ENZYMATIC HYDROLYSIS OF CELLULOSE (II): X-RAY PHOTOELECTRON SPECTROSCOPY STUDIES ON CELLULASE ADSORPTION. EFFECT OF THE SURFACTANT TWEEN 85

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The adsorption of proteins on particulate and fibrous celluloses was studied by means of X-ray photoelectron spectroscopy (XPS). The presence of protein (bovine serum albumin and Cellulase, a commercial cellulase from Trichoderma reesei) adsorbed on the fibres was detected by the N1s signal in the wide-field spectrum. The proteins adsorbed onto several types of cellulose could be compared, irrespective of the fibres' specific surface area. The fractional monolayer coverage of cellulose fibres could also be calculated.

The influence of the surfactant Tween 85 on cellulase adsorption and enzymatic activity was also investigated. At low enzyme concentrations, the surfactant reduces the amount of adsorbed enzyme, simultaneously improving the reaction rate. The effect of the surfactant depends on the structural properties of the substrate. The higher the crystallinity of the substrate, the less effective is the enhancement of the reaction and the reduction of the amount of adsorbed enzyme by the surfactant.

Keywords: Cellulase; Adsorption; Surfactant; XPS; Cellulose

INTRODUCTION

Cellulases are a consortium of hydrolytic enzymes which adsorb on and hydrolyse insoluble cellulose (Béguin and Aubert, 1994; Enari and Niku-Paavola, 1987). Adsorption precedes the hydrolytic reaction, and is a major determinant of cellulase activity (Nidetzky and Claeyssens, 1994). In

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turn, adsorption depends markedly on the cellulose surface area and on the surface properties (Lee et al., 1982; Walker and Wilson, 1991). Thus, the site specificity of cellulase adsorption (Nieves et al., 1991), and the influence of the adsorption force on the reaction kinetics are important factors in the enzymes' action (Klyosov, 1990; Park and Kajijuhi, 1995). When studying the adsorption of cellulases onto different substrates, the amount of enzyme adsorbed per unit of surface area cannot be calculated, unless the accessible area is accurately measured.

The cellulose surface area is usually calculated by measuring nitrogen adsorption or by the solute exclusion technique. The drawbacks of each technique have been discussed elsewhere (Gama et al., 1994). Some authors estimate the surface area accessible to cellulases by measuring the amount of enzyme adsorbed at high enzyme to substrate ratio, i.e., when the cellulose is saturated (Gilkas et al., 1992; Wang and Converse, 1992). This approach is useful, but should be considered with caution, because:

- the fibres may undergo dispersion or fragmentation during hydrolysis, which may drastically increase the surface area (Woodward et al., 1992; Nidetzky and Claeyssens, 1994).
- several reports show that cellulose saturation is not always reached, even with high enzyme concentrations (Lee et al., 1982; Wang and Converse, 1992).
- when utilising purified enzymes, the maximum adsorbable amounts differ greatly, suggesting the existence of both common and distinct adsorption sites (Kyriacou et al., 1988; Beldman et al., 1987).
- the enzymes may form multilayers on the cellulose surface, owing to their aggregation (Dominguez et al., 1992; Nieves et al., 1991). This hypothesis was raised to explain the decay of the overall hydrolytic activity at high enzyme concentrations (Wang and Converse, 1992).

Bovine serum albumin (BSA) adsorption on cellulose fibres has been studied previously, as a tool to measure the accessible surface area, assuming that this protein does not have the drawbacks mentioned above for cellulases (Gama et al., 1994). However, it is not known whether the affinity of this protein for different celluloses with different crystallinity will be the same. Moreover, the maximal amounts of adsorbed BSA (0.19–2.90 mg·g⁻¹) are quite small compared to the values obtained when the adsorbate is a cellulase (9–83 mg·g⁻¹) (Beldman et al., 1987; Ooshima et al., 1990; Wang and Converse, 1992). X-ray photoelectron spectroscopy (XPS) has been used in the current work to estimate directly the BSA and cellulase concentration on the cellulose fibres following adsorption equilibrium.
XPS is a surface sensitive technique. Although X-rays can penetrate deeply into a sample, only the photoelectrons generated by the interaction of the X-ray photons with molecules/atoms near the surface (within 10–100 Å) can escape from the surface with a characteristic kinetic energy for each chemical element, and reach the electron analyser. The escape depth is governed by the attenuation length \( \lambda \) (depending on the inelastic mean free path) of the photoelectrons, which is a function of the sample density (Andrade, 1983; Defossé, 1984; Seah, 1990). The theoretical background necessary for a quantitative analysis is also presented, allowing the calculation of the fractional coverage of the cellulose surface by the proteins.

