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Bio-Ethanol Production from Cassava Waste Peels using Acid Hydrolysis and Fermentation Process

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ABSTRACT

In this research study, cassava peel waste was used as a sole carbon source for ethanol production using the process of fermentation and co-culture techniques. Production of bioethanol from cassava was examined using co-culture of *Saccharomyces cerevisiae*. Sulfuric acid with concentration of 2%, 6% and 10% was used to hydrolyze the substrates. Saccharomyces cerevisiae was further used to ferment the substrates at 35^{0}C for 48 hours. The fermented liquid was distilled at 78^{0}C and quantity of ethanol produced was determined. The findings proved that 10%H_2SO4 acid pretreated sample resulted into maximum ethanol yield (37.5 g/ml), pH 4.5, sugar content (15.5%) and alcohol content (8.5%) after 2 days. This study further revealed that bioethanol can be produced from cassava peels with maximum yield obtained using 10%H_2SO4 for hydrolysis and *Saccharomyces cerevisiae* for fermentation.

Keywords: Bioethanol; Cassava peels; Acid hydrolysis; Fermentation; Saccharomyces cerevisiae

INTRODUCTION

Bio fuels [1] are increasingly recognized as important forms of renewable energy. Bioethanol is produced by hydrolysis and fermentation of carbohydrate feedstocks [2]. This ethanol may be used as a fuel as is or in a mixture with fossil fuels, using various proportions. Biodiesel [3] is produced from oil plants like *Jatropha curcas* where the oil is blended with diesel to produce fuel. Bio fuels (especially second generation bio fuels) hold the promise of sustainable and environmentally friendly energy forms and consequently, production has increased by 8% from the levels in 2005 to 33 million liters in 2009. It has established that production of bioethanol and other domestic forms of energy is economically viable and feasible with available technologies. Crops grown for energy production may be sugarcane, cassava, corn, and sweet potato as well as other high sugar and high biomass producing crops and nontraditional food or cash crops. In countries such as Uganda, cassava is at present predominantly used for food and production of cassava remains low in terms of yield per hectare compared to its potential. The volume presently produced may not meet the demand of ethanol production as well as reducing food security in situations of food deficit. This calls for exploitation of alternative forms of feed stocks. In terms of cassava, the above ground biomass, including stem and leaf residues is often not utilized for economic purposes, apart from being a source of planting material, and the unintended use of cyanogenic glycosides may be exploited for energy production taking into account their role in nutrient recycling. The quest by many countries for energy independence as well as the widespread awareness of the need to reduce green-house gas emissions have heightened the search for alternative energy sources. Bio-fuels are expected to reduce dependence on imported petroleum with associated political and economic vulnerability, reduce greenhouse gas emissions and other pollutants, and revitalize the economy by increasing demand and prices for agricultural products. There is an increasing demand for bio-ethanol as alternative source of energy and Nigeria currently depends on the importation of ethanol to meet its local demand. Bio-ethanol is a microbiological way of converting simple sugar into ethanol and carbon dioxide (CO_2). It is a principal fuel that can be used as petrol substitute for vehicle, and also a renewable energy source produced mainly by sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam.

