



Cellulase catalyzed bioconversion of different waste paper materials into fermentable sugars

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Abstract

The search and development of alternative and renewable energy resources are issues that should become more topical as the negative effect of fossil fuels on the environment is experienced. Also of environmental importance is the management of huge volumes of solid waste produced annually. Waste paper is the major component of organic solid waste and during this investigation the relative saccharification of eight different waste paper materials with cellulase from *Trichoderma viride* has been concluded. Different sugar releasing patterns have revealed the difference in susceptibility of different organic waste materials for cellulase catalyzed bioconversion into sugars. The highest extent of degradation was observed with brown envelope paper followed by cardboard while the least susceptibility for this process of degradation was experienced with newspaper. The pH value of all incubation mixtures changes between values of pH 5.0 to pH 7.0 during the 51 hours of cellulase catalyzed bioconversion.

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Introduction

The accumulation of excess volumes of solid waste such as waste paper and the search for clean energy are topical global issues. The aim of this study was to investigate the degradation of various waste paper materials into fermentable sugars such as glucose by hydrolyzing the cellulose content of each paper material with cellulase enzymes. Cellulase is a hydrolytic enzyme isolated from micro-organisms such as *Trichoderma viride*. Glucose obtained during the saccharification of cellulose could be fermented into bioethanol, a process that is essential for the developed of bioenergy that is clean form of energy. The process will also limit the amount of global wastepaper produced annually. Waste paper is a well-known potential resource of bioenergy and the aim of this investigation was to compare the relative saccharification of different wastepaper materials, a process that would be of future importance during further investigations to optimize the bioconversion of wastepaper into sugars.

Currently the global population is confronted with two major environmental issues that could have a drastic effect on the quality of life as is known to human kind. The effect of climate change as a result of extensive air pollution has not yet been fully realized by all communities of the globe (Ekholm *et al.*, 2014). Similar is the concerning effect of the accumulation of huge volumes of non-biodegradable as well as biodegradable waste materials especially in highly densely populated areas. Solid waste does not only occupy valuable land but also contributes largely towards environmental pollution (Meizah *et al.*, 2015). Most scientists agree that to prevent a catastrophe something needs to be done to limit environmental pollution produced by fossil fuels and fossil based substances (Chandra *et al.*, 2012).

An analysis of the amount solid waste produced annually revealed that waste paper is a major component of solid waste materials (Kumar and Singh, 2013). Also contributing to solid waste are other organic waste materials such as garden waste, kitchen waste and agricultural waste (Wani and Rao,

2013). All these waste materials are classified as organic waste as they are derived from plant materials. These organic waste substances are also described as lignocellulosic materials that comprise of about 10% - 25% lignin, 20% - 30% hemicellulose and 40% - 50% cellulose (Igbal *et al.*, 2011; Irshad *et al.*, 2013). Cellulose present in renewable lignocellulosic materials is considered to be the most abundant organic substrate on the earth that could be developed as a chemical feedstock (Krishna, 1999). Cellulose is a branched glucose biopolymer composed of glucose units linked by 1,4- β -D-glucosidic bonds (Kumar *et al.*, 2009). The destruction of cellulose into fermentable sugars can be achieved by acid hydrolysis as well as enzymatic catalyzed hydrolysis. Enzymatic hydrolysis is however more preferred because it produces fewer bi-products and proceeds under milder conditions (Taherzaden and Karimi, 2007). The hydrolysis of cellulose can be achieved by a multicomponent enzyme system known as cellulase which could be of bacterial (Yang *et al.*, 2011; Maki *et al.*, 2009) or fungal origin (Sanaa *et al.*, 2014; Maki *et al.*, 2009). The degradation of cellulose into reducing sugars is however hampered by a number of variables such as the heterogeneous nature of the catalytic process (Van de Vyver *et al.*, 2011), crystalline nature of cellulose (Joeh *et al.*, 2007) and the type of cellulase (Dashtban *et al.*, 2010) used during the saccharification process. Chemical (Swatloski *et al.*, 2002) or physical (Agbor *et al.*, 2011) pretreatment of the cellulose material is often applied to render cellulose more susceptible for enzyme catalyzed degradation.

