



Research Article

Optimization of Reducing Sugar Production from *Sida cordifolia* Biomass Using Response Surface Methodology

M.A. Ahmad¹, A.D. Tambuwal^{1,2}, A.M. Sokoto¹, and A. Sanda¹

¹Department of Pure and Applied Chemistry, Usmanu Danfodiyo University

²Sokoto Energy Research Center, Usmanu Danfodiyo University

*Corresponding author: awwalsharp@gmail.com, doi.org/10.55639/607.807978

ARTICLE INFO:

Keywords:

Reducing Sugar,
Sida cordifolia,
Optimization,
Hydrolysis,
Response Surface

ABSTRACT

This study is aimed at evaluating the potential of *Sida cordifolia*, for the production of reducing sugars via acid hydrolysis. Effect of temperature (60°C-100°C), acid concentration (1%-6%) and time(30min-60min) was investigated using response surface methodology (RSM) experimental design. Reducing sugars yield from acid hydrolysis was optimized using RSM. The interaction of (acid concentration)² and (temperature)² were found to be the most significant quadratic terms affecting reducing sugars yield. The optimum parameter conditions were found to be temperature (80°C), reaction time (60 min) and acid concentration (3.50%) with a reducing sugar yield of 4.931%. The findings suggest that the biomass can be harnessed as a potential feedstock for the production of bioethanol and other bio-based chemicals.

Corresponding author: M.A. Ahmad Email: awwalsharp@gmail.com

Department of Pure and Applied Chemistry, Usmanu Danfodiyo University

INTRODUCTION

Lignocelluloses are composed of cellulose, hemicellulose, lignin and other complex molecules. These molecules intertwined nested with each other on the spatial distribution, formed different anti-degradation barrier layers, thus challenging the use of lignocellulosic material resources. Conducting

research on essential structural properties of lignocelluloses is a key to forming high efficiency preprocessing method of breaking down the anti-degradation barrier, improving the conversion efficiency of raw materials, promoting the comprehensive utilization of cellulose (Dvoretzky *et al.*, 2017; Mustapha *et al.*, 2017). The use of stalk and other

lignocellulosic raw materials need to undergo a certain pretreatment to improve its degradation, conversion efficiency and thus improving its utilization as raw materials. At present, common pretreatment methods include acid treatment, alkali treatment, puffing treatment, steam explosion and oxidation treatment (Oliveira, 2017). In recent years, microwave pretreatment has become a research hot-spot (Oliveira, 2017), it focuses on pretreatment by auxiliary chemical reagents, reducing the amount of chemical reagents and follow-up costs (Mushtaq *et al.*, 2015). Lignocellulosic materials can be derived from wood, grasses, agricultural residues, and waste materials.

Sida cordifolia is one of the biomass available in large quantities in Sokoto state, Nigeria and can be used for biofuel production (Ahmad *et al.*, 2015). According to the local people of Sokoto, there are several uses of this weed such as solid fuel and herb. So, the

explorations of the uses of these weed as a main raw material in producing reducing sugar will be investigated. Moreover, *Sida cordifolia* has been shown to be a promising non-edible feedstock for bioethanol production because of its low cost, drought-resistant and has a short growth cycle, and therefore it can be grown on marginal lands.

Pretreatment is a crucial process step for the biochemical conversion of lignocellulosic biomass into e.g. bioethanol. It is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars (Mosier *et al.*, 2005; Byadgi, 2016). Pretreatment has been recognized as one of the most expensive processing steps in cellulosic biomass-to-fermentable sugars conversion and several recent literature review provide a general overview of the field (Xu *et al.*, 2013)

