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Amperometric method for the determination of cellulase activity and its optimization using response surface method

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Abstract

Cellulases are a group of enzymes, which catalyse different steps of cellulose hydrolysis, and are broadly used in industry as unpurified mixtures of several enzymes. The total activity of cellulase is defined as the ability of the enzyme to produce glucose, which is the final product of cellulose hydrolysis, and is expressed in cellulase units. However, common strategies for the determination of the cellulolytic activity of industrial cellulase preparations are based on the assessment of different steps of cellulose hydrolysis, and the results obtained with different methods are not similar. The aim of the present study was to develop an assay for the determination of cellulase activity that relies on the amperometric determination of the final product of cellulose hydrolysis glucose. The assay conditions were optimized using response surface methodology (RSM) combined with Box-Behnken design. The detection limit of the proposed method was 1.71 ± 0.06 U. We compared the results of the amperometric method with the ones obtained with the spectrophotometric method and viscosimetry in a commercial cellulose preparation.

Keywords: Cellulase activity, Amperometric detection, Response surface method, Optimization of assay conditions

Introduction

Cellulolytic enzymes, commonly known as cellulases are mainly produced by cellulolytic bacteria, fungi and protozoa (Annapure and Pratisha 2022). The name "cellulase" is commonly used for a natural mixture of enzymes that in general includes three major glycosylases: endoglucanase (EC 3.2.1.4), exoglucanase (cellulase 1,4-beta-cellobiosidase (non-reducing end), EC 3.2.1.91), and cellobiase or β-D-glucosidase (EC 3.2.1.21) (Anoop Kumar et al. 2019). The enzyme mixture acts synergistically on the hydrolysis of cellulose which is gradually broken down from crystalline and/or amorphous cellulose to smaller, soluble fragments and finally to glucose (Dimarogona et al. 2012; Jayasekara and Ratnayake 2019; Percival Zhang et al. 2006; Pino et al. 2018; Yunus and Kuddus 2021). It has been calculated that the natural cellulose crystalline area has 8 and the amorphous area at an average 5.3 hydrogen bonds per glucose unit (Kobayashi and Fukuoka 2013). During cellulose hydrolysis, endoglucanases first attack the amorphous area of the polysaccharide chain, breaking the β-1,4-glycosidic bonds releasing cellodextrin and creating new chain ends. Released cellodextrins are hydrolysed by exoglucanases producing tetrasaccharides and disaccharides such as cellobiose. In the final step, β-glucosidases cleave exocellulase products releasing soluble individual monosaccharides such as glucose. These three hydrolysis steps can occur simultaneously (Percival Zhang et al. 2006). The mechanism of cellulolytic action is illustrated in Fig. 1.

Commercial cellulases which are widely used in the food industry (Ejaz et al. 2021), are mostly complex mixtures of several enzymes, because the separation of individual enzymes is not feasible (Wang et al. 2012). Cellulase preparations are used for maceration of plant material to improve the extraction yield of juice, and

