International Journal of Chemical, Materials and Biomolecular Sciences
ISSN: 2415-6620
Vol:6, No:4, 2012

Characterization of Corn Cobs from Microwave and Potassium Hydroxide Pretreatment

Boonyisa Wanitwattanarumlug, Apanee Luengnaruemitchai, and Sujitra Wongkasemjit

***Abstract*—**The complexity of lignocellulosic biomass requires a pretreatment step to improve the yield of fermentable sugars. The efficient pretreatment of corn cobs using microwave and potassium hydroxide and enzymatic hydrolysis was investigated. The objective of this work was to characterize the optimal condition of pretreatment of corn cobs using microwave and potassium hydroxide enhance enzymatic hydrolysis. Corn cobs were submerged in different potassium hydroxide concentration at varies temperature and resident time. The pretreated corn cobs were hydrolyzed to produce the reducing sugar for analysis. The morphology and microstructure of samples were investigated by Thermal gravimetric analysis (TGA, scanning electron microscope (SEM), X-ray diffraction (XRD). The results showed that lignin and hemicellulose were removed by microwave/potassium hydroxide pretreatment. The crystallinity of the pretreated corn cobs was higher than the untreated. This method was compared with autoclave and conventional heating method. The results indicated that microwave-alkali treatment was an efficient way to improve the enzymatic hydrolysis rate by increasing its accessibility hydrolysis enzymes.

***Keywords*—**Corn cobs, Enzymatic hydrolysis, Microwave, Potassium hydroxide, Pretreatment.

I. INTRODUCTION

IN the last several decades, there has been an argument over the energy policy in many countries. Due to energy consumption has increased and many countries have become industrialized countries; therefore, the world energy consumption is projected to increase by 50%, by the year 2020. In addition, they are not a renewable energy resource and the combustion of fossil fuels results in greenhouse gas emissions and accelerates the global climate change. Therefore, the reduction of greenhouse gas emissions has become a primary focus of environmental solution in countries around the world.

There are many types of biofuel produced from lignocellulosic biomass such as bioethanol and biobutanol. However, butanol has several advantages over ethanol. It is used in industrial feedstock not only for use as a solvent, but also as a fuel that can replace gasoline. Moreover, butanol is less corrosive and more suitable for distribution through existing pipelines.

Corn is one of the most important agricultural crops in Thailand and it produce corn cob residue around 1.1 million ton per year, which it is normally used as animal feed and the elimination of residue leads to an environmental problem. In order to increase its value, the most attractive is to convert it to fermentable sugar. Corn cob is mainly composed of cellulose, hemicellulose, and lignin. This natural structure makes it difficult to hydrolyze into fermentable sugar.

The enzymatic hydrolysis is an interesting way to produce sugars from cellulose wastes because of its mild operating condition, regarding pH and temperature, and the absence of by-products [1]. Therefore, efficient of pretreatment method has improved enzymatic hydrolysis to remove lignin and hemicellulose, disordered the crystalline structure of cellulose, and increased the porosity of the materials to make cellulose more accessible to the enzyme for a maximal reducing sugar production.

There are many research work related with pretreatment methods; the microwave irradiation is widely used because of high heating efficiency, easy operation, and taking short time. Microwave heating pretreatment can break down lignin-hemicellulose complex and expose more accessible surface area of cellulose with cellulase enzyme [2]. Microwave irradiation can be easily combined with chemical reaction and, in some case, accelerate the chemical reaction [3]. Recently, a few reports combined microwave with alkali pretreatment, which could operate process at low temperature. Alkali pretreatment removed lignin and increased the biodegradability of cellulose owing to cleavage of the lignin bond. In my studies, the chemical composition, morphology and microstructure were catalyzed by HPLC, TG-DTA, SEM, XRD and BET.

The objective of this work was to characterize the pretreated corn cobs from microwave and potassium hydroxide pretreatment.

II. EXPERIMENTAL

A. *Pretreatment of Corn Cobs by Microwave Assisted Alkali*

Microwave radiation system was used in this study and combine with alkali pretreatment. This process used 2 g of corn cobs suspended in 30 mL of different potassium hydroxide concentrations (0.75 % to 3 %) and then transferred to microwave oven to treat corn cobs at 60-120 °C for 10 min to 30 min. After this process was completed, the residues were collected by filter paper and washed with tap water until neutral pH, dried at 65 °C.

