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Utilization of Banana Peel as a Substrate for Single Cell Protein and Amylase Production by *Aspergillus niger*

*C.E. Oshoma, S.O. Eguakun-Owie and I.S. Obuekwe

Department of Microbiology, Faculty of Life Sciences, University of Benin, P.M.B. 1154 Nigeria.
*Correspondence Author E-mail: cyprian.oshoma@uniben.edu, Tel: +2349099727664

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Abstract: The suitability of banana peel medium for *Aspergillus niger* Single Cell Protein (SCP) and amylase production was studied. Single Cell Protein and amylase production was investigated using media designated as Supplemented Banana Peel (SBP), Glucose Banana Peel (GBP) and Unsupplemented Banana Peel (UBP). Biomass of *A. niger* protein content, amylase production and pH were determined using standard techniques in the course of submerged fermentation. Analyses of the various media showed that SBP medium produced the highest yield of *A. niger* biomass 3.05± 0.03 g/L while the least (2.04±0.01 g/L) was from UBP medium at 6 days of fermentation. The highest yield of protein content (0.68±0.00 g/L) and amylase (728.00±18.20 U/mL) was produced from SBP medium. Statistically, the protein content and amylase yield from SBP medium were significant different from UBP medium ($p < 0.05$). Thus, the study demonstrated that *A. niger* biomass and amylase production can be enhanced through nutrient supplementation of banana peel medium.

Keywords: Single Cell Protein, Amylase, Banana peel, *Aspergillus niger*, Fermentation

Introduction

In Nigeria, enormous quantities of fruits and vegetables are produced annually. The peels generated from fruit processing industries and agricultural practices are discarded into the environment as wastes. Waste generated from the consumption of oranges, banana, pineapple and water melon are considered by a large proportion of the populace as unfit for consumption. A huge quantity of fruit and vegetable wastes (FVW) and by-products from the fruit and vegetable processing industry are available throughout the world. For example fruit and vegetable processing, packing, distribution and consumption in the organized sector in India, the Philippines, China and the United States of America generate a total of approximately 55 million tonnes of FVW. A large proportion of these wastes are dumped in landfills or rivers, causing environmental hazards (Wadhwa and Bakshi, 2013). These discarded peels can also result in the spread of infectious diseases due to the proliferation of pathogenic microorganisms (Yazid *et. al.*, 2017).

Banana peels are rich in organic matters that are natural substrates to support the growth of microorganisms (Essien *et al.*, 2005; Azam *et al*., 2014). The utilization of this cheap available substrate by microorganisms and their conversion into valuable added products such as ethanol, enzymes and animal feeds (SCP) enhances food security and sustainable development (Amande and Itah, 2011; Ghosh *et al*., 2016). The rapidly increasing world population poses serious threat to food security and a challenge of providing necessary food sources. Increasing protein demands and high prices of soyabean meal have further necessitated the search for alternative means of cheap and economic agro residues (Oshoma and Ikenebomeh, 2005).

The term SCP refers to dead, dry microbial cells or total proteins extracted from pure microbial cell culture including that of bacteria, fungi and algae (Anupama and Ravindra, 2001). Microorganisms like bacteria, yeast, fungi and algae utilize inexpensive feedstock and waste to produce biomass, protein concentrate or amino acids. Conventional substrates such as starch, molasses, fruit and vegetable wastes have been used for SCP production, as well as unconventional sources such as petroleum by-products, natural gas, ethanol, methanol and lignocellulosic biomass (Gour *et al.*, 2015). Fungi have been used as sources of protein in specialty food for centuries and *Aspergillus* sp. has been widely used for single cell protein production (Ravinder *et al*., 2003).

