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Pretreatment of Switchgrass by Ammonia Fiber Explosion (AFEX)

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Abstract

The effects of ammonia fiber explosion (AFEX) pretreatment of switch grass using its major process variables are reported. The optimal pretreatment conditions for switchgrass were found to be near 100°C reactor temperature, and ammonia loading of 1:1 kg of ammonia: kg of dry matter with 80% moisture content (dry weight basis [dwb]) at 5 min residence time. Hydrolysis results of AFEX-treated and untreated samples showed 93% vs 16% glucan conversion, respectively. The ethanol yield of optimized AFEX-treated switchgrass was measured to be about 0.2 g ethanol/g dry biomass, which is 2.5 times more than that of the untreated sample.

Index Entries: Ammonia fiber explosion (AFEX); switchgrass; enzymatic hydrolysis; simultaneous saccharification and fermentation.

Introduction

Switchgrass (*Panicum virgatum*, L., Poaceae) is a sod-forming, warm season grass, which combines good forage attributes and soil conservation benefits (*1*), along with a wide range of other environmental benefits, as an alternative energy crop (*2*). Development of a significant national capacity to utilize perennial forage crops such as switchgrass as raw materials for biofuel production could benefit our agricultural economy by providing an important new source of income for farmers. In addition, lignocellulosic biomass is a potentially attractive sustainable energy resource to meet US energy demands as well as raw material needs.

One way of generating ethanol from pretreated lignocellulosic material is through the SSF process (*3*), which is a combination of chemical and/or physical digestion followed by microbial fermentation. In this process biomass is broken down to less complex species that can be enzymatically hydrolyzed to fermentable sugars. AFEX-treated samples are completely

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solubilized in the SSF process, thus increasing the interaction of yeast and enzymes with biomass, thereby leading to higher ethanol yield.

There are two major processing impediments to producing economically viable commercial ethanol from biomass such as switchgrass. One obstacle is the inherent resistance of lignocellulosic materials toward conversion to fermentable sugars (*4*). In order to improve the efficiency of enzymatic hydrolysis, a pretreatment step is necessary to make the structural carbohydrate fraction accessible to cellulase enzymes. A unique and effective biomass pretreatment called ammonia fiber explosion (AFEX) is under development (*5*). Previous work (*6–8*) has demonstrated that the AFEX process substantially enhances the digestibility of lignocellulosic biomass.

Another economically important issue in biomass conversion is the cost of enzyme. High cost of the enzymes has been a major obstacle to the commercialization of biomass hydrolysis. One way to reduce this cost is to use as little enzyme per unit of biomass hydrolyzed as possible without sacrificing the ethanol yield. Previous work has shown that effective enzymatic hydrolysis of AFEX-treated biomass at enzyme loadings as low as 7 FPU/g of glucan was achieved by adjusting the pretreatment parameters (*9*).

The main focus of this article has been to optimize all the major factors that influence the effectiveness of the pretreatment of switchgrass in AFEX process. Ammonia loading, sample moisture content, and reactor temperature have all been optimized to maximize the conversion of glucan and xylan to fermentable sugars using a fixed amount of enzyme.

Materials and Methods

Substrate

The biomass material studied was switchgrass. Moisture content of the samples at the time of pretreatment was measured to be about 5.5% dwb. National Renewable Energy Laboratories (NREL) provided samples of switchgrass with composition of 34.2% glucan, 22.1% xylan, and 3.1% arabinan plus galactan. Liquid anhydrous ammonia was obtained from AGA (Lansing, MI).

AFEX Treatment

The bench-top reactor consists of a 300 mL stainless-steel pressure vessel (PARR Instrument Co., IL) (Fig. 1). The vessel was loaded with switchgrass adjusted to the desired moisture content. The vessel was topped up with 1 mm-diameter steel spheres to occupy the void space, thus minimizing transformation of the ammonia from liquid to gas phase during ammonia loading. The lid was then bolted shut. Using the precalibrated ammonia sample cylinder, the predetermined amount of liquid ammonia was charged to the vessel. A 400 W PARR heating mantle heated the vessel

