



Actions of Cellulase Isolated from the Hemolymph of Giant African Snail (*Archachatina marginata*) on Solid Wastes

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ABSTRACT

Solid wastes generation is a ginormous and ever-expanding problem affecting the world, majorly developing countries. This is because the world populace produces million tonnes of municipal solid wastes each day and this number has been projected to triple in the next few years. Drastic increase in wastes especially solid wastes could be directly proportional to increase in pollution and this could pose deleterious effect to mankind in general. Cellulose which is a chief constituent of solid wastes could be degraded by cellulase, a hydrolytic enzyme presents in the genetic make-up of giant African snail. In this study, cellulase was isolated from the hemolymph of *Archachatina marginata* and partially purified by draining the hemolymph into a sterilized beaker. The drained hemolymph was centrifuged at 4000g for 20 min to obtain a crude fraction of the enzyme. The actions of isolated cellulase on different agricultural and industrial wastes were examined using standard procedure. The enzyme had a specific activity of 1.39U/mg. Biodegradation study showed that the enzyme has highest activities on agricultural and industrial wastes such as; Leaves (approximately 125.32-426.40%), Plastics (2.95-91.14%), Wrappers (21.29-72.65%), Kitchen wastes (3.89-65.71%), Woods (12.34-40.77%) and the lowest activity was recorded on Nylons (3.26-9.64%). The degree of biodegradation of solid wastes signifies that the enzyme could play a key role in efficient, reliable and non-hazardous means of waste management and material recycling.

Keywords: Solid Wastes, Pollution, Cellulose, Cellulase, *Archachatina marginata*, Waste Management.

Introduction

Cellulase, an enzyme complex produced primarily by bacteria, fungi, and protozoans catalyses the breakdown of cellulose (Chapin III *et al.*, 2002). The enzyme consists of at least three components generally classified by their hydrolysing functions i.e. the breakdown of β -1,4-glycosidic bonds in cellulose into the followings: endo- β -1,4-glucanase (E.C. 3.2.1.4) randomly hydrolysing the

central of cellulose chains, exo- β -1,4-cellobiohydase (E.C. 3.2.1.91) hydrolysing the non-reducing end of cellulose chains into a disaccharide, cellobiose units and β -glucosidase (EC 3.2.1.21) hydro-lysing cellobiose or longer chains from non-reducing end to glucose units (Watanabe *et al.*, 2001; Bala *et al.*, 2008). On the other hand, there are also cellulases produced by

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other types of organisms such as plants and animals (Byrne *et al.*, 1975; Davies, 1998, Fagbohunka *et al.*, 2021ab) these variations in cellulose species makes them differ mechanistically and structurally (Chapin III *et al.*, 2002). Cellulases are used commercially for food processing, coffee production, in textile industry and in laundry detergents. They have also been used in the pulp and paper industry for various purposes, and in pharmaceutical applications (Chapin III *et al.*, 2002). The beauty of these enzymes is that they are involved in the conversion of cellulosic materials to fermentable sugars (Bayer *et al.*, 2007) 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). Furthermore, cellulase dominates 20% of the total enzyme market in all over the world, because of its massive demand in various industries such as in biofuel production, pulp, paper, textile, food, beverages, detergent industries as well as in bioremediation of wastes (Srivastava *et al.*, 2015a). This makes cellulase the third largest industrial enzyme distributed globally and of key importance in the enzyme market (Yoon *et al.*, 2014).

In many industrial applications of cellulose, enzymatic hydrolysis is desirable owing to the mild processing conditions, high specificity of the reactions and better control of the processes (Teeri, 1997). Cellulase has been shown to exist not only in plants, mould, fungi, bacteria and protozoa, but also in herbivorous invertebrates such as arthropods, nematodes and mollusc (Suzuki *et al.*, 1993).

The management of solid waste continues to be a significant challenge in urban areas throughout the globe notably within the growing cities of the developing world (Foo, 1997). The dynamic economic trends and speedy urbanization complicate solid waste management. Consequently, solid waste is not only increasing in composition but also changing in quantity from a

few kilograms to tonnage proportions. Thus, this research focuses on how the problem posed by these wastes could be biodegraded or recycled to benefit the rapidly increasing populace.

