

AN UPDATE ON SULFITE PRETREATMENT (SPORL) OF LIGNOCELLULOSIC BIOMASS FOR EFFECTIVE PRODUCTION OF CELLULOSE ETHANOL

Xuejun Pan^{1*}, and Junyong Zhu^{1,2}

4. Department of Biological Systems Engineering, University of Wisconsin-Madison, 460 Henry Mall, Madison, WI 53706, USA;

5. USDA Forest Service, Forest Products Laboratory, Madison, WI 53726, USA.

*Corresponding author: xpan@wisc.edu

ABSTRACT

SPORL (Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose) has been demonstrated as an effective and robust pretreatment technology for ethanol production from lignocellulosic biomass, in particular woody biomass^[1-7]. The process consists of a short chemical treatment of feedstock with sulfite followed by mechanical size reduction (fiberization) with disk mill. Studies indicated that SPORL process is applicable to all types of feedstocks and directly deals with wood chips with regular size, and no extensive size reduction is required prior to the pretreatment. SPORL produced a readily digestible cellulose substrate because of the removal of hemicellulose and lignin, partial sulfonation of lignin (increased hydrophilicity and therefore reduced hydrophobic interaction with enzymes), depolymerization (prehydrolysis) of cellulose, and size reduction (increased surface area). The process has a high recovery yield of fermentable sugars with limited formation of fermentation inhibitors, which facilitates the fermentation of dissolved hemicellulosic sugars in pretreatment liquor. Lignin was partially dissolved in form of lignosulfonate with great potential for co-product development. In addition, the SPORL can directly adapt existing infrastructure and equipment in paper industry, which reduce the cost for equipment R&D and the risk of scale-up. This paper provides an update on our research in SPORL process.

Keywords: pretreatment, sulfite, SPORL, enzymatic hydrolysis, lignocellulosic biomass

INTRODUCTION

A typical bioconversion of lignocellulosic biomass to fuel ethanol consists of three major steps: feedstock pretreatment to remove the recalcitrance of the biomass to enzymes, enzymatic saccharification of cellulose to

glucose, and the fermentation of the glucose to ethanol. Among the three operations, the pretreatment is the most critical and also the most cost- and energy-intensive one, which not only directly affects the enzymatic digestibility of the resultant substrate, but also impacts the recovery of hemicellulose sugars, potential of lignin utilization, and downstream wastewater treatment. The pretreatment is a cannot-skip operation, which is one of the major barriers retarding the commercialization of cellulosic ethanol.

An ideal pretreatment method should have following features: applicable to all types of biomass from easy agricultural residues to tough softwood, requiring minimum size reduction, producing readily digestible cellulosic substrate, maximizing overall sugar recovery, minimizing the formation of fermentation inhibitors, providing high potential of coproducts from lignin and/or hemicellulose, and more importantly, having good scalability for commercialization. In the last several decades, research and development efforts have made significant progress in pretreatment technologies for lignocellulosic feedstocks^[8-12]. Many pretreatment technologies, such as lime, dilute acid, ammonia, hot water, steam explosion, and organosolv pretreatments, have achieved varying levels of success, but they still have technical and economical barriers to overcome before commercialization^[8, 13-20].

ABOUT SPORL PRETREATMENT

SPORL refers to Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose. It is a pretreatment technology of lignocellulosic biomass for producing cellulose ethanol, recently developed at the University of Wisconsin-Madison and the USDA Forest Service, Forest Products Laboratory^[1, 5]. As shown in Fig. 1, the SPORL process consists of two steps, chemical treatment of feedstock using sulfite to remove the recalcitrance of hemicellulose and lignin followed by a mechanical size reduction to increase access surface area of the substrate. In the process, wood chips first react with a solution of sodium (or other bases like calcium and magnesium) sulfite at 160~190 °C and pH 2~5 for about 10~30 min. The pretreatment liquor to wood ratio can be as low as 2~3, which ensures relatively concentrated hemicellulosic sugar stream and low thermal energy consumption for heating. The pretreated wood chips, after separation from pretreatment liquor by filtration, are then fiberized through mechanical milling using a disk refiner to generate fibrous substrate for subsequent enzymatic saccharification and fermentation. The dissolved hemicellulosic sugars (a mixture of hexoses and pentoses) in the pretreatment liquor are fermentable because of limited formation of fermentation inhibitors during the pretreatment. Our studies indicated the SPORL pretreatment liquor is easier to ferment than spent sulfite liquor due to high sugar and low lignosulfonate concentrations^[2, 21]. The dissolved

