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The influence of substrate concentration on *T. viride* catalyzed saccharification of various waste paper materials

Khomotso M. P. Mokatse and J. Pieter H. van Wyk*

Department of Pharmacology and Therapeutics, Sefako Makgatho Health Sciences University, **South Africa**.

***Correspondence:** bioenergy.res@gmail.com **Accepted:** 16 Aug. 2019 **Published online:** 27 August 2019

In everyday life a massive amount of paper materials is dumped by the global population which contributes towards environmental pollution. Environmental pollution caused by municipal solid waste (MSW) is a major issue worldwide especially in developing countries. An important step that can help reduce the accumulation of waste on land is through the conversion of cellulosic waste into fermentable sugars. Waste paper forms a major part of MSW and it can be converted into high-value added bio-products such as biopharmaceuticals by saccharification and fermentation processes. In this study the influence of substrate concentration on the saccharification of waste paper materials by *T. viride* cellulase was investigated. Substrate concentrations ranging from five disc pieces to forty-five disc pieces were used to determine the best substrate concentration that is able to yield the highest amount of sugars. The results revealed that hydrolyzing 15 pieces of brown envelope paper with *T. viride* cellulase enzyme yields the highest amount of sugar of 21.0 $mg.ml^{-1}$. The lowest amount of sugar was yielded by Pick 'n Pay advertising paper with a sugar concentration of 10 $mg.ml^{-1}$ when 20 pieces of paper were incubated. Newspaper resulted with the highest degree of saccharification of 50% and Pick 'n Pay with the lowest degree of saccharification of 27%. It was also discovered that by increasing the substrate concentration beyond a certain value resulted in a decrease in the yield of sugar concentration due to end-product inhibition of cellulase enzyme and recrystallization of sugar in solution.

Keywords: Substrate concentration, *Trichoderma viride*, waste paper, saccharification, bio-products.

INTRODUCTION

Economically, the most important industrial material other than foodstuffs affected by microorganisms are cellulose as well as wood. Used paper materials amount to being a major component of organic waste dumped annually (Rathnan et al., 2015). Paper itself contains cellulose which forms about 40-50% of plants composition making it the most abundant polymer worldwide with an estimated synthesis rate of 10^{10} tons year (Singh and Hayashi, 1995; Lynd et al., 2002). Cellulose is made up of a glucose polymer of 8000 to 12 000 units, composed of anhyro-β-1,4-glucosidic bonds. Cellulose-rich plant biomass such as wheat straw, sawdust and wood are one of the sustainable sources of bio-products such as biofuels and bio-pharmaceuticals. Cellulose is degraded by a hydrolytic enzyme known as cellulase that exist in multiple forms and catalyze reactions that degrade insoluble cellulose to soluble carbohydrates (Moser et al., 2008). There is an increased interest in the use of cellulases to hydrolyze waste materials such as sawdust, wheat straw, cotton waste, banana waste, wood scraps and waste paper because of their many potential applications (Kuhad et al., 2011). Cellulases are used in research and in the production of bioenergy, biofuel as well as in the

food, textile, laundry, pulp paper and agriculture industries (Akhtar et al., 1994; Bhat, 2000).

A complete cellulose system consists mainly of three classes of enzymes (Zhang et al., 2006; Deswal et al., 2011), namely endo-glucanases (1,4-β-D-glucan-4-glucanohydrolase; EC 3.2.1.4), cellobiohydrolases (1,4-β-D-glucan glucohydrolase; EC 3.2.1.740) and β-glucosidases (β-D-glucosidase glucohydrolase; EC 3.2.1.2). These three classes of enzymes work synergistically together to hydrolyze cellulose into fermentable sugars (Bayer et al., 1994). The enzymatic catalyzed saccharification of cellulose is affected by different types of factors such as the amount of cellulase enzyme, the substrate concentration, pH value, reaction temperature, reaction time, enzyme inhibition and activation (Bensah and Mensah, 2013).

An increase in cellulase dosage could enhance the yield of glucose from cellulose and rate of cellulose hydrolysis however this serves as a disadvantage because this would mean an increase in the cost of the saccharification process (Martin et al., 2012). Cellulase dosing in cellulose hydrolysis can vary over a wide range, depending on the type and concentrations of cellulose substrates. Normally increasing the amount of substrate would result in an increase in hydrolysis yield and rate of the reaction. However, a high substrate conversion rate can cause an inhibition of substrate degradation. When the substrate concentration is too high, the substrate-enzyme interaction can be more complicated. In this case, substrates are competing for the active site or enzyme. The molecular structure of the enzyme, the catalytic rate can be influenced by the substrate concentration in many different ways (Jayasekara and Ratnayake, 2019). The aim of this study was to determine the influence of the amount of waste paper materials during saccharification with *T. viride* cellulase enzyme.

