
Production of yeast biomass in maize and millet within Sokoto State, Nigeria

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Abstract: The research was aimed at determining the yield in yeast biomass production using *Saccharomyces cerevisiae* as the test organism. Maize and millet bran were used to enhance the growth of the organism using control and complex media. Biomass yield was determined based on the concentration of the organism in the culture at 24 hours interval for the period of four (4) days. The results obtained for millet in control media were 0.765, 0.922, 0.765 and 0.713 cell densities for the period of 24, 48, 72 and 96 hours respectively. The results obtained for maize in control media were 0.766, 0.927, 0.766 and 0.713 cell densities for the periods of 24, 48, 72 and 96 hours respectively. The results for millet in complex media were recorded as 0.767, 0.925, 0.767 and 0.712 cell densities for the periods of 24, 48, 72 and 96 hours respectively. The results of maize in complex media were recorded as 0.769, 0.928, 0.769 and 0.713 cell densities for the periods of 24, 48, 72 and 96 hours. From the results obtained in this work it could be seen that the maximum yield in yeast biomass was achieved using maize bran which was supported with complex media. Therefore, the use of maize bran for cheaper biomass production in large quantity is highly encouraged.

Keywords: Production, Yeast, Biomass, Maize, Millet

1. Introduction

Vast quantities of agricultural and agro-industrial residues that are generated as a result of diverse agricultural processes represent one of the most important energy rich resources. Accumulation of these biomass in large quantities yearly result not in deterioration of the environment but a huge loss of potential valuable nutritional materials which when processed yield good seed, good variety of chemicals for man and animals. Biomass currently contributes about twenty five percent of the world energy requirement equivalent to twenty million barrels of fuels of oil per day. It is currently the economic force of Brazil and United States where biomass contributes three percent of their total energy. (Bassaria, 2002)

Yeast belongs to the group of organisms called fungi. They are divided into two classes from the phylum Eumycetes. These two classes are Ascomycetes and fungi imperfecti. The organisms are spherical to ovoid in shape with budded cells, some are rounded or elongated and may form pseudomycelium. They possess ascus with four ascospores. Reproduction is by multiple budding or by ascospores

formation which may follow conjugation or may develop from diploid cells when these represent the vegetative stage. The ascospores contain one to four ascus which are usually round or ovate. Some yeast appears creamy while others may be chromogenic. (Crosse *et al.*, 2007). *Saccharomyces cerevisiae* belongs to the class of top and bottom fermented yeast respectively. This is because the top fermented yeast always remains at the top of the medium and the other at the bottom of the fermenter after fermentation. The bottom fermented yeasts are used in making beer, while the top yeasts are employed for leavening of bread, ale, fermentation of wine, alcohol production, glycerol and mutase. Top fermenting yeast sporulates more rapidly than bottom fermenting yeast. The bottom fermented yeast has the ability to ferment raffinose completely, top fermenting yeast ferment only fructose mostly of the trisaccharide and they lack metabolase (Negoda *et al.*, 2009).

Yeast biomass are widely used in good processing feed, drugs and many laboratory culture media. Appreciable amount of yeast biomass are product of brewing industries. Propagation of yeast for biomass production has become a common practice in many part of the world. The choice of

