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Sustainable Solid Waste Management: Isolation of Cellulolytic Microorganisms from Dumpsites in Lagos, Southwest Nigeria

Ojo-Omoniyi^{1*}, Olusola Abayomi, Okwa², Omolade Olayinka, Junaid¹,
Iswat Olaide and Ikuoye¹, Abosede Oyebola

¹Department of Microbiology, Lagos State University, P.M.B. 0001 LASU Post Office,
Lagos-Nigeria

²Department of Zoology and Environmental Biology, Lagos State University, P.M.B. 0001
LASU Post office, Lagos-Nigeria

*Corresponding author

ABSTRACT

This research was to carry out an environmental surveillance for cellulose-degrading microorganisms present in wastes dumpsites in Lagos, southwest Nigeria. Solid waste has become a heavy burden to both government and citizens in developing countries all over the world due to increase in population. The presence of cellulolytic microorganisms and their potential to degrade the complex polymer; cellulose into simple sugars like glucose under optimum environmental conditions were also evaluated. Soil and leachate samples were collected (0 -10cm depth) from three dumpsites in Lagos following aseptic procedures and the microbial species were isolated using Nutrient agar and Potato-dextrose agar media respectively. A total of 34 microorganisms were isolated from the three different sampling locations in Lagos. The cellulase-producing microbial species were characterized following conventional and standard microbiological methods. They were then screened for cellulase activity using the Cellulose Congo-Red plate technique. The diameters of clear zone of inhibition were measured in millimeters (mm). All isolates were cellulase producers, with *Bacillus licheniformis* having 42.5% occurrence, as well as the highest cellulase activity and hydrolytic value (34mm and 8.5 respectively) among the bacterial species while *Aspergillus sp.*(40.0% occurrence) had the highest cellulase activity (63mm) and hydrolytic value (15.8) among the fungal species. This result suggested that these microorganisms utilized the available sources of cellulose present in wastes both for growth, biomass production and biodegradative processes. Consequently, the introduction of these microorganisms into waste recycling processes will enhance the sustainable solid waste management practices for megacities as well as the quality of public health in developing economies.

Keywords

Solid Waste
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Introduction

Municipal solid wastes is composed of 40-50% cellulose, 9-12% hemicelluloses 10-15% lignin on a dry weight basis (Rani and

Nand, 2000; Gautam *et al.*, 2010). These waste materials could be turned from liabilities into assets. Microorganisms which

utilize cellulosic materials for their carbon and energy source could be exploited for the conversion of these wastes into products that are beneficial to man (Belewu and Banjo, 1999; Banjo and Kuboye, 2000; Belewu and Afolabi, 2000).

Microorganisms perform their metabolic processes rapidly with remarkable specificity under ambient conditions catalyzed by their diverse enzymes-mediated reactions. The introduction of microbial enzymes as an alternative to harsh chemical technologies has led to intensive exploration of natural microbial biodiversity to discover microbial enzymes with possible application in waste recycling under appropriate conditions (Gautam *et al.*, 2010).

Cellulase is the key enzyme for the conversion of cellulosic materials into simple sugars which can serve as feed-stock for the production of different chemicals and fuels via anaerobic fermentation (Ryu and Mandels, 1980). Cellulolytic enzymes play an important role in natural biodegradation processes in which plant ligno-cellulosic materials are effectively degraded by cellulolytic fungi, bacteria, Actinomycetes and protozoa.

Many fungi capable of degrading cellulose synthesize large quantities of extracellular cellulases that are more efficient in depolymerising the cellulose substrate. Cellulases produced by bacteria, appear to be bound to the cell wall and are unable to hydrolyze native ligno-cellulose preparation to any significant extent.

Examples of bacteria and fungi capable of producing cellulase include *Trichoderma sp.*, *Humicola sp.*, *Penicillium sp.*, *Aspergillus sp.*, *Pseudomonas sp.*, *Bacillus sp.*, *Staphylococcus sp.*, *Cellulomonas sp.*, etc., (Gautam *et al.*, 2010).

The objectives of the current study is to isolate, characterize and identify the native microbial population in waste dump sites in Lagos as well as to evaluate the potentials of these microorganisms isolated for cellulase production.

