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RESEARCH PAPER

Utilization of Corn (*Zea Mays*) wastes in Bioethanol production by Separate Hydrolysis and Fermentation

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Abstract. The study investigates the feasibility of producing bioethanol from corn (*Zea mays*) wastes. Corn cobs, husks and grains were collected from Githurai market and analysis was done in the Kenyatta university laboratory. Wastes were physically pre-treated to increase their surface area for enzymatic reactions. Separate Hydrolysis and Fermentation were carried out by using *Aspergillus niger* for enzymatic hydrolysis and *Saccharomyces cerevisiae* yeast for fermentation at different incubation temperatures (28°C, 30°C, 32°C) and times (24, 48, 72 and 96 hours). Fermentation was carried out in 150 ml cotton- plugged conical flasks containing 100 ml sample hydrolysates inoculated with 2 ml cultured yeast suspension. Ethanol concentration was determined by potassium dichromate oxidation method after each incubation time and expressed as % v/v whilst ethanol yield (l/kg) was derived from the ethanol concentration divided by the quantity of substrates used. Maximum yields of 1.84 l/kg, 1.76 l/kg and 2.05 l/kg were obtained from 50 g of pre-treated corn cobs, corn husks and corn grains respectively. The optimum temperature for maximum ethanol yield in all corn substrates was 30°C whilst incubation time 96 hours, 48 hours and 24 hours were optimum for ethanol production in corn cobs, corn husks and corn grains respectively. Results show that bioethanol can easily be produced from corn wastes and is recommended that they are used wisely for energy generation specifically bioethanol to add value to them rather than landfilling.

Keywords: Bioethanol; corn wastes; enzymatic hydrolysis; fermentation

1. Introduction

Maize (*Zea mays*) belongs to the tribe Maydae of the family Poaceae. It has a genome size of 2.3 gigabase and contains over 32,000 genes on ten chromosomes. It was first grown in a form of wild grass called *teosinte* in Central America. The term 'maize' was derived from a word 'mahiz' meaning 'source of life' of Tano language of the people of the Caribbean islands and it later became 'maiz' in Spanish. It is commonly called 'corn' in English speaking countries (Singh & Kumar, 2016). The use of corn rather than maize in United States is due to the referral of maize as *Indian corn* during the arrival of early European settlers in the New World. Corn originated from the Germanic word 'korn' which referred to any edible grass. The spread of maize to Africa and East Asia was due to colonial conquests and trade (Abbassian, 2006).

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The annual global production of corn is about 520 Tera grams. Major production regions are North America (42%), Asia (26%), Europe (12%) and South America (9%). Leading producers are USA with about 40% of global production followed by China with 20%. Leading producers of corn in Africa are South Africa, Nigeria, Ethiopia, Tanzania, Egypt, Malawi, Kenya, Zambia, Uganda and Ghana (Vlaams Interuniversitair Instituut Voor Biotechnologie, 2017). In Africa, 63.43% of corn is used as food, 24.27% is used as animal feed and 8.61% of wastes are generated. The estimated maize consumption in Kenya is 171g/person/day. The high rate of consumption has led to the increase in quantities of corn wastes (Ran'um et al., 2014). Corn wastes are made up of large amount of sugars that can be utilized to produce bioethanol. Corn grains contain high amount of starch which is easily converted to monosaccharides upon pre-treatment and hydrolysis. Corn cobs, husks, stalk and leaves also contain glucan in different forms (Tambuwal et al., 2018; Yesmin et al., 2020).

Ethanol is a volatile, flammable and colourless organic compound with a molecular weight of 46.07g/mol. It is derived from feedstocks that contain sufficient quantities of sugar or materials that can easily be converted into sugars (Gerlach, 2012; Endalew, 2015). Sugars are metabolised by yeast cells through glycolysis pathway to gain energy for biosynthesis to produce bioethanol under anaerobic conditions (Talebnia, 2008). Corn waste products are rich in lignocellulose which comprises of two main structures known as cellulose and hemicellulose which are easily hydrolysed to produce fermentable sugars (Tropea *et al.,* 2014).

Bioethanol production from corn wastes will reduce the quantities of wastes in addition to fuel generation. Corn wastes are cost effective, renewable and abundant in quantity making them suitable substrates for bioethanol production rather than energy crops. Ethanol production from wastes would allow agricultural lands to be used more efficiently and also prevent competition in food supplies (Khamala & Alex, 2013; Shrivastava et al., 2014; Braide et al., 2016).