MATERIALS AND METHODS

Cellulosic materials

Filter paper (Whatman no.1), newspaper, office paper, brown envelope paper, foolscap paper as well as Pick 'n Pay and Woolworths advertising papers were used as substrates for saccharification into fermentable sugars by the *T. viride* cellulase enzyme. The materials were cut into small circular discs of 6.0 mm in diameter and the mass for the number of pieces of paper is represented in figure 1.

Cellulase enzyme and incubation procedure

Crude *T. viride* cellulase enzyme (0.20 g) was dissolved in 50.0 ml of tris buffer (0.05 $ml.dm^{-3}$), pH 5 resulting in an enzyme concentration of 2 $mg.ml^{-1}$. The enzyme solution (200 µl) was transferred to a test tube filled with tris buffer (800 µl) and paper materials ranging from five pieces to forty-five pieces for each incubation. This reaction mixture was incubated at 50^{o}C for 2 hours.

Determination of total reducing sugars

After cellulase catalyzed bioconversion of the various waste paper materials with *T. viride* cellulase the reaction mixtures were cooled to room temperature. The cooled solutions were mixed with the DNS reagent according to the method described by Miller (Miller, 1959). The resulting mixtures were heated in a boiling water bath for a period of 10 min, with the resulting colour intensity determined on a spectrophotometer (Shimadzu UV-1800) at 520 nm. A sugar calibration curve was constructed using glucose standards ranging from 0.5 $mg.ml^{-1}$ to 8.0 $mg.ml^{-1}$. The constructed calibration curve was used to determine the concentration of the various sugar solutions obtained during the cellulase catalyzed degradation of the different waste paper materials.

Table 1. Masses (mg) of waste paper materials saccharified with *T. viride* cellulase.

Number of disc pieces	Wool worths	Newspaper	Brown envelope	Office	Pick 'n Pay	Foolscap	Filter
5	9.1	6.7	15.6	10.9	5.8	7.3	8.6
10	20.0	13.2	31.7	22.3	13.4	14.9	17.4
15	30.1	20.2	47.5	33.2	21.1	20.9	25.7
20	40.0	27,0	63.7	44.0	27.6	28.9	34.6
25	49.8	33.7	79.7	55.0	34.0	36.6	43.6
30	60.0	40.1	96.7	66.5	41.5	44.3	51.2
35	70.1	4.6	113.0	77.5	46.4	52.0	59.5
40	79.8	53.6	129.5	88.1	52.8	59.7	68.9
45	90.1	59.7	145.8	98.8	58.8	68.1	78.5

Calculation of percentage saccharification

The concentration of the released reducing sugar was calculated by using a standard calibration curve of glucose, and expressed as mg/ml. The percentage saccharification was calculated as follows: %Saccharification = Reducing sugars ($mg.ml^{-1}$) / initial substrate concentration ($mg.ml^{-1}$) x 0.9 x 100. The factor 0.90 was used to convert polysaccharide to monosaccharide accounting for water uptake during hydrolysis (Alrumman, 2016).

RESULTS AND DISCUSSION

Waste paper is a major component of solid waste and is classified as organic waste due to the presence of cellulose a structural component of waste paper materials. Glucose the monomer of this biopolymer is an important feedstock for the synthesis of many biochemicals and bio-pharmaceuticals (Babu et al., 2013) and a number of factors complicate the bio-conversion of cellulose into fermentable sugars. Variables such as cellulase composition (Juturu and Wu, 2014) as well as the structural features of cellulose (Trudeau et al., 2014) and catalytic properties (Sibiya and van Wyk, 2016) of the cellulase enzyme are major determinants of an effective saccharification of waste cellulose. Another important factor is the heterogenicity of the saccharification process of waste paper as it is not a soluble substance. The amount of paper material mixed with a fixed incubation volume and enzyme concentration is thus another variable to be investigated in this already complicated enzyme catalyzed bioconversion process.

During the saccharification of filter paper (figure 1) the amount of sugar production increased from the lowest mass of filter paper (8.6 mg) to a mass of 51.2 mg of filter paper, incubated. During this process the sugar concentration increased from 2.27 $mg.ml^{-1}$ to 10.2 $mg.ml^{-1}$. The concentration of sugar production decreased when masses higher than 51.2 mg of filter paper was degraded and the highest degree of filter paper saccharification was obtained during the degradation of 25.7 mg of filter paper to an extent of 25.6%. When Pick ‘n Pay paper was degraded with *T. viride* cellulase (figure 2) the amount of sugar production increased strongly from a mass of 5.8 mg to 27.6 mg with the amount of sugar production varied between 9.02 $mg.ml^{-1}$ to 8.9 $mg.ml^{-1}$ when the mass of Pick ‘n Pay paper degraded was increased from 34.0 mg to 58.8 mg. Maximum percentage saccharification was obtained at 33.3 % when 27.6 mg of this paper material was degraded. A relative high degree of saccharification of 30.9 % was also calculated during the degradation of the lowest mass of 5.8 mg Pick ‘n Pay paper, incubated. The percentage saccharification decreased gradually from 33.3 % when 27.6 mg was degraded to 13.6 % when the highest mass of 58.8 mg of Pick ‘n Pay was degraded.

