École Polytechnique Fédérale de Lausanne

ChE-320: Bioreactor modeling and simulation

Project 4: Fed-batch Reactor

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Introduction

In chemical engineering, fed-batch reactors are an ubiquitous type of reactors specifically used in the pharmaceutical industry. This type of reactor is characterized by a specific operating mode that allows dosing substrates, nutrients, diluent, or any other components of the system throughout the process [1]. It is generally preceded by an initiation phase with different feeding rate concentrations as during the process. Fed-batch reactors are also efficient in addressing substrate inhibition issues. Cells consume the substrate and form the product; however, when the substrate reaches a high concentration, a change in the metabolism of the cells, related to the change in the physical environment, can lead to a dramatic slowdown of the reaction rate. To tackle this challenge, we harvest the product, and the substrate feed rate is maintained below a fixed threshold. The aim of the following study is to model the behavior of a fed-batch reactor, with an initiation phase and a series of filling and harvesting cycles of the tank. The enhancement of the production rate with varying feed flow is also studied to avoid reaching toxic substrate concentrations while ensuring maximum efficiency of the process.

Exercise 1

a)

The quantities, and their corresponding concentrations, of each component at each time-point are found by solving the system of ordinary differential equations, defined in our "model" function and reported in Figure 4. As such, we import and use the "solve_ivp" function from the "scipy.integrate" sub-package. This function integrates the ODEs from the initial conditions "y_0" and over the chosen time span: "t_eval" = np.linspace(0, 1 + t_cycle, 1000)", which generates an interval of 1000 evenly-spaced time-points. Integration is here performed using the "Radau" method, which is an implicit method suitable for stiff problems.

The thus obtained temporal evolution in substrate (S), biomass (X), and product (P) concentrations after being initiated and spending the first cycle of processing and harvesting within a fed-batch bioreactor is represented in Figure 1.

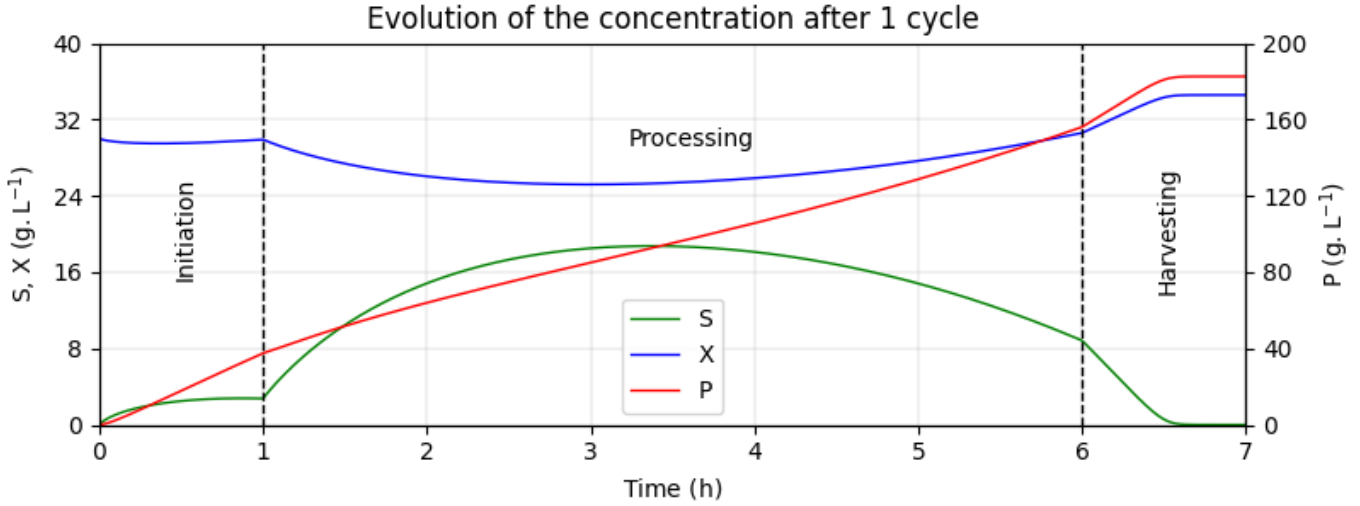

Figure 1: Temporal evolution of concentrations in the bioreactor after initiation and a single processing and harvesting cycle under fed-batch operation

