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Effect of alkali pretreatment on cellulase hydrolysis of wheat straw: Kinetic study

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Abstract

The most efficient way to hydrolyse cellulose from wheat straw is by enzymic hydrolysis, which can be improved by different pretreatments of the substrate. The enzyme kinetics of alkali (sodium hydroxide) pretreated wheat straw was studied using different concentrations of a commercial cellulase enzyme (6.25–75 g/L). This pretreatment increased hydrolysis compared to untreated wheat straw. The influence of enzyme concentration on the production of reducing sugars was studied from two different theoretical approaches. In the first, the hydrolysis model and kinetic parameters (maximal velocity, V_{emax}, and half-saturation constant, K_e) were determined from initial velocities by an alternative approach to the classical Michaelis–Menten equation. In the second, the Chrastil approach is used, which is the study of all the time values from the rate of product formation, taking into account that in a heterogeneous system, these reactions are diffusion limited and the time curves depend strongly on the heterogeneous rate-limiting structures of the enzyme system.

Keywords: Cellulase; Wheat straw; Cellulose; Alkali pretreatment; Hydrolysis; Kinetics

1. Introduction

Agricultural residues such as wheat straw represent large renewable resources for lignocellulosic bioconversion. Wheat straw is a widely available substrate and its disposal entails an environmental problem. Transformation of this agricultural by-product is, therefore, desirable. Bioconversion of wheat straw is favoured because of its relatively low lignin content (<20%, w/w) and high carbohydrate content (hemicellulose (50%) and cellulose (30%)) [1].

Enzymic hydrolysis is an interesting way to produce sugars from cellulosic wastes because of its mild operating conditions, regarding pH and temperature, and the absence of by-products [2,3]. Xylan-rich cell walls (wheat straw up to 20% (w/w) of xylans), which contain significant amounts of lignin, are also generally resistant to enzymic hydrolysis and require severe chemo-mechanical pretreatments [4] (including steaming, radiation, acid hydrolysis and alkali digestion) before the polysaccharides become accessible to enzymes and can be hydrolysed to monomeric sugars in high yield [5]. Compared to acid pretreatments, alkaline processes have less sugar degradation, furan derivative formation is avoided and many of the caustic salts can be recovered [6].

Optimization of lignocellulosic bioconversion by cellulase enzymes requires good knowledge of the kinetic reaction. The complexity of the enzymic hydrolysis of lignocellulosic wastes stems from the fact that they are heterogeneous insoluble substrates, and thus, their enzymic hydrolysis is always limited.

The kinetics of enzymes has been usually studied by the Henri–Michaelis–Menten equation [7–9] from initial velocity kinetics. It has been shown that the Henri–Michaelis–Menten equation is not suitable for the analysis of enzymic hydrolysis of heterogeneous structures, especially when the enzymtic reaction is diffusion limited [10,11]. In this regard, alternative kinetic models have been proposed to study enzymic

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catalytic reactions. Baley [12] proposed an alternative approach by taking into account that the measurement of initial reaction velocity, v_0, as a function of substrate concentration (Henri–Michaelis–Menten equation), is a very artificial convention when the reaction takes place on a hydrated solid within which it is impossible to change the concentration of substrate sites. This alternative approach to the classical Henri–Michaelis–Menten equation suggests that the initial hydrolysis velocity, v_0, should be expressed as a function of the initial enzyme concentration, $[E]_0$. Accordingly, it is convenient to define a maximal velocity, V_{emax}, and a corresponding half-saturation constant, K_e, as Eq. (1) shows:

$$v_0 = \frac{V_{emax}[E_0]}{K_e + [E_0]} \quad (1)$$

On the other hand, in the case of heterogeneous systems, it is possible to study the enzymic kinetics using all time data in front of the Henri–Michaelis–Menten initial velocity kinetics, as Chrastil stated out in several papers [10,11,13]. Also, it is possible to consider that these enzymic reactions are diffusion limited, and therefore, the time curves depend strongly on the heterogeneous rate-limiting structures of the substrate–enzyme system. In this case, the influence of substrate surface modification, as the alkali pretreatment studied in the present work, will be pointed out for every enzyme concentration in all time curves. The initial enzyme concentration will act as driving force due to the existence of a concentration gradient and the substrate surface will influence strongly the adsorption step. In order to reach the active centres (some of them promoted by the alkali pretreatment hydrolysis), the reactant should be diffused through the limiting external layer while part of the reactant disappears by adsorption on closer centres [11]. The products formed in activated centres (quantified as reducing sugars in this work) will be desorbed from the surface to be incorporated to the main bath. If not, they will act as inhibitors [14,15] of the transport to further reaction centres along or across the high molecular structures of the substrate. Therefore, there will be two gradients modifying the whole behaviour: first step enzyme concentration and final time surface product concentration.

Eq. (2) shows the diffusion-limited kinetic model proposed by Chrastil [11]:

$$P = P_\infty[1 - \exp(-kE_0t)]^n \quad (2)$$

where P and P_∞ are the products which diffused at every considered time t and at equilibrium, respectively, k a rate constant proportional to the diffusion coefficient as is defined by Fick's law [16], E_0 the initial enzyme concentration and n a structural diffusion resistance constant dependent on sterical structure of the system [10,11]. The parameter n shows reaction order characteristics. When diffusion resistance is small, n tends to 1 (for low-resistance films $n = 0.9$–1.0) and the reaction is of first order. If the system is strongly limited by diffusion resistance, n is small (high-resistance structures $n = 0.5$–0.6). In addition, when $n > 1$, a consecutive reaction order may be expected.

The objective of this work was to study the enzyme kinetics of alkali-pretreated wheat straw in order to analyze the effect of the pretreatment conditions on the reaction kinetics. An alternative approach to the classical Henri–Michaelis–Menten equation and a kinetic model proposed for diffusion-limited enzymic reactions have been applied and compared to analyse the experimental data.

2. Materials and methods

2.1. Materials

Wheat straw was obtained from a farm in a local harvest. It was milled and a fraction (<6 mm) was separated using a sieve shaker of 6 mm. The substrate was washed with water at room temperature for 2 h (solid–liquid ratio 5%, w/w), filtered and dried at 40 °C.

2.2. Alkali pretreatment

Washed wheat straw was pretreated during 6 h at 80 °C with a 0.5 M NaOH solution in a thermostated 2 L batch stirred reactor (300 rpm) using a solid–liquid ratio of 5% (w/v). After cooling down, the wheat straw was glass filtered and neutralised to pH 5.3 (optimum catalytic activity of the enzyme) with a concentrated H_2SO_4 solution.

2.3. Enzymic hydrolysis

A commercial cellulase enzyme solution supplied by Novozymes A/S was used. The product is standardized to a declared activity of 400 EGU/g (Novo Nordisk AF 275 test method). Enzyme activity obtained was of 428.8 FPU/g of crude enzyme, measured by the filter paper method (FP) as described by Mandel et al. [17].

The pretreated wheat straw was hydrolysed with the enzyme in a thermostated stirring reactor (300 rpm) for 8 h at 50 °C and pH 5.3 (maximum enzymic activity). The pH was maintained at 5.3 with 0.05 M sodium acetate buffer. A liquid–solid ratio of 5% (w/v) was always used. Initial enzyme concentrations of 6, 12.5, 25, 37.5, 50 and 75 g/L were tested for different hydrolysis times. To follow the hydrolysis reaction, after reaching the desired time, a sample solution of 1 mL was taken out from the reactor, filtered through a glass filter and deactivated by increasing the temperature to 80 °C for 15 min. Finally, the concentration of product formation (quantified as total reducing sugars) was determined by the dinitrosalycilic acid method [18], using pure glucose for calibration. Experiments were carried out by triplicate. From the product formation curve the hydrolysis rate was calculated.