Detecting modes in fed-batch fermented process. Application to *Escherichia Coli* Cultivation

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Abstract - This paper presents a strategy based on residual generation to detect acetate formation during a culture of *Escherichia coli* as producer of recombinant proteins. Indeed protein productivity is both affected by acetate concentration that inhibits cell growth and by acetate production that reduces protein production. Formation of acetate occurs when the specific glucose uptake exceeds a critical value. This situation must consequently be avoid. The proposed approach deeply uses a model that gathers the knowledge about the process. It aims to study the possibility to identify the current biological behaviour from this model and from a reduced instrumentation. Depending on the instrumentation, specialized residuals are generated for the different biological modes. These residuals, calculated from the available measurement, have to be close to zero when the mode they have been design for is active. According to the residuals that trigger a deviation from zero test, it is then possible to recognize the current mode and, that way, to determine the strain biological activity.

Keywords: Biotechnology, Fed-batch fermented process, Escherichia coli cultivations, residual generation

I. INTRODUCTION

Monitoring fermentation processes has been address for a long time. First, measurements errors were concerned and data reconciliation techniques from stoechiometric considerations were used [WAN, 83]; [VAN, 94]). Another approach utilized data miming or data analysis to deeper exploit information that is contained in repeated fed-batch cultures ([STE, 97]; [KAM, 00]; [LEN, 01]; [NOM, 95]). The origin of the deviation between expected behavior and the actual was then difficult to establish.

Using a dynamical model of the fermentation process observers have also be design to detect changes in the values of model parameters such as specific growth rate or conversion ratio [KAB, 01,02].
This paper aims to explore the possibility to monitor changes in the metabolism of the cell during the culture. This topic has seldom been addressed. In [AKE, 01], a heuristic procedure using the dissolved oxygen probe measurement allows to distinguish two modes: production or non-production of acetate by *Escherichia coli*. This information is used to drive the feeding rate and the global procedure is called "probing control".
A deeper analysis of the used model ([XU,99]; [AKE,01]), leads to set five different metabolisms which represent the modes of the cultivation of *Escherichia coli*. This paper aims to recognize the actual mode, including the modes related to acetate production, from a reduced instrumentation. It implements a method used for Fault detection and Isolation: Analytical Redundancy Relations [CAS, 98]. Theses relations have to be verified by the available measurements in the mode they have been designed for. The residuals of these relations can be tested to recognize the actual biological mode.

This paper is structured as follows: the next section deals with the fed-batch fermentation process including the description of the cell metabolism and its model. The following section presents the method of residual generation and its application to the detection of modes of the considered fermentation process. Simulations are proposed in the last section to illustrate the efficiency of the proposed method.

II. PROCESS DESCRIPTION

The fed-batch fermentation process considered in this study involves a genetically modified *Escherichia coli* strain that is cultivated in a bioreactor [AKE, 01]. Figure 1 gives a basic scheme of such a plant. The liquid medium contains cells and the substrates which are

needed for the cell growth and production. The feed is used to control the substrate concentration. Air is spurged into the liquid in order to supply the culture with oxygen. Well-mixed conditions are obtained through agitation with a mechanical stirrer. The stirrer speed N is used to control oxygen transfer rate. Temperature and pH are controlled to remain within operating conditions.

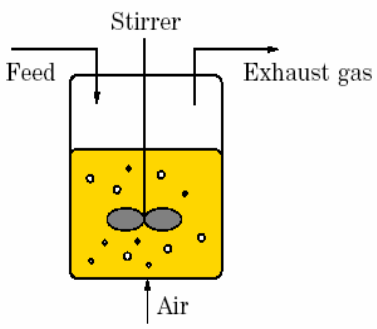

Fig. 1. Bioreactor basic scheme

The cell metabolism is described by the specific rates of growth, glucose uptake, oxygen uptake and net production of acetate [AKE, 99]. The model equations include these rates and are derived from mass balance in the liquid medium:

