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RESEARCH ARTICLE

PROGRESS IN AEROBIC BIOPROCESS MODELING AND CONTROL. CASE STUDY

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Abstract

The overview presents the most important and recent issues regarding the modelling and the control of the aerobic bioprocess. First part is dedicated to the discussion about instruments and techniques for bioprocess variables determination and monitoring: (a) the standard direct physical determinations: temperature, pressure (over pressure), agitator shaft power and rate of stirring, foam, gas and liquid flow, weight; (b) the regular chemical determinations: pH, redox potential, dissolved oxygen concentration, exit-gas analysis, on-line analysis of other chemical factors (ion-specific sensors, enzyme electrodes, microbial electrodes, mass spectrometers, fluorimeters). In the second part the mathematical modelling of the aerobic bioprocess is presented with several types of models: unstructured global models; structured models; segregated models; metabolic modelling. The bioprocess control has different goals and objectives, function of bioprocess characteristics and imposed performances. A case study based on authors' original research presents the bioprocess modelling and the intelligent structure design to control the fed batch cultivation of *Hansenula polymorpha* CBS-4732 yeast for alcoholoxidase-containing cellular mass.

Key words: bioprocess, modeling, control, case study

Introduction

The bioprocess advancement is determined by the living cells capabilities and characteristics, the bioreactor performance as well as by the cultivation media composition and the main parameters evolution. The high metabolic network complexity inside the cells often determine very sophisticated, non-linear growth and product formation kinetics, with further consequences on the bioprocess behavior, but at the same time on the product quality and yield.

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The key issue of this rather complicated situation is the use of modeling and further on of computer assisted control as a powerful tool for bioprocess improving (Chirvase, 2007).

The process models, as relationships of the input, output and inner variables, though incomplete and simplified, can be effective to describe the phenomena and the influences of great importance for control, optimization and better theoretical knowledge. The function of any biological model is to describe the metabolic reactions rates and their stoichiometry on the basis of bioreactor conditions, with the main difficulties-the identification of principal factors affecting cellular growth and bioproduct formation, and the building up of a suitable model structure for the intracellular processes. Moreover the scheduling, supervision and automatic control in modern bioprocessing is done by advanced process control systems, where all the functions are implemented in software (in accordance with the Figure 1).

The main bioprocess control attributes are: handling of off-line analyses; recipe and scheduling; high level overall control; state and parameters estimation; simulation; prediction; optimization.