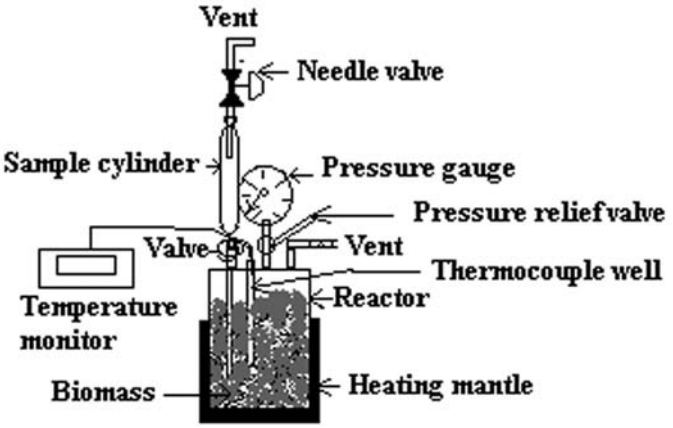

Fig. 1. Schematic diagram of laboratory AFEX apparatus.

to a set temperature with a heating up time of about 30 min. After holding the vessel at the target temperature for 5 min residence time, the vent valve was rapidly opened to explosively relieve the pressure. The treated samples were removed and allowed to stand overnight in a fume hood to evaporate the residual ammonia.

Enzymatic Hydrolysis

For enzymatic hydrolysis the NREL standard protocol (LAP-009) was followed. All the samples were hydrolyzed in a pH 4.8 citrate buffer with the desired cellulase enzyme (Spezyme Cp provided by NREL, CAS 9012-528) at a loading of 15 FPU/g of glucan and β-glucosidase (Sigma, St. Louis, MO) at a loading of 40 IU/g of glucan. All the samples were hydrolyzed at 50°C with gentle rotation (75 rpm) for a period of 168 h. At predetermined time intervals (0, 3, 6, 24, 48, 72, and 168 h), 1 mL of hydrolysate was taken for sugar analysis. The Waters (Milford, MA) High Performance Liquid Chromatograph (HPLC) system is used for sugar analysis. This unit equipped with a Bio-Rad (Richmond, CA) Aminex HPX-87P carbohydrate analysis column and a Bio-Rad Deashing Cartridge Micro-Guard column. The mobile phase used was degassed HPLC water at a flow rate of 0.6 mL/min and 85°C column temperature. The injection volume was 20 µL with a run time of 21 min.

Simultaneous Saccharification and Fermentation (SSF)

SSF experiments were conducted according to NREL standard protocol (LAP-008). The SSF flasks were equipped with water traps to maintain anaerobic conditions and were incubated at 37°C with gentle rotation (130 rpm) for a period of 168 h.

At time intervals of 0, 3, 6, 24, 48, 72, 96, and 168 h, 2 mL was removed aseptically from each flask. The sample was centrifuged; and the supernatant

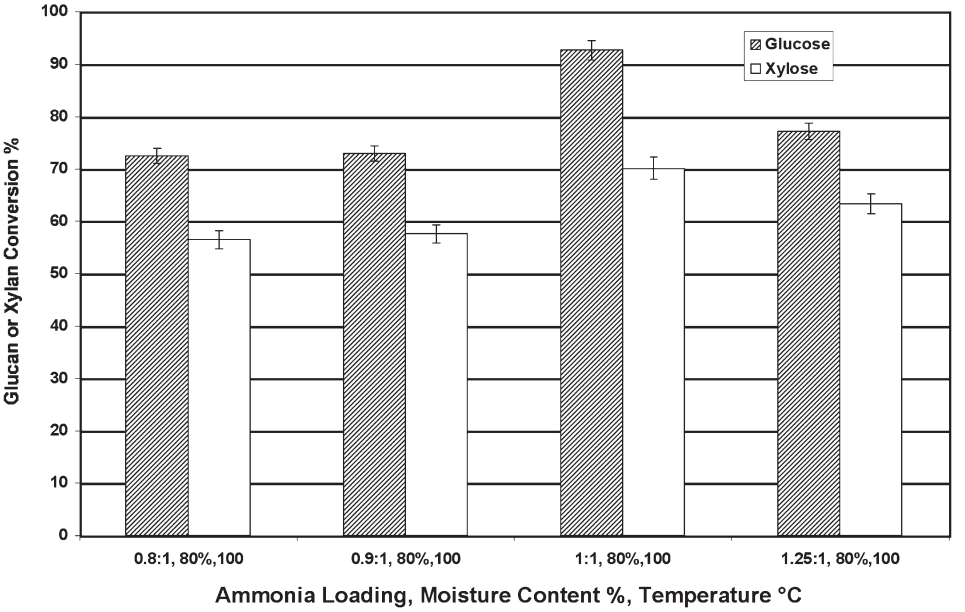

Fig. 2. Effects of ammonia loading on enzymatic conversion of glucan and xylan for AFEX treatment of switchgrass at 100°C reactor temperature and 80% (dwb) sample moisture content (168 h of hydrolysis at 15 FPU/g glucan).

