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Optimization of Reducing Sugar production from Acid Pretreatment of *Ipomoea repens*

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ABSTRACT

Ipomoea repens is a weed and abundant bio-resources for reducing sugar production. The cellulose of Ipomoea repens can be converted into reducing sugar and biofuels. The research reports the evaluation of Ipomoea repens to produce reducing sugars through acid hydrolysis. The effects of temperature (60–100 °C), acid concentration (1–6%), and time (30–60 min) were investigated using a statistical experimental design. Scanning Electron Microscopy was used in elemental and morphological analysis. The reducing sugar yield was influenced by the interaction of (acid concentration. acid concentration), which was found to be the most significant quadratic term. The optimal parameter conditions were found to be temperature (100 °C), reaction time (45 min), and acid concentration (3.50%), with a reducing sugar yield of 6.203%.The result of elemental analysis showed , Oxygen(%) 38.98 ±1.717 , carbon(%) 60.18 ±2.19 , Calcium (%) 2.0 ±0.00 , Silver (%)0.26 ±0.00 , Potassium (mg/kg) 14100 ±229 , Sodium (mg/kg) 228±291, Nitrogen (%) 2.10±0.02 and tellurium(%) 0.68±0.00 According to the findings, biomass can be used as a potential feed stock for the production of reducing sugar, biofuels and other biological derived chemicals.

Keywords: Reducing sugar, *Ipomoea repens*, optimization, hydrolysis,

INTRODUCTION

Lignocellulose, the most available renewable biomass synthesize by plants through photosynthesis . Annually, 200 billion metric tons generated approximately globally (Ahmad et al., 2023 ;Ragauskas et al., 2006; Zhang et al., *2006).* Lignocellulosic biomass is the main raw material for biorefineries and bioethanol production. (Lin and Tanaka, 2006). The composition of lignocelluloses biomass depends on plant species, plant parts, growth conditions and method of process the biomass . etc. (Ahmad et al., 2023 ;Ding and Himmel, 2006; Zhang and Lynd, 2004). Chemically , the composition of lignocelluloses structure are crystalline cellulose, amorphous hemicelluloses, lignin, and other plant chemicals (Ahmad et al., 2023 ;Percival et al., 2006; Sanchez, 2009). Cellulose microstructure are covered with hemicelluloses ,holocellulose structures and coated by lignin .The structures is highly lignified, which prevent accessibility to cellulose. To optimize reducing sugars production , a pretreatment process known as acid hydrolysis is required. Acid hydrolysis will increase the production of reactive cellulosic fibre, prevent the destruction of hemicelluloses and cellulose, consume little or no chemicals, and use cheap chemicals (Ahmad et al., 2023 ;Yat et al., 2008 ; Saha et al., 2000).

A reducing sugar in chemistry is define as a carbohydrate that is oxidized by oxidizing agent such as tollen reagent. Must reducing sugar are monosaccharide , others are disaccharides, oligosaccharides, and polysaccharides (Saha, 2005).

Monosaccharide is further divided into aldoses and ketoses. , in which the Ketoses isomerizes into aldoses before acting as reducing sugars.

Optimization in simple term mean the conditions that will give the best yield from a system during a reaction. it is applicable in scientific and Engineering research. Must scientific and engineering research are carried out by optimization of the process conditions This research , statistically optimize reducing sugar yield from *Ipomoea repens* biomass .

Ipomoea repens is a weed locally known as Duman Kadaa" and is largely available in large parts of Sokoto State, Nigeria. The weed is inedible by humans and domestic animals, and hence, the significant economic importance of Ipomoea repens has not been established. This paper aimed at exploring the use of this weeds are the main substrate for reducing sugar production via acid hydrolysis.

MATERIALS AND METHODS

Sample Collection

The samples of *Ipomoea repens* biomass was collected in Tamaje Area of Sokoto State Nigeria. The biomass was dried under the sun and crushed with the aid of mortar and pestle , sieved and stored at room temperature prior to its usage.

