# Saw Dust to Bioethanol: An Alternative Fuel for Future

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**Abstract:**

Due to over use of traditional fossil fuels leading to its scarcity and the fast increasing price of petroleum together with environmental concerns, the search for alternative renewable fuels has attracted great attention in recent years. Lignocellulosic materials provide an alternative source for bioethanol production. These materials are cheap and easily available throughout the year as a waste material from agriculture and forestry. The present study includes the development of better saccharification method to liberate maximum fermentable sugars for effective ethanol production. Saccharification was carried out at different NaOH concentration (1%-10%) for different period of time. Similarly, enzymatic treatment by crude cellulose was given to NaOH treated saw dust after neutralizing the pH for different period of time. Liberated sugar was measured using 3, 5-dinitrosalicyclic acid. Treated broth was used as fermentation medium and fermentation was carried out for 96 h by *Saccharomyces cerevisiae*. Highest saccharification was observed at 3% NaOH concentration and at 18 h enzymatic treatment. Ethanol estimation was carried out using iodometric method.

**Key words:** Bioethanol, Saw dust, Saccharification, Fermentable sugar

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## Introduction:

The use of sugar and starch as raw material for fuel production competes with their use as foods (Pimental *et al.,* 2008). Moreover supply is not sufficient to meet the increasing public demand. Lignocellulose is the major structural component of woody and non woody plants which provides an alternative attractive fuel source for biofuel production and also replaces the conventional fossil fuels (Wan and Li., 2011). Lignocellulosic materials are less expensive and easily available throughout the year. On worldwide basis, terrestrial plants produce $2.3 \times 10^{11}$ metric tons/year (dry weight basis) of wood which is equivalent to $7 \times 10^{10}$ metric tons of coal. Available cellulosic feedstock from agriculture and other sources are about 180 million tons/year (Lynd *et al.,* 2003).

Cellulosic biomass can be used as a substrate for ethanol production but it requires extensive pretreatment to release the fermentable sugars. Difficulties arise due to (1) Resistant nature of biomass to breakdown (2) The need for genetically engineered organisms to ferment the variety of sugars which are release by breaking cellulosic and hemicellulosic materials (3) The cost of collection and storage of low density biomass feedstocks. Many pretreatment methods are employed for breaking the cellulosic materials like physical pretreatment, chemical pretreatment, physicochemical pretreatment and biological pretreatment. The main goal of pretreatment is to overcome the recalcitrant nature of material and to separate the cellulose from the matrix polymers to make it more accessible for enzymatic hydrolysis. Pretreatment processes are selected by aiming certain criteria like: (i) To produce highly digestible solids which enhances sugar yields during enzymatic hydrolysis, (ii) It must avoid the degradation of sugars (mainly pentose) including those derived from hemicelluloses, (iii) It minimizes the formation of inhibitors for subsequent fermentation steps, (iv) Recovery of lignin should be possible for conversion into valuable co products, and (v) It must be cost effective by operating in reactors of moderate size and by minimizing heat and power requirements.

In addition to acid treatment NaOH can be widely employed to lignocellulosic material. It is applied in dilute as well as in concentrated form. Dilute NaOH separate the bonds between lignin and carbohydrate, thus it increase the surface area and disrupt the lignin structure. It

also decreases the degree of polymerization and crystallinity. Concentrated form of alkali increased the delignification. Increased pH from 10 to 13 removes lignin from 40 to 80% w/w at 140°C dry wheat straw (Pedersen *et al.,* 1998). NaOH has been reported to increase hardwood digestibility from 14% to 55% by reducing lignin content from 24-55% to 20% (Kumar *et al.,* 2009). Zhao *et al.,* in 2009 recorded an increase in delignification, from 52.3% to 75.5%. Positive results have come out from combined treatment of NaOH and other agents such us per acetic acid, urea, hydrogen peroxide and polyelectrolyte. Normally, NaOH treatment is carried out at room temperature or at elevated temperature but recently cold NaOH (-5°C) or NaOH/urea (-20°C) are used to treat plant fiber and cotton cellulose respectively. Bamboo was subjected to ultrasound irradiation and NaOH/urea pretreatment at low temperature (-12°C) which break the recalcitrant material and produce high reactive cellulose (Li *et al.,* 2010). NaOH treated sawdust have highest cellulose and hemicelluloses fraction i.e. 63.1% and 19.5% respectively (Sridevi A. *et al*., 2015).

