BIOETHANOL FROM CASSAVA FOR A COMMUNITY PROJECT

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ABSTRACT: Although the North-West Province of South Africa is renowned for its platinum industry, wildlife tourism and Casino resorts, its people are among the poorest in South Africa. The Thusanang community project concept was initiated to stimulate job creation and skills development in our local communities through biofuels production. Cassava roots contain up to 80% starch and it is not considered to be staple food due to the presence of hydrogen cyanide in the raw roots. A study was undertaken to assess the conditions for optimal production of ethanol from Cassava roots in a community project. Different hydrolysis and fermentation parameters were varied to assess the effect of the change on the final glucose or ethanol yield. It was found biomass loading, biomass form, pH and enzyme loading all had a significant effect on glucose concentration and yield during hydrolysis. The SSF process route produced the highest ethanol yield (530 $L.ton^{-1}$) within 48 hours. The results from this study was implemented to design and built a community ethanol demonstration plant.
Keywords: Cassava, biomass form, process route, hydrolysis, fermentation.

1 INTRODUCTION

The North West Province is world renowned for the Sun City Palace and Casino complex, many wildlife game farms in the area as well as one of the world's largest precious metal producers. Despite all this economic activity, the rural areas of the North West Province rank among the poorest in South Africa. Unemployment and illiteracy is high in the province and young people flock to the big cities in search of work opportunities. The South African government has opted to use biofuels production as a vehicle for job creation and community upliftment in especially rural areas (Department of Minerals and Energy Affairs, 2007).

The Thusanang community project concept was initiated to stimulate job creation in the energy sector and to empower impoverished communities to first economic status.

Maize is produced mainly in three provinces in South Africa and although the North West province is one of the largest maize producing provinces, the Industrial Biofuels Strategy of South Africa prohibits the use of maize for energy production [1].

Sugarcane and sugarbeet has been suggested as suitable crops for bioethanol production in South Africa, but sugarcane cannot be cultivated on the arid, marginal land in the North West while sugarbeet is prone to disease and needs irrigation to grow well in arid regions. An alternative crop thus needed to be found to produce bioethanol for economic empowerment in provinces with large marginal land areas.

Cassava (Manihot esculenta) is a tuberous root plant that is native to South America and is cultivated around the world as a primary source of starch as well as a low-grade animal feed [2]. Cassava is considered to be the sixth most important staple food in the world [3]. Cassava is not considered to be a staple food in South Africa and is thus also not commercially cultivated for food purposes. Cassava can be grown in arid, marginal soil where other crops such as sugarcane and sugarbeet fail [4, 5]. Dai *et al.* [6] and De Vries et al. [7] showed that production of bioethanol from cassava is energy and renewable energy efficient. Various studies [8, 9, 10, 11] have shown that production of ethanol from cassava is both economical and sustainable. Cassava is thus a good crop to be considered for ethanol production in arid regions in South Africa without compromising food security.

Amutha and Gunasekaran [12] investigated the use of co-immobilized yeast cells to ferment cassava starch to ethanol. It was shown [12] that co-immobilized yeast cells of Zymomonas mobilis and Saccharomyces diastaticus could retain their activity during a continuous fermentation cycle of cassava and a final ethanol yield of approximately 0.3 $g.g^{-1}$ could be obtained. Kosugi *et al.* [13] showed that ethanol yields as high as 0.46 $g.g^{-1}$ could be obtained by fermenting cassava pulp (starch and peels) to ethanol with a surface-engineered strain of Saccharomyces cerevisiae. Nitayavardhana *et al.* [14] used ultrasound to try and increase the ethanol yield and overall ethanol conversion efficiency when converting cassava starch to ethanol using Saccharomyces cerevisiae, but an ethanol yield of only 0.43 $g.g^{-1}$ could be obtained although an overall ethanol conversion efficiency of 95.7 % with sonification was reported.

In this study the improvement of the ethanol yield from cassava using Saccharomyces cerevisiae was investigated for application to a community bioethanol plant.

2 EXPERIMENTAL

2.1 Cassava

Raw cassava roots were obtained from the Agricultural Research Council (ARC) of South Africa. A complete compositional analysis of cassava used in this study was done according to AACC methods by the South African Grain Laboratory (SAGL) and is presented in Table I.

