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Enhancing the Production of Reducing Sugars from Cassava Peels by Pretreatment Methods

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ABSTRACT

Cassava peel is one of the major biomass wastes in Nigeria obtained from production of cassava tuber for human consumption, starch production and industrial uses. The objective of this work was to investigate the optimal condition for pretreating cassava peel with dilute sulphuric acid, methanol with catalyst (organosolv) and alkali prior to microbial enzymatic hydrolysis for the production of fermentable sugars. The pretreated samples reducing sugar yield was measured after enzymatic hydrolysis. The result shows that acid hydrolysis using sulphuric acid at a concentration of 0.1M at 120^{o}C for 30 min gave a maximum reducing sugar yield of 88.8% and 98%, followed closely by methanol treated peels (78 and 98%) while alkali pretreated peels produce the least (66 and 88%) for *Pseudomonas flourescens* and *Aspergillus terreus* respectively. In this study, H_2SO_4 and methanolysis treated peels prior to enzymatic hydrolysis had a greater capacity for hydrolyzing cassava peels than NaOH and also combination of pretreatments method with enzymatic treatment is an alternative to improve efficiency of reducing sugar production from cassava peel.

Keywords: *Acid hydrolysis, Alkali hydrolysis, organosolv, Cassava peels, enzymatic hydrolysis, Pretreatment.*

1. INTRODUCTION

Modernisation has open up an access to efficient utilisation of agro-industrial by-products, aiming at the obtainment of value added products like biofuels, biochemicals and biomaterials.Bioprocessing of agro- industrial residues can help solve environmental problems associated with the disposal of these materials. Cassava (Manihot *esculenta Crantz*) is a perennial woody shrub, grown as an annual mainly for its starchy roots. It is a cheap source of carbohydrates for human populations in the humid tropics [1, 2, 3, and 4]. The largest producer of cassava world-wide is Nigeria, followed by Brazil, Thailand, Zaire, and Indonesia [5, 6]. It is the staple food for over 500 million people in western and central Africa [7, 8, and 9] with an average consumption of approximately 500 cal/day [10]. In the processing of cassava, the roots are normally peeled to rid them of two outer coverings, *i.e.* a thin brown outer covering and a thicker leathery parenchymatous inner covering.

The peels constitute about 20-35% of the weight of the tuber, especially in the case of hand peeling [11]. Consequently, a large amount of cassava peel waste is generated annually. In Nigeria, cassava peels produced were about 450,000 tons annually with an increasing trend [6]. These peels are regarded as waste and are usually discarded and allowed to rot. Vegetation and soil around the heaps of the peels are rendered unproductive and devastated due to biological and chemical reactions that take place between the continuously fermenting peels, soil and the surrounding vegetation.
Besides their pollution and hazardous aspects, in many cases, these peels might have potentials for recycling raw materials or for conversion into useful products of higher value or even as raw material for other industries or for their use as food or feed after biological treatment [12]. Cassava peel is a lignocellulolytic material and because of this it is of interest to be used as an alternative substrate for ethanol production. To achieve this maximally ,cassava peel has to be pretreated so as to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the lignocellulosic materials and also to enhance sugar production by reducing the formation of byproducts that have inhibitory to the enzymatic hydrolysis and reducing the possibilities of loss of carbohydrates [13]. A mature cassava root possesses three distinct regions: a central vascular core, the cortex (flesh), and the phelloderm (peels). The peel is 1-4 mm thick and may account for 10-12% of the total dry matter of the root [14]. Cassava peels have been evaluated as a feedstuff for animals [15, 16 and 17]. The aim of the study was to obtain soluble reducing sugars by using different pretreatment methods prior to microbial enzymatic hydrolysis of cassava peels and to determine the optimal conditions of each treatment and measure the reducing sugar produced.

2. MATERIALS AND METHODS

A. Substrate Preparation

Cassava peels from the factory of cassava processing site in Odogbo barracks, Ojo, Ibadan, Oyo State, Nigeria were collected and washed thoroughly in tap water. It was then air dry for 24hours after which it was milled by a blender machine and dried overnight at 55^{o}C in a hot-air oven. The moisture content was found to be 11.2%.

