Optimizing Alkali-cellulase Processing of Biomass into Glucose

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The alkali-cellulase processing of biomass into glucose near where it is grown has already been demonstrated at laboratory scale. Glucose can be fermented locally or transported to distant facilities for the production of bioethanol as fuel. This renewable energy process uses materials and methods that are readily available and that can be implemented at local or regional sites near growing fields. This study evaluated the effects on glucose production of different durations and amounts of NaOH pretreatment as well as different lengths of time for adsorption of cellulase. The pretreatment of corn stover (CS) with NaOH at 0.1 g/g CS for 6 h at a temperature of 100 °C resulted in the most acceptable glucose release following enzymatic hydrolysis. The exposure of pretreated CS solids to cellulase for 1 h resulted in the most acceptable release of glucose following the volume expansion at 10-fold dilution. The residual solids remaining after 3 h of enzymatic hydrolysis can be recycled to increase yields. The resulting glucose solution can be concentrated to minimize transportation costs when delivered to conventional grain fermentation facilities. This study introduced new conditions that enhanced practicality of the alkali-cellulase processing of biomass by allowing the processing time to be reduced to 10 h.

Keywords: Biomass conversion; Cellulose; Pretreatment; Cellulase; Alkali-cellulase; Alkcell; Bioethanol

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INTRODUCTION

Increasing attention continues to be paid to the local processing of biomass to facilitate its conversion to fuel ethanol at fermentation facilities at a distance from the growing fields (Digman *et al.* 2010; Eranki *et al.* 2011). Advantages include enhanced transportation feasibility and optimization of fermentation operations, both of which are considered necessary for the cost-effective production of bioethanol (Richard 2010; Spatari *et al.* 2010). Alkali-cellulase (Alkcell) processing is one approach that appears feasible for implementation near growing sites or at regional centers (Savarese 2013). This process involves pretreatment of biomass with strong bases followed by enzymatic hydrolysis that has been identified as one of the possible approaches to cellulose saccharification (Wyman *et al.* 2011; McIntosh and Vancov 2011). However, the Alkcell Process for practical local implementation requires better characterization. Alkcell processing utilizes NaOH or other strong alkalis to pretreat biomass under mild conditions. This is followed by cellulase adsorption to the pretreated solids and the release of glucose using a volume expansion technique (Savarese 2013). Glucose and other products of the enzymatic hydrolysis have been shown to inhibit cellulase activity (Holtzapple *et al.* 1990; Xiao *et al.* 2004.). Using volume expansion, the effects of these inhibitors are reduced, resulting in an enhanced release of glucose.

The Alkcell process has been demonstrated in the laboratory using materials and methods that are not complex and that can be readily implemented on a larger scale at local sites. Optimizing the Alkcell processing conditions will favor cost reduction. The current work examined various conditions that could increase process efficiency. A full cost analysis has not been done, but the savings implications are evident. Some technical features of this current work were fully described in a previous report (Savarese 2013); however, all relevant information is presented here.

The glucose solution produced by the Alkcell Process will need to be concentrated to reduce transportation cost. Membrane or thermal concentration can be performed locally and will require further study (Jiao *et al.* 2004; Garcia-Castello *et al.* 2009). Even drying the glucose solution to produce a glucose powder for transportation is possible, but complete drying is energy-intensive and would require additional equipment that probably render drying not economically feasible. However, the prospect of delivering fermentation-ready glucose as concentrate to any conventional grain fermentation facility should result in a meaningful cost reduction, enough to offset the current high cost of lignocellulose bioethanol production (Pimentel *et al.* 2009).

EXPERIMENTAL

Materials

Corn stover (CS) was used in these experiments because it is readily susceptible to Alkcell processing. The corn stover was harvested locally and shredded coarsely using an electric garden shredder (McCulloch Model MCS1400). To accommodate laboratory glassware, the shredded material was further comminuted into centimeter-size particles using a kitchen blender.

