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Optimization and Production of Liquid Biofertilizer from Agro-Wastes using Microbial Cells

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ABSTRACT

Biofertilizer is an important byproduct of fermentation and components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. This study is designed to optimize the production of liquid biofertilizer and enhance soil quality and plant productivity through the use of selected microorganisms. The organisms used for the liquid biofertilizer production were *Aspergillus niger, Penicillium chrysogenum, Bacillus cereus, Bacillus licheniformis, Pseudomonas fluorescens* and *Azotobacter chroococcum.* The proximate compositions of the substrates (plantain peel and poultry waste) were ascertained. In the single factor optimization process (w/v); 3% zinc sulphate was the optimum concentration for growth of *Bacillus cereus* and *Pseudomonas fluorescens,* and 4% for *Aspergillus niger, Penicillium chrysogenum, Bacillus licheniformis* and *Azotobacter chroococcum.* With regards to carbon source, 15% of plantain peel was the optimum concentration for growth of all organisms whereas, 1% of poultry waste was the optimum nitrogen source for proliferation of *Pseudomonas fluorescens.* For *Aspergillus niger* and *Bacillus cereus,* 3% of poultry waste was optimal whereas, 4% was optimal for *Penicillium chrysogenum, Bacillus licheniformis* and *Azotobacter chroococcum*. Chemical composition of the fermentation broths including the consortium ranged from 0.019±0.00 - 0.042±0.00 (%) [organic nitrogen]; 0.074±0.00 - 0.119±0.00 (%) [inorganic nitrogen); 0.417±0.00 - 4.510±0.03 (mg/L) [total phosphate]; 409.8±1.80 - 892.8±52.8 (mg/L) [total sulphate]; 77.50±0.01 - 97.73±0.75 (%) [total organic matter]; 217.25±0.22 - 319.82±1.03 (mg/L) [potassium]; 24.13±1.08 - 151.33±0.92 (mg/L) [calcium]; and 30.05±0.06 - 110.15±0.08 (mg/L) [magnesium]. Plantain peel could be used as alternative and cheap substrate for microbial fermentation processes and as a means of converting waste to wealth. The microbial cultures and the rich fermentation broth produced in this study have the potential to enhance plant growth and increase food security, and its affordability and environment friendliness makes it preferable to chemical fertilizer.

Keywords: Liquid biofertilizer, poultry waste, plantain peel, bacteria, fungi, sustainable agriculture.

Introduction

Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants' uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil (Laditi *et al*., 2012). They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants (Ram *et al*., 2014). Very often, microorganisms are not as efficient in natural surroundings as one would expect them to be and therefore artificially multiplied cultures of efficient selected microorganisms play a vital role in accelerating the microbial processes in soil.

Use of biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture (Bákonyi *et al*., 2013).

Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers (Meena *et al*., 2014). The study of Sharma *et al*. (2011), Kaechai and Hyde (2009) and Amal and Heba (2023) deduced that there are groups of bacterial and fungal species that have beneficial effects on plant growth and can be used as biofertilizers and some of these organisms are *Alcaligenes*, *Bacillus*, *Azotobacter, Enterobacter*,

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Pseudomonas; mycorrhizal fungi, *Penicillium*, *Chaetomium* and *Trichoderma,* etc. Agro wastes and some industrial wastes have been utilized as substrates for the production of biofertilizers. For example, animal wastes and plant residues are effective organic materials used for the production of biofertilizer.
Thus, wastes from agro allied industries and other industrial effluents are cheap, rich in nutrient and might have potentials for use as substrate for the production of fertilizer in a large scale; a viable option for sustainable development of the agro industry and protection of the environment (Deng and Tabatabai, 1994). Though, for the commercial production of biofertilizer, carbon, phosphorus and nitrogen sources are necessary for optimum growth and sporulation of the microorganisms, therefore, the carbon to nitrogen (C:N) ratio is of major importance in the optimization of the medium nutrient supply (Dubois *et al.,* 1956).

Furthermore, nitrogen, phosphorus, calcium, etc. could be in complex forms and hence, not bioavailable to plants (Panjanapongchai *et al.,* 2017). Agricultural-biotechnology has to play a major role in producing liquid biofertilizer using effective soil microorganisms that increase the effective absorption capacity in plants, improve soil quality, reduce chemical residues and increase productivity so that perturbed soils can be rehabilitated the natural way ensuring sustainability in soil and the environment (Panjanapongchai *et al.,* 2017). In fact, biofertilizers are formulated products containing one or more microorganisms that enhance the nutrient status and health of the plants by either replacing soil nutrients and/or by making nutrients more available to plants and/or by increasing plant access to nutrients or by producing specific metabolites (Malusa and Vassilev, 2014). The lack of success of biofertilizers to exert their specific functions reflects problems related with production and formulation of the inocula. Thus, this study is designed to optimize the production of liquid biofertilizer and enhance soil quality and plant productivity through the use of selected microorganisms.

Materials and Methods

Bacteria Isolates Used for the Study

The bacterial and fungal isolates previously isolated and molecularly identified by Uzah *et al*. (2024) were used for this study of liquid biofertilizer production. The isolates were identified and assigned with their accession numbers as follows; *Bacillus cereus* (OP970172), *Bacillus licheniformis* (OP970169), *Pseudomonas fluorescens* (OP970170), *Azotobacter chroococcum* (OP970171), *Aspergillus niger* (OP970215) and *Penicillium chrysogenum* (OP970216).

Substrate Collection, Processing and Proximate Analysis

Poultry dropping and plantain peel were used as substrates for biofertilizer production. Poultry waste was collected from a poultry farm in Erema Town, ONELGA, Rivers State while plantain peels were collected from bole sellers in Port Harcourt metropolis. All substrates were transported in a black polythene bag to Industrial Microbiology Laboratory, Department of Microbiology, University of Port Harcourt for processing and analysis.

Poultry waste and plantain peels were air dried for 3–5 days. The substrates were further dry-milled with an electric blender to obtain fine particles and stored in a plastic container under aseptic conditions until they were used. During optimization and production of biofertilizer, the substrates were measured and hydrolyzed by acid and heat methods. Hydrochloric acid was used with the addition of distilled water for the hydrolysis of the substrate and sterilized in an autoclave at 121 $^{\circ}$C for 15 minutes at 15 psi. The method described by A.O.A.C (1995) was used to ascertain the proximate composition of the substrates.

Inoculum Development

Aspergillus niger and *Penicillium* sp. inoculum were prepared according to the method of Odu *et al.* (2020). Spores from a 48–96 hours old slant cultures were used for the inoculation. The suspension of the two (2) fungal isolates were prepared separately by adding 10 ml of sterile water containing 2 drops of 0.1% Tween 80 to the surface of the slant having copious spore growth.

With a sterile inoculating needle, the spore clumps were carefully scraped under aseptic conditions and the tubes were vigorously shaken to obtain a homogenous mixture of the suspension. Then, 5 mL each of the fungal suspension was transferred into 500 mL Erlenmeyer flasks containing 250 mL of yeast extracts and incubated for 96 hours at 28 ± 2 $^{\circ}$C.

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Bacillus licheniformis and *Bacillus cereus* inocula were prepared separately in nutrient broth enriched with salts (gL^{-1} in distilled water) consisting of 0.002 g $FeSO_4$, 0.02 g $ZnSO_4$, 0.02 g $MnSO_4$, 0.3 g $MgSO_4$ and 2 g glucose with the pH adjusted to 7.2. Three loopfuls of each of the bacterial culture were transferred from a 24-hour nutrient agar slant into 250 mL of nutrient broth in 500 mL Erlenmeyer flask. The culture flask containing the inocula was incubated aerobically on a rotary shaker at 250 rpm and at 37 °C until an optical density (OD_{600nm}) of 0.6-0.8 was obtained. Consecutively, *Azotobacter* sp. and *Pseudomonas fluorescens* inocula were prepared separately in Ashby and Cetrimide (supplemented with 10% v/v glycerin) broths respectfully. Three loopfuls of each of the bacterial culture was transferred from a 24 hour- Ashby and Cetrimide agar slant into 250 mL of the broths in 500 mL Erlenmeyer flasks. The flasks were incubated aerobically on a rotary shaker at 250 rpm, and at 37 °C until the culture broth attained an optical density (OD_{600nm}) of 0.6–0.8.

Experimental Design for the Optimization of Biofertilizer Production

One Factor at a Time (OFAT) was used for medium optimization by four (4) levels for each organism with a total number of 24 runs per variable (carbon source, nitrogen source and trace element). The fermentation media contained salts in (g/L); Sucrose (150 g), KH_2PO_4 (2.5 g), $MgSO_4.7H_2O$ (0.23 g), NH_4NO_3 (3.0 g) and the pH was adjusted to a constant using HCL/NaOH based on the growth requirement of the organism. One hundred milliliter (100 mL) of the substrate and 50 mL of the fermentation medium were dispensed into each of the Erlenmeyer flasks (250 mL) and sterilized in an autoclave at 121 °C for 15 min at 15 psi, and allowed to cool at room temperature before inoculation of 15 mL of the inoculum suspension and incubated at 28 ±2 °C (fungal inocula) and 37°C (bacterial inocula) for 7 days.

Determination of Medium Optimization Response

Determination of Cell/Spore Number

The spore density was measured using Nuebauer counting chamber following a modified procedure described by Blessing *et al*. (2018). Using the formula stated in equation 1 below, the number of spores was calculated.

