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# Studies on Reducing Sugar Yields Produced from Corn Cob and Corn Stalk Hydrolysis Using Aspergillus Niger

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***Abstract:*** *Over the years, managing agricultural wastes after harvest in developing countries like Nigeria has been a major challenge and continue to generate environmental concerns. To address this concerns, these agricultural wastes in the form of lignocellulosic biomass are burned. This approach in turn, had led to a greater environmental interest as green house gases an emission to the atmosphere is increased. The attendant implications of this are significant contribution to ozone layer depletion and elimination of soil micro flora present. Interestingly, these waste are potential precursors for biofuel production. This study was undertaken to investigate the reducing sugar yields of two lignocellulosic biomass wastes (corn stalk and corn cob) as biofuel precursors. The findings of the study showed that the optimal reducing sugar yields as product of hydrolysis using Aspergillus Niger isolated from soil after 72 hours of hydrolysis were 2.9 mg/ml and 3.0 mg/ml for corn cob and corn stalk, respectively.*

***Keywords****: Aspergillus Niger, Reducing Sugar, agricultural waste and hydrolysis, lignocellulosic biomass.*

## 1. INTRODUCTION

Long-term economic and environmental concerns are some of the key factors attributing research focus in the past couple of decades on alternative and renewable sources of fuels. In recent times, the bioconversion of agricultural and industrial wastes to biofuels has led to extensive research on cellulolytic enzymes produced by fungi and bacteria [1]. One area that generates these wastes (lignocellulosic biomass) is the agricultural sector. Most of the agro-wastes after harvest are allowed to rot away and are not utilized [2]. Lignocellulosic biomass comprising forestry, agricultural and agro-industrial wastes are abundant, renewable and inexpensive energy sources. The main components of lignocelluloses are celluloses (35–50%), hemicelluloses (25–30%), and lignin (25–30%) [3]. Cellulose constitutes the highest percentage because it is a strong elastic material that makes up the cell wall of nearly all plants [4]. The cellulose can be hydrolyzed to produce simple sugars for human needs. The simple sugars are then used as substrates for fermentation to produce biofuels [3].

## 2. PRODUCTION

Production of reducing sugars for fermentation purposes is still a costly process [5] and bioconversion of agricultural wastes biomass to produce value-added fuels and chemicals offers potential economic, environmental and strategic advantages over traditional fossil-based products [6]. The digestibility of cellulose present in lignocellulosic biomass is hindered by many physicochemical, structural, and compositional factors. In the conversion of lignocellulosic biomass to fuel, the biomass needs to be treated so that the cellulose in the plant fibers is exposed. Pretreatment uses various techniques, including ammonia fiber explosion, chemical treatment, biological treatment, and steam explosion. This is to alter the structure and composition of the cellulosic biomass to make cellulose more accessible [4] [7]. It also removes lignin and hemi-cellulose, reduce cellulose crystallinity and increase its porosity [8].

Many microorganisms have been evaluated for the production of enzymes for hydrolysis of lignocelluloses. The bacteria species use for hydrolysis includes Bacillus licheniformis, Corynebacterium spp. (Kapoor et al., 1983) [9]. While fungi species use for hydrolysis include such as Aspergillus Niger, A. awamori, A. foetidus, Penicillium restrictum [10] [11]. However, Aspergillus Niger a filamentous fungus remains the organism of choice for hydrolysis. This is due to ease of handling, its ability to hydrolyze a variety of cheap raw materials, and high yields [12]. Corncob and corn stalk consist of polymers of mainly two types of sugars: glucose and xylose. Both sugars can be obtained, in monomeric forms, with high yield after pretreatment and subsequent hydrolysis [13]. The main aim of this study was to produce reducing sugars for fermentation from a cheap commonly available agricultural waste (corn cob and corn stalk) using Aspergillus Niger and to compare and evaluate the two lignocellulosic biomass in terms of reducing sugar yields after hydrolysis.

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## 3. MATERIAL AND METHODS

### 3.1 COLLECTION OF SAMPLES

Corn cobs and corn stalks were obtained from farmlands in Zaria, Nigeria. They were sun dried for 7 days.

### 3.2 ISOLATION OF ASPERGILLUS NIGER FROM SOIL

Isolation of Aspergillus niger from soil was carried out according to the method described by Ijah [14]. Serial dilution of soil samples was carried out and dilutions of $10^{-6}$ to$10^{-9}$ were inoculated onto solidified Sabouraud's Dextrose Agar (SDA) prepared according to manufacturer's instructions. The medium was supplemented with 100mg/100ml of chloramphenicol to suppress bacteria growth using sterile bent glass rod and incubated at room temperature for a period of seven days. The growth of fungal colonies was observed after the incubation period. Discrete colonies were sub cultured and transferred to Sabouraud's Dextrose Agar (SDA) slants and stored at $4^0$C for further study.

