

Studies on degradation of waste papers using microflora/microbial consortia isolated from refuse dumpsites in Ilorin metropolis

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Abstract. A total of ninety samples of domestic solid waste from five domestic waste dumpsites at Taiwo, Agbo Oba, Baboko, Unilorin and Tanke in Ilorin metropolis were collected over a six month period at two weeks interval, characterized and analyzed. Baboko has the highest mean waste composition of paper and cardboard with the lowest being from Tanke. Five fungal genera and five bacterial species were identified from the wastes. Food wastes have the highest percentage of 63.30% while wood has the lowest of 0.08%. Glass and paper had the highest mean percentage of non-combustible component of 99.98% each, while leather had the highest ash of 18.44%. *Escherichia coli* and *Aspergillus niger* had the highest occurrence in both the rainy and dry seasons of the bacterial isolate and fungal isolates respectively. *Bacillus*, *Aspergillus* and *Mucor* species were found to be the most widely distributed on all the wastes. Degradation was monitored through glucose production in enrichment media where *Bacillus cereus* was identified as the only bacterial isolate that can degrade paper while *A. niger* was found to be the most active degrader of the waste papers of the fungal isolate. All fungal isolate except *Mucor mucedo* and *Rhizopus stolonifer* were able to degrade waste papers to different degrees. In mixed culture, glucose production was highest with *Aspergillus* and *Penicillium* consortium. It is concluded that microorganisms present in domestic wastes could be used either singly or in consortium for waste degradation thereby proving to be a key to a clean and safe future world.

Keywords: Wastes, enrichment media, degrader/biodegradation, glucose production, consortium.

INTRODUCTION

Waste is any unavoidable material resulting from domestic activities or industrial operations for which there is no economic demand and which must be disposed off (Sridhar, 1996). Waste is any material which though may no longer be needed somewhere but may become a feedstock or raw material elsewhere (Odocha, 1994). Getting rid of household rubbish has always been a problem but only recently has it reached epidemic proportions.

For thousands of years, people managed solid waste by gathering it up, carting it out and dumping or burying it in isolated places. Crude as it was, this system worked because most of the waste consisted of biodegradable organic compounds that decomposed easily. In addition, the volume of rubbish was much lower than now because there were fewer people and less packaging materials (Kujawa, 2002; Sabiiti, 2011; Adeyemo and Sani, 2013).

Over the last few decades, new synthetic and hazardous materials have been introduced into the waste stream, this has complicated the problem since many of these materials are not biodegradable and some produce toxic residues that has led to tighter environmental controls on landfills and during incineration. With open space in shortage, many communities are literally drowning in municipal solid wastes.

Among the industrialized nations, Americans are by far the world's largest waste makers. New Yorkers hold the record for producing the most garbage per person per day. In Nigeria, average waste generated in big cities is approximately 0.46 kg/person/day, while in Ilorin, a person generates 0.11 kg on the average with a maximum household waste output of 0.77 kg/person. The waste generation for major hotels in Ilorin varies from 10 to 50 kg/day, supermarket generate 3 to 5 kg/day on the average while institutions generate between 17 and 20 kg/day. The university teaching hospital Ilorin generates nearly 230 kg/day while over 1000 kg/day is generated at the Ipata Abattoir (Ciwat, 2000). In Nigeria with reference to Ilorin Township, there are so many sub-urban areas which are mostly for residential purposes and fairly thickly populated. Generally, there is lack of proper housing and community planning and this invariably leads to an increase improper waste disposal due to inadequate disposal systems in these areas. Most people dump their garbage and other domestic solid wastes on the street and roadsides and around their houses, this practice constitute not only a nuisance but also a hazard to public health as they increase air and water pollution and also lead to increase in rodent and insect infestation which are often vectors of causative agents of many diseases which adversely affect community life and development. The wastes dumped into open drains causes blockage of drainage systems leading to stagnant pools which harbors the breeding of vectors like mosquitoes. During decomposition, toxic gases like sulphur (IV) oxide are released to the atmosphere which can give rise to health and environment hazards with health implications as bronchitis, tuberculosis, food poisoning and yellow fever (Sridhar, 1996; Adeyemo and Sani, 2013).

Biodegradation is the complete microbial breakdown or mineralization of complex materials into simple inorganic constituents such as CO₂, water and mineral components. This allows the recycling of such necessary building blocks such as carbon, hydrogen, oxygen, nitrogen and sulfur which might be tied up as complex non-biodegradable substances and thus unable to enter the natural cycles without the activities of the microorganisms (Bridgewater and Bollag, 1992; Omojasola et al., 2009).

