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Enzymatic Hydrolysis of Cotton Fibers in Supercritical CO_2

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Abstract A study was carried out on the application of supercritical fluid to the hydrolysis of boll fibers of cotton (cultivar Tashkent-6 of *Gossypium hirsutum* L.) by cellulase enzymes from *Trichoderma viride*, *Trichoderma reesei* and *Aspergillus niger*. Conditions of the enzymatic process were optimized. The stabilities of cellulase enzymes were sustained at the pressure of up to 160 atm for 48 hours at 50°C in supercritical carbon dioxide.

Keywords: cellulase, cotton fibers, glucose, supercritical carbon dioxide

INTRODUCTION

Bioconversion of cellulose-containing waste matter belongs to topical problems of biotechnology. Utilization of cellulose materials is known to produce great amounts of wastes whose processing can yield usable products, including carbohydrates.

Cellulase enzymes process cellulose under certain conditions, cleave glucoside bonds, reduce the degree of cellulose polymerization, and eventually cleave it to glucose. The majority of natural substrates are resistant to enzymatic cleavage, because of their crystallinity [1]. Therefore, development of technologies for decrystallization is a hard task, and use or readly hydrolyzable matter is of prime importance.

Cotton processing industry wastes (such as a cotton lint, linter, bur, stalks, cottonseed hulls, chiganak, cotton dust, linter waste of oil works) have been found to be little prone to hydrolysis due to their highly crystalline state or the high content of lignin in some of them [2,3]. For this reason, we choose kind of raw material not investigated previously: cotton fibers from bolls of early autumn picking. This matter is discarded unprocessed, although it is rich in cellulose (75-80%). Note that cellulose fibers are amorphous at this age, and their enzymatic degradation requires no pretreatment [4].

Supercritical fluids are materials at temperature and pressure above their critical points. Above the critical point, molecular thermal energy exceeds the attractive forces between molecules, and a gas-like/a liquid like states exists. Consequently, the properties of supercritical fluids bridge the gap between the properties of liquids and gases. Supercritical fluids offer several advantages for enzymes. It may allow better control on reaction rates and selectiveness, and simplification of product separation and recovery may be made possible.

Supercritical fluids serve as a particularly interesting class of solvents which may be used for enzyme-catalyzed reactions [5]. The enzymatic hydrolysis reaction in supercritical carbon dioxide to produce glucose from cellulose was investigated in 1996 by Zheng and Tsao [6]. In comparison with the result from the enzymatic hydrolysis reaction of cellulose without carbon dioxide introduced as a reaction medium, the reaction rate and glucose concentration are increased.

Substantial improvement in the yield was possible in supercritical medium as compared to that obtained in the hydrolysis carried out in atmospheric condition [7].

We carried out the enzymatic hydrolysis of cotton fibers from bolls of early autumn picked in supercritical carbon dioxide and the result was compared with that in atmospheric condition.

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MATERIALS AND METHODS

Materials and Enzymes

We studied cotton cultivars Tashkent-6, Omad, 149-F, Termez-31, C-6037, Ashhabad-25 of species *G. hirsutum* L., *G. barbadense* L., *G. arboreum* L. and *G. herbaceum* L. maintained at the Laboratory of Cytology and Genetics of Zaitsev Agricultiral Research Institute, Republic of Uzbekistan. We used 30-day-old fibers of Tashkent-6 for a comparative study of enzymatic hydrolysis of cotton fibers in a supercritical carbon dioxide and atmosphere conditions.

The cultivar Tashkent-6 belongs to the early group (115-120 days from sprouting to ripening); its raw cotton yield is 39.5-45.0 ton/ha. The bulk of the harvest (85-90%) ripens in September. Fibers are of Type V. The raw cotton yield per boll is 6.0-6.5 g.

