International Journal of GEOMATE, Nov., 2018 Vol.15, Issue 51, pp.31-38
Geotec., Const. Mat. & Env., DOI: https://doi.org/10.21660/2018.51.24851
ISSN: 2186-2982 (Print), 2186-2990 (Online), Japan

EFFECT OF COMBINATION OF LIQUID HOT WATER SYSTEM AND HYDROGEN PEROXIDE PRETREATMENT ON ENZYMATIC SACCHARIFICATION OF CORN COB

Supawan Upajak [1], Navadol Laosiripojana[2], Verawat Champreda[3,4], Torpong Kreethachart[1] and *Saksit Imman [1]

[1]School of Energy and Environment, University of Pahyao, Thailand;
[2]The Joint Graduate School for Energy and Environment (JGSEE), King Mongkut's University of Technology Thonburi, Thailand
[3]Enzyme Technology Laboratory, National Center for Genetic Engineering and Biotechnology, Thailand
[4]BIOTEC–JGSEE Integrative Biorefinery Laboratory, National Center for Genetic Engineering and Biotechnology, Thailand

*Corresponding Author, Received: 30 March 2018, Revised: 11 April 2018, Accepted: 14 May 2018

ABSTRACT: Alkaline hydrogen peroxide pretreatment is an effectively enhance the increasing enzymatic digestibility of lignocellulosic biomass for conversion to fuels and chemicals in the biorefinery processes. In this study, effects of H_2O_2 on monomeric sugar in the liquid fraction during hydrogen peroxide pretreatment and sugar after enzymatic hydrolysis from corncobs were studied under varying reaction conditions. The temperature (30-120ºC) and H_2O_2 concentration (2.5-10%) efficiently promoted sugar yield in the piqued fraction and improved enzymatic hydrolysis of pretreated solids. The optimal condition for H_2O_2 pretreatment of corncob (H_2O_2 concentration of 5% using 60 ºC for 2 h) increased hemicellulose solubilization into the aqueous phase, resulting into the maximized pentose yield of 61.88% (xylose + arabinose) in the aqueous phase. H_2O_2 pretreatment under the optimal conditions at 60ºC for 2 h, leading to the enhance glucose yield from enzymatic hydrolysis of the pretreated biomass using 10 FPU/g CelluclastTM (85.66 %) and small amount of formation of inhibitory by-products. Combined with glucose in the aqueous phase, this resulted in the maxima 95.61% glucose recovery from the native corncob. This was related to changes in crystallinity and surface area of the pretreated biomass. Scanning electron microscopy (SEM) showed disruption of the intact biomass structure resulting increasing enzyme's accessibility to the cellulose microfibers which showed higher crystallinity index compared to the native biomass as shown by X-ray diffraction with a marked increase in surface area as revealed by BET measurement. The results provided efficiency of H_2O_2 pretreatment on increasing sugar recovery and an efficient approach for its processing in biorefinery industry.

Keywords: Corncob, Enzymatic hydrolysis, Lignocelluloses, Liquid hot water, Pretreatment

1. INTRODUCTION

The variation of crude oil price and increasing concerns on greenhouse gas release to global warming are main drivers of finding renewable resources for the production of fuels and chemicals. The northern of Thailand has been suffered from smog problems. The combustion of corn residues after cultivation is the major cause of smog which occurs every year. A corn residue is considered promising underused available biomass which can be used as starting materials for the production of bioethanol [1]. Fuel ethanol production from biomass involves pretreatment of the feedstock, followed by enzymatic hydrolysis of the substrates to sugars which are then subsequently fermented to ethanol [1,2].

Lignocelluloses mainly consists of cellulose, hemicellulose, and lignin, and are considered as potential source of biofuels [3]. Cellulose, a linear homopolymer of D-glucose formed into a highly organized crystalline fibrous structure which are associated with hemicellulose, an amorphous branched heteropolymer of pentoses, hexoses, and sugar acids, which acts as an interconnecting substance, and lignin, a heteropolymer of phenolic alcohols (p-coumaryl, coniferyl, and sinapyl alcohols), which acts as a protective shield giving strength to plant cells [4]. These biopolymers are organized into a multicomponent complex structure of the plant cell wall, which is a major limitation to their efficient utilization. [5]. This leads delignification pretreatment of biomass to be one of the keys in the field of biofuel production or biorefinery technology [6].

