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Ethanol Production from Corn Cobs By Co-cultures of *Saccharomyces cerevisiae* and *Aspergillus niger*

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***Saccharomyces cerevisiae* and *Aspergillus niger* were used in a co-culture for the simultaneous saccharification and fermentation (SSF) of 1% and 10% (w/v) dry pre-treated corn cobs to ethanol. Positive controls of glucose of same concentrations in a synthetic medium were also fermented. At 1% substrate concentration, the complex medium containing corn cobs had a maximum ethanol yield of 4.17%, while the synthetic medium with glucose gave 3.45%. At a higher concentration of 10% the synthetic medium gave a yield of 6.23% which is comparable to 6.17% by the complex medium. Residual sugar concentration was observed to decrease with increase in ethanol production and cell growth increased with time of fermentation.**

Key words: *Saccharomyces cerevisiae*, *Aspergillus niger*, corn cobs, glucose, ethanol.

The demand for ethanol has been on the increase due to it various uses such as, chemical feedstock and more importantly as an alternative source of liquid fuel for automobiles. One of the ways of producing ethanol is through fermentation of crops which are rich in sugar or starch such as sugarcane, sugar beet, sweet sorghum, corn and cassava (Abouzeid and Steinkraus, 1983; Okolo *et al*., 1995). However, the major disadvantage of this process is that most of these crops are food crops and tend to increase the cost of production. In order to make the fermentation method cost effective and to meet the great demand for ethanol, research studies are now being directed in two areas namely, the production of ethanol from cheaper raw materials and the study of new microorganisms or yeast strains efficient in ethanol production (Favela-Torres *et al*., 1986; Pandey *et al*., 2000; Akin-Osanaiye *et al*., 2008). In this respect, in expensive raw materials like wastes, such as agricultural wastes, cellulosic wastes, fruit wastes, vegetable wastes, municipal and industrial wastes can be used to produce ethanol cheaply (Abouzeid and Reddy, 1986; Green and Shelef, 1989; Park and Baratti,1991; Schugerl, 1994; Joshi *et al*., 2001; Akin-Osanaiye *et al*., 2008).

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Nigeria produces large quantity of corn and large amount of waste in the form of corn cobs is generated during the processing of the corn. Most of these wastes end up in the environment thereby constituting environmental pollution problem. However, the corn cobs are made up of carbohydrates which can be processed into sugars and subsequently fermented to ethanol. The aim of this study therefore is to convert corn cobs to ethanol in a simultaneous saccharification and fermentation process using co-cultures of *Saccharomyces cerevisiae* and *Aspergillus niger*.

MATERIAL AND METHODS

Source of Organisms

Saccharomyces cerevisiae and *Aspergillus niger* strains were obtained from culture collection of Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria. The organisms were maintained on potato dextrose agar slants at 4°C.

Sample Preparation and Pretreatment

The corn cobs samples were collected from milling centers in Zaria, Nigeria. The samples were converted into fine powder by hammer milling and sieving. Pretreatment of the sample was then carried out by refluxing the powder with 0.2M NaOH for 2hours and then neutralized with HCL. The pretreated sample was dried in an oven at 65°C.

Culture Condition for Ethanol Production

A synthetic medium containing yeast nitrogen base glucose broth and a complex medium containing the pretreated corn cobs were used for ethanol production. The synthetic medium was prepared by dissolving 6.7g of yeast nitrogen and 10g of glucose in a litre of distilled water. The complex medium was prepared with corn cobs at a concentration of 10g/litre supplemented with 0.1% $FeNH_4 (SO_4)_2$, 0.25% $(NH_4)_2 HPO_4$, 0.3% urea and 0.5% peptone. The pH of the medium was adjusted to 5.0, sterilized in an autoclave and filtered. Another set of media containing 100g/L of glucose and corn cobs were similarly prepared. The media were dispensed into 500ml Erlenmeyer flasks each containing 200ml of the medium. The flask containing synthetic medium were inoculated with *Saccharomyces cerevisiae* while those containing complex medium were inoculated with both *Saccharomyces cerevisiae* and *Aspergillus niger*. The flasks were incubated at ambient temperatures on an orbital shaker set at 250rpm for 5 days.

