

Enzymatic hydrolysis ability and mechanical strength of cellulose fibres

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Abstract

The correlation between the mechanical strength and the enzymatic hydrolysis of cotton fibers was studied. In the course of research, it was proved that not strong, defective fibers (not suitable for industry) are more exposed to enzymes. Moreover, the stronger the fiber, the higher its technological properties, the higher the resistance of the fiber to biodegradation. It was also found that the fibers of diseased cotton plants are hydrolyzed at the highest rate compared to fibers of healthy plants. Moreover, the efficiency of enzymatic hydrolysis for some studied fiber samples can reach up to 77% in the yield of sugar substances. In addition, for the first time it was established that enzymatic hydrolysis is a characteristic feature of the strength of cotton fiber, which allows us to develop new biological criteria for assessing the strength of fiber in industry.

Keywords: Mechanical strength of the fiber, enzyme, enzymatic hydrolysis, technological properties of the fiber, bio degradation, the effectiveness of enzymatic hydrolysis, sugar substances

1. Introduction

Biocatalysis is attracting more and more attention to obtain low-molecular substances - reagents for directed synthesis [1]. It is known that the type of plant feedstock and the method of cellulose extraction from it determine the physical and chemical properties of the substrate, therefore, determine the reactivity to enzymatic hydrolysis [2-4]. That is why the dependence of fermentolysis of cellulose substrates on the characteristics of cellulose (degree of crushing, crystallinity, polymerization, the presence of non-cellulose impurities, etc.) is the subject of many studies [3, 5-6, 7].

2. Biodegradation of crystalline cellulose

During hydrolysis of cotton cellulose, a joint action occurs between "strongly" and "weakly" adsorbing enzymes. The amount of adsorbed cellulose depends not only on the specific surface area, but also on other structural properties of the cellulose-containing material itself for example, on the crystallization and degree of polymerization. According to the data, there is a linear relationship between the crystallinity index of cellulose substrates that do not contain lignin and their reactivity. The higher the crystallinity index, the lower the rate of enzymatic hydrolysis. It is believed that cellulolytic enzymes more easily hydrolyze more amorphous sites than crystalline sites of cellulose. In the crystalline part of the substrate almost all hydroxyl groups are included in the formation of intramolecular and inter molecular hydrogen bonds.

3. Materials and methods of research

The objects of research were samples of fibres of genetically different cotton lines taken from the collection of the laboratory of private genetics of cotton of the National University of Uzbekistan. The samples differed in their genetic characteristics and appearance.

Enzymes. In the work, there were used technical-commercial preparations of enzymes from fungi of the genus *Aspergillus*, in particular, active cellulose *Aspergillus foetidus* and *Tricholomataceae*, in particular, *Trichoderma viride*, made at the Volga Biochemical Enzyme Plant (Russia). The content of enzymes in mentioned preparations was as follows:

- Celloviridin GZH (exoglucanase - 83 unit/g, cellobiase - 10 units/g, endoglucanase 50 units/g)
- Pectophytidine GZKH (cellobiase - 3200 units/year, pectinase - 80 unit/year),
- Pectinase 500 (cellobiase - 64 units/year, pectinase - 90 unit/year)

Reagents: To determine glucose, horseradish peroxidase (activity 350-400 thousand units/year) of the firm, Reanal (Hungary), and glucose oxidase (Lviv, Ukraine) with a specific activity of 74000 units/year.

The kinetics of enzymatic hydrolysis of cotton fibre was studied at 40°C, at pH 4.5 (acetate buffer) in a stirred reactor, which is a thermostatic cell with a volume of 50 ml, equipped with a magnetic stirrer. Solutions of enzymatic preparations dissolved in the acetate buffer were added to the fibre suspension [B.T. Yuldashev., 1991]. Determination of glucose in the mixture during hydrolysis was carried out using spectrophotometric methods [Klesov A.A., Rabinovich M.L. et al., 1980]. The total amount of reducing sugars was determined by the method of Shomodi-Nelson [Klesov A.A., Rabinovich M.L. et al., 1977]. Cellular activity by cotton fibre was determined by the method [Chose T., Montencour B.S. 1981].

Statistical processing of results was carried out with the help of the computer program "Microsoft Excel" using generally accepted statistical criteria [Lakin G., 1990].

