École Polytechnique Fédérale de Lausanne

ChE-320: Bioreactor modeling and simulation

Project 3: Design of fed-batch bioreactors

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Introduction

Reactor types are commonly discussed in biochemical engineering. The achievement and performance of a reaction involving microoragnisms and living cells are greatly influenced by the environment in which the reaction takes place. A very commonly used type is the fed-batch reactor. Unlike batch reactors that are only filled before the start and then left isolated, fed-batch are provided with substrate of choice, nutrients, diluents or any other component during the process. It enables precise control of the growth medium, and directly influence system dynamic, while enhancing metabolic activity and potentially increasing productivity [1]. Its widespread use makes fed-batch reactor a great topic of study. A variety of setups and reactions will be explored in this sequence of problems, in order to shed light over its performance and dynamics, as well as optimize its use in the industry.

Exercise 1

a)

The mass balance for the cell concentration X is derived as follow (where the volume V of the tank is constant with time):

$$\frac{d(X_1 \cdot V)}{dt} = V \cdot \frac{dX_1}{dt} = FX_0 + \alpha FCX_1 - (1+\alpha)FX_1 + V\mu X_1$$

For a steady state situation, the derived mass balance can be simplified:

$$V \cdot \frac{dX_1}{dt} = 0 \Rightarrow F(X_0 + \alpha C X_1) + V\mu X_1 = (1+\alpha)FX_1$$

As mentioned in the description of the problem, the feed does not contain any cell, i.e X_0=0. Hence one can isolate the specific growth rate μ and introduce D=F/V:

$$\mu = \frac{(1+\alpha)FX_1 - F\alpha CX_1}{VX_1} = D(1+\alpha(1-C))$$

To operate at a dilution factor D larger than the growth rate, the system must verify:

$$0 \leq (1+\alpha(1-C)) \leq 1$$

As α is a fraction, it ranges by definition in [0;1]. Hence the following relations must be verified:

b)

$$-1 \leq \alpha(1-C) \leq 0 \Rightarrow -\frac{-1}{\alpha} \leq (1-C) \leq 0 \Rightarrow 1 \leq C \leq \frac{\alpha+1}{\alpha}$$

The numerical values can be inserted into the expression for μ:

$$\mu = D(1+\alpha(1-C)) = \frac{1500}{1000} \cdot (1 + 0.5 \cdot (1-2)) = 0.75\ h^{-1}$$

c)

The Monod equation provides the growth rate μ as a function of the maximum growth rate μ_m and the K_s constant. It is expressed as:

$$\mu = \frac{\mu_m S}{S + K_s}$$

Substituting in the expression for μ found in the previous question:

$$\mu = D(1+\alpha(1-C)) = \frac{\mu_m S}{S+K_s} \Rightarrow S = \frac{DK_s(1+\alpha(1-C))}{\mu_m - D(1+\alpha(1-C))} = 0.03\ g/L$$

d)

The mass balance for the substrate is obtained in a similar manner than for the cell concentration. First, the rate of substrate consumption is derived from the yield Y:

$$Y_{X/S} = \frac{\mu X}{\mu_S S} \Rightarrow \mu_S = \frac{\mu X}{Y_{X/S} S}$$

$$\frac{d(SV)}{dt} = V \cdot \frac{dS}{dt} = FS_0 + \alpha FS - V\mu_S S - (1+\alpha)FS$$

Again, at steady state, the substrate concentration does not vary with time. Thus the concentration in the recycle stream μ_S can be isolated:

$$\mu_S = \frac{DS_0 + \alpha DS - (1+\alpha)DS}{S} = \frac{D(S_0 - S)}{S} = 98.5h^{-1}$$

And therefore:

e)

$$X_1 = \frac{Y_{x/s}\mu_s S}{\mu} = 1.97\ h^{-1}$$

Using a mass conservation balance:

$$(1+\alpha)FX_1 = FX_2 + \alpha FCX_1$$

Therefore X_2 can be isolated:

f)

$$X_2 = X_1(1 + \alpha(1 - C)) = 0.985\ g/L$$

Using the equation from part a) along with the requirement to have $\mu \leq$D and the fixed C value of 2, a range of possible α values can be found:

$$0 \leq 1 + \alpha(1 - C) \leq 1 \Rightarrow 0 \leq 1 - \alpha \leq 1 \Rightarrow 0 \leq \alpha \leq 1$$

