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pH-Values for Optimum Saccharification of Various Waste Paper Materials by Cellulase from *Trichoderma viride*.

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ABSTRACT

Organic waste, a major section of solid waste is composed of various types of paper materials of which cellulose, a glucose-based biopolymer, is the major structural component. When exposed to the hydrolytic action of *Trichoderma viride* cellulase a number of seven different waste paper materials have been saccharified at different pH-values in order to determine the optimum pH-value at which each paper material is maximally degraded into glucose. From this investigation it was observed that certain paper materials such as office paper and filter paper were maximally degraded at two pH-values of pH 4,5 and pH 5,0 whilst materials such as foolscap paper, brown envelope paper, Woolworths advertising paper, Pick 'n Pay advertising paper and newspaper were maximally degraded at a pH-value of 4,5. The relative percentage saccharifcation of each paper material at the various pH-values were also determined.
KEY WORDS: Waste cellulose, Saccharification, Bio-products, Biodegradation, Optimum pH

INTRODUCTION

The accumulation of municipal wastes is a major concern worldwide [1]. The main component of municipal wastes is paper of various types generated from food, packing and commercial materials. Currently used paper materials are either recycled to a limited extent but eventually all are dumped or burnt, thus generating even further environmental pollution [2]. A substantial constituent of daily life is paper and when used it is classified as the most abundantly cellulosic biomass wastes produced, annually [3]. The biodegradation of cellulosic wastes into high value bio-products by saccharification processes is an important step that could be applied to reduce environmental pollution [4].

Paper materials can be classified as versatile or multipurpose commodities because they can be applied as a protective agents or be used as packaging materials. The enzymatically catalysed bioconversion of organic based waste such as used paper materials into soluble sugars like glucose is extremely important in the production of bio-pharmaceuticals and bioethanol [5]. Biomass cellulose is described as the greatest abundant renewable organic resource with approximately 200 billion tons accessible for bioconversion into biofuels and other value-added products such as pharmaceuticals, organic acids and industrial enzymes [6]. Waste biomass such as paper are structural polysaccharides of plants that comprise of cellulose (~ 50%), hemicellulose (~30%) and lignin (~20%) [7]. Cellulose is a polysaccharide of a high molecular weight consisting of 3000 or more glucose components, bonded by β-1,4-glycosidic bonds and cellulose is the building block for all fiber derived papers and textiles [8,9].

Cellulase is the most significant group of lignocellulolytic enzyme that could be used during the biodegradation of cellulose into fermentable sugars such as glucose. Cellulase is categorized as a hydrolytic enzyme which hydrolyse the β-glycosidic bonds of cellulose and related cellooligosaccharides. It is described as an enzyme system with the potential to be used for industrial conversion of cellulosic materials into simple sugars [10]. Cellulases are mostly substantiated in fungi and bacteria however fungi is the most common cellulase producer [11]. Among the cellulolytic fungi, *Trichoderma* and *Aspergillus* are the best cellulase enzyme producers [12]. *Trichoderma,* a filamentous ascomycetes class is used widely industrially because of its high secretary capacity and inducible promoting features [13].

The effect of pH on saccharification of various waste paper materials was studied during this investigation. Seven waste paper materials, office paper, foolscap paper, filter paper, newspaper, brown envelope paper as well as advertising paper from retailers Woolworths and Pick 'n Pay were exposed to the *Trichoderma viride* cellulase enzyme activity at different pH values.

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MATERIALS AND METHODS

USED PAPER MATERIALS

Foolscap paper (0.0478 g), Woolworth paper (0.0673 g), brown envelope (0,1g), filter paper (0.0541 g), newspaper (0.0415 g), Pick 'n Pay paper (0.0445 g) and office paper (0.0638 g) were used as substrate for saccharification and the determination of optimum pH value for maximum bioconversion of these paper material. Thirty pieces were prepared from the paper materials as circular discs with a diameter of 6.0 mm and these were transferred to a test tube resulting in a total mass as indicated before. Each type of paper was investigated in triplicate for its ability to be saccharified by the cellulase enzyme at different pH values.

