IJPSR (2021), Volume 12, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 July 2020; received in revised form, 09 May 2021; accepted, 15 June 2021; published 01 July 2021

TEMPERATURE DEPENDENT BIOCONVERSION OF WASTE PAPER BY GARDEN SNAIL (*CORNU ASPERSUM*) CELLULASE INTO GLUCOSE A FEEDSTOCK FOR BIO-PRODUCT DEVELOPMENT

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Keywords:

Biomass, Waste paper, Cellulase, Saccharification, Garden Snails

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ABSTRACT: The global production of solid waste and lack of its management is a concern for many global populations. Also of major concern is the production of fossil fuel-based materials and substances such as medicines. Another unease observation is the destructive action of garden snails on plant materials, of which many are found in gardens and fields of farmers. The search, however, continues for procedures that could contribute to the production of bio-based products such as bio-medicines as well as actions to limit the production of solid waste, which is a major contributor to environmental pollution. Glucose a fermentable sugar, has been identified as an important feedstock for the synthesis of many bio-products. Cellulose a glucose-based biopolymer, and structural components of paper material can be degraded into glucose by cellulase a hydrolytic enzyme system. The cellulolytic action of garden snails has been used to saccharify various waste papers at different incubation temperatures, and the optimum sugar formation from these materials and % saccharification of each paper material was determined. Optimum degradation of office paper, newspaper, filter paper, Pick 'n Pay paper, filter paper, Woolworth's paper, and brown envelope paper was recorded at temperatures of 30 ºC, and 40 ºC whilst the extent of degradation differs with brown envelope paper exhibiting the highest degree of degradation producing a sugar concentration of 9.67 $mg.ml^{-1}$ at an incubation temperature of 30 ºC and 18% saccharification. The lowest degree of saccharification at optimum incubation temperature was at 30 ºC when newspaper produced a sugar concentration of 1.71 $mg.ml^{-1}$ and 7.6% saccharification, respectively.

INTRODUCTION: High economic growth and consumption of natural resources and production and lack of effective solid waste management are global difficulties.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(7).3985-93

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(7).3985-93

Although systems for the treatment of solid waste have been developed, it remains an issue for middle and low-income countries because of the lack of infrastructure. Waste management is also a challenge for high-income countries because of increasing levels of consumption [1].

Waste paper, such as newspaper, is one of the largest components of municipal solid waste, and the production of bioethanol from organic waste materials such as waste newspaper has been carried out by Byadgi and Kalburgi, which is a process of

utilizing solid waste to benefit the environment [2]. Presently biofibers are attracting increasing interest because of their use in the biomedical sciences, and these substances reduce the use of petroleum-based synthetic polymers because they are safe, have a low production cost, and are biodegradable [3].

Cellulose is a major structural component of waste paper, and the hydrolysis of this bio-polymer is a crucial step in the development of bio-substances from waste paper. A challenge is a time taken for hydrolysis and the necessity of large quantities of costly commercially available cellulase enzymes [4]. There is thus a need for affordable enzymes from naturally available sources, and the synergetic nature of protozoa and bacteria is a good example to illustrate and explain cellulose digestion in invertebrates and herbivores cattle. Cleveland first published in 1924 his work with termites, which established the possibility of *Reticulitermes flavipes* functional elimination of protozoa from the hindgut, thus concluding that a hindgut protozoan fauna was responsible for the cellulose digestion [5]. Numerous cultures of bacteria from the termites' gut have been isolated and classified based on their similarities, dissimilarities, and biochemical tests by Upadhyaya *et al.* [16].

The African giant land snail (*Archachatina marginata*) has also been discovered to be a good source of digestive enzymes, and activities of digestive enzymes in the gut regions of the African giant land snail has been investigated by Ademolu *et al.*, where protease, lipase, α-glucosidase, amylase and cellulase were detected in all the gut regions of the alimentary canal of African giant land snail when it was active and when it was aestivated [6, 7]. The cellulose catalyzed enzymatic hydrolysis of lignocellulosic biomass for production of fermentable sugars has already been investigated by numerous researchers to be the most important step for the production of biofuels and other bio-based products [8, 9, 10, 11]. Investigations to improve the efficiency of the cellulase enzymes in hydrolyzing cellulose with the production of glucose is a continuous process [11]. Various factors that have an influence on the performance of the cellulose enzyme are the crystallinity of cellulose biomass, environmental pH, and temperature to which the cellulose is exposed during the hydrolytic process. Kumar and Wyman previously studied the access of cellulase to cellulose and lignin for poplar solids produced from cellulose by leading pre-treatment technologies at 4 ºC [12]. Zheng *et al.*, investigated the temperature sensitivity of cellulase adsorption on lignin and its impact on enzymatic hydrolysis of lignocellulosic biomass where adsorption behaviors were examined at both 4 ºC and 50 ºC, with the latter preferred for the enzymatic hydrolysis of cellulose. It was reported that an increase in temperature usually improves the adsorption kinetics of cellulase on lignocellulosic materials as it increases the diffusion coefficient of the protein in solution to reach the adsorption surface [13].