Microorganisms are capable of degrading various wastes and therefore have been developed to be of use in recycling domestic solid waste as well as toxic substances, the paramount role of microorganisms in the global recycling of carbon and other elements has long been recognized (Layokum et al., 1990; Raji and Okeniyi,

1998; Bukoye, 2002). The diverse metabolic activities of microorganisms and in particular their ability to interact with complex organic and inorganic substrate are now being exploited in the treatment of solid wastes (David et al., 1992). Biological technologies specifically those on the activities of microorganisms have been viewed with increasing favour as a method for treatment of hazardous and domestic solid wastes. These technologies have a number of advantages. Firstly, microorganisms tend to be specific both with their respect to the reactions involved allowing a process based on their ability to be fairly predictable; secondly, they are selective in allowing the treatment of a specific component within heterogeneous wastes; and thirdly they are flexible in that they carry out their attack despite fluctuations in the waste composition.

MATERIALS AND METHODS

Sample collection

All the samples were collected by the use of shovels, filling the bucket with refuse materials after which the materials were then overturned from the bucket into the polythene bags. 15 samples were collected on each trip from the five dumpsites at two weeks interval. Altogether, a total of 90 samples were collected from the dumpsites, which were then transported to the laboratory for further analysis.

Sample characterization

Each bagful of waste was weighed and the components were separated by handpicking. The wastes were also separated to putrescible and non-putrescible. The samples were dried and reweighed and the weights recorded.

Isolation and purification of seed bacteria and fungi

Spot inoculation of materials like polythene bags, clothing etc, were made on Potato Dextrose Agar and incubated at room temperature 27°C for 72 h, for the isolation of fungi. Samples were placed in universal bottle containing sterile distilled water and shaken thoroughly. The mixture was allowed to settle and the supernatant decanted off into separate tubes, this served as stock solution. Serial dilutions of the stock were made and 0.1 ml aliquot was spread on PDA plate containing 1% streptomycin (to inhibit bacterial growth) using a sterile glass spreader. The inoculated plates were incubated at 27°C for 72 h for fungi. From the stock culture, 0.1 ml aliquot were taken and spread on Nutrient Agar plate and incubated at 37°C for 24 to 48 h for bacterial growth. Representative fungal and bacterial colonies were transferred on fresh PDA and NA, respectively. The purified isolates were transferred to PDA and NS slants which were incubated at room

temperature for 24 h, for fungi and bacteria. They were then stored at 4°C.

Characterization and identification of fungal isolates

Isolated colonies were observed macroscopically and microscopically. Macroscopic examination was based on colonial characteristics such as colony, shape, elevation and edge. For microscopic identification, scrapings from the pure cultures were teased on clean grease free slides and stained with lactophenol cotton blue solution. The prepared slides were covered with clean grease free cover slips and examined microscopically (using ×40 objectives) for their characteristic features such as type of hyphae (whether septate, coenocytic or branched), asexual reproductive structures (whether borne on sporangia, conidia, in chains or singly) isolated fungal colonies were identified according to the scheme of McGinnins (1980) for both microscopic and macroscopic examinations.

Characterization and identification of bacterial isolates

The bacterial isolates were differentiated first on the basis of colonial morphology (shape, colour, edge, translucency, elevation, size and surface texture) followed by microscopic examination. All the isolates were identified according to Bergey's manual of determinative bacteriology.

Cell morphology

The following staining tests were carried out on each of the bacterial isolates

Gram staining

A smear of each isolate from 24 h old culture was made on slide and heat fixed. The slide was then flooded with the primary stain (crystal violet) for 60 s washed off and Longol's iodine was added for another 60 s and washed off. The smear was decolorized rapidly with Gram's alcohol and then counter stained with 1% safranin for 40 s after which the stain was washed off in running water. Dried smear was examined microscopically under the oil immersion objective (×100). The gram reaction and morphology of the organisms were observed.

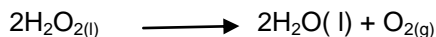
Motility test

A hanging drop preparation of each isolate from an overnight broth culture of the organism inside peptone

water medium was made using a cavity slide. The slides were then examined microscopically for motility of bacteria.

Catalase test

This was used for detecting presence of catalase enzyme in the isolates. The catalase enzyme catalyse the breakdown of hydrogen peroxide to release free oxygen and water.



A few drop of hydrogen peroxide was added to smear of 24 h old culture on a slide. Production of white froth shows a catalase positive reaction while absence of the white froth indicates a negative reaction.

Spore stain

This was done using the Schaeffer-Fulton method. Bacteria smear was heat fixed and flooded with malachite green. The stained slide was gently heated till it just started to bubble. Stain was washed off and counter stained with safranin. Bacterial spores stained green while vegetative cells stained red on microscopic examination.

