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Bio-Ethanol Yield from Selected Lignocellulosic Wastes

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Abstract: Developing nations are experiencing energy deficit because of overdependence on fossil-based fuels. Countries such as Nigeria have abundant raw materials for biofuels, yet these have not been explored. This study was designed to evaluate the bioethanol production potentials of lignocellulosic-based wastes. The mean glucose yield and TRS obtained from the 13.1M H_2SO_4 were significantly higher than those of 9.4M and 5.6M H_2SO_4 hydrolysis. The mean glucose yield and TRS obtained from the 13.1M H_2SO_4 hydrolysis were: CP (85.1±5.7, 209.8±3.7mg/kg), YP (269.2±11.2, 541.3±7.8 mg/kg), PP (304.0±6.1, 461.2±3.6 mg/kg) and SD (343.2±4.8, 535.9±5.0 mg/kg). The 13.1M hydrolysate was used for the ethanol production and the maximum production was obtained at 48hours of fermentation, the mean ethanol yield being: CP - 160.0±15.1 mL/kg, YP -211.7±15.3 mL/kg, PP - 265.0±20.5 mL/kg and SD - 280.0±11.5 mL/kg. A linear relationship exists between the ethanol yield and fermentation time ($R^2 = 0.711$). Sawdust produced the highest glucose and ethanol yield among the substrates; hence ethanol production from sawdust should be explored and optimized.

Keywords: Bioethanol Production, Glucose Yield, Lignocellulosic Wastes, *Saccharomyces Cerevisiae*, Total Reducing Sugars (TRS)

1. Introduction

The world's energy supply is mainly dependent on non-renewable, crude oil-derived (fossil) liquid fuels, of which almost 90% are employed for energy generation and transportation. The problem of rapidly increasing population has caused many developing countries to expand their Industrial base, resulting in increased energy demands (1). It is inevitable that fossil fuels such as oil, coal and natural gas will be exhausted with time. Hence, there is need to explore the possibilities of using alternative energy source, which are as efficient as oil; ethanol fermentation is one such option (2).

Many industrialized countries are pursuing the development of expanded or new biofuels industries for the transport sector, and there is growing interest in many developing countries for similarly "modernizing" the use of biomass in their countries and developing greater access to clean liquid fuels while helping to address energy costs, energy security and global warming concerns associated with fossil fuels (3). Biofuels are considered as a replacement for fossil fuels and the answer to poverty and even the climate crisis. They are presented as being both renewable and environment friendly (4). Increasing attention is being focused on the production of biofuels as the alternatives that will contribute to global reduction in greenhouse gas emissions (5).

In Nigeria, the use of biofuels is anticipated to make significant impact on petroleum products quality enhancement in view of the current limitations of the fossil-based fuels which have not kept pace with the increasing demand for environmentally friendly fuel. Furthermore Nigeria recently adopted an ethanol production policy with cassava as its main feedstock, in response to the global initiative (bio-fuel production), which promises a harmonious correlation with sustainable development, efficient and energy conservation. Although fuel ethanol is currently produced from sugarcane and other starch rich grains, ethanol also can be made from cellulosic materials such as wood, grass and agro-residue (6). This would in turn reduce the pressure on food security due to excessive use of food crops for bio-fuel produce and reduce dependence on imported petroleum for vehicle, ensure environmental sustainability, sound public health and create wealth and opportunities.

Ethanol production from cellulose biomass material instead of traditional feedback is known as bio-ethanol: a carbon neutral compound. Bio-ethanol is a fuel derived

from renewable resources like locally grown crops and even waste product/waste paper or grass and tree trimmings etc (6). These materials contain lignocelluloses which has cellulose, hemicelluloses and lignin in its compound. The lignocellulosic structure is more resistant to decay by organism and it is not perishable like soluble sugar and starch. The complex substance may be broken down into sugars by either acid treatment at various temperatures or by enzymatic treatment (7).

Alcohol fermentation was done by using the mash of dried sweet potato with its dregs as substrate (8). In another study, cellulosic pyrolysate – containing levo – glucosan was chemically hydrolyzed and a maximum glucose yield of 17.4% was obtained through hydrolysis with 2mol/litre H_2S0_4 at 121^0C for 20minute. The total initial glucose level was maintained at 41.9g/litre by diluting the hydrolysate. The hydrolysate was neutralize with $Ca(OH)_2$ (to bring to about pH 6.0 or 10.4) and, which was completely fermented by *S.cerevisiae* and *Pichia sp.* Yz – 1. A maximum ethanol yield of 0.45/g glucose was obtained by *S. cerevisiae*(9). Another substrate, liquefied cassava starch, was used for ethanol production by co – immobilized cells of *Z. mobilis* and *S. diastaticus*. The co – immobilized cells produce 46.7 g/litre ethanol from 150 g/litre liquefied cassava starch, while the immobilized cells of yeast *S. diastaticus* alone produced 37.5g/litre ethanol. Thus, co-immobilized cells of *S.diastaticus* and *Z.mobilis* produced a high ethanol concentration as compared to the immobilized cells of *S. diastaticus* during batch fermentation of liquefied cassava starch (10).

