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CHEMICAL AND BIOPROCESS ENGINEERING DEPARTMENT
BIOSYSTEM ANALYSIS – IIQ3733

Report 1

Simulation and selection of models of microbial bioreactors operating in high cell densities

Group 3

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April 17, 2021

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1 Introduction

Biotechnological applications have increased in recent years and offers an enormous potential for the sustainable production of many products. However, to produce desired bioproducts on industrial scale with high productivity, it is necessary to keep in mind several strategies, such as the correct selection of host strain, improve host tolerance to the product, select the most plausible operating conditions, etc. Lee and Kim (2015).

Escherichia coli is one of the most employed host organism in bioprocess because it is a widely studied prokaryotic model organism. To obtain high productivities in the production of different bioproducts using *E. coli*, it has been necessary the development of high cell density culture (HCDC) techniques, which in addition to achieve high productivities, they allows to add advantages of increased cost effectiveness, reduced culture volume, enhanced downstream processing, reduced wastewater, lower production costs and a reduced investment in equipment Jong Hyun Choia and Lee (2006).

Fed-batch culture has been used frequently to achieve HCDC since allows to reach cell concentrations greater than 50 g(DCW) l-1, but a maximum cell concentration of 200 g(DCW) l-1. The implementation of this culture seems to be a promising strategy for enhance production of bioproducts. However, it has several limitation, including: substrate inhibition, limited oxygen transfer capacity, the formation of growth-inhibitory by-products, and limited heat dissipation Lee (1996).

One of the major issues of HCDCs is the production of acetate when *E. coli* is grown under anaerobic conditions, however, acetate can also be produced by cultures growing in the presence of excess glucose, even under aerobic conditions Lee (1996). This process is known as glucose overflow metabolism and some studies have suggested that it is triggered by high specific growth rate, high specific glucose uptake rate, bottlenecks in the Krebs cycle, limited respiratory capacity, or a combination of any of the above Xu et al. (1999). A high concentration of acetate (above 5 gl-1 at pH 7) reduces growth rate, biomass yield and maximum attainable cell densities in HCDCs Lee (1996).

To overcome this drawback, different strategies have been developed to reduce the formation by controlling the specific growth rate by limiting essential nutrients and controlling the variables such as temperature, mixing, dissolved oxygen, and accumulation of CO_2. Even It is possible to reduce acetate formation through metabolic engineering Lee (1996).

Another critical strategy to the success of HCDC is the selection of nutrient feeding because it affects the metabolic pathway fluxes, and consequently affects the maximum attainable cell concentration, the specific productivity and formation of products. Simple feeding methods such as constant-rate feeding, stepwise increased feeding rate and exponential feeding have been successfully used to obtain high cell density of *E. coli* Jong Hyun Choia and Lee (2006). While Constant-rate feeding and stepwise increased allow to decreased the specific growth rate, exponential feeding let the specific growth rate to remain at a constant level. There are other more sophisticated feeding methods that incorporate feedback control, e.g. pH-star, DO-stat, Carbon dioxide evolution rate (CER) or Cell concentration Lee (1996).

This work focused on the comparison of 3 models designed by different authors which used some of the strategies described above to address the aforementioned problems of high density crops. These models correspond to those proposed by Xu et al., Anane et al. and Dewasme et al.

2 Models

2.1 Xu model

In this work by Xu et al. (1999) a kinetic model of aerobic growth was explained, where the metabolism corresponded to the glucose overflow of the *E. Coli* W3110 strain, which was developed in batch and fed-batch cultures. This model considers the consumption of glucose, as a limiting substrate, for the generation of biomass and energy for the maintenance of the system, and also considers the consumption and generation of acetate.

In addition, this work presented simulations describing the transient accumulation of acetate, respiration and growth of the strain using unlimited glucose as substrate. It was also shown that in the presence of glucose the rate of acetate consumption was faster than in the absence of glucose, and there was no variation in the source of acetate (endogenous or exogenous). What is important to note about this model is that endogenous acetate (produced by glucose overflow) had inhibitory effects on the cells, i.e., the higher the concentration, the lower the maximum specific growth rate, but the biomass yield with glucose was not affected. Finally, the most sensitive parameters to highlight from the model were the maximum pyruvate flux to respiration and the maximum rate of glucose uptake.

Glucose consumption is modeled by Monod-type kinetics with acetate (fermentation product) inhibition:

$$q_S(S,A) = q_{S_{max}} \cdot \frac{S}{K_s + S} \cdot \frac{1}{1 + \frac{A}{K_{I,A}}} \tag{1}$$

Where S and A are the concentrations of glucose and acetate respectively, q_s and $q_{S_{max}}$ are the specific rate of glucose consumption and maximum glucose consumption respectively. finally, Ks corresponds to the average rate constant of glucose consumption and KIA to the inhibition constant for acetic acid in glucose consumption.

It should be noted that, consumption of the carbon source can occur entirely oxidatively ($q_{S_{ox}}$) or fermentative ($q_{S_{of}}$), subject to the following restriction on the specific rate of oxygen consumption:

$$q_{O_S} \leq \frac{q_{O_{max}}}{1 + \frac{A}{K_{i,o}}} \tag{R1}$$

Where $q_{O_{max}}$ is the specific rate of maximal oxygen consumption, and $k_{i,o}$ is the inhibition

constant exerted by acetic acid on oxygen consumption. The overflow kinetic model of the *E. coli* strain used in this work starts with glucose consumption according to the Monod model described above, to give way to fermentative metabolism, in case constraint (R1) is active, or to oxidative metabolism in case constraint (R1) is not activated, since, at low sugar consumption rates, all sugar is channeled through the oxidative pathway ($q_{S_{ox}} = q_S$) Xu et al. (1999). The flux from the oxidative pathway is further divided into a flux used for anabolism and the remainder is used in a flux for oxidative energy metabolism. The sugar flux to oxidative anabolism is described as:

$$q_{S_{ox,an}} = (q_{S_{ox}} - q_m) Y_{X/S,ox} \frac{C_X}{C_S} \tag{2}$$

While aerobic energy metabolism is described as:

$$q_{S_{ox,en}} = q_{S_{ox}} - q_{S_{ox,an}} \tag{3}$$

And this is oxidized through respiration, so the oxygen used for glucose oxidation (q_{O_S}) is defined as:

$$q_{O_S} = q_{S_{ox,en}} Y_{O/S} \tag{4}$$

Replacing equation (2) and (3) in (4) we have that:

$$q_{O_S} = \left(q_S - (q_S - q_m) \cdot Y_{S_{ox}X} \cdot \frac{C_X}{C_S} \right) \cdot Y_{SO} \tag{4.1}$$

