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Pretreatment and Hydrolysis of Cassava Peels for Fermentable Sugar Production

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ABSTRACT

Fermentable sugars are important prerequisite for ethanol production. These sugars are insufficient for production of the required amount of ethanol for Nigeria consumption in spite availability of raw materials. This study is aimed at producing fermentable sugars by pretreatment and hydrolysis of cassava peels using *Aspergillus niger* and their crude enzymes (amylase, cellulase and pectinase), as well as comparing the reducing sugar yield. Cassava peels were pretreated by soaking and a combination of soaking and boiling at varying time. Hydrolysis of pretreated cassava peels with *Aspergillus niger* and crude enzymes were carried out for 5 and 15 days, respectively. The result showed that pretreatment by soaking and boiling for up to 120 min removed the highest amount of cyanide and increased amount of carbohydrate produced but reduced the fibre content (37.04±0.01 mg g^{-1}, 71.42±0.02 and 10.38±0.42%). Hydrolysis using *Aspergillus niger* yielded up to 95.44±0.11 mg g^{-1} reducing sugar while hydrolysis using enzymes yielded up to 72.38±0.06 mg g^{-1} reducing sugar. The study revealed the potentials of cassava peels in reducing sugar production. Soaking and boiling of cassava peels for 120 min removed more cyanide and yielded high carbohydrate needed for reducing sugar production. Microbial cells are better tools that could be used for hydrolysis of carbohydrate to reducing sugars than their enzymatic products.

Key words: Pretreatment, hydrolysis, *Aspergillus niger*, reducing sugar

INTRODUCTION

The importance of reducing sugar cannot be overemphasized because it is an essential raw material for the production of ethanol, a preferable alternative transportation fuel in this generation. Ethanol is beneficial to mankind; it can be used as solvent, germicide, anti-freeze and intermediate for other organic chemicals (Otulugbu, 2012). It is a renewable biofuel that could be produced from plant biomass and burn effectively in automobile engines without emission of hazardous gases to the environment (Adelekan, 2010). The search for alternative energy has become paramount due to anthropogenic emission of greenhouse gases which originate from combustion of fossil fuel as coal, oil and natural gas (Oyedepo, 2012).

Cassava peels has been chosen as plant waste for this research because it contain high amount of starch deposit constituting 20-35% of the tuber (Nwabueze and Otunwa, 2006), it offer numerous advantages in comparison to other crop residues such as rice straw, wheat and sugarcane bagasse and can easily be attacked by micro-organisms (Wongskeo *et al.*, 2012). This makes it a good choice of raw material for reducing sugar production. However, Nigeria is the highest producer of Cassava in the world, producing higher than Brazil, Thailand and Indonesia (NCMP, 2006). Industrial and local processing of cassava to food and other products has led to generation of enormous wastes that are dumped in drainages rather than transforming them to useful products. These wastes end up polluting the surface and underground water (Olanbiwoninu and Odunfa, 2012). For example, about 2.96 million metric tons of cassava peels are generated and discarded annually in Nigeria from about 10 million metric tonnes of Cassava processed for Garri alone (Aro *et al.*, 2010). The present annual ethanol production rate of 134 million liters in Nigeria is grossly inadequate and the companies producing the ethanol in the country import their raw materials from Brazil in spite of abundance of plant wastes that could serve as raw materials (Elijah, 2010). Nigeria needs to explore the abundant agricultural wastes to produce enough ethanol for consumption and exportation. This will serve as a source of employment and income to the citizenry and the country in general. It will also curtail spending Nigeria's scarce resources in importation of ethanol.

Pretreatment is to enhance the release of carbohydrate from the biomass for easy conversion to reducing sugar by hydrolysis (Lucas, 2012). Having known that cassava contain high cyanide concentration (Lucas, 2012), it is therefore, necessary to pretreat the peels before hydrolysis to remove the cyanide content that could hinder microbial and enzyme activities and invariably affect the final reducing sugar yield. This will increase the porosity of the lignocellulosic materials and also enhance sugar production by reducing the formation of byproducts that are inhibitory to the enzymatic hydrolysis and reducing the possibilities of loss of carbohydrates (Olanbiwoninu and Odunfa, 2012). The aim/objective of this research work is to pretreat cassava peels to remove high amount of cyanide. Then to hydrolyzed the pretreated cassava peels using microbial cells and their enzymes to produce reducing sugars. Finally, to compare the fermentable sugar yield of the microbial cells and their enzymes.

MATERIALS AND METHODS

Sample collection and preparation: Large quantity of fresh cassava peels were collected in clean polythene bag from Cassava waste dump site, Kasuwan Gwari Market, Minna, Niger State, Nigeria. The cassava peels were washed and sun dried on a large clean mat for three days and finally milled into powder using motar and pistil.

Pretreatment of cassava peels

Soaking for 24 h: Five grams each of the milled Cassava peels was soaked with 50 mL distilled water in a beaker for 24 h and samples were taken every 6 h for the determination of crude fibre, cyanide and total carbohydrate.

