

Part two

BIOPROCESS CONTROL STATEMENT

In the first part of this section, the most usually literature models regarding the bioprocess kinetic will be presented. Hence, based on a general admitted classification, different biomass growth and product models in addition with bioprocess parameter evolution will be analyzed and the conceptual limitations (if necessary) will be set up. In #2.2, the general control objectives will be established, with a special emphasize on the economic performance criteria. Finally, a general presentation on the control procedures will be made, and representative point of views will be shown in order to point out the state of the art in the field and the outcoming tendencies.

2.1. BIOPROCESS MODELING

The knowledge about bioprocess behavior and modes of operation (Doran, 1986; Young, Bungay, 1973), allows the metabolic routes considerations in view of bioprocess optimal control. If that knowledge is carried out in different kinetic equations, than:

- The bioprocess mathematical representation can be the basis for adequate optimization and control technique applications (Moser, 1992; Wucherer *et al.*, 1992);
- The model provides the necessary information about the characteristics of the chosen procedure (Chen, Bastin, 1991; Bastin *et al.*, 1992);
- A good model synthesizes the physiology and the genetic determinations of the specified microorganism. Hence, this is the best technique to predict the process efficiency (Mosrati *et al.*, 1991).

The mathematical models, which describe the living cell evolution, must show the complex biosystem attributes, must be as possible extensive and non-speculative and must be based on cell's biochemistry (Freyer *et al.*, 1989). Hence, the bioprocess model must be an acceptable compromise between the presentation of detailed internal processes (i.e. with considerable number of parameters) and the consideration of a short parameter number, easy to use and estimate (Ljubenova, Ignatova, 1994).

Based on living system specificity, the bioprocesses are characterized by *non-linearity, multivariability and parameter time variance* (Eiki, Osono, 1990; Cushing, 1991; Stanbury, Whitaker, 1994; Kurtanek, 1992). Consequently, the variables, which describe the bioprocess evolution, demonstrate a strong interdependency, which make impossible the correlative influences study (Turner *et al.*, 1988). The general equation presented below:

$$\bullet \\ X = f(X, S, O_2, pH, T, \dots, t)$$

is only a theoretical assumption.

The attempts to realize such global models were not successful (firstly, due to the impossibility to measure on-line the great number of bioprocess parameters, and secondly, due to the high degree of complexity, which characterizes the cell mechanisms). The deadlock was surmounted by the implementation of the models depending of few variables, or by the use of the linear models for restricted sections (periods of time) (Stanbury, Whitaker, 1994). This last one is functionally taking into account that the bioprocesses are generally characterized by high time constants (hours or tens hours), hence, the bioprocess should be considered quasi-linear (Stanbury, Whitaker, 1994).

A general review of the bioprocess kinetic models was done by Moser (Moser, 1988). According to his classification, for the modeling of the biosystems, the deterministic models are preferred to the probabilistic ones. Several kinds of deterministic models were used (Moser, 1988):

- Unstructured models – the cells are considered as black box. In these conditions the cell concentration is the most important parameter and the cell, as an entity, is significantly influenced by the environmental conditions.
- Structured models – based on the assumption of the cell structure, considered at the level of the main chemical components, or having in mind the cellular morphology (age, dimension, shape etc.).

From a point of view neglecting the recognition/omission of the above

classification, Moser has proposed another classification (Moser, 1988):

- Unsegregated (continuous, distributive) models – which consider individual identical cells;
- Segregated (corpuscular) models – based on individual cells consideration, but with distinctive features.

The segregated models are useful to describe biosystems characterized by average states. If the biosystem contains cells obtained after genetically modification, some characteristics are different from a cell group to another, and as a consequence, the correlation with the product formation is more complex.

The simplest and most used examples are the unstructured, unsegregated models (e.g. the Monod model for the microbial growth). Only structured models are able to predict the biological phenomena in unequilibrated conditions of growth (the growth is equilibrated for a period of time if each extensive property of the biosystem is proportional by increasing) (Campbell, 1977). The Monod model is applied only for the biosystems with equilibrated growth (e.g. the exponential growth in batch cultures and the steady state phase in continuous cultivation).

Finally, the postulates defined by Edwards and Wilke (Edwards, Wilke, 1968) in the bioprocess-modeling domain establish the biological kinetic norms; hence, a biological model:

- Must be able to represent all culture phases;
- Must be enough flexible to approximate different data types without the insertion of significant distortions;
- Each model parameter must have a physic significance;
- Must be continuously derivable;
- The parameters must be easy to evaluate;
- The model must be easy to operate, once the parameters evaluated.

2.1.1. Unstructured and unsegregated models

Conforming to the scientific literature (Kossen, Oosterhuis, 1985), an unstructured model can be conceived as *black box* and the structured one as *gray box*. A gray box is build as a black box gathering in addition with their relationships, which define the biosystem *structure*. The unstructured mathematical models can be:

- Constitutive equations:
 - Kinetic equations.
 - Transport equations.
 - Thermodynamic equations.
- Balance equations (stoichiometry)

2.1.1.1. Balance equations

The balance equations were utilized (Cooney *et al.* 1977) to design an algorithm to keep up the monitoring of bioprocess performance indicators. The main parameters X , $Y_{X/S}$ and $Y_{X/O}$ were determined using the stoichiometric relationship relating to product formation and growth and by the continuous monitoring of the oxygen flow rate, OUR, CPR and of the carbon and natrium source consumption. The estimation algorithm was tested on a batch process of yeast (*Saccharomyces cerevisiae*) growth using glucose substrate.

Through the next step, the proposed algorithm was used for optimal control of the feed rate in a fed-batch bioprocess in order to increase the substrate consumption yield ($Y_{X/S} = 0,5$) and to maintain a high value of the cell mass productivity ($dX/dt = \max$).

2.1.1.2. Unstructured kinetic models for growth depending on substrate concentration

Generally speaking, one can consider that the specific growth rate (i.e. $\mu = \frac{1}{X} \frac{dX}{dt}$) is the key variable for cell growth, substrate consumption and product formation (Bastin, Dochain, 1990). The specific growth rate is

time dependent and, moreover, is dependent on different physical, chemical and/or biological parameters (i.e. substrate concentration, S , cell concentration, X , product concentration, P , pH, temperature, T , dissolved oxygen concentration, pO_2 , and different inhibitors, I).

$$\mu(t) = \mu(S)\mu(X)\mu(P)\mu(pH)\mu(T)\mu(C)\mu(I) \quad 2.1.1.2.1$$

a) $\mu=\mu(S)$ Kinetic models with growth limitation through substrate concentration (without inhibition)

The Monod equation (Monod 1942, Monod, 1949) is the most used relation from this category. It is empirically derived from the Michaelis & Menten equation (Michaelis, Menten, 1913; Sakamoto, 1986) and can be considered as a formal kinetic equation (Moser, 1988; Sivakumar *et al.*, 1994; Luong, 1987; Han, Levenspiel, 1988):

$$\mu(S) = \frac{\mu_{\max} S}{K_S + S} \quad 2.1.1.2.2$$

where : μ_{\max} = maximum specific growth rate [1/h]
 K_S = saturation constant [g/L]

The following figure shows the typical dependence of the specific growth rate upon substrate concentration, without inhibition, conforming to Monod equation (kinetics with saturation).

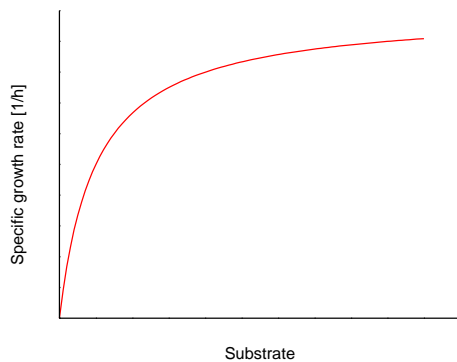


Fig. 2.1.1.1 $\mu=\mu(S)$ dependence, cf. Monod law

Conforming to literature assumptions ((Bastin, Dochain, 1990), the specific growth rate dependence upon different process parameters can be designed as follows:

Moreover, the substrate consumption rate is:

$$q_S = \frac{q_{S_{\max}} S}{K_S + S} \quad 2.1.1.2.3$$

These dependencies are linked together through the yield coefficient, $Y_{X/S}$, conforming to the following expression:

$$Y_{X/S} = \frac{\Delta X}{\Delta S} = \frac{r_X}{r_S} = \frac{dX}{dS} = \frac{\mu}{q_S} \quad 2.1.1.2.4$$

Other models were proposed alternatively. Some of they are presented below:

- Teissier equation (Teissier, 1936):

$$\mu(S) = \mu_{\max} \left(1 - e^{-\frac{S}{K_S}}\right) \quad 2.1.1.2.5$$

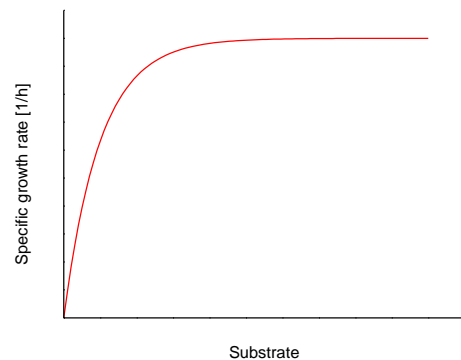


Fig. 2.1.1.2 $\mu=\mu(S)$ dependence cf. Teissier equation

- Moser equation (Moser, 1988):

$$\mu(S) = \frac{\mu_{\max} S^n}{K_S + S^n} \quad 2.1.1.2.6$$

through analogy with a Hill kinetic ($n>0$)

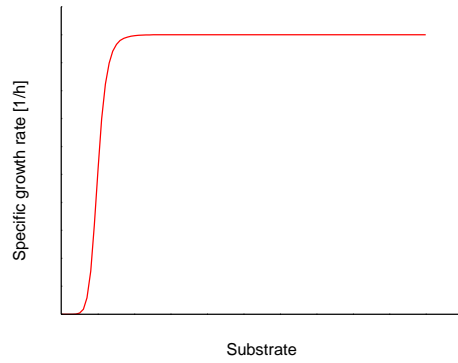


Fig. 2.1.1.3 $\mu=\mu(S)$ dependence cf. Moser equation

- Blackman equation (Blackman, 1905):

$$\mu = \begin{cases} \frac{\mu_{\max}}{K_m} S(t) & \text{if } S(t) \leq K_m \\ \mu_{\max} & \text{if } S(t) > K_m \end{cases} \quad 2.1.1.2.7$$

