

# Bioethanol from Waste Lignocelluloses Waste Papers through Active Acidic Hydrolysis

Ashade Noah.O<sup>1</sup>, Okibe Ora. P<sup>2</sup>, Opeyemi Adegboyo.O<sup>3</sup>, Olabimtan Olabode.H\*<sup>4</sup>

<sup>1</sup>Research and Development, Outstation Coordination Department, National Research Institute for Chemical Technology Zaria Kaduna State, Nigeria

<sup>2,4</sup>Department of Industrial and Environmental Pollution, National Research Institute for Chemical Technology, Zaria, Kaduna State, Nigeria.

<sup>3</sup>Department of Science Laboratory Technology, Federal Polytechnic ilaro Ogun State, Nigeria.

**Corresponding author email\*: [Olabode4angel@gmail.com](mailto:Olabode4angel@gmail.com)**

**Abstract:** *The environmental disadvantages of fossil-based fuels have necessitated some serious demands for safer and sustainable energy alternatives. Bioethanol was identified and recognized as a promising option with a sustainable choice of biofuel that was chiefly from first-generation feedstocks that threaten food security while Second generation bioethanol is produced from lignocellulosic biomass, one of the abundant renewable bio resources on earth with the adoption of active acid pretreatment to isolate the components of cellulose from hemicellulose and lignin for fermentation with the activity of enzymatic fermentation. The Paper industry has the biggest income of biomass for non-food-chain production, and, simultaneously generates a high amount of residues specifically as waste papers that are quite rich in mono- and poly-saccharides. Technically, they were utilized as a proper feedstock for second-generation bioethanol production. 100g of shredded cellulosic enzyme pretreated (2.55g initial sugar contents), acidic hydrolyzed, and *Saccharomyces cerevisiae* fermented (72hours) waste paper samples were actively converted into 58.50% molecular sieve dehydrated ethanol. Classical characterizations (ceric ammonium nitrate & combustion tests) with GCMS –FID conditions were employed in the qualitative analysis of the generated bioethanol. This innovation reinforced the point that bio refineries must be interconnected with the technology that will be productive in the production of ethanol processing with the existing paper manufacturing plants. This will lead to added ecological advantages with product diversification and improved productivity for the pulp and paper industries.*

**Keywords:** bioethanol, waste lignocellulosic papers, acidic hydrolysis, cellulose enzyme and fermentation.

## 1.0 INTRODUCTION

The world relies on non - renewable fossil fuels for power generation and transportation as fossil resources are essentially the primary sources of energy, offering approximately 78.4 percent of the total energy supply [1]. With the increasing energy demands and effects with the use of fossil fuels on human health and the climate, there is an urgent need for substitutes, as the sustaining rate of fossil fuels will not equal the rate of present consumption [1]. Biofuels are potential renewable energy resource to substitute fossil fuels, in particular, because of the much fewer emissions of greenhouse gasses (GHGs) and that they are developed from diverse sources of biomass that are geographically more distributed equally than renewable resources that encourage an independent and secure supply of energy [1]. Among these factors, the scientific community is growing interest in biofuels as global energy demand is rising where energy deficits would be a global problem. Therefore, bioethanol is regarded as a major renewable fuel that partially can substitute fossil-based fuels as world bioethanol production rose through 50 million m<sup>3</sup> in 2007 to almost 100million m<sup>3</sup>. In 2012, the United States with Brazil accounted for about 80 % of the world's supply, mostly with sugar cane and corn [2]. In emerging economies, feedstuffs associated with food are ideally substituted by non - edible

feedstock like sorghum and cassava. Through the adoption of widely accepted biomass, the production of bioethanol can significantly increase as bioethanol based on lignocellulose is presently receiving attention. Industrially, ethanol is produced with the acid-catalyzed process of hydration on ethylene, while for applications in alcoholic drinks, and as biofuel, direct fermentation with specific yeast species, such as *Saccharomyces cerevisiae* or bacteria such as *Zymomonas mobilis*, activates sugars under de-oxygenated situations to yield ethanol and carbon dioxide as by-products. The key factors for bioethanol's development are its use as a beneficial close to zero-carbon alternative energy, thereby reducing Greenhouse gas emissions and related global warming; its application as an octane enhancer with unleaded gasoline; and as an oxygenated fuel mixture for cleaner oxidation, thereby controlling the pollutant emissions from pipes with the ambient air quality remediation. Ethanol's octane numbers as the number of motor octane (MON) and the number of the research octane (RON) are 90 and 109, respectively, on average 99 compared to 91 for the regular gasoline [3]. Ethanol is not efficiently burned by compression ignition due to its low cetane number and is also not easily miscible with diesel fuel. Measures can be taken to optimize the use of ethanol in engine compression ignition systems, such as the

