Conversion of Sorrel (Hibiscus Sabdariffa) Calyces To Glucose

O. A. Ajayi, Olawale, A.S., S. S. Adefila

Abstract:- The utilization of agrowaste for the production of bioethanol has attracted worldwide attention as a strategy for reducing global warming and improving global energy security. Sorrel drink, popularly known as "zoborodo" in Nigeria is a non-alcoholic drink, which is obtained from the calyces of *Hibiscus sabdariffa*, by solid-liquid extraction process, leaving the calyces pulp as the raffinate. The calyces obtained after extraction being rich in carbohydrate, is used in this work as raw material for glucose production. The rate of glucose production and its concentration using hydrochloric acid was found to increase with increase in temperature, acid concentration and reaction contact time and reduction in calyces' particle sizes. The conversion after 120 minutes was found to be 25% at 60^0C with 6% w/v acid strength. The results suggest that acid strength greater than 6%w/v would be more effective from 60^0C and above. The finding also shows a shift in the influence of mass transfer control to reaction control mechanism, with increase in temperature. It is established that under favorable condition sorrel calyces could serve as alternative source of energy and raw material for food and pharmaceutical industries.

Keyword: zoborodo, acid hydrolysis, Sorrel calyx, cellulose, bioethanol, glucose

INTRODUCTION

The calyx of *Hibiscus sabdariffa* is widely used by humans, as food, jams, jellies, juice drinks, wine and as medicinal syrups (Akanya et al, 1997; Daltziel and Burkill, 1985). The solid residue obtained after leaching of the calyces constitutes solid waste. This solid waste could increase soil acidity owing to its characteristic low pH (Olawale and Ajayi, 2008). Additionally, due to its high organic material content and high biogradability (Babalola *et al*, 2005) it could constitute a serious environmental problem, if not properly dispose, as is the current practice in Nigeria. The composition of sorrel calyces reported by Samy (1987), Duke(1983) and www.hort.purdue.edu (2007), suggest that this waste could serve as source of glucose (a major source of energy), food and/or raw material for the production of many organic chemicals. Conversion of abundant cellulosic biomass to useable product presents an important opportunity to improve energy security, reduce the trade deficit, reduce green house gas emission, and improve price stability (Wyman, 1999), while reducing waste arising from agricultural produce. The cellulose and hemicellulose content in any biomass can be hydrolyzed chemically or enzymatically (Karimi *et al*, 2006). Cellulose normally has strongly resistance to attack both by enzymes and chemical agents and various workers have attempted to hydrolyse cellulose using enzymatic and/or chemical hydrolysis under mild or harsh conditions. Enzymatic hydrolysis of cellulose is a slow process and is not likely to be applied in industrial practice in the immediate future (Gan *et al*, 2003; Sun *et al*, 2009). In the meantime, Chosdu and co-workers (1993) upgraded cellulosic waste material via radiation and chemical pretreatment, while Gregg and Sadder (1996) investigated factors affecting cellulose enzymatic hydrolysis.

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Imai and others (2004) employed ultrasonic approach to enhance hydrolysis of cellulose. Yet enzymatic hydrolysis with/without steam explosion have also been employed in conversion of ethanol, from cellulosic materials, to glucose (Highina *et al*, 2006; Grous *et al*, 1986). Acid hydrolysis of cellulose is a relatively faster process with higher yield and the conversion is usually strongly affected by acid concentration, temperature and reaction time (Liao *et al*, 2006; Iranmanhboob *et al*, 2002; Choi and Matthew, 1996). These types of application commonly utilize either concentrated acid at low temperature or dilute acid at high temperature (Liao *et al*, 2006; Sun and Cheng, 2002). Grahmann *et al*, (1985) reported that concentrated acid hydrolysis is much more effective than dilute acid hydrolysis, while Herrera *et al*, (2004) reported on use of hydrochloric acid for sorghum straw hydrolysis. Despite, its effectiveness, concentrated acid hydrolysis major drawback is the associated serious environmental concerns (Sun *et al*, 2009). Interestingly though, various authors have adopted means of minimizing this negative effect through neutralization (Alves *et al*, 2002). However, the economics of such process *viz-a-viz* product yield makes it a less profitable approach. Different lignocellulosic materials exhibit varying hydrolytic behaviors due to differences in composition and other properties. Thus each material needs to be studied to establish its hydrolysis parameter. No related work has been reported on the production of glucose from sorrel calyces, using either acid (dilute or concentrated) or enzymatic hydrolysis route. This paper presents results of investigation carried out on production of glucose from sorrel calyces, using dilute hydrochloric acid, under different operating conditions.

2. METHODOLOGY

2.1 Preparation of Sorrel Calyces Residue

The *Hibiscus sabdariffa* calyces procured from a local market in Zaria, Nigeria, was beneficiated and reduced in size, using mortar and pestle. A solute to solvent (water) ratio of 1:10 at 100^oC for 20 mins was employed for the extraction purpose. The resulting mixture was filtered to obtain the juice and the residue, which was later refrigerated at about -3^0C, awaiting analysis and hydrolysis reaction. Prior to analysis and hydrolysis, the refrigerated calyces were dried to constant temperature and weight. The

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dried calyces were sieved to obtain particle sizes of 2mm, 300µm and 75µm.

