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Saccharification of Various Wastepaper Materials by Cellulase from Brown Garden Snail (*Cornu aspersum*) at Different Incubation pH Values

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ABSTRACT

Increased solid waste pollution and the negative effect of fossil fuel consumption on the environment are issues that would require more scientific attention and application to deal effectively with these phenomena. Wastepaper, a major component of solid waste, is classified as organic waste due to the presence of cellulose, a glucose-based biopolymer that is part of its structural composition. The saccharification of cellulose into glucose, a fermentable sugar, can be achieved with a hydrolytic enzyme known as cellulase. Although cellulase from fungal species such as *Trichoderma*, *Aspergillus,* and *Penicillium* are well described, knowledge about cellulase isolated from the brown garden snail is limited as it has not been the subject of many research endeavors. The waste paper has been described as a suitable resource for bio-energy development due to cellulose, a structural component of this bio-material that can be degraded into glucose, a fermentable sugar. Although paper materials such as newspaper, office paper, filter paper, Woolworths and Pick and Pay (retailers) advertising paper, as well as foolscap paper, were saccharified by different cellulases, the degradation of these paper materials by garden snail cellulase is a novel investigation from our laboratory. With the effects of temperature and incubation time on this cellulase action when degraded paper materials have already been investigated and reported, this study dealt with the garden snail cellulase action when degraded paper materials at different pH values. Most of the paper materials were degraded optimally at a pH value of 6.0, while optimum saccharification was observed at pH 4.5 when newspaper and brown envelope paper were degraded, with office paper showing maximum bioconversion at pH 7.0. The difference in the structural composition of the paper materials also affects the degree of saccharification, as the amount of sugar released from the various paper materials at optimum pH values is not similar. Together with other catalytic parameters, the pH value of this enzymatic catalysis is also to be considered when designing the development of waste paper as a bio-product resource, with limiting environmental pollution as an additional advantage of this process.

INTRODUCTION

The bio-conversion of biomass to valuable bioproducts, biofuels, and biochemicals has become an important area of current research. Biomass can include agricultural waste, natural herbaceous plants, forest residues, and industrial and municipal wastes. Also included as biomass derivatives or cellulose-related products are paper materials, and it was reported (Loelovich 2014) that the world pulp and paper industries produced about 300-350 million tons of various types of paper and board. This statement was supported by (Tiseo 2021), who indicated that in 2018, the global production of paper and cardboard stood at 419.72 million metric tons. With such an enormous volume of paper produced annually, it can be concluded that the amount of wastepaper is also extremely high and that this waste cellulose material should be considered a potential resource for developing renewable substances. The use of organic waste materials as a resource for the biotechnological production of biofuels should be economically and environmentally advantageous, and the development of lignocellulosic matter as raw materials for the hydrolytic production of fermentable sugars has already become a feasible option (Vladimir et al. 2014).

Snails are organisms that can use cellulose as an energy resource since they can produce cellulase enzymes by bacteria in their digestive tract, and the cellulase can decompose cellulose into simple sugars such as glucose by breaking the chemical bonds between glucose units in the cellulose polymer. These chemical bonds are known as □-1,4-glycosidic bonds and can be broken by the cellulase enzyme components known as endoglucanase, exoglucanase, and

-glucosidase (Dini et al. 2019). The degradation of cellulose into sugars can also be done by exposing the cellulose material to high temperatures or treating it with acidic or alkaline agents, which are not environmentally friendly. As an alternative, an environmentally friendly procedure like an enzyme-based treatment of cellulose with a cellulase enzyme as the biocatalyst has become a major focus of research (Navarro et al. 2018).

The in-situ pH, oxygen, and hydrogen profiles of the helicid snails (gastropoda: pulmonata) gut microenvironment have been described by Charrier & Brune (2003), who concluded that the degradation of cellulose by snails is subjected to two factors that link all animals feeding on a lignocellulosic diet. The first of these two variables is the enlarged gut region, and the second is the gut microbiota that provides the host with a battery of digestive enzymes to hydrolyze plant food, mainly its lignocellulosic components. The pH profiles of *Cornu aspersum* (commonly known as the brown garden snail) investigated by Charrier and Brune were discovered to be acidic in the crop, close to neutrality in the distal intestine, and above pH 7 in the glands. The pH of the digestive system of slugs and snails decreases shortly after they have been fed, with the main change in pH after feeding in the crop, which is significantly more acidic than the digestive gland and salivary gland (Walker et al. 1996). The crop pH values of snails were 7.7 when empty and 5.8 when full, whereas the corresponding values for the digestive gland were 7.0 and 6.3, respectively, reflecting information of the pH value when the cellulase is saccharifying cellulose in the digestive system of the snail. It was suggested that the gut pH in slugs is characteristic of mollusks rather than determined by their diet preferences, and the proteolytic activity of crude gut homogenates of snails and slugs has been reported to have a pH far from the physiological pH (Walker et al. 1996). In comparison, a close relationship between the pH optimum for proteolytic activity and the in-situ pH of the alimentary tract for crude gut homogenates has been reported to be pH 6.0 ± 0.1 (Evans & Jones 1961).

