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Screening Among Constructive and Operating Alternatives when Designing an Enzymatic Reactor

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Abstract: Optimal design and operation of enzymatic processes is a fundamental engineering problem to be solved when laboratory-scale kinetic data and enzyme characteristics are available. The final decision is based on a comparative analysis of reactor performances by accounting for various optimal/sub-optimal operating alternatives, enzyme activity and stability, materials and operation costs, purification steps, and product value. Due to the high complexity of the engineering problem, development of a library of quickly adaptable reactor models allows evaluation of process scaling-up alternatives, in terms of reactor type (well-mixed vs. plug-flow), enzyme use (free-enzyme vs. immobilized enzyme), or operation mode (simple batch, batch with intermittent addition of enzyme following certain optimal policies, semi-batch with uniform or optimal enzyme feeding policy, fixed-bed or fluidized-bed continuous reactors with time-optimal feeding policies). Analysis of process dynamics under various operating conditions for fast, moderate fast, or slow deactivating enzyme leads to choose the most suitable reactor and operation mode based on several performance indices (enzyme specific consumption and stability, reactor productivity, operating time, easy operability and control, etc.). Two case studies exemplify this comparative analysis, that is the design of an industrial reactor for D-glucose enzymatic oxidation (using free pyranose oxidase), and the design of an industrial reactor for inulin enzymatic hydrolysis (using free or immobilized inulinase). Model-based simulations of the enzymatic reactors suggest optimal operation policies according to the enzymes variable characteristics.

Keywords: enzymatic reactors; optimal design and operation; D-glucose oxidation; inulin hydrolysis

1. Introduction

Enzymatic reactions, displaying a high selectivity and specificity, compete with the chemical synthesis in terms of consumed energy and waste minimization, by using low concentrations of catalyst and moderate reaction conditions. [1, 2] Industrial developments cover a wide range of applications in the food, pharmaceutical, detergent, and textile industry, environmental engineering, biochemical synthesis, medical-tests, production of bio-sensors, or bio-renewable sources of energy. [3, 4]

Current researches are focus on modifying enzyme characteristics by using protein / genetic engineering, and on developing nano-structures used as carrier materials. Such efforts lead to improve the enzyme stability and its catalytic efficiency, trying to overcome the biocatalysts' disadvantages, that is the high costs of producing stable enzymes, their high sensitivity to operating conditions and impurities, and their variable characteristics leading to a difficult process control.

Enzymatic reactions can be conducted in two alternatives: free-enzyme or immobilized enzymes on solid supports.

If the enzyme is cheap, and the product can be easily separated, or when enzyme deactivates rapidly, and its immobilization does not report a significant increase in stability, the use of simple batch (BR), batch with intermittent addition of enzyme (BRP), and semi-batch reactors (SBR) can be a good choice for scaling-up the process. [4 - 7]

Alternatively, the use of stable immobilized enzymes on various supports (ceramics, alumina, silicates, clay, gels, natural / synthetic polymers, etc.) is advantageous, offering an easy product separation with less enzyme loss, and a better control of the process. Fixed-bed (FXBR) or fluidized-bed (FLBR) continuous reactors with time-optimal feeding policies are usually used for such purposes. [5, 8]

In this context, the right choice of the enzymatic reactor and derivation of optimal operating policies continue to be subjects of current interest. However, the choice of the enzymatic reactor constructive solution and derivation of optimal operation policies based on a process model is not an easy task due to various reasons: I) multiple objectives to be accounted for (product yield maximization, enzyme loss and waste minimization, operating time and utilities minimization), in the presence of technological constraints; ii) process low reproducibility due to the variability in raw-material and enzyme characteristics; iii) enzyme high sensitivity to operating conditions; iv) nonlinear process dynamics characterized by a small number of observed variables; v) limited validity of the process model, due to multiple sources of uncertainty, requiring frequent parameter up-dating.

The scope of this paper is to present a model-based rule to scale-up an enzymatic process of known kinetics, by comparing various alternatives, that is free-vs.-immobilized enzyme, under various constructive and operation choices: BR, BRP, SBR, FXBR, or FLBR.

The examined case studies are those of the enzymatic oxidation of D-glucose (DG) to 2-keto-D-glucose (kDG) using pyranose oxidase (POx), and the inulin enzymatic hydrolysis to D-fructose.

