

Bioethanol Production from Watermelon (*Citrulus lanatus*) Waste Using Alkaline and Acid Hydrolysis in Dutse, Nigeria

Mustapha U. I.^{1*}, Adamu, M.², Zaharaddeen, U.³, and Mustapha, T.²

¹Department of Biological Sciences, Nigeria Police Academy Wudil, Kano State P.M.B. 3474

²Department of Biological Sciences, Federal University Dutse, P.M.B. 7156 Jigawa State Nigeria.

³Department of Biology, Jigawa State College of Education Gumel, Jigawa State Nigeria

*Corresponding author email: musteengumel@gmail.com

Abstract

*The depletion of fossil fuels and escalating greenhouse gas emissions necessitate extensive research in renewable and environmentally friendly energy sources. This study explores the potential of watermelon waste, an agricultural residue, as a feedstock for second generation fuels, which avoids competition with food crop production. Focusing on Dutse, Nigeria, the research investigates bioethanol production through yeast fermentation processes utilizing watermelon waste as a substrate. Two distinct hydrolysis methods, alkaline (NaOH) and acid (H₂SO₄), were employed to break down the blended watermelon waste. *Saccharomyces cerevisiae* was then utilized for yeast fermentation to generate bioethanol. The characterization of the produced bioethanol involved assessing flammability and boiling points. The findings revealed that the percentage yield of ethanol, based on alkaline and acid pretreatments, was 10% and 8%, respectively. The boiling point of the generated bioethanol was found to be 78 °C. In conclusion, the research indicates that alkaline pretreatment outperforms acid pretreatment under the conditions studied. Moreover, the study establishes that watermelon waste can effectively serve as a substrate for bioethanol production, contributing to the development of sustainable and cleaner energy alternatives.*

Keywords: Alkaline, Acid, Bioethanol, *Citrulus lanatus*, Fermentation, Watermelon.

1. Introduction

Energy plays a vital role in driving global advancements and is classified into two main categories: non-renewable and renewable sources (Boontome *et al.*, 2017). Non-renewable sources, which comprise fossil fuels, coal, and gas (Osueke *et al.*, 2011), face various challenges such as diminishing reserves, price instability, and significant environmental consequences (Aden *et al.*, 2002). Consequently, there has been a noticeable surge in research focused on sustainable renewable energy derived from biological origins.

Biomass, however, an energy source that is renewable in nature and characterized by its high carbon content (Watanabe *et al.*, 2002), exhibits potential as a viable alternative to the depleting reserves of fossil fuels. Unlike fossil fuels, the utilization of biomass-derived energy does not contribute to the emission of greenhouse gases (Watanabe, 2002; Gumel *et al.*, 2018; Takase *et al.*, 2023). Various technologies for the conversion of biomass have attracted significant attention, with energy-intensive processes such as pyrolysis and fermentation of sugars extracted from crops like wheat, sugar beets, corn, cane, bagasse, and molasses being of particular interest (Duraisam *et al.*, 2017; Veza *et al.*, 2021). Watermelon, renowned for its utilization in nutraceuticals and its elevated sugar content, generates considerable waste streams during the process of conversion (Choudhary *et al.*, 2009; Artés-Hernández *et al.*, 2010; Fish 2014). This waste, encompassing pulp, juice, and rind, emerges as a valuable raw material for the process of ethanol fermentation (Shetty *et al.*, 2012; Salih & Paranjothi 2019; Rico *et al.*, 2023).

Ethanol has been suggested as a possible substitute for petrol in vehicles, presenting advantages such as the ability to achieve energy self-sufficiency, foster national economic growth, manage waste effectively, and decrease

greenhouse gas emissions (Shyamkishore *et al.*, 2022). The existing global energy scenario is heavily reliant on limited and politically precarious reserves of fossil fuels, which are contributing to the phenomenon of global warming (Bhan *et al.*, 2020; Naved *et al.*, 2022). The escalating demand for fossil fuels, driven by population expansion and industrialization, underscores the necessity of seeking long-term renewable energy alternatives (Dyatlov *et al.*, 2022). Ethanol, acting as a biofuel, has the capacity to alleviate the dependence on petroleum and reduce greenhouse gas emissions by as much as 43% in comparison to gasoline (Meussen, 2018). Furthermore, the production procedures for ethanol are less susceptible to geopolitical factors, thereby enabling nations to domestically manufacture it and diminish their reliance on the global petroleum market. By embracing ethanol as a substitute fuel, countries can attain energy security, stimulate economic progress, and promote environmental sustainability.

