TECHNICAL NOTE

COMBINED HEAT TREATMENT AND ACID HYDROLYSIS OF CASSAVA GRATE WASTE (CGW) BIOMASS FOR ETHANOL PRODUCTION

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ABSTRACT. The effect of combined heat treatment and acid hydrolysis (various concentrations) on cassava grate waste (CGW) biomass for ethanol production was investigated. At high concentrations of H_2SO_4 (1–5 M), hydrolysis of the CGW biomass was achieved but with excessive charring or dehydration reaction. At lower acid concentrations, hydrolysis of CGW biomass was also achieved with 0.3–0.5 M H_2SO_4, while partial hydrolysis was obtained below 0.3 M H_2SO_4 (the lowest acid concentration that hydrolysed CGW biomass) at 120°C and 1 atm pressure for 30 min. A 60% process efficiency was achieved with 0.3 M H_2SO_4 in hydrolysing the cellulose and lignin materials present in the CGW biomass. High acid concentration is therefore not required for CGW biomass hydrolysis. The low acid concentration required for CGW biomass hydrolysis, as well as the minimal cost required for detoxification of CGW biomass because of low hydrogen cyanide content of CGW biomass would seem to make this process very economical. From three litres of the CGW biomass hydrolysate obtained from hydrolysis with 0.3 M H_2SO_4, ethanol yield was 3.5 (v/v %) after yeast fermentation. However, although the process resulted in gainful utilization of CGW biomass, additional costs would be required to effectively dispose new by-products generated from CGW biomass processing. © 1997 Elsevier Science Ltd

INTRODUCTION

Cassava is a very important source of carbohydrate in the diet of people from the eastern part of Nigeria. It has an enlarged root (a root tuber). Apart from being a major source of starch, it is also rich in cellulose and hemicellulose.[1] In the eastern part of Nigeria, cassava is converted to food material by various fermentation processes. 'Garri' is one of the food products from cassava. It is obtained by removing the peel, grating the whole tuber, bagging and expressing out the liquid under pressure for about 3–4 days, during which fermentation occurs. It is then sieved to obtain the starch which is roasted lightly in a heated pan to introduce flavour and reduce the moisture content for effective storage. The product is called 'garri'. The waste material obtained after sieving, whose composition is mainly cellulose,

lignin and hemicellulose is discarded. As cassava is a major source of food material for millions of people in that part of Nigeria, the waste material from its processing is abundant. Dunlap and Chiang[1] reported that cassava cellulose and corn stover are the most abundant biomass generated in Nigeria and the U.S.A. each year. The technologically advanced world has developed efficient recycling of waste into useful products, while this technology is lacking in the developing world, hence waste materials constitute serious health hazards in these countries.

Chemical hydrolysis, especially acid hydrolysis of biomass, is one of a number of viable technologies being developed as a biomass conversion process. A great deal of effort has been devoted to the investigation of the kinetics,[2–5] and reactor design of the processes.[6–8] Abaseed and Lee[9] studied the effect of transient heat transfer and particle size on acid hydrolysis of hardwood cellulose in the reaction conditions of 1–3% sulphuric acid at temperatures of 98–215°C. Cellulose is known to be highly resistant to enzymatic hydrolysis but these difficulties can be overcome by employing a suitable chemical or mechanical pre-treatment prior to hydrolysis.[9]

Various pre-treatment methods are available in the literature.[10-12] However, amongst the available methods, three attract most attention: 'Organosolv' pre-treatment using a water–ethanol mixture, dilute acid pre-hydrolysis of hemicellulose, and steam pre-treatment at 180–210°C for 1–30 min.[13] Brownwell and Saddler[14,15] however, reported that steam explosion does not enhance hydrolysis rate. But the studies by Nidetzky *et al.*[16] revealed that steaming releases uronic acids and acetyl groups in the form of acetic acid and under these acid conditions, the hemicelluloses are hydrolysed[13] with concomitant removal of the 'shielding effect' of the hemicellulose–lignin matrix.

This paper presents the results of our study aimed at finding a solution to the environmental pollution and hazards caused by the uncontrolled massive dumping of cassava grate waste (CGW) biomass obtained after cassava processing. Although steam/enzymatic processes have been used in hydrolysis of cellulose biomass from other sources than from cassava with some recorded successes, enzymes are very expensive. In this study, a less expensive method of combined steam/acid hydrolytic process was employed in obtaining a more useful product—ethanol from CGW biomass.