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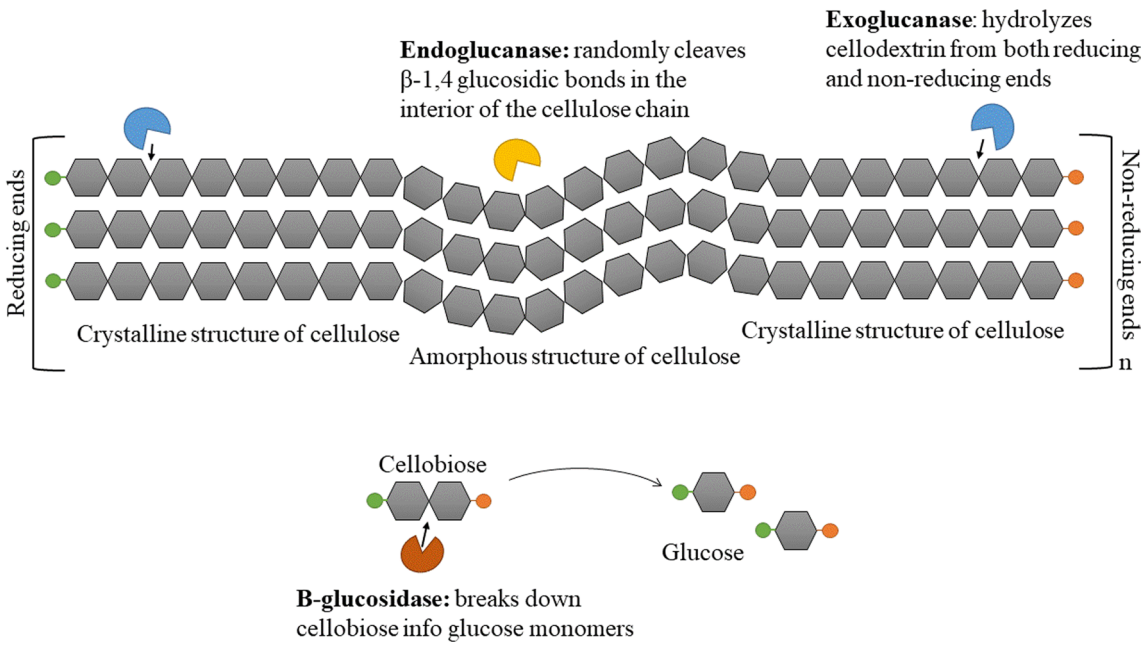

Fig. 1 Action mechanism of cellulolytic enzymes: endoglucanase randomly cleaves internal bonds at amorphous sites that create new chain ends; exoglucanase cleaves two to four units from the ends of the exposed chains produced by endocellulase, resulting in disaccharides, such as cellobiose; and β-glucosidase hydrolyses disaccharides into individual monosaccharides, such as glucose

clarification of fruit and vegetable juices increasing juice cloud stability and reducing its viscosity (Soares et al. 2016). Following the same principle, cellulase, hemicellulase, and pectinase are used for the extraction and maceration of the must during winemaking to enhance juice extraction as well as the release of phenolic compounds, anthocyanins, and fragrance precursors that contribute to the sensory quality of wine (Ottone et al. 2020). Another application of cellulases is the valorisation of food waste and by-products via the conversion of cellulose fibres into soluble sugars to be converted by fermentation to bio-energy, mainly bio-ethanol (Zou et al. 2020).

The standard technique for the assessment of total cellulase activity, which was first proposed by Mandels and acknowledged by the International Union of Pure and Applied Chemistry (IUPAC), is the filter paper activity (FPA) method, measuring the decomposition degree of filter paper (Mandels et al. 1976). The value of 2.0 mg of reducing sugar as glucose from 50 mg of filter paper (4% conversion) in 60 min has been designated as the intercept for calculating filter paper cellulase units (FPU) by IUPAC (International Union of Pure and Applied Chemistry 1987). The main disadvantage of this method is that cellulase must be diluted until the amount of product plotted against cellulase concentration is reasonably linear. Although this analysis can be carried out in most laboratories, it is time-consuming, labour-intensive, requires large quantities of reagents, and it is difficult to obtain adequate sensitivity and reproducibility when characterizing newly isolated cellulases (Dashtban et al. 2010). The sensitivity and reproducibility of FPA method is often affected by the fact that most natural cellulase complexes tend to have a shortage of β-glucosidase activity (Breuil et al. 1986; Coward-Kelly et al. 2003). The other widely used method for the detection of cellulase activity is determining reducing sugars released during cellulose hydrolysis (Xi et al. 2013; Yunus and Kuddus 2021). The reducing sugars can be evaluated by different methods such as high-performance liquid chromatography (HPLC) (Ferrari et al. 2014; Fujita et al. 2002) or spectrophotometry with different dyes or reagents (Brescia and Banks 2011; Gusakov et al. 2011; Hernández-López et al. 2020; Lone et al. 2012; Shuangqi et al. 2011; Vancov and Keen 2009). The main shortcoming of this detection principle is the fact that it is not possible to differentiate between cellobiose and glucose, which are formed during different steps of cellulose hydrolysis. In addition, cellulose activity has been assessed by the change in viscosity of cellulose solution upon incubation with cellulase (Hendel and Marxsen 2020; Joos et al. 1969). One cellulase unit is the enzymatic activity that results in a relative fluidity change of one in 5 min in a defined CMC substrate at 50 °C (pH 4.5) (Pomeranz 1991). Viscosimetry is specific to endocellulase, since exocellulase enzymes produce little or no change in viscosity.