Materials and Methods

Materials

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

Methods

Collection and Preparation of Samples

Solid wastes samples such as wood, maize, cashew and kitchen wastes 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. Giant African Snail (*Archachatina marginata*) was acquired from Sagamu Local Market, Sagamu, Ogun State. They were taken to the laboratory in a well-ventilated container.

Crude Extraction

The Snails were properly rinsed with distilled water. The pointed tips of the shell of the snail were carefully broken, so as not to puncture the gut. The hemolymph was drained carefully into a sterilized beaker, centrifuged at 4000g for 20 min and then filtered with a loose plug of glass wool to obtain a crude fraction. The supernatant was transferred to a

clean reagent bottle. This served as the crude extract of the cellulose enzyme and this was stored inside the refrigerator.

Determination of Protein Concentration

Protein concentration was determined using Biuret method (Gornall, 1959) using bovine serum albumin (BSA) as a standard. During the purification, protein concentration was measured by taking the absorbance at 280nm and 260nm (Warburg and Christian, 1942).

Determination of Cellulase Activity

The activity of cellulase was determined by the method of Miller *et al.*, 1959. The reducing sugars were determined by estimating 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, 1959).

Biodegradability Test

Biodegradability test of the samples were 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 to the mixture and incubated overnight. A control with Carboxymethylcellulose (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 was read at 540nm.

Results

Biodegradability potential Test

A. *marginata* hemolymph cellulose was purified with a specific activity of 1.39 U/mg protein. The results for the action of the enzyme on various agricultural and industrial waste samples are presented in Tables 1 – 6. The cellulase from the hemolymph of *Archachatina marginata* showed significant degradability potential on all solid wastes tested. The highest degradation activity was observed on Cashew leaf waste (*Anacardium occidentale*) (426.40%), followed by Maize (*Zea mays*) (125.32%), Ijagbe (*Swieteria macrophylla*), a hard wood (40.77%) and the least activity was recorded in Aga (*Musanga cecropiodes*), which is also a hard wood (12.34%). The activity of cellulase on kitchen wastes was high in onion peel (*Allium cepa*) (65.71%) while the least activity was seen in eggshell (3.89%); on different wrappers, dufils indomie wrapper showed the highest degradability (72.65%) whilst the least degraded was Cornflakes wrappers (Infinity Foods Ltd) (23.21%); Leo Smart Pen plastic was highly degraded (91.14%) while the least degraded was Dana Plast Bucket (2.95%); on different nylons cellulose from the hemolymph of *Archachatina marginata* degraded Black Nylon (Just supplies and packaging Limited) the most (9.64%) while the least degraded was White Nylon (Tokyo packaging solutions) (3.26%).

Table 1: Action of Cellulase from the hemolymph of *Archachatina marginata* on different leaves

Leaves	Botanical Name	Activity (%CMC)
Cashew	<i>Anacardium occidentale</i>	426.40
Pear	<i>Pyrus communis</i>	416.23
Guava	<i>Psidium guajava</i>	411.69
Mango	<i>Magnifera indica</i>	393.94
Kolanut	<i>Cola acuminata</i>	322.29
Orange	<i>Citrus sinensis</i>	306.06
Lemon	<i>Citrus limon</i>	297.62
Banana	<i>Musa acuminata</i>	255.41
Cassava	<i>Manihot esculenta</i>	188.74
Maize	<i>Zea mays</i>	125.32

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

Table 2: Action of Cellulase from the hemolymph of *Archachatina marginata* on Wood Wastes

