



Research Article

Effect of Delignification on Reducing Sugar Recovery from Crocodile Gourd for Bioethanol Production

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ABSTRACT

A central composite experimental design was used to optimize the key parameters affecting biomass delignification process of crocodile gourd for maximum reducing sugar recovery. The effects of temperature (70 °C to 90 °C), residence time (2 hrs. to 6 hrs.), concentration of sodium chloride (0.05 g/dm³ to 0.1 g/dm³) and volume of acetic acid (0.50 mL to 3 mL) were studied. The result obtained showed that optimum reducing sugar yield for crocodile gourd was achieved at temperature, time, sodium chloride and acetic acid of 70 °C, 6 hrs. 3 g and 3 mL respectively. The results indicate that X₃, X₄, X₁² interactions and X₂X₄ interaction has linear effect on reducing sugar yield for crocodile gourd. The result of the proximate analysis showed moisture content of 6.50±0.50 %, Ash Content 11.00±0.28 %, Carbohydrate 64.40±0.62 %, Organic carbon 12.95±0.13 (%), Nitrogen 2.10±0.02 (%), Carbon/Nitrogen 6.16±0.08 %, Potassium 14100±23 mg/kg, Sodium 228±29 mg/kg, and Fixed carbon 7.4±0.80 %. The result of proximate analysis and compositional analysis revealed that the substrate could be utilized for biofuel production.

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INTRODUCTION

Bioethanol is an alternative source of fuel obtained from biomass and is environmentally and less hazardous (Rawayaua, *et al.*, 2021). It is a fuel consisting of fatty acid methyl ester produced from chemical reaction between vegetable oil and alcohol (Novita *et al.*, 2014). Lignocellulosic biomass being the most abundant renewable biomass obtained from agricultural residues or plants for production of bioethanol and is largely available and cheaper although still its growth is still facing technological challenges (Abo *et al.*, 2018). This is believed to be safer and could replace fossil fuel as a source of energy. Source of Lignocellulosic biomass include; Agricultural residue (leaves, stovers, straws), agro-waste (solid cattle manure), forest biomass (cedar, spruce, willow), forest waste (saw dust, wood chips), industrial wastes (chemical pumps), municipal solid wastes (food waste, kraft paper) (Abo *et al.*, 2018).

In general, the lignocellulose structure is composed of crystalline cellulose, amorphous hemicellulose and non-sugar lignin. Cellulose is a linear glucose homopolymer molecule having mass from 50kDa at 2500000 kDa or between 300 to 15000 glucose residues having repeated cellobiose units (Lavoine *et al.*, 2012). Recycling lignocellulosic waste materials as a byproduct or through composting, biofuel production, energy valorization, synthesis of glucose and other high-valued products and the synthesis of biocomposites, nanofibers, biopolymers and nanoparticles are interesting alternatives that align well with the circular economy (Blasi *et al.*, 2023).

The analysis of several agricultural waste streams revealed rich composition of cellulose, lignin as well as hemicellulose content. These components serve as valuable feedstock for the production of ethanol and bio-oil, which can be employed as alternative energy sources that are ecofriendly. Still, the extraction and transformation of lignocellulosic components into furfural, levulinic acid and hydroxymethylfurfural, offer potential for the synthesis of various high-value biochemical (Blasi *et al.*, 2023).

Most processes require high chemical charges to attain the complete lignin removal, because the single pretreatment process is not effective when performed at low chemical charges. In

some cases, rearrangement of the lignin structure occurs during the pretreatment process (Kumar and Wyman, 2009). Products released by the degradation of polysaccharides and hemicellulose extracts strongly inhibit the cellulose hydrolysis by cellulase (Jing *et al.*, 2009). The presence of lignin limits the maximum utilization of cellulose and hemicelluloses to simple fermentable substrate that can later be converted to fuel. Therefore, this research was aimed at removing lignin which will enhance the hydrolysis of substrate into fermentable sugar.

MATERIALS AND METHODS

Sample Collection and Treatment

The fresh sample of crocodile gourd was collected in Gidan-kuka area of Sokoto metropolis. The sample was dried under the sun for three weeks to reduce the moisture content. The dried sample was crushed with the aid of a mortar and pestle, sieved through an 80 mm mesh and stored in a clean polyethene until required for use.

Proximate Analysis of the Plant Substrate

The powdered sample was analysed for proximate and ultimate contents in accordance with method described by Thiammah (1999) and Akin-Osanaiye (2005).

