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Laboratory and field production of bioethanol from cassava peel-waste using dilute acid low temperature hydrolysis and fermentation processes

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Abstract: In tropical countries, agricultural wastes such as cassava peel are potential raw materials for production of bioethanol because they are renewable, cheap and widely available. This study investigated the potential of cassava peels as raw materials for ethanol production following dilute acid hydrolysis and fermentation using *Saccharomyces cerevisiae* (baker's yeast). The experiments were conducted both under laboratory and field conditions. In the laboratory experiments, dry cassava peel flour samples (1.0g) were hydrolysed with sulphuric acid (50mL; 0.1M) at different temperatures ranging from 25°C to 70°C and hydrolysates were analysed for reduced sugars. The optimum result of the acid of hydrolysis was fermented by baker's yeast for a period of six days at room temperature, distilled and the alcohol yield was determined using an alcoholimeter. In the field experiment, 6 kg of dry cassava peel flour were hydrolysed with 30L of 0.1M battery acid in a Solar Still for hydrolysis for three days, after which it was neutralised and anaerobically fermented with 150g of baker's yeast at room temperature for four days. The fermented mixture was filtered using mutton cloth, distilled using the local distillation apparatus and the alcohol content of the filtrate determined. In the laboratory experiment, maximum reducing sugars yields of 45% were obtained at 70°C over a period of two days and the maximum obtainable yield of bioethanol was 12%. In the field experiment, the highest ethanol yield was 7.5% ethanol. The results suggest that it is possible to hydrolyse cassava peels using dilute acid. However, there is need to maintain higher temperatures under field conditions throughout the year and more specifically during the period of cassava harvest.

Keywords: *Acid hydrolysis, Bioethanol, Cassava peels, Reducing sugar, Baker's Yeast*

1. Introduction

Inadequate energy supply has been identified as one of the major problems confronting Malawi and limiting its socio-economic and industrial development (Gamula et al., 2013). The country uses hydropower, biomass, fossil fuels, coal and other renewable energy sources to meet its energy demands. However, with growing human population now at 18,628,747 and annual growth rate of 3 % and urbanisation at 17 %, demand for energy is ever increasing (National Statistics Office, 2019). Apart from hydropower, biomass, coal and other renewable energy sources, Malawi imports all fossil fuels to meet its energy demands spending at least 10% of its foreign currency reserves (Government of Malawi, 2019). There is therefore a need to find alternative and cheaper sources of fuel to replace or supplement the imported fossil fuels which are proving too costly for the county's economy. Realising the need for alternative and cheaper sources of fuel, and to save foreign currency, the government of Malawi has since the 1980s been promoting the use of ethanol produced from sugarcanes as an alternative source of energy. This is manifested through National Energy Policy where the Malawi government has made a commitment to support, encourage and promote the production of bioethanol for blending or stand-alone use in vehicles, as well as cooking, lighting, by increasing local capacity to produce bioethanol without threatening food security, especially through the collaboration of farmers' cooperatives, women farmers' coalitions, and other marginalized groups. (Government of Malawi, 2019). Production of ethanol in Malawi started in the early 1980s, and ethanol has since then been used as alternative motor fuel i.e. for gasoline blending and cooking fuel (Gasparatos et al., 2017; Nyambane et al., 2020; Zabed et al., 2014).

Around the globe, looming energy crisis due to continuous depletion of fossil fuels, economic and political crises, and growing concerns on environment safety have led to an increased interest in biofuels as alternative source of fuel (Baadhe et al., 2014; Dincer & Zamfirescu, 2016; Okudoh et al., 2014). Biofuels are of particular interest because they can be produced from renewable materials such as agricultural residues. These biofuels are obtained from mainly three types of raw materials; sugar juice, starchy crops, and lignocellulosic materials. Bioethanol is an alcohol compound obtained by hydrolysis and fermentation of carbohydrate rich biomass with the help of microorganisms and cassava (*Manihot esculenta*) is one such starchy crop that can be utilised for this purpose (Niphadkar et al., 2018; Nuwamanya et al., 2012). Cassava is an important food crop in the tropics consumed in various forms as a major carbohydrate staple by humans and its usage as a source of ethanol for fuel among other uses is increasing (Taiwo, 2006). Several researchers have explored the potential of cassava as a raw material for bioethanol production using various parts of the cassava plant such as the tubers, peels and stems as well as its products, starch and flour (Han et al., 2011; Mushimiyimana & Tallapragada, 2016; Nuwamanya et al., 2012; Ogunsuyi et al., 2016; Osemwengie et al., 2021; Wangpor et al., 2017). Cassava peel is an important agricultural residue that can be used to produce bioethanol. It is rich in carbohydrates such as starch and cellulose, thereby potentially a raw material for bioethanol production and cassava peels are renewable, cheap and widely available wastes in tropical countries (Aruwajoye et al., 2020; Bušić et al., 2018; Femi et al., 2018; Zhang et al., 2016).