Local Name	English Name	Botanical Name	Type	Activity (%CMC)
CMC + Enzyme	-	-	-	100
Ijagbe	Mahogany	<i>Swieteria macrophylla</i>	Hard	40.77
Igbo	Black Afara	<i>Terminalia ivorensis</i>	Hard	30.13
Akomu	African Nutmeg	<i>Pycnanthus angolensis</i>	Soft	30.13
Utara	Sugar-Plum	<i>Uapaca quineensis</i>	Hard	27.97
Ayinre	Somb Wood	<i>Entada Africana</i>	Hard	27.12
Ahun	Cheese Wood	<i>Alstonia boonie</i>	Soft	26.08
Opoto	Fig Tree	<i>Ficus capensis</i>	Hard	20.43
Ekun	Sap Wood	<i>Brachystegia eurycoms</i>	Hard	20.43
Oro	Back-Cloth Wood	<i>Antiatia Africana</i>	Soft	18.46
Aga	Cork Wood of Umbrella Tree	<i>Musanga cecropiodes</i>	Soft	12.34

Table 3: Action of Cellulase from the hemolymph of *Archachatina marginata* on Kitchen Wastes

Substrates	Botanical Name	Activity (%CMC)
CMC + Enzyme	—	100
Onion peel	<i>Allium cepa</i>	65.71
Yam peel	<i>Dioscorea alata</i>	44.00
Beans peel	<i>Canavalia ensiformis</i>	37.88
Cassava granule Chaff	<i>Manihot esculenta</i>	32.18
Groundnut peel	<i>Arachis hypogaea</i>	27.38
Cassava flour Chaff	<i>Manihot esculenta</i>	14.96
Orange peel	<i>Citrus sinensis</i>	8.19
Corn cob	<i>Zea mays</i>	7.90
Potato peel	<i>Dioscorea bulbifera</i>	5.36
Egg shell		3.89

Table 4: Action of Cellulase from the hemolymph of *Archachatina marginata* on different wrappers

Wrappers	Manufacturer	Activity (%CMC)
CMC + Enzyme	—	100
Indomie wrappers	Dufils	72.65
Biscuit wrappers	P1 Pardee Food Nig Ltd	71.43
Dano wrappers	Fan Milk Nig Ltd	70.99
Pepsi wrappers	Pepsi-Cola	68.38
Minime wrappers	Dufils	66.74
Salt wrappers	Dangote	64.57
Cheeseball wrappers	Sunlight Resources Ltd	63.74
Spaghetti wrappers	Honeywell Food Plc	63.35
Detergent wrappers	Unilever Brand	47.97
Cornflakes wrappers	Infinity Foods Ltd	21.29

Table 5: Action of Cellulase from the hemolymph of *Archachatina marginata* on different Plastic

Plastics	Manufacturers	Activity (%CMC)
CMC+ Enzyme	_____	100
Pen	Leo smart company	91.14
Shaving stick	Dorco company	75.19
Shampoo container	Franemm Industrial Ltd	32.20
Soap case	Winbo	29.99
Toothbrush	Wisdom	18.61
Bournvita cover	Cadbury Nigeria Plc	15.81
Keg	Nipimco	14.18
Custard cover	isabi Mills (NIG) Ltd	7.24
Comb	Made in China	6.65
Bucket	Dana Plast	2.95

Table 6: Action of Cellulase from the hemolymph of *Archachatina marginata* on Nylon Wastes

Nylons	Manufacturers	Activity (%CMC)
CMC + Enzyme	_____	100
Black nylon	Just supplies and packaging Ltd.	9.64
Right option bread nylon	Right option bakery industry	8.66
Cotton wool nylon	Bex Investment	6.37
Transparent nylon	Abafem factory	6.04
Bigi package drinks nylon	Rite foods Ltd.	5.71
Pure water nylon	Tunde Sho Nig. Ltd.	5.55
Black and white nylon	Abafem factory	4.41
Polythene bag nylon	Innoplas packaging solutions	3.75
Vinkay pharmaceutical nylon	Vinkay pharmacy store	3.75
White nylon	Tokyo packaging solutions	3.26

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

Discussion

In this study, cellulase isolated from Giant Africa snail had a specific activity of 1.39 U/mg. This value is lower than that obtained for cellulase from other sources. *Oryctes rhinoceros larva* cellulase was purified with a specific activity of 2.38U/mg

(Apena *et al.*, 2018). Fagbohunka *et al.* 2018 reported a specific activity of 3.78 U/mg for beetle gut cellulose. Also, termite (*Amitermes eveuncifer*) Silvestri soldier cellulase was reported to have a specific activity of 7.08U/mg (Fagbohunka *et al.*, 2018). Although lower values of specific activity have been reported from termite (*Amitermes eveuncifer*) Silvestri worker which showed a specific activity value of 0.25U/mg (Ezima *et al.*, 2014). Also, fungi (*Aspergillus niger*) cellulase showed a specific activity of 0.0452U/mg (Hurst *et al.*, 1977).

