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Original Research Article

Production of Bioethanol from Watermelon and Banana peels

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Abstract
Bioethanol, an ethanol liquid that is known to be a clean fuel for combustion engines, is a readily available substitute since it can be derived from plant-based materials. is the most commonly used alternative transportation fuel to petrol. It can help to mitigate climate change and reduce environmental pollution. In this study, pretreated fruits peels were enzymatically hydrolysed using *Aspergillus niger* from soil. *Saccharomyces cerevisiae* from palm wine was used for fermentation. The hydrolysate was used to produce bioethanol which was determined by HPLC analysis. HPLC analysis was carried out to determine the presence of bioethanol produced. Peak area of the distillated bioethanol produced was compared with that of a standard ethanol. Bioethanol produced from the hydrolysate of fruits peels using *Saccharomyces cerevisiae* showed highest yield of (1.36%) from biologically pretreated fruit peels and hot water pretreatment produced a low bioethanol concentration of (0.16%). Bioethanol was produced in the fermentation broth with High Performance Liquid Chromatography (HPLC) peak of 2.548min for watermelon and 2.234min for banana. highest reducing sugar yield was achieved with sample of Bacillus subtilis pretreatment.

Introduction
Fossil fuels are the major source of energy worldwide and the use of fossil fuel is associated with global warming, climate change, and a variety of energy and security problems. Moreover, fossil fuel is not-evenly distributed within nations, and equally non-renewable. Bioethanol, an ethanol liquid that is known to be a clean fuel for combustion engines, is a readily available substitute since it can be derived from plant based materials. The total consumption of bioethanol in 2008 in Nigeria was more than 65,000 million liters and the usage is growing rapidly because it has already replaced 5.4 % gasoline usage in 2013 (Kim *et al.*,2008). The use of bioethanol as an alternative either as an octane enhancer or main fuel tend to reduce the problems associated with fossil fuels (Kim *et al*.,2008). However, deriving bioethanol from food sources is not a viable alternative, because we have to make the choice between food and ethanol (El Zawawy *et al*.,2011).

Ethanol also called alcohol is a colorless, flammable, volatile liquid with a molecular formula of C_2H_6O. It has a molar mass of 46.07 g/mole, a density of 0.789 g/cm3, a melting point of −114 °C, and a boiling point of 78.37 °C (El Zawawy *et al*.,2011). It is widely used as a solvent, a fuel, and as a raw material for the production of other useful chemicals that have wide applications in the industry. It is also consumed as alcohol beverage, for household heating, and applied as an antiseptic. It is produced from ethylene hydration and fermentation of sugars, starch, lignocellulosic materials, or hydrocarbon-based ethanol production. The use of lignocellulosic biomass for bioethanol production is a recent alternative with great promise and still under research. It is an efficient, cost-effective, and a food security-wise alternative. Such biomass includes residues from agriculture or forest, industrial and municipal wastes, and dedicated energy crops. The substances that have been used previously to produce bioethanol include sugarcane bagasse, corn curb, newspapers, sawdust, rice straw, wood, wheat etc (Curreili *et al*.,1997).

The biochemical conversion of lignocellulosic biomass into bioethanol via microbial fermentation is appealing due to its mild operating conditions. However, lignocellulosic biomass, with its complex and resistant structure, cannot be directly utilized as a carbon source for microbial growth and bioethanol production. Therefore, it must undergo

pretreatment before the saccharification steps and fermentation (Kumar et al, 2017). Pretreatment alters the physical, chemical, and rheological properties of lignocellulosic biomass, enhancing sugar yield through the hydrolysis of structural carbohydrates, which in turn improves bioethanol titer, yield, and productivity. This additional step in bioethanol production significantly increases costs, making it less economically competitive with conventional fossil fuels or first-generation bioethanol produced from sugar- or starch-based feedstocks (Robak *et al.*,2018). Following pretreatment, the lignocellulosic biomass undergoes cellulase hydrolysis and fermentation by the yeast Saccharomyces cerevisiae (Ambat *et al*.,2018)

MATERIALS AND METHODS

Study Area

The study was conducted at Kasuwar daji market situated adjacent to Pepsi Area and Sokoto modern abattoir Sokoto North local government area, with coordinates $13^0$03N $5^0$14E. Sokoto state is located at 13.0533^{0}N 5.3223^{0}E.

