

## Review

# Determination methods of cellulase activity

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**There are lots of assay methods used for cellulase activity that have not been unified. According to their experimental determination purpose, many research institutions adopt different methods, and do some changes according to their own experience, making determination to be more diversified. Summarizing the determination methods of cellulase activity and problems, it can provide references and assistance for the selection of methods based on different experiment purposes.**

**Key words:** Cellulase, cellulase activity, cellulose, determination method, enzyme.

## CELLULOSE AND CELLULASE

### Cellulose

Cellulose is the main component in the cell walls of plants and it is most widely distributed on earth. As the most abundant carbohydrate, cellulose is one of the most important carbon sources in the biosphere (Zhang et al., 2006). Cellulose is polysaccharide with the formula  $(C_6H_5O_{10})_n$ , where n ranges from 500 to 5,000, depending on the source of the polymer, consisting of a linear chain of several hundred to over ten thousand  $\beta(1\rightarrow4)$  linked D-glucose units (Spence et al., 2010). Due to the strong hydrogen bonds that are found between cellulose chains, through the association of hydrogen bonds, cellulose chains can form cellulose base fiber. Cellulose fibers are composed of long parallel chains of these molecules. The chains are interlinked to each other by hydrogen bonds between the hydroxyl groups of adjacent molecules, leading to the formation of crystalline regions of cellulose. A parallel orientation of adjacent chains is also favored by intermolecular hydrogen bonds. Although, an individual hydrogen bond is relatively weak, many of such bonds acting together can impart great stability to certain conformations of large molecules. As the density decreases in the pretreatment process, the combination declines, which increases the gap between molecules, orientation variation and the formation of amorphous regions of cellulose (Filson and Dawson-Andoh, 2009). In

addition to the existence of hydrogen bonds between cellulose chains, the intramolecular hydrogen bonds also exist. The geometry of the short, carbon-hydrogen bonds minimizes the distance between layers and, therefore, Van der Waals forces are maximized. These bundles are then crystallized into fibers by the same side-to-side hydrogen bonding and layer-to-layer Van der Waals forces. So cellulose is resistant to degradation due to its robust crystal structure (Pérez and Samain, 2010).

### Cellulase

Cellulase refers to a group of enzymes which, acting together hydrolyze cellulose including exoglucanase, endoglucanase and  $\beta$ -glucosidase (cellulase complex). In detail, it subdivides  $\beta$ -1,4-endoglucanases (EG I, II, III and V),  $\beta$ -1,4-cellobiohydrolases (CBH I and II), xylanases (XYN I and II),  $\beta$ -glucosidase,  $\alpha$ -L-arabinofuranosidase, acetyl xylan esterase,  $\beta$ -mannanase and  $\alpha$ -glucuronidase (Lenting and Warmoeskerken, 2001). Cellulase molecules generally have a similar structure, via the catalytic domain, cellulose binding domain and the connecting bridge (linker). As a result, cellulose can be degraded to glucose with this enzyme in synergistic action. However, a large number of bacteria, fungi and actinomycetes are known to degrade cellulose (Nagaraju et al., 2009).

In addition to the cellulase hydrolysis of cellulose into glucose and other active ingredients, plant cell contents

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improve the extraction rate by increasing the permeability of plant cell walls. So cellulose is widely used in plant-based raw materials for industrial and agricultural production.

## **DETERMINATION METHODS OF CELLULASE ACTIVITY**

### **Traditional determination methods of cellulase activity**

Cellulase activity reported a lot of determination methods. The representative and extensive methods that are widely used are subsequently shown.

#### **Thread cutting method**

With constant temperature oscillator, one end of the fine cotton thread was immersed in a test tube containing the enzyme solution (that is, the optimal temperature and pH), being micro-oscillation. Thus, the enzyme activity can be obtained by determination of the required time cut for fine cotton-thread (Thörig et al., 1984).

#### **Filter paper collapsing method**

With the constant temperature oscillator, that is, utilizing certain rules of the filter paper as substrate and adding a tube containing enzyme solution, micro-oscillation were carried on an optimal temperature and pH. According to the determined time required for the complete collapse of the filter paper, we can obtain the enzyme activity. Since determining the filter paper collapse time is difficult, so the error is large.

