Biotechnology Letters **22:** 491–494, 2000.

Saccharification of used paper with different cellulases

J.P.H. van Wyk*, M.A. Mogale & T.A. Seseng
Department of Chemistry and Biochemistry, Medical University of Southern Africa (Medunsa), South Africa
**Author for correspondence (Fax: + 27 12 345 1445; E-mail: jphvwyk@mcd4330.medunsa.ac.za)*

Received 14 December 1999; Revisions requested 24 December 1999; Revisions received 1 February 2000; Accepted 2 February 2000

Key words: A. niger, cellulase, paper, *P. funiculosum, T. reesei, T. viride*

Abstract

Various used paper materials have been exposed to the action of cellulases from *Penicillium funiculosum*, *Trichoderma reesei*, *Trichoderma viride* and *Aspergillus niger*. A 2 h incubation period showed cellulase from *T. viride* the most active except for office paper that was maximally degraded by *A. niger* cellulase. Cellulase mixtures increased saccharification while sequential treatment with cellulases from *T. reesei* and *P. funiculosum* increased biodegradation at values between 15% and 190%. The maximum increase of saccharification (190%) was obtained when *T. reesei* cellulase initiated the sequential treatment of newspaper relative to the sole action of *P. funiculosum* cellulase on this non-pretreated and pretreated material.

Introduction

The effort to develop bioethanol technology gained significant momentum in the late 1970s as a result of the then energy crises. To accomplish this process, different cellulase enzymes were identified as potential biocatalysts that could degrade cellulose to fermentable sugars like glucose. Since that time much applied research in the area of biomass conversion has answered most of the major challenges on the road to commercialization but as with any new technology, there is still room for performance improvement or change of application focus. Extensive simulation of the bioconversion process were conducted to identify the effects of operating conditions, pretreatment effectiveness, microorganism parameters and enzyme characteristics on the bioconversion process. In particular, the digestibility of the substrate as a result of pretreatment and the cellulase enzyme dosage, specific activity and composition had a profound effect on the process.

With the energy crises mastered, a few other problems are now facing the global population making the development of bioproducts including bioethanol quite topical (Block 1999). Increasing air pollution due to fossil fuel combustion as well as the massive amount of solid waste produced annually is a major concern for many countries (Agunwamba 1998). Used paper is the major component of the organic waste section (Gupta 1998) with cellulose the major structural component of it. Cellulase can biodegrade these waste materials to sugars (Van Wyk & Botha 1997) resulting in fermentation products, ethanol (Nguyen *et al.* 1999) and lactic acid (Schmidt & Padukone 1997).

This paper describes the saccharification of various used paper materials by cellulases from *Penicillium funiculosum*, *Trichoderma reesei*, *Trichoderma viride*, and *Aspergillus niger*. To increase bioconversion all materials were pretreated and exposed to cellulase mixtures as well as sequential cellulase treatment which is a more effective way of degrading waste cellulose fibers (Jeffries & Schartmann 1999).

Materials and methods

Source of enzymes and paper materials

Cellulases (Sigma; E.C. 3.2.1.4.) from *P. funiculosum*, *T. reesei*, *T. viride* and *A. niger* were prepared separately at 0.5 mg ml^{-1} in 50 mM sodium acetate/NaOH buffer, pH 4.5. Used, non-pretreated (0.5 cm × 0.5 cm) and fine, milled (pretreated) foolscap paper

Table 1. Formation of reducing sugars from used paper materials after a 2 h saccharification period with different cellulases as well as mixtures thereof (*P. funiculosum*:*A. niger*:*T. viride*).

