GSC Advanced Engineering and Technology

Cross Ref DOI: 10.30574/gscaet

Journal homepage: https://gsconlinepress.com/journals/gscaet/

(RESEARCH ARTICLE)

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Comparative assessment of the co-production of bioethanol and single cell proteins from Sugarcane Bagasse, Rice Husks, and cassava peels using *Saccharomyces cerevisiae*

Otoikhian SK * and Amune OU

Edo State University, Uzairue, Edo State, Nigeria Km 7, Auchi-Abuja Road, Iyamho-Uzairue Edo State, Nigeria.

GSC Advanced Engineering and Technology, 2022, 03(02), 001–009

Publication history: Received on 19 March 2022; revised on 25 April 2022; accepted on 27 April 2022

Article DOI: https://doi.org/10.30574/gscaet.2022.3.2.0034

Abstract

This work compares various waste sources of feedstock (cassava peels, sugarcane bagasse, and rice husks) for the co-production of bioethanol and single cell protein using *Saccharomyces Cerevisiae* as fermentation enzyme. Sulphuric acid solution was used for the pre-treatment, dilute hydrochloric acid was used for hydrolysis, temperature, time, and pH of 131.8 °C, 1 hr, and 5.3 respectively. Sodium hydroxide (NaOH) was used to adjust the pH of the hydrolysate to neutral (6.7). The primary component of the single-cell protein was ammonia (NH_3), which was quantified using Kjeldahl's technique of analysis, while the bioethanol produced was assessed using the colorimetric and colored reaction method using a spectrophotometer. Sugarcane bagasse showed the highest amount of bioethanol (36.57 %) while Rice husks and Cassava peels showed 30.97 % and 29.31 % respectively. The greatest bioethanol production (47.85 %) was achieved by combining feedstock, cassava, and sugar cane feedstocks. The percentage of single cell protein generated from individual feedstock was the highest using sugarcane bagasse (29.4 %) while a sugarcane bagasse and cassava peels combination gave the highest concentration for single cell protein (43.6 %). The study has demonstrated efficient bioethanol and single cell protein (SCP) co-production from *Saccharomyces Cerevisiae* using sugarcane bagasse, cassava peels, and rice husk as feedstock.

Keywords: Acid hydrolysis; Comparison; Fermentation; Hydrolysate; Saccharification

1. Introduction

Alternative energy sources are a critical consideration because of their environmental friendliness, renewable nature, and sustainability. Greenhouse gas (GHG) levels in the Earth's atmosphere have risen dramatically as a result of fossil fuel consumption, resulting in alternative energy sources being a critical consideration because of their environmental friendliness, renewable nature, and sustainability [1]. Bioethanol as a renewable energy source could substitute petrol in function in its purest form among many other functions. One of the environmental advantages of bioethanol is the reduction of pollutant emission from 80-90 % to about 40-60 % of 2nd generation bio-sourced fuels [2].

Ethanol is typically made from sucrose-based feedstock starch processing, enzymatic liquefaction, and saccharification. As a result, a rather clean glucose pool is produced [3]. However, the direct conflict between energy production from food crops and the world's acute food problem has necessitated the development of bioethanol from sources other than feedstock with direct food and feed benefits [4].

Agricultural wastes such as potato peels, yam peels, maize wastes, sugarcane bagasse, and cassava peels have all been utilized as lignocellulosic feedstock in the manufacture of bioethanol [5]. Nigeria, a significant producer of agricultural

* Corresponding author: Otoikhian SK
Edo State University, Uzairue, Edo State, Nigeria Km 7, Auchi-Abuja Road, Iyamho-Uzairue Edo State, Nigeria.

waste across the world, especially sugar cane, has over 500,000 hectares of excellent sugarcane fields and produced over 3.4 million metric tons of sugar in 2020 [6]. Cassava, particularly an interesting feedstock for bioethanol production is produced in large quantities in Nigeria and the country remains the largest producer of cassava in the world since 2005 [7]. Bioethanol is produced by the metabolic activity of the yeast *Saccharomyces Cerevisiae*, which is capable of converting sugar from feedstock such as sugarcane juice, molasses, etc. into bioethanol. In any case, with the help of the yeast cell digestive system, that component of the sugar is compromised, and other non-ethanol by-products are finally generated [8]. Dry yeast which may be commercialized as SCP is a by-product of bioethanol production. It can be produced from remaining streams from diverse industries giving the plausibility of cheap production [9].

