IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 10, Issue 6 Ver. II (Jun. 2016), PP 56-62
www.iosrjournals.org

Bioethanol Production from Banana Peels

Gaddafi I. Danmaliki*[1], Auwal M. Muhammad[2], Abdullahi A. Shamsuddeen[3]
Bashir J. Usman[4]

[1](Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria)
[2](Central Drug Control Laboratory, National Agency for Food and Drug Administration and Control, Lagos, Nigeria)
[3](Department of Chemistry, Sokoto State University, Sokoto, Nigeria) [4](Department of Chemistry, Kebbi State University of Science and Technology, Aliero, Nigeria)

Abstract: *Banana peels are lignocellulosic agricultural waste that has the potential to produce bioethanol as a renewable form of energy. Pretreatment and hydrolysis of lignocellulosic biomass are crucial steps in bioethanol production. Our study determined the efficiency of three pretreatment techniques namely water, alkaline, and acidic pretreatments on the production of bioethanol. We applied the same hydrolysis technique using H_2SO_4 to all the pretreatment techniques. Our results showed highest reducing sugar concentrations when banana peels were pretreated with acid; pretreatment using water alone gave the lowest reducing sugar concentrations. We used an activated strain of S. cerevisiae to* convert *the reducing sugars to bioethanol via a three-day fermentation process. The bioethanol concentrations detected by GC-*FID *from* the *water, acidic, and alkaline pretreatments were 40 ppm, 60 ppm, and 80 ppm respectively. It was evident that obtaining a high concentration of reducing sugars after hydrolysis does not guarantee bioethanol production. Moreover, our results have confirmed that the ultimate production of ethanol was pretreatment dependent.*

Keywords: *Banana Peels, Bioethanol, Fermentation. Hydrolysis, Pretreatment. S. cerevisiae*

I. Introduction

Fossil fuels are the major source of energy worldwide. The use of fossil fuel is associated with global warming, climate change, and a variety of energy and security problems. Moreover, fossil fuel is non-evenly distributed within nations, and equally non-renewable. Bioethanol, an ethanol liquid that is known to be a clean fuel for combustion engines, is a readily available substitute since it can be derived from plant based materials. The total consumption of bioethanol in 2008 was more than 65,000 million liters and the usage is growing rapidly because it has already replaced 5.4 % gasoline usage in 2013. The use of bioethanol as an alternative either as an octane enhancer or main fuel tend to reduce the problems associated with fossil fuels [1] [2]. However, deriving bioethanol from food sources is not a viable alternative, because we have to make the choice between food and ethanol [3].

Ethanol also called alcohol is a colorless, flammable, volatile liquid with a molecular formula of C_2H_6O. It has a molar mass of 46.07 g/mole, a density of 0.789 g/cm^3, a melting point of −114 °C, and a boiling point of 78.37 °C. It is widely used as a solvent, a fuel, and as a raw material for the production of other useful chemicals that have wide applications in the industry. It is also consumed as alcohol beverage, for household heating, and applied as an antiseptic. It is produced from ethylene hydration and fermentation of sugars, starch, lignocellulosic materials, or hydrocarbon-based ethanol production. The use of lignocellulosic biomass for bioethanol production is a recent alternative with great promise and still under research [4] [5]. It is an efficient, cost-effective, and a food security-wise alternative. Such biomass includes residues from agriculture or forest, industrial and municipal wastes, and dedicated energy crops. The substances that have been used previously to produce bioethanol include sugarcane bagasse, corn curb, newspapers, sawdust, rice straw, wood, wheat etc.