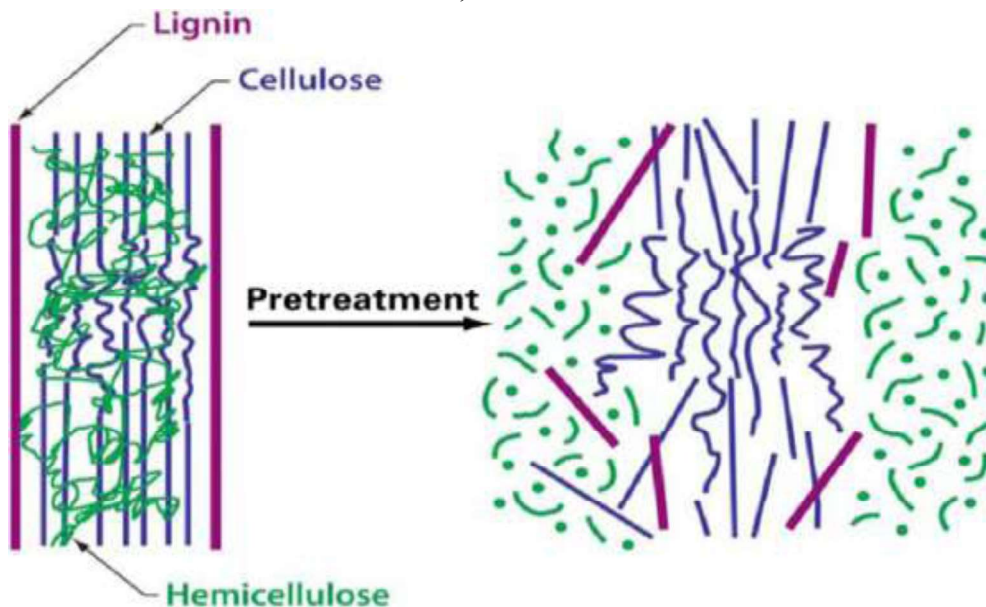


Figure 1: Schematic presentation of effects of pretreatment on lignocellulosic biomass (Hsu *et al.*, 1980)

Pretreatment involves the alteration of biomass so that (enzymatic) hydrolysis of cellulose and hemicellulose can be achieved

more rapidly and with greater yields. Possible goals include the removal of lignin and

disruption of the crystalline structure of cellulose (Figure 1).

Sida cordifolia is one of the biomass available in large quantities in Sokoto State, Nigeria and can be used for biofuel production. According to the local people of Sokoto, the weed is used as solid fuel and as herb. Moreover, *Sida cordifolia* has been shown to be a promising non-edible feedstock for bioethanol production because of its low cost, drought-resistant and has a short growth cycle, and therefore it can be grown on marginal (Ahmad *et al.*, 2015). Thus, the exploration of the uses of this weed as a raw material for production of reducing sugar is considered important.

In an ideal case, the pretreatment employed leads to a limited formation of degradation products that inhibit enzymatic hydrolysis and fermentation, and is also cost effective. In this study, the potential of *Sida cordifolia*, for reducing sugar production was investigated.

MATERIALS AND METHODS

Sample Collection and Treatment

The sample of *Sida cordifolia* leaf was collected from Rinjin Sambo area of Sokoto metropolis, Sokoto State, Nigeria, on 23 July 2017. The fresh sample was dried under the

sun for three weeks to reduce the moisture content. The dried sample was crushed with the aid of mortar and pestle, then sieved through (80 mm) mesh and stored in a polyethene bag under room temperature until required for use.

Proximate Analysis of the Plant Substrate

The powdered samples were used to determine the physico-chemical quantities in accordance with method of Thiammaih (1999), Akin (2005), AOAC (2005) and NREL LAP.

Determination of Moisture Content

An empty porcelain crucible was dried in an oven at 105°C for 3 hours after which it was cooled in desiccators. It was then weighed using an electrical weighing balance by adding the test sample (1.0g) which is represented by (W_1). The sample in the crucible was dried in an oven at 105°C for 3hrs; crucible containing the dried sample was then cooled in a desiccator, until a constant mass was attained and weighed (W_2). This procedure was in triplicate; average of W_1 and W_2 were calculated. The percentage of the moisture content of the sample was calculated using equation (1)

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \dots\dots\dots (1)$$

Determination of Ash Content

An empty crucible was weighed (W_0), then sample(2.0g) was placed into it and weighed again (W_1). This was then placed into a muffle furnace at 500°C for 3hrs. This was

repeat until a constant weight is gained. The sample was cooled in a desiccator and the crucible with the dried sample was weighed W_2 . The ash content was calculated using the equation (2):

$$\text{Ash (\%)} = \frac{w_2 - w_0}{w_1 - w_0} \times 100 \dots\dots\dots (2)$$

Determination of Crude Fibre

The sample (2.0g) was placed in conical flask (1000 mL) and weighed (W_0). H_2SO_4 (200 cm^3 , 1.25% v/v) was added to the sample and boiled gently for 30 minutes. The mixture was

