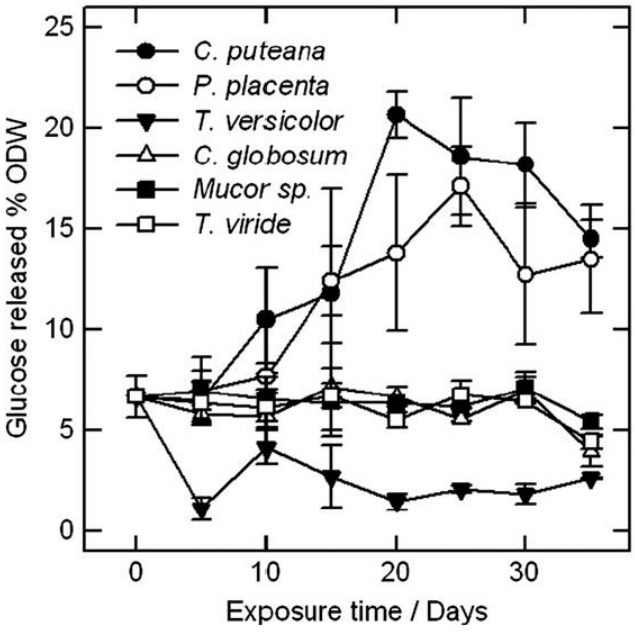


Fig. 1.3 Glucose yields from Pine sapwood pretreated with six different fungi [9]

Song *et al.* [10] developed the biological pretreatment of corn stover by adding metal ions for improving subsequent enzymatic hydrolysis. White rot fungus *Irpex lacteus* and metal ion (K^+, Ca^{2+}, Cu^{2+}, Fe^{2+}, Mg^{2+}, and Mn^{2+}) were used in fungal pretreatment. Their results showed that the efficiency of fungal pretreatment was greatly improved with the manganese addition in corn stover. After enzymatic hydrolysis of 28 days pretreated corn stover, maximum glucose yield was 308.98 mg/g corn stover with manganese addition, which increased by 61.39% compared to the conventional fungal pretreatment. Furthermore, manganese also enhanced the production of ethanol (83.39% of ethanol conversion). Manganese greatly improved the delignification especially of *Irpex lacteus*. This study showed a promising effect of manganese on fungal pretreatment and the production of biofuels. However, the main drawbacks of biological pretreatment are loss of polysaccharides (hemicelluloses and cellulose) and the longer pretreatment duration compared to other technologies. It is necessary to continue studying and testing more fungi for improving the biological pretreatment of the lignocellulosic material at shorter pretreatment time with higher efficiency.

B.) Physical Pretreatments

Mechanical Pretreatment

The objective of the mechanical pretreatment is the reduction of particle size and crystallinity of lignocellulosic biomass to increase the specific surface area and reduce the degree of polymerization (DP). Different mechanical processes, such as chipping, milling, or grinding, are selected depending on the desired particle size on the further process. Hideno *et al.* [11] developed a new type of wet disk milling for rice straw pretreatment. Wet disk milling showed higher hydrolysis yields (glucose and xylose) than conventional dry milling. In addition, Buaban *et al.* [12] presented high ethanol yields that obtained from mechanical ball milling pretreatment with enzymatic hydrolysis and fermentation from sugarcane bagasse. However, the mechanical pretreatment has a barrier in high energy consumption that is unlikely economically feasible.

Extrusion

Extrusion is a novel and promising physical pretreatment method for biomass conversion to ethanol production. In the extrusion method, the biomass is heated, mixed, sheared, and resulted in physical and chemical modifications during the path through the extruder. The extruder has many advantages, such as the ability to provide high shear, rapid heat transfer with an effective and rapid mixing process. Extrusion has the potential to become an interesting option for lignocellulosic pretreatment. It has been recently employed for increasing the enzymatic hydrolysis yields of switchgrass [13], corn stover [14], wheat bran [15], and soybean hull [15].

C.) Chemical Pretreatments

Chemical pretreatments have been originally developed and extensively used in the paper industry for delignification of cellulosic materials to produce high quality paper products. Chemical pretreatments that have been studied to date have had the primary goals on improving the biodegradability of cellulose by removing lignin and/or hemicellulose, and decreasing the degree of polymerization (DP), and crystallinity of the cellulose component. Chemical pretreatment were the most widely studied method compared to other pretreatment categories. Currently, seven promising chemical pretreatment techniques have been reported including catalyzed steam-explosion, acid, alkaline, ammonia fiber/freeze steam-explosion, organosolv, pH-controlled liquid hot water, and ionic liquids pretreatments [16].

Acid Pretreatment

Acid is added to the raw material and the mixture is held at an elevated temperature for a short period of time. Acid pretreatment employs acids as catalysts, which can significantly solubilize the hemicellulose of biomass and makes the cellulose more accessible to enzymes. Acid pretreatment can be classified into two groups; concentrated acid pretreatment and diluted acid pretreatment. Nevertheless, concentrated acid pretreatment has important drawbacks in inhibiting compound formation, equipment corrosion, and the difficulty in acid recovery. Diluted acid pretreatment has been successfully developed and appeared to be a favorable method in industrial scale [17]. The most widely used acids in this process are dilute sulfuric acid (H_2SO_4), hydrochloric acid (HCl), nitric acid (HNO_3), and phosphoric acid (H_3PO_4). They can offer effective hemicellulose solubilization and provide better cellulose availability [18].

Chen *et al.* [19] studied the pretreatment efficiency of rice straw by an integrated process of dilute acid and steam explosion. The optimum operational conditions for the first

dilute acid were determined at 165 °C for 2 min with 2% H_2SO_4 and for the second step (steam explosion) was carried out at 180 °C for 20 min. They found that rice straw pretreated by the two-step process of dilute acid/steam explosion had a higher xylose yield and a greater degree of enzymatic hydrolysis when compared to the acid-catalyzed steam explosion as shown in Fig. 1.4.

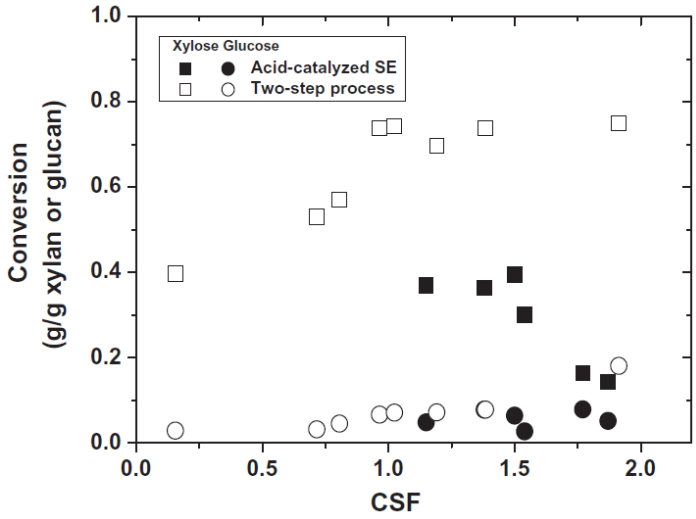


Fig. 1.4 The obtained xylose and glucose conversions for pretreatment versus combined severity factor (CSF) of the dilute acid hydrolysis (the first step) and the acid-catalyzed steam explosion [19]

Baadhe *et al.* [20] studied the influence of dilute acid and alkali pretreatment on reducing sugar production from corncobs by the crude enzymatic method. Firstly, solid (biomass) to liquid (acid/alkali) ratio was examined. 1:20 S/L ratio was the optimum ratio that released maximum sugar in both acid and alkali pretreatment. In enzymatic hydrolysis, the maximum sugar yield released during 36 h from both acid and alkali pretreatment as shown in Fig. 1.5. The highest amount of sugars released during enzymatic hydrolysis of 0.25 M H_2SO_4 treated corncob was 398.5 mg/mL whereas corncob pretreated with 1 M NaOH released fewer amounts of sugars that was 320.75 mg/mL. This indicated that acid pretreatment (H_2SO_4) is more suitable for the subsequent fermentation process for the value-added chemicals production.

Some sugar degradation compounds, such as furfural, HMF, and aromatic lignin degradation compounds, are normally detected during concentrated acid pretreatment. To

solve this problem, dilute acid pretreatment could generate lower degradation products than concentrated acid pretreatments.

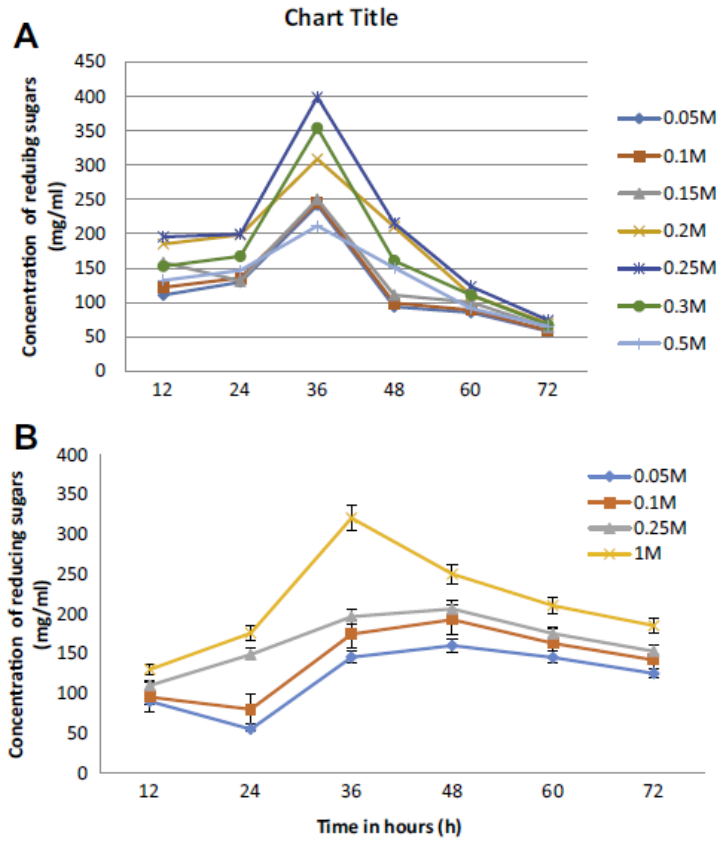


Fig. 1.5 Enzymatic hydrolysis of (A) H_2SO_4 and (B) NaOH pretreated corncobs with S/L 1:20 [20]

Alkaline Pretreatment

Alkaline pretreatment employs various bases, such as sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$), potassium hydroxide (KOH), and ammonia hydroxide (NH_4OH) [8]. Alkaline pretreatment increases cellulose digestibility and is effective for lignin solubilization. In addition, the alkaline pretreatment can swell a plant cell wall to increase the internal surface of cellulose and decrease the degree of polymerization that improves the accessibility for the subsequent enzymatic hydrolysis [21]. Normally, alkaline pretreatment can be performed at room temperature. Kumar *et al.* [3] described that alkaline pretreatment caused less sugar degradation than acid pretreatment and was more effective on agricultural residues than on wood materials.

Toquero and Bolado [22] compared four different pretreatment methods, including thermal, dilute acid, dilute basic, and alkaline peroxide, on enzymatic hydrolysis and ethanol fermentation of wheat straw. Hydrolysates of washed alkaline peroxide pretreated wheat straw provided the highest sugar concentrations (31.82 g/L glucose), xylose (13.75 g/L), and ethanol concentrations (17.37 g/L). Thus, alkaline-peroxide was considered the most suitable pretreatment for wheat straw.

Yang *et al.* [23] presented the dilute alkali pretreatment of Sawtooth Oak shell waste for the first time. Three parameters, temperature, time, and alkalinity, were varied during the pretreatment process. The optimal pretreatment conditions were 2% NaOH at 121 °C/15 psi for 60 min which achieved the highest delignification (39.34%), the highest sugar release (426.36 mg/g pretreated material), the maximum sugar recovered from the holocellulose fraction (494.5 mg/g oak shell, 78.8% conversion) This study provides a new effective pretreatment for fuel/chemicals production using Sawtooth Oak shell waste. Inhibitory compounds probably formed during the alkaline pretreatment from the loss of sugars. Moreover, cost of operations and energy requirement are significant factors in the economic viability of an industrial scale process.

Organosolv Pretreatment

The organolsolvation method is a promising pretreatment strategy and is more attractive because of the potential for utilization in lignocellulosic material. In the organosolvation process, numerous organic/aqueous solvent mixtures can be utilized. The solvents, usually used in this process are methanol, ethanol, acetone, ethylene glycol, triethylene glycol, and tetrahydrofurfuryl alcohol. Typically, acids such as HCl, H_2SO_4, oxalic acid, salicylic acid, and acetylsalicylic acid can be added as catalysts. Organosolv pretreatment provides good lignin solubility of biomass and yielded suitably treated cellulose for further enzymatic hydrolysis. A benefit of the organosolv pretreatment is the relatively pure lignin as a by-product. However, the high cost of solvents is the important disadvantage for economic reasons [3, 17].

Amiri *et al.* [24] studied the organosolv pretreatment of rice straw for efficient acetone, butanol, and ethanol production (ABE). Pretreatment of the straw with 75% (v/v) aqueous ethanol containing 1% w/w sulfuric acid at 150 °C for 60 min resulted in the highest total sugar concentration of 31 g/L in the enzymatic hydrolysis. However, the highest ABE concentration and productivity (10.5 g/L and 0.20 g/L h, respectively) were obtained from

the straw pretreated at 180 °C for 30 min. Thus, the organosolv pretreatment at 180 °C for 30 min can be applied for the efficient production of the solvents from rice straw.

Organosolv pretreatment provides good solubility of lignin from biomass and yields suitably treated cellulose for further enzymatic hydrolysis. Nevertheless, the high cost of solvents should be considered for industrial applications.

Ozonolysis Pretreatment

Ozonolysis pretreatment has been used as a technique for the delignification of biomass, which oxidatively degrades the lignin polymer structure through the process that slightly affects hemicellulose. The ozonolysis process is normally performed at room temperature and normal pressure which is difficult for inhibitory compound formation. The performance of enzymatic digestibility depends on feedstock type with the optimum conditions such as biomass moisture content (25-35%), ozone concentration (2-6%) and reaction time (1-2 h). There are no toxic residues left out because the ozone in the system will quickly decompose back to O_2 due to its short half-life [17, 24].

Ozonolysis has been applied on several agricultural residues, such as wheat straw and rye straw, increasing in the enzymatic hydrolysis yield after ozonolysis pretreatment [25]. Travaini *et al.* [26] also studied ozonolysis pretreatment of sugarcane bagasse to increase lignocellulosic material digestibility. Bagasse was ozonated at room temperature by varying ozone concentration and sample moisture. Ozonolysis significantly increased fermentable sugars released during enzymatic hydrolysis. Glucose and xylose yields increased from 6.64% and 2.05% (for raw bagasse, to 41.79%, and 52.44%) under the best experimental conditions (3.44 % (v/v) Ozone concentration, 40 % (w/w) moisture of raw bagasse)**.**

Nevertheless, its important drawback is the high amount of ozone needed, which can make the process economically unfeasible.

Ionic Liquids (ILs) Pretreatment

Ionic liquids (ILS) are salts that exist in liquid phase at relatively low temperature or room temperature. The characteristics of ILS are typically comprised of large organic cations with heterogeneous molecular structure and small inorganic anions, such as N-methylmorpholine-N-oxide monohydrate (NMMO), 1-allyl-3-methylimidazolium chloride, 3-methyl-N-bytylpyridinium chloride (MBPCl), and benzyldimethyl (tetradecyl) ammonium chloride, used for pretreatment. The advantages of ILs are their thermal and chemical stability, wide liquid temperature range, nonflammability, and good solvating properties of various types of materials. ILs pretreatment leads to the opening of the hydrogen bonds between molecular chains of the cellulose and creates the cellulose dissolution. Several imidazolium-based ILs were originally reported as good methods to dissolve large amounts of cellulose. The recovered cellulose has increased porosity and decreased crystallinity [17, 27].

Nasirpour *et al.* [28] developed the ionic liquid pretreatment of sugarcane bagasse for enhancing enzymatic hydrolysis by surfactant addition. 1-butyl-3-methyl imidazolium chloride ([BMIM]Cl) was used as an ionic liquid (IL). Different concentrations of Tween 80 (TW) and polyethylene glycol 4000 (PEG) were used to determine the optimum concentration of surfactant for the highest percentage of cellulose conversion. Both TW and PEG increased lignin removal by 12.5% over the IL-only pretreated sample. The 3% (w/w) PEG showed a significant increase in enzymatic digestibility with an efficiency of 96.2% after 12 h of hydrolysis; this was 23% higher than the efficiency of SCB pretreated with IL. The increase in digestibility of surfactant assisted IL pretreatment method can be attributed to the decrease in cellulose crystallinity, changes in the cellulose lattice, and delignification, which was confirmed by FT-IR, XRD, and FE-SEM analysis. However, there are still many challenges for the large-scale application of ILs pretreatment, such as the high cost of solvent, energy-efficient recycling methods, and lack of detailed toxicity of enzymes and fermentative microorganisms.

D.) Physicochemical Pretreatment

Wet Oxidation

Wet oxidation is an oxidative pretreatment method that employs oxygen or air as a catalyst. Oxidative pretreatment is performed at temperature 170-200 °C, pressure 10-12 bar O_2 for a short period of time. The temperature, followed by reaction time and oxygen pressure, are the most important parameters in wet oxidation [29]. The addition of oxygen at temperatures above 170 °C makes the process exothermic reducing the total energy demand. Wet oxidation method is an efficient method for solubilization of hemicellulose and lignin. The soluble sugars are produced from hemicellulose and oligomer. During wet oxidation pretreatment, there is low formation of inhibitors e.g. phenolic compounds, furfural and 5-hydroxymethyfurfural (HMF). Furthermore, Na_2CO_3 addition has been shown to decrease the formation of inhibitory compounds by maintaining pH from medium to base range [30].

This technology has been widely used for ethanol production followed by simultaneous saccharification and fermentation (SSF). Martin *et al.* [31] studied wet oxidation pretreatment of clover (*Trifolium repens*)-ryegrass (*Lolium perenne*) mixtures. The highest conversion efficiency (93.6%) was achieved for the sample pretreated at 195 °C, 1.2 MPa for 10 min. The simultaneous saccharification and fermentation of the pretreated material yielded cellulose conversions of 87.5 and 86.6%, respectively.

Haagensen *et al.* [32] studied pretreatment and ethanol fermentation potential of olive pulp at different dry matter concentrations. The combination of wet oxidation and enzymatic hydrolysis resulted in the glucose and xylose concentration increases in 138 and 444%, respectively, compared to 33 and 15% with only enzymes added. However, costs of oxygen and catalyst are considered as the main disadvantages for wet oxidation development technologies.

Ammonia Fiber Explosion (AFEX)

During AFEX pretreatment, the biomass is treated with liquid anhydrous ammonia at a temperature range of 60 to 100 °C and high pressure for a period of time. Then the pressure is released, vaporizing the ammonia and allowing its recovery and recycling. The ammonia has a marked effect on lignocellulose causing swelling and physical disruption of biomass fibers, partial decrystallization of cellulose, and breakdown of lignin-carbohydrates linkages. Herbaceous and agricultural residues are well suited for AFEX. However, this method works only moderately well on hardwoods, and is not attractive for softwoods. The

low formation of inhibitors for the downstream biological processes is one of the main advantages of the AFEX pretreatment, even though some phenolic fragments of lignin and other cell wall extracts may remain on the cellulosic surface [8, 33].