The generation of carbon-neutral bioethanol from agrarian wastes (cassava peels, sugarcane bagasse, and rice husks) addresses the financial, natural/environmental, and food-scarcity concerns of bioethanol production and is anticipated to extend within the future, bringing about an expansive volume of leftover or waste streams [10]. SCP production is an interesting alternative to biogas however, the use from bioethanol production is associated with several challenges including the complexity of residual streams and degradation of products. Some of these degrading products can inhibit the formation of SCP. It is therefore essential to find micro-organisms suitable for these specific residual streams [10].

This paper compares bioethanol production by *Saccharomyces Cerevisiae* utilizing sugarcane bagasse, cassava peels, and rice husks as feedstock by discussing production strategies such as pre-processing, pre-treatments, and hydrolysis, fermentation, and separation procedures.

2. Literature Review

Bioethanol is utilized in cosmetics, thermometers, solvents, preservatives, and most vitally, as an engine fuel (additive for gasoline). These irreplaceable employments of bioethanol have driven an amazingly high request for the item [2] and consequent research for various feedstock and alternatives for its production.

Adesanya et al., produced ethanol using *Saccharomyces cerevisiae* from cassava peel hydrolysate. By day seven, they obtained the largest yield of simple sugar (0.88 mg/ml), and three days, later, the ethanol produced after separating the cell-free extracts with S. cerevisiae was 1.05 percent.

Braide et al. investigated the production of bioethanol from different agricultural waste. *Saccharomyces cerevisiae* was utilized as an enzyme to ferment agricultural-waste from sugarcane plant (*Saccharum officinarum*) i.e. samples from the bagasse and its bark and also from maize plant (*Zea mays*) i.e. cornstalk, corncob and the husk for 5 days, with acid hydrolysis as a pretreatment. The results revealed that the specific gravity, sugar content, and pH of sugarcane bagasse, sugarcane bark, cornstalk, corncob, and corn husk declined with time giving maximum ethanol yields of 6.72, 6.23, 6.17, 4.17, and 3.45 respectively at 72 hrs of Fermentation. Ethanol yields were highest at pH 3.60, 3.82, 4.00, 3.64, and 3.65. Finally, they concluded that their findings demonstrated the potential of the aforementioned agricultural wastes in the ethanol production process and consequently wealth generation [11].

Chibuzor et al., [12] investigated bioethanol production from cassava peels using different microbial inoculants. They used *Saccharomyces cerevisiae*, *Rhizopus nigricans*, *Spirogyra Africana*, and Aspergillus niger in different combinations as inoculants while using *Saccharomyces cerevisiae* as control only. Using *S. Africana*, *S. cerevisiae*, and *R. nigricans* alone, they discovered that Cassava peels sourced from TME 4779 produced the greatest ethanol quantity of 14.46 ± 2.08 g/cm3. Similarly, employing the same combination of *S. Africana*, *S. cerevisiae*, and *R. nigricans*, cassava peels from TME 0505 produced the second-highest ethanol yield of 13.33 ± 0.67 g/cm3. With *S. cerevisiae alone*, low yields of ethanol (4.82 ± 1.00, 6.43 ± 0.58, and 7.77 ± 0.88) g/cm3 were achieved from peels of TME 419, TME 0505, and TME 4779, respectively. They claimed that their yields were comparable to yields reported by other studies from potato peels, millet husks and cassava peels, using several other inoculant treatments and that the inoculants used in this work suggested significant promise for bioethanol generation from cassava peels [12].