CELLULASE SOLUTION

Crude *T. viride* (0.12 g) cellulase enzyme was dissolved in 50.0 ml of Tris buffer pH 3,5 ; 4,0 ; 4,5 ; 5,0 ; 5,5 and 6,0. A homogenous solution of the cellulase enzyme-buffer at a concentration of 2,0 $mg.ml^{-1}$ was prepared. Aliquots from this cellulase stock solution were mixed with the paper materials to bioconvert waste paper into glucose.

INCUBATION PROCEDURE AND DNS DETERMINATION OF SUGARS PRODUCED BY CELLULASE ACTION

To select the suitable pH value for maximum saccharification of each waste paper material, the enzyme (100 µl) was transferred to a test tube filled with Tris buffer (800 µl), methanol (100 µl) and pieces of the various paper materials. This reaction mixture was incubated at 40^{o}C for 2 hours. The incubation mixtures were analysed to determine the amount of sugars released during saccharification of the various paper materials by the cellulase enzyme. Once the incubation between the enzyme and paper was completed, the mixture was cooled to room temperature and followed by the addition of DNS reagent to determine the amount of reducing sugars described by Miller [14]. The sugar concentration obtained during the saccharification was determined from a standard calibration curve using glucose as the standard sugar solution.

RESULTS AND DISCUSSION

During this investigation seven different waste paper materials were exposed to the degradation action of cellulase from *T. viride*. In order to determine the optimum bio-action of the cellulase enzyme on each paper material, each material was incubated with the cellulase enzyme at different pH-values. Table 1 reflects information regarding the optimum pH-values as well as the percentage saccharification which results during saccharification of these paper materials at each pH-value. Besides the optimum pH-value it was also concluded that certain paper materials produced maximum amounts of sugar at two pH-values such as filter paper and office paper that could be maximally degraded at pH 4, 5 as well as pH 5, 0. The incubation masses of the various paper materials differ and equal number of identical paper discs were used during the degradation process. From the results it was also evident that the highest relative saccharification was observed during the bio-conversion of newspaper and Woolworth paper. Figures 1-7 illustrate the bioconversion profiles of the seven different waste paper materials when saccharified with *T. viride* cellulase at different pH-values as well as the percentage saccharification of these papers at the different incubation pH-values.

Table 1: Sugar concentration ($mg.ml^{-1}$) and saccharification (%) of the various waste paper materials during degradation by cellulase from *T. viride* at optimum catalytic pH-values.

Paper	Optimum pH	Mass incubated (mg)	Sugar concentration ($mg.ml^{-1}$)	Saccharification (%)
Newspaper	4,5	41	0,5	1,2
Pick n Pay advertising paper	4,5	44	0,4	0,9
Woolworths advertising paper	4,5	67	0,7	1,2
Brown envelope	5,0	100	0,9	0,9
Office paper	4,5 ; 5,0	64	0,4	0,6
Foolscap	4,5	48	0,4	0,8
Filter paper	4,5 ; 5,0	54	0,4	0,8