- Powell equation (Powell, 1958); the influence of cell permeability, substrate diffusion and cell dimensions are showed through K_D parameter:

$$\mu(S) = \mu_{\max} \frac{S}{K_s + K_D + S} \quad 2.1.1.2.8$$

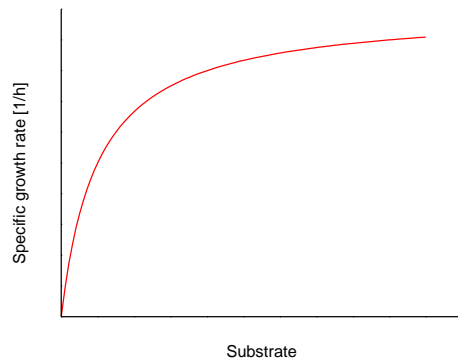
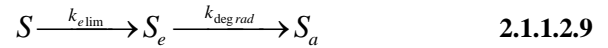


Fig. 2.1.1.4 $\mu=\mu(S)$ dependence cf. Powell equation

Conforming to literature references, the Monod kinetic slowly tills to the asymptotic value; i.e. it fit not well the experimental data, in simplest cases. Hence, the Teissier and Blackman kinetics represent better the data evolution because the saturation level is faster attempted.

There are also some models, which utilize the substrate concentration in more complex structures. Hence, Verhoff (Verhoff *et al.*, 1972), introduces two steps in the cell behavior: assimilation and ingestion, but the resulting equation is too complex. Moreover, Nyholm (Nyholm, 1976), introduces a dual function for substrate utilization: consumption (including assimilation and desassimilation in the liquid phase) and growth (substrate utilization for growth):



where S_e is the substrate utilized for growth and S_a the substrate used for consumption. The growth rate is linked to the intracellular concentration of limiting substrate (S_{int}/X) and to *preserved* substrates (i.e. inorganic ions or vitamins, which are not decomposed through cell metabolism):

$$\mu = \mu \frac{\frac{S_{int}}{X}}{dS_{int}} = r_{S_e\lim} - r_{S_{deg\ rad}} \quad 2.1.1.2.10$$

This kinetic model has a practical application in the wastewater treatment field.

One of the most important objectives for developing a general kinetic model is to establish a conceptual basis for microorganism growth description (O'Neil, Fyeras, 1986). Therefore, the general dependence of the cell growth upon substrate concentration can be considered (Neubert *et al.*, 1984) as a difference $\mu_{\max} - \mu$. Hence, Konak kinetics (Konak, 1974) can be taken into consideration:

$$\frac{d\mu}{dt} = k(\mu_{\max} - \mu)^p \quad 2.1.1.2.11$$

where: k = kinetic constant
 p = reaction order

This equation designates an analogy with the well-known *power law*, which is utilized in the chemical kinetics field. If the relative growth rate notion (in a biological sense) is introduced:

$$r_{rel} = \frac{\mu}{\mu_{max}} \quad 2.1.1.2.12$$

then equation (2.1.1.2.11) can be written:

$$\frac{d\mu}{dS} = k\mu_{max}^{p-1} \left(1 - \frac{\mu}{\mu_{max}}\right)^p \quad 2.1.1.2.13$$

Konak has demonstrated that this equation can be reduced to a Monod kinetic (for $p = 2$) and to a Teissier kinetic (for $p = 1$). An attractive dependence between μ_{max} and K_S can be written:

$$K_S = \frac{1}{\mu_{max} k} \quad 2.1.1.2.14$$

Based on above (differential) equations, Kargi and Shuler (Kargi, Shuler, 1979) has obtained the following relationship:

$$\frac{d\mu}{dS} = K \left(\frac{\mu}{\mu_{max}}\right)^m \left(1 - \frac{\mu}{\mu_{max}}\right)^p \quad 2.1.1.2.15$$

where $K, m, p = \text{constants}$

Meanwhile, the general equation (2.1.1.2.15) can be reduced to the following simple models:

Table 2.1.1 Specific cases for eq. 2.1.1.2.15

Model	K	m	p
Monod	$1/K_S$	0	2
Teissier	$1/K_S$	0	1
Hill-Moser	$n/K_S^{1/n}$	$1-1/n$	$1+1/n$

b) $\mu=\mu(X,S)$ The influence of cell and substrate concentrations upon the specific growth rate, μ

Because the dependence $\mu=f(X)$ also involves (Yamane, 1993) a dependence upon the substrate concentration, there are few models which express a double influence; the most commonly being written as $\mu = f(X,S)$.

From the cell growth point of view (Chattaway *et al.*, 1992), the culture evolution rate decreases if the cell concentration increases (Mulchandani, Luong, 1988; Matanguihan *et al.*, 1994). A simple kinetic model which describes these situations was proposed by Verhulst (Verhulst, 1845) through a linear dependence:

$$\mu(X) = \mu_{max} (1 - k_x X) \quad 2.1.1.2.166$$

This model is also named the *growth logistic model* (fig.2.1.1.6).

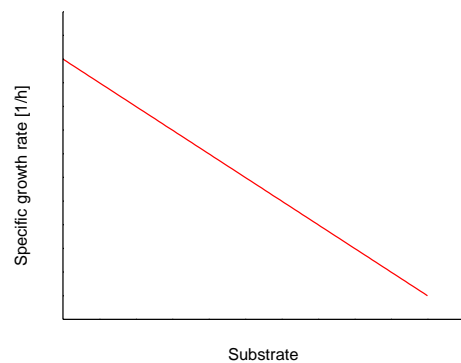


Fig. 2.1.1.5 $\mu=\mu(X)$ dependence cf. Verhulst equation

Meyrath (Meyrath, 1973) introduced a more realistic equation, which defines the growth

limitation through substrate concentration. This model is based on Monod kinetic:

$$\mu(X, S) = \mu_{\max} \frac{S_0 - \frac{X}{Y}}{K_s + S_0 - \frac{X}{Y}} \quad 2.1.1.2.17$$

where Y = the substrate/cell yield.

Based on the above model, the Verhulst – Pearl kinetic was proposed:

$$N = N_0 \exp(\mu_{\max} t) \\ = \frac{N_0 \mu_{\max}^0 \exp(\mu_{\max}^0 t)}{\mu_{\max}^0 + m_x N_0 (\exp(\mu_{\max}^0 t) - 1)} \quad 2.1.1.2.18$$

The most known model in the related field ($\mu = f(X, S)$) is the Contois (Contois – Fujimoto) equation (Contois, 1959):

$$\mu = \mu_{\max} \frac{S}{K_x X + S} \quad 2.1.1.2.19$$

As it can be seen, if S = constant, the only dependence remains $\mu = f(X)$.

Kono and Asai (Kono, Asai, 1968) built a growth equation based on chemical kinetic concepts. Hence, a *growth consumption activity coefficient* Φ was introduced. This coefficient was redefined by Bastin as *apparent growth activity coefficient*.

$$\mu(X) = K_x \Phi \quad 2.1.1.2.170$$

where K_x = growth rate constant (has different values depending on the growth step in a batch culture);

$\Phi = 0$, induction phase;

$\Phi = \varphi$, cu $0 < \varphi < 1$, transition phase,
 $\varphi = \alpha t$;

$\Phi = 1$, exponential phase

The basic idea of Kono was a general growth equation:

$$\frac{dX}{dt} = k_1^i k_2^j C_1^i C_2^j - k_3 C_3 \quad 2.1.1.2.21$$

where: C_1 = limiting substrate concentration
 C_2 = co-substrate concentration;
 C_3 product concentration;
 X_{crit} = critical cell concentration.

The reaction order changes if $X = X_{\text{crit}}$. Hence, $i = 1$ and $j = 0$ if $X < X_{\text{crit}}$, $i = 0$ and $j = 1$ if $X > X_{\text{crit}}$.

Other corresponding interesting kinetics are presented below:

- Nihtila and Virkkunen model (Moser, 1988)

$$\mu(X, S) = K_1 \frac{C(t)}{X(t)} \quad 2.1.1.2.22$$

$$\frac{dC}{dt} = K_2 S(t)(X(t) - C(t)) - K_3 C(t)$$

where: $C(t)$ = cell-substrate group concentration;

K_1, K_2, K_3 = constants

- Kishimoto model (Kishimoto, 1978):

$$\mu(X, S) = \bar{\mu} + Q_1(X(t) - \bar{X}) + Q_2(S(t) - \bar{S}) \quad 2.1.1.2.23$$

where $\bar{\mu}, \bar{X}, \bar{S}$ = corresponding mean values;
 Q_1, Q_2 = regression coefficients.

- Staniskis and Levisauskas model (Moser, 1988):

$$\mu(X, S) = k_1 S(t) - k_2 X(t) \quad 2.1.1.2.24$$

c) Growth kinetic with substrate inhibition

In most cases, the kinetic model equations are derived (like the Monod model) from the

inhibition theory of enzymatic reactions. Hence, these equation types are not generally valid. They can be applied in connection with experimental acceptability.

The most usually kinetic models with substrate inhibition are presented below:

- Andrews model (Andrews - Noack), substrate inhibition in a chemostat (Andrews, 1968):

$$\mu = \mu_{\max} \frac{1}{1 + \frac{K_S}{S} + \frac{S}{K_i}} = \frac{S}{K_S + S} \frac{1}{1 + \frac{S}{K_i}} \quad 2.1.1.2.25$$

where: K_i = inhibition constant (considering with the substrate influence).