Mustafa et al. utilized cassava waste peels to generate bioethanol by acid hydrolysis nd fermentation. After 4 days, they discovered that using 10% H_2SO_4 concentration pretreatment yielded the highest ethanol produced (37.35 g/ml) with a pH of 4.55, sugar content of 15.5 %, and alcohol content of 8.5 %. They subsequently concluded that cassava peels may be used to generate maximum bio-ethanol yield, when using 10 % sulphuric acid for hydrolysis and *Aspergillus niger* and *Saccharomyces cerevisiae* for fermentation [13].

Cassava pulp was also employed as a feedstock in an enzymatic hydrolysis method by Djuma'ali et al to produce biofuel. They discovered that glucoamylase enzyme was the most effective for hydrolyzing cassava pulp for 10 minutes at temperatures of 65 °C and 95 °C, giving around 86.22 percent and 90.18 percent dextrose equivalent, respectively. They

also discovered that the optimal conditions for enzymatic pretreatment of 30 % (w/v) cassava pulp by a potent cellulolytic/hemicellulolytic enzyme were 50 °C for 3 hours, and the that for liquefaction and saccharification using a thermo-stable –amylase, optimality can be achieved at 95 °C for 1 hour or 50 °C for 24 hours, respectively. They finally reasoned that the high glucose output suggests that enzymatic-hydrothermally processed cassava pulp may be used as a low-cost ethanol substrate [14].

Ezebuiro et al., explored bioethanol production utilizing sugarcane bagasse and cassava peels as feedstock by an ethanol-tolerant *bacillus cereus* strain gbps9. Total carbohydrate and lignin concentrations (percent dry weight) for cassava peels were 69.6 ±1.2 and 13.9±0.4, respectively, and 70.3±1.9 and 16.2±1.2 for sugarcane bagasse, according to the chemical composition analysis. Steam explosion, acid and alkali pretreatments were also used to enhance cellulose content and therefore reduce lignin content in the feedstock. They observed that steam explosion for sugarcane bagasse and acid for cassava peels produced the best pretreatment procedures, boosting total carbohydrate content to 85.4±2.33 percent and 80.4±2. Percent, respectively, for sugarcane bagasse and cassava peels. Sugarcane bagasse and cassava peels had lignin levels of 4.2±0.44 and 4.8±0.8, respectively, following pretreatment. The cassava peels and sugarcane bagasse substrates had ethanol concentrations of 17.80 and 18.40 g/L, respectively, according to gas chromatography-mass spectrometry (GC-MS) analyses. They concluded that by utilizing sugarcane bagasse and cassava peels as feedstock, Bacillus cereus can effectively generate bioethanol [4].

Kaur et al. proposed the generation of bio-ethanol with rice husk as feedstock and using simultaneous saccharification and fermentation method, as well as pretreatment optimization procedure while comparing the acid and alkaline pretreatment processes (Kaur et al., 2017). They discovered that pretreatment with 2 percent HCl (acid) and 3 percent NaOH (alkaline) resulted in the highest ethanol yields of 6.34 percent and 5.89 percent, respectively, using 3,5-Dinitrosalicylic Acid (DNSA), FTIR, and GC [15]

Saccharomyces cerevisiae growth, physiology, and metabolism in alcoholic beverage fermentations were discussed by Walker et al. They came to the conclusion that yeasts are critical in supplying the alcohol content and sensory profiles of these drinks.

Maiorella et al., studied the inhibition effects of by-product on ethanolic fermentation by *saccharomyces cerevisiae*. They found out amongst several other products that although ethanol can be inhibited by Direct Interference with Ethanol Production or Cell Growth Pathways, it was not observed in their experiment [16].

Andrietta et al. explored Yeast from Ethanol-sourced SCP (Single Cell Protein). They monitored the cell (Yx/s) and protein yield in the mass of four distinct strains of *Saccharomyces cerevisiae* isolated from the industrial production of ethanol in a growth medium prepared from labratory prepared sugarcane molasses. They concluded that there is a link between the two factors investigated based on their findings. The amount of protein produced is proportional to the amount of cell mass generated [17].

