POTENTIALS OF MICROORGANISMS ASSOCIATED WITH PLANTAIN PEELS IN THE LAGOS METROPOLIS FOR BIODEGRADATION AND BIOCONVERSION.

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ABSTRACT

The role of microbes in the degradation of plantain derived-wastes and their potential to produce cellulolytic enzymes was assessed. Soil samples of decomposing waste piles were collected from two major plantain markets in the Lagos metropolis and analyzed for physicochemical properties, toxic heavy metal content and microbial populations. Findings revealed that the values of moisture content of the two soils varied between 7.27±0.04 and 8.06±0.19 %. M-12 site had the highest organic matter content of 6.89±0.14 %. A similar pattern was observed for nitrate, phosphate and chloride levels while some heavy metals were also detected in varying and high amounts. The highest viable bacterial counts was 58.0±2.9 x 10^4 cfu/g at MU and there were no fungi at the site whereas M-12 had a fungal count of 40.0±3.3 x 10^3 cfu/g. Out of the total of 34 isolates encountered, 8 isolates having maximum cellulase activities were selected for further studies by the primary screening technique. These test organisms were then evaluated by secondary screening for enzyme production. The test organisms were phenotypically and biochemically characterized and identified as *Klebsiella pneumoniae* spp *pneumoniae* (2 strains), *Klebsiella pneumoniae* spp *ozaenae, Enterobacter aerogenes, Providencia alcalifaciens, Aspergillus flavus, Aspergillus fumigatus and Aspergillus niger* respectively. Both the bacteria and moulds were found to be capable of utilizing lignin and cellulosic substrates for growth and for production of cellulolytic enzymes. It is suggested that such microorganisms could be useful in bioconversion of cellulosic substrates like plantain-derived wastes for biotechnological applications.

Keywords: Plantain derived-wastes, Soil, Heavy metals, Bacteria, Cellulolytic enzymes, Fungi.

INTRODUCTION

Plantain (*Musa paradisiaca*) is a member of Musaceace family. The plant is one of the earliest cultivated crops, originating from South East Asia (Purseglove, 1978). Today they are found in many countries that have the tropical rainforest type of climate; such as in West Africa, India, Jamaica, Cameroon and some other African countries. The fruits are eaten when unripe and in ripe form and are very important in the international trade (Adewole *et al*., 2012). In Nigeria, plantain which are grown together with banana (*Musa sapientum*) are found mostly in the Southern States , where the fruits form the staple food for the people as well as an important article of trade with the northern states and other West African Countries. The green pseudostem contains water that fills up the intercellular openings. The pseudostem except the core is made up of cellulosic filaments which are bonded together into cellulosic films by lignin and hemicelluloses (Akpabio *et al*., 2012). Thousands of tons of these agricultural wastes are produced annually in Nigeria and a large quantity rot away due to inadequate preservation methods.

Most nations of the world, that are economically advanced or which are at different stages of development are faced with serious challenges and problems of disposal and treatment of wastes (Itelima *et al*., 2013). The biological methods have been the best option to get rid of this type of wastes from the environment and to meet sanitary standards. In Nigeria, many food crops have been specifically grown for the production of bio-ethanol. However, bio-ethanol generated from fruit wastes is limited. The greatest potential to increase the use of biomass in energy production seems to lie in forest residues and other biomass resources such as agro biomass and fruit biomass (Kramer, 2002; Eija *et al*., 2007). Several forms of biomass resources exist (starch or sugar crops, weeds, oils plants,

agricultural, forestry and municipal wastes) but of all of them, cellulosic resources represent the most abundant global source (Park and Barratti, 1995; Joshi *et al.*, 2001; Kadar *et al.*, 2004; Pimentel and Patzek, 2005). Plantain wastes are readily generated especially in the market places and homes in the tropics constituting a nuisance to the environment. However, these wastes can be microbially degraded and at the same time be converted to a renewable energy source such as ethanol which has been a sustainable option for transportation fuel (El-Naggar *et al.*, 2014).

