



Dedicated to Prof. Ion Grosu
on the occasion of his 70th anniversary

IN-SILICO DERIVED OPTIMAL OPERATION POLICIES OF A FED-BATCH BIOREACTOR TO MAXIMIZE THE mAbs PRODUCTION BY USING PARETO OPTIMAL TECHNIQUES

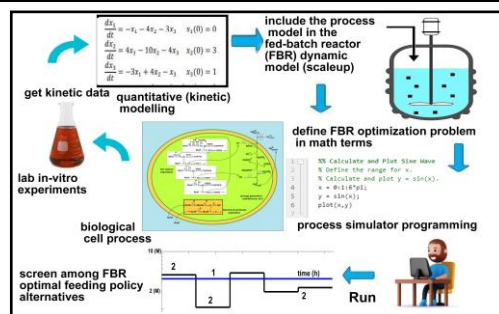
Laura RENE^a, Gheorghe MARIA^{a,b,*} and Daniela GHEORGHE^a

^aDept. Chemical and Biochemical Engineering, National University of Science and Technology Politehnica Bucharest, G. Polizu str. 1–7, zip 011061, Bucharest, Roumania

^bRoumanian Academy, Calea Victoriei 125, zip 010071, Bucharest, Roumania

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Intense efforts have been spent to maximize the **mAbs** production in bioreactors using hybridoma cell cultures. This engineering problem is difficult to solve due to the high nonlinear process dynamics, involving a large number of decision (control) variables, and multiple nonlinear process constraints, which often translates into a multi-objective non-convex optimization problem. Based on an adequate kinetic model from literature, this paper is aiming at *in-silico*, off-line deriving and comparing optimal operating policies of a batch bioreactor (**BR**), or a fed-batch bioreactor (**FBR**) with using the biomass immobilized on a porous support (alginate). The optimal **FBR** operation is derived by using a constant, or a variable feeding, with considering a few number of control variables (feed flow rate, GLC/GLN substrates, viable biomass) seeking maximization of **mAbs** production with raw-materials minimization. The optimally operated **FBR** with a variable time stepwise feeding using a small number of time-arcs (<10), and wide feasible ranges for setting the control variables can lead to high performances of the bioreactor. The **FBR** with constant feeding obtained from using a multi-objective Pareto-optimal technique is also an attractive alternative, requiring a much simpler process control.



INTRODUCTION

Today, special attention is paid to the biosynthesis of pharmaceutical products. In this context, any mean or device able to improve the bioprocess yield is considered. Thus, in a production chain of monoclonal antibodies (**mAbs**), different key-operations are managed and optimized (*i.e.*, cultivation, purification, filtration, capture, polishing steps, bioreactor operation, etc.). This work is focused on the engineering part,

seeking for the bioreactor productivity optimization of hybridomas expressing these **mAbs** (*i.e.* maximize the molecule production in a minimum of time, with minimizing the raw-materials consumption), by using *in-silico* engineering techniques.^{1–3} Bioreactors with microbial cultures are currently used to produce a large variety of valuable molecules, being constructed and operated in multiple alternatives as reviewed in literature.^{3–5} In spite of their larger volumes, continuously mixed aerated tank reactors,

* Corresponding author: gmaria99m@hotmail.com

operated in **BR** (batch), or **FBR** (fed-batch) modes, are the most used because they ensure a high oxygen transfer, and a rigorous temperature/pH control, as also the case here for the **mAbs** production.

Concerning the used bioreactor, an essential engineering problem to be solved is referring to the development of *optimal operating policies* seeking for production maximization, raw-material consumption minimization, with obtaining a product of high quality (less by-products). This engineering problem is *in-silico* solved, based on a bioprocess dynamic (kinetic) mathematical model derived from on-/off-line measurements. Eventually, the **BR** optimal operation can be performed in two alternatives: (a) **off-line** (or ‘run-to-run’), the optimal operating policy being determined by using an adequate kinetic model previously identified based on experimental data (this paper;^{1,2,6-11}

(b) **on-line**, by using a simplified, often empirical math model to obtain a state-parameter estimator based on the on-line recorded data” (such as the classical Kalman filter).^{10,12-19}

“Even if the bioprocess kinetics and biomass characteristics (inactivation rate) are known, *in-silico* solving this off-line engineering problem is not an easy task, due to multiple contrary objectives, and a significant degree of uncertainty of the model/constraints originating from multiple sources.^{12,20-24} Due to such reasons, the bioreactor optimal operating policies are determined by using heuristic, stochastic, or deterministic optimization rules.^{3,6,13,25,26} In the deterministic alternative (this paper), single-/multi-objective criteria, including the productivity, operating and (raw-)materials costs, product quality, etc., are used to *in-silico* obtain feasible optimal operating (control) policies for the analyzed bioreactor²¹ by using specific numerical algorithms.^{11,14,19,20,27}

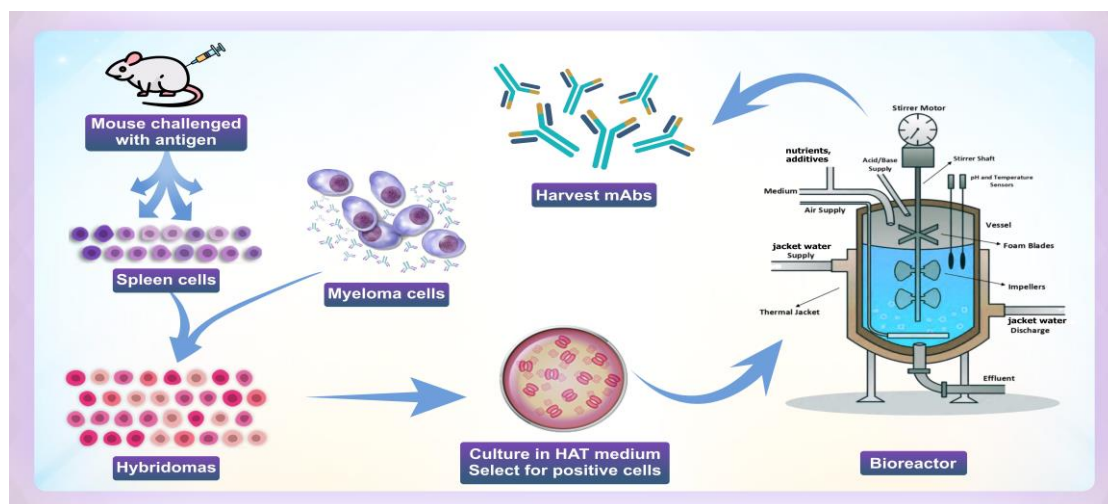


Fig. 1 – A simplified representation of the hybridoma technology used to produce monoclonal antibodies (**mAbs**)/vaccines [adapted from wikipedia, 2023]. [Right] Oversimplified “scheme of a **BR** or a **FBR** used to conduct biological processes. In the **BR** operating mode, substrate(s), biocatalyst, and additives are only initially loaded in recommended amounts (concentrations). In the **FBR** operating mode, the substrate(s)/biocatalyst, and additives (nutrients, pH-control substances, etc.) are continuously fed, following a certain (optimal) policy, to be determined”.³ The **BRP** presents a similar construction to a **BR**, and similar modelling hypotheses.

However, unlike **BR**, the reactants and/or biocatalyst are added during the batch, following a time-step-wise variable (optimal) policy, to be determined *off-line*,³ or *on-line*.¹⁸

To be consistent, the *in-silico* derived optimal operation policy of a bioreactor should be based on a simulation model that must include most of the key variables (particularly the biomass viability) in an adequate bioprocess kinetic model. The use of a deterministic dynamic model (based on the process mechanism), and macroscopic (measurable) state-variables, as the case here, is preferred due to the physical significance of the terms/parameters, which make possible their validation *vs* experimental and literature data, even if repeated

model updating is often necessary due to the high variability of the bioprocess.” Typical optimization objective functions were reviewed by Engasser.²⁸

One of the a-priori *in-silico* analysis advantages it is that which makes it possible to compare performances of various bioreactor constructive / operating alternatives.³ In the engineering practice, such an *off-line* numerical approach allows obtaining an optimal operating policy of the analyzed bioreactor leading to important economic benefits.^{1,2,14,20,29-32} That is because the used

bioprocess kinetic models of moderate complexity are very flexible. Thus, when running the bioreactor, if significant inconsistencies are observed between the model-based predicted key-species dynamics and the experimental evidence, an intermediate model updating step (between batches) is applied, by re-estimating the rate constants for improving the model adequacy.

