Modular Simulation to Determine the Optimal Operating Policy of a Batch Reactor for the Enzymatic Fructose Reduction to Mannitol with the *in situ* Continuous Enzymatic Regeneration of the NAD Cofactor

MARA CRISAN, GHEORGHE MARIA*
Politehnica University of Bucharest, Department of Chemical and Biochemical Engineering, 1-7 Polizu Str., 011061, Bucharest, Romania

*Novel coupled enzymatic systems reported important applications in the industrial bio-catalysis. Multi-enzymatic reactions can successfully replace complex chemical syntheses, using milder reaction conditions, and generating less waste. For such systems acting simultaneously, the model-based engineering calculations (design, reactor operation optimization) are difficult tasks, because they must account for interacting reactions, differences in enzymes optimal activity domains and deactivation kinetics. The determination of the optimal operating mode (enzyme ratios, enzyme feeding policy, temperature, pH) often turns into a difficult multi-objective optimization problem with multiple constraints to be solved for every particular system. The paper focuses on applying a modular screening procedure that can identify the optimal operating policy of an enzymatic reactor, which minimizes the enzyme consumption, given the process kinetic model, and an imposed production capacity. Following an optimization procedure, the process effectiveness is evaluated in a systematic approach, by including simple batch reactor (**BR**), batch with intermittent addition of the key-enzyme following certain optimal policies (**BRP**). Exemplification is made for the case of the enzymatic reduction of D-fructose to mannitol by using suspended MDH (mannitol dehydrogenase) and NADH (Nicotinamide adenine dinucleotide) cofactor, with the in-situ continuous regeneration of the cofactor by the expense of formate degradation in the presence of suspended FDH (Formate dehydrogenase).*

Keywords: D-fructose reduction; Enzymatic reactor optimization; D-Mannitol; Mannitol dehydrogenase; Formate dehydrogenase; Coenzyme regeneration

Remarkable progresses made in the development of new enzymes and in realizing complex coupled enzymatic systems, able to in-situ recover the main reaction cofactor(s), reported important applications in the industrial biocatalysis, presenting important advantages. Thus, multi-enzymatic reactions can successful replace complex chemical syntheses, using milder reaction conditions, and generating less waste. Multi-enzymatic systems with parallel or sequential reactions are successfully applied for [1]: recovering the main reaction co-factor, shift equilibrium of the main reaction, remove the excess of one product, etc.

Even if the multi-enzymatic systems are advantageous, the engineering part when developing such a process is not an easy task because it must account for the interacting reactions, differences in enzymes optimal activity domains and deactivation kinetics, the presence of multiple and often contrary objectives, technological constraints, and an important degree of uncertainty coming from multiple sources: model inaccuracies, constraint uncertainty, presence of disturbances in the operating parameters and nonlinear process dynamics. The determination of the optimal operating mode (enzyme ratios, enzyme feeding policy, temperature, pH) turns into a difficult multi-objective optimization problem with multiple constraints to be solved for every particular system [2]. The problem to be solved is the model-based selection of the best reactor type, and its optimal operation mode, allowing the best use of the enzyme (free vs. immobilized) leading to screening among various reactor types, that is: simple batch (**BR**), batch with intermittent addition of enzyme following certain optimal policies (**BRP**), and others [3-5].

In the both suspended or immobilized enzyme operation alternatives, all engineering calculations are tremendously facilitated if a simple and adequate kinetic model of the process is available. This model, developed on an experimental basis is used: i) in the design stage to decide (on a cost basis) on the optimal choice of the most suitable reactor type (BR, BRP, SBR, FXBR, or MACR), and ii) in the reactor operation stage to determine (on a cost/productivity basis) the optimal operating policy [4,6]. As some examples, it is to mention the large number of biosynthesis processes used to produce fine-chemicals, or organic compounds in food, pharmaceutical, or detergent industry, such as: the production of monosaccharide derivatives, organic acids, alcohols, amino-acids, etc., by using single- or multi-enzymatic reactors [7,8].

To facilitate the evaluation of the reactor operation alternatives, Maria [3] proposed a computational modular platform (fig. 1) that allows simulating and comparing the optimal operating policies of various enzymatic reactors in respect to certain formulated objectives. The essential part of such calculations is the availability of a simple but adequate kinetic model of the enzymatic main process and also for the enzyme deactivation. A weak model adequacy (especially of the enzyme deactivation model) may lead to wrong conclusions / decisions in both reactor design or operation stages. One of the major advantages of this simulation platform is the possibility to indicate the best operating alternative and its limits of efficiency as the process or the enzyme characteristics change.

It is well documented in the literature [3,4,6,9] that the use of simplified models for enzyme deactivation (like the *default* 1-st-order [10]), for multi-enzymatic systems in the engineering calculations involves a high cost, leading to

* email: gmaria99m@hotmail.com

important negative consequences, namely inadequate process design solutions, and/or sub-optimal reactor operating policies. For instance, while for a first-order enzyme deactivation process the optimal operating batch alternative is to add the whole substrate and enzyme at the beginning of the batch, for a higher-order deactivation or for bi-enzymatic cases, it was proved that a semi-continuous operation (SBR), or a BRP operation with addition of the key-enzyme following a certain policy can be more advantageous.

This paper is aiming at determining the optimal operating alternative of a batch reactor (BR, or BRP operation) for a complex bi-enzymatic system with suspended enzymes and cofactor regeneration. Exemplification is made for the case of the enzymatic reduction of D-fructose to mannitol by using suspended MDH (mannitol dehydrogenase) and NADH (Nicotinamide adenine dinucleotide) cofactor, with the in-situ continuous regeneration of the cofactor by the expense of formate degradation in the presence of suspended FDH (Formate dehydrogenase).