The lignocellulosic materials include agricultural residues, municipal solid wastes (MSW), pulp mill refuse, switch grass and lawn, garden wastes (Pimentel and Patzek, 2005). Currently, there is a growing interest in ecologically sustainable bio-fuels all over the world. This study therefore investigated the activity of the microorganisms involved in the degradation of agricultural wastes generated from plantain and their potentials to produce cellulolytic enzymes which could be used for possible biotechnological applications.

MATERIALS AND METHODS

Chemicals, Reagents and Suppliers

Carboxymethylcellulose was obtained from Sigma Aldrich, Germany. Unless otherwise stated, all other chemicals and disposable Petridishes were obtained from Esota Laboratory and Chemical Supplier, Oshodi, Lagos, Nigeria.

Collection of Samples and Microbiological Analyses

Samples of decomposing plantain wastes were collected from two major plantain markets within the Lagos metropolis in sterile screw cap bottles for microbiological analyses while polyethylene bags were used for soil samples designated for physicochemical analysis. The sampling points were Mushin and Mile 12, both in Lagos metropolis (Fig. 1). A ten-fold dilution of soil suspensions were aseptically spread on various selective and non-selective media for enumeration and isolation of various microorganisms. Nutrient Agar plates were used for total bacterial counts while moulds were enumerated on Potato Dextrose Agar plates.

Determination of Physicochemical Parameters of Decomposed Plantain Waste Soils

The physicochemical properties of soil samples were determined by standard methods. Soil pH was measured electrometrically with Orion 3 Star bench top pH meter (Thermoscientific, USA). The pH of the each sample was determined by adding $CaCl_2$ solution to a measured quantity of the soil in ratio 1:2, soil solution. The resulting mixture was allowed to stand for 30 minutes after which the pH was measured electrometrically with Orion 3Star pH Benchtopmeter (Thermoscientific, USA).

The moisture content was determined by gravimetric method as previously described (Michael *et al.*, 1994 and Black, 1965). Each soil sample (2.0 g) was weighed into a crucible of known weight. The soil was then dried in the oven at 105°C for 2 hours. The crucible and its content was allowed to cool in a dessicator and the weight of crucible+soil determined. This was repeated until a constant weight was achieved. The difference in weight of the crucible alone and the crucible +soil was then used to calculate the moisture content of the soil as follows:

$$\% \text{ moisture content} = (w_2-w_3) \times 100 / (w_2-w_1)$$

where w_1 – weight of dried crucible

w_2 – weight of dried crucible + soil

w_3 – weight of dried crucible + dried soil

The total organic carbon and total organic matter were determined by titrimetric method as previously described by Walkley and Black (1934). Each soil sample (1.0 g) was weighed into a 250 ml conical flask, 10 mL of $K_2Cr_2O_7$ and 20 mL conc. H_2SO_4 were added. The mixture was adequately mixed by gently swirling the flask and was then left to stand for 30 minutes. Distilled water (100 ml) was then added, followed by 3 drops of ferroin indicator. This was then titrated to a reddish brown end point with ferrous ammonium sulphate ($(NH_4)_2Fe(SO_4)_2.6H_2O$). A blank containing all reagents used but without any sample in equal measure was also titrated to the same end point with ferrous ammonium sulphate ($(NH_4)_2Fe(SO_4)_2.6H_2O$). The TOC and TOM were then calculated.

The PO_4^{3-} content in the soil samples was determined as total available phosphate as previously described by Olsen *et al.* (1954). Two grams of each soil sample was placed in a conical flask and 40mL of 0.5M sodium bicarbonate was added. The flask and its content was shaken for 30 minutes (reciprocating shaker 150 rpm), after which the resulting mixture was filtered (Whatman filter paper cat No 1001 090 Maidstone, England). The PO_4^{3-} content in the filtrate was then determined colorimetrically using the ascorbic acid method (Murphy and Rifey, 1962).

The metal contents of the soils were determined as previously by Michael *et al.* (1999). Soil sample (1.0g) was placed in a kheljhal flask, 10 mL aqua regia was added. The resulting mixture was then digested at 60°C for two hours after which the digest was cooled and transferred into a 50 mL standard flask. This was then filtered into an acid pre-washed polyethylene bottle. The total heavy metal contents in each digest was determined by Atomic Absorption Spectrophotometry (Perkin-Elmer AAnalyst 200, Shelton, Connecticut, USA).