Table I: Compositional analysis (wt% dry basis) of cassava roots used in this study

Component	Unpeeled cassava (starch and peels)	Cassava starch	Cassava peels
Moisture	9.5	10.4	9.2
Protein	2.5	2.6	5.1
Starch	81.4	82.0	67.0
Fat	0.6	0.5	1.1
Ash	2.5	2.5	7.0
Crude Fiber	3.5	2.0	10.6

The moisture content of the raw cassava roots were determined to be between 55 and 62 wt% as measured by a Mettler-Toledo HR 83 moisture analyzer according to standard methods. The raw cassava roots were dried in the sun for 3 days and then milled into fine flour and sieved with a +1.5 mm screen. The cassava was prepared such that the cassava starch and peels could be fermented separately.

2.2 Chemicals, enzymes and microorganisms

The enzyme mixtures Termamyl® SC (α-amylase enzyme mixture), Spiritzyme Fuel® (gluco-amylase enzyme mixture) and Celluclast® 1.5L (cellulase enzyme mixture) were obtained from Novozymes SA and used without further modification. Saccharomyces cerevisiae was obtained from Anchor Yeast South Africa and was revived from the dormant state using the fermentation broth as a growth medium for ten minutes before use in batch fermentation experiments..

2.3 Hydrolysis

Enzymatic hydrolysis of cassava samples were done according to the methods described by Ayernor *et al.* [15] and Mojovic [16] with modifications. Milled cassava flour (100 g) was weighed in Scott Duran sterile bottles and the bottles were filled to 500 ml with distilled sterile water resulting in a 20 wt% biomass loading. Termamyl® SC was added according to the experiments to be conducted and pH was monitored throughout hydrolysis with a calibrated hand-held pH meter. Saccharification was done in the same container by adding the correct dosage of Spiritzyme Fuel ® and Celluclast® 1.5L. Saccharification was done for 2 hours. The hydrolysis steps for converting cassava starch into glucose were optimized by varying different process parameters and assessing their influence on the final glucose concentration after hydrolysis. All experiments were performed with unpeeled cassava roots (starch and peels)

2.3.1 Influence of temperature

The influence of temperature on the final glucose concentration after hydrolysis was investigated by varying the temperature between 85°C and 95°C for the liquefaction step and between 55°C and 65°C for the saccharification step. Liquefaction was done at a pH of 6 for 60 minutes with a Termamyl® SC loading of 7.5 $\mu L.g^{-1}$. Saccharification of the liquefied starch was done at a pH of 4.5 for 48 hours with a Spiritzyme Fuel® loading of 7.5 $\mu L.g^{-1}$.

2.3.2 Influence of pH

The influence of pH on the final glucose concentration after hydrolysis was investigated by varying the pH between 5.5 and 6.5 during the liquefaction step and between 4 and 5.5 during the saccharification step. Liquefaction was done at 95°C for 60 minutes at a Termamyl® SC loading of 7.5 $\mu L.g^{-1}$. Saccharification was done at a temperature of 55°C for 48 hours at a Spiritzyme Fuel ® loading of 7.5 $\mu L.g^{-1}$.

2.3.3 Influence of biomass loading

The effect of biomass loading on the final glucose concentration after hydrolysis was investigated by liquefaction (95°, pH 6, Termamyl® SC loading of 7.5 $\mu L.g^{-1}$) and saccharification (55°C, pH 4.5, Spiritzyme loading of 7.5 $\mu L.g^{-1}$) of cassava at 10 wt% and 20 wt% biomass loadings respectively.

2.3.4 Influence of cellulase enzymes

The extent to which cellulase enzymes were able to convert available cassava cellulose from the peels into glucose was assessed by adding Celluclast® 1.5L to the saccharification step during hydrolysis and comparing the obtained glucose concentration with a sample to which only Spiritzyme Fuel ® was added during saccharification. Liquefaction was done for both samples at 95°C at a pH of 6 and with a Termamyl® SC loading of 7.5 $\mu L.g^{-1}$. Saccharification was done for both samples at 55°C, with a pH of 4.5 and a Spiritzyme Fuel® loading of 7.5 $\mu L.g^{-1}$. The Celluclast® 1.5L loading was 4 $\mu L.g^{-1}$.