In addition, the maximum value of D, D_m can be obtained as a function of α:

$$D_m = \frac{\mu_m}{1 + \alpha(1 - C)}$$

The cell concentration as a function of the dilution rate with different α are plotted below:

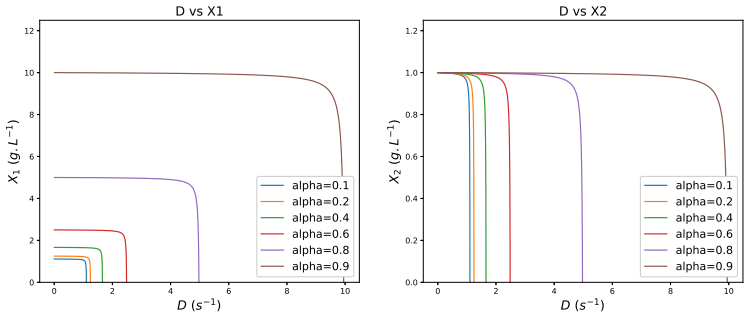

Figure 1: Impact of the recycling ratio α on the cell concentrations X as a function of the dilution rate D.

For X_1, the concentration is higher at higher α values, and stays approximately constant until the dilution rate reaches its maximum, then plunges to 0. For X_2, the concentration is independent of the value of *alpha*, but as for X_1, using a higher α allow for a bigger maximum dilution rate.

The productivity as a function of the dilution rate with different α are plotted below:

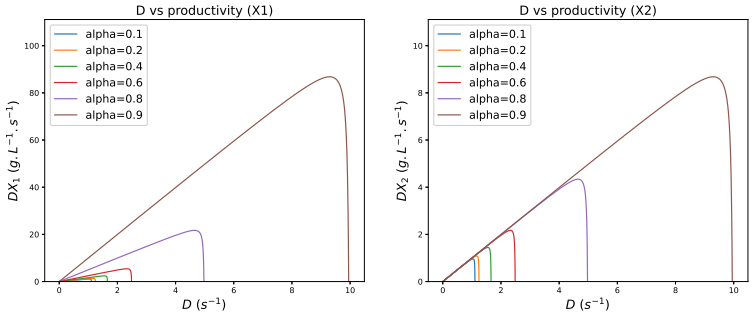

Figure 2: Impact of the recycling ratio α on the productivity DX as a function of the dilution rate D.

For the productivity of X_1, a higher α augments the productivity at the same dilution rate and allows the use of higher dilution rate, increasing the maximum productivity further. For X_2, the productivity stays the same when using the same dilution rate, but as the maximum dilution rate increases with higher value of α, higher maximum productivity are obtained when using a higher α value. When α equals 0, the behavior of the reactor would equate the one of a fed batch with no recycling system.

Exercise 2

a) The fed-batch differential equation for a single cycle is derived below. One defines the total amount of biomass in reactor X^t as $[X] \cdot V(t)$, the change of volume f as $\frac{dV}{dt}$, the dilution rate D as $\frac{f}{V}$ and the total amount of cells in the reactor as $\frac{dX^t}{dt} = \mu \cdot X^t = r_X$. The general mass balance is:

$$\text{Accumulation} = \text{In} - \text{Out} + \text{Reaction} \iff \frac{dN_A}{dt} = F_{\text{A0}} - F_A + \int_0^V R_A \, dV \tag{1}$$

The influx into the semi batch is composed of only substrate (no biomass). The growth rate μ for uncompetitive substrate inhibition is Haldene's equation $\frac{\mu_{max} \cdot [S]}{K_S + [S] + \frac{[S]^2}{K_I}}$. Haldene's (or Andrew's) equation describes a single-substrate inhibition of enzymatic reaction rate.

$$\begin{aligned} \frac{dX^t}{dt} &= \frac{d[X]}{dt} \cdot V(t) + \frac{dV}{dt} \cdot [X] \quad \text{(chain rule)} \\ &\rightarrow \frac{d[X]}{dt} \cdot V(t) = \mu \cdot X^t - f \cdot [X] = \mu \cdot [X] \cdot V(t) - f \cdot [X] \\ &\rightarrow \frac{d[X]}{dt} = [X] \cdot (\mu - D) = [X] \cdot \left(\frac{\mu_{max} \cdot [S]}{K_S + [S] + \frac{[S]^2}{K_I}} - D\right) \end{aligned} \tag{2}$$

