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Acid Pre-Treatment and Enzymatic Saccharification of Sorghum Stalk for Cellulosic Ethanol Production

By

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Abstract

The aim of this study is to investigate the effect of process variables during dilute acid pre-treatment and enzymatic saccharification of sorghum stalk for cellulosic ethanol production. The sorghum stalk was pre-treated at different reaction temperature (160 – 220 ℃), dilute acid concentration (1 – 3 %v/v), solid/liquid ratio (0.06 – 0.15 g/ml) and time (5 – 15 mins). This was followed by fermentation to production and characterization of ethanol. Optimum conditions for pretreatment were reaction temperature of 210 ℃, acid concentration of 3 % v/v, solid/liquid ratio of 0.1 g/ml and a reaction time of 10 mins. Maximum percentage reducing sugar at this condition was 14 %. The result of ethanol characterization revealed that the ethanol production meets the ASTM standard. Hence, we conclude and recommend that sorghum stalk is a potential feedstock for the production of bioethanol.

Keywords: Bioethanol, delignification, renewable energy, sorghum stalk

INTRODUCTION

Renewable energy is anticipated to be the fastest growing contributor to world energy demand in a couple of decades. The global demand for these sustainable forms of energy is estimated to rise by an average annual compounded growth rate of about 7.3 % between 2007 and 2030[1]. This sustainable form of energy at the present time contributes about 13 % of the total global energy consumption out of which approximately 10 % is derived from biomass[2]. Biomass fuels like bio-diesel, bio-ethanol and bio-hydrogen are typical examples of renewable energy that can be potentially used as possible substitute to petroleum derived fuel. For an alternative fuel to be a feasible replacement of the fossil fuel, the fuel must be environmentally friendly, economically competitive with the fossil fuel and also be produced in sufficient quantity to create a significant influence on energy requirements. It must give a net energy gain greater than the energy sources from which it is derived[3].

Global bioethanol production is reported to be about 31 billion litres in the year 2001 and it is expected to grow to about 39 billion litres by 2007; the volume is however anticipated to grow above than 100 billion litres by the year 2015[4]. Currently, bioethanol is regarded as the most

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[1] Sadorsky, P. (2011). Some future scenarios for renewable energy. *Futures*, 43, 1091–1104

[2] Ho, D. P., Ngo, H. H., Guo, W. (2014). A mini review on renewable sources for biofuel. *Bioresource Technology*, 169, 742–749

[3] Jason, H., Erik N., David T., Stephen, P. and Douglas, T. (2006). Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Journal of Biotechnology*, 103 (30), 11206 - 11210.

[4] Taherzadeh M. J. and Karimi K. (2007). Acid based hydrolysis processes for ethanol from lignocellulose

widely used liquid biofuel alternative. The synthesis and usage of bioethanol have numerous advantages in respect of the environment, energy security and rural economic improvement. Bioethanol contribution to global warming is negligible when compared to fossil derived fuels due to sequestration thus maintaining natural carbon cycle balance[5]. Bioethanol is an alcohol produced via microbial fermentation procedure, in contrast to ethanol produced by synthesis mode from petrochemical sources. It is produced by distillation of the ethanolic mash emerging from fermentation of biomass-extracted sugars, it can be used as a liquid (fluid) fuel in internal combustion engines, either clean or as blends with petroleum-based fuel[6]. Bioethanol has become one of the most promising in the category of renewable energy sources[7]. The production of bioethanol production in the main term (short term) is purely dependent on the use of starchy food crops such as corn in USA and sugarcane in Brazil as feedstock[8]. The choice of this biomaterial for bioethanol production is not economical and these feedstocks are required for a number of food applications. A viable bioethanol project must adequately address seven key national concerns, and these includes renewability, global climate change mitigation, biodegradability, metropolitan air pollution, carbon capture, national energy security, and agricultural farm economy. Lignocellulose biomass is envisaged to provide a significant portion of the raw materials for bio-ethanol production in the medium and long-term due to its low cost and high availability[9].