Ethanol is a chemical frequently employed daily for personal or domestic consumption or cooking, lighting, heating, and steam production[4]. Additionally, ethanol is utilized to create vinegar and a variety of alcoholic beverages [5]. Alternately, in academic studies, medicinal production. It is used in various therapeutic medicines administered orally, topically, or intravenously [6]. Ethanol is a powerful solvent in the pharmaceutical industry. Ethanol is used in personal care products because it has skin-cleansing properties at low doses. As a preservative, it aids in the cosmetic's homogenization of its ingredients. Its incorporation into hair sprays improves the adhesion of the spray to the hair [7]. Ethanol possesses antimicrobial properties, exhibiting effectiveness against bacteria, fungi, and viruses [8]; it is highly effective at a concentration of 70-80% against germs and most viruses related to hospitals [9]. One of the viruses sterilizing with ethanol is the Coronavirus (COVID-19); human endemic coronaviruses can persist on non-living surfaces such as metal, glass, or plastic for up to 9 days but can be effectively inactivated by 62-71% surface disinfection procedures. When it comes to household items like paint, ethanol is used as a solvent to help bring paint's components together [10]. It may also be used as fuel for vehicles and engines when mixed with other compounds [11]. An estimated 30 million gallons of ethanol are used worldwide as a motor fuel. Ethanol can be combined with gasoline or employed as a fuel additive in any proportion. Presently, modern gasoline-fueled automotive engines can ingest a mixture of (bioethanol/gasoline) with ethanol concentrations of up to 15% [12]. Agricultural and cellulosic waste can produce ethanol, such as wood, weeds, and local garbage. That has contributed to its popularity as a car fuel. Residue-Derived Ethanol Production Since bio-ethanol has a wide range of applications, its output is predicted to expand significantly because of rising investment and its economic and environmental benefits in decreasing carbon emissions from energy, which contribute to climate change [13]. New ethanol facilities will be erected in the next several years, and production will reach 110 billion liters by 2023. Besides being used in various sectors, numerous methods for producing raw materials as renewable resources have been devised [14]. According to [15], Brazil and the United States produce the most bioethanol worldwide. Since 1975, Brazil has made ethanol from sugarcane as automobile fuel, whereas the United States has produced ethanol from corn [16]. Most of the bioethanol generated worldwide is derived from sugar cane and corn [17]. Many countries seek to increase the use of bio-ethanol and reduce their dependence on fossil fuels through their politicians in the search for renewable energy sources [18], to address environmental and economic issues affecting our planet. Raw materials for bioethanol synthesis include sugar- and starch-rich crops.

These raw materials may include rice, Sweet corn, potatoes, wheat straw, barley, wheat, and sugar by-products such as molasses and date residues, as well as forest remnants. Ethanol production from agricultural waste is cost-effective and ecologically benign. As a result of their inappropriate disposal, agricultural wastes are seen as a significant environmental burden more agricultural waste results in increased health and ecological and economic harm. Many methods have been used to estimate the presence of ethanol in solutions [19]; an HPLC (High-performance liquid chromatogram) system with a suitable column should be used to achieve high precision in detecting the concentration of bio-ethanol produced [20]. Monochromatic light interactions with the molecules in the sample, photon scattering, and vibration are used to create diagrams illustrating the measurements[21]. Quantitative optical spectroscopy relies on Beer's law and Lambert's rule, which asserts that the intensity of light absorbed by a sample is proportional to the concentration of the chemical [22]. The wavelengths 1200 and 950 cm-1are used to determine ethanol concentrations. Beer assessment using a Gas–liquid chromatography (GLC) device is currently used as a standard protocol in the U.S[23]. and has been the most popular technique for assessing ethanol and blood. This device is associated with a high analytical speed and provides a high accuracy in measuring GLC [24]. Gas chromatography analysis (GC) was employed to determine the quantity of ethanol in alcoholic drinks by injecting the sample straight into the GC apparatus after adding an adequate amount of acetonitrile solution [24]. This approach yielded a result less than 8 minutes after the sample was inserted. In addition to dichromate oxidation, an enzyme oxidation approach uses alcohol dehydrogenase. GC is the most often utilized approach since it provides results more expediently. Dichromate oxidation is the simplest and most appropriate approach for determining ethanol concentration for research applications, such as choosing a high-yielding strain, designing bio-ethanol manufacturing techniques, monitoring processes and managing alcoholic drinks, and measuring the ethanol concentration in clinical samples; this technique determines the amount of ethanol solvent removed from the fermentation medium in this procedure. Its solution's color of the solution changes, the color may change from orange to green when there is an abundance of ethanol in the water. Due to the standard curve of absolute ethanol,

to determine the ethanol content, the absorbance at a wavelength of 584 nm was measured using the chromic acid procedure, which involved the use of a spectrophotometer.

Cassava peels, a byproduct of cassava processing, are rich in starch but also contain complex carbohydrates like cellulose and lignin, which complicate their conversion into fermentable sugars. The conventional approach of bioethanol production from cassava focusses mainly on cassava tubes which is the food in Uganda. This imposes food insecurity; therefore, the alternative use of the cassava waste peels is an area which has not largely been studied in Uganda. Bioethanol production from cassava peels using acid hydrolysis and fermentation presents a promising approach to leveraging agricultural waste for renewable energy. Acid hydrolysis is used to break down these complex carbohydrates into simpler sugars, but this process must be carefully optimized to maximize sugar yield and minimize inhibitory by-products that can affect fermentation. The subsequent fermentation of these sugars into ethanol depends on selecting efficient yeast strains and optimizing fermentation conditions to achieve high ethanol yields. Despite its potential, the economic and environmental feasibility of this process needs thorough evaluation to ensure it is both cost-effective and sustainable. Thus, addressing the challenges of optimizing hydrolysis and fermentation processes while assessing economic viability and environmental impact is crucial for the successful commercialization of bioethanol production from cassava peels.

