1. Muniafu, M.M., Kahindi, J.H. and Kwena, M.O. (2015) Bio-ethanol production from cassava (Mannihot esculenta Crantz) at the...

Research Journal of Agriculture and Environmental Management. Vol. 4(7), pp. 299-306, July, 2015
Available online at http://www.apexjournal.org
ISSN 2315 - 8719© 2015 Apex Journal International

Full Length Research

Bio-ethanol production from cassava (*Mannihot esculenta Crantz*) at the coast region in Kenya

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Received 15 June, 2015; Accepted 29 June, 2015; Published 31 July, 2015

High yielding cassava varieties were developed over the years by the Kenya Agricultural Research Institute (KARI) research station in Mtwapa, Kilifi County several which have improved the yield from three tonnes per hectare to five tones per hectares. The Sustainable Development Initiatives Centre (SUDIC) linked with a farmers group in the Tezo area of Kilifi County by the name Basi Mwangaza. The aim was using cassava tubers (toxic cassava) and cassava peelings as waste from cassava to produce ethanol which was to be used as a biofuel which would then be a substitute for fossil fuels. Ultimately, this would be a less polluting fuel and thus mitigate against climate change and other negative environmental impacts associated with the transport industry exhaust. A shortage in the sources of fuel and increased environmental degradation has necessitated the search for biofuel for use in vehicles and machineries. Karemb*o and* Siri varieties produce higher percentage ethanol while Nzalauka *and* Shibe produced relatively lower percentages of ethanol in all method used. Ethanol extracted from the cassava tubers is roughly twice the percentage of that extracted from the cassava peelings. The use of cassava ethanol as a second-generation bio-fuel provides a starting-point for improvements in cultivation and adoption of cassava and improving food security.

Key words: Saccharification, natural fermentation, brix, cassava varieties and yeast.

INTRODUCTION

As the world population steadily increases over the years, the demand for alternative sources of fuel has become an urgent need. Additionally, such fuel must preferably fulfill the “green” condition in having a minimal adverse impact on the environment and the organisms therein. A shortage in the sources of fuel and increased environmental degradation has necessitated the search for biofuel for use in vehicles and machineries. This present study seeks to find out the ways in which ethanol could be extracted from cassava tubers and cassava peelings and also the percentage of ethanol that could be obtained for use as a biofuel. Cassava (study subject of the project) was discovered to have certain properties that made it an ideal source of ethanol for use as bio-fuel and it can be grown and harvested throughout the year. It has a high starch content of high quality (70 – 85% dry base) and dried chips which can be stored for a long time with correspondingly small losses of starch. A lot of fossil fuel reserves (especially oil) have been over-exploited, leading to a great shortage in fuel sources available to run many economies. Of greater concern is the increase in the severity of environmental degradation that accompanies the use of fossil fuel such as the emissions of pollutant and green house gases as well as their attendant impacts. It is for this reason that the project exploited ways of generating biofuels from cassava in the coastal region of Kenya. Bio-fuels (carbohydrate based or oil based; Chandel *et al. 2007*) are increasingly recognized as important forms of renewable energy (Londo *et al., 2010*). Bio-ethanol is produced by hydrolysis and fermentation of carbohydrate feedstock. This ethanol may be used as a fuel as is, or in a mixture with fossil fuels, using various proportions. Biodiesel is produced from oil plants like *Jatropha curcas* where the

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oil is blended with diesel to produce fuel (Davies, *2006*). Bio-fuels (especially second-generation bio-fuels) hold the promise of sustainable and environmentally friendly energy forms (Chandel *et al., 2007*) and consequently, production has increased by 8% from the levels in 2005 to 33 thousand million liters in 2009. It has been established that production of bio-ethanol and other domestic forms of energy is economically viable and feasible with available technologies (Londo et al., 2010; Tillman et al., 2009). Crops grown for energy production purposes may be sugar cane (Hall *et al., 2009*), cassava, corn, and sweet potato (Ziska *et al., 2009*) as well as other high sugar and high biomass producing crops and nontraditional food or cash crops. In addition, municipal and agricultural wastes may be used for energy production (Rist et al., 2009). However, the choice of feedstock is based on availability, competition between food and non-food products, and cost (Londo et al., 2010), thus making the search for an optimal feedstock of uttermost importance. The high productivity and yield of cassava (Ziska *et al., 2009)*, along with its ability to grow on marginal soils (Dixon *et al., 2002*), requiring a minimum of labor (Chiwona-Karltun *et al., 1998*) and management costs (Jannson *et al.,* 2009), have placed it among the candidates for bio-ethanol production.