At optimal conditions, AFEX can achieve more than 90% conversion of cellulose and hemicellulose to fermentable sugars. Wyman *et al.* [34] indicated that despite the removal of only small amounts of lignin or hemicellulose in the AFEX process, enzymatic digestion is very high at low enzyme loadings compared to other pretreatment. This may suggest that ammonia affects lignin and possibly hemicellulose differently from other chemicals. It reduced the ability of lignin to adsorb enzyme and make its access to cellulose more difficult.

Recently, Jin *et al.* [35] showed that AFEX pretreatment can be successfully used in SSCF processes with recombinant *S. cerevisiae* and *Escherichia coli* strains which resulted in high ethanol yields from switchgrass and corn stover, respectively.

Steam Explosion

Steam explosion is one of the most widely employed technologies for pretreating lignocellulose for bioethanol production. It is a hydrothermal pretreatment in which the biomass is subjected to pressurized steam for a period of time and then suddenly depressurized. It combines mechanical forces and chemical effects due to the hydrolysis (autohydrolysis) of acetyl groups present in hemicellulose. The most important factors affecting the effectiveness of steam explosion are particle size, temperature, residence time, and Overend and Chornet [36] found the combined effect of both temperatures (T) and time (t), which is described by the severity factor ($\log R_0$) as [$\log R_0 = \log(t*e^{[T-100/14.75]})$]. The steam explosion process offers several attractive features when compared to other pretreatment technologies and has been successfully proven in ethanol production from a wide range of raw materials.

Boluda-Aguilar *et al.* [37] studied the steam explosion pretreatment of lemon (*Citrus limon* L.) citrus peel wastes to obtain bioethanol, galacturonic acid and other co-products. The steam explosion pretreatment showed an interesting effect on lemon peel wastes for obtaining ethanol and galacturonic acid. The SSF processing of steam-exploded lemon citrus peel wastes with a low enzymatic concentration produced more than 60 L ethanol/1000 kg fresh lemon citrus peel wastes. In addition, it has been shown that the minimum inhibitory concentration of lemon citrus essential oils on yeast is lower than that obtained from orange and mandarin citrus essential oils.

Wang *et al.* [38] developed a two-step process based on steam explosion pretreatment followed by alkaline ethanol solution post-treatment to fractionate Lespedeza stalks. It was found that the content of glucose in cellulose rich fractions was gradually increased from 73.7 to 86.9% as the result of raising steaming pressure. The scanning electron microscopy images of the cellulosic residues in Fig. 1.6 shown that steam explosion mainly resulted in breakage of the fibers, and extraction post-treatment led to the solution of lignin (and hemicelluloses) and significant defibrillation. Thereby, significantly improving the enzymatic hydrolysis efficiency.

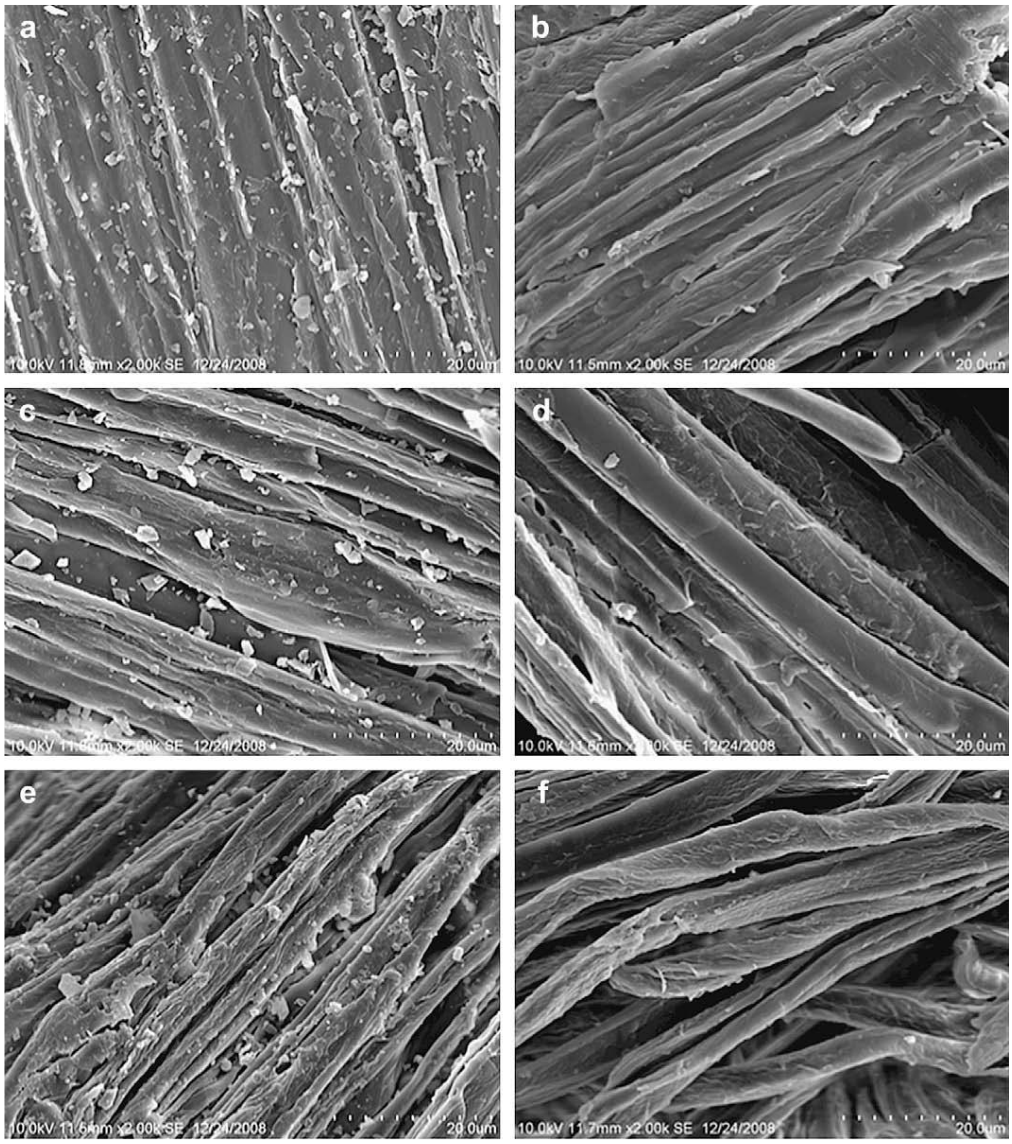


Fig. 1.6 Scanning electron micrographs of steam-exploded and alkaline ethanol extracted Lespedeza stalks (2000 magnification). (a) steam-exploded at 15 kg/m^2 for 4 min, (b) extracted after steam explosion at 15 kg/m^2 for 4 min, (c) steam-exploded at 20 kg/m^2 for 4 min, (d) extracted after steam explosion at 20 kg/m^2 for 4 min, (e) steam-exploded at 25 kg/m^2 for 4 min, (f) extracted after steam explosion at 25 kg/m^2 for 4 min. [38]

Singh *et al.* [39] reported the steam explosion of sugarcane bagasse, which eventually showed the enzymatic hydrolysis efficiency of 100 % after 24 h of incubation by using the cellulases from *Penicillium pinophilum* with an enzyme loading of 10 FPU/g. To compare its potential use with commercially available cellulose (Accellerase^{TM} 1000), the results indicated that using the Penicillium cellulase and Accellerase^{TM} 1000 showed that the

saccharifying potential are comparable towards the treated substrates, such as steam exploded sugarcane bagasse and ball milled cellulose powder.

Kemppainen *et al.* [40] compared three different pretreatments of spruce bark, including steam explosion (SE), hot water extraction (HWE) at 80 °C, and sequential hot water extraction and steam explosion (HWE + SE) for ethanol production. The best steam explosion conditions were at 190 °C for 5 min without acid catalyst based on the efficiency of enzymatic hydrolysis. However, the hydrolysis rate and yield of HWE bark was as good as that of SE and HWE + SE barks when pectinase was added in the enzyme mixture. Ethanol was efficiently produced from the pretreated and hydrolyzed materials suggesting that of the hot water extraction was suitable for spruce bark pretreatment.

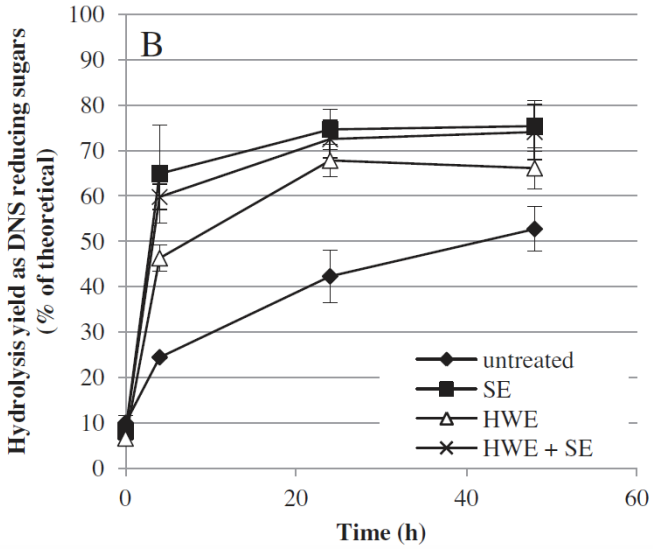


Fig. 1.7 Hydrolysis of pretreated spruce bark cellulase and β-glucosidase, high dosage of pectinase

Liu *et al.* [41] studied effects of biomass particle size on steam explosion pretreatment for improving the enzyme digestibility of corn stover. The particle sizes of corn stover at 2.5, 2.0, 1.5, 1.0 and 0.5 cm were compared. The highest sugar recovery reached 99.6% for glucan and 67.0% for xylan at the particle size of 1.0 and 0.5 cm, respectively, but the highest sugar conversion (100% for glucan and 83% for xylan) was obtained at the particle size of 2.5 cm. The enzymatic hydrolysis rate and conversion of pretreated biomass obviously increased with increasing biomass particle size. The results indicated the larger

corn stover particles were better mixed and reacted with steam explosion pretreatment that would be more suitable to reduce process cost compared with smaller ones.

Martin *et al.* [42] studied the fermentability of the enzymatic hydrolyzates of sugarcane bagasse pretreated by a steam explosion method with different impregnating agents. Three experimental conditions were (1) impregnated with sulfur dioxide (SO_2), (2) impregnated with sulfuric acid (H_2SO_4), and (3) without any impregnation. The H_2SO_4-impregnated bagasse presented the highest glucose yield (35.9 g/100 g), but the lowest sugar yield (42.3 g/100 g) among three impregnating conditions, as shown in Tables 1.2 and 1.3.

Table 1.2 Yields after pretreatment, g/100 g dry bagasse [42]

Impregnating agent	Glucose yield	Xylose yield	Arabinose yield	Acetic acid yield	Fiber yield
None	1.0 (0.07)	6.1 (0.00)	0.6 (0.07)	3.5 (0.21)	72.1 (1.48)
SO_2	1.0 (0.14)	8.2 (0.14)	0.9 (0.07)	4.3 (0.14)	69.2 (0.92)
H_2SO_4	22.6 (0.84)	3.6 (0.42)	0.4 (0.14)	7.0 (0.42)	58.2 (1.77)

[a]Mean values from two replicates are indicated. The standard deviation is shown in parentheses.

Table 1.3 Sugar yields after pretreatment and enzymatic hydrolysis, g/100 g dry bagasse [42]

Impregnating agent	Glucose	Xylose	Arabinose	Total sugar
None	33.0 (0.42)	13.1 (0.07)	1.3 (0.14)	47.4
SO_2	35.2 (0.56)	16.2 (0.61)	1.5 (0.20)	52.9
H_2SO_4	35.9 (0.93)	5.9 (0.18)	0.5 (0.15)	42.3

[a]Mean values for at least three replicates. The standard deviation is shown in parentheses.

After enzymatic hydrolysis, the low total sugar yield from the H_2SO_4-impregnated bagasse was largely due to by-product formation, as the dehydration of xylose to furfural. H_2SO_4 impregnation led to an increase in the concentration of the fermentation inhibitors including furfural, 5-hydroxymethylfurfural (HMF), formic acid, acetic, acid and levulinic acid compared to the other two impregnating agents.

Subcritical Water Pretreatment

Ahmed *et al.* [43] studied subcritical water and dilute acid pretreatments for bioethanol production from *Melaleuca leucadendron* shedding bark (Paper-bark Tree, PBT). Dilute sulfuric acid concentration (0%, 0.5%, 1%, and 2% v/v), pretreatment temperature (120, 140, and 160 °C), and pretreatment time (15, 30, and 60 min) on yield of sugars and inhibitors, and to identify optimal pretreatment conditions. Overall, dilute acid pretreatment showed a better effect on xylan solubilization in comparison with the subcritical water for PBT bark. Thus, the optimum pretreatment conditions for PBT shedding bark is 1% (v/v) H_2SO_4, 140 °C, and 30 min, which is confirmed by high dissolution of hemicellulose and small loss of cellulose. Moreover, the formation of degradation products of pentose and hexose sugar, including furfural and 5-hydroxymethylfurfural (HMF), was also investigated. Subcritical water prehydrolysate was free of detectable inhibitors while the accumulation of furfural and HMF increased with increasing process conditions in dilute acid prehydrolysate. However, fermentation trials confirmed the feasibility to convert the hydrolysate into ethanol with high yield (91%) at lower inoculums, which implies that paper bark tree shedding is a promising feedstock for bioethanol production.

Table 1.4 Effect of alkali and subcritical water treatments on the solubilization (%) of raw Taiwanese sugarcane bagasse [44]

Fraction	*Alkali treatment, NaOH (M)*			
	0	1	2	4
Soluble (liquid fraction)	19.97 ± 1.48	31.08 ± 0.60	65.21 ± 0.77	69.55 ± 0.91
Residue (solid fraction)	80.03 ± 1.48	68.92 ± 0.60	34.21 ± 0.77	30.45 ± 0.91
Fraction	*Subcritical water treatment*			
	120 °C	140 °C	160 °C	180 °C
Soluble (liquid fraction)	31.16 ± 0.98	50.99 ± 1.17	60.34 ± 0.52	66.86 ± 2.62
Residue (solid fraction)	68.84 ± 0.98	49.01 ± 1.17	39.66 ± 0.52	33.14 ± 2.62

* Each experimental variable was run three times, and the results were expressed via mean value and standard deviation. A two-tailed p value of less than 0.05 was considered to be statistically significant.

Ju *et al.* [44] studied soluble and insoluble fractions of sugarcane bagasse pretreated by alkali and subcritical water. As shown in Table 1.4, the yields of the insoluble extraction residue are closed to the cellulose content of bagasse, indicating a substantial removal of hemicelluloses and lignin during alkali and subcritical water extractions of sugarcane bagasse. Evidently, the extractability of hemicelluloses in sugarcane bagasse was increased

by alkali and subcritical water treatments, which are thought to be due to the chemical and thermal degradation of cell walls resulting in cell wall disruption. Such effects of alkaline delignification, acidic, and subcritical water treatments were shown to improve the isolation of lignin, hemicelluloses, and cellulose extracts from various plant materials.

Abdelmoez *et al.* [45] studied subcritical water technology for the hydrolysis of wheat straw to produce reducing sugars. They found the optimum hydrolysis conditions at the temperature of 190 °C, 30 min hydrolysis time, 6/1 of water/wheat straw ratio, and 180-355 µm of a wheat straw particle size that obtained 51.5% of the maximum yield of total reducing sugars (TRS). Under these optimum conditions, the maximum yield of TRS was 32.6% for 1.2% of H_2SO_4 hydrolysis. They inferred that the subcritical water hydrolysis was better than acid hydrolysis. They also analyzed the collected wheat straw sample and showed that the hydrolysis product consists of 3.2% of glucose (0.33 g), 7.6% of xylose (0.79 g) and 89.2% of remaining reducing sugars (arabinose, galactose, etc.). On the other hand, the compositions of fructose and mannose were insignificant. They compared the total yield of hydrolysis (including glucose, fructose and xylose) with a new combined supercritical and subcritical hydrothermal technology gave glucose yield of 6.7% while the total yield was 10.8% in their work.

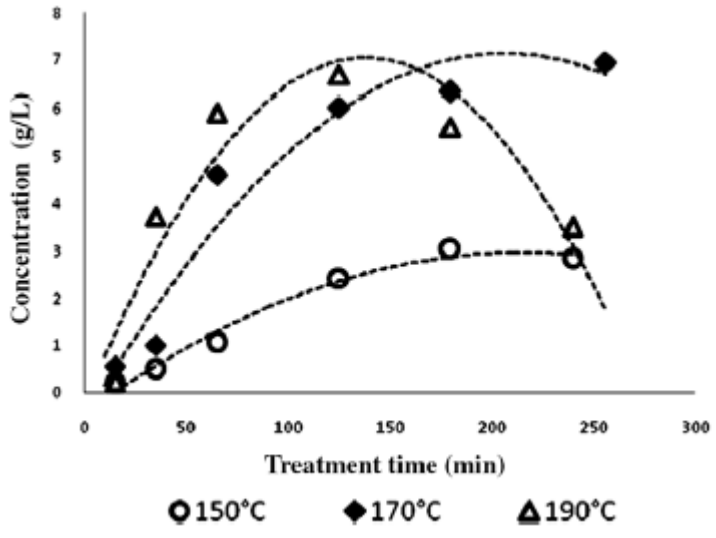


Fig. 1.8 HPAEC analysis of the xylose composition of hydrolysates obtained by hydrothermal treatment of sugarcane bagasse at different temperatures (150 °C, 170 °C, and 190 °C) [46]

Boussarsar *et al.* [46] studied the optimization of sugarcane bagasse conversion by hydrothermal treatment for the recovery of xylose to the production of xylitol. Hydrothermal treatment optimization step was achieved by varying reaction time and temperature to select operational conditions that allow obtaining of a high xylan solubilization with minimum of impurities. The results shown in Fig 1.7 indicated that there was a minimal extraction and low solubilization at 150 °C and xylose concentration seemed to decrease after 2 hours at 190 °C. For this reason, the temperature at 170 °C was selected as optimal temperature for sugarcane bagasse pretreatment. Furthermore, maximum yield was obtained 55±0.22% (w/w) after 4 hours, but 2 hours permitted to reach 48.8±0.22% (w/w) of extraction. Therefore, they were selected at 2 hours as a reaction time. Xylose and dry mass analysis allowed supporting 170 °C and 2 h as optimum pretreatment conditions.

Goh *et al.* [47] studied the hot compressed water (HCW) pretreatment of oil palm fronds (OPF) to enhance sugar recovery in enzymatic hydrolysis. A quadratic polynomial equation was used to model glucose yield by multiple regression analysis, using response surface methodology (RSM). The optimum conditions for HCW pretreatment of OPF were found at 178 °C, 11.1 min, and a liquid-solid ratio of 9.6. Under this optimum conditions, HCW pretreatment of OPF showed good performance with a 92.78 % wt. of glucose yield, which was consistent to the calculated value using the model.

1.2.2 Hydrolysis

Hydrolysis is the subsequent step following pretreatment. The objective of hydrolysis is to break down polysaccharides into monosaccharides. Cellulose is broken down to glucose and hemicellulose is broken into pentose and hexose sugars, mainly xylose. The hydrolysis can be divided into two groups, including chemical and enzymatic. This study will be focused in enzymatic hydrolysis. Enzymes are added to the biomass, acting as catalysts to destroy the glycosidic bonds of the polysaccharides. The mechanism involved in the enzymatic degradation of lignocellulose depends on the chemical nature and physical structure of the substrate.

