Bioresource Technology 101 (2010) 5426–5430

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Comparison of different pretreatment methods based on residual lignin effect on the enzymatic hydrolysis of switchgrass

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ARTICLE INFO

Article history:
Received 8 September 2009
Received in revised form 4 February 2010
Accepted 7 February 2010
Available online 9 March 2010

Keywords:
Holocellulose
Pretreatment
Lignocelluloses
Switchgrass

ABSTRACT

Three different pretreatment methods for switchgrass were studied. Aqueous sodium hydroxide (0.5–10% w/v, 90 °C, 1 h), dilute H_2SO_4 (0.5–6.0% v/v, 121 °C, 1 h) and hot water (100 °C, 1 h) were employed in this study to determine how each method affected the digestibility of switchgrass during enzymatic hydrolysis. Switchgrass pre-treated with 0.5% w/v sodium hydroxide generally produced glucose in higher concentrations than sulfuric acid and hot water pre-treated samples. SEM studies on the pre-treated samples revealed a great deal of pore formation in the NaOH pre-treated samples and little or no physical changes on the acid and hot water pre-treated samples. Lignin analysis carried out on the pre-treated samples showed a considerable decrease in lignin content in the NaOH pre-treated samples and only a slight decrease in lignin content for the other pretreatment methods studied.

1. Introduction

Ethanol made from lignocellulosic biomass is extensively accepted as a substitute or as an additive to fossil fuel due to its negligible impact on the environment (Lugar and Woolsey, 1999; Wyman, 1999; Schell et al., 1998). Cellulose, hemicellulose and lignin make up about 90% of the entire composition of lignocelluloses (Kumar et al., 2009). Ethanol production from lignocelluloses requires the conversion of the holocellulosic component (cellulose and hemicellulose) of the biomass into simple monomeric sugars that can be further converted, either microbially or chemically, into energy sources such as ethanol, butanol, and various other products such as organic acids, acetone, or glycerol (Wyman, 2002; Palonen et al., 2004).

Unfortunately a great deal of enzyme is required to achieve the conversion holocellulose into fermentable sugars. A recent modeling study (Zhu et al., 2008) concluded that lignin content and crystallinity are the key factors in determining biomass digestibility during enzymatic hydrolysis. To enhance the overall efficiency of this hydrolysis step, the crystalline structure of the holocelluloses needs to be disintegrated and the lignin content reduced in order to increase the accessibility of the enzymes to the substrates (holocelluloses). Consequently, there arises the need to pretreat the biomass in such a manner that disrupts the internal structure of the holocelluloses, increases the reaction surface area, increases the porosity of the biomass, and simultaneously reduces the percentage lignin content. The desirable properties required of pre-treated lignocelluloses (Adsul et al., 2005; Lynd, 1996) material are (a) residual fibers must be reactive, (b) yield pentose in non-degradable form, (c) should not produce compounds that would inhibit hydrolysis and fermentation, (d) work in reactors of reasonable size with moderate cost, (e) produce no solid residues, (f) have high degree of simplicity, and (g) be effective at low moisture contents.

One abundant and low cost feedstock that has generated considerable interest and attention to researchers as a potential substrate for ethanol production is switchgrass (*Panicum virgatum* L.). It is rich in hollocellulose, capable of growing throughout the country in a range of climates, resistance to drought, pest and plant diseases. It can also thrive well on degraded and contaminated soils. It also helps to reduce soil erosion. Most important is that switchgrass regenerates its self after it has been cut for harvest. It requires little fertilizers and pesticides to flourish. Moreover it helps trap CO_2 in the ground (Epplin, 1996; Pimentel and Patzek, 2005). Several methods such as alkali, dilute acid, steam explosion, hot water and ammonia fiber explosion are potential candidates for the pretreatment of lignocellulosic materials (Hsu, 1996; Kitani and Hall, 1989). Many of the prior studies focusing on the pretreatment of lignocellulosic materials used corn stover, wood samples or other lignocellulosic materials for biomass sources. Very few of these studies actually used grass as a biomass source. Some of the more recent studies on switchgrass pretreatment have focused on techniques such as ammonia fiber explosion, radio frequency (RF) dielectric heating (Hu et al., 2008), microwave radiation (Hu et al., 2008), pulse electric field pretreatment (Kumar et al., 2009) and the use of calcium hydroxide (Chang et al., 1997). Methods involving the use of sodium hydroxide (alkali), dilute acid and hot water were employed in this study. Although prior studies

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doi:10.1016/j.biortech.2010.02.031

have demonstrated the merit of techniques such as AFEX, microwave and RF radiation to pretreat switchgrass in the laboratory, the use of alkali (NaOH), acid and hot water have been routinely used to pretreat raw materials for the pulp and paper industry. Moreover scaled up versions of these processes are in place and commonly used by the paper industry. Hence if these methods are effective, it is anticipated that the technical issues regarding the large scale design of these processes for switchgrass pretreatment would be minimal. This study examines the impact of NaOH, dilute acid and hot water on the surface morphology of the grass and the lignin content. These data were correlated with the enzymatic conversion of the cellulosic component of the switchgrass into fermentable sugars. Each pretreatment method was analyzed with enzymatic hydrolysis, SEM images and lignin content.