filtered through muslin cloth stretch over 9cm Buchner funnel, and then rinsed with hot distilled water. The material was scraped back into the flask with spatula and boiling NaOH (200 mL, 1.25% v/v) was added and allowed

to boil gently for 30minute.The material was again filtered through muslin cloth. The sample was then thoroughly washed with hot distilled water and rinsed with HCl (10%v/v) and methylated spirit. it was again petroleum ether (BP 40-60°C). The sample was allowed to dried and then scraped into a crucible and

dried overnight at 150°C in the oven. It was then cooled in desiccators and weighed (W₁). The Sample was ashed at 500°C for 90 minutes in a muffle furnace, cooled in a desiccator and weighed (W₂) (Rabah, *et al.*, 2011). The crude fibre was calculated using the formula:

$$\%Crude\ Fibre = \frac{w_1 - w_2}{w_0} \times 100 \dots\dots\dots (3)$$

Determination of Crude Lipid

The crude lipid content was determined using soxhlet extraction method with petroleum ether as extracting solvent. The sample (2g) was weighed into a porous thimble and covered with a clean white cotton wool. Petroleum ether (200 mL) was added into the flask. The covered porous thimble was placed

in the extraction chamber, for extraction at a temperature (80°C) for 6hrs. Then, the thimble was removed from extractor. The extraction flask containing the oil or fat was dried at Temperatures (105 °C) in the oven for 1hr, cooled in the dissiccator and weighed (Rabah, *et al.*, 2011). The crude fat was calculated using equation (4)

$$Crude\ Lipid\ (\%) = \frac{w_2 - w_1}{w_{ieght\ of\ sample}} \times 100 \dots\dots\dots(4)$$

W₁= weight(g) of empty extraction flask

W₂=weight of flask + oil (lipid) extract

Carbohydrate

The carbohydrate was obtained by subtracting the total percentage (%) of moisture, ash, fat, and

Protein from 100 (Rabah *et al.*, 2011).

$$\%Carbonhydrate = 100\% - (\%ash + \%moisture + \%fat + \% protein) \dots\dots\dots(5)$$

Optimization of Pretreatment Process

The pretreatment optimization for the *Sida cordifolia* substrate was carried out by using different concentrations of acid (1-6%), heating period (30- 60 min.) and temperature (60– 100°C). One gramme (1g) of each *Sida cordifolia* substrate was added 10 mL of dilute Sulfuric acid ratio (1:10). The flask containing the mixture was covered with cotton wool, wrapped in aluminium foil, heated on water bath for (30- 60 min.) at temperatures ranges of (60– 100°C). The product was then autoclaved for 15 min. at 121°C. The flask was

allowed to cool, filtered through No. 1 Whatman filter paper.

Determination of Reducing Sugar

The reducing sugars was estimated using dinitrosalicylic (DNS) reagent. DNS (10g) was dissolved in 2M NaOH (200 mL) with shaking and heated for 15min. Sodium potassium tartrate tetrahydrate (40) was dissolved in distill water. The two solutions were mixed and make up to 1L with distilled water. 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 min. to develop a red brown colour then, cooled to room temperature under running water tap. The absorbance of the sample was measured at 540nm using UV-VIS spectrophotometer. The reducing sugar content was subsequently determined by making reference to a standard curve of known glucose concentration (Ahmad *et al.*, 2015).

Yield Optimization of Reducing Sugar from Acid Hydrolysis of *Sida cordifolia* Weeds using RSM

The effect of hydrolysis on reducing sugar yield was investigated. Temperature (60-100°C), residence time (30-60min) and Acid concentration (1-6%) were selected as independent process variables. The ranges of

variables were chosen based on information from a prior study (Xue *et al.*, 2017; Raghavi *et al.*, 2016). Experiments for the optimization of process variables were set up according to the central composite design (CCD). The 20 treatments were run at random order to minimize the effect of any unexplained variability in the observed responses due to extraneous factors. The response measured was reducing sugar yield (%). Statistical analysis was performed using the statistical software package of Design Expert version 6.0.6 (Stat-Ease., Inc., MN) to generate a regression model and a significance test on the regression models and individual model coefficient were analyzed using one-way analysis of variance (ANOVA).

