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The Ammonia Freeze Explosion (AFEX) Process

A Practical Lignocellulose Pretreatment

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ABSTRACT

The Ammonia Freeze Explosion (AFEX) process treats lignocellulose with high-pressure liquid ammonia, and then explosively releases the pressure. The combined chemical effect (cellulose decrystallization) and physical effect (increased accessible surface area) dramatically increase lignocellulose susceptibility to enzymatic attack. There are many adjustable parameters in the AFEX process: ammonia loading, water loading, temperature, time, blowdown pressure, and number of treatments. The effect of these parameters on enzymatic susceptibility was explored for three materials: Coastal bermudagrass, bagasse, and newspaper. Nearly quantitative sugar yields were demonstrated for Coastal bermudagrass and bagasse, using a very low enzyme loading (5 IU/g). Newspaper proved to be much more resistant to enzymatic hydrolysis.

Index Entries: Lignocellulose; pretreatment; Ammonia Freeze Explosion; cellulase; sugar.

INTRODUCTION

The use of lignocellulose has the potential to solve or mitigate problems, such as global warming, balance of trade, Third World debt, air pollution, trash disposal, soil erosion, herbicide/pesticide accumulation,

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Table 1
Weaknesses of Various Lignocellulose Pretreatments

CHEMICAL	REACTIVITY	ENERGY	CAPITAL	CHEMICAL RECOVERY	DEGRADATION
ORGANOSOLV	X				X
NaOH				X	
NH_3	X				
CONC ACID				X	X
DIL ACID				X	X
SOLVENTS				X	
AUTOHYDROLYSIS					X
PHYSICAL					
BALL MILL		X	X		
TWO-ROLL MILL		X	X		
PHYSICOCHEMICAL					
STEAM EXPLOSION					X
AFEX					

X= WEAKNESS

and deforestion. However, since native lignocellulose is chemically recalcitrant, the key to using lignocellulose is to identify an effective pretreatment that enables rapid and complete enzymatic conversion to fermentable sugars. Pretreatments may be categorized as chemical, physical, or physicochemical (*1*). Table 1 summarizes these pretreatments and shows that all of them—except Ammonia Freeze Explosion (AFEX))—have one or more severe weaknesses. The AFEX process uses a volatile reagent (ammonia) that is easily recovered. The processing conditions are mild, which minimizes biomass degradation. Capital and energy requirements are modest, and, as will be shown, the enzymatic reactivity is quite high for a number of biomass materials.

In the AFEX process (*2,3*), lignocellulose is soaked with high-pressure (ca. 15 atm) liquid ammonia at moderate temperatures (ca. 50°C) for about 15 min, causing cellulose to decrystallize. Then, the pressure is instantaneously released, causing the ammonia to flash violently and disrupt the fibrous structure. The combined chemical effects (cellulose decrystallization, hemicellulose prehydrolysis, lignin alterations) and physical effect (increase in accessible surface area) markedly increase the susceptibility of lignocellulose to enzymatic hydrolysis. Figure 1 shows a schematic diagram of an industrial-scale AFEX process. All the ammonia, except that chemically bound as ammonium ions, will be recovered for reuse.

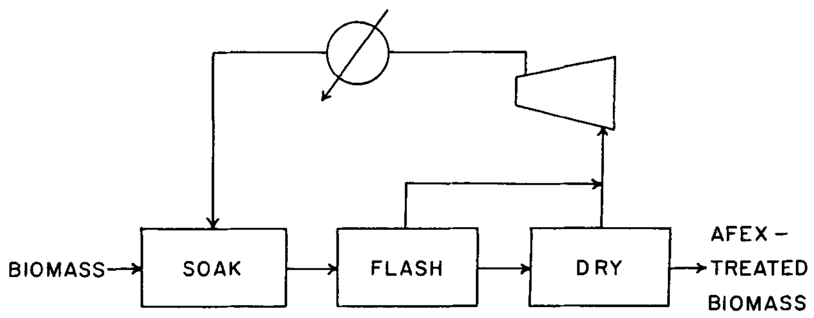

Fig. 1. Schematic diagram of an industrial AFEX process.

Typically, about 0.5% to 1% ammonia remains in the lignocellulose (2), which serves as a nitrogen source for the microbes that use the sugars enzymatically hydrolyzed from the lignocellulose.

The explosive pressure release of AFEX invites comparisons with the stream explosion process (4). The processes are very different, however. Steam explosion operates at substantially higher temperatures (ca. 235°C) and pressures (ca. 32 atm), which degrade about 10% to 20% of the biomass. This is particularly detrimental to protein-containing biomass, such as grass. Feed-grade protein is worth about four times more per unit weight than carbohydrate, so its destruction by steam explosion cannot be tolerated. The mechanical disruption of lignocellulose fibers by steam explosion does *not* contribute to the efficacy of the process (4). Rather, the increased reactivity from steam explosion results from autohydrolysis of the lignocellulose by organic acids released by the high temperatures. A recent comparison of AFEX with steam explosion (5) shows that in general, AFEX-treated biomass has greater enzymatic reactivity than steam exploded biomass. In the eight trials in which sugar yields were expressed per gram of cellulose in the raw feed, AFEX performed better five times, whereas steam explosion was better only three times. When AFEX was better, it was dramatically better (18%–236%). When steam explosion was better, it was only marginally better (6%–47%). It should be emphasized that the AFEX treatment was not optimized in this comparison.