For direct and efficient ethanol production from cellulosic materials, a novel cellulose – degrading yeast strain was developed by genetically modifying two cellulolytic enzymes on the surface of *S.cerevisiae*. This could grow in a synthetic medium containing glucan as the sole source of carbon and could directly ferment 45g of glucan per litre to produce 16.5g of ethanol per litre within 50 hours. Thus, 0.48g of ethanol was produced per gram of carbohydrate utilized, which corresponded to 93.3% of the theoretical yield. This result indicates that efficient and simultaneous sacharification and fermentation of cellulose to ethanol was carried out by recombinant yeast cells displaying cellulolytic enzymes (11).

Alfenore *et al.,* (2002) described a nutritional strategy that allowed *S.cerevisiae* to produce a final ethanol litre of 19% (V/V) ethanol in 45hours in a fed – batch culture at 30^0C. This performance was achieved by implementing exponential feeding of vitamins throughout the fermentation process. A maximum instantaneous productivity of 9.5g/litre/hour was reached in the best fermentation. These performances resulted from improvements in growth, ethanol production rate, and concentration of viable cells in response to the nutritional strategy (12).

In other studies, (13) introduced new genes into a cyanobacterium in order to create a novel pathway for fixed carbon utilization, which results in the synthesis of ethanol. The coding sequences of the PDC and ADH II from the bacterium *Z. mobilis* were cloned into the shuttle vector pCB4 and were then used to transform the cyanobacterium *Synechococcus* sp strain PCC 7942. The PDC and ADH genes were expressed at high levels, as demonstrated by Western blotting and enzyme activity analyses. The transformed cyanobacterium synthesized ethanol, which diffused from cells into the culture medium. As cyanobacteria have simple growth requirements and use light, CO_2, and inorganic elements efficiently, production of ethanol by cyanobacteria is a potential system for bioconversion of solar energy and CO_2 into a valuable resource. Metabolic engineering of *Z. mobilis* strains was tried to maximize the ethanol production from mixtures of hexose and pentose sugars through the application of metabolic flux control techniques (14).

Currently about 90% of the world ethanol is produced from food substances such sugar cane and other starch grains. This process may lead to global food crisis while achieving energy security. Hitherto, little attention had been paid by researchers and policy makers in energy sector to the viability of lignocellulosic based wastes in ethanol production. Hence in our study we explored bioethanol production from selected Lignocellulosic wastes using *Saccharomyces cerevisiae* as the ethanologenic organisms.

2. Materials and Methods

2.1. Sample Source

The different lignocellulosic wastes utilized in this study were collected from the following sources in Ibadan: The Cassava Peels (CP) was obtained from International Institute of Tropical Agriculture (IITA). The Institute has a Cassava Processing Plant (CPP) where large quantity of Cassava Peels (CP) is generated. Yam Peels (YP) was collected in Abadina Quarters (AQ) of the University of Ibadan (UI). Plantain Peels (PP) was obtained from the Ajose Building Canteen (ABC) which is located within the University College Hospital (UCH) a sub campus of UI. The Sawdust (SD) was obtained from the Bodija Timber Processing Centre (BTPC) in Ibadan.

2.2. Biomass Sampling

Sampling of the Biomass

A representative sample of each biomass was obtained from the parent substrates. From each heap of biomass wastes, a grab sample was collected into a polythene bag ready for physical and biochemical characterization.