MATERIALS AND METHODS

Sample Collection and Preparation
CGW—18–20% moisture was collected from freshly processed 'garri' waste in the Enugu area of Nigeria and dried at 32°C in a hot-air oven (Gallenkamp, U.K.) for 4 days to 3–4% moisture after which it was milled to obtain the flour. The ground flour was sieved to produce fine particles of less than 0.6 mm dia.

Reagent Preparation
Unless otherwise stated, all chemicals used in this study were either BDH or Sigma Chemicals. Concentrated sulphuric acid (mol. wt, 98.07; purity, 97 w/w %; sp. gr. 1.849) was used to prepare the various acid concentrations classified as high concentrations (1–5 M) and low concentrations (0.2–0.5 M) for use in the CGW biomass hydrolysis.

Hydrogen Cyanide Content of Cassava Peel, Grate and Grate Waste Biomass
The colorimetric determination of cyanide by modified Koenig reaction method of Lambert *et al.*[17] and Williams'[18] method which uses KCN as standard were used in the estimation of hydrogen cyanide content of cassava.

Cellulose Determination
A modified method of Crampton and Maynard[19] was employed in the determination of cellulose materials

present in the CGW biomass. Sample, 1 g (4% moisture), and 20 ml of 80% acetic acid containing 2 ml concentrated HNO_3 in a reflux condenser were heated gently. After boiling for 15 min, the solution was cooled and mixed with 25 ml ethanol. The slurry was centrifuged (Denley BS 400 Centrifuge, U.K.) at maximum speed for 20 min to remove non-cellulose materials. After washing several times with alcohol and ether, the residue was dried. The dried sample, contained in a porcelain boat, was ignited in a muffle furnace at 550°C for 3 h. Cellulose material was calculated as loss in ignition.

Lignin Determination
The method of Morrison[20] was employed for this determination. Sample, 5 g, was suspended in 25% acetylbromide in acetic acid to remove any interfering materials (mainly phenols). Treated sample, 0.1 g, was mixed with 25 ml of distilled water and heated for about 15 min and filtered, hot, through a Whatman No 1 filter paper with the aid of a suction pump. After washing several times with water and ethanol, the material was dried at 105°C for 10 min. The dried sample was transferred to a 250 ml volumetric flask and mixed with 25 ml of acetic acid containing 5 ml of 2 M NaOH. An additional 200 ml acetic acid and 8 ml 5 M hydroxylammonium chloride were added and the mixture allowed to stand for 60 min to precipitate protein materials. The absorbance of the sample and blank (distilled water) were read in a Bausch and Lomb Spectronic 21 spectrophotometer at 280 nm. A standard lignin curve was used to estimate the lignin content present in the CGW biomass.

Ash Determination
This was determined by heating 5 g of the dry sample contained in a porcelain boat in a muffle furnace at 550°C for 3 h.

CGW Biomass Hydrolysis
Biomass flour (0.6 mm dia.) from CGW, 25 g, was hydrolysed with 250 ml (1:10 w/v) of the various concentrations of H_2SO_4 for 30, 60 and 90 min at 120°C in a pressure vessel (1 atm) after which each hydrolysate was neutralized (pH 6.8) by mixing with 1 M NaOH solution and allowed to stand for 24 h at 28°C. The neutralized hydrolysates were centrifuged (Denley BS 400 Centrifuge, U.K.) at maximum speed for 20 min to remove any suspended or unhydrolysed materials. The substrate, hydrolysed for 30 min, was used for the study. The hydrolysates obtained after 60 or 90 min were discarded (poor results).

Reducing Sugar Determination
The reducing sugars (as glucose) present in the hydrolysates were determined quantitatively by the methods of Nelson Somogyi as outlined by Robyt

and Whelan.[21] After mixing the samples with the assay reagents, the absorbance was measured at 600 nm (Spectronic 21, Bausch and Lomb Spectrophotometer) against the appropriate blank solution. The amount of reducing sugars were related to glucose using standard curves.

Hydrolysis and Preparation of CGW Biomass for Fermentation

The lowest acid concentrations which hydrolysed the CGW biomass (for economy of process) were used for the study. Approximately 250 g CGW biomass were hydrolysed with 2.5 l of 0.3, 0.5 and 1 M H_2SO_4 and neutralized as described earlier. The hydrolysates were further diluted to 3 l with 500 ml water and heated for 30 min. After cooling, the carbon-rich media were fortified with ammonium sulphate (3 g l^{-1}) and potassium dihydrogen phosphate (3 g l^{-1}).

Yeast Propagation and Fermentation

Yeast, Saccharomyces cerevisiae, was propagated in 50 ml of the enriched hydrolysate contained in 250 ml Erlenmeyer flask, agitated at 28°C on a rotary shaker (Gallenkamp orbital incubator, U.K.) for 24 h after which the yeast was inoculated at the rate of 3 g fresh weight per litre of the hydrolysate. Fermentation was carried out in a 5-l flask at 30°C.

