IDENTIFICATION OF A REACTION MECHANISM FOR A CLASS OF ANIMAL CELL CULTURES*

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Abstract. A reaction mechanism for an animal cell culture of adherent cells (VERO cells) is proposed. This model is validated in different experimental conditions (including batch and renewed cultures) with a model-based estimator of the biomass. An important original feature of the model is to take a dozen of amino acids into account instead of glutamine only as usual in animal cell cultures modeling.

Keywords. modeling of animal cell culture, VERO cell cultures

INTRODUCTION

Animal cell culture is a domain of growing importance in health care industry. Stationary cultures are nowadays replaced by the culture in bioreactors which offer reproducibility and more reliable control of the growth. The optimization of these cultures is the keypoint of the development of products or vaccines to increase the yield in a quantitative but also in a qualitative way. The first step of the optimization is the modeling of the culture. The model must be a copy of the behaviour of the culture and has to hold even when the experimental conditions are varied. The complexity of animal cell cultures makes the modeling issue a really difficult task compared to microorganisms culture modeling. The available models of the literature describe hybridomas cultures (see [1] to [7]). These models take only two substrates into account, the glucose and the glutamine. We believe that these two substrates are not enough to render the behaviour of the animal cell culture for varied experimental conditions. Therefore, we involve all the amino acids or more precisely a selection among all of them in the model. We focus here on VERO cell cultures which are adherent cells and not on hybridomas as in other studies. In this paper, we will propose a basic model of VERO cell culture (model A) and an interesting improvement of it (model B). Then, we will validate these two models with data obtained in different experimental conditions and compare the performances of both models.

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RESULTS AND DISCUSSION

Model A Amino acids are the building blocks of the cells. Traditionally only one amino acid is taken into account in the models, the glutamine. We believe that it is necessary to also involve other amino acids. As a matter of fact, we observe that in batch culture (see Fig 3) when the glutamine is totally consumed, the growth continues. There is a shift of the metabolism to other amino acids and therefore, we can not restrict the choice of the amino acids to glutamine only. We have selected 13 amino acids varying in parallel with the biomass and we have constructed the variable A which is the sum of these amino acids. The selected amino acids are aspartate, glutamine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, tryptophane. These are significantly present in the culture and their consumption appears to be associated with the growth. The other 7 amino acids are not taken into account for the following reasons : 1) alanine and serine : either consumed or accumulated for different experimental conditions; 2) glutamate : intermediate product for the degradation of other amino acids; 3) glycine, proline, asparagine, threonine : measurements noisy or not available.

We propose the following reaction scheme to de-

scribe the culture:

$$
\begin{aligned}
c_1A + En &\rightarrow X && \text{(A.1)}\\
c_2A &\rightarrow N + En && \text{(A.2)}\\
G &\rightarrow 2P + En && \text{(A.3)}\\
P &= L && \text{(A.4)}\\
P + 3O_2 &\rightarrow 3CO_2 + 3H_2O + En && \text{(A.5)}
\end{aligned}
$$

where En represents energy, X biomass, A amino acids, G glucose, N ammonia, P pyruvate, L lactate. c_1 and c_2 are biological stoichiometric coefficients that have to be calibrated from the data. This reaction scheme is motivated as follows: Amino acids are the building blocks of the cells: eq. (A.1). The energy is mainly supplied by the transformation of glucose into pyruvate in the glycolysis: eq. (A.3) and the consumption of the pyruvate in the Kreb's cycle: eq. (A.5). Some energy is also provided by the degradation of the amino acids: eq. (A.2). The pyruvate is transformed in lactate and we also suppose that this reaction is reversible: eq. (A.4). This last hypothesis is quite unusual in mammalian cell modeling but it corresponds to our experimental observations: lactate can be consumed when the glucose is totally consumed (see Fig 1).

Assuming that the process takes place in a stirred tank, we can write the dynamical equations of the mass balances from this reaction network:

$$
\begin{aligned}
\dot{X} &= r_1 && (1)\\
\dot{A} &= -c_1r_1 - c_2r_2 + D(A_{in} - A) && (2)\\
\dot{N} &= r_2 - DN && (3)\\
\dot{G} &= -r_3 + D(G_{in} - G) && (4)\\
\dot{P} &= 2r_3 + r_4 - r_4' - r_5 - DP && (5)\\
\dot{L} &= -r_4 + r_4' - DL && (6)
\end{aligned}
$$

where r_1, r_2, r_3, r_4, r_4' and r_5 are the reaction kinetics; r_4 for the transformation of pyruvate in lactate and r_4' for the reverse reaction, D is the renewal rate:

$$D = \frac{renewed\ volume}{total\ volume} \cdot \frac{1}{\Delta t} \qquad (7)$$