The cellulase used in these experiments was Accellerase 1500, supplied by Genencor. Accellerase 1500 is composed of endoglucanase (2200 to 2800 CMC U/g) and beta-glucosidase (450 to 775 pNPG U/g). Each experiment used 2 mL of Accellerase 1500 and 5 g of dry CS in accordance with information given by the product data sheet. The enzymatic hydrolysis was done at 60 °C and at pH 4.5. Gentle agitation was provided by magnetic stirring bars at the lowest setting.

Methods

The work was done at laboratory scale, but could be adapted to pilot operation scale using readily available equipment and materials. For the alkali pretreatment, 5 g of dry CS and varying amounts of NaOH were suspended in 200 mL of tap water in 500 mL Erlenmeyer flasks. The alkali pretreatment was conducted at 100 °C, as this and other mild temperatures have proven effective with lime pretreatments of switchgrass (Xu *et al.* 2010). Furthermore, boiling can be maintained without strict temperature control, and the resulting thermal agitation provides sufficient mixing. Because short exposure to alkali has been reported as a possibility (McIntosh and Vancov 2011), the alkali pretreatment was conducted for durations of 12, 6, 4, and 2 h. After the NaOH pretreatment, the solids were separated using a 1 mm wire mesh. The solids were washed twice with 100 mL of 30 mM citrate buffer at pH 4.5, and the wash liquid was drained. The solids were then suspended in 50 mL of citrate buffer at pH 4.5, and cellulase was added using a pipette.

Glucose was measured using a hand held meter with glucose oxidase assay strips (Reveal glucose meter). The use of this measurement method on standard glucose

solutions conducted in 30 mM citrate buffer at pH 4.5 at 40 °C demonstrated the accuracy, precision, and reliability of the method. When standard glucose was tested, this method assayed 20% between repeat samples. Previously, standard deviation of repeat experimental samples varied from 13 to 30% (Savarese 2013). Often, both Nelson-Somogyi (NS) and 3,5-dinitrosalicylic acid (DNS) assays are used to determine the quantity of reducing sugars such as glucose. However, these assays have been found to provide substantially different results when used to determine cellulase activities (Gusakov *et al.* 2011) and will test positive for other reducing sugars that may accompany glucose. HPLC avoids this problem, but is not suited for the immediate determination of glucose levels as might be done locally near a growing site.

A glass thermometer was dipped into the liquid to be assayed. When the temperature reached 40 °C, the tip was touched to the assay strip. This method requires a drop or less of each sample and is specific to glucose. It does not test positive for the xylose or glucan polymers that may be released into the liquid phase during enzymatic hydrolysis. The measurements were done in duplicate.

In a previous report, controls were used to determine the effects of NaOH pretreatment and enzymatic hydrolysis (Savarese 2013). The Alkcell process was carried out beginning with pretreatment, and including enzymatic hydrolysis as well as volume expansion. The glucose production was absent or minimal when the NaOH pretreatment was omitted. Likewise, the glucose release was absent or minimal when the pretreated biomass underwent a mock enzymatic hydrolysis step but cellulase was omitted.

RESULTS AND DISCUSSION

Effects of NaOH Pretreatment Loading

Five grams of CS were pretreated with NaOH at loadings of 1.0 g of NaOH/5 g CS, 0.75 g NaOH/5 g CS, and 0.5 g NaOH/5 g CS for 12 h at 100 °C. The third loading was the highest that had been tested in previous work on the pretreatment of corn stover for enzymatic saccharification; it was found that lower NaOH loadings were inferior (Chen *et al.* 2013). After washing, the solids were suspended in 50 mL of citrate buffer and enzymes added. The time course of glucose production is shown in Fig. 1. Before enzyme addition, washed NaOH-pretreated solids when suspended in 50 mL of citrate buffer (0 h 50 mL) produced no measurable glucose. One hour after the cellulase was added (1 h 50 mL), glucose release was nearly the same for all three NaOH loadings used in the pretreatment. At this time the suspension was diluted to 500 mL (0 h 500 mL), and the glucose increased by a factor of 3.75 for all three NaOH pretreatment loadings. At three hours (3 h 500 mL) the glucose had increased by nearly five times for all three loadings. At 24 h there was a continued release of glucose from the 1.0 g NaOH/5 g CS loading, whereas for the two lower loadings of NaOH, there was a tapering off of glucose production. The greater production of glucose from the 0.5 g NaOH pretreatment compared with the 0.75 g NaOH pretreatment is anomalous. However, the amount of glucose released combined with the lower NaOH usage identified the 0.5 g NaOH/5 g CS as the superior pretreatment.

