Local Biomass Processing is Practical for Facilitating Fermentation to Bioethanol

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Abstract: Local processing of biomass prior to fermentation at another site has advantages in transportation savings and in fermentation facility operation. To evaluate the feasibility of treating biomass locally to produce fermentation ready glucose an alkali-cellulase process was evaluated at laboratory scale using 5 g of three types of biomass. After pretreatment with an aqueous solution of NaOH (0.5% w/v, 20% w/w-biomass), T=100°C x 12 h, corn stover and wheat straw were shown to undergo cellulase hydrolysis to glucose; however, pine chips were not as susceptible. Commercially available cellulase was capable of producing glucose within three hours from corn stover and wheat straw. The NaOH pretreated biomass was mixed with enzymes in a small volume (50 mL) to conserve water. However, glucose inhibition of cellulase appeared to limit hydrolysis. Volume expansion by ten-fold dilution (500 mL) resulted in rapid release of glucose presumably by decreasing end product inhibition. Application at a commercial level will require increased water management. The resulting glucose solution could be concentrated by thermal or membrane technology for delivery to grain fermenting facilities to be used without further processing. Solids remaining after enzyme hydrolysis can be recycled locally to produce additional glucose. Scale-up of this alkali-cellulase process for local application appears feasible given the materials and conditions evaluated in this study. Local treatment of biomass using the alkali-cellulase process to produce glucose to be transported to existing grain fermenting facilities is a novel approach based on reliable technology and has been demonstrated at laboratory scale.

Keywords: Biomass, alkali, cellulase, alkali-cellulase, pretreatment, bioethanol.

1. INTRODUCTION

Enzyme hydrolysis of cellulosic biomass as a renewable energy source to produce glucose for fermentation to bioethanol, that is, ethanol for fuel or conversion to other products is an approach that has received much attention worldwide [1]. During the last quarter century the potential of cellulosic biomass rather than petroleum as a source of liquid fuel has sparked research into cost-effective techniques for converting cellulose to ethanol [2]. Attempts to find economies in biomass conversion to fuel ethanol span decades and early on included novel techniques such as combined saccharification and fermentation [3]. Efforts to further consolidate process steps continue to be explored; for example, consolidated bioprocessing in which cellulase production, cellulose hydrolysis, and fermentation are combined has been evaluated [4]. However, even with progress challenges remain including biomass sourcing, biomass pretreatment, cellulase efficiency, and fermentation processes [5].

While the technology is feasible, delivering biomass to the conversion facility is now recognized as a major roadblock due to transportation cost and lack of infrastructure [6, 7]. One approach to offset biomass transportation difficulty is to pretreat biomass locally where it is produced, that is, near the growing site. On-farm acid pretreatment with anaerobic storage has been shown to enhance subsequent combined saccharification and fermentation to ethanol although modestly [8]. Microbiologic treatment of sorghum during harvesting and anaerobic storage has also shown promise [9]. Various other methods of biomass pretreatment have been shown to allow cellulases to hydrolyze cellulose more effectively [10].

Most pretreatments utilize severe, inconvenient, or expensive applications involving high temperature, high pressures, and corrosive chemicals [11]. Most of these approaches are not practical for local use. However, alkali pretreatment has shown promise; for example, 2% NaOH for 60 min at 121°C/15 psi is effective in removing lignin from pretreated solids while maintaining a high level of glucan [12]. This work also demonstrated that NaOH was more effective than sulfuric acid in removing lignin from biomass (cotton stalks) and rendering the pretreatment solids more susceptible to cellulose hydrolysis. Recently 2% (w/v) NaOH at 50°C for 96 h was shown to be effective in rendering switchgrass susceptible to enzyme hydrolysis [13]. They also reported that at 0.10 g/g switchgrass, calcium hydroxide at 50°C for 24 h was an effective pretreatment [14]. Even shorter times have been reported effective as pretreatment of wheat straw, 2% NaOH at 60°C for 90 min [15]. These results support the feasibility of alkali-cellulase processing under conditions achievable near the growing site or at regional centers.