Mannitol is a natural hexitol with important applications in medicine and food industry. It is produced on an industrial scale, ca. 50,000 tons/year being currently produced by the chemical hydrogenation alone around the world via hydrogenation of 50% fructose/50% glucose syrup with a high cost at high pressures and temperatures using a Raney nickel catalyst [11]. Mannitol has found extensive applications in the food and pharmaceutical industry due to some other favorable known properties. This sugar alcohol is best known for its sweet taste, about half as sweet as sucrose being not metabolized by humans, and having a low caloric value (1.9 kcal g^{-1}). Over the last decades, several production alternatives have been developed [11,14,15]:

i) The simplest but the most expensive is its direct extraction from plants.

ii) Nowadays mannitol is primarily produced by catalytic hydrogenation of fructose, sucrose or high fructose corn syrup (fructose/glucose mixture) by using a high-pressure catalytic reaction using nickel as a catalyst. Because only á-fructose is converted to mannitol, while â-fructose is converted to sorbitol, the process presents a poor selectivity of only 25%wt in mannitol. At this point the production costs are high due to the severe reaction conditions, and because it is relatively difficult to separate mannitol from sorbitol. If sucrose is used instead of the glucose/fructose mixture, and the pH is alkaline during the hydrogenation, then higher yields of mannitol can be obtained (aprox. 30%) [15]. However, the process is still expensive because of the high pressure of ca. 50 atm used because hydrogen has an extremely low solubility in water even at high pressures [16]. Some improvements have been reported when using Ni with Cu/silica catalysts [17].

iii) Combinations of glucose isomerase immobilized on silica and copper-on-silica-catalysts reported good yields (62-66%) under slight milder conditions (buffer of *p*H~ 7, 70°C, 50 atm [18]).

iv) One attractive and economic alternative is the biological production of mannitol by fermentation using lactic acid bacteria, yeasts, and fungi, with important advantages: good yields, less by-products, no need of ultra-pure, and expensive raw materials. The process occurs with a high selectivity (with no sorbitol formation), while the used living cell cultures possess their own *built-in* cofactor regeneration machinery. However, the process

Table 1

THE NOMINAL REACTION CONDITIONS FOR THE ENZYMATIC REDUCTION OF D-FRUCTOSE TO MANNITOL*.

Parameter	***Value***
Temperature	25°C
Pressure / pH (buffer solution)	Normal / 7
Molar initial concentrations	
Fructose, $[F]_o$ (note a)	0.1 M
$[NADH]_o$	0.0002 M
Formate $[HCOO]_o$	0.1 M
Others: $[NAD]_o = [M]_o = [CO2]_o = 0$	none
[CO2] saturation level at 25°C and pH= 7	CO2*= 0.0313 M [25,26]
Volumetric dilution of the reactor content due to the addition of the enzyme solution (note b)	10% V_o
Reaction time (BR, BRP, SBR)	48 hrs.
Initial FDH (referred to the reactor volume)	0.1 kU/L, (to be optimized)
Injected MDH (in the injected solution)	10 kU/L, (to be optimized)
Number of enzyme additions over the batch time (N_{inj})	1 (**BR**); 20 (**BRP**)

** Using MDH (mannitol dehydrogenase) and NADH cofactor, with the in-situ continuous regeneration of the cofactor by the expense of formate degradation in the presence of FDH (Formate dehydrogenase). These conditions are those used in the batch tests of Slatner [21]. The used FDH (EC 1.2.1.2) from Candida boidinii has a specific NAD-dependent activity of 2.4 U/mg, measured at 25°C and pH 7.0. The MDH (EC 1.1.1.67) from Pseudomonas fluorescens DSM 50106 was over-expressed in E. coli JM 109. The NADH-dependent FDH and MDH typical activity in D-fructose reduction is of 1-2 U/mL, that is 1-2 kU/L.*

(a) Higher initial concentrations of fructose are possible, but have not been checked due to the kinetic model validity range and due to higher amounts of required co-factor and formate (see also Slatner [21] for an extensive discussion).

(b) The injected enzyme solution volume is maximum 10% of the reactor liquid initial volume V_o.

suffers of some important disadvantages, that is: a low volumetric productivity and high costs achieved with yeasts and filamentous fungi, and a too high variability of product quality due to the variability of the cell culture characteristics (bacteria or fungi) from batch to batch, leading to by-product formation (lactic or gluconic acid) [19].

v) A modern and very attractive alternative and of high productivity is the enzymatic production of mannitol. The first enzymatic alternative was proposed by Kulbe [20] by using a cheap fructose/glucose substrate mixture with combined purified enzymes to produce D-mannitol and D-gluconic acid. The coupled enzyme system consisted of NADH - dependent MDH and NAD-dependent glucose dehydrogenase. However, the achieved productivity was lower than those achieved through fermentation. Another alternative was proposed by Slatner [21]. A higher productivity was achieved using only D-Fructose as substrate. The enzymatic reduction of D-fructose to D-mannitol is made using MDH (mannitol dehydrogenase) by using NADH as cofactor, with the in-situ continuous regeneration of the cofactor by the expense of formate degradation in the presence of FDH (Formate dehydrogenase). The advantage of using NADH cofactor is that it is relatively cheap [22], and much more stable than the NADPH [23].

Even if the reactions occur under mild conditions (*p*H = 7. 25°C), the engineering part approached in the present paper is a difficult task, because it has to cope with two problems: minimize the consumption of the costly enzymes, and efficiently couple the main reaction with the quick regeneration of the exhausted cofactor NAD^+ .

Theory and calculation

The modified kinetic model of the bi-enzymatic reduction of D-fructose to mannitol with the in-situ regeneration of the NADH cofactor by the expense of formate ($HCOO^-$) decomposition in the presence of FDH

The studied process involves two concomitant interfering reactions (table 2): i) the enzymatic reduction of D-fructose to mannitol by using suspended MDH (mannitol dehydrogenase) and NADH cofactor, and ii) the in-situ continuous regeneration of the NADH by means of the reaction of NAD^+ with the (initially added) formate in the presence of suspended FDH (Formate dehydrogenase).

Based on experimental data collected at 25°C and *p*H 7.0, Slatner [21] proposed a Haldane kinetic model with incompetitive inhibition in respect to reactants even if the mannitol inhibition might be significant. For the concomitant reaction for recovering NADH from NAD^+ by the expense of formate degradation in the presence of suspended FDH (table 2), a simple Michaelis-Menten kinetics was suggested.

In the present study, because in the main reaction, the fructose concentration is relatively small, while the quick recovering of NADH is maintaining a high NADH level during the batch, the main reaction rate R1 inhibition with NADH was considered. For the NADH regeneration reaction, only inhibition with NAD^+ was taken into account. The enzymes MDH and FDH inactivation during the reaction has been neglected due to lack of available data.

Due to such assumptions, some the rate constants suggested by Slatner [21] under the nominal conditions of table 1 have been re-estimated to match the experimental data / kinetic curves given by Slatner [21], that is; the turnover numbers kc1= kc2, KM1, and K_i. The values of the rate constants are given in table 2.

The mathematical models of the checked enzymatic reactors

For the analyzed bi-enzymatic process with in-situ cofactor regeneration, an inspection of the reactor operation alternatives of the simulation platform of Maria [3] in figure 1, indicates as being suitable to be tested only the batch reactor in both simple operation **BR** (initial addition of enzymes and substrates), or **BRP** with intermittent addition of the key-enzyme (MDH) following a certain optimal policy to be determined, and for an optimal initially added amounts of FDH to be determined.

The **BR** and **BRP** used models, presented in table 3, correspond to a perfectly mixed isothermal **BR**. In the **BRP** case the mass balance accounts for an intermittent addition

Reactions:	***Rate expressions:***
$F + NADH\ (+H^+) \xrightarrow[(kc1, KM1, Ki)]{MDH} M + NAD^+$	$R1 = \frac{kc1 \cdot C_{MDH} \cdot C_F \cdot C_{NADH}}{KM1 + C_{NADH} + C_{NADH}^2 / Ki}$
$HCOO^- + NAD^+ \xrightarrow[(kc2, KM2)]{FDH} CO_2 + NADH$	$R2 = \frac{kc2 \cdot C_{FDH} \cdot C_{HCOO} \cdot [NAD^+]}{KM2 + [NAD^+]}$
$\frac{dc_F}{dt} = -R1\ ;\ \frac{dc_{NADH}}{dt} = -R1+R2$ $\frac{dc_{NAD}}{dt} = +R1-R2\ ;\ \frac{dc_{HCOO}}{dt} = -R2$ $\frac{dc_M}{dt} = +R1\ ;\ \frac{dc_{CO2}}{dt} = +R2$	
Rate constants at 25°C and pH= 7 are estimated to match the experimental data/ kinetic curves of Slatner [21]: $kc1 = kc2 = 3 \times 10^{-3}$; 1/h//(U/L) $KM1 = 25 \times 10^{-3}$ M $KM2 = 1.5 \times 10^{-4}$ M $Ki = 1.4$ M	
Notations: k_j , K_j = rate constants; r_j = species *j* reaction rate. *Abbreviations:* F= D-fructose, NADH= Nicotinamide adenine dinucleotide, NAD+= Nicotinamide adenine dinucleotide (oxidized form), HCOO- = formate, M=Mannitol, MDH= mannitol dehydrogenase, FDH= Formate dehydrogenase.	
* Using MDH (mannitol dehydrogenase) and NADH cofactor, with the in-situ continuous regeneration of the cofactor by the expense of formate degradation in the presence of FDH (Formate dehydrogenase). The rate constants are, with some exceptions, those suggested by Slatner [21] under the nominal conditions of Table 1.	

Table 2
MODIFIED KINETIC MODEL FOR THE ENZYMATIC REDUCTION OF D-FRUCTOSE TO MANNITOL*

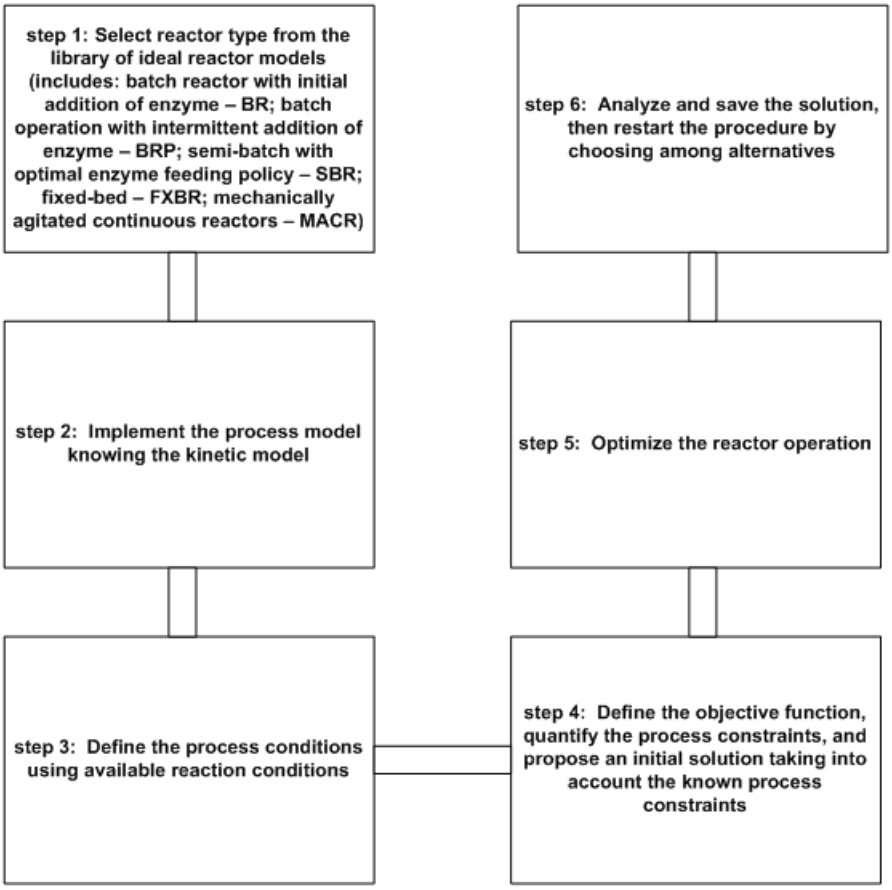

Fig. 1. Modular platform for enzymatic reactor selection and optimization (adapted from Maria [3])

