# **Composition and Ethanol Production Potential of Cotton Gin Residues**

# FOSTER A. AGBLEVOR,\* SANDRA BATZ, AND JESSICA TRUMBO

Department of Biological Systems Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, Email: fagblevo@vt.edu

## **Abstract**

Cotton gin residue (CGR) collected from five cotton gins was fractionated and characterized for summative composition. The major fractions of the CGR varied widely between cotton gins and consisted of clean lint (5–12%), hulls (16–48%), seeds (6–24%), motes (16–24%), and leaves (14–30%). The summative composition varied within and between cotton gins and consisted of ash (7.9–14.6%), acid-insoluble material (18–26%), xylan (4–15%), and cellulose (20–38%). Overlimed steam-exploded cotton gin waste was readily fermented to ethanol by *Escherichia coli* KO11. Ethanol yields were feedstock and severity dependent and ranged from 58 to 92.5% of the theoretical yields. The highest ethanol yield was 191 L (50 gal)/t, and the lowest was 120 L (32 gal)/t.

**Index entries**: Cotton gin waste; steam explosion; characterization; summative composition.

## Introduction

Raw cotton processing generates cotton gin residue (CGR), which is composed of immature bolls, cottonseed, hulls, sticks, leaves, and dirt. This material could potentially be used for ethanol production. The major advantages of this feedstock over other lignocellulosics include high cotton cellulose content and concentration of large volumes of this material at cotton-ginning plants. Further, conversion of this feedstock to ethanol could reduce particulate emission, reduce fire hazards from spontaneous combustion of CGRs, and also create jobs in rural America.

However, because CGR is an agroindustrial byproduct, its chemical composition varies considerably because of several factors including season, harvesting, and processing protocols. Several studies have been conducted on CGRs (1-6), some of which showed that the fuel value of the

<sup>\*</sup>Author to whom all correspondence and reprint requests should be addressed.

wastes varied by machine and type of cotton (5). Other studies showed that the ash content of gin wastes from six cotton gins in Texas ranged from 11 to 17% while the carbon content ranged from 41 to 43% (6). A few studies investigated the summative composition of cotton gin waste, but the samples were collected from only one gin, and therefore the data did not show between-gin variations (1,2). The amount of gin residue produced also varied widely between gins and between crop years (3). Estimates of ethanol yield were based on burr trash composition of 40% cellulose, 30% hemicellulose, and 25% lignin (3). Although some of the cited studies addressed between-gin fuel value variability, they did not address variation in summative composition and its impact on potential bioconversion product yield. In the present study, we analyzed freshly discharged and old discharged CGRs from five cotton gins in southeastern Virginia in order to assess the impact of ginning practices on feed-stock composition and yield of ethanol.

#### Materials and Methods

Sampling and Fractionation of CGR

CGR samples were collected from five cotton gins in southeastern Virginia on three consecutive days. The cotton gins are located in Emporia, Franklin, Suffolk, Wakefield, and Windsor. The CGR samples were collected by taking 5-kg samples around the perimeter of the piles as they were discharged from the gins. These samples were labeled "fresh discharge." Additionally, material that had been piled by bulldozers for several weeks after discharge were also collected and labeled "old discharge." All samples were transported by truck on the same day to Virginia Tech, Blacksburg, VA. Because of the continuous spraying of the material with water during discharge, the samples were wet and thus were dried at room temperature to equilibrium moisture content and stored before chemical analysis. About 1-kg grab samples of dried material were first hammer milled to pass through a 1-mm screen and then knife milled (Wiley mill model 4) until all the material passed through a 20-mesh screen. These materials were stored at room temperature until the time of analysis.

About 1-kg dry CGR samples from each of the five gins were shipped to the USDA Cotton Ginning Laboratory (Stoneville, MS) where they were fractionated into eight fractions consisting of clean lint, hulls, sticks and stems, grass, seeds, small leaves, and pin trash. About 150 g of each sample was fractionated according to Shepherd (7).

