

# **Activity and stability prediction of enzymatic reactions as a tool for thermal optimization: The case of inulin hydrolysis**

E. Ricca\*, V. Calabrò, S. Curcio, G Iorio

Università della Calabria, Dipartimento di Modellistica per l'Ingegneria  
Via P. Bucci – Cubo 39/C – 87030 Rende (CS) – ITALY. \* [ericca@unical.it](mailto:ericca@unical.it)

In the present work a relevant problem for enzymatic process temperature optimization is formulated, evidencing the need for an accurate choice of the reaction temperature. A reaction kinetics model and a deactivation model of the reaction of inulin enzymatic hydrolysis are coupled to predict reaction performance; the result is a complete model able to predict reaction performances for substrate concentrations ranging between 10 and 40 g/l and reaction temperature up to 60 °C, even on a long time scale.

The model is used to predict optimal conditions in different situations of industrial interest, proving to be not only a predictive tool, but also a means to reduce reaction times through thermal optimization of enzymatic processes. From a different point of view, the model could be used to minimize the enzyme loading needed for a reaction with reaction time and final conversion constraints.

## **1. Introduction**

An increasing interest is being given, in the specific literature, to the search for the best operating conditions in fructose production by inulin enzyme hydrolysis (Ricca et al. 2007), in terms of substrate origin (van Loo et al. 1995), inulinase provenience (Pandey et al. 1999), immobilization technique (Kochar et al. 1999; Nakamura et al., 1995; Wenlig et al., 1999), reactor type (Diaz et al., 2006; Gill et al. 2006). Many of the above mentioned works report experimental results proving good productivity and versatility of the process under study. However, stability and performance data over vast ranges of operating conditions are not proposed in a comprehensive work. The knowledge of reaction kinetics and deactivation rates and their overall effect over the reaction progress could be a very useful tool to further improve the potentialities of the fructose production process by inulin enzymatic hydrolysis.

In the present work a complete model, accounting for reaction kinetics of the Michaelis–Menten type and enzyme deactivation, is proposed as a tool to find the optimal temperature as a compromise between high temperatures favoring kinetics and low temperatures lowering deactivation rates. Optimization results can be extended to a vast set of operating conditions in terms of substrate initial concentration, enzyme loading and reaction progress which are the most important variables together with temperature that is the objective of optimization.

## 2. Theoretical

### 2.1 Mathematical model

The mathematical model adopted to describe the system relies on reaction rate modelled through Michaelis-Menten kinetics with parameters' temperature dependence of Arrhenius type (Ricca et al., 2009a) and deactivation kinetics of first order with deactivation constant related to temperature through Arrhenius relationship (Ricca et al. 2009b). The model equations are the following:

$$-\frac{dS}{dt} = V[S, T, e_a(t, T)] \quad (1)$$

$$t = 0, \quad S = S_0 \quad (2)$$

$$x = \frac{S_0 - S}{S_0} \quad (3)$$

$$V(S, T) = \frac{k_2(T)e_a S}{K_m(T) + S} \quad (4)$$

$$-\frac{de_a}{dt} = r_d[e_a(t, T)] \quad (5)$$

$$t = 0, \quad e_a = e_0 \quad (6)$$

$$r_d(e_a, T) = k_d(T)e_a \quad (7)$$

$$k_2(T) = \exp\left(\ln k_{20} - \frac{E_{a2}}{RT}\right) \quad (8)$$

$$K_m(T) = \exp\left(\ln K_{m0} - \frac{E_{am}}{RT}\right) \quad (9)$$

$$k_d(T) = \exp\left(\ln k_{d0} - \frac{E_{ad}}{RT}\right) \quad (10)$$

The model equations must be simultaneously solved to determine the exact relationship between reaction time (t) and substrate initial concentration ( $S_0$ ), enzyme loading ( $e_0$ ) and substrate conversion (x). When the final conversion  $x_f$  is given the reaction time corresponds to the duration of the reaction process.