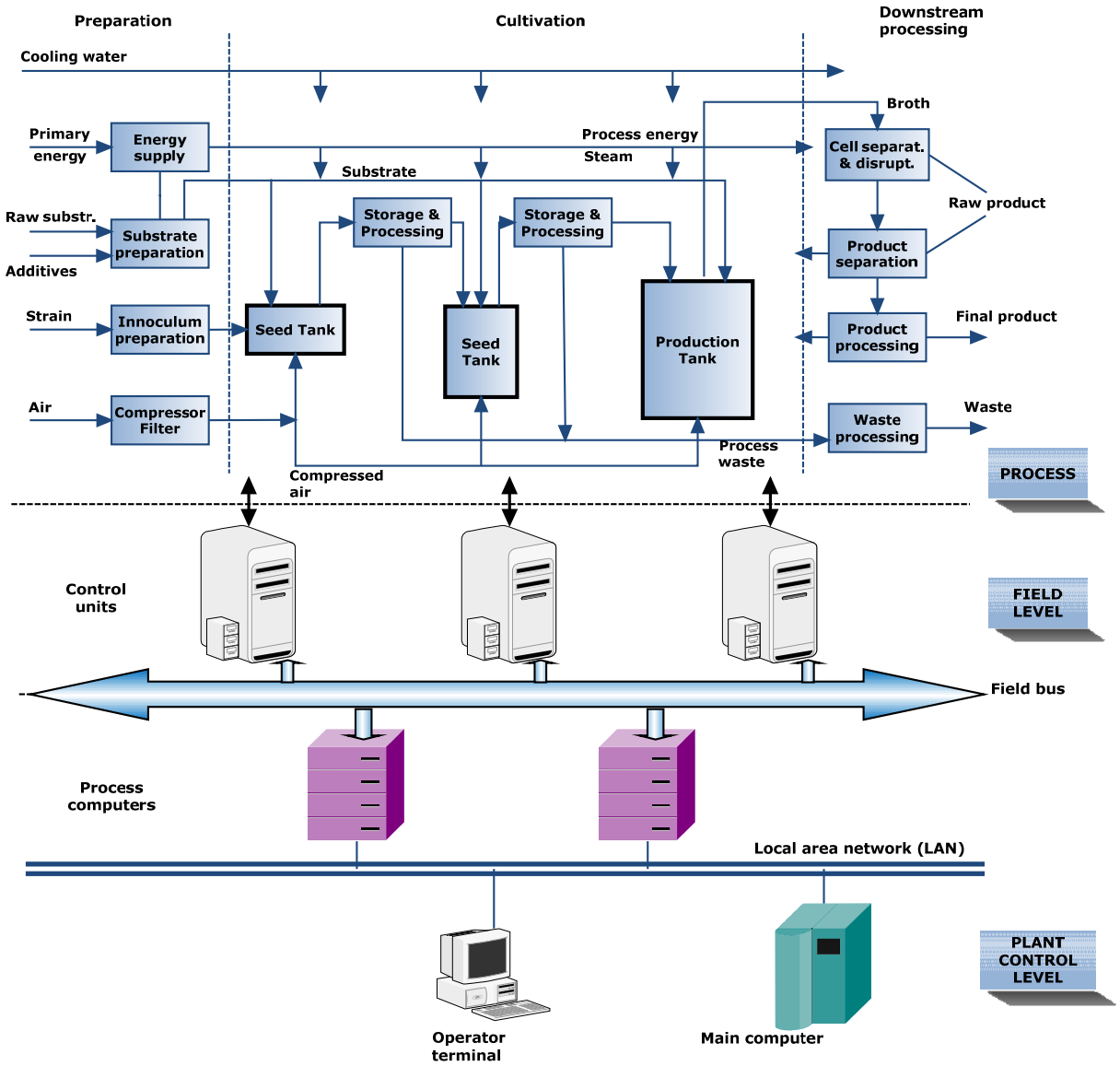

Figure 1. *General presentation of the computerized bioprocess control (Bellgardt, 2000)*

For the industrial developments the central and manifold objective of the computer control is the realization of the economic interests in assuring high operational stability, process reproducibility and increased product yield together with the maintaining of rigorous safety and the implementation of the GMP or environmental regulations, important requests in modern biomanufacture imposed by the product quality improving needs.

Materials and methods

To achieve the biological potential of cells, the optimal environmental conditions must be maintained in the bioreactor for cell growth / product formation, at least with regard to the key parameters. Generally speaking, biological systems are influenced by different process variables, which have a direct influence on cell metabolism. Sensors for these variables are (typically) inserted into specially designed ports on the bioreactor. As bioreactors increase in size (i.e. in the industry field), the mixing problems become usual and probe location becomes problematic. To accurately outline large fermenters, probes may be collected from several locations.

The mathematical modeling of the aerobic bioprocess

The aerobic bioprocess modeling is an useful tool to accomplish several important tasks (Bellgardt, 2000): (a) it can be the basis for adequate optimization and control technique applications; (b) it can provide the necessary information about the features of the chosen bioprocessing system; (c) it synthesizes the characteristics of the specified living cells' evolution and hence, it is the best technique to predict the process efficiency. The models show the complex biosystems attributes; so they must be as possible as extensive and non-speculative. Moreover the models are an acceptable compromise between the presentation of processes in detail, with considerable number of parameters, and the use of few parameters, easy to apply and estimate.

Most important properties of a biological mathematical model were defined in the Edwards and Wilke' postulates (Bellgardt, 2000): (a) it is capable to represent all the culture phases; (b) it is flexible enough to approximate different data types without the insertion of significant distortions; (c) it must be continuously derivable; (d) it must be easy to operate, once the parameters evaluated; (e) each model parameter is to have a physic significance and must be easy to evaluate. The attempts to realize high 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. Finally several types of models can represent the evolution of the aerobic bioprocess. The most important categories will be presented further on.

1. The unstructured global models are in use nowadays as the main tool for both the bioprocess modeling, but also for being applied in overall computer control (Schugerl, 1991). Their limit is they are a simplified representation of the bioprocess behavior: conforming to this concept the bioprocess evolution depends directly and only on the macroscopic variables representing the working conditions in the bioreactor. Therefore the unstructured models are essentially kinetic equations that describe the variation of substrate or product concentrations and of a unique biological state variable-the cell concentration, and can also express the influences of some important process variables (pH, pO_2, temperature, and others), and only sometimes they are balance equations.