Morphology Analysis of *Ipomoea repens* Biomass

The sample of *Ipomoea repens* biomass was transferred into a samples holder ,then transferred into the SEM machine (JEOL JSM-IT500) and then scan to optical image to then SEM images and the SEM image was capture and stored in a flash .The principle involved the use of electrons to form an image.

Elemental Analysis of *Ipomoea repens* Biomass

The sample of *Ipomoea repens* biomass was transferred into a samples holder ,then transferred into the SEM machine (JEOL JSM-IT500) and then scan for elemental analysis. The principle involved electron interact with the sample .

Optimization of Reducing Sugar yield

The effect of temperature (60-100 ^oC), residence time (30-60min) and acid concentration (1-6%) were selected as independent process variables. The independent process variables were optimized using design of experiment. Design Expert software, version 6.0.6 (Stat-Ease., Inc., MN) was used in designing the experimental. The ranges of variables were selected based on previous information on the study (Raghavi *et al*, 2016; Xue *et al*, 2017). Reducing sugar yield(%) was selected as response variable. Statistical analysis was calculated using Design Expert software .

Model Validation and Confirmation

The maximum reducing sugar yield (%) predicted by RSM-CCD was validated by performing actual acid hydrolysis experiments. Validation was performed in triplicate.

RESULTS AND DISCUSSION

The composition of *Ipomoea repens* biomass is presented in Table 1. The Results show that about 60% of *Ipomoea repens* biomass is carbohydrate and about40% are protein, phytochemicals and other element.

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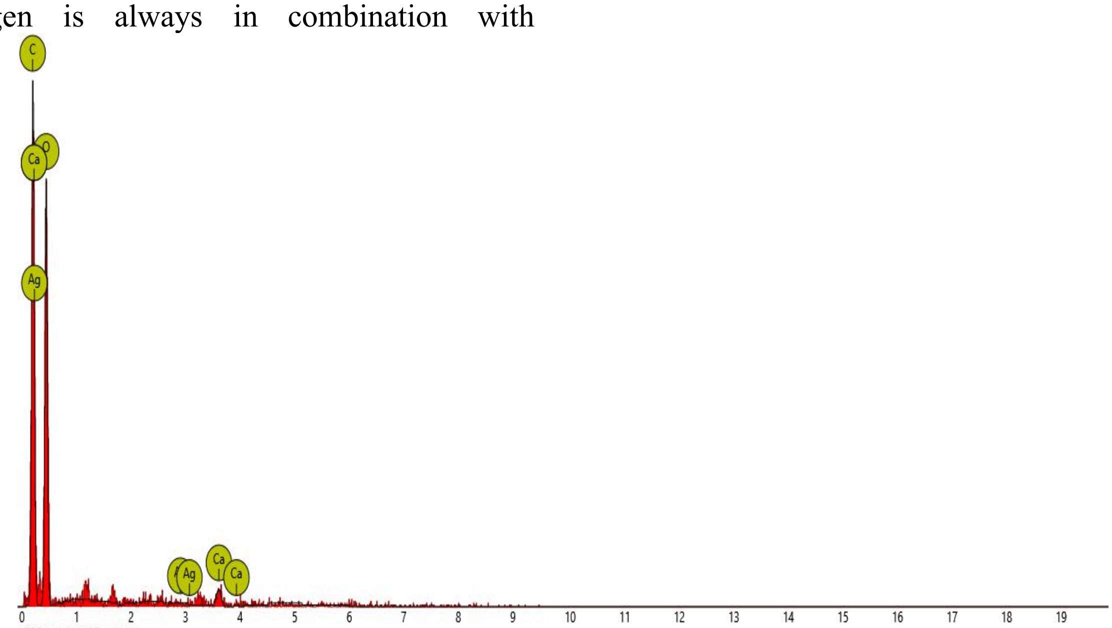
Table 1: Ultimate Analysis of the Substrates

