Chemical Engineering and Processing 89 (2015) 54–61

Contents lists available at ScienceDirect

Chemical Engineering and Processing: Process Intensification

journal homepage: www.elsevier.com/locate/cep

# A model of kinetics of the enzymatic hydrolysis of biopolymers – a concept for determination of hydrolysate composition

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ARTICLE INFO

*Article history:*
Received 27 November 2014
Accepted 26 January 2015
Available online 29 January 2015

*Keywords:*
Biopolymer decomposition
Enzymatic hydrolysis
Intermediate product composition
Model analysis

ABSTRACT

A mathematical model for the enzymatic hydrolysis of biopolymers was proposed. The model was used to determine the quantitative and qualitative composition of a reaction mixture. Assumptions of the model were characterized, model parameters were defined and a method for their determination was specified. The model was positively verified using the data obtained for β-lactoglobulin and trypsin.

Independent of the kind of kinetic equation, the model allows choice of such a reaction time in which the desired decomposition of the fractions is achieved.



## 1. Introduction

Biopolymers are the polymers that occur in living organisms and are produced by the living organisms. The most important groups of biopolymers are polysaccharides (including cellulose, starch, pectin, etc.), proteins, nucleic acids and lignins. Of interest are both biopolymers as such (for instance pectins are widely used in the food industry, lignins in the production of cotton candy, albumin in medicine, etc.) and products of their hydrolysis. The use of hydrolysates of natural polysaccharide biopolymers (cellulose, hemicellulose, etc.) as renewable energy sources is widely discussed in the literature [1,2]. Protein hydrolysates (short peptides) are known as compounds with therapeutic, regulatory or food-flavoring properties [3,4].

Hydrolysates with a desired chain length and composition are obtained by means of the enzymatic reaction. Chemical hydrolysis usually leads to complete hydrolysis of the biopolymer, and its course (hence, the concentration of intermediate products) is random. Through the selection of biocatalysts with defined specificity, particular bonds in the biopolymer are cleaved in a controlled manner.

The more diversified the monomeric structure of a biopolymer, which may also affect the variety of bonds between monomeric units, the more difficult the selection of enzymes and control of the hydrolysis process. Most difficulties appear in the hydrolysis of many chemically and structurally diversified hemicelluloses and proteins in which the combination of 20 naturally occurring amino acids is arbitrary. The arrangement of individual amino acids in the peptide chain and the specificity of the applied protease determine the quantitative and qualitative composition of intermediate and final products that will be formed.

In the literature, there are not so many papers describing the quantitative and qualitative composition of protein hydrolysates. The progress of proteolysis is usually described by the overall degree of hydrolysis (DH) of the protein and depending on its run, a kinetic equation that referred or did not refer to inhibition was attributed to it. Further on, based on experiments, kinetic constants were determined [5–7]. Such an approach is insufficient if the goal is to obtain a specific fraction of intermediate products, e.g., only peptides composed of a few or several amino acids to which therapeutic properties are attributed.

A kinetic model that allows the determination of the qualitative and quantitative composition of the reaction mixture for the hydrolysis of β-lactoglobulin was presented by Fernández and Riera [8]. For β-lactoglobulin, a protein with a relatively short chain of amino acids (162 monomer units), 13 products were found in its hydrolysis in reaction with trypsin. On the basis of the identification of these products, the places of the enzyme cuts were indicated. The kinetics of the process was described by 19 equations, assuming that all the reactions are first order. Eight rate constants were found by fitting to the model. Additionally, six constants describing the availability of the individual bonds were introduced to the model. A good agreement of the model and the experimental run of the mass fraction of selected polypeptides was

*Abbreviations:* E, enzyme; *h*, inhibitor fraction; *i,j*, number of monomer units in the chain; *m*, position of in the chain.

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http://dx.doi.org/10.1016/j.cep.2015.01.008
0255-2701/

**Nomenclature**

| | |
|---|---|
| $A_0$ | Avogadro's number ($mol^{-1}$) |
| $c_0$ | Initial concentration of biopolymer ($g\,L^{-1}$) |
| $c_E$ | Enzyme concentration ($g\,L^{-1}$) |
| DH | Degree of hydrolysis |
| $i$ | Number of monomeric units in the oligomer |
| $K_I$ | Inhibition constant ($g\,L^{-1}$) |
| $k$ | 1st order reaction rate constant ($s^{-1}$) |
| $k'$ | 2nd order reaction rate constant ($L\,s^{-1}\,g_E^{-1}$) |
| $M$ | Average molar weight of the monomeric unit ($g\,mol^{-1}$) |
| $N$ | Number of units in the biopolymer |
| $r$ | Reaction rate constant ($g\,L^{-1}\,s^{-1}$) |
| $S_i$ | Amount of oligomer containing $i$ monomeric units in a chain ($L^{-1}$) |
| $t$ | Time (s) |
| $y$ | Mass fraction |

Greek symbols

| | |
|---|---|
| $\beta$ | Reactivity coefficient |

presented. The main inconvenience of the method presented is the very large number of constants (in this case 14). Their experimental designation in many cases is very complex, particularly for proteins of higher molecular weight.