$$\begin{cases} \dfrac{dX}{dt} = \mu(G,A)X - \dfrac{F}{V}X \\ \dfrac{dG}{dt} = \dfrac{FG_{in}}{V} - \dfrac{FG}{V} - q_g(G)X \\ \dfrac{dA}{dt} = q_a(G,A)X - \dfrac{FA}{V} \\ \dfrac{dC_0}{dt} = K_{La}(N)(C_0^* - C_0) - \dfrac{FC_0}{V} - q_0(G)X \end{cases} \quad (1)$$

where V, X G, A and C_o are respectively the liquid volume, the cell concentration, the glucose concentration, the acetate concentration and the dissolved oxygen concentration. Further, F, G_{in} and C_O^* denote the feed rate, the glucose concentration in the feed, and the dissolved oxygen concentration in equilibrium with the oxygen in gas bubbles. F represents the variation of volume: $F = \dfrac{dV}{dt}$.

In practice, most sensors do not measure the oxygen concentration but the dissolved oxygen tension which is related to the dissolved oxygen concentration through Henry's law: $O = HC_o$.

The cell metabolism is described by the specific rates of growth μ, glucose uptake q_g, oxygen uptake q_o and production of acetate q_a. The volumetric oxygen transfer coefficient K_{La}(1/h) depends on the stirrer speed N. This coefficient was approximated by the following linear expression:

$$K_{La}(N) = \alpha(N - N_0) \quad (2)$$

For the 3 liters laboratory bioreactor, $\alpha = 0{,}92 h^{-1} rpm^{-1}$ and $N_0 = 323$ rpm give a good approximation for stirrer speeds between 400 rpm to 1200 rpm [AKE, 01].

The model given by (1) depends on specific rates. The glucose consumed by the cells provides energy and raw material for the cell growth.

The specific glucose uptake q_g is supposed to be of Monod type:

$$q_g(G) = q_g^{\max} \frac{G}{k_s + G} \quad (3)$$

A part of the glucose uptake q_g is assumed to be required for non-growth associated maintenance purpose and this is modeled by the flux $q_m = \min(q_g, q_{mc})$.

The net acetate production is given by: $q_a = q_a^p - q_a^c$

Where q_a^p and q_a^c denotes respectively acetate production and consumption. The mathematical expression used for q_a^c is:

$$q_a^c = \begin{cases} \min\left\{q_a^{c,pot}, \left(q_o^{\max} - (q_g - q_m)Y_{og} - q_m Y_{om}\right)/Y_{oa}\right\} & \text{if } q_g \le q_g^{crit} \\ 0 & \text{if } q_g > q_g^{crit} \end{cases} \quad (4)$$

with $q_a^{c,pot} = \dfrac{q_a^{c,\max} A}{k_a + A}$

The mathematical expression used for q_a^p is:

$$q_a^p = \begin{cases} 0 & \text{if } q_g \le q_g^{crit} \\ (q_g - q_g^{crit})Y_{ag} & \text{if } q_g > q_g^{crit} \end{cases} \quad (5)$$

The specific rates of growth μ are given by:

$$\mu = \begin{cases} (q_g - q_m)Y_{xg}^{ox} + q_a^c Y_{xa} & \text{if } q_g \le q_g^{crit} \\ (q_g^{crit} - q_m)Y_{xg}^{ox} + (q_g - q_g^{crit})Y_{xg}^{fe} & \text{if } q_g > q_g^{crit} \end{cases} \quad (6)$$

And the oxygen uptake q_o:

$$q_o = \begin{cases} (q_g - q_m)Y_{og} + q_m Y_{om} + q_a^c Y_{oa} & \text{if } q_g \le q_g^{crit} \\ q_o^{\max} = (q_g^{crit} - q_m)Y_{og} + q_m Y_{om} & \text{if } q_g > q_g^{crit} \end{cases} \quad (7)$$

III. RESIDUAL GENERATION

The procedure of the evaluation of the redundancy given by model of the system is based on the generation of the residuals [FRA, 97]. According to the already quoted metabolic relations, there are two modes and each mode product some residuals.