On this basis, a weight loss method of the filter paper was developed. In the best conditions of the temperature and pH, the filter paper and enzyme reaction lasted for a longer period of time, and then drying and determining the quality of the filter paper were done before and after the change of reaction. Consequently, while the weight loss of the filter paper was calculated, the enzyme activity was also calculated (Toyama et al., 2007).

#### **Spectrophotometric method**

Generated carbohydrates in enzymatic reaction with the chromogenic agents occur chromogenically in the reaction, and the absorbance is measured with the spectrophotometer at wavelength around 500 nm. The spectrophotometric method greatly reduces the time required for the determination of enzyme activity, and has a higher accuracy as the most widely used method (Coleman et al., 2007). According to their experimental

purposes in many research institutions, different substrates and chromogenic reagents are used, and thus many methods are derived from the spectrophotometric method.

#### **Flat band method**

In the strain selection, we expect to screen the bacteria producing cellulase in the flat, which can greatly improve the efficiency. Phosphate expansion cellulose was divided into two uniform layer plates, so that the cellulase can form clear and transparent circle in the plate. Hence, this method was widely used in screening the fungal that produced cellulase (Jang et al., 2007).

#### **Branch and swain method**

With salicylic acid as substrate, according to the amount of the hydrolyzed salicylic alcohol and  $\beta$ -D-glucose, we can determine  $\beta$ -glucosidase activity by colorimetry. This method is commonly used in  $\beta$ -glucosidase activity determination (Bhat, 2000).

#### **CMC method**

The determination of the CMC viscosity reduction method makes use of the rotating viscometer. The method is based on the function of enzymes which shorten the lengths of cellulose molecules in a viscous solution of pretreated biomass and cause it to become less viscous. The effect of the cellulase enzymatic activity on the viscosity of CMC is determined at  $35.0 \pm 0.05^\circ\text{C}$  by comparing the flow rate in a viscometer with that of deionized water. Several types of automated or semi-automated viscometers are available to survey the rheological changes of a pretreated cellulose solution. Viscosity is one of the fundamental rheological parameters that characterize the resistance of the fluid to flow. The viscosity of a pretreated cellulose solution is related to the hydrolysate concentration, the extent of the CMC-solvent interaction and the cellulose structure such as molecular shape, weight, molecular conformation and molecular flexibility (Lee et al., 2007).

The method of reducing sugar increase is done through the substrate and the enzyme reaction under certain conditions, with the increase of glucose to calculate the enzyme activity (Zhou et al., 2004). This method is currently used widely to measure the activity of cellulase.

#### **Determination of CMC cellulase activity liquefaction method**

With chrome alum as a cross linking agent of the

carboxymethyl cellulose that is cross-linked to higher viscosity of the gel, produced through observing the extent and speed of the liquid gel, we can determine the extent of the enzyme activity (Liu et al., 2008). This method is simple and visual, thus, screening bacteria is very convenient.

### **Main methods used for the determination of cellulase activity**

In recent years, most of the new methods used to determine cellulase activity via the DNS principle was that after the enzymatic hydrolysis of the cellulose was formed, the reducing sugar can reduce the nitro of 3,5-dinitrosalicylic acid (DNS) to amino, thereby generating a reddish brown colour for amino compounds (Vancov and Keen, 2009). In a certain concentration range, the amount of reducing sugar and the color of brown present a positive correlation. It can be detected by the spectrophotometric method.

### **CMCase activity**

Through the amorphous cellulose as substrate, CMCase activity can be characterized by a formation of reducing sugar. Amano method uses sodium carboxymethyl cellulose, while the International Pharmaceutical Federation provided hydroxyethyl cellulose, although, Merz method introduces phosphate to expand the cellulose (Mehmet et al., 2006).

### **PNPCase activity**

p-Nitrophenol-D-cellobioside (PNPC), as the substrate and scale of nitrobenzene formation, evaluate enzyme activity, and sometimes when avicel acts as the substrate, it is called avicelase activity. However, endoglucanase has high degree of avicel hydrolysis, in terms of the original enzyme solution, which reflects the synergistic effect of the cellulase components' results (Bhat et al., 1997). As a single component, it reflects the exoglucanase activity.