The purification of cellulase from garden snails and the relative activity on different waste paper materials has recently been published [14, 15]. With this research, the activity of the cellulase enzyme from a brown garden snail on different waste paper materials was investigated under controlled conditions at temperatures ranging from 24 ºC to 60 ºC, and the aim was to conclude the sugar production, as well as the saccharification, extend of these paper materials. Information obtained from these temperature profiles would be an important variable to consider when performing large-scale bio-conversion of waste paper materials into glucose that could be utilized as feedstock for the bio-synthesis of substances such as bio-medicines.

MATERIALS AND METHODS:

Preparation of Solutions: A mass of 0.6 g of tris (hydroxymethyl) aminomethane (Merck, Darmstadt, Germany) was dissolved in 1.0 dm^3 distilled water to prepare a 0.005 M buffer solution with pH 5.0 adjusted by using hydrochloric acid (30%) and 0.5 M potassium hydroxide solutions. The Tris-HCl buffer solution was used during the purification of the enzyme as well as during waste paper saccharification with the cellulase enzyme. A dinitrosalicylic acid (DNS) solution was prepared by dissolving 10.0 g DNS, 2.0 g phenol, 0.5 g sodium sulphite, 200.0 g potassium sodium tartrate, and 10.0 g sodium hydroxide in 1.0 dm^3 of distilled water. The DNS solution (60.0 ml) was finally diluted by mixing it with 140.0 ml of distilled water and used to determine the amount of sugar produced during saccharification of waste paper with cellulase from garden snails from a calibration

curve constructed with glucose solutions with different concentrations. Isolation and purification of the enzyme from garden snails: Garden snails were collected from a garden in Pretoria, South Africa, during a rainy season and drowned in water for a period of 24 h. Based on the validated method by Ndlovu and Van Wyk, shells of snails were removed with the foot cut off from each snail. The visceral sections were weighed and cut into small pieces and transferred into a glass beaker filled with 15.0 ml of 1.0% methanol-tris-HCl buffer. The mixture was homogenized using a hand blender, and the homogenate was then stirred for 1 hour, transferred into a test tube, and centrifuged for 30 min at 4000 rpm using a Beckman, GP Centrifuge (UK, Marca). The supernatant was collected as crude cellulase enzyme and transferred into a dialysis tube (Sigma, St Louis, Switzerland) that was soaked in distilled water at 4 ºC for a period of 4 h. The enzyme solution in the dialysis tube was immersed in distilled water (stirring) for a period of 18 h where after the protein content was determined by the Buiret reagent method [15, 16].

Saccharification of Waste Paper Materials: Filter paper, office paper, foolscap paper, newspaper, Pick 'n Pay advertising paper, Woolworths advertising paper, and brown envelope (kraft) paper were prepared as round discs with a diameter of 6 mm each. Twenty pieces of each paper material were transferred into test tubes where after 800 µl of Tris-HCl buffer and 200 µl of dialyzed snail enzyme (cellulase) were added. The mass of paper materials was determined and used to calculate the percentage saccharification of each paper material. These test tubes were incubated for 2 h at 24 ºC, cooled down to room temperature where after 1500 µl of diluted DNS solution was added and the test tubes then placed in a boiling water bath for 10 min. All tubes were cooled in ice water, and the determination of reducing sugars released as a result of the cellulase action performed according to the DNS assay method using a Shimadzu, UV 1800 spectrophotometer at 520 nm [17]. The same waste paper–cellulose saccharification procedure was performed at 30 ºC, 40 ºC, 50 ºC, and 60 ºC, and all incubations were performed in triplicate.

