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Thermodynamics of enzymatic hydrolysis of cellulose

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Abstract

In this research, the thermodynamical analysis of the enzymatic hydrolysis of cellulose samples with different crystallinity has been performed. Calorimetric methods were used to determine the combustion enthalpies of cellulose samples and enthalpies their interaction with water. As a result, the standard formation enthalpies of dry and wet cellulose samples as well as of glucose solutions were calculated. In addition, also the standard entropies were found. Based on the obtained parameters, the thermodynamic functions of the hydrolysis reaction at the standard temperature (298 K) were calculated. It was established that the hydrolysis process of semi-crystalline cellulose samples 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 at standard temperature. Moderate enhancement in temperature to optimal value (323 K) increases the negative Gibbs potential and thereby promotes the hydrolysis of cellulose substrates. A correlation was found between the negative value of the Gibbs potential and the concentration of the resulting glucose solutions. It was also shown that the enzymatic hydrolysis of completely crystalline cellulose cannot be performed even at optimal temperature due to the zero value of the Gibbs potential.

Keywords: Cellulose; Crystallinity; Hydrolysis; Glucose; Enthalpy; Entropy; Gibbs potential; 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 cellulosic feed 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 glucose production.

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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 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-food 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]. In particular, it was established that cellulosic materials have a relatively low enzymatic digestibility. This is explained by the cellulose crystallinity, which is considered the most important structural factor impeding enzymatic hydrolysis. As is known, the theoretical basis for the feasibility of various reactions is chemical thermodynamics. Unfortunately, the thermodynamics of enzymatic hydrolysis of cellulose with different crystallinity has been poorly studied. Only Popovic et al. [16] investigated the thermodynamics of a hypothetical hydrolysis case of completely amorphous and completely crystalline celluloses at temperatures from 273 to 373 K using a low mass ratio of an aqueous catalyst solution to cellulose (SCR) of 0.6 to 1.1 to obtain saturated glucose solutions followed by their dilution. However, in practice, due to such a low SCR, the liquid medium is almost completely soaked up into porous cellulose samples and disappears, so the implementation of enzymatic hydrolysis becomes impossible. It is also known that a saturated glucose solution cannot be obtained by enzymatic hydrolysis of cellulose due to the inhibition of enzymes by highly concentrated glucose. In addition, crystalline cellulose is resistant to hydrolysis. It can also be noted that making thermodynamic calculations of cellulose hydrolysis at temperatures above 328 K is meaningless due to the inactivation of cellulolytic enzymes at elevated temperatures [17].

In this study, to further elucidate the effect of cellulose crystallinity on its conversion to glucose during enzymatic hydrolysis, the thermodynamic characteristics of the starting cellulose substrates and final glucose solutions were determined, and the thermodynamical analysis of the actual hydrolysis process at standard and moderate temperatures was performed.

2 Materials and Methods

2.1 Materials

Various semicrystalline cellulose samples having crystalline structure of CI were used. The Avicel MCC PH-101 (AV) sample was purchased from FMC. Refined and bleached cotton cellulose (CC) and Kraft pulp (KC) were supplied 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, rinsed with absolute ethanol, and dried in a vacuum chamber at 378 K to constant weight. The cellulose of Switchgrass (SC) was isolated from the biomass by the Kürschner-Hoffer method followed by extraction with boiling 2% NaOH; then samples were washed with deionized water to a neutral pH value, rinsed with absolute ethanol, and dried in a vacuum chamber at 378 K to constant weight. Decrystallized cotton cellulose (DC) was prepared by ball-milling of CC with ceramic balls for 10 h and then it was recrystallized in water and dried in a vacuum chamber at 323 K to constant weight. The main characteristics of the used cellulose samples are shown in Table 1.

Table 1 Characteristics of the cellulose samples

Sample	Abbreviation	α-Cellulose, %	DP	X	Y
Avicel PH-101	AV	99	220	0.76	0.24
Cotton cellulose	CC	98	2700	0.71	0.29
Kraft cellulose	KC	97	1200	0.64	0.36
Cellulose of Switchgrass	SC	95	740	0.51	0.49
Decrystallized cellulose	DC	93	610	0.25	0.75

In addition, chemically pure crystalline glucose (GL) purchased from Sigma-Aldrich was used.

