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Comparative Study of Bioethanol Production from Wheat Straw and Rice Straw

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ABSTRACT

The study was carried out to determine the proximate and comparative analyses in the production of bioethanol from lignocellulosic biomass, rice straw and wheat straw. The proximate composition were determined in accordance with ASTM analytical methods. Ethanol production from agro waste was optimized using 2% NaOH solution to chemically pretreat milled straw. Pretreated and un-pretreated straws were subjected to hydrolysis and fermented. The hydrolysate and fermented filtrate were analyzed using UV-spectrophotometer and FTIR on complete hydrolysis, the straw hydrolysate gave a higher fermentable sugar concentration for the pretreated milled straw than un-pretreated straw. An increase in the reducing sugar amount of 36.00% and 28.85% for rice and wheat straw respectively were the result of difference and improvement due to alkaline pretreatment. Obtained fermentable sugar due to alkaline pretreatment of milled rice straw and wheat straw are 4.42g/l and 4.60 g/l respectively relative to a lower value of the un-pretreated rice straw and wheat straw of 3.25g/l and 3.57g/l respectively. Alkaline pretreatment prior to downstream processes improved the efficiency of the bioconversion of cellulose to glucose, hence it optimizes the bioconversion process by increasing the ethanol yield of room fermented rice straw from 57.65% to 61.55% and wheat straw from 61.80% to 64.02%, and also increased the yield of straws fermented in the incubator from 55.77% to 58.46% for rice and 60.16% to 62.64% for wheat.

Keywords: Bioethanol, Pretreatment, Hydrolysis, Lignocellulosic biomass, Bioconversion.

1.0 Introduction

The growing depletion in the world's non-renewable energy source and its deleterious environmental impact on the globe mandates the search for more accessible and cleaner alternatives [1]. Fossil fuel have been the main source of world's energy, but it is subject to depletion and it requires thousands of years to replenish in the earth [2]. Sustainable alternative by the use of waste from plants organism have been explored and utilized by some countries to generate biofuel [3]. The world's readily available terrestrial biomass energy resource excluding the aquatic biomass is estimated to 100 times output capacity of the world's total annual energy requirement [4].

Lignocellulose biomass, the woody inedible part of plant biomass having energy from the sun stored in it as chemical energy through the process of photosynthesis is the most abundant form of biomass which defines 87% of the total biomass energy [5]. It is the carbon resource that is renewable over time and large enough to serve the same purpose as fossil fuels [2]. Lignocellulosic biomass abounds in tree stumps, branches, stalks, stems, sawmills residuals, sawdust, tree bark, shrubs, seaweeds, straw. The discovery of lignocellulosic biomass potential to meet the same need the first generation category of biomass (food crops) have provides a better and self-sustaining source which does not constitute threat to the food security of any Nation [6]. Lignocellulosic biomass composition comprising of hemicellulose, cellulose, and lignin [7] vary in percentage composition in the range of 40 – 60% cellulose, 20 – 40% hemicellulose and 10

– 25% lignin [8]. Lignin, the component responsible for chemical and biological degradation resistance is undesirable of the three components [9]. The desired hemicellulose and cellulose by pretreatment are separated from lignin and easily converted to fermentable sugars and subsequently fermented to yield biofuel [10]. Straw, a lignocellulosic waste generated from cereal crop cultivation have been minimally utilized for further use but to dispose-off [11]. The larger the cultivation of cereals, the more the prospect of an industrial scale production of bioethanol from its straw as a kilogram harvest of rice is associated with 1.5kg straw generation [12].