In spite of its low productivity, **BRs** are commonly used for slow bioprocesses (as also the case here), because they are highly flexible and easy to operate,²⁹ in various alternatives:³ (i).— simple **BR**, when substrate(s), biocatalyst, and additives are initially loaded in the recommended amounts (concentrations)(Fig. 1).^{33–36} Usually, a single- or multi-objective **BR** optimization is off-line performed to determine the best batch time, and substrate/biocatalyst initial load;^{12,15,25,37,38–42} (ii).— a batch-to-batch (**BR-to-BR**) optimization, by including a model updating step based on acquired information from the past batches (‘tendency modelling’, not approached here) to evaluate the optimal load of the next **BR**;^{1,8,9,13,27,43–45} (iii).— an optimally operated serial sequence of **BR-s** (**SeqBR**).⁴⁵

The **SeqBR** includes a series of **BRs** of equal volumes. For every **BR**, its content is transferred to the next one, with adjusting the reactants and/or biocatalyst(s) concentrations of the latter, at off-line determined levels, to ensure its optimal operation.^{9,27} (iv).— **BRP**, that is a **BR** with reactants and/or biocatalyst added during the batch in a pulse-like addition of equal / uneven solution volumes, with a certain frequency to be determined;^{33,46} (v).— **Semi-Batch** (that is “**Fed-Batch**”) **Reactor (SBR or FBR)** (Fig. 1), with an optimally varied feeding policy of biocatalyst/substrate(s).^{2,3,14,20,21,33,44,47} Usually, **FBRs** reported better performances compared to other batch operating alternatives. However, they are difficult to operate. “That is because they need previously prepared stocks of cell-cultures, and substrate(s), of different concentrations (to be a-priori *in-silico* determined), to be fed for every ‘time-arc’ of the batch (that is a batch-time division in which the feeding composition is constant; self-understood, the feeding of time-‘arcs’ usually differ between them). This is the price paid for achieving **FBR** best performances.”^{3,20,26,33,48} Other constructive solutions can be adopted, such as: (vi).— **FiXed-Bed continuous Reactor (FXBR)** with immobilized biocatalyst.^{3,33} (vii).— **Mechanically Agitated Semi-Continuous Reactors (MASCR)** with immobilized biocatalyst.²⁰

Due to such difficulties, trials to optimally operate **BRs** in a simple approach, or a cyclic use, or even a parallel disposal have been reported in

the literature, by using 2–5 biological **FBR**,^{49–51} 14-times cyclic **BRs**,⁵² or 24 parallel **BRs**,⁵³ or even 48 parallel **BRs**.⁵⁴ “As expected, optimized **FBR** reported better performances compared to the simple **BR**, due to a higher operating flexibility.^{3,26,48} Only **SeqBR** reported similar performances. That is because by optimizing the initial load of every **BR** from the series of twin reactors, the **SeqBR** reproduces somehow the **FBR** operation with time step-wise feeding variation.⁴⁵ On the other hand, the flexibility of an optimally operated **FBR** involves a more considerable modelling and computational effort to *in-silico* determine the time-varying optimal feeding policy with substrates and/or biocatalyst.³ By contrast, the **FBR** presents a higher productivity with a smaller raw-materials consumption compared to **BR** as discussed in this paper, and in the literature.^{20,33,48}

Sometimes, **FBR** optimization translates into a constrained multi-objective problem, as is also the case here.^{20,44,47} A comparative discussion of the all mentioned bioreactor types and operating alternatives is provided by Koller,⁵⁵ and Maria,^{3,45} with comparative examples.

The approached case-study in this paper refers to optimization of the industrial production of **mAbs**, in concerned bioreactors, that is a molecule of major importance in medicine, by using the so-called ‘Hybridoma technology’ (with using antibody-secreting hybridoma cell cultures) (Fig. 1).^{56,57} However, the large-scale production of **mAbs** by using mammalian cells in optimized initial load of **BRs**, or **FBRs** {with continuous feeding of glucose (GLC) and glutamine (GLN) substrates, and of necessary nutrients, of optimally varied concentrations/feeding rates^{31,58}} is limited by the engineering problems associated to the *in-silico* optimization, that is: (i) the presence of multiple/opposite objectives; (ii) the significant degree of model/constraints uncertainty originating from multiple sources, and (iii) the unwanted decline of biomass viability during the batch.^{1,10,15,28,58,59} Also, the bioprocess inhibition by several by-products (such as ammonia AMM, lactate LAC, etc.) and/or hyper-osmotic stress related to nutrient feeds and base additions to control pH, all raise serious issues regarding the optimal operation of the **BRs** and **FBRs**. To reduce the by-product production, the use of adapted CHO (Chinese Hamster Ovary) cells was suggested by Freund and Croughan.⁶⁰

By using an adequate kinetic model adopted from literature, the *in-silico* analysis of this paper is aiming to evaluate and compare the performances of several optimally operated **BRs**, with those of various alternatives of optimally operated **FBRs**.”

The **FBR** optimal feeding policy will be given in two options: *(a)* an optimal variable feeding (of substrates and biomass concentrations, and also of the feed flow rate) for every ‘time-arc’ (that is over N_{div} equal time-intervals in which the batch-time is divided;³ $N_{div} = 5$ here); *(b)* a constant feeding, by keeping constant all the feeding characteristics at their optimal values (to be *in-silico* determined). Comparison will be made with an optimally operated **BR** (to be determined), in terms of raw-materials consumption and **mAbs** productivity. These operating alternatives have been obtained by using several optimization algorithms, including the classical Nonlinear Programming **NLP**, but also the Pareto-optimal front technique. This computational analysis presents a significant number of novelty elements, reviewed in ‘Conclusions’ section.

MATERIALS AND METHODS (BIOREACTOR AND NUMERICAL RULES)

The experimental bioreactor

The studied **BR** / **FBR** is those of Liu and Gunawan⁶ with the characteristics of Table 1. This bioreactor was used in the **BR** or **FBR** mode by Kontoravdi *et al.*^{31,61} to obtain the experimental data necessary to estimate and validate the rate constants of the hybrid (mixed apparent and structured) kinetic model for the studied **mAbs** synthesis with using *hybridoma* cells. The completely automated **FBR** of up to 3 L capacity includes a large number of facilities, similarly to those described in detail for a regular commercial bench-scale bioreactor by Chen.⁶²

Table 1

Nominal operating conditions (SPBR), and some properties of the raw-materials for the BR with suspended mammalian hybridoma cell culture of Liu and Gunawan,⁶ and of Kontoravdi *et al.*^{31,61} LGM denotes the adopted kinetic model and data for mAbs production of Liu and Gunawan⁶

Parameter	Nominal value	Remarks
Total cell initial density ($X_{t,o}$)	2 10^8 Cell L ⁻¹	LGM; referred to reactor-lq. Adopted equal to ($X_{v,o}$) in this paper
Viable cell initial density ($X_{v,o}$)	2 10^8 Cell L ⁻¹	LGM; referred to reactor-lq.
	$[2 \cdot 10^7 - 10^{10}]$ (cells L ⁻¹)	Variable, to be optimized in this paper within imposed limits ³
Glucose initial concentration, [GLC] _o , or in the feeding solution, $C_{GLC,inlet,j}$	29.1, mM	LGM
	20–300, mM	Variable, to be optimized in this paper within imposed limits ³
Glucose solution properties	Solubility 5M (25°C), 7M (30°C), 10.2M (40°C)	[63], (M = 180.15 g mol ⁻¹)
	1000 cps (4.5M, 30°C), compared to 1094 cps (molasses, 38°C) Ca. 1–3 cps (up to 300 mM)	[64,65]
Glutamine initial concentration, [GLN] _o	4.9, mM	LGM
	3–50, mM	optimized variable, or constant policy in this paper within imposed limits
Glutamine solubility in water	36 g/L (250 mM)(25°C)	(M = 146.1 g mol ⁻¹) [66]
Lactate initial concentration, [LAC] _o	0, mM	LGM
Ammonia initial concentration, [AMM] _o	0.31, mM	LGM
Monoclonal antibodies initial concentration, [mAbs] _o	80.6, mg/L	LGM Referring to reactor-lq.
Temperature	35–37°C	[59]
pH (buffer, using CO ₂ injection)	7	See an optimal policy given by Li <i>et al.</i> ⁵⁹
Aeration in excess	nutrients in sufficient amounts; other additives in recommended amounts	[59,62,67]
Initial volume of the liquid in the bioreactor ($V_{L,o}$)	1 L (up to 3 L capacity)	LGM
Batch time (tf)	approx. 100 h.	LGM

In the present study, one assumes that this bioreactor can be used to investigate a **BR** operation mode, with an optimal initial load to be determined. But, it also can be adapted to be operated in a semi-continuous **FBR** mode, with a constant or a variable addition (feed flow rate) of the substrates (GLC, GLN) solution, and of the viable biomass (X_v). In this paper, the biomass is assumed to be immobilized on alginate porous beads, being added during the batch according to an optimal policy to be further *in-silico* determined. A reduced bioreactor scheme can be found in the right side of (Fig. 1).

Bioprocess kinetics and bioreactor dynamic model

To support the engineering calculations, several kinetic models of this bioprocess have been reviewed, by choosing the one, of a reasonable size, but able to fairly predict the key-species dynamics in the bulk-phase as a function of extra-cellular substrates/metabolites/biomass concentrations. Generally, the dynamics models employed in engineering calculations are of two types: (A) Reduced apparent models including the dominant factors involved in the **mAbs** production.^{1,6,9,44} (B) More sophisticated kinetic models that also explicitly include intra-cellular factors related to the cellular metabolic fluxes,^{31,32,37,58,61,68} associated to the central carbon metabolism (**CCM**).⁶⁹ It is here to mention that the adopted bioprocess model should be inserted in the bioreactor dynamic model. For the (B) case, a large math model is thus obtained able to predict the dynamics of the macro-scopic state-variables (in the bulk phase) linked to the dynamics of the nano-scopic state-variables of the cell metabolism. Due to their large size, the models of (B) type are too difficult to be identified, and their use for *in-silico* engineering studies is considerably limited.

In the present study, to obtain rapid engineering evaluations, the bioprocess kinetic model **LGM** of Liu and Gunawan⁶ for the **mAbs** production was adopted. That is because **LGM** presents an acceptable compromise between its complexity (number of considered key-species) and its adequacy. The **LGM** is presented in Table 2, together with the associated rate constants, and included in the **BR** model respectively. The hypotheses used to develop the kinetic expressions of **LGM** have been discussed by Liu and Gunawan,⁶ and by Kontoravdi *et al.*,^{31,61} and are

not repeated in this paper. Only a short observation is to be made here: “the **mAbs** mass balance describes the net rate of **mAbs** secretion by the hybridoma cells upon excess production over what is needed for the biomass generation, *i.e.* when $(2 - \gamma \mu)$ is larger than 0. The values of all model parameters, were determined by fitting the experimental data with using a maximum likelihood parameter estimation rule.”⁶

Here, it is worth to notice that **LGM** was validated vs. extensive experiments by Kontoravdi *et al.*^{31,61} In fact, **LGM** is the reduced form (19 rate constants, and 7 key-species) of a previously published extended structured kinetic model [31 rate constants, with 8 additional intermediates belonging to the **CCM**], experimentally validated by Kontoravdi *et al.*,^{31,61} and Kiparissides *et al.*³² In the **LGM**, the essential terms account for the inhibition/limitation effects on the reaction rates due to the presence of various intra-/extra-cellular metabolites, substrates, or by-products in a similar way with other reported models.^{37,58,67,70–72} It is assumed that the rate constants determined from kinetics data using free biomass experiments remain the same for the immobilized biomass case studied in this paper. Being of a reduced form and enough adequate, **LGM** is preferred in engineering analyses, due to a fewer number of rate constants (parsimonious principle).

“The **BR** or **FBR** reactor is equipped with a lot of control systems (see a simplified scheme in Fig. 1). Consequently, the **BR** and **FBR** adopted model is a classical one, of ideal type,^{73,74} developed with the following main hypotheses: (i) Isothermal, iso-pH, and iso-DO (DO denotes the dissolved oxygen); (ii) Nutrients (that is, compounds playing roles of sources of carbon, nitrogen, and phosphorus;⁶⁷) are added initially and during the bioreactor operation in recommended quantities for ensuring, together with an appropriate DO (air/oxygen sparger) an optimally biomass maintenance, and to avoid any growth limitation. Other additives are added to maintain the constant pH, and for other purposes (vitamins, antibodies, etc.⁶²); (iii) The liquid phase is perfectly mixed (with no concentration gradients), by using continuous mechanical agitation and aeration. (iv) While the liquid volume is quasi-constant in the **BR** case, this volume continuously increases in the **FBR** case according to the liquid feed flow rate time-varying policy Eq.(1-FBR). The limits of the volumetric liquid feed flow rate ($F_{L,j}$ in Eq.(1-

FBR)) are adjusted (footnote [e] of Table 3) to ensure a limited dilution of the reactor content to not increase separation costs significantly; (v) There is a negligible mass resistance for the transport of nutrients/substrates/products/oxygen into the liquid and inside the porous alginate beads; (vi) The substrates GLC/GLN, and the solid carrier (of a millimetre size) including the immobilized biomass (Xv) are initially added to the **BR** in a

concentration to be determined by *in-silico* off-line optimization. In the **FBR** case, the control species (GLC, GLN, Xv) are loaded initially, and then continuously added during the batch according to an optimal variable feeding policy to be *in-silico* determined; (vii) Solid particles of alginate are considered of equal size,⁷⁵⁻⁷⁷ and uniformly distributed in the homogeneous liquid phase (perfect mixing conditions).

Table 2

The **batch bioreactor BR model**, including the key-species of the bioprocess kinetic model **LGM** of Liu and Gunawan,⁶ and the associated rate constants

Species	Parameters	Remarks
<p>Biomass balance: Viable biomass balance: $\frac{dX_v}{dt} = (\mu - \mu_d) X_v ; [X_v](t=0) = [X_v]_0 =$ control variable is to be optimized.</p> <p>Total biomass balance: $\frac{dX_t}{dt} = \mu X_v - K_{lysis} (X_t - X_v) ;$ adopted $X_{t,0} = X_{v,0}$, where:</p> $\mu = \mu_{max} \left(\frac{[GLC]}{K_{glc} + [GLC]} \right) \left(\frac{[GLN]}{K_{gln} + [GLN]} \right) \left(\frac{K_{I_{lac}}}{K_{I_{lac}} + [LAC]} \right) \left(\frac{K_{I_{amm}}}{K_{I_{amm}} + [AMM]} \right)$ $\mu_d = \frac{\mu_{d,max}}{1 + (K_{d,amm} / [AMM])^2}$	$K_{lysis} = 0.0551 \text{ h}^{-1}$ $\mu_{max} = 0.058 \text{ h}^{-1}$ $K_{glc} = 0.75 \text{ mM}$ $K_{gln} = 0.075 \text{ mM}$ $K_{I_{lac}} = 172 \text{ mM}$ $K_{I_{amm}} = 28.5 \text{ mM}$ $\mu_{d,max} = 0.03 \text{ h}^{-1}$ $K_{d,amm} = 1.76 \text{ mM}$	LGM
<p>Balance of substrate species: $\frac{d[GLC]}{dt} = -Q_{glc} X_v ; [GLC](t=0) = [GLC]_0 =$ control variable to be optimized;</p> <p>where: $Q_{glc} = \frac{\mu}{Y_{x,glc}} + m_{glc}$</p>	$Y_{x,glc} = 1.06 \cdot 10^8 \text{ cell} \cdot \text{mmol}^{-1}$ $m_{glc} = 4.85 \cdot 10^{-14} \text{ mmol} \text{ (cell.h)}^{-1}$	LGM
$\frac{d[GLN]}{dt} = -Q_{gln} X_v - K_{d,gln} [GLN]$ <p>where: $[GLN](t=0) = [GLN]_0 =$ control variable to be optimized;</p> <p>where:</p> $Q_{gln} = \frac{\mu}{Y_{x,gln}} + m_{gln}$	$K_{d,gln} = 0.0096 \text{ h}^{-1}$ $Y_{x,gln} = 5.57 \cdot 10^8 \text{ cell/mmole}$ $m_{gln} = \alpha_I = -0.00067 \text{ mmol (cell.h)}^{-1}$	LGM

Species	Parameters	Remarks
Balance of the other key-species:		
$\frac{d[LAC]}{dt} = + Q_{lac} X_v ;$ $[LAC](t=0) = [LAC]_0 = 0 \text{ mM}$ where: $Q_{lac} = Y_{lac, glc} Q_{glc}$	$Y_{lac, glc} = 1.4 \text{ L}$	LGM
$\frac{d[AMM]}{dt} = + Q_{amm} X_v + K_{d, gln} [GLN]$ $[AMM](t=0) = [AMM]_0 = 0.31 \text{ mM}$ where: $Q_{amm} = Y_{amm, gln} Q_{gln}$	$K_{d, gln} = 0.0096 \text{ h}^{-1}$ $Y_{amm, gln} = 0.427 \text{ L}$	LGM
$\frac{d[mAb]}{dt} = + (2 - \gamma \mu) \lambda X_v$ $[mAb](t=0) = [mAb]_0 = 80.6 \text{ mg/L}$	$\gamma = 0.1 \text{ h}$ $\lambda = 7.21 \times 10^{-9} \text{ mg (cell h)}^{-1}$ μ expression is given in the biomass balance	LGM
Liquid volume dynamics:		
$\frac{dV_L}{dt} = 0$. In the BR operation mode, V_L is assumed to be quasi-constant.		LGM

The **BR** dynamic model is those given by Table 2, and Eq. (1-BR). The **FBR** dynamic model is presented in the Eq.(1-FBR), the reaction rates being the same as those from Table 2. They include the differential mass balances of the bioprocess considered key-species. The **BR** initial conditions and time stepwise values of the control variables for the **FBR** operating mode will be further *in-silico* determined by solving the associated optimization problems.