4. Finding

In heterogeneous systems, such factors as the reactivity of glucoside bonds, the degree of crystallinity and polymerization of substrates, the size of available surface, the polydispersibility of cellulose, the presence of foreign components in the substrate (e.g., lignin, etc.) have the most important influence on the hydrolysis rate of cellulose fibres. The study of such systems is complex because of the large number of factors that have a direct impact on the enzymatic process and its kinetics. Nevertheless, in experiments on hydrolysis of cellulose substrates, a direct relationship between the hydrolysis rate and the mechanical strength of the fibre is found: the higher it is, the slower the enzymatic process.

Figure. 1 shows the results of kinetics on the effect of substrate concentration on the hydrolysis rate of cellulose fibres in Linunwer-Berk coordinates. From the presented data, it is visible that at the hydrolysis of cotton fibres with low mechanical durability value S_{max} is high (fig.1, curve 1) and the substrate is hydrolyzed with high speed. With an increase of mechanical strength of fibres, the speed of hydrolysis decreases.

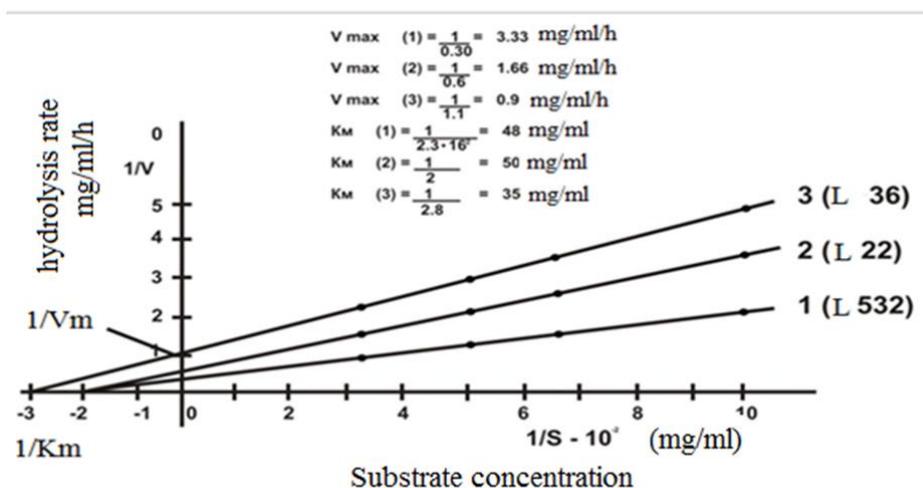


FIGURE-1. Effects of Substrate Concentration on Hydrolysis Speed at Linunwer Burke Coordinates

Thus, for example, at the hydrolysis of cotton fibres of L-532, L-22 and L-36 lines the maximum hydrolysis speed $V(m)$ was 3.33; 1.66 and 0.9 mg/ml/hour, respectively (Fig 1, curves 1.2 and 3). The value of $K(m)$ increases with increasing mechanical strength of fibres of genetically different cotton lines and is 35.50 and 48 mg/ml for fibres of lines L-532, L-22 and L-36, respectively.

It is known that the strength of fibres depends not only on cotton varieties but also on the accumulation and formation of cellulose in it. The higher the strength of fibres, the greater their orderliness and crystallinity. Fibres with lower strength arising from diseases, fibres of immature, fallen cotton boxes contain more amorphous areas of cellulose. These cotton line samples also have

lower levels of crystallinity and polymerization. Studies have shown that ripened cotton fibres with high orderliness and high strength undergo less enzymatic hydrolysis. As can be seen from the data in Fig.2, the lower the strength of the fibres, the higher the hydrolysis of cellulose fibres. Thus, for example, cotton fibres with a strength of 5.2 g.s. (L-36), hydrolysis at a lower speed than cotton fibres with 3.2 g.p. strength. (JI-532). Due to their enzymatic hydrolysis in the reaction medium, the content of glucose and BC is 1.054 and 4.9 g/l, against 3.069 and 9.2 g/l, respectively (Fig. 2). Comparative analysis of the obtained data allowed revealing a direct correlation between strength and hydrolysis capacity of fibres with different strength parameters.

However, in some cases there is a discrepancy, so, for example, if the mechanical strength of the fibres is relatively high, they are subjected to fermentative hydrolysis with high speed.