B. Cassava Peel Pretreatments

i. **Alkali pretreatment by NaOH**: Ten grams of cassava peel was suspended in 90 ml of 0.01M to 0.25M sodium hydroxide (NaOH) and placed in an autoclave for fifteen minutes at 121^0 C. The solid residues were collected and washed extensively with tap water until neutral pH was reached prior to enzymatic hydrolysis.

ii. **Acid pretreatment by sulfuric acid (H_2S0_4)**: Ten grams of cassava peel was suspended in 90 ml 0.01M to 0.25M sulphuric acid (H_2SO_4) and heated at 121 ºC for 15 min in a 500 ml beaker. The solid residues were collected and neutralize with 2M NaOH and then washed extensively with tap water until neutral pH was reached prior to enzymatic hydrolysis.

iii. **Organosolv pretreatment**: Ten grams of cassava peel was suspended in 100mls of methanol with varying concentration of Sodium Acetate (0.01M to 0.25M).Both hot and cold treatment was applied, after which the solid residue were collected and washed extensively with tap water prior to enzymatic hydrolysis.

C. Microorganism

Aspergillus terreus SC1 and *Pseudomonas fluorescens B9* used in this research work were obtained from rotten cassava peels. The fungi was maintained on PDA agar slant and kept at 4^oC for further use while the bacteria was maintained on nutrient agar slant and kept the same way.

D. Enzymatic Hydrolysis

Pretreated cassava peels (1.5%w/v) were placed in a jar containing 100mls of minimal basal medium (1g/l $CaCl_2.7H_2O$, 1g/l $MgSO_4$, 2g/l $(NH_4)_2SO_4$, and 0.5g/l KH_2SO_4), the medium was sterilized and inoculated with $3.0x10^8$ cfu of *Pseudomonas flourescens B9* and 9mm plug of *Aspergillus terreus SC1* respectively. The mixture was then incubated at 50 ºC for 24 h for bacteria and 72hrs for fungi. After the appropriate timing, the reducing sugar content of the hydrolysates was measured quantitatively using the DNS method [18]

The concentration that gave a maximum reducing sugar was chosen. The selected concentration was applied to determine the optimum temperature for cassava peel hydrolysis. A temperature range used in this study was between 115^0C and 130^0C and hydrolysis time was from 15 to 60 min.

$$\% \text{ Peel Hydrolysis} = \frac{\text{Reducing Sugars produced by growth} - \text{Reducing sugar in control}}{\text{Reducing sugar in control}} \times 100$$

3. RESULTS AND DISCUSSION

Cassava peels pretreated with dilute acid, alkali and methanol with sodium acetate as catalyst (organosolv) were hydrolyzed using microbial crude enzymes under varying conditions and the % reducing sugar yield was determined for both bacteria and fungi used. Figure 1 shows that optimal % reducing sugar yields for 0.01M alkali and organosolv treated peels were 55.5 and 66.6% respectively while 0.1M dilute acid treated peels produce optimal % reducing sugar of 77.7% using *Pseudomonas flourescens B9*. Figure 2 shows that optimal reducing sugar yield for 0.05M alkali and organosolv treated peels were 66.6 and 94.4% respectively while 0.1M dilute acid treated peels produce optimal % reducing sugar of 88.8% using *Aspergillus terreus*.

The concentration of acid, alkali and methanol that gave a maximum reducing sugar was chosen to determine the optimum temperature and hydrolysis time. For organosolv, both isolates produce high concentration of reducing sugar (78% and98% for bacteria and fungi respectively) after 45 minutes of exposure at 0.05M concentration. For dilute acid treated peels, the highest concentration of reducing sugar was produced at 120^0 C after 30 minutes of exposure; the same is applicable to NaOH treated cassava peel. From this study, dilute acid pretreated peels was found to be suitable for highest reducing sugar production and this was supported by the work of Teerapart and Palmarola [19, 20].