Xiao *et al.* [48] reported the enhanced enzymatic hydrolysis of bamboo (*Dendrocalamus giganteus* Munro) culm pretreated by hydrothermal pretreatment. The pretreatment temperatures were varied at 140 °C, 160 °C, 180 °C, and 200 °C with 10 min, 30 min, 60 min, and 120 min of the reaction time. The main fraction of the degraded component during the hydrothermal process was hemicellulose. Xylan (expressed as hemicelluloses) was almost completely removed at 200 °C. Hemicellulose degraded first,

followed by lignin decomposition at intermediate temperatures during the hydrothermal process, whereas cellulose was decomposed at a relatively high temperature (> 230 °C) and only a few degradation products were detected at low temperatures (< 200 °C). As expected, yield of glucose and the hydrolysis efficiency were significantly affected by the hydrothermal pretreatment as shown in Fig. 1.9. The maximum glucose yield of 75.7% with an acceptable solid residue (65.2%) and cellulose content (49.9%) was achieved from the residue obtained by the hydrothermal pretreatment at 200 °C for 120 min (Fig. 1.9 D). It indicated that the hydrothermal pretreatment could effectively enhance the enzymatic hydrolysis efficiency.

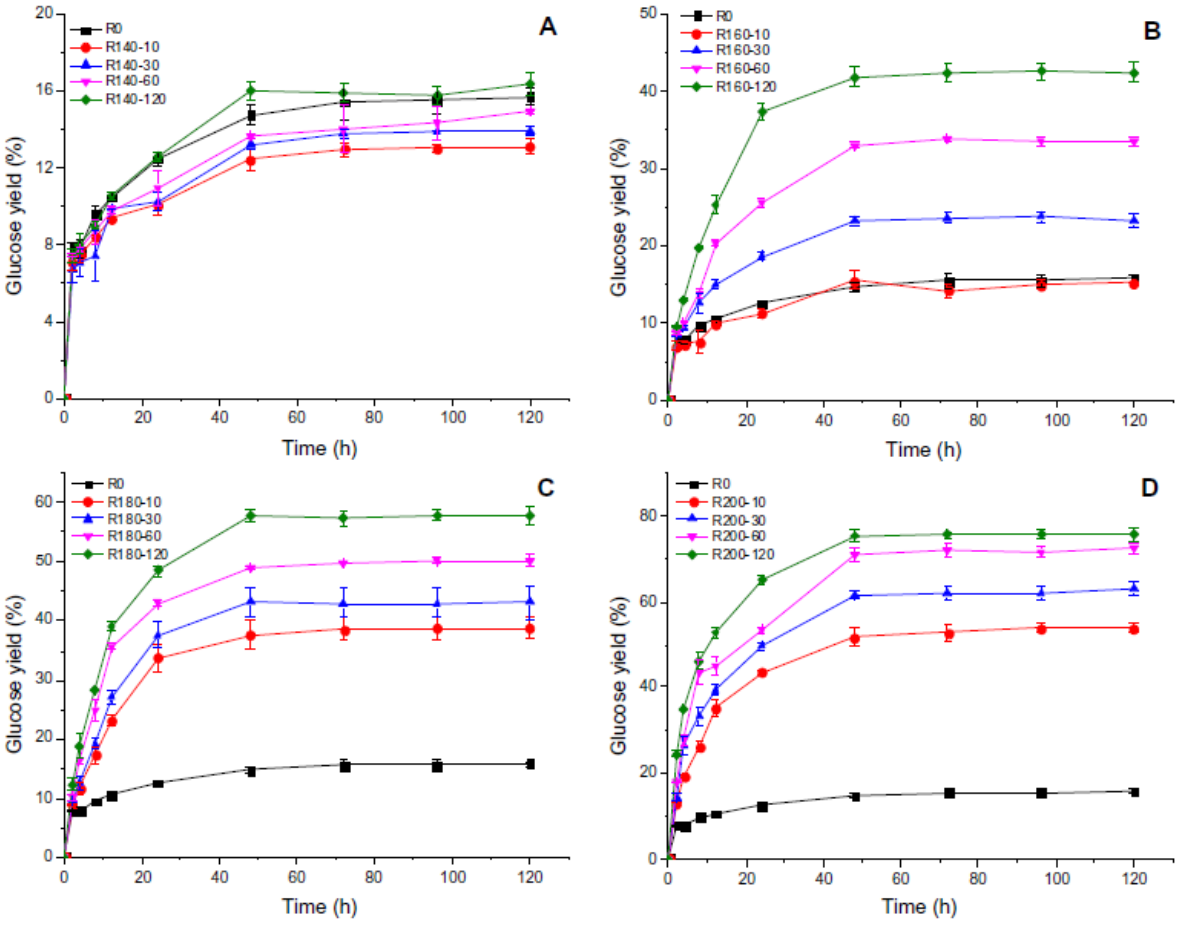


Fig. 1.9 Glucose yields of enzymatic hydrolysis of the raw material and the residues under different pretreatment conditions (A) 140 °C, (B) 160 °C, (C) 180 °C, (D) 200 °C [48]

It has been established that digestibility of a biomass is depending on the type of pretreatment, enzyme efficiency, and loading. The recent results indicate that the mixing is

another important factor in integrating pretreatment and hydrolysis [49]. In Fig. 1.10, acid pretreated corn stover (PCS) and hot water wheat straw (HWS) were hydrolyzed with a mixture of Celluclast and Novozym 188 (a weight ratio 5:1) using two different types of mixing: tumbling (lift and drop) and shake flask orbital mixing.

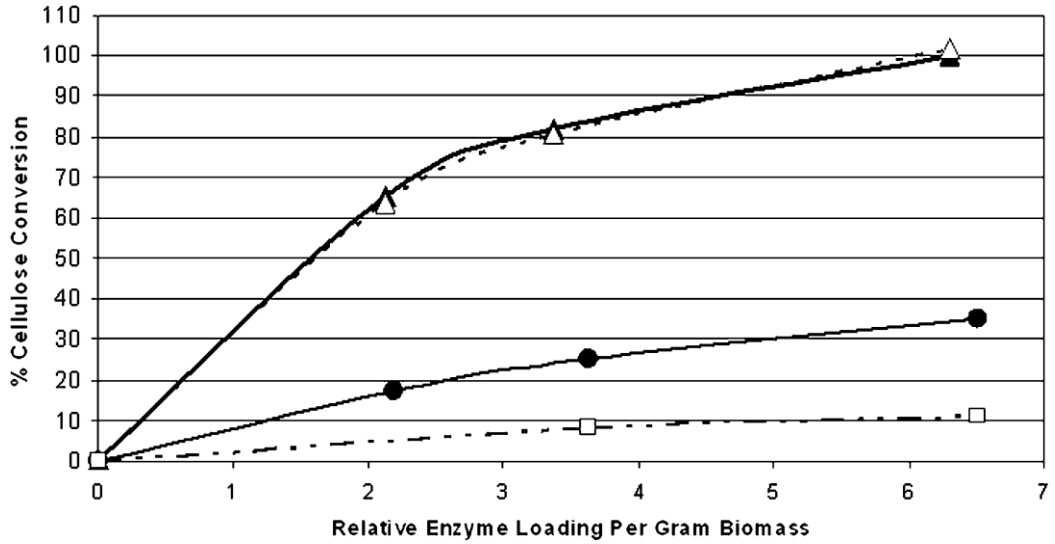


Fig. 1.10 Comparison of the impact of tumbling and shake flask orbital mixing on enzymatic hydrolysis. PCS tumbling (▲), PCS in a shaker (Δ), HWS tumbling (●), and HWS in a shaker (□) [49]

The cellulose in both PCS can be absolutely hydrolyzed with no difference in either the rate or extent of hydrolysis. While, the pretreated HWS shows a little improvement in hydrolysis from tumble mixing as compared to shake flask orbital mixing.

These results indicate that the type of mixing during hydrolysis may allow fewer severe pretreatments to be applied that could be decrease capital and operating costs during pretreatment. In addition, this type of vigorous mixing can be operated at higher solid levels during pretreatment and hydrolysis, resulting in a more concentrated sugar and higher ethanol fermentation.

1.3 Objectives

1. To study the subcritical water and steam explosion pretreatment of sugarcane bagasse and identify the optimum conditions to increase the enzymatic digestibility of the cellulose fraction for subsequent sugar conversion.
2. To test the developed process under optimum conditions on the large scale to study the technical feasibility for application in the biorefinery industry.

1.4 Scopes of Research Work

The subcritical water and steam explosion pretreatments were firstly carried to fractionate cellulose compounds from sugarcane bagasse. Several operating conditions for pretreatment, including pretreatment temperature, time, and bagasse to water ratio were evaluated for achieving high cellulose recovery. Subcritical water and steam explosion pretreatment were compared systematically and had the optimum conditions of an effective pretreatment to the further enzymatic digestion. Then, the enzymatic hydrolysis was studied with an aim to achieve a high sugar product condition. In this process, biomass compositions, reducing sugars, glucose yield (%), sugar degradation products, and activation energy from different pretreatment processes and conditions were determined.

CHAPTER 2
THEORIES

2.1 Composition of Lignocellulosic Materials

Lignocellulosic biomass is mainly composed of cellulose, hemicellulose, and lignin in the different ratios that varies with different types of lignocellulosic materials, such as wood, agricultural residues, industrial residues, municipal solid wastes, and energy crops. Remainder contains fewer amounts of minerals, oils, and other components [50]. The composition of lignocellulosic biomass varies greatly by type of species but contains approximately 30-50% cellulose, 20-30% hemicellulose, and 10-25% lignin as shown in Table 2.1.

Table 2.1 Composition of common agricultural residues and wastes

Lignocellulosics materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Corn stover [a]	37.5	22.4	17.6
Corn fiber [a]	14.28	16.8	8.4
Pine wood [a]	46.4	8.8	29.4
Popular [a]	49.9	17.4	18.1
Wheat straw [a]	38.2	21.2	23.4
Switch grass [a]	31.0	20.4	17.6
Office paper [a]	68.6	12.4	11.3
Hardwood stems [b]	40-55	24-40	18-25
Softwood stem [b]	45-50	25-35	25-35
Nut shells [b]	25-30	25-30	30-40
Cotton seed hairs [b]	80-95	5-20	0

a: [33] b: [51]

2.1.1 Cellulose

Cellulose is the main structural component of the plant cell walls and consists of a linear polymer of glucose molecules ($C_6H_{10}O_5$), linked by β-(1-4)-glycosidic bonds. Cellulose consists of two different parts of structures: crystalline structure and amorphous

structure. The crystalline structure comprises highly ordered cellulose molecules that are tightly bundled and bound together by strong inter-chain hydrogen bonds, whereas the molecules in the amorphous structure are not well ordered. The long-chain cellulose polymers are linked together by hydrogen and van der Waals bonds, which leads the cellulose to form microfibrils. Several microfibrils are united and become macrofibrils or cellulose fibers as shown in Fig. 2.1. The crystallinity of cellulose is depending on the type of biomass. For example, cotton cellulose is more crystalline than the cellulose in wood. [52]

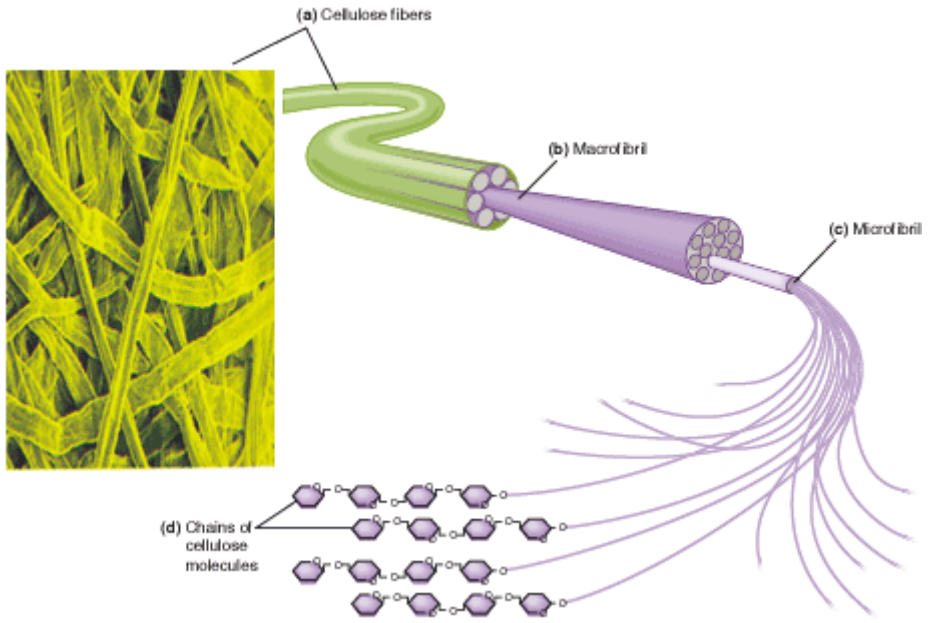


Fig. 2.1 Cellulose fibers [52]

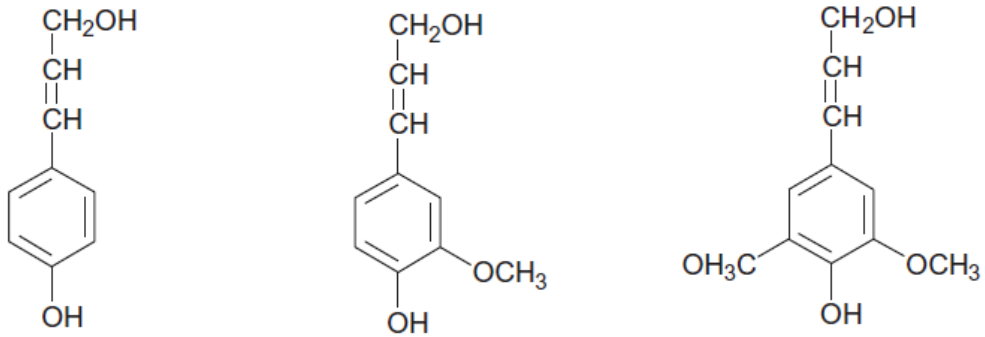
2.1.2 Hemicellulose

Hemicellulose is a branched polymer of different 5-carbon sugars (D-xylose, L-arabinose), 6-carbon sugars (D-glucose, D-galactose and D-mannose), small amounts of L-rhamnose, and acid sugars such as D-glucuronic acid, 4-O-methyl-D-glucuronic acid, and D-galacturonic acid. Hemicellulose can be divided into three main forms: xylans, mannans, and glucans. Glucuronoxylans and glucomannans are dominant hemicellulose sugars in hardwoods and agricultural residues, while galactoglucomannans and arabinoglucuroxylans are dominant hemicellulose sugars in softwoods [53]. Hemicellulose links between the cellulose fibers and the lignin form rigid networks. The molecular weight of hemicellulose is less than that of cellulose (with the degree of polymerization of hemicellulose is only 100-

200 units) and its branched structures of hemicellulose is more readily hydrolyzed compared to cellulose [8].

2.1.3 Lignin

Lignin is an amorphous heterogeneous complex polymer composed of three aromatic alcohols, including p-coumaryl alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S) linked through carbon-carbon and ether bonds to form a layer of the cell walls. The proportion of these three units varies with the species of plants. For example, S and G units mostly form in hardwoods, whereas softwood contains only G units. Lignin is hydrophobic and highly resistant to physical, chemical and biological degradation, resulting in rigidity and strength of the plants. The solubility of lignin in water is around 180ºC under neutral conditions; however, it also dissolves in alkaline, neutral or acid solvent depending on the precursor of the lignin (p-coumaryl, coniferyl, and sinapyl alcohol) [54].



p-coumaryl alcohol coniferyl alcohol sinapyl alcohol

Fig. 2.2 The structural basis of the lignin polymer [8]

2.2 Pretreatment of Lignocellulosic Materials

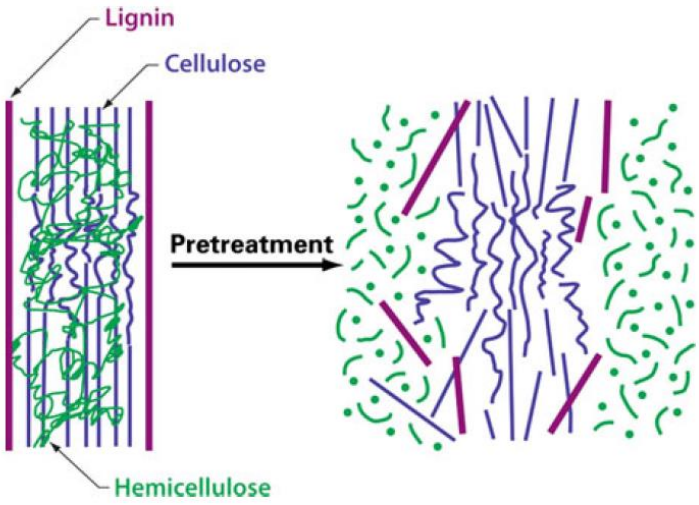


Fig. 2.3 Schematic of the effects of pretreatment on lignocellulosic biomass [3]

The pretreatment is an essential step to alter structural characteristics of biomass for the further conversion process, as shown in Fig. 2.3. In general, the effectiveness pretreatment technologies enhance the formation of sugars directly or subsequently by hydrolysis and minimize formation of degradation of sugars and inhibitory products. The pretreatment has been attributed to a modification in the degree of polymerization and crystallinity index, to a disruption of the lignin-carbohydrate linkages, to lignin and hemicelluloses removal, and to an increase of the porosity of the material. Physical, chemical, physicochemical, and biological treatments are the four fundamental types of pretreatment techniques employed [3].

2.2.1 Subcritical Water Pretreatment

Subcritical Water and Its Utilizations [56]

According to Fig. 2.4, water maintaining its liquid state in the temperature range of 100 ºC to 374 ºC under pressurized conditions is called subcritical water or compressed hot water.

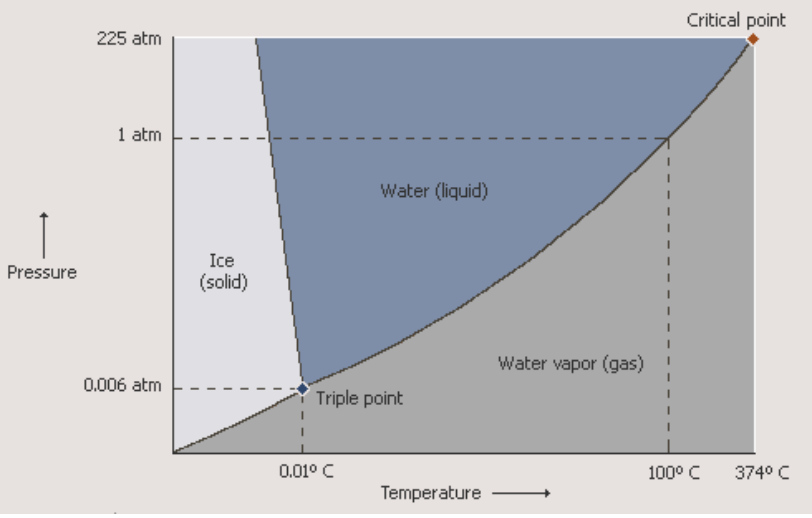


Fig. 2.4 Phase diagram of water [55]

The thermodynamic properties of liquid water change with temperature due to the disturbance of hydrogen bonding. At critical points of the phase equilibrium, the water properties, such as density, thermal conductivity, and viscosity, are controlled by temperature and pressure. The fluid becomes non-polar, highly reactive, and miscible for organic components. As a result, the water has two unique properties: (1) a high ion product at elevated temperatures. Water can act as an acid or base catalyst. Degradation kinetics of saccharides and lipids in the subcritical water were examined. It was also shown that the water catalyzed the condensation of peptides and dicarboxylic acids, and isomerization of fatty acids and saccharides. (2) A low relative dielectric constant, Ɛ. The relative dielectric constant at temperatures of 200 to 300 ºC of water is almost equivalent to those of ambient acetone and methanol. ($Ɛ_{water,\ 200\ °C}$ = 35.5, $Ɛ_{water,\ 300\ °C}$ = 20.7, $Ɛ_{methanol,\ 25\ °C}$ = 32, $Ɛ_{acetone,\ 25°C}$ = 21) It indicates that the water can be used for extracting hydrophobic substances from natural materials. Solubility of fatty acids in water was shown that the hydrogen bond between water molecules became weak at temperatures higher than 150 ºC. A novel method for preparing nanoemulsions having a narrow distribution of oil-droplet size was proposed based on the high solubility of lipid in the subcritical water. Defatted rice bran was treated by the subcritical water to produce functional materials that possessed emulsifying and emulsion-stabilizing abilities and antioxidant activity. The functionalities were experimentally authenticated and the conversion process of other by-products of the subcritical water also presented useful materials.