Cellulosic ethanol production from inexpensive and abundant second-generation feedstock (lignocellulosic biomass) is now being accepted across the world as a viable substitute for traditional petroleum based fuels[5]. The second-generation biofuels produced from lignocellulose biomass have a favourable life cycle, negligible greenhouse gases emission and offer greater socio-economic and environmental benefits[10]. This lignocellulosic biomass is the most available potential feedstock for the production of bioethanol[11]. Lignocellulose materials in the form of forestry, agricultural, and agro-industrial wastes is accumulated in large quantities every year, they are the most abundant biomass produced from photosynthesis and has a yearly supply of 200 billion tonnes[12]. There are about 8 to 20 billion tonnes of lignocellulose biomass wastes; that are potentially available for conversion to valuable end products, although these materials are structurally more complex. Their processing into bioethanol involves collection of biomass, pre-

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Materials: A Review. *Bioresources*, 2(3), 472-499.

[5] Kim T. H. and Kim T. H. (2014). Overview of technical barriers and implementation of cellulosic ethanol in the U. S. *Energy*, 66, 13-19, http://dx.doi.org/10.1016/j.energy.2013.08.008

[6] Graeme, M. Walker (2010). *Bioethanol: Science and Technology of fuel alcohol*. University of Abertay Dundee, Scotland, pp. 8-111

[7] Almodares, A., Hadi, M (2009). Production of Bioethanol from sweet Sorghum- A Review, *African Journal of Agricultural Research*, 4(9), 772 – 780.

[8] Saldivar, S., Christina, C., Ether, P and Erick, H. (2010). Sorghum as a Multifunctional crop for the production of fuel Ethanol: Current status and future trends. www. Intechopen,com

[9] Balat, M and Balat, H (2009). Recent trends in global production and utilization of bio-ethanol fuel, Applied Energy, 86 2273–2282, doi:10.1016/j.apenergy.2009.03.015

[10] Ko Chun-Han, Ya-Nang Wang, Fang-Chih Chang, Jia-Jie Chen , Wen-Hua Chen, Wen-Song Hwange (2012). Potentials of lignocelluloses bioethanols produced from hardwood in Taiwan. *Energy*, 44, 329-334, http://dx.doi.org/10.1016/j.energy.2012.06.026

[11] Canakci, M., Necati, O. A (2011). Performance and combustion characteristics of alcohol- gasoline blends at wide-open throttle. *Energy*, 36, 2747 – 2752.

[12] Heng, K., Pituan, W., P., Day, D., Montogomoery, W., Micheal, M., Aita, G.(2012). Ethanol production potential of sweet sorghum assessed using Forage Fiber analysis procedures. *GCB Bioenergy*, 1, 9.

treatment, hydrolysis of pre-treated biomass and the fermentation of the hydrolysate[13]. The pre-treatment step constitutes the most challenging step, since it has to cope with many factors limiting enzymatic hydrolysis, such as cellulose crystallinity, lignin and hemicellulose barrier and fibre accessibility[12]. Pretreatment process invo1ves de1ignification of the feedstock in order to destroy 1ignin shell protecting ce11u1ose and hemice11u1oses, increase porosity, decrease crysta11inity of ce11u1ose to access substrate (sugar) via a pre-treatment step[14,15]. Irrespective of the feedstock type, biomass biotransformation into bioethanol has been limited by two major factors; the cost and efficiency of hydrolysis.