The first mode which corresponds to $q_g \le q_g^{crit}$ is noted mode I and the second one is noted mode II which corresponds to $q_g > q_g^{crit}$. The figure 2 shows the different situations and shows that four cases in Mode I and one case in mode II can arise.

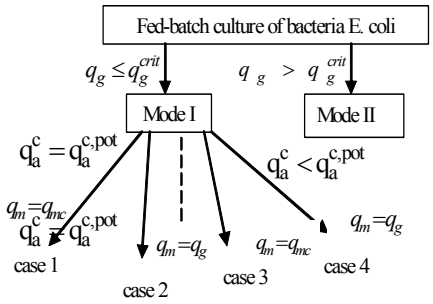

Fig. 2. Different cases of culture of *E. coli*

Two sensors are used for the measure of G and A.

The biomass X is deduced by using the second equation of (1) and we obtain:

$$X = \frac{(k_s + G)}{q_g^{\max} G}\left(\frac{FG_{in}}{V} - \frac{FG}{V} - \frac{dG}{dt}\right) \qquad (8)$$

In case 1, two residuals starting from the first and the third equation of (1) are obtained:

$$\begin{cases} r1X = \dfrac{dX}{dt} - \left(q_g^{\max}\dfrac{G}{k_s+G} - q_{mc}\right)Y_{xg}^{ox}X \\ \quad - \dfrac{q_a^{c,\max} A}{k_a + A}Y_{xa}X + \dfrac{F}{V} \\ \mathrm{rI1A} = \dfrac{\mathrm{dA}}{\mathrm{dt}} + \dfrac{q_a^{c,\max} A}{k_a + A}X + \dfrac{F}{V}A \end{cases} \qquad (9)$$

In case 2, two residuals starting from the first and the third equation of (1) are obtained:

$$\begin{cases} \mathrm{rI2X} = \dfrac{\mathrm{d\,X}}{\mathrm{dt}} - \left[\dfrac{q_a^{c,\max}\mathrm{A}}{\mathrm{k_a + A}}\mathrm{Y_{xa}} - \dfrac{\mathrm{F}}{\mathrm{V}}\right]\mathrm{X} \\ \mathrm{rI2A} = \dfrac{\mathrm{d\,A}}{\mathrm{dt}} + \dfrac{q_a^{c,\max}\mathrm{A}}{\mathrm{k_a + A}}\mathrm{X} + \dfrac{\mathrm{F}}{\mathrm{V}}\mathrm{A} \end{cases} \qquad (10)$$

In case 3, in the same way, 2 residuals starting from the first and the third equation of (1) are deduced:

$$\begin{cases} rI3X = \dfrac{dX}{dt} + \dfrac{F}{V}X - \left(q_g^{\max}\dfrac{G}{k_s+G} - q_{mc}\right)Y_{xg}^{ox}X \\ \quad + \left(q_o^{\max} - \left(q_g^{\max}\dfrac{G}{k_s+G} - q_{mc}\right)Y_{og} - q_{mc}Y_{om}\right)\dfrac{Y_{xa}}{Y_{oa}}X \\ rI3A = \dfrac{dA}{dt} + \dfrac{F}{V}A \\ \quad + \left[(q_o^{\max} - (q_g^{\max}\dfrac{G}{k_s+G} - q_{mc})Y_{og} - q_{mc}Y_{om})/Y_{oa}\right]X \end{cases} \qquad (11)$$

In case 4, one can obtain 2 residuals starting from the first and the third equation of (1):

$$\begin{cases} rI4X = \dfrac{dX}{dt} + \dfrac{F}{V}X \\ \quad - \left[(q_o^{\max} - q_g^{\max}\dfrac{G}{k_s+G}Y_{om})Y_{xa}/Y_{oa}\right]X \\ rI4A = \dfrac{dA}{dt} + \dfrac{F}{V}A \\ \quad + \left[(q_o^{\max} - q_g^{\max}\dfrac{G}{k_s+G}Y_{om})/Y_{oa}\right]X \end{cases} \qquad (12)$$