The saccharification of cellulose by cellulase enzymes is a process investigated for the degradation of various types of waste celluloses by cellulases from different origins. Different types of waste paper, such as newspaper (Mokatse & Van Wyk 2017), office paper (Van Wyk & Sibiya 2016), foolscap paper (Mokatse et al. 2016), and filter paper (Van Wyk et al. 2000) have been investigated as a possible resource for bioenergy development as it has been subjected to the saccharification action of cellulase from *Trichoderma viride* and *Aspergillus niger* (Zhao et al. 2020). The hydrolytic action of cellulase from garden snails on cellulose has not yet been investigated in detail or depth, as is the case with cellulases from fungal resources. All enzymatically catalyzed reactions are sensitive to changes in many catalytic properties, such as pH, and to ensure an optimum yield of the reaction product, these properties must be optimized. Due to the cellulase enzyme's complexity and the structural differences of the various waste cellulose materials, it cannot be accepted that the enzyme would act optimally at the same pH value when degrading the paper materials. This investigation attempted to determine the bioconversion of various waste paper materials at different incubation pH values and to conclude the pH value for optimum saccharification of each paper by a cellulase enzyme isolated from garden snails.

MATERIALS AND METHODS

Isolation of the Cellulase Enzyme from Garden Snail

The isolation of cellulase from the brown garden snail was carried out according to the method described by Ndlovu and Van Wyk (2019). A dialysis tube (Sigma, St Louis, Switzerland) was soaked in water and cooled in the fridge at 4°C for four hours. A snail's foot was removed, and the organs inside the shell were cut into small pieces. Tris-buffer (0.005M, 30mL; Merck, Darmstadt, Germany) was added to form a homogeneous mixture by stirring the mixture for one hour on a magnetic stirrer. The snail sample and tris-buffer mixture were centrifuged (Beckman, Indianapolis, United States) at 4000 rpm for 30 min. The supernatant was collected and transferred into the soaked dialysis tube that was immersed into a glass container filled with water and left for 24 h in a cold room at 4°C with the water continuously stirred. After 24 h of stirring, the dialyzed cellulase sample was transferred from the dialysis tube into a clean glass container. Samples of this dialyzed enzyme solution have been used to saccharify various waste paper materials at different pH values.

Incubation of Wastepaper Materials with Brown Garden Snail at Different pH Values

Filter paper, office paper, newspaper, foolscap paper, brown envelope paper, Pick 'n Pay, and Woolworth's advertising paper were cut into round discs with a diameter of 6 mm (Woolworths and Pick 'n Pay are two local retailers). Twenty pieces of each paper were transferred in triplicate into separate test tubes and mixed with 800 µL tris-buffer at different pH-values of 3.5; 4.0; 4.5; 5.0; 5.5; 6.0; 6.5, 7.0 adjusted with 32% hydrochloric acid (HCl) or 0.5M potassium hydroxide (KOH). A fixed volume of the dialyzed garden snail cellulase solution (200 ul) was added to each test tube with the well-mixed content. The filled test tubes were incubated for 2 hours at 50°C and centrifuged for 15

minutes at 4000 rpm. The supernatant was transferred into a clean test tube to determine the sugar released from each paper material when saccharified at different pH values with cellulase from the brown garden snail.

Sugar Determination and Percentage Saccharification

The amount of sugar released from each paper material when saccharified with garden snail cellulase at different pH values was determined according to a method described by Miller (1959). During this analysis, 1500 µL of dinitrosalicylic acid solution (DNS) was added to each tube. The tubes were placed in a boiling water bath for 10 minutes; whereafter it was cooled in ice water. The cooled samples were read on a spectrophotometer (Shimadzu, Kyoto, Japan) at 520 nm to determine the concentration of the produced reducing sugars from each paper material using a calibration curve constructed with glucose standards solutions prepared at different concentrations. To determine the percentage saccharification of each paper material, the mass of the paper disks incubated with the cellulase enzyme was determined (weighed) before the paper was saccharified. With the sugar concentration determined after the bioconversion of the paper materials, the total mass of sugar in the incubation mixture was determined. This mass of sugar produced during the degradation of the paper materials was expressed as a percentage of the total mass of paper material incubated with the garden snail cellulase.

Statistical Analysis

All the experimental analyses were performed in triplicate, and the mean values with standard deviations were determined with Microsoft Excel.

RESULTS AND DISCUSSION

The pH of a biological environment is an important parameter that could affect the activity of enzymes operating in this milieu by changing the shape and structure of these bio-catalysts. Each enzyme, irrespective of its nature or action, performs optimally at specific pH values, and to ensure maximum catalysis, these pH values must be maintained while enzymes are in contact with substrates. Certain enzymes could exhibit optimum activity over a broad pH range, such as extracellular alkaline protease from *Bacillus licheniformis* NCIM-2042 (Bhunia et al. 2011), while peak activity of others could be executed at narrower pH values as described for the maximum activity of the enzyme, phosphoserine aminotransferase from *Bacillus alcalophilus* at a pH-value of 9.0 (Dubnovitsky et al. 2005). With enzyme-like reactions responsible for the conversion of substrates into products, a change in the pH value of these reactions will influence the yield of the reaction. Cellulase-catalyzed reactions do not differ and are also pH-dependent for optimum bioconversion of cellulose into glucose.