Enzymes improve the efficiency of hydrolysis by means of synergistic advantages. Cellulase are set of enzymes involved in the complete hydrolysis of cellulose and are made from organisms that reside on cellulosic materials which are either generated in a separate reactor or purchased from suppliers from industries. These enzymes are normally produced from bacteria or fungi. *Aspergillus niger* is one of the fungi mostly used in the production of cellulase enzymes. It belongs to the family *Trichocomaceae*. It is a very important organism in biotechnology because of its use in the production of enzymes. Cellulase has high capability of hydrolysing specifically bonds of β-1-4-glucosidic which make them highly utilised in ethanol production (Li et al., 2007; Neagu et al., 2012).

Separate Hydrolysis and Fermentation require isolating the hydrolysis process from the fermentation process. The isolation enables enzymes to function at their required temperature for better performance while fermentation organisms can be operated at optimum temperature to optimise the use of sugar (Azhar et al., 2017). This method was employed in the study because it can be operated at different optimal conditions for hydrolysis and fermentation (Williams, 2017). Baker's yeast is made from *Saccharomyces cerevisiae* strains which consist of roughly 30-33% dry materials, 6.5%- 9.3% nitrogen, 40.6-58.0% lipids, 5.0-7.5% minerals and various quantities of vitamins depending on the type and conditions required for its growth. *Saccharomyces cerevisiae* is yeast in the order *Saccharomycetales* that produces maximum ethanol productivity, yields and has high ethanol inhibition resistance which make it more desirable to be used in ethanol production (Bekatorou et al., 2006; Williams, 2017).

The study reports on bioethanol produced at different incubation temperatures and times by separate hydrolysis and fermentation using corn cobs, corn husks and corn grains collected from Githurai market, Nairobi, Kenya. The study aimed at producing bioethanol from cheap and

easily available substrates and also from simple, reliable and effective methods which can easily be utilized by other researchers and ethanol manufacturing companies to produce bioethanol.

2. Methodology

2.1. Description of study area

Githurai is situated on the eastern part of Nairobi between latitudes 36º54'49.77''E, 1º12'10.07''S and longitudes 36º55'10.62''E, 1º12'10.21''S (see Figure 1). Average daily temperatures range from 29ºC in the dry season to 24ºC during the rest of the year with average annual rainfall of 1000 mm.