It is the purpose of this paper to present optimized conditions for the AFEX treatment of three substrates of industrial concern: Coastal bermudagrass, bagasse, and newspaper. The AFEX process has many degrees of freedom: ammonia loading, water loading, temperature, time, blowdown pressure, and multiple AFEX treatments. If optimized AFEX treatment conditions were employed in the comparison to steam explosion, it is expected that in all cases, AFEX would have rendered the biomass more reactive. As will be shown, multiple AFEX treatments significantly improve the enzymatic reactivity, compared to a single treatment. Multiple steam explosions are not a viable option, since too much of the biomass would be degraded.

Table 2
Preliminary Cost Estimate for AFEX Process

	$/tonne	$/year
Raw Materials		
Ammonia ($0.22/kg)	1.20	384,000
Utilities		
Electricity ($0.07/kWh)	6.00	1,919,904
($0.02/kWh)	(1.71)	(548,571)
Steam ($0.0044/kg)	2.23	714,813
Cooling Water ($0.013/m^3)	0.51	164,192
Labor		
Plant Manager (1 @ $60,000/year)	0.19	60,000
Supervisors (4 @ $40,000/year)	0.50	160,000
Workers (12 @ $30,000/year)	1.13	360,000
Fixed Charges		
Depreciation (10 years)	5.31	1,700,000
Local Tax (0.03 X FCI)	1.60	512,629
Insurance (0.007 X FCI)	0.37	119,613
Maintenance (0.04 X FCI)	2.14	683,505
Interest (0.15 X FCI)	7.97	2,550,000
	29.15	9,328,646
	(24.86)	(7,957,313)

Note: Fixed Capital Investment (FCI) = $17 million

Assumptions:

1. Plant capacity = 40 tonne/h
2. Ammonia loading = 1 kg NH_3/kg dry biomass
3. Water loading = 0.11 kg H_2O/kg dry biomass
4. Reaction time = 15 min
5. Blowdown pressure = 1 atm absolute
6. Number of treatments = 1
7. Reaction temperature = 21°C

Table 2 shows a preliminary cost estimate for an AFEX plant that processes 40 dry tonne biomass/h (*6*). AFEX is estimated to cost $25 to $29/tonne, depending on the cost of electricity. The higher cost results from purchasing electricity from the local utility, whereas the lower cost results from cogeneration. Since the final blowdown pressure is assumed to be 1 atm, the energy to recompress the ammonia vapors is quite substantial. As shown in Fig. 2, significant savings result by not blowing down to 1 atm. For example, a 3-atm blowdown uses only about 40 to 60% of the compression energy required by a 1-atm blowdown.

MATERIALS AND METHODS

Sample Preparation

Coastal bermudagrass was fertilized and cut at two-week intervals, so it had a high protein content (15%). It was air dried and hammermilled to

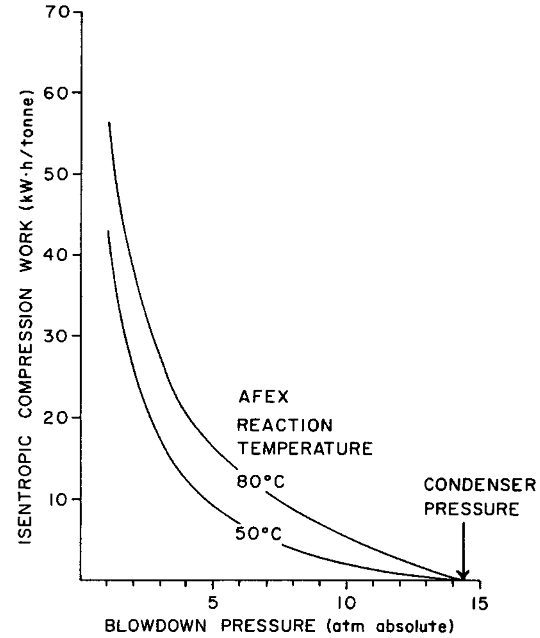

Fig. 2. Ideal isentropic energy required to recompress flashed ammonia vapors. (Assumptions: ammonia loading = 1 kg ammonia/kg dry biomass, water loading = 0.25 kg water/kg dry biomass, condenser temperature = 38 °C)

reduce the particle size to approx 0.5 mm × 10 mm. Bagasse was supplied from both Lousiana and South Texas. The samples were air dried and ground in a blender to a particle size of approx 1 mm × 15 mm. Newspaper (Houston Chronicle) was pulped in water, using a blender. The pulp was filtered and air dried. The dry pulp was again shredded in a blender, reducing the particle size to approx 0.03 mm × 1 mm.

