Liquid Hot Water Pretreatment of Rice Straw for Enzymatic Hydrolysis

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Abstract: Liquid hot water is one of promising lignocellulosic biomass pretreatment technologies for improving biomass digestibility for enzymatic hydrolysis. This work studied the optimization of liquid hot water pretreatment (LHW) on rice straw based on sugar production from autohydrolysis and inhibitory product formation in the liquid fraction and sugar yield from enzymatic hydrolysis of the pretreated rice straw. The LHW conditions were in the temperature range of 160 to 200°C with the pretreatment time for 5 to 20 min. The optimal pretreatment condition for rice straw was at 200°C for 5 min, which led to the reducing sugar yield of 602.3 mg/g substrate after enzymatic hydrolysis using the commercial cellulose mixtures. Glucose was the main product from hydrolysis of the pretreated rice straw with the lower amounts of released xylose and arabinose. The formation of inhibitory by-product from degradation of sugars increased with temperature. The highest concentrations of 5-hydroxymethyl-2-furaldehyde (HMF) (0.74 mg/ml) and furfural (2.5 mg/ml) were found for the biomass pretreated at 200°C for 10 min. Based on the experimental result, the optimal pretreatment conditions for rice straw was at 200°C for 5 minutes, which led to the low glucose concentration from autohydrolysis and high sugar yield from enzymatic hydrolysis with relatively low inhibitory by-product formation.

Keywords: Pretreatment, Liquid Hot Water, Rice Straw, Enzymatic Hydrolysis.

1. INTRODUCTION

Nowadays, energy and environmental crisis is an important problem worldwide due to the deficit of petroleum resources and the dramatic rising of the oil price. These problems exist from the rising of energy demand of the world especially for industry and transportation. The demand for fuels in Thailand has been rising rapidly due to a population growth and an increasing of the manufacturing and transportation sectors. Imported oil supplies have also risen substantially to fulfill increasing fuel demands because Thailand can supply only 10% of the required fuel [1]. The imported oil, which is likely to increase in price in the long term, leads to widening the unbalance of the trade deficit and unstability of energy supply.

Lignocellulosic biomass has been considered a potent alternative energy resource for Thailand. Biomass is a renewable resource produced from organic matter including agriculture residues and is the main renewable energy resource available, especially in agricultural countries like Thailand. Thailand has many kinds of biomass, for example, rice husk, rice straw, corn cob, coconut shell, palm shell, cassava pulp and sugarcane bagasse. With advances in chemical engineering and biotechnology, many processes have been established for energy production from biomass. These materials can be used as fuel to heat water to produce steam or processed into liquids and gases, which can be converted to energy and fuel via thermochemical and biochemical conversion. The products at the end of the conversion routes are diverse, but can be divided into four main groups: biodiesel mainly fatty acid methyl ester (FAME), alcohols (ethanol/methanol), synthetic fuel (BTL) and hydrogen.

However, the lignocellulosic biomass need to be pretreated to change their physicochemical properties and fractionated for their components for subsequent processing and utilisation. The objective of the pretreatment is to remove lignin and hemicellulose, increase the porosity of the materials, and reduce cellulose crystallinity. In general, pretreatment must follow these requirements: firstly, improving the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis; secondly, avoiding the degradation or loss of carbohydrates; minimizing the formation of by-products inhibitory to the subsequent hydrolysis and fermentation processes; and finally, being cost-effective. Physical, physico-chemical, chemical, and biological processes have been used for pretreatment of lignocellulosic materials [2].

Liquid hot water is an environmental friendly process due to does not require rapid decompression and does not employ any catalysts or chemicals. Pressure is applied to maintain water in the liquid state at elevated temperatures (160-240°C) and provoke alterations in the structure of the lignocellulose. The aim of the liquid hot water is to solubilize mainly the hemicellulose fraction, to make the cellulose swelled and to keep away the inhibitory formation. After pretreatment, the slurry can be filtered divide into two phases: first is solid cellulose-enriched fraction and second phases is a liquid fraction rich in hemicellulose derived sugars. Efficiency of liquid hot water has been shown to remove the
hemicelluloses up to 80%, leading to improved enzymatic digestibility of pretreated lignocellulosic feedstocks, such as corn stover [3], sugarcane bagasse [4] and wheat straw [5]. Lignin is partially depolymerized and solubilized as well during hot water pretreatment but complete delignification is not possible using hot water alone, because of the recondensation of soluble components originating from lignin. In general, liquid hot water pretreatments are attractive because of following a cost-savings potential: no catalyst requirement and low-cost reactor construction due to low-corrosion potential. It has also the major advantage that the solubilized hemicellulose and lignin products are present in lower concentration, due to higher water input, and subsequently concentration of degradation products is reduced. In comparison to steam explosion, higher pentosan recovery and lower formation of inhibitors are obtained, however, water demanding in the process and energetic requirement are higher and it is not developed at commercial scale.

