Standard Scientific Research and Essays Vol2 (9): 431-437, September 2014 (ISSN: 2310-7502)
http://www.standresjournals.org/journals/SSRE

Research Article

Bioethanol production from lignocellulosic biomass wastes by fermentation of the hydrolyzates

[*1]Etonihu AC and [2]Idoko O

[1]Chemistry Department, Nasarawa State University, PMB 1022, Keffi, Nigeria
[2]Chemistry Advanced Laboratory, Sheda Science and Technology Complex (SHESTCO) Abuja, Nigeria

*Corresponding Author E-mail: criseto@yahoo.com, Tel; +234(0)8036863275

Accepted 23 September 2014

Abstract

Lignocellulosic biomass wastes from *Moringa oleifera* seed pods, sawdust and office waste papers were treated using four different hydrolytic methods with 18M H_2SO_4, 0.6M H_2SO_4 and 0.5M alkaline pretreatment/dilute acid and enzymatic hydrolysis. The hydrolyzates were fermented at room temperature using *Saccharomyces cerevisiae* at varied fermentation periods between 1 – 10 days. At intervals of 1, 3, 5, 7 and 10 days, the fermented samples were taken for ethanol quantification. The results of the analyses showed increased ethanol yield (day 1 to day 7) but decreased thereafter. Among the lignocellulosic biomass wastes, the highest yield of ethanol was in the order waste paper > *Moringa oleifera* seed pods > sawdust. The enzymatic method yielded significantly (P > 0.10) more ethanol than the chemical methods, and increased as the fermentation period increased (from day 1 to day 10). Since lignocellulosic biomass wastes are renewable and readily available, such conversions could lead to reduction of green house gas emission thereby ameliorating the problem of global warming, conserving Nigeria's overstretched fossil fuel, solving some energy crises, curbing food supply shortage, and conserve foreign exchange.

Keywords: Agro-waste, Bioethanol, Biomass, Hydrolyzate, Lignocellulosic

INTRODUCTION

In contemporary times, a great deal of interest has been generated worldwide regarding the use of biofuels namely biogas, bioethanol and biodiesel for energy supply (World Bank, 2008). A large variety of feedstock is currently available for producing ethanol from cellulosic biomass. Although producing bioethanol from starch, such as cassava, is relatively cheap and simple, it is a crop crucial for food security especially in Nigeria. The implication is that threat to food security exists in the face of growing fuel ethanol demand (Cadena, 1998; Srinorakutara *et al*., 2006). With the increasing demand for ethanol production through fermentation processes (Equation 1) all over the globe as oil reserves are dwindling and prices are soaring (Brooks, 2008), it has become imperative that the searchlight be turned at the use of non-food starch and lignocellulosic materials (Akponah and Akpomie, 2011). Lignocellulosic biomass including forest residue, agricultural residue, yard waste, wood products, animal and human wastes, etc is a renewable resource that stores energy from sunlight (McKennedy, 2002). Lignocellulosic biomass has great potentials for the production of affordable ethanol because it is less expensive than starch (e.g corn) and sucrose (e.g sugarcane) and it is available in large quantities (Lin and Tanaka, 2006).

$$C_6H_{12}O_6 \longrightarrow C_2H_5OH + CO_2 \quad \ldots \quad 1$$

Prasad *et al*. (2007) observed that with world reserves of petroleum fast depleting, ethanol has in recent years emerged as the most important alternative resource for liquid fuel and has generated a great deal of research interest in

ethanol fermentation. Production of ethanol from cellulosic biomass corn leaves and stalks has the potential to augment the feedstock in the existing industry and become the technology of the future for ethanol production (Figure 1). Today, many countries of the world including Brazil, France, the United States, Indonesia, Philippines, Guatemala, Costa Rica, Argentina, South Africa, Kenya, Thailand and Sudan use a significant amount of ethanol with government or private ethanol fuel programmes. The programmes are designed to reduce the country's dependence on costly imported fuel and to assist in creating a new domestic fuel industry (Cliff and Ken, 1984).

