Bioprocesses parameters control in the case of a BIOSTAT A PLUS bioreactor

Ionuț-Aurelian Nisipeanu*, Elena Bunciu (Stanciu), Roxana Stanică*****

**S.C. CORD ROMANIA S.R.L., Slatina, Romania*
(e-mail: ionut.nisipeanu@pirelli.com)}
***Department of Control, University of Craiova, A.I. Cuza 13, Craiova, Romania*
(e-mail:bunciu.elena@yahoo.com)
****S.C. Polystart Security S.R.L., Craiova, Romania*
(e-mail:roxana_batm@yahoo.com)

Abstract: In this paper some bioprocesses parameters control methods in the case of a continuous BIOSTAT A PLUS bioreactor are presented and analysed. More exactly, three experiments regarding the control of temperature, pH and pO_2 are developed and analysed. For the temperature control it was used a PID controller whose parameters were tuned by using Nichols method and for the stirrer speed control to adjust the controller parameters the Ziegler-Nichols method was used. In the case of pH control the parameters of a PI controller were designed and tuned by using the Hokushin method. All the three tuning control methods were confirmed by practical experiments.

Keywords: bioprocesses, bioreactors, temperature control, pH control, pO_2 control.

1. INTRODUCTION

The control of biotechnological processes has been and remains an important problem attracting wide attention, the main engineering motivation being the improvement of the operational stability and production efficiency of such living processes.

From the engineering point of view, the control of bioprocesses poses a number of challenging problems. These problems arise from the presence of living organisms, the high complexity of the interactions between the micro-organisms, as well as the high complexity of the metabolic reactions. Moreover, for monitoring and control applications, only a few measurements are available, either because the measuring devices do not exist or are too expensive, or because the available devices do not give reliable measurements. Therefore, we can deduce that the main difficulties arising in the on-line monitoring and control of bioprocesses arrive from two main sources: the process complexity and the difficulty to have reliable measurements of bioprocess variables (Bastin and Dochain, 1990; Bernard and Gouze, 2002; Dochain, 2008; Selisteanu and Petre, 2004, Selişteanu *et al*., 2007a; Schugerl, 2001).

The bioreactors can operate in three modes: the continuous mode, the fed-batch mode and the batch mode (Dochain, 2008; Bastin and Dochain, 1990; Petre and Selisteanu, 2005). For example, in a Continuous Stirred Tank Bioreactor (CSTB), the substrates (the nutrients) are fed to the bioreactor continuously and an effluent stream is continuously withdrawn such that the culture volume is constant.

The phenomena occurring in the bioreactor are complex, because of their interdependence with the biochemical transfer. The analysis process must be aware of (in addition to actual biochemical reactions and physical phenomena with which they interact) the following: flow, mass transfer, and heat transfer present in any bioreactor. The quantitative description of these phenomena provides some equations which can be used to analyzed, optimized, sized, and adjusted the bioreactor (Petre and Selisteanu, 2005).

In practice, the bioprocess control is often limited to regulation of temperature and pH at constant values favourable to the microbial growth. There is however no doubt that the control of biological state variables (concentrations of biomass, substrates, products etc.) can help to increase the performance. To develop and apply any control or advanced control strategies for these biological variables, it is necessary to obtain useful dynamical models and to design estimation and identification strategies.

In this paper some bioprocesses parameters control methods in the case of a continuous laboratory BIOSTAT A PLUS micro-bioreactor are presented and analysed. It must be noted that this type of bioreactor can be used only for the development of vegetable cells culture. More exactly, three experiments regarding the control of temperature, pH and pO_2 are developed and analysed. For the temperature control a PID controller was used tuned by using the Nichols method and for the stirrer control the Ziegler-Nichols method was used. In the case of pH control a PI controller tuned by using the Hokushin method was used.

In the analyzed bioreactor, the transformation of raw materials is performed by the enzyme system of living micro-organisms, animals, and plant cells or isolated enzymes (Bowman *et al*., 1988).