### 3.3 PRESERVATION OF FUNGAL ISOLATES

After the incubation period, pure fungal colonies was obtained by subculturing distinct colonies from the culture plate onto fresh solidified Sabouraud's Dextrose Agar (SDA) plates and incubated for another four days after which each pure culture of the fungal colonies was subcultured onto freshly prepared Sabouraud's Dextrose Agar (SDA) slant supplemented with 100mg/100ml of chloramphenicol to suppress bacteria growth and preserved for further analysis [15].

### 3.4 IDENTIFICATION AND CHARACTERIZATION OF ASPERGILLUS NIGER

Pure colonies from the preserved Sabouraud's Dextrose Agar (SDA) slant were used to identified and characterized. Aspergillus niger. Macromorphological characteristics such as colour, texture of the reversed side of the colony were observed and recorded. Also, micromorphological characteristics was observed by mounting a small portion of the fungal growth on a clean grease free slide with a drop of lactophenol cotton blue stain and covered with cover slip. The slide was observed microscopically using x40 objective lens [16]. Characteristics of the sexual reproductive structures, presence or absence of septation, spore and chlamydospore were observed and recorded. Finally, the features of fungal isolate obtained was carefully compared with appropriate taxonomic guide as described by [17]; [18]; [19]; [20].

### 3.5 PRE-TREATMENT OF SUBSTRATES

The corn cobs and stalks were oven dried at $50^0$C to reduce the moisture content to make them more susceptible for crushing. They were then reduced to very small sized particles by grinding. The particles were then sieved to obtain an average particle size of 300μm for each sample. Fifty grams (50g) of the substrates were soaked individually in 500ml of 20% concentrated sulphuric acid in a 1000ml flask. The flasks with the contents were then autoclaved at $121^0$C for 20 minutes after which it was cooled to room temperature. The treated substrates were then filtered and the hydrolysates were allowed to dry [8].

### 3.6 PREPARATION OF HYDROLYSIS MEDIUM

Freshly prepared inoculum of Aspergillus niger (with a count of $8.9x10^8$) was inoculated into 100 ml of optimized Mandel and Sternberg's mineral medium $(NH_4)_2SO_4$ 0.14 gm, $KH_2PO_4$ 0.2 gm, $CaCl_2$ 0.03 gm, $MgSO_4$ 0.03 gm, $FeSO_4$ 0.50 gm, $MnSO_4$ 0.16 gm, $ZnSO_4$ 0.14gm, Substrate 5gm, Distilled water 100 ml). The substrates were inoculated in separate flasks. All flasks were incubated at $30^0$C on a rotary shaker at 150rpm for 14days. The reducing sugar concentration was measured at regular intervals [8].

### 3.7 ESTIMATION OF GLUCOSE STANDARD

A glucose standard curve was prepared by dissolving 100mg of D-glucose in 100ml of distilled water. From the working solution, 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1.0ml were collected and made up to 1ml using distilled water this gives a concentrations of (0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1.0mg/ml). The mixture was boiled for 5 minutes, then, cooled and 10ml of distilled water was added. After the homogenization of reaction mixture, the absorbance was determined at 540nm. The relationship between glucose concentration and absorbance at 540nm was plotted to determine the glucose standard curve.

### 3.8 REDUCING SUGAR DETERMINATION BY DINITROSACLICYLIC ACID METHOD (DNS METHOD)

The concentration of the reducing sugar present in the samples was determined by adding 1ml of DNS acid to 1ml of each of the samples and boiled for 5 minutes after which 10ml distilled water was added. The absorbance of each of sample was determined at 540nm using HACH portable spectrophotometer. Thus, the concentration values were extrapolated from the glucose standard curve [8].

## 4. RESULTS

### 4.1 IDENTIFICATION AND CHARACTERIZATION OF THE ASPERGILLUS NIGER

Identification and characterization of the Aspergillus niger was carried out using taxonomic guide as described by [17]; [18]; [19]; [20]. Macroscopic features of the Aspergillus niger isolates revealed colonies that appeared wooly, at first whitish and later turn black. The microscopic features revealed conidiophores that appeared smooth and long with biserate that covers the entire vesicle to form a radiate head.