Applications of Subcritical Water

Subcritical water can be used to break down organic materials at temperatures ranging from 250-374 °C with pressurization. It has been found that cellulose reacts 2-3 times faster than lignin in the liquid hot water. Subcritical water can be used to release glucose from cellulose. It seems that longer reaction times provide similar yields at reduced temperatures. The temperature has been found to play an important role in the liquefaction of some particular feedstock.

Subcritical vs. Supercritical Water

Subcritical water has similar potential to supercritical but is less expensive. Subcritical water requires less energy input because of the lower temperature and pressure. High temperature liquid water has potential to be more manageable, less corrosive with wider applications in industry than supercritical water systems. Water has been shown to display unique qualities characteristic of supercritical water down to temperatures of 300 °C. Overall, subcritical water has potential as an effective alternative to supercritical water.

Subcritical Water Reaction Mechanism [56]

The major reaction pathways for cellulose degradation in subcritical water are hydrolysis, retro-aldol condensation, keto-enol tautomerism, and dehydration. The hydrolysis reaction is important to the production of monomeric sugars as the cellulose and hemicellulose are hydrolyzed into glucose and xylose monomers, respectively. The main products of the subcritical reaction are hydrolyzed products, aqueous degradation products of glucose, and organic acids. The subcritical liquification of pure cellulose produces phenols, cyclopentanones, and hydroquinones

2.2.2 Steam Explosion

Steam Explosion and Its Mechanism

The use of steam for the pretreatment of lignocellulosic biomass is extensively demonstrated and implemented methods in research and commercial facilities. Among the physicochemical processes, steam explosion is the most widely applied pretreatment processes for lignocellulosic biomass and also referred to “auto-hydrolysis” method owing to water acts as an acid at high temperatures. In general, steam explosion process is treated with high-pressure saturated steam 1 to 3.5 MPa under temperature 160 to 260 °C in a batch or continuous reactor followed by an explosive decompression of the biomass. During the steam pretreatment, some of the lignin is solubilized and repolymerized on cooling step and forms a part of the acid-soluble lignin fraction. Most of the hemicellulose solubilized after

the application of the steam pretreatment and is subsequently recovered in the aqueous fraction or further degraded to other compounds i.e. furfural, 5-hydroxymethylfurfural (HMF). In addition, the cellulose content is preserved in the solid fraction and hydrolyzed to glucose under high steam pretreatment temperature conditions (more than 200 °C). The hydrolysis of the hemicelluloses is proposed to be brought about mainly by the action of acetic acid formed from the acetyl groups released during the steam pretreatment. Furthermore, other acids i.e. formic, levulinic, and pyromucic acids produced during the steam pretreatment process may also play an important role in the acid catalyzed breakdown of the hemicellulosic glycosidic bonds. Water at high temperatures has been demonstrated to possess some acidic properties that could also enhance the hemicelluloses hydrolysis. The acidic conditions provided by the steam pretreatment could thus also lead to the degradation of available sugars in the biomass materials

Advantages of Steam Explosion

General advantages of the steam explosion processes as compared to other pretreatment technologies for chemical utilization of lignocellulose are according to Garrote *et al.* (1999):

- No other chemicals added except water
- Good yield of hemicelluloses with low degraded by-products
- Minimal equipment corrosion due to a mild pH of reaction media.
- Avoiding stages of acid handling and acid recycling
- Disruption of the solid residues from bundles to individual fibers due to the explosion effect

Factors Influencing and Optimizing the Steam Explosion [57]

The major factors influencing the steam explosion processes are residence time, temperature, moisture content, and particle size. Normally, the optimum process conditions are considered with the result in the greatest biomass for hydrolysis and the smallest amount of sugar degradation compounds, which is the cause of lost sugar.

An optimum solubilization and hydrolysis of the hemicellulose was considered by using a combination of high temperatures and short residence times (i.e. 270 °C, 1 min) or lower temperatures with longer residence times (i.e. 190 °C, 10 min).

At lower steam pretreatment temperatures, the recovery of the obtained hemicellulosic sugars in the hydrolysate is maximized, with the acid-labile biomass polysaccharides partially converted to water soluble sugars.

Normally, an increase in the steam temperature coincides with a decrease in the sugar yields while a longer reaction time causes increased lignin condensation and a degradation of pentosan with acid hydrolysis leads to over degradation reactions with the use of shorter exposure times. On the contrary, higher steam temperatures would most likely facilitate an enhanced accessibility of the macromolecules of the biomass material, but with undesired sugar losses. The high steam temperatures have also been demonstrated to lead to an increase of acid-insoluble lignin in the pretreated materials.

With the use of the excessive steam temperatures of 220–240 °C and optimum residence times, condensation reaction relating to the by-products are derived from the lignin and hemicellulose. The acid-soluble lignin was formed and accumulated to acid-insoluble polymeric materials. This condensation reaction driven alteration of the lignin during steam pretreatment has two important effects: (1) the formed polymeric materials could increase in the overall lignin yields (2) these by-products are possible to remain in the pretreated material even after washing steps (i.e. alkaline washing). Thus, the selection of the best temperature and residence time could be changed to the other parameters such as the further conversion steps and the targeted products (fuel/chemical).

Furthermore, the lignocellulosic biomass with different moisture contents could vary in the steam pretreatment process efficiency. The utilization of naturally dry biomass (5–15% moisture content) or cheap unprocessed biomass (higher moisture content) is preferred to minimize the moisture content reduction process costs.

2.3 Enzymatic Hydrolysis

2.3.1 Enzymatic Hydrolysis and Its Applications [58]

The hydrolysis process is the basis of the biorefinery. The aim of hydrolysis is to cleave cellulose and hemicellulose into their monomers. After hydrolysis, cellulose is converted to glucose, whereas the hemicellulose is converted to pentoses and hexoses. Generally, hydrolysis can be divided into two groups, including chemical and enzymatic hydrolysis. Chemical hydrolysis is related to exposing the lignocellulosic biomass into some chemicals with a wide range of acid concentration for a period of time at a specific temperature, and hence results in reducing sugars from both cellulose and hemicellulose. For enzymatic hydrolysis of cellulose, the main component in lignocellulosic biomass is carried

out by cellulolytic enzymes, which are highly specific. The hydrolysis product is mainly glucose, which can be further converted into bioethanol and other chemicals.

Enzymatic hydrolysis is the promising process for converting cellulosic compounds into fermentable sugar for subsequent conversion processes due to the potential for higher yields, higher selectivity, lower energy costs, and milder operating conditions than are chemical processes. Enzymatic hydrolysis is influenced by both structural features of cellulose and the mode of enzyme action. The enzymatic hydrolysis reaction is carried out by means of enzymes that act as catalysts to break the glycosidic bonds. Theoretically, enzymes are proteins that reduce the activation energy (E_a) of the chemical reaction, thus increasing the rate of the reaction. Therefore, reaction reach their equilibrium state and products are form more rapidly. Moreover, enzymes are selective to their specific substrates with the several metabolic pathways.

It is generally accepted that three types of enzymes are required to hydrolyze cellulose into glucose monomers: endo-b-1,4-glucanases (EG, EC 3.1.2.4) attack the endogenous part of cellulose chain, and cellobiohydrolases (CBH, EC 3.2.1.91) attack the ends of the polymer, releasing cellobiose that is ultimately cleaved into two glucose molecules by β-glucosidases (BG, EC 3.2.1.21). In addition, accessory or ‘helper’ enzymes including hemicellulases and ligninases may also play a role in hydrolysis by clearing access to cellulose for the main enzymes [59].

However, the high costs of enzyme production and the excessive enzymatic dosages necessary to hydrolyze pretreated biomass are often considered to be the major bottleneck on the path to a commercial lignocellulosic industry.

CHAPTER 3
METHODOLOGY

3.1 Raw Material Preparation

Sugarcane bagasse obtained from the PTT Global Chemical Public Company Limited, Thailand was used as the feedstock in this work. The sugarcane bagasse is 0.85 mm sieved size. The bagasse was dried at 60 °C for 24 h before processing. A portion of the sugarcane bagasse was characterized according to the methodologies of the National Renewable Energy Laboratory [60] followed by high performance liquid chromatography analysis (HPLC) to determine carbohydrate content (mostly cellulose and hemicellulose), acid insoluble lignin (AIL), acid soluble lignin (ASL), and ash content. Both untreated and treated sugarcane bagasse were analyzed and compared.

3.2 Pretreatment

Sugarcane bagasse was pretreated with subcritical water and steam explosion. The parameters tested were reaction temperature, reaction time, and bagasse to water ratio. A flow chart for the experiments is shown in Fig. 3.1

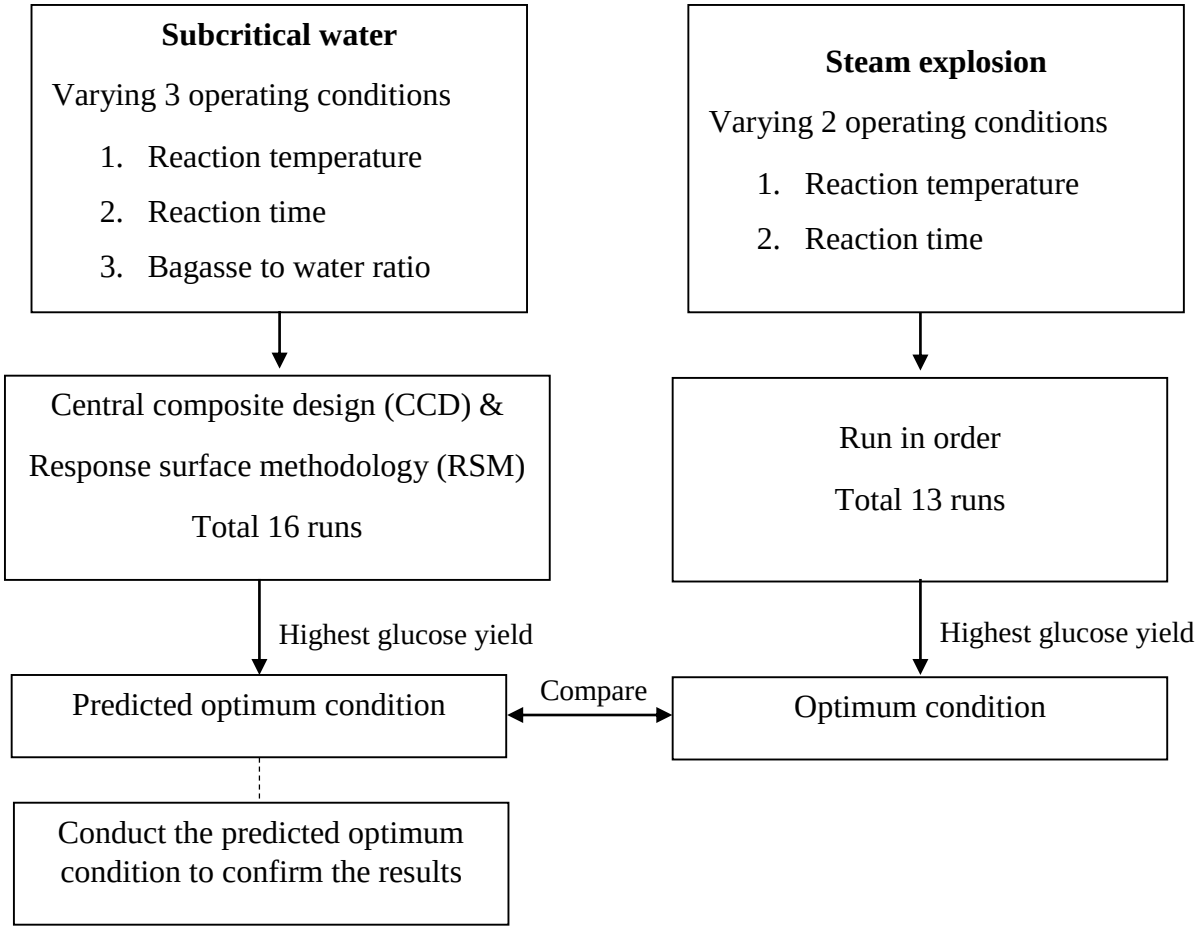


Fig. 3.1 Experimental procedures for subcritical water and steam explosion of sugarcane bagasse

3.2.1 Subcritical Water Pretreatment of Sugarcane Bagasse

Subcritical water pretreatment was performed in an autoclave reactor consisting of a 600 mL reactor and 1000 L reactor with a heater. The central composite design and response surface methodology were used to optimize the pretreatment conditions: reaction temperature, reaction time, and biomass to water ratio, as shown in Table 3.1. The sugarcane bagasse and de-ionized water were added to the reactor at different bagasse to water ratios, which are 1:4.64, 1:6, 1:8, 1:10, and 1:11.36. The hydrolysis was carried out at 126.36 °C, 140 °C, 160 °C, 180 °C, and 193.64 °C for 3.18, 10, 20, 30, and 36.82 min. When the desired reaction time was reached, the reactor was cooled down by immersing into a cold water bath. All experiments were run in triplicate. Then, the pretreated slurries obtained after subcritical water pretreatment was separated by filtration. The solid fraction was washed with de-ionized water until pH value became in the range of 3 to 5. The sample was dried at 60 °C

for 24 h and weighed for calculation of the solid residue before subjecting to enzymatic hydrolysis to evaluate the improvement in biomass digestibility.

Table 3.1 Experimental design for subcritical water pretreatment of sugarcane bagasse

Run	Coded value			Experimental value		
no.	X_1	X_2	X_3	Temp. (°C)	Time (min)	Bagasse to water
1	-1	-1	-1	140	10	1:6
2	1	-1	-1	180	10	1:6
3	-1	1	-1	140	30	1:6
4	1	1	-1	180	30	1:6
5	-1	-1	1	140	10	1:10
6	1	-1	1	180	10	1:10
7	-1	1	1	140	30	1:10
8	1	1	1	180	30	1:10
9	-1.682	0	0	126.36	20	1:8
10	1.682	0	0	193.64	20	1:8
11	0	-1.682	0	160	3.18	1:8
12	0	1.682	0	160	36.82	1:8
13	0	0	-1.682	160	20	1:4.64
14	0	0	1.682	160	20	1:11.36
15	0	0	0	160	20	1:8
16	0	0	0	160	20	1:8

3.2.2 Steam Explosion Pretreatment of Sugarcane Bagasse

Steam explosion pretreatment was provided at the Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI). Sugarcane bagasse was loaded into a 2 L pressurized vessel reactor, which was connected to a boiler supplying saturated steam. The material will be heated up in the range of 190 – 210 °C for 1 - 15 min and followed by an explosive decompression. The pretreated slurry obtained after the steam explosion pretreatment was separated by filtration. Analytical methods were the same as those for the subcritical water pretreatment. Then, subcritical water and steam explosion pretreatment

were compared systematically and the optimum conditions of an effective pretreatment for further enzymatic digestion were summarized.

3.3 Enzymatic Hydrolysis

The enzymatic assay was provided by the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. After pretreatment, solid residue (5% of 5 ml total reaction volume) was hydrolyzed with 50 mM of citrate buffer (pH 5). Then, the enzyme (Cellic® CTec2) was added at 10 FPU/g dry treated bagasse. Next, the mixture was incubated at 50 °C, and 200 rpm for 72 h and collected for determination of reducing sugars by DNS methods.

3.4 Analytical Method

3.4.1 Analysis of the Solid Fraction

The chemical compositions of solid residue were analyzed according to the NREL method followed by the High Performance Liquid Chromatography (HPLC) analysis to determine carbohydrate content, acid insoluble lignin, acid soluble lignin, and ash content. The HPLC (Shimadzu, Japan) system composed of an organic acid column, Aminex HPX-87H (Bio-Rad, Hercules, CA, USA) with a refractive e index and UV-Vis detectors (210 nm). The mobile phase consisted of 5 mM aqueous sulfuric acid, which was set to a flow rate of 0.6 cm^3/min. The column was operated at 45 °C. The analysis for a sample was completed in 60 min. Each component in the sample was identified by comparing with the retention time of those pure compounds. And the concentrations of each component in the sample were analyzed using calibration curves to achieve form standard solution with exactly known concentration.

Morphological structures of the raw material and pretreated material were characterized using a scanning electron microscope, SEM (S-3400N, Hitashi). SEM technique was taken at 1,000 X magnification.

CHN measurement (CHN 628, Leco) was employed for the elemental analysis.

Thermal gravimetric analysis, TGA (Pyris 1 TGA, PerkinElmer) was used to measure quantities of energy of raw and pretreated materials at Advanced Fuel Processing Laboratory (AFPL) of JGSEE, Thailand. Nitrogen with the volumetric flow rate of 50

ml/min was blown into the TG for giving a pyrolytic environment and the heating rate of the TG was 10 °C min^{-1}

3.4.2 Analysis of the Liquid Fraction

HPLC with a refractive index detector was used to analyze the liquid products for free sugars, organic acids, and furan aldehydes. The conditions of the HPLC system were the same as mentioned above. UV-Vis was used as quantification tool for solubilized lignin. Total organic carbon (TOC) was used as an indication of biomass at different conditions.

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 Raw Sugarcane Bagasse

The components of sugarcane bagasse in this research were determined according to the standard methods of National Renewable Energy Laboratory [60], as shown in Table 4.1.

Table 4.1 Compositions of sugarcane bagasse

Source	%Composition			
	Cellulose	**Hemi-cellulose**	**Lignin**	**Ash**
Sugarcane bagasse[(a)]	44.32	23.75	23.20	4.90
Rice straw[(b)]	38.89	23.21	20.65	17.25
Corn stover[(b)]	40.00	27.70	23.10	6.50

(a) Current study (b) From the review paper (NREL methods)

The results in Table 4.1 showed that the main compositions of sugarcane bagasse were 44.32% cellulose, 23.75% hemicellulose, 23.20% lignin and 4.90% ash. According to the component analysis, sugarcane bagasse contained a relatively higher content of cellulose and hemicellulose with lower ash content compared to other biomass e.g. rice straw and corn stover.

4.2 Effect of Bagasse and Water Volume in 0.6 L Reactor

Bagasse and water volume per total volume of subcritical water pretreatment was varied at 4/5, 4/5 (N_2 purged), 3/5, and 2/5 at 160 °C, 20 min, 1:8 bagasse to water ratio. The compositions of pretreated sugarcane bagasse were analyzed according to the NREL method, as shown in Table 4.2. Subcritical water pretreatment significantly removed hemicellulose from the solid phase with higher content of cellulose. The highest reducing sugars is obtained from 4/5 bagasse and water volume per total volume, which is 285.07

mg/g pretreated. Thus, 4/5 bagasse and water volume per total volume will be performed in further experiments of subcritical water pretreatment.