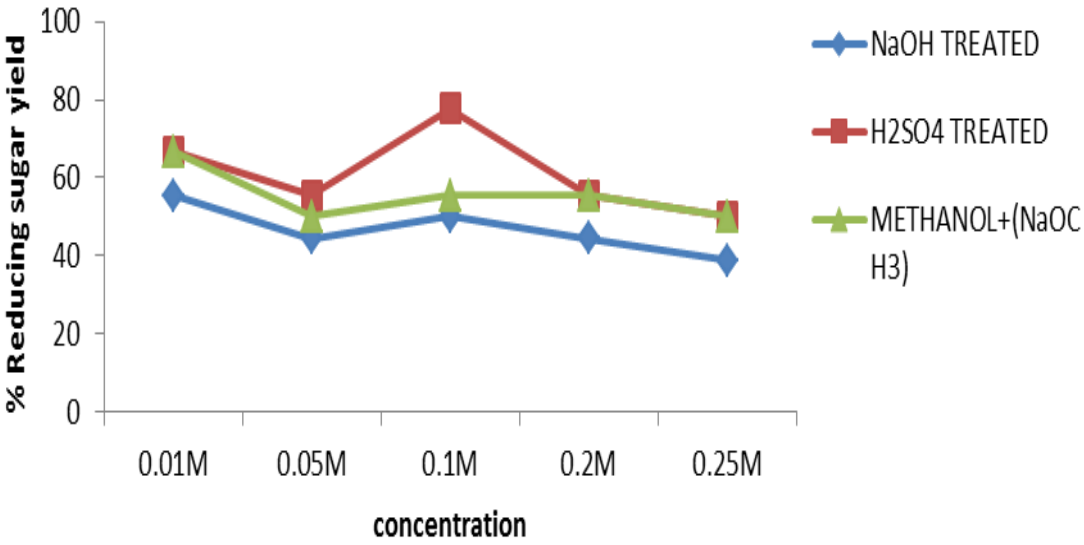

Fig.1: % Peel hydrolysis of *Pseudomonas fluorescensB9* inoculated on cassava peels under different pretreatment methods after 24hrs.

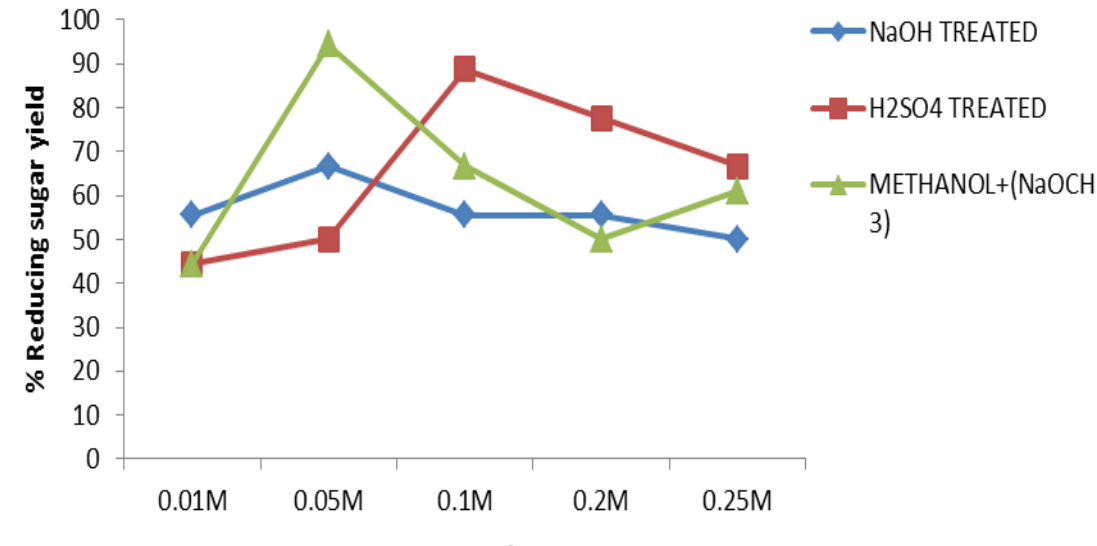

Fig 2: % Peel hydrolysis of *Aspergillus terreusSC1* inoculated on cassava peels under different pretreatment methods after 72hrs.