Snails are rich in a wide range of digestive enzymes, particularly carbohydrates, of which cellulases are a major component (Okeniyi et al. 2015). Although the pH of the gut of snails is important for the optimum activity of its cellulases producing maximum glucose as an energy resource from cellulose, the acid-base balance can be changed by chemical substances produced during bacterial fermentation (Dar et al. 2017). Thus, mechanisms must exist within the gut of snails to maintain a pH value that would ensure a relatively high cellulase activity. Cellulose is a major component of organic waste, such as waste paper. If this bio-polymer could be resolved effectively into glucose by cellulase enzymes, many cellulose-related waste materials could be developed as a resource of bio-energy. During this investigation, the effect of changing pH values on the degradation of various wastepaper materials into glucose by cellulase isolated from the brown garden snail has been concluded.

Fig. 1 reflects the concentration of sugar released from filter paper when degraded with cellulase from the brown garden snail at different incubation pH values as well as the percentage saccharification of this paper material. The saccharification of filter paper showed a gradual increase in sugar production from pH 3.5 and reached the highest amount of sugar production and saccharification at pH 6.0. The highest level of filter paper bioconversion was at pH 6.0 with 6.35% saccharification, while a 65 % increase in sugar concentration was observed from pH 3.5 (0.70 $mg.mL^{-1}$) to pH 6.0 (1.98 $mg.mL^{-1}$). Filter paper is regarded as pure cellulose, and the maximum concentration of sugar produced from this material (1.98 $mg.mL^{-1}$) at pH 6 was the lowest sugar concentration compared to the amount of sugar released from the other paper materials degraded with cellulase from the brown garden snail. During the saccharification of office paper (Fig. 2) at different pH values, a maximum sugar concentration of 3.39 $mg.mL^{-1}$ was obtained at pH 7.0, while at the same pH value, the highest percentage of saccharification was calculated at 9.11%. The amount of sugar released from this paper material when degraded with cellulase from garden snails increased by 34% as the incubation pH increased from 3.5 (2.25 $mg.mL^{-1}$) to pH 7.0 (3.39 $mg.mL^{-1}$).

Fig. 3 reflects the extent of sugar production and percentage saccharification of foolscap paper when degraded with brown garden snail cellulase. The profiles reflected in Fig. 3 indicate that this paper material was increasingly degraded as the incubation pH value increased from a value of 3.5 to 6.0, with a decrease in the degradation when the pH value was increased to higher pH values. At the incubation

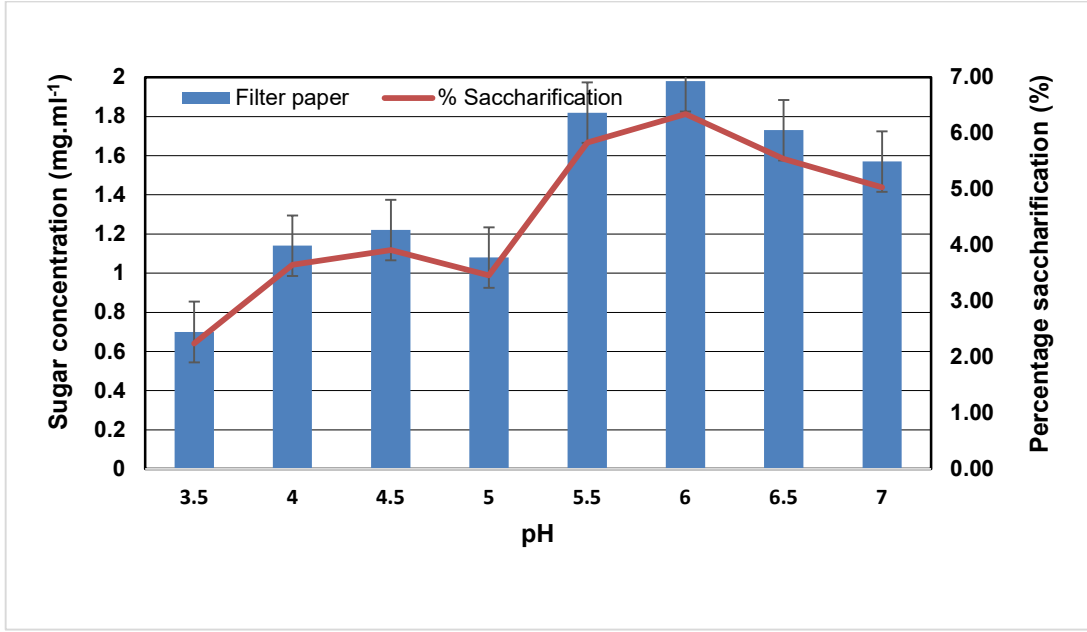

Fig. 1: Concentration of sugar (mg.mL^{-1}) produced from and percentage saccharification (%) of filter paper when degraded with cellulase from the brown garden snail (*Cornu aspersum*) at different pH-values.

