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Biodegradation of Wastes using Partially Purified Cellulose-Enzyme from Gut of *Oryctes rhinoceros* larvae from Rafia Palms in Itokin, Lagos State, Nigeria

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ABSTRACT: Conversion of urban wastes to utilizable sugars is very important as a vital step towards reduction of environmental pollution. Consequently, this work was aimed at obtaining cellulase from the gut of *Oryctes rhinoceros* larvae from raffia palms in Itokin, Lagos State, Nigeria to evaluate its degradability potential on different plant leaves and selected plastic bottles. Cellulase from *Oryctes rhinoceros* larvae was partially purified by a combination of precipitation with ammonium sulphate and fractionation with gel filtration on Biogel P- 100. The cellulase degradability potential was examined on tree leaves from cashew, banana, maize, lemon, cassava, pawpaw, mango, almond and palm respectively, while the plastics were from bottles of teem,pepsi, eva, mirinda, cascade, still, coke, devana and nestle respectively. Data obtained show that the enzyme- cellulase has a specific activity of 2.38 Unit/mg with 32% yield with higher degradability potential on samples from plant origin than the materials from industrial wastes.

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Cellulase is the general term for any enzyme that has the ability to hydrolyze the polysaccharide cellulose. Hydrolysis of cellulose can be done by microorganisms that produce enzymes known as the cellulase systems (Gusakov *et al.*, 2007). The enzymes work randomly by cleaving the β-1,4 bonds in the cellulose strands to minimize the effect of polymerization on the cellulose chains being converted into smaller subunits. Subunits are removed at the non-reducing ends of cellulose by the enzymes, releasing cellobiose or glucose. Cellobiose can be hydrolyzed to glucose by the enzymes (Clarke, 1997). Due to inhibition feedback from cellobiose onto EG enzymes, β-glucosidase activity is very important in the complete degradation of cellulose (Gruno *et al.*, 2004). Cellulases are produced mostly by microorganisms (bacteria and fungi) (Lynd *et al.*, 2002) but also by other organisms such as insects, molluscs, nematodes and protozoa (Watanabe, et al., 1997). Cellulases have been the target of active research for over five decades and are presently the enzymes are amongst the largest industrial enzyme globally (by dollar volume) since they are commonly used in cotton processing, paper recycling, detergent enzymes, juice extraction and as animal feed additives (Wei and Lee, 2005). *Oryctes rhinoceros*, because of its resemblance to the rhino is a pest of coconut in major parts of the world, for example, it can be found in Asia and Africa, Nigeria in particular; it lives and feeds mostly on oil and raffia palms. The larva, also

called grub, is called ipe by the Yorubas, osori by the Ijaws, tam by the Ogonis and utukuru by the Ibos, all of Southern Nigeria. They can be eaten raw or when they are processed. It may be consumed as part of a meal or as a complete meal (Okaraonye and Ikewuchi, 2009). More than 300 species of *rhinoceros* beetles are known. Some are known for their shapes and large sizes. Some common species are, the Atlas beetle (*Chalcosoma atlas*). Another common speceis of rhinoceros beetle are (*Xylotrupes ulysses*), elephant beetle (*Megasoma elephas*). European rhinoceros beetle (*Oryctes nasicornis*), Hercules beetle (*Dynastes hercules*), Japanese rhinoceros beetle or *kabutomushi* (*Allomyrina dichotoma*), ox beetle (*Strategus loeus*) and the unicorn beetle (*Dynastes tityus*) (Okaraonye and Ikewuchi, 2009). Considering the threats of pollution and famine, the major problems of developing countries like Nigeria is the conversion of cellulosic wastes such as agricultural wastes into useful materials. The commonest ways which these wastes are disposed of, especially in urban cities, are incineration and decomposing. The resulting odour, the smoke and the particulate materials in the atmosphere contribute largely to environmental pollution. Therefore, the objective of this paper is to obtain cellulose from the gut of *Oryctes rhinoceros* Larva collected from raffia palms in Itokin Lagos state, Nigeria to degrade different plant leaves and selected plastic bottles.

