Comparative Study on Single Cell Protein (SCP) Production by *Trichoderma viride* from Pineapple Wastes and Banana Peels

Chioma Ogugua Anichebe[a], Bright Obidinma Uba[b], Ebere Linda Okoye[c] and Charles C. Onochie[d]

[a, c, d] Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, P.M.B. 5025 Awka, Anambra State, Nigeria.
[b]Department of Microbiology , Chukwuemeka Odumegwu Ojukwu University P.M.B. 02 Uli, Anambra State, Nigeria.

ABSTRACT:

Single cell protein (SCP) are microbial cells or a mixed culture grown in mass culture and harvested for use as protein source for food and animal feed using agricultural wastes as substrates. 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 isolate of *Trichoderma viride* was investigated. The methods employed were proximate analysis, growth determination using mycelia dry weight, absorbance (sporulation) and pH as parameters whereas protein yield determination was done using proximate protein analysis. The result revealed that the nutrient found in pineapple and banana peel extracts were 7.43 % and 9.04 % of crude protein, 6.72 % and 7.68 % of fibre, 5.04 % and 6.61% of fat, 40.12 % and 48.16 % of carbohydrate, 5.84 % and 6.15 % of 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 g) – Glucose (0.45 g) and cellulose (3.05 g) – fructose (0.44 g) mycelial dry weight; maltose (3.620) – mannose (0.275) and saccharose (7.590) – maltose (0.918) for absorbance; cellulose (7.08) – glucose (6.18) and cellulose (7.24) – maltose (6.20) for pH and fructose(18.35 %) – glucose (9.57 %) and saccharose (30.96 %) – sucrose (13.20 %) for protein yields. Also, the media supplemented with nitrogen sources using sucrose as carbon source revealed: potassium nitrate (1.13 g) – ammonium oxalate (0.62 g) and sodium nitrite (1.04 g) – potassium nitrate (0.71 g) for mycelial dry weight; ammonium oxalate (1.288) – sodium nitrite (0.200) and ammonium oxalate (2.643) - ammonium sulphate (0.155) for absorbance; ammonium oxalate (6.61) – ammonium nitrate (5.99) and ammonium oxalate (6.57) – potassium nitrate (6.25) for pH and potassium nitrate (20.31 %) – ammonium nitrate (9.20 %) and sodium nitrate (27.72 %) – ammonium nitrate (18.04 %) for protein yield on pineapple and banana extracts, respectively after 5 – 7 days incubation period. 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 reveal that both banana and pineapple wastes 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 could also be exploited for effective waste management.

Keywords: Banana peel wastes; pineapple peel wastes; single cell protein; *Trichoderma viride;* waste management

* Corresponding author. Tel.: +2348069693773, +2348065059593.
E-mail address: Ubabright4real@yahoo.com and Ogugua.chioma31@gmail.com

1.1 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 (Khan and Dahot, 2010). For this reason, in 1996, new 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 (Parajo *et al*., 1995). Single cell protein (SCP) represents microbial cells (primary) grown in mass culture and harvested for use as protein sources in foods or animal feeds (Dhanasekaran *et al.,* 2011). 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, upon the amino acid composition (Dhanasekaran *et al.,* 2011). 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 (Dhanasekaran *et al.,* 2011).

Microorganisms (fungal species) can utilize a variety of substrate like agricultural wastes and effluents, industrial wastes, natural gas like methane, etc. that also help in decomposing pollutants (Huang and Kinsella,

