Model-Based Optimization of Mannitol Production by Using a Sequence of Batch Reactors for a Coupled Bi-Enzymatic Process—A Dynamic Approach

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Abstract: Multi-enzymatic reactions can successfully replace complex chemical syntheses, using milder reaction conditions, and generating less waste. The present model-based analysis compares the performances of several optimally operated Batch Reactors (BR) with those of an optimally operated serial Sequence of BRs (SeqBR). In multi-enzymatic systems, SeqBR could be more advantageous and flexible, allowing the optimization of costly enzymes amounts used in each BR in the series. Exemplification was made for the bi-enzymatic reduction of D-fructose to mannitol by using MDH (mannitol dehydrogenase) and the NADH cofactor, with the in situ continuous regeneration of NADH at the expense of formate degradation in the presence of FDH (formate dehydrogenase). For such coupled enzymatic systems, the model-based engineering evaluations are difficult tasks, because they must account for the common species’ initial levels, their interaction, and their dynamics. The determination of optimal operating modes of sole BR or of a SeqBR turns into a multi-objective optimization problem with multiple constraints to be solved for every particular system. The study presents multiple elements of novelty: (i) the proof of higher performances of an optimal SeqBR (including N-BRs) compared to a sole optimal BR operated for N-number of runs and (ii) the effect of using a multi-objective optimization criteria on SeqBR adjustable dynamics.

Keywords: D-fructose reduction with NADH; D-mannitol production; sequential batch reactors; enzymatic reactor optimization; mannitol dehydrogenase; formate dehydrogenase for NADH regeneration

1. Introduction

“Remarkable progresses made in the development of new enzymes and in realizing complex coupled enzymatic systems, able to in-situ recover the reaction cofactor(s), reported important applications in the industrial biocatalysis, presenting important advantages. Examples include the large number of biosynthesis processes used to produce fine-chemicals, or organic compounds in food, pharma, or detergent industry, such as: the production of monosaccharide derivatives, organic acids, alcohols, amino-acids, etc., by using single- or multi-enzymatic reactors [1,2].

Thus, “multi-enzymatic reactions can successfully replace complex chemical syntheses, using milder reaction conditions, and generating less waste. Multi-enzymatic systems with parallel or sequential reactions are successfully applied for: (i) recovering the main reaction co-factor, (ii) shift equilibrium of the main reaction, (iii) remove the excess of one product, (iv) prolongs the life of the main reaction enzyme, by degrading its inhibitor, etc.” [3]

Even if the multi-enzymatic systems are advantageous, the engineering part for developing and/or optimizing such a process is not an easy task because it must account for the interacting enzymatic reactions, differences in enzymes optimal activity domains, deactivation kinetics (if significant), the presence of multiple and often opposed optimization
objectives, technological constraints, an important degree of uncertainty coming from multiple sources (model/constraints inaccuracies, the presence of disturbances in the control variables), and the highly nonlinear process dynamics. [4–7].

The classic and simplest technology involves the use of batch reactors (BR) to efficiently conduct complex (multi-enzymatic) processes. BR are simple, relatively cheap, easy to operate and control, flexible, and adaptable, thus being suitable for the multi-product operation.

Even if an optimal pH and temperature is used, the determination of the optimal operating mode (enzyme(s), substrate(s), cofactor) feeding policy of the BR (or its derivatives) often turns into a difficult multi-objective optimization problem with multiple constraints to be solved for every particular system [4–6,8,9].

Depending on the suspended or immobilized enzyme(s) operation alternatives, the model-based problem to be solved consists of the selection of the most suitable reactor type and its optimal operation mode for a given (multi-)enzymatic process of the already known kinetic model and enzyme(s) characteristics. To facilitate the evaluation of the reactor operation alternatives, Maria [4] proposed a computational modular platform allowing to simulate and to compare the optimal operating policies of various enzymatic reactors with respect to certain formulated objectives, thus providing the best use of enzyme(s), by screening among various reactor alternatives, that is:

i. Simple Batch Reactor (BR) (Figures 1 and 2). Substrate(s), biocatalyst, and additives are initially loaded in the recommended amounts (concentrations) [4,6,10–12];

ii. Sequential Batch-to-batch Reactors (SeqBR) (Figure 2) consist of a certain number of (usually identical) BR operated in series. The BR content is transferred from every BR to the next one, with adjusting the reactants and/or biocatalyst(s) amounts (concentrations) at the beginning of each BR, to reach optimal levels (off-line determined in this paper) [13,14];

iii. BRP, that is a BR with reactants and/or biocatalyst(s) added during the batch in a Pulse-like addition of equal/uneven solution volumes, with a certain frequency (to be determined) (Figure 2) [4–6,8];

iv. Semi-Batch (or Fed-Batch) Reactor (SBR or FBR), with an optimal feeding policy of enzyme(s)/substrate(s) (Figure 2) [4–7,15–21];

v. Fixed-Bed continuous Reactor (FXBR) with immobilized enzyme(s) (no figures here; see [4–6]);

vi. Mechanically Agitated (Semi-)Continuous Reactors (MA(S)CR) with immobilized enzyme(s) (no figures here; see [5,7]);

As roughly represented in Figure 2, the simple BR can be operated in various modes: simple (repeated) batch, BRP, FBR, SBR, serial batches (SeqBR), cyclic or repeated batch, etc.

By using an adequate kinetic model from the literature [10], the in silico analysis of this paper aims to evaluate and compare the performances of several optimally operated Batch Reactors (BR), of a single or repeated use (with using the same optimal or non-optimal initial conditions), with those of an optimally operated serial Sequence of BRs (SeqBR). As proved in this paper, in multi-enzymatic systems, SeqBR is more flexible than a simple BR even if optimally operated. Thus, SeqBR could be more advantageous than the use of simple BR (even if repeatedly operated for the same number of runs as many BR-s are in the SeqBR series), thus allowing optimization of the costly enzymes quantities used in each BR in the series.

The economical superiority of an optimal SeqBR compared to a sole optimal BR (even if repeatedly operated) is due to the possibility of adjusting the dynamics of each BR in the series by adjusting the costly enzymes and the key substrates amounts used in each BR. This study is dealing with investigating such a superiority of optimally operated SeqBR vs. BR in a practical multi-enzymatic system case.
Due to the multi-enzymatic system complexity, several multi-objective optimization criteria have been used in this regard, such as (Section 4.3): (i) minimum initial concentration of enzymes in every BR from the SeqBR (for a given or an optimized substrate initial concentration \([F_o]\)); (ii) maximum substrate conversion for optimized amounts of \([\text{NADH}_o]\) and \([F_o]\). See [5–7,10,18,22–25] for an extended discussion on optimization multi-objectives in biochemical or biological reactors.