During the bioconversion of newspaper with *T. viride* cellulase (figure 3) the concentration of sugar production increased from 2.4 $mg.ml^{-1}$ when 6.7 mg of the paper material was degraded to 14.8 $mg.ml^{-1}$ when 40.1 mg of this waste paper was bioconverted. A decreasing sugar concentration was calculated when newspaper masses higher than 40.1 mg was degraded. The highest degree of saccharification of 45 % was obtained when 20.2 mg of the paper material was degraded, followed by a decreasing tendency of relative saccharification when increasing masses were degraded. The lowest degree of saccharification was obtained at 11.9 % when 59.6 mg of newspaper was bioconverted by the cellulase enzyme. A relatively high percentage of 32.2 % was obtained when the lowest mass of 6.7 mg of newspaper was degraded by *T. viride* cellulase.

Figure 4 reflects the changing sugar production from Woolworths paper when different masses of this material was exposed to *T. viride* cellulase. A strong increase in sugar production was obtained from 2.94 $mg.ml^{-1}$ to 13.3 $mg.ml^{-1}$ during the degradation of a 9.1 mg to 60.0 mg of this paper material. A decrease in sugar production was also calculated when masses of Woolworths paper higher than 60.0 mg was degraded. The highest relative percentage saccharification was calculated at 31 % when 30.1 mg of Woolworths paper was degraded. A decreasing tendency of percentage saccharification was obtained when masses higher that 30.1 mg of paper was degraded. Opposite to all the other paper materials that were treated with *T. viride* cellulase a relatively low percentage saccharification of 2.9 % was obtained when the lowest mass of Woolworths paper was degraded.

A maximum sugar concentration of 21.0 $mg.ml^{-1}$ was obtained when 47.4 mg of brown envelope paper was degraded. This sugar concentration was two times higher than the lowest sugar concentration of 6.8 $mg.ml^{-1}$ when 15.6 mg of brown envelope paper was degraded.

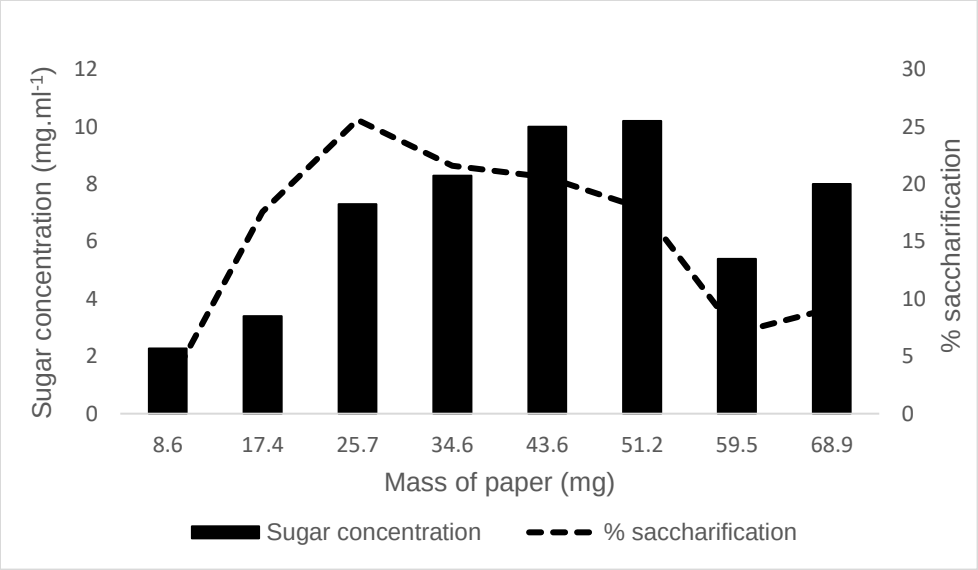

Figure 1; Saccharification of different masses of filter paper with a fixed amount of *T. viride* cellulase.

