Operating Alternatives for the (semi)batch Reactor used for D-glucose Enzymatic Oxidation with Free-enzymes

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Batch and semi-batch reactors are very suitable for conducting enzymatic reactions within a wide range of applicative areas including food, pharmaceutical, textile industry, and biochemical synthesis. Free enzyme operation can be often preferred, by taking advantage of enzyme full activity leading to high yields. Such an alternative is suitable when the product separation does not raise special problems, or when its contamination with the enzyme is not very important. Optimal and flexible operation of such reactors are subjects of continue interest, due to the possibility of improving their productivity, with a considerable reduction in enzyme consumption. A "real life" example is provided for the case of a design industrial reactor used for D-glucose enzymatic oxidation in the rare sugar production lines. By investigating several operating alternatives, including simple batch, batch with intermittent addition of enzyme, or semi-batch with constant or optimal enzyme feeding policy, the study proves how considerable savings in enzyme consumption can be obtained without any productivity or conversion loss. Advantages and drawbacks of the checked operating alternatives are comparatively discussed to derive relevant conclusions of general use.

Keywords: semi-batch reactor, optimization, D-glucose, oxidation, pyranose oxidase, catalase

Recent improvements in the synthetic biotechnology and production of modified enzymes, exhibiting desired functions, allowed a considerable progress in industrial enzyme technologies and various other applications. Enzymatic reactions, displaying a high selectivity and specificity, are attractive bioengineering routes to obtain a wide range of products in food, pharmaceutical, detergent, and textile industry, biochemical synthesis, or presenting challenging applications in medical-tests, bio-sensor production, or emerging bio-renewable energy industries [1,2]. Enzymatic routes are also competing against classical chemical synthesis pathways in terms of efficiency, while new efforts are invested in protein engineering (molecular level design) coupled with bioactive nanostructure fabrication, leading to new biocatalytic systems, new devices, or modification of industrial bioreactors.

Such efforts are trying to overcome most of difficulties related to industrial use of biocatalysts, that is the high costs of producing enough stable and long lifetime enzymes, their high sensitivity to operating conditions and impurities, too high substrate specificity, and difficult process controllability.

In this context, optimization of enzymatic reactors continues to be an intensively investigated subject. In particular, optimal operation of batch (BR) and semi-batch (SBR) reactors is a classical but still of interest problem due to several reasons. On one hand, it is the economic interest to increase the plant productivity, the reactor being the main equipment on which the optimization efforts are focused due to the high value of raw materials and products related to the production cost, but also due to its high sensitivity to operating conditions affecting the product quality [3]. On the other hand, it is the current trend to move production of a lot of industrial products from the stable continuous plants to multi-product (semi)batch reactors, by using alternative / biocatalytic ways of synthesis, as being more efficient, flexible and easily adaptable to market requirements [3,33].

In general, the use of SBR is particularly attractive, due to increased possibilities to control the process by controlling the addition of co-reactants (chemicals, enzymes, substrate, nutrients, etc.), thus resulting a higher reactor productivity using a reduced reaction volume [12,31,32]. Such a SBR flexibility makes it very suitable for a large area of bio/chemical applications: limit the thermal sensitivity and risk for highly exothermal chemical reactions; adapt the bioprocess efficiency by certain feeding policies of nutrients, substrate, additives in fed-batch bioreactors; adapt the enzymatic process efficiency by adjusting the enzyme loading policy over the batch.

In particular, free-enzyme SBR, exhibiting a high enzyme activity and leading to high process yields, can be preferred if the product can be easily separated or its contamination with the enzyme is not important. In contrast, the use of immobilized enzymes offers an easy product separation, an increased enzyme stability, and protection against harmful environmental stress. However, enzyme immobilization leads to a considerable activity decrease due to the matrix-enzyme interactions (changing the enzyme structure) and due to resistance introduced by the diffusional transport through support. Moreover, when the enzyme deactivation rate is high enough, the use of fixed-bed reactors with immobilized enzymes becomes too costly, requiring frequent biocatalyst replacement.

Derivation of optimal operation and control policies for an enzymatic reactor based on a process model is a difficult task due to various reasons [4]: high complexity of process kinetics (often poorly understood) and constraints difficult to be characterized; modest process reproducibility due to the variability in raw-material and enzyme characteristics; high particular enzyme sensitivity to operating conditions (temperature, *p*H, impurities, species levels, flow oscillations); the nonlinear process dynamics including a wide range of time constants; the small number of observed variables of varied observability; limited validity of the process and bioreactor models requiring frequent parameter up-dating.

Even if a process model is available, finding the optimal operating solution, sometime in the presence of multiple objectives, is still not an easy task due to multiple sources of uncertainty to be correspondingly treated [4-9]: model

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inaccuracies in both structure and parameters coming from a large variety of sources (experimental data quality, adopted hypotheses, material variability, etc.); constraint uncertainty due to their simplified formulation and uncertainty of other variables; presence of disturbances in the operating parameters making accurate implementation of the optimal policy questionable; limited on-line corrective actions due to the finite duration of a (semi-)batch.

In spite of being intensively investigated, derivation of the optimal policy of a BR/SBR remains a complex problem to be solved for every particular case. There are several important aspects to be considered in formulation of an optimization objective function: maximization of the SBR productivity and product quality, using minimum necessary raw materials and utilities; meeting the product quality requirements by limiting side-reactions; meeting technological and ecological constraints; scale-up the laboratory information to get a process model and optimal SBR operating conditions; ensure a certain flexibility of the optimal SBR operating policy, to become readily adaptable to the available equipment in a multipurpose-plant and multi-product development strategy.

Both indirect (heuristic) and direct (finite-dimensional) optimization methods are successful used to derive optimum batch operating policies. In spite of the mentioned limitations, mechanistic dynamic models are proved to be effective tools in optimising enzymatic reactors. Multi-objective performance criteria, including economic benefits, investment and operating costs, quality and control aspects, are currently used to derive feasible solutions for a wide range of industrialized processes, sometime employing methods from chemical engineering [4,10-12,30].

The purpose of this paper is to investigate various alternatives to operate a free-enzyme industrial BR/SBR, for which a known process model is available. The examined process is the enzymatic oxidation of D-glucose (DG) to 2-keto-D-glucose (kDG) using pyranose oxidase (POx). The process is of industrial interest for production of various monosaccharide-derivatives of great value. For instance, the kDG can be used for the free of aldose D-fructose production (the Cetus process; [15]). Because the free enzyme is relatively quickly deactivated by some of the products (H_2O_2, HO•, keto-derivatives; [13]), addition of catalase decomposes the hydrogen peroxide, thus prolonging the POx enzyme activity.

The developed computing methodology can offer a quickly adaptable optimal/sub-optimal control policy of the added enzyme during the batch under several operating constrains (referring to the volumetric dilution, oxygenation rate, operation time, enzyme consumption, realized productivity). The developed systematic approach can be easily extended to determine optimal operating policies for BR/SBR of industrial interest, for carrying out a satisfactory compromise between the productivity and low-cost goals.

