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Dedicated to Professor Dumitru Oancea
on the occasion on his 75th anniversary

MODULAR SIMULATION TO CHECK PERFORMANCES OF VARIOUS REACTORS FOR THE ENZYMATIC D-GLUCOSE OXIDATION

Mara CRIȘAN and Gheorghe MARIA*

Dept. of Chemical and Biochemical Engineering, Politehnica University of Bucharest, P.O. 35-107, Polizu Str. 1-7, 011061, Bucharest, Roumania

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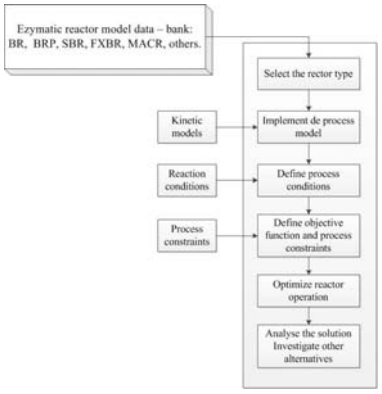
Due to the ongoing progress in the development of new enzymes and improvement of the enzyme activity, there is currently a major interest that provides successful applications in different areas of industrial biocatalysis and medicine. Multi-enzymatic reactions can successfully replace complex chemical syntheses, using milder reaction conditions, and generating less waste. The paper is focused on applying a modular screening procedure that can identify the optimal enzymatic reactor type and operating policies which minimize the enzyme consumption, given the process kinetic model, and an imposed production capacity. Exemplification is made for the case of the specific oxidation of mono- and disaccharides in the presence of pyranose oxidase (P2Ox) at C-2 position that leads to 2-keto-sugars. This reaction is actually of great interest for the industrial production of rare sugars or sugar derivatives starting from D-glucose. Specifically, the present study refers to the complex oxidation of D-glucose (DG) to 2-keto-D-glucose (kDG) and H_2O_2, in the presence of P2Ox and catalase (to prevent the earlier inactivation of P2Ox). The kDG can be further used for the production of fructose, manitol, and other derivatives.



INTRODUCTION

Over the last century a considerable progress in industrial enzyme technologies has been made. Before enzymes were accepted as alternative catalysts in the chemical/food industry, there were some technological advances of a major impact on enzymatic biotransformations, such as: the development of large-scale techniques for the release of enzymes from the microorganism cells; techniques for efficient stabilization of enzymes via their immobilization; biocatalysis in organic media; biosynthesis of enzymes with superior properties using "the recombinant DNA technology, which becomes part of industrial microbial transformations" [1]. All these recent improvements in the synthetic biotechnology and production of modified enzymes took place because enzymes exhibit a series of remarkable advantages and because nature provides a large number of enzyme resources. Furthermore, industrial enzymatic reactors are competing these days with those used for complex chemical synthesis, which often involve a large number of intermediate reaction steps and byproducts. The reason why enzymatic routes are a real competitor

* Corresponding author: gmaria99m@hotmail.com; website: https://sites.google.com/site/gheorghemariasite/

against classical chemical pathways is that enzymatic reactions display high selectivity and specificity, they consume less energy and generate less environmental pollution. All of these advantages have made enzymes very attractive for different practical applications in several domains such as: food, pharmaceutical, detergent, textile industry; environmental engineering; bio-renewable energy industries; biochemical synthesis; medical tests, bio-sensor production. However, there are some difficulties related to the industrial use of enzymes, related to their high costs, difficulties to produce stable enough enzymes immobilized on suitable supports; their high sensitivity to operating conditions; the low reproducibility of bioprocesses used to obtain the industrial enzymes, etc. In spite of that, the enzyme superior characteristics present a highly engineering interest for new process development.

