American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)
ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

Production of Ethanol from Cassava and Yam Peels Using Acid Hydrolysis

Samson Olayemi[a*], Adeola Ibikunle[b], Joy Olayemi[c]

[a]*Analytical Research Division, Department of Production, Analytical Services, and Laboratory Management, Federal Institute of Industrial Research Oshodi, Lagos, Nigeria*

[b]*Chemical Sciences Department, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria*

[c]*Biochemistry Department, Lagos State University, Ojo, Lagos, Nigeria*

[a]*Email: samprof81@yahoo.com,* [b]*Email: adeolaalliance@yahoo.com,* [c]*Email: joy01forreal@yahoo.com*

Abstract

Energy from fossil fuels has played a very important role in our lives, but such an important role has been clouded out due to the environment hazards caused from fossil emissions. This has led to a new dimension in energy utilization known as renewable energy fuels. To fully support this type of energy from biological mass, adequate biomass source must be harnessed. This work thus was carried out with a view of utilizing some locally available biomass wastes as an alternative source of ethanol. The production of ethanol from cassava and yam peels was examined using acid hydrolysis at two different temperatures and fermenting with two different strains of *saccharomyces cerevisiae* (baker′s yeast and freshly isolated yeast).Fermentation was allowed for about 5 days after which ethanol was recovered by distillation (at 78 ^{O}C). Iodoform test and gas chromatography were used, just to confirm that the distillates were ethanol. Cassava peels hydrolyzed at room temperature produced higher yields of ethanol (11.30% and 8.63%). Fermentation with freshly isolated yeast produced more yield of ethanol both at room temperature (11.30%) and at 80 ^{O}C (6.15%) than those fermented with baker′s yeast. Yam peels also produced more ethanol at room temperature than at 80 ^{O}C using either of the two enzymes for fermentation (21.72% and 27.08%). Moreover, the use of baker′s yeast for fermentation produced more yield of ethanol from yam peels. For the mixtures by proportion, only the ratio of 2:1 of cassava to yam peels (C_2Y_1) produced a higher yield of ethanol at room temperature (60.52% and 13.39% at room temperature using baker′s yeast and freshly isolated enzymes respectively). The other mixtures [(C_1Y_2) and (CY) sets of samples] gave higher yields of percentage ethanol at 80 ^{O}C than at room temperature. Every other sets of samples gave higher yields at room temperature. Generally, most of the samples hydrolyzed better at room temperature except for C_2Y_1 and CY sets of samples. The highest yield of ethanol was produced by (C_2Y_1) when hydrolyzed at room temperature using baker′s yeast (60.52%).

Keywords: Ethanol yield; Hydrolysis; Energy; Temperature; *Saccharomyces cerevisiae.*

----

* Corresponding author.

1. Introduction

The rapid growth of industries and technological advancement in the world call for development in the chemical sector. The production of industrial chemicals will enhance the economic progress of any nation. Ethanol, one of the important industrial chemicals, can be produced extensively from biomass such as cassava peels and yam peels. The main constituents of this class of crop by-product are cellulose [1] and hemicelluloses, making them lignocelluloses [2] that can be excellent energy sources. Mechanized farming has led to extensive discharge of agricultural wastes that have had negative effects on the environment. As a result of the environmental mess caused the agricultural activities of farmers, the utilization of such wastes has become very important to researchers [3,4,5].

This work was designed to look into the possibility of converting some of such by- products into industrial chemicals of economic importance. Ethanol is one such chemical. Ethanol produced from regeneration sources is an attractive petrochemical feedstock in petroleum for poor countries [6]. The various uses of ethanol and the importance of ridding the environment of the harmful effects of these agricultural by-products (biomass) underscore the significance of this work. Ethanol is produced from palm wine by fermentation process [7]. Fermentation is one of the oldest processes known to man, and it is used in making a variety of products including foods, flavorings, beverages, pharmaceuticals, and chemicals. Ethanol is made from a variety of products such as grain, molasses, fruit, cobs, and shell; its production, excluding that of beverages, has been declining since the 1930s because of the low cost [8]. In 1975, only 76×10^6 L of proof industrial ethanol were produced by fermentation compared to 7.95×10^6 L by synthesis.