2.4 Fermentation

All fermentation experiments were conducted for 48 hours using a liquefied hydrolyzate that was treated at 95°C at a pH of 6 with a Termamyl® SC loading of 7.5 $\mu L.g^{-1}$. A 20 wt% biomass loading was used in all instances. Fermentation was carried out in Scott Duran bottles that were lightly capped to allow the carbon dioxide that formed during the fermentation to escape while allowing the minimum of air to come into contact with the fermentation broth.

2.4.1 Separate hydrolysis and fermentation (SHF)

The SHF process involved a two step fermentation process. Unpeeled cassava roots were first hydrolyzed (liquefaction and saccharification) and then fermented with *Saccharomyces cerevisiae*. Both Spiritzyme Fuel ® (7.5 $\mu L.g^{-1}$) and Celluclast® 1.5L (4 $\mu L.g^{-1}$) were added during the saccharification step.

2.4.2 Simultaneous saccharification and fermentation (SSF)

In the SSF process, the saccharification step and fermentation step was carried out simultaneously for 48 hours. This shortened the overall conversion of cassava to ethanol with 48 hours. During the saccharification and fermentation step, Spiritzyme Fuel® (7.5 $\mu L.g^{-1}$), Celluclast® 1.5L (4 $\mu L.g^{-1}$) and *Saccharomyces cerevisiae* (8 $g.L^{-1}$) was added simultaneously to the prepared hydrolyzate.

2.5 Analysis

The presence of residual starch in hydrolyzed samples was detected with an iodine solution according to the method described by Morrison and Laignelet [17]. All hydrolyses proceeded until the iodine test showed complete conversion of all amylase in the feedstock sample.
Glucose and ethanol analyses were done with calibration

curves using high performance liquid chromatography (HPLC) with a Shodex column fitted to a refractive index detector. A water and acetonitrile mixture was used as mobile phase and samples were prepared for analysis according to standard procedures.

3 RESULTS AND DISCUSSION

3.1 Hydrolysis

3.1.1 Influence of temperature on hydrolysis

Cassava slurries were subjected to liquefaction (pH 6, biomass loading of 20 wt%, Termamyl ® SC loading of 7 $\mu L.g^{-1}$) and saccharification (pH 4.5, Spiritzyme Fuel loading of 7 $\mu L.g^{-1}$, Celluclast® 1.5L loading of 4 $\mu L.g^{-1}$) at different temperatures and the glucose concentration was measures over time. The influence of varying liquefaction and saccharification temperatures on the glucose yield (gram glucose per gram milled cassava) after 60 minutes of liquefaction and 2 hours of saccharification is presented in Figures 1 and 2 respectively.

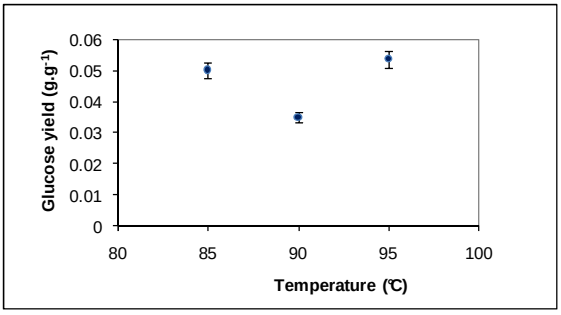

Figure 1: Influence of temperature in glucose yield during liquefaction of milled cassava

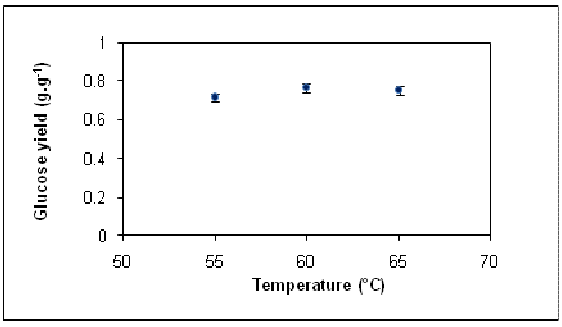

Figure 2: Influence of temperature on glucose yield during saccharification of milled cassava