Cell (spore mL^{-1}) = No of Cells / No of Squares x Dilution Factor x 10^4 …… Equation 1

Determination of Cell Biomass

Wet weight measurement method was used in determining the cell biomass of the fermentation broth. This was done by drying in an oven an empty weighing pan made of aluminum foil/eppendorf. They were weighed and stored in a desiccator lined with Drierite (anhydrous $CaSO_4$). Ten milliliters of the broth culture was dispensed into centrifuge tube and the cells were separated from the broth by centrifugation at 10,000 rpm for 5 minutes. The supernatant was carefully discarded and the cell paste was scraped from the centrifuge tube into a weighing pan and the centrifuge tube was rinsed with a small volume of water and poured into the weighing pan, as well. The wet weight of the culture was measured immediately after all the water has been pulled through and expressed in g/L.

Fermentation Technique for the Biofertilizer Production

Batch culture and submerged fermentation technique were used for the production of liquid biofertilizer as adopted by Panjanapongchai *et al.* (2017). A ten-liter capacity plastic container was used as the fermenter containing 4 liters of sterilized processed substrate and 1 liter of fermentation medium containing (g/L); Sucrose (150 g), KH_2PO_4 (2.5 g), $MgSO_4.7H_2O$ (0.23g), and NH_4NO_3 (3.0g). A total of six (6) batch cultures were setup, each containing 100 mL per inoculum. The fermentation medium components were adjusted specifically for each of the microorganisms based on the OFAT optimization model and were carried out for 10 days at ambient temperature.

Quality Analysis of the Biofertilizer

Physico-chemical analysis of the biofertilizer

The temperature and pH of the fermentation broths of the respective set-ups were determined using a pocket-size multiple meter (HANNA® products, USA) at a constant interval of 24 hours all through the period of fermentation. The mean of the readings was calculated and taken as the pH and temperature reading of the batch culture.

Organic and inorganic nitrogen content, phosphorus, potassium, calcium, sulphate, magnesium and total organic matter content were determined by the Kjeldahl method as described by Sluiter *et al.* (2011).

Stabilization of Fungal and Bacterial Spores

The fermentation broths from the different batches of the six (6) microorganisms were aseptically mixed together and the fungal and bacterial spores in the fermentation broth were stabilized to increase the shelf-life of the biofertilizer according to the method adopted by Santhosh (2015). The cell protectants or stabilizers viz., glycerol (0.5%) and polyvinyl pyrrolidone (PVP, 0.5%) were added to the liquid biofertilizer, packaged and stored under ambient temperature for applications. Furthermore, the shelf-life of the liquid was estimated by checking the cell viability quarterly for 12 months.

Results

The proximate compositions of the plantain peel and poultry dropping are shown in Figure 1. The mean value of carbohydrate, protein, lipid, moisture, ash, fibre and nitrogen concentrations (%) of plantain peel are 33.25±0.06, 10.53±0.04, 27.63±0.5, 5.9±0.07, 8.01±0.01, 14.69±0.5. and 1.70±0.02 respectively. While the mean value of carbohydrate, protein, lipid, moisture, ash, fibre and nitrogen concentrations (%) of poultry dropping are 8.01±0.01, 8.33±0.02, 4.88±0.04, 1.2±0.07, 76.55±0.01, 1.03±0.03 and 1.35±0.02 respectively. Statistically, there is significant difference between the proximate compositions of plantain peel and poultry dropping at $p<0.001$ which shows that plantain peel has more proximate content than poultry waste.

The proliferation in microbial counts in fermenters during inoculum development is shown in Figure 2. The result shows an optimum cell population (Log_{10}CFU or spore/mL) of *Aspergillus niger*, *Bacillus cereus, Bacillus licheniformis, Penicillium chrysogenum* and *Pseudomonas fluorescence* at 192 hours of fermentation with a mean value of 6.3±0.01, 6.3±0.00, 6.3±0.01, 6.1±0.01 and 6.4±0.02 respectively while there was a decline of microbial population (CFU or spore/mL) of the above organisms at 240 hours.

Azotobacter chroococcum had a linear progression and optimal population (CFU/mL) at 240 hours of culture with a mean value of 5.3±0.01. Statistically, there is a significant difference at $p<0.001$ which shows that time has great impact on fermentation and microbial cell growth.

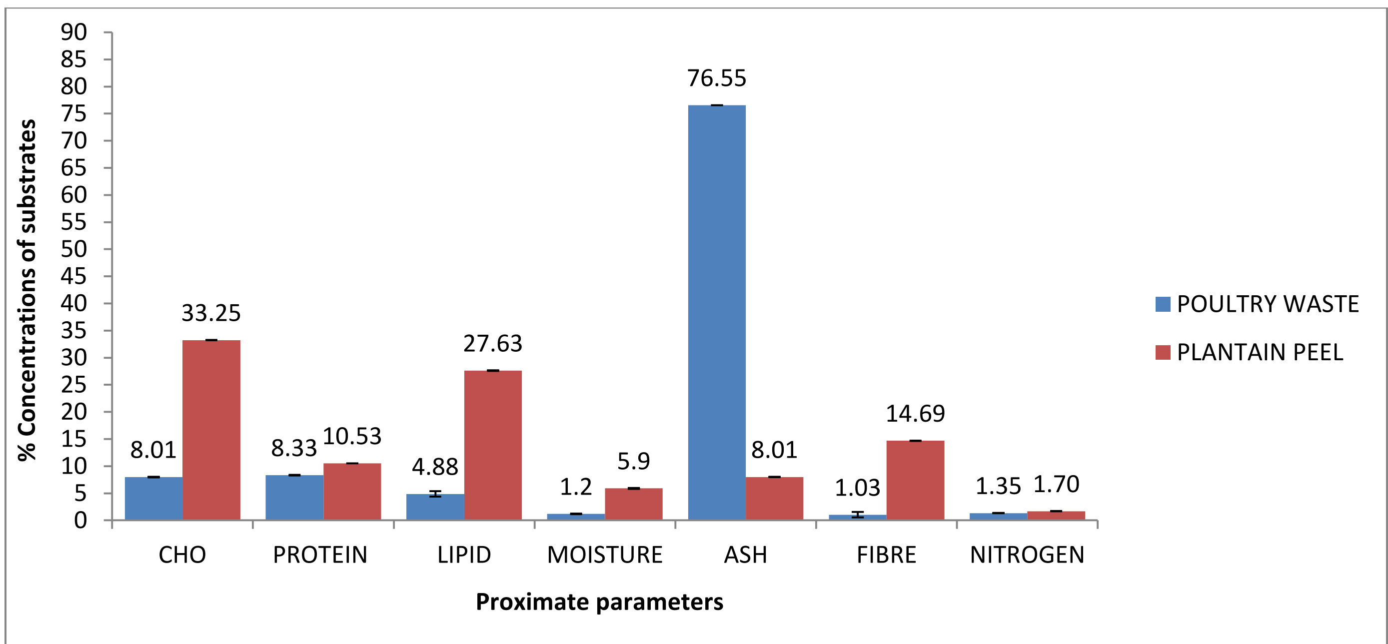

Fig. 1: Proximate composition of Substrates (poultry waste, plantain peel)

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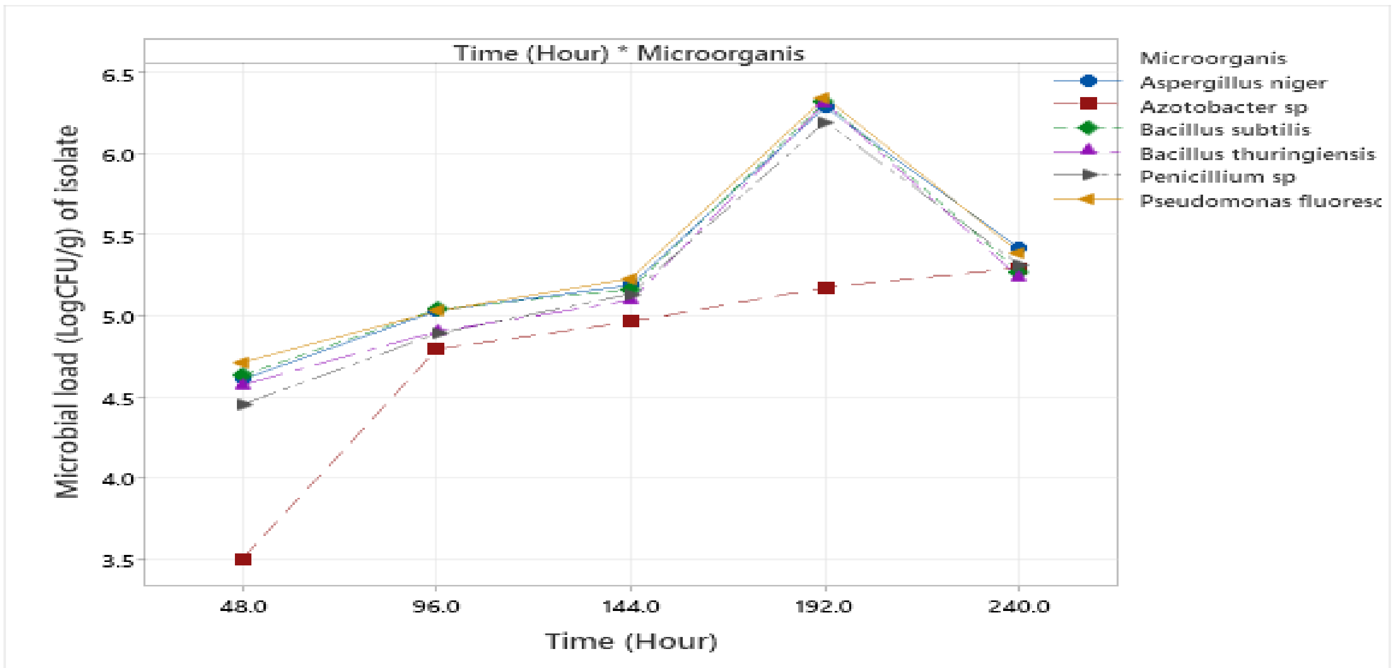

