

## Effects of Different Incubation Methods on Ethanol Production from Selected Food Wastes Products

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### Abstract

The study describes the potential of producing bioethanol from corn (*Zea mays*) cobs, collard greens (*Brassica oleracea*) waste and banana (*Musa acuminata*) peels using different methods of incubation. Wastes were pre-treated by grinding into smaller particles and enzymatic hydrolysis was carried out using commercial cellulase from *Aspergillus niger*. Anaerobic fermentation was done using cultured *Saccharomyces cerevisiae* yeast suspension. Different incubation conditions (incubator, dark room and under soil) at different temperatures 30°C, 21°C and 19°C respectively were optimised for bioethanol production at different incubation times of 48, 96 and 144 hours. Maximum bioethanol percentages of corn cobs, banana peels and collard greens were (0.48%), (0.39%) and (0.15%) respectively. The optimum conditions for maximum ethanol concentration in corn cobs was the incubator conditions at temperature 30°C and 144 hours; banana peels was under soil conditions at 19°C and 48 hours whilst collard greens was dark room conditions at 21°C and 48 hours incubation times. Results obtained show the potential of producing bioethanol from corn cobs, collard greens and banana peels under different incubation conditions. Use of incubators for fermentation especially in collard greens wastes and banana peels can successfully be replaced with dark room and soil which are more economically feasible.

### Keywords

Bioethanol, hydrolysis, fermentation, corn cobs, collard greens wastes, banana peels

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## 1. INTRODUCTION

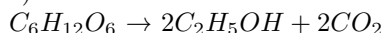
Bioethanol is known globally as a transportation fuel with economic and environmental merits. It is mainly used in inks and coatings as carrier solvents; in cosmetic preparations like hair setting sprays; in pharmaceutical and personal care products like mouthwashes and also in detergent preparations (Gashaw and Getachew, 2014). Bioethanol was first utilized as a motor fuel in internal combustion engine that was invented by Nikolaus Otto in 1897 but its establishment as an alternate fuel was in the 1970s during the oil crises. It is predominantly generated currently from corn and sugarcane derived feed stocks. These feed stocks are used to produce first generation bioethanol (Lohri et al., 2017). Drawback associated with use of energy crops for bioethanol production is their inadequacy in meeting higher fuel demands which may eventually lead to deforestation in order to obtain enough farmland. This negative impact has led to the involvement of second-generation bioethanol which involves the use of agricultural residues, wood, paper and municipal solid wastes (Saini et al., 2015). These materials consist of

lignocellulose which is considered as a good raw material for the production of bioethanol. Globally, lignocellulose is recognized as preferred biomass for fuels and chemicals production. It is the most abundant and widespread carbon source in nature and is known to be the only source capable of providing enough feedstock to meet the world's energy and chemical needs in a renewable manner. Lignocellulose materials can produce bioethanol up to 442 billion litres per year (Woldesenbet et al., 2016).

Increased global per-person food supply has led to the generation of high quantities of waste in recent years (Nair et al., 2017). Leachate and uncontrolled anaerobic decomposition of food waste in landfills result in ground water contamination and greenhouse gases emissions (Ali et al., 2016). High water quantity in food waste and possibility of dioxin formation make incineration also impractical. Food wastes contain soluble sugar, starch, lipids, proteins, cellulose and other compounds making them good substrates for bioethanol production (Moukamnerd et al., 2013). Ethanol production from food waste will reduce the amount of waste in landfills and thus minimise environmental problems re-

lated to landfills (Byadgi and Kalburgi, 2016).

Bioethanol production from lignocellulose materials involves three main stages which are pre-treatment; hydrolysis and fermentation (Sarkar et al., 2012). Pre-treatment is used to separate bonds and release cellulose and hemicellulose to make them susceptible for the activities of chemical and biological agents (Triana, 2016). Hydrolysis converts feed stocks into fermentable sugars which are subsequently converted into bioethanol using micro-organisms (bacterium, yeast or fungi) under anaerobic conditions during fermentation (Azhar et al., 2017); (Woldesenbet et al., 2016); (Li, 2008). The overall chemical formula for fermentation is:



The main objective of the study is to determine the amount of bioethanol produced from corn cobs, collard greens wastes and banana peels under different fermentation conditions.

## 2. EXPERIMENTAL SECTION

### 2.1 Materials

Substrates used in this research were collard greens, banana peels and corn cobs obtained from Nairobi market, Kenya. Micro-organisms used were baker's yeast (*Saccharomyces cerevisiae*) purchased from Nairobi market and cellulase from *Aspergillus niger* purchased from (Sigma Aldrich, Kobian Scientific Limited, Nairobi, Kenya). All chemicals used were analytical grade procured from Biochemistry department in Kenyatta University.