### 4.2 ESTIMATION OF REDUCING SUGAR IN CORN COB AND CORN STALK

Table 1.0 present result of the level of reducing sugar yield of the two agricultural substrates (corn cob and corn stalk) over a period of 14 days. The level of reducing sugar yields of the two substrates was extrapolated from the glucose standard curve presented in figure 1.0 below.

The hydrolysis of corn cob and corn stalk using cellulase enzyme produced by the Aspergillus niger yielded the following ranges of reducing sugar; corn cob 0.216 to 2.896mg/ml and corn stalk 0.360 to 2.977mg/ml. Similar reducing sugar yields of 0.0276-2.400mg/ml was reported by [21] using cellulase produced by Aspergillus niger and saw dust as a substrate.

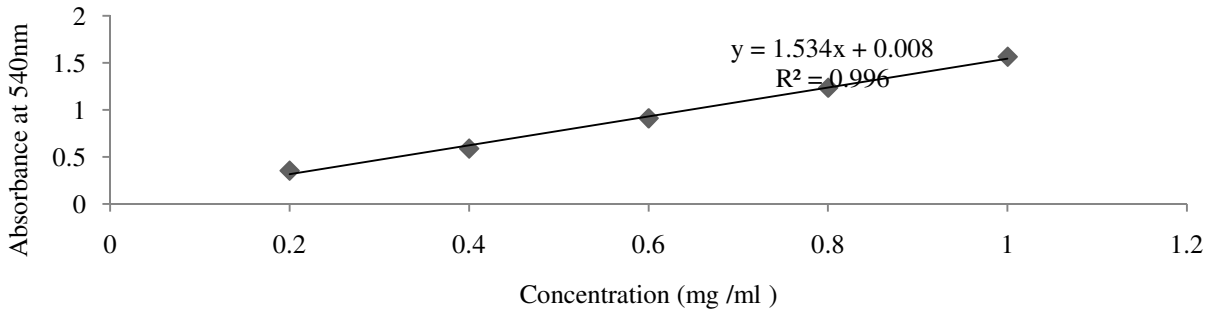


**Fig. 1. Glucose Standard Curve**

**TABLE 1: Result of Mean Reducing Sugar Yield from Hydrolysis of Corn Cob and Corn Stalk Using Aspergillus niger**

| Hydrolysis (Days) | Substrates | | | |
|---|---|---|---|---|
| | Corn cob | | Corn stalk | |
| | Absorbance (540nm) | Concentration (mg/ml) | Absorbance (540nm) | Concentration (mg/ml) |
| 2 | 3.600 | 2.342 | 3.470 | 2.257 |
| 3 | 4.450 | 2.896 | 4.575 | 2.977 |
| 5 | 3.240 | 2.107 | 3.200 | 2.081 |
| 7 | 2.290 | 1.488 | 2.785 | 1.810 |
| 10 | 0.846 | 0.546 | 1.075 | 0.696 |
| 12 | 0.322 | 0.216 | 0.561 | 0.360 |
| 14 | 0.399 | 0.254 | 1.396 | 0.905 |

*t-test ($p \leq 0.05$) =0.623*

The high yields of reducing sugar recorded in this study using these two substrates suggest that the two substrates can serve as suitable cheap substrates for production of reducing sugars that can be used for biofuel production.

The level of the reducing sugar (RS) yields of corn stalk was found to be slightly higher than that of corn cob. However, using T-test (at $p \leq 0.05$), significance difference was not observed between the two agricultural substrates (p value = 0.623). This may be due to the fact that the two substrates have almost the same cellulose, lignocellulose and simple sugar compositions.

The effect of incubation time on the level of reducing sugar yields showed that 72hours was the optimum for hydrolysis of the two substrates (2.896mg/ml for corn cob and 2.977mg/ml for corn stalk). From a similar study conducted by [21] they observed that the optimum reducing sugar yield was recorded at the fourth day of the fermentation. Beyond 72hours of hydrolysis, the level of reducing sugar yields started declining. This might be because the Aspergillus niger cells might have reached decline phase and displayed low reducing sugar yields.

### 5. CONCLUSIONS

The production of reducing sugars from corn cob and corn stalk (hydrolysis) under submerged fermentation were studied by Aspergillus niger. The two substrates had reducing sugar yields that were comparable to other substrates used for biofuels production. The corn cob and corn stalk used in this research work had similar reducing sugar yields which makes them suitable substrates for reducing sugar production. The research findings

demonstrated that corn cob and corn stalk are indeed a cheap agricultural waste with potential as substrates for reducing sugar production which will reduce the cost of fermentable sugar for biofuel production.

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