Materials and Methods

Collection of soil samples and leachates

Soil and leachate samples were collected from three dumpsites under the control of Lagos State Waste Management Authority (LAWMA), Lagos state. The dumpsites were; Hotel Soluos –Igando Landfill site in Alimosho Local Government Area, Olushosun Landfill site in Kosofe Local Government Area and Ibeshe -Ikorodu in Ikorodu Local Government Area. These dumpsites are labeled A, B and C respectively.

The soil and leachate samples were collected in the morning from each dumpsite between 10-11am. Soil samples were collected aseptically from a depth of 0- 20cm with sterile soil auger. The temperature of the refuse dump in each location was monitored with a thermometer and the mean temperature range was recorded for each dumpsite. The soil samples were labeled A, B and C while the leachate samples were labeled AL, BL, and CL respectively. The samples were transported to the laboratory immediately for microbiological analysis.

Isolation of Microorganisms from Soil Samples and Leachates

The collected soil and leachate samples were serially diluted until 10^{-6} dilutions was achieved. However, 1ml sample was taken from diluent 10^{-4} and 10^{-6} and this was inoculated on sterile Nutrient agar media (NA) plates and Potato dextrose agar (PDA)

plates in duplicates using spread plate technique and this was followed by incubation at $30 \pm 2^\circ\text{C}$ for 24-48 hours for the Nutrient agar plates and the PDA plates were incubated at $30 \pm 2^\circ\text{C}$ left for 4-8 days. After incubation, the plates were examined for growth and the numbers of colonies were counted to give the viable count of microorganisms in the samples. Representative colonies were purified by repeated streaking on NA and PDA plates, the isolated bacteria and fungi were identified on the basis of their colonial morphology, microscopic morphology and biochemical tests. The identified strains were maintained on PDA and NA slants at low temperature (4°C) (Gautam *et al.*, 2012).

Biochemical Characterization

Biochemical tests were performed in order to identify the isolates using Gram staining, catalase test, citrate test, indole test, Urease test, oxidase test, MetyhlRed-Voges-Proskauer test, gelatinase test, starch hydrolysis test and sugar fermentation.

Microscopic examination of fungal cells

Each fungus isolated was sub-cultured on Potato dextrose agar (PDA) and characterized. Thee spore head, color of mycelium, characteristics of hyphae were observed. Pieces of young mycelium from the periphery of each culture were cut with sterile razor blade and placed on clean glass slides. Cut section were flamed briefly to melt the agar and then stained with lacto phenol cotton blue, cover slips was then placed over each slide and examined under the microscope by the x40 objective lens (Cheesbrough, 2004).

Screening for Cellulolytic Activity

Confirmation of cellulose degrading ability of the bacteria and fungi isolates was

performed by inoculating their pure cultures in the center of Cellulose Congo Red agar with the following composition: KH_2PO_4 , MgSO_4 , Cellulose, agar, gelatin, and distilled water at pH 6.8-7.2. Isolates showing discoloration of Congo-Red were taken as positive cellulose- degrading bacteria and the diameter of the clearance zone was measured with the aid of a calibrated meter rule and recorded. Cellulose-degrading potential of the positive isolates was also qualitatively estimated by calculating hydrolysis capacity (HC), that is, the ratio of diameter of the clear zone and colony (Lu *et al.*, 2005).

Results and Discussion

The standard and conventional methods for microbiological analysis revealed through morphological and biochemical tests that bacterial isolates were of eight different (8) genera (Table 1).

The colonial and microscopic characterization of the fungal isolates showed that they belong to the genera; *Penicillium*, *Aspergillus* and *Trichoderma* (Table 2). Pure culture of fungal isolates are presented in Figure 1.

The percentage of occurrence of the bacterial and fungal isolates from the three dumpsites studied were recorded with *Bacillus* sp. having the highest value (42.5%) among the bacterial isolates while *Aspergillus* sp. ranked the highest among the fungal isolates (40.0%) (Tables 3 and 4).

Bacillus licheniformis showed the highest zone of clearance (34mm) and hydrolytic value (8.5) while *Streptococcus* sp. showed the least values (7mm and 2.3 respectively) (Table 5). However, the fungal isolates with the least clearance zone and hydrolytic value was *Penicillium* sp. (39mm and 7.8

respectively) while *Aspergillus* sp. (63mm and 15.8 respectively) had the highest cellulase-producing potential and hydrolytic value (Table 6).

