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Enzyme kinetics modeling as a tool to optimize food industry: a pragmatic approach based on amylolytic enzymes

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Abstract

Modeling is an important tool in the food industry since it is able to simplify explanation of phenomena and optimize processes that cover a broad field from manufacture to by-products treatment. The goal of the current article is to explore the development of enzyme kinetic models and their evolution over the last decades. For this reason, corresponding simulations were classified in deterministic, empirical and stochastic models, prior investigating limitations, corrections and industrial applications in each case. The ultimate goal is to provide an answer to a major problem: how can we develop an intermediate complexity model that achieves satisfactorily representation of the main phenomena with a limited number of parameters?

Keywords

Multienzyme kinetics, amylases, empirical models, Michaelis Menten, Monte Carlo, Neural Networks

1. Introduction

Enzyme kinetics is important in food manufacturing design since it is able to estimate the extent of substrate conversion under known conditions and evaluate reactor performance (Gekas & Lopez-Leiva, 1985). The new trend in food processing requires the development of tailor-made, low cost products and for this reason modeling has found a role as an engineering practice in accelerating enzymatic reactions, revealing the behavior of depolymerizing enzymes and reducing operational cost (Torgerson et al., 1979; Vasic-Racki et al., 2003; Buckow et al., 2006). In particular, enzyme kinetics modeling uses standard compound information to explore pathways and reaction mechanisms of complex macromolecular substrates prior to developing innovative process control strategies to ensure stability and desired efficacy (McDermott & Klein, 1986; Sin et al., 2009). The enzymatic hydrolysis of starch and the simultaneous production of derivatives with defined saccharide composition represent a typical example (Marchal et al., 2001). Indeed, available kinetic models using hydrolases vary widely in the assumed simplifications and consequent complexity. The simpler models are restricted to the operational conditions of their assay, while the practical implementation of the more complex models is more difficult since a large number of parameters have to be determined experimentally (Zanin & Moraes, 1996).

One of the key elements of enzyme kinetics modeling is the assembly of model compound reaction pathways and kinetics into a prediction of a real system (McDermott & Klein, 1986). This procedure might not be so simple in practice. For example, simulation of enzymatic starch degradation kinetics may lack of description when dealing with substrate composition and amylolytic enzymes action models. This is happening due to the fact that at different hydrolysis

intervals, multiple linear and branched dextrins are formed and subsequently reactions of several rates are obtained (Bryjak et al., 2000). Similar problems may arise during prediction of other processes like pectin hydrolysis. Pectin includes a family of polysaccharides with common features, but extremely diverse fine structures and thereby modeling of hydrolysis procedure should consider the conversion of dominant forms (homogalacturonans, rhamnogalacturonans I and II) to multiple oligomers (arabinooligosaccharides, oligogalacturonides etc) (Martínez et al., 2010). Besides, estimation of cellulose hydrolysis to glucose molecules is subjected to limitations such as the dramatic deceleration at high conversion degrees (Bansal et al., 2009).

The purpose of the current review is to highlight the basic aspects that should be taken into account during the development of an enzyme kinetic model destined to food industry. Specifically, representative models concerning the enzymatic depolymerization of biopolymers are categorized and analyzed according to the selected criteria of simulation method. Thereafter, limitations and applicability of the models are discussed, while corresponding industrial applications are explored. The latest concerns both manufacture and waste treatment stages. Finally, outcomes of the review were discussed in a pragmatic approach.

2. Complexity of models over the years

Enzyme kinetics modeling arises from the late 60's, when the so-called "subsite mapping" was developed to describe proteases activity (Schechter & Berger, 1967; Morihara & Oka, 1968). The denoted theory was based on the hypothesis that the catalytic site is surrounded with a number of independent subsites and each of them was assumed to accommodate one amino acid chain with a certain affinity. The unitary free energy of subsite association with the catalytic

site is assumed to be independent of the presence or absence of contiguous interactions. Subsequently, when *n* contiguous sites are occupied, the sum of lost subsite energy will be proportional to the relative population density for a specific binding mode. Following this consideration, the binding region of proteases was mapped by numbering the subsites, locating the catalytic amino acids position within them and finally determining the subsite-substrate-monomer-unit binding energies as well as hydrolytic rate coefficients (Thoma et al., 1970; Allen & Thoma, 1976). The respective subsite structure was useful in the interpretation of substrate specificity, theoretical prediction of product distribution and characterization of subsites (Hiromi, 1970).

Thereafter, subsite theory was also used to describe the hydrolysis of saccharides by α-amylases and in this case amino acid chain was replaced with a glucose unit. Up to the end of 80's, several studies (Thoma et al., 1970; Hiromi, 1970; Thoma, 1976; Torgerson et al., 1979; Hiromi et al., 1983; MacGregor & MacGregor, 1985; Nikolov et al., 1989) have been involved in theoretical investigations and quantitatively analysis of *α*-, *β*- and gluco-amylases' action patterns in terms of their subsite structure and affinities. In particular, they dealt with hydrolytic rate dependence on depolymerization degree of linear substrates and the cleavage pattern of malto-oligosaccharides. Since the component residue of a substrate is identical for homopolymer-degrading enzymes, only one subsite affinity can be assigned for a certain subsite and thereby kinetics prediction is much simpler.