This study is focused on one crucial engineering problem when developing a new and optimized multi-enzymatic process at an industrial scale, of known kinetics. Development of a sustainable biochemical process has to consider several aspects related to biocatalyst design, process integration and cost minimization. If the enzymatic process characteristics are known, then the problem is finding the optimal constructive and operating policy for the enzymatic reactor ensuring "the productivity maximization and cost reduction".[2] In most of cases, this is not an easy task because there are different operation modes, phases, constructive solutions, and complex optimization criteria (plant complexity and controllability, mass transfer resistance, enzyme stability, and sensitivity, plant flexibility), which have to be taken into consideration. The key point in screening among reactor alternatives and operating modes is the chemical stability of the enzymes and their deactivation characteristics. All of these aspects are related to the free or immobilized enzyme costs and the possibility to regenerate and reuse the enzyme. For multi-enzyme systems acting simultaneously, reactor optimization must account for interacting reactions, differences in enzyme optimal activity domains, and deactivation kinetics. The optimal operation mode turns into a difficult problem "to be solved for every particular system".[3]

Recently Maria[4] proposed a systematic computing methodology based on a modular simulation platform that is capable of optimizing and finally compare the performances of the main types of enzymatic reactors for a given enzymatic process of known kinetics (Fig. 1). By using ideal reactor models, this approach demonstrated that the right choice of the operating alternative could lead to a significant saving in the enzyme consumption, by preserving an imposed conversion and reactor productivity. The most significant advantage of the developed computing methodology is its adaptability. "The platform can basically offer a quickly adaptable optimal policy of the added enzyme/substrate during the process under several operating constraints".[4] Maria & Crișan[5] "extended application of such a systematic strategy by screening among optimized reactors for the case of a bi-enzymatic system of high complexity", that is the DG oxidation to kDG using P2Ox and catalase in the temperature range of 25 - 30°C.

The present paper aims at extending optimization of this bi-enzymatic process by using the modular simulation platform of Fig. 1 by checking different reactor alternatives at the optimal reaction temperature of 30°C only, with considering the P2Ox enzyme complex inactivation, and several simultaneous optimization objectives.

PROBLEM FORMULATION

Usually, the bi-enzymatic systems are more complex than the single-enzyme ones, due to interactions between reactions in which different enzymes are involved. In all cases, the multi-objective optimization problem can be formulated as follows: given the kinetic model of the process and the optimal running conditions for the enzymes (temperature, pH), determine what is the optimal reactor type and operating policy to get maximum reactor productivity (*e.g.* substrate conversion, 99% here), with a minimum enzyme consumption (P2Ox and catalase, here), over an imposed reaction time.

The considered enzymatic reactors in further model-based simulations and optimization are the following: simple batch (BR) with suspended P2Ox and catalase enzymes, batch with intermittent addition of one of enzymes (*i.e.* P2Ox here) following certain optimal feeding policies (BRP), semi-batch with optimal P2Ox enzyme feeding policy (SBR), fixed-bed (FXBR) or mechanically agitated continuous reactors (MACR) with co-immobilized enzymes (P2Ox and catalase). The process kinetic model and the rate constants are taken from literature[6,11] and are given in Table 1. The parameter values for several [Catalase]/[P2Ox] ratios are displayed in Table 1. For other ratios, the values can be easily obtained by simple interpolation. The differential mass balance of the BR, BRP, SBR, and MACR reactor models[5] are given in Tables 1 and 2.

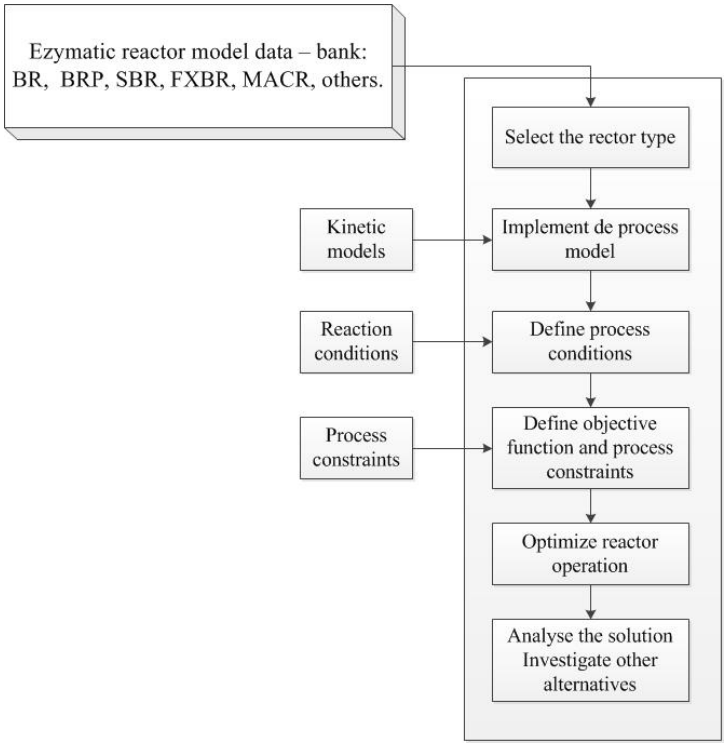


Fig. 1 – Modular platform for enzymatic reactor selection and optimization (adapted from Maria[4]).