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E-ISSN: 1929-6002/13

The work reported here examined alkali-cellulase processing at laboratory scale using conditions that could be scaled locally for large quantity use. Pretreatment of biomass with NaOH at a practical temperature and for a reasonable duration was explored. The alkali treated biomass was then subjected to hydrolysis with commercially available enzymes. This process was evaluated for its potential to produce glucose locally near growing sites for transport to fermentation facilities in a ready to use state.

2. MATERIALS AND METHODS

2.1. Biomass

Biomass consisted of corn stover, wheat straw, and pine chips that were harvested locally and shredded coarsely with an electric garden shredder (McCulloch Model MCS1400). To accommodate laboratory glassware the shredded material was further comminuted with a kitchen blender to centimeter size particles Five grams of air dried biomass were used in all experiments and results are presented per 5 g corn stover (CS), wheat straw (WS), or pine chips (PC).

2.2. Experimental Conditions

Work was done at laboratory scale that could reasonably be scaled to pilot operation using readily available materials and equipment. For alkali pretreatment 5 g of biomass were suspended in 200 mL of tap water in 500 mL Erlenmeyer flasks. 1 g of NaOH per 5 g of corn stover and wheat straw was chosen based on supporting literature [12, 13]. Given the recalcitrance of woody biomass 4 g of NaOH were used for each 5 g of pine chips [14]. Alkali pretreatment was conducted at 100°C (boiling) since even mild temperatures have proven effective with lime pretreatment of switchgrass [15]. Thermal mixing at boiling was the only agitation. Flasks were heated for twelve hours; however, shorter exposure to alkali might be possible [16]. After heating, solids were separated using a 1 mm wire mesh. Solids were washed twice with 100 mL of 30 mM citrate buffer pH 4.5 and the wash liquid drained. Solids were then suspended in 50 ml of citrate buffer pH 4.5 and enzymes added by pipette.

2.3. Enzymes

Cellulose hydrolyzing enzymes supplied by Genencor were Accellerase 1500, Accellerase XC, and Accellerase XY. Accellerase 1500 is a cellulase composed of endoglucanase (2200-2800 CMC U/g) and beta-glucosidase (450-775 pNPG U/g). Accellerase XC is composed of endoglucanase (1000-1400 CMC U/g) and a xylanase (2500-3800 ABXU/g). Accellerase XY is xylanase (20,000-30,000 ABXU/g). Xylanase has been found to augment the activity of cellulases [17]. Amounts of enzymes used were based on the product data sheet. Enzymatic hydrolysis was done at 60°C and pH 4.5. Gentle agitation was provided with magnetic stirring bars at lowest setting. At scale-up minimal mixing or no mixing might be possible [18].

2.4. Glucose Determination

Glucose was measured with a glucose meter using glucose oxidase assay strips (Reveal meter and strips). Validation with standard glucose solutions done in citrate buffer pH 4.5 at 40°C demonstrated the accuracy, precision, and reliability of the method. Samples reading LO contained less than 20 mg/dL and were considered undetectable in this study. Samples reading HI, that is, above 600 mg/dL were diluted and rerun. Often Nelson-Somogyi (NS) and 3,5-dinitrosalicylic acid (DNS) assays are used to determine reducing sugars such as glucose. These methods were not used since both assays have been found to provide substantially different results when used to determine cellulase activities [19]. HPLC avoids this problem but is not suited for 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 T = 40°C the tip was touched to the assay strip. This method requires a drop or less of sample and is specific for glucose. It does not test for xylose or glucan polymers that may be released into the liquid phase by enzymatic hydrolysis. Therefore, the glucose reported here underestimates the total glucan content including cellobiose potentially available for fermentation.

3. RESULTS AND CONCLUSION

3.1. NaOH Pretreatment

CS, WS, and PC were pretreated with NaOH as described above. The solution darkened quickly due to the release of lignin. Xylose, other non-glucose monomers, and carbohydrate polymers released into the alkaline solution were not measured. Glucose released into the NaOH solution is shown in Table **1**.