This present research revealed the presence of various bacterial and fungal species known to be associated with cellulose degradation in wastes dumpsites. A total of 34 microorganisms were isolated. However, 19 of these isolates were bacterial species and 15 fungal species, these species belonged to the genera: *Bacillus*, *Corynebacterium*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Enterobacter*, *Aeromonas*, *Klebsiella*, *Trichoderma*, *Aspergillus*, and *Penicillium*. All isolates reported in this study have also been reported to be associated with waste and waste biodegradation (Obire *et al.*, 2002).

The temperature of soil samples from all dumpsites ranged between 30⁰C and 34⁰C and this falls within the mesophilic temperature (20 - 45⁰C). The temperature range obtained in this study is within the range reported by Lu *et al.*, (2005) who showed that 15 cellulose degrading bacteria isolated from vegetable wastes were within mesophilic temperature range. All isolates were screened for cellulase activity by using the Cellulose Congo Red plate technique. The result showed that all tested isolates were cellulase producers but their ability to degrade cellulose differs from organism to organism, and this was indicated by the diameter of the clearance zone presented by each isolate on the cellulose Congo red plates (Lu *et al.*, 2005).

In the bacterial group, *Bacillus* species had the highest occurrence (42.5%) in all dumpsites compared to other isolates because they are indigenous to soil environment and have the ability to survive harsh environmental conditions. This observation agrees with the findings of Holt

(1994) who reported that the abundance of strains of *Bacillus* genus in refuse samples is not surprising since this ubiquitous genus is known to include cellulolytic species and are commonly found in soil and plant litter as well as compost where they play major role in biodegradation and bio-conversion of macromolecules. In addition, Beg and Gupta (2003) reported them to be the most important source of several enzymes aside from *B. cereus* and *B. anthracis*. They have been considered safe to humans.

The cellulolytic bacteria isolates encountered in this study showed a hydrolytic capacity value (HC) between 2.3 and 8.5 and this is similar to that reported by Lu *et al.*, (2005) for mesophilic cellulase degrading bacteria. Fungal isolates presented a higher HC value than the bacteria with value between 7.8 and 15.8.

Maximum cellulase activity was also observed in *Bacillus* species with diameter of clearance zone ranging between 20mm-34mm in respect to other bacteria isolates. Among these, *Bacillus licheniformis* presented the largest zone (34mm), followed by *Bacillus sphaericus* (27mm) and *Bacillus subtilis* (26mm). This finding is in line with the report of Das *et al.*, (2010) who isolated 8 bacteria strains from cow dung samples and observed maximum cellulase activity among *Bacillus* species as well as *Pseudomonas*, *Klebsiella*, *Staphylococcus*, *Coryne-bacterium*, *Aeromonas* and *Enterobacter* were found to be moderate cellulase producers with diameter of clearance zone ranging between 18mm and 25mm while *Streptococcus* sp. showed the lowest value between 7mm and 9mm.

Cellulolytic fungal isolates encountered in this study showed a higher diameter of clearance zone than the bacteria isolates with *Aspergillus* sp. having the highest value (63mm), followed by *Trichoderma* sp.

(58mm) and *Penicillium* sp. (48mm). This agrees with the findings of Kang *et al.*, (1999) who reported that *Aspergillus* strains have the ability to produce β -glucosidase with significantly higher yields than *Trichoderma* species and they have a number of characteristics which makes them the ideal organisms for industrial applications such as fermentation capabilities, ability to assimilate various organic substrates and suppress development of other organisms and high sporulation capacity.

Gautam *et al.*, (2010) studied on the isolation and screening of cellulolytic fungi from municipal solid waste. Out of 20 fungal culture isolates from environmental sources including 8 different zones, 16 fungi were found to possess cellulose degrading ability. Result obtained during this study clearly suggested that cellulase activity of *Aspergillus fumigatus* and *Trichoderma* sp. were found relatively towards the higher side and *A. niger*, *A. flavus*, *A. nidulans*, *Alternaria* sp., and *Penicillium* sp. showed moderate range activity while *Fusarium* sp., *Humicola* and *Torulasp.*, showed low cellulase activity.

This result suggested that fungi are better cellulase producers than the bacterial species and this was in accordance with the findings of Guatam *et al.*, (2009) who discovered that many fungi capable of degrading cellulose synthesize large quantities of extracellular cellulases that are more efficient in depolymerizing the cellulose substrate unlike cellulases produced by bacteria which appear to be bound to the cell wall and are unable to hydrolyze native lignocelluloses preparation to any extent.

