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Kinetic model of enzymatic hydrolysis of steam-exploded wheat straw

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ABSTRACT

The hydrolysis kinetics of steam-exploded wheat straw treated with cellulase NS 50013 enzyme complex in combination with β-glucosidase NS 50010 is studied. The time dependence of the reducing sugars amount is followed at varying the temperature value and the amount of the enzyme introduced. The activation energy determined on the ground of the rate temperature dependence stays unchanged in the course of the process. The preexponential factor decreases with the increase of the degree of hydrolysis and is responsible for the process rate decrease. A new expression for the dependence of degree of hydrolysis of one of carbohydrate polymers (cellulose) in wheat straw on the time, the enzyme concentration and the temperature is obtained. It is of practical importance as well because it provides estimation of the degree of hydrolysis required at predetermined values of the temperature, the enzyme concentration and the time used. The expression can be used for control of the enzyme hydrolysis of cellulose in the wheat straw.

1. Introduction

Agricultural residues such as straw represent large renewable resources for lignocellulosic bioconversion. Wheat straw is a widely available substrate and transformation of this agricultural by-product is desirable. Bioconversion of wheat straw is favored because of its relatively low lignin content (<20% w/w) and high carbohydrate content (hemicellulose (50%) and cellulose (30%)) (Carrillo, Lis, Colom, Lopez-Mesas, & Vallderas, 2005; Harper & Lynch, 1981). Due to the close connection of cellulose and hemicellulose with lignin in the plant cell wall, pre-treatment is necessary to make these carbohydrates available for enzymatic hydrolysis and fermentation. The thermochemical pre-treatment such as dilute acid hydrolysis and steam explosion, which solubilise the hemicellulose components and increase cellulose accessibility, are commonly used to prepare lignocelluloses for enzymatic saccharification (El-Zawawy, Ibrahim, Abdel-Fattah, Soliman, & Mahmoud, 2011; Meunier-Goddik & Penner, 1999). While steam explosion has been tried and tested for agro residues like corn stover and rice straw, an additional acid hydrolysis step is needed for achieving high sugar yield from soft wood (Cardona & Sanchez, 2007). Steam explosion (autohydrolysis) is one of the most cost effective and widely used pretreatment methods for wheat straw (Alfani, Gallifouco, Saporosi, Spera, & Cantarella, 2000). According to this method size-reduced biomass is rapidly heated by high pressure steam for a short period of time and then the pressure is suddenly reduced which makes the materials undergo an explosive decomposition (Jurado, Prieto, Martinez-Alcala, Martinez, & Martinez, 2009).

Hydrolysis using appropriate enzymes represents the most effective method to liberate simple sugars from cellulosic materials. Cellulose hydrolysis is catalyzed by class of enzymes known as cellulases. Three major groups of enzymes namely, endo-gluconase, exo-gluconase and β-glucosidase are involved in hydrolysis of cellulose. The enzymatic hydrolysis can be influenced by substrate and end product concentration, enzyme activity and reaction conditions. β-glucosidase plays a significant role in the hydrolysis process, since cellobiose is an end-product inhibitor of many cellulases (Galbe & Zacchi, 2002; Talebnia, Karakashev, & Angelidaki, 2010). The products of the hydrolysis are usually reducing sugars including glucose. The degraded by cellulases reducing sugars can be fermented by yeasts or bacteria to ethanol (Sun & Cheng, 2002). The susceptibility of cellulosic substrates to cellulases depends on the structural features of the substrate including cellulose crystallinity, degree of cellulose polymerization, surface area, and content of lignin. Lignin interferes with hydrolysis by blocking access of cellulases to cellulose and by irreversibly binding hydrolytic enzymes. Therefore, removal of lignin can dramatically increase the hydrolysis rate (McMillan, 1994).