The graphical presentation is the following:

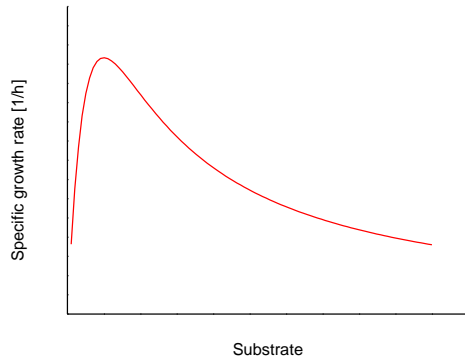


Fig. 2.1.1.6 $\mu=\mu(S)$ dependence cf. Andrews equation

- Webb model (Webb, 1963):

$$\mu = \mu_{\max} \frac{S(1 + \frac{S}{K_S^I})}{S + K_S \frac{S^2}{K_S^I}} \quad 2.1.1.2.26$$

where: K_S^I = inhibition constant (considering the substrate influence).

- Yano model (Yano *et al.*, 1966)

$$\mu = \mu_{\max} \frac{1}{1 + \frac{K_S}{S} + \sum_j (\frac{S}{K_{i,S}})^j} \quad 2.1.1.2.27$$

where: $K_{i,S}$ = inhibition constant (considering the substrate influence).

- Aiba model (Aiba, Hara, 1965):

$$\mu = \mu_{\max} \frac{S}{K_S + S} e^{-\frac{S}{K_{i,S}}} \quad 2.1.1.2.28$$

The graphical presentation is given below:

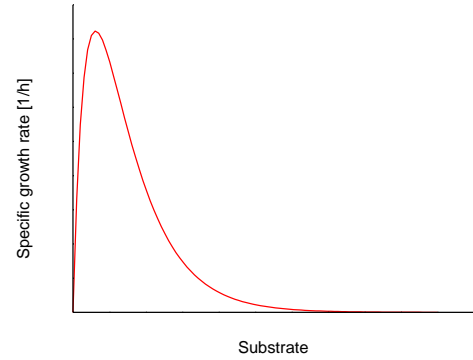


Fig. 2.1.1.7 $\mu=\mu(S)$ dependence cf. Aiba equation

Edwards (Edwards, 1970) analyzed eqs. (2.1.1.25 - 2.1.1.28) based on different experimental data sets. He demonstrated that it is not an objective criteria to classify these equations regarding the generalization potential. Hence, he recommended the Andrews model as the simplest and easy to use.

Wayman and Tseng (Wayman, Tseng, 1976) proposed a different equation type in order to introduce a substrate inhibition kinetic:

$$\mu = \mu_{\max} \frac{S}{K_S + S} - K_{i,S}(S - S_C) \quad 2.1.1.2.29$$

where: S_C = limiting substrate concentration (a threshold value).

This equation can be used whether the specific growth rate has a linear reduction trend (fig. 2.1.1.9).

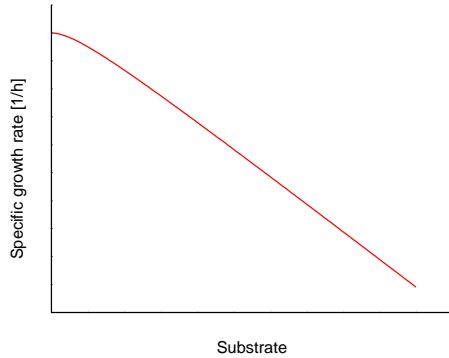


Fig. 2.1.1.8 $\mu=\mu(S)$ dependence cf. Waymann & Tseng equation

d) $\mu = f(S,P)$ Growth kinetic with product inhibition

Hinshelwood (Hinshelwood, 1946) detected different product inhibition influences upon the specific growth rate: linear decrease, exponential decrease, growth sudden stop, and linear/exponential decrease in comparison with a threshold value of P. The first type (Hinshelwood - Dagley model) is presented below:

$$\mu(S,P) = \mu_{\max} \frac{S}{K_S + S} (1 - kP) \quad 2.1.1.2.30$$

where: k = inhibition constant (considering the product concentration influence)..

This equation was modified as follows:

- Holzberg model (Holzberg *et al.*, 1967):

$$\mu(P) = \mu_{\max} - K_1(P - K_2) \quad 2.1.1.2.31$$

where: $K_1, K_2 = \text{constants } (>0)$.

The graphical presentation of Holzberg model is presented below:

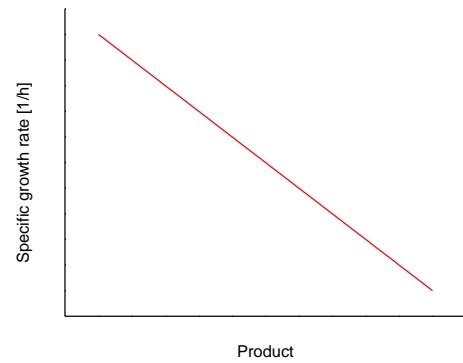


Fig. 2.1.1.9 $\mu=\mu(P)$ dependence cf. Holzberg equation

- Ghose and Tyagi model (Ghose, Tyagi, 1979) fig. 2.1.1.11:

$$\mu(P) = \mu_{\max} \left(1 - \frac{P}{P_{\max}}\right) \quad 2.1.1.2.32$$

where: P_{\max} = maximum product concentration.

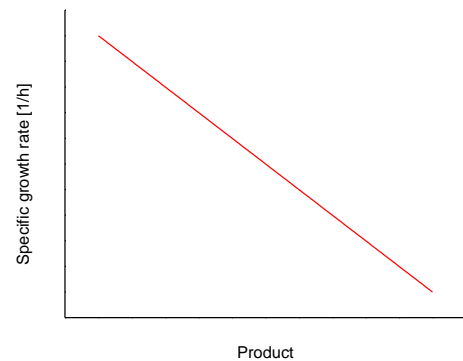


Fig. 2.1.1.10 $\mu=\mu(P)$ dependence cf. Ghose & Tyagi equation

A different equation type was introduced by Aiba (Aiba, 1982), fig. 2.1.12:

$$\mu(P) = \mu_{\max} e^{-K_1 P(t)} \quad 2.1.1.2.33$$

where: $K_1 = \text{constant}$



Fig. 2.1.1.11 $\mu=\mu(P)$ dependence cf. Aiba equation

The Aiba and Shoda model (Aiba, Shoda, 1989) presents a variant of the above equation:

$$\mu(S, P) = \mu_{\max} \frac{S}{K_S + S} e^{-KP} \quad 2.1.1.2.34$$

Also Ierusalimsky (Ierusalimsky, 1967) recommended a model type describing the dependence $\mu = f(P, S)$ which is similar with an enzymatic kinetic:

$$\mu(P) = \frac{\mu_{\max} P(t)}{K_{i,P} + P(t)} \quad 2.1.1.2.35$$

or, more developed:

$$\mu(S, P) = \frac{\mu_{\max} P(t)}{K_{i,P} + P(t)} \frac{S}{K_S + S} \quad 2.1.1.2.36$$

where: $K_{i,P}$ inhibition constant (considering the product concentration influence).

The last equation represents the most useful kinetic model regarding growth kinetic with product inhibition.

e) The influence of dissolved oxygen (as a second substrate) upon the specific growth rate

Usually, the dissolved oxygen can be considered as a second substrate (Eiki, Osono, 1990). Hence, the most used equation is the kinetic model with double growth limitation, $\mu(S, C)$, i.e. Olsson model (Moser, 1988):

$$\mu(S, C) = \mu_{\max} \frac{S}{K_S + S} \frac{C}{K_C + C} \quad 2.1.1.2.37$$

where: K_C = saturation constant (vs. oxygen), or, more complex, the Williams model (Williams, 1969):

$$\mu(S, C, P) = \left(\frac{K_1 S}{K_S + S} \frac{K_2 P}{K_P + P} \right) \cdot \left(\frac{C}{K_C + C} + K_3 C - K_4 \right) \quad 2.1.1.2.38$$

f) The influence of environmental factors upon the specific growth rate

There are simple models proposed to predict the pH effect (Ben-Hassan *et al.*, 1991) on the process behavior:

- Andreyeva and Biriukov (polynomial model) (Andreyeva, Biriukov, 1973):

$$\mu(pH) = a \cdot (pH)^2 + b \cdot (pH) + c$$

2.1.1.2.39

- Jackson and Edwards model (with inhibition) (Moser, 1988):

$$\mu(H^+) = \frac{H^+}{K_H + H^+ + K_i (H^+)^2} \quad 2.1.1.2.40$$

In order to model the simultaneous effect of substrate concentration and pH, $\mu = f(S, pH)$, there are different variants of the previous equations:

- Andreyeva and Biriukov (Andreyeva, Biriukov, 1973):

$$\mu(S, H^+) = \mu_{\max} \frac{SH^+}{K_S + SH^+ + \frac{(SH^+)^2}{K_i}} \quad 2.1.1.2.41$$

or:

$$\mu(S, H^+) = \mu_{\max} \frac{S}{K_S + S} \frac{K_H}{K_H + H^+} \quad 2.1.1.2.42$$

- Jackson and Edwards (Moser, 1988):

$$\begin{aligned} \mu(S, H^+) &= \\ &= \mu_{\max} \frac{S}{\left(1 + \frac{K_2}{H^+} + \frac{H^+}{K_1}\right)(K_H + S + \frac{S^2}{K_i(1 + \frac{K_3}{H^+})})} \end{aligned} \quad 2.1.1.2.43$$

The **temperature** influence is introduced through an Arrhenius equation type, conforming to Topiwala and Sinclair model (Topiwala, Sinclair, 1971):

$$\mu(T) = \begin{cases} A_1 e^{\frac{-E_1}{RT}} - A_{21} e^{\frac{-E_{21}}{RT}} - A_3, & T \in [T_1, T_2] \\ 0 & T \notin [T_1, T_2] \end{cases} \quad 2.1.1.2.44$$

Finally, Constantinides (Constantinides *et al.* 1970) defines the dependence $\mu(T, X)$ using the following equation:

$$\mu(T, X) = b_1(T) \left(1 - \frac{X}{b_2(T)}\right) \quad 2.1.1.2.45$$

where:

$$b_1(T) = K_1 - K_2(T - K_3)^2 \quad 2.1.1.2.46$$

and:

$$b_2(T) = K_4 - K_5(T - K_6)^2 \quad 2.1.1.2.47$$

g) $\mu(S_1, S_2)$ Kinetic models based on different substrates

The dissolved oxygen was commonly considered as a second substrate (cf. e)). However, there are many cases when two or more carbon sources are taken into consideration (Chiu *et al.*, 1972). There are two primary cases regarding these aspects:

- The cells grow through the sequential (consecutive) substrate consumption (diauxy); hence, a simple Monod model can be utilized;
- The cells grow through the simultaneous consumption of substrates (e.g. wastewater treatment); in this case, the mathematical modeling is more complex (Chiu *et al.*, 1972).