Table 4.2 The compositions of pretreated materials at varied bagasse and water volume per total volume [C: Cellulose, H: Hemicellulose, L: Lignin, and A: Ash]

No.	Bagasse and water volume per total volume	%Composition				Reducing sugars	
		C	H	L	A	mg/g pretreated	mg/g raw mat.
1	4/5	58.62	8.98	27.46	4.94	285.05	200.70
2	4/5, N_2 purged	59.64	7.63	28.60	4.13	217.32	148.71
3	3/5	53.37	15.61	26.87	4.14	241.14	186.03
4	2/5	60.26	4.07	30.42	5.25	276.50	171.69

4.3 Subcritical Water Pretreatment of Sugarcane bagasse

4.3.1 Optimization of Subcritical Water Pretreatment Conditions in 0.6 L Reactor

In this part, response surface methodology (RSM) was used for optimizing pretreatment parameters to achieve maximum glucose yield. The study was conducted based on a central composite design (CCD) with a quadratic model to evaluate the combined effect of three independent variables, i.e. reaction temperature, reaction time, and bagasse to water ratio. Each variable was varied at five levels including –α (axial point), -1 (low level), 0 (central level), +1 (high level), and +α (axial point) as shown in Table 4.3. The code and actual values of independent variables can be calculated from Equation 4.1.

$$x_i = \frac{X_i - X_0}{\Delta X} \tag{4.1}$$

Where x_i is the dimensionless value of an independent variable, X_i is the real value of an independent variable, X_0 is the value of X_i at the central point and ΔX is the step change. In this study, a total 16 experimental runs with different conditions of three parameters and 2 duplicates of the central point were carried out randomly according to CCD configuration, as shown in Table 3.1.

Table 4.3 Parameter and code level for CCD

Parameter	Code level				
	Axial point (-1.682)	**Low level (-1)**	**Central point (0)**	**High level (+1)**	**Axial point (+1.682)**
Reaction Temperature (°C)	126.36	140	160	180	193.64
Reaction Time (min)	3.18	10	20	30	36.82
Bagasse to water ratio	1:4.64	1:6	1:8	1:10	1:11.36

The experimental data was processed using STATISTICA 8.0 (Statsoft, USA) software to acquire results from the analysis of variance (ANOVA), regression coefficients, and regression equation. The experimental data obtained from the CCD model experiments can be represented in the following equation:

$$Y = b_o + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n} b_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} x_i x_j + e_i \quad (4.2)$$

Where Y is the predicted response; n is the number of factors; x_i and x_j are the coded variables; b_o is the offset term; b_i, b_{ii}, and b_{ij} are the first-order, quadratic, and interaction effects, respectively; i and j are the index numbers for factors; and e_i is the residual error.

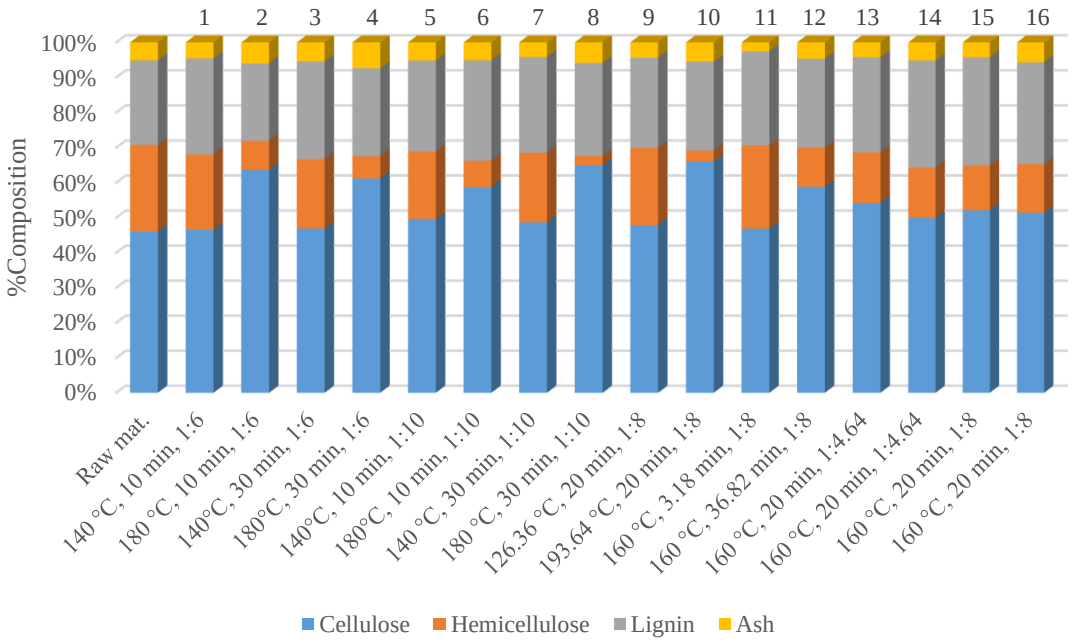


Fig. 4.1 Compositions of raw bagasse and subcritical water pretreated bagasse

The compositions of raw material and subcritical water pretreated bagasse are shown in Fig. 4.1. In severe conditions especially for temperatures above 180 °C and 20 min i.e. experimental run No. 4 (180 °C, 20 min, 1:6), No. 8 (180 °C, 30 min, 1:10), and No. 10 (193.64 °C, 20 min, 1:8), the hemicellulose content was obviously decreased from raw material which has 24.70% of hemicellulose. The percentage of hemicellulose removal was ranged from 12.71% to 93.02% for experimental run No. 11 (160 °C, 3.18 min, 1:8) and No. 8 (180 °C, 30 min, 1:10), respectively. The hemicellulose solubilization was corresponded with enzymatic hydrolysis results as shown in Fig. 4.2. The highest reducing sugars was obtained from run No. 4 which is 273.36 mg/g pretreated. It can be seen that more solubilization of hemicellulose was made greater accessibility to hydrolytic enzymes, which increases the conversion of cellulose to glucose.

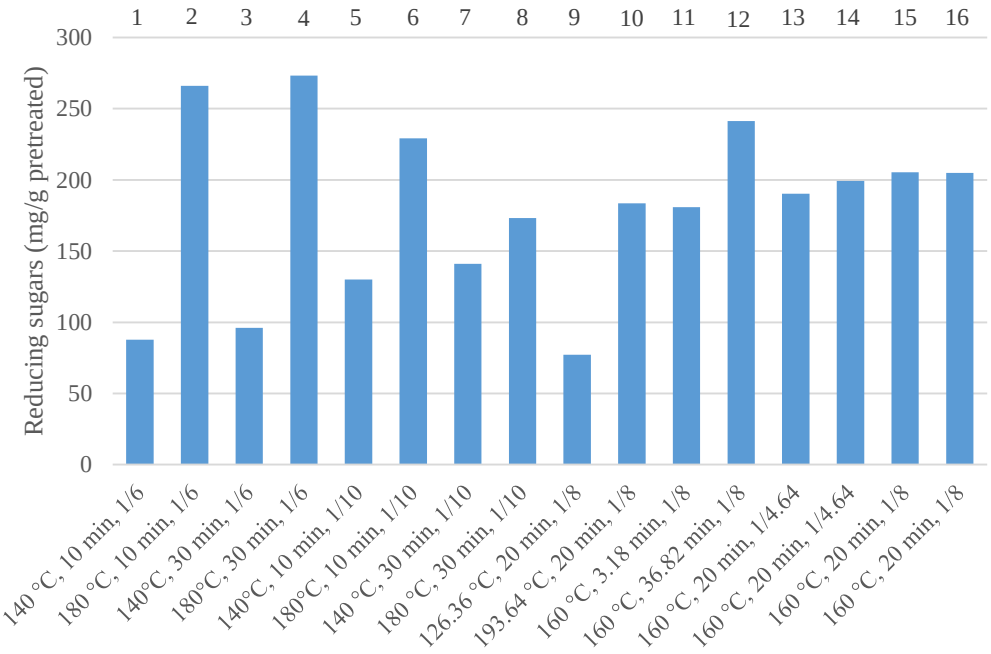


Fig. 4.2 Reducing sugars of subcritical water pretreated bagasse

Glucose yield (%) from enzymatic hydrolysis is the main indicator for optimizing the pretreatment conditions in subcritical water pretreatment of sugarcane bagasse. The glucose yield (%) was calculated according to Equation 4.3.

$$\text{Glucose yield (\%)} = \frac{\text{amount of glucose released from enzymatic hydrolysis} \times \text{\% solid residue}}{\text{initial glucose content in raw material}} \quad (4.3)$$

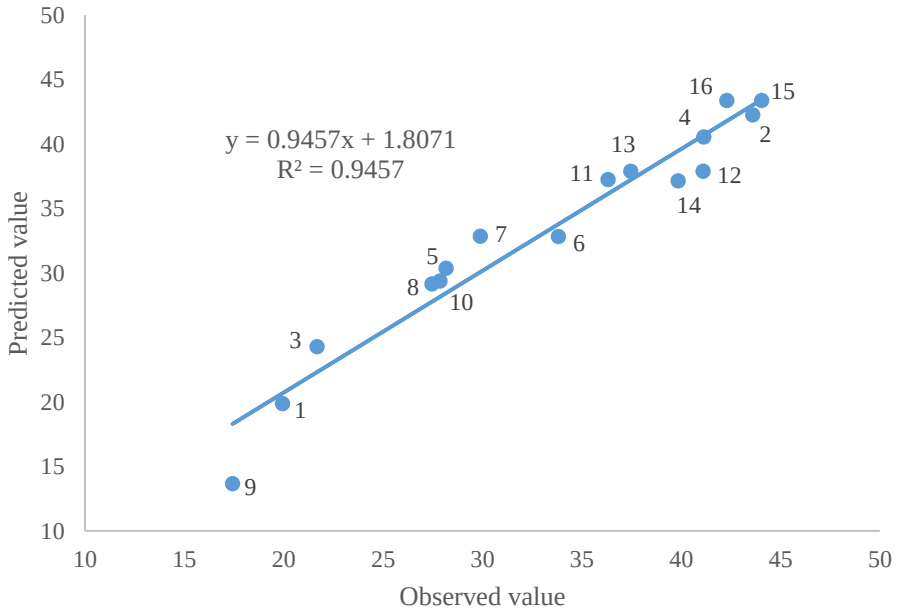


Fig. 4.3 The linear plot of mathematical model predicted values and experimental results (observed values) on glucose yield

As shown in Fig 4.3, the R^2 value of 0.9457 indicates a close agreement between the experimental results and the theoretical values predicted by the model. This high R^2 value was acceptable for giving a decent prediction on glucose yield with appropriate pretreatment conditions.

The resulted response function in terms of coded factors A, B and C, together with their corresponding coefficients after the elimination of insignificant terms derived to predict the glucose yield as shown in Equation 4.4.

$$\text{\% Glucose yield} = 43.37131 + 9.35715*A - 15.45607*A^2 + 0.39346*B - 4.10241*B^2 - 0.44014*C - 4.14904*C^2 - 3.07060*AB - 9.97888*AC - 0.97920*BC \quad (4.4)$$

Where A is the coded value of reaction temperature, B is the coded value of reaction time, and C is the coded value of bagasse to water ratio. In the equation, A, B, and C are the main effects while AB, AC, and BC are the interactions, where A^2 , B^2 and C^2 are the quadratic terms involved in the pretreatment process.

Table 4.4 Analysis of variance (ANOVA) for the regression equation of glucose yield.

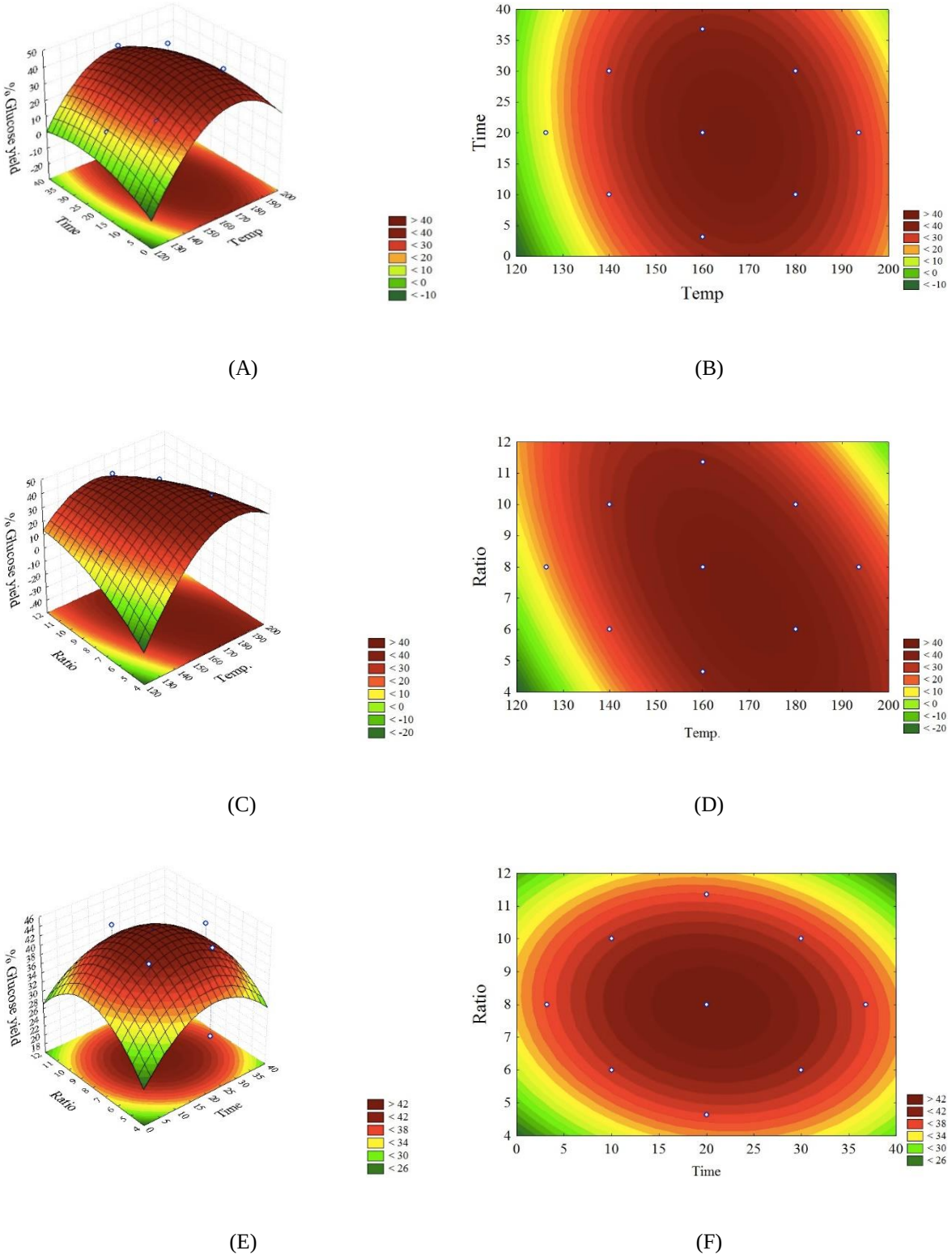
Source	Sum of squares	Degree of freedom	Mean square	F Statistic	p-Value
A – Reaction temperature	298.966	1	298.9665	28.41841	0.001777
A^2	553.692	1	553.6917	52.63146	0.000349
B – Reaction time	0.529	1	0.5286	0.05025	0.830073
B^2	39.007	1	39.0075	3.70788	0.102470
C - Ratio	0.661	1	0.6608	0.06282	0.810460
C^2	39.735	1	39.7355	3.77708	0.099960
AB	18.857	1	18.8571	1.79247	0.229118
AC	199.156	1	199.1559	18.93087	0.004818
BC	1.918	1	1.9177	0.18229	0.684312
Residual	63.121	6	10.5202		
Total	1161.556	15			

The regression coefficients for the coded factors are presented in Table 4.4. Statistical significance of the model equation to the fitted model was evaluated by the ANOVA. Normally, the p-value less than 0.05 indicates that a model is statistically valid and acceptable. The linear term A is found to be the most significant factor in the regression with the p-value of 0.001777. The interaction and the quadratic coefficient AC and A^2 are also important terms. The effect of increasing reaction parameters i.e. reaction temperature in subcritical pretreatment process has an influence on enhancing glucose content released from enzymatic hydrolysis. Moreover, the increase of glucose yield is observed at both higher temperature and bagasse to water ratio. For example, the increasing reaction temperature and bagasse to water ratio in experimental runs, Nos. 3 and 8 (140 °C to 180 °C and 1:6 to 1:10 bagasse to water ratio) resulted in positive effect on the glucose yield (21.68% to 27.46%).

Table 4.5 Predicted optimal conditions for maximizing glucose yield on subcritical water pretreated bagasse

Factor	Observed Minimum	Critical Values	Observed Maximum
Reaction Temperature (°C)	126.36	170.59	193.64
Reaction Time (min)	3.18	19.31	36.82
Bagasse to water ratio	4.64	6.64	11.36
Predicted at solution : 44.67858			

Fig. 4.4 are plots of two-dimensional contours and three-dimentional response surface after pretreatments. These plots were made to illustrate results from the modelled equations in the investigation of interactions from two chosen independent variables, while the third variable is fixed constant at a central point value to visualize the optimal results from these variables for a desired response (glucose yield). In Fig. 4.4, the fixed central point value for the plots in (A) and (B) is the bagasse to water ratio of 1:8, the fixed central point value for the plots in (C) and (D) is the reaction time of 20 min, and the fixed central point value for the plots in (E) and (F) is the reaction temperature of 160 °C. It can be seen that the interaction between reaction temperature and bagasse to water ratio significantly influences the amount of glucose content produced. Under the combined conditions, the desired glucose yield increased from 17.42% to 44.05%. The best predicted conditions providing the highest glucose yield are the reaction temperature of 170.59 °C, the raction time of 19.31 min, and the bagasse to water ratio of 1:6.64 as shown in Table 4.5.



(A) (B)

(C) (D)

(E) (F)

Fig. 4.4 Contour and 3D response surface plot showing interactions between variables affecting glucose yield: (A), (B) interaction of reaction temperature and reaction time (C), (D) interaction of reaction temperature and bagasse to water ratio; (E), (F) interaction of reaction time and bagasse to water ratio

Table 4.6 Enzymatic hydrolysis results of subcritical water optimum condition in 0.6 L raector

Condition	Reducing sugars		%Glucose yield
	mg/g pretreated	mg/g raw mat.	
170.59 °C, 19.31 min, 1:6.64	297.09	207.63	48.76

To confirm the predicted optimum condition, subcritical water pretreatment of sugarcane bagasse at 170.59 °C, 19.31 min, and 1:6.64 bagasse to water ratio was performed. The enzymatic hydrolysis are shown in Table 4.6. This predicted optimum condition obtained the highest reducing sugars and %glucose yield which is 297.09 mg/g pretreated and 48.76%, respectively.

4.3.2 Test of Subcritical Water Pretreatment in 1000 L Reactor

The results shown in this section mainly focused on the effect of reaction temperature in subcritical water pretreatment in 1000 L reactor by varying temperatures from 130 to 160 ºC. Bagasse to water ratio was fixed at 1:12 which is convenient for the slurry suction into the reactor. The pretreatment time was maintained at 240 min at all temperatures.