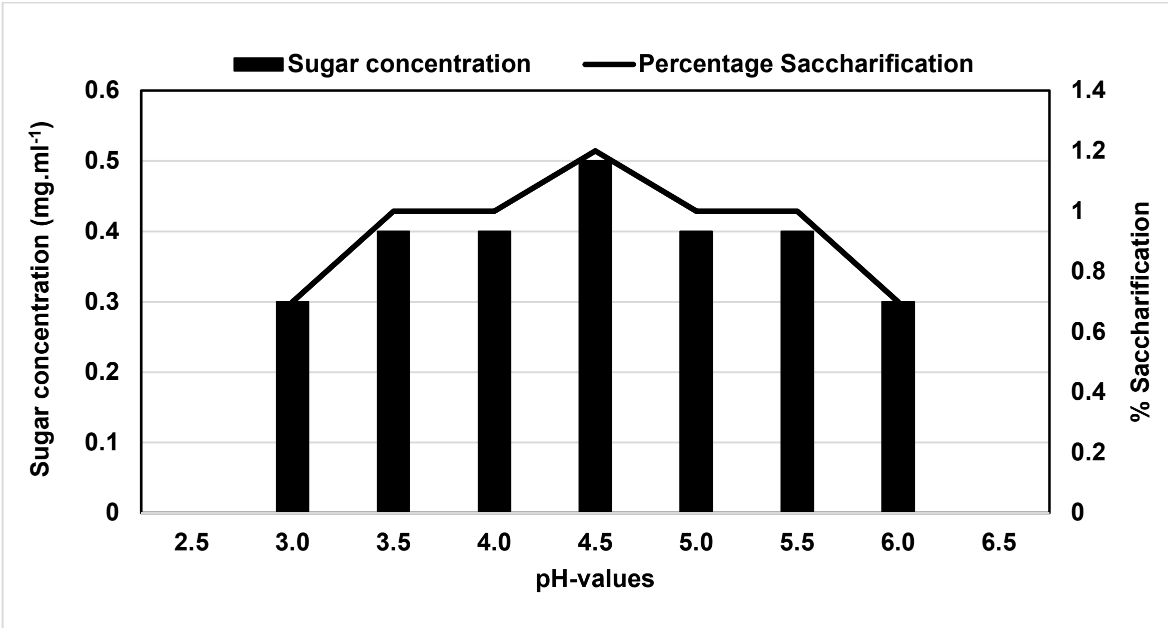

Figure 1: The effect of pH on enzymatic catalysed saccharification of newspaper by cellulase from *T. viride*.

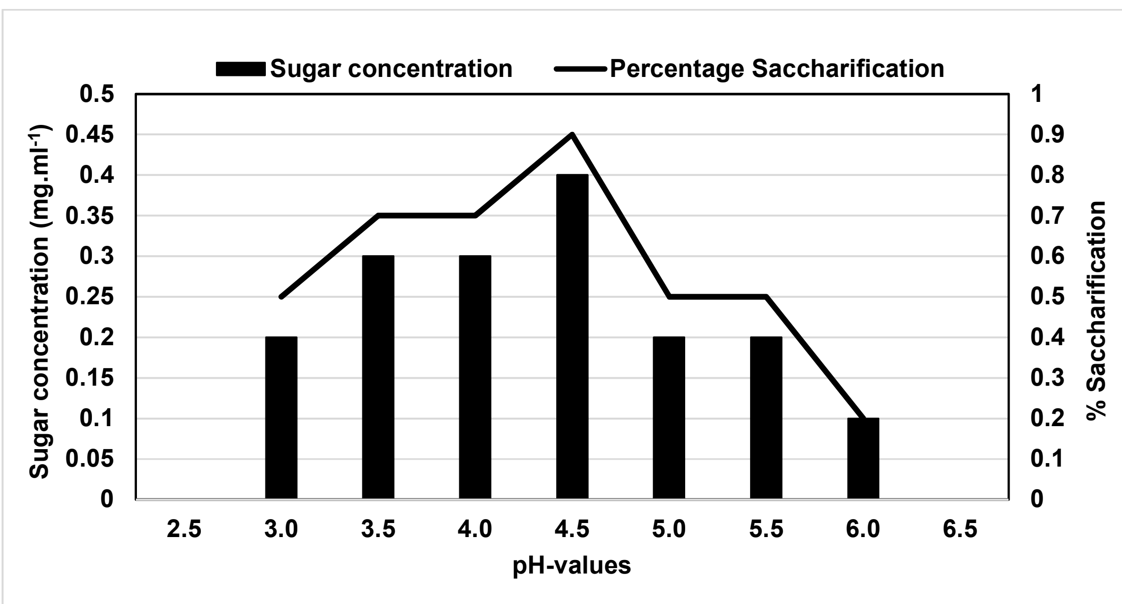

Figure 2: The effect of pH on enzymatic catalysed saccharification of Pick 'n Pay advertising paper by cellulase from *T. viride*.