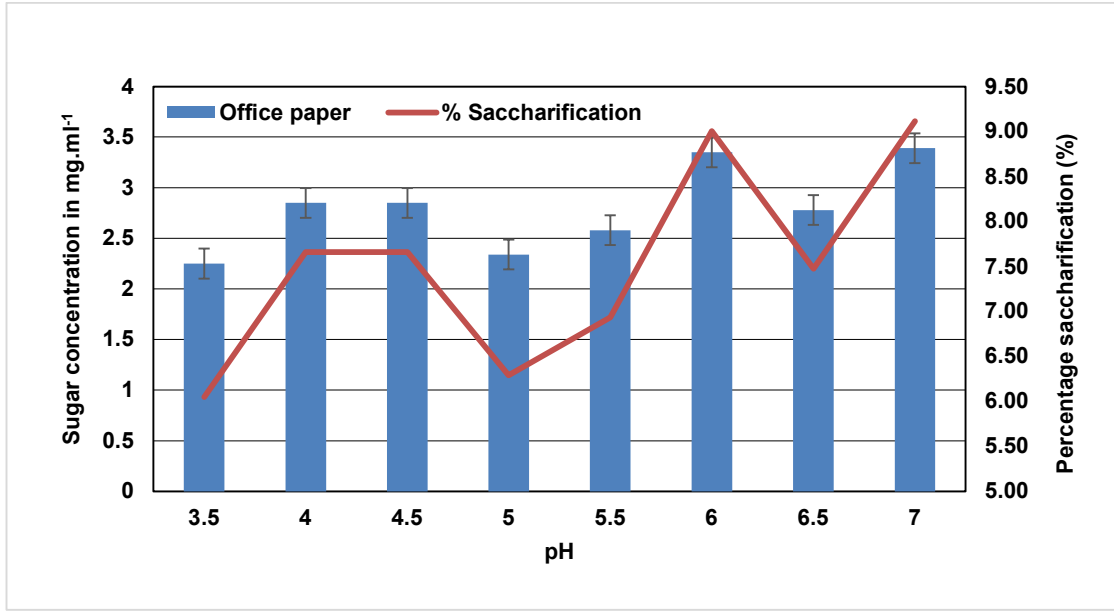

Fig. 2: Concentration of sugar (mg.mL^{-1}) produced from and percentage saccharification (%) of office paper when degraded with cellulase from the brown garden snail (*Cornu aspersum*) at different pH-values.

pH-values less than 6.0, the amount of sugar produced varies between concentrations of 2.13 mg.mL^{-1} and 2.35 mg.mL$^{-1,}$ while the maximum sugar concentration at pH 6.0 was calculated at a concentration of 2.92 mg.mL^{-1}. At pH-values higher than 6.0, the amount of sugar decreased to a value of 2.49 mg.mL^{-1} and 2.62 mg.mL^{-1} at the pH-values of 6.5 and 7.0, respectively. The percentage saccharification at the pH value of maximum saccharification was 10.81%, while the lowest degree of saccharification was obtained at 7.89%, calculated at an incubation pH of 4.0. The degree of saccharification increased by 37% from the lowest sugar yield to the maximum amount of sugar produced.

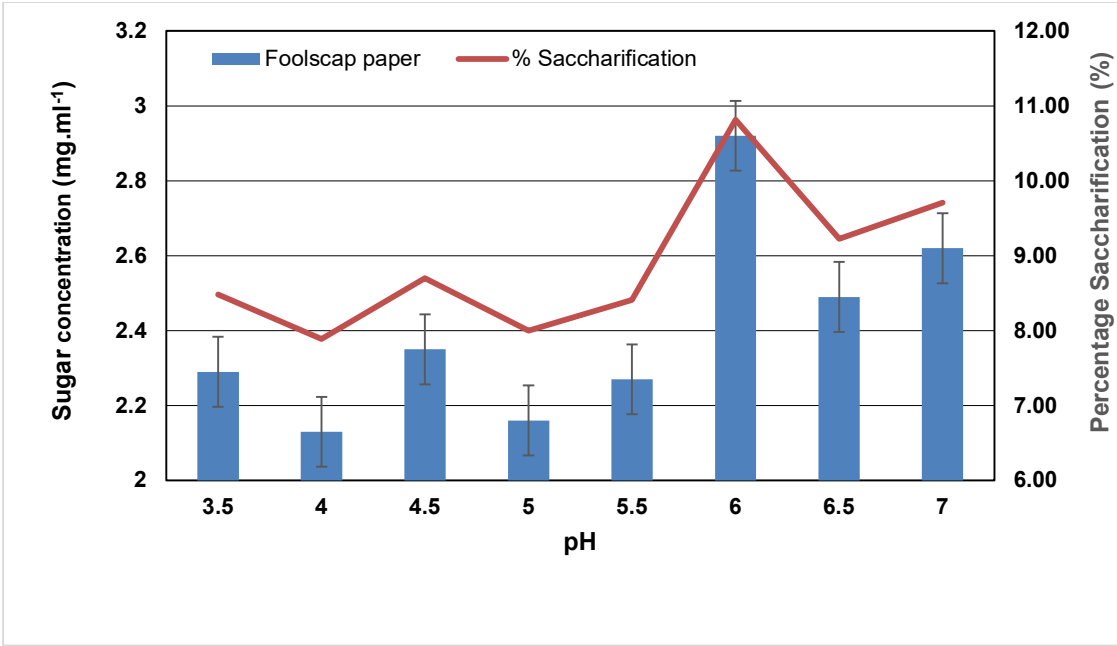

Fig. 3: Concentration of sugar (mg.mL^{-1}) produced from and percentage saccharification (%) of foolscap paper when degraded with cellulase from the brown garden snail (*Cornu aspersum*) at different pH-values.