Enzymes are defined as biological catalysts, protein in nature and produced by living cells to bring about specific biochemical reactions, generally forming parts of the metabolic processes of the cell. (Abd-Elhalem *et. al.,* 2015; Ali *et. al.,* 2017). Amylases are hydrolytic enzymes which has found its application in a range of industries including food, brewing, detergent, distilling industry, textile, paper pharmaceutical and bioconversion of solid waste (Pandey *et al.,* 2000; Gupta *et al*., 2003). Large range of applications is the triggering factor for the industrialization of α-amylase production. Amylases have been reported to be produced by plant, animal and microbial sources, although the microbial amylase production has been reported to be most effective because of its plasticity and vast availability (Jahir and Sachin, 2011). Use of microorganisms for the production of amylase is economical as microbes are easy to manipulate to obtain enzyme of desired characteristics (Aiyer, 2005). They are mainly employed for starch liquefaction, production of maltose, oligosaccharide mixtures, high fructose syrup and maltotetraose. They are also applied during detergent production to improve cleaning effect and for starch de-sizing in the textile industry (Krishna *et al.,* 2012).

Amylase of fungal origin was found to be more stable than the bacterial enzymes on a commercial scale. Many attempts have been made to optimize culture conditions and suitable strains of fungi (Abu *et al*., 2005). Studies on fungal amylases especially that of *Aspergillus niger* has gained much attention because of their ubiquitous nature, ability to tolerate acidity and non-fastidious nutritional requirement (Abu *et al*., 2005; Kumar and Duhan, 2011; Kumar *et. al*., 2011; Krishna *et al.,* 2012). Ikenebomeh and Chikwendu (1997) reported high amylolytic activity and biomass production using *A. niger*.

Single Cell Protein and amylase production through fermentation processes have the advantage of not being controlled by weather conditions, therefore, they can be carried out in any geographical location (Hafiza *et al*., 2011). This study was aimed at the utilization of banana peel under different nutrient supplementations as a substrate for Single Cell Protein (SCP) and amylase production by *Aspergillus niger*.

Materials and methods

S*ample collection and preparation of fermentation medium*: Banana fruit wastes were collected from the fruit section of Uselu market in Ugbowo area of Benin City, Edo State, Nigeria. These fruit wastes (peels) were washed several times with sterile, distilled water and dried before they were weighed and blended with distilled water in the ratio 1:4. The blended fruit wastes were passed through muslin cloth to trap solids leaving behind the banana peel broth designated as Banana peel medium (BPM) used in this study.

Isolation and Inoculum Preparation of Aspergillus niger: The fungus used for this study was isolated from onions left at room temperature to undergo spoilage. The fungal isolate was identified based on cultural and microscopy characterization following standard methods (Barnet and Hunter, 1972; Larone, 1986) and maintained on potato dextrose agar (PDA) slant and stored at 4ºC. Inoculum was prepared from a subcultured *A. niger* on potato dextrose agar (PDA) plates and incubated for 5 d. The *A. niger* cultured plates were flooded with 10 mL of sterile 1% (v/v) Tween 80 solution to dislodge the spores from the hyphae. The solution with spores was filtered with a sterile muslin cloth to remove any hyphal fragments present (Ikenebomeh and Chikwendu 1997). The number of spores was counted using a haemocytometer and inoculum size of 10^6 spore/mL was used to inoculate all the media.

Fermentation process: Submerged fermentation was carried out at room temperature of 28±2 ºC on an orbital shaker at a speed of 120 rpm using three trial media. The media used were designated as follows: Supplemented Banana Peel (SBP) medium [$(NH_4)_2SO_4$ (2.0 g/L), KH_2PO_4 (1.0 g/L), $MgSO_4.7H_2O$ (0.5 g/L) and $ZnSO_4.7H_2O$ (0.1 g/L) made up to 1 litre with BPM]; the second medium was Glucose Banana Peel (GBP) [Glucose (2.0 g/L) made up to 1 litre with BPM] while the third was Unsupplemented Banana Peel (UBP) medium which contained BPM only. 100 mL of each medium was transferred in to 250 mL Erlenmeyer flasks and autoclaved at 121 ºC for 15 min. The medium in each flask was inoculated with 500 uL of *A. niger* inoculum (10^6 spores/ mL). The media were left to ferment on an orbital shaker at 120 rpm at temperature of 28±2 ºC for 8 d. Fungal biomass, crude protein content, amylase production and pH values were determined at every 2 d interval.