From Figure 1 and 2 it can be seen that temperature had a significant influence on the glucose yield during liquefaction, but that the influence during saccharification was smaller. The highest glucose yield was obtained at a temperature of 95°C for liquefaction and a temperature of 55°C for saccharification. Starch swells initially when heated in water (called gelatinization) and thus the enzymes need to diffuse through the swelled starch granules to get to active sites to liquefy the starch. Water starts to boil at approximately 90 to 92 °C at Potchefstroom. At 85°C, it is thus fair to assume that the starch as not swelled completely and the enzymes would thus have a shorter route to travel to active sites than at 90°C when the starch granules is fully cooked and swelled. At a temperature of 95°C, the starch is also completely swelled and cooked, but now the enzymes have sufficient energy to diffuse faster than at 90°C. This would explain the low glucose yield at 90°C observed during liquefaction. Temperature did not have a significant influence on the glucose yield during saccharification, but the highest glucose yield (0.75±0.02 $g.g^{-1}$) was recorded at a pH of 5.5.

3.1.2 Influence of pH on hydrolysis

Cassava slurries were subjected to liquefaction (temperature of 95°C, biomass loading of 20 wt%, Termamyl® SC loading of 7 $\mu L.g^{-1}$) and saccharification (temperature of 55°C, Spiritzyme Fuel ® loading of 7 $\mu L.g^{-1}$, Celluclast® 1.5L loading of 4 $\mu L.g^{-1}$) at different pH levels and the glucose concentration was monitored over time. The pH was adjusted to the desired level by using either sulfuric acid (H_2SO_4) or calcium hydroxide ($Ca(OH)_2$). The pH of the control sample was not adjusted and no enzymes were added to the control sample. The influence of pH on the final glucose concentration during liquefaction and saccharification is shown in Figures 3 and 4 respectively.

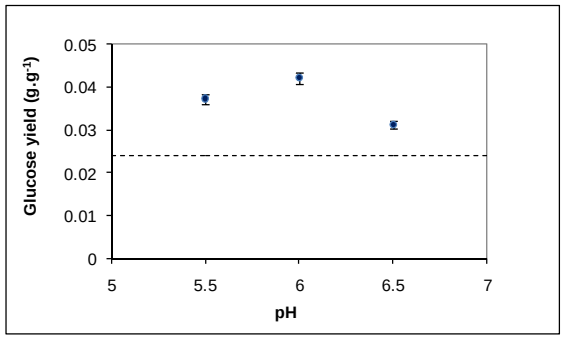

Figure 3: Influence of pH on glucose yield during liquefaction
(• - experimental results, ----- control sample)

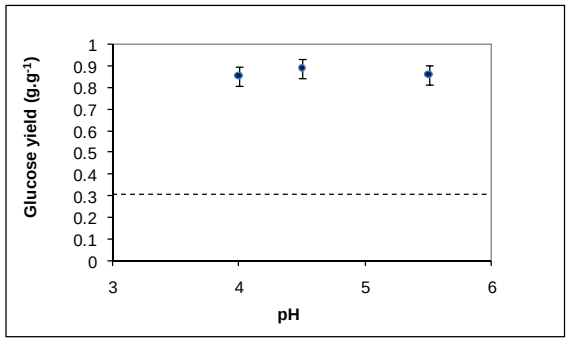

Figure 4: Influence of pH on glucose yield during saccharification
(• - experimental results, ----- control sample)

From Figure 3 and 4 it can be seen that all samples showed glucose yields higher than that of the control samples, validating the activity of the enzymes added during liquefaction and saccharification. During liquefaction, pH had a significant effect on the glucose yield with the highest yield of 0.04±0.001 $g.g^{-1}$ obtained at a pH of 6. The lower glucose yields at a pH of 6.5 and

5.5 is attributed to the lower enzyme activity at these pH values as stated by the supplier's specification sheet for the Termamyl® enzyme mixture. Glucose yield did increase with an increase in pH during saccharification with the highest significant glucose yield of 0.94±0.03 $g.g^{-1}$ obtained at a pH of 5.5.

3.1.3 Influence of biomass loading on hydrolysis

Two different biomass loadings were used during liquefaction (temperature of 95°C, pH of 6, Termamyl® SC loading of 7 $\mu L.g^{-1}$) and saccharification (temperature of 55°C, pH of 4.5, Spiritzyme Fuel® loading of 7 $\mu L.g^{-1}$, Celluclast® 1.5L loading of 4 $\mu L.g^{-1}$) i.e. 10 wt% and 20 wt%. The influence of the biomass loading in the final glucose concentration and glucose yield after 60 minutes of liquefaction followed by 2 hours of saccharification is presented in Figures 5 and 6 respectively.