The study of the cellulolytic activities of cellulase from the gut of Giant African Snail (*Archachatina marginata*) showed an astonishing result specifically with Dried Cashew leaves (*Anacardium occidentale*) 426.40%, Plastic pen (Leo Smart Company) 91.14%, Indomie wrapper (Dufil) 72.65%, Onion peel from kitchen wastes 65.71%, although it showed least activity with Wood waste (Ijagbe) 40.77% and Black Nylon (Just Supplies and packaging Ltd) 9.64%. The fluctuating degrees of activities of the enzyme on various solid wastes could be attributed to the texture of the materials, susceptibility of the materials to the cellulosic activity of cellulase or due to the conformation of cellulose present in the materials. Also, it has been noted that varied degree of cellulase activities on the different polymers could be as a result of the chemical and physical properties of plastics and the surface conditions of waste (Brueckner *et al.*, 2008; Eberl *et al.*, 2009; Tokiwa *et al.*, 2009). The existence of a cellulolytic enzyme capable of hydrolysing soluble cellulose (CMCase) principally may be because of the adaptation of the snail to majorly a vegetarian diet. Thus, it facilitates the animal to grow on the moist leafy vegetables, which form its chief food sources. We conclude that cellulase from the Gut of giant African snail (*Archachatina marginata*) showed a considerable degradability potential could serve as a key tool in waste management and biodegradation of wastes.

Conflict of interest

The authors declare they have no any conflict of interest.

References

Apena, A., Adeyemi, O. E. and Fagbohunka, B. S. (2018). Biodegradation of Wastes using Cellulase from the *Oryctes rhinoceros* Larva. (In the press)

Bala, M., Appelbaum, L. and Almogy, G. (2008). Unexpected cause of large bowel obstruction: colonic bezoar. *Isr. Med. Assoc. J.* 10: 829-30.

Bayer, E. A., Lamed, R. and Himmel, (2007). The potential of cellulases and cellulosomes for cellulosic waste management. *Curr. opin Biotech.* 18: 237-245.

Brueckner, T., Eberl, A., Heumann, S., Rabe, M. and Guebitz, G. M. (2008). Enzymatic and chemical hydrolysis of poly (ethylene terephthalate) fabrics. *Journal of Polymer Science A-Polymer Chem.*46:6435–6443.

Byrne, H., Chrstou, N., Desh, P. and Maclachlan, G. (1975): Purification and characterization of Two Cellulases from Auxin-treated Pea Epicotyls, *J. Biochem* 250: 1012.

Chandra, M., Kalra, A., Sharma, P. K., Kumar, H. and Sangwan, R. S. (2010). Optimization of Cellulases Production by *Trichoderma citrinoviride* on marc of *Artemisia annua* and its application for bioconversion process. *BIOENERG.*34:805–811.

Chapin III, F. S., Matson, P. A. and Mooney, H. A. (2002): Principles of Terrestrial Ecosystem Ecology. Springer-Verlag New York, NY. The Merck Manual of Diagnosis and Therapy, Chapter 24.

Conn, E. E. and Stumpf, P. K. (1976). Outlines of biochemistry, 4th edition, in E.J. Wood, (Eds.), John Wiley and Sons, Inc. London

and New York, Pp. 630.
<https://www.sciencedirect.com/science/article/pii/030744127790019X>

Davies, G. (1998): Structural Studies on Cellulases; *Biochem Soc Trans* 26: 167.