Sugar fermentation tests

The sugars used are lactose, sucrose, glucose and fructose. 1 g of the desired sugar was added to 100 ml of peptone water broth in a conical flask. 1 ml of Andrade's indicator was added to each flask. The solutions were then dispersed into labeled tubes. Durham's tubes were put in inverted positions in each of the sugars solutions which were then sterilized by free steaming for 10 min. The preparations were allowed to cool and then inoculated overnight. Presence of acid was shown by a pink colour and gas production indicated by gas bubbles in the Durham tube. Negative results were the normal straw colour of the peptone water broth with no gas in the Durham tube.

Substrate pretreatment

A modified method of Ali et al. (1991) was used which involves alkaline and steam treatments of the substrates

Alkaline and steam pretreatment of waste paper

Waste papers were washed thoroughly with water to remove dirt and then dried at 70°C and cut into tiny pieces and

thereafter autoclaved for one hour at 121°C with 5% (w/v) NaOH in separate conical flasks for delignification. The autoclaved materials were filtered through muslin cloth, neutralized with dilute acids (0.1 M H₂SO₄), and then washed with water. They were finally washed in distilled water and dried at 70°C in a regulated oven (Gallenkamp). It was then grinded with domestic blender (Nakai, Japan Mx- 736) for increased surface area.

Cultivation of selected organisms for cellulase production

Mineral salts media (MSM) for cultivation of fungal isolates was prepared with compositions as shown below (g/L). KH₂PO₄, 10 g; (NH₄)₂SO₄, 10.5 g; MgSO₄·7H₂O, 0.3 g; CaCl₂, 0.5 g; FeSO₄, 0.013 g; MnSO₄·H₂O 0.04; ZnSO₄·7H₂O 0.04; Yeast extract 0.5 g; treated waste paper (40 g). One hundred and fifty milliliter of each medium was dispersed into conical flask and sterilized in the autoclave at 121°C for 15 min. The final pHs of the medium was adjusted to 5.0 with 0.1 M NaOH and 0.1 M HCl using a pH meter.

Inoculum development

The inoculum was developed by transferring a loopful of the respective organism into one hundred milliliter of the mineral salt glucose medium. This was inoculated at room temperature (27 to 32°C) in an orbital shaker (Gallenkamp, England) at 100 rpm for 72 h for fungi and at 37°C for bacteria.

Hydrolysis

Five millimeter of the developed inoculum of each organism was transferred into 150 ml of each of the hydrolyzing medium containing treated waste paper in five conical flasks while the sixth flask contained a mixed culture of five cellulose degrading isolates. The culture media were incubated at room temperature (27 to 32°C) in an orbital shaker (Gallenkamp, England) at 100 rpm for seven days. Four consortia were also prepared and degree of degradation monitored by glucose production.

Determination of glucose content using glucose-oxidase (enzymatic) method

Five milliliter of the supernatant obtained after suction was employed for the determination of its glucose content. The glucose-oxidase enzymatic method promotes the oxidation of glucose to gluconic acid with the production of an equivalent amount of hydrogen peroxide. In the presence of peroxidase, oxygen (nascent) from peroxide is transferred to a suitable chro-

mogen acceptor with the production of a coloured end product which was measured colourimetrically at 550 nm. The intensity of the colour is proportional to the concentration of the glucose. The glucose content was evaluated by comparing with values from standard glucose curve.

Determination of moisture content of sample

Moisture was determined by the loss in weight that occurs when the sample was dried to a constant weight in a regulated oven (Gallenkamp). Five gram of the sample was weighed into a silica dish previously dried and weighed. It was then dried in a regulated oven (Gallenkamp) at 135°C for 2 h, cool in a desiccator and weighed again. The drying and weighing continued until a constant weight was achieved (Cullison, 1982; AOAC, 2000).

$$\% \text{ Moisture} = \frac{\text{Weight of sample + dish before drying} - \text{weight of sample + dish after drying}}{\text{Weight of sample taken}} \times 100$$

The dry matter (DM) was calculated as:

$$\% \text{ DM} = 100 - \% \text{ Moisture.}$$

RESULTS AND DISCUSSION

Experimental evidence presented in this work showed that the larger percentage of waste generated in Ilorin is vegetable and putrescible (Figure 1). This is a characteristic feature of a developing nation. This result is in agreement with earlier results by researchers who posited that industrialized nations are known to throw away more packaging materials while developing nations generate more of the organic wastes hence the wastes generated in a community may be used to determine the standard of living in such communities (Hagerty, 1973; Egun and Atinmo, 1993; Falomo, 1995). From the result, it is obvious that Nigeria is fast developing and packaging material are on the increase, this was obvious as the percentage of packaging materials were far higher than that obtained in the 80s (Bridgewater and Mumford, 1979). Results revealed that wastes generated in Ilorin is made up of over 70% food waste, hence if composting plant are designed and given high priority, the compost produced can then be used to fertilize farm lands, tennis lawn and pitches where grasses are grown; this would then ensure that much wealth is made out of wastes as also suggested by Jeunesse (2000). Masses and volumes occupied by these wastes were found to reduce averagely by 70% for non-putrescible wastes when dried while those of putrescible were found to be reduced to between 55 and 60% when sundried and burnt properly (Figure 2). This result is in accordance with that of bridgewater who posited that wastes will reduce averagely by 60% if properly sundried and burnt (Bridgewater and