Saccharification of newspaper (Fig. 4) at different pH values yielded the highest amount of sugar at a concentration of 2.18 mg.mL^{-1} when degraded at a pH of 4.5, with the second highest amount of sugar released at a concentration of 2.11 mg.mL^{-1} obtained at a pH-value of 6.0. The lowest amount of sugar was released at a pH of 3.5 when the produced sugar concentration was 1.5 mg.mL$^{-1,}$ and this amount was increased 1.45 times to produce the maximum concentration of 2.18 mg.mL^{-1}. When incubated at pH values higher than pH-6.0, the amount of sugar released from the newspaper decreased with a concentration of 1.77 mg.mL^{-1} produced during incubation at a pH of 7.0. The degree of saccharification at the optimum pH value of saccharification was 9.60%.

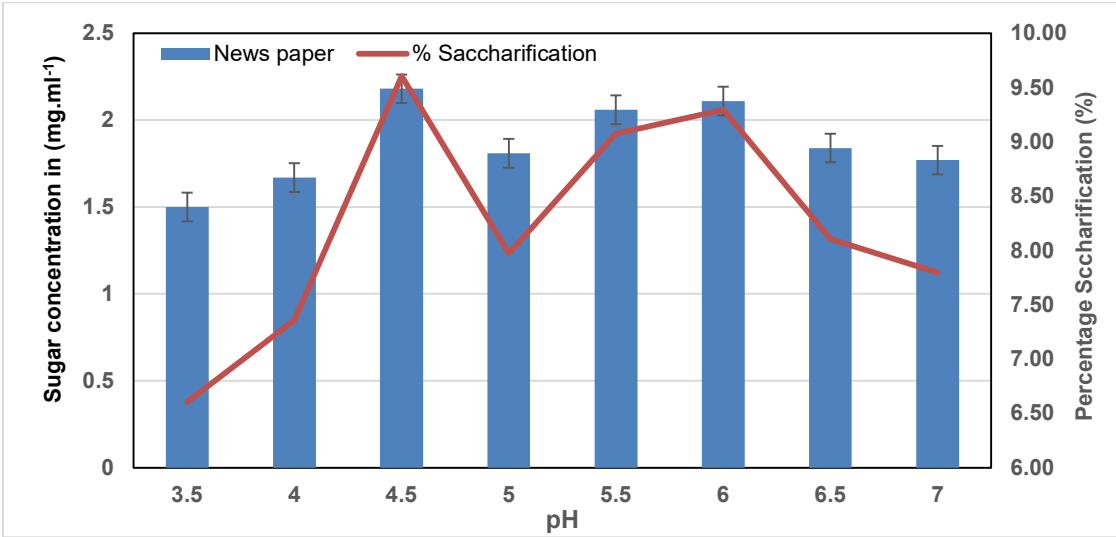

Fig. 4: Concentration of sugar (mg.mL^{-1}) produced from and percentage saccharification (%) of the newspaper when degraded with cellulase from the brown garden snail (*Cornu aspersum*) at different pH-values.

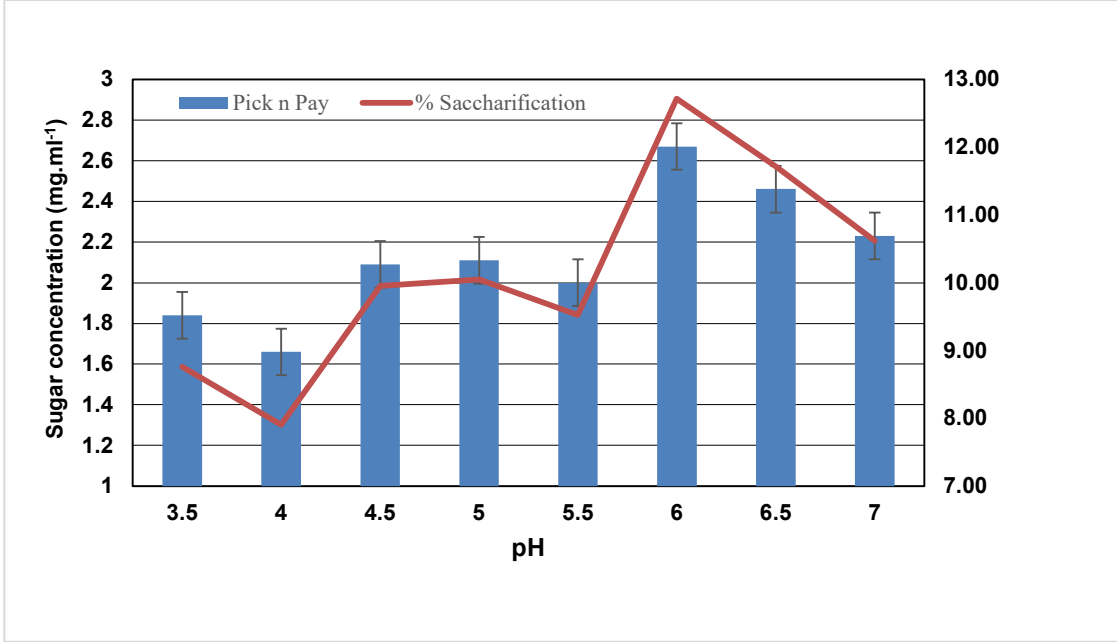

Fig. 5: Concentration of sugar (mg.mL^{-1}) produced from and percentage saccharification (%) of Pick 'n Pay advertising paper when degraded with cellulase from the brown garden snail (*Cornu aspersum*) at different pH-values.