MATERIALS AND METHODS

Materials

Weighing balance, Conical flasks, Beakers, Measuring cylinder, Spatula, Funnels, Filter paper, Cotton wool, Aluminum foil, Water bath, Autoclave, Distillation set-up, pH meter, Refractometer, Polythene bag, Pestle, Mortar, Gloves, Chemicals, Sodium hydroxide (NaOH), Sulfuric acid (H2SO4), Potassium dichromate (K2Cr2O7), Anti-foam.

Samples Collection

Cassava peels (CP) were collected from domestic wastes dump site located at Lugazi town in Buikwe district. The samples were collected into a polythene bag and taken to chemistry laboratory of Sugar Cooperation of Uganda (SCOUL) for further analysis. The cassava peels samples were washed thoroughly with distilled water to eliminate adhering soil and dust. The peels were sun dried and then grounded into powdered form using pestle and mortar. The grounded powder was then sieved through a 1mm screen to standardize the particle size range of 1mm. The sample was kept in a tightly close container at room temperature. The organisms used were Saccharomyces cerevisiae and were collected from stock cultures of Microbiology Laboratory at SCOUL.

Bio-ethanol Production

The methods used for Bio-ethanol production includes; acid hydrolysis, filtration, neutralization of the filtrate, fermentation and distillation process.

Acid hydrolysis: Exactly, 20 g each of the cassava peel samples was weighed and was poured in a 500cm^3 conical flasks, then distilled water, and 2 %, 6 % and 10 % of sulphuric acid were added separately to respective conical flasks. Sterile distilled water was added to make up to 200 cm^3 mark and the flasks were plunged with sterile cotton wool wrapped in aluminium foil to avoid contamination, the samples were sterilized by heating in an autoclave at 80 degree Celsius for 10minutes and the samples were allowed to cool and were filtered through a Whatman filter paper. The pH of the filtrate sample was adjusted to pH of 4.5 using 10 % NaOH. The samples were labeled as follow C1 = Untreated cassava peels samples (control), C2 = Pretreated cassava peels sample with 2v/v % H_2SO_4., C6 = Pretreated cassava peels sample with 6v/v % H_2SO_4., and C10 = Pretreated cassava peels samples with 10v/v % H_2SO_4.

Fermentation Process

The fermentation was carried out along with simultaneous saccharification and fermentation process (SSF), as described by [20] The conical flask containing the hydrolyzed samples were covered with cotton wool, wrapped in aluminium foil, and autoclaved at 80^{0}C for 10 minutes, and the samples were cooled to the required optimum temperature of 35^{0}C. Co-cultures of Saccharomyces cerevisiae were inoculated into each flasks containing the hydrolyzed samples while the control set still served as control. The flasks were corked using cotton wool, shaken and incubated at the temperature of 35^{0}C. The flasks were shaken at interval to produce a homogenous solution and even distribution of the yeast in the substrates mixture.

Distillation Process

Distillation was carried out by using distillation apparatus setup. The fermented liquid was transferred into round bottom flask and placed on a heating mantle fixed to a distillation column enclosed to a running tap water. Another flask was fixed to the other end of distillation column to collect the distillate at 78 oC (standard temperature for ethanol production).

Analytical methods for bio-ethanol production

Different analytical methods were used for further analysis of bioethanol after distillation such as pH test, determination of quantity of ethanol produced, determination of percentage sugar content, analysis of the strength of ethanol in the distillate, and confirmatory test for bio-ethanol produced.

pH Test: pH meter was first calibrated and was inserted separately into each of the filtrate. The readings were then taken.

Determination of Quantity of Ethanol produced from distillation of fermented wash.

The distillate collected from C1, C2, C6 and C10 were measured using a measuring cylinder and expressed as quantity of ethanol produced in g by multiplying the volume of the distillate by the density of ethanol (0.8033 g/cm3).

Determination of percentage sugar content

Refractometer was used to determine the percentage of total sugar content of the cassava hydrolysate after hydrolysis. This was carried out by placing a drop of cassava hydrolysate on the graduated hand Refractometer glass slide and expressing the brix reading in percentage. The brix (%) was determined using a hand Refractometer according to AOAC [25].