Given the aforementioned research completed amongst others, very little has been done to selectively compare three distinct sources of agricultural feedstock for the co-production of bioethanol and single cell protein using the very popular enzyme (*Saccharomyces cerevisiae*).

3. Material and methods

3.1. Feedstock Sample collection

Sugarcane bagasse, cassava peels, and Rice husks were collected from a local farmer in Iyamho, Edo State, Nigeria. The agricultural wastes were carefully washed to remove all sand and dirt present, dried naturally for approximately three days, and then ground with a machine to reduce the surface area. The prepared sample was then kept in an air-tight container.

Saccharomyces Cerevisiae which was used as the yeast was cultured to ferment the sugar-rich liquid. The essence of preparing the yeast culture is to grow the yeast population.

3.2. Acid Hydrolysis of Feedstock Sample

50 g for each sample of ground wastes were used for the individual experimental feedstock, 25 g each for two samples experimented together while 16.7 g was weighed for all three wastes processed together. Dilute hydrochloric acid (HCl) with concentration running at 1.2 v/v%, the temperature of 131.8 °C and pH of 5.3 time (an hour) was used for acid

hydrolysis of the sample. After filtering the hydrolyzed sample to remove solids from liquids, the hydrolysate was fermented by *Saccharomyces Cerevisiae*, a cultivated yeast. According to Hashem & Darwish [18], the pH of the hydrolysate was adjusted using NaOH to a neutral 6.7 to prepare the hydrolyzed sample as a growth and fermentation medium of the yeast to ensure the production of single cell protein (SCP) hydrolysate and bioethanol.

3.3. Fermentation

The fermentation process was carried out in an anaerobic condition (air-tight container) at approximately room temperature. The yeast that was utilized to ferment the sugar-rich hydrolysate was cultured to make the fermentation medium. This was done to grow the yeast population. 5 g of Ammonium Sulphate (NH_4SO_4), 1.5 g of KOH, and 0.2 g $CaCl_2.H_2O$ were dissolved in 500 ml of distilled H_2O. The media was then autoclaved for 15mins at a temperature of 121 °C and pressure of 15 psi. 15 mg/10 ml of yeast was further added to the yeast culture for continuous growth. The hydrolysate was placed in a flask fit into an orbital shaker and 1 g of yeast was measured and added to the liquid media and left for about four days at 30 °C.

3.4. Analysis

3.4.1. Bioethanol determination

The spectrophotometric technique was used to determine the content of bioethanol after the fermentation time. After measuring 800 mL of distilled water in a suitable container, 7.721 g of sodium acetate was added to the solution, followed by 0.353 g of Acetic Acid. The pH is then adjusted using NaOH with distilled water until volume is 1 litre.

In a 50 ml volumetric flask, 5 ml potassium dichromate solution, 5 ml acetate buffer (pH 4.3), and 25 ml 1N sulphuric acid were added to an aliquot of a standard stock solution containing 1.6 mg/ml. The mixture was gently shaken for 1 minute before being incubated at room temperature for 120 minutes, resulting in the production of a green-colored reaction product. After which the absorbance at 600nm was read on a 562 UV-VIS spectrophotometer - model 752.

Ethanol in the sample (%) was given by:

$$Percentage\ absorbance\ (\%) = Cs \times \frac{A_u}{A_s} \times 100\%\ (Eq.1)$$

Where Cs = Concentration of standard, Au = Standard Absorbance, and As = Absorbance of sample.