2. Process Characteristics

2.1. D-glucose oxidation with POx

D-glucose oxidation to kDG is a reaction of high interest for producing sugar-derivates, such as D-fructose (Cetus process) [9], D-mannitol, D-sorbitol, etc. [10 - 12] The current way of producing fructose is based on enzymatic isomerisation of glucose in the presence of salts. However, this process suffers of a large number of disadvantages: low equilibrium conversion (ca. 50% at 50-60^{o}C), significant amounts of impurities (e.g. allergenic aldose), high process temperature, difficult and costly separation of fructose on large chromatographic columns, poor stability of isomerase, raw-material (glucose) purification to remove calcium ions from the previous starch hydrolysis. [4]

Even if costly, due to the subsequent kDG enzymatic reduction to D-fructose (using recyclable NADPH), the Cetus process was becoming attractive, by presenting several advantages: high conversion and selectivity, low temperature, and absence of aldose in the final product. D-glucose oxidation using POx occurs under the following optimal conditions [11]: 25-30^{o}C, pH= 6.5-7, $[DG]_o$ = 200-250 mM. The oxygen is supplied through both liquid surface and bubbles-liquid interface (by using a mixing-sparging equipment). Separate experiments indicated an overall mass transport coefficient of $k_{oxl}a$ = 0.02-0.04 s^{-1}. The overall reaction is presented in Table 1 together with the kinetic model proposed by Treitz et al. [11]. To hinder the quick inactivation of POx by H_2O_2, catalase is added in the reactor to quickly decompose H_2O_2 (see reaction kinetics in Table 1, proposed by Maria & Cocuz [12]).

2.2. Inulin hydrolysis

Inulin is a natural polyfructan present in many plants, containing a *m* = 20-70 molecules of D-fructose linked to a terminal glucose [13]. The inulin solubility in water varies with the temperature, from 60 g L^{-1} at 10^{o}C to 330 g L^{-1} at 90^{o}C [14, 15], but its diluted solution properties are similar to those of the water. The optimal conditions of the inulin enzymatic hydrolysis are the following [16]: 50-60^{o}C, pH= 5, [S]o = 40-100 g L^{-1} (S= inulin). The activity of free-inulinase is high at 50-60^{o}C and pH=4-6, but it decreases sharply at higher temperatures [17]. Even if several enzyme immobilization possibilities have been reported, they are still not very successful (half-life of $t_{0.5}$ =7.2 hrs at 50^{o}C in calcium alginate, [18] $t_{0.5}$ =1.1 days at 55^{o}C on Amberlite-support, [19]. To quickly simulate the process, the reduced kinetic model of Table 2 has been adopted from literature [20], by considering an average fructose polymerisation degree in inulin of *m*= 29.

TABLE 1. Reduced kinetic model of the DG oxidation using POx, (from Peniophora gigantea) and of the H_2O_2 decomposition using catalase (kinetic parameters for 25^{o}C, pH=7; [11, 12] Notations: DG= D-glucose; DO= dissolved oxygen; kDG= 2-keto-D-glucose; POxox= inactive form of enzyme POx.

Reaction pathway:

$$DG + Y_{ox}O_2 \xrightarrow[(water)]{POx} kDG + H_2O_2$$

$$Y_{POx}POx + H_2O_2 \xrightarrow[(water)]{Fe\ traces} POxox$$

$$H_2O_2 \xrightarrow[(water)]{catalase} H_2O + 0.5O_2$$

Rate parameters:	*Rate expressions:*
μ_m = 0.0891 mM mL/ s·U K_{DG} = 63.523 mM K_{DO} = 0.2613 mM k_d = 9.2827·10^{-6} (1 + 6.95 $c_{Fe}^{0.36}$), mL/ s·U Y_{POx} = 1 U/mL mM; Y_{ox} =1; $k_c = \begin{cases} 1.9648\cdot10^{-5}\ 1/s, & \text{for } [Catalase] \le 1\ kU/mL \\ 3.3987\cdot10^{-3}\ 1/s, & \text{for } [Catalase] > 1\ kU/mL \end{cases}$	$r_{our} = \mu_m \frac{c_{DG}}{K_{DG}+c_{DG}} \frac{c_{DO}}{K_{DO}+c_{DO}} c_{POx}$ $r_d = k_d c_{POx} c_{H_2O_2}$ $r_c = k_c c_{H_2O_2}$

3. Enzymatic Reactor Models

To compare the efficiency of the two investigated enzymatic processes conducted in various reactors, standard batch, batch with intermittent enzyme addition, semi-batch, or continuous operated reactors (fixed-bed or fluidized-bed) have been checked.