Elements	Oxygen (%)	Carbon (%)	Calcium (%)	Silver (%)	Potassium (mg/kg)	Sodium (mg/kg)	Nitrogen (%)	Telluriu m(%)
Ipomoea repen	38.983±1.717	60.18±2.19	2.0±0.00	0.26±0.00	14100±229	228±291	2.10±0.02	0.68±0.00

The nitrogen content of biomass as shown in Table 1, is low. Nitrogen is an essential nutrient in biofuel production and has been a significant contributor to the biochemical process.During fermentation, yeast growth and metabolic activity are facilitated by nitrogen as nutrient (Mahmoudi *et al* .,2010).The Nitrogen content has no calorific value and consequently does not contribute to the calorific value of the substrate ,but is useful when converting the substrate to biofuels.

Table 1, Shows that weight concentration of oxygen is 38.983±1.717.In plant substrates, oxygen is always in combination with hydrogen and carbon. The value obtained from elemental analysis of oxygen implies that the substrate is rich in cellulose. Carbon content is a key factor to consider when producing bioethanol from a substrate.. The value of carbon content will give an inside on sugar or cellulose. Carbon in plant substrate always occur in cellulosic form. In term of calorific value, the higher carbon content, the high the calorific value.

Apart from carbon and oxygen content ,other trace minerals content such as calcium, cesium ,tellurium and indium are present in the substrate.



Element Number	Element symbol	Element Name
6	C	Carbon
8	O	Oxygen
20	Ca	Calcium
47	Ag	Sliver

Figure 1: Spectral distribution of element in *Ipomoea repens* substrate (region 1)

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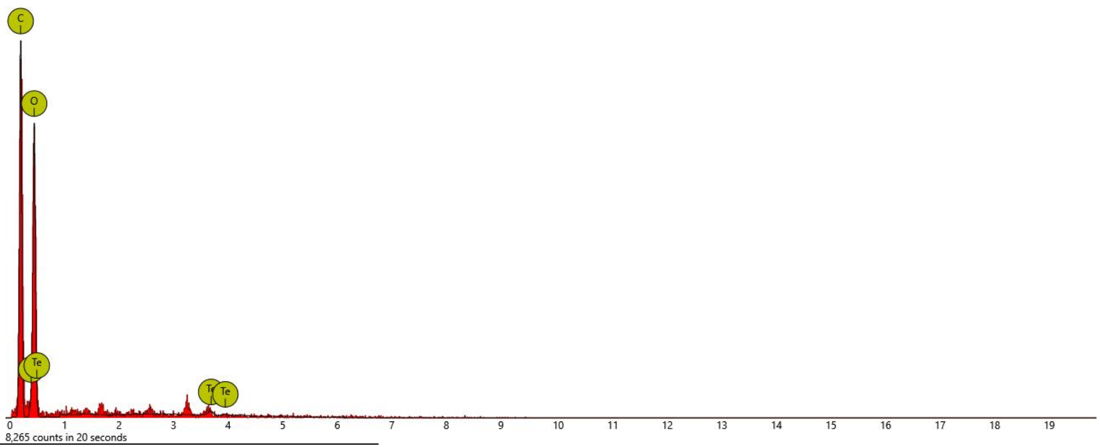

Element Number	Element symbol	Element Name
6	C	Carbon
8	O	Oxygen
52	Te	Tellurium

Figure 2: Spectral distribution of element in *Ipomoea repens* substrate (region 2)

Minerals are acquired from the soil by plant roots. The uptake of elements by plant root and their distribution within the plant varies due to lignin content distribution of the plant. The trace metal in the plant are integral part of the ash content. The element calcium found in *Ipomoea repens* can be traced to weathering of rock around the sampling area.

Tellurium and silver are present in *Ipomoea repens*in trace quantity but they are considered as heavy metals because of their atomic number (Ahmad et al*.,* 2023)

Morphological Analyses of Weedy Biomass

The morphological structure of *Ipomoea repens* at different magnifications before pretreated with an acid was shown in Figure 3.

