# MYCELIAL PROTEIN PRODUCTION BY *Aspergillus niger* USING BANANA PEELS

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**ABSTRACT**

The ability of *Aspergillus niger* to produce mycelia protein from pretreated banana peels as substrate was studied with yeast nitrogen base glucose broth as control. Banana peels gave the highest yield of mycelia protein with optical density of 0.28 (O.D) compared to the yeast nitrogen base glucose broth 0.08 (O.D). The residual glucose content in the banana peel medium decreased from 1800 µg/ml on the first day to 320 µg/ml on the 7th day. In the yeast nitrogen base glucose broth, it decreased from 2440 µg/ml on day one to 420 µg/ml on the 7th day. The mycelia protein from banana peals had a crude content of 20.4% and a lipid content of 33.3%. The results indicate the possibility of using banana peels as substrates for mycelia protein production with *A. niger.*

***Keywords:*** Mycelial Protein, Banana Peels, *A. niger*.

## INTRODUCTION

Numerous agricultural residues generated due to diverse agricultural practices and food processing such as rice straw, yam peels, cassava peels, banana peels among others represents one of the most important energy resources. The major components of these are cellulose and hemicellulose (75-80%) while lignin constitutes only 14% (Bowen & Harper, 1989).

Yearly accumulation of these agricultural residues causes deterioration of the environment and huge loss of potentially valuable nutritional constituents which when processed could yield food, feed, fuel, chemicals and minerals (Bisaria, 1991). Agricultural residues when dumped in open environment constitute health hazard due to pollution and support for the growth of microorganisms such as actinomycetes, fungi and bacteria (Barton, 1979). If these residues are industrially developed, a vast bulk of them could be rendered economically useful and could help control pollution and elimination of waste disposal problem.

Recycling of agricultural residue can be achieved naturally and artificially by microorganisms. Aerobic organisms such as fungi, bacteria, and some anaerobic organisms have been shown to be able to degrade some constituents of these residues. Fungi play a significant role in the degradation of cellulose under aerobic conditions (Schlegel, 1999). Microorganisms as potential cell matter are rich in B-group vitamins and in protein that contain essential amino acids. They therefore constitute the potential enrichment for deficient diets.

Increasing world population results in a rising demand for protein for both human and animal consumption. The escalating prices of traditional protein ingredients for animal feeds have intensified the problem (Saquido *et al.,* 1983). There is the problem of finding new source of protein that will not require agricultural land, costly and tedious means of production. The single cell protein has been widely and wisely used to overcome protein shortage in most parts of the world such as Taiwan, India and China. These microbial proteins can be cultivated on industrial wastes as nutrients and can yield a large cell biomass that is very rich in protein (Pelczar *et al.,* 1982)

Microbial protein means microbial cell grown and harvested for use as a protein source for human and animal consumption (Senez, 1987; Frazier & Westhoff, 1988). This microbial protein is referred to as a whole microbial biomass which can be derived from a variety of microorganisms both unicellular and multicellular namely bacteria, yeast, fungi and microscopic algae (Dunlap, 1975).

These potentially important food substances are dehydrated cells consisting of mixtures of proteins, lipids, carbohydrates, nucleic acids and a variety of other non-protein nitrogenous compounds, vitamins and organic compounds. It is a non traditional protein, not a palatable food and must be incorporated directly into other foods (Saquido *et al.,* 1983).

In most developing countries such as Nigeria and Niger, agricultural residues are generated in large quantities yearly constituting environmental pollution problem. This work is therefore undertaken with the objective of producing mycelial protein by *A. niger* using banana peels which is one of the most important agricultural residues.

## MATERIALS AND METHODS

**Sample Collection:** The samples used were banana peels collected in clean polythene bags from the open market in Samaru, Zaria, Kaduna State, Nigeria.