Modelling the process

Enzymes

The enzyme used for the D-glucose oxidation is the pyranose oxidase (POx, E.C. 1.1.3.10), which specifically catalyses the oxidation of some pyranose saccharides at C-2 position to keto derivates. The POx molecular mass is about 322 800 ± 18 300 Da, containing four identical subunits and four molecules of FAD (flavine-adenosine-dinucleotide) per tetramer [14]. Several studies revealed similarities of POx obtained from various white-rot fungi [15,16]. In [13] it was studied the stability of POx from *Peniophora gigantea,* and it was proposed a reduced kinetic model for D-glucose oxidation. The stoichiometric production of H_2O_2 and of peroxide radicals $HO^{\cdot}$ in the reaction medium (due to traces of ferrous impurities) causes a rapid deactivation of the POx, its activity being practically annihilated after 1 day of reaction at 20-30°C [13,15]. Recent researches identify the binding structure responsible for the POx specific activity, and suggest enzyme modifications by mutagenesis to increase its stability [17]. As revealed by [18], immobilisation of POx considerably increases its stability (from 90% to only 4% deactivation over 1 day of batch reaction) with the expense of losing 50% of its activity.

To make feasible the industrial use of POx in both free-/immobilised enzyme alternatives, catalase enzyme addition in the reaction medium has been suggested by several authors [15] (e.g. from bovine liver, E.C. 1.11.1.6, [19]) in order to rapidly decompose H_2O_2 to oxygen and water, thus preventing its attack on POx. A catalase-to-POx ratio of about 1000 seems to ensure the POx quasi-'stabilization' during the oxidative process. The use of fungal catalase seems to be more expedient, being more stable vs. hydrogen peroxide action. Other additives may have a positive effect on POx and catalase stability during the oxidative process [15].

Process description

D-glucose oxidation to 2-keto-D-glucose (kDG) is a reaction of high interest for both large and small-size production of rare sugars or sugar-derivates, such as D-fructose, synthetic carbohydrates, 2-keto-D-gluconic acid (and then D-isoascorbic acid), D-mannitol, D-sorbitol, diaminosorbitol, diaminomannitol, etc. [15,20,21]. The subsequent enzymatic reduction of kDG to D-fructose (the Cetus process, [15]) has become very attractive over the last years due to several advantages, including a high conversion and selectivity, and the absence of aldose in the final product (presenting a potential allergenic effect). The alternative way of fructose production, by means of enzymatic glucose isomerisation in the presence of salts, is industrially developed, even if the equilibrium conversion is quite low (ca. 50% at 50-60°C), and significant amounts of impurities are also present [1,22,23]. Other alternatives, such as innuline enzymatic hydrolysis, still do not exhibit a satisfactory productivity [24-26].

Experiments [13] and from [15] suggest the following conditions for oxidation of D-glucose over free POx: 25-30°C (ensuring high reaction rates, POx being very stable in the absence of H_2O_2), *p*H= 6.5-7 (buffer solution), atmospheric pressure for the supplied air (oxygen), initial DG concentrations of 200-250 mM (ca. 4.5%wt). In the present study the same conditions have been used to design a (semi)batch industrial reactor, that is 25°C, *p*H= 6.5-7, and possible traces of ferrous ions (of pM order).

The oxygen is supplied by using a mixing-sparging equipment for enzymatic reactors, the oxygen diffusion through both liquid surface and bubbles-liquid interface being separately studied by various researchers (pure oxygen feeding is used in this paper, but the results can be easily extrapolated for the aerated reactor case). Free reaction experiments indicated an overall mass transport coefficient $k_{oxl}a$ between 0.02 s^{-1} (absence of bubbles, [13]) and 0.04 s^{-1} (presence of bubbles and mixing with turbine stirrers and baffles, [27]).

The mol-to-mol main reaction of DG with oxygen leads to the stoichiometric synthesis of kDG and also of H_2O_2, other secondary products being produced in negligible amounts (table 1). Inactivation of POx by H_2O_2, HO radicals,

Table 1
REDUCED KINETIC MODEL OF THE DG OXIDATION USING POx [13], AND OF THE H_2O_2 DECOMPOSITION USING CATALASE (KINETIC PARAMETERS FOR 25°C, pH=7)

Reaction schema	Parameters, Reference
$C_6H_{12}O_6 + Y_{ox}O_2 \xrightarrow[(water)]{POx} C_6H_{10}O_6 + H_2O_2$ Abbreviation: $DG - DO \xrightarrow[(water)]{POx} kDG - H_2O_2$ Rate expression: $r_{our} = \mu_m \frac{c_{DG}}{K_{DG} + c_{DG}} \frac{c_{DO}}{K_{DO} + c_{DO}} c_{POx}$ $Y_{POx}POx + H_2O_2 \xrightarrow[(water)]{Fe\ traces} POxox$ (Inactivated form) Rate expression: $r_d = k_d c_{POx} c_{H_2O_2}$	Ref. [13]: $\mu_m = 0.0891$ mM mL $s^{-1}U^{-1}$ $K_{DG} = 63.523$ mM $K_{DO} = 0.2613$ mM $k_d = 9.2827 \cdot 10^{-6}$ $(1+6.95 c_{Fe}^{0.36})$, mL $s^{-1}U^{-1}$ $Y_{ox} = 1.0$ $Y_{POx} = 1.0$ U mL^{-1} mM^{-1}
$H_2O_2 \xrightarrow[(water)]{catalase} H_2O + 0.5O_2$ Rate expression: $r_c = k_c c_{H_2O_2}$	Present study: $k_c = \begin{cases} 1.9648 \cdot 10^{-5}\ s^{-1}, \\ \quad \text{for [Catalase]} \le 1\ \text{kU mL}^{-1} \\ 3.3987 \cdot 10^{-3}\ s^{-1}, \\ \quad \text{for [Catalase]} > 1\ \text{kU mL}^{-1} \end{cases}$

and possible kDG probably occurs through successive steps involving several educts, of increased inactivity. However, an overall deactivation reaction can be assumed to simplify the subsequent numerical analysis, of a reaction rate dependent on (ferrous) impurities that amplify the production of free radicals [13]. H_2O_2 is quickly decomposed in the presence of catalase, the resulting water contributing to the slight dilution of the reactor content [15].

Kinetic model for D-glucose oxidation with POx

A reduced kinetic model was considered to quickly simulate the complex enzymatic process. The adopted model of [13], indicated in table 1 (upper part), fairly represent the process dynamics under the mentioned operating conditions. The main reaction rate expression (r_{our}), of Michelis-Menten type (M-M), accounts for rate limitations at high substrate and/or oxygen levels. Thus, the substrate M-M rate constant of 64 mM also implicitly considers operation at higher substrate levels, while the oxygen M-M rate constant of 0.26 mM includes both operation using fresh loaded POx (at low DO levels), or using partially inactivated POx (when DO is close to the saturation level of 1.21 mM). The influence of (ferrous) impurities on the POx deactivation rate has been included in the expression of k_d rate constant, based on the experimental datafrom [13]. This kinetic model has been elaborated for process operating with only POx. Consequently, for the industrial reactor design and operation purposes, the model must be completed with terms accounting for the catalase action on the hydrogen peroxide.