Table 1: Total Glucose (Mean and Standard Deviation from four Experiments) in 200 mL Liquid Above NaOH Treated Biomass for 12 hours at T= 100°C

	1 g NaOH/5 g CS	1 g NaOH/5 g WS	4 g NaOH/5 g PC
Mean	200 mg	252 mg	1105 mg
SD	60 mg	16 mg	143 mg

The release of more glucose from pine chips is probably due to the greater amount of NaOH used in pretreatment. Glucose in the alkaline solution will be lost from the amount produced by enzymatic hydrolysis of the remaining solids but might be captured after processing of the spent solution. It is anticipated that the NaOH solution could be reused for additional pretreatments until the alkalinity is reduced to below effective concentration. The residual liquid could then be neutralized with acid and used for irrigation or processed to recover lignin and other organic components including glucose.

3.2. Enzyme Hydrolysis and Volume Expansion

The release of glucose is shown in Figure **1** at one hour after adding enzymes (1 h 50 mL). This demonstrates the binding of cellulase and xylanase to the alkali treated solids in 50 mL buffer. However, the amount of glucose produced was small and only slightly higher than that for biomass not treated with NaOH. While using small volumes for enzyme hydrolysis has the advantage of less water use, it is probable that enzyme inhibition by glucose reduces its release since it is known that glucose can inhibit cellulase activity [20]. This was tested by diluting the 50 mL with citrate buffer by ten-fold, that is, by a rapid volume expansion.

Figure **1** shows that volume expansion does have a marked effect on the release of glucose presumably by decreasing the concentration of glucose and possibly other products thereby decreasing cellulase inhibition. At the time of dilution to 500 mL, that is, at 0 h 500 mL, the amount of glucose produced from CS was 3.75 times that produced in 50 mL after one hour exposure to enzymes. Comparing the amount of glucose released at 1 h 50 mL with that after volume expansion for one hour, that is, at 1 h 500 mL, demonstrated a 5.75 times greater release of glucose from CS.

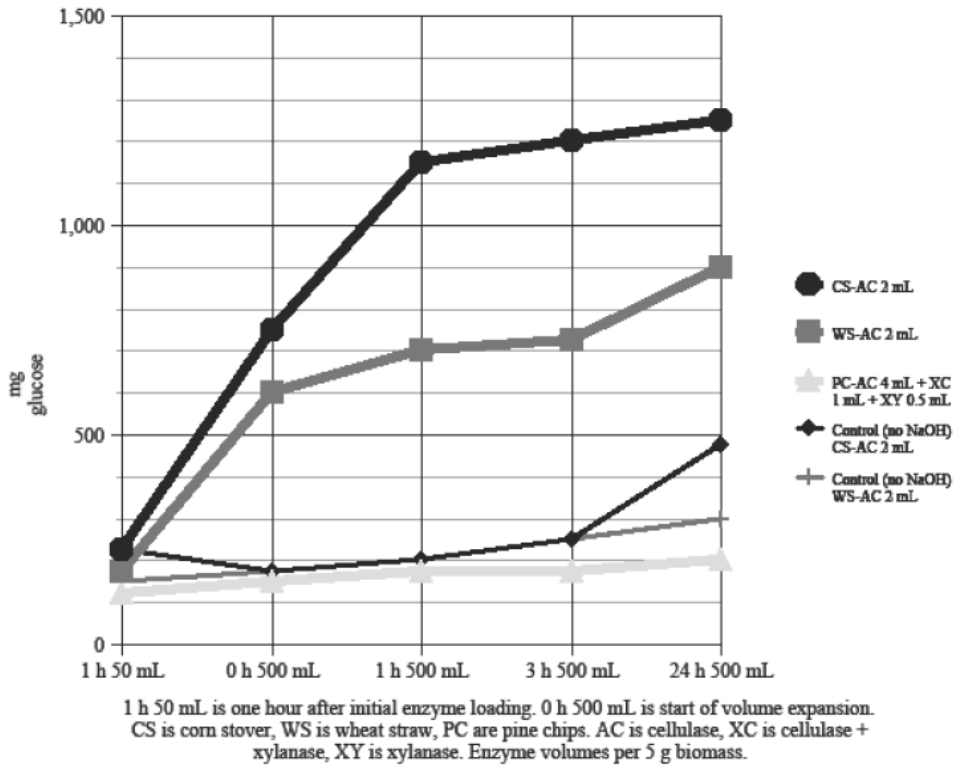

Figure 1: Time and volume of enzyme treatment. Glucose as mean of three experiments from 5 grams each of three types of NaOH treated biomass after one hour exposure in 50 mL citrate buffer, and at the time of dilution and thereafter.

