

## ALKALINE PRETREATMENT OF SPRUCE AND BIRCH TO IMPROVE BIOETHANOL AND BIOGAS PRODUCTION

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Alkaline pretreatment with NaOH under mild operating conditions was used to improve ethanol and biogas production from softwood spruce and hardwood birch. The pretreatments were carried out at different temperatures between minus 15 and 100°C with 7.0% w/w NaOH solution for 2 h. The pretreated materials were then enzymatically hydrolyzed and subsequently fermented to ethanol or anaerobically digested to biogas. In general, the pretreatment was more successful for both ethanol and biogas production from the hardwood birch than the softwood spruce. The pretreatment resulted in significant reduction of hemicellulose and the crystallinity of cellulose, which might be responsible for improved enzymatic hydrolyses of birch from 6.9% to 82.3% and spruce from 14.1% to 35.7%. These results were obtained with pretreatment at 100°C for birch and 5°C for spruce. Subsequently, the best ethanol yield obtained was 0.08 g/g of the spruce while pretreated at 100°C, and 0.17 g/g of the birch treated at 100°C. On the other hand, digestion of untreated birch and spruce resulted in methane yields of 250 and 30 l/kg VS of the wood species, respectively. The pretreatment of the wood species at the best conditions for enzymatic hydrolysis resulted in 83% and 74% improvement in methane production from birch and spruce.

*Keywords:* Alkaline pretreatment; Spruce; Birch; Enzymatic hydrolysis; Bioethanol; Biogas

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### INTRODUCTION

One of the most important prerequisites for sustainable development is the production of appropriate fuels, which can be applied as alternatives to the current fossil fuels. Bioethanol and biogas from lignocellulosic materials can definitely be proper substitutes for conventional fuels (Demirbas and Balat 2006). Lignocellulosic materials are the most plentiful renewable resources on Earth, and they can be hydrolyzed and then fermented to ethanol, or digested anaerobically to biogas (Wingren 2005; Zhang and Lynd 2004). However, efficient and successful ethanol and biogas production from these materials requires pretreatment, since these materials are resistant to enzymatic and bacterial hydrolyses.

Several pretreatment processes have been investigated to change the crystalline structure of these materials (Taherzadeh and Karimi 2008). It has frequently been proven that by decreasing the crystallinity, one can increase the accessibility of cellulose to

enzymatic attack and improve the yield of subsequent enzymatic hydrolysis (Takashi et al. 1979). Changing the crystalline structure consequently can improve the yield of ethanol from lignocelluloses (Taherzadeh and Karimi 2008). Alkaline pretreatment, with e.g. NaOH, is one of the most effective processes among the different proposed pretreatment methods (Zhao et al. 2008).

Alkaline pretreatment of lignocelluloses with NaOH can remove or modify its lignin by fracturing the ester bonds that form cross-links between xylan and lignin, thereby increasing the porosity of the biomass (Tarkow and Feist 1969). However, the process is very complicated, involving several reactive and nonreactive phenomena, e.g. dissolution of nondegraded polysaccharides, peeling-off reactions (referred to as formation of alkali-stable in end-groups), hydrolysis of glycosidic bonds and acetyl groups, and decomposition of dissolved polysaccharides (Fengel and Wegener 1984). Therefore, the efficiency of NaOH pretreatment depends greatly on the process conditions, e.g. temperature, concentration of NaOH, and the treatment time, as well as the inherent characteristics of the lignocellulose used (Zhao et al. 2008; Sharma et al. 2007; Wanapat et al. 1985).