2.2 Methods

2.2.1 Characterization of samples

The content of alpha-cellulose in the samples was determined by the NREL LAP 002 method [18]. The average degree of polymerization (DP) was measured by the viscosity method using diluted cellulose solutions in Cadoxen [19]. The crystallinity (X) and amorphicity (Y) degrees of the samples were determined by the thermochemical method [20].

2.2.2 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 [21]. Before starting the experiments, the air-dry 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. Thereafter, 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 ± 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:

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

$$Y = 1 - X \quad \text{..... (2)}$$

where $\Delta_w H_{am}$ = -27.2 kJ/mol is the standard wetting enthalpy of completely amorphous cellulose (AC) [20].

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

2.2.3 Enthalpies of combustion and formation

Combustion of the dry samples was carried out in a stainless-steel calorimetric bomb having a volume of 0.320 dm^3 at an oxygen pressure of 3.05 MPa with 1.00 cm^3 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 ±0.001 K. The value of the energy equivalent of the calorimeter determined by standard benzoic acid was 15802.3 ± 0.9 J/K. The true mass of the sample used in each experiment was determined from the mass of the produced CO_2. 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 $C_aH_bO_c$, can be calculated from the known Hess equation:

$$\Delta_f H = a\,\Delta_f H\,(CO_2, g) + 0.5b\,\Delta_f H\,(H_2O, l) - \Delta_c H \quad \text{..... (3)}$$

where $\Delta_c H$ is the measured standard enthalpy of combustion; $\Delta_f H\,(CO_2, g)$ = -393.51 kJ/mol and $\Delta_f H\,(H_2O, l)$ = -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

Currently, to implement effective hydrolysis of cellulose, enzyme preparations were used, which include at least three types of specific enzymes [10, 12]:

- Endo-1,4-β-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-β-glucanases that attack the reducing or non-reducing ends of the depolymerized cellulose particles by forming oligomeric products containing di- and tetra-saccharides;
- β-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 β-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 10-40 mg of protein per 1 g of cellulose substrate.

As is known, at the beginning of hydrolysis, dry semi-crystalline cellulose (CEL)is wetted with an aqueous solution of cellulolytic enzymes to obtain a wet cellulose substrate containing m moles H_2O, sorbed by one mole of AGU:

$$CEL \rightarrow CEL \cdot m\ H_2O \text{……..}(4)$$

Standard thermodynamic (TD) functions of the wet cellulose substrate (CW), namely, the standard formation enthalpy (Δ_fH) and standard entropy (S), were calculated by the following equations:

$$\Delta_fH(CW) = \Delta_fH(C) + m\ \Delta_fH(H_2O, l) + \Delta_wH \text{ ……..}(5)$$

$$S(CW) = S(C) + m\ S(H_2O, l) + \Delta_wS \text{ ……..}(6)$$

where $\Delta_fH(C)$ and S(C) are standard TD functions of dry cellulose (Table 2), Δ_wH and Δ_wS are standard wetting enthalpy and entropy of dry cellulose (Table 3), and **m** is the number of moles of H_2O molecules sorbed by one mole of AGU of cellulose after saturation with water [22] (Table 4),

Table 2 Standard TD functions of dry celluloses and glucose

Cellulose	$-\Delta_cH$, kJ/mol	$-\Delta_fH$, kJ/mol	S, J/mol K
CR*	2810.6	979.6	183.0
AV	2819.5	970.7	184.7
CC	2821.0	969.0	185.1
KC	2824.0	966.2	185.5
SC	2828.8	961.4	186.4
DC	2838.5	951.7	188.3
AC*	2847.8	942.4	190.0
GL	2803.0	1273.0	210.0

*Note: CR denotes CI crystallites, while AC denotes completely amorphous cellulose, the standard thermodynamic functions of which were determined in [23] and [24].