At the same period, other models based on Michaelis-Menten equation were developed to estimate amylases action in terms of linear substrates concentration as well as mono- and di-saccharide equivalents of the product (Kusunoki et al., 1982; Cabral et al., 1983; Beschkov et al.,

1984; Yankov et al., 1986). They included reversibility and inhibition parameters of the kinetics, which were not described adequately via subsite mapping. Since starch is composed of linear amylose and branched amylopectin that differ among the botanical sources, researchers in the middle of 90's simulated corresponding depolymerization process by developing a "two-phase" model based on hydrolysis of both polysaccharide fractions and Michaelis-Menten equation (Park & Rollings 1994; 1995).

These control techniques offered satisfactory solutions on time, but the complexity of data processing in practice and the dramatic increase of kinetics equations doomed classical modeling to fail. For this reason, scientists tried later to approach hydrolytic processes with stochastic models such as Monte Carlo (McDermott & Klein, 1986; Pinto & Kaliaguine, 1991; Nakatani et al., 1996; 1997; Carbonell et al., 1998; Wojciechowski et al., 2001; Marchal et al., 2001; 2003) and more recently empirical multivariable methods like neural networks (Bryjak et al., 2000; Huang et al., 2002; Ciesielski et al., 2004; Ng et al., 2004; Rashid et al., 2006).

3. Classification of models

Enzyme kinetics models were herein categorized in three major groups (deterministic, empirical and stochastic) according to the implemented methodology. Classification was conducted in order to identify the crucial points that should be considered during modeling optimization.

Table 1 shows the main characteristics of several deterministic models applied for hydrolytic or depolymerization processes as well as respective compatibility with experimental data (inadequate < moderate < reasonable < good < excellent). Almost all of these methods were

based on Michaelis-Menten equation including or not subsite mapping. Enzyme inhibition has typically been introduced to describe retardation of the process due to the high concentration of the substrate. Other parameters such as reverse reactions, competition phenomena and inactivation constants may be included in a deterministic model. Allen & Thoma (1976) proposed a subsite model for depolymerization processes (in general) using inhibition constants and the so-called "microscopic" and "macroscopic" events. The "microscopic" events described the processes associated with one particular binding mode (i.e. positional isomer of the polymer substrate), whereas the "macroscopic" events referred to the net interactions of the enzyme with polymer substrate. This method is suitable for endo-amylases (i.e. α-amylase), but cannot be applied to exo-amylases (β-amylase) (Gyémánt et al., 2002). Inhibition constants have been suggested by other authors, too, while Kusunoki et al. (1982) applied Runge-Kutta-Gill method to solve a differential equation expressing the consumption rate of starch during its hydrolysis by glucoamylase.

Most of the other early studies concerned the action of both α- and β-amylase for the hydrolysis of insoluble or soluble starch, but they did not consider the influence of essential control aspects like temperature nor allowed the estimation of the final sugar composition. Marc et al. (1983) were among the first trying to introduce Arrhenius equation in a deterministic model with a final purpose of predicting the effect of temperature in hydrolysis procedures. Later, Koljonen et al. (1995) improved this model by analyzing in details the parameter estimation problem and final performance. In particular, 6 model parameters regarded the dissolution and denaturation of both amylases, while 10 of them related to the breakdown of starch. Model predictions were compared with the determined values of the state variables and corresponding

parameters were modified in order to minimize the prediction error. Thereafter, this model was applied to describe β-glucanase activity and glucanolysis progress (Kettunen et al., 1996). Besides, Langmuir-Hinshelwood kinetics, which is traditionally used in chemical engineering for solid catalysts, have been applied in more specific estimations, i.e. modeling of starch hydrolysis by immobilized glucoamylase (Cabral et al., 1986).

Although deterministic models provide at least reasonable compatibility with experimental data, in most cases it is rather impractical to obtain a mathematic solution since the number of demanded equations can possess double digits. Deterministic methods predominantly assume a homogenous structure of a substrate, predict only one or two final products and do not examine the intermediate product (Wojciechowski et al., 2001). Besides, calculating and predicting intermediate and final product concentration using differential equations is much more complicated for multienzymatic and multisubstrate systems (Bryjak et al., 2000). Subsequently, minimum assumptions about the molecular mechanism of the reaction and the kinetics have to be made. This is the reason why empirical equations for product concentration versus time are proposed (Paolucci-Jeanjean et al., 2000a; b). Most of these models predict only the breakdown and formation of a limited number of carbohydrates since simulation requires a differential or empirical reaction.