Enzymatic degradation of cotton fibers was done by cellulase enzymes from *Trichoderma viride* (6.9 units/mg), *Trichoderma reesei* (10.4 units/mg) and *Aspergillus niger* (1.18 units/mg). These enzymes were purchased from Sigma Chemical Co., USA (one unit liberate 1.0 mmole of glucose from cellulose in one hour at pH 5.0 and at 37°C)

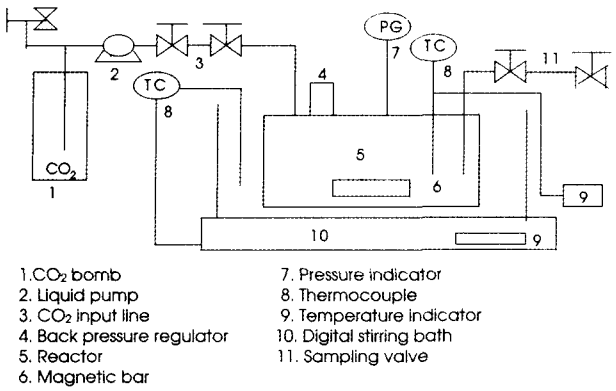

Fig. 1. Shematic diagram of apparatus.

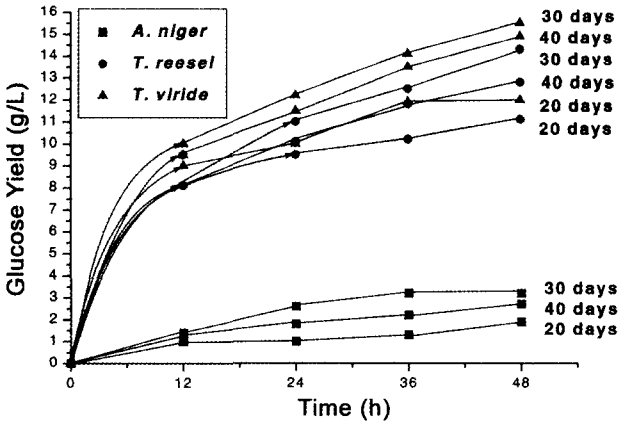

Fig. 2. Comparative 48-h hydrolysis of "Omad" cultivar cotton fibers by cellulases of various origin; 20 g/L of substrate, 6 g/L of cellulase, 100 rpm, pH 5.0, and 50°C.

Apparatus and Analytical Methods

The experiments were performed in Shaking Incubator (Korea Manhattan Co.) with 15-20 mL temperature-controlled (50°C) cells, speed control (100 rpm) at pH 5.0 and 0.1 M acetate buffer and in the batch reactor system, shown in Fig. 1.

The reaction vessel (250 mL working volume) made of SS 316 and designed to sustain the pressure of up to 250 atm, was placed in a digital water stirring bath equipped with a temperature controller. The constant temperature inside the vessel was maintained within the accuracy +0.5°C. The reaction vessel was filled with 0.1 M acetate buffer (pH 5.0) and flushed out air with carbon dioxide (99.99% of purity) from a dip-tube container. The reaction vessel was enclosed and carbon dioxide was immediately pumped into the reaction vessel using a LDC Analytical Minipump (Gearmotor, USA) until the desired pressure was reached and the pressure was maintained with a back pressure regulator (Tescom Co., USA). The reaction mixture was agitated by a magnetic stirrer. After a designated length of time kept at a certain pressure and temperature, the sample was immediatly taken out through the sampling line and then analyzed to measure the glucose concentration. The stability of enzymes in supercritical carbon dioxide was determined by incubating an enzyme in each supercritical condition. The relative enzyme activity was measured at optimum condition of atmospheric pressure.

Determination of Glucose and Reducing Sugars

The concentration of glucose in the solution was determined by glucose oxidase-peroxidase method [8] and a glucose analyser (YSI 1500, USA). The content of total reducing sugars (RS) was determined by a modified Somogyi-Nelson method [8]. Optical density was measured in a spectrophotometr (Shimadzu UV-1201, Japan). RS were assayed at 620 nm; glucose, at 480 nm. The concentrations were: substrate, 2%; enzyme, 0.6%. Temperature was 50°C.

