

Evaluation of Banana Peels and Pineapple wastes as substrates for Single Cell Protein (SCP) Production.

Onochie C.C¹, Onwa Ndubuisi Collins² and Anichebe, Chioma Ogugua¹

¹Enugu State University of Science and Technology Enugu Nigeria

² Ebonyi State University Abakaliki, Ebonyi Nigeria.

ABSTRACT

Single cell protein (SCP) are microbial cells (primary) algae, fungi, yeast and bacteria or a mixed culture grown in mass culture and harvested for use as protein source for food and animal feed using agricultural wastes as substrate. The production of single cell protein (SCP) using pineapple and banana peel wastes as substrates in the presence of different carbon and nitrogen sources by a local isolates of *Trichoderma viride* was investigated. The methods employed were proximate analysis; growth determination using mycelia dry weight, absorbance and pH as parameters whereas protein yield determination was done using proximate protein analysis. The results revealed that the nutrient found in pineapple and banana peel extract were 7.43% and 9.04% crude protein, 6.72% and 7.68% fibre, 5.04% and 6.61% fat, 40.12% and 48.16% carbohydrate, 5.84% and 6.15% ash. The highest and lowest values for pineapple and banana media supplemented with carbon sources using sodium nitrate as nitrogen sources were cellulose (3.28) - glucose (0.45), cellulose (3.05) - fructose (0.44) mycelial dry weight; maltose (3.620) - mannose (0.275), saccharose (7.590) - maltose (0.918) absorbance; cellulose (7.08) - glucose (6.18), cellulose (7.24) - maltose (6.20) pH and fructose(18.35) - glucose (9.57); saccharose (30.96) - sucrose (13.20) protein yield. Also, the media supplemented with nitrogen sources using sucrose as carbon source revealed: potassium nitrate (1.13) - ammonium oxalate (0.62) and sodium nitrite (1.04) - potassium nitrate (0.71) mycelial dry weight; ammonium oxalate (1.288) - sodium nitrite (0.200) and ammonium oxalate (2.643) - ammonium sulphate (0.155) absorbance; ammonium oxalate (6.61) - ammonium nitrate (5.99) and ammonium oxalate (6.57) - potassium nitrate (6.25) pH and potassium nitrate (20.31) - ammonium nitrate (9.20) and sodium nitrate (27.72) - ammonium nitrate (18.04) protein yield for pineapple and banana extracts respectively after 5 - 7 days incubation period. Banana extract protein yield increase significantly at p (<0.05) than pineapple extract. There was no significant differences detected in the biomass content of both banana and pineapple extracts. The present findings revealed that both banana and pineapple waste could be used as effective alternative carbon and energy source for SCP production but banana offers a better option. Thus, the potential of *Trichoderma viride* to consume the substrates.

Keywords: Single cell protein, banana, microbial cells and substrates.

INTRODUCTION

The growing shortage of protein and other protein rich food supplies has stimulated the effort in searching new and alternate source of protein rich food and feed [1]. For this reason, in 1996, sources mainly yeast, fungi, bacteria and algae named single cell protein (SCP) as coined to describe the protein production

from biomass, originating from different microbial sources [2]. Single cell protein (SCP) represents microbial cells (primary) grown in mass culture and harvested for use as protein sources in foods or animal feeds [3]. The protein obtained from microorganisms such as algae, fungi, yeast and bacteria is cheap and competes

well with other sources of protein and may provide good nutritive value depending, however, on the amino acid composition [4]. The single cell protein (SCP) is a dehydrated cell consisting of mixture of proteins, lipids, carbohydrates, nucleic acids, inorganic compounds and a variety of other non- protein nitrogenous compounds such as vitamins [5].

Microbial biomass has been considered an alternative to conventional sources of food or feed. Large-scale processes for SCP production show interesting features, including: the wide variety of methodologies, raw materials and microorganisms that can be used for this purpose; high efficiency in substrate conversion; high productivity, derived from the fast growth rate of microorganisms and independence of seasonal factors [6] [7].