### 2.2 Methods

#### 2.2.1 Waste Preparation and Pre-treatment

Collard greens (*Brassica oleracea*), banana (*Musa acuminata*) peels and corn (*Zea mays*) cobs were oven dried at 110°C for two days after which they were physically pre-treated by grinding using a grinder. 100 g of each pre-treated wastes were dissolved in 500 ml distilled water. pH was adjusted to 4.5 and sterilization was done using an autoclave at 120°C for 15 minutes.

#### 2.2.2 Enzymatic Saccharification

Enzyme solution containing 2 g of commercial cellulase from *Aspergillus niger* and 20 ml of 0.05 M citrate buffer solution at pH 4.8 was added to each sterilized substrate and incubated at 30°C on an incubating shaker with agitation rate of 200 rpm for 24 hours. The samples were centrifuged after incubation at 10,000 rpm for 10 minutes to obtain the hydrolysates. The concentration of glucose present in each food hydrolysate was determined by high performance liquid chromatography using the method described by (Kim et al., 2011).

#### 2.2.3 Preparation of Yeast inoculum and Fermentation Procedure

*Saccharomyces cerevisiae* inoculum was prepared in 250 ml cotton-plugged conical flask which contained 100 ml YPD

broth solution consisting of 20 g/l of dextrose, 4 g/l of yeast extract and 3 g/l of peptone at pH 7.0. Solution was sterilized and 0.6 g baker's yeast was added and incubated at 30°C for 48 hours on an incubating shaker with agitation rate of 150 rpm. Fermentation was carried out in 50 ml cotton-plugged conical flasks containing 20 ml of hydrolysates with pH 4.5. Sterilization was done and hydrolysates were inoculated with 2 ml cultured yeast suspension and shaken at agitation rate of 200 rpm for 30 minutes. The methods of incubation used for the fermentation process were an incubator, dark room and under soil at different temperatures of 30°C, 21°C and 19°C respectively and at durations of 48, 96 and 144 hours. Dark room and under soil temperatures were determined by the use of a thermometer.

#### 2.2.4 Analytical Procedure

Aqueous phase samples were collected from each flask after 48, 96 and 144 hours of fermentation and centrifuged at 5000 rpm for 10 minutes to remove yeast cells and other solids present in the samples. Supernatant fluid was filtered with a filter paper and the filtrate was used to determine the concentration of bioethanol.

#### 2.2.5 Bioethanol Analysis

Biochemical method involving the use of potassium dichromate and sulphuric acid together with UV-vis spectrophotometer set at 620 nm was used in the analysis of bioethanol. Standards were prepared using different volumes of 2% absolute ethanol together with 4 ml distilled water, 1 ml of 10% potassium dichromate solution and 2 ml concentrated sulphuric acid. A curve was drawn using the prepared standard solutions and their corresponding absorbance values. Ethanol solutions from filtrates were also prepared using the same procedures for the standards and their absorbance values were also determined. The concentrations of bioethanol (%v/v) from collard greens wastes, banana peels and corn cobs were extrapolated from the standard ethanol curve. Ethanol productivity was obtained from ethanol concentration divided by fermentation time and expressed as percentage ethanol per time in hours (%/hr).

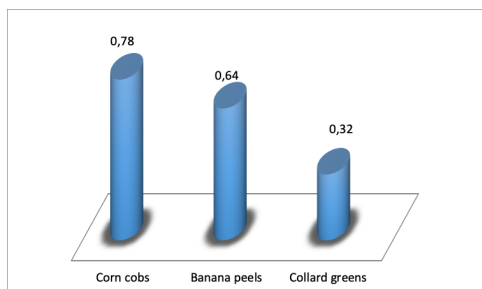
#### 2.2.6 Statistical Analysis

One way ANOVA was used to analyse data and significant differences among means were separated at 5% level of probability ( $P < 0.05$ ) using Genstat statistical package (Discovery version 4)

## 3. RESULTS AND DISCUSSION

### 3.1 BIOETHANOL PRODUCTION AT DIFFERENT INCUBATION CONDITIONS AND TIMES

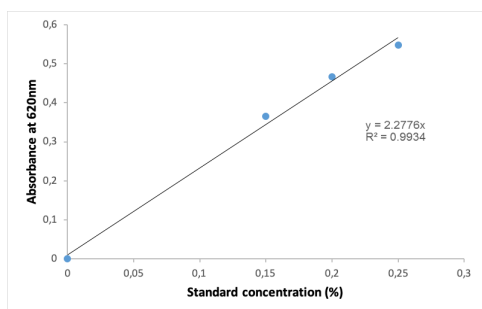
The highest ethanol productivity and concentration was achieved in corn cobs followed by banana peels with collard greens wastes recording the lowest. The high glucose content in corn cobs and banana peels as seen from Figure 1 is