Collection of Samples

Twenty Kilogram of watermelon and 20kg of banana fruit peels were collected in a new polythene bag throughout the research from Kasuwar Daji Market, Sokoto State on 18th November, 2019 where it was transported to the Laboratories of Microbiology and Biochemistry Departments, Usmanu Danfodiyo University, Sokoto for analysis.

Sample Processing

The banana and watermelon peels were washed with distilled water to remove dirt and other contaminants. The samples were chopped into small pieces using sterile knife and allowed to air dry. It was grounded to powdered form and transported to Microbiology research lab UDUS inside a new polythene bag.

Study Design

The sampling method employed was a laboratory experiment which involves isolation fungi, fermentation, Reducing sugar and HPLC. All samples (watermelon peels, banana peels, and soil) were collected at Kasuwan Daji market Sokoto and transported to Usmanu Danfodiyo University, Sokoto for analysis.

Serial dilution

Serial dilution of the soil samples collected from Kasuwar Daji Market was carried out, the 3rd and 4th dilution factors were inoculated on sabraud dextrose agar (SDA) to isolate *Aspergillus niger* for hydrolysis of the fruit peels.

Inoculation and incubation

The sample was inoculated on SDA using pour plate method. 0.1ml of the sample was inoculated on petri plates and SDA media was poured and allow to solidify. The SDA plates were incubated at room temperature for 7 days for colony development. Identification of the fungal isolate was based on macroscopy techniques, i.e., the growth rate, colour, texture and colonial morphology. For microscopy, which comprised of spores and presence of special structures such as conidiophores, etc according to Sourza-motta *et al.* (2003) using Atlas in Mycology laboratory of Usmanu Danfodiyo University, Sokoto.

Subculture

Different Fungal colony grown on the SDA plates were sub cultured by cutting a small portion of the fungal mycelium using inoculation needle and transferring it onto sterile Sabrouds dextrose agar plate. The plates were labelled properly and incubated at room temperature for 48-72 hours (Sourza-motta *et al.*, 2007).

Macroscopy/Microscopy examination

Plate of pure was observed macroscopically i.e the growth rate, colour, texture and colonial morphology and also the appearance of the reverse of the plate was observed. For Microscopic examination a slide of pure fungal isolate was prepared on a slide using lacto phenol cotton blue and covers slip. A prepared slide was placed on the staged of microscope and viewed under x40 objective lens according to Sourza-motta *et al.* (2003) using Atlas in Mycology laboratory of Usmanu Danfodiyo University, Sokoto.

Enzymatic Saccharification of Pretreated Fruits Peels

The enzymatic saccharification of fruit peels was perfomed using *Aspergillus niger* as described by Gupta (2006). In this method, the pretreated fruit peels samples were inoculated with 0.5 ml suspension of 96 hours culture of *Aspergillus niger* at room temperature for 7 days. Samples were taken daily for reducing sugar determination using 1,4-dinitro salicylic acid (DNS) method to determine the net yield of fermentable sugars. The samples were then filtered using Whatman filter paper No. 1 and the filtrates were used for fermentation.

Isolation of *Saccromyces cereviceae* from Palm Wine for Fermentation

Isolation and identification of yeast (*Saccharomyces cerevisiae*) from palm wine was carried out by the standard morphological and physiological test and identification keys described by Barnett *et al*. (1990). One ml of serially diluted palm wine was inoculated on plates of SDA containing streptomycin by spread plate method and incubated at 28^0C for 48 hrs. The colonies that developed were isolated and purified by further streaking on SDA.

Fermentation of the Hydrolysate for Bioethanol Production

The fermentation studies were carried out using *Saccharomyces cerevisiae* isolated from palm wine. The hydrolysates were autoclaved at 121^OC for 15 min and the flasks were then cooled to room temperature. The pH of the fermentation medium which differs differently depending on the pretreatment used was adjusted to 6.5 by addition of H_2SO_4 or NaOH and then 1ml of prepared suspension of yeast isolated was added in the hydrolysate. The fermentation was allowed for 7 days and samples from the medium were withdrawn periodically at 24 hrs interval from the flasks to determine ethanol concentration in the broth (Mojovic *et al.*, 2006).

Distillation of the Fermented Broth

The fermented broth was filtered using whatman filter paper. Each sample was then heated at 78^0C on a distillation chamber until the solution turned colorless.

