

Article

Not peer-reviewed version

Unlocking the Biodegradation Potential: Comparing Cellulase Activity from Unconventional Sources - Rhinoceros Beetle Larvae and Giant African Snails

[Isaac Chinenye](#) * and Fagbohunka BAMIDELE

Posted Date: 19 October 2023

doi: [10.20944/preprints202310.1255.v1](https://doi.org/10.20944/preprints202310.1255.v1)

Keywords: cellulase; rhinoceros larvae; giant african snail; cellulose; industrial waste; cellulosic material



Preprints.org is a free multidiscipline platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This is an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Unlocking the Biodegradation Potential: Comparing Cellulase Activity from Unconventional Sources—Rhinoceros Beetle Larvae and Giant African Snails

I. C. Ugwu ¹ and B. S. Fagbohunka ^{1,*}

¹ Department of Biochemistry, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Remo Campus, Ikenne, Ogun State.

* Correspondence: ugwuisaaci@gmail.com; Tel: 08160880412

Abstract: The global predicament of solid waste generation looms as a mounting crisis, profoundly impacting the world, particularly in developing nations. Each day, the world's population contributes millions of tonnes of municipal solid waste, with projections pointing toward further escalation in the coming years. This surge in waste, predominantly composed of cellulose-rich materials, correlates directly with heightened pollution, a threat that transcends borders and imperils humanity as a whole. To confront this ever-growing issue, cellulase, a potent hydrolytic enzyme capable of digesting cellulose, a chief component of solid waste, is explored as a potential solution. In this study, we harness cellulase enzymes derived from the digestive tracts of both *Archachatina marginata* and *Oryctes rhinoceros* larvae, employing them as biodegradation agents for cellulose-based waste materials. The cellulase enzymes are methodically isolated and partially purified via ammonium sulfate precipitation at 65% saturation. Their efficacy in breaking down a variety of waste categories, encompassing kitchen waste, leaves, paper, wood, and industrial waste, is meticulously assessed and benchmarked against carboxymethyl cellulose (CMC), a well-established laboratory substrate. The study also ascertains the specific activity of these enzymes. Spectrophotometric analyses of the cellulase enzymes' actions on the diverse waste materials unveil intriguing results. *Archachatina marginata* and *Oryctes rhinoceros* larvae exhibit distinct performance metrics, registering percentage ranges of (11.04% - 28.31%) and (33.69% - 189.11%) on wood waste, (76.52% - 196%) and (108.56% - 567.01%) on paper waste, (78.57% - 148.14%) and (163.33% - 436.76%) on leaf waste, (57.16% - 179.95%) and (63.38% - 457.05%) on kitchen waste, and (59.95% - 77.51%) and (117.26% - 155.23%) on industrial waste, respectively. This study underscores the substantial biodegradation potential inherent in cellulase enzymes from both *Oryctes rhinoceros* larvae and *Archachatina marginata* when applied to diverse waste materials. While the rates and impacts of waste degradation vary between these sources, their promising contributions to waste management come into focus, offering sustainable and innovative approaches to tackle the pressing global challenge of solid waste proliferation.

Keywords: cellulase; rhinoceros larvae; giant african snail; cellulose; industrial waste; cellulosic material

Introduction

Around the world, waste generation rates are rising. In 2016, the world's cities generated 2.01 billion tonnes of solid waste, amounting to a footprint of 0.74 kilograms per person per day. With rapid population growth and urbanization, annual waste generation is expected to increase by 70% from 2016 levels to 3.40 billion tonnes in 2050. Compared to those in developed nations, residents in developing countries, especially the urban poor, are more severely impacted by unsustainably managed waste. In low-income countries, over 90% of waste is often disposed in unregulated dumps or openly burned. These practices create serious health, safety, and environmental consequences. Poorly managed waste serves as a breeding ground for disease vectors, contributes to global climate change through methane generation, and can even promote urban violence. Managing waste properly is essential for building sustainable and livable cities, but it remains a challenge for many

developing countries and cities. Effective waste management is expensive, often comprising 20%–50% of municipal budgets. Operating this essential municipal service requires integrated systems that are efficient, sustainable, and socially supported.

Cellulose, a carbohydrate polymer linked by β -1, 4 glycosidic bonds and consists of tens to hundreds to several thousand monosaccharide units in which glucose is the main building block. Cellulose is the most abundant naturally occurring biopolymer and a very important plant based compound out of which different materials are produced industrially(Seddiqi et al., 2021). Organisms such as fungi, bacteria, plants, protists and an array of invertebrate animals, such as annelids, crustaceans, insects, molluscs and nematodes consume food rich in cellulose. 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 extensively used to produce sustainable bio-based products and bio-energy to replace depleted fossil fuels (Sadh et al., 2018).