Ethanol combustion yields water vapor and CO₂, both of which can be readily assimilated by plants via photosynthesis, thereby rendering it an ecologically sound fuel (Breux, 2012). The utilization of domestically produced ethanol holds the potential to supplant a fraction of petroleum, thereby enabling savings in foreign exchange (Iag *et al.*, 2021). An escalation in ethanol production has the capacity to contribute to sustainable development by fostering the cultivation of watermelons, agro-processing, and manufacturing, thereby aligning with initiatives such as the Greenbelt Initiative (Jamuwa *et al.*, 2016). This investigation addresses the exigent requirement for recycling agricultural waste while furnishing an alternate source of biofuel to alleviate the exorbitant cost of fossil fuels in Nigeria (Sharif *et al.*, 2021). Therefore, the study investigates the transformation of watermelon waste into bioethanol through the means of yeast fermentation, with the objective of converting waste into resources while concurrently producing environmentally sustainable fuels.

2. Materials and Method

2.1 Sample Collection

The watermelon waste that was obtained from the prominent markets of Dutse metropolis, located in the state of Jigawa, Nigeria, was collected and subsequently used as the primary raw material for the production of bio-ethanol. In order to optimize the utilization of the watermelon waste, it underwent a series of processing procedures in accordance with the methodology described by Biose *et al.* (2020). The collection of the watermelon waste was carried out with great care and precision, ensuring that it accurately represented the waste produced within the major markets of Dutse metropolis.

2.2 Alkali Hydrolysis

Watermelon waste was subjected to different treatments prior to hydrolysis to obtain maximum yield of ash and increase cellulose content. Thus, watermelon waste (lignocellulose material) was grinded in to liquid to reduce the cellulose crystallinity (Zabed *et al.*, 2017). This was followed by chemical treatment, where the lignocelluloses substrate was treated with aqueous solution of 2 % (w/v) NaOH. Briefly, a 100 g of the substrate was mixed with 250 ml NaOH and allowed to soak at room temperature (37 °C) for 6 days. After this period, the mixture was autoclaved at 121 °C for 30 minutes; upon autoclaving, the mixture was allowed to cool and filtered until residue was obtained (Ravindran, 2018).

2.3 Acid Hydrolysis

A total of 100 g of the grinded watermelon waste was added into two separate conical flasks of 500 ml capacity followed by the addition of 250 mL of 0.2 M Sulfuric acid (H₂SO₄) in each case, tightly plugged with cotton wool and aluminium foil and allowed to stand for 6 days. After this period, the mixtures from the two flasks were filtered using Whatman No 1 filter paper and the residue was collected.

2.4 pH Adjustment

Before inoculation, the pH of the filtrate was adjusted to a pH level of 5.5 to 6 according to (Gupta, 2009). Where the predetermined pH was acidic, NaOH solution was added drop wise to the obtained filtrate with constant stirring until the pH is adjusted to the range of 5.5 to 6. For pH level greater than 6, few drops of concentrated Sulphuric acid was added until the pH drops to a range of 5.5 to 6. After pH adjustment, the filtrates from the two flasks were kept in an autoclave for 30 min at 121°C.

2.5 Yeast Fermentation and Distillation

Four grams of yeast (*Saccharomyces cerevisiae*) was added into each autoclaved filtrates aseptically in a Laminar flow chamber, plugged with sterile cotton and Aluminium foil and placed in the shaking incubator at a temperature of 35 °C and 120 rpm and was allowed to ferment for 6 days (Ueyama *et al.*, 2019). After 144 hours, the farmented samples subjected to the different hydrolysis ware centrifuged to separate the ethanol from the residue. The bioethanol generated was collected through distillation technique at 78-80°C. A refractometer was used to measure the ethanol content of the fermented samples.

2.6 Collection of Ethanol Samples and Test for Flammability

Samples of the distilled ethanol were collected in clean and labeled collection vessels. The flammability test was performed by introducing a small amount of each ethanol sample to an open flame. The flammability of the ethanol was confirmed by the presence of a visible flame. To detect the generated bioethanol, a few drops of potassium dichromate solution were added to each ethanol sample and color changes in the samples were observed and recorded.

3. Results

Results obtained from the hydrolysis of the water melon sample, as presented in Table 1, exhibited significant alterations in the physical properties of the substrate upon acid treatment, manifesting in a distinct shrinkage of its appearance. Conversely, the application of an alkaline treatment induced a noticeable swelling effect on the water melon substrate. Moreover, the analysis of the substrate's coloration revealed a brownish colored hue (Figure 1a), while no discernible change in color (Figure 1b) was observed in the substrate subjected to hydrolysis with H₂SO₄.

Table 1: Physical Characteristics of Water Melon (*C. lanatus*) Substrate Hydrolysed with NaOH and H₂SO₄.