Quantification of bioethanol Produced

Ethanol concentrations in the fermentation broth was determined daily for 7 days. 1 ml of ethanol was poured into a test tube, 7 ml of distilled water was then added and 2 ml of acidified potassium dichromate was added. It was then heated at 40^0C in water bath. The absorbance was read at 580 nm using UV-visible spectrophotometer (Ibrahim *et al.*, 2011).

Determination of Bioethanol Properties

Measurements of Density of the Biothanol Produced

The density of the Bioethanol produced was measured using density bottle according to the methods described by Amenaghawon *et al*. (2012). The density bottle was filled with distilled water and the weight of the water at 20^OC and note as X_3. The density bottle was then made empty, clean, and dry and note as X_1. The density bottle was then made empty, clean, dry and then filled with sample (Bioethanol) and note as. X_2. The specific density of the bioethanol was calculated according to the following formula given below.

$$\text{Density} = (X_2 - X_1) / (X_3 - X_1) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{eqn (8)}$$

Where: X_1=weight (g) of empty density bottle

X_2=

weight (g) of empty density bottle + sample

X_3 = weight (g) of empty density bottle + water. (Adelekan, 2010)

Measurements of Viscosity of the Bioethanol Generated

Fifty ml each of the Bioethanol produced was placed in to the capillary tube of the viscosity instrument and the level of the test sample was adjusted with the help of pressure pump to the designated mark position on the capillary arm of the instrument, about 5 mm ahead of the first timing mark with sample flowing freely (Adelekan, 2010). Time was measured in seconds to within 0.25 the time needed for the meniscus to pass from the first to the next timing mark (in triplicate) and took average measurements as the flow time (Adelekan, 2010). The following parameters were recorded during the experiment: Spindle number, temperature, and time, revolution per minute (RPM), % tague and viscosity. Viscosity was calculated using the formula below;

$$\text{Viscosity} = CT \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{eqn}(9)$$

Where C = calibration constant of the viscosity T = flow time, sec

High Performance Liquid Chromatography (HPLC)

HPLC analysis was carried out to determine the presence of bioethanol produced. Peak area of the distillated bioethanol produced was compared with that of a standard ethanol. The HPLC analysis was carried out with on Agilent technologies 1200 series HPLC instrument (Shimatzu, Japan) equipped with solvent delivery module LC-10Ai, Degassing unit 20A3R, column oven CTO-20A, UV-VIS detector speed 20A and automatic sample injector SIL-IDA CBB-20A lite. The mobile phase consists of methanol pumped at a flow rate of 1.0 ml/min. 20µl of sample were injected into the thermostatic column of ambient temperature. The eluent was monitored at 254nm wavelengths. The chemstation

integration software was used for data analysis and integration. The analysis was carried out at Central advanced science complex (CASLAC) lab Usmanu Danfodiyo University, Sokoto.

Result:

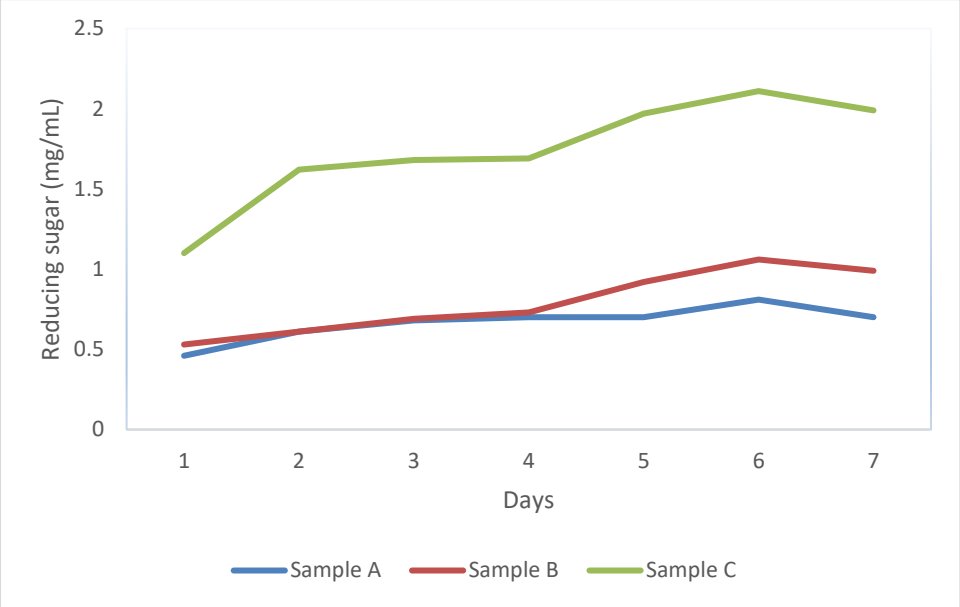


Figure 1: Concentration of reducing sugar produced (mg/L) from microbial hydrolysis