Lignocellulose biomass is characterized by its carbohydrate content, serving as a prerequisite for ethanol production. The biomass is composed of cellulose (40–50%), hemicellulose (25–35%) and lignin (15–20%) [6]. Cellulose is the dominant polymer among the three components. The efficient conversion of lignocellulosic biomass to fermentable sugars is the rate limiting step for efficient ethanol production [6] [7]. Cellulose is a structural polymer of glucose joined together by β-1, 4 glycosidic linkages. Due to this linkage, cellulose is conferred with a high resistant and compacted crystalline structure that is able to sustain some biological and chemical hydrolysis. Hemicellulose is found almost together with cellulose in plant cell wall. It consists of several heteropolymers with main chain xylan backbone (β-1, 4 linkages), glucuronoxylan, arabinoxylan, glucomannan, xyloglucagan and other sugars like mannose, xylose, galactose, rhamnose, and arabinose. It is not as resistant to hydrolysis as cellulose due to its little strength amorphous nature. Hemicellulose can be hydrolyzed easily by acidic treatment or enzymatic activity. The type of plant greatly influences the content of hemicellulose to be found. The last component in the lignocellulosic biomass is the

lignin, a complex polymer of aromatic alcohols known as monolignols that provide support to the plants. Overcoming the compactness and the strength of the lignocellulosic biomass in the production of ethanol is the overall aim of all pretreatments and hydrolysis processes [8].

Conversion of cellulose polymer to ethanol involves two different processes 1. Converting cellulose to glucose units via hydrolysis and the sugars produced from the hydrolysis process can then be converted to ethanol by 2. Fermentation using *Saccharomyces cerevisiae*. Pre-treatments are normally applied to separate the mixed polymers of lignin, hemicellulose and cellulose to provide the sugars needed for the hydrolysis and the fermentation processes [9]. The basic steps involved in the conversion of lignocellulosic biomass to ethanol are shown in **Fig 1**.

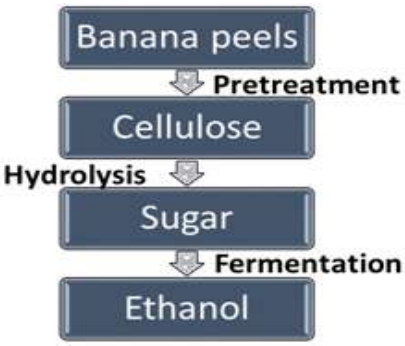

Fig 1. The steps involved in the conversion of lignocellulosic biomass to ethanol

The aim of the pretreatment step is to destroy lignin shell protecting cellulose and hemicellulose within the plant materials. The methods used for the pretreatment include 1. Chemical pretreatments by the use of compounds such as H_2SO_4, HCl, ammonia, or NaOH, 2. Physical pretreatment involving the use of steam explosion or liquid hot water 3. Biological pretreatment that involves the use of microorganisms such as white rot fungi, brown rot, Bacillus, Trichoderma, Aspergillus etc. The second step from **Fig 1** is the hydrolysis step which helps break down polysaccharides into monomers for effective fermentation and distillation. It involves two methods namely acid hydrolysis and enzymatic hydrolysis. Enzymatic hydrolysis is substrate specific, reacting directly with cellulose using enzymes such as cellulase, endo-β-gluconase, exo-β-gluconase or β-glucosidase [10]. Cellulase enzyme is the most common type of enzyme applied for hydrolysis purposes, and it can be derived from bacteria or fungi. Hydrolysis using dilute acid requires a high amount of temperature which is uneconomical since a significant amount of energy is required. Moreover, hydrolysis using concentrated acid requires a large volume to achieve an effective hydrolysis. The last step in **Fig 1** is the paramount fermentation step which helps convert sugars to ethanol using microorganisms. S. cerevisiae (yeast) is the most common microorganism employed for this process.

There are limitations to the production of bioethanol from lignocellulosic materials, these limitations range from the choice of pretreatment, hydrolysis, and fermentation technologies, to the economic and commercial scale application of the resource. Despite the presence of different pretreatment techniques, not all are suitable for different classes of lignocellulosic biomass. A steam explosion has been widely used for the pretreatment of agricultural related biomass residues e.g. sugarcane bagasse, rice straw or corn stover, but acid hydrolysis is otherwise required in addition for the treatment of wood to produce high ethanol content. Due to this drawback, research is needed to determine the pretreatment technique that will be cost effective, and at the same time efficient to achieve the desired goal [11] [12] [13].