RESULTS AND DISCUSSION: The utilization of renewable feedstocks to synthesize various chemical-related commodities such as bio-pharmaceuticals and bio-chemicals would become more topical as the impact of climate change as a result of fossil fuel combustion is realized. Also, a major concern is the cost of many catalysts needed during bio-synthetic procedures, and the accumulation of solid waste in many cities around the globe is also problematic. The cellulose component of organic solid waste could be developed as a suitable renewable feedstock for many bio-synthetic procedures due to the synthetics potential of glucose (a building block of cellulose) as fermentable sugar. Many populations such as farmers and dedicated gardeners consider garden snails as a pest due to their destructive action on plants which act as a source of energy for these snails. The isolated cellulase enzyme that is a major degrading system in snails can thus be used effectively to saccharify the cellulose content of waste paper producing glucose that could assist the process of developing bio-feed stocks for pharmaceutical and chemical procedures. An important incubation variable to optimize saccharification of waste cellulose is to determine the temperature at which optimum sugar production from each waste paper material could take place when bio-converted by cellulase isolated from garden snails. **Fig. 1** reflects garden snails used for the extraction of cellulase activity that was used for the bio-degradation of various paper materials into glucose, a fermentable sugar.

During saccharification of filter paper with garden snail cellulase **Fig. 2**, optimum degradation was obtained at 30 ºC and 40 ºC resulting in a sugar concentration of 1.72 $mg.ml^{-1}$ and 1.79 $mg.ml^{-1}$, respectively. The percentage saccharification at these temperatures was calculated at 5.5% for 30 ºC and 5.7% obtained at 40 ºC. The lowest extent of sugar production obtained at an incubation temperature of 24 ºC was 0.50 $mg.ml^{-1}$ that was 71% less than the highest concentration produced at 30 ºC and 72% less than the highest concentration produced at 40 ºC. Optimum sugar production of 5.67 $mg.ml^{-1}$ was produced during the degradation of office paper **Fig. 3** at a temperature of 30 ºC that was 78% higher than the amount of sugar released from this paper material when exposed to the cellulase enzyme system at 24 ºC.

GARDEN SNAILS EATING GRASSS

GARDEN SNAIL EATING PAPER

VISCERAL SECTION OF SNAIL USED TO PREPARE EMULSION USED FOR CELLUASE ACTION

PAPER MATERIALS SACCHARIFIED WITH SNAIL CELLULASE

FIG. 1: VICERAL SECTION OF GARDEN SNAIL USED FOR BIO CONVERSION OF PAPER MATERIALS INTO GLUCOSE

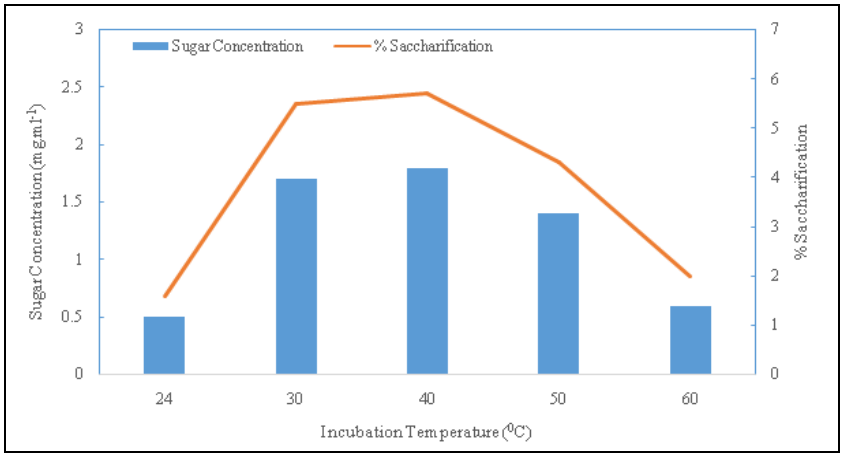

FIG. 2: RELATIVE AMOUNT OF SUGAR PRODUCED AND PERCENTAGE SACCHARIFICTION OF FILTER PAPER WHEN SACCHARIFIED BY GARDEN SNAIL CELLULASE AT DIFFERENT INCUBATION TEMPERATURES