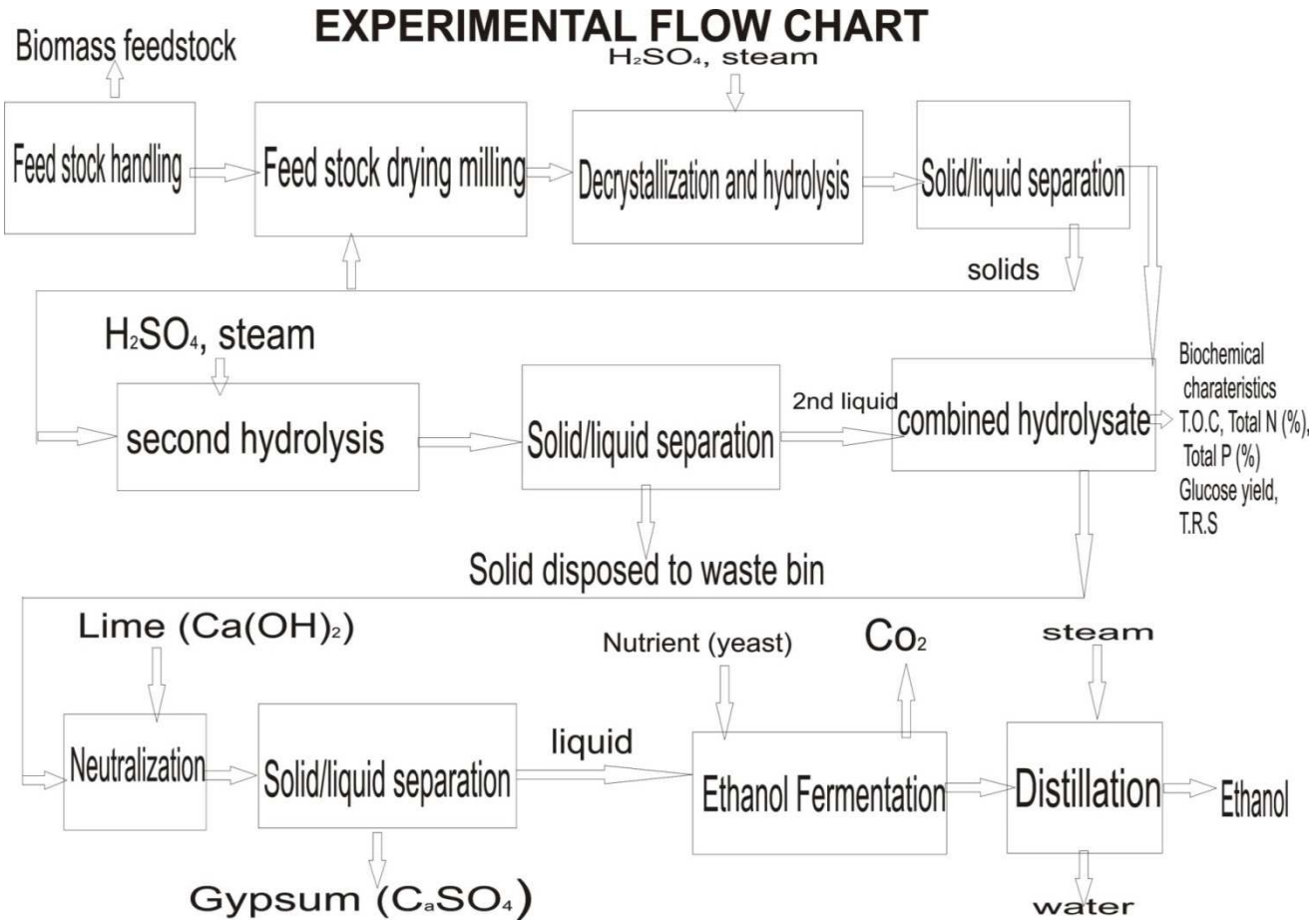


Figure 1. *Flow chart illustrating the various stages involve in the bio-ethanol production process (Farone and Cozen, 1996).*

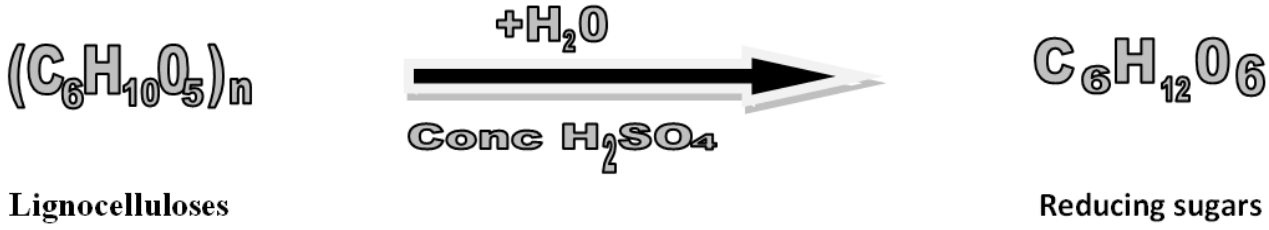
2.3. Pre-treatment of Biomass

The various samples were sun-dried for about 3-5 days to reduce the moisture content to about 10%. The dried samples were pulverized to a size of about 15mm. This allowed for a large surface of the substances to facilitate chemical hydrolysis.