Waste lignocellulosic materials are mostly treated like normal solid waste by which it is dumped (Mtui, 2009), incinerated (Barrett and Lawlor, 1995) or used as a source for "informal burning" (Ofori-Boateng *et al.*, 2013). Different waste paper materials are available according to their specific need and the recycling of used paper is an option to deal with waste paper but the process can only be repeated a few times whereafter paper loses its characteristics that is required for a good quality paper. Most waste paper becomes part of organic solid waste and as a result its

potential to be developed as a renewable chemical feedstock or a bioenergy resource is neglected.

During this investigation the relative saccharification of eight different waste paper materials treated with cellulase from *Trichoderma viride* was concluded. The change in the pH value of the various incubation mixtures was also recorded during an incubation period of 51 hours. Waste paper materials used during this investigation included foolscap paper, office paper, cardboard, newspaper, paper towel, brown envelope paper as well as advertising papers from the retailers Woolworths and Pick-n-Pay.

Materials and methods

Waste paper materials

Various waste paper materials such as office paper, newspaper, brown envelope paper, cardboard, foolscap paper, and 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 bioconversion of waste paper into glucose is classified as a heterogeneous catalytic process that needs to be performed at an optimum pH-value allowing maximum degradation of waste cellulose materials by the cellulase enzyme. A tris buffer solution (pH 5.0) was prepared by dissolving tris (1.2 g) in distilled water (2000 ml). The pH of the buffer solution was adjusted with concentrated hydrochloric acid and potassium hydroxide (2%).

Cellulase solution

Crude *T. viride* (0.12 g) cellulase enzyme was weighed and dissolved in 50.0 ml of the tris buffer. The cellulase enzyme-buffer solution was mixed with a magnetic stirrer until a homogenous solution was obtained at a cellulase concentration of 2.00 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 with the *T. viride* cellulase was calculated from a standard glucose calibration curve, using the method of Miller (1959). Samples from a standard glucose solution (20 mg.ml⁻¹) were taken and diluted to prepare 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. These diluted sugar solutions were used to construct a calibration curve.

Incubation mixture and sampling

The various waste paper materials were bio-treated with cellulase from *T. viride* 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 Erlenmeyer 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 saccharification process. To prevent microbial growth in the incubation mixture a fixed volume of methanol (10.00 ml) was added to the flask content. The cellulase enzyme (10.00 ml of the stock solution) was added to each incubation container and the incubations were performed in a water bath at 40°C during a period of 51 hours. Samples of 500 µl were taken on regular time intervals and kept in a refrigerator at 4°C. 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

Environmental benign biocatalytic processes to

convert lignocellulosic biomass into fermentable sugars suitable for conversion into bioethanol is a topical endeavor involving many scientists. The aim

of this bioconversion procedure is to limit the negative effect of fossil fuels on the environment (Dincer and Zamfirescu, 2014).