Exemplification is made for the case of the enzymatic reduction of D-fructose to mannitol by using suspended MDH (mannitol dehydrogenase) and \(\text{NADH}\) (nicotinamide adenine dinucleotide) as the cofactor, with the in situ continuous regeneration of the cofactor at the expense of formate degradation in the presence of suspended FDH (formate dehydrogenase) [6].

**Paper’s Novelty**

The paper presents a significant number of novelty aspects. Among these, the following are to be mentioned:

The in silico (math-model-based) engineering analysis of a complex bi-enzymatic process, leading to the optimization of the related industrial plant, that is a sequence (SeqBR) of BRs. The previously experimentally validated dynamic model of the bi-enzymatic process allows determining the optimal operating policy of SeqBR which was proved to be economically superior to other operating modes and reactor types. From our knowledge, in the literature, there does not exist any systematic analysis of SeqBR in a direct comparative approach, that is vs. simple or repeatedly operated BRs for a certain multi-enzymatic process.
Except for ref. [10], there are very few multi-enzymatic processes analyzed in the literature from the engineering point of view. This approached bi-enzymatic process is already known, but systematic engineering analyses are missing in the literature. Even if the approached enzymatic system in this study is the same with those of [10], the subject is different, while the formulated optimization objectives are similar but not identical.

Moreover, a direct comparison of the multi-enzymatic process performances when conducted in an optimized SeqBR or in an optimized BR is missing in the literature. As proved in this paper, in multi-enzymatic systems, SeqBR is more flexible than a simple BR even if optimally operated.

Even if for the approached bi-enzymatic process, this study uses a kinetic model imported from the literature [10], the goal of this paper is different: that is, optimization of the SeqBR dynamics and the comparison of its efficiency with an optimally BR (repeatedly operated). It is worth mentioning that the optimization objectives in this paper are similar to those of [10] but not identical, being more comprehensive.

Except for ref. [10], there are very few studies dealing with the BR multi-objective optimization for this process. It is worth also underlining the originality of the used numerical methodology to solve this SeqBR multi-objective engineering problem.

The scientific value of this paper is not “virtual”, as long as the numerical analysis is based on a kinetic model imported from the literature [10] constructed and validated by using the extensive experimental datasets of [26].
2. Process Kinetics

Mannitol is a natural hexitol with important applications in medicine and the food industry due to some other favorable known properties [27], being currently produced via the hydrogenation of 50% fructose/50% glucose syrup with a high cost, at high pressures and temperatures, using a Raney nickel catalyst [28]. Over the last decades, several more profitable production alternatives have been developed [27]. The most attractive are (i) the biological production by fermentation using lactic acid bacteria, yeasts, and fungi, with important advantages such as good yields, less by-products, no need for ultra-purity, and expensive raw materials [29], and (ii) the enzymatic production of mannitol. The most promising is that proposed by [26]. A higher productivity was achieved using only D-fructose as substrate. This method (Figure 3) consists in the enzymatic reduction of D-fructose to D-mannitol in the presence of the MDH enzyme and using NADH as cofactor (proton donor). The advantage of using NADH instead of others is that it is relatively cheap [30] and much more stable than the NADPH [31].

![Figure 3. Simplified reaction scheme of the two coupled enzymatic reactions: (Up) D-fructose (F) reduction to mannitol (M) by using suspended MDH (mannitol dehydrogenase) and the cofactor NADH (nicotinamide adenine dinucleotide). (Down) NADH cofactor continuous regeneration by the expense of formate (HCOO) degradation in the presence of suspended FDH (formate dehydrogenase) [26]. The use of the NADPH cofactor is not recommended, being much more expensive [30] and very unstable. [31].](image)

By far, the bi-enzymatic alternative of [26], also approached in this paper, is more advantageous, the process occurring under mild conditions (pH = 7, 25 °C) and generating no waste. However, due to the costly enzymes, solving the engineering part (this study) is important. Recent advances try coupling the two reactions not in the same BR but in the same genetic modified micro-organism (Bacillus megaterium), which is used as host for both enzymes synthesis and cofactor regeneration [32].

The Kinetic Model

The adopted kinetic model to be used in this study is presented in Table 1 and belongs to [10]. As mentioned above, the approached bi-enzymatic process involves two concomitant interfering enzymatic reactions (denoted by R1 and R2 in Table 1): (R1) enzymatic reduction of F to M by using suspended MDH and the NADH cofactor, and (R2) in situ continuous regeneration of NADH by means of the reaction of NAD(+) with the formate in the presence of suspended FDH enzymes (both initially added).
Table 1. The kinetic model of [10] referring to the two coupled enzymatic reactions, that is: (R1) reduction of D-fructose to mannitol using the MDH enzyme and NADH cofactor and (R2) in situ continuous regeneration of the cofactor NADH at the expense of formate degradation in the presence of FDH (Figure 3). Rate constants have been estimated under the nominal conditions of Table 2 to match the experimental kinetic data of [26].

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Rate Expressions</th>
</tr>
</thead>
<tbody>
<tr>
<td>F + NADH (+H⁺) → M + NAD⁺</td>
<td>$R_1 = \frac{k_{c1} C_{MDH} C_F C_{NADH}}{K_{M1} + K_F C_F + K_{NH} C_{NADH}}$</td>
</tr>
<tr>
<td>HCOO⁻ + NAD⁺ → CO₂↑ + NADH</td>
<td>$R_2 = \frac{k_{c2} C_{FDH} C_{HCOO} C_{NAD}}{K_{M2} + K_{HC} C_{HCOO} + K_{NAD} C_{NAD}}$</td>
</tr>
</tbody>
</table>

Species rate stoichiometry

\[
\begin{align*}
\frac{dc_F}{dt} &= -R_1; \quad \frac{dc_{NADH}}{dt} = -R_1 + R_2; \quad \frac{dc_{NAD}}{dt} = +R_1 - R_2; \quad \frac{dc_{HCOO}}{dt} = -R_2 \\
\frac{dc_M}{dt} &= +R_1; \quad \frac{dc_{CO₂}}{dt} = +R_2
\end{align*}
\]

Rate constants

\[
\begin{align*}
k_{c1} &= 2 \times 10^{-3}; \quad k_{c2} = 8.3259 \times 10^{-3}; \quad 1/h/(U/L) \\
K_{M1} &= 7.2367 \times 10^{-2} \text{ M}; \quad K_{M2} = 8.8047 \times 10^{-2} \text{ M}; \\
K_F &= 1; \quad K_{NH} = 1; \quad K_{HC} = 5.0061 \times 10^{-2}; \quad K_{NAD} = 90.181
\end{align*}
\]

Based on experimental data collected at 25 °C and pH 7.0, a Michaelis–Menten-type kinetic model was suggested, with non-competitive inhibition with respect to reactants, even if the mannitol inhibition might be significant [26]. Based on the large experimental dataset collected at 25 °C and pH 7.0 by [26] in a BR presented in (Table 2), and using their qualitative observations, a simple Michaelis–Menten kinetic model of Ping-Pong-Bi-Bi type was proposed by [10] for both reaction rates R1 and R2 (Table 1) by analogy with a similar process of pseudo second-order kinetics [33]. The inactivation of MDH and FDH enzymes during the reaction has been neglected due to a lack of available data. The rate constants have been estimated by using an effective nonlinear least squares procedure [34], adopting a simple BR model (Tables 1 and 3). The adequacy of the resulted kinetic model of Table 1 was proved to be very good vs. the experimental data [10], which are roughly illustrated here by the comparative results of (Table 4) for three (experimentally tested) different initial [F]₀.