Cellulase nature and mixtures (by vol.)	Formation of reducing sugars (mg mg^{-1} protein)							
	Filter paper		Newspaper		Foolscap paper		Office paper	
	Non-pretreated	Pretreated	Non-pretreated	Pretreated	Non-pretreated	Pretreated	Non-pretreated	Pretreated
P. funiculosum	1.2	1.9	1.0	1.6	1.3	2.3	0.7	1.0
A. niger	0.48	1.1	1.1	1.8	0.3	1.8	1.1	1.8
T. viride	2.6	3.6	1.6	1.8	1.8	4.3	0.8	1.7
(4:1:1)	1.7	2.9	1.7	1.8	1.9	4.3	0.8	1.3
(1:4:1)	1.2	2.4	1.7	1.8	1.1	3.4	1.1	2.0
(1:1:4)	2.3	3.2	1.8	2.3	2.7	5.0	0.6	1.8
(3:2:1)	1.6	2.8	1.7	2.0	2.4	3.6	1.0	1.7
(1:3:2)	1.7	2.6	1.9	2.0	3.0	3.6	1.1	2.2
(2:1:3)	1.7	4.1	2.4	2.6	3.0	5.4	1.1	2.0
(2:2:1)	1.9	2.6	1.7	2.0	2.3	4.1	1.0	1.7
(1:2:2)	1.7	3.6	1.9	2.4	3.1	4.3	1.2	2.4
(2:1:2)	1.9	4.0	2.0	2.4	2.8	5.2	1.0	2.0

(unglazed ruled manuscript paper), office paper (used for photocopying and printing), filter paper (Whatman No. 1) and newspaper were exposed to cellulase treatment.

Cellulase treatment

All incubations were at 50 °C for 2 h with each paper material (20 mg, dry weight) incubated separately with the individual cellulases (1.0 ml). Mixtures of the different cellulases were prepared according to the ratios of *P. funiculosum*:*A. niger*:*T. viride* (by vol.), resulted in a final incubation concentration of 0.5 mg protein ml^{-1}. During sequential treatment these materials were firstly treated with cellulase form *P. funiculosum* for 1 h succeeded by *T. reesei* cellulase for another hour, and vice versa. After each incubation period the mixtures were centrifuged at 5000 rpm for 10 min and the supernatant removed to determine the total reducing sugars, produced. During sequential treatment the residual paper material left after the first hour of incubation has been mixed with fresh cellulase to perform the second period of hydrolysis. All incubations were performed in triplicate.

Analytical methods and unit of activity

Total reducing sugars produced were calculated by using glucose as standard (Miller 1959) while the enzyme concentrations were determined with the Lowry test using BSA as standard. A unit specific enzyme activity is taken as the number of mg reducing sugars produced mg^{-1} protein under these conditions.

Results and discussion

Pretreatment and cellulase mixtures

Of various cellulases, that from *T. viride* was the most active on all non-pretreated and pretreated paper materials (Tables 1 and 2) except for office paper that was maximally hydrolyzed by *A. niger* cellulase. Of the non-pretreated materials, filter paper was the best hydrolyzed while pretreated foolscap paper exhibited the highest extent of bioconversion. By comparing the individual enzyme action on the various paper materials it was observed that cellulase from *P. funiculosum* was the most active on both non-pretreated and pretreated foolscap paper. *T. viride* cellulase showed maximum activity on non-pretreated filter paper and pretreated foolscap paper with *T. reesei* cellulase being most active on non-pretreated foolscap paper and pretreated newspaper. Although *A. niger* cellulase caused maximum degradation of office paper this enzyme displayed similar maximum biodegradation rates for non-pretreated and pretreated newspaper as well as

Table 2. Increased saccharification of used paper materials due to sequential treatment with cellulases from *P. funiculosum* and *T. reesei*.