The **FBR** is considered here with using immobilized biomass, for several reasons: (a) a

higher biomass stability was reported,⁷⁵⁻⁷⁷ and (b) **FBR** operation with an on-line addition of the viable biomass (of variable concentration, coming from different cell-cultures stocks, previously prepared to be fed for every ‘time-arc’) should eventually be considered in our analysis (an option seldom described in the literature”).

From a math point of view, the **BR** dynamic model translates to a set of differential mass balances written for every considered species ‘i’, and for every time-arc ‘j’ in the following general form:

$$\frac{dC_i}{dt} = \pm r_i(C(t), C_0, \mathbf{k}); C_{i,0} = C_i(t=0); V_L \approx \text{constant} \quad (1\text{-BR})$$

Similarly, the **FBR** dynamic model can be written as followings:

$$\frac{dC_i}{dt} = \frac{F_{L,j}}{V_L} (C_{inlet,i,j} - C_i) \pm r_i(C(t), C_0, \mathbf{k}); C_{i,0} = C_i(t=0)$$

$$\frac{dV_L}{dt} = F_{L,j}; V_{L,0} = V_L(t=0); F_{L,0} = F_L(t=0);$$

$j = 0, 1, \dots, (N_{div} - 1)$ (time step-wise ‘arcs’); i = species index, that is (X_v , X_t , GLC , GLN , LAC , AMM , mAb). The variable feeding concerns the species $i = GLC$, GLN , X_v , and the feed flow rate F_L , that is:

$$C_{inlet,i,j} = \begin{cases} C_{i,0} & \text{if } 0 \leq t < T1 \\ C_{i,1} & \text{if } T1 \leq t < T2 \\ C_{i,2} & \text{if } T2 \leq t < T3 \\ C_{i,3} & \text{if } T3 \leq t < T4 \\ C_{i,4} & \text{if } T4 \leq t < t_f \end{cases} ; F_{L,j} = \begin{cases} F_{L,0} & \text{if } 0 \leq t < T1 \\ F_{L,1} & \text{if } T1 \leq t < T2 \\ F_{L,2} & \text{if } T2 \leq t < T3 \\ F_{L,3} & \text{if } T3 \leq t < T4 \\ F_{L,4} & \text{if } T4 \leq t < t_f \end{cases} \quad (1\text{-FBR})$$

where index ‘*i*’ relates to the key-species X_v , X_i , GLC, GLN, LAC, AMM, and $mAbs$ from the abbreviation list. For the adopted $N_{div} = 5$, the $j = 1, (N_{div} - 1)$ time-arcs switching points are: $T1 = 20$ h. ; $T2 = 40$ h. ; $T3 = 60$ h. ; $T4 = 80$ h.; $t_f = 100$ h. The reaction rate r_i expressions together with the associated rate constants and other details are given in Table 2. In Eqns. (1-BR) and (1-FBR), C = vector of the species concentrations; C_0 = initial vector C at time $t = 0$; k = the model rate constant vector.³

The viable biomass (X_v) dynamics model in Table 2 adopted from literature are of a Monod-type and includes terms accounting for the growth and death inhibition, respectively, caused by the bulk-phase species, such as [GLC], [GLN], [LAC], [AMM],^{6,31,58,70,71,72} or intra-cellular metabolites related to the CCM.⁵⁸

To determine the species dynamics over the batch time (t_f), the BR / FBR model Eqns. (1-BR) / (1-FBR), and Table 2 are solved (section 4.5) with a proposed initial condition of $C_{i,0} = C_i(t = 0)$, and using the best medium conditions (of Table 1). The nominal BR run (denoted by SPBR) used by Liu and Gunawan⁶ is displayed in Table 1.

BR or FBR optimization problem

Control variables selection

By analyzing the BR, and FBR dynamic models of Table 2 and Eq. (1-BR), and Eq. (1-FBR), “the natural option is to choose as control

variables those that are related to the reactor feeding with raw materials (GLC, GLN) and biomass (X_v), whose concentrations play the major role in $mAbs$ production. Additionally, in the FBR case, the liquid feed flow rate F_L will also be considered as a control variable, being responsible for the reactor content dilution. Consequently, the selected control variables are as follows:”

BR case, see Eq. (1-BR). Involves finding the initial $[GLC]_0, [GLN]_0, [X_v]_0$ (see the first guess in Table 1), by using a common optimization rule (the nonlinear programming, NLP), seeking to determine the extreme of an objective function in the presence of multiple constraints, Eq. (4-BR).

FBR case, see Eq. (1-FBR). The batch time is divided in N_{div} equal time-intervals. For every $j = 1, \dots, N_{div}$ time-‘arcs’ are to be determined the following control variables:

The continuously added substrates solution (including the immobilized biomass), with the time stepwise liquid feed flow rate $F_{L,j}$ ($j = 0, \dots, N_{div} - 1$); $F_{L,0} = F_L(t=0)$;

The time stepwise added inlet solutions present variable concentrations of $[GLC]_{inlet,j}$; $[GLN]_{inlet,j}$; $[X_v]_{inlet,j}$ ($j = 0, \dots, N_{div} - 1$). The initial load is also an unknown to be determined, that is $[Ci]_0 = [Ci](t=0) = [Ci]_{inlet,0}$; $i = GLC, GLN, X_v$.

The FBR time-‘arcs’ are defined in Eq. (1-FBR). For an adopted $N_{div} = 5$, the FBR optimal operation is determined in two alternatives:

Alternative A.- A constant feeding along the entire batch, that is:

$$\begin{aligned} F_{L,0} &= F_{L,1} = F_{L,2} = F_{L,3} = F_{L,4}; \\ [GLC]_0 &= [GLC]_1 = [GLC]_2 = [GLC]_3 = [GLC]_4; \\ [GLN]_0 &= [GLN]_1 = [GLN]_2 = [GLN]_3 = [GLN]_4; \\ [X_v]_0 &= [X_v]_1 = [X_v]_2 = [X_v]_3 = [X_v]_4. \end{aligned} \quad (1)$$

The 4 variables to be optimized are the initial values of the chosen control variables, that is: $F_{L,0}$; $[GLC]_0$; $[GLN]_0$; $[X_v]_0$. For a single objective function case, this problem can be solved by applying a NLP procedure in the presence of multiple constraints, Eq. (4-FBR). Details on the used NLP optimization algorithm are given by Maria.³

Alternative B. A time step-wise variable uneven feeding along the batch, with the following control variables to be optimized:

$$F_{L,j}; [GLC]_{inlet,j}; [GLN]_{inlet,j}; [X_v]_{inlet,j} \\ j = 0, 1, \dots, (N_{div} - 1) \text{ of Eqn. (1-FBR).}$$

In this optimization alternative, there are in total $4 \times N_{div}$ unknowns to be determined, that is $4 \times 5 = 20$ unknowns for the adopted $N_{div} = 5$ here.

Single objective function (Ω) optimization (NLP)

“By considering the above mentioned control variables, the BR optimization consists in determining the initial load leading to the maximization of $[mAbs]$ produced during the batch. On the contrary, FBR optimization consists in determining the initial conditions together with the optimal feeding policy for every time-interval during the batch that leads to the maximization of the $[mAbs]$ production, that is:

Max Ω , where $\Omega = \text{Max} [\mathbf{mAbs}(t)]$,

While feasible searching ranges are imposed to the control/decision variables, as specified in Eq. (4) and the footnote (e) of Table 3 (2)

In the above formulation, the time-varying $[\mathbf{mAbs}(t)]$ is in fact a multi-variable function “ $mAbs(C(t), C_o, k)(t)$ ” (in **BR** case), or “ $mAbs(C(t), C_o, C_{inlet}, k, F_L)(t)$ ” (in **FBR** case), evaluated by using the process/bioreactor models over the whole batch time ($t \in [0, t_f]$). As an observation, the (Fig. 5) reveals that, in the present case study, the maximum $[\mathbf{mAb}]$ is reached at the batch end.