Initially, yeast was used for human food, but then research has been directed towards using it as a protein supplement in animal feeds due to acute shortage of both soybean and fish meal in many countries [8]; [9] [10].

Various microorganisms used for the production of SCP are bacteria (*Cellulomonas*, *Alcaligenes*, etc.), algae (*Spirulina*, *Chlorella*, etc.), molds (*Trichoderma*, *Fusarium*, *Rhizopus*, etc.) and yeast (*Candida*, *Saccharomyces*, e.t.c.) [11]. Many fungal species are used as protein-rich food. Single cell protein from mixed cultures of *Trichoderma reesei* and *Kluyveromyces marxianus* are reported to contain essential amino acid which compares favourably with FAO guidelines and soya bean meal. They can also provide the B-complex vitamins as well as low level of nucleic acid content. [12]; [13].

Microorganisms can utilize a variety of substrates like agricultural wastes and effluents, industrial wastes, natural gas like methane, etc. so also help in decomposing pollutants [14]. Agricultural wastes are useful substrates for production of microbial protein, but must meet the following criteria: it should be non-toxic, abundant, totally regenerable,

non- exotic, cheap and able to support rapid growth and multiplication of the organisms resulting in high quality biomass [15]. Several studies have been conducted using agricultural waste as a substrate including mango kernel meal [16], Hyacinth bean (*Lablab purpureus*) [17], Leaf meal (*Ipomeoea asarifolia*) [18], Breadfruit (*Treculia africana*) hulls [19], Papaya (*Carica papaya L.*) [20], Rice bran [21], Pineapple waste [22] and Banana waste peel [23]. The use of such a cheap and readily available substrate is desirable to lower the cost of production, reduce waste disposal and management problems, conserve natural resources and provide feed for live stock purpose [24]. Banana (*Musa spp.* AAA or ABB group) are one of the world's most important food crops [25]. Bananas (*Musa spp.*) fruit peel is an organic waste that is highly rich in carbohydrate content and other basic nutrients that could support microbial growth [26]. In Nigeria, it is an important staple crop as well as a source of income for subsistence farmers with large-scale production in traditional humid and sub-humid rain forest areas of Nigeria [27]. Pineapple (*Ananas comosus*), is an important fruit crop leading member of the family *Bromeliaceae* comprises about 2,000 species mostly epiphytic and many strikingly ornamental and varies from nearly white to yellow in color [28]. It is a herbaceous perennial plant which grows from 1.0 to 1.5 m tall with 30 or more trough-shaped and pointed leaves, 30 cm long, surrounding a thick stem. It is a multiple fruit, forming what appears to be a single fleshy fruit [29]. Until recently, about 80% of pineapple produced in Nigeria came from small scale farms managed under mixed cropping systems and current production figures shows that Nigeria is the 6th largest producer of pineapple in the world [30]; [31]. The skin waste was found to contain both carbohydrate and protein nutrients that are suitable and favourable for the growth of microorganisms, [32].

Objectives

- To analyze or determine the chemical composition of the extract.
- To establish the fermentation and bioconversion of the pineapple and banana substrates into single

cell protein (SCP) using carbon and nitrogen sources by *Trichoderma viride*.

- To compare the effects of various carbon and nitrogen sources on the growth and protein yield of *Trichoderma viride*.

MATERIALS AND METHODS

Collection of Soil Samples

The soil samples, containing decaying woods were collected top 2-5cm depth of soil from different corners of Anambra State University, Uli and adjoining areas. Soil samples were mixed together, put in a properly labeled sterilized polyethene bag and taken to the laboratory for microbial analysis [33].

Isolation and Identification of Microorganism

One gram of the soil sample was added to 100ml of sterilized distilled water. This suspension was then subjected to serial dilutions and a dilution of 10^{-5} was attained. One millilitre of each dilution viz., 10^{-3} to 10^{-4} was poured onto Saboraud Dextrose Agar (SDA) and incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 4-5 days. The microbial growth was identified on the basis of their morphological characters. Cultural characteristics comprising growth rate, colour and colony appearance were examined. The microscopic examination of the shape, arrangement and development of conidiophores or phialides, and conidia were made from slide preparations stained with lactophenol-cotton blue. Microbial growth observed was compared with reference materials of [34]; [35] [36]. The purified and identified cultures of *Trichoderma viride* were maintained on Saboraud Dextrose Agar (SDA) agar slants and stored at 4°C for further use. The sub-culturing was done once in every fourth night to ensure proper growth [37].

Inoculum Development

A spore suspension was prepared by adding sterile distilled water to stock culture to get 80×10^6 spores/ml [38].

Preparation of Banana and Pineapple Peels Extracts

500g ripe banana and pineapple fruits were obtained from Coca-cola market,

Onitsha, Anambra Nigeria. The fruits were washed with several changes of sterile water and peeled off. The peels were cleaned initially with 2% solution of H_2SO_4 , cut into small pieces, rinsed in sterile water and pulverized into slurry using a sterilized electric blender. The extracts were obtained from slurry filtered with the help of sterilized sieve. The extracts were placed into a sterile container and proximate composition was determined [39].

Determination of Carbohydrate Content

5ml of the sample was added into a beaker, this was followed by the addition of 15ml perchloric acid. It was stirred continuously for 30 minutes and the mixture was filtered using Whatman No. 1 filter paper. 2ml of the filtrate was mixed with 8ml of Anthrone reagent in a test tube and the absorbance of the mixture was measured using spectrophotometer at wavelength of 620nm. The total soluble CHO was estimated using the standard curve of glucose [39].

Determination of Protein Content

0.9ml of the sample was mixed with 2.7ml of Biuret reagent in a test tube and the mixture was shaken thoroughly and allowed for 5 minutes. The absorbance was determined at wavelength of 540nm against a blank containing Biuret reagent and distilled water but no protein [40].

Determination of Moisture Content

The weight of the dish (W_1) was taken and then the sample was added and the total weight was taken (W_2). The dish and the content was placed in an oven and dried for 4 hours at 125°C and the weight (W_3) was taken. The weight lost was determined by measuring the difference in the initial weight (W_2) and final weight after oven drying (W_3).

Moisture content (%)

$$= \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Determination of Crude Fibre

A given quantity of moisture and fat free sample was treated with 20ml of 1.25% H₂SO₄. This was filtered using Whatman No. 1 filter paper. After filtration and washing, the residue was treated with 1.25% NaOH. Then washed with hot distilled water and diluted with 1% HNO₃, filtered and washed with hot distilled water again. The residue was ignited and the weight was taken as the fibre content of the sample [41].

Determination of Ash Content

According to [42], a given quantity of the sample was added into a clean dried dish of weight (W₁) and the weight of the dish and its content was taken (W₂). The dish and its content was placed in an oven and heated until it completely charred. Then it was further heated for 5 hours at 600°C. Then the weight of the dish plus the content was re-weighed (W₃).

Weight of ash = W₃ - W₁.

Percentage of Ash

$$= \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Determination of Crude Fat

A given quantity of the sample was weighed into a heat stable conical flask and heated at 125°C for 4 hours. The fat content was extracted using solvent extraction method. The heated sample was saturated with acetone, filtered to obtain the filtrate. Then, the filtrate was evaporated to obtain the residue. The residue was dissolved in ethanol and subjected to evaporation to obtain the fat [43]; [44].

Effect of Carbon and Nitrogen

From the banana and pineapple extracts, 100ml each was measured into sterile 250 ml conical flasks. To each different

carbon sources were added i.e. sucrose, glucose, fructose, lactose, starch, mannose, maltose, cellulose and galactose at 3 g/100ml only, sodium nitrate at 0.2g/100ml was used as nitrogen source. Similarly, for studying nitrogen sources i.e. sodium nitrate, potassium nitrate, ammonium nitrate, sodium nitrite, ammonium sulphate and ammonium oxalate at 0.2 g/100ml only, sucrose at 3g/100 ml was used as carbon source supplement. The pH of the medium was adjusted to 6.5 with 0.1M NaOH. The conical flasks were plugged with sterile cotton wool and aluminium foil and autoclaved at 121°C for 15 min. On cooling, the media in the flasks were inoculated with 1ml of inoculum (80 x 10⁶ spores/ml). The flasks were incubated at room temperature (28 ± 2°C) for 5-7 days. The culture broth was separated from mycelium after incubation period by filtration through Whatman No. 1 filter paper [45].