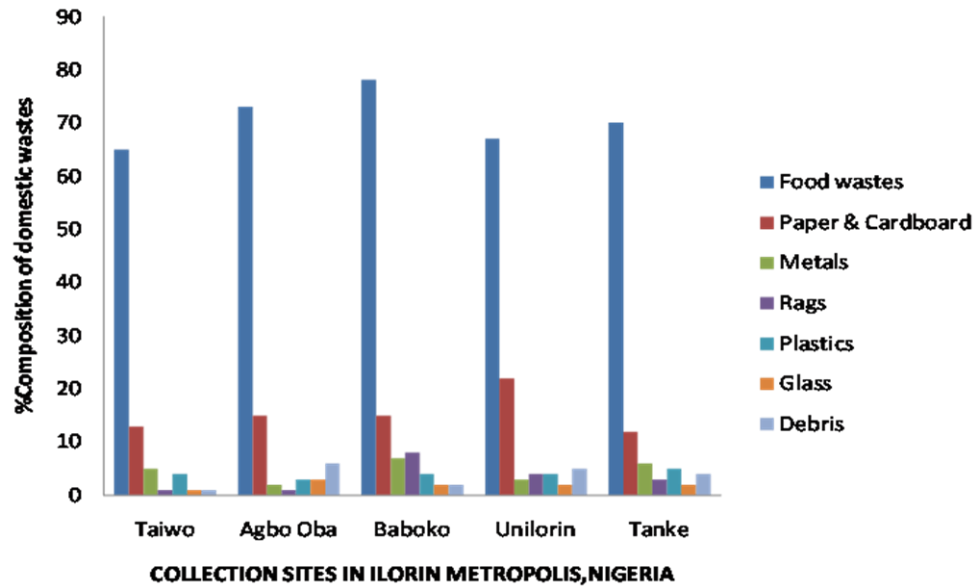


Figure 1. Mean waste composition (in percentages) of the selected sites.

