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Abstract. *Switchgrass is a promising lignocellulosic biomass for fuel-ethanol production. However, pretreatment of lignocellulosic materials is necessary to improve its susceptibility to enzymatic hydrolysis. The objectives of this study were to examine the feasibility of microwave pretreatment to enhance enzymatic hydrolysis of switchgrass and to determine the optimal pretreatment conditions. Switchgrass samples immersed in water, dilute sulfuric acid and dilute sodium hydroxide solutions were exposed to microwave radiation at varying levels of radiation power and residence time. Pretreated solids were enzymatically hydrolyzed and reducing sugars in the hydrolysate were analyzed. Microwave radiation of switchgrass at lower power levels resulted in more efficient enzymatic hydrolysis. The application of microwave radiation for 10 minutes at 250 watts to switchgrass immersed in 3% sodium hydroxide solution (w/v) produced the highest yields of reducing sugar. Results were comparable to conventional 60 minute sodium hydroxide pretreatment of switchgrass. The findings suggest that combined microwave-alkali is a promising pretreatment method to enhance enzymatic hydrolysis of switchgrass.*

Keywords. Microwave pretreatment, chemical pretreatment, enzymatic hydrolysis, switchgrass, ethanol production, lignocellulosic biomass

Introduction

Given the steady increase in energy consumption and current dependence on crude oil to meet these needs, there is considerable interest in exploring alternative energy sources. Campbell and Laherrere (1998) stated that there would be a steady decline in crude oil production in the coming decades. Ethanol is one of the alternative renewable energy sources that could address the growing energy challenge. In the United States, fuel-ethanol is primarily produced from corn. However, to meet increasing demand, corn-based ethanol may not be practical as limited arable lands are also required for growing corn for food and feed applications (Sun & Cheng, 2002). In lieu of this, lignocellulosic materials have been identified as promising feedstock for fuel-ethanol production. These materials include agricultural residues, cellulosic waste such as newsprint and office paper and forage and woody crops. Switchgrass (*Panicum virgatum L.)*, a native perennial warm-season forage grass, is a potential lignocellulosic feedstock for ethanol production. Switchgrass shows promise due to high productivity across a wide geographic range, suitability for marginal land quality, low water and nutritional requirements, positive environmental benefits, and flexibility for multipurpose uses (McLaughlin, 1993).

The conversion of lignocellulosic materials into ethanol involves hydrolysis of cellulose into fermentable sugars and the subsequent fermentation of these sugars into ethanol. The hydrolysis is typically carried out by cellulase enzymes. Lignocellulosic materials contain cellulose, hemicellulose and lignin in a complex crystalline structure. As a result, the efficiency of the hydrolysis is reduced due to limited accessibility of the enzymes to cellulose (McMillan, 1994). Hence, pretreatment processes are essential in improving the efficiency of enzymatic hydrolysis. The purpose of pretreatment is to remove lignin and hemicellulose, reduce crystallinity of cellulose and increase porosity of the biomass. Several physical, chemical and biological pretreatment methods have been studied in detail. In regards to switchgrass, previously examined pretreatments include dilute sulfuric acid pretreatment (Chung et al., 2005, Dien et al., 2006 and Fenske et al., 1994), lime pretreatment (Chang et al., 1997), ammonia fiber explosion (Alizadeh et al., 2005), fractionation using ammonia and hydrogen peroxide percolation (Kim and Lee, 1996 and Iyer et al., 1996) and ammonia water pretreatment (Kurakake et al., 2001).