The aim of the present study was to develop an assay for the determination of the cellulase activity of commercial preparations, based on the amperometric determination of glucose, the end product of the hydrolysis of cellulose. The assay conditions were optimized using the response surface methodology (RSM) based on the Box-Behnken design (BBD). The activity of a complex cellulase preparation sample assessed by the proposed amperometric assay was compared to the results obtained with spectrophotometric and viscosimetric methods.

Materials and methods

Materials

Cellulase (EC 3.2.1.4, from A*spergillus niger*, 1 U/mg, one unit liberates 1.0 µmole of glucose from cellulose in 1 min at pH 5.0 at 37 °C), glucose oxidase (GOD, EC 1.1.3.4, from *Aspergillus niger,* 17 300 U/g, one unit oxidizes 1.0 µmole of β-D-glucose to D-gluconolactone and H_2O_2 per min at pH 5.1 at 35 °C, equivalent to an O_2 uptake of 22.4 µl per min) and carboxymethyl cellulose (CMC) were purchased from Sigma-Aldrich, Germany; complex cellulase preparation (from A*spergillus niger* 29 000 U/g, 1 unit is defined as enzyme activity liberating 10 µg of glucose from CMC per 1 min at 40 °C) from TCI Chemicals, USA; and 3,5-dinitrosalicylic acid (DNS, 98%) from Acros Organics, Geel, Belgium. The CMC solution (10 g/l) and cellulase solution (100 U/ml) were prepared into 50 mM sodium acetate buffer (pH 5.0). All chemical reagents and solvents used were of analytical grade.

Optimization of the assessment conditions

For the selection of the optimal conditions for the assessment of cellulase activity, response surface methodology (RSM) coupled to Box-Behnken design (BBD) was used. The optimization with Design Expert 12 software was carried out for 3 independent variables: a) incubation time of the studied sample with CMC (X_1); b) GOD concentration (X_2); and c) time of glucose oxidation (reaction depth) (X_3). The variation ranges of these input factors were 10–70 min, 4–12 U/ml and 300–600 s, respectively, and these were coded for the design and analyses. The complete experimental design included 17 runs with five runs for central points (Table 1). Other assay conditions were fixed at their most favourable levels described afterwards.

Amperometric assessment of cellulase activity

One unit of cellulase activity can be defined as the amount of enzymes that liberates 1 µmol glucose from CMC per minute at pH 5.0 and 37 °C (Sigma-Aldrich 2021). Although different temperatures are used to define cellulose units, this unit corresponds to approximately 5 FPU calculated on the basis of glucose released. For the amperometric assessment of cellulase activity, cellulase samples were first incubated in 35 ml of 1% CMC solution (in 0.1 M of acetate buffer, pH 5.0) for 70 min at 25 °C. The sample activity was ranging from 0.5 to 20 U, defined as in (Sigma-Aldrich 2021); and this definition of the enzyme activity unit will be used throughout the

Table 1 Box-Behnken experimental design and response values for normalized output signal change

Run	Incubation time of the studied sample with CMC (X_1, min)		GOD concentration (X_2, U/ml)		Assessment time (reaction depth) (X_3, sec)		Normalized sensor signal change ΔI
	Real value	Coded level	Real value	Coded level	Real value	Coded level	
1	40	0	12	1	300	−1	0.56
2	40	0	12	1	600	1	0.64
3	40	0	4	−1	600	1	0.47
4	40	0	8	0	450	0	0.46
5	10	−1	8	0	300	−1	0.34
6	10	−1	4	−1	450	0	0.27
7	70	1	12	1	450	0	0.73
8	70	1	8	0	600	1	0.71
9	40	0	8	0	450	0	0.39
10	70	1	8	0	300	−1	0.60
11	10	−1	8	0	600	1	0.26
12	40	0	8	0	450	0	0.48
13	40	0	4	−1	300	−1	0.48
14	40	0	8	0	450	0	0.51
15	40	0	8	0	450	0	0.46
16	70	1	4	−1	450	0	0.70
17	10	−1	12	1	450	0	0.33