Soaking overnight and boiling for 120 min: Another set of the milled cassava peels were soaked for 24 h and boiled for 120 min and samples were taken every 30 min to determine crude fibre, cyanide and total carbohydrate.

Having observed that soaking+boiling removed more cyanide, the chunk of the sample was subjected to soaking overnight and boiling for 120 min being the best treatment that removes high cyanide and extract more carbohydrate from the cassava peels.

Identification of microorganism

Aspergillus niger: Macro culture method was used to identify the organisms (Steinbach and Stevens, 2003). The organism from the stock was subcultured on saboraud dextrose agar plates at 28°C for 3 days to obtain distinct colonies. The physical observation of initial white growth that later turns black at the top with pale yellow color at the bottom identify the organism to be *Aspergillus niger*.

Microbial Hydrolysis: One hundred grams of the pretreated cassava peels were soaked into 1000 mL distilled water in a conical flask and inoculated with 100 mL of 3 days growth of *Aspergillus niger* in a Saboraud Dexterous Broth (SDB). The pH was adjusted to 3.5 and hydrolysis carried out for 5 days at room temperature. Samples were taken daily for reducing sugar determination. The organisms were obtained from the stock in the laboratory of Federal University of Technology, Minna. The fungi was maintained on SDA agar slant and kept at 4°C for further use.

Enzyme hydrolysis: Two hundred grams of the pretreated cassava peels was soaked in 2000 mL water in a conical flask. Two hundred milliliter of each of the crude enzymes were introduced one after the other and the pH adjusted for optimum hydrolysis. For cellulase the pH was adjusted to 4.5, for amylase the pH was adjusted to 4.0 and for pectinase the pH was adjusted to 5.5. Hydrolysis was carried out for 5 days for each of the enzyme at room temperature. Samples were taken daily for reducing sugar determination.

All results are presented as triplicates of the mean standard values each analyzed using SPSS at significance of $p \geq 0.05$.

RESULTS AND DICUSSION

Two methods (soaking and soaking+boiling) were used to pretreat cassava peels and the end product were hydrolyzed to reducing sugar using *Aspergillus niger* and crude enzymes (cellulase, amylase and pectinase) produced from the same organism. The larger sample was subjected to soaking+boiling being for 120 min being the best method that yielded more carbohydrate and liberate more cyanide from the cassava peels. Figure 1 show the % yield of carbohydrate (71.42±0.02%), % reduction in crude fibre (10.38±0.42%) and reduction in cyanide content (37.04±0.01 mg g^{-1}) after soaking+boiling of the cassava peels for 120 min. The carbohydrate yield almost conform to 72.50±0.4% reported for fermented cassava peels by Okpako *et al*. (2008), although, it is lower than 193.1% carbohydrate yield reported by Adamafio *et al*. (2012) for the treatment of groundnut shell with potash. The reduction in the cyanide content was higher than 6.2 mg kg^{-1} reduction of cyanide in fermented cassava peels reported by Oboh (2005). The study reveals that treatment will improve the carbohydrate content of samples with concomitant reduction in the cyanide levels allowing the microorganisms needed for hydrolysis to attain optimum level in the degradation of the sample to reducing sugars. Figure 2 show reducing sugar yield (95.44±0.11 mg g^{-1}) obtained after five days hydrolysis of pretreated cassava peels with Aspergillus niger, while Fig. 3 shows reducing sugar yield of

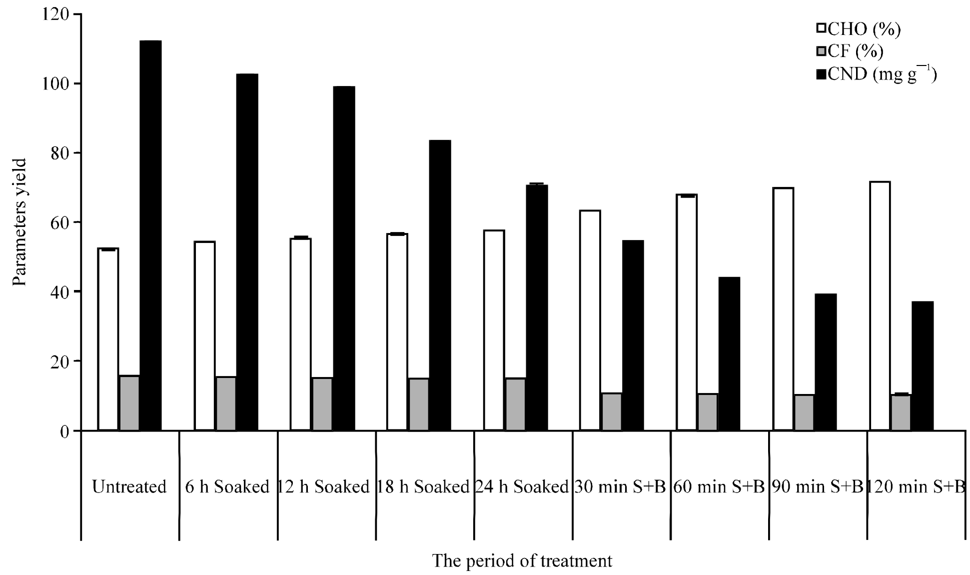

Fig. 1: Carbohydrate, crude fibre and cyanide contents of the pretreated cassava peels CHO: Carbohydrate, CF: Crude fibre, CND: Cyanide, S+B: Soak+boiling, Level of significance: $p \geq 0.05$