The result of isothermal resolution of the above system is the following:

$$t = -\frac{1}{k_d(T)} \ln \left\{ \frac{k_d(T)}{k_2(T)e_0} [K_m(T) \ln(1-x) - S_0 x] + 1 \right\} \quad (11)$$

### 2.2 Process temperature optimization

In enzymatic studies the term "optimal temperature" is generally used to indicate the temperature of maximal enzyme activity, since the curve of Activity vs. Temperature

often shows a maximum; in this sense the optimal temperature is an indirect measure of enzyme thermo-stability: the higher it is, the more stable the enzyme.

However, the optimal temperature is found experimentally by means of initial velocity tests at different temperatures. In these conditions the effect of irreversible enzyme thermal deactivation is negligible. In fact, if the optimal temperature were used as the operating temperature, a rapid enzyme deactivation would occur and the process would not be optimized, because although at the beginning the reaction would be very fast, enzyme deactivation would be fast as well and the reaction rate would fall down quickly: in brief, higher temperatures favour kinetics but thwart activity retention by augmenting the deactivation rate. In terms of “real” optimization, it could be convenient to lower down the temperature in order to have a slower but much more enduring process. An optimization procedure is necessary to determine which is the value giving the best compromise between the two requirements.

The optimization problem has been defined as follows: with other conditions unchanged, the optimal temperature is the temperature value for which the reaction time is minimum. By definition the problem is unconstrained and its solution is given by minimization of eq. 11 with respect to T (eq. 12). The derivative of t with respect to T is obtained analytically, while the optimization equation (12) can be solved numerically.

$$\frac{d[t(T)]}{dT} = \frac{d \left[ -\frac{1}{k_d(T)} \ln \left\{ \frac{k_d(T)}{k_2(T)e_0} [K_m(T) \ln(1-x) - S_0x] + 1 \right\} \right]}{dT} = 0 \quad (12)$$

### 2.3 Activity and stability data

As a numerical example, simulations will be referred to the reaction of inulin from chicory roots (Sigma Aldrich, Italy; average degree of polymerization DP of 28) catalysed by Fructozyme L<sup>TM</sup> from *Aspergillus niger* (Novozymes A/S, Denmark) is considered.

The enzyme activity is 1070 U per gram of Fructozyme L<sup>TM</sup> per minute of reaction, under the following conditions: T=40°C, S<sub>0</sub>=10 g l<sup>-1</sup>, pH=5.0, t=30 min.

The values of activation energies and pre-logarithmic constants defined in eqs. 8, 9, 10 are the following (Ricca et al. 2009b):

$$k_2(T) = \exp \left( \ln k_{20} - \frac{E_{a2}}{RT} \right) = \exp \left( 21.4 - \frac{9450}{T} \right) \quad (8')$$

$$K_m(T) = \exp \left( \ln K_{m0} - \frac{E_{am}}{RT} \right) = \exp \left( 27.4 - \frac{7630}{T} \right) \quad (9')$$

$$k_d(T) = \exp \left( \ln k_{d0} - \frac{E_{ad}}{RT} \right) = \exp \left( 111 - \frac{37700}{T} \right) \quad (10')$$

### 3. Results and discussion

#### 3.1 Process temperature optimization results

The predictive model determined above is a valuable tool to determine optimal conditions and its potentiality is shown below where optimal temperature values are calculated at different operating conditions from those used for validation.

Simulations have been run assuming the following conditions: initial substrate concentration  $S_0=40\text{g/l}$  and pH is always equal to 5.0.

Many theoretical curves of reaction time  $t$  vs. temperature  $T$  (eq. 12) are reported in Fig. 1 for different final conversion  $x$ , in the case of a substrate/enzyme initial mass feed rate equal to 400 ( $E_0=107\text{ U/l}$ ), and in fig. 2 at final conversion  $x=0.9$  with different substrate/enzyme initial mass feed rate values, that corresponding to different  $E_0$  values, taken as a parameter.