There are various pretreatment technologies for biomass materials, and these can be divided as physical (milling, thermal, etc) [7], chemical (acid, alkaline, oxidation, etc) [8], and

biological methods [9]. Hydrogen peroxide pretreatment is one of the most promising chemicals for delignification of the biomass. It was reported that the phenolic acids of lignin had a high tendency to dissolve in alkaline hydrogen peroxide solution [10]. The peroxide plays the role of oxidants during alkaline hydrogen peroxide condition [11], which takes part in the natural delignification process and has been widely used to bleach high lignin wood pulps in the pulp and paper industry. The role of alkaline is to reduce or to remove lignin, acetyl, and other uronic substitutions in hemicellulose fraction via swelling, solvation, and saponification, so that the accessibility and digestibility of hemicellulose and cellulose will be increased [12]. Corn cob is one of the most agricultural residues in Thailand with the annual availability more than 200,000 tons [13]. Most are used as animal feed while the rest is burned which results in air pollution problem [13]. In this study reports the use liquid hot water system combine with hydrogen peroxide for pretreatment of corncob. The effect of the hydrogen peroxide concentration, temperature and pretreatment time for increasing cellulose digestibility was tested via measuring sugar recoveries in the liquid phase and saccharified solid phase. The experiments were carried out at temperature range of 30-120ºC for 1-4 h with various H2O2 conditions. The work gives an insight on the efficiencies and potential of liquid hot water system together with hydrogen peroxide as an effective pretreatment technology aiming for a more competitive and economically viable biorefinery industry.

2. MATERIALS AND METHODS

2.1 Materials

Corncobs were obtained from Suwan farm, Nakorn Ratchasrima province, Thailand. The biomass was physically processed using a cutting mill (Retsch SM2000, Hann, Germany) and sieved to a particle size of 250–420 μm (0.21–0.35 mesh). The processed biomass was then used for experimental studies. The biomass contained 43.2% cellulose, 27.2% hemicellulose, 16.6% lignin, and 4.2% ash on a dry-weight basis according to the standard NREL method [14].

2.2 Alkaline hydrogen peroxide pretreatment

The pretreatment was performed in 50 ml reactor in a temperature-controlled jacket with vertical shaking system to provide optimal mixing. The individual stainless steel reactor vessel was installed with a thermocouple to record the temperature inside the reactor. The reaction containing 2.5-10% hydrogen peroxide solution. A solution diluted from a commercial 30% stock. The pH was adjusted to 11.5±0.2 with added of 5 M NaOH solution and mixed with the biomass. The biomass was pretreated under a set of conditions with varying temperatures (30-120°C) and residence times (1, 2, and 4 h), while substrate loading was fixed at 10 % (w/v) and the initial pressure was at 20 bars under nitrogen. The reactor was quenched in a water bath after heating at the desired conditions. The pretreated solid biomass was separated from the liquid phase by filtration and then thoroughly washed with DI water on a Bushner funnel. The sample was dried at 60 °C before subjecting to enzymatic hydrolysis to evaluate the improvement in biomass digestibility. The liquid fraction was collected for analysis of sugar and inhibitory by-products by HPLC.

2.3. Enzymatic hydrolysis

The pretreatment efficiencies were assessed based on digestibility of the pretreated solid residues using a commercial cellulase. The hydrolysis reactions of 1-mL total volume contained 5% (w/v) pretreated substrate with 10 FPU/g Trichoderma reesei cellulase (CelluclastTM 1.5L, Novozymes AS, Bagvaerd, Denmark) supplemented with 330 IU/g Aspergillus niger b-glucosidase (Novozym 188, Novozymes AS) and 120 IU/g based on endo-xylanase activity of Humicola insolent hemicellulase (Optimash® BG, Danisco AS, Copenhagen, Denmark) in 50 mM sodium acetate buffer, pH 5.0. The reactions were incubated at 50oC for 72 h with vertical mixing at 30 rpm. The experiments were done in triplicate. Filter paper unit (FPU) for cellulase was analyzed according to a standard method (15). β-glucosidase activity was determined using p-nitrophenyl-β-D-glucopyranoside (PNPG) as the substrate (16). One international unit (IU) was defined as the amount of enzyme which produced 1 μmole of reducing sugar or p-nitrophenolate in 1 min. The fermentable sugars profiles were analyzed on a Waters e2695 high-performance liquid chromatography, equipped with a differential refractometer, using a Biorad Aminex HPX-87P column (17). Sugar yields were reported based on the amount of sugars obtained from enzymatic hydrolysis of the solid residues on a dried weight basis. Sugar recoveries were reported as the percentage of glucose or pentose recovered based on the available cellulose (x1.11) and hemicellulose (x1.13) in the native biomass.