Key: Sample A: watermelon

Sample B: banana

Sample C: mixture

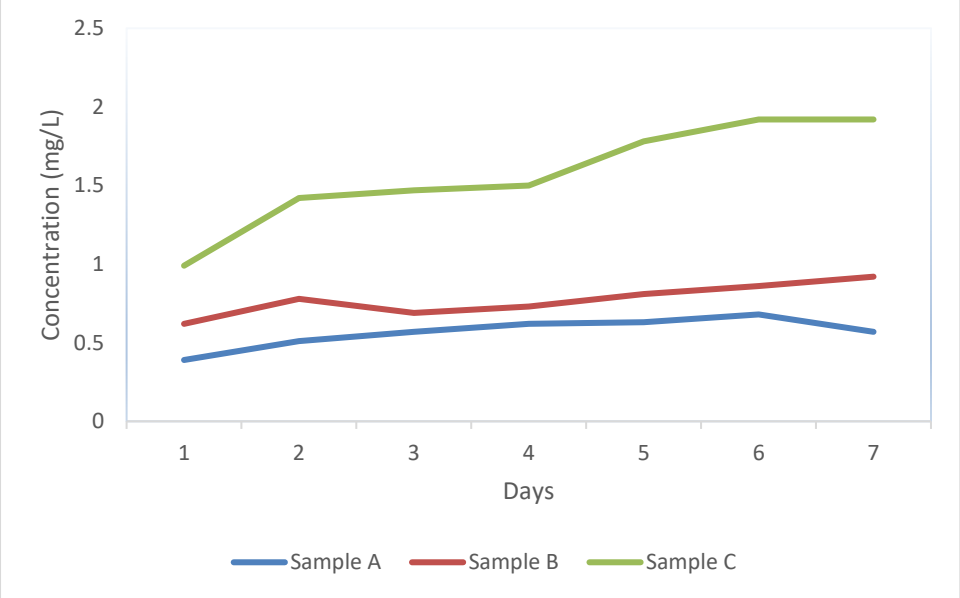


Figure 2: Concentration of reducing sugar produced LHW from day 1-7

KEY: **Sample A**: watermelon

Sample B: banana

Sample C: mixture

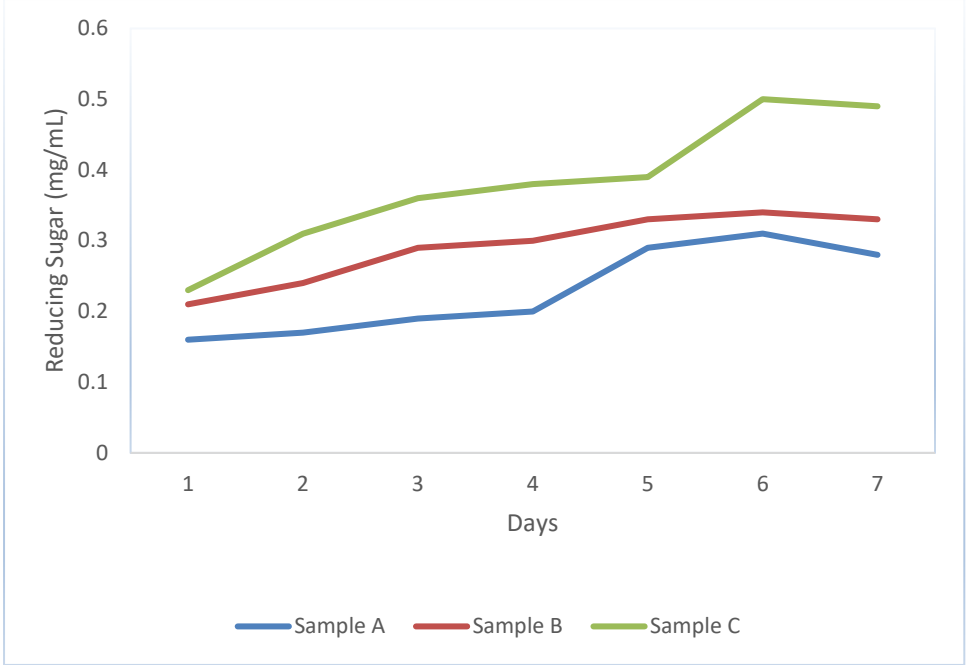


Figure 3: Concentration of bioethanol produced from sample pretreated with LHW

KEY: **Sample A**: watermelon

Sample B: banana

Sample C: mixture

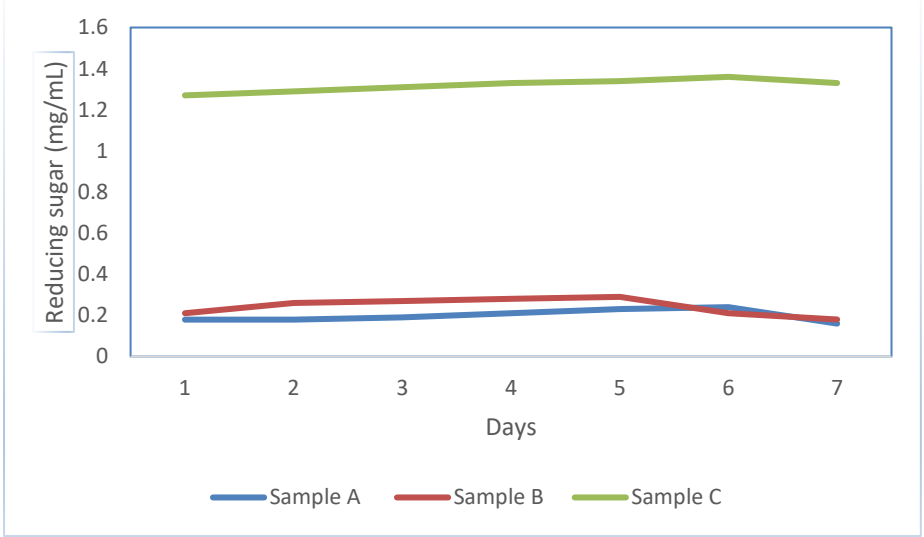


Figure 4: Concentration of bioethanol produced from samples pretreated with *Bacillus subtilis*

KEY: **Sample A**: watermelon