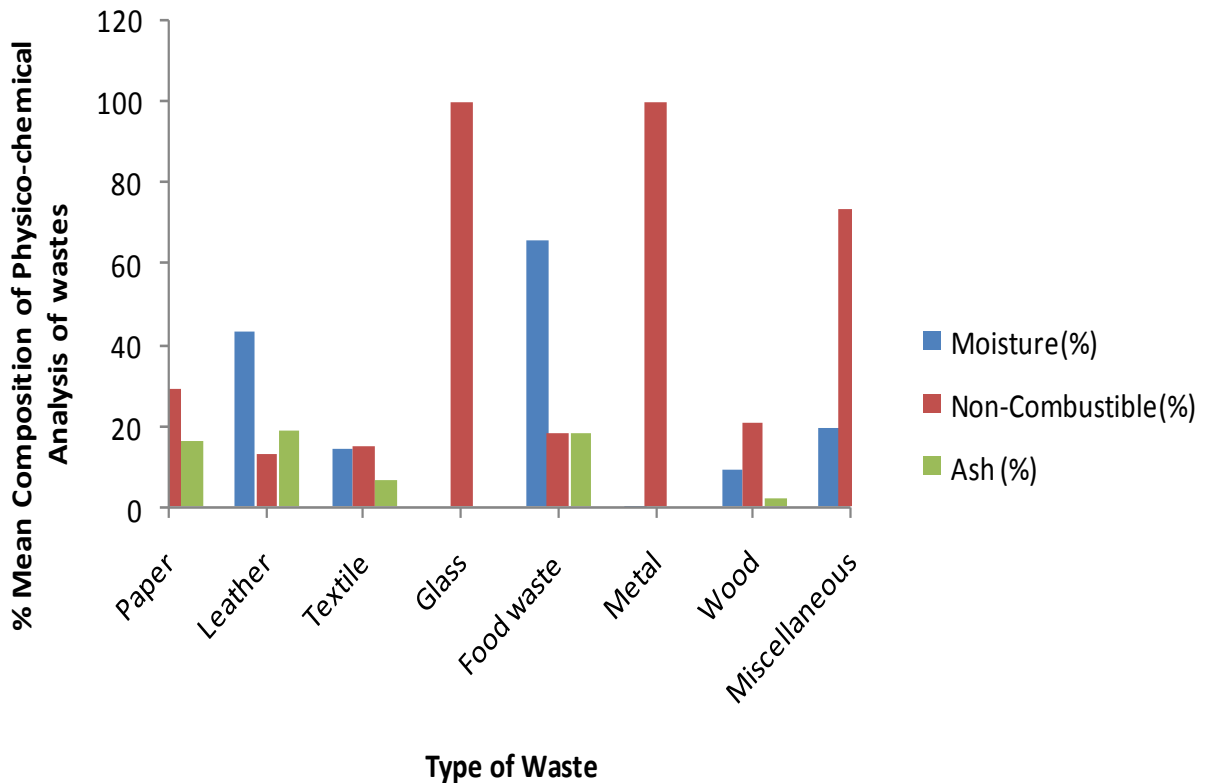


Figure 2. Results of physico-chemical analysis of collected wastes in Ilorin Metropolis.

Mumford, 1979). However, burning of waste as a disposal method is not acceptable as harmful gases such as SO_2 , CO and CO_2 have been reported in some other works to be released during burning thereby polluting the environment and even impairing the respiratory tract of

man (Bremser, 1975).

In Ilorin, there is poor hygienic condition, faecal matters are even thrown on the dumpsites near the houses, it is therefore easy to contact diseases through surface run off when rainfall carry some of the wastes to nearby

Table 1. Incidence of fungi on the collected wastes.

Type of waste	<i>Aspergillus niger</i> ($\times 10^8$)	<i>Aspergillus flavus</i> ($\times 10^8$)	<i>Aspergillus fumigatus</i> ($\times 10^8$)	<i>Penicillium chrysogenum</i> ($\times 10^8$)	<i>Penicillium italicum</i> ($\times 10^8$)	<i>Mucor mucedo</i> ($\times 10^8$)	<i>Rhizopus stolonifer</i> ($\times 10^8$)	<i>Fusarium solani</i> ($\times 10^8$)
Polyethene	2	1	-	-	-	3	2	-
Bags	5	3	-	-	-	7	3	-
Papers	7	5	2	1	1	1	1	-
Food Items	8	7	5	4	2	12	9	4
Clothing	6	8	6	3	4	8	5	4
Bottles	3	-	-	-	-	6	6	-
Egg shells	6	4	2	2	2	5	2	1
Tin can	3	-	-	-	-	3	-	-
Leaves	8	6	5	-	-	6	4	2
Shoes/leathers	5	3	1	-	-	3	3	1
Cassava peel	6	4	4	8	9	-	-	4
Metal scraps	1	-	-	-	-	2	2	-
Floor tiles	-	-	-	-	-	-	-	-
Maize husks	3	2	-	-	5	-	-	-

Table 2. Incidence of bacteria on the collected wastes.

Type of waste	<i>Bacillus cereus</i> ($\times 10^8$)	<i>Proteus vulgaris</i> ($\times 10^8$)	<i>Escherichia coli</i> ($\times 10^8$)	<i>Klebsiella pneumoniae</i>	<i>Streptococcus faecalis</i>
Polyethene	3	2	2	-	-
Bags	4	-	3	1	-
Papers	2	-	2	1	-
Food Items	2	-	1	-	1
Clothing	2	1	-	-	1
Bottles	2	1	-	-	-
Egg shells	-	-	1	-	1
Tin can	1	1	-	-	-
Leaves	1	2	-	-	-
Shoes/leathers	1	-	-	-	-
Cassava peel	3	1	1	-	-
Metal scraps	-	2	-	-	-
Floor tiles	2	-	-	-	-
Maize husks	1	-	-	-	-

streams which serves as drinking water for some communities. Also, domestic animals feed on these refuse dumps and possibly contact one of the diseases, these animals are thereafter slaughtered for human consumption to the effect that they may transfer such disease to the consumers or the animals may die before slaughtering causing economic loss to the nation. The result also indicated that there is a large number of fungi and bacteria present on the various wastes (Tables 1 and 2), therefore they may be used for the biodegradation of these wastes but their large number also pose a health risk as some of them are pathogenic, hence they may cause disease outbreak when they come in contact with food or water of man as earlier been reported (Joshua et

al., 1992; Manaham, 1993; Raji and Okeniyi, 1998; Kujawa, 2002). Among the microorganisms isolated, *Bacillus*, *Aspergillus* and *Mucor* species were found to be more widely distributed on the wastes; this may be due to their ubiquitous nature. In general, microbes degrade waste to generate energy and nutrients for growth, hence a usual consequence of biodegradation of a compound is an increase in the number of the number of organisms degrading the waste (Joshua et al., 1992; Raji and Okeniyi, 1998; Okafor, 1987). It was discovered that more fungi and bacteria were isolated from waste collected after rainfall compared to those collected on hot sunny days; this may be due to the fact that the spores which are dormant in the absence of water germinate in