2.4. Scanning electron microscopy analysis

The native and pretreated biomass microstructures were analyzed by scanning electron microscope (SEM) using a JSM-6301F Scanning Electron Microscope (JEOL, Tokyo, Japan). The samples were dried and coated with gold for analysis. An electron beam energy of 5 kV was used for analysis.

2.5. X-ray diffraction analysis

The crystallinity of the native and pretreated biomass was determined by X-ray diffraction (XRD) using an X'Pert PRO diffractometer (PANalytical, Almelo, The Netherlands). The samples were scanned in a range of 2θ=10°–30° with a step size of 0.02° at 500 kV, 30 mA and radiation at Cu Kα (λ=1.54 Å). Crystallinity was calculated according to the following equations for crystallinity index below (18).

$$CrI(\%) = \left[\frac{I_{002} - I_{amorphous}}{I_{002}} \right] \times 100$$

in which I_{002} is the intensity for the crystalline portion of biomass (i.e., cellulose) at 2Θ = 22.4 and $I_{amorphous}$ is the peak for the amorphous portion (i.e., cellulose, hemicellulose, and lignin) at 2Θ = 18.0.

2.6 BET surface area measurement

The method of Brunauer, Emmett, and Teller (BET) was used to determine the total surface area of materials. Raw and pretreated biomass samples were analyzed for the BET surface area using a Belsorp-max TPDpro (BEL Japan, Tokyo, Japan) with thermal conductivity detector (Semi-diffusion type, 4-element W-Re filament) at the National Nanotechnology Center, Thailand.

3. RESULTS AND DISCUSSION

3.1 Influences of H_2O_2 concentration on Sugar Releases in Liquid Fraction

The effect of H_2O_2 concentration on the efficiency of pretreatment of corncob at various concentrations from 2.5% to 10% was first studied at 60ºC (Fig.1). The Pentose in the form of xylose and some arabinose was the major sugar released into the liquid phase. The maximum pentose yield in the liquid phase of 49.6% was achieved under the pretreatment condition of 60ºC for 1 h using the H_2O_2 concentration of 10%. Glucan solubilization in the range of 3.50-4.31% was obtained with varying concentration of H_2O_2 from 2.5-10%. The H_2O_2 pretreatment induces an increase in surface area, breaking structural intermolecular bonds between carbohydrates and lignin, disordering the lignin structure, and isolating lignin from the biomass

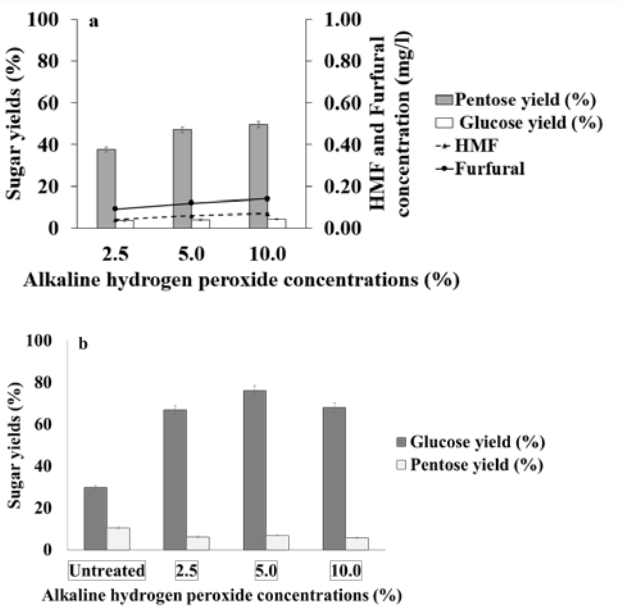

Fig. 1 Fig. 1 Effect of H2O2 concentration on sugar releases into the liquid phase (a) sugar and inhibitory by-products (b) sugar after enzymatic hydrolysis

