Jour. Korean For. Soc. Vol. 99, No. 5, pp. 744~749 (2010)

JOURNAL OF KOREAN FOREST SOCIETY

Enzymatic Hydrolysate from Non-pretreated Biomass of Yellow Poplar (*Liriodendron tulipifera*) is an Alternative Resource for Bioethanol Production

Ji Young Jung[1], Myung Suk Choi[1], Ji Su Kim[1], Mi Jin Jeong[1], Young Wun Kim[2], Byeng Tae Woon[2], Jin Ki Yeo[3], Han Na Shin[3], Young Bon Goo[3], Keun Ok Ryu[3] Chandrakant S. Karigar[4] and Jae Kyung Yang[1]*

[1]*Division of Environmental Forest Science, Gyeongsang National University, Jinju, 660-701, Korea*
[2]*Korea Research Institute of Chemical Technology, Daejeon 305-600, Korea*
[3]*Departments of Forest Resources Development, Korea Forest Research Institute, Suwon 441-847, Korea*
[4]*Department of Biochemistry, Bangalore University, Bangalore, 560001, India*

Abstract : Enzymatic hydrolysate from non pre-treated biomass of yellow poplar (*Liriodendron tulipifera*) was prepared and used as resource for bioethanol production. Fresh branch (1 year old) of yellow poplar biomass was found to be a good resource for achieving high saccharification yields and bioethanol production. Chemical composition of yellow poplar varied significantly depending upon age of tree. Cellulose content in fresh branch and log (12 years old) of yellow poplar was 44.7 and 46.7% respectively. Enzymatic hydrolysis of raw biomass was carried out with commercial enzymes. Fresh branch of yellow poplar hydrolyzed more easily than log of yellow poplar tree. After 72 h of enzyme treatment the glucose concentration from Fresh branch of yellow poplar was 1.46 g/L and for the same treatment period log of yellow poplar produced 1.23 g/L of glucose. *Saccharomyces cerevisiae* KCTC 7296 fermented the enzyme hydrolysate to ethanol, however ethanol production was similar (~1.4 g/L) from both fresh branch and log yellow poplar hydrolysates after 96 h.

Key words **:** *yellow poplar, non-pretreatment, enzymatic hydrolysate, bioethanol, fermentation*

Introduction

Bioethanol derived from plant biomass is one of many renewable energy alternatives to fossil fuels. As a substitute for gasoline bioethanol has great potential since the distribution system for liquid fuel already exists (Sorensen *et al*., 2007).

Lignocellulosic biomass serves as a cheap and abundant feedstock, for the production of bioethanol at reasonable costs. Inexpensive waste products from the forestry industry as well as from agricultural residues can be utilized as raw materials (Najafi *et al*., 2009). Extensive research has been directed towards the conversion of lignocellulosic biomass to ethanol in the past two decades (Dale *et al*., 1984; Cadoche and Lopez, 1989; Reshamwala *et al*., 1995; Bjerre *et al*., 1996; Duff and Murray, 1996; Wright, 1998).

The sugar component from hemicelluloses can be fermented into ethanol after saccharification of the biomass by hydrolysis. The presence of lignin and hemicelluloses generally hinders the access of hemicelluloses enzymes to cellulose, thereby reducing the efficiency of the hydrolysis. However, the cost of ethanol production from lignocellulosic biomass is relatively high with current technologies. The presently employed hydrolytic processes including enzymatic pretreatments still leave room for improvement of efficiency and economy. This is because these processes usually require the use of chemicals such as sulfuric acid and ammonia that enhance the cost of the process. In addition these chemicals also require a neutralization or recovery step to reduce the loads on the environment. Hence, currently a new pretreatment technology is needed for an efficient and economical ethanol production from lignocellulosic biomass.

Important energy related characteristics of woody biomass feedstock include: bark quality, moisture content, specific gravity (density), amount of extractives, and inorganic elements including alkali metals, ash/residue and cellulose/lignin ratio (Kenney *et al*, 1990). These characteristics are influenced by several factors including changes in the cambium as it ages, genetic controls and

*Corresponding author
E-mail: jkyang@gnu.ac.kr

environmental factors. Plant age is an important factor that influences the wood composition like variations in lignin, hemicelluloses, cellulose and other chemicals of the biomass (Berrocal *et al*., 2004) and therefore the efficiency of hydrolysis.