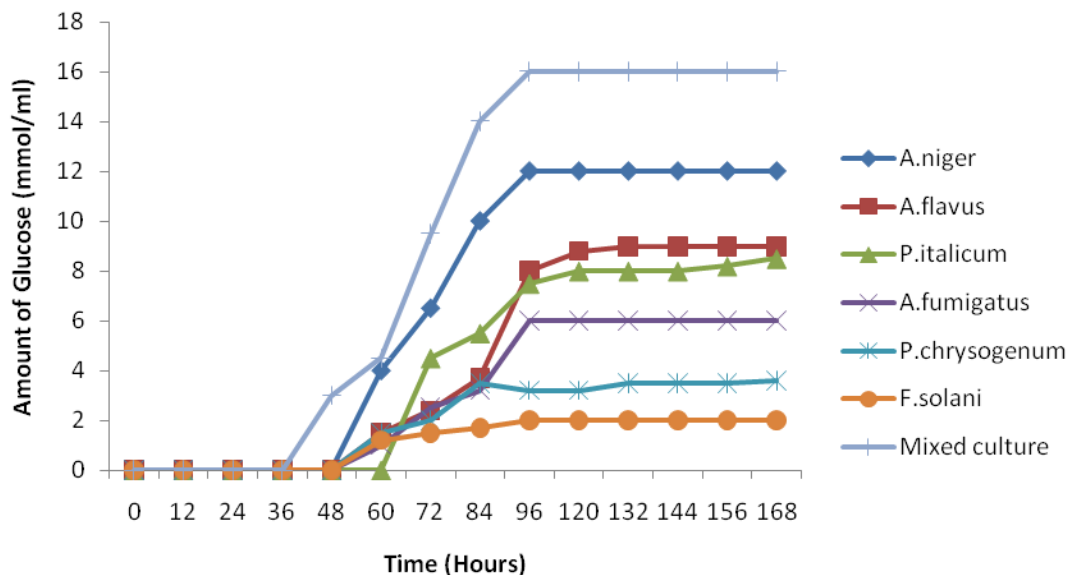


Figure 3. Glucose production (mmol/ml) with time for various hydrolyzed wastes at 30°C and pH 5.0.

the presence of water. Fungal species were found to be more than bacteria because having a larger surface area and being saprophytic, they are able to produce larger quantity of extracellular enzymes than bacteria with which they are able to survive on the wastes as earlier reported by Bolin and Cook (1983). Also, Pelczar et al. (1977) concluded that since the composition of waste varies at every dumpsite, types and number of microorganism will also fluctuate.

Production of glucose by fungi decreased from *A. niger*, *Aspergillus flavus*, *Penicillium italicum*, *Aspergillus fumigatus*, *Penicillium chrysogenum* and *Fusarium solani* respectively (Figure 3), while *Rhizopus stolonifer* and *mucor mucedo* were unable to utilize the grounded paper as carbon source being unable to produce cellulase, the enzyme needed to hydrolyze cellulose to glucose. This result agree with earlier reports in literature that cellulose degrading organisms include *Aspergillus*, *Penicillium*, and *Fusarium* (Raji and Okeniyi, 1998; Roosmalen et al., 1998; Bukoye, 2001; Adeyemo and sani, 2013). Among the bacteria, only *B. cereus* was able to hydrolyze the paper to glucose being the only cellulose degraders among the isolates. *A. niger* produced the highest glucose followed by *B. cereus* while the least glucose production was by *F. solani*, this might be due to the fact that cellulase activity *in vivo* is not mediated by a single enzyme, rather it is a complex of several enzymes which act in concert (Eveleigh, 1987; Lamed et al., 1987; Gbekeloluwa and Moo-young, 1991; Bukoye, 2001). The cellulase complex known as “cellulosome” which brings about the complete enzymatic hydrolysis of cellulose complex macromolecules to glucose require the synergistic participation of at least three groups of enzymes namely Endo-1,4- β -glucanase which cleaves the cellulose biopolymer thereby affecting the

solubilization of crystalline cellulose; Exo- glucanase of two types: Exo-cellobiohydrolase which releases cellobiose units; Exo-glucohydrolase which releases glucose units from the non reducing termini. β -glucosidase which hydrolyses cellobiose and various soluble cellodextrins into glucose (Eveleigh, 1987; Boisset et al., 2000). Therefore, any organism that will biodegrade cellulolytic materials such as paper must possess these enzymes in the right proportion; hence an organism that cannot produce all three will be regarded as non-cellulose degrader when it acts alone although it may be able to degrade cellulolytic materials when it acts in consortium with other organisms (Eveleigh, 1987; Boisset et al., 2000).