We may classify the NaOH pretreatment processes into “high concentration” and “low concentration” processes, in terms of NaOH. In low-NaOH concentration processes, typically 0.5-4% NaOH at high temperature and pressure is used, and no recycling of NaOH occurs. Its mechanism is reactive destruction of lignocelluloses, while NaOH at high temperatures disintegrates the lignin and hemicellulose and removes them from the solid phase. This property of NaOH is used in pulping processes. For instance, the treating of hemp with 3.5% NaOH and 14% Na<sub>2</sub>SO<sub>3</sub> for 2 h at 180°C can efficiently remove its lignin (Gümüşkaya and Mustafa 2006). On the other hand, in high-concentration NaOH pretreatment, usually 6 to 20% NaOH is used at “ambient” pressure and low temperatures. The mechanism of this process is dissolution of cellulose, which occurs in at least 6-8% NaOH solution at low temperatures and ambient pressure. In this process, lignin is not significantly removed from the cellulose. In addition, since the NaOH solution can be reused in this process, its economy and environmental impact may not be so critical. This work was dedicated to the latter pretreatment process, while no previous study on the effects of temperature was detected in the literature.

In this work, sodium hydroxide was used to pretreat hard- and softwoods at different temperatures. After the pretreatments, the untreated and treated wood species were subjected to separate enzymatic hydrolysis and fermentation (SHF) to ethanol, and anaerobic digestion to biogas.

## EXPERIMENTAL

### Raw Materials and their Analysis

Native species of spruce (*Picea abies*) and birch (*Betula*) were obtained from the forest around the city of Borås in Sweden. They were debarked and ground by a combination of chipping and milling with a ball mill (MM 400, Retsch GmbH, Hann, Germany) and then screened to attain a particle size of less than 0.8 mm. The dry weight content of the selected samples was measured, using a convection drying oven at 105°C

until a constant weight was achieved. These wood species were analyzed for carbohydrate and lignin fractions according to NREL methods (Ehrman 1994; Ruiz and Ehrman 1996; Templeton and Ehrman 1995). The acid-soluble lignin was measured by UV-vis spectroscopy at 205 nm with absorbency of 30 (l/g cm).

### **Pretreatment**

The milled wood species were pretreated with 7%w/w sodium hydroxide solution at different temperatures of -15, 0, 5, 50, 80, and 100°C for 2 h. The suspension of the wood species in NaOH solutions was equilibrated at the desired temperature before starting the pretreatment. A laboratory freezer was used for -15 and 0°C, and a refrigerator for 5°C. The freezer and refrigerator were opened every 15 min, and the mixture were mixed manually. The experiments at 50, 80, and 100°C were performed in a water bath and mixed every 15 min for 2 h. The treated wood species were subsequently washed with deionized water to eliminate chemicals and neutralize to pH 7. The materials were kept at 5°C for further use.

### **Enzymatic Hydrolysis**

The untreated and pretreated wood species were enzymatically hydrolyzed with 5%w/v dry matter in 50 mM sodium citrate buffer at pH 4.8. The mixtures were autoclaved at 121°C for 20 minutes before adding the enzymes. After cooling to room temperature, 20 FPU cellulase (Celluclast 1.5L, Novozyme, Denmark) and 50 IU  $\beta$ -glucosidase (Novozyme 188, Novozyme, Denmark) per gram of the wood species were added. The hydrolyses were carried out at 45°C and 120 rpm in a water bath shaker for 96 h, and their progress were followed by analyses of the sugar formed.

### **Fermentation**

The fermentations were performed in 120 ml anaerobic bottles using a flocculating yeast strain of *Saccharomyces cerevisiae* CCUG 53310 (Culture Collection University of Gothenburg, Sweden). A volume of 20 ml of each hydrolyzate was supplemented with 5 ml of all other nutrients (Taherzadeh et al. 2003), inoculated with  $1.0 \pm 0.1$  g/l yeast, and incubated at 30°C in a shaking bath for 24 h under anaerobic conditions. Samples were withdrawn periodically for sugar and ethanol analyses by HPLC. All experiments were performed in duplicate and average values were reported.