lignin (lignosulfonate) can be recovered and directly marketed as a co-product, such as dispersants in the existing market. If necessary, pretreatment chemicals, including sulfite and metal base (sodium or magnesium or calcium) in the pretreatment liquor, can be recovered using existing technologies in paper industry. In addition, the SPORL technique can be integrated with steam explosion pretreatment by using sulfite and acid as catalysts. In this case, the mechanical size-reduction step of disk-milling is not needed since the “explosion” can achieve the size reduction.

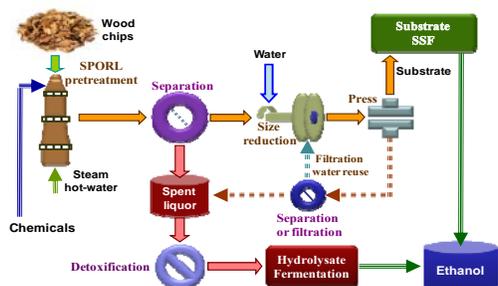


Fig. 1. Flowchart of SPORL pretreatment

Table 1. Mass balance of spruce SPORL pretreatment

	SPORL	Dilute acid
Unpretreated spruce /g	100	100
Glucose	46.7	46.7
Galactose	2.6	2.6
Mannose	10.8	10.8
Arabinose	1.2	1.2
Xylose	5.5	5.5
Substrate /g	60.5	64.1
Glucose	40.3	33.3
Mannose	7.1	--
Pretreatment liquor/g		
Glucose	2.9	3.0
Galactose	1.3	0.4
Mannose	4.5	0.9
Arabinose	0.4	0.1
Xylose	2.2	0.2
Inhibitor in liquor /(g/L)		
Acid-soluble lignin	16.6	4.8
Formic acid	1.9	7.4
Acetic acid	2.7	5.3
Furfural	1.3	2.9
Hydroxymethylfurfural	2.0	4.7
Levulinic acid	3.2	11.4

Note: pretreatment conditions, sulfuric acid 5% on wood, sulfite 9% (0% for dilute acid pretreatment) on wood, 180 °C for 30 min at 5 liquor to wood ratio.

The removal of the strong recalcitrance of the biomass through SPORL is achieved by the combined effects of dissolution of hemicelluloses, depolymerization of cellulose, partial delignification, partial sulfonation of lignin, and increased surface area by fiberization using disk-mill^[2, 5]. The dissolution of hemicellulose and lignin removed the physical barriers to enzymes, and pores generated after the removal of hemicellulose and lignin generated greater surface area for enzymes.

Depolymerization of cellulose at low pH value and high temperature shortened cellulose chains and generated more ends for cellobiohydrolases, making cellulose hydrolysis faster. Lignin sulfonation increased the hydrophilicity of SPORL pretreated substrates and reduced non-productive hydrophobic adsorption of enzymes on lignin. For example, cellulose-to-glucose conversion over 90% can be easily achieved within 24 to 48 h with enzyme loadings of 15 and 7.5 FPU/g substrate for softwoods and hardwoods, respectively. The arrangement of sulfite treatment followed by disk-milling significantly reduced mechanical energy consumption for size reduction, as low as 20 Wh/kg (<0.1 MJ/kg)^[22]. Furthermore, SPORL produced lower amounts of fermentation inhibitors, such as acetic acid, hydroxymethylfurfural (HMF), and furfural than dilute acid due to a higher pretreatment pH caused by sulfite buffering, when compared with dilute acid pretreatment^[1,2,5].

