Comparison of the Determination Methods on Mannanase Enzyme Activity

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Abstract

Two determination methods were employed in this paper to measure the enzyme activity of mannanase. The mechanism of one method was to measure the reducing sugar releasing rate, while the mechanism of the other method was to measure the apparent viscosity change. Experiment results showed that both two method can measure the enzyme activity of mannanase and the results of two methods have obvious linear relationship of y=23.085x+4.01. In addition, the enzymatic degradation mechanism, merits and drawbacks and the application was discussed in the paper. In conclusion, the enzyme activity measured by reducing sugar releasing rate and viscosity reduce efficiency can be convert with the linear regression equation. Also, the former method can be used to research in the applications which is relate to the reducing sugar like food or feed and the latter method can be used to research in the applications which is relate to viscosity such as gel-breaker of hydraulic fluids.

Keywords

Mannanase, guar gum, enzyme, determination method.

1. Introduction

Mannanase is the enzyme to hydrolyze the complex polysaccharides of plant tissues into simple molecules such as manno-oligosaccharides and mannoses. [1] It has been widely discovered from animals, plants, fungi, yeasts and bacteria. Inside, microbial mannanase was the main source, such as *Aspergillus sp*., Bacillus *sp*. *Thermotoga sp*., *Caldicellulosiruptor sp*., and *Dictyoglomus sp*. [2] The enzyme was widely applied in the industry of paper and pulp, food and feed, coffee extraction and so on. Morover, the most important application of mannanase is the enzyme breaker to degrade the guar-based fracturing fluid after the hydraulic fracturing. [3] As the unconventional resources development, hydraulic fracturing has been a commonly used technology to stimulate the oil and gas production. [4] After hydraulic fracturing, the fracturing fluid in the oil or gas formation must be flow back to the surface through viscosity reducing. Guar gum acted as the thickening agent in the hydraulic fracturing. It was also a natural polysaccharides extracted from plants. Therefore, mannanase, used as gel-breaker, is employed to degrade fracturing fluids to reduce the fluid viscosity to facilitate fluid flow-back. [5]

According to the extensive use, the evaluation method of mannanase was important in the enzyme application. Generally, enzyme activity was determined through the measurement of catalytic products increase or the decrease of substrate. Usually, the enzyme activity of mannanase was accurately measured through the releasing rate of reducing-sugar. However, the reducing sugar was not the concerned parameter in many industry. In the hydraulic fracturing, the viscosity was the most crucial parameter. Therefore, the viscosity change was used to measure the enzyme activity of mannanase as the gel-breaker. Nevertheless, the difference of these two methods was unaware.

The objective of this study was to investigate the relationship of two determination methods on mannanase enzyme activity. Based on the mechanism of enzyme hydrolysis, the reducing sugar-releasing rate was determined by reducing sugar-DNS method. In addition, the viscosity-reducing efficiency was determined by the change of apparent viscosity. Also, the relationship of these

methods and the different applications of these measurement methods was discussed. To the knowledge of authors', there is no related reports on the different of these two method. The researches in this paper will supply the more accurate and convenient measurement method for the researchers and worker in the enzyme industry.

2. Materials and Methods

2.1 Materials

Substrate: the hydrolytic substrate is guar gum supplied by Wanbo Chemical Products Co., Ltd., Henan, China and other reagents were analytical grade and produced by Chinese Medicine Group Chemical Reagent Co., Ltd. Deionized (DI) water was used for the preparation of all aqueous solutions.

Mannanase: the enzyme was produced by liquid fermentation from bacterial strain of Bacillus sp. isolated by the lab of microbial enhanced oil recovery in the China University of Petroleum (East China). The enzyme fluid was centrifuged at 25 °C 3000 rpm for 10 min to remove the impurity and filtered through the 0.22 μm filter membrane to remove the bacteria. The liquid can be used as the crude enzyme solution in the following tests.

3, 5-dinitrosalicylic acid agent: 500 mL DNS solution was prepared containing 3.25 g DNS, 162.5 mL 2.0 mol·L-1 NaOH and 7.5 mL propanetriol.

Reducing sugar standard solution: D-mannose was diluted in the DI water at the different concentrations of 0.0, 0.4, 0.8, 1.2, 1.6, 2.0 mg/L.

2.2 Rheology Curves

Brookfield DV-III viscometer was used to measure the the rheology curves of guar gum solution at the guar gum concentration of 1.0~8.0 g/L.

2.3 Enzyme Activity Measurements

2.3.1 Reducing-sugar Release Rate

The measurement of reducing-sugar release rate was used as the typical method to determine the enzyme activity. [6] The concentration of reducing sugar was assayed using the 3, 5-dinitrosalicylic acid agent (DNS).

Before the measurement of enzyme activity, the standard curve of reducing sugar and absorbance must be plotted first. The reaction was 2.0 mL DNS solution and 2 mL reducing sugar standard solution. And then the mixed solution was heated at at 100 °C for 2.0 min. Thereafter, the tubes were cooled in the cold water to room temperature and 6 mL DI water was added in the reaction system to dilute the solution. The absorbance of solutions can be measured at 540 nm using the spectrophotometer (721, produced by Shanghai Precision Scientific Instrument Co., Ltd.). The standard curve was plotted using reducing sugar content against absorbance.