Pretreatment	Acidic	Alkaline
Physical change of the substrate	Shrinkage of the substrate	Swelling of the substrate
Color change	No change	Yellowish-brown

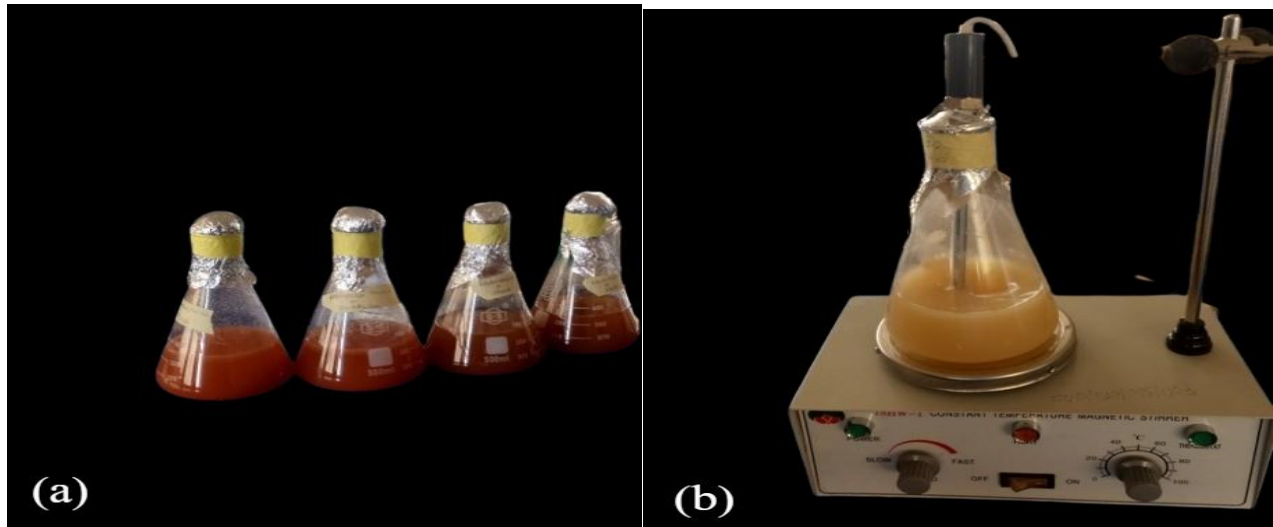


Figure 1: Color Change of the Hydrolysed Watermelon (*C. lanatus*) Waste before Subjecting to Yeast Fermentation.

In order to ascertain the production of ethanol subsequent to the process of fermentation, careful observation was undertaken to detect the presence of carbon dioxide (CO₂), which has the ability to induce a transformation in lime water, rendering it milky in appearance, coupled with the emergence of an unpleasant odor and the formation of bubbles within the flasks. The outcome derived from this investigation, revealed that the treatment of watermelon waste with acid and alkali yielded bioethanol at a rate of 8% and 10% respectively, subsequent to the process of distillation, while the production of absolute ethanol was found to be 20% (Figure 2).

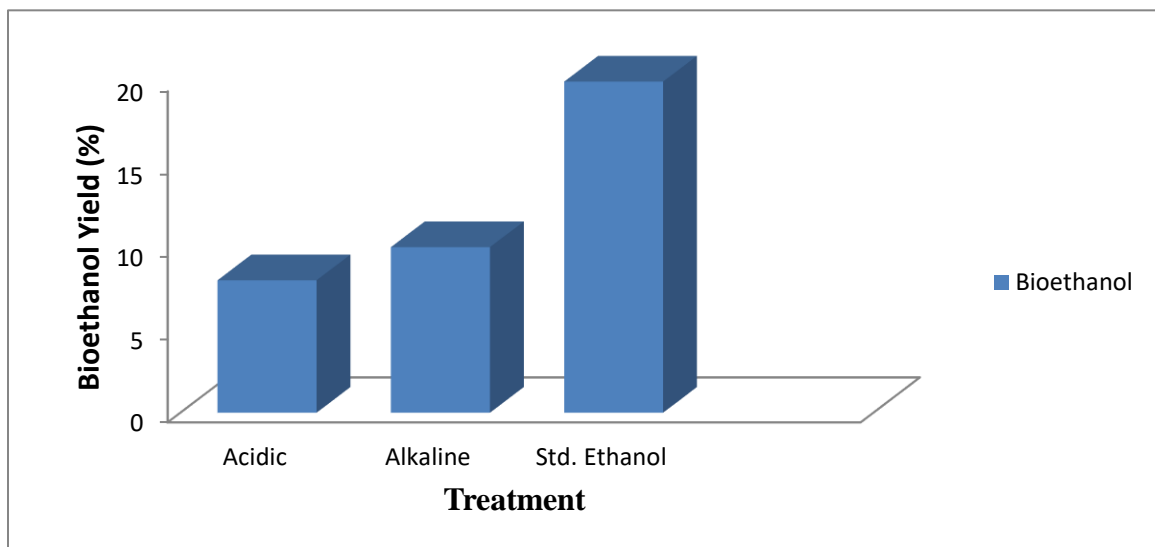


Figure 2: Bioethanol Yield from Water Melon (*C. lanatus*) Waste Subjected to Acid and Alkaline Pretreatment.