Sweet sorghum is a crop with high photosynthetic efficiency. It can grow well in most sub-tropical and temperate regions of the world. Sweet sorghum is known to be drought resistant, tolerate water logged soil, has tolerance and resistance to salinity. Hence, the development of sweet sorghum will play an important role in promoting agricultural production, livestock husbandry and bioenergy[7]. Numerous features make sweet sorghum suitable raw material for bioenergy production and these include : 1) short growth life cycle of about four months which permits double cropping; 2) easy seed propagation; 3) potential for full mechanization; 4) both stem sugar and grain starch can be harnessed from it; 5) efficient water and nutrient usage; 6) utilization of resulting byproduct (bagasse and forage) for energy generation; 7) widespread adaptability to different environments[16]. Sorghum can be harvested in one single season thereby yielding a better return than sugarcane on a unit land area basis[16]. Sorghum has high sugar content, rapid growth, and high carbohydrate content among others[8]. Carbohydrates available in sweet sorghum can be either nonstructural (sugars and starch) or structural (hemicellulose, cellulose and pectic substances). Monosaccharide's glucose and fructose are main sugars present in the biomass while the disaccharides group primarily includes sucrose, maltose and trisaccharide raffinose. Depending upon stalk sugar type, sorghum is further classified as saccharin- type sweet sorghum and syrup-type sweet sorghum. The sucrose containing Saccharin-type can be used for refining crystal sugar while the Syrup- type sweet sorghum, made up of glucose are used for producing syrup. The sweet sorghum stalk juice sugars content typically includes sucrose and invert sugars (glucose, fructose, maltose and xylose). The presence of high amount and ease of conversion of carbohydrates (sucrose and invert sugar) in sorghum stalk has made it a potential feedstock for bioethanol production. Massoud and Abd El-Razek[17] studied the suitability of Sorghum bicolor L. stalks and grains obtained from Egypt for bio ethanol production[18]. Shen *et al.* reported the production of bioethanol from Chinese sweet

[13] Lin, Y and Tanaka, S. (2006). Ethanol fermentation from biomass resources: Current state and prospects. *APPI Microbiol Biotechnology*, 69, 627 – 642.

[14] Cheng, J (2002). Hydrolysis of lignocellulosic materials for ethanol production: A Review. *Bioresource Technology*, 83, 1 – 11.

[15]

[16] Rutto, L.K., Xu, Y., Brandt, M., Ren, S., Kering, M. K. (2013). Juice, Ethanol, and Grain Yield Potential of Five Sweet Sorghum (Sorghum bicolor [L.] Moench) Cultivars. *Journal of Sustainable Bioenergy Systems*, 3,113-118, http://dx.doi.org/10.4236/jsbs.2013.32016

[17] Shen, F., Yongmei Zeng, Y., Deng, S. and Liu, R. (2011). Bioethanol production from sweet sorghum stalk juice with immobilized yeast. *Procedia Environmental Sciences*, 11 (2011) 782 – 789.

[18] Massoud, M. and Abd El-Razek, A. M (2011). Suitability of Sorghum bicolor L. stalks and grains for bioproduction of ethanol. Annals of Agricultural Science, 56(2), 83–87.

sorghum stalk juice with immobilized yeast. Yu *et al.* [19] investigated the optimization and synthesis of Bioethanol from NaOH-Pretreated Solid State Fermented China Sweet Sorghum Bagasse using engineered strain of Z. *mobilis*. In another related study Jin *et al.* [20] reported the Kinetics of Batch Fermentations for Ethanol Production using Immobilized Saccharomyces cerevisiae growing on Sweet Sorghum Stalk Juice.

The major challenge however with the wide spread commercial bioethanol production from sweet sorghum on industrial scale is the efficiency of conversion of the fermented sweet sorghum bagasse into bioethanol. The production of bio ethanol production from sweet sorghum bagasse basically involves pretreatment of the feedstock, acid or enzymatic hydrolysis, fermentation and ethanol distillation. Pretreatment step contribute to 30 – 40 % of total cost of production[19]. Acid hydrolysis is one of the most potentially viable pretreatment techniques for large scale production of bioethanol. It is often carried out with the aid of mineral acids and organic acids. Dilute sulfuric acid pretreatment has been used for appreciable number of lignocellulosic biomass in spite of its drawback which include high cost of reactor construction materials, the formation of gypsum and inhibitory by-products. This process compared to other methods is associated with high hemicellulosic sugars recovery from the pretreatment liquid and enhanced enzymatic convertibility of solid cellulose fraction into bioethanol[21]. The sugar content of sweet sorghum juice varies from one variety to another thereby resulting into varying yield and concentration of bioethanol[22]. Hence the need to attempt the documentation of the potential of a typical Nigeria Sorghum stalks for bioethanol. This research attempts to investigate the dilute acid pre-treatment, enzymatic saccharification of a typical Nigerian sorghum stalk for bioethanol production using *aspergillus Niger* and *Saccharomyces cerevisiae.*

2. MATERIALS AND METHOD

Materials

Sorghum stalks were obtained from a sorghum farm in Gidan-Kwano, Minna, Niger state, and sun-dried. The dried sorghum stalks were shredded, grinded and sieved to obtain uniform sized biomass. *Aspergilius Niger* and *Saccharomyces Cerevisiae* microorganisms were cultured in the microbiology laboratory, Federal University of Technology, Minna, Niger state.