Table 2. Nominal reaction conditions of [26] for the enzymatic reduction of D-fructose to mannitol using MDH and the NADH cofactor in an experimental BR, with the in situ continuous regeneration of the cofactor at the expense of formate degradation in the presence of FDH. 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 varies within the range of 0.5–2 kU/L.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value [26]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>25 °C</td>
</tr>
<tr>
<td>Pressure/pH (buffer solution)</td>
<td>Normal/7</td>
</tr>
<tr>
<td>Molar initial concentrations</td>
<td></td>
</tr>
<tr>
<td>Fructose, [F]₀ **</td>
<td>0.1–1 M (tested by [26])</td>
</tr>
<tr>
<td>Formate, [HCOO]₀</td>
<td>0.1–3 M (this paper)</td>
</tr>
<tr>
<td>[NADH]₀</td>
<td>[HCOO]₀ = [F]₀</td>
</tr>
<tr>
<td>[NAD]₀</td>
<td>0.008 M (0.1–0.5 M) (this paper)</td>
</tr>
<tr>
<td>Others:</td>
<td></td>
</tr>
<tr>
<td>[M]₀ = [CO₂]₀ = 0</td>
<td>none</td>
</tr>
<tr>
<td>$c_{CO₂}^*$</td>
<td>CO₂ saturation level at 25 °C and pH=7</td>
</tr>
<tr>
<td></td>
<td>0.0313 M [35,36]</td>
</tr>
</tbody>
</table>
Table 2. Cont.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value [26]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction time</td>
<td>48 h</td>
</tr>
<tr>
<td>Initial FDH (referred to the reactor liquid)</td>
<td>0.1−2 kU/L (to be optimized)</td>
</tr>
<tr>
<td>Initial MDH (referred to the reactor liquid)</td>
<td>0.1−2 kU/L (to be optimized)</td>
</tr>
</tbody>
</table>

* Higher initial concentrations of fructose are possible, but have not been checked by [26] due to the side-effects (process inhibition by the products) [10].

Table 3. The ideal models (species mass balances) for the BR and SeqBR enzymatic reactors. BR hypotheses [37]: isothermal, iso-pH; (ii) additives (for the pH control) are added initially and during the BR operation in recommended quantities; (iii) perfectly mixed liquid phase (with no concentration gradients). Indices: “o” = initial; “f” = final (at the batch time); “i” = reaction; “j” = species; “k” = BR number. NBR = No. of BR in the SeqBR series.

\[
\frac{dc}{dt} = \sum_{i=1}^{\nu_{ij}} \nu_{ij} r_i; \quad j = \text{species index (F, M, HCOO, NADH, NAD, CO}_2^2); \quad \nu_{ij} \text{ given in Table 1.}
\]

Initial conditions \(c_{j,o} = c_j(t = 0)\) are given in Table 2.

\[
\frac{dc_{E}}{dt} = 0, \quad \text{(negligible inactivation of MDH and FDH); } \quad E = \text{enzymes (MDH and FDH)}; \quad \text{if } c_{CO_2} > c_{CO_2}^* \text{, then } c_{CO_2} \approx c_{CO_2}^* \text{ (excess being removed from the liquid phase).}
\]

SeqBR, that is a Series of \((k = 1, \ldots , N_{BR})\) Simple BR

\[
\frac{dc}{dt} = \sum_{i=1}^{\nu_{ij}} \nu_{ij} r_i; \quad j = \text{species index (F, M, HCOO, NADH, NAD, CO}_2^2); \quad \nu_{ij} \text{ given in (Table 1); } \quad t_f = 48 \text{ h (batch time; this paper).}
\]

Initial conditions: \([F]_{o,k} = [F]_{o,0}; [HCOO]_{o,k} = [HCOO]_{o,0}; [NADH]_{o,k} = [NADH]_{o,0}; [MDH]_{o,k} = [MDH]_{o,0}; [FDH]_{o,k} = [FDH]_{o,0}; [NAD]^+_{o,k} = 0.0005 \text{ M (for the first BR, as recommended in Table 2).}

The condition of reactors connected in series leads to the following constraints:

\[
[NAD]^+_{o,k} = [NAD]^+_{f,k-1}; k = 2, \ldots , N_{BR};
\]

\[
[CO_2]_{o,k-1} = 0 \text{ (for the first BR); } [CO_2]_{o,k} = [CO_2]_{i,k-1}; k = 2, \ldots , N_{BR};
\]

\[
[M]_{o,k-1} = 0 \text{ (for the first BR); } [M]_{o,k} = [M]_{i,k-1}; k = 2, \ldots , N_{BR};
\]

Other adopted hypotheses:

\[
\frac{dc_{E}}{dt} = 0, \quad \text{(negligible inactivation of MDH and FDH); } \quad E = \text{enzymes (MDH and FDH)}; \quad \text{if } c_{CO_2} > c_{CO_2}^* \text{, then } c_{CO_2} \approx c_{CO_2}^* \text{ (excess being removed from the liquid phase).}
\]

Table 4. The high adequacy of the used kinetic model [10] (see Table 1), proved by the perfect match of some predicted BR performances compared to the experimental data of [26]. Initial conditions: \([HCOO]_o = [F]_o; [NADH]_o = 0.008 \text{ M; } [NAD]_o = 0.0005 \text{ M; Batch time = 48 h.}

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model Validation by [10]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>([F]_o = 0.1−1 \text{ M})</td>
</tr>
<tr>
<td>[F]_o</td>
<td>0.1 M 1 M 1 M</td>
</tr>
<tr>
<td>FDH (U/L)</td>
<td>1000 1000 1000</td>
</tr>
<tr>
<td>MDH (U/L)</td>
<td>1000 1000 1000</td>
</tr>
<tr>
<td>F conversion</td>
<td>1 0.95 0.68</td>
</tr>
<tr>
<td>Model [10]</td>
<td></td>
</tr>
<tr>
<td>F conversion</td>
<td>0.99 0.95 0.68</td>
</tr>
<tr>
<td>Experimental [26]</td>
<td></td>
</tr>
</tbody>
</table>

3. Optimal BR

3.1. Reactor Model

The analyzed BR is that of [26] with the adopted operating conditions of Table 2. The reactor model used in the simulations is presented in Table 3, corresponding to a perfectly
mixed isothermal reactor [37]. The reactor model includes the studied process kinetic model adopted from the literature [10] (Table 1).