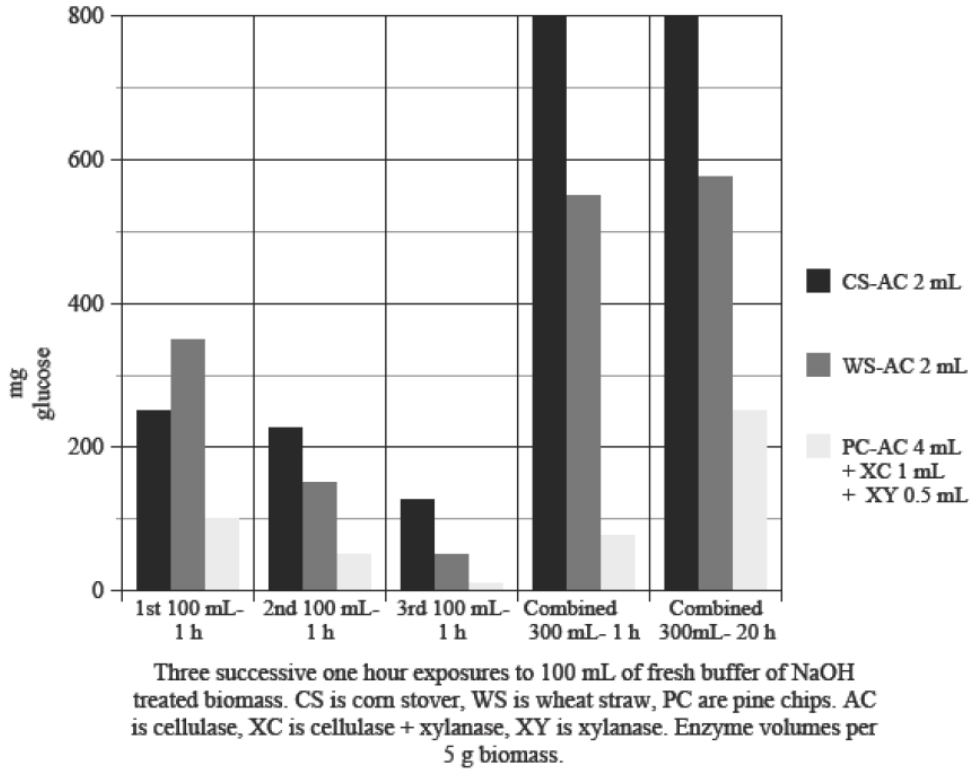

Figure 2: Effect on glucose production of successive exposures to fresh buffer. Glucose as a mean of three experiments from 5 g each of three types of NaOH treated biomass after initial enzyme loading following buffer changes and combination.

Woody biomass may not be suitable for this alkali-cellulase treatment since glucose yields are minimal for PC as shown in Figure **1** in spite of high enzyme loading that included cellulase and xylanase. The effect of bypassing NaOH pretreatment is shown by the CS and WS control groups. Little glucose was produced with cellulase treatment when the biomass was not pretreated with NaOH. Glucose produced from PC without NaOH pretreatment was undetectable and not shown in the figure. Also, CS (5 g) when pretreated with 2 g NaOH under the same experimental conditions but without enzyme treatment did not produce glucose. Since corn stover was the most susceptible to hydrolysis it is reasonable to assume wheat straw and pine chips would also not produce glucose without enzymes.

Corn stover contains approximately 40% cellulose and wheat straw 35% cellulose [21]. In this study glucose yields at 24 h of enzyme hydrolysis were approximately 60% and 50% of theoretical for CS and WS, respectively. This does not include glucose lost by alkali pretreatment or that remaining in the residual solids. Also, not included are cellobiose or glucans released by enzyme hydrolysis. Important to commercial operation, most initial enzyme activity occurred within three hours. Reports show an initial burst of cellulase activity that achieves 50% conversion of cellulose within 24 hours [22]. The less time required for enzyme hydrolysis the greater will be the economic feasibility of the alkali-cellulase process. However, this gain will be mitigated by the need for greater use of water.