Note that, here, the first 3 equations (1), (2), (3) are totally decoupled from the last 3 equations (4), (5), (6) and therefore the variables X, A and N are totally decoupled from the variables G, P, and L. The coupling exists only through the energy (En) and the mass balance does not involve this value. We eliminate the two unknown r_1 and r_2 from the first 3 equations :

$$c_1\dot{X} = -\dot{A} + D(A_{in} - A) - c_2(\dot{N} + DN) \qquad (8)$$

We now integrate and discretize this equation and we define the following values, at time t: X_{tot_t}, the total amount of biomass produced; N_{tot_t}, the total amount of ammonia produced; A_{tot_t}, the total amount of amino acids consumed and G_{tot_t}, the total amount of glucose consumed :

$$
\begin{aligned}
X_{tot_t} &= X_t - X_o\\
N_{tot_t} &= N_t - N_o + \sum_{k=o}^{t-1} \frac{V_{ren_k}}{V_{tot_k}} N_k\\
A_{tot_t} &= A_o - A_t + \sum_{k=o}^{t-1} \frac{V_{ren_k}}{V_{tot_k}} (A_{in_k} - A_k)\\
G_{tot_t} &= G_o - G_t + \sum_{k=o}^{t-1} \frac{V_{ren_k}}{V_{tot_k}} (G_{in_k} - G_k)
\end{aligned}
$$

where indices 'tot' and 'ren' are respectively total and renewed. It follows, if we omit the indices k:

$$
\begin{aligned}
& c_1X_{tot} = A_{tot} - c_2N_{tot}\\
\Rightarrow\ & X_{tot} = k_1A_{tot} - k_2N_{tot} && (9)
\end{aligned}
$$

where $k_1 = \frac{1}{c_1}$ and $k_2 = \frac{c_2}{c_1}$.
The stoichiometric coefficients c_1 and c_2 are thus identifiable from the measurements of X, A, N.

Model B In other studies, cell growth is provided by consumption of both glucose and glutamine (see [1], [2], [4], [5], [6], [7]). Inspired by this idea, we have considered in model B that cell growth can result from consumption of glucose and amino acids together, as expressed in eq. (B.2) here after. On the other hand, we observe that growth can continue even when glucose is totally consumed (see Fig 1): this implies that we must keep equation (B.1) in the model which expresses this fact (growth on amino acids without glucose). The model B is thus the following:

$$
\begin{aligned}
c_1A + En &\rightarrow X && \text{(B.1)}\\
c_2A + c_3G &\rightarrow X + 2c_3P + En && \text{(B.2)}\\
c_4A &\rightarrow N + En && \text{(B.3)}\\
P &= L && \text{(B.4)}\\
P + 3O_2 &\rightarrow 3CO_2 + 3H_2O + En && \text{(B.5)}
\end{aligned}
$$

where $c_i (i = 1, ..., 4)$ are biological stoichiometric coefficients. As previousely, we can write the dynamical equations of the mass balances in a stirred tank:

$$
\begin{aligned}
\dot{X} &= r_1 + r_2\\
\dot{A} &= -c_1r_1 - c_2r_2 - c_4r_3 + D(A_{in} - A)\\
\dot{N} &= r_3 - DN\\
\dot{G} &= -c_3r_2 + D(G_{in} - G)\\
\dot{P} &= 2c_3r_2 + r_4 - r_4' - r_5 - DP\\
\dot{L} &= -r_4 + r_4' - DL
\end{aligned}
$$

Note that we do not have decoupling any more. We eliminate r_1, r_2 and r_3. from the first 4 equations :

$$
\begin{aligned}
\dot{X} = \ & \tfrac{1}{c_1}(-\dot{A} + D(A_{in} - A)) - \tfrac{c_4}{c_1}(\dot{N} + DN)\\
+ \ & \tfrac{1}{c_3}(1 - \tfrac{c_2}{c_1})(-\dot{G} + D(G_{in} - G))
\end{aligned}
$$

We then integrate and discretize as above. It follows, if we omit the indices k:

$$X_{tot} = k_3 A_{tot} + k_4 G_{tot} - k_5 N_{tot} \qquad (10)$$

where $k_3 = \frac{1}{c_1}$, $k_4 = \frac{1}{c_3}(1 - \frac{c_2}{c_1})$ and $k_5 = \frac{c_4}{c_1}$
It appears now that the stoichiometric coefficients c_i are no longer identifiable. From equations (9) and (10) we can derive estimators of the biomass.
Model A: $\dot{X}_{tot_A} = k_1 A_{tot} - k_2 N_{tot}$ (11)
Model B: $\dot{X}_{tot_B} = k_3 A_{tot} + k_4 G_{tot} - k_5 N_{tot}$ (12)
We can now validate the models A and B by the study of the reliability of these estimators in varying experimental conditions. Five different experiments covering a variety of experimental conditions have been carried out : 1) three batch cultures (duration 9 days) with different initial glucose concentrations of 1 g/l, 2 g/l and 3 g/l (experiments labelled respectively G1, G2 and G3: see Fig 1, 2, and 3 and 4); 2) two periodically renewed cultures (duration 17 days), either with daily renewals (experiment R5, Fig 5) or with 3 renewals at 6th, 9th and 12th day (experiment R6, Fig 6). We identify the coefficients k_i (i = 1, ..., 5) by linear regression with experiments G1, G3 and R5 and we cross-validate these values by the estimation of the biomass of experiments G2 and R6. The results of estimation of the biomass are reported in Fig 7a to 7e. The estimators can be compared using the following criterion which expresses the relative estimation error over an experiment:

$$E = \frac{\{\sum_{k=t_o}^{t_{final}} |X_{tot_k} - \hat{X}_{tot_k}|^2\}^{\frac{1}{2}}}{\sum_{k=t_o}^{t_{final}} X_{tot_k}} \qquad (11)$$

The final forms of the estimators (11) and (12) are different only in the term in N_{tot}. This point suggests to study the importance of each of the variables A_{tot}, N_{tot} and G_{tot} in the estimation as presented in Table 1.

Table 1: errors of estimation, E, calculated for the experiment R6. The errors have been calculated for 5 different models. These models are based on the variables of the columns 2 to 4, with the coefficients k_i (i = 1, ..., 5) identified by linear regression with data of experiments G1, G3 and R5.

model	variables used for the estimation			coefficients			E (*100)
A	a a		NH_3	k_1		k_2	4.67
A'	*aa*			k_1		0	4.43
B	*aa*	*gl*	NH_3	k_3	k_4	k_5	3.17
B'	*aa*	*gl*		k_3	k_4	0	2.95
C		*gl*	NH_3		k_4	k_5	8.53
D	*GLN*	*gl*		k_3'	k_4	0	6.46

aa stands for amino acids; *gl* for glucose

As shown in Table 2, three conclusions can be drawn from the Table 1. We call E_i, where i = A, A', B, B', C, D, the estimation error for model i.

Table 2 : conclusions from Table 1.

	Observations	Conclusions
1°)	$E_C \gg E_A, E_{A'}, E_B, E_{B'}, E_D$	the estimation must involve the amino acids
2°)	$E_B < E_A$ $E_{B'} < E_{A'}$	the estimation is better when the glucose is included in the estimator
3°)	$E_A \approx E_{A'}$ $E_B \approx E_{B'}$	the estimation does not require the ammonia

These comparisons indicate that models B or B' are better than models A or A'. The Fig 7a to 7e represent the biomass estimates obtained with model B' compared to the measured values of the biomass. The coefficients for the estimation in the 5 cases are the same, the estimator is: $\dot{X}_{tot_{B'}} = 5.13\ 10^6\ A_{tot} + 0.162\ 10^6\ G_{tot}$. We have also represented the biomass estimate based on a model including only glutamine and glucose for the experience R6 (Fig 7e). Two conclusions can be drawn from these figures: 1°) the estimation performed with the model B is really reliable in the 5 different experimental conditions studied here. 2°) the estimation performed only with glutamine and glucose is less good than when the 13 amino acids are included in the estimator.

CONCLUSION

We have developed a model of animal cell culture and we have validated this model for 5 different experimental conditions including batch and renewed cultures. We have compared the reliability of different biomass estimators based on amino acids, glucose and/or ammonia and we have shown that the best estimation is obtained by an estimator including amino acids and glucose. We have identified a subset of 13 amino acids appropriate for the biomass estimation and we have shown that the biomass estimation is improved when these amino acids are taken into account instead of only the glutamine as usually described in the literature. We have identified a set of parameters valid for the five experimental conditions studied here. It is worth to notice that this method does not require the knowledge nor the modeling of reaction kinetics.

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MATERIEL AND METHODS

VERO cells (passage 138-150) were cultured in spinner flasks (250 ml) on Cytodex 1 (3 g/l) using M199 medium supplemented with foetal calf serum (10 % at the inoculation and 5 % for medium renewals) and antibiotics. Cell counting was performed with haemacytometer using crystal violet staining (the cell density is expressed in cells per ml). Lactic acid and glucose were measured with a Yellow-Spring analyser (YSI 2000). Ammonia was determinated with Sigma kit (n° 170). The amino acids were performed by ion exchange HPLC method.

