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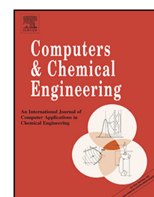

Quick identification of a simple enzyme deactivation model for an extended-Michaelis–Menten reaction type. Exemplification for the D-glucose oxidation with a complex enzyme deactivation kinetics

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ABSTRACT

One essential engineering problem when developing an industrial enzymatic process concerns the model-based design and optimal operation of the enzymatic reactor based on the process and enzyme inactivation kinetics. For a complex enzymatic system, the "default" used first-order enzyme deactivation model has been proved to lead to inadequate process design or sub-optimal operating policies. The present study investigates if a complex enzyme deactivation can be approximated with simple 1st, 2nd, or a novel proposed model with variable deactivation constant. The approached complex enzymatic system is those of the oxidation of D-glucose to 2-keto-D-glucose in the presence of pyranose 2-oxidase. The necessary "simulated experimental data" have been generated by means of an extended kinetic model from literature used to simulate a batch reactor under well-defined nominal conditions. The proposed enzyme deactivation model has been found to be the best lumping alternative, presenting several advantages: simplicity, flexibility, and a very good adequacy.

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1. Introduction

When developing a new enzymatic process, they are crucial engineering decisions to be taken based on the available information on the process kinetics, enzyme characteristics [activity, stability/half-life, temperature and pH optimal activity range, interactions with products and intermediates, carrier loading capacity if immobilized, enzyme recovery possibilities; Domach, 1984, etc.].

In the both suspended and immobilized enzyme-operating alternatives, all engineering calculations are tremendous facilitated if a simple but adequate kinetic model of the process is available. This model, developed on an experimental basis is used: (i) in the design stage to decide (on a cost basis) on optimal choice of the most suitable reactor type (batch, semi-batch/fed-batch, continuous fixed-bed, or mechanically agitated reactors; Maria, 2007, 2012; Maria and Crisan, 2015; Wang, 2009), and (ii) in the reactor operation stage to determine the optimal operating policy (Maria, 2007, 2012; Maria and Crisan, 2015). Examples include the large number of biosynthesis processes used to produce fine-chemicals, or organic compounds in food, pharmaceutical, or detergent industry (Liese et al., 2006; Straathof and Adlercreutz, 2005), such as: the production of monosaccharide derivatives, organic acids, alcohols, amino-acids, etc., by using single- or multi-enzymatic reactors.

All these engineering calculations depend on the quality of the process kinetic model, and especially on the right characterization of the enzyme deactivation during the reaction due to process intermediates and/or products. In most of the cases, the "default" adopted model is those of first-order enzyme inactivation ($dc_E/dt = -k_d c_E$; Blanch and Clark, 1997), even if there are a large number of enzymatic processes which do not obey this law, such those reviewed in Table 2. While the n-th deactivation models ($n = 1 - 3$) are relatively easily estimable from the recorded enzyme activity kinetic curves, more complex deactivation cases involving complex reaction pathways are more difficult to be clarified and modelled, lumped models being preferred.

However, the use of a simplified (lumped) first-order enzyme deactivation model instead of the real complex model in the engineering calculations involves a high cost, leading to important negative consequences well documented in the literature (Maria, 2007, 2012; Maria and Crisan, 2015). For instance, while for a first-order enzyme deactivation process the optimal operating batch

Abbreviations: arg, argument of ...; ABTS, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid; BR, batch reactor; DG, D-glucose; DO, dissolved oxygen; kDG, 2-keto-D-glucose; E,ENZ, enzyme; M, molar; MINLP, mixed integer nonlinear programming; NAD(P)H, nicotinamide adenine dinucleotide (phosphate); NLP, nonlinear programming; P2Ox, pyranose oxidase.

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Nomenclature

c_j	Species j concentration, in mM (DG, kDG, H_2O_2, DO), and (U/mL) for the enzyme P2Ox
D	Reactor content dilution rate, 1/s
d_r	Reactor diameter, m
$\boldsymbol{H}$, H	Model function vector, or reactor length, m
k_j, kc, kd, Kj	Rate constants, units given in Table 3
$k_{oxl}a$	Overall gas-liquid mass transfer coefficient, 1/s
M	Molecular weight, g/mole
m	Number of observed variables
n	Yano-Koya exponent
N	Number of measured experimental points
p	Number of model parameters
r_j	Species j reaction rate, mM/s, or (U/mL)/s
s_y^2	The model error variance (in relative terms)
t	Time, s
V	Liquid volume, m^3
Y, Y_E	Stoichiometric coefficients, (U/mL), or (U/mL)/mM

Greek symbols

α, β	Deactivation model parameters, α of k_d units; β in 1/s
$\boldsymbol{\phi}$	Model parameter vector
μ_m	Turnover number of the main reaction, units given in Table 3
ρ	Density, g/L
σ	Standard deviations of the measured data, units of c_j

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app	Apparent
in	Inlet
min	Minimum
o	Initial
r	Reaction
w	Water

Superscipts

$\hat{}$	Estimated value (model prediction)
*	Saturation

alternative is to add the whole substrate and enzyme at the beginning of the batch, for a higher-order deactivation case, it was proved that a semi-continuous operation with addition of enzyme following a certain policy is more advantageous. Consequently, the improper use of the first-order enzyme deactivation model for complex inactivation cases leads to inadequate process design solutions or sub-optimal reactor operating policies.