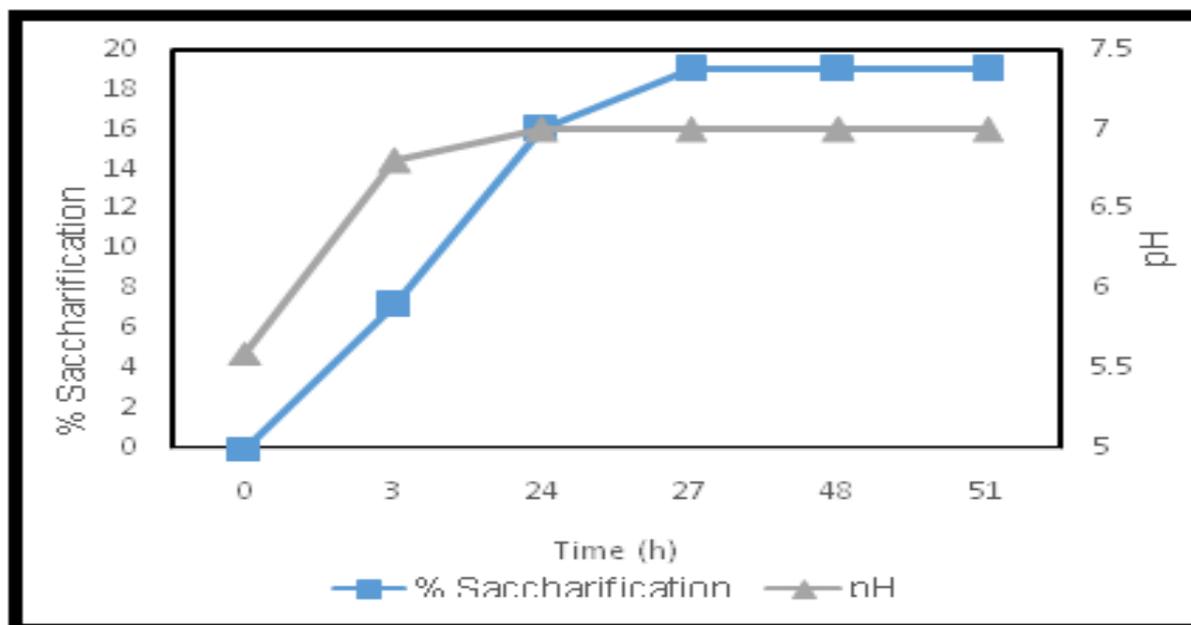


Fig. 1. Saccharification of office paper by cellulase from *T. viride* and change in the pH-values of the incubation mixture during the bioconversion process.

Waste paper is part of lignocellulosic waste and its accumulation is a major problem in many countries such as China (Zhao *et al.*, 2013), India (Zhao *et al.*, 2014), UK (Cesaro and Belgiorna, 2014) and also most countries in Africa (Tonn, 2002). Cellulase enzymes are described as suitable biocatalysts that could be used for the effective bioconversion of lignocellulosic waste such as waste paper into fermentable sugars and reports on the bioconversion of newspaper (Van Wyk *et al.*, 1999), office paper (Ikeda *et al.*, 2006) and cardboard (Van Wyk and Mohulatsi, 2003) have already been described. Of major importance is the relative saccharification of different waste paper materials by cellulase enzymes from different organisms. Information obtained could be used to design a waste cellulose bioconversion procedure to be performed by a specific cellulase enzyme on a specific organic waste substance. The current investigation relates the relative saccharification of eight different waste paper materials while degraded with cellulase from *T. viride*.

During the bio-treatment of office paper (Figure 1) with cellulase from *T. viride* the maximum degree of saccharification of 19% was obtained after 27 hours of incubation. The degree of saccharification did not change for the rest of incubation period. Maximum saccharification was 171% higher than the initial rate of 7% saccharification after 3 hours of incubation. The incubation pH during the saccharification of office paper changed from the initial pH-value of 5.60 to 7.04 after 24 hours of incubation and this pH-value was maintained until the end of the saccharification process. When newspaper was degraded (Figure 2) the sugar production increased gradually during the first 3 hours of cellulase catalyzed degradation. After 27 hours a 20% saccharification of this paper was calculated with the degradation slowly increasing until a 21% saccharification rate was confirmed after 48 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. Maximum degradation of newspaper was 425% higher after initial saccharification rate of 4% that was obtained than the first 3 hours of

incubation. The initial pH-value of 5.20 was maintained during the complete cellulase catalyzed saccharification period.

Pick-n-Pay advertising paper (Figure 3) showed a relatively low susceptibility during degradation by cellulase from *T. viride*, with a maximum rate of 17% saccharification reached after 27 hours of incubation.

Maximum saccharification of this paper was 193% higher than the 5.8% saccharification obtained after 3 hours of enzyme treatment. The pH-value of the incubation mixture when Pick-n-Pay advertising paper was degraded changed from pH 5.4 to pH 7.0 after 24 hours of incubation and this pH-value was maintained for the rest of the incubation period.