We proposed a less complex (with a smaller number of constants) model to determine the qualitative (the length of chain and sequence of monomeric units in them) and quantitative composition of the hydrolysate for any reaction kinetics. The model is general and can be used for any biopolymer and biocatalyst. We have adopted it to the description of the hydrolysis of β-lactoglobulin with trypsin in a reaction carried out by Fernández and Riera [8].

Based on this model, it is possible to design a continuous process and intensify the hydrolysis of biopolymers depending on the demand for a specified (of the given length) product. The model may be particularly useful for the calculation of the membrane reactor, allowing for a controlled recovery from the reaction mixture of the low molecular weight fractions [9,10], also a low weight inhibitor of the reaction.

## 2. A mathematical model of the biopolymer hydrolysis

Enzymatic depolymerisation is a very specific series-parallel reaction. A number of oligomers are formed from a specific polymer. The oligomers constitute a substrate for further depolymerisation. For biopolymers containing hundreds of monomeric units, the reaction system is very complex. In the simplest case, when the original polymer is composed of N identical monomeric units, the reaction system contains N − 1 substrates. In our model, the main assumption is that the substrates of the hydrolysis are particular chemical mer–mer bonds contained in the biopolymer.

In the case of proteins, individual monomeric units (amino acids) differ in the side chain and hence, bonds between them are not equivalent (equally susceptible to hydrolysis by a given protease). Hence, the number of bonds with a specific sequence of amino acids and the position in the chain of the substrate determine the description of the reaction kinetics. For a given chain length, there are a few or several different sequences of amino acids susceptible to a different extent to hydrolysis further in the reaction.

Description of such a complex reaction system requires a number of assumptions:

**Assumption 1.** There is a known sequence of monomeric units in the hydrolysed biopolymer and substrate specificity of the applied enzyme;

**Assumption 2.** As a result of a single reaction, one bond in the polymer chain is cleaved;

**Assumption 3.** Each chemical bond in the biopolymer chain has a defined reactivity coefficient, depending on the specificity of the enzyme used;

**Assumption 4.** Hydrolysis of all bonds is described by one kind of kinetic equation that takes into account their reactivity coefficient ($\beta$);

**Assumption 5.** Each monomeric unit in the biopolymer has the same molar weight, average for the biopolymer considered.

In equations describing the reaction kinetics, the amount of a given reagent ($S_i$) was expressed as molar concentration ($c_o y_i$) – Eq. (1), requiring an assumption of the averaged molecular weight of the monomeric unit $M$ (Assumption 5).

$$S_i = \frac{A_0 c_0 y_i}{Mi} \tag{1}$$

A general form of the kinetic equation for hydrolysis of a reagent containing $i$ monomeric units can be written as a combination of four functions:

$$r_i = k f_1(S_i) f_2(\beta_{ij}) f_3(S_h) f_4(t) \tag{2}$$

$f_1$ – function taking into account the amounts of a given reagent with $i$ monomeric units,

$f_2$ – function describing the reactivity ($0 \le \beta \le 1$) of a bond in position $j$ in the reagent with $i$ monomeric units,

$f_3$ – function describing inhibition by the particular product(s); this effect often occurs in the enzymatic hydrolysis of proteins usually caused by short peptides (their amount is denoted as $S_h$),

$f_4$ – the function describing the enzyme inactivation in time.

Because of the very large number of bonds that occur in a biopolymer molecule and their diversity in terms of reactivity, an additional Assumption 6 was introduced:

**Assumption 6.** Bonds occurring in the biopolymer can be classified into several groups that differ in the value of the reactivity coefficient. The number of groups is chosen depending on the specificity of the applied enzyme in relation to a given substrate, as follows.

- Highly reactive bonds with coefficient $\beta = 1$.
- Moderately reactive bonds with coefficient $\beta \in (0,1)$; there may be several subgroups.
- Nonreactive bonds with coefficient $\beta = 0$.

Simultaneously with the hydrolysis of the reactant of a given chain length, its formation from the reactants with longer chains could take place. The oligomer containing $i$ monomeric units can be formed from the reactant with a longer chain in two ways as shown in Fig. 1 according to Assumption 2. The sequence of monomeric units in these two oligomers (at $i = 8$) is different, hence the oligomers are qualitatively different and consequently have various susceptibilities to further hydrolysis. Thus, in the balancing of changes in the amount of the oligomer with a given sequence of monomeric units, on the side of its formation one should take into account only the oligomers with a longer chain