**Sample Preparation and Pretreatment:** The samples were prepared by air dying in the sun to remove the moisture content. The peels were hammer milled to a fine power and sieved with a wire mesh of 80-100 pores (Roy *et al.,* 1993), pretreated through

the combined alkaline and steam by autoclaving the ground peels at 121$^{o}$C for one hr with a swelling agent by refluxing the powder with 0.2M NaOH solution in Erlenmeyer flask (500ml). The autoclaved material was then filtered through a muslin cloth and the residues (substrate) washed thoroughly with distilled water, neutralized with dilute HCL and dried in an oven at 65 $^{o}$C.

**Test organism**: The test organism used was *Aspergillus niger*, obtained from culture collections of Department of Microbiology, Ahmadu Bello University, Zaria. It was maintained on potato dextrose agar (PDA) under refrigerated condition.

**Culturing for mycelial protein production:** Yeast nitrogen base glucose broth (a synthetic medium) served as control. Pretreated banana peels (a complex medium) were used for mycelia protein production. The synthetic medium was prepared by dissolving 6.7 g yeast nitrogen base and 10g glucose in a litre of distilled water. The content was thoroughly mixed, filtered and sterilised and 200 ml dispensed aseptically into sterile 500 ml Erlenmeyer flask.

The complex medium was prepared using the pretreated sample at a concentration of 10g/litre supplemented with 0.1 % $FeNH_4$ $(S04)_2$, 0.25 % $NH_4$ $HP0_4$; 0.3 % Urea and 0.5 % peptone. The pH of the medium was adjusted to 6.5 and 200 ml sterilised in 500 ml Erlenmeyer flask. After sterilisation, samples were cooled to room temperature and 200 ml of each medium were inoculated with 1ml of 72 hrs PDA slant culture of the test organism. The flasks were incubated at room temperature on an orbital shaker at 200 rpm for 7 days.

Biomass yield was determined based on the concentration of the organism in the culture at 24 hrs interval for a period of 7 days. 10 ml of the cultural samples were taken from each sample and a dilution of 1: 10 made with sterile distilled water. Same procedure carried out on the blank sample containing the pretreated sample only. This was used to blank the spectrophotometer and their absorbance were measures at 650 nm. The values were used to represent the cell density (optical density) which represents the yield of the biomass. The cultures were centrifuged at 2500 rpm to obtain the filtrate which was subsequently used for residual sugar determination.

**Determination of Residual Sugar**: Different concentrations of glucose ranging from 300 µg/ml to 3000 µg/ml were prepared. From the stock solution 0.3 g of glucose in 100 ml equivalent to 300 µg/ml, various concentrations were made using sterile distilled water as diluents. To 1ml each, 1ml of 3, 5-dinitrosalicyclic acid reagent (DNSA) was added in test tubes. A blank containing 1ml DNSA and 1ml of water was also prepared. The tubes containing the mixture were heated in boiling water bath for 5 min, 10 ml of distilled water added to the tubes. The absorbances of the coloured reactions were measured using spectrophotometer at 540 nm. The absorbance values in each tube were plotted against the corresponding glucose concentrations serves as the standard curve.

To determine the glucose content in the culture filtrates, the liberated glucose in the filtrate were estimated by 3, 5-dinitrosalicyclic acid (DNSA) method (Raji *et al.,* 1998) as above. The absorbance values were used to estimate the amount of glucose produces in the culture filtrate from the standard curve prepared.

**Determination of Protein Content of the Mycelia Protein:** The mycelia sample was weighed and placed into a Micro-Kjedahl flask and 5 mls nitrogen free concentrated $H_2SO_4$ was added. This was heated to digest for 30-45 min until the content became clear. The digest was allowed to cool and transferred into a volumetric flask. 10 ml was then put into a Micro still flask with 10 ml of 40 % NaOH solution added. The mixture was steam distilled to liberate ammonia into 5 ml of boric acid solution in a conical flask containing 4 drops of mixed indicators methyl red and bromocresol green. The indicator turned green and distillation continued for two more minutes. The distillate was removed and titrated against standard HCl. The end point was reached when a colour change from green to grey to pink was observed. A blank was also run. The amount of acid consumed represents the titer value and is used to calculate percentage nitrogen.