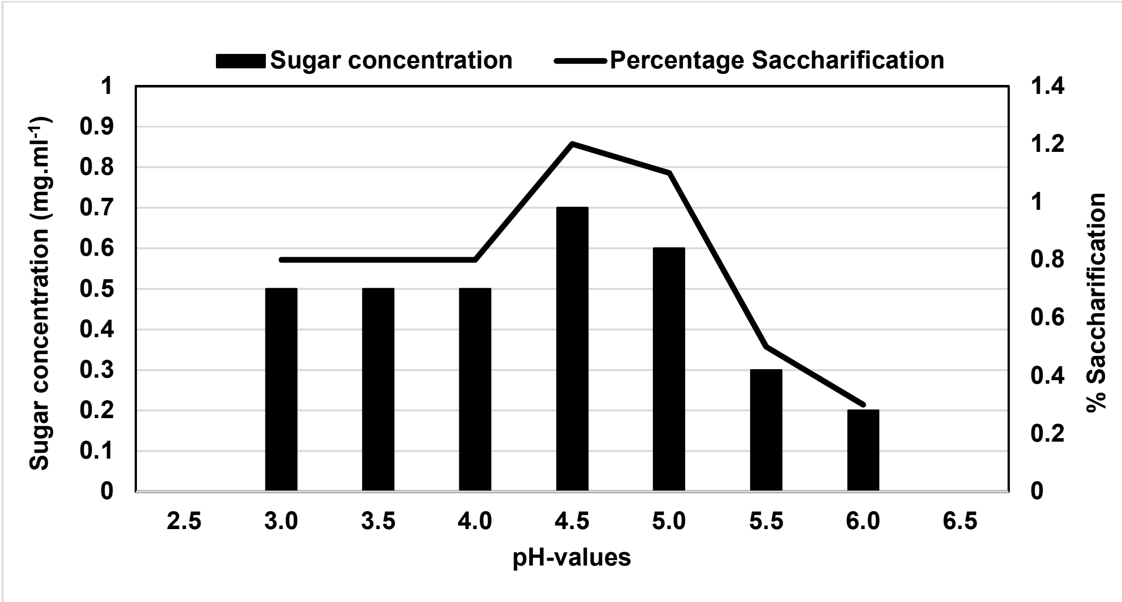

Figure 3: The effect of pH on enzymatic catalysed saccharification of Woolworths advertising paper by cellulase from *T. viride*.

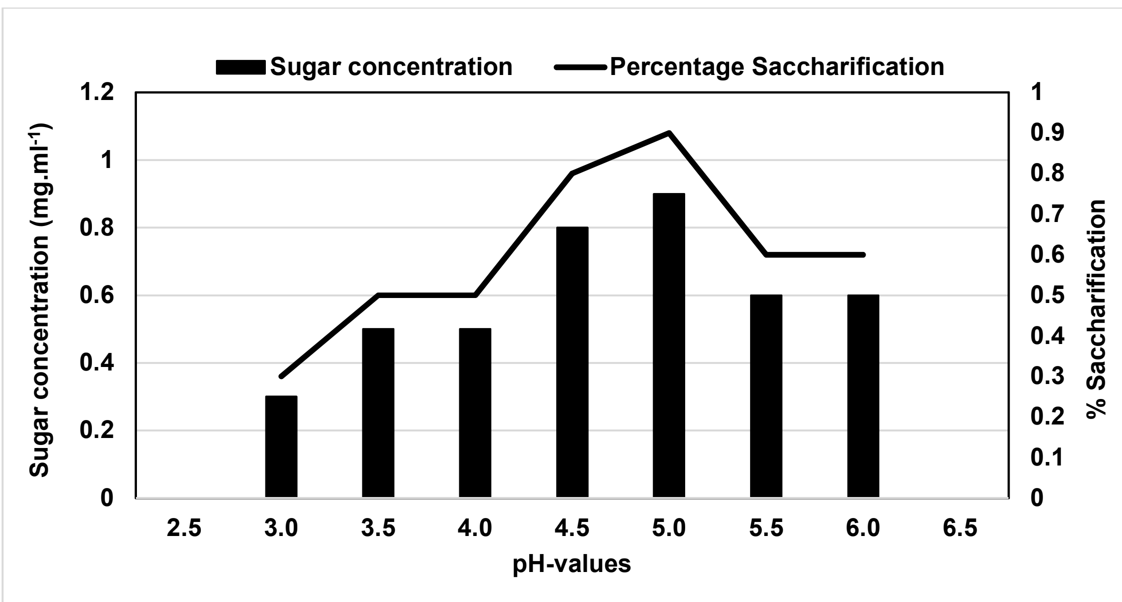

Figure 4: The effect of pH on enzymatic catalysed saccharification of brown envelope by cellulase from *T. viride*