From the microscopic view of untreated substrates *Ipomoea repens*, the surface of weedy biomass is relatively open, there is not many empty spaces on it, which signified no damage. Careful examination of Figure 3 indicates that the milling of the *Ipomoea repens* biomass didn't damage the structure of the substrates, it implies that the carbohydrate are in close contact with lignin. At the same time, analysis of the images shows that, the structure are closely tight and no accessibility to sugar , Thus, the structure need to be crack via suitable pretreatment process such as acid hydrolysis.

Pretreatment would expose the fiber structure by creating holes in the substrate; the chemical used for the pretreatment will interact with the structure, as a result altering the structure. As a result of holes, the chemical use for pretreatment will have access to the bond (glycosidic bond) and also break the bond to release reducing sugar (Ahmad, 2015)

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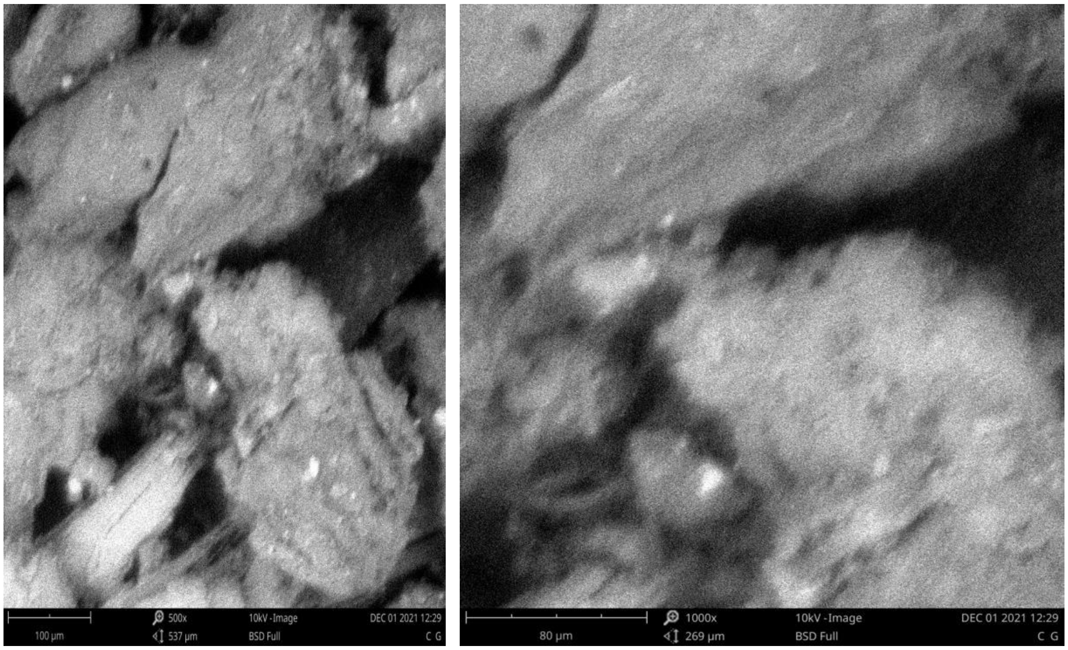

Figure 3: SEM image of *Ipomoea repens* at 1000 um

Reducing Sugar Yield (%) of *Ipomoea repens*

In table 2, the experimental Design Matrix and Reducing sugar yield for *Ipomoea repens biomass.* The concentration of the sugar yield was6.203% at a experimental conditions of: temperature-100^{o}C,time-45 min and acid concentration-3.50%. These results are simlar with that of Albarico *et al.* (2017). From the results of reducing sugar yield, it proves that *Ipomoea repens* substrates have little reducing sugar content.

Optimization of Acid Pretreatment of *Ipomoea repens*

The result (ANOVA) present in Table 3 revealed that the Model is significant. Since the Model F-value (6.01) and p – value (0.05). it mean that the model is a good predictor of response variable. In this case, the ANOVA revealed a significant quadratic terms of acid concentration and acid concentration interaction ,F(22.37),p=0.05.