Fossil fuels, mainly petroleum, coal, and natural gas, were the main energy sources for most industries during the 20th century, and are still the most important feedstocks to produce energy in the world. However, these sources are no longer regarded sustainable, and their availability is much lower. Hence, the need for more research in the production of Bioethanol which can be used as petrol substitute for vehicles.

The objective of this study is to produce ethanol from cassava peels and yam peels using acid hydrolysis at two different temperatures and fermented with two strains of *saccharomyces cerevisiae* (baker's yeast and freshly isolated yeast).

2. Materials and methods

2.1 Reagents and apparatus

REAGENTS:

Concentrated sulfuric acid, diethyl ether, sodium hydroxide, distilled water, freshly isolated and industrially made yeasts.

APPARATUS AND EQUIPMENT:

250ml conical flasks, beakers, funnels, whatzman filter paper (could be ashless or any other), measuring cylinders, volumetric flasks (or other collector bottles), distillation sets, round bottomed flasks, retort stands, heating source (heating mantles, plates or other sources), water bath, thermometers, refractometer, weighing balance, specific gravity (SG) bottle,

2.2 Methodology (procedure for production)

Collection and Processing of Substrate Used:

COLLECTION OF SAMPLES:

Cassava peels and wastes were collected from the old pilot plant building of the Federal Institute of Industrial Research, Oshodi, (FIIRO) Lagos state. Yam peels and wastes were also collected from the market premises of a local suburb in Ogba-iyo Ijoko, Ogun state.

SORTING AND DRYING OF SAMPLES:

The collected samples were taken to a convenient place where they are sorted. The peels of cassava and yam were separately sorted to have mainly the peels for drying. The drying was carried out in open air but due to the weather which delays drying for weeks as a result of frequent rainfall, drying was completed in an oven.

MILLING OR GRINDING OF SAMPLES:

The well dried samples were then milled using a hammer mill in the new pilot plant building of the federal institute of industrial research, oshodi, FIIRO, lagos state. A serrated disc grinder could also be used to reduce the yam and cassava peels to very small particle sizes.

ISOLATION OF CELLULOSE (PRETREATMENT):

From the milled cassava and yam peels, 120g of five different samples were weighed as follows:

120g of cassava peels

120g of yam peels

120g of a mixture of cassava and yam peels in the ratio of 2:1

120g of a mixture of cassava and yam peels in the ratio of 1:2 and

120g of a mixture of cassava and yam peels in the ratio of 1:1

The cellulose was isolated by the procedure described by [9]. To 30g of each sample of the agricultural waste was added 60ml of diethyl ether in a 250ml conical flask in order to remove extractives and the residue left was washed with distilled water.

Each of the five weighed samples are done in quadruplet giving rise to twenty different samples [C_{1-4}, Y_{1-4}, $(CY)_{1-4}$, $(C_1Y_2)_{1-4}$, $(C_2Y_1)_{1-4}$].

HYDROLYSIS:

30g of the washed residues are then weighed into separate conical flasks and divided into two sets of samples. Each quadruplet being split into duplicates. 150ml of 4.5M sulfuric acid is then added to all the samples in the conical flasks and hydrolysis is allowed to take place at two different temperatures. One set of duplicates (10 samples) are placed in a water bath at 80 ^{O}C while the other set of ten samples are allowed at room temperature. The stirred 250ml conical flasks serve as reactors. Hydrolysis is then allowed to proceed for about two and half hours. A hand refractometer is used to monitor and analyze the glucose level during the reaction.

The resulting hydrolyzed samples were filtered with filter papers leaving filtrates with high percentage of glucose. These filtrates act as the substrates in the fermentation process. Each filtrate is poured into a portable one liter plastic container which acts as the fermentation medium (fermentation jar).

FERMENTATION:

The substrates in the fermentation media were inoculated with *saccharomyces cereviciae* as the starter culture. To one of each duplicate, 3g of baker′s yeast was added and to the other, 5ml of freshly isolated yeast was added. This was to determine which of the enzymes was more potent and will ferment which sample best. The sterilized containers were well capped to avoid air entering the reactor medium. The fermentation process was allowed to go on for about five days. At intervals within the fermentation period, the conversion rate of glucose to ethanol was being monitored using a hand refractometer which determined the glucose and ethanol concentration in the sample.