AFEX Treatment

The reactor consisted of a 4-L Autoclave Engineers pressure vessel (*see* Fig. 3). The vessel was charged with prewetted lignocellulose, and the lid was bolted shut. The amount of liquid ammonia added to the reactor was determined from the weight loss of the ammonia supply cylinder. Heating tape warmed the biomass to the desired temperature. After the ammonia soak was complete, the 2.85 cm ball valve was quickly opened, releasing the pressure to the blowdown tank. An in-line filter captured any biomass that was entrained in the high-velocity ammonia vapors. The final blowdown pressure was regulated by charging the blowdown tank with a desired pressure of nitrogen gas.

Saccharification

7.5 g AFEX-treated biomass were added to 150 mL of 0.05 *M* citrate buffer, pH 4.8, that was prewarmed to the reaction temperature of 50 °C in a 100 rpm shaking water bath. Genencor 300P cellulase with a filter

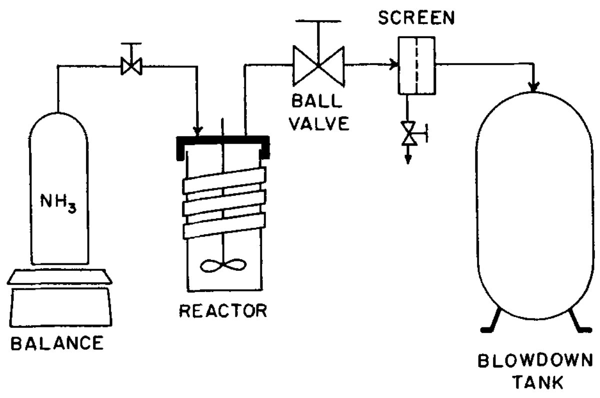

Fig. 3. Schematic diagram of laboratory AFEX apparatus.

paper activity (7) of 132 IU/g dry powder was added, so the cellulase loading was 5 IU/g dry biomass. Since *T. reesei* cellulase has a specific activity of about 0.7 IU/mg protein, this enzyme loading corresponds to 7 kg cellulase protein/tonne dry biomass. Novo 188 cellobiase with an activity (*8*) of 250 CBU/mL was added, so the cellobiase loading was 28.4 CBU/g dry biomass. One-milliliter liquid samples were taken, using a mechanical pipet with an enlarged tip. The samples were boiled in a sealed test tube for 30 min to denature the enzyme. The boiled samples were filtered through 0.22 μm nylon membrane filters. The reducing sugar concentration was measured, using the DNS assay (*9*). (Note: All reducing sugar yields are expressed as mg *equivalent* glucose/g dry substrate since glucose was used as the calibration standard.) The hydrolysis was performed for 3 d, with samples taken at 0, 3, 6, 24, and 72 h.

RESULTS AND DISCUSSION

Coastal Bermudagrass

Including the control, a total of 16 different Coastal bermudagrass samples were hydrolyzed. In all cases, the curves that describe sugar production vs time had a similar shape; only the scale was different. Figure 4 shows sugar production vs time, with the data normalized to the 3-d yield. The 3-d sugar yield is a convenient measure of the enzymatic susceptibility of the AFEX-treated biomass. The normalized curve allows determination of sugar yields at times other than 3 d. For example, about 50% of the 3-d sugars were released in 4 h, whereas 85% were released in 24 h.

Multiple AFEX treatments of Coastal bermudagrass were performed by repeatedly applying the conditions described in Table 3. Four different blowdown pressures were used: 1.4, 2.3, 3.0, and 4.0 atm. Figure 5 shows that the additional AFEX treatments (up to three) always increased the reactivity of the bermudagrass. In general, increasing the blowdown pressure decreased the enzymatic reactivity of the grass. The results from

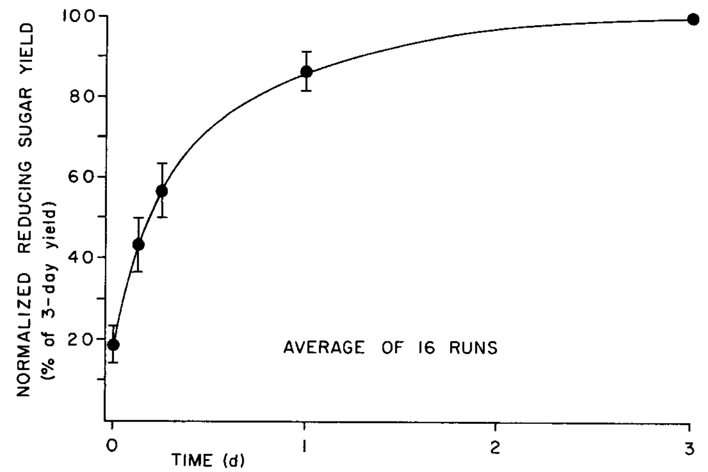

Fig. 4. Normalized hydrolysis profile for Coastal bermudagrass. (3-d yield = 100, error bars are ±1 Sd)