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 (*FAO 2005–2008*). The volume presently produced may not meet the demand of ethanol production as well as reducing food insecurity in situations of food deficit. This calls for exploitation of alternative forms of feedstock. In terms of cassava, the above ground biomass, including stem and leaf residues, is often not utilized for economical purposes (Ahamefule*, 2005)*, apart from being a source of planting material (Pattiya *et al., 2007*) and the unintended use as fertilizer (Fermont *et al., 2008*). Root residues, especially peels, which are poisonous due to high levels of cyanogenic glycosides (Guo *et al.,* 2008*;* Pattiya *et al.,* 2007), may be exploited for energy production taking into account their role in nutrient recycling (Fermont et al. 2008). Sustainability may be achieved if energy production is linked to food production and if energy production is harmonized with the livelihoods of people. Livelihood diversification would require the understanding of society dynamics in terms of domestic energy consumption as well as investigating possible ways of producing energy from available resources (Amigun *et al., 2008*). Non-food parts of the cassava may play a very significant role in the production of energy since they produce relatively high amounts of biomass, are easily hydrolysable and have a high content of dry matter (Kosugi *et al., 2009*). Furthermore, starch extraction industries produce lignitic and cellulosic material that may be used for generating ethanol (Akpan *et al., 2004*). Using cellulosic and lignocellulosic material as feedstock in production of bio-ethanol is as efficient as starch-based feed stocks and is important since the greenhouse gas emission from this bio-ethanol is reduced by up to 80% compared to the 40–60% of the first generation bio-fuels (Londo *et al., 2009*). The aim of the present study was to explore the feasibility of using non-food parts of cassava for bio-fuel production. The inexpensive nature of cassava stems and peel bio-mass as well as their abundance, create an opportunity for cassava exploitation in the production of bio-ethanol.

MATERIALS AND METHODS

In order to achieve the aims and objectives of this study, the project was divided into a 3 sections which were; first to undertake a survey of cassava growing regions at the coast and recruit farmers and/ their groups to participate in the project. The second section was to carry out laboratory trials that would establish simple, affordable and replicable protocols for ethanol extraction. The laboratory trials would then be taken to the field for adoption by farmers. And the third section was to train the farmers on the processes developed in the labs.

Survey of the coast region

In November 2010, the SUDIC research team visited the Kenya Agricultural Research Institute Mtwapa, at the coast, to establish the best modalities that could be used in obtaining cassava waste peelings from the farmers in the region. The visit revealed that KARI had developed a number of high yielding cassava varieties whose yields were proving a challenge given taste preferences among the locals. Contact was established with two active farmer groups, one in Basi Village, Tezo, Kilifi County and another in Lunga Lunga, Kwale County. Given the organization level of the Basi village farmers, a decision was made to use the Basi village farmers as a pilot group. This group is registered as the Basi Mwangaza Group. It was established in 2000. It comprised of 30 members - 5 men and 25 women. Its notable assets include a cassava grating machine and a milling plant. Five varieties of cassava were then collected from Basi Village for laboratory trials and these included Shibe, Siri, Tajirika, Nzalauka, Karibuni and Karembo.