Here, we tried using banana waste as a substrate for bioethanol production. We aimed to produce bioethanol from banana peels by the least expensive pretreatment technologies available i.e. hydrolysis, pulping using dilute sodium hydroxide, and acidic pretreatment using sulfuric acid. We selected banana waste due to its availability and accessibility. The composition of banana waste is given as lignin 20.31 %, holocellulose 57.76 %, and ash 13.63 %. Different pretreatment techniques were used for the experiment, however, only acid hydrolysis was used for the hydrolysis which was later followed by fermentation using S. *cerevisiae*. The sugar content obtained from banana peels was estimated using the Benedict's solution and the result indicated that banana peels are an excellent source of bioethanol though the yield was pretreatment dependent.

II. Experiment

2.1 Sample Collection

Lignocellulosic residues of banana peels were obtained from the student cafeteria at Usmanu Danfodiyo University Sokoto (UDUS) and the residues were used as the source of sugars for the bioethanol production. Sodium hydroxide, sulfuric acid, and yeast were used for the alkaline pretreatment, acid hydrolysis, and fermentation respectively.

2.2 Pretreatment

Pretreatment of lignocellulosic biomass prior to hydrolysis is a prerequisite for bioethanol production because it determines the yield of bioethanol that would be obtained after fermentation. The aim of pretreatment is to reduce the compactness, strength and crystalline nature of cellulose aiding in hydrolyzing the lignocellulosic biomass to simple sugar units. **The alkaline pretreatment** was carried out using electrically heated autoclave by the use of 10 % (wt/wt) NaOH and liquor to fiber ratio of 6:1. We cooked the fiber at 120 °C for six hours prior to the discharge of pressure into the atmosphere. The peels were washed with water and air-dried at 45 °C. We subjected the peels to **water pretreatment** as a pretreatment process in a closed autoclave. The banana waste was cooked at 120 ◦C using water to liquor ratio of 1:10 for six hours. The pressure was released into the atmosphere and the pulped fiber was washed with water and air-dried at 45 °C. For **acid pretreatment**, 40 g of banana sample was mixed with 200 mL of five percent H_2SO_4 and kept at 120 °C for six hours. The mixture was filtered to separate the solid residues from the filtrate fraction. The solid residues were thoroughly washed with tap water to neutral pH and dried at 45 °C. The setup we used for the pretreatment techniques is depicted in **Fig 2**.

Fig 2. The setup for the pretreatment techniques

2.3 Hydrolysis

The aim of hydrolysis is to further degrade the polysaccharides present in the pretreated lignocellulosic biomass of banana waste into monosaccharides subunits. The monosaccharides that will be produced upon hydrolysis will enhance the fermentation process by S. *cerevisiae*. This study used sulfuric acid due to its availability and ease of handling. 10 % sulfuric acid was prepared and mixed with the lignocellulosic biomass of banana waste produced from the various pretreatment processes. We used a sulfuric acid to fiber ratio of 6:1. The set up was heated at a temperature of 120 °C for six hours and allowed to cool. Glucose assay was carried out using Benedict's test to confirm the presence of reducing and non-reducing sugars prior to the fermentation process. There was a color change observed after hydrolysis and the intensity was pretreatment dependent. The image is given in **Fig 3**.

Fig 3. The color change observed after hydrolysis from different pretreatment techniques

2.4 Glucose Assay

Total reducing sugar produced from the hydrolysis of banana waste was estimated prior to the fermentation. Confirming the presence or absence of sugar prior to fermentation saves a considerable amount of time and effort. Benedict's test for reducing sugars was used to determine the amount of sugars generated from

the pretreatment techniques. The principle behind Benedict's test is that reducing sugars convert blue copper sulfate present in the Benedict's reagent to red copper oxide precipitate. Samples from acid, alkaline and water pretreatments that were under hydrolysis were collected at 0 h, 24 h, 48 h, and 72 h. Color changes were monitored from the samples after adding a few drops of Benedict's solution and heating in a water bath for five minutes. The color change from Benedict's reaction gives a semi-quantitative or a rough estimate of the reducing sugars present within a sample. The amount of reducing sugar present in a sample can be quantified using the following color change: Blue (no sugar), Green (0.5 % sugar), Yellow (1 % sugar), Orange (1.5 % sugar), Red (2 % sugar), Brown (highest level of sugar).