Table 3
Summary of AFEX Treatment Conditions

Exp	NH_3 * Loading (kg/kg)	H_2O * Loading (kg/kg)	Reactor Temp. (°C)	Reactor Press. (atm abs)	Blowdown Press. (atm abs)	Reactor Time (min)	Number of Treat.
Coastal Bermudagrass							
1	1.0	0.43	48	11.0	1.4	15	1 to 3
	1.0	0.43	48	11.0	2.3	15	1 to 3
	1.0	0.43	48	11.0	3.0	15	1 to 3
	1.0	0.43	48	11.0	4.0	15	1 to 3
2	1.0	0.43	48	11.0	1.4	15	1 to 6
Bagasse							
1	1.0	0.25	52	14.0	1.5	15	1 to 6
	1.0	0.25	52	14.0	3.0	15	1 to 6
2	1.0	0.25	52	10.3	1.6	15	1
	1.5	0.25	52	16.6	1.6	15	1
	2.0	0.25	52	19.5	1.6	15	1
	2.5	0.25	52	18.6	1.6	15	1
3	1.5	0.00	52	21.5	1.6	15	1
	1.5	0.10	52	18.8	1.6	15	1
	1.5	0.25	52	16.0	1.6	15	1
	1.5	0.50	52	12.3	1.6	15	1
4	1.5	0.25	13	7.1	1.3	10	1
	1.5	0.25	38	13.7	1.5	10	1
	1.5	0.25	66	20.3	1.6	30	1
	1.5	0.25	93	32.2	1.7	50	1
5	1.5	0.25	93	32.2	1.7	50	1 to 3
Newspaper							
1	1.0	0.25	52	13.0	1.4	15	1 to 3
	1.0	0.25	52	13.0	3.0	15	1 to 3
2	2.5	0.50	87	19.4	1.6	180	1 to 3
3	2.5	0.50	85 to 175	?	2.4	120	1

* Note: Loadings are expressed on a dry weight basis.

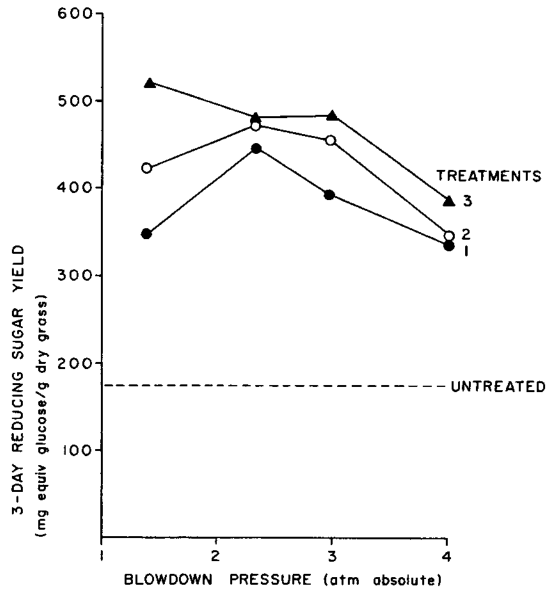

Fig. 5. Coastal Bermudagrass Exp 1: Sugar yields from multiple AFEX treatments, using various blowdown pressures.

blowdown pressures of 1.4, 2.3, and 3.0 atm were similar, whereas the blowdown to 4.0 atm was significantly less reactive. Using three treatments to a blowdown pressure of 1.4 atm, 520 mg reducing sugars/g dry grass were produced in 3 d. This roughly corresponds to 90% hydrolysis of the cellulose and hemicellulose.

Since repeated AFEX treatments were shown to increase the reactivity of bermudagrass, up to six treatments were applied, using a final blowdown pressure of 1.4 atm. As shown in Fig. 6, the maximum reactivity was reached after three treatments; the fourth, fifth, and sixth treatments had no additional beneficial effect.

Bagasse

Bagasse is the residue remaining after sucrose is extracted from sugar cane. Only about one-third of sugar cane dry matter is sucrose, so the remaining two-thirds is frequently wasted. Since sugar cane is the world's most abundant agricultural product—outproducing milk, wheat, corn, and rice—significant quantities of bagasse are available for use (*10*).

Including controls, a total of 31 bagasse samples were hydrolyzed. Figure 7 shows the normalized sugar yields for bagasse. The normalized curve is nearly identical to Coastal bermudagrass, with about 50% of the sugars released in 2 h and 85% released in 24 h.