Enzymatic treatment on treated sawdust leads to a better saccharification and can produce good amount of fermentable sugars.

## Method and Materials:

**Materials:** Sawdust was collected from a local mill and dried in oven at 60 °C for 24 h. Dried saw dust was milled to minimize the size to provide effective pretreatment. The milled sawdust was stored at room temperature in closed vessel until it was used. Extra pure NaOH was procured from Fisher Scientific Pvt. Ltd.

**Pretreatment to Sawdust:** Dried sawdust was mixed with the different concentration of NaOH. 100 mL broth was prepared by mixing the fine dried particles of sawdust into the distilled water. NaOH concentration ranges from 1% to 10% were selected for the present study. All the broths were kept at 37°C for 2 h to check the effectivity of NaOH on hydrolysis process. After pretreatment, filtration was carried out and treated sawdust was washed to remove the traces of NaOH with the help of running tap water until pH becomes neutral or near to neutral.

**Time Standardization for pretreatment process:** 100 mL broth with 3% NaOH in the proportion of 1:10 (Sawdust: NaOH) and kept at 37 °C for different period of time. After 2 h,

4 h, 6 h, 12 h, 18 h and 24 h, sawdust was washed with tap water. It was dried in oven at a60 °C and then was proceed for enzymatic saccharification.

**Enzymatic Saccharification:** Crude cellulase enzyme was produced by cowdung isolated bacteria *Bacillus subtilis* using Carboxy Methyl Cellulose as a substrate. This crude enzyme was used for the saccharification purposes. In 100 mL crude cellulase enzyme 10 gm sawdust was soaked and kept on shaker for different time period i.e. 2 h, 4 h, 6 h, 12 h and 24 h at 120 rpm. Again filtration was carried out and filtrate was used for the determination of enzyme activity in terms of sugar liberation. Treated broth was used for the alcohol production after adjusting the pH 6.5 with the help of HCl.

**Sugar Estimation Assay:** The DNS assay was carried out by taking 0.2 mL of culture filtrate. It was mixed with 1.8 mL of 1% CMC which was prepared in 0.05 M citrate buffer having pH 4.8 in a test tube and incubates at 60 °C for 30 minutes. Reaction was terminated by adding 3 mL of DNS reagent and tube was incubated at 100 °C for 15 minutes. After cooling 1 mL of 40% Rochelle salt solution was added and optical density was measured at 575 nm against blank. One unit of the cellulase activity defines the amount of enzyme that release 1 µM of glucose.

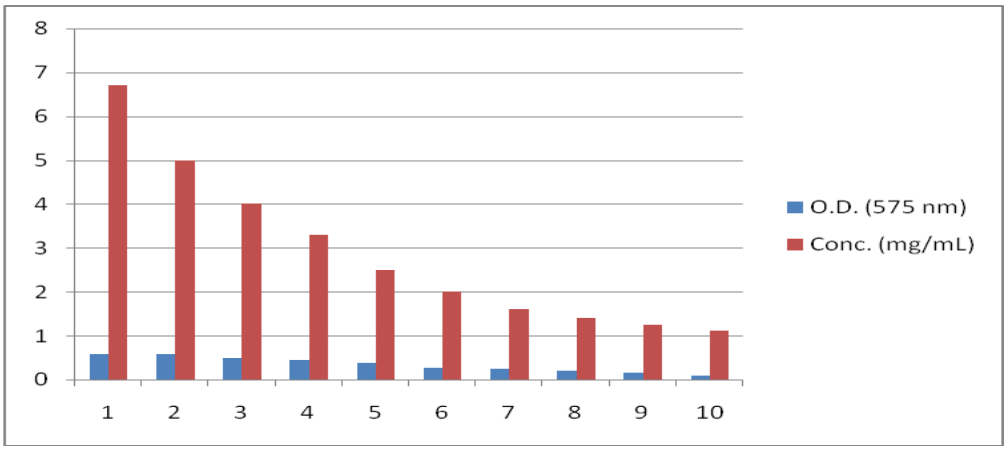
**Iodometric Assay for Alcohol Determination:** 1 mL broth was taken after centrifugation and mixed with 4 mL of distilled water. 10 mL of 0.2 N Potassium dichromate ($K_2Cr_2O_7$) was added into the 5 mL system and incubate for 30 minutes in boiling water bath. After cooling 4 mL of 20% KI was added and was titrated against 0.1 N sodium thiosulphate solution using 1% starch as an indicator. Ethanol content was measured from the standard curve.

