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Research Article

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Production of Biofertilizer from Coconut Coir Pith by Solid State Fermentation Using Micro-organism (*Aspergillus Niger*)

Kenneth K Dagde, Saviour A James

Department of Chemical/Petrochemical Engineering, RiversState University of Science and technology Port Harcourt, Nigeria

Abstract The industrial and commercial applications of the coir pitha waste product obtained from coir industries have so far been very limited. Disposing coir pith has been found to be very expensive and difficult. Thus, a laboratory scale study was conducted to determine the possibility of Converting coir pith into bio-fertilizer using the microorganism for solid state fermentation. The first step in the conversion of coir pith to bio-fertilizer was delignification using microorganism (*Aspergillus Niger*). The nitrogen content of the biofertilizer was enhanced by using the nitrogen fixing bacteria, (Azotobacter). The results obtained showed 3.30% (wt.%) nitrogen,16.60% moisture content, 46.0% volatile matter , 10.0% carbon content, 4.02% phosphorus,4.37% potassium, 4.60% conductivity and a pH of 6.60 within the period of 45 days of sub-culturing there by making it a better soil conditioner and a slow releasing nitrogenous fertilizer.

Keywords Coir pith, delignification, solid state fermentation, *Aspergillus Niger*, Azotobacter, biofertilizer, subculturing

Introduction

Keeping in view the increasing population, the production of food grains needs to be increased continuously. The coir waste by-product from coconut processing industries is a potential wealth and can be converted into valuable organic manure, biofertilizer, using bio-technological method [1]. Bio fertilizers are eco-friendly that supplies all nutrient input of biological origin for plant. Normally plant needs nitrogen and other nutrients for its growth. The soil micro-organisms used in biofertilizers are phosphate solubilizing microbes, mycorrhizae, azospirillum, azotobacter, Rhizobium, Sesbania, Blue green algae, and Azolla. Coconut is grown in more than 93 countries around the world in an area of 12.167 million hectares producing 59,569 million nuts [2]. It is socially, culturallyand religiously associated with millions of people around the world. Apart from food and drink, it provides sustainable income to millions who are involved in its cultivation and product utilization, and it is estimated that 5.7 million metric tons of coconut are produced in the world [3-6]. Coir as a naturally made reinforcement fiber designed to cushion the blow when coconut drops, served as the protective husk. Coir or coconut fiber is the course, stiff outside fiber extracted from the husk of coconuts and account for 50 – 60% of the total weight of the hush [7-9]. Coir pitch is a biomass residue generated during the coir extraction of coir fiber [2]. The husk coir weighs about 300 grams and is interwoven with the soft, peat moss-like coir pith in a 1:2 weight ratio; the resulting "cushion" is covered by a thin glossy skin. Coir by far dominates other 'hard fiber", such as sisal and abaca in several ways. It is the thickest, stiffest and most resilient of all commercial natural fibers; it cellulous structure makes it weaker and more elastic and the cell walls of fiber and pith contain more lignin than others which impart longevity. These exceptional qualities made it to be used in twin and ropes, brooms and bushes, mattresses, upholstery, car seat, doormats, etc. Above all, the retention of moisture is up to nine times its own volume, with maintenance of excellent air filled porosity, provides vital oxygen to the roots and soil. It fibrous and sponge-like structure is ideal for any soil condition, whether breaking up the heaviest of clay or retaining moisture in sandy soils. It is by far the most efficient and economical way to re-habilitate degraded soil without the risk of contamination [10-11]. Having a slow degradation rate, it conditions the soil and promotes the development of an optimum pH level. `With the natural pH of 5.7 to 6.5 plus an unusually

high cation exchange capacity and 27% of easily available water assures that coir will hold and release nutrient in solution over extended period without dewatering [12].

Previous studies in this field dealt with the delignification of coir pith using different micro-organisms. Israel et al. [6] studied the characterization of coconut coir dust for the physiochemical properties. Kanmani et al.[9] performed experiments on lignocelluloses biodegradation of coir waste in solid state fermentation. Krishna et al. [13]; Mishra and Dadhick, [14]; Marino et al. [15]; Yunchen et al. [5] conducted a comparative evaluation of different organic fertilizers on soil fertility. This research is a feasibility study of converting coir pitch into bio-fertilizer. *Aspergillus Niger* was sub-cultured and acclimatized for delignification of the coir pith. The variation of pH, conductivity, moisture content, volatile matter content, ash content carbon content, lignin content, and nitrogen content were studied using the strain

Materials and Methods

Materials

The following instruments were used in the experiment: pH meter (Unicam,9450), conductivity meter, volumetric flask, conical flask, pipette, burette, beaker, Aluminum dry dish, digestion flask, digital balance, stop watch, retort stands, Thermometer, test tubes, fume cupboard, Kjedahl apparatus, microwave oven, desiccators, muffle furnace, pair of tong, Porcelain crucible, lid, plate, wire gauge, Atomic Absorption, Spectrometer(model: Philip PU9100X)

Micro-Organisms

The fungal culture was prepared from a Potato Dextrose Agar, PDA slants and was procured from the culture collection unit in Department of Chemical/Petrochemical Engineering, Rivers State University of Science and Technology, Port Harcourt, Nigeria. The strain was maintained as pure culture on PDA slants.

Preparation of PDA Slants

40 gm of PDA (comprising 200 gm of Potato in fusion form, 20 gm of Dextrose and 15 gm of Agar) was accurately weighed and dissolved in 1 L of distilled water. The solution was well shaken and heated to dissolve the PDA. The solution was poured into the nine test tubes and sterilized at 1.5 kg/cm^2 gauge pressure for 20 minutes. The test tubes were then kept in a slant position in aseptic conditions to allow the medium to cool and solidify. The slants were then properly labeled and stored.

Sub-culturing

The micro-organism strains were sub-cultured on the thus prepared PDA slants. The sub-culturing environment was maintained under aseptic conditions by first using UV radiation in the inoculation chamber to kill any foreign micro-organism present and then the surface was disinfected using ethanol. Then, the microorganism transferring was taken from the master stock with the help of the loop and inoculated on the PDA slant in a zig-zag manner, to maximized growth. It was incubated at 30 ^{o}C for 2–3 days. After visual observation for satisfactory growth, the subcultures were stored at 50 ^{o}C. The sub-culture was used for further culturing and experimentation.

Substrate

Coconut husk was obtained from MkpatEnin Local Government Area coconut Plantation site in Akwa Ibom State, Nigeria on June, 2015 and later cleaned and dried. The dried husk was screened for homogeneity. The husk thus obtained was ready for experimentation.

Media Preparation

Table 1 shows the Composition of nutrient media, used for inoculums preparation. The nutrient media components were dissolved in 100 ml of distilled water in a conical flask.

Table 1: Composition of nutrient media, used for inoculums preparation

S/No	Nutrient media	Quantity
1.	Distilled water	100 ml
2.	$N(NH_4)_2SO_4$	1.4 gm
3.	$KHPO_4$	2.0 gm
4.	Urea	0.3 gm
5.	$CaCl_2$	0.4 gm
6.	$MgSO_4$	0.6 gm
7.	Glucose	7.5 gm
8.	Peptone	0.75 gm
9.	$MnSO_4.5H_20$	20 gm
10.	$FeSO_4.7H_20$	5.0 mg
11.	$ZnSO_4$	1.4 mg

The flask was plugged with cotton and was sterilized at 1.5 kg/cm^2 steam pressure for 15 minutes and cooled at room temperature. The nutrient media was then ready for inoculation.

Preparation of Liquid Inoculums

The micro-organism was transferred from the slants to the liquid media under aseptic conditions, using the same procedure as outlined in sub-culturing. The liquid media flask was stored in sterile conditions for 7–8 days for optimum growth of the micro-organism.

Inoculation of Substrate

To the 5 kg batch of Coir pith, 3000 ml distilled water and 1000 ml liquid nutrient media were added for nourishment of the microorganisms. The prepared substrate was then inoculated with the liquid inoculums. The inoculated substrate was kept in a moist state by adding distilled water every alternate day or as per requirement. The inoculation was followed by analysis of the then formed bio-fertilizer.

Inoculation with Nitrogen Fixing Bacteria

After satisfactory decrease in lignin content, the substrate was inoculated with the solid inoculums of Azotobacter obtained from the culture. The substrate was inoculated with solid in three batches of 2, 4, and 6% of inoculums; the three batches were studied

Method

Determination of the pH

Procedure: 10 gm of the sample was accurately weighed into a beaker and100 ml of distilled water was added to it. The sample was shaken manually for homogeneity and left to stand for 125 minutes. The pH was then determined with the aid of a previously standardized digital pH meter (Unicam, 9450 model), the pH meter calibrated using pH 4.0 and 7.0 buffer.