It should be noted that if the constraint (R1) is active, $q_{O_S} = q_{O_{max}}$, and combining both cases we have the following restriction:

$$q_{O_S} = \min \left(\left(q_S - (q_S - q_m) \cdot Y_{S_{ox}X} \cdot \frac{C_X}{C_S} \right) \cdot Y_{SO}, q_{O_{max}} \right) \tag{R2}$$

It is important to mention that overflow metabolism is initiated when the glucose concentration increases because the rate of glucose uptake progressively increases, observing a maximal respiration rate ($q_{O_{max}}$), which is inhibited by acetate Kleman and Strohl (1994),Xu et al. (1999), so in this investigation the term of non-competitive inhibition with a constant $K_{i,o}$ was included. Therefore, to satisfy the boundary condition $q_{O_{S_S}/(1+A/K_{i,o})}$, the values for oxidative energy metabolism ($q_{S_{S_{ox,en}}}$) and anabolism ($q_{S_{ox,an}}$) are reduced proportionally, so the resulting glucose flux is described as:

$$q_{S_{ox}} = q_{S_{ox,an}} + q_{S_{S_{ox,en}}} \tag{5}$$

The specific rate of oxidative consumption ($q_{S_{ox}}$) is obtained from replacing equations (2), (3) and (4) in (5) as a function of the constraint (R2), where the following relation remains:

$$q_{S_{ox}} = \frac{\frac{q_{O_S}}{Y_{SO}} - q_m \cdot Y_{S_{ox}X} \cdot \frac{C_X}{C_S}}{1 - Y_{S_{ox}X} \cdot \frac{C_X}{C_S}} \tag{5.2}$$

As for the rate of glucose directed to overflow metabolism is obtained from the difference between total glucose consumption (q_S) and total glucose oxidative flux ($q_{S_{ox}}$) shown below:

$$q_{S_{of}} = q_S - q_{S_{ox}} \tag{6}$$

Where $q_{S_{of}}$ represents the specific rate of glucose consumption in fermentative regime.

The contribution to growth of this excess glucose flux is obtained from equation (2) and derives to an anabolic flux for biomass generation, while the remaining is used in energy generation, this is represented respectively according to the following equations:

$$q_{S_{of,an}} = q_{S_{of}} \cdot Y_{X/S,of} \cdot \frac{C_X}{C_S} \tag{7}$$

$$q_{S_{of,en}} = q_{S_{of}} - q_{S_{of,an}} \tag{8}$$

The rate of acetate formation can be obtained from this flux by the stoichiometry of conversion of glucose to acetate represented below in equation (9), and replacing equations (7) and (8) in (9) gives a relation for the specific rate of acetate generation according to equation (9.1)

$$q_{A_p} = q_{S_{of,en}} \cdot Y_{A/S} \tag{9}$$

$$q_{A_p} = (q_{S_{of}} - q_{S_{of}} \cdot Y_{S_{of,x}} \cdot \frac{C_X}{C_S}) \cdot Y_{SA} \tag{9.1}$$

When glucose uptake is very low, and acetate is present in the medium, it is reconsumed. The specific rate of acetate consumption is assumed to follow the Monod model and is represented as follows:

$$q_{A_c} = q_{A_{c,max}} \frac{A}{A + K_A} \tag{10}$$

The authors of this paper explain that a hypothetical flux to anabolism can be obtained from mass balances on carbon fixation in the cell by analogy with equation (2) according to the following equation,

$$q_{A_{c,an}} = q_{A_c} \cdot Y_{X/A} \cdot \frac{C_X}{C_A} \tag{11}$$

The acetate flux for respiratory combustion is obtained from the subtraction of the anabolic flux from the total acetate uptake restricted to the remaining respiration capacity released by glucose metabolism,

$$q_{A_{c,en}} = q_{A_c} - q_{A_{c,an}} \leq (q_{O_{max}} - q_{O_S}) / Y_{O/A} \tag{12}$$

It is important to mention that replacing equations (10) and (11) in (12) gives the oxygen restriction, which together with the kinetics of acetate consumption according to Monod was used to obtain the specific rate of acetate consumption by calculating the minimum between these 2

parameters.

$$q_{A_c} = \min\left(\frac{q_{A_{c,max}} \cdot A}{A + K_A}, \frac{(q_{O_{max}} - q_{O_S}) \cdot Y_{OA}}{1 - Y_{AX} \cdot \frac{C_X}{C_A}}\right) \tag{10.1}$$

Finally, the total oxygen consumption rate is calculated as a sum of the parts used for glucose and acetate oxidation, which is represented in equation (13). And the specific growth rate is obtained from the sum of the 3 substrate fluxes by the related performance coefficients (equation 14) as explained by the authors of this research .

$$q_O = q_{O_S} + q_{A_{c,en}} \cdot Y_{O/A} \tag{13}$$

$$\mu = (q_{S_{ox}} - q_m) \cdot Y_{S_{ox}X} + q_{S_{of}} \cdot Y_{S_{of}X} + q_{A_c} \cdot Y_{AX} \tag{14}$$

The description of this whole kinetic overflow model is summarized in the figure below where the number of each equation coincides with the order in which they must be executed.