## Results & Discussion:

### ❖ Standard Graph for Sugar Estimation:

| No. | Glucose Stock (mL) | Citrate Buffer (mL) | O.D. (575 nm) | Conc. (mg/mL) |
|---|---|---|---|---|
| **1** | 1.0 | 0.5 | 0.579 | 6.7 |
| **2** | 1.0 | 1.0 | 0.572 | 5.0 |
| **3** | 1.0 | 1.5 | 0.493 | 4.0 |
| **4** | 1.0 | 2.0 | 0.440 | 3.3 |
| **5** | 1.0 | 3.0 | 0.382 | 2.5 |
| **6** | 1.0 | 4.0 | 0.274 | 2.0 |
| **7** | 1.0 | 5.0 | 0.242 | 1.6 |
| **8** | 1.0 | 6.0 | 0.191 | 1.4 |
| **9** | 1.0 | 7.0 | 0.153 | 1.25 |
| **10** | 1.0 | 8.0 | 0.099 | 1.11 |

**Table-1: Standard graph readings for Sugar estimation**

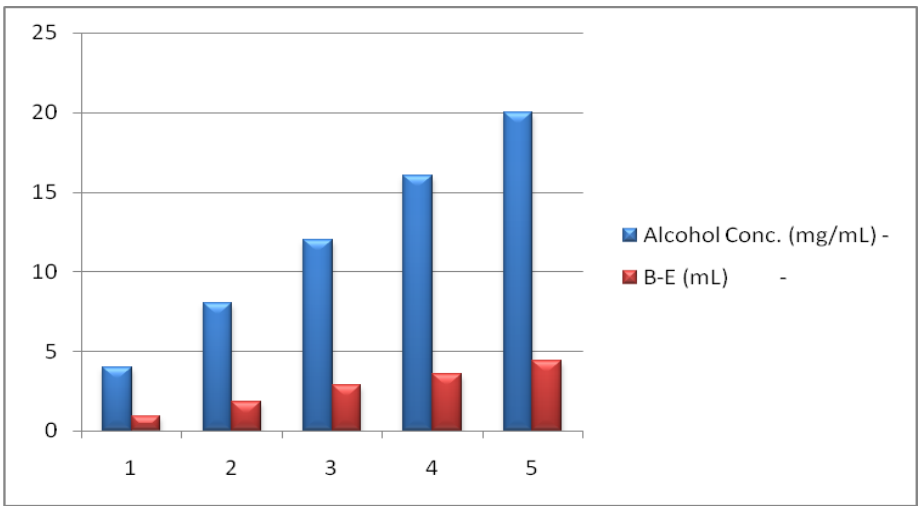



**Graph 1: Standard graph of Sugar estimation**

❖ **Standard Graph for Ethanol Estimation:**

| No. | Alcohol Aliquots (mL) | Alcohol Conc. (mg/mL) | Final Vol. with D.W. | $K_2Cr_2O_7$ (mL) | Incubation | 20% KI (mL) | 1% Starch | 0.1 N $Na_2S_2O_3$ (mL) | B-E (mL) |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 0.0 | - | 5.0 | 10.0 | | 4.0 | | | |
| **2** | 1.0 | 4 | 4.0 | 10.0 | 30 Minutes | 4.0 | Few | Until | 0.9 |
| **3** | 2.0 | 8 | 3.0 | 10.0 | & | 4.0 | Drops | Color | 1.8 |
| **4** | 3.0 | 12 | 2.0 | 10.0 | At Room Temperature | 4.0 | | Changes | 2.9 |
| **5** | 4.0 | 16 | 1.0 | 10.0 | | 4.0 | | To light blue | 3.6 |
| **6** | 5.0 | 20 | 0.0 | 10.0 | | 4.0 | | | 4.4 |