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Table 2: Experimental Design Matrix and Reducing Sugar Yield form *Ipomoea repens.*

Run	Acid Conc (%)	Time (minute)	Temperature (°C)	ReducingSugar Yield (%) *Ipomoea repens*
1	3.50	45.00	80.00	4.312
2	3.50	45.00	80.00	3.921
3	1.00	60.00	100.00	1.212
4	3.50	45.00	80.00	3.769
5	6.00	45.00	80.00	2.376
6	6.00	30.00	60.00	2.712
7	3.50	45.00	60.00	3.215
8	3.50	45.00	80.00	4.317
9	6.00	60.00	60.00	2.159
10	1.00	30.00	60.00	0.826
11	3.50	30.00	80.00	2.491
12	3.50	45.00	100.00	**6.203**
13	6.00	60.00	100.00	2.126
14	3.50	60.00	80.00	3.513
15	1.00	60.00	60.00	2.412
16	1.00	30.00	100.00	0.921
17	3.50	45.00	80.00	3.71
18	6.00	30.00	100.0	2.312
19	1.00	45.00	80.00	1.051
20	3.50	45.00	80.00	4.312

Table 3: Anova for the model Regression For *Ipomoea repens*

Source	**Sum of Squares**	**DF**	**Mean Square**	**F Value**	**Prob > F**	
Model	30.83	9	3.43	6.01	0.0049	significant
A	*2.77*	*1*	*2.77*	*4.86*	*0.0520*	
B	*0.47*	*1*	*0.47*	*0.82*	*0.3868*	
C	*0.21*	*1*	*0.21*	*0.37*	*0.5571*	
A^2	*12.74*	*1*	*12.74*	*22.37*	*0.0008*	
B^2	*2.05*	*1*	*2.05*	*3.60*	*0.0868*	
C^2	*1.95*	*1*	*1.95*	*3.43*	*0.0938*	
AB	*0.86*	*1*	*0.86*	*1.50*	*0.2486*	
AC	*0.056*	*1*	*0.056*	*0.099*	*0.7594*	
BC	*0.11*	*1*	*0.11*	*0.19*	*0.6730*	
Residual	5.70	10	0.57			
Lack of Fit	*5.28*	*5*	*1.06*	*12.58*	*0.0074*	*significant*
Pure Error	*0.42*	*5*	*0.084*			
Cor Total	36.53	19				

Where A= acid concentration in percentage (%)
B=time in minute and C=temprature in °C

By Removing the insignificant variable and rearranging the equation , we get the following: Reducing Sugar Yield=+1.20774-0.34444 X_1^2 eqn .(iii) Where X_1,X_2,X_3 are independent vairable.

The interaction of the key variables on the hydrolysis of *Ipomoea repens* was plotted and represented in 3-Dimensional plots (Figure 4).

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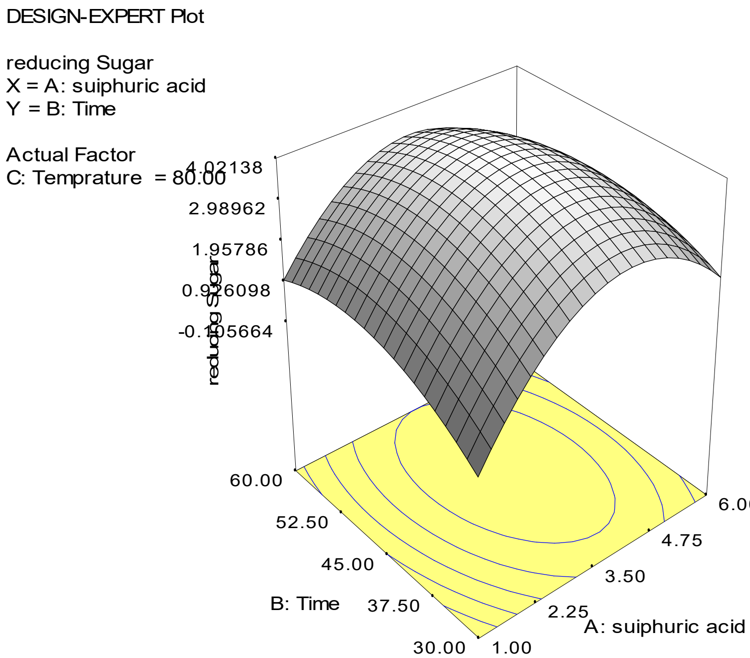