### **Cellobiase activity**

Cellobiose as the substrate and amount of glucose formation of enzymatic reaction, indicates the cellobiase activity, and because both glucose and cellobiose have reductive sugar, it is difficult to distinguish them (Ghorai et al., 2010).

### **Filter paper activity**

The International Association of Theoretical and Applied

Chemistry Commission (Fermentation Committee) determined the standard method of filter paper activity in 1984. The filter paper (as the natural crystalline cellulose that forms the substrate of the reducing sugar by cellulose hydrolysis) has been widely used and it indicates the total saccharification of the cellulose enzyme capacity (Olivova et al., 2009). However, due to the structural heterogeneity of the filter paper and the method palaver, which leads to larger error with determination results, it is difficult to accurately calculate the results quantitatively.

### **Differential gravimetry**

A crucible of sand cores as the enzymatic reactor, avoiding deviation of the hydrolyzate produced during the transfer, but not having to reflect the termination of the enzyme solution added agents, can direct filtrate and terminate the reaction. It has lots of advantages, such as saving reagents and avoiding interference of the reagent. The measured rate of enzymatic hydrolysis of the reducing sugar content is higher than the conventional measure of enzyme hydrolysis, while the test operation is simple, well reproducible and suitable for the small sample test of enzyme.

## **PROBLEMS AND PROSPECTS**

### **Problems in the determination of cellulase activity**

There are many determination methods of cellulase activity which have not been unified, but there are many difficulties in the determination; so the development and utilization of cellulase brought lots of disadvantage effects (Parsiegla et al., 2008). Three main problems exist in the process of determination: (1) According to the General Principles of enzyme kinetics, enzyme activity should be measured in the presence of excess substrate conditions, where the initial velocity of enzymatic reaction stands for cellulase activity. However, the various components of a multi-component cellulase enzyme synergistically form a variety of end products, involving multiple feedback control mechanism. The principle of initial velocity measurements would not reflect the substrate characteristics, thereby making the standardized method of determining the enzyme activity difficult; (2) Cellulose does not have high-glycans solubility in water, so cellulase and its reaction is carried out in the solid interface, where the enzymatic reaction rate is being affected by the substrate of the enzyme protein adsorption rate and the diffusion rate of the product. Different sources of the same type of cellulose, whose composition and proportion of each component are quite different, makes the determination of the inconsistent results; (3) Due to the complexity of the structure of cellulase composition and biochemical characteristics and enzymatic effects as well as the

presence of topoisomerase, the obtained purity of each component is inconsistent under different test conditions, resulting in the composition and nature of the classification of cellulase system, which is not completely unified in view.

### Option in the determination of cellulase activity

Methods of determining cellulase activity are heterogeneous thereby making the selection of specific methods to be considered as the specific purpose of the determination. For example, in the biochemical study, the determination of the cellulase activity is done for a single component, so it is necessary to select a specific substrate and buffer. In microbial test, the operation is mainly screened for enzyme production strains and mutant strains, on account of the larger number of determination, thus a more simple method can be used without the need for a very high accuracy. However, in industrial production, the conversion of cellulose to glucose is mainly done for industrial fermentation, where glucose is the most important indicator of output, so we can choose high-purity substrates such as filter paper, cotton, etc (Nóra et al., 2004). On the other hand, the enormous amount of work also needs to be considered in industrial production. For this reason, some of the more simple method can be used.

### Prospect in the determination of cellulase activity

Cellulose is the most important world's annual output of renewable resources. If we can work out successful development and utilization of the resources, sustainable development will have great significance (Malešič et al., 2005). The environmental and ecological being pointed at issues in the interest of today's society, like how the cellulase can be successfully used to fulfill cellulose degradation is the main objective of the researchers, while the determination of cellulase activity is the focus of the study (Aisien et al., 2009). Nonetheless, how to find one kind of simple, rapid and accurate determination of the enzyme activity will become a strong point of the entire cellulase research, so cellulase application shifts into a new era.

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