For the analyzed bi-enzymatic process with in situ cofactor regeneration, a comparative analysis of the optimal BR (with the initial addition of enzymes and substrates) against the BRP with the intermittent addition of the key enzyme MDH following various optimal policies was made by [6]. Simulations with the present kinetic model revealed that the best operating alternative is the BR, requiring less MDH enzyme to obtain an imposed fructose conversion (99%) over 48 h batch time.

3.2. Optimal BR Operation

Starting from such an approximate result, and by using the kinetic model of Table 1, the optimal operation of the BR of (Table 2) was analyzed by [10] by using for simulations the BR model given in Table 3. The present study was aiming at determining the optimal BR operation in a more systematic way, on a wider range of initial \([F]_o\) and \([NADH]_o\) while concomitantly fulfilling several optimization criteria: (i) minimizing the enzymes (MDH and FDH) consumption while (ii) realizing a high fructose conversion (>0.80) at the batch end. The relevant BR optimization results further presented will be derived for different initial concentrations of fructose \([F]_o\), that is 0.1 M, 1 M, and 3 M, which are to be directly compared with the below SeqBR optimization results. To solve the multi-objective (i–ii) BR optimization problem, the same exhaustive numerical algorithm of [10] has been used. As a general conclusion, in all the tested alternatives, the model-based predicted performances of an optimally operated BR are much better in terms of enzymes consumption (2x less for FDH, and 2–5x less for MDH), compared to the experimental trials of [26] to obtain a high conversion in a non-optimally operated BR. The species dynamics for three optimal BR operations are represented in Figure 4 for \([F]_o = 0.1\) M, in Figure 5 for \([F]_o = 1\) M, and in Figure 6 for \([F]_o = 3\) M. Such an optimization with different \([F]_o\), and \([NADH]_o\) initial conditions has not been tested by [10]. These optimal BR set-point dynamic plots lead to several conclusions:

i. The optimal BR values for \([F]_o = 0.1\) M and for \([F]_o = 1\) M have been experimentally validated by [26] (see Table 4).

ii. There is a close connection between the coupling reactions, enzyme concentrations, and the quasi-stationarity of the NADH/NAD ratio over the batch. For all the optimal conditions of Figures 4–6, the two enzymatic reactions are well coupled. Thus, the ratio of the high reaction rates \(R1\) and \(R2\) reaches a quasi-stationary level, leading to a quasi-constant NADH/NAD ratio much higher than 10, thus maintaining the process efficiency.

iii. The cofactor NADH regeneration is very efficient, as formate decomposition is quasi-complete (Figures 4–6) and leads to saturation \([CO_2]^*\) in a short time (after ca. 10 h or even earlier), with removal of the CO\(_2\) excess from the system over the rest of the batch-time [10].

iv. As revealed by the repeated simulations of [10] and the results of (Table 4), the BR performances are more sensitive to the \([MDH]_o\) than to the \([FDH]_o\).
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Trials of [26] to obtain a high conversion in a non-optimally operated BR. The species dynamics for three optimal BR operations are represented in Figure 4 for $[F_o] = 0.1 \text{ M}$, in Figure 5 for $[F_o] = 1 \text{ M}$, and in Figure 6 for $[F_o] = 3 \text{ M}$. Such an optimization with different $[F_o]$ and $[\text{NADH}_o]$ initial conditions has not been tested by [10]. These optimal BR set-point dynamic plots lead to several conclusions:

i. The optimal BR values for $[F_o] = 0.1 \text{ M}$ and for $[F_o] = 1 \text{ M}$ have been experimentally validated by [26] (see Table 4).

ii. There is a close connection between the coupling reactions, enzyme concentrations, and the quasi-stationarity of the NADH/NAD ratio over the batch. For all the optimal conditions of Figures 4–6, the two enzymatic reactions are well coupled. Thus, the ratio of the high reaction rates $R_1$ and $R_2$ reaches a quasi-stationary level, leading to a quasi-constant NADH/NAD ratio much higher than 10, thus maintaining the process efficiency.

iii. The cofactor NADH regeneration is very efficient, as formate decomposition is quasi-complete (Figures 4–6) and leads to saturation $[\text{CO}_2]^*$ in a short time (after ca. 10 h or even earlier), with removal of the CO$_2$ excess from the system over the rest of the batch-time. [10]

iv. As revealed by the repeated simulations of [10] and the results of (Table 4), the BR performances are more sensitive to the $[\text{MDH}_o]$ than to the $[\text{FDH}_o]$. 

---

**Figure 4.** Simulated dynamics of species concentrations and of the reaction rates $R_1$–$R_2$ (Table 1) for the optimal BR with $[F_o] = 0.1 \text{ M}$, $[\text{MDH}_o] = 880 \text{ U/L}$, $[\text{FDH}_o] = 500 \text{ U/L}$, $[\text{NADH}_o] = 0.01 \text{ M}$, and $[\text{NAD}_o] = 5 \times 10^{-4} \text{ M}$. The realized F-conversion is 0.95 over a batch time of 48 h. Note: the transformation of fructose (F) into mannitol (M) is quantitative, so $[\text{M}] = [F_o] - [F]$ at any moment.

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**Figure 5.** Simulated dynamics of species concentrations and of the reaction rates $R_1$–$R_2$ (Table 1) for the optimal BR with $[F_o] = 1 \text{ M}$, $[\text{MDH}_o] = 1388 \text{ U/L}$, $[\text{FDH}_o] = 1000 \text{ U/L}$, $[\text{NADH}_o] = 0.01 \text{ M}$, $[\text{NAD}_o] = 5 \times 10^{-4} \text{ M}$. The realized F-conversion is 0.82 over a batch time of 48 h.
Figure 6. Simulated dynamics of species concentrations and of the reaction rates R1–R2 (Table 1) for the optimal BR with \([F]_o = 3\, M\), \([MDH]_o = 800\, U/L\), \([FDH]_o = 500\, U/L\), \([NADH]_o = 0.5\, M\), and \([NAD]_o = 5 \times 10^{-4}\, M\). The realized F-conversion is 1 over a batch time of 48 h.