Field training for the community on technology adoption

The fabricated distillation apparatus for ethanol extraction was set up in the village. The SUDIC research team took one year to carry out training for the farmers. Emphasis was placed on the fermentation and distillation processes since these were keys to ethanol production. The group members were taken through one of the processes that

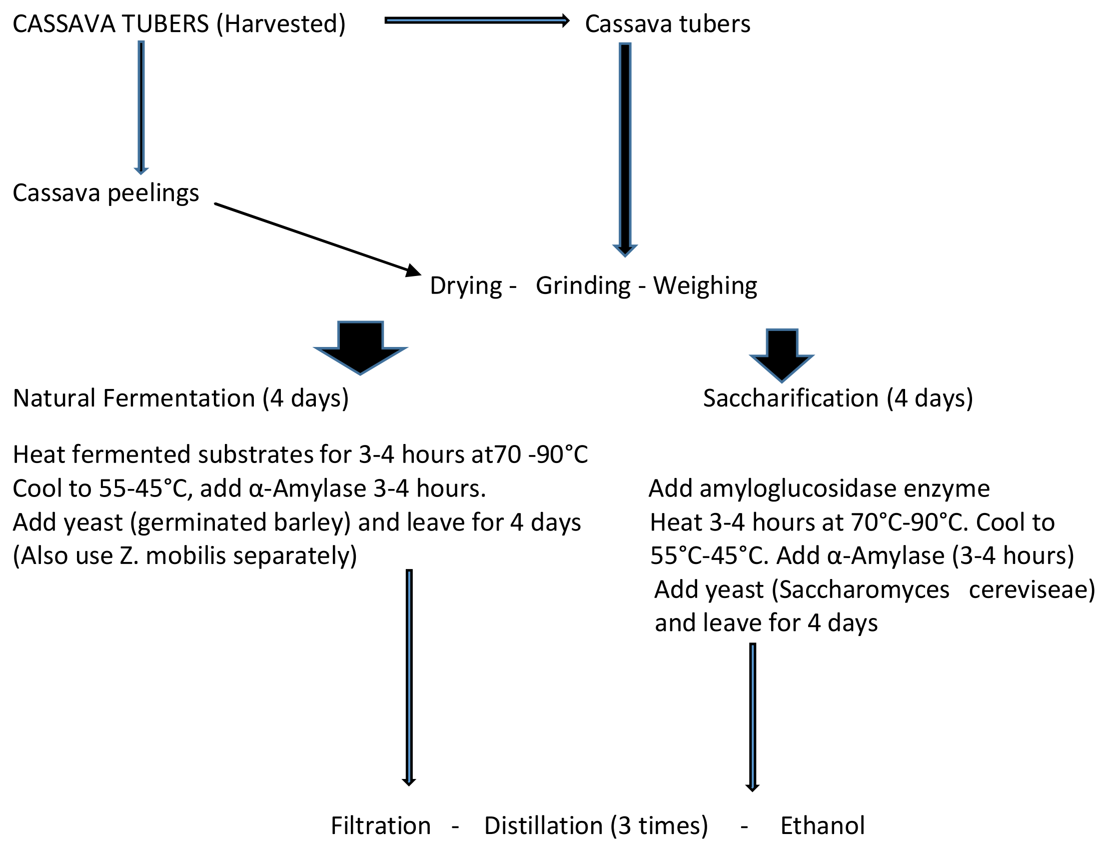

Figure 1. Diagram of the processes leading to fermentation processes.

were adapted in the USIU labs. It involved the use of amylase enzyme and yeast as a mixture of the natural fermentation and saccharification methods. Training centered on setting up the distillation process without any leakages. A few members were also taught the procedures of lighting of the biofuel stove. An alcoholmeter was provided to the group to determine percentage purity of ethanol produced.

Production of ethanol from cassava tubers and peelings using Natural fermentation and saccharification method

Natural fermentation: Harvesting freshly harvested cassava roots were peeled; the peelings and tubers were sun-dried separately, the dried peelings and tubers were ground separately. The flour and water were mixed in the ratio 1:3 for tuber and peelings, the mixture was left to ferment in a warm place for 3 days; the mixture was placed in hot water (70-90℃) for 3-4 hours and then cooled to 45-55℃. A teaspoonful of alpha-amylase enzyme was measured to every 3 liters of the flour/ water mixture and the enzyme was dissolved in it. Yeast was sprinkled to the liquefied mixture and the mixture was allowed to stand in a warm place and ferment for 4 days. The fermented products were filtered using a clean cotton cloth. The residue was collected for drying and packing in sacks. The filtrate was distilled and samples obtained at 76-80℃ were collected. The specific gravity reading (finishing gravity) was read using a hydrometer. Distillation was done again until an alcohol percentage of close to 100% was obtained.