Optimization of lignocellulosic bioconversion by cellulase enzymes requires good knowledge of the reaction kinetics. The complexity of the enzymatic hydrolysis of lignocellulosic wastes

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come from the fact that they are heterogeneous insoluble substrates, and thus, their enzymatic hydrolysis is always limited (Carrillo et al., 2005). The enzyme kinetics has been usually studied by the Henri-Michaelis–Menten equation (Henri, 1902). The chosen model provides equations, witch express a specific mathematical function between the product concentrations and the hydrolysis time at different initial enzyme and substrate concentrations (Shen & Agblevor, 2008). The equations are not subjected to the time limit. It is desired that they could describe the initial characteristics of enzymatic hydrolysis of cellulose.

It has been shown that the Henri-Michaelis–Menten equation is not suitable for the analysis of enzymatic reactions of heterogeneous system (Baley, 1989; Chrastil & Wilson, 1982; Chrastil, 1988a). The alternative approach suggests that the initial hydrolysis velocity should be expressed as a function of the initial enzyme concentration (Chrastil, 1988b). According a kinetic model based on the second order reaction of enzyme deactivation Zhang, Xu, Xu, Yuan, and Guo (2010) have obtained a complex equation presenting a three-parameter mathematical function that directly expresses the relationship between glucose concentration and two hydrolysis conditions – the time and the initial enzyme concentration.

The heterogeneous nature of the system wheat straw – cellulase allows to apply the models of heterogeneous catalytic reactions for description of the kinetics of enzymatic hydrolysis, which is the purpose of this study.

2. Experimental

Kinetic investigations were performed on wheat straw. The chemical composition was as follows: 44% of cellulose (Kurschner & Hoffer, 1933); 33.5% of pentosans (Standard:TAPPI T19m-54); 24.3% of lignin (Standard: TAPPI-T222 om-88); easily hydrolysable polysaccharides and water-soluble substances – 20.7% (Obolenskaya et al., 1965) and 4.6% of ash.

The steam explosion method is used as a pre-treatment of wheat straw. The steam explosion was performed in 2 L stainless steel laboratory installation at the following conditions: a hydromodul ratio 1:10; an initial temperature of 100 °C; a maximum temperature of 190 °C; pressure of 12.8 bar; heating time of 60 min followed by additional 10 min at the maximum temperature.

The residue was washed with distillated water and the obtained hydrolysate was filtered. The solid residue produced in the course of steam explosion pretreatment was subjected to enzymatic hydrolysis as a second stage of treatment.

The cellulase complex NS 50013 and β-glucosidase NS 50010 of Novozymes AS were used for the enzymatic hydrolysis. The enzyme charge of NS 50013 was 5%, while that of NS 50010 was 0.5%, both referred to the mass. The specific enzyme action consists in the hydrolysis of the carbohydrate polymer under consideration (cellulose) in the wheat straw.

The cellulasic hydrolysis was carried out in polyethylene bags in a water bath previously heated to the desired temperature.

The kinetics of the cellulasic hydrolysis was examined at temperatures of 30 °C, 40 °C and 50 °C, consistency of 10%, $pH_{initial}$ 5.0–6.0 and pH_{final} 4.2–4.6 and reaction time of 1 to 24 h.

To follow the concentration dependence of the hydrolysis process solutions of 1%-, 2%-, 3%- and 5%-enzyme concentration referred to the mass were used.

The reducing sugars in the hydrolysates obtained were determined according to the DNS method (Miller, 1959) and calculated in % with respect to the mass.

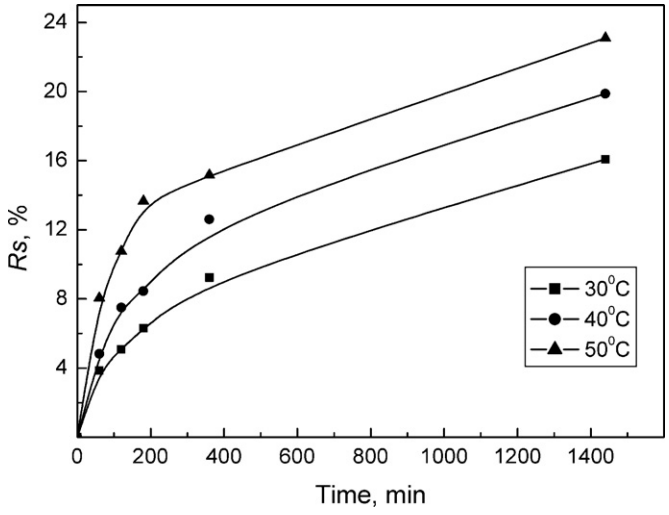

Fig. 1. Kinetic curves referring to different temperature values and 5%-enzyme concentration.