2.1.1.3. Unstructured kinetic models for product formation

The product formation kinetic is taken into account in conjunction with the growth kinetic. Nowadays, the Gaden classification (Gaden, 1959) is still useful. Based on this categorizing, four kinetic types can be defined (Moser, 1988):

Type 0: This production type occurs even in resting cells that use only a little substrate for their own metabolism. The microbial cells function only as enzyme carriers. Some examples are provided by steroid transformation and vitamin E synthesis by *Saccharomyces cerevisiae*.

Type 1: Type-1 situations include processes in which product accumulation is directly associated with growth; in this case the product formation is linked to the energy metabolism. Examples include fermentation to produce alcohol and gluconic acid and situations in biological wastewater treatment.

Type 2: Type-2 bioprocesses include fermentations in which there is no direct

connection between growth and product formation (for example, penicillin and streptomycin synthesis).

Type 3: This production type includes those having a partial association with growth and thus, an indirect link to energy metabolism (e.g. citric acid and amino acid production)

Recognition of the production type is done with the aid of plots r_s , r_X , $r_P = f(t)$, and expressly, tacking into consideration the specific rates q_s , μ , q_p .

The diagram of the time dependence of the bioprocess specific rate is called *quantification diagram* (Moser, 1988). It gives the best insight into the bioprocess and a basic for designing mathematical models.

The following figure shows the formal kinetic linear relationship between specific rates (product formation rate, q_p , vs. specific growth rate, μ).

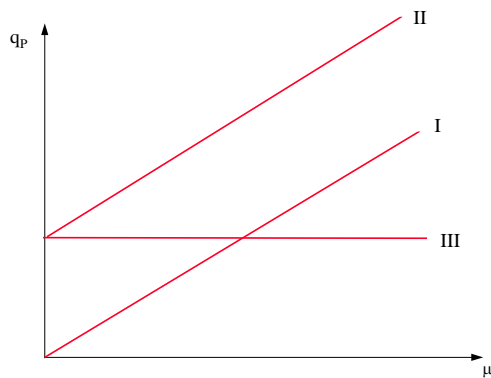


Fig. 2.1.1.13 – Product formation rate vs. specific growth rate cf. Gaden equations (type I, II, III)

Type 1: Product formation linked to microbial growth can be described by (Moser, 1988):

$$r_P = Y_{P/X} r_X \quad 2.1.1.2.48$$

and:

$$q_P = Y_{P/X} \mu \quad 2.1.1.2.49$$

In the same way, Constantidines (Constantinides *et al.* 1970):

$$r_P = Y_{P/S} r_S \quad 2.1.1.2.50$$

Finally, the following relationship exists between yield factors:

$$\frac{Y_{P/S}}{Y_{X/S}} = Y_{P/X} \quad 2.1.1.2.51$$

Substituting a Monod-type equation into eq. 2.1.1.2.50 results in a hyperbolic function (Moser, 1988) for production in the case of growth association:

$$r_P = q_P X = q_{P_{\max}} \frac{S}{K_S + S} X \quad 2.1.1.2.52$$

Type 3: Non-growth-linked product formation is more difficult to quantify because no direct relationship to growth exists. As an alternative, the dependence $r_P = f(X)$ is often successfully used:

$$r_P = k_P X \quad 2.1.1.2.53$$

Product formation can also be quantified by the dependence of substrate utilization (cf. eq. (2.1.1.2.50)).

Type 2: When product formation is partly growth linked and partly independent of growth, a combination of eqs. (2.1.1.2.49) and (2.1.1.2.53) is valid, as it was proposed by Luedeking and Piret (Luedeking, Piret, 1959):

$$q_P = Y_{P/X} \mu + k_{pr} \quad 2.1.1.2.54$$

Hence, the general forms of eq. (2.1.1.2.54) – with eqs. (2.1.1.2.49) and (2.1.1.2.53) as boundary cases – suggests a logistic equation (Luedeking, Piret, 1959, Moser, 1988).

Kono and Asai (Kono, Asai, 1968) introduced a generalized concept, using the consumption coefficient Φ (as an apparent coefficient of growth activity):

$$r_P = k_{P_1} X \Phi + k_{P_2} X (1 - \Phi) \quad 2.1.1.2.55$$

This equation can be linked to eq. (2.1.1.2.20) which describes the specific growth rate evolution. Hence, the Gaden type product formations can be particularized through different values of the parameter $k_{P,j}$: $k_{P,j} > 0$ or $k_{P,j} < 0$, $j = 1, 2$

In special cases, different kinetic equations were proposed, according to experimental data (Shu, 1961, Fishman, Birjukov, 1974, Ryu, Humphrey, 1972, Brown, Vass, 1981).

2.1.2. Structured models

The most important structured models are correlated with the chemical cell structure. Williams and Ramkrishna has proposed models with two components. Williams (Williams, 1975) has considered the concentration of R-components (principally ARN) and D-components with structural-genetic part (ADN and proteins). Ramkrishna (Ramkrishna, 1969) has divided the cell in G-mass (ADN and ARN) and D-mass (proteins). Fredrickson (Fredrickson, 1970) has analyzed later on these models and has demonstrated their invalidity, due to kinetic expressions which don't utilize the intrinsic concentrations of structural components.

A rigorous structured model involves kinetic equations, which take into account the intrinsic concentrations of components (i.e. the component quantity / cell unity of volume).

Hence, Frederickson builds up the general balance of a discontinuous reactor:

$$\frac{d(m\hat{V}c_j)}{dt} = m\hat{V}\sum_i r_{ij} \quad 2.1.2.1$$

where: m = total biomass (at time t);

\hat{V} = volume filled by the cells / cell mass unit;

C_j = mass of component j / cell volume unit (intrinsic concentration of component j);

r_j = appearance/disappearance of component j through process i / cell volume.

If \hat{V} is constant and one consider (cf. the unstructured kinetic) that:

$$\mu = \frac{1}{m} \frac{dm}{dt} \quad 2.1.2.2$$

the following equation can be obtained:

$$\frac{dc_j}{dt} = \sum_i r_{ij} - \mu C_j \quad 2.1.2.3$$

In the most structured models, the expression μC_j can be ignored.

Moreover, the term C_j can be substituted with the concentration X_j (mass of component j / cell mass unit), in conformity with the following relation:

$$X_j = C_j \hat{V} \quad 2.1.2.4$$

The structured models can be applied for the mixed cultures, too. In these cases, the chemical structure is substituted with component specie divisions (population models).

Harder and Roels (Harder, Roels, 1981) surveyed the biotechnology applications of simple structured models. Moreover, they presented the main principles of structured model design (derived from molecular biology):

- The modifications of substrate/intermediate concentrations induce modifications of the reaction rates;
- The enzyme interactions with some small molecules generate modifications of enzyme conformation;
- The concentration of some cell macromolecules accommodate oneself to environment conditions through synthesis rate modification;
- The natural selection is an adaptation modality;

- The environment changes can induce some modifications in respect of different species in a mixed culture.

Following these principles and according to the *time relax* concept, the authors proposed some structured models:

- Biomass grow conforming to a bi-compartmental model;
- Biomass grow conforming to a tri-compartmental model;
- The synthesis of enzymes, which are genetic controlled.

Moreover, Ryu and Kim (Ryu, Kim, 1992) studied a recombinant cell bioprocess (i.e. with/without plasmid carrying cells).

For product formation kinetic, the following equation can be proposed:

$$q_p = k_0 \varepsilon G_p (\mu^+ + b) \quad 2.1.2.5$$

where: k_0 = constant of the global biosynthesis rate;

ε = genetic expression efficiency;

G_p = ADN concentration in plasmids;

μ^+ = specific growth rate of plasmid carrying cells;

b = constant.

This equation can be related to the unstructured model of Luedeking - Piret (eq. 2.1.1.2.54), which can be written as follows:

$$\frac{dP}{dt} = A \frac{dX^+}{dt} + BX^+ \quad 2.1.1.2.54'$$

where: X^+ = concentration of plasmid carrying cells;

Hence, the parameters A and B get a biological significance:

$$\begin{aligned} A &= k_0 \varepsilon G_p \\ B &= Ab \end{aligned} \quad 2.1.2.6$$

2.1.3. Segregated kinetic models

The scientific literature doesn't clearly present other segregated kinetic models, except those using the composition based on chemical structure.

Shuler (Shuler, 1985) defines as segregated (but unstructured) models, the models based on the presumption that an unique variable (i.e. cell age, cell dimensions) can completely describe the cell state (i.e. all cells with the same age or dimensions have the same chemical composition and the same productive potential).

Also, Bley (Bley, 1987) proposed a specific model for yeast cells, which comprises two different physiological states – budding /unbudding cells. Moreover, he proposed a model taking into consideration the yeast cells, which are characterized by two physiological states. The two states differ through μ , dS/dt , biomass production efficiency, etc. Hence, for a continuous process, in which the state transition is (mainly) influenced by the substrate concentration (S), the following equations are valid:

$$\begin{cases} \frac{dX_1}{dt} = (\mu_1(S) - k_1(S) - D)X_1 + k_2(S)X_2 \\ \frac{dX_2}{dt} = (\mu_2(S) - k_2(S) - D)X_2 + k_1(S)X_1 \\ \frac{dS}{dt} = (S_0 - S)D - \alpha_1(S)X_1 - \alpha_2(S)X_2 \end{cases}$$

2.1.3.1

where: X_1 = biomass concentration for budding cells;

X_2 = biomass concentration for unbudding cells;

D = dilution rate.