Analytical methods: Determination of fungi biomass, crude protein content, amylase production and pH values were carried out at every 2 d interval for 8 d. For fungal biomass quantification, fermenting broths were pasteurized at 65 ºC for 30 min in a water bath. The fungal mycelia were collected through filtration using a pre-weighed Whatman No. 1 Filter paper and washed twice with 50 mL sterile distilled water. The collected fungal biomass on the filter paper were dried al 90 ºC in a Genlab hot air oven (YIA 110 model, England) to a constant weight. Determination of dried biomass protein content was by a modified biuret procedure of Herbert *et al.* (1971) and Tietz (1986).

Amylase activity was assayed by measurement of glucose released from starch as described by Ramakirshna *et al*. (1982) using a reaction mixture comprising of 1 mL of crude enzyme (from the fermented broth), 1 mL of 1% starch solution and 0.1 mL of citrate buffer solution (pH 4.5). The reaction mixture in a 10 mL test tube was

incubated at 60°C for 1 h and the reaction was terminated by immersing the tube in boiling water (100 °C) for 2 min. The reducing sugars liberated were estimated by the DNS methods (Miller, 1959). One (1) unit of amylase activity (U) was defined as the amount of enzyme that liberated 1.0 µmole of D-glucose from starch in 1.0 µL reaction mixture under the assay conditions. Determination of pH was through the use of pH meter (3305 Jenway, England).

All assays were carried out in duplicates. Means and standard deviations (SD) were determined using SPSS version 23. However, t-test was used for statistical comparison of the data for *A. niger* biomass and amylase production from the different media.

Results

The isolated fungus obtained from onions was related mainly to the generic nomenclature *Aspergillus* known as *Aspergillus niger*. The fermentation was carried out in defined media; Supplemented Banana peel (SBP), Glucose Banana peel (GBP) and Unsupplemented Banana waste (UBP) in order to study the potential of banana wastes as an available substrate for fungal biomass, single cell protein (SCP) and amylase production. From the media there was a gradual increase in pH with time. The highest pH (6.60±0.28) was obtained from GBP at Day 8 of fermentation while the least (6.35±0.07) was from UBP medium.

Table 1: pH of the different media during time course fermentation period at 120 rpm and 28 ± 2ºC

DAY	UBP	SBP	GBP
0	5.35 ± 0.07	5.35 ± 0.21	5.20 ± 0.00
2	5.60 ± 0.00	5.50 ± 0.00	5.50 ± 0.00
4	5.75 ± 0.07	5.75 ± 0.21	5.85 ± 0.07
6	6.00 ± 0.14	6.55 ± 0.07	5.90 ± 0.28
8	6.35 ± 0.07	6.55 ± 0.07	6.60 ± 0.28

Values are means ± standard deviations of duplicate determination. SBP: Supplemented Banana peel medium, GBP: Glucose Banana peel medium and UBP: Unsupplemented Banana peel medium.

The *A. niger* biomass cropped in all the fermentation media is shown in Fig.1. It was observed that biomass increased gradually as the fermentation progresses. The highest biomass was recorded after Day 6 of fermentation in SBP medium (3.05±0.03 g/L) while the least was from UBP medium (2.04±0.01 g/L) and the difference in values were statistically significant ($p< 0.05$).

The protein content of *A. niger* biomass in supplemented SBP medium gave a higher yield (0.68±0.00 g/L) compared to that obtained from GBP medium (0.67±0.05 g/L) on Day 6 of fermentation. On the other hand, the unsupplemented medium (UBP) gave the least protein yield of 0.57 ±0.01 g/L on the same day (Fig. 2).