Fig. 2: Microbial cell count during inoculum development of microorganisms

The results of the interaction between the microorganisms and optimization parameters are shown in Figures 3.1 to 5.2. The response of microbial cell counts (Log_{10} CFU or spore/mL) to the trace element (zinc sulphate) indicates that there was a linear increase of microbial population from 1.0% concentration of zinc sulphate. However, 3.0% zinc sulphate concentration gave the optimum microbial population of 5.13±0.03 and 5.43±0.01 (Log_{10} CFU or SFU/mL) for *Bacillus cereus* and *Penicillium chrysogenum* respectively while 4.0% zinc sulphate concentration gave optimum microbial populations (Log_{10} CFU or SFU/mL) of 5.51±0.01, 5.65±0.00, 5.40±0.01 and 4.76±0.01 for *Aspergillus niger*, *Pseudomonas fluorescence, Bacillus licheniformis* and *Azotobacter chroococcum* respectively.

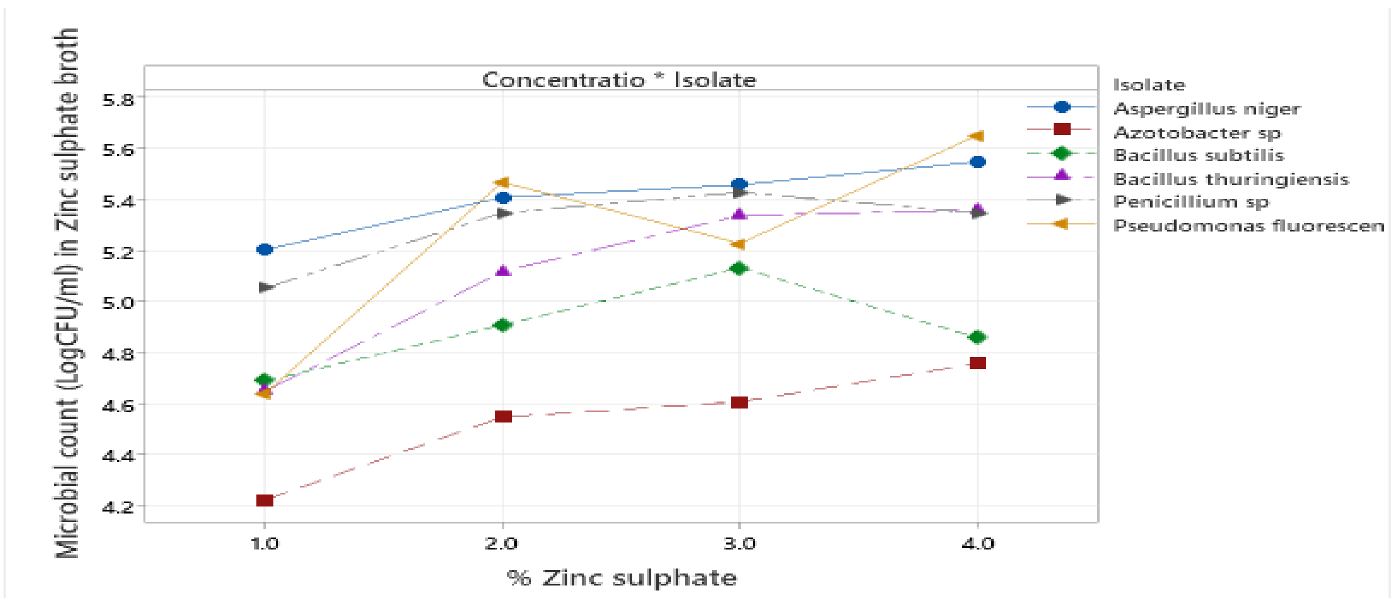

Fig. 3.1 Microbial Cell Count (Log_{10} CFU/ml) using Zinc Sulphate in OFAT Optimization

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Figure 3.2 shows that there was a linear increase in microbial cell biomass (g) from 1.0% concentration of zinc sulphate. However, 3.0% gave optimal microbial cell biomass (g) of 0.57±0.01 and 0.60±0.01 (g) for *Bacillus cereus* and *Penicillium chrysogenum* respectively while 4.0% zinc concentration gave optimum microbial cell biomass (g) of 0.77±0.01, 0.39±0.02, 0.61±0.01 and 0.59±0.02 for *Aspergillus niger, Pseudomonas fluorescence, Bacillus licheniformis* and *Azotobacter chroococcum* respectively. Statistically, there was significant difference of (p<0.001) between counts obtained at various zinc sulphate concentrations which suggests that trace element (zinc sulphate) had a profound impact on the growth of microorganisms in liquid medium.

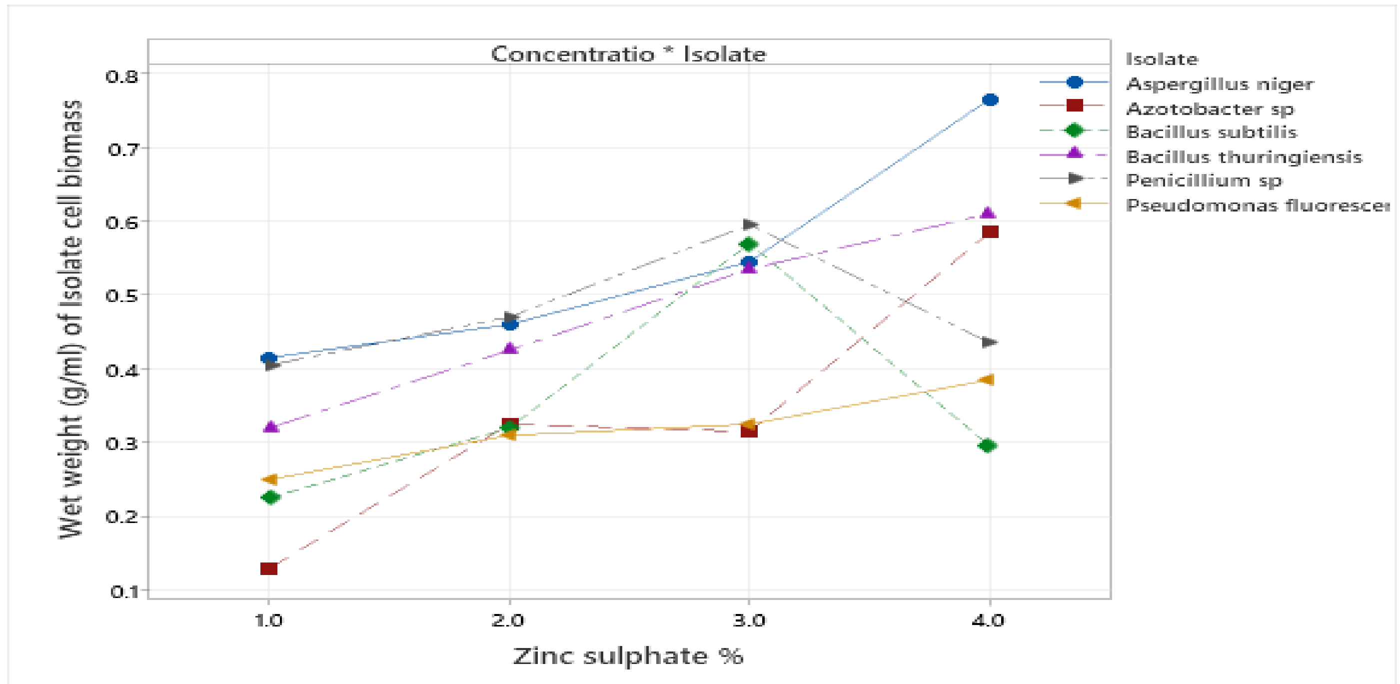

Fig. 3.2 Microbial Cell Biomass (g/10ml) using Zinc Sulphate in OFAT Optimization

Furthermore, Figures 4.1 and 4.2 reveals the response of microbial populations to the provided carbon substrate (plantain peel). The result shows that there was a linear increase in microbial counts from 5.0% concentration of plantain peel with optimal performance obtained at 15% carbon substrate concentration.

As shown in Figure 4.1, at 15% plantain peel substrate concentration, microbial counts (Log_{10} CFU or SFU/mL) of 5.95±0.00, 5.41±0.00, 5.75±0.00, 5.89±0.00 and 5.83±0.00 for *Aspergillus niger*, *Bacillus cereus*, *Bacillus licheniformis, Penicillium chrysogenum*, and *Pseudomonas fluorescence* respectively were obtained whereas, 10.0% substrate concentration gave the optimal microbial counts of 5.19±0.00 Log_{10} CFU/mL for *Azotobacter chroococcum.*

When compared to the control set up, there was a linear increase in microbial cell biomass (g/10mL) from 5% concentration of plantain peel with increase in plantain peel substrate concentration as shown in Figure 4.2. Generally, 15% gave an optimum microbial cell biomass (g/10ml) of 1.34±0.02, 1.28±0.01, 1.05±0.01, 1.63±0.00, 0.71±0.01 and 2.19±0.05 for *Aspergillus niger, Azotobacter chroococcum, Bacillus cereus, Bacillus licheniformis, Penicillium chrysogenum* and *Pseudomonas fluorescens* respectively.