From the mathematical point of view, the single objective (BR, BRP cases), or multi-objectives (SBR, MACR cases) reactor optimization problem consists in finding the manipulated variable (control) vector $\boldsymbol{u}$ of enzyme feeding policy to get an imposed DG conversion ($x_{f,imp}$= 0.99), with minimum enzyme consumption, for an imposed final batch (t_f = 10 hr, BRP, SBR), or residence time (V_L/f_o = 10 hr, MACR), and given the reactor volume V_L = 10 m^3, that is (see notations in the footnotes of Tables 1-2; "sup"= support; "in"=inlet; "inj"=injected; o= initial):

$\boldsymbol{u}$= argument of Min Ω; $\Omega = \{[E]_{sup,o}^{MACR}; [E]_{inj}^{BR}; [E]_{inj}^{BRP}; [E]_{inlet}^{SBR}\}$, $Max\, t_{onstream}^{MACR}$, and Max x_{DG} =0.99 at t_f; with a 0.0001 tolerance; (1)

where:

$\boldsymbol{u}^{BRP} = [V_{inj,1},..,V_{inj,Ninj},[E]_{inj}^{BRP}]$, for the BR ($N_{inj}$=1) and BRP case ($N_{inj}$=20);

$\boldsymbol{u}^{SBR} = [f_1,..,f_{Ndiv},[E]_{in}^{SBR}]$, for the SBR case;

$\boldsymbol{u}^{MACR} = \{f_1,...,f_{Ndiv}, [E_{sup,o}^{MACR}]\}$, for the MACR case ($N_{div}$=20);

subjected to the following constraints: $d[c,V]/dt = H(c,f,f,V,t)$ (dynamic process model); $[c,V](t_0) = [c_0,V_0]$ (initial conditions: $[DG]_o$= 0.25 M, oxygen saturation $[DO^*]$= 0.2484 mM); $[c,V_{inj,u},f_u] \geq 0$ (physical significance constraints); $\{[E]_{sup,o}, [E]_{inj}, [E]_{in}\} \in [[E]_{min}, [E]_{max}]$, (i.e. [0.1,5] U/g-support (MACR), or [0,500] U/mL (BR, BRP, SBR)); $[E]_{sup} \geq 0.1[E]_{sup,o}$, $[Catalase]/[E]_{sup,o} \in [0,300]$ (kinetic model validity range); [kDG] ≤ 1 M (MACR - due to product separation reasons); $f_u^{MACR} \in$ [0.5,5] m^3/h $\wedge$ $f_u^{MACR} \geq 0.9\, f_o$ (due to reactor productivity reasons[1]); maximum reactor content dilution of $\sum_u V_{inj,u} = 0.1V_0$ (BRP); $\int_0^{t_f} f\, dt = 0.1\, V_0$ (SBR); $t_{onstream}^{MACR}$ ≥ 2 days; minimum capacity of 360 t DG/yr; no. of injections per batch: BRP (N_{div} = 20); t_f = 10 hr, N_{inj} =20. (V_o = 10m^3).

The used notations are: $\boldsymbol{c}$ is the species concentration vector, $V_{inj,u}$ are the injected volumes of enzyme (E) solution over N_{inj} = 20 injections during the BRP batch at equal time-intervals; $f_u(t)$ are the feed flow rates of inlet substrate DG (MACR) or enzyme E (SBR) solution, over $u = 1,...,N_{div}$ equal time-intervals (SBR, MACR) (for MACR an adopted small Δt = 10 min is used to check the $f_u(t)$ constancy); ϕ is the model

parameter vector, DO is the dissolved oxygen; the indices are: 'o'=initial, 'in'=inlet, 'sup'= support, 'inj'= injected.