Nkhotakota district of Malawi is one district with high cassava production and therefore peels are in abundance during harvest seasons. During this time, the cassava peel wastes generated are generally disposed of in the environment where they generate greenhouse gases (GHG) as they biodegrade. Initial studies have estimated that the actual peels produced to be approximately 6.1 metric tons per year (Mkandawire, 2016). If not utilised, these peels can pose danger to the environment as decomposition of the wastes will negatively affect it. There is therefore a need to explore the use of these cassava peels in bioethanol production. With bio-ethanol production potential estimated based on exploitation of 20 % of the available peels, and the ethanol being concentrated to 40 % alcohol, these available peels have potential of producing 222,252 litres of bio-ethanol per year (Mkandawire, 2016). This study was therefore aimed at investigating the potential of cassava wastes generated in Nkhotakota for bioethanol production under laboratory and field conditions using dilute acid hydrolysis of cassava wastes.

2. Materials and Methods

2.1.Materials

For the laboratory studies, fresh and fermented cassava peel samples were collected from Nkhotakota Cassava Processors Association in Nkhotakota district. The peels were dried to a constant weight for one week in a solar drier constructed at a workshop of the MUST Institute of Industrial Research and Innovation (MIIIRI) of the Malawi University of Science and Technology (MUST). The dried cassava peels were then ground using a heavy duty blender and sieved using a 2.0 mm sieve. Sieved samples were stored in plastic bags before chemical analysis. For the field studies, fresh cassava peels were collected from Nkhotakota Cassava Processors Association members and were sun-dried for one week. After drying, the cassava peels were milled into flour using a maize mill and stored in sacks at room temperature prior to being utilised for bioethanol production.

2.2. Laboratory Experiment

A triplicate of 1.0g of ground samples were weighed using an analytical balance and were mixed with 50mL of 0.1M Sulphuric acid in a 250mL reagent bottle. The mixtures were then shaken for about 1min before being placed in a water bath that was set at temperatures of 25, 30, 50, 55, 60, 65 and 70°C for the specified period of time. The total amount of reducing sugars was estimated by anthrone method (Hodge, 1962) as follows: At the specified time interval, aliquots of the mixture were taken and filtered using whatman paper no. 42 before being neutralised with solid sodium carbonate. Using a micropipette, exactly 5mL of the sample solution was transferred into a 100mL volumetric flask that was then made up to the mark with distilled water. Using a micro-pipette, exactly 1ml of the sample solution was transferred into a test tube which was followed by addition of 4mL ice cold Anthrone reagent. The test tubes were then put in boiling water for exactly 10 minutes before being cooled in cold water. The absorbance of the samples was measured at 630nm using UV-Visible spectrophotometer (Hova Labs, Ambala, Haryana). The amount of total glucose was calculated using glucose standard graph.

Fermentation of the optimum result of the acid of hydrolysis process was conducted as follows: After sulphuric acid hydrolysis, samples were cooled to about 30°C and filtered using Whatman No. 1 filter papers. The filtrate was neutralised using sodium hydroxide to pH 5 and baker's yeast (*Saccharomyces cerevisiae*) at 2.5 % was added. Samples were then left in open bottles to 1 hour to allow the yeast to grow after which the sample bottles were closed and left on the benches at room temperature for fermentation. Fermentation process was done for 6 days and after fermentation, alcohol was distilled at a range of between 75 and 80°C and collected as a distillate. Alcohol content of the distillate was measured using an alcoholmeter as follows: 100mL of distillate was measured into a measuring cup, then alcohol meter was dipped into the distillate. The immersed limit on the distillate surface indicated the alcohol content of the sample under test (Waisnawa & Sudana, 2021).