The aim of this paper therefore is to carry out the proximate analysis of rice straw and wheat straw in accordance to the ASTM analytical method, and compare relative methods of bioethanol production from rice (*oryza sativa)* straw and wheat (*triticum aestivum)* straw. Utilizing chemical pretreatment to pretreat straw, the resulting effect are compared with the un-pretreated. Goel and Wati [13] in their work, "Ethanol Production from Rice (*oryza sativa*) Straw by Simultaneous Saccharification and Cofermentation" pretreated rice straw with dilute alkali (2%) NaOH prior to fermentation with combination of 3 yeast strain. Pretreatment of the straw resulted in efficient delignification. Sun *et al*. [14] in their work "Influence of Alkaline Pretreatments on the Cell-Wall Components of Wheat-Straw" discovered alkaline pretreatment as most effective in the delignification and dissolution of hemicellulose from wheat straw. They found out that the optimal method for alkaline pretreatment involves the use of 1.5% NaOH for 144 hours at 20 ^oC. This yields 80% hemicellulose and 20% lignin release. In contrast with other pretreatment processes, alkaline pretreatment utilizes lower pressure and temperature [15].

2.0 Materials and Methods

The biomass: rice straw and wheat straw were sampled from farmland in Kaduna North Local government area of Kaduna state, Nigeria. The crops were removed from the plant head. Debris on the straw was rinsed off with tap water, sample (rice straw and wheat straw) were cleansed separately, dried, chopped to pieces with knife and pulverized using a blender [16].

Determination of Percentage Volatile Matter (PVM): Pulverized sample of 2 g was weighed into a pre-weighed crucible and placed in an oven heated to a temperature of 105 ^oC. Weight of the sample was monitored with analytical balance till a constant weight was obtained. The sample in crucible was then covered with a crucible lid, placed in a furnace at a temperature of 550 ^oC for 4 hours and weighed after cooling in a desiccator [17].

PVM was calculated using the equation:

PVM = $\frac{A-B}{A}$ x 100

Where, **A** = weight of oven dried sample,

B = weight of the sample after 4 hours in the furnace at 550 ^oC.

Determination of Moisture Content: Cleaned petri-dish was dried for 30 minutes in an oven at 104 ^oC and cooled in a desicator. The petri-dish was weighed and a 5 g mass of milled sample was weighed into it and placed in the oven at 105 ^oC. It was removed and cooled in the desicator after heating for 2 hours. It was weighed and subsequently heated in the oven until an observed constant mass. Percentage moisture was then calculated from the difference in initial weight of sample and weight after heating using the formula below [17].

Moisture content (%) = 100 $\frac{(B-A)-(C-A)}{(B-A)}$

Where, **A** = weight of empty petri dish (g)

B = weight of petri dish + sample (g)

C = weight of petri dish + dried sample (g)

Determination of Dry Matter:

Dry Matter (%) = 100% **-** % Moisture content [17].

Determination of Ash Content: Pulverized sample of 2.0 g was weighed into a pre-weighed crucible. The sample containing crucible was placed in a muffle furnace burning at 550 ^{o}C. Sample containing crucible was removed after 4 hours and placed in the desicator to cool. When cooled, the crucible and ash of sample was weighed to determine the ash weight [17].

$$\textbf{Ash (\%)} = \frac{\text{Ash weight}}{\text{Sample weight}} \times 100$$

Determination of Percentage Fixed Carbon (PFC): PFC was computed by subtracting the sum of percentage volatile matter (PVM) and percentage ash content (PAC) from 100% as shown:

PFC = $100\% - (PAC+PVM)$ [17].

Ethanol Production

Pretreatment: Weighed 10 g of the pulverized and sieved sample was saturated with 25 ml 2% NaOH solution in a 250 cm^3 conical flask. Saturated solution was autoclaved at 15 psi for 1 hour and left to cool [13]. The un-pretreated was saturated in water for 24 hours.

Hydrolysis: The medium (broth of pretreated and un-pretreated straw) were made to attain pH 9 with 10% w/v NaOH in order to detoxify with $Ca(OH)_2$ against action of fermentation inhibitors [18]. The pH of the mediums were reduced to 5 with 10% v/v H_2SO_4 to a slightly acidic state suitable for the action of fermenting microorganism. The mediums (broth) were thereafter autoclaved at 121 ^{o}C/15 psi for 30 minutes and allowed to cool overnight [19].