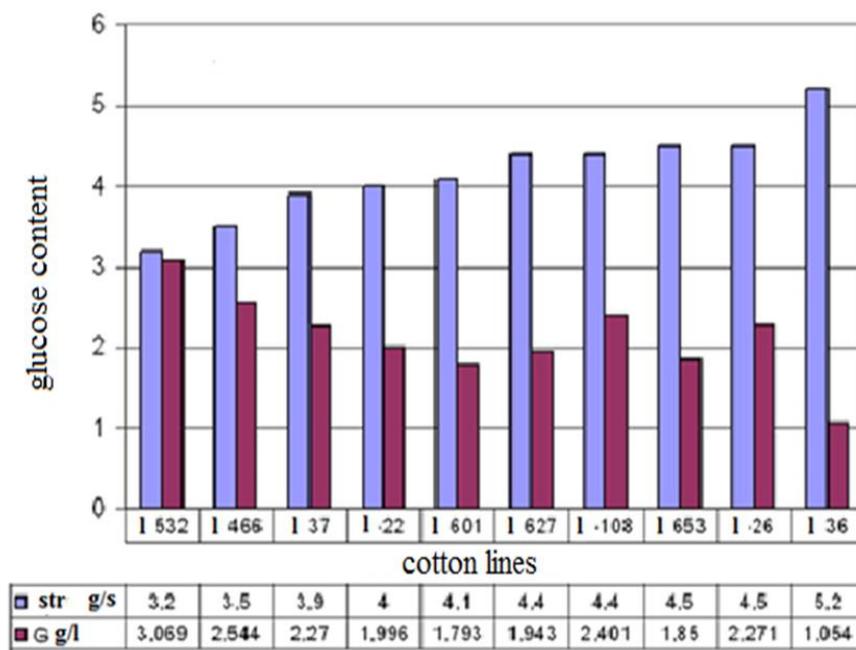


FIGURE 2. Correlation between strength and biohydrolysis properties of cotton fibres

The collected data showed that there is a direct correlation between their hydrolyzability and mechanical strength. Further, there were studied some mechanical and physical properties of fibres of genetic different lines of cotton and their enzymatic hydrolysisability. From the data of Table 1, we can see that the strength of the fibres of lines L-627 and F-108 is the same (4.4 g.p.), but the hydrolysisability (after glucose yield) is different. The fibre of F-108 line has a linear density of 6020 teks, relative breaking load 26,5 g.p. teks, accumulation of hydrolysis product - glucose is equal to 2,401 g/l. Meanwhile, for L-627 with a higher linear density of 7640 teks and a relative breaking load of 33,8 g.p. teks, biodegradation is less expressed and glucose yield at the end of the process is only 1,94 g/l. It is possible to observe similar regularities for lines L - 653 and L - 26 by results of hydrolysis

Nº	Lines	Strength g.s	Linear density tex	Relative breaking load g.s. tex	Glucose output g.l	VS Concentration g/l
1	L-532	3,2	6080	19,5	3,069	11,2
2	L-466	3,5	7200	25,2	2,544	8,80
3	L-37	3,9	6390	24,9	2,270	7,73
4	L-22	4,0	6920	27,7	1,996	7,23
5	L-601	4,1	6330	26,0	1,793	7,19
6	L-627	4,4	7640	33,8	1,941	6,32

7	F-108	4,4	6020	26,5	2,401	5,57
8	L-653	4,5	5600	25,2	1,850	5,70
9	L-26	4,5	5440	24,5	<u>2,271</u>	6,01
10	L-36	<u>5,2</u>	4380	22,8	<u>1,054</u>	4,90

TABLE 1. The relationship between mechanical and physical properties and enzymatic hydrolysisability of fibres of different cotton lines.

5. Conclusion

Based on the obtained data, we can conclude that the technological properties of fibre can significantly affect the process of fibre destruction. It should be noted that the strength of the fibre has great importance and plays a major role in the process of enzymatic hydrolysis.

The following characteristic features of hydrolysis are grouped, which determine the strength of cellulose fibres by an accumulation of hydrolysis product - glucose in the reaction mixture. So,

- a) resistant - (L - 36, L - 501, L - 525, L - 602),
- b) medium resistant - (L - 12, L - 12-1, L - 654),
- c) unstable - (L - 468, L - 469, L-532).

The analysis of the number of common reducing sugars showed identical results both for the degree of hydrolysis and for evaluation of fibre strength estimation in the tested samples.

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