

## Specific features of enzymatic hydrolysis of cellulose

Michael Ioelovich \*

*Designer Energy Ltd, 2 Bergman Str., Rehovot 7670504, Israel.*

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### Abstract

In this article, the features of enzymatic hydrolysis of cellulose samples with different structural characteristics were studied. It was discovered that outer paracrystalline layers of crystallites are accessible and susceptible to hydrolysis along with the poorly ordered amorphous domains of cellulose. The thermodynamic analysis showed that the hydrolysis process of cellulose samples at 298 K is exothermic. Since reaction enthalpy is negative and the temperature-entropy factor is positive, the Gibbs potential of this process becomes negative, which contributes to the implementation of enzymatic hydrolysis of cellulose. Moderate enhancement in temperature to optimal value, 323 K, increases the negative value of Gibbs potential and thereby promotes the hydrolysis of cellulose substrates. The most negative Gibbs potential was observed for the hydrolysis of the most accessible amorphous cellulose. Thus, amorphization of cellulose substrates facilitates enzymatic hydrolysis. On the other hand, the enzymatic hydrolysis of high-ordered crystalline domains of cellulose cannot be performed even at optimal temperature since the Gibbs potential of this process is close to zero.

**Keywords:** Cellulose; Structural characteristics; Enzymatic hydrolysis; Features; Thermodynamic analysis

### 1. Introduction

Glucose is the most abundant monosaccharide in nature [1]. This monomeric carbohydrate is produced in all land plants and most algae through photosynthesis using sunlight, water, and carbon dioxide, after which it is converted into polysaccharides such as cellulose and starch [2-4]. In addition, glucose is an integral part of disaccharide molecules such as sucrose of sugar cane and milk lactose. Glucose is the main energy source for the cells of living organisms.

Glucose enters the organisms of herbivores and omnivores as a result of enzymatic and bacterial hydrolysis of cellulose and starch feeds in the digestive system of these animals [5]. Humans do not contain active cellulolytic enzymes and bacteria, and therefore, they obtain glucose mainly through the enzymatic breakdown of starch-rich foods or sucrose [6]. The normal concentration of glucose in the fasting blood of humans should not exceed 5.6 mmol/L or 100 mg/dL [7]. Higher glucose level in the blood indicates the onset of diabetes.

In addition to its physiological significance, glucose is of great importance due to its widespread use in medicine, food, and chemical industries. Currently, glucose is produced mainly from starch by enzymatic hydrolysis [8], which has practically replaced the less profitable and harmful acid hydrolysis. Various crops can be used as a starch source for glucose production such as maize, wheat, barley, potato, etc. In the USA, maize corn starch is used almost exclusively as a feedstock for enzymatic production of glucose.

However, the use of various starch sources as feedstocks for glucose production creates an acute problem because these crops are required by the food and feed industry. Moreover, further expansion of glucose production in large volumes

\* Corresponding author: Michael Ioelovich

could cause a shortage of agricultural land areas, increased expenses, a deficit of food and feed products, and a rise in their prices [9, 10].

Production of glucose from non-edible cellulose substrates has been regarded as a promising way to obtain this valuable bioproduct without competing with the food industry. In particular, purified scraps of pulp, paper, cellulose fabrics and fibers, as well as cotton residues (e.g. linter, fuzz, etc.) and some other cellulose wastes can be used as promising and relatively cheap raw materials for enzymatic hydrolysis to produce the glucose [10]. Besides, huge amounts of pretreated cellulose-enriched plant materials (forest and agricultural wastes, bushes, grasses, etc.) can be used for enzymatic hydrolysis to convert cellulose into glucose.

Features of enzymatic hydrolysis of cellulose and cellulose-enriched materials have been discussed in many studies [11-15]. Currently, to implement effective hydrolysis of cellulose, enzyme preparations were used, which include at least three types of specific enzymes:

- Endo-1,4- $\beta$ -glucanases that cleave chemical glycoside bonds mainly in the amorphous domains of cellulose fibrils; as a result, the fibrils are split with the formation of small particles with a reduced degree of polymerization;
- Exo-1,4- $\beta$ -glucanases that attack the reducing or non-reducing ends of the depolymerized cellulose particles by forming oligomeric products containing di- and tetra-saccharides;
- $\beta$ -glucosidases that hydrolyze the oligosaccharides and convert these into glucose.

These enzymes act synergistically because endo-acting enzymes generate new chain ends for the exo-acting enzymes, which release the oligosaccharides that are converted into glucose by  $\beta$ -glucosidases.

Enzymatic hydrolysis of cellulose is usually carried out at pH 4.5-5.0 and temperature 298-328 K using a dose of enzyme preparation of 10-40 mg of protein per 1 g of cellulose substrate. Hydrolysis of wet cellulose is carried out faster and more completely than dried cellulose [11]. It has been found that to achieve maximum glucose concentration during enzymatic hydrolysis, the optimal loading of cellulose substrate in the aqueous enzyme system should be at least 150 g/L [16-18]. At a higher substrate loading, enzymatic hydrolysis ceases due to a significant reduction in mass transfer and inhibition of cellulolytic enzymes by a large amount of formed glucose [19].

Numerous studies have shown that when using cellulose substrates, a problem arises due to their low enzymatic digestibility. This is explained by the crystallinity of cellulose, which is considered the most important structural factor that prevents enzymatic hydrolysis [11, 20, 21]. Therefore, it should be expected that a decrease in the crystallinity and an increase in the amorphicity of cellulose should contribute to a rise in the hydrolyzability degree of the substrate.