Primary Screening of Microorganisms for Cellulolytic Activity

Carboxymethyl cellulose medium recommended by Pye *et al.* (1977) was adopted for preliminary screening. It has the following composition (g/l): Carboxymethylcellulose (CMC), 20; K_2HPO_4, 1.394; $MgSO_4.7H_2O$, 0.756; $CaCO_3$, 0.005; $FeSO_4.7H_2O$, 0.0182; $ZnSO_4.\ 7H_2O$, 0.0176; NH_4NO_3, 1.006; Agar, 15 and pH adjusted to 7. All the plates were incubated at 37 °C for 3-5days. The plates were flooded with 1.0 % Congo red and 1M NaCl to determine the cellulolytic activity of isolated strains. The formation of a clear zone of hydrolysis indicated cellulose degradation. The ratio of the clear zone diameter to colony diameter was measured in order to select for the highest cellulase producer. The highest ratio was assumed to show the highest activity.

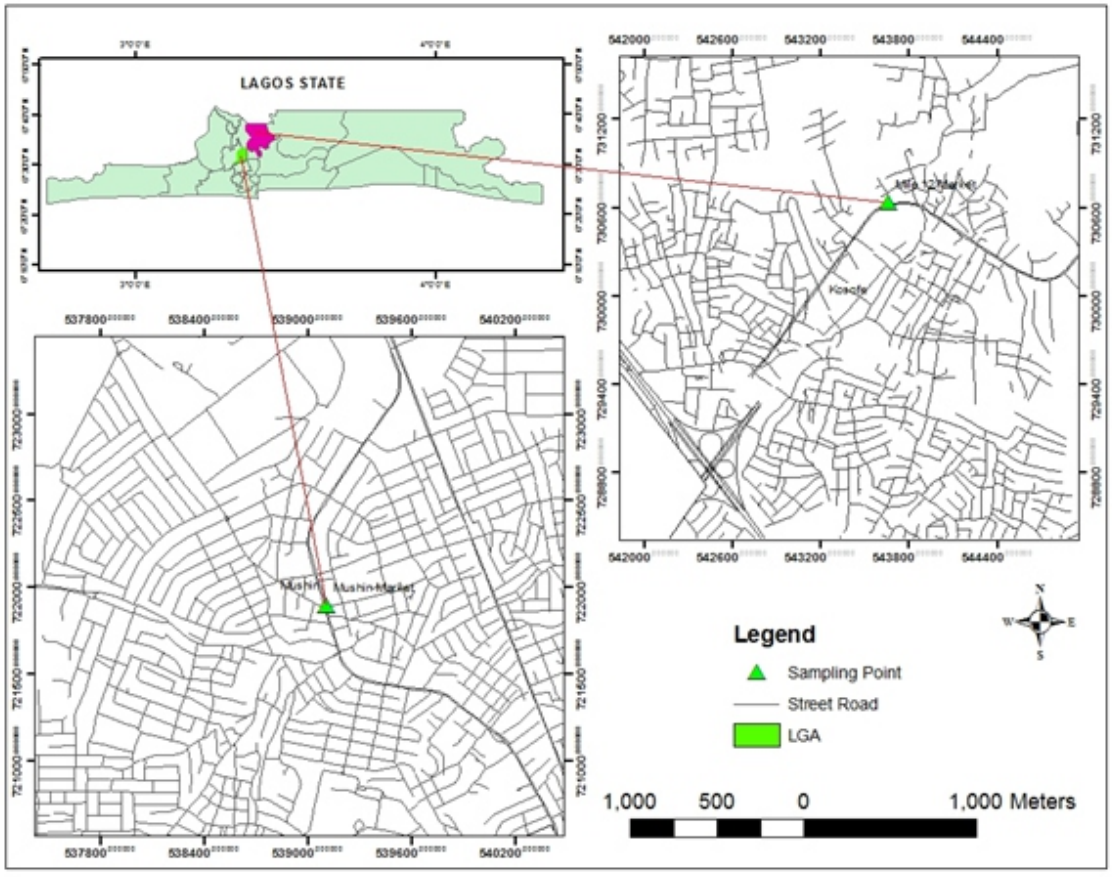

Fig.1: Map of the Lagos City showing Mushin(Idi-Oro) and Mile 12 Plantain Markets and sampling points.