Yellow poplar (*Liriodendron tulipifera*) has been the main alternative regarding the utilization of short-rotation forest for the production of bioenergy like heat and power. Over the years, more yellow poplar varieties with considerably higher productivities have been developed through breeding, selection, and plantation management (Yoo *et al*., 2003). Because of its rapid growth at preferred sites, yellow poplar may have an additional potential as a source of fiber for biologically based products, biofuels, and chemicals (Nagle *et al*. 2002) in the future. In this study, we have evaluated the potential of fast growing yellow poplar as a bioresource for ethanol production. We also, report the effects of enzymatic hydrolysis and ethanol production from yellow poplar raw materials.

Materials and Methods

1. Plant material

Yellow poplar (*L. tulipifera)* plant was collected from an experimental plot in Korea Forest Research Institute, Seoul, Korea. The fast-growing woods were baled and stored at room temperature after harvest. The branches (1 year old) and logs (12 years old) were debarked, chipped and grounded with a Wiley mill. The poplar powder between 80-20 mesh size was collected for further use.

2. Chemical analysis of the plant material

Chemical composition of the biomass was determined according to the National Renewable Energy Laboratory (NREL Golden, CO) analytical methods for biomass (NREL, 1996). Carbohydrates, lignins (acid-insoluble, acid soluble), extractives and ash content of raw materials were determined according to NREL procedures (Sluiter *et al*., 2004). The structural carbohydrates and lignin, extractives and ash contents were also determined (Sluiter *et al*., 2005). The raw biomass was extracted with ethanol and the residue was subjected to carbohydrates and lignin determination based on monomer content measured after a two-step acid hydrolysis procedure to fractionate the fiber. A first step with 72% (w/w) H_2SO_4 at 30°C for 60 min was used. In a second step, the reaction mixture was diluted to 4% (w/w) H_2SO_4 and autoclaved at 121°C for 1 h. This hydrolysis liquid was then analyzed for sugar content by HPLC. The remaining acid-insoluble residue was considered as acid-insoluble lignin.

3. Enzymatic hydrolysis

The enzymes used for biomass hydrolysis were Celluclast 1.5® L (Novo Co., Denmark), cellulase from *Trichoderma reesei*, and Viscozyme® L (Novo Co., Denmark) as β-glucosidase. One gram (1.0 g) of biomass was transferred to a 250 mL Erlenmeyer flask containing 50 mL of 0.1 M citrate buffer (pH 4.8). To this appropriate aliquots of cellulase (65 FPU/g) and β-glucosidase (24 CBU/g) were added. Later the flask was placed on a shaking incubator (IS-97IR, Jeio-Tech Co., Korea) maintained at 50°C and 150 rpm for 96 h. Samples were withdrawn after 0, 24, 48, 72 and 96 h to monitor the progress of hydrolysis. The sugar was analyzed in supernatants obtained after denaturing of enzymes by heating the aliquots to 100°C for 10 min immediately after withdrawing from the reaction flask.

4. Ethanol fermentation

Yeast, *Saccharomyces cerevisiae* KCTC 7296, was used throughout in fermentation study. Inoculum of *S. cerevisiae* was prepared by transferring the yeast maintained on GPYA medium (glucose, 40 g/L peptone, 5 g/L yeast extract, 5 g/L agar, 15 g/L) into 100 mL flask. The growth was carried out at 35°C on an orbital shaker for 24 h. An inoculum containing about 1.5×10^8 cells/mL was seeded at 5% (v/v) of SHF medium.