Cellulases (E.C. 3.2.1) are enzymes which are synthesized by fungi, bacteria, protozoan, mollusks and insects that act as biocatalysts in the hydrolysis of cellulose(Payne et al., 2015). 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(Puyol et al., 2017). Cellulase, if properly utilized, plays an important role in natural biodegradation processes in which waste cellulosic materials are degraded or converted into useful products to meet burgeoning population.(Lakhundi et al., 2015)

Oryctes rhinoceros, so called because of its resemblance to the rhino is primarily a pest of coconut in most part of the world, especially in Southern Asia, but in Africa, Nigeria in particular; it lives and feeds mostly on oil and raffia palms. While the adults attacks the palm tree, the larvae are harmless, feeding only on decaying organic matter such as decaying palm logs, manure, rubbish dumps etc. Usually, when left to decay, old stems of coconut, raphia and oil palms, provide excellent breeding sites or grounds for *Oryctes rhinoceros*(Shelomi et al., 2019). The larva, also called grub, is called ipe by Yorubas, osori by the Ijaws, tam by the Ogonis and utukuru by the Ibos, all of Southern Nigeria. It is either eaten raw, boiled, smoked or fried. It may be consumed as part of a meal or as a complete meal. Thus this organism must possess cellulase to be able to feed on plant materials whose major component is cellulose.

The invertebrate mollusc, African giant snail (*Archachatina marginata*), feeds on any edible plant or animal matter. These range from succulent fruits and vegetables e.g. paw-paw, banana and their leaves to ripe or overripe fruits, decaying organic material and grains. The nature of their food will require an effective cellulase system for degradation and digestion.

Some have reported possible endogenous enzyme sources are the hepatopancreas, gastric teeth and crystalline styles (needle-like structures made of crystalline proteins forming a motor organ in the stomach of bivalves)(BLIGH & DYER, 1959)

AFRICAN GIANT SNAIL (ARCHACHATINA MARGINATA)

Archachatina marginata is the largest of the *Archachatina* snails and is found in West Africa. It appears to be a mainly terrestrial snail. In Cameroon it can be found aestivating under ground during the drier months, having a closed aperture, sealed with a solid, calcareous, white epiphragm. It is to be noted that when a crawling snail is disturbed it produces a peculiar screaming noise, caused by the expulsion of air as the shell is rapidly retracting by the powerful columella muscle. Natively, this species does not cause any appreciable damage to native crops and is actually considered an economic asset among many native peoples who include it in their diet. In many parts of West Africa, it is considered the second best snail to eat after *Achatina achatina*. They have been known to stow away on banana shipments and make it to Germany (Boettger, 1938).

An albino-bodied form can be found and is actually becoming more common. This is because natives prefer to eat the dark-skinned ones, based on the belief that they are tastier and that there is something undesirable or freakish about the white-skinned ones. It must be noted that in captivity, it is known that captive snails tend to be lighter than their wild counterparts and this is not the same

thing. The shells of *Archachatina marginata* are used for domestic purposes by locals for salt holders and cups etc.

IDENTIFICATION

Archachatina have a raised V ridge on their tail that you can feel clearly if you run a wet finger over it. The snail's skin to be more finely textured than *Achatina*, although this is less apparent near the tail. Unfortunately this can't tell you what type of *Archachatina* you have but it is the first step. In addition to geographic locale, there are two main features distinguish the several variations of *Archachatina marginata* from the related members of the genus:

"The first is the subsutural, usually strongly marked engraved line, separated from the suture by a low narrow depressed area covered with irregular, low, vertical folds, the suture itself being straight or very slightly wavy, not crenulate. The engraved line starts on the fourth and fifth whorl and it is often deep and prominent, particularly on the body-whorl; but occasionally it is weak or almost lacking, especially in subsp. *eduardi* Pilsbry. The second feature is a peculiar microsculpture of the body-whorl, only visible with the proper magnification. It consists of numerous extremely fine, close-set, criss-cross or anatomising lines, making the surface of the periostracum look as if it had been pressed with a very finely woven cloth. This "weave" type of microsculpture is more pronounced in some forms or races than in others. It is particularly conspicuous when the periostracum is well developed and preserved, as is more common in some of the Cameroon races, such as subsp. *egregia*. In old shells, even when taken alive, the microsculpture is sometimes almost completely worn off, but traces of it may generally be detected in a few spots. The nepionic whorls, when well preserved, as in newly hatched or very young shells, are densely covered with regular spiral and vertical rows of minute granulations, which become coarser on the first, post-nepionic whorl.

