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Short Communication

A Spectrophotometric Method for Determination of the Solubilizing Activity of Cellulase Complexes

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Summary. A standardized spectrophotometric method for determination of the solubilizing activity of microbial cellulase complexes has been developed. It is based on the release of coloured compounds from microcrystalline cellulose (Avicel SF®) dyed with Levafixbrilliantrot E-2B®.

Keywords. Cellulase complex; Spectrophotometric method; Solubilizing activity; Dyed microcrystalline cellulose; Levafixbrillantrot E-2B®; Avicel SF®.

Eine spektrophotometrische Methode zur Bestimmung der solubilisierenden Aktivität des Cellulase-Komplexes (Kurze Mitteilung)

Zusammenfassung. Es wurde eine standardisierte spektrophotometrische Methode zur Bestimmung der Aktivität des Cellulase-Komplexes von Mikroorganismen ausgearbeitet. Die Methode beruht auf der Möglichkeit, mit Levafixbrillantrot E-2B® gefärbte mikrokristalline Cellulose (Avicel SF®) als Substrat zu verwenden.

Different methods have been developed for the determination of the solubilizing activity of microbial cellulase complexes (e.g. methods using insoluble cellodextrines [1], hydroxyethylcellulose [2] and cellulose substrates [3–7]) preliminary dyed with reactive dyes.

Comparative studies of some dyed insoluble substrates have shown that the microcrystalline cellulose (Avicel SF®) is the most suitable cellulose substrate for preliminary dyeing and subsequent determination of the solubilizing activity of the cellulase complexes [4].

We have investigated the solubilizing activity of cellulase complexes produced by some microorganisms (*Oxyporus sp.* – kindly donated by "E. Merck", FRG; *Aspergillus niger* – "Hemicellulase"®, "Koch-Light", U.K.; *Aspergillus oryzae* – "Luizym"®, "Luitpoldwerk", FRG; and *Trichoderma sp.* – the cellulase preparation "Cellulase CB"® obtained in our laboratory) using as substrate insoluble microcrystalline cellulose "Avicel SF"® dyed with Levafixbrillantrot E-2B® according to Stamm [8]. The spectrum of the acid hydrolysate of the dyed substrate has the same absorption maximum at 520 nm as that of the free dye and the corresponding molar absorptivity is $2.00 \cdot 10^4 \, l \, mol^{-1} \, cm^{-1}$.

We have optimized the influence of the following factors on the solubilizing activity of cellulase complexes with dyed substrate: rate of stirring (350–400 rpm), temperature of filtration (90 °C), and size of the pores in the glass filter (16 μm).

On the basis of the results obtained the following standard procedure for determination of the solubilizing activity of microbial cellulase complexes has been proposed:

One milliliter appropriately diluted enzyme solution is added to a suspension of 50 mg dyed substrate in 4 ml 0.1 *M* citrate-phosphate buffer $pH = 5.0$. The suspension is incubated for 60 min at 50 °C while stirring (400 rpm). The reaction is stopped by heating the sample in a boiling water bath for 5 min. The suspension is filtered through glass filter with a diameter of pores less than 16 μm. The filtrate is cooled and its absorbance is measured at 520 nm against a blank. The solubilizing enzymatic activity is evaluated using a calibration curve.

The calibration curve expressing the relationship between the absorbance (A) measured at 520 nm on one hand and the activity and the concentration of the cellulase preparation from *Oxyporus sp.* on the other is shown in Fig. 1. The mean relative error in measuring the absorbance at 520 nm ($A = 0.100 \div 0.300$) ranges from ± 0.8% to ± 1.8%. For absorbance exceeding 0.300 the calibration curve is not linear. In such cases the enzyme solution should be diluted.