2.3 Pretreatment and Optimization of Pretreatment Parameters

The sorghum stalk sample was pre-treated with 1.0-3.0 v/v% H_2SO_4 and 5-15 %w/v solid liquid ratio. The acid treated sample was placed in an oven set at 160 - 220 ^oC for 6-14 min reaction time. After heating, the samples were neutralized with 1.0 M NaOH before filtration to separate

[19] Yu, M., Li, J., Chang, S., Du, R., Li, S., Zhang, L., Fan, G., Yan, Z., Cui, T., Cong, G., and Zhao, G (2014). Optimization of Ethanol Production from NaOH-Pretreated Solid State Fermented Sweet Sorghum Bagasse. *Energies*, 7, 4054-4067, doi:10.3390/en7074054

[20] Jin, H., Liu, R and He, Y. (2012). Kinetics of Batch Fermentations for Ethanol Production with Immobilized *Saccharomyces cerevisiae* Growing on Sweet Sorghum Stalk Juice. Procedia Environmental Sciences, 12, 137 – 145, doi: 10.1016/j.proenv.2012.01.258

[21] Jönsson, L.J., Martín, C. (2015). Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresour. Technol.*, http://dx.doi.org/10.1016/j.biortech.2015.10.009

[22] Almodares A, Sepahi A, Dalilitajary H, Gavami R (1994). Effect of phenological stages on biomass and carbohydrate contents of sweet sorghum cultivars. Ann. Plant Physiol. 8: 42-48.

liquid from residual solid particles. The liquid fraction (filtrate) was analysed using refractometer for determination of the percentage of the reducing sugar present. The substrate was stored in a desiccator before subsequent enzymatic saccharification and fermentation. The effect of pretreatment parameters on the ethanol yield was studied by varying one parameter while others are kept constant. Solid/liquid ratio was varied as: 5, 8, 10, 12 and 15 g/L, pre-treatment temperature as: 160, 180, 200, 210, 220 ^{o}C, acid concentration: 1.0, 1.5, 2.0, 2.5, 3.0 v/v % and reaction time as: 6, 8, 10, 12 and 14 min.

2.4 Enzymatic Hydrolysis/Saccharification and Fermentation

An enzyme (*Aspergillius niger*) to pre-treated sample ratio of 20 FPU/g was used for hydrolysis. The *Aspergillius niger* treated samples pH was adjusted to pH 5 using a buffer (sodium citrate) solution, placed in a water bath set at 50 ^{o}C, 150 rpm for 48 h. After hydrolysis, the sample was withdrawn for glucose and other monomeric sugar concentration determination using refractometer. The hydrated sample was fermented by inoculation with baker's yeast (*saccharomyces cerevisae*) and placed in a water bath for 72 h. Thereafter, the sample was distilled, and the ethanol was characterized.

2.5 Characterization of Bioethanol

The bioethanol produced was characterized for some of its fuel properties according to the method reported by Ademiluyi[23].

3 RESULTS AND DISCUSSION

3.1 Effect of Temperature

Figure 1 shows the data of percentage reducing sugar with increasing pre-treatment reaction temperature from 160 – 210 ^{0}C. As the temperature increased from 160 – 175 ^{0}C, the temperature increase did not show any significant effect on the sugar yield. Further increase in temperature beyond 175 ^{0}C resulted into a gradual increase in total sugar yield. This result is in agreement with the work of Dogaris *et al*[24]on sorghum bagasse and Saha [25] reported that increasing temperature during dilute acid pre-treatment of sorghum bagasse led to significant increase in xylan hydrolysis. The highest percentage reducing sugar obtained was 13.5 % at a pre-treatment temperature of 210 ^{0}C for solid/liquid ratio of 0.1 g/ml, acid concentration of 2 v/v and a pre-treatment time period of 10 min. At temperatures higher than 210 ^{0}C the percentage reducing sugar remained constant. The result also showed that sorghum stalk delignification had been accomplished providing easier access for cellulose and hemicellulose thereby yielding higher percentage of reducing sugar at this high pre-treatment temperature. This observation is in agreement with the report of literature which stated that high pre-treatment temperature of lignocellulose material enhances cellulose digestibility and increases the porosity of the lignocellulose material[13,14].