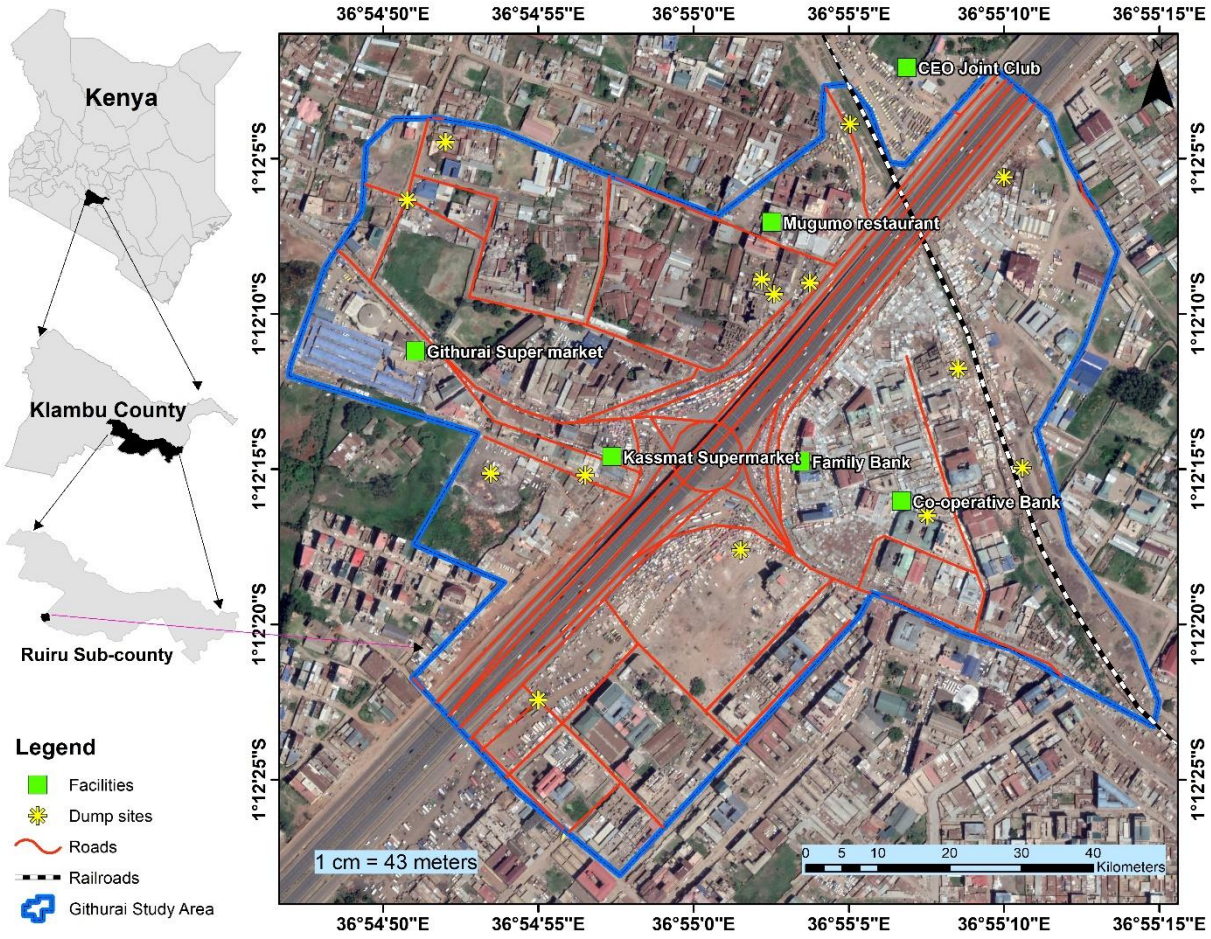

Figure 1. Map shows Githurai market where corn cobs, husks and grains were collected. Source: ArcGIS Platform, 2019.

2.2. Sample collection and Pre-treatment of corn wastes

Corn cobs, husks and grains were collected from sellers in Githurai market. They were stored in the oven overnight at 110ºC after which they were physically pre-treated separately by the use of a grinder in order to increase their surface area for enzymatic hydrolysis. 50 g of each pre-treated corn wastes was mixed with 200 ml distilled water and pH adjusted to 4.5. Pre-treated substrates were sterilized at 120ºC for 15 minutes by the use of an autoclave to avoid microbial contamination.

2.3. Enzymatic hydrolysis of pre-treated wastes

Enzyme solution was prepared by dissolving commercial cellulase from *Aspergillus niger* purchased from (Kobian scientific limited, Nairobi, Kenya) in citrate buffer solution for 30 minutes. The solution was added to each pre-treated substrate and incubated at 30°C using an incubating shaker with agitation rate of 150 rpm for 24 hours. Sample hydrolysates were obtained after centrifugation at 5000 rpm for 20 minutes. The glucose percentage in each corn hydrolysates was determined by high performance liquid chromatography (HPLC) according to the methods described by (Kim et al., 2011). Experiment was done in triplicates.

2.4. Fermentation Process

Anaerobic batch fermentation was carried out using cultured *Saccharomyces cerevisiae* yeast to convert the released sugars into bioethanol. Yeast was cultured according to the methods described by (Osei *et al*., 2020). Different incubation temperatures (28°C, 30°C and 32°C) and times (24, 48, 72 and 96 hours) were optimised at pH 4.5 for bioethanol production. Fermentation was carried out in 150 ml cotton- plugged conical flasks containing 100 ml sample hydrolysates. Hydrolysates were sterilised and inoculated with 2 ml cultured yeast suspension. Samples were incubated at different temperatures (28°C, 30°C and 32°C) in an incubating shaker with agitation rate of 150 rpm at different incubation times (24, 48, 72 and 96 hours). Centrifugation was done after each incubation time at 4000 rpm for 10 minutes in order to remove yeast cells. The supernatant was used to determine the concentration of ethanol.