Statistically, there were significant differences (p<0.001) between counts of microbes obtained at various concentrations and substrate concentrations which indicate that the plantain peel substrate had an impact on the growth of microorganisms in liquid medium.

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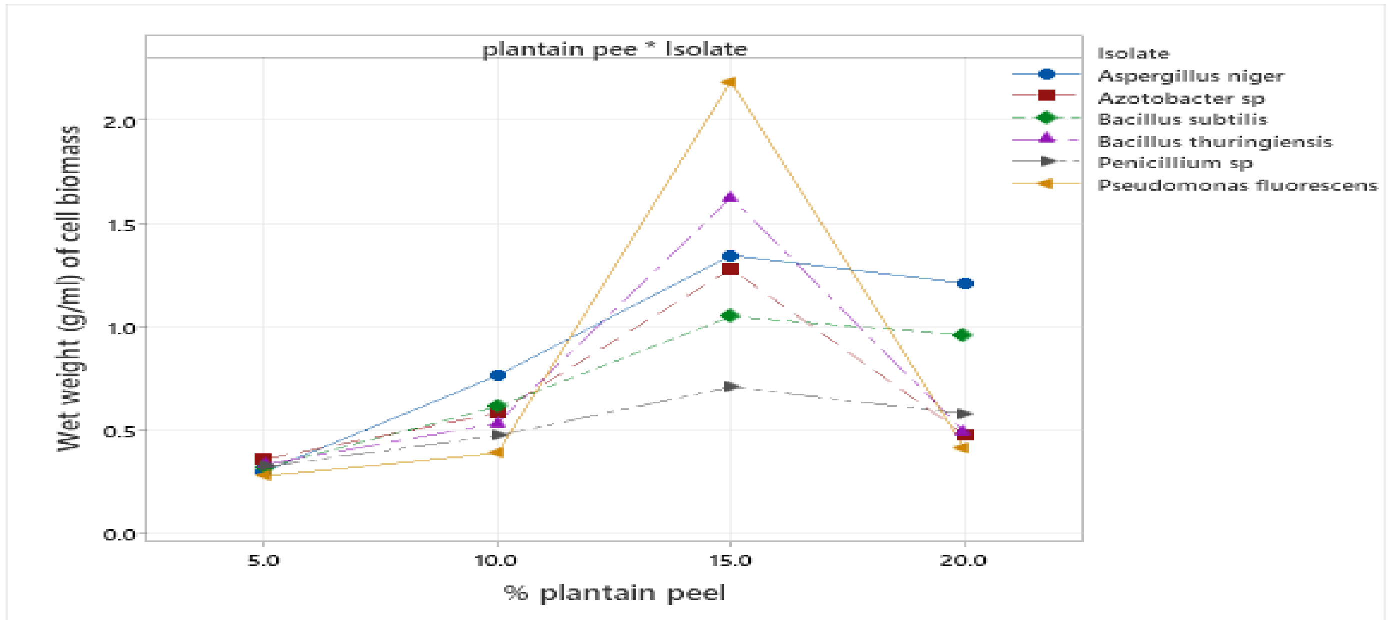

Fig 4.1 Microbial Cell Count (Log_{10} CFU/ml) using Plantain Peel in OFAT Optimization

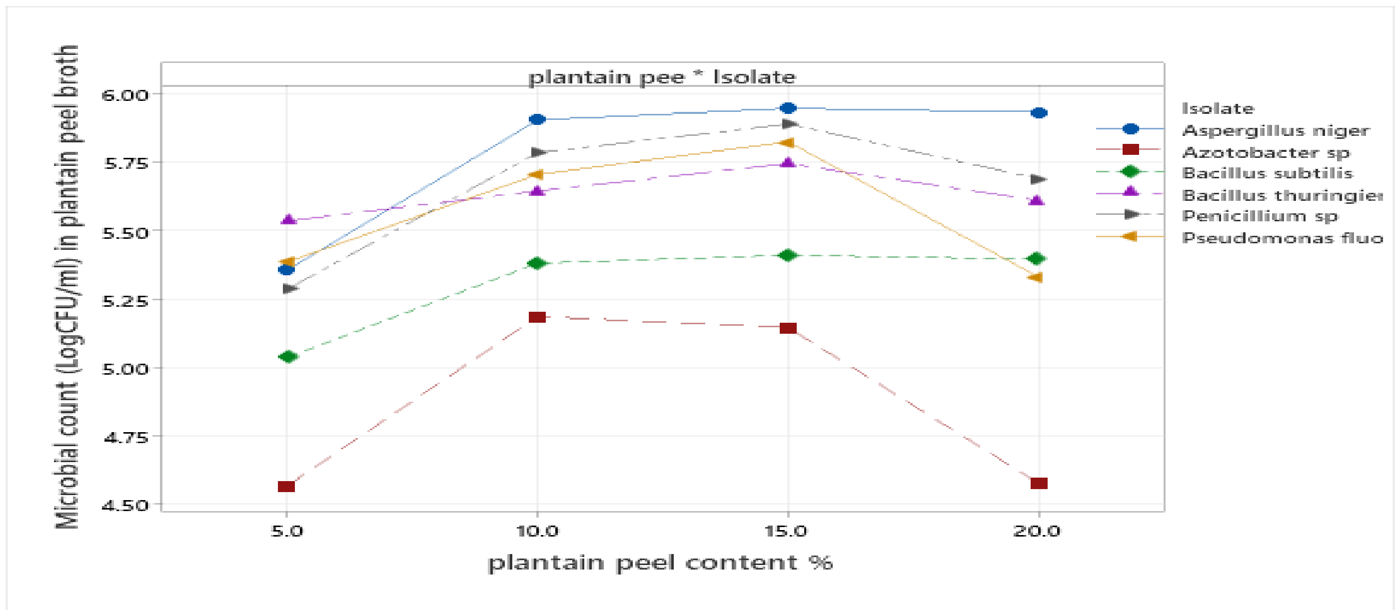

Fig. 4.2 Microbial Cell Biomass [Wet Weight (g/10ml)] using Plantain Peel in OFAT Optimization

The application of poultry dropping (PD) resulted in a linear increase in microbial counts starting from 1.0% PD concentration in which the optimal microbial counts (Log_{10} CFU/ml) of 5.42±0.00, 5.36±0.00 for *Azotobacter chroococcum* and *Bacillus cereus* was achieved at 4.0% PD concentration as indicated in Figure 5.1. At 3.0% PD concentration, optimal microbial populations of 5.55±0.00 and 5.89±0.00 SFU/mL for *Aspergillus niger* and *Penicillium chrysogenum* was obtained; for *Bacillus licheniformis* and *Pseudomonas fluorescence*, 1.0% PD concentration gave optimal microbial counts (Log_{10} CFU/ml) of 4.91±0.00 and 5.48±0.01 respectively. The impacts of PD concentrations on the microbial cell biomass (g/10mL) were different from that of cell count.

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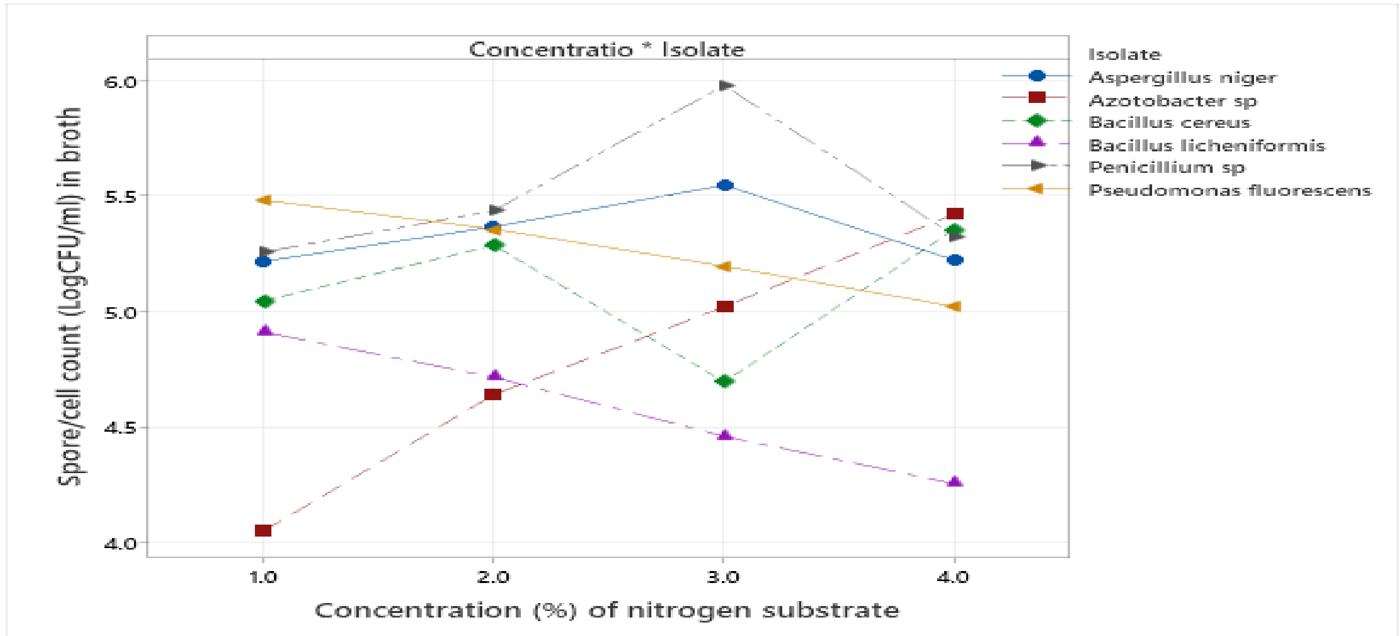

