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Saccharification and change of incubation pH during the bioconversion of various waste paper materials with cellulase from *Aspergillus niger*

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ABSTRACT

Waste paper contains a high content of cellulose, a biopolymer that could be hydrolyzed into glucose a fermentable sugar. The cellulose content of eight different waste paper materials have been bio-converted into glucose by cellulase from Aspergillus niger. During this enzyme catalyzed degradation different saccharification profiles have been concluded for the various paper materials. Brown envelope paper showed the highest susceptibility for degradation by these cellulase followed by foolscap paper and cardboard. Newspaper showed the lowest degree of degradation by this enzyme system. Waste paper from local retailers, Wool worths and Pick 'n Pay were also included in the study. The change in the pH values during the bio-conversion of each paper material was also recorded.

Keywords: *Waste paper, Cellulose, Cellulase, Saccharification, A. niger, Incubation pH*

INTRODUCTION

The search and development of alternative and renewable energy resources will become more topical as the effect of climate change is experienced by the global population. Also of universal concern is the increased production of solid waste especially the accumulation of therof on valuable land and the release of dangerous gases from these waste materials during natural fermentation of the organic part of solid waste. The amount of solid waste production has constantly increased during the last century[1] with organic waste a major component of solid waste and composed mainly of waste paper[2], garden waste[3], kitchen waste[4] and other forms of waste materials.

Currently fossil fuels are the major source for the synthesis of bio-chemicals, pharmaceuticals and textiles[5]. The link between fossil fuels and climate change has been widely discussed, described and published[6]. A replacement for fossil fuels with a renewable resource is essential to limit future degradation of the environment and depletion of natural resources[7]. Bio-ethanol has been described as a suitable replacement for fossil fuels, not for its energy potential only but also as a valuable renewable substance in the bio-chemical and pharmaceutical industries[8]. The production of bio-ethanol from sugar cane is a well-established process already applied with major environmental advantages in countries such as Brazil[9].

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An analysis of waste paper, a major component of organic waste has indicated that it is composed of cellulose acting as a structural component of paper. Cellulose is composed of glucose molecules that are linked together by means of β-1,4-glucosidic bonds. If isolated from waste paper the cellulose part could act as a renewable resource for bio-energy development when the glucose released from the waste cellulose is fermented into bio-ethanol. Cellulase a multi-component enzyme system has the ability to hydrolyze the β-1,4-glucosidic bonds thus releasing glucose from the complexed bio-polymer, cellulose[10]. Cellulase enzymes are present in many bacterial and fungal organisms where it hydrolyzes cellulose into glucose that serves as a resource of energy for the microorganisms[11]. Cellulase isolated from different microorganisms have been implicated in the degradation of waste paper such as newspaper, office paper, filter paper and foolscap paper into fermentable sugars mainly glucose[12]. The effectiveness of the bio-conversion of waste paper into glucose is determined by the type of cellulase used as well as the nature of the organic waste material degraded, such as different types of waste papers. Cellulases are composed of various enzyme components of which each play a unique role during the degradation of cellulose.The association of cellulose with other structural components such as lignin and hemi-celluloses has also an effect on the saccharification of waste cellulose[13].

During this investigation various waste paper materials have been treated with cellulase from *Aspergillus niger* in order to hydrolyze the cellulose components of waste paper into glucose[14]. Eight different waste paper materials, office paper, foolscap paper, newspaper, brown envelope paper, paper towel, cardboard as well as advertising paper from current retailers, Woolworths and Pick n Pay were bio-converted into glucose. These waste paper materials were saccharified during an incubation period of 51 hours with samples taken regularly to determine the amount of sugars produced. The activity of cellulases is pH dependent and the pH was recorded at regular intervals during the incubation period[15].