A promising pretreatment method involves the application of microwave radiation to biomass in an aqueous environment. The rationale for microwave pretreatment stems from two aspects. First from a physical aspect, microwave radiation supplies internal heat to the biomass resulting from the vibrations of polar bonds in the biomass and surrounding aqueous medium. The radiation generates a continuously changing magnetic field causing the polar bonds to vibrate as they align with the magnetic field. This disruption and shock to the polar bonds accelerates chemical, biological and physical processes (Sridar, 1998). Secondly, the thermal treatment of lignocellulosic materials in an aqueous medium is known to release acetic acid, hence providing an acidic environment for auto-hydrolysis (Lora and Wayman, 1978). The earliest known study involving microwave pretreatment examined the effect of microwave radiation on rice straw and baggase immersed in water (Ooshima et al., 1984). Other studies on sugar cane baggase and rice straw involved extraction of hemicellulose and lignin following application of microwave radiation (Azuma et al., 1984 and Kitchaiya et al., 2003). Recent published work in this area combined microwave radiation with alkali reagents in the pretreatment of rice straw and wheat straw (Zhu et. al., 2005 and 2006).

The objectives of this study were to identify different methods of using microwave radiation for the pretreatment of switchgrass, assess the feasibility of these methods and define optimal pretreatment conditions.

Safety Emphasis

Several experiments in this study involved microwave heating of switchgrass. For safety considerations, a microwave leak detector (Model MLD 15 from Environmental Concepts, Ft. Lauderdale FL) was used to ensure measure microwave leakage was within 5 milliwatts (mW) per square centimeter at a distance of 2 inches as prescribed by the Center of Devices and Radiological Health (FDA, 2006).

Materials and Methods

Biomass

Switchgrass samples were obtained from NC State University's Central Crops Research Station in Clayton, North Carolina. The switchgrass cultivar named "Performer" was harvested until the end of the growing season in October 2005. Post-harvesting operations included oven drying and grinding the biomass to particles of 1 mm in diameter. The biomass was stored in a sealed plastic bag at room temperature.

Pretreatments

For all pretreatments described, experiments were done in triplicate and average values are reported. The collected solids from all pretreatments were washed with 200 ml of deionized water to remove excess acid, base or other pretreatment by-products that may inhibit enzymatic hydrolysis. Wet pretreated samples were stored in a sealed bag at 4°C for enzymatic hydrolysis.

The microwave based pretreatments were carried out in a general purpose laboratory microwave oven made by Panasonic Corporation (Model NN-S954). The apparatus provided microwave radiation at variable power levels ranging from 125 to 1250 watts. Biomass samples were immersed in dilute sulfuric acid (H2SO4) and dilute sodium hydroxide (NaOH) at concentrations of 1, 2 and 3 % (w/v) and at a solid loading of 10%. The mixtures were placed in an open 250 ml glass beaker and exposed to microwave radiation at 250 watts. Pretreatments were conducted at residence times of 5, 10, 15 and 20 minutes. In addition, preliminary experiments to investigate several different options to use microwave radiation were conducted. These experiments along with relevant results are described in the results and discussion section.

To compare results from the combined microwave-chemical pretreatments, conventional dilute H2SO4 pretreatment and dilute NaOH pretreatments were also conducted. These involved immersing the biomass in 2% (w/v) solutions (in both cases) at 10% solid loading. The biomass mixtures were placed in sealed serum bottles and pretreated in an autoclave at 121°C and 15 psi for 60 minutes.

Enzymatic Hydrolysis

Enzymatic hydrolysis of pretreated samples were carried out in 250 ml Erlenmeyer flasks in a controlled environment incubator shaker set at 55°C and 150 rpm. 1 g (dry basis) of wet pretreated biomass was immersed in 30 ml of 50 mM sodium citrate buffer to maintain a pH of 4.8. Cellulase from *Trichoderma reesei* (E.C. 3.2.1.4) was added at an enzyme loading of 25 FPU/g dry biomass. The cellulase activity was supplemented with cellobiase (Novozyme 188) from *Aspergillus niger* (E.C. 3.2.1.21) at a ratio of 1:1.75, as reported by Silverstein et al. (2006). The additional cellobiase was necessary to mitigate cellobiose inhibition of cellulase (Ryu and Mandels, 1980). Sodium azide (0.3% (w/w)) was added to the hydrolysis mixture to inhibit microbial growth. The hydrolysis was carried out for 72 hours after which the hydrosylate was centrifuged and the supernatant was stored at -20°C for reducing sugar analysis. As with the pretreatments, experiments were done in triplicates and average values are reported.