**Figure 1.** Percentage Glucose content in corn cobs, banana peels and collard greens

**Table 1.** Optical Density of Ethanol Standard Solutions

Serial number	Standard ethanol (ml)	O.D at 620 nm
1	0.00 (blank)	0
2	0.2	0.188
3	0.4	0.278
4	0.6	0.365
5	0.8	0.466
6	1	0.547



**Figure 2.** Ethanol standard curve

as a result of their high ethanol content. In anaerobic fermentation, glucose is converted to pyruvic acid through the Embden-Meyerhof-Parnas (EMP) pathway and pyruvic acid into acetaldehyde by the enzyme pyruvate decarboxylase and finally into ethanol with the assistance of the enzyme dehydrogenase (Syawala et al., 2013). Hence the higher the glucose content, the higher the quantity of ethanol produced. Also leafy vegetables in contrast with the other substrates contain high amounts of antioxidants like phenolic components which inhibit yeast activity in producing bioethanol. (Utama et al., 2019) reported low amount of ethanol in napa cabbage as compared to bananas and papayas wastes. The study by (Cutzu and Bardi, 2017) reported maximum amount of ethanol from corn threshing residue as compared to apple and kiwi fruits. (Singh and Singh, 2015) also reported high ethanol content from corn cob as compared to banana peels.

**Table 2.** Maximum Bioethanol Concentration and Productivity of Collard greens, Banana peels and Corn cobs

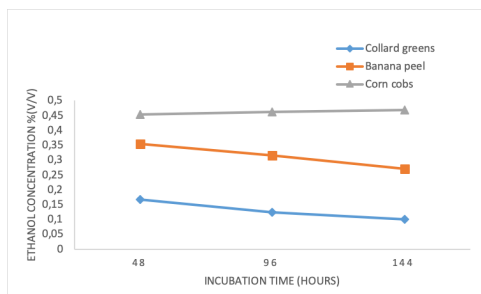
Selected food wastes	Ethanol productivity (%/hr)	Ethanol concentration (%)
Collard greens	0.001820 a	0.1470 a
Banana peels	0.004326 b	0.3877 b
Corn cobs	0.006032 c	0.4787 c
P value	***	***
l.s.d (p<0.05)	0.000774	0.01507

\*\*\*= highly significant at P<0.001. l.sd = least significant difference among different substrates used.

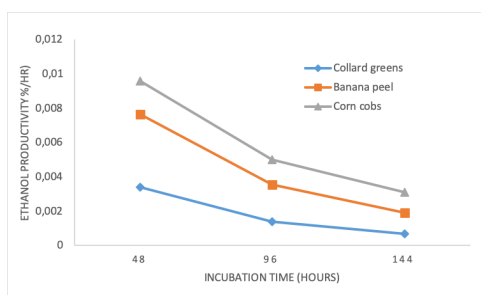
Amounts of bioethanol produced from collard greens, banana peels and corn cobs were low as compared to studies of (Utama et al., 2019) who reported 7.38% and 1.18% bioethanol from banana wastes and leafy vegetable respectively and (Gawande and Patil, 2017) who also reported 4.3% maximum ethanol from damaged corn grains. This variation is due to low amount of fermentable sugars present in collard greens, banana peels and corn cobs as compared to the whole banana fruit wastes and corn grains used.

### 3.2 EFFECTS OF DIFFERENT FERMENTATION TIMES ON ETHANOL PRODUCTION

Optimum fermentation time for maximum ethanol concentration and productivity for banana peels and collard greens was 48 hours. This is due to early entry of yeast cells into the exponential phase because of high initial inoculum of yeast suspension. The decrease in ethanol with increase in fermentation time can be attributed to the accumulation of inhibitors and toxic metabolic by-products during fermentation which affect yeast growth leading to decrease in yeast cell biomass (Gawande and Patil, 2017). Reduction in their hydrolysate sugar levels as incubation time increases can also lead to decrease in bioethanol because availability



**Figure 3.** Effects of different incubation times on ethanol concentration

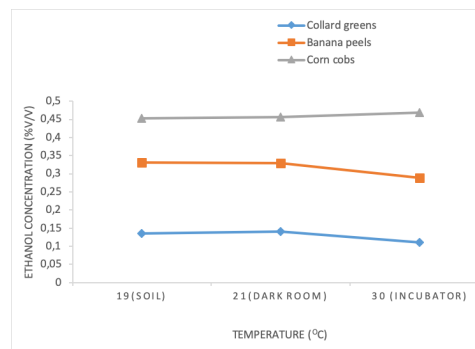


**Figure 4.** Effects of different incubation times on ethanol productivity

of limited sugars causes progression of yeast cells into the stationary phase (Braide et al., 2018).