Saccharification method: Harvesting freshly harvested cassava roots were peeled; the peelings and tubers were sun-dried separately, the dried peelings and tubers were ground separately. The flour and water were mixed in the ratio 1:3 for tuber and peelings, the mixture was placed in hot bath water at (70-90℃) for 3-4 hours and then cooled to 45-55℃. A teaspoonful of alpha-amylase enzyme was measured to every 3 liters of the flour/ water mixture and the enzyme was dissolved in it. Yeast was then added to the mixture and was allowed to stand in a warm place and ferment for 4 days; the fermented products were filtered using a clean cotton cloth. The residue was collected for drying and packing in sacks and filtrate was distilled and samples obtained at 76-80℃ were collected. The specific gravity reading (finishing gravity) was read using hydrometer and distillation was done again until an alcohol percentage of close to 100% was obtained (Figure 1).

Adoption of the ethanol extraction process to community level

A meeting was held with the community preceding the transfer of the extraction and distillation technology developed at the USIU laboratories. It sought to establish the following: After some investigations, the group leaders were assured by the local administration that there would be no obstacles provided the location of the distillation still was disclosed for regular inspection. The group affirmed that they would use the same member's land on which the grating and milling plant was located. They were unanimous in stating that the plant should be community-based for the purposes of maximizing benefits from it since they would be in control of all the processes that go with ethanol extraction. The coordinator of basi Mwangaza and ward Agricultural Officer managed to make an exchange trip to a group in Machakos County where they use fast growing tree species as wood fuel. Subsequently, as explained, this was superseded by a suitable biofuel being found. The group gave the assurance that there were more than 100 members of combined groups that each had no less than an acre of cassava each. Some of this acreage had non-preferred varieties in quantities that would run the distillation plant without compromising food security. As the community considered the aforementioned questions, the research team proceeded to design a distillation still that would suit local conditions such as lack of running water, absence of grid electricity, lack of a building in which to locate the distillation plant and the use of materials that would be available and repairable by the local artisans. It took about four designs before a suitable system was finally in place after some failing the test in field conditions. A notable feature of the adoption process was that the research team was able to obtain a bio-fuel extracted from castor oil that produced heat to a suitable degree to run the distillation plant. This removed the query on the source of energy to be used by the community.

Ethanol distillation plant

There was moderate success by the farmer's group in the setting up of the apparatus and carrying out the fermentation process. However there was 0 – 5% ethanol content in all distillates for the entire period of the trials. These due inefficient technology and innovation of equipment's at remote level, it was hard to use some equipment like water bath and centrifuge to be used by farmers, therefore these reasons made saccharification method not to be adopted. Sufficient, effective and low costing method was developed at USIU labs and was adopted by Basi mwangaza in Kilifi count. The farmers were trained on the method and able to produce 40% ethanol purity for first distillation, then improved on efficiency and managed to produce 60% then final 80% purity.

Determination of alcohol content in cassava varieties ethanol obtained using the titration method

To obtain accurate alcohol content a hydrometer was used before fermentation and after fermentation. The hydrometer measures the density of the liquid by floating at a given level corresponding to the specific gravity of the fluid being measured. It should be read by noting the level at which the surface of the fluid contacts the glass when the hydrometer is floating in the liquid. The first reading should be taken before the addition of yeast, and at 60°F. The second reading should be taken after fermentation is complete, that is, before bottling, and before adding priming sugar. The sample for determining alcohol by weight (a.b.w.) and alcohol by volume (a.b.v.) are shown:

The first reading (Original gravity [O.G.]) = 1.045

The second reading (Final or finishing gravity) = 1.010

Subtract final from original: 1.045-1.010= 0.035

Multiply this figure by 105: 0.035*105= 3.68% a.b.w.