Table 3 Standard wetting enthalpy and entropy of dry cellulose substrates

Cellulose	$-\Delta_wH$, kJ/mol	$-\Delta_wS$, J/mol K
CR	0	0
AV	6.5	21.8
CC	7.9	26.5
KC	9.8	32.9
SC	13.3	44.6
DC	20.4	68.4
AC	27.2	91.3

Table 4 Standard TD functions of wet cellulose substrates

Wet Cellulose	m H_2O/AGU	$-\Delta_fH$, kJ/mol	S, J/mol K
CRW	0	979.6	183.0
AVW	1.1	1292.0	239.9
CCW	1.3	1348.5	249.6
KCW	1.6	1433.3	264.6
SCW	2.2	1603.5	295.8
DCW	3.4	1943.9	357.9
ACW	4.5	2255.8	413.7

The wet semi-crystalline cellulose substrate was then treated with an aqueous enzyme system. As a result, the amorphous cellulose domains hydrolyze and form a final glucose solution containing n moles H2O per mole of glucose (GL), while the stable crystalline domains of cellulose (CR) remain unhydrolyzed.

$$CEL \cdot m\ H_2O + (Yn - m + Y)\ H_2O \rightarrow Y(GL \times n\ H_2O) + X\ CR \quad \text{.....(7)}$$

where X and Y are degrees of crystallinity and amorphicity of cellulose, respectively.

It has been found that to achieve maximum glucose yield during enzymatic hydrolysis, the optimal loading of cellulose substrate in the aqueous enzyme system should be at least 150 g/L [25-27]. 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 [28].

After complete hydrolysis of amorphous cellulose (AC) at a loading of 150 g/L, the final concentration of the formed glucose solution will be maximum, about 170 g/L, in which there are about n=59 moles of H_2O per one mole of GL. However, for semicrystalline cellulose samples, the final GL concentration is lower because only amorphous domains of cellulose substrates are hydrolyzed to glucose. Standard TD functions of the final glucose solutions, namely, the standard formation enthalpy (Δ_fH) and standard entropy (S), were calculated, as follows:

$$\Delta_fH(GL \times n\ H_2O) = \Delta_fH(GL) + n\Delta_fH\ (H_2O, l) + \Delta_{dis}H \quad \text{..... (8)}$$

$$S(GL \times n\ H_2O) = S(GL) + n\ S(H_2O, l) + \Delta_{dis}S \quad \text{..... (9)}$$

where Δ_fH (GL) and S(GL) are standard TD functions of dry crystalline glucose (Table 2), Δ_fH (H_2O, l) and S(H_2O, l) 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 temperature T_s=298 K.

The calculation results are shown in Table 5.

Table 5 Standard TD functions of final GL solutions having concentrations C (GL)

Cellulose	C (GL), g/L	n H_2O/GL	$-\Delta_fH$, kJ/mol	S, J/mol K
AV	41	244	71003.9	17328.9
CC	49	203	59285.3	14461.7
KC	61	164	48137.5	11728.9
SC	83	120	35561.0	8648.9
DC	128	78	23556.1	5708.9
AC	170	59	18125.4	4378.9

After the determination of standard TD functions of the wet cellulose substrates, (Table 3), and final glucose solutions (Table 5), the standard TD functions of the hydrolysis reaction can be calculated, as follows:

$$\Delta_r H = X\,\Delta_f H(CR) + Y\,\Delta_f H(GL \times n\,H_2O) - \Delta_f H(CW) - (Yn - m + Y)\,\Delta_f H(H_2O, l) \quad \ldots\ldots\ldots (10)$$

$$\Delta_r S = X\,S(CR) + Y\,S(GL \times n\,H_2O) - S(CW) - (Yn - m + Y)\,S(H_2O, l) \quad \ldots\ldots\ldots (11)$$

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

$$\Delta_r G = \Delta_r H - T_s\,\Delta_r S \quad \ldots\ldots\ldots (12)$$

The values of standard TD functions of the hydrolysis of cellulose substrates having various crystallinity degrees (X) are presented in Table 6.

