Original Research Article

Comparative study on ethanol Production from sweet potato flour and sugarcane juice using thermotolerant *Kluyveromycesmarxianus*

Abstract

Renewable energy is widely acknowledged as the fastest growing energy source due to its environmental friendly. The demand for ethanol is increasing and the cost and availability of substrate are the major challenges facing bioethanol production. Therefore, the aim of this research was to compare bioethanol production from sugarcane juice and sweet potato flour. Bioethanol production was carried out using liquefied and pretreated sweet potato flour and sugarcane juice in 250 ml conical flaskinoculatedwiththermotolerant *Kluyveromycesmarxianus*. The result showed that the sugar concentration of sweet potato flour and sugarcane juice were 18 and 25 % respectively. During fermentation the sugar concentrationdecreased while ethanol concentration increased to maximum of 5.273 and 3.886 % respectively from sugarcane juice and sweet potato flour after 48 hours of fermentation. In conclusion, this study has demonstrated that sweet potato and sugarcane, which are efficiently and abundantly cultivated in most parts of Nigeria, can be efficiently utilized for bioethanol production.

Keywords: Ethanol, glucose, sweet potatoes, sugarcane juice and thermotolerant

INTRODUCTION

Energy is an index for global technological advancement. The present world energy supply derived from non-renewable sources cannot satisfy the increasing world demand arising from population explosion and the rapid depletion of the source of non-renewable energy (García *et al*., 2011).The global climate changes resulting from atmospheric pollution is the key consequences associated with the use of petroleum derived fuels (Quinteroa et al., 2008, Kim, 2014).

Renewable energy is widely acknowledged as the fastest growing energy source due to its environmentally-friendly nature and renewability (Maity *et al*., 2011). According to the International Energy Agency (IEA), the world demand for renewable energy is expected to grow continuously between 2007 and 2030 at a compound average rate of 7.3% annually (Sadorsky, 2011). Bioethanol production level in the world has been identified to account for about 10% of the world energy produced (Balat and Balat, 2009). Bioethanol has been identified as one of the most common liquid biofuels that can be

produced from sugar (sugar cane, sugar beets and sorghum), starchy (maize, wheat, barleys, sweet potatoes etc.) or cellulosic (crop residues, hard wood, softwood) raw materials (Ko *et al*., 2012; Zegada-Lizarazu and Monti 2012). Among these feedstock, wheat, barley, corn, sweet potatoe, cassava, sugarcane, sugar beet are examples of first generation biofuel feedstock; whereas, cassava waste (roots, peels, stem and leaves), Miscanthus, straw, wood, and grass constitute the sources of second generation biofuels raw materials (Musa, 2012, Bala, 2014). Sugar and starchy raw materials are presently the major feedstock for bioethanol production (Theuretzbachera*et al*., 2013).

Today, many countries have outstanding record of bioethanol production. The Nigeria's production capacity of 1st generation bioethanol currently stood at 134 000m3 per annum coming from five major commercial scale ethanol distilleries located in Lagos, Sango Ota and Bacita. In order to meet the nation's local domestic demand of 5.14 Mm3 per annum, over $3.86 billion has been invested in the feedstock plantation and construction of 19 ethanol bio-refineries with an expected annual capacity of over 2.66 Mm3 of fuel grade ethanol annually (Iye and Bilsborrown 2013). The target feedstocks are mainly sugar cane, cassava and sweet sorghum. However, to meet the 10% ethanol replacement (E10) in petroleum motor spirit will require about 1 million hectares of land which is 3% of the 34 million hectares under cultivation (Iye and Bilsborrown 2013). These land under cultivation represent only about 8% of the Nigeria's arable land underutilized with potentials of providing in excess of Nigeria's and West Africa food demand. The use of particular crop depends on its domestic availability and level of production in addition to sustainability, favourable soil and climatic conditions of the region peculiar to the energy crop (Escobar *et al*., 2009). Today, sugar cane is the best known crop for the production of biofuel with high biomass content of about 12-17% total sugars constituted by 90% sucrose and 10% glucose/fructose (Limtong*et al*., 2007).