matrix [19]. In the liquid phase, small amounts of inhibitory by-products were obtained under the H_2O_2 pretreatment. The maximum formation of HMF (0.07 mg/L) and furfural (0.14mg/L) were obtained in the liquid phase and decreased which could be due to further degradation of furans to aldehydes and organic acids such formic and acetic acids [20]. The separated hemicellulose streams rich in C_5 sugars and dehydration products can be further converted into a variety of commodity and specialty chemicals e.g. furans, sugar alcohols and butanediol by catalytic or fermentation processes [21,22]. The influent of H_2O_2 pretreatment on enzymatic hydrolysis of the pretreated solid obtained under varying pretreatment H_2O_2 concentration were determined based on sugar released after enzymatic hydrolysis using a commercial enzyme mixture. The native biomass was relatively resistance to enzymatic hydrolysis resulting in a low glucose yield (30%). Increasing H_2O_2 concentration (2.5-5.0% H_2O_2 concentration) led to higher sugar yield after enzymatic hydrolysis of the pretreated solid (Fig. 1b). The highest glucose yield of 76.15% was achieved under 5.0% H_2O_2 concentration for 1 h, equivalent to 80.18% glucose recovery from the native corncob. The maximum pentose yield of 6.9% was observed under 5.0% H_2O_2 concentration for 1 h, equivalent to 53.9% pentose recovery from the native corncob. The further increasing dosage of

hydrogen peroxide (10% H_2O_2 concentration) induced low yield of glucose. The oxidative action of the $H_2O_2^-$ derived radicals (HO• and O_2^-) is thought to depolymerize lignin by attacking lignin side chains and acting only in the aliphatic part of the macromolecule [23]. However, excessive usage of peroxide concentration could also lead to the low recovery of cellulose [24]. It might be the reason why the yield of glucose yield after enzymatic hydrolysis was decreased at higher concentration of hydrogen peroxide (10% H_2O_2 concentration). Consequently, 5% hydrogen peroxide was selected as the optimum concentration for further experiments due to showed lower amounts of by-products in the liquid phase and achieved highest glucose yield from enzymatic hydrolysis of the solid residues. Normally, hydrogen peroxide concentration was higher when it is applied in different lignocellulosic biomass due to its high content of lignin [25]. Such as Rabelo et al. (2014) reported the optimum hydrogen peroxide dosage was 7.36% for the pretreatment of sugarcane bagasse [26]. Alvarez-Vasco and Zhang (2013) reported the optimum hydrogen peroxide dosages of 4% for softwood [27].

3.2 Effect of temperature on sugar released in liquid fraction and enzymatic hydrolysis

The effect of temperatures on H_2O_2 pretreatment of corncob was studied by analysis of the released sugars, degradation products in the liquid fraction and sugar released after enzymatic hydrolysis. H_2O_2 pretreatment led to extensive solubilization and hydrolysis of the hemicellulose in the native corncob. A trend was observed of increasing pretreatment temperature with increasing sugar yield, partially pentose in the liquid fraction. The highest pentose yield of 52.51% was found at 120ºC with a concentration of 5% (Fig. 2a). Small amounts of glucose yield were observed (3.31% to 7.47%) in the liquid fraction. Inhibitory by-products (HMF and furfural) from degradation of the released pentose and glucose were found as a minor degraded product. The maximum HMF and furfural concentration were observed at 120ºC with a concentration of 5% of H_2O_2 concentration for 1 h in the range of 0.05-0.10 mg/l and 0.19-0.11mg/l, for furfural and HMF respectively. These levels of byproducts are not inhibitory to Saccharomyces cerevisiae or Candida guilliermondii, which tolerate HMF and furfural up to 2 mg/mL [28]. The effect of H_2O_2 pretreatment on digestibility of the pretreated solid was determined based on sugar released after enzymatic hydrolysis. The results showed that increasing pretreatment temperature from 30ºC to 60ºC led to increasing digestibility of the pretreated solids (Fig. 2b). The glucose yield was decreased from 86.15% to 73.07% for pretreatment temperature of 60ºC to 120ºC, which may be due to higher temperature resulted in an excessive quantity of radicals led to decrease the recovery of cellulose [29]. The maximum glucose yields were achieved at 60ºC for 1 h with H_2O_2 concentration of 5% after 72 h of enzymatic hydrolysis. These yields were 2.87 fold higher than the glucose yields obtained from untreated biomass. Pentose yield increased from 6.54% to 6.90% for pretreatment temperature 30ºC to 60ºC. A trend of decreasing pentose yield was observed with increasing pretreatment temperature from 60ºC to 120ºC. Higher operations temperature led to lower sugar yield, probably due to solubilization of cellulose and hemicellulose into the liquid phase during H_2O_2 pretreatment and degradation of monomeric sugars to inhibitory by-products [30]. The pretreatment conditions of 60ºC and H_2O_2 concentration of 5% were selected for further experiments. The optimal conditions based on the maximized overall sugar yield and lower by-products formulation.