DISTILLATION: After the fermentation process, ethanol was recovered using a simple batch distillation method. Distillation sets were mounted and ethanol was recovered at about 78 ^{O}C. Thermometers were attached to ascertain the temperature of distillation.

Confirmatory tests were then carried out to ascertain that the distillates were actually ethanol (iodoform test and gas chromatography).

DETERMINATION OF QUANTITY OF ETHANOL PRODUCED

The distillate collected was measured using measuring cylinder and expressed as quantity of ethanol produced in g/L by multiplying the volume of distillate by the density of ethanol (0.8033g/ml)

DETERMINATION OF ETHANOL CONCENTRATION

First Method:

Ethanol concentration was determined by comparing the density of the ethanol produced with the standard ethanol density curve.

Standard ethanol curve was obtained by taking series of percentage ethanol (10, 20, 30, 40 & 50 percent) solution which was prepared in a 100ml volumetric flask and the weight was measured. The density of each of the prepared ethanol was calculated and a standard curve of density against percentage ethanol was plotted.

Second Method Used:

The percentage yield of ethanol was also calculated using the specific gravities of the ethanol solutions produced. Each product (ethanol) was measured using a measuring cylinder and the volume was recorded. It was then weighed with a specific gravity bottle on a weighing balance and the weight noted. The corresponding volume of distilled water was also weighed with the specific gravity bottle and the weight was also recorded. Specific gravity (SG) = weight of ethanol produced (for each sample)/weight of distilled water Percentage ethanol (v/v) = $[8610.6 - (16584 \times SG)] + (7973.3 \times SG \times SG)$. Both the above stated methods were used in this work to calculate the percentage yield of ethanol (with emphasis on the second method). The SG (second) method is used to report the results in this research work

3. Results and discussion

DEFINITION OF TERMS

For proper understanding, the terms used in this chapter are defined below:

RT - room temperature

C_1 and C_3 - cassava peels samples hydrolyzed at 80 ^{O}C and RT respectively (using baker′s yeast)

Y_1 and Y_3 - yam peels samples hydrolyzed at 80 ^{O}C and RT respectively (using baker′s yeast)

$(CY)_1$ and $(CY)_3$ - mixture of cassava and yam peels in the ratio of 1:1 hydrolyzed at 80 ^{O}C and RT respectively (using baker′s yeast)

$(C_1Y_2)_1$ and $(C_1Y_2)_3$ - mixture of cassava and yam peels in the ratio of 1:2 hydrolyzed at 80 ^{O}C and RT respectively (using baker′s yeast)

$(C_2Y_1)_1$ and $(C_2Y_1)_3$ - mixture of cassava and yam peels in the ratio of 2:1 hydrolyzed at 80 ^{O}C and RT respectively (using baker′s yeast)

C_2 and C_4 - cassava peels samples hydrolyzed at 80 ^{O}C and RT respectively (using freshly isolated enzyme)

Y_2 and Y_4 - yam peels samples hydrolyzed at 80 ^{O}C and RT respectively (using freshly isolated enzyme)

$(CY)_2$ and $(CY)_4$ - mixture of cassava and yam peels in the ratio of 1:1 hydrolyzed at 80 ^OC and RT respectively (using freshly isolated enzyme)

$(C_1Y_2)_2$ and $(C_1Y_2)_4$ - mixture of cassava and yam peels in the ratio of 1:2 hydrolyzed at 80 ^OC and RT respectively (using freshly isolated enzyme)

$(C_2Y_1)_2$ and $(C_2Y_1)_4$ - mixture of cassava and yam peels in the ratio of 2:1 hydrolyzed at 80 ^OC and RT respectively (using freshly isolated enzyme)

RESULTS

Tables I and II show the volume (cm^3), mass (g/cm^3), specific gravity and percentage yield of ethanol; (%) of ethanol produced from cassava peels, yam peels and the various mixtures by proportions when hydrolyzed at room temperature and 80 ^OC respectively, and fermented with baker′s yeast. Maximum yield of 3.695g/cm3 with a concentration of 60.52% was produced from the mixture of cassava and yam peels with the ratio 2:1 when hydrolyzed at room temperature $(C_2Y_1)_3$.