Optimization was performed by using a random search MMA optimization procedure[7,8] for single Ω objective (*i.e.* minimum P2Ox consumption for BRP, SBR), or a genetic algorithm optimization procedure[9] for two Ω objectives (i.e. minimum P2Ox consumption, with maximum time-on-stream for MACR).

RESULTS

1. Characteristics of the bi-enzymatic oxidation of D-glucose to keto-D-glucose

The approached DG oxidation to kDG is the first step of "the Cetus process for DG conversion to D-fructose of high purity".[10] The reaction occurs at 25-30°C and pH=6-7 using recombinant P2Ox (EC 1.1.3.10) from *Coriolus sp.*[10,11] with more than 99% DG conversion (xDG) and selectivity leading to kDG product of high purity and free of allergenic compounds. The process is still very costly because of the expensive and fast deactivating P2Ox due to the resulted H_2O_2. To prolong P2Ox life, catalase (EC 1.11.16, from bovine liver) has been added in large [Catalase]/[P2Ox] ratios (up to 300/1-1000/1 U/U) to decompose H_2O_2.[10,11]

For the investigated temperature of 30°C (optimal for P2Ox activity), Maria *et al.*[11] proposed a kinetic model accounting for three main reactions (displayed in Table 1), with the estimated rate constants correlated with the catalase / P2Ox ratio: The P2Ox deactivation by H_2O_2 is completed in this paper with the enzyme slow inherent degradation, and the H_2O_2 decomposition by catalase. In further calculations, no significant change in rate constants by the co-immobilization of P2Ox and catalase on porous gel beads will be assumed as reported by some literature results.[12]

Table 1

The kinetic model for DG oxidation using commercial P2Ox and catalase (pH= 6.5, 25-30°C, $[DG]_o$ = 0.1 M, $[P2Ox]_o$ = 0.25 U/mL, [Catalase]/[P2Ox]= 0-300 U/U), and the BR and BRP reactor models[5,6,11]

Reactions:	Rate expressions:
$DG + O_2 \xrightarrow[(water)]{P2Ox} kDG + H_2O_2$	$r_{our} = \dfrac{\mu_m c_E c_{DG} c_{DO}}{K_{DG} c_{DO} + K_{DO} c_{DG} + c_{DG} c_{DO}}$
$Y_E E + H_2O_2 \xrightarrow[(water)]{Fe\ traces} Eox$	$r_d = k_d c_E c_{H_2O_2}$, (Fe traces)
$H_2O_2 \xrightarrow[(water)]{catalase} H_2O + 0.5O_2$	$r_c = k_c c_{catalase} c_{H_2O_2} / (1 + K_{OH} c^n_{H_2O_2})$

Rate constants at 30°C [6,11]: n = 2.58; Y_{P2Ox} = 0.01019, (U mL^{-1} mM^{-1}); $k_{oxl}a$ = 0.01-0.02 (s^{-1}) (experimental); c^*_{DO} = 0.2484 mM (fed air, 30°C).

Parameter	[Catalase]/[P2Ox] (U U^{-1})		
	0	100	300
μ_m, [mM s^{-1} (U mL^{-1})$^{-1}$]	0.988	0.369	0.049
K_{DG}, (mM)	0.519		
K_{DO} (mM)	14.545		
k_d, [s^{-1} (U mL^{-1})$^{-1}$]	$1.42 \cdot 10^{-3}$	$6.07 \cdot 10^{-4}$	$1.15 \cdot 10^{-4}$
k_c, [s^{-1} (U mL^{-1})$^{-1}$]	-	$5.55 \cdot 10^{-5}$	10^{-7}
K_{OH}, (mM^{-n})	-	0.58	

BR and BRP models:

$$\frac{dc_{DO}}{dt} = k_{oxl} a (c^*_{DO} - c_{DO}) - Y_{ox} r_{our} + 0.5\, r_c - D\, c_{DO};$$

$$\frac{dc_{DG}}{dt} = -r_{our} - D\,c_{DG}\,;\quad \frac{dc_{E}}{dt} = -Y_E r_d - D\,c_{E}\,;$$
$$\frac{dc_{kDG}}{dt} = r_{our} - D\,c_{kDG}\,;\quad \frac{dc_{H_2O_2}}{dt} = r_{our} - r_d - r_c - D\,c_{H_2O_2}\,;\quad \frac{1}{V}\frac{dV}{dt} = D$$