Completing the process model with the catalase action

By assuming an overall decomposition reaction of H_2O_2 by catalase, the associated rate constant k_c (table 1) depends on the catalase concentration in the reactor. To determine its value, the experimental data from [15] have been considered, that is the residual POx activity (%) plot vs. catalase concentration under the following imposed operating conditions: 24 h batch run, 100 mM initial DG, 0.5 U mL^{-1} POx initial activity, buffer solution of *p*H=6.5, 25°C (fig. 1). The catalase decomposition seems to reflect low HOOH in the medium, thus the reaction is assumed to be of first order. Catalase is fairly cheap, so having a fair amount around is reasonable and thus so is the assumed kinetics.

The mass balance equations for the considered species in the batch operation are those presented in table 2. Water resulting from the process has lead to add a dilution term to the mass balance, even if the volume growing rate $D = dLn(V)/dt$ is quite small (the final volume growth being of ca. 0.1%V_0 for the mentioned conditions). This term accounts for the sum of volumes of H_2O_2 and H_2O resulted from DG oxidation and hydrogen peroxide decomposition respectively. The oxygen coming from H_2O_2 decomposition was neglected, being in much smaller amounts vs. incoming oxygen. The reactor model corresponds to ideal mixing conditions, and intense aeration (of an overall $k_{oxl}a = 0.04\ s^{-1}$). By applying a standard statistical estimator, the unknown k_c rate constant has been determined for various catalase concentrations. Then, a simple power-law correlation, of type $k_c = k_{c0}[\text{Catalase}]^{\gamma}$ has been found to fairly represent the experimental data in the range of

Mass balance equations	Parameters
$\frac{dc_{DO}}{dt} = k_{oxl}a(c_{DO}^{*} - c_{DO}) - Y_{ox}r_{our} - Dc_{DO}$ $\frac{dc_{DG}}{dt} = -r_{our} - Dc_{DG}$ $\frac{dc_{kDG}}{dt} = r_{our} - Dc_{kDG}$ $\frac{dc_{POx}}{dt} = -Y_{POx}r_d - Dc_{POx}$ $\frac{dc_{H_2O_2}}{dt} = r_{our} - r_d - r_c - Dc_{H_2O_2}$ $\frac{dV}{dt} = DV$	Content dilution due to the reaction water: $D = \frac{1}{V}\frac{dV}{dt} = \frac{d[H_2O]_{reaction}}{dt}\frac{M_w}{\rho_w}$; D = average logarithmic volume growing rate, s^{-1} ; $M_w = 18$ g mol^{-1}; $\rho_w = 997$ g L^{-1} c_{DO}^{*} = saturation concentration of DO at the reaction temperature (1.21 mM at 25°C)

Table 2
MASS BALANCE IN THE BASIC BATCH OPERATION MODE

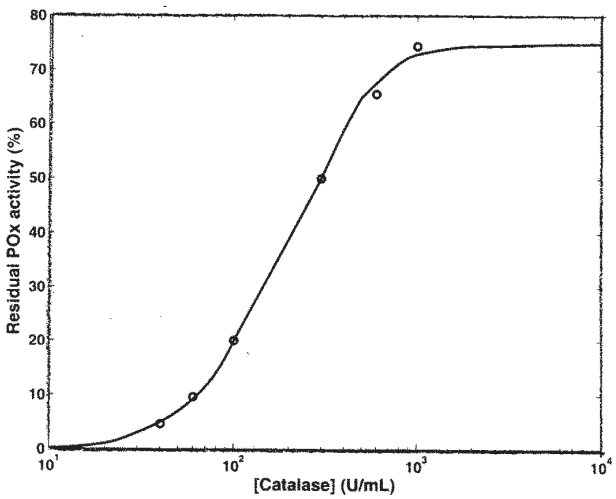


Fig. 1. Effect of catalase on the POx activity after a 24 h batch operation: (—) residual activity determined experimentally, by using bovine liver catalase and POx from *Trametes multicolor* (re-built curve from Leitner et al. [15]); (o) predictions using the proposed H_2O_2 decomposition model with the rate constant $k_c = 1.9648 \times 10^{-5}$ [Catalase]$^{0.746}$ (valid for [Catalase]<1000 U mL^{-1}). Conditions: [POx]$_o$ = 1 U ml^{-1}; *p*H = 6.5 (50 mM phosphate buffer solution), 25°C

0≤[Catalase]≤1000 mM, as represented in figure 1. The fitted k_{c0} and γ are indicated in table 1, while a fixed value of $k_c = k_{c,lim}$ is established for higher catalase concentrations of [Catalase]>1000 mM.

Operating alternatives

Semi-batch reactor - alternative operation and models

The design of isothermal well-mixed free-enzyme reactor (with sparger system) has been considered in four operation alternatives:

- batch operation with initial addition of POx and catalase enzymes ($V_{inj}c_{POx,inj}$);
- batch operation with intermittent addition of POx solution, of volumes $V_{inj,u}$ and given concentration $c_{POx,inj}$, over N_{inj} uniformly distributed addition times over the batch (u=1,...,N_{inj});
- semi-batch operation with a constant feed flow rate F of the POx solution, of known concentration $c_{POx,in}$;

iv) semi-batch operation with a time step-wise variable feed flow rate $F(t)$ of the POx solution, of known concentration $c_{POx,in}$. The inlet flow rates F_u are assumed as being constant over each time-interval Δt (the so-called 'arcs'), u=1,...,N_{div}, calculated by division of the batch time in N_{div} equal parts, i.e. $\Delta t = t_f / N_{div}$. Individual values of F_u can be heuristically established, or by using a model-based optimization procedure [31].

The adopted pseudo-homogeneous model of the batch reactor is presented in table 2. Species concentration dynamics is determined by simple integration of the mass balance equations, starting from the initial conditions (initial load of substrate and enzymes).