3.4.2. Single Cell Protein (SCP) determination

For single cell protein determination, Kjeldahl's technique was employed. This procedure was carried out following AOAC International Method 981.10. [19]. 1 gram of sample material was hydrolyzed in 15 mL concentrated sulphuric acid (H_2SO_4) with copper catalyst tablets and 7 grams of potassium sulfate. It was then cooked for 60–90 minutes at 370 °C. Before neutralization and titration, 250 mL of H_2O was added to the hydrolysates after cooling. The amount of total nitrogen in the samples was determined using simple titration and molarity calculations.

$$Moles\ of\ Acid = Molarity\ of\ acid \times Volume\ acid\ used\ (Eq.2)$$

$$Moles\ of\ Base = Molarity\ of\ base \times Volume\ of\ base\ titre\ (Eq.3)$$

$$Amount\ of\ Nitrogen\ (gms) = Moles\ of\ Nitrogen \times Atomic\ Mass\ (Eq.3)$$

$$Percentage\ Nitrogen\ (\%) = {}^{Amount\ of\ Nitrgen}/_{Amount\ of\ Sample}\ (Eq.4)$$

4. Results

Results from Figures 1 – 7 in the figures section showed the absorbance of bioethanol produced from Sugarcane bagasse, Cassava peels, and Rice husks respectively. It also shows the percentage of bioethanol absorbed from cassava peels and sugarcane bagasse combined, Cassava peels and Rice husks combined, Sugarcane bagasse and Rice husks combined and finally a combination of the three checked samples. This is summarized in Table 1 below.

Table 1 Summarized Bioethanol and Single Cell Protein (SCP) concentration (%) for all Waste samples studied in different modes (Cassava peels, Sugarcane bagasse, and Rice husks)

S/N	Sample	Response Bioethanol concentration %	Response Single Cell Proteins (SCP) concentration %
1	Cassava	29.31	24.5
2	Sugarcane	36.57	29.4
3	Rice husks	30.97	19.6
4	Cassava + Rice husks	28.99	39.6
5	Cassava + Sugarcane	47.85	43.6
6	Sugarcane + Rice husks	36.25	40.9
7	Cassava + Sugarcane + Rice husks	47.06	27.1

5. Discussion

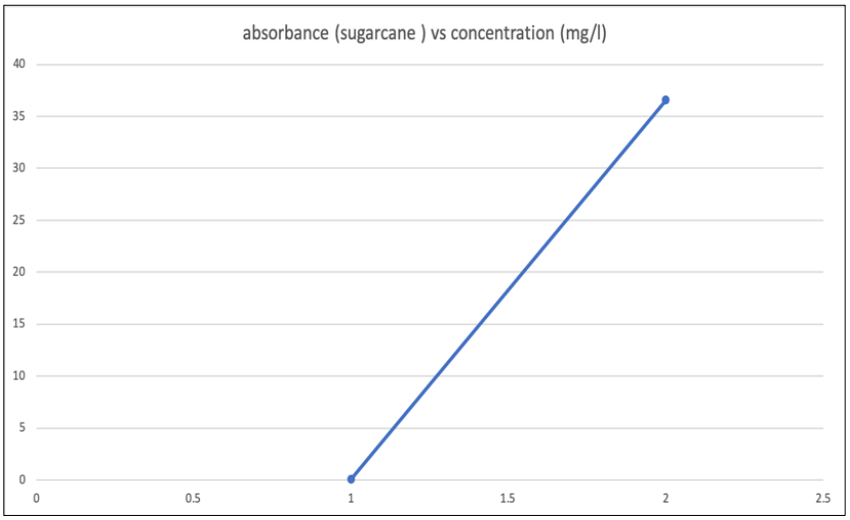

Figure 1 A plot of bioethanol absorbance from Sugarcane bagasse sample (Y-axis) against the concentration of standard (X-axis)

From figures 1 – 3 as shown above, the graph showed that sugarcane gave the highest absorption percentage (36.57 %). This co-relates with the result gotten by [11] where different waste materials got from the sugarcane plant and Maize plant were investigated. It's also due to the high glucose and starch content of the food. in the feedstock (60 – 80 %) [4]. Rice husks and Cassava peels followed respectively i.e. 30.97 % and 29.31 %. Investigations by Kaur et al., and Mustafa et al., [13], [15] respectively proved the above result in order as they obtained maximum bioethanol products at varying conditions – 10 % H_2SO_4 for hydrolysis and 4 days for fermentation for cassava peels while 2 % H_2SO_4 acid hydrolysis and 3 days' fermentation for rice husks. This showed that maximum bioethanol production will be reached with rice husks before cassava peels considering the operating conditions used in this study.