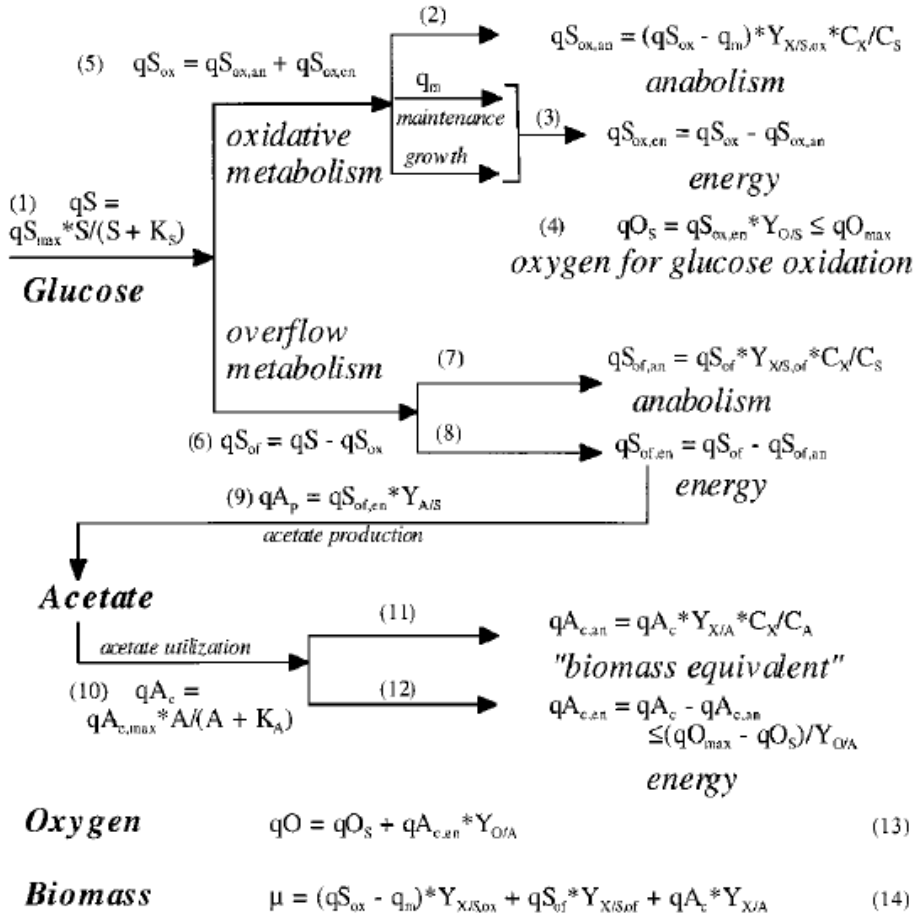


Figure 1: Kinetic model of metabolic overflow in *E. coli* W3110, Xu et al. (1999)

The differential equations for this model are:

$$\frac{dS}{dt} = \frac{F}{V} \cdot (S_{feed} - S) - q_S \cdot X \tag{15}$$

$$\frac{dA}{dt} = (q_{A_p} - q_{A_c}) \cdot X - \frac{F}{V} \cdot A \tag{16}$$

$$\frac{dX}{dt} = \mu \cdot X - \frac{F}{V} \cdot X \tag{17}$$

$$\frac{dV}{dt} = F \tag{18}$$

For the substrate balance in equation (13), the consume term is formed by q_S representing the usage of it for the growth, that could be on the respirative or the respiro-fermentative regime. Also, there's an inlet of substrate and a dilution term on the balance.

For the product present in equation (14) we have the difference of the rates for production and consume of it, plus a dilution term.

In equation (15) we see the typical mass balance of the biomass where it has a growth term and a dilution term. The growth term is composed by all the rates explained before.

Finally the equation (16) refers to the volume of the reactor. The parameters are presented in the Table 2 in the appendix.

2.2 Dewasme Model

In this model the overflow metabolism is described by non-linear expressions for the oxidation and fermentation rates of the microorganism. Three key reactions for growth are distinguished:

Substrate oxidation (associated with the growth of the microorganism):

$$k_{S1}\ S + k_{O1}\ O \xrightarrow{r_1\ X} k_{X1}\ X + k_{C1}\ C \tag{19}$$

Substrate fermentation (associated with the overflow metabolism):

$$k_{S2}\ S + k_{O2}\ O \xrightarrow{r_2\ X} k_{X2}\ X + k_{P2}\ P + k_{C2}\ C \tag{20}$$

Byproduct oxidation (associated with the growth of the microorganism):

$$k_{P3}\ P + k_{O3}\ O \xrightarrow{r_3\ X} k_{X3}\ X + k_{P2}\ P + k_{C2}\ C \tag{21}$$

The rate r_1 is defined as:

$$r_1 = \frac{\min(r_S, r_{S_{crit}})}{k_{S1}} \tag{22}$$

With:

$$r_S = \mu_S \frac{S}{S + K_S} \tag{23}$$

$$r_{S_{crit}} = \frac{r_O}{k_{os}} = \frac{\mu_O}{k_{OS}} \frac{O}{O + K_O} \frac{K_{iP}}{K_{iP} + P} \tag{24}$$

Where we can see that r_S follows the Monod laws and $r_{S_{crit}}$ follows an inhibitory effect of the byproduct. This relation with the min function illustrates the threshold between a respirative regime and a respiro-fermentative regime. The respirative regime means that the substrate is used on it's entirety for the growth, and this is only when the substrate becomes limiting ($r_S < r_{S_{crit}}$). The respiro-fermentative regime activates when there's an excess of substrate, and the microbe uses it for the generation of the byproduct ($r_S > r_{S_{crit}}$).

The rate r_2 is defined as:

$$r_2 = \frac{\max(0, r_S - r_{S_{crit}})}{k_{S2}} \tag{25}$$

This rate represent it's activation when $r_S > r_{S_{crit}}$, which means that the respiro-fermentative regime is present on the growth, and there will be production of the byproduct on the reactor.

