

# Production of Ethanol and Biomass from Rice Husk Using Cultures of *Aspergillus flavus*, *Aspergillus eamarii* and *Saccharomyces cerevisiae*

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**Abstract** Microbial degradation/conversion of agricultural/industrial wastes to useful bio-products such as bioethanol has become increasingly a popular alternative to gasoline worldwide and has been proven to be useful in many industrial and pharmaceutical processes. In this work, microbial hydrolysis of rice husk into fermentable sugars using *Aspergillus flavus* and *Aspergillus eamarii* and subsequent fermentation of sugars to ethanol was achieved using *Saccharomyces cerevisiae*. The rice husks were ground to fine powder and microbially hydrolyzed by incubating with *Aspergillus flavus* and *Aspergillus eamarii* in peptone water for 7 days. Determination of reducing sugar using Fehlings' reagents confirmed the presence of fermentable sugar in both media. Approximately 6.89° Brix in *Aspergillus flavus* medium was optimized to 24.41° Brix while 7.12° Brix in *Aspergillus eamarii* was optimized to 24.10° Brix by adding 300g of sucrose and then fermented to ethanol using *Saccharomyces cerevisiae* isolated from palm wine at room temperature. The results showed a reduction in pH (6.5-4.1 and 6.8-4.1), an increase in temperature (28-30°C and 28-30°C), increase in percentage titratable acidity (0.33-2.96% and 0.27-2.90%), significant increase in alcohol content (0.00-14.89% and 2.42-14.47%), increase in biomass (0.00-2.16g/l and 0.00-2.08g/l) and decrease in specific gravity (1.112-1.005 and 1.114-1.007) in *Aspergillus flavus* and *Aspergillus eamarii* media respectively over a period of 7 days of fermentation. *Aspergillus flavus* and *Aspergillus eamarii* hydrolysates yielded 14.89% and 14.47% ethanol respectively after distillation. The results of the experiment conducted shows that rice husk and other cellulosic agricultural wastes could be potential substrates which can be exploited by industries for production of bioethanol and other biotechnological products.

**Keywords:** rice husk, ethanol, biomass, *Aspergillus*, *saccharomyces*, hydrolysis

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## 1. Introduction

The sharp increase in the price of petroleum products, the finite nature of fossil fuels, growing concerns especially related to greenhouse gas emissions and health safety considerations have resulted in increased search for new energy sources and alternatives to power the worlds motor vehicles as well as generating electricity [1]. Ethanol is a clean-burning renewable resource that can be produced from fermented cellulose biomass. Ethanol does not add to a net carbon(iv)oxide atmosphere increase thus these is no contribution to global warming. Combustion of ethanol results in relatively low emissions of volatile organic compounds, carbon monoxide and nitrogen oxides. The importance of the biomass based ethanol production has undergone a huge increase in the last few years [2]. However, further cost reduction is still essential for the development of this new technology.

Lignocellulosic materials represent a promising option as a feed stock for ethanol production considering their

output/ input energy ratio, their great availability and their ethanol yields one of the advantages of the use of lignocellulosic biomass is that it is not directly related to food production. This implies the production of bioethanol without the need of employing vast extension of fertile cultivable land. In addition, lignocellulosic is a resource that can be processed in different ways for production of many other products like synthesis gas, hydrogen, and electricity [3].

Rice is the seed of the grass species *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice). As a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. Rice is the most important grain with regard to human nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide by humans. Genetic evidence has shown that rice originates from a single domestication 8,200-13,500 years ago in the Pearl River valley region of China. Rice is the agricultural commodity with the third highest worldwide production after sugarcane and maize, Nigeria being seventeenth in the world [4].

Ethanol from biomass has become an increasingly popular alternative to gasoline. However, the production of bioethanol from food crops such as grains (first generation biofuels) has resulted in an undesirable direct competition with food supply. A switch to a more abundant inedible plant material should help to reduce pressure on the food crops. Large parts of these plant materials are made up of complex carbohydrates such as cellulose and hemicelluloses which can be converted to fermented sugars. Ethanol-fermenting microorganism can utilize these sugars and convert into ethanol [5].