B. *Enzymatic Hydrolysis*

A hydrolysis mixture consisted of 0.5 g of pretreated corn cobs and 15 mL of 0.1 mol L^{-1} citrate buffer (pH 4.8). The mixture was added with 0.1 mL of the commercial cellulese enzymes that was incubated at 50 °C in an incubator shaker

B. W. is with The Petroleum and Petrochemical College; and Center for Petroleum, Petrochemical, and Advanced Materials, Chulalongkorn University, Soi Chula 12, Phyathai Road, Pathumwan, Bangkok 10330, Thailand (e-mail: boonyisa.w@gmail.com)

A. L. is with The Petroleum and Petrochemical College; and Center for Petroleum, Petrochemical, and Advanced Materials, Chulalongkorn University, Bangkok 10330, Thailand (Phone: +66(0)221 84148;e-mail: apanee.l@chula.ac.th)

S. W. is with The Petroleum and Petrochemical College; and Center for Petroleum, Petrochemical, and Advanced Materials, Chulalongkorn University, Bangkok 10330, Thailand(Phone: +66(0)221 84133;e-mail: sujitra.d@chula.ac.th)

at 150 rpm for 48 h. Thereafter the hydrolysis solution was heated to 100 °C immediately for 3 minutes to denature the enzymes, cooled to room temperature, and then centrifuged for 20 min at 8000 rpm [3]. Then, the sample from the reaction was stored for sugar analysis.

C. Component Analysis of the Biomass Samples

Neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), and acid insoluble ash (AIA) of corn cobs before and after pretreatment were determined by the Nakhonratchasima Animal Nutrition Research and Development Center (Nakhonratchasima province, Thailand). The difference between NDF and ADF estimated detergent hemicellulose. Detergent cellulose was calculated by subtracting the values for (ADL + AIA) from ADF.

D. Thermal Gravimetric Analysis

For TG-DTA work, the untreated corn cobs were loaded with approximately 5 mg in high purity alumina pan in Perkin Elmer/Pyris Diamond. Nitrogen was used as a carrier gas for creating the inert environment. The heating rate was 10 °C/min from 50 °C to 1000 °C. In general, weight change of a sample was recorded as a function of time or temperature and characterized by a TG curve. DTG emphasized the zone of reaction where various reaction steps are taking place over the entire temperature range [4].

E. Monosaccharide Analysis

Glucose, xylose, and arabinose were determined using an HPLC system equipped with a refractive index detector (Model 6040 XR, Spectra-Physics, USA). An organic acid column (Aminex HPX- 87H column, Bio-Rad Lab, USA) was used with 0.005 M sulfuric acid solution as a mobile phase. The flow rate was controlled at 0.6 mL min^{-1} and the column temperature was 65 °C.

F. Crystallinity Measurement

X-ray diffraction (XRD) was used for phase identification of the untreated and pretreated corn cobs. Samples were scanned and recorded by using Rigaku X-Ray Diffractometer system (RINT-2200) with Ni filter and Cu K_{α} radiation (1.5406 Å) that generated at 30 mA and 40 kV. The scan speed of 5^{o} (2θ)/min with scan step of 0.02 (2θ) was used for the continuous run in 5 to 90 ^{o}C (2θ) range.

The crystalline indices of cellulose samples were calculated from the X-ray diffraction patterns by the following equation [5]:

$$CrI = \frac{I_{002} - I_{amophous}}{I_{002}} \times 100\%$$

Where I_{002} was the intensity for the crystalline portion of biomass (i.e., cellulose) at about 2h = 22.5 and $I_{amorphous}$ was the peak for the amorphous portion (i.e., cellulose, hemicellulose, and lignin) at about 2h = 19.

G. BET Surface Area Analysis

BET surface area of corn cobs before and after pretreatment was measured by N_2 adsorption/desorption measurements (Quantachrome/Autosorb1). The dried sample (0.1-0.5 g) was put into the sample tube and outgassed to remove the humidity and volatile adsorbents adsorbed on surface under vacuum at 150 °C for 4 h prior to the analysis. Then, N_2 was purged to adsorb on surface, and the quantity of gas adsorbed onto or desorbed from their solid surface at some equilibrium vapor pressure by static volumetric method will be measured. The solid sample was maintained at a constant temperature of the sample cell until the equilibrium is established. The BET surface area and pore volume were obtained from the N_2 adsorption/desorption curves.