A similar equation is derived for the substrate, while considering the substrate input (S_F) and reaction rate $r_S = \frac{r_X}{Y_{X/S}} + m \cdot [X]$ is considered, where the cell maintenance term (m) is neglected.

$$\begin{aligned}
\frac{dS^t}{dt} &= \frac{d[S]}{dt} \cdot V(t) + \frac{dV}{dt} \cdot [S] = f \cdot S_F - r_S \\
&\rightarrow \frac{d[S]}{dt} \cdot V(t) = f \cdot (S_F - S) - r_S \\
&\rightarrow \frac{d[S]}{dt} = \frac{f}{V} \cdot (S_F - S) - \frac{\mu \cdot X}{Y_{X/S}} = D \cdot (S_F - S) - \frac{\frac{\mu_{max} \cdot [S]}{K_S + [S] + \frac{[S]^2}{K_I}} \cdot X}{Y_{X/S}}
\end{aligned} \tag{3}$$

For the product, on the other hand, one defines q_r, where q_r is the specific rate of product formation. The rate of product formation thus becomes $r_P = q_P \cdot X \cdot V$

$$\begin{aligned}
\frac{dP^t}{dt} &= \frac{d[P]}{dt} \cdot V(t) + \frac{dV}{dt} \cdot [P] = r_P = q_P \cdot X \cdot V \\
&\rightarrow \frac{d[P]}{dt} \cdot V(t) = q_P \cdot X \cdot V - f \cdot [P] \rightarrow \frac{d[P]}{dt} = q_P \cdot X - D \cdot f
\end{aligned} \tag{4}$$

Finally, as the volume is not constant, one should not overlook the following equation:

$$\frac{dV}{dt} = f \text{ if V} < 800 \textbf{ or } \frac{dV}{dt} = -600 \text{ if V} >= 800 \tag{5}$$

The initial conditions for the system are $X_0 = 30g/l$, $S_0 = 0g/l$, $P_0 = 0g/l$ and $V_0 = 200L$.

b) From the information above, one understands that the cycle ends when the volume reaches 800 L. Integrating Equation 5, one finds $V(t) = V_0 + f \cdot t \rightarrow t = \frac{600L \ h}{50L} = 12 \ h$.

c)

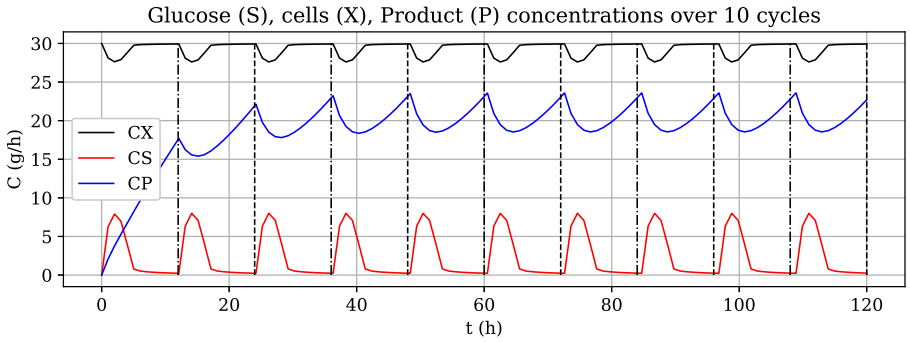

Figure 3: Dashed and Dashdotted lines show the passage of to a new cycle

Consider the concentration in products found at the end of each cycle. One multiplies each concentration by the volume of outflow per cycle (600 L) and sums said values. Thus, after 120 h (10 cycles of 12 h), a total of 136.9 kg of products was produced.

d)

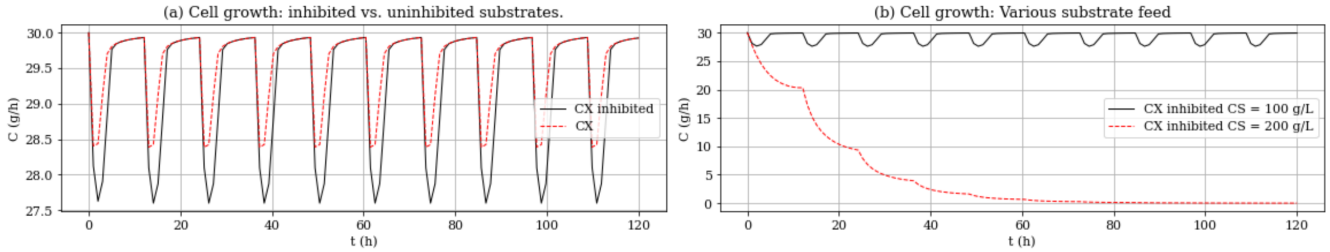

Figure 4: (a) Cell growth concentration evolution (X) for inhibited and non inhibited substrate feed and (b) the effect of increasing initial substrate feeds.