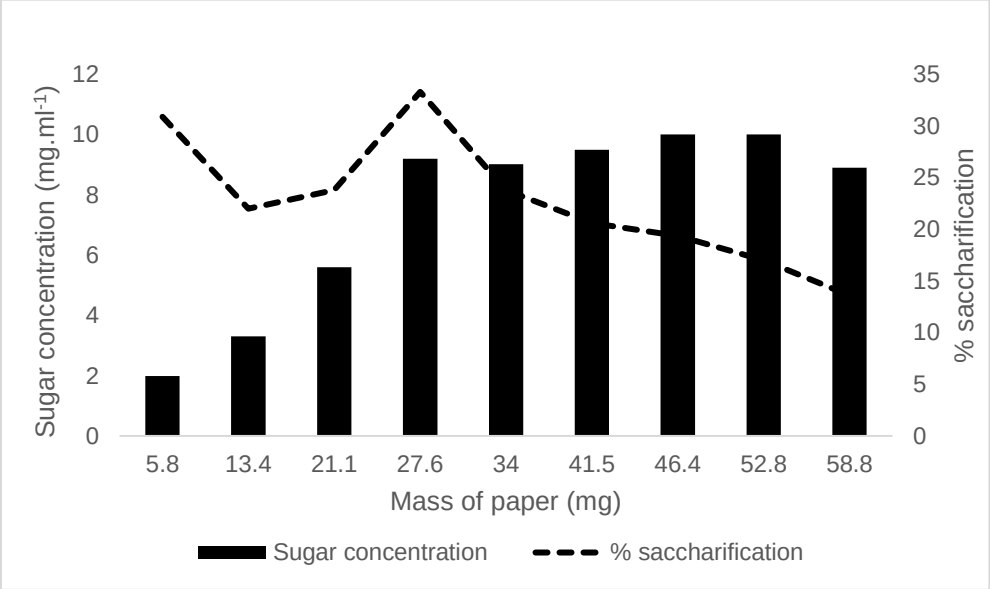

Figure 2; Saccharification of different masses of Pick 'n Pay paper with a fixed amount of *T. viride* cellulase.

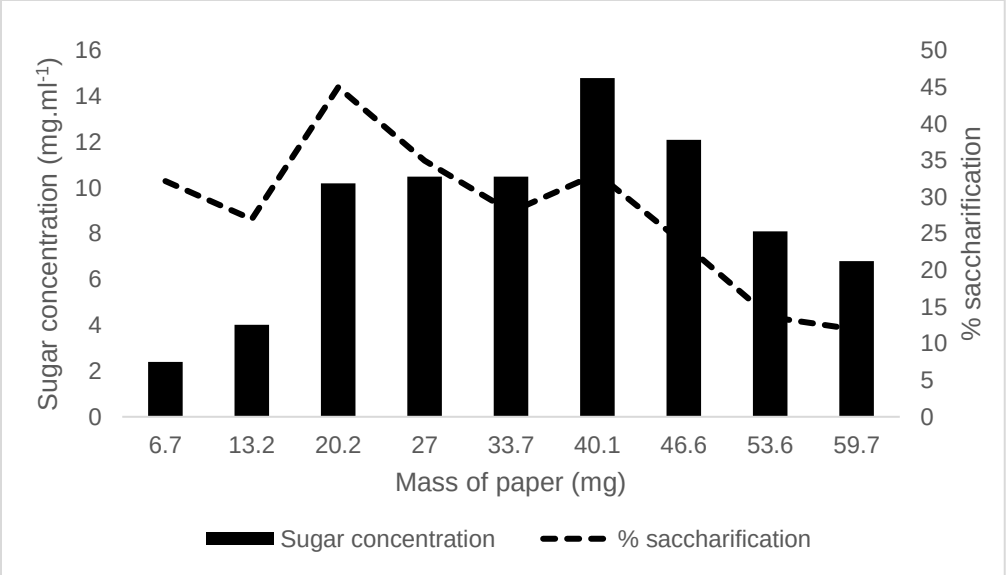

Figure 3; Saccharification of different masses of newspaper with a fixed amount of *T. viride* cellulase.

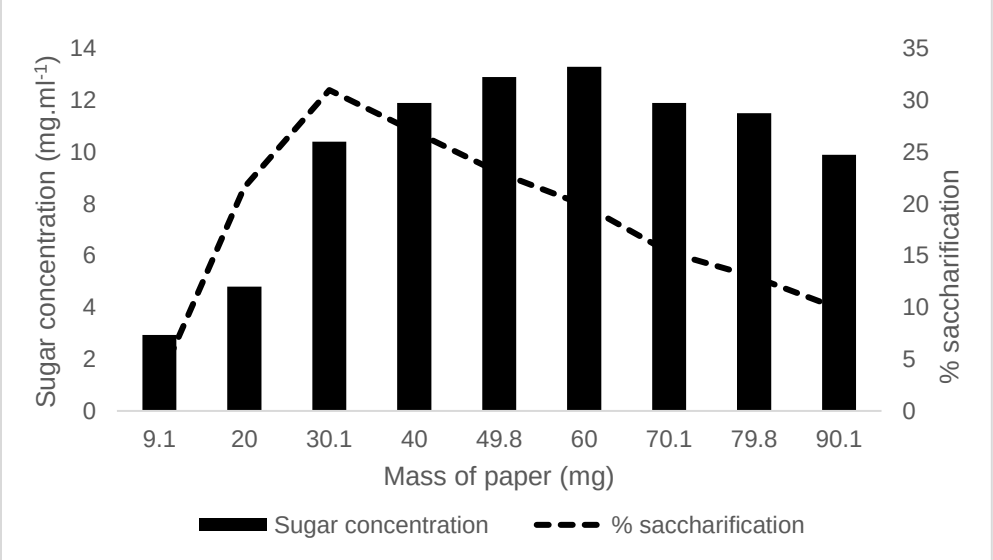

Figure 4; Saccharification of different masses of Wool worths paper with a fixed amount of *T. viride* cellulase.

