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Full Length Research Article

# SUSTAINABLE PRODUCTION OF BIOETHANOL FROM MAIZE HUSK BY SIMULTANEOUS SACCHARIFICATION AND FERMENTATION USING *ACREMONIUM BUTYRI* AND *ZYMOMONAS MOBILIS*

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**ABSTRACT**
A green bio-energy from bio-polymeric resources with the potential in maintaining ecological balance brings about a search for elements and techniques to ease liquid fuel production. Bioethanol was produced using maize husk by simultaneous saccharification and fermentation. Test organisms include fungi and bacteria. The fungi *A. butyri* was isolated from dead plant matter roots of *Piliostigma reticulatum* using sabouraud dextrose agar (SDA). The observed cultural and morphological characteristics were identified and compared with those already known and reported fungi taxa. The bacteria used (*Acremonium butyri*) were obtained from the department of microbiology, Udusok, Nigeria. Proximate compositions of maize husk included protein (6.9%), Ash (5.02%), Moisture (7.6%), Fat (17.0%), Fibre (3.25%), and Carbohydrate (65.52%). The maize husk was pretreated using an $H_2SO_4$ dilute acid solution. Post-pretreated maize husk, the sample was inoculated with $10^7$ fungal spore cells of *A. butyric*, incubated at 30°C on a rotary shaker for 72h. The bacteria *Z. mobilis* $1\times10^8$ cells were subsequently inoculated for the next 48h in the same flask. In each duplicate experiment, samples were analyzed for reducing sugar and bioethanol quantity up to 120h. The highest mean of reducing sugar was between 72 (3.865±0.077) and 96h (3.865±0.063). Bioethanol was determined quantitatively using potassium dichromate. About 1% of Standard ethanol concentration was obtained. The diluted 1% was re-diluted with distilled water again and obtained 0, 0.2, 0.4, 0.6, and 0.8. These concentrations added with potassium dichromate reagent were used for the development of a standard ethanol curve using UV-VIS spectrophotometer 588nm. About 2.5ml of sample products was mixed with 1Ml potassium dichromate and absorbance was also taken at 588nm using a spectrophotometer. The quantity of bioethanol in the presence of *A. butyri* and *A. butyri* plus *Z. mobilis* was peak at 72h (0.98%) and 120hrs (1.65%) respectively. A successive increase across the different periods in the quantity of bioethanol was observed. Therefore, *A. butyri* is a potential agent that can both hydrolyze and ferment sugars. Maize husk is a vital waste with potential sugars that can be used to produce bioethanol.

**Keywords:** Bioethanol, Maize husk, Hydrolysis, and Fermentation.

## INTRODUCTION

Bioethanol is an alcoholic liquid fuel made from sugar via a fermentation process with a significant function in the area of environmental conservation, protecting from the harm of fossil fuel. Production of bioethanol is another means of reducing greenhouse gases (GHG) and or environmental pollution as there is zero $CO_2$ emission to the atmosphere (Oyeleke and Jibrin, 2009, Krishnan, *et al*., 2020). It was believed worldwide that, bioethanol can replace crude oil in the transportation sector. Bioethanol, the first generation can be partly or fully blended with gasoline as a suitable substitute. However, there are considerable drawbacks to the raw materials thus, second-generation bioethanol production that uses agro-waste biomass has procured much-added attention nowadays (Naik *et al*., 2010, Amornraksa, *et al*., 2020).
The bioethanol obtained from agro-wastes of cereal crops possesses huge potential due to the accessibility of substrate and technological proficiency, cost-effective simpler approach for the growth of biorefinery, inexpensive and eco-friendly (Sage *et al*., 1999; Banerjee *et al*., 2019; Kumar *et a*l., 2020). Furthermore, there has been an increase in agro-waste generated nowadays, as a result of boosted cereal crop yield via advances in seed production and plant-breeding activities thus, this turn out to be a feasible resource for bioethanol production. The cereal crops maize is one of the promising feedstocks with high biomass yields (Anwar *et a*l., 2014; Kumar *et a*l., 2020).

Simultaneous saccharification and fermentation is a process that carries two organisms performing different functions one after the other in a single vessel. In this process, little amount of sugar can be accumulated, there is a limited chance of microbial contamination, require less time consumption, and it's a cost-effective and efficient process (Pinaki *et al*., 2015).
The present study was intended to produce bioethanol from maize husk using simultaneous saccharification and fermentation.