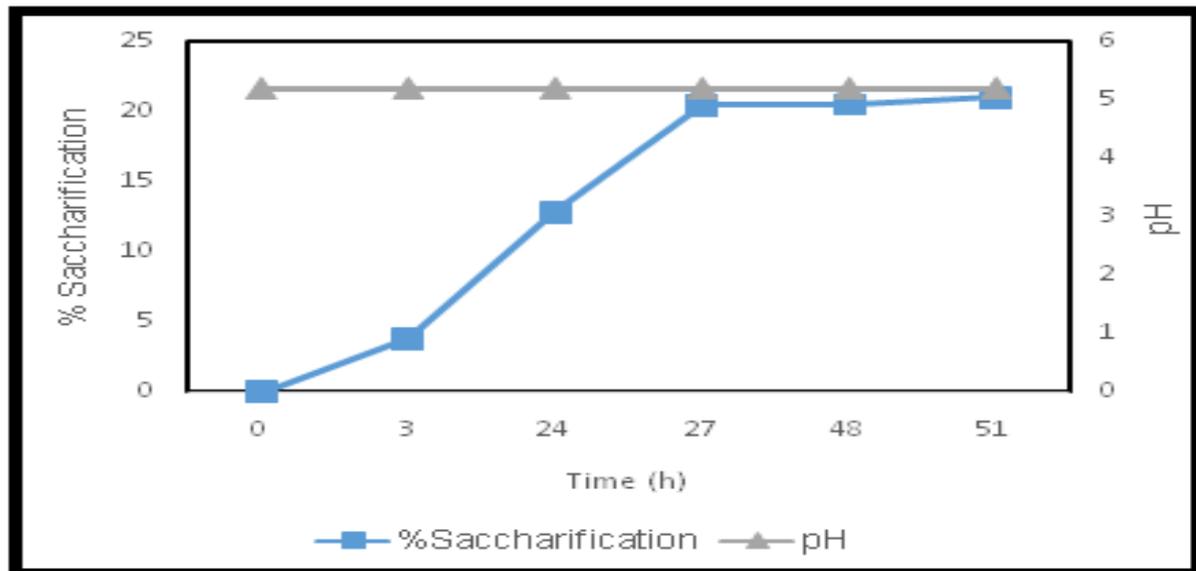


Fig. 2. Saccharification of newspaper by cellulase from *T. viride* and change in the pH-values of the incubation mixture during the bioconversion process.

The pH-value of the incubation mixture when foolscap paper (Figure 4) was degraded changed from pH 5.5 to pH 7.3 after 24 hours of incubation, a value that did not change during the rest of the incubation period. The saccharification of this paper material increased rapidly during the first 3 hours of incubation resulting in a sugar concentration of 14% saccharification. Maximum degradation was achieved after 24 hours of enzyme treatment that resulted in 26% saccharification of foolscap paper and this degree of degradation was 86% higher than the sugar concentration produced after 3 hours of enzyme treatment.

Brown envelope paper, of all bioconverted waste paper materials degraded with *T. viride* cellulase (Figure 5) produced the highest rate of saccharification at 28% that was reached after 27 hours. Saccharification of brown envelope paper also

increased rapidly during the first 3 hours of degradation that resulted in a saccharification rate 13.7%. This maximum saccharification was 18.9% higher than the amount of sugar produced after 3 hours of cellulase catalyzed degradation. Brown envelope paper produced 65% more sugar than Pick-n-Pay advertising paper that showed the lowest degree of saccharification of all waste paper materials degraded. The initial pH-value changed from pH 5.6 to pH 7.5 after 24 hours of enzyme action. Papertowel (Figure 6) showed the second highest degree of saccharification at a rate of 27% saccharification that was reached after 27 hours of incubation. An initial relative high rate of sugar formation was also produced during the first 3 hours of incubation which resulted in a 16% saccharification after 3 hours. The initial pH of 5.3 was maintained during the complete saccharification period.