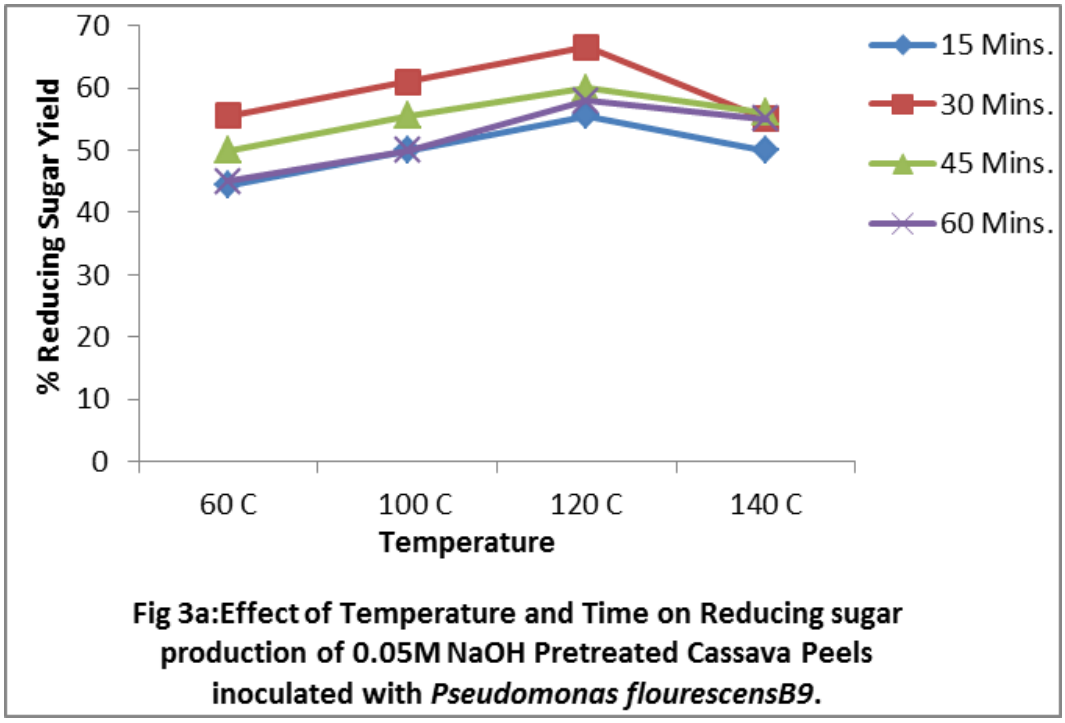

Fig 3a:Effect of Temperature and Time on Reducing sugar production of 0.05M NaOH Pretreated Cassava Peels inoculated with *Pseudomonas flourescensB9*.