For **BRP** the mass balance after each enzyme addition at times $t_{inj,u}$, $u = 1,...,N_{inj}$, is given by the mixing equations[5]:

$$\left(V/V_o\right)_{t_{inj,i+}} = 1 + \sum_{u=1}^{i}\left(V_{inj,u}/V_o\right);$$

$$c_j(t_{inj,u+}) = \left(1 - \frac{V_{inj,i}/V_o}{V/V_o}\right)c_j(t_{inj,u-}) + \frac{V_{inj,i}/V_o}{V/V_o}c_{j,inj}(t_{inj,u})\,;$$

$$\Delta t_{inj,u} = t_{inj,u+1} - t_{inj,u} = t_f/N_{inj}\,,\quad u = 1,...,(N_{inj}-1)\,.$$

For the enzyme uniform addition policy: $V_{inj,u}/V_o$, $u = 1,...,N_{inj}$:

$$\frac{V_{inj,u}}{V_o} = \frac{V_{inj,tot}}{V_o}\frac{1}{N_{inj}}\,;\; t_{inj,u} = \frac{t_f}{N_{inj}}(u-1)$$

Notations: c_j = species *j* concentration; c_j^* = saturation level; *D*= dilution rate; k_j, k_c, k_d, K_j = rate constants; $k_{oxl}a$ = gas-liquid mass transfer coefficient (0.04 s^{-1})[6,11]; N_{inj} = number of enzyme injections over the batch; r_j = species *j* reaction rate; *t* = time; Δt = time interval; *V* = liquid volume; Y_E, Y_{ox} = stoichiometric coefficients; Index: 'o'= initial; 'inj'=injected, 'f'=final batch. *Abbreviations:* BR= batch reactor; BRP= BR with intermittent addition of enzyme solution; DG = D-glucose; DO = dissolved oxygen, E = P2Ox = pyranose-oxidase; Eox = inactive form of E; kDG = 2-keto-D-glucose.

2. The mathematical models of the checked enzymatic reactors

Even if the simulation platform of Maria[4] includes the main types of enzymatic reactors, that is (Fig. 1): BR, BRP, SBR, FXBR, MACR, only the batch with intermittent addition of one of enzyme (P2Ox) following certain optimal policies (BRP), the semi-batch reactor (SBR) with continuous addition of the suspended enzyme solution (P2Ox and catalase), and the mechanically agitated continuous reactor (MACR) with co-immobilized P2Ox and catalase on porous alginate beads have been checked for determining the optimal process development alternative as being known to be more efficient in terms of enzyme consumption.

The BRP model (Table 1) corresponds to a perfectly mixed isothermal batch reactor, with intermittent addition of suspended enzyme P2Ox solution, of volumes $V_{inj,u}$ and concentration $c_{E,inj}$, over N_{inj} uniformly distributed addition times $t_{inj,u}$ during the batch. Parameters $\{V_{inj,u}, t_{inj,u}\}$ are evaluated to get imposed nominal conditions of 30°C, $[DG]_o$ = 0.25 M, pH = 6.5, $V_{inj,tot}$ =0.1 V_o (V_o = 10 m^3), x_{DG}= 0.99, batch time t_f = 10 hr, N_{inj} =20.

The SBR model (Table 2) is similar to BRP, except for the continuous addition of P2Ox solution over the batch, with an adjustable time step-wise feed flow rate f_u over $u = 1,...,N_{div}$ equal time-intervals $\Delta t = t_f/N_{div}$, determined to ensure optimal nominal conditions similar to BRP (N_{div} = 20).

The MACR ideal model (Table 2) corresponds to an isothermal, perfectly mixed reactor of constant volume (V_L = 10 m^3), with aeration and mechanical stirring, continuously fed with DG substrate solution (adjustable f_u^{MACR} to get a minimum capacity of 360 t DG/yr., with a fluid residence time of 10 hr.), and including suspended solid particles (spherical beads of 0.1 mm diameter, 1035 kg/m^3 density, and 0.5 porosity), with uniformly co-immobilized P2Ox and catalase. The enzyme initial load and feed flow rate are adjusted to get optimal conditions similar to BRP (the solid volumetric fraction in the MACR is 0.2). FXBR was not checked due to the very rapid P2Ox inactivation for a plug-flow fluid circulation[5].

