Knowledge System to Automate a Batch and Fed-Batch Fermentation Process of B.t.

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Abstract. In this paper we describe an Intelligent Control System (ICS) that operates in real time a batch or fed batch fermentation of *Bacillus thuringiensis* (*Bt*) for a specific growth rate. The ICS follows an analytical approach based on Life Cycle of Systems and on Task Model in order to organize the knowledge required by an Expert System that supervises and controls the *Bt* fermentation. The model control was a recurrent neural network. It predicts the nutrient requirements to growth *Bt* at a specific growth rate. The ICS was developed with the Expert System Shell Chronos™ and validated with a fed batch fermentation of *Bt*. The success of our research was to arrive to control the protein toxicity of Cry1A(c), manipulating the nutrient concentration into the fermentor.

1. Introduction

The increase interesting of the biotechnology in several domain of the science and industry, has conduced to develop new technologies with the intention of optimizing the yield and production of the products generated during the microorganism metabolism [1]. Another factor of interest is to reduce the operation costs, since it is current that mistakes during the microorganisms cultivation conduce to lost the fermentation.

The development of fermentation control systems has not been currently applied because the complexity relation between the microorganism metabolism and its environment, which avoid the development of feasible model for describe the static and dynamic characteristics of the microorganism cultivation. Another reason is the lack of sensors able to detect the evolution of microorganisms, nutrients and metabolic products [2]. In practice, the development of biotechnology process has been done in an empirical sense, which increase the cost and time consumption.

In the last two decades, there have been appears many publications about alternative technologies that offer to resolve the biotechnology problems of control. Those technologies are based on Expert Systems, Neural Networks, Fuzzy Logic and Genetic Algorithms [3]. Observing the results reported on these researches, the Artificial Intelligence had improve the biotechnology processes. An inconvenient of works reported is the fact that they had used analytical cultures in where it is possible to measure the microorganism population using sensors based on optical density [4].

When industrial cultures are used, alternative technologies must be applied.

In this paper it is presented an Intelligent Control System (ICS) that let it to supervised and control the spores produced by *Bacillus thuringiensis* (*Bt*) as a function of its specific growth rate [5]. *Bt* is a microorganism that synthesizes toxic proteins known as Cry, commonly used to fight insect pest immature stages [6]. *Bt* is the most important commercial microbial pesticide worldwide, so it has become of interest the improvement of culture methods to efficiently produce Cry proteins. The importance of the work presented here is the kind of variables involved in the ICS developed, the specific growth rate, which determined the protein toxicity of Cry1A(c). The growth rate depends of the nutrients available into the fermentor during the exponential phase.

The paper is organized as follows: Section 2 presents a short description of the *Bt* fermentation process, Section 3 presents the characteristics of the Intelligent Control System developed, Section 4 describes in detailed the analytical procedure applied to implement the ICS and Section 5 some conclusions.

2. Fermentation Process.

A 5 liter stirred tank fermentor (Chemap Fermentor model FZ 3000) was used for the Bt fed-batch fermentations (Figure 1). The fermentor is provided with standard instrumentation to control temperature, pH and agitation, variables which are usually control in any microorganism cultivation. The dissolved oxygen concentration and foam production are other interesting variables. The former is usually controlled increasing the agitation (rpm) and airflow rate. The second one is controlled by feeding a 1 % ethylene glycol solution at the end of the fermentation stating.

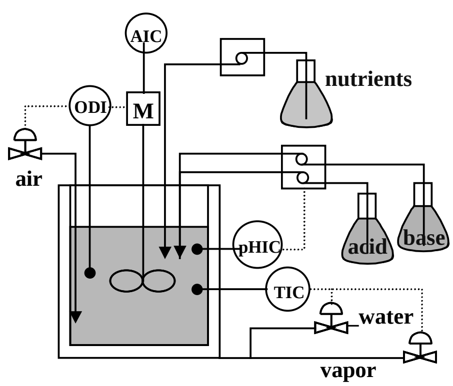

Fig. 1. Experiment setup. DOI: Indicator of dissolved oxygen; AIC: Indicator and controller of agitation; pHIC: Indicator and controller of hydrogen potential; TIC:Indicator and controller of temperature; M: motor.

3. Intelligent Control System (ICS)

Due to the lack of reliable mathematical models for predicting the Bt fermentation kinetics and the requirements of nutrients into the fermentor, it was decided to develop an Intelligent Control system based on Expert System and Neural Networks (Figure 2). The development of the Expert System (ES) was carried out with the Expert System Shell Chronos [7]. This shell was selected since it works in real time and it let to carried out multitasks under a certain priority previously assigned. The Neural Network (NN) implemented is of recurrent type. It was trained with the algorithm of error backpropagation [8]. The function of the ES is to control the sequence of actions that must be carried to cultivate Bt. The function of the NN is predict the glucose (limiting nutrient) available into the fermentor in order to control the specific growth rate of *Bt*.