Determination of Lignin

The sample (3g) was weighed and transferred into a beaker and H₂SO₄ (72%, 40 mL) solution was added gradually while stirring and macerating the material with a glass rod. After the specimen was dispersed, the beaker was covered for 2 hrs. at room temperature. Water (400 mL) was added to the flask and then the mixture from the beaker was transferred into the flask. Sulphuric acid (3%) was added to make up the volume (1540 mL). The solution was boiled for 4 hrs. and constant volume was maintained by frequent addition of hot water. The insoluble material (lignin) was allowed to settle then filtered. Then 3g of lignin was weighed out and placed in an oven set at 105 ± 3°C for 2 hrs.; crucible containing the dried sample was cooled in a desiccator and weighed. To determine acid-soluble lignin (ASL) the content of acid-soluble lignin in the first filtrate was subjected to spectrophotometry at 205 nm.

Delignification of the Substrate

The sample (110 g) was wash several times with hot distilled water (80 °C) to remove free non-structural sugar that may be present in

significant amount. The washed Sample substrate was squeezed by hand to remove excess water and then dried at room temperature (35 °C) for three days in an oven. Each treated sample was heated at 70±2 °C on a hotplate, the sample (5.0 g) was weighed into the flask (250 mL) and water (160 mL) was added followed by sodium chloride (3g) and also acetic acid (5 mL). The slurry was thoroughly mixed by shaking the flask. Sodium chloride and acetic acid were added to the reaction every 2 hrs. for 6 hrs. during delignification. After delignification, the slurry was filtered through a filter paper to separate the liquid from the residue and the residue was washed with distilled water and dried for 3 hrs. at room temperature and weighed.

Acid Hydrolysis of Delignified Substrate

This was carried out according to the method described by Humphrey and Caristas (2007); Gupta *et al.* 2009; Oyeleke and Jibrin (2009). Each of the delignified sample (5 g) was weighed into a conical flask (250 mL), sulphuric acid (5.0%, 150 mL) was added into the conical flasks. The flask was covered with cotton wool, wrapped in aluminum foil, heated in a water bath for 2 hrs. at temperature 50 °C. The flask was allowed to cool, filtered through No.1 Whatman filter paper. The pH of the hydrolysate was adjusted to 4.5 with sodium hydroxide (0.4M).

Determination of Reducing Sugar from Delignified Substrate

The reducing sugars were estimated by using DNS reagent in accordance with Rabah *et al.* (2011). The DNS reagent was prepared by adding sodium hydroxide (10 g) to distilled water (1 L). In a separate container, potassium sodium tartarate (40 g) was dissolved into distilled water (100 mL). The reducing sugar content of the hydrolysate was assayed by adding DNS reagent (3 mL) into the sample (3 mL). The mixture was heated in boiling water for 10 minutes to develop a red brown colour. Then, potassium sodium tartarate solution (1 mL of 40%) was added to stabilize the colour and was cooled to room temperature under running water tap. The absorbance of the sample was measured using UV-VIS spectrophotometer (520 nm). The reducing sugar content was subsequently determined by making reference to a standard curve of known glucose concentration.

Experimental Design and Data analysis

A central composite experimental design was used to optimize the temperature (X_1), time (X_2), sodium chloride concentration (X_3) and acetic acid volume (X_4) on reducing sugar recovery from delignified crocodile gourd. The reducing sugar recovery was used as dependent output variables. Twenty-one (21) experiments were performed for crocodile gourd. A quadratic model (Box *et al.*, 1978) was used to evaluate the optimization of key parameters using the following equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 \dots \dots \dots (1)$$

Where, Y = predicted response; X_1 , X_2 , X_3 and X_4 = parameters and β is to be found. The response variable (Y) was fitted using a predictive polynomial quadratic equation in order to correlate the response variable to the

independent variables (Lay, 2000). The Y values were regressed with respect to temperature, time, sodium chloride concentration and acetic acid concentrations.

Table 1: Independent Variables and their Coded Levels for the Central Composite Design used for Delignification

Independent Variable	Coding	Lower levels	Upper level
Temperature (°C)	X ₁	70	90
Time (hours)	X ₂	2	6
Sodium chloride (g/dm ³)	X ₃	0.05	0.1
Acetic acid (mL)	X ₄	0.50	3

RESULTS AND DISCUSSION

Proximate and Ultimate Composition of the Substrate

Proximate and ultimate composition analysis gave moisture, ash, lipid, fiber, carbohydrate and organic matter on percentage weight basis while ultimate analysis gives its chemical constituents such as nitrogen, carbon among others (Khan *et al.*, 2020). Table 2 showed the result of proximate and ultimate analysis of the substrates.

