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Comparative study of Protein enrichment of Lignocellulose Wastes using Baker's Yeast (*Saccharomyces cerevisiae*) for Animal Feeds

[*1]Kutshik J.R., [2]Usman, A.M and [2]Ali-Dunkrah U
[1]*Department of Biochemistry, University of Jos, P.M.B 2084, Jos, Plateau State, Nigeria.*
[2]*Department of Biotechnology, School of Pure and Applied Sciences, ModiboAdama University of Technology, Yola, Adamawa State, Nigeria.*

Abstract: *Obtaining an economical product for food and feed for animals could solve worldwide food protein deficiency. And these can be possible through the bioconversion of fruit wastes into certain valuable products like single cell protein (SCP). However, using wastes as substrate for the production of high nutritious product may also alleviate environmental pollutant up to some extent. In this study, we attempt to use two different wastes as carbon source to see which support Saccharomyces cerevisiaefor the production of SCP more. The sugarcane bagasse and groundnut shells were used as sole carbon source for preparation of fermentation media on which Saccharomyces cerevisiae was grown. S. cerevisiaebiomass was produced by solid state fermentation.A comparative study of single cell protein produced on these fruit wastes reveals that maximum protein content was found with Saccharomyces cerevisiaeto be 9.3 mg per 2 g of substrate on groundnut shells. The present research work can helps in SCP production from inexpensive and cheap agro waste material.*

Keywords: *Single Cell Protein, Sugarcane bagasse, Groundnut shells, Saccharomyces cerevisiae*

I. Introduction

In developing countries, human population growth is increasing at an alarming rate and animal need for protein is also on the high side, but it would have been better, if this growth had been proportionate with food supply for humans and animals. Generally, the world demands for protein rich foods have make the need to search for alternative sources of proteins to supplement the already existing sources. And Single Cell Protein (SCP) has offered this alternative (Najafpur, 2007).Single Cell Proteins refers to microbes grown on a suitable substrate, dead, dried and total protein extracted from the cell culture. And these are produced using different microorganisms including bacterium, fungus and algae (Anupama and Ravindra, 2000). It is also called biomass, bioprotein or microbial protein. Single Cell Protein also contains other nutrients like carbohydrates, fats, vitamins and minerals (Jamelet al., 2008; Jamel et al., 2005). Single Cell Protein are also rich in some essential amino acids that are limiting in most plants and animals proteins like methionine and lysine (Gad et al., 2010; Anupama and Ravindra, 2000). These SCP are produce through the process of fermentation.The Fibrous waste Sugar bagasses and groundnuts shells are agricultural wastes that are inexpensive and renewable feedstock for bioethanol production through conversion to fermentable sugars (Li et al., 2010; Mussatto&Teixeira, 2010). Both wastes are of lignocellulose and made up of lignin, hemicellulose and cellulose. The lignin provides the crystalline structure of the lignocellulose of which the complex chemical structure hinders direct enzymatic digestion of the cellulose. The enzymatic degradation process of lignocellulose is in two steps: firstly, the lignocelluosic material is pre-treated to destroy its complicated natural make up; secondly, the material is enzymatically converted to fermentable sugars. The pre-treatment of lignocelluloses is necessary to break down the crystalline structure of the cellulose, remove hemicellulose, eliminate or modify lignin to increase cellulose accessibility and digestibility by the cellulolytic enzymes (Yu et al., 2013, Zhang et al., 2013, Macrelli et al., 2012). Among the methods of scarification of lignocellulose wastes to release fermentable sugars, cellulolytic enzymes appear to be the most promising for large-scale application (DeMartini et al. 2013).

Most of these agricultural waste with high carbohydrate content are very good substrate for microbial growth, for they provide the required carbon for energy production. When these microbes have grown and increase in number and harvested also serve as source of single cell protein. Most of the agricultural wastes used for single cell protein production are rice husk, spent millet, sweet potatoes peels, etc. In this study we used baker's yeast *Saccharomyces cerevisiae* because they produce cellulase that breakdown cellulose. Finally they act the monosaccharaides and disaccharides through the process of fermentation to produce by products that include amino acids and they multiple and also served as source of Single Cell Protein (Jaganmoham, et al., 2013; Mondal et al., 2012). Although much attention has been paid on how these wastes could be used for biofuel production (Macrelli et al., 2012) but our attention is on how to produce Single Cell Protein from these

wastes and use them as feed for livestock. The quest for proper harnessing of these wastes and also converting it to useful product is part of the main reason for this study. Since it is well documented that these wastes could and are use as feed for animals and chickens, improving its nutritional quality is the focus of this study.