Initially introduced in negligible amounts, the substrate concentration undergoes a concave increase and mild stabilisation at 2.80 g.L^{-1} after the first hour of its initiation. This is consistent with the relatively low glucose feed rate (0.2 L.h^{-1}) and the negligible initial glucose concentration. The glucose introduced is thus quickly consumed by the cells, but said low feed rate allows a slight accumulation of glucose until it stabilises when the input and consumption are balanced. It then has a sharper concave increase and reaches a global maximum of 18.74 g.L^{-1} at 3 hours and 22 minutes; i.e., 2 hours and 22 minutes into the processing step. This can be attributed to the feed rate being more than twice as high as before (0.5 L.h^{-1}), which introduces more glucose. The cellular consumption is known to follow a Monod kinetics here modified by substrate inhibition, and the input initially exceeds the consumption, which leads to the observed increase. The fact that we reach a global maximum indicates that the consumption rate starts to catch up with the inflow.

As the biomass further adapts to its environmental conditions and increases, more cells become available to consume the glucose. Although growth is not exponential due to substrate inhibition, it is sufficient for the total glucose consumption by the biomass to become faster than the continuous glucose input. Consequently, the glucose is gradually depleted, as shown by the concave decrease to a value of 8.84 g.L^{-1} at 6 hours. This latter time-point is also marked by an inflection point which gives rise to a sharp convex decrease and subsequent stabilisation at around 0 g.L^{-1} after 6 hours and 30 minutes. This is expected as the harvest flow rate is five times higher than the processing rate, leading to a rapid elimination of residual glucose until it reaches negligible concentrations.

Product production starts as soon as cells begin to metabolize glucose. Indeed, we observe that the initially zero product concentration continuously increases before eventually stabilising at about 182 g.L^{-1} after 7 hours. It sharply increases, between 0 and 1 hour of initiation, to a value of 37.52 g.L^{-1}. This rapid increase suggests that the growth rate is initially high and glucose is consumed quickly. It then undergoes a less steep increase, during five hours, to reach 156 g.L^{-1} after processing. This slower variation is likely a consequence of a reduced cell growth rate and a more pronounced manifestation of uncompetitive substrate inhibition.

During harvesting, there is a resurgence of a more pronounced increase starting from 6 hours, which can be explained by the rapid dilution (2.5 L.h^{-1}) of other components, causing a decrease in the culture's volume and concentration of the remaining products. It then gradually reaches a plateau of around 182 g.L^{-1} after about 30 minutes because the substrate is completely depleted and the cells can no longer generate product.

The initial concentration is said to be very low and the feed rate is low (0.2 L.h^{-1} with 80 g.L^{-1} of glucose). Thus, there would be a balance between the substrate consumption rate by the cells and its addition rate into the medium. The glucose would therefore be consumed as it is added, which would not allow significant accumulation leading to appreciable biomass growth. This is precisely what we observe, since the biomass concentration stays relatively constant at 30 g.L^{-1} during initiation. From 1 to 2.99 h, it then starts to significantly decrease, to reach a global minimum of 25.19 g.L^{-1}. This decline in biomass is probably due to a lag phase where the cells adapt to the increased availability of the substrate, combined with the inhibitory effect of glucose at high concentration, which reduces the net growth rate.

After adapting, cells begin to grow more rapidly, and the biomass gradually increases again. Due to the continuous substrate supply, it even slightly surpasses its initially introduced concentration at

the end of the processing step by attaining 30.59 g.L^{-1}. During harvesting, the cell concentration rapidly increases for 30 minutes. This could be rationalized by the dilution, which can temporarily concentrate the cells due to the effect of the high harvest rate. Once the substrate is depleted, the biomass concentration stabilizes at 34.56 g.L^{-1}. This could be explained by the fact that we reach a steady state where the components' input and output rates are balanced.

b)

Using a Python differential equation solver on a modulo three step model (initiation, production and harvesting), a simulation is run for a fed batch initiation and ten production and harvesting cycles (Figure 2). In particular, the modulo value (time step %total time) allows for the selection of the appropriate differential equations for the specific step one wants to model.

Figure 2 shows an oscillatory behaviour, related to the cycling from filling to partial emptying of the tank. When harvesting occurs, sudden decreases in cell biomass and product occurs. The decrease of substrate, on the other hand, is directly correlated to the increase in cells. During initiation and the first cycle, the product concentration increases steadily. One sees that the cells must initially adjust to their new fed batch environment, thus their growth during the first cycle is smaller and their consumption of substrate is lower. It takes three cycles for the system to reach the start of the quasi-equilibria; product, cells and substrate concentration vary in the same manner regardless of the cycles number.