3. Results and discussion

3.1. Temperature dependence of the hydrolysis process

The temperature effect on the hydrolysis process is followed in the range from 30 °C to 50 °C at a constant amount of 5% (referred to the mass) of the enzyme introduced. The experimental data concerning the reducing sugars amount, R_s, in case of enzyme complex hydrolysis provide to obtain the kinetic curves shown in Fig. 1. It is seen that the reducing sugars amount increases with time and is favored by temperature increase.

The dimensionless quantity α is used as a kinetic variable in this investigation. It is determined by the relative change of the reducing sugars amount and is calculated in correspondence with Eq. (1):

$$\alpha = \frac{R_s}{R_{s,\max}} \tag{1}$$

where R_s is the current amount of the reducing sugars, while $R_{s,\max}$ (equal to 35%) is the maximum quantity of the reducing sugars obtained in course of enzyme treatment for 48 h. It is evident that α can be treated as enzymatic hydrolysis degree.

The kinetics of the process is described by the exponential kinetic equation valid for processes taking place at uniformly inhomogeneous surfaces. According to the model of uniformly inhomogeneous surfaces the active centers on the surface are distributed linearly referring to their energy and entropy.

$$v = v_0 e^{-a\alpha} \tag{2}$$

where $v = (d\alpha/dt)$ is the current rate and v_0 is the initial rate of enzymatic hydrolysis. The kinetic coefficient of inhomogeneity a accounts for the energy and the entropy inhomogeneity of the system. When this coefficient does not depend on the temperature it characterizes only the entropy inhomogeneity of the surface. The same coefficient is related to the number of active centers, their accessibility and spatial orientation (Radeva, Bikov, & Valchev, 2009; Radeva, Valchev, & Tsekova, 2010; Radeva, Valchev, & Valcheva, 2011; Radeva, Valcheva, & Veleva, 2009).

All kinetic curves are linearized in coordinates α vs. $\ln t$ in correspondence with the approximate integral form of the exponential kinetic equation:

$$\alpha = \frac{1}{a}\ln(v_0 a) + \frac{1}{a}\ln\ t \tag{3}$$

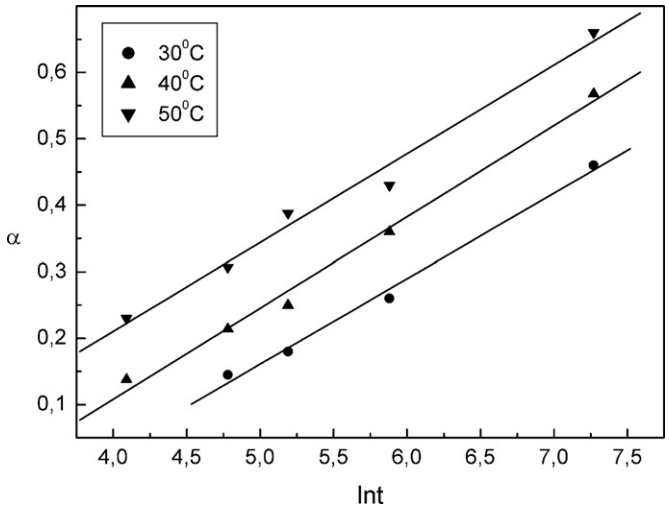

Fig. 2. Linear dependences of the kinetic variable α vs. ln t.

The linear dependences obtained are presented in Fig. 2.

The coefficient of inhomogeneity is estimated from the slope of the lines in Fig. 2. It is found equal to 7.7 for temperature values of 30 °C, 40 °C and 50 °C. The coefficient a accounts only for entropy inhomogeneity in cases where it is temperature independent (Valcheva, Veleva, Radeva, & Valchev, 2003).