Determination of the Conductivity

The conductivity of the sample was determined using the conductivity meter. The sample prepared for pH was used and thereafter readings were taken and recorded accordingly.

Determination of the Moisture Content

5.0 gm of the sample was weighed into a pre-weighed aluminum dry dish. The dish and its contents was then transferred into an oven at a temperature 105 ^{o}C and dried for four hours. This was then allowed to cool in desiccators and weighted. The dish was then returned into the oven for another half an hour and again cooled and weighed. The process was repeated until a constant weight was reached.

$$X(moisture\ content\ \%) = \frac{M_1 - M_2}{M_1 - M_0} x100 \quad (1)$$

where $M_1 is$ the weight of the sample + dish is before drying, M_2 is the weight of the sample + dish after drying, and M_0 is the weight of the aluminum dish.

Determination of the Ash Content

5.0 gm of the sample was weighed into a Porcelain crucible previously ignited and weighed organic matter was charred by igniting the material on a hot plate in a fume cupboard until no fume was seen. This was then transferred into a muffle furnace (55 ^{o}C) using a pair of tongs and ignited for 3 hours. It was then cooled in a dedicator and weighed immediately.

$$\%\ Ash\ Content = \frac{(Wt\ of\ Crucible + Ash) - (Wt\ of\ Crucible)}{Weight\ of\ Sample}\ x\ 100 \quad (2)$$

The pith in the crucible obtained from the above procedure was burned to ash completely. It was cooled and weighed. Heating, cooling and weighing was repeated for constant weight.

Determination of Volatile Matter

5.0 gm of the sample was weighed into a crucible, covered, and thereafter heated, for about 7 minutes, cooled and weighed. The procedure was repeated until a constant weight was obtained.

$$\%\ Volatile\ Matter = \frac{Weight\ Loss}{Initial\ \ Weight\ of\ Sample}\ x\ 100 \quad (3)$$

Determination of Fixed Carbon Content

This was obtained by subtracting from 100, the sum addition of moisture, volatile matter and ash content. The remainder value is the carbon content in the sample.

$$Carbon\ Content\ (\%) = 100 - (\%\ moisture + Volatile\ Matter + Ash) \quad (4)$$

Determination of Nitrogen Content

This was carried out using the Kjedahl nitrogen method as follows:

1 gm of sample was weighed into a digestion flask. Kjedahlcatalyst (5 selenium tablets) was added to the sample. 20 ml concentrated tetra-oxosulphate (VI) acid (H_2SO_4) was also added and then fixed for 5 hours in the digestion units at (45 ^{o}C) in a fume cupboard. The digest pure yellow colouration after cooling changed into colourless liquid transferred into 100 ml volumetric flask and diluted with distilled water. 20ml of 4% Boric

acid solution was pipette into conical flask, 5 drops of methyl red was added to each flask as indicator. The mixture was distilled for 15 minutes and the filtrate was then titrated against 1 N HCl.

$$\%\, Total\ Nitrogen = Titre\ Value\ x\ Normality\ of\ Acid\ x\ 0.0014\ x\ 0.01\ wt\ of\ sample \quad (5)$$

Determination of Phosphorus and Potassium content

The phosphorus and potassium content were determined using the Atomic absorption Spectrophotometer (AAS), Model: Philip PU 9100X). The concentration of the element in the sample was calculated as follows:

$$Conc.(mg/100g) = \frac{Standard\ \ Conc.x\ Sample\ \ Absorbance}{Standard\ \ Absorbance\ \ x\ Weight\ of\ Sample}\ x\ 100 \quad (6)$$

1 gm of air dried sample was taken in a beaker and 100 ml of distilled water was added to it. 25 ml of 0.1 N $KMnO_4$ was added to the beaker containing the sample. The reaction was allowed to take place for about 10 minutes. At the end of the 10^{th} minute, the reaction was turned by adding 5 ml of KI solution. The liberated iodine was titrated against 0.1 N $Na_2S_2O_3$ using starch as an indicator toward the end point.