### **Digestion**

Digestion experiments were performed in serum batch flasks with 118 ml working volume containing 0.25 g dry weight of the treated or untreated wood species according to Hansen et al. (2004). The bacterial inoculum was taken from a 3000-m<sup>3</sup> municipal solid waste digester operating at thermophilic (55°C) conditions (Borås Energi och Miljö AB, Sweden). A volume of 20 ml inoculum and 5 ml distilled water was added to each flask. Moreover, inoculum and deionized water were applied as a blank, to be able to determine the gas production of the inoculum alone. Anaerobic conditions were provided by purging the flasks with a gas mixture containing 80% N<sub>2</sub> and 20% CO<sub>2</sub> for 1 min. The flasks were then put in incubator at 55°C. The experiment was continued until

the gas production stopped and the accumulated gas production remained at a stable level. All these digesting experiments were carried out in triplicates.

### **Analytical Methods**

High performance liquid chromatography (HPLC) was used to quantify sugars and fermentation products. The HPLC (Alliance 2695, Waters, Milford, MA) was equipped with an RI detector (Waters 2414). An ion-exchange column (Aminex HPX-87H, Bio-Rad, Hercules, CA) at 60°C, using 5 mM H<sub>2</sub>SO<sub>4</sub> as eluent at flow rate 0.6 ml/min, was utilized for the analyses of ethanol and sugars. All experiments were carried out in duplicate, and all the reported results were the mean values. The average standard deviation of the achieved results was less than 4.0%.

Gas samples from the headspace of each digesting bottle were withdrawn regularly and analyzed by a gas chromatograph (Auto System Perkin Elmer, Waltham, MA) in order to measure the methane production. The GC was equipped with a packed column (Perkin Elmer, 6'×1.8" OD, 80/100, Mesh) and a thermal conductivity detector (Perkin Elmer) with inject temperature of 150°C. The carrier gas applied was nitrogen with a flow rate of 23 ml/min at 60°C. The results of methane production for each sample were calculated by using a standard gas containing 100% methane.

The crystallinity of the pretreated wood species were examined using a Fourier transform infrared (FTIR) spectrometer (Impact 410, Nicolet Instrument Corp. Madison, WI). The spectra were obtained with an average of 64 scans and a resolution of 4 cm<sup>-1</sup>, in the range from 600 to 4,000 cm<sup>-1</sup> according to Carrillo et al. (2004) monitored with “Nicolet OMNIC 4.1” software. The baselines of the spectra were adjusted and normalized by the software, and the absorption bands at 1,427 and 898 cm<sup>-1</sup> were used for to calculate the crystallinity.

## **RESULTS AND DISCUSSION**

Pretreatments of spruce and birch species with 7% NaOH were performed for 2 h at different temperatures from -15 up to 100°C, and the effects of the pretreatments on composition, crystallinity and the subsequent enzymatic hydrolysis of the lignocelluloses, and their ethanol and biogas yields were investigated. The results of the current work show that the pretreatment with sodium hydroxide can significantly improve the yield of both biogas and bioethanol production from softwood spruce and hardwood birch, although it was more effective on the hardwood. Generally, alkaline pretreatment can successfully increase the yield of enzymatic conversion of lignocellulosic materials by increasing the surface area and reducing the crystallinity and the chain length of cellulose (Fengel and Wegener 1984; Fan et al. 1982). The results here show that the temperature of the NaOH pretreatment is a key factor for the process, and it can affect the composition of both hard- and softwoods.

### **Effects of Pretreatment on Composition of Spruce and Birch**

The compositions of spruce and birch wood species before and after pretreatment were analyzed and the results are presented in Table 1. The other components, such as

extractives and acetyl content, were not analyzed. The dominant type of lignin in the woods was acid-insoluble lignin. No significant change in lignin content was observed during all the pretreatment of spruce. However, there were some acid-insoluble lignin removals in pretreatment of the hardwood birch, but not the acid-soluble lignin (Table 1). It should be noticed that NaOH at lower concentrations (e.g. less than 4%) and higher temperature is able to remove lignin as it occurs in e.g. alkaline sulfite pulping. However, the lignin could not be significantly removed from the lignocelluloses under the conditions used in this work.