The values of the hydrolysis process initial rate are estimated with the application of Eq. (3). It is found that the initial rate increases with temperature increase. The temperature dependence according to Arrhenius equation (Eq. (4)) is presented in Fig. 4.

The current rate of the hydrolysis process is estimated at different values of α following Eq. (2). The temperature effect is outlined in Fig. 3. It is seen that the process rate is the highest at α values less than 0.2. Then it decreases significantly most probably because of exhaustion of the accessible active centers on the surface.

The temperature dependence of the initial and the current rate is described by the Arrhenius equation:

$$v = Ae^{-(E/RT)} \tag{4}$$

The values of the activation energy E and the preexponential factor A are calculated at different constant values of α in accordance with the logarithmic form of Eq. (4). The linear dependences obtained are shown in Fig. 4.

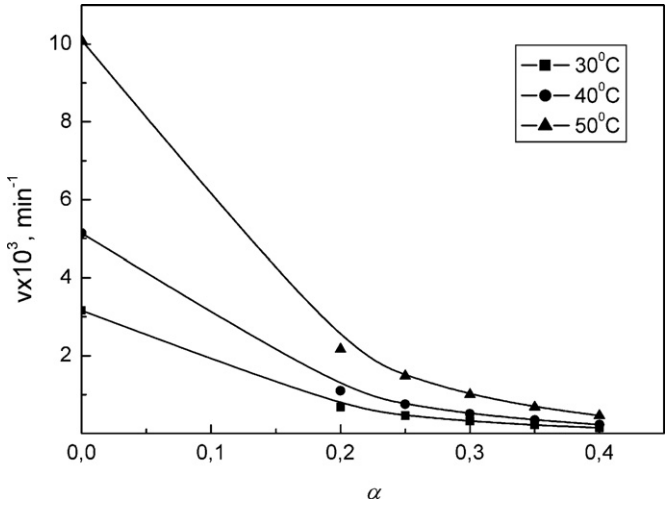

Fig. 3. Dependence of the enzymatic hydrolysis current rate v on α at various temperature values.

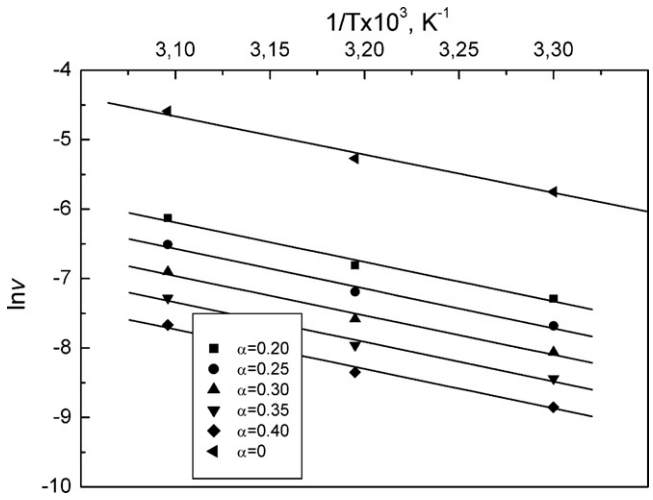

Fig. 4. Temperature dependence of the initial and current rate of the process at α = *const.*

The activation energy determined on the ground of the temperature dependence of the initial rate is equal to 46 kJ mol^{-1}. The activation energy estimated on the ground of the current rate data stays unchanged in the course of the process ($E_0 = E =$ to 46 kJ mol^{-1}). This means that the system studied is homogeneous from energetic point of view but inhomogeneous when its entropy is concerned.

Unlike the activation energy, the preexponential factor A decreases with the increase of the degree of hydrolysis, α. This dependence is described by Eq. (5):

$$\ln A = \ln A_o - a\alpha \tag{5}$$

It is presented in Table 1.

The preexponential factor accounts for entropy factors of the system discussed. They are determined by enzyme molecules spatial orientation and the accompanying steric hindrances. The decrease of ln A in the course of the process can be explained with the decreased accessibility and exhaustion of the active centers on the surface. The results obtained show that the preexponential factor decreases is responsible for the process rate decrease, i.e. not energetic, but complex entropy factors have a determining effect.