Fermentation ability tests were performed for hydrolysates using, *S. cerevisiae* KCTC 7296, which ferments glucose and mannose, but not xylose or other pentoses. The pH of the medium was adjusted to 5.5 with 20% (w/w) $Ca(OH)_2$. Fermentation was carried out in 100 mL flasks with containing 20 mL medium consisting of 18.5 mL hydrolysate, 0.5 mL nutrients, and 1 mL inoculum (yeast) (Taherzadeh *et al*., 1996). The flasks were sealed with rubber stoppers pierced with hypodermic needles for removal of the CO_2 produced, as well as for withdrawal of samples. The concentration of fermentable sugars was adjusted by the addition of glucose to a total concentration to 5 g/L to avoid the influence of variation in sugar concentrations between filtrates. A reference solution was prepared with 5 g/L glucose to serve as control fermentation. The fermentation flasks were incubated at 35°C for 96 h, and the samples for monitoring the progress were withdrawn at regular intervals to quantify ethanol and sugars.

5. Analysis of sugars and ethanol

Enzymatic hydrolysate was analyzed for monomeric sugar composition with a HPLC (Shimadzu, Kyoto, Japan) fitted with a refractive-index detector (Shimadzu). The column used for the separation of the sugars glucose, xylose, galactose, arabinose, and mannose was an Aminex HPX-87P (Bio-Rad, Hercules, CA, USA) operated at 85°C with water as an eluteant, at a flow rate of 0.6 mL/min. The ethanol in the fermented samples was determined

with HPLC using an Aminex HPX-87 H column (Bio-Rad) operated at 65°C, with 5 mM H_2SO_4 as eluent, at a flow rate of 0.6 mL/min (Sluiter and Hames, 2004). All samples were filtered through a 0.2 μm filter before analysis to remove coarse particles. All analytical determinations were performed in duplicate and average results are shown.

Results and Discussion

1. Chemical composition of yellow poplar

Chemical analysis of fresh branch yellow poplar showed significant differences in the chemical composition (Table 1). The cellulose portion of the fresh branch comprised of 44.7% glucose, and 14.2% xylose. The same branch showed 18.2% of hemicellulosic sugars with xylose (14.2%) as the main sugar, galactose (2.0%), arabinose (1.0%), and mannose (1.0%) were other additional available carbohydrates. Lignin content in this sample was 24.7%. Acid insoluble lignin accounted for 24.2%, however soluble lignin was 0.5%. Ash content was 2.1% and extractives were 9.6% of raw material.

The log yellow poplar Carbohydrate contained glucose (46.7%), and xylose (14.9%), galactose (2.1%), arabinose (1.1%), and mannose (1.1%) respectively. The total lignin content was 28.1%. Hemicellulosic sugars were 19.2% of the raw material with xylose as the main sugar (77.6%). Ash content of the log yellow polar was 1.2% and extractives were 3.3%.

Cellulose (as glucose) was higher in log yellow poplar log than fresh branch yellow poplar. Cellulose content is higher than that reported for other woods like eucalyptus, aspen and spruce (Ramos *et al*., 1992). Lignin content in Fresh yellow poplar was 24.7%, which included acid-insoluble lignin (0.5%) and soluble lignin (24.2%). Considering acid-soluble lignin content that refers to the small fraction of lignin that is solubalized during the hydrolysis process (NREL, 1994). Lignin content is comparable and/or higher than that reported for other woods and grasses like birch wood, spruce wood, pine wood, switchgrass (Hayn *et al*., 1993; Wiselogel *et al*., 1996). The accessibility of these sugars vary among plant species, but are often difficult to access due to the lignin seal in the lignocellulose structure. The factors that have been identified to affect the hydrolysis of cellulose include porosity (accessible surface area) of the waste materials, cellulose fiber crystallinity, and lignin and hemicellulose content (McMillan, 1994).

Content of extractives varied between fresh branch and log yellow poplars. The extractive contents may include non-structural components of biomass, like waxes, fats, tannins, sugars, some resins and colouring matters. Content of extractives in fresh branch yellow poplar was high compared to log yellow poplar. This result indicated that fresh branch yellow poplar is rich in non-structural components as fat, resin etc. In this study, a high value of 15.4% was found among the fresh branch and log yellow poplar biomass.