In conclusion, the conversion of cellulolytic biomass by microorganism is a potential sustainable approach to develop novel bio-processes and products. Microbial cellulases are now commercially produced by several industries globally and are widely used in food, animal feed, fuel, paper industry and also various chemical industries. Cellulases are important in waste recycling processes for efficient waste to energy systems.

This study showed that waste dumpsites are source of cellulolytic microorganisms. Among all the isolates, *Bacillus* species, *Aspergillus* sp., and *Trichoderma* sp. have shown high cellulase activity compared to other isolates. These organisms are recommended as source of cellulases which may be harnessed for industrial production of the enzyme as well as management of solid wastes containing cellulose.

The result of this investigation is useful to industries that use cellulases (textile, laundry, detergents, pulp and paper industries) since microorganisms are cheap natural resource and also environmental agencies that are concerned with solid wastes management.

Environmental agencies can take advantage of the result of this investigation and then invest in further studies which could lead to the isolation and characterization of specific high yielding bacterial and fungal strains found in the local soil environments. This will go a long way in reducing the cost of management of solid wastes. However, further studies needed to be carried out to determine quantitatively the catalytic activity of the cellulases produced by each of these organisms.

Table.1 Cultural morphology and biochemical characteristics of bacterial isolates

Isolate Code	Colour	Gram reaction	Cellular morphology	Catalase test	Oxidase test	Indole test	Citrate utilisation	Urease test	Methyl red test	Vogesprosk auer test	Gelatin hydrolysis	Starch hydrolysis	glucose	sucrose	lactose	Probable Identity
Aa 10 ⁻⁶	Cream-white	+	cocci	+	+	-	-	-	-	+	+	-	+	+	+	<i>Staphylococcus albus</i>
Aa 10 ⁻⁴	Brown-green	+	cocci	-	-	+	+	+	-	-	+	+	+	+	+	<i>Streptococcus</i>
Ba 10 ⁻⁶	cream	+	rods	+	+	-	+	-	-	+	+	+	+	+	+	<i>Bacillus subtilis</i>
Ba 10 ⁻⁴	cream	+	rods	+	-	-	+	-	-	-	+	+	+	+	-	<i>Bacillus licheniformis</i>
Ca 10 ⁻⁶	cream	+	rods	+	+	-	-	+	-	+	+	-	+	-	-	<i>Bacillus sphaericus</i>
Ca 10 ⁻⁴	Green	-	rods	+	+	-	-	-	-	-	+	-	+	+	-	<i>Pseudomonas cepaciae</i>
AL10 ⁻⁴ A	Cream	-	rods	+	-	-	+	-	-	+	+	-	+	+	+	<i>Klebsiella aerogenes</i>
AL10 ⁻⁴ B	Cream	-	rods	+	+	-	+	-	-	+	+	-	+	-	-	<i>Bacillus coagulans</i>
AL10 ⁻⁴ C	White	-	rods	+	-	-	+	-	-	+	-	-	+	+	+	<i>Enterobacter aerogenes</i>
AL10 ⁻⁶ A	cream	-	rods	+	-	-	+	-	-	+	+	+	+	+	-	<i>Bacillus licheniformis</i>
AL10 ⁻⁶ B	Cream	+	rods	+	-	-	+	-	-	+	+	+	+	+	-	<i>Bacillus licheniformis</i>
AL ₁ 10 ⁻⁶ C	Cream	-	rods	+	+	+	-	-	-	+	+	+	+	-	+	<i>Aeromonas</i>
BL10 ⁻⁴ A	Cream	+	Coccoid rods	+	+	-	-	-	-	+	+	+	+	+	+	<i>Corynebacterium stratum</i>
BL10 ⁻⁴ B	Yellow	+	cocci	+	-	-	-	+	-	+	-	-	+	+	+	<i>Staphylococcus aerus</i>
BL10 ⁻⁶ A	Cream	+	Rods	+	+	-	+	-	-	+	+	+	+	+	+	<i>Bacillus substilis</i>
BL10 ⁻⁶ B	Cream	+	rods	+	-	-	+	-	-	-	+	+	+	+	-	<i>Bacillus licheniformis</i>
CL10 ⁻⁴ A	Cream			+	-	-	+	-	-	-	+	-	+	+	+	<i>Klebsiella aerogenes</i>
CL10 ⁻⁴ B	Green	-	rods	+	+	-	+	-	-	-	+	-	+	-	-	<i>Pseudomonas aeruginosa</i>
CL10 ⁻⁶	Cream	+	cocci	-	-	+	+	+	-	-	+	+	+	+	+	<i>Streptococcus</i>