The glucose yield at 3 h after volume expansion appeared acceptable. The incremental increase in glucose production after 24 h did not warrant extending the time beyond 3 h, given the cost of continuing the hydrolysis.

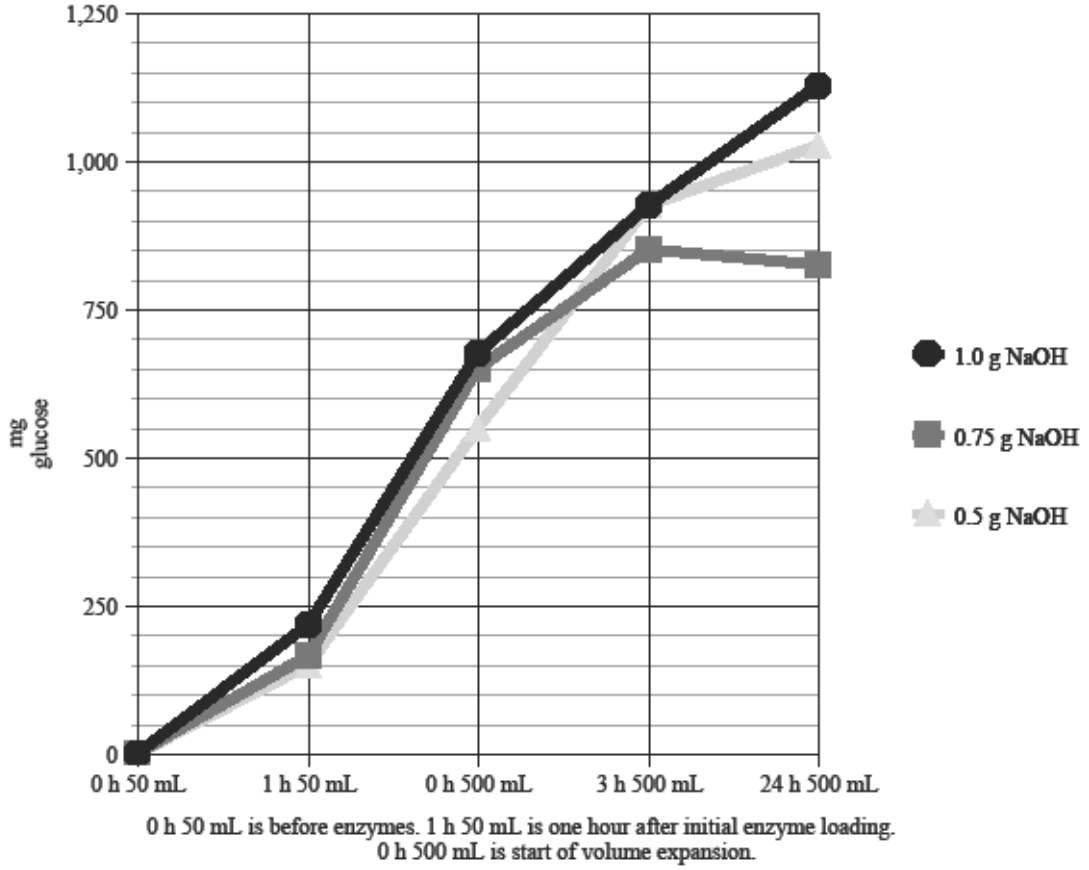

Fig. 1. Glucose produced from 5 g of corn stover. Duration and volume of NaOH pretreatment lasting 12 h at 100 °C and subsequent enzymatic hydrolysis. Glucose is the mean of duplicate measurements while in 50 mL citrate buffer and before enzymatic hydrolysis (0 h, 50 mL), after one hour of enzyme exposure (1 h, 50 mL) [2 mL of Accellerase 1500], immediately after dilution [volume expansion] (0 h, 500 mL), and 3 h and 24 h thereafter.

Effect of Duration of NaOH Pretreatment

NaOH pretreatment lasting 12 h at 100 °C is costly in energy input and time. Lower temperature is one possibility for increasing cost-effectiveness. In one study, 2% (w/v) NaOH at 50 °C for 96 h was effective in rendering switchgrass susceptible to enzymatic hydrolysis (Xu *et al.* 2010). However, pretreatment for a shorter length of time at 100 °C seems more practical, as well as providing thermal agitation for mixing. Pretreatments were conducted for 2, 4, and 6 h, and then the specimens were subjected to enzymatic hydrolysis.

The results shown in Fig. 2 demonstrated that the three shorter times were not as productive as the 12-h pretreatment shown in Fig. 1. However, the savings in energy and time should offset the 20% or so lower yield of glucose for the 6 h NaOH pretreatment. Again, continuing the hydrolysis to 24 h provided some additional glucose, but would be more costly. Furthermore, the recycling of residual solids will produce more glucose. Therefore, pretreatment with NaOH for 6 h at 100 °C optimized the process in terms of glucose yield, processing time, and cost of materials.