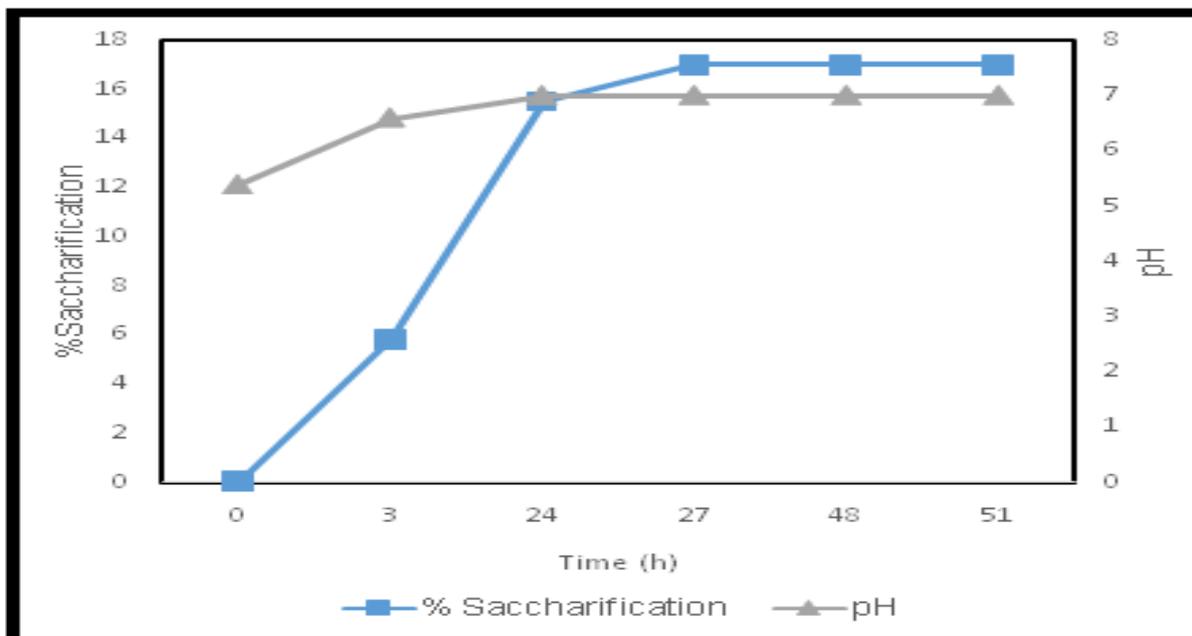


Fig. 3. Saccharification of Pick n Pay by cellulase from *T. viride* and change in the pH-values of the incubation mixture during the bioconversion process.

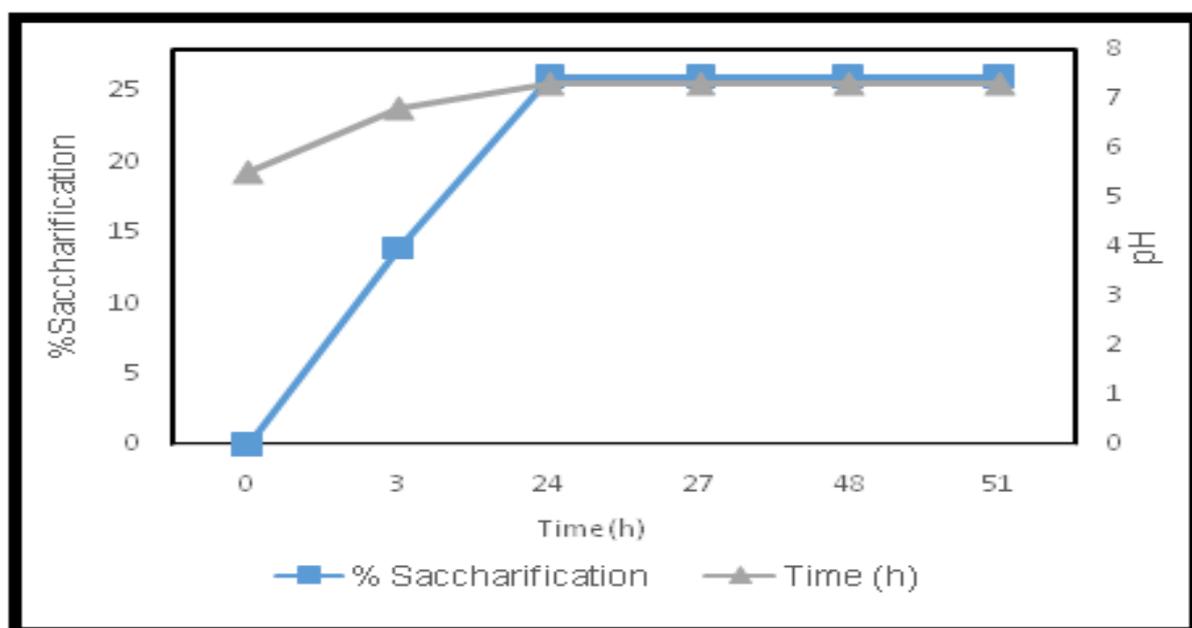


Fig. 4. Saccharification of foolscap by cellulase from *T. viride* and change in the pH-values of the incubation mixture during the bioconversion process.