In consortium, *A. niger*, *Penicillium italicum* and *A. flavus* produced the highest amount of glucose being 22 mmol/ml (Figure 4, Mixed A) while the least amount of glucose was produced from a consortium of *Penicillium chrysogenum*, *F. solani* and *A. fumigatus* being 7 mmol/ml (Figure 4, Mixed D). The mixed culture designated as B is a consortium of *A. flavus*, *P. italicum* and *P. chrysogenum* that produced 12 mmol/ml of glucose, while the consortium C is a mixture of *A. fumigatus*, *F. solani* and *P. chrysogenum* producing 10 mmol/ml of glucose. The varying degree of glucose produced may not be due to ability of isolates to produce degrading enzyme alone but some member of the consortia might have made use of the glucose produced being faster grower than others while others might have released waste products toxic to others thereby inhibiting their growth and or glucose production (Sani et al., 1992).

Figure 5 shows the glucose production by *B. cereus* being the only bacterium of the isolates that is able to produce glucose cultured at 37°C and pH 8.0 since bacteria grow under a higher temperature and pH than

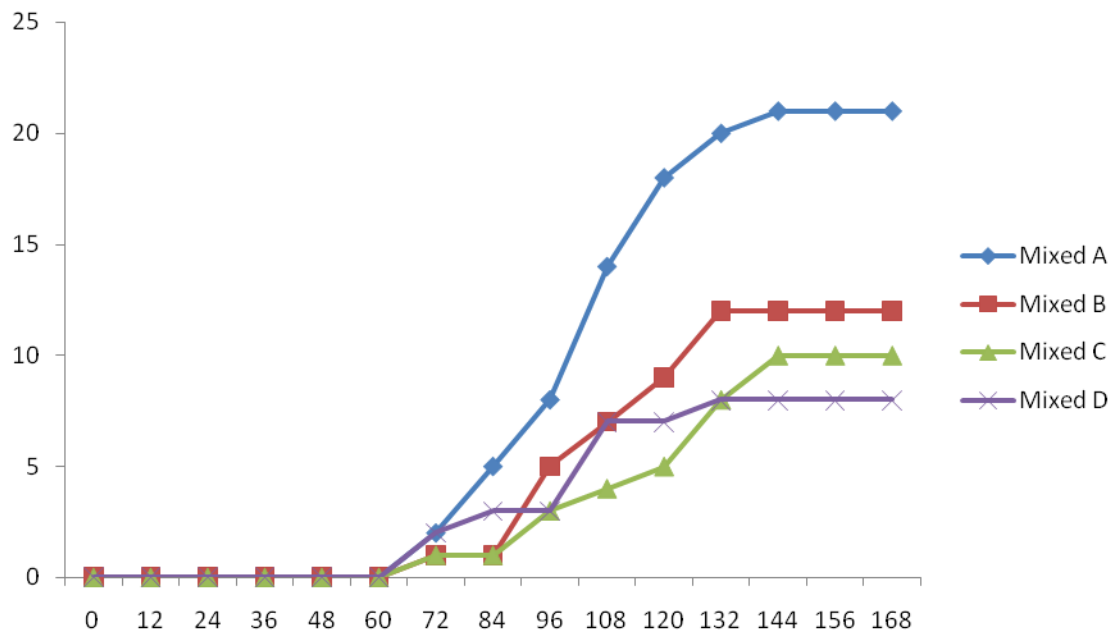


Figure 4. Glucose production (mmol/ml) by Consortia of fungal isolates at 30°C and pH 5.0.