MATERIALS AND METHODS

Waste paper materials

Various wastepaper materials such as office paper, newspaper, brown envelope paper, cardboard, foolscap paper, paper towel as well as the advertising paper from Woolworths and Pick-n-Pay were collected for degradation with cellulase enzymes. These paper materials were prepared in pieces of 2 cm x 2 cm. A mass of approximately 5.0 g of each paper was transferred to a 250 ml conical flask and each material was transferred in triplicate to a total of three different flasks with the precise mass content of each paper material recorded.

Buffer solution

The cellulase catalyzed degradation of waste paper is classified as a heterogeneous catalysis and the process needs to be performed at a pH-value allowing maximum degradation of waste cellulose materials by the cellulase enzyme. A pH 5.0, tris buffer solution was prepared by dissolving 1.2 g of tris in 2000 ml of distilled water and the pH adjusted with concentrated hydrochloric acid and potassium hydroxide (2%).

Cellulase solution

Crude *A. niger* (0.12 g) cellulase enzyme was weighed and dissolved in 50 ml of the tris buffer. Thecellulase enzyme-buffer solution was mixed with a magnetic stirrer until a homogenous solution was obtained at acellulase concentration of 2.0 $mg.ml^{-1}$. Aliquots from this cellulase stock solution were taken and mixed with the paper materials to degrade waste paper into glucose.

Glucose standard stock solution and DNS analyses

The concentration of sugars released from each waste paper material during degradation was determined from a standard glucose calibration curve by using the DNS method[16]. A standard glucose solution at concentration of 20.00 $mg.ml^{-1}$ was prepared. Samples were taken from the stock solution to prepare diluted glucose solutions at concentrations of 0.50 $mg.ml^{-1}$, 2.00 $mg.ml^{-1}$, 4.00 $mg.ml^{-1}$, 6.00 $mg.ml^{-1}$ and 8.00 $mg.ml^{-1}$.

Incubation mixture and sampling

The different wastepaper materials were bio-treated with cellulase from *A. niger* in order to degrade the cellulosic component of these waste cellulose materials into fermentable sugars, mainly glucose.

A mass (5.00 g) of each waste paper material was transferred in triplicate to conical flasks. Tris buffer (190.00 ml, pH 5.0) was transferred to each flask, with the pH of the reaction mixture measured at the beginning of the incubation and at regular intervals during the bio-conversion process. To prevent growth of microorganisms in the incubation mixture a volume of 10.00 ml acetonitrile was added to the content of each conical flask. The cellulase enzyme (10.00 ml of the stock solution) was added to each incubation container. The incubations were performed in a water bath at 40^0C during a period of 51 hours. Samples of 500 ul were taken on regular time intervals and kept in a refrigerator at 4^0C. All incubations were done in triplicate with three samples taken from each flask at a specific time interval. When all samples were collected the sugar content of each sample was determined with the DNS method. The pH-values of each incubation mixture was also recorded when samples were taken from the various flasks.

RESULTS AND DISCUSSION

Waste paper is a major component of the large volumes of organic waste produced annually by the global population[17]. Currently organic waste is dumped and is not considered for its potential to be bio-recycled into useful products. The cellulose content of organic waste is a suitable bio-polymer that could be hydrolyzed into glucose during the cellulase catalyzed bio-conversion of waste paper such as newspaper, office paper and foolscap paper which have already been reported[18]. Organic waste such as waste paper could be considered as a renewable energy resource and this initiative would not only address the negative effect of fossil fuels on the environment but could also limit the large amounts of organic waste. The complexity of cellulose in waste paper results from its interaction with lignin which requires that each type of waste paper should be studied individually for its potential to be developed as a resource of bio-energy. Eight different waste paper materials were treated separately with cellulase from *A. niger* during an incubation period of 51 hours with the pH-value of the incubation mixture regularly recorded.