2.2 Proximate Analysis of the Residue

The dried calyx was subjected to various treatments, in order to determine moisture, ash, crude protein, fat and fibre contents, following the methodologies detailed in the literature (AOAC, 1990). The absorbances of standard glucose solution read at 550nm using UV-spectrophotometer (model Perkin-Elmer Lambda 6UV/Vis with PECS 5 software) were correlated to concentrations of glucose (g/l), for the purpose of determining concentrations of glucose in the hydrolytic matrix. The carbohydrate content was estimated following Pearson's method, after which its various components, namely, soluble carbohydrate, cellulose, hemicellulose and lignocellulose were determined using wet method analysis.

2.3 Hydrolysis of the Calyces and glucose assay

Three different particle sizes (i.e. 2mm, 300µm and 75µm) were subjected to hydrolysis using 6% w/v acid concentration at 60^0C, set for reaction times varying between 15 and 120mins (at intervals of 15mins). The established optimal particle size (among the investigated ones) was later subjected to hydrolysis, for reaction time varying from 30 to 210mins and at temperatures ranging from 25^0C to 80^0C, using same acid concentration. The detailed descriptions of the procedure follow herewith. Four standard acid solutions having concentrations of 1, 2, 4 and 6% w/v were prepared, in accordance with the works reported by Aguilar et al (2002) and Bustos et al (2003). Ten gramme (10g) of the sorrel calyces was weighed and introduced into a 250 ml round bottom pyrex flask containing 100 ml of 1% w/v acid solution; to give a solute-solvent (mass/volume) ratio of 1:10. The flask was immersed in a glycerol bath set at 40^oC. After a reaction time of 30mins, about 4 ml of the content was introduced into a 50 ml beaker, which was neutralized with 6 ml of 0.5N NaOH. The content of the beaker was allowed to cool and then filtered. To 1 ml of the filtrate, about 3 ml of DNS reagent was introduced, boiled in a water bath for 5mins and cooled to room temperature. The absorbance of the solution was read at 550nm. The same procedure was repeated at every 30mins until the variation in absorbance became negligible. The aforementioned procedure was repeated on the same quantity of calyces with 2, 4 and 6% w/v solutions at 40^0C. These steps were repeated at 50^0C and 60^0C. A relatively lower temperature range ($40-60^0$C) was selected to avoid formation of competitive and inhibiting side products (Herrera *et al*, 2004).

3 RESULTS AND DISCUSSION.

3.1 Proximate Composition Analysis

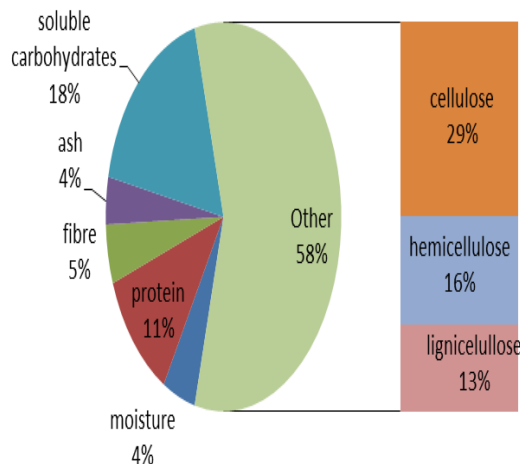The results of the proximate analysis are presented in Figure 1.

Figure 1: Proximate analysis of sorrel calyx residue

The composition of calyces from *Hibiscus sabdariffa,* shows relatively high carbohydrate content. This value (75.56 %) is close to that (74.5 %) reported for American *Hibiscus sabdariffa*. The relatively high percentage composition of carbohydrate in the calyces serves as a good justification for embarking on this project. As indicated in www.hort.purdue.edu (2007), the carbohydrate content of American *Hibiscus sabdariffa* calyces consists of glycoside (63.5%) and cellulose (29%) in dried form, which is observed to be very close to our findings as depicted in Figure 1. The concentration of glucose solution was correlated to the absorbance as y=0.488x; where y is concentration of glucose (g/l) and x is the absorbance at 550nm with R^2=0.993.

3.2 Hydrolysis of Calyces

3.2.1 Effect of particle sizes on product yield

Smaller particle size leads to larger interfacial area between the solid and liquid, and therefore higher rate of material transfers and hence smaller distance through which the solute must diffuse within the solid. This implies that less time is required for solubility, chemical reaction and product yield, as shown in Figure 2.
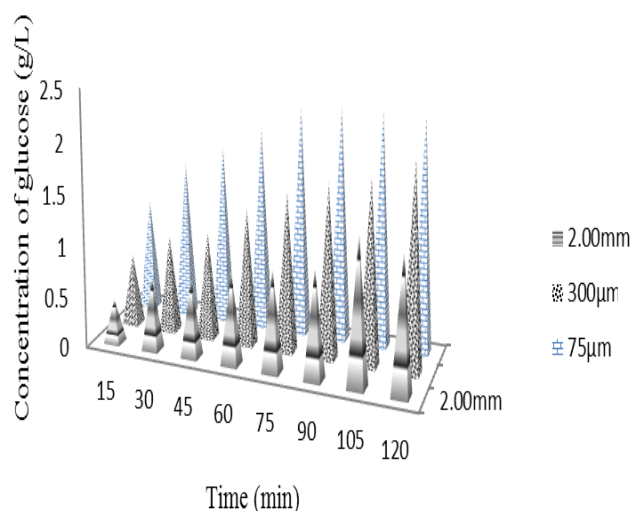
Figure 2: Particle size effect on glucose produced from sorrel calyces.