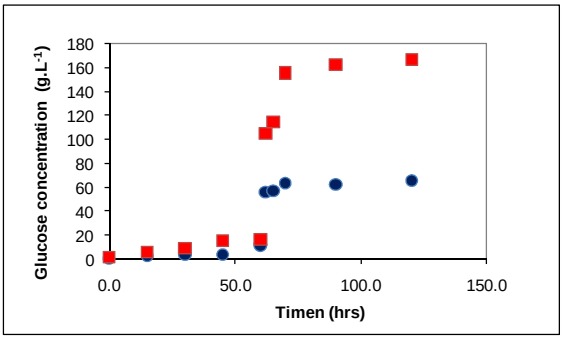

Figure 5: Influence of biomass loading on final glucose concentration after hydrolysis of unpeeled milled cassava (• - 10 wt% biomass loading ■ - 20 wt% biomass loading)

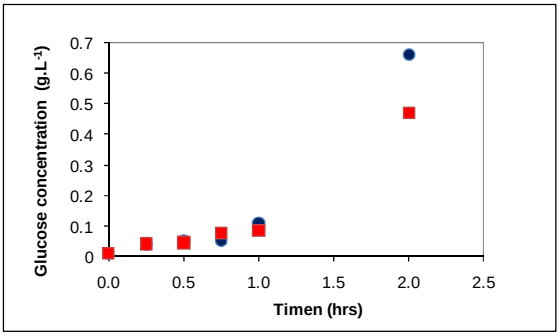

Figure 6: Influence of biomass loading on final glucose yield after hydrolysis of unpeeled milled cassava (• - 10 wt% biomass loading ■ - 20 wt% biomass loading)

Biomass loading had a significant effect on the final glucose concentration. The glucose concentration more than doubled from 65±1.9 $g.L^{-1}$ to 167±5 $g.L^{-1}$ with a doubling in the biomass loading. The enzymes that are added during liquefaction and saccharification are added per mass of biomass used and thus it was expected that more biomass should yield more glucose. From Figure 6 it is clear however that the 10 wt% biomass loading produced more glucose per gram of biomass used than was expected. At a lower biomass loading, the viscosity of the mixture is significantly lower than at a biomass loading of 20wt% and it was shown by Herrera-Gomez et al. [18] that starch cooked in limited amounts of water results in a significant amount of agglomeration betweens starch molecules. The high state of agglomeration at 20wt% biomass loading will thus result in longer diffusion times for the enzymes to get to active sites and thus lower overall conversion to glucose in the same amount of time as for the 10wt% biomass loading.

3.1.4 Influence of addition of cellulase enzymes during hydrolysis

Unpeeled, milled cassava roots contain approximately 3.5 wt% crude fiber (see Table 1). It is believed that milling of the dried cassava roots have liberated enough of the cellulose in the crude fiber component that it should be accessible to cellulase enzymes for conversion to glucose. The influence of adding cellulase enzymes (Celluclast® 1.5L) to the hydrolysis mixtures was investigated by performing a complete hydrolysis with and without Celluclast® 1.5L using a 10 wt% biomass loading and noting the final glucose yield. The glucose yield obtained with and without the presence of cellulase enzymes is presented in Figure 7.

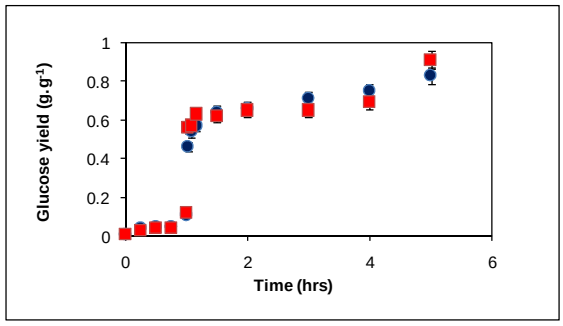

Figure 7: Glucose yield obtained from hydrolysis of unpeeled cassava roots with (■) and without (•) the addition of cellulase enzymes

Addition of Celluclast® 1.5L did significantly improve the glucose yield from 0.83±0.04 $g.g^{-1}$ to 0.91±0.05 $g.g^{-1}$. The slight increase is attributed to the conversion of the available cellulose in the crude fiber. The additional 8 wt% glucose yield gained by the addition of Celluclast® 1.5L will results in an additional 40 L of ethanol per ton of unpeeled cassava roots, which is significant in monetary terms.