Therefore, you have a brew of 3.68% alcohol by weight. To get alcohol by volume simply multiply this figure for % a.b.w. by 1.25: 3.69* 1.25= 4.6% a.b.v.

Back titration method

The burette was filled with standard solution of acidified potassium dichromate. $25cm^3$ of unknown concentration then ethanol was pipetted into conical flask. The acidified potassium dichromate was titrated with ethanol until the color changed from orange to brown. The moles of ethanol were calculated using mole ratio. The concentration or molarity of ethanol was then calculated.

To get the % purity of ethanol:

%= (Concentration/ molarity* RMM) 100 or Mass* 100

Mass/Density *100= (Volume*100)

Mass= Molarity *RMM

RESULTS

Table 1 and Figure 1 show that the mean percentage purity of ethanol for cassava tuber in all varieties was very signficant difference ($p<0.001$) combared to cassava peelings using saccharification method.The different in production is as result in significant different in percentage starch content between cassava tuber and peeling.

Table 1. Percentage purity of ethanol in cassava varieties obtained from different methods and yeast developed in laboratory.

Cassava varieties	Saccharification method using Saccharomyces cerevisiae as yeast		Natural fermentation (germinated) millet yeast)	Natural fermentation. (*Z. mobilis* from coconut palm)
	Cassava tuber	Cassava peeling	Cassava tuber	Cassava tuber
Karibuni	57.70%	30.00%	57.00%	54.40%
Nzalauka	46.30%	18.00%	44.60%	58.40%
Karembo	63.90%	29.70%	61.20%	49.10%
Siri	60.90%	25.90%	59.10%	61.80%
Tajirika	54.9 %	16.90%	51.50%	60.10%
Shibe	58.00%	18.00%	55.00%	52.50%

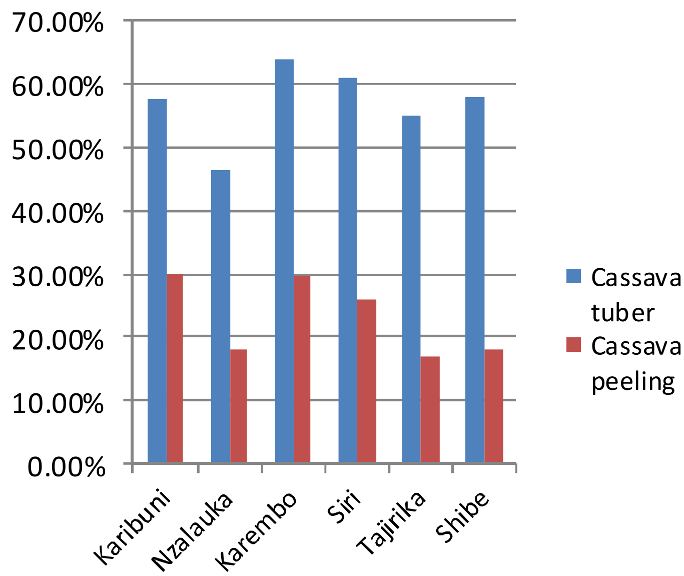

Figure 1. Means percentage purity of ethanol for both cassava tuber and cassava peeling varieties using saccharification methods.

Figure 2 and Table 2 indicate that the mean percentage purity of ethanol for cassava tuber in Karembo and Siri varieties shows no signficant difference between them,using three different methods and yeast fermentation respectively**.** The different in production in Nzalauka was significant as combared to other cassava varietie.

DISCUSSIONS

The extraction of ethanol from the different varieties of cassava produced the following results: *Karembo and Siri* varieties produce higher ethanol quantities in all the methods used; *Nzalauka and Shibe* produced relatively lower percentages of ethanol. Ethanol extracted from the cassava tubers is roughly twice the percentage of that extracted from the cassava peelings. There is not much apparent difference in the ethanol percentages produced between the three methods. Kenya has about 207,200 farm households involved in growing cassava covering approximately 90,000 ha with an annual output of 540,000 tons. Approximately 60% of this production comes from Western Kenya mainly Busia Tezo, Siaya and Bungoma districts and the Coastal districts of Kwale, Kilifi and Malindi. The extraction of ethanol from cassava has a great potential at the coastal region where it is the second main staple food and is becoming a major cash crop (Munga, 2000). A previous dilemma for the first phase of the project lay in the twin competing factors of meeting energy needs and food security with the latter naturally having a greater priority especially in Africa. An alternative therefore would have been to use cassava peelings with a challenge of using a much lower starch content substrate. Tests done in the laboratories at USIU (United States International University) showed that the