Identification of Cellulolytic Test Microorganisms

Bacterial isolates from growths on various media were identified by the conventional taxonomic techniques as described by Buchanan and Gibbons (1974) and by high throughput biochemical characterizations (using the biomérieux vitek®2 system, Hazelwood, MO, USA). The fungal colonies described according to the procedures described by Gilman (1959), Alexopoulus (1962) and Harrigan and McCance (1976). The isolates were tested for their ability to utilize hemicellulose (xylan), carboxymethylcellulose and plantain peel (naturally-occurring cellulosic substrate).

Assay of Cellulolytic Enzymes

Bacterial and fungal isolates were grown in mineral salts medium containing carboxymethylcellulose (2.0 % w/v) as the sole carbon source using the method of Pye *et al.* (1977). Cellulase activity was determined in culture filtrates using xylan, carboxymethylcellulose and plantain peel as substrates. The assay tubes contained the substrate solution (2.0 ml, 0.5 % w/v), acetate buffer (2.0 M, 2.0 m1) and the enzyme solution (2.0 ml). The mixtures were incubated at 50 °C for 30 min. Progressive changes in cellulase activities of bacterial and fungal cultures were assayed on a daily basis for 16 days at 2 days interval. Reducing sugar released in the assay tubes, was measured by the Nelson-Somogyi method (Nelson, 1944; Somogyi, 1952). Enzyme activity was expressed as the amount of reducing sugar released per ml of culture filtrate.

RESULTS

Physicochemical Characteristics of Decomposed Plantain Waste Soil Samples

Table1a shows the results of various physicochemical parameters including pH, moisture content, total organic matter and carbon and the levels of phosphorus, nitrate, chloride, iron, nickel and zinc. High levels of these physico-chemical parameters were observed in the soil samples. The values of parameters at the sampling sites were compared and Figure 2a&2b clearly show distinct variations in values obtained from the two sites. The soil physico-chemical parameters like moisture content, total organic matter, total carbon, phosphate, nitrate, iron, chloride, zinc and nickel obtained from the two sites has direct relation to the population of cellulose-utilizing bacteria and fungi. However, the phosphate ion was high for the two sites with slight differences in values. Table1b shows the concentration of heavy metals obtained from the plantain waste piles. Of all the elements measured, the average concentration of these metals at the two sites are 44.49±0.96 and 52.74±1.08 mg/kg for iron, 26.60±0.86 and 34.88±0.45 mg/kg for chloride, 8.26±0.27 and 9.46±0.44 mg/kg for nickel and 50.36±0.62 and 47.69±1.09 mg/kg for zinc while average concentration of 17.50±0.55 and 24.36±0.87 mg/kg for nitrate was obtained and 6.03±0.06 and 6.99±0.32 mg/kg was recorded for phosphate.

Table 1a**:** Physicochemical Parameters of Soils from Plantain-derived Waste Piles

Sites	pH	MC (%)	TOC (%)	C (%)	TOM (%)	NO_3^- (mg/kg)	Cl^- (mg/kg)	PO_4^{3-} (mg/kg)
Mushin	5.26± 0.08	7.27±0.04	3.23±0.09	2.44±0.07	5.67±0.07	17.50±0.55	26.60±0.86	6.03±0.06
Mile12	10.18±0.16	8.06±0.19	4.03±0.11	2.97±0.10	6.89±0.14	24.36±0.87	34.88±0.45	6.99±0.32

MC-Moisture content, TOC-Total organic carbon, C-Carbon, TOM-Total organic matter, NO_3^-Nitrate, Cl^--Chloride, PO_4^{3-}-Phosphate

Table 1b: Heavy Metal Contents of the Soils from Plantain-derived Wastes

Sites	Soil Parameter (Heavy metal) (mg/kg)		
	Fe^{2+}	Ni^{2+}	Zn^{2+}
Mushin	44.49±0.96	8.26±0.27	50.36±0.62
Mile12	52.74±1.08	9.46±0.44	47.69±1.09