Examples of empirical models and corresponding characteristics of certain enzymatic reactions are shown in **Table 2**. Typically, reaction time is the independent and product concentration is the predicted variable that defines the hydrolysis rate. Empirical models are faster to develop and require fewer equations, while they are very useful for providing local trends in optimization and control without detailed knowledge of the system. Early studies did

not use the popular Michaelis-Menten equation, but preferred differential equations based on cubic spline functions, first-order and double exponential kinetics to describe starch and protein hydrolysis procedures (Rollings & Thompson, 1984; González-Tello et al. 1996; Margot et al., 1997; Paolucci-Jeanjean et al. 2000b). A similar approach was recently proposed by Martínez-Araiza et al. (in press). According to this study, modeling complex systems such as alcalase hydrolysis of whey proteins require a combination of theoretical models and their empirical evaluation in specific experimental conditions. Particularly, they applied Michaelis-Menten equation by assuming that the formation of one enzyme-substrate complex operates under mass action law in a quasi-steady-state and plotting the initial velocity as a function of substrate concentration. The latest yields the linear version Lineweaver–Burk plot in which a hyperbolic relationship is typically held.

Over the last decade, most of the empirical models used artificial neural networks to implement experimental results. These networks have the ability to map non-linear relationships without demanding prior information about the process or the model system (Bryjak et al., 2000). In other words, they could identify arbitrary discriminant functions directly from experimental data (Almeida, 2002). Their advantage over the classical mathematical models is the fact that they allow the simultaneous identification of structure parameters, while they possess the ability to adapt by examples (Bryjak et al., 2004). For example, Bryjak et al. (2000) applied this method in combination with a second order equation in order to predict both product concentration and substrate conversion degree using enzyme concentration and reaction time as independent variables. Huang et al. (2002) used alternatively principal component analysis and mean hypothesis testing together with neural networks with a final purpose of predicting

generally product amount as a function of reaction time and substrate concentration in fermentation procedures. More recently, artificial neural networks in combination with response surface methodology were utilized to model immobilized cellulase activity depending on pH alteration and coupling time (Zhang et al., 2010). Specifically, using the trained neural networks as the fitness function, a genetic algorithm was additionally implemented to optimize the immobilization conditions for maximum activity yield. Genetic algorithms use evolutionary natural selection processes, where selection results in species that fit best to the model. Specifically, a population of individuals is generated and a fitness value is assigned to each of them by specific fitness functions. Thereafter, individuals with higher fitness values are selected and undergo under genetic operation, i.e. crossover and mutation. The newly generated "child" population is utilized as a "parent" population for the next generation and this process is repeated continuously until a stop criterion has been met (He et al., 2008; Izadifar & Jahromi, 2007).

An empirical kinetic model without the application of neural networks has been developed by Åkerberg et al. (2000), too. According to this study, enzymatic wheat saccharification was simulated taking into account glucose inhibition as well as pH dependence of product concentration. A similar model based on inhibition and condensation reactions for free enzymes (Zanin & Moraes, 1996) was described by Morales et al. (2008) considering temperature dependence of glucose production. On the other hand, Muller (2000) provided a simple calculation that requires minimal analytic data interpretation. Particularly, the referred model predicted wort fermentability during high-temperature mashing for the production of low alcohol beers using only three equations. Although it just provided information about one aspect of brewing, the model represented a compromise between accuracy and ease of use. Nevertheless, it

is not appropriate for predictions of fermentability from standard mashes that are usually limited more by starch structure and less by enzyme activity.

The effect of other parameters such as acoustic power during lactose hydrolysis has been simulated using a single-step non-first-order enzyme inactivation kinetic model (Sadana & Henley, 1987) to predict enzyme stability as a function of processing time, while nonlinear least-squares method of Marquardt-Levenberg has been proposed to minimize error between experimental and calculated values (Demirhan & Özbek, 2009). Lately, catalytic efficiency of cellulase as a function of reaction time and substrate conversion has been implemented using differential equations based on molar enthalpy change and cumulative heat (Olsen et al., 2011).

Except from the deterministic and empirical models, it is easier in some cases to apply the stochastic methods instead of analytical procedures (Kurtz, 1972). Particularly, the latest models can be used to describe the formation and breakdown of all carbohydrates without determining numerous parameters and evaluating a large numbers of dependent equations. A brief presentation of stochastic models is shown in **Table 3**. The classic Michaelis-Menten equation, subsite mapping as well as reversibility, inhibition and competition constants have been applied in different procedures. Compatibility with experimental data has been shown to be reasonable, although it is lower compared to deterministic models. A general characteristic of stochastic simulations is that the prediction is performed by converting each hydrolysis reaction in a discrete event and thereby the probability of hydrolysis has to be translated to a discrete action (Marchal et al., 2003).