Table I: Samples hydrolyzed at room temperature and fermented with baker′s yeast

Samples	Volume of Ethanol Produced (cm^3)	Mass of Ethanol Produced (g/cm^3)	Specific Gravity	Percentage Yield of Ethanol (Conc.) (%)
C_3	8	6.4264	0.98808	8.63
$(CY)_3$	5.3	4.2575	0.99205	5.47
$(C_1Y_2)_3$	7.7	6.1854	0.97434	21.51
$(C_2Y_1)_3$	4.6	3.6952	0.94405	60.52
Y_3	4	3.2132	0.96921	27.08

Table II: Samples hydrolyzed at 80 ^OC and fermented with baker′s yeast

Samples	Volume of Ethanol Produced (cm^3)	Mass of Ethanol Produced (g/cm^3)	Specific Gravity	Percentage Yield of Ethanol (Conc.) (%)
C_1	18	14.4594	0.99341	4.45
$(CY)_1$	8.8	7.0690	0.96555	31.32
$(C_1Y_2)_1$	11	8.8363	0.94851	53.86
$(C_2Y_1)_1$	4.6	3.6952	0.95561	43.91
Y_1	11.5	9.2380	0.97146	24.59

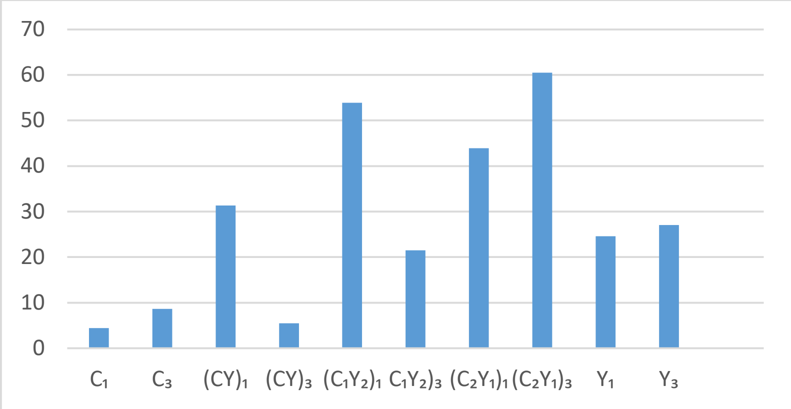

Figure 1: Samples fermented with baker′s yeast and their corresponding percentage yields of ethanol

Tables III and IV show the volume (cm^3), mass (g/cm^3), specific gravity and percentage yield of ethanol; (%) of ethanol produced from cassava peels, yam peels and the various mixtures by proportions hydrolyzed at room temperature and 80 ^OC respectively, and fermented with freshly isolated enzyme (*saccaharomyces cerevisiae).* Maximum yield of 4.8198g/cm3 with a concentration of 31.70% was produced from the mixture of cassava and yam peels with the ratio 1:2 when hydrolyzed at 80 ^OC $(C_1Y_2)_2$.

Table III: Samples hydrolyzed at room temperature and fermented with freshly isolated enzyme (*saccaharomyces cerevisiae*).

Samples	Volume of Ethanol Produced (cm^3)	Mass of Ethanol Produced (g/cm^3)	Specific Gravity	Percentage Yield of Ethanol (Conc.) (%)
C_4	9.2	7.3904	0.98495	11.30
$(CY)_4$	6.8	5.4624	0.97944	16.38
$(C_1Y_2)_4$	9.3	7.4707	0.98915	7.75
$(C_2Y_1)_4$	6.4	5.1411	0.98262	13.39
Y_4	6.3	5.0608	0.97414	21.72

Table IV: Samples hydrolyzed at 80 ^OC and fermented with freshly isolated enzyme {*saccaharomyces cerevisiae*).