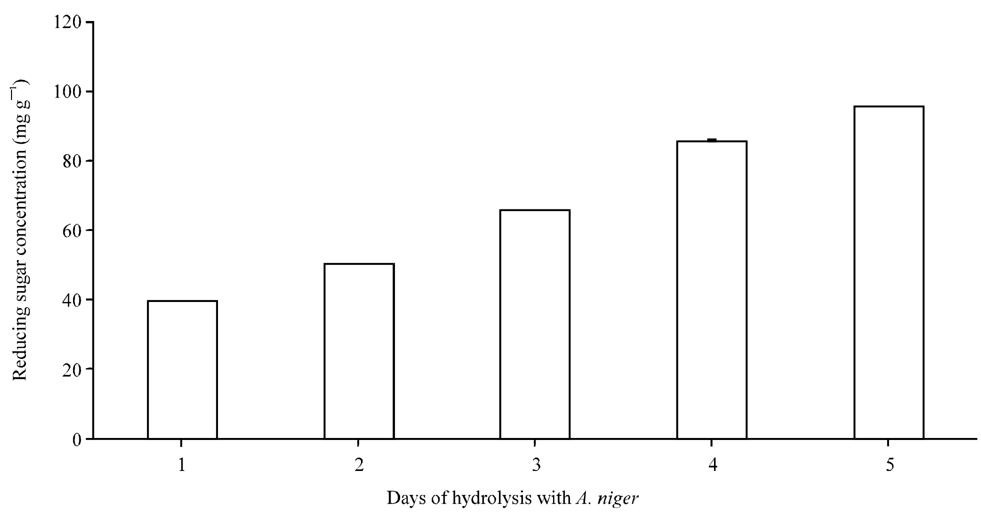

Fig. 2: Reducing sugar yield from hydrolysis of treated cassava peels using *Aspergillus niger*, Level of significance: $p \geq 0.05$

(40.58±0.10, 55.73±0.05 and 72.38 mg g^{-1}) within 15 days hydrolysis of pretreated cassava peels with amylase cellulase and pectinase, respectively. The study reveal that microbial cells yielded more reducing sugar in five days compare to the crude enzymes in 15 days probably because degrade the substrate as their source of carbon and energy and also liberate various enzyme during the process. The reducing sugar yield by microbial cells is almost in agreement with the 98% reducing sugar for methanol treated cassava peels reported by Olanbiwoninu and Odunfa (2012) and higher than 88% from alkali treatment reported by the same work.

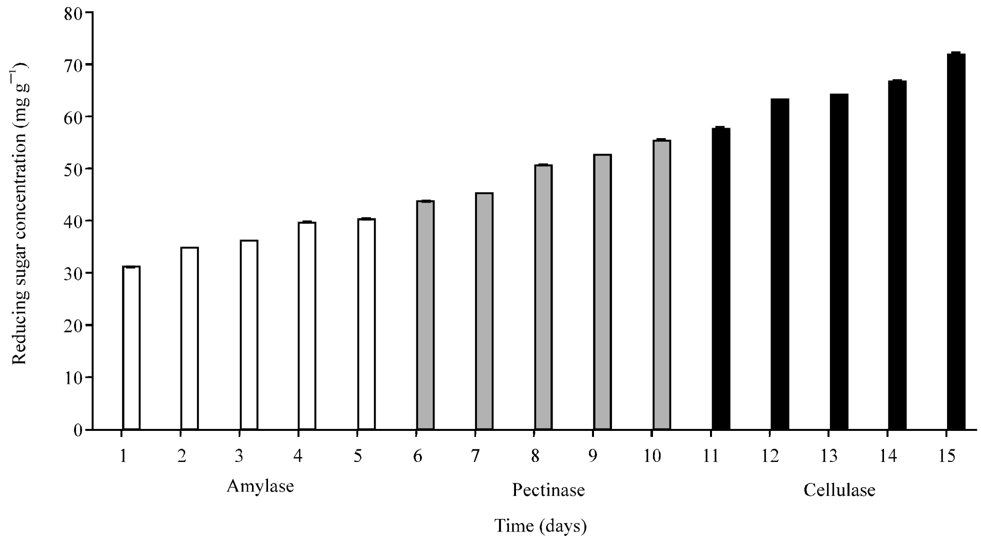

Fig. 3: Reducing sugar yield from enzymatic hydrolysis of treated cassava peels, Level of significance: $p \geq 0.05$

CONCLUSION

Soaking and boiling of cassava peels can be used in place of acid treatment to remove cyanide from plant biomass to enhance carbohydrate yield. This method can be used as an alternative to safeguard the environment against the dangers of chemicals. Microbial hydrolysis yielded more reducing sugar compare to the product of their enzymes. This revealed the potentials of microbial cells, confirming them as better tools for reducing sugar production compared to crude enzymes produced from the same cells.

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