2.5 Fermentation

Fermentation is the final stage of bioethanol production. We used S. *cerevisiae* to convert the monosaccharides and some disaccharides produced during hydrolysis into ethanol with the help of invertase and zymase enzymes present in S. *cerevisiae*. An activated yeast strain was obtained from the biology lab at UDUS. The S. *cerevisiae* cells were suspended in deionized water and the pretreated banana waste was used as the only carbon source for the yeast cells. Out of the six bottles used, three served as controls (deionized water plus banana peels but without yeast cells) while the remaining three were supplemented with both banana peels and the yeast cells in the deionized water. **Fig 4** illustrates the yeast cells prior to activation and the fermentation set up we applied. We allowed the fermentation process to continue for three days because S. *cerevisiae* grows in three days, and finally, the samples were centrifuged and bioethanol was analyzed from the filtrate using chromatography.

Fig 4. The activated yeast strain used for the fermentation experiment and the setup applied for the fermentation process. We had a blank for each pretreatment technique.

2.6 Ethanol Analysis

Ethanol analysis was carried out using the gas chromatography technique with a Varian 450-GC (Gas chromatography model CP-8400) equipped with flame ionization detector (FID). GC equipped with FID is the most common detector in gas chromatographic separation. It has several advantages over other detectors such as high sensitivity, wide linear range, the ability to detect most organic compounds, and a low noise level.

III. Result and discussion

The selection of a good pretreatment technique determines the success of every bioethanol production, so it is imperative to select the best pretreatment technique available [14]. It helps open up and disintegrate the biomass constituents into individual components i.e. cellulose, hemicellulose, and lignin. The breakdown helps achieve an efficient hydrolysis and fermentation [15] [16]. We used three different pretreatment techniques to process the banana peels namely water, alkaline, and acidic pretreatments using distilled water, sodium hydroxide and sulfuric acid respectively. We obtained a varying degree in color and biomass debris at the end of the pretreatment relative to the technique applied. The color change followed the sequence water pretreatment > alkaline pretreatment > acidic pretreatment. However, the debris generated by the acid pretreatment was the highest compared to the other pretreatment techniques. We attributed the high amount of debris generated by acid pretreatment largely to the heat applied during the process. We believe high temperature helped in breaking down the lignocellulosic biomass structure, however, we did not determine the composition of the biomass quantitatively. Further studies are therefore needed to help characterize the biomass to provide a useful insight into the structural composition of the polymer chains. We speculate that acid pretreatment will have a significant impact on the hydrolysis.

Hydrolysis is the second stage of processing bioethanol from lignocellulosic biomass. Two different methods are commonly employed during hydrolysis: acid hydrolysis and enzymatic hydrolysis. The sole aim of

hydrolysis is to convert lignocellulosic biomass constituents into accessible reducing sugars that will serve as substrates during fermentation by S. cerevisiae. This study used 10 % sulfuric acid for the hydrolysis; we autoclaved the mixtures for five hours at 120 ℃ and allowed the hydrolysis to proceed for three days. Samples were retrieved at 0 h, 24 h, 48 h and 72 h and we applied Benedict's test for reducing sugars to the samples to ascertain the presence of sugars prior to fermentation.

The result from the Benedict's test for reducing sugar is shown in **Fig 5** with bar charts having different colors signifying the level of sugars after each day of sampling. Benedict's reagent contains blue copper (II) ions which are reduced to copper (I) ions in the presence of sugars. The ions will be precipitated as a red copper (I) oxide which is insoluble in water. The scale of colors starts with blue signifying (no sugars) < green (0.5 % sugars) < yellow (1 % sugar) < orange (1.5 % sugar) < red (2 % sugar) < brown signifying highest level of sugars. The result showed that the reducing sugar concentration varies dramatically with the pretreatment technique and the number of hours used for the hydrolysis. The concentration of reducing sugar obtained from the pretreatment techniques applied followed the order acid pretreatment > alkaline pretreatment > water pretreatment. The hydrolysis time also played a significant role in the production of reducing sugars, the sequence followed the order 0 h < 24 h < 48 < 72 h.