Amylase production by *A. niger* had a steady increase during fermentation and peaked at Day 4 (Fig.3). The medium SBP gave the highest amylase production of 728.00±18.20 U/mL while UBP medium had the least 253.00±11.11 U/mL and these differences were statistically significant ($p< 0.05$).

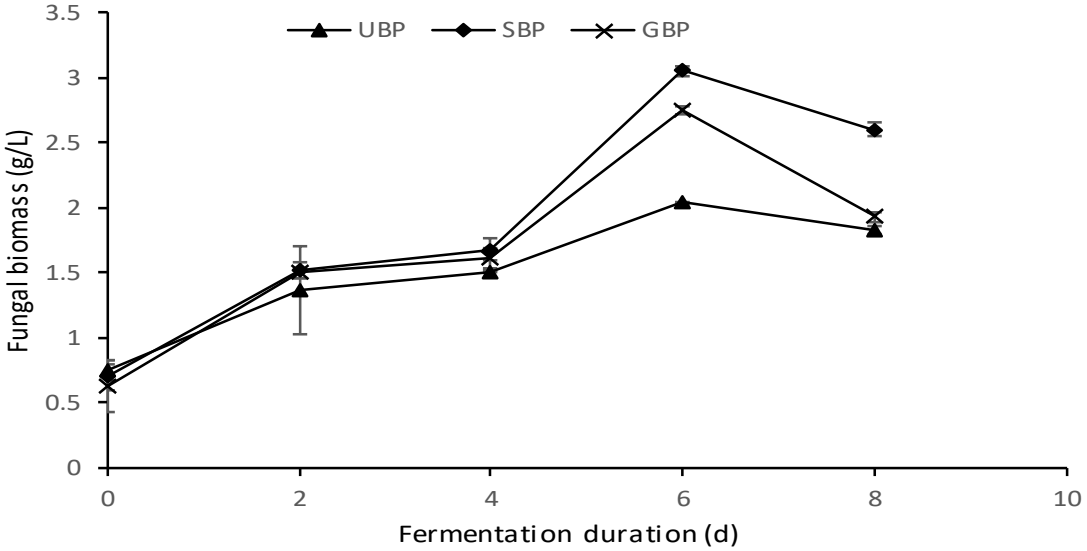

Figure 1: Biomass yield of *Aspergillus niger* produced from the different media during 8 d fermentation pertod. (SBP: Supplemented Banana peel medium, GBP: Glucose Banana peel medium and UBP: Unsupplemented Banana peel medium).