#### Determination of Ash

The moisture content of the samples was determined by an oven-dry method as in ASTM standard method E 1756-95 (8). The ash content of the CGR was determined using ASTM standard method E 1755-95 (9). Thus, 1 g of knife-milled CGR was weighed in ceramic crucibles that had been

preheated and ignited at 575°C. The crucible and the CGR were heated at 575°C in a muffle furnace for 3 h and cooled to room temperature in a desiccator and weighed. The process was repeated until a constant weight was achieved. The ash content was calculated on a moisture-free basis for triplicate samples.

#### Determination of Extractives

The ethanol extractives content of the CGR was determined using ASTM standard method E 1690-95 (10). The CGR samples,  $10\,\mathrm{g}$  of  $-20\,\mathrm{mesh}$ , were weighed into dry cellulose extraction thimbles and extracted for 8 h with 350 mL of 95% ethanol in a Soxhlet extraction apparatus. After extraction, the samples were cooled to room temperature and the residual solids were vacuum filtered using a Buchner funnel. The filtrate was added to the ethanol extract and vacuum evaporated to dryness on a Buchii rotary evaporator at  $40\,^\circ\mathrm{C}$  and  $84\,\mathrm{kPa}$ . The final product was dried overnight in a vacuum oven, weighed, and the extractives content calculated on an oven-dry biomass basis.

## Determination of Acid-Insoluble Residue

The acid-insoluble residue of the samples was determined using ASTM standard method E 1721-95 (11). Samples of extractives-free CGR (0.3 g) were hydrolyzed with 72%  $\rm H_2SO_4$  for 2 h at 30°C. The hydrolyzed samples were diluted with deionized water to 4%  $\rm H_2SO_4$  and autoclaved at 121°C for 1 h using a liquid cycle. The hydrolysates were filtered through preignited ceramic filtering crucibles. The filtrates were collected and used as stock samples for carbohydrate analysis. The residues were dried overnight at 105°C in a laboratory oven and weighed. The oven-dried residues were ashed as described earlier. The acid-insoluble residue content was calculated on an oven-dry biomass basis.

## Determination of Carbohydrates

Carbohydrate content of the CGR samples was determined as alditol acetates. The acid-hydrolyzed samples saved from the acid-insoluble residue analysis procedure were used for these analyses. The analytes were prepared according to ASTM standard method E 1821-96 (12). This method describes the procedure for converting sugars to alditol acetates.

The derivatized sugar samples were analyzed by gas chromatograph (Shimadzu GC 14A) using a Restek (Bellefonte, PA) Rtx-225 capillary column (15 m, 0.25- $\mu$ m ID, 0.2- $\mu$ m film thickness). The following conditions were used for the gas chromatographic analysis: carrier gas: helium; total gas flow rate: 64 mL/min; column gas flow rate: 0.6 mL/min; programmed column temperature: initial temperature of 190°C for 5 min, heating rate of 10°C/min, final oven temperature of 210°C, total run time of 20 min; injection port temperature: 240°C; detector: flame ionization at 220°C; sample size: 3  $\mu$ L; split ratio: 33:1.

## Steam Explosion Treatment of CGR

Steam explosion of the CGR samples was carried out in a 25-L batch reactor located at the Recycling Laboratory, Thomas M. Brooks Forest Products Center, Blacksburg, VA. The CGR samples were steam exploded at two severity parameters (3.5 and 4.5) as defined by Overend and Chornet (13). The samples were treated in two modes: In the first case, the samples were soaked with their own weight of water and left overnight in covered 10-gal buckets before the steam explosion treatment. The second set of samples was steam exploded "as received" without any further addition of water. About 2 kg of each sample was weighed and loaded into a 25-L batch steam explosion gun. Saturated steam was admitted into the chamber, and the biomass temperature was raised to 237°C (this usually took  $20 \pm 5$  s). The run times for the severities 3.5 and 4.5 were 20 and 200 s, respectively. The steam explosion chamber was washed with water between runs to recover any residual fiber trapped in the unit. All samples were bagged and stored in a cold room until the time of analysis or hydrolysis and fermentation.