Table 1: Experimental Range and Level

Independent Variables	Symbols	Actual Levels
Acid Concentration (%)	X ₁	1- 6
Time (Min)	X ₂	30-60
Temperature (°C)	X ₃	60-100

The percentage of reducing sugar yield was calculated using equation 1.

$$\% \text{ reducing sugar yield} = \frac{\text{Mass of reducing sugar (g dm}^{-3}\text{) of the sample}}{\text{mass of the sample}} \times 100 \dots \dots \dots \text{eqn1}$$

Model Validation and Confirmation

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



Plate 1: Experimental set up for acid hydrolysis

RESULTS AND DISCUSSION

Characterization of *Sida cordifolia* biomass

The result of proximate composition of *Sida cordifolia* weed is summarized in Table 2. Result show that 57% of the *Sida cordifolia*

weed is composed of carbohydrate and 19% is ash. Due to *Sida cordifolia* nature, lipid content was already in little quantity. The result is in agreement with the findings of Rabah. *et al.* (2011); Ahmad *et al.* (2015).

Table 2: Proximate Composition of *Sida cordifolia*

Parameter	% Composition
Moisture	7.50± 0.50
Ash	18.50± 0.57
Crude Fat	1.50± 0.29
Crude fiber	6.0±0.76
Total Carbohydrate	56.875±5.58

Reducing Sugar

Reducing sugar production from *Sida cordifolia* weeds was optimize using Central Composite Design based on Response Surface methology (CCD-RSM). The reducing sugar is any sugar that is capable of acting as a reducing agent and can donate electrons to

another molecule. (Ahmad, *et al.*,2015). Specifically, a reducing sugar is a type of carbohydrate or natural sugar that contain free aldehyde or ketone group. Little quantity of reducing sugar was obtained from the hydrolysates of *Sida cordifolia* feedstock. RSM evaluate the relationship between

dependent (reducing sugar yield) variable and independent variable (Acid concentration, Time and Temperature). Table 3 shows the experimental Design Matrix and Reducing sugar yield for *Sida cordifolia*.

The result of the Response Surface analysis revealed that *Sida cordifolia* has a reducing sugar yield of 4.931% at experimental conditions of temperature-80°C, time-60 min and acid concentration-3.50%. These results are in agreement with the report of Albarico

et al. (2017). According to Albarico *et al.* (2017), the optimum parameters for reducing sugar production is temperature-90°C, time-60 min and acid concentration-3%. From the results of reducing sugar yield, it proves that the *Sida cordifolia* substrate have little reducing sugar content. To produce a higher yield of reducing sugar from the substrate, the substrates have to be delignify before hydrolysis.

Table 3: Experimental Design Matrix and reducing sugar yield of *Sida cordifolia*

Run	Acid Conc. (%)	Time(minute)	Temperature (°C)	Reducing Sugar Yield(%) <i>Sida cordifolia</i>
1	3.50	45.00	80.00	3.117
2	3.50	45.00	80.00	3.120
3	1.00	60.00	100.00	1.129
4	3.50	45.00	80.00	3.470
5	6.00	45.00	80.00	2.126
6	6.00	30.00	60.00	1.054
7	3.50	45.00	60.00	2.148
8	3.50	45.00	80.00	3.835
9	6.00	60.00	60.00	2.457
10	1.00	30.00	60.00	1.031
11	3.50	30.00	80.00	3.081
12	3.50	45.00	100.00	3.217
13	6.00	60.00	100.00	2.471
14	3.50	60.00	80.00	4.931
15	1.00	60.00	60.00	1.212
16	1.00	30.00	100.00	0.835
17	3.50	45.00	80.00	3.297
18	6.00	30.00	100.0	2.302
19	1.00	45.00	80.00	3.505
20	3.50	45.00	80.00	3.271

Analysis of Variance (ANOVA) of *Sida cordifolia*

The analysis of variance presented in Table 5 indicate that the values of Prob \geq less than 0.0500 indicate the model is significant. In this

case (acid concentration)², (temperature)² are significant quadratic terms. By Removing the non significant terms and refitting, we can get the reducing sugar yield and the regression equation of all factors as follows:

Reducing Sugar Yield
 = -0.15789X₁²-2.79955E-0.03X₃².....eqn 2

Where X₁,X₂,X₃ are independent variable

The effect of interaction on the hydrolysis of *Sida cordifolia* was analyzed and plotted using Design-Expert 6.0.6 software based on the regression model. By analyzing Figure 4, with

the increase of Temperature and acid concentration, the yield of reducing sugar is gradually increasing