MATERIALS AND METHODS

Materials: Citric acid, sodium citrate, ammonium sulphate (enzyme grade), ethylenediamine tetraacetic acid (EDTA), Coomassie Brilliant-Blue, Blue Dextran, Reactive Blue-2 Agarose and Bovine Serum Albumin (BSA) were obtained from Sigma Chemical Company, St. Loius, Mo., USA. Disodium hydrogen phosphate and monosodium dihydrogenphosphate were products of Kermel Reagent Company Limited, Tianjin, China. N, N-methylene bisacrylamide (MBA), N,N,N^1,N^1-tetramethylenediamine (TEMED) and ammonium persulphate were products of Feinbiochemica, Heidelberg, Germany. Biogel P-100 was purchased from Bio-Rad Laboratories Inc., Benicia Ca., USA.

Methods: Collection of Larvae of *Oryctes rhinoceros:*- Larvae of *Oryctes rhinoceros* were collected from rafia palms in Itokin, Lagos state, Nigeria. They were taken to the laboratory together with their moist feed in a well-ventilated plastic container which was used within few hours of collection. The Larvae of *Oryctes rhinoceros* were identified and authenticated at the Department of Zoology, Obafemi Awolowo University, Ile-Ife.

Collection of Waste Materials: - Leaves and plastic materials were collected as presented in table I.

Table 1: Leaves and plastic materials used

Plastics	Leaves
	Cashew
Teem	Banana
Pepsi	Maize
Eva	Mgelina
Mirinda	Lemon
Cascade	Cassava
Still	Pawpaw
Coke	Mango
Devana	Almond
Nestle	Palm

Enzyme Extraction and Preparation of Crude Enzyme: The larvae were collected and gently washed to remove surface dirt and then cut open transversely to remove the gut content. One hundred grams (100 g) of the gut content was homogenized in three volumes of 0.05 M citrate buffer, pH 4.8 with a Warring Blender. The homogenate was filtered with a double layer of cheese cloth and then centrifuged at 12, 000 rpm at 10°C for 30 min using Centurion cold centrifuge (R-1880). The pellets were discarded and an aliquot of the supernatant was then assayed for cellulase activity by a method described by Zhang *et al,* (2006) and d protein concentration was determined by the method of Bradford (1976) using Bovine Serum Albumin (BSA) as the standard. The supernatant was then precipitated with 80% ammonium sulphate. The ammonium sulphate precipitates were dialysed against several changes of 0.05 M citrate buffer (pH 4.8) for 18 h. The dialysates were centrifuged at 12,000 rpm at 10°C for 30 min to remove insoluble materials and the supernatants were assayed for cellulase activity and protein concentration.

Biogel P-100 gel filtration resin was prepared by swelling 20 g of the resin in distilled water for 3 days. The resin was washed with several changes of 0.05 M citrate buffer, pH 4.8. This was then packed into the column (1.5cm × 25cm) and equilibrated with 0.05 M citrate buffer, pH 4.8. 10 ml of the samples were applied to the Biogel P-100 column. Fractions of 2 ml were collected at a flow rate of 10 ml per hour. Proteins were monitored using Bradford method and cellulase activity was monitored using the assay method described earlier.

Actions of the partially purified cellulase on some selected Agricultural wastes and industrial wastes: The different wastes materials were dried and cut into small pieces. In the case of leaves and plastic materials were used. 0.1g of the various waste materials was

added 1ml of 0.1mM sodium acetate buffer (containing 1mMEDTA) pH 5.0, 0.2ml of partially purified enzyme was added and incubated overnight a control with CMC as substrate was run along each waste. The enzyme activity was assayed.

RESULTS AND DISCUSIONS

The purification of *Rhinoceros larva* Cellulase is presented in Table 1. Cellulases are being studied because of their applications in the hydrolysis of cellulose, the most abundant biopolymer and a potential source of utilizable sugars. These serve as raw materials in the microbial production of a wide variety of chemicals, food and fuel.

The cellulase from *rhinoceros* larva was tested on waste samples from plant origin such as Leaves and plastic materials, have degradability potential while samples with the lowest degradability is plastic (Industrial wastes). This is in line with the work of Ghose (1997) 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 Wie, and Smith, (2005) submission that agricultural products have higher cellulose content. The different degrees of activities of the enzyme on different leave samples could be as a result of the texture of the leaves and the type of cellulose components the leaves are made of as the susceptibility of a material to the cellulolytic activity of cellulase depends on the cellulose makeup of that material (Klysov, *et al*., 1990, Potrykus and Shillito. 1986).