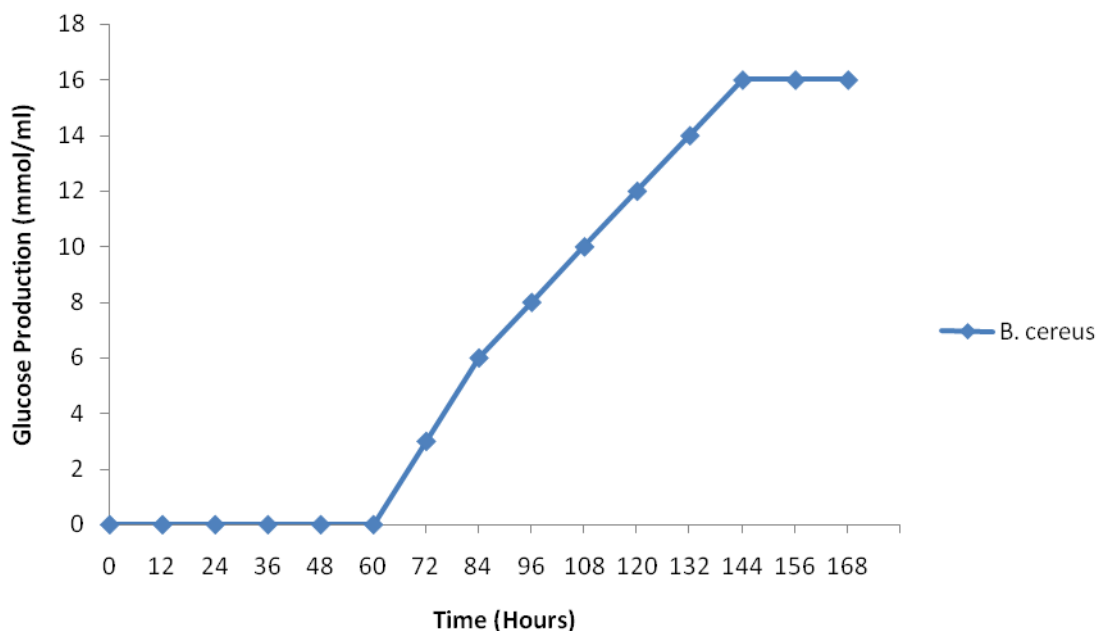


Figure 5. Glucose production (mmol/ml) by *Bacillus cereus* at 37°C and pH 8.0.

fungi, it produced 16 mmol of glucose per milliliter.

CONCLUSION

It is obvious from the discussion of the result obtained in this work and other earlier write ups that microbial

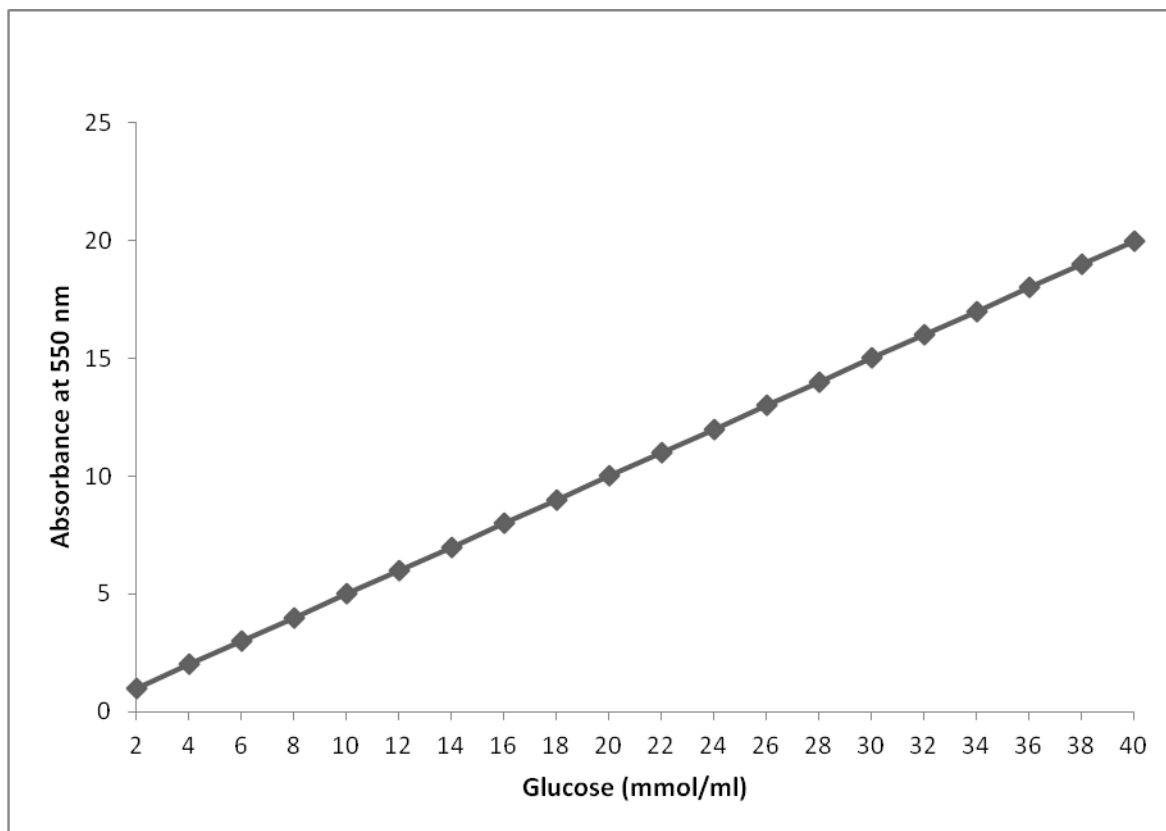
degradation is a necessary and important process for preserving a clean environment. Nevertheless, it should be apparent that, although a significant body of knowledge exists about this process, much additional information could still be obtained by further research to fully use the degradative ability of microorganism. Once these information are available, it will be possible to

biodegrade manufactured synthetic materials and help solve the problem and menace associated with the domestic solid wastes thereby making Nigeria and the world a better and clean safe environment.

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APPENDIX



Standard glucose curve used in determining the glucose content of cultured filtrate.