Table 6 Standard TD functions of the hydrolysis reaction of cellulose substrates

Cellulose	X	$\Delta_r H$, kJ/mol	$T_s\,\Delta_r S$, kJ/mol	$\Delta_r G$, kJ/mol
AV	0.76	-1.04	5.69	-6.73
CC	0.71	-1.63	7.15	-8.78
KC	0.64	-2.17	8.61	-10.78
SC	0.51	-2.95	11.68	-14.63
DC	0.25	-4.47	17.88	-22.35
AC	0	-6.04	23.9	-29.94

Although crystallites of cellulose are resistant to enzymatic hydrolysis, actual cellulose substrates can be hydrolyzed due to the presence of amorphous domains. The obtained result showed that the hydrolysis reaction of the studied semi-crystalline celluloses is exothermic. Since reaction enthalpy ($\Delta_r H$) is negative and the temperature-entropy factor ($T_s\Delta_r S$) is positive, the Gibbs potential ($\Delta_r G$) of this process becomes negative, which contributes to the implementation of enzymatic hydrolysis of cellulose at standard temperature.

$$\text{If } \Delta_r H < 0 \text{ and } T_o\,\Delta_r S > 0, \text{ then } \Delta_r G = (\Delta_r H - T_o\,\Delta_r S) < 0$$

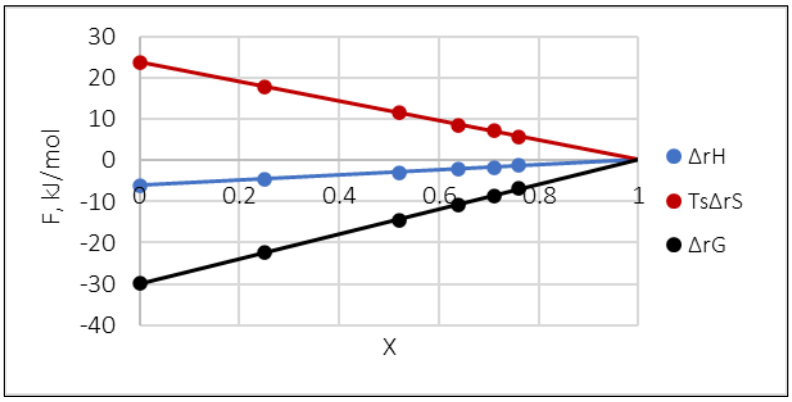

Figure 1 Dependence of TD functions of hydrolysis process on crystallinity degree of cellulose substrates at standard temperature

With an increase in cellulose crystallinity, the entropy-temperature factor decreases, while the enthalpy and Gibbs potential of the hydrolysis reaction become less negative (Figure 1), which should complicate the hydrolysis process and reduce the yield of glucose. Moreover, when extrapolated to X = 1, the Gibbs potential and other thermodynamic functions of the reaction become zero, which confirms the resistance of cellulose crystallites to enzymatic hydrolysis.

As is known, the optimal temperature for enzymatic hydrolysis of cellulose is T_o= 323 K [17]. Therefore, it is advisable to perform a thermodynamic analysis of the hydrolysis process also at the mentioned optimal temperature. For this purpose, the averaged values of the specific heat capacity ($\hat{C}_p$) for dry and wet celluloses [24], as well as for water and aqueous solutions of glucose indicated in reference books were used. The value of formation enthalpy at the optimal temperature was calculated by Kirchhoff's equation:

$$\Delta_f H\ (T) = \Delta_f H\ (T_s) + \Delta\hat{C}_p\ (T_o - T_s) \quad \text{.....(13)}$$

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

$$S\ (T) = S\ (T_s) + \Delta\hat{C}_p \ln(T_o/T_s) \text{..... (14)}$$

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 between the $\hat{C}p$ values of final hydrolysis products (part of glucose solution and remaining part of crystalline cellulose) and the $\hat{C}p$ values of starting substances (cellulose substrate and part of water).