Standard ideal reactor models have been used to rapidly simulate and compare the performance, as followings:

BR – free-enzyme, isothermal, homogeneous, perfectly mixed batch reactor:

$$dc_j / dt = r_j \ , \qquad (1)$$

(r_j = species *j* reaction rate; c_j = species *j* concentration; *t* = time).

BRP – free-enzyme, isothermal, homogeneous, perfectly mixed batch reactor with intermittent addition of enzyme solution (see [7, 12] for determining the injected volumes of enzyme solution over the batch).

SBR – free-enzyme, isothermal, homogeneous, perfectly mixed semi-batch reactor [12]:

$$dc_j / dt = (f / V)(c_{j,in} - c_j) + r_j \ ; \ dV / dt = f(t) \ , \qquad (2)$$

(*V* = liquid volume; *f* = enzyme solution feed flow rate, constant or optimized; 'in' = inlet).

FLBR – isothermal, perfectly mixed continuous fluidised-bed reactor, of constant volume, with immobilized enzyme in spherical beads [21, 22]. The apparent reaction rate $r_{j,app}$ results from solving the steady-state liquid-solid (L-S) mass transfer equation:

$$r_{j,app} = k_s a_s (c_j - c_{j,s}) = \eta_j \, r_j(c_{j,s}), \qquad (3)$$

(k_s = L-S mass transfer coefficient on liquid side; a_s = specific interfacial area; $c_{j,s}$ = concentration at L–S interface). The effectiveness factor η_j was calculated for every reaction j with the relationships corresponding to Michaelis-Menten rate expression [23].

FXBR – isothermal, continuous plug-flow reactor, with immobilized enzyme in spherical beads [21]. The apparent reaction rates results from solving the steady-state L-S mass transfer equation (3).

TABLE 2. Reduced kinetic model for inulin enzymatic hydrolysis using a commercial inulinase from Aspergillus ficuum [20]. Concentrations c_S, c_F, c_W, c_G are in g/L, c_E in U/L-liquid, r_j in g/L·min. Notations: S= inulin (substrate); F= fructose; W= water; G= glucose; E= enzyme; M_W, M_F, M_G are the molecular weights.

Overall reaction: $S + (m-1)H_2O \xrightarrow{E} (m-1)F + G$	
Rate expressions:	*Rate parameters:*
$r_j = \frac{v_{m,j} c_S}{K_m + c_S}$, j= S,F,W,G	$k_2 = \exp(23.22 - \frac{9450}{T})$, g/(U·min)
$r_E = k_d c_E$;	$K_m = \exp(27.4 - \frac{7630}{T})$, g/L
$v_{mS} = k_2 c_E$;	$k_d = \exp(125 - \frac{42300}{T})$, 1/h
$v_{mF} = \alpha v_{mS}$;	$\alpha = \frac{1}{m/(m-1) - M_W / M_F}$;
$v_{mW} = \alpha v_{mS} \frac{M_W}{M_F}$;	m= 29; (T = temperature, K).
$v_{mG} = \alpha v_{mS} \frac{1}{m-1}$	

Stationary and dynamic simulations are derived, by using the mass balance differential equations for the considered isothermal reactors. In the BR case, the enzyme is initially loaded as a solution of volume not exceeding 10% of the initial volume (V_o) of the reactor. For the BRP, two feeding policies have been considered: equal volumes of enzyme solution uniformly injected over the batch (BRP-uni; N_{inj} = 20 injections of total volume $0.1V_o$); an exponential decrease of the added volumes of enzyme solution over the batch (BRP-exp; 20 injections of total volume $0.1V_o$), determined by using the rule of Maria [7]. The SBR was operated with a constant feed flow rate (SBR-uni) of the enzyme solution of known concentration, or with an optimum feeding policy (to be determined, SBR-opt), i.e. a time step-wise variable feed flow rate over ca. N_{div} time-interval ('arcs'), calculated by division of the batch time (t_f) in N_{div} equal parts. For the continuous FLBR and FXBR, dynamic operation was considered to account for the enzyme deactivation, while the feed flow rate was diminished during N_{div} = 1000 equal time-intervals to maintain constant the output conversion at the initial value, until 0.9 f_o was reached.