3.2.2 Selection of reaction temperature

Figure 3 gave the effect of reaction temperature on the concentration of glucose. It was observed that for temperatures ranging between 60 and 80^0C, there is little or no difference in the product yield. The yield noticed for 25^0C and 30^0C were observed to be too small for consideration. Accordingly, temperatures ranging between 40^0C and 60^0C, were choosen for the investigation.
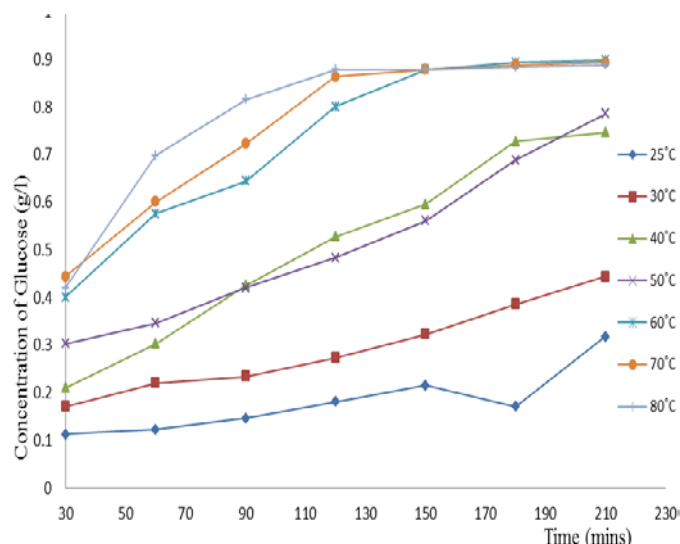

Figure 3: Temperature effect on glucose yield.

3.2.3 Effect of reaction temperature and acid concentration on glucose yield

Figures 4 (a-c) show the effect of acid strength on glucose concentration produced. In general, the concentration of glucose was observed to increase with increase in acid strength, with the highest value recorded with 6%w/v HCl at all temperatures between 0 and 75mins. However, the maximum glucose concentration (0.6 and 0.9g/l) were obtained between 150 and 180 mins, for all acid strength employed.
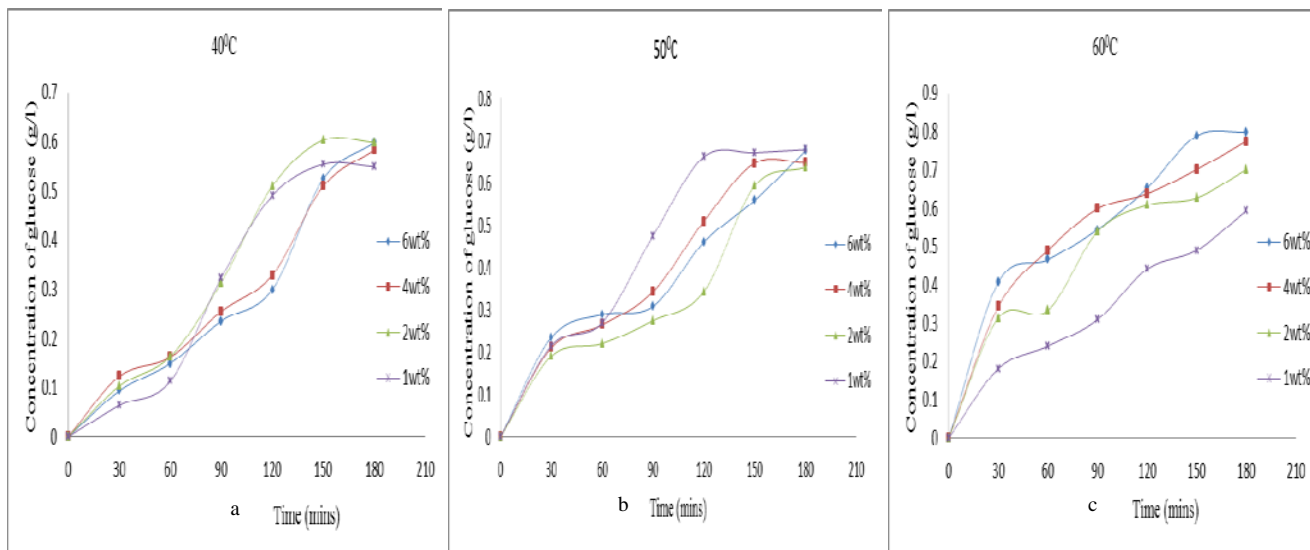