was filtered for sugar analysis by HPLC and ethanol analysis by an AutoSystem Perkin Elmer GC (Boston, MA) unit equipped with an Altech (Deerfield, IL) Econo-Cap EC-1000 column. The injection temperature was set at 250°C and the detector temperature at 275°C. The column temperature was held at 110°C with a run time of 2 min for each sample.

Results and Discussion

Effects of Ammonia to Biomass Ratio on the Enzymatic Hydrolysis of AFEX-Treated Switchgrass

Figure 2 shows the effect of ammonia to biomass ratio (0.8:1, 0.9:1, 1:1, and 1.25:1 kg of anhydrous ammonia: kg of dry biomass) on the subsequent enzymatic hydrolysis of AFEX-treated switchgrass. This figure compares the effect of ammonia loading on conversion of glucan to glucose of AFEX-treated sample. Both glucan and xylan conversions reach their maximum level at approx 1 kg ammonia: 1 kg dwb biomass.

It is known that ammonia can react with lignocellulosic materials by ammonolysis of ester crosslinks of some uronic acids with the xylan units and by cleaving the bonds linking hemicellulose and lignin (*10*). However, it is evident from Fig. 3 that further increases in ammonia loading beyond about 1:1 ratio decrease glucan conversion. It is possible that extra liquid

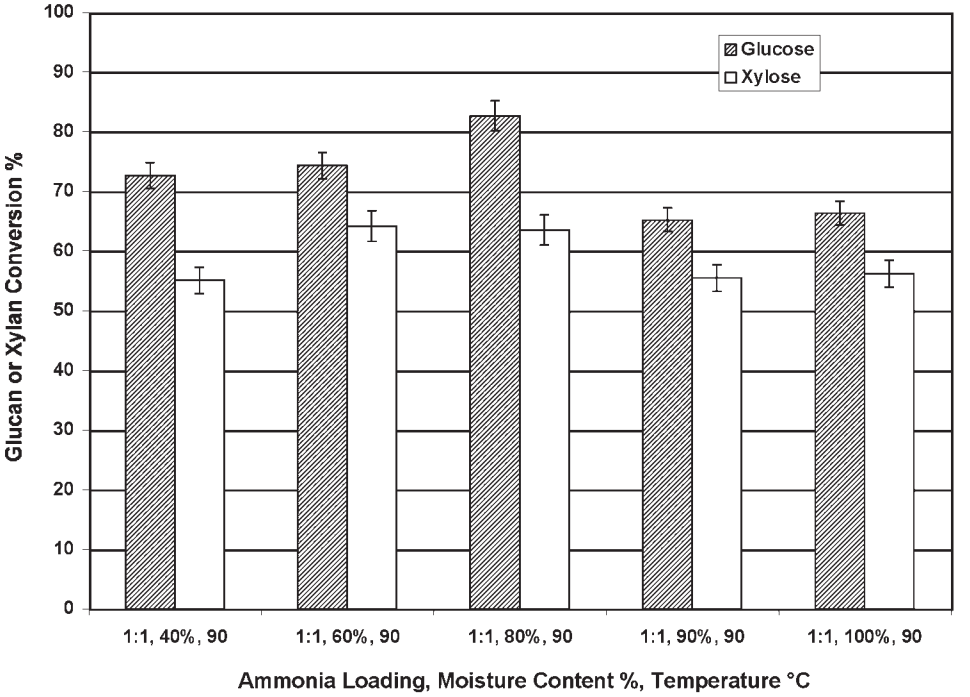

Fig. 3. Effects of sample moisture content on glucan and xylan conversion of AFEX treated switchgrass at 90°C reactor temperature and 1:1 kg of NH3: kg of dry biomass ammonia loading (168 h of hydrolysis at 15 FPU/g glucan).

ammonia plasticizes (*11*) the cellulose and thereby reduces the disruptive effect of sudden pressure release.