1986). Agricultural wastes are useful substrate 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 (Dhanasekaran *et al.,* 2011). Several studies have been conducted using agricultural waste as a substrate including mango kernel meal (Diarra and Usman, 2008), hyacinth bean (*Lablab purpureus)* (Rasha *et al*., 2007), leaf meal (*Ipomeoea asarifolia)* (Madubuike and Ekenyem, 2006), breadfruit (*Treculia Africana)* hulls (Nwabueze and Otunwa, 2006), papaya (*Carica papaya L.)* (Ojokoh and Uzeh, 2005), rice bran (Oshoma and Ikenebomeh, 2005), pineapple waste (Dhanasekaran *et al.*, 2011) and banana waste peel (Sankar *et al*., 2011). Single cell protein from mixed cultures of *Trichoderma Reessei* and *Kluyveromyces marxianus* are reported to contain essential amino acid which compares favourably with FAO guidelines and Soya bean meal (Frazier and Westhoff, 1990; Ghanem, 1992). The yeast, *Sacchromyces cerevisiae* was grown with molasses as the carbon source and ammonium salts as the nitrogen source for the consumption as protein supplement (Litchfield, 1983). 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 livestock purpose (Sanker *et al*., 2011).

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 (Morton, 1987). It is an herbaceous perennial plant which grows to 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 (Idise, 2012). 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 (Fawole, 2008; FAO /World Bank, 1999). The skin waste was found to contain both carbohydrate and protein nutrients that are suitable and favourable for the growth of microorganisms (Dhanasekaran *et al*., 2011).

Banana (*Musa* spp. AAA or ABB group) are one of the world's most important food crops (Ferris *et al.,* 1997). 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 (Sanker *et al*., 2011). 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 (Baiyeric and Ajayi, 2000). Although literatures abounds on the production of SCP using pineapple and banana wastes, there little information on the optimization of production using different carbon and nitrogen sources and hence validate this study. This study was undertaken to compare the single cell protein (SCP) production by *Trichoderma viride* from pineapple wastes and banana peels using carbon and nitrogen sources

1.2 Materials and Methods

1.2.1 Collection of Soil Samples

The soil samples were collected in different points of Chukwuemeka Odumegwu Ojukwu University (COOU) Uli Campus and adjoining areas. Soil samples were collected from different corners of the school containing decaying woods. The samples were collected from top 2 - 5 cm depth of soil and mixed together to make a composite sample. The composite soil sample was placed into sterilized polyethene nylon bag, labelled appropriately and immediately taken to the Microbiological Department Laboratory COOU, for microbial analysis (Mishra *et al*. 2011).

1.2.3 Isolation and Identification of Microorganisms

One gram of the soil sample was taken and added to 1 mL of sterilized distilled water to make a dilution of 10^{-1}. This suspension was then subjected to serial dilutions and a dilution of 10^{-5} was attained. One millilitre of each dilutions viz., 10^{-3} to 10^{-4} were pipetted onto Sabouraud Dextrose Agar (SDA) plates and incubated at 28 ± 2 °C for 4-5 days. They were 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. The pure cultures were compared with known taxa (Oyeleke and Manga 2008; Mishra *et al*. 2011; Shaiesta *et al*. 2012). The purified and identified cultures of *Trichoderma*

viride were maintained on Sabouraud Dextrose Agar (SDA) agar slants and stored at 4 °C for further use. The sub-culturing was done once in every two weeks (Sanker *et al*., 2011).

1.2.4 Inoculum Development

A spore suspension was prepared by adding sterile distilled water to stock culture to get 80 x 10^6 spores /mL (Sanker *et al*., 2011).

1.2.5 Preparation of Banana and Pineapple Peels Extracts

Five hundred grams of ripe banana and pineapple fruits were obtained from the Ihiala Township Market, local vendors and Modern Uli Market, Ihiala Local Government Area, Anambra State, Nigeria. The fruits were washed thoroughly with 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 sterile blender. The peel extracts were obtained from slurry filtered with the help of sterile sieve and the filtrate was evaporated to obtain the crude residue. The crude extracts were placed into a sterile plastic containers and proximate analysis of each samples were determined (Sanker *et al*., 2011).