When foolscap paper was degraded (Figure 1) the sugar production increased rapidly during the first 3 hours of cellulase catalyzed degradation. After 3 hours a 20% saccharification of this paper was calculated with the degradation slowly increased until a 25% saccharificationrate was calculated after 24 hours of enzyme treatment. No more increase of bio-degradation was observed during the rest of the incubation period that was terminated after 51 hours. The initial pH-value of 5.7 of the incubation mixture has increased gradually to a value of 7.0 after 48 hours of incubation. This pH was maintained for the next 3 hours of incubation until the end of the enzyme treatment.During the bio-treatment of office paper (Figure 2) with cellulase from *A. niger* the maximum degree of saccharification of 16.2% was obtained after 24 hours of incubation. This maximum saccharification was 35% less than the maximum saccharification of foolscap paper.The maximum sugar production was 38% higher than the sugar produced after 3 hours of degradation of this paper. The incubation pH during the saccharification of office paper changed from the initial pH 5.5 to pH 7.2 after 27 hours of incubation. This pH-value was maintained during the rest of the incubation period. Newspaper (Figure 3) showed a relative low susceptibility for degradation by cellulase from *A. niger*. Maximum saccharification at 4% was reached after 27 hours of saccharification that was 60% higher than the 2.5% saccharification obtained after 3 hours of incubation. The maximum degradation of foolscap paper was 525% higher than the maximum amount of sugar produced by newspaper. The pH of the incubation mixture when newspaper was degraded changed from pH 5.0 to pH 6.9 after 27 hours of incubation and this pH-value was maintained for the rest of the incubation period.

The pH-value of the incubation mixture when Woolworthsadvertising paper (Figure 4) was degraded changed from pH 5.5 to pH 7.0 after 24 hours of incubation. This pH-value was maintained for the rest of the incubation period. The saccharification of this paper material also increased rapidly during the first 3 hours of the incubation resulting in a paper bio-conversion of 15% saccharification. Maximum degradation was achieved after 24 hours of incubation that resulted in a 20% saccharification of Woolworths advertising paper. This maximum saccharification was 30% higher than the amount produced after 3 hours of enzyme treatment. The maximum degree of sugar formation from Woolworths paper was 25% less than sugars produced from foolscap and 19% more than obtained from office paper while a 400% higher value than the maximum amount of sugars released from newspaper was calculated.

From all the paper materials treated with cellulase from *A. niger* the saccharification of brown envelope paper (Figure 5) produced the highest rate of saccharification at 29% that was reached after 24 hours of incubation. After 3 hours of incubation a 18% saccharification was obtained that was 61% less than the maximum rate of degradation. Brown envelope produced 625% more sugar than newspaper that showed the lowest degree of saccharification of all waste paper materials. The pH–value of the incubation mixture during the saccharification of brown envelope paper changed from pH 5.1 to pH 7.2 after 48 hours. Card board **(**figure 6) together with foolscap paper showed the second highest degree of saccharification at 25% that was reached after 48 hours of incubation. An initial high rate of sugar formation was also produced during the first 3 hours of incubation which resulted in a 12% saccharification after 3 hours. Other than the rest of the paper materials a relative high degree of sugar formation during the next 45 hours was calculated. The maximum saccharification was achieved after 48 hours of bio-treatment with the initial pH of 5.2 increased rapidly to a value of pH 6.8 after 3 hours of incubation that slowly increased to pH 7.0 during the rest of the incubation period.

In contrast to the rest of the paper materials paper towel (Figure 7) showed an initial increased sugar formation during the first 24 hours. During the last part of the incubation period the sugar formation increased to a maximum of 26% saccharification after 48 hours. The maximum saccharification of this paper was 20% higher than the sugars produced after 24 hours of the incubation. The pH-value changes from pH 5.3 to pH 5.7 when maximum bio-degradation took place. The maximum saccharification of Pick n Pay (Figure 8) advertising paper was also relatively low and was 27% higher than newspaper that showed the highest resistance towards *A. niger* cellulase degradation. The initial increase in sugar production resulted in a 3.2% degradation after 3 hours of incubation with the maximum degree of saccharification 60% higher than the amount of sugars produced after 3 hours. The pH of this incubation mixture changed from a value of pH 5.4 to pH 7.0 during the first 27 hours. The minimum time for maximum saccharification was 24 hours as observed with foolscap paper, office paper, Woolworth's paper and brown envelope paper. After 27 hours of incubation maximum saccharification was obtained from newspaper and Pick n Pay paper. The longest incubation period of 48 hours was needed for maximum saccharification of cardboard and paper towel (Table 1). As observed with incubation mixtures the pH values increased during the incubation period and changed between an initial value of pH 5.7 and a maximum value of pH 7.2.