4. Optimal SeqBR

The high productivity potential of the optimally operated BR, as underlined in the Section 3, raises a legitimate question: Is it more profitable to repeatedly use a certain number of batches with the same BR by preserving every time the same (optimal) initial conditions, or is it more profitable to use a series of a certain number of BRs (that is, a SeqBR) with the optimal initial conditions of each BR to be determined? This section is dedicated to investigating this kind of engineering problem.

4.1. Control Variable Choice

The SeqBR ideal model approached here is presented in Table 3. Thus, at the batch end of each BR, its content is transferred into the next BR of the series. However, the concentrations of the following compounds (control variables) are initially adjusted to match the optimal values (to be determined, see below): \([F]_o\), \([NADH]_o\), \([MDH]_o\), and \([FDH]_o\). The initial concentrations of the rest of the substances (that is, \([M]_o\), \([NAD]_o\), and \([CO_2]_o\)) are those from the end of the previous BR. Only \([HCOO]_o\) is adjusted to be equal with \([F]_o\). The resulted optimization problem (see below) corresponds to a Nonlinear Programming Problem (NLP) with \(4 \times N_{BR} = 40\) searching variables, which are subjected to multiple nonlinear implicit and explicit constraints.

Here, it is to observe that there are many other operating variables that could be considered when optimizing the SeqBR (e.g., the batch number and time). However, the number of variables would become too large (it is already large), and the NLP problem would become too multi-modal, which would not only greatly increase the computation time for finding a feasible problem solution but would also greatly increase the difficulty of finding the global optimum of the problem.
4.2. The Choice of $N_{BR}$

A series of a moderately large number $N_{BR} = 10$ BRs connected in series are considered here with an equal batch time of 40 h for each BR. It is worth mentioning, by analogy with the time stepwise optimal feeding policies of FBR [15,38], that the use of a SeqBR with a higher number of BRs suffers from a series of disadvantages, that is: (a) as the $N_{BR}$ increases, the number of optimization (control) variables (that is, the initial load of substrate(s)/enzyme(s) to be determined for every BR) is higher, thus increasing the computational effort and the number of local solutions of the SeqBR optimization problem due to its high nonlinearity, thus decreasing the chance to quickly locate the global optimum of the problem; (b) as the $(N_{BR})$ increases, the optimal operating policy of SeqBR is more difficult to implement since the optimal policy requires physical operations to adjust the substrate(s)/enzyme(s) concentrations at the beginning of each BR from the series of BRs; (c) SeqBR operation with using a larger number of $(N_{BR})$ can raise special operating problems when including PAT tools (Process Analytical Technology) [39], and it also involves a higher investment and process control cost.

In spite of such difficulties, some trials to operate SeqBR, or cyclic BR, or even parallel BR have been reported in the literature, by using two to five biological FBR [40–42], 14 cyclic BR [43], 48 parallel BRs [44], or 24 parallel BRs [45]. As expected, optimized FBR reported better performances compared to the simple BR due to a higher operating flexibility [5,15,22,46]. In a way, it can be said that by varying the initial condition of every BR, the SeqBR tries to reproduce somehow the FBR operation with time stepwise varying the control variables [15]. On the other hand, the chemical reactor theory demonstrated that a series of a large number of CSTRs tends to have the best performances of a plug-flow reactor [47]. Consequently, it is expected that an optimal SeqBR will present better performance than a repeated operation of a BR with the same initial conditions.

From a practical point of view, the implementation of the optimal operating policy of the SeqBR including a series of 10 BRs, in fact, can also be made with a physical number of less than 10 BRs if a fast enough circulation of materials between batches is ensured (that is discharge, loading, adjustment of the next BR initial conditions; the use of immobilized enzymes can help in this respect). This implementation flexibility of the SeqBR optimal operation policy is another advantage compared to the repeated use of the same BR with optimal initial conditions, as further proved.

4.3. Optimization Problem Formulation for the SeqBR

While optimization of BR was already approached in Section 3, this section is dedicated to the presentation of a novel methodology proposed to approach the multi-objective optimization of a series of BR (SeqBR) by using the adequate dynamic model of the approached bi-enzymatic process.

The SeqBR to be optimized in this paper includes a series of $N_{BR} = 10$ identical BRs (with the reaction general conditions of Table 2) for the approached bi-enzymatic process described in Section 2. The novelty of the model-based off-line optimization approached here consists of solving of at least two simultaneously economic objectives:

i. **Determine the optimal initial conditions** for every BR from the series to ensure the highest productivity in mannitol at the SeqBR output, which is the output of the last $(N_{BR})$-th BR; and

ii. **Minimize the costly enzymes' overall consumption** (MDH and FDH) while preserving the best connection of the two enzymatic reactions to ensure a quick regeneration of the cofactor and a high fructose reduction rate.

Other optimization objectives can be formulated as well, depending on the reactor and process type [7,18], but these are beyond the scope of this paper.

From the mathematical point of view, this optimization problem consists of the following:
Find:

\[
\{[F]_{o,k}: [NADH]_{o,k}; [FDH]_{o,k}; [MDH]_{o,k}\}, \text{ with } (k = 1, \ldots, N_{BR}) =
\]

\[= \arg \text{ Min } W(c, c_o, k); \]

with the following composite objective function:

\[
W = (F_{obj2} + F_{obj3})/F_{obj1}, \text{ where: } 
\]

\[
F_{obj1} = [M(t_f)]_{k = 1}^{N_{BR}}, \text{ with } [M] \text{ in M units.} 
\]

\[
F_{obj2} = \sum_{k = 1}^{N_{BR}} [MDH]_{o,k}, \text{ with MDH conc. in kU/L units.} 
\]

\[
F_{obj3} = \sum_{k = 1}^{N_{BR}} [FDH]_{o,k}, \text{ with FDH conc. in kU/L units.} 
\]

An alternative objective function that gives more weight to the M-production maximization in the detriment of enzymes consumption is shown below:

\[
\text{Min } W = F_{obj2} + F_{obj3} - F_{obj1}, 
\]

with the same definition of Fobj1–3, and the same searching variables as those used in Equation (1).

As an observation, the formulated composite objective function Equation (2), or Equation (1) corresponds to the so-called “simple, or inverted utility function method”, respectively [48].