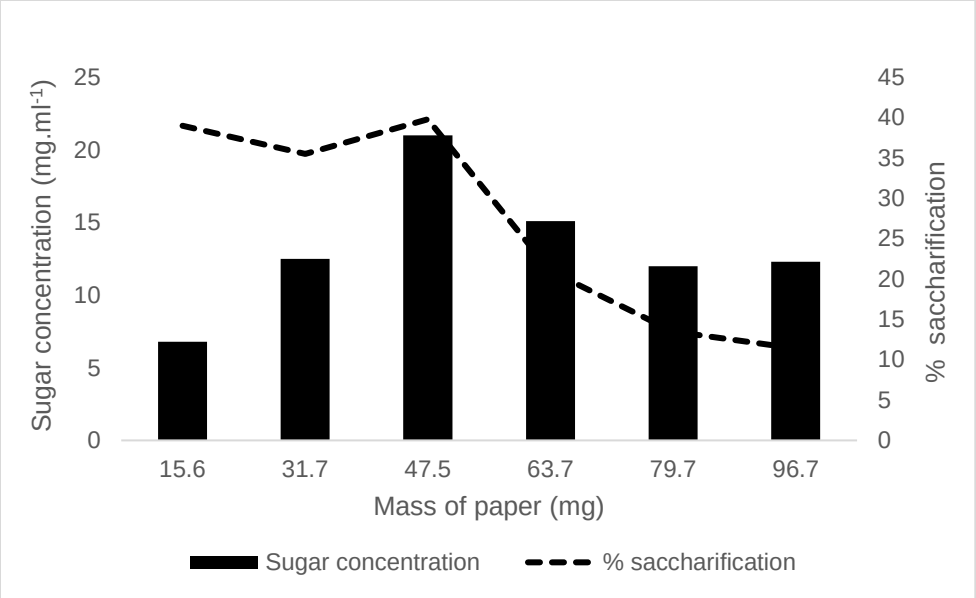

Figure 5; Saccharification of different masses of brown envelope paper with a fixed amount of *T. viride* cellulase.

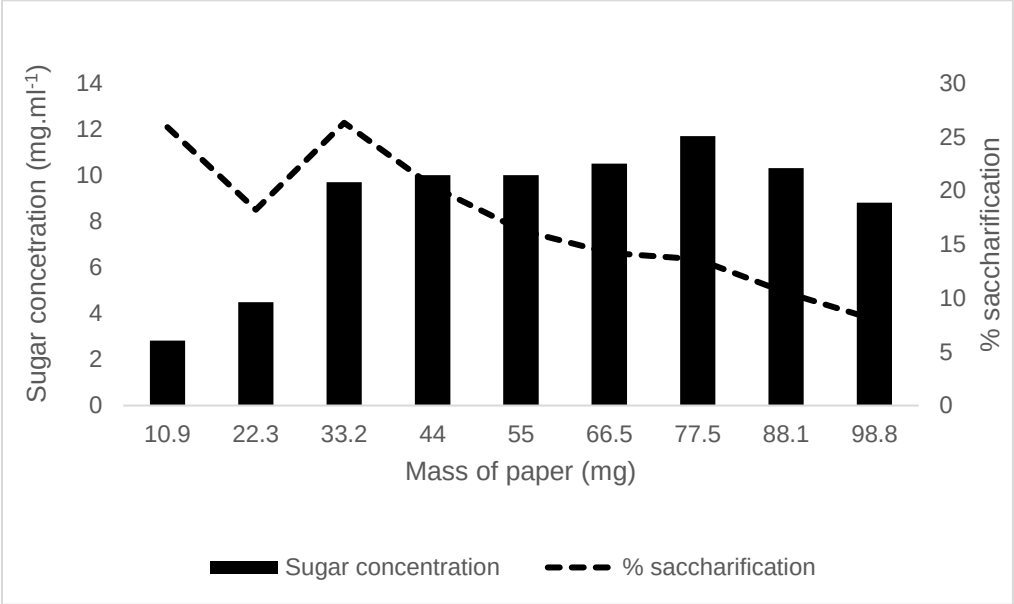

Figure 6; Saccharification of different masses of office paper with fixed amount of *T. viride* cellulase.