substrates and yeast strains is dependent on locality as well as other consideration its quite reasonable to ensure that isolates are from edible substrate and be non-pathogenic, non-oxygenic and should be able to produce the desired product in large quantity. (Emejuiwe *et al.*, 2000). Yeast are single cell fungi which are eukaryotic in nature. They are good sources of biomass due to their fermentative ability on wide range of substrate such as fruit juices, leavening of bread and by making certain food palatable and nutritive. Today much importance have been attached to their use in a wider range of fermentative process for synthesizing certain vitamins, fat and proteins from simple carbohydrate residues and ammonium nitrogen (Pelezar, 2011). Commercially today in Sokoto market items of yeast desired biomass are on display but little attempt has been made toward utilizing agricultural residues. Considerable records have shown that local strains of yeast and bacteria could be used for possible single cell protein, vitamin production as biomass (Emejuiwe *et al.*, 2000). Of all the yeast tested in the course of study by camel, the *ogi* yeast (Nigerian locally fermented food) produced one of the highest yield of biomass on the substrate *ogi*. Probably on account of novelty of single cell protein, it has met with very strong opposition especially in some countries notably in Japan and Italy. The public in the former country has become aware of the health hazards of environmental pollution and in particular the "minimata disease" which is due to the consumption of contaminated sea food with biomass (Bassaria, 2003). The government was concerned with the possibility of the presence of carcinogenic compound in petroleum grown single cell protein with its limited and non-renewable nature because it was not conventional (Okafor, 2006). The AI-company, which had been working on the single cell protein from petroleum substrate in Italy the concern with over the safe content nucleic acid in protein (SCP), polycyclic aromatic hydrocarbon, fatty acids containing odd numbered carbon skeleton and the presence of a paraffin is carried out from petroleum grown yeast feed to farm animals. (Okafor, 2006).

Since the availability of agricultural residues in Sokoto for biomass production is unquestionable there is the need to explore the ever growing pyramid of agricultural residues as well as perishable agricultural produce for biomass production. This will go a long way to alleviate the problems of storage and environmental pollution posed by these residues.

2. Materials and Methods

2.1. Samples Collection

The samples used for this study includes maize and millet bran. The samples were obtained from milling center in Sokoto central market. Samples were collected in sterile polythene bags and were air dried. One local isolate of yeast organism was used, which was *Saccharomyces cerevisiae*, the test organism was obtained from the stock cultures of Mycology laboratory, of the Department of Biological

Sciences, Usmanu Danfodiyo University Sokoto.

2.2. Samples Preparation and Pretreatments.

Samples of maize and millet were prepared by complete air drying in the sun for 48 hours to completely remove moisture contents. They were milled into fine powder with the use of a mortar and pestle. The powdered material was then sieved with a wire mesh of 100 pores. The samples were then neutralized with hydrochloric acid and finally washed with distilled water. The pre-treated samples were then dried at 65°C in an oven for an hour. (Roy *et al.*, 2002).

2.3. Saccharification of Samples

The pretreated samples were saccharified by acid saccharification with dilute hydrochloric acid under high pressure and temperature. This was achieved by autoclaving at 121°C for 15 minutes. The hydrolyses were filtered with muslin cloth and the hydrolysates were neutralized with sodium hydroxide solution. (Roy *et al.*, 2002).

2.4. Culture Condition for Biomass Production

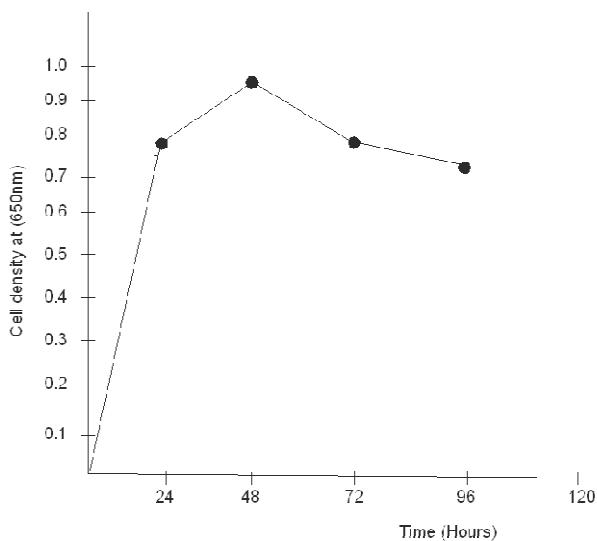
Yeast Nitrogen base glucose broth (a synthetic medium) served as control and complex media consisting of saccharified maize bran and millet bran were used for biomass production. The synthetic medium was prepared by dissolving 6.7g of yeast nitrogen base and 10g of glucose in a liter of distilled water. The contents were thoroughly mixed, filter sterilized and 100ml amount was dispensed aseptically into sterile 500ml Erlenmeyer yeast. The complex media were also prepared with the saccharified samples at a concentration of 10g/litre. The media were supplemented with 0.1% FeNH₄(SO₄)₂, 0.25% (NH₄)₂, HPO₄, 0.3% urea and 0.5% peptone. The supplements were introduced into 100mls of the saccharified samples prior to sterilization. The pH of the media was adjusted to 6.5 and then filtered. The media were then sterilized in 500ml Erlenmeyer flasks. After sterilization, the samples were cooled to room temperature and the Erlenmeyer flask (500 ml) containing each of the medium were inoculated with 1ml aliquot the 24 hours broth culture of the test organisms. The flask was incubated at room temperature on an orbital shake at 650 rpm (revolution per minute) for 4 days. (Roy *et al.*, 2002).