3.1 Glucose concentration obtained from water pretreatment hydrolysis

The blue bars in **Fig 5** indicate the amount of reducing sugars obtained from hydrolysis of water pretreated samples as a function of time. The samples tested at 0 h prior to the start of hydrolysis showed a pale blue color signifying little or no sugar from the sample or (0.1 % of the sugars). After 24 h of hydrolysis, a sample was retrieved to confirm the amount of reducing sugar by Benedict's reagent and a pale greenish-yellow color was observed indicating a little amount of reducing sugar present (0.3 % of the total sugars). At exactly 48 h of hydrolysis, we retrieved a sample and tested further, a pale yellow color was observed signifying a little improvement from the previous results (0.8 %). The last sample was evaluated after three days of hydrolysis and a pale-orange color was observed indicating the presence of reducing sugars (1 % of the total sugars). In general, the amount of reducing sugars produced by this pretreatment was, therefore, minimal.

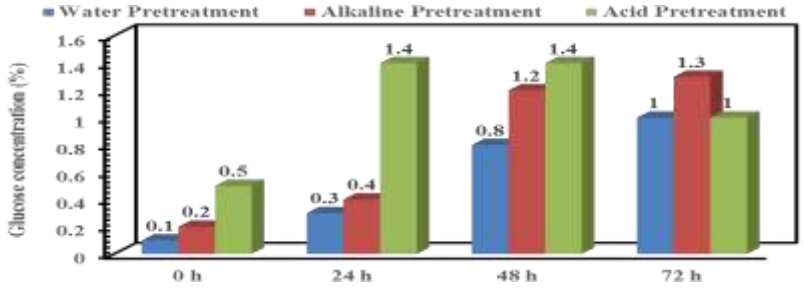

Figure 5: The amount of reducing sugars produced from different pretreatment techniques between 0 – 72 h

3.2 Glucose concentration from alkaline pretreatment

The brown bars in **Fig 5** above indicate the amount of reducing sugars obtained after the hydrolysis of the alkaline pretreated samples. The sample tested at 0 h of hydrolysis showed a pale blue coloration which signifies the absence of the reducing sugars (0.2 % from the rating scale). The sample tested at 24 h showed a pale green coloration signifying the presence of little sugar from the sample (0.4 %) while at 48 h of sampling the concentration increased compared to the previous samples tested to (1.2 % of the total sugars). The last sample tested after three days showed a dark orange coloration indicating the highest concentration of the reducing sugars from the pretreatment (1.3 % of the total sugars).

3.3 Glucose concentration from acid pretreatment hydrolysis

The green bars in **Fig 5** above indicate the amount of reducing sugars obtained from the hydrolysis of the acid pretreated samples. The sample taken at 0 h of hydrolysis showed a little amount of sugar (pale green color or 0.5 % from the rating scale) in contrast to the other pretreatments that showed little or no sugar at all at 0 h of sampling. The concentration of reducing sugar increased significantly after 24 h of sampling which showed a dark coloration (orange-red coloration or 1.4 % of the total sugars). We obtained the highest amount of sugar after 48 h of sampling, the Benedict's test during that time showed an orange to red coloration signifying a high amount of sugar in the sample (1.4 %). However, after 72 h of hydrolysis, the result showed a dramatic change, instead of the sugar concentration to increase, it decreased from the observed coloration (yellow-orange or 1 % of the total sugars). We attributed this drop largely to the accumulation of other metabolites unknown to us which might have interfered with the production of reducing sugars. In general, we obtained the highest concentration of reducing sugars after hydrolysis from the acid pretreated samples.

3.4 Ethanol Analysis

S. *cerevisiae* is the main organism used for the production of ethanol from a variety of substrates. It produces ethanol in a large quantity and has the advantage over other organisms of resisting multiple inhibitors such as furans, phenolic compounds and organic acids [17]. The result obtained from the glucose analysis showed a great promise of producing bioethanol from all the pretreatment techniques employed. The result of the ethanol analysis is presented in **Fig 6**. The chromatogram obtained from GC-FID confirmed the presence of bioethanol in all the pretreatment techniques. The blue, pink, and green chromatograms represent alkaline, water, and acid pretreatments respectively.

