Kinetic Parameters Measured during Cellulase Processing of Cotton

A. Cavaco-Paulo and L. Almeida

Department of Textile Engineering, University of Minho, Guimarães, Portugal

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Kinetic parameters were measured during cellulase treatment of cotton. A recent approach was used instead of the classical Michaelis–Menten equation to calculate kinetic constants relating the initial reaction rate with the enzyme concentration. The calculated parameters are discussed for two commercial cellulases with both low and high levels of mechanical action. Further comparisons are also made on scoured fibres and mercerized, raised, and original fabrics.

1. INTRODUCTION

Cellulase enzymes have a specific catalytic action on 1,4 β -D glycosidic bonds of the cellulose polymer [1], the principal constituent of cotton fibres. Cellulase is a multi-enzymatic system and a mixture of at least endoglucanases (EG) (EC 3.2.1.4), cellobiohydrolases (CBH) (EC 3.2.1.91), and cellobiases (EC 3.2.1.21) [1]. A general model for the action of these enzyme components on cotton cellulose is that the endoglucanases (EGs) cause random hydrolytic-chain scission at the most accessible points of long cellulose polymers, whereas the cellobiohydrolases (CBHs) split cellobiose from the ends of cellulose molecules. The cellobiase hydrolyzes cellobiose to glucose. A synergism between the different components has been observed, but the detailed mechanism of their action is not yet fully understood [2]. However, cellulase mixtures can be comparatively characterized by measuring their activity towards substrates such as substitute celluloses (carboxymethylcellulose or hydroxyethylcellulose) and crystalline or amorphous celluloses by quantifying endoglucanase activity and total activity (EG + CBH), respectively [3].

Cellulases have been used in recent years in the processing of cotton [4,5]. Reduction of pilling in knitted fabrics and the ageing of garments, to produce, for example, the washed look of denim fabrics, are widely used applications in textile finishing [5]. Cellulases are applied in textile processes in which mechanical friction is always present, for example, in jets, winches, or washing machines. It has been verified that increasing mechanical action increases the weight loss during a cellulase-hydrolysis process [6].

Kinetic parameters measured during a textile process can provide useful mechanistic information. By applying the classical Michaelis–Menten equation, Equation (1), we can calculate the rate of substrate turnover at saturation ($V_{\max}$) and a half-saturation constant (K_m), which can be interpreted as an apparent dissociation constant of all enzyme-bound species [7]. However, expressing the reaction rate (V) as a function of the substrate concentration ($[S]$) is a very artificial convention when the reaction takes place on hydrated cellulose, where it is impossible to change the concentration of the substrate sites [7]. Moreover, it was verified that the EG–CBH synergism disappears at high enzyme concentrations, i.e. it is competitive synergism [8]. For these reasons, it was suggested that V should be expressed as a function of the enzyme concentration ($[E]$) [7,9]. It has been suggested that an analogous equation, Equation (2), which was proved later to apply

in cellulase hydrolysis of crystalline cellulose [9,10], should be used. The calculated parameters $V'_{\max}$ and K_e are analogous to the Michaelis–Menten parameters.

$$V = \left(V'_{\max} [S] \right) / \left(K_m + [S] \right) \quad (1)$$

$$V = \left(V'_{\max} [E] \right) / \left(K_e + [E] \right) \quad (2)$$

In this communication, kinetic parameters are calculated during the cellulase processing of cotton. Parameter differences between two different cellulases, two levels of mechanical action, and different cotton fibres and fabrics are discussed. Comparisons are also made between raised, mercerized, and original fabrics. A brief characterization of both cellulases is given.

2. EXPERIMENTAL

2.1 Cellulases

Cellusoft L (total cellulase, used for reducing pilling and for softening; maximum activity at pH 5) and Denimax T (ageing cellulase, used for 'second-hand' effects; maximum activity at pH 7) were both kindly supplied by Novo Nordisk A/S*.

2.2 Enzyme Activity

The activities per gram of crude enzyme were measured in carboxymethylcellulose (CMC), phosphoric-acid-swollen Avicel (PASA), and cellobiose, as described previously [11]. The activity on scoured cotton fabric was also determined by measuring the weight loss and the reducing sugars liberated, as a cuprous neocuproine complex [11]. From the values of weight loss and the reducing sugars liberated, the mean chain length was calculated. The pH values used were 4.8 and 7 for the total cellulase and for the ageing cellulase, respectively.

2.3 Cotton Substrates

The substrates were 100% scoured cotton fibres and 100% bleached and scoured cotton fabrics. There were three types of fabric: raised, mercerized, and original (unraised and unmercerized). The specifications of all the fabrics are shown in Table I.