introduction of an emulsifying agent to enhance the solubility of ethanol-diesel; the introduction of ethylhexyl nitrate or diterbutyl peroxide to increase the level of cetanes; the adoption of a combined fuel system in which ethanol and diesel are loaded independently into the cylinder; or the modification for the auto-ignition engines [3] The fermentation of raw materials based on sugar is called "first generation" bioethanol, while the use of raw materials for lignocellulose is typically known as "the second generation." The algal bioethanol "third-generation" is now at an early stage of inquiry. Compared to the first generation, bioethanol of the second generation can be derived from solid biomass, such as woods, chemical, or environmental waste [1]. These materials characteristically do not generate food sustainability challenges and do not require substantial land areas. Such feedstock include lignocellulosic biomass like agricultural residues, crops, and forest materials as woody materials [4]. The world paper industry is among the world's largest industries, processing around 400 million tons of paper products, with 188 million tons of natural pulp in 2015[1]. It is the world's largest consumer of woody biomass as it subsequently provides the facilities that generate a significant quantity of waste. Industrial plants develop solutions that can fractionate and convert biomass [4]. Therefore advanced bio refineries for the production of bioethanol from waste and by-products could be incorporated in modern pulp and paper mills. This is an effective option for waste management with by-product diversification which enhances paper industry profitability. Also, since one of the key constraints with the development of second-generation cellulosic ethanol is the high capital and operating cost, the process's economics can be strengthened by using the existing pulp and paper industry equipment [4]. Low-quality Kraft pulp and pulp and paper sludge are among the numerous waste materials from the pulp and paper industry that can be used for bioethanol processing [4] [14]. Overall, Lignocellulosic Biomass is composed of cellulose (30–60%), hemicellulose (20–40%), and lignin (15%–25%), and limited portions of extractives and ashes [1]. Cellulose and hemicelluloses are polymers that can be digested into fermentable simple sugars and processed into bioethanol. Lignin is not used in the manufacture of bioethanol but an origin of aromatic high value-added products [1]. Cellulose is a continuous, repeating unit homopolysaccharide consisting of D-glucose subunits connected by  $\beta$ -(1, 4)-glycosidic chains. The fibers are bound by hydrogen bonds intra-and inter-molecular bonds that lead to a highly complex crystalline form. Hemicelluloses are strongly transitioned and shorter heteropolysaccharides consisting of various monomers like hexoses, uronic acids, and pentoses. Also by various polysaccharides, such as glucomannan, galactoglucomannan, and xylan. Lignin is an amorphous, strongly hydrophobic, polymer matrix which is non-polysaccharide. It is a polyphenol of undefined molecular structure and is

composed primarily of p-hydroxyphenyl, guaiacyl, and syringyl structural units connected by etheric bonds and also of carbon-carbon bonds in between structural units, making lignin a complex unstable structure [5]. Cellulose, hemicelluloses, and lignin are joined by noncovalent interactions and covalent cross-linkages, integrating directly in a complex system [4]. Broadly speaking, the processing of Lignocellulosic materials to bioethanol typically begins with an initial step of feedstock pretreatment, which entails cleaning, size reduction by milling, grinding or chopping, with a significant amount of energy inputs[1]. This is then followed by four critical steps that degrade lignocellulosic framework to obtain fermentable sugars that convert sugars into ethanol, and conservation and dehydration to separate and purify the ethanol generated.

## **2.0 MATERIALS AND METHODS**

Waste papers, blender, distilled water, water bath, test tubes, syringe, cellulose enzyme, ceric ammonium nitrate, bunsen burner, round-bottom flask, distillate-capturing flask, fractionating column, Liebig condenser, thermometer, oven., 0.4M H<sub>2</sub>SO<sub>4</sub>, saccharomyces cerevisiae, 0.01M Ca(OH)<sub>2</sub>

### **2.1 Physical pretreatment**

To enhance the reactivity of lignocellulosic biomass, various size-reduction procedures are employed. Shredded and pre-weighed (100g) waste papers were blended with a small amount of distilled water Shredded and pre-weighed waste papers were blended with a small amount of distilled water [6]. This method of pretreatment is expected to decrease the stability of cellulose, increase the total surface, and increase the amount of ethanol after delignification [7].