further description and discussion of the results. Before adding cellulase-containing samples, the CMC solution was saturated with compressed air for at least 1 h at 25 °C. At the end of the incubation, 100 µl of GOD (420 U) was injected to catalyse the oxidization of the produced glucose by dissolved oxygen (final concentration of GOD 12 U/ml). We did not use quenching to terminate the glycolytic reaction, because this reaction was followed by the enzymatic determination of the released glucose. Temperature 25 °C was applied to secure a sufficient level of dissolved oxygen (8.27 mg/L) for the glucose oxidation, and to make the procedure as simple as possible. The decrease of dissolved oxygen concentration (DOC) due to glucose oxidation was followed using a Clark-type oxygen sensor (Helox 10–15, Elke Sensor, Estonia) (Kivirand and Rinken 2009). The signal was registered under constant stirring at 1 s interval for 600 s. The normalized data was analyzed with GraphPad Prism software. The principle scheme for the amperometric assessment of cellulase activity is shown in Fig. 2.

Assessment of cellulase activity by 3,5-dinitrosalicylic acid method

The 3,5-dinitrosalicylic acid (DNS) assay is a commonly used method for the quantification of reducing sugars derived from cellulose hydrolysis based on the reduction of 3,5-dinitrosalicylic acid to the corresponding 3-amino-5-nitrosalicylic acid which results in a colour change from yellow to brick red (Deshavath et al. 2020). Cellulase preparations (100 µl) with an activity ranging from 3–20 U (0.06–0.4 U/ml) were mixed with 4.9 ml of 1% CMC solution (in 0.1 M of acetate buffer, pH 5.0) and incubated for 70 min at 25° C. At the end of the incubation, 3 ml of DNS reagent (1%) was added to 1 ml of cellulase reaction mixture and incubated for 10 min at 90 °C. This high temperature is required to increase the rate of the development of a stable red-brown colour associated with the reduction of DNSA to 3-amino,5-nitrosalicylic acid in the presence of reducing sugars. The 1% DNS reagent was prepared as follows: 1.6 g of NaOH was added to 75 ml of distilled water under continuous stirring and then 1 g of DNS was added to it. Finally, 3 g of sodium potassium tartrate was added and the remaining volume was filled with distilled water to make 100 ml (Deshavath et al. 2020). After cooling down to room temperature, the absorbance at 540 nm was measured using a UV–vis spectrophotometer (UV-1800, Shimadzu, Japan).

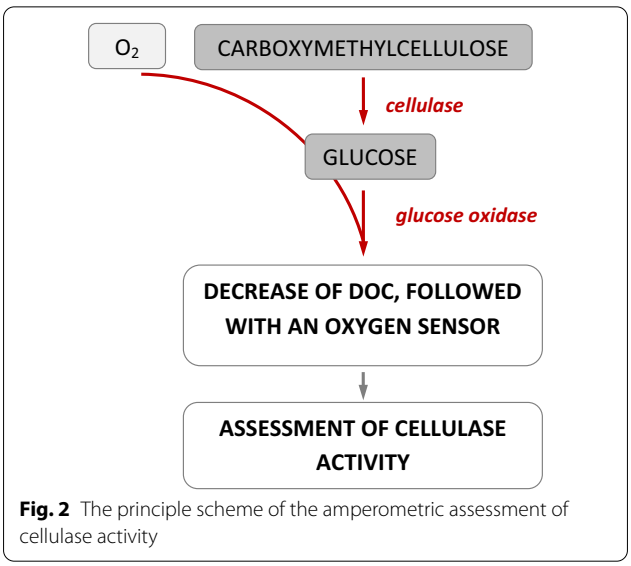

Fig. 2 The principle scheme of the amperometric assessment of cellulase activity

Assessment of cellulase activity by viscosity

Viscosity assesses mainly the endoglucanase activity cleaving randomly β-1,4-glycosidic bonds of cellulose, but also the exoglucanase activity reducing the chain size with chain-end-cleaving. To assess the dependence of viscosity on the activity of cellulases, cellulase preparations (in 0.1 M acetic buffer pH 5.0) were mixed with 1% CMC solutions (final cellulase activity ranging from 0.06 to 0.4 U/ml), and incubated for 10 min at room temperature (20 °C). After that, the viscosity of the mixture was determined using Ostwald U-Tube viscometer. All samples were measured in triplicates. The sample kinematic viscosity was calculated as following:

$$\eta = \eta_w \times t \times \frac{d}{t_w \times d_w} \quad (1)$$

where η_w is the viscosity (1.0034 mm^2/s), d_w is the density (998.2 kg/m^3) and t_w is the flow time of water; and η, d and t are the same parameters of the sample, respectively. The density of 1% CMC solution was 1004 kg/m^3.

Results and discussion

Optimization of the protocol for the amperometric detection of cellulase activity

The efficiency of the amperometric evaluation of the cellulase activity is influenced by several factors, such as the hydrolysis temperature and the pH value, the amount and concentration of CMC, the incubation time with CMC, but also by the amount of glucose oxidase used, the concentration of dissolved oxygen (DOC), rate of stirring and the depth of the glucose oxidation reaction (follow-up time of the reaction-related decrease in the oxygen concentration, further referred as assessment time). In the present study, the effect of three independent input variables such as incubation time, assessment time and glucose oxidase concentration, was optimized using surface response method with Box-Behnken design to achieve maximum normalized signal change ΔI of the

oxygen sensor. This approach allowed the determination of optimal conditions from a minimal number of runs, helped to reveal the effect of independent variables, and provided information for estimating the results to design an assay. The other abovementioned factors were kept unchanged: temperature 25 °C, pH 5.0 (0.1 M acetate buffer), the concentration of CMC 1% and the stirring speed of the magnetic stirrer was set to a constant speed to ensure uniform stirring and avoid formation of bubbles and vortices, that affect the oxygen sensor signal. All used solutions were air-saturated: the DOC level at 100% saturation at 25 °C is 8.26 mg/l (Dissolved oxygen saturation calculator 2021). The temperature 25 °C was selected as optimal to make the experimental procedure as simple as possible, although the rate of enzymatic catalysis increases along with the increase of temperature. The pH value 5.0 was chosen based on the definition of the cellulase unit (Sigma-Aldrich 2021) as the most favourable in order to achieve the highest cellulase activity. In order to maximize the glucose produced during the CMC hydrolysis, which leads to larger changes in the measured signal and consequently a higher system sensitivity, a relatively high cellulase concentration (0.57 U/ml) was used in optimization studies.

The optimization results are shown in Table 1. Based on the summary statistics of model fitting, the best model to maximize the sensitivity of the assessment was the linear model. The final equation in terms of actual factors was as follows:

$$y = 0.114363 + 0.006417 \times X_1 + 0.010625 \times X_2 + 0.000083 \times X_3 \quad (2)$$

where X_1, X_2 and X_3 are the incubation time, (GOD) concentration and assessment time, respectively. The fitting characteristics ($p < 0.0001$, $F = 34.30$) imply that the model was significant. The ANOVA test indicated that the incubation time and glucose oxidase (GOD) concentration were significant ($p < 0.05$) while the effect of the assessment time was not significant ($p = 0.5321$). The predicted by the model and experimental values of the normalized biosensor signal change were in good correspondence (Additional File 1: Fig. S1) confirmed by the small difference (~0.06) between the predicted R^2 and adjusted R^2 values (0.8020 and 0.8619, respectively). The signal to noise ratio (adequate precision) had a value of 17.59 indicating adequate signals in the entire design space.

Based on the statistical analysis and the purpose of this study to maximize the sensitivity of the system, we chose the maximum values of all input factors to build the calibration curves for the assessment of cellulase activity: the sample incubation time with CMC was 70 min, GOD concentration 12 U/ml, and the measurement time 600 s. The latter was chosen considering that by registering the biosensor output signal for a longer time, it was possible to characterize and analyse the system sensitivity (dependencies between the assessed cellulase activity and biosensor signal) at various selected time points.