The choice of (Ndiv) for the FBR case. The **FBR** operating policy with a variable feeding (alternative B) implies a time step-wise variable feeding of the bioreactor, over an adopted ($N_{div} = 5$ here) equal time-arcs that covers the whole batch time. Each time-arc ‘j’ ($j = 1, \dots, N_{div}$) is characterized by optimal levels of the feed flow-rate $F_{L,j}$, and of the $C_{inlet,i,j}$, with $i = \text{GLC, GLN, Xv}$ [Eqn.(1-FBR)]. It is self-understood that, over a time-interval, the control variables are kept constant. Of course, optimal values on various intervals may differ from each other. The time-intervals of equal lengths $\Delta t = t_f/N_{div}$ are obtained by dividing the batch time into “Ndiv” equal parts.

“A brief survey of the **FBR** optimization literature¹⁸ reveals that a small number (N_{div}) < 10 is commonly used due to multiple advantages, extensively discussed in literature.^{3,26} Thus, to not complicate the computational analysis, (N_{div}) = 5 was adopted, with equal time-arcs covering the batch time $t_f = 100$ h.” An extended discussion

about the N_{div} choice alternatives, and why the present option is preferred was made by Maria.³

Multi-objective optimization by using the Pareto optimal front

When more than one objective functions are simultaneously considered, the optimization problem is more difficult to be solved. “For multi-objective optimization, several alternatives can be followed.^{78,79} One elegant option is to obtain the set of Pareto optimal solutions, also called Pareto-front for the case of at least two adverse objectives.⁸⁰ A Pareto solution is one where any improvement in one objective can only take place at the cost of the other objective.” For the case study of a **FBR** operated with a constant optimal feeding (in terms of feed flow rate FL, inlet [GLC], inlet [GLN], and inlet [Xv]), several opposite objectives can be considered at the batch end, such as: maximum **mAbs** production, minimum substrates [GLC + GLN] consumption, minimum necessary viable immobilized biomass [Xv], minimum feed flow rate FL (to avoid a too high dilution of the bioreactor content). Of course, the Pareto-optimal fronts can be obtained by using any pair of these opposite objective functions. Such an approach is also applied here. In math terms, the Pareto optimal fronts correspond to the following four opposite objectives considered two-by-two, based on their adverse effect in the process model (given by the model equation form, and their sensitivities⁶), that is:

$$\begin{aligned} & \text{Max. } \mathbf{mAbs} \text{ production} -vs- \text{Min. substrates [GLC + GLN] consumption;} \\ & \text{Max. } \mathbf{mAbs} \text{ production} -vs- \text{Min. necessary viable immobilized biomass [Xv];} \\ & \text{Max. } \mathbf{mAbs} \text{ production} -vs- \text{Min. feed flow rate FL.} \end{aligned} \quad (3)$$

The Pareto optimal-front solutions for every opposite pairs of objective functions given in Eq.(3), must also to fulfill the problem constraints Eq. (4-BR), and footnote (e) of Table 3. Due to the limited efficiency of the used Pareto-optimizer, the rough (slightly oscillating) obtained Pareto-curves will be further smoothed in order to be better interpreted.

Optimization problem constraints

The above formulated nonlinear optimization problem (**NLP**) Eq. (2), or the Pareto-optimal front

problem (section 2.3.3), must account for the followings constraints:

(a). – The **BR** model Eqn.(1-BR), or the **FBR** model Eq. (1-FBR) including the bioprocess kinetic model (Table 2), depending on the considered case;

(b). – The initial $[\mathbf{mAb}]_0$, $[\text{AMM}]_0$, $[\text{LAC}]_0$, adopted values from Table 1, as recommended by Liu and Gunawan⁶ in their trials;

(c). – To limit the excessive consumption of raw-materials, or an excessive dilution of the reactor content, feasible searching ranges are

imposed to the control/decision variable, as specified in Eq. (4), with numerical limits specified in the footnote (e) of Table 3, as

$$C_{i,o,\min} \leq C_{i,o} \leq C_{i,o,\max} ; i = \text{GLC, GLN, Xv} \quad (4\text{-BR})$$

In the **FBR** optimization case (both alternatives A-B), the search boundaries are:

$$\begin{aligned} C_{i,\text{inlet},\min} \leq C_{i,o} ; C_{i,\text{inlet},j} \leq C_{i,\text{inlet},\max} ; \\ F_{L,\min} \leq F_{L,j} ; F_{L,o} \leq F_{L,\max} ; \end{aligned} \quad (4\text{-FBR})$$

$j = 0, 1, \dots, (\mathbf{N}_{\text{div}} - 1)$ (time step-wise ‘arcs’); $i =$ species index, that is (GLC, GLN, Xv).

As an observation, in the **FBR** of variable feeding case, “the optimization problem consists in finding the optimal values of the initial and of the input levels of the **4** selected control variables of section 2.3.1, over \mathbf{N}_{div} time-intervals (‘arcs’) of equal lengths $\Delta t = t_{\text{r}} / \mathbf{N}_{\text{div}}$ and under the specified operating constraints of this section, that maximize the chosen objective function Eq. (2). Thus, in total, there are $4 \times \mathbf{N}_{\text{div}}$ searching variables. In Eq. (4-FBR), and Eq. (1-FBR), the time-intervals of equal lengths $\Delta t = t_{\text{r}} / \mathbf{N}_{\text{div}}$ are obtained by dividing the batch time into \mathbf{N}_{div} parts $t_{j-1} \leq t \leq t_j$, where $t_j = j\Delta t$ are switching points ($j = 1, \dots, \mathbf{N}_{\text{div}} - 1$, where the reactor input is continuous and differentiable).” Time-intervals are displayed in eq.(1-FBR). To limit the excessive consumption of raw-materials, or an excessive dilution of the reactor content, feasible searching ranges are imposed to the control/decision variable, as specified in Eq. (4), with numerical limits specified in the footnote (e) of Table 3, as recommended in literature.^{1,6,9,75}

The used numerical solvers

“The [**mAb**] time-evolution in Eq. (4-BR), or Eq. (4-FBR) is determined by solving the bioreactor dynamic model Eqs. (1-BR,1-FBR), with the initial condition of $C_{j,0} = C_j(t=0)$ searched during optimization iterative numerical rule, and the optimal medium conditions of Table 1. The dynamic model Eq. (1) solution was obtained with enough precision, by using the low-order stiff integrator (“ode23s”) of the MATLAB™ computational package.

Because the **BR** / **FBR** model Eq. (1) is a nonlinear one, and the problem constraints Eq. (4) are all nonlinear, the formulated problem Eq. (2) translates into a nonlinear optimization problem (**NLP**) with a multimodal objective function and a non-convex searching domain. To obtain the global feasible solution with enough precision, the multi-modal optimization solver **MMA** of

recommended in literature.^{1,6,9,75} For the **BR** optimization case these constraints are the followings:

Maria^{81,82} has been used, as being proved in previous works to be more effective compared to the common (commercial) algorithms. The computational time was reasonably short (minutes) using a common PC, thus offering a quick implementation of the obtained **FBR** optimal operating policy.”

The rough Pareto-optimal fronts have been generated by using the dedicated algorithm (“GAMULTIOBJ”) of the MATLAB™ math computational package. Due to the limited efficiency of the used optimizer, the obtained “rough” Pareto-curves are difficult to be interpreted. To better point-out their monotony, approximate Pareto-curves have been generated by using the cubic smoothing spline procedure of Matlab™ (function “CSAPS”), with a suitable smoothing constant.

RESULTS AND DISCUSSION

The results obtained by solving the **NLP** optimization problem, and the obtained Pareto-optimal fronts, are presented in the following forms:

The Pareto optimal fronts for several opposite objectives (Figs. 2–4);

The **NLP** optimal operating policies for the **BR** and **FBR** in the all operating alternatives are comparatively displayed in (Figs. 6–8);

A comparison of all **FBR** operating alternatives in terms of **mAbs** production and raw-materials consumption in Table 3.

It is to mention that optimal / non-optimal **BRs** are compared to the optimally operated **FBR** in two alternatives: *i*) **FBR** with a time step-wise variable feeding (set-point denoted **SP2/large** in Table 3 and Figs. 6–8), or *ii*) **FBR** with a constant but optimal feeding (Table 3 and Figs. 6–8). In Table 3, the substrate consumption for **FBR** case

was evaluated with the formula $\sum_j^{N_{div}} F_{L,j}[species]_{inlet,j} \Delta t_j$. For the **BR** case, the raw-materials consumption is based on the only initial load.

By analyzing these results, with comparing the performances of the **BR** and **FBR** operating alternatives in Table 3, several conclusions can be derived, as followings:

(1) – In the Pareto optimal front case, four opposite objectives have been considered, according to Eq. (3). The most important Pareto-front, is those indicating the dependence of the

{Max. **mAbs** production – vs – Min. substrates [GLC + GLN] consumption} (Fig. 2). Based on the findings of Maria,^{80,83} the “break-points” in the exponential-like increasing curve can be considered as being the preferred solution of the optimization problem. Thus, the marked “break point” in (Fig. 2) was chosen as being the solution of this optimization problem (also displayed in Table 3). Such a set-point (SP) of the **FBR** with an optimal but constant feeding, corresponds to the marked SP in the similarly obtained Pareto-fronts of (Figs. 3–4) (in terms of FL, [GLC], [GLN], [Xv], see Eq. (3)).