RESULTS AND DISCUSSION

Cassava waste processing is difficult because it is high in toxic materials.[22] However, in the process described for the conversion of raw cassava to food materials (see Introduction), the fermentation step, which is an integral part of the process, is important because fermentation reduces to a large extent the hydrogen cyanide levels in cassava.[23,24] Table 1 shows the results of hydrogen cyanide levels found in cassava peel and the peeled/grated cassava. It is clear from Table 1 that cassava peel contains higher levels of hydrogen cyanide assessed by both methods. It is also obvious from Table 1 that although the peeled/grated cassava has a small amount of hydrogen cyanide, the hydrogen

cyanide level was drastically reduced by over 78% following the fermentation step of the process.

This confirms the important role the fermentation step described (see Introduction) could play in detoxification of cassava. In other studies, fermentation also reduced to a large extent (by about 95%) the hydrogen cyanide level of cassava peel.[23,24] It is, however, interesting to note that the hydrogen cyanide level of the fermented cassava grate assayed by both methods are extremely low, and that the sieved-grate waste used in this study was virtually free of hydrogen cyanide (Table 1) and would reduce waste processing cost. Although Lambert's method for determining hydrogen cyanide level gave higher values of hydrogen cyanide than Williams' method, the similar and low levels of hydrogen cyanide found in the fermented material confirms that fermentation detoxifies hydrogen cyanide, thus making the food prepared from cassava safe for consumers of cassava products.

In Table 2, the composition of the CGW biomass is presented. It can be seen from Table 2 that the CGW biomass is rich in cellulose and lignin materials. For ethanol production from CGW biomass, which was the main focus of this study, CGW biomass was prepared as described (see Materials and Methods). Although CGW biomass is an excellent substrate for biogas production,[25] the interest in ethanol production was because of the important industrial use of ethanol. The dwindling economy of the developing countries, including Nigeria, is making it increasingly difficult for the procurement of many basic materials, including chemicals, required to sustain the existing industries in Nigeria.

In this study, the process temperature, particle size and hydrolysis time were maintained at constant values, while the acid concentrations were varied to obtain the lowest acid concentration that would hydrolyse the CGW biomass. High acid concentrations (1–5 M H_2SO_4) were used in this study because cellulose materials are reported to be highly resistant to enzymatic hydrolysis.[9,26] At the high acid concentrations used for the CGW biomass hydrolysis, although the solubilized materials increased with increasing acid concentrations (Fig. 1), a lot of charring or browning or dehydration reactions occurred to a varying degree (Table 3). Hydrolysis of CGW biomass does not require high H_2SO_4 concentrations, as shown again in Table 3, where acid concentrations, of 0.3–0.5 M H_2SO_4 also hydrolysed the CGW

TABLE 1
Hydrogen Cyanide (mg/100g) of Cassava

	Method 1*	Method 2†
Cassava peel	52	44
Whole cassava grate before fermentation	18	9
Whole cassava grate after fermentation	3	2
Reduction in (HCN) level after fermentation (%)	83.3	77.8
Sieved CGW biomass	—	—

*Lambert et. al. method.
†Williams' method.

TABLE 2
Percentage Composition of CGW Biomass

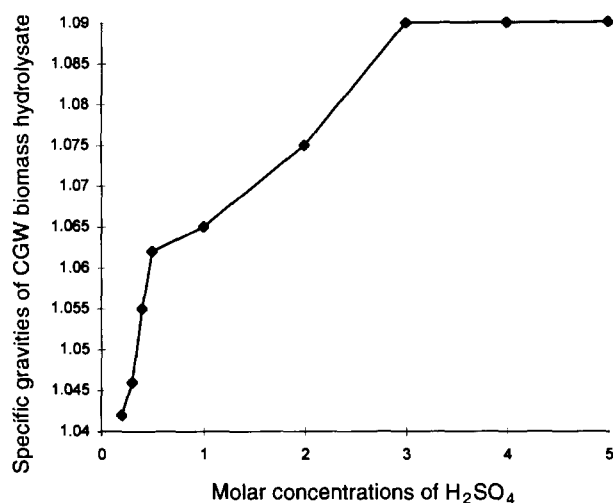|	Cellulose	Lignin	Ash
Before acid–steam hydrolysis	58	22	6.6
After acid–steam hydrolysis	23	9	—
Process efficiency (%)	60.3	59.1	—

FIGURE 1. Specific gravities of CGW biomass hydrolysates at the different acid concentrations.

biomass. At acid concentration of 0.3 M H_2SO_4, 60% hydrolysis of cellulose or lignin material present in the CGW biomass was achieved (Table 2). However, below 0.3 M H_2SO_4, hydrolysis of the CGW biomass was less effective, suggesting that 0.3 M H_2SO_4 is the minimum economical acid concentration required to hydrolyse the CGW biomass (Table 3). It has been reported elsewhere[27] that high yields of sugar release from biomass hydrolysis occur by operating under atmospheric pressure, at a relatively moderate temperature, and with dilute acids.