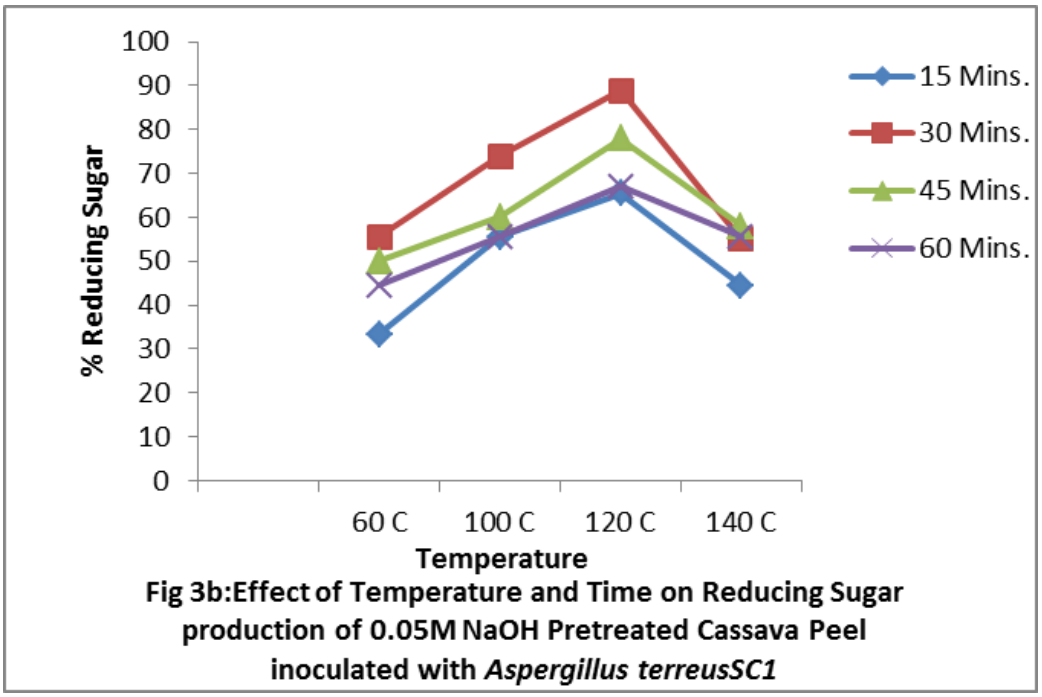

Fig 3b:Effect of Temperature and Time on Reducing Sugar production of 0.05M NaOH Pretreated Cassava Peel inoculated with *Aspergillus terreusSC1*

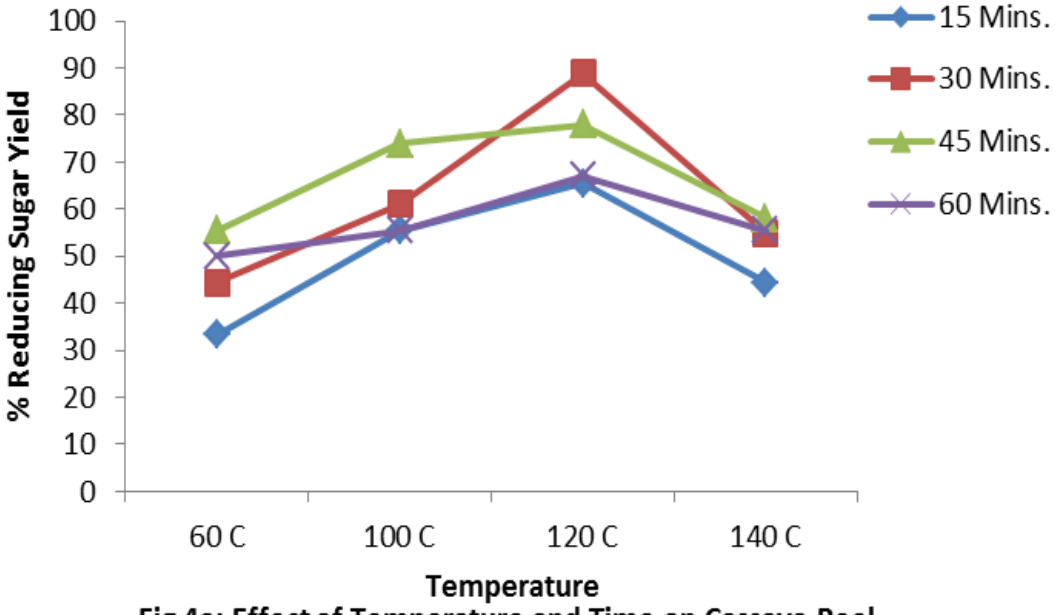

Fig 4a: Effect of Temperature and Time on Cassava Peel Pretreated with 0.1M H_2SO_4 Inoculated with *Pseudomonas flourescensB9.*