Monte Carlo technique is very popular in stochastic considerations. In principle, a pseudorandom number pointing out the polymer linkage attacked by an enzyme is generated to

simulate numerically the polymer hydrolysis. On the strength of defined enzyme specification, it is assumed that the enzyme can split off substrates from the attacked point (Wojciechowski et al., 2001). For example, according to the Monte Carlo simulation described by Marchal et al. (2003), there only two possibilities with regard to α-amylolysis of amylopectin: a linkage can be hydrolyzed or not. Thereby, a random number is selected using generator software. If the latest number is smaller or equal to the calculated chance of hydrolysis, the glucose linkage between two subsites is hydrolyzed and the enzyme moves to the next point randomly. If it is decided not to hydrolyze, the enzyme simply moves to the next random point within the matrix without cleavage of the polymer. Earlier, Nakatani (1996; 1997) provided a simple Monte Carlo combined with subsite mapping to describe the multiple-attack mechanism of α-and β-amylase. Indeed, depolymerization of a single substrate molecule possessing a certain molecular weight was repeatedly simulated. Finally, subsite position and the branched reaction paths from the enzyme-product complex were selected by random number and probabilities. Wojciechowski et al. (2001) predicted time evolution of all constituents in the starch hydrolysis system by applying both multienzymatic and multisubstrate reactions.

Later, Besselink et al. (2008) considered all the available information obtained from previous articles and developed a stochastic model to predict the formation and breakdown of all carbohydrates involved in time using a minimum number of experiments. This model used subsite theory instead of Monte Carlo technique and simulated the saccharide composition as well as esterification degree in time for wheat starch hydrolysis by α-amylase. Particularly, the subsite maps of α-amylase from Kandra et al. (2002) and Allen & Thoma (1976) were used as input for the model, while a method for the simulation of starch structure was developed, too.

Thereby, the association constant for the enzyme-substrate complex was calculated from the binding energies of the occupied subsites. At this case, in order to improve moderate compatibility with experimental data, it seems to be necessary to obtain a subsite map that can be used for longer hydrolysis times and larger carbohydrates. Such a subsite map could be obtained by fitting the estimated with experimental data similarly to the methodology described by Demirhan & Özbek (2009). Moreover, if the experimentally observed repetitive-attack mechanism for α-amylases was included in the model, compatibility of model and experimental data would eventually be better.

The classification presented above showed that there is a large multiplicity in simulating depolymerization processes of several macromolecules and specifically starch by amylolytic enzymes. Additionally, models were always modified from time to time in order to include aspects that were not considered in previous reported studies. Despite the omnipresence of developed models, an important gap still remains between theory and reality as it can be seen by comparing compatibility of stochastic and deterministic model presented in Table 3 and 1, respectively. For instance, the well-developed stochastic model of Basselink et al. (2008) showed a moderate compatibility. Specifically, although the order of magnitude of the predictions was comparable with the experimentally determined values but there was still a differentiation between the absolute and the experimental results. On the other hand, Wojciechowski et al. (2001) predicted more accurately the theoretical values, but in practice the simulation of more complex multisubstrate and multienzymatic systems might not be as easy as it has been claimed by the authors. For this reason, the basic limitations that a model should encompass are presented in the next section. Depending on the simulation, most of the accounted limitations and factors

are crucial for the enzyme's behavior in a certain reaction. In simpler words, the goal is to provide all the available information in order to develop a valid and realistic model.

4. Limitations and criteria of applicable models

Although there is a general agreement for a simple-to-use and accurate model, however, there are some basic parameters that should not be omitted. Besides, a better understanding of the enzymatic process enables the identification of the structural factors limiting enzymatic hydrolysis (Colonna et al., 1992). For a certain enzymatic reaction, researchers should focus in three major factors affecting the whole process: (a) the enzyme, (b) the substrate and (c) the mechanisms between them. Subsequently, substrate hydrolysis in a productively manner depends on enzyme and substrate characteristics. Herein, most of the involved mechanisms and prospective limitations are covered in order to obtain a coherent picture of biotechnological processes. As the most typical example among enzymatic reactions, starch enzymatic hydrolysis is selected for further exploitation.

To be more specific, two major categories of hydrolases are chosen: endo-amylase (i.e. α-amylase) and exo-amylase (i.e. β-amylase). As it is known from the literature, α-(1,4) linear bonds in the near vicinity of a branch point are not hydrolysable. This information can be used to "correct" the total number of kinetically available linear bonds using simple mass balance relationships (Park & Rollings, 1995). On the other hand, some glucidic molecules such as maltose, maltotriose and their derivatives inhibit strongly and competitively the action of α-amylases (Colonna et al., 1992). Indeed, it has been shown that enzymes can recognize substrate's ternary and quaternary structures and that their catalytic activities can reflect this

recognition (Park & Rollings, 1989). Thoma et al. (1971) mentioned that the hydrolytic rate constant of α-amylase is dependent on the polymerization degree of a substrate and proposed to add the effect of occupation (chain length) as a correcting factor. Park & Rollings (1995) developed a relative model and concluded that branching characteristics affect the action patterns of random-acting enzyme (α-amylase). Actually, it was shown that substrate branching characteristics strongly influence both the observed enzymatic activity as well as the enzyme's action pattern. As it is obvious, depending on the enzyme's origin, there are few limitations (i.e. inhibition, competition, branching or polymerization degree), which are independent of the enzyme's basic mode of action. Other mechanistically analogous limitations (i.e. mass transfer due to the high viscosity of high solid samples) may also reduce hydrolysis rate (Komolprasert & Ofoli, 1991; Vidal et al., 2009; Olsen et al., 2011).