The success of biotechnological experiments depends on the optimal growth conditions of the micro-organisms in the created bioreactor. The cells are constantly struggling with changes in the culture, in order to obtain and maintain optimal growth conditions. (Petre, 2008).

The bioreactor control must ensure the continuous supply of cells with appropriate resources used for growth or production of metabolites, ensuring also optimum values for pH, temperature, substrate concentration, mineral salts concentration, growth factors and oxygen concentration (Petre and Selisteanu, 2005).

The paper is organized as follows. In Section 2 the technical features of the BIOSTAT A PLUS bioreactor are presented. Section 3 contains some experiments regarding the control of the temperature, section 4 details the problems regarding the control of the stirrer speed and section 5 deals with pH and pO_2 control.

2. THE TECHNICAL FEATURES OF BIOSTAT A PLUS BIOREACTOR

The reactor used in this paper is a Biostat A Plus bioreactor, a product from Sartorius Sepadim Company.

In Fig. 1 the entire package Biostat A Plus bioreactor is presented. The equipment contains three components: an autoclaved bioreactor, a control unit and a software application that can control different parameters like temperature, pH, pO_2, stirrer velocity or different gas flows (BIOSTAT, 2006).

Fig. 1. Biostat A Plus Bioreactor (BIOSTAT, 2006).

The communication between the control unit and the laptop that contains the microDCU MFCS/DA software (Multi Fermenter Control System – Data Acquisition) is realised by using the Ethernet TCP/IP protocol (BIOSTAT, 2006). Ethernet is a broadcast network designed for local area networks. Ethernet became the most popular technology based on Layer 2 (can take forwarding decisions based on MAC address) because it is best suited for the occasional network traffic (Iancu, 2004).

2.1 Bioreactor description

The culture vessel used for experiments has a volume of 5 litres and it is made of borosilicate glass that can be heated up to approximately 80°C by an electrical blanket (BIOSTAT, 2006).

Autoclave sterilization of the vessel and its components is done at a temperature of 121°C (BIOSTAT, 2006).

The vessel lid is made of stainless steel and has holes of different diameters for introducing the stirrer, tubes for introducing the substances and probes for pH, pO_2, temperature, antifoam/level measurement (BIOSTAT, 2006).

The homogeneity of culture is realised by the stirrer and consists in a drive that rotates an arbour. A helicoidally paddle is attached to the arbour. All components that are in direct contact with the culture are made of stainless steel (BIOSTAT, 2006).

The stirrer of the cell culture version bioreactor consists of three coaxial segments and ensures a slide and effective mixture (BIOSTAT, 2006).

In the bioreactor's field the temperature can be controlled in several ways. For example, in the case of double-wall vessels the temperature is controlled by a system of serpentines through which the water runs (BIOSTAT, 2006).

In the case of the presented bioreactor with a single wall vessel, the control of temperature is done with the help of a heating blanket that consists of an electrical serpentine covered with a layer of silicone rubber foam. When the temperature in the vessel decreases bellow the reference value, the blanket starts heating. The major disadvantage is the necessity of a separated system for cooling the bioreactor (BIOSTAT, 2006).

2.2 Control Unit

The control unit is characterized by an integrated control system, integrated pumps, gas flow controllers, control for pH/DO and foam/level (BIOSTAT, 2006).

For the control unit it is necessary to use an external computer. This also offers the possibility of a touch panel attachment and development of software for managing / monitoring process (BIOSTAT, 2006).

In the frontal part of the unit thee exist three peristaltic pumps that can be linked to the acid, base and antifoam/level bottle (BIOSTAT, 2006).

3. TEMPERATURE CONTROL

To control the process temperature, first we must determine the process model, in particular the transfer function that describes this process. To do this we developed an experiment, more exactly a bioreactor heating test that gives us the possibility to identify the parameters of the transfer function. The dynamics of the system is studied by using signal acquisition from the temperature sensor inside the vessel and from its graphical representation by using the micro-DCU.