The BR model with intermittent addition of POx solution is the same as for the simple batch operation, excepting for the addition times $t_{inj,u}$ when species concentrations have to be re-calculated using the mixing solution equations of table 3 (low part). Concerning the volume $V_{inj,u}$ of each POx solution injection in the reactor, only equal volumes (uniform policy), or exponential-like decreasing volumes have been checked. Calculation of these injected volumes is presented in table 3, with imposing a certain limit for the total added *POx* solution volume over the batch ($V_{inj,tot}$), usually taken as 10%V_o in industrial practice (to avoid excessive dilution of the reactor content). For both uniform or exponential-like injected POx-solution volumes, determination of {$V_{inj,u}$, $t_{inj,u}$} requires the knowledge of only final batch time t_f, the number of injections N_{inj}, and the total POx solution amount $V_{inj,tot}$. However, the exponential-like addition policy $V_{inj,u}$ also depends on two empiric parameters {a,b}. The parameter b is determined by successive trials (starting from $b>0$) and reactor simulations to get maximum DG conversion, while the parameter a results from the $V_{inj,tot}$ constraints in the form of a simple algebraic relationship (table 3). In [12,30] it was proved that for a pseudo-first-order deactivation of the biocatalyst (POx in the present case), the exponentially decreasing intermittent addition policy of the enzyme in the reactor is very close to the precise optimal feeding policy, with the advantage of not requiring steady computational steps to derive it, but only simple algebraic

POx addition policy	**Injected volume function** $V_{inj,u}/V_o$, $u = 1,...,N_{inj}$	**Injected volume function parameters**	**Imposed constraints**
Uniform	$\frac{V_{inj,u}}{V_o} = \frac{V_{inj,tot}}{V_o}\frac{1}{N_{inj}}$ $t_{inj,u} = \frac{t_f}{N_{inj}}(u-1)$	N_{inj}	$N_{inj} > 1$; Δt_{inj} = constant; $c_{POx,inj,u}$ = constant (given); $\sum_{u=1}^{N_{inj}} V_{inj,u} = V_{inj,tot} = 0.1 \times V_o$; $t_f = 7$ h
Exponential (increased, s>0) (decreased, s<0)	$\frac{V_{inj,u}}{V_o} = \frac{a}{V_o} \times exp[sb(u-1)]$ $t_{inj,u} = \frac{t_f}{N_{inj}}(u-1)$	$s = \pm 1$; Optimal $b > 0$, $exp(b) \neq 1$, chosen to ensure: Max $x_{DG}(t_f)$: $\frac{a}{V_o} = \frac{V_{inj,tot}}{V_o}\frac{exp(b)-1}{exp(b \times N_{inj})-1}$	
Mass balance of the batch reactor after each enzyme addition for $t = t_{inj,u}$, $0 \le t_{inj,u} \le t_f$, $u = 1,...N_{inj}$: $c_j(t_{inj,u+}) = \left(1 - \frac{V_{inj,i}/V_o}{V/V_o}\right) c_j(t_{inj,u-}) + \frac{V_{inj,i}/V_o}{V/V_o} c_{j,inj}(t_{inj,u})$, $j = 1,...,n_s$, $C_{DO,inj} \approx C_{DO}\big\|_{t_{inj}}$; $\frac{V}{V_o}\Big\|_{t_{inj,t+}} = 1 + \sum_{u=1}^{i} \frac{V_{inj,u}}{V_o}$.		

Table 3
CALCULATION OF THE POx SOLUTION ADDED VOLUMES $V_{inj,u}/V_o$,u = 1,...,N_{inj} OVER N_{inj} INJECTIONS FOR A BATCH REACTOR WITH INTERMITTENT ADDITION OF POx [12]. THE APPROACHED CASES CORRESPOND TO UNIFORM OR EXPONENTIAL INCREASING/ DECREASING ADDITION POLICIES [$\Delta t_{inj,u} = t_{inj,u+1} - t_{inj,u} = t_f / N_{inj}$, u= 1,...,($N_{inj}$ -1)]

Mass balance equations	Parameters
$\frac{dc_{DO}}{dt} = k_{oxl}a(c^*_{DO} - c_{DO}) - Y_{ox}r_{our} + \frac{F(t)}{V(t)}(c_{DO,in} - c_{DO}) - Dc_{DO}$	Content dilution due to the reaction water: $D = \frac{1}{V}\frac{dV}{dt} = \frac{d[H_2O + H_2O_2]}{dt}\frac{M_w}{\rho_w}$;
$\frac{dc_{DG}}{dt} = -r_{our} - \frac{F(t)}{V(t)}c_{DG} - Dc_{DG}$	D = average logarithmic volume growing rate, s^{-1};
$\frac{dc_{kDG}}{dt} = r_{our} - \frac{F(t)}{V(t)}c_{kDG} - Dc_{kDG}$	M_w = 18 g mol^{-1}; ρ_w = 997 g L^{-1}
$\frac{dc_{POx}}{dt} = -Y_{POx}r_d + \frac{F(t)}{V(t)}(c_{POx,in} - c_{POx}) - Dc_{POx}$	c^*_{DO} = saturation concentration of DO at the reaction temperature (1.21 mM at 25°C);
$\frac{dc_{H_2O_2}}{dt} = r_{our} - r_d - r_c - \frac{F(t)}{V(t)}c_{H_2O_2} - Dc_{H_2O_2}$	$c_{DO,in} = c^*_{DO}$
$\frac{dV}{dt} = F(t) + DV$	

Table 4
MASS BALANCE IN A SEMI-BATCH REACTOR WITH CONTINUOUS ADDITION OF PO_X ENZYME SOLUTION

relationships. These two intermittent feeding policies of the batch reactor (uniform, and exponential-like) will be further tested.

The model of the semi-batch reactor, with a continuous addition of POx enzyme, is displayed in table 4. The supplementary terms account for the input flowrate $F(t)$, maintained constant or varied, according to a certain (optimal) policy.

Optimization criteria for the industrial batch/semi-batch reactor

Optimization of BR/SBR implies derivation of operating conditions ensuring maximization of an economic/performance criterion (such as conversion, yield, productivity, benefit, operating time, utilities, etc.) in the presence of technological and safety constraints. Derivation of such an optimal policy is not an easy task, due to the biochemical process complexity, multiple influencing factors to be considered, raw-materials/catalyst variable characteristics, and the high sensitivity of the process to the operating conditions [12,30]. In the absence of a process model, the problem is solved by using the available information about the process, the past experience (batch-to-batch optimization), heuristic rules, or the operating experience from a similar process.

Alternatives consider one-time BR/SBR optimization (using the off-line derived process model), run-to-run optimization (using additional information from past batches), and on-line optimization (solution being adapted based on the on-line acquired information and model updating). In mathematical terms, derivation of an optimal running policy consists in determining the manipulated variable vector $\boldsymbol{u}$, and sometimes of the final batch time t_f, from minimization of a suitable objective function:

$$(\hat{\boldsymbol{u}}, \hat{t}_f) = arg\ Min\ \Phi(\boldsymbol{x}, \boldsymbol{u}, \boldsymbol{\phi}, t_f)$$
$$\text{s.t. } \dot{\boldsymbol{x}} = \boldsymbol{f}(\boldsymbol{x}, \boldsymbol{u}, \boldsymbol{\phi}, t),\ \boldsymbol{x}(t_o) = \boldsymbol{x}_o, \text{ (process dynamic model)} \quad (1)$$
$$\boldsymbol{g}(\boldsymbol{x}, \boldsymbol{u}, \boldsymbol{\phi}, t) \le \boldsymbol{0}, \text{ (constraint)}$$

where:

ϕ= operating parameter vector;

x = state variable vector).

