Chapter 4

Data Acquisition Systems in Bioprocesses

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http://dx.doi.org/10.5772/48466

1. Introduction

Data acquisition systems in bioprocesses have distinct working specification. These processes normally involves microbial cells, vegetable cells, mammalian cells, microalgae cells or even enzymatic biochemical reactions, which implicates in time required for biochemical transformations and biomolecules production. Monitoring bioprocesses do not require real time monitor, because low times intervals (minutes or seconds) do not present significant differences on process parameters values. Bioprocesses are important area in the biotechnology and they are applied in many industries. It has many reaction routes available at any time, each one permitting a different distribution of biomolecules and products based on the conditions under which the fermentation process takes place. Control techniques are indicated to improve the productivity, yield and efficiency of the biotechnological processes. In the bioprocesses, it is very important to have a real time analysis of the process for creating a product with high speed, quality and economy. Knowing the parameters which affect biomolecule formation as well as the concentration of the nutrients, and develop or chose a cheap and simple system capable of on line measurements are required for bioprocesses control.

This chapter is separated into four parts for better understanding of the systems described. First, the data acquisition system Fersol$_1$ is described and its application in different bioprocesses are also presented. Second, the Fersol$_2$ system is also presented describing its functional components such as sensors, controllers, interface and the software and their functions in this system. Examples of application of Fersol$_2$ are described in the second item. Third part presents other systems already developed and the final part presents biosensors and its applications.

Data acquisition system as a source of information about the process behavior and further processing of generating the parameters allows a comparative analysis. Besides the main

softsensors and biosensors in biotechnology and applicability to several processes and respective microorganisms, through the use of acquisition system data, sensors and computational tool is presented.

2. Data acquisition systems developed in DEBB

2.1. Fersol1

Fersol1 is a software that was developed in 1987, which runs only on DOS operational systems, using the methods described by [1]. It was developed and used to manipulate solid-state fermentation (SSF) parameters to solve the problem of difficult separation of biomass from the solid substrate and its heterogeneous characteristics [2]. SSF is characterized by the development of microorganisms in a low-water activity environment on a non-soluble material which is used as nutrient source and physical support [3].

Several authors used respirometry to follow the gas effluents from the bioreactor (CO_2 and O_2) in order to control fermentation and to evaluate different microorganisms' activities [4-13]. O_2 consumption and CO_2 production are the result of metabolic activity of microorganisms from which they obtain the necessary energy for growth and maintenance. Besides, the metabolic activity is associated to the growth and it can be employed for biomass biosynthesis estimation [2,3].

2.1.1. Biomass determination in a fermentation process

One of the most important factors in bioprocesses evaluation and control, both in laboratory and industrial scale, is the estimation of biomass. In a submerged fermentation process this is normally done through measurement of biomass at a particular time by the so-called direct methods. Such methods include direct cell counting, dry biomass determination or optical density determination [2,3].

Considering all the above facts, several methods have been developed for biomass determination that can be divided as direct or indirect measurements. Direct measurements are based on direct separation of biomass followed by normal standard procedures as established for submerged biomass determination (cell counting, etc.). The main problem with this method is the necessity of a whole extraction of biomass from the remained solid substrate. The employment of innocuous detergent in order to guarantee the whole extraction of the biomass from the sample was attempted. However, in the case of mycelium production, the method is not feasible due to the impossibility of a complete separation of biomass. Indirect measurements are based on the determination of a particular component of the cell, or the mycelium that is not present in the solid substrate [2,3,4,8].

It includes:

a. Glucosamine content determination: The method is based on the fact that glucosamine is a monomer component as acetylglucosamine of chitin. Chitin is an insoluble polymer present in the mycelium. The process consists in the depolymerisation of chitin, followed by the liberated glucosamine determination. Principal difficulties with this

method are the lengthy analytical procedure, which takes about 24 hours and the sample adequacy as statistically representative.

b. Ergosterol method: The method is based as the former one due to the presence of ergosterol in the biomass but presents same difficulties such as time-consuming procedure as glucosamine content determination.
c. DNA determination: The basis of this method is the precise increase of the DNA content in the medium as the biomass develops due to the fact that DNA is a constant cellular component. The principal errors that could be made are related with sample adequacy and the possible consumption of cell DNA containing during the process. Another difficulty encountered is related with the DNA isolation and procedures with determination, which takes a long time.
d. Protein determination: This may be the most intended method for direct estimation of biomass. The principal problems of this determination are how exactly protein content is determined and which part of the protein present in the substrate are not consumed, or transformed. It seems that when the solid matrix has no or high protein content, the method could work reliable. For protein estimation, it is usual to determine the N content by the Kjeldahl determination, previous precipitation of the present protein, but a more accurate procedure could be obtained by using an amino acid analyzer.
e. Metabolic gas method Method: This method overcomes the sample adequacy, damage of biomass, or mycelium and is an on-line and fast delivery method. As a matter of fact, this method could be considered a direct measurement of the process kinetics. O_2 consumption and/or CO_2 involved during the process are linearly related with biomass synthesis in an aerobic system. But it is also an indirect method for biomass estimation. In an anaerobic process, CO_2 evolved is a direct indication of biomass synthesis and associated product formation as it occurs in the alcoholic fermentation. The method considers the determination of exhaust gas (exhaust air) composition determination from the fermenter during fermentation. The procedure implies a balance for O_2 and CO_2 considering the airflow through the fermenter.

As has been pointed out previously, all these methods are subject to an appropriate sampling. This is more significant at the initial stages of the process, or when the problems with gradients are not solved. Besides, in particular processes biomass, or mycelium could be damaged by sample acquirement [2].

However, current methods used in liquid fermentation cannot be applied in solid-state processes. This fact is due, for example, to partial or complete adhesion of filamentous fungus mycelium to the solid substrate/support in solid-state fermentation (SSF) system heterogeneity. This causes difficulty in the measurement of whole biomass. Besides, the heterogeneous character of the system demands a more precise acquirement of the samples, which is not faced in submerged or homogeneous fermentation processes [8].

Regarding the software solution, the O_2 consumed and/or CO_2 evolved seems to be more adequate because of on-line measurement possibility and fast results [8]. Besides this method could be considered as a direct measurement of process kinetics, although in true sense it is still an indirect measure of biomass synthesis.

2.1.2. Application of respirometry analysis using Fersol[1]

2.1.2.1. Estimation of growth by respirometry analysis

The respiratory metabolism of the microorganism can be evaluated by determining the O_2 consumption and CO_2 production. This indirect method is used to estimate the biomass biosynthesis by the fungal culture [8]. The exhausted saturated air from the bioreactors passes through silica gel column and then is analyzed by gas chromatography in order to determine the oxygen uptake rate and the CO_2 evolved during the process.

Biomass analytical determination is made by subtracting the quantity of protein in a certain time of the initial quantity of protein present in the substrate.

A mass balance is carried out for the estimation of oxygen uptake rate (OUR) and CO_2 evolved in terms of volumetric flow (L/h). If exhausted airflow (F_{out}) is known and the inlet airflow is F_{in}, the following equations are considered:

$$V_{O_2 out} = \left(\frac{\%O_{2out}}{100} \right) \times F_{out}$$

$$V_{CO_2 out} = \left(\frac{\%CO_{2out}}{100} \right) \times F_{out}$$

$$F_{out} = V_{O_2 out} + V_{CO_2 out} + V_{N_2 out}$$

$$V_{N_2 out} = \left(\frac{100 - \%O_{2out} - \%CO_{2out}}{100} \right) \times F_{out}$$

Considering the air composition (79% N_2 and 21% O_2), it can be written:

$$V_{N_2 in} = \frac{79}{100} \times F_{in}$$

$$V_{N_2 in} = V_{N_2 out}$$

Then, the follow equation relates the inlet and the outlet airflow:

$$F_{in} = \frac{\left(100 - \%O_{2out} - \%CO_{2out}\right) \times F_{out}}{79}$$

The mass balance for oxygen is given in order to evaluate the volumetric flow of O_2 uptake rate:

$$V_{O_2 uptake} = \left(\frac{21}{100} \right) \times F_{in} - \left(\frac{\%O_{2out}}{100} \right) \times F_{out}$$

For the estimation of OUR and CO_2 evolved in mass flow units (mol/h), it is considered that the air was an ideal gas at the respective volumetric flow ($V_{O_2 uptake}$ and $V_{CO_2 out}$) and the proper corrections for temperature conditions.

Considering the balance of OUR, the following equation is obtained [8]:

$$X_n = \left\langle Y_{X/O}\Delta t\left\{\frac{1}{2}\left[\left(\frac{dO_2}{dt}\right)_{t=0} + \left(\frac{dO_2}{dt}\right)_{t=n}\right] + \sum_{i=1}^{i=n-1}\left(\frac{dO_2}{dt}\right)_{t=i}\right\} + \left(1-\frac{a}{2}\right)X_0 - a\sum_{i=1}^{i=n-1} X_i \right\rangle \Big/ \left(1+\frac{a}{2}\right)$$

Where:

$$a = m_X Y_{X/O}\Delta t$$

From the results of the OUR and CO_2 production, some bioprocess parameters are estimated. The estimation of biomass in a certain time (X_n) consists of assuming values for its yield based on oxygen consumption ($Y_{X/O}$) and biomass maintenance coefficient (m_X). Fersol[1] software [1] is then used in the calculations.

2.1.2.2. Acquisition and manipulation of fermentation data

A SSF system, developed by Raimbault and Alazard [14], can be employed in different bioprocesses for diverse biomolecules production [4-13, 15, 16]. This system composed by ten glass column bioreactors with 20 cm length, 4 cm diameter, work volume of 250 cm^3 (Fig. 1). The columns are closed at both ends with cotton plugs, connected to humidifiers and aerated according to the different processes (value set with the aid of a rotameter). The production of CO_2 and O_2 consumption by the cultures is generally measured through a GC (Shimadzu GC-8A, Shimadzu Co., Japan), which is linked to a program for chromatograph control and integration.

The software Chroma (Biosystèmes, Ltd., France) can be employed. There are also other programs that are available such as PeakSimple (SRI Instruments, USA), Base Line N2000 (BaseLine, China), PCChrom (H&A Scientific, USA) and others. The column used in the GC was a Porapak 80/100 at 60ºC, with 2 m length, with helium as carrier gas and a thermal conductivity detector.

Case 1: Respirometry analysis of biomass production during citric acid production by SSF

The relationship between citric acid production, an organic acid, by solid-state fermentation (SSF) of cassava bagasse and the respiration of *Aspergillus niger* LPB 21 was studied. SSF was employed using column fermenters at laboratory scale (Figure 1) and a horizontal drum (HD) bioreactor at semi-pilot scale (Fig. 2), which was coupled with the gas chromatography system to evaluate the release of CO_2 evolved and the O_2 consumption.

SSF was conducted in glass columns with 80 g of humid substrate (laboratory scale) and horizontal drum bioreactor (semi-pilot scale) with 10 kg of humid substrate. Treated and inoculated substrate was placed inside the fermenters. As shown in Fig. 2, the horizontal drum bioreactor consisted of a shovel coupled to a motor axis that rotated with controlled

speed. During fermentation the material was revolved 3 to 4 times a day. After 20 hours of fermentation, saturated air was inserted continually into drum in order promote growth and to control substrate temperature and moisture [17].

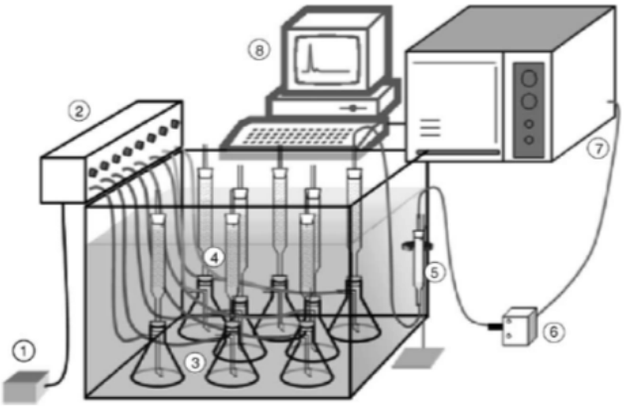

Figure 1. Column set-up for respirometry studies. (1) Air pump; (2) air distribution system; (3) humidifiers; (4) fermentation columns—these are immersed in a water bath with controlled temperature; (5) drying column attached to a column exit; (6) sampling valve; (7) gas chromatograph; (8) computer with data acquisition and control software. Source: [16].

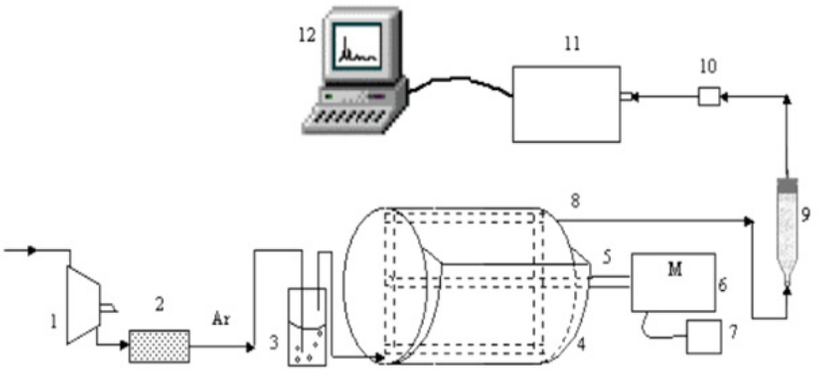

Figure 2. Outline of the horizontal drum bioreactor and auxiliary equipments: (1) compressor, (2) air filter, (3) humidifier, (4) horizontal drum bioreactor, (5) axis, (6) motor, (7) speed controller, (8) air discharge, (9) silica gel column, (10) automatic injector, (11) gaseous chromatograph, (12) computer. Source: [9]

Citric acid production and other characteristic parameters, such as substrate consumption, pH evolution and biomass, were followed during SSF of *A. niger* with CB as substrate. A higher citric acid production was reached in column fermenters (309.7 g/kