European Journal of Biotechnology and Bioscience

E J B B

Volume: 3, Issue: 8, 44-46
Aug 2015
www.biosciencejournals.com
ISSN: 2321-9122
Impact Factor: 3.742

K. Mashadi P. Mokatse
Department of Pharmacology and Therapeutics, Sefako Makgatho Health Sciences University, South Africa

J. Pieter H. van Wyk
Department of Pharmacology and Therapeutics, Sefako Makgatho Health Sciences University, South Africa

Correspondence:
K. Mashadi P. Mokatse
Department of Pharmacology and Therapeutics, Sefako Makgatho Health Sciences University, South Africa.

Relative Saccharification of Various Waste Paper Materials by Cellulase from *Aspergillus niger*

K. Mashadi P. Mokatse, J. Pieter H. van Wyk

Abstract
Waste paper is a major component of organic solid waste with cellulose, composed of numerous glucose units, a structural component of waste paper. Cellulase a hydrolytic enzyme can hydrolyse cellulose into glucose thus initiate a possibility of developing cellulose as a renewable energy resource. Eight different waste paper materials have been biodegraded into glucose with cellulase from *A. niger*. Non-pretreated paper materials as well as pulped paper materials were treated with the cellulase enzyme system. Pulping proved to be an effective physical pretreatment method for increased cellulose catalyzed degradation of cellulose associated with waste paper. It was also shown that different waste papers released different amounts of sugar during cellulase catalyzed saccharification.

Key words: Waste paper, Cellulose, Cellulase, Saccharification, Pretreatment

1. Introduction

Large volumes of solid waste produced annually is currently a major concern for many countries and especially to communities with limiting land available to manage post utilized materials. An analysis of solid waste has revealed that the organic part constitutes a major section of used materials [1]. Included in organic waste are substances such as waste paper, kitchen-, garden-, agricultural- and food waste. A major structural component of these organic materials is cellulose a biodegradable glucose biopolymer. If hydrolysed into glucose waste cellulose materials could be developed as a potential resource for bioenergy or bioproduct development [2, 3].

Cellulose with a complex chemical structure could be hydrolysed by cellulase a multicomponent enzyme system into fermentable sugars such as glucose [4, 5]. The enzyme catalyzed degradation of cellulose is however hampered by a structural feature of cellulose known as the crystalline section. Various pretreatment methods such as milling, heat and pulping could be used to make cellulose more susceptible for cellulase catalyzed degradation into sugars [6, 7].

Waste paper contributes largely to organic waste and can only be recycled for a number of times before it is managed as solid waste. Current management procedures of waste paper indicate that these materials are either dumped or burned with both procedures contribute largely to environmental pollution [8, 9]. The cellulase catalyzed bioconversion of the cellulose content of waste paper has already been investigated and papers that have been investigated to be developed as a renewable energy resource include office paper, newspaper and filter paper [10].

Cellulase from various microorganisms have been implicated in the saccharification of different waste cellulose materials such as agricultural waste, waste paper and garden waste. To increase the extent of sugar formation from waste cellulose, treatment with different cellulase cocktails have also been introduced in the bioconversion process. The latest technology suggests the use of more effective cellulase enzymes [11].

Currently waste paper, a potential bioenergy resource, is considered as waste and if correctly treated it could be developed as a resource for bioproduct development with a less negative effect on the environment. During this investigation eight different waste paper materials were treated with cellulase from *Aspergillus niger* in order to biohydrolysed their cellulose content into sugars such as glucose. To increase the sacharification the various paper materials were pulped prior to enzymatic treatment. The amount of sugars produced before and after pulping of these paper materials were determined and the relative increase in sugar formation calculated.

2. Materials and Methods

2.1 Materials, Incubation Procedure and Sugar Determination

The paper materials used during this investigation were, foolscap paper, office paper, paper towel, cardboard, newspaper, brown envelope paper as well as advertising paper from retailers, Woolworths and Pick n Pay. These materials were prepared as circular discs with a diameter of 6.0 mm. Each material (0.05 g) were transferred in triplicate into glass test tubes. A volume of 5.0 cm^3, 0.5 $mol.dm^{-3}$ Tris buffer pH 5.0 was transferred to each test tube. A crude cellulase solution was prepared at a concentration of 2.0 $mg.cm^{-3}$ and 1.0 cm^3 of this enzyme solution was transferred to each test tube containing the paper materials. The test tubes with their content were incubated at 50 °C during a period of 2 h. After the incubation period 1500 cm^3 of the DNS reagent [12] was transferred into each test tube and heated in a boiling water bath for a period of 10 min. The coloured solutions were then centrifuged for a period of 15 min at 4000 rpm. The colour intensity of the supernatant was read on a spectrophotometer (Shimadzu UV-1800) at 520 nm and the sugar concentration of each sample concluded from a glucose calibration curve.

2.2 Pretreatment of Waste Paper Materials

To increase the extent of cellulase catalyzed saccharification all paper materials (0.05 g) were mixed with the Tris buffer (5.0 cm^3) and blend into a pulp. These mashed papers were thoroughly mixed with the enzyme solution (1.0 cm^3) and incubated at 50 °C for a period of 2 h. DNS (1.5 cm^3) was added to the post incubated mixtures, boiled for 10 min and centrifuged (4000 rpm, 15 min). Finally the supernatant was read at 520 nm and the sugar concentration concluded from a glucose calibration curve.