### **2.2 Culturing saccharomyces cerevisiae:**

Saccharomyces cerevisiae is applied to ferment sugar and must be cultured 48 hours before the experiment. Exactly 10 g of potato dextrose agar is fully dissolved in a conical flask in 250ml of water. The solution is protected with cotton wool and foil paper and was sterilized for 5 minutes in an autoclave at 121<sup>0</sup> C. It is allowed to cool on removal and then poured into Petri dishes, and is set aside and allowed to solidify. With the help of a sterilized inoculating loop, the Saccharomyces cerevisiae is then inserted into the Petri dishes. The Petri dishes are then sealed and held at a temperature of 25<sup>0</sup> C in an incubator for 48 hours [10]

### **2.3 Hydrolysis of the cellulosic paper biomass**

To 100ml of 0.4 M H<sub>2</sub>SO<sub>4</sub>, 100 g of waste paper was added. The mixture's pH was 3.4, as 0.01 M Ca (OH)<sub>2</sub> was applied in drops until a pH of 4.61. The mixture was put in an autoclave and was subjected to a temperature of 120<sup>0</sup>C for 10minutes. The sugar content was tested to be 24.7g. After which the 2.5g of cellulose was added and Saccharomyces cerevisiae was added. [11]

**2.4 Fermentation and distillation of ethanol**

The mixture was held at 150rpm for 72 hours in a shaker incubator at 30<sup>0</sup> C to allow it to ferment fully. The mixture was checked for sugar content on a four hourly basis to determine fermentation rate with time and time required. In a distillation bath, the sample (100ml) was distilled and 6.3 ml of ethanol was obtained at 78<sup>0</sup>C [11]

**2.5 Dehydration of ethanol**

Ethanol and water form an azeotrope that limits the quantity of water that conventional distillation can extract. The vogelbusch molecular sieve method enables dehydration of ethanol further than purity of 95 percent [13].

**2.6 Combustion test**

5ml of ethanol to a large test tube, add a boiling pin, keep with a tube holder, and heat up until the liquid boils. Hold the open end of the test tube to the flame, and ignite the

**3.0 RESULTS AND DISCUSSION**

ethanol vapors. Ethanol burns with a light blue flame, without smoke [7].

**2.7 Ceric ammonium nitrate test**

1ml of ceric ammonium nitrate reagent was added to 5 drops of ethanol and thoroughly blend. The yellow color of the reaction changes to a red [8].

**2.8 GCMS analysis**

This was conducted according to Nicholas B.Tiscione et al (2011). [9]

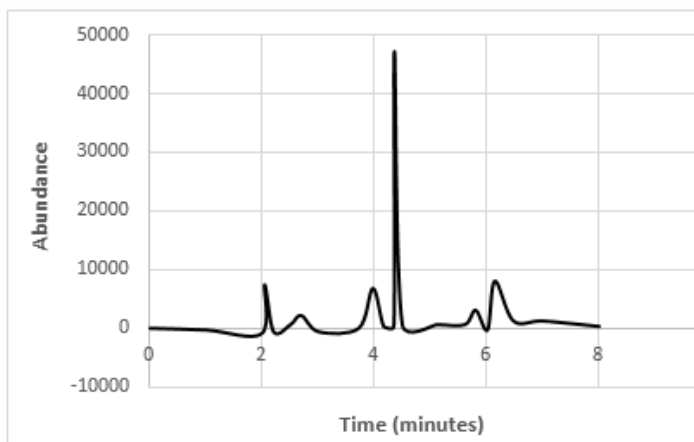
**2.9 Brix determination**

The sugar content of the fermentable mixture was conducted accordingly [12]

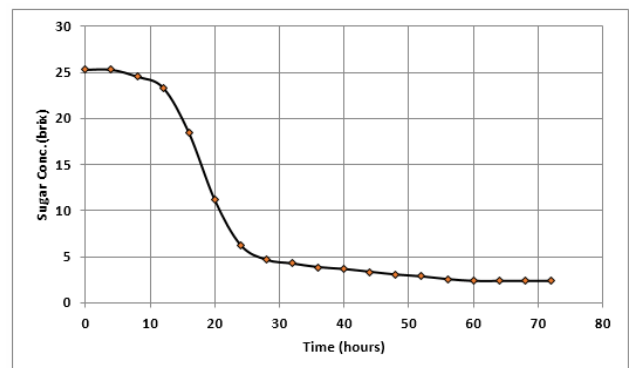
**Table 2. Sugar concentration determinations of the hydrolyzed And fermentable paper solution mixture**

Table 1. Classical Alcohol Tests	
Test	Remark
Cerric Ammonium Nitrate	Positive
Combustion	Positive

Time (hr.)	Sugar Conc.(Brix)
0	25.30
4	25.30
8	24.55
12	23.35
16	18.45
20	11.15
24	6.25
28	4.65
32	4.25
36	3.85
40	3.65
44	3.35
48	3.05
52	2.85
56	2.55
60	2.35
64	2.35
68	2.35
72	2.35



**Figure 1. GCMS of dehydrated ethanol**



**Figure 2. Plot of sugar concentration (brix) against the time periods of fermentation**

Basic classical tests for ethanol (alcohol) with ceric ammonium and combustion (flame) were positive. The GCMS profile with the appearance of a sharp peak with the retention time of 4.37 minutes declares the specific identity of the dehydrated and extracted ethanol (Figure 1). The sugar concentration plot against the respective fermentation periods (72hours) with the interval of 4hours was well defined (Figure 2 and Table 2). As observed, the completion of the fermentation process was attained after 52 hours with the maximum conversion of 2.55g sugar content into ethanol with 58.50 % recovery.