From Figure 4 (a), one observes a clear local inhibition effects, as the drops in the inhibited spectra are approximately 25% deeper compared to cells relying on non-inhibited substrate. As substrate is consumed, biomass can be created to replace the dying biomass. When a substrate undergoes uncompetitive inhibition (ES + S $\rightleftharpoons$ ESS), the inhibitor (excess substrate) binds to enzyme-substrate complexes. It thus becomes temporarily unavailable for cellular consumption, reduces the conversion of substrate to product, and slows down growth. Before the substrate is released from the complex, a larger death due to starvation is seen. Nevertheless, one notes that the cell concentration at the end of each cycle is independent of substrate inhibition. The system itself is thus not affected by inhibition. This could be rationalized by the reversible nature of the process. In fact, the consumption of free substrate triggers the release of additional substrate as the system seeks equilibrium, in accordance with the equation for reversible inhibition (Le Chatelier's Principle)[2].

Figure 5 (b) shows that increasing the amount of substrate in the feed would induce a degeneration and decay of the cell's biomass number, leading to the death of the colony by 5 cycles (60 h). One explains this by the fact that an increase in free substrate translates into more substrate getting sequestered in inactive complexes. This strongly shifts the equilibrium towards enzyme-substrate-inhibitor complexes, rendering the substrate unavailable for cellular consumption.

e)

In the physical case studied, substrate inhibition refers to when the rate of cell growths diminishes due to high substrate concentrations. In this context, substrates are the nutrients necessary for the survival of the cell. Fed batches allow to add the substrate slowly to the medium, alleviating the strong excess in nutrients and thus the substrate inhibition.

f)

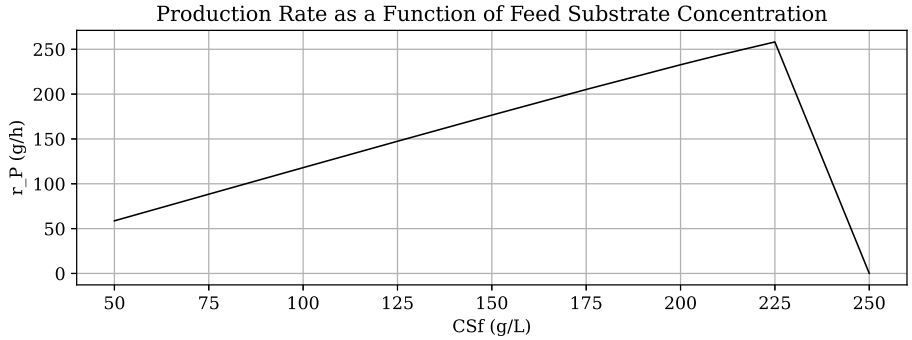

Figure 5: Production rate at the exit of the bioreactor for 10 cycles ($\sum Cs \cdot \frac{600\ L}{120\ h}$)

The production rate increases linearly to a maximum of 250 g/h at a substrate feed concentration of about 225 g/L. This increase can be attributed to the greater availability of substrate, which provides cells with sufficient resources to grow and produce the desired product. However, production

then plummets to 0 g/h when the substrate concentration reaches 250 g/L. This indicates a threshold beyond which production shifts from highly efficient to severely inhibited. This dramatic drop can be explained by the fact that too much substrate could inhibit enzymatic activity, which is crucial for product formation. Indeed, an excess of substrate likely leads to uncompetitive inhibition, where the substrate binds only to the enzyme-substrate complex rather than the free enzyme. Thus, adding even more substrate beyond said threshold would only exacerbate the formation of the inhibited complex and decrease the reaction rate leading to products; which explains the observed sharp decline.