3.2. Concentration dependence of the hydrolysis process

The effect of the enzyme complex concentration on the hydrolysis process kinetics is followed. Solutions of 1%-, 2%-, 3%- and 5%-enzyme concentration, c_E, referred to the mass are used. The experiments are carried out at 40 °C and 50 °C.

The reducing sugars amount R_s obtained in the course of the enzymatic hydrolysis varies with time. The corresponding curves referring to 40 °C and 50 °C are shown in Fig. 5.

Fig. 5 shows that the amount of the reducing sugars, R_s, increases with the increase of enzyme concentration and this effect is better outlined at the higher temperature. The kinetics of the process is described by the exponential kinetic equation (Eq. (2)) using the degree of hydrolysis α (Eq. (1)) as a kinetic variable. The applicability of the exponential equation has been already verified. All kinetic

Table 1
Dependence on prexponential factor on degree of hydrolysis.

α	0	0.20	0.25	0.30	0.35	0.40
ln A	12.46	11.40	11.17	10.63	10.33	9.86

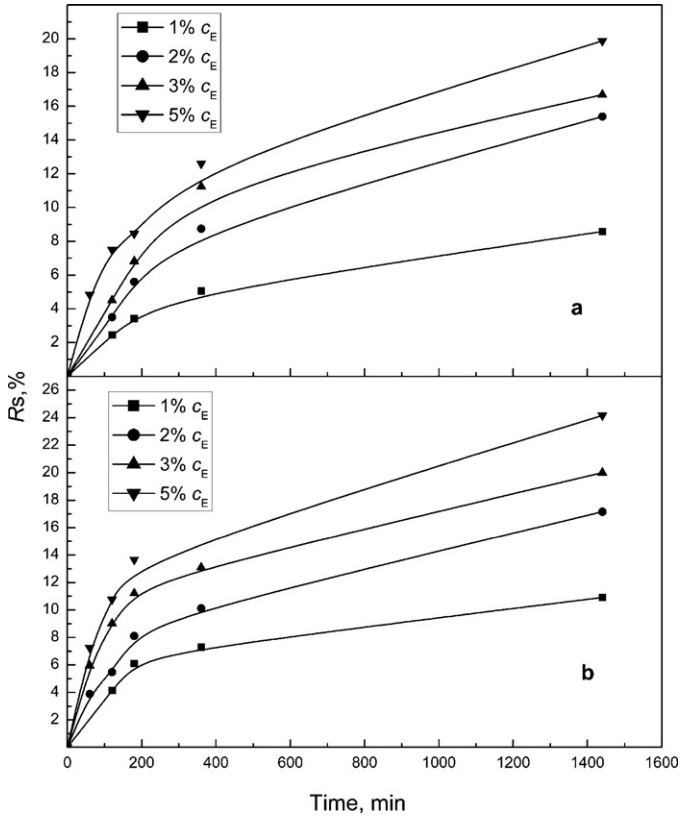

Fig. 5. Kinetic curves of reducing sugars amount, *Rs*, referring to the different enzyme concentrations: (a) the data at 40 °C; (b) the data at 50 °C.