Rice husk has several characteristics that make it a potential feedstock for biofuel production. It has high cellulose and hemicellulose content that can be readily hydrolyzed into fermentable sugars.

The aim of this research was to utilize rice husk (waste) as a source of single cell (biomass) for ethanol production using *Aspergillus flavus*, *Aspergillus eamarii*, and *Saccharomyces cerevisiae*.

To achieve this aim, the following objectives were sought:

1. To saccharify rice husk (cellulose) using cultures of *Aspergillus flavus* and *Aspergillus eamarii*.
2. To generate biomass and ethanol using *Saccharomyces cerevisiae*.

## 2. Materials and Methods

### 2.1. Materials

Laboratory facilities and reagents were sourced from the Central Laboratory Services Unit, Natural Root Crops Research Institute, Umudike.

### 2.2. Methods

#### 2.2.1. Collection of Materials

The rice husk was obtained from a Rice mill in Bende Local Government Area of Abia State, and Palm wine yeast was isolated in the Central Laboratory Services Unit of NRCRI, Umudike. The test organisms were obtained from the Department of Biotechnology, Federal Institute of Industrial Research, Oshodi, Lagos State, Nigeria.

#### 2.2.2. Pre-treatment of Lignocellulosic Material

The rice husk substrates were washed with distilled water and sun dried for 2 days to reduce moisture content and ease grinding. They were finely grounded to powder using a mechanical grinding machine.

#### 2.2.3. Sample/Media Preparation

The media used in the pre culture were prepared in accordance with manufacturer's instruction (Biomark India). 20grammes of peptone powder was dissolved in 1000ml of distilled water and sterilized by autoclaving. It was allowed to cool to room temperature before it was used. The same was done to 20grammes of sugar. After cooling, a loopful each of *Aspergillus flavus* and *Aspergillus eamarii* were respectively inoculated into the sterilized sugar solution and peptone water. They were incubated for 7days.

#### 2.2.4. Preparation of Fermentation Medium

The test organism, *Saccharomyces cerevisiae*, was activated, a sterile loop each in 100ml of sugar solution and 100ml of peptone water.

#### 2.2.5. Hydrolysis of Rice Husk Substrate

The test rice husks were weighed out in 500g portions into different plastic vats and labeled accordingly. They were sterilized by autoclaving at 121°C for 15minutes (to get rid of microbial impurity in the husk). The sterile rice husks were allowed to cool to room temperature and incubated with the different enzyme producing organisms, *A. flavus* (Hydrolysate A) and *A. eamarii* (Hydrolysate B), in different fermentation vats. They were incubated for 8days during which they were observed daily.

#### 2.2.6. Specific Gravity

Specific gravity was determined by the pycnometer gravimetric method [6]. The pycnometer (specific gravity bottle) was weighed while it was clean and dry but with its stopper in place-  $W_a$ . It was then filled with water, up to the capillary of the stopper. The outside was wiped with a dry blotting paper. It was weighed filled with water-  $W_w$ . The water was poured out and the flask was dried without heating. It was then filled with the sample solution and weighed-  $W_s$ . The specific gravity was determined as shown below.

$$S.G = \frac{W_s - W_a}{W_w - W_a}$$

Where  $W_a$  = weight of empty specific gravity bottle.

$W_s$  = weight of bottle + sample.

$W_w$  = weight of bottle + water.

#### 2.2.7. Determination of Reducing Sugar and Glucose Optimization

The presence of reducing sugar in the hydrolysates was determined using Fehling's solution and Anthrone reagent as described by Onwuka [7]. After glucose determination, Brix contents of 6.89 and 7.12 were respectively increased to a concentration of 24.41°Brix and 24.10°Brix by adding 100g sucrose.