In the present study, various operating alternatives of the enzymatic reactor will be compared in terms of required amount of POx enzyme (an expensive raw-material) necessary to be spent over one batch for reaching an imposed DG conversion, under the same initial conditions and for the same final batch time. The conclusions of the numerical analysis will be generalized to be useful when solving similar free-enzyme operating problems.

For the BR studied case, with an initial addition of POx, or with N_{div} =20 imposed enzyme additions at equal time intervals, the optimum will correspond to the minimum POx amount necessary to obtain $x_{DG}(t_f)$= 0.9990 over t_f = 7 h of operation, under nominal conditions of temperature and *p*H, and for a maximum $V(t_f) / V_o$ = 10% dilution of the reactor content (to prevent increased costs of subsequent transport, separation, and concentration steps).

For the SBR case with a constant feed flow rate F, only the POx inlet concentration must be determined to meet the same requirements as for the BR case.

For the SBR case with variable feed flow rate $F(t)$, the derived feeding policy is given on time intervals, obtained by dividing the batch time t_f in N_{div} equal parts, i.e. the so-called 'arcs', of length $\Delta t = t_f / N_{div}$, where the reactor input is continuous and differentiable [9]. By considering constant feeding characteristics over every such interval, that is constant $F_u(t)$ and $c_{POx,in,u}(t)$, over $t_{u-1} \le t < t_u$, u=1,...N_{div}, $t_u = u\Delta t$ (switching points), then an optimal policy [F_1, F_2,...,F_{Ndiv}] (for constant $c_{POx,in}$), or [$F_{POx,1}$, $F_{POx,2}$,...,$F_{POx,Ndiv}$] (for both flow rate and adjustable $c_{POx,in}$) can be derived by maximizing the reactor performance index in the presence of various technological constraints. In the present study, the optimal solution corresponds to the minimum inlet POx concentration and to a suitable feeding policy over N_{div} =20 time intervals arcs, ensuring a maximal DG-conversion which equals the imposed value of $x_{DG}(t_f)$ = 0.9990 over t_f= 7 h of operation, under given initial conditions, and mentioned constraints (of *p*H, temperature, and initial volume dilution). In other words such a criterion corresponds to finding:

$$\{\text{Min } c_{POx,in},\ F_1, F_2, ..., F_{N_{div}}\} = arg\{Max\ x_{DG}(\boldsymbol{c}, \boldsymbol{F}, \boldsymbol{\phi}, V, t_f) = 0.9990\}, \text{ (imposed).}$$
$$\text{s.t.} \quad d[\boldsymbol{c}, V]/dt = \boldsymbol{f}(\boldsymbol{c}, \boldsymbol{F}, \boldsymbol{\phi}, V, t), \quad \text{(process dynamic model)}$$
$$[\boldsymbol{c}, V](t_o) = [\boldsymbol{c}_o, V_o], \quad \text{(imposed initial conditions)} \quad (2)$$
$$t_f = 7 \text{ h}, \quad \text{(imposed final batch time)}$$
$$[\boldsymbol{c}, \boldsymbol{F}, \boldsymbol{\phi}, V] \ge \boldsymbol{0}, \quad \text{(constraints)}$$

Table 5

NOMINAL OPERATING CONDITIONS OF THE INDUSTRIAL PROCESS. ONE UNIT (U) OF POx ACTIVITY IS DEFINED AS THE AMOUNT OF ENZYME NECESSARY FOR THE OXIDATION OF 1μmol OF ABTS PER MINUTE UNDER THE GIVEN CONDITIONS (ABTS= [2.2'-AZINOBIS(3-ETHYLBENZTHIAZOLINE-6-SULFONIC ACID); [13])

Operating conditions	Value	Operating conditions	Value
Initial liquid volume (V_o)	75000 L	Initial D-glucose (DG) concentration	1000 mM
Liquid physical properties	water	Initial catalase concentration	1000 U mL^{-1}
Temperature (isothermal operation)	25°C	POx concentration (Note b)	to be establish by optimization
Pressure	normal	Iron traces concentration	$3 \cdot 10^{-5}$ mM
pH (phosphate buffer solution)	6.5-7	Dissolved oxygen (DO) saturation concentration	1.21 mM
Batch (reaction) time	7 h	Number of POx additions over the batch, N_{inj}	1-20
Maximum volumetric dilution	10% V_o	Number of time-arcs over the semi-batch run time, N_{div}	1-20
Oxygen overall mass transfer coefficient, $k_{ox}a$ (Note a)	0.04 s^{-1}	Imposed DG conversion, x_{DG}	99.90%

(a) [13,27].

(b) Maximum POx activity is reported as being of ca. 240 U mL^{-1} (from *Peniophora gigantea*, [13]), and of ca. 5 U mL^{-1} (from *Trametes multicolor*, [15]).

The solving procedure implies a computational loop: for every tried $c_{POx,in}$, an optimization sub-problem is solved, finding the feed flow rates F_1, F_2,...,F_{Ndiv} ensuring a maximum of DG conversion, and checking if this $M_{ax}\, x_{DG}$ equals the imposed level. The search starts from small values, and checks for increased values, stopping when the obtained Max x_{DG} equals 0.9990.

Results and discussions

Nominal conditions of the batch reactor

The chosen nominal conditions of the process are presented in table 5. They correspond to the recommended conditions for an industrial (well-mixed and aerated) batch reactor. The set initial liquid volume corresponds to a production of 10'000 tonnes D-fructose / year by using the Cetus alternative. The DG initial concentration was taken 1000 mM (ca. 180 g L^{-1}), by considering the current possibilities of getting raw-materials from natural resources (from the starch hydrolysis). A higher concentration is not recommended due to the substrate-limitation of the reaction rate, and due to an increased viscosity of the manipulated solutions. As the effective [POx] in the reactor ranges from 1 mM to 2 mM, a concentration of 1000 mM catalase was used (as recommended [15]), and a buffer solution of *p*H=7. Even if purified water was used, the presence of some ferrous impurities has also been considered.

Comparing optimal operating alternatives of the BR/SBR

Following the imposed optimization objective, the derived operating solutions for the (semi-)batch reactor can be directly compared.

Species concentration evolution in the BR operated with initial addition of POx (fig. 2), is similar to those obtained for the BR operated with imposed enzyme additions at equal time intervals (fig. 3 for uniform addition policy, and figure 4 for exponential decreasing injected volumes of enzyme solution). However, the last operating alternative (fig. 4) seems to be more advantageous because the POx concentration is maintained for longer time at an average moderate level (of around 1-2 U mL^{-1}) with consuming less enzyme.