Effects of Sample Moisture Content on the Enzymatic Hydrolysis of AFEX-Treated Switchgrass

Figure 3 shows the effect of moisture content (40%, 60%, 80%, 90%, and 100% dwb) on the glucan and xylan conversion at a fixed temperature and ammonia loading. Glucan conversion increased with increasing moisture content and attained its maximum value at approximately an 80% moisture content. Xylan conversion also showed the same trend as glucan conversion in response to moisture content. Even though at higher moisture content ammonia is more diluted, apparently the affinity of ammonia for cellulose and hemicellulose is still sufficiently strong so that the ammonia reacts adequately with these macromolecules. Previous studies have postulated that the moisture in the biomass allows formation of ammonium hydroxide, which hydrolyzes hemicellulose and thereby enhances the overall effect of AFEX treatment. Based on these data 80% (dwb) is selected as the optimum moisture content for AFEX treatment of switchgrass.

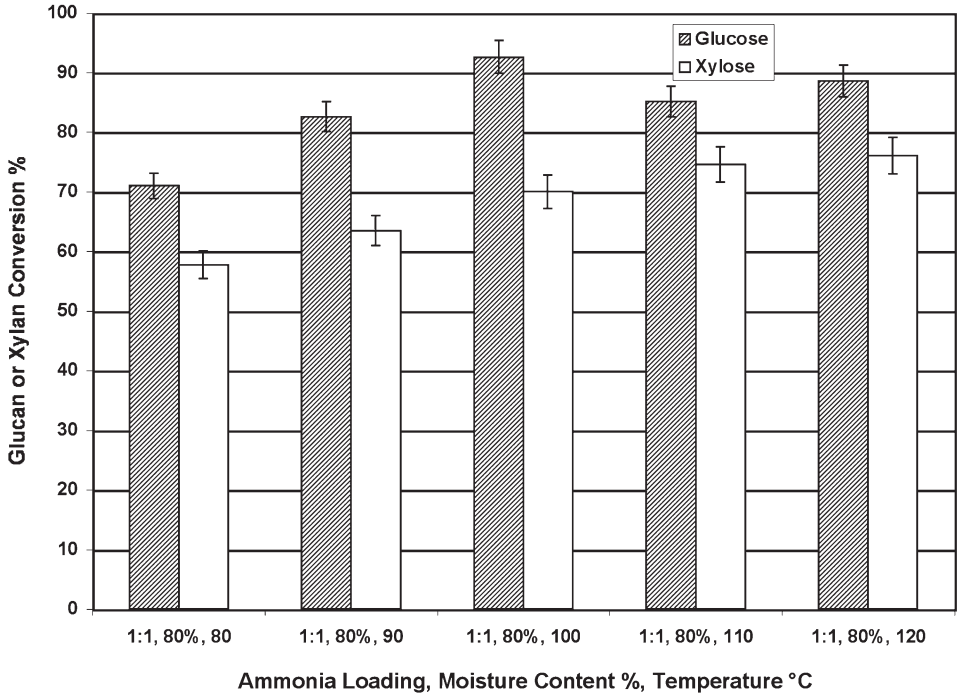

Fig. 4. Effects of reactor temperature on enzymatic conversion of glucan and xylan for AFEX-treated switchgrass at 80% (dwb) sample moisture content and 1:1 kg of NH_3: kg of dry biomass ammonia loading (168 h of hydrolysis at 15 FPU/g glucan).

Effects of Reactor Temperature on the Enzymatic Hydrolysis of AFEX-Treated Switchgrass

As is apparent from Fig. 4, at optimum conditions of 1:1 ammonia loading and 80% dwb moisture content, cellulose conversion peaks at a reactor temperature of 100°C. Both glucan and xylan conversions increase as the temperature is increased from 80 to 90°C and 100°C. Further increases in temperature decrease glucan conversion. The exact mechanisms by which temperatures beyond 100°C reduce glucan conversion are unclear and are under investigation at this time.

Enzymatic Hydrolysis Time Profile

Figure 5 illustrates glucan and xylan conversions versus time for optimized AFEX conditions of 80% sample moisture content, 100°C reactor temperature, and 1:1 kg ammonia: kg switchgrass, ammonia loading. Both the initial rate and final extent of conversion are increased by AFEX treatment. As these data suggest, AFEX treatment increases glucan conversion by about six fold and xylan conversion by almost 23 fold.