Furthermore, from Figures 4 – 6 above, the largest bioethanol produced was from the combination of cassava peels and sugarcane bagasse i.e. 47.85 % followed by those of Sugarcane bagasse and Rice husks (36.25 %) before Cassava peels and Rice husks (28.99 %). This could be as a result of the abundance of glucose in sugarcane bagasse and the high amount of starch in cassava peels as discussed by Adiotomre [2]. Rice husk on the other hand naturally has a higher Lignin content than the other waste materials [20] which needs to be controlled at more extreme and specific conditions using specific methods before fermentation. Kaur and Singh explored similar results in-depth after generating bioethanol sourced from rice husk utilizing Simultaneous Saccharification and Fermentation (SSF) and optimization of pre-treatment methods in 2017. Also, Chambon et al., in 2019 [20] looked for ways to efficiently fractionate Lignin content in several agricultural wastes including sugarcane bagasse, rice husks, etc. Another cause of this low ethanol production could include the high formation of ethanol inhibitory compounds e.g. aliphatic acids, phenolic compounds,

or furan derivatives [10] which affect the performance of enzymes during hydrolysis and fermentation periods of the production process.

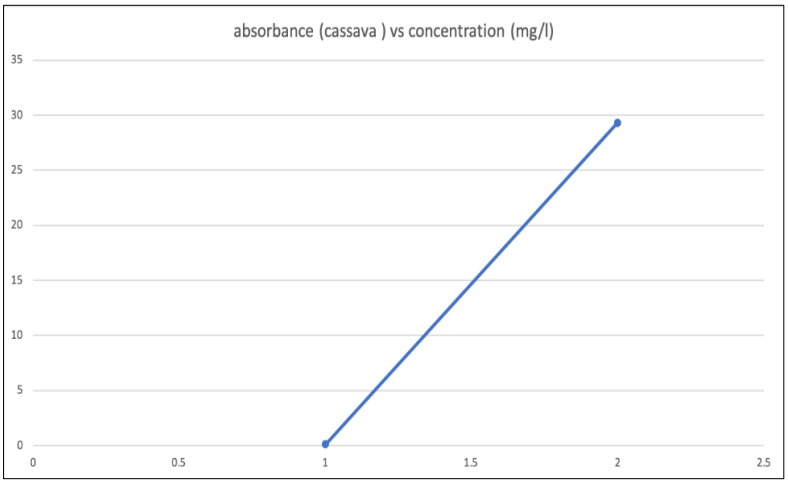

Figure 2 A plot of bioethanol absorbance from Cassava peels sample (Y-axis) against the concentration of standard (X-axis)

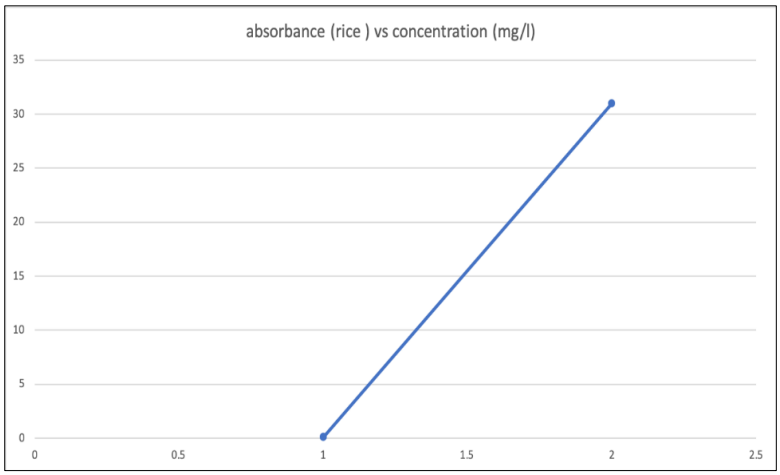

Figure 3 A plot of Bioethanol absorbance from Rice husk sample (Y-axis) against the concentration of standard (X-axis)