#### 2.2.8. Determination of Titratable Acidity

Exactly 5ml of each hydrolysate was mixed with 45ml of distilled water and 3drops of phenolphthalein was added as indicator, as described by Mbajuka *et al.* [8]. It was titrated against 0.1M NaOH solution to an end point marked by a pink coloration which lasted for 15seconds and beyond. The formula below was used to calculate the TTA.

$$\% TTA = \frac{100 \times N \times \text{Titre}}{V}$$

Where N = normality of titrant

V = volume of sample used

## 2.3. Production of Alcohol/Biomass

The prepared fermentation media were used to inoculate the hydrolysates. *S. cerevisiae* prepared in sugar solution was used to inoculate *A. flavus* hydrolysate in Vat

A while *A. eamarii* hydrolysate in Vat B was inoculated with *S. cerevisiae* in peptone water. Both vats were allowed to incubate for 7 days at room temperature during which samples were aseptically collected and analyzed for alcohol content, biomass, and sugar. Sampling was done at 24hours interval beginning from the second day until the end of the fermentation. After the 7day period, distillation was carried out to recover the alcohol produced.

**2.3.1. Assessment of Alcohol Quality**

The produced alcohol was analyzed to determine its physical properties, which may confirm its identity as ethanol. The properties tested included boiling point and specific gravity.

**2.3.2. Determination of Temperature and pH**

Temperature of the sample was determined using a thermometer while pH was allowed to equilibrate for 10minutes. It was calibrated with buffered solutions of pH 4.0 and 7.0. To take reading, the electrode of the pH meter was inserted into the ethanol in a beaker and its pH value was read on the screen of the meter. Reading was taken when the figures were steady.

**2.3.3. Sugar Uptake**

Changes in fermentable sugar were monitored by method of specific gravity and Anthrone reagent. A fall in the specific gravity indicates sugar uptake. Values in Brix were read from a table.

**2.3.4. Determination of Biomass**

Biomass formation was monitored using a spectrophotometer. The sample was diluted with distilled water and intensity of light in the medium was measured at a wavelength of 620nm as described by Onwuka [7]. The biomass concentrations of the media were recorded from the calibration curve read-out of the electronic reader.

**2.3.5. Determination of Alcohol Yield**

Alcohol yield was determined volumetrically as the ratio of the volume of alcohol produced to the quality of fermented substrate distilled [8]. A measured quantity of each fermented rice husk hydrolysate was mixed with equal value of distilled water and transferred into a large bottom distillation flask. The flask was connected to the condenser and its thermometer was slotted into position while the flask stood in the heating mantle. As the mixture boiled, the temperature was noted and the thermometer was constantly observed for any change. Meanwhile the recovery outlet tube (through which the alcohol flows) was inserted into a receiver flask with the tube passing through a stopper with large barrier. The receiver flask was corked so that escape of received alcohol will be to the minimum. When the temperature started rising and the base of the condenser became misty, the distillation was stopped and the volume of alcohol was measured. The alcohol yield was calculated using the formula below.

$$\% \text{ yield} = \frac{\text{VE} \times 100}{\text{VF}}$$

Where VE = volume of alcohol distilled off.  
VF = volume of fermented hydrolysate distilled.

**3. Results**

The alcohol content and biomass of fermenting Rice husk produced were recorded as shown in Table 1 and Table 2. Table 3 shows the rate of glucose uptake during fermentation. The results show a mean value of 7.12 °Brix as sugar content after hydrolysis with pH 6.8 for *Aspergillus eamarii* (Table 1), and 6.89 °Brix as sugar content and pH 6.5 for *Aspergillus flavus* (Table 2). The pH of the medium during fermentation period decreased from 6.5 to 4.1 in *Aspergillus flavus* hydrolysate and 6.8 to 4.1 in *Aspergillus eamarii* hydrolysate with increased acidity as shown in Figure 1.