The present work examined in detail the characteristics of HCW pretreatment of the potential local lignocellulosic agricultural (Rice straw) including reducing sugar and inhibitor formation in the liquid fraction as well as enzymatic hydrolysis. Variable parameter in this study including pretreatment time, temperature and substrates.

2. MATERIALS AND METHODS

2.1 Raw materials
In this project, rice straw was obtained locally farmer. The rice straw was physically processed using a cutting mill (Retsch SM2000) and sieved to the size 250-420 μm for biomass pretreatment study.

2.2 Liquid hot water pretreatment
In this project, a multi-reactor thermal pretreatment system will be constructed. This reactor is designed for multi-purpose thermo-chemical pretreatment. In this stage, the reactor will be used for reaction parameter optimization for liquid hot water pretreatment. The system will contain 6 small-scale reactors (10 ml) in a temperature-controlled jacket with vertical shaking system to provide optimal mixing in the system. The reactor vessel will be constructed with high-grade stainless steel (Stainless steel 316). Each reactor will be installed with a thermocouple. The operated temperature range of the reactor will be up to 500°C with the maximal pressure limit at 10 MPa.

2.3 Enzyme formulation and optimization of the reaction conditions
The optimally pretreated biomass will be used for development of the efficient enzyme mixtures for the hydrolysis of each lignocellulosic substrate. Commercial enzymes from major enzyme suppliers (e.g. Celluclast® 1.5 L: cellulases from Trichoderma reesei; and Novozym® 188: β-glucosidase from Aspergillus niger from Novozyme AG, Bagsvaerd, Denmark; and Optimash BG: β-glucanase/ hemicellulase from T. reesei from Genencor, Danisco A/S, Copenhagen, Denmark) or the recently introduced cellulases from Novozymes AS will be used as the basic components for enzyme formulation. The hydrolysis efficiency will be analysed based on the total released reducing sugars using dinitrosalicylic acid method (DNS) [6].

For analysis of biomass hydrolysis efficiency in this project, the native and pretreated agricultural residues (5% w/v) described was enzymatically hydrolyzed by 10 FPU/g Celluclast 1.5 L, 5% (v/w) Novozyme 188, and 100% Optimash BG. Hydrolysis was carried out for up to 72 h at 200 rpm, 50°C, and pH 4.8 in a rotator. Upon completion of the hydrolysis, the hydrolysate was boiled for 10 min to deactivate the enzymes. The reducing sugars released were determined by the DNS method [6]. The analysis of redining sugars was done in triplicate.

2.4 Sugars and inhibitory by-product analysis
Sugar profile in the liquid fraction was analyzed quantitatively by a Waters HPLC model 2695 system equipped with a 2414 refractive detector and a Waters 2695 autosampler using Millenium32 chromatography manager 3.2 software (Waters Co.,Milford, MA). A Bio-Rad Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA) was employed for sugar analysis. The clarified samples were then filtered through 0.45-μm nylon membrane filter. The filtered samples were injected (20 μL) into column and eluted with 50 mM H₂SO₄ at a flow rate of 0.6 mL/min and a constant temperature of 40°C. Glucose, xylose and arabinose (Sigma-Aldrich,USA) were used to as standards for preparation of calibration curves.

HMF and Furfural in liquid fraction was analyzed by HPLC. A Shodex RSpak KC811 column with UV detector was employed to detect the HMF and furfural. A solution of 5 mM H₃PO₄ was used as the eluent with a volumetric flow rate of 0.6 mL/min at 60°C. Deionized water was used as the eluent with a volumetric flow rate of 1.0 mL/min at 85°C. The sample injection volume was 10 μL. The amount of each compound in the liquid products was determined using calibration curves obtained by analyzing standard solutions with known amounts.
2.5 Analysis of biomass components
The composition of biomass was determined using the standard analysis procedures provided by the National Renewable Energy Laboratory [7]. Two-stage sulfuric acid hydrolysis was used to determine carbohydrates (glucan and xylan). Insoluble lignin was the insoluble residue remaining after first swelling biomass in 72% sulfuric acid at 25°C for 2 h and then boiling the sample in 3% sulfuric acid for 4 h. The soluble fraction was used to determine the acid-soluble lignin using a spectrophotometric method. The ash content was determined as the weight of the biomass residue remaining after ignition at 575±25°C minimum for 3 h.

3. RESULTS AND DISCUSSION

3.1 Raw materials
The composition of rice straw used in this study was determined to be (on a dry weight basis): 35.27% glucan, 21% xylan, 3.07% arabinan, 18.56% insoluble lignin, 3.6% acid soluble lignin, 14.27% ash and 4% extractive. The chemical composition differences may suggest a significant structural and would give rise to unique characteristics of HCW pretreatment of rice straw.