Finally the mode II generates 2 residuals given by the relation (13):

$$\begin{cases} rIIX = \dfrac{dX}{dt} - (q_g^{crit} - q_{mc})Y_{xg}^{ox}X + \dfrac{F}{V}X \\ \quad - \left[(q_g^{\max}\dfrac{G}{k_s+G} - q_g^{crit})Y_{xg}^{fe}\right]X \\ rIIA = \dfrac{dA}{dt} - \left[(q_g^{\max}\dfrac{G}{k_s+G} - q_g^{crit})Y_{ag}\right]X + \dfrac{F}{V}A \end{cases} \qquad (13)$$

IV. SIMULATION AND RESULTS

The model was simulated using the parameters proposed by [AKE, 01]. Initial values in the simulation were fixed to V(0)=2.5l, G(0)=10g/L, X(0)=10g/L, A(0)=0g/L and O(0)=30%. G_{in} was chosen equal to 60 g/L and the feed rate was constant and equal to 0.82 $l.h^{-1}$. The evolution of the obtained dilution factor is given in figure 3.. Figures 4 also provide the main state variables evolution.

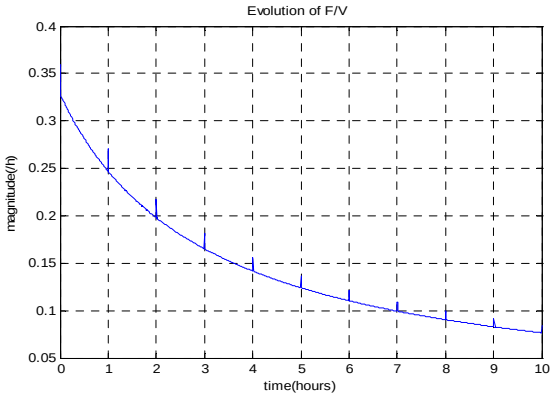

Fig. 3. Evolution of the dilution factor F/V

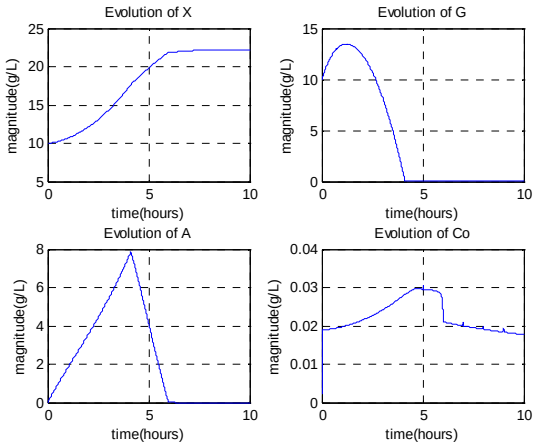

Fig. 4. Evolution of biomass, acetate, glucose and dissolved oxygen concentration

In order to allow better interpretation of the residuals, figure 5 gives the time evolution of q_g and the limit values of this variable that govern the switching between the modes. However, with the restricted instrumentation, this information is not available and the switching can't be directly checked.

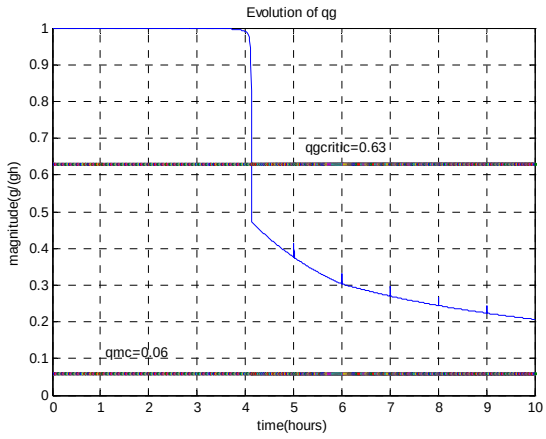

Fig. 5. Evolution of q_g

In this figure 5, an abrupt change in the q_g value is observed at time 4.15h. Before this instant, the q_g value is upper q_g^{crit} and acetate is produced as it can be verified (see Fig. 4).