2.3. Field Experiment

A sample of 6 kg of the cassava peels flour was thoroughly mixed with 30L of 0.1M acid prepared by dilution of battery acid locally purchased with ground water. The mixture was then placed in a plastic container in a solar still for hydrolysis for three days after which the flour-acid mixture was neutralised using 200g of NaOH to pH of 4.8 and left to cool to a temperature of 35^{o}C. After cooling 150g of baker's yeast was added to the neutralised mixture, thoroughly mixed, and left to stand for three hours to allow the yeast to grow. Then the container was closed with a black plastic sheet to enable anaerobic fermentation and left to ferment at room temperature (28-35^{o}C) for four days. The fermented mixture was then filtered using mutton cloth and the filtrate was distilled using the local apparatus normally used for local liquor (*kachasu*) distillation. The distillate was collected, stored in glass bottles and its alcohol content was determined using an alcoholmeter as previously described.

3. Results and Discussion

Results from the laboratory studies revealed that the amount of reducing sugars increased with increasing temperature of hydrolysis as depicted in Figure 1.

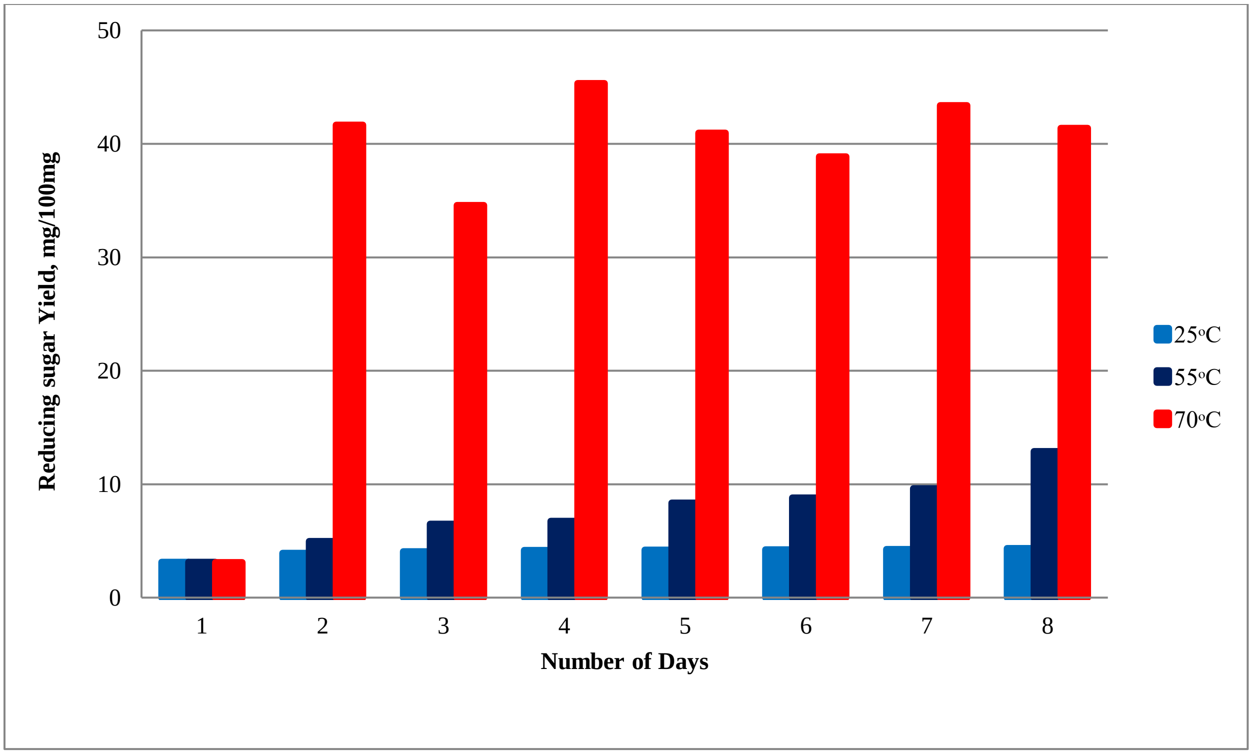

Figure 1- Reducing sugar yields with duration in days at 25, 55 and 70 ℃

At 0.1M acid solution concentration, the hydrolysis was insignificant at temperatures below 50 ℃ but yielded significantly higher amounts of reducing sugar at temperatures above 50 ℃: No significant change in the amount of reducing sugars was observed at 25 ℃.