## Fermentation of Steam-Exploded Cotton Gin Waste

Steam-exploded CGR samples were overlimed and hydrolyzed according to Jeoh and Agblevor (1). Commercial cellulase enzyme preparations, Spezyme<sup>TM</sup> CP (94 IU/mL; Genencor) and Econase<sup>TM</sup> (105 IU/mL; New York Enzyme Development), were used to hydrolyze the steam-exploded samples at  $50^{\circ}$ C, pH 4.7, and 24 h incubation time. About 0.25 mL of each enzyme preparation was added to 100 g of steam-exploded sample, which was slurried in 1000 mL of citrate buffer solution.

Escherichia coli KO11 (provided by L. O. Ingram, Department of Microbiology and Cell Science, University of Florida), a recombinant organism with genes (pdc, adhB) from Zymomonas mobilis incorporated into its chromosome for enhanced ethanol production, was used for fermentation. The wild-type microorganism was E. coli ATCC11303. For long-term storage, stock cultures were prepared by adding of 20% glycerol (v/v) to concentrated E. coli KO11 cultures and stored at –70°C.

The growth medium was prepared according to Asghari et al. (14): 5 g/L of yeast extract, 10 g/L of tryptone, 5 g/L of NaCl, 50 g/L of xylose, and 40 mg/L of chloramphenicol. Fresh colonies of *E. coli* KO11 from agar plate (5 g/L of yeast extract, 10 g/L of tryptone, 5 g/L of NaCl, 20 g/L of xylose, and 15 g/L of agarose) were used to inoculate 50 mL of the growth medium in 250-mL Erlenmeyer flasks. The cultures were grown in a shaker bath at 35°C and 150 rpm for 18 h. The cells were harvested, centrifuged at 11,000g under sterile conditions, and resuspended in 2 mL of deionized water.

The 2-L flasks containing 65 g of enzyme-hydrolyzed steam-exploded CGR samples were supplemented with  $5\,g/L$  of yeast extract and inoculated with  $E.\ coli$  KO11 at an optical density of 0.2. After inoculation, the flasks were flushed aseptically with nitrogen to establish near-anaerobic condi-

Concetted Burning Two Shifting Seasons									
Gin Name	Clean lint (%)	Hulls (%)	Stick/ stems (%)	Grass (%)	Seed (%)	Motes (%)	Small leaf (%)	Pin trash (%)	Total (%)
Franklin	10.4	19.7	7.1	0.4	5.6	19.5	30.3	5.0	98.0
Emporia	5.3	35.6	7.1	0.4	12.7	16.1	21.3	0.6	99.1
Windsor	9.0	16.8	3.6	0.2	6.9	23.9	34.6	1.6	96.6
Suffolk	12.5	15.9	5.4	0.3	24.0	18.6	18.5	2.2	97.4
Wakefield	7.1	48.1	6.1	0.4	7.7	15.6	13.9	0.6	99.5

Table 1 Fractional Composition of Cotton Gin Waste from Samples Collected During Two Ginning Seasons

tions and sealed. The initial pH of the fermentation broth was 6.0. Fermentation was carried out at 35°C and 120 rpm for 72 h. There was no pH control during fermentation and no antibiotic was added to the fermentation broth. Samples (1.5 mL) of the culture broth were withdrawn at 24-h intervals and centrifuged at 11,000g for 10 min. The supernatants were collected and analyzed by gas chromatography for ethanol and sugar contents.

## **Results and Discussion**

## Fractional Composition of CGR

Because CGR is a heterogeneous material, fractionation into various components will aid in the interpretation of the summative compositional and fermentation data. The fractional composition of the CGR is given in Table 1. The sticks/stems and grass fractions were relatively uniform, which indicated that the harvesting methods were quite similar. Clean lint content ranged from 5 to 12%, seed from 6 to 24%, and hulls from 16 to 48%. The other fractions of the CGR varied widely among ginning plants probably because of the different ginning protocols at each plant. This explanation agrees with the findings of Schacht and LePori (6), who reported significant variation in the ash, carbon, and energy contents of cotton gin wastes from six cotton gins in Texas.

#### Mass Balance

The mass closure for the compositional analysis of the CGR varied considerably within ginning plants and among plants. Mass closures were as high as 98% for some samples and as low as 69% for others (Tables 2–6). The large variation in mass closure could be attributed to the heterogeneity of the feedstocks, the ginning method, and perhaps the sampling method as well.