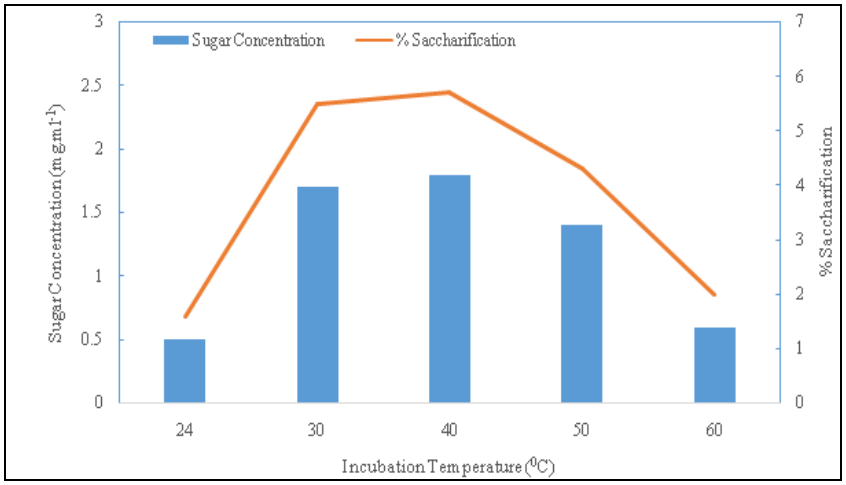

FIG. 3: RELATIVE AMOUNT OF SUGAR PRODUCED AND PERCENTAGE SACCHARIFICTION OF OFFICE PAPER WHEN SACCHARIFIED BY GARDEN SNAIL CELLULASE AT DIFFERENT INCUBATION TEMPERATURES

The amount of sugar produced during degradation at an incubation temperature higher than 30 ºC decreases gradually until a sugar concentration of 2. 62 mg.ml^{-1} was produced during incubation at 60 ºC. The percentage saccharification at an optimum incubation temperature of 30 ºC was 15.2% whilst 3.3% saccharification was calculated during the bioconversion process at 24 ºC. The percentage saccharification at 40 ºC, 50 ºC, and 60 ºC was 13.0%, 10.6%, and 7.1%, respectively. When bio-converted by snail cellulase, the amount of sugar released from foolscap paper **Fig. 4** exhibited a relatively high sugar concentration at temperatures of 30 ºC, 40 ºC, and 50 ºC. The amount of sugar released during incubation at these temperatures were 3.94 mg.ml^{-1} at 30 ºC, 3.85 mg.ml^{-1} at 40 ºC and 3.54 mg.ml^{-1} at 50 ºC. The lowest amount of sugar was released at a concentration of 0.96 mg.ml^{-1} at 24 ºC whilst a sugar concentration of 1.69 mg.ml^{-1} was released when incubated at 60 ºC. The relative percentage of saccharification changed from 3.6% when this waste paper material was incubated at 24 ºC to 14.6% at a temperature of 30 ºC, resulting in a 75.6% increase in saccharification. **Fig 5** reflects the sugar production tendency and percentage saccharification of the newspaper when degraded with garden snail cellulase. The amount of sugar released from this paper material varied between 1. 79 mg.ml^{-1} when incubated at 40 ºC and 1.14 mg.ml^{-1} when incubated at 60 ºC. The percentage saccharification varied between 5.0% for minimum degradation to 7.9% bioconversion when degraded at 40 ºC resulting in the maximum amount of sugar produced that was 33.3% higher than the lowest sugar concentration.

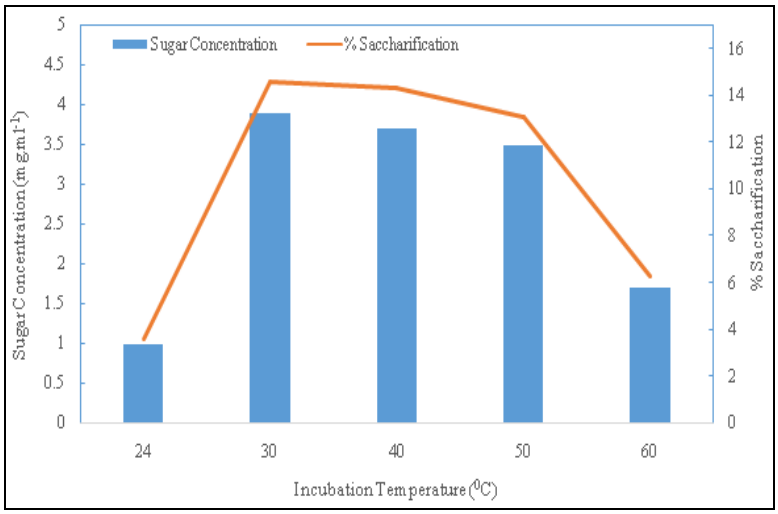

FIG. 4: RELATIVE AMOUNT OF SUGAR PRODUCED AND PERCENTAGE SACCHARIFICTION OF FOOLSCAP PAPER WHEN SACCHARIFIED BY GARDEN SNAIL CELLULASE AT DIFFERENT INCUBATION TEMPERATURES