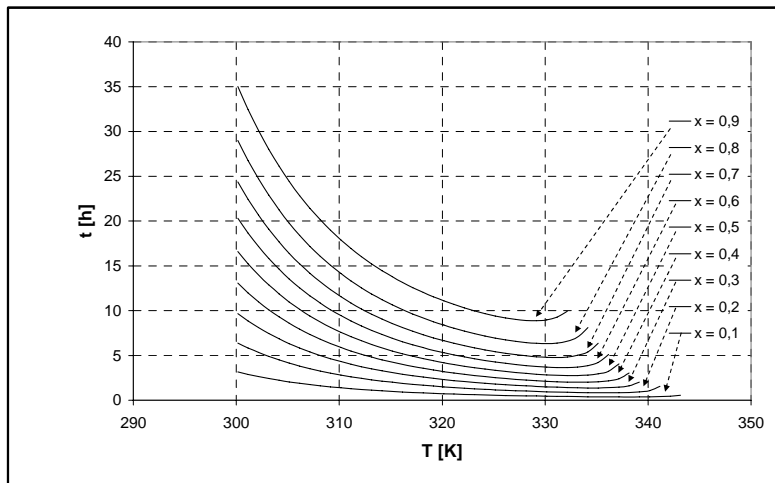


Fig.1. Plot of “ $t$  vs.  $T$ ” and “ $T_{opt}$  locus”.  $S_0=40\text{g l}^{-1}$ ,  $E_0=107\text{ U l}^{-1}$ .

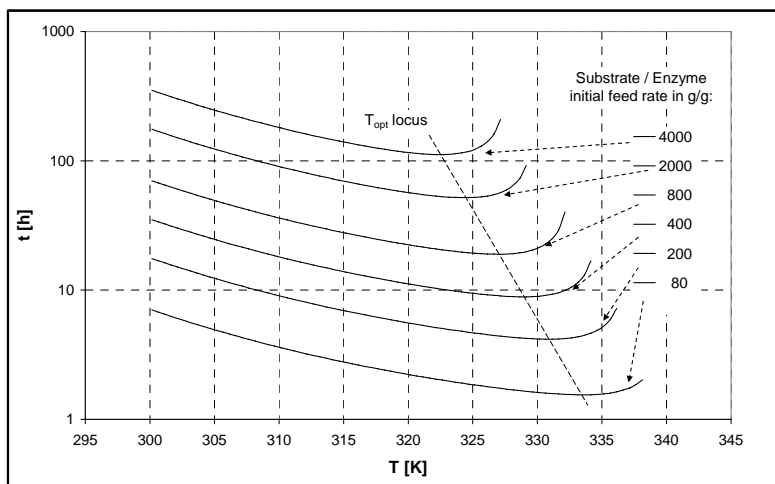


Fig.2. Semi-log plot of “ $t$  vs.  $T$ ” and “ $T_{opt}$  locus”.  $S_0=40\text{g l}^{-1}$ ,  $x=0.9$ .

As it was supposed on the basis of physical knowledge of the process, the model predicts the existence of a minimum of the function  $t(T)$ ; the value of  $T$  such that  $t(T)=\min$  is  $T_{\text{opt}}$ . As a result of simulations,  $T_{\text{opt}}$  is not equal to  $60^{\circ}\text{C}$  and, moreover, it is not constant, since it depends on  $E_0$ . In particular, when  $E_0$  increases, in addition to an expected decrease of the reaction time, an increase of  $T_{\text{opt}}$  is observed, as highlighted by the  $T_{\text{opt}}$  locus; to quantify this tendency in Fig. 3, the values of  $T$  satisfying eq.10 (i.e. the  $T_{\text{opt}}$  locus) at each enzyme loading  $E_0$  are reported.

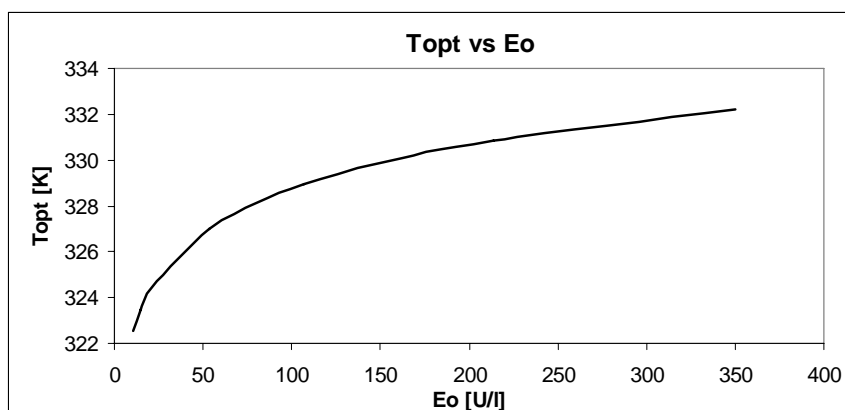


Fig. 3.  $T_{\text{opt}}$  vs.  $E_0$ : optimal temperature values suggested at each enzyme loading.  $S_0=40\text{g l}^{-1}$ ,  $x=0.9$ .