of suspended MDH enzyme solution of volumes $V_{inj,u}$ and concentration $c_{E,inj}$, over N_{inj} uniformly distributed addition times $t_{inj,u}$ during the batch. The **BRP** alternative is more flexible, also allowing a linearly increasing/decreasing injected volumes $V_{inj,u}$ policy during the batch, with $V_{inj,u}$ determined with the relationships of table 3 to ensure the maximum of the substrate conversion ($x_F(t_f)$). If the parameter N_{inj} is adopted, and $t_{inj,u}$ is defined by table 3 relationships, then the parameters $\{c_{MDH,inj}, c_{FDH,o}, V_{inj,u}\}$ have to be determined in respect to a defined objective criterion (if FDH is added at the beginning of the batch with $c_{FDH,o}$).

Reactor optimal operation problem formulation

The engineering part of the approached bi-enzymatic process of D-fructose reduction to mannitol by using suspended MDH and NADH, with the in-situ continuous recover of the NADH by the expense of formate degradation in the presence of suspended FDH, has to solve two essential aspects:

i)determine the operating conditions leading to preserve a maximum reactor productivity with a minimum enzyme consumption and,

ii)determine the MDH and FDH initial/injected concentrations (ratio) leading to a best connection of the two enzymatic reactions to ensure a quick regeneration of the exhausted cofactor NAD^+ thus preserving a high reaction rate of the main reaction (fructose reduction).

Three batch-operating alternatives have been checked in this study:

1)the *simple batch* **BR** with initially added substrate (fructose), NADH, formate, and FDH. The initial concentration of FDH and MDH has to be determined from optimizing a certain criterion.

2)A modified batch **BRP** with an *UNIFORM addition* of MDH with initially added substrate (fructose), NADH, formate, and FDH. The MDH solution is evenly added during the batch at equal time intervals $\Delta t_{inj,u} = t_{inj,u+1} - t_{inj,u} = t_f / N_{inj,u}$, u=1, ...,($N_{inj-1}$) and in equal volumes .

The initial concentration $[FDH]_o$, and of the injected $[MDH]_{inj}$ concentration have to be determined from optimizing a certain criterion.

3)A modified batch **BRP** (with an *LINEARLY increasing addition* of MDH) with initially added substrate (fructose), NADH, formate, and FDH. The MDH solution is added during the batch at equal time intervals $t_{inj,u} = t_f(u-1) / N_{inj}$, u=1,...,$N_{inj,}$ but in uneven linearly increased volumes $v_{inj,u}$ determined with the relationships of table 3 to ensure maximum of substrate conversion ($x_F(t_f)$). The initial concentration $[FDH]_o$, and the injected $[MDH]_{inj}$ concentration have to be determined from optimizing a certain criterion. Only the increasingly enzyme addition policy was tested here.

In all three alternatives, the total added MDH-enzyme solution volume $V_{inj,tot}$ is maximum 10% of the initial reactor volume to not dilute too much the reactor content.

To formulate the problem in mathematical terms, let us assume that the parameter N_{inj} is adopted, and FDH is added at the beginning of the batch with $c_{FDH,o}$ then, if the batch time t_f is defined, the injection times of the MDH solution is $t_{inf,1} = 0$ for the **BR** case, and $t_{inj,u}$ computed with table 3 relationships for the **BRP** case (with an uniform or linearly increasing added enzyme volume policy), then the parameters $\{c_{MDH,inj}, c_{FDH,o}, V_{inj,u}\}$ have to be determined in respect to a defined objective criterion.

Such a bi-enzymatic system is more complex than a single-enzyme one, due to interactions between the reactions in which the two enzymes are involved. 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, *p*H), determine what is the optimal operating policy of the batch reactor (that is simple batch **BR** with initial addition of FDH and MDH**, BRP** with an *UNIFORM addition* of MDH, or **BRP** with an *LINEARLY increasing addition* of MDH) to get the maximum reactor productivity (an imposed substrate F conversion of 99% here), with a minimum

Table 3

THE IDEAL MODELS FOR THE BATCH **(BR)**, AND **BRP** ENZYMATIC REACTORS (AFTER MARIA [3])

$\frac{dc_j}{dt} = \sum_{i=1}^{n_r} \nu_{ij} r_i$; j= species index (for both **BR** and **BRP** enzymatic reactors)

$\frac{dc_E}{dt} = 0$ (negligible inactivation of MDH and FDH); *E*= enzymes (MDH and FDH);

If $c_{CO_2} > c^*_{CO_2}$, then c_{CO_2} in the liquid is $c^*_{CO_2}$.

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

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

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

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

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

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

For the ***linearly variation*** of the added volume, $V_{inj,u} / V_o$, $u = 1,..., N_{inj}$; $N_{inj} > 1$

$$\frac{V_{inj,u}}{V_o} = \frac{a}{V_o} + s\frac{b}{V_o}(u-1); \; t_{inj,u} = \frac{t_f}{N_{inj}}(u-1);$$

$s = +/-1$ increasingly/ decreasingly volume addition policy.