Samples	Volume of Ethanol Produced (cm^3)	Mass of Ethanol Produced (g/cm^3)	Specific Gravity	Percentage Yield of Ethanol (Conc.) (%)
C_2	10	8.033	0.99117	6.15
$(CY)_2$	5.2	4.1772	0.97358	22.31
$(C_1Y_2)_2$	6.0	4.8198	0.96523	31.70
$(C_2Y_1)_2$	6.7	5.3821	0.99126	6.08
Y_2	7.1	5.7034	0.97571	20.09

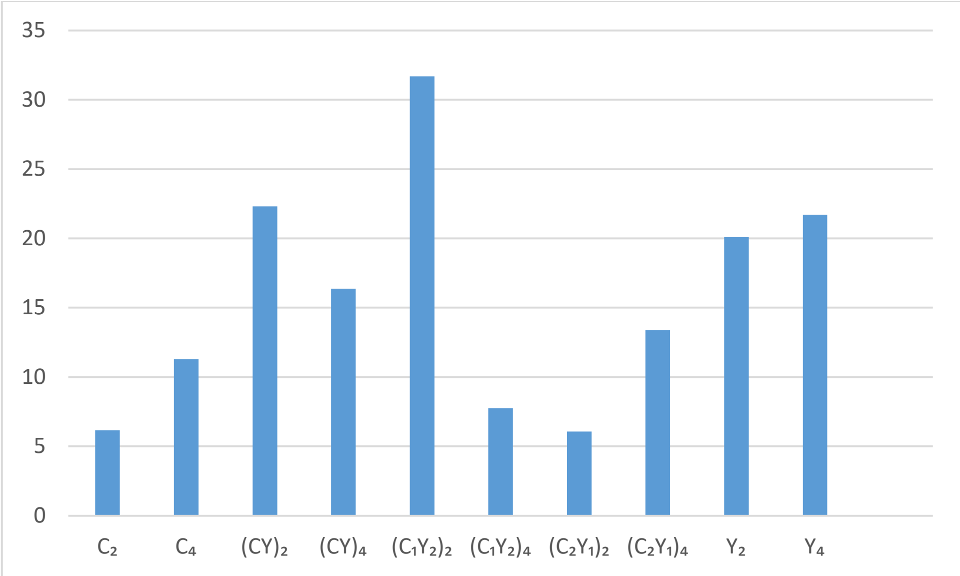

Figure 2: Samples fermented with freshly isolated enzyme (*saccaharomyces cerevisiae)* and their corresponding percentage yields of ethanol

DISCUSSION

Acid hydrolysis of cassava and yam peels at different temperatures using two different sources of the same enzyme (freshly isolated and baker′s yeast) gave various yields of ethanol.

TEMPERATURE EFFECT

Cassava peels when hydrolyzed at room temperature gave higher yields of ethanol using either freshly isolated yeast or baker′s yeast for fermentation (11.30% and 8.63% respectively). It was also noted that for cassava peels, fermentation with freshly isolated yeast produced more yield of ethanol both at room temperature (11.30%) and at 80 ^{O}C (6.15%) than those fermented with ready-made enzymes (baker′s yeast) which produced 8.63% and 4.45% at room temperature and at 80 ^{O}C respectively.

Yam peels also produced more ethanol at room temperature than at 80 ^{O}C using either of the two enzymes for fermentation (21.72% and 27.08%). But in this case, the use of baker′s yeast for fermentation produced more yield of ethanol from yam peels. Using baker′s yeast, 27.08% and 24.59% were produced at room temperature and at 80 ^{O}C respectively while 21.72% and 20.09% ethanol were realized using freshly isolated enzyme.

For the mixtures by proportion, only the ratio of 2:1 of cassava to yam peels (C_2Y_1) produced a higher yield of ethanol at room temperature. (C_2Y_1) gave 60.52% and 13.39% at room temperature using baker′s yeast and freshly isolated enzymes respectively while at 80 ^{O}C, it produced 43.91% and 6.08% using baker′s yeast and freshly isolated enzymes respectively. The other mixtures [(C_1Y_2) and (CY) sets of samples] gave higher yields of percentage ethanol at higher temperature (80 ^{O}C) than at room temperature as can be seen in tables 3 and 6 above. Every other sets of samples gave higher yields at room temperature. This implies that most of the samples hydrolyzed better at room temperature than at higher temperatures (except for (C_2Y_1) and (CY) sets of samples)