Sugarcane is widely grown in the northern region of Nigeria. Its juice has sufficient minerals and organic nutrient that make it suitable for the production of ethanol (Karuppaiya *et al*., 2012). The complexity of the production process depends mainly on the feedstock used (Sanchez and Cardona 2008). The sugar content of the cane does not require modification during fermentation (Ranković*et al*., 2009). However, the optimization of bioethanol produced from Nigerian sugarcane has not been adequately investigated.

Sweet potato (Ipomea batatas) has been considered a promising substrate for alcohol fermentation since it has a higher starch yield per unit land cultivated than grains (Duvernay *et al*., 2013; Lee et al., 2012; Srichuwong*et al*., 2009). Industrial sweet potatoe are not intended for use as a food crop. They are bred to increase its starch content, significantly reducing its attractiveness as a food crop when compared to other conventional food cultivars (visual aspect, color, taste). Therefore, they offer potentially greater fermentable sugar yields for industrial conversion processes and the planted acreage (even on marginal lands) can be increased beyond what is in place for food. It has been reported that some industrial sweet potato breeding lines developed could produce ethanol yields of 4500–6500 L/ha compared to 2800–3800 L/ha for corn (Zhang *et al*., 2011). Sweet potato has several agronomic characteristics that determine its wide adaptation to marginal lands such as drought resistance, high multiplication rate and low degeneration of the propagation material, short growth cycle, low incidence and plagues, cover rapidly the soil and therefore protect it from the erosive rains and controlling the

weed problem (Cao *et al*., 2011; Vilaró *et al*., 2009). Previous transformation of the raw material into chips or flour (powder) can be done in order to facilitate its transport and/or plant conservation.

However, the demand for ethanol is increasing faster than its production and current ethanol production is not enough to meet increasing demand due to the cost of substrate. Therefore, this research focuses on the comparative study of bioethanol from sugarcane juice and sweet potato flour through fermentation using *Kluyveromycesmarxianus* as the fermenting organism.

MATERIALS AND METHODS

Sample Collection

Fresh sweet potatos were collected from a local market (Ogbete Market) Enugu State peeled and washed. Sugarcane stem was collected from New Artisian market Enugu State and the back cover was peeled and washed then taken to a milling machine.

Preparation of Sweet Potatoes and Sugarcane Juice

The sweet potatoe were cut into pieces and sundried. The dried sweet potatoe were ground using a clean milling machine and sieved into a fine powder with muslin cloth and packed in an air tight container before transporting to the laboratory.

The sugarcane juice extraction was done by peeling, washing, cutting and shredded into pieces before grinding it with an electric grinding machine and sieved with muslin cloth to get the juice.

Sources of pure microbial enzymes

The microbial enzymes used in the work were purchased from Cornrows Nigeria Limited, Enugu State. The Company name is SIGMA ALDRICH, thermostable α-amylase from *Bacillus Licheniformis* and glucoamylase from *Aspergillus niger*.

Preparation of α-amylase and glucoamylase

A 0.1g of α-amylase was dissolved in 100ml of distilled water before substrate addition and then boiled. A 0.4g of glucoamylase was dissolved in 100ml of the substrate after boiling when the temperature was cooled down.

Pretreatment of Sweet Potatoe and Sugarcane

A 20% of sweet potato flour was dissolved in 100ml of distilled water and was pretreated by liquefaction in the presence of thermostable α-amylase in 250ml conical flakes and boiled for 10min and allowed to cool. Thereafter, glucoamylase was added, while sugarcane was boiled for 10min only without enzyme.

Collection of Yeast and Inoculum Preparations

Kluyveromycesmarxianus was collected from Prof. J. C. Ogbonna's Laboratory, Department of Microbiolgy University of Nigeria, Nsukka. The yeast cell was prepared by inoculating three loops of 24 hours yeast culture from slant into a 10 ml of sterile PDA broth in a test tube. Thereafter the test tube culture was incubated at room temperature for 24 hours, estimated using hemocytometer to 3. 0 x 10^7 Cells/ml and was used to inoculate the fermentation medium.