Generally speaking, one considers that the specific growth rate (Bastin *et al.,* 1990)

$$(\mu = \frac{1}{X}\frac{dX}{dt})$$

is the key variable for cell growth, substrate consumption and product formation. The specific growth rate is time dependent and dependent on different physical, chemical and/or biological parameters (substrate concentration-S, cell concentration-X, product concentration-P, pH,

temperature-T, dissolved oxygen concentration-C, and different inhibitors-I). Conforming to the literature assumptions (Bastin *et al.*, 1990), the specific growth rate dependence upon different process parameters can be considered as follows:

$$\mu = f(S, X, P, pH, C, I, ..., t) \qquad (1)$$

a) $\mu=\mu(S)$ *Kinetic models with growth limitation through substrate concentration (without inhibition)* (Bellgardt, 1991, 2000)

b) $\mu=\mu(X, S)$ *The influence of cell and substrate concentrations upon the specific growth rate* (Bellgardt, 1991, 2000)

c) Growth kinetics with substrate inhibition: In most cases, the kinetic model equations are derived (like the Monod model) from the inhibition theory of enzymatic reactions. Consequently they are not generally valid and can be applied in connection with experimental acceptability (Bellgardt, 1991, 2000).

d) $\mu = f(S, P)$ *Growth kinetic with product inhibition* (Bellgardt, 1991, 2000) Hinshelwood (Hinshelwood, 1946) detected 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.

g) μ *(*S_1*,* S_2*) Kinetic models based on different substrates* Besides the case when the dissolved oxygen is considered as a second substrate, there are many cases when two or more carbon sources are taken into consideration. There are two typical situations: (1) the cells grow through the sequential (consecutive) substrate consumption (diauxic growth), where a simple Monod model can be applied; (2) the cells grow through the simultaneous consumption of substrates (e.g. wastewater treatment); in this case, the mathematical modeling is more complex.

h) Unstructured kinetic models for product formation: The product formation kinetic is taken into account in conjunction with the growth kinetic. Nowadays, the Gaden (Schugerl, 1991) classification is still useful. Based on this categorizing, four kinetic types can be defined:

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)

Afterward there are now more advanced models, the structured and the segregated models.

2. In case of the structured models the biotic phase is not any more viewed as a homogenous component, but they provide information about the physiological state of the cells, their composition and regulatory adaptation to the environment (Boudreau *et al.*, 2006; Henzle *et al.*, 2006). Conforming to this concept the cell mass is structured in several intracellular compounds and functional groups, which are connected to each other and to the environment by fluxes of material and information. The structured models can be: multi compartment models, genetically structured models, and biochemical structured models.

3. The segregated models can describe more complex phenomena like: alterations or disturbances in the physiology and cell metabolism; cells 'morphological differentiation; genome mutations; spatial segregations of growth regions; cells aggregation; mixed cultures (including the competition between two or more species for the same substrate) (Boudreau *et al.*,

2006; Henzle et al., 2006). On the contrary the unstructured and structured models have the limit to consider a homogenous population of cells and only one species in the bioreactor. The segregated models can be built by using ordinary differential equations to describe the behavior of several classes of independent/correlated cells. Each cell class behavior can be described by both unstructured and structured models.

Aerobic bioprocess control

The bioprocess control has different goals and objectives, function of bioprocess characteristics and imposed performances. In spite of high non-linearity linear control theory and basic controllers (on/off, PID) are still applied in most industrial applications.

More sophisticated control should rely on models able to correctly represent the biosystems behavior. Due to the complexity of the biological systems, basic models, which are nice to use and help to simplify the underlying mathematics, are not able to reflect the real situations. The large sets of parameters from the complex models need to be experimentally identified, and consequently the experiments should be carefully designed to provide this valuable information. Taking into account the time-to-market, which must be as short as possible the accepted control solution could be suboptimal based on classical robust control. Bioprocess reproducibility and living cell systems variability reduction from run to run is to be carefully studied. The media composition optimization and the successful application of PAT (process analytical technologies combining the techniques for in-process monitoring, data-based modeling process control) will contribute to the quality of production improvement.