Constant ***a*** is determined with the relationship: $0 < \frac{a}{V_o} < \frac{V_{inj,tot}}{V_o} \frac{1}{N_{inj}}$,

such that to ensure maximum of substrate conversion ($x_F(t_f)$);

Constant ***b*** results from: $\frac{b}{V_o} = \frac{2}{N_{inj}(N_{inj}-1)}\left[\frac{V_{inj,tot}}{V_o} - \frac{a}{V_o} N_{inj}\right]$

For **BR** $N_{inj} = 1$. The volume dilution, and enzyme overall balance are:

$$\sum_{u=1}^{N_{inj}} V_{inj,u} = V_{inj,tot} = 0.1\, V_{batch,o}; \quad \sum_{u=1}^{N_{inj}} m_{E,inj,u} = m_{E,tot} = V_{inj,tot} c_{E,inj}$$

Notations: c*j = saturation level; N_{inj} = no. of enzyme (*MDH*) injections over the batch; Δt= time interval; Index: o= initial; inj=injected, f=final batch, tot=total, E = enzymes

enzyme consumption (FDH and MDH here), over an imposed reaction time.

From the mathematical point of view, the multi-objectives reactor optimization problem (multiple optimization objectives, often contrary) consists in finding the manipulated variable (control) vector of enzyme feeding policy to get an imposed fructose conversion ($x_{F,f}$ = 0.99), with a minimum enzyme FDH and MDH consumption, for an imposed final batch (t_f = 48 h), and given the reactor volume maximum dilution by the enzyme solution of $0.1V_o$, that is (see notations in the footnote of table 3):

$\hat{u}$ = Argument of {Min Ω1∧ Min Ω2∧ $\Omega Max_{xF,f}$ = 0.99 with a 0.0001 tolerance}with:Ω1=$[MDH]_{inj}$ (in the injected solution in **BR**, or during **BRP**); ΩW2=$[FDH]_o$ (initially added in **BR**, or in **BRP**) (1)

where:

$\hat{u}$= $[V_{inj,1},...V_{inj,Ninj},[MDH]_{inj,}$ $[FDH]_o]$, for the **BR** (N_{inj}=1) and **BRP** case (N_{inj}=20); subjected to the following constraints: d[c, V]/ dt = H(c,V,θ,t) (dynamic process model); $[c,V](t_o) = [c_o, V_o]$ (initial conditions of table 1); $[c,c_{inj,u}, V_{inj,u}] \geq 0$ (physicalsignificance constraints); $0 \leq [MDH]_{inj} \leq 30$ kU/L; $0 \leq [FDH]_o] \leq 1\, kU/L$ (kinetic model validity range); maximum reactor content dilution of $V_{inj,tot} = \sum_u V_{inj,u} = 0.1\, V_0$ (**BR** and **BRP**); no. of injections per batch: BRP N_{inj}=20 (2)

The used notations are: ***c*** is the species concentration vector, $V_{inj,u}$ are the injected volumes of enzyme (MDH) solution over N_{inj}= 20 injections during the **BRP** batch at

equal time-intervals; θ is the model parameter vector, the indices are: *in*=inlet; *inj* =injected; o= initial; *tot*=total.

This multi-objective problem can be solved by means of various algorithms. The most common, but not the most effective, is the so-called *weighting function method* [24]. The method consists in summation of the chosen objectives multiplied by suitable weights (w). The chosen weighting factors $w_J \in [0,1]$ associated to the objective functions are chosen depending on the relative importance given to each objective. This rule is very subjective and questionable.

Instead, we have applied a much simpler procedure, that is a simple heuristic exhaustive search method of optimal $[MDH]_{inj}$ and $[FDH]_o$ in the assumed ranges of constraint (2). For every tried $[FDH]_o$, the optimum $MDH]_{oinj}$ is searched such that Max $x_{F,f} = 0.99$ to be obtained under constraints (2). Local optima of the hybrid objective (1) are possible, but the entire inspection of the searching interval for $[MDH]_{inj}$ and $[FDH]_o$, with a small step, ensures localization of the global optimum.

Results and discussions

The solution of the optimization problem (1-2) for the simple batch BR case is presented in figure 2, while for the modified batch BRP (with an UNIFORM addition of MDH, the results are presented in figure 3, while for the modified batch BRP (with an LINEARLY increasing addition of MDH, the results are presented in figure 4. From the comparative analysis of these results several conclusions can be derived, as follows:

i)as expected in all three cases (the up plots), the required MDH optimal concentration in the injected solution in the batch reactor exponentially decreases with the increase on the FDH initial concentration (for getting 99%