The rate r_3 is defined as:

$$r_3 = \frac{\max\left(0, \frac{k_{os}(r_{S_{crit}} - r_S)}{k_{op}} \frac{P}{P + K_P}\right)}{k_{P3}} \tag{26}$$

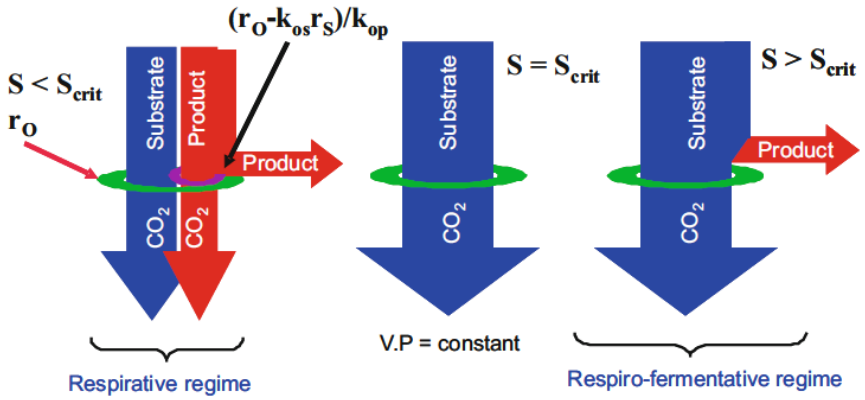


Figure 2: Bottleneck assumption for cells limited respiratory capacity and the relation of the substrate concentration on the regime (Dewasme et al., 2011).

This expression illustrates the usage of byproduct when the growth is on the respirative regime, and it's maximum consume rate is determined by the oxygen availability.

Then, the growth rate of the microorganism is represented by:

$$\mu = k_{X1} r_1 + k_{X2} r_2 + k_{X3} r_3 \tag{27}$$

The optimal condition where we want to operate the reactor is at the boundary of this two regimes, meaning that the fermentation and byproduct oxidation rates are equal to zero. If we look at the expressions of the rates we can conclude that:

$$r_1 = r_S = r_{S_{crit}} = \frac{r_O}{k_{os}}$$

$$r_2 = 0$$

$$r_3 = 0$$

From the value of r_1 on this optimal condition, we can see how the respiratory capacity of the microorganism has a crucial influence on the critical substrate concentration, so we can assure oxygen availability is important for the optimal growth. For modeling purposes, it will be assumed that all oxygen needed for growth will be supplied to the culture, via an oxygen controller.

The differential equations for this model are:

$$\frac{dX}{dt} = (k_{X1}r_1 + k_{X2}r_2 + k_{X3}r_3)X - DX \tag{28}$$

$$\frac{dS}{dt} = -(k_{S1}r_1 + k_{S2}r_2)X + DS_{in} - DS \tag{29}$$

$$\frac{dP}{dt} = (k_{P2}r_2 - k_{P3}r_3)X - DP \tag{30}$$

$$\frac{dO}{dt} = -(k_{O1}r_1 + k_{O2}r_3)X - DO + k_L a(O_{sat} - O) \tag{31}$$

$$\frac{dC}{dt} = (k_{C1}r_1 + k_{C2}r_3)X - DC + k_L a(C - C_{sat}) \tag{32}$$

$$\frac{dV}{dt} = F_{in} \tag{33}$$

In equation (10) we see the typical mass balance of the biomass where it has a growth term and a dilution term. The growth term is composed by all the rates explained before.

For the substrate balance in equation (11), the consume term is formed by $k_{S1}r_1$ and $k_{S2}r_2$ representing the usage of it for the growth, that could be on the respirative or the respiro-fermentative regime. Also, there's an inlet of substrate and a dilution term on the balance.

For the product present in equation (12), we have the difference of the rates for production and consume of it, plus a dilution term.

For the oxygen balance in (18) we have the consume rates of it by the microorganism, a dilution term and a mass transfer rate from the interface between fluid and gas. The equation (19) it's analogous to the equation (18) but with a term of generation of CO_2.

Finally the equation (20) refers to the volume of the reactor. The parameters are presented in the Table 3 in the appendix.

2.3 Anane Model

Anane et al. (2017) modeled the metabolism in *Escherichia coli* using the concept acetate cycling with an initial batch phase following by a fed batch phase applying a exponential feed rate and then a constant feed rate. In contrast with previous authors who describe acetate profiles using functions of discontinuous nature, they included the novel of define a set of differentiable and continuous equations, which is better for complex optimization problems since is not necessary to deal with min or max restrictions. Furthermore, they include the effect of the sensor in the measure of the dissolved oxygen adding a new differential equation with the effect of the response time related to the sensors, the authors added glucose pulses and the model can react faster with the addition of the last equation.

Firstly, the state variables X (biomass), S (substrate) and F (feed) are modelled as in a conventional fed-batch fermentation process. Considering that inlet concentration of biomass is zero, the biomass balance in the fed-batch is expressed as follows

$$\frac{dX}{dt} = \frac{F}{V} \cdot (0 - X) + \mu X \tag{34}$$

With X being the concentration (cell dry weight) of cells ans μ is the non-inhibited Monod-type specific growth rate, which take the form:

$$\mu = (q_{sox} - q_m) Y_{em} + q_{sof} Y_{xsof} + q_{sA} Y_{xa} \tag{35}$$

Where q_{sox}, q_{sof} and q_{sA} represent the uptake rates of the substrate for oxidation, substrate metabolized through the overflow route and acetate, respectively, q_m represents the glucose expended for cell maintenance and Y is defined as the respective yield. Also, q_{sox}, q_{sof} and q_{sA} are related with growth of the culture by contributing as energy equivalents.

The mass balance for substrate is given as

$$\frac{dS}{dt} = \frac{F}{V} \cdot (S_i - S) - q_s X \tag{36}$$

The susbtrate concentration S is modelled taking acetate inhibition into account, therefore the specific substrate uptake rate is modelled with Mono-type kinetics with non-competitive inhibition:

$$q_S = \frac{S}{K_s + S} \cdot \frac{q_{Smax}}{1 + \frac{A}{K_{I,A}}}$$

Where $K_{I,A}$ and K_s are the acetate inhibition and the substrate affinity constants respectively. The model considers that not all the substrate consumed is metabolized in the TCA cycle, thus a portion goes to the overflow path q_{sof}

$$q_{sox} = (q_s + q_{sof}) \cdot \frac{DOT}{DOT + K_o}$$

With

$$q_{sof} = \frac{P_{Amax} q_s}{q_s K_{ap}}$$

The parameter k_o is a dimensionless constant set to 0.1 to increase the stability of the numeric simulation, P_{Amax} and K_{ap} are the maximum acetate production and the production affinity constants, respectively. Acetate production and consumption is a cyclic process, considering that inlet concentration of acetate is zero in the feed, its mass balance is:

$$\frac{dA}{dt} = \frac{F}{V} \cdot (0 - A) + q_{sA} X \tag{37}$$

The equilibrium $q_{sA} = 0$ when the production of acetate through the overflow route p_A is equal to the consumption of acetate q_{sA}:

$$q_A = p_A - q_{sA}$$

With

$$p_A = q_{sof} Y_{sA}$$

Y_{sA} is the gram of acetate per gram of substrate consumed through the overflow route. The specific acetate consumption rate is modelled as

$$q_{sA} = \frac{q_{Amax}}{1 + \frac{q_s}{K_{is}}} \cdot \frac{A}{A + K_{sa}}$$

With q_{Amax}, K_{is} and K_{sa} being constant parameters that represents the maximum acetate uptake rate, the acetate uptake inhibition and acetate affinity constant, respectively. Furthermore, the acetate uptake is inhibited in a non-competitive way by the glucose in the medium because of *E. coli* preference for glucose over acetate.

Lastly, the model include a balance of actual dissolved oxygen (DOT_a) which is calculated in % of saturation with the assumption that feed solution in the fed-batch phase is fully saturated with dissolved oxygen. This balance is given as

$$\frac{dDOT_a}{dt} = K_{La}(DOT^* - DOT_a) - q_o X H \tag{38}$$

Where DOT^* is the saturation value of dissolved oxygen in the medium, K_{La} the volumetric mass transfer coefficient, H the Henry equilibrium constant, and q_o the oxygen rate which are described with

$$q_o = (q_{sox} - q_m) Y_{os} + q_{sA} Y_{oa}$$

Where Y_{os} and Y_{oa} are the yield coefficients for the substrate and acetate to oxygen consumption respectively. With the probe response, the measure DOT is described by

$$\frac{dDOT}{dt} = K_p(DOT_a - DOT) \tag{39}$$

Where K_p is the state gain of the sensor, which is the inverse of the probe response time t.

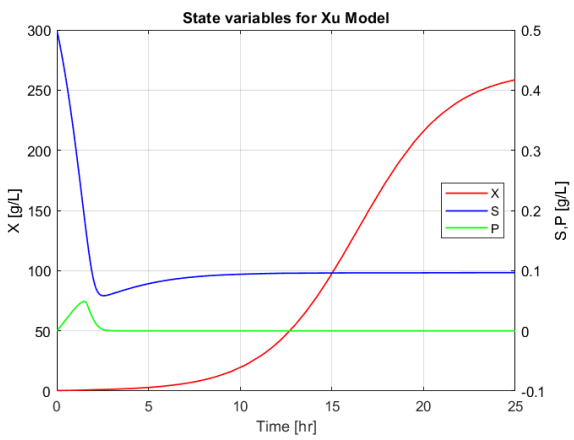
3 Simulation Results & Discussion

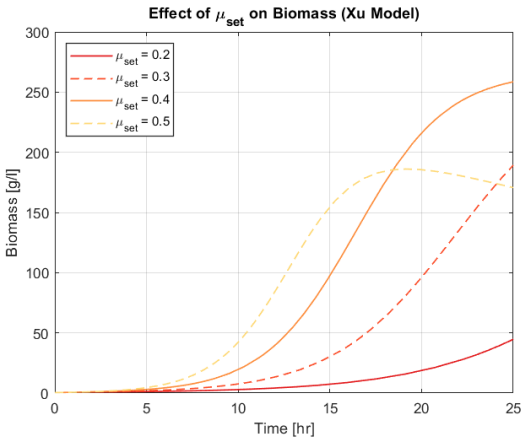
The three models were tested with an exponential feed rate from $t = 0$ to the final time declared in the model's papers. The feed rate expression is:

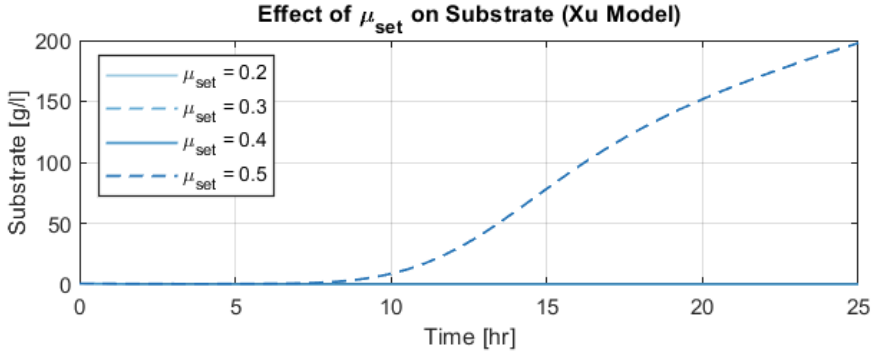
$$F(t) = \frac{\mu_{set}}{Y_{xs}\, S_{in}} (XV)_0 \exp\left(\mu_{set} \cdot t\right) \tag{40}$$

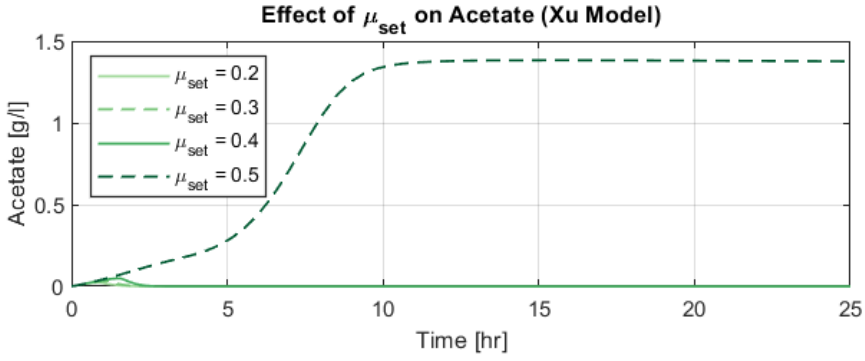
Where the value of μ_{set} is chosen to obtain that value for the growth rate, since the overflow metabolism is modeled, there will be an optimal μ_{set} to obtain the highest amount of biomass.