Fig 5.1 Microbial Cell /Spore Count (LogCFU/ml) using Poultry Waste in OFAT Optimization

Figure 5.2 show that 4.0% gave an optimal microbial cell biomass (g/10m) of 0.61±0.01, 0.52±0.01 and 0.52±0.00 for *Azotobacter chroococcum, Bacillus licheniformis* and *Penicillium chrysogenum* whereas, 3.0% gave an optimum of 0.55±0.00 and 0.37±0.00 for *Aspergillus niger* and *Bacillus cereus* respectively. However, there was a linear decrease of cell biomass of *Pseudomonas fluorescens* with 1.0% concentration of PD at 0.77±0.02 g/10ml.

Statistically, there were significant differences (<0.001) between data obtained for microbial counts at various PD concentrations which shows that the nitrogen source (PD) had an impact on the growth and proliferation of microorganisms in liquid medium.

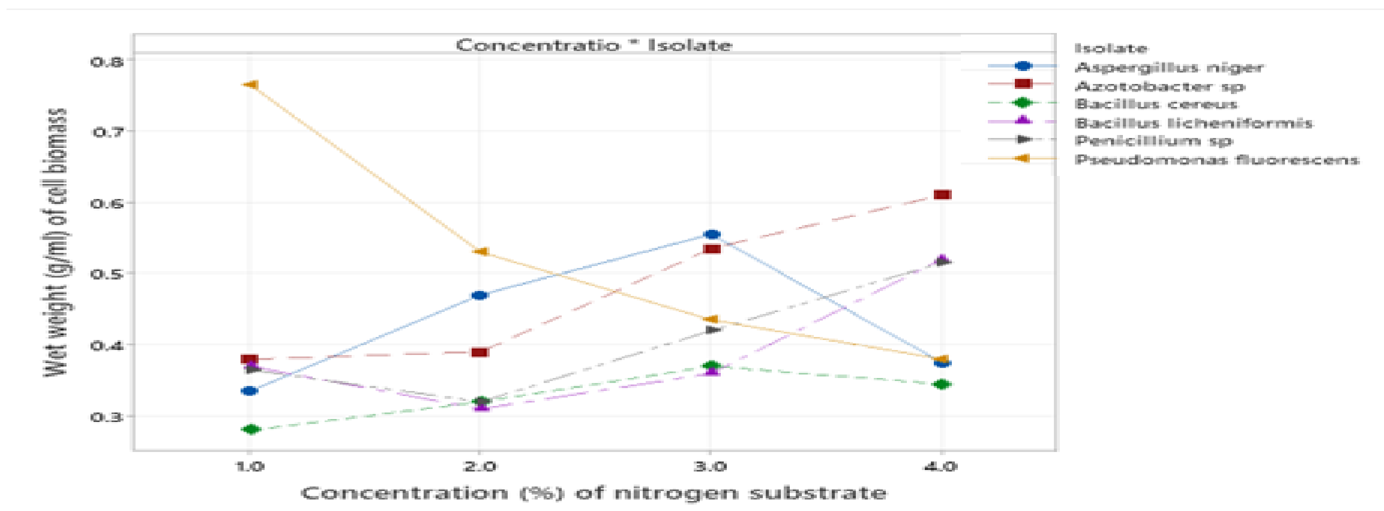

Fig. 5.2 Microbial Cell Biomass [Wet Weight (g/10ml)] using Poultry Waste in OFAT Optimization

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The viable cell count during the large scale production of liquid biofertlizer as shown in Figure 6.1. The result indicates an exponential increase of microbial cell count (Log_{10} CFU/ml) from 0 hour to 168 hours of fermentation in which 168hours of fermentation gave a peak viable microbial cell count of 6.55±0.00, 6.93±0.00, 6.79±0.00, 6.81±0.00, 6.62±0.00 and 6.93±0.00 for *Aspergillus niger, Azotobacter chroococcum, Bacillus cereus, Bacillus licheniformis, Penicillium chrysogenum* and *Pseudomonas fluorescens* respectively. Statistically, there was a significance difference of P<0.001 which shows that time or fermentation period have great impacts on microbial growth. Furthermore, Figure 6.2 demonstrates the microbial cell biomass of the different fermentation broths of the liquid biofertilizer production.

The result reveals that *Penicillium chrysogenum* had the lowest cell biomass of 1.3 g/10ml at 168 hours of fermentations while *Pseudomonas flourescens* had the highest cell biomass of 2.1g/10ml at 168 hours of fermentation. The pH variations of the fermentation broth as shown in figure 6.3 indicate a linear decrease of pH value from the initial as fermentation period progresses. At 168hours of fermentation, the pH values were 4.05±0.00, 4.25±0.00, 5.05±0.00, 5.30±0.00, 4.40±0.00 and 5.15±0.00 for *Aspergillus niger, Azotobacter chroococcum, Bacillus cereus, Bacillus licheniformis, Penicillium chrysogenum* and *Pseudomonas fluorescens* respectively. Statistically, there is significance difference of P<0.001 which shows that the reduction of pH is directly proportional to fermentation period in a fermentation process.

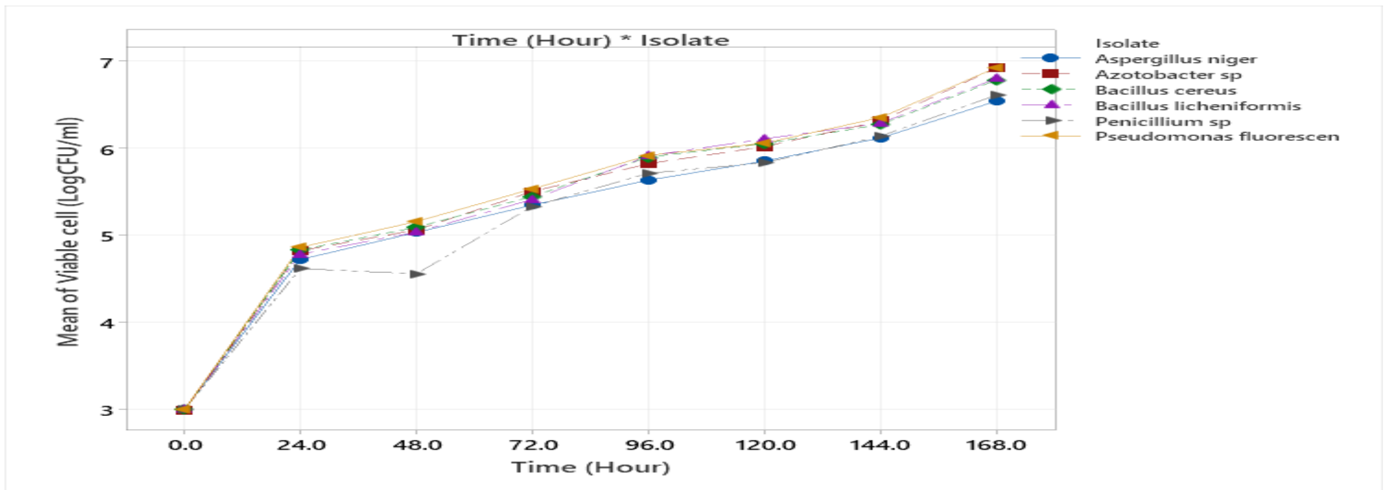

Fig. 6.1: Viable Microbial Cell Count (Log_{10}CFU/ml) for Large Scale Liquid Biofertilizer Production