Results and Discussion

Table 2-10 shows the results of various constituents of the Coir pith and the trend of the content of inoculated coconut husk during the experimental period (45 days).

Table 2: Experimental Results

Time (Days)	pH Value	Conductivity Value	Moisture Content (%)	Ash Content	Volatile matter (%)	Carbon Content (%)	Nitrogen Content (%)	Phospho-rus& Content (ppm)	Potas-sium
0	4.32	2.80	10.40	22.0	36.0	31.6	1.79	1.01	0.99
15	5.20	4.30	12.60	23.0	40.0	24.8	1.83	1.68	2.54
30	6.50	4.50	14.60	23.2	46.0	16.2	1.88	3.58	3.84
45	6.60	4.60	16.60	27.4	46.0	10.0	3.30	4.02	4.37

Table 3: pH Variation

Time (Days)	0	15	30	45
pH value	4.32	5.2	6.5	6.6

Table 4: Conductivity

Time (Days)	0	15	30	45
Conductivity value	2.8	4.3	4.5	4.6

Table 5: Moisture Content

Time (Days)	Wt. of Surplus (g)	Wt. of Sample + dish before drying (g)	Wt. of Sample + Dish grinding	% Moisture
0	5.0	30.91	30.39	10.4%
15	5.00	24.79	24.18	12.2%
30	5.00	47.34	46.61	14.6%
45	5.00	29.49	28.66	16.6%

Table 6: Ash Content

Time (Days)	Wt. of Surplus (g)	Wt of empty Crucible (g)	Wt. of Crucible + Ash (g)	% Ash
0	5.00	65.00	66.10	22%
15	5.00	65.00	66.15	23%
30	5.00	65.00	66.16	23.2
45	5.00	65.00	66.37	27.4%

Table 7: Volatile Matter

Time (Days)	Wt. of Surplus (g)	Wt of Sample + before Heating (g)	Wt. of Sample + after heating	% Volatile
0	5.00	5.00	3.2	36.0%
15	5.00	5.00	3.0	40.0%
30	5.00	5.00	2.7	46.0%
45	5.00	5.00	2.7	46.0%

Table 8: Fixed Carbon Content

Time (Days)	Wt. Moisture	% Volatile matter	% Ash	% Fixed Carbons (100 sun addition)
0	10.4	36.0	22	31.6
15	12.2	40.0	23	24.8
30	14.6	46.0	23.2	16.2
45	16.6	46.0	27.4	10.0

Table 9: Total Nitrogen Content

Time (Days)	Wt. of Sample	1st Reading (m/s)	2nd Reading (m/s)	Average Reading (m/s)	% Nitrogen
0	1.00	12.9	12.7	12.8	1.79
15	1.00	13.2	13.0	13.1	1.83
30	1.00	13.4	13.4	13.4	1.88
45	1.00	23.6	23.6	23.6	3.30

Table 10: Phosphates and Potassium Content

Time (Days)	Stand Conc. = 2ppm K Standard Absorption = 0.179	Standard Conc. = 2ppm p. Standard Adsorption = 0.142
0	1.01	0.99
15	1.68	1.54
30	3.58	2.54
45	4.02	4.37

Figure 1 shows the variation in pH of inoculated coconut husk during the bio-fertilizer production. The pH value increased from an acidic state towards a neutral value. The increase might probably be as a result of the breakdown of the cellulose by Cellulose enzyme produced by the *Aspergillus Niger*, which further increase in the number of days would have no effect on the neutral state of the pH of the inoculants.

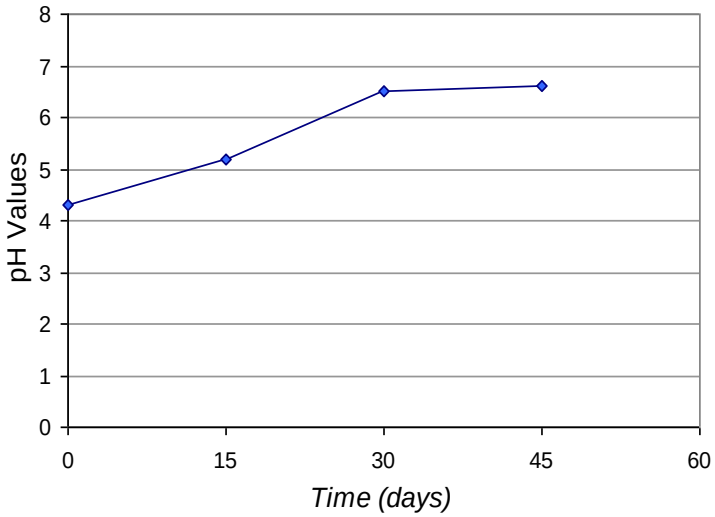

Figure 1: Variation in pH of Inoculated coconut husk during bio-fertilizer Production