**Table-2: Standard graph readings for Ethanol estimation**

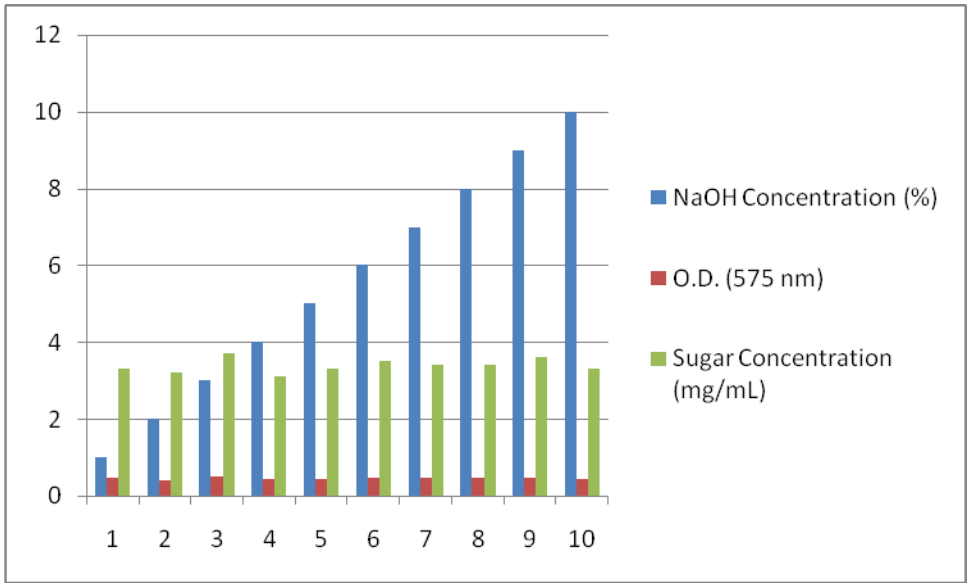



**Graph 2: Standard graph of Ethanol estimation**

❖ **Different Concentration of NaOH Treatment to Sawdust:**

| No. | NaOH Concentration (%) | O.D. (575 nm) | Sugar Concentration (mg/mL) |
|---|---|---|---|
| **1** | 1 | 0.454 | 3.3 |
| **2** | 2 | 0.413 | 3.2 |
| **3** | **3** | **0.490** | **3.7** |
| **4** | 4 | 0.421 | 3.1 |
| **5** | 5 | 0.447 | 3.3 |
| **6** | 6 | 0.471 | 3.5 |
| **7** | 7 | 0.456 | 3.4 |
| **8** | 8 | 0.458 | 3.4 |
| **9** | 9 | 0.481 | 3.6 |
| **10** | 10 | 0.446 | 3.3 |