RESULTS AND DISCUSSION

Experiments on stability of cellulases were carried out in supercritical carbon dioxide. The activity of cellulase was found sustained at the pressure of up to 160 atm. No change of activity was observed at the temperature of up to 50°C, which was an optimum temperature for the cellulase activity at ambient pressure. The activity, however, decreased down to 50% at 70°C and the pressure of 200 atm. The cellulase activity was mostly maintained at the pressure of 120 atm for 48 h and 50°C. This observation is also in accordance with the experimental results [9]: in the hydrolysis of cellulose the glucose yield showed its maximum at 50°C, the temperature at which activity of cellulase was the highest.

For enzymatic hydrolysis of cotton fibres used commercial enzymes of a various origin of Sigma Chemical Co., USA. In Fig. 2, results of comparative hydrolysis of a fibres of a cotton cultivar "Omad" are submitted under optimum conditions by cellulase enzymes from *Trichoderma viride, Trichoderma reesei* and *Aspergillus niger* during 48 h.

As it is well visible from the diagram, the maximal yield of glucose is observed at 48 hour hydrolysis of 30-days-old fibres by cellulase from *Trichoderma viride* and makes 15.5 g/L. These data also prove to be true calculations on a degree of conversion of an enzyme-substrate complex of 20, 30 and 40-days-old cotton fibres on weight (Table 1). Thus the degree of conversion varied from 33.9% (*A. niger*) up to 92.3% (*T. viride*). And only in a case cellulase enzyme from *Trihoderma viride* the degree of conversion about 90% was observed.

In this work various cultivars and species of a cotton plant 20, 30 and 40-day's age were used. As it was revealed in the previous researches, these fibres in view of more amorphous structure in them, than crystalline, with the maximal degree of conversion are exposed to hydrolysis. Besides 20-40-days-old fibres are compound waste products of cotton-growing the autumn-winter

Table 1. Degree of conversion after 48-hourly hydrolysis of "Omad" sort cotton fibers at different days of maturation by cellulases of various origin

Days of maturation	*A. niger*	*T. reesei*	*T. viride*
20	53.9%	90%	92.3%
30	33.9%	80.8%	91.5%
40	38.4%	77.8%	87.7%

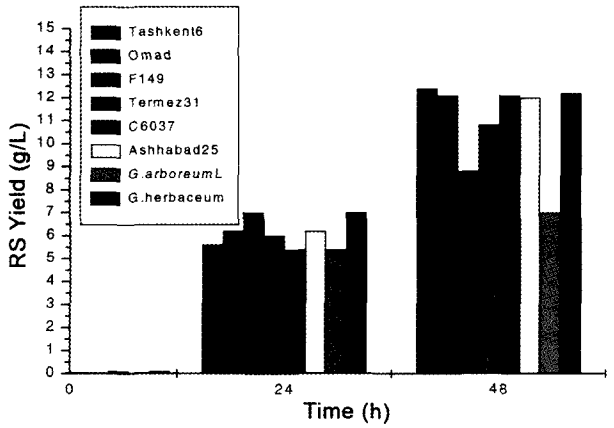

Fig. 3. Comparative 48-h hydrolysis of different cotton fibers by cellulases from *Aspergillus niger*: 20 g/L of substrate, 6 g/L of cellulase, 100 rpm, pH 5.0, and 50°C.

collecting [4]. For the first time we carry out a comparative hydrolysis of cotton fibres cultivars and species most sowed in republic of Uzbekistan in supercritical carbon dioxide. In Fig. 3, the results of reducing sugrars (RS) yields are shown at 2, 24, 48 h hydrolysis of such fibre by cellulase from *Aspergillus niger*. Despite of some distinctions in reducing sugars (RS) yields which speak first of all distinction in precocity and qualitative parameters of a fibre, significant deflections are not observed. Therefore for continuation of experiments in supercritical carbon dioxide we used the 30-days-old fibres of cultivar Tashkent - 6 (*G. hirsutum* L.).