The thermodynamics theory claims that the heating process taking place inside an enclosure can be

approximated by a first order transfer function with the input voltage and output power of the mantle represented by the temperature inside the vessel.

The transfer function, denoted $G_h(s)$, can be expressed by the following equation (Marin 2004):

$$G_h(s) = \frac{Y(s)}{U(s)} = \frac{K \cdot e^{-\tau \cdot s}}{T \cdot s + 1}. \quad (1)$$

The value of the gain, K, is obtained by measuring the value Δu and the value $[y(\infty) - y_{st}(0)]$ corresponding to graphic segments in Fig. 2 (Marin, 2004). Then, K is given by:

$$K = \frac{[y(\infty) - y_{st}(0)]}{\Delta u}. \quad (2)$$

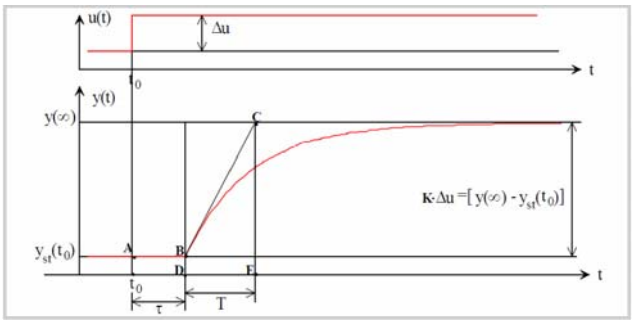

Fig. 2. The step response of a first order aperiodic element with time delay (Marin, 2004).

A method for estimating the time constant T is the tangent method (Marin, 2004). In the initial point B, a tangent can be drown to the response that intersects the $y(\infty)$ axis in the point C. DE is the projection of segment BC on the time axis and represents the time constant T (Marin 2004).

In Fig. 3 the experimental results obtained for a reference value of 35°C and an initial value inside the culture vessel of 25.6°C are represented. The duration of the experiment was 440 minutes. We can observe that the process is a slow one, having a high time constant and time delay. The steady state error is also higher and for that reason it is necessary to design a controller.

Fig. 3. The step response without the controller

Using the method described above the parameters of the transfer function can be determined as follows:

$$K = 0.53; \quad \tau = 11\,[\text{min}]; \quad T = 187\,[\text{min}]$$

Now, since we obtained the transfer function, in order to develop a control method for temperature control various standard methods can be used (see, Marin 2004):

- Methods that start from the transfer function of the plant (Oppelt, Chien-Hrones-Reswick, Kopelovici, Cohen – Coon, Nichols).
- Methods using direct practical tuning on the plant (Ziegler-Nichols, Hokushin, Pessen).

Note that, usually, the direct tuning methods on the plant are used in practice for the cases were the transfer function can be determined from the step response. For slow processes this type of method can be an advantageous solution because of the high response time to the parameters changes of the control law. Since the biotechnological system chosen in this paper represents a slow process, we chose a method from the first class meaning that the controller is obtained from the transfer function parameters.

For a plant described by the transfer function

$$H_f(s) = \frac{K_f}{T_f \cdot s + 1} e^{-\tau \cdot s} \quad (3)$$

To obtain an appropriately control law, Nichols proposed the following parameters (Marin 2004):

Table 1

	K_{Ropt}	T_{iopt}	T_d
P	$\frac{T_f}{\tau}$	–	–
PI	$0.9 \cdot \frac{T_f}{\tau}$	$3.3 \cdot \tau$	–
PID	$(1.2 \div 2) \cdot \frac{T_f}{\tau}$	$2 \cdot \tau$	$0.5 \cdot \tau$