**Table 1.** Composition of Untreated and Pretreated Wood Species with NaOH \*

Material	Pretreatment Temperature (°C)	Cellulose (%)	Other sugars (%)	Acid-insoluble lignin (%)	Acid-soluble lignin (%)
Spruce	Untreated	43.0	20.8	28.3	0.53
Spruce	-15	47.1	15.1	27.0	0.47
Spruce	0	47.9	14.8	27.2	0.56
Spruce	5	50.0	15.9	27.7	0.54
Spruce	50	50.8	15.7	27.3	0.52
Spruce	80	51.1	14.5	28.4	0.48
Spruce	100	52.6	13.9	27.6	0.54
Birch	Untreated	41.0	27.9	27.0	2.68
Birch	-15	55.8	14.9	24.6	2.73
Birch	0	55.3	15.8	25.1	2.18
Birch	5	52.0	17.5	24.4	2.74
Birch	50	52.0	12.5	25.6	2.70
Birch	80	55.2	12.0	22.2	2.62
Birch	100	56.1	8.0	22.2	3.04

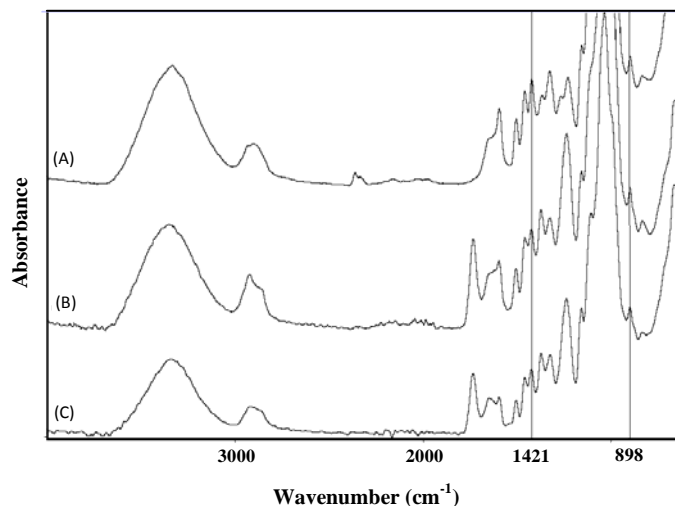
\* The data are the average of the carbohydrate and lignin content of the wood species pretreated for 2 h.

The cellulose contents of the native spruce and birch were 43% and 41%, respectively. The alkali pretreatments increased the cellulose content to 47-53% for spruce and 52-56% for birch. The highest cellulose content was obtained by treatment at the highest temperature. Generally, a considerable decrement in other sugars' content (hemicelluloses content) was detected after pretreatment of both spruce and birch. The hemicelluloses' content in untreated spruce was detected as approximately 20.8%, while it was decreased to less than 16% after different conditions of pretreatment. The hemicelluloses' content for untreated birch was about 28%, which decreased to less than 17.5% after treatment. Higher treatment temperature resulted in higher hemicellulosic sugar removal. The lowest hemicellulose content, which was 13.9 and 8.0% for spruce and birch, respectively, was achieved by pretreatment at 100°C. Therefore, it may be concluded that increased cellulose content is a result of decreased hemicellulosic part of the woods. Hemicelluloses, which are branched and heterogeneous polymers of pentoses and hexoses, can relatively easily hydrolyzed to their monomers. On the other hand, cellulose is a crystalline polymer that cannot easily be hydrolyzed by chemicals (Taherzadeh and Karimi 2008).

The hemicellulose removal was probably an important factor for improvement of enzymatic hydrolysis and biogas production in this work, besides decreasing the cellulose crystallinity. Lower hemicellulose and higher cellulose content is also preferable in enzymatic hydrolysis processes. While the native spruce contains 43% cellulose, the treatment enhanced it to 47-52%. The cellulose content of birch was also increased from 41% to more than 52%.