2.5. Ethanol Analysis

Potassium dichromate oxidation method was carried out using 10% potassium dichromate solution, concentrated sulphuric acid, distilled water and UV-vis spectrometer set at 575 nm to determine the amount of ethanol produced. Standard curve was drawn from known standard concentrations and their corresponding absorbance values (see Figure 2). Ethanol concentration of each corn hydrolysate was extrapolated from the standard curve and expressed as % v/v and ethanol yield is determined from the concentration divided by amount of substrate used and expressed as l/kg.

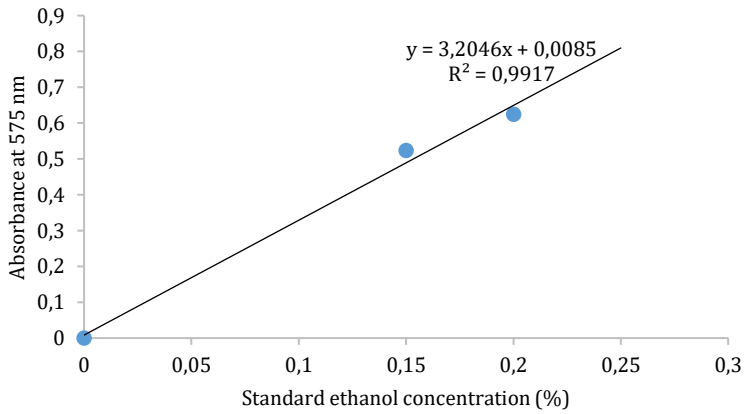

Figure 2. Standard ethanol curve

2.6. Statistical analysis

Data were analysed by analysis of variance using Genstat statistical package (Discovery version 4) and the significant differences among means were separated by using Fisher's unprotected least significant difference method at 5% level of probability ($P<0.05$).

3. Results and Discussion

3.1. Bioethanol production at different fermentation conditions

The maximum amount of glucose as shown in Figure 3 was achieved in corn grains. This is due to the presence of high starch content available in corn grains. Starch is a term for saccharides consisting of complicated molecules which comprise of thousands of glucose molecule chains. The molecule chains are easily convertible to glucose as compared to cellulose which is present in high quantities in corn cobs and husks (Virginie et al., 2018). Table 1 also shows the maximum ethanol content as obtained in corn grains followed by corn cobs and corn husks respectively. The high amount of glucose produced in corn grains as seen from Figure 3 resulted in their high bioethanol content as compared to the other substrates. Schwietzke et al. (2009) reported higher ethanol yields in corn grains compared to corn cobs. Braide et al. (2016) also reported maximum ethanol yield in corn cobs as compared to corn husks when different agricultural wastes (sugarcane bagasse, sugarcane bark, corn cob, corn stalk, corn husks) were utilized for ethanol production.

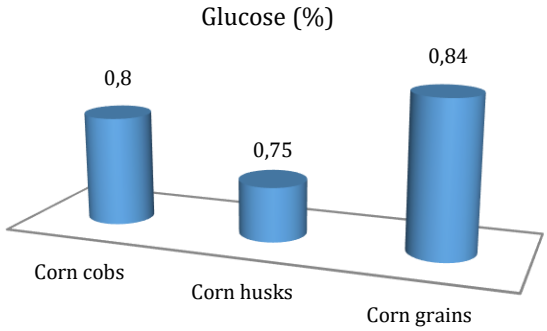

Figure 3. Percentage glucose content in corn cobs, corn husks and corn grains

Table 1. Maximum ethanol concentration and yield of corn cobs, husks and grains

Substrates	Ethanol concentration (% v/v)	Ethanol yield (l/kg)
Corn cobs	0.4588b	1.835b
Corn husks	0.4403a	1.761a
Corn grains	0.5122c	2.049c
P value	***	***
l.s.d (P<0.05)	0.01293	0.0517

***=highly significant at P<0.001; l.s.d = least significant difference among substrates used

3.2. Effects of different incubation times on ethanol production

Optimum incubation time in corn grain hydrolysates was 24 hours as shown in Table 2 and Figure 4. The decrease in ethanol with increasing fermentation time was due to reduction in sugar level in the corn grain hydrolysate causing yeast cells to progress to the stationary phase (Braide et al., 2018). According to Gibson *et al.* (2007), depletion in sugar level causes yeast cells to experience diauxic shift leading to reduction in growth as cells metabolism is modified to use non-fermentable carbon sources which in effect decrease amount of ethanol produced. Ali and Kemat (2017) reported decrease in ethanol yield with increased fermentation time from *Moringa oleifera* seeds husk. Shahzad et al. (2019) also reported decrease in ethanol concentration with increase fermentation time from 48 to 96 hours using cotton stalk.