Ethanol Production from sweet potato flour and sugarcane juice

Ethanol production was carried out using liquefied and pretreated 20% sweet potato flour (20g of sweet potatoes in 100 ml of distilled water); and 20 % v/v sugarcane (20 ml of juice in 80 ml of distilled water) in a 250 ml conical flask. α-amylase and glucoamylase were added only in the medium containing sweet potato flour. Approximately, 10 ml of yeast cell containing 3.0 x 107 cells/ml was added and incubated at 40^{o}C for 96 hours using simultaneous saccharification and fermentation (SSF). The samples were removed every day and analyzed for ethanol.

Determination of glucose and ethanol Concentration

After fermentation, the fermented medium was added into 1.5ml eppendorf tubes and centrifuged at 12000 rpm for 10mins using micro-centrifuge. Therefore, a 100µl of the fermented sample was injected into HPLC auto sampler vials containing 800µl of HPLC grade water. Ethanol concentration was determined using High Performance Liquid Chromatography (Shimazu) with pump ALC-20AT; oven model CTO-20A; Detector A RID-20A; Aminex HPX-87H Ion Exclusion column, column ID 431686, column length 300mm, column diameter 0.1mm; maximum usable temp. 75ºC and mobile phase 10Mm H2SO4 under the following conditions: injection volume 20µl, flow rate 0.800ml/min and oven temperature 65ºC.

RESULTS

Sugar concentration of sweet potato flour and sugarcane juice is shown in Fig 1, Sugar concentration were analyzed at 0 hrs on both substrates with maximum concentration of 18 and 25 %, respectively, from sweet potato flour and sugarcane juice. Sugar concentration depreciated as time increased up to 96 hours in sweet potato and sugarcane juice.

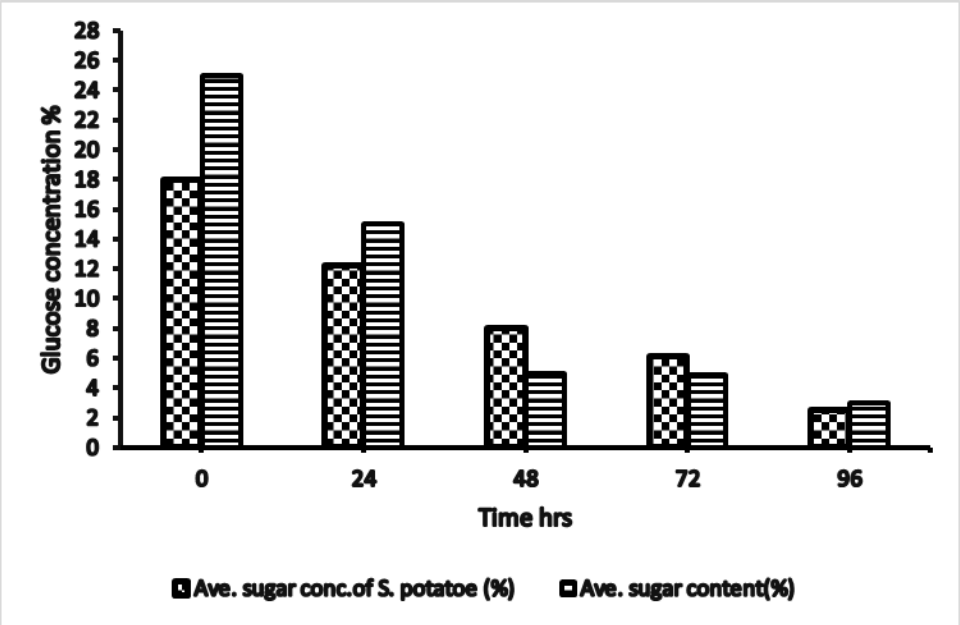

Fig 1: Glucose concentration of sweet potato flour and sugarcane juice

Fig. 2 shows ethanol and glucose concentration from sweet potato flour after 96 hours of fermentation. As the glucose concentration decreased during, fermentation ethanol Concentration increased up to 3.000 % after 48 hours of fermentation.