2.5. Determination of Biomass yield

Biomass yield was determined based on the concentration of organism in the cultures at 24 hours intervals for the period of four days. 1ml of the culture sample was collected from each medium and 1:10 dilution was made with sterile distilled water. Same procedures were done to the blank sample containing the saccharified samples. This was used to blank the spectrophotometer and the absorbance of the culture media was measured at 650nm wavelength. The absorbance values were used to derive the cell density (optical density) which represents the yield of the yeast biomass. (Roy *et al.*, 2002).

3. Results

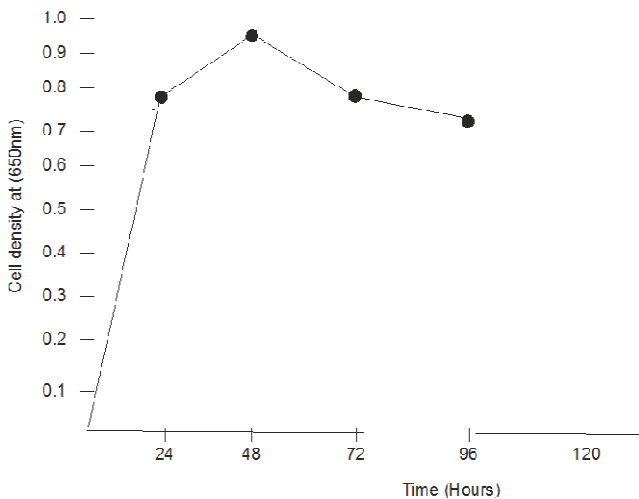
The results obtained from the study are shown in figures 1 to 4.



Cell Density at (650nm)	Time (Hours)
0.765	24
0.922	48
0.765	72
0.765	96

Figure 1. Millet in Control Media (YNB)

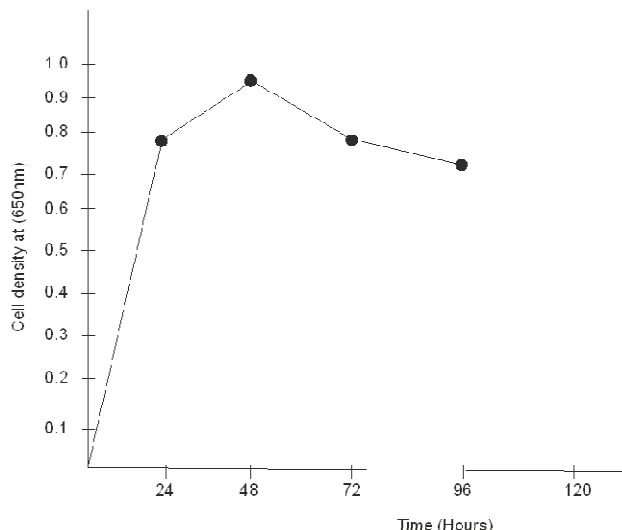
Key:
YNB = Yeast Nitrogen broth
nm= nanometer