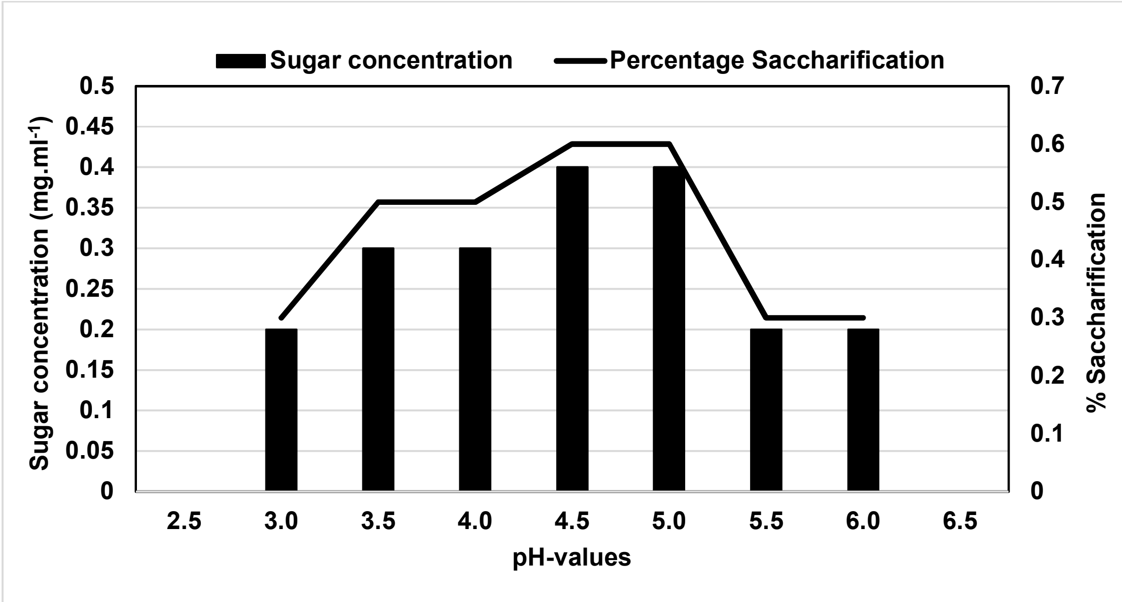

Figure 5: The effect of pH on enzymatic catalysed saccharification of office paper by cellulase from *T. viride*.

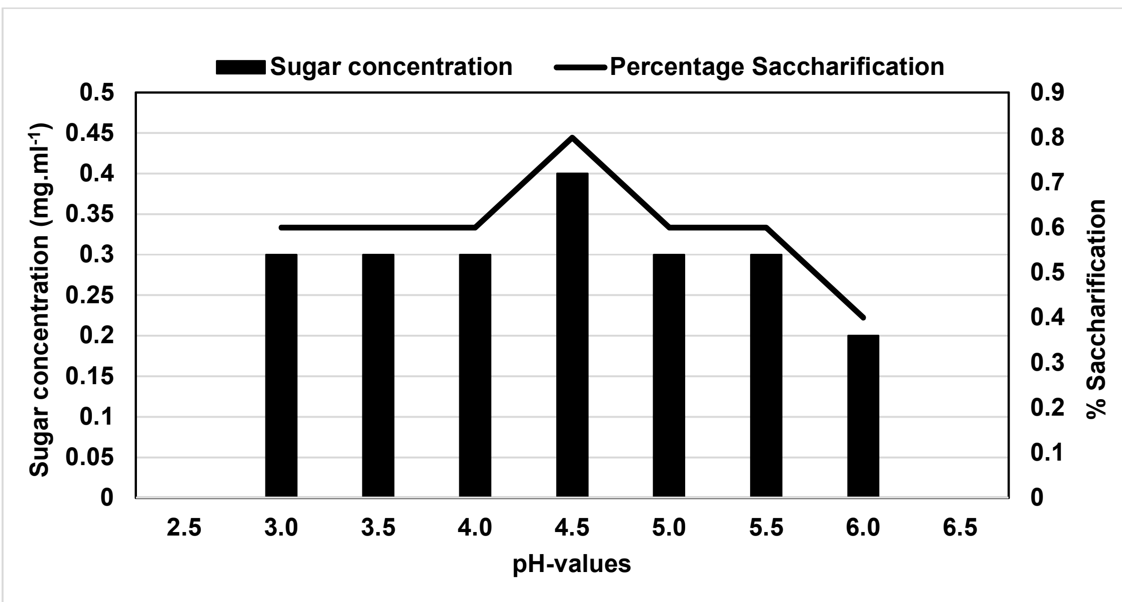

Figure 6: The effect of pH on enzymatic catalysed saccharification of foolscap by cellulase from *T. viride*.