Table I
Specifications of the Fabrics

Fabric Type	Fabric Count (ends/picks per cm)	Weight per Unit Area (g/m ²)	Fabric Thickness at 2.5 gf/cm ² (mm)
Cotton fabrics (plain-woven)	60/32	100	0.5
Cotton raised fabrics (plain-woven)	21/15	160	1.0
Cotton mercerized fabrics (plain-woven)	60/30	100	0.5

2.4 Kinetic Measurements

A 10.0-g sample was treated with 100 mL of solutions of 0.1, 0.5, 1, and 2 g/L of cellulase in a buffer of pH 4.8 (acetate, 0.5M) for Cellusoft L and in a buffer of pH 7.0 (phosphate, 0.5M) for Denimax T, at a temperature of 50°C. After 60 min, 0.1 mL of the reaction

*The names of commercial products are provided only for specific information. Their mention does not constitute a recommendation.

solution was taken out, and the concentration of reducing sugars as glucose was determined by the cuprous neocuproine method [11]. The experiments were run three times, and the final result was the average of the three values obtained. The value of the reducing sugars determined after 60 min was taken as the initial reaction rate. The reaction kinetic was carried out with a low level of mechanical action in the 500-mL stainless-steel pots of a Linitest machine rotating at 65r/min. A higher level of mechanical action was provided by twelve steel disks used in determining dye fastness to dry-cleaning.

2.5 Kinetic Calculations

The approach of relating the initial reaction rate and the initial enzyme concentration was used to calculate the maximum rate of enzyme saturation ($V_{\max}^*$) and a half-saturation constant (K_c) by using Equation (2). The parameters were calculated by using non-linear regression (NLR) in an SPSS 6.0 for Windows.

3. RESULTS AND DISCUSSION

The activities of both cellulases are shown in Table II. The total cellulase is the more active on all cellulosic substrates, but the ageing cellulase has a higher activity in CMC. The mean lengths of the sugars produced from cotton hydrolysis are shorter for the total cellulase than for the ageing cellulase. Thus the total cellulase is a complete cellulase, and the ageing cellulase is an enzyme with higher endo-activity. Recent SEM studies showed that the total cellulase has a deeper action on fibers, whereas the ageing cellulase has merely a superficial action [6].

Table II
Enzyme Activity

Enzyme	Ageing Cellulase	Total Cellulase
Cellobiose*	0.85 U/g	0.75 U/g
CMC ⁺	115 U/g	159 U/g
PASA ⁺	144 U/g	361 U/g
Cotton ⁺	10 U/g	51 U/g
Weight loss	0.91%	1.33%
Mean chain length	2.4	1.7

*One unit (U) is the amount of enzyme that produces in the cellobiose hydrolysis 2 μ mol of glucose per minute.

⁺One unit (U) is the amount of enzyme that produces 1 μ mol of reducing sugars as glucose per minute.

Both enzymes have less activity in cellobiose (Table II) and their hydrolysis extent on cotton processing will therefore be shortened by the inhibiting presence of large amounts of cellobiose [2]. It is desirable during cellulase processing of cotton to have a hydrolysis-extent controller such as cellobiose to prevent excessive damage to the treated fabrics. Thus, this lower cellobiose activity is possibly 'tailor-made' by the enzyme producer.

The relation between reaction rate and enzyme concentration is shown in Fig. 1. The solid lines of these plots are obtained by NLR, and the calculated kinetic parameters are shown in Table III.

The maximum rate is represented in Fig. 2 for all processes with all substrates. We can confirm the activities of both enzymes by the higher turnover of the total cellulase. As verified previously, increasing mechanical action increases the reaction rate at saturation for both enzymes and all substrates. These facts are more significant for the fabric substrates than for the fibre substrates [6].

Fig. 1 Relation between reaction rate and enzyme concentration. The solid lines on the plots are obtained by non-linear regression (TCLMX—total cellulase with low mechanical action; TCHMX—total cellulase with high mechanical action; ACLMX—ageing cellulase with low mechanical action; ACHMX—ageing cellulase with high mechanical action; X=R—raised fabrics; X=M—mercerized fabrics; X=O—original fabrics; X=S—scoured fibres)

Fig. 1 (cont.) Relation between reaction rate and enzyme concentration. The solid lines on the plots are obtained by non-linear regression (TCLMX—total cellulase with low mechanical action; TCHMX—total cellulase with high mechanical action; ACLMX—ageing cellulase with low mechanical action; ACHMX—ageing cellulase with high mechanical action; X=R—raised fabrics; X=M—mercerized fabrics; X=O—original fabrics; X=S—scoured fibres)