The objective of this study was to determine the carbohydrate content of the feedstock and thus be able to assess theoretical and practical ethanol yields. The analytical method was therefore optimized for the carbohydrate fraction at the expense of other fractions such as lipids,

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Component (%)	Fresh	Fresh	Fresh	Old		
	discharge	discharge	discharge	discharge		
	10-21-00	10-22-00	11-26-00	10-21OD-00		
Arabinan	$1.84 \pm 0.36$	$2.46 \pm 0.28$	$1.34 \pm 0.10$	$1.65 \pm 0.48$		
Xylan	$8.69 \pm 0.76$	$12.56 \pm 0.95$	$6.08 \pm 0.95$	$9.57 \pm 0.95$		
Mannan	$1.13 \pm 0.04$	$1.09 \pm 0.10$	$0.89 \pm 0.36$	$1.31 \pm 0.06$		
Galactan	$1.65 \pm 0.13$	$1.50 \pm 0.18$	$1.34 \pm 0.37$	$1.77 \pm 0.13$		
Glucan	$36.64 \pm 0.21$	$38.54 \pm 3.03$	$30.72 \pm 2.40$	$38.21 \pm 0.98$		
Total carbohydrates	49.95	56.16	40.37	52.51		
Extractives	$7.7 \pm 0.6$	$8.6 \pm 0.2$	$8.8 \pm 0.3$	$7.6 \pm 1.6$		
Acid-insoluble residue	$26.8 \pm 2.1$	$22.3 \pm 1.0$	$22.2 \pm 1.1$	$26.5 \pm 1.5$		
Ash	$7.9 \pm 0.1$	$8.6 \pm 0.9$	$8.8 \pm 0.8$	$12.0 \pm 0.3$		
Grand total	92.35	95.66	80.17	98.61		

Table 2
Summative Composition of Cotton Gin Waste from Emporia, VA (moisture-free whole-biomass basis)

which could constitute an appreciable fraction of the feedstock especially if it contained cottonseed. The feedstock may also contain residual pesticide and herbicides, uronic residues, acetyl groups, and other extraneous materials, which were not amenable to the method of analysis. Thus, samples that contained large fractions of cottonseed could have low mass closure because the lipids content was not determined and vice versa.

# NonCarbohydrate Components

The extractives, acid-insoluble material, and ash contents of the samples from the five cotton gins are presented in Tables 2–6. Within each plant, there was no significant difference between the compositions of the fresh discharge materials on different days. However, the composition of the old discharge materials from all the gins was significantly different (p < 0.05) from those of the fresh discharge samples.

The ash contents of the old discharge feedstocks were 15–70% higher than those for the fresh discharge samples. The wide range of ash composition may be due to the differences in the microbial degradation of the old discharge samples from different plants or the method used to move the piles from the point of discharge. After the initial discharge of the CGR, the material was moved away from the discharge area with a bulldozer and compacted. It is plausible that this operation introduced more dirt into the CGR, which could account for the increased ash content for all old discharge materials.

Microbial degradation could have contributed to the increased ash content of the old discharge feedstocks. It has been reported (15) that the

Table 3
Summative Composition of Cotton Gin Waste from Franklin, VA (moisture-free whole-biomass basis)

Component (%)	Fresh discharge 10-21-00	Fresh discharge 10-22-00	Fresh discharge 11-25-00	Old discharge 10-21OD-00
Arabinan Xylan Mannan Galactan Glucan	$2.39 \pm 0.57$ $10.46 \pm 3.54$ $1.28 \pm 0.25$ $1.73 \pm 0.17$ $30.27 \pm 7.31$	$2.59 \pm 0.41$ $10.33 \pm 0.72$ $0.73 \pm 0.04$ $1.24 \pm 0.19$ $35.47 \pm 2.21$	$2.75 \pm 0.26$ $10.62 \pm 0.98$ $1.19 \pm 0.34$ $1.48 \pm 0.19$ $27.75 \pm 2.17$	$2.03 \pm 0.17$ $7.76 \pm 0.47$ $0.95 \pm 0.16$ $1.41 \pm 0.14$ $30.08 \pm 2.50$
Total carbohydrate	46.13	50.36	43.79	42.23
Extractives Acid-insoluble residue Ash	$5.1 \pm 1.0$ $24.1 \pm 1.0$ $11.6 \pm 0.3$	$6.4 \pm 1.3$ $26.2 \pm 1.5$ $11.7 \pm 0.3$	$9.6 \pm 1.2$ 22.9 ± 1.4 $9.6 \pm 0.2$	$8.0 \pm 0.3$ $22.0 \pm 7.6$ $13.3 \pm 0.9$
Grand total	86.93	94.66	85.89	85.53