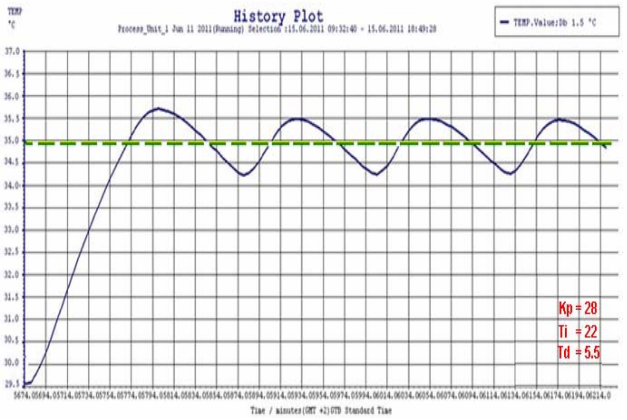

Fig. 4. System behaviour for a PID controller tuned by using Nichols method.

Note that, in this experiment, the heating of the vessel was done by using the electrical blanket and its cooling was natural.

4. STIRRER SPEED CONTROL

To control the speed of the stirrer, the parameters of the PID controller can be set by using the Ziegler-Nichols method based exclusively on the limit of the system stability that operates in closed loop in the absence of the controller. It is necessary to determine the limit gain i.e. the gain that assures the plant operation as a self-oscillating system in closed circuit (Marin 2004). It is also necessary to establish the oscillation period. If the controller integration time $T_i = \infty$ and the derivative time $T_d = 0$, the proportionality factor K_R increases until oscillations become self-maintained ones. If we denote $K_{\lim}$ the gain value of the proportionality factor the for which the system operates at the limit of stability and $T_{\lim}$ is the system self-oscillation period, then the controller parameters can be determined using the relations presented in Table 2 (Marin 2004).

Table 2

	K_R	T_i	T_d
P controller	$0.5\,K_{R\lim}$	–	–
PI controller	$0.45\,K_{R\lim}$	$0.85\,T_{\lim}$	–
PID controller	$0.75\,K_{R\lim}$	$0.6\,T_{\lim}$	$0.1\,K_{R\lim}$

The presented method is very simple and easy to apply. Unfortunately, such a method does not provide information about system performance.

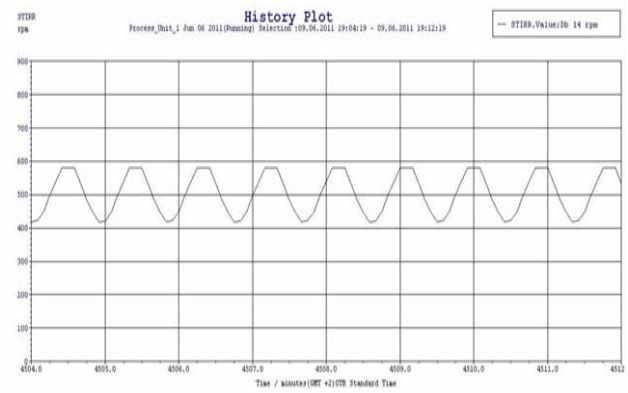

Fig. 5. The process self-maintained oscillations when the proportionality factor increases.

Once the synthesis operation is completed, it is recommended to evaluate the system behaviour in closed loop circuit.

For the above case, the self-maintained oscillations occur when the proportionality factor is $K_{\lim} = 3.5$, that is a proportionality band $BP_{\lim} = 28\%$.

The next step is to determine the oscillation period. Based on the Fig. 5, $T_{\lim}$ can be determined as $T_{\lim} = 60s$.

Based on the relationships presented in Table 2, the following parameters can be calculated.

Table 3

	$BP\%$	T_i	T_d
P	56%	–	–
PI	62.1%	51 sec	–
PID	37.2%	36 sec	6 sec

Fig. 6 presents the system response for all 3 regulators parameterized according to the Ziegler-Nichols method.

For P and PI regulators, there is a good development of the stirrer, unlike in the case of the PID regulator, whose derivative component provides oscillations around the reference.