Table 2

Semi-batch (SBR) and mechanically agitated continuous reactor (MACR) models with co-immobilized P2Ox and catalase on porous alginate beads[4]

SBR reactor model[(a)]:

$$\frac{dc_{DO}}{dt} = k_{oxl}a(c^{*}_{DO} - c_{DO}) + \frac{f(t)}{V(t)}(c_{j,in} - c_j) - Y_{ox}r_{our} + 0.5\,r_c - D\,c_{DO}$$

$$\frac{dc_j}{dt} = \frac{f(t)}{V(t)}(c_{j,in} - c_j) + r_j - D\,c_j \;, (j \neq DO)\;; \quad \frac{dV}{dt} = f(t)\;.$$

MACR reactor model[(a,b)]:

$$\frac{dc_{DO}}{dt} = k_{oxl}a\left(c^{*}_{DO} - c_{DO}\right) + \frac{f}{V}(c_{j,in} - c_j) - Y_{ox}r_{our} + 0.5\,r_{c.app}\;;$$

$$\frac{dc_j}{dt} = \frac{f}{V}(c_{j,in} - c_j) + r_{j,app}\;; (j \neq DO, E)\;;$$

$$\frac{dc_E}{dt} = -Y_E r_{d,app} - k_{Ld}\,c_E\;.$$

Apparent rate $r_{j,app}$ is the solution of the quasi-steady-state liquid-solid mass transfer relationships: $r_{j,app} = k_s a_s(c_j - c_{j,s}) = \eta_j\, r_j(c_{j,s})$.

Remarks: (a) At t=0, $c_j = c_{j,o}$, $V = V_o$; (b) Biocatalyst spherical particle effectiveness factors are calculated with the relationships: $r_{our} \approx \eta_{our}\frac{v_m c_{DG}}{K_m + c_{DG}}$; η_{our} (Thiele moduli for Michaelis-Menten rate[13,14]); $r_{d,app} = \eta_d r_d \approx \frac{c_{H_2O_2}}{1/k_s a_s + 1/\eta_d k_d c_{P2Ox}}$; η_d (Thiele moduli for pseudo-first order rate[13]); $r_{c,app} \approx \eta_c r_c$; $\eta_c \approx 1$ (adopted[5]); $k_s a_s$ is evaluated using criterial relationships[11]; adopted k_{Ld} = 9.8·10^{-7} 1/s (enzyme biodegradation constant[15]).

Notations (see also Table 1): f = enzyme solution feed flow rate; 'in' = inlet; 'app'= apparent; $k_s a_s$ = liquid-solid mass transfer coefficient (on liquid side); N_{div} = number of equal divisions of the batch/running time to implement the optimal step-wise feed flow rate policy $[f_1, f_2, ..., f_{N_{div}}]$; η_{our}, η_d, η_c = effectiveness factors of spherical porous support for the process reactions of Table 1.

DISCUSSION

The resulted BRP optimal policy represented in Fig. 2 indicates an increase in the required and recovered P2Ox amount with the catalase content in the reactor, up to an optimum net consumption of 2.3 MU/hr batch for [Catalase] = 40 U/mL. However, this uniform intermittent addition of P2Ox does not efficiently use the second half of the batch time when no much increase in conversion is achieved. Finally, 85% of enzyme (Fig. 5) is still active and can be recovered.

The SBR optimal feeding policy of P2Ox solution is derived for getting the minimum enzyme consumption over the batch (Fig. 3). The enzyme utilization is better than for BRP; however, only 62% of the fed P2Ox can be finally recovered (Fig. 5).

For the MACR case, minimization of the initially P2Ox load on support, concomitantly with maximization of the running time until the constraints of (1) is done until certain constraints are violated (e.g. [kDG] > 1 M). Finally, the obtained optimal operating policy displayed in Fig. 4 indicates that larger MACR productivities require larger P2Ox and catalase consumption, but will shorten the running time, which in turn will increase the operating costs.

