# Biodegradation of Wastes Using Cellulase from the Gut of Rhinoceros (*Oryctes rhinoceros*) Beetle

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***ABSTRACT***
*Recycling of wastes in Africa has not been fully embraced and practised; therefore, these waste materials are seen as physical pollutants that may affect the ecosystem, cause pollutions and create various environmental hazards. The use of synthetic substances in degrading wastes could further damage the ecosystem and cause global warming which makes the use of biocatalysts a better option. Most of the wastes generated daily are rich in cellulosic materials. Oryctes rhinoceros beetles feed on plant materials especially palm leaves whose major component is cellulose. Thus, it is reasonable to assume that the beetle will contain cellulase, a cellulose degrading enzyme. This work explored the use of cellulase from Oryctes rhinoceros beetle as a biodegrading agent on the cellulosic components of wastes. Cellulase isolated from the gut of Oryctes rhinoceros beetle was partially purified by precipitation with Ammonium sulphate at 80% saturation. Its actions on some industrial and agricultural wastes were examined and the results were compared with that of carboxyl methyl cellulose (CMC) the laboratory substrate. The enzyme had a specific activity of 3.78 U/mg. Spectrophotometric assessment of the enzyme on the waste materials showed the following activities; (36.30%-99.10%) on wood wastes, (9.36%-98.32%) on nylons, (10%-95.9%) on dry leaves, (14.97%-95.14%) on papers, (28.5%-90.5%) on wrappers, (10%-86%) on food wastes and (14.78%-73.63%) on plastics. This study suggests that cellulase from the Oryctes rhinoceros beetle has biodegrading potential on waste materials and could be relevant in waste management.*

**Key words**: Cellulase, *Oryctes rhinoceros* beetle, cellulose, waste management, cellulosic materials, biodegradation

## 1.0 Introduction

Solid Waste Management (SWM) is a major challenging issue in developing countries and some developed countries due to its adverse environmental effects (Zamorano *et al.,* 2009; Jalil, 2010; Adekunle *et al.,* 2011; Quadri *et al.,* 2017). Accumulation of increased solid wastes and careless disposal of waste materials in the natural environment affects the natural system, creates various environmental hazards and leads to global warming (Davis &Song, 2006; Song *et al.,* 2009; Dey *et al.,* 2012). Cellulose, the most abundant organic compound on earth and most common form of biologically fixed carbon is a fibrous, insoluble, crystalline polysaccharide constituent of plant cell walls which is made up of β, 1-4-polyacetal of cellobiose (4-O- β -D-glucopyranosyl-D-glucose). Cellulose is obtained either directly or indirectly as forest production or in wastes such as straw, paper waste, municipal solid waste and other industrial wastes. Cellulose-based waste materials could also be widely used to produce sustainable bio-based products and bio-energy to replace depleted fossil fuels (Angenent *et al.,* 2004).

Cellulases (E.C. 3.2.1) are enzymes which are synthesised by fungi, bacteria, protozoan, mollusks and insects that act as biocatalysts in the hydrolysis of cellulose (Lee, 2001;Watanabe &Tokuda, 2001). A principal component of plant cell wall and potential source of utilizable sugars which serve as raw materials in the microbial production for a wide variety of chemicals, food and fuel in several agricultural and waste management processes (Ekperigin, 2007; Chandra *et al.,* 2010). Cellulase, if properly utilised, plays an important role in natural biodegradation processes in which waste cellulosic materials are degraded or converted into useful products to meet burgeoning population (Bennet *et al.,* 2002; Ojo-Omoniyi*et al.,* 2016).*Oryctes rhinoceros,* agood source of the cellulase enzymeisan herbivorous beetle which feeds mainly on coconut tender leaves whose major component is cellulose. The cellulose and lignin contents present in the leaf fibers are the significant factors for the pest attack. The rhinoceros beetlehas the digestive enzymes produced either by the digestive glands of the digestive tract or by the symbiotic bacteria living in the digestive tract to utilise these carbohydrate sources (Haripriya & Thirumalai, 2017).The general aim of this research work is to evaluate the biodegrading potential of cellulase from the gut of the Rhinoceros beetle on some waste materials

## 2. Materials and Methods

### 2.1 Materials

Dinitrosalicylic acid (DNSA) and Potassium sodium tartrate were obtained from Trust Chemical laboratories. Carboxymethyl cellulose (CMC), bovine serum albumin (BSA) and sodium hydroxide were obtained from Sigma-Aldrich Chemical Company, Limited, St. Louis, MO., U.S.A. Ethylenediaminetetraacetic acid (EDTA) was obtained from NAAFCO Scientific Supplies Ltd., New York. Sodium acetate and ammonium sulphate were purchased from BDH Laboratory Supplies Poole, England. All other reagents were of analytical grades and were obtained from reputable sources. Glass distilled water was used for all preparations of solutions.