Low moisture content in the substrate is an indication that microbe's activities would be reduced and thereby increases the shelf life of sample (Khan *et al.*, 2020). A low moisture level in the substrate is usually preferable because high-moisture in the substrate burn less readily and provide less useful heat per unit mass (Demirbas, 2001). From the result in Table 2, Crocodile gourd has a low moisture content, it implies that the substrate is a good substrate for biofuel production. The moisture content for crocodile gourd was less than 12 percent recommended for good storability and this implies that the substrates can be stored on till the need arises (Demirbas, 2004; Mahmoudi *et al.*, 2010.). Moisture content also lower the rate of combustion and takes away appreciable amount of the liberated heat in form of latent heat of vaporization (Demirbas, 2001; 2004).

Ash content of a biomass is the solid remain after oxidation (Mahmoudi *et al.*, 2010.). As

shown in Table 2, crocodile gourd has a low ash content. The result indicated that the substrate is a good substrate for bio ethanol production. Researchers have shown that high ash concentrations are unfavorable for bioethanol production and also ash does not contribute to the calorific value of the fuel (Demirbas, 2001; 2004; Mahmoudi *et al.*, 2010).

The nitrogen content of crocodile gourd as shown in Table 2, nitrogen is an important nutrient in biofuel production and have contribute significantly to biochemical process (fermentation). Nitrogen serves as nutrient for the growth and metabolic activity of the yeast during fermentation (Mahmoudi *et al.*, 2010.). The higher the percentage fixed carbon, greater is the calorific value (Mahmoudi *et al.*, 2010). From Table 2, crocodile gourd has the low percentage fixed carbon (7.4 ± 0.80), these implies that crocodile gourd has low heat value.

Starch, a polymer of glucose units, functions as a sugar store in many plants. It is a mixture of two polysaccharides: amylose and amylopectin. Amylose is the polysaccharide equivalent of the disaccharide maltose and consists of alpha (1-4) linked D-glucopyranose units (Wyman *et al.*, 2005). Previous research has revealed that a substrate with high carbohydrate content is suitable for bio ethanol production. From Table 2, crocodile gourd has a carbohydrate content of (64.40 ± 0.62).

Table 2: Proximate and Ultimate Analysis of the Substrate

Parameters	Crocodile gourd
Moisture (%)	6.50± 0.50
Ash (%)	11.00± 0.28
Carbohydrate (%)	64.40±0.62
Organic carbon (%)	12.95±0.13
Nitrogen (%)	2.10± 0.02
Carbon/Nitrogen (%)	6.16±0.08
Potassium (mg/kg)	14100±23
Sodium (mg/kg)	228± 291
Fixed carbon (%)	7.4±0.80

± standard deviation n=3

Lignin Content of the Substrate

Table 3 result shows lignin content of crocodile gourd. According to the literature, there are two type of lignin. They are acid-insoluble lignin and acid-soluble lignin. Acid soluble lignin is lignin that are soluble in acid during determination of lignin content while acid insoluble lignin is lignin that are insoluble in acid during determination of lignin content. Lignocellulose, the principal component of the plant cell walls, is mainly composed of cellulose (40–60% of the total dry weight), hemicellulose (20–40%), and lignin (10–25%) (Mahmoudi *et al.*, 2010). Lignin are phenolic compounds which are formed by

polymerization of three types of monomers (p-coumaryl, coniferyl, and synapyl alcohols) (Wyman *et al.*, 2005). Critical literature review revealed that high lignin content is unfavorable for bioethanol production (Demirbas, 2001). The results of Table 3 indicate that crocodile gourd (Acid-insoluble lignin + Acid-soluble lignin) has a high lignin contents of (111.13 mg/g). Studies have shown that lignin content varies from plant to plant these may be due to some factors like plant species, plant parts, growth conditions (Permata *et al.*, 2021). Similar result was reported by Mahmoudi *et al.*, (2010).

Table 3: Lignin Content of the Substrate

Lignin Type	Concentration of Lignin (mg/g)
Acid-Insoluble Lignin	103.33± 3.79
ASL=Acid-Soluble Lignin	7.81± 0.24
Total Lignin Content	111.13 mg/g

n=3± SD

Statistical Optimization for Delignification Process

Delignification process was optimized using central composite design. Table 4 shows the reducing sugar recovery from the delignified crocodile gourd.