Amperometric determination of cellulase activity

For the determination of cellulase activity, we used CMC. CMC is a water-soluble derivative of cellulose, which major difference is the presence of some anionic carboxymethyl groups replacing hydrogen atoms from hydroxyl groups in the pristine cellulose molecule (Rahman et al. 2021). The properties of CMC depend on the degree and uniformity of substitution, but also the degree of polymerization (DP): the solubility increases with the decreased DP and increased substitution rate, while the viscosity increases with the increased DP (Ergun et al. 2016). However, CMC is widely used to characterize endoglucanase enzyme activity due to the presence of amorphous sites which are ideal for the assessment of endoglucanase action (Nagl et al. 2021).

For the amperometric assay, we followed the decrease of DOC corresponding to the normalized output signal change ΔI (calculated as $1-I/I_0$) [35] at different cellulase concentrations (Fig. 3). As indicated above, the sensitivity of the system is determined by the difference in the normalized output signal at different CMC concentrations. Considering that, the DOC curves decrease for different cellulase concentrations (in Fig. 3) at different times (marked with grey dashed lines); we can see that the difference in ΔI increases along with the increase in the measurement period. Analysis of the slopes of the calibration plots based on the data collected at different times between 50 and 600 s revealed a hyperbolic correlation (Fig. 4). The correlation clearly indicated that the sensitivity of the assessment system does not change if we increase the assessment time over 300 s, when the sensor signal has reached 97% of the steady-state and the signal is only controlled by diffusion (Rinken and Tenno 2001).

It is also important to mention that while the shorter assessment times lead to a lower sensitivity of the system, the use of a shorter assessment time enables the detection range of the cellulase activity in the samples to be extended.

Based on these considerations, the normalized output signal change value ΔI_{300} was used to evaluate the cellulase activity The ΔI_{300} value was linearly dependent on the cellulase activity of the cellulase (between 0.5 and 15 U) with the slope $s = 0.057 \pm 0.002$ 1/U (Fig. 5). This value, showing the sensitivity of the assay, was similar to the slope of the dependence of the reaction depth (calculated with the biosensor dynamic model

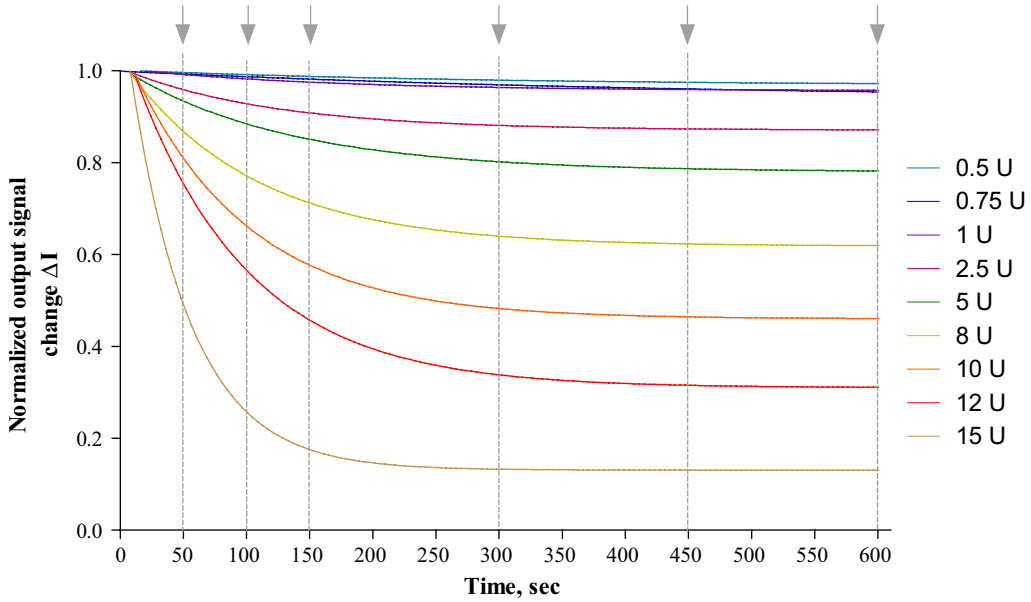

Fig. 3 The normalized biosensor output signal change (ΔI) at different cellulase activity. The cellulose samples were incubated in air-saturated 1% CMC solution in 0.1 M acetate buffer (pH 5.0) at 25 °C; the final concentration of glucose oxidase added was 12 U/ml; measurement time at constant stirring was 600 s