This work involved the production and estimation of bioethanol from lignocellulosic biomass wastes from *Moringa oleifera* seed pods, waste papers and sawdust by using four hydrolysis methods. The amount of ethanol produced was estimated at intervals of 1, 3, 5, 7 and 10 days for each of the samples using *Saccharomyces cerevisiae* for fermentation.

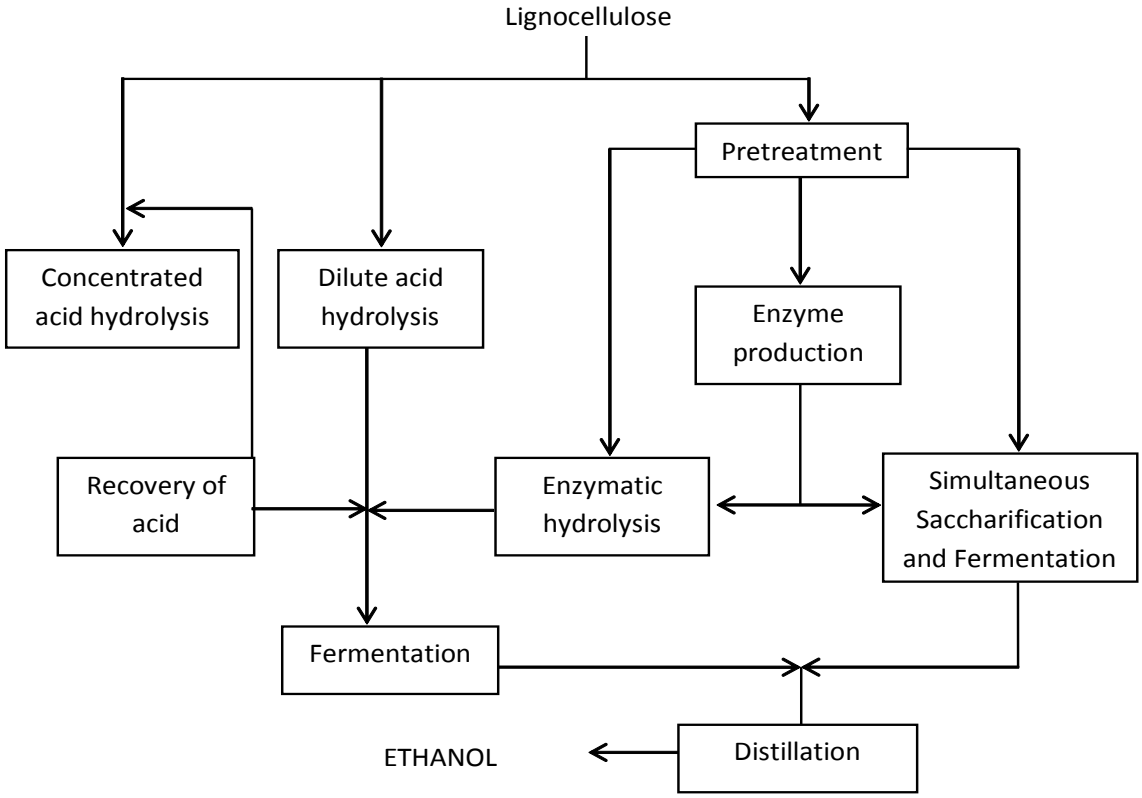

Figure 1. Cellulosic ethanol production process (Zacchi and Galbe, 2002)

MATERIALS AND METHODS

Collection of Lignocellulosic Materials

The lignocellulosic biosolid wastes identified and used are *Moringa oleifera* seed pod, office waste papers and sawdust. The *Moringa oleifera* seed pods and waste papers were collected from Sheda Science and Technology Complex (SHESTCO), while sawdust was collected from Timber processing facilities at Kwali Area Council of the Federal Capital Territory (F.C.T), Abuja. The samples were sun-dried for 24 hours. Then the *Moringa oleifera* seed pods were crushed with mortar and pestle and ground using KANCHAN Twister Laboratory Blender. The office waste papers were reduced to smaller sizes using a pair of scissors.