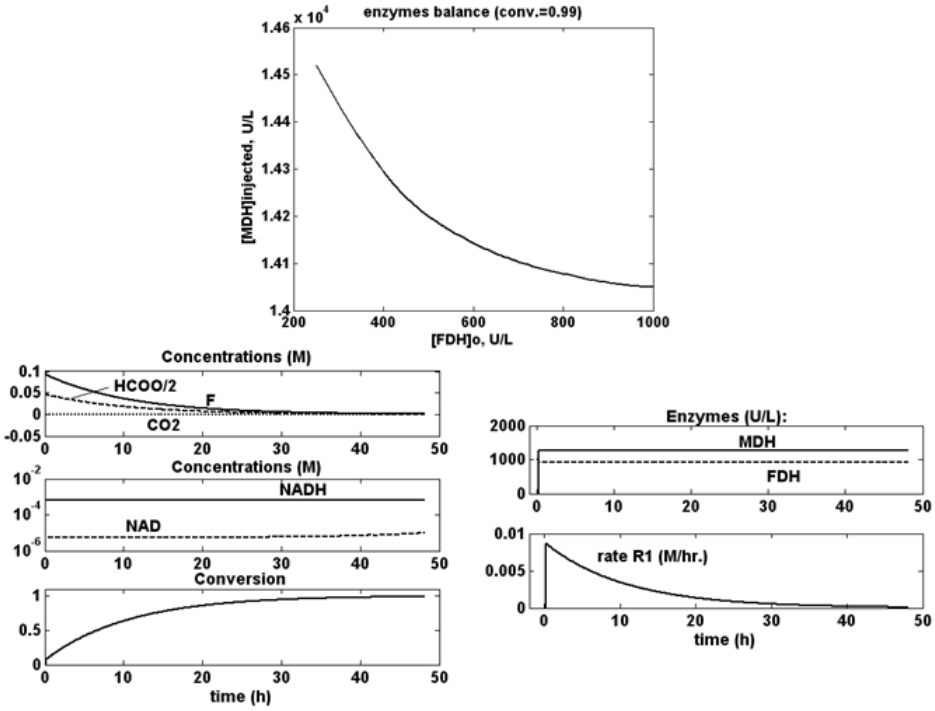

Fig. 2. (Up) Optimal feedings with MDH (concentrations before injection and dilution) for various FDH initial concentrations for getting an imposed fructose conversion of 0.99. (Down) Species concentrations, and the reaction rate R1 (table 2) dynamics for the BR with the optimal setpoint of: $[FDH]_o$ = 1 kU/L (referred to the reactor volume), and $[MDH]_{o.}$ = 14 kU/L (concentration before injection and dilution) to get the final imposed conversion x_F = 0.99, with Ninj=1, over 48 hrs. of batch under the reaction conditions of Table 1. Transformation of fructose (F) in mannitol (M) is quantitative so, $[M] = [F]_o - [F]$ at any moment

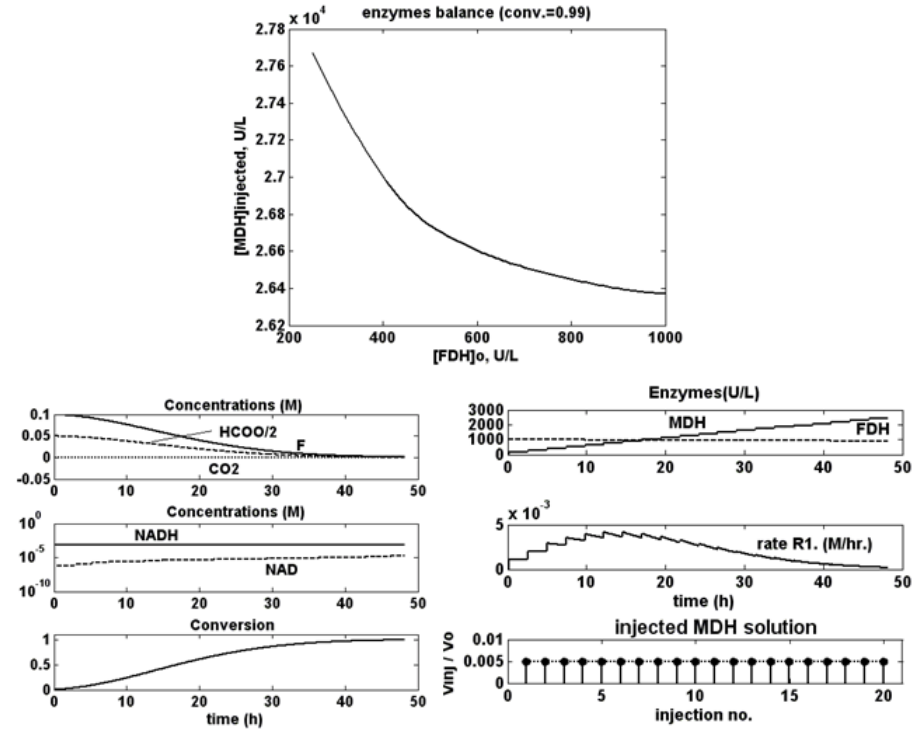

Fig. 3. (Up) Optimal feedings with MDH (concentrations before injection and dilution) for various FDH initial concentrations for getting an imposed fructose conversion of 0.99. (Down) Species concentrations, and the reaction rate R1 (table 2) dynamics for the BRP with an UNIFORM addition of MDH for the optimal setpoint of: $[FDH]_o$ = 1 kU/L (referred to the reactor volume), and $[MDH]_{o.}$ = 26.3 kU/L (concentration before injection and dilution) to get the final imposed conversion x_F = 0.99, with Ninj=20, over 48 hrs. of batch under the reaction conditions of table 1. Transformation of fructose (F) in mannitol (M) is quantitative so, $[M] = [F]_o - [F]$ at any moment.