More favourable evolutions of species concentrations are obtained for the SBR operation case, in both

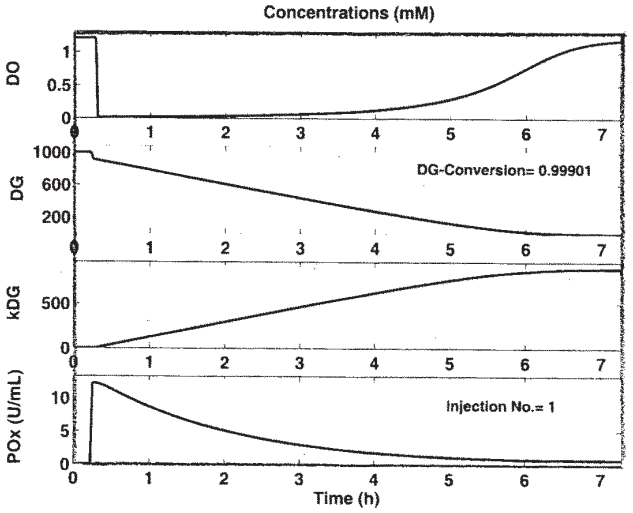


Fig. 2. Key species concentration dynamics in the industrial batch reactor initially loaded with D-glucose (DG), POx, and catalase enzymes. Initial POx amount was ranged [POx]= 135 U ml^{-1} to ensure a final DG conversion (x_{DG}) of 99.90% over a 7 h runtime. Nominal conditions correspond to: [DG]= 1000 mM; [Catalase]= 1000 U ml^{-1}; pH = 6.5, 25°C. Residual POx activity is 59.33%

alternatives: using a constant feed flow rate of POx solution (fig. 5), or using an optimal feeding policy (fig. 6). While getting the same conversion over the same batch time, the optimal SBR alternative appears to be the best one, the POx concentration in the reactor displaying a quasi-constant profile.

The summary of results displayed in table 6 reveals significant differences in POx consumption for every operating alternative to get the same DG conversion over the same reaction time and similar conditions. The number of time-arcs (20) in the semi-batch mode was kept the same with the number of enzyme injections (20) over the batch running time. An increased number of time-arcs might lead to an improvement of reactor productivity or, equivalently, to a reduction of POx consumption for the same reactor productivity. However, such increased reactor flexibility will require additional computational efforts to derive the complex feeding policy, and additional implementation costs. The required POx consumption was evaluated with the formula:

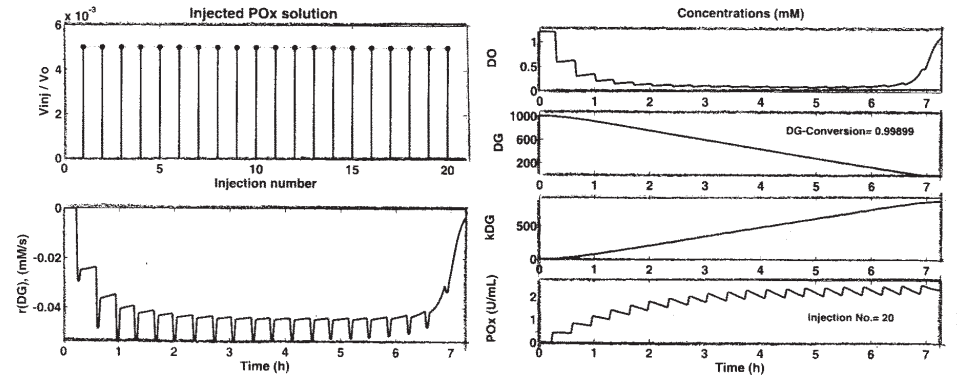


Fig. 3. (Left column) uniform POx solution addition policy ($V_{inj,u}/V_o$, u=1,...,N_{inj}), and DG-reaction rate (r_{DG}) over the batch. (Right column) Key species concentration dynamics in the BR under nominal conditions. An injected solution of [POx]= 86 U ml^{-1} was found to ensure a final x_{DG}= 99.90% over a 7 h runtime, with an overall dilution of 10% (excluding reaction water). Residual POx activity is 93.98%.

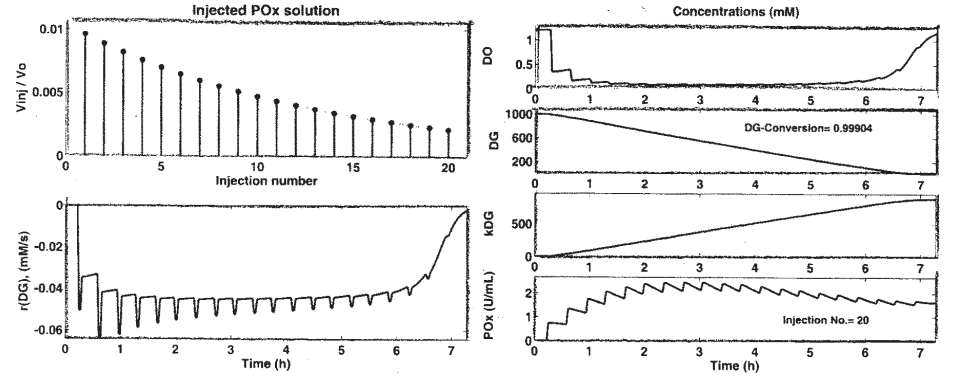


Fig. 4. (Left column) Exponentially decreasing addition policy of POx solution ($V_{inj,u}/V_o$, u=1,...,N_{inj}), and DG-reaction rate (r_{DG}) over the batch. (Right column) Key species concentration dynamics in the BR under nominal conditions. An injected solution of [POx]= 77 U ml^{-1} was found to ensure a final x_{DG}= 99.90% over a 7 h runtime, with an overall dilution of 10% (excluding reaction water). Residual POx activity is 67.31%