Several authors tried to explain the possible mechanisms by which endo-amylase and exo-amylase act, taking into account the properties and limitations of each enzyme. For example, Wojciechowski et al. (2001) defined substrate specificity of α- and β-amylases. Subsequently, certain hydrolysis susceptible bonds were correlated with the action pattern of α-amylase by the following limitations:

i. minimal length of substrate was 5 glucose units,
ii. minimal distance from the reducing end was two glucose units,
iii. minimal length from the non-reducing end was 3 glucose units,
iv. minimal distance from a branched-point was 3 glucose units in major chain towards the reducing end,

v. minimal distance from a branched-point was 2 glucose units towards the non-reducing end, and

vi. minimal distance from a branched-point was 3 glucose units in subordinate chain towards the non-reducing end.

Expect from the above limitations, the action of α-amylase may be restricted by high starch concentrations. At this case, an empirical first-order model may describe better the hydrolysis profile (Komolprasert & Ofoli, 1991). With regard to the action pattern of β-amylase, limitations included (Wojciechowski et al. 2001):

- minimal length of substrate was 4 glucose units,
- productive attack linkage should be localized 2 glucose units from a non-reducing end,
- hydrolysis ceased in the vicinity of branched points, and
- minimal distance from reducing end and branched point was 2 glucose units.

Such structural information is fundamentally important since it is well known that starch degrading enzyme actions are influenced by substrate. The latest will potentially affect kinetic processes (Park & Rollings, 1995). The action patterns of other amylolytic enzymes should also be considered in detail. For example, the conversion degree of immobilized glucoamylase is known to be dependent on linear velocity due to internal and external mass transfer limitations (Cabral et al., 1986). The prevailing action patterns of the rest amylolytic enzyme during starch degradation are:

- the single-chain attack,
- the multi-chain attack, and
- multiple attack (an intermediate state between single- and multi-chain attack)

In the case of single-chain action, a polymer molecule is hydrolyzed completely by the productive enzyme-substrate complex before another is attacked. At this case, a serious limitation is related to the fact that even complete enzymatic reactions may be reversible (Tzafriri & Edelman, 2004). The multi-chain attack implies that one bond is hydrolyzed in a single enzyme-substrate encounter. The term multiple attack describes the situation by which several units are removed from the polysaccharide at a single encounter of enzyme and substrate (Banks & Greenwood, 1975). The degree of multiple attack may be defined as the average number of catalytic events, while the amount of multiple attack shown by an enzyme appears to be closely related to the kinetic constants. The latest govern the lifetime of the enzyme-substrate complex as compared with the catalytic turnover time. Thus, for a high degree of multiple attack, this lifetime may be large compared to the catalytic turnover time (Robyt & French, 1967).

Following the aforementioned limitations and corrections, the key to success is to develop an intermediate complexity model that represents adequately the main phenomena with a minimum, but significant number of assayed parameters. With regard to the overall strategy, the target is to blend the experimental measurements with the modeling of the enzyme kinetics in order to analyze an enzyme reactor as much as possible. By coupling different mathematical formulas with modern computer techniques, the experimental data could fit ideally with the predicted ones, while developed models will be effective in searching optimal operating conditions, ensuring biocatalyst microenvironment and finally enhancing productivity. For instance, initial rate data of forward and reverse enzymatic reaction at different substrate or product concentrations could be fitted to the kinetic equations using a non-linear least-squares regression technique and algorithms like Marquardt, Nelder-Mead, Gauss-Newton, Rosenbrock

etc. Thereafter, the estimated parameters represent the values of initial estimates of the parameter determination through the fitting procedure to the overall reaction rate (Vasic-Racki et al., 2003). In any case, it is unrealistic to expect that an exact mathematical model of a complex depolymerization process such as starch hydrolysis would be constructed in a way that it contains only experimentally obtained parameter values. Some of the available complex models exist in the literature predicted the formation of different species and contained parameters whose values have to be assumed (Zanin & Moraes, 1996, Åkerberg et al. 2000 and Wojciechowski et al. 2001). Nevertheless, most of the respective kinetic studies express only the average decrease of molecular weight and not the exact synthesis of the oligomers. This is understandable since in a process like α-amylase catalyzed hydrolysis, several hundreds to thousands of different saccharides make up this average mass number (Marchal et al., 2001).

Conclusively, the basic parameters that should be accounted for during the development of an enzyme kinetics model are:

(a) the basic action mode and catalytic site of the assayed enzyme in order to detect substrate subsites (when it is necessary),

(b) the structural characteristics of the substrate in order to determine accurately the total number of kinetically available bonds,

(c) limitations such as inhibition or competition that are independent of the enzyme action,

(d) limitations such reversibility that are strongly related to the enzyme action,

(e) and finally aspects such as mass transfer that affect directly reaction kinetics. The latest parameter could either restrict (i.e. in the case of high viscosity) or accelerate (i.e. in the case of ultrasonics treatment) enzymatic process.