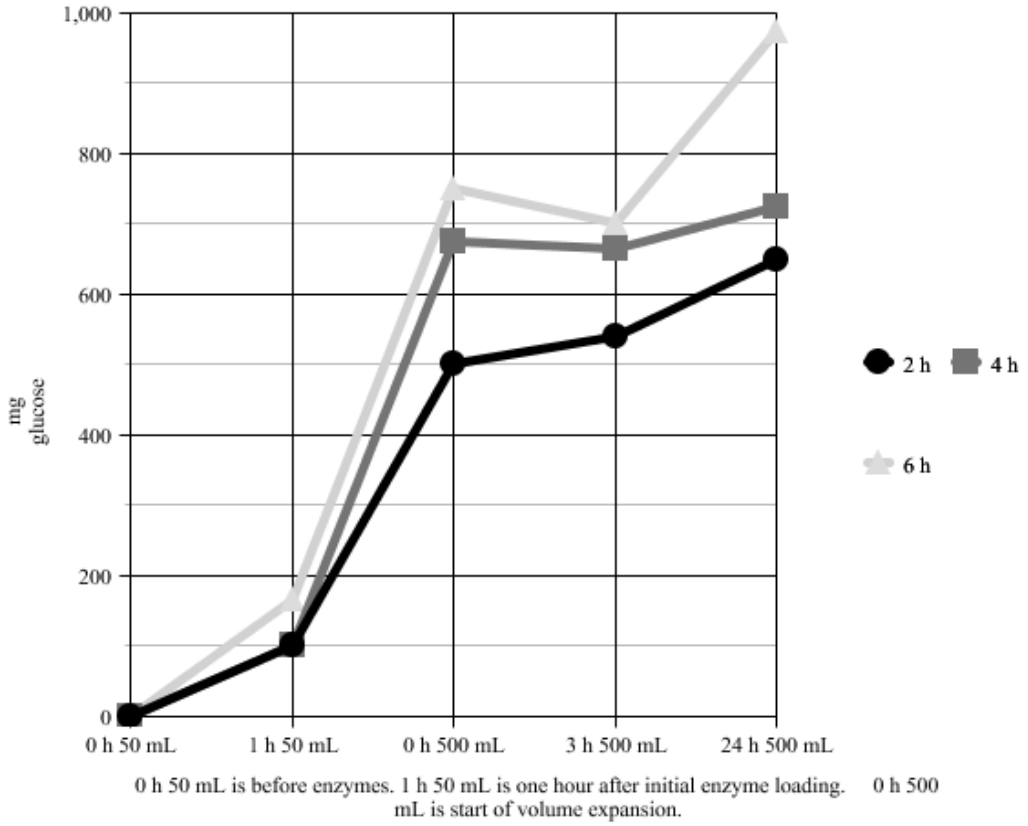

Fig. 2. Glucose produced from 5 g of corn stover. Duration and volume of NaOH pretreatment for 2, 4, and 6 h at 100 °C and subsequent enzymatic hydrolysis. Glucose is the mean of duplicate measurements while in 50 mL citrate buffer and before enzymatic hydrolysis (0 h 50 mL), after one hour of enzyme exposure (1 h, 50 mL) [2 mL of Accellerase 1500], immediately after dilution [volume expansion] (0 h, 500 mL), and 3 h and 24 h thereafter.

Effect of Duration of Cellulase Adsorption to Pretreated Biomass

Before the 10-fold volume expansion, the exposure of NaOH-pretreated corn stover to cellulase was kept to one hour. To determine if less time for enzyme adsorption to the biomass would be feasible, pretreated corn stover was exposed to cellulase for 15, 30, and 45 min before volume expansion. These results are shown in Fig. 3. The 45 min exposure appears to have been the most effective and most comparable to the one hour of exposure shown in Figs. 1 and 2. Exposure for less time resulted in less production of glucose. Therefore, cellulase does require a minimum time to saturate the biomass and adsorb to cellulose sites where hydrolysis occurs. Less time will allow less cellulase to adsorb to the cellulose, resulting in release of less glucose when undergoing volume expansion. Submitting biomass to cellulase for longer than the minimum required time for maximal adsorption does not increase glucose release when volume expanded and may result in greater release of glucose in the cellulase bath that will be lost to recovery.