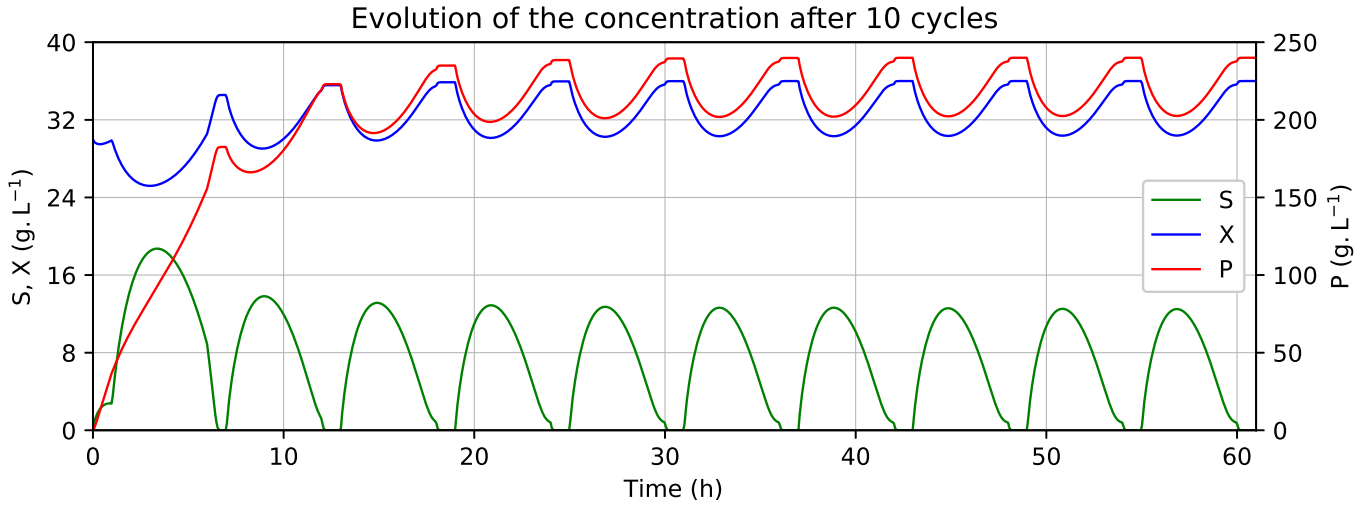

Figure 2: Initiation followed by ten complete cycles of cells processing and harvesting

Two methods were used to calculate production rates. When one considers the cell production during harvesting to be negligible with respect to the outflow, one subtracts the mass remaining after harvesting from the mass gathered at the end of the production and divides by the cycle time to find 98.11 g/h. Thus, one would consider the cell harvesting flow rate to be much greater than the production rate. When such an assumption is not valid, one considers Equation 1.

$$P_{\mathrm{h}} = \int \frac{F_h N_h^P}{V_h} dt \tag{1}$$

Where F_h is the harvesting flow rate, V_h is the fluctuating volume, N^P is the mass of the product in the tank and P is the product concentration. One integrates the infinitesimal concentration of

product in the tank during a harvesting time dt and divides with a cycles time, yielding 97.66 g/h. Thus one considers both the product mass produced during harvesting and the mass thrown out due to the output. In the given case, during harvesting one produces 38.40 g of product, while 585.98 g are taken out. The first method entails neglecting those 38.40 g, thus generating an error of approximately 6.6% in mass generation.

Exercise 2

In order to maximize the production rate of the product P, the feed concentration of substrate during the processing time (S_p) can be optimized. For S_p ranging from 0 to 200 $g_{glucose}.L^{-1}$, the evolution of the production rate of product during the cycle is plotted in Figure 3.