3.1 Confirmatory Test for flammability of the Produced Bioethanol

The experimental procedure of assessing the samples flammability was successfully conducted and yielded affirmative results, conclusively establishing that the distilled samples possessed the characteristic of being easily ignited. Furthermore, the utilization of the ethanol detection test effectively corroborated the presence of ethanol in each and every sample, as the observed alteration in color from an initial state of being colorless to a final state of exhibiting a vibrant green hue subsequent to the addition of potassium dichromate reaffirmed this fact. Additionally, the determination of the average boiling point for the bioethanol was carried out, yielding a value of 78°C, which inherently serves as an additional piece of evidence further substantiating the generation of ethanol within the samples. Consequently, it can be confidently concluded that the conducted experiments successfully confirmed the flammability of the distilled samples, as well as the presence and generation of ethanol within said samples.

4. Discussion

Table 1 presents an overview of the alterations in the physical properties of the substrates observed during the hydrolysis process. It is noteworthy that the acid treated watermelon waste experienced a reduction in size, indicating a shrinking effect. Surprisingly, there was no discernible change in its color. Conversely, the alkali treated watermelon waste exhibited a swelling phenomenon, accompanied by noticeable modifications in its coloration. These findings are consistent with the research conducted by Punnapayak and Emert (1986) on acid and alkali-pretreated rice straw. Acid-treated rice straw underwent shrinkage and a color change, while alkali-pretreated rice straw displayed swelling and significant alterations in coloration (Ju *et al.*, 2023; Cutrim *et al.*, 2019).

During the process of fermentation, the existence of carbon dioxide (CO₂) results in a chemical reaction that causes lime water to transform into a milky consistency, which is accompanied by an unpleasant odor, and it is in line with Mokomele *et al.* (2023). Additionally, the presence of bubbles was observed within the flasks leading to the suspecting of ethanol production (Stanzer *et al.*, 2023). This particular study aligns with the research conducted by Niu *et al.* (2009), where they investigated sugarcane bagasse. In their study, they also identified the presence of CO₂, an unpleasant odor, and the occurrence of bubbles (Al-Mudhafar & Alkhafaji, 2023).

The flammability test conducted confirmed that the samples that underwent distillation were flammable (Angraini & Yusmaita, 2023). This research is similar to a study conducted by Lu and Minoru (1993) on the effects of radiation pretreatment on rice straw in the presence of NaOH solutions (Benko *et al.*, 2019). Both studies observed color transformations in the samples after the addition of potassium dichromate, indicating the presence of ethanol (Chen *et al.*, 2019; Stanescu *et al.*, 2020). The findings of this research align with the study by Tewari *et al.* (1986) on the production of ethanol from banana peels, which also noted changes in color following the introduction of potassium dichromate (Almengor & Monaghan, 2015).

In conclusion, the flammability test results affirm the potential combustibility of the distilled samples. The similarities between this research and the studies conducted by Lu and Minoru (1993) and Tewari *et al.* (1986) shed light on the consistent color changes observed when potassium dichromate was introduced, indicating the presence of ethanol. These observations contribute to the growing body of knowledge surrounding the production and analysis of ethanol, particularly in relation to various organic sources.

The determination of the mean boiling point of bioethanol was successfully achieved and recorded as 78 degrees Celsius, a crucial finding that aligns impeccably with the previous investigations conducted by Galbe and Zacchi (2012). In a comprehensive examination encompassing the manufacturing process of ethanol derived from softwood, it was also ascertained that the mean boiling point reached 78 °C, further reaffirming the consistency and reliability of the obtained results.

5. Conclusion

From the findings of this research, it can be confidently stated that alkaline pretreatment emerges as the most optimal method for the purpose of achieving both effective and efficient biofuel production. The results obtained from this study clearly indicate that this particular treatment technique boasts a noteworthy biofuel yield of 10% when compared to the bioethanol yields obtained from alternative acidic pretreatment methods, which only amounted to 8% of the original ethanol. It is worth highlighting that these outcomes solidify the superiority of alkaline pretreatment in terms of its ability to generate substantial quantities of biofuel, thereby positioning it as the preferred approach for bioethanol production.

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