3. Results and Discussion

The bioconversion of waste cellulose into glucose a fermentable sugar is an important step in the development of waste cellulose for renewable purposes. Utilized paper is one of the major contributors towards solid waste. A global increase in paper production since 1960 is currently a major issue to be dealt with by waste managers [13]. Over decades paper has been made available in endless numbers of grades, types, shapes and colours and is used generally as well as for highly specialized purposes. Too often paper products are discarded soon after their purchase and only a part is recovered for recycling while the rest is managed by dumping (legal and illegal) as well as burning.

To investigate the relative amount of sugars that could be released from various paper materials these materials were biohydrolysed by cellulase from *A. niger*. In order to increase the amount of sugars released all paper materials were pulped to increase their surface area. When the non-pulped papers were exposed to cellulase the highest amount of sugar was released by paper towel at a concentration of (2.09 $mg.ml^{-1}$) followed brown envelope paper (1.29 $mg.ml^{-1}$), foolscap paper (1.26 $mg.ml^{-1}$) and paper from the retailer Woolworths (1.15$mg.ml^{-1}$). The highest resistance towards cellulose catalyzed degradation was observed with newspaper (0.35 $mg.ml^{-1}$) and paper from the retailer Pick n Pay (0.39 $mg.ml^{-1}$). The amount of glucose produced from paper towel the most degraded non-pulped material was 50% higher than the sugar produced from newspaper which showed the highest resistance towards cellulase activity. Pulping had at aim to make the cellulose component of waste paper more susceptible for cellulase catalyzed degradation. As experienced with non-pulped paper the pulped towel paper was also maximal degraded (3.26 $mg.ml^{-1}$) followed by Woolworths paper (2.54 $mg.ml^{-1}$), office paper (2.47 $mg.ml^{-1}$) and Pick n Pay paper (1.98 $mg.ml^{-1}$). Brown envelope paper (1.29 $mg.ml^{-1}$) showed the lowest degree of saccharification and this pulped material also exhibited the highest resistance towards bioconversion. No increase in sugar production could have been obtained from pulped brown envelope paper relative to the non-pulped material. The sugar released from pulped paper towel was 82% higher than the amount of sugar released from pulped Woolworths paper which produced the second highest amount of sugar. The pulped paper towel also produced 50% more sugar than the sugar produced from the brown envelope paper that was least degraded. The relative amount of sugar produced from the pulped and non-pulped papers are reflected in figure 1.

The relative increase in sugar formation from the pulped materials compared to the non-pulped materials is indicated in table 1. From these results it can be concluded that office paper showed the highest increase in sugar formation of 308% followed by newspaper with an increase of 271% and cardboard that produced a 218% increase in sugar formation. The paper material from the two retailers, Pick n Pay and Woolworths showed a relative low increase of 151% and 120%, respectively. Brown envelope paper showed the highest resistance and pulping could not result in an increased degradation of this cellulose waste resource.

Although cellulose is a biopolymer composed of glucose units the interaction between various cellulose chains resulted in cellulose exhibiting a crystalline section that is difficult to hydrolyze [14]. Cellulose has also an amorphous section that is more susceptible to degradation [15]. The aim of pulping was to destroy the chemical interaction between cellulose chains thus limiting the crystalline section making the material more susceptible for enzyme catalyzed degradation. The ratio of crystallinity to the amorphous part is not uniform in a specific waste paper and is also not identical for ll waste paper materials. Although pulping prove to be effective at increasing sugars from cellulose present in waste paper materials it could not render brown envelope paper more susceptible for cellulase catalyzed bioconversion.

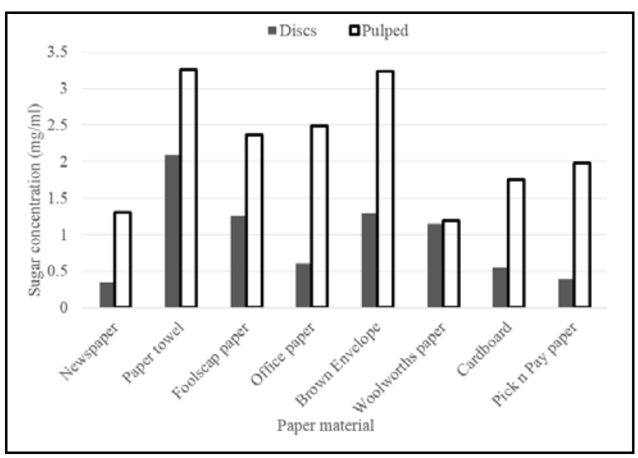

Fig 1: The relative saccharification of pretreated waste paper materials

Table 1: Percentage increase in saccharification of paper materials after pulping

Paper material	Percentage (%) sugar increase
Newspaper	271
Paper towel	73
Foolscap paper	88
Office paper	308
Brown envelope	0.00
Woolworths paper	120
Cardboard	218
Pick n Pay paper	151

4. Conclusions

Increased solid waste production with waste paper as a major component will become a concern for many communities as the global population increases. Linked to waste production is the negative environmental effect of fossil fuel combustion to satisfy the global energy demand. The cellulose component of waste paper can be developed as a bioenergy resource thus limiting the amount of solid waste and making available green energy. Pulping is a physical method that could be applied to make waste cellulose more degradable and should thus be considered in the pretreatment of any waste cellulose for increased glucose production. It has been concluded that waste paper could be developed as a resource for bioenergy which could limit environmental pollution.

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