The obtained results are shown in Table 7.

Table 7 TD functions of the hydrolysis reaction of cellulose substrates at optimal temperature

Cellulose	X	$\Delta_r H$, kJ/mol	$T_o \Delta_r S$, kJ/mol	$\Delta_r G$, kJ/mol
AV	0.76	-2.32	4.85	-7.17
CC	0.71	-3.18	6.14	-9.23
KC	0.64	-4.1	7.33	-11.43
SC	0.51	-5.55	9.95	-15.5
DC	0.25	-8.4	15.29	-23.7
AC	0	-11.27	20.46	-31.73

Analysis of TD characteristics shows that the process of enzymatic hydrolysis at the optimal temperature is more exothermic than at the standard temperature (Figure 2). However, as cellulose crystallinity increases, the hydrolysis process becomes less exothermic, and for completely crystalline cellulose with X = 1, the heat release stops.

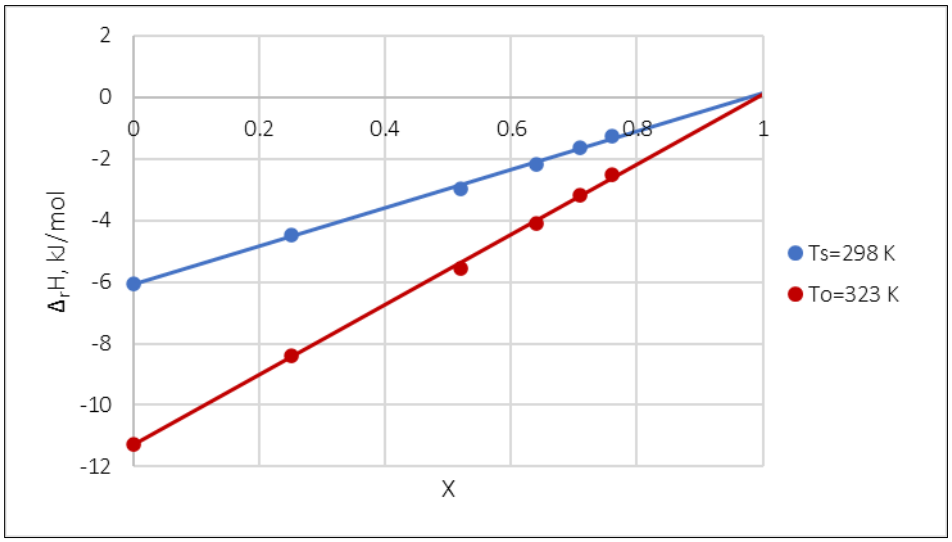

Figure 2. Dependence of enthalpy of hydrolysis process on crystallinity degree of cellulose substrates at standard (T_s) and optimal (T_o) temperatures

At optimal hydrolysis temperature, the Gibbs potential becomes more negative than at standard temperature simultaneously with the increase in the exothermicity of the reaction (see Tables 6 and 7). When X = 1, the Gibbs potential and other thermodynamic functions of the reaction become zero (Figure 3), which indicates the resistance of cellulose crystallites to enzymatic hydrolysis.