In order to develop an intermediate complexity model that achieves satisfactorily representation of the main phenomena with a limited number of parameters, a simple methodology is proposed and illustrated in **Fig. 1**. Particularly, the first step is to define the assayed system (hydrolysis or depolymerization procedure) in terms of respective linearity and enzyme's action. Specifically, the number of substrate monomers in linear and branched polymer as well as the polymerization degree of the second should be defined. With regard to the enzyme, substrate preference and productivity cycles should be identified. Thereafter, the appropriate model should be selected prior determining initial reaction rate and/or formulating respective mathematical expressions. In the case of selecting a theoretical (stochastic) model, changes in substrate and intermediate product structures should be described. The next steps include sequentially parameter identification and mathematic formulation of the overall reaction rate. At this point, limitations should be taken into account, whereas the gap between accuracy and ease of use could be filled using the algorithms referred above. This is a key step of model's applicability since the efficiency of the developed algorithm is able to restrict the number of the necessary equations. Thereby, coupling between theoretical and experimental values (verification) is the next stage, while the choice of the appropriate algorithm is directly connected to each individual application. For example, the Levenberg-Marquardt algorithm is very popular in solving generic curve-fitting problems, but it finds only a local (not a global) minimum, i.e. compared to Rosenbrock function. This means that it can be used only in well-defined conditions. The final stage consists of combining the developed model with the reactor configuration. The latest aims relations identification between reaction rates and reactant. Validation is conducted using the data from batch reactor experiments at different initial

substrate or enzyme concentrations, which should be in good correlation with simulated data obtained by means of numerical integration (Vasic-Racki et al., 2003). Ultimately, calculation and prediction on the basis of the developed model is conducted.

5. Industrial Applications

Enzyme kinetics modeling has found a broad range of applications in the food industry. Indeed, since most of the investigated cases concern carbohydrates hydrolysis, modeling has been utilized to optimize the viscoelastic behavior of hydrolysates during the production of non-dairy products from starch and β-glucan containing materials, i.e. oat grains (Patsioura et al., 2011). With regard to bioprocessing, empirical models based on neural networks have been proposed to improve yield and monitor fermentations (Petrova et al., 1997; Karim et al., 1997), enhance inulinase and delivered fructooligosaccharides production (Mazutti et al., 2009; 2010), separate bioproducts (Patnaik, 1999) and estimate biomass concentration on-line (Wolf et al., 2000). Other empirical models have been successfully applied in the production of pharmaceuticals such as L-carnitine. At this case, kinetics modeling was able to evaluate three different fermentation modes and select fed-batch process (in comparison with 1-stage or 2 stage continuous processes) for the necessary biotransformation from γ-butyrobetaine (Hoeks et al., 1996). On the other hand, deterministic models have been used to design immobilized glucoamylase reactors (Cabral et al., 1986), while differential equations have optimized alcoholic fermentation conducted by immobilized cells (Galanakis et al., in press).

Food by-products treatment is another area of kinetics modeling applicability and for this reason several examples exist in the literature, i.e. optimization of enzymatic hydrolysis of

molasses (Najafpour & Shan, 2003), whey proteins (Margot et al., 1997), cellulose from dairy waste (Liao et al., 2008) and visceral waste proteins of yellowfin tuna (Ovissipour et al., 2012). Kinetic models have also been used to simulate biodegradation processes of multienzymatic system based on cultures, i.e. hydrolysis of starch wastes (Del Re et al., 2003) and suspensions of bovine serum albumin (Confer & Logan, 1997). Besides, they have been applied in membrane bioreactors and immobilized enzymes (i.e. thermolysin or tyrosinase) with a final purpose of removing of peptides or phenols, respectively, from food waste streams (Trusek-Holownia & Noworyta, 2008; Calabrò et al., 2009).

Conclusion and future trends

In spite of designing food manufacturing and by-products treatment processes based on enzymatic hydrolytic reactions, different kind of modelling can be applied. Given a set of assumptions, a theoretical model can be formulated prior validating with experimental data. The so-called “forward modeling” had been successfully used for a long time in the description of basic mechanisms in enzyme kinetics prediction (Chow & Voit, 2009). However, rigorous theoretical models are too complicated and require plenty of data, especially for the multienzymatic systems and respective fermentation procedures. Empirical models meet obstacles when working requirements demand many parameters and there is an additional task to interpret them physically. The scheme of developing the model first and then move to the interpretation of the experimental results or the opposite (i.e. to start from the experimental results and then create the model) does not always work. Therefore, it is recommended that the practical scientist and engineer should combine the modeling ideas in a mutli-step interactive

scheme between theory and experimental approach. For instance, Wojciechowski et al. (2001) approached ideally this scheme by defining clearly the system of starch in terms of structure and different enzyme's action and calculating accurately the discrete reaction time for each of them. The second was conducted using Monte Carlo simulation that pointed out randomly the attacked molecule and linkage. This approach may be applied in modelling of other enzymatic polymer degradation processes and the non-linear least-squares regression technique could be used to fill in the gap between experimental and predicted values like in the case of lactose or starch hydrolysis modeling referred by Demirhan & Özbek (2009) and Bryjak et al. (2000), respectively. Finally, a choice among the various types of modeling, the rigorous mathematical, the non-deterministic and the empirical ones supply additional degrees of freedom in order to provide both pragmatic and creative solutions to the problems to be encountered.