Both optimization problems (1 or 2) are subjected to the following constraints:

(i) \[\frac{dc_i}{dt} = \sum_{i=1}^{n_x} v_{ij} r_i \] (dynamic model of the process; Tables 1 and 3); J = species index (F, M, HCOO⁻, NADH, NAD⁺, CO₂, MDH, FDH)

(ii) Initial conditions of:

\[
[c]_{i,0} (t = 0) = [c]_{i,k-1} (t = t_f); k = 2, \ldots, N_{BR} 
\]

except for the “J” species, which are the four search variables, that is, \([F]_{o,k}; [NADH]_{o,k}; [FDH]_{o,k}; [MDH]_{o,k}\)

with \(k = 1, \ldots, N_{BR}\)

(iii) \[c_i(t) \geq 0, \text{ for all } t \text{ (physical significance constraints)} \]

(iv) Searching ranges suggested in Table 2 (experimentally validated), that is:

\[
[M]_{o,k} \leq [F]_{o,k} \leq [F]_{o,k} \leq 0.1 - 2 \text{ kU/L; } k = 1, \ldots, N_{BR} 
\]

\[
0.1 \leq [F]_{o,k} \leq 3 M; 0.01 \leq [NADH]_{o,k} \leq 0.5 M; 
\]

(v) \[V_1 = V_2 = \ldots = V_{N_{BR}} \] (BR of equal volumes);

(vi) The main reaction R1 occurs quantitatively, that is

\[
[M(t)] = [F]_{o} - [F(t)], \text{ at any moment in each BR. } t_f = 40 h \text{ for every BR of the SeqBR.} 
\]

(vii) One excludes the trivial solution (infeasible):

\[
W = F_{obj2} = F_{obj3} = F_{obj1} = 0 
\]

(viii) The SeqBR model of Table 3 imposes that the mannitol [M] produced in each BR is passed (at the batch end) to the next BR of theses. So, the mannitol continuously accumulates, reaching a maximum concentration in the last BR of theses.

Concerning the formulation of the optimization problem (1 or 2), some observations are necessary: (i) the chosen units for M, MDH, and FDH allow the direct comparison of Fobj1, Fobj2, and Fobj3, and their concomitant use in the W objective function because they present the same order of magnitude; (ii) the way in which \(W\) was built implicitly ensures the simultaneous realization of the following derived objectives: Max(Fobj1), Min(Fobj2), and Min(Fobj3); (iii) equal volumes of BR make possible the consistency of the summative objectives (Fobj2 and Fobj3); (iv) other formulations of the W function are also possible, depending on the weight given to each individual sub-objective (Fobj1, Fobj2, Fobj3) [18].

The math formulation of the SeqBR optimization problem corresponds to two alternatives, that is (1+3) denoted by problem (I), which means the optimization function (1) subjected to the associated constraints (3). Similarly, notation (2+3), below denoted by the problem (II), it means the optimization function (2) subjected to the associated constraints (3).
The resulted optimization problems (1+3) or (2+3) correspond, in fact, to two Nonlinear Programming Problems (NLP) [34,49], each with $4 \times N_{BR} = 40$ searching variables defined in (3), subjected to multiple nonlinear implicit and explicit constraints. To avoid local sub-optimal solutions, a very effective multi-modal adaptive random search procedure has successfully been used, which is the MMA of [34,49].

5. Results and Discussion

The SeqBR optimization problem (1+3) was solved in two alternatives, by setting different upper limits of the key substrate(s):

Alternative (a)—Max $[F]_{o,k} = 1$ M, and Max $[NADH]_{o,k} = 0.1$ M,

$[MDH]_o$; $[FDH]_o \in [0.1–1]$ kU/L;

Alternative (b)—Max $[F]_{o,k} = 3$ M, and Max $[NADH]_{o,k} = 0.5$ M,

$[MDH]_o$; $[FDH]_o \in [0.1–1]$ kU/L;

In the Table 5 the optimal policies of the BR obtained in the Section 3.2. are compared to the experimental data of [26].

Table 5. Some optimal BR predicted by the kinetic model of Table 1 ([10]) compared to the experimental data of [26]. Initial conditions: $[HCOO]_o = [F]_o; [NAD]_o = 0.0005$ M; Batch time = 48 h.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Optimal BR</th>
<th>Experimental [26]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$[F]_o = 0.1$ M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$[NADH]_o = 0.008$ M</td>
<td></td>
</tr>
<tr>
<td>FDH (U/L)</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>MDH (U/L)</td>
<td>214</td>
<td>385</td>
</tr>
<tr>
<td>F conv.</td>
<td>0.89</td>
<td>0.93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Optimal BR</th>
<th>Experimental [26]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$[F]_o = 1$ M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$[NADH]_o = 0.008$ M</td>
<td></td>
</tr>
<tr>
<td>FDH (U/L)</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>MDH (U/L)</td>
<td>1192</td>
<td>1192</td>
</tr>
<tr>
<td>F conv.</td>
<td>0.58</td>
<td>0.76</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Optimal BR</th>
<th>Experimental [26]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$[F]_o = 3$ M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$[NADH]_o = 0.5$ M;</td>
<td></td>
</tr>
<tr>
<td>FDH (U/L)</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>MDH (U/L)</td>
<td>500</td>
<td>100</td>
</tr>
<tr>
<td>F conv.</td>
<td>1</td>
<td>0.99</td>
</tr>
</tbody>
</table>

The resulted SeqBR optimal operation policy is plotted in Figures 7 and 8, respectively, while the system’s performances are comparatively summarized in Table 6. The analysis of these results reveals several conclusions.

The direct comparison is made between the sole optimal BR (operated over 10 runs with the same initial load) and one run of an optimally operated SeqBR including a series of the same number of 10 BR, of the same size, but each BR operated in a different way. Such a direct comparison between a sole BR repeatedly operated $10 \times$ with the same optimal initial conditions and an optimal SeqBR with the same number of BRs operated only one time is justified because the three optimization objectives explained in Section 3.2 (for a single BR) and in Section 4.3 (for a SeqBR) are basically the same, even if the optimized operating policy is obtained by applying different algorithms (see Sections 3.2 and 4.3).
Note: the transformation of fructose (F) in mannitol (M) is quantitative, so $[M] = [F]_o - [F]$ at any moment.

Figure 7. Species dynamics (a) and the feeding policy (b) for the optimal SeqBR with $\max[F]_o = 1 \text{ M}$, $\max[NADH]_o = 0.1 \text{ M}$, $[\text{HCOO}]_o = [F]_o$, $[\text{NAD}]_o = 5 \times 10^{-4} \text{ M}$ (for BR = 1). Solution of the optimization problem (1+3).

5.1. Optimization Problem (I), Equations (1+3) of Section 4.3

When solving the SeqBR optimization problem denoted by (I), that is eqn. (1+3) of section 4.3, in both tested alternatives (a,b), the optimal SeqBR with $N_{\text{BR}} = 10$ identical BRs reported better performances compared to a single optimal BR $10\times$ repeatedly operated under the same constraints. More specifically, (see Table 6):

(IIa) For the SeqBR optimization problem (2+3) with the searching variables limits of Alternative (a), the resulted operating policy reported an enzyme consumption roughly 3–12× smaller (1.12 kU/L and 3 kU/L for the MDH and FDH, respectively, in Table 7) compared to the equivalent $10\times$ runs of the optimal BR (13.88 kU/L and 10 kU/L for the
MDH and FDH, respectively) to obtain a too-small increase in the M production (8.2 M in Table 7) over a longer reaction time (480 h) compared to an optimal SeqBR with a series of 10 BRs (6.8 M in Table 7) over a shorter reaction time (400 h). The 10 individually optimally operated BRs of [26] (the third line) reported a huge consumption of enzymes to obtain a modest M production.