Table 1 Chemical composition of yellow poplar biomass.[a]

Composition	Biomass (%)	
	Fresh branch	Log
Extractives	9.6 ± 0.3[b]	3.3 ± 0.2
Cellulose as glucose	44.7 ± 0.2	46.7 ± 0.5
Hemicellulose as	18.2	19.2
Xylose	14.2 ± 0.3	14.9 ± 0.5
Galactose	2.0 ± 0.2	2.1 ± 0.2
Arabinose	1.0 ± 0.2	1.1 ± 0.1
Mannose	1.0 ± 0.1	1.1 ± 0.2
Acid insoluble lignin	24.2 ± 0.5	26.9 ± 0.4
Acid soluble lignin	0.5 ± 0.1	1.2 ± 0.2
Ash	2.1 ± 0.3	1.5 ± 0.1

[a]Data in the table are based on oven dry samples.
[b]Values are mean ± S.D of three separate experiments.

2. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out on non-delignified raw material of the fresh branch and log yellow poplar as the substrates (Table 2). The hydrolysate showed differences in carbohydrate composition among fresh branch and log yellow poplar biomass. The reducing sugars in fresh branch and log yellow poplars were 2.6 and 2.2 g/L respectively. Among reducing sugars, glucose was 1.46 and 1.23 g/L respectively in fresh branch and log yellow poplars.

The time course of enzymatic hydrolysis of fresh branch and log yellow poplar is shown (Figure 1). The hydrolysis rate was high for fresh branch yellow poplar than log yellow poplar. Saccharification of biomass was enhanced in direct relation to time. Initially the saccharification increased until 24 h of incubation thereafter leveling off. Among the two poplars old sample showed 24% digestibility in 72 h whereas the fresh branch yellow poplar was digested to the extent of 19%.

The concentration of free glucose significantly increased with hydrolysis time (Figure 2). The highest concentration of glucose reached 1.46 and 1.23 g/L after 72 h of enzymatic hydrolysis for fresh branch and log yellow poplar respectively. However, concentration of xylose was lower than that of glucose. Xylose did increase after a lag of 24 h with stable production after 24 h.

The observed higher rate of hydrolysis in case of fresh branch poplar than log yellow poplar may be due to various reasons. The low hydrolysability of biomass may arise from the high content of inert components (lignin, ash and

Table 2 Carbohydrate composition of the enzyme hydrolysate hydrolysis.*

Substrates**	Reducing sugar (g/L)	Glucose (g/L)	Xylose (g/L)	Galactose (g/L)	Arabinose (g/L)	Mannose (g/L)
Fresh branch	2.60	1.46	0.84	0.16	0.10	0.05
Log	2.20	1.23	0.71	0.14	0.08	0.04

*Digestibility at 96 h, Enzymatic hydrolysis conditions : pH 4.8, 0.1 M citrate buffer, Digestibility at 50°C and 150 rpm.
**The substrates were used non pretreated fresh branch and log yellow poplar

the like). After enzymatic hydrolysis, glucose in fresh branch and log yellow poplar was 1.46 and 1.23 g/L respectively. In this study, hydrolysis rate in yellow poplar was low compared to other species. However, these values represent higher than those obtained with pretreatment that use chemicals such as sulfuric acid and ammonia.

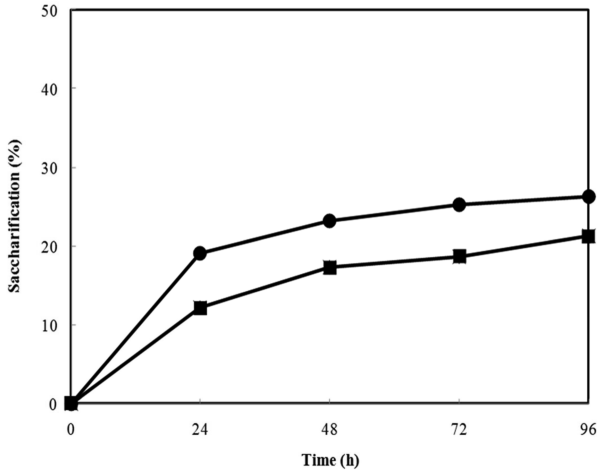

Figure 1. Time course of saccharification of non pretreated fresh branch and log yellow poplar. Saccharification in fresh branch (●) and old log of yellow poplar (■). Enzymatic hydrolysis conditions: 72 h, Cellulase (65 FPU/g), β-glucosidase (24 CBU/g), pH 4.8-5.0, 50°C, 120 rpm.

