Preliminary approach to predictive modelling of a process for depolymerisation of cassava non-starch carbohydrate using oxalic acid and ionic salt

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Abstract

A study on preliminary approach to the development of a predictive modelling of a process for depolymerisation of cassava non-starch carbohydrate using oxalic acid and ionic salt (potassium iodide) is reported. The effects of three independent variables namely; oxalic acid (0.0278 M), potassium iodide (0.241 M) at volumetric (mL) range of 2-10 and 0.05-1.00 for oxalic acid and potassium iodide respectively with heating range time (min) of 60 to180 were studied using the central composite rotatable design in a 23 factorial experiments. Hydrolysate markers namely: solubility index and reducing sugar were evaluated as a measure of depolymerisation, consequently used as responses in the study. Equations for predicting the responses were developed and their adequacy confirmed using analysis of variance and residual assessment. The predictive model provides insight to the potentials of using oxalic acid as depolymerisation agent for cassava non-starch carbohydrate in the presence of potassium iodide salt. This would open a new opportunity for depolymerisation of cassava non-starch carbohydrate using foodgrade depolymerisation agent with view to yield product that its application will not be limited by safety concern.

Introduction

Cassava non-starch carbohydrate is the major by-product during the process of extraction of starch from cassava tubers. The by-products are mainly, hemicellulose and cellulose and some lignins. These are substrates for production of commodity chemicals. The hemicellulose and cellulose in lignocellulosic biomass require pre-treatment to hydrolyse the polymers to fermentable sugars or oligosaccharides prior to fermentation (Lee and Jeffries, 2011). Pre-treatment is essential for saccharification because native lignocelluloses is highly recalcitrant due to lack of porosity, due to the presence of side chains, the crystallinity of cellulose and the high molecular weights and impermeable nature of the heterogeneous matrix (Lee and Jeffries, 2011). The choice of a pre-treatment lies in the fulfilment of three condition that are first, production of substrates with high susceptibility towards enzymatic hydrolysis. Second, limited or minimal production of undesirable degradation products such as phenolic compounds, furfural or hydroxymethyl furfural. Third, allowances for high recovery of valuable derived products.

Broadly, there are three pre-treatment methods namely, physical, chemical and biological. Among which chemical is the most versatile and most studied. Although, two acids namely sulphuric and hydrochloric used are the traditional solvents used for pre-treatment of lignocelluloses biomass. However, application of the two acids is associated with disadvantages such as production of inhibitory products and corrosion of equipment. In order to overcome these limitations, phosphoric acid, dicarboxylic acids such as oxalic acid have been suggested as alternative to the use of sulphuric acid for pre-treatment of ligno- cellulosic materials (Lu and Mosier, 2007; Kootstra *et al*., 2009; Lee *et al*., 2009). The bases for the suggestion are: dicarboxylic organic acids exhibits two pH values, which may impart more efficient hydrolysis on the substrate over a range of temperature and pH values. In addition both phosphoric acid and oxalic acid are less toxic to yeasts and other microbes, does not inhibit glycolysis and does not produce obnoxious odour when compared to application of sulphuric acid and hydrochloric acid (Mosier *et al*., 2001).

Previous studies had shown that dicarboxylic organic acids can hydrolysed β-(1, 4)-bonds selectively than sulphuric acid. While the relatively weaker ionization potential of oxalic acid relative to sulphuric acid could reduce subsequent dehydration reactions and its unwanted products. The process of using oxalic acid is sustainable since it is fruit

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chemical. In addition, ionic salt such as potassium iodide, magnesium chloride and ferrous chloride had been shown to enhance depolymerisation of starch (Zuwei *et al.*, 2001). The rationale for using salt was that on addition of the salt to solvent conductivity increases, consequently increase rate of heating. Therefore, the objective of this study was to accomplish statistical modelling of a process for the depolymerisation of cassava non-starch carbohydrate using oxalic acid in the presence of potassium iodide salt.

Materials and Methods

Non-starch carbohydrate isolation

The protocol was adapted from Baik and Dalgetty (2003) and modified as reported in earlier study by Daramola (2014). After cassava mash was sieved using muslin cloth to eliminate starch, the residues were screened in 2 L of water using sieves with opening ranging from 50 µm to 90 µm. The recovered non-starch-carbohydrate (NSC) residues were thoroughly rinsed using water till run-off water was clear colourless. The sample fractions obtained were pooled together and dried using tray dryer (supplied by Armfield), then milled using Laboratory blender. These were packaged for subsequent treatment and analysis.

Depolymerisation procedure

Depolymerisation procedure was adapted from the method of Fontana *et al.* (2008). Simply, the solid (g) to solvent (mL) was 3:100 and aqueous oxalic acid was added independently to give final pH values from 1.80 to 3.76 and potassium iodide was added appropriately in accordance with the scheme shown in Table 1. The suspensions were heated using pressure-pot (aluminium pressure cooker MC-C 5300) for the stated holding time.