Determination of Final Ph Value

The filtrates of each extracts were poured into beaker after calibration with standard buffer solution. The final pH value of the culture broth was determined using pH meter [46].

Determination of Mycelial Biomass

At the end of incubation, the mycelial biomass was recorded after filtering the mycelial mats on Whatman No 1 filter paper and dried at 60°C for 24 h in a hot air oven.

Determination of Protein

0.9ml of the sample was mixed with 2.7ml of Biuret reagent in a test tube and the mixture were shaken thoroughly and allowed for 5 minutes. The absorbance was determined at wavelength of 540nm against a blank containing Biuret reagent and distilled water but no protein [47].

RESULTS ANALYSIS

The results were expressed as mean ± standard deviation (mean ± s. d.) of three different replicates. Statistical analysis was performed on data generated from the study using microsoft excel and SPSS soft ware. One way analysis of variance (ANOVA) and "T test" analysis were used

to compare differences in mean results of the different sample groups

Results

Test organism

The cultural and morphological characteristics of the *Trichoderma viride* isolated are shown on table 1.

Proximate Analysis

Table 2 shows the results of the chemical analysis of pineapple waste crude extract Table 3\4 shows the results of the chemical analysis of banana waste crude extract

Effects Of Carbon And Nitrogen Sources On Growth And Protein Yield

The results of the effect of different carbon sources on the mycelial dry mass, sporulation, pH and protein yield of *Trichoderma viride* of pineapple and banana extracts. The highest and the lowest protein yield of both extracts were observed in the media supplemented with fructose (18.35%) and glucose (9.57%) for pineapple extract and saccharose (30.96%)

and sucrose (13.20%) for banana extract respectively. Sporulation, pH and biomass yield were also maximum in maltose (3.620) and cellulose (7.08 and 3.28) for pineapple extracts, while that of banana extract were saccharose (7.590) and cellulose (7.24 and 3.05). Poor growth and biomass yield were observed in media supplemented with mannose (0.275) and glucose (6.18 and 0.45) for pineapple extract and maltose (0.918 and 6.20) and fructose (0.44) for banana extract.

The results of the effect of different nitrogen sources on the mycelial dry mass, sporulation, pH and protein yield of *Trichoderma viride* of pineapple and banana extracts are shown in Figure 5 and 6 respectively.

Table 1. Cultural and morphological characteristics of *Trichoderma viride*

Colonial morphology	Microscopy
Fast growing mycelium forming compact cluster or more effuse, light green conidia over the entire medium. A single concentric ring was found around the point of inoculum.	The conidia of <i>T. viride</i> were globose to ellipsoidal and bluish-green colour. Phialides of <i>T. viride</i> are short flask shaped arranged in divergent groups of 2-4. The whole conidiophores system are usually not extensively branched

Table 2: Proximate chemical composition of pineapple extract

Ingredients	Percent (%) / mg/l	
	Pineapple	Banana
Carbohydrate	40.12 ± 0.02	48.16± 0.01
Proteins	7.43 ± 0.01	9.04± 0.02
Fats	5.04 ± 0.02	6.61± 0.03
Moisture	32.14 ± 0.03	21.26 ± 0.02
Ash	5.84 ± 0.01	6.15± 0.02
Fibre	6.72 ± 0.01	7.68± 0.02
Nutritional value (J)	235.56 ± 0.01	288.29±0.01
Total suspended solids	188.50± 0.02	296.25±0.03
Calcium	208.46 ± 0.02	1,045.1± 0.01
Sodium	20.05± 0.02	21.20± 0.00
Magnesium	28.17± 0.01	28.74± 0.02
pH	5.71	7.23

Table 3: Effect of various carbon supplements on growth and protein yield of *Trichoderma viride* on pineapple extract