(Ia) For the SeqBR optimization problem (1+3) with the searching variables limits of Alternative (a), the resulted operating policy reported an enzyme consumption roughly 6–12x smaller (1.112 kU/L and 1.755 kU/L for the MDH and FDH, respectively, in Table 6) compared to the equivalent 10× runs of the optimal BR (13.88 kU/L and 10 kU/L for the MDH and FDH, respectively) to obtain a too-small increase in M production (8.2 M in Table 6) over a longer reaction time (480 h) compared to the optimal SeqBR with a series of 10 BRs (5.06 M in Table 6) over a shorter reaction time.

Similarly, the 10× repeated BR optimal runs by using the initial conditions of [26] reported a huge consumption of enzymes (10 kU/L for both enzymes (see Table 6) to obtain a modest M production (6.8 M) compared to the optimal SeqBR (1.112 and 1.755 kU/L for the MDH and FDH, respectively, in Table 6) for getting a close 5.06 M mannitol production over a smaller reaction time.

(Ib) For the SeqBR optimization problem (1+3) with the searching variables limits of Alternative (b), the enzyme consumption is smaller (1.112 kU/L and 1.755 kU/L for the MDH and FDH, respectively, in Table 6) compared to (1 kU/L and 3 kU/L for the MDH and FDH, respectively, in Table 6) the sole BR 10× optimal runs for obtaining only a 40% increase in M production (26.1 M) compared to the one optimal SeqBR run (of a series of 10 BRs) leading to close to 17.7 M mannitol production over a smaller reaction overall time.

Figure 8. Species dynamics (a) and the feeding policy (b) for the optimal SeqBR with max[F]₀ = 3 M, max[NADH]₀ = 0.5 M, [HCOO]₀ = [F]₀, [NAD]₀ = 5 × 10⁻⁴ M (for BR = 1). Solution of the optimization problem (1+3).

(Ia) For the SeqBR optimization problem (1+3) with the searching variables limits of Alternative (a), the resulted operating policy reported an enzyme consumption roughly 6–12x smaller (1.112 kU/L and 1.755 kU/L for the MDH and FDH, respectively, in Table 6) compared to the equivalent 10× runs of the optimal BR (13.88 kU/L and 10 kU/L for the MDH and FDH, respectively) to obtain a too-small increase in M production (8.2 M in Table 6) over a longer reaction time (480 h) compared to the optimal SeqBR with a series of 10 BRs (5.06 M in Table 6) over a shorter reaction time (400 h). Similarly, the 10× repeated BR optimal runs by using the initial conditions of [26] reported a huge consumption of enzymes to obtain a modest M production.
enzymes (10 kU/L for both enzymes (see Table 6) to obtain a modest M production (6.8 M) compared to the optimal SeqBR (1.112 and 1.755 kU/L for the MDH and FDH, respectively, in Table 6) for getting a close 5.06 M mannitol production over a smaller reaction time.

Table 6. Performances of the optimal SeqBR policy obtained by solving the optimization problem (I), that is, Equations (1+3) compared with some optimal BR operating policies of Table 5 derived in Section 3.2, and with the experimental data of [26]. Initial conditions: [HCOO]¢ = [F]¢; [NAD]¢ = 0.0005 M; Batch time = 48 h for individual BR and 40 h for each of the 10 serial BRs included in the SeqBR.

<table>
<thead>
<tr>
<th>Initial Conditions (Maximum Amount)</th>
<th>Mannitol Production (Total Operating Time) (M/h)</th>
<th>MDH Total Consumption (kU/L)</th>
<th>FDH Total Consumption (kU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SeqBR (10 BR)</td>
<td>max [F]¢ = 1 M [Alternative (a)]</td>
<td>5.059/400</td>
<td>1.112</td>
</tr>
<tr>
<td>10 repeated runs of the optimal BR</td>
<td>[F]¢ = 1 M; [NADH]¢ = 0.008 M (from Table 5)</td>
<td>8.2/480</td>
<td>13.88</td>
</tr>
<tr>
<td>10 repeated runs of the experimental BR of [26]</td>
<td>[F]¢ = 1 M; [NADH]¢ = 0.008 M (from Table 5)</td>
<td>6.8/480</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>max [F]¢ = 3 M [Alternative (b)]</td>
<td>17.692/400</td>
<td>1.112</td>
</tr>
<tr>
<td>10 repeated runs of the optimal BR</td>
<td>[F]¢ = 3 M; max [NADH]¢ = 0.5 M (from Table 5)</td>
<td>26.1/480</td>
<td>1</td>
</tr>
</tbody>
</table>

(Ib) For the SeqBR optimization problem (1+3) with the searching variables limits of Alternative (b), the enzyme consumption is smaller (1.112 kU/L and 1.755 kU/L for the MDH and FDH, respectively, in Table 6) compared to (1 kU/L and 3 kU/L for the MDH and FDH, respectively, in Table 6) the sole BR 10× optimal runs for obtaining only a 40% increase in M production (26.1 M) compared to the one optimal SeqBR run (of a series of 10 BRs) leading to close to 17.7 M mannitol production over a smaller reaction overall time.

5.2. Optimization Problem (II), Equations (2+3) of Section 4.3

When solving the SeqBR optimization problem denoted by (II), that is eqn. (2+3) of Section 4.3, in both tested alternatives (a,b), this objective function gives more weight to the M production maximization to the detriment of enzymes’ consumption. The obtained results are presented in Table 7. The optimal SeqBR with NBR = 10 identical BRs reported better performances compared to a sole BR 10× repeatedly operated even if under optimal conditions. More specifically, see Table 7.

Similarly, the 10× repeated BR optimal runs by using the initial conditions of [26] reported a huge consumption of enzymes (10 kU/L for both enzymes in Table 7) to obtain a modest M production (6.8 M) compared to the one optimal SeqBR run (1.12 and 3 kU/L for the MDH and FDH, respectively, in Table 7) for getting a close 6.8 M mannitol production over a smaller reaction time.