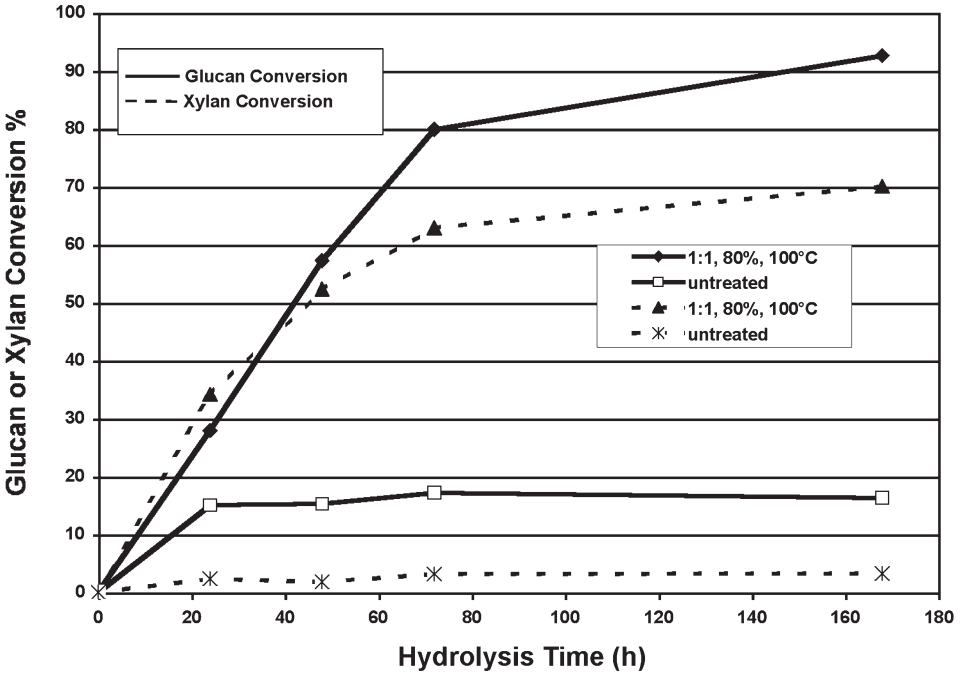

Fig. 5. Glucan and xylan conversion time profile for AFEX treated and untreated switchgrass samples.

Simultaneous Saccharification and Fermentation (SSF)

The ultimate goal of the AFEX pretreatment is to increase the yield of products such as ethanol by increasing the digestibility of the biomass. Therefore, the optimally AFEX-treated switchgrass samples were subjected to SSF analysis to evaluate ethanol production potential. As Fig. 6 shows, throughout the SSF process, glucose produced by the cellulase enzyme was almost completely consumed by the yeast and converted to ethanol. The rate of ethanol production was quite rapid during the first 24 h of the fermentation. The AFEX-treated sample attained the maximum amount of ethanol after 96 h of the SSF process. As seen in Fig. 6, AFEX-treated sample produced almost 2.5 times more ethanol than untreated sample.

On the other hand, xylose concentration levels off after sharply increasing during the first 24 h of fermentation. The yeast (*Saccharomyces cerevisiae* D_5A) used in this SSF study does not have the ability to utilize xylose to convert to ethanol. In addition, as shown in Fig. 7, the AFEX treated switchgrass sample is almost completely solubilized by SSF compared to untreated sample. An essentially fluid sample compared to high solids samples not only increases the interaction of enzymes and microorganisms with the substrate, thus maximizing the yield, but also increases the ease of materials handling in the whole process.