To answer the question of whether the crystallinity and amorphicity of cellulose are the main characteristics influencing enzymatic hydrolysis or whether this process is determined by other structural factors, additional studies are needed. Therefore, in this research, the enzymatic hydrolysis of cellulose samples with different structural characteristics was studied. In addition, thermodynamic analysis was performed to predict the enzymatic hydrolyzability of various cellulose substrates.

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## 2. Materials and Methods

### 2.1. Materials

Various semicrystalline cellulose samples having crystalline structure of CI were used. Refined and bleached cotton cellulose (CC), Kraft pulp (KC), and Sulfite Pulp (SP) were purchased from Buckeye Technologies, Inc. The cellulose samples were additionally purified by extraction with boiling 2% NaOH and boiling water; then samples were washed with deionized water to a neutral pH value and squeezed to and squeezed to remove water excess. Microcrystalline cellulose (MC) was prepared by hydrolysis of CC with boiling 2.5 N HCl for 1h [22]; then MC sample was washed with deionized water to a neutral pH value and squeezed to remove water excess. The cellulose of Switchgrass (SC) was isolated from the biomass by the K $\ddot{u}$ rschner-Hoffer method followed by extraction with boiling 2% NaOH, washing with deionized water to a neutral pH value, and squeezing to remove water excess. Amorphous cellulose (AC) was prepared by dissolving MC in cold 7% NaOH/12% Urea solvent with following regeneration [23]; then, the regenerated AC sample was washed with deionized water to a neutral pH value and squeezed to remove water excess.

The main characteristics of the used cellulose samples are shown in Table 1.

**Table 1** Some characteristics of cellulose samples

Sample	Abbreviation	$\alpha$ -Cellulose, %	DP
Microcrystalline cellulose	MC	99	180
Cotton cellulose	CC	98	2700
Kraft pulp	KP	97	1200
Sulfite pulp	SP	98	1100
Cellulose of Switchgrass	SC	95	740
Amorphized cellulose	AC	93	170

## 2.2. Methods

### 2.2.1. Characterization of samples

The content of alpha-cellulose in the samples was determined by the standard TAPPI T203 method [24]. The average degree of polymerization (DP) was measured by the viscosity method using diluted cellulose solutions in Cadoxen [25].

### 2.2.2. Enzymatic hydrolysis

Wet cellulose samples were hydrolyzed by a commercial enzyme preparation Cellic CTec-3 (Novozymes A/S, Bagsvaerd, Denmark) containing endo-1,4-glucanases, exo-1,4-glucanases (EXG), and  $\beta$ -glucosidases. The dosage of Cellic CTec-3 was 30 mg per gram of dry sample. Samples containing 1 g of solids and 1 mL of 50 mM acetate buffer (pH 4.8) were placed into 50-mL polypropylene tubes and supplemented with the required amount of the enzyme and an additional buffer volume to provide the substrate loading of 150 g/L. The tubes covered with caps were incubated for 10-120 h at 323 K with constant shaking. Glucose concentration was determined by HPLC using an Agilent 1200 Infinity Series system (Agilent Technologies, United States) with an Amines HPX-87H column. The mobile phase was 0.005 M sulfuric acid; the flow rate was 0.6 mL/min at 318 K. Hydrolyzed samples were preliminarily filtered through a 0.45- $\mu$ m nylon filter. The hydrolyzability degree (H) of the samples was calculated using the equation:

$$H = C_f/C_m \quad (1)$$

where  $C_f$  is the final concentration of glucose after the finish of hydrolysis of cellulose sample;  $C_m = 169.5$  g/L is the maximum concentration of glucose achieved after the complete hydrolysis of cellulose substrate at loading of 150 g/L.

### 2.2.3. Wide-angle X-ray scattering (WAXS)

Pre-dried cellulose samples were pressed into tablets with a diameter of 16 mm and a thickness of 2 mm. The experiments were carried out on a Rigaku-Ultima Plus diffractometer (CuK $\alpha$ -radiation,  $\lambda = 0.15418$  nm) in the  $\varphi = 2\theta$ -angle range from 5 to 50  $^\circ$  using a reflection mode. Collimation included a system consisting of vertical slits and Soller slits. The procedure of 0.02  $^\circ$  step-by-step scanning was used to determine the exact position of the peaks. After recording the diffraction patterns, the incoherent background was subtracted and the peak intensities were corrected. Corrections include absorption and LP coefficients, and Rietveld refinement. The lateral size of crystallites was calculated using the following equation:

$$D = \lambda / \cos \theta_{200} (B^2 - b^2 - \Delta^2)^{1/2} \quad (2)$$

where B is the width of the (200) peak (in radians);  $\theta_{200}$  is Bragg's angle at the (200) peak maximum; b is the instrumental factor, and  $\Delta$  is the correction on lattice distortion.

Then, the paracrystallinity degree of crystallites was calculated [26]:

$$p = 4h (D - h)/D^2 \quad (3)$$

where  $h \approx 0.4$  nm is the thickness of external paracrystalline layers of crystallites.

#### 2.2.4. Enthalpy of interaction with water

The standard enthalpy of the interaction of the dry cellulose samples with water, i.e., wetting enthalpy ( $\Delta_w H$ ) of the samples was measured using a TAM Precision Solution Calorimeter. Before starting the experiments, the wet sample was put into a special glass ampoule and dried in a vacuum at 378 K to constant weight. The glass ampoule containing the dry sample was sealed and introduced into the calorimetric cell filled with the liquid. The calorimeter was thermostated at 298 K to achieve an equilibrium state. After that, the sealed ampoule with the dry sample was broken to ensure that the cellulose sample to contact with the liquid. The released exothermic heat effect was measured with accuracy  $\pm 0.01$  J. Three of the same samples were tested to calculate a reliable enthalpy value and standard deviation.