Determination of liquefaction index

Non-starch carbohydrate biomass liquefaction yield was determined according to the method of Liang *et al.* (2006). The amount of residue in the hydrolysate after depolymerisation process subsequent to filtration and rinsing of residue using distilled water was measured to evaluate the liquefaction in index. After depolymerisation process, the hydrolysate was filtered from the liquefied biomass and the non-liquefied residue was obtained using Whatman No. 45 filter paper. The solid residue was rinsed with distilled water repeatedly until a colourless filtrate was obtained. The rinsed solid residue was dried afterwards in an air oven (HS 121 A) at 105°C for 24

h to obtain the dry mass of the residue.

$$\text{Liquefaction yield} = \left[1 - \frac{\text{dry mass of solid residue}}{\text{dry mass of feedstock}} \right]$$

Measurement of reducing sugar

The concentration of soluble reducing sugars were measured using a 3,5 dinitrosalicylic acid (DNS) method (Chaplin, 1986). Immediately after heating, the solution was diluted with water 10 times and 0.2 mL of the diluted sample was transferred to a test tube where it was mixed with 2 mL of the DNS reagent. After mixing it with a vortex, the samples were heated in the water bath at 100°C for 10 min followed by rapid cooling in iced water. The absorbances of the samples were measured at 570 nm using a (Bausch and Lomb Rochester NY, USA) spectrophotometer. Glucose was used for the preparation of a standard curve.

Statistical analysis

The central composite orthogonal designed was analysed as repleted by Cochran and Cox (1957). Each of the X-matrix was multiplied by the Y-column (response) to obtain corresponding sums of products that is 0y to 13y for X_0 to $X_1 X_3$. Consequently, the coefficients b_0 to b_{13} were calculate as:

$$b_0 = 0.166338(0y) - 0.056791 \, \Sigma \, (iiy) \quad \quad (1)$$

$$b_i = 0.073224(iy) \quad \quad (2)$$

$$b_{ii} = 0.062500(iiy) + 0.006889\Sigma \, (iiy) - 0.056791(0y) \quad \quad (3)$$

$$b_{ij} = 0.125000(ijy) \quad \quad (4)$$

The quadratic model was fitted using the regression coefficients and the predicted response calculated for each of the observed values. The model was assessed for adequacy on subjection to analysis of variance and residual analysis.

Results and Discussion

Pre-treatment in any of its form offers depolymerisation, a mandatory process for a carbohydrate substrate to be useful for fermentation, preparation of sugars/sweeteners or production of commodity-chemicals. As required by improved processing, less severe but effective chemicals such as phosphoric acid (Gamez *et al.*, 2004; Fontana *et al.*, 2008) had been used to depolymerise sugar bagasse. Daramola (2014) used pyruvic acid, a food grade chemical to depolymerise cassava non-starch carbohydrate in the presence of salt. This paper reports on the use of oxalic acid a dicarboxylic and

Table 1. Process variables used in the central composite rotatable design (K=3)

Independent variables	Code	Levels				
		-1.682	-1	0	1	1.682
Oxalic acid (0.0278 M)	X_1 (ml)	2	4	6	8	10
KI (0.241 M)	X_2 (ml)	0.05	0.13	0.25	0.5	1.0
Time	X_3 (min)	60	90	120	150	180

Note: 40 g of oxalic acid was dissolve in 160 mls of H_2O and the stated volume was used accordingly 20 g of KI was dissolve in 50 mls of H_2O and the above stated volume was used accordingly

Table 2. Regression coefficients for the quadratic model equations

Sum of products	% liquefaction	Reducing sugar	Regression coefficients	% liquefaction	Reducing sugar
0y	1128	96.65	bo	66.1158329	5.61055431
y	66.82	12.55	b1	4.89282768	0.9189612
2y	41.46	12.44	b2	3.03586704	0.91090656
3y	127.87	7.613	b3	9.36315288	0.5574543
11y	699.08	73.51	b11	-5.62763	0.37509866
22y	704.74	58.17	b22	-5.27388	-0.5836513
33y	735.84	52.608	b33	-3.33013	-0.9312763
12y	18	1.26	b12	2.25	0.1575
13y	-12	-0.98	b13	-1.50	-0.1225
23y	18	5.08	b23	2.25	0.635
Σ (iiy)	2139.66				

organic acid and potassium iodide, an ionic salt to depolymerise cassava non-starch carbohydrate. Two polymerization markers evaluated are liquefaction index and carbohydrate hydrolysates measured in terms of reducing sugar.

Liquefaction index

Liquefaction is primarily an index of dissolution of amorphilic structure of carbohydrate. It is an effective method to convert lignocellulosic biomass to intermediates rich in hydroxyl groups (Liang *et al.*, 2006). The central composite orthogonal design to fit the polynomial model of the depolymerisation of cassava non-starch carbohydrate was accomplished as elicited by Cochran and Cox (1957). The computed sums of products and regression coefficients to fit the model are presented in Table 2. Therefore, the quadratic model for the liquefaction index takes the form:

$$L^I = 66.1158329 + 4.89282768X_1 + 3.03586704X_2 + 9.36315288X_3 - 5.62763X_1^2 - 5.27388X_2^2 - 3.33013X_3^2 + 2.25X_1X_2 - 1.50X_1X_3 + 2.25X_2X_3.$$

The predicted liquefaction index for each of the experimental runs as well as the residuals is shown in Table 3. A cursory look at the residuals appears to suggest that the fitted model was quite adequate in characterization of the depolymerisation process. The

analysis of variance to test the fitness of the model is shown in Table 4. In agreement with the conjectural statement, the first order and second order terms were quite significant as revealed by the higher calculated F-ratio compared with tabulated values. Since the calculated F-ratio for the lack of fit was lower than the tabulated value, adequacy of the fitted model is established.