DISTRIBUTION

This species occurs in Western Africa (Cameroon to the Democratic Republic of the Congo) and the Caribbean (Martinique). [1] How the species reached Martinique is unknown, but they may have been intentionally introduced as "pets" or by workers returning from West Africa. [2] The natural spread of this species is very slow; however, unintentional spread by individuals for food and as folk medicine is very common. [3] The USDA routinely checks for the species in the luggage of travelers from West Africa, Nigeria particularly, Ghana, and Cameroon. This species has not yet become established in the United States, but it is considered to represent a potentially serious threat as a pest, an invasive species that could negatively affect agriculture, natural ecosystems, human health, or commerce. Therefore, this species may be given top national quarantine significance in the United States (Agbelusi & Adeparusi, 1999).

LIFESPAN OF THE ARCHACHATINA MARGINATA

Healthy, genetically-strong *Archachatina marginata* will live at least 4 to 6 years on average, and up to 10 years with exceptional care. The wide range of reported life spans is a result of different habitat conditions. For the first 6 months, they grow really fast, after that the rate at which they grow is reduced and is not as pronounced as before (Miladinov, 2020).

DIET

Snails eat during the day, but they prefer to eat at night. Wild snails are known to eat up to 500 different species of plants. Snails that have become domesticated typically consume food that is high in protein and low in fats. Studies of domesticated snails have shown that poultry droppings have been the most effective meal to both grow and gain weight. [8] Captive individuals are easily fed with a variety of fruit and vegetables including tomato, lettuce, carrot, cucumber, beans, squash, banana and more. Captive individuals should also be supplemented with a source of calcium and other vitamins (such as is found in reptile turtle pellets, etc.) (Agbelusi & Adeparusi, 1999)

ORYCTES RHINOCEROS

The coconut palm rhinoceros beetle (*Oryctes rhinoceros*) is a species of rhinoceros beetle of the family Scarabaeidae. *O. rhinoceros* attacks the developing fronds of raffia, coconut, oil, and other palms in tropical Asia and a number of Pacific islands. Damaged fronds show typical triangular cuts.[Bed 1] The beetle kills the palms (particularly newly planted ones) when the growing point is destroyed during feeding.[5] They also infest dead trunk debris(Bedford, 2013).

LARVAE

Larvae are 'grubs' with brown heads and legs and a C-shaped creamy-white body that grow up to 100 mm.Preferred locations for larval development are dead or dying palm logs, either standing or on the ground, or in the stumps of palms.In 8-12 days the eggs hatch. There are three larval life stages (instars).Grubs at the end of the 3rd instar tunnel down below where they have been feeding and make a hollow in the soil (or palm trunk) lined with liquid faecal material (frass or insect droppings).The grub moults to the pupal stage in this hollow before emerging as an adult(Stünzner, 2021).

IMPACTS

Historically, uncontrolled populations of CRB was estimated to have killed 50% of palms over a 10 year period, with some islands suffering more than this. Where pest populations are generally under control, the damage CRB causes to palms results in reduced leaf area, early death of flowers and early nut fall, consequently reducing coconut yields.

Pest control is needed when CRB infest palms. The combination of direct losses (reduced yield) and costs of control results in economic losses. Infestations of CRB result in replanting costs and costly control measures. It has been estimated CRB has caused losses of \$1.1 million in South Pacific Territories in 1968 alone(Palanivel & Shah, 2021).

DIET

As with many beetles, adults and larvae have different feeding preferences. In the case of *Oryctes rhinoceros*, damage to plants is caused by adults (especially young adults) and not larvae, which feed on already rotting material (Giblin-Davis 2001).Larvae live in decaying material including: *Cocos nucifera*, *Artocarpus* sp. (breadfruit), *Calophyllum inophyllum* (Alexandrian laurel), *Mangifera* sp. (mango), and *Pandanus* sp.

Adults are a major pest of *Cocos nucifera* (coconut palm) and *Elaeis guineensis* (African oil palm) (Giblin-Davis 2001) but are a minor pest of many other palms and plant species. By feeding on healthy leaves, *Oryctes rhinoceros* causes physical damage, which can stunt growth and lead to secondary infections from bacteria or fungi(Marshall et al., 2017).

DISTRIBUTION

Oryctes rhinoceros (L.), the coconut rhinoceros beetle, is a pest species occurring throughout many tropical regions of the world. Adults can cause extensive damage to economically important wild and plantation palms.*O. rhinoceros* is endemic to the coconut-growing regions of South and South-East Asia from Pakistan to the Philippines (CIE, 1967). It was accidentally introduced into parts of Papua New Guinea in the Bismarck Archipelago (New Britain, New Ireland, Manus Island); Western and American Samoas, Tonga, Fiji, Wallis Island, Nigeria, Micronesia, Mauritius and the Cocos Islands (Bedford, 1974, 1980). It has recently been found in Guam and Saipan (Moore, 2007) and Hawaii (Hawaii Department of Agriculture, 2014).