The concentration of sugar released also decreased to a concentration of 12.3 mg.ml^{-1} when the highest mass of 96.7 mg of brown envelope paper was degraded (figure 5). During bio-conversion of the lowest mass of brown envelope paper 15.6 mg a relative high percentage of saccharification was obtained at 39% that was the same degree of saccharification when 47.5 mg of this waste cellulose material was bio-converted into sugars. When office paper (figure 6) was degraded with *T. viride* cellulase the sugar concentration increased from 2.8 mg.ml^{-1} to 9.7 mg.ml^{-1} between 10.9 mg to 33.2 mg of the substrate degraded. This shows a 2.5 times increase in sugar production. The produced sugar concentration varied between 9.7 mg.ml^{-1} to 8.8 mg.ml^{-1} when higher masses of office paper between 33.2 mg and 98.8 mg was saccharified. The percentage saccharification showed maximum values of 25.9 % and 26.3 % during the

degradation of 10.9 mg and 33.2 mg, respectively. When masses higher than 33.2 mg was degraded the % degradation decreased from 8% when 98.8 mg of this paper material was degraded.

During the degradation of foolscap paper (figure 7), the amount of sugar increased from 2.9 $mg.ml^{-1}$ when 7.3 mg paper was treated with the cellulase enzyme to a sugar concentration of 14.5 $mg.ml^{-1}$ when 52.0 mg of the paper material was degraded. The sugar concentration also decreased when masses of foolscap paper higher than 52 mg was bio converted. The highest percentage saccharification was 41 % when 28.9 mg of paper was degraded with the lowest amount of paper 7.3 mg also resulting in a relative high percentage of degradation of 36.7%. The percentage degradation also decreased when masses higher than 28.9 mg of the foolscap paper was degraded. A general observation when all waste paper materials were saccharified was that an initial increase in sugar production was observed when increasing masses of the various materials were degraded. A tendency of decreasing sugar formation was also evident with each paper material during degradation of masses higher than the distinctive masses producing the highest sugar concentration with exception of Woolworths paper when a relative high percentage sugar was produced from the degradation of the higher masses of the cellulose materials. The relative percentage of saccharification was also lower during bio-conversion of the higher masses of each paper material. Table 2 reflects the mass of each paper material which results in the highest sugar concentration and optimum percentage saccharification when degraded with *T*. *viride* cellulase. The mass for optimum sugar production when degraded with the same cellulase concentration varied between 20.9 mg for foolscap paper to 47.0 mg when brown envelope was treated with the cellulase enzyme. The highest sugar concentration was obtained from brown envelope paper at a concentration of 21.0 $mg.ml^{-1}$ and percentage saccharification of 44 %. A higher saccharification of 50 % was calculated for newspaper at a concentration of 14.8 $mg.ml^{-1}$ when 40.0 mg of this material was degraded. The sugar concentration varied between 10.0 $mg.ml^{-1}$ and 21.0 $mg.ml^{-1}$ when the paper materials at optimum masses were degraded, with the percentage saccharification varied between 26.5 % for Pick 'n Pay paper to 50 % for newspaper. When degraded at their optimum masses for degradation the rate of increase in sugar production when the lowest mass was degraded until maximum sugar concentration was obtained as reflected in table 3. From this calculation it was concluded that the highest rate of 1.57 $mg.ml^{-1}$ sugar produced per mg paper hydrolysed was obtained during degradation of Woolworths paper followed by a rate of 0.91 $mg.ml^{-1}$sugar produced per mg paper hydrolysed during degradation of newspaper. The slowest rate of sugar formation was 0.2 $mg.ml^{-1}$ sugar produced per mg paper hydrolysed when newspaper was saccharified and this rate was 6 times slower than the degradation rate experienced during degradation of Woolworths paper.

Cellulosic biomass is an abundant renewable resource that can serve as a substrate for the production of alternative fuels, such as ethanol, bio-products and bio-pharmaceuticals. Substrate concentration affects the primary rate and yield of enzymatic hydrolysis. High substrate concentrations often result in substrate inhibition, which drastically lowers the hydrolysis rate (Liaw and Penner, 1990). High solid loading has caused problems such as reduction in heat and mass transfer efficiency, rheological problems, and increased inhibitor concentration (Balan, 2014).

The influence of different masses of substrate of different waste paper materials during the saccharification of these materials were investigated and illustrated in figure 1-7. The results show a significant trend of increase in sugar concentration when the amount of the substrate was increased from 5 pieces to 35 pieces however when the amount exceeds 40 pieces the sugar yield decreased. Pick 'n Pay yielded 10.0 $mg.ml^{-1}$ of sugar at 35 pieces of substrate incubation. The sugar yielded by Pick 'n Pay is low as compared to the other paper materials with office paper yielding the second least high amount of 11.7 $mg.ml^{-1}$. Brown envelope paper yielded the highest amount of sugar of 21.0 $mg.ml^{-1}$ with only 15 pieces degraded making it the most effective degraded paper material by yielding high amounts of sugars. The yield of reducing sugar started to show a downward trend when the substrate concentration continued to increase after adding more than 35 pieces of paper.

This could happen due to several factors such as if the substrate loading is too high, a problem with enzyme accessibility could inhibit the conversion of cellulose. An increase of viscosity material could also lead to affect enzyme mobility (Dahnum et al., 2015).