In this model, $k_1(S)$ represents the specific rate to pass from the state X_1 to X_2 , and $k_2(S)$ the specific rate for the inverted process; $\alpha_1(S)$ and $\alpha_2(S)$ represent the coefficients of yield

conversion ($S \rightarrow X_1$ and $S \rightarrow X_2$, respectively).

Nowadays, the segregated and structured models are insufficiently applied. They were taken over from molecular biology and physiology, where they play an important role in cell mechanism investigations (Sonnleitner, Fiechter, 1989). For a fast increase of their applications in bioprocess control it is necessary:

- To develop the non-destructive analytical techniques (Sonnleitner, 1992);
- To link the mathematical modeling to the understanding of the internal cell mechanisms (van Breusegem, Bastin, 1992).

2.2. CONTROL STRUCTURE CONFIGURATION

2.2.1. Control statement

The bioprocess control (and optimisation) strategies are based on three main implementations (Pokkinen *et al.*, 1992):

- *Algorithmic optimisation*: the optimum can be analytically reached (due to relationship defined between process parameters); hence, the algorithmic optimisation is cheaper than the adaptive one;
- *Adaptive optimisation*: this kind of optimisation requires not *a priori* knowledge regarding the existing relationships between bioprocess parameters;
- *Intelligent technique optimisation*: requires knowledge transfer from a human expert to the control structures (expert system, neural nets, fuzzy structure, etc).

Generally speaking, the first solution offers the answer based on peak parameter values estimation, i.e. process optimisation is performed in connection with a (lot of) specified parameter(s). The second one utilises uncomplicated kinetic models with periodical (i.e. the simple period) parameter adjustments. Hence, the optimum indices are continuously adjusted.

The control optimisation difficulties come from continuous variation of optimal bioprocess conditions, due to living cells and metabolic cycles.

The major bioprocess control difficulties are presented below (DECHEMA, 1984):

- The impossibility to access on-line all process variables;
- The measurements are noise influenced;
- The process has high value of time delay;
- The process parameters are strongly interconnected;
- The bioprocess evolution curves are time varying and depend upon initial conditions.

The first obstacle still to be removed through the large-scale use of biosensors (e.g. glucose sensor). The standard method is based on *observation schema* (Nogai, 1979), which utilises secondary measurements. For example, it is easy to determine the RQ values (in a fed-batch bioprocess) through the substrate addition varying. In this case, the main problem is to fix the set-point value of the secondary variable in order to attempt the desired value of the primary process variable.

The general configuration of a bioprocess control structure is shown in fig. 2.2.1.

2.2.2. Performance criteria

The scientific literature (Moser, 1988, Richards, 1988, Trilli, 1977) recommends, from the bioprocess control point of view, three performance indices, which must be maximised:

- the productivity;
- the conversion;
- the profit.

The productivity and the conversion rate look upon technological aspects, and the last one considers the economic point of view.

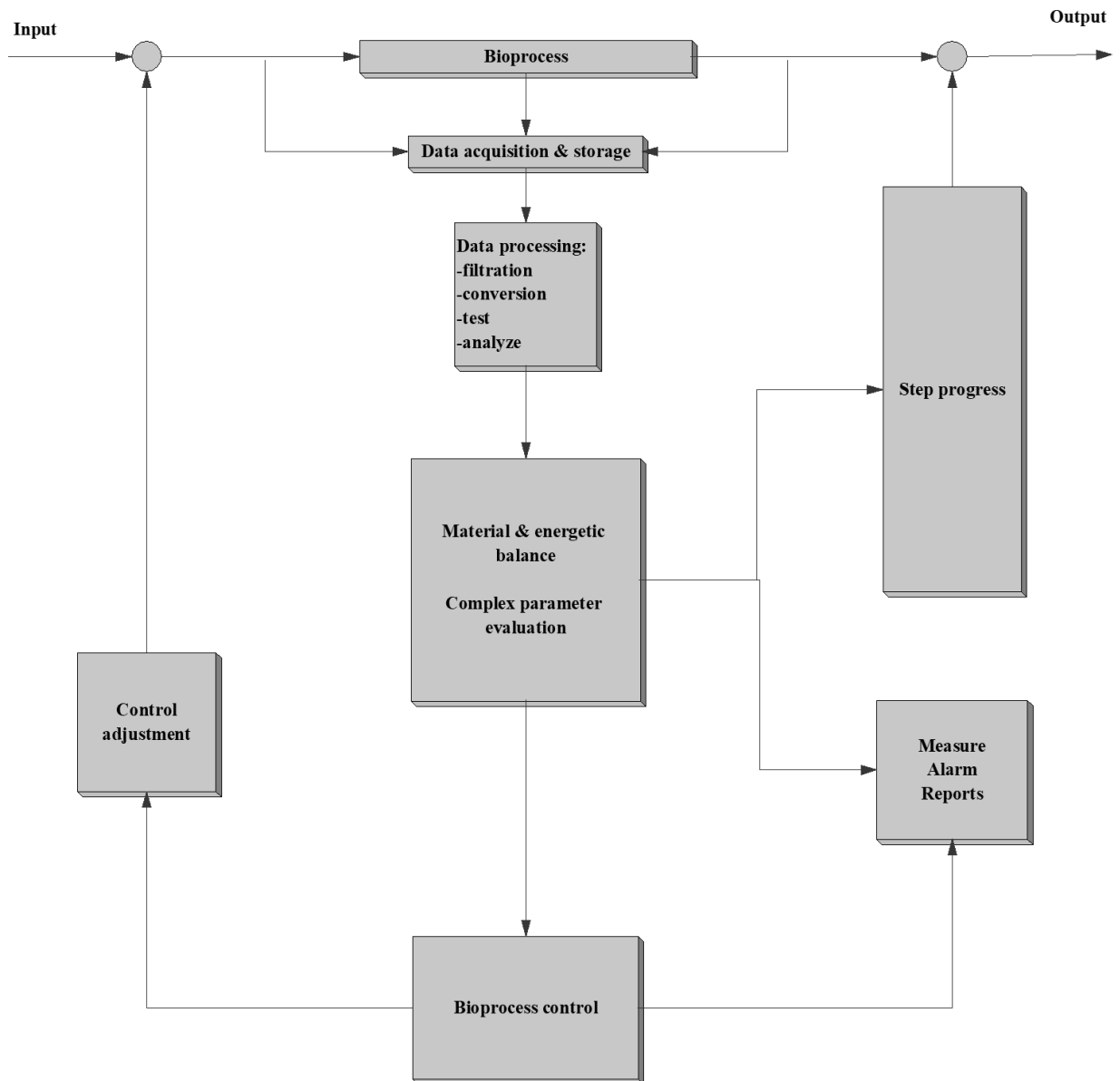


Fig. 2.2.1 The general structure of bioprocess control

To clarify the productivity concept (r_j) (dimensions $\text{kg} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$), the curve from the considering fig. 2.2.2 can be used demonstrates for discontinuous processes. Hence, a certain lag time, t_0 , is necessary between production cycles due to harvesting, emptying and refilling operations (Moser, 1988).

Drawing a tangent from this point to the concentration/time curve, the value of the product concentration is obtained at point 2; this level can be reached in the whole production time ($t_{\text{tot}} = t_0 + t_r$).

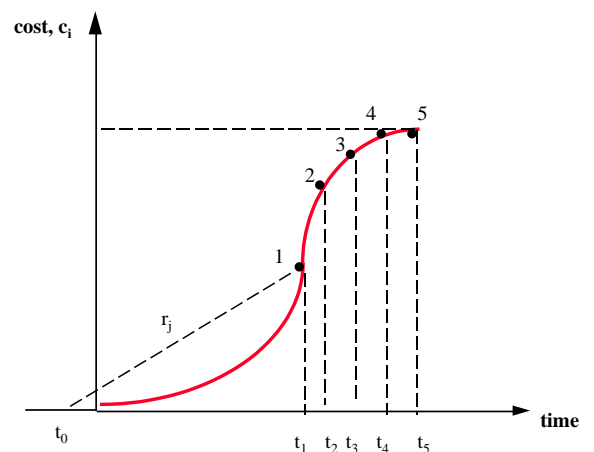


Fig. 2.2.2 Schematic representation of optimal operating point for a process based on different economic criteria (from Moser, 1988)

The maximum productivity attainable in a discontinuous bioprocess can be calculated from:

$$r_{j \max} = \frac{C_{\max j} - C_{0j}}{t_0 - t_r} \quad t_r = t_2 - t_0 \quad 2.2.1$$

Point **1** (fig. 2.2.2) gives the maximum productivity of an equivalent continuous bioprocess (because a continuous process has no dead time, the slope of the tangent is greater than in a batch process).

Moreover, at point **5** the productivity is 0. This point may be of interest when very expensive substrates are being used (Moser, 1988): in these cases, the process may be run to complete substrate utilisation.

The conversion, ξ_i , is defined ($V = \text{constant}$):

$$\xi_i = \frac{C_{i0} - C_{it}}{C_{i0}} \quad 2.2.2$$

and can also be given as a relative quantity:

$$\xi = \frac{\xi_i}{\xi_{i \max}} \quad 2.2.2'$$

Moreover, a yield, $Y_{i/j}$, can be determined as follows:

$$Y_{i/j} = \frac{C_{jt} - C_{j0}}{C_{i0}} \quad 2.2.3$$

The yield compares the total amount of a product j yielded from an amount of material i consumed. A relative yield can also be defined:

$$Y_{i/j \text{ rel}} = \frac{Y_{i/j}}{Y_{i/j \max}} \quad 2.2.3'$$

The output of the reactor (dimensions tons/day) can be calculated from the relationship:

$$\text{Output} = \xi_i n_i = Y_i n_i \quad 2.2.4$$

where n_i is the mass flow of component i [t/d] (Moser, 1988). If $\xi_i \rightarrow 1$ or $Y_i \rightarrow 1$, the output will be equal to n_i .

Point **4** in fig 2.2.2 represents the point where the minimum costs are reached (Moser, 1988). The accumulated cost at any time can be formulated according to:

$$C_{\text{tot/Kg}} = \frac{C_B}{W} + \frac{C_R}{W} \quad 2.2.5$$

where: C_B = batch costs (sterilisation, materials, etc.);

C_R = running costs (stirring & aeration power, etc.)