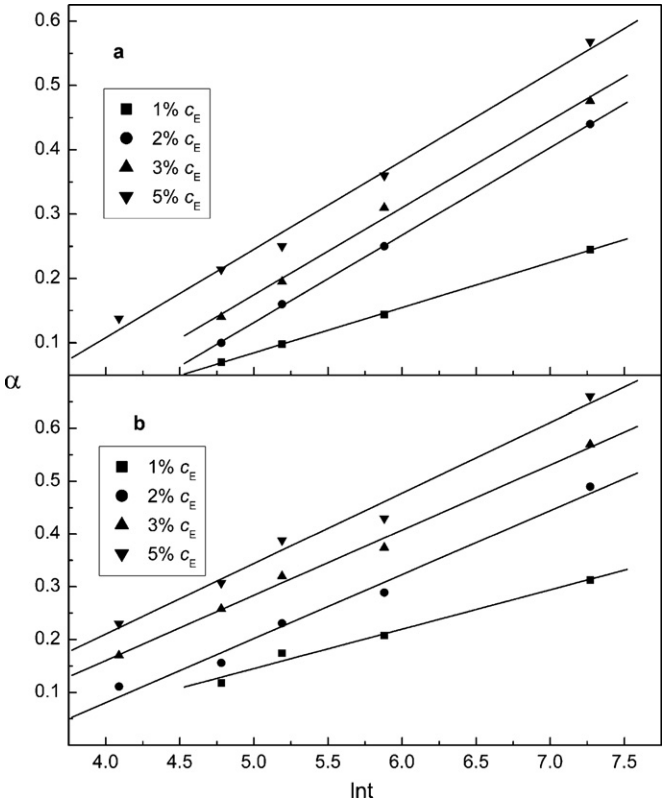

Fig. 6. Linear dependences α vs. $\ln t$ referring to different enzyme concentrations: (a) the data at 40 °C; (b) the data at 50 °C.

curves are linearized in coordinates α vs. $\ln t$ at the temperatures studied and the enzyme concentrations used (Fig. 6).

It is found that the kinetic coefficient of inhomogeneity a is equal to 7.7 for both temperatures studied and besides, it stays unchanged in case of enzyme concentration of 2%, 3% and 5%. That of 1% is an exclusion as $a = 14.2$. The latter value indicates hindrances in the course of the process, most probably determined by enzyme insufficiency.

The values of the initial rate of the process, v_0 (Eq. (3)), are calculated. They are found dependent on the temperature and the enzyme concentration (Table 2).

Fig. 7 presents the dependence of the logarithm of the initial rate on enzyme concentration at the temperatures studied.

The dependence of the initial rate v_0 increase on enzyme concentration c_E increase can be described by the following equation valid for both temperatures investigated:

$$v_0 = ke^{bc_E} \tag{6}$$

where b is a coefficient equal to 0.23 (it does not depend on the temperature and the enzyme concentration), while k has the meaning of an apparent rate constant. It is equal to 1.83×10^{-3} min^{-1} at 40 °C and to 3.2×10^{-3} min^{-1} at 50 °C. The apparent activation energy, E_c, is found equal to 46.7 kJ mol^{-1}. It is calculated on the ground of the rate constants values obtained in correspondence with Arrhenius equation in its classic form:

$$k = \lambda e^{-(E_c/RT)} \tag{7}$$

and coincides with that found on the ground of the temperature dependence of the rate in 5%-presence of enzyme. The preexponential factor λ in the Arrhenius equation (Eq. (7)) has a value of 8×10^4 min^{-1}.

Another expression of the current rate of the process is obtained on the ground of Eqs. (2) and (6):

$$v = ke^{(bc_E - a\alpha)} \tag{8}$$

Table 2
Values of the initial rate v_0 at the temperatures and enzyme concentrations studied.

c_E,%	$v_0 \times 10^3$, min^{-1}	
	$T = 40$ °C	$T = 50$ °C
1	2.30	4.03
2	2.88	5.07
3	3.62	6.37
5	5.74	10.10

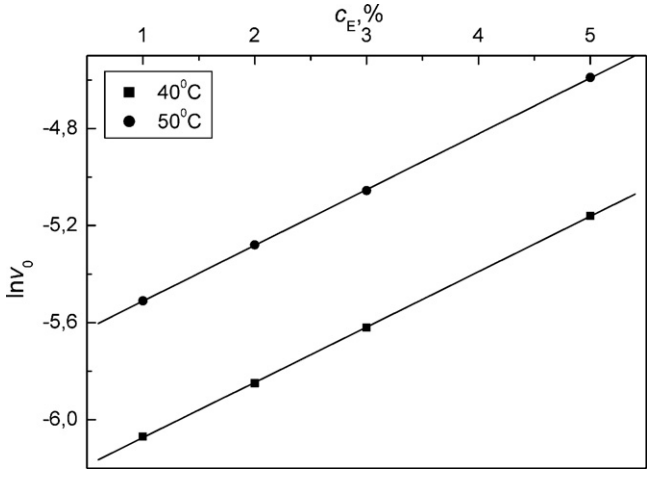

Fig. 7. Dependence of $\ln v_0$ on enzyme concentration, c_E.