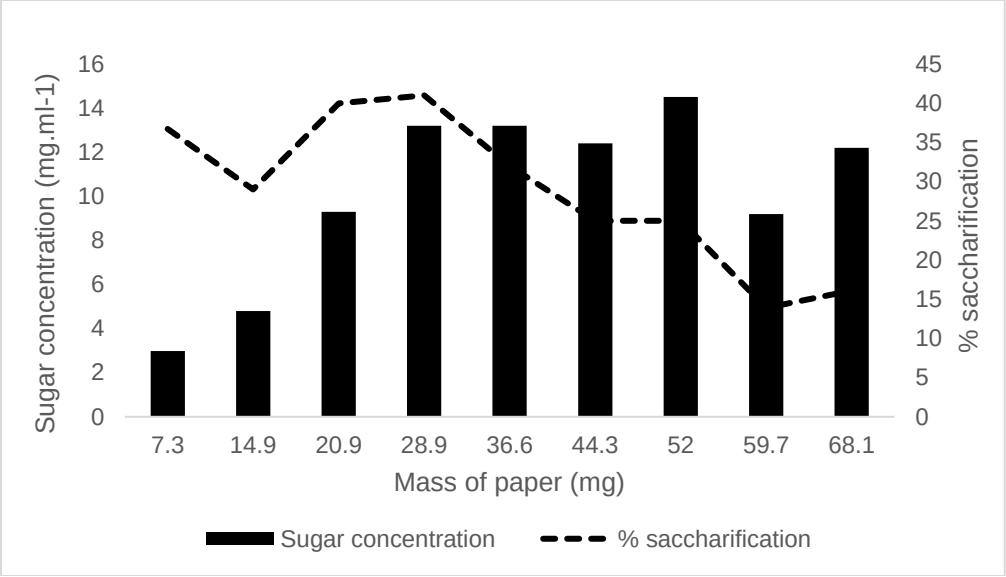

Figure 7;Saccharification of different masses of foolscap paper with a fixed amount of *T. viride* cellulase.

Table 2;Sugar concentration (mg.ml^{-1}) and saccharification (%) of the various waste paper materials during optimum degradation by cellulase from *T. viride* at different concentrations.

Paper materials	Optimum paper mass (mg)	Optimum sugar concentration mg.ml^{-1}	Maximum saccharification (%)
Filter paper	25.7	11.9	40.8
Pick 'n Pay	27.6	10.0	26.5
Foolscap	20.9	14.5	44.0
Woolworths	30.0	13.3	35.0
Brown envelope	47.0	21.0	44.0
Newspaper	40.0	14.8	50.0
Office paper	33.0	11.7	29.0

Table 3; Rate of increase in sugar production for maximum sugar production.

Paper materials	Rate of increase sugar production expressed in change concentration of sugar produced per mg of substrate
Filter paper	0.22
Pick 'n Pay	033
Newspaper	0.91
Brown envelope	0.44
Office paper	0.31
Foolscap	0.47
Woolworths	1.57

At high substrate concentrations, the decreasing sugar yield and reaction rates are reduced and this could be because of end-product inhibition of cellulase enzyme but at low concentrations, the decreasing sugar yield and reaction rates are improved (Nikolic 2010; Liu et al., 2013; Madadi et al., 2017; Mardawati et al., 2019). It has been reported that avoiding substrate inhibition, lowering substrate concentration is the most suitable measure (Wang et al., 2011). The increase in substrate concentration decreased the saccharification percentage and this might be caused by poor stirring, enzyme inhibition by saccharification products, and decreased synergistic action between cellulase enzymes, as mentioned by Wen et al., 2004 (Alrumman, 2016).

From this investigation it was discovered that paper discs lower than 35 pieces yield high amounts of sugars for six paper materials and paper discs lower than 15 yield more sugars for brown envelope and this could be associated with the mass of brown envelope. It could be said that brown envelope paper is the best paper to yield high amounts of sugars at lower substrate concentration.

CONCLUSION

Increasing the substrate concentration above thirty-five pieces leads to a decrease in sugar yield and this is affected by factors such as end-product inhibition, if the substrate loading is too high, a problem with enzyme accessibility could inhibit the conversion of cellulose and an increase of viscosity material could also lead to affect enzyme mobility. Another important factor is that the bioconversion process was environmentally friendly as no dangerous side products were formed, and the energy demand for the process is relatively low as it was performed at 50^{o}C.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

JPHvW designed and supervised experiments. KMPM conducted research laboratory work. KMPM wrote the manuscript with JPHvW. JPHvW reviewed the manuscript. All authors read and approved the final copy of the manuscript.

REFERENCES

Akhtar, M, 1994. Biochemical pulping of aspen wood chips with three strains of *Ceriporiopsis subvermispora*. Holzforschung 48 (1994): 199-202.

Alrumman SA, 2016. Enzymatic saccharification and fermentation of cellulosic date palm wastes to glucose and lactic acid. Braz J Microbiol 47 (1): 110-119.

Babu RP, O'Connor K, Seeram K. 2013. Current progress on bio-based polymers and their future trends. Prog Biomater 2 (8): 1-16.