The bagasse samples were treated according to the conditions described in Table 3. Figure 8 shows the results from multiple AFEX treatments, using two different blowdown pressures. The results were nearly identical for the two blowdown pressures, although the lower pressure (1.5 atm)

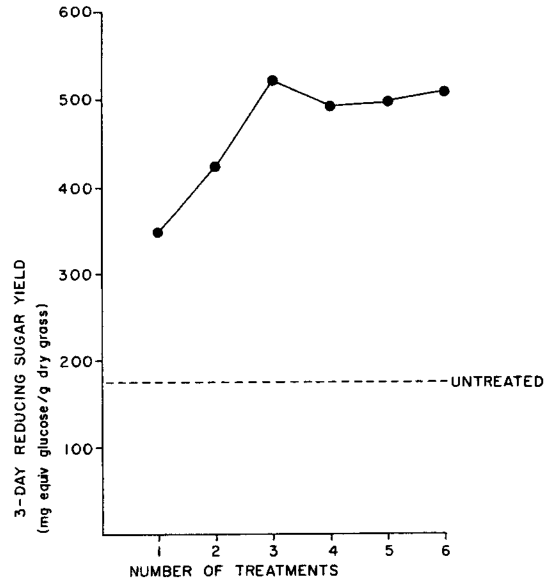

Fig. 6. Coastal Bermudagrass Exp 2: Sugar yields from multiple AFEX treatments.

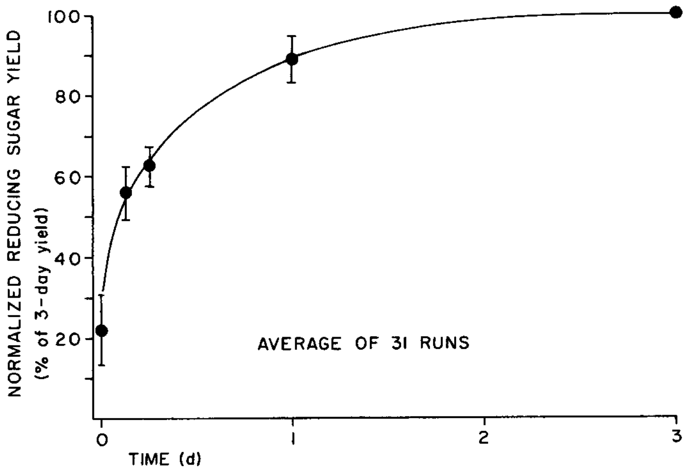

Fig. 7. Normalized hydrolysis profile for bagasse. (3-d yield = 100, error bars are ±1 Sd)

caused the material to be slightly more reactive than the higher blowdown pressure (3.0 atm). There was nearly a linear increase in reactivity with each treatment for the first three treatments. Although the latter three treatments also were beneficial, there were diminishing returns, since the improvement was not as great.

Figure 9 shows the effect of ammonia loading on biomass reactivity. Above 1 kg ammonia/kg dry bagasse, the reactivity is fairly constant, although 1.5 kg ammonia/kg dry bagasse is best. This loading was used in subsequent bagasse experiments.

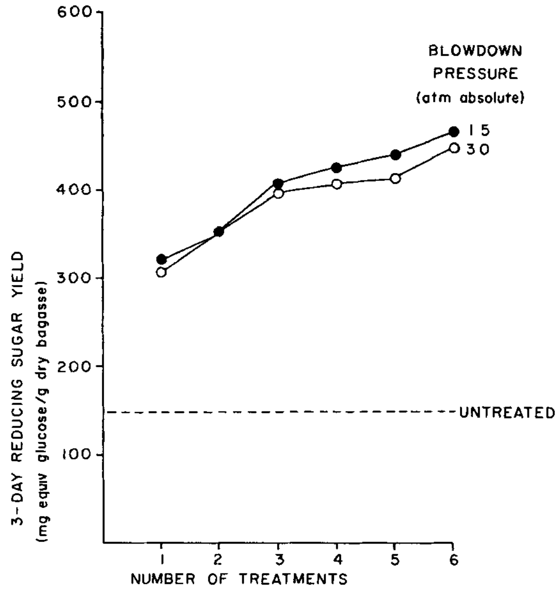

Fig. 8. Bagasse Exp 1: Sugar yields from multiple AFEX treatments, using two different blowdown pressures. (Louisiana bagasse)

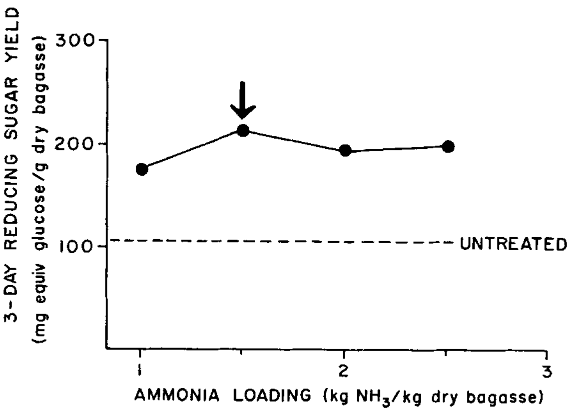

Fig. 9. Bagasse Exp 2: Effect of ammonia loading on sugar yields. (South Texas bagasse)

Figure 10 shows the effect of water loading on biomass reactivity. The maximum reactivity was achieved with 0.25 kg water/kg dry bagasse, so this was used in subsequent experiments. This optimum is similar to the optimum determined for alfalfa (0.3 kg water/kg dry alfalfa) (*2*).