In the enzyme activity measurement, the substrate was 1mL 4.0 g·L-1 guar gum solution and the enzyme fluid was 1 mL. The mixture was incubated at 50 °C for 60 min, and the reducing sugar change can be measured by the standard curve. Therefore, the crude enzyme activity can be defined as Eq. (1).

$$E = \frac{m_1 - m_0}{t \times V} \quad (1)$$

Where, E was enzyme activity, $\mu g \cdot mL^{-1} \cdot min^{-1}$; m_1 and m_0 were the reducing sugar content after enzyme reaction and control treatment, respectively, μg; t was the reaction time, min; V was the volume of crude enzyme fluid, mL.

2.3.2 Viscosity-reduce Efficiency

Fluids viscosity was the more concerned parameter in many industries, especially in the oil field development industry. The concentration of substrate was 4.0 $g \cdot L^{-1}$, duo to the usage of guar gum in the fracturing fluid. The volume ratio of substrate and enzyme was 10:1 and the reaction temperature

was 50℃, and the reaction time was 2h.The enzymatic viscosity-reduce efficiency defined as Eq. (2). The apparent viscosity was measured by a rotating viscometer (NDJ-1-1B, Shanghai Changji Co., Ltd., China). [7]

$$\omega = \frac{\mu_0 - \mu}{\mu_0} \times 100\% \quad (2)$$

Where, μ_0 and μ were the initial and real-time apparent viscosity of guar gum solution, mPa·s; ω was viscosity-reduce efficiency, %.

3. Results and Discussion

3.1 Rheology of Guar Gum Solution

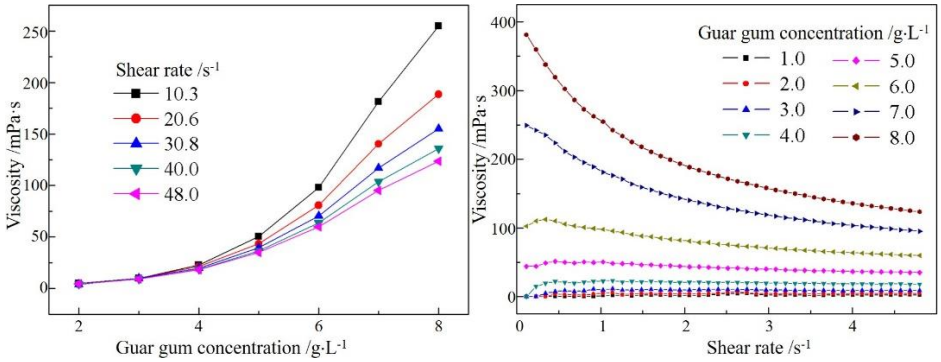

Fig. 1 Effect of concentration on the rheology curve of guar gum solution

Fig.1 showed the rheology curve of guar gum solution at different guar gum concentrations. The guar gum has good thickening ability. As the concentration increase, the viscosity increase significantly. Also, when the concentration is lower than 4.0 g/L, the rheology curve demonstrated the characteristic of Newtonian fluids. The viscosity was almost changed as the increasing of shear rate. However, when the concentration was higher than 4.0 g/L, the rheology curve showed the features of non-Newtonian fluids and the viscosity will decrease as the shear rate increasing. [8] Therefore, the apparent viscosity of the guar gum solution will be measure in the same shear rate.

3.2 Standard Curve

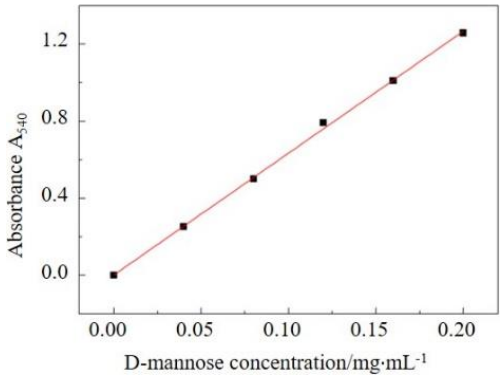

Fig. 2 Standard curve of D-mannose concentration and absorbance

Fig.2 showed standard curve of D-mannose concentration and absorbance. The linear regression equation was y=6.322x+0.003 and the correlation coefficient was 0.9990. The linear relation showed that the DNS method has high sensitivity to measure the reducing sugar, which implied that this measurement method of enzyme activity will be also sensitive through the measurement of reducing sugar releasing rate.