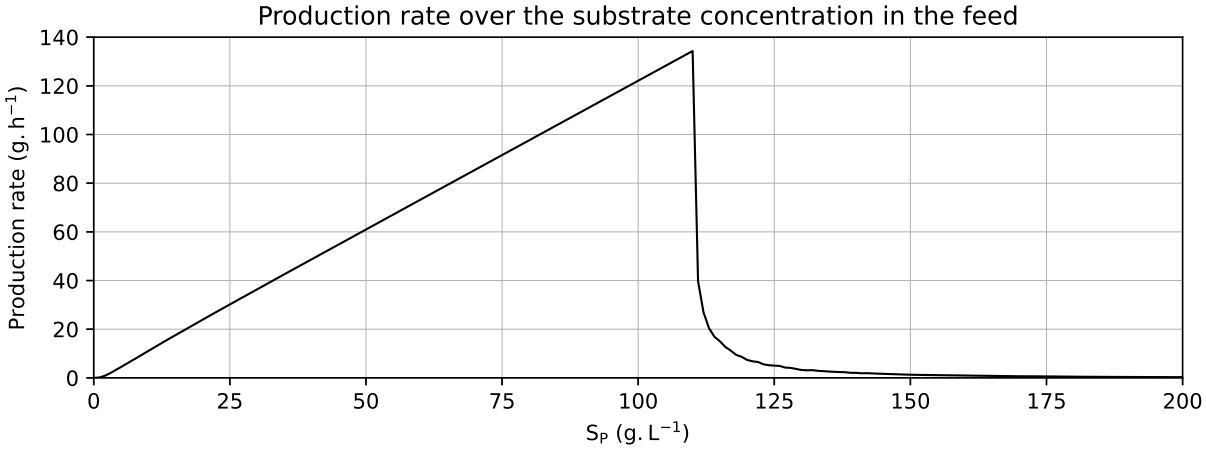

Figure 3: Evolution of the production rate (g.h^{-1}) with the substrate feed concentration (g.L^{-1})

It is seen that for S_p included between 0 and 110 g.L^{-1}, the production rate of product linearly increases with a slope of 1.23. However when P reaches 134 g.h^{-1}, for S_p=110 g.L^{-1}, it suddenly undergoes a drop to 13 g.h^{-1} for S_p=116 g.L^{-1}. This was an expected result, as this system is subject to substrate inhibition. When the substrate surpasses a threshold concentration, the reaction rate rapidly decreases, often due to changes in the cells' metabolism in such environments. Given that the substrate concentration can vary during the process, the optimized choice of S_p should not be too close to the dropping limit. To avoid reaching a toxic substrate concentration that would lead to a poor production rate, S_p can be chosen around 102 g.L^{-1}, allowing for deviations while still maintaining a fair production rate of approximately 125 g.h^{-1}.

Conclusions

In conclusion, through a nested modelling exercise we simulated and optimised the operation of a fed-batch reactor during initialisation, processing and harvesting phases. A three part model was developed, which, according to the times modulo with the full cycle period, selects the system's appropriate differential equation. For a single cycle, one saw a mild substrate and cell stabilisation at respectively 2.80 g.L^{-1} and approximately 29.50 g.L^{-1}. During processing, a dilution of cell biomass is witnessed, as substrate is introduced in larger quantities reaching a maximal concentration of 18.74 g.L^{-1} at 3 hours and 22 minutes. During harvesting, substrate feed is paused, leading to its concentration plummeting as biomass consumes the remaining substrate. One saw that product and

cell concentration increase became inhibited when the substrate is depleted at the end of the cycle (6 hours and 30 minutes). When running the model for an initialisation and ten full cycles, the system adapts during the first three cycles, before reaching a quasi-steady state. Production rates were found to be 98.11 g.L^{-1} when neglecting growth during harvesting, and 97.66 g.h^{-1} otherwise. Optimizing the process to yield the largest product production rate without intoxication of the cells allowed to choose an optimal substrate input value (S_p) of 102 g.L^{-1}, yielding products at 125 g.h^{-1}.

References

[1] Mears, L., Stocks, S.M., Sin, G., & Gernaey, K.V. (2017). A review of control strategies for manipulating the feed rate in fed-batch fermentation processes. Available at: https://www.sciencedirect.com/science/article/pii/S0168165617300251?casa_token=sZwIH9bvuuAAAAAA:49TsIhckPeUk49EpTnWOVGWFhTfW8xjW6VPTgkKIfq6353vj4NcgcE04eqnyqTF4dgeVegRORO2- [Accessed in April 2024].

Annexes

The system of ODEs modeling our system is represented by the following Figure:

Differential Equations	Initiation	Processing	Harvesting
$\frac{dN_X}{dt} =$	$\mu_{net} XV$	$\mu_{net} XV$	$- F_H X + \mu_{net} XV$
$\frac{dN_S}{dt} =$	$F_I S_0 - \frac{\mu_{net} XV}{Y_{X/S}}$	$F_P S_P - \frac{\mu_{net} XV}{Y_{X/S}}$	$- F_H S - \frac{\mu_{net} XV}{Y_{X/S}}$
$\frac{dN_P}{dt} =$	$\frac{\mu_{net} XV}{Y_{X/P}}$	$\frac{\mu_{net} XV}{Y_{X/P}}$	$- F_H P + \frac{\mu_{net} XV}{Y_{X/P}}$
$\frac{dV}{dt} =$	F_I	F_P	$- F_H$

Figure 4: Differential equations modeling the temporal evolution of the mass of cells, substrate, and product, as well as the volume