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Research Article

Production of Bioethanol from Rotten Watermelon (*Citrullus lanatus*) and Banana (*Musa sapientum)* Using Enzymatic Approach

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ABSTRACT

Fossil fuels have been a major source of energy for our life, but this vital role has been overshadowed by the risks these emissions pose to the ecosystem. A sufficient biomass supply must be used if this form of energy from biological mass is to be supported adequately. This work thus was carried out with a view of utilizing some locally available biomass wastes as alternative sources of ethanol. The samples (watermelon and Banana) were pre-treated (washed thoroughly) using distilled water to obtain right fermentable sugar hydrolysates. The substrates were then subjected to enzymatic hydrolysis. Fermentation took place with the aid of *Sacchromyces cerevisea* as the fermentation organism with optimisation of the reaction by fermenting with two strains of *saccharomyces cerevisiae* (baker's yeast and freshly isolated yeast). Fermentation was allowed for about 5 days after which ethanol was recovered by distillation (at 78°C). Confirmatory test such as flammability test, density, specific gravity, refractive index, boiling point and Fourier Transform Infrared Spectroscopy (FTIR) were conducted to ascertain the presence of right bioethanol extract. The use of the two substrates (water and banana) in bioethanol production was compared. The results showed that both substrates yielded sufficient amounts of bioethanol, with the rotten banana being more efficient. The rotten banana yielded higher quantities of bioethanol (13.17%) than the rotten watermelon (10.36%). In an effort to lower cost and improve yield, purity and turnaround time for these biofuels, efforts should be made to employ additional organic waste and to pursue further process optimisation solutions.

Keywords: Bioethanol, Enzymatic Hydrolysis, Banana, Watermelon, Fermentation

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INTRODUCTION

One of the main foundational elements of contemporary society is energy. Along with the rapid population expansion and urbanization, there is a constant rise in the demand for energy and the resources that supply it. Renewable and non-renewable energy sources can be categorized into two groups (Dombek & Ingram, 2022).According to the FAO (2008), bioenergy is defined as energy derived from biomass, which is the biodegradable portion of goods, waste, and leftovers from forestry and allied industries, as well as the biodegradable portion of industrial and municipal trash. A variety of biomass sources, including agricultural waste, can be used to create various types of bioenergy (Hossain *et al.,* 2011).

In the traditional definition, biotechnology refers to a method of industrial production where needed products are produced using live creatures or their components, such as enzymes. According to Lin & Tanaka (2006), fermentation is a biotechnological term for an anaerobic cellular process in which organic compounds are broken down into smaller molecules and chemical energy (ATP) is produced.

The definition has been the fermentation of sugar by yeasts to produce alcohol, carbon dioxide, and energy (Pasteur, 2007). Non-renewable energy is not renewable and is derived from taxed energy reserves that are kept bound unless they are released through human contact (Dombek & Ingram, 2022). Coal, oil, and natural gas are the three non-renewable energy sources that make up fossil fuels and account for 75 percent of the world's energy production (Boyle, 2004). Through the emission of carbon monoxide (CO), carbon dioxide (CO_2), nitrogen oxides (NOx), sulfur oxides (SOx), and particulate matter, the continuous use of energy could have a serious negative impact on the environment, indirectly causing global warming and other issues. To tackle the issues, efforts must be made to discover a fossil fuel alternative that is environmentally beneficial (Kumar *et al.,* 2009). Continuous use of this energy does not significantly deplete it, and it does not result in large pollution emissions or other environmental issues. According to Sener, (2018), bioenergy, which is regarded as a renewable energy source, is energy produced from biological resources like wood, straw, or animals that were once living things. The substance can be directly burned to generate heat or power, or it can be turned into biofuels (Moser, 2009). Biofuel is characterized as fuel that is solid, liquid, or gaseous and is made from recently decomposed biological material. Unlike fossil fuels, which are made from long-dead organic stuff, this is different. Any biomass, or biological carbon source material, can be used to make biofuel. Biofuels are fuel sources that can replace petroleum and have a number of benefits, such as sustainability, a decrease in greenhouse gas emissions, regional development, social structure, agriculture, and supply security (Demirbas, 2009).