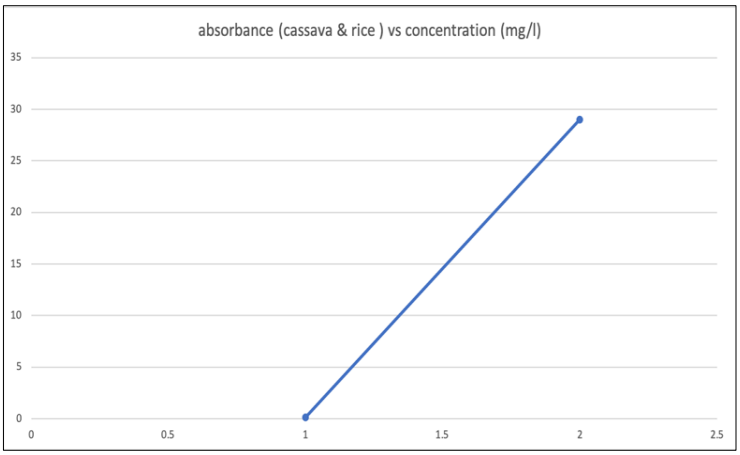

Figure 4 A plot of Bioethanol absorbance from Cassava peels and Rice husk (combined) (Y-axis) against the concentration of standard (X-axis)

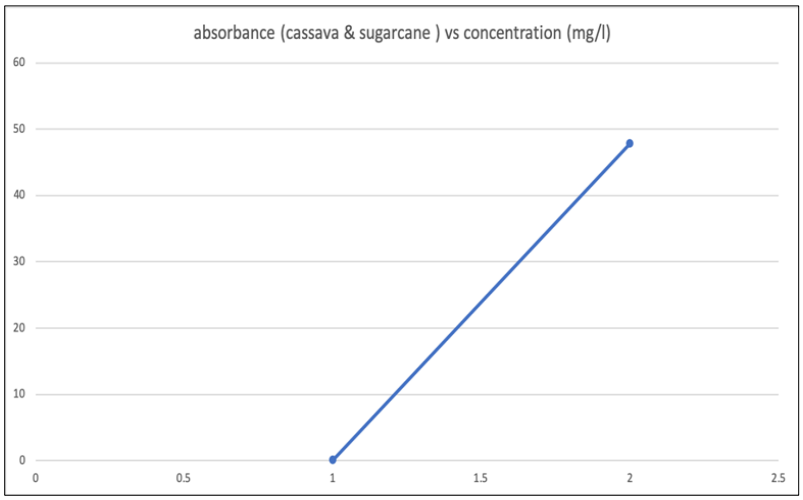

Figure 5 A plot of Bioethanol absorbance from Cassava peels and Sugarcane bagasse (combined) (Y-axis) against the concentration of standard (X-axis)