A comparison of the required P2Ox enzyme, and the residual P2Ox activity at the end of the batch for different operating alternatives is given in Fig. 5. The plots reveal that the MACR operation is the most efficient, by consuming 143 times less enzyme than BRP or SBR, even if the recovering possibilities are modest (only 50% of initial P2Ox comparatively to 85% for BRP).

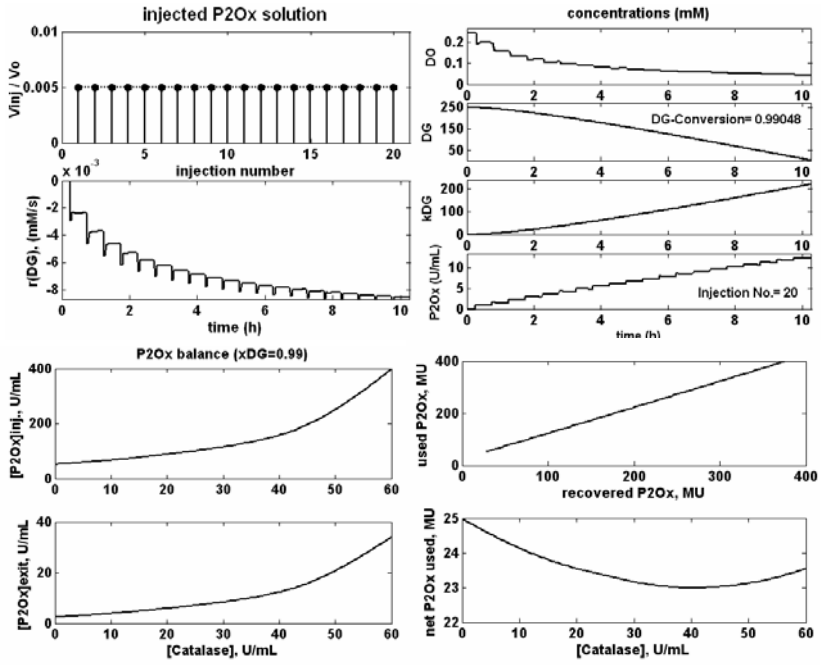


Fig. 2 – Feeding policy and conversion rate (up-left), species conc. dynamics (up-right), injected and final P2Ox enzyme (down-left), total and net P2Ox consumption (down-right) in the **BRP** for a uniform P2Ox solution addition policy, and a final x_{DG}= 0.99 over 10 hr batch (30°C, $[DG]_o$ = 0.25 M, pH = 6.5, optimal setpoint of $[P2Ox]_{inj}$ = 158.5 U/mL, 20 P2Ox injections, [Catalase] = 40 U/mL).

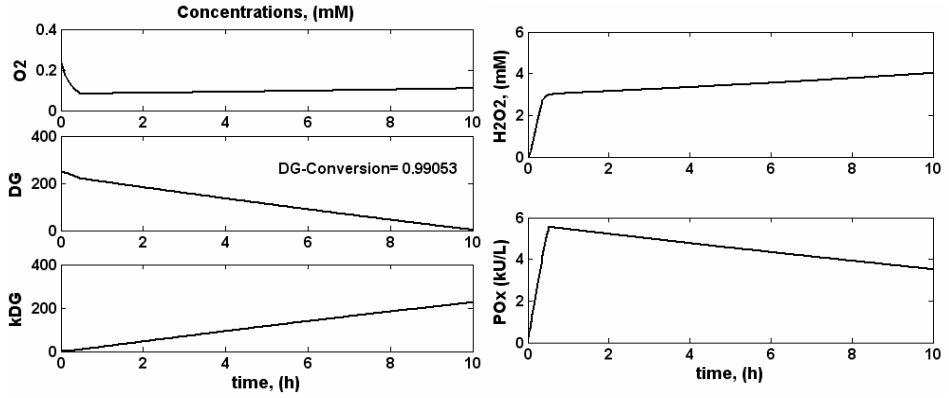


Fig. 3 – Key species concentration dynamics in the **SBR** for an optimized feeding policy with P2Ox solution (*vs.* minimum amount of added P2Ox), for an imposed x_{DG}= 0.99 over 10 hr batch (30°C, $[DG]_o$ = 0.25 M, pH = 6.5, optimal setpoint of $[P2Ox]_{inj}$ = 62 U/mL, [Catalase] = 40 U/mL).