CELLULASE AND WASTE MANAGEMENT

The recognition that environmental pollution is a worldwide threat to public health has given rise to a new massive industry for environmental restoration. Despite the massive utilization of

lignocellulose materials, there are still ample cellulose-containing raw materials and waste products that are not exploited or that could be used more efficiently. The problem in this respect is, however, to develop sustainable processes that are economically profitable. Biological degradation, for economic and ecological reasons, has become an increasingly popular alternative for the treatment of agricultural, industrial, organic, as well as toxic wastes. These wastes have been insufficiently disposed leading to environmental pollution(Lohri et al., 2017). Lignocellulose is the most abundant plant cell wall component of the biosphere and the most voluminous waste produced by our society. It consists of 70% moisture and 30% solid; of which holocellulose accounts for 65.5%, lignin 21.2%, ash 3.5%, hot water-soluble substances 5.6%, and alcohol-benzene soluble 4–1% (Zhang et al., 2015). Lignin is an integral cell wall constituent, which provides plant strength and resistance to microbial degradation (Saranraj et al., 2012). Cellulose Containing wastes may be agricultural, industrial, or urban in origin, and sewage sludge might also be considered a source of cellulose, since its cellulosic content provides the carbon needed for methane production in the anaerobic digestion of sludge. Biological degradation with enzymatic hydrolysis of cellulosic biomass requires low volumes of chemicals and are conducted at mild conditions, in comparison with chemical hydrolysis. Moreover, chemical hydrolyzates need to be detoxified before carrying out fermentation. Therefore, enzymatic hydrolysis of lignocellulosic substrates is an efficient process (Zoghlami & Paës, 2019). A variety of microorganisms take part in enzymatic hydrolysis of cellulose with the aid of a multienzyme system. Cellulases are inducible enzymes synthesized by a large diversity of microorganisms including fungi, bacteria, and actinomycetes during their growth on cellulosic materials. These microorganisms can be aerobic, anaerobic, mesophilic, or thermophilic(Hyde et al., 2019). But, relatively few fungi and bacteria produce high levels of extracellular cellulase capable of solubilizing crystalline cellulose extensively(Li et al., 2019). Cellulase production by different organisms in fermentation has received more attention and is found to be cost- prohibitive because of the high cost of process engineering. Therefore, its production using readily available sources will help reduce importation costs. A portion of pretreated biomass can be used to feed a fungus or other organisms that produce cellulase that can then be added to pretreated solids to release glucose from cellulose(Novy et al., 2019). Cellulases are responsible for the hydrolysis of the β -1,4-glucosidic bonds in cellulose. They are members of the glycoside hydrolase families of enzymes, which hydrolyze oligosaccharides and/or polysaccharides(Lynd, 1996). In nature, complete enzymatic hydrolysis of cellulose is mediated by a combination of the three main types of cellulases: (1) endo-1,4- β glucanase (CMCase), (2) cellobiohydrolase or exoglucanases (Avicelase), and (3) β -glucosidase (cellobiase), which act synergistically in the hydrolysis of cellulose (Klein-Marcuschamer & Blanch, 2015). Waste recycling has been advanced as a method for preventing environmental decay and increasing food supply. The potential benefits from a successful recycling of lignocellulosic wastes are enormous. Cellulose and hemicellulose are sugar-rich fractions of interest for use in fermentation processes, since microorganisms may use the sugars for growth and production of value-added compounds, such as bioethanol, animal feed, compost, flavor, bioactive compounds, organic acids, and others. Nature solves the problem of removing recalcitrant plant cell wall material from the environment through the action of broad consortia of bacteria in the various cellulosic ecosystems, but over extended time periods. But the development of scientific and/or engineering approaches to the cost-effective conversion of plant cell wall biomass to biofuels is more beneficial(Briški & Vuković Domanovac, 2019)

Materials and Methods

Materials

Sodium chloride, potassium chloride, lead chloride, barium chloride, manganese chloride, nickel chloride and ammonium sulphate, were purchased from BDH Chemical Limited, Poole England. EDTA and Carboxymethyl-cellulose were purchased from Sigma Chemical Company, St. Louis, USA. All other reagents were of high analytical grade and used without further purification.

The agro-industrial wastes (banana peel, rice husk, orange mesocarp, pineapple peel, maize cob) used were obtained locally, ground to powder using mechanical grinder, sieved and dissolved in the assay buffer. These wastes were used without prior pre-treatment. Rhinoceros Larvae and the Gaint african snail were collected from South-Western part of Nigeria. Apparatus used include, mortar and pestle, weighing balance (Mettler PN1210), pH meter (PHS-25), Centrifuge (Helmreasinn 80-2) and UV/VIS Spectrophotometer (Bosch 752N).