**4.0 CONFLICT OF INTEREST: No**

**5.0 CONCLUSION**

The cellulosic bioethanol production process involves specific processing steps, especially in the pretreatment and hydrolysis. As various pretreatment methods have been developed for the delignification of lignocellulose biomass to obtain ethanol. However, the physical size reduction method which is economically practicable and environmentally friendly for the complete biomass delignification was adopted for this work. The pulp and paper industry certainly has one of the biggest incomes of biomass for non-food-chain production, and, at the same time, generates a high amount of residues. Waste residues from this industry that are rich in monosaccharide and polysaccharides, are direct feedstock for ethanol production with the high level of technological infrastructures that are capable of fractionating the woody biomass. In other words, the production of bioethanol must be integrated into all of the industrial paper mill plants, with other processes that could lead to continuous bio refinery implementation. Exploiting the technologies and equipment that are already present in the pulp and paper mills to produce ethanol and converting these mills into integrated bio refineries seems promising to valorize the wastes generated and also to diversify the products and to increase the profitability and acceptability of pulp and paper industry. Decreasing investment cost and increasing the opportunity of success of large-scale second-generation bioethanol production are other potential advantages of this process integration.

**6.0 REFERENCE**

1. Rita H. R. Branco, et al (2018). Second Generation Bioethanol Production: On the Use of Pulp and Paper Industry Wastes as Feedstock. Mdpi.com. <https://www.mdpi.com/2311-5637/5/1/4/pdf>.
2. Kumar, S. et al (2018). De-construction of major Indian cereal crop residues through chemical pretreatment for improved biogas production: An overview. *Renewable and Sustainable Energy Reviews*, 90, 160–170. doi:10.1016/j.rser.2018.03.049
3. Kang, Q et al (2014). Bioethanol from Lignocellulosic Biomass: Current Findings Determine Research Priorities. *The Scientific World Journal*, 2014, 1–13. doi:10.1155/2014/298153
4. Branco, R. et al (2018). Second Generation Bioethanol Production: On the Use of Pulp and Paper Industry Wastes as Feedstock. *Fermentation*, 5(1), 4. doi:10.3390/fermentation5010004
5. Silveira, M. H. L. et al. (2015). Current Pretreatment Technologies for the Development of Cellulosic Ethanol and Bio refineries. *ChemSusChem*, 8(20), 3366–3390. doi:10.1002/cssc.201500282
6. Chakraborty et al (2019). Valorization of paper and pulp waste: Opportunities and prospects of bio refinery. *Industrial and Municipal Sludge*, 623–656. doi:10.1016/b978-0-12-815907-1.00027-1
7. Karimi, K., & Taherzadeh, M. J. (2016). A critical review on analysis in pretreatment of lignocelluloses: Degree of polymerization, adsorption/desorption, and accessibility. *Bio resource Technology*, 203, 348–356. doi:10.1016/j.biortech.2015.12.035
8. Ahpa.org. (2017). <http://www.ahpa.org/DesktopModules/EasyDNNews/DocumentDownload.ashx?portalid=0&moduleid=1244&articleid=875&documentid=369>.
9. Nicholas B. Tiscione et al (2011). Ethanol Analysis by Headspace Gas Chromatography with Simultaneous Flame-Ionization and Mass Spectrometry Detection. *Journal of Analytical Toxicology*, Vol. 35, September 2011.
10. Dymond, J. S. (2013). *Saccharomyces Cerevisiae Growth Media. Laboratory Methods in Enzymology: Cell, Lipid and Carbohydrate*, 191–204. doi:10.1016/b978-0-12-420067-8.00012-x
11. Kingsley Otulugbu (2012). Production of Ethanol from Cellulose (sawdust) <https://www.theseus.fi/bitstream/handle/10024/42578/Otulugbu.pdf?sequence=1>.

12. Magwaza, L. S., & Opara, U. L. (2015). Analytical methods for determination of sugars and sweetness of horticultural products—A review. *Scientia Horticulture*, 184, 179–192. doi:10.1016/j.scienta.2015.01.001
13. PSA TSA Ethanol Dehydration Process Molecular Sieve -Pingxiang Gophin Chemical

Co., Ltd. Gophinchem.com. (2020). <https://www.gophinchem.com/article/ethanol-dehdryation-molecular-sieve-i00006i1.html>.