The relative susceptibility of the various waste paper materials bio-converted into fermentable sugars by *A. niger* cellulase can be concluded from the sugar concentration produced by each paper material at the end of saccharification (Table 1) as well as degradation during the initial 3H of enzyme treatment (Table 2). From the sugar concentration it could be concluded that brown envelope paper was the most degraded followed by paper towel, foolscap paper, cardboard, Woolworth's paper, office paper, paper from Pick n Pay with newspaper showing the lowest degree of degradation. The initial rate (3H) of sugar formation reflects an almost similar order of decreasing susceptibility with brown envelope paper exhibiting the highest susceptibility followed by foolscap paper, paper towel, office paper and Woolworth's paper with cardboard exhibiting the 3rd lowest amount of sugar formation. Pick n Pay paper produced the second lowest amount of sugar with the lowest amount of sugar released from newspaper.

The bio-conversion of waste cellulose into glucose can be achieved with cellulase a multi-component enzyme system. The degradation of waste cellulose is a complicated procedure, not due to its chemical structure but also due to the interaction between cellulose and other bio-polymers such as hemi-cellulose and lignin. The complexity of the saccharification process is further illustrated by different degradation profiles obtained when eight different waste cellulose materials were bio-converted by cellulose from *A. niger*. A common observation was the increase in the pH value of all incubation mixtures during the saccharification of the various waste paper materials. The rate of sugar formation during the initial period of 3h is indicated in table 2. From these calculations it can be concluded that brown envelope paper showed the highest initial rate of cellulose bio-conversion at a rate of 0.033 mg.ml.min^{-1} followed by foolscap paper at a rate of 0.025 mg.ml.min^{-1}. The lowest rate of sugar formation during this initial stage of bio-conversion was observed with newspaper at a rate of 0.003 mg.ml.min^{-1}. The unique susceptibility

of each type of waste paper is well concluded by this investigation showing brown envelope paper to be the most susceptible for degradation and newspaper the least when exposed to the cellulase from *A. niger*.

The search for raw materials to be developed as resources for bio-technological development of bio-fuels, bio-chemicals and bio-pharmaceuticals is currently investigated by numerous scientists. Waste lignocellulosic materials appear to be a promising substance due to its potential to be hydrolyzed into fermentable sugars[19]. Waste paper has been identified as a suitable lignocellulosic material which instead of being dumped as solid waste or burned could be developed as a resource of bio-energy. Cardboard[20], office paper[12] and newspaper[8] are amongst the most popular waste papers that are currently investigated as a potential resource of bio-energy. Besides acid catalyzed hydrolysis, waste cellulose can also be degraded with a cellulase enzyme system that is an important bio-chemical part of many bacterial and fungal organisms[13]. Lignocellulosic material consists of mainly three different types of polymers, namely cellulose, hemicellulose and lignin which are associated with each other[21]. Different waste paper materials exhibit chemical interaction of different strength between cellulose chains and lignin resulting in non-similar extents of sugar production when treated with cellulose enzymes. In order to achieve maximum cellulose bio-conversion different degradation procedures should be design for various waste cellulose materials and variables that should be optimized for each substance include, type of cellulase enzyme[22], incubation pH and temperature[23], cellulase loading[24], as well as pretreatment[25] of cellulose.