METHODS

Materials

The cellulase from *Trichoderma reesei*, Celluclast, was a kind gift from Novo, Denmark. Three kinds of cellulose were used: Sigmacell type 100; Avicel PH-101; fibrous Whatman CF-11. A characterisation of these substrates (degree of polymerisation, particle size, porosity, surface area, crystallinity) is presented in the first part of this paper, and in previous work (Gama et al., 1994).

Enzymatic Hydrolysis

The reaction mixture, containing 50 mg of cellulose, 3.0 ml of 50 mM acetate buffer, pH 5.0, with and without Tween 85 at 0.02% (w/v), and 1.0 ml of diluted enzyme (protein final concentration in the reaction mixture ranging from 2 to 250 \( \mu \)g \( \cdot \) ml\(^{-1}\), was incubated for 18 h in a 50 ml flask at 50°C in a reciprocating shaker (100 rpm). The reaction medium was then centrifuged (5000 rpm, 3 min), and the supernatant analysed for soluble sugars by the DNS method.

Adsorption Experiments

**BSA Adsorption**

One ml BSA solution in 50 mM acetate buffer pH 5.0, with initial concentrations between 2.0 \( \mu \)g \( \cdot \) ml\(^{-1}\) and 2.5 mg \( \cdot \) ml\(^{-1}\), was mixed with 50 mg cellulose in a 2 ml eppendorf tube. The suspension was incubated for 16 h in an orbital shaker (100 rpm, 20°C), after which it was centrifuged
(5000 rpm, 5 min). The centrifuged cellulose particles were resuspended twice in 1 ml of buffer and prepared for XPS analysis as described below.

**Enzyme Adsorption**

The suspension containing 50 mg of cellulose and 1.0 ml of enzyme (with concentrations of 5 μg·ml⁻¹, 0.1, 1.5 mg·ml⁻¹) diluted in 50 mM acetate buffer pH 5.0, with and without Tween 85 at 0.02% (w/v), in a 2 ml eppendorf, was incubated for 30 min in an orbital shaker (100 rpm, 20°C). The particles were then washed as above and prepared for XPS analysis.

**XPS Analysis**

The washed particles were dried in a SpeedVac, and 0.013 m diameter pellets were prepared with a hydraulic press at a pressure of 0.6 kg·m⁻² for 1 min. The pellets were then analysed in an Escalab 200A (VG Scientific-Fisons) XPS analyser equipped with a MgKα radiation source at 1253.6 eV.

**Theoretical Background**

The surface sensitivity of XPS allows the determination of the protein film thickness using the relationship

\[
I = \gamma I_0 \left(1 - \exp^{-d/(\lambda_{N1s} \sin \alpha)}\right),
\]

where \( I \) is the N1s photoelectron signal from an overlayer of thickness \( d \) and fractional coverage (monolayer) \( \gamma \), \( I_0 \) is the intensity for a thick overlayer \( (d = \infty) \), \( \lambda_{N1s} \) is the attenuation length of the N1s photoelectron, and \( \alpha \) is the angle between the surface and the analyser. For a uniform and flat surface, the sampled thickness is given by

\[
d = 3\lambda_{N1s} \sin \alpha
\]

and is maximum when \( \alpha = 90° \). In the case of a substrate with a uniform thin overlayer, the angular variation of intensities enhances the surface sensitivity, and the overlayer thickness can be determined (Andrade, 1983; Defossé, 1984; Fitzpatrick et al., 1991; Seah, 1990). However, the surface roughness of the pellets analysed in this work leads to an averaging of electron exit angles and also to shadowing effects (Fulghum and Linton, 1988), and therefore the surface enhancement effect could not be observed.