Table 2. Effects of different incubation times on ethanol concentration and yield of corn cobs, husks and grains

Substrates	Incubation time (hrs)	Ethanol concentration (%v/v)	Ethanol yield (l/kg)
Corn cobs	24	0.4238b	1.696b
Corn cobs	48	0.4535d	1.814d
Corn cobs	72	0.4669e	1.869e
Corn cobs	96	0.4908g	1.963g
Corn husks	24	0.4522 d	1.808d
Corn husks	48	0.4659e	1.864e
Corn husks	72	0.4332c	1.731c
Corn husks	96	0.4077a	1.630 a
Corn grains	24	0.5364j	2.144 j
Corn grains	48	0.5232i	2.093i
Corn grains	72	0.5008h	2.003h
Corn grains	96	0.4883f	1.953f
P value		***	***
l.s.d P(<0.05)		0.003094	0.01190

***=highly significant at P<0.001; l.s.d = least significant difference among substrates used

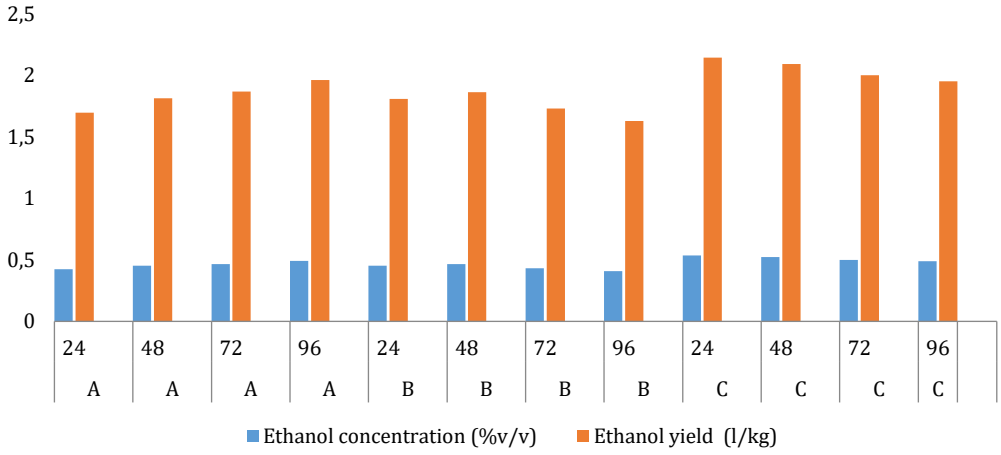

Figure 4. Effects of different incubation times on ethanol concentration and yield
*A= Corn cobs; B= Corn husks C= Corn grains

Maximum ethanol content in corn husk hydrolysates was achieved at 48 hours. The increase in ethanol from 24 hours to 48 hours is a result of yeast cells reaching their exponential growth phase after 24 hours but progress to the stationary phase after 48 hours causing the gradual decreases after the 48 hours. Agrawal et al. (2019) also reported increase in ethanol from 24 hours to 48 hours but decrease after 48 hours to 96 hours using de-oiled rice bran. The 96 hours was optimum for ethanol production in corn cobs. Increase in ethanol with incubation time in corn cobs was because yeast cells were still in their logarithm growth phase due to the availability of nutrients in the corn cob hydrolysates. According to Fahrizal et al. (2013), the velocity of yeast cells logarithm growth rate is influenced by the availability of nutrients in the media.

Akpan et al. (2008) reported increase in ethanol concentration from 12 to 144 hours using maize and old waste papers. Irfan et al. (2014) also reported increases in ethanol production in sugarcane bagasse, rice straw and wheat straw with increase in incubation time from 24 to 72 hours. Different substrates yielded different results due to differences in their chemical

constituents necessary for ethanol production under different conditions. According to Nuwamanya et al., (2012), differences in characteristics of constituents of feedstocks highly affect their hydrolysis process which in effect influences the kinds of sugars produced and thus control the metabolic activities carried out by yeast under different treatments and conditions.