The degrees of cellulose crystallinity ( $X$ ) and amorphicity ( $Y$ ) were calculated as follows:

$$X = 1 - (\Delta_w H / \Delta_w H_{am}) \quad (4)$$

$$Y = 1 - X \quad (5)$$

where  $\Delta_w H_{am} = -27.2$  kJ/mol is the standard wetting enthalpy of completely amorphous cellulose (AC) [27].

In addition, the standard enthalpy of dissolution ( $\Delta_{dis} H$ ) of dry crystalline glucose in water was determined.

#### 2.2.5. Enthalpies of combustion and formation

Combustion of the pre-dried samples was carried out in a stainless-steel calorimetric bomb having a volume of 0.320 dm<sup>3</sup> at an oxygen pressure of 3.05 MPa with 1.00 cm<sup>3</sup> of deionized water added to the bomb. The combustion measurements were carried out by an isothermal water calorimeter at 298 K with an accuracy of  $\pm 0.001$  K. The value of the energy equivalent of the calorimeter determined by standard benzoic acid was 15802.3  $\pm$  0.9 J/K. The true mass of the sample used in each experiment was determined from the mass of the produced CO<sub>2</sub>. The correction of combustion energy for ignition and some other corrections were considered. To adjust the experimental combustion energy to standard conditions, T=298 K and P= 0.1 MPa, the Washburn correction was introduced. Finally, to calculate the standard enthalpy of combustion ( $\Delta_c H$ ), the correction for the change in the number of moles of gases before and after combustion was introduced. For each sample, five experiments were performed to calculate the reliable value of combustion enthalpy and standard deviation.

The standard enthalpy of formation ( $\Delta_f H$ ) of one mole of the repeating anhydroglucose unit (AGU) of cellulose or one mole of glucose (GL), having the general formula C<sub>a</sub>H<sub>b</sub>O<sub>c</sub>, can be calculated from the known Hess equation:

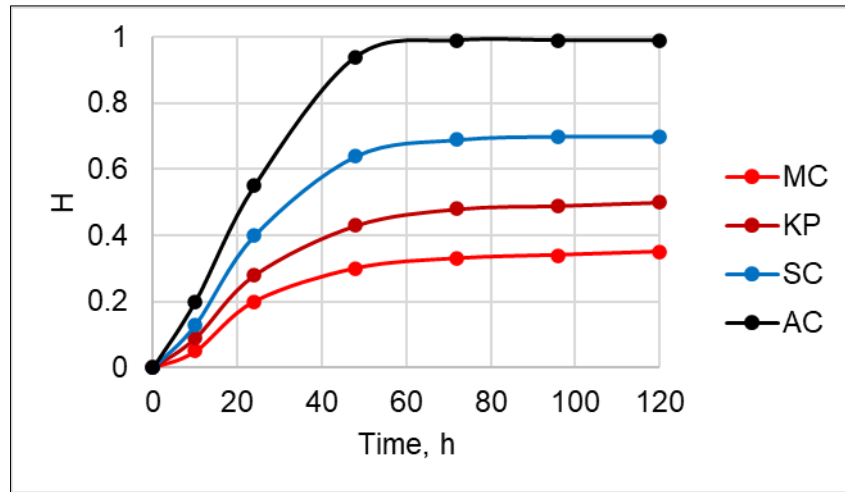
$$\Delta_f H = a \Delta_f H (\text{CO}_2, g) + 0.5b \Delta_f H (\text{H}_2\text{O}, l) - \Delta_c H \quad (6)$$

where  $\Delta_c H$  is the measured standard enthalpy of combustion;  $\Delta_f H (\text{CO}_2, g) = -393.51$  kJ/mol and  $\Delta_f H (\text{H}_2\text{O}, l) = -285.83$  kJ/mol are standard enthalpies of the formation of carbon dioxide and liquid water, respectively, the values of which are given in reference books.

## 3. Results and Discussion

### 3.1. Enzymatic hydrolyzability of cellulose

Kinetic studies of enzymatic hydrolysis of cellulose samples have shown that at least three days are required to achieve the final glucose concentration and hydrolyzability degree (Figure 1).

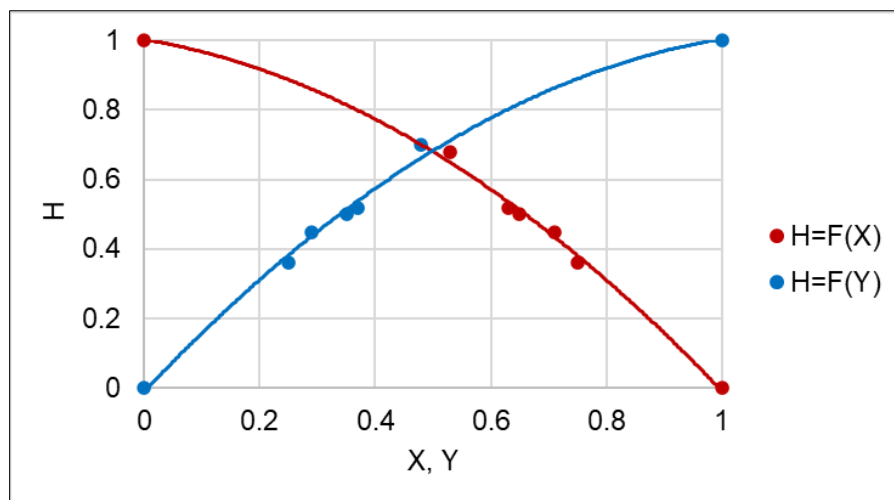


**Figure 1** Kinetical curves of enzymatic hydrolysis of some wet cellulose samples

Moreover, the higher the crystallinity degree ( $X$ ) of the samples, the lower the final concentration of glucose ( $C_f$ ) and hydrolyzability degree ( $H$ ) of these substrates (Table 2). Vice versa, the increase in amorphicity degree ( $Y$ ) of cellulose leads to a rise in  $C_f$  and  $H$  values.