4. Comparison of Reactor Optimized Performances

To compare the performances of various reactors in the case of the same studied enzymatic process, the same production capacity and reaction / residence times have been imposed, the simulated reactors being optimally operated vs. a formulated optimization criterion.

For the D-glucose oxidation case, a 10000 t fructose / year production capacity and optimal reaction conditions were adopted. For the BR and BRP, the optimum will correspond to the minimum POx amount necessary to obtain a 99.90% conversion over t_f = 7 h of operation, under nominal conditions. For the SBR with a constant feed flow rate, only the POx inlet concentration must be determined to meet the same requirements. For the SBR with variable feed flow rate, the optimal solution corresponds to the minimum inlet POx concentration and to a suitable feeding policy over N_{div} time intervals, ensuring a maximal DG-conversion that equals the imposed value of 99.90% over 7 h of operation, under given initial conditions and mentioned constraints.

The results are presented in Fig. 1 for the BRP-exp case and in Fig. 2 for the SBR-opt case. It is to observe that the required enzyme amount in the SBR-opt case ($[POx]_{in}$= 57 U ml^{-1}), to get the imposed 99.90% over the batch, is much lower than those required by the BRP-exp operation ($[POx]_{in}$= 77 U ml^{-1}), for the same amount of injected enzyme solution in the reactor. Such a result is explained by the adaptation of the feeding to the enzyme deactivation characteristics, which is more difficult to be realised in the exponentially decreasing pulse-like BRP. In both cases, the low concentration of DO reveals the control of the process by the aeration rate, as long as the POx concentration in the reactor is maintained to an optimal value (ca. 2 U/mL, see also [9]).

A comparison of free-enzyme operating alternatives for kDG production in batch or semi-batch reactors is presented in Fig. 3. Under optimal conditions, the required enzyme amount to get the same conversion at the same production capacity is significantly different from one reactor to another. The best results are obtained with the SBR with an optimal feed flow rate of the enzyme solution.

For the inulin hydrolysis case, a 5000 t fructose / year production capacity and optimal reaction conditions were adopted. For the BR and BRP, the optimum will correspond to the minimum ENZ (inulinase) amount necessary to obtain a 99.00% conversion over t_f = 13 h of operation, under nominal conditions. For the SBR, the

optimal solution corresponds to the minimum inlet ENZ concentration and to a suitable feeding policy (constant or optimal) over N_{div} time intervals, ensuring a maximal inulin hydrolysis conversion that equals the imposed value of 99.00% over 13 h of operation, under given initial conditions. For the continuous FLBR and FXBR, a similar optimization problem is solved, by determining the minimum concentration of enzyme on the solid support and the decreasing feeding policy of the reactors that compensate the continuous enzyme deactivation by ensuring a quasi-constant exit conversion (99%) for a residence time in the reactor of 13 hrs.