Table 4
Summative Composition of Cotton Gin Waste from Windsor, VA
(moisture-free whole-biomass basis)

Component (%)	Fresh discharge 10-21-00	Fresh discharge 10-22-00	Fresh discharge 11-25-00	Old discharge 10-21OD-00
Arabinan Xylan Mannan Galactan Glucan	$1.75 \pm 0.39$ $5.41 \pm 0.50$ $1.01 \pm 0.23$ $1.57 \pm 0.12$ $26.95 \pm 1.11$	$1.40 \pm 0.38$ $6.20 \pm 1.45$ $0.92 \pm 0.17$ $1.51 \pm 0.14$ $30.50 \pm 1.55$	$1.32 \pm 0.39$ $4.45 \pm 1.14$ $1.01 \pm 0.04$ $1.79 \pm 0.41$ $29.05 \pm 0.82$	$1.26 \pm 0.50$ $5.23 \pm 1.61$ $0.81 \pm 0.22$ $1.28 \pm 0.15$ $32.65 \pm 1.59$
Total carbohydrates	36.69	40.53	37.62	41.23
Extractives Acid-insoluble residue Ash	$8.2 \pm 0.5$ $22.4 \pm 0.2$ $10.4 \pm 0.2$	$7.2 \pm 0.3$ $25.2 \pm 1.6$ $9.9 \pm 0.1$	$13.9 \pm 1.1$ $21.6 \pm 1.1$ $8.4 \pm 0.7$	$6.4 \pm 1.8$ $20.6 \pm 0.7$ $12.3 \pm 0.9$
Grand total	77.69	82.83	81.52	80.53

hemicellulose component of biomass is more susceptible to microbial degradation than the cellulose and lignin components. Thus, if the increase in ash was partly due to microbial degradation, there should be an inverse correlation between the ash and cellulose, or ash and hemicellulose. A plot of ash vs hemicellulose and ash vs cellulose did not show any correlation (data not shown). Thus, the increase in the ash content of the old discharge samples was probably due to the incorporation of dirt into the feedstock as the result of pile movement and compaction by the bulldozer.

Table 5
Summative Composition of Cotton Gin Waste from Suffolk, VA
(moisture-free whole-biomass basis)

Component (%)	Fresh discharge 11-24-00	Fresh discharge 11-25-00	Fresh discharge 11-26-00	Old discharge 11-24OD-00
Arabinan Xylan Mannan Galactan Glucan	$3.03 \pm 0.46$ $11.80 \pm 2.53$ $1.16 \pm 0.14$ $0.23 \pm 0.07$ $26.31 \pm 0.78$	2.25 12.11 1.04 1.56 21.62	2.91 13.22 2.61 3.17 26.08	$1.42 \pm 0.48$ $8.24 \pm 1.37$ $0.38 \pm 0.04$ $0.79 \pm 0.00$ $24.22 \pm 1.76$
Total carbohydrates	42.53	38.58	47.99	35.05
Extractives Acid-insoluble residue Ash	$10.8 \pm 0.9$ $22.3 \pm 0.5$ $6.8 \pm 0.4$	$11.6 \pm 0.3$ $22.5 \pm 0.3$ $8.3 \pm 0.1$	$13.2 \pm 0.4$ $18.1 \pm 0.7$ $8.9 \pm 0.1$	$10.1 \pm 1.2$ $21.9 \pm 1.6$ $10.3 \pm 0.5$
Grand total	82.43	80.98	88.19	77.35

Table 6
Summative Composition of Cotton Gin Waste from Wakefield, VA (moisture-free whole biomass basis)