Figure 4: 3-Dimensional plots of pretreatment time and concentration of sulphuric acid for *Ipomoea repens.*

The concentration predicted by the software was plotted against experimental reducing sugar concentrations, as shown in Figure 5. It can be showed that most of data points lie close to the regression line, and three of data points are on the regression line, which implies the model is significant.

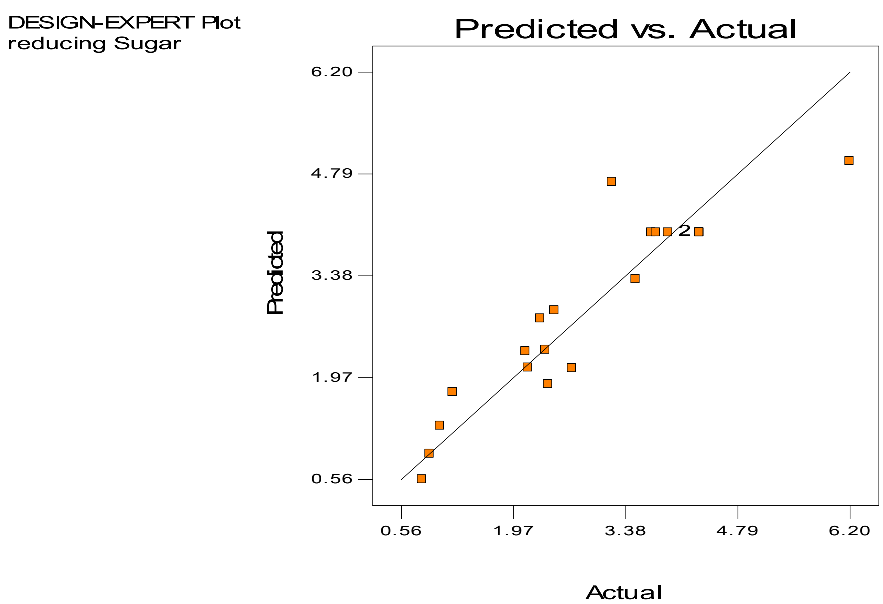

Figure 5: Predicted versus experimental of reducing sugar concentration.for *Ipomoea repens*

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Model Validation and Confirmation For *Ipomoea repens*

The predicted optimum process parameters for the acid hydrolysis of *Ipomoea repens* were confirmed through experimental verification. Using the optimum acid concentration (4.87%) and reaction time (57.44min), acid hydrolysis of *Ipomoea repens* biomass was again performed at 68.30 ºC. Results are summarized in Table 3 Results show that the mathematical model generated has good predictive ability.

Table 4: Optimization experiment and result

No	Acid concentration (%)	Reaction time (min)	Temperature (ºC)	Reducing sugar yield (%)
1	4.87	57.44	68.30	6.921
2	4.87	57.44	68.30	6.713
3	4.87	57.44	68..30	7.217

From the result, it is showed that the model was useful to predict the reducing sugar yield as well as optimize the experimental conditions.

Table 5: Summary of key statistic

Terms	*Ipomoea repens*
F-value	6.01
P>F	0.05
R^2	0.8440
Adjusted R	0.7036

CONCLUSION

The study is focused on optimizing the reducing sugar yield from *Ipomoea repens* biomass . .From this Optimization, the observed optimum conditions for reducing sugar yield was at a 100 ºC, 45 min and acid concentration of 3.50% and a maximum reducing sugar yield of 6.203%. Therefore, this optimal condition of acid hydrolysis is useful for producer to scale-up reducing sugar production. In team of scalability, the quantity of reducing sugar can be increase with increase in substrate concentration at the optimal conditions.

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Conflicts of Interest: We, the authors declare no conflict of interest.

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