The role of water in the AFEX process is uncertain, but it probably promotes hydrolysis of the hemicellulose. At 52°C, the importance of this effect seems to be minor, since the bone-dry bagasse (prepared by drying in an oven at 135°C) had 90% of the reactivity of the optimal water loading.

It is known that steam autoclaving biomass *after* AFEX treatment has a beneficial effect, since sugar yields from wheat straw were increased about 15% compared to AFEX treatment alone (*11*). To test the effect of

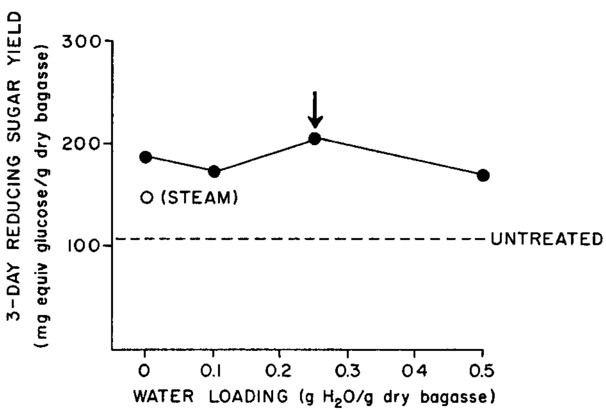

Fig. 10. Bagasse Exp 3: Effect of water loading on sugar yields. (South Texas bagasse)

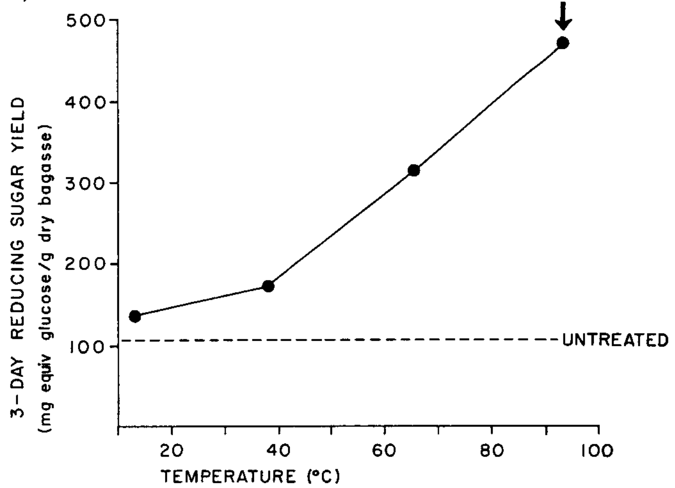

Fig. 11. Bagasse Exp 4: Effect of temperature on sugar yields. (South Texas bagasse)

steaming *before* AFEX treatment, one of the samples was wetted with 20% moisture and steam autoclaved for 15 min at 128°C. It was subsequently dried at 135°C in the drying oven and then treated by the AFEX process. As shown in Fig. 10, this treatment detrimentally affected biomass reactivity. Although more steaming conditions must be tried before definitive conclusions can be made, it appears that treatment with steam prior to AFEX is not beneficial.

Reactor temperature is an important variable, since it determines the amount of ammonia vaporized during the explosive flash. Fig. 2 shows that operating the reactor at 80°C compared to 50°C increases the compressor load by 40% to 100%. At higher reactor temperatures, more ammonia vapors flash, causing greater disruption of the fibrous structure. Also, chemical reactions, such as alkaline hydrolysis of hemicellulose, will be accelerated at higher temperatures. Figure 11 shows the dramatic effect

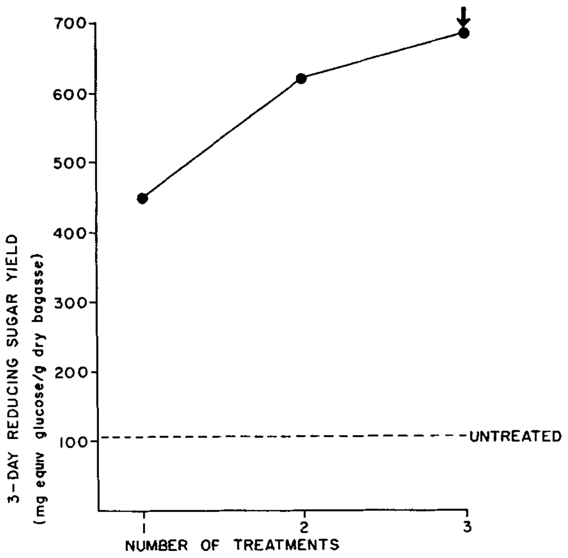

Fig. 12. Bagasse Exp 5: Effect of multiple treatments on sugar yields. (South Texas bagasse)

of increasing temperature. The sugar yields from a 93°C treatment were 3.5 times greater than a 13°C treatment. Therefore, a 93°C treatment temperature was used in subsequent experiments. Further, temperature increases above 93°C may be beneficial, although this must be demonstrated.