In comparison, the lowest extent of saccharification was 7.36% when degraded at an incubation pH-value of 4.0, with a 7.80% saccharification when degraded at a pH-value of 7.0.

When Pick 'n Pay (Fig. 5) advertising paper was saccharified with cellulase from the brown garden snail, the maximum amount of sugar was released at a pH of 6.0, producing a sugar concentration of 2.67 mg.mL^{-1} and a percentage of saccharification equal to 12.71%. The degradation of this paper material at pH values higher than 6.0 was more than the amount of sugar produced at pH values less than 6.0. The lowest degree of saccharification was calculated at a concentration of 1.66 mg.mL^{-1} and 7.90% saccharification at an incubation pH of 4.0. At the pH-values higher than 6.0, the rate of saccharification was 11.71% and 10.62 % at pH-values of 6.5 and 7.0, respectively, while the corresponding sugar concentrations were 2.46 mg.mL^{-1} and 2.23 mg.mL^{-1}. The highest amount of sugar produced during the degradation at pH 6.0 was 60.8% higher than the lowest amount of sugar released at the pH value of 4.0.

Other than degradation of the other paper materials was, the saccharification of Woolworth's paper highly pH-specific, and maximum bioconversion was observed at a pH-value of 6.0 with a strong decline in sugar production at pH-values higher and lower than the optimum pH-value of cellulase action. When maximally degraded, the sugar concentration was 3.86 mg.mL^{-1} with a degree of saccharification of 8.85%. At pH values higher and lower than the optimum pH value for saccharification of this paper material, the amount of sugar produced varied between 2.67 mg.mL^{-1} and 2.97 mg.mL$^{-1,}$ with the percentage saccharification varied between 6.12% and 6.81% (Fig. 6). The increase in saccharification from the lowest yield of 2.67 mg.mL^{-1} to the highest concentration was 44.5%.

No unique optimum pH value was identified for the degradation of brown envelope paper (Fig. 7) as the amount of sugar produced varied between 5.29 mg.mL^{-1} and 5.91 mg.mL^{-1} during incubation between pH values of 3.5 and 6.5. Incubation at a pH value of 7.0 resulted in the lowest sugar production from this paper material at a concentration of 4.76 mg.mL^{-1} and 8.90% saccharification. In contrast, the percentage of saccharification at the lower pH values varied between 9.89% and 11.05%.

These findings of changing pH values on the catalytic effect of cellulase from garden snails are supported by work done by Gautam et al. (2011), who concluded that the optimum pH for cellulase activity is at pH 6.5 and also noted that the enzyme activity was stable at pH range between 5.0 and 8.0. During the current investigation, the optimum degradation of brown envelope paper was obtained at pH values of 4.5 as well as pH 6.0, which supports the findings of Mokatse and Van Wyk (2017), who concluded that certain paper materials could be saccharified optimally by