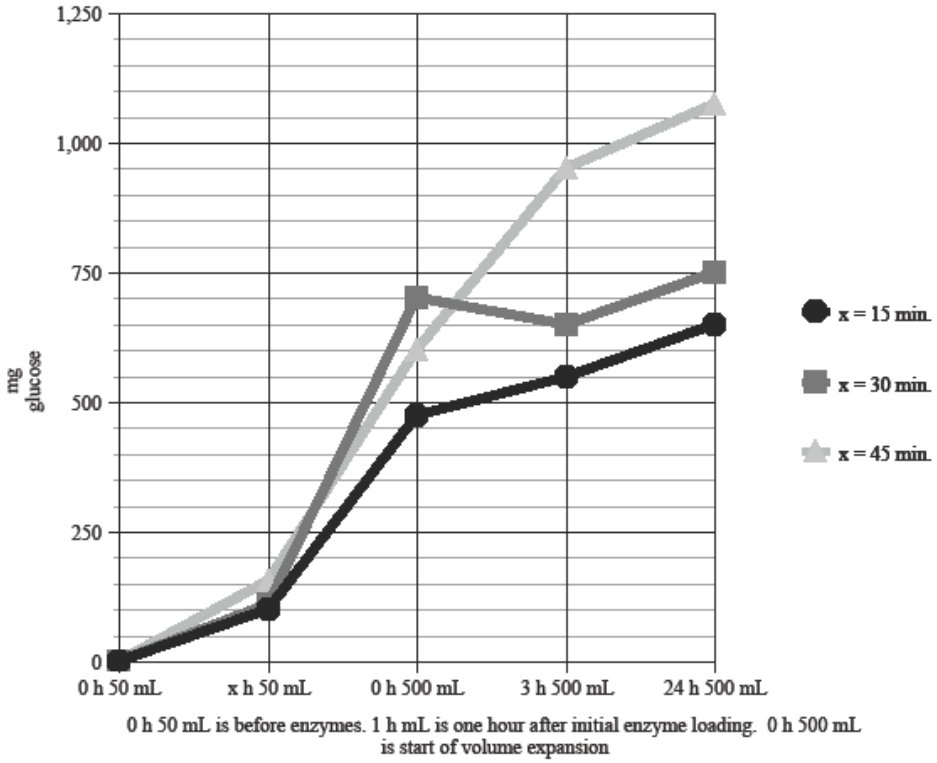

Fig. 3. Glucose produced from 5 g of corn stover. Duration and volume of cellulase exposure for 15 min, 30 min, and 45 min following 0.5 g NaOH pretreatment at 100 °C for 4 h. Glucose is the mean of duplicate measurements while in 50 mL citrate buffer before enzyme exposure (0 h, 50 mL), after 15 min, 30 min, and 45 min of enzyme exposure (1 h, 50 mL), immediately after dilution [volume expansion] (0 h, 500 mL), and 3 h and 24 h thereafter.

Loss of Glucose in Pretreatment Liquid and in Residual Solids

NaOH pretreatment causes the release of some glucose that will be lost from enzyme conversion. Likewise, residual solids from the Alkcell Process contain cellulose that could undergo further hydrolysis to glucose. Table 1 shows the amount of glucose lost in the NaOH pretreatment liquid. It also shows the amount of residual solids left behind under various conditions.

Table 1. Total Glucose in a 200-mL Water Suspension of 5 g of Corn Stover with NaOH at 1.0 or 0.5 g Heated at 100 °C*

NaOH/5 g CS	1.0 g	0.5 g	0.5 g	0.5 g	0.5 g
Time at T=100°C	12 h	4 h	4 h	4 h	6 h
Glucose in NaOH solution after heating	130 mg	106 mg	110 mg	100 mg	105 mg
Time for cellulase adsorption	1 h	45 min	30 min	15 min	1 h
Residual solids	0.7 g	1.1 g	1.5 g	1.8 g	1.2 g
Residual solids as % of original biomass	14%	22%	30%	36%	24%

Note: *Subsequent cellulase treatments are done to vary the times of adsorption. Residual solids (dry) are those remaining after NaOH and enzyme treatments.

