Bulgarian Chemical Communications, Volume 47, Special issue A (pp. 30 – 33) 2015

Kinetic aspects of enzyme hydrolysis of cellulose fiber material

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Received June 30, 2014; Accepted January 29, 2015

The kinetic dependences characterizing enzyme hydrolysis of cellulose fiber material (bleached hardwood pulp) by cellulasic enzyme complexes are studied. It is found that the topochemical mechanism provides a good interpretation of the cellulase action. The kinetics of the process is described by the modified topochemical equation of Prout – Tompkins. It is applied to the processes starting on the easily accessible outer surface of the pulp to continue further through by a gradual penetration to the capillary system of the fiber. The structural features of the system pulp – enzyme control the rate of the process. The activation energy of the hydrolysis is found constant indicating that the energy characteristics of the cellulose are identical on the surface and in the bulk of the fiber matrix. The pre-exponential factor accounts for the accessibility and the varying dimensions of the reaction area, which is formed by the cellulose–enzyme complexes and change during the process. The rate decrease in the course of the process is determined not only by the pre-exponential factor decrease but by the enzyme inhibition as well.

Keywords: *bleached hardwood pulp, enzyme hydrolysis, kinetics.*

INTRODUCTION

The enzyme hydrolysis of cellulose to glucose by cellulases is one of the major steps involved in the conversion of cellulose and lignocellulose material to yield biofuel. The susceptibility of cellulose substrates to cellulase depends on the structural features of the substrate including the cellulose crystallinity, the degree of cellulose polymerization, the surface area, and the lignin content [1]. Cellulose is known to have relatively easily accessible amorphous regions with a few lateral interactions between the cellulose chains as well as crystalline domains that are much harder to hydrolyze. In addition, the hydrolysis product of the cellulase reaction, cellobiose (glucosyl β-1-4 glucose), inhibits severely the cellulase [2]. The optimization of lignocellulosic bioconversion by cellulase enzymes requires good knowledge of the reaction kinetics.

The enzyme processes are generally characterized by relations analogous to those of the heterogeneous catalytic and topochemical reactions. This concept is based on the fact that a specific chemical interaction proceeds between the substrate and the enzyme. It can be limited to the accessible contact surface or can proceed in the bulk of the fiber material. The heterogeneous nature of the macromolecular structure of the cellulose and the lignocellulose matrices hampers the thorough elucidation of the mechanism of the enzyme-substrate interaction [3-6]. Our former investigations on the kinetics of cellulase hydrolysis of wheat straw and maize stalks show that the kinetic model of inhomogeneous surfaces is applicable [7,8]. The explanation refers to the specific structural peculiarities and the highly developed contact surface which result from the hydrothermal pretreatment of these lignocellulose materials. The same kinetic model is also found valid in case of enzyme hydrolysis of steam exploded *Paulownia Tomentosa*. It is worth adding that the cellulase product in the latter case is of lower activity and which is why it attacks only the accessible active centers of the cellulose. Thus the process taking place cannot reach the bulk of the material, which in turn results in low efficacy [9]. According to M. Ioelovich and E. Morag [1] the more active cellulasic enzyme can generate new nano-holes (meso-pores) mainly in the non-crystalline amorphous domains of the cellulose and hence provide access of the enzyme molecules to the inner cellulose structure. The application of a highly active cellulasic complex in the hydrolysis of bleached hardwood pulp preserving its pulp fiber structure, irrespectively of the pretreatment carried out, requires the consideration of the enzyme process from the topochemical point of view.

The aim of the present communication is to present the kinetic relations observed in the course of the enzyme hydrolysis of bleached

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hardwood pulp which in turn provide to elucidate the mechanism of action of the highly active cellulase complex.

EXPERIMENTAL

The investigations were carried out on bleaching hardwood pulp from "Slilocell" AD - Svishtov. The cellulasic hydrolysis was carried out in polyethylene bags in a water bath preheated to the desired temperature. The cellulase complexes NS 22086, with activity 1000 BHU.g^{-1} and β-glucosidase NS 22118, with activity 250 CBU.g^{-1}, were used in a ratio of 10:1 for the enzymatic hydrolysis. The kinetics of the latter was examined at: temperature values of 30°C, 40°C, 50°C; a consistency of 10%; pH range of 5.0–6.0; reaction time of 1h to 48 h and 5% enzyme charge. The glucose content was analyzed by a Dionex HPLC system in accordance with the NREL standard biomass analytical procedure [10].

RESULTS AND DISCUSSION

The amount of glucose obtained, G (%), at different temperatures and reaction times is presented in Table 1.

Table 1. The amount of glucose, G (%) at different temperatures and reaction times

Time, min	G, %		
	T=30^{0}C	T=40^{0}C	T=50^{0}C
60	7.63	9.52	12.94
120	10.20	12.58	16.63
180	12.00	15.90	20.00
1440	26.90	34.00	40.80
2880	33.10	42.28	49.20

The dimensionless quantity α is used as a kinetic variable. It has the meaning of an degree of hydrolysis, i.e. an extent of conversion of cellulose to glucose. It is determined by the relative change of the glucose amount and is calculated in correspondence with Eq. 1:

$$\alpha = \frac{G}{G_{\max}} \quad (1)$$

where G (%) is the current amount of glucose, while G_{max}=87% is the maximal amount.