Filtering and Fermentation: After hydrolysis, the broth was filtered with Whatman's filter paper to collect the filtrate for fermentation. Yeast (*Saccharomyces cerevisiae*) cell was used to inoculate the filtered hydrolysate. A set of the pretreated and un-pretreated hydrolysate were fermented at room temperature and another set was fermented in the incubator at 35^{o}C.

Determination of Reducing Sugar Concentration: The hydrolysate filtrate was collected and analyzed for the reducing sugar content by using DNS (dinitrosalicylic) test. DNS reagent of 3 cm^3 was added to 3 cm^3 of hydrolysate filtrate in a lightly capped test tube. The mixture was heated at 90 ^{o}C for 5-15 minutes to develop a red-brown colour. 1 cm^3 of 40% Potassium tartrate was added to stabilize the colour. After cooling, the absorbance was taken with a UV-spectrophotometer at 540 nm. Concentration (g/l) of reduced sugar was calculated with the line equation of the standard curve of known glucose concentration [20].

Determination of Ethanol by dichromate assay: Fermented hydrolysate was centrifuged to obtain clear ethanol filtrate. With FTIR and UV-spectrophotometer the filtrates were analysed. The ethanol concentration was determined by Dichromate assay in the test tube and absorbance at wavelength 595 nm was taken on the UV-spectrophotometer [21]. Gunasekaran and Kamini [22] proposed modified formula was used to determine the ethanol yield.

$$\text{Ethanol Yield \%} = \frac{\text{Produced Ethanol}}{\text{Utilized TRS}} \times 100$$

3.0 Result and Discussion

Table 1.0: Proximate Result for Rice Straw and Wheat Straw

Biomass	Rice Straw	Wheat Straw
Moisture Content (%)	5.42	7.98
Volatile Matter (%)	65.55	76.93
Volatile Matter (%)	18.27	4.00
Fixed Carbon (%)	16.18	19.06
Dry Matter (%)	94.58	92.02

Table 2.0: Reducing Sugar Concentration of Hydrolyzed Rice and Wheat Straw

S/No	Sample	Reduced Sugar Conc. (g/l)
1	RHb (Pretreated Rice Hydrolysate)	4.42
2	WHb (Pretreated Wheat Hydrolysate)	4.60
3	RH (Un-pretreated Rice Hydrolysate)	3.25
4	WH (Un-pretreated Wheat Hydrolysate)	3.57

Table 3.0: Concentration of Ethanol Produced from Rice and Wheat Straw

Samples at condition fermented	Ethanol Concentra-tion (g/l)
IRR (Incubator Fermented Pretreated Rice straw)	32.02
IR (Incubator Fermented Un-pretreated Rice straw)	19.51
IWR (Incubator Fermented Pretreated Wheat straw)	36.26
IW (Incubator Fermented Un-pretreated Wheat straw)	25.28
RRR (Room Incubated Pretreated Rice straw)	36.24
RR (Room Incubated Un-pretreated Rice straw)	28.28
WRR (Room Incubated Pretreated Wheat straw)	37.27
WR (Room Incubated Un-pretreated Wheat straw)	35.33