Fig. 1: Saccharification of foolscap paper by cellulase from *A. niger* and change in the pH-values of the incubation mixture during the bioconversion process

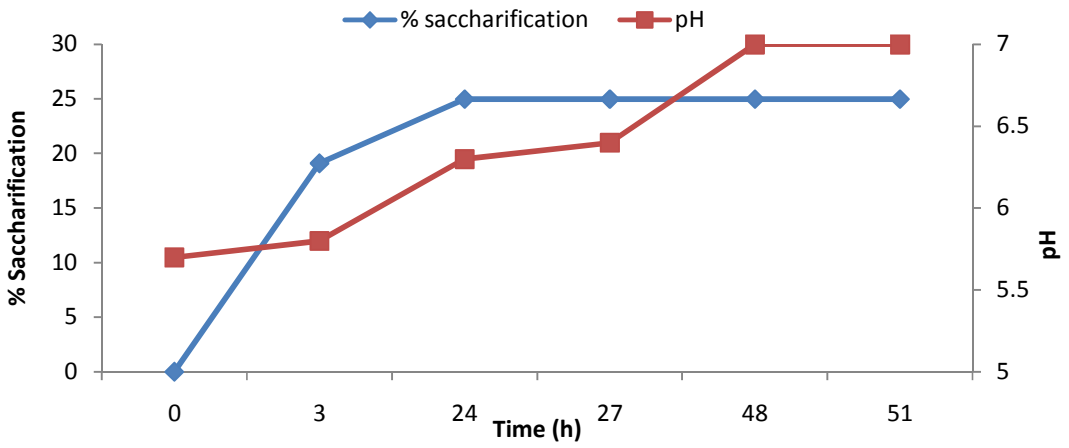

Fig. 2: Saccharification of office paper by cellulase from *A. niger* and change in the pH-values of the incubation mixture during the bioconversion process

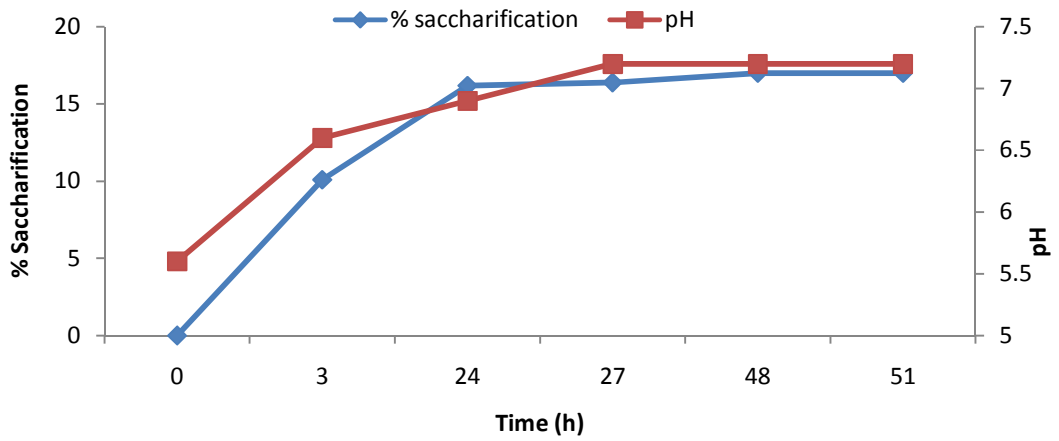

Fig. 3: Saccharification of newspaper by cellulase from *A. niger* and change in the pH-values of the incubation mixture during the bioconversion process