Fe^{2+}-Iron, Ni^{2+}-Nickel, Zn^{2+}-Zinc

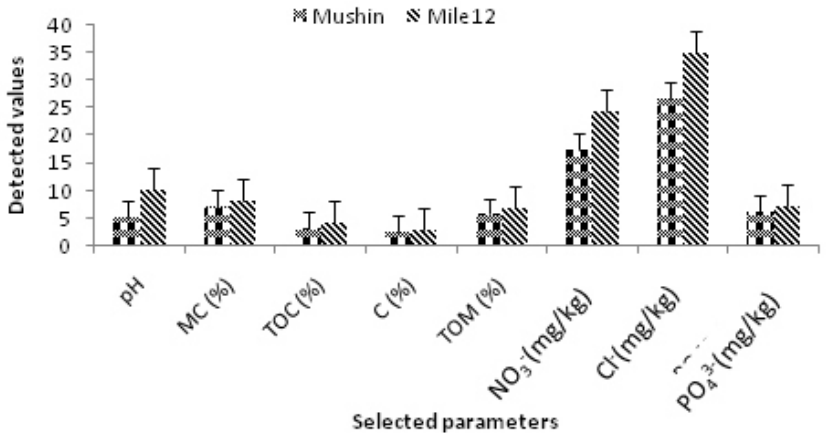

Fig.2a: The Physicochemical Analysis Results Obtained for the Two Sampling Sites (Mushin (Idi-Oro) and Mile12)

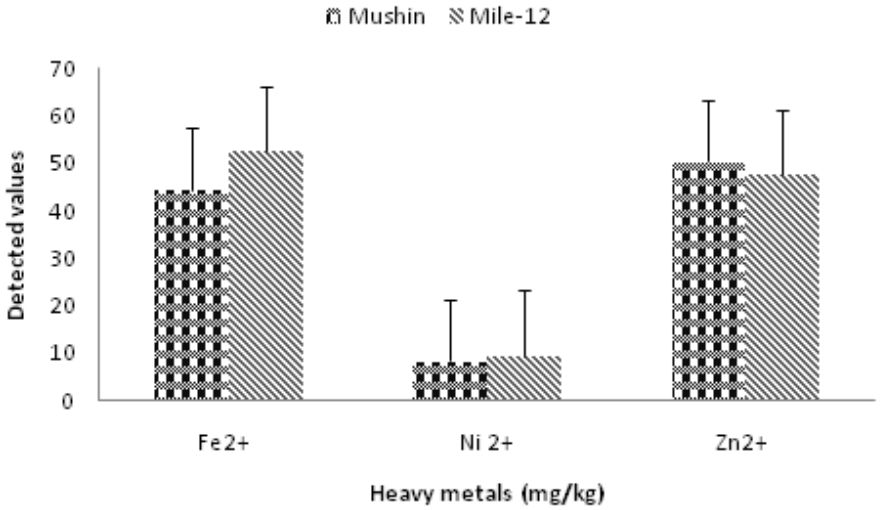

Fig.2b: The Physicochemical Analysis Results Obtained for the Two Sampling Sites (Mushin (Idi-Oro) and Mile12)

Microbial Dynamics and Distribution

Table 2 shows the population levels of bacteria and fungi in the soil samples collected from the decomposed plantain waste piles. Mushin site had the highest viable bacterial counts of 58.0±8.2 x10^4cfu/g recording no fungal isolate. The bacteria counts at Mile12 was 34.0±6.5 x 10^4 cfu/g and fungi count was 40.0±3.3 x 10^3 cfu/g respectively. MU-4B had highest occurrence of 13.0±2.9 x 10^4 cfu/g followed by MU-11B with 8.0±1.4 x 10^4 cfu/g and MU-6B had least of 3.0±0.8 x 10^4 cfu/g. At Mile12, M12-11B had 6.0±2.2 x 10^4cfu/g as the only bacterial isolate while M12-4F had highest occurrence of 7.0±2.5 x 10^3 cfu/g followed by M12-5F with 6.0±1.6 x 10^3 cfu/g and M12-6F had the lowest occurrence of 4.0±2.8 x 10^3cfu/g (Table2).