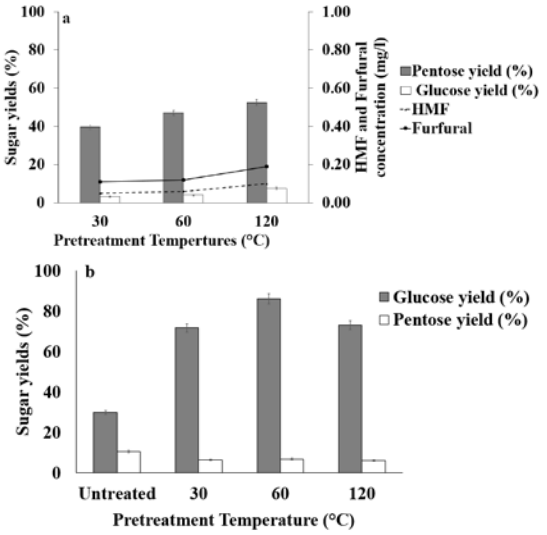

Fig. 2 Effect of pretreatment temperature on sugar releases into the liquid phase (a) sugar and inhibitory by-products in (b) sugar after enzymatic hydrolysis

3.3 Effect of reaction time on sugar released in liquid fraction and enzymatic hydrolysis

In this study, the further H_2O_2 pretreatment was studied for 1, 2, and, 4 h, respectively, using pretreatment temperature of 60ºC and H_2O_2 concentration of 5%, to optimize pretreatment time. Pentose was detected as the main product in the liquid fraction. The pentose yield of 47.04-56.18% was obtained in after H_2O_2 pretreatment. The maximum pentose yield of 56.18% was observed at 60ºC, for 2 h with H_2O_2 concentration of 5% and decreased due to degradation of pentose to

inhibitory by-products for 4 h pretreatment time. Glucose yield of 4.03-10.82 % was released into the liquid fraction. Increasing of pretreatment time led to increasing solubilization of hemicellulose and cellulose from the solid. Degradation products of HMF and furfural were also observed in the liquid fraction. HMF and furfural concentration of 0.06-0.31 mg/l and 0.12-0.35 mg/l for HMF and furfural, respectively, were obtained after H_2O_2 pretreatment with varying pretreatment time from 1-4 h (Fig. 3a). Increased pretreatment time generated higher inhibitory by-product compared with mild conditions. After hemicellulose solubilization from corncob, the remaining solid (mainly of cellulose) is easily hydrolyzed by cellulases into glucose. To evaluate the effect of reaction time on consequent enzymatic hydrolysis, the solid after pretreatment were hydrolyzed by cellulase of 10 FPU/g for 72 h (Fig. 3b). The composition of sugars in the hydrolysate, also as a result of the pretreatment were measured. The maximum glucose yield of 85.66% was obtained at 60°C, for 2 h with 5% of H_2O_2 concentration. However, no significant (p>0.05) of glucose yield after enzymatic hydrolysis between pretreatment temperature of 2 h (85.66%) and 4 h (82.81%). In this work, the corncob at a solid loading of 10% (w/v) was pretreated under the optimized conditions (60°C for 2 h with 5% of H_2O_2 concentration) and the remaining insoluble fraction was separated from the pretreatment hydrolysate prior to enzymatic saccharification. A minority of the glucose was released into the liquid fraction, while a substantial fraction of hemicelluloses was present as pentoses in the liquid fraction. Although longer pretreatment time would provide more extensive delignification, it could also induce the destruction of cellulose and thereby decrease enzymatic hydrolysis efficiency [29]. Overall, a high glucose yield from the optimal condition of 85.66% from the starting corncob was obtained after enzymatic hydrolysis of the solid.

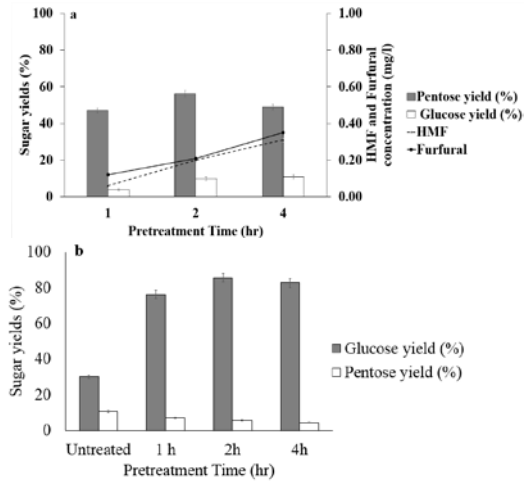

Fig. 3 Effect of pretreatment time on sugar releases into the liquid phase (a) sugar and inhibitory by-products in (b) sugar after enzymatic hydrolysis