Cell Density at (650nm)	Time (Hours)
0.766	24
0.927	48
0.766	72
0.713	96

Figure 2. Maize in Control Media (YNB)

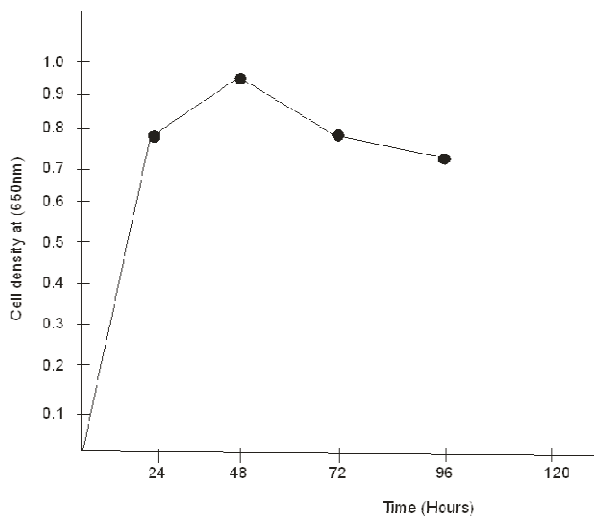
Key:
YNB = Yeast Nitrogen broth
nm= nanometer



Cell Density at (650nm)	Time (Hours)
0.767	24
0.925	48
0.767	72
0.712	96

Figure 3. Millet in Complex Media

Key:
YNB = Yeast Nitrogen broth
nm= nanometer



Cell Density at (650nm)	Time (Hours)
0.769	24
0.928	48
0.769	72
0.713	96

Figure 4. Maize in Complex Media

Key:
YNB = Yeast Nitrogen broth
nm= nanometer

Figure 1 indicated the yield of yeast Biomass using millet bran with control media. A sharp increase in cell density was observed for *Saccharomyces cerevisiae* within period of 24 to 48 hours and a slight decrease in cell density at 72 hours, it

further decrease slightly at 96 hours. The maximum growth of the organism was at 48 hours which was 0.922 cell density, while the minimum growth of the organism was at 96 hours which was at 0.713 cell density. Figure 2 indicated the yield in yeast biomass using maize bran with control media. A sharp increase in cell density was observed for *Saccharomyces cerevisiae* within a period of 24 to 48 hours and a slight decrease in cell density at 72 hours, it further decreased slightly at 96 hours. The maximum growth of the organism was at 48 hours which was 0.925 cell density while the minimum was 96 hours which was 0.713 cell density. Figure 3 indicated the yield in yeast biomass using millet bran with complex media. A sharp increase in cell density was observed for *Saccharomyces cerevisiae* within a period of 24 to 48 hours and a slight decrease in cell density at 72 hours, it further decreased slightly at 96 hours. The maximum growth of the organism is at 48 hours which was 0.928 cell density while the minimum is at 96 hours at 0.713 cell density. Figure 4 indicated the yield in yeast biomass using maize bran with complex media. A sharp increase in cell density was observed for *Saccharomyces cerevisiae* within the period of 24 to 48 hours and a slight decrease in cell density at 72 hours, it further decreased slightly at 96 hours. The maximum growth of the organism was at 48 hours which was 0.928 cell density while the minimum was at 96 hours that is 0.712 cell density.