Table 4.7 Enzymatic hydrolysis results of subcritical water pretreatment in 1000 L reactor

Reaction temperature (ºC)	Reducing sugars (mg/g pretreated)
130	94.88
140	150.71
160	196.30

Increasing temperature from 130 to 160 ºC resulted in higher enzymatic digestibility of cellulose fractions from pretreated bagasse, as shown in Table 4.7. The reducing sugars were 94.88, 150.71 and 196.30 mg/g pretreated for the temperature of 130, 140, 160 ºC, respectively, that is not really different from 0.6 L at the same reaction temperature. This would be the great potential for scaling up in industry section but several issues must be addressed before a commercial technology.

4.3.3 Carbon Balance of Subcritical Water Pretreatment System

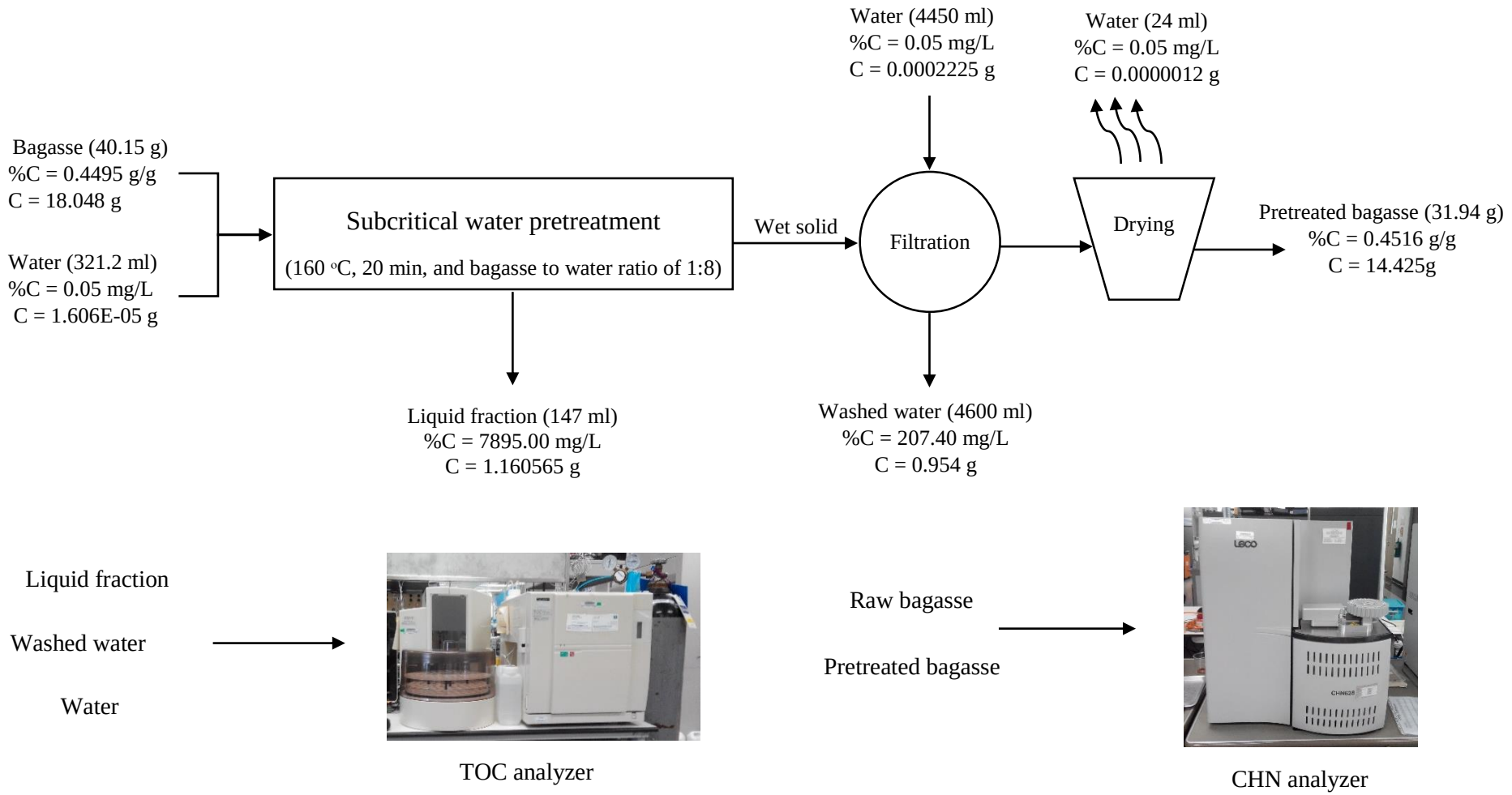


Fig. 4.5 The example of carbon balance in run No. 15 (160 °C, 20 min, and bagasse to water ratio of 1:8) for the subcritical water pretreatment

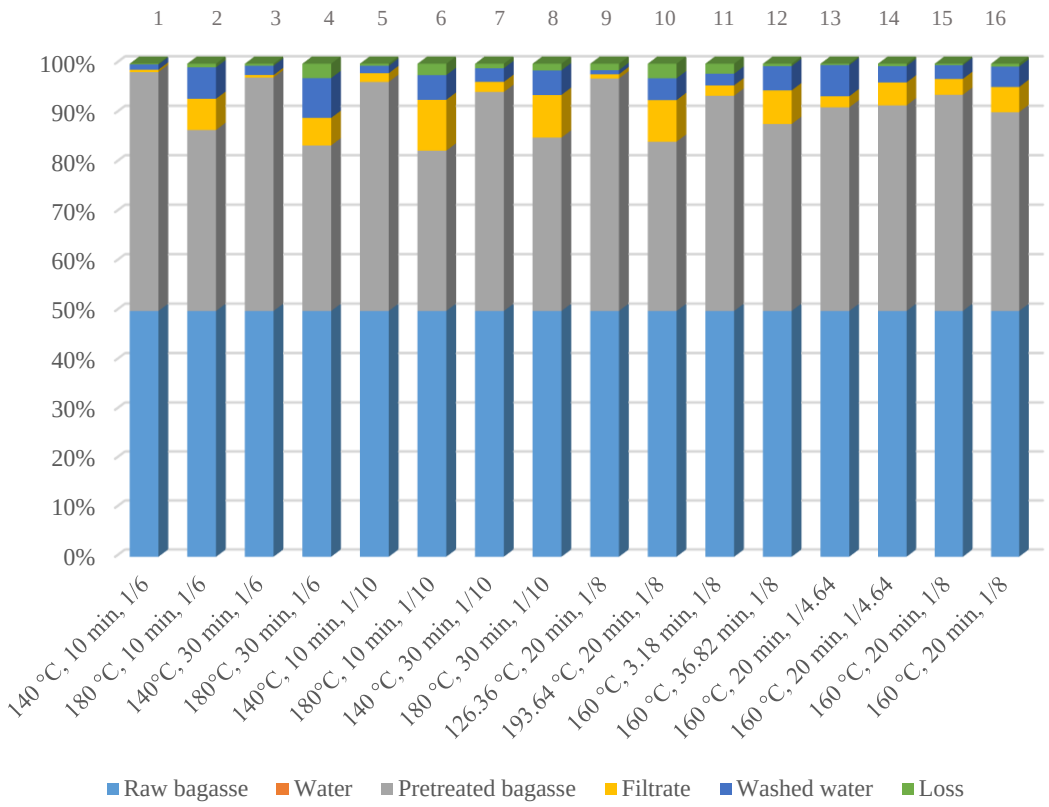


Fig. 4.6 Carbon balance for whole process of subcritical water pretreatment

To evaluate the process performance and the possibility of filtrate utilization, a carbon balance of each step in subcritical water pretreatment was calculated, as shown in Fig. 4.6. The carbon balance in experimental run No. 15 (160 °C, 20 min, and bagasse to water ratio of 1:8) was selected as an example in block diagram as shown in Fig. 4.5. The liquid sample was analyzed with total organic carbon analyzer (TOC) and the solid sample was examined with CHN analyzer. The results indicated that the carbon contents in liquid products increasing with raising reaction temperature, reaction time and bagasse to water ratio. The highest carbon loss was observed at 5.88% in run No. 10 (193.64 °C, 20 min, and bagasse to water ratio of 1:8). The compositions of filtrate and washed water were analyzed with the HPLC system and the chromatograms indicated that the main components in the liquid fraction were glucose, xylose, and furfural, as shown in Table 4.8.

4.4 Steam Explosion of Sugarcane Bagasse

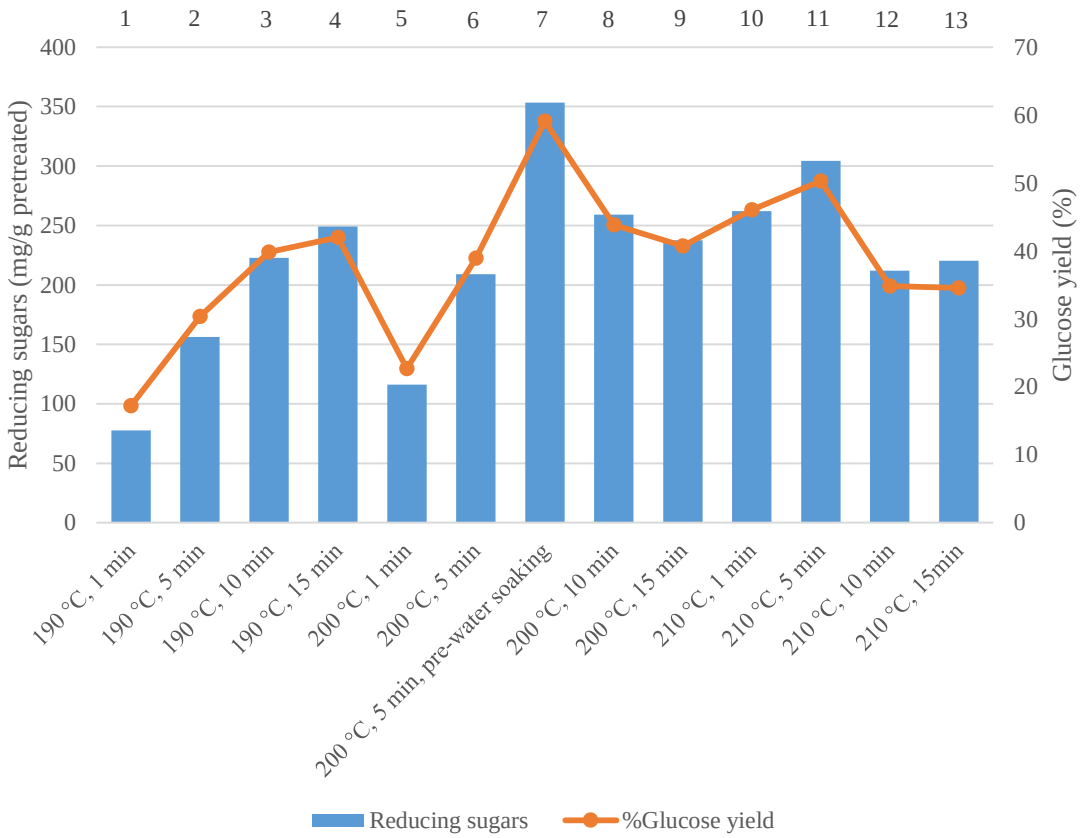


Fig. 4.7 Reducing sugars and glucose yield (%) of steam exploded bagasse

Steam explosions were performed with 190 °C, 200 °C, and 210 °C and time at 1 min, 5 min, 10, and 15 min. Moreover, pre-water soaking for 1 h at 200 °C and 5 min was also selected to perform. The results of glucose yield and reducing sugars are shown in Fig. 4.7. The percent glucose yield availability ranged from 17.23% to 59.12% and closely agreed with the reducing sugars which in the range of 77.75 to 353.37 mg/g pretreated. The best glucose yield was obtained from 200 °C for 5 min with pre-water soaking which is 59.12%. Pre-water soaking had marked a positive effect on glucose yield and could be an increasing enzyme accessibility to the cell wall structure and removal of enzyme inhibitors such as lignin-based compounds, sugar degradation products, xylose, and xylo-oligomers. [61]

During steam explosion, some portion of sugarcane bagasse was removed, therefore % compositions of steam exploded bagasse are summarized in Fig 4.8. The most significant change was the decrease of the content of hemicellulose, which was 24.70% in the raw

bagasse, declined 4.07% at 210 °C for 15 min. In the overall, the percentage of solubilized hemicellulose was obviously increased, while cellulose and lignin were less preserved. It confirmed that more solubilization of hemicellulose led to greater accessibility of the cellulose fraction to hydrolytic enzymes, which enhances the conversion of cellulose to glucose.

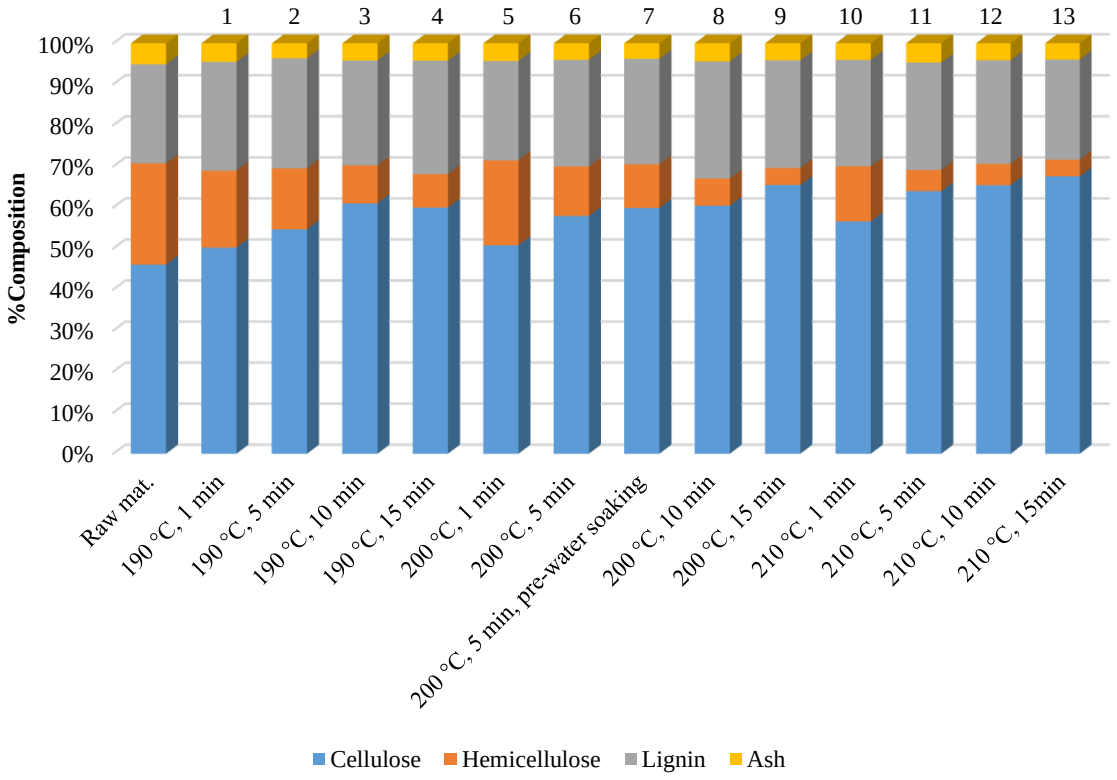


Fig. 4.8 Compositions of raw bagasse and steam exploded bagasse

4.5 Liquid Fraction of Subcritical Water Pretreated and Steam Exploded Sugarcane Bagasse

Table 4.8 Compositions in liquid fraction of pretreated bagasse

(A) Subcritical water

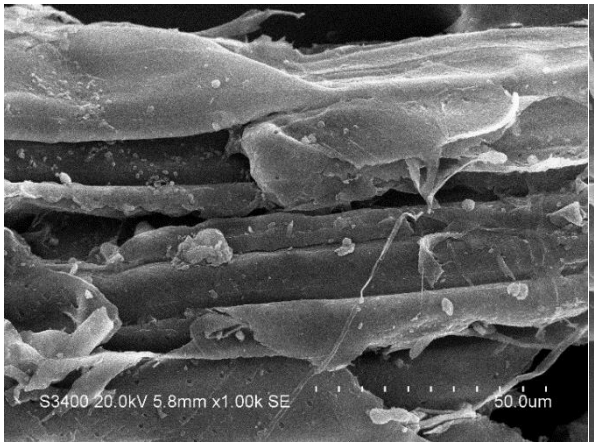
No.	Conditions	Concentrations (mg/g biomass)				
		Glucose	Xylose	Furfural	Succinic acid	Lactic acid
1	140 °C, 10 min, 1:6	-	0.54	0.26	-	-
2	180 °C, 10 min, 1:6	-	1.61	4.13	-	-
3	140 °C, 30 min, 1:6	0.04	0.57	0.18	8.69	-
4	180 °C, 30 min, 1:6	0.12	5.61	18.40	-	-
5	140 °C, 10 min, 1:10	-	1.00	0.18	-	-
6	180 °C, 10 min, 1:10	0.10	3.20	0.60	-	-
7	140 °C, 30 min, 1:10	0.41	0.92	0.90	3.50	4.50
8	180 °C, 30 min, 1:10	0.76	5.61	1.20	-	-
9	126.36 °C, 20 min, 1:8	-	-	0.08	-	-
10	193.64 °C, 20 min, 1:8	1.35	8.96	49.08	-	-
11	160 °C, 3.18 min, 1:8	-	0.77	0.88	-	-
12	160 °C, 36.82 min, 1:8	0.08	2.64	5.99	-	-
13	160 °C, 20 min, 1:4.64	1.66	0.70	1.11	-	-
14	160 °C, 20 min, 1:4.64	1.49	0.77	1.13	-	-
15	160 °C, 20 min, 1:8	3.09	0.85	0.63	1.72	0.78
16	160 °C, 20 min, 1:8	4.75	1.75	0.32	-	-

(B) Steam explosion

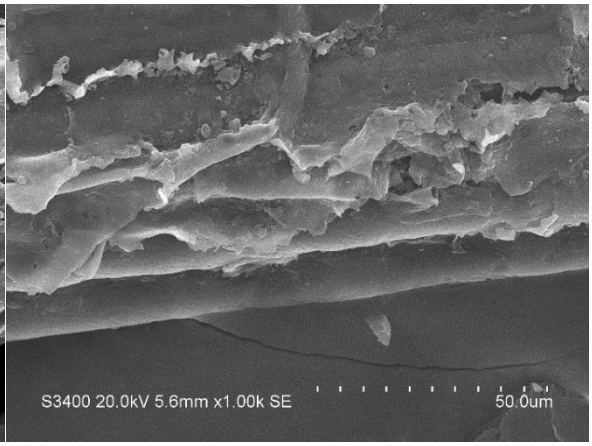
No.	Conditions	Concentration (mg/g biomass)		
		Glucose	Xylose	Furfural
1	190 °C, 1 min	-	0.47	-
2	190 °C, 5 min	-	3.50	1.40
3	190 °C, 10 min	4.32	14.80	8.02
4	190 °C, 15 min	16.80	28.20	31.80
5	200 °C, 1 min	-	0.34	-
6	200 °C, 5 min	-	3.24	2.16
7	200 °C, 5 min, pre-water soaking	9.52	19.04	12.69
8	200 °C, 10 min	4.75	36.15	23.90
9	200 °C, 15 min	8.86	89.61	46.37
10	210 °C, 1 min	0.89	1.87	0.27
11	210 °C, 5 min	17.14	45.02	16.03
12	210 °C, 10 min	13.15	45.63	88.16
13	210 °C, 15 min	24.85	73.78	80.77