For multi-enzyme systems acting simultaneously (e.g. Maria and Crisan, 2015), reactor operation optimization is even more difficult, because it must account for interacting reactions, differences in enzymes' optimal activity domains and deactivation kinetics. Determination of the optimal operating mode (enzyme ratios, enzyme feeding policy, temperature, pH) turns into a difficult multi-objective optimization problem with multiple constraints to be solved for every particular system (Ghose et al., 1978). The computational modular platform of Maria (2012) can successfully be used in this respect. The essential part of such calculations is the availability of a simple but adequate kinetic model of the enzymatic main process but also for the enzyme deactivation. A weak adequacy especially of the enzyme deactivation model may lead to wrong conclusions/decisions in both reactor design or operation stages.

The present study is aiming at investigating in what extent it is possible to approximate enzyme deactivation complex models with simple 1-st, 2-nd order deactivation models (of constant deactivation parameter k_d), or with a novel proposed pseudo 1-st order model (of variable deactivation constant k_d), easier to be used in further engineering calculations (design, optimization). The advantage of the proposed pseudo 1-st deactivation model is tested in the case of a complex enzymatic system used for the oxidation of D-glucose (DG) to 2-keto-D-glucose (kDG) in the presence of P2Ox (pyranose 2-oxidase, EC 1.1.3.10), by using "simulated experimental data" generated by means of an adequate extended model adopted from literature and used to simulate a pilot batch reactor operated under well-defined nominal conditions. (30 °C, pH = 6.5, no added catalase, 15 h reaction time; 250 mM initial DG, under continuous aeration).

2. The bi-enzymatic system for D-glucose oxidation

The approached process in the present paper is the enzymatic transformation of β-D-glucose (DG) in 2-keto-D-glucose (kDG) in the presence of P2Ox (pyranose 2-oxidase, EC 1.1.3.10, commercial product number P4234 Sigma–Aldrich of 10.4 U mg-protein^{-1} activity, obtained from *Coriolus* sp. expressed in *E. Coli*), with or without catalase (EC 1.11.16, commercial product number C1345 Sigma–Aldrich of 2860 U mg-protein^{-1} activity from bovine liver) (see details of Maria et al., 2012). The role of catalase is to quickly decompose the resulted H_2O_2 from the main reaction, thus preventing the quick deactivation of P2Ox enzyme.

This specific reaction has been intensively studied, being of high interest for the industrial small/large-size production of rare sugars or sugar- derivatives, such as: D-fructose (by enzymatic hydrogenation of kDG using NAD(P)H and aldose reductase; see the kinetic model and reaction pathway of Maria and Ene, 2013); D-mannitol (by enzymatic hydrogenation of D-fructose using in-situ regenerable NADH; Slatner et al., 1998); 2-keto-D-gluconic acid (and then D-isoascorbic acid); precursors of some vitamins (thiamine and pyridoxol); derivatives of D-sorbitol and D-galactose used as low caloric sweetener, and other synthetic carbohydrates (Maria et al., 2012).

However, the most important application at a large industrial scale is the very specific conversion of DG to D-fructose of high purity following the two-enzymatic steps of Cetus process (Leitner et al., 1998): (I) in the first step, DG is oxidized on P2Ox at 25–30 °C and pH = 6–7 with more than 99% conversion and selectivity leading to the kDG product of high purity and free of allergenic compounds (such as aldoses); (ii) then, the kDG is reduced to D-fructose by NAD(P)H-dependent aldose reductase at 25 °C and pH = 7, the $NAD(P)^+$ being in-situ continuously regenerated and re-used (Maria and Ene, 2013; Slatner et al., 1998). The process drawbacks are related to the very costly and fast deactivating P2Ox by H_2O_2, and to the continuously regeneration of the very costly and unstable $NAD(P)^+$ (Chenault and Whitesides, 1987). The current efforts try to increase the P2Ox half-life by including catalase in the system (Leitner et al., 1998; Treitz et al., 2001), by modifying P2Ox structure (Bannwarth et al., 2006), or by co-immobilizing P2Ox and catalase on a suitable support (Huwig et al., 1994; Sukyai et al., 2008). More details on the kinetics of these two processes are offered by Maria et al. (2012), and Maria and Ene (2013), while more details on the alternative technologies to produce mannitol from D-fructose are offered by Ghoreishi and Shahrestani (2009).

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