Hence, a typical time course for the accumulation of costs in a batch bioprocess is shown in fig. 2.2.3; the time when costs are minimal (t_4) may be calculated.

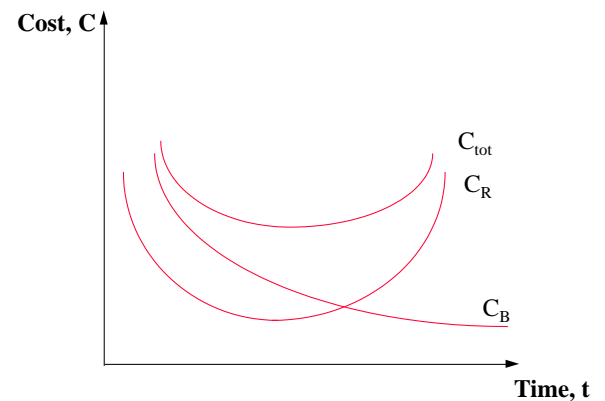


Fig. 2.2.3 Typical cost time evolutions for a discontinuous bioprocess over time (from Moser, 1988)

Finally, point **3** from fig. 2.2.2 is that of maximum profitability. From an empirical point of view (Moser, 1988), this point lies between the point of maximum productivity (for a batch bioprocess) and the point

representing minimum costs. The profit is calculated through the following analytical relationship (Geyson, Gray, 1972, Moser, 1988):

$$\text{Profit}[\$/\text{tona}] = W(\text{price} - C_{pe}) - C_R t - (C_B - C_e)$$

2.2.6

where: W = mass, [kg];

C_{pe} = the extraction cost for product isolation, [\$];

C_e = the extraction running costs, [\$].

The maximum profit can be graphically obtained from eq. 2.2.6 as it is shown in fig. 2.2.4. Hence, the maximum profit represents the slope of the tangent to curve drawn from a starting point A.

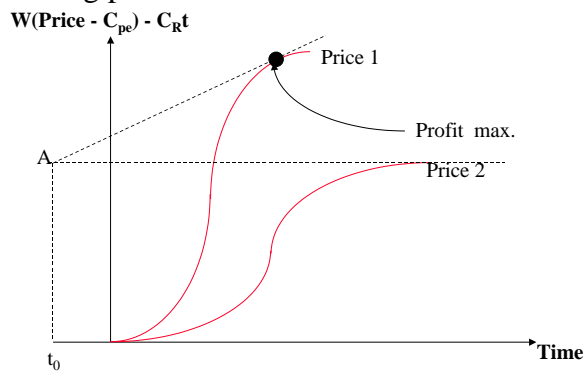


Fig. 2.2.4 Graphic method for evaluating the maximum profit at a given selling price and starting point (from Moser, 1988)

Finally, one can appreciate that the use of economic analysis of processing costs is a powerful tool in establishing control performances criteria.

2.3. BIOPROCESS CONTROL STRATEGIES

2.3.1. Bioprocess control using *a priori* model

Good knowledge about physiological states and operation modes which characterize a biosystem, allow the design of control algorithms dedicated to optimize the bioprocess (Vanichsriratana *et al.* 1996). If this knowledge is enough for model design (i.e. mathematical description of cell metabolic processes), then:

- The process mathematical description is used for the design of the control and optimization algorithms (Abidi *et al.*, 1993; Isaacs, Thoma, 1992);
- The model (based on the cellular metabolic reactions) can produce more information about the specificity of the applied procedure (Rohner, Meyer, 1995; Eaton, Rawlings, 1992).

A good mathematical model (i.e. comprehensive, with skillful microbial kinetics integration) is able to give predictions relative to procedure efficiency (Moser, 1984). Moreover, it represents the best way for strategy design of optimal control (Slimes *et al.*, 1995).

Mathematical models, which describe the living cells evolution, must represent the dynamic nature of the biosystem, as general as possible (and more complex as a consequence), less empirical, reflecting the biochemistry of the microorganism culture. In these conditions, the model should be set up based on a compromise between the detailed description of the bioprocess (which means the use of a great number of parameters, often undeterminable/ uncontrollable) and the use of a limited number of parameters easy to estimate and control. (Patwardhan *et al.*, 1992; Henson, Seborg, 1992).

As a general rule for the bioprocesses control with *a priori* model, the deterministic models

are preferred to the probabilistic ones (Cazzador, 1988). For the time being, the unstructured deterministic models (the cells are considered as black-box units) are very used in the bioprocess control. In the future an increase of the structured models role is expected, as a consequence of modern analysis methods development, as well as of the capacity to more adequately describe the phenomena.

These characteristics can be accomplished taking into account the cellular structure, at the level of the chemical components (chemical structured models) or from the point of view of cellular morphology (size, dimension and cellular age).

Also it is necessary to remark that the control bioprocess can be done, besides the kinetic equations, using the mass balance equations (Weinrich, Lapidus, 1972; Balzer *et al.*, 1984) and the typical elements introduced by the bioprocess stoichiometry (yield coefficients $Y_{X/S}$, $Y_{P/S}$, $Y_{X/O}$, etc.).

2.3.1.1. Main characteristics of the control with mathematical model

The development of a global model for the bioprocess evolution offers, besides the advantage of the analytical determination of the optimum value, the means to change the parameters during the process.

In these conditions, after setting up the model, the maximizing/minimizing strategy must be established as well as the performance index (Cardello, San, 1988; Dovi, Reverberi, 1993).

If the mathematical rules, which characterize the bioprocess, are defined, the bioprocess control consists of an algorithm put into operation in order to maintain the parameter evolution all around the optimum values. In

fact this represents the simplest case for the use of a control algorithm.

The major problems connected to the *a priori* model control are determined by the nonlinear and non-stationary characteristics of the bioprocess (Shields, Kao, 1994). Moreover, the access to the information (on-line and estimative determinations) is limited and powerfully affected by several errors, as it has already presented.

In a recent study, Proell (Proell *et al*, 1994) have presented the solution of a bioprocess control using *a priori* model. He proposed the following equations:

$$\frac{dX}{dt} = \mu X - \frac{X}{V} \frac{dV}{dt} \quad 2.3.1.1$$

$$\frac{dS}{dt} = \frac{FS_0}{V} - \mu \frac{X}{Y_{X/S}} - \frac{S}{V} \frac{dV}{dt} \quad 2.3.1.2$$

$$\frac{dC_t}{dt} = q^3 \sqrt{S} - \frac{C_t}{V} \frac{dV}{dt} \quad 2.3.1.3$$

$$\frac{dV}{dt} = F - F_v \quad 2.3.1.4$$

where: X = cell concentration;
S = substrate concentration;
V = volume of culture;
C_t = inhibitor (toxin);
μ = specific growth rate;
F = feed rate;
S₀ = substrate concentration in the feed;
Y_{X/S} = yield coefficient (S → X);
F_v = evaporation loss rate.

Conforming to the above conditions, the analytical expressions of the parameters Y_{X/S} and μ are:

$$Y_{X/S} = \frac{\mu y}{My + \mu} \quad 2.3.1.5$$

$$\mu = \mu_{\max} \frac{S}{K_s + S + \frac{S^2}{K_i}} \frac{K_t}{K_t + C_t^2} \quad 2.3.1.6$$

where the values of parameters y, M, μ_{max}, K_i, K_s and K_t are obtained by estimation.

In this case the control problem comprises two levels:

- the determination of the time minimum value at the end of the process for which the cell concentration attains the maximum value (after this moment the process can be described as a stationary system);
- the optimization of the global productivity in a fixed time.

Hence, the authors define the objective function:

$$\max_F F = \int_0^{t_f} (\mu X) dt \quad 2.3.1.7$$

The system is characterized by cell concentration, substrate concentration, toxin concentration and culture volume, the following restrictions being necessary:

$$\begin{aligned} F_{\min} &\leq F \leq F_{\max} \\ 0 &\leq V \leq V_{\max} \\ C_t &\leq C_{t \max} \end{aligned} \quad 2.3.1.8$$

The optimum feed flow rate is obtained by maximizing the functional Hamiltonian (Proell *et al*, 1994):

$$F = \begin{cases} F_{\max} & \text{if } H_F = \frac{\partial H}{\partial F} > 0; \\ F_{\min} & \text{if } H_F < 0; \\ F_S & \text{if } H_F = 0 \end{cases} \quad 2.3.1.9$$

where: F_S = solution on the singular arc.

The control solution must be circumscribed to the control conditions afforded by the growth specific rate:

$$F(t+1) = F(t) + K\varepsilon(t) \quad 2.3.1.10$$

$$\varepsilon(t) = \mu_{ref} - \mu(t) \quad 2.3.1.11$$

where: μ_{ref} = optimal value of the specific growth rate (set-point value);
K = constant

Taking into account the linear dependence $\mu = f(S)$, the relationship can be written:

$$\varepsilon(t) = S_{ref} - S(t) \quad 2.3.1.12$$

The set-point value, S_{ref} , can be analytically determined:

$$S_{ref} = \sqrt{\frac{K_S K_i}{2}} \quad 2.3.1.13$$

In the authors' opinion, in order to modify these models into linear equivalents, it is better to use the nonlinear modifications of the coordinates. In this way, the fermenter productivity is indirectly controlled by the cell concentration, substrate and metabolic product concentrations adjustment; the obtaining results are sub-optimal.

2.3.1.2. Bioconversion control using *a priori* model

In many cases, in the bioconversion process for complex chemical substance obtaining, strong inhibition exercised by substrate or product has been recorded.

To control such processes, it has been established, as a general rule, the use of the optimum domains (without inhibition) for the major bioprocess parameters.

For the bioprocesses characterized by substrate inhibition, the fed-batch fermentations are preferred, the substrate and other products being introduced during the process, according to a specific protocol.

Meanwhile, for the bioprocess performance improving, the repeated fed-batch system has been used, which means that at the end of the process about 5-10% of the medium volume is kept as inoculum for the next cycle. In these culture conditions, the system is safe as regarding the sterility and the apparatus fiability.

The control of the bioprocesses when the cell growth and product formation take place at the same time, is based on the modeling with balance equations including kinetic expressions. In this way, by solving the differential equation system, the optimum concentrations of cells and products, as well as the specific process rates can be determined.