Literature

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Table 1: Deterministic models and corresponding parameters of certain enzymatic reactions.

Simulated procedure	Enzyme	Number of presented equations	Methodology			Compatibility with experimental data	Reference
			Michaelis-Menten equation	Subsite mapping	Other		
Depolymerization	Depolymerase	18	Yes	Yes	Inhibition, microscopic & macroscopic events	–	Allen & Thoma (1976)
Polymeric substances (up to 12 monomers) hydrolysis	α-amylase	6	Yes	Yes	No	Reasonable	Torgerson et al. (1979)
Starch hydrolysis	Glucoamylase	11	Yes	No	Inhibition & Runge-Kutta-Gill	Good	Kusunoki et al. (1982)
Starch hydrolysis	α- & β-amylase	42	Yes	No	Arrhenius	Good	Marc & Engasser (1983)
Starch hydrolysis	Glucoamylase	25	Yes	Yes	Inhibition & competition	Excellent	Hiromi et al. (1983)
Starch hydrolysis	Glucoamylase	2	Yes		Reversibility	Good	Cabral et al. (1983)
Maltose & maltotriose	Glucoamylase	7	Yes	No	Inhibition,	Excellent	Beschkov et al. (1984)

hydrolysis					reversibility		
Starch hydrolysis	α-amylase	8	Yes	Yes	No	Reasonable	MacGregor & MacGregor (1985)
Starch hydrolysis	Glucoamylase	3	Yes	No	Langmuir-Hinshelwood	Good	Cabral et al. (1986)
Starch hydrolysis	Glucoamylases I & II	23	Yes	Yes	No	Good	Nikolov et al. (1989)
Starch hydrolysis	Amyloglucosidase	14	Yes	Yes	Inhibition	Good	Lee et al. (1992)
Amylose hydrolysis	α-amylase	7	Yes	Yes	No	Reasonable	MacGregor et al. (1994)
Starch hydrolysis	α- & β-amylase	24	Yes	No	Arrhenius	Good	Koljonen et al. (1995)
β-glucan hydrolysis	β-glucanase	12	No	No	Arrhenius	Reasonable	Kettunen et al. (1996)
Starch saccharification	Amyloglucosidase	11	Yes	No	Inhibition, reversibility & competition	Reasonable	Zanin & Moraes (1996)
Depolymerization of homopolymers	Endo-enzymes	27	Yes	No	No	Good	Sendra & Carbonell (1998)
Lactose hydrolysis	β-galactosidase	11	Yes	No	Inhibition & competition	Reasonable	Ladero et al. (2000)
Starch hydrolysis	β-amylase & debranching enzyme	12	Yes	No	Runge-Kutta	Perfect	Zhou (2000)
Maltodextrins	Glucoamylase	8	Yes	No	Inhibition &	Good	Cepeda et al. (2001)

hydrolysis					competition		
Cellulose hydrolysis	Cellulase	20	Yes	No	Inhibition & reversibility	Moderate	Gan et al. (2003)
Starch hydrolysis	α- & β-amylases	14	Yes	No	No	Reasonable	Brandam et al. (2003)
Starch saccharification	Glucoamylase	1	Yes	No	Inhibition	Excellent	Polakovic & Bryjak (2004)

Table 2: Empirical models and corresponding parameters of certain enzymatic reactions.

Simulated procedure	Enzyme	Number of presented equations	Methodology			Predicted variable	Independent variable	Reference
			Neural Networks	Michaelis-Menten equation	Other			
Starch hydrolysis	α-amylase	8	No	No	Differential equations	Hydrolysis rate	Reaction time	Rollings & Thompson (1984)
Starch hydrolysis	α-amylase	10	No	Yes	No	Dextrose equivalents	Reaction time	Komolprasert & Ofoli (1991)
Starch hydrolysis	Glucoamylase	13	No	No	Cubic spline functions & differentiation	Hydrolysis rate	Initial concentration & reaction time	González-Tello et al. (1996)
Starch hydrolysis	Maltogenase	8	No	Yes	No	Maltose formation	Reaction time	Gaouar et al. (1997)
Whey protein hydrolysis	Trypsin (serin protease)	1-5	No	Yes	Double exponential kinetics & inhibition	Substrate conversion degree	Reaction time	Margot et al. (1997)
Starch hydrolysis	Exo-α-amylase	15	No	No	First-order reaction	Product concentration	Reaction time	Paolucci-Jeanjean et al. (2000b)
Starch saccharification	α-amylase & amyloglucosidase	13	No	Yes	Inhibition	Product concentration	pH, starch & enzyme concentration	Åkerberg et al. (2000)