(IIb) For the SeqBR optimization problem (1+3)with the searching variables limits of Alternative (b), the enzyme consumption (1.72 kU/L and 5.34 kU/L for the MDH and FDH, respectively, in Table 7) is comparable to those of (1 kU/L and 3 kU/L for the MDH and FDH, respectively, in Table 7) the sole BR 10× optimal runs. However, the SeqBR production (28.93 M) is slightly higher than those of the sole BR 10× optimal runs (26.1 M), being obtained over a smaller reaction overall time (400 h).
Table 7. Performances of the optimal SeqBR policy obtained by solving the optimization problem (II), that is Equations (2+3) compared with some optimal BR operating policies derived by [10] and with the experimental data of [26]. Initial conditions: \([\text{HCOO}]_0 = [\text{F}]_0; \ [\text{NAD}]_0 = 0.0005 \text{ M}; \) batch time = 48 h for individual BR and 40 h for each of the 10 serial BRs included in the SeqBR.

<table>
<thead>
<tr>
<th>Initial Conditions (Maximum Amount)</th>
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<th>MDH Total Consumption (kU/L)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>SeqBR (10 BR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>max ([\text{NADH}]_0 = 0.1 \text{ M})</td>
<td>6.79/400</td>
<td>1.12</td>
<td>3</td>
</tr>
<tr>
<td>10 repeated runs of the optimal BR ([\text{F}]_0 = 1 \text{ M}; \ [\text{NADH}]_0 = 0.008 \text{ M}) (from Table 5)</td>
<td>8.2/480</td>
<td>13.88</td>
<td>10</td>
</tr>
<tr>
<td>10 repeated runs of the experimental BR of [26] ([\text{F}]_0 = 1 \text{ M}; \ [\text{NADH}]_0 = 0.008 \text{ M}) (from Table 5)</td>
<td>6.8/480</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>SeqBR (10 BR)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>max ([\text{NADH}]_0 = 0.5 \text{ M})</td>
<td>28.93/400</td>
<td>1.72</td>
<td>5.34</td>
</tr>
<tr>
<td>10 repeated runs of the optimal BR ([\text{F}]_0 = 3 \text{ M}; \ max \ [\text{NADH}]_0 = 0.5 \text{ M}) (from Table 5)</td>
<td>26.1/480</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

5.3. Additional Conclusions about the Results of the Optimization Problems (I) and (II)

III. In the all alternatives (a–b), the optimal SeqBR proved to be more effective vs. a sole BR uniformly operated with an optimal or non-optimal initial load, but of a number of runs equal to the number of BRs from the SeqBR series.

IV. All searching variables ([\text{F}]_0, [\text{NADH}]_0, [\text{MDH}]_0, [\text{FDH}]_0) present a large influence on the objective functions (1 or 2), proving their correct choice as control variables.

V. In the SeqBR, passing the content of one BR to the next BR leads to the accumulation of mannitol, but with preserving the NAD(+) reserve, while the systematic initial adjustment of the [\text{NADH}] ensures its availability during all the batches (Figures 7 and 8).

VI. The requirement of the SeqBR optimal operating policies (Figures 7 and 8) to sometimes decrease the concentration of one of the enzymes in the next BR of the SeqBR series involves an intermediate physical separation operation, the separated enzymes being further re-used. Adjustments of the enzymes levels at the initial state of each BR from the series could be very much facilitated if immobilized enzymes are used.

VII. The direct comparison of the SeqBR with an optimized FBR may be an interesting problem to be studied in a separate numerical analysis, which is beyond the scope of this paper.

VIII. In all tested alternatives, the optimal SeqBR reports incomparably better performances than the experimentally tested optimal BR of [26] (Table 5), even if operated in a repetitive manner.

IX. The superiority of the optimal SeqBR vs. the use of a sole BR (even if optimally operated for the same number of runs as the number of the BRs in the SeqBR) are due to a greater flexibility in the operation of each BR from the series of SeqBR. Positive results with SeqBR have also been reported by [13,14,50] etc.

In fact, a SeqBR is even more economic than the repeatedly operated BRs. Not only is the enzymes consumption much smaller to get the same high productivity, but the cumulated reaction time is also smaller. From a practical point of view, if the material handling is done quickly enough, the number of BRs in the SeqBR series can be even
smaller than 10 by keeping the same productivity, once the first BRs from the series remain empty as soon as the working front moves forward to the reactors from the tail of the series.

6. Conclusions

The numerical/engineering analysis of this paper, based on an experimentally validated kinetic model from literature, demonstrates that optimally operated SeqBR can lead to high productivities with a substantially lower consumption of costly enzymes compared to the repeated use of sole BR even if optimally operated.

The relatively simple but relevant case study analyzed in this paper proves that for the coupled multi-enzymatic systems, derivation of the optimal operating conditions (minimum enzyme consumption with maximum reactor productivity) is not a trivial engineering problem even for a simple BR case [9,10].

Solving this optimization engineering problem by only using an experimental approach/procedure, as tried by [26], may not be the best choice because it involves high costs and a large number of experimental separate tests.

The use of an adequate process model can offer an approximate if not exact solution to the problem (depending on model quality) with a moderate computational effort. In addition, the paper proves in a simple yet suggestive way how a lumped but adequately dynamic model can successfully support in silico engineering evaluations aiming to optimize the BR or SeqBR operation, thus saving considerable experimental effort.

Author Contributions: Conceptualization, data analysis, investigation, writing—original draft preparation, writing—review and editing, supervision, project administration, G.M. Data analysis, investigation, manuscript revision, data curation, I.M.P. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Nomenclature

c_j species / concentration

c_j^* species / saturation level

k_j, K_j rate constants

Min/Max minimum/maximum

r_j, R1, R2 species / reaction rate; reaction rates

t time

t_f the batch time

V the BR volume

x_F fructose conversion

W the objective function of the optimization problem

Greek Symbols

ε accepted tolerance to achieve the target conversion

v_ij stoichiometric coefficient of species j in the reaction i

Index

o initial

f final

Abbreviations

arg the argument of a function

BR batch reactor

BRP BR with intermittent addition of enzyme solution

CSTR continuous stirred-tank reactor
Conv. conversion
DO dissolved oxygen
E enzyme
F D-fructose
FDH formate dehydrogenase
FXBR fixed-bed solid–liquid continuous reactor
GFS fructose/glucose syrup
HCOO⁻ formate
M mannitol
MACR/MASCR mechanically agitated solid–liquid (semi-)continuous reactor
MDH mannitol dehydrogenase
NAD(P)H nicotinamide adenine dinucleotide (phosphate)
NAD, NAD⁺ nicotinamide adenine dinucleotide (oxidized form)
NLP nonlinear Programming Problem
NBR number of BR connected in series
SeqBR sequential batch-to-batch reactor
SBR semi-batch reactor
[X] concentration of X

References
13. Scoban, A.G.; Maria, G. Model-based optimization of the feeding policy of a fluidized bed bioreactor for mAb production using a hybridoma cell culture. Molecules 2020, 25, 5648. [CrossRef]