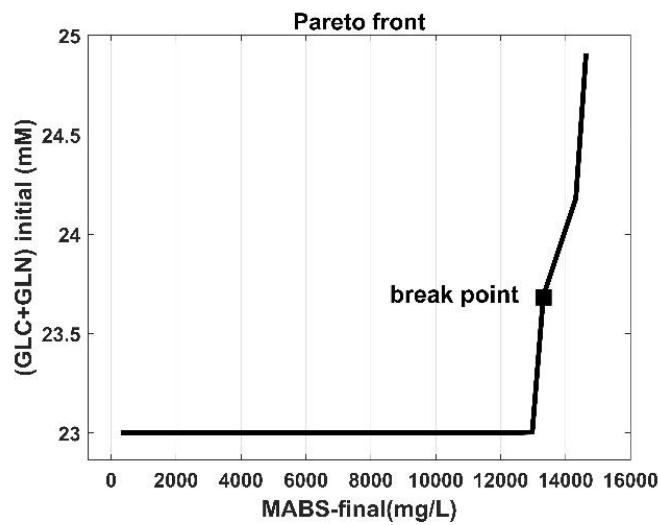


Fig. 2 – The Pareto-optimal front for the analyzed FBR of Table 1 in terms of two opposite objectives, that is maximum mAbs production vs.- minimum substrate (GLC+GLN) consumption (added constantly during the batch). This problem Eqn. (3) solution was obtained by imposing the control variable limits given in the footnote (e) of Table 3. The marked “break point” was chosen as being the most favorable solution of this optimization problem, according to the suggestions of Dan and Maria.^{80,83}

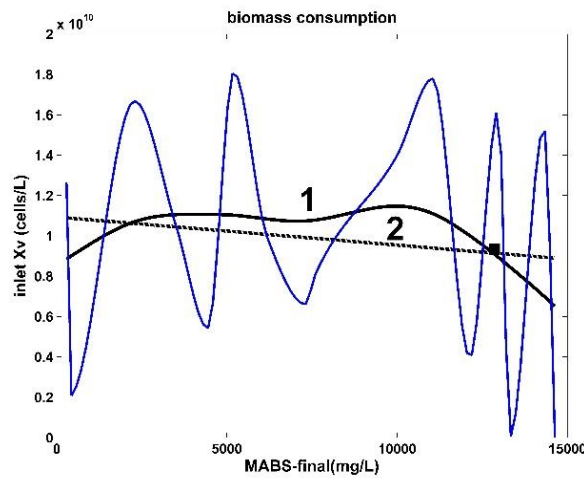


Fig. 3 – The Pareto-optimal operating policy of the **FBR** in terms of required constant feed of [Xv], for various maximum **mAbs** produced. The marked point corresponds to those of the Pareto-optimal curve of Fig. 2. The blue curve is the “rough” Pareto-optimal front. The curves “1-2” illustrate the approximate Pareto-optimal front with using the cubic spline procedure of Matlab™ (function “CSAPS”), with a smoothing constant 1e-10 (curve “1”), or 1e-20 (curve “2”), respectively.

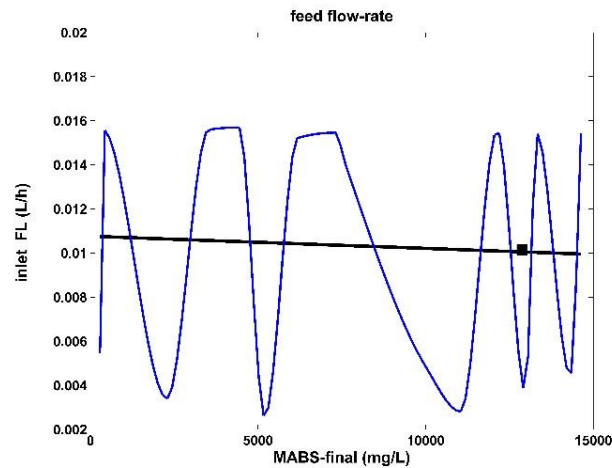


Fig. 4 – The Pareto-optimal operating policy of the **FBR** in terms of required constant feed flow rate FL, for various maximum **mAbs** produced. The marked point corresponds to those of the Pareto-optimal curve of **Fig. 2**. The blue curve corresponds to the “rough” Pareto-optimal front. The black curve illustrates the approximate Pareto-optimal front with using the cubic spline procedure of Matlab™ (function “CSAPS”), with a smoothing constant 1e-20.

(2) – The optimally operated **BR** reported much better performances (10x in terms of produced mg **mAb/L**) compared to the non-optimal **BR** of Liu and Gunawan⁶ in Table 3. However, such a better performance implies an approx. 10x higher consumption of raw-materials (GLC, GLN, Xv).

(3) – The best operating alternative is the Pareto-optimal SP of Table 3 (and Figs. 2–4), for a **FBR** operated with an optimal but constant feeding during the whole batch, thus realizing a **mAbs** production of (13,314 mg **mAbs/L**), in spite of a final higher liquid volume of 2.5 L.

(4) – By comparison, almost as efficient are those of the **NLP** optimal variable feeding **FBR** (11,978 mg **mAbs/L**), or the **NLP** optimal constant feeding **FBR** (11,965 mg **mAbs/L**). The slightly advantage is given by the much lower increase (10-18%) of the liquid volume, leading to a smaller dilution of the product, compared to the optimal **BR** (12,424 mg **mAbs/L**), these **FBR** two optimal operating alternatives being superior by reporting a much lower (10x) consumption of raw-materials, in spite of a slightly lower productivity.

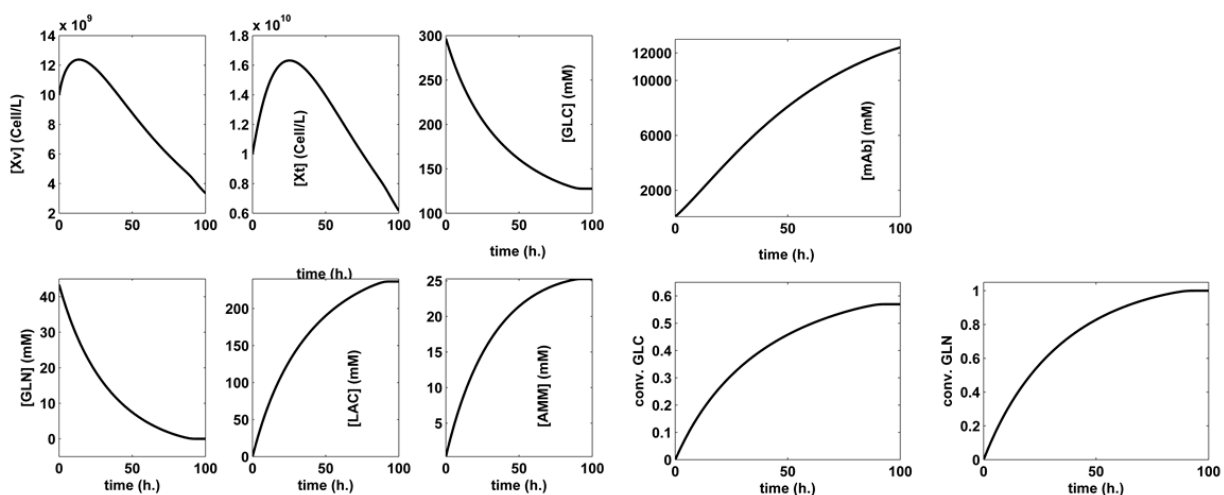


Fig. 5 – Simulated species dynamics in the **BR**, by using the **LGM** model of Table 2, for the optimum operating policy (set point SP of Table 3). Overlapping trajectories have been obtained for the **FBR** operated with an **NLP** optimal but constant feeding policy (optimal control variables of Table 3). For both cases, the same large search intervals for the control variables have been used (see footnote (e) of Table 3).

(5) – The optimally operated **FBR** with a constant feeding (Figs. 6–8) reported close performances compared to the **FBR**, operated

with an optimal variable feeding. The raw-materials consumption is similar. It is also to remark in (Fig. 5) that the key-species dynamics

for the optimum operated **BR** case, is very similar (basically superimposed curves) to those of the **FBR** operated with an optimal but constant feeding policy (see the optimal control variable values of Table 3). For both **FBR** cases, the same large search intervals for the control variables

have been used (footnote (e) of Table 3). However, the **FBR** with an optimal constant feeding requires a 10x smaller amount of immobilized biomass (X_v) which, is an important advantage due to the difficulties to prepare and maintain its viability.