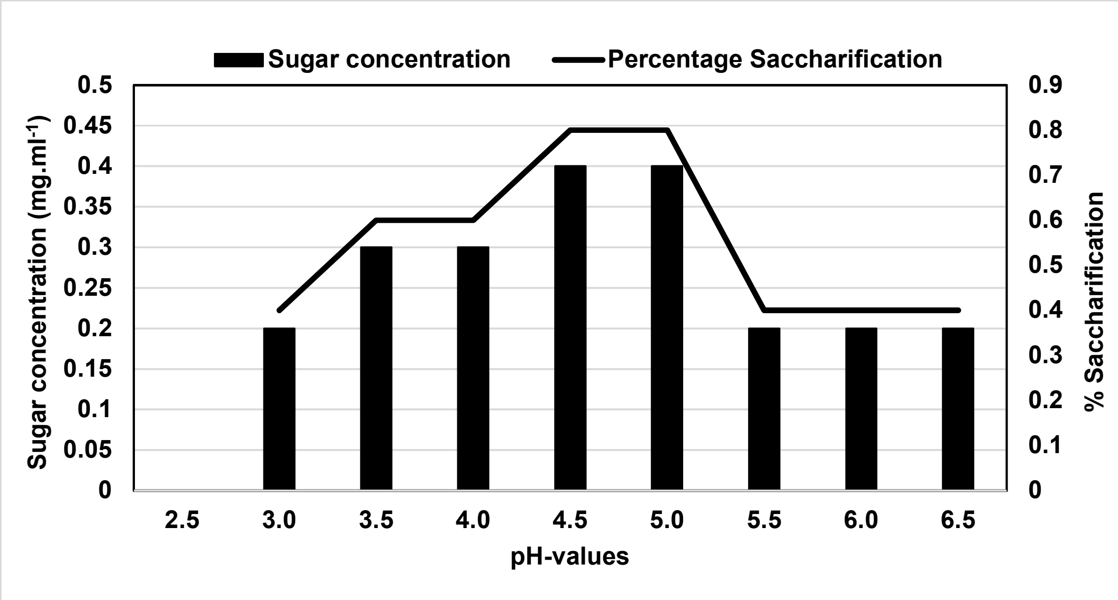

Figure 7: The effect of pH on enzymatic catalysed saccharification of filter paper by cellulase from *T. viride*.

The incubation pH of an enzyme catalysed reaction is an important variable that has to be optimized in order to secure maximum catalytic action of the enzyme while acting on the substrate. Cellulase is a multi-component enzyme system that is responsible for the degradation of cellulose into its building block, glucose a fermentable sugar [15]. Cellulose is a structural component of paper materials and the relative amount in each type of paper is determined by the purpose of the paper, its quality as well as how many times the paper has been recycled. For maximum cellulase catalysed bioconversion of the cellulose component of waste paper into glucose it is important to determine the optimum pH-value of cellulase that would result in the maximum degradation of the various waste paper materials into glucose. Different waste paper materials are a major component of solid waste and the bioconversion of the cellulose component of these waste materials into glucose a fermentable sugar could support the process of developing alternative and renewable energy resources. These renewable resources would not only be suitable for combustion purposes but could also produce chemical substances that could be utilized in the pharmaceutical industry as a renewable feedstock for synthetic purposes [16].