The analysis of variance presented in Table 5 indicates that model F-value (16.52) and p-value (0.05) implies that the model is significant. In this case X_3 , X_4 , X_1^2 , X_2 , X_4 has a linear relationship with reducing sugar

recovery. It implies that the model is a good predictor of the response variable. The fit of the model was checked by using regression coefficient. The regression square was calculated to be 0.9747. Similar result was reported by Mansfield *et al.* (1999) using same model. The result of the analysis revealed that crocodile gourd has a maximum reducing sugar recovery of 0.863 g/dm³ at an experimental condition of: temperature-70 °C, time-6 hrs., sodium chlorite-0.1g/dm³ and acetic acid 3 mL.

Table 4: Experimental Design for Reducing Sugar Recovery from Delignified Crocodile Gourd

Run	X_1 (°C)	X_2 (hrs.)	X_3 (g/dm ³)	X_4 (mL)	Reducing sugar recovery (g/dm ³)
1	70.00	2.00	0.050	0.50	0.515
2	80.00	4.00	0.075	1.75	0.312
3	80.00	4.00	0.075	0.50	0.244
4	80.00	4.00	0.100	1.75	0.317
5	90.00	2.00	0.100	3.00	0.721
6	80.00	4.00	0.075	1.75	0.311
7	90.00	4.00	0.075	1.75	0.596
8	90.00	6.00	0.100	0.50	0.214
9	70.00	2.00	0.100	0.50	0.224
10	70.00	4.00	0.075	1.75	0.421
11	70.00	6.00	0.100	3.00	0.863
12	80.00	4.00	0.050	1.75	0.514
13	80.00	4.00	0.075	3.00	0.516
14	80.00	6.00	0.075	1.75	0.342
15	70.00	6.00	0.050	3.00	0.861
16	80.00	4.00	0.075	1.75	0.274
17	80.00	4.00	0.075	1.75	0.310
18	90.00	6.00	0.050	0.50	0.312
19	90.00	2.00	0.050	3.00	0.831
20	80.00	2.00	0.075	1.75	0.512
21	80.00	4.00	0.075	1.75	0.273

Table 5: ANOVA for Reducing Sugar recovery from Delignified Crocodile Gourd

Sources	Sum of Squares	DF	Mean Square	F-Value	P-Value Prob>F
Model	0.89	14	0.064	16.52	0.0012
X ₁	0.015	1	0.015	3.97	0.0933
X ₂	0.014	1	0.014	3.75	0.1010
X ₃	0.048	1	0.048	12.50	0.0123
X ₄	0.037	1	0.037	9.60	0.0212
X ₁ ²	0.047	1	0.047	12.13	0.0131
X ₂ ²	7.396E-003	1	7.396E-003	1.92	0.2153
X ₃ ²	4.573E-003	1	4.573E-003	1.19	0.3179
X ₄ ²	1.189E-004	1	1.189E-004	0.031	0.8664
X ₁ X ₂	0.021	1	0.021	5.53	0.0570
X ₁ X ₃	8.201E-004	1	8.201E-004	0.21	0.6609
X ₁ X ₄	0.010	1	0.010	2.65	0.1548
X ₂ X ₃	0.012	1	0.012	3.02	0.1331
X₂ X₄	0.029	1	0.029	7.64	0.0327
X ₃ X ₄	9.870E-003	1	9.870E-003	2.56	0.1607
Residual	0.023	6	3.855E-003		
Lack of Fit	0.021	2	0.011	25.37	0.0053
Pure error	1.6903E-003	4	4.225E-004		
Cor Total	0.91	20			

CONCLUSION

Using CCD model, Optimum conditions for delignification were: temperature-70 °C, time-6 hrs., sodium chloride-0.1g/dm³ and acetic acid 3 mL. By optimizing with coded factor the maximum reducing sugar yield observed by the model was 0.863g/dm³. Since conventional methods of optimization for delignification are extremely time-consuming and expensive. Thus, statistic methodology is efficient and easy to handling large number of design parameters and maximizes the reducing sugar yield, reduce the cost of production. Proximate composition of substrates of crocodile guard with more lignin content and higher values of ligninolytic activities show that these enzymes have potential in industrial applications especially biofuel production.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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