### 2.2 Methods

**Collection and Preparation of Samples:**
Wastes samples were collected randomly in sterile polythene bags from various dumpsites and industries in Ikenne, Ogun State, Nigeria. The samples were washed and macerated into smaller particles for biodegradability study. Life adult rhinoceros beetles were collected from oil palm plantation sites at Itokin, Ijebu Ode. They were taken to the laboratory in a well ventilated container. The beetles were immobilised in a -80°C freezer and were used within 24 hours of collection.

**Enzyme Extraction:**
The beetles were washed and rinsed with distilled water. The whole guts from the beetles were dissected and weighed. The sample was gently homogenised with a pre-frozen mortar in 10 mM sodium acetate buffer, pH 5.0 in 5 volumes of the buffer. The homogenates was centrifuged at 4,000 rpm for 20 minutes at room temperature. The pellets were discarded, and the supernatant was collected as crude enzyme and stored at $4^0$C.

**Ammonium Sulphate Precipitation:**
The crude enzyme was brought to 80% (w/v) Ammonium Sulphate Saturation. The suspension was refrigerated overnight and centrifuged at 4,000 rpm for 20 minutes. The supernatant was discarded and the precipitate was re-suspended in 1/5 (w/v) 10 mM Sodium Acetate

**Determination of Cellulase Activity:**
Protein concentration was determined using Biuret method (Gornall *et al*., 1949) using Bovine Serum Albumin (BSA) as a standard. During the purification, protein concentration was measured by taking the absorbance at 280nm and 260nm (Warburg & Christian, 1942).



**Determination of Cellulase Activity:**
The activity of cellulase was determined by using the method of Miller *et al*.(1959). The reducing sugars were determined byestimating amount of sugars released from 1% solution of CM-cellulose. The reaction mixture consisted of 0.5ml of crude enzyme and 0.5 ml of CMC in 10 mM Sodium Acetate buffer and incubated at 50˚C for 30 minutes. After the incubation time, reaction was stopped by adding 1ml of DNSA reagent and boiled at 100˚C in a water bath for 5 minutes. The reaction mixture was allowed to cool to room temperature. The enzyme activity was determined by the measurement of absorption at 540 nm and compared with a blank sample containing all components except enzyme solution which was replaced with 0.5ml distilled water. One unit (IU) of enzyme activity is expressed as the quantity of enzyme which is required to release 1μmol of glucose per minute under standard assay conditions (Miller *et al*., 1959).

**Biodegradability Test:**
Biodegradability test of the samples was carried out in triplicate. 0.1 g of the waste materials were added into 10 ml of 0.1 mM Sodium Acetate buffer (containing 1 mM EDTA) pH 5.0, 0.1ml of partially purified enzyme was added and incubated overnight. A control with Carboxymethyl cellulose (CMC) as substrate was run along the wastes.The blank was prepared by replacing the enzyme with distilled water while the activity of the enzyme remained as carried out.0.1ml of distilled water was added to the blank sample containing all components except enzyme solution. The activity of the enzyme was then assayed as described above. The samples were thoroughly mixed by covering the tube with parafilm and inverting it several times. The absorbance of the samples were read at 540nm.

## 3. Results

The summary of the purification procedure for cellulase from the rhinoceros beetle is shown in Table 1. The results of the partial purification procedure provided evidence for the existence of cellulase in the *Oryctes rhinoceros* beetle. The enzyme was partially purified by precipitating with ammonium sulphate at 80% saturation and gave a specific activity of 3.78 U/mg of protein.