### Crystallinity

Crystallinity of cellulose is one of the most important properties of lignocelluloses to make the cellulose fibers resistant to the cellulytic enzymes. Crystallinity of the treated and untreated woods was studied by FTIR spectroscopy (Colom et al. 2003; Carrillo et al. 2004). The spectra for untreated and pretreated birch at 80 and 100°C are presented in Fig. 1. The 1,427 and 898  $\text{cm}^{-1}$  absorption bands, which were assigned to the respective crystalline cellulose I and cellulose II, were used to study crystallinity changes. The absorbance ratio  $A_{1427}/A_{898}$  or crystallinity index (CI) is illustrated in Fig. 2. The data showed that pretreatment with NaOH resulted in reducing the absorption band at 1,427  $\text{cm}^{-1}$ , and increasing the band at 898  $\text{cm}^{-1}$ . All the treatments reduced the CI of the woods compared with untreated woods. This reduction in crystallinity might be a main factor for improving the yield of subsequent enzymatic hydrolysis of the lignocelluloses and also the biogas yield in the bacterial digestion.



**Fig. 1.** FTIR spectra of untreated birch (A), and birch treated with 7% NaOH at 80°C (B), and 100°C (C).

### Separate Enzymatic Hydrolysis and Fermentation (SHF)

The enzymatic hydrolysis was performed at 45°C and 120 rpm for 96 h using 20 FPU cellulase and 50 IU  $\beta$ -glucosidase per gram of the woods. The results are summarized in Fig. 3. The glucose yield was calculated based on the percentage of theoretical yield. It is based on the total glucose which can be produced from glucan in the biomass. Each gram of glucan can be hydrolyzed to 1.111 grams glucose.

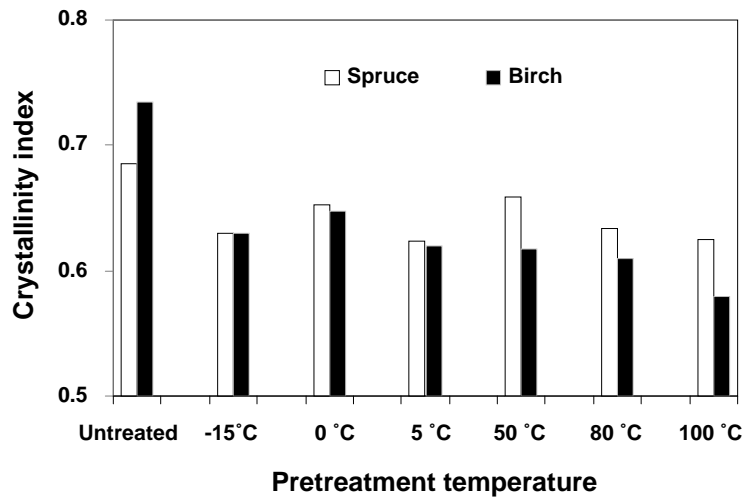


Fig. 2. Total crystallinity index of pretreated and untreated spruce and birch species