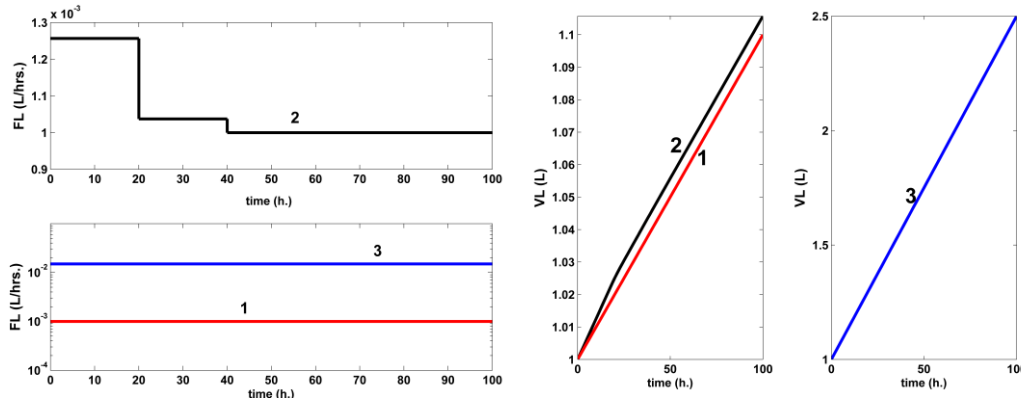


Fig. 6 – The in-silico determined optimal operating policies of **FBR** defined by eqn.(1-FBR), in terms of feed flow rate (FL)[left], and liquid volume increase (VL)[right]. The **FBR** is operated in several alternatives, as followings: **Curve 1(red)**. The optimal set-point (SP) for an optimal but constant feeding policy ($[GLC]_{in}$, $[GLN]_{in}$, FL_{in}), obtained by using the large search intervals for the control variables (FL , GLC , GLN , X_v) given in Table 3, footnote (e). **Curve 2(black)**. The optimal operation policy (denoted **SP2/large** in Table 3) derived in this paper, that is the optimal time step-wise variable feeding policy, in terms of flow-rate $FL(t)$, substrates $[GLC]_{in}(t)$, $[GLN]_{in}(t)$, and biomass $[X_v]_{in}(t)$, with using large search intervals for the control variables, as given in Table 3 (footnotes (e)). **Curve 3, blue)**. Constant, but Pareto-optimal **FBR** feeding derived in this paper (concerning the control variables including inlet flow-rate $FL(t)$, inlet substrates $[GLC]_{in}(t)$, $[GLN]_{in}(t)$, and biomass $[X_v]_{in}(t)$), with using the large search intervals for the control variables, as given in Table 3 (footnote (e,f)).

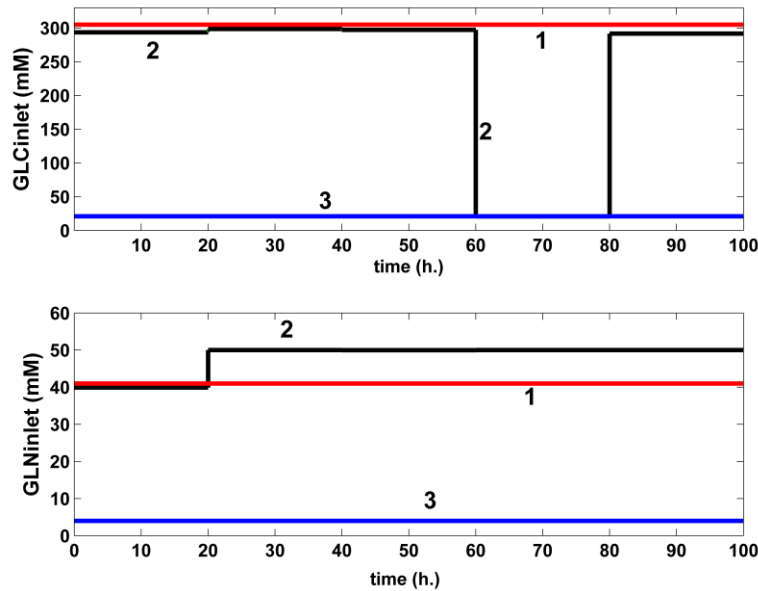


Fig. 7 – The in-silico determined optimal operating policy of **FBR** defined by eqn.(1-FBR), in terms of GLC , and GLN concentrations in the feeding solution. The **FBR** is operated in several alternatives, as followings: **Curve 1(red)**. The optimal set-point (SP) for an optimal but constant feeding policy ($[GLC]_{in}$, $[GLN]_{in}$, FL_{in}), obtained by using the large search intervals for the control variables (FL , GLC , GLN , X_v) given in Table 3, footnote (e). **Curve 2(black)**. The optimal operation policy (denoted **SP2/large** in Table 3) derived in this paper, that is the optimal time step-wise variable feeding policy, in terms of flow-rate $FL(t)$, substrates $[GLC]_{in}(t)$, $[GLN]_{in}(t)$, and biomass $[X_v]_{in}(t)$, with using large search intervals for the control variables, as given in Table 3 (footnotes (e)). **Curve 3, blue)**. Constant, but Pareto-optimal **FBR** feeding derived in this paper (concerning the control variables including inlet flow-rate $FL(t)$, inlet substrates $[GLC]_{in}(t)$, $[GLN]_{in}(t)$, and biomass $[X_v]_{in}(t)$), with using the large search intervals for the control variables, as given in Table 3 (footnote (e,f)).

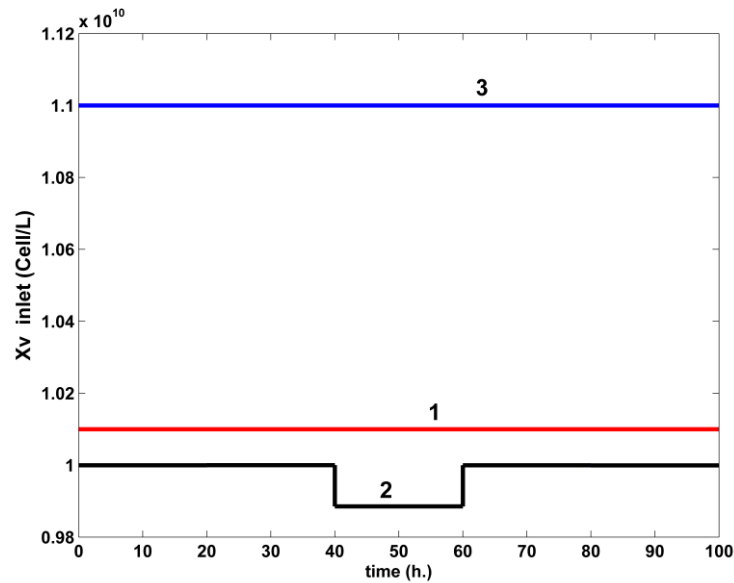


Fig. 8 – The *in-silico* determined optimal operating policy of **FBR** defined by eqn.(1-FBR), in terms of viable biomass (X_v) concentration in the feeding solution. The **FBR** is operated in several alternatives, as followings: **Curve 1 (red)**. The optimal set-point (SP) for an optimal but constant feeding policy ($[GLC]_{in}$, $[GLN]_{in}$, FL_{in}), obtained by using the large search intervals for the control variables (FL , GLC , GLN , X_v) given in Table 3, footnote (e). **Curve 2 (black)**. The optimal operation policy (denoted **SP2/large** in Table 3) derived in this paper, that is the optimal time step-wise variable feeding policy, in terms of flow-rate $FL(t)$, substrates $[GLC]_{in}(t)$, $[GLN]_{in}(t)$, and biomass $[X_v]_{in}(t)$, with using large search intervals for the control variables, as given in Table 3 (footnotes (e)). **Curve 3 (blue)**. Constant, but Pareto-optimal **FBR** feeding derived in this paper (concerning the control variables including inlet flow-rate $FL(t)$, inlet substrates $[GLC]_{in}(t)$, $[GLN]_{in}(t)$, and biomass $[X_v]_{in}(t)$), with using the large search intervals for the control variables, as given in Table 3 (footnote (e,f)).