During the saccharification of newspaper (figure 1) with *T. viride* cellulase at different incubation pH-values the concentration of sugar produced has increased from an incubation at pH 3,0 until the maximum concentration was obtained at pH 4,5. At pH-values higher than pH 4,5 the amount of sugar production has decreased until newspaper was incubated at pH 6,0. The sugar production profile produced a symmetrical image on both side of pH 4,5 when maximum bioconversion took place. Identical sugar concentrations of 0,3 $mg.ml^{-1}$ was produced during degradation at pH 3,0 and pH 6,0 whilst a sugar concentration of 0,4 $mg.ml^{-1}$ was produced during bioconversion at pH-values of 3,5 ; 4,0 ; 5,0 and 5,5. Sugar of maximum concentration (0,5 $mg.ml^{-1}$) was obtained during incubation at pH 4,5 and this amount of sugar produced was 1,6 times more than the minimum concentration (0,3 $mg.ml^{-1}$). The relative saccharification of newspaper at the various pH-values follows the same profile tendency as observed with the formation of sugars and the maximum degree of saccharification was 1,2 % calculated for the bioconversion at pH 4,5. At the lowest saccharification rate a percentage saccharification of 0,7 % was calculated during the degradation of newspaper at pH-values of 3,0 as well as 6,0.Like newspaper, maximum degradation of Pick 'n Pay paper (figure 2) was also maximally converted at incubation pH-value of 4,5. The lowest extent of degradation was observed at pH-values of 3,0 resulting in a sugar concentration of 0,2 $mg.ml^{-1}$ and at pH-value of 6,0 that produced the lowest amount of sugar at a concentration of 0,1 $mg.ml^{-1}$. At pH-values of 3,5 and 4,0 sugar concentrations of 0,3 $mg.ml^{-1}$ was obtained while a concentration of 0,2 $mg.ml^{-1}$ was calculated during the degradation of this paper at pH-values of 5,0 and 5,5. The maximum sugar concentration of 0,4 $mg.ml^{-1}$ was recorded during the degradation at an incubation pH of 4,5.

Maximum bioconversion of Pick 'n Pay paper resulted in 0,9 % saccharification that is lower than the 1,2 % that was obtained with newspaper during maximum degradation. The lower rate of saccharification was 0,2 % at an incubation pH of 6,0 that was followed by a 0,4% during incubation at pH-values of 3,0; 5,0 and 5,5. The maximum bioconversion of Woolworths paper (figure 3) is similar obtained at the pH-value of 4,5 where Pick 'n Pay paper and newspaper showed maximum degradation. With Woolworths paper the maximum amount of sugar produced was obtained at a concentration of 0,7 $mg.ml^{-1}$. Incubations at pH-values lower than pH 4,5 produced the same amount of sugar at a concentration of 0,5 $mg.ml^{-1}$. At pH-values higher than 4,5 the concentration of sugar has decreased until the lowest sugar concentration of 0,3 $mg.ml^{-1}$ was obtained at pH-values of 6,0. The highest amount of sugar produced from Pick 'n Pay was 2,3 times higher than the lowest amount produced. The percentage saccharification when maximum sugar from Woolworth paper was produced was 1,2 % equal to the number obtained during degradation of newspaper at its optimum pH-value. During the formation of the lowest sugar concentration at pH 6,0 a saccharification rate of 0,2 % was calculated that was equal to the lowest degree of saccharification of Pick 'n Pay paper but less than the lowest rate of 0,7 % observed with newspaper.

Different to newspaper, Pick 'n Pay paper and Woolworths paper that were maximally degraded at a pH-value of 4,5, brown envelope paper (figure 4) was maximally degraded at an incubation pH of 5,0 producing a sugar concentration of 0,9 $mg.ml^{-1}$ that was 3 times higher than the lowest sugar concentration obtained at a pH-value of 3,0. The sugar concentration obtained at pH 3,5 and pH 4,0 was both 0,5 $mg.ml^{-1}$ whilst a level of 0,6 $mg.ml^{-1}$ was calculated for the amount of sugar produced during an incubation at pH 5,5 and pH 6,0.