Starch hydrolysis	Glucoamylase		Yes	No	Levenberg-Marquart network weight (second order optimization)	Products concentration & substrate conversion degree	Enzyme concentration & reaction time	Bryjak et al. (2000)
Fermentation	−	15	Yes	No	Principal component analysis & mean hypothesis testing	Product amount	Reaction time & substrates amount	Huang et al. (2002)
Protein hydrolysis	Alcalase	1	Yes	No	No	Antioxidant properties of hydrolysates	Reaction time, pH & temperature	Li et al. (2006)
Cellobiose hydrolysis	β-glucosidase	12	Yes	Yes	Multilayer perceptrons & inhibition	Maximum reaction rate, Michaelis-Menten & substrate inhibition constants	Initial substrate and product concentrations	Corazza et al. (2005)
Whey protein hydrolysis	Protease	3-5	Yes	No	Moving average & smoothing algorithm	Substrate concentration	Reaction time	Pinto et al. (2005)
Starch hydrolysis	α-amylase, amyglucosidase & glucoamylase	20	Yes	No	Gauss-Newton method	Product concentration	Enzyme dosage & temperature	Rashid et al. (2006)
Pectin hydrolysis	Pectolytic enzymes	4	No	No	Response surface methodology & second order	Substrate conversion & endopectolytic	Temperature, pH Enzyme & substrate	Rodríguez-Nogales et al. (2007)

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					model	productivity	concentration	
Whey protein hydrolysis	Alcalase	3	Yes	No	Multilayer perceptrons & differential mass balances	Substrate conversion & production rate of four pseudocomponents	Temperature & pH	Pinto et al. (2007)
Starch hydrolysis	*α*-amylase	8	No	Yes	Inhibition	Glucose production	Temperature & time	Morales et al. (2008)
Starch hydrolysis	*α*-amylase & glucoamylase	6	No	No	Asymptotic limit of product concentration	Product concentration	Reaction time	Vidal Jr. et al. (2009)
Lactose hydrolysis	β-galactosidase	2	No	No	Marquardt-Levenberg nonlinear least-squares	Enzyme activity	Acoustic power & processing time	Demirhan & Özbek (2009)
Egg white protein hydrolysis	Pepsin	20	No	Yes	No	Hydrolysis degree	Temperature, pH, reaction time, substrate & enzyme concentrations	Ruan et al. (2010)
Activity of immobilized cellulase	Cellulase	4	Yes	No	Genetic algorithm	Activity yield	pH & coupling time	Zhang et al. (2010)
Lignocellulosic material hydrolysis	Cellulase	5	No	No	Inhibition	Cellulose conversion rate	Substrate concentration	Smith et al. (2010)
Lignocellulosic material hydrolysis	Cellulase	3	No	No	Molar enthalpy change & cumulative heat	Catalytic efficiency	Reaction time & substrate conversion	Olsen et al. (2011)

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* "MAM" for "multiple attack mechanism". ** "SRAM" for "single- and random- attack mechanism".

Table 3: Stochastic models and corresponding parameters of certain enzymatic reactions.

Simulated procedure	Enzyme	Number of presented equations	Methodology				Compatibility with experimental data	Reference
			Monte Carlo	Michaelis-Menten equation	Subsite mapping	Other		
Lignin depolymerisation	Depolymerase	6	Yes	No	No	Reversibility	Not determined	McDermott et al. (1990)
Cellulose degradation	Exo-enzymes	31	No	Yes		Inhibition & competition	Reasonable	Nassar et al. (1991)
Cellobiose hydrolysis		10	Yes	No	No	−	Reasonable	Pinto et al. (1991)
Depolymerization of amylase &amylopectin	α-amylase (MAM*)	5	Yes	No	Yes	−	Inadequate	Nakatani et al. (1996)
Maltose hydrolysis	α-amylase	5	No	No	Yes	−	−	Suganuma et al. (1996)
Depolymerization of maltooligosaccharides, amylose & amylopectin	β-amylase (MAM*)	6	Yes	No	Yes		Reasonable	Nakatani et al. (1997)
Depolymerization of β-glucan	β-glucanase (SRAM**)	13	Yes	Yes	No	−	Excellent	Carbonel et al. (1998)

Amylopectin branched structure	α- & β amylase, glucoamylase	1	Yes	No	No		Reasonable	Marchal et al. (2001)
Starch hydrolysis	α- & β-amylase	4	Yes	No	No	Iteration model	Excellent	Wojciechowski et al. (2001)
	α-amylase	1	No	No	Yes	Iteration model	-	Gyémánt et al. (2002)
Maltooligosaccharides (up to 10 monomers) hydrolysis	α-amylase	−	No	No	Yes	No	-	Kandra et al. (2002)
Starch hydrolysis	α-amylase	11	Yes	No	Yes	Inhibition	Moderate	Marchal et al. (2003)
Starch hydrolysis	α-amylase	10	No	Yes	Yes	Inhibition	Moderate	Besselink et al. (2008)
Starch hydrolysis	α-amylase	11	No	Yes	Yes	Inhibition & competition	Inadequate	Baks et al. (2006)
Cellulose	cellulase	22	Yes		No	─	Not determined	Ting et al. (2009)

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Figure 1. Steps of enzymatic kinetics modeling procedure based on the initial ideas proposed by Wojciechowski et al. (2001) and Vasic-Racki et al. (2003).