Fig. 3 Sugarcane juice was fermented for 96 hours. During fermentation, glucose concentration decreased while ethanol Concentration increased up to 5.000 % after 96 hours of fermentation with maximum time of 48hours from sugarcane juice.

Ethanol production from sweet potato flour and sugarcane juice was compared and the result is shown in Fig 4. Ethanol concentration increased within 48hrs on both substrates with maximum concentration of 3.886% and 5.273% respectively from sweet potato flour and sugarcane juice.

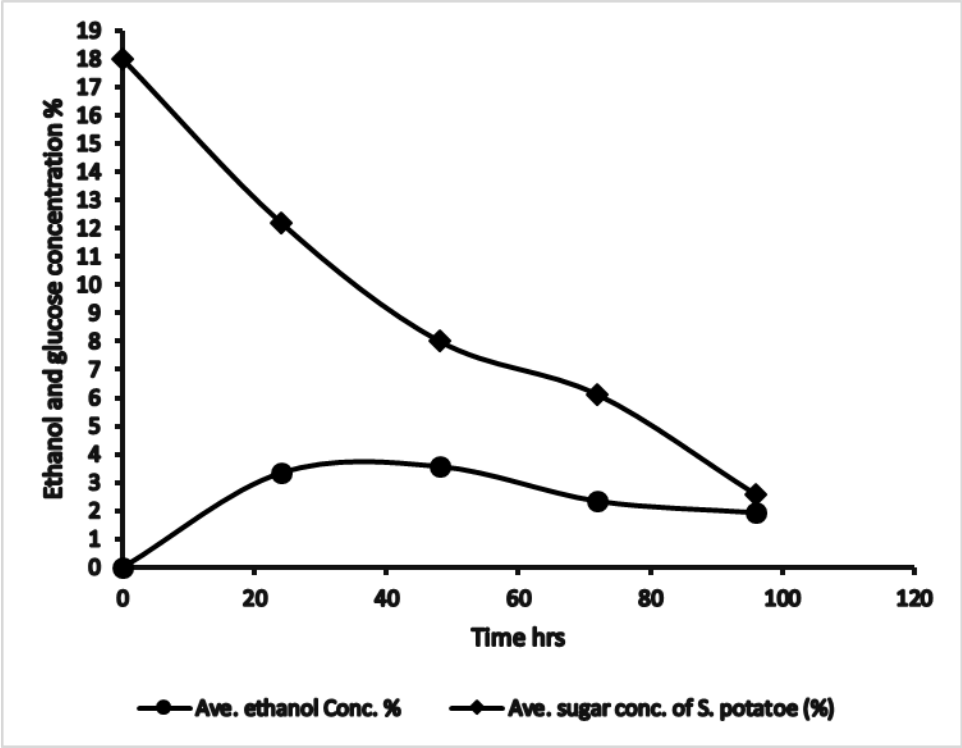

Fig 2: Ethanol and glucose concentration from sweet potato flour.