When Pick 'n Pay advertising paper, **Fig. 6** was saccharified with garden snail cellulase the maximum amount of sugar was produced at a concentration of 1.63 mg.ml^{-1} when the paper material was degraded at an incubation temperature of 40 ºC resulting in a saccharification percentage of 7.8%. Saccharification at incubation temperature of 24 ºC, 50 ºC and 60 ºC resulted in almost identical sugar concentration of 0.93 mg.ml^{-1}, 0.89 mg.ml^{-1} and 0.99 mg.ml^{-1}, respectively. The maximum degree of saccharification was calculated at 7.8% whilst the lowest degree of saccharification was 4.4 % (24 ºC), 4.2% (50 ºC), and 4.7% (60 ºC), which was 45.4% higher than the lowest degree of saccharification. When Woolworth's advertising paper **Fig. 7** was bio-treated with garden snail cellulase, maximum sugar production was obtained at a concentration of 3.81 mg.ml^{-1} at 30 ºC with percentage saccharification of 8.8 %. When this material was degraded at temperatures higher than 30 ºC the amount of sugar produced decreased gradually until a concentration of 2.44 mg.ml^{-1} sugar was obtained at 60 ºC. At this highest incubation temperature, the percentage saccharification was 5.6% whilst the lowest sugar concentration of 1.29 mg.ml^{-1} and 3.0 % saccharification was calculated at 24 ºC. The sugar formation profile when brown envelope paper **Fig. 8** was degraded exhibited a profile almost similar to the degradation pattern of office paper **Fig. 3**.

Maximum degradation, which resulted in a sugar concentration of 9.67 mg.ml$^{-1,}$ was released during incubation at 30 ºC with percentage saccharification of 18.1%. The amount of sugar released at 60 ºC was 4.59 mg.ml^{-1} at a degree of saccharification of 8.6%. When incubated at 24 ºC, the lowest degree of saccharification of 4.9% was achieved with sugar produced at a concentration of 2.63 mg.ml^{-1}. **Table 1.** reflects the amount of sugar produced and the extent of saccharification of various paper materials at optimum catalytic temperatures when bio-converted with cellulase from the garden snail.

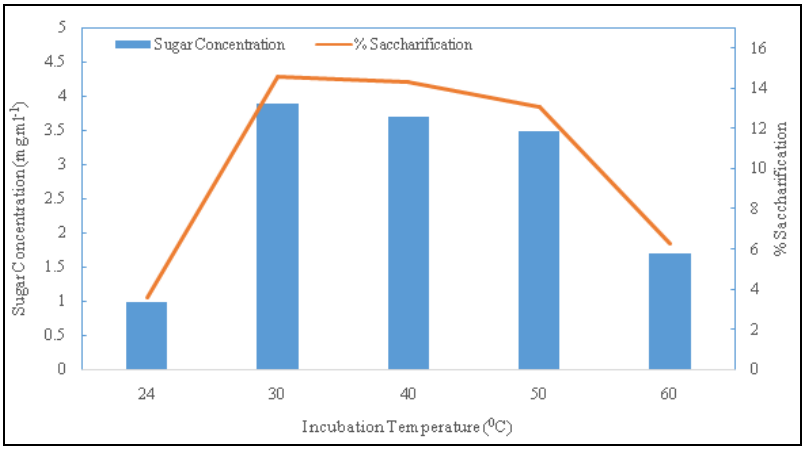

FIG. 5: RELATIVE AMOUNT OF SUGAR PRODUCED AND PERCENTAGE SACCHARIFICTION OF NEWSPAPER WHEN SACCHARIFIED BY GARDEN SNAIL CELLULASE AT DIFFERENT INCUBATION TEMPERATURES