Reducing Sugar Analysis

The reducing sugar content of the supernatant from the enzymatic hydrolysis was measured using the 3, 5-dinitrosalicylic acid reagent (DNS) method (Miller, 1959). The reducing sugar levels were calculated as mg of equivalent glucose units per ml of hydrosylate (henceforth expressed as mg/ml).

Data Analysis

Statistical analysis of experimental data was conducted using the PROC GLM functionality in SAS, version 9.1.3 (SAS Institute Inc., Cary, NC).

Results and Discussion

Preliminary Experiments

Several preliminary experiments were conducted to investigate different options for using microwave radiation as a means of pretreatment. The first experiment involved the microwave radiation of switchgrass immersed in water at 10% solid loading in a capped glass vessel. This treatment was applied for 1, 2 and 5 minutes at 1250 watts (W) of radiation, after which the collected solids were prepared for enzymatic hydrolysis. The results of this experiment (shown in Table 1) indicate that the 1 and 2 minute pretreatments produced higher reducing sugar yields after 72 hours of hydrolysis than the 5 minute pretreatments. However, in comparison with hydrolysis yields of untreated switchgrass, the improvement was minimal. The reducing sugar yield from the 5 minute pretreatment was much less. Visual inspection of the solids collected for this pretreatment condition indicated some charring of the biomass. This implies that degradation of some sugars may have occurred, and subsequently, a reduction in yield from the hydrolysis was observed.

Table 1: Results from Preliminary Experiments 1 and 2

Pretreatment Conditions	Vessel Type	Reducing Sugar Yield (mg/ml)
1250 W - 1 min	capped glass	5.18
1250 W - 2 min	capped glass	4.96
1250 W - 5 min	capped glass	3.32
1250 W - 1 min	sealed ptfe tube	5.99
Untreated Biomass - Control	-	4.87

The second preliminary experiment involved loading the switchgrass samples immersed in water at 10% solid loading inside a microwave-safe one-half inch polytetrafluoroethylene (ptfe) tube. One end of the tube was fitted with a ptfe plug and the other end with a polypropylene ball valve. The tube and its contents were subjected to microwave radiation at 1250 watts for 1 minute. The idea involved a rapid increase in pressure inside the sealed tube for 1 minute, then a sudden release of the pressure by opening the ball valve. The contents of the tube were released into a collection bag and prepared for enzymatic hydrolysis. The hydrolysis yields from this enhanced pressure pretreatment were better than those from the pretreatments in the first preliminary experiment (refer Table 1). However, the improvement over hydrolysis yields of untreated biomass was still marginal.

The results presented in Table 1 indicated that microwave radiation at higher power levels did not improve hydrolysis yields relative to the control. A subsequent experiment showed that microwave radiation at reduced power levels was more effective. Consequently, 250 W was selected for subsequent work. In addition, a review of published studies indicated that microwave pretreatment in the presence of chemical reagents would be more effective. Further, Sridar (1998) states that efficient and rapid heating by microwave radiation can accelerate chemical reactions.

Combined Microwave-Chemical Pretreatments

The reducing sugar yields from enzymatic hydrolysis of microwave pretreatments combined with varying levels of H2SO4 and dilute NaOH, are shown in Figures 1 and 2, respectively. As the data clearly indicates, combined microwave-NaOH pretreatments yielded significantly higher reducing sugars than combined microwave-H2SO4 pretreatments ($p<0.001$). The mean value for yields from microwave-NaOH pretreatments were approximately four times that of microwave-H2SO4 pretreatments.