## MATERIALS AND METHODS

### Collection of samples

The samples collected include maize husk from grains commercial milling center (Kara central market) and the roots of *Piliostigma reticulatum* from a commercial herbalist (at Shehu Shagari central market), Sokoto north local government, Sokoto state, Nigeria. These were collected using clean and sterile polyethylene bags. They were immediately transported to the laboratory for the subsequence process.

### Isolation and Identification of the Test Organism

*Piliostigma reticulatum* roots were maintained by adequate

moistening with sufficient distilled water in a clean beaker for 8 days. The growth seen from the surface of the root matter was picked using a sterile wire loop and inoculated on sterile, solidified sabouraud dextrose agar (SDA) plates. The plates were incubated at room temperature, 25°C for 5 days. The colony was subcultured repeatedly to obtain a pure culture. The pure colony was maintained in tubes contained with SDA media, and these were stored at 4°C for further use (Abdullahi *et al*., 2020). The fungi were identified based on cultural and morphological characteristics and compared with those fungi of known taxa as described by Watanabe (2010), and Bello *et al*. (2020).

A pure colony of *Zymomonas mobilis* (AX101) was obtained from the microbiology Department, at Usmanu Danfodiyo University Sokoto. The colony was re-cultured on Luria Bertani media g/l (tryptone, 10.0; yeast extract, 5.0; NaCl, 10.0) at pH 7.0 and maintained on slants tubes at 4°C for further use.

**Proximate Analysis**

Proximate analysis was carryout using the standard AOAC method (2005) as adopted by Wu and Wu (2017). Moisture content was determined by direct wet-weight procedure; a known mass of the sample was dried in the oven at 105°C for 120mins until a constant mass was obtained. Protein content was obtained based on the Kjeldahl procedure (method No. 978.04). Crude fat was based on the soxhlet extract procedure (method No. 930.09), using petroleum ether as an agent. Ash content was determined after incinerating the mixture in a muffle furnace (method No. 930.05). The total fiber was determined using the method described by Van Soest and Wine (1967); Arora (1981); Kochhar *et al*. (2006). Total carbohydrate was determined based on the method described by BeMiller (2010).

**Dilute acid Solution Pretreatment**

The pretreatment process was based on the method described by Nikzad *et al*. (2013); Sanusi *et al*. (2021). About 7g of pretreated maize husk and 133 mL of 0.5 to 1.0% (v/v) $H_2SO_4$ solution was mixed in an Erlenmeyer's flask with 500mL capacity. The mixture was covered using aluminum foil, autoclaved for 20mins at 121°C, and allowed to cool and filtered via Whatman filter paper. The residual product was washed using distilled water, pH was adjusted using NaOH, air dried, and stored at 4°C for further use.

**Simultaneous Saccharification and Fermentation**

This process was achieved based on the procedure adopted by Shittu *et al*. (2016) and Sanusi *et al*. (2020). The treated maize husk product was 5g mixed with 50ml sterile water, inoculated with $10^7$ *Acremonium butyri* spore cells (as determined by hemocytometer), and incubated on a rotary shaker 120rpm at 30°C for 72h. After then, Inoculated were about $1\times10^8$ cells (0.5 McFarland standards) of *Z. mobilis* in the same flask for subsequent 48 hours for further process. All the experiments were in duplicate. These were sampled every 24h for analysis.

**Separation and Purification**

The fabric filter was used to separate the solid and liquid blend products. To attain a clear product, the liquid parts were centrifuged before being put via a syringe filter. Purification was achieved using a vacuum evaporator at 65°C for 50mins (Chongkhong and Lolharat, 2013; Shittu *et al*., 2016).

**Bioethanol Determination**

Bioethanol was determined qualitatively based on the method described by Shubhra *et al*. (2019). The sample products 3ml were added with 2% of 1ml $K_2Cr_2O_7$ and 5ml of $H_2SO_4$. This was incubated at 40°C for 5mins. The appearance of green coloration was observed after the incubation.

Quantitative determination of bioethanol was observed following the method described by Rabah *et al*. (2020). This was achieved by alcohol quantitative analysis with chromium VI reagents using a UV-VIS spectrophotometer. A standard ethanol curve was developed from the reading obtained at 1% ethanol diluted to 10ml distilled water with chromium reagents using a 588nm spectrophotometer.

**Statistical data analysis**

The generated data were analyzed in form of mean and standard deviations. These were presented in bars and tables.