Reducing sugar

Reducing sugar is an index of depolymerisation of carbohydrates expressing the cleavage of inter-chain hydrogen bond yielding carbonyl functional group of sugars. It is important for giving insight to the extent to which hydrolysate would be useful for fermentation by organisms. The computed sums of products and regression coefficients to fit the model are presented in Table 2. The quadratic model takes the form:

$$R^S = 5.6105543 + 0.9189612X_1 + 0.91090656X_2 + 0.5574543X_3 + 0.37509866X_1^2 - 0.5836513X_2^2 - 0.9312763X_3^3 + 0.1575X_1X_2 - 0.1225X_1X_3 + 0.635X_2X_3.$$

The predicted amount of reducing sugar formed in each of the experimental runs and respective residuals are shown in Table 3. An examination of the residuals suggested that the model was adequate

Table 3. Residual analysis of assessed parameters

Run	Liquefaction			Total reducing sugar		
	Observed	Predicted	Residual	Observed	Predicted	Residual
1	30	37.332	-7.332	1.5	2.7534	-1.2534
2	38	45.6124	-7.6124	3.21	4.52138	-1.31138
3	25	34.672	-9.672	2.1	2.9902	-0.8902
4	48	51.9515	-3.9519	5.38	5.388193	-0.008133
5	52	59.3201	-7.3201	1.61	2.84325	-1.23325
6	60	57.1	2.9	3.77	4.121176	-0.351176
7	62	60.892	1.108	5.69	5.6201196	0.06988
8	73	72.1719	0.8281	7.54	7.528039	0.04961
9	50	41.969	8.031	6.5	5.12564	1.37436
10	60	58.419	1.581	8.61	8.21702	0.39298
11	52	46.106	5.894	4.3	2.427845	1.872155
12	60	57.1864	2.8136	5.38	5.492135	-0.112135
13	55	40.9555	14.0445	3.5	2.03927	1.46073
14	68	72.4425	-4.4425	4.21	3.91453	0.29547
15	64	66.116	-2.116	6.05	5.61055	0.43945
16	60	66.116	-6.116	5.2	5.61055	-0.41055
17	60	66.116	-6.116	4.5	5.61055	-1.11055
18	67	66.116	0.884	5.5	5.61055	-0.11055
19	74	66.116	7.884	6.7	5.61055	1.08945
20	70	66.116	3.884	5.4	5.61055	-0.21055

Table 4. Analysis of variance (ANOVA) for the predictive model equations

Independent variable	Statistical term	DF	SS	MS	F_{cal}	F_{Tab}
Liquefaction %	First order	3	1650.08	550.02	17.5349	5.41
	Second order	6	957.15	159.52	5.085	4.95
	Lack of fit	5	580.814	116.163	3.70	5.05
	Error	5	156.836	31.367		
	Total	19	3344.8			
Reducing Sugar	First order	3	27.1085	9.03617	16.0102	5.01
	Second order	6	23.37	3.895	6.90	4.95
	Lack of fit	5	13.5625	2.7125	4.8059	5.05
	Error	5	2.822	0.5644		
	Total	19	66.863			

in representing the depolymerisation process. The claim was confirmed by testing the predicted model. The analysis of variance (ANOVA) to test the fitness of the model is shown in Table 4. In agreement with the stated prediction, the first order and second order terms were quite significant as shown by the higher calculated F-ratio in comparison with the tabulated values. As argued earlier, since the calculated F-ratio for the lack of fit was lower than the tabulated value, adequacy of the model is established. Comparatively, it can be seen that the values of liquefaction indices are higher than the value of reducing sugar because more of intra hydrogen bonds are susceptible to breakage than inter chain hydrogen bond. The liquefaction index is a reflection of dissolution of both intra and inters hydrogen bonds within the cellulose polymers strands (Varendel *et al*., 2011). While the reducing sugar value gives insight to the possibility of derivatives such as alcohols, sorbitol, hydroxymethyl furfural, levunic acid that could be obtained from the monomers or oligomers of the hydrolysates.

Conclusion

The potentials of using halide salt assisted oxalic

acid as an agent for depolymerisation of cassava non-starch carbohydrate were demonstrated. Predictive model was developed for the process and adequacy of the model was confirmed using analysis of variance and residual assessment. This study provides insight to the possibility of depolymerising cassava non-starch carbohydrate which could serve as a feed stock for fermentation and production of commodity green chemicals.

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