Dilute Acid Hydrolysis

The hydrolysis was carried out using the method described by Akponah and Akpomie (2011), with some modifications. 100 g of each sample was weighed into a flat-bottomed flask and o1 L of 0.6M H_2SO_4 was added and placed on a water bath at 100^oC for 2 h. 100 ml of distilled water was added followed by filtration. The filtrate was autoclaved at 121^oC for 15 mins and the pH was adjusted to 4.62 with 1M NaOH.

Concentrated Acid Hydrolysis

To 100 g each of the samples was added 500 ml 18M sulphuric acid and allowed to stand for 24 h. After which 200 ml of distilled water was added and filtered. The filtrate was autoclaved at 121^{o}C for 15 mins and the pH adjusted to 4.65 with 10M NaOH (Mike, 1983).

Alkali Pretreatment/Dilute Acid Hydrolysis

Each of the samples (100 g) was soaked with 500 ml of 0.5M NaOH and autoclaved at 121^{o}C for 12 mins. The pretreated samples were neutralized using 1M HCl. 10% HCl was added and placed in water bath shaker and a stirrer was used to mix the solid with a speed of 300 rpm for 1 h at 45^{o}C. This was followed by filtration and the filtrate was autoclaved and pH adjusted to 4.60 with 1M NaOH (Ghasem *et al.,* 2007).

Enzymatic Hydrolysis

Each of the samples (100 g) was weighed into a beaker and 500 ml of 1.5 % H_2SO_4 was added and the mixture was autoclaved at 121^{o}C for 30 mins. It was allowed to cool to room temperature, filtered and washed with hot water. The pH of the filtrate was adjusted to 4.54 with 1M NaOH prior to fermentation. 200 ml of distilled water was added to the residue and 10 g of cellulase enzymes was added and the beaker placed in a water bath shaker at 50^{o}C for 48 h at 100 rpm. After the hydrolysis, it was allowed to cool to room temperature, filtered and the pH adjusted to 4.58 with 1M NaOH (Gerhardt, 2008; Yesun, 2005).

Fermentation Medium

Fermentation medium comprising of glucose-10 g/l, yeast extract-0.1 g/l, potassium hydrogen phosphate-0.5 g/l and magnesium sulphate-0.1 g/l was prepared and autoclaved at 121^{o}C for 15 mins. The flask was brought to room temperature and pH adjusted to 4.70 with 1M NaOH. The *Saccharomyces cerevisiae* were inoculated (10^8 cells/ml) aseptically into the medium and incubated at 28^{o}C for 24 h (Ravikumar *et al.,* 2011).

Fermentation of the Hydrolysates

One hundred (100) ml of the fermentation medium was added to each of the filtrate aseptically and incubated at room temperature for a period between 1 - 10 days. Each of the fermented samples was taken for ethanol estimation at the intervals of 1, 3, 5, 7 and 10 days (Ravikumar *et al.,* 2011).

Test for Simple Sugars (Fehling's Test)

After hydrolysis, the presence of fermentable sugars was investigated using Fehling's test as follows: 2ml of the Fehling's reagent was added to a clean test tube and 3 drops of the hydrolyzed sample was added and place in a water bath at 60^{o}C for 5 mins. A positive test was indicated by a green suspension and a brick-red precipitate (Prout *et al.,* 1997).

Test for Ethanol (Iodoform Test)

To 1 ml of the fermented sample in a test tube, 6 drops of iodine solution were added followed by 1M NaOH in drops with shaking until the pale yellow triiodomethane, CHI_3 (iodoform) separated as yellow crystals (Aboh and Ewelukwa, 1999).