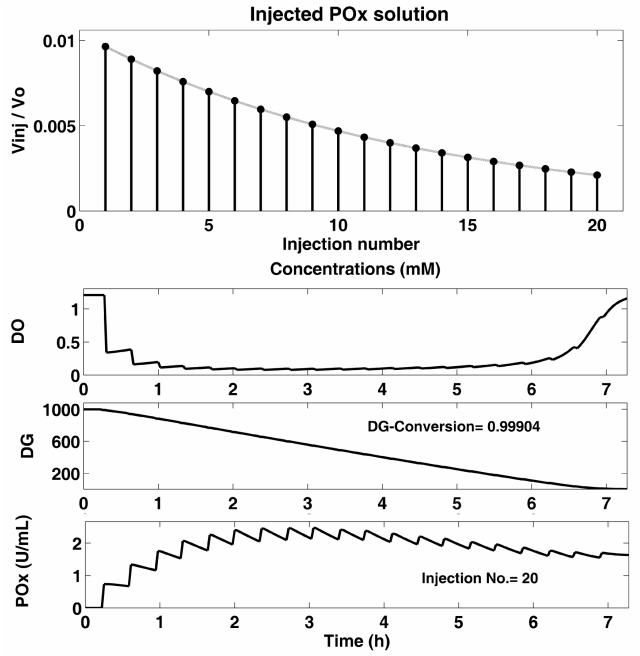

Figure 1. Addition policy of POx solution (up), and key species concentration dynamics in the BRP-exp under nominal conditions (center, down). An injected solution of [POx]= 77 U ml^{-1} was found to ensure a final x_{DG}= 99.90% over 7 h runtime, with an overall dilution of 10% [12]

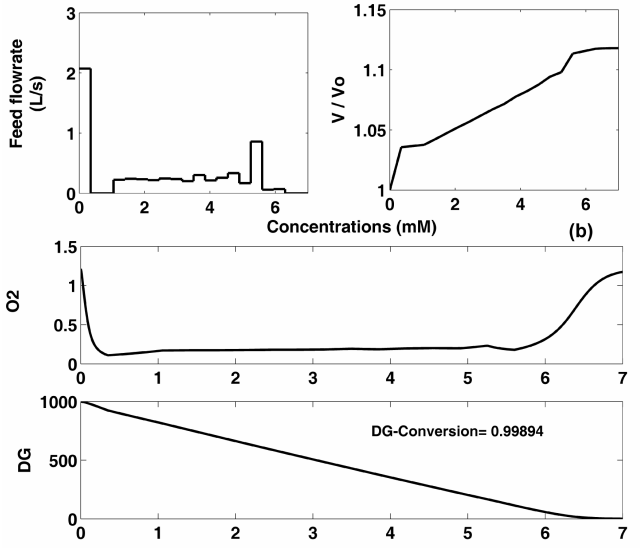

Figure 2. Optimal feeding policy of SBR-opt with POx, and liquid volume dynamics (up); [DO] and [DG] evolution under nominal conditions (center, down). A feeding with [POx]= 57 U ml^{-1} was found to ensure a final x_{DG}= 99.90% over 7 h runtime, with an overall dilution of 10% [12]

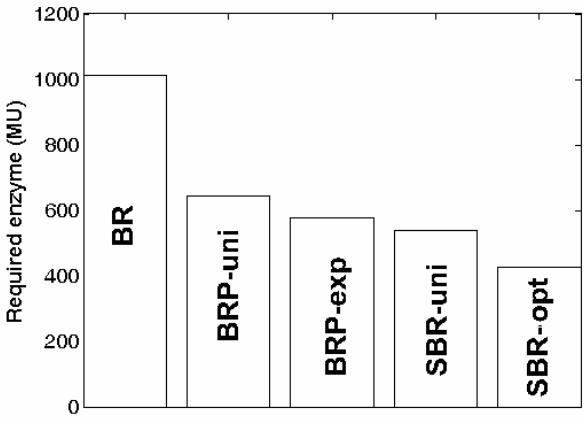

Figure 3. Free-enzyme operating alternatives for kDG production. Conditions for production of 10000 t fructose / year: 25^{o}C, pH=7; $[DG]_o$= 1 M; [Catalase]= 1 kU/mL; 10% liquid volume increase; initial volume = 75 m^3; reaction time = 7 hrs; sparging using oxygen; imposed DG conversion of 99.90%. BR= batch reactor; BRP-uni= batch reactor with uniform addition of POx (20 injections); BRP-exp= batch reactor with exponential decreasing addition of POx (20 injections); SBR-uni= semi-batch reactor with constant fed POx solution; SBR-opt= semi-batch reactor with optimal feedflowrate of POx solution (20 time-arcs) [12]

A comparison of free-enzyme operating alternatives for fructose production in batch or semi-batch reactors is presented in Fig. 4.