A slight steady increase in the amount of reducing sugars was observed at 55 °C reaching a level of 13 mg/ 100 mg after 8 days of hydrolysis while at 70 °C, yields of above 13 mg/ 100 mg were achieved. Both temperature and reaction time are important factors in a hydrolysis reaction. The higher the temperature and the longer the reaction time, the more the degradation (Scordia et al., 2010). Thus the reducing sugar yields could be attributed to increased temperatures and reaction time.

A comparison of fresh/unfermented and fermented cassava peels showed that reducing sugar yields were higher following hydrolysis of fresh cassava peels; at 70 ˚C the amount of reducing sugars obtained over the 8 days of hydrolysis averaged 36 mg/100 mg for fresh cassava peels and 16 mg/100 mg for fermented peels. Thus reducing sugars yields from fermented cassava peels were found to be less than 50% of yields from non-fermented peels (Figure 2).

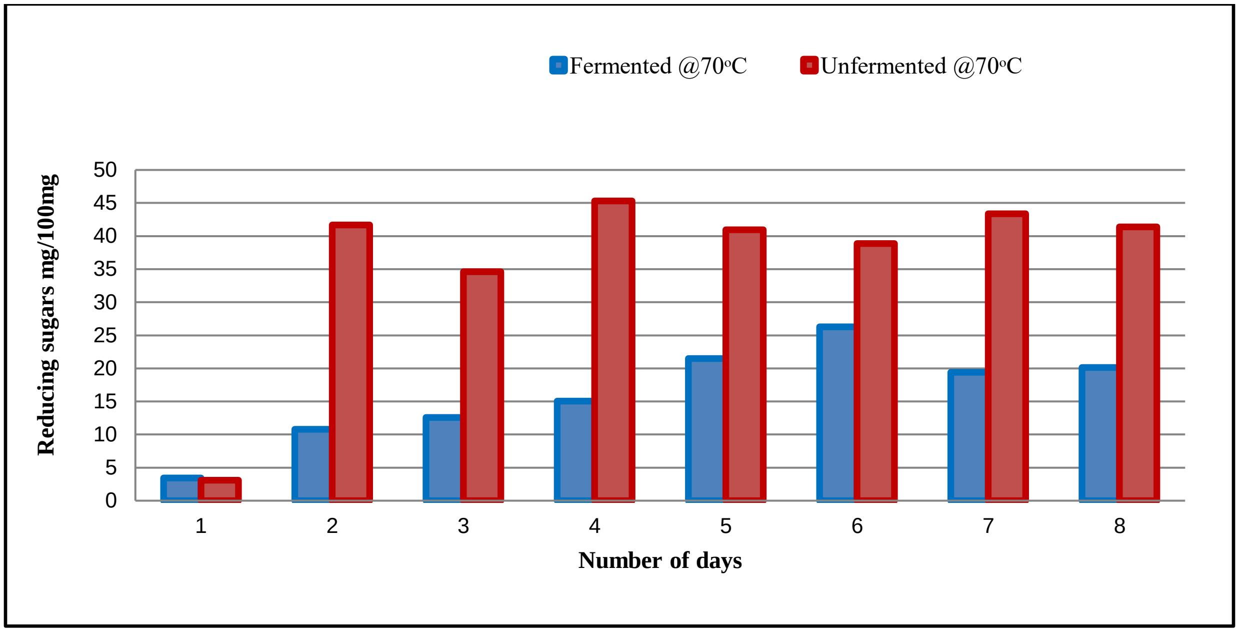

Figure 2- Reducing sugar yields for fresh and fermented cassava peels at 70 ° C with duration in days

Acid hydrolysis of agricultural waste is influenced by type and concentration of the acid, and temperature and duration of acid hydrolysis. Several researchers have studied the use of different acids for optimal production of reducing sugars and bioethanol from cassava peels and found sulphuric acid to be more effective acid for the degradation of cassava peels into fermentable sugars (Ogunsuyi, et al., 2016; Adesanya et al., 2008; Yoonan & Kongkiattikajorn, 2004). This study used sulphuric acid for hydrolysis of cassava peels because the acid is readily available in Malawi and commonly sold as 'battery acid' in all areas including rural areas of the country. Further the study used sulphuric acid of 0.1 M concentration. The use of dilute acid in this study was influenced by the fact that this research project is local community based, aimed at empowering local communities through decentralised production of bioethanol from cassava wastes, hence the economic status and financial capacity of the beneficiaries needed to be considered as this would be one of the major contributing factors affecting its adoption. Use of dilute acids entails reduced expenditure in the production process. Our baseline survey revealed that the production of ethanol at local level involves sugar, maize bran, yeast and water. Sugar alone constitutes about 71% of the total production costs with firewood and labour being other components of the cost. Water and maize bran are usually supplied free of charge and yeast is recycled (Mkandawire 2016). Thus replacing sugar with reducing sugars obtainable after fermentation should translate into reduced costs.