Figure 2 shows the variation of conductivity of inoculated coconut husk during the bio-fertilizer production. There was an increased from 2.8 at initial day to 4.3 in the first 15 days, the following 15 days showed minimal increase in conductivity to 4.5 and no feasible changed was observed toward the end of the 45 days due to the complete breakdown of cellulose enzymes.

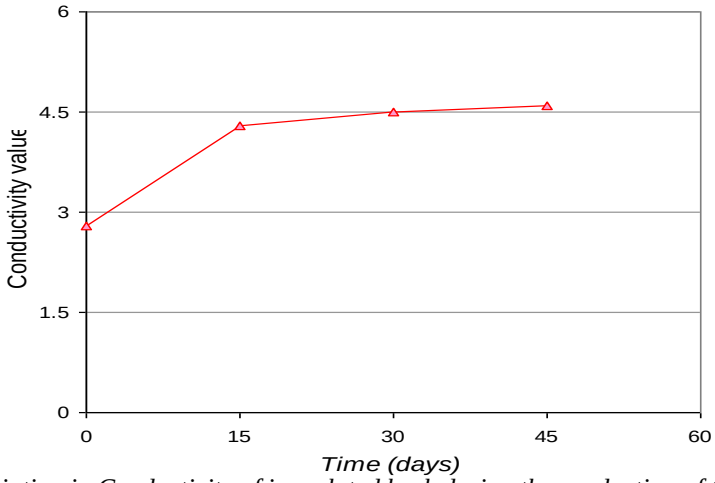

Figure 2: Variation in Conductivity of inoculated husk during the production of the biofertilizer.

Figure 3 shows the variation in the moisture content of the inoculated coconut husk during the production of bio-fertilizer. The graph revealed that there was a gradual increase in moisture content from 10.4% at the initial day to 16.6% on the 45th day.

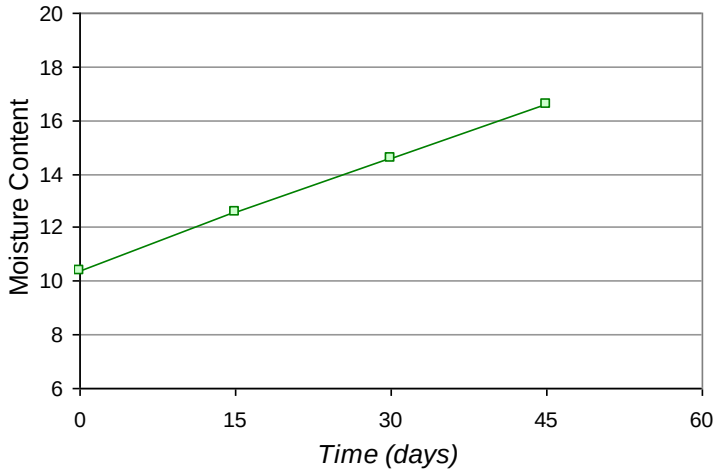

Figure 3: Moisture content of inoculated coconut husk during bio-fertilizer production

The enhanced moisture content was as a result of fermentation of the substrate brought about as a result of enzymatic hydrolysis of the cellulose by the cellulose enzyme product by *Aspergillus Niger*. Thus, appropriate moisture content allows for proper growth and activity of the fermenting micro-organism.