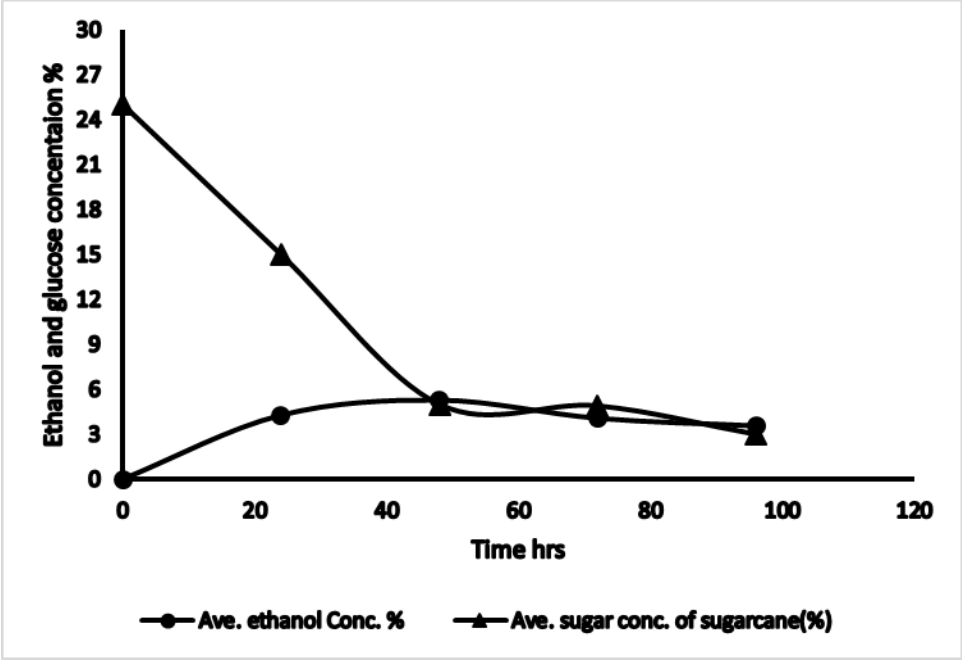

Fig 3: Ethanol and Glucose concentration from sugarcane juice.

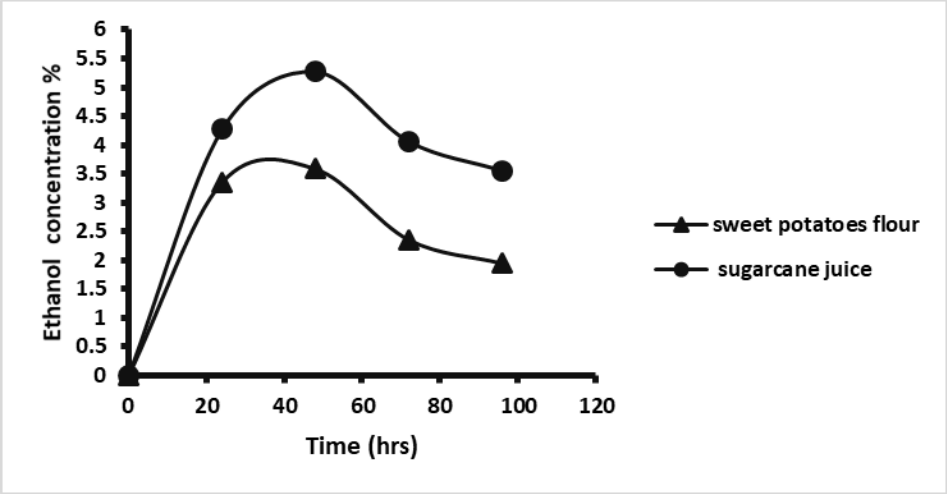

Fig 4 Ethanol production from sweet potato flour and sugarcane juice

DISCUSSION

There have been great progresses in development and commercialization of bioethanol in the last decade. However, ethanol selling cost is still higher than that of fossil fuel which can be attributed to the high cost of raw materials that are presently used for bioethanol production (Ezea, 2023). This research work has demonstrated that sweet potato flour and sugarcane juice can be converted to ethanol. With the concentrations of ethanol obtained from this work, the price of ethanol can be reduced if sweet potato flour and sugarcane juice can be harnessed for bioethanol production.

Production of ethanol from sugarcane juice was significantly higher than sweet potato flour using microbial enzymes (α-amylase and glucoamylase) and*Kluyveromycesmarxianus*. In this study, the sugar concentration of sweet potato flour and sugarcane juice was analysed at 18 and 25%. The maximum amount of ethanol (5.273 with ethanol yield of 0.264g/g) was produced from sugarcane juice at concentration of 20% sugarcane juice which was higher than the value obtained from sweet potato flour (3.586 with the ethanol yield of 0.179g/g) from the same 20% substrate concentration.