Component (%)	Fresh discharge 11-25-00	Fresh discharge 10-26-00	Old discharge 11-25OD-00
Arabinan	$0.69 \pm 0.17$	$0.65 \pm 0.11$	$0.66 \pm 0.13$
Xylan	$3.02 \pm 0.39$	$4.07 \pm 0.47$	$4.29 \pm 0.18$
Mannan	$2.37 \pm 0.99$	$1.33 \pm 0.56$	$2.79 \pm 0.15$
Galactan	$2.26 \pm 0.27$	$1.75 \pm 0.43$	$3.48 \pm 0.29$
Glucan	$25.63 \pm 0.50$	$25.90 \pm 1.05$	$22.57 \pm 2.27$
Total carbohydrates	33.97	33.70	33.79
Extractives	$9.1 \pm 0.9$	$7.5 \pm 0.4$	$8.4 \pm 1.2$
Acid-insoluble residue	$19.4 \pm 0.9$	$19.7 \pm 0.7$	$22.0 \pm 0.9$
Ash	$8.8 \pm 0.2$	$8.1 \pm 0.1$	$14.6 \pm 0.4$
Grand total	71.27	69.00	78.79

The most surprising data were the high acid-insoluble material content of the samples (Tables 2–6). The fraction of cotton gin waste that was insoluble in 72% H<sub>2</sub>SO<sub>4</sub> was comparable with that found in woody biomass (16,17). The acid-insoluble material from woody biomass is normally classified as lignin. However, it would be erroneous to classify the material from the CGR as lignin. Since CGR is a complex mixture of organic and inorganic materials, there could be other acid-insoluble material apart from lignin. A probable source of nonlignin acid-insoluble material is the cottonseed. A typical cottonseed is composed of 32% hull, 23% protein,

12% fibers, 20% oil, and 14% carbohydrates (18). Our analysis of the cottonseed from the Emporia gin showed that about 34% was acid-insoluble material (data not shown). However, the hull, which is mostly lignocellulosic, constitutes only 32% of the ginned cottonseed. Thus, it is obvious that the acid-insoluble material is composed of lignin and other condensable compounds. It is known (19) that proteins condense and become insoluble in concentrated  $H_2SO_4$ . Thus, one could attribute the high acid-insoluble material content of the cottonseed to the condensation of some of the protein on the lignin. Similarly, it could be argued that perhaps the large fraction of acid-insoluble material in the cotton gin residue is a combination of lignin and condensed proteins from the cottonseed.

The 95% ethanol extractives content of the cotton gin waste was relatively low and was comparable with contents for agroindustrial residues such as wheat straw and sugarcane bagasse (17).

## Carbohydrate Component

Carbohydrates are the most important component of the feedstock with respect to ethanol production. Carbohydrate analyses of the CGR are presented in Tables 2–6. It is clear from the data that variation in carbohydrate composition was more pronounced than variation in the noncarbohydrate fraction discussed earlier. The total carbohydrate contents ranged from 33 to 56%, which is relatively low compared with that for woody (16) or herbaceous biomass (17). For woody biomass, the total carbohydrate content ranges from 67 to 82% for softwoods and 49 to 85% for temperate-zone hardwoods (16). The total carbohydrate content varied within and among ginning plants.

Mannan, arabinan, and galactan contents were very low, as expected from an agroindustrial residue. Within any ginning plant, these sugars did not vary much and neither was there much variation among ginning plants. Because of their relatively low concentrations, their potential influence on ethanol yield is expected to be small.

Glucan and xylan components constituted between 80 and 90% of the carbohydrates. The surprising result was the low xylan content of the feedstock, which was lower than those reported for most agroindustrial residues and hardwoods (15–35%) (17). Since xylan is a typical plant cell wall component that derives mostly from the seed coats and stems, the large variation among ginning plants is probably a reflection of the different ginning protocols, as shown by the data in Table 1. Feedstocks with relatively high seed and mote fractions had higher xylan content. However, this explanation needs to be investigated further.