In bioindustry, bioprocesses are subject to a number of local and / or supervisory control structures. Local controllers are used to get the set-point control of different physical / chemical parameters (e.g. temperature, pH and dissolved oxygen concentration), while supervisory control is necessary for optimizing the feed in a fed batch process or the dilution rate in a continuous one.

I. Bioprocess control with a priori model (model based process control)

The bioprocess control based on *a priori* model (BCAPM) can be seen as the on-line application of optimal control, where control actions are regularly re-calculated based on a global process model and process information (Brosilow *et al.*, 2002). The global model is used to calculate optimal control actions by a prediction of future outputs over a limited time horizon. The basic concepts of BCAPM consider two main ideas (Ryckaert *et al.*, 1998, Wouwer *et al.*, 2005): (1) the explicit use of an *a priori* model to predict the process output(s); (2) the calculation of the future control actions by minimizing a global objective function.

The problem can be solved in different ways: (a) for a linear, time-invariant model, and in the absence of constraints, an explicit analytic solution of the above optimization problem can be obtained; (b) with linear constraints, the above optimization problem is a Quadratic-Programming problem, which can be numerically solved; (c) in the presence of a nonlinear model or nonlinear constraints, a non-convex optimization problem must be solved at each sampling period. So iterative optimization algorithms, (e.g. the Nelder-Mead method) can be used in order to converge to local minima.

Recent developments in on-line measurement techniques, parameter and state estimation, in addition to the search of improved quality control, motivated the development of BCAPM. Now the technique was upgraded with better results. For instance (Dunn *et al.*, 2003) the applied BCAPM for feed control in the production of monoclonal antibodies allows to improve the yield with 43%.

II. Bioprocess adaptive control

When the process characteristics change during time, the operation conditions must also be changed: controller parameters and set point values. Moreover, optimal bioprocess evolution is commonly determined off-line, the process conditions are not perfectly known, and the process model is not well defined. Furthermore, it can be a

lot of changes in process conditions in conjunction with different microorganisms' life cycles (when the cell concentration increase in time in a batch bioprocess, the oxygen set point must be increased). Hence, there is a need for some feedback mechanisms based on on-line measurements. On-line adaptation is possible when the state variables can be measured online (Galvanauskas *et al.*, 2004) (directly using *hardware sensors* or indirectly by *soft sensors*). The adaptive control structures 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 (the *minimal modeling* concept) (Dunn *et al.*, 2003; Jadot *et al.*, 1998, Lant *et al.*, 2004, Kay, 2006).

There are two classes of adaptive control (where the adaptation is attained on the basis of on-line parameter observers) (Dunn *et al.*, 2003): (1) the process changes can be measured – therefore it is possible to systematically adjust the controller settings, based on the measured / anticipated bioprocess changes; (2) the process changes cannot be measured / predicted – hence the controller settings are automatically adjusted by a loop optimizer.

III. Bioprocess control using Artificial Intelligence (AI)

The limitations of the bioprocess control systems do not concern only the measurements or models, but at the same time much valuable human knowledge is only available in a qualitative heuristic form. Hence, it has been found that the knowledge-based control structures using the human decisional factor (i.e. a subjectively element) offer sometimes better results.

Moreover, the computer performances are developed in the detriment of the general knowledge concerning life phenomena and do not promote advanced comprehension upon the metabolic routes of bioprocesses.

Consequently, the intelligent techniques (i.e. neural nets, fuzzy structures, genetic algorithms or expert systems) are capable of simulating human expert-like reasoning and decision making, dealing with uncertainties and imprecise information (Jadot *et al.*, 1998).

As the human perception about the bioprocess is commonly altered by the psychological factors, the intelligent control systems founded (only) on the human subjective knowledge is less valuable than the control systems who utilize the objective information fitted by a conceptual model.

Hence, the literature recommends the intelligent control techniques utilization only if the control structure based on quantitative models fails.

Frequently, different process parameters are controlled in order to follow predefined transitory trajectories. Such control strategies can be designed by a *trial-and-error* approach in combination with operator's experience and statistical analysis of historic data.

Results. A Case Study

The research objective of this case study was to develop an appropriate control method for a bioprocess and to implement it on a laboratory plant, namely the control of the fed batch cultivation of *Hansenula polymorpha* yeast for alcoholoxydase-containing biomass (Launt et al., 2004).