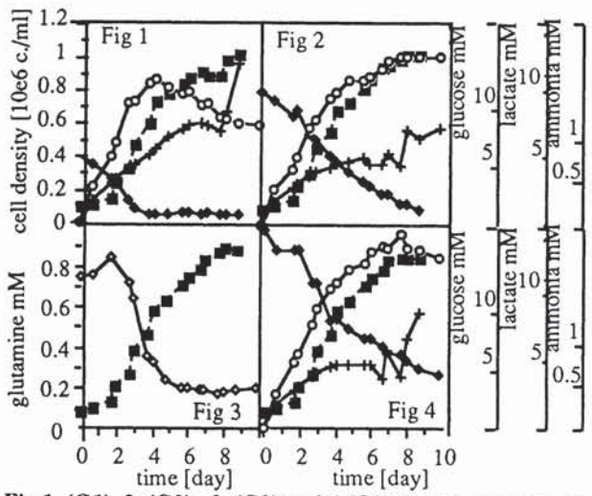

Fig 1 (G1), 2 (G2), 3 (G3) and 4 (G3) : batch cultures with initial glucose concentration of 1 (G1), 2 (G2) and 3 g/l (G3) : cell density (-■-), glucose (-◆-), lactate (-○-), ammonia (-+-) and glutamine (-◇-) concentrations.

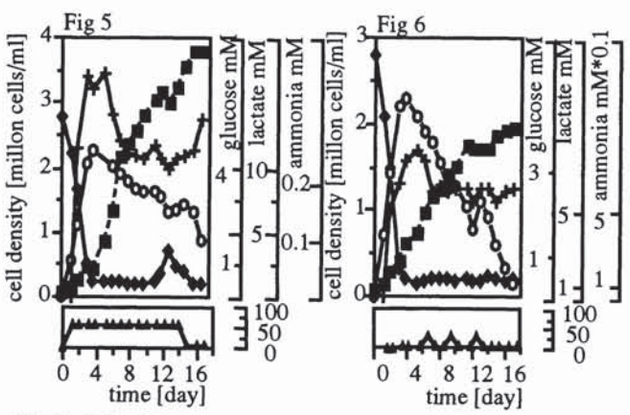

Fig 5 (R5), 6 (R6) : renewed cultures (see text) : cell density (-■-), glucose (-◆-), lactate (-○-), ammonia (-+-), concentrations and renewal rate [%] (-▲-).

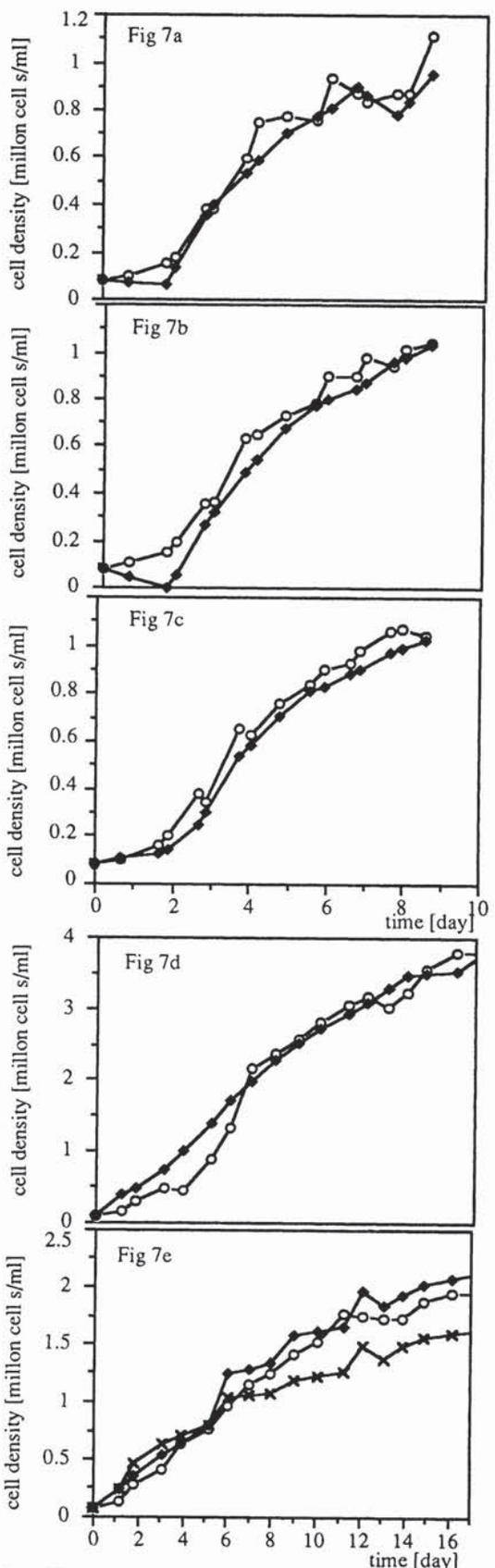

Fig. 7a to 7e comparison of estimated (-◆-) and measured (-○-) values of the biomass for the 5 different experiments G1 (a), G2 (b), G3 (c), R5 (d) and R6 (e). The estimator is based on model B'. Fig 7e presents also the biomass estimate based on glutamine and glucose (-✱-).