Figure 4: Glucose concentration produced as a function of acid concentration at: (a) 40^0C, (b) 50^0C, (c) 60^0C

The hydrolysis progression with 4%w/v and 6%w/v HCl appeared to be similar, while the progression for 1 and 2%w/v, seemed to follow a common trend. It appears that for the first 75mins, hydrolysis occurred at the interface of the liquid-solid reacting mixture, with little or no diffusion resistances. However, beyond 75mins, mass transfer seemed to control the conversion rate, resulting from ease or difficulty of penetration of the calyces pores by the acid. Between 75 and 150mins, it was observed that concentration of glucose produced for 4 and 6%w/v HCl

was lower compared with 1 and 2%w/v at 40^0C. There are two probable reasons for the observed trend here: the first is incidence of mass transfer control due to higher viscosity at low temperature (40^0C) and higher acid concentration (4 and 6% w/v) and second is the existence of conditions more favorable formation of competitive products as noted by Herrera *et al*, (2004).
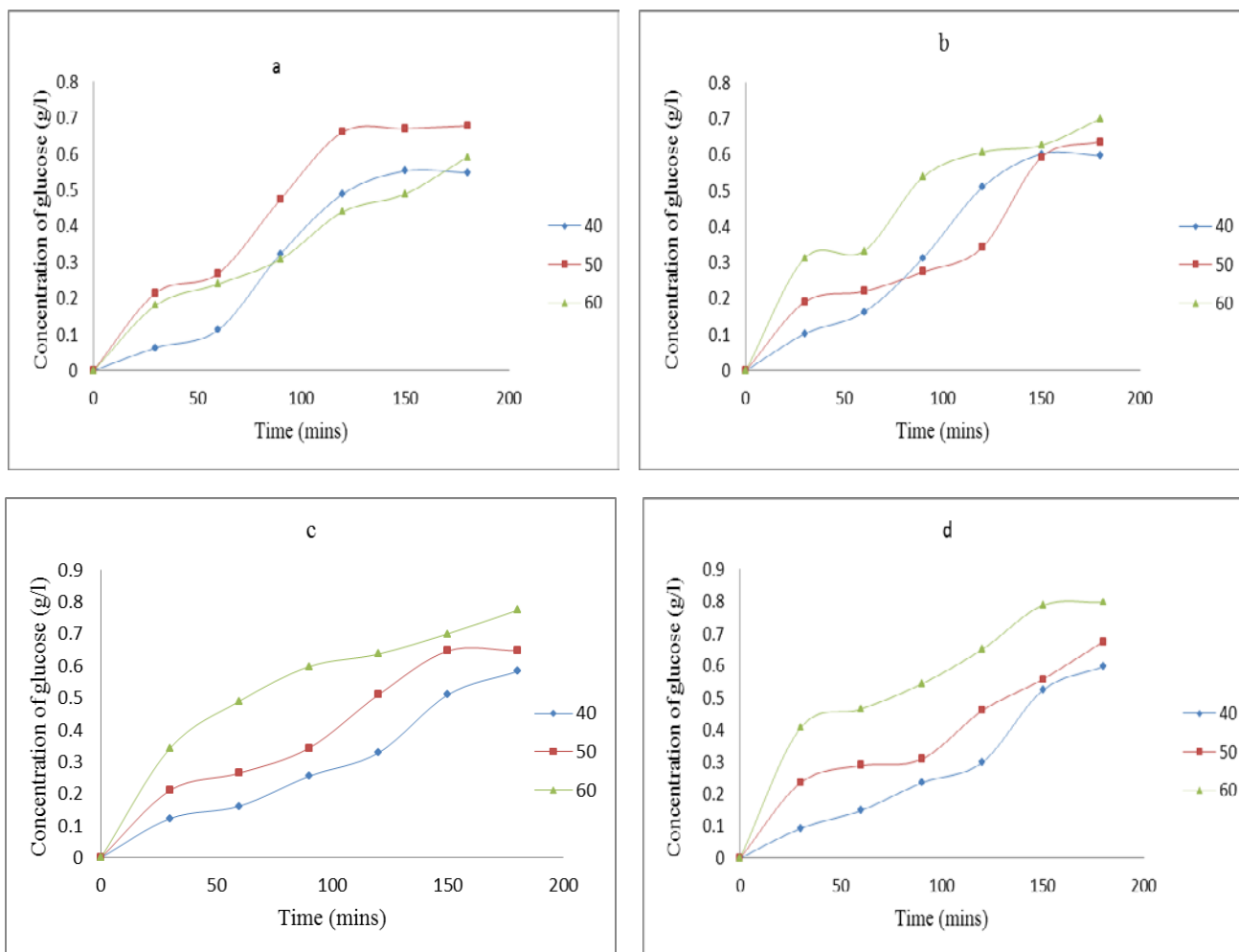
Figure 5: Glucose concentration as a function of temperature at: (a) 1wt%, (b) 2wt%, (c) 4wt%, (d) 6wt%