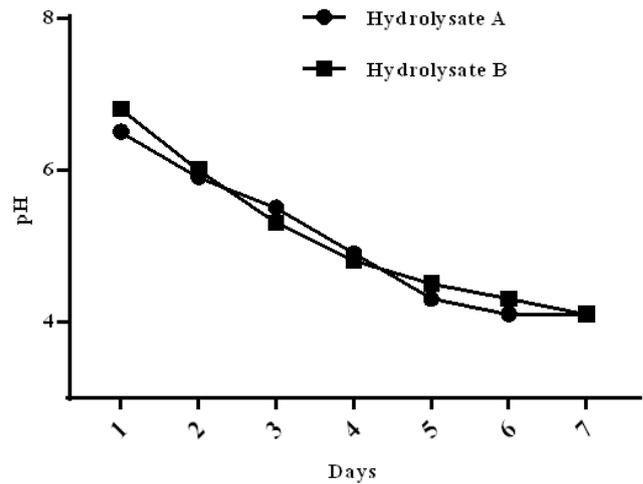


Figure 1. Changes in pH during the fermentation period

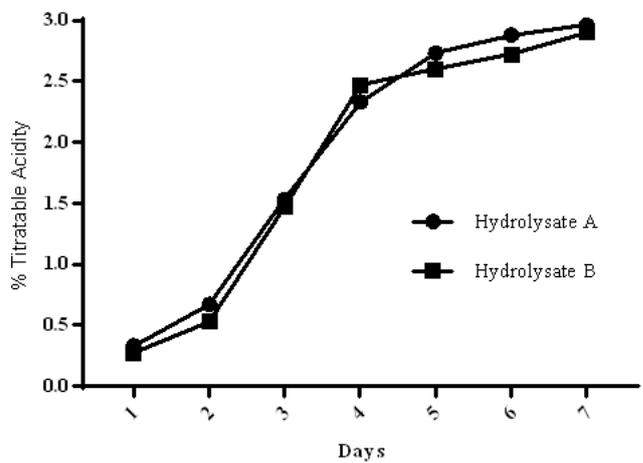


Figure 2. Changes in Titratable acidity

Table 1. Change in parameters during fermentation in Hydrolysate A

Days	PH	TTA(%)	A.C(%)	Biomass (g/l)	TEMP (°C)	S.G
1	6.5	0.33	0.00	0.00	29	1.112
2	5.9	0.67	2.68	0.72	28	1.085
3	5.5	1.53	5.94	1.18	30	1.061
4	4.9	2.33	8.69	1.64	30	1.058
5	4.3	2.73	11.68	1.91	30	1.032
6	4.1	2.88	13.88	2.04	29	1.021
7	4.1	2.96	14.89	2.16	30	1.005

TTA= Titratable acidity, SG= Specific gravity  
TEMP= Temperature, A.C = Alcohol Content.

**Table 2. Change in parameters during fermentation in Hydrolysate B**

Days	PH	TTA(%)	A.C(%)	Biomass (g/l)	TEMP (°C)	S.G
1	6.8	0.27	2.42	0.00	28	1.114
2	6.0	0.53	6.18	0.58	29	1.095
3	5.3	1.47	9.52	0.96	29	1.082
4	4.8	2.47	12.12	1.33	30	1.073
5	4.5	2.60	13.64	1.52	30	1.042
6	4.3	2.72	13.93	1.73	29	1.028
7	4.1	2.90	14.47	2.08	30	1.007