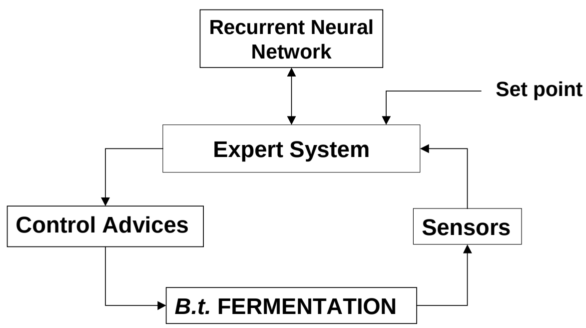

Fig. 2. Intelligent Control System

4. Knowledge required to design the ICS

The specification of the knowledge required to implement the knowledge base of the Intelligent Control System was determined applying an analytical procedure based on the Lifecycle of Systems Design and on the Task-Oriented Approach. The Lifecycle of System Design was selected because it is a general method that let it design structural computer systems [9],[10]. The Task Oriented Approach was selected because it can be used at any level of abstraction. That means, to define the system objectives, to describe methodologies or to represent the actions to be performed on the system itself [11]. The analytical approach proceeds as follows: 1. Delimitation of the problem, 2. Conceptualization (comprehension of the problem and proposition of alternative solutions), 3. Conception (introduction of technologic aspects that resolve the problem), 4. Implementation (physical implementation of the control system and devices) and 5. Verification and validation of the ICS performance.

4.1. Delimitation of the problem.

The sequence of tasks involved in the Bt fermentation process is presented in figure 3. According to the characteristics of these tasks, they can be classified as: strictly manually, semi automatic and tasks that actually are carried out in an automatic way. The tasks strictly manually are: reactor cleaning, verification of services (air, water and electricity) supply, elimination of leaks (if they exist), calibration of sensors and installation of connections for feeding the acid, base, antifoam and nutrient solutions. All these tasks are include in the fermentation starting. The semi-automatic tasks are: sterilization process, setting of the operational conditions and the inoculation. The tasks able to be automated are: control, monitoring and registering of the operational conditions.

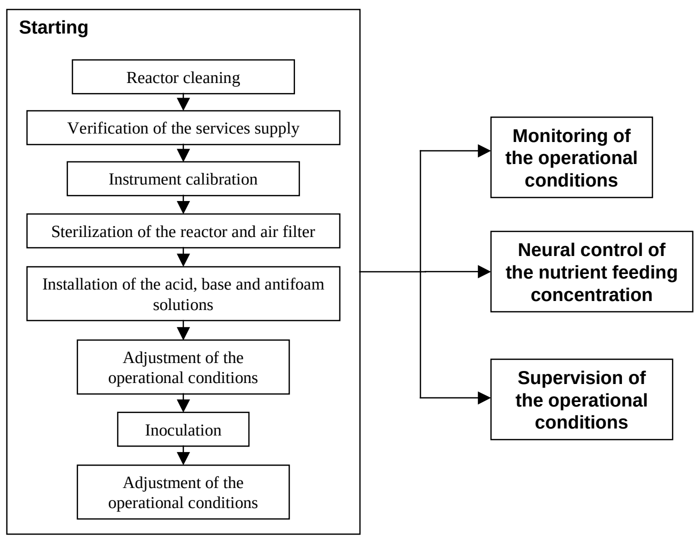

Fig. 3. Fermentation process of *Bacillus thuringiensis*.

For developing a control system, it is well known that it is required a model able to predict the statistic and dynamic characteristics of the process under study. Due to the complexity involved in the Bt metabolism, a feasible mathematical model can not be formulate [12]. Thus, the control is commonly carried out over the temperature, pH and dissolved oxygen concentration. This is done, having in mind that growing the microorganisms under an adequate environment, the cell and metabolite production will be as it is desired. In this work, it is use a neural network model for predicting the Bt fermentation kinetics (cells, glucose and spore) and the nutrients required to produce Bt at a specific growth rate [8].

Analyzing the Bt fermentation process shown in figure 1, the potential tasks to be automated are (1) sterilization, (2) operational conditions adjustment, (3) inoculation of the fermentor, (4) monitoring of the operational conditions, (5) surveillance of the operational conditions and (6) control of the nutrient concentration into the fermentor. The first three tasks included in the starting block (Figure 1) must be carried out in a sequential procedure and the last three ones in parallel. A block diagram of the ICS indicating the order as the tasks must be performed, is presented in figure 4.