Exercise 3

The first reactor can be modeled as found in project 2:

$$\mu_1 = D_1 \tag{6}$$

$$S_1 = D_1 \cdot \frac{K_S}{\mu_{max} - D_1} \tag{7}$$

$$X_1 = Y_{X/S}(S_0 - S_1) \tag{8}$$

The second reactor is different, because the feed contains some biomass. Starting from mass balances for the substrate and biomass, as well as the Monod's equation, it follows that:

$$\mu_2 = \mu_{max} \cdot \frac{S_2}{K_S + S_2} \tag{9}$$

$$X_2 = X_1 \cdot \frac{D_2(K_S + S_2)}{D_2(K_S + S_2) - \mu_{max} S_2} \tag{10}$$

$$\tag{11}$$

And S_2 is a second order polynomial with:

$$a = Y_{X/S}(\mu_{max} - D_2) \tag{12}$$

$$b = Y_{X/S}(D_2(S_1 - K_S) - \mu_{max} X_1 \tag{13}$$

$$c = Y_{X/S} D_2 S_1 K_S \tag{14}$$

Reactor 1				**Reactor 2**			
V_1 (L)	μ_1 (h^{-1})	S_1 ($g.L^{-1}$)	X_1 ($g.L^{-1}$)	V_2 (L)	μ_2 (h^{-1})	S_2 ($g.L^{-1}$)	X_2 ($g.L^{-1}$)
800	0.125	0.107	4.946	200	0.005	0.004	4.998
200	0.500	0.750	4.625	800	0.009	0.007	4.996
900	0.111	0.094	4.953	100	0.009	0.007	4.997
100	1.000	10.000	0.000	900	0.111	0.094	4.953
1000	0.100	0.083	4.958	0	-	-	-

Table 1: Results for different reactor volumes

The volume dependency for the substrate concentration can be illustrated as follows:

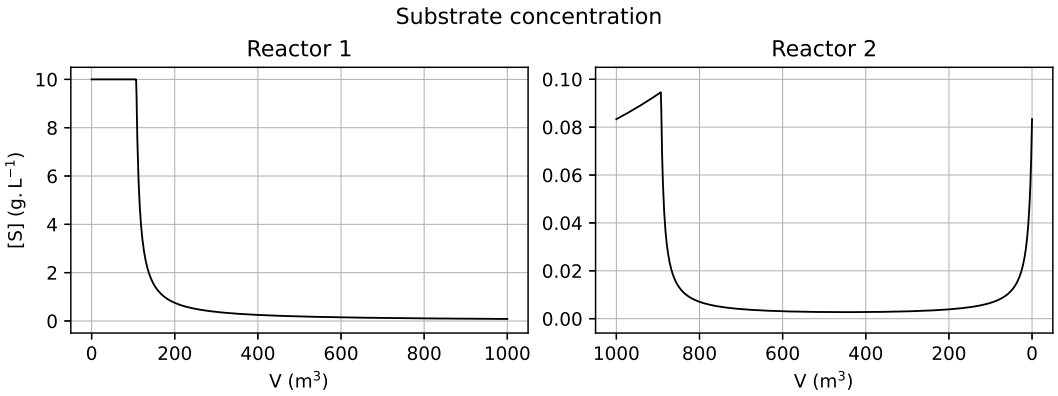

Figure 6: Volume dependency of the substrate concentration in each reactor

This graph shows that the substrate consumption in the second reactor will always be lower than in the first (for a total volume of 1000 m^3 and that in order to optimize the consumption of the substrate, the second reactor should be between a volume range from approximately 200 to 600 m^3, agreeing with Table 1, where the lowest substrate consumption was found to be when the volume was 200 m^3. Volumes outside this range will yield a higher substrate concentration, again seen in Table 1 when the volume was 100 and 800 m^3. It also shows that operating the second reactor with a volume between 1000 and 900 m^3 will yield a higher substrate concentration than using a single reactor of 1000 m^3, which is shown in Table 1 when the volume tested was 900 m^3.