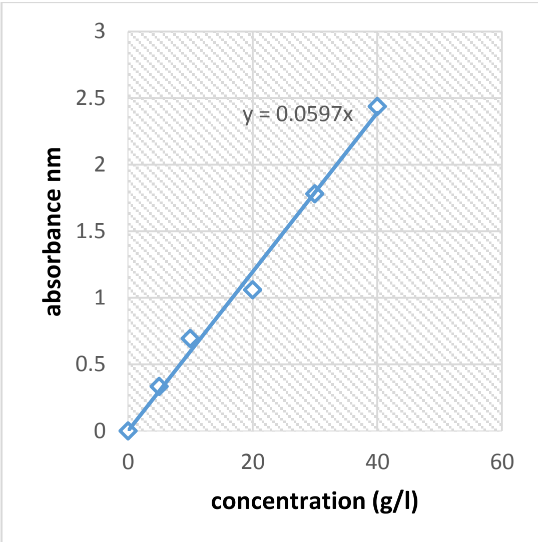
Discussion

Proximate: Table 1.0 shows proximate result of rice straw and wheat straw. Their low moisture indicates they can easily burn off when used as sources of heat. Moisture content obtained is comparable to wheat straw moisture obtained by Lam *et al.* [23]. Low ash content obtained indicates wheat straw will yield lower residue compared to rice straw on complete combustion. The resulting PVM are closely in agreement with 75.27% and 65.47% obtained by Jenkins *et al.* [24] for wheat straw and rice straw respectively. The high PVM values signifies easy ignition of the biomass and proportionate increase in flame length [25]. Dry matter of rice straw is comparable to the value obtained by Jekayinfa and Omisakin [26] for rice husk briquette of 87.33%. High dry matter indicates high percentage of biomass will combust. Fixed carbon is the measure of solid combustible material in solid fuel after expulsion of volatile matter i.e. estimate amount of coke obtainable on carbonization [27].

Reducing Sugar Content: Reduced sugar were determined with the aid of a standard concentration plot. Method which precludes alkaline pretreatment gave a reducing sugar value of 3.25 g/l and 3.57 g/l for rice straw and wheat straw respectively. Method that applies alkaline pretreatment before hydrolysis gave a higher reducing sugar value of 4.42 g/l and 4.60 g/l for rice and wheat straw respectively. These values obtained are comparable to Chapla *et al.* [28] results. Alkaline pretreated straw is therefore more susceptible to hydrolysis and hence increases yield of fermentable sugar.

Ethanol Concentration of Rice Straw and Wheat Straw: The Ethanol concentration was determined using the standard ethanol concentration plot (Figure 1.0). The result obtained show samples subjected to alkaline pretreatment gave higher alcohol concentration relative to samples un-pretreated. Un-pretreated samples gave concentration values of 19.51 g/l (IR), 28.28 g/l (RR), 25.28 g/l (IW), and 35.33 g/l (WR). Pretreated samples gave concentration values of 32.02 g/l (IRR), 36.24 g/l (RRR), 36.26 g/l (IWR), and 37.27 g/l (WRR). This observation substantiates alkaline pretreatment a means of optimizing fermentation. Takano and Hoshino [29] pretreated rice straw with alkali solution and obtained a comparable result of 30.5 g/l for ethanol concentration after 36 hours. Goel and Wati [13] produced ethanol using rice straw. In their comparative analysis, they conclude a varying ethanol concentration of 5.30 g/l to 24.94 g/l after 96 hours of incubation.

Figure 1.0: Ethanol Calibration Curve



Ethanol Yield of Straw: Incubated pretreated wheat straw sample gave high yield of 62.64% (IWR) relative to 60.16% (IW) for un-pretreated wheat straw fermented in the incubator. In wheat straw incubated at ambient temperature; pretreated straw gave an ethanol yield of 64.02% (WRR) relative to 61.80% (WR) of un-pretreated wheat straw. Incubator fermented pretreated rice straw gave a yield of 58.46% (IRR) compared to 55.77% (IR) yield of un-pretreated straw sample. Rice straw incubated at room temperature gave yield of 61.55% (RRR) for the pretreated straw relative to 57.65% (RR) yield of the un-pretreated straw.

Fourier Transform Infrared Spectrophotometer Analysis (FTIR): FTIR Spectra of the fermented filtrate notify various functional groups in the ethanol produced from pretreated straws. A control sample of fermented mixture of corn and millet (first generation biomass) was analyzed. Their spectra (including the control sample) have highest peak per cm^{-1} within the range of 3333 cm^{-1} to 3348 cm^{-1}. The peaks at the band frequency estimate of 3400 cm^{-1} signifies the presence of produced alcohol.

4.0 Conclusion: The bioethanol conversion of the straw were found to take place optimally in samples pretreated with hydroxide of sodium relative to un-pretreated samples. Pretreatment of the straws with alkali solution resulted to increase of fermentable sugar content. Improving obtained amount of reduced sugar of rice straw by 36% and wheat straw by 29%. This improves the ethanol yield compared to the un-pretreated. A higher and stable temperature (35 ^{o}C) facilitates quick fermentation, hence, obtaining fast yield within shorter 24 hours period compared to room fermentation at a longer timeframe of 168 hours. It is established that prior to downstream hydrolysis and fermentation, alkaline pretreatment optimizes bioethanol production irrespective of the period of incubation or additional microorganism used (cofermentation) for inclusive fermentation of other simple sugar along with glucose.