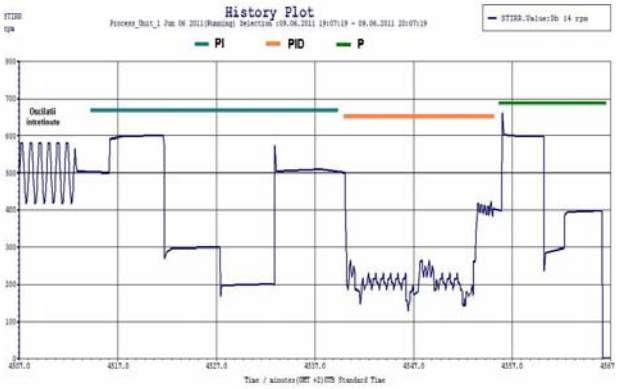

Fig. 6. Behaviour of the stirrer system (controlled with the Ziegler-Nichols method)

5. pH CONTROL

Besides temperature and dissolved oxygen concentration, pH is one of the major influential factors in fermentation processes. Deviations from the optimal values can lead to loss of activity by irreversible denaturing of proteins. Also, enzymatic activity is highest at a certain value of pH in culture medium, which determines the need of implementing some rules to ensure the process performance requirements (Petre, 2008).

The system dynamic is studied by using the signal acquisition from the pH sensor inside the vessel and its graphical representation by using micro-DCU (BIOSTAT, 2006) also. The pH control is done by pressing the two peristaltic pumps (20 RPM) adequate for supplying 7% citric acid and 88% sodium hydroxide (see, Fig. 7).

For pH control a PI controller is chosen with $K_R = 10$ and $T_i = 5\,\text{sec}$. It should be noted that all types of classic controllers (P, PI, PID) were tested and very good results in terms of tracking reference were obtained in all three cases. The main differences between these controllers were the duration of the transitional regime and the mode to switch on and off the pump (at low values of the constant of integration we could see fast switching on and off in the actuators level).

In Fig. 7 the strong dependence of the pH loop of the stirrer action can be observed.

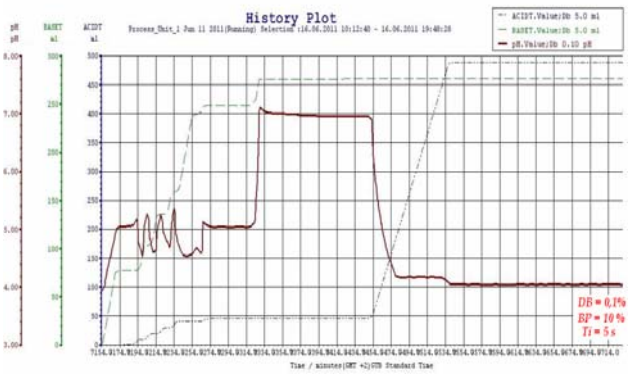

Fig. 7. pH control with a PI controller tuned by using the Hokushin method

The continuous line represents the pH values, the interrupted line represents the acid values and the dotted line is the base value.

Fig. 8 presents the evolution of pH in the culture medium according to the mixing status of the system. It is noted that a priori to mixing, the pH value oscillates around the reference value ($pH = 5$), leading to an intensive consumption of remedial solutions, as shown in Fig. 7. Once started the process of homogenization (Stirrer - 100 rpm), the pH stabilizes, the system behaves well for reference changes.

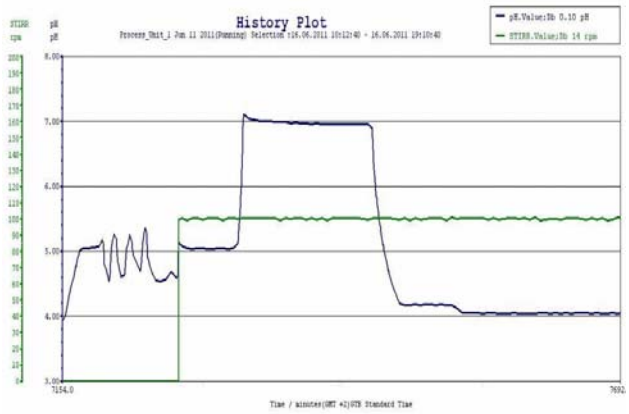

Fig. 8. pH values before and after mixing the culture

The first y-axis represents the stirrer velocity and the second one represents the pH values. After starting the stirrer we change the pH reference from 5 to 7 and then from 7 to 4.