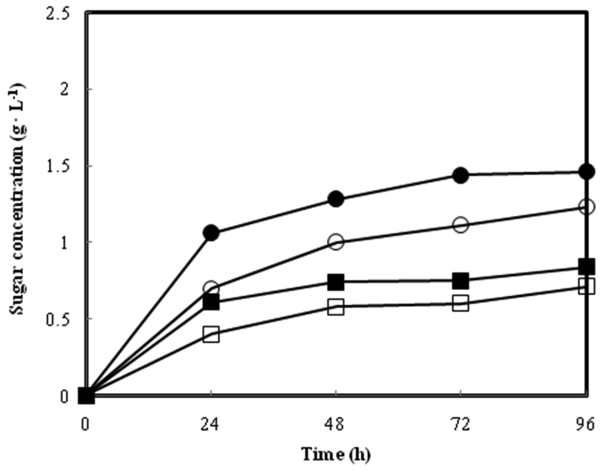

Figure 2. Concentration of glucose and xylose released by enzymatic hydrolysis from non pretreated fresh branch and log yellow poplar. glucose in fresh branch (●), glucose in old log (○), xylose fresh branch (■), xylose in old log of yellow poplar (□). Enzymatic hydrolysis conditions : 72 h, Cellulase (65 FPU/g), β-glucosidase (24 CBU/g), pH 4.8-5.0, 50°C, 120 rpm.

3. Ethanol production by fermentation

The time course of ethanol production using the enzymatic hydrolysates from fresh branch year and log yellow poplar are shown (Figure 3). Correlation between ethanol production and reducing sugar content was high. However, ethanol production was inversely correlated with sugar consumption. The glucose was not detected after 24 h of fermentation suggesting that any glucose released then was directly converted into ethanol.

The ethanol productivity was similar whether the substrates were derived from fresh branch or log yellow poplar (Figure 4), ethanol production reaching 50 and 52.6%, respectively.

The time course of ethanol production showed similar

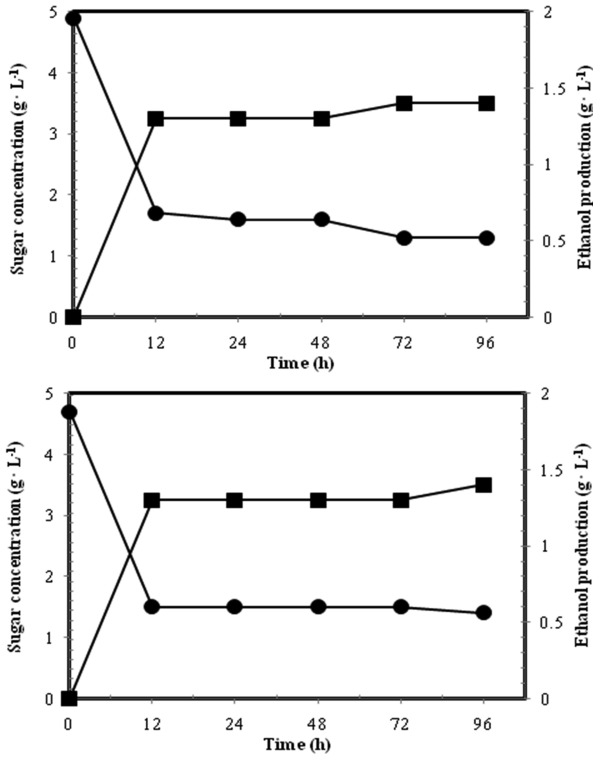

Figure 3. Ethanol production and sugar consumption pattern on *S. cerevisiae* fermentation using hydrolysates of non pretreated fresh branch (upper) old log (buttom) yellow poplar. Sugar consumption (●), ethanol production (■).