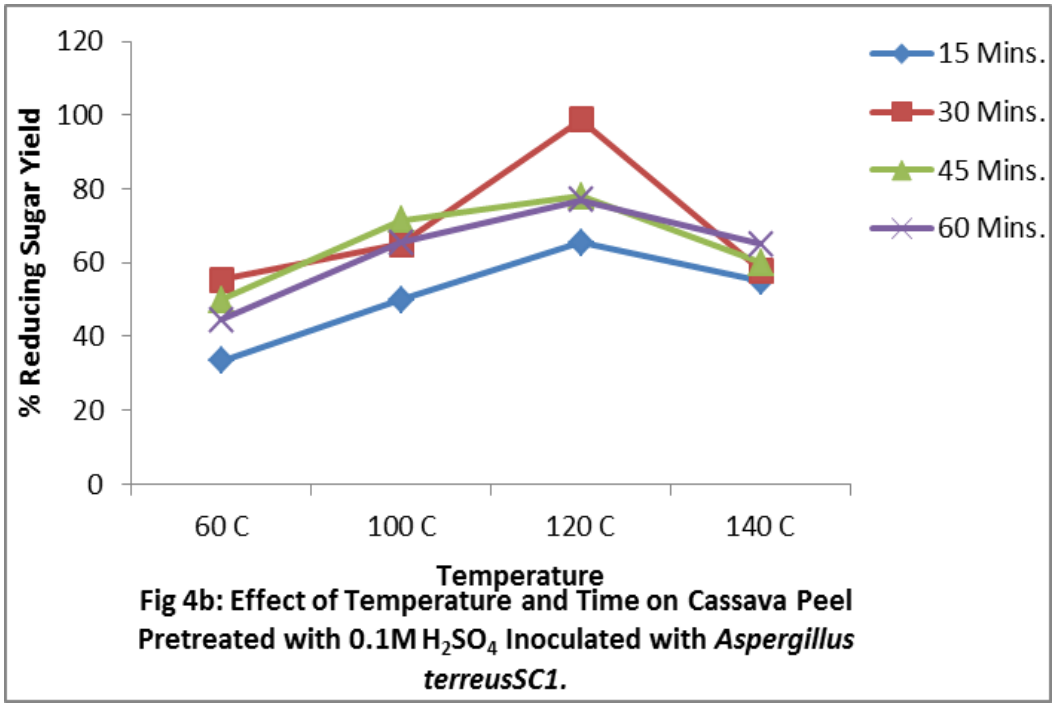

Fig 4b: Effect of Temperature and Time on Cassava Peel Pretreated with 0.1M H_2SO_4 Inoculated with *Aspergillus terreusSC1.*

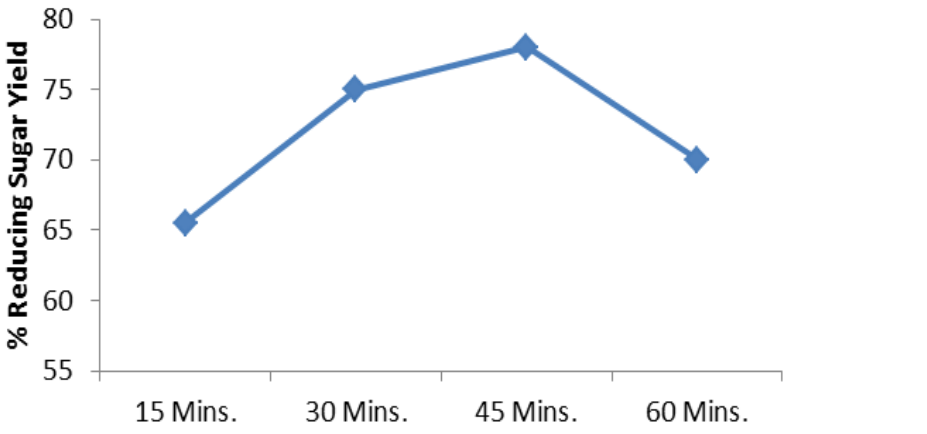

Fig 5a: Effect of Time on Reducing Sugar Production of Cassava Peel Pretreated with 0.05M Methanolysis inoculated with *Pseudomonas flourescensB9*.