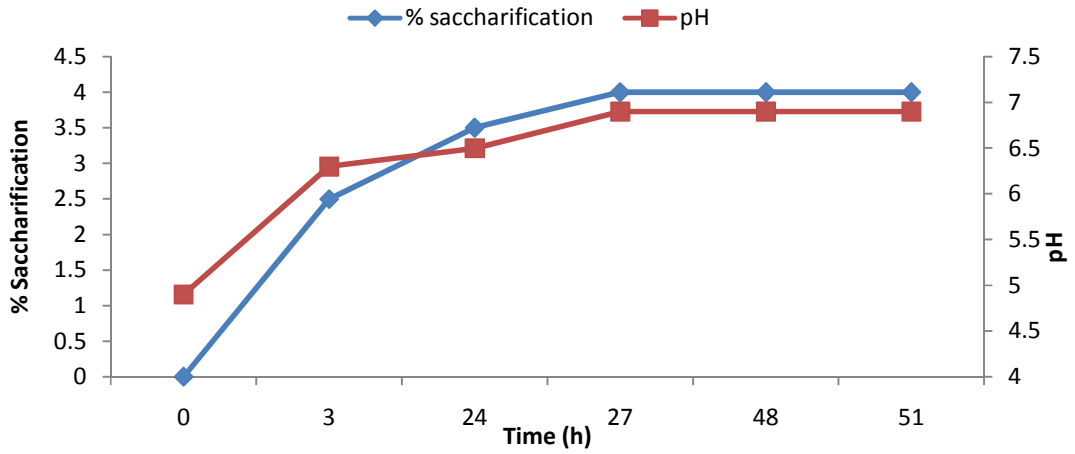

Fig.4: Saccharificationof Woolworths paper by cellulase from *A. niger* and change in the pH-values of the incubation mixture during the bioconversion process

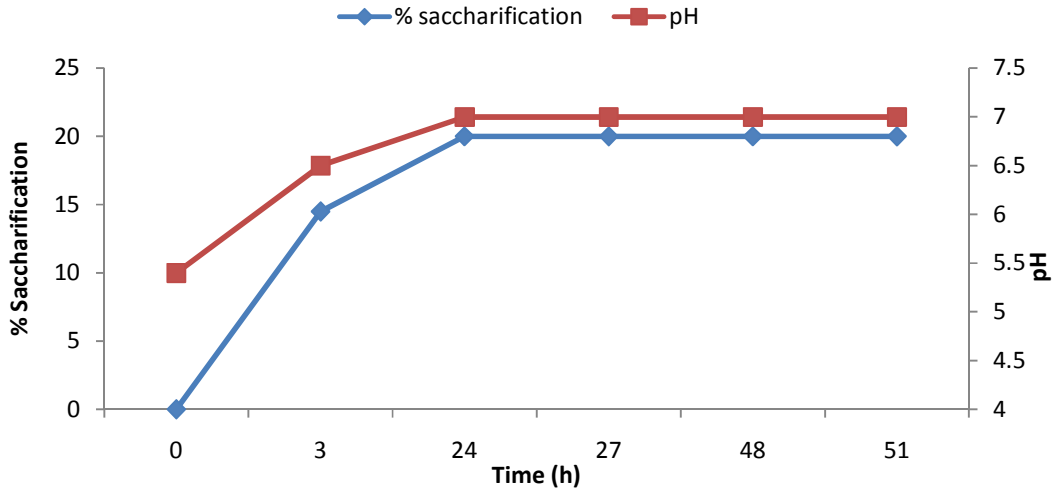

Fig. 5: Saccharification of brown envelope by cellulase from *A. niger* and change in the pH-values of the incubation mixture during the bioconversion process