Extraction of enzyme

Enzyme Extraction and Purification Rhinoceros Larvae and the Gaint african snail were washed and rinsed with distilled water. The Gut of Rhinoceros Larvae and the Gaint african snail were gently homogenized in 10 mM sodium acetate buffer, pH 5.0, containing 1 mM EDTA. The mixture collected was centrifuged at 15,000 rpm for 15 minutes at room temperature. The supernatant, which served as the crude enzyme, was collected and stored in a refrigerator. The supernatant was salted out by bringing the crude extract to 65% (w/v) saturation with Ammonium sulphate.

Assay Method

The activity of cellulase was determined by the method of Nelson, 1944) for reducing sugar. To 0.1ml of the enzyme solution was added 0.5ml of 1% CM-cellulose in 10ml of 0.1mM Sodium Acetate buffer (containing 1mM EDTA) pH 5.0. The solution was incubated at 40oC for 30 minutes before 0.4ml of distilled water was added to the mixture. 1 ml of combined copper reagent was added to the reaction mixture and heated at 100oC for 20 minutes to stop the reaction. The mixture was allowed to cool to room temperature. 1.0 ml of Arsenomolybdate reagent was added and mixed thoroughly to dissolve all precipitate of CU2O. 7.0 ml of distilled water was then added to dilute the mixture. Absorbance was then read at 540nm. A unit of enzyme activity is taken as the amount of enzyme that produces a 0.1 change in absorbance at 540nm under specific conditions (Hurst et al., 1977). Protein concentration was estimated using the method of Bradford (1976) using bovine serum albumin as the standard

Actions of the partially purified cellulase on some wastes

The different waste materials were dried and cut into small pieces. In the case of wood materials, the saw dust was used. To 0.1 g of the various waste materials was added 1 ml of 0.1 mM Sodium Acetate buffer (containing 1 mM EDTA) pH 5.0, 0.2 ml of partially purified enzyme was added and incubated overnight. A control with CMC as substrate was run along each waste. The activity of the enzyme was then assayed as described above.

Results

LARVA

Table 1. Summary of the purification of Gut Rhinoceros Larvae cellulase.

Purification step	Total Protein (mg)	Total Activity (μ mol/min)	Specific Activity	% Yield	Purification fold
Crude Extract	1031.05	712.63	0.69	100	1
65% Ammonium sulphate	582.10	591.09	1.02	56.46	1.47

Table 2. Summary of the purification of archachatina marginata gut.

Gut cellulase					
Purification step	Total Protein (mg)	Total Activity (μmol/min)	Specific Activity	% Yield	Purification fold
Crude Extract	1162.61	805.21	0.69	100	1
65% Ammonium sulphate	538.26	637.24	1.18	46.32	1.72

ARCHACHATINA MARGINATA HEAMOLYMPH**Table 3.** Summary of the purification of archachatina marginata Heamolymph cellulase.

Purification step	Total Protein (mg)	Total Activity (μmol/min)	Specific Activity	% Yield	Purification fold
Crude Extract	652.33	812.32	1.25	100	1
65% Ammonium sulphate	241.12	629.11	2.61	37	2.09

KINETIC PARAMETERS**Table 4.** Kinetic Parameters of Cellulase from Rhinoceros Larvae, African giant Snail Gut and African giant Snail Haemolymph.

	African giant Snail Gut	African giant Snail Haemolymph	Larvae
K_m	3.43	23.76	11.63
V_{max}	112.02	998.50	648.01

Action of Rhinoceros Larvae and the Gaint african snail cellulase on some wastes**The results of the action of cellulase on some wastes are presented in Tables 2–6.****WOOD****Table 5.** Action of Cellulase from Rhinoceros Larvae and the Gaint african snail on different Woods.

	African giant Snail Gut	African giant Snail Haemolymph	Larvae

Fig tree	15.02	18.22	33.69
Oak tree	11.84	16.27	116.24
Palmplum tree	13.36	17.62	108.47
Kick tree	25.35	173.09	189.11
African balsam tree	16.87	28.65	67.85
Maple tree	28.31	16.32	70.17
Kapok tree	17.41	53.56	102.42
Mugwort tree	19.88	14.37	45.70
Kola tree	15.81	14.17	98.84
Ohugbo tree	17.86	1.17	102.51

PAPER

Table 6. Action of Cellulase from Rhinoceros Larvae and the Gaint african snail on different Paper.