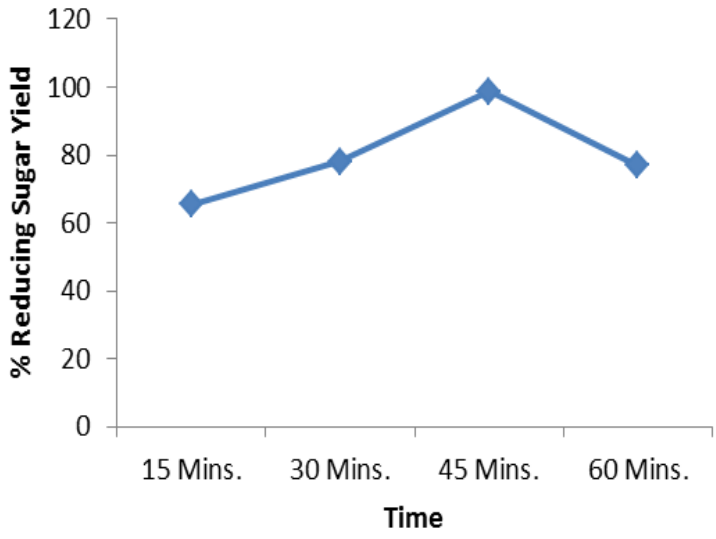

Fig5b: Effect of Time on the Reducing Sugar Production of Cassava Peel Pretreated with 0.05M Methanolysis inoculated with *Aspergillus terreusSC1*.

4. CONCLUSION

Experimental study to determine the appropriate method for pretreatment of cassava peels was carried out using acid, alkali and organosolv prior to microbial enzymatic hydrolysis. Different pretreatment methods were evaluated for their ability to produce reducing sugars from cassava peels. Hydrolysing cassava peels with dilute sulphuric acid pretreatment gave a higher reducing sugar production than organosolv and alkali pretreatment method. Pretreatment of cassava peels using 0.1M H_2SO_4 at 120^{O}C for 30minutes prior to enzymatic hydrolysis yielded % reducing sugar of 88% and 98% for *Pseudomonas fluorescens* and *Aspergillus terreus* respectively, this is in agreement with the work of Jirasak and Kalaya, 2006 [21] who obtain 68% of reducing sugar using 0.15M dilute sulphuric acid. Organosolv pretreated cassava peels using methanol with 0.05M Na_2CO_3 as catalyst for 45 minutes prior to enzymatic hydrolysis yielded % reducing sugar of 78 and 98% for bacteria and fungi isolates respectively, this is close to the results obtain from dilute acid, therefore this method of pretreatment could be apply so as reduce the environmental hazard of using acid for pretreatment. While 0.05M NaOH treated peels produced 66 and 88% reducing sugar at 120^{O}C for 30minutes for bacteria and fungi isolate respectively. It can thus be concluded that organosolv pretreatment method can be used as an alternative to acid pretreatment method in combination with *Aspergillus terreus SC1* crude enzymes for the bioconversion of cassava peels. Also, using microbial crude enzymes for saccharification of cassava peels produced more soluble sugars effectively than the pretreatment method alone and also makes the process cost effective in comparison to using commercial enzymes.

REFERENCES

[1] **Lin HJ, Xian L, Zhang QJ, Luo XM, Xu QS, Yang Q, Duan CJ, Liu JL, Tang JL, Feng JX**. Production of raw cassava starch-degrading enzyme by *Penicillium* and its use in conversion of raw cassava flour to ethanol. J Ind Microbiol Biotechnol. 2011;38(6):733–742. doi: 10.1007/s10295-010-0910-7.[PubMed][Cross Ref]

[2] **Henry, G. and C. Hershey, 2002**. Cassava in South America and the Caribbean. In: Hillocks, R.J., J.M. Thresh and A.C. Bellotti (eds.). *Cassava: Biology, Production and Utilization*. CABI Publishing Oxon, UK and New York, USA. 17-40p.

[3] **Hillocks, R. J., 2002**. Cassava in Africa. In: Hillocks, R.J., J.M. Thresh and A.C. Bellotti (eds.). *Cassava: Biology, Production and Utilization*. CABI Publishing Oxon, UK and New York, USA, 40-54p.

[4] **Onwueme, I.C., 2002**. Cassava in Asia and the Pacific. In: Hillocks, R.J., J.M. Thresh and A.C. Bellotti (eds.). *Cassava: Biology, Production and Utilization*. CABI Publishing Oxon, UK and New York, USA, 55-65p.