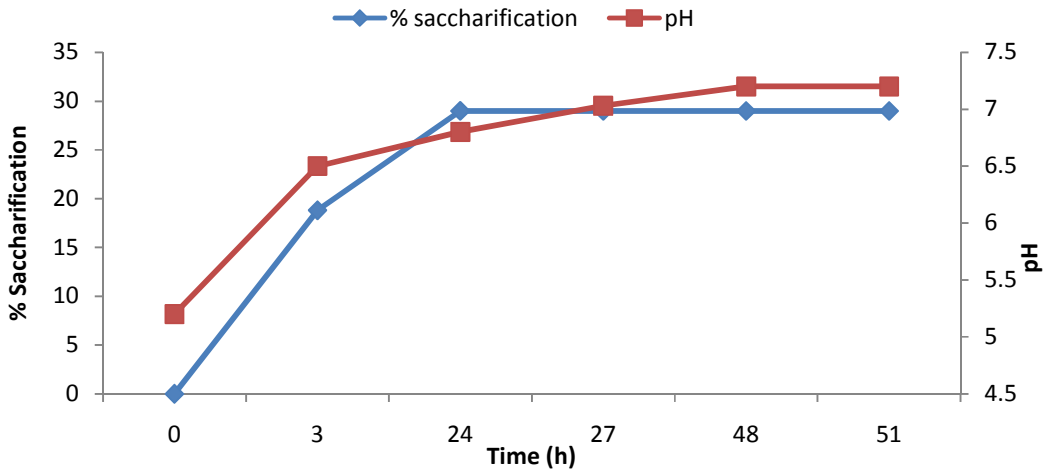

Fig. 6: Saccharification of card board by cellulasefrom *A. niger* and change in the pH-values of the incubation mixture during the bioconversion process

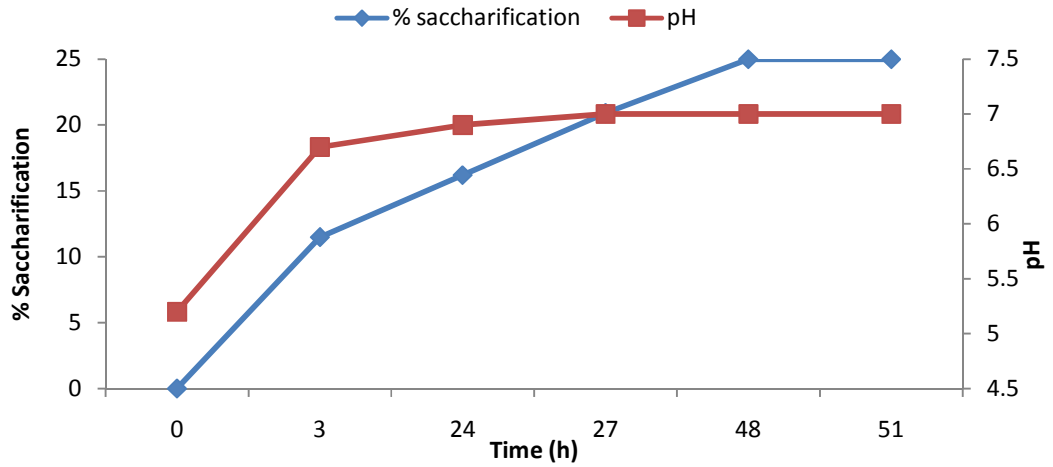

Fig. 7: Saccharification of paper towel by cellulase from *A. niger* and change in the pH-values of the incubation mixture during the bioconversion process

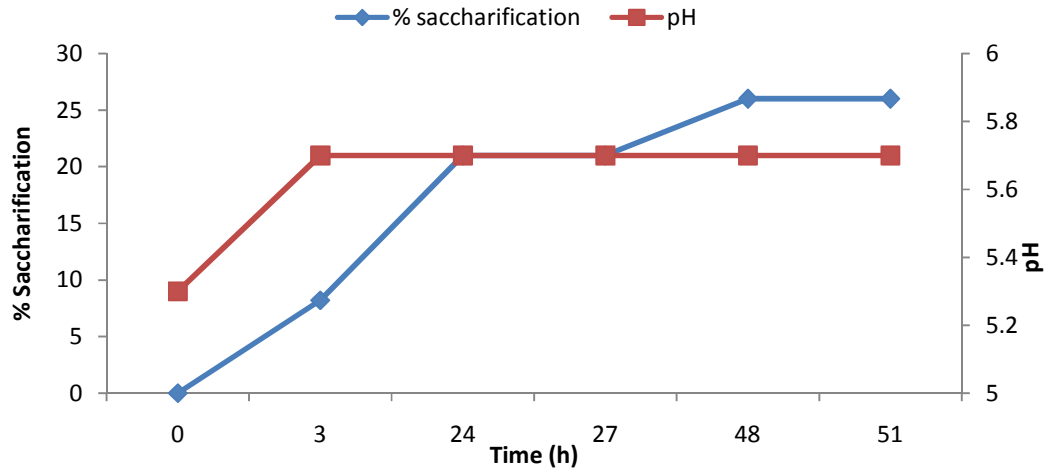

Fig. 8: Saccharificationof Pick n Pay paper by cellulase from *A. niger* and change in the pH-values of the incubation mixture during the bioconversion process