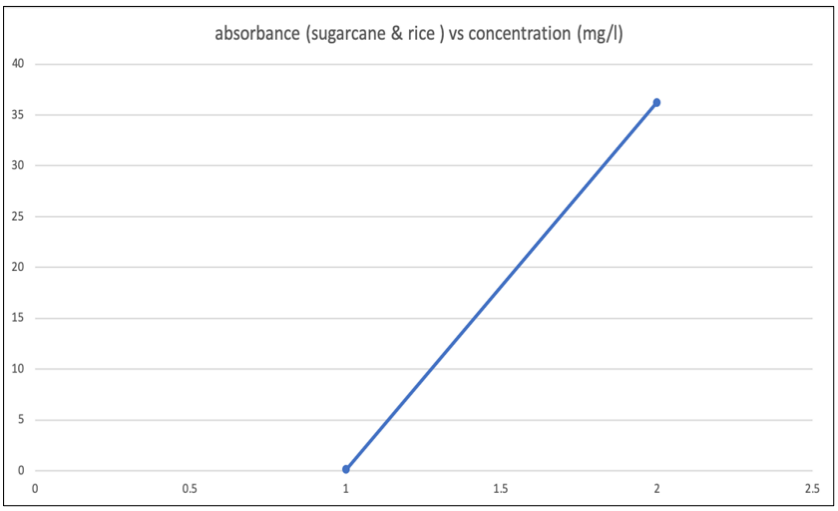

Figure 6 A plot of Bioethanol absorbance from Sugarcane bagasse and Rice husk (combined) (Y-axis) against the concentration of standard (X-axis)

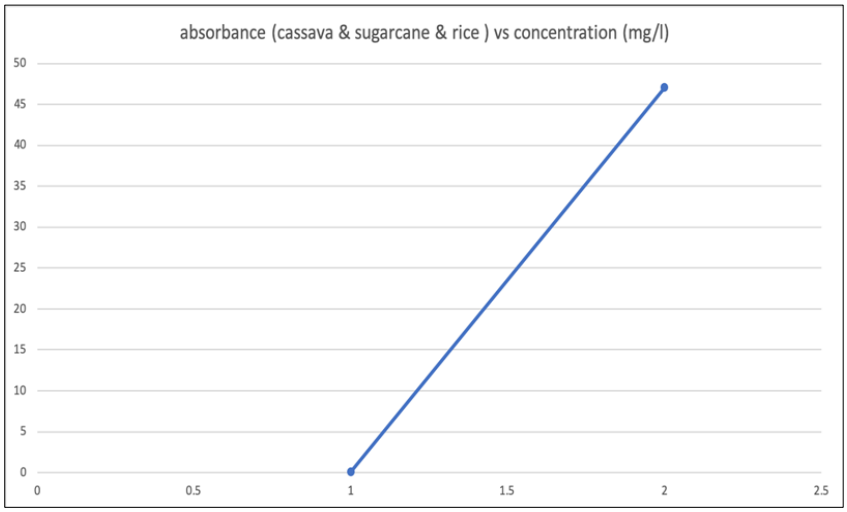

Figure 7 A plot of bioethanol absorbance from Sugarcane bagasse, Cassava peels, and Rice husks (Y-axis) against the concentration of standard (X-axis)

Figure 7 as shown above gives the bioethanol production from the three feedstock combined. The resulting bioethanol absorbance (47.06 %) was unexpectedly shown to be less than that of cassava peels and sugarcane bagasse combined (47.85 %). Due to the large quantity of lignin in rice husks, the high amount of furan derivatives produced, and the inability of the extremely weak concentration of acid during hydrolysis pretreatment to break down the lignin walls, this outcome was a possibility, as stated by Chambon [20].

Finally, from the results obtained in comparing the protein content of all wastes differently, sugarcane also has the highest protein concentration of 29.4 % of Single Cell Proteins produced. This result though pre-treated with acid hydrolysis still correlates with Magalhaes et al., [21]. They discovered that the single cell proteins obtained from sugarcane bagasse were higher than the normal or expected yield. Cassava peels which followed in SCP yield (24.5 %) could be a result of the low formation of furans that normally inhibit the microbial growth in fermentation processes [22].

The concentration of the protein content as expected was highest (43.60 %) in the co-processing of cassava and sugarcane for Single Cell Proteins production. This is most likely also a result of the low quantity of lignin and furans which normally will inhibit the growth of *Saccharomyces Cerevisiae* [22].

6. Conclusion

The findings of this study demonstrate that sugarcane bagasse, rice husk, and cassava peels, all of which contain sugar, are suitable substrates for ethanol synthesis. As a result, the findings of this study show that ethanol may be generated from agricultural wastes rather than contributing to environmental pollution.

Compliance with ethical standards

Acknowledgments

The authors hereby acknowledge the technologists from the Department of Chemical Engineering Laboratory, Edo State University, Uzairue for their availability and valuable support during the experiments aspects of this research.

Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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