The trend changes as hydrolysis temperature increases from 40 to 60^{0}C, as depicted in Figures 5a-d. Thus, hydrolysis with 6%w/v acid gave the highest glucose concentration at 60^{0}C (0.9g/l) at 120mins [27]. The other three acid strengths produced their highest glucose concentration at 180mins, which is found to be lower than that of 6%w/v. Figure 5(a) shows 50^{0}C to be an optimum temperature when the acid concentration is minimal. This observation is in agreement with the work done by Wen *et al*, (2004) on hydrolysis of animal manure. Increase in reaction temperature and acid concentration, expectedly increases the glucose production proportionally, as depicted in Figures 5b to 5d. This observation was attributed to enhanced solvent mobility, low viscosity and increase in number of colliding molecule (reduced activation energy-E_A-) resulting from increase in temperature. The observed trend for glucose production was seen to compare favorably with the work of Sun *et al* (2009). A cumulative dependence of glucose concentration on acid strength and reaction time employed was done using table curve 3D as depicted in Figure 6(a-c). The 3D plot was done for temperatures of 40, 50 and 60^{0}C using the generalized model shown in equation 1.

$$C_{Glu} = a + bt + \frac{c}{C_{HCl}} + dt^2 + \frac{e}{C_{HCl}^2} + \frac{ft}{C_{HCl}} + gt^3 + \frac{h}{C_{HCl}^3} + \frac{it}{C_{HCl}^2} + \frac{jt^2}{C_{HCl}} \qquad Eqn.1$$

The regression for this model was determined to be 0.9793, 0.9528 and 0.9759 for temperature of 40, 50 and 60^{0}C respectively. Figure 6 gave a better insight into the behavior of the interdependency of the parameters investigated, i.e. glucose concentration, concentration of HCl, time and temperature.

T=40^{0}C
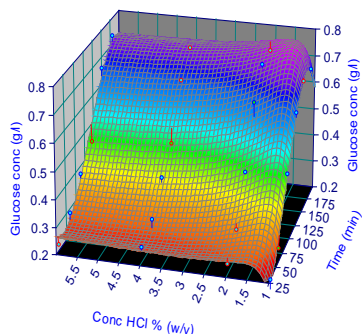
r^2 Coef Det	DF Adj r^2	Fit Std Err	F-value	
0.9792547551	0.9632968745	0.0359528457	73.42816078	
Parm	Value	Std Error	t-value	99.90% Confidence Limits
a	0.472195312	0.147212492	3.207576371	-0.1373358	1.08172642
b	-0.00880096	0.002716834	-3.23941979	-0.02004997	0.002448044
c	-0.6794867	0.95397834	-0.71226638	-4.62941966	3.270446254
d	0.000109704	2.66969e-05	4.10924865	-8.3391e-07	0.000220242
e	1.114152723	1.995709876	0.558273894	-7.14905397	9.37735942
f	0.009486408	0.003323797	2.854087766	-0.00427572	0.02324854
g	-2.9598e-07	8.27089e-08	-3.57854612	-6.3843e-07	4.64773e-08
h	-0.78128	1.187757821	-0.65777719	-5.69917338	4.136613379
i	-0.00118601	0.002119128	-0.55967052	-0.00996023	0.007588203
j	-3.6548e-05	1.00659e-05	-3.63092424	-7.8226e-05	5.12916e-06

T=50^{0}C
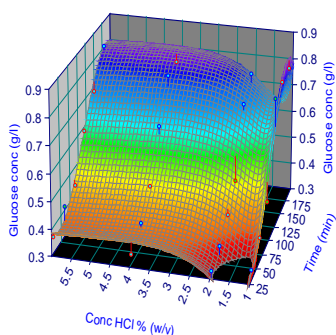
r^2 Coef Det	DF Adj r^2	Fit Std Err	F-value	
0.9528413771	0.9165655133	0.0512283992	31.430046223	
Parm	Value	Std Error	t-value	99.90% Confidence Limits
a	0.438348838	0.209759761	2.089766107	-0.43015829	1.30685597
b	-0.0091609	0.003871155	-2.36645122	-0.02518936	0.006867559
c	1.141829925	1.359302225	0.840011812	-4.48634047	6.770000325
d	0.000115838	3.80398e-05	3.04518223	-4.1665e-05	0.000273341
e	-3.50198189	2.843642	-1.23151293	-15.2760388	8.272075003
f	0.004408463	0.004736003	0.930840396	-0.01520089	0.024017814
g	-3.1984e-07	1.1785e-07	-2.71395755	-8.078e-07	1.68116e-07
h	2.317797333	1.692409336	1.369525258	-4.68959805	9.325192713
i	0.000468175	0.003019497	0.155050523	-0.01203401	0.012970357
j	-2.0975e-05	1.43426e-05	-1.46242536	-8.036e-05	3.84103e-05