Estimation of Ethanol and Calculations

In a 250 ml conical flask, 10 ml of acidified dichromate solution was added and 1 ml of the fermented sample was pipette into a sample holder which was suspended over the acidified dichromate solution and held in place with a string and rubber stopper and stored overnight at 25^{o}-30^{o}C. The following morning, the flask was cooled to room temperature and the sample holder removed carefully. The walls of the flask were rinsed with distilled water and 100 ml of the distilled water was added followed by 1 ml of 1.2M potassium iodide and then swirled to mix. A blank was also prepared by adding 10 ml of acidified dichromate to a conical flask and 100 ml of distilled water with 1 ml of potassium iodide and swirl to mix. The burette was filled with 0.03M sodium thiosulphate and titrated with each of the flask until the brown

color faded to yellow, then 1 ml of starch solution was added and the titration continue until the blue colour disappeared (Paschen, 2011). The analysis was repeated trice. The equation for the ethanol estimation is as follows:

In equation (2), ethanol is oxidized to ethanoic acid by reacting with an excess of potassium dichromate in acid.

$$2Cr_2O_7^{2-} + 3C_2H_5OH \longrightarrow 4Cr^{3+} + 11H_2O + 3CH_3COOH \quad \ldots 2$$

The amount of unreacted dichromate is then determined by adding KI solution which is oxidized by the acidified dichromate to form Iodine in equation (3).

$$Cr_2O_7^{2-} + 14H^+ + 6I^- \longrightarrow 2Cr^{3+} + 3I_2 + 7H_2O \quad \ldots 3$$

The iodine was then titrated with a standard solution of sodium thiosulphate (Equation 4).

$$2S_2O_3^{2-} + I_2 \longrightarrow S_4O_6^{2-} + 2I^- \quad \ldots 4$$

The titration results are used to calculate the ethanol content of the original solution (g/l) using the relationships:

6 mol of $S_2O_3^{2-} \equiv$ 1 mol of $Cr_2O_7^{2-}$

2 mol of $Cr_2O_7^{2-} \equiv$ 3 mol of C_2H_5OH

1 mol of $S_2O_3^{2-} \equiv$ 0.25 mol of C_2H_5OH (Paschen, 2011).

Statistical Analysis

The analysis of variance (ANOVA) using the Turkey's Post Hoc Test was conducted to determine which specific group means pairs is significantly different in the Tables.

RESULTS AND DISCUSSION

The Fehling's test confirmed the presence of fermentable sugars after each hydrolysis method on the samples, while the test for ethanol (Iodoform test) indicated that after the fermentation process ethanol was present in all the samples. Table 1 shows that the maximum ethanol yield for MO, WP, and SWD was 3.47 g/l, 3.60 g/l and 2.14 g/l respectively on the 7th day.

Table 1. Ethanol yield from dilute acid hydrolysis (H_2SO_4) of MO, WP and SWD at various fermentation times

Fermentation	Ethanol yield (g/l)						
Time [day(s)]	MO	WP	SWD	Mean($\bar{X}$)	$\Sigma(X - \bar{X})^2$	SD	CV
1	1.23	1.89	1.19	1.44	0.3090	0.3209	0.1030
3	1.90	2.60	1.36	1.95	0.7730	0.5075	0.2576
5	2.81	2.88	1.62	2.44	1.0027	0.5781	0.3342
7	3.47	3.60	2.14	3.07	1.3058	0.6596	0.4352
10	3.27	3.45	2.00	2.91	1.2491	0.6452	0.4163

F = 2.9543, $p<0.10$. MO = *Moringa oleifera* seed pod, WP = waste paper, SWD = sawdust, CV = Coefficient of Variation, SD = Standard deviation

For the ethanol yield from waste paper, a similar work reported 3.7 g/l (Ravikurma *et al.,* 2011). Subashini *et al*. (2010) used 0.3M H_2SO_4 on sago wastes and obtained ethanol yield of 5.8 g/l and 6.4 g/l on the 5th and 10th day respectively. The amount of ethanol production for WP was higher compared to MO and SW within the period. After the 7th day, ethanol yield declined in all the samples. This reduction could be related to the inhibitory activities of both ethanol produced and other byproducts formed on the growth and transport metabolism of the yeast (Xu *et al.,* 1996; Akponah and Akpomie, 2011).