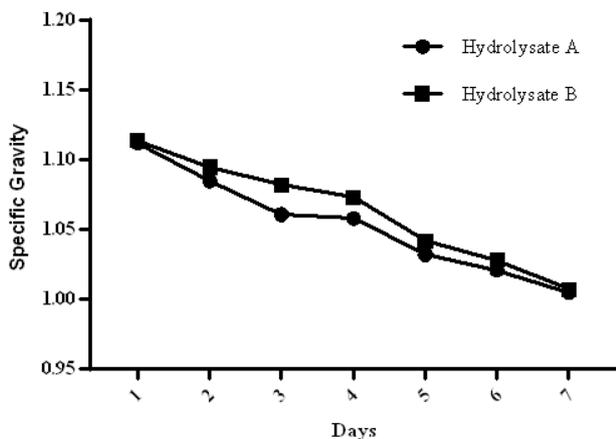
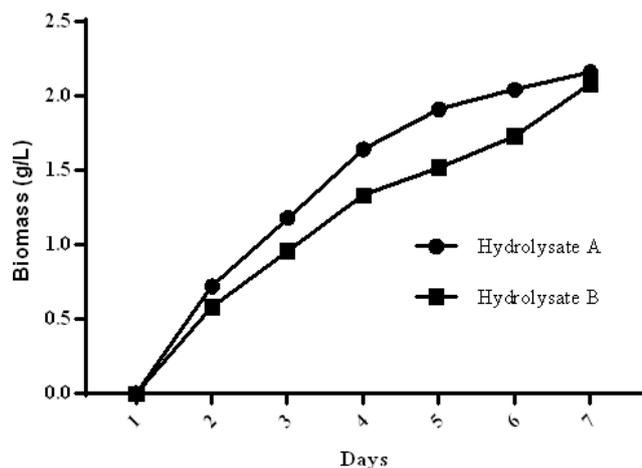
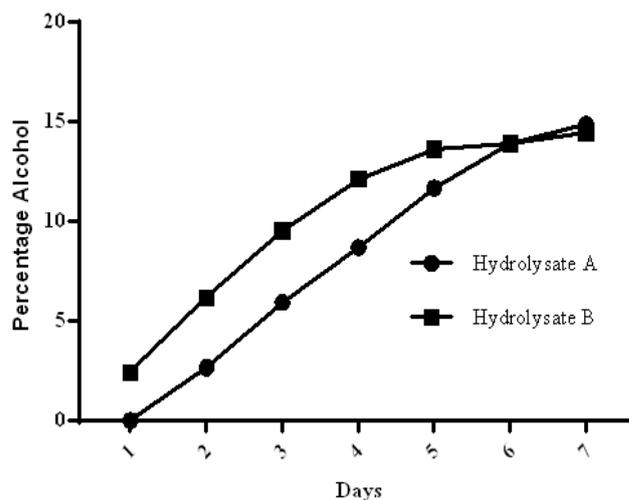
TTA= Titratable acidity, SG= Specific gravity  
TEMP= Temperature, A.C = Alcohol Content.

**Table 3. Concentration of Glucose (Brix Content) During Fermentation**

Days	1	2	3	4	5	6	7
Hydrolysate A (%)	24.41	18.67	15.59	10.15	6.42	4.13	1.92
Hydrolysate B (%)	24.10	18.15	15.79	9.95	2.36	2.15	1.38

In the *Aspergillus flavus* hydrolysate, total titratable acidity increased during fermentation from 0.33% to 2.96% while it increased from 0.27% to 2.90% in the *Aspergillus eamarii* hydrolysate, as shown in Figure 2.

Changes in specific gravity, biomass and alcohol concentration of both medium are shown in Figure 3, Figure 4 and Figure 5 respectively.

**Figure 3. Changes in Specific Gravity****Figure 4. Changes in Biomass Production****Figure 5. Changes in Alcohol Production**

## 4. Discussion

In this work, the production of bioethanol from agricultural waste was carried out. A constant weight obtained after drying the rice husks under the sun for two days suggests that sun-dry method is good in removing the moisture content of the rice husks though blasting in hot air oven will be more efficient in large scale business and during wet seasons.

Patel *et al.* [9] observed that insufficient drying of the rice husk would encourage fungal growth and cause the husks to lose some of its sugars. Enzyme-producing microorganisms are capable of breaking down cellulose as also observed by Sun and Cheng [10]. Hydrolysis of ground rice husks with *Aspergillus flavus* and *Aspergillus eamarii* gave a sugar yield of 6.89°Brix and 7.12°Brix respectively.