MASS BALANCE OF SPORL PRETREATMENT

An ideal pretreatment is not only able to produce a readily digestible substrate, but also to maximally recover all available sugars in fermentable form with limited formation of inhibitors. Our studies indicated that the SPORL was an effective and robust pretreatment and also achieved high total sugar recovery with reduced formation of fermentation inhibitors^[2, 3, 7]. For example, mass balance results from pretreatment of spruce with SPORL and dilute acid (DA) methods are summarized in Table 1. From 100 g of oven-dry spruce wood (containing 46.7 g glucose, 2.6 g galactose, 10.8 g mannose, 1.2 g arabinose, and 5.5 g xylose), 64.1 g (containing 33.3 g glucose) and 60.5 g (containing 40.3 g glucose and 7.1 g mannose) substrates were obtained from DA and SPORL pretreatments, respectively. High substrate yield from DA pretreatment was due to the retention of almost all of the original lignin. Total detected sugars in the pretreatment liquors were 4.6 g (3.0 g glucose, 0.4 g galactose, 0.9 g mannose, 0.1 g arabinose, and 0.2 g xylose) for DA pretreatment and 11.3 g (2.9 g glucose, 1.3 g galactose, 4.5 g mannose, 0.4 g arabinose, and 2.2 g xylose) for SPORL, respectively. The calculations from these data indicated that total sugar recovery was 56.7% (DA) and 87.9% (SPORL), and glucose recovery was 77.7% (DA) and 92.5% (SPORL). Comparing the recovery of hexoses and pentoses, it was found that the recovery of hexoses was much higher than that of pentoses, 62.8% vs. 4.5% for DA and 93.7% vs. 38.8% for SPORL, respectively, indicating the pentoses were easier to further degrade at high temperature and low pH than were the hexoses. The results above clearly indicate that under the same acid loading, temperature and reaction time, SPORL is superior to DA for the recovery of total sugars – hexoses and pentoses. This is likely because of the higher pH value of the SPORL pretreatment liquor formed by the addition of sulfite. High sugar recovery in

SPORL process implies that fewer sugars were degraded and limited inhibitors were generated, which will benefit the fermentation of the liquors.

Potential fermentation inhibitors formed during the DA and SPORL pretreatments are listed in Table 1, including the soluble lignin, acetic acid released from acetyl groups on hemicelluloses, furfural derived from pentoses, HMF from degradation of hexoses, and levulinic and formic acids from successive decomposition of HMF. The data in Table 1 clearly indicated that fewer inhibitors were formed from degradation of saccharides during the SPORL pretreatment than the DA pretreatment. The total of known inhibitors (furfural, HMF, and formic, acetic and levulinic acids) formed in SPORL were only 35% of those formed in DA pretreatment. As discussed above, this was owing to the addition of sulfite in the SPORL pretreatment, which increased the pH value of the pretreatment liquor, limiting extensive degradation of saccharides. The high lignin concentration in SPORL liquor is due to the formation of water-soluble lignosulfonate. Low traditional inhibitor and high sugar concentrations suggest that good fermentability can be expected for SPORL pretreatment liquor.

ENERGY EFFICIENCY OF SPORL PROCESS

Table 2. Energy efficiency of different pretreatments

Pretreatment	$\eta_{\text{pretreatment}} / (\text{kg/GJ})$
SPORL	
Aspen	395
Lodgepole pine	270
Beetle infested lodgepole pine	227
Dilute acid	
Aspen	339
Lodgepole pine	135
Steam explosion	
Spruce	260
Organosolv	
Lodgepole pine	310

Note: (1) Aspen: sulfuric acid 1.1% on wood, bisulfite 3% on wood, 170°C for 30 min at 3 liquor to wood ratio; enzyme loadings, 7.5 FPU and 11.3 CBU/ g substrate. (2) Spruce: sulfuric acid 5% on wood, sulfite 9% (0% for dilute acid pretreatment) on wood, 180°C for 30 min at 5 liquor to wood ratio; enzyme loadings, 15 FPU and 30 CBU/ g cellulose. (3) Lodgepole pine: sulfuric acid 2.2% on wood, sulfite 8% (0% for dilute acid pretreatment) on wood, 180°C for 25 min at 3 liquor to wood ratio; enzyme loadings, 15 FPU and 22.5 CBU/ g substrate. (4) Mountain-pine beetle infested lodgepole pine, dead for 4 years, pretreatment conditions: sulfuric acid 2.2% on wood, bisulfite 8% on wood, 180°C for 20 min at 3 liquor to wood ratio; enzyme loadings, 15 FPU and 22.5 CBU/g substrate.