The initial increase in sugar production from cardboard (Figure 7) resulted in a 13% saccharification that was reached after 3 hours of cellulase treatment with the maximum degree of saccharification of 26% recorded after 27 hours of degradation. The maximum degree of saccharification was 100% higher than the amount of sugars produced after 3 hours. The pH of this incubation mixture

changed from pH 5.4 to pH 7.2 during the first 3 hours with no pH change observed during the rest of incubation period. A maximum saccharification of 17.5% was obtained after 24 hours during the degradation of Woolworths paper (Figure 8). This maximum saccharification was 130% higher than the initial value of 5.8% saccharification. The maximum degradation of Woolworths advertising paper was 3%

higher than the maximum amount of sugar produced from Pick- n-Pay advertising paper. The pH-value increased rapidly from a value of pH 5.4 to pH 7.1

during the first 3 hours, with no change in pH-value during the rest of incubation period.

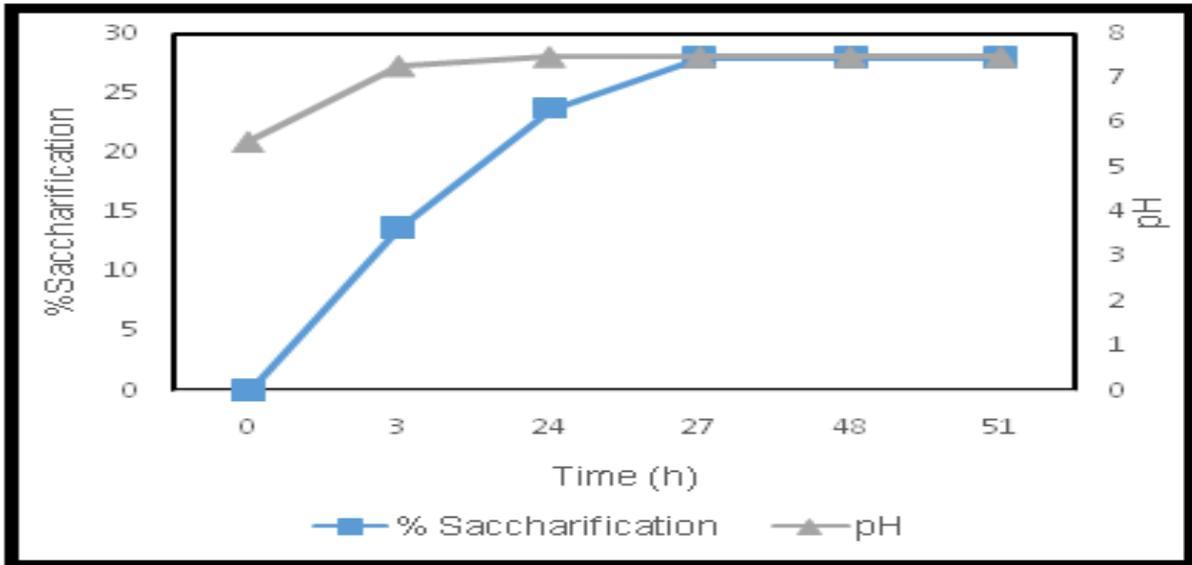


Fig. 5. Saccharification of brown envelope paper by cellulase from *T. viride* and change in the pH-values of the incubation mixture during the bioconversion process.