Sample B: banana

Sample C: mixture

Table 1: Densities and viscocity of the Bioethanol Produced from Sample Pre-treated with microbial hydrolysis using *Bacillus subtilis*

Sample	Density (g/cm^3)	Viscosity (mili pasca)
Sample A	0.97	17.1
Sample B	0.98	13.6
Sample C	0.98	17.6

KEY: **Sample A**: watermelon

Sample B: banana

Sample C: mixture

Table 2: Densities and viscocity of the Bioethanol Produced from Sample Pre-Treated by LHW

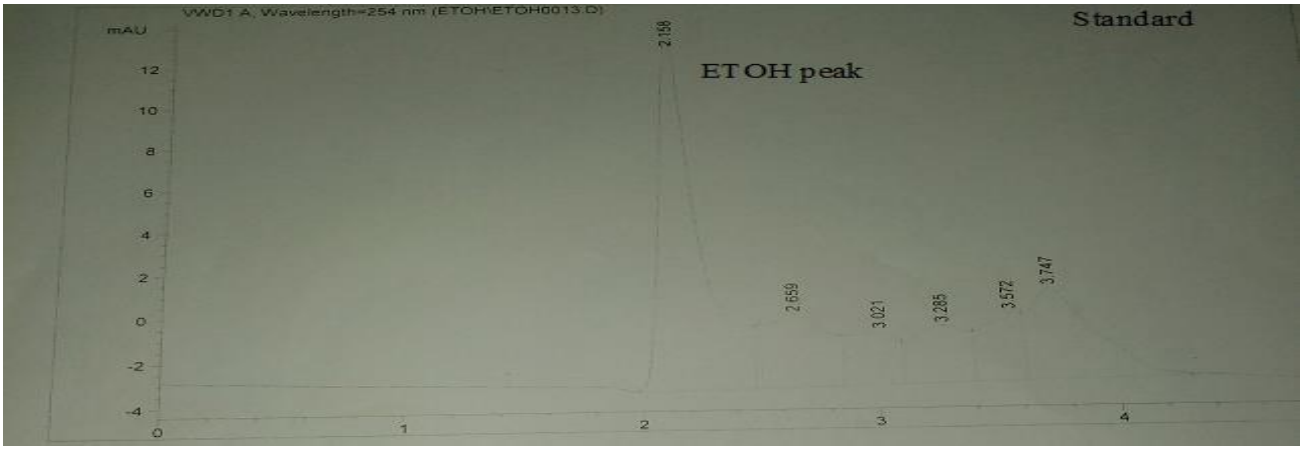
Sample	Density (g/cm^3)	Viscosity (mili pasca)
Sample A	0.97	16.1
Sample B	0.99	14.9
Sample C	0.98	14.8