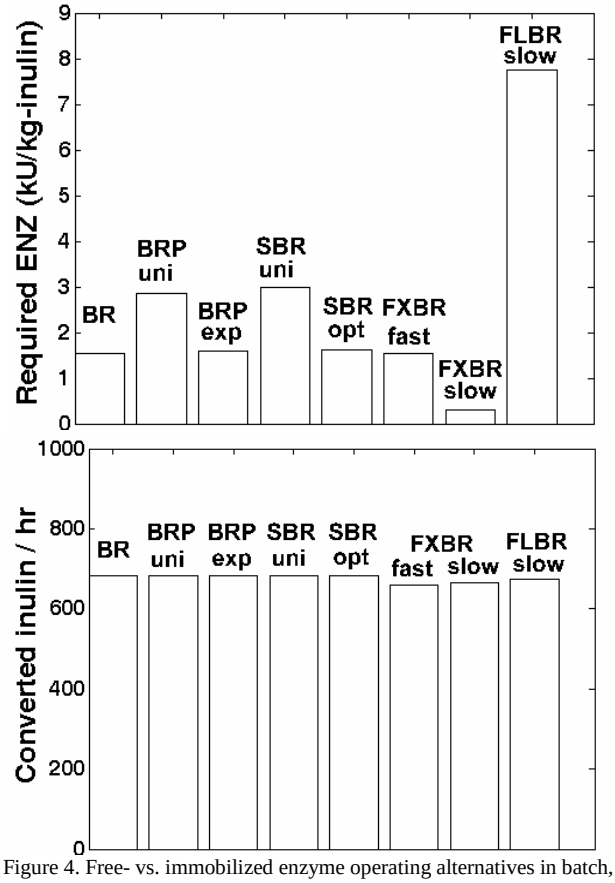

Figure 4. Free- vs. immobilized enzyme operating alternatives in batch, semi-batch and continuous reactors for inulin hydrolysis. Operating conditions for 5000 t fructose / year: 55^{o}C, pH=5; $[S]_o$= 100 g/L; optimized enzyme conc.; 10% liquid volume increase, or initial flow-rate decrease; initial volume = 88.66 m^3 (6 parallel FXBR); reaction time = 13 hrs; imposed inulin conversion of 99.00%; particle diameter 1-2 mm; solid fraction 0.47 (FXBR), and 0.3 (FLBR); FLBR = fluidized-bed lq.-solid continuous reactor; FXBR = fixed-bed lq.-solid continuous reactor (other notations from Fig. 3); fast / slow refer to the enzyme deactivation rate.

While the reactor performance (expressed in kg hydrolysed inulin per hour) is practically the same, the amount of consumed enzyme to get the same conversion at the same production capacity is very different from one reactor to another. Because the enzyme is not very rapidly deactivating ($t_{0.5}$ = 36.5 hrs. at 55^{o}C; [16]), the best choice of free-enzyme reaction is the BR, requiring only 1.55 kU kg-inulin^{-1}. This was not the case of kDG production, when the rapidly deactivating POx indicate the SBR-opt operation as being the best choice. Concerning the continuous operation, the FXBR is from far the best alternative. However, by checking the operating solutions for fast ($t_{0.5}$ = 36.5 hrs) vs. moderate slow ($t_{0.5}$ =182.5 hrs.) deactivating immobilized enzyme, it appears that FXBR is as more efficient (requiring less enzyme amounts) as the enzyme is more stable.

In general, repeated simulations of various reactor type and operating alternative efficiency can indicate what is the 'threshold' of the immobilized enzyme half-life which make the use of FXBR more efficient than the use of BR / SBR operating mode.

5. Conclusions

Derivation of the most suitable enzymatic reactor type and operating alternative is a difficult task, requiring steady experimental efforts to get enough information on the process kinetics and enzyme characteristics, but also steady computational steps to simulate and compare optimal operating alternatives in various reactors.

When the enzyme is fast deactivating, the SBR operation mode with free-enzyme is the best alternative. As the deactivation rate is smaller, various other alternatives become efficient. If the enzyme is cheap, and the product can be easily separated, the BR is a suitable choice. When immobilization improves very much the enzyme stability, then operation in continuous reactors become very effective, especially in the FXBR. It is also to notice that the global efficiency of a certain alternative has to be based not only on enzyme consumption to get an imposed conversion / selectivity, but also on other implementation costs, such as: the required process control complexity; implementation costs of the optimal operating policy (i.e. on-line measurements to on-line adjust the feeding policy); fulfilment of technological and ecological constraints; flexibile / multi-product reactors, easily adaptable to market requirements.

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