3.4 Effect of H_2O_2 pretreatment on structural properties of corncob

Enhancement of enzymatic hydrolysis using the H_2O_2 pretreatment reported contributes to removing some compositional impediments and modifies the physicochemical structure and properties [30]. The changes in structural properties were analyzed for dries pretreated corncob using SEM, XRD, and BET. Changes in corncob microstructures pretreated under optimal condition were analyzed using SEM (Fig. 4). Comparison of the SEM images of the raw material and pretreated residues shows that microstructures of the pretreated solid are disrupted by H_2O_2 pretreatment process. The Texture of raw corncob was compact, which might be the wax layer commonly found in lignocellulosic biomass [31]. After H_2O_2 pretreatment at optimal condition, cavities and cracks in the plant cell wall were observed in the pretreated solid, which reflect the removal of hemicellulose and modification of the surface lignin [32]. These structural modifications led to an increase in an available surface area related using BET analysis (Table 1). Substantial increases in accessible surface area from 3.7 m^2/g to 8.1 m^2/g were observed after H_2O_2 pretreatment. The accessible surface area is regarded as one of the most important factors affecting the effectiveness of enzymatic digestibility [33]. The crystallinity of the corncob is also considered an important factor influencing the efficiency of enzymatic hydrolysis. According to the analysis of XRD patterns (Fig. 5), higher CrI (63.7%) was obtained for pretreated samples under the H_2O_2 pretreatment conditions for highest sugar production compared with native corncob (56.2%). The increases in CrI were revealed to effects of H_2O_2 pretreatment on the removal of the amorphous hemicellulose and lignin fractions in the biomass, while it showed less effect on the disruption of the cellulose, which containing highly crystalline structures. Increases in biomass crystallinity were reported in different biomasses pretreated by other pretreatment technologies, e.g., Tamarix ramosissima pretreated under LHW pretreatment [34] and also other pretreatment processes, e.g., microwave-assisted acid pretreatment [35] and steam explosion [36].

Table 1 BET surface area and crystallinity index of untreated and pretreated at optimal condition

Biomass	Surface area (m^2/g)		Crystallinity index (%)	
	Native	Pretreated	Native	Pretreated
Corncob	3.7	8.1	56.2	63.7

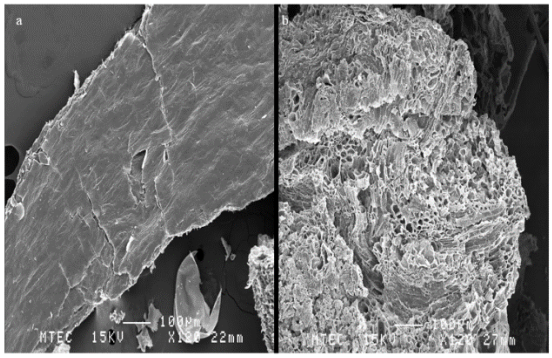

Fig. 4 X- Scanning electron micrographs of native and solid residues from pretreatment of corncob under optimal condition. (a) Native corncob; (b) pretreated corncob at 5% H_2O_2 concentration at 60°C for 2 h.

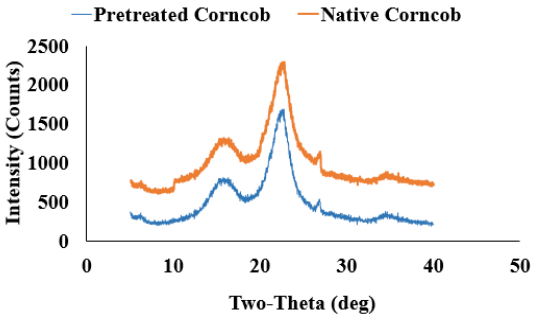

Fig. 5 X-ray diffraction profiles of the native (-) and pretreated biomass (-)

4. CONCLUSION

In conclusion, alkaline hydrogen peroxide pretreatment of corncob has been reported in this study. The results showed a modification of the biomass structural and chemical properties of corncob during pretreatment reaction, which led to remarkable enhance in enzymatic hydrolysis of pretreated corncob with high sugar yields from the starting native corncob. Moreover, H_2O_2 pretreatment has been able to effectively pretreat biomass, however, there are certain challengers that need to be addressed such as high cost of H_2O_2 solution, difficulty in recycling and reuse. In this study shows the potential for implementation of alkaline hydrogen peroxide pretreatment as an efficient pretreatment method for corncob.