Table III
Calculated Kinetic Parameters*

Process	V_{max} (mg/mL/h)	K_c (mg/mL)	V_{max}/K_c (1/h)
TCLMR	2.4 ± 0.2	1.5 ± 0.3	1.6 ± 0.4
TCHMR	6.9 ± 0.9	5.4 ± 0.9	1.4 ± 0.3
ACLMR	0.9 ± 0.2	0.7 ± 0.3	1.3 ± 0.6
ACHMR	2.2 ± 0.1	2.0 ± 0.2	1.1 ± 0.1
TCLMM	3.4 ± 0.4	1.7 ± 0.4	2.0 ± 0.5
TCHMM	8.9 ± 1.7	5.1 ± 1.3	1.7 ± 0.6
ACLMM	1.1 ± 0.1	1.0 ± 0.2	1.1 ± 0.2
ACHMM	1.1 ± 0.1	0.7 ± 0.2	1.6 ± 0.5
TCLMO	1.3 ± 0.1	0.3 ± 0.1	4.3 ± 1.5
TCHMO	2.6 ± 0.5	1.0 ± 0.4	2.6 ± 1.2
ACLMO	0.5 ± 0.1	0.2 ± 0.1	2.5 ± 1.3
ACHMO	1.0 ± 0.2	0.5 ± 0.3	2.0 ± 1.3
TCLMS	1.6 ± 0.1	0.3 ± 0.1	5.3 ± 1.8
TCHMS	2.7 ± 0.3	1.1 ± 0.3	2.5 ± 0.7
ACLMS	0.9 ± 0.1	0.5 ± 0.2	1.8 ± 0.8
ACHMS	1.4 ± 0.2	0.6 ± 0.2	2.3 ± 0.9

*See caption to Fig. 1 for explanation of the abbreviations in the 'Process' column

On comparing both cellulase turnovers of all fabrics, the mercerized fabrics have the maximum reaction rate. Raised fabrics also have a higher turnover than the original fabrics. These differences can be explained by the more accessible structure of mercerized cellulose and by the damaged fibres on the surface and more open structure (Table I) of raised fabrics. Scoured fibres have a slightly higher turnover than the original fabrics because the cellulose on a bundle of fibres is more accessible than that on a fabric [6].

The calculated half-saturation constants are represented in Fig. 3. This half-saturation constant can be interpreted as an apparent dissociation constant of all enzyme-bound species. The apparent dissociation constant is higher for the total cellulase than for the ageing cellulase. This seems to indicate that, in general, EG bind more strongly to cellulose than other cellulase species.

Fig. 2 Maximum rate for all substrates and treatments (see caption to Fig. 1 for explanation of the legends on the horizontal axis)

Fig. 3 Half-saturation constants for all substrates and treatments (see caption to Fig. 1 for explanation of the legends on the horizontal axis)

Increasing mechanical action on all substrates will increase dissociation of all enzyme-bound species. Raised and mercerized fabrics have a higher dissociation constant because they have a more accessible cellulose structure. This means that the enzyme is possibly greater on the non-associated form, i.e. it is ready for catalysis. This fact is in accordance with the higher turnover verified for these fabrics.

Dividing V_{max} by K_e , we obtain the catalytic specificity, which is represented in Fig. 4. The ageing cellulase has a lower catalytic specificity, hence confirming endo action, than the total cellulase. If, by definition, endo action is a random scission of a cellulose polymer, it will be less specific than exo action on the polymer ends. The total cellulase therefore has a higher exo action, whereas the ageing cellulase has a higher endo action when both catalytic specificities are compared.

Increasing mechanical action will usually reduce the catalytic specificity of the cellulase

Fig. 4 Catalytic specificity for all substrates and treatments (see caption to Fig. 1 for explanation of the legends on the horizontal axis)

action, but these changes are probably not significant owing to the error limits associated with them. The less accessible cotton, such as the original fabric and the scoured fibres, has a specific action of both enzymes higher than that of the other substrates. It seems that, on a more accessible cellulose structure, the hydrolysis is of a random type, whereas, on more crystalline cellulose, the hydrolysis starts at the polymer ends. This seems to indicate that CBHs are the first enzymes to attack crystalline cellulose.

4. CONCLUSIONS

The calculation of kinetic parameters provides important mechanistic information about cellulase hydrolysis in a simulation of a textile process.

It is confirmed that the increase in mechanical friction is more synergistic with cellulase action on fabrics than on a bundle of fibres.

The synergism between increasing mechanical friction and cellulase action is to increase the dissociation of the enzyme-bound species, leaving more free enzyme for catalysis.

Mercerized and raised fabrics are more accessible to enzymatic attack because they have more accessible points, a more open structure of mercerized cellulose, and more damaged pill fibres on the surface of raised fabrics.

Catalytic specificities give important information about the endo/exo character of cellulase mixtures.

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