The compositions of filtrates from both pretreatments mainly consisted of glucose, xylose, and furfural, as shown in Table 4.8. The xylose and furfural were found in relatively high concentrations, which agree with high degree of hemicellulose solubilization as discussed above. In accordance with other studies, the degradation of pentoses was significantly occurred at severe condition i.e. the high furfural formation especially above 180 °C was released in subcritical water. Glucose was found in low concentrations in the filtrate, which is in agreement with the relative low degree of hydrolysis observed for cellulose, as shown in Fig. 4.1. [62]

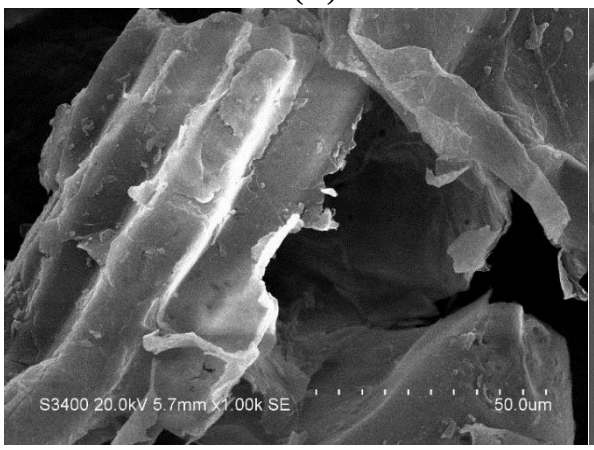
4.6 SEM Images of Subcritical Water Pretreated and Steam Exploded Sugarcane Bagasse



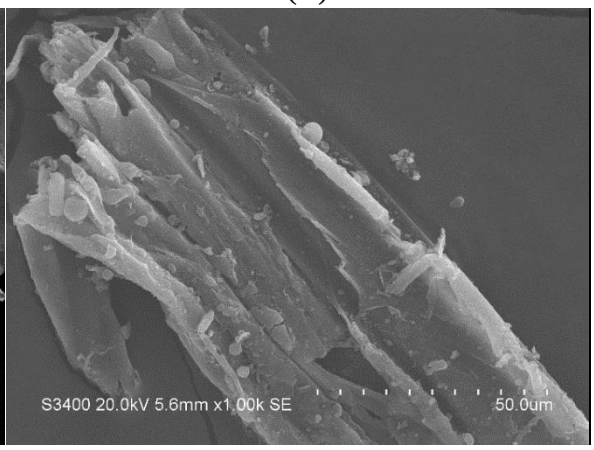
(A)



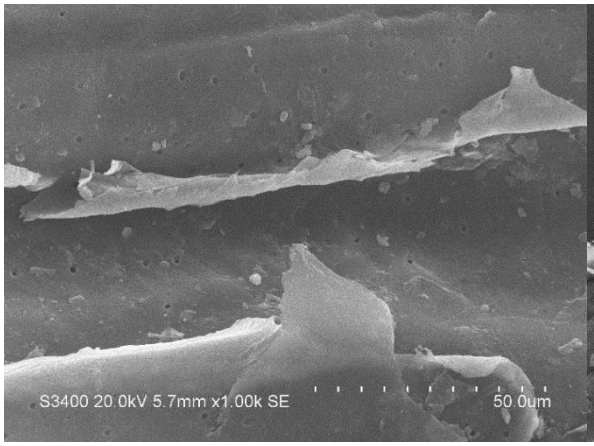
(B)



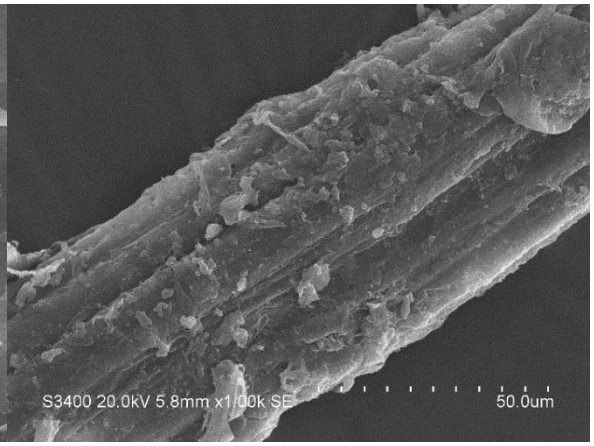
(C)



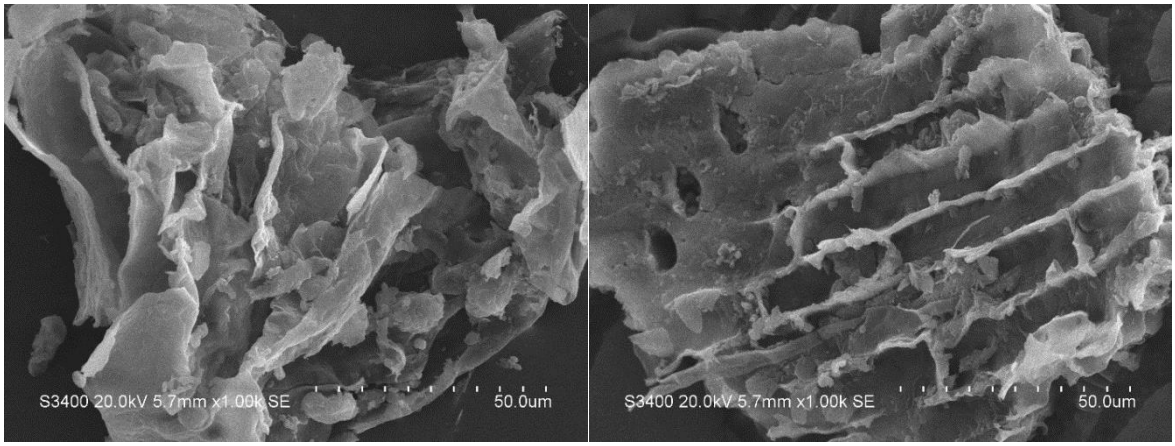
(D)



(E)



(F)



(G) (H)

Fig. 4.9 SEM images of sugarcane bagasse; (A) Raw bagasse; (B), (C), and (D) Subcritical water pretreated bagasse at 126.36 °C 20 min 1:8 bagasse to water ratio, 160 °C 20 min 1:8 bagasse to water ratio, and 193.64 °C 20 min 1:8 bagasse to water ratio, respectively; (E), (F), (G), (H) Steam exploded bagasse at 190 °C 5 min, 200 °C 5 min, 210 °C 5 min, and 200 °C 5 min with pre-water soaking, respectively

The use of scanning electron microscopy (SEM) as an analytical technique proved to be of great importance and versatility for studying the biomass structure. Fig. 4.9 shows the morphological characteristics of the subcritical water pretreated and steam exploded bagasse as well as the raw bagasse, obtained by using SEM technique taken at 1,000 X magnification. Raw bagasse exhibited a rigid and compact fiber structure as shown in Fig. 4.9 (A). After being pretreated in subcritical water and steam explosion, a large amount of hemicellulose and part of lignin and cellulose were removed from bagasse as discussed above. Pretreated bagasse presented more disordered morphology with greater exposure of the fibers. The microfibrils were separated from the initial connecting structure resulting in higher external surface area and porosity leading to greater accessibility of hydrolytic enzymes, which enhances the conversion of cellulose to glucose.

4.7 TGA and Kinetic Parameters

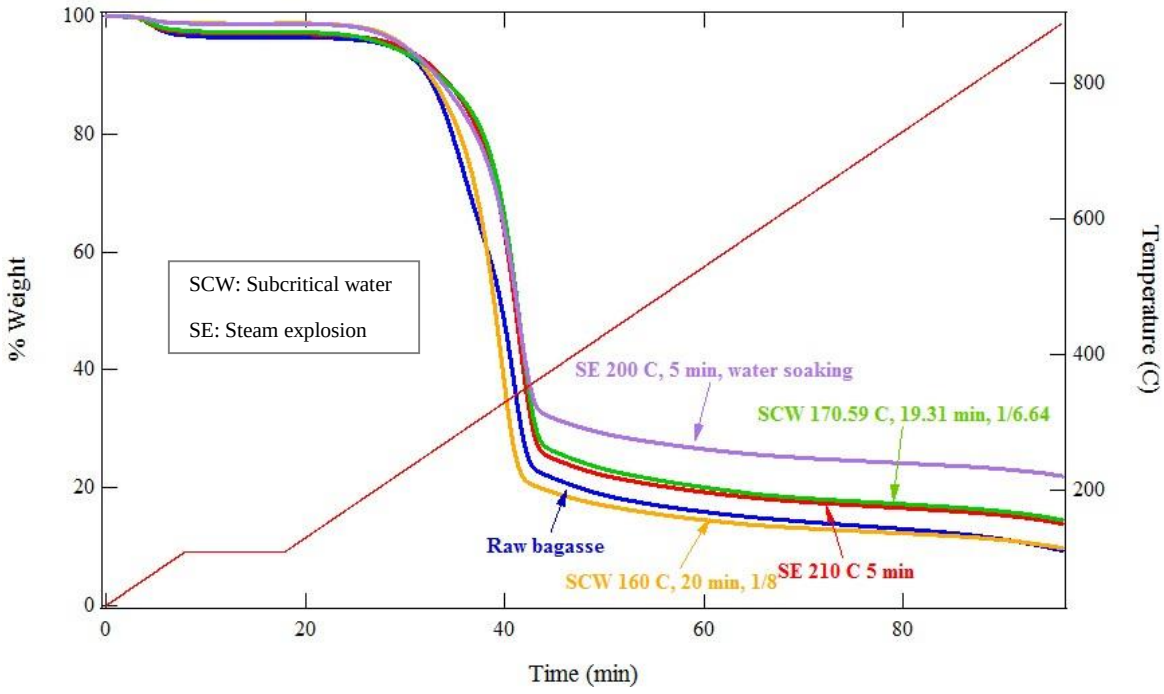


Fig. 4.10 TGA distributions of raw sugarcane bagasse and pretreated bagasse

In the experiments of TGA, the initial weight of each sample was around 4.7 mg. Nitrogen at flow rate 50 ml/min was carried into the TG to giving a pyrolytic environment. The heating rate of the TG was 10 °C min^{-1}. Fig. 4.10 shows the TGA curves of raw bagasse and pretreated bagasse obtained from subcritical water and steam explosion. The decomposition of sugarcane bagasse took place in several steps and the losses of moisture was firstly occurred. The distributions of TGA for the hemicellulose and cellulose was investigated, their profiles were found to be similar with each other. The hemicellulose and cellulose were decomposed at 220 °C to 350 °C. Lignin was decomposed at temperature varies from 300 to 500 °C. The remaining components from severe conditions i.e. SE 200 °C, 5 min, and pre-water soaking were cellulose and lignin. A shift of decomposition temperature of cellulose and lignin could be described by the increase in order structure of cellulose and the formation of more thermo-labile lignin after pretreatment. This indicated that the steam-exploded bagasse had higher thermal stability than did the raw materials. [63]

Table 4.9 Kinetic parameters in pyrolysis procedure at 10 °C/min

Sources	Temp. range (°C)	E (kJ/mol)	A (s^{-1})	R^2
Raw bagasse	250-350	67.64	10538.60	0.97
SCW optimum condition	220-320	58.63	1187.85	0.98
SCW 160 °C, 20 min, 1:8	220-320	65.39	6060.21	0.99
SE 210 °C, 5 min	250-320	66.31	5283.80	0.94
SE 200 °C, 5 min, pre-water soaking	250-320	45.79	85.07	0.96

A wide range of kinetic schemes have been used to analyze the pyrolysis kinetics of lignocellulosic biomass. Parameters of the reaction kinetics were determined using the procedure applied by the Coats-Redfern. The pylolysis of linocellulosic biomass is frequently described by a single reaction:

$$\text{Solid} \xrightarrow{k} \text{volatiles} + \text{char}$$

Where the rate of mass loss depends on mass and temperature according to the following equation:

$$\frac{d\alpha}{dt} = k(T) f(\alpha) \qquad (4.5)$$

α, t, T, and K defines the reacted fraction, time, temperature, and the rate constant, respectively. The variation of the rate constant with temperature is calculated approximately by the Arrehenius Equation:

$$k(T) = A \exp(-E/RT) \qquad (4.6)$$

where A, E, R, T denotes the frequency (pre-exponential) factor, activation energy, gas constant and temperature, respectively. f(α) is approximated by:

$$f(\alpha) = (1 - \alpha)^n \qquad (4.7)$$

where n is the formal reaction order. α is calculated from the corresponding TGA curve by:

$$\alpha = \frac{m_0 - m}{m_0 - m_{char}} \tag{4.8}$$

where m_0, m, and m_{char} denotes the initial sample mass, the actual sample mass and the relative char yield, respectively. By assuming a first order rate of reaction (n = 1), Equations (4.5) to (4.7) are written by:

$$\frac{d\alpha}{dt} = A \exp\left(-\frac{E}{RT}\right)(1-\alpha) \tag{4.9}$$

Based on the Arrhenius equation, the heating rate can be derived by:

$$T = T_0 + \beta t \qquad \rightarrow \qquad \beta = \frac{dT}{dt} \tag{4.10}$$

When Equation (4.9) is taken by the natural logarithm and rearranged, the pyrolysis kinetic equation obtains:

$$\ln\left[\frac{d\alpha/dt}{1-\alpha}\right] = \ln A - \frac{E}{RT} \tag{4.11}$$

A plot of ln[(dα/dt)/(1-α)] versus 1/T should ideally give a straight line with a slope of (-E/RT) and an intercept of ln (A) by using Microsoft Excel. The results of the kinetic parameters (A and E), the determination R^2 are reported in Table 4.9.

Activation energy (E) can be defined as the minimum energy required to start a chemical reaction. Table 4.9 shows a good fit of the activation energy to the enzymatic results. The activation energy of both subcritical water and steam exploded bagasse were lower than the raw bagasse. The interesting found is E of steam exploded at 200 °C, 5 min, pre-water soaking, which was reduced 32% of energy required when compared with the raw bagasse and was obtained the highest reducing sugars (353.37 mg/g pretreated bagasse). [64, 65]

CHAPTER 5
CONCLUSION

In this thesis, sugarcane bagasse was firstly pretreated in 0.6 L reactor by two methods: subcritical water and steam explosion. The optimum conditions of pretreatment were identified and compared systematically to enhance the enzymatic digestibility of the cellulose fraction for subsequent sugar conversion. Moreover, the process in 1000 L reactor was primarily investigated for the scale up possibility. The results are summarized as follows:

Without pretreatment, raw bagasse gave very low reducing sugars. Both of subcritical water and steam explosion have been shown significantly increasing in hemicellulose solubilization and resulting in higher amount of sugars when compared to the raw bagasse. The optimal conditions for the maximum enzyme digestibility of subcritical water pretreated sugarcane bagasse was 170.59 °C 19.31 min 1:6.64 bagasse to water ratio that obtained reducing sugars 297.09 mg/g pretreated (48.76% glucose yield) while the optimal conditions of steam exploded bagasse was 200 °C 5 min with pre-water soaking and had led to the highest amount of sugars as 353.37 mg/g pretreated (59.12% glucose yield) This could be suggest that using steam explosion pretreatment could be suitable for increasing enzymatic digestibility of sugarcane bagasse.

In large scale applications, the enzymatic hydrolysis results show that the subcritical water pretreatment in 1000 L reactor can be operated with relatively high amounts of sugars. (196.30 mg/g pretreated) from 160 °C, 20 min, 1:12 bagasse to water ratio. This would be the great potential for scaling up in industry section, but several issues must be addressed before a commercial technology.

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APPENDIXES

APPENDIX A: Experimental results

Table A-1 The overview conditions and percentage of components in subcritical water pretreatment of sugarcane bagasse [C: Cellulose, H: Hemicellulose, L: Lignin, and A: Ash]

No.	Conditions	%Composition				%Solid residue	%Relative composition				%Hemi-cellulose removal
		C	H	L	A		C	H	L	A	
	Raw mat.	44.32	23.75	23.20	4.90	-	46.08	24.70	24.12	5.10	-
1	140 °C, 10 min, 1:6	41.35	19.06	22.89	4.04	97.02	46.60	21.48	27.37	4.56	19.77
2	180 °C, 10 min, 1:6	41.06	5.37	18.91	3.90	69.70	63.60	8.32	22.04	6.04	77.38
3	140°C, 30 min, 1:6	39.87	16.82	21.81	4.59	96.05	46.93	19.79	27.88	5.40	29.20
4	180°C, 30 min, 1:6	37.45	3.88	22.85	4.53	64.05	61.20	6.35	25.04	7.41	83.65
5	140°C, 10 min, 1:10	42.86	16.59	22.69	4.43	92.34	49.66	19.23	25.98	5.14	30.13
6	180°C, 10 min, 1:10	32.11	4.16	21.87	2.80	62.64	58.66	7.60	28.64	5.11	82.49
7	140 °C, 30 min, 1:10	39.99	16.38	22.61	3.42	90.31	48.64	19.92	27.28	4.16	31.05
8	180 °C, 30 min, 1:10	40.62	1.66	22.82	3.68	67.36	64.97	2.65	26.49	5.89	93.02
9	126.36 °C, 20 min, 1:8	41.30	18.94	20.69	3.74	96.02	47.95	21.99	25.72	4.34	20.25
10	193.64 °C, 20 min, 1:8	40.08	1.90	22.38	3.32	64.62	66.03	3.14	25.36	5.47	91.98
11	160 °C, 3.18 min, 1:8	40.87	20.73	21.95	2.26	96.16	46.90	23.79	26.72	2.59	12.71
12	160 °C, 36.82 min, 1:8	40.27	7.70	22.54	3.22	72.49	58.80	11.24	25.26	4.70	67.60
13	160 °C, 20 min, 1:4.64	41.54	11.06	22.67	3.25	83.86	54.19	14.43	27.13	4.24	53.42
14	160 °C, 20 min, 1:4.64	36.49	10.41	22.55	3.74	85.05	50.00	14.26	30.61	5.13	56.17
15	160 °C, 20 min, 1:8	41.04	10.10	22.84	3.35	91.39	52.13	12.84	30.78	4.25	57.46
16	160 °C, 20 min, 1:8	36.85	9.95	19.24	4.09	88.03	51.41	13.88	29.01	5.70	58.11

Table A-2 Enzymatic hydrolysis results in subcritical water pretreatment of sugarcane bagasse

No.	Conditions	Reducing sugars		%Glucose yield
		mg/g pretreated	mg/g raw mat.	
	Raw mat.	-	65.09	-
1	140 °C, 10 min, 1:6	87.78	85.16	19.94
2	180 °C, 10 min, 1:6	266.01	185.41	43.60
3	140 °C, 30 min, 1:6	96.12	92.32	21.68
4	180 °C, 30 min, 1:6	273.36	175.08	41.14
5	140 °C, 10 min, 1:10	129.94	119.98	28.18
6	180 °C, 10 min, 1:10	229.15	143.54	33.82
7	140 °C, 30 min, 1:10	141.12	127.45	29.90
8	180 °C, 30 min, 1:10	173.27	116.71	27.46
9	126.36 °C, 20 min, 1:8	77.29	74.21	17.42
10	193.64 °C, 20 min, 1:8	183.62	118.65	27.87
11	160 °C, 3.18 min, 1:8	180.80	173.86	36.41
12	160 °C, 36.82 min, 1:8	241.40	174.99	41.10
13	160 °C, 20 min, 1:4.64	190.33	159.61	37.47
14	160 °C, 20 min, 1:4.64	199.25	169.47	39.84
15	160 °C, 20 min, 1:8	205.36	187.68	44.05
16	160 °C, 20 min, 1:8	205.01	180.47	42.30