An average error of about 10% may be expected from the quantitative analysis of XPS. An overall accuracy of about 20% may result if corrections for instrumental parameters are made and if an approximate evaluation of
atomic cross sections (sensitivity factors) and attenuation length values is utilised (Defossé, 1984). In this work constant settings were used, and hence corrections were unnecessary.

RESULTS AND DISCUSSION

As the cellulose fibres contain carbon and oxygen, and proteins have high levels of nitrogen, the amount of adsorbed protein can be calculated. The major advantage of XPS relies on the fact that only the surface of a sample pellet with adsorbed protein is analysed, and therefore the measured N1s signal is independent of the specific surface area of each particulate cellulose. Thus, the protein adsorbed on different celluloses can be compared on a surface area basis. If one simply measures the amount of adsorbed enzyme, a sample with high surface area and low affinity for the enzymes, and another sample with low surface area but high affinity for the enzymes might display similar adsorption properties. In this analysis, it must be assumed that the enzymes do not penetrate the substrate, implying that only the external surface of the fibres is accessible. Previous work has shown that this assumption is likely to be correct, since the pores of the particles are smaller than 50 Å.

Figure 1 displays a typical XPS spectrum of cellulose fibres with adsorbed protein. The results of BSA adsorption on the cellulose fibres, as measured by XPS, are shown in Fig. 2 and Table I. These results generally
confirm the depletion isotherms previously presented (Gama et al., 1994). Whatman and Avicel particles become saturated by BSA at concentrations of 50 and 200 µg·ml⁻¹, respectively. Sigmacell has a higher adsorbing capacity, a saturation plateau being not well defined in the range of concentrations used. For low BSA concentrations, the XPS signal follows the reverse order of the specific surface area (which is as follows: Sigmacell > Avicel > Whatman), as expected. Since Sigmacell has a higher surface area than the other samples, the concentration of adsorbed protein on the surface of this cellulose was expected to be lower. With increasing protein concentration, the signal obtained approaches roughly the same value (between 6 and 9 keV/S) for the three cellulosics, although in the case of Sigmacell a higher value was reached for the protein concentration of 2.5 mg/ml.

The intensity of the N1s signal displayed by the fibres following cellulase adsorption was higher than with BSA adsorption (Fig. 2). Therefore, either the BSA molecules do not cover the surface of the fibres completely, or the cellulases are organised in a tighter fashion, perhaps forming multilayers (Steiner et al., 1988; Wang and Converse, 1992). To find out which of these possibilities is true, a quantitative analysis of the results is necessary. In either case, this result can be explained as a consequence of a different affinity of the proteins towards cellulose, the cellulase affinity being higher because of the cellulose binding domain.
<table>
<thead>
<tr>
<th></th>
<th>BSA (mg/ml)</th>
<th>Cellucl. (mg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.002 0.01 0.2 1.0 2.5</td>
<td>0.005 0.1 1.5</td>
</tr>
<tr>
<td>Avicel</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.860 (2.1) 3.869 (9.3) 4.980 (12.0) 6.191 (14.9) 7.743 (18.6)</td>
<td>2.024 (4.1) 4.483 (9.1) 18.362 (37.1)</td>
</tr>
<tr>
<td></td>
<td>(2.5) (11.1) (14.3) (17.8) (22.2)</td>
<td></td>
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<tr>
<td></td>
<td>Tw</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3.145</td>
</tr>
<tr>
<td></td>
<td>Tw</td>
<td>16.964</td>
</tr>
<tr>
<td></td>
<td>Tw</td>
<td></td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>(6.3) (34.2)</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>(7.1) (38.1)</td>
</tr>
<tr>
<td>SigmaCell</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.865 (4.5) 3.429 (8.2) 8.146 (19.6) 14.52 (34.9)</td>
<td>1.645 (3.3) 9.570 (19.3) 28.569 (57.7)</td>
</tr>
<tr>
<td></td>
<td>(5.4) (9.9) (23.4) (41.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tw</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.522</td>
<td>3.995</td>
</tr>
<tr>
<td></td>
<td>Tw</td>
<td>14.724</td>
</tr>
<tr>
<td></td>
<td>Tw</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(1.1) (8.1)</td>
<td>(29.7)</td>
</tr>
<tr>
<td></td>
<td>(1.2) (9.0)</td>
<td>(33.1)</td>
</tr>
<tr>
<td>Whatman</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.923 (2.2) 7.955 (19.1) 6.315 (15.2) 7.310 (17.6) 9.353 (22.5)</td>
<td>3.497 (7.1) 14.064 (28.4) 34.32 (69.2)</td>
</tr>
<tr>
<td></td>
<td>(2.6) (22.8) (18.1) (21.0) (26.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tw</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.036</td>
<td>13.18</td>
</tr>
<tr>
<td></td>
<td>Tw</td>
<td>30.67</td>
</tr>
<tr>
<td></td>
<td>Tw</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4.1) (26.6)</td>
<td>(61.9)</td>
</tr>
<tr>
<td></td>
<td>(4.6) (29.6)</td>
<td>(68.9)</td>
</tr>
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</table>
In order to estimate the $\gamma$ values of the protein films adsorbed on the cellulose fibres, $I_0$ was experimentally determined by preparing and analysing a pellet of lyophilised BSA. As expected, using the VG5250 quantitative analysis software, taking into account experimental conditions and the equipment characteristics, including Scofield atomic cross sections and corrections for analyser transmission and escape depth, an atomic nitrogen composition of 13.64% was obtained. This corresponds to the average N-composition of proteins. Thereafter, the $I$ value expected for a BSA or cellulase monolayer was calculated making use of Eq. (1), considering the thickness $d$ equal to be the dimensions of a side-on adsorbed protein.