Table.2 Colonial and microscopic characteristics of fungal isolates

Fungal Isolates	Macro- culture (colonial characteristics)	Microscopy (morphological features)
<i>Penicillium species</i>	Blue green or ash flaky colonies	Septate hyphae, branched conidiophores, a brush like colonial head
<i>Aspergillus niger</i>	Large black head (thick black colonies)	Septate hyphae, non- septate conidiophores, mop- like conidial head
<i>Trichoderma species</i>	White colonies	Branched conidiophores and aerial hyphae

Table.3 Occurrence of bacterial isolates in dumpsites

Isolated organism	A	AL	B	BL	C	CL	Total number of isolates	% of occurrence
<i>Staphylococcus</i>	1	ND	ND	1	ND	ND	2	10.5
<i>Streptococcus</i>	1	ND	ND	ND	ND	1	2	10.5
<i>Bacillus</i>	ND	3	2	2	1	ND	8	42.5
<i>Enterobacter</i>	ND	1	ND	ND	ND	ND	1	5.2
<i>Aeromonas</i>	ND	1	ND	ND	ND	ND	1	5.2
<i>Corynebacterium</i>	ND	ND	ND	1	ND	ND	1	5.2
<i>Pseudomonas</i>	ND	ND	ND	ND	1	1	2	10.5
<i>Klebsiella</i>	ND	1	ND	ND	ND	1	2	10.5

KEYS:

% = Percentage

ND = Not determined

B = Olushosun soil sample

C= Ibeshe soil sample

A= Hotel soluos soil sample

AL= Hotel soluos leachate sample

BL= Olushosun leachate sample

CL= Ibeshe leachate sample

Table.4 Occurrence of fungal isolates in dumpsites

Isolated organism	A	AL	B	BL	C	CL	TOTAL NO. ISOLATES	% of OCCURRENCE
<i>Penicillium</i>	ND	1	1	1	ND	1	4	26.7
<i>Aspergillus</i>	1	ND	2	1	1	1	6	40.0
<i>Trichoderma</i>	1	ND	1	1	2	ND	5	33.3

Keys:

% = Percentage

ND = Not determined

B = Olushosun soil sample

C= Ibeshe soil sample

A= Hotel soluos soil sample

AL= Hotel soluos leachate sample

BL= Olushosun leachate sample

CL= Ibeshe leachate sample

Table.5 Mean diameter of clearance zone and hydrolytic capacity value for cellulolytic bacteria

Isolate code	Mean diameter of clearance zone (mm)	Hydrolytic capacity
Aa 10 ⁻⁶	18	6.0
Aa 10 ⁻⁴	7	2.3
Ba 10 ⁻⁶	24	8.0
Ba 10 ⁻⁴	24	8.0
Ca 10 ⁻⁶	27	6.8
Ca 10 ⁻⁴	18	3.6
AL 10 ⁻⁴ A	18	4.5
AL 10 ⁻⁴ B	20	6.7
AL 10 ⁻⁴ C	25	8.3
AL 10 ⁻⁶ A	31	7.8
AL 10 ⁻⁶ B	34	8.5
AL ₁ 10 ⁻⁶ C	24	4.5
BL10 ⁻⁴ A	25	6.3
BL 10 ⁻⁴ B	23	5.8
BL 10 ⁻⁶ A	26	6.5
BL 10 ⁻⁶ B	33	8.3
CL 10 ⁻⁴ A	20	4.0
CL 10 ⁻⁴ B	16	5.3
CL 10 ⁻⁶	9	2.3

KEY:

A= Hotel soluos soil sample

AL= Hotel soluos leachate sample

B = Olushosun soil sample

BL= Olushosun leachate sample

C= Ibeshe soil sample

CL= Ibeshe leachate sample

Table.6 Mean diameter of clearance zone and hydrolytic capacity value for cellulolytic fungi

Isolates code	Diameter of zone of Clearing(mm)	Hydrolytic capacity
CL 10 ⁻⁶	39	7.8
AL 10 ⁻⁴	41	10.3
Cb 10 ⁻⁴	63	15.8
Ca 10 ⁻⁴	55	11.0
Ab 10 ⁻⁶	53	13.3
Ca 10 ⁻⁶	58	11.6