4. Discussion

From the result obtained it was observed that there is sharp increase in the growth of the organism at 48 hours and this is observed both in the control media (Yeast Nitrogen base Glucose broth) and complex media and also in the millet and maize bran, and the organism attained its highest growth at 48 hours. After the 48 hours the growth of the organism continued the declined in both the control and complex media and also both the maize and millet bran up to 96 hours. The higher growth of the organism was observed in complex media with maize bran in complex media having a cell density of 0.928 at 48 hours while maize bran in control media (Yeast Nitrogen base glucose broth) having a cell density of 0.927 at 48 hours. In the case of millet the complex media has a cell density of 0.925 at 48 hours while the control media containing millet bran has a cell density of 0.922 at 48 hours. The highest growth of the organism is observed in maize bran than in millet bran with maize bran in complex media having the highest growth of 0.928 at 48 hours while the maize bran control media has a cell density of 0.927 at 48 hours which even higher than the cell density of millet in complex media that is 0.925 cell density in 48 hours the least growth is millet with control media that has the cell density of 0.922 at 48 hours.

The increase in the growth of the organism at 48 hours could be due to availability of nutrient present in the substrate and also the availability of carbon and energy source for metabolic process while the decrease could be due to decline in the availability of carbon and energy sources for

metabolic process. The higher growth of the organism in the complex media recorded is due to addition of nutrient supplements to the medium which provide available nitrogen sources for the organism and therefore enhance their growth. Although the nutrient composition of the tested substrates was not determined, the results showed that most of them contained sufficient nutrients for yeast biomass production. Since the Yeast Nitrogen Base Glucose (YNB) is a defined or synthetic medium, it appears that the complex media containing the tested substrate have comparable or better nutritional composition than YNB. The use of agricultural wastes for yeast biomass production has been reported by other workers. (Emejuiwe *et al.*, 2000) reported appreciable yield of yeast biomass using aqueous extract of spoiled and discard oranges, grapes and tomatoes compared to yeast nitrogen base (YNB). (Okafor, 2006) reported comparable and higher growth of yeast using an aqueous infusion of the waste pulp of the locust bean plant (*Parkia SP*) compared to potato dextrose agar.

The effect of pre-treatment by milling reduces the fibrous residues to smaller size making its accessible to acid base hydrolysis. The Sodium hydroxide used in pre-treating the sample gives maximum protein yield and also with high conversion efficiency. He further reported that it does not lead to the formation of toxic compounds that may be harmful to the protein consumer. Acid saccharification of the sample lead to the breakdown of the cellulose and the starchy materials to the assailable glucose utilized by the organism for biomass production. (Gumbria *et al.*, 2011). The metabolic activities taking place on these classes of substrates are fermentative in nature. Since glucose makes the largest portion of the substrates fermentation occurs with the production of energy and other metabolic end products for example ethanol. The glucose generated as a result of acid saccharification is utilized for energy and carbon, which therefore increases the growth of organisms. The introduction of nutrient supplements in the media help to boost yield of biomass compared to non supplemented media (Emejuiwe *et al.*, 2000). The increase in growth observed for most of the substrates used might be due to the fermentative ability of yeast and the presence of the enzymes, for glucose utilization to produce the required energy for growth and reproduction. This study demonstrated yeast biomass production using *Saccharomyces cerevisiae*.

Saccharomyces cerevisiae is dependent on the type of substrate and the need of nutrient supplement in the complex media which is not unusual in industrial fermentation, therefore for large scale production of yeast biomass the use of agricultural residues like corn bran and maize bran in place of the expensive commercially available media such as Yeast Nitrogen Base becomes necessary since agricultural and food processing residues are recognized cheap sources of fermentation substrates, residues arising from agricultural produce could be utilized to support yeast biomass production. This will go a long way in conserving the starch foreign exchange which is used in importing the synthetic commercial media (Roy *et al.*, 2002). The recycling of these residues would minimize

obvious environmental pollution as well as breeding of harmful micro-organisms and potential vectors for disease transmission (Emajuaiwe *et al.*, 2000).

5. Conclusion

From the result of this work it can be seen that maximum yield of yeast biomass was achieved using maize bran which was supported with complex media. Therefore maize bran could be used as alternative cheap substrate for large scale production of yeast biomass after further studies.

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