DESIGN-EXPERT Plot

reducing Sugar
 X = A: sulphuric acid
 Y = B: Time

Actual Factor
 C: Temperature = 80.00

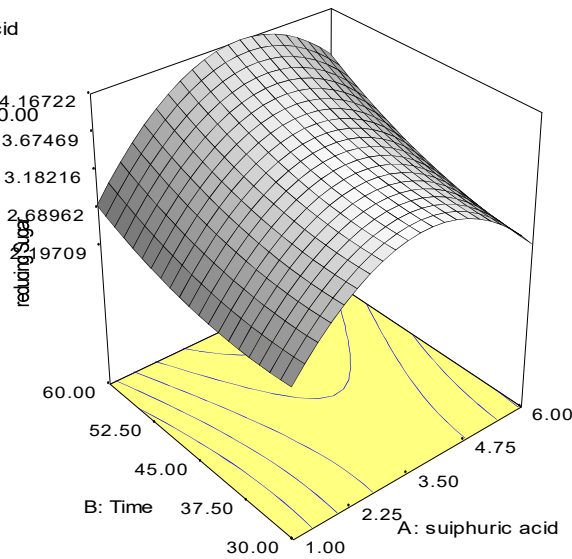


Figure 2: Response surface graphs showing the interactive effect of pretreatment time and concentration of sulphuric acid for *Sida cordifolia*

DESIGN-EXPERT Plot

reducing Sugar
 X = A: sulphuric acid
 Y = C: Temperature

Actual Factor
 B: Time = 45.00

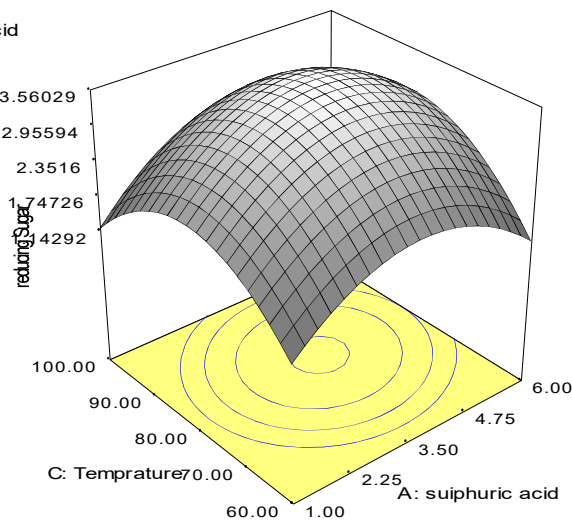


Figure 3: Response surface graphs showing the interactive effect of temperature and concentration of sulphuric acid for *Sida cordifolia*

Table 5: Analysis of variance for the model Regression (*Sida cordifolia*)

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	18.67	9	2.07	4.56	0.0133	significant
A	0.73	1	0.73	1.60	0.2347	
B	1.52	1	1.52	3.34	0.0977	
C	0.42	1	0.42	0.93	0.3588	
A²	2.68	1	2.68	5.88	0.0357	
B ²	0.11	1	0.11	0.25	0.6274	
C²	3.45	1	3.45	7.58	0.0204	
AB	0.15	1	0.15	0.33	0.5780	
AC	0.30	1	0.30	0.65	0.4381	
BC	0.16	1	0.16	0.35	0.5699	
Residual	4.55	10	0.46			
Lack of Fit	4.19	5	0.84	11.44	0.0091	significant
Pure Error	0.37	5	0.073			
Cor Total	23.23	19				

Where A= Acid concentration in percentage (%)

B=Time in minute and C=temperature in °C

Model validation and confirmation

Using Design-expert 6.0.6 software to optimize the design, the target value is the

highest yield of reducing sugar, the verification test and the results shown in Table 6, set three parallel test groups.

Table 6: Optimization Experiment

S/No	Acid concentration (%)	Reaction time(min)	Temperature	Reducing Sugar Yield (%)
1	5.79	56.74	67.00	5.031
2	5.00	56.74	67.00	5.211
3	5.79	57.00	67.00	4.970

CONCLUSION

The Use of RSM based CCD model, for the Optimum conditions of reducing sugar yield at a temperature-80°C, time-60 min and acid concentration-3.50% at a maximum reducing sugar yielded of 4.931%. Therefore, this optimal condition of acid hydrolysis is useful for producer to operate the process for

converting lignocellulose waste to reducing sugar. Conventional methods of hydrolysis are extremely time-consuming and expensive. RSM is efficient and easy to handle large number of design parameters and maximize the reducing sugar yield, reduce the cost of production.