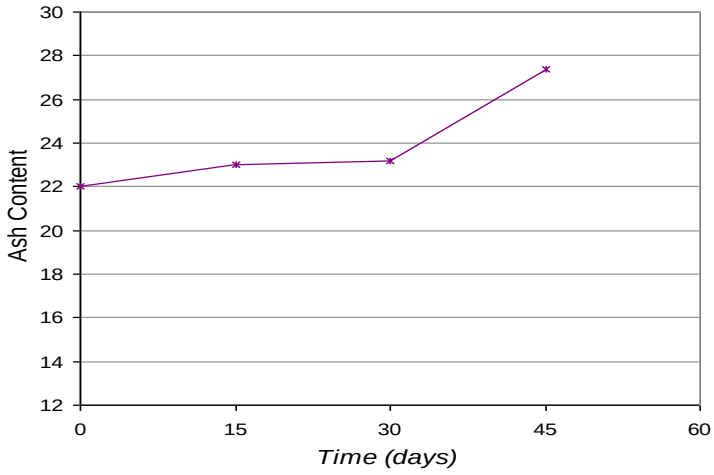

Figure 4: Ash content of inoculated coconut husk during fermentation for bio-fertilizer production

Figure 4 shows the ash content of inoculated coconut husk during fermentation of biofertilizer. The result revealed a gradual increase in the ash content with time from an initial value of 22% at 0 day to 27.4% at the 45th day of the experiment. The ash content is a measure of the mineral content in a product sample [13, 15, 14].

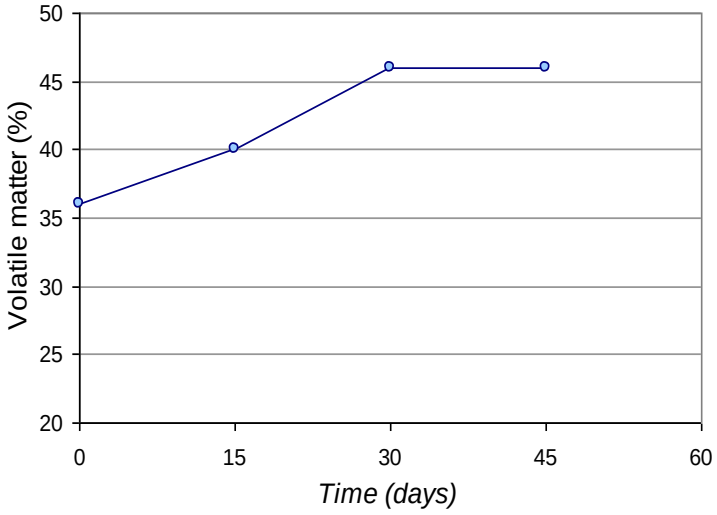

Figure 5: Volatile Matter of inoculated coconut husk during fermentation of Bio-fertilizer production

Figure 5 shows the volatile matter of the inoculated coconut husk during fermentation for bio-fertilities production. The result showed a gradual increase in the volatile matter from 36% to 46% in the first 30 days. However, there was no further increase in volatility even with the increase in the numbers of days to 45, the volatility remain constant of 46% showing the completion of the fermentation process and the end of microbial activities.

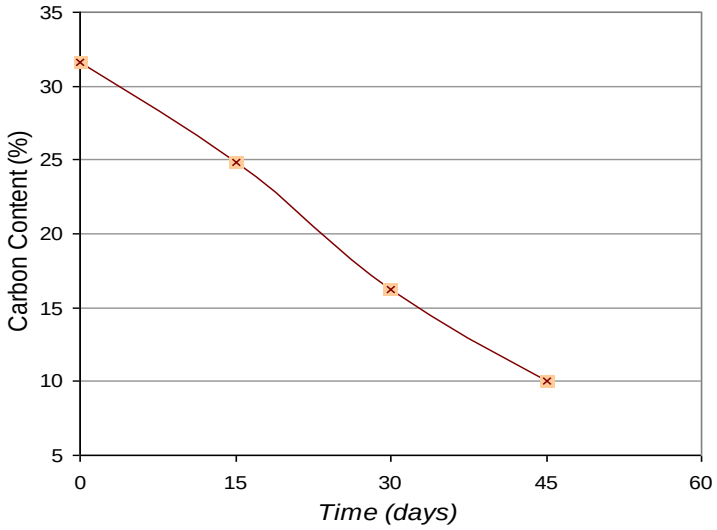

Figure 6: The Fixed Carbon content of inoculated coconut husk during fermentation for Product of Bio-fertilizer

The results of fixed carbon content for the sample during fermentation of the husk sample for the production of bio-fertilizer is represented in Figure 6. The result revealed a gradual decrease in the fixed carbon content from 31.6% to 10.0%. The decrease was due to increase in volatility of culture and increasing activities of the microbe on the substrate

Figure 7 depicts the variation of total nitrogen in the inoculated coconut husk for bio-fertilizer production. The result revealed that Nitrogen content increased as the number of days increased from 1.79% in 0 day to 3.30% in the 45th days. This divulged the ability of the bio-fertilizer to condition the soil as it stayed longer without the risk of being contaminated as the nitrogen in very essential for plants growth.