Carbon sources	Mycelial dry wt. (g)	Absorbance (550nm)	pH	Total protein (%)
Fructose	0.64 ± 0.63	0.530 ± 0.53	6.32 ± 6.32	18.35 ± 18.35
Glucose	0.45 ± 0.45	1.708 ± 1.71	6.18 ± 6.18	9.57 ± 9.57
Mannose	0.64 ± 0.64	0.275 ± 0.28	6.21 ± 6.21	11.46 ± 11.46
Lactose	1.04 ± 1.04	1.317 ± 1.32	6.83 ± 6.83	16.31 ± 16.31
Sucrose	0.52 ± 0.52	0.918 ± 0.91	6.39 ± 6.39	12.05 ± 12.05
Cellulose	3.28 ± 3.28	1.247 ± 1.25	7.08 ± 7.08	10.30 ± 10.30
Saccharose	1.02 ± 1.02	0.596 ± 0.60	6.33 ± 6.33	15.33 ± 15.33
Maltose	1.50 ± 1.51	3.620 ± 3.62	6.42 ± 6.42	12.22 ± 12.22
Galactose	1.24 ± 1.23	0.550 ± 0.55	6.98 ± 6.98	12.11 ± 12.11

Table 4: Effect of various carbon supplements on growth and protein yield of *Trichoderma viride* on banana extract

Carbon sources	Mycelial dry w.t. (g)	Absorbance (550nm)	pH	Total protein (%)
Fructose	0.44 ± 0.44	1.118 ± 1.12	6.50 ± 6.17	29.76 ± 29.79
Glucose	1.84 ± 1.84	1.076 ± 1.08	6.78 ± 6.78	18.88 ± 18.88
Mannose	0.87 ± 0.87	1.483 ± 1.48	6.38 ± 6.39	19.14 ± 19.14
Lactose	0.63 ± 0.63	1.113 ± 1.11	6.50 ± 6.51	18.01 ± 18.01
Sucrose	0.94 ± 0.94	0.985 ± 1.33	6.53 ± 6.53	13.20 ± 13.20
Cellulose	3.05 ± 3.05	1.047 ± 1.04	7.24 ± 7.24	21.28 ± 21.28
Saccharose	0.72 ± 0.72	7.590 ± 7.60	6.68 ± 6.69	30.96 ± 30.96
Maltose	1.64 ± 1.04	0.918 ± 0.92	6.20 ± 6.20	22.55 ± 22.54
Galactose	0.81 ± 0.81	1.276 ± 1.28	7.09 ± 7.09	17.07 ± 17.07