2.4. Acid Hydrolysis

Twenty grammes (20g) each of the powdery biomass was hydrolyzed separately with 100ml (1:5w/v) of various concentration of H_2S0_4 of 5.6M, 9.4M and 13.1M in a two stage hydrolysis. In the first hydrolysis, the mixture of acid and biomass was heated to 100^0C for 60 mins to hydrolyze the lignocelluloses. This resulted in the formation of a thick gel, which was pressed on a sieve to obtain an acid-sugar stream. The solids remaining after the first hydrolysis was again hydrolyzed with H_2SO_4 at 100^0C for 50min. The resulting gel was again pressed to obtain a second acid-sugar stream. The stream from the two hydrolysis steps was combined. The mixed hydrolysates were analyzed for glucose and total reducing sugar (TRS) to determine which of the acid hydrolysis gave best yield of glucose and TRS.

Equation of the reaction:



The left over solid which is lignin, the most recalcitrant to degradation out of the 3 component of lignocelluloses material (lignin, hemicelluloses and cellulose) was discarded.

2.5. Glucose Yield and TRS Determination

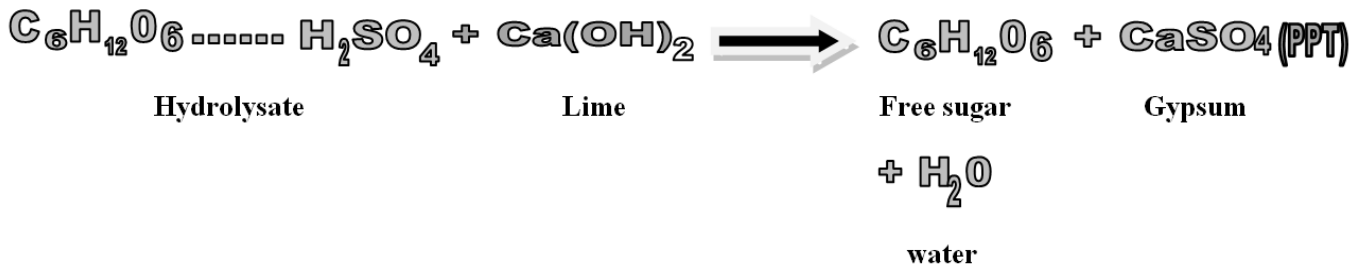
The AOAC method (15) was employed in the determination of glucose yield and TRS. The glucose yield

in the hydrolystate was determined by using the ferric cyanide method while the total reducing sugar content was determined quantitatively by using the Phenol-sulphuric acid method as outlined by Dubois *et al*, (1956). The amount of reducing sugar released was colorimetrically determined using UV spectrophotometer at a wavelength of 420 nm. A calibration curve was obtained using D- glucose as standard.

2.6. Neutralization Process

The sugar-acid stream/hydrolysate obtained from the acid hydrolysis was neutralized by adding lime [$Ca(OH)_2$], which forms a gypsum precipitate. The $CaSO_4$ was removed by filtration using a Whatman No1 filter paper and then discarded. The filtrate which is a free sugar stream was tested for the presence of reducing sugar using Fehling solution before subjected to ethanol fermentation.

Equation of the reaction



Hydrolysate **Lime** **Free sugar** **Gypsum**

water

2.7. Glucose Fermentation

The 13.1M H_2SO_4 gave the best yield of glucose and TRS hence was fermented with *Saccharomyces cerevisiae* in a 250ml fermenter at 30^0C for 72 hrs to ensure maximum ethanol production. Samples were taken from the fermenting broths every 24hours to test for the presence of ethanol and ethanol yield determination.

Equation of the reaction

Glucose **Ethanol** **Carbon dioxide**

2.8. Bio-Ethanol Yield Determination

The ethanol yield (v/w) was determined by using the AOAC methods (15). The ethanol was distilled from the sample and collected in an acid solution of potassium dichromate where it is oxidized by acetic acid at 60^0C. The residue dichromate was determined by back titration with ferrous sulphate in a strong acid solution using feroin indicator (1, 10-phenathroline ferrous sulphate complex).

2.9. Statistical Analysis

All data was summarized using descriptive statistics such as proportions, mean and standard deviation. The result obtain from the biochemical analysis were subjected to one – way ANOVA at 5% level of Significance. A Simple Linear Regression Model was used to indicate the relationship between the ethanol yield of the substrates and the fermentation time.