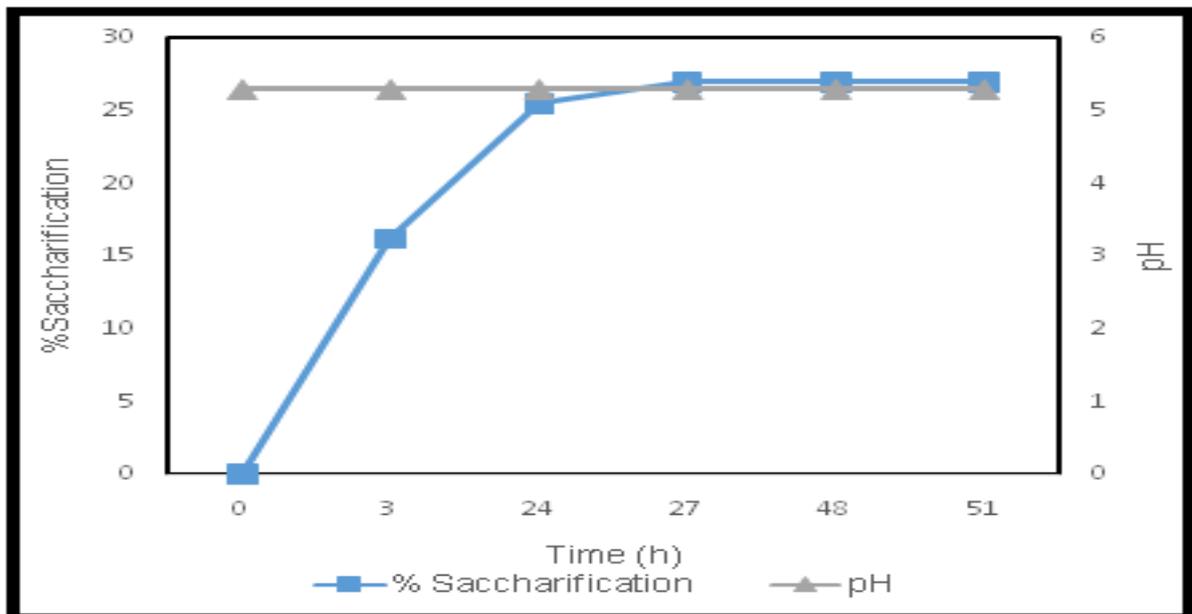


Fig. 6. Saccharification of papertowel by cellulase from *T. viride* and change in the pH-values of the incubation mixture during the bioconversion process.

The minimum time for maximum saccharification was 27 hours as observed with cardboard, brown envelope paper, foolscap paper, paper towel and office paper. After 24 hours of incubation maximum saccharification was obtained from foolscap paper. The longest incubation period of 51 hours was needed

for maximum saccharification of newspaper. The pH-values increased during the incubation period and changed between an initial value of pH 5.1 and a maximum value of pH 7.5 for all waste paper materials.