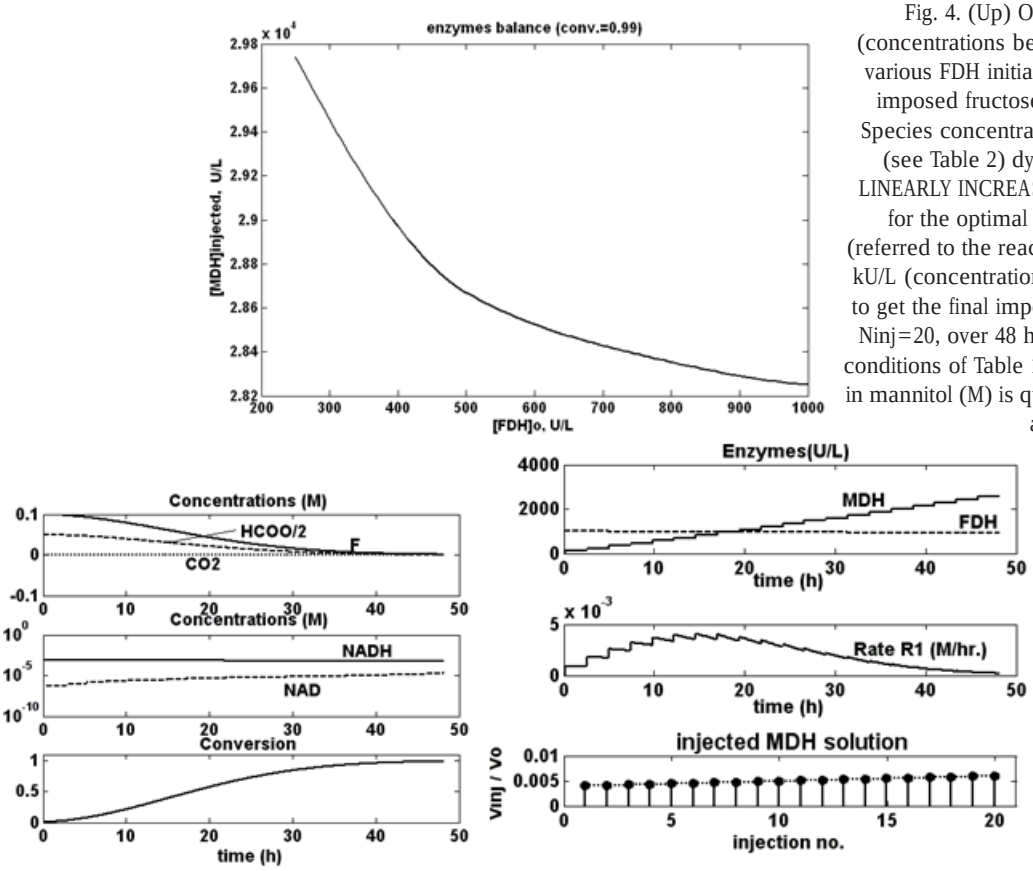

Fig. 4. (Up) Optimal feedings with MDH (concentrations before injection and dilution) for various FDH initial concentrations for getting an imposed fructose conversion of 0.99. (Down) Species concentrations, and the reaction rate R1 (see Table 2) dynamics for the BRP with an LINEARLY INCREASING addition of MDH- solution for the optimal setpoint of $[FDH]_o$ = 1 kU/L (referred to the reactor volume), and $[MDH]_{o.}$ = 28.2 kU/L (concentration before injection and dilution) to get the final imposed conversion x_F = 0.99, with Ninj=20, over 48 hrs. of batch under the reaction conditions of Table 1. Transformation of fructose (F) in mannitol (M) is quantitative so, $[M] = [F]_o - [F]$ at any moment.

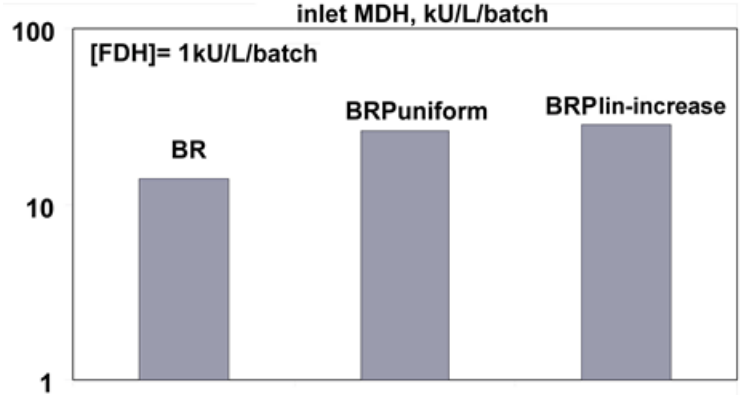

Fig. 5. MDH enzyme consumption in various batch operating alternatives for the optimum running policies plotted in the figs. 2-4, that is: I) *Simple* BR (only initial addition of enzymes), $[MDH]_{o.}$ = 14 kU/L (concentration before injection and dilution); ii) BRP with an *UNIFORM addition* of MDH-solution, $[MDH]_{o.}$ = 26.3 kU/L. (concentration before injection and dilution); iii) BRP with an *LINEARLY INCREASING addition* of MDH-solution, $[MDH]_{o.}$ = 28.2 kU/L. (concentration before injection and dilution). In all the cases an initial amount of $[FDH]_o$ = 1 kU/L is used to get the final imposed conversion x_F = 0.99, over 48 hrs. of batch under the reaction conditions of table 1

fructose conversion). That is because a high level of FDH maintains a NADH high recovering rate R2 during the batch thus compensating the reaction rate R1 reduction due to smaller amounts of MDH.

ii)The objective Min Ω2 =$[FDH]_o$ leads to choose the minimum $[FDH]_o$ = 1 kU/L (referred to the reactor volume) (see upper plots of figures 2-4) as the preferred operating policy. Such a choice corresponds for the *simple batch* **BR** case to the optimum $[MDH]_{inj}$=14 kU/L (concentration before injection and dilution), for the modified batch **BRP** (with an *UNIFORM addition* of MDH) to the optimum $[MDH]_{inj}$ = 26.3 kU/L. (concentration before injection and dilution), while for the modified batch **BRP** (with an *LINEARLY increasing* addition of MDH), to the optimum $[MDH]_{inj}$ = 28.2 kU/L. (concentration before injection and dilution).

iii)The analysis of the above results clearly indicates the modified batch **BRP** operation with an *UNIFORM addition* of MDH as being the best alternative because the optimum $[MDH]_{inj.}$ = 26.3 kU/L. concentration in the injected solution is minimum, while realizing the imposed x_{Ef} = 0.99, due to the way by which optimal values of $[FDH]_o$ and $[MDH]_{inj}$ have been derived. The advantage of this operating alternative also results from analyzing figure 5 where the optimal $[MDH]_{inj}$ derived in the three batch operation are directly compared.

iv) If a larger number of enzyme MDH injections during the batch (not checked here due to the required very large computational effort) are adopted, the BRP is getting close to the **SBR** operation (for N_{inj}-∞ the operation is tending to **SBR**). Thus, the **SBR** operation remains an optimal operation alternative to be investigated, even if the **BRP** operating alternative remains a valuable option, being simple to be implemented.