Glucan, which derived from both the cotton fiber and the cell wall of the lignocellulose, was the largest fraction of the feedstocks. However, glucan was lower than those reported for wood and other agroindustrial residues (17). Glucan varied widely among ginning plants (20–38%) compared to within ginning plants (Tables 2–6). The variation in glucan among ginning plants could be attributed to the ginning methods, because some

	Severity – 3	3.5	Severity – 4.5 <sup>a</sup>		
Sample	Ethanol	Ethanol	Ethanol	Ethanol	
	yield	yield	yield	yield	
	(% theoretical)	(L/t)	(% theoretical)	(L/t)	
Emporia fresh discharged	78.4	155	17.1	31	
Franklin fresh discharged	85.8	152	ND	ND	
Suffolk fresh discharged	92.5	191	21.7	53	
Windsor fresh discharged	81.0	133	19.4	40	
Wakefield fresh discharged	58.0	120	24.4	52	

Table 7
Ethanol Production from Steam-Exploded Cotton Gin Waste at Two Severities

gins had higher cotton lint content than others (Table 1). Consequently, this could have influenced the glucan content.

## Hydrolysis and Fermentation of Steam-Exploded CGR

When some samples of steam-exploded CGR were enzymatically hydrolyzed and fermented without overliming there was no cell growth and no ethanol was produced. However, when the steam-exploded substrate was overlimed and hydrolyzed, the *E. coli* KO11 grew very rapidly and fermented the substrate to ethanol.

Ethanol yields were determined as a percentage of the theoretical yield. The ethanol yield reported here is a combination of the fermentation of xylose and glucose by *E. coli* KO11. Although other minor sugars were present in the steam-exploded substrates, it is not known that *E. coli* KO11 can ferment these sugars into ethanol.

Ethanol yield was a function of both steam explosion severity and the source of feedstock (Table 7). At the higher severity (4.5), ethanol yields were very low and similar for all samples from all the cotton gin plants. The low yields at the high severity were attributed to inhibitory compounds produced during the steam explosion. Although overliming of lignocellulosic hydrolysates reduces enzyme and microbial inhibitors, it does not necessarily restore cell conversion efficiency. It has been reported (J. D. McMillan, personal communication, 2001; [21]) that although cells may grow in overlimed lignocellulosic hydrolysates, the product yield may be reduced considerably. In the case of the CGR, the samples that were steam exploded at a severity parameter of 4.5 and overlimed with calcium hydroxide supported cell growth, but the ethanol yields were very low. Ethanol yields were reduced from 92.5 to 21.7% for the Suffolk gin CGR. Clearly, the choice of severity for CGR treatment is very important for CGR fermentation and ethanol production.

The highest ethanol yield was achieved for samples treated at a severity 3.5 and overlimed with calcium hydroxide. It can be seen that the etha-

<sup>&</sup>lt;sup>a</sup>ND, not determined.

nol yields were considerably higher for all feedstocks at this severity compared to the samples at a severity of 4.5 (Table 7). Interestingly, ethanol yield was different for feedstocks from different cotton gins. This result is of practical significance because product yield and therefore profitability could be considerably affected by the source of feedstock.

Ethanol yield was highest (92.5%) for the feedstock from the Suffolk, or equivalent to 191 L/t of CGR, while that from the Wakefield cotton gin was the lowest (58%), or equivalent to 120 L/t of CGR. Ethanol yield for most feedstocks was better than those estimated by Beck and Clements (3) for an acid hydrolysis process.

## Conclusion

Production of ethanol from cotton gin waste appeared to be influenced by several factors including feedstock origin, steam explosion severity, sample heterogeneity, feedstock composition, and other unknown factors. To optimize ethanol yield from this resource, some or all of these factors have to be addressed. Feedstock composition appeared to vary considerably even for samples taken within a ginning season. These feedstocks need to be analyzed over a few years to establish an average compositional data on which to base the expected ethanol yield for practical applications.

High steam explosion severities tended to create more inhibitory compounds and these resulted in low ethanol yields. It appears that a severity of 3.5 is adequate for ethanol production at a reasonable yield. Although the process was not optimized, the highest ethanol yield for these feedstocks was 191 L (50 gal)/t, considerably higher than the 142.8 L (37.8 gal)/t estimated by Beck and Clements (3) for an acid hydrolysis process.

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