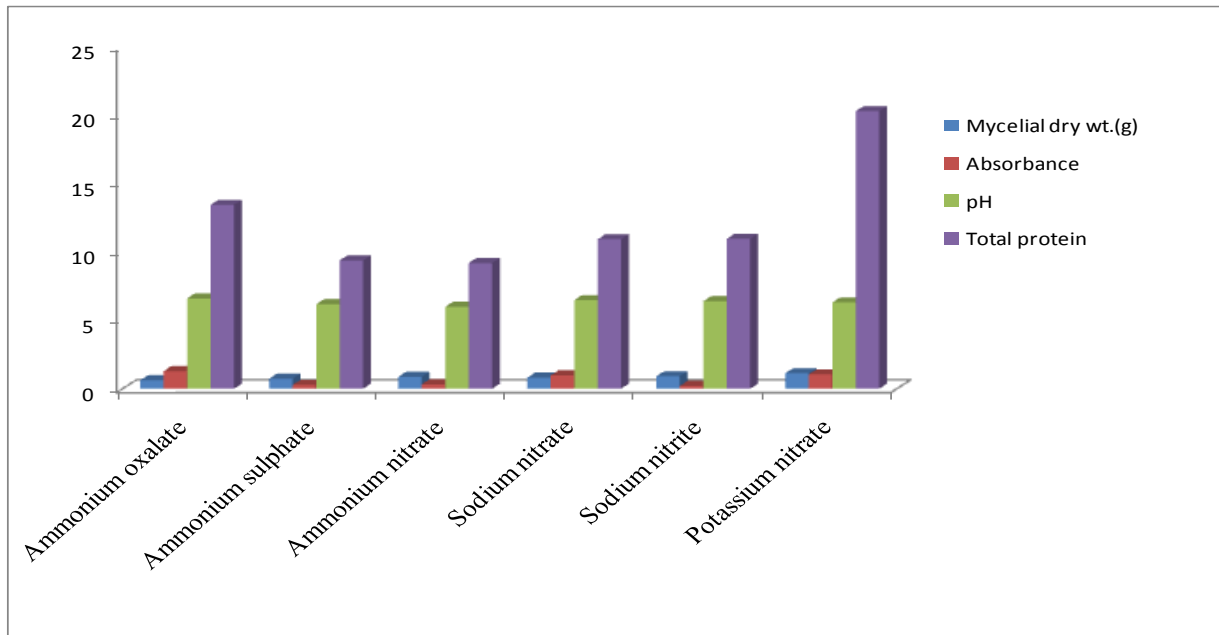


Figure 1: Effect of various nitrogen supplements on growth and protein yield of *Trichoderma viride* on pineapple extract

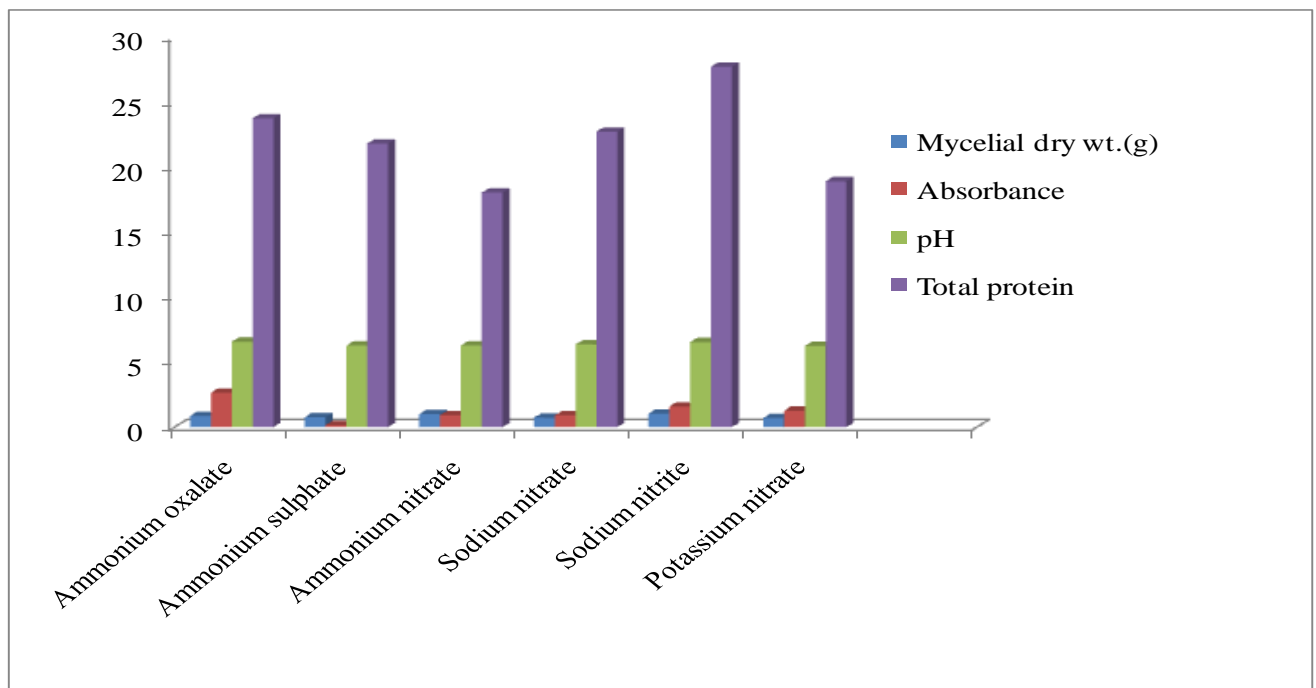


Figure 2: Effect of various nitrogen supplements on growth and protein yield of *Trichoderma viride* on banana extract