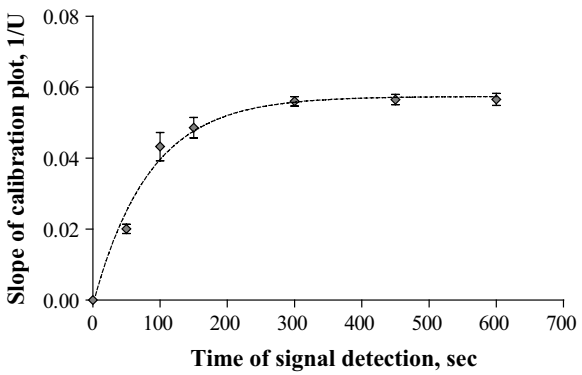

Fig. 4 The dependence of the slope of calibration plots on time of signal detection. The cellulose samples were incubated in air-saturated 1% CMC solution in 0.1 M acetate buffer (pH 5.0) at 25 °C; the final concentration of glucose oxidase added was 12 U/ml

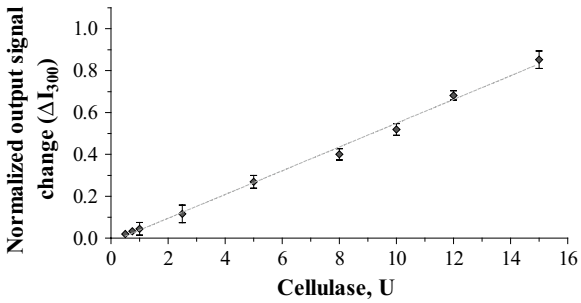

Fig. 5 Calibration plot: for the assessment of cellulase activity. All measurements were carried out in air-saturated 1% CMC solution in 0.1 M acetate buffer (pH 5.0) at constant stirring at 25 °C. Experimental conditions: CMC was incubated with cellulase for 70 min; glucose oxidase concentration was 12 U/ml; measurement time was 300 s

(Rinken et al. 1996)) on cellulase activity (Additional file 1: Table S1), which was 0.055 ± 0.001 1/U, giving an additional indication that using the optimal protocol, the maximum sensitivity of the assay can be achieved in a limited time period using the transient phase data of the process. The reproducibility of the assay was very good, as reflected by the R^2 value of 0.9928, and the relative standard deviation of the y-intercept σ was 0.013.

The limit of detection (LOD) and limit of quantification (LOQ) of the amperometric assessment were calculated as following (ICH 2005):

$$\mathrm{LOD} = \frac{3.3 * \sigma}{s} \tag{3}$$

$$\mathrm{LOQ} = \frac{10 * \sigma}{s} \tag{4}$$

where σ is the standard deviation of the y-intercept and s is the slope of the calibration curve. The LOD and LOQ values were 1.21 ± 0.06 U and 3.66 ± 0.17 U, respectively.

For testing, the results of the amperometric assessment were compared with those of the DNS method and

viscosimetry. A commercial cellulase preparation from TCI Chemicals with a declared cellulase activity 29,000 cellulase U/g was used as a model sample for this test. The apparent cellulase activity of this preparation, evaluated in triplicates with different measurement methods and expressed in units of cellulase activity per gram of solid preparation, is shown in Table 2.

Apparent cellulase activity as determined by various methods was substantially different. The amperometric assay, which measured the total glucose produced by complete hydrolysis of CMC, gave an apparent cellulase activity of 4053 ± 7 U/g, which is ~ 2 and 3 times lower than the DNS and viscosimetric methods. This lower apparent activity is likely to be caused by the low β-glucosidase activity compared to other cellulase enzymes in the commercial preparation examined. It has also been shown that an inappropriate ratio of endoglucanase, exoglucanase and β-glucosidase can lead to an accumulation of cellobiose, resulting in an inhibition of the cellulase activity (Saritha Mohanram 2015). As expected, the apparent activity obtained by the DNS method (Additional File 1: Fig. S2) was higher than the activity measured by the amperometric method, which measures the levels of reducing sugars, including both cellobiose and glucose (Gusakov et al. 2011). The apparent cellulase activity obtained with the DNS method is also explained by the fact that cellobiose, one of the main products of cellulases, is broken down by the DNS itself and measured as approx. 1.5 glucose molecules rather than one reducing end group (Gusakov et al. 2011). The viscosimetry, which mainly focuses on the assessment of endocellulase activity, showed the highest apparent activity of 12,236 ± 350 U/g (Additional File 1: Fig. S3), however, a synergistic effect between the components is required for the complete and effective hydrolysis of cellulose.