As shown previously, multiple AFEX treatments substantially increase the biomass reactivity. Figure 12 shows the effect of using up to three treatments of bagasse. The first treatment increased the sugar yields by about 4.5 times, compared to the control, the second treatment increased yields about 6 times, and the third treatment increased yields by almost 7 times. With three treatments, the sugar yield was 680 mg sugar/g dry bagasse, which represents over 90% conversion of the cellulose and hemicellulose.

Newspaper

Newspaper is an important substrate, since it comprises approx 10% of municipal waste (*12*). In addition, it is the most recalcitrant paper fraction of garbage, since it has a high lignin content (18–30%) (*13*). The main component is mechanically ground softwood that is reputed to be much more difficult to treat than hardwood.

Including controls, a total of 22 newspaper samples were enzymatically hydrolyzed. Figure 13 shows the normalized sugar yields. The results are very similar to the other biomass materials, since 50% of the sugars were released in about 4 h and 80% were released in 24 h.

The recalcitrance of newspaper is confirmed by Fig. 14. Even with three treatments to a final blowdown pressure of 1.4 atm, the sugar yields were only 18% higher than the control. As demonstrated with the other

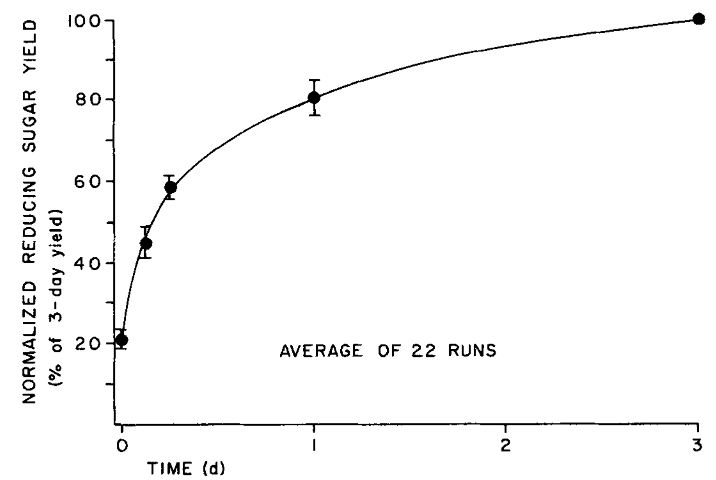

Fig. 13. Normalized hydrolysis profile for newspaper. (3-d yield = 100, error bars are ±1 Sd)

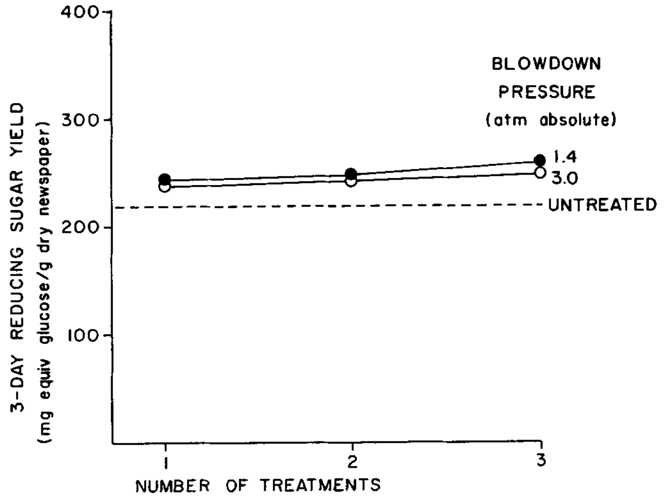

Fig. 14. Newspaper Exp 1: Effect of blowdown pressure and number of treatments on sugar yields.

materials, a higher reactivity was obtained with a lower blowdown pressure, although the effect was relatively slight, since the newspaper was so nonreactive.

Since newspaper was nonresponsive to treatment conditions used in the first experiment, more aggressive treatment conditions were employed in the second. The ammonia loading was increased from 1 to 2.5 kg ammonia/kg dry newspaper, the water loading was raised from 0.25 to 0.5 kg water/kg dry newspaper, and the temperature was increased from 52 to 87°C (see Table 3). Figure 15 shows that the more aggressive treatment conditions were fairly successful, since the sugar yields increased

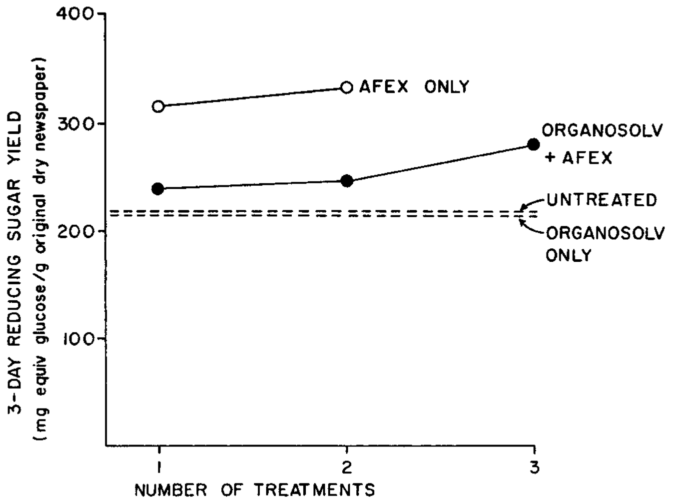

Fig. 15. Newspaper Exp 2: Sugar yields from AFEX treatment of raw and organosolv-treated newspaper.

by 50%, compared to the control. The sugar yield of 330 mg sugar/g dry newspaper, using two AFEX treatments, represents about 40% conversion of the cellulose and hemicellulose.