3. ACKNOWLEDGMENTS

This project was financially supported by research grants from the Thailand Research Fund and the Office of the Higher Education Commission (MRG6080162) and Higher Education Research Promotion and Thailand's National Research Universities (2559A31962002).

4. REFERENCES

[1] Adepu K., and Shaishav S., Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. Bioresources and bioprocessing, Vol. 4:7, 2017, https://doi.org/10.1186/s40643-017-0137-9

[2] Xin Z., Qipeng Y., and Gang C., Deconstruction of corncob by steam explosion pretreatment: Correlations between sugar conversion and recalcitrant structures. Carbohydrate Polymers, Vol. 156, 2017, pp. 351-356.

[3] Sambusti C., Monlau F., Ficara E., Carrere H., and Malpei F., A comparison of different pretreatments to increase methane production from two agricultural substrates. Applied Energy, Vol. 104, 2013, pp. 62-70.

[4] Cara C., Ruiz E., Ballesteros I., Negro M.J., and Castro E., Enhanced enzymatic hydrolysis of olive tree wood by steam explosion and alkaline peroxide delignification. Process Biochemistry, Vol. 41, 2006, pp. 423-429.

[5] Pauly M., and Keeqstra K., Plant cell wall polymers as precursors for biofuels. Current Opinion in Plant Biology, Vol. 13, 2010, pp. 305-312.

[6] Kaparaju P., Serrano M., Thomsen A.B., Kongian P., and Anqelidaki I., Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept. Bioresource Technology, Vol. 100, 2009, pp. 2562-2568.

[7] Hendriks A.T.W.M., and Zeeman G., Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource. Technology, Vol. 100, 2009, pp.10-18.

[8] Wongthanate J., Chinnacotpong K., Khumpong M., Impacts of pH, temperature, and pretreatment method on biohydrogen production from organic wastes by sewage microflora. International journal of renewable energy and environmental engineering, Vol. 5, 2014, pp. 1-6.

[9] Kamcharoen A., Champreda V., Eurwilaichitr L., and Boonsawang P., Screening and optimization of parameters affecting fungal pretreatment of oil palm empty fruit bunch (EFB) by experimental design. International Journal of Energy and Environmental Engineering. Vol. 5, Issue 4, 2014, pp. 303-312.

[10] Alvarez-Vasco C., and Zhang X., Alkaline hydrogen peroxide pretreatment of softwood:

Hemicellulose degradation pathways. Bioresource Technology, Vol. 150, 2013, pp. 321-327.

[11] Kuman A.K., and Sharma S., Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. Bioresources and Bioprocessing, Vol. 4, 2017, pp. 1-19.

[12] Chaturvedi V., and Verma P., An overview of key pretreatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value-added products. 3 Biotech, Vol. 3, 2013, pp. 415-43.

[13] Kerdsuwan S., and Laohalidanond., Approach of using corn residue as an alternative energy source for power production: A case study of the northern plain area of Thailand. Energy. Procedia. Vol. 79, 2015, pp. 125-130.

[14] Sluiter A., Hames B., Ruiz R., Scarlata C., Sluiter J., Templeton D., and Crocker D., Determination of structural carbohydrates and lignin in biomass. Laboratory analytical procedure, Technical Report NREL/TP-510-42618, National Renewable Energy Laboratory, Department of Energy, USA (2008)

[15] Adney B., and Baker J., Measurement of cellulase activities, chemical analysis and testing task. In Laboratory Analytical procedure LAP-006. Denver: The National Renewable Energy Laboratory (NREL), 1996.

[16] Ruiz H. A., Silva D. P., Ruzene D. S., Lima L. F., Vicente A. A., and Teixeira J. A., Bioethanol production from thermal pretreated wheat straw by a flocculating Saccharomyces cerevisiae strain— the effect of process conditions. Fuel, Vol. 95, 2012, pp. 528–536.

[17] Imman S., Laosiripojana N., and Chaampreds V., Effects of Liquid Hot Water Pretreatment on Enzymatic Hydrolysis and Physicochemical Changes of Corncobs. Applied Biochemistry and Biotechnology, Vol. 184, 2018, pp. 432-443.

[18] Sindhu R., Kuttiraja M., Binod P., Janu K.U., Sukumara R.J., and Pandey A., Dilute acid pretreatment and enzymatic saccharification of sugarcane tops for bioethanol production. Bioresource Technology, Vol. 102, 2011, pp. 10915–10921.