The optimum fermentation time for ethanol production was found to be 48hrs. Sweet potato flour and sugarcane juice were fermented up to 96hrs in conical flask and length of time for maximum production was 48hrs. Hossain *et al.* (2014) and Mohd *et al*. (2017) reported that higher fermentation time causes toxic effect on microbial growth especially in batch mode due to the high concentration of ethanol in the fermentation broth. Murata *et al*. (2015) also reported maximum yield after 24 hrs of fermentation during ethanol fermentation by thermo- tolerant yeast, *Kluyveromycesmarxianus* TISTR5925 isolated from extracted sap of old oil palm trunk. Rani *et al.* (2010) has reported that Saccharomyces cerevisae at 30^{o}C for 48 hrs resulted in maximum yield of bioethanol of 56.8g/l. Arumungam *et al*. (2011) found that yield of bioethanol of 35.86% is equal to the rate of fermentation efficiency of 70.31 % at 48 hrs of fermentation time.

CONCLUSION

Bioethanol production from sweet potato flour and sugarcane juicewas successfully done with appreciable sugar and ethanol yield. Sugarcane juice had more ethanol yield than sweet potato flour. Comparison of bothsubstrates have shown that sweet potato flour and sugarcane juice have the potential for bioethanol production. However, technology development for bioethanol production from agricultural crops with bioethanol production potential should be encouraged by increasing more agricultural input of such crops in the farm.

REFERENCES

Arumugam. R, and Manikandan. M., (2011), Fermentation of pretreated hydrolysates of banana and mango fruit wastes for ethanol production. *Asian Journal of Experimental Biological Sciences, 2*(2): 246-256.

Bala, E.J. (2014), Energy and power development in Nigeria: prospects, challenges, and way forward, 21st, 22nd and 23rd Federal University of Technology Convocation Lecture, Minna, March.

Balat, M. and Balat, H. (2009), Recend trends in global production and utilization of bioethanol fuel. *Applied Energy*, 86: 2273-2282.

Cao Y, Tian H, Yao K, Yuan Y., (2011). Simultaneous saccharification and fermentation of sweet potato powder for the production of ethanol under conditions of very high gravity. *Frontiers of Chemical Science and Engineering, 5*(3):318–324.

Duvernay W.H, Chinn M.S, Yencho G.C. (2013). Hydrolysis and fermentation of sweet potatoes for production of fermentable sugars and ethanol. *Industrial Crops and Products, 42*: 527–537.

Escobar, J.C., Lora, E.S., Venturini, O.J., Yanez, E.E., Castillo, E.F. and Almazan, O. (2009). Biofuels, technology and food security. *Renewable and Sustainable Energy Review, 13*: 1275-128.

Ezea, I. B. (2023). Technologies and Factors Affecting Bioethanol Fermentation and its Commercialization. *Archives of Ecotoxicology, 5* (1): 32 - 36.

García, A.E., Carmona, R.J., Lienqueo, M.E. and Salazarc, O. (2011). The current status of liquid biofuels in Chile. *Energy*, *36*: 2077-2088.

Hossain Z., Golam F., Jaya N.S., Mohd S.A., Rosli H., and Amru N.B. (2014). Bioethanol Production from Fermentable Sugar Juice. Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia Department of Petroleum and Chemical Engineering, Faculty of Engineering.

Iye, E. and Bilsborrown, P. (2013). Cellulosic ethanol production from agricultural residues in Nigeria. *Energy Policy,63*: 207-214.

Karuppaiya, M., Viruthagiri, T. and Manikandan, K. (2012). Statistical screening of medium components on ethanol production from cashew apple juice using saccharomyces diasticus, International Journal of Biological Veterinary and Agricultural. *Food Engineering, 6*: 64-67.

Kim, T.H. (2014). Overview of technical barriers and implementation of cellulosic ethanol in the U.S, *Energy*, *66*: 13-19.

Ko, C.H., Wanga, Y.N., Chang, F.C., Chen, J.J., Chen, W.H. and Hwange, W.S. (2012). Potentials of lignocellulosic bioethanols produced from hardwood in Taiwan. *Energy,44*: 329-324.