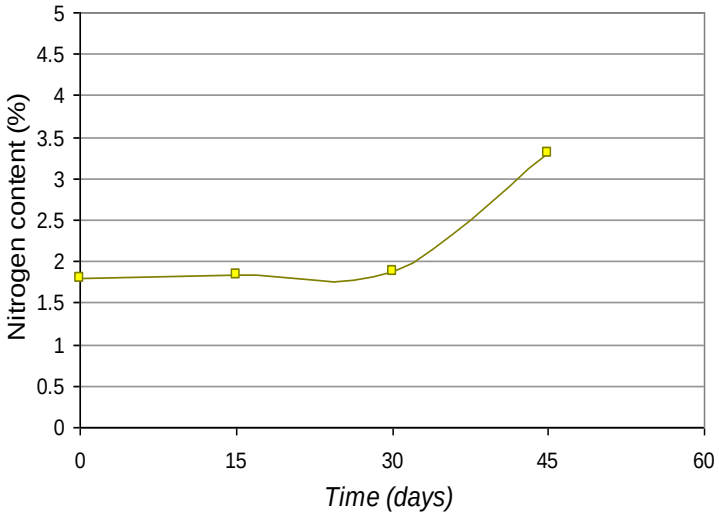

Figure 7: Total Nitrogen content of Inoculated Coconut husk during the Production of Bio-fertilizer

The results of phosphorus and potassium content of the inoculated coconut husk during production of bio-fertilizer are shown in Figure 8. The results depict an enhanced level of both mineral elements. The continuous increase was probably as a result of the conversion of the cellulose components during the process.

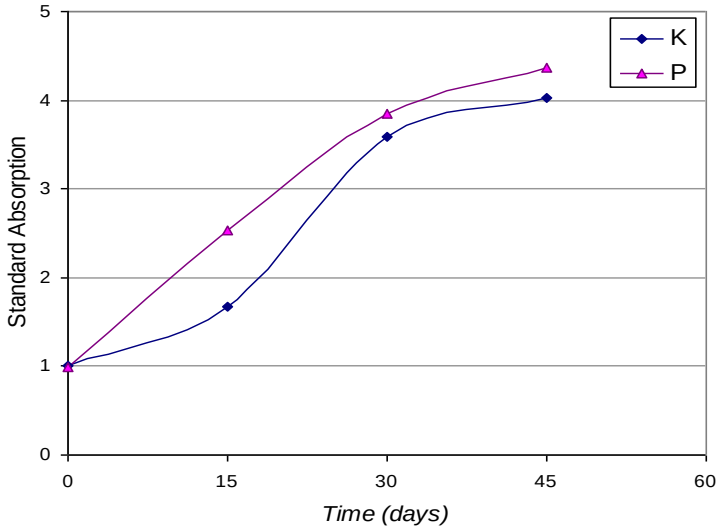

Figure 8: Phosphorus and Potassium content of Inoculated Coconut husk during bio-fertilizer Production

Conclusion

Coconut coir pitch, obtained as a by-product of coir industries is considered as a solid waste due to the high lignin content, low bulk density, low biodegradability, high moisture retention and low calorific value. This research is a feasibility study of converting coir pitch into bio-fertilizer. *Aspergillus Niger* was sub-cultured and acclimatized for delignification of the coir pith. The variation of pH, conductivity, moisture content, volatile matter content, ash content carbon content, lignin content, and nitrogen content were studied using the strain. The maximum percent of nitrogen fixed was found to be 3.30% (wt. %). In addition to the nitrogen content, the release of nitrogen from the bio-fertilizer to the plant was very slow, unlike any inorganic fertilizer. Hence, this bio-fertilizer was proved to be not only a good soil conditioner but also a slow releasing nitrogenous fertilizer. Also, the moisture content of the final product was almost 400%. Hence, this fertilizer is more suited for pot plants, which can be watered bi-weekly and also environmentally friendly.

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