In most of previous studies^[10, 11, 20], people only used sugar yield as a metric to evaluate and compare pretreatments. It should be pointed out that net energy production is the ultimate goal of all biofuel technologies simply because the whole idea of biofuel is about net energy output. We believe that the technical performance of any pretreatment technologies needs to be evaluated based not only on the total fermentable sugar production, but also on the energy consumed for unit sugar production

Therefore, we define a term of pretreatment process energy efficiency as the total sugar yield divided by energy consumption in pretreatment as shown below^[4]:

$$\eta_{\text{pretreatment}} (\text{kg sugar/GJ}) = \frac{\text{Total monomeric sugar yield (kg)}}{\text{Total energy consumption (GJ)}}$$

Energy efficiency of SPORL pretreatment of different feedstocks is summarized in Table 2 and compared with that of DA and other pretreatments. It is apparent that the SPORL pretreatment has better energy efficiency than the alternatives.

ENZYMATIC DIGESTIBILITY

Enzymatic digestibility is greatly dependent on substrate characteristics, such as hemicellulose content; lignin structure, distribution and content; cellulose crystallinity and degree of polymerization; and surface area, pore size and particle size of the substrate^[24]. Generally speaking, removing hemicellulose and lignin; swelling cellulose to destroy crystallinity; prehydrolyzing cellulose to shorten chain length (increasing the number of chain ends for enzymes to attack); and increasing surface area or decreasing particle size, are favorable for enzymatic digestibility of cellulosic substrates.

The enzymatic hydrolysability of SPORL-pretreated red pine is shown in Fig. 2. The enzymatic digestibility of SPORL substrate is similar to that of organosolv substrate, which is believed as one of the most readily digestible substrates^[13], and 24h hydrolysis achieved more than 90% cellulose-to-glucose conversion. The SPORL substrate displayed much better hydrolysability than DA substrate. For example, the former was completely hydrolyzed in 48 h, while the later was hydrolyzed only by 70% under the same conditions.

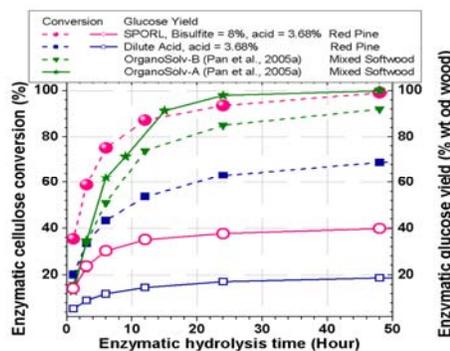


Fig. 2. Enzymatic digestibility of SPORL substrate

The digestibility of SPORL and DA pretreated substrates from different feedstocks, including switchgrass, hardwood (aspen and eucalyptus), and softwood (lodgepole pine) was compared in Table 3. As you can see, all SPORL

substrate showed significantly better digestibility than DA substrate. It was out of our expectation that the digestibility of switchgrass SPORL substrate was poorer than that of woody biomass (both hardwood and softwood). The observation is consistent with previous reports that switchgrass had poor response to pretreatment^[25,26].

Table 3. Enzymatic digestibility of SPORL substrates

Pretreatment	Cellulose to glucose yield at 48 hour/%	
	SPORL	Dilute acid
Grass		
Switchgrass	83	74
Hardwood		
Aspen	90	85
Eucalyptus	90	82
Softwood		
Lodgepole pine	90	42

Note: (1) Switchgrass: sulfuric acid 6% on switchgrass, sulfite 6% on switchgrass, 180 °C for 30 min at 7 liquor to switchgrass ratio; enzyme loadings, 15 FPU and 30 CBU/ g cellulose. (2) Aspen: sulfuric acid 1.1% on wood, bisulfite 3% on wood, 170 °C for 30 min at 3 liquor to wood ratio; enzyme loadings, 7.5 FPU and 11.3 CBU/ g substrate. (3) Eucalyptus: sulfuric acid 1.84% on wood, bisulfite 4% (0% for dilute acid pretreatment) on wood, 180 °C for 30 min at 5 liquor to wood ratio; enzyme loadings, 15 FPU and 22.5 CBU/ g substrate. (4) Lodgepole pine: sulfuric acid 2.2% on wood, sulfite 8% (0% for dilute acid pretreatment) on wood, 180 °C for 25 min at 3 liquor to wood ratio; enzyme loadings, 15 FPU and 22.5 CBU/ g substrate.