3. Results

3.1. Levels of Glucose Yield

Table 1 shows the levels of Glucose Yields (mg/kg) of the substrate hydrolysates at different acid concentrations of 5.6M, 9.4M and 13.1M respectively. In each of the acid concentration hydrolysis, the SD hydrolysates gave the highest mean glucose yield followed by PP, then YP and CP hydrolysate being the least. The mean glucose yield of the substrate hydrolysates were significantly different from each other for each of the acid concentration hydrolysis ($p<0.05$). The mean glucose yield obtained from the 13.1M H2SO4 were significantly higher than those obtained from the 9.4M and 5.6M H2SO4 hydrolysis ($p<0.05$).

Table 1. *Levels of Glucose Yield from Substrate Hydrolysates at different acid concentrations.*

Sample Description	5.6M H_2SO_4				9.4M H_2SO_4				13.1M H_2SO_4			
	1st	2nd	3rd	Mean ±S.D	1st	2nd	3rd	Mean ± S.D	1st	2nd	3rd	Mean ±S.D
SD Hydrolysate	286.0	280.6	290.5	285.7±5.0	300.0	292.5	309.5	300.7±8.6	343.0	338.5	348.0	343.2±4.8
PP Hydrolysate	257.0	249.5	260.0	255.7±5.4	275.0	273.8	285.5	278.1±6.5	305.0	297.5	309.6	304.0±6.1
YP Hydrolysate	230.0	228.0	235.0	231.0±3.6	240.0	235.0	245.0	240.0±5.0	271.5	257.0	279.0	269.2±11.2
CP Hydrolysate	65.0	41.0	45.5	50.5±12.8	70.0	69.5	75.0	71.5±3.0	84.0	80.0	91.2	85.1±5.7

* The hydrolystate obtained from the 13.1M hydrolysis gave the highest yield or mean value of Glucose Yield throughout the three (3) trials than the 5.6M and 9.4M hydrolysis. Hence it was used for the ethanol production.

3.2. Levels of Total Reducing Sugars

From Table 2, the mean Total Reducing Sugars (TRS) increased as the concentration of the acid increased and vice-versa. Among the substrates, YP hydrolysates recorded the highest mean TRS (mg/kg) at different acid concentrations while the least mean TRS was found in CP hydrolysates. The mean TRS obtained from the 13.1M H_2SO_4 were significantly higher than those obtained from the 9.4M and 5.6M H_2S0_4 hydrolysis ($p<0.05$). At 13.1M hydrolysis, mean TRS of PP was significantly higher than those of CP, YP and SD ($p<0.05$). Hence the 13.1M hydrolysate was used for ethanol production, since glucose and reducing sugars serve as a precursor for ethanol production.

Table 2. *Levels of Total Reducing Sugars (TRS) from Substrate Hydrolysates at different acid concentrations.*

Sample Description	5.6M H_2SO_4				9.4M H_2SO_4				13.1M H_2SO_4			
	1st	2nd	3rd	Mean ±S.D	1st	2nd	3rd	Mean ±S.D	1st	2nd	3rd	Mean ±S.D
SD Hydrolysate	375.0	367.0	381.5	374.5±7.3	450.0	448.8	460.8	453.2±6.6	537.5	530.3	540.0	535.9±5.0
PP Hydrolysate	315.0	309.5	319.5	314.7±5.1	395.0	391.3	403.0	396.4±6.0	460.5	458.0	465.0	461.2±3.6
YP Hydrolysate	390.0	381.3	395	388.8±6.9	460.0	455.6	465.0	460.2±4.7	544.5	532.5	547.0	541.3±7.5
CP Hydrolysate	91.5	89.0	95	91.8±3.0	125.0	117.5	127.0	123.2±5.0	209.0	206.5	213.8	209.8±3.7

* The hydrolystate obtained from the 13.1M hydrolysis gave the highest yield or mean value of Total Reducing Sugars (TRS) throughout the three (3) trials than the 5.6M and 9.4M hydrolysis. Hence it was used for the ethanol production.

3.3. Ethanol Yield

Figure 2 - 3 shows the ethanol production of the fermenting broths of the various substances every 24 hours. The mean ethanol yields at 24 hours of fermentation were: CP (123.3 ± 11.1mL/kg), YP (172.0 ± 17.5ml/kg), PP (217.7 ± 13.5 mL/kg) and SD (240.3±14.0mL/kg) ($p<0.05$) respectively. The maximum ethanol production was obtained at 48 hours, the mean ethanol yield being: CP - 160.0±15.1mL/kg, YP – 211.7±15.3mL/kg, PP – 265.0±2.0mL/kg and SD – 280.0±11.5mL/kg. Mean ethanol yield at 48 hours of fermentation were significantly different from those obtained at 24 hours. A simple linear Regression established a linear relationship between the ethanol yield of the substrates and the time of fermentation ($R^2=0.711$) as shown in Figure 4.