$$\text{Percentage Nitrogen (N \%)} = \frac{(A-B)N \times 14}{W} \times 100$$

Where:

A = Quantity of hydrochloric acid used to neutralize the test sample
B = Quantity of hydrochloric acid used to neutralize blank
W = Weight of the sample in mg
14 = Relative atomic mass of nitrogen
N = Normality of hydrochloric acid
% Protein = % Nitrogen x Protein Factor (6. 25)

**Determination of Lipid Content:** A Soxhlet extractor with reflux condenser and a small (500 ml) round bottom flask was used. The sample was weighed into previously weighed filter paper. The filter paper (W1) and the weighed sample with the filter paper (W2) was folded and placed in the extractor thimble. The petroleum ether was poured into the extractor.

The condenser was placed and joints were tightened. The set up was placed on the heating mantle. The heat source was adjusted so that solvent boiled gently and refluxed several times for 6 hours until the ether extracted all the oil and there was no more fat left. The sample was then placed in an oven at 52 $^{o}$C and dried to constant weight (W3). The percentage lipid (fat) was calculated:

$$\text{\% lipid} = \frac{W2-W3}{W2-W1} \times 100$$

**RESULTS**

Table 1 shows the yield of mycelia biomass using banana peels and yeast nitrogen base glucose broth which was used as control. There was an increasing exponential growth for the first 3 days for the banana peels. It remained stationary for the next 24 hrs before resuming slight growth till it reached the peak on the 7th day with a maximum cell density of 0.28 (OD). There was a higher yield of biomass compared with that of the yeast nitrogen base glucose broth which gave a maximum cell density of 0.08 (OD) on the 7th day.

**TABLE 1. MYCELIAL BIOMASS YIELD FROM BANANA PEELS (GROWTH OPTICAL DENSITY VALUE AT 650 NM)**

| Time (days) | Synthetic Medium | Banana Peels |
|---|---|---|
| 1 | 0.01 | 0.07 |
| 2 | 0.03 | 0.13 |
| 3 | 0.04 | 0.17 |
| 4 | 0.05 | 0.17 |
| 5 | 0.06 | 0.23 |
| 6 | 0.07 | 0.26 |
| 7 | 0.08 | 0.28 |

**TABLE 2. RESIDUAL GLUCOSE CONCENTRATION FROM BANANA PEELS AND SYNTHETIC MEDIUM DERIVED FROM THE GLUCOSE STANDARD CURVE**

| Time (Days) | Banana Peels (Absorbance nm) | Residual Sugar (µg/ml) | Synthetic Medium (Absorbance nm) | Residual Sugar (µg/ml) |
|---|---|---|---|---|
| 1 | 0.31 | 1800 | 0.43 | 2440 |
| 2 | 0.29 | 1640 | 0.40 | 2320 |
| 3 | 0.26 | 1520 | 0.27 | 1560 |
| 4 | 0.21 | 1220 | 0.20 | 1160 |
| 5 | 0.12 | 720 | 0.16 | 940 |
| | 0.09 | 560 | 0.11 | 660 |
| 7 | 0.05 | 320 | 0.07 | 420 |

Table 2 shows the concentration of glucose from banana peels and yeast nitrogen base glucose broth using *A. niger* as inoculum. The glucose concentration from banana peels was 1800 µg/ml which decreases slowly until the fourth day. A drastic decline occurred on the fifth day followed by gradual decrease until the 7th day with a concentration of 320µg/ml.

For the control, the initial concentration of 2440µg/ml was obtained on the first day which drastically reduced up to the 4th day. Thereafter, the concentration decreased gradually until the 7th day with a concentration of 420µg/ml.