**Table 1: Summary of the purification Steps of Cellulase from the Rhinoceros (*Oryctes rhinoceros*) Beetle**

| Purification Step | Volume (ml) | Total Activity (Units) | Total protein (mg) | Specific Activity (Units/mg) | Yield (%) | Purification Fold |
|---|---|---|---|---|---|---|
| Crude Extract | 55 | 59.95 | 21.33 | 2.81 | 100 | 1.00 |
| 80% Ammonium sulphate | 40 | 76.40 | 20.16 | 3.78 | 51 | 1.34 |

**Biodegradability Test**
The results for the action of the enzyme on various agricultural and industrial waste samplesare presented in Tables 2 – 8. The cellulase from the *Oryctes rhinoceros* beetle showed significant degradability potential on all the agricultural wastes tested. The highest activity of the enzyme on wood wastes was seen in Opoto (*Ficuscapensis)*, a hard wood (99.10%) and the least activity was seen in Utara (*Uapacaguineesis)*, also a hard wood (36.30%). Likewise, activity of the enzyme on kitchen waste shows that the orange peels (86%) were highly susceptible while yam flour (10%) was least susceptible. The mango leaves (95.9%) were highly degraded by cellulase while the least degraded leaves were the cashew leaves (10%). The activity of the cellulase enzyme on papers was highly seen in Infinity cornflakes pack (95.14%)

while the least activity was shown in Familiar tissue paper (14.97%).

**Table 2: Action of Cellulase from the Rhinoceros (*Oryctes rhinoceros*) Beetle on Wood Wastes**

| LOCAL NAME | ENGLISH NAME | BOTANICAL NAME | TYPE | ACTIVITY |
|---|---|---|---|---|
| Opoto | Fig trees | *Ficuscapensis* | Hard | 99.10% |
| Asasa | Nasturtium tree | *Marcarangabartoi* | Hard | 98.88% |
| Akomu | African nutmeg | *Pycnanthusanglensis* | Soft | 89.53% |
| Oro | Back cloth wood | *Antiatisfricana* | Soft | 85.96% |
| Eku | Sap wood | *Brachystegiaeurycoms* | Hard | 84.85% |
| Ayinre | Somb tree | *Entadaafricana* | Hard | 72.93% |
| Aga | corkwood tree or umbrella tree | *Musangacecropiodes* | Soft | 66.48% |
| Ijagbe | Mahogany | *Swieteriamacrophylla* | Hard | 59.79% |
| Ahun | Cheese wood | *Alstoniaboonie* | Soft | 59.46% |
| Utara | Sugar Plum | *Uapacaguineesis* | Hard | 36.30% |

All results are presented as % activity of the enzyme on CM-cellulose.

**Table 3: Action of Cellulase from the Rhinoceros ( *Oryctes rhinoceros*) Beetle on Dry Leaves**

| Leaves | Botanical names | Activity |
|---|---|---|
| Mango | *Magniferaindica* | 95.90% |
| Lemon | *Citrus limon* | 58.50% |
| Orange | *Citrus sinensis* | 45.60% |
| Guava | *Psidiumguajava* | 44.40% |
| Banana | *Musa acuminate* | 40.30% |
| Cassava | *Manihot esculenta* | 34.00% |
| Pear | *Pyruscommunis* | 33.40% |
| Kolanut | *Cola acuminate* | 33.00% |
| Maize | *Zea mays* | 24.00% |
| Cashew | *Anacardiumoccidentale* | 10.00% |

All results are presented as % activity of the enzyme on CM-cellulose.



**Table 4: Action of Cellulase from the Rhinoceros ( *Oryctes rhinoceros* ) Beetle on Paper Wastes**

| Papers | Manufacturers | Activity |
|---|---|---|
| Cornflakes Pack | Infinity Cornflakes Pack | 95.14% |
| A4 Paper | Vera Multi-use A4 Paper | 61.94% |
| Exercise book cover | Onward Exercise book cover | 55.87% |
| Noodles Cartons | Indomie Instant Noodles Cartons | 49.59% |
| Exercise Book Sheet | Onward Exercise Book Sheet | 44.93% |
| Brown Envelope | Excellence Brown Envelope | 34.14% |
| Newspaper | Punch Newspaper | 27.53% |
| Office File | Onward White Office File | 27.12% |
| Cardboard | Blue Cardboard | 20.65% |
| Tissue paper | Familiar Inner roll tissue paper | 14.97% |

All results are presented as % activity of the enzyme on CM-cellulose.