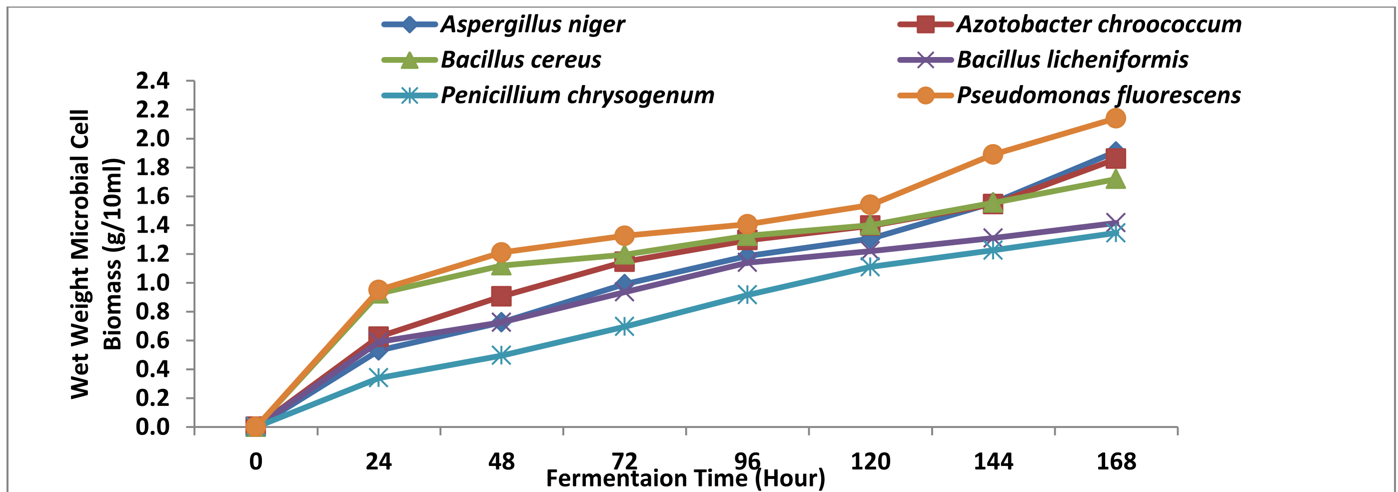

Fig. 6.2 Microbial Cell Biomass (g/10ml) of Large Scale Liquid Biofertilizer Production

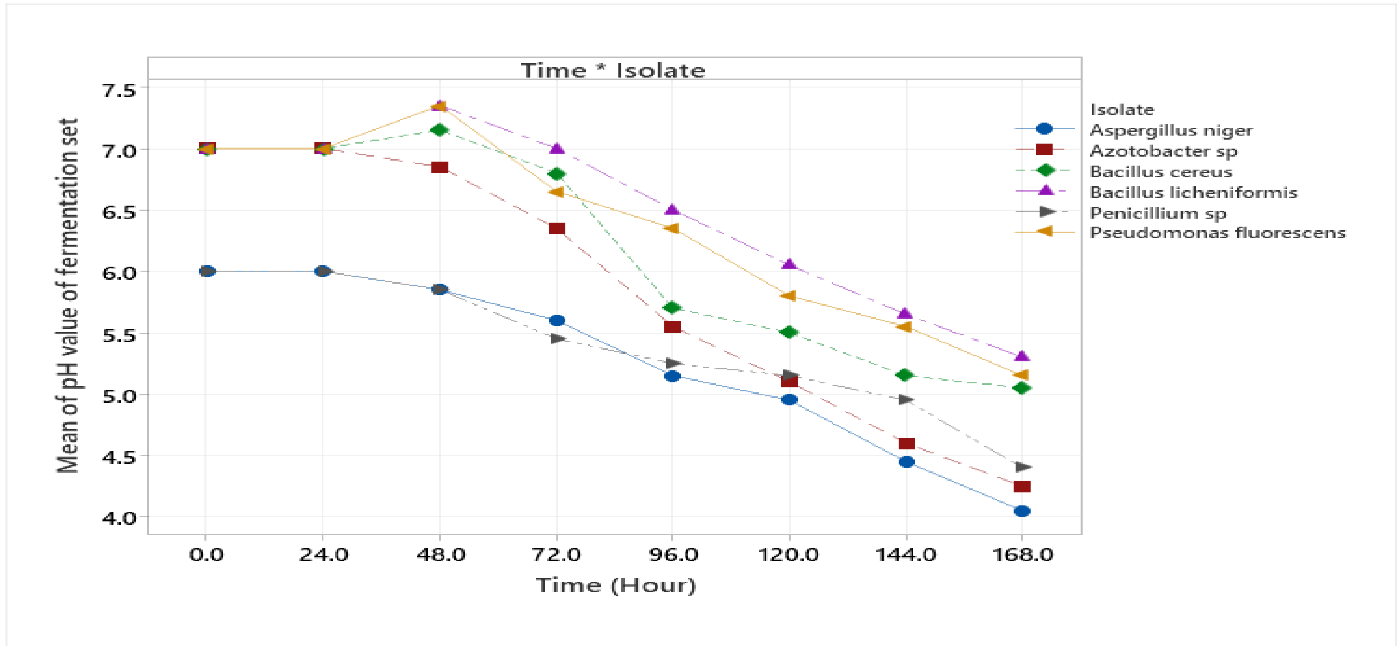

Fig. 6.3: pH Variation for Large Scale Liquid Biofertilizer Production

Figure 7 demonstrates the stability and viability of the microbial cells of the liquid biofertilizer. The result reveals that the liquid biofertilizer contains a mixed viable cell count of 6.91 Log_{10} CFU/ml from the initial month after production and progressively reduced to 6.56 Log_{10} CFU/ml after 12 months.

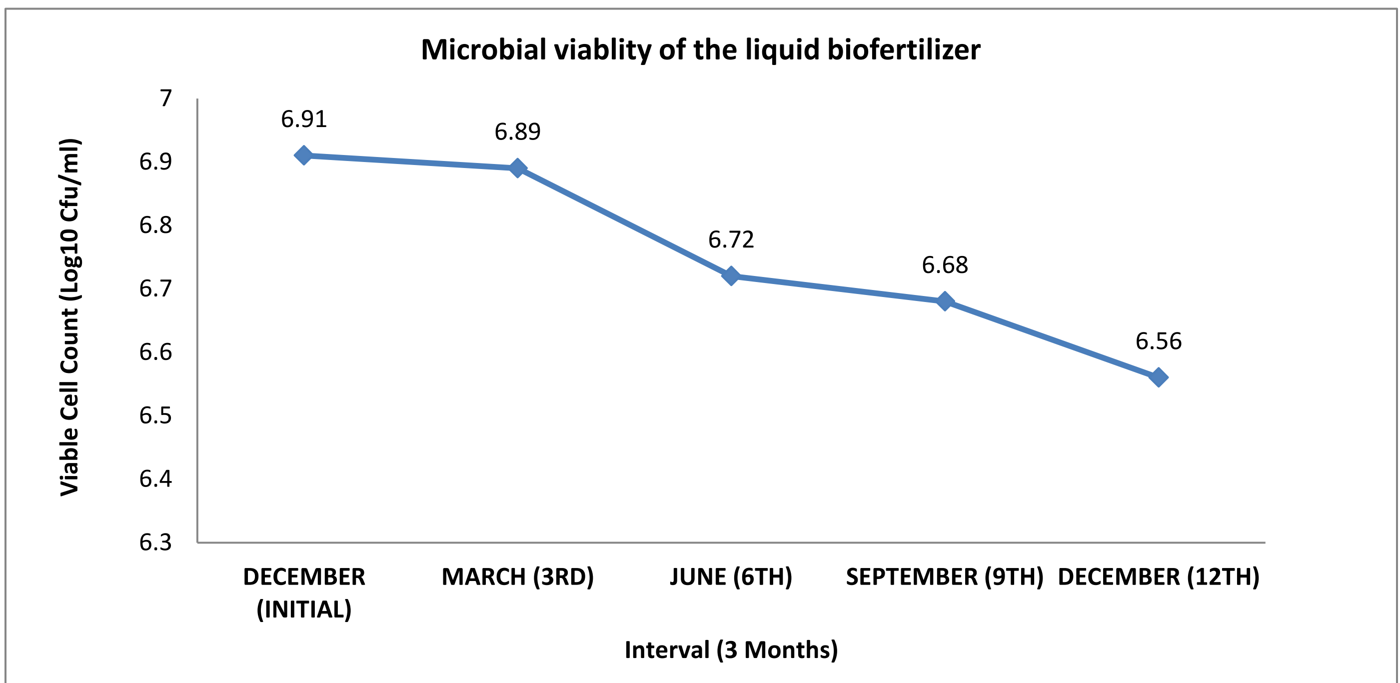

Figure 7: Stability of Microbial Cells of Liquid Biofertilizer at three months interval for 12 Months Duration

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The chemical and mineral content of the fermentation broths of the different fermenters and consortium are stated in Table 1. The result indicates that *Bacillus cereus* broth has the highest organic nitrogen content (%) of 0.042±0.00 while *Azotobacter chroococcum* broth has the lowest organic nitrogen content (%) of 0.019±0.00 whereas the microbial consortium has 0.035±0.00 % of organic nitrogen content. The result reveals that *Bacillus cereus* broth has the highest inorganic nitrogen content (%) of 0.119±0.00 while *Pseudomonas fluorescens* broth has the lowest inorganic nitrogen content (%) of 0.074±0.00 and the microbial consortium has 0.092±0.00 % of inorganic nitrogen content. Furthermore, *Bacillus cereus* broth has the highest total phosphate content (mg/L) of 4.510±0.03 while *Penicillium chrysogenum* broth has the lowest total phosphate content (mg/L) of 0.417±0.00 and the microbial consortium contains 0.883±0.00 (mg/L) of total phosphate.