The following figures give the evolutions of the residuals. The left figure shows the evolution of residual associated with the biomass variable and the right figure, the evolution of residual associated with the acetate variable. Their comments will explain how the information on the mode they provide fit with the expected mode derived from q_g.

Figure 6 shows that the 2 residuals associated with mode II are null until 4.15h. This mode corresponds to acetate production and is indeed active during this period as shown by the evolution of acetate concentration. .

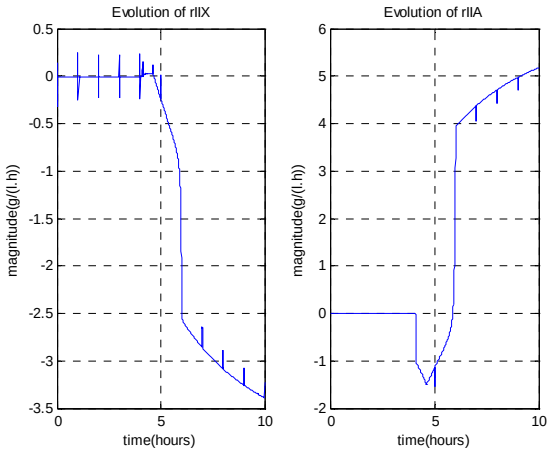

Fig. 6. Residuals relating to the mode II

After 4.15h, figure 7 shows that the residual design to refer to mode I (case 3) are both null during a very short period. This period corresponds to acetate consumption that can be correlated with the decrease of acetate concentration in figure 4. In this phase, acetate consumption related to q_a^c starts from its maximal value to be zeroed due to the lack of acetate.

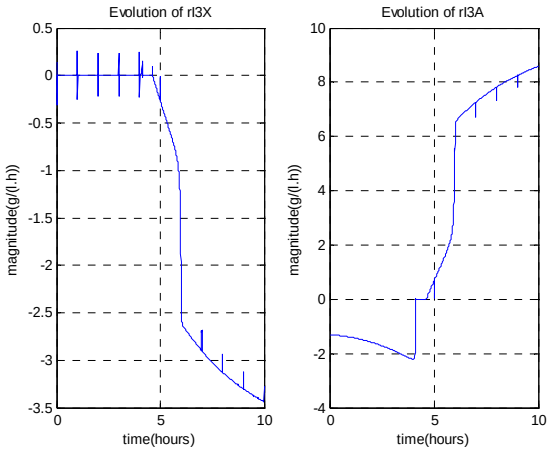

Fig.7. Residuals relating to the mode I (case 3)

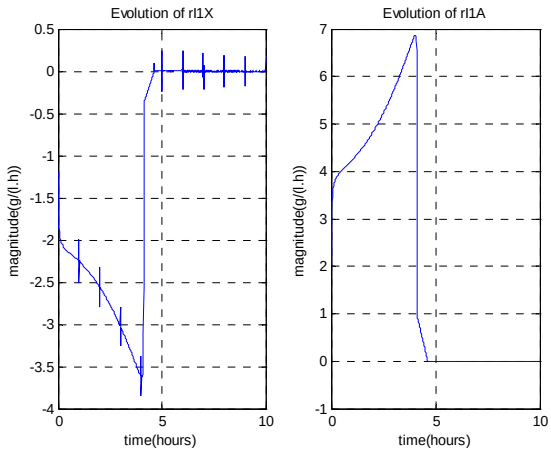

Fig. 8. Evolution of residuals according to mode I (case 1)

During the end of the culture the mode I (case 1) is active as the residuals associated with this mode are both null starting from 4.75 h.. In this mode glucose is limited and no more acetate is available. However the concentration is upper than the level needed for maintenance of biomass. Indeed, the lower value of q_g is over than q_{mc} For this reason, that leads q_m to be always equal to q_{mc} and never equal to q_g. The reactional metabolism does not fire case 2 and case 4 of mode I. Thus, the residuals relating to these cases were found to never be null as it can be verified in figures 9 and 10.