EFFECT OF ENZYME

It was noticed that in almost all the samples, the percentage yield of ethanol by the samples fermented with baker′s yeast are much higher than the ones gotten from the samples fermented with freshly isolated yeast. The exceptions are for purely cassava (C) samples, $(CY)_3$ and $(CY)_4$ which seemed to produce higher yields with the freshly isolated enzyme (yeast). For the other samples, the baker′s yeast proved more effective and fermentation was better with it than with the freshly isolated enzymes while it was vice versa for the purely cassava peels samples and the mixture of cassava and yam peels with ratio 1:1 which was hydrolyzed at room temperature $(CY)_3$ and $(CY)_4$. Hence, it implies that baker′s yeast is more effective in the fermentation of most of the samples used for the production of ethanol in this work.

PRETREATMENT

The conversion of cellulose to ethanol requires: pre-treatment (or delignification) to liberate cellulose and hemicelluloses from their complex with lignin; hydrolysis of the carbohydrate polymers to produce free sugars; and fermentation of free sugars (hexose and pentose) to produce ethanol [10]. All the samples were pretreated and they produced appreciable yields of ethanol.

YIELDS

This research work shows that yam peels produce higher concentration (yield) of percentage ethanol either at room temperature or at 80 ^{O}C using any of the two enzymes (freshly isolated yeast or baker′s yeast). This invariably shows that it could be more productive using yam peels for ethanol production than cassava peels.

However, the mixture of yam and cassava peels in different proportions also produced good yields of ethanol (in percentage v/v of ethanol). The mixture of cassava and yam peels with ratio 2:1 when hydrolyzed at room temperature and fermented with baker′s yeast gave the highest yield (60.52%) of all the samples worked on in this work. It should be noted that ratio 1:2 of the mixture at 80 ^{O}C using same enzyme also gave close value (53.86%).

Based on the results above, it can therefore be seen that production of ethanol from yam and cassava peels (wastes) was most productive when a mixture of both cassava and yam peels are used at ratio 2:1 hydrolyzed at room temperature or ratio 1:2 hydrolyzed at 80 ^{O}C using ready-made (baker′s) yeast for fermentation.

4. Conclusion

Ethanol can be produced from biomass in different quantities using either enzyme or acid hydrolysis. However, due to the high cost of production and preservation of enzymes, it is more advisable to carry out the production on a larger scale using acid hydrolysis.

This research work has also shown that it could be more productive producing ethanol from mixture of different sources rather than from the same source. The mixtures of cassava and yam at ratio 1:2 hydrolyzed at 80 ^{O}C and 2:1 hydrolyzed at either 80 ^{O}C or room temperature gave excellent yield of ethanol when compared to previous works done on the production of ethanol from biomass. This implies that mixtures of different sources of ethanol could be more productive (perhaps, not in all cases).

It can also be concluded from this work that for different samples, hydrolysis is best carried out at different temperatures. Hydrolysis of a particular sample might be complete at a particular temperature while that of the other is best at higher or lower temperatures.

Also, this research work showed that some substrates are best fermented with some enzymes while other substrates will be better fermented using other enzymes as in the case of this work which shows some samples fermenting better with the industrial yeast while others underwent better fermentation with the freshly isolated yeast.

It is also very important to note the right temperature of hydrolysis for each sample. It should be seen from this work that hydrolysis of different samples is better at different temperatures. Some samples hydrolyze better at lower temperatures to produce more glucose while others hydrolyze better at higher temperatures. Hence, temperature of hydrolysis is very important in other to get the best yield of ethanol from the waste cassava, yam or any other peels to be used for ethanol production.

References

[1]. M.M. Chang; J.Y. Chou; and G.T. Tsao. “Structure, pretreatment and hydrolysis of cellulose”. Adv. Biochem. Engin. 1981, 16: 40-5.

[2]. E.B. Cowling. “Physical and chemical constraints in the hydrolysis of cellulose and lignocellulose’s material”. Biotech. Bioengin. Symp. Series 5:163-81. 1976.

[3]. V.A. Oyenuga. Nigeria Foods and Feeding Stuffs, 2nd ed. Revised. Univ. Press, Ibadan, Nigeria, 1959, pp. 56-7, 71.