Balan V, 2014. Current challenges in commercially producing biofuels from lignocellulosic biomass. ISRN Biotechnol 2014: 1-31.

Bayer EA, Morag F and Lamed R, 1994. The cellulosome-a treasure-trove for biotech-nology. Trends Biotechnol 12 (9): 379-386.

Bensah EC and Mensah M, 2013. Chemical pretreatment methods for the production of cellulosic ethanol: Technologies and Innovations. Int J Chem Eng 2013: 1-13.

Bhat MK, 2000. Cellulases and related enzymes in biotechnology. Biotechnol Adv 18 (2000): 355-383.

Dahnum D, Tasum SO, Triwahyuni E, Nurdin M and Abimanyu, H, 2015. Comparison of SHF and SSF processes using enzyme and dry yeast for optimization for bioethanol production from empty fruit bunch. Energy Procedia 68: 107-116.

Deswal D. Khasa YP and Kuhad RC, 2011. Optimization of cellulase production a brown rot fungus *Fomitopsis* sp. RCK2010 under solid state fermentation. Bioresour Technol 102 (10): 6065-6072.

Jayasekara S and Ratnayake R, 2019. Microbial Cellulases: An overview and application. Intechopen 1-18.

Juturu V, Wu JC, 2014 Microbial cellulases: Engineering, production and applications. Renew Sust Energ Rev 33:118–203.

Kuhad RC, Gupta R and Singh A, 2011. Microbial cellulases and their industrial applications. Enzyme Res 2011:1-10.

Liaw E, Penner MH, 1990. Substrate velocity relationships for the *Trichoderma viride* cellulase-catalyzed hydrolysis of cellulose. J Appl Environ Microbiol 56 (8): 2311-2318.

Liu G, Zhang L, Qin Y, Zou G, Li Z, Yan X, et al, 2013. Long-term strain improvements accu-mulate mutations in regulatory elements responsible for hyper-production of

cellulolytic enzymes. Sci. Rep 3:1569.
Lynd LR, Weimer PJ, van Zyl WH and Pretorius IS, 2002. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiol Mol Biol Rev 66 (3): 506-577.
Madadi M, Tu Y and Abbas A, 2017. Recent status on enzymatic saccharification of lingo-cellulosic biomass for bioethanol production. eJBio 13 (2):135-143.
Mardawati E, Putri AV, Yuliana T, Rahimah S, Nurjanah S and Hanidah I, 2019. Effects of substrate concentration on bioethanol production from oil palm empty fruit bunches with simultaneous saccharification and fermentation (SSF). ESS. 230: 1-7.
Martin C, de Moraes Rocha GJ, Alves dos Santans, JB, de Albuquerque Wanderley, MC and Gowela, ER 2012. Enzyme loading dependence of cellulose hydrolysis of sugarcane agasse. Quim Nova. 35 (10): 1927-1930.
Miller GL, 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. J Anal Chem 31: 426-428.
Moser F, Irwin D, Chen SL and Wilson DB, 2008. Regulation and characterization of Thermo-bifida fusca carbohydrate-binding module proteins E7 and E8. Biotechnol Bioeng 100:1066–1077.
Nikolic S, Mojovic L Pejin D, Rakin M and Vukasinovic M, 2010. Production of bioethanol from corn meal hydrolyzates by free and immobilized cells of *Saccharomyces cerevisiae var. ellipsoideus*. Biomass Bio-energ 34 (10): 1449-1456.
Rathnan RK, Balasaravanan T, Anto SM, Tony AK, Anamika P and Ambili M, 2015. Bio-conversion of waste paper by co cultures of fungi isolated from lignocellulosic waste. IJCMAS 4 (6) 326-333.
Sibiya JBM and Van Wyk JPH, 2016. Bioconversion of waste newspaper into fermentable sugars at different temperatures with *Aspergillus niger* cellulase components. Journal of Applied Biology and Biotechnology 4 (4): 69-74.
Singh A and Hayashi K, 1995. Microbial cellulases: protein or architecture, molecular properties and biosynthesis. Adv Appl Microbiol 40: 1-44.
Trudeau DL, Lee TM, Arnold FH, 2014 Engineered thermo stable fungal cellulases exhibit efficient synergistic cellulose hydrolysis at elevated temperatures. Biotechnol and Bioeng 111 (12): 2390-2397.
Wang W, Kang L, Wei H et al, 2011. Study on the decreased sugar yield in enzymatic hydrolysis of cellulosic substrate at high solid loading. Appl Biochem Biotechnol 164 (7): 1139-1149.
Wen Z, Liao W, Chen S, 2004. Hydrolysis of animal manure lignocellulosics for reducing sugar production. Bioresour Technol 91 (1):31–39.
Zhang PYH, Himmel ME and Mielenz JR, 2006. Outlook for cellulase improvement: screening and selection strategies Biotechnol Adv 24 (5): 452-481.