Eberl, A., Heumann, S., Bruckner, T., Araujo, R., Cavaco-Paulo, A. and Kaufmann, F. (2009). Enzymatic surface hydrolysis of poly (ethylene terephthalate) and bis(benzoyloxyethyl) terephthalate by lipase and cutinase in the presence of surface-active molecules. *J. of Biotech.*143: 207–212.

Ekperigin, M. M. (2007). Preliminary Studies of Cellulase Production by *Acinetobacter anitratus* and *Branhamella* sp. *Afr J. Biotech.* 6:28–33.

Ezima, E. N., Adeyanju, M. M., Odufuwa, K. T., Aremu, T. A. and Fagbohunka, B. S. (2014). Biodegradation of Wastes using Cellulase from the Termite, *Amitermes eveuncifer* (Silverstri) Workers: A Clue to the Application of Termites Cellulase in Waste Management. *Sci. focus.*19:93-98

Fagbohunka, B. S., Agboola, F. K. and Afolayan, A. (2012). Characterization of a Cellulase from The Haemolymph of the Giant African Snail (*Archachatina marginata*). *African J. Biotech.*11: 9254-9264.

Fagbohunka, B. S., Babasanya-Craig, O., Adenuga, G. A. and Agboola, F. K. (1997). Partial purification and characterization of cellulase 1 from the gut and heamolymph of the giant African snail *Archachatina marginata*. *Niger. J. of Nutr. Sci.*18: 28-34.

Fagbohunka B. S., Ezima E. NI., Okonji, E., Adegbesan B. O. and Itakorode B. O. (2021) Biodegradation of Wastes Using Cellulase from Termite, *Amitermes Eveuncifer* (Silverstri) Soldier: A Clue to

The Application of Termites Cellulase in Waste Management. FUOYE (Int.) *J. Pure and Appl. Sci.*: 10 – 19.

Fagbohunka, B. S., Nwolisah, O. S., Adeyanju M. M., Fasanya T. A., Adelegan A. A. (2021) Biodegradation of Wastes Using Cellulase from the Gut of Rhinoceros (*Oryctes Rhinoceros*) Beetle. *International J. Environ. Stud and Sustain. (IJESS)*. In press.

Foo, T. S. (1997). Recycling of domestic waste: early experience in Singapore. *Habitat Int.* 21, 277-289.

Gornall, A. G., Bardawill, C. J. and David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *J. biol. Chem.*, 177, 751-766.

Hurst, P. L., Wielson, J., Sullivan, P. A. and Shepherd, M. G. (1977): Purification and properties of a cellulase from *A. niger* *Biochem. J.* 165: 33-41

Klyosov, A. A. (1990). Trends in Biochemistry and Enzymology of cellulose degradation. *J. Biochem.* 29:10577-10589.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem.* 31: 426-428.

Olutiola, P. O. and Ayres, P. G. (1977). A cellulase complex in culture filtrates of

Rhychosporium secalis, *Trans. Br. Mycol. Soc., Great Britain*, 60: 273-282.

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

Srivastava, N., Rawat, R., Oberoi, H. S. and Ramteke, P. W. (2015a). A review on fuel ethanol production from lignocellulosic biomass. *Int. J. Green Environ.* 12:949–960.

Teeri, T. T. (1997). Crystalline cellulose degradation: new insight into the function of cellobiohydrolases. *Trends Biotech.*, 15; 160-167.

Tokiwa, Y., Calabia, B. P., Ugwu, C. U. and Aiba, S. (2009). Biodegradability of plastics. *International J. Mol. Sci.* 10: 3722-3742.

Warburg, O. and Christian, W. (1942) *Biochem. Z* 310, 384-421.

Watanabe, H., Noda, H. and Tokuda, G. (2001). Animal cellulases. *CMLS, Cell. Mol. Life Sci.* 58: 1176-1178.

Yoon. L. W., Ang, T. N., Ngoh, G. C. and Seak, M. C. A. (2014). Fungal solid-state fermentation and various methods of enhancement in cellulase production. *Biomass and Bioenerg.* 67:319–338.