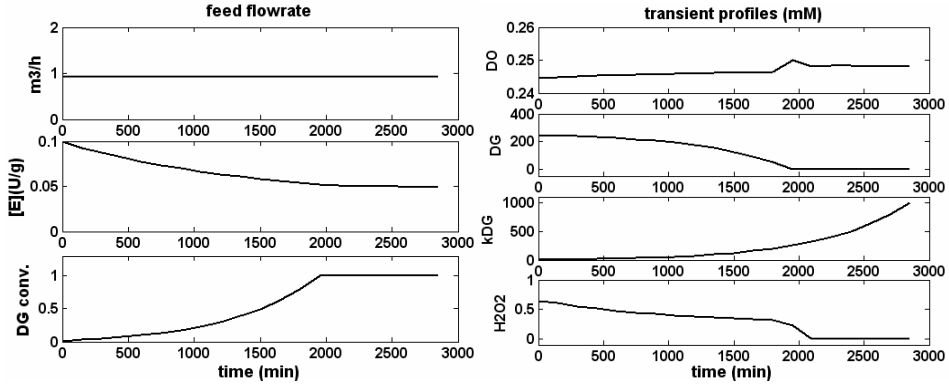


Fig. 4 – **MACR** feeding policy over time, xDG, enzyme P2Ox dynamics (left), and species conc. dynamics (right) for a variable feeding policy with DG solution to ensure a quasi-constant output xDG= 0.99 for 10 hr residence time (30°C, $[DG]_o$ = 0.25 M, pH = 6.5, stopped operation for [E] < 0.1 $[E]_{sup,o}$, or [kDG] > 1 M; optimal setpoint: f_o = 0.9125 m3/h, $[P2Ox]_o$ = 0.1 U/g-supp, $[Catalase]/[P2Ox]_o$ = 300).

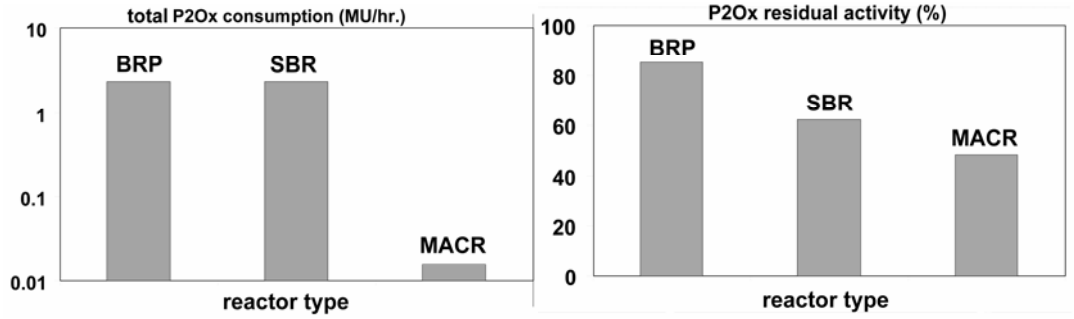


Fig. 5 – P2Ox enzyme consumption in various operating alternatives (left), and residual P2Ox activity at the end of each batch (right).

CONCLUSIONS

This study has demonstrated that, if the process kinetics and enzyme deactivation characteristics are specified, then by using a modular reactor simulation platform a large number of possibilities can be obtained in a systematic way by assessing the operating alternatives for different enzymatic reactors. The employed comparative computational approach allows a quick selection of reactor type and design solution based on a direct comparison of optimized operating policies with an imposed criterion. It can also determine the optimal operating policy *vs.* imposed performances, but most importantly, the operation can be quickly adapted according to process variable characteristics in order to obtain a satisfactory productivity *vs.* cost trade-off.

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ABBREVIATIONS

BR = simple batch;
BRP = batch with intermittent addition of enzyme following certain optimal policies;
DG = D-glucose;
DO = dissolved oxygen;
FXBR = fixed-bed reactor with immobilized enzymes on support (column reactor), continuously operated;
kDG = 2-keto-D-glucose;
MACR = mechanically agitated continuous reactors with immobilized enzyme, continuously operated;
P2Ox = pyranose-oxidase;
SBR = semi-batch with uniform or optimal enzyme feeding policy.

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