**TABLE 3. PROTEIN AND LIPID CONTENT PRODUCED FROM BANANA PEELS**.

| Substrate | % Protein | % Lipid |
|---|---|---|
| Banana Peels | 20.4 | 33.3 |

Table 3 shows the amount of protein and lipid content produced from banana peals by *A. niger.* The product had a crude protein content of 20.4% and a lipid content of 33.3%.

**DISCUSSION**

The substrate tested for mycelia protein production can be used in place of some synthetic commercial media such as yeast nitrogen base glucose broth. There was higher yield of mycelia protein or biomass using banana peels with a cell density of 0.28 (OD) on the seventh day than the yeast nitrogen base glucose broth which gave a cell density of 0.08 (OD). This is because the substrate contains cellulose, starch, pectin which were broken down to glucose and other sample sugars by carbohydrate hydrolyzing enzymes such as cellulase, amylase and pectinase known to be produced by *A. niger* (Anderson *et al.,* 1975).

The persistent growth of the organism was due to the availability of nutrients in the substrate and the stability of the growth after a period of time was due to decline in the available carbon and energy sources for the metabolic processes. Increased growth after stationary phase was due to the fact that the organism was able to utilize its metabolic end products which if accumulated may be inhibitory or toxic to the organism.

The addition of nutrient supplements provided available nitrogen source for the organism thereby enhancing its growth (Emejuaiwe *et al.,* 1988). There was a higher growth in banana peels substrate due to the presence of lipids, proteins, mineral, pectin, vitamins and other soluble carbohydrates which served as sources of nutrients (Bowen & Harper, 1989). Other workers Saquido *et al.,* (1983) reported that since carbohydrates are the main carbon sources for organisms, it would be reasonable to predict that fungal and yeast growth on banana wastes which are largely compose of carbohydrates would be substantial. Essien *et al.,* (2003) reported banana fruit peels could support microbial growth thus it can be

used as substrate for the production of valuable microfungi biomass.

The high residual glucose concentration at the initial growth phase was attributed to increasing activity of starch hydrolyzing enzymes such as amylases, pectinase which converts the substrate to simple sugar. The drastic decrease in the sugar concentration and increase in growth after stationary phase shows that the organism is capable of using its metabolic end products for growth.

Taken the crude protein content of the final product into consideration, the value obtained in this study (20.4%) agreed with the protein content value obtained in the study by Saquido *et al.,* (1983) on banana rejects using *A. niger.* Essien *et al.,* (2003) reported the high protein of mycelia protein on banana peels as an indication that the waste could serve as a possible alternative substrate for the cultivation of fungi. The lipid obtained in this study (33.3%) is high due to the fact that fats are vital to the structure and biological functions of cells and are used as alternative energy source (Essien *et al.,* 2003)

Microfungal protein when used at level of 10.5%, 21.0% and 42.0% for feeding rats and chicks showed results comparable to these obtained in animals fed with casein. The economic feasibility of any mycelia protein process depends on its being able to produce a protein feed supplement of comparable quality at a competitive price with alternative protein feed supplement such as soya beans meal or fish meal (Duthie, 1975).

It is concluded that banana peels offer a good medium for production of mycelia protein and *A. niger* can be used for large scale production of mycelia protein using banana peels as substrate. The technique adopted for measuring the growth performance was adequate when compared to growth on yeast nitrogen base glucose broth. The need to explore organic materials as alternative source of microbial growth medium is essential due to an ever increasing high cost of substrate for microbial cultivation. Banana peel offers a good option, if researches on the possibilities of augmenting its nutritional status are carried out, otherwise the potential of the moulds to utilize the substrate could be harnessed for effective waste management. It is recommended that the study on mycelia protein production by *A. niger* using banana peels should be conducted on a large scale. Extensive toxicological and acceptability tests should be performed before the product is approved for large scale consumption Low technology fermentation must be put in place where agricultural products are produced so that the waste will be directly used for microbial growth. Mixed culture gives high protein content. For high production of protein from mycelia, a mixed culture of micro-organisms is recommended.

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