To evaluate the fractional coverage $\gamma$ for each analysed pellet, we determined

$$\gamma = I / \left[ I_0 \left( 1 - \exp^{-d/\lambda_{N1s} \sin \alpha} \right) \right], \quad (3)$$

where $\gamma = 1$ corresponds to complete coverage by a side-on adsorbed monolayer. The corresponding expected signal intensity $I$ was calculated from Eq. (1), using the $I_0$ value experimentally obtained with lyophilised BSA as described previously, $d$ was made equal to 27 Å, considering the dimensions of the BSA molecule (112 × 27 × 27 Å), and the attenuation length was calculated by the equation (Andrade, 1983)

$$\lambda_{N1s} = (M/\rho n) E_k / (13.61 n(E_k) - 17.6 - 1400/E_k), \quad (4)$$

$E_k$ is the kinetic energy (eV) of the photoelectrons generated by the nitrogen in the protein, $M$ is the molecular weight of the molecule, $n$ the number of valence electrons in the molecule and $\rho$ is the density. Therefore, the ratio $(M/\rho n)$ will be nearly constant for every protein ($= 2.25$). The value for $\lambda_{N1s}$ will thus be nearly the same for BSA and the cellulases ($= 26.45$ Å).

The theoretical value of N1s intensity ($I$) for the particles with a monolayer of adsorbed cellulases was also calculated, by using the same parameters, but applying in this case a $d$ value of 45 Å, considering the dimensions of the CBH I molecule (67 × 45 × 45 Å), which accounts for the major part of the protein excreted by the fungi Trichoderma reesei (Abuja et al., 1988).

Therefore, the fractional coverage in each experimental case could be calculated by Eq. (3). In the $\gamma$ calculation we assume that multiple layers are not possible until a complete protein monolayer is formed.

The results obtained from these calculations are shown in Table I. The values in brackets correspond to the $\gamma$ value obtained using two different average exit angles in the calculations, 42° (above) and 90° (below). It can be seen that uncertainty in the exit angle, which cannot be predicted exactly
for the rough surface of the pellets, introduce a maximum possible variation of about 20% in the value of $\gamma$, therefore allowing the discussion below. An angle of 42° is considered, because it is a fair representation of the electrons escape angle from the round surface presented by the spherical bundles of microfibres, according to the theoretical analysis by Deslandes et al. (1993).

For high protein concentration (2.5 mg·ml$^{-1}$) the fraction of surface coverage of the cellulose particles by BSA is relatively low, as compared to the $\gamma$ values obtained with Celluclast. These low $\gamma$ values could be the result of washing the BSA off the fibres. To rule out this possibility, Avicel particles were equilibrated with a BSA solution (2.5 mg·ml$^{-1}$) and pellets were prepared without washing the fibres. As expected, higher amounts of nitrogen were detected in this case (N1s peak intensity of 19.049 kCeV/S), but nevertheless full coverage of the particles was not reached ($\gamma$ of 45.8% for an average exit angle of 42°). Also, the supernatants of the washed fibres were tested for the presence of protein by the Coomassie blue G-250 method (Bradford, 1976), the resulting being negative. Hence, it was concluded that BSA does not saturate the cellulose surface and therefore the adsorption isotherms do not allow the determination of the absolute surface area accessible to the enzymes.

