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BIOCONVERSION OF COTTON CELLULOSE TO GLUCOSE BY SUPERCRITICAL CO_2

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Cellulose-containing wastes from cotton production and reprocessing of crude cotton are known to be valuable sources of raw material for enzymatic hydrolysis (EH) to produce sugars and glucose [1, 2]. Immature fibers (unopened bolls with fiber) from the fall cotton harvest have been proposed as an alternate raw material for their production [3]. However, yields of reducing sugars (RS) and glucose were low. Therefore, the search for methods of increasing the yield of RS and glucose is an important problem of modern biotechnology. Recent publications in this area indicate that the use of supercritical (SC) liquids in the EH processing of various cellulose-containing substrates increases substantially the yields of RS and glucose [4, 5]. Herein we communicate results from a study of the effect of supercritical CO_2 on the yields of glucose obtained from EH of cellulose fiber from various cotton varieties and species.

The accumulation of cellulose and the degree of crystallization during growth of cotton fiber are important factors in carrying out EH. Table 1 shows that the investigated cotton fibers differ sharply in cellulose contents, i.e., they ripen early and accumulate cellulose during fiber growth and development. Investigations of the change of yield during various growth stages showed that it decreased, especially during ripening, as the fibers grew regardless of the early ripening and cellulose content. This phenomenon was observed for reactions at both atmospheric and SC pressures. Thus, for example, fiber of Tashkent-6 variety with 12-h hydrolysis and atmospheric pressure gave glucose yields for 30-day growth of 7.2 mg/mL; for 40- and 60-day growth, 6.5 and 3.9 mg/mL, respectively.

Analogous trends were noted for fibers hydrolyzed at SC pressure with the difference that the glucose yield in this instance was slightly higher. Thus, whereas the glucose yield for 30-day fiber was 11.2 mg/mL, those for 40- and 60-day were 7.3 and 5.8 mg/mL, respectively. Also, fiber of Termez-31 variety with 48-h hydrolysis and 120 atm pressure had glucose yields for 30-day fiber of 20.8 mg/mL; for 40- and 60-day, 19.7 and 8.8 mg/mL, respectively. This is explained not by the content of cellulose synthesized during plant growth (Table 1) but by the compaction of the supermolecular structure from fiber growth. The amount of cellulose in 30-day fiber was less for all studied fibers than for 40- and 60-day fiber. Nevertheless, the glucose yields for them were greater than for the others due to denser packing, close to crystalline, for the molecules of 40- and 60-day fiber [6]. Crystalline cellulose is known [7] to be resistant to EH, which is also evident from the glucose yields for 60-day fiber. At this age glucose yields drop sharply due to structural factors, the crystalline structure. The described mechanism is typical of fibers of all studied varieties and species.

Glucose yields from SC pressure were measured at 1, 100, 120, 140, and 160 atm. We found that glucose yields increased at SC pressure up to 120 atm and decreased at higher pressures. Apparently the substrate and enzyme surfaces were in good contact up to the optimal (120 atm) pressure. Then both the enzyme activity and the structural deformation of the enzyme surface decreased. Glucose yields from EH of fiber from cotton varieties and species at SC conditions in the presence of CO_2 were 1.3-1.4 times greater than at atmospheric conditions. Analogous results have been reported before [8]. A sharp contrast in glucose yields from SC pressure was observed by comparing them with the length of the EH. As the hydrolysis time increased, the glucose yields increased. Glucose was produced for up to 36 hours of hydrolysis. The maximum yield at both atmospheric and SC pressure occurred at 48 h of EH for 30-40-day fiber.

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TABLE 1. Glucose Yield from EH of Cotton Fiber at Atmospheric and SC Pressures by Celluloses from *Trichoderma viride*, mg/mL (50°C)

Cotton variety	Fiber age, d	Cellulose content, %	Pressure, 1 atm				SC pressure, 120 atm			
			Hydrolysis time, h							
			12	24	36	48	12	24	36	48
					G. hirsutum L.					
Tashkent-6	30	78.3	7.2	8.8	9.5	10.8	11.2	12.6	13.5	15.5
	40	83.9	6.5	7.1	8.3	9.3	9.3	10.8	11.9	13.9
	60	92.9	3.9	4.3	5.2	5.8	6.0	6.5	7.8	8.4
149-F	30	75.3	8.7	9.1	9.7	10.5	12.5	13.0	13.9	15.0
	40	89.2	7.4	8.4	9.1	9.8	11.1	12.0	13.0	13.9
	60	94.7	3.8	4.5	4.8	5.3	5.5	6.5	7.0	7.7
					G. barbadense L.					
Termez-31	30	74.1	9.6	11.6	13.0	14.3	13.8	16.6	18.5	20.8
	40	83.5	9.1	10.1	11.4	13.1	13.0	15.7	17.6	19.7
	60	94.0	3.7	4.4	6.9	5.7	5.8	6.7	7.8	8.8
Ashkhabad-25	30	71.3	10.4	12.0	13.3	14.9	14.7	18.0	19.8	22.3
	40	86.0	9.4	11.6	12.7	14.1	13.9	17.1	19.0	21.4
	60	93.1	3.9	4.6	5.9	6.1	6.1	7.1	8.0	9.0
					G. arboreum L.					
C-7059	30	75.0	9.4	11.7	12.7	15.3	13.3	17.1	18.8	21.6
	40	87.0	8.4	10.5	13.3	14.7	12.6	16.2	18.0	21.3
	60	91.7	3.7	4.3	5.3	6.0	5.8	6.7	7.6	8.5
HCP_{05}	-	-	0.21	0.24	0.31	0.37	0.21	0.24	0.31	0.37

The results indicate that both the hydrolysis conditions (SC pressure, reaction time, temperature, etc.) and the fiber age have a great influence on the EH and degree of conversion of cellulose from cotton fiber of different age and varieties (different species) into glucose. The maximum glucose yield for the same age occurs at 48 hours of hydrolysis for 30-40-day fiber. Therefore, immature 30-40-day cotton fiber with cellulose of amorphous structure can be recommended as the substrate for producing sugars without their preprocessing. SC pressure of CO_2 has a substantial effect on the EH, increasing the reaction rate and glucose yield by 1.3-1.4 times.

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