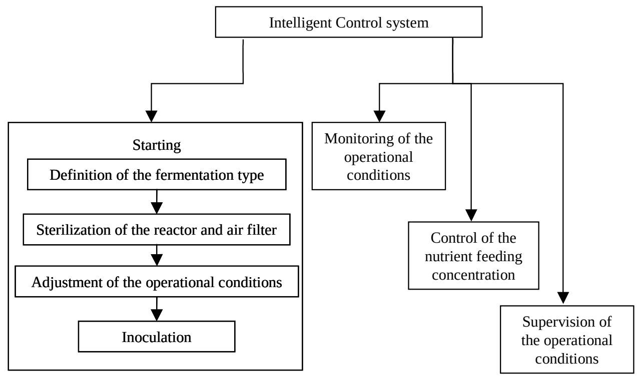

Fig. 4. Potential tasks of the Bt fermentation to be automated

4.2. Conceptualization of the ICS

The surveillance of the experimental setup safety and the regulation of input/output data are two tasks (procedures) of extreme importance when a process is going to be automated. The ICS presented here included these two tasks as well as an user interface (Figure 5). This last task (or procedure) was included in order to have the flexibility of change the operational conditions, according to the characteristics of the microorganism to be cultivated. The specific functions of the *surveillance of the experimental setup safety* and *regulation of input/output data* are described in the next paragraphs.

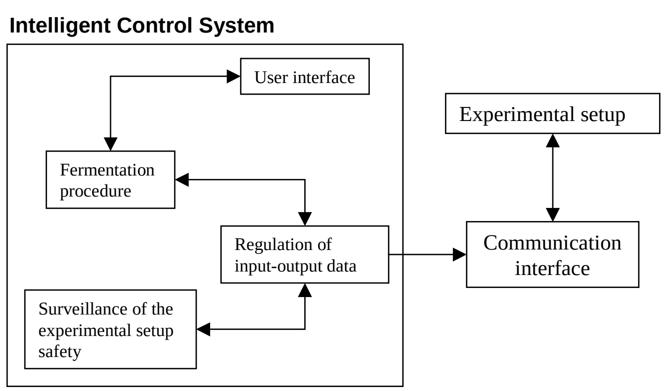

Fig. 5. General Structure of the Intelligent Control System.

Surveillance of experimental setup safety. The objective of this task is to test the functional state of instruments, diagnose instrument malfunctions, and repair some electrical failures. The knowledge used to perform this task was acquired from a study of the experimental setup malfunctions, as well as from the failures detected during the construction of the ICS.

Regulation of input-output data. The task has as objective to avoid the lost of electrical signals or the activation of tasks which could conduce to lost the fermentation. For examples: If the culture temperature is higher than 30°C and the task inoculation is activated, the microorganism died. Another example is when the foam peristaltic pump is activated and the stop signal is lost. The exceeding of foam kill the microorganism.

4.3. Conception of the ICS

The tasks describing the Bt fermentation procedure if Figure 4 involve actions related to the adjustment and monitoring of the operating variables. Thus, in order to eliminate the conflict among the activation of tasks, it is suggested to manipulate the input/output data, send towards the experimental setup, through blocks named adjustment and monitoring. The specific instructions of each task could be maintain in a new block of instructions having the same name, which we do in this work. A diagram showing the tasks interactions is shown in Figure 6.

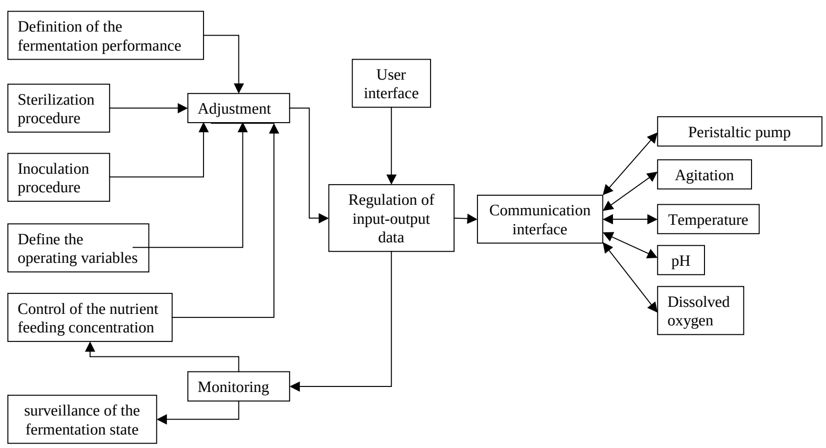

Fig. 6 Structure of tasks for the implementation of the knowledge base system

4.4. Implementation of tasks

The ICS can be implemented using a structural language or an Expert System Generator (ESG) developed to work on real time. From those two alternatives it was decided to use the ESG Chronos™. Chronos™ was constructed by Euristic Systèmes (SAGEM) [7]. It manipulates information on real time, executes multitasks, runs external computational programs and executes simple arithmetic operations. This last property had let us to resolve our problem with few rules. The problem of sequence the rule activation was resolved sending messages among tasks.