v) In all the previous calculations, the MDH and FDH enzyme inactivation were neglected. Otherwise, for a 1-st-order enzyme inactivation, the best operating alternative is the **BR** with all substrate and enzyme added initially [9], while for a high-order / complex enzyme inactivation, the best operating alternative can be a **SBR** operation with addition of the key-enzyme following a certain policy [3,4,6].

vi)As $[FDH]_o$ the is smaller, as the MDH consumption is higher and, as higher as the MDH is injected increasingly during the batch.

vii)As revealed in figure 4, the only advantage of a modified batch **BRP** operation with an *LINEARLY increasing* addition of MDH is the relatively constant and well distributed reaction rate R1 level during the batch, with a favourable effect when reaction condition small fluctuations are present.

vi)The previous conclusions suggest that, if successfully immobilized enzymes are available, the continuous **SBR** or **MACR** might be better operating options to be investigated.

Conclusions

When conducting a complex multi-enzyme process (or with a complex inactivation kinetics of enzymes in interfering reactions), derivation of the optimal operating policy of an enzymatic reactor is a difficult engineering task.

This study has demonstrated that, if the process kinetics is adequately specified, then by using a modular reactor simulation platform like those of Maria [3] a relatively large number of operating possibilities can be investigated in a systematic way by assessing and comparing the optimal operating alternatives for different enzymatic reactors vs. a formulated multi-objective criterion. The employed comparative computational approach allows a quick selection of the design solution. 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.

Such reduced adequate kinetic models are of tremendous importance when integrated in complex engineering simulation platforms employed to facilitate decisions concerning operating alternatives, by determining the *level* of enzyme deactivation rate below the point where the operation of a certain reactor (**BR**, **BRP**, **SBR**, **FXBR**, or **MACR**) becomes economically efficient, by getting a satisfactory productivity vs. cost trade-off [3,4].

References

1. XUE, R., WOODLEY, J.M., Bioresour. Technol., **115**, 2012, p. 183.
2. GHOSE, T.K., FIECHTER, A., BLAKEBROUGH, N., Advances in Biochemical Engineering, **10**, Springer-Verlag, Berlin, 1978.
3. MARIA, G., Comput. Chem. Eng., **36**, 2012, p. 325.
4. MARIA, G., CRI'AN, M., Asia-Pac. J. Chem. Eng., **10**, 2015, p. 22.
5. WANG, P., Appl. Biochem. Biotechnol., **152**, 2009, p. 343.
6. MARIA, G., Comput. Chem. Eng., **31**, 2007, p. 1231.
7. LIESE, A., SEELBACH, K., WANDREY, C., Industrial biotransformations, Wiley-VCH, Weinheim, 2006.
8. STRAATHOF, A.J.J., ADLERCREUTZ, P., Applied biocatalysis, Harwood Academic Publ., Amsterdam, 2005.
9. MOSER, A., Bioprocess technology: Kinetics and reactors, Springer-Verlag, New York, 1988.
10. BLANCH, H.W., CLARK, D.S., Biochemical engineering, Marcel Dekker, New York, 1997.
11. BHATT, S.M., MOHAN, A., SRINIVASTAVA, S.K., ISRN Biotechnology, 2013, Article ID 914187.
12. KORAKLI, M., VOGEL, R.F., FEMS Microbiol. Lett., **220**, 2003, p. 281.
13. LEE, J.K., KOO, B.S., KIM, S.Y., HYUN, H.H., Appl. Environ. Microbiol., **69**, nr. 8, 2003, p. 4438.
14. GHOREISHI, S.M., GHOLAMI SHAHRESTANI, R., Trends Food Sci. Tech., **20**, 2009, p. 263.
15. SAHA, B.C., RACINE, F.M., Appl. Microbiol. Biotechnol., **89**, 2011, p. 879.
16. BARANENKO, V.I., Kirov, V.S., At. Energ., **66**, nr. 1, 1989, p. 30.
17. MAKKEE, M., KIEBOOM, A.P.G., VAN BEKKUM, H., Carbohydr. Res., **138**, 1985, p. 225.
18. MAKKEE, M., KIEBOOM, A.P.G., VAN BEKKUM, H., Carbohydr. Res., **138**, 1985, p. 237.
19. VON WEYMARN, N., Process development for mannitol production by lactic acid bacteria, Laboratory of Bioprocess Engineering, Espoo, 2002.
20. KULBE, K.D., SCHWAB, U., GUDERNATSCH, W., Ann. N.Y. Acad. Sci., **506**, 1987, p. 552.
21. SLATNER, M., NAGL, G., HALTRICH, D., KULBE, K.D., NIDETZKY, B., Biocatal. Biotransform., **16**, 1998, p. 351.
22. CHENAULT, H.K., WHITESIDES, G.M., Appl. Biochem. Biotechnol., **14**, 1987, p. 147.
23. MARIA, G., ENE, M.D., Chem. Biochem. Eng. Q., **27**, nr. 4, 2013, p. 385.
24. RAO, S.S., Engineering optimization – Theory and practice, Wiley, New York, 2009.
25. CARROLL, J.J., MATHER, A.E., J. Solut. Chem., **21**, 1992, p. 607.
26. REID, R.C., PRAUSNITZ, J.M., POLING, B.E., The Properties of Gases & Liquids, 4 ed., McGraw-Hill, Boston, 1987.

Manuscript received: 8.09.2016

REVISTA DE CHIMIE
SI
REVISTA MATERIALE PLASTICE
POT FI ACCESATE GRATUIT
LA URMATOARELE ADRESE:

http://www.revistadechimie.ro/
http://www.revmaterialeplastice.ro/

FREE WEB ACCESS FOR
REVISTA DE CHIMIE
AND
MATERIALE PLASTICE

http://www.revistadechimie.ro/
http://www.revmaterialeplastice.ro/