Fig. 9 is showing the production and consumption of the dissolved oxygen from chemical reactions taking place between citric acid and sodium hydroxide during the experiment.

ACKNOWLEDGMENT

This work was supported partially by CNCSIS–UEFISCDI, Romania, project number PNII–IDEI 548/2008, and partially by the strategic grant POSDRU/88/1.5/S/50783, Project ID50783 (2009), co-financed by the European Social Fund – Investing in People, within the Sectorial Operational Programme Human Resources Development 2007–2013.

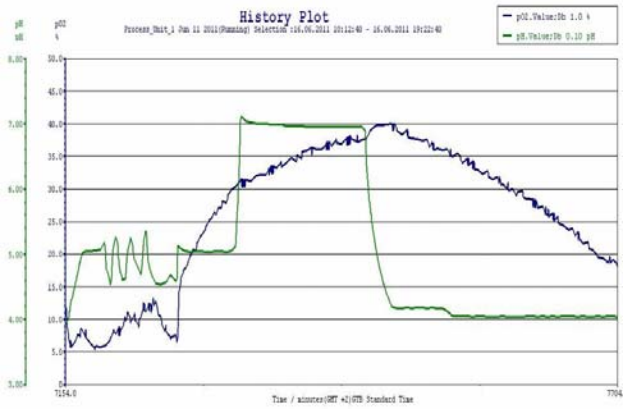

Fig. 9. pO_2 variation according to the pH value

REFERENCES

Bastin, G. and Dochain, D. (1990). *On-line estimation and adaptive control of bioreactors*. New York, NY: Elsevier.

BIOSTAT A Plus Operating Manual (2006), Sartorius BBI Systems GmbH.

Bernard, O. and Gouze, J. L. (2002). State estimation for bioprocesses. *Mathematical Control Theory*, *ICTP lecture notes*.

Bowman, E. J., Siebers A., and Altendorf K. (1988). *Bafilomycins: a class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells*. Proc. Natl. Acad. Sci. USA 85:7972-7976.

Dochain, D. (Ed.) (2008). *Automatic control of bioprocesses*. UK: ISTE and J. Wiley & Sons.

Iancu, E. (2004). *Data Transmision Theory*. Ed. Universitaria, Craiova.

Marin, C. (2004). *Control Engineering – Elements of analysis and synthesis*, Ed. SITECH, Craiova.

Petre, E. and Selisteanu, D. (2005). *Modelling and identification of wastewater treatment bioprocesses* (in Romanian). Craiova, RO: Universitaria.

Petre, E. (2008). *Nonlinear Control Systems - Applications in Biotechnology* (in Romanian, 2nd Edition). Craiova, RO: Universitaria.

Selisteanu, D. and Petre, E. (2004). Some on adaptive control of a wastewater biodegradation process. *Journal of Control Engineering and Applied Informatics*, Vol. 6, No. 3, pp. 48-56.

Selişteanu, D., Petre, E., Popescu, D. and Bobaşu, E. (2007). High frequency control strategies for a continuous bioprocess: sliding mode control versus vibrational control. *Proceedings of the 13th IEEE/IFAC Int Conf. on Methods and Models in Automation and Robotics MMAR 2007*, Szczecin, Poland, August 2007, pp. 77-84.

Schugerl, K. (2001). Progress in monitoring, modelling and control of bioprocesses during the last 20 years. *J. Biotechnology, vol. 85, no. 2*, pp. 149-173.