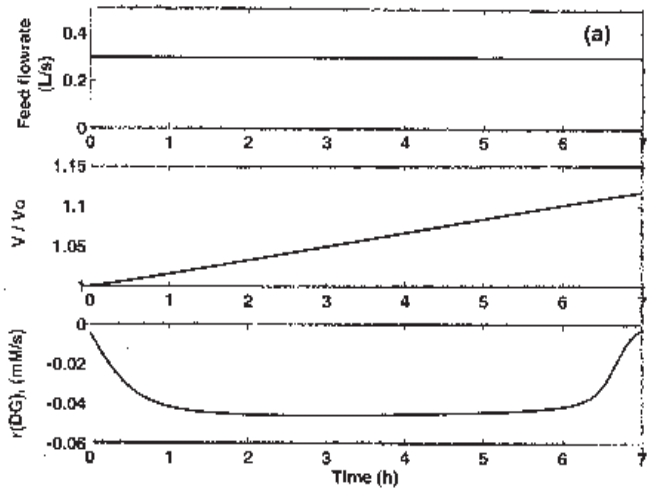
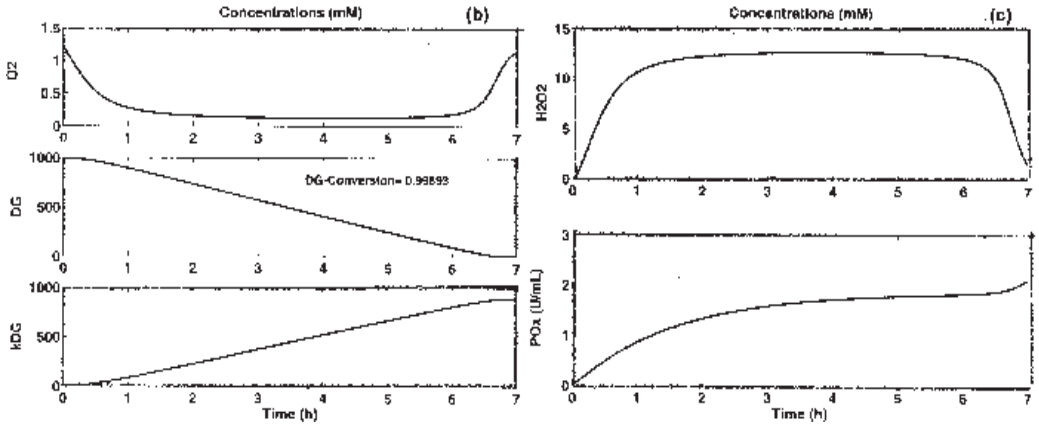


Fig. 5. (a) The uniform feeding policy with POx solution of the SBR, reactor liquid volume dynamics, and the DG-reaction rate (r_{DG}) evolution over the batch. (b) Key species concentration dynamics in the SBR and, (c) H_2O_2 and POx concentration evolution, under nominal conditions. A constant [POx]= 72 U ml^{-1} in the inlet solution was found to ensure a final x_{DG}= 99.90% over a 7 h runtime, with an overall dilution of 10% (excluding reaction water)



$$m_{POx,tot} = \sum_{u=1}^{N_{inj}} V_{inj,u} c_{POx,inj,u} \quad \text{(batch operation mode)}$$

$$m_{POx,tot} = \int_0^{t_f} F c_{POx,in} dt \quad \text{(semi-batch operation mode)} \qquad (3)$$

As expected, the results indicate the semi-batch operating mode with an optimal feeding policy as being the best alternative (fig. 7). The required POx consumption per batch is with ca. 58% smaller than of the classic batch operation case with initial enzyme addition. Such a running solution also offers a higher degree of flexibility in operation, with the expense of reactor feeding costs, and of supplementary computational steps to derive the optimal feeding solution. However, the considerable reduction in

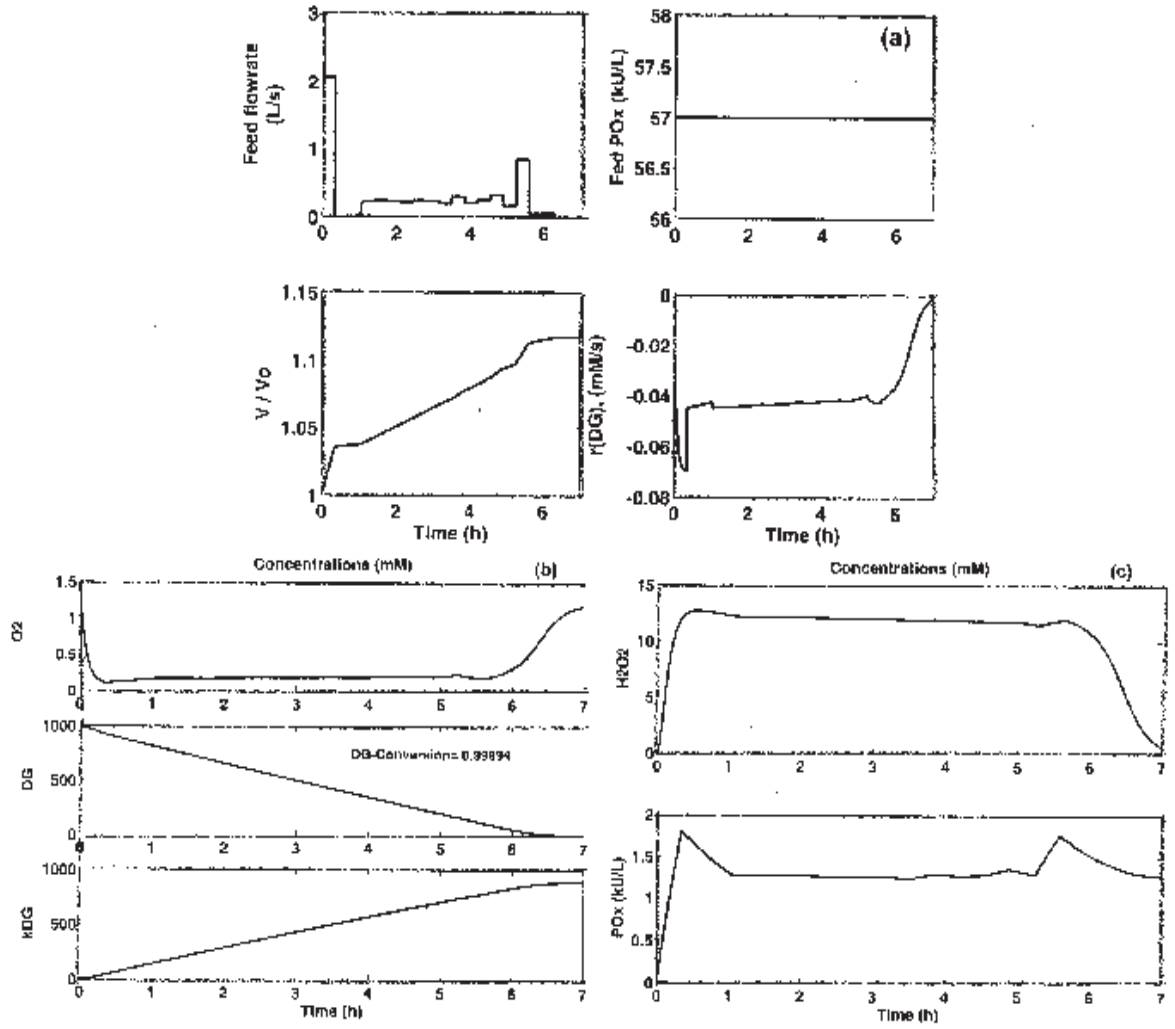


Fig. 6. (a) The optimal feeding policy with POx solution of the SBR, reactor liquid volume dynamics, and DG-reaction rate (r_{DG}) evolution over the batch. (b) Key species concentration dynamics in the SBR and, (c) H_2O_2 and POx concentration evolution under nominal conditions. A constant [POx]= 57 U ml^{-1} in the inlet solution was found to ensure a final x_{DG}= 99.90% over a 7 h runtime, with an overall dilution of 10% (excluding reaction water)