**Table 2** Structural characteristics and hydrolyzability of cellulose samples

Cellulose	X	Y	D, nm	p	A	$C_f$ , g/L	H
MC	0.75	0.25	10.2	0.15	0.36	61.0	0.36
CC	0.71	0.29	8.1	0.19	0.43	72.0	0.42
KP	0.65	0.35	7.0	0.22	0.49	84.7	0.50
SP	0.63	0.37	6.1	0.25	0.53	88.5	0.52
SC	0.53	0.47	3.5	0.41	0.69	118.0	0.70
AC	0	1	-	0	1	169.5	1



**Figure 2** Dependence of final hydrolyzability of wet cellulose substrates on their crystallinity ( $X$ ) and amorphicity ( $Y$ ) degree

However, the hydrolyzability value of all studied semi-crystalline cellulose samples is higher than their amorphicity degree (Figure 2). This means that not only accessible amorphous domains undergo hydrolysis, but also cellulose

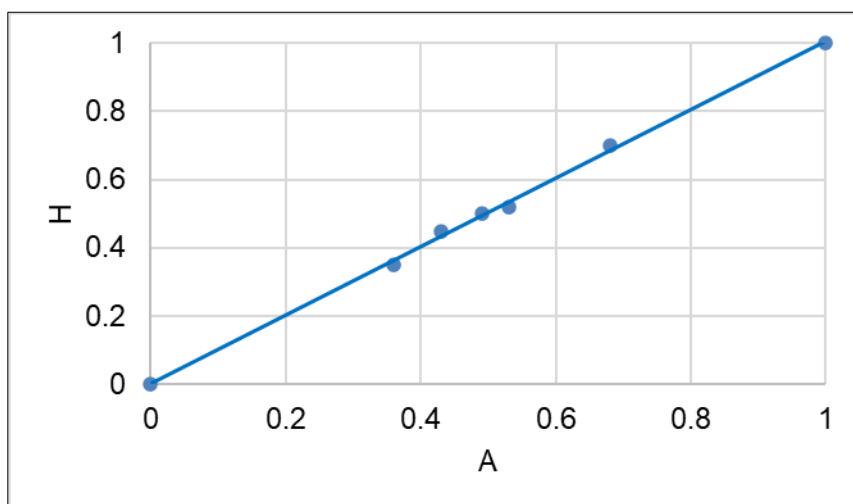
crystallites can be partially hydrolyzed. Such a phenomenon can be explained by the presence of accessible paracrystalline layers on the surface of cellulose nanocrystallites [3, 26].

Since hydrolyzability is related to the accessibility of amorphous domains of cellulose and distorted paracrystalline layers of crystallites, the degree of accessibility of the supramolecular structure of cellulose can be expressed, as follows:

$$A = Y + pX \tag{7}$$

where  $p$  is the paracrystallinity degree of crystallites.

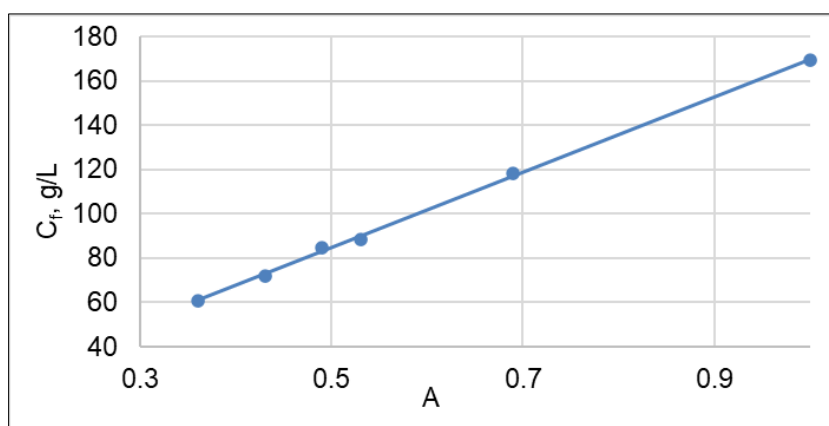
Unlike the nonlinear correlations of  $H$  from  $X$  and  $Y$  (Figure 2), the correlation of the final hydrolyzability degree from the accessibility degree,  $A$ , is linear (Figure 3). In this case, a complete congruence between  $A$  and  $H$  values is observed. Moreover, these values are almost identical.



**Figure 3** Dependence of final hydrolyzability of wet cellulose substrates on their accessibility degree

Thus, although the inner high-ordered domains of crystallites are resistant to enzymatic hydrolysis, outer paracrystalline layers of crystallites are distorted and therefore susceptible to hydrolysis along with the poorly ordered amorphous domains of cellulose.