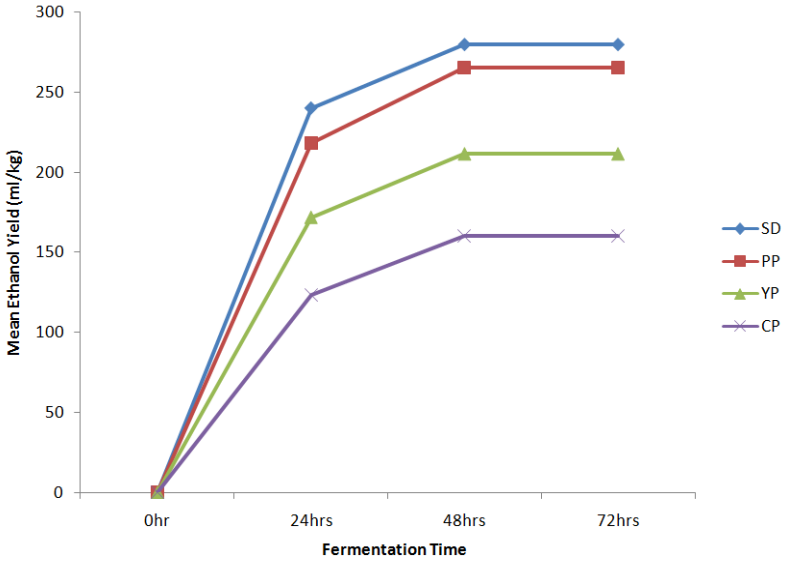


Figure 2. *Mean Values of Ethanol Yield of the Substrates at various Fermentation Time.*

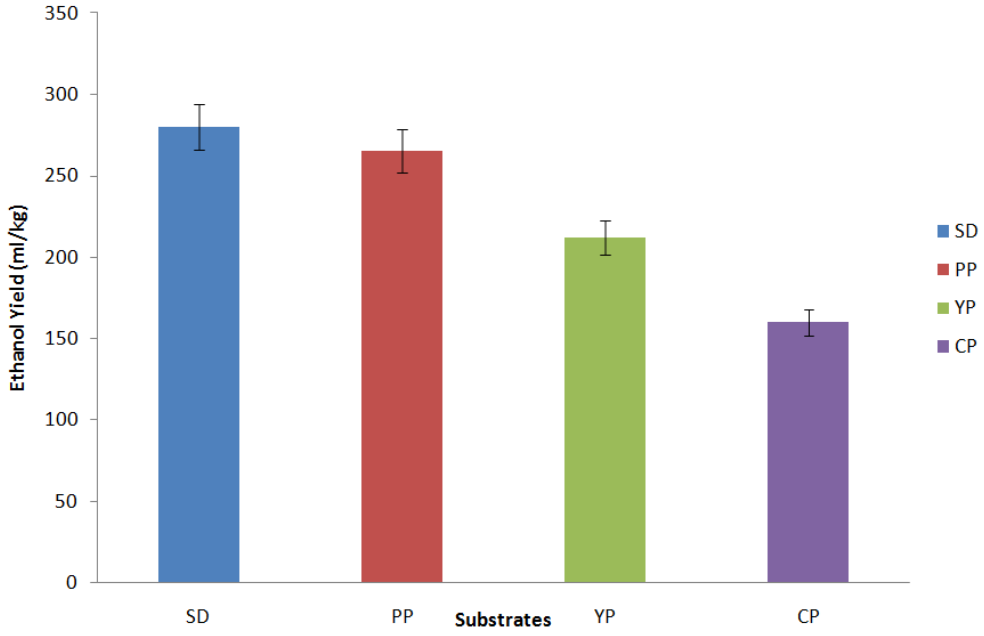


Figure 3. *Maximum Ethanol Yield (ml/kg) obtained from the various Fermenting Broths at 48hrs of Fermentation.*