**RESULTS AND DISCUSSION**

The fungi *A. butyri* culturally appeared in the plates, yellow which were very light, soft, and cottony at the lateral edge of the colony. Some of the colonies branched in the plates. The standing conidiophores were branched with more than two phialides. The fungi were bearing septate hyphae with egg-shaped conidia at the top of conidiophores. The genus *Acremonium* growth was observed on dead plants as mentioned above, especially if the moisture on the matter has been kept constant. Related to the report by Park *et al*. (2017) that saprobic moldy fungi growing on dead plants matter, physically seen with cottony growth on dead plants can also be found in soil habitats. The organism was previously reported to be isolated from dead plants of *Piliostigma reticulatum* (Sanusi *et al*., 2020). Since this organism can produce metabolites, the enzyme cellulase can break down complex polymers into simpler monomers which may be potential for fuel production (Sanusi *et al*., 2020). This can reduce the lengthy stages and energy used during production which saves time.

Table 1 shows the proximate breakdown of used maize husk. The contents observed include protein 6.9%, fat 17.0%, and carbohydrate 65.52%. This result shows maize husk contains high amounts of sugar molecules because the percentage of carbohydrates was higher. This favors the area of biofuel production because it relies on sugar conversion. Similarly, the content of fat was much greater than that of protein in the maize husk. The result correlated with the proximate composition of maize husk reported by Madika *et al*. (2017).

Figure 1 shows reducing sugar contents at varied periods after 24h intervals. The highest yield observed was 3.76 after 5 days whereas, the least was between zero, 2.175 to day one, 3.175. There was a significant increase in the sugar amount released after each 24h interval. This showed the ability of *A. butyri* to split apart bonds between sugars in the biomass. Though, acid solution pretreatment was carried out to ease the work by *A. butyri* unlike the study report by Sanusi *et al*. (2020) who observed both pretreatment and hydrolysis work done by this particular organism in a given lengthy period of days. Also, greater amounts of reducing sugar obtained may be attributed as a result of acid solution pretreatments done before *A. butyri* was introduced. Therefore, it became easy for the introduced organism to break down the reducing sugar from its structural components.

Table 2 shows Bioethanol produced quantitatively using 24h intervals. There was an increase in the quantity of bioethanol

produced after every 24h. *A. butyri* alone was able to ferment maize husk and produced up to 0.98% bioethanol quantity. A greater increase in quantity was observed after the introduction of known fermentative bacteria *Z. mobilis* from 1.5 to 1.65% bioethanol quantity. Though, the quantity produced by the co-fermentative organism was not enough as expected. These might be attributed to the reports by Rabah *et al.* (2020) that hexose sugars can only be fermented by *Z. mobilis* but not pentose sugars. Also, the reducing sugar at 72 and 96h was the same (3.865) while the quantity of bioethanol was observed to be increasing between such hours. This may be a result of technical problems or otherwise.

**Table 1**: Proximate Composition of Maize husk

| Parameters | Percentage (%) |
|---|---|
| Moisture | 7.6 |
| Ash | 5.02 |
| Protein | 6.9 |
| Carbohydrate | 65.52 |
| Fat | 17.00 |
| Fibre | 3.25 |

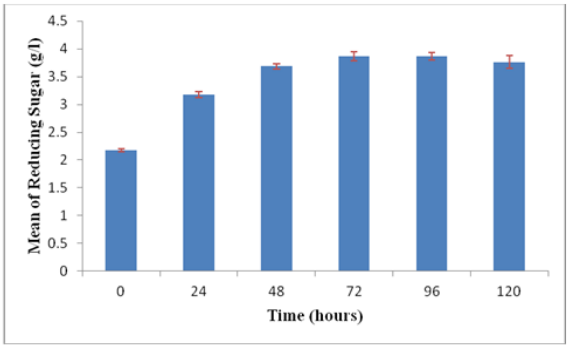


**Figure 1**: Mean and Standard Deviation of Reducing Sugar (g/l) after five (5) days

**Table 2**: Quantity of Bioethanol Produced

| Time (hours) | Used Organism | Volume of Bioethanol (%) |
|---|---|---|
| 0 | *A. butyri* | 0.76±0.042 |
| 24 | *A. butyri* | 0.90±0.028 |
| 48 | *A. butyri* | 0.95±0.014 |
| 72 | *A. butyri* | 0.98±0.014 |
| 96 | *A. Butyri* + *Z. mobilis* | 1.5±0.282 |
| 120 | *A. butyri* + *Z. mobilis* | 1.65±0.212 |

**Conclusion**

Some mold fungi like *Acremonium butyri* can break down the bonds across the sugar molecule in maize husk and convert the sugar via fermentation to bioethanol. Knowing its potential will shorten the production stages though; *Zymomonas mobilis* potential in biofuel production was already established. The work by two individual microbes had shown a better capacity to produce bioethanol. Another approach may be used to highlight the *Acremonium butyri* potential in the production of biofuel.

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