Regarding different celluloses, the fraction of surface coverage by the enzymes was higher in the case of Whatman, due to the comparatively low surface area (Gama et al., 1994). The enzymes display a higher affinity for Sigmacell than for Avicel, probably due to the lower crystallinity and hence higher wettability of the former. Further experiments would be necessary to verify whether cellulose saturation corresponds to the calculated intensity for a monolayer or not. Assumptions had to be made in this calculation. Namely, it was considered that the degree of compactness of the protein in the lyophilised pellet is comparable to that on the surface of the fibres. However, it must be stressed that, due to their geometry and the fitting on the irregular surface of the fibres, the enzymes are not expected to cover the particles homogeneously, and voids must exist among the molecules when surface saturation is reached. Therefore, an homogeneous layer of protein on the fibres surface is unlikely. The surface concentration of the enzymes on the saturated surface of the different celluloses may also be different, because of the different morphology of the particles. The effect of the surface morphology is not predictable in a quantitative manner. The calculated intensity corresponding to a protein monolayer may therefore have been overexpressed, and, in this case, the real $\gamma$ values under the experimental conditions of this work are higher.
TABLE II  Hydrolysis rate (μg glucose equivalent/ml·h) of Avicel (Av), Sigmacell (Sigm) and Whatman (Whatm), with and without surfactant Tween 85 (Surf).

<table>
<thead>
<tr>
<th>Enz. conc. (μg/ml)</th>
<th>2.2</th>
<th>4.4</th>
<th>8.8</th>
<th>17</th>
<th>50</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av + Surf</td>
<td>12</td>
<td>25</td>
<td>44</td>
<td>65</td>
<td>117</td>
<td>225</td>
</tr>
<tr>
<td>Av</td>
<td>3</td>
<td>13</td>
<td>34</td>
<td>65</td>
<td>115</td>
<td>223</td>
</tr>
<tr>
<td>Sigm + Surf</td>
<td>24</td>
<td>46</td>
<td>89</td>
<td>150</td>
<td>278</td>
<td>400</td>
</tr>
<tr>
<td>Sigm</td>
<td>3</td>
<td>16</td>
<td>39</td>
<td>104</td>
<td>254</td>
<td>408</td>
</tr>
<tr>
<td>Whatm + Surf</td>
<td>7</td>
<td>14</td>
<td>22</td>
<td>38</td>
<td>61</td>
<td>111</td>
</tr>
<tr>
<td>Whatm</td>
<td>5</td>
<td>14</td>
<td>29</td>
<td>40</td>
<td>67</td>
<td>103</td>
</tr>
</tbody>
</table>

The rate of enzymatic hydrolysis for the several celluloses, with and without Tween 85, is presented on Table II. It should be noted that the enzymatic hydrolysis and adsorption experiments were conducted at different temperatures. A lower temperature was chosen for the adsorption experiments in order to reduce the enzymatic reactivity. As temperature influences the adsorption process, the protein adsorbed on the enzymatic experiments is possibly different from that detected by XPS. Therefore, the comparison between the enzymatic reactivity of the substrates and the adsorption of the enzymes, in the discussion below, is merely qualitative.

The surfactant enhanced the enzyme activity on Avicel and Sigmacell, especially at low enzyme concentration (from 3 to 12 and 3 to 24 μg glucose equivalent·ml⁻¹·h⁻¹, respectively for Avicel and Sigmacell, for an enzyme concentration of 2.2 μg·ml⁻¹), simultaneously reducing its adsorption (γ drops from 4.1 to 0, 7.1 to 4.1 and 3.3 to 1.1 respectively for Avicel, Whatman and Sigmacell, for an enzyme concentration of 5.0 μg·ml⁻¹) (Table I). In the case of Whatman, both hydrolysis rate and cellulase adsorption were less strongly affected by the surfactant (reaction rate increase from 5 to 7 and γ vary from 7.1 to 4.1, conditions and units as above). For the higher enzyme concentrations (>50 μg·ml⁻¹), the hydrolysis rate was slightly accelerated by the surfactant only in the case of Sigmacell. Interestingly, adsorption also seems to be influenced only in this case, and to a similar extent (for enzyme concentrations >100 μg·ml⁻¹). These results suggest a cause–effect relationship between the depletion of the enzymes from the fibre surface and the kinetics of hydrolysis.