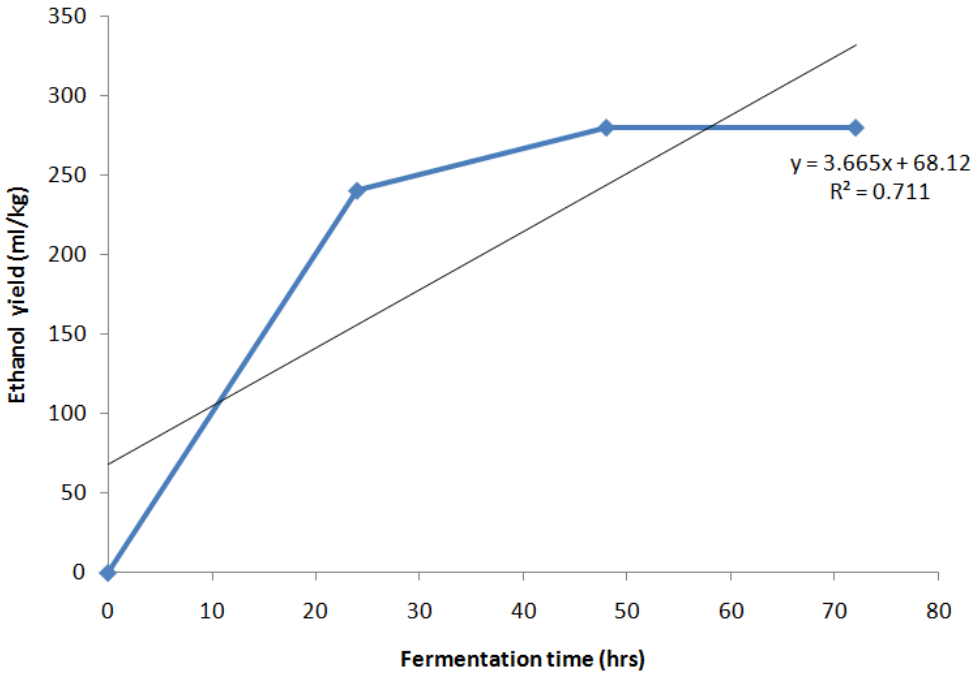


Figure 4. *Shows the Simple Linear Regression curve between the Ethanol Yield and Fermentation Time.*

4. Discussion

With increased population growth there is a corresponding demand for energy resources especially for non-renewable forms. This over dependence has result in the depletion of the resource base and gross degradation of the environment. This has led to the search for alternative and renewable energy sources (16). In the present investigation, we explored the production of bio-ethanol from selected Lignocellulosic wastes commonly found within Nigeria's south-western region.

In this study, the optimization of sugar production from cellulose hydrolysis under different acid strengths was assessed. The result showed that hydrolysis at 13.1M (70%) provided the maximum sugar content in the substrate. This agrees with the concentrated acid technology of using 70% conc. H_2SO_4 for sugar production from cellulosic material developed by (17). At over 70% H_2SO_4 concentration, a lot of charming or dehydrating reactions occurred to a varying degree. Similar result at a higher acid concentration was reported by (18) on cassava granted waste (CGW) biomass at 120^0C for 30mins and using a high concentration of H_2SO_4 (1-5M) hydrolysis was achieved but with excessive charring

or dehydrating reactions. Other chemical reactions reported in previous studies include the formation of furfural from xylose. Furfural was reported to inhibit activities of some glycolytic enzymes particularly dehydrohygenase in *S.cerevisiae* for ethanol production (19).

The finding of this study revealed that hydrolysis at 13.1M H_2SO_4 gave the best glucose and TRS yield for all the substrates when steam at 100^oC for 60mins and 50mins respectively. Jeffries and Lee (1999) also reported auto-hydrolysis (steam explosion) as an effective pretreatment method for lignocelluloses materials for hydrolysis (20). In fact, (21) reported an increasing glucose concentration in hydrolysate as the severity of steam explosion increases.

Among the substrates, the highest glucose yield was obtained from sawdust. The high amount of glucose yield in sawdust is due to the lignocelluloses content of hard and softwood stem as reported by (22 – 23) from which it is produced. Ojumu *et al.,* (2003) also reported that sawdust obtained from the tree *Triplochiton scleroxylon* contained 69.5 – 80% cellulose and hemicelluloses and 25 – 30% lignin. The high cellulose content of SD is responsible for its high mean glucose yield; since cellulose is a homogenous polymer of glucose (24). Badmus (2002) also produced glucose from palm tree trunk using auto hydrolysis prior to acid hydrolysis (25). The lowest amount of glucose yield and TRS found in CP can be attributed to its containing cellulose and hemicelluloses at levels of 24.99% and 6.67% (w/w) respectively as reported by (26). This agrees with previous study done by (26) who reported that the maximum reducing sugar of 6.09% (w/v) was recovered from cassava waste after pretreatment with 0.6M H_2SO_4 at 120^OC for 30mins. At concentration of H_2SO_4 higher than 0.6M, the reducing sugar was lower than 6.09%.