**Table-3: Results of Pretreatment with different conc. of NaOH**

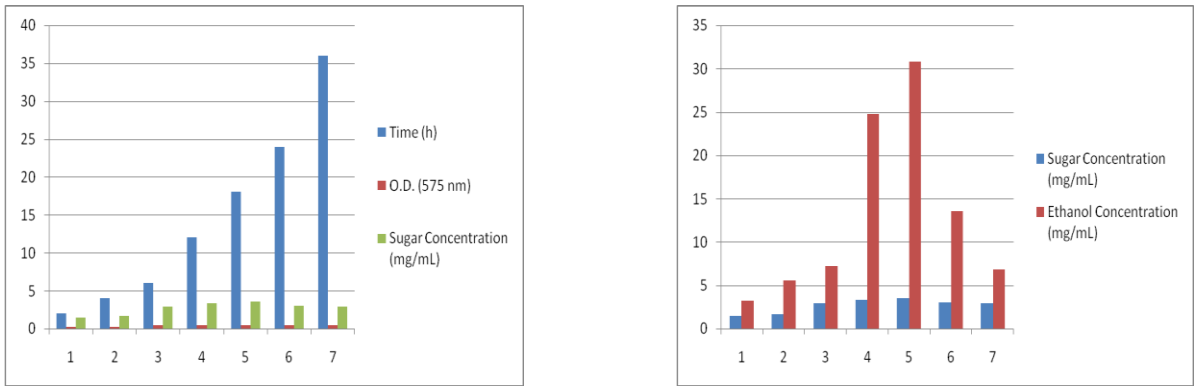



**Graph 3: Results of Pretreatment with different concentration of NaOH**

❖ **Time Standardization for Effective Saccharification and Ethanol Production**

| No. | Time (h) | O.D. (575 nm) | Sugar Concentration (mg/mL) | Ethanol Concentration (mg/mL) |
|---|---|---|---|---|
| **1** | 2 | 0.205 | 1.50 | 3.2 |
| **2** | 4 | 0.235 | 1.70 | 5.6 |
| **3** | 6 | 0.400 | 2.95 | 7.2 |
| **4** | 12 | 0.440 | 3.30 | 24.8 |
| **5** | **18** | **0.480** | **3.55** | **30.8** |
| **6** | 24 | 0.405 | 3.00 | 13.6 |
| **7** | 36 | 0.390 | 2.90 | 6.8 |

**Table-4: Effectiveness of Saccharification process for different time and Ethanol production**




**Graph 4: Effectiveness of Saccharification process for different times and Ethanol production**

The results of pretreatment to sawdust with different concentration of NaOH show that treatment with 3% NaOH was found to be effective for the hydrolysis of cellulosic materials i.e sawdust and after enzymatic treatment with crude cellulase yields sugar concentration of 3.7 mg/mL. The results of time standardization for effective saccharification are shown in the **table-4** which indicate that after 18 h of enzymatic treatment maximum fermentable sugars were released and was responsible for the production of 30.8 mg/mL of ethanol which is similar to the result of J.N. Nwakaire *et al.* who had noted the production of ethanol 30.9 mg/mL.

**Conclusion:**

It can be concluded that the ethanol production from sawdust is possible which would replace the edible ethanol producing sources. Ethanol production from sawdust was found to be around 30.8 mg/mL which is low compared to ethanol production from starchy materials. So it cannot replace saccharine and starchy materials as the substrate for ethanol production but can be act as a substitute for the grain based ethanol production because of its abundance and easy accessibility. Moreover, ethanol production from cellulosic feed stocks would reduce green house gas emission of about 80% which is very high compared to corn based ethanol i.e. 20-30%.

**References:**


G.L. Pederson, G.A. Screws, D.M. Cedroni. (**1992)**.Can. Textile J., 31-35.

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

Lone M.A., Wani M.R., Bhat N.A., Sheikh S.A., Reshi M.A. (**2012**).Evaluation of cellulase enzyme secreted by common and stirring Rhizosphere fungi of Juglans regia L. by DNS method. Journal of enzyme research,Vol. 3, issue 1.

Lynd LR, Jin H, Michels JG, Wyman CE, Dale B. (**2003**). Bioenergy: background, potential and policy. Centre for Strategic and International Studies, Washington, D.C.

Miller GL. (**1959**).Use of Dinitrosalicyclic acid for the determination of reducing suga. Anal chem. 31: 426-428.

Nwakaire J.N., Ezeoha S.L., Ugwuishiwu B.O. (**2013).**Production of cellulosic ethanol from wood sawdust. Agric Eng Int: CIGR Journal; Open access at http://www.cigrjournal.org Vol. 15, No.3.

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

Sridevi A, Narasimha G, Ramanjaneyulu G, Dileepkumar K, Rajasekhar B, Suvarnalatha Devi P.( **2015**). Saccharification of pretreated sawdust by Aspergillus niger cellulose, Springer**.**

Wan C, Li Y. (**2011**).Effectiveness of microbial pretreatment by *Ceriporiopsis subvermispora* on different biomass feedstocks. Bioresour Technol; 102:7507–7512.

Zhao X, Cheng K, Liu D. (**2009).**Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. Appl Microbiol Biotechnol.82(5):815–827.