Paper material	Cellulase treatment (mg total reducing sugars produced mg^{-1} protein)							
	A	B	C	D	E	F	G	H
Filter paper: non-pretreated	1.2	1.6	0.9	1.0	1.9	1.0	1.3	2.3
Filter paper: pretreated	1.9	2.5	1.1	1.9	3.0	1.4	1.9	3.3
Foolscap paper: non-pretreated	1.3	2.5	1.2	1.8	3.0	1.7	1.9	3.6
Foolscap paper: pretreated	2.3	2.6	1.4	2.3	3.7	1.9	2.8	4.7
Office paper: non-pretreated	0.7	1.1	0.7	0.8	1.5	0.9	1.1	2.0
Office paper: pretreated	1.0	1.2	0.9	0.9	1.5	1.0	1.2	2.2
Newspaper: non-pretreated	1.0	1.6	0.9	1.1	2.0	1.2	1.6	2.8
Newspaper: pretreated:	1.6	3.2	1.1	2.6	3.7	1.4	3.0	4.4

A: Treatment with *P. funiculosum* cellulase for 2 h.
B: Treatment with *T. reesei* cellulase for 2 h.
C: Treatment with *P. funiculosum* cellulase during the 1st h of sequential treatment.
D: Treatment with *T. reesei* cellulase during the 2nd h of sequential treatment.
E: Total of C and D.
F: Treatment with *T. reesei* cellulase during the 1st h of sequential treatment.
G: Treatment with *P. funiculosum* cellulase during the 2nd h of sequential treatment.
H: Total of F and G.

pretreated foolscap. Increased used paper saccharification with cellulase mixtures composed of two cellulases such as, *P. funiculosum* and *A. niger* (Van Wyk & Mogale 1998), *P. funiculosum* and *T. reesei* (Van Wyk 1999), *P. funiculosum* and *T. viride* (Van Wyk 1998) have been described. This investigation showed the increased bioconversion with cellulase mixtures consisting of the three cellulases from *P. funiculosum*, *T. viride* and *A. niger*. With non-pretreated filterpaper (Table 1) no combination could exceed degradation caused by *T. viride* cellulase while with the pretreated material only two combinations (2:1:3 and 2:1:2) could increase the relative saccharification.

With *T. viride* cellulase being the most active on newspaper all enzyme combinations exceeded this enzyme's performance on the non-pretreated material. During degradation of this pretreated substrate no cellulase combination showed a bioconversion magnitude lower than observed with the sole *T. viride* cellulase and the ratio of 2:1:3 was determined to be the most active on both newspaper structures (Table 1). With the exception of one cellulase combination (1:4:1) all other caused an increased saccharification relative to the individual action of *T. viride* cellulase on non-pretreated foolscap paper with three combinations (1:1:4; 2:1:3; 2:1:2) more active than this sole enzyme acting on the pretreated material (Table 1). During saccharification of non-pretreated office paper only the combination of 1:2:2 could exceed the bioconversion caused by *A. niger* cellulase. Five mixtures were more effective on this pretreated material with the combination of 1:2:2 the most active resulting in a 33% saccharification increase relative to the *A. niger* cellulase action (Table 1).

Sequential treatment

Successive treatment, irrespective of the cellulase order, caused a higher sugar production from all paper materials than the prolonged incubation with both individual enzymes (Table 2). Applying *T. reesei* cellulase during the first incubation period produced more reducing sugars form all non-pretreated and pretreated materials than obtained when cellulase from *P. funiculosum* was initiating the sequential treatment (Table 2). The highest saccharification yield, due to sequential treatment, was obtained with pretreated foolscap paper at a rate of 12% biodegradation. This bioconversion was 2.5 fold more than obtained with *P. funiculosum* cellulase and 86% higher than when this enzyme from *T. reesei* acted on non-pretreated foolscap paper. The best increase in saccharification for a pretreated used paper material exposed to sequential treatment relative to the corresponding non-pretreated substrate was calculated at 190% when *P. funiculosum* cellulase acted on newspaper.

An important factor to consider during optimization of waste cellulose degradation to make it economical viable is that various paper materials are

differently manufactured and as a result exhibit non-similar susceptibilities for cellulases. Variables such as pretreatment, cellulase mixtures and sequential cellulase treatment enhance bioconversion and illustrate the uniqueness of each used paper material toward different cellulase catalyzed saccharifications in the development of these biomass components as a resource of bioenergy.

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