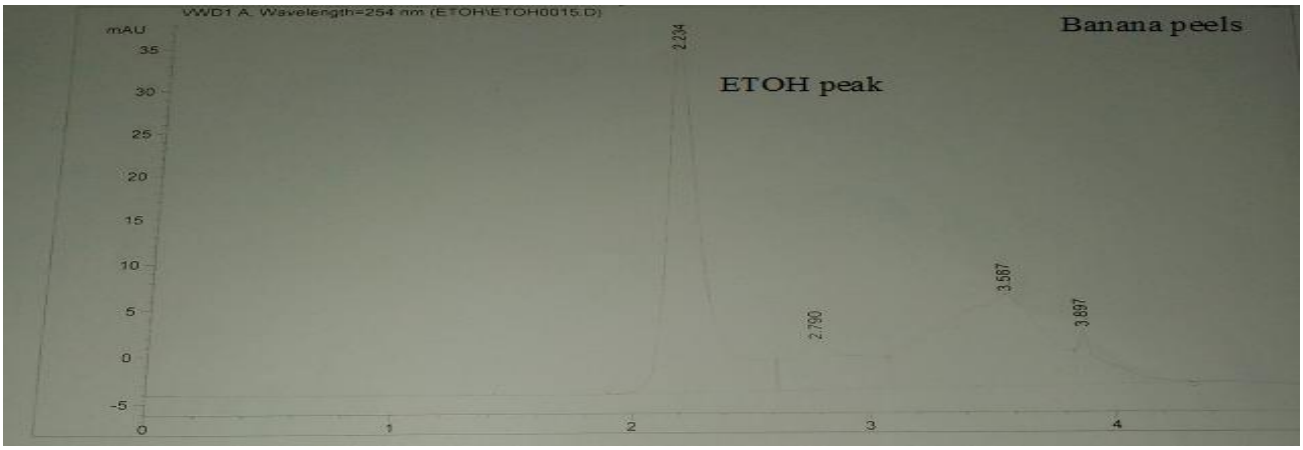
KEY: **Sample A**: watermelon

Sample B: banana

Sample C: mixture

Figure 6: HPLC Chromatogram of the fermented broth. Bioethanol was produced in the fermentation broth with an HPLC peak of 2.548min for watermelon and 2.234min for banana