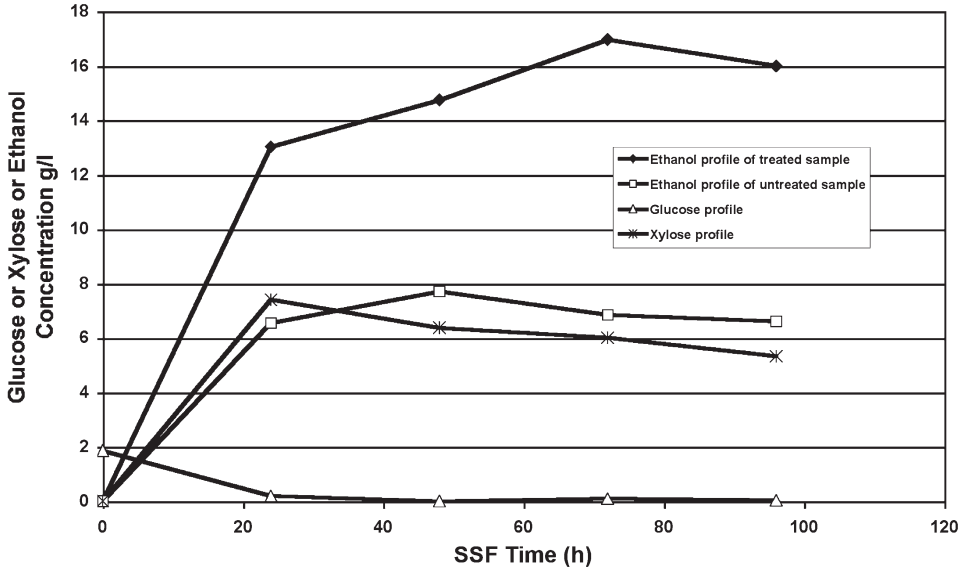

Fig. 6. Ethanol, glucose, and xylose concentration time profile in yeast SSF.

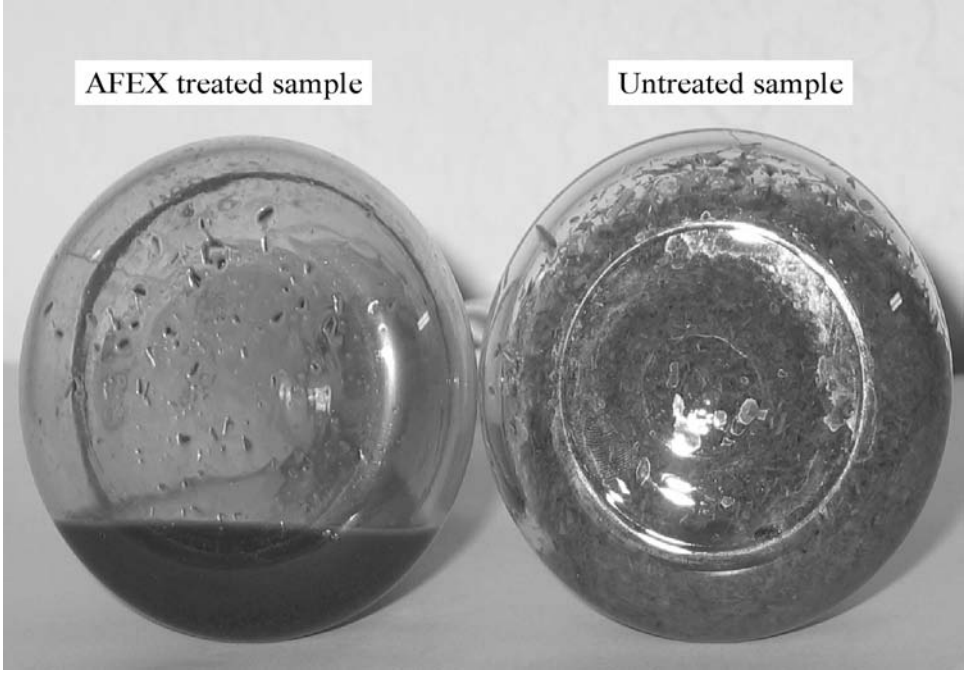

Fig. 7. Flasks of AFEX treated and untreated switchgrass samples after 168 h of SSF.

Conclusion

Increasing ammonia loading along with increased reactor temperature and switchgrass moisture content enhance the effect of the AFEX treatment

up to a point. Our experimental results show that 1:1 kg ammonia: kg biomass loading at 80% dwb moisture content and 100°C reactor temperature for a residence time of 5 min, provide most effective conditions for AFEX treatment of switchgrass. Furthermore, enzymatic hydrolysis of AFEX-treated switchgrass showed almost 93% glucan conversion and 70% xylan conversion vs 16% and 3% for untreated samples, respectively.

AFEX-treated switchgrass sample produced almost 2.5 times more ethanol than untreated sample in SSF process. The ethanol yield of optimized AFEX-treated switchgrass was measured to be about 0.2 g ethanol/g dry biomass.

At the end of the SSF period, while there were still considerable amounts of essentially intact solids in the flask containing untreated sample, the AFEX-treated sample was all solubilized and had lost its characteristic structure (Fig. 7). This solubilization characteristic not only increases the interaction between enzyme and yeast with the treated biomass, but a fluid process stream makes engineering of materials handling equipment much easier.

Acknowledgments

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