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[23] Ademiluyi, F. T. (2013). Yield and properties of ethanol biofuel from different whole cassava flours. Biotechnol., 2, 1-5.

[24] Dogaris, I., Olga, G., Mamma, D., Kekos, D (2012). Bioconversion of dilute-acid pre-treated sorghum bagasse to ethanol by *Neurospora crassa*, *Applied Microbiology and Biotechnology*, Vol. 1, 18.

[25] Saha, B. C. (2005). Hemicellulose bioconversion. Journal of Industrial Microbiology & Biotechnology, 30, 279 – 291.

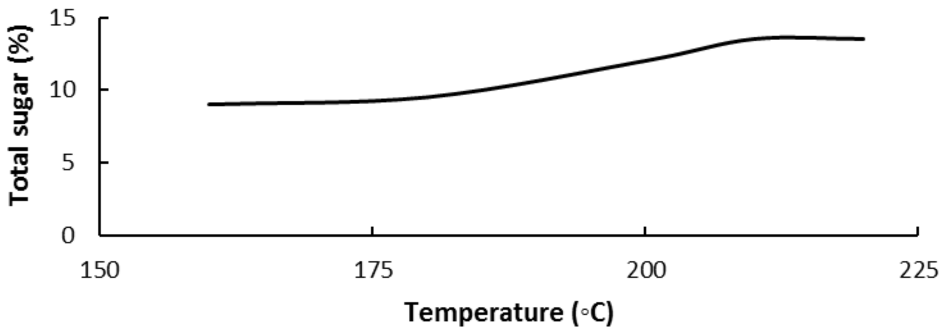

Figure 1: Percentage reducing sugar against Pre-treatment temperature

3.2 Effect of Time

Pre-treatment reaction time is an important process parameter when pre-treating lignocellulose material with dilute acid[26]. The result of the effect of reaction time on percentage of reducing sugar is presented in Figure 2.

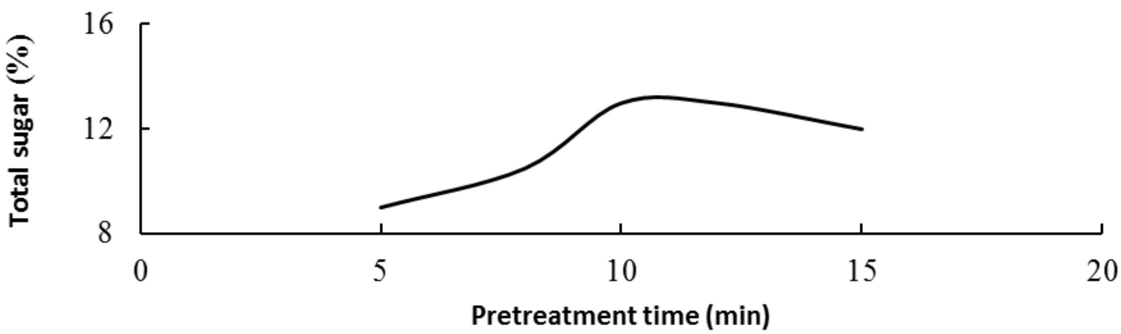

Figure 2: Percentage Reducing Sugar against Pre-treatment Reaction Time

The results revealed that increasing pre-treatment reaction time led to increase in the percentage reducing sugar on till a pretreatment time of 10 mins was attained and thereafter, the percentage sugar began to decrease. This decrease in percentage reducing sugar is in agreement with the study reported by Saha[25]. However, at the pretreatment time of 10 min, the highest percentage reducing sugar was 13.0 % for a solid/liquid ratio of 0.1 g/ml, acid concentration of 1.8 v/v and for a pre-treatment reaction temperature of 210 ^{0}C. The maximum percentage reducing sugar obtained at the pre-treatment time of 10 min corresponds to 19.2 % increase when compared to the reducing sugar percentage at the pre-treatment time of 8 min.