1.2.6 Nutrient Evaluation of Banana and Pineapple Crude Extracts
1.2.6.1 Determination of Carbohydrate Content

Five millilitres of the samples was added into a beaker, followed by the addition of 15 mL perchloric acid. It was stirred continuously for 30 minutes and the mixture was filtered using Whatman No. 1 filter paper. Two millilitres of the filtrates was mixed with 8 mL of Anthrone reagent in a test tube and the absorbance of the mixture was measured using spectrophotometer (Astell UV- Vis Grating, 752 W) at wavelength of 620 nm. The total soluble carbohydrate was estimated using glucose standard curve (Orji *et al.* 2013).

1.2.6.2 Determination of Protein Content

Zero point nine millilitre of the samples was mixed with 2.7 mL 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 540 nm against a blank containing Biuret reagent and distilled water but no protein (Pearson, 1982).

1.2.6.3 Determination of Moisture Content

Following the standard method of AOAC (1999), 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 hrs at 125 ^{0}C and the weight (W_3) was taken. The weight lost was determined by measuring the difference in weight and percentage moisture content evaluated using the formula below:

$$\text{Moisture content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

1.2.6.4 Determination of Crude Fibre

A given quantity of moisture and fat free sample was treated with 20 mL of 1.25 % H_2SO_4. 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 % HNO_3, filtered and washed with hot distilled water again. The residue was ignited and the weight was taken as the fibre content of the sample (Ismaila *et al*., 2011).

1.2.6.5 Determination of Ash Content

According to Ismaila *et al*., (2011), a given quantity of the sample was added into a clean dried dish of weight (W_1) and the weight of the dish and its content was taken (W_2). The dish and its content was placed in an oven and heated until it completely charred. Then it was further heated for 5 hrs at 600 °C. Then, the weight of the dish plus the content was re-weighed (W_3). The percentage moisture content was evaluated using the formula below:

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

1.2.6.6 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 hrs. 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 (Kwather, 2007; Ismaila *et al*., 2011).

1.2.7 Effect of Carbon and Nitrogen

From the banana and pineapple extracts, 100 mL each were 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 3.0 g/100 mL using sodium nitrate at 0.2 g/100 mL as the only nitrogen source supplement. Similarly, for studying nitrogen sources, 0.2 g/100 mL of sodium nitrate, potassium nitrate, ammonium nitrate, sodium nitrite, ammonium sulphate and ammonium oxalate were added using sucrose at 3.0 g/100 mL was used as the only carbon source supplement. The pH of the medium was adjusted to 6.5 with 0.1 M NaOH. The conical flasks were plugged with sterile cotton wool and aluminum foil and autoclaved at 121 °C for 15 minutes. On cooling, the media in the flasks were inoculated with 1 mL of inoculum (80 x 10^6 spores /mL). The flasks were incubated at 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 (Sankar *et al.,* 2011).

1.2.8 Determination of 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 (Khan and Dahot, 2010).

1.2.9 Determination of Mycelial Biomass

At the end of incubation, the mycelial dry weight were determined after filtering the mycelial mats on Whatman No. 1 filter paper and dried at 60 °C for 24 hrs in a hot air oven. The extent of sporulation was determined by UV Spectrophotometric method (Astell UV - Vis Grating, 752 W) (Waghunde *et al*., 2010).

1.2.10 Determination of Protein

Zero point nine millilitre of the sample was mixed with 2.7 mL 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 540 nm against a blank containing Biuret reagent and distilled water but no protein (Pearson, 1982).

1.2.11 Statistical Analysis of Data

The results were expressed as mean ± standard deviation (mean ± S.D) of three different replicate. Statistical analysis was performed on data generated from the study using Microsoft Excel and SPSS software. One - way Analysis of Variance (ANOVA) and T - TEST analysis were used to compare differences in mean result of the different sample groups.

1.3 Results and Discussion

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 *T. viride* were globose to ellipsoidal and bluish-green colour. Phialides of *T. viride* are short flask shaped arranged in divergent groups. The whole conidiophores system are usually not extensively branched.