Table 2 summarizes some results of experiments on comparative 48 h enzymatic hydrolysis of 30-days-old cotton fibres of cultivar Tashkent-6 performed in supercritical and atmospheric conditions. Despite of significant deviations at use of various enzymes at different pressures, the yields of glucose in supercritical carbon dioxide almost always is higher than at atmospheric condition. Distinctions in a glucose yields depending on used enzymes speak differences in structure of enzymes of cellulase complex and activities of its each separate component. The maximal yields of glucose was observed at use of cellulases from *Trichoderma viride* during 48 h at supercritical condition (120 atm, 50°C) and has made 13.9 mg/mL.

As shown on Fig. 4, glucose yield at 50°C and 160 atm are more than 6 g/L for 24 h of reaction time in super-critical condition, whereas 48 h of reaction time was required for such a yield at atmospheric condition.

Table 2. Glucose yields (g/L) at 48 h hydrolysis of 30 days-old cotton fibers of cultivar Tashkent-6 (*G. hirsutum* L.) in supercritical and atmospheric conditions by cellulases from *Aspergillus niger* and *Trichoderma viride*; 20 g/L of substrate, 41 units/mL of cellulase, 100 rpm, pH 5.0, and 50°C

Enzyme reaction	Hours of hydrolysis	*A. niger*	*T. viride*	*T. viride* + *A.niger*
Atmosphere	6	3.0	7.0	6.4
	12	4.1	8.6	7.2
	24	5.4	9.8	9.5
	36	5.9	10.6	10.9
	48	6.0	11.0	10.6
Pressure		160 atm	120 atm	100 atm
Supercritical CO_2	6	3.3	8.2	8.5
	12	4.4	11.2	9.5
	24	6.4	12.6	9.9
	36	7.1	13.5	10.7
	48	7.2	13.9	10.7

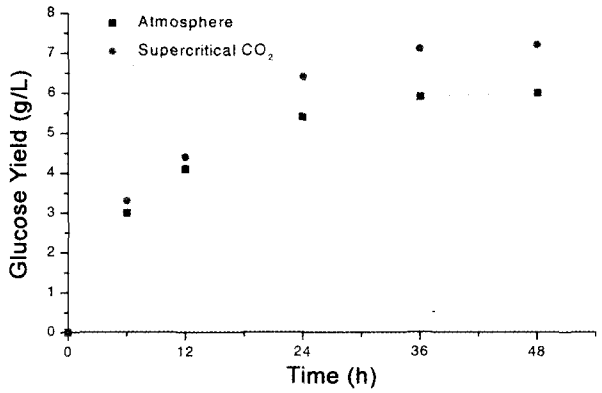

Fig. 4. Profiles of 48-h hydrolysis of 30 days-old cotton fibers of cultivar Tashkent-6 (*G. hirsutum* L.) in supercritical (160 atm) and atmospheric conditions by cellulases from *Aspergillus niger*; 20 g/L of substrate, 41 units/mL of cellulase, 100 rpm, pH 5.0, and 50°C.

In the work [10], where the hydrolysis of starch was performed, the same trend was observed. Glucose yield in supercritical carbon dioxide was increased by 1.2 times as compared to that in atmospheric condition.

CONCLUSION

The purpose of the present work was to improve the current methods of cellulose biodegradation and study of possibility of using cellulases from different sources on their activity toward cotton fibres of different species as well as conducting of enzymatic hydrolysis of cotton-growing wastes in supercrtical fluids in compare to atmosheric condition. The results of our experiments are as follows:

1) Optimal condition for conducting this experiment is: pressure 120 atm at 50°C for 48 h.

2) The highest activity was showed by cellulase enzymes from *T. viride* (degree of conversion up to 92.3%).

3) The best substrate is 30-days old cotton fibres from Tashkent-6 (*G. hirsutum* L.), although there is no big difference in compare to fibres from other cultivars and species.

4) The total productivity of glucose in supercritical carbon dioxide was about 1.2 times of that obtained at atmospheric pressure.

These results indicate that the method of supercritical carbon dioxide can be considered further as one of potential ways of bioconversion of wastes of cotton growing. But at the same time the given problem demands further more detailed studying depending on concrete conditions of realization of the hydrolysis, used enzymes, and substrates.

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