[26] Gomez, S., Rafael, A., Santander, C., Costa, A., Maciel, F (2011). Pretreatment of sugar cane baggase with phosphoric and sulfuric diluted acid for fermentable sugars production by enzymatic hydrolysis. School of Chemical Engineering, Thesis, TUNICAMP, 1- 6.

3.3 Effect of Solid/Liquid Ratio

The effect of Solid to liquid ratio on ethanol yield was studied at different ratios. The result in Figure 3 shows that an increase in the solid (sorghum stalk) to liquid (dilute acid) ratio from 0.06 to 0.1 g/ml resulted into an increase in the reducing sugar yield and a Total sugar increase from 11 to 13 %. However, beyond 0.1 g/ml, a decrease in the total sugar was observed. This decrease may be attributed to insufficient volume of acid to facilitate the reducing sugar release.

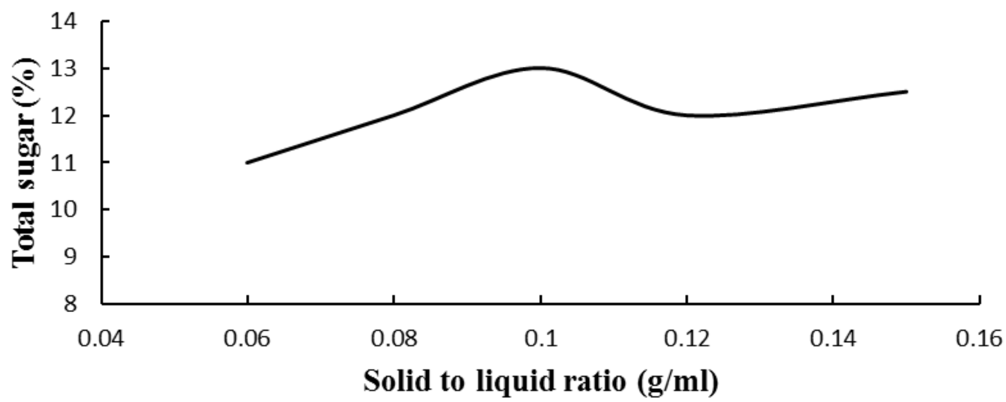

Figure 3: Percentage Reducing Sugar against Solid Liquid Ratio

The results of this study show quantitative agreement with work of Ko *et al.*[10]. The maximum reducing sugar of 13 % was obtained at solid liquid ratio of 0.1 g/ml which represents 7.7 % increase when compared with the sugar released at 0.8 g/ml solid/liquid ratio. This proportional increase in reducing sugar released with the corresponding solid liquid ratio increase is attributed to increase in the concentration gradient between the solid and liquid phase[24].

3.4 Effect of Acid Concentration

Concentration of H_2SO_4 used for pre-treatment was varied from 1 to 3 v/v% in order to determine its effect on the release of reducing sugar and the results are presented in Figure 4.