T=60^{0}C
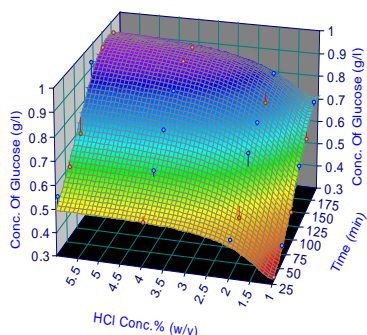
r^2 Coef Det	DF Adj r^2	Fit Std Err	F-value	
0.9758737114	0.9573150279	0.0376057387	62.919987425	
Parm	Value	Std Error	t-value	99.90%	Confidence Limits
a	0.402872541	0.153980426	2.616388006	-0.2346811	1.040426179
b	0.001907446	0.002841737	0.671225406	-0.00985872	0.013673616
c	0.428241327	0.997836457	0.429169854	-3.7032855	4.559768154
d	3.99072e-05	2.79242e-05	1.429125121	-7.5713e-05	0.000155527
e	-1.06482211	2.087460467	-0.51010409	-9.70792075	7.578276525
f	-0.0061271	0.003476605	-1.76238029	-0.02052193	0.008267731
g	-1.8361e-07	8.65113e-08	-2.12243784	-5.4181e-07	1.74584e-07
h	0.592470667	1.242363695	0.476889874	-4.55151751	5.736458845
i	0.001378632	0.002216552	0.621971558	-0.00779897	0.010556233
j	1.98009e-05	1.05286e-05	1.880672949	-2.3793e-05	6.33945e-05

Figure 6: 3D plot model for the dependence of glucose concentration on HCl concentration and time for 40, 50 and 60^{0}C respectively.

3.2.3   Determination of kinetics and thermodynamic data

Eqn. 2
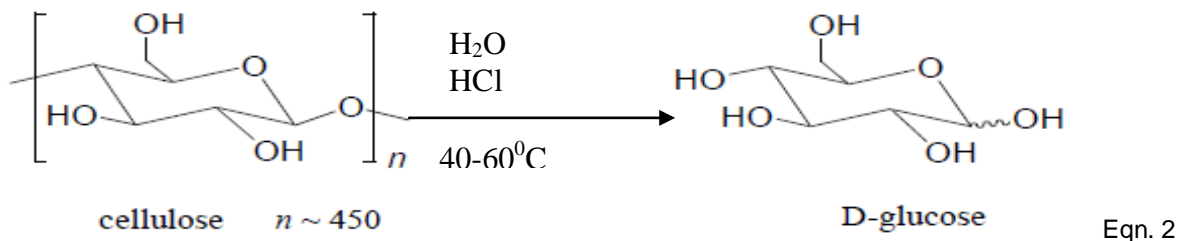
Ordinarily, the reaction depicted in Equation 2, should follow a first order reaction, but with the presence of hemicellulose and lignin in the calyces, a second order reaction was fitted and tested for conformity, since the initial material was not pretreated for removal of any form of impurities. These impurities and other forms of cellulose *viz-a-viz* soluble carbohydrate were believed to have taken part in the reaction. Figures 7 to 10 testify to the proposed second order reaction model, with the best fitted points coming from calyces treated with 6wt% HCl.
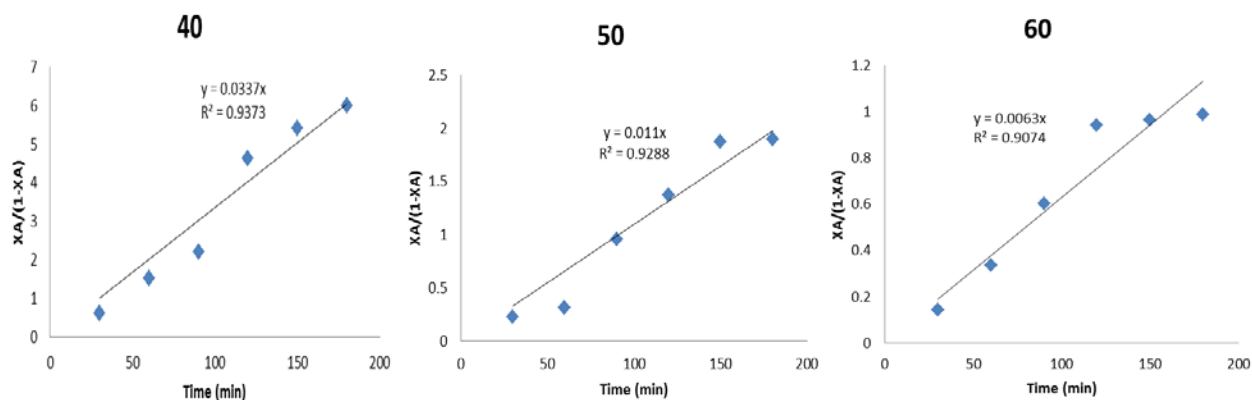