Table2: Microbial Dynamics and Distribution of Test Organisms within Microbial Populations of Soils from Plantain-derived Waste Piles.

Sites	Isolate code	Occurrence (cfu/g)	Identity
Mushin	MU-4B	$58^{*4}\pm2.9(13^{*4}\pm2.9)$	*Klebisella pneumoniae spp pneumoniae* (strain 1)
	MU-6B	$58^{*4}\pm2.9(3^{*4}\pm0.8)$	*Klebisella pneumoniae spp ozaenae*
	MU-11B	$58^{*4}\pm2.9(8^{*4}\pm1.4)$	*Providencia alcaliciens*
Mile 12	M12-9B	$34^{*4}\pm6.5(8^{*4}\pm4.2)$	*Enterobacter aerogenes*
	M12-11B	$34^{*4}\pm6.5(6^{*4}\pm2.2)$	*Klebisella pneumoniae spp pneumoniae* (strain 2)
	M12-4F	$40^{*3}\pm3.3(7^{*3}\pm2.5)$	*Aspergillus flavus*
	M12-5F	$40^{*3}\pm3.3(6^{*3}\pm1.6)$	*Aspergillus fumigatus*
	M12-6F	$40^{*3}\pm3.3(4^{*3}\pm2.8)$	*Aspergillus niger*

$^{*3}= x\ 10^3$, $^{*4}= x\ 10^4$, ±-standard error

Isolation and Primary Screening for Cellulase Producing Bacteria and Fungi

A total of thirty-four isolates were obtained from the two plantain waste disposal sites (Table 3), out of which 14 isolates were removed due to similar colonial and morphological characteristics. The resulting 20 isolates were then tested on CMC agar for cellulase activity and their CMCase activity was shown in Table4. Among them 11 isolates gave the maximum ratio of cleared zone diameter to colony diameter on the CMC agar plate as compared to plates cultured with the other strains. The selected bacteria identified were *Klebsiella pneumoniae* ssp *pneumoniae* (strain 1 & 2), *Klebsiella pneumoniae* ssp *ozaenae, Enterobacter aerogenes* and *Providencia alcalifaciens,* Furthermore, *Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus niger* were identified amongst fungal isolates.

Table 3: Sites for sample collection for cellulase producers.

Sites	Sample no.	Total no. of isolates	Labeled as
MU	1	12	MU-1B,2B,3B, 4B, 5B, 6B 7B,8B, 9B,10B,11B &12B
M 12	1	22	M12-1B, 2B,3B, 4B, 5B, 6B 7B, 8B, 9B, 10 B,11B, 12B, 1F,2F, 3F, 4F, 5F, 6F,7F,8F, 9F & 10F

MU-Mushin (Idi-Oro), M12-Mile 12

Table 4: Zone of Hydrolysis of Different Isolates

S/N	Isolate code	Colony diameter (x) (cm)	Zone diameter (y) (cm)	$(y/x)\ 10^{-3}$
1	MU-4B	2.7	0.6	222
2	MU-6B	3.8	0.4	105
3	MU-11B	2.9	0.8	276
4	M12-9B	3.6	0.4	111
5	M12-11B	3.4	0.4	118
6	M12-4F	5.1	1.2	236
7	M12-5F	4.2	1.3	310
8	M12-6F	5.8	2.2	379

MU-Mushin (Idi-Oro), M12-Mile 12

Secondary Screening and Production of Cellulase Enzyme

On the basis of primary screening the isolates were subsequently evaluated for their enzyme production potential in a submerged fermentation process. For the enzyme activity study, the crude enzyme samples from MU and M12 were assayed by cellulase enzyme assay method.