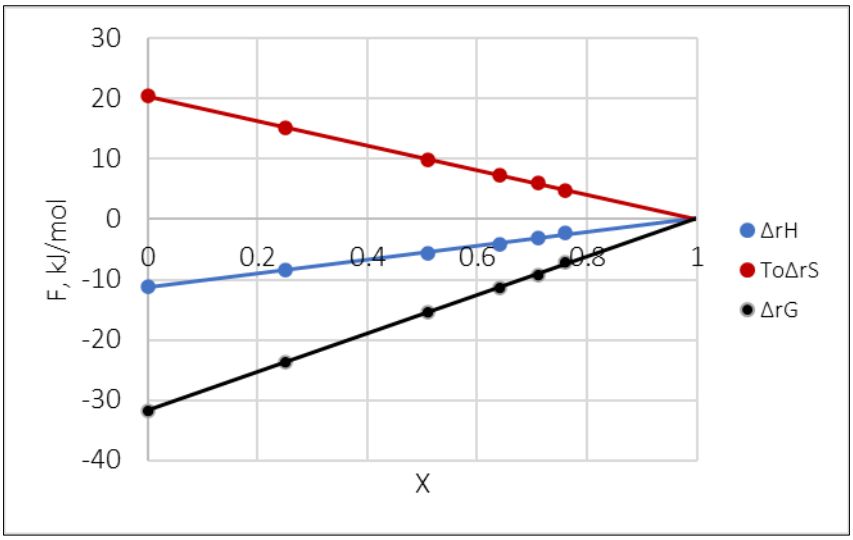

Figure 3 Dependence of TD functions of hydrolysis process on crystallinity degree of cellulose substrates at optimal temperature

Studies have shown that the most negative value of the Gibbs potential, characteristic of hydrolysis of amorphous cellulose, provides the obtaining of the most concentrated glucose solution, 170 g/L if a substrate loading is 150 g/L. As the negative value of the Gibbs potential decreases, the concentration of the resulting glucose solution reduces and becomes equal to zero at the zero value of Gibbs potential, which is characteristic of completely crystalline cellulose (Figure 4). Thus, enzymatic hydrolysis of such crystalline cellulose cannot be implemented even at the optimum temperature.

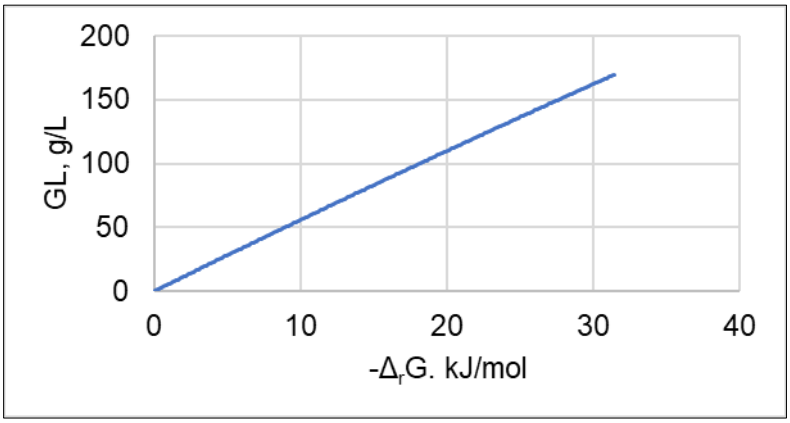

Figure 4 Dependence of glucose concentration on the value of Gibbs potential after hydrolysis of cellulose substrates at optimal temperature

The performed thermodynamic analysis explains the experimental results of the enzymatic hydrolysis of cellulose, according to which, to obtain the maximum yield and concentration of glucose, the cellulose substrate must be amorphized to destroy its crystalline structure impending the enzymatic hydrolysis of cellulose to glucose [11, 29, 30].

4 Conclusion

The thermodynamic analysis of the enzymatic hydrolysis of cellulose substrates with different crystallinity was performed. The obtained result showed that the hydrolysis reaction of the studied semi-crystalline cellulose substrates

at standard temperature (298) 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 at standard temperature

Increasing the temperature of enzymatic hydrolysis of cellulose substrates to the optimal value (323 K) leads to an increase in exothermic enthalpy and negative Gibbs potential, which should improve the efficiency of the hydrolysis process. It was found that an increase in the negative value of the Gibbs potential leads to a rise in the concentration of the resulting glucose solution. Thus, a moderate enhancement of temperature increases the negative Gibbs potential and thereby promotes the hydrolysis of cellulose substrates. It was also shown that the enzymatic hydrolysis of completely crystalline cellulose cannot be implemented even at optimal temperature due to the zero value of the Gibbs potential. The performed thermodynamic analysis explains the experimental results of the enzymatic hydrolysis of cellulose, according to which the cellulose substrate must be amorphized to achieve the maximum concentration of formed glucose.

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