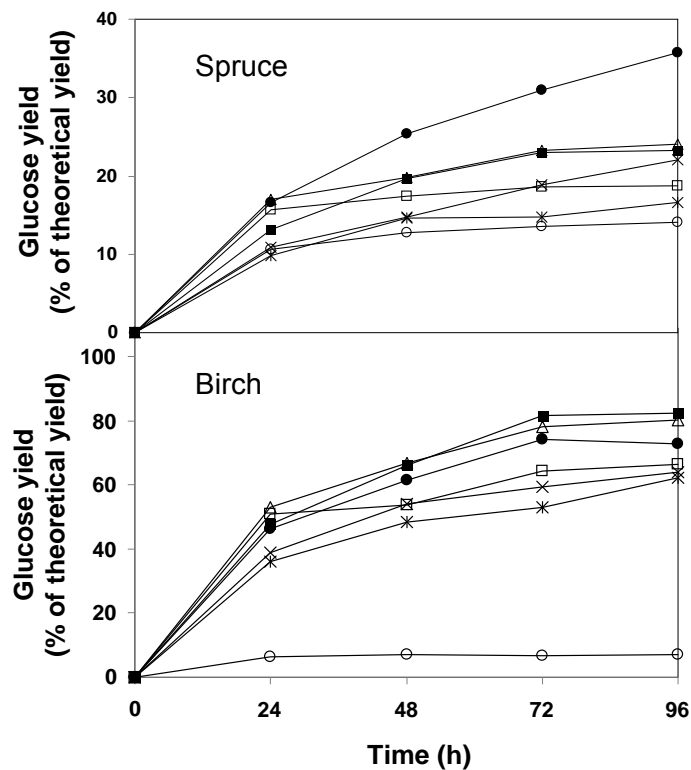


Fig. 3. Effect of NaOH pretreatment at different temperatures on enzymatic hydrolysis of spruce and birch. The symbols represent: (○) untreated wood, and pretreatment at (□) -15°C, (×) 0°C, (●) 5°C, (\*) 50°C, (Δ) 80°C, (■) 100°C. The glucose yield was calculated based on the percentage of theoretical yield [produced glucose/ (1.111\* biomass (g/l) \*F), where F is cellulose fraction in biomass].

The pretreatments under all conditions significantly improved the hydrolyses of both the wood species compared to the untreated ones. However, the pretreatment was more effective on the hardwood birch compared to the softwood spruce. The enzymatic hydrolysis of untreated spruce and birch for 4 days resulted in only 14.1% and 6.9% of the theoretical yield. In other words, the enzymatic hydrolysis of untreated spruce was approximately two times as successful as that of untreated birch. The pretreatment at different temperatures (-15, 0, 5, 50, 80, and 100°C) resulted in improving the conversion yield of cellulose to glucose (Fig. 3). A gradual increase in glucose production yield was observed under all the conditions during 96 h. The pretreatment enhanced the yield of hydrolysis up to 16.6%-35.7% of the theoretical value for spruce and 62.3%-82.3% for birch. In general, the pretreatment at higher temperatures (80 and 100°C) resulted in higher yield of saccharification (around 80%) for birch. However, the best saccharification yield for spruce, 35.7%, was obtained after pretreatment at 5°C. This indicates that the alkaline pretreatment of spruce and birch improved the efficiency of their enzymatic hydrolyses by factors of 2.5 and 11 times, respectively.

Some selected enzymatic hydrolyzates were fermented by *S. cerevisiae* for 30 h. The most important results are summarized in Table 2. The fermentation of untreated spruce and birch resulted in 10.9% and 11.3% of the theoretical yield of ethanol, respectively (Table 2). The pretreatment with sodium hydroxide enhanced this yield to 18.9-26.1% for spruce and 47.0-54.8% for birch. The results showed a significant effect of the NaOH pretreatment of woody materials on the ethanol yield.

It was previously shown that the efficiency of NaOH pretreatment was higher for straws than for hardwoods, while no effect was observed for softwoods, and this was related to the lignin content of these materials. The softwoods are usually more difficult to hydrolyze than hardwoods (Gregg and Saddler 1995; Palonen et al. 2004). The results of this work showed that both bioethanol and biogas production from spruce and birch can be enhanced by sodium hydroxide pretreatment. However, pretreatment had a significant effect only on birch, while little improvement was observed for the softwood spruce.

### Biogas Production

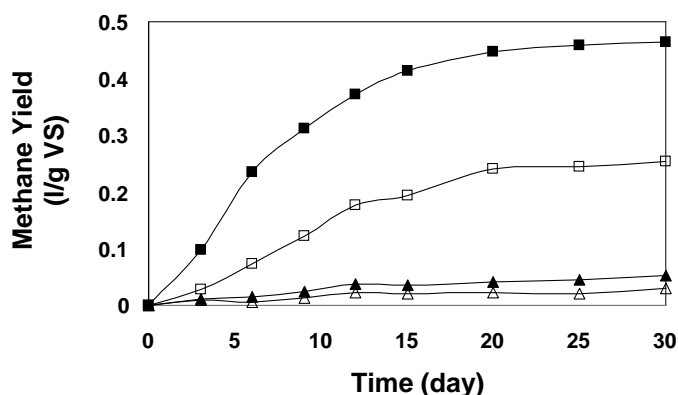
The results of anaerobic digestion of pretreated birch and spruce species, compared to untreated samples, are presented in Fig. 4. The birch pretreated with NaOH at 5°C and spruce pretreated at 100°C were chosen for digestion, since they showed the best results in glucose yield during the enzymatic hydrolysis. The anaerobic digestions were performed for 30 days under thermophilic conditions (55°C) until the gas production was stabilized.