[5] **Phillip, T.A., D.P. Taylor, L.O. Sanni, M. Akoroda, C. Ezedinma, R. Okechukwu, A. Dixon, E. Okoro and G. Tarawali, 2005.** The Nigerian cassava industry statistical handbook. IITA, Nigeria. International Institute of Tropical Agriculture, Ibadan, Nigeria, 56p.

[6] **FAO, 2006**. Statistical database of the Food and Agricultural Organization of the United Nations. Available at http://faostat.fao.org/ (accessed 14 August 2007; verified 2 August 2008). FAO, Rome

[7] **FAO, 1996**. *Food requirements and population growth*. Technical Background Document. No.4. Rome.

[8] **FAO, 2005**. Statistical database of the Food and Agricultural Organization of the United Nations. Available at http://faostat.fao.org/ (accessed 14 August 2006; verified 2 April 2007). FAO, Rome.

[9] **Egesi, C.N, P. Ilona, F.O. Ogbe, M. Akoroda and A. Dixon, 2007a**. Genetic variation and genotype x environment interaction for yield and other agronomic traits in cassava in Nigeria. *Agronomic Journal* **99**: 1137-1142.

[10] **Iglesias, C., J. Mayer, L. Chávez and F. Calle, 1997**. Genetic potential and stability of carotene in cassava roots. *Euphytica* **94**: 367-373.

[11] **Obadina, A.O., O.B. Oyewole, L.O. Sanni and S.S. Abiola**. 2006. Fungal enrichment of cassava peels proteins**. African J. Biotechnol.** 5(3): 302–304.

[12] **Ajao,A.T, Abdullahi H.J, Atere,T.G, Kolawole, O.M,** 2009**:** Studies on the biodegradation and utilisation of selected tuber wastes by *Penicillium expansum*,Bioresource Research Communication, Vol.21, No 5.

[13] **Godliving Y. S. Mtui Recent advances in pretreatment of lignocellulosic wastes and production of value added products** African Journal of Biotechnology Vol. 8 (8), pp. 1398-1415, 20 April, 2009

[14] **Nartey,F**. 1979. Studies on cassava cynogenesis, and biosynthesis of cynogenic glucoside in cassava (*Manihot* spp.), In: Nestel, B., and R. Maclntyre. (Eds.), **Chronic cassava toxicity**, IDRC 010e: 73-87.

[15] **Adegbola, A.A.,and V.O. Asaolu**. 1986. Preparation of cassava peels for use in small ruminant production in Western Nigeria. In: Toward Optimum Feeding of Agricultural By-products to Livestock in Africa. Preston, T.R., Nuwanyakpa, M.Y.(Eds.), pp. 109-115.Proc. of Workshop held at the University of Alexandria, Egypt. Oct. 1985. ILCA, Addis Ababa, Ethiopia.

[16] **Obioha, F.C. and P, C.N. Anikwe**. 1982. Utilization of ensiled and sun-dried cassava peels by growing swine. **Nutr. Rep. Int.** 26: 961-972.

[17] **Osei, S.A., M. Asiamah and C.C. Atuahene**. 1990 Effects of fermented cassava peel meal on the performance of layers. **Animal Feed Sci. Technol.** 29(3–4): 295–301.

[18] Miller **L.G.,,** "Use of Dinitrosalisalic acid reagent for determination of reducing sugar," *Analytical Chemistry*, vol 31, Mar. 1959.

[19] Teerapart Srinorakutara, Lerdluk Kaewvimol and La-aied Saengow, 2005: Approach of Cassava waste pretreatments for fuel ethanol production in Thailand.

[20] Palmarola B, Choteborska P, Galbe M and Zacchi G, 2005: Ethanol production from non starch carbohydrates of wheat bran. Bioresource Technology, vol 96, pp 843-850.

[21] Jirasak Kongkiattikajorn* and Kalaya Yoonan 2006. Conversion of Cassava Industry Waste to Fermentable Sugars*The 2nd Joint International Conference on "Sustainable Energy and Environment (SEE 2006)" E-031 (P) 21-23 November 2006, Bangkok, Thailand.*