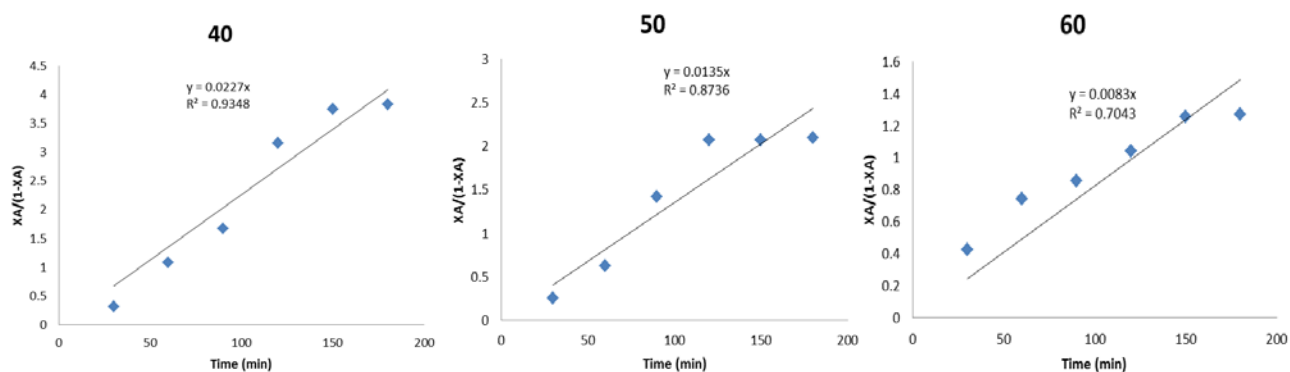**Figure 7:** Test of the order of reaction for 6wt% HCl

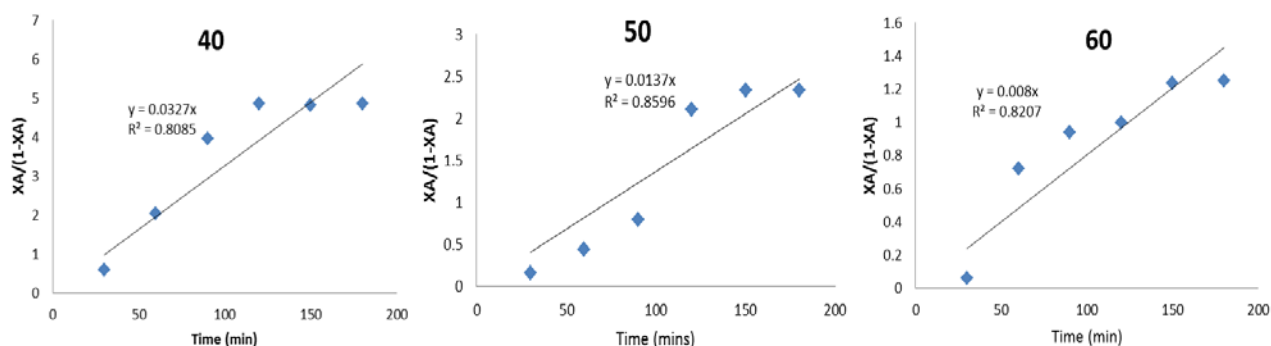**Figure 8:** Test of the order of reaction for 4wt% HCl

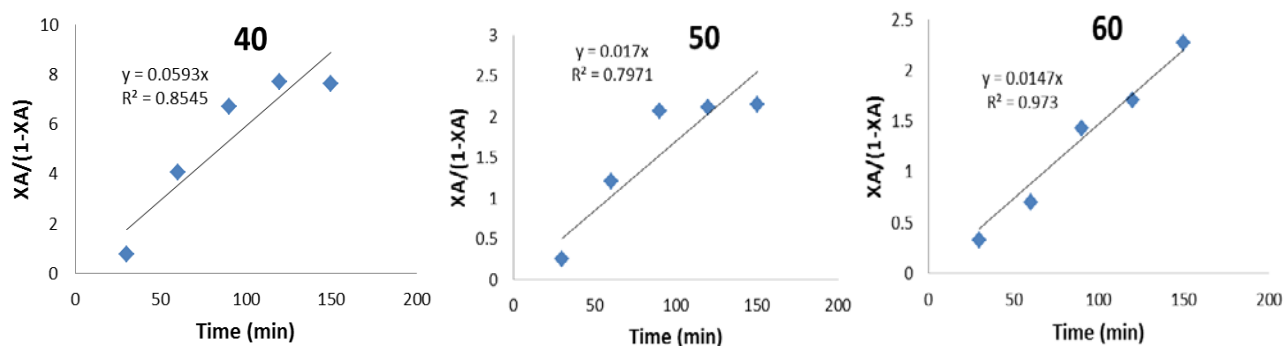**Figure 9:** Test of the order of reaction for 2wt% HCl