The high mean TRS found in YP, SD and PP may be attributed to the high amount of hemicelluloses content. Hemicelluloses are macromolecules often polymers of pentoses (xylose and arabinose), hexoses (mostly mannose) and a number of sugar acids. Hemicelluloses are particularly industrial interest since they are readily available bulk source of xylose from which xylitol and furfural can be derived (27 - 28).

Ethanol produced from cellulosic biomass materials instead of traditional feedstock is known as bioethanol: a carbon-neutral compound. The traditional process of ethanol production is through fermentation of sugars with a species of yeast called *Saccharomyces cerevisiae*. However, the changing needs, energy demands, and technological advances to overcome the general limitations in yeast-based ethanologenic fermentations have led to an exploration of different methods using a broad range of substrates and novel organisms, indigenous or genetically modified. New technologies are being developed that convert the fibrous portion of plant material to bioethanol. These feedstock materials are abundant and inexpensive (29).

In the present study, the Simultaneous Saccharification and Co-Fermentation (SSCF) was employed which involves the fermentation of both six-carbon hexoses (glucose, mannose, and galactose) and five–carbon pentoses (xylose and arabinose) sugars to ethanol. This is in line with several authors who reported that the SSCF is superior to the Simultaneous Saccharification and Fermentation (SSF) technology in terms of cost effectiveness, better yields, and shorter processing time (6, 30). A complete conversion of glucose and xylose mixture was obtained by a respiratory deficient mutant of *S.diastaticus* co-cultivated with *Pichia stipilis* in continuous culture (31).

Of all the substrates, SD gave the highest ethanol yield. This may be attributed to its high glucose yield and TRS, since glucose is a precursor for ethanol production. According to (32), the total sugar content is important for the ethanol yield; a key economic parameter depending upon the sugar content. The maximum ethanol production was obtained at 48hours of fermentation for all the substrates and after which the level remained constant. This outcome corroborates previous findings in which different substrates were used to assess the efficiency of the strain *klebsiella oxytoka* viz., mixed office paper (33 – 35) and sugar beet pulp (36). The best strains of the transformants converted 10% glucose and 10% cellobiose into 44-45g/litre of ethanol within 48 hours. Integrating cellulose components like extracellular endoglucanase can reduce the ethanol production costs (37). When a comparative study was done, in which galacturonic acid-rich sugar beet pulp was fermented, K011 produced significantly higher quantities of ethanol production due to *E.coli* K011 affinity for the substrate. Dien *et al.,* (1998) developed a novel hexose and pentose utilizing the ethanologenic *E.coli* strain FBR3 by incorporating the plasmid pL01297. An ethanol yield of 4.38% - 4.66% (w/v) with 90-91% theoretical conversion in 70-80 hours was achieved (38).

Mixing has an important role in fermentation. The influence of mixing (from 100-110 rpm [revolutions per minute]) on the performance of *Z.mobilis* anaerobic continuous culture was studied. It was found that the biomass yield and ethanol productivity were improved at higher stirring intensities along with a decrease in the by-product formation. Vigorous mixing led to a better coupling between catabolism and anabolism (39). In another study (40) used immobilized *S. cerevisiae* cells and found that the maximum fermentation capacity of the system was at 30^0C and was relatively pH – sensitive. A packed column reactor was used to test this biocatalyst's operational sensitivity to key fermentation variables. Results of this study as well as characteristics of the polymer, prepared by an epoxy resin and di-amino polyethylene oxide polymerization establish the suitability of this method for ethanol production.

Although several microorganisms, including Clostridium sp., have been considered as ethanologenic microbes, the yeast *S. cerevisiae* and the facultative bacteria *Z. mobilis* are better candidates for the industrial alcohol production (41). The feedstock typically account for more than one third of the production costs, thus maximizing the ethanol yield is imperative. A high ethanol yield means using strains of bacteria that can produce fewer side products and metabolize

all major sugars, which typically include glucose, xylose, arabinose, and mannose (42).

5. Conclusions

The purpose of this study was to determine the ethanol yielding capacity of some selected lignocellulosic based-wastes. The results show that sawdust produced the highest glucose and ethanol yield among the substrates. Bioconversion offers a cheap and safe method of not only disposing the agricultural residues, but also it has the potential to convert lignocellulosic waste into usable forms such as reducing sugars that could be used for ethanol production. Hence the conversion of lignocellulosic "wastes" into biofuels such as ethanol will help reduce environmental pollution, contribute toward the mitigation of greenhouse gases emissions and serve as a sustainable solid waste management strategy.

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