[19] Ko J.K., Bak J.S., Jung M.W., Lee H.J., Choi I.G., Kim T.H., and Kim K.H., Ethanol production from rice straw using optimized aqueous-ammonia soaking pretreatment and simultaneous saccharification and fermentation processes. Bioresource Technology. Vol. 100, 2009, pp. 4374-4380.

[20] Yu Q., Zhuang X., Yuan Z., Wang Q., Qi W., Wang W., Zhang Y., Xu J., and Xu H., Two-step liquid hot water pretreatment of Eucalyptus grandis to enhance sugar recovery and enzymatic digestibility of cellulose. Bioresource Technology, Vol. 101, 2010, pp. 4895–4899.

[21] Carvalheiro F., Duarte L.C., and Grio FM., Hemicellulose biorefineries: a review on biomass Pretreatments. Journal of Scientific and Industrial Research, Vol. 67, 2008, pp. 849-864.

[22] Saha B.C., Hemicellulose bioconversion. Journal of Industrial Microbiology and Biotechnology, Vol. 30, 2003, pp. 279-91.

[23] Cabrera E., Muñoz M.J., Martín R., Caro I., Curbelo C., and Díaz A.B., Alkaline and alkaline peroxide pretreatments at a mild temperature to enhance enzymatic hydrolysis of rice hulls and straw. Bioresource Technology, Vol. 167, 2014, pp. 1–7.

[24] Ramadoss G., and Muthukumar K., Influence of dual salt on the pretreatment of sugarcane bagasse with hydrogen peroxide for bioethanol production. Chemical Engineering Journal, Vol 260, 2015, pp. 178–187.

[25] Ross A.B., Jones J.M., Kubacki M.L., and Bridgeman, T., Classification of macroalgae as fuel and its thermochemical behavior. Bioresource Technology, Vol 99, 2008, pp. 6494-6504.

[26] Rabelo S.C., Andrade R.R., Filho R.M., and Costa, A.C., Alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis and fermentation of sugarcane bagasse to ethanol. Fuel, Vol. 136, 2014, pp. 349–357.

[27] Alvarez-Vasco C., and Zhang X., Alkaline hydrogen peroxide pretreatment of softwood: hemicellulose degradation pathways. Bioresource Technology, Vol. 150, 2013, pp. 321–327.

[28] Sanchez B., Effects of furfural and 5-hydroxymethylfurfural on the fermentation of Saccharomyces cerevisiae and biomass production from Candida guilliermondii. Enzyme and Microbial Technology. Vol. 10, 1998, pp. 315–318.

[29] Li M., Pattathil S., Hahn M.G., and Hodge D.B., Identification of features associated with plant cell wall recalcitrance to pretreatment by alkaline hydrogen peroxide in diverse bioenergy feedstocks using glycome profiling.RSC Advances, Vol. 4, 2014, pp. 17282-17292.

[30] Mosier N., Dale B., Elander R., Lee Y.Y., Holtzapple M., Ladisch M., Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology, Vol. 96, Issue, 6, pp. 673-686.

[31] Yan L.F., Li W., Yang J.L., and Zhu Q.S., Direct visualization of straw cell walls by AFM. Macromolecular Bioscience, Vol, 4, Issue. 2, 2014, pp. 112-118.

[32] Pan X., Xie D., Gilkes N., Gregg D.J., and Saddler N.J., Strategies to enhance the enzymatic hydrolysis of pretreated softwood with high residual lignin content. Applied Biochemistry and Biotechnology, Vol. 124, 2005, pp. 1069-1079.

[33] Kumar S., Kothari U., Kong L., Lee Y.Y., Ram B., and Gupta R.B., Hydrothermal pretreatment of switchgrass and corn stover for production of ethanol and carbon microspheres Biomass and Bioenergy, Vol. 35, 2011, pp. 956-968.

[34] Xiao L.P., Shi Z.J., Xu F., and Sun R.C., Hydrothermal treatment and enzymatic hydrolysis of Tamarix ramosissima: Evaluation of the process as a conversion method in a biorefinery concept. Bioresource Technology, Vol. 135, 2013, pp. 73-81.

[35] Binod P., Satyanagalakshmi K., Sindhu R., Janu K.U., Sukumaran R.K., and Pandey A., Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse, Renewable Energy, Vol. 37, 2012, pp. 109-116.

[36] Pang F., Xue S., Yu S., Zhang C., Li B., and Y. Kang., Effects of combination of steam explosion and microwave irradiation (SE–MI) pretreatment on enzymatic hydrolysis, sugar yields and structural properties of corn stover, Industrial Crops and Products, Vol. 42, 2013, pp. 402-408.