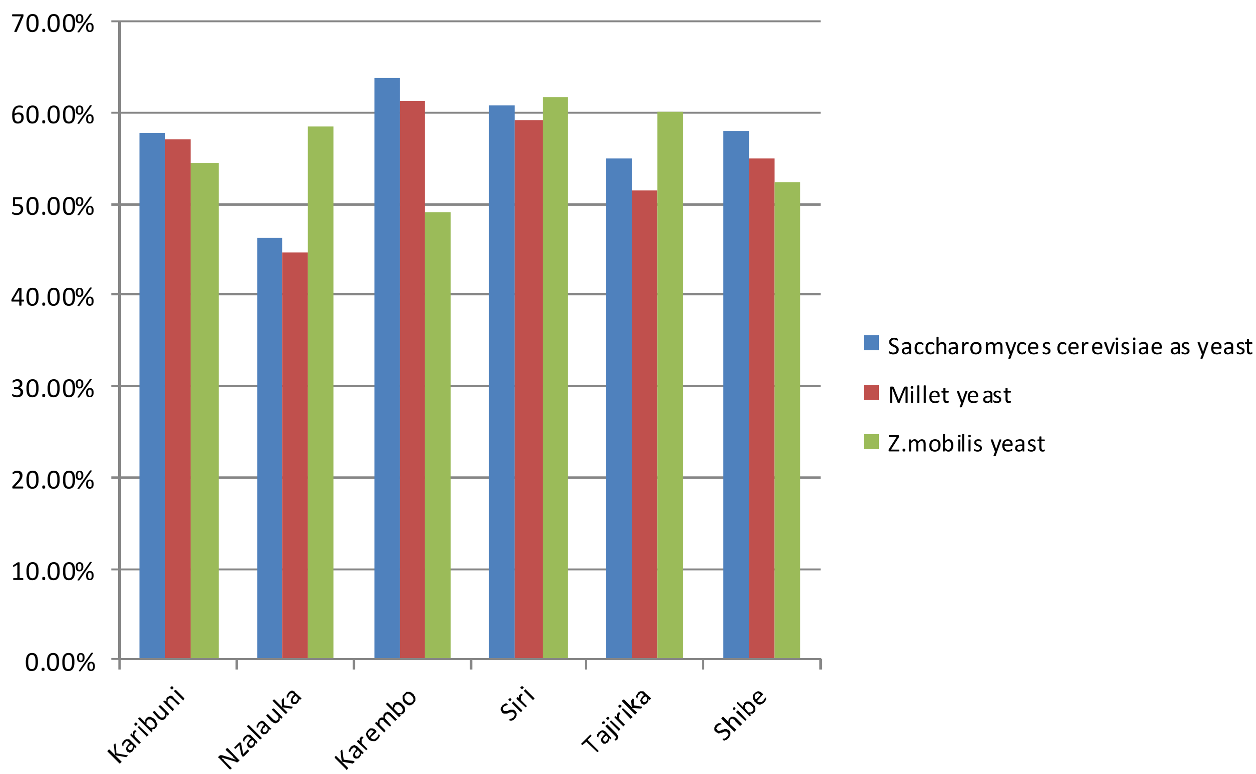

Figure 2. Mean percentage purity of ethanol for both cassava tuber varieties using three different methods and yeast fermentation.

Table 2. Percentage of ethanol in brix obtained in varieties of cassava using a calibrated refractometer.