Table 2. Proximate chemical composition of pineapple extract

Ingredients	Percent (%) / mg /L
Carbohydrate	40.12 ± 0.02
Proteins	7.43 ± 0.01
Fats	5.04 ± 0.02
Moisture	32.14 ± 0.03
Ash	5.84 ± 0.01
Fibre	6.72 ± 0.01
Nutritional value (J)	235.56 ± 0.01
Total suspended solids	188.50 ± 0.02
Calcium	208.46 ± 0.02
Sodium	20.05 ± 0.02
Magnesium	28.17 ± 0.01
pH	5.71 ± 0.02

Table 3. Proximate chemical composition of banana extract

Ingredients	Percent (%) / mg /L
Carbohydrate	48.16 ± 0.01
Proteins	9.04 ± 0.02
Fats	6.61 ± 0.03
Moisture	21.26 ± 0.02
Ash	6.15 ± 0.02
Fibre	7.68 ± 0.02
Nutritional value (J)	288.29 ± 0.01
Total suspended solids	296.25 ± 0.03
Calcium	1,045.1± 0.01
Sodium	21.20 ± 0.00
Magnesium	28.74 ± 0.02
pH	7.23 ± 0.02

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

Carbon source	Mycelial dry weight (g)	Absorbance (550 nm)	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 5. Effect of various carbon supplements on growth and protein yield of *Trichoderma viride* on banana extract

Carbon source	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

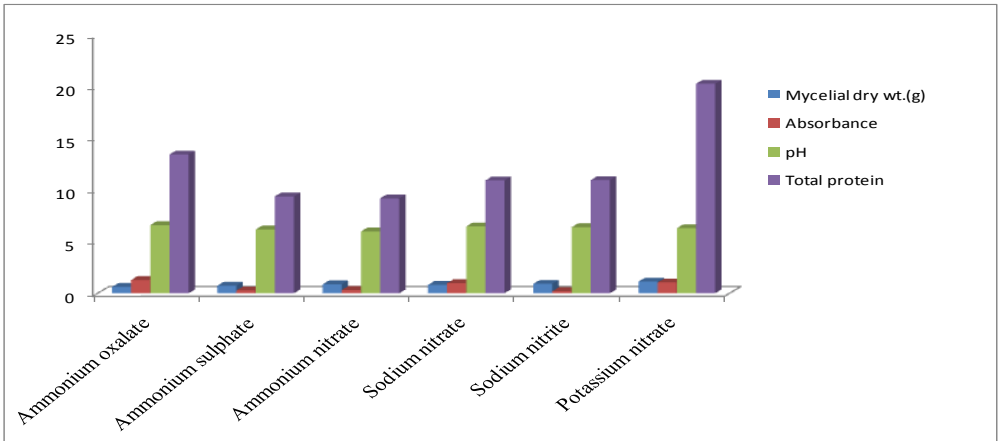

Figure 1: Effect of various nitrogen supplements on growth and protein yield of *Trichoderma viride* on pineapple extract