At first, the process is described and a mathematical model is proposed and then the control strategy is defined and the intelligent control structure is designed.

Hence, a discontinuous fed-batch bioprocess for alcoholoxydase-containing biomass with the methylotrophic yeast *Hansenula polymorpha* CBS - 4732 was operated in an airlift lab - bioreactor

The intracellular enzyme, to be separated further on, is used for obtaining a high-specialized kit for methanol/ethanol determination.

The yeast was cultivated on a complex medium with $(NH_4)_2SO_4$, KH_2PO_4, Na_2HPO_4, $MgSO_4*7H_2O$, $CaCl_2$, yeast extract or autolysed residual beer yeast as organic N source and microelements (Fe, B, Cu, I, Mn, Zn, Mo).

$$\frac{dV}{dt} = -\frac{E_S}{\rho_S} - \frac{E_M}{\rho_M}$$
$$\frac{dX}{dt} = \frac{\mu_{\max} S}{K_S + S} X + \frac{X}{V}\left(\frac{E_S}{\rho_S} + \frac{E_M}{\rho_M}\right) \quad (1)$$
$$\frac{dS}{dt} = -\frac{\mu_{\max} S}{K_S + S}\frac{X}{Y_{X/S}} - \frac{E_S \rho_S}{V} + \frac{S}{V}\left(\frac{E_S}{\rho_S} + \frac{E_M}{\rho_M}\right)$$

where: E_S and E_M are the substrate and medium loss by evaporation [g/h]; ρ_S and ρ_M are the substrate and medium densities [g/L]; $Y_{X/S}$ is the substrate conversion yield referred to the biomass [g dry matter/ g substrate]; μ is the specific growth rate [1/h]; V is the volume of the cultivation medium in the bioreactor [L]; X and S are the biomass and substrate concentrations [g/L] and *t* is the time [h], μ_{max} represents the maximum specific growth rate [1/h] and K_S is the saturation constant [g/g]. The main process parameters were: continuous temperature control 37°C; a minimal level of pO_2 - 10% from the saturation concentration was maintained during the exponential growth; continuous pH control between 4.5-5.0 by addition of NH_4OH (12.5%); no foam control, if the main parameters are optimally controlled. The unique C source, the methanol was introduced function of the yeast growth rate in connection with the substrate consumption rate for avoiding the growth inhibition by substrate concentration. The developed model (1) is based on the mass-balance principle and on the hypothesis of a non-inhibitive substrate effect (i.e. the specific growth rate is defined by the Monod equation). In line with the operation mode (fed-batch with discontinuous substrate feeding), there are discontinuous variations of the main variables due to: substrate feeding, medium feeding (to overcome the loss by evaporation or sample collection) or samples withdraws. That is why the following mass-balance equations are to be added to express each discontinuous modification for volume, and substrate or biomass concentrations:

$$V_k + A_{Sk} + A_{Mk} = P_{Mk} + V_{k+1} \quad (2)$$
$$S_k \rho_M V_k + A_{Sk} \rho_S = P_{Mk} \rho_M S_k + S_{k+1} \rho_M V_{k+1}$$
$$X_k V_k = P_{Mk} X_k + X_{k+1} V_{k+1}$$

where: V_k, V_{k+1}= volume before / after modification [L]; A_{Sk}, A_{Mk}= substrate volume and respectively medium volume adding [L]; P_{Mk}=sample withdraw [L]. The same notations are used for S_k, S_{k+1} and X_k, X_{k+1}. We use: ρ_S = 800[g/L]., respectively ρ_M = 1000[g/L]. The identification of the model parameters was carried out based on measured values in order to minimize the modeling error. The identification procedure (i.e. Nelder-Mead algorithm) determines the optimum values for the following process parameters: E_S, E_M, μ_{max}, K_S and $Y_{X/S}$. For this bioprocess, the overall control objective is to obtain large biomass quantities, based on the assumption that high biomass concentration will assure the obtaining of important alcoholoxydase-active biomass. In this paper a control system based on fuzzy logic is proposed. It is well known that Fuzzy Control Systems (FCS) can manipulate incomplete and uncertain information about the process assuring high control performances (Bellgardt, 1991, Boudreau *et al*., 2006, Heinzle *et al*., 2006). The proposed FCS receives information about the state of the bioprocess expressed by the biomass and substrate concentrations. Based on this information, FCS computes the quantity of substrate to be added into the reactor. According to these observations the inputs of FCS are the biomass (X) and substrate (S) concentrations, and the output is the correction to be applied on the substrate addition. The rules are presented in Table 1.