As shown in Fig. 4, the pretreated birch at 100°C showed the highest methane production yield in comparison with the pretreated spruce and untreated samples. Methane production yield for treated birch was 0.46 liter per gram of volatile solid (l/gVS), while the yield of the untreated birch was 0.25 l/gVS (Fig. 4). Pretreatment of spruce with NaOH at 5°C also improved the yield of methane production from 0.03 to 0.05 l/gVS. However, these results indicate that the yield of both treated and untreated spruce was far less than that of the hardwood birch.

**Table 2.** Yield of Ethanol from Untreated and Pretreated Lignocelluloses after 24 and 30 h Fermentation, as Percentage of Theoretical Yield (0.56 g/g cellulose)

Raw materials	Pretreatment temperature (°C)	Maximum ethanol Yield (%)
Spruce	Untreated	10.94
	5	23.03
	80	18.90
	100	26.05
Birch	Untreated	11.35
	0	47.03
	80	52.95
	100	54.76

The results indicate 50% enhancement in yield of methane after the pretreatment of birch, compared to the untreated condition. However, the pretreatment was not so successful in increasing biogas in digesting spruce. These facts might lead to a hypothesis that the pretreatment with NaOH at the conditions used in this work is very successful for hardwood but not for the softwood. Teghammar et al. (2010) pretreated paper tube residuals by NaOH to improved biogas production. Under the best conditions, pretreatment with 2% NaOH and 2% H<sub>2</sub>O<sub>2</sub> at 220°C and 23 bar, they observed that the yield of methane increased from 0.24 to 0.49 (l/gVS). This result is comparable with the results of the current work with birch, which was pretreated at higher concentration of NaOH (7%), 100°C, and only atmospheric pressure.



**Fig. 4.** Effect of NaOH pretreatment on yield of methane for spruce and birch species. The symbols represent: (□) untreated birch, (■) treated birch at 100°C, (Δ) untreated spruce, (▲) treated spruce at 5°C.

## CONCLUSIONS

Pretreatment with 7% NaOH under mild conditions (atmospheric pressure, moderate temperatures) is an alternative for pretreatment of both hardwoods and softwoods to enhance bioethanol and biogas production. In this process, very limited or no destruction of lignin occurs, and the alkali solution can be reused. This pretreatment method was very successful for the hardwood birch for either enzymatic hydrolysis and fermentation to ethanol, or bacterial digestion to biogas. However, the pretreatment may have difficulties to compete with other pretreatment methods in the case of softwood spruce.