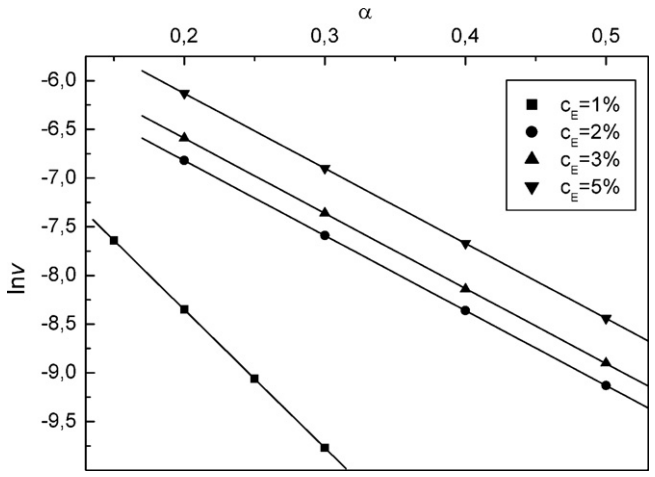

Fig. 8. Dependence of the current rate, v on the degree of hydrolysis α at 50 °C.

It is used to estimate the current rates v of the hydrolysis process at different values of the degree of hydrolysis,α, at the enzyme concentrations used. The dependences obtained are shown in Fig. 8.

As Fig. 8 shows the current rate decreases in the course of the process. The treatment of the rates at a given degree of hydrolysis shows that they increase with the enzyme concentration increase. Analogical dependence is obtained at 40 °C.

The substitution of Eq. (7) into Eq. (8) leads to the following generalized expression of the current rate, v, which accounts for the effect of the temperature, T, of the degree of hydrolysis and that of the enzyme concentration, c_E:

$$v = \lambda e^{(bc_E - a\alpha)} e^{-(E/RT)} \tag{9}$$

The enzyme–substrate system has the specific behavior of an energetically homogeneous one as the activation energy, E, does not vary in the course of the process. The preexponential factor, A, depends on the degree of hydrolysis α and the enzyme concentration. It is described by:

$$A = \lambda e^{(bc_E - a\alpha)} \tag{10}$$

where A is the preexponential factor obtained on the ground of the temperature dependence of the rate, while λ is the preexponential factor obtained by the temperature dependence of the rate constant k.

Eq. (3) can be rewritten on the ground of Eqs. (6) and (7) in the form:

$$\alpha = \frac{1}{a}\ln(\lambda a t) + \frac{b}{a}c_E - \frac{E}{aRT} \tag{11}$$

Thus a new expression for the dependence of degree of hydrolysis α on the time (t), the enzyme concentration (c_E) and the temperature (T) is obtained. It is of practical importance as well because it provides estimation of the degree of hydrolysis required at predetermined values of the temperature, the enzyme concentration and the time used.

This expression can be used for an easy and precise control of the enzyme hydrolysis of cellulose part of the wheat straw.

4. Conclusions

The kinetics of the cellulasic hydrolysis of the cellulose part of wheat straw after steam explosion was investigated. It is found that the exponential kinetic equation provides a good interpretation of cellulase action. The time dependence of the reducing sugars amount is followed at varying the temperature value and the amount of the enzyme introduced. The activation energy determined on the ground of the rate temperature dependence stays unchanged in the course of the process. The preexponential factor decreases with the increase of the degree of hydrolysis and is responsible for the process rate decrease. A new expression for the dependence of degree of hydrolysis on the time, the enzyme concentration and the temperature is obtained. It is of practical importance as well because it provides estimation of the degree of hydrolysis required at predetermined values of the temperature, the enzyme concentration and the time used.

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