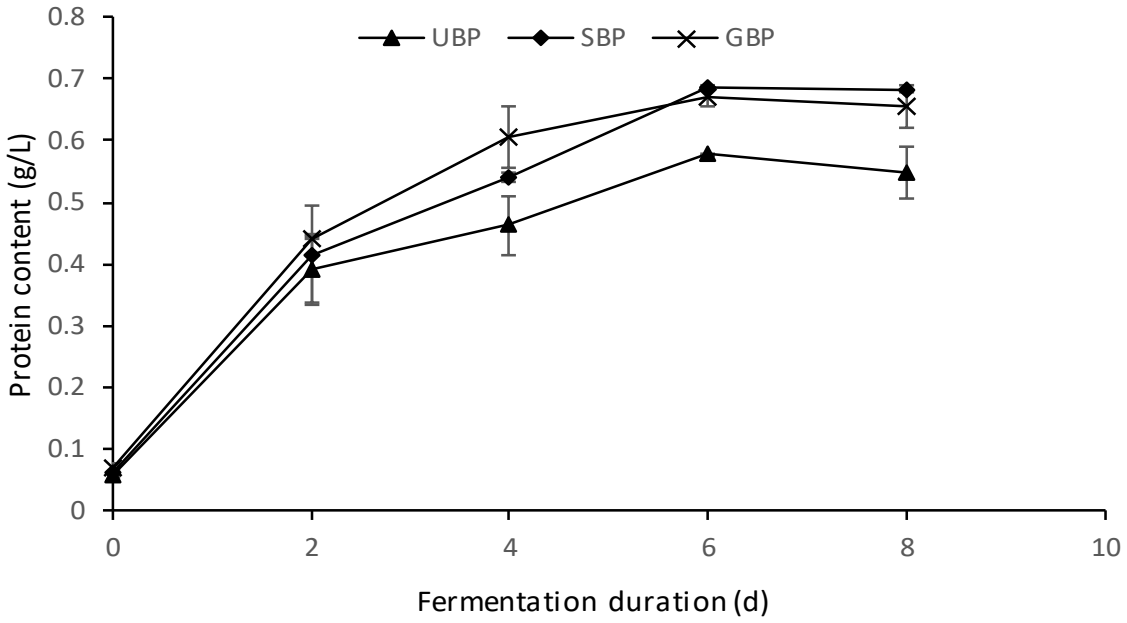

Figure 2: Protein content of *A. niger* biomass produced from the different media during 8 d fermentation period. (SBP: Supplemented Banana peel medium, GBP: Glucose Banana peel medium and UBP: Unsupplemented Banana peel medium).

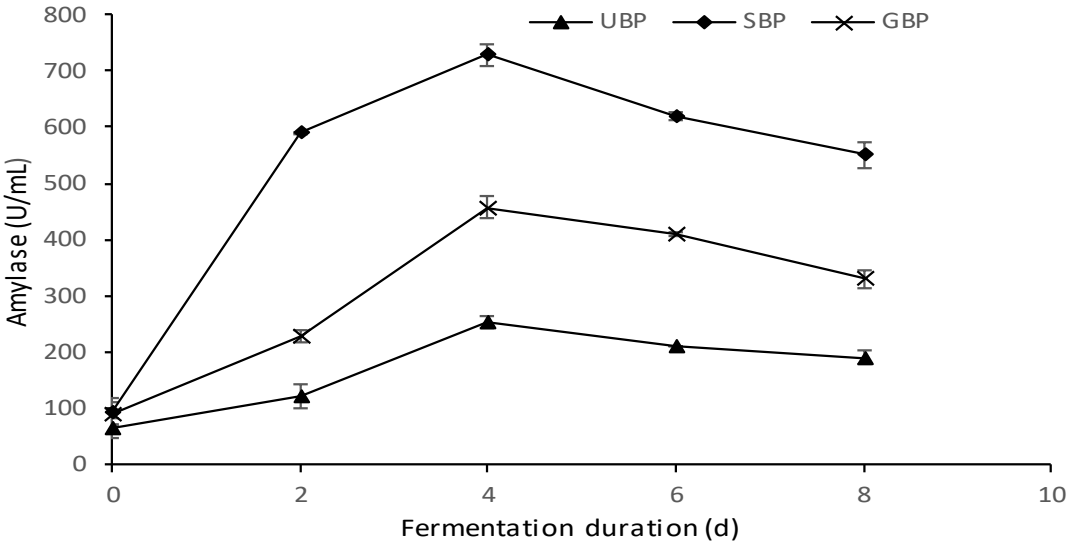

Figure 3: Amylase yield of *A. niger* produced from the different media during 8 d fermentation period. (SBP: Supplemented Banana peel medium, GBP: Glucose Banana peel medium and UBP: Unsupplemented Banana peel medium).