3.2 Fermentation

Liquefaction during the SHF process was done at pH 6 and 95°C using Termamyl® SC (7.5 $\mu L.g^{-1}$) α-amylase enzymes. Saccharification was done at pH 4.5 and 55°C using Spiritzyme Fuel ® (7.5 $\mu L.g^{-1}$) and Celluclast® 1.5 L (4 $\mu L.g^{-1}$). Yeast (Saccharomyces cerevisiae) was added to the hydrolyzate at a loading of 8 $g.L^{-1}$. During the SSF process, liquefaction was at the same conditions as for the SHF process. After liquefaction, both yeast (8 $g.L^{-1}$) as well as Spiritzyme Fuel® (7.5 $\mu L.g^{-1}$) and Celluclast® 1.5 L (4 $\mu L.g^{-1}$) was added simultaneously at pH 4.5 and 30°C. The fermentation was allowed to continue for 72 hours. The ethanol yield (gram ethanol per gram unpeeled cassava) for both fermentation processes is presented in Figure 8.

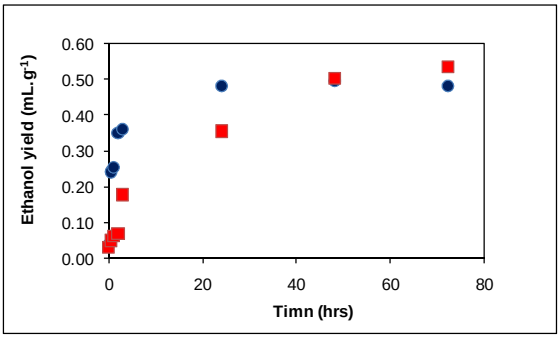

Figure 8: Ethanol yield obtained for SHF (•) and SSF (■) processes

From Figure 8 it can be seen that the SSF process ultimately produced a significantly higher ethanol yield (0.53±0.03 $mL.g^{-1}$) than the SHF process (0.48±0.02 $mL.g^{-1}$). After 48 hours both processes produced approximately the same amount of ethanol (0.5±0.02 $mL.g^{-1}$). There is an increase in ethanol yield after 48 hours for the SSF process, while the ethanol yield for the SHF process decreases slightly. The SHF process requires the additional 2 hours of saccharification at 55°C prior to fermentation and if that is taken into account in interpreting the above results, it is clear that the SSF process produces the same amount of ethanol than the SHF process in a shorter time. The finale ethanol yield for the SSF process translates to 530 L of ethanol per ton of unpeeled cassava roots.

4 CONCLUSIONS

In this study on the production of bio-ethanol from Cassava for a community project, it was found that temperature had a significant effect on glucose yield during liquefaction, but not saccharification. The best operating temperature was found to be 95°C and 55°C for the liquefaction and saccharification step respectively. The pH during hydrolysis was found to have a significant effect on glucose yield during both liquefaction and saccharification. The optimum operating pH for liquefaction and saccharification was found to be 6 and 5.5 respectively. Biomass loading also had a significant effect on the glucose yield and glucose concentration during hydrolysis. It was found that a 10 wt% biomass loading performed significantly better than a 20 wt% biomass loading due to the agglomeration of starch molecules at the higher biomass loading. Celluclast® 1.5 L was found to increase glucose yield significantly if added during saccharification. The glucose yield increase with 8% when Celluclast® 1.5L was added to co-convert the cellulose in the unpeeled roots to glucose. Finally, the SHF and SSF process for producing ethanol from unpeeled cassava roots were compared. It was found that the SSF process can produce the same amount of ethanol in a shorter time than the SHF process at a lower temperature. A final ethanol yield of 530 L of ethanol per ton of unpeeled cassava roots was obtained. This yield is high enough to produce ethanol economically from unpeeled cassava roots in a community scale bio-ethanol plant. A demonstration plant was designed and built based on the results of this study.