Under the strongest pretreatment conditions tested (1.0 g NaOH for 12 h at 100 °C), there was a 30% greater loss of glucose in the NaOH pretreatment liquid than under milder conditions (0.5 g NaOH for 6 h at 100 °C). Following enzyme exposure for less than one hour, the amount of residual solids observed for the strongest pretreatment conditions was about half that observed for the milder conditions. The additional loss of potential glucose under the milder conditions is a cost consideration; however, residual solids can be reprocessed for additional glucose (Weiss *et al.* 2013; Savarese 2013). These results support the superiority of pretreatment for 6 h, and perhaps even 4 h, at 100 °C.

Corn stover contains approximately 40% cellulose so that 5 g of CS will contain approximately 2 g cellulose (Walker 2011). The lowest to highest amount of glucose released in the NaOH pretreatment liquid shown in Table 1 is 5 to 7% of the available cellulose for the 0.5 g NaOH pretreated for 6 h and the 1.0 g NaOH, respectively.

Comparison of Original Biomass with Residual Solids

The effects of the Alkcell processing on the original CS are shown in Fig. 4. The set of strongest processing conditions was compared with that of the milder conditions. The former produced a residual solid of homogenous paste, while the milder conditions resulted in a solid that still contained fibers. Under all conditions, after the removal of the residual solids, the glucose-containing liquid was clear and straw-colored.