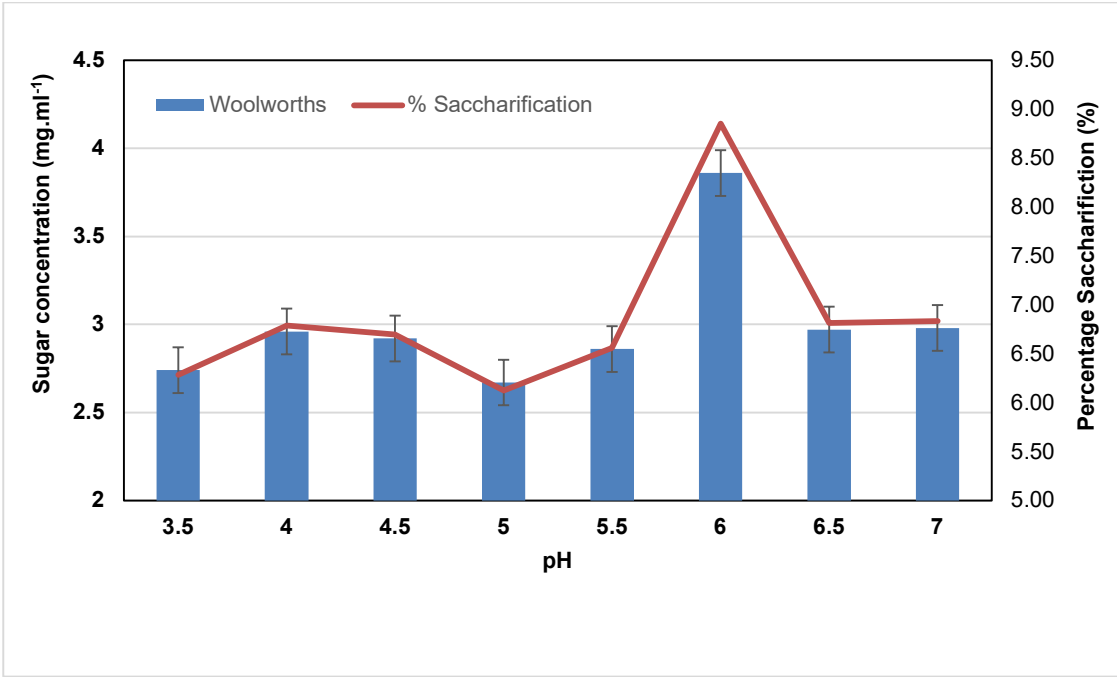

Fig. 6: Concentration of sugar (mg.mL^{-1}) produced from and percentage saccharification (%) of Woolworth's advertising paper when degraded with cellulase from the brown garden snail (*Cornu aspersum*) at different pH values.

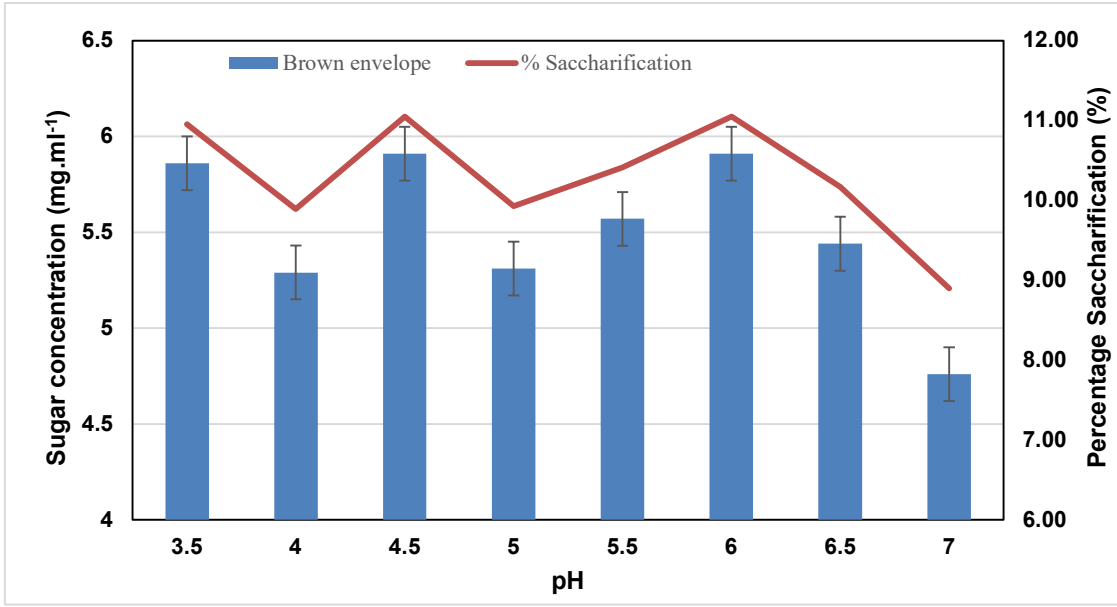

Fig. 7: Concentration of sugar (mg.mL^{-1}) produced from and percentage saccharification (%) of brown envelope paper when degraded with cellulase from the brown garden snail (*Cornu aspersum*) at different pH values.

cellulase enzymes at two different pH-values. The optimum pH value of 6.0 is also consistent with the value published by Abdulsattar et al. (2020) when performing a cellulase-related investigation.

Cellulose is described as the most abundant renewable substance on the planet, and it is present in many waste substances, such as paper materials, kitchen and garden waste, and agricultural waste. With the rate at which

solid waste is produced daily without an effective waste management system in place, the accumulation of solid waste is already a major environmental concern to many countries, and the future effects of waste on the environment do not appear to be very positive. Dumping at landfills, incineration, and illegal burning are still popular ways of dealing with organic waste, including cellulose-derived materials. Chemically, cellulose is described as a biopolymer composed of glucose units that have the potential to be developed as a resource for bio-energy production. Waste cellulose could also be used as an alternative feedstock to fossil fuels for the biosynthesis of bioproducts. To utilize waste cellulose effectively as a resource with renewable potential, it must be degraded into glucose, a fermentable sugar. This saccharification process could be performed with a hydrolytic enzyme system such as cellulase, which exhibits the ability to break the chemical bonds between glucose units in the cellulose molecule, releasing free glucose units. The degradation of cellulose by cellulase enzymes from bacteria (Hosny & El-Sheshtawy 2022) and mostly fungi (Sarsaiya et al. 2018) are well described with many references to the optimum catalytic properties such as pH, temperature, enzyme as well and substrate concentration. The saccharification effect of cellulase from *Trichoderma reesei* and *Aspergillus niger* on various paper materials has been published to a great extent by laboratories where the current research is performed.