A strategy to minimize water use would be to remove glucose by draining smaller volumes of liquid and replacing with fresh buffer. Results of this approach are shown in Figure **2**. Following NaOH treatment and washing, enzymes were added to the solids in 100 mL of buffer. At one hour of enzyme treatment glucose was measured (1st 100 mL–1 h) and the liquid replaced with another 100 mL of buffer. This procedure was repeated in another hour (2nd 100 mL-1 h) and then for a third time (3rd 100 mL-1 h).

As expected, replacement of the 100 mL aliquots resulted in decreasing yields of glucose. After the third aliquot was tested for glucose all three 100 mL volumes were combined along with the remaining solids. One hour later glucose was measured (Combined 300 mL- 1 h) and at 20 hours (Combined 300 mL- 20 h). The combined volumes had more glucose than the sum of the three volumes since enzyme hydrolysis continued during the hour following

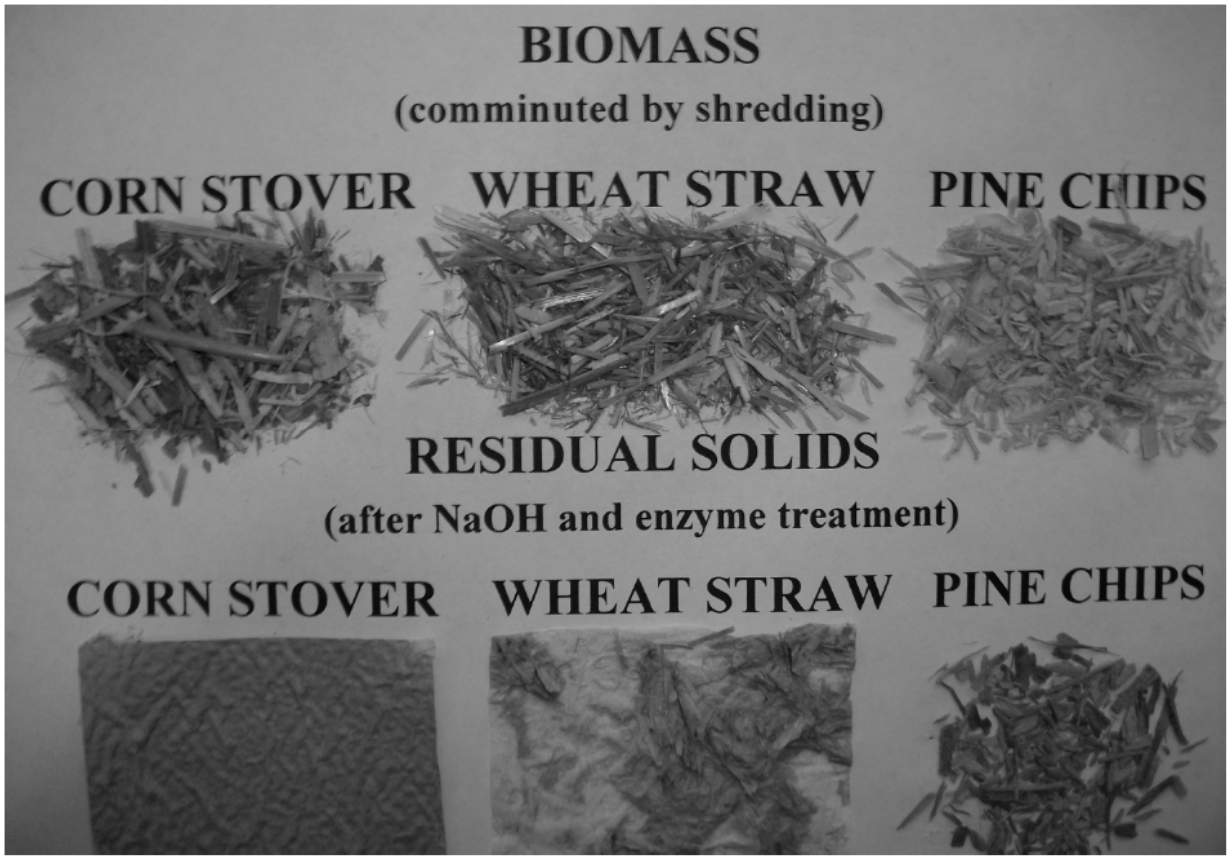

Figure 3: Original three types of biomass before processing and residual solids remaining after processing.