3.3 Enzyme Activity

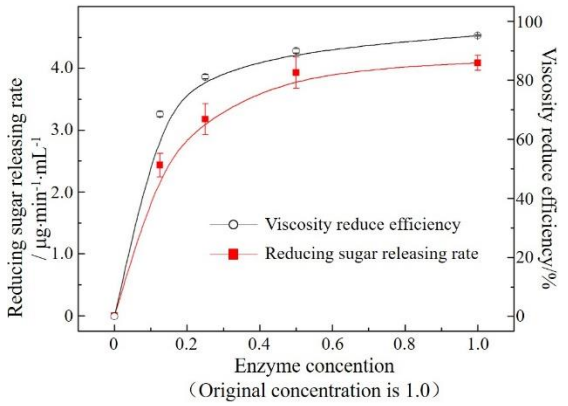

Fig. 3 Measurement results of enzyme activity using two methods at different enzyme concentrations

Fig.3 showed that the measurement results of two enzyme activity methods. The original enzyme fluid was diluted into 1/2, 1/4, 1/8 and then measurement the enzyme activity. The enzyme activity will reduced as the dilution and the change has the synchronization. In Fig. 4, two enzyme activity method showed the obvious linear relationship and the linear regression equation was y=23.085x+4.01 and the correlation coefficient was 0.9779. Therefore experiment results implied that two enzyme activity method has the strong correlation and both can be used to measure the enzyme activity. However, Fig.3 also showed that the error bar of reducing sugar releasing rate was larger than the other, which means these measurement result can be influenced more easily.

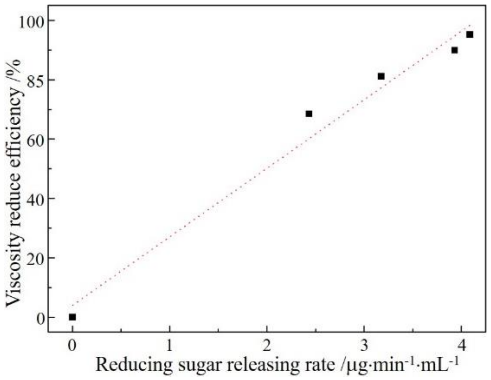

Fig. 4 Relationship of the enzyme activity determination by two methods

4. Discussion

As the second most abundant heteropolymer, hemicellulose has the two most important and representative hemicelluloses: hetero-1,4-β-D-xylans and the hetero-1,4-β-D-mannans. The former is comprise the major hemicellulose component in hardwoods and grasses, while the latter are more prominent in the hemicelluloses of softwoods, plant seed etc. For example, guar gum is a kind of representative hemicellulose and is widely used in many industries, especially in the oil or gas development. Guar gum is extracted from the seeds of *Cyamopsis tetragonolobus*. The guar gum molecule consists of a chain of (1→4)-linked β-D-mannopyranose units with α-D-galactopyranose units connected to the mannose backbone through (1→6) glycosidic linkages, with a mannose-to-galactose ratio of 1.2 to 1.8. [9]

The excellent thickening property of guar gum results from the its long polysaccharide chain and high molecular weight. Therefore, mannanase is often used as the enzyme gel-breaker to reduce the viscosity at the end of hydraulic fracturing. The mechanism of enzymatic degradation of mannanase was that: In the enzyme reaction the backbone chain of guar gum was cut by the β-mannanase. [10] The viscosity depends on the dimensions of the polymer chain and lower intrinsic viscosity means shorter chain. [11] In addition, in the enzymatic degradation, the backbone chain cut result from the enzyme attack on the endo- and exo-β-1, 4 linkages of backbone chain. The cleavage of these bonds,

especially the β-1, 4 linkages in the D-mannose backbone reduces the polymer molecular weight and the thickening ability. In this process, the sugar units will be released. Therefore, these dissociative sugar will be the new product and can be used to indicate the enzyme activity.

The viscosity reduce efficiency method depends on the viscosity parameter, so this method cannot be used in the low concentration solution. And the usage of substrate relays on the apparent viscosity measurement, so in this paper, the usage of guar gum solution is higher than 30 mL. However, the viscosity measurement was more convenient and faster than the measurement of reducing sugar. Moreover, the viscosity measurement can be successive with no suspension of enzymatic reaction, which has important significance in the enzyme research and industrial application.

The reducing sugar releasing rate method is more accurate to measure the enzyme activity. Also, the error bar in Fig.3 showed that the experimental result was more easily influenced by the measurement environment duo to the low concentration of reducing sugar released. The measurement is also more complex to prepare regents and to measure the standard curve. In addition, this method can be used in all the concentration theoretically, however, it is difficult to accurately draw 1 mL high viscosity solution accurately, so this method is not available for the high viscosity substrate solution. Besides, this method is not successive and the enzymatic reaction has to end at some time point. However, the requirement of substrate usage is far less than viscosity measurement. According to the mechanism, it was more attractive in the application relate to the sugar release such as food or feed. [12]

5. Conclusion

In the present study, we came to the following conclusions:

(1) The enzyme activity measured by reducing sugar releasing rate and viscosity reduce efficiency can be convert with the linear regression equation of y=23.085x+4.01.

(2) The reducing sugar releasing rate method can be used to research in the applications which is relate to the reducing sugar like food or feed. Also, this method was suitable for the low usage of substrate.

(3) The latter method can be used to research in the applications which is relate to viscosity such as gel-breaker of hydraulic fluids. Also, this method was available for the continuous determination.

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