In the present study one "relative cellulase solubilizing unit" (1 RCSU) has been defined to be the quantity of multienzyme cellulase complex in 1 ml which

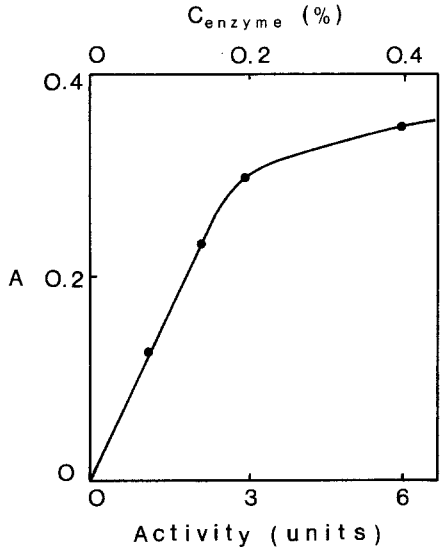

Fig. 1. Calibration curve for the determination of the solubilizing activity and the concentration of the cellulase preparation from *Oxyporus sp.*

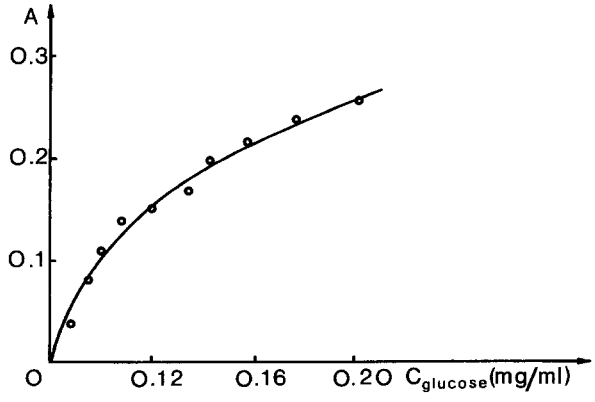

Fig. 2. Absorbance vs. concentration of the hydrolysis products of the dyed substrate determined as glucose [9]

Table 1. Solubilizing activity of microbial cellulase complexes

No.	Cellulase preparation	Activity (RCSU)
1	*Oxyporus sp.*	1.51
2	*Trichoderma sp.*	2.11
3	*Aspergillus niger*	0.00
4	*Aspergillus oryzae*	0.00

under the standard conditions of determination catalyzes the release of colour products giving at 520 nm absorbance equal to 0.150.

The hydrolysis products of the dyed substrate have been determined formally as glucose according to the method of Dubois et al. [9]. In Fig. 2 the correlation between the hydrolysis products expressed as glucose and the absorbance of the filtrated hydrolysate at 520 nm is illustrated.

According to the proposed method (Figs. 1 and 2) 1 RCSU ml^{-1} is equal to 0.37 FPU (Filter paper unit) ml^{-1}.

The method developed in the present communication has been used for the determination of the solubilizing activity of microbial cellulase complexes produced by *Oxyporus sp., Trichoderma sp., Aspergillus oryzae* and *Aspergillus niger* (Table 1). The results presented in Table 1 confirm the literature data that the cellulase complexes produced by *Oxyporus sp.* and *Trichoderma sp.* contain all enzyme components while those produced by *Aspergillus sp.* do not have the enzyme 1,4-β-*D*-Glucan cellobiohydrolase (EC.3.2.1.91).

References

[1] Fernley H. N. (1963) Biochem. J. **87**: 90
[2] Biely P., Mislovičová D., Toman R. (1985) Anal. Biochem. **144**: 142
[3] Poincelot R. P., Day P. R. (1972) Appl. Microbiol. **22**: 875
[4] Leisola M., Linko M. (1976) Anal. Biochem. **70**: 592
[5] Leisola M., Kauppinen V. (1978) Biotechnol. Bioeng. **20**: 837
[6] Ng T. K., Zeikus J. G. (1980) Anal. Biochem. **103**: 42
[7] Mullings R., Parish J. H. (1984) Enzyme Microbiol. Technol. **6**: 491
[8] Stamm O. A. (1963) Helv. Chim. Acta **46**: 3008
[9] Dubois M., Gilles K., Hamilton J. K., Rebers P. A., Smith F. (1956) Anal. Chem. **28**: 350

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