After removing the outer layers, new layers should form on the surface of the crystallites and the hydrolysis process can theoretically continue slowly until the entire substrate is completely converted to glucose. However, in practice, as the hydrolysis process continues, the adsorption of glucose molecules on the developed surface of the nanocrystallites significantly increases, which leads to the cessation of hydrolysis due to the inhibition of enzymes by the saturated adsorption layers of the sugar [19].



**Figure 4** Calculated dependence of final glucose concentration on accessibility degree (line) with experimental points

After determining the accessibility degree, it is possible to calculate the final glucose concentration ( $C_f$ ):

$$C_f = A C_m \quad (8)$$

where  $C_m = 169.5$  g/L is the maximum concentration of glucose achieved after the complete hydrolysis of cellulose substrate at the loading of 150 g/L.

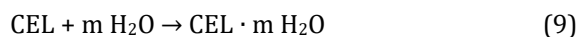
The resulting dependence  $C=F(A)$  is shown in Figure 4.

The disclosed features of enzymatic hydrolysis of cellulose substrates were considered in the thermodynamic analysis of this process.

### 3.2. Thermodynamic analysis of enzymatic hydrolysis of cellulose

An attempt to perform a thermodynamic study of the enzymatic hydrolysis of completely crystalline and completely amorphous cellulose at temperatures from 273 to 373 K using a small mass ratio of an aqueous catalyst system to cellulose (SCR) to obtain saturated glucose solutions followed by their dilution was made by Popovic et al. [28]. However, this theoretical study did not take into consideration such specific features of enzymatic hydrolysis as the negative impact of cellulose crystallinity on the hydrolysis process, limited cellulose accessibility, cessation of enzymatic hydrolysis at small SCR, the inhibition of the hydrolysis by a saturated glucose solution, low hydrolysis rate below room temperature and the inactivation of cellulolytic enzymes at temperatures above 328 K [29]. These factors were taken into account in this research when studying the thermodynamics of the real enzymatic hydrolysis process of cellulose.

As is known, for enzymatic hydrolysis, it is preferable to use wet cellulose substrates (CW). After cellulose (CEL) wetting, such substrates can contain different moles ( $m$ ) of water molecules sorbed by one mole of repeating anhydroglucose units (AGUs) of cellulose:



Standard thermodynamic (TD) functions of the wet cellulose, namely, the standard formation enthalpy ( $\Delta_f H$ ) and standard entropy ( $S$ ), were calculated by the following equations:

$$\Delta_f H(\text{CW}) = \Delta_f H(\text{CEL}) + m \Delta_f H(\text{H}_2\text{O}, l) + \Delta_w H \quad (10)$$

$$S(\text{CW}) = S(\text{CEL}) + m S(\text{H}_2\text{O}, l) + \Delta_w S \quad (11)$$

where  $\Delta_f H(\text{CEL})$  and  $S(\text{CEL})$  are standard TD functions of dry cellulose (Table 3),  $\Delta_w H$  and  $\Delta_w S$  are standard wetting enthalpy and entropy of dry cellulose (Table 4), and  $m$  is the number of moles of  $\text{H}_2\text{O}$  molecules sorbed by one mole of AGUs of cellulose after saturation with water [27] (Table 5).

**Table 3** Standard TD functions of dry celluloses and glucose

Cellulose	$-\Delta_c H$ , kJ/mol	$-\Delta_f H$ , kJ/mol	S, J/mol K
*CR	2810.0	980.2	180.0
MC	2818.8	971.4	182.5
CC	2820.2	970.0	182.9
KP	2822.4	967.8	183.5
SP	2823.1	967.1	183.7
SC	2826.6	963.6	184.7
*AC	2845.1	945.1	190.0
GL	2803.0	1273.0	210.0

\*CR denotes CI crystallites, while AC denotes completely amorphous cellulose, the standard thermodynamic functions of which were determined in [30] and [31].

The entropy values of the studied semi-crystalline cellulose samples were calculated using the additivity rule:

$$S = X S_{cr} + Y S_{am} \quad (12)$$

where  $S_{cr}$  and  $S_{am}$  are standard entropies of CR and AC, respectively.

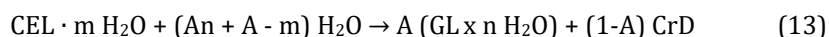
**Table 4** Standard wetting enthalpy and entropy of dry cellulose substrates

Cellulose	$-\Delta_w H$ , kJ/mol	$-\Delta_w S$ , J/mol K
CR	0	0
MC	6.8	22.8
CC	7.9	26.5
KP	9.5	31.9
SP	10.1	33.8
SC	12.8	43.0
AC	27.2	91.3

**Table 5** Standard TD functions of wet cellulose substrates

Wet Cellulose	m H <sub>2</sub> O/AGU	$-\Delta_f H$ , kJ/mol	S, J/mol K
CR (W)	0	980.2	180.0
MC (W)	1.1	1292.6	236.2
CC (W)	1.3	1349.5	247.0
KP (W)	1.6	1434.6	264.1
SP (W)	1.7	1463.1	269.1
SC (W)	2.1	1576.6	289.0
AC (W)	4.5	2258.5	413.7

The wet semi-crystalline cellulose substrate was then treated with an aqueous enzyme system. As a result, the accessible amorphous domains of cellulose and outer paracrystalline surface layers of crystallites hydrolyze and form a final glucose solution containing  $n$  moles of H<sub>2</sub>O per mole of glucose (GL), while the inner high-ordered crystalline domains (CrD) remain unhydrolyzed:



where  $A$  is the accessibility degree of cellulose.