Analysis of the strength of ethanol in the fermented wash :The brix table was used to determine the percent alcohol of the Fermented wash according to AOAC [25].

Confirmatory Test for Bio-ethanol Produced

Confirmatory test was carried out on the extracted bio-ethanol sample using potassium dichromate test. 5 mL of the distillate sample was taken and 2 drops of potassium dichromate was added into the distillate, heated in a water bath for 30 minutes.

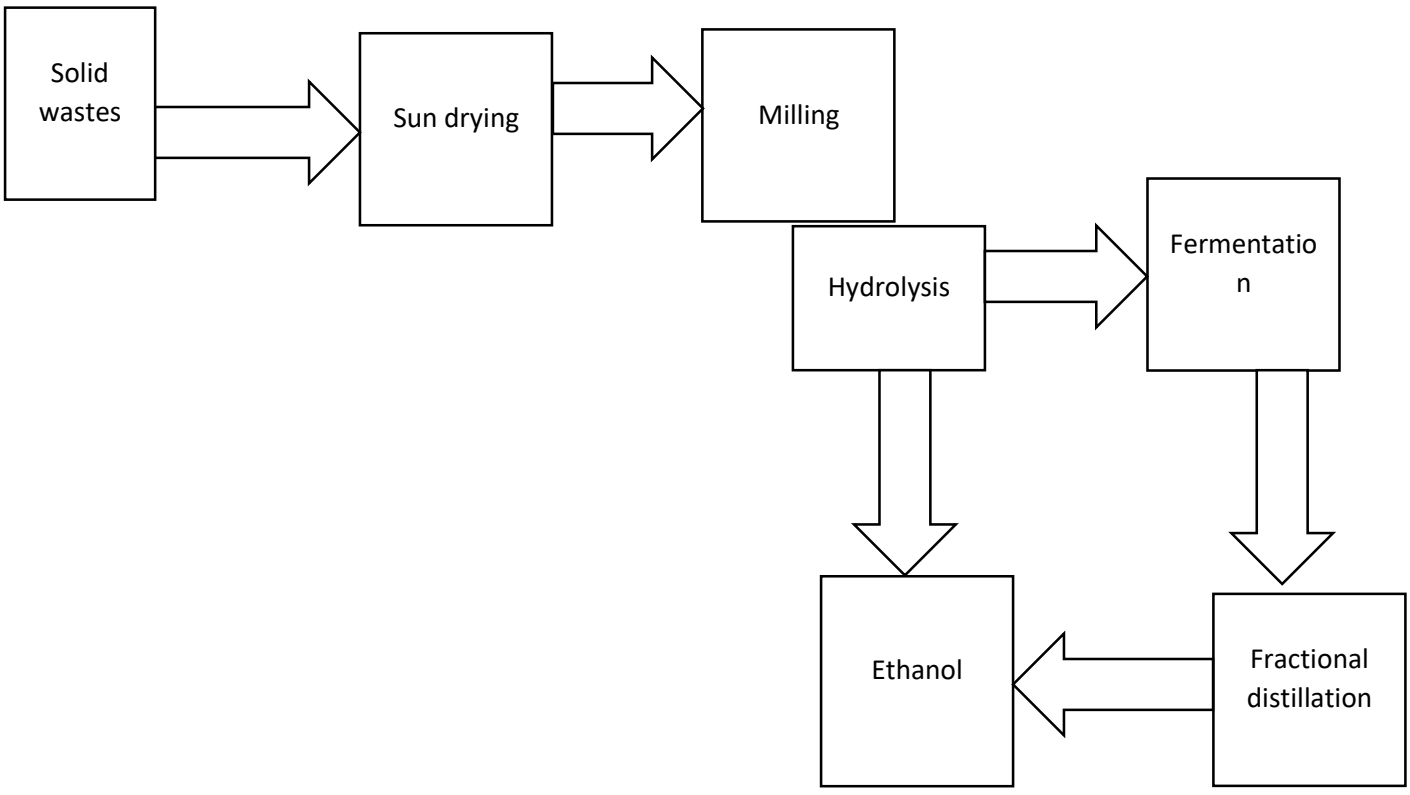

Figure 1: Flow chart of Ethanol production from cassava waste peels

RESULTS

Calculations

Determination of the quantity of ethanol produced

Quantity of the distillate (g) =volume of the distillate * the density of ethanol. Density of ethanol = 0.8033g/cm^3

Table 1: showing the volume of the distillate and quantity of ethanol produced (distillate).