Kilifi (basi mwangaza) Cassava varieties	Saccharification method using Saccharomyces cerevisiae as yeast		Natural fermentation (germinated)millet yeast)	Natural fermentation. (*Z. mobilis* from coconut palm)
	Cassava tuber	Cassava peeling	cassava tuber	cassava tuber
Karibuni	15%	5 %	11%	14%
Nzalauka	10%	4%	12%	9%
Karembo	17%	6%	17%	14%
Siri	15%	6%	16%	14%
Tajirika	13%	3%	15%	13%
Shibe	13%	4%	13%	8%

ethanol generated was up to 50% lower in percentage than that distilled from starchy tubers. During the field surveys carried out in Basi Village, Tezo, Kilifi County, Kenya, it was found that the Kenya Agricultural Research Institute (KARI) had introduced several high yielding cassava varieties in the region. Two notable ones were Karembo and Tajirika in which on-site harvest tests established yields of upto 15 to 50 tonnes per acre respectively for the two varieties in comparison to 7 tonnes per acre for the local Kabanda Meno variety. Of the two new varieties, Karembo has a bitter taste that threatened the success of KARI's introductions as it was slow moving on the market and local consumption fronts. The ethanol project thus offered a much needed alternative use for such varieties some of which had remained unharvest for almost a year. The high content of dry matter observed in the different parts of cassava plants may be hydrolyzed into fermentable sugars. This has a bearing on the final yield of reducing sugars, since high contents of dry matter are desirable in ethanol production (Ziska et al., 2009). Fermentation efficiency depended on the ability of the yeast to utilize particular feedstock based on their characteristics and compositional differences. The absence of differences in fermentation efficiency during some hours was due to the yeast establishing itself in the fermenting solution, growing to a certain colony volume able to utilize the existing sugars. The variations in fermentation efficiency may be attributed to the type of sugars produced as well as substrate preferences by the fermenting organism, since different parts of the cassava plant produce different sugar types apart from glucose (Brauman et al., 1996). Different compositional characteristics of feed stocks affect their hydrolysis, consequently affecting the type of reducing sugars produced and hence moderating the type of metabolism carried out by yeast under such circumstances (Van Dijken et al., 1993).

CONCLUSION AND RECOMMENDATIONS

The present study has demonstrated a potential for exploitation of cellulosic and starch ethanol from cassava non-food parts. Taking into account that the simultaneous need for energy and food need to be met without compromising the environment, they provide a viable option, holding great potential for acquiring a sustainable system. The use of cassava ethanol as a second-generation bio-fuel provides a starting-point for improvements in cultivation and adoption of cassava as well as improving food security. In addition, this may mitigate the human effects on climate change by producing efficient, clean, and renewable energy.

To achieve a sustainable food and energy system, it is important that investments be made in developing, improving, and making technologies available for feedstock preparation, hydrolysis, and fermentation. Comprehensive guiding policies on exploitation of the bio-fuel sector are urgently needed for this purpose. Policies would ensure the use of both edible and nonedible components of the plant rather than a competition between food and energy, thus facilitating dual plant utilization. In particular, seed availability in communities where the stems provide the bulk of cassava seeds, care should be taken not to generate a shortage of adequate planting material. This is particularly crucial in cassava-farming communities with poor households, mainly comprising women and children. The project so far met the first two objectives as set out earlier with a modification of using the cassava tubers rather than the peelings. The project was to move into the next phase with an up-scaling of the ethanol distillation process. The production process was enhanced and tested against fixed parameters to determine its efficiency and profitability with a view of making improvements. A micro-organization of the Basi Mwangaza group was done on the basis of working groups at various levels starting from growers to harvesters, preparers of cassava tubers and peelings, fermentors, distillers and marketers. Training was done at each level to maximize on the cassava ethanol extraction process. While this was going on, laboratory research was and will continue to be done at the USIU laboratories with frequent trips to the field.

ACKNOWLEDGMENTS

I would wish to thank Kenya Agricultural Research Institute (KARI) research station in Mtwapa developed several high yielding varieties of cassava and Basi Mwangaza community group by assisting and supporting researchers led by Dr Maina Muniafu director Sustainable development initiative Centre (SUDIC) under school of science and technology of United States International University Africa (USIU). We also thank the National Commission for Science, Technology and Innovation, Kenya for funding the project.

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