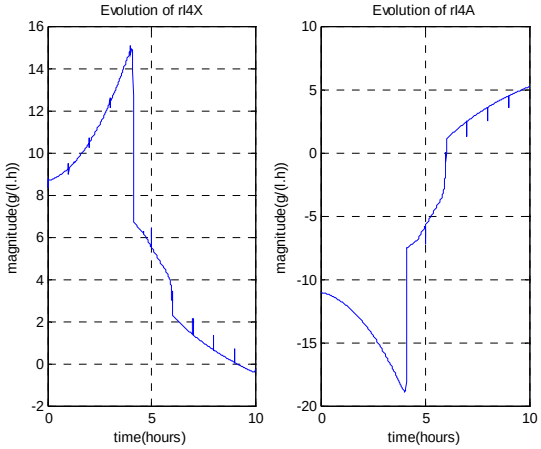

Fig. 9. Residuals relating to the mode I (case 4)

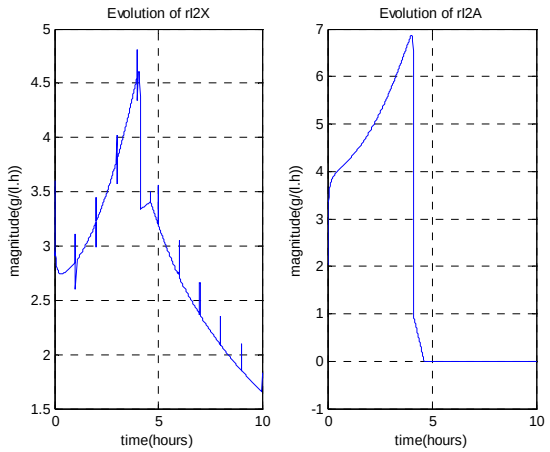

Fig. 10. Evolution of residuals according to mode I (case 2)

V. CONCLUSION

This paper shows through the results of simulations how the various modes that occur during a fermentation of bacteria can be detected or not from residuals generated from the non-linear dynamical model of this process and a reduced instrumentation. The recognized modes correspond to the actual ones derived from the q_g values provided by the simulator. However, when glucose concentration is not measured from the physical process, a direct recognition is not possible. In this case, the residual based recognition approach must be used to detect the mode changes.

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Acknowledgments

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NOMENCLATURE

Parameters used in simulation

Symbol	Value	Description
G_{in}	60g/l	Glucose concentration in feed
H	$14x10^3$(l%)/g	Henry's constant
k_s	0.01g/l	Saturation constant for glucose
k_a	0.05g/l	Saturation constant for acetate uptake
O^*	100%	Equilibrium dissolved oxygen tension
$q_a^{c,\max}$	0.20g/(gh)	Maximum specific acetate consumption rate
$q_g^{\max}$	1.0g/gh	Maximum specific glucose uptake rate
q_g^{crit}	0.63g/(gh)	Critical specific glucose uptake rate
q_{mc}	0.06g/(gh)	Maintenance coefficient
$q_o^{\max}$	0.35g/(gh)	Maximum specific oxygen uptake rate
Y_{ag}	0.55g/g	Acetate/glucose yield
Y_{oa}	0.55g/g	Oxygen/acetate yield
Y_{og}	0.50g/g	Oxygen/glucose yield for growth
Y_{om}	1.07g/g	Oxygen/glucose yield for maintenance
Y_{xa}	0.40g/g	Biomass/acetate yield
Y_{xg}^{fe}	0.15g/g	Fermentative biomass/glucose yield
Y_{xg}^{ox}	0.51g/g	Oxidative biomass/glucose yield