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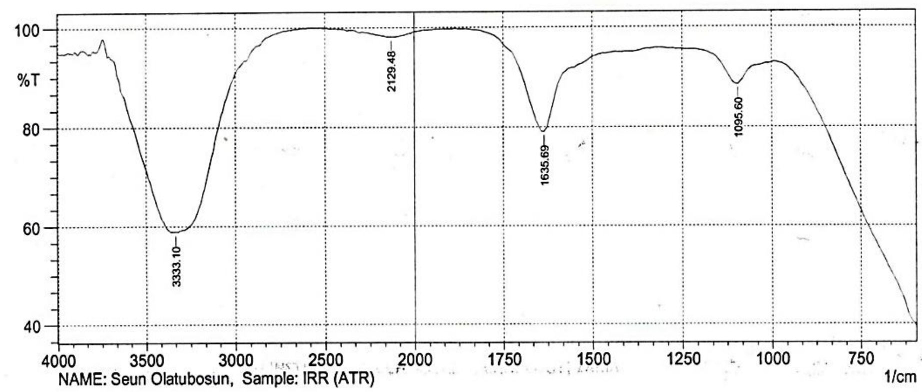
FTIR Spectrum of Rice Straw (Sample IRR)

FTIR ANALYSIS RESULT

NATIONAL REASEARCH INSTITUTE FOR CHEMICAL TECHNOLOGY, ZARIA

SHIMADZU

FTIR-8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1095.6	88.53	5.135	1226.77	1018.45	7.179	1.596
2	1635.69	79.005	18.136	1882.59	1435.09	14.99	9.787
3	2129.48	98.083	1.268	2291.51	1882.59	2.108	1.079
4	3333.1	58.884	0.431	3340.82	2546.12	57.564	-33.734

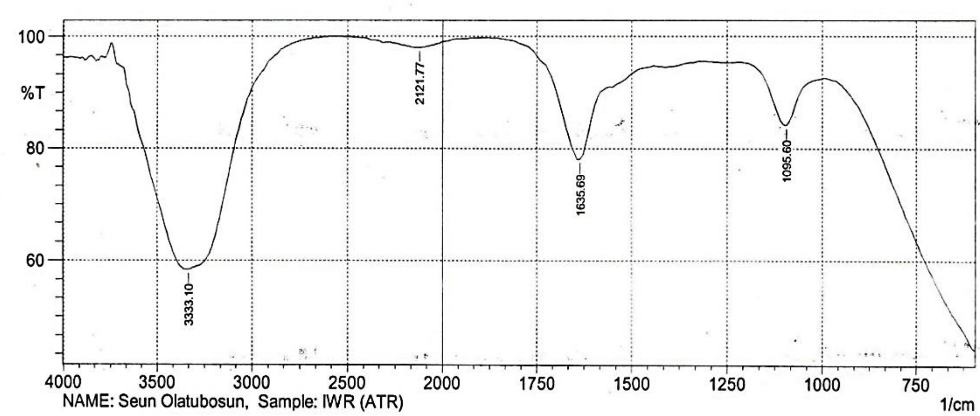
FTIR Spectrum of Wheat Straw (Sample IWR)

FTIR ANALYSIS RESULT

NATIONAL REASEARCH INSTITUTE FOR CHEMICAL TECHNOLOGY, ZARIA

SHIMADZU

FTIR-8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	1095.6	84.199	9.725	1211.34	995.3	9.305	3.513
2	1635.69	78.085	15.023	1882.59	1558.54	12.165	5.388
3	2121.77	98.012	0.068	2129.48	1928.88	1.085	0.056
4	3333.1	58.391	0.478	3340.82	2669.57	57.732	-21.084