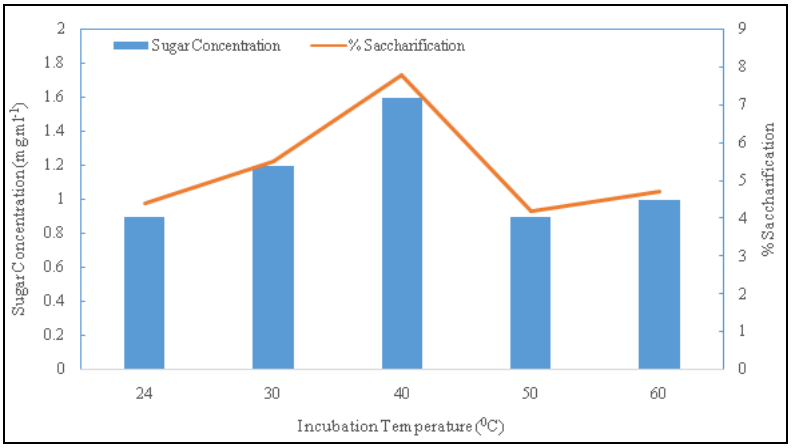

FIG. 6: RELATIVE AMOUNT OF SUGAR PRODUCED AND PERCENTAGE SACCHARIFICTION OF PICK 'N PAY PAPER WHEN SACCHARIFIED BY GARDEN SNAIL CELLULASE AT DIFFERENT INCUBATION TEMPERATURES

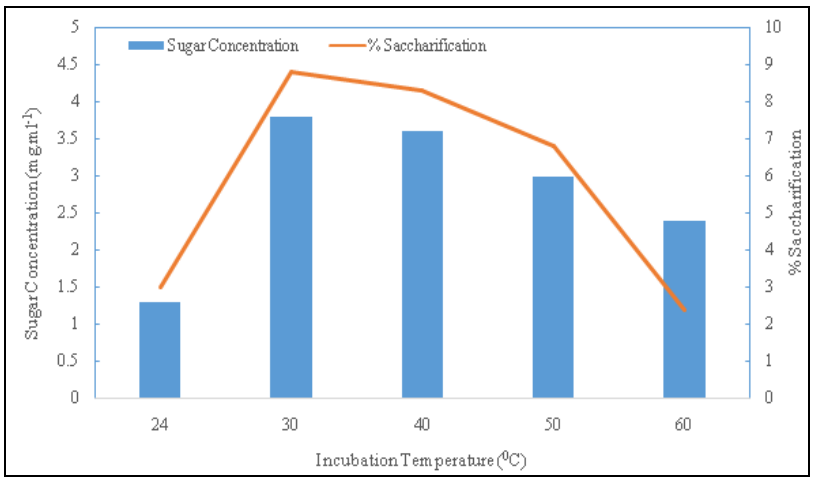

FIG. 7: RELATIVE AMOUNT OF SUGAR PRODUCED AND PERCENTAGE SACCHARIFICTION OF WOOLWORTHS PAPER WHEN SACCHARIFIED BY GARDEN SNAIL CELLULASE AT DIFFERENT INCUBATION TEMPERATURES

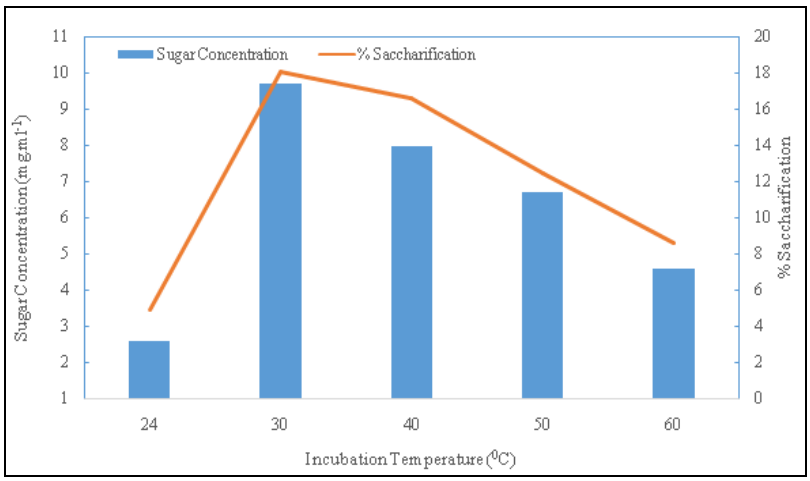

FIG. 8: RELATIVE AMOUNT OF SUGAR PRODUCED AND PERCENTAGE SACCHARIFICTION OF BROWN ENVELOPE PAPER WHEN SACCHARIFIED BY GARDEN SNAIL CELLULASE AT DIFFERENT INCUBATION TEMPERATURES

TABLE 1: SUGAR PRODUCTION AND RATE OF SACCHARIFICATION OF VARIOUS PAPER MATERIALS AT OPTIMUM INCUBATION TEMPERATURE WITH GARDEN SNAIL CELLULASE

Paper Material	Optimum Incubation Temperature (ºC)	Sugar Concentration ($Mg.Ml^{-1}$)	Percentage Saccharification (%)
Office paper	30	5.67	15.2
Foolscap paper	30	3.94	14.6
Woolworths paper	30	3.81	8.8
Brown envelope paper	30	9.67	18.1
Filter paper	30, 40	1.72	5.5
Newspaper	30, 40	1.79	5.7
Pick 'n Pay paper	40	1.71	7.6