Table A-3 The overview conditions and percentage of components of steam explosion pretreatment of sugarcane bagasse [C: Cellulose, H: Hemicellulose, L: Lignin, and A: Ash]

No.	Conditions	%Composition				%Solid residue	%Relative composition				%Hemi-cellulose removal
		C	H	L	A		C	H	L	A	
	Raw mat.	44.32	23.75	23.20	4.90	-	46.08	24.70	24.12	5.10	-
1	190 °C, 1 min	43.46	16.23	22.93	3.91	94.35	50.22	18.76	26.50	4.52	31.65
2	190 °C, 5 min	42.78	11.64	20.96	2.86	82.70	54.68	14.88	26.78	3.65	50.98
3	190 °C, 10 min	44.29	6.71	18.55	3.03	76.11	61.02	9.25	25.56	4.18	71.73
4	190 °C, 15 min	39.37	5.35	18.15	2.77	71.82	59.98	8.15	27.65	4.22	77.48
5	200 °C, 1 min	41.10	16.77	19.60	3.49	83.28	50.77	20.72	24.21	4.31	29.38
6	200 °C, 5 min	44.45	9.26	19.93	3.09	79.32	57.93	12.07	25.97	4.03	61.01
7	200 °C, 5 min, pre-water soaking	41.03	7.31	17.61	2.58	71.24	59.87	10.67	25.70	3.76	69.22
8	200 °C, 10 min	39.07	4.29	18.43	2.86	71.91	60.44	6.64	28.51	4.42	43.83
9	200 °C, 15 min	42.65	2.70	17.08	2.70	72.97	65.47	4.15	26.23	4.15	88.62
10	210 °C, 1 min	36.72	8.73	16.82	2.61	74.79	56.59	13.45	25.93	4.03	63.26
11	210 °C, 5 min	39.06	3.15	15.98	2.86	70.36	63.98	5.16	26.18	4.68	86.73
12	210 °C, 10 min	42.51	3.38	16.40	2.67	72.52	65.43	5.21	25.25	4.12	85.75
13	210 °C, 15 min	40.42	2.44	14.57	2.37	66.86	67.60	4.07	24.37	3.96	89.75

Table A-4 Enzymatic hydrolysis results in steam explosion of sugarcane bagasse

No.	Conditions	Reducing sugars		%Glucose yield
		mg/g pretreated	mg/g raw mat.	
	Raw mat.	-	65.09	-
1	190 °C, 1 min	77.75	73.36	17.23
2	190 °C, 5 min	156.31	129.26	30.36
3	190 °C, 10 min	222.85	169.61	39.83
4	190 °C, 15 min	249.04	178.86	42.00
5	200 °C, 1 min	116.01	96.62	22.69
6	200 °C, 5 min	208.96	165.74	38.92
7	200 °C, 5 min, pre-water soaking	353.37	251.75	59.12
8	200 °C, 10 min	259.19	186.38	43.83
9	200 °C, 15 min	237.56	173.34	40.71
10	210 °C, 1 min	262.16	196.07	46.04
11	210 °C, 5 min	304.24	214.07	50.27
12	210 °C, 10 min	212.05	153.77	34.86
13	210 °C, 15 min	220.20	147.23	34.57

Table A-5 Carbon balance for whole process of subcritical water pretreatment

No.	Conditions	Inlet Carbon (g)		Sum (g)	Outlet Carbon (g)			Sum (g)	Carbon loss (g)	Carbon loss (%)
		Bagasse	Water		Solid	Filtrate	Washed water			
1	140 °C, 10 min, 1:6	19.804	0.0003	19.804	19.129	0.200	0.415	19.744	0.0603	0.30
2	180 °C, 10 min, 1:6	20.258	0.0004	20.258	14.834	2.550	2.577	19.961	0.2974	1.47
3	140 °C, 30 min, 1:6	20.279	0.0003	20.279	19.146	0.206	0.763	20.115	0.1643	0.81
4	180 °C, 30 min, 1:6	20.275	0.0005	20.276	13.575	2.258	3.263	19.096	1.1795	5.82
5	140 °C, 10 min, 1:10	14.876	0.0001	14.876	13.782	0.522	0.447	14.751	0.1251	0.84
6	180 °C, 10 min, 1:10	17.094	0.0004	17.094	11.085	3.516	1.71	16.311	0.7834	4.58
7	140 °C, 30 min, 1:10	14.863	0.0004	14.863	13.167	0.606	0.816	14.589	0.2744	1.85
8	180 °C, 30 min, 1:10	15.784	0.0005	15.785	11.068	2.720	1.572	15.36	0.4245	2.69
9	126.36 °C, 20 min, 1:8	18.057	0.0003	18.057	16.982	0.303	0.302	17.587	0.4703	2.60
10	193.64 °C, 20 min, 1:8	18.003	0.0005	18.004	12.323	3.034	1.588	16.945	1.0585	5.88
11	160 °C, 3.18 min, 1:8	20.282	0.0004	20.282	17.656	0.849	0.957	19.462	0.8204	4.04
12	160 °C, 36.82 min, 1:8	20.267	0.0004	20.267	15.328	2.756	1.99	20.074	0.1934	0.95
13	160 °C, 20 min, 1:4.64	22.525	0.0004	22.525	18.552	1.012	2.843	22.407	0.1184	0.53
14	160 °C, 20 min, 1:4.64	13.506	0.0003	13.506	11.233	1.251	0.894	13.378	0.1283	0.95
15	160 °C, 20 min, 1:8	18.137	0.0002	18.137	15.857	1.162	1.015	18.034	0.1032	0.57
16	160 °C, 20 min, 1:8	18.021	0.0003	18.021	14.49	1.832	1.493	17.815	0.2063	1.14

APPENDIX B: HPLC Standard curve

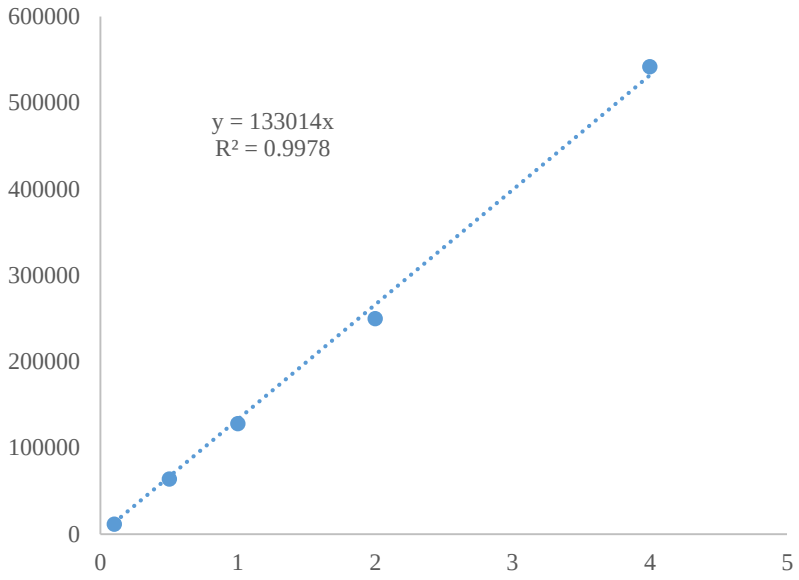

Fig. B-1 Standard calibration curve of glucose

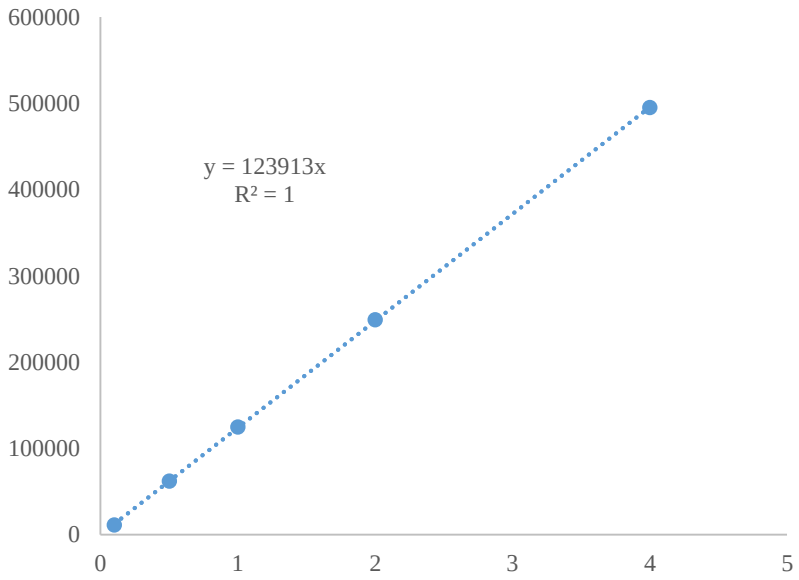

Fig. B-2 Standard calibration curve of xylose

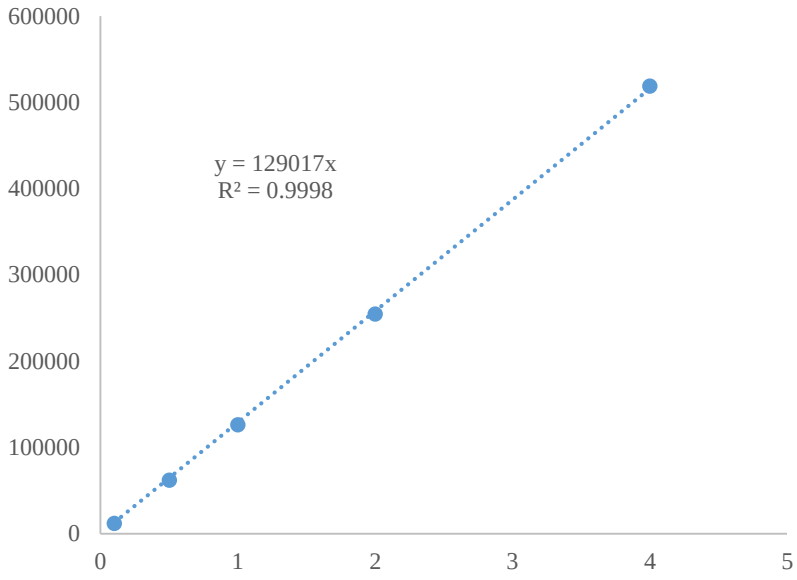

Fig. B-3 Standard calibration curve of arabinose

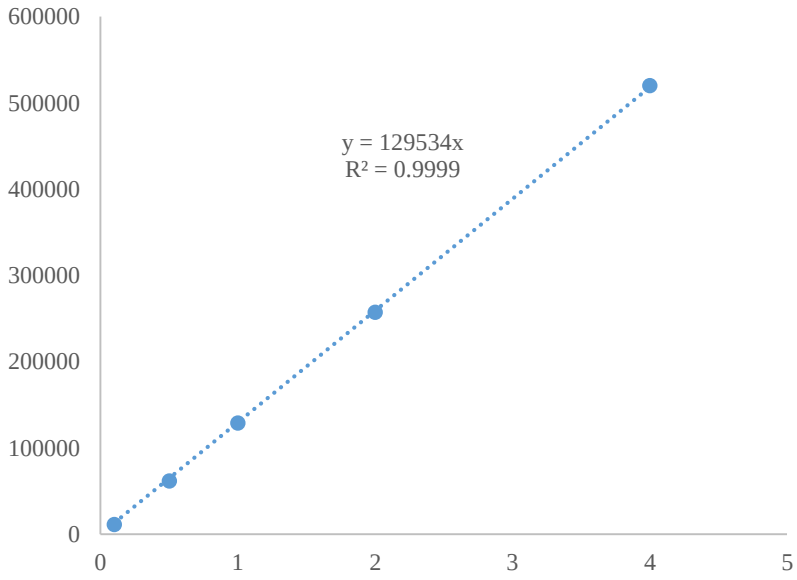

Fig. B-4 Standard calibration curve of galactose

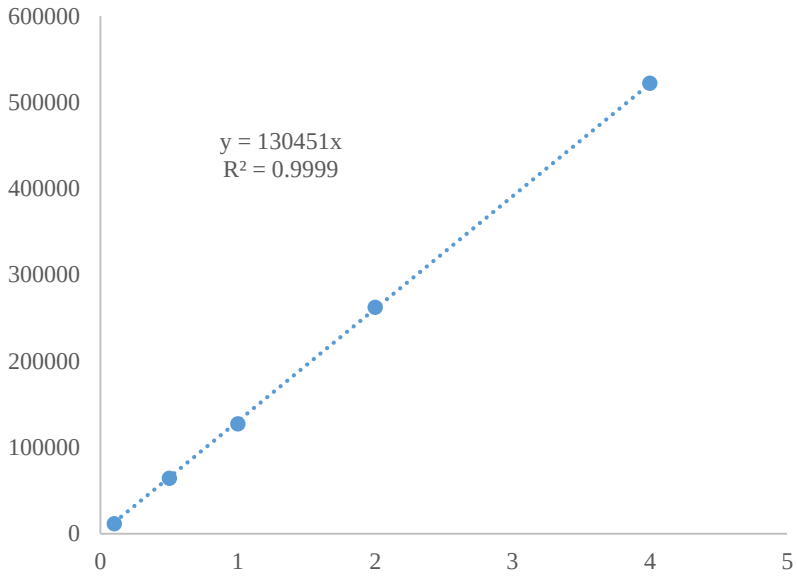

Fig. B-5 Standard calibration curve of mannose

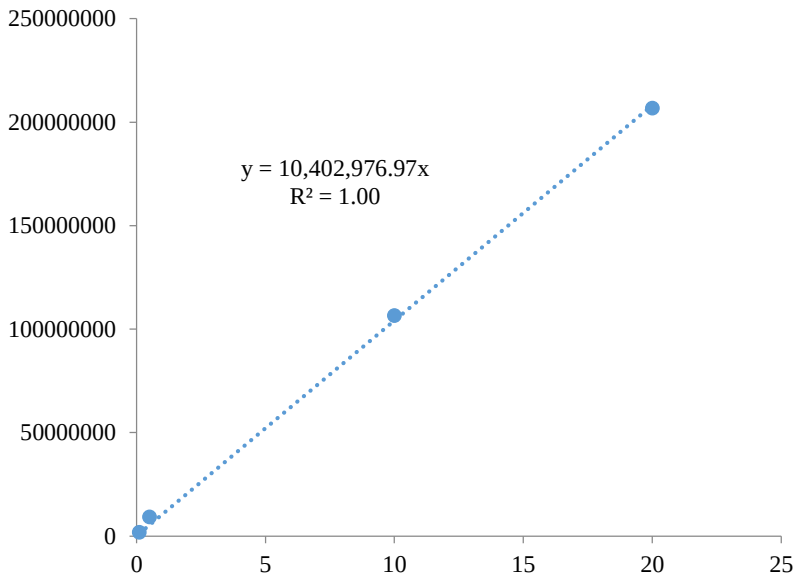

Fig. B-6 Standard calibration curve of furfural

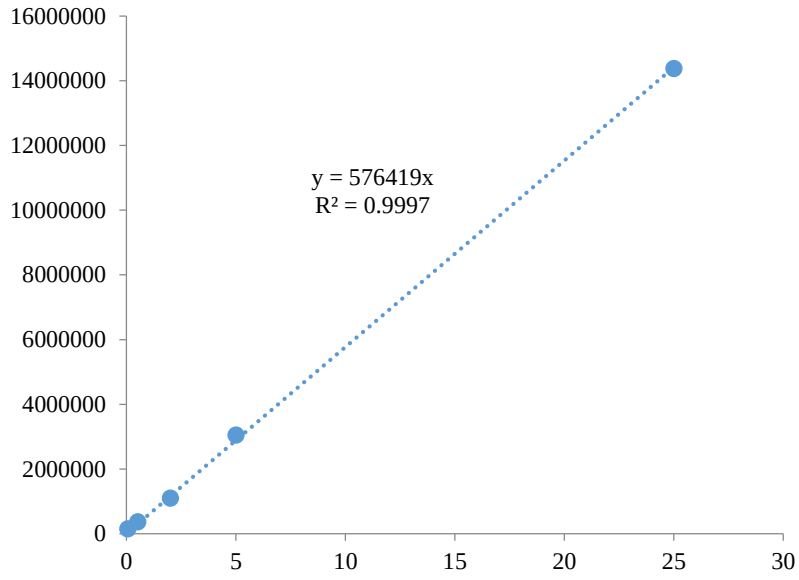

Fig. B-7 Standard calibration curve of succinic acid

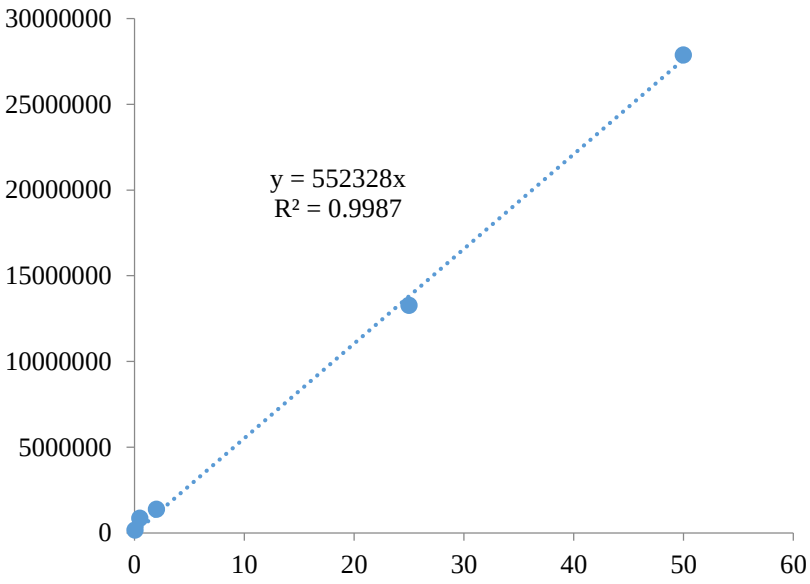

Fig. B-8 Standard calibration curve of lactic acid

APPENDIX C: DNS method standard curve

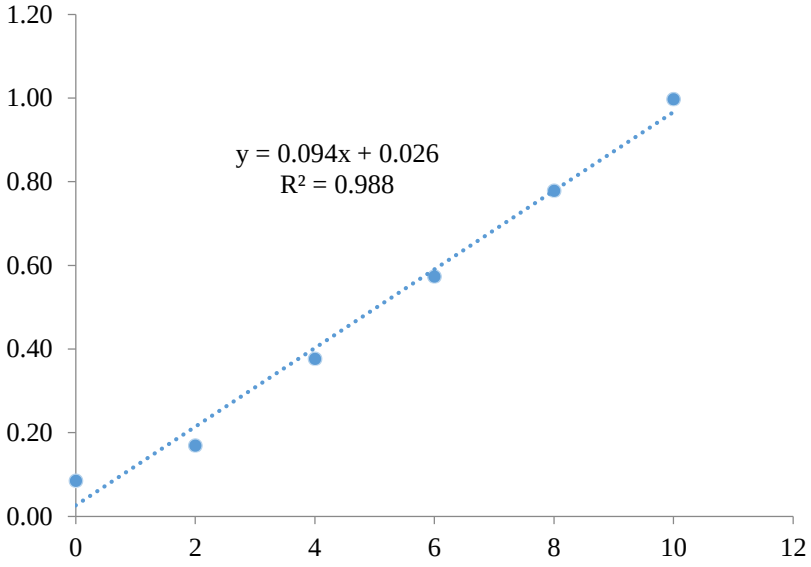

Fig. C-1 Standard curve for analysis of the concentration of reducing sugar by the DNS method (OD 540 nm)