II. Materials and Methods

2.1 Samples collection

Sugarcane bagasse was collected from JagaJaga market, Girel, in Yola, Adamawa State of Nigeria. The groundnut shells were collected after consuming the groundnuts and baker's yeast was bought from Jimeta Modern market also in Adamawa State of Nigeria.

2.2 Samples Preparation

2.3 The Sugarcane bagasse and groundnut shells were dried and ponded to powdered form with pistle and mortar. For the powdered sugarcane bagasse, 40 g each was weighed and placed into 250 ml biodegradable flasks marked (bagasse, 0, 3, 5, 6 and 7 days). For the powdered groundnut shell, 40 g each was weighed and placed into 250 ml biodegradable flasks marked (shell, 0, 3, 5, 6 and 7 days).

2.3 Pre-treatment of sugarcane bagasse

In each of the biodegradable flask 100 ml of 25% NaOH was added and plugged with cotton wool, wrapped with aluminium foil paper. The pH of the semi-solid paste was adjusted to 4.6 and autoclaved at 121 °C for 15 minutes.

2.4 Preparation of Innoculum

2.5 The *Saccharomyces cerevisiae* was bought in Terminus Market, Jos, Plateau State, Nigeria and culture in the laboratory to validate its viability. The baker's yeast was cultured on potato dextrose agar using inoculation needle and incubated at room temperature for 4-7 days in order to obtain distinct colonies. The colonies obtained were then inoculated in a liquid broth for 3 days for fermentation and fixing on slant.

2.5 Fermentation Process

The autoclaved samples were allowed to cool down, and then 1 ml of the inoculum was added to the conical flasks with the semi-solid paste of sugarcane bagasse and groundnut shell. The flasks were then labelled according to the days of fermentation (0, 3, 5, 6 and 7 days) with one flask labelled bagasse and the shell, where these are the flasks without inoculum added to them. All the biodegradable flasks with the exception of the ones with bagasse and shell only, and flasks of zero days, were all incubated at 30 °C.

2.6 pH determination

The pH of the fermented bagasse and groundnut shells in the semi-solid form was determined using pH metre for the periods of fermentation (0, 3, 5, 6, and 7 days).

2.6 Moisture Content determination

Empty aluminium dishes were weighed and recorded; 2 g of the fermented samples were poured into the dishes. The dishes containing the samples were placed in a hot air oven and dried at 105 °C for three hours, after which they were removed and allowed to cool in a desiccator. Then the dishes containing the dried samples were reweighed.

2.8 Reducing Sugar and non-reducing sugar determination

Total sugars, reducing and non-reducing sugar in the fermented sugarcane bagasse and groundnut shell were determined the method of Lane and Erynon.

2.9 Crude Protein Content determination

After stopping the fermentation, the semi-solid samples were dried in an oven at 40 °C. The crude protein content was then determined at each end of the fermentation period using the Kjedahl method after defatting (A.O.A.C, 2006).

III. Results

The result of pH, moisture content, reducing sugar, non-reducing and crude protein content of fermented sugar bagasse at various period of fermentation is presented in in table 1 below.

Table 1: The result of pH, moisture content, reducing sugar, non-reducing and crude protein content of fermented sugar bagasse at various period of fermentation.

Fermentation Period (days)	pH	Moisture Content (%)	Reducing Sugar (%)	Non-reducing Sugar (%)	Crude Protein Content (%)
Bagasse					3.9±0.2
0	4.5±0.3	55.0±2.0	42.0±1.3	5.7±1.5	4.7±0.4
3	4.1±0.2	50.0±3.0	36.4±2.1	3.8±0.9	6.5±0.1
5	4.0±0.1	45.0±4.0	32.3±1.7	1.9±0.5	8.5±0.1
6	4.4±0.2	65.0±1.0	30.3±2.2	1.3±0.3	8.3±0.2
7	6.1±0.1	60.0±2.0	28.3±1.9	1.1±0.2	7.9±0.1

Each of these data is repeated three times.

The pH, moisture content, reducing sugar, non-reducing and Crude Protein Content of fermented Groundnut shells after 0, 3, 5, 6, and 7days of fermentation with *Saccharomyces cerevisiae* (baker's yeast). As shown in table 2 below.