Within the various microwave-H2SO4 pretreatment conditions, there was no statistical difference among the different concentrations of H2SO4. Residence times of 15 and 20 minutes gave significantly higher yields than residence times of 5 and 10 minutes ($p<0.005$). The highest yields from combined microwave-H2SO4 pretreatments were obtained at a concentration of 3% sulfuric acid and a residence time of 15 minutes.

Within the various microwave-NaOH pretreatment conditions, each concentration gave significantly higher reducing sugar yields than the corresponding lower concentrations ($p<0.01$). In regards to the effect of time, the only significant difference was between the mean values at 5 minutes and 10 minutes ($p<0.01$). The highest yields from the combined microwave-NaOH pretreatment were obtained at a concentration of 3% NaOH and a residence time of 10 minutes.

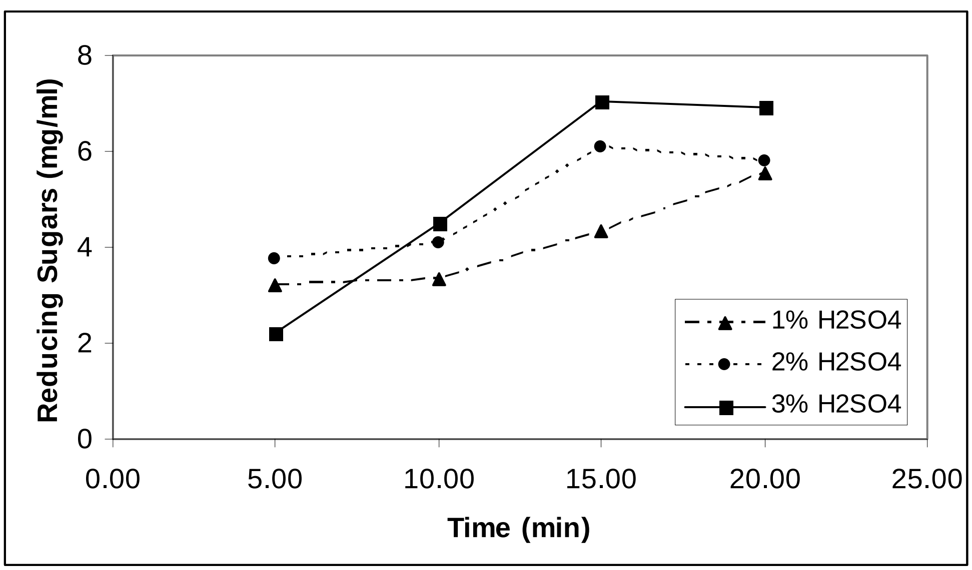

Figure 1. Reducing Sugar yields from combined microwave-H2SO4 pretreatment at different concentrations and times.