combination and this is the value given (Combined 300- 1 h) and not that when the aliquots were initially combined. The combined volumes resulted in release of more glucose from corn stover and wheat straw after one hour and from all three biomasses at 20 hours. However, if there is proportionality, calculation shows that the concentration of glucose in the 300 mL volume was the same as in the 500 mL shown in Figure **1**. Therefore, this approach does not appear to provide an advantage over conducting the enzymatic hydrolysis in an increased fixed volume, that is, with volume expansion with buffer.

These results support the feasibility of an alkali-cellulase protocol that can produce glucose using readily available materials over reasonable processing time and with equipment not requiring pressure, high temperatures, or having concerns of corrosion. However, the production of large volumes of dilute glucose concentration probably requires a concentration procedure. Concentrating sugars with membranes is a common procedure in the food industry [23]. Thermal concentration is an alternative that has the advantage of producing steam for heating other stages of the process.

The lignin containing alkali solution can be used to treat additional batches of biomass until its effect on biomass cellulose decreases. The spent alkali solution when neutralized would be suitable for irrigation or shipped for processing to recover dissolved organics particularly lignin. The residual solids could be recycled through enzyme hydrolysis for additional glucose.

3.3. Recycling Residual Solids

Since residual solids may still contain hydrolyzable cellulose, further enzyme treatment could produce additional glucose. Figure **3** shows dried residual solids following alkali-cellulase treatment.

Corn stover appeared most susceptible to the alkali-cellulase treatment as demonstrated by the resulting homogeneous dried paste. Wheat straw was less susceptible and pine chips appeared to undergo little change.

Residual solids were suspended in buffer and enzymes added. Results shown in Table **2** indicate that additional glucose can be obtained especially from corn stover residual solids although higher levels of enzymes were needed.

Table 2: Glucose in Five Experiments Using Additional Enzyme Treatment on Residual Solids (RS) Obtained After NaOH and Enzyme Treatment of Three Types of Biomass

Residual Solids(RS)	%RS from starting 5 g biomass	Enzyme treatment (added to starting 100 mL)*	Glucose mg/ g RS [then buffer added to reach 500 mL]		
			0 h (100 mL)	8 h (100 mL)	24 h (500 mL)
1) CS	22%	2 mL AC[a]	27	61	LO[d]
2) WS	36%	2 mL AC	22	49	LO
3) CS + WS combined	24%	4 mL AC + 1 mL XC[b] + 0.5 mL XY[c]	26	59	340
4) CS	10%	4 mL AC+ 1 mL XC + 0.5 mL XY	83	167	375
5) WS	34%	4 mL AC + 1 mL XC + 0.5 mL XY	30	40	LO

*100 mL rather than 50 mL to enhance glucose release; [a]AC = cellulase; [b]XC = xylanse + cellulase; [c]XY = xylanase; [d]LO = not detected presumably due to dilution.

It appears that recycling the residual solids provides additional glucose and should enhance economic feasibility.

4. CONCLUSION

Pretreatment with NaOH (0.5%w/v-H_2O, 20% w/w-biomass) at 100°C for 12 h rendered biomass susceptible to enzyme hydrolysis. Cellulase hydrolysis for three hours or less was sufficient to produce glucose. Volume expansion substantially enhanced glucose production. Repeated processing of residual solids can produce additional glucose that can maximize yields. Allkali-cellulase processing can be done locally or at regional depots. The concept of regional biomass processing depots for initial pretreatment is currently being explored [24]. Concentrating the resulting glucose solution will be necessary to allow economical transport. Local treatment of biomass using the alkali-cellulase process to produce glucose for ethanol production at existing grain fermenting facilities is a novel approach that has been demonstrated at laboratory scale using in this study using materials and methods that can be easily scaled-up.

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Received on 05-06-2013 Accepted on 01-08-2013 Published on 31-08-2013

DOI: http://dx.doi.org/10.6000/1929-6002.2013.02.03.5