Table 2: Showing values for pH, moisture content, reducing sugar, non-reducing sugar and Crude Protein Content of fermented Groundnut shells.

Fermentation Period (days)	pH	Moisture Content (%)	Reducing Sugar (%)	Non-reducing Sugar (%)	Crude Protein Content (%)
Shells					03.5±0.3
0	04.6±0.1	50.00±3.0	45.0±1.2	05.7±0.7	04.4±0.5
3	03.7±0.2	44.00±1.0	39.0±0.9	04.7±0.6	07.2±0.4
5	04.5±0.3	55.00±1.0	32.0±0.7	03.6±0.6	08.8±0.1
6	04.6±0.1	60.00±6.0	25.0±0.9	02.2±0.8	09.4±0.2
7	06.2±0.1	62.00±1.0	24.0±0.8	01.4±0.5	07.5±0.2

Each data is repeated three times

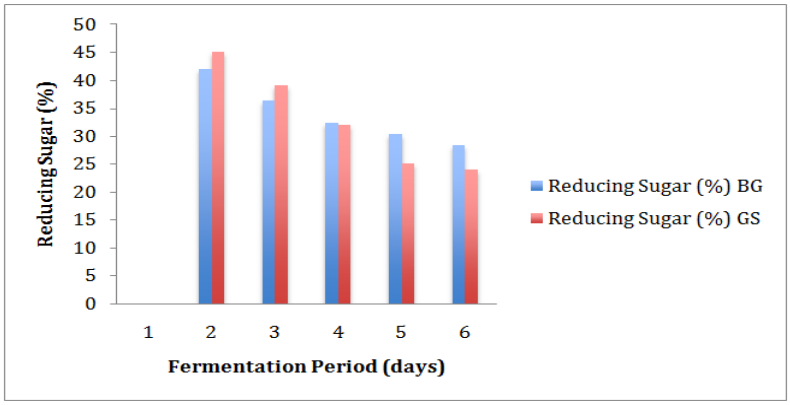

Figure 1: Bar chart comparing the concentration of reducing sugar in the two different substrates during fermentation with *Saccharomyces cerevisiae*.

Key: BG = Bagasse, GS = Groundnut Shell.

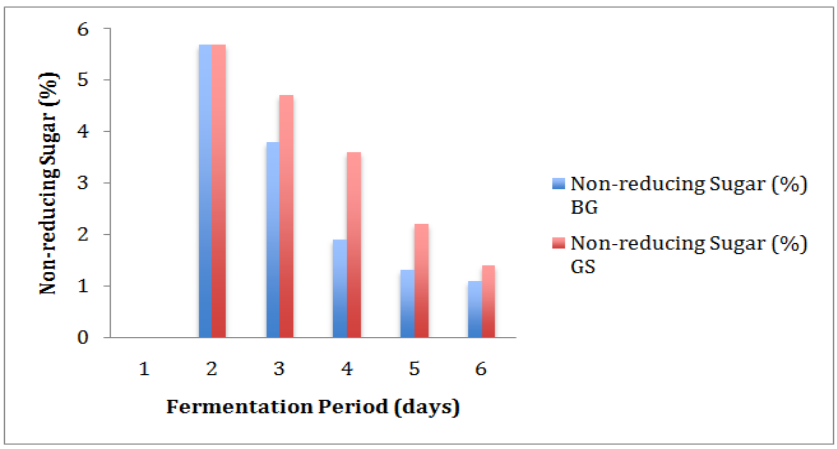

Figure 2: Bar chart comparing the concentration of Non-reducing sugar in the two different substrates during fermentation with *Saccharomyces cerevisiae*.

Key: BG = Bagasse, GS = Groundnut Shell.