	African giant Snail Gut	African giant Snail Haemolymph	Larvae
Carton paper	177.43	353.96	567.01
Brown paper	109.84	133.10	175.29
Tissue paper	76.52	56.76	108.56
Art paper	142.30	259.36	353.37
Jotter paper	136.35	243.33	328.62
News paper	156.52	107.68	209.45
Office file	161.87	133.10	262.82
Note book	196.08	108.58	183.81

LEAVES

Table 7. Action of Cellulase from Rhinoceros Larvae and the Gaint african snail on different Leaves.

	African giant Snail	African giant Snail Haemolymph	Larvae

	Gut		
Orange Leaf	113.09	180.87	163.33
Mango Leaf	148.14	348.32	301.40
Kolanut Leaf	117.41	218.62	436.76
Cassava Leaf	78.57	162.30	192.23
Lemon Leaf	84.79	148.02	169.57

KITCHEN WASTE

Table 8. Action of Cellulase from Rhinoceros Larvae and the Gaint african snail on different Kitchen Waste.

	African giant Snail Gut	African giant Snail Haemolymph	Larvae
Garri	57.16	137.89	97.91
Egg shell	82.73	61.85	63.38
Yam flour	74.76	49.97	100.47
Yam peel	65.42	91.16	94.09
Orange peel	179.95	452.37	457.05
Pineapple peel	129.10	94.90	138.72
Onion peel	175.41	428.0	382.60
Beans peel	90.98	122.06	174.36

INDUSTRIAL WASTE

Table 9. Action of Cellulase from Rhinoceros Larvae and the Gaint african snail on different Industrial Waste.

	African giant	African giant	Larvae

	Gut	Haemolymph	
Groundnut shell	59.95	63.55	121.36
Sorghum	67.10	67.59	124.34
Millet	70.68	73.33	122.85
Rice hulls	68.19	101.39	120.47
Wheat	77.51	111.93	155.23
Soluble starch	76.50	107.63	117.26

Discussion

The results of our study reveal intriguing insights into the potential application of cellulase enzymes derived from the gut of *Archachatina marginata* and *Oryctes rhinoceros* larva in the biodegradation of various waste materials. While the enzymatic activities of these sources were evaluated and compared against a common laboratory substrate, carboxymethyl cellulose (CMC), the discussion that follows delves into the implications and significance of these findings.

1. Waste Material Specificity:

Our study demonstrates the varying degrees of enzymatic activities exhibited by *Archachatina marginata* and *Oryctes rhinoceros* larva cellulases when applied to different waste materials. Notably, cellulases from *Oryctes rhinoceros* larva show higher activity levels on wood waste, paper waste, and industrial waste, while *Archachatina marginata* cellulases exhibit more balanced activities across the waste types. This specificity suggests the potential for tailored waste management strategies depending on the waste composition.

2. Role in Waste Management:

The observed biodegradation potentials of cellulase from both sources underscore the relevance of these enzymes in waste management efforts. In a world grappling with the mounting challenge of solid waste, finding effective and sustainable methods for waste degradation is imperative. Cellulase enzymes, such as those from *Archachatina marginata* and *Oryctes rhinoceros* larva, offer promise in addressing this issue.

3. Environmental Impact:

As traditional waste disposal methods contribute to environmental pollution, the application of cellulase enzymes for waste degradation holds potential for reducing this environmental burden. These enzymes can contribute to more eco-friendly waste management practices, diminishing the environmental repercussions associated with waste accumulation and landfilling.

4. Rate of Degradation:

Our study reveals variations in the rates at which the cellulases from *Archachatina marginata* and *Oryctes rhinoceros* larva degrade different waste materials. This insight is crucial for optimizing the application of these enzymes in real-world waste management scenarios, allowing for efficient and controlled waste degradation processes.

Conclusion:

In conclusion, the findings of this study provide compelling evidence for the biodegrading potential of cellulase enzymes sourced from the gut of both *Oryctes rhinoceros* larva and *Archachatina marginata* when applied to a range of waste materials. While the enzymatic activities

exhibit variations and specificities based on the waste type, the broader implication is that these cellulases hold promise as valuable assets in the realm of waste management.

The world faces an ever-mounting crisis of solid waste generation, one that carries severe environmental and public health consequences. By harnessing the biodegradation capabilities of cellulase enzymes, we can unlock innovative solutions that contribute to a more sustainable and eco-friendly approach to waste disposal. These enzymes, derived from unconventional sources, have the potential to play a pivotal role in shaping the future of waste management, offering a path toward reducing waste, mitigating pollution, and safeguarding the environment. Further research and application of these enzymes may lead to more effective waste management strategies, paving the way for a cleaner and more sustainable world.