## REFERENCES CITED

- Carrillo, F., Colom, X., Suñol, J. J., and Saurina, J. (2004). "Structural FTIR analysis and thermal characterisation of lyocell and viscose-type fibres," *Eur. Polym. J.* 40, 2229-2234.
- Colom, X., Carrillo, F., Nogués, F., and Garriga, P. (2003). "Structural analysis of photodegraded wood by means of FTIR spectroscopy," *Polym. Degrad. Stab.* 80, 543-549.
- Demirbas, M., and Balat, M. (2006). "Recent advances on the production and utilization trends of bio-fuels: A global perspective," *Energy Convers. Manage.* 47, 2371-2381.
- Ehrman, T. (1994). "Standard method for determination of total solids in biomass," Laboratory analytical procedure No. 001, National Renewable Research Laboratory (NREL), Golden, CO.
- Fan, L., Lee, Y., and Gharpuray, M. (1982). "The nature of lignocellulosics and their pretreatments for enzymatic hydrolysis," *Adv. Biochem. Eng.* 23, 157-187.
- Fengel, D., and Wegener, G. (1984). *Wood: Chemistry, Ultrastructure, Reactions*, Walter de Gruyter, Berlin.
- Gregg, D., and Saddler, J. (1995). "Bioconversion of lignocellulosic residue to ethanol: process flowsheet development," *Biomass Bioenergy* 9, 287-302.
- Gümüşkaya, E., and Mustafa, U. (2006). "Dependence of chemical and crystalline structure of alkali sulfite pulp on cooking temperature and time," *Carbohydr. Polym.* 65(4), 461-468.
- Hansen, T. L., Schmidt, J. E., Angelidaki, I., Marca, E., Jansen, J. I. C., Mosbæk, H., and Christensen, T. H. (2004). "Method for determination of methane potentials of solid organic waste," *Waste Manage.* 24, 393-400.
- Palonen, H., Thomsen, A., Tenkanen, M., Schmidt, A., and Viikari, L. (2004). "Evaluation of wet oxidation pretreatment for enzymatic hydrolysis of softwood," *Appl. Biochem. Biotechnol.* 117, 1-17.
- Ruiz, R., and Ehrman, T. (1996). "Determination of carbohydrates in biomass by high performance liquid chromatography," Laboratory Analytical Procedures No. 002, National Renewable Research Laboratory (NREL), Golden, CO.
- Sharma, K., Kuhar, S., Kuhad, R., and Bhat, P. (2007). "Combinatorial approaches to improve plant cell wall digestion: Possible solution for cattle feed problems," in

- Lignocellulose Biotechnology: Future Prospects*, Kuhad, R.C., and Singh, A. (eds), IK International Publishing House Pvt. Ltd., New Delhi.
- Taherzadeh, M. J., Fox, M., Hjorth, H., and Edebo, L. (2003). "Production of mycelium biomass and ethanol from paper pulp sulfite liquor by *Rhizopus oryzae*," *Bioresour. Technol.* 88, 167-177.
- Taherzadeh, M. J., and Karimi, K. (2008). "Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review," *Int. J. Mol. Sci.* 9, 1621-1651.
- Takashi, S., Noriko, N., Takashi, T., Yohko, S., and Keiji, K. (1979). "Correlation between X-ray diffraction measurements of cellulose crystalline structure and the susceptibility to microbial cellulase," *Biotechnol. Bioeng.* 21(6), 1031-1042.
- Tarkow, H., and Feist, W. (1969). "A mechanism for improving the digestibility of lignocellulosic materials with dilute alkali and liquid ammonia," *Adv. Chem. Ser.* 95, 197-218.
- Templeton, D., and Ehrman, T. (1995). "Determination of acid-insoluble lignin in biomass," Laboratory Analytical Procedures No. 003, National Renewable Research Laboratory (NREL), Golden, CO.
- Teghammar, A., Yngvesson, J., Lundin, M., Taherzadeh, M. J., and Horváth, I. S. (2010). "Pretreatment of paper tube residuals for improved biogas production," *Bioresour. Technol.* 101(4), 1206-1212.
- Wanapat, M., Sundstøl, F., and Garmo, T. H. (1985). "A comparison of alkali treatment methods to improve the nutritive value of straw. I. Digestibility and metabolizability," *Anim. Feed Sci. Technol.* 12, 295-309.
- Wingren, A. (2005). *Ethanol from softwood, Techno-Economic evaluation for development of the enzymatic process*, Department of Chemical Engineering, Lund University, Lund, Sweden.
- Zhang, Y. H., and Lynd, L. R. (2004). "Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplexed cellulase systems," *Biotechnol. Bioeng.* 88, 797-824.
- Zhao, Y., Wang, Y., Zhu, J., Ragauskas, A., and Deng, Y. (2008). "Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature," *Biotechnol. Bioeng.* 99(6), 1320-1328.

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