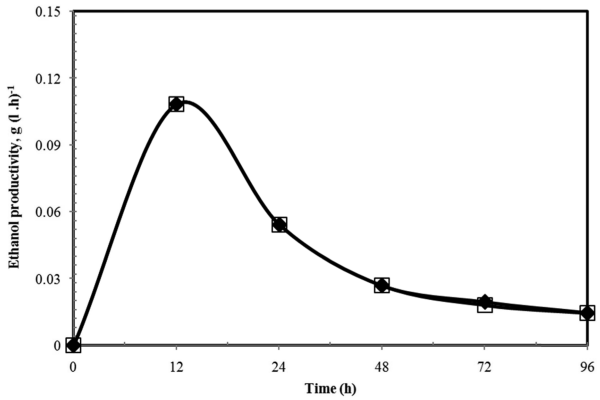

Figure 4. Ethanol productivity vs. time in separate hydrolysis and fermentation process using yellow poplar. Fresh branch (●) and old log (□) of yellow poplar.

Table 3. Ethanol yields in from fresh branch and log yellow poplar hydrolysates.

Substrates*	Ethanol yield, %
fresh branch	50.4
Log	52.6

*The substrates used were non pretreated fresh branch and old log of yellow poplar.

pattern for both hydrolysates from yellow poplar (Figure 4). Production of ethanol drastically increased in the initial fermentation process which then decreased after 96 h. Production of ethanol increased and reached a maximum level after 12 h yielding 0.14 g/L of ethanol, however after 96 h ethanol production was same for both hydrolysates.

Difference in the chemical composition and degree of hydrolysis are due to cell wall structure, configuration and compositions. The structure, configuration and composition of cell walls vary depending on plant taxa, tissue, age and cell type, and also within each cell wall layer (Bothast *et al*., 2005; Ding *et al*., 2006).

Lignocellulosic biomass is of various types, from grass and soft plants to hardwood etc. Lognocellulose consists of cellulose, hemicellulose and lignin in various proportions depending on the type of biomass, its age and various conditions. Especially, plant age is very important factor with respect to bioethanol production. Of the variables investigated in this study the yellow poplar age proved to be an important factor affecting ethanol production. The production of ethanol and hydrolysis was influenced in different patterns by yellow poplar age. Studies focused on the harvest timing on bioethanol production must be performed in the future, in order to clarify the roles of factors such as fertilization, genotype and environmental conditions involved in biomass production. We found that the bioethanol production is possible by combining non-preteatments using early grown biomass with a low cost pretreatment such as hydrothermal hydrolysis.

Conclusion

Enzyme processing of yellow poplar biomass for providing new substrates for bioethanol production has been investigated. These results indicated two concerns in connection with bioethanol production. The enzymatic hydrolysis of biomass depends on plant age and chemical treatments. Also for biomass to function as substrate for bioethanol production must be low in lignin, and high in easily fermentable hexose sugars. The study indicated that fresh branch tree of yellow poplar was found to be a good biomass resource to achieve a high saccharification yield and bioethanol production. This study represents that delignification of raw material can be advantageous for ethanol production with cost and environmental benefits. These results will thus contribute to the design of developed process for an industrial development.

Acknowledgements

This work was partially supported by the Korea Forest Research Institute Grant.

Literature Cited

1. Berrocal, J., Baeza, J., Rodriguez, M., Espinosa J. and Freer, J. 2004. Effect of tree age on variation of *Pinus radiata* D. Don chemical composition. Journal of the Chilean Chemical Society 49: 251-256.
2. Bjerre, A.B., Olesen, A.B. and Fernqvist, T. 1996. Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose. Biotechnology and Bioengineering 49: 568-577.
3. Bothast, R.J. and Schlicher, M.A. 2005. Biotechnological processes for conversion of corn into ethanol. Applied Microbiology and Biotechnolgy 67: 19-25.
4. Cadoche, L. and Lopez, G.D. 1989. Assessment of size reduction as a preliminary step in the production of ethanol from lignocellulosic wastes. Biology Wastes 30: 153-157.
5. Dale, B.E., Henk, L.L. and Shiang, M. 1984. Fermentation of lignocellulosic materials treated by ammonia freeze-explosion. Developments in Industrial Microbiology 26: 223-233.
6. Ding, S.Y. and Himmel, M.E. 2006. The maize primary cell wall microfibril: a new model derived from direct visualization. Journal Agricultural and Food Chemistry

54: 597-606.