Solubilization of the CGW biomass materials increased up to 3 M H_2SO_4 and then levelled off, suggesting that maximum digestion of CGW biomass was achieved with 3 M H_2SO_4 (see Fig. 1). Although the mechanism for the acid hydrolysis of CGW biomass is not known, it might be similar to that reported for steam treatment of wheat straw.[13,16] Steaming not only removes the 'shielding effect' of the hemicellulosic–lignin matrix,[13] it has also been described as a pre-treatment 'severity factor',[28,29] probably because steaming exposes the cellulosic materials to either acid-hydrolysis, enzyme-hydrolysis, or a combination of

both. The various colours shown by the solutions of the CGW biomass hydrolytic products in H_2SO_4 solution (Table 3) arise from dehydrating and oxidizing actions of sulphuric acid on the different hydrolytic products. The oxidation products are complex acids including aldaric acids whose salts are known to possess characteristic properties which serve in the identification of sugars. These observations agree with the report of Parisi.[13] Other chemical reactions reported include the formation of furfural from xylose.[27]

When the CGW biomass hydrolysates obtained from 0.3, 0.5 and 1 M H_2SO_4 were fermented, the sugar levels dropped progressively for the respective acid concentration and remained constant after 4 days of fermentation (Fig. 2). The pH was in the acidic region (Table 4). Similar results of enzymatic hydrolysis of wheat straw after steam pre-treatment at 170°C have been reported elsewhere.[16] The low pH could be related to accumulation of acetic acid,[16,27] and uronic acid,[16] in the fermentation medium as the nutrients are utilized by yeast. Although the sugars present in the fermentation broth were not determined, the high final gravities after fermentation (Table 4) may be as a result of the presence of unassimilated sugars such as xylose, which is not metabolized by the yeast—*S. cerevisiae*, used in this study or other inhibitory compounds usually obtained from acid hydrolysis of biomass.[30-32] The release of a high percentage of xylose from acid hydrolysis of biomass has been reported elsewhere.[16,23] It is also possible that high acid concentrations released more of these unassimilated soluble materials (Fig. 1). This argument is further supported by the results of fermentation of CGW biomass hydrolysates prepared from 0.3, 0.5 and 1 M H_2SO_4, where higher percentages of unfermented extracts were found to increase with hydolysate prepared from higher acid concentrations (Fig. 2 and Table 5).
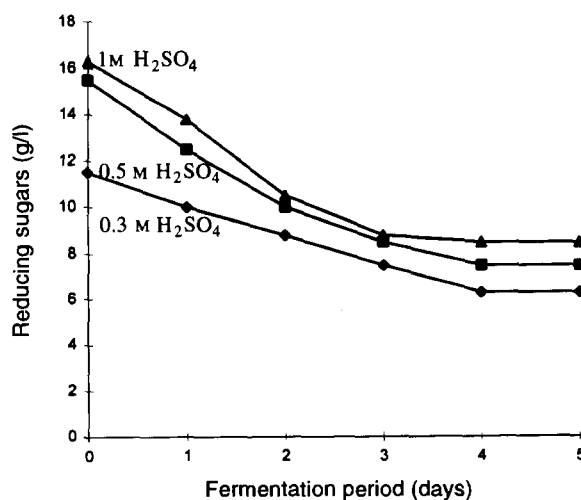
TABLE 3
Effect of Acid Concentrations on CGW Biomass Hydrolysis

H_2SO_4 conc. (M)	Reducing sugars (g l^{-1})	Colour of hydrolysate	Iodine reaction
5.0	18.5 ± 4.2	Dark brown	Faint yellow
4.0	18.4 ± 3.3	Brick red	Faint yellow
3.0	18.5 ± 2.4	Brown	Faint yellow
2.0	17.8 ± 0.9	Light brown	Faint yellow
1.0	16.3 ± 1.2	Pale yellow	Faint yellow
0.5	15.5 ± 0.9	Pale yellow	Faint yellow
0.4	13.8 ± 0.3	Pale yellow	Faint yellow
0.3	11.5 ± 0.6	Pale yellow	Faint yellow
0.2	10.5 ± 0.3	Light green	Blue–black

Values are mean of two replicate determinations, ± SD.