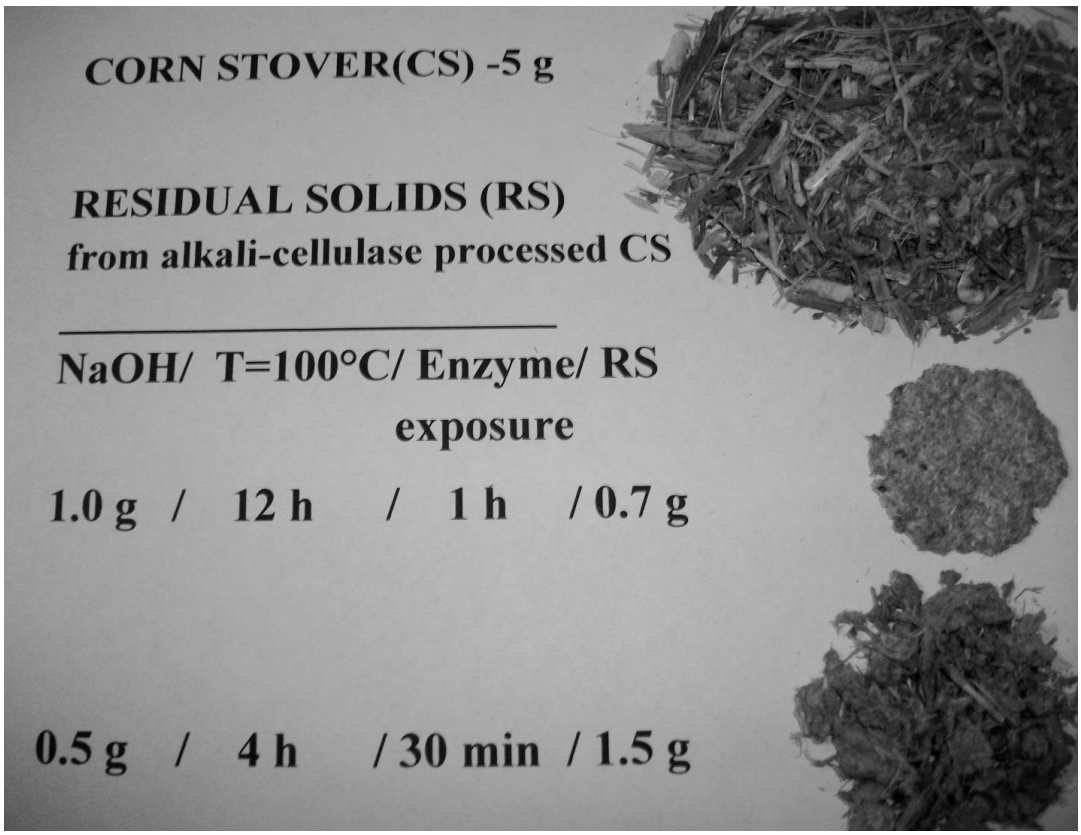

Fig. 4. 5 g corn stover before processing; residual solids resulting from Alkcell processing under two sets of conditions

CONCLUSIONS

1. This report supports the use of a new set of operating conditions that optimize the Alkcell Process and make it more practical for local implementation.
2. Corn stover pretreated with an NaOH loading of 0.1 g/g CS for 6 h at 100 °C provided the highest glucose yields following enzymatic hydrolysis under the conditions tested.
3. Glucose loss due to pretreatment was 5 to 7% of the cellulose available in corn stover.
4. Exposure to cellulase for 45 to 60 min prior to the 10-fold volume expansion provided the optimal glucose production of all the pretreatment conditions described in this report.
5. Three hours of enzymatic hydrolysis at 60 °C in 30 mM citrate buffer at pH 4.5 produced acceptable glucose yields. Hydrolysis for 24 h gave incrementally more glucose but at the cost of more time. Since residual solids can be reprocessed to yield additional glucose, extending hydrolysis to 24 h appears not to be cost effective.
6. The optimal operating conditions for the Alkcell Process determined in this work improve the feasibility at a laboratory level of converting cellulose to glucose at the biomass growing site. Glucose in a concentrated solution, or possibly dried, can be economically transported to existing grain fermentation facilities and used directly without further pretreatment.

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