7. Duff, S.J.B. and Murray, W.D. 1996. Bioconversion of forest products industry waste cellulosics to fuel ethanol: a review. Bioresource Technology 55: 1-33.
8. Hayn, M., Steiner, W., Klinger, R., Steinmüller, H., Sinner, M. and Esterbauer, H. 1993. Basic Research and Pilot Studies on the Enyzmatic Conversion of Lignocellulosics. pp. 33-72. In: Saddler, J.N. (Ed.), Bioconversion of Forest and Agricultural Plant Residues. CAB International, Wallingford, UK.
9. Kenney, W.A., Sennerby-Forsse, L. and Layton, P. 1990. A review of biomass quality research relevant to the use of y reseaand willow for energy conversion. Biomass 21: 163-88.
10. McMillan, J.D. 1994. Pretreatment of lignocellulosic biomass. pp. 292-324. In: Himmel, M.E., Baker, J.O. and Overend, R.P. (Eds.), Enzymatic Conversion of Biomass for Fuels Production. American Chemical Society. Washington. DC.
11. Nagle, N.J., Elander, R.T., Newman, M.M., Rohrback, B.T., Ruiz, R.O. and Torget, R.W. 2002. Efficacy of a hot washing process for pretreated yellow-poplar to enhance bioethanol production. Biotechnology Progress 18: 734-738.
12. Najafi, G., Ghobadian, B., Tavakoli, T. and Yusaf, T. 2009. Potential of bioethanol production from agricultural wastes in Iran. Renewable and Sustainable Energy Reviews 13: 1418-1427.
13. National Renewable Energy Laboratory (NREL). Chemical Analysis and Testing Laboratory Analytical Procedures: LAP-002 (1996). LAP-003 (1995). LAP-004 (1996). LAP-005 (1994). LAP-010 (1994) and LAP 017 (1998). NREL, Golden, CO, USA.
14. Reshamwala, S., Shawky, B.T. and Dale, B.E. 1995. Ethanol production from enzymatic hydrolysates of AFEX-treated coastal Bermuda grass and switchgrass. Applied Biochemistry and Biotechnology 51/52: 43-55.
15. Ramos L. P., Breuil, C. and Saddler, J.N. 1992. Comparison of steam pretreatment of eucalyptus, aspen, and spruce wood chips and their enzymatic hydrolysis. Applied Biochemistry and Biotechnology 34/35: 37-48.
16. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J. and Templeton, D. 2004. In: NREL laboratory analytical procedures: determination of structural carbohydrates and lignin in biomass. National renewable energy laboratory. Boulevard, Golden, Colorado.
17. Sørensen, A., Teller, P.J., Hilstrom, T. and Ahring, B.K. 2008. Hydrolysis of *Miscanthus* for bioethanol production using dilute acid presoaking combined with wet explosion pre-treatment and enzymatic treatment. Bioresource Technology 99: 6602-607.
18. Taherzadeh, M.J., Liden, G., Gustafsson, L. and Niklasson, C. 1996. The effects of pantothenate deficiency and acetate addition on anaerobic batch fermentation of glucose by Saccharomyces cerevisiae. Applied Microbiology and Biotechnology 46: 176-82.
19. Wright, J.D., 1998. Ethanol from biomass by enzymatic hydrolysis. Chemical Engineering Progress 84: 62-74.
20. Wiselogel, A., Tyson, S. and Johnson, D. 1996. Biomass Feedstock Resources and Composition. pp. 105-118. In: Wyman CE, (Ed.) Handbook on Bioethanol: Production and Utilization (Applied Energy Technology Series), CRC press, Washington DC.
21. Yoo, K.O. and Kim, H.E. 2003. Development of techniques and handling for seedling production of yellow poplar (*Liriodendron tulipifera*). Journal of Korean Forestry Society 92: 236-245.

(Received September 8, 2010; Accepted October 25, 2010)