Treatment of cellulosic materials with *Aspergillus* species disrupts the hydrogen bonding between cellulose chains, releasing glucose. About 300g of sugar added to the hydrolysate increased the concentration of fermentable sugar from 6.89°Brix to 24.41°Brix and 7.12°Brix to 24.10°Brix in hydrolysates A and B respectively. *Saccharomyces cerevisiae* from palm wine were capable of growing in the medium and fermenting the sugar to ethanol and carbon dioxide (CO<sub>2</sub>). The glucose in the medium is catabolized via the Embden Meyerhof-Parnas (EMP) pathway or glycolytic pathways to pyruvate as indicated by Hough *et al.* [11]. The pyruvate is then decarboxylated by pyruvate decarboxylase with the formation of aldehyde and CO<sub>2</sub> and enzyme use a co-factor thiamine pyrophosphate (TPP) for activity. The acetaldehyde thus acts as an electron acceptor and is used to oxidize NADH with the formation of ethanol. The fermentation was observed to be taking place by formation of bubbles after 24hours of set up which indicates the release of CO<sub>2</sub> and an alcoholic flavor. These observations were also reported by Mbajiuka *et al.* [8] on fermentation of Sorghum using *S. cerevisiae* for Burukutu production. By the end of fermentation, sugar content reduced from 24.41°Brix to 1.92°Brix in hydrolysate A and 24.10°Brix to 1.38°Brix in hydrolysate B (Table 3). When specific gravity was determined, it was found to be decreasing in

both hydrolysates and this is a direct indication of the metabolic activities of the yeast carrying out fermentation (Figure 3). The release of CO<sub>2</sub> caused the pH of each medium to decrease from 6.5 to 4.1 and 6.8 to 4.1 in hydrolysates A and B respectively (Figure 1) and was within the range of pH changes during beer and wine production as reported by Idise [12] and Karimi *et al* [13]. The sweet wine flavor produced by yeast during fermentation suggests that the rice husks could be fermented to produce sweet wine. A close observation of the temperatures revealed fluctuations in mediums A and B between 28°C (Table 1) and 30°C (Table 2) and is widely accepted as the optimal temperature for growth of yeasts. A fall in the temperature was observed during fermentation and could be attributed to environmental factors and other factors that may affect fermentation [14]. However, the temperature range is important in regulating the enzymatic activity of the yeast because too low temperature slows down their metabolic activities while too high temperatures may denature the enzymes [14]. The continual increase in biomass in A and B, from 0.72 to 2.16 (Table 1), and 0.58 to 2.08 (Table 2) respectively (Figure 4), is in agreement with the definition of fermentation by Taouda *et al.* [15]. These organisms, when recovered, could serve as fuel, animal feeds, or single cell proteins (SCPs). Due to high solubility of these cells in liquids, immobilization could serve a better means of recovering them for effective use [5].

The fermentation was completed on the 7th day as no observable change was noticed on the fermentation medium such as bubbles of CO<sub>2</sub>. This observation suggested maximum ethanol production and corresponds with observations of Patel *et al.* [9]. Following the method described by Hough *et al.* [11], 14.89% and 14.47% ethanol was produced in hydrolysates A and B respectively (Figure 5) by the end of fermentation. This corresponds with the findings of Mbajiuka *et al.* [16]. The general short comings of batch fermentation described by Reed [17], may also affect yield, suggesting continuous fermentation, increase of the yeast population by recycling and the removal of ethanol during the fermentation as a better method. Separating the ethanol from the fermentation liquid by partial distillation recovered about 50% ethanol by volume which was reported by Mbajiuka *et al.* [8] on cassava waste.

Further distillation of the sample under controlled conditions could produce a higher percentage of ethanol.

## 5. Conclusion

The utilization of lignocellulosic biomass for bioethanol production necessitates the production technology to be cost effective and environmentally sustainable. Rice husk appears a promising and potent candidate for production of bioethanol due to its abundant availability and attractive composition. Biological conversion of waste into fermentable sugars, employing microorganisms and hydrolyzing enzymes is at present the most attractive alternative due to environmental concerns. Rice husks, a

waste material can therefore be utilized for production of ethanol, biomass, and other value added products.

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