Table 6
COMPARISON OF FREE-ENZYME OPERATING ALTERNATIVES FOR kDG PRODUCTION

Operating mode	Batch with initially added POx enzyme	Batch with intermittent addition of POx	Semi-batch with POx solution constant feed flowrate	Semi-batch with POx solution optimal feed flowrate
Necessary enzyme, $m_{POx,tot}$ (kU)	1 012 500 [a]	645 000 [a,b] 577 500 [a,c]	540 000 [d]	427 500 [d]
Added liquid to initial load (%V_o)	10	10	10	10
No. injections / No. of feeding arcs	1	20	20	20
Batch time, t_f (h)	7	7	7	7
Imposed final conversion, x_{DG}	99.90	99.90	99.90	99.90
Dilution due to the reaction water (%V_o)	1.8	1.8	1.8	1.8

(a) $m_{POx,tot} = \sum_{u=1}^{N_{inj}} V_{inj,u} c_{POx,inj,u}$; (b) uniform addition policy; (c) exponential decreasing addition policy; (d) $m_{POx,tot} = \int_0^{t_f} F c_{POx,in} dt$.

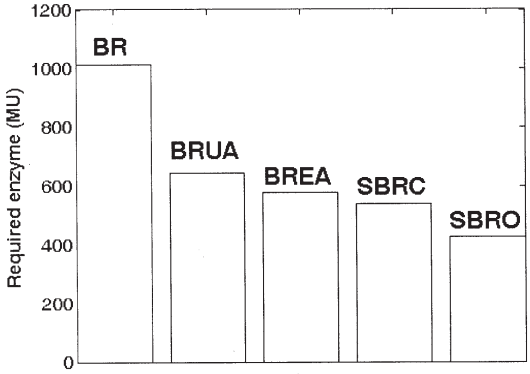


Fig. 7. Free-enzyme batch operating alternatives for kDG production. Operating conditions: 25°C, *p*H=7; $[DG]_o$= 1 M; [Catalase]= 1 kU/mL; 10% liquid volume increase; initial volume = 75 m^3; reaction time = 7 h; sparging using oxygen; imposed DG conversion of 99.90%. BR= batch reactor; BRUA= batch reactor with uniform addition of POx (20 injections); BREA= batch reactor with exponential decreasing addition of POx (20 injections); SBRC= semi-batch reactor with constant fed POx solution; SBRC= semi-batch reactor with optimal feedflowrate of POx solution (20 time-arcs)

the enzyme consumption obtained without any productivity or conversion loss fully justifies such efforts when the raw-materials and enzymes cost is considerable.

Even if being a sub-optimal solution, the SBR operation with a constant feed flow rate is still a good alternative, the POx consumption being with only 26% higher than for the optimal feeding policy case, but still with 47% less than of the basic batch operation. The advantage consists in the lack of computational steps, the optimal feeding being derived by means of a few number of SBR simulations.

The optimal batch operation with intermittent addition of POx, following an exponential decreasing policy, is very close to the optimal SBR operation with a constant feeding flow rate. This alternative is also computational inexpensive, requiring only few number of batch process simulations.

The classic batch operation mode, with an initial load of all ingredients is by far, the less economic alternative. Such an operation not only lacks of flexibility during operation, but also of any possibility to on-line adjusts the process performances.

Conclusions

Derivation of the most suitable (optimal) operating alternative for a (semi)batch enzymatic reactor is a difficult problem, requiring steady experimental effort to get enough information on the process kinetics, but also steady computational efforts to derive feasible solutions of the nonlinear optimization problem.

A decision on choosing a suitable SBR feeding policy, that performs a satisfactory productivity vs. costs compromise, depends on raw-materials, products, and other batch costs. However, our study proves that the semi-batch operating mode with an optimal feeding policy is the best alternative in terms of flexibility, productivity, and material consumption. Such a solution requires a supplementary effort to derive it, but also an advanced control to be implemented for keeping the process under the defined conditions and in an economic operating region [28,29]. It is also to be mentioned, that the optimal operating policy is not always easily to be implemented, due to the required large number of state variables to be on-line measured and to the large number of control function parameters. This is why sub-optimal policies, based on a sufficiently appropriate reduced dynamic model (accounting for the essential parts of the process), can lead to economic benefits by accounting for the main process characteristics and requiring a reduced computational effort.

Interesting sub-optimal operating alternatives can be obtained with a much less computational effort, based on a reduced process model and using constant feeding policies, or an intermittent addition of the enzyme following an exponentially decreasing function. The former alternative can be easily implemented, without requiring special control equipment. The enzyme injection policy must be derived for each studied enzymatic process by checking its agreement with the enzyme deactivation function and process kinetic characteristics. The number of injections is limited by the reactor-regulatory possibilities, while the injected solution must be enough concentrated to not considerably dilute the reactor content.

Such sub-optimal solutions can be easily adapted when the process model and its parameters change, involving only few steps to re-set the feeding policy parameters (i.e. feed flow rate and inlet POx concentration in the SBR mode, or the volume and concentration of POx injections in the BR mode). Thus many difficulties associated with the localization of the global optimal control solution are bypassed, leading to reliable results with reduced analysis, computational and implementation costs.

In a more systematic approach, several sources of uncertainty would be necessary to be investigated and considered during the BR or SBR optimization [5,7,8], especially for cases presenting large fluctuations in the operating conditions, or large uncertainties in model parameters.

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Symbols

a,b - $V_{inj,u}$ control function constants (table 3)
c_j - species j concentration
D - reactor content dilution rate
F - enzyme solution feed flow rate in SBR
f - model function vector
g - constraint function vector
K_j - Michaelis-Menten constants
k_{oxl} - oxygen overall mass transport coefficient
k - rate constants
M_w - water molecular weight
m - mass
N_{div} - number of equal divisions of the batch time
N_{inj} - number of enzyme injections over a batch
n_s - number of species considered in the model
r_j - species j reaction rate
s - switch constant (table 3)
t - time
Δt - time interval
t_f - final batch time
u - control variable vector
V - liquid volume
x, **x** - conversion, or state variable vector

Y - stoichiometric coefficient

Greeks

Δ - finite difference

Φ - optimisation objective function

ϕ - operating parameter vector

γ - kinetic constant

μ_m - kinetic constant

ρ_w - water density

Index

in - inlet

inj - injected

o - initial

our - oxygen uptaking rate

tot - total

- / + - just before / immediately after

Superscipts

* - saturation

^ - estimated

Abbreviations

BR - batch reactor

DG - D-glucose

DO - dissolved oxygen

FAD - flavine-adenosine-dinucleotide

kDG - 2-keto-D-glucose

mM - milli-molar concentration

M-M - Michelis-Menten

pM - pico-mo - pyranose oxidase

SBR - semi-batch reactor

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