DISCUSSION

Banana and pineapple crude extracts contain variable ingredients with major amount of carbohydrates, small amount of protein, lipid and ash (Table 2). The results of banana extract increased significantly at $p (<0.05)$ than the pineapple crude extract. The differences in these results could be attributed to the different types of carbon sources with sacchrose being the most favourable for the growth of the microorganism and production of mycelial biomass. The result agreed with the observation of [48] [49] that pineapple and banana crude extracts contain variable ingredients and may be used as carbon and energy sources for the growth of fungi in the production of single cell protein. The carbohydrate and protein content of banana peels are an indication that the waste could serve as a possible alternative substrate for cultivation of fungi [4].

From the results, there were indications that *Trichoderma viride* had variabilities in the consumption of the different carbon sources of both extracts (Tables 10 and 11). The highest and the lowest protein yield of both extracts were observed. highest growth and protein yield increased significantly at $p (<0.05)$ than pineapple extract but the biomass yield were not significant. These differences could be attributed to the variable nutritional values which could serve as sources of nutrients for the growth of the mould in the production of single cell protein. These findings were in agreement with the observation of [15] that there was a higher growth in banana

CONCLUSION AND RECOMMENDATION

On the whole, the bioconversion effect of pineapple and banana waste into single cell protein was evaluated using *Trichoderma viride*. The supplementation of their extracts with different carbon and nitrogen sources increased significantly the growth and protein yield of *Trichoderma viride*. The highest biomass content of banana extract media was recorded with cellulose as the carbon source and sodium nitrite as the

peels substrate due to the presence of proteins, minerals, vitamins and other soluble carbohydrates which served as source of nutrients.

The media supplemented with potassium nitrate gave the highest protein of 20.31% followed by ammonium oxalate (13.45%) for pineapple extract while sodium nitrite gave the highest protein of 27.72% followed by ammonium oxalate with 23.75% for banana extract (Fig. 5 and 6).

In the same vein, sporulation, pH and biomass yield were also maximum in ammonium oxalate (1.288 and 6.61) and potassium nitrate (1.13) for pineapple extract while that of banana extract were ammonium oxalate (2.643 and 6.57) and sodium nitrite (1.04) respectively. Poor growth and protein yield were observed with media supplemented with ammonium oxalate (0.62), sodium nitrite (0.200) and ammonium nitrate (5.99 and 9.20) for pineapple extract while banana extract have potassium nitrate (0.71) and (6.25), ammonium sulphate (0.155), and ammonium nitrate (18.04). Statistical significant difference at $p (<0.05)$ were detected in the highest growth and protein yield of banana extract than pineapple extract but the biomass yield were not significant. These differences could be as a result of nitrogenous sources which tend to supplement the nutritional status of the extracts and support the growth of *Trichoderma viride*. These findings were in agreement with the report of [38], that the addition of nutrient supplements provided available nitrogen source for the organism thereby enhancing its growth.

nitrogenous source while that of pineapple extract was recorded with cellulose as the carbon source and potassium nitrate as nitrogenous source. The highest protein content of banana extract media was recorded with saccharose as the carbon source and sodium nitrite as the nitrogenous source, while that of pineapple extract was recorded with fructose as the carbon source and potassium nitrate as the

nitrogen source of single cell protein production.

Comparatively, banana extract protein yield increased significantly at $p < 0.05$ than pineapple extract. There was no significant differences detected in the biomass content of both banana and pineapple extracts. The present findings revealed that both banana and pineapple waste could be used as effective alternative carbon and energy source for SCP production but banana offered a

better option. Moreso, the potential of *Trichoderma viride* to consume the substrates could be exploited for effective waste management.

It was recommended that the study on single cell protein production by *Trichoderma viride* using pineapple and banana wastes should be conducted on large scale. Extensive toxicological and acceptability tests should be performed before the product is approved for large scale consumption.

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