REFERENCES

Ahmad, M.A., Bagudo, B.U., Tambuwal, A. D., and Uba, A. (2015). Effect of key parameter on bio ethanol production from country mallow and water

hyacinth. *International Journal of Applied Chemistry*, 11(3). 277 -289.
Akin-Osanaiye, B. C., Nzelibe, H.C., and Agbaji, A.S. (2005). Production of ethanol from *Carica papaya* (paw paw) agrowastes: effects of

- saccharization and different treatments on ethanol yield. *African Journal of Biotechnology*, 4(7):657-659
- Albarico, J.S., Detras, M.C., Sanchez, P.R., Alfafara, C.C., Borires, M.G., Nayue, F.P., Dcarado, A.A., Escobar, C.E and Ventura, J.S. (2017). Yield optimization of reducing sugar from acid hydrolysis of *Chlorella vulgaris* waste biomass. *Philippine e-Journal for Applied Research and Development*, (7) 21-33.
- AOAC (2005). Fiber (Crude) in Animal Feed and Pet Food. Fritted Glass Crucible Method. AOAC International 18th ed., Gaithersburg, Maryland.
- Dvoretzky, D.S., Dvoretzky, S., Temnov, M., Markin, I., and Fu, P. (2017). The technology of pre-purification treatment of Municipal Wastewater using microalgae *Chlorella vulgaris*, *Chemical Engineering Transactions*, 57, 49-54.
- Mosier, N., and C. Wyman (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, 96(6), 673-686.
- Mushtaq, F., Tuan Amran, T.A., Mat, R., and Ani, F.N. (2015). Optimization and characterization of bio-oil produced by microwave assisted pyrolysis of oil palm shell waste biomass with microwave absorber, *Bioresource Technology*, 190, 442-450.
- Mustapha, M.A., Manan, Z.A., and Wan Alwi, S.R. (2017) Sustainability assessment of a Municipal Sewage Treatment Plant using a single green performance indicator, *Chemical Engineering Transactions*, 56, 127-132.
- NREL LAP 005. Standard Method for Ash in Biomass. National Renewable Energy Laboratory, Colorado, USA. NR.
- NREL LAP 002. Two Stage Sulfuric Acid Hydrolysis for Determination of Carbohydrates. National Renewable Energy Laboratory, Colorado, USA.
- NREL LAP 005. Standard Method for Ash in Biomass. National Renewable Energy Laboratory, Colorado, USA.
- Narkprason, N., Assavarachan, Rittichain A., and Wongputtisin, P. (2013). Optimization of Reducing sugar production from Acid hydrolysis of sugar cane bagasse by Box behnke design. *Journal of Medical and Bioengineering*, 2, 238-241.
- Oliveira, R.A.D. (2017) Hybrid Short Path Evaporation as an Option to Lactic Acid Recovery from Fermentation Broth. *Chemical Engineering Transactions*, 57, 37-42.
- Pataro, G., Goettel, M., Straessner, R., Gusbeth, C., Ferrari, G. Frey, W. (2017). Effect of PEF Treatment on Extraction of Valuable Compounds from Microalgae *C. vulgaris*. *Chemical Engineering Transactions*, 57, 67-72.
- Raghavi, S., Sindhu, R., Binod, P., Gnansounou, E., and Pandey, A. (2016). Development of a novel sequential pretreatment strategy for the production of bioethanol from sugar cane trash. *Bioresource Technology*, 199, 202-212.
- Rabah, A.B., Oyeleke, S.B., Manga, S.B. and Hassan, L. G (2011). Utilization of millet and guinea corn husks for bioethanol production. *African Journal of Microbiology Research*, 5(31), 5721-5724.
- Thimmaiah, S.K. (1999). *Standard Methods of Biochemical Analysis*. Kaliani publishers New Delhi.pp1032-1040.
- Xue Z., Liu Q., and Zhang, M. (2017). Study on optimization of reducing sugar yield from corn stalk based on micro wave-assisted acid pretreatment process. *Chemical Engineering Transactions*, 62, 2017.
- Xue, F., Yu, J.M., Tesso, T., Dowell, F. and Wang, D.H. (2013). Qualitative and quantitative analysis of lignocellulosic biomass using infrared techniques. *Applied Energy*, 104,