KEY:

A= Hotel soluos soil sample

AL= Hotel soluos leachate sample

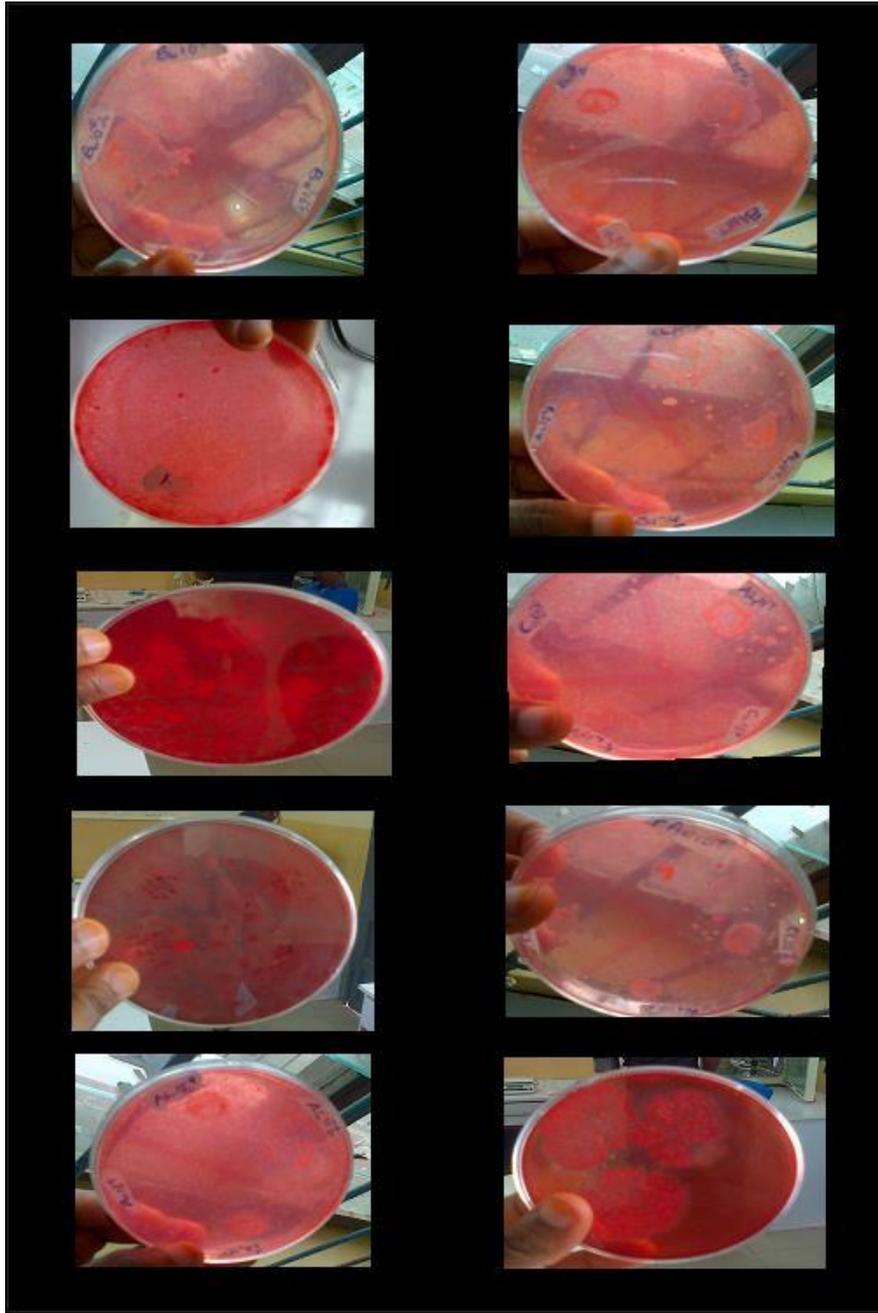
C= Ibeshe soil sample

CL= Ibeshe leachate sample

Fig.1 Pure Culture of Fungal Isolates



Fig.2 Bacterial Isolates with cellulase activity



Also, future research would seek to extract the DNA sequence of these cellulolytic microorganisms to develop a DNA probe from that for enhanced environmental surveillance for this group of organisms as well as their integration into waste-to-energy programme.

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