(Sharma et al., 2007) reported decrease in bioethanol concentration after 48 hours using citrus and banana wastes. (Arumugam and Manikandan, 2011) also reported decrease in ethanol content after 48 hours of incubation from banana and mango wastes. A study by (Gutierrez et al., 2015) reported decrease in ethanol percentage as fermentation time increased using ripe carabao mango peelings.

The increase in ethanol concentration as incubation time increases in corn cobs is as a result of gradual increase in the number of yeast cells due to the availability of nutrients (Tahir et al., 2010). (Siddesh and Kavya, 2019) reported increase in ethanol concentration as incubation time increases using corn and sugarcane bagasse feedstock. (Akpan et al., 2008) also reported increase in bioethanol concentration with increase in incubation time from maize and old waste papers. Decrease in ethanol productivity in corn cobs as seen from figure 4 is due to decrease in amount of substrate but increase in the amount of products which acts as inhibitory agent. (Thapa et al., 2019) also reported increase in ethanol concentration with time but decrease in productivity. The optimum fermentation time for maximum ethanol concentration in corn cobs was 144 hours whilst 48 hours was optimum for maximum ethanol productivity.



**Figure 5.** Effects of different incubation conditions on ethanol concentration

### 3.3 EFFECTS OF DIFFERENT INCUBATION CONDITIONS ON ETHANOL PRODUCTION

Optimum incubation conditions for ethanol concentration in corn cobs, banana peels and collard greens were incubator (30°C), under soil (19°C) and dark room (21°C) respectively. Yeast growth under different temperature conditions depends on the composition of the media (Raines-Casselmann, 2005). Low ethanol concentration from corn cobs hydrolysate under soil and dark room conditions as compared to incubator is due to the low temperatures in the soil and dark room and the presence of other unfavourable conditions causing stresses in yeast growth and fermentation performance. (Kumar and Tantwai, 2019) also reported maximum bioethanol content from corn wastes using an incubator set at temperature 30°C.

For collard greens and banana peel substrates, dark room and biological conditions under the soil were favourable for their maximum ethanol production. Also, abiotic factors like temperature, soil organic matter, pH, conductivity, availability of water and macronutrients like nitrogen, phosphorus, potassium, sodium and magnesium present in soils might have influenced proper yeast growth in their hydrolysates.

Their low ethanol production under incubator conditions at 30°C can be attributed to the process of heat formation occurring during glucose catabolism to ethanol might have caused a further increase in the set temperature leading to low yeast activity due to extremely high temperature (Tiukova, 2014). Aside heat formation, there can also be the possibility of 30°C temperature being extremely high for effective yeast growth and metabolism in their hydrolysates leading to low ethanol production.

## 4. CONCLUSIONS

Findings from the study suggest that banana peels, corn cobs and collard greens can be used efficiently to produce bioethanol and is recommended that they are utilized instead of energy crops that threaten food security. The study has also proven the potential of incubation using soil and dark room and should therefore be incorporated as part

**Table 3.** Effects of different Incubation times on Bioethanol Concentration and Productivity of Selected Food Wastes Products

Treatments	Incubation duration (hours)	Ethanol productivity (%/hr)	Ethanol concentration (%)
Collard greens	48	0.003479d	0.1670 c
Collard greens	96	0.001301b	0.1249b
Collard greens	144	0.000693a	0.0998 a
Banana peels	48	0.007390f	0.3547 f
Banana peels	96	0.003300d	0.3168 e
Banana peels	144	0.001881.c	0.2709d
Corn cobs	48	0.009467 g	0.4544 g
Corn cobs	96	0.004819 e	0.4626 g
Corn cobs	144	0.003251 d	0.4681 g
P value		***	***
l.s.d (P<0.05)		0.0003621	0.02212

\*\*\*= significant at P<0.001. l.sd = least significant difference among different substrates used

**Table 4.** Effects of different Incubation methods on Bioethanol Concentration of Collard greens, Banana peels and Corn cobs

Treatments	Temperature (°C)	Ethanol Concentration (%)
Collard greens	19	0.1346ab
Collard greens	21	0.1410b
Collard greens	30	0.1112a
Banana peels	19	0.3309d
Banana peels	21	0.3285d
Banana peels	30	0.2882c
Corn cobs	19	0.4533 e
Corn cobs	21	0.4564 e
Corn cobs	30	0.4678 e
P value		*
l.s.d(P<0.05)		0.02529

\*= significant at P<0.05 l.sd = least significant difference among different substrates used.

\*19°C= soil conditions; 21°C= dark room conditions; 30°C= incubator conditions.

of incubation techniques for fermentation especially in low income countries.

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