III. RESULT AND DISCUSSION

A. Chemical Composition of Biomass

The main component of corn cobs was 41.27% cellulose, 46% hemicellulose and 7.4% lignin as shown in Table I. The others may include some organic compounds (uronic acid and acetyl groups) and other trace components such as minerals, waxes, fats, starches, resins, and gums [6]. Cellulose is the β-1,4-polyacetal of cellobiose, which is considered as the polymer of the glucose. The common polymer of hemicellulose is xylan that is mainly composed of five carbon sugar monomers such as xylose and arabinose, and six carbon sugar monomers such as glucose, mannose and galactose. Lignin is a complex polymer which consists of three types of phenolic acids: *p*-coumaryl alcohol, coniferyl alcohol, and synapyl alcohol. It plays an important role in the cell's endurance and development, as it affects the transport of water, nutrients, and metabolites in the plant cell [7].

TABLE I
CHEMICAL COMPOSITION OF CORN COBS

Composition	Dry solid (%, w/w)
Cellulose	41.27
Hemicellulose	46.00
Lignin	7.40

B. Thermal Gravimetric Analysis (TG-DTA)

The thermal decomposition behavior of untreated corn cobs was investigated by thermal gravimetric analysis TG-DTG, as shown in Fig. 1.

The first step of decomposition begins with moisture which was removed by 5.5% mass loss occurs at about 100 ºC. The second mass loss step was hemicellulose starting decomposition at 200-320 ºC by 32% mass loss. Hemicellulose was constructed an amorphous structure and linear polymer structure with short side chains which were easier to remove from the main stem than cellulose and degraded to volatiles such as CO, CO_2, and some hydrocarbon at the lower temperature. The third thermal decomposition of cellulose took place from 320 °C to 700 °C by 56.6% mass loss. Since cellulose was mainly consisted of semicrystalline arrangement chains associated with other which its structure was strong and produced it thermally. As for lignin, it was steadily decomposed at 250 to 500 °C. However, some groups reported that decomposition of heavier volatiles such as lignin occurs from temperature range 150 up to 900°C since it is more thermally stable in contrast to cellulose and hemicelluloses [4]. Lignin was the most difficult to decompose because it was complex structure of phenolic polymer covering the polysaccharides of the cell walls which made strong and durable composite material.

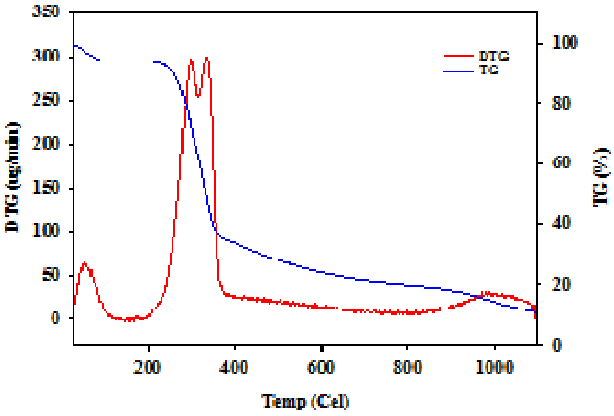

Fig. 1 TG-DTG curves of corn cobs

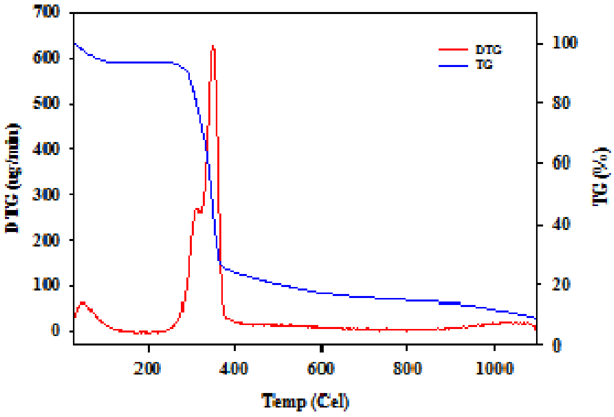

Fig. 2 TG-DTG curves of pretreated corn cobs
(2% KOH 120 °C 25 min)

Fig. 2 shows that the microwave/KOH pretreatment could remove hemicellulose and lignin. The weight loss of hemicellulose decreased from 32.1% to 19.2 %. Due to the overlapped peak of lignin involved with another peak that could not prove the percentage of lignin removal. For DTG peak of cellulose degradation temperature of pretreated corn cobs had left shifted comparing to the untreated control because of the lower crystallinity [8].