3.1 Xu Results

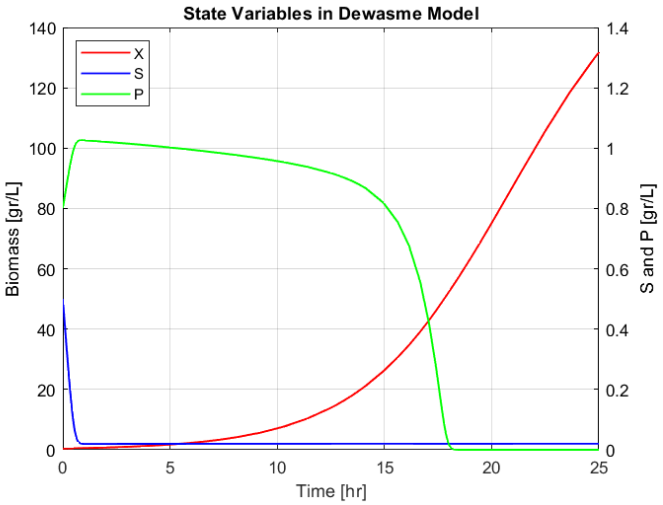


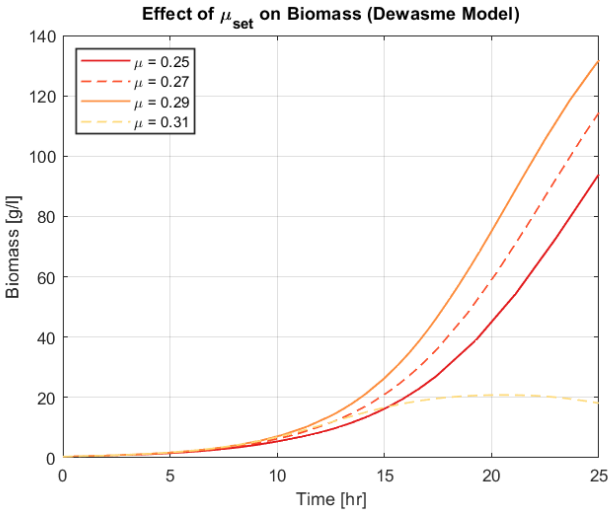


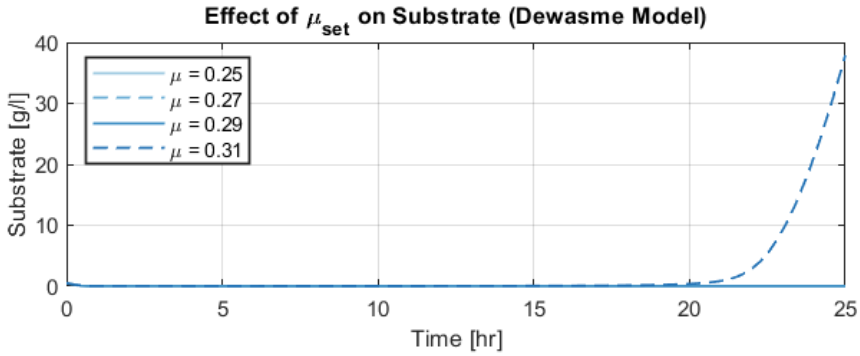


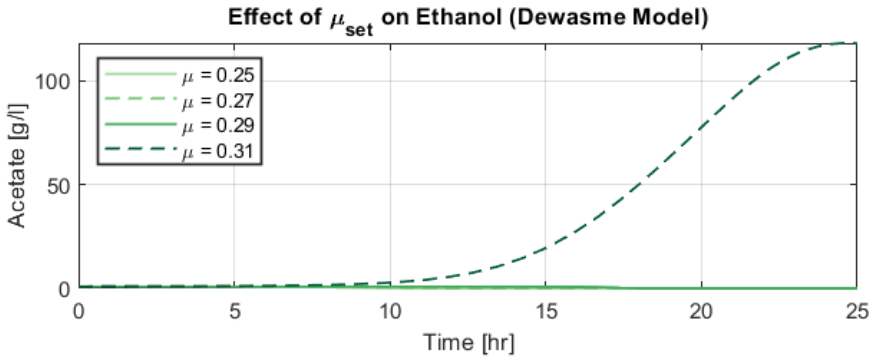


3.2 Dewasme Results

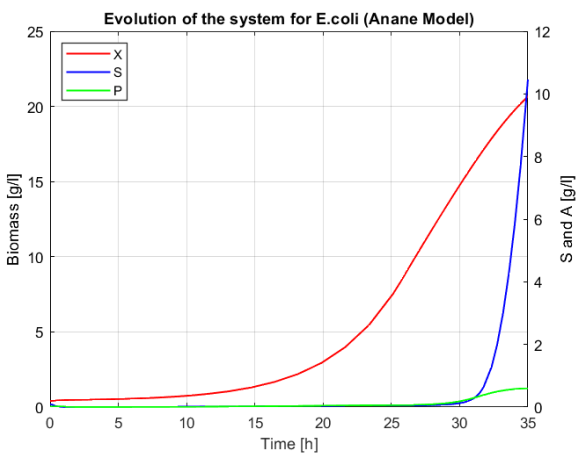


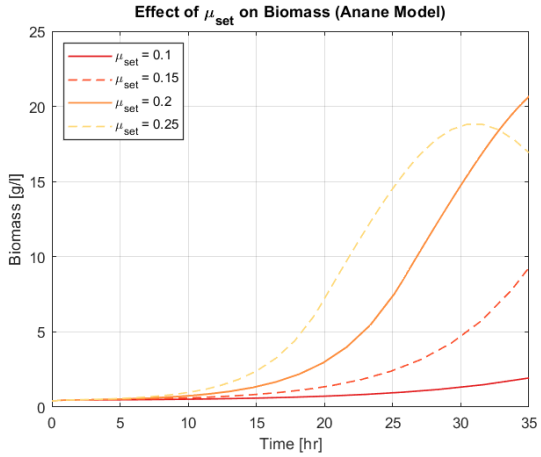


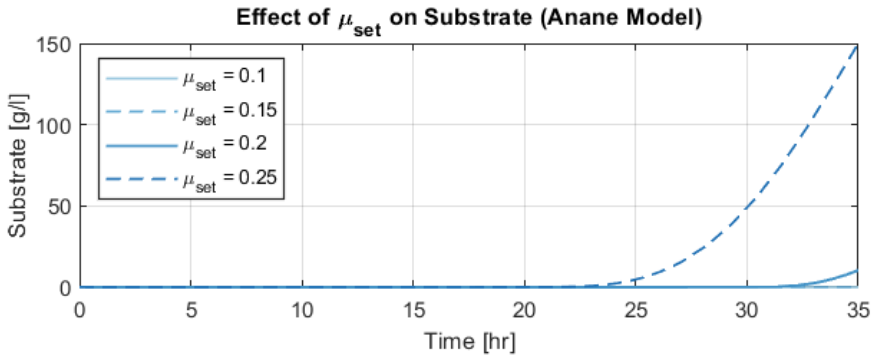




3.3 Anane Result







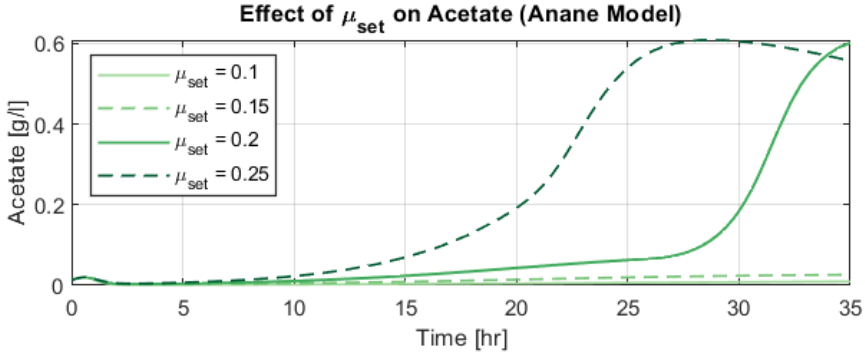


Table 1: Simulation summary

Model	$t_{end}[hr]$	$X\ [g/L]$	$P\ [g/L]$	V [L]
Anane	35	20.65	0.602	8.3677
Dewasme	25	131.71	0	29.166
Xu	25	258.56	0	220.56

The code of the simulations can be found on this Github Repository.

3.4 Discussion

We observe that the models capture the essence of a fed-batch reactor and most important the overflow metabolism. We can appreciate from the graphs the importance of keeping the byproduct as low as possible so that it doesn't affect the growth of the microbial culture, since the main objective is to obtain as much biomass as possible.

The Xu and Anane models use parameters for an *Escherichia coli* culture, while Dewasme models for *Saccharomyces cerevisiae*. We observed differences in the final biomass values, however, our interest is to capture the behavior of this type of growth, so that we can then adjust our own parameters using the university fermentation laboratory.

There's an optimal value of μ_{set} for each type of culture that we can predict with this model, only characterizing values for the yield coefficients of the microorganism, that can be determined using a continuous bioreactor.

We assumed that the oxygen control was implemented on each simulation, using a high value of $k_L a$, so that oxygen would not become a constraint in modeling the reactor.

4 Conclusions & Proposed Future Work

From the Sonnleitner and Käppeli (1986) "bottleneck" modelation for prokariotic cells in fed batch reactors with the three different metabolic paths many new models with several modelations are developed, with broad application in *E. coli* and *S. Cereviseae* cultures. Between them, the most highlighted are the models of Anane et al. (2017), Dewasme et al. (2011) and Xu et al. (1999).