Also, *Azotobacter chroococcum* broth has the highest total sulphate content (mg/L) of 892.8±52.8 while *Bacillus cereus* broth has the lowest total sulphate content (mg/L) of 409.8±1.80 and the microbial consortium has total sulphate content of 570.4±10.40 mg/L. *Penicillium chrysogenum* broth has the highest total organic matter content (%) of 97.73±0.75 while *Aspergillus niger* broth has the lowest total organic content (%) of 77.50±0.01 and the microbial consortium has 93.70±0.3 % of organic matter. *Azotobacter chroococcum* broth has the highest potassium content (mg/L) of 319.82±1.03 while *Penicillium chrysogenum,* broth has the lowest total potassium content (mg/L) of 217.25±0.22 and the microbial consortium has 294.48±1.39 mg/L. However, *Azotobacter chroococcum* broth has the highest calcium content (mg/L) of 151.33±0.92 while

Bacillus licheniformis broth has the lowest calcium content (mg/L) of 24.13±1.08 and the microbial consortium contains 77.59±0.86 mg/L of calcium content. Furthermore, *Azotobacter chroococcum* broth has the highest magnesium content (mg/L) of 110.15±0.08 while *Bacillus cereus* broth has the lowest magnesium content (mg/L) of 30.05±0.06 and the microbial consortium has 57.38±0.87 mg/L of magnesium. Statistically, there is a significance difference of chemical compositions among the different fermentation broths of microorganisms with P<0.001.

Table 1: Chemical Composition of the Liquid biofertilizer produced by various microorganisms from plantain peel and poultry waste

Starter cultures of fermentation broth	Chemical Content							
	Organic nitrogen (%)	**Inorganic nitrogen (%)**	**Total phosphate (mg/L)**	**Total sulphate (mg/L)**	**Total organic matter (%)**	**Potassium (mg/L)**	**Calcium (mg/L)**	**Magnesium (mg/L)**
Azotobacter chroococcum	0.019±0.00	0.081±0.00	0.447±0.00	892.8±52.8	92.55±0.3	319.82±1.03	151.33±0.92	110.15±0.08
Aspergillus niger	0.035±0.00	0.113±0.00	0.517±0.00	730.2±0.60	77.50±0.01	218.98±0.16	32.32±0.33	41.92±0.09
Bacillus cereus	0.042±0.00	0.119±0.00	4.510±0.03	409.8±1.80	88.28±0.4	224.79±0.01	25.74±0.01	30.05±0.06
Bacillus licheniformis	0.037±0.00	0.086±0.00	0.537±0.01	523.2±1.20	95.34±0.3	289.22±0.03	24.13±1.08	31.39±0.89
Penicillium chrysogenum	0.030±0.00	0.102±0.00	0.417±0.00	769.2±1.20	97.73±0.75	217.25±0.22	30.32±0.79	36.95±0.99
Pseudomonas fluorescens	0.029±0.00	0.074±0.00	0.797±0.00	623.4±0.60	88.33±0.4	236.55±0.14	28.56±0.96	38.50±0.69
Microbial consortium	0.035±0.00	0.092±0.00	0.883±0.00	570.4±10.40	93.70±0.3	294.48±1.39	77.59±0.86	57.38±0.87
P- value	***P<0.001***	***P<0.001***	***P<0.001***	***P<0.001***	***P<0.001***	***P<0.001***	***P<0.001***	***P<0.001***

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Discussion

The findings from this study reveals the proximate compositions of plantain peels and poultry waste as shown in Figure 1 which confirmed that plantain peels are rich source of nutrient and cheap substrate for microbial fermentation processes. The carbohydrate content (%) of the plantain peel was 33.25±0.06 which is lesser than the content value of other reporters who reported (54.01%), (68%) and (48.18%) respectively. However, the protein content (%) of the plantain peel was 10.53±0.04 which is higher than (2.93, 6.8, and 2.3%) as reported by other researchers. The lipid content obtained in this study was 27.63±0.5 which demonstrated a higher content as compared to other reports of (6.00, 7.3, and 0.9%).

Furthermore, the moisture level (%) of the plantain peel was 5.9±0.07 which is less than the moisture values (33.53, 9.5, and 7.8%) as reported by other researchers. The ash content also has a value of 8.01±0.01 which shows a higher ash content as compared to some reports which indicates an ash content value of 1.00 and 5.9% but less than the ash content value of 8.9 and 10.12%. The crude fibre content of the plantain peel was 14.69±0.5 which is higher than 2.53, 7.97, 8.36 and 10.4% as reported by other researchers. The nitrogen content (%) of the plantain peel was 1.70±0.02 which is yet to be reported by other researchers (Adamu *et al.,* 2017; Okareh *et al.,* 2015; Ighodaro, 2012; Tsado *et al.,* 2021). These variations can be attributed to the mode of processing, change in variety, geography, climate, soil fertility, application of fertilizers and even time of harvesting. In addition to the rich proximate composition and essential minerals, plantain peels have been proven to be a cheap substrate endowed with high carbon source for proliferation of microorganisms in fermentation processes. The carbohydrate, protein, lipid, moisture, ash, fibre and nitrogen contents (%) of poultry waste were 8.01±0.01, 8.33±0.02, 4.88±0.04, 1.2±0.07, 76.55±0.01, 1.03±0.03 and 1.35±0.02 respectively which is lesser than the reports of Usman *et al.* (2019) who determined the proximate composition of poultry waste to be 9.62±0.02% moisture content; ash content: 28.83±0.29%, crude protein: 21.34±0.16%, fat content: 2.61±0.13%, crude fibre:16.09±0.04, and carbohydrate: 21.53±0.03. However, the nitrogen content of poultry waste is yet to be reported.

The moisture content of a substrate significantly influences microbial growth, affecting factors like nutrient availability, oxygen diffusion, and metabolism. A balance is crucial for successful culture or fermentation. Protein and nitrogen are essential components in diets, providing amino acids and aiding in sporulation. High fiber content in diets removes potential mutagens, steroids, and xenobotics, benefiting microbial cultures, livestock, and fish farming (Adepoju and Adeniji, 2008; Okareh *et al.,* 2015).

The biofertilizer production starter cultures were developed for 240 hours in a broth medium. The optimal fermentation period was 192 hours, resulting in high microbial cell yields for *Aspergillus niger*, *Bacillus cereus, Bacillus licheniformis, Penicillium chrysogenum* and *Pseudomonas fluorescence*. However, *Azotobacter chroococcum* showed linear growth up to 240 hours, indicating that optimal inoculum size depends on strain, medium composition, fermentation time, and culture type. The larger inoculum size with active seed culture minimizes the length of adaptation (lag period) phase and facilitates the biomass concentration with a short fermentation time leading to higher production of any microbial product such as biofertilizer and exopolysaccharides. This is in line with the observations of Paul and Dubey (2014), who stated that culture that is used for large scale production or fermentation process is incubated until maximum cell population of 10^{10} to 10^{11}cfumL-1 is produced.

Under optimum conditions this population level could be attained within 4 to 10 days of incubation. According to Zywicka *et al.* (2021), regardless of the type of culturing, biosynthesis process is carried out as semi-continuous, continuous, or fed-batch fermentation and it must always be preceded by inoculum preparation. Inoculum preparation is a process in which dormant microbial cells are introduced from a stock culture to a favorable environment and grow to form a metabolically active microbial population.

In other words, the quantity and quality of the inoculum have a significant impact on the quantity and quality of the final product (Blasco *et al.* 2020).

The optimization parameters; Carbon compound (plantain peel), Nitrogen compound (poultry waste) and Trace Element (Zinc Sulphate) used in this study to optimized the microbial cell yield for liquid biofertilizer production were in agreement with other authors who have confirmed that when the isolates are obtained, better cell proliferation can be achieved with richer culture media, containing higher concentrations of nutrients (de Castro *et al.,* 2013). The minimal concentration of zinc sulphate for the increase of microbial cell count and biomass of all the microorganisms utilized in the production of the biofertilizer was 1.0%, as demonstrated in this study. On the other hand, 4.0% zinc concentration produced optimal microbial cell counts (Log10 CFU/ml) for *Aspergillus niger*, *Pseudomonas fluorescence*, *Bacillus licheniformis*, and *Azotobacter chroococcum*. 3.0% zinc concentration yielded optimum microbial cell counts and biomass for *Bacillus cereus* and *Penicillium chrysogenum*. This finding demonstrates the impact of trace element in microbial growth in which the minimum and optimal tolerance to each trace element depends on the individual organism as well as the growth. This is in line with the findings of other researchers that a number of divalent metal ions have profound influence on growth of microorganism liquid culture or fermentation and synthesis of microbial products and when in excess, the presence of trace elements in the media can negatively influence the growth of microorganisms and microbial products such as biofertilizer, citric acid (Adham, 2002). They play an important role in biological processes, acting as co-factors of enzymes (Wintsche *et al*., 2016), forming functional complexes with secondary metabolites and promoting the detoxification of reactive oxygen species (ROS) (Locatelli *et al*., 2016). Though metal ions are essential for many biological processes, they can be toxic at high concentrations (Puri *et al*., 2010). Furthermore, the optimization responses for the proliferation of microbial cell growth and biomass using carbon compound (Plantain peel) as revealed in this study, indicate that 15% was the optimal concentration of plantain peel for the growth of *Azotobacter chroococcum, Aspergillus niger*, *Bacillus cereus*, *Bacillus licheniformis, Penicillium chrysogenum*, and *Pseudomonas fluorescence.* This is in agreement with other studies which state that 14% to 18% of carbon compound is required to achieve high yields of microbial growth or microbial products (Soccol *et al*., 2006). Carbon is a fundamental element for the growth and survival of microorganisms, just as it is for all living organisms. Microorganisms, including bacteria and fungi, play a crucial role in recycling carbon and returning it to the atmosphere through various metabolic processes. Microorganisms utilize a variety of carbohydrates and its products are intermediate of carbohydrate metabolism (Wayman and Mattey, 2003). The presence of carbohydrate or carbon compound has been found necessary for microbial growth. Also, the nature and concentration of the carbon source can regulate the secondary metabolism through phenomena such as catabolic repression (Khani *et al.,* 2016).