The results obtained clearly show the need to identify the method used to evaluate of cellulase activity, since commercial cellulase preparations differ extensively in the amounts of the various enzymes, which leads to fluctuations in the rate and extent of hydrolysis of cellulose substrates.

Compared to other currently available methods for the assessment of cellulase activity, the amperometric method allows a quick and specific detection of glucose, which is the final product of the hydrolysis of celluloses, in the presence of the hydrolysis intermediate cellobiose. The proposed method is simple, does not require the quenching of the glycolytic reaction, and depending on the enzymatic activity of the sample and aim of analysis, it allows the modification of the sensitivity and detection range of the assay.

Table 2 The apparent cellulase activity of a commercial cellulase preparation (TCI Chemicals)

	Assessment method	Apparent cellulase activity, U/g solid
1	Data on producer's datasheet	29 000
2	Amperometric assessment	4 053 ± 7
3	DNS method	6 590 ± 61
4	Viscosimetry	12 236 ± 350

Conclusions

The aim of the present study was to develop an assay for the determination of the cellulase activity of commercial preparations, based on the amperometric determination of glucose with a biosensor based on glucose oxidase. Glucose is the end product of the hydrolytic cascade of cellulose degradation by various synergistic enzymes. The assay conditions were optimized using the response surface methodology and the Box-Behnken design. The optimized protocol for determining the cellulase activity comprised 70-min incubation of the sample with air-saturated 1% CMC solution at 25 °C (pH 5.0), consequent injection of glucose oxidase (final concentration 12 U/ml) and registration of the decrease in dissolved oxygen due to the oxidation of the glucose produced with an oxygen sensor for at least 300 s. The modification of the assessment time allows easy variation of the detection range: shorter time results in wider detection range with lower sensitivity. The application of this protocol allowed achieving the limit of detection and quantification values of 1.21 ± 0.06 U and 3.66 ± 0.17 U, respectively. The slope of the calibration plot (system sensitivity) was 0.057 ± 0.002 1/U. The apparent cellulase activity of a model commercial cellulase preparation, which contains different synergistic cellulase enzymes, was approximately 2 times and 3 times lower with the proposed amperometric assay compared to the activities assessed spectrophotometrically, which measures the amount of reducing sugars, and to the change of viscosity, which measures the cleaving of internal bonds at amorphous sites of cellulose chains.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-022-00331-8.

Additional file 1. Figure S1. The correlation between predicted and experimental normalized biosensor output signal change ΔI = 1-I/Io. **Figure S2**. Cellulose calibration plot using 3,5-dinitrosalicylic acid (DNS) assay.

A commercial cellulase preparation from TCI Chemicals with a declared cellulase activity 29 000 cellulase U/g was used as a model sample for this testing. The sample was assessed at two concentrations: 0.36 and 0.76 U/ml. **Figure S3**. Cellulosecalibration plot using viscosimetry. A commercial cellulase preparation from TCI Chemicals with a declared cellulase activity 29 000 cellulase U/g was used as a model sample for this testing. The sample was assessed at two concentrations: 0.34 and 0.69 U/ml.

Author contributions

GN contributed to experimental work, methodology and analysis of results; KK contributed to experimental work, methodology, validation, writing and editing; SBO contributed to experimental work, methodology, validation, writing and editing; TR contributed to conceptualisation, supervision, methodology, validation, writing, and editing. All authors have read and approved the final manuscript.

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Availability of data and materials

The data and materials will be available on a reasonable request.

Declarations

Competing interests

The authors have no conflicts of interest to declare. We certify that the submission is original work and is not under review at any other publication.

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