The biomass concentration in both reactors is plotted below:

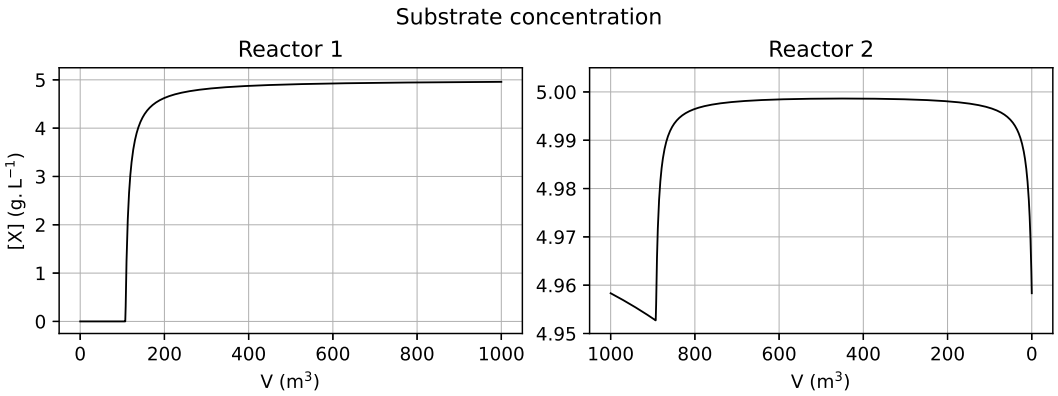

Figure 7: Volume dependency of the biomass concentration in each reactor

The same rationale applied to substrate concentration can be extended to explain biomass concentration in each reactor. Given that the biomass concentration in the second reactor can never be lower than that of the first, employing two reactors is best for optimizing biomass output concentration. The ideal volume range for the second reactor falls between 600 and 200 m^3, as seen with Table 1. However, operating with a volume range between 1000 and 900 m^3 for the second reactor would result in inferior outcomes compared to using a single reactor of 1000 m^3.

Exercise 4

a)

Knowing the equations describing the rate of product (r_p) and biomass (r_x) formation, the productivity of product formation (PD) is expressed as a function of r_p and the dilution rate (D) as:

$$PD = r_p \cdot D = (\alpha \cdot r_x + \beta \cdot X) \cdot D = (\alpha \cdot \frac{\mu_m \cdot S}{K_S + S} \cdot X + \beta \cdot X) \cdot D = (\alpha \cdot D + \beta) \cdot X \cdot D \tag{15}$$

To find the optimal dilution rate (D_{opt}) that maximizes PD, we differentiate PD with respect to D, set it to zero, and apply the product rule:

$$\frac{dPD}{dD} = \frac{d[(\alpha \cdot D + \beta) \cdot X \cdot D]}{dD} = 0 \iff \frac{dX}{dD} \cdot (\alpha \cdot D^2 + \beta \cdot D) + X \cdot (2D \cdot \alpha + \beta) = 0 \tag{16}$$

Since growth rate (μ_g) in a chemostat is limited by at least one substrate, we substitute into the Monod equation [3] and relate the effluent substrate concentration (S) to D, for D $< \mu_m$ (no washout):

$$\mu_g = D = \frac{\mu_m \cdot S}{K_S + S} \iff S = \frac{K_S \cdot D}{\mu_m - D} \tag{17}$$

Using (17), we express the steady-state cell concentration (X) as a function of the yield coefficient ($Y_{X/S}$) and feed substrate concentration (S_0). We then differentiate it with respect to D:

$$X = Y_{X/S} \cdot (S_0 - S) = Y_{X/S} \cdot (S_0 - \frac{K_S \cdot D}{\mu_m - D}) \implies \frac{dX}{dD} = -Y_{X/S} \cdot (\frac{K_S \cdot (\mu_m - D) + K_S \cdot D}{(\mu_m - D)^2}) \tag{18}$$

Substitution of (18) into (16) thus gives:

$$-Y_{X/S} \cdot (\frac{K_S \cdot (\mu_m - D) + K_S \cdot D}{(\mu_m - D)^2}) \cdot (\alpha \cdot D^2 + \beta \cdot D) + Y_{X/S} \cdot (S_0 - \frac{K_S \cdot D}{\mu_m - D}) \cdot (2D \cdot \alpha + \beta) = 0 \tag{19}$$

Numerically, the root of (19) – i.e. the optimal value of dilution rate that maximizes product formation productivity – is found to be: $D_{opt} = 1.73 \cdot 10^{-4}\ s^{-1}$ which converts to $6.23 \cdot 10^{-1}\ h^{-1}$. Graphically, we plot Equation (15) versus D, in Figure (8), and return D_{opt} corresponding to PD_{max}.