Production of Cellulolytic Enzymes by Bacterial and Fungal Isolates

Tables 5-7 show the progressive release of reducing sugars from three cellulosic substrates by culture filtrates of *Klebsiella pneumoniae* ssp *pneumoniae* (strain 1&2), *Klebsiella pneumoniae* ssp *ozaenae, Enterobacter aerogenes, Providencia alcalifaciens, Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger*. Generally enzyme activities recorded for all the strains using plantain peel as substrate were significantly higher than those for xylan and carboxymethylcellulose. Furthermore, enzyme activities in culture filtrates increased with time and the age of the culture. Maximum activity peaks were observed between day 6 and 10 as a general pattern, and also between day 6 and 12 of incubation in some strains. The enzyme showed the highest relative activities on plantain peel (987 x 10^{-3} mg/ml at day 10 by *Klebsiella pneumoniae* ssp *pneumoniae* strain 1) followed by CMC (916 x 10^{-3} mg/ml at day 6 by *Klebisella pneumoniae* ssp *pneumoniae* strain 2) and xylan (778 x10^{-3} mg/ml at day 10 by *Aspergillus fumigatus*).

Table 5: Release of Reducing Sugar (mg/ml) from Carboxymethyl-cellulose by Enzymes in the Culture Filtrate of Cellulosic Substrates of *Klebisella pneumoniae* ssp *pneumoniae*(strain l &2), *Klebsiella pneumoniae* ssp *ozaenae, Providencia alcalifaciens, Enterobacter aerogenes*, *Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger*.

Days	Test Microorganisms							
	K.pne.1	*K.pne.2*	*K. Pne.3*	*E. aer*	*P.alc.*	*A.fum.*	*A.flav.*	*A. nig*
2	159	207	263	200	150	131	167	134
4	283	301	326	224	469	233	312	148
6	655	916	661	760	857	373	804	673
8	581	494	445	635	525	451	304	293
10	684	583	657	416	503	690	287	345
12	436	363	375	301	283	281	486	196
14	250	309	204	174	171	141	251	109
16	186	143	145	112	108	108	112	148

*Kl. Pne.*1 =*Klebsiella pneumoniae* ssp *pneumoniae (strain 1)*; *Kl. Pne.*2 =*Klebsiella pneumoniae* ssp *pneumoniae (strain 2); Kl.pne 3= Klebsiella pneumoniae* ssp *ozaenae; E.aer. = Enterobacter aerogenes; P. alc. = Providencia alcalifaciens*; A *nig = Aspergillus niger*; *A flav = Aspergillus flavus and A fum = Aspergillus fumigatus.*

Table 6: Release of Reducing Sugar (mg/ml) from Xylan by Enzymes in the Culture Filtrate of Cellulosic Substrates of *Klebisella pneumoniae*ssp *pneumoniae* (strain l &2), *Klebsiella pneumoniae* ssp *ozaenae, Providencia alcalifaciens, Enterobacter aerogenes*, *Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger*.

Days	Test Microorganisms							
	K.pne.1	*K.pne.2*	*K. Pne.3*	*E. aer*	*P.alc.*	*A.fum.*	*A.flav.*	*A. nig*
2	176	179	108	134	100	75	175	104
4	339	375	421	395	291	218	376	355
6	378	326	587	507	668	519	460	579
8	216	411	487	410	522	653	683	769
10	568	442	547	611	478	778	474	599
12	408	265	328	435	323	505	336	330
14	291	302	237	250	207	270	153	163
16	202	176	147	133	119	140	105	100

*Kl. Pne.*1 =*Klebsiella pneumoniae* ssp *pneumoniae*; *Kl. Pne.*2 =*Klebsiella pneumoniae* ssp *pneumoniae (strain 2); Kl.pne2= Klebsiella pneumoniae* ssp *ozaenae; E.aer. = Enterobacter aerogenes; P. alc. = Providencia alcalifaciens*; A *nig = Aspergillus niger*; *A flav = Aspergillus flavus and A fum = Aspergillus fumigatus.*

Table 7**:** Release of Reducing Sugar (mg/ml) from Plantain Peel by Enzymes in the Culture Filtrate of Cellulosic Substrates of *Klebisella pneumoniae* ssp *pneumoniae*(strain l &2), *Klebsiella pneumoniae* ssp *ozaenae, Providencia alcalifaciens, Enterobacter aerogenes*, *Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger.*