The growth of  $T_{\text{opt}}$  with  $E_0$  is explained by the objective function of optimization: to minimize the time required to reach a fixed conversion. In this context, the use of a higher amount of enzyme permits low reaction times, preventing the enzyme from a long permanence in the deactivating environment; this, in turn, allows adopting higher operating temperatures. At the extreme, when  $E_0$  is very high, reaction times are so small that deactivation does not occur at all and  $T_{\text{opt}}$  approaches  $60^{\circ}\text{C}$ , the temperature of maximal activity, classically referred to as optimal. On the other hand, it is evident that when low enzyme loadings are adopted, low operating temperatures are suggested. It must be also noticed that, for process purposes, the problem must be reversed assessing that the procedure shown in this paper is able to establish minimum amounts of enzyme to be adopted in order to reach desired conditions of final conversion and reaction time. This tool could, thud, represent a valuable tool for reducing operation costs of enzymatic processes whereas the enzyme cost is highly incident on total process costs.

#### 4. Conclusion

In the present work a deactivation model and a kinetic model have been implemented to predict reaction performances at different operating conditions and to optimize the reaction in terms of reaction time minimization. It has been shown how this model can be used to clear up some aspects of the reaction performance and to determine optimal operating conditions for enzymatic hydrolysis of inulin.

Beside the use showed above regarding the optimization of a batch reactor, the complete model could be of great help in designing continuous reactors.

Economical benefits could be derived from this approach, through which the enzyme loading could be set to the minimum value, without releasing constraints of reaction time and substrate conversion. In the case of costly enzymes, this could definitely contribute to process economy.

## References

- Diaz EG, Catana R, Ferriera BF, Luque S, Fernandes P and Cabral JMS, 2006, Towards the development of a membrane reactor for enzymatic inulin hydrolysis. *Journal of Membrane Science*. 273, 152–158
- Gill PK, Manhas RK and Singh P, 2006, Hydrolysis of inulin by immobilized thermostable extracellular exoinulinase from *Aspergillus fumigatus*. *J Food Eng* 76, 369–375.
- Kochhar A, Gupta AK and Kaur N, 1999, Purification and immobilisation of inulinase from *Aspergillus candidus* for producing fructose. *J Sc Food Agric* 79, 549–554
- Nakamura T, Ogata Y, Shitara A, Nakamura A, Ohta K, 1995, Continuous production of fructose syrups from inulin by immobilized inulinase from *Aspergillus niger* Mutant 817, *J. Ferment. Bioeng.* 80, 164-169.
- Pandey A, Soccol CR, Selvakumar P, Soccol VT, Krieger N, Fontana JD, 1999, Recent developments in microbial inulinases, *Appl. Biochem. Biotechnol.* 81, 35–.
- Ricca E, Calabrò V, Curcio S, Iorio G, 2007, The state of the art in the production of fructose from inulin enzymatic hydrolysis, *Crit. Rev. Biotechnol.* 27, 1-17.
- Ricca E, Calabrò V, Curcio S and Iorio G, 2009, Fructose production by chicory inulin enzymatic hydrolysis: a kinetic study and reaction mechanism. *Proc Biochem* 44, 466-470.
- Ricca E, Calabrò V, Curcio S and Iorio G, 2009, Optimization of inulin hydrolysis by inulinase accounting for enzyme time- and temperature-dependent deactivation. *Biochem. Eng. J.* 48, 81–86.
- van Loo J, Coussement P, de Leenheer L, Hoebregs H, Smits G, 1995, On the presence of inulin and oligofructose as natural ingredients in the western diet, *Crit. Rev. Food Sci. Nutr.* 35, 525-
- Wenling W, Huiying WWL, Shiyuan W, 1999, Continuous preparation of fructose syrups from Jerusalem artichoke tuber using immobilized intracellular inulinase from *Kluyveromyces* sp. Y-85, *Process Biochem.* 34, 643-646.