Action of Oryctes rhinoceros cellulase on some selected wastes: The results of Biodegradation activities of *Oryctes rhinoceros* larvae are presented in Table 1. All wastes tested were appreciably degraded by the enzyme with the greatest biodegradability by agricultural wastes. The cellulase from *rhinoceros* larva was tested on waste samples from plant origin such as Leaves, also with plastics.

Table 2: Purification steps of *Rhinoceros larva* Cellulase

Fractions	Total Protein (mg)	Total Activity (Unit)	Specific Activity (Unit/mg)	Purification Fold	Yield (%)
Crude	26325.23	9266.48	0.352	1	100.0
Ammonium Sulphate	17637.90	13034.41	0.739	2.1	67.0
Gel Filtration	8424.07	20040.87	2.379	6.76	32.0

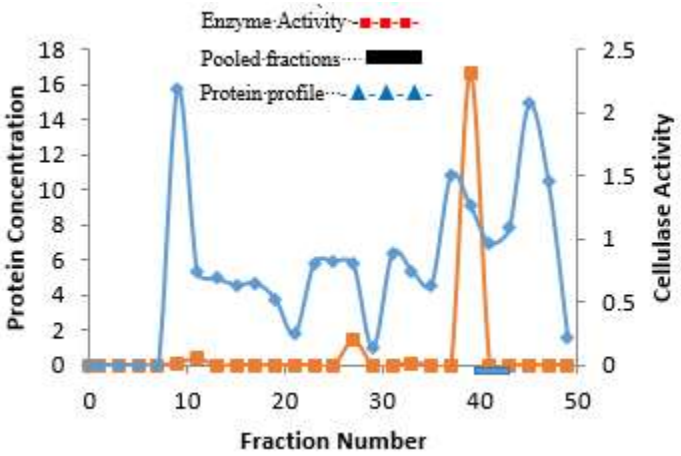

Fig 1: Gel Filtration Chromatography on Biogel-P-100 (column 1.5 x 25 cm) of *Oryctes rhinoceros* larva Gut.

The fact that agricultural wastes samples are more susceptible to the enzyme more than CMC its laboratory substrate, implies that biodegradation of solid wastes ie native cellulose using crude or purified cellulase from the guts of *Rhinoceros* larvae would be an efficient and more productive means of handling the problem of waste management and material cycling. For about 80% of the leave wastes tested, the enzyme showed above 50% activity. The study of the cellulolytic activities of cellulase from *Oryctes rhinoceros* larva showed amazing results especially with Plastic samples while the plastic sample (Teem) showed the highest activity of 19.62%. This may be attributed to the precursors of the polymer which shows greater biodegradability as compared to other

synthetic polymers (Sen and Raut 2015) or due to the conformation of cellulose present in the plastic.

Table 3: Action of cellulase from *Oryctes rhinoceros* larva on different Plastics Materials.

Plastics	Activity/ (% CMC)
Teem	19.62± 1.99
Pepsi	17.60± 0.81
Eva	17.19± 0.46
Mirinda	17.07± 0.49
Cascade	15.95± 0.85
Still	15.95± 0.48
Coke	15.65± 0.34
Devana	15.35± 0.57
Nestle	13.99± 0.25

All results are presented as % activity of the enzyme on CM-cellulose and value SEM

Table 4: Action of cellulase from *Oryctes rhinoceros* larva on different Leaves

LEAVES	Activity/ (% CMC)
Cashew	131.24± 0.19
Banana	108.40± 8.21
Maize	74.92± 3.29
Mgelina	74.80± 2.08
Lemon	60.54± 9.64
Cassava	57.43± 10.84
Pawpaw	54.41± 6.66
Mango	41.30± 6.60
Almond	38.42± 15.74
Palm	7.05± 2.95

All results are presented as % activity of the enzyme on CM-cellulose and value SEM

Cellulose exists as a crystalline form and a small amount chains form amorphous cellulose. In the amorphous conformation, cellulose is more susceptible to enzymatic degradation (Perez *et al.*, 2002). Polymers also contain both crystalline and amorphous regions, the latter being more susceptible to microbial and enzymatic attach (Tokiwa *et al.*, 2009; Loredo-Trevino *et al.*, 2012; Restrepo- Flores *et al.*, 2014). The variable degrees of activities of the enzyme on different samples could be as a result of the chemical and physical properties of the plastics, the surface condition ie (surface area, hydrophilic and hydrophobic properties), the first-order structures (chemical structure, Molecular weight and Molecular weight distribution) and the high order structures.

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