Maximum saccharification of brown envelope paper was obtained at a degree of 0,9 % with the lowest degree equal to 0,3 %. The maximum saccharification of brown envelope paper was lower than the maximum saccharification of newspaper and equal to the maximum rate of saccharification of Pick 'n Pay paper and higher than the maximum saccharification calculated for Woolworth paper.Office paper (figure 5) is maximally degraded at two pH-values of 4,5 and 5,0 both producing a sugar concentration of 0,4 $mg.ml^{-1}$. The lowest sugar concentration of 0, 2 $mg.ml^{-1}$ was obtained during bioconversion of office paper at pH-values of 3,0, 5,5 as well 6,0. Maximally saccharification produced sugar at a concentration 2 times higher than the lowest sugar concentration. The amount of saccharification during maximally degradation equals 0,6% whilst the lowest extent of saccharification of 0,3 % was calculated at the pH-value of lowest sugar formation.Foolscap paper (figure 6) showed a relative wide pH-range for maximum degradation by *T. viride* cellulase into fermentable sugars at pH-values between 3,0 to 5,0 although the highest amount of sugar was released at a concentration of 0,4 $mg.ml^{-1}$ when degraded at pH-value of 4,5. At the other relative high sugar producing pH-values a sugar concentration of 0,3 $mg.ml^{-1}$ was concluded except for the sugar concentration of 0,2 $mg.ml^{-1}$ that was released during bioconversion of foolscap paper at a pH value of 6,0. Sugar produced at the optimum pH-value for foolscap paper degradation was 100 % higher more than sugars produced at the lowest sugar producing pH-value.Maximum saccharification of 0,8 % was calculated for foolscap paper that was higher than the maximum saccharification of office paper but less than the maximum saccharification of newspaper, Pick 'n Pay paper, Woolworth paper and brown envelope paper.

Similar to the degradation of office paper the maximum degradation of filter paper (figure 7) occurred at two pH-values of 4,5 and 5,0. Minimum degradation occurred at pH-values of 3,0; 5,5; 6,0 and 6,5. The maximum amount of sugar produced resulted at a sugar concentration of 0, 4 $mg.ml^{-1}$ that was 100 % more than the lowest sugar concentration produced. Maximum saccharification was calculated at a percentage value of 0,8% at the pH-value responsible for maximum filter paper bioconversion by cellulase from *T. viride*. The lowest saccharification was calculated at 0,2 % that was equal to the lowest degree of saccharification observed with Pick 'n Pay paper.

The issue of environmental pollution [17], climate change [18] and the decline of natural source [19] will become more topical as the global population is increased. The amount of solid waste produced annually is increased exponentially and this observation is evident in densely populated areas. Although the effect of non-managed solid waste on the environment is well documented [20] the organic part of solid waste offers a sustainable option to be developed as a renewable energy resource. As a renewable resource it would not only have a positive effect on the environment, but could also be utilized as feedstock for synthetic routes such as the development of bio-drugs in the pharmaceutical industry.

The pH-values for optimum degradation of the seven waste paper materials compare well with the values reported by Mahamud and Gomes who concluded that the optimal pH for saccharification of alkali treated sugar cane bagasse by *Trichorderma sp* was pH 5,0 [21] while a study performed by Rathnan *et al*., reported that the maximum pH-value for saccharification of waste paper by fungal cultures from *A. oryzae*, *P. citrinum* and *T. viride* was pH 5,5. A further increase in pH resulted in a gradual reduction of saccharification by the organism [22]. A pH of 6,0 was reported by Baig *et al*. for optimal enzymatic saccharification of steam-treated leaves and pseudo-stem of banana by cellulase from *T. lignorum* [23]. *Rhizopus oryzae* PR 7 cellulase, as reported by Karmakar and Ray, shown optimal saccharification at pH 7,0 of various agro-wastes, such as orange peel, sugarcane bagasse, dried flower, water hyacinth, and coconut shell [24].

CONCLUSIONS

Waste paper a major component of organic solid waste contains cellulose that could be resolved into glucose a fermentable sugar and building block of cellulose. In order to perform a cellulase catalysed saccharification of waste paper it is however important that the cellulase-cellulose incubation takes place at a pH-value that would result in the maximum action of the cellulase enzyme on the cellulose substrate thus producing the maximum amount of glucose. Seven different types of waste paper materials have been treated at different pH-values with *T.viride* cellulase and it was concluded that each paper material was maximally degraded at a unique pH-value whilst a number of papers were susceptible for maximum degradation at two different pH-values. The biodegradation of waste biomass such as used paper into a fermentable sugar such a glucose would not only support efforts to develop renewable energy resources for bio-product development but will also establish procedures to limit environmental pollution.

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