2.3.1.3. Conceptual restrictions of the control based on *a priori* model

The control of the bioprocesses according to *a priori* model represents an optimal method, due to the possibility of analytical determination of the extreme values of the coordinates.

As it has already presented in #2.1 a great variety of analytical models can describe the culture evolution steps. If the parameter

influence is considered, the models are able to realize a proper fit between model and experiment. Despite these aspects, the models mentioned in the literature can not describe accurately enough all the evolution steps of a complex bioprocess.

This fact is a consequence of the lack of measurement methods for on-line obtaining the variable levels from the system, (cf. #1.3.). On the other hand, the theoretical studies presented in the literature can not cover all the biological, chemical and physical phenomena taking place during micro-organism cultivation.

Hence, there are a great number of kinetic models, each one trying to better present the parameter interdependence and the bioprocess evolution. Therefore, in the future, the control structure based on *a priori* model will be replaced by more flexible configurations using the adaptive or artificial techniques in addition with the previous control structures.

2.3.2. Adaptive control of bioprocesses

At the same time with the developing of the bioprocess control based on *a priori* model, other alternative control systems were designed (Liu *et al.*, 1993; Jorgensen *et al.*, 1992). The most attractive was the adaptive control strategy (Ferreira, de Azevedo, 1996, Vanichsriratana *et al.*, 1996).

The adaptive control structures (cf. to the Belgian School Bastin, Dochain, 1990; Chen *et al.*, 1991), are based on the design of different estimation algorithms which are able to determine the off-line parameter values. Many control algorithms were developed based on *minimal* knowledge about bioprocess kinetics (i.e. the *minimal modeling* concept). Hence, the general state space model which describe the dynamics of bioreactors can be described as follows: the bioprocess reactions which occur in the reactor are assumed to be encoded into a reaction network of M reactions involving N components ($N > M$).

For instance, the following equation describes a simple growth microbial process, with a single substrate (with concentration S):



This relationship represents an auto-catalyzed reaction, with φ_g = growth rate, X = biomass concentration. Note that biomass production requires an initial biomass concentration.

The enzyme-catalyzed reactions can be described as follows:

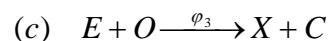
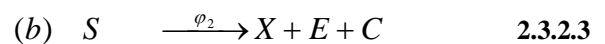
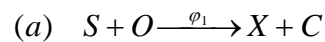


where: φ_c = enzymatic catalysis rate;
P = product.

The enzymatic catalysis is conceivable with a biomass, which grows on the same substrate.

An usual example for this kind of complex reaction is the yeast growth on glucose substrate. This bioprocess is characterized by three metabolic reactions:

- Respiratory growth on glucose (a);
- Fermentative growth on glucose (b);
- Respiratory growth on ethanol (c).



This example (Bastin, 1992), involves three reactions with five components; O represents dissolved oxygen concentration, E, ethanol concentration and C, dissolved carbon dioxide.

Hence, the above example can be generalized, and the general dynamic model of the bioprocess can be written: the mass balance dynamics (of each component) results from

two mechanisms – i.e. reaction kinetics and exchange of material with the environment:

$$\frac{d\xi_i}{dt} = \sum_{j=i} \pm k_{ij} \varphi_j - D\xi_i - Q_i + F_i \quad 2.3.2.4$$

where:

- a) $\sum_{j=i}$ represents the rate of consumption and/or production of component ξ_i in the bioreactor, according to the reaction network; the summation is taken over the reactions with index j which involve the component ξ_i .
- b) K_{ij} represents the yield coefficients (strictly positive) of the component ξ_i in reaction j (e.g. $Y_{X/S}$ or $Y_{P/S}$);
- c) Q_i represents the rate of removal of component ξ_i from the reactor in gaseous form per unit of volume;
- d) $D\xi_i$ represents the dilution of component ξ_i in the bioreactor due to the increase in volume (D = dilution rate);
- e) F_i represents the feed rate component ξ_i into the reactor per unit of volume.

If the following matrix notations are introduced:

$$\begin{aligned} \xi^T &= [\xi_1, \dots, \xi_N] \\ \varphi^T &= [\varphi_1, \dots, \varphi_M] \\ F^T &= [F_1, \dots, F_N] \\ K &= [K_{ij}], \text{matrix } N \times M \end{aligned} \quad 2.3.2.5$$

then the bioprocess dynamic can be represented as follows:

$$\frac{d\xi}{dt} = K\varphi(\xi, t) - D\xi + F - Q(\xi) \quad 2.3.2.6$$

This General Dynamical Model of biological reactors (proposed by Prof. Bastin) is the fundament of the control algorithms, which will be presented below.

Hence, cf. to eq. (2.3.1.1) it can be obtained:

$$\varphi_j(\xi) = \mu_j(\xi) X_i \quad \text{or} \quad \varphi_g = \mu X \quad 2.3.2.7$$

where $\mu(\xi)$ = specific growth rate.

The dynamic model become:

$$\frac{d}{dt} \begin{pmatrix} S \\ X \end{pmatrix} = \begin{bmatrix} -k_1 \\ k_2 \end{bmatrix} \varphi - D \begin{bmatrix} S \\ X \end{bmatrix} + \begin{bmatrix} F \\ 0 \end{bmatrix} \quad 2.3.2.8$$

and φ can be written as follows:

$$\begin{aligned} \varphi(S, X) &= \alpha(S, X) SX \\ \varphi(S, X) &= \mu(S, X) X \end{aligned} \quad 2.3.2.9$$

Conforming to the second relationship from (2.3.2.8), the dynamic model is identical with the classical one whether $k_2 = 1$ and $F = DS_0$ (S_0 = substrate concentration in the feed rate):

$$\begin{aligned} \frac{dS}{dt} &= D(S_0 - S) - k_1 \mu X \\ \frac{dX}{dt} &= (\mu - D)X \end{aligned} \quad 2.3.2.10$$

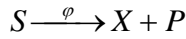
Moreover:

$$q_s = \frac{\mu}{Y_{X/S}} \equiv k_1 \mu \quad 2.3.2.11$$

Hence:

$$\frac{dS}{dt} = D(S_0 - S) - q_s X \quad 2.3.2.12$$

Moreover, for an autocatalytic reaction, with one substrate and one gaseous product, the following equation can be used:



2.3.2.13

Consequently, the following situations are to be considered:

and the global dynamic model becomes:

$$\frac{d}{dt} \begin{bmatrix} S \\ X \\ P \end{bmatrix} = \begin{bmatrix} -k_1 \\ 1 \\ k_2 \end{bmatrix} \varphi - D \begin{bmatrix} S \\ X \\ P \end{bmatrix} + \begin{bmatrix} DS_0 \\ 0 \\ 0 \end{bmatrix} - \begin{bmatrix} 0 \\ 0 \\ Q_1 \end{bmatrix} \quad 2.3.2.14$$

The design of the kinetics $\varphi(\xi)$ is complex, as it depends on the following factors:

- The adequate choice of biological and physic-chemical factors;
- The founding of an analytical relationship for correctly describing the phenomena;
- The determination of kinetic coefficients based on experimental data.

Consequently, Prof. Bastin recommends the introduction of a minimal modeling of the bioprocess kinetic $\varphi(\xi)$ using the expression done below:

$$\varphi(\xi) = H(\xi)\rho(\xi) \quad 2.3.2.15$$

where: $H(\xi)$ is a known matrix (i.e. it represents the known component of the kinetics) and $\rho(\xi)$ represents the unknown component. The above equation is flexible and involves many realistic situations. The limiting situation comprises a bioprocess kinetics entirely unknown:

$$\varphi(\xi) = \rho(\xi) \quad 2.3.2.16$$

The enzymatic catalysis bioprocess offers a simple example of the minimal modeling concept application:



I If the kinetic is *a priori* completely unknown, the parameters can be defined as follows:

$$\begin{aligned} \rho_1(\xi) &= \varphi_g(S, X, P) \\ \rho_{21}(\xi) &= \varphi_c(S, X, P) \end{aligned} \quad 2.3.2.19$$

II Moreover, the following expression can be added:

$$\varphi(\xi, t) = \alpha(\xi, t)G(\xi) \quad 2.3.2.20$$

where: $\alpha(\xi, t)$ = specific growth;

$G(\xi)$ = diagonal matrix of the product $\prod_{n=j} \xi_n$

Taking into account the above expression (if the specific reaction rates are unknown), two relationships can be written:

$$\begin{aligned} \rho_1(\xi) &= \alpha_g(S, P) \\ \rho_2(\xi) &= \alpha_c(S, X) \end{aligned} \quad 2.3.2.21$$

In this case, the matrix $H(\xi)$ becomes

$$H(\xi) = G(\xi) = \begin{bmatrix} SX & 0 \\ 0 & SX \end{bmatrix} \quad 2.3.2.22$$

Finally:

$$\begin{bmatrix} \varphi_g(S, X, P) \\ \varphi_c(S, X, P) \end{bmatrix} = \begin{bmatrix} SX & 0 \\ 0 & SX \end{bmatrix} \begin{bmatrix} \rho_1 \\ \rho_2 \end{bmatrix} \quad 2.3.2.23$$

III If the specific growth rate $\mu(S, P)$ is an unknown parameter of the model, the following variables can be defined:

$$\rho_1(\xi) = \mu(S, P)$$

$$\rho_2(\xi) = \alpha_c(S, X) \quad 2.3.2.24$$

$$H(\xi) = \begin{bmatrix} X & 0 \\ 0 & SX \end{bmatrix}$$

Hence, using the state variables, which are continuously monitored (through standard instrumentation), on-line estimation algorithms can be developed and useful information will be send to the human expert.