3.2 Effects of hydrothermal pretreatment on biomass hydrolysis
Enzymatic hydrolysis of the pretreated biomass from HCW pretreatment under different conditions was carried out in 50 mM citrate (pH 4) at 45°C for 72 h. The analysis of reducing sugars from the hydrolysis reactions of the rice straw is shown in Fig 1. Enzymatic hydrolysis of the pretreated biomass showed that the digestibility of all pretreated biomass was improved in comparison to the control with no pretreatment. The optimal pretreatment condition for rice straw was 200°C for 20 min which led to the reducing sugar yield of 602.3 mg/g substrate. Optimal pretreatment condition increase rice straw digestibility. The result thus indicates remarkable increase of rice straw digestibility was 4 folds after the hydrothermal pretreatment.

![Fig. 1](image)

**Fig. 1** Effects of HCW pretreatment on rice straw was saccharified using the commercial enzyme mixture at 50°C for 72 h.

3.3 Analysis of sugar profile from hydrolysis of pretreated biomass
The reducing sugar profile from enzymatic hydrolysis of rice straw after HCW pretreatment was shown in Fig. 2. Glucose was the main product from hydrolysis of the pretreated rice straw with lower amount of released xylose and arabinose. An increasing trend of glucose was obtained with increasing pretreatment temperature and time. The highest glucose yield was obtained for rice straw pretreated at 200°C for 5 min (≈472 mg/g).

It is found that pretreatment temperature basically dominated the pattern of sugar production for an HCW process, whereas the length of pretreatment time exerted an influence on the amount of the produced sugars. For glucose production, pretreatment for 20 min tended to generate a higher amount of glucose than pretreatment for 10 min. However, at 180°C and 200°C pretreatment for 10 min tended to generate a higher amount of xylose and arabinose than pretreatment for 20 min, which suggested that xylose and arabinose were degrade rapidly when the substrate was pretreated for longer time and high temperature.
3.5 Formation of Inhibitors in Liquid Fraction

HCW pretreatment can lead to the formation of by-products from the disruption of lignocellulosic structure and the degradation of pentose, hexoses, and lignin, which would inhibit subsequent enzymatic hydrolysis and ethanol fermentation. The formation of inhibitors in liquid fraction from HCW pretreatment of rice straw is shown in Fig. 3.

As expected, the glucose degradation was increased with temperature, which led to an increase yield of 5-hydroxymethyl-2-furaldehyde (HMF). The highest HMF concentration was found for the biomass pretreated at 200°C for 10 min. HMF concentration was decreased at higher process severity as it can be further degraded under harsh conditions such as 200°C for 20 min. Similar trend for furfural formation was found at different pretreatment temperature and time. The formation of furfural increased when temperature increased from 160 to 200°C; however with a significant decrease at 200°C for 20 min. Furfural production achieved the maximum (25 mg/g) at 200°C for 10 min (Fig. 3b), corresponding to increasing degradation of xylose and arabinose. The decrease in furfural concentration at high severity pretreatment conditions was probably due to its further degradation into aldehydes and acid component [8].
Based on the experimental result, the optimal pretreatment conditions for rice straw was 200°C for 5 minutes, which led to the high production sugars after enzymatic hydrolysis with relatively low inhibitory by-products.

4. CONCLUSIONS

Bioconversion of lignocellulosic plant biomass to value-added products, including biofuels, biodegradable plastics and chemicals, as an alternative to petroleum-based processes is the basis of the sustainable green biotechnology. Establishment of an economically viable and technologically practical hydrolysis process for efficient saccharification of biomass is thus considered as a platform for applicability of the bioconversion process in industry. In this research project, an integrated pretreatment and enzymatic hydrolysis process for the TOP 5 local lignocellulosic agricultural by-products, including sugarcane bagasse, rice straw, corn cob, corn stover, and empty palm fruit bunch will be studied aiming to produce composite biomass sugars which are the key starting point for further conversion to biofuels and value-added chemicals in biorefinery. The potential “one-for-all” biomass pretreatment technology, based on thermochemical pretreatment with the focus on liquid hot water (LHW) method will be investigated for optimal conditions for the target feedstock. Preliminary study on LHW pretreatment using water as the solvent showed a significant improvement on biomass digestibility after pretreatment for all biomass i.e. rice straw, bagasse, corn cob, corn stover, and empty palm fruit bunch. The optimal pretreatment conditions for all biomass were in the range of 200°C for 5 min at the initial pressure of 2.5 MPa. This led to the reducing sugar yield of 580 mg/g. Sugar profile from rice straw hydrolysis showed that glucose was the major products while xylose and arabinose from hemicelluloses were obtained. The effects of external acids and bases on LHW pretreatment will be further investigated in order to lower the energy used for the pretreatment process.
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6. REFERENCES