Lee W-S, Chen I-C, Chang C-H, Yang S-S. (2012). Bioethanol production fro sweet potato by co-immobilization of saccharolytic molds and Saccharomyces cerevisiae. Renewable Energy, 39: 216–222.

Limtong, S., Sringiew, C. and Yongmanitchai, W. (2007). Production of fuel ethanol at high temperature from sugar cane juice by a newly isolated *Kluyveromycesmarxianus*, *Bioresources Technology,8*: 3367-3374.

Maity, J.P., Hou, C.P., Majumder, D., Bundschuh, J., Kulp, T.R., Chen, C.Y., Chuang, L.T., Chen, C.N.N., Jean, J., Ojeda, T.K., Sánchez, E. and Kafarov, V. (2011). Sustainable ethanol production from lignocellulosic biomass - application of energy analysis. *Energy*, *36*, 2119-2128.

Mohd Azhar S. H., Abdulla R., Jambo S. A., Marbawi H, Gansau J. A.,, Mohd Faik A. A. , Rodrigues K. F. (2017). Yeasts in sustainable bioethanol production: A review. *Biochemistry and Biophysics Reports*, *10*: 52-61.

Murata M., Nitiyon S., Lertwattanasakul N., Sootsuwan K., Kosaka T., Thanonkeo P., Limtong S., Yamada M., (2015). High-temperature fermentation technology for low cost bioethanol. Journal of Japanese Institution. *Energy*, *94*:1154–1162.

Musa, U. (2012). Extraction and transesterification of Jatropha caucus oil for biodiesel production, M.Eng. Thesis, Federal University of Technology, Minna.

Quinteroa, J.A., Montoyaa, M.I., Sancheza, O.J., Giraldoc, O.H. and Cardonaa, C.A. (2008). Fuel ethanol production from sugarcane and corn: comparative analysis for a colombian case. *Energy*, *33*: 385-399.

Rani. P, Sharma, S, Garg, F.C, Kushal Raj, and Leela Wati., (2010). Ethanol production from potato flour by Saccharomyces cerevisiae. *Indian Journal of Science and Technology, 3* (7): 733-736.

Ranković, J., Dodić, J., Dodić, S., Popov, S., Jevtić-Mučibabić, R. (2009). Thin juice from sugar beet processing as medium for bioethanol production, *ZbornikradovaTehnološkogfakulteta u Leskovcu*, *19*: 44-51.

Sadorsky, P. (2011). Some future scenarios for renewable energy. *Features*, *43*: 1091-1104.

Sánchez, O. J, Cardona CA. (2008). Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresources Technology, 99*(13):5270–95.

Srichuwong S., Fujiwara M., Wang X. (2009). Simultaneous saccharification and fermentation (SSF) of very high gravity (VHG) potato mash for the production of ethanol. *Biomass- Bioenergy, 33*: 890–898.

Theuretzbachera, F., Bauera, A., Lizasoain, J., Beckerc, M., Rosenau, T., Potthast, A., Fried, A., Piringera, G. and Gronauera, A. (2013), Potential of different Sorghum bicolor (L. moench) varieties for combined ethanol and biogas production in the pannonian climate of Austria. *Energy, 55*: 107-113.

Vilaró F, Vicente E, Rodríguez G, Pereira G, Spina W, Reggio A, Ibañez F. (2009). Nuevasvariedades de boniato para ususdiversos. *Revista INIA, 20*:44–47.

Zegada-Lizarazu, W. and Monti, A. (2012). Review: are we ready to cultivate sweet sorghum as a bioenergy feedstock? A review on field management practices. *Biomass Bioenergy, 40*: 1-12.

Zhang L, Zhao H, Gan M, Jin Y, Gao X, Chen Q, Guan J, Wang Z. (2011). Application of simultaneous saccharification and fermentation (SSF) from viscosity reducing of raw sweet potato for bioethanol production at laboratory, pilot and industrial scales. *Bioresources Technology, 102*: 4573–4579.