Rules evaluation by the inference engine is made according to the min-max inference rule and the output defuzzyfication is made based on the centroid defuzzyfication method.

Table 1. *The rule base*

S_k \ X_k	S	M	L
S	Z	PZ	P
M	NZ	Z	PZ
L	N	NZ	Z

The control loop was implemented in MATLAB, version 6.5. For control loop simulation the proposed mathematical model was used and the simulation results were compared with the experimental data.

The simulation results show that the proposed fuzzy control system is capable of computing the substrate feedings needed for cell growth according to the biomass concentration increase. The evolution of the substrate concentration marks the substrate consumption and additions, as well as the increase of the additions along with cell growth. The biomass concentration obtained by simulation follow closely the experimental data. As a conclusion of this case-study, it can be accepted that the success of such a control implementation is critically dependent upon the technical operating conditions of the process.

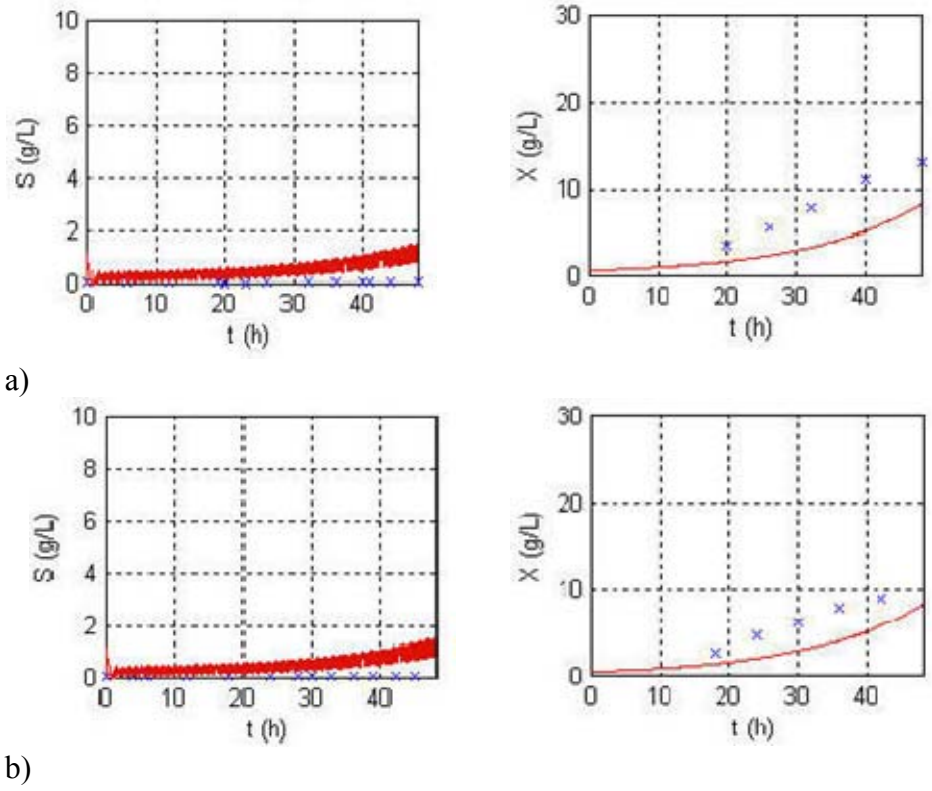

Figure 3. *Simulation results of the control loop: a) first experiment; b) second experiment; ('-' – simulation results; 'x' – experimental data)*

Conclusions

The overview on the current status of bioprocess modeling and control focuses on three main topics: (i) unstructured versus structured and metabolic modeling; (ii) control based on common technique (model based control and adaptive control); (iii) control based on artificial intelligence.

It is finally to underline that the framework of bioprocess modeling & control still offers interesting perspectives to obtain robust control solutions for the aerobic bioprocess. Moreover the future of bioprocesses' optimal control will rely on applying the same concept: the use of different modeling methods in conjunction with intelligent control techniques.

If a simplified representation of the bioprocess exists (i.e. an *a priori* model), this optimal profile can serve as an initial trajectory for intelligent control algorithms when the complexity of the process representation is described in a subjective mode (by human expert).

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