**Table 5: Action of Cellulase from the Rhinoceros (*Oryctes rhinoceros*) Beetle on Kitchen Wastes**

| Samples | Botanical Names | Activity |
|---|---|---|
| Orange peel | *Citrus sinensis* | 86% |
| Corn cob | *Zea mays* | 82% |
| Yam peel | *Dioseoreacayenensis* | 70% |
| Cassava granule | *Manihot esculenta* | 59% |
| Potato peel | *Ipomoea batatas* | 54% |
| Beans shaft | *Phaseolus vulgaris* | 53% |
| Onion peel | *Allium cepa* | 43% |
| Egg shell | | 37% |
| Groundnut peel | *Arachishypogaea* | 11% |
| Yam flour | *Dioscoreacayenensis* | 10% |

All results are presented as % activity of the enzyme on CM-cellulose.

**Table 6: Action of Cellulase from the Rhinoceros (*Oryctes rhinoceros*) Beetle on Plastic wastes**

| Plastics | Manufacturers | Activity |
|---|---|---|
| Plastic Chair | TafPlast | 73.63% |
| Plastic Electric kettle | GLEN Electrical Ltd. | 58.29% |
| Plate | Luvleen (lovpack) | 56.78% |
| Spoon | Ok Plast | 50.85% |
| Bucket | Stallion Plast | 48.85% |
| Yellow Keg | Union Plast | 48.12% |
| Plate cover | Luvleen (lovpack) | 47.27% |
| Sprite PET bottle | Coca-cola Bottling Company | 46.89% |
| Cooler | Thermos Weekend | 22.79% |
| Sewage Pipes | Tigre | 14.78% |

All results are presented as % activity of the enzyme on CM-cellulose.

**Table 7: Action of Cellulase from the Rhinoceros ( *Oryctes rhinoceros*) Beetle on Nylons wastes**

| Nylons | Manufacturers | Activity |
|---|---|---|
| White bread nylon | Goodluck nylon | 98.32% |
| Polythene black nylon | Abafem nylon | 96.65% |
| Juice pack nylon | Viju milk pack nylon | 68.89% |
| Toilet tissue pack nylon | Rose tissue pack | 68.56% |
| Value bread nylon | Right Options bread nylon | 68.23% |
| Pure water satchet nylon | Manatime water | 66.22% |
| Pure water pack nylon | Manatime water | 56.86% |
| White and black nylon | Abafem nylon | 46.15% |
| White nylon | Abafem nylon | 11.71% |
| Black nylon | Abafem nylon | 9.36% |

All results are presented as % activity of the enzyme on CM-cellulose.



**Table 8: Action of Cellulase from the Rhinoceros ( *Oryctes rhinoceros*) Beetle on Wrappers**

| Wrappers | Manufacturers | Activity (%CMC) |
|---|---|---|
| Beveragewrappers | Friesland Campina WamcoNig.Plc | 90.50 |
| Tomato paste wrappers | Vital Products Plc | 67.80 |
| Soft drink wrappers | Coca-cola Bottling Company | 56.00 |
| Cheese balls wrappers | Sunlight Resources | 47.40 |
| Minimie wrappers | Pure Flour Mills | 42.30 |
| Cornflakes wrappers | NASCO foods | 39.40 |
| Biscuit wrappers | P1 pardee food night | 33.20 |
| Sausage roll wrappers | UAC foods | 32.00 |
| Spaghetti wrappers | Golden pasta | 28.50 |

All results are presented as % activity of the enzyme on CM-cellulose.