Why SPORL substrate had substantially better enzymatic digestibility than DA substrate under the same hydrolysis conditions can be explained below. First, DA pretreatment dissolved almost all hemicellulose, but kept all lignin, resulting in DA substrate with very high lignin content. In addition, the lignin was highly condensed, extremely hydrophobic, and covered the surface of the substrate, all of which enhanced the effect of lignin as physical barrier and non-productive enzyme adsorbent. On the other hand, the SPORL substrate contained less lignin, and the partial sulfonation made the residual lignin in substrate less hydrophobic, which is believed to reduce the non-productive hydrophobic adsorption of enzymes onto the lignin. For example, with the removal of hemicellulose, approximately one third (~33%) of the SPORL substrate from spruce^[2] was lignin, but this lignin retarded the hydrolysis little. The observation implies that the residual lignin in the SPORL substrate was enzyme-friendly and behaved differently from the acid-condensed DA lignin. This suggests that costly delignification is not the only way to remove the recalcitrance (attributable to lignin) to enzymatic degradation of cellulose; less expensive lignin modification may be more promising. The results also suggest that the action of lignin as a purely physical barrier played a less important role in its impacts on enzymes, compared to other interactions such as non-productive adsorption, which agrees with our previous results^[27].

ACKNOWLEDGEMENTS

The authors acknowledge Gaosheng Wang, R. Gleisner, Li Shuai, Wenyuan Zhu, Shen Tian, Dongsheng Zhuang, and Xiaolin Luo for their excellent contributions to this work. The authors appreciate the assistances from Drs. Fachuang Lu, John Ralph, Paul Weimer, and Bruce Dien in NMR and fermentation experiments. The financial supports for the research were provided by USDA Forest Service, Biomass Marketing and Utilization Program, USDA McIntire-Stennis fund, and Graduate School of University of Wisconsin-Madison.

REFERENCES

1. Wang, G. S.; Pan, X. J.; Zhu, J. Y.; Gleisner, R.; Rockwood, D. Sulfite Pretreatment to overcome recalcitrance of lignocellulose (SPORL) for robust enzymatic saccharification of hardwoods. *Biotechnol. Prog.* 2009, 25(4): 1086.
2. Shuai, L.; Yang, Q.; Zhu, J. Y.; Lu, F. C.; Weimer, P. J.; Ralph, J.; Pan, X. J. Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production. *Bioresour. Technol.* 2010, 101(9): 3106.
3. Luo, X.; Gleisner, R.; Tian, S.; Negron, J.; Zhu, W.; Horn, E.; Pan, X. J.; Zhu, J. Y. Evaluation of mountain beetle-infested lodgepole pine for cellulosic ethanol production by sulfite pretreatment to overcome recalcitrance of lignocellulose. *Ind. Eng. Chem. Res.* 2010, 49(17): 8258.
4. Zhu, J. Y.; Pan, X. J. Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation. *Bioresour. Technol.* 2010, 101(13): 4992.
5. Zhu, J. Y.; Pan, X. J.; Wang, G. S.; Gleisner, R. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. *Bioresour. Technol.* 2009, 100(8): 2411.
6. Zhu, J. Y.; Pan, X. J.; Zalesny, R.S. Pretreatment of woody biomass for biofuel production: energy efficiency, technologies, and recalcitrance. *Appl. Microbiol. Biotechnol.* 2010, 87(3): 847.
7. Zhu, J. Y.; Zhu, W. Y.; OBryan, P.; Dien, B. S.; Tian, S.; Gleisner, R.; Pan, X. J. Ethanol production from SPORL-pretreated lodgepole pine: preliminary evaluation of mass balance and process energy efficiency. *Appl. Microbiol. Biotechnol.* 2010, 86(5): 1355.
8. Chandra, R. P.; Bura, R.; Mabee, W. E.; Berlin, A.; Pan, X.; Saddler, J. N. Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics? *Biofuels* 2007, 108: 67.
9. Lynd, L. R.; Weimer, P. J.; van Zyl, W. H.; Pretorius, I. S. Microbial cellulose utilization: fundamentals and