Information regarding cellulase isolated from brown garden snails is not available to the same extent as information related to other cellulase enzymes, as the scientific community has not studied this cellulase extensively. A method to isolate cellulase from brown garden snails has been described, as well as its hydrolytic effect on the degradation of certain waste paper materials (Ndlovu et al. 2019). To secure maximum cellulose conversion into glucose, it is essential that the catalytic parameters, such as the pH of the enzyme action, be optimized. The various paper materials have non-identical structural compositions. They would exhibit different susceptibilities towards the cellulase enzyme, thus the observed difference in relative sugar production from the different paper materials when saccharified with cellulase from the brown garden snail. The pH value of an enzyme-catalyzed reaction is a catalytic parameter that would affect the effective catalysis of the reaction, and such is the case with the bioconversion of waste paper into sugars by cellulase from the brown garden snail. This investigation found that maximum saccharification was obtained at pH 6 with almost all the paper materials. However, the sugar-releasing patterns at higher and lower pH values than 6.0 differ for the various materials. The pH-value of 6 for optimum activity is more acid than the pH-value of 7.4 observed for the cellulase isolated from *B. licheneniformis* used to degrade carboxymethyl cellulose (Zanab et al. 2022). A more acidic pH value of 4.8 was recorded for the degradation of corn straw, wheat straw, and sugarcane bagasse by cellulase from *Aspergillus niger* (De Aguiar et al. 2022). Cellulase with a wide optimum pH range between pH 5.0 to pH 9.0 has also been reported for the degradation of cellulose by cellulase from *Geobacillus* sp. isolated from hot spring water (Khadka et al. 2022).

Although waste paper is rich in cellulose, not all types treated with the garden snail cellulase have been degraded with cellulase from other bacterial or fungal cellulases. The degradation of Woolworths and Pick 'n Pay paper is a unique attempt by researchers in our laboratories, as any other researchers have not reported its bio-conversion potential. These two paper materials have been bio-converted into sugars by cellulase from *Trichoderma viride,* and both were maximally degraded to a value of pH 4.5. During the same investigation, it was concluded that newspaper and foolscap paper also showed maximal degradation into sugars by the same cellulase at pH 4.5. Brown envelope paper, office paper, and filter paper showed optimum degradation by the *T. reesei* enzyme at a pH of 5.0 (Mokatse & Van Wyk 2017). Of the paper materials, filter paper is the utmost studied, mostly to demonstrate the action of cellulase and at optimum values of pH 5.5 when degraded with *T. viride* cellulase (Rathman et al. 2015) and pH 4.8 when treated with *T. reesei* cellulase (Chu et al. 2012). The presence of dyes or ink on paper material is a physical factor that needs to be considered when degrading the cellulose content of used paper with cellulase enzymes. Ink or dyes covering the paper materials act as a barrier between the enzyme and the cellulose substrate, thus preventing the effecting interaction between enzyme and substrate. This observation was made during an investigation in our laboratory when paper covered to different extents was degraded with cellulase from *T. viride*. It can be assumed that cellulase from the garden snail would experience the same difficulty (Van Wyk & Sibiya 2014).

Environmental pollution is a global concern, and associated with this singularity is climate change, whose effects are already experienced by many countries in terms of excessive heat, drought, and flooding. The use of fossil fuels is a major contributor to this change in natural processes, and replacing this energy resource as a fuel and feedstock in many chemical syntheses with an environmentally friendly substance becomes more urgent. Bioenergy is classified as environmentally friendly, and waste cellulose materials such as wastepaper contain cellulose as a structural component that could be resolved into glucose by cellulase, a biological catalyst. Glucose is a fermentable sugar that can be used as a feedstock for synthesizing bioethanol (Byadgi &

Kalburgi 2016). To optimize the saccharification of different wastepaper materials effectively, catalytic parameters such as incubation pH must be optimized, and this writing does not only reveal the effect of pH on cellulase-catalyzed degradation of various wastepaper materials but also describes the action of a cellulase isolated from brown garden snails an enzyme which has not received much scientific attention during the past.

CONCLUSIONS

Environmental pollution and climate change are two issues that already influence global activities, and corrective scientific procedures will have to be investigated and applied more aggressively to counteract these occurrences. Developing organic waste as a renewable energy resource and utilizing it as a feedstock for the biosynthesis of chemical commodities could assist in controlling the negative environmental forces caused by pollution and climate change. Using cellulase isolated from garden snails as biocatalysts contributes further to the value of the waste bioconversion process, as snails are regarded by many communities, such as farmers, as a pest due to the destructive effect of snails on their crops. The development of wastepaper as a renewable energy resource is contributing to the process of dealing with solid waste in an environmentally friendly manner, and the optimization of incubation pH-values for various paper materials when degraded by garden snail enzyme is contributing to the effectiveness of the process. The observation that most paper materials are optimally degraded at a pH value of 6.0 indicates the similarity between these materials. However, the difference in relative sugar yield, From the investigation, it can be concluded that cellulase isolated from garden snails can degrade the cellulose component of various wastepaper materials into glucose. That waste cellulose can be developed as a renewable feedstock for producing glucose-related fermentation bio-products. Identifying the optimum incubation pH values is also an important catalytic parameter for optimizing bioconversion.

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