C. Total Sugar in Enzymatic Hydrolysis

The corn cobs were treated with microwave and KOH solution. Hydrolysis experiment contained a mixture of buffer solution and pretreated corn cobs. The enzyme used in this work is Novozyme 50013. The enzyme solution consisted of three components: endo-β-glucanase (EG) which attach to regions of low crystallinity in the cellulose fiber, generating free chain ends; exo-β-glucanase or cellobio-hydrolase (CBH) which degrade cellulose molecules further by removing cellobiose units from the free chain; and, β-glucosidase that hydrolyze cellobiose to produce glucose [9].

The maximum total sugar concentration in each pretreatment temperature obtained from enzymatic hydrolysis of pretreated corn cobs is shown in Fig. 3. At low temperature, 3% KOH gave the highest total sugar concentration. And as pretreatment time and KOH concentration increased, glucose concentration slightly increased. On the other hand, high sugar concentration was obtaind by 2% KOH at high temperatures. At high temperature and high KOH concentration during pretreatment resulted in higher solid loss, which leading to less total sugar released.

These results indicated that the optimal condition for enzymatic hydrolysis was 2% KOH at 120 °C for 25 min.

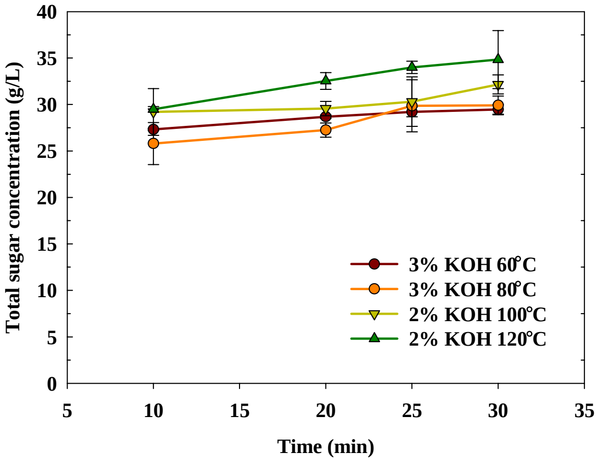

Fig. 3 The total sugar concentration obtained from enzymatic hydrolysis of pretreated corn cobs at different conditions.

D. Surface Morphology by SEM

SEM images of the untreated and the pretreated corn cobs in different conditions are shown in Fig. 4. The untreated corn cobs were clear and no pore (Fig. 4a). After the pretreatment with 0.75% KOH at 60°C for 25 min, the structure was damaged, looked soft and pores were appeared (Fig. 4b). When the temperature was 120 °C, the structure become more porous (Fig. 4c). This temperature would certainly increase the external surface area. With the KOH concentration was 2%, the surface area of corn cobs had many micropores which were favorable for cellulase to hydrolyze the cellulose into reducing sugar.

From the SEM result, the surface area of corn cobs increased when pretreatment temperature, pretreatment time and KOH concentration increased that was consistent with BET analysis, as shown in Table II. At optimal pretreatment condition surface area of corn cobs was increased from 3.926 m^2/g to 5.719 m^2/g.

TABLE II
BET SURFACE AREA, TOTAL PORE VOLUME, AND AVERAGE PORE DIAMETER OF SAMPLES

Sample	Surface area (m^2/g)	Total pore volume (cm^3/g)	Average pore diameter (nm)
Untreated corn cobs	3.926	0.0078	7.930
Pretreated corn cobs with 2% KOH at 120 °C for 25 min	5.719	0.0096	8.158