Sigmacell hydrolysis at a low enzyme loading (2.2 μg·ml⁻¹) and without surfactant was slow compared to the rate of Whatman and Avicel hydrolysis (data partially shown in Table II), and to the relative rate obtained at higher enzyme concentration. This effect can be explained as a consequence of irreversible adsorption of the enzyme to Sigmacell, or, at least, a slow desorption rate. On the other hand, in the case of Whatman, multiple
attack of the same enzyme molecule on the cellulose surface may occur. This hypothesis is supported by the results obtained with the surfactant. In fact, the surfactant had little effect on the Whatman digestion, while in the case of Sigmacell a positive and strong effect could be detected, even at the highest enzyme concentrations.

Surfactant enhancement of the hydrolysis rate, and reduction in the adsorption level, has been demonstrated by several authors (Castanon and Wilke, 1981; Helle et al., 1993; Ooshima et al., 1986; Park et al., 1992). According to Castanon and Wilke (1981), by hindering the adsorption, the surfactant prevents the immobilisation of the enzyme on the surface of the substrate. As demonstrated by Tilton et al. (1993) for the case of the lysozyme adsorption on mica surfaces, in the absence of surfactant, the protein adsorbs irreversibly. In its presence, protein desorption may occur by direct interaction of the surfactant with the surface, displacing the protein. Surfactant binding to the protein may also modify the strength of interaction force between the protein–surfactant complexes and the surface.

It has been shown that the enzymes quickly adsorb at the beginning of the reaction, then gradually returning to the liquid phase (Moloney and Coughlan, 1983; Nidetzky and Steiner, 1993; Nutor and Converse, 1991). However, apparently irreversible binding, even after extensive digestion or dilution with buffer, has often been reported (Beldman et al., 1987; Kyriacou et al., 1989; Lee et al., 1982; Nutor and Converse, 1991; Otter et al., 1989). These irreversibly bound enzymes are likely to form the so-called non-effective complex, which severely reduces the enzyme efficiency, unless the enzymes diffuse laterally on the cellulose surface, performing several catalytic events without leaving the fibres. Ryu et al. (1984) and Kyriacou et al. (1989) also demonstrated the reversibility of the adsorption, providing evidence for competition of the various cellulase components for the adsorption sites. However, visualisation of adsorbed cellulases using antibody-colloidal gold conjugates (Nieves et al., 1991) suggests the existence of site specific adsorption. Hence, the competition would occur only to a limited extent.

Sigmacell is the least crystalline of the three substrates analysed in this work (Crl: Sigmacell = 79%; Avicel = 91%; Whatman = 95%) (Gama et al., 1993). Apparently, this is also the substrate with more non-effective binding sites. However, considering other results in the literature, the amorphous areas cannot be simply associated with the non-effective binding sites (Helle et al., 1993; Nutor and Converse, 1991). In fact, in the works by Helle et al. (1993) and Ooshima et al. (1986), the digestion of rather amorphous substrates was only poorly surfactant enhanced.
The nature of the non-effective interaction, which this paper presents indirect evidence of, should be studied further, since it is a major drawback in the hydrolytic effectiveness and recyclability of cellulases. These studies should be done by utilising a larger number of substrates with well characterised surfaces (crystallinity, surface tension, wettability, lignin content) and analysing the enhancing effect of surfactants on the reaction rate, as well as on the adsorption, by XPS.

XPS is a powerful technique for studies of heterogeneous biocatalysis. In this paper, the usefulness of XPS as a tool to analyse the cellulose–cellulase interaction has been demonstrated, and for the first time it has been shown that the positive effect of the surfactant on the reaction rate is dependent on the surface properties of the substrate and on the surface concentration of the non-effective sites.

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References