The CHRONOS™ language is based on production rules: **rule** *name*: **as soon as** <*antecedents*> **then** <*actions*> **end rule**. An example of the implementation of tasks is shown in figure 7, as a tree of tasks for a batch fermentation of Bt.

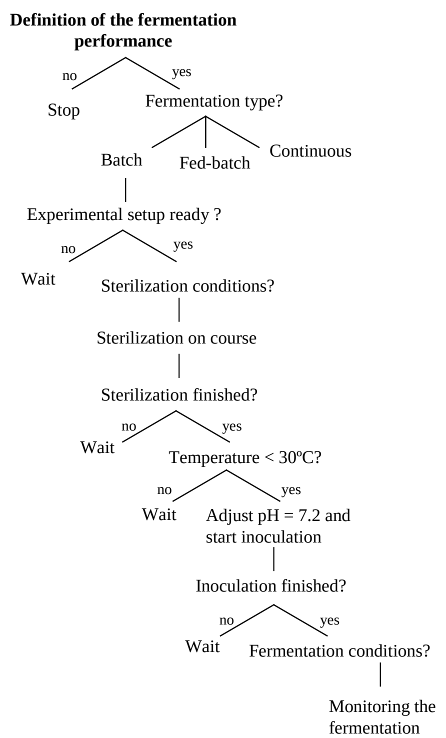

Fig. 7 Tree of tasks of a batch fermentation

4.5. Verification and validation of the Intelligent Control System

The verification was focus to determine the rule implementation congruence. The verification consist in comparing the instruction sequence emitted by the ICS, with that followed in the experimental procedure. The results observed were satisfied. The test of the ICS to control the specific growth rate of the Bt fermentation was carried out on line. The testing consist in growth Bt at a specific growth rate. The ICS was in charge to determine the time where the nutrient feeding must be initiated as well as the nutrient flux. The nutrient concentration of the flux was maintained constant. The results obtain are shown in Figure 8

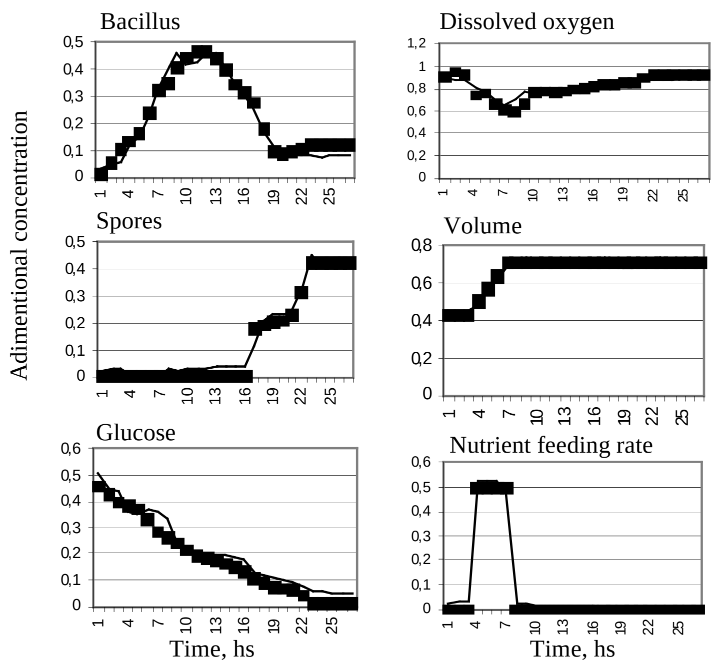

Fig. 8 Fed-batch fermentation of Bt for a specific growth rate of 1.14 h^{-1} . Initial cell concentration, $1.08*10^8$, initial glucose concentration, 12.6 g/l, initial volume, 3l, volume added, 2 liters in 5 hours of 12.6 g/l glucose. Full line represent the ICS prediction and the symbols experimental data.

5. Conclusions

The great sensibility of microorganisms face to variations in the operating conditions demands experience and concentration from the operators in order to avoid mistakes, which could conduce to lose the fermentations. The lose of fermentations increases the product cost and the spending time. The contribution of the Intelligent Control System (ICS) developed here is to give fail-safe, by continuously supervision, and to guide the operator during all the fermentation. In our case, it was possible to control and supervise continuously, during 2-3 days, different fermentations of *Bt*. The yield and production of Cry1A(c) protein was satisfied. It is important to say that the ICS can be applied to cultivate another kind of microorganism.

Concerning the organization of the knowledge required to resolve different kind of biotechnology problems, we think that the analytical approach based on the Life

Cycle of Systems and the Task Model is a well heuristic that combined with the artificial intelligence techniques (ES and RNN), give better results.

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