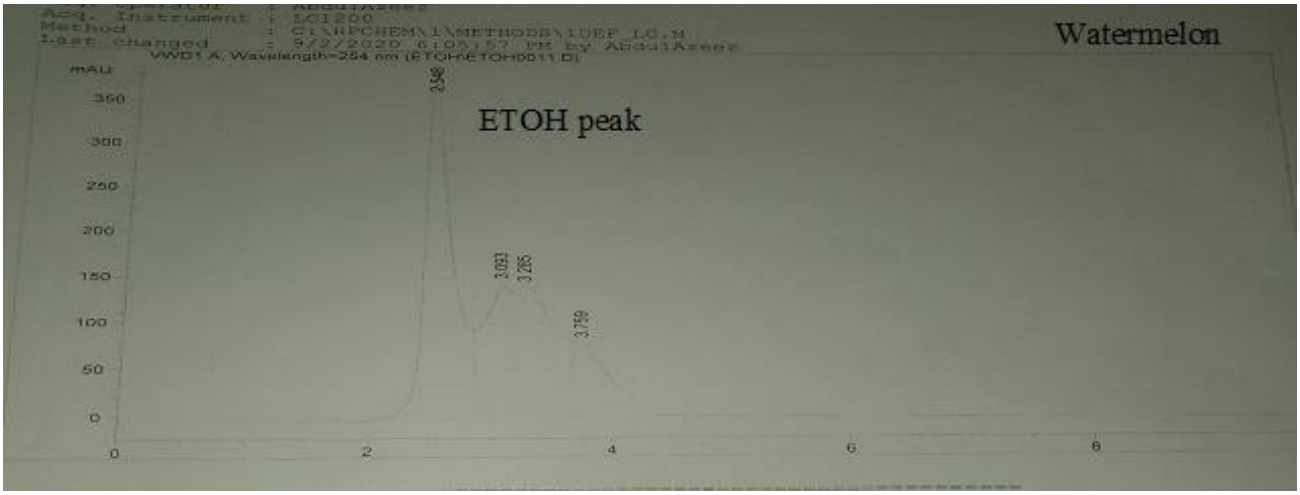


Figure 1:HPLC Chromatogram of the fermented broth of watermelon and banana peels.

Discussion:

The study results showed that the highest reducing sugar yield of 1.92% was achieved on day 6 of microbial hydrolysis when 6 x 10^8 CFU/ml of Bacillus subtilis was used to pretreat 3g of the sample. In contrast, when the sample quantity was reduced to 1g, the lowest yield of 0.39% was observed on day 1. This indicates that the higher the sample quantity, the greater the bioethanol production, and conversely, the lower the sample quantity, the lower the bioethanol yield.

The densities of the distilled bioethanol produced by the various pretreatment methods used in this research indicated that samples with lower densities yielded a higher percentage of bioethanol, with densities being less than 1. Specifically, the results showed that bioethanol produced by pretreatment with Bacillus subtilis had the lowest density of 0.97, while bioethanol produced by liquid hot water (LHW) pretreatment had the highest density of 0.99.

The viscosities of the bioethanol produced by different pretreatment methods also revealed that samples with lower viscosities yielded higher percentages of bioethanol compared to those with higher viscosities. Viscosity, which measures the ease of fluid flow, has a standard value of 1.200 cP at 20°C for ethanol. The study found that bioethanol produced by pretreatment with Bacillus subtilis had the lowest viscosity of 13.6 (mPa·s), while bioethanol produced by LHW pretreatment had a viscosity of 14.8 (mPa·s).

Bioethanol was confirmed in the fermentation broth, with HPLC peaks at 2.548 minutes for watermelon and 2.234 minutes for banana, which differ from the ethanol peak of 12.13 minutes reported in previous research (Iram et al., 2018). This variation may be due to differences in the column and flow rate used. These findings confirm that ethanol was successfully produced in the fermentation broth.

Conclusion

In conclusion, the study demonstrates a clear correlation between the quantity of the sample and the yield of bioethanol, with larger sample sizes leading to higher yields. The highest reducing sugar yield was achieved on day 6 using a 3g sample of *Bacillus subtilis* pretreatment, emphasizing the importance of sample size in maximizing bioethanol production. Furthermore, the study highlights that lower density and viscosity in the produced bioethanol are associated with higher ethanol content, with Bacillus subtilis pretreatment yielding the lowest density and viscosity. The successful identification of ethanol in the fermentation broth, despite variations in HPLC peak times compared to previous studies, confirms the effectiveness of the fermentation process. These findings underscore the potential of optimizing pretreatment conditions and sample quantities to enhance bioethanol production efficiency.

Outlook

The clear relationship between sample size, pretreatment method, and bioethanol yield suggests that further refinement and scaling of these techniques could significantly enhance production efficiency. By optimizing the conditions such as increasing sample quantities, fine-tuning pretreatment methods like microbial hydrolysis with Bacillus subtilis, and controlling factors like density and viscosity industrial-scale bioethanol production could become more viable and competitive.

The successful fermentation of bioethanol from diverse feedstocks like watermelon and banana peels indicates a potential for utilizing agricultural waste as a sustainable and cost-effective raw material. Future research might focus on

further improving yield and process efficiency, exploring other microbial strains or pretreatment techniques, and addressing any remaining challenges related to scalability and economic feasibility.

Overall, the advancements made in this study pave the way for more sustainable and efficient bioethanol production processes, contributing to the broader goal of reducing dependence on fossil fuels and supporting renewable energy initiatives.

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