In the coming decades, non-renewable energy sources like fossil fuels will run out, prompting an increase in interest in biomass-based biofuel production as a solution to the anticipated global energy problem. The most popular liquid biofuel is ethanol, which is produced through the fermentation of sugars, starches, or cellulosic biomass, such as fruit waste. In this study, rotting bananas and watermelon were used to produce bioethanol. *Saccharomyces Cerevisiae* yeast is used in the fermentation process to create bioethanol from banana trash. The specific objectives of the study were to produce bioethanol from rotten banana and rotten watermelon through enzymatic and acidic hydrolysis processes, characterize the produced bioethanol, quantify and compare the volume of bioethanol obtained from the various substrate and finally compare the produced bioethanol with the standard ethanol. The ethanol produced from lignocellulose is sulfate-free and oxygenated. Since the carbon in ethanol is of vegetative origin, it won't contribute to the emission of CO_2 during combustion (Khawla *et al.,* 2014). The content of renewable ethanol, made from discarded bananas, aids in a net decrease in the emissions of CO_2, CO, and hydrocarbons. Because ethanol burns more thoroughly and cleanly than gasoline on its own, it can be combined with gasoline (Dombek & Ingram, 2022).

MATERIALS AND METHODS

Pre-treatment of Samples: Sample Collection and Preparation

The fruits (rotten watermelon and rotten banana) were collected from Danrimi maket, Dutsinma, Katsina state on a day prior of the experiment. They were put in a polyethene bag and transported to the FUDMa chemistry laboratory on the day of the experiment.

The rotten watermelon was washed thoroughly with distilled water. A sterile knife was used to take the peel off and the pulp was taken out and 400 g of the pulp was weighed in a beaker on an electrical scale. 400 grams of pulp were blended with 40 mL of distilled water in a sterilized juice blender. The watermelon matrix was poured into two fermenting vessels so that the experiment could be carried out in duplicates.

The rotten banana was also cut using sterile knife and 400 g were weighed on a beaker on an electrical scale and blended with 40 mL of distilled water in a juice blender. The banana matrix was poured into two fermenting vessels so that the experiment could be carried out in duplicates.

400 g of the washed rotten water melon and rotten banana were mixed and blended with 40 mL of distilled water. The mixture was poured into two fermenting vessels so that the experiment could be carried out in duplicates.

Hydrolysis of the pre-treated sample

Different 500ml conical flasks were filled with 220ml (per setup) of the pre-treated samples, and 1ml of the enzyme (cellulases) was added. It was heated to 65^{o}C for 2 hours, then cooled down to room temperature. Citric acid was added in a drop-wise

manner to get the pH down to 4.5, which is ideal for fermentation. The remaining three samples from each setup were used as controls. The initial and final sugar concentrations of the samples were measured. (Shrinivas, 2019).

Fermentation in Yeast

Fresh cultures of *Saccharomyces cerevisiae* were obtained from Department of Microbiology, Federal University, Dutsinma. The yeast cells were inoculated in sterile peptone water for three days, thereafter, the culture was centrifuged at 3000rpm for 5minutes, and the supernatant was discarded leaving the fresh centrifuge which will be used in the hydrolysate. The commercial yeast required (*Saccharomyces cerevisiae*) was obtained from the market, using an electrical scale, 25 grams of baker's yeast were prepared with 50 milliliters of distilled water and autoclaved for 15 minutes at 121^{o}c in a sterilized beaker.

10ml of the activated baker's yeast and the fresh isolated yeast cell was added into the hydrolysate respectively and stirred with a sterilized stirrer for a few minutes, shaked properly for the proper dissolution of the cells. The pH was adjusted to 4.5-5 using pH meter for proper fermentation using citric acid. The fermenting vessels were tightly sealed and left for approximately three days (Jagesser, 2017).

The fermented matrix for each sample was filtered into a conical flask using clean sieving cloth. A sample of the filtrate was measured to determine Brix and the final sugar concentration.

Distillation of bioethanol

Distillation was carried out following the method of Jagessar (2017), with slight modifications using distillation apparatus. The fermented liquid was transferred into a round bottom flask and placed on a heating mantle fixed to a distillation column enclosed in running tap water. Another flask was fixed to the other end of the distillation column and the ethanol was collected at 78^{o}C. A thermometer was attached to ascertain the temperature of distillation.