The three models were tested with a similar exponential feed rate setting the μ_{set} according to the μ_{crit} of each model and the values reported in the paper of Annane were 0.1, 0.15, 0.2 and 0.25 $[h^{-1}]$. These different value results in different profile for biomass, substrate and acetate production. Regarding Dewasme model, the values reported were 0.25, 0.27, 0.29 and 0.31 $[h^{-1}]$, and as well as Annane model, different value results in different profile for the same state variables. Xu have similar results, in this paper the values reported were 0.2, 0.3, 0.4 and 0.5 $[h^{-1}]$, and with each value different profiles were obtained.

It is possible to observe from the model results that Dewasme and Xu models were able to simulate High density cultures since both reach biomass concentration higher than 50 g/L, which is the minimum value used to consider a HCDC. After 25 hours of simulation, Dewasme and Xu reach approximate biomass concentrations of 125 g/L and 255 g/L, respectively.

In future work is expected to control variables like the dissolved oxygen (DO), which wasn't covered in the current report. Furthermore, is expected include pH variable with additional equations. Later, with data that will be provided by DIQB and with the developed model and fits parameters. It is important to emphasize that being a pre-fed strategy it is very sensitive to the parameters and therefore these must be correctly estimated. Then iterate and optimize the feeds rate with different feed rates how is described in Varmaa and Kumar (2017) with special emphasis in exponential feed rate due it is the most useful in the fed batch research.

Moreover, it is expected to apply an extended Kalman Filter (EKF) as Dewasme et al. (2013) who used the model described in the section 2.2. This is because for dynamic systems as fed batch can be difficult to control due the inefficient sensibility of probes of substrate (i.e glucose sensor) and EKF appears a suitable option to measure substrate and others variables.

The Xu model was substantially higher in biomass concentration and total biomass production than the other two models. This was in part, due to the fact that this model did not considered dissolved oxygen as a state variable. This is specially important because the high amount of final mass and density in the reactor causes a shift in the partial pressure of the gas dissolved in the liquid. Also it's important to notice that it's more difficult to maintain oxygen control as the reactor volume increases, because the stirring gets more expensive and cost efficient, and the global mass transfer coefficient decreases. Thus the Xu model does not have necessarily the best performance.