Serial no	Samples	Volume of the distillate (g/cm^3)	Quantity of ethanol produced (g)
1	20 g cp + distilled H2O	25.40	20.40
2	20 g cp + 2v/v%H_2SO_4	29.19	23.45
3	20 g cp + 6v/v%H_2SO_4	37.10	29.80
4	20 g cp + 10v/v%H_2SO_4	46.50	37.35

Concentration of ethanol in the fermented wash

The percent alcohol was obtained from the brix results that is brix after hydrolysis and brix after fermentation by calculating using Brix to ABV (Alcohol by Volume) conversion formula provided in AOAC (2000). ABV (%) = (Initial Brix – final Brix)*0.59.Where 0.59 is the approximation factor.

Table 2: showing initial Brix, final brix and percent alcohol.

Serial no	Sample s	Initial Brix	Final Brix	Percent alcohol (v/v%)
1	20 g cp + distilled H2O	4.0	0.6	2
2	20 g cp + 2%H2SO4	8.3	1.0	4.3
3	20 g cp + 6%H2SO4	11.6	1.3	6.1
4	20 g cp + 10%H2SO4	15.5	1.1	8.5

Table 3: showing quantity of ethanol produced, sugar content, and percent alcohol of the filtrate

Serial no	Samples	Quantity of ethanol (g)	Sugar content (%)	Percent alcohol (v/v%)
1	20 g cp + distilled H_2O	20.40	4.0	2.0
2	20 g cp + 2%H_2SO_4	23.45	8.3	4.3
3	20 g cp + 6%H_2SO_4	29.80	11.6	6.1
4	20 g cp + 10%H_2SO_4	37.35	15.5	8.5

Quantity of Ethanol Produced

The distillate collected was measured using a measuring cylinder, and expressed as the quantity of ethanol produced. The result Proved that the quantity of ethanol increased with concentration. The sample with maximum ethanol was that treated with 10 % H2SO4.