(6) – One major advantage of the **FBR** with a constant but optimal feeding, consists in a much simpler operation compared to the **FBR** with a variable optimal time step-wise feeding policy. That is because the latter requires different stocks with feeding substrate solutions or biomass of different concentrations, “and different stocks with cell-cultures to be fed for every (N_{div}) ‘time-arc’ over the batch. This is the price paid by **FBR** for usually achieving a better operating flexibility compared to the others alternatives discussed here. Besides, **FBR** operation with using N_{div} time-arcs can raise special operating problems when including PAT (Process Analytical Technology) tools”.⁸⁴

(7) – To conclude, despite some drawbacks, the **FBR** operated in both alternatives, that is a time step-wise variable optimal feeding policy, or a constant but optimal feeding policy, presents multiple advantages. And finally, the **FBR** operated with a constant but Pareto-optimal optimal feeding policy reported the best performances in spite of a higher dilution of the bulk phase.

Besides, the here approached rule to *in-silico*, *off-line* derive the **FBR** optimal operation policies, by employing various algorithms, and multiple

objective functions, and control variables during the batch, is proved to be more flexible and reported better performances compared to the optimal policies of similar **FBRs** from literature. For instance, Amriht *et al.*² used an exponential trajectory of the liquid feed flow rate, by varying only the inlet $[GLC]$ and $[GLN]$ concentrations, the optimal feeding policy being obtained by using a hybrid deterministic–empiric dynamic model of a lower quality.

(8) – The rough classification given in Table 3 for the different **FBR** operating alternatives (*i.e.*, ‘fairly good’, ‘enough good’, ‘best’) is based on the operating policy performance (*i.e.*, mAbs production maximization, minimum raw material (GLC , GLN , X_v) consumption, and the fulfillment of the variation limits for the control variables given in the Footnote (e) of Table 3.

The present *in-silico* derived results have not been experimentally validated. However, as long as the used **LGM** bioprocess model was experimentally validated in a multiple and independent manner by Liu and Gunawan,⁶ by Kontoravdi *et al.*,^{31,61} and by Kiparissides *et al.*,³² the adopted kinetic model presents sufficient credibility and adequacy to be used for the

engineering evaluations of the bioreactor / process, as performed in this work.

It is also to remark, that the **FBR** optimal control strategy is very adaptable. That is because the employed bioprocess kinetic model of moderate complexity is enough flexible, due to its large number of rate constants. Thus, if significant inconsistencies are observed between the model-

predicted bioreactor dynamics and the recorded data, then an intermediate numerical-analysis step will be applied to improve the model adequacy (*i.e.* a ‘model updating’ step), and the bioreactor optimization is applied again with the novel model. This evolutionary successful adaptation of the bioprocess model is the so-called ‘tendency modelling’.⁸⁵

Table 3

BR and **FBR** productivity and raw-materials consumption when operated in various modes. The equal time-arcs of the **FBR** set-point **SP2/large** are of 20 h each. The initial volume is 1 L, and 100 h. batch time in all cases. SP = set-point

Bioreactor operation				Raw-material consumption (b)			Max [mAbs] (b)	FBR final VL
Type	Ndiv			GLC, mmoles	GLN, mmoles	Xv, (cells L ⁻¹) (c)	(mg L ⁻¹)	(L) (a)
BR Liu and Gunawan ⁶ model	0	Nominal initial load SPBR (d,g)		29.1	4.9	2×10⁸	1,231	1
		[GLC]o	29.1					
		[GLN]o	4.9					
		[Xv]o	2 10 ⁸					
FBR variable feeding	5 (e,g) Figs. 6–8	Optimal SP2/large (e)		25.8	5.04	10¹⁰	11,978 (fairly good)	1.18
FBR Constant NLP optimal feeding (this paper)	1 (e–h) Figs. 6–8	Optimal inlet conc.		30.5	4.1	10⁹	11,965 (enough good)	1.1
		[GLC]in	305					
		[GLN]in	41					
		[Xv]in	1.01 10 ¹⁰					
		FL,in	0.001					
FBR Constant Pareto optimal feeding (this paper)	1 (e–h) Figs. 6–8	Optimal inlet conc.		31.5	6	1.65×10¹⁰	13,314 (best)	2.5
		[GLC]in	21					
		[GLN]in	4					
		[Xv]in	1.1 10 ¹⁰					
		FL,in	0.015					
BR NLP optimal (this paper)	0 (g,h)	Optimal initial load		300	43.4	10¹⁰	12,424	1
		[GLC]o	300					
		[GLN]o	43.4					
		[Xv]o	10 ¹⁰					

Footnotes:

(a). – Referring to the reactor liquid initial volume.

(b). – The displayed digits come from the numerical simulations.

(c). – Referred to the FBR initial volume (Table 1).

(d). – The BR nominal set-point (Table 1) of Liu and Gunawan.⁶ Notation: SPBR = the BR nominal set-point (SP).

(e). – The FBR optimal time step-wise variable feeding policy obtained by using *larger* search

intervals for the optimal control variables compared to those used by Maria,³ that is: FL_j ∈ [0.001 — 0.05](L h⁻¹); [GLC]inlet,j ∈ [20–300](mM); [GLN]inlet,j ∈ [3–50] (mM); [Xv]inlet,j ∈ [2· 10⁷ — 2·10¹⁰](cells L⁻¹); VL < 3 L. In the case of the time step-wise variable feeding of **FBR**, the control variables (section 2.3.1, alternative (b)), that is: FL_j; [GLC]inlet,j; [GLN]inlet,j; [Xv]inlet,j; j = 0,1,...(Ndiv -1) of Eqn.(1-FBR) and Table 2, follow an uneven policy to be optimized (that is 20 unknowns for Ndiv = 5). Details on the used optimization rule are given

by Maria.³ The resulted optimal control variables policy is given in Figs. 6–8.

(f). – The FBR operation with a constant over time feeding for all the control variables, as mention in the section 2.3.1 (alternative (a)), and (Figs. 6–8). The only 4 variables to be optimized are the initial values FL_o ; $[GLC]_o$; $[GLN]_o$; $[Xv]_o$ of Maria,³ under the same large searching intervals and constraints of (e). The resulted FBR optimal operating policy is given in Figs. 6–8.

(g). – The units are: $[GLC]_{in}$, mM; $[GLN]_{in}$, mM; FL_{in} , L h⁻¹; $[Xv]_{in}$, (cells L⁻¹).

(h). – Search intervals used to obtain the optimal SP are those from the footnote (e).

CONCLUSIONS

To conclude, the **FBR** operation with an optimal time stepwise control of the feeding policy, or even an optimal constant feeding, but using multiple control variables, reported better performances than the simple **BR** operation due to its higher flexibility in using the biomass and substrates, even if a small number of equal time-arcs is used. “The major drawback of the **FBR** of variable feeding is coming from its difficult operation, as long as the time step-wise optimal feeding policy requires different feeding substrate solution stocks of different concentrations, and separate different cell-Abbreviations and Notations

AMM	Ammonia	GLC	Glucose
BR	Batch reactor	GLN	Glutamine
RP	Batch reactor with intermittent addition of biomass/substrates	LAC	Lactate
CCM	Central carbon metabolism	LGM	Liu and Gunawan model ⁶
DO	Dissolved oxygen	Lq.	Referring to the liquid phase
FBR	Fed-batch bioreactor	MMA	The multi-modal optimization solver of Maria ^{81,82}
mAb (mAbs)	Monoclonal antibody (antibodies)	NLP	Nonlinear programming (numerical rules for solving nonlinear optimization problems)
SPBR	BR nominal setpoint (SP) of Liu and Gunawan model ⁶ (Table 1)	[x]	“x” species concentration
MASCR	Mechanically agitated semi-continuous reactor	Ω	The optimization objective function in Eqn.(2)
SBR/SeqBR	Semi-batch reactor / Sequential BR	Δ	Discrete interval
C_i	Species “i” concentration	α₁, γ, λ	Rate constants of the kinetic model
FL, F_L	Liquid feed flow rate	PAT	Process Analytical Technology tools ⁸⁴

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cultures stocks to be fed over the batch. This is the price paid for achieving improved **FBR** performances. An economic global evaluation (not approached here) accounting for the product/raw-material relative value can give a more accurate answer to such a sensitive issue.

The present optimization analysis proves its worth by including multiple elements of novelty. Among others it is to mention: (i) An optimally operated **FBR** with a variable feeding using a small number of time-arcs (<10), and using wide but feasible ranges for setting the control variables can lead to high performances of the bioreactor. (ii) The major role played by the variable feeding with the viable biomass, leading to consider (Xv) as a control variable during **FBR** optimization (an option seldom discussed in the literature).” (iii) The **FBR** with an optimal constant feeding is also an attractive alternative, requiring a much simpler process control.

“The in-silico analysis of the paper proves that the optimal operating policies of **FBR** or **BR** are simple to be implemented, and more effective and flexible (in spite of a moderate small Ndiv) compared to some optimal policies reported in the literature,² that uses an exponential trajectory of the feeding liquid flow rate, obtained by using a hybrid structured – empiric dynamic model, and with by considering but only the inlet $[GLC]$ and $[GLN]$ as control variables.”

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