RESULTS AND DISCUSSIONS

From the substrates of banana, watermelon, and (banana + watermelon mixture), the average amount of bioethanol produced is 28.98 ml with a concentration of 6.02 and a yield of 13.17, 22.79 ml with a concentration of 4.37 and a yield of 10.36, and 25.98 ml with a concentration of 5.20 and a yield of 11.81, respectively. The remainder acts as the substrate's negative control.

Confirmatory Test

The test is carried out by adding pure drops of potassium dichromate and then observe the change in colour of the sample (Fletcher, et al., 2003).The result for confirmatory test for the bioethanol is shown in Table 2.

Ethanol Characterization

At 0.929 g/ml, watermelon has the highest density, followed by bananas at 0.918 g/ml and the combination of bananas and watermelon at 0.911 g/ml. The distilled samples' boiling points were determined to be 79^{o}C for watermelon, 79^{o}C for banana, and 79^{o}C for (watermelon + banana), respectively. Additionally, 96.52 percent purity was found in all of the substrates, including watermelon and banana. It was discovered that each sample had a refractive index of 1.38. It was discovered that every bioethanol that was produced had a viscosity of 1.3.

The samples with the highest yield of bioethanol in this work was obtained from banana substrate (28.98ml) followed a higher yield of 25.98ml obtained from a combination of watermelon and banana, and a least yield of 22.79ml was recorded from watermelon substrate. The highest yield recorded in the banana substrate could be due its high sugary content than what is obtainable in watermelon because the more the sugary content the more the ethanol yield. From table 1 it was confirmed that the substance produced is 96% ethanol with the highest purity of 96.52. The flammability test verified the flammability of the distilled sample materials.

The study revealed that high concentrations (%v/v) of bioethanol from the three substrates 6.02, 4.37, 5.2 from banana, watermelon and a combination of both banana and watermelon, respectively. The 6.02 concentration of bioethanol recorded from banana substrate in the study correlates with the concentrations of bioethanol obtained from banana substrate reported by Shrinivas, (2019). The 3.58 (%v/v) concentrations of bioethanol obtained from watermelon substrate in the study is in line with the findings of Ezejiofor *et al.,* 2018, who reported 3.58 and 5.42 (%v/v) concentrations of bioethanol from watermelon through acidic and enzymatic hydrolysis, respectively.

From table two, The confirmatory test revealed colour changes in all of the samples after the addition of potassium dichromate, from colourless to green, indicating the presence of ethanol. This study is comparable to that of Tewari *et al*. (1986). From table 3, Ethanol from banana peels changed colour once potassium dichromate was added. The boiling point of the extracted ethanol was found to be 79^{o}C, and when compared to the boiling point of pure commercially available ethanol, which is 78^{o}C, indicating that it conformed to the boiling point of absolute ethanol. This study is consistent with that of Galbe and Zacchi (2012). An analysis of the ethanol generation from soft wood revealed that the typical boiling point is 76°C.

The crucial method of Fourier transform infrared spectroscopy (FTIR) makes it simple to determine whether a particular functional group is present in an organic molecule. The vibration frequencies of functional groups are exclusive to that functional group. The infrared (IR) frequency range includes these vibrational frequencies. As a result, the functional groups vibrate at particular frequencies when an IR signal is passed through the organic complex. In other words, an infrared signal will be absorbed at these distinctive frequencies when it passes through an organic component and can then be converted into a distinct spectrum. For researchers, one of the most crucial analytical methods is Fourier transform infrared (FTIR). Characterizing samples in the forms of liquids, solutions, pastes, powders, films, fibers, and gases can be done using this type of analysis. The material on the substrate's surfaces can also be analyzed using this method (Fan *et al*., 2012). FTIR is relatively common in comparison to other kinds of characterization analysis. This characterization study is relatively sensitive, accurate, and quick (Fan *et al*., 2012).