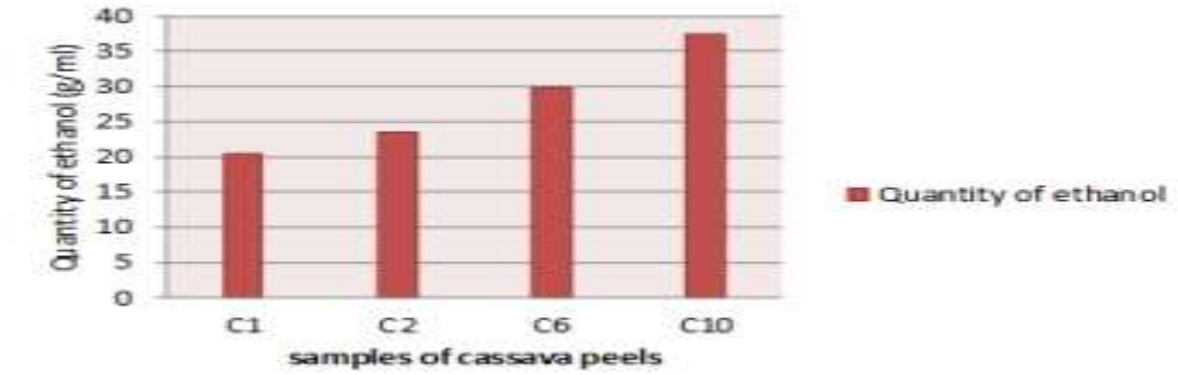

Figure 2: Ethanol yielded after distillation of fermented wash

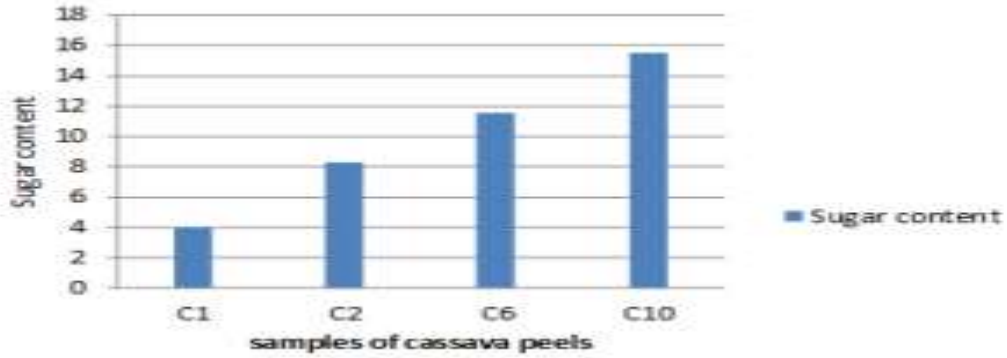

Figure 3: Estimation of percentage sugar content after hydrolysis

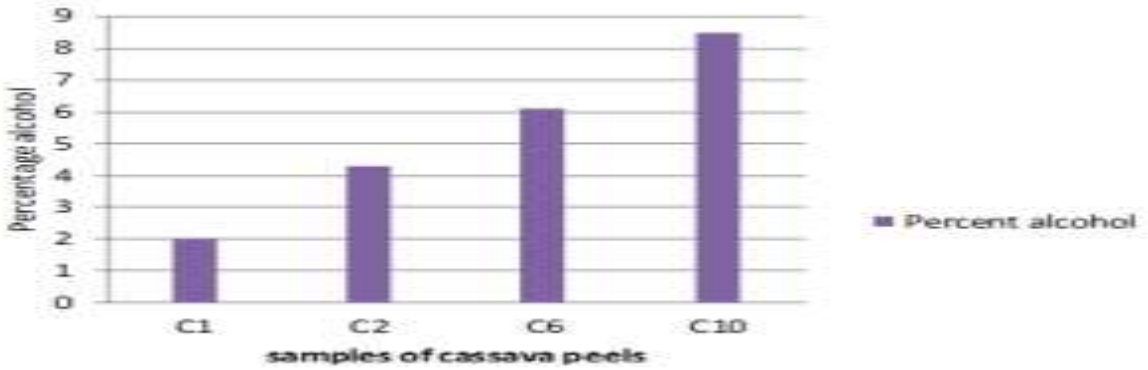

Figure 4: Estimation of percent alcohol after hydrolysis

Table 4: confirmatory test for bio-ethanol produced

Test	Observation	Conclusion
5 ml of distillate + 2 drops of potassium dichromate heated in a water bath for 30 minutes	The formation of green color	Ethanol present

DISCUSSION

Cassava peels are rich in carbohydrates, particularly starch, which can be hydrolyzed into fermentable sugars and then converted to ethanol via fermentation. The use of sulfuric acid as a catalyst helps to break down the cellulose and hemicellulose components of the cassava peels releasing more fermentable sugars, which ultimately influence ethanol production. Saccharomyces cerevisiae was

used to carry out fermentation of cassava peels at 35^{0}C, pH 4.5 and 20 g substrate for 2 days. From the results obtained, there was a gradual increase in ethanol yield as a result of higher concentration of sulfuric acid but a decrease in yield was observed with lower concentration of sulphuric acid indicating that under the condition of temperature (35^{0}C), pH (4.5) and substrate concentration (20 g); the maximum ethanol yield was obtained with 10 % H2SO4, followed by 6 % and 2 % sulphuric acid treatment as shown in table 3. The sample without sulfuric acid serves as the control. The ethanol yield is relatively low because the cassava peels lignocellulosic structure remains largely intact as water does not assist significantly in breaking down the complex polysaccharides in cassava peels. This limiting the availability of fermentable sugars for saccharomyces cerevisiae. Only the naturally occurring starches or simple sugars in the cassava peels are available for fermentation. The slight increase in ethanol yield is observed with 2% sulfuric acid treatment. The dilute acid helps to break down of the hemicellulose and cellulose components of the cassava peels, releasing more fermentable sugars. However, the break down is not extensive hence the ethanol yield is only moderately higher than the control. The 6% sulfuric acid treatment results in a more significant increase in ethanol yield. The higher acid concentration more effectively hydrolyzes the complex carbohydrates (cellulose and hemicellulose), converting them into simpler sugars like glucose and xylose. This sugars are readily fermentable by saccharomyces cerevisiae leading to higher ethanol production.

The highest ethanol yield is observed with 10% sulfuric acid treatment. At this concentration, there is extensive hydrolysis of the cellulose and hemicellulose, maximizing the release of fermentable sugars. More sugars available for fermentation results in higher ethanol production. The variation in the sugar content of cassava peels solutions treated with different concentrations of sulfuric acid result from the effectiveness of the acid in breaking down the lignocellulosic structure of the cassava peels, thereby releasing fermentable sugars. In the absence of sulfuric acid, the cassava peel's complex carbohydrates such as cellulose and hemicellulose remain mostly intact. Therefore, only the naturally occurring sugars (starch or simple sugars) in cassava peels are available in the solution, resulting in a low sugar content (2.0%). The lack of pretreatment process limits the breakdown of complex polysaccharides into fermentable sugars.