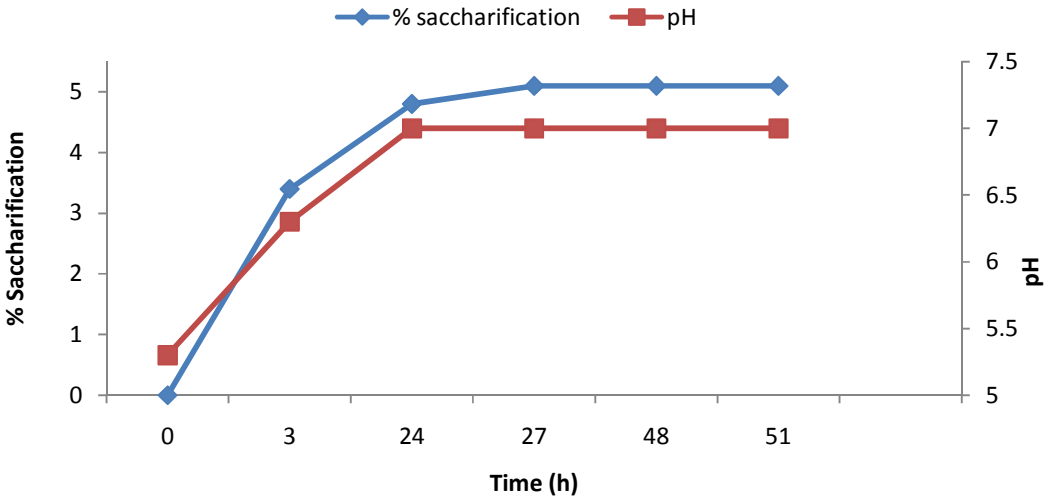

Table 1: The relative and maximum saccharification of different waste paper materials

Type of paper	Time for maximum saccharification (h)	pH at maximum saccharification	Maximum saccharification (%)
Foolscap paper	24	6.3-7.0	25
Office paper	24	6.9-7.2	16.2
Newspaper	27	6.9	4
Woolworths	24	7.0	20
Brown envelope	24	6.8-7.2	29
Cardboard	48	7	25
Pick n Pay	27	7.0	5.1
Paper towel	48	5.7	26

Table 2: The rate of sugar formation from different waste paper materials during the initial phase of bio-conversion with cellulase from *A. niger*

Type of paper	Sugar concentration ($mg.ml^{-1}$)	Rate of sugar formation ($mg.ml.min^{-1}$)
Office paper	2.31	0.018
Newspaper	0.51	0.003
Foolscap	4.41	0.025
Woolworths	3.2	0.018
Brown envelope	5.93	0.033
Cardboard	2.45	0.014
Paper towel	3.73	0.021
Pick n Pay	0.85	0.005

CONCLUSIONS

From this study it was concluded that different waste paper materials showed different rates of saccharification with brown envelope paper maximally bio-converted with newspaper showing the lowest degree of saccharification. All waste paper materials showed an initial strong increase in sugar formation during the first 3 hours of enzyme treatment. The unique susceptibility of each type of waste paper is well concluded by this investigation showing brown envelope paperto be the most susceptible for degradation and newspaper the least when exposed to the hydrolytic action of cellulase from *A. niger.*

The accumulation of waste paper as a major component of solid waste could be limited if considered and treated as a resource for bio-energy development. Although not yet optimized the bio-development of waste cellulose as a resource of bio-energy should become more topical as the need for alternative renewable energy resource are recognized by the global scientific community. From this investigation it was also concluded that an unique bio-conversion process should be designed for each waste paper material during degradation with cellulase enzymes.

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