Days	Test Microorganisms							
	K.pne.1	*K.pne.2*	*K. Pne.3*	*E. aer*	*P.alc.*	*A.fum.*	*A.flav.*	*A. nig*
2	207	278	176	141	167	135	459	240
4	491	722	593	358	487	294	359	243
6	814	865	905	645	932	993	668	515
8	952	987	946	792	743	774	683	643
10	987	710	826	910	866	890	903	736
12	817	481	603	619	531	613	806	959
14	471	252	376	470	409	374	579	832
16	162	143	204	232	141	180	139	222

*Kl. Pne.*1 =*Klebsiella pneumoniae* ssp *pneumoniae (strain 1)*; *Kl. Pne.*2 =*Klebsiella pneumoniae* ssp *pneumoniae; Kl.pne2= Klebsiella pneumoniae* ssp *ozaenae; E.aer. = Enterobacter aerogenes; P. alc. = Providencia alcalifaciens*; A *nig = Aspergillus niger*; *A flav = Aspergillus flavus and A fum = Aspergillus fumigatus.*

DISCUSSION

The wastes from a variety of sources especially agriculturally derived-streams find their way into the soil. These wastes end up interacting with the soil system thereby changing its physical and chemical properties (Piccolo and Mbagwu, 1997). As a consequence, the physicochemical properties of soils vary greatly. This in turn affects the "in situ" microbiology thereby having a profound implication on the biodegradation rate of organic matter. It is not surprising therefore that indigenous bacterial and fungal species in decomposed plantain soils were mainly *Klebsiella pneumoniae* ssp *pneumoniae* (strain 1&2)*, Klebsiella pneumoniae* ssp *ozaenae, Enterobacter aerogenes, Providencia alcalifaciens, Aspergillus flavus, Aspergillus fumigatus, and Aspergillus niger*. Yang *et al.* (2009) had reported the adaptation of *Aspergillus niger* tolerating high concentration of heavy metals for bioleaching of fly ash. This study showed that not only fungi proved to be the best cellulase producers but bacteria demonstrated high potentials within the same context.

Ogunyemi *et al.* (2010) had reported on physico-chemical properties of municipal refuse in the Lagos metropolis and cellulolytic activities of resident microorganisms associated with organic matter degradation and established that the test fungi were the only best candidates for cellulolytic activity than bacteria which were mildly cellulolytic. There are limited studies on the physicochemical characteristics of plantain-derived wastes from the Nigerian environment. Reports stated that much of the physical and chemical parameters in such wastes were within the limits specified by relevant local and international regulatory environmental agencies as evident in our results. Iron and Nickel levels were considerably below the permissible level for soils as recommended by Bowen (1979). In a similar study, Kabata-Pendias and Pendias (1994) reported a lower range 30-300 mg/kg for both metals. Nickel and zinc were found to be below the critical permissible concentrations in soils.

The higher population counts of bacteria at the Idi-Oro, Mushin sampling site and higher fungal counts at Mile 12 was an indication that these sites presented a conducive environment for microbial proliferation and biological activity. However, the bulk of cellulolytic activities are attributable to the bacterial and fungal populations. However this is does not preclude the possible existence of other cellulose-decomposing unculturable bacteria and fungi at these sites that evaded detection in this study while it is also possible that some could occur in low numbers.

The enzymatic hydrolysis of cellulosic materials has been widely studied (Wen *et al.*, 2005; Okafoagu and Nzelibe, 2006; Foyle *et al.*, 2007). The production of sugars, biofuels and single cell protein are potential uses of such hydrolysis. Studies on fungal growth on cellulose and hemicellulose waste materials have been carried

out using sawdust, paper, filter paper, corn bran and straw. However, the rate of hydrolysis of cellulosic materials is still relatively slow and much of the present work in this regard is aimed at increasing these rates. In this wise, the cellulolytic activities of the bacterial and fungal strains from this study using shake-flask cultures exhibited a relatively higher rate of cellulase and hemicellulase activities and the same observations were reported by Pye *et al.* (1977).

Cellulosic biomass abundantly provided by nature is a renewable resource for production of fuels and other useful products. This study therefore reports that decomposing plantain-derived wastes may provide a reliable source of microorganisms that could be used in the bioconversion of cellulosic substrates and as a potential source of cellulolytic enzymes for biotechnological application.

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