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6 Annexes

Table 2: Parameters and its value in modified Xu model

Parameter	Description	Value
$Y_{S_{ox}X}$	Oxidative yield of glucose in biomass	$0.51\left(\frac{g\,DCW}{g\,S}\right)$
$Y_{S_{of}X}$	Fermentative yield of glucose in biomass	$0.15\left(\frac{g\,DCW}{g\,S}\right)$
Y_{AX}	Yield of biomass in acetate	$0.4\left(\frac{g\,DCW}{g\,A}\right)$
Y_{OA}	Yield of acetate in oxygen	$1.067\left(\frac{g\,A}{g\,O_2}\right)$
Y_{SA}	Yield of acetate in glucose	$0.667\left(\frac{g\,A}{g\,S}\right)$
Y_{SO}	Yield of oxygen in glucose	$1.067\left(\frac{g\,O_2}{g\,S}\right)$
C_X	Molar carbon content per gram of biomass	$1/30\left(\frac{mol_C}{g\,DCW}\right)$
C_A	Molar carbon content per gram of acetate	$1/30\left(\frac{mol_C}{g\,A}\right)$
C_S	Molar carbon content per gram of glucose	$1/30\left(\frac{mol_C}{g\,S}\right)$
q_m	Specific rate of glucose consumption for cell maintenance	$0.04\left(\frac{g_S/g_{DCW}}{h}\right)$
q_{Smax}	Specific maximum glucose consumption rate	$1.25\left(\frac{g_S/g_{DCW}}{h}\right)$
q_{Acmax}	Specific maximum acetate consumption rate	$0.2\left(\frac{g_A/g_{DCW}}{h}\right)$
q_{Omax}	Specific maximum oxygen consumption rate	$0.43\left(\frac{g_{O_2}/g_{DCW}}{h}\right)$
K_S	Average glucose consumption rate constant	$0.05\left(\frac{g_S}{L}\right)$
K_iA	Inhibition constant for acetic acid in glucose consumption	$5\left(\frac{g_A}{L}\right)$
K_A	Average acetate consumption rate constant	$0.05\left(\frac{g_A}{L}\right)$

Table 3: Parameters and its value in Dewasme model

Parameter	Description	Value
k_{X_1}	Yield coefficient for biomass	$0.49\left(\frac{g \text{ of } X}{g \text{ of } S}\right)$
k_{X_2}	Yield coefficient for biomass	$0.05\left(\frac{g \text{ of } X}{g \text{ of } S}\right)$
k_{X_3}	Yield coefficient for biomass	$0.72\left(\frac{g \text{ of } X}{g \text{ of } E}\right)$
k_{S_1}	Yield coefficient for substrate	$1\ (-)$
k_{S_2}	Yield coefficient for substrate	$1\ (-)$
k_{P_2}	Yield coefficient for ethanol	$0.48\left(\frac{g \text{ of } E}{g \text{ of } S}\right)$
k_{P_3}	Yield coefficient for ethanol	$1\ (-)$
k_{O_1}	Yield coefficient for oxygen	$0.3968\left(\frac{g \text{ of } O_2}{g \text{ of } S}\right)$
k_{O_2}	Yield coefficient for oxygen	$0\left(\frac{g \text{ of } O_2}{g \text{ of } S}\right)$
k_{O_3}	Yield coefficient for oxygen	$1.104\left(\frac{g \text{ of } O_2}{g \text{ of } E}\right)$
k_{C_1}	Yield coefficient for carbon dioxide	$0.5897\left(\frac{g \text{ of } CO_2}{g \text{ of } S}\right)$
k_{C_2}	Yield coefficient for carbon dioxide	$0.4621\left(\frac{g \text{ of } CO_2}{g \text{ of } S}\right)$
k_{C_3}	Yield coefficient for carbon dioxide	$0.6249\left(\frac{g \text{ of } CO_2}{g \text{ of } E}\right)$
μ_O	Maximum value of specific growth rate	$0.256\left(\frac{g \text{ of } O_2}{g \text{ of } X \cdot h}\right)$
μ_S	Maximum value of specific growth rate	$3.5\left(\frac{g \text{ of } S}{g \text{ of } X \cdot h}\right)$
K_O	Monod constant for oxygen	$0.0001\left(\frac{g \text{ of } O_2}{L}\right)$
K_S	Monod constant for substrate	$0.1\left(\frac{g \text{ of } S}{L}\right)$
K_E	Monod constant for ethanol	$0.1\left(\frac{g \text{ of } E}{L}\right)$
K_{iE}	Inhibition constant for ethanol	$10\left(\frac{g \text{ of } E}{L}\right)$

Table 4: Parameters and its value in Anane model

Parameter	Description	Value
K_{ap}	Monod-type intracellular acetate production	0.5052 $(-)$
K_{sa}	Affinity constant, acetate consumption	0.0134 $\left(\frac{g}{L}\right)$
K_o	Affinity constant, oxygen consumption	0.001 $\left(\frac{g}{L}\right)$
K_s	Affinity constant, substrate consumption	0.0370 $\left(\frac{g}{L}\right)$
K_{ia}	Inhibition of glucose uptake by acetate	1.2399 $\left(\frac{g}{L}\right)$
K_{is}	Inhibition of acetate uptake by glucose	2.1231 $\left(\frac{g}{L}\right)$
p_{Amax}	Maximum spectate of acetate production rate	0.2268 $\left(\frac{g}{g\,h}\right)$
q_{Amax}	Maximum spectate of production rate	0.1148 $\left(\frac{g}{g\,h}\right)$
q_m	Spectate maintenance coefficient	0.0129 $\left(\frac{g}{g\,h}\right)$
q_{Smax}	Maximum spectate of glucose uptake rate	0.6356 $\left(\frac{g}{g\,h}\right)$
Y_{as}	Yield of acetate on substrate	0.9097 $\left(\frac{g}{g}\right)$
Y_{oa}	Yield of oxygen on acetate	0.5440 $\left(\frac{g}{g}\right)$
Y_{xa}	Yield of biomass on acetate	0.5718 $\left(\frac{g}{g}\right)$
Y_{em}	Yield exclusive maintenance	0.5333 $\left(\frac{g}{g}\right)$
Y_{os}	Yield of oxygen on glucose	1.5620 $\left(\frac{g}{g}\right)$
Y_{xsof}	Biomass Yield from the overflow route	0.2268 $\left(\frac{g}{g}\right)$