2. Methods

2.1. Materials

Switchgrass samples used in this experiment were a generous gift from the University of Tennessee. The grass was received as air dried with an average total solid content of 94% dry matter as determined by Laboratory Analytical Procedure (LAP) 012 of the National Renewable Energy Lab (NREL). The switchgrass was milled with a laboratory blender. The sizes of the milled particles used in this study varied between 1–7 mm in length and 0.02–0.6 mm in width. Chemicals used in this experiment include sodium hydroxide, sulfuric acid, ethanol and tetracycline. These were all purchased from Fisher Scientific. Cellulase from *Aspergillus niger* (activity = 1.0 U/mg) and β-glucosidase from almonds (activity = 30 U/mg) was purchased from Sigma–Aldrich in St. Louis, MO.

2.2. Compositional analysis

The initial chemical composition of the untreated switchgrass was determined by the procedures outlined by the National Renewable Energy Laboratory (NREL). The total solids content of our switchgrass was determined as the percentage mass of solids remaining after the volatile component (moisture) was removed by heating. Each sample was heated to 105 °C until a constant weight was obtained (NREL LAP 001). Percentage extractive was determined as the ethanol soluble material after extraction in a Soxhlet extractor for 24 h (NREL LAP 010). Percentage ash content was determined by weight measurements of the initial and final weights after igniting the sample at 575 °C in a muffle furnace for 24 h (NREL LAP 005). The results indicated our grass was comprised of 15% extractives, 3.7% ash, 14.81% acid insoluble lignin. About 1.60% acid soluble lignin and the balance being cellulose (37%) and hemicellulose (28%).

2.3. Alkali pretreatment

Samples were soaked in NaOH solutions of concentrations ranging from 0.5% to 10% w/v in 800 ml beakers at a biomass loading of 0.15 g/ml. They were treated at 85–90 °C for 1 h. Samples were thoroughly washed with de-ionized water to neutralize the base, air dried and stored. Wet samples were refrigerated for further SEM analysis.

2.4. Dilute acid pretreatment

Switchgrass samples were soaked in dilute acid solutions of different concentrations ranging from 0.5% to 6% v/v in capped 250 ml Duran bottles at a biomass loading of 0.15 g/ml. Pretreatment was done in an autoclave at 120 °C for 1 h. Samples were cooled, washed thoroughly with de-ionized water to neutralize the acid. Samples were air dried and stored. Wet samples were refrigerated for further SEM analysis.

2.5. Hot water pretreatment

Switchgrass samples were immersed in boiling water in 800 ml beakers at a biomass loading of 0.025 g/ml and allowed to boil for 1 h. They were washed thoroughly with de-ionized water, air dried and stored for further analysis. Wet samples were refrigerated for further SEM analysis.

2.6. Enzymatic hydrolysis

The enzymatic hydrolysis of the untreated and pre-treated switchgrass samples was carried out adhering to the analytical procedures (LAP 009) of the NREL. The only modification was that the reaction volume was scaled up by a factor of 5. The biomass samples were hydrolyzed using cellulase and β-glucosidase in a 0.1 M sodium acetate buffer with a pH of 4.7–4.9 at a biomass loading of 0.027 g/ml of buffer. The cellulase and β-glucosidase loadings were 60 FPU/g and 64 U/g of cellulose respectively. The reaction was carried out in 250 ml capped Duran bottles in a shaker maintained at 50 °C at 100 rpm for 48 h. About 40 μl/ml of reaction mixture of tetracycline was added to inhibit the growth of microbes that could inhibit the reaction. 0.5 ml samples were taken and filtered through Acrodisc syringe filters containing a Tuffryn membrane with 0.45 μm pores before being analyzed for reducing sugars. Results are expressed as grams of reducing sugar per gram of biomass.

2.7. Sugar measurement

Reducing sugar concentrations from hydrolysis experiments were determined using the dinitrosalicylic (DNS) acid method (Ghose, 1987; Miller, 1959).

2.8. Acid soluble/insoluble lignin test

After each pretreatment the lignin content in each sample was assessed using sulfuric acid (H_2S0_4) treatment. Approximately 0.3 g of sample was mixed and hydrolyzed with 3 ml of 72% H_2SO_4 in 16×100 mm glass test tubes for 2 h. The mixture was diluted to 4% with de-ionized water and subsequently autoclaved at 121 °C for 1 h. The hydrolysis solution was cooled to room temperature and vacuum filtered through already ignited filtering crucibles. The crucible and its contents were dried at 105 °C to a constant weight in a drying oven and subsequently cooled in a desiccator before the weight was recorded. The crucible was further ignited at 575 °C in a muffle furnace for about 3 h after which it was cooled and reweighed. The percentage total solids experiment was performed alongside this experiment. The values obtained from weight measurements and total solids experiments were used to calculate the percentage acid insoluble lignin using the procedures given in NREL LAP 003. Acid soluble lignin was determined by UV spectrophotometry analysis of the filtrate obtained from the acid insoluble lignin experiment. The UV absorbance reading was taken at 205 nm and the values obtained were used to calculate the acid soluble lignin using the procedures in NREL LAP 004. All experiments were repeated for result confirmatory purposes. All analysis was carried out in triplicates and the average values are reported in this text.

2.9. SEM sample preparation

To determine the extent at which the aforementioned objectives of pretreatment were met, SEM images were taken for all samples used in this study. Prior studies (Wong et al., 1998; Est-