The ethanol yield increased considerably for all the samples when concentrated acid was used for the hydrolysis (Table 2), with the maximum ethanol yield for MO, WP, and SWD of 3.79 g/l, 4.10 g/l, and 2.5 g/l respectively on the 7th day. This is comparative to a similar work by Ijogbemeye and Sideso (2011) who recorded 2.9 g/l and 3.6 g/l at the fermentation temperatures of 20°C and 30°C respectively, for sawdust. Furthermore, ethanol yield from the concentrated acid hydrolysis of the samples between 1 – 7 day was higher compared to ethanol yield of SWD between 3rd – 5th day. The concentrated acid hydrolysis method produced more ethanol in all the samples compared to the dilute acid hydrolysis. This could be attributed to the fact that concentrated acid hydrolysis of biomass produces higher fermentable sugars compared to dilute acid hydrolysis (Taherzadeh and Karimi, 2008).

Table 2. Ethanol yield from concentrated H_2SO_4 hydrolysis on MO, WP and SWD at various fermentation time

Fermentation	Ethanol yield (g/l)						
Time [day(s)]	MO	WP	SWD	Mean($\bar{X}$)	$\Sigma(X - \bar{X})^2$	SD	CV
1	1.29	2.70	1.26	1.75	1.3542	0.6718	0.4514
3	1.93	3.05	1.89	2.29	0.8672	0.9983	0.9968
5	2.93	3.10	2.36	2.79	0.3066	0.3165	0.1002
7	3.79	4.10	2.50	3.46	1.4401	0.6928	0.4800
10	3.60	4.05	2.40	3.35	1.4550	0.6964	0.4850

F = 2.9515, p < 0.10., MO = *Moringa oleifera* seed pod, WP = waste paper, SWD = sawdust, CV = Coefficient of Variation, SD = Standard deviation

The alkali pretreatment/dilute acid hydrolysis (Table 3) shows that maximum ethanol yield for MO, WP and SD was 3.55 g/l, 3.64 g/l and 2.16 g/l respectively on the 7th day and produced higher ethanol yield compared to dilute acid hydrolysis. Table 3 showed that pretreatment before hydrolysis produced higher ethanol yield compared to dilute acid hydrolysis (Table 1). This may be due to the fact that pretreatment process breaks down the lignin – hemicelluloses – complex, disrupt and loosen up the crystalline structure of cellulose, thereby increasing the porosity of the biomass (Mosier *et al.,* 2005). The other reasons could be that dilute NaOH pretreatment was found to be more effective for the hydrolysis of lignocelluloses with low lignin content (Bjerre *et al.,* 1996). Alkali pretreatment processes are more effective on agricultural residues as compared to wood materials (Kumar and Wyman, 2009).

Table 3. Ethanol yield from NaOH pretreatment/dilute HCl hydrolysis on MO, WP and SWD at various fermentation times

Fermentation	Ethanol yield (g/l)						
Time [day(s)]	MO	WP	SWD	Mean($\bar{X}$)	$\Sigma(X - \bar{X})^2$	SD	CV
1	1.26	2.00	1.20	1.49	0.3968	0.3625	0.1322
3	1.93	2.48	1.38	1.93	0.6050	0.3174	0.1008
5	2.86	2.93	1.72	2.50	0.9229	0.5546	0.3076
7	3.55	3.64	2.16	3.12	1.3767	0.6774	0.4589
10	3.40	3.57	2.07	3.01	1.3491	0.6705	0.4497

F = 3.1518, p < 0.10. MO = *Moringa oleifera* seed pod, WP = waste paper, SWD = sawdust, CV = Coefficient of Variation, SD Standard deviation