Analytical Methods

At 24hour intervals, samples were taken aseptically from the fermentation media to determine growth, residual sugar and ethanol concentrations. The growth was determined by measuring the cell density (optical density) at 650nm, the residual sugar was determined using dinitrosalicylic acid (DNS) method described by Miller, (1959) and ethanol was determined after standard distillation using the method described by AOAC (1974).

RESULTS AND DISCUSSION

The results of the ethanol yield from the synthetic medium containing glucose and complex medium containing corn cobs are shown

Table 1. Ethanol Yield from Synthetic and Complex Media

Fermentation time (Hrs)	Ethanol yield (%)			
	SM (Glucose)		CM (Corn cobs)	
	1%	10%	1%	10%
24	1.76	3.47	2.21	2.77
48	2.38	5.21	3.44	4.80
72	3.45	6.23	4.17	6.17
96	2.85	6.01	3.89	5.11
120	2.67	5.62	3.57	4.89

SM = Synthetic medium with glucose; CM = Complex medium with corn cobs

Table 2. Residual Sugar Obtained During Fermentation of the Synthetic and Complex Media

Fermentation time (Hrs)	Residual Sugar (mg/ml)			
	SM (Glucose)		CM (Corn cobs)	
	1%	10%	1%	10%
24	690	680	650	660
48	635	490	430	520
72	486	350	360	480
96	390	230	300	320
120	280	195	180	200

SM = Synthetic medium with glucose; CM = Complex medium with corn cobs

Table 3. Growth (Cell density) Obtained During Fermentation of the Synthetic and Complex Media

Fermentation time (Hrs)	Cell Density (OD) at 650nm			
	SM (Glucose)		CM (Corn cobs)	
	1%	10%	1%	10%
24	0.01	0.08	0.03	0.08
48	0.02	0.13	0.07	0.20
72	0.04	0.33	0.12	0.35
96	0.05	0.33	0.17	0.37
120	0.06	0.34	0.26	0.40

SM = Synthetic medium with glucose; CM = Complex medium with corn cobs

in Table 1. At 1% and 10% glucose concentration, the synthetic medium gave a maximum ethanol yield of 3.45% and 6.23% respectively. While at the same concentrations of corn cobs, the complex medium yielded 4.17% and 6.17% ethanol. It was observed that at all concentrations of substrates; the ethanol yield increased steadily reaching the peak after 72 hours of fermentation and then declined. The corn cobs at 1% concentration gave a better ethanol yield compared to glucose, while at 10%, the ethanol yield was comparable to that of glucose. This shows that the corn cobs can serve as one of the cheaper substrates for ethanol production (Schugerl, 1994; Joshi *et al*., 2001; Akin-Osanaiye *et al*., 2008).

The results in Table 2 shows the pattern of residual sugar during the fermentation period. The residual sugar in the fermentation media was observed to decrease with increase in fermentation time. This could be attributed to the utilization of the sugar as carbon source for growth and subsequent ethanol production.

The growth (cell density) of the organisms was also observed to increase steadily during the fermentation period (Table 3). This may be due to the fact that the organisms are utilizing the nutrients present in the media for growth and ethanol production. However, the media with 10% substrate concentrations supported more growth of the organisms. Also the corn cobs seem to support higher growth of the organisms compared to glucose. This may be due to the presence of other compounds in the corn cobs such as non starch carbohydrates, proteins, amino acids and other compounds which support more growth (Achi and Njoku-Obi, 1992).

CONCLUSION

The results of this study show that corn cobs are good substrate for ethanol production compared to glucose. The substrates at 10% concentrations supported higher yield of ethanol and ethanol production increases with fermentation time and peaked at 72 hours. Therefore the findings of this work suggest that ethanol can be produced from agricultural wastes such as corn cobs.

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