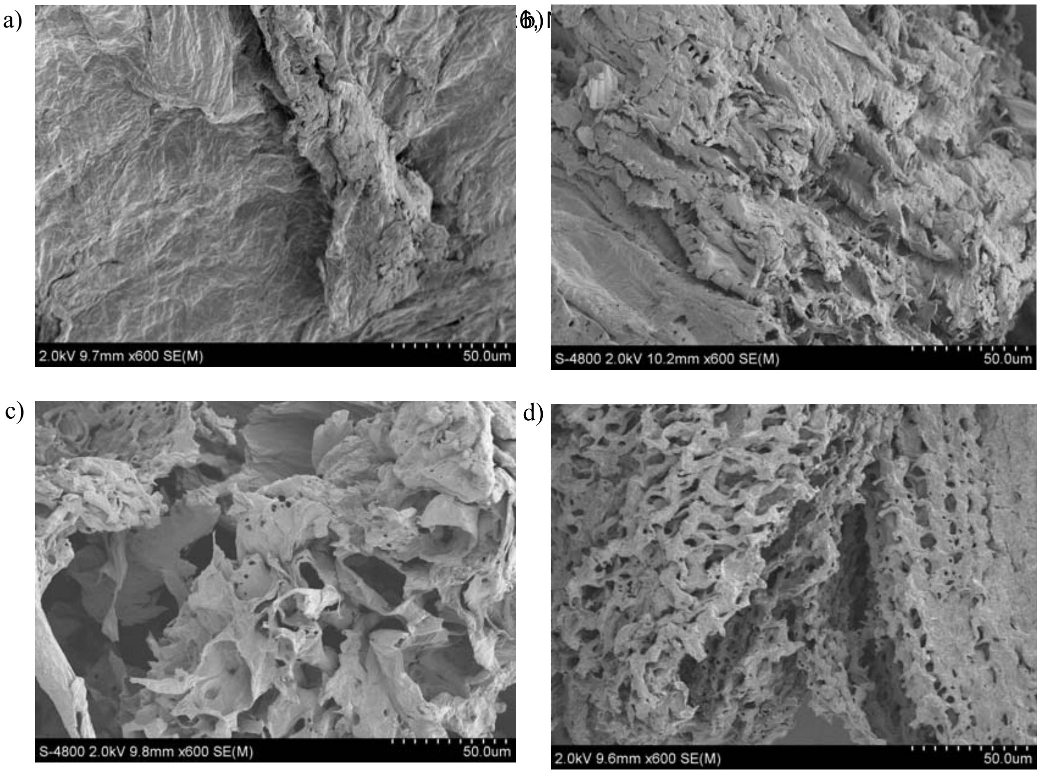

Fig. 4 Scanning electron microscope images of corn cobs. (a) Raw corn cobs without treatment; (b) Corn cobs after pretreatment with 0.75% KOH at 60°C for 25 min; (c) Corn cobs after pretreatment with 0.75% KOH at 120°C for 25 min; (d) Corn cobs after pretreatment with 2% KOH at 120°C for 25 min.

E. *X–ray Diffraction Analysis*

Crystallinity of cellulose is one of the main factors influencing enzymatic hydrolysis [10]. The cellulose crystallinity value of untreated sample was 24.5 % while the pretreated sample was up to 57.28 %. The crystallinity index in pretreated corncobs with KOH and microwave increased due to lignin and hemicellulose removal, which was good evidence that the amorphous portion of the corn cobs was more removed than the crystalline portion. Moreover, higher KOH concentration, pretreatment temperature and time which leaded to higher lignin removal, resulting in more crystallinity index which raised the enzymatic digestibility.

TABLE III
CRYSTALLINITY INDEX (%) OF UNTREATED AND TREATED CORN COBS

Sample	Crystallinity index
Untreated corn cobs	24.50
Pretreated corn cobs with KOH of 0% for 25 min at 120 °C	39.78
Pretreated corn cobs with KOH of 0.75% for 25 min at 120 °C	40.07
Pretreated corn cobs with KOH of 2% for 25 min at 60 °C	45.39
Pretreated corn cobs with KOH of 2% for 10 min at 120 °C	56.06
Pretreated corn cobs with KOH of 2% for 25 min at 120 °C	57.28

F. *Comparison of Total Sugar Concentration Obtained from Different Pretreatment Methods*

This part, the pretreatment by microwave and KOH solution was compared with other pretreatment methods. From Table IV shown that the total sugar obtained from enzymatic hydrolysis of pretreated corn cobs with 2% KOH with microwave 120 °C for 25 min was 34.79 g/L much was higher than total sugars of pretreated corn cobs with 2% KOH with autoclave 121 °C for 60 min and 2% KOH with conventional heating 120°C for 25 min. Because microwave radiation can heat uniformly inside the sample, it breaks down the lignin and hemicellulose structure. Furthermore, the partial crytallinity of cellulose was destroyed and hemicellulose was degraded into reducing sugar. Microwave also saved the pretreatment time and increased its accessibility to hydrolytic enzymes.