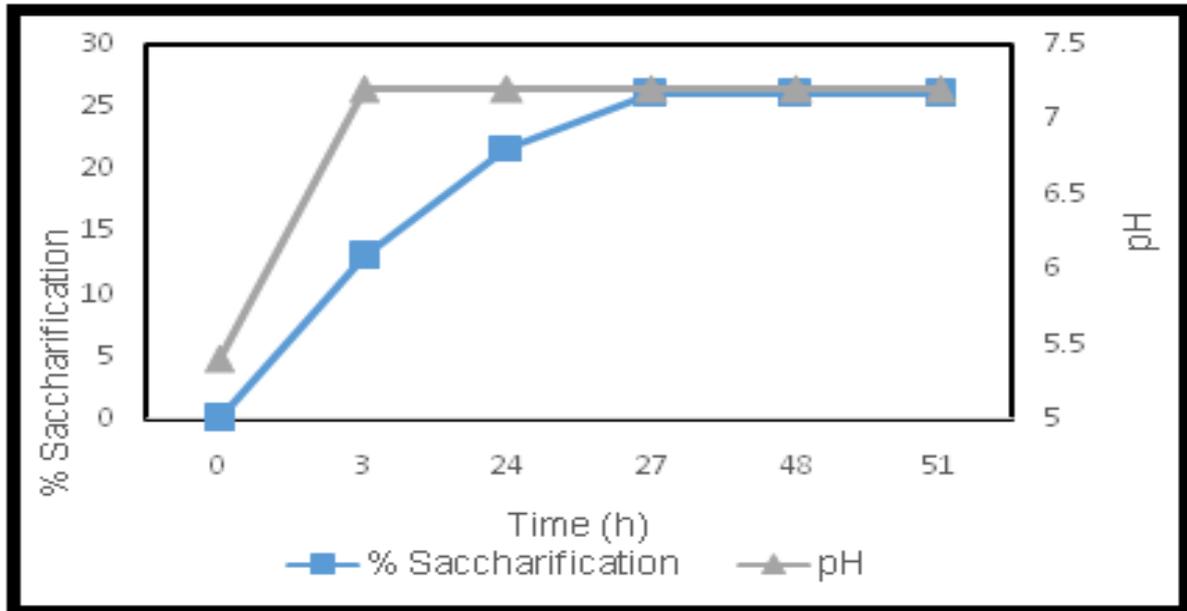


Fig. 7. Saccharification of cardboard by cellulase from *T. viride* and change in the pH-values of the incubation mixture during the bioconversion process.

Waste to energy is a concept that would become more topical in the future as the negative effect of fossil fuel consumption on the environment is realized (Hasanbeigi and Price, 2015). Certain countries such as Sweden (Lucia and Ericsson, 2014) is already majorly dependent on the burning of solid waste for their energy demands. The effective use of liquid bioenergy such as bioethanol is well developed and successfully used in Brazil (Singh *et al.*, 2016). Certain types of wastepaper have also been investigated by numerous scientists as a possible

resource for bioenergy purpose. An optimized cellulase catalyzed bioconversion process of waste cellulose into fermentable sugars needs to be finalized and this process would not only address the issue of fossil fuels but will also limit the amounts of accumulated organic solid waste. The cellulase catalyzed bioconversion of waste cellulose into sugars could also be applied to other forms of organic waste such as kitchen waste, garden waste and agricultural waste.

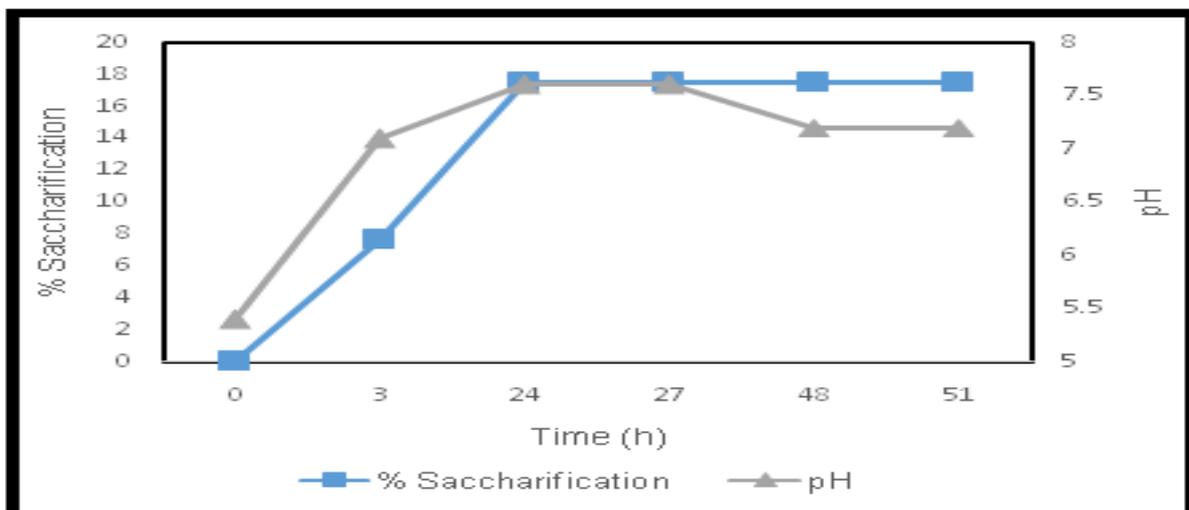


Fig. 8. Saccharification of Woolworths paper by cellulase from *T. viride* and change in the pH-values of the incubation mixture during the bioconversion process.

Conclusion

From this study it was concluded that different paper materials showed different rates of saccharification when degraded with cellulase from *T. viride* with brown envelope paper maximally bio-converted. Wastepaper due to its cellulose component exhibits the ability to be developed as a resource of bioenergy. An optimized bioconversion procedure could also be applied during the bioconversion of other types of organic solid waste. Another environmental advantage of the bioconversion process would be to limit the accumulation of solid waste materials on valuable land.

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