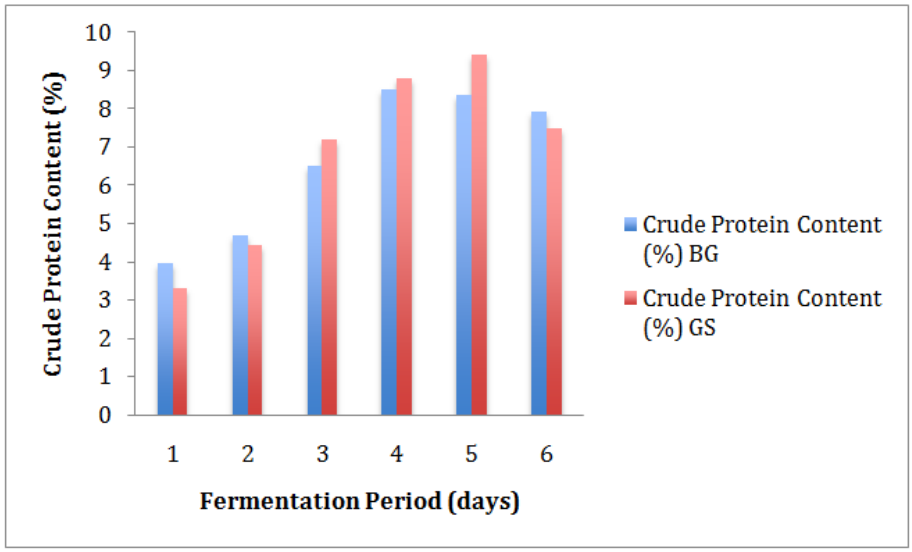

Figure 3: Bar chart comparing the concentration of Crude Protein Content in the two different substrates during fermentation with *Saccharomyces cerevisiae*.
Key: BG = Bagasse, GS = Groundnut Shell.

IV. Discussion and Conclusion

Lignocellulosic biomass comprising forestry, agricultural and agro-industrial wastes are abundant, renewable and inexpensive energy sources. We have these lignocellulose wastes in abundance in our environment and these could be source of wealth and employment. But yet to be harnessed, this study attempt to show how much of thiswaste such as sugarcane bagasse and groundnut shells can be useful. Especially, protein need is on the increase because of competition between humans and animals.Although, in this study we try to compare two lignocellulose wastes to see which one support growth of *Saccharomyces cerevisiae* more for SCP production (Jaganmoham, et al., 2013; Mondal et al., 2012).Single Cell Protein (nitrogen content) increased significantly ($p < 0.05$) from 3.5% to 9.4% for groundnut shells. While for the sugarcane bagasse, there was also SCP (nitrogen content) increased significantly ($p < 0.05$) from 3.9% to 8.5%. The same period of fermentation was observed for both substrates but groundnut shells gives higher SCP (nitrogen content) compared to sugar cane bagasse. This is evident because the composition of sugarcane bagasse as compare to the composition of groundnut shell. This simply means *Saccharomyces cerevisiae* having access to the monosaccharaides in ground shells more compared to sugarcane bagasse even with the pretreatment (Dhanasekaran et al., 2011; Kutshik et al., 2010). The highest SCP produce by *Saccharomyces cerevisiae* using sugarcane bagasse as substrate was found to be on day five (5) which has the lowest pH and moisture content (see table 1). Whereas, for groundnut shell was on day six (6) with pH of 4.6 and moisture 60% with the lowest reducing and non-reducing sugar (see table 2). The pH range for optimization was selected(4.0, 4.1, 4.5, 4.4 and 6.1) for the fermentation of sugarcane bagasse. *Saccharomyces cerevisiae*yielded high amount of protein at the pH of 4.0 with sugarcane bagasse. The protein content of *S. cerevisiae*in sugarcane bagasse increased gradually from the pH range 4.0 to 6.1 and then decreased rapidly (Table 1). The pH range for optimization was selected (4.6, 3.7, 4.5, 4.6 and 6.2) for the fermentation of groundnut shells. *Saccharomyces cerevisiae*yielded high amount of protein at the pH of 4.6 with groundnut shells. The protein content of *S. cerevisiae*in groundnut shells increased gradually from the pH range 3.7 to 6.2 and then decreased rapidly (Table 2). In this study, we found that groundnut shells gave the mycelial protein in compares' with sugarcane bagasse (Li et al., 2009).

In conclusion, the development of processes for reuse of these wastes is of great interest. Since these wastes are rich in sugars, which are easily assimilated by microorganisms, they are very appropriate for use as raw materials in the production of industrially relevant compounds by fermentation. Products such as food, pharmaceutical and biofuels industries may be produced by fermentation of lignocellulose, by solid-state fermentation systems. Solid-state fermentation systems are interesting option to reuse lignocellulose and have as advantage the no need of raw material fractionation previous the use in the fermentation stage. Besides to serve as low-cost raw materials for the production of important metabolites, the lignocellulose reuse in fermentation processes is an environment friendly method of waste management (Mussatto and Teixeira, 2010).

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