Because newspaper is highly lignified, removal of some lignin may dramatically increase the response to AFEX treatment. The organosolv process—which treats biomass with a solvent/water/catalyst mixture—is fairly selective at removing lignin from biomass (*14*). Typically, organosolv treatment uses a temperature above 190°C. However, the goal here was not complete delignification, but rather, partial removal. Therefore, a lower temperature (128°C) was employed. (The organosolv treatment conditions are as follows: ethanol loading = 1.7 kg EtOH/kg dry newspaper, water loading = 1.3 kg H_2O/kg dry newspaper, ammonia loading = 0.57 kg NH_3/kg dry newspaper, reaction time = 180 min, ethanol/water wash = 60 kg wash liquid/kg dry newspaper.) After the organosolv treatment, the solids were washed with copious amounts of a water/ethanol mixture that removed 7.6% of the material. Figure 15 shows that the organosolv treatment actually had a detrimental effect on biomass reactivity, so this treatment was abandoned.

Since increasing temperature improved bagasse reactivity, higher temperatures were attempted with newspaper. As shown in Fig. 16, high temperatures (up to 175°C) actually had a detrimental effect on biomass reactivity. Under these high temperatures, there appeared to be significant degradation of the newspaper, as evidenced by a dark color and bad odor.

Following the AFEX process with a methanol wash is beneficial for aspenwood (*5*). Here, we washed with warm (50°C) 190 proof ethanol until all the color was extracted from the AFEX-treated newspaper. As shown in Fig. 16, the ethanol wash was actually detrimental to the enzymatic reactivity.

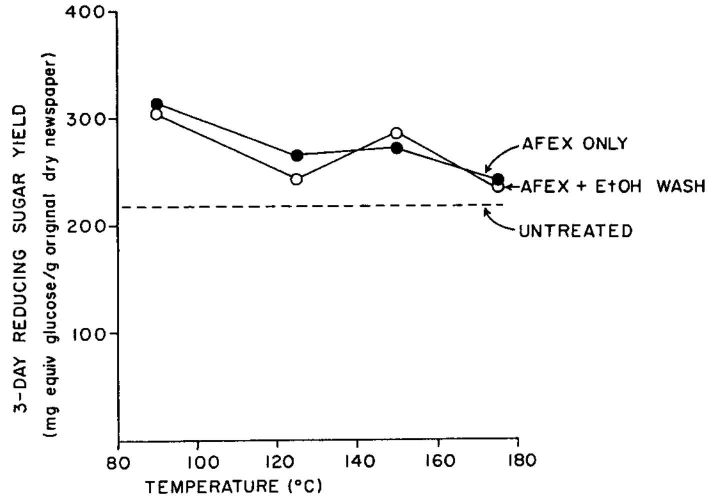

Fig. 16. Newspaper Exp 3: Sugar yields, using high temperatures.

Considering the difficult nature of newspaper, additional treatments may be required. Alternately, an attrition bioreactor (*15*) may be required to increase the reactivity.

CONCLUSIONS

Nearly quantitative sugar yields have been produced from Coastal bermudagrass and bagasse. Although this has been demonstrated in previous AFEX studies (*2,3*), the cellulase loadings employed here were substantially less (5 IU/g vs 80 IU/g). We believe that the cellulase loadings employed in this study are economically viable, even if cellulase is not recycled.

Of the three lignocellulosics studied, Coastal bermudagrass was the easiest to treat. This is expected, since it was harvested young and had a low lignin content (about 5%). Bagasse was more difficult to treat, since it has a fairly high lignin content (about 20%). However, multiple treatments, using high temperatures, allowed nearly theoretical yields of sugars to be produced. Newspaper was the most difficult to treat, since it is a highly lignified (up to 30%) softwood.

Ammonia and water loading had relatively minor effects on the reactivity of biomass, whereas temperature and number of treatments had the greatest effect. Previous studies with AFEX always used a 1-atm blowdown pressure (*2,3*). Here, we showed that excellent treatment results from blowdown pressures up to 3 atm, with good results demonstrated at 4 atm. The ability to use a higher blowdown pressure results in a tremendous energy saving, compared to 1-atm blowdowns (*see* Figure 2). This savings will partially offset the costs of multiple explosions, so the AFEX costs shown in Table 2 are approximately valid for two to three multiple explosions. Further work is required to determine the exact cost of the various treatment conditions explored in this paper.

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