Figure 10: Test of the order of reaction for 1wt% HCl

The regression factor obtained for acid strength of 1wt% was observed to be relatively poorer compared with the other acid strength; hence the decision was taken not to consider its thermodynamic properties. The reason for this observation is not conclusive. The activation energy for all the acid strength used was determined graphically (Figure 11 (a-c)) to be 135, 88, 109 and 103kJ/mol, respectively, for 6, 4 and 2wt% of HCl. The pre-exponential or frequency factor for 6, 4 and 2wt% of HCl were respectively determined to be 6.28E-24, 3.60E-16 and 1.61E-19.
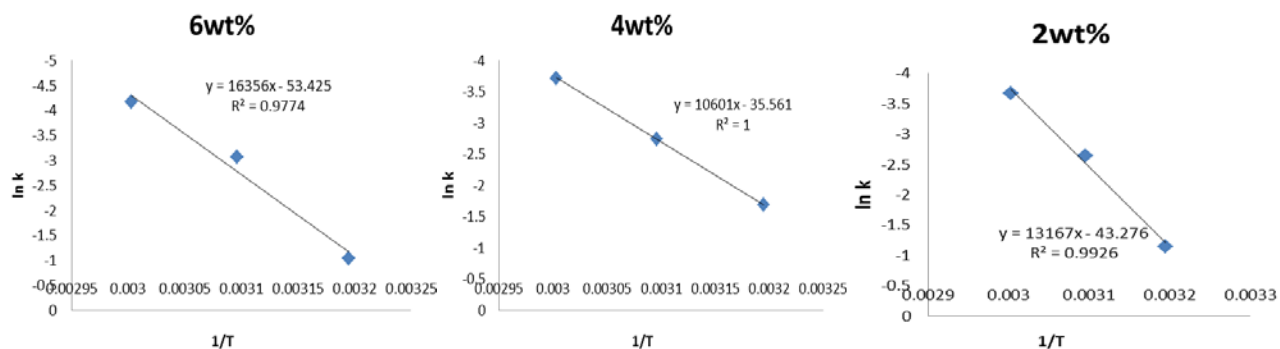

Figure 11: Temperature dependency of the hydrolysis reaction rate

Figure 12 (a and b) gave a column chart representation of how ΔG and ΔH change with reaction temperature and acid strenght. Hydrolysis conducted using 4wt% HCl was found to have not only the lowest EA but also the lowest ΔG and ΔH, suggesting that its an energy and environmental optimum concentration, within the acid strength ranges considered, *viz-a-viz* yield of glucose produced.
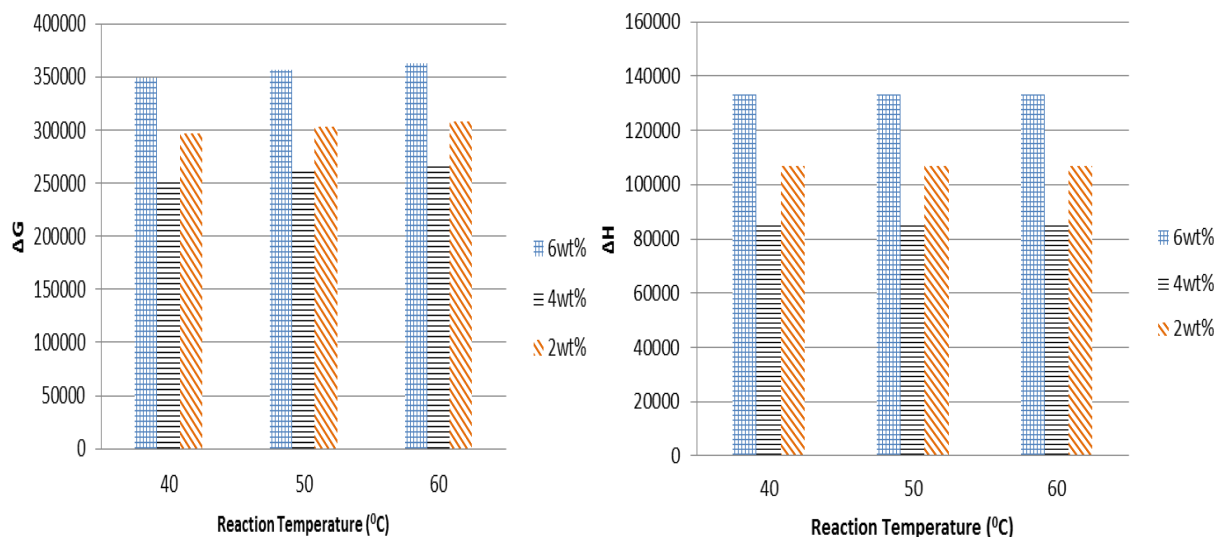
Figure 12: Enthalpy and Gibb's energy value for the hydrolysis

4   CONCLUSION

Sorrel calyces were converted to fermentable glucose via a single stage low temperature-dilute acid hydrolysis. The selected operating condition was noted not to favour formation of competitive products and /or camelization of glucose formed. The resulting glucose can be further converted into value added products such as ethanol, fillers for drugs and other petrochemical products. This is a welcome idea now, when alternative sources of revenue to oil and gas are being sought – an environmental strategy. This novel use of sorrel calyx – *hibiscus sabdariffa* – may also assist in solving the problem of solid waste disposal occasioned by the careless discarding of calyces after preparation of "zoborodo" drink. Future studies with this material should focus on measuring mixture effects on pretreatment on yield, double stage hydrolysis and development of kinetic model for design purpose.

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