Standard TD functions of the final glucose solutions (GL  $\times$   $n$  H<sub>2</sub>O), were calculated, as follows:

$$\Delta_f H(GL \times n H_2O) = \Delta_f H(GL) + n \Delta_f H(H_2O, i) + \Delta_{dis} H \quad (14)$$

$$S(GL \times n H_2O) = S(GL) + n S(H_2O, i) + \Delta_{dis} S \quad (15)$$

where  $\Delta_f H$  (GL) and  $S$ (GL) are standard TD functions of dry crystalline glucose (Table 3),  $\Delta_f H$  (H<sub>2</sub>O,  $i$ ) and  $S$ (H<sub>2</sub>O,  $i$ ) are standard TD functions of liquid water, while  $\Delta_{dis} H = 11.6$  kJ/mol and  $\Delta_{dis} S = \Delta_{dis} H/T_s$  are the enthalpy and entropy of dissolution for dry crystalline glucose in water at standard temperature,  $T_s=298$  K.

The calculation results are shown in Table 6.



**Table 6** Standard TD functions of final GL solutions having final concentrations  $C_f$ 

Cellulose	$C_f$ , g/L	n H <sub>2</sub> O/GL	$-\Delta_fH$ , kJ/mol	S, J/mol K
MC	61.0	164	48137.5	11728.9
CC	72.0	139	40991.8	9978.9
KP	84.7	118	34989.3	8508.9
SP	88.5	113	33560.2	8158.9
SC	118.0	85	25557.0	6198.9
AC	169.5	59	18125.4	4378.9

After the determination of standard TD functions of the wet cellulose substrates (Table 5) and final glucose solutions (Table 6), the standard TD functions of the hydrolysis reaction can be calculated, as follows:

$$\Delta_rH = (1-A) \Delta_fH(\text{CrD}) + A \Delta_fH(\text{GL} \times n \text{ H}_2\text{O}) - \Delta_fH(\text{CW}) - (An + A - m) \Delta_fH(\text{H}_2\text{O}, i) \quad (16)$$

$$\Delta_rS = (1-A) S(\text{CrD}) + A S(\text{GL} \times n \text{ H}_2\text{O}) - S(\text{CW}) - (An + A - m) S(\text{H}_2\text{O}, i) \quad (17)$$

In addition, the standard Gibbs potential of the hydrolysis reaction at temperature  $T_s=298$  K was calculated:

$$\Delta_rG = \Delta_rH - T_s \Delta_rS \quad (18)$$

The values of standard TD functions for the hydrolysis of cellulose substrates having various accessibility degrees are presented in Table 7.

**Table 7** TD functions of the hydrolysis reaction of cellulose substrates at 298 K

Cellulose	A	$\Delta_rH$ , kJ/mol	$T_s \Delta_rS$ , kJ/mol	$\Delta_rG$ , kJ/mol
MC	0.36	-0.21	6.08	-6.29
CC	0.43	-0.22	7.01	-7.23
KP	0.49	-0.50	8.18	-8.68
SP	0.53	-0.50	8.70	-9.20
SC	0.69	-0.62	11.09	-11.71
AC	1	-3.34	23.86	-27.20

The obtained results showed that the hydrolysis reaction of the studied cellulose samples is exothermic. Since reaction enthalpy ( $\Delta_rH$ ) is negative and the temperature-entropy factor ( $T_s \Delta_rS$ ) is positive, the Gibbs potential ( $\Delta_rG$ ) of this process becomes negative, which promotes the implementation of enzymatic hydrolysis of cellulose substrates.

As is known, the optimal temperature for enzymatic hydrolysis of cellulose is  $T_o= 323$  K [32]. Therefore, it is advisable to perform a thermodynamic analysis of the hydrolysis process also at the mentioned optimal temperature. For this purpose, reference data on the average values of the specific heat capacity ( $\hat{C}_p$ ) of wet cellulose, water, and aqueous solutions of glucose were used. The enthalpy of cellulose hydrolysis at the optimal temperature was calculated by Kirchhoff's equation:

$$\Delta_rH(T_o) = \Delta_rH(T_s) + \Delta \hat{C}_p (T_o - T_s) \quad (19)$$

On the other hand, the entropy of hydrolysis at the optimal temperature was calculated, as follows:

$$\Delta_rS(T_o) = \Delta_rS(T_s) + \Delta \hat{C}_p \ln(T_o/T_s) \quad (20)$$

where  $T_s$  is the standard temperature (298 K),  $T_o$  is the optimal temperature (323 K), and  $\Delta\hat{C}_p$  is the difference in specific heat capacities of the final hydrolysis product (GL solutions) and starting substances (Table 8).