## 4. Discussion

Pollution of the environment and the threat of famine are two of the most important problems facing the world today. In view of the high consumption of disposable cellulosic materials in developed societies, it is not surprising that more than half the total wastes of major urban settlements consist of cellulosic materials of various types. These materials must be disposed of by reutilization, compositing or by incineration, of which the latter method is the more common (Bassis, 1999). The resulting stench from the dumping grounds, the smoke and particulate materials that enter the atmosphere as a result of burning, all contribute largely to urban environmental pollution. Disposal by biological means using cellulolytic organism or isolated cellulose, if properly carried out, avoids this pollution. Waste management has become increasingly important as the increasing population is accompanied with a relative increase in waste generation. Formerly, waste bodies were a resort for waste disposal but have now become protected resulting in inland waste disposal. The land sites themselves are also becoming difficult to find as waste disposal is alarmingly on the increase (Baum & Parker, 1974; Daniel, 1993; Bryon, 2006).Considering the tremendous worldwide activity in seeking food stuffs from conventional sources, utilisation of wood cellulose and cellulosic waste materials as sources of food for animals or even for human consumption may become imperative. Food processors are already experimenting with fermentation methods and treatment with cellulolytic enzymes as means of increasing the nutritive value of food and other refractory food materials or waste products (Goel & Wood, 1978). The enzyme from the *Oryctes rhinoceros* beetle is highly potent and useful in the conversion of wastes to re-utilisable compounds. This definitely will alleviate problem of famine and environmental pollution.

The results of the partial purification procedure provide evidence for the existence of cellulase in the *Oryctes rhinoceros* beetle. The enzyme was partially purified by precipitating with ammonium sulphate at 80% saturation and gave a specific activity of 3.78 U/mg of protein. This value is higher than the cellulase purified in this laboratory from the *Oryctes rhinoceros larva* with a specific activity of 0.739U/mg (Apena *et al.*, 2018). bacterium *Bacillus cereuss* (0.104 U/mg)(Nema, *et al.*, 2015), the fungi Aspergillus niger with a specific activity of 0.0452U/mg (Hurst *et al.*, 1977) and from termite (*Amitermeseveuncifer*)Silvestri worker also purified in this laboratory with a specific activity of 0.25U/mg (Ezima *et al.,* 2014). However, it is lower than cellulases obtained by the same author from the gut of African giant snail (*Archachatinamarginata*) with specific activity of 69.5 U/mg (Fagbohunka *et al*., 1997), the heamolymph of African giant snail (*Archachatinamarginata*) with specific activity of 1359.09 U/mg (Fagbohunka *et al*., 2012), the termite (*Amitermeseveuncifer*)Silvestri soldier purified in this laboratory with a specific activity of 7.08U/mg (Fagbohunka *et al.,* 2018), *Trichoderma longibrachiatum* (30.0 unit/mg) (Pachauri *et al*., 2017), *Bacillus pantothenticus* (55.28 unit/mg) (Quadri*et al*.,2017) and digestive tracts (1822.87unit/mg) of the *Achatinaachatina* (Ozioke *et al*., 2013).

The cellulase from the *Oryctes rhinoceros* beetle showed significant degradability potential on all the agricultural wastes tested. The highest activity of the enzyme on wood wastes was seen in Opoto (*Ficuscapensis)*, a hard wood (99.10%) and the least activity was seen in Utara (*Uapacaguineesis)*, also a hard wood (36.30%). The orange peels (86%) were highly susceptible to the enzymatic attack while the least kitchen waste susceptible to the enzymatic attack was the yam flour (10%). The mango leaves (95.9%) were highly degraded by cellulase while the least degraded leaves were the cashew leaves (10%). The activity of the cellulase enzyme on papers was highly seen in Infinity cornflakes Pack (95.14%) while the least activity was shown in Familiar tissue paper (14.97%). The values obtained from the enzymatic attacks on the agricultural wastes tested were slightly similar to that obtained from the *Oryctes rhinoceros larva* (Apena *et al.,* 2018) and higher than that obtained from the termite (*Amitermeseveuncifer*) Silvestri worker (Ezima *et al.,* 2014).The varying degrees of activities of the enzyme on these wastes could be attributed to the texture of the materials, susceptibility of the materials to the cellulosic activity of cellulase (Potrykus&Shillito, 1986; Klysov, 1990) or due to the conformation of cellulose present in the materials. Cellulose exists as a crystalline form and a small amount of cellulose chain forms amorphous cellulose. In the amorphous conformation, cellulose is more susceptible to enzymatic degradation (Perez *et al*., 2002).

The results showed that leaves, papers, food wastes and wood (all of plant origin) have very high degradability potential. This is in accordance with the work of Bisaria & Ghose (1981) who reported that wastes of plant origin have the highest degradability potential and the highest yield of useful products. This is also in agreement with Wiegel's submission (1982) that agricultural products have higher cellulose content.

