

# In-Silico Optimal Operating Policies of A Batch or A Fed-Batch Bioreactor for mAbs Production Using A Hybridoma Cell Culture



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## Abstract

Production of monoclonal antibodies (mAb) is a well-known method to synthesize a large number of identical antibodies, of huge importance in medicine. In this context, huge efforts have been spent to maximize the mAb production in industrial bioreactors by using hybridoma cell cultures. However, the optimal operation of these bioreactors is an engineering problem difficult to solve due to the highly nonlinear bioprocess dynamics, and a bioreactor involving a large number of decision (control) variables, subjected to multiple nonlinear process constraints, which often translates into a non-convex optimization problem. Based on an adequate kinetic model adopted from literature, this paper is aiming at in-silico, off-line deriving and comparing the optimal operating policies of a batch bioreactor (BR), and a fed-batch bioreactor (FBR) operated in several feeding alternatives (including substrates and the viable biomass) with using a hybridoma culture immobilized on a porous support (alginate) for mAb production. FBR with a variable time stepwise optimal feeding policy proved to reach better performances in terms of mAb production maximization with a minimal raw-material consumption.

**Keywords:** monoclonal Antibodies (mAbs); Hybridoma cell culture; Batch or fed-batch bioreactors; Production maximization; Raw material consumption minimization

**Abbreviations:** mAbs: monoclonal Antibodies; BR: Batch Bioreactor; FBR: Fed-Batch Bioreactor

## 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) (Figure 1), 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 productivity optimization in a FBR used for the mAbs synthesis, by employing a hybridoma cell culture (i.e. maximize the molecule production in a minimum of time, with minimizing the raw-materials consumption), by using *in-silico* engineering techniques.

Generally, bioreactors with microbial cultures are currently used to produce a large variety of valuable molecules, being constructed and operated in multiple alternatives as reviewed [1,2]. In spite of their larger volumes, continuously mixed aerated

tank reactors, operated in BR (batch), or FBR (fed-batch) modes (Figure 1), 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. Production of mAbs is a well-known method to synthesize a large number of identical antibodies, of huge importance in medicine. Intense efforts have been spent to maximize the mAbs production in bioreactors. However, the optimal operation of these bioreactors is an engineering problem 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 non-convex optimization problem [3,4].

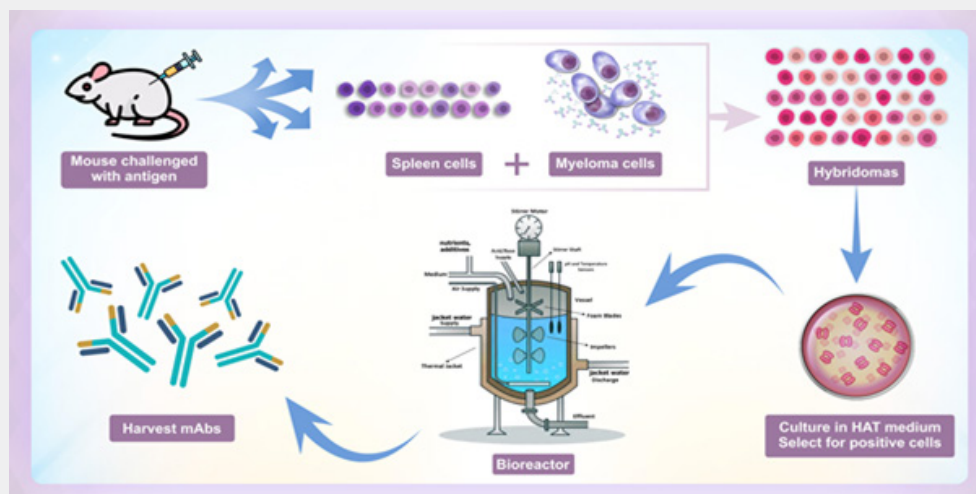
Concerning the used bioreactor, an essential engineering problem to be solved is referring to the development of *optimal operating policies* seeking for production maximization, raw-material consumption minimization, with obtaining a product

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, and [2,3,7-12]);

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) [13,11,14 -20].



**Figure 1:** A simplified representation of the hybridoma technology used to produce monoclonal antibodies (mAbs)/vaccines [adapted from wikipedia, 2023]. [Down] 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 [1]. 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 [1] or on-line [13].

## Discussion and conclusions

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 [14,21-25]. Due to such reasons, the bioreactor optimal operating policies are determined by using heuristic, stochastic, or deterministic optimization rules [1,7,15,4]. 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 bioreactor [23] by using specific numerical algorithms [12,16,20,21,26].

Based on an adequate kinetic model adopted from literature [7], this paper is aiming at *in-silico*, off-line deriving and comparing the optimal operating policies of a batch bioreactor (BR), and a fed-batch bioreactor (FBR) with using a hybridoma culture immobilized on a porous support (alginate). The optimal operation of the FBR is derived in several alternatives, by using a constant, or a variable feeding, with considering a few number of control variables (feed flowrate, GLC/GLN substrates, viable biomass) seeking for the mAb production maximization. The

results [3] indicated that the FBR with a variable time stepwise optimal feeding policy, or with a constant optimal feeding, proved to reach better performances in terms of maximum productivity and raw-material consumption minimization. The present optimization analysis proves its worth by including multiple elements of novelty, as follows [3]:

i) An optimally operated FBR with a small number of time-arcs (that is 5 here), and using wider 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 ( $X_v$ ) as a control variable during FBR optimization (an option seldom discussed in the literature).

iii) The *in-silico*, off-line derived optimal operation of bioreactors is proved as being a very effective engineering tool.

By concluding, 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 a constant optimal feeding obtained from using a multi-objective Pareto-

optimal technique is also an attractive alternative, requiring a much simpler process control. More details are given by Maria et al. [3].

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