**Table 8** Values of  $\Delta\hat{C}_p$  for hydrolysis of various cellulose substrates

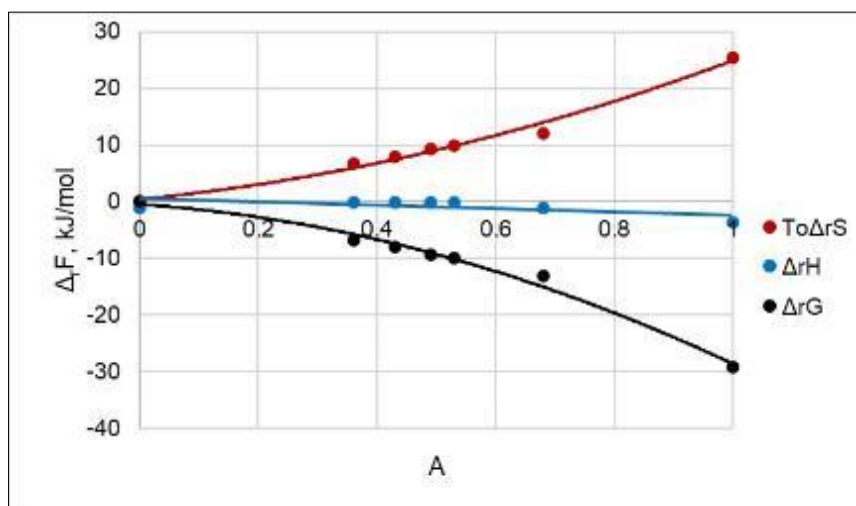
Cellulose	$\Delta\hat{C}_p$ , J/mol K
MC	6.0
CC	14.3
KP	17.2
SP	16.3
SC	-21.0
AC	-15.4

The obtained results are shown in Table 9.

**Table 9** TD functions of the hydrolysis reaction of cellulose substrates at 323 K

Cellulose	A	$\Delta_rH$ , kJ/mol	$T_o \Delta_rS$ , kJ/mol	$\Delta_rG$ , kJ/mol
MC	0.36	-0.06	6.77	-6.83
CC	0.43	-0.07	7.96	-7.90
KP	0.49	-0.07	9.34	-9.41
SP	0.53	-0.09	9.85	-9.95
SC	0.69	-1.15	11.85	-13.0
AC	1	-3.73	25.50	-29.23

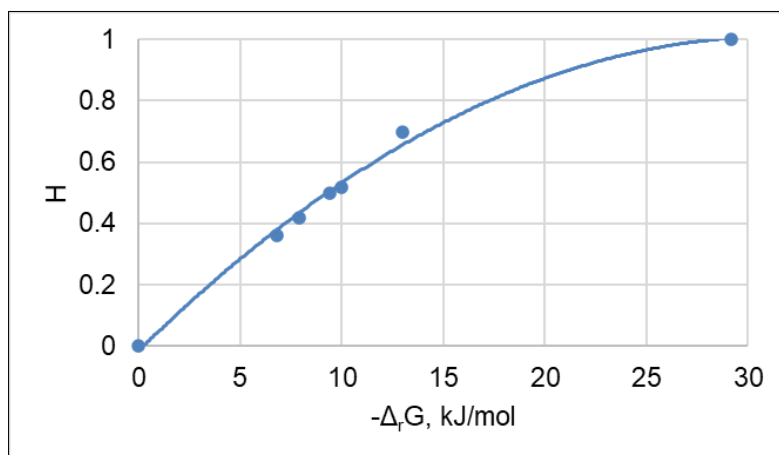
Analysis of TD characteristics shows that at the optimal hydrolysis temperature of 323 K, the contribution of the temperature-entropy component to the Gibbs potential is predominant, while the Gibbs potential becomes more negative than at the temperature of 298 K. Thus, a moderate increase in temperature should promote enzymatic hydrolysis of cellulose, which is confirmed by literature data [32].



**Figure 5** Dependence of TD functions of hydrolysis process on accessibility degree of cellulose substrates at optimal temperature of 323 K

The most negative Gibbs potential was observed for the hydrolysis of the most reactive and accessible amorphous cellulose (Figure 5). With a decrease in cellulose accessibility, the Gibbs potential of the hydrolysis reaction becomes less negative. Moreover, when extrapolated to  $A = 0$ , the Gibbs potential and other thermodynamic functions of the reaction tend to be zero due to the resistance of high-ordered crystalline domains of cellulose to enzymatic hydrolysis.

The Gibbs potential determines also the hydrolysability degree of cellulose, namely an increase in the negative value of Gibbs potential promotes the archive of a higher value of enzymatic hydrolysability (Figure 6).



**Figure 6** Dependence of cellulose hydrolysability on Gibbs potential of enzymatic hydrolysis at optimal temperature

The performed thermodynamic analysis explains the experimental results, according to which amorphization of cellulose substrates facilitates enzymatic hydrolysis.

#### 4. Conclusions

It was discovered that the hydrolyzability of studied semi-crystalline cellulose samples is higher than their amorphicity degree. It is explained by the fact that outer paracrystalline layers of crystallites are distorted and susceptible to hydrolysis along with the poorly ordered amorphous domains of cellulose.

The disclosed features of enzymatic hydrolysis of cellulose substrates were used for the thermodynamic analysis of this process. The thermodynamic analysis showed that the hydrolysis process of cellulose samples at 298 K is exothermic. Since reaction enthalpy is negative and the temperature-entropy factor is positive, the Gibbs potential of this process becomes negative, which contributes to the implementation of enzymatic hydrolysis of cellulose.

Moderate enhancement in temperature to an optimal value of 323 K increases the negative value of the Gibbs potential and thereby promotes the hydrolysis of cellulose substrates. The most negative Gibbs potential was observed for the hydrolysis of the most accessible and reactive amorphous cellulose. It was also shown that the enzymatic hydrolysis of high-ordered crystalline domains of cellulose cannot be performed even at optimal temperature since the Gibbs potential of this process is close to zero.