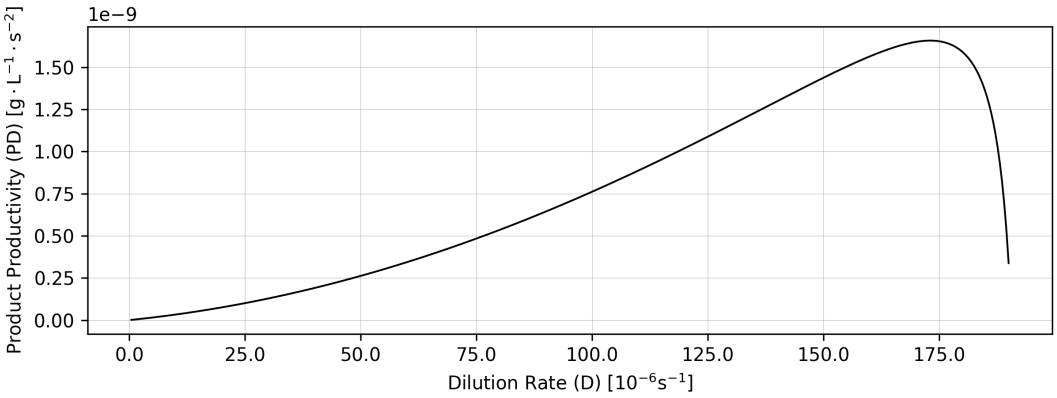

Figure 8: Product Formation Productivity (PD) as a function of Dilution Rate (D)

We get: $D_{opt} = 1.71 \cdot 10^{-4}\ s^{-1}$ ($6.17 \cdot 10^{-1}\ h^{-1}$). Results from both approaches only differ by 1.17%.

b)
The productivity of biomass formation (XD) is given by:

$$XD = r_X \cdot D = D \cdot X \cdot D = X \cdot D^2 \tag{20}$$

We inject(18) into (20), differentiate it with respect to D, and set it to zero to find $\tilde{D}_{\text{opt}}$ [4]:

$$XD = Y_{X/S} \cdot (S_0 - \frac{K_S \cdot D}{\mu_m - D}) \cdot D^2 \implies \tilde{D}_{\text{opt}} = \mu_m(1 - \sqrt{\frac{K_S}{K_S + S_0}}) \tag{21}$$

We therefore compute D_{opt} to be:

$$\tilde{D}_{\text{opt}} = 0.7 \cdot (1 - \sqrt{\frac{20 \cdot 10^{-3}}{20 \cdot 10^{-3} + 1}}) \approx 1.67 \cdot 10^{-4} s^{-1}$$

The optimal dilution rate ($\tilde{D}_{\text{opt}}$) maximizing the productivity of cell formation is therefore about $1.67 \cdot 10^{-4}\ s^{-1}$; i.e. $6.02 \cdot 10^{-1}\ h^{-1}$.

Operating a chemostat at $\tilde{D}_{\text{opt}}$ can jeopardize its stability, unless the flow rate and liquid volume can be maintained exactly constant [3]. We should thus choose a dilution rate slightly less than $\tilde{D}_{\text{opt}}$ to reach a common ground between stability and biomass productivity.
It is also worth mentioning that the $\tilde{D}_{\text{opt}}$ for maximizing biomass formation only differs by 3.00% from the D_{opt} determined to maximize product formation. Such a result is not always valid [3], since the optimal dilution rate for one might not be the most suitable for the other.

Conclusions

In conclusion, these exercises deepened our understanding of fed-batch bioreactors by simulating their dynamics. We first performed mass balances on a fermenter to show that fed-batch systems with cell recycle can operate at dilution rates surpassing the microorganism's specific growth rate. We also explored how varying the parameter α can affect their productivity. Subsequently, we demonstrated that fed-batch systems, unlike batch reactors, effectively mitigate substrate inhibition and provide better control over substrate concentrations, which enhances cell growth and productivity. Our comparative analysis of various bioreactor configurations then confirmed that using two reactors maximizes biomass output, as the biomass concentration in the second reactor always matches or exceeds that of the first. The optimal volume for the second reactor was identified as between 200 and 600 m^3; whilst volumes between 900 and 1000 m^3 should be avoided as they result in higher substrate concentrations at the outlet than a single reactor of 1000 m^3. On another note, we determined the optimal dilution rates for maximizing productivity of product and of cell formation to be approximately 0.620 h^{-1} and 0.602 h^{-1}, respectively. The mere 3.00% difference between the latter values indicate the possibility of having a dilution rate maximizing both species simultaneously within our system, which is not always achievable in all chemostats.

References

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