5 REFERENCES

[1] South Africa, Department of Minerals and Energy Affairs, Biofuels industrial strategy of the Republic of South Africa (2007), pag. 4

[2] S. Putthacharoen, R.H. Howeler, S Jantawat, V. Vichukit, Nutrient uptake and soil erosion losses in cassava and six other crops in a Psamment in eastern Thailand, Field Crops Research. 57 (1998), pag.113 – 126.

[3] K. Sriroth, K. Pijachomkwan, S. Wanlapatit, C.G. Oates, Cassava starch technology: The Thai experience, Starch. 52 (2000), pag. 439 – 449

[4] K. Sriroth, K. Piyachomkwan, S. Wanlapatit, S. Nivitchanyong, The promise of a technology revolution in cassava bioethanol: From Thai practice to the world practice, Fuel, (2010) doi:10.1016/j.fuel.2009.17.008

[5] C. Zhang, W.J.. Han, G.Q. Pu, C.T. Wang, Life cycle economic analysis of fuel ethanol derived from cassava in south west China, Renewable and Sustainable Energy, 7 (2003), pag. 353 – 366.

[6] D. Dai, Z. Hu, G. Pu, H. Li, C. Wang, 2006. Energy efficiency and potentials of cassava fuel ethanol in Guangxi region on China, Energy Conversion and Management. 47 (2006):1686 – 1699.

[7] S.C. De Vries, G.W.J. Van de Ven, M.K. Ittersum, K.E. Giller, Resource use efficiency and environmental performance of nine major biofuels crops processed by first generation conversion techniques, Biomass and Bioenergy. (2010) doi:10.1016/j.biomass.2010.01.001.

[8] R. Leng, C. Wang, C. Zhang, D. Dai, G. Pu, Lifecycle inventory and energy analysis of cassava-based fuel ethanol in China, Journal of Cleaner Production, 16 (2008), pag. 374 – 384.

[9] T.L.T. Nguyen, S.H. Gheewala, S. Garivait, Energy balance and GHG-abatement cost of cassava utilization for fuel ethanol in Thailand, Energy Policy. 35 (2007), pag.4585 – 4596.

[10] S. Yu, S. J. Tao, Energy efficiency assessment by life cycle simulation of cassava-based fuel ethanol for automotive use in Chinese Guangxi context, Energy. 34 (2009), pag. 22 – 31.

[11] Z. Zhou, H. Jiang, L. Qin, Life cycle sustainability assessment of fuels, Fuel. 86 (2007), pag. 256 – 263.

[12] R. Amutha, P. Gunasekaran, Production of ethanol from liquefied cassava starch using co-immobilized cells of Zymomonas mobilis and Saccharomyces diastaticus, Journal of Bioscience and Bioengineering. 92(6) (2001), pag. 560 – 564.

[13] A. Kosugi, A. Kondo, M. Ueda, Y. Murata, P. Vaithanomsat, W. Thanapase, T. Arai, Y. Mori, Production of ethanol from cassava pulp via fermentation with a surface-engineered yeast strain displaying glucoamylase, Renewable Energy. 34 (2009), pag. 1354 – 1358.

[14] S. Nitayavardhana, P. Shrestha, M.L. Rasmussen, B.P. Lamsal, J.H. Van Leeuwen, S.K. Khanal, Ultrasound improved ethanol fermentation from cassava chips in cassava-based ethanol plants, Bioresource Technology. 101 (2010), pag. 2741 – 2747

[15] G.S. Ayernor, T.K. Hammond, A. Graffham, The combination of rice and malt and amyloglucosidase

for the production of sugar syrup from cassava flour, African Journal of Science and Technology, 3(1) (2002), pag. 10 – 17.
[16] L. Mojovic, S. Nikolic, M. Rakin, M. Vukasinovic, Production of bioethanol from corn meal hydrolyzates, Fuel. 85(12-13) (2006), pag. 1750 – 1755.
[17] W. Morrison, B. Laignelet, An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches, Journal of Cereal Science. 1 (1983), pag. 9 -20.
[18] A. Herrera-Gómez, M. Canónico-Franco, G. Ramos, R.C. Pless, Aggregation in cooked maize starch, Carbohydrate Polymers. 50 (2002), pag. 387-392.

6 ACKNOWLEDGEMENTS

- This research was supported by the South African National Energy Research Institute (SANERI).
- The authors would like to gratefully acknowledge the Agricultural Research Council in supplying the cassava roots for this research.