#### References

- [1] Domb AJ, Kost J, Wiseman D. Handbook of Biodegradable Polymers. *CRC Press: Boca Raton*; 1998.
- [2] Kamide K. Cellulose Products and Cellulose Derivatives: Molecular Characterization and its Applications. *Elsevier: Amsterdam*; 2005.
- [3] Ioelovich M. Models of supramolecular structure and properties of cellulose. *J. Polym. Sci. A*, 2016; 58: 925–943.
- [4] Pfister B, Zeeman SC. Formation of starch in plant cells. *Cell. Mol. Life Sci.*, 2016; 73: 2781–2807.
- [5] Kauter A, Epping L, Semmler T, et al. The gut microbiome of horses: current research on equine enteral microbiota and future perspectives. *Anim. Microbiome*, 2019; 1:14.
- [6] Tappy L. Metabolism of sugars: A window to the regulation of glucose and lipid homeostasis by splanchnic organs. *Clinical Nutrition*, 2021; 40: 1691-1698.

- [7] Güemes M, Rahman SA, Hussain K. What is a normal blood glucose? *Archives of Disease in Childhood*, 2016; 101:569-574.
- [8] Fellows PJ. *Food Processing Technology*. Woodhead Publishing: Cambridge; 2016.
- [9] Lagi M, Bar-Yam Y, Bertrand K. The food crises: a quantitative model of food prices including speculators and ethanol conversion. *ArXiv*, 2011; 1109.4859: 1-56.
- [10] Ioelovich M. Recent findings and the energetic potential of plant biomass as a renewable source of biofuels – a review. *Bioresources*, 2015; 10: 1879-1914.
- [11] Ioelovich M, Morag E. Effect of cellulose structure on enzymatic hydrolysis. *Bioresources*, 2011; 6: 2818-2834
- [12] Horn SJ, Vaaje-Kolstad G, Westereng B, Eijsink VG. Novel enzymes for the degradation of cellulose. *Biotech. Biofuel.*, 2012; 5: 45-57.
- [13] Bansal P, Vowell BJ, Hall M, et al. Elucidation of cellulose accessibility, hydrolysability and reactivity as the major limitations in the enzymatic hydrolysis of cellulose. *Biores. Technol.*, 2012; 107: 243-250.
- [14] Amândio MST, Rocha JMS, Xavier AMRB. Enzymatic hydrolysis strategies for cellulosic sugars production to obtain bioethanol from eucalyptus globulus bark. *Fermentation*, 2023; 9, 241: 1-19.
- [15] Reis CER, Junior NL, Bento HBS, et al. Process strategies to reduce cellulase enzyme loading for renewable sugar production in biorefineries. *Chem. Eng. J.*, 2023, 451 (2),138690, 1-10.
- [16] Ioelovich M, Morag E. Study of enzymatic hydrolysis of pretreated biomass at increased solids loading. *Bioresources*, 2012; 7: 4672-4682.
- [17] Pino MS, Rodríguez-Jasso RM, Michelin M, et al. Bioreactor design for enzymatic hydrolysis of biomass under the biorefinery concept. *Chem. Eng. J.*, 2018; 347: 119–136.
- [18] Da Silva AS, Espinheira RP, Teixeira RSS, et al. Constraints and advances in high-solids enzymatic hydrolysis of lignocellulosic biomass: A critical review. *Biotechnol. Biofuels*, 2020; 13, 58: 1-28.
- [19] Xiao Z, Zhang X, Gregg DJ, Saddler JN. Effects of sugar inhibition on cellulases and beta-glucosidase during enzymatic hydrolysis of softwood substrates. *Appl. Biochem. Biotechnol.*, 2004;113-116:1115-1126.
- [20] Hall M, Bansal P, Lee J, et al. Cellulose crystallinity – A key predictor of the enzymatic hydrolysis rate. *FEBS Journal*, 2010; 277: 1571-1582.
- [21] Park S, Baker JO, Himmel ME, et al. Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. *Biotechnol. for Biofuels*, 2010; 3: 1-10.
- [22] Ioelovich M. Microcellulose vs nanocellulose - a review. *World J. Adv. Eng. Technol. Sci.*, 2022; 5: 1-15.
- [23] Ioelovich M. Preparation, characterization and application of amorphized cellulose - a review. *Polymers*, 2021; 13, 4313: 1-21.
- [24] TAPPI. Standard method for determination of alpha, beta, and gamma cellulose in pulp. T 203.
- [25] Ioelovich M, Leykin A. Nano-cellulose and its application. *SITA*, 2004; 6: 17-24.
- [26] Ioelovich M, Leykin A, Figovsky O. Study of cellulose paracrystallinity. *Bioresources*, 2010; 5: 1393-1407.
- [27] Ioelovich M. Application of thermochemical method to determine the crystallinity degree of cellulose materials. *Appl. Sci.*, 2023; 13, 2387: 1-11.
- [28] Popovic M, Woodfield BF, Hansen LD. Thermodynamics of hydrolysis of cellulose to glucose from 0 to 100 °C: cellulosic biofuel applications and climate change implications. *J. Chem. Thermodynamics*, 2019; 128: 244-250.
- [29] Ioelovich M. Thermodynamics of enzymatic hydrolysis of cellulose. *World J. Adv. Res. Rev.*, 2024; 21: 577–586.
- [30] Ioelovich M. Thermodynamic analysis of cellulose nitration. *World J. Adv. Res. Reviews*, 2024; 21: 485–494.
- [31] Goldberg RN, Schliesser J, Mittal A, et al. A thermodynamic investigation of the cellulose allomorphs: Cellulose(am), cellulose Iβ(cr), cellulose II(cr), and cellulose III(cr). *J. Chem. Thermodynamics*, 2015; 81: 184-226.
- [32] Kabir F, Lu-Kwang Ju. On optimization of enzymatic processes: temperature effects on activity and long-term deactivation kinetics. *Process Biochem.*, 2023; 130: 734-746.