All the paper materials with the exception of Pick 'n Pay paper was optimally degraded at 30 ºC. Pick 'n Pay paper was optimally degraded at 40 ºC whilst filter paper and newspaper showed optimum sugar production when degraded by garden snail cellulase at incubation temperatures of 30 ºC and 40 ºC. Four paper materials exhibited relatively high amounts of sugar concentration, with brown envelope paper producing the highest sugar concentration of 9.67 $mg.ml^{-1}$ and a relative saccharification percentage of 18.1%. The second-highest amount of sugar was released from office paper at a concentration of 5.67 $mg.ml^{-1}$ (15.2% percentage saccharification), which was 41.4 % less than the amount of glucose released from brown envelope paper. The third highest amount of sugar was released from

foolscap paper at a sugar concentration of 3.94 $mg.ml^{-1}$ and 14.6% of saccharification. The fourth highest amount of sugar was produced from Woolworth's advertising paper at a concentration of 3.81 $mg.ml^{-1}$ and 8.8% percentage saccharification. Filter paper, newspaper, and Pick 'n Pay paper produced the lowest amount of sugar during saccharification at their respective optimum incubation temperatures producing sugar concentrations that varied between 1.63 $mg.ml^{-1}$ to 1.79 $mg.ml^{-1}$ with percentage saccharification varying between 5.5 % and 7.9%. The global use of paper is increased, and such is the contribution of post-consumed paper as a major component of solid waste [18, 19]. Although the potential of waste cellulose as a renewable feedstock has been realized the effective utilization of this bio-polymer is far from optimized.

The saccharification of waste paper by cellulase enzymes from different fungal and bacterial sources has been described indicating optimum catalytic properties such as incubation temperature. The optimum incubation temperature for degradation of most cellulose materials varies between 25ºC and 70ºC, for example, the bioconversion of sugarcane bagasse by *Aspergillus niger* and *Aspergillus oryzae* at 28-30 ºC, esculine by *Issatchenkia orientalis* at 50 ºC, carboxymethyl cellulose by Penicillium sp. LMI01 at 60 ºC [20]. When waste paper is degraded by cellulase enzymes from other sources such as *Trichoderma viride* and *Aspergillus niger*, the optimum incubation temperature is higher than the mostly 30 ºC experienced during saccharification of waste paper with garden snail cellulose [10, 21]. The relative low optimum incubation temperature obtained when garden snail cellulase was used to saccharify various waste paper materials could be attributed to the body temperature of snails when cellulase act optimally in the snails' gut. The body temperature of the snail is dependant on its environment [22].

CONCLUSION: Cellulose obtained from waste paper is a resource for bio-product development, and in addition, the utilization of cellulase from garden snails to saccharify waste cellulose could address many issues such as environmental pollution, renewable feedstock development, and the utilization of less expensive biocatalysts. By optimizing these variables could not only limit environmental pollution but could also assist farmers by protecting their crops against the destructive action of garden snails and provide glucose that could be utilized as feedstock for the synthesis of renewable substances such as bio-medicines. The relatively low incubation temperature when bio-converting waste paper cellulose into fermentable sugars by using cellulase from garden snails has another positive effect on the environment as less generated energy is needed during the incubation procedures when cellulose is saccharified.

ACKNOWLEDGEMENT: Appreciation to the department of Pharmacology and Therapeutics, Sefako Makgatho Health Sciences University, for allowing the research to be conducted in the departmental laboratories.

CONFLICTS OF INTEREST: None declared.

REFERENCES:

1. Singh J, Laurenti R, Sinha R and Frostell B: Progress and challenges to the global waste management system. Waste Management Research 2014; 32(9): 800-12.
2. Byadgi S and Kalburgi P: Production of bioethanol from waste newspaper. Proceedings Environmental Sciences 2016; 35: 55-562.
3. Mostafa N, Farag A, Abo-dief H and Tayeb A: Production of biodegradable plastic from Agricultural wastes. Arabic Journal of Chemistry 2018; 11: 546-53.
4. FenilaF and Yogendra S: Optimal control of enzymatic hydrolysis of lignocellulosic biomass. Resource of Efficient Technology 2016; 2: S96-S04.
5. Cleveland LR: The physiological and symbiotic relationships between the intestinal protozoa of termites and their host, with special reference to Reticulitermes flavipes Kollar. Biological Bulletin 1924; 46(5): 117-27.
6. Upadhyaya S, Manandhar A, Mainali H, Pokhrel A, Rijal A, Pradhan B and Koirala B: Isolation and characterization of cellulolytic bacteria from gut of termite. Rentech Symposium 2012; 1: 14-18.
7. Ademolu K, Fakeye O, Dedeke G, Ajayi O and Idowu A: Digestive enzymes in African giant land snail (*Archachatina marginata*) during aestivation. Archivos de Zootecnia 2013; 62(237): 73-77.
8. Paz A, Outeirino D, Guerran NP and Domínguez JM: Enzymatic hydrolysis of brewer's spent grain to obtain fermentable sugars. Bioresource Technology 2019; 275: 402-09.
9. Li H, Chen X, Xiong L, Luo M, Chen X, Wang C, Huang C and Chen X: Stepwise enzymatic hydrolysis of alkaline oxidation treated sugarcane bagasse for the co-production of functional xylo-oligosaccharides and fermentable sugars. Bioresource Technology 2019; 275: 345-51.
10. Mokatse K and Van Wyk JPH: Identification of optimum incubation temperature for saccharification of various waste paper materials by cellulase from Trichodermaviride. Bioscience Research 2017; 14(4): 1269-78.

11. Chatterjee S, Sharma S, Prasad RK, Datta S, Dubey D, Meghvansi MK, Vairale MG and Veer V: Cellulase enzyme based biodegradation of cellulosic materials: An overview. South Asian J of Exp Bio 2015; 5(6): 271-82.
12. KumarR and Wyman C: Access of cellulase to cellulose and lignin for poplar solids produced by leading pretreatment technologies. Biotechnology Progress 2009; 25(3): 807-19.
13. Zheng Y, Zhang S, Miao S, Su Z and Wang P: Temperature sensitivity of cellulase adsorption on lignin and its impact on enzymatic hydrolysis of lignocellulosic biomass. Journal of Biotechnology 2013; 166: 135-3.
14. Ndlovu TM and Van Wyk JPH: Saccharification of waste paper with cellulase from garden snails (*Cornu aspersum*). International Journal of Environmental Science and Techn 2018: https://doi.org/10.1007/s 13762-018-1934-1.
15. Ndlovu TM and Van Wyk JPH: Isolation of cellulase enzyme from brown garden snail (*Cornu aspersum*) for the saccharification of waste paper materials. Elsevier B.V. Methods X 2019; 6: 1030-35.
16. Janairo G, Lindley SY, Yap L, LIanos-Lazaro N and Robles J: Determination of the sensitivity range of biuret test for undergraduate biochemistry experiments. E Journal of Science and Technology 2011; 5(6): 77-83.
17. Miller G: Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 1959; 31: 426-28.
18. Elias P and Boucher D: Planting for the future how demand for wood products could befriendly to tropical forests.Union of Concerned Scientists 2014; www.ucsusa. org/futurewooduse.
19. Haggith M, Kinsella S, Baffoni S, Anderson P, Ford J, Leithe R, Neyroumande E, Murtha N and Tinhout B: The state of the global paper industry shifting seas New challenges and opportunities for forests, people and the climate. Environmental Paper Network 2018; http://epd. canopyplanet.org.
20. Srivastava N, Rathour R, Jha S, Pandey K, Srivastava M, Thakur VK, Sengar RS, Gupta VK, Mazumder PB, Khan AF and Mishra PK: Microbial beta glucosidase enzymes recent advances in biomass conversation for biofuels application. Bio molecules 2019; 9(220): doi:10.3390/ biom 9060220.
21. Sibiya JBM and Van WykJPH: Bioconversion of waste newspaper in to fermentable sugars at different temperatures with different *Aspergillus nigercellulase* concentrations. Journal of Applied Biology and Biotechnology 2016; 4(4): 69-74.
22. Knigge T, De Lellies MA, Köhler HR and Monsinjon T: Relevance of body size and shell colouration for thermal absorption and heat loss in white garden snails, Thebapisana (Helicidae), from Northern France. Journal of Thermal Biology2017; 69: 54-63.

How to cite this article:
Ndlovu TM and Van Wyk JPH: Temperature dependent bioconversion of waste paper by garden snail (*Cornu aspersum*) cellulase into glucose a feed stock for bio-product development. Int J Pharm Sci & Res 2021; 12(7): 3985-93. doi: 10.13040/IJPSR.0975-8232.12(7). 3985-93.