References

1. B.S. Fagbohunka, R.E. Okonji, and Z. A. Ayinla (2017) Purification and Characterization of Cellulase from Termite *Ametermes eveuncifer* (Silverstri) Soldiers. *International Journal of Biology*. 9(1):1-9.
2. B. S. Fagbohunka, S. E. Edorh, M. M. Adeyanju, E.N. Ezima, M. A. Alabi, and O.O. Ogunlabi (2015). Activities of a Cellulase of the Termite, *Ametermes eveuncifer* (Silverstri) Soldier: Clue to Termites Salt Intolerance. *Journal of Natural Sciences Research*, 5(11): 117 –123.
3. E. N. Ezima, M. M. Adeyanju, K. T. Oduduwa, T. A Aremu and B. S. Acharya S. and Anita C. (2012). Bioprospecting Thermophiles for Cellulase Production: A Review. *Brazilian Journal of Microbiology*: 844-856
4. Awosusi A.O (2010). Assessment of Environmental Problems and Methods of waste Management in Ado-Ekiti Nigeria. *African Research Review*.4(3b): 331-343.
5. Bayer E.A, Lamed R, Himmel M.E (2007). The Potential of Cellulase and Cellulosomes for Cellulosic Waste Management. *Current Opinion in Biotechnology*.18:237-245.
6. Bhaumik P and Dhepe, P.L (2015). Conversion of Biomass into Sugars. Biomass Sugars for Non-Fuel Applications. *Green Chemistry Series. Royal Society of Chemistry*.1-53.
7. Bignell, D. E., & Eggleton, P. (2000). Termites in ecosystems. In T. Abe, D. E. Bignell, & H. Higashi (Eds.), *Termites: Evolution, sociality, symbiosis, ecology* (pp. 363–387). Dordrecht:kluwer Academic Publishers.
8. Blumer-Schuette S.E, Lewis D.L and Kelly R.M (2010). Phylogenetic, Microbiological, and Glycoside Hydrolase Diversities within the Extremely Thermophilic, Plant Biomass-Degrading Genus *Caldicellulosiruptor*. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*,76(24):8084–8092.
9. Chandra R.P, Bura R, Mabee W.E, Berlin A, Pan X, Saddler J.N (2007). Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics. *Advances in Biochemical Engineering/ Biotechnology*.108:67–93.
10. Deborah P.D and Yehudit A (1995), Cellulose Biosynthesis. *American Society of Plant Physiologists*.7:987-1000.
11. Dustin Severtson (2006). A feasibility study of employing Australian subterranean termites in the Management of discarded paper (lignocellulosic waste). *Bioconversion of waste paper by termites*:1-60.
12. Ebikapade Amasuo & Jim Baird (2016). The concept of waste and waste management. *Journal of Management and Sustainability*; 6(4):88-96.
13. Ebna F.M, Md. Rokon H, Md. Sayed R, Salma A, Sm. Arifur R, Tanisa T.S (2013), Solid Waste Management Strategy & Improvement of Existing Scenario Based on Market Waste. *Global Journal of Researches in Engineering*.13(4)
14. Edward A.B, Henri C, Raphael L and Yuval S (1998). Cellulose, Cellulases and Cellulosomes. *Current Opinion in Structural Biology*.8:548-557.
15. Eggleton, P. (2000). Global patterns of termite diversity. In T. Abe, D. E. Bignell, & M. Higashi (Eds.), *Termites: Evolution, sociality, symbiosis, ecology* (pp. 25–51). Dordrecht: Kluwer Academic Publisher.
16. Eggleton, P. (2011). An introduction to termites: Biology taxonomy and functional morphology. In D. E. Bignell, Y. Roisin, & N. Lo (Eds.), *Biology of termites: A modern synthesis* (pp. 1–26). Dordrecht: Springer.
17. Ezima et al (2014). BIODEGRADATION OF WASTES USING CELLULASE FROM THE TERMITES, *Ametermes eveuncifer* (Silverstri) WORKERS: A CLUE TO THE APPLICATION OF TERMITES CELLULASE IN WASTE MANAGEMENT. *Science Focus*.19 (2): 93 – 98.
18. Fagbohunka (2014). Biodegradation of wastes using cellulase from the termite *Ametermes eveuncifer* (Silverstri) Workers: A clue to the application of termites cellulase in waste management. *Science Focus* 19(2): 93-98.
19. B. S. Fagbohunka, E. N. Ezima, M. M. Adeyanju, M. A. Alabi, D. E. Oyedele and A. A. Adeneye, (2014) Inhibition Studies Of Some Key Enzymes of the Termite, *Ametermes Eveuncifer* (Silverstri) Workers: Clue to Termites Salt Intolerance. *Science Focus*, 19 (1): 81- 87.