Moreover, if an adaptive control algorithm is used, the controller must determine, at each time period, the best-input flow in the system, based on measured and estimated variables. From the point of view of the General Dynamic Model, the control objective is the regulation of the output variable (i.e. a scalar variable) which represents a linear combination of the state variables:

$$y = \sum_{i=1}^N C_i \xi_i = C^T \xi \quad 2.3.2.25$$

where: $C^T = [C_1, C_2, \dots, C_N]$ represents a vector of known constants. In this case the variable u designates the substrate flow:

$$u = F_i$$

$$F = bu + f$$

$$b^T = [b_1, b_2, \dots, b_N] \quad b_i = 1 \quad b_j = 0 \quad \forall j \neq i$$

$$f^T = [f_1, f_2, \dots, f_N] \quad f_i = 0 \quad j_j = F_j \quad \forall j \neq i$$

2.3.2.26

Conforming to these notations, the General Dynamic Model (2.3.2.6) becomes:

$$\frac{d\xi}{dt} = K\varphi(\xi) - D\xi + bu + f - Q \quad 2.3.2.27$$

with f and Q on-line measurable. The state variables, ξ , can be on-line measured/estimated, too.

The principle of the Linear Control involves the design of a control law $u(\varphi, Q, f, y^*)$, which is a multivariable, non-linear function. This control law must be designed so that the error (y^*-y) respects a pre-specified nonlinear differential equation.

The design of the Linear Control procedure involves three steps:

Step I: An input/output model can be obtained (through derivation) from the general Dynamic Model:

$$\frac{d^\delta y}{dt^\delta} = f_0(t) + u(t)f_1(t) \quad 2.3.2.28$$

where: δ = relative degree

The I/O model is linear according to $u(t)$; at the mean time, $f_0(t)$ and $f_1(t)$ can be complex functions, depending on ξ , Q , F and theirs derivatives.

Step II: A linear reference model of the error $[y^*(t)-y(t)]$ will be selected, conforming to the following equation:

$$\sum_{j=0}^{\delta} \lambda_{\delta-1} \frac{d^j}{dt^j} [y^*(t) - y(t)] = 0 \quad \text{with} \quad \lambda_0 = 1$$

2.3.2.29

The coefficients λ are arbitrary selected so that the above differential equation becomes stable. Moreover, the reference model gets independent, if the working point is considered.

Step III: Finally, the control variable $u(t)$ is to be calculated, so that the I/O model becomes identical with the reference model:

$$u(t) = \frac{1}{f_i(t)}[-f_0(t) + \sum_{j=0}^{\delta-1} \lambda_{\delta-j} \frac{d^j}{dt^j}(y^*(t) - y(t)) + \frac{d^\delta y^*(t)}{dt^\delta}] \quad 2.3.2.30$$

The last equation designates *the control law with exact linearization*.

Conforming to author conceptions, the design of an adaptive control algorithm must focus on the entire bioprocess stability. Hence, two general aspects must be outlined:

- a) The main objective of a fed-batch bioprocess control is to maintain the control upon an unstable evolution trajectory; in a continuous bioprocess, the main objective is to preserve the global stability;
- b) In a minimal modeling context (and depending on modeling strategies) the same linear controller design method can generate stable or unstable closed loops.

Finally, the following conclusions can be set up:

- The matrix conceptualization of bioprocess variables offers easy-to-use in control algorithm implementations (Hinde, Cooper, 1995; Meszaros, *et al.*, 1995), but does suggest nothing about the corresponding real variables;
- The optimization is made during a sample time period (Estler, 1995; Andrews, 1995); thus, it not corresponds to a *global* optimization (Harmon, *et al.*, 1987; Modak, Lim, 1987; Modak, Lim, 1989); *ipso facto*, the control strategy is sub-optimal (Morningred *et al.*, 1992; Modak, Lim, 1992);
- The control structures based on these model typologies don't produce basic knowledge about the bio/chemical mechanisms, which control the cell metabolism (Lee, Ramirez, 1996).

2.3.3. Intelligent control of bioprocesses

The impact of intelligent techniques in the bioprocess application field represents, on one hand, a quasi-failure of the standard control strategy applications, and on the other hand, a pragmatic approach tentative in opposition with the knowledge bioprocess uncertainty. Hence, it has been found (Narendra, Parthasarathy, 1992) that the control structures based on the human decisional factor (i.e. a subjectively element) offer better results as the standard control systems based on algorithmic determinations. Moreover, it has been observed that a general stoppage exists in the bioprocess-modeling field (without the structured models); so that, the computer performances are developed in the detriment of the general knowledge concerning life phenomena (Hinde, Cooper, 1994; Cooper *et al.*, 1992). Consequently, through intelligent techniques (i.e. neural nets, fuzzy structures, genetic algorithms or expert systems) the uncertainties regarding the living phenomena can be mathematically formalized. Furthermore, the (subjective) human knowledge is used and different simulation techniques are adopted in order to find out the evolution types. These kind of solutions offers better results as the standard control techniques but don't promote advanced comprehension upon the metabolic routes of bioprocesses.

2.3.3.1. Bioprocess control using expert systems

The development in the field of fermentation process control makes necessary the integration of some research disciplines. Moreover, the control of biotechnological processes is based on the knowledge of experts and human operators as well as on analysis and processing of numerical data. Also, Expert Systems (ES) can handle this knowledge, acquired by human experts – but the specified knowledge are able to describe not more than the conscious thinking and decisions.

The ES applicability starts with the selection of a specialized algorithm dedicated to the

designated bioprocess (ESDT). This selection is achieved following a well-defined set of rules.

Hence, it must focus on the structure, functions, implementation structure of ES and ESDT algorithm characteristics (i.e. bioprocess limitations, etc.)

ES – functions and implementation

The specificity of an ES algorithm must be correlated with the functions, to be fulfilled in real conditions:

- Identification of the cell population state (i.e. on-line evaluation of the physiological state, cell behavior interpretation, future state prediction and the diagnostic of biological phenomena);
- Identification of the entire equipment state (i.e. fault detection);
- Supervision of the conventional control equipment (i.e. set-point values modification, parameter control revision, etc.);
- Advanced communication with the human operator (i.e. parameter monitoring, etc).

The most used control implementation in the bioprocess field is the indirect control; two procedures are available:

- The ES algorithm runs on an independent computer; it is interfaced with conventional controllers (this is, nowadays, the standard control procedure);
- The ES and the conventional control algorithms run together on the same computer; this solution can be the best one in the bioprocess control field.

ESDT characteristics

The usual characteristics of all applications for bioprocess control are:

- High run speed (i.e. ESDT must be developed in Assembler and/or C);
- Rules activation at different time period;
- Continuous cyclic operation;

- Human operator intervention relating to bioprocess evolution;
- Integration with other external algorithms (through information exchanges);
- Powerful mechanism for knowledge organization during process evolution;
- On-line integration of new information regarding process evolution; inexact knowledge rejection (i.e. advanced control system flexibility);
- Continuous control system learning through neural nets and/or other structures.

An useful ESDT involves the following features:

- Limiting number of on-line measurable/estimable variables and relationships;
- Powerful mechanisms for qualitative/subjective information inclusion, and for confidence-level definition;
- Continuous system supervision (i.e. modification of time sample period, sensor periodical re-calibrations, measure apparatus tests, etc).

The design of an ES for fermentation process control is based on the database and knowledge resources formalization, i.e. it is preferably to define a specific ESDT for each kind of bioprocess

2.3.3.2. Bioprocess control using neural nets

The control and optimization of a continuous stirrer tank fermenter using a neural network (Thibault *et al.*, 1994) is based on the presumption that the control set-point is not known explicitly, but it is calculated considering the goal (through neural nets, NN) to optimize an objective criterion. Hence, the final results show that NN can model very accurately the bioprocess dynamics and can predict the state variable over a long prediction horizon.

For instance, it is assumed that the bioreactor volume remains constant and the biomass growth kinetics can be appropriately described

with a Haldane substrate inhibition model. A general objective cost function (J) is expressed by the following equation:

$$J = \alpha FX + (1 - \alpha)X \quad 2.3.3.1$$

where: F = the flow rate
 X = biomass concentration

If it is desired to optimize the productivity, the value of α would be set to one. However, the process economics dictate the double condition to have at the same time the highest possible biomass productivity and a high biomass concentration. Hence, the authors resolve this conflicting objective by weighting accordingly both contributions to the objective function.

The authors propose a NN to be used to predict the biomass and the substrate concentrations one sample period in the future, being given the inlet substrate flow rate, the biomass and substrate concentrations at the current sampling instant. A set of 200 samples, obtained by randomly changing the inlet substrate flow rate was considered as the learning data set. The NN weights were obtained using the backpropagation algorithm in order to minimize the sum of errors squares on both biomass and substrate concentrations.

The results clearly demonstrates (Thibault *et al.*, 1994) the ability of the neural model to indirectly represent the complex relationship occurring in the bioprocess. In addition, the NN was able to reproduce with an outstanding accuracy the variation of the objective function as a function of flow rate.

Moreover, the use of the NN in conjunction with an optimizing routine can determine, at each sample instant, the inlet substrate flow rate that will maximize the steady state objective performance criterion. The authors note that the presence of noise increases the time required to find a proper NN; however, a

moderate level of noise contributes to produce a more informative data set.

CONCLUSIONS

1. The literature model investigation indicates that it is impossible to design conceptual models conforming to Edwards & Wilke postulates. Hence, the mathematical expressions of bioprocess parameter evolution are built using some conceptual simplifications (e.g. the multivariable character of bioprocess). Moreover, a dichotomy regarding the model classes can be seen: on one hand, it is necessary to increase the knowledge about the cell metabolic mechanisms, and on the other hand, the synthetic approach is acceptable from a macroscopic point of view (i.e. for the control design);
2. The objective control criterion is to be made from an economic point of view, taking into account the (final) industrial requirements. Hence, different ways for profit determination are presented, linked with the bioprocess type and evolution phase.
3. The analysis of different literature methods for bioprocess control proves that it is not possible, in this moment, to globally optimize this kind of processes. Hence, the control with *a priori* model is not efficient due to the non-existence of global bioprocess models; the adaptive control strategies are (theoretically speaking) sub-optimal: the optimization during each sample time period cannot agree (in most cases) with the global optimum. Finally, the intelligent control technique solutions offer an economic answer (i.e. an increasing of efficiency) *versus* the expansion of basis knowledge regarding the process general evolution. In addition, this solution is based on human experience, i.e. it involves strong subjective characteristics augmented by computer speed.