Normally, in ethanol CH_3CH_2OH, the prominent bonds are O-H bond stretching vibrations (appearing at around 3600 to 3200 cm^{-1}), C-O bond stretching vibrations (appearing at around 1200 cm^{-1} to 1020 cm^{-1}), C-H bond bending vibrations (appearing at around 1300 cm^{-1} to 1600 cm^{-1}). From this study, the spectra have the characteristic O-H bond of aliphatic alcohol (one of which is Ethanol) could clearly be seen at exactly 3257 cm^{-1} for all the samples. The peak is broad and strong usually due to hydrogen bonding attributed to the polar O-H bond. C-O bond of alcohol was clearly shown at 1048 $cm^{-1}$from all the extracts, which confirmed the C-OH of ethanol. The spectra showed that the CH_3 and CH_2 chains of ethanol from all three extracts exhibit equal C-H bending at a wavelength of about 1640 cm-1. These obvious peaks in the spectrum provide further evidence that the distillates produced were most likely ethanol, considering the FTIR data which confirmed that the extract produced was ethanol. The spectra presented in the result is similar to the existing spectra from the related literature.

Table 1: Average Quantity of Bioethanol Produced from the Six Samples

Sample	WM	BN	(WM+BN)	WMC	BNC	(WM+BN)C
Volume (ml)	22.79	28.98	25.98	9.00	7.23	9.92
Concentration (ppm)	4.37	6.02	5.20	0.36	0.82	0.72
Yield (%)	10.36	13.17	11.81	4.09	3.29	4.51

WM= watermelon, BN= banana, (WM+BN) = combination of watermelon and banana
WMC= watermelon control, BNC= banana control, (WM+BN) = combination of watermelon and banana control

Table 2. Ethanol Confirmatory Tests

SN	Test	Observation	Inference
1	Physical appearance of the sample collected	The sample was colorless, and volatile at room temperature	The sample collected may be Ethanol
2	Few drop of concentrated H_2SO_4 was added to sample	Very volatile, sweet smelling liquid when perceived	Presence of Ethanol
3	Few drop of potassium dichromate was added in drop wise	Instantaneous appearance of yellowish green color was observe	Presence of Ethanol

Table 3. Ethanol Characterization

Sample	WM	BN	WM+BN	WMC	BNC	(WM+BN)C
Density (g/ml)	0.929	0.918	0.911	0.921	0.923	0.921
Specific Gravity	0.812	0.811	0.811	0.852	0.833	0.835
Boiling Point	79	79	79	80	80	79
% purity	96.52	96.52	96.52	92.4410	92.4410	96.5231
Refractive Index at 25°C	1.38	1.38	1.38	1.35	1.34	1.35
Viscosity (cp)	1.3	1.3	1.3	1.3	1.3	1.3

Table 4: FTIR of the Produced Bioethanol

S/N	Frequency (cm^{-1})	Functional group	Remarks
1.	3252.7	O-H vibration	Stretching
2.	1640.0	C-H vibration	Stretching
3.	1043.7	C-O vibration	Stretching

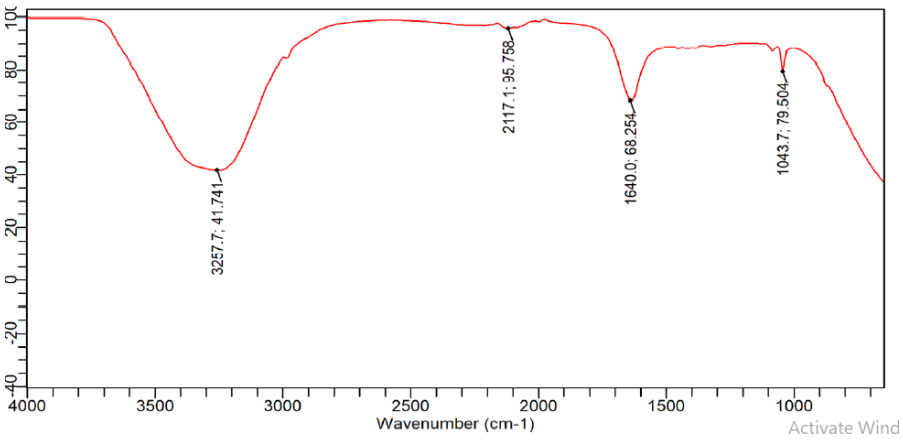

Figure 1. FTIR of produced bioethanol (BN)

CONCLUSION

According to the study, it was concluded that rotten bananas and rotten watermelons both produced significant amounts of bioethanol by enzymatic hydrolysis, with rotten bananas producing more in comparison to rotten watermelons of excellent quality and purity. This bioethanol can be used to power vehicles, reducing the amount of CO and CO_2 emissions. Furthermore, it can be applied as a waste management procedure for environmental recycling.

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