[4]. U.G. Akpan. “Acid demethylation of agricultural waste (citrus peel)”. Paper presented at the 8th Annual Sci. Conf. Nigeria Society for Biol. Conserv., University of Uyo, Uyo Nigeria, 1999.

[5]. A.O. Amosun. “Gasification of biomass for methanol production”. An unpublished B.Eng. Chemical Engineering Dept., Federal Univ. of Technology, Minna, Nigeria. 2000.

[6]. G.B. Gordon; and S. Michael. Food Science. Pergamon Int. Popular Sci. Series, Oxford, UK. 1979, pp. 21, 37-97.

[7]. J.F. Harris. Wood as a Chemical Raw Material. The Chemistry of Wood. Interscience Publ., New York, NY, USA. 1963.

[8]. Othman, Kirk. Encyclopedia of Chemical Technology, Vol. 2, John Wiley, New York, NY, USA. 1981, pp. 393-6.

[9]. S.K. Layokun. “Kinetics of acid hydrolysis of cellulose from sawdust,” Proc. 11th Annual Conf. Nigeria Soc. Chem. Engin., 1981, pp. 63-8.

[10]. Y. Lin; and S. Tanaka. "Ethanol fermentation from biomass resources: current state and prospects". Applied Microbiology Biotechnology, 69(6): 627-642. (2006).

[11]. Z. Adeeb. "Glycerol delignification of poplar wood chips in aqueous medium". Energy Education Science and Technology 13/2, 2004, pp. 81-88.

[12]. O. D. Adeniyi; A. S. Kovo; A. S. Abdulkareem; and C. Chukwudozie. "Ethanol Production from Cassava as A Substitute for Gasoline". J. Dispersion Sci. Technol 28:501-504. (2007),

[13]. U. G. Akpan; "Acid Demethylation of Agricultural Waste (Citrus Peel)". A. M. S. E 46(6): (2003), pp. 33-42.

[14]. U. G. Akpan; A. S. Kovo. M. Abdullahi; and U. J. J. Ijah, "Production of Ethanol from Maize Cobs and Groundnut Shell". AU J. T. 9(2): 106-110. (2005).

[15]. APA: Ethanol fuel | Fuel Flow Meter. (n.d.). Retrieved from http://www.fuelflowmeter.net/ethanol-fuel/ Chicago: Ethanol fuel | Fuel Flow Meter, http://www.fuelflowmeter.net/ethanol-fuel/ (accessed November 12, 2011).

[16]. C. Arato; E.K. Pye; and G. Gjennestad. "The lignol approach to biorefining of woody biomass to produce ethanol and chemicals". Applied Biochemistry and Biotechnology 123/1-3, (2005), pp. 871-882.

[17]. S.K. Black; B.R. Hames; and M.D. Myers. "Method of separating lignocellulosic material into lignin, cellulose and dissolved sugars": Anon., patent number US 5730837, (1994).

[18]. B.L. Browning. The Chemistry of Wood, Interscience, New York, 1967, pp 703.

[19]. C.A. Cardona; and O.J. Sanchez. "Fuel ethanol production: Process design trends and integration opportunities". Bioresource Technology 98(12): 2007, pp.2415-2457.

[20]. J.O.B. Carioca; P.V. Pannirselvam; E.A. Horta; and H.L. Arora. "Lignocellulosic biomass fractionation: I - solvent extraction in a novel reactor". Biotechnology Letters 7/3, 1985, pp. 213-216.

[21]. F. Carrillo; M.J. Lis, et al. "Effect of alkali pretreatment on cellulase hydrolysis of wheat straw: Kinetic study". Process Biochemistry 40(10): 3360-3364. (2005).

[22]. F. Carvalheiro; L.C. Duarte, et al. "Hemicellulose biorefineries: A review on biomass pre- treatments". Journal of Scientific and Industrial Research 67(11): 849-864. (2008).

[23]. Y. Chen; R.R. Sharma-Shivappa, et al. "Potential of agricultural residues and hay for bioethanol production". Applied Biochemistry and Biotechnology 142(3): 276-290. (2007).