Hydrolysis ability of waste is a function of combined acid concentration, temperature and reaction time (Alfonsín et al., 2019). Several researchers have studied conditions for optimum reducing sugar yields from cassava and its wastes using different methods (Akaracharanya & Kesornsit, 2011; Candra & Murdianto, 2019; Gaewchingduang & Pengthemkeerati, 2010; Jeddou et al., 2014; Olanbiwoninu & Odunfa, 2012; Srinorakutara & Kaewvimol, 2006). Akaracharanya & Kesornsit (2011) found that pretreatment of 6% (w/v) cassava pulp (dry weight basis) by 2% (w/v) H_2SO_4 for 30min yielded a glucose level of 79.8% (w/w). Candra et al (2019), found that hydrolysis by H_2SO_4 was more effective than HCl. Hydrolysis solution of 0.58 M H_2SO_4 gave an optimum reducing sugar in hydrolysate (5.6%), which equivalent to a yield of 28.18%. Gaewchingduang & Pengthemkeerati (2010) investigated the optimal condition for hydrothermally pretreating cassava baggasses with or without acid addition and found that pretreating cassava baggasses with sulfuric acid at 120 °C for 60 min gave a maximum reducing sugar yield. They also found that sulfuric acid had a greater capacity for hydrolysing cassava baggasses than phosphoric acid and enzymatic hydrolysis combined hydrothermal pretreatment. Sinorakutara et al (2006) obtained maximum reducing sugar at 6.1% (w/v) using a cassava waste to acid ratio of 1:2 (w/v) at 0.6 M H_2SO_4 and 120 °C. Other researchers have also achieved optimum reducing sugar yields using 0.1 M H_2SO_4 acid solutions for hydrolysis ranging from 60 to 70% (Olanbiwoninu & Odunfa, 2012; Yoonan & Kongkiattikajorn, 2004; Abidin et al., 2014; Mustafa et al., 2019). In their study on enhancing the production of reducing sugar from cassava peels, Olanbiwoninu & Odunfa, 2012 found that acid hydrolysis using sulphuric acid at a concentration of 0.1M at 120°C for 30 min gave a maximum reducing sugar yield of 88.8% and 98%. Yoonan & Kongkiattikajorn (2004) achieved reducing sugar yield of 60.7% at 135°C after 90 minutes of acid hydrolysis whereas while Abidin et al. (2014) achieved only 5.24% of reducing sugars following hydrolysis of cassava peels with 0.5 M sulphuric acid solution at 100°C for 60 min. Mustafa et al. (2019) used 2 %, 6 % and 10 % sulphuric acid solutions to hydrolyse cassava peel waste and found that 10 % H_2SO_4 concentrated acid pre-treated sample resulted into maximum sugar yield of 15.5%.

These results clearly indicate that maximum reducing sugar values are achievable using 0.1M sulphuric acid. However, it is also important to note that these maximum reducing sugar yields are achievable at temperatures around and above 100°C. The results of this study have shown that reducing sugar yields increased with increasing temperature. The maximum reducing sugar yield of 45% obtained in this study could be improved if temperatures higher than 70°C were employed. Nevertheless, in the laboratory study, lower temperatures were chosen because higher temperatures are unachievable in the field; maximum temperature during the day in the field was around 30°C. Thus even lower reducing sugar yields could be expected under field condition, subsequently resulting in lower alcohol yields.

An innovation to achieve higher temperatures for hydrolysis under field conditions was explored by developing a Solar Still (Figures 3 and 4).

Figure 3- Solar still design at project site

Figure 4- Inside layout of the solar still design at project site

Temperature data collected in the month of March as read inside the solar still ranged on average from 26°C at 6:00 am to 63°C at 12:00 noon as opposed to average range of from 24°C at 6:00 am to 31°C outside the solar still (Figure 5).