The degree of conversion α increases with the increase of time and temperature, (Fig. 1).

The applicability of different kinetic equations referring to diffusion, topochemical and other heterogeneous catalytic processes is examined. It is found that the enzyme process is best described by the modified Prout – Tompkins topochemical (P-T) equation:

$$\frac{\alpha}{1-\alpha} = (kt)^{\chi} \quad (2)$$

where k has the meaning of an apparent rate constant, while the power factor χ is an invariable quantity characteristic for the cellulose – enzyme system.

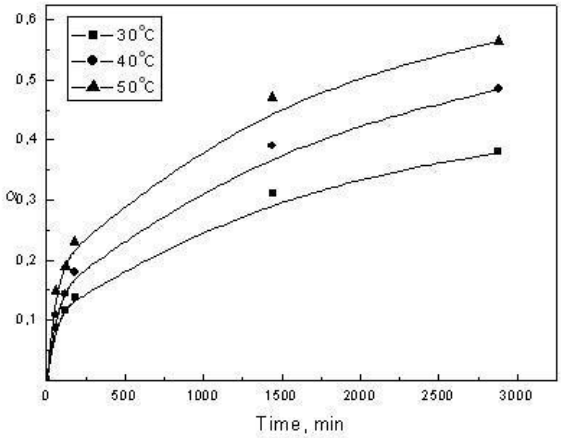

Fig. 1. Kinetic curves of the extent of enzyme hydrolysis at different temperatures.

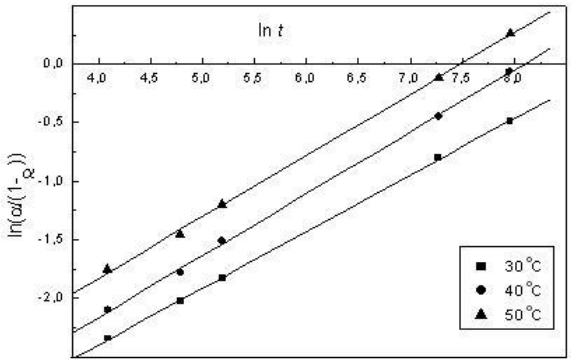

Fig.2. Linearized form of Prout – Tompkins equation for different temperatures.

It is known that Eq. (2) is successfully applied to chain topochemical reactions of chain mechanism [10]. A similar equation can be used in case of diffusion controlled heterogeneous processes. Analogous results have been reported for the xylanase action on kraft pulp [11].

All kinetic curves are linearized in coordinates $\ln\frac{\alpha}{1-\alpha}$ vs. ln t in correspondence with the logarithmic form of Eq. (2):

$$\ln\frac{\alpha}{1-\alpha} = \chi \ln k + \chi \ln t \quad (3)$$

The linear dependences obtained are illustrated by Fig. 2. The apparent rate constant k and power factor χ are calculated. The latter is temperature independent and has an average value of 0.5. The apparent rate constant k depends on the temperature in correspondence with the Arrhenius equation.

On the ground of the linear relation between ln *k* and 1/*T* (Fig. 4), the values of the activation energy *E* and pre-exponential factor ln λ are calculated: *E*=47.5 kJ/mol and ln λ=10.05.

It is of interest to determine the current rate $v = \frac{d\alpha}{dt}$, which varies during the process. The derivative form of the modified Prout-Tompkins equation is applied:

$$v = \chi k \alpha^{\frac{\chi-1}{\chi}} (1-\alpha)^{\frac{\chi+1}{\chi}} \qquad (4)$$

It outlines the dependence of the rate *v* (min^{-1}) on both the amount of the substrate left $(1-\alpha)$ and that of the product formed, α.

The factor $(1-\alpha)^{\frac{\chi+1}{\chi}}$ decreases with α increase. The factor $\alpha^{\frac{\chi-1}{\chi}}$ decreases also with α increase, because the exponent is negative (χ<1). This factor takes account of the glucose inhibiting effect on cellulase, especially near the end of the hydrolysis process when the rate decreases significantly [1]. In case of χ=0.5, Eq. (3) becomes:

$$v = k\frac{(1-\alpha)^3}{2\alpha} \qquad (5)$$

Eq. (5) provides the calculation of the current rates *v* (mim^{-1}) at different α and temperature values. It is shown in Fig. 3.

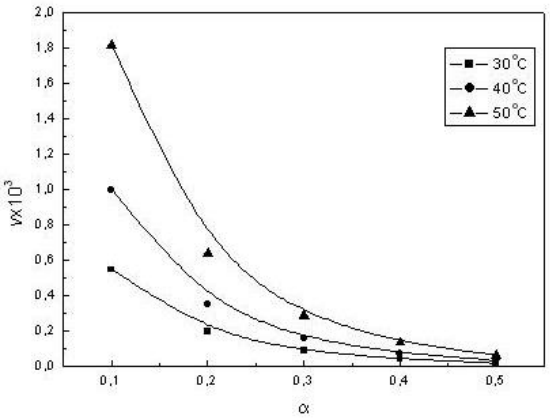

Fig. 3. Dependence of the current rate *v* (mim^{-1}) as a function of α at different temperatures.