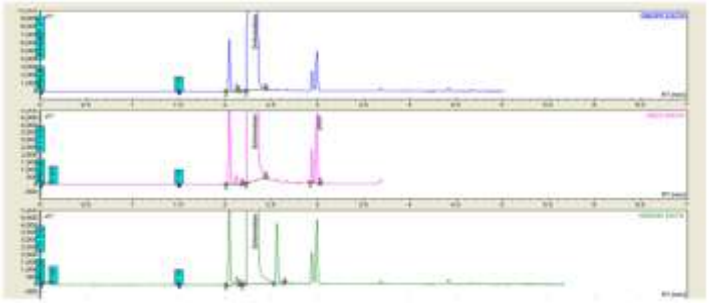

Fig 6. GC-FID chromatogram showing the concentration of ethanol obtained from various pretreatment techniques. The blue, pink and green chromatograms represent alkaline, water, and acid pretreatments respectively.

The fermentation step lasted for three days and the result obtained by water pretreatment gave the least bioethanol concentration (40 ppm) followed by the acid pretreatment that produced the highest glucose concentration during hydrolysis, however, it yielded a 60 ppm ethanol concentration. Lastly, alkaline pretreatment yielded an ethanol concentration of 80 ppm. The result indicates that obtaining a high amount of sugar after hydrolysis would not guarantee high ethanol production after fermentation. We attributed this trend largely due to the accumulation of other toxic compounds or secondary metabolites such as phenolic compounds, organic acids, and furans that were produced during the fermentation of the acid pretreated sample. These toxic compounds may have induced the death phase of S. *cerevisiae* early during the microbial growth. Additionally, our results have confirmed that production of bioethanol is pretreatment dependent. A bar chart showing the amount of ethanol produced from the banana peels by the various pretreatment techniques is given in **Fig. 7.**

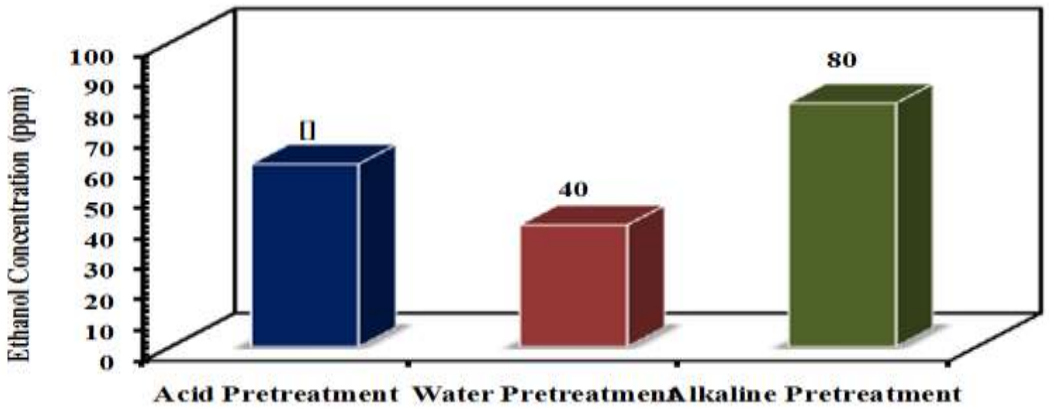

Figure 7: A bar chart indicating the amount of ethanol produced from banana peels by different pretreatment techniques.

IV. Conclusion

This study investigated the feasibility of producing bioethanol from banana peels as a source of lignocellulosic biomass. Three different pretreatment techniques were employed as the first step in the experimental design. The pretreatment techniques were water, alkaline, and acidic pretreatments. The techniques produced different concentrations of reducing sugar after hydrolysis with sulfuric acid. Pretreatment with acid produced the highest reducing sugar concentration followed by alkaline pretreatment and finally water pretreatment as confirmed by Benedict's test. However, alkaline pretreatment achieved the best conversion of reducing sugars to ethanol. The result clearly indicates that each pretreatment technique has its inherent advantages. In general, pretreatment using water alone without chemical enhancement yielded low result from

both the glucose and ethanol experiments. The best method to produce ethanol from banana waste is to employ alkaline pretreatment followed by acid hydrolysis and then carry out the subsequent fermentation with S. *cerevisiae* as e have confirmed in this study. In general, other enzymatic techniques should be employed for the hydrolysis because acid hydrolysis is rather highly expensive. More specific methods to maximize the yield of bioethanol are therefore required.