- biotechnology. *Microbiol. Mol. Biol. Rev.* 2002, 66(3): 506.
10. Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y.Y.; Holtzapple, M.; Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* 2005, 96(6): 673.
 11. Wyman, C. E.; Dale, B. E.; Elander, R. T.; Holtzapple, M.; Ladisch, M. R.; Lee, Y. Y. Coordinated development of leading biomass pretreatment technologies. *Bioresour. Technol.* 2005, 96(18): 1959.
 12. Hendriks, A. T. W. M.; Zeeman, G. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour. Technol.* 2009, 100(1): 10.
 13. Pan, X.J.; Arato, C.; Gilkes, N.; Gregg, D.; Mabee, W.; Pye, K.; Xiao, Z. Z.; Zhang, X.; Saddler, J. Biorefining of softwoods using ethanol organosolv pulping: Preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products. *Biotechnol. Bioeng.* 2005, 90(4): 473.
 14. Nguyen, Q. A.; Tucker, M. P.; Keller, F. A.; Eddy, F. P. Two-stage dilute-acid pretreatment of softwoods. *Appl. Biochem. Biotechnol.* 2000, 84(6): 561.
 15. Kim, Y.; Mosier, N. S.; Ladisch, M. R. Enzymatic digestion of liquid hot water pretreated hybrid poplar. *Biotechnol. Prog.* 2009, 25(2): 340.
 16. Sendich, E.; Laser, M.; Kim, S.; Alizadeh, H.; Laureano-Perez, L.; Dale, B.; Lynd, L. *Recent process improvements for the ammonia fiber expansion (AFEX) process and resulting reductions in minimum ethanol selling price.* *Bioresour. Technol.* 2008, 99(17): 8429.
 17. Sierra, R.; Granda, C.; Holtzapple, M.T. Short-Term Lime Pretreatment of Poplar Wood. *Biotechnol. Progr.* 2009, 25 (2): 323.
 18. Wingren, A.; Galbe, M.; Zacchi, G. Energy considerations for a SSF-based softwood ethanol plant. *Bioresour. Technol.* 2008, 99(7): 2121.
 19. Monavari, S.; Galbe, M.; Zacchi, G. Impact of impregnation time and chip size on sugar yield in pretreatment of softwood for ethanol production. *Bioresour. Technol.* 2009, 100(24): 6312.
 20. Wyman, C. E.; Dale, B. E.; Elander, R. T.; Holtzapple, M.; Ladisch, M. R.; Lee, Y. Y.; Mitchinson, C.; Saddler, J. N. Comparative sugar recovery and fermentation data following pretreatment of poplar wood by leading technologies. *Biotechnol. Prog.* 2009, 25(2): 333.
 21. Tian, S.; Luo, X. L.; Yang, X. S.; Zhu, J. Y. Robust cellulosic ethanol production from SPORL-pretreated lodgepole pine using an adapted strain *Saccharomyces cerevisiae* without detoxification. *Bioresour. Technol.* 2010, 101(22): 8678.
 22. Zhu, W.; Zhu, J. Y.; Gleisner, R.; Pan, X. J. On energy consumption for size-reduction and yields from subsequent enzymatic saccharification of pretreated lodgepole pine. *Bioresour. Technol.* 2010, 101(8): 2782.
 23. Zhu, J. Y.; Wang, G. S.; Pan, X. J.; Gleisner, R. Specific surface to evaluate the efficiencies of milling and pretreatment of wood for enzymatic saccharification. *Chem. Eng. Sci.* 2009, 64(3): 474.
 24. Mansfield, S. D.; Mooney, C.; Saddler, J. N. Substrate and enzyme characteristics that limit cellulose hydrolysis. *Biotechnol. Prog.* 1999, 15: 804.
 25. Hu, Z. H.; Wang, Y. F.; Wen, Z. Y. Alkali (NaOH) pretreatment of switchgrass by radio frequency-based dielectric heating. *Appl. Biochem. Biotechnol.* 2008, 148(1-3): 71.
 26. Hu, Z. H.; Wen, Z. Y. Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment. *Biochem. Eng. J.* 2008, 38(3): 369.
 27. Pan, X. J.; Xie, D.; Gilkes, N.; Gregg, D. J.; Saddler, J. N. Strategies to enhance the enzymatic hydrolysis of pretreated softwood with high residual lignin content. *Appl. Biochem. Biotechnol.* 2005, 121: 1069.

In: Proceedings of the 16th international symposium on wood, fiber and pulping chemistry. 2011 June 8-10. Tianjin, China. Beijing, China: China Light Industry Press: 968-972.