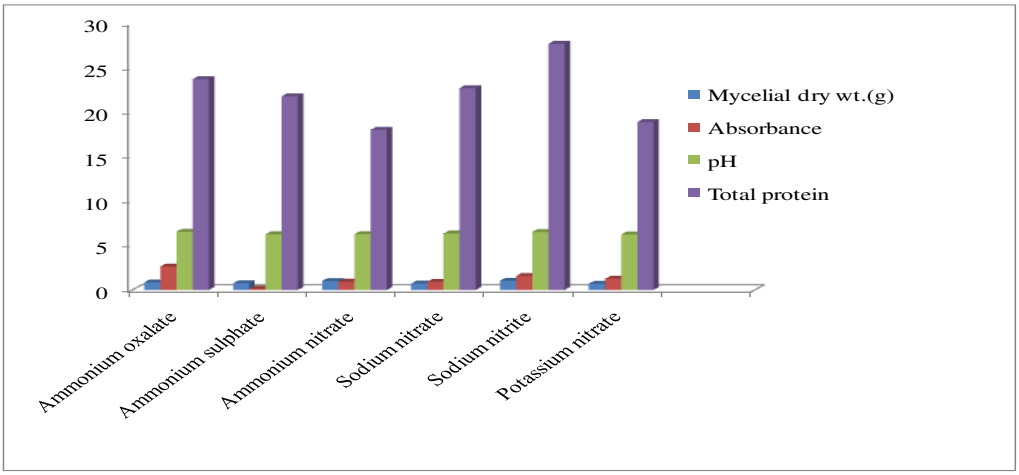

Figure 2: Effect of various nitrogen supplements on growth and protein yield of *Trichoderma viride* on banana extract

The isolated fungal culture (Table 1) was obtained from decaying woods and identified to the generic nomenclature *Trichoderma viride* using atlas of mycological gallery. These characteristics were regarded as taxonomically useful characteristics for *Trichoderma viride.* This organism has been characterized and documented by Sankar *et al*. (2009), Mishra *et al*. (2011) and Shaiesta *et al*. (2012).

The results of the chemical analysis of the pineapple and banana waste crude extracts are presented in Tables 2 and Table 3. From these results, banana and pineapple crude extracts contain variable ingredients with major amount of carbohydrates, small amount of protein, lipid and ash. The result of banana extract increase significantly at p (< 0.05) than pineapple crude extract. The differences in these results could be attributed to the nutritional value with carbohydrate being the most favourable for the growth of microorganism and production of mycelial biomass. The result agrees with the observation of Dhanasekaran *et al*. (2011) and Sankar *et al*. (2011) that pineapple and banana crude extracts contains 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 (Essien *et al*., 2003).

The effects of different carbon supplements on growths and protein yields of *Trichoderma viride* on pineapple and banana extracts are presented in Tables 4 and 5. From the results, there were indications that *Trichoderma viride* had variability in the consumption of the different carbon sources of both 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. Absorbance (sporulation), pH and biomass yield were also maximum in maltose (3.62) and cellulose (7.08 and 3.28) for pineapple extracts, while that of banana extract were saccharose (7.59) and cellulose (7.24 and 3.05). Poor growth and biomass yield were observed in media supplemented with mannose (0.28) and glucose (6.18 and 0.45) for pineapple extract and maltose (0.92 and 6.20) and fructose (0.44) for banana extract. The result of banana extract with highest growth and protein yield increase significantly at $p (< 0.05)$ than pineapple extract but the biomass yield were not significant. These differences could be attributed to the variable ingredients which could serve as source nutrients for the growth of the mould in the production of single cell protein. These findings were in agreement with the observation of Bowen and Harper (1989) that there was a higher growth in banana peels substrate due to the presence of proteins, minerals, vitamins and other soluble carbohydrates which served as source of nutrients.

Also, the results of the effects of different nitrogen supplements on growths and protein yields of *Trichoderma viride* on pineapple and banana extracts are shown in Figures 1 and 2. From these results, 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. In the same vein, the absorbance (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 was 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 Emejuaiwe *et al*. (1998), that the addition of nutrient supplements provided available nitrogen source for the test organism thereby enhancing its growth.

1.4 Conclusion

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 were recorded with cellulose as the carbon source and sodium nitrite as the nitrogenous source while that of pineapple extract were recorded with cellulose as the carbon source and potassium nitrate as nitrogenous source. The highest protein content of banana extract media were recorded with saccharose as the carbon source and sodium nitrite as the nitrogenous source, while that of pineapple extract were 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 pineapple and banana wastes could be used as effective alternative carbon and energy source for SCP production but banana offers a better option. Moreso, the potential of *Trichoderma viride* to consume the substrates could be exploited for effective waste management.

1.5 Recommendation

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

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