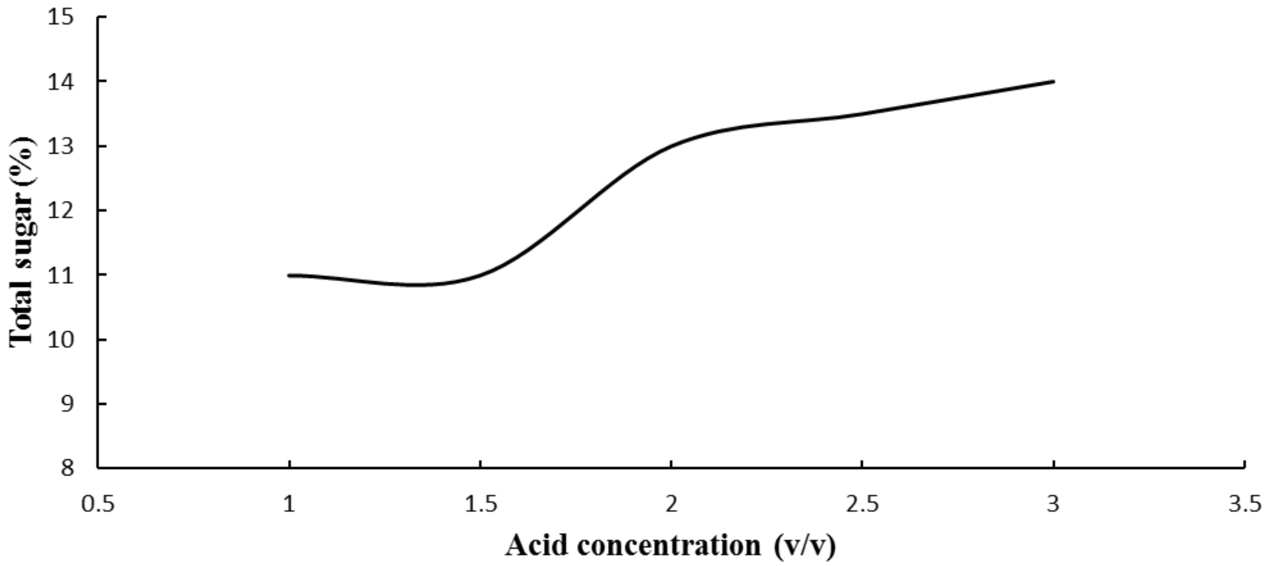

Figure 4: Percentage Reducing Sugar against Acid Concentration

The results showed that there was an insignificant increase in the concentration of sugar as the concentration of acid increased from 1.0 - 1.5 v/v%. This simply implies that these relatively low concentrations were not adequate to excite the solute molecules. However, beyond 1.5 v/v% a gradual increase in total sugar yield was observed. The highest percentage (14 %) of reducing sugar was obtained at an acid concentration of 3 v/v %, pre-treatment reaction temperature of 210 ^{o}C and reaction time of 10 min. These results have shown acid concentration is an important process parameter when pre-treating lignocellulose material with dilute acid[26].

3.5 Characterization of the Bioethanol

Table 1: Characterization of Bioethanol Produced

Property	**ASTM Standard**	**This work**	**Bugaje and Moh'd[27]**	**Abdulkareem *et al.*[28]**
Boiling point (^{o}C)	78.5	79	-	-
Density (g/cm^3)	0.99	0.79	0.79	0.987
Specific Gravity	0.87	0.79	-	0.922
Viscosity (cP)	1.2	1.2	1.22	1.34
Refractive Index	1.36	1.3	-	1.356
Flash Point (^{o}C)	18.6	17	21.00	19.2

[27] Bugaje, I. M. and Mohammed, I.A (2008). Biofuel Production Technology. Science and Technology Forum (STF), Zaria, Nigeria, 35-78.
[28] Abdulkareem, A S., Afolabi, A.S and Ogochukwu, M.U (2015). Production and Characterization of Bioethanol from Sugarcane Bagasse as Alternative Energy Sources. Proceedings of the World Congress on Engineering (WCE) Vol II, July 1 – 3, London, UK.

Bioethanol produced was characterized and the result shown in Table 1.
The result of the characterization for viscosity and refractive index shows an agreement with the ASTM Standard and the reported literature.

The difference in the value might be due to difference in the feedstock. The boiling point was obtained as 79 ^{0}C, which was quantitatively in agreement with the Standard and other reported literature. The slight difference might be due to presence of impurities which in this case might be water. The density and specific gravity differ from the value of 0.99 and 0.87 while flash point shows close proximity to the Standard and related literature.

4. Conclusion

Sorghum stalk is a promising feedstock that can be employed to produce substantial amount of bioethanol by dilute acid pretreatment and saccharification using *saccharomyces cerevisae*. In this study, the optimum process condition for dilute acid pre-treatment were determined to be a pre-treatment reaction time of 10 mins, acid concentration of 3 v/v, solid/liquid ratio of 0.1 g/ml and a reaction temperature of 210 ^{o}C. The maximum percentage reducing sugar obtained was 14 %. The characterization of ethanol produced showed similarities with ASTM Standard for ethanol, hence suggesting that the bioethanol produced can serve as an alternate fuel to petroleum-based gasoline.