[24]. C. Crofcheck; M.D. Montross; A. Berkovich; and R. Andrews. "The effect of temperature on the mild solvent extraction of white and red oak". Biomass and Bioenergy 28/6, pp. 572-578. (2005).

[25]. T. Eggeman; and R.T. Elander. "Process and economic analysis of pretreatment technologies". Bioresource Technology 96(18 SPEC. ISS.): 2019-2025. (2005).

[26]. M.P. Garcia-Aparicio; I. Ballesteros, et al. "Effect of inhibitors released during steam-explosion pretreatment of barley straw on enzymatic hydrolysis". Applied Biochemistry and Biotechnology 129(1-3): 278-288. (2006).

[27]. T.K. Ghose; P.V. Pannir Selvam, et al. "Catalytic solvent delignification of agricultural residues: Organic catalysts". Biotechnology and Bioengineering 25(11): 2577-2590. (1983).

[28]. R.D. Girard; A.R.P. van Heiningen. "Delignification rate of white birch chips during ethanol- water cooking in a stirred batch reactor with rapid liquor displacement": Journal of pulp and paper science

26/1, pp. 1-7. (2003).

[29]. A.R. Gonçalves; D.S. Ruzene. "Influence of pressure in ethanol/water pulping of sugarcane bagasse". Applied Biochemistry and Biotechnology 105-108, pp. 195-204. (2003).

[30]. G. González; J. López-Santín, et al. "Dilute acid hydrolysis of wheat straw hemicellulose at moderate temperature: A simplified kinetic model". Biotechnology and Bioengineering 28(2): 288-293. (1986).

[31]. J.W. Groenestijn; J.H.O. van; R.R. Hazewinkel; Bakker. "Pre-treatment of lignocellulose with biological acid recycling (the Biosulfurol process)". International Sugar Journal 110 (1319). - p. 689 - 692. (2008).

[32]. E.E. Harris; G.J. Beglinger; Hajny and Sherrard EC "Hydrolysis of Wood: Treatment with Sulfuric Acid in a stationary digester" Industrial and Engineering Chemistry, 37(1): 12- 23. (1945).

[33]. I. Hasegawa; K. Tabata; O. Okuma; and K. Mae. "New pretreatment methods combining a hot water treatment and water/acetone extraction for thermo-chemical conversion of biomass". Energy & Fuels 18/3, pp. 755-760. (2004).

[34]. A.T.W.M. Hendriks; and G. Zeeman. "Pretreatments to enhance the digestibility of lignocellulosic biomass". Bioresource Technology 100(1): 10-18. (2008).

[35]. Jesper Norgard, "Ethanol Production from Biomass", Optimization of Simultaneous Saccharification and Fermentation with respect to stirring and heating, 2005, pp 1-5.

[36]. T.H. Kim; and Y.Y. Lee. "Pretreatment of corn stover by soaking in aqueous ammonia". Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology 124(1-3): 1119-1131. (2005).

[37]. E. Kiran; and H. Balkan. "High-pressure extraction and delignifcation of red spruce with binary and ternary mixtures of acetic acid, water, and supercritical carbon dioxide". Journal of Su- percritical Fluids 7, 75. (1994).

[38]. H.B. Klinke; A.B. Thomsen, et al. "Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass". Applied Microbiology and Biotechnology 66(1): 10-26. (2004).

[39]. H.B. Klinke; B.K. Ahring, et al. "Characterization of degradation products from alkaline wet oxidation of wheat straw". Bioresource Technology 82(1): 15-26. (2002).

[40]. O.R. Inderwildi. Energy and Environmental Science 2, 2008, pp. 343.

[41]. P. Obileku. "Production of cellulosic ethanol from wood sawdust" Unpublished Bachelor Project work. Department of Agricultural & Bioresources Engineering, University of Nigeria, Nsukka, Enugu State, Nigeria. 2007.

[42]. X. Pan; N. Gilkes; J.F. Kadla; K. Pye; S. Saka; D. Gregg; K. Ehara; D. Xie; D. Lam; and J.N. Saddler. "Bioconversion of hybrid poplar to ethanol and co-products using an organosolv fractionation process: optimization of process yields". Biotechnology and Bioengineering 94/5, pp. 851-861. (2006).