This study found that poultry waste can be an effective source of organic nitrogenous compound for microbial growth in submerged cultures. The optimal concentrations for *Bacillus licheniformis* and *Pseudomonas fluorescence, Aspergillus niger* and *Penicillium chrysogenum*, and *Azotobacter chroococcum* and *Bacillus cereus* were 1.0%, 3.0%, and 4.0% respectively. This suggests that poultry waste can be a valuable source of organic nitrogenous compound for microbial growth in submerged cultures. It is generally believe that poultry waste encompassed with nitrogenous compound but have not been used as substrate for growth of microorganisms in a submerged culture or fermentation processes utilizing it as a source of organic nitrogenous compound. This study tends to confirm the potentiality of poultry waste as a good source of organic nitrogenous compound for microbial growth in submerged culture in which other researchers have reported the impact of various nitrogen sources on microbial growth. For example, Hungund and Gupta (2010a) investigated that the effect of different nitrogen sources such as peptone, casein hydrolysate, beef extract, malt extract, sodium nitrate, ammonium chloride, ammonium sulphate, potassium nitrate, ammonium nitrate and urea on microbial growth and by-product. Nitrogen compound increases and enhance the growth of bacteria and other microorganisms (Kazim, 2015). Nitrogen compounds in combination of carbon compounds helps to provide some growth factors such as amino acids and vitamins, especially vitamin B complex, which is required by bacteria and fungi for growth and also serves to stimulate the productivity of microbial growth (Kazim, 2015).

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In addition, Nitrogen compound plays an important role in the biosynthesis of essential molecules such as protein and nucleic acids. Embuscado *et al.* (1994) stated that organic nitrogen sources enhanced higher microbial yield than inorganic nitrogen sources. It was similar with the results of the research carried out by Abdelhady and coworkers (2015). Organic nitrogen sources support rapid growth and high cell yields of bacteria than inorganic nitrogen sources (Costa *et al,* 2010).

The final production of the liquid biofertilizer in this study demonstrates that 168hours of fermentation were the maximum time to produce viable microbial cells for the production of liquid biofertilizer with a reduction in pH. This is in correlation with the fact that optimal growth or fermentation conditions of microorganisms increase the yield of microbial cells and its by-product as reported by Akdeniz *et al.* (2012), who observed that if the input parameters (pH, Carbon, Trace element, Nitrogen and Methanol) were maintained at optimal level, there would be a consequential increase in microbial cells and its by-products. There is significance difference of $P<0.001$ which shows that the reduction of pH is directly proportional to fermentation period in a fermentation process which is in agreement with other researchers. This is in line with the report of Yusuf and Nazif (2023), who stated that the pH of biofertilizer is slightly acidic. This supported the study by other researchers which states that the ability of fungi and bacteria to solubilize phosphate in vitro is generally associated with the release of organic acids which decreases the pH of the growth medium (Okolie *et al*., 2018).

The findings from this study reveal the chemical composition of the fermentation broths of the microorganisms and its consortia at various concentrations. Consequently, *Bacillus cereus* stands out with the highest organic nitrogen content (%) at 0.042±0.00, while *Azotobacter chroococcum* broth has the lowest organic nitrogen content at 0.019±0.00, and other microorganisms, namely *Aspergillus niger*, *Bacillus licheniformis, Penicillium chrysogenum, Pseudomonas fluorescens*, and the microbial consortium, fall in between with values ranging from 0.029 to 0.037±0.00, which could be of interest for specific applications where higher nitrogen levels are desirable.

Azotobacter chroococcum, with the lowest organic nitrogen content, might be less efficient in nitrogen metabolism or might have different metabolic pathways compared to the other microorganisms. The microbial consortium's organic nitrogen content (0.035±0.00) could indicate synergistic effects of different microorganisms working together. Such consortia are often explored for enhanced efficiency in fermentation or other biotechnological processes.

Microbial consortia and specific strains like *Bacillus cereus, Bacillus licheniformis, and Pseudomonas fluorescens,* with higher chemical contents, may be of interest in agricultural applications. Farmers and researchers could potentially choose or engineer microbial formulations based on these findings to optimize nutrient availability in soils. The findings open avenues for further research to understand the mechanisms behind mineral production or mobilization such as phosphate, sulphate, calcium etc., in these microorganisms. Additionally, assessing the impact of these variations on plant growth and soil health would be valuable for practical applications.

Consequently, the differences in sulfate content among these microorganisms could have implications for their potential use as biofertilizers, especially in sulfur-deficient soils. The microorganisms with higher sulfate content, such as *Azotobacter chroococcum*, *Penicillium chrysogenum*, and *Aspergillus niger*, may be of interest in agricultural applications. Total organic matter content is a crucial parameter as it represents the overall organic compounds present in the fermentation broths. However, Understanding the magnesium, phosphate, sulphate calcium, potassium, and organic matter content is crucial for optimizing fermentation conditions, as these chemical contents are involved in several biochemical processes essential for microbial growth and product formation.

The pH value of the biofertilizer produced in this study is similar to the study done by Mario and Agripina (2020), it was found that liquid biofertilizers (LBFs) have moderate acidity, with pH values ranging from 5.81 to 5.93. Pig ingesta LBFs had the highest macronutrient content (0.31%), followed by cow-pig ingesta LBFs (0.27%). LBFs also contained sulfur, calcium, magnesium, iron, copper, manganese, zinc, sodium, and boron. The study also found that LBFs contain small amounts of micronutrients.

Yusuf and Nazif (2023) studied the physicochemical properties of liquid biofertilizer in various setups and a control set up, KL. They found that nutrient release was slower in KL due to the absence of microorganisms. However, the addition of *Azotobacter spp*. decreased pH and total nitrogen concentrations, and increased nitrates, carbon oxide, total organic carbon, potassium, and phosphates concentrations, indicating effective microorganisms' synergy. This means that when the heterotrophic microbial population grows, minerals, enzymes, hormones, organic acids, amino acids, vitamins, and the relative enrichment of solid organic substrates rise (Okolie *et al*., 2018).

Therefore, findings from this study revealed that the differences in chemical compositions among the microorganisms may have implications for their use in fermentation processes, as the micro and macro nutrients is crucial for microbial growth and metabolism. Understanding the nutrient content can be essential for optimizing fermentation conditions and yields in various industrial and biotechnological applications. Thus, liquid biofertilizers are considered complete of both macro and micronutrient elements which are essential for the growth and development of crops (Johnson and Mirza, 2020; Gao *et al* (2023).

The stability of the liquid biofertilizer as observed in this study reveals that the microbial cells were viable up to 12 months at the rate of 6.56 Log_{10} CFU/ml with the aid of cell protectants applied. This observation confirmed a longer shelf life than that of solid state biofertilizer which is in line with the findings of Santhosh (2015), who studied the effect of different cell protectants viz., glycerol (0.5%), polyvinyl pyrrolidone (PVP, 0.5%), polyethylene glycol (PEG, 0.5%), gum arabic (GA, 0.5%) and sodium alginate (SA, 0.1%) on shelf life of different liquid biofertilizer inoculants viz., *Rhizobium, Azotobacter, Azospirillum* and PSB (*Bacillus megaterium*). The experiment showed that, liquid biofertilizer inoculants developed using 0.5% PVP in addition to 0.5% glycerol (T3) increased the shelf-life of all the biofertilizer inoculants tested when the liquid formulations were stored for 180 days. This confirmed the findings of other researchers that liquid biofertilizer formulations can improve shelf-life by incorporating more nutrients, cell protectants, and inducers. They can tolerate temperatures up to 55°C, extending their shelf-life from six months to two years, compared to solid carrier-based biofertilizers (Mahdi *et al.,* 2010).

Polyvinylpyrrolidone is a high molecular weight compound (40000), water soluble compound with stabilization and adhesive properties, with high water holding capacity that appears to slow down the drying rate of media, thus maintaining the moisture level in the media (Deaker *et al*., 2004). However, the production and application of liquid biofertilizer is under-utilized compared to the solid formulation and other kinds of fertilizers. Thus, successful commercialization of less expensive liquid biofertilizer is a challenge and requires more stakeholders' engagement.

In conclusion, the findings from this study confirmed that that plantain peel has more proximate content than poultry waste and also tend to demonstrate the impact of carbon compound (plantain peel), Nitrogen compound (Poultry waste) and trace element (zinc sulphate) on microbial growth in which the minimum and optimal tolerance to each optimization parameter depends on the individual organism as well as the growth. This also indicates that the microorganisms work effectively in synergy to break down the wastes, thereby increasing the nutrients which led to the growth and proliferation of the microbial cells.

The observed differences in mineral content suggest potential variations in the physiological characteristics and requirements of the starter cultures which reveal that different microorganisms produce distinct mineral contents in their fermentation broths. The stability of the liquid biofertilizer as observed in this study reveals that the microbial cells were viable up to 12 months at the rate of 6.56 Log_{10} CFU/ml with the aid of cell protectants; glycerol (0.5%), polyvinyl pyrrolidone (PVP, 0.5%) applied. Therefore, plantain peel could be used as alternative and cheap substrate for microbial fermentation processes and as a means of converting waste to wealth. The microbial cultures and the rich fermentation broth produced in this study have the potential to enhance plant growth and increase food security with its affordability as compared to chemical fertilizer.

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