The activities of the enzyme on the industrial wastes; nylons, wrappers and plastics are higher than the values obtained from *Oryctes rhinoceros larva* (Apena*et al.,* 2018), termite (*Amitermeseveuncifer*) Silvestri soldier (Fagbohunka *et al.,* 2018) and from termite (*Amitermeseveuncifer*) Silvestri worker (Ezima *et al.,* 2014). These polymers were highly degraded by cellulase from the *Oryctes rhinoceros* beetle. This might be pointing to a different cellulase from this organism. This calls for a further probe in future works. The demand of cellulase is gradually increasing in industries and therefore, developing cost-effective methods to produce cellulase at large scale is needed. The results obtained from this study suggest that cellulase isolated from *Oryctes rhinoceros* beetle would be a good choice for the recycling and management of cellulosic waste materials.


Adekunle, I.M. (2011). Recycling of organic wastes through composting for land applications: A Nigerian Experience. *Waste Management and Research, 29*, pp. 582-593.

Angenent, L.T., Karim, K., Al-Dahhan, M.H., Wrenn, B.A. &Domiguez-Espinosa, R. (2004). Production of bioenergy and biochemicals from industrial and agricultural wastewater. *Trends in Biotechnology*, pp. *22*, 477–485.

Apena, A., Adeyemi, O.E. &Fagbohunka, B.S. (2018). Biodegradation of wastes using cellulase from the *oryctes rhinoceros* larva. (In the press)

Bassil, L. (1999). *Waste disposal*.http://www.solidwastemag.com/library/garbage.htm, www.google.com.

Baum, B. & Parker, C.H. (1974). *Solid waste disposal.* Ann Arbor. http://www.epa.gov, www.google.com.

Bennet, J.W., Wunch, K.G. & Faison, B.D. (2002).*Use of Fungi Biodegradation.* Washington, DC: USA: ASM Press

Bisaria, & Ghose. (1981).*Biodegradation of cellulosic material: substrates, microorganism, enzymes and products*.

Bryon, D. (2006). *Advances in Environmental Sciences and Technology Series* Wiley, NY:

Chandra, M., Kalra, A., Sharma, P.K., Kumar, H., Sangwan, R.S. (2010). Optimization of cellulases production by *trichodermacitrinoviride*on marc of *artemisiaannua*and its application for bioconversion process. *Biomass and Bioenergetics*, *34*.pp. 805–811.

Daniel, D. E. (1993). *Geotechnical Practice for Waste Disposal*. London. http://www.solidwastemag.com/library/garbage.htm, www.google.com.

Davis, G. & Song, J.H. (2006).Biodegradable packaging based on raw materials from crops and their impact on waste management. *Industrial Crops Production*, *23*(2).pp. 147–161.

Dey, U., Mondal, N.K., Das, K. &Dutta, S. (2012).An approach to polymer degradation through microbes. *Journal of Pharmacology*, *2*(3).pp. 385–388.

Ekperigin, M.M. (2007). Preliminary studies of cellulase production by*Acinetobacteranitratus*and*Branhamella sp. African Journal Biotechnology*,*6*.pp. 28–33.

Ezima, E.N., Adeyanju, M.M., Odufuwa, K.T., Aremu, T.A. &Fagbohunka, B.S. (2014). Biodegradation of wastes using cellulase from the termite, *Amitermeseveuncifer*(silverstri) workers: a clue to the application of termites cellulase in waste management. *Science* focus, *19*(2).pp. 93-98.

Fagbohunka, B.S., Agboola, F.K. &Afolayan A (2012). Characterization of a cellulase from the haemolymph of the giant African Snail (A*rchachatinamarginata*).*African Journal of Biotechnology*, *11*(38).pp. 9254-9264.

Fagbohunka, B.S., Babasanya-Craig, O., Adenuga, G.A. &Agboola, F.K. (1997). Partial purification and characterization of cellulase from the gut and heamolymph of the giant African snail *Archachatinamarginata*. *Nigeria Journal of Nutrition Sciences*,*18*.pp. 28-34.