FIGURE 2. Drop in sugars during fermaentation of cassava (CGW) biomass hydrolysates.

TABLE 4
Fermentation Profiles of CGW Biomass Hydrolysates from 0.3, 0.5, and 1 M H_2SO_4

Days	0.3 M		0.5 M		1.0 M	
	Sp. gr.	pH	Sp. gr.	pH	Sp. gr.	pH
0	1.046	4.0	1.062	4.2	1.065	4.2
1	1.040	4.0	1.050	4.0	1.055	4.2
2	1.035	4.0	1.040	4.0	1.042	4.0
3	1.030	4.0	1.038	3.8	1.035	3.9
4	1.025	4.0	1.030	3.8	1.032	3.9
5	1.025	4.0	1.030	3.8	1.032	3.8

TABLE 5
Some Properties of the Fermented Broth

	Acid concentrations		
	0.3 M H_2SO_4	0.5 M H_2SO_4	1.0 M H_2SO_4
Broth volume (l)	3.0	3.0	3.0
Original sp. gr.	1.046	1.062	1.065
Final sp. gr.	1.025	1.030	1.032
Ethanol (v/v%)	3.5	3.3	3.3
pH	4.0	3.8	3.8
Unfermented extract (%)	2.01	3.01	3.1
Fermented extract (%)	97.99	96.99	96.9

Although studies by Delgenes et al.[27] on acid hydrolysis of wheat straw suggested that Pichia stipitis—a xylose metabolizing yeast was apparently less sensitive to inhibitors in acid hydrolysates, Detroy et al.[30] reported that Pachysolen tannophilius was totally inhibited when grown on a crude hemicellulose hydrolysate from wheat straw. Clark and Mackie[31] also reported some inhibitory effect on S. cerevisiae used in their fermentation study of hydrolysate derived from soft wood Pinus radiata. The slow or incomplete fermentation of the CGW biomass hydrolysate observed in the present study agree with similar studies reported elsewhere.[30,33] Poor fermentation performance from crude hemicellulosic hydrolysate, when compared to those from synthetic medium[27] was related to the presence of biomass degradation by-products—mainly furfural, acetic acid and compounds from lignin and extractives.[31] Furfural was reported to inhibit activities of some glycolytic enzymes, particularly dehydrogenases in S. cerevisiae.[32]

CONCLUSION

This study showed that the hydrolysate made from 0.3 M H_2SO_4 which released the least level of sugar, gave a higher alcohol yield (Table 5). Although the reason for this observation is not clear, it is possible that the higher levels of sugars found in the CGW

biomass hydrolysates made from 0.5 or 1.0 M H_2SO_4 were caused by the presence of other sugars which are not assimilated by yeast during fermentation of CGW biomass hydrolysate. The higher final extract levels found in the broth prepared by hydrolysing CGW biomass with 1.0 M H_2SO_4, in contrast to that found in broth prepared by hydrolysing CGW biomass with 0.3 M H_2SO_4 suggest that higher acid concentrations released more of these unfermentable materials. In all the fermentation studied, although more than 97% of the original extract was utilized during fermentation (Table 5), the yeast performance was poor in relation to ethanol production. This may be related to the inhibitory effect of biomass hydrolytic by-products on the yeast as reported by other workers.[30-32] In this regards it is possible that much of the nutrients (sugars) are being used for yeast growth rather than for ethanol production. However, it would seem that if a mixed yeast flora containing both hexose and xylose fermenting strains[27] are used to inoculate the CGW biomass hydrolysate, higher ethanol yields would be achieved. The poor ethanol yields notwithstanding, the process reported in this study resulted in gainful utilization of CGW biomass by a simple acid–steam hydrolytic process, considering that CGW biomass is usually accumulated as heaps of waste material after cassava processing, with all the associated environmental pollution and health hazards.

Although a process efficiency of 60% achieved with the lowest acid concentration and the low cyanide level of CGW biomass (see Tables 1 and 2), would suggest that this process is economical, other problems are apparent from the study. These include the disposal of unhydrolysed biomass after the primary hydrolytic process prior to yeast fermentation. High biological oxygen demand (BOD) values are usually encountered in the treatment of molasses-based stillage.[34] Another problem is the disposal of the high levels of unutilized materials in the broth after yeast fermentation.[35] These additional processes may result to an increase in the cost of production of ethanol from CGW biomass. However, experimental studies to optimize this process and reduce cost due to biological treatment of the residue are still under investigation.

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