Discussion

In this study, banana peels as food wastes were chosen as a potential substrate for SCP and amylase production. The bioconversion of these substrates is mostly due to their availability and affordability (Rahman *et al*., 2016). Banana peels are one of the locally available nutrient-rich agro-wastes that can be used as carbon and energy sources for fungal growth and subsequent amylase and SCP production (Sankar *et al*., 2011). Researchers have used varieties of fruit wastes such as mango, apple, banana, carrot and orange wastes as a substrates for SCP production (Khan *et al*., 2010; Bacha *et al*., 2011). The utilization of food and other agricultural wastes for fungal biomass production, not only help to combat pollution but could also solve the problem of malnutrition by providing protein supplements to the malnourished individuals at affordable prices. Banana waste has high carbohydrate and protein content that may also have a useful effect on the *A. niger* biomass production (Bacha *et al*., 2011; Mondal *et al*., 2012).

The fermentable sugars from the banana peels were supplemented with minerals for *A. niger* growth thereby producing single cell protein. Amylotic activity and biomass production of *A. niger* was reported to be superior to other *Aspergillus* strains using different agricultural wastes (Yigitoglu, 1992; Oshoma and Ikenebomeh, 2005). Appreciable biomass yield was observed in supplemented banana peels media compared to the unsupplemented medium, highlighting the need for supplementation. Agricultural wastes are usually low in nutrients such as nitrogen (Linde *et al*., 2006). Supplementation of media with nitrogen source and other trace elements for microbial metabolism improve fungal growth thus, increase in protein content. The low biomass and protein content yield observed from UBP medium in this study may have be due to nutrient limitation particularly nitrogen sources required for fungal growth and biomass formation (Mondal *et al*., 2012).

Biomass cropped had maximum yield at Day 6 of fermentation and decreased thereafter. The decrease in biomass yield may be as a result of nutrient depletion in the growth media (Said *et al.*, 2014). Earlier investigations recorded maximum biomass yield after 6 days of fermentation (Khan *et al*., 1992; Oshoma and Ikenebomeh, 2005). In contrast, maximum biomass was recorded after 4 days of fermentation when Brewer spent grain was used as substrate (Aregbosola and Omafuvbe 2014).

The effect of supplementing banana peel medium with inorganic compound was studied to ascertain the impact on amylase production. Supplemented Banana Peel medium was chosen because of the ability to serve as a cheap nitrogen source and other trace elements for *A. niger* growth (Krishna *et al*., 2012). The high nutritional composition of banana peels made it a good source of carbohydrate mostly in starch form (Krishna *et al*., 2012). The peel also contains minerals such as calcium (19.2 mg/g) required for amylase activity, thus making banana peel a good substrate for the production of amylase from *A. niger* (Sankar *et al.,* 2011; Krishna *et al*., 2012; Mishra *et al*., 2016; Salman *et al*., 2016).

Fermentation time for obtaining maximum yield of enzyme is governed by cultural characteristics. Optimum fermentation duration for high amylase yield was 96 h, this was similarly reported by Said *et al*. (2014). After

96 h, there was a gradual decrease in amylase and this may be attributed to depletion in essential nutrients needed for microbial growth and enzyme production.
Among the fermentation parameters, pH of a medium plays a vital role by inducing changes in the microbial morphology and enzyme secretion (Gupta *et al*., 2010). Amylase production is sensitive to changes in pH of the medium. The optimum pH for SCP and amylase production is in the range of 5.0 – 6.0 (Said *et al*., 2014). Similarly, Singh *et al*. (2014) observed that the optimal pH for microbial growth and enzyme production is about 6.0. In contrast however, Varalakhshmi *et al*. (2009) reported that *A. niger* and *A. flavus* had maximum SCP and amylase production at pH between 7 0 – 7,5.

Conclusion

This study revealed that agro-wastes such as banana peels can be safely utilized as substrates for the production of single cell protein and amylase. Supplementation of banana peel medium with minerals enhanced *A. niger* utilization of the substrate. The results showed that *A. niger* can be used as a potential fungal strain for SCP and amylase production. Banana peel was successfully utilized for the enrichment of protein production hence, the possibility of converting the agricultural wastes to proteinaceous food and enzyme. Also, our data confirmed that banana peel medium can be enriched through nutrient supplementation to enhance product yield.

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