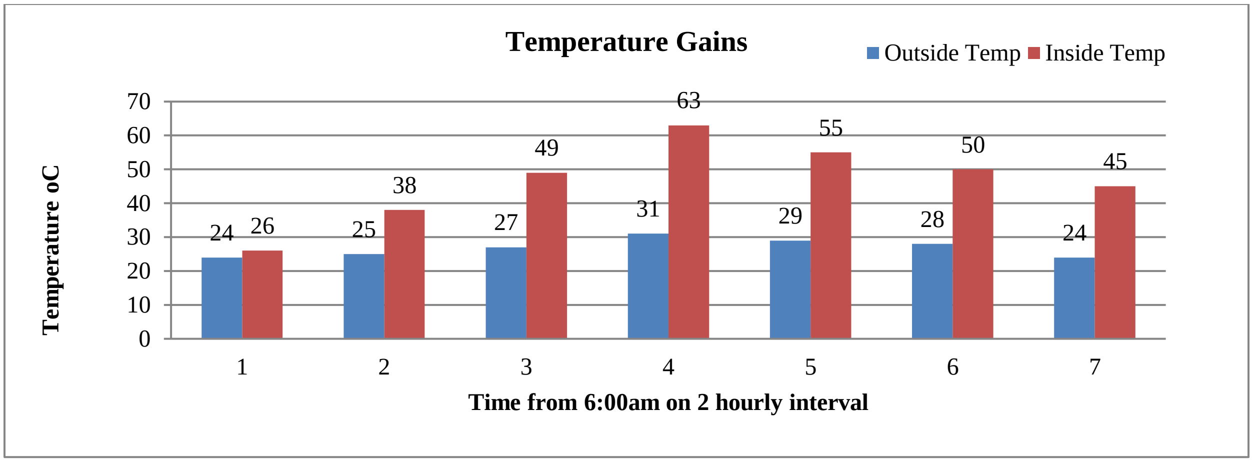

Figure 5-Temperature gains using solar still design at project site

The month of March falls within the autumn season in Malawi which is characterized by cool temperatures. The use of solar still demonstrated that higher temperatures could be achieved under field conditions and that for even higher temperatures field production could achieve better results if carried out during summer season (usually from October to December).

The use of fresh and fermented cassava peels for hydrolysis revealed that higher reducing sugars are obtainable with fresh peels. The amount of reducing sugars over the 8 days following hydrolysis at 70˚C was twice as much as that obtained for fermented peels. This indicates that extended peels storage will result in lower yields of reducing sugars. Therefore, use of fresh or unfermented cassava peels is highly recommended to achieve higher reducing sugar yields.

In the laboratory studies, a maximum yield of 12% alcohol was obtained following distillation that was conducted at temperatures ranging between 75 and 80˚C which translated into 1L of 12% alcohol for every 2kg of cassava peels. Under field studies, the concentration of the alcohol obtained was lower than what was obtained under laboratory studies: 6kg of cassava peel flour yielded 1.18 litres of 7.5% ethanol. The lower alcohol yield under field conditions would be attributed to the fact that it is very difficult to control distillation temperatures using local Kachasu distillation unit as compared to controlled distillation in the laboratory. However, the results are comparable with those reported by other researchers (Nuwamanya et al., 2012; Ogunsuyi et al., 2016; Chibuzor et al., 2016). Ogunsuyi et al (2016) reported alcohol yield of 20.7% following acid hydrolysis using H_2SO_4 whereas Nuwanyama et al (2012) reported an alcohol yield of 11%. Chibuzor et al (2016) obtained bioethanol yields ranging from 4.8 to 7.8% for peels of different cassava cultivars whereas Abidin et al (2014) reported even lower bioethanol yield of 3.59%. Hence our results fall within those reported results.

4. Conclusions

Results of the study have shown it is possible to hydrolyse cassava peels in Nkhotakota using dilute acid. However, temperature of hydrolysis remains a big concern. There is need to explore ways of achieving and maintaining higher temperatures under field conditions throughout the year and more specifically during the period of cassava harvest when cassava peels are generated. Further, unfermented cassava peels gave higher reducing sugar yields than fermented peels. Therefore, cassava peels to be used for bioethanol production should not be stored for extended periods as this may result in low reducing sugar yields. In addition, the results from field studies have also shown that the laboratory results are not reproducible at field conditions. Hence there is need to explore increasing concentration of acid.

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