With the addition of 2% sulfuric acid, the acid starts to hydrolyze some of the hemicellulose and cellulose in the cassava peels, leading to the release of more fermentable sugars. The sugar content increases to 4.3% because the mild acid treatment breaks down the portion of the complex carbohydrates into simple sugars like glucose, though the effect is still moderate due to the relatively low acid concentration. At 6% sulfuric acid, the sugar content rises significantly to 11.6%. This increase is due to the stronger acid concentration which is much more effective in hydrolyzing the cellulose and hemicellulose in the cassava peels. As a result, more complex carbohydrates are broken down into simpler, fermentable sugars. This shows that a 6% sulfuric acid treatment substantially improves the breakdown of lignocellulosic structure leading to the release of the great amount of sugar.

The highest sugar content (15.5%) is observed with the 10% sulfuric acid treatment. At this concentration, the acid effectively breaks down most of the cellulose and hemicellulose into fermentable sugars, maximizing the release of sugars from the cassava peels. The strong acid treatment causes intensive hydrolysis, allowing for the highest conversion of polysaccharides into simple sugars, hence the highest sugar content in this sample.

The ethanol yield from Figure I shows that there was increase in yield and this may be because ethanol yield increase with increase in concentration. For the control sample where no control sample is used, the ethanol is relatively low (2.0%) because only the naturally occurring sugars and starches in the cassava peels are available for fermentation. The complex carbohydrates (cellulose and hemicellulose) remain largely intact, limiting the amount of the fermentable sugars accessible to saccharomyces cerevisiae. The ethanol percentage increases to 4.3% with the addition of 2% sulfuric acid. The acid helps to hydrolyze some of the hemicellulose and cellulose present in the cassava peels, releasing more fermentable sugars like glucose. This increase in available sugars allows saccharomyces cerevisiae to produce more ethanol during fermentation resulting in a higher alcohol percentage.

At 6% sulfuric acid, the ethanol percentage rises further to 6.1%. This higher concentration of sulfuric acid is more effective in breaking down the cassava peels' lignocellulosic structure, leading to a greater release of fermentable sugars. As more sugars become available, saccharomyces cerevisiae is able to convert them into more ethanol, resulting in a higher alcohol yield. The highest alcohol percentage (8.5%) is observed with 10% sulfuric acid. This strong acid concentration causes extensive hydrolysis of the cellulose and hemicellulose, maximizing the release of fermentable sugars from the cassava peels. With an abundance of fermentable sugars, the yeast can produce the highest quantity of ethanol in this sample, resulting in the highest alcohol percentage.

CONCLUSION

The results show that sulfuric acid pretreatment enhances the breakdown of cassava peel's lignocellulosic material, increasing the availability of fermentable sugars for ethanol production. As the concentration of sulfuric acid increases, more complex carbohydrates are hydrolyzed, leading to higher ethanol yields. Hence, the sugar content, the quantity of ethanol and alcohol percentage produced increase with the concentration of sulfuric acid. However, very high acid concentrations can also lead to the formation of inhibitory byproducts like furfural and hydroxymethylfurfural, which can affect fermentation efficiency, although this was not directly tested in the experiment. Therefore, treating cassava peels with sulfuric acid, particularly at concentrations of 6% and 10% significantly enhances the breakdown of complex carbohydrates hence higher yields. This suggests that sulfuric acid hydrolysis is an effective method to maximize ethanol production from cassava peels' biomass.

RECOMMENDATIONS

1. Implement detoxification methods such as activated carbon treatment to remove fermentation inhibitors from the hydrolysate produced by acid hydrolysis. This will enhance yeast performance and ethanol yield.
2. Evaluate the environmental footprint of bioethanol production from cassava peels in comparison to conventional fossil fuels. Analyze carbon emissions, waste management to determine the sustainability benefits of this approach.
3. Advocate for policies that promote the use of agricultural waste like cassava peels for bioethanol production. This could include subsidies, tax incentives and the establishment of frameworks supporting renewable energy resources, waste reduction and bio economy development.

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