TABLE IV
COMPARISON OF TOTAL SUGAR CONCENTRATION OBTAINED FROM DIFFERENT PRETREATMENT METHODS

Method	Glucose (g/L)	Total Sugar (g/L)
2% KOH with microwave 120°C for 25 min	19.49	34.79
2% KOH with autoclave 121°C for 60 min	15.88	27.64
2% KOH with conventional heating 120°C for 25 min	15.59	27.24

IV. CONCLUSION

The results showed that the potassium hydroxide pretreatment with microwave on corn cobs was an effective method in improving enzymatic hydrolysis accessibility. The optimum conditions were found at 2% KOH at 120 °C for 25 minutes which could increase in surface area by 45.67% and the cellulose crystallinity index up to 57.28 %. And the highest glucose concentration can reach up 19.49 g/L and total sugar 34.79 g/L was released. Moreover, microwave assists KOH can produce high total sugar concentration at shorter pretreatment time compared with autoclave and conventional methods.

ACKNOWLEDGMENT

The authors would like to thank the National Research University Project of CHE and the Ratchadaphiseksomphot Endowment Fund (EN269B) and the Sustainable Petroleum and Petrochemicals Research Unit, the Center of Excellence on Petrochemical and Materials Technology, Chulalongkorn University, Thailand. The authors also sincerely thankful to B&C Pulaski Corporation Limited, Novozymes Inc., and Boon Rawd Brewery Co., Ltd. for providing material support such as corn cobs, cellulase enzyme, and Beverage Cooler. Their contribution to this thesis work is highly appreciated.

REFERENCES

[1] Carrillo F, Lis MJ, Colom X, Lo´pez-Mesas M, Valldeperas J. Effect of alkali pretreatment on cellulase hydrolysis of wheat straw: Kinetic study. Process Biochemistry 2005;40;3360–3364.

[2] Cheng J, Su H, Zhou J, Song W, Cen K. Microwave-assisted alkali pretreatment of rice straw to promote enzymatic hydrolysis and hydrogen production in dark- and photo-fermentation. International Journal of Hydrogen Energy 2011;36;2093-2101.

[3] Zhu S, Wu Y, Yu Z, Liao J, Zhang Y. Pretreatment by microwave/alkali of rice straw and its enzymic hydrolysis. Process Biochemistry 2005; 40;3082–3086.

[4] Abdullah SS, Yusup S, Ahmad MM, Ramli A, Ismail L. Thermogravimetry Study on Pyrolysis of Various Lignocellulosic Biomass for Potential Hydrogen Production. International Journal of Chemical and Biological Engineering 2010;72;129-133.

[5] Cao Y, Tan H. Study on crystal structures of enzyme-hydrolyzed cellulosic materials by X-ray diffraction. Enzyme and Microbial Technology 2005;36;314–317.

[6] Wang Z, Keshwani DR, Redding AP, Cheng JJ. Sodium hydroxide pretreatment and enzymatic hydrolysis of coastal Bermuda grass. Bioresource Technology 2010;101;3583–3585.

[7] Harmsen PFH, Huijgen WJJ, Bermúdez López LM, Bakker RRC. Literature Review of Physical and Chemical Pretreatment Process for Lignocellulosic Biomass. Food & Biobased Research 2010;Accessed on 9 May 2011http://www.ecn.nl/publicaties/PdfFetch.aspx?nr=ECN-E--10-013

[8] Zeng J, Singh D, Chen S. Biological pretreatment of wheat straw by Phanerochaete chrysosporium supplemented with inorganic salts. Bioresource Technology 2011;102; 3206–3214.

[9] Champagne P, Li C. Enzymatic hydrolysis of cellulosic municipal wastewater treatment process residuals as feedstocks for the recovery of simple sugars. Bioresource Technology 2009;100;5700–5706.

[10] Kuila A, Mukhopadhyay M, Tuli DK., Banerjee R. Production of ethanol from lignicellulosics: an enzymatic venture. Experimental and Clinical Sciences Journal 2011;85-96;1611-2156.