The enzymatic hydrolysis (Table 4) shows that the maximum ethanol yield for MO, WP and SWD was 3.62 g/l, 4.21 g/l and 2.59 g/l respectively on the 10th day. Kanokphorn (2011) reported a yield of 21.02 g/l of the ethanol production from pretreated office paper using cellulase from *Aspergillus niger* and *Trichoderma viride* and *sacharomyces cerevisiae* for saccharification and fermentation process. The low ethanol yield recorded in this work could be as a result of the fermentation conditions, acidity, the reaction vessel, pH value and temperature which if not controlled can affect the yield of ethanol (Ijogbemeye and Sideso, 2011). The ethanol yield increased with increase in the fermentation periods (Akponah and Akpomie, 2011) as shown in Table 4.4, with the ethanol yield increasing from 1st – 10th day. A similar work reported by Nirmala and Sumitra (2009) varied the incubation periods between 1-9th day and with increasing ethanol yield as incubation period is increased. This hydrolysis method may produce higher ethanol yield if the fermentation period is extended beyond the 10th day. This is not the same in the case of acid and alkali treatments (Tables 1, 2 and 3) where the ethanol yield increased within 1st – 7th day and suddenly decreased. The enzymatic hydrolysis is considered the prospective viable strategy to offer advantage over other chemical conversion routes of minimal byproducts formation, low energy requirements, mild operating condition and environmentally friendly processing (Saha, 2000; Wingren *et al.,* 2005).

Table 4. Ethanol Yield from enzymatic (Cellulase) hydrolysis on MO, WP, and SWD at various fermentation time

Fermentation	Ethanol yield (g/l)						
Time [day(s)]	MO	WP	SWD	Mean(X)	$\Sigma(X - X)^2$	SD	CV
1	1.26	2.76	1.27	1.76	1.4900	0.7046	0.4966
3	1.83	2.98	1.76	2.19	0.9386	0.5592	0.3128
5	2.71	3.23	2.31	2.75	0.4256	0.3765	0.1418
7	3.43	4.09	2.38	3.30	1.4874	0.7041	0.4958
10	3.62	4.21	2.59	3.47	1.3443	0.6694	0.4481

F = 3.3836, p < 0.10. MO = *Moringa oleifera* seed pod, WP = waste paper, SWD = saw dust, SD = Standard deviation, CV = Coefficient of variation

The dilute acid hydrolysis produced the lowest ethanol yield from all the samples and could be related to the fact that dilute acid hydrolysis often lead to the formation of more byproducts such as furfural, hydroxyl methyl furfural (HMT) and phenolic acid (Wingren et al., 2005) compared to the other hydrolytic methods. The ethanol yield from the concentrated acid hydrolysis and enzymatic hydrolysis is higher compared to the dilute acid hydrolysis and the alkali pretreatment/dilute acid hydrolysis. The analysis of variance (ANOVA) using the Turkey's Post Hoc test shows significant difference at P > 0.10 between groups mean of day 1 to 10 in Tables 1 to 4. The ethanol yield for WP was the highest in all the four hydrolytic methods used and the reason could be that substrates with smooth surfaces produce high amounts of fermentable sugars at the end of hydrolysis and that waste paper contains high amount of cellulose and hemicelluloses and low amount of lignin (Akponah and Akpomie, 2011; Jorgensen *et al.,* 2007).

CONCLUSION

The conversion of some lignocellulosic biomass wastes into bioethanol with concentrated H_2SO_4 and enzymatic hydrolysis produced the highest ethanol yield from all the samples on the 7th and 10th day of the fermentation periods. Among the lignocellulosic biomass wastes, the highest yield of ethanol was in the order waste paper > *Moringa oleifera* seed pods > sawdust. The ethanol yield from the concentrated acid hydrolysis and enzymatic hydrolysis is significantly higher compared to the dilute acid hydrolysis and the alkali pretreatment/dilute acid hydrolysis. Since lignocellulosic biomass wastes are renewable and readily available, such conversions could lead to reduction of green house gas emission thereby ameliorating the problem of global warming, conserving Nigeria's overstretched fossil fuel, solving some energy crises, curbing food supply shortage, and conserve foreign exchange.

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