References

[1] T. H. Kim, F. Taylor, and K. B. Hicks, Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment, Bioresour. Technol., 99(13), 2008, 5694–702.

[2] W. K. El-Zawawy, M. M. Ibrahim, Y. R. Abdel-Fattah, N. A. Soliman, and M. M. Mahmoud, Acid and enzyme hydrolysis to convert pretreated lignocellulosic materials into glucose for ethanol production, Carbohydr. Polym., 84(3), 2011, 865–871.

[3] S. Sakai, Y. Tsuchida, H. Nakamoto, S. Okino, O. Ichihashi, H. Kawaguchi, T. Watanabe, M. Inui, and H. Yukawa, Effect of lignocellulose-derived inhibitors on growth of and ethanol production by growth-arrested Corynebacterium glutamicum R., Appl. Environ. Microbiol., 73(7), 2007, 2349–53.

[4] N. Curreli, M. B. Fadda, A. Rescigno, A. C. Rinaldi, G. Soddu, F. Sollai, S. Vaccargiu, E. Sanjust, A. Rinaldi, C. Biologica, U. Cagliari, and V. Pineta, Mild alkaline / oxidative pretreatment of wheat straw, Process Biochemoistry, 32(8), 1997, 665–670.

[5] B. Yang and C. E. Wyman, Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn stover cellulose., Biotechnol. Bioeng., 86(1), 2004, 88–95.

[6] K. A. Gray, L. Zhao, and M. Emptage, Bioethanol., Curr. Opin. Chem. Biol., 10(2), 2006, 141–146.

[7] A. E. Farrell, R. J. Plevin, B. T. Turner, A. D. Jones, M. O. Hare, and D. M. Kammen, Ethanol can contribute to energy and environmental goals, Science, 311(5760), 2006, 506-508.

[8] C. E. Wyman, Potential synergies and challenges in refining cellulosic biomass to fuel, chemicals, and power, Biotechnol. Prog. 19, 2003, 254-262.

[9] A. Endo, T. Nakamura, A. Ando, K. Tokuyasu, and J. Shima, Genome-wide screening of the genes required for tolerance to vanillin, which is a potential inhibitor of bioethanol fermentation, in Saccharomyces cerevisiae, Biotechnol. Biofuels, 1(1), 2008, 3

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

[11] A. T. W. M. Hendriks and G. Zeeman, Pretreatments to enhance the digestibility of lignocellulosic biomass, Bioresour. Technol., 100(1), 2009, 10–18.

[12] C. A. Cardona, J. A Quintero, and I. C. Paz, Production of bioethanol from sugarcane bagasse: Status and perspectives, Bioresour. Technol., 101(13), 2010, 4754–66.

[13] N. Mosier, C. Wyman, B. Dale, R. Elander, Y. Y. Lee, M. Holtzapple, and M. Ladisch, Features of promising technologies for pretreatment of lignocellulosic biomass, Bioresour. Technol., 96(6), 2005, 673–86.

[14] P. Alvira, E. Tomás-Pejó, M. Ballesteros, and M. J. Negro, Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review., Bioresour. Technol, 101(13), 2010, 4851–61.

[15] M. Chen, J. Zhao, and L. Xia, Comparison of four different chemical pretreatments of corn stover for enhancing enzymatic digestibility, Biomass and Bioenergy, 33(10), 2009, 1381–1385.

[16] L. Da Costa Sousa, S. P. S. Chundawat, V. Balan, and B. E. Dale, "'Cradle-to-grave' assessment of existing lignocellulose pretreatment technologies.," Curr. Opin. Biotechnol., 20(3), 2009, 339–47.

[17] B. Hahn-Hägerdal, K. Karhumaa, C. Fonseca, I. Spencer-Martins, and M. F. Gorwa-Grauslund, "Towards industrial pentose-fermenting yeast strains.," Appl. Microbiol. Biotechnol., 74 (5), 2007, 937–53.