Fagbohunka, B.S., Ezima, E.N., Adeyanju, M.M., Alabi, M.A. & Idowu, G.O. (2018). Biodegradation of wastes using cellulase from the termite, *Amitermeseveuncifer* (silverstri) soldiers: a clue to the application of termites cellulase in waste management.

Goel, S. K. & Wood, B. J. B. (1978). Technical note: Cellulaseabd Exoamylase in experimental soy-sources fermentation. *Journal of Food Technology*, 13. pp. 243-247.

Gornall, A. C., Bardawill, C. J. & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem*, *177*.pp. 751-767.

Haripriya, R. &Thirumalai, V.P. (2017). Cellulose degrading capabilities of cellulolytic *Bacillus sp.* Isolated from the intestinal tract of the beetle, *Oryctes rhinoceros*. *International Journal of Advanced Research*, *5*(4).pp. 2070-2075.

Hurst, P.L., Nielson, J., Sullivan, P.A. &Shepherd, M.G. (1977). Purification and properties of cellulase from *Aspergillus niger. J. Biochem*, *165*.pp. 33-41.

Jalil, A. (2010). Sustainable Development in Malaysia: A Case Study on Household Waste Management. *Sustainable Development*, *3*. pp. 91-102.

Klyosov, A.A. (1990). Trends in Biochemistry and Enzymology of cellulose degradation. *Journal of Biochemistry, 29*(47pp. 10577-10589.

Lee, S.M. & Koo, Y.M. (2001). Pilot-scale production of cellulose using *Trichoderma reesei*Rut C-30 in fed-batch mode. *Journal of Microbiology and Biotechnol*ogy, *11*.pp. 229-233.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*.pp. 426-428.

Nema, N., Alamir, L., &Mohammad, M. (2015).Partial purification and molecular weight determination of cellulase from *Bacillus cereus.International Food Research Journal*, ***23***(2).pp. 894-898.

Ojo-Omoniyi, O.A., Okwa, O.O., Junaid, I.O. &Ikuoye, A.O. (2016). Sustainable solid waste management: Isolation of cellulolytic microorganisms from dumpsites in Lagos, Southwest Nigeria.Retrieved fromhttp://dx.doi.org/10.20546/ijcmas.511.096

Ozioko, P.C., Eze, S.O.O. &Chilaka, F.C.(2013). Partial purification and characterization of cellulases from digestive tracts of the African giant snail (*Achatinaachatina*). *Turk. J. Biol*, *37*pp. 1205-1211.

Pachauri, P., More, S., Aranganathan, V., Sullia, S.B. &Deshmukh, S. (2017). Kinetic study and characterization of cellulase enzyme from isolated *Aspergillus niger subsp. awomori*for cellulosic biofuels. *Journal of scientific and industrial research*, *77*, pp. 55-60.

Pérez, J., Munoz-Dorado, J., de la Rubia, T.D.L.R. & Martinez, J. (2002). Biodegradation and biological treatments of cellulose, hemicellulose and lignin: *An overview International Journal Microbiology*,*5*(2).pp. 53-63.

Potrykus, J. &Shillito, R.D. (1986). Protoplasts: Isolation, culture, plant regeneration. *Methods Enzymol, 118*.pp. 549-578.

Quadri, H.O., Ademakinwa, A.N., Adejumo, A.L. &Agboola, F.K. (2017). Partial purification and characterization of cellulolytic enzyme from *bacillus pantothenticus* isolated from a dumpsite. *Journal of Microbiology and Biotechnology*,*6*(2)

Song, J.H., Murphy, R.J., Narayan, R. &Davies, G.B.H. (2009). Biodegradable and compostable alternatives to conventional plastics. *Philos Trans R SocBiol*,*364*pp. 2127–2139.

Warburg, O. & Christian, W. (1942). Isolation and Crystallization of Enolase. *Biochemistry, 310*.pp. 384-421.

Watanabe, H. & Tokuda, G. (2001). Animal Cellulases. *Cell Molecular Life Science*, *58*.pp. 1167–1178.

Wiegel, J. (1882). Ethanol from cellulose. *Experimential*, *38*.pp. 151-156.

Zamorano, M. (2009). A planning scenario for the application of geographical information systems in municipal waste collection: a case of churriana de la vega (granada, spain). *Resource Conservation and Recycle*, *54*.pp. 123-133.