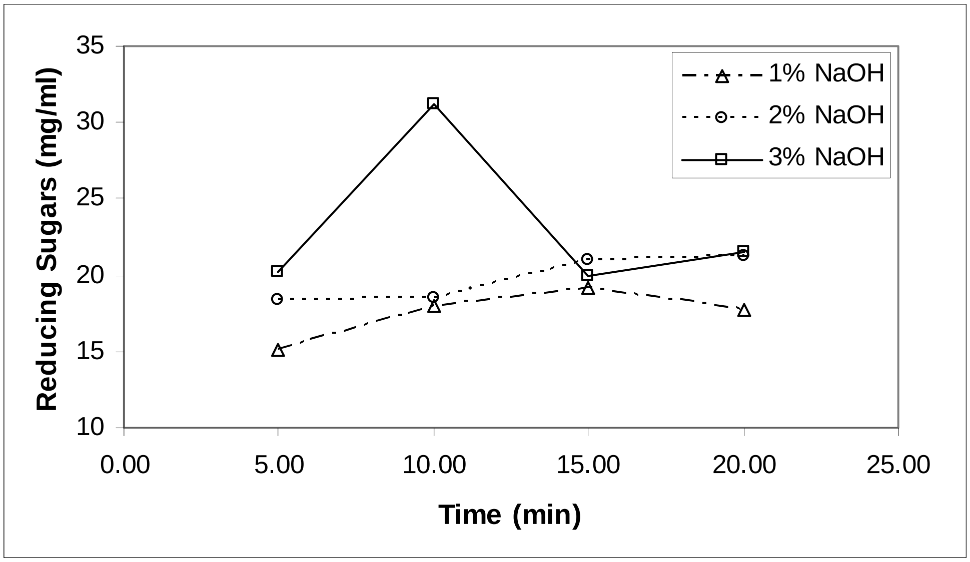

Figure 2. Reducing Sugar yields from combined microwave-NaOH pretreatment at different concentrations and times.

Comparison with Chemical Pretreatment and Published Data

Switchgrass samples were subjected to conventional chemical pretreatment using 2% NaOH and 2% H2SO4 solutions. The results in table 2 compare the enzymatic hydrolysis of these samples with optimal values from combined microwave-chemical pretreatments at 2% w/v concentrations. Although the values are slightly less for microwave-based pretreatments, the significant reduction in residence times should be noted. For instance, microwave-2% NaOH pretreatment gave sugar yields that were 78% of the yields from conventional-2% NaOH pretreatment but only required one-sixth the residence time.

Table 2: Comparison of conventional chemical and combined microwave-chemical pretreatments

Pretreatment Conditions	Reducing Sugar Yield (mg/ml)
2% H2SO4 - 60 minutes	8.53
2% NaOH - 60 minutes	27.03
Microwave-2% H2SO4 -15 min	6.08
Microwave-2% NaOH -10 min	21.23

Microwave pretreatment of switchgrass has not been previously reported in literature. However, combined microwave-NaOH pretreatment of rice straw have been reported by Zhu et al. (2005, 2006). While their pretreatment and enzymatic hydrolysis conditions are slightly different, a comparison is possible for one scenario. At a concentration of 1% NaOH and a pretreatment time of 6 minutes with 300 W of microwave radiation, Zhu et al. (2006) reported a 72 hour hydrolysis yield of approximately 6 mg/ml. In this study, at a concentration of 1% NaOH and a pretreatment of 5 minutes with 250 W of microwave radiation, the 72 hour hydrolysis yield was 15.08 mg/ml. In contrast to Zhu et al. (2006), the pretreated biomass in this study was not oven dried prior to hydrolysis. This could account, in part, for the significantly higher reducing sugar levels reported in this study, since the standard laboratory procedure for enzymatic hydrolysis of lignocellulosic biomass states that pretreated biomass should never undergo any drying prior to hydrolysis since irreversible pore collapse may occur and limit enzymatic hydrolysis (DOE, 1996).

Conclusion

This study examined the potential of microwave-based pretreatment of switchgrass. The results show that a combined microwave-alkali pretreatment is a promising approach to enhance enzymatic hydrolysis. It was determined that the most efficient method for utilizing microwave radiation as a pretreatment process was at lower power levels in combination with dilute NaOH. The highest reducing sugar yields were obtained for microwave-NaOH pretreatment at 250 W for 10 minutes with the biomass immersed in 3% NaOH solution. Results comparable to conventional NaOH pretreatments were obtained at only one-sixth of the residence time.

Future plans include applying the optimal conditions to other switchgrass cultivars obtained from NC State University's Central Crops Research Station in Clayton, North Carolina. The effect of microwave-alkali pretreatment on biomass composition and structure will be determined by quantifying removal of lignin and hemicellulose. The combined effect of microwave radiation with lime (calcium hydroxide) pretreatment will also be investigated as it is cheaper, safer to handle and easier to recover than NaOH (Chang et al., 1997). Since the enzyme loading in this study was excessive, optimal conditions will be determined to minimize enzyme requirements while maintaining high sugar yields. Finally, fermentation parameters for hydrolysates obtained under ideal pretreatment and hydrolysis conditions will be determined.

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