It is seen that the process rate is the highest at α values less then 0.2. Then it decreases significantly, probably because of the inhibiting effect of the accumulated glucose at the final stage of the process.

The temperature dependence of the hydrolysis rate is followed in accordance with Arrhenius equation presented in the following form:

$$v = Ae^{-\frac{E}{RT}} \qquad (6)$$

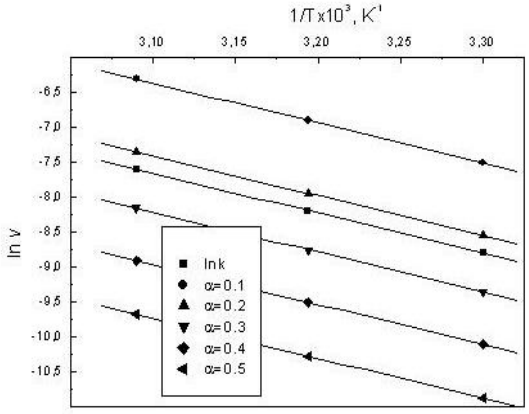

Fig. 4. Temperature dependence of the current rate and the rate constant at α=*const*.

The activation energy *E* and the pre-exponential factor *A* are calculated at different constant values of α (Fig. 4). The activation energy is *E* = 47.5 kJ/mol, i.e. a value which coincides with that found from the temperature dependence of the rate constant. It is evident that the activation energy stays constant during the process, which in turn shows that the cellulose active centers do not change their activity.

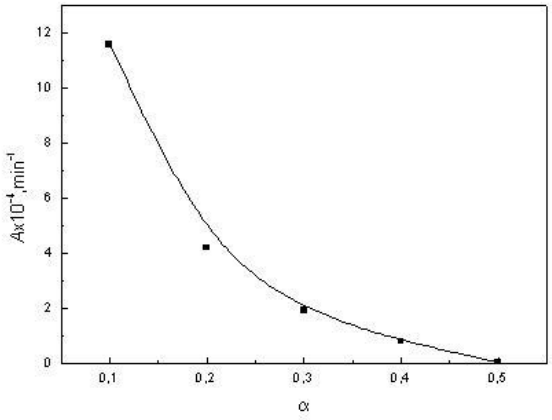

Fig. 5. Dependence of pre-exponential factor *A* (min^{-}1) on the degree of hydrolysis α.

Unlike the activation energy, the pre-exponential factor *A* decreases with the increase of the degree of hydrolysis α (Fig. 5) following the equation:

$$A = \lambda\frac{(1-\alpha)^3}{2\alpha} \qquad (7)$$

The factor *A* can be related to the dimensions and the accessibility of the reaction area, which is formed on the basis of the cellulose-enzyme complexes and changes during the process. It accounts also for the applicability of the

topochemical kinetic model. The rate decrease is due not only to *A* decrease, but also to the inhibition of the enzyme by the product obtained.

The hydrolysis process is starting to take place on the most accessible outer surface of the bleached hardwood pulp to proceed further gradually penetrating the capillary system of the fiber pulp matrix. The structural features of the system pulp – enzyme control the rate of the process. The constant activation energy shows that the energy characteristics of the cellulose are identical on the outer and the inner surface of the capillaries system, i.e. the enzyme interacts chemically with one and the same cellulose active sites. The rate decrease in the course of the process is determined not only by the pre-exponential factor decrease but by the enzyme inhibition as well.

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КИНЕТИЧНИ АСПЕКТИ НА ЕНЗИМНАТА ХИДРОЛИЗА НА ЦЕЛУЛОЗЕН ВЛАКНЕСТ МАТЕРИАЛ

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Постъпила на 30 юни, 2014 г.; Приета на 29 януари, 2015 г.

Изучени са кинетичните зависимости на ензимна хидролиза на целулозен влакнест материал (избелена широколистна целулоза) с целулазен ензимен комплекс. Установено е, че процесът на ензимна хидролиза може да се интерпретира с топохимичен кинетичен модел. За описание на кинетиката на процеса е използвано модифицираното топохимично уравнение на Праут – Томпкинс. То е приложимо за процеси, които започват от най-лесно достъпните външни центрове и постепенно проникват в структурата на целулозното влакно. Определящи за скоростта на процеса са структурните изменения на повърхността. Установено е, че активиращата енергия не се променя в хода на процеса, което показва, че енергетичните характеристики са идентични и на повърхността и във вътрешността на целулозната матрица.

Пред-експоненциалният фактор, който отчита достъпността и особеностите на реакционната зона на системата целулоза – ензим, се променя в хода на процеса. Установено е, че намалението на скоростта се дължи не само на намалението на пред-експоненциалния множител, но и на инхибирането на ензима.