3.3. Effects of different incubation temperatures on bioethanol concentration and yield

The optimum temperature for maximum ethanol production in all substrates was 30°C (see Table 3 and Figure 5). This shows that temperature 30°C was conducive for proper yeast growth and fermentation performance in all the different corn hydrolysates. Corn husks and grains achieved lowest ethanol content at temperature 32°C whilst corn cob was at 28°C. Temperature 32°C was high for yeast cells growth and metabolism in corn grain and husk hydrolysates hence, causing low ethanol content in them compared to temperature 30°C and 28°C. Toxic effects of ethanol are influenced by high temperatures which reduce yeast cells viability causing low fermentation rate (Gibson et al., 2007). Temperature 28°C was low for maximum ethanol production in corn cob hydrolysates leading to low fermentation rate. Gibson et al. (2007) again stated that, low temperature conditions cause reduction in membrane fluidity of yeast cells resulting in stresses in yeast growth.

Table 3. Effects of different temperatures on ethanol concentration and yield of corn cobs, husks and grains

Substrate	Temperature (°C)	Ethanol concentration (%v/v)	Ethanol yield (l/kg)
Corn cobs	28	0.4704 d	1.881d
Corn cobs	30	0.4879 f	1.952 f
Corn cobs	32	0.4808 e	1.923 e
Corn husks	28	0.4476 b	1.790 b
Corn husks	30	0.4584 c	1.834c
Corn husks	32	0.4345 a	1.738a
Corn grains	28	0.5231 h	2.092 h
Corn grains	30	0.5285 I	2.115 I
Corn grains	32	0.5096 g	2.038g
P value		***	***
l.s.d (P<0.05)		0.000177	0.001768

***=highly significant at P<0.001; l.s.d = least significant difference among substrates used

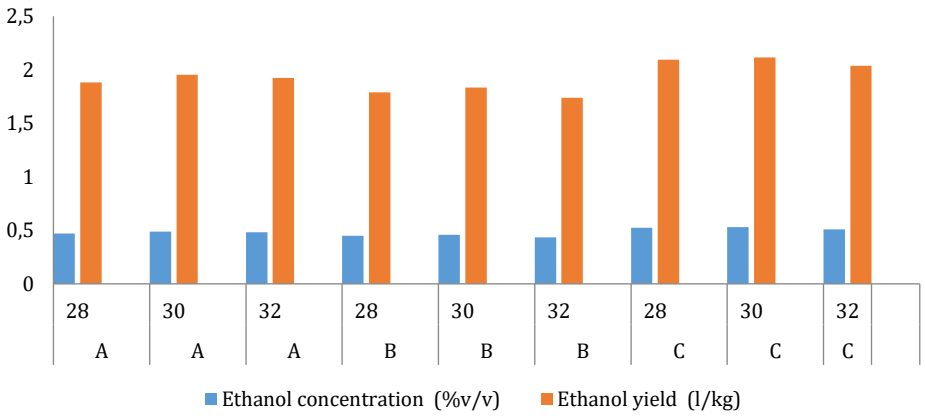

Figure 5. Effects of different temperatures on ethanol concentration and yield. *A= Corn cobs; B= Corn husks C= Corn grains

Kumar *et al.* (2019) reported maximum bioethanol yield at temperature 30°C from corn cobs. Tahir et al. (2010) also reported maximum ethanol at 30°C when different temperatures (10°C-40°C) were optimised for ethanol production using yeast *Saccharomyces cerevisiae* Bio-07. Agrawal et al. (2019) also reported 30°C as the optimum temperature for ethanol production from de-oiled rice bran when different temperatures (20°C, 25°C, 30°C and 35°C) were optimised for ethanol production using yeast *Saccharomyces cerevisiae* MTCC 4780.

4. Conclusion

Findings from the study has proved that corn husks, corn cobs and corn grains which are abundant in market places, dumpsites etc. in most developing countries can be widely utilized in bioethanol generation rather than allowing them to pollute our environment and cause public health hazards. When corn grains, cobs and husks were subjected to different temperatures and times, maximum ethanol was achieved at temperature 30°C in all the substrates. Incubation times at 96 hours, 48 hours and 24 hours were optimum for maximum ethanol in corn cobs, corn husks and corn grains respectively.

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