20. B. S. Fagbohunka, F. K. Agboola, and A. Afolayan, (2012). Characterization of a cellulase from the haemolymph of the Giant African Snail (*Archachatina marginata*). *Africa Journal of Biotechnology* 11(38): 9254-9264.
21. F. K. Agboola, B. S. Fagbohunka, and G. A. Adenuga (2008) Activities of *Archachatina (Calachatina) marginata* haemolymph enzymes: clues to terrestrial snails salt intolerance. *International Journal of Biological and Chemical Sciences*, 2(1): 66-71.
22. B. S. Fagbohunka., C.O. O. Babasanya, G. A. Adenuga, and F. K. Agboola (1997). Partial purification and characterization of cellulase I from the haemolymph and gut of the giant African snail, *Archachatina marginata*. *Nigeria Journal of Nutrition Sciences* 18(2):28-34.
23. Agboola, F. K., Fagbohunka, B. S. and Adenuga, G. A. (2008). Activities of *Archactina (Calachatina) marginata* haemolymph enzymes: clue to terrestrial snails' salt intolerance. *Int. J. Biol. Chem. Sci.*, 2(1): 66-71.
24. Agboola, F. K., Fagbohunka, B. S., and Adenuga, G. A. (2006). Activities of Thiosulphate and 3-Mercaptopyruvate-cyanide-sulphurtransferases in Poultry Birds and the Fruit Bat. *Journal of Biological Sciences* 6(5): 833-839.
25. Agboola, F. K., Kuku, A. Okonji, R. E. and Fagbohunka, B. S. (2004). Tissue distribution of thiosulphate and mercaptopyruvate sulphur transferases in the human. *Science Focus* 7: 81-84. Alabi, M.A. and Daini, O.A. (2009).
26. Enzymatic properties of purified serine protease from *Aspergillus fumigatus* grown on sabaraud dextrose agar. *Int. J. Biol. Sci.* 2(1): 51-59.
27. Cleveland, L. R. (1925): The ability of termites to live perhaps indefinitely on a diet of pure cellulose. *Biol. Bull.* 48: 289-293.
28. Ezima, E. N., Fagbohunka, B. S. and Adeyanju, M. M. (2011). The Effects of Partially purified Cellulase from Worker Termite (*Ametermes eveuncifer Silvestri*) on some wastes. *International Journal of Biochemistry* 3(1):15-20
29. Fagbohunka, B. S., Okonji, R. E., Adenuga, G. A., and Agboola, F. K. (2004) Properties of Rhodanese (thiosulphate sulphur transferase) from the hepatopancreas of giant snail, *Archachatina marginata*. *Science Focus* 7: 76-80.
30. Fagbohunka, B. S., Edorh, S. E., Adeyanju, M. M., Ezima, E.N., Alabi, M. A. and Ogunlabi, O. O. (2014). Activities of a Cellulase of the Termite, *Ametermes Eveuncifer (Silverstri) Soldier*: Clue to Termites Salt Intolerance. *IISTE-Journal of Natural Science Research*.
31. In Press Gay and Calaby (1970). Termites of the Australian region. in; Krishna K Weesner FM eds. *Biology of Termites*, Vol. II Academic Press NY, Pp. 401.
32. Hurst, P. L., Nielson, J., Sullivan, P. A. and Shepherd, M. G. (1977). Purification and Properties of Cellulase from *Aspergillus niger*. *J. Biochem.* 165, 33-41.
33. Malik, K. A., Kauser, F. And Azan, E. (1980). Effect of Sodium Chloride on the Cellulolytic Ability os some Aspergilli. *Mycologia*, 72(2), 322-328.
34. Montgomery, R. D. (1980) Cyanogens. In *Toxic Constitution of Plants Food Stuff*, Liener, I.E. (Ed) 2nd Edn., New York, Academic Press, pp: 143-161.
35. Nakashima, K., H. Watanabe, H. Saitoh, G. Tokuda and J. I. Azuma (2002a). Dual cellulose-digesting system of the wood-feeding termite, *Coptotermes formosanus Shiraki*. *Insect Biochem. Mol. Biol.*, 32, 777-784.
36. Nakashima, K; Watanabe, H; Azuma, J. (2002b). Cellulase genes from the parabasalian symbiont *Pseudotrichonympha grassii* in the hindgut of the wood-feeding termite *Coptotermes formosanus*. *Cell. Mol. Life. Sci.*, 59, 1554-1560.
37. Smith, R. L. (2007). Termites. Arizona-Sonora Desert Museum. email: info@desertmuseum.org
38. Umezurike, G. M. (1976). The beta glucosidase in the gut content of the snail (*Archatina achatina*). *Biochem. J.* 157: 381-387.
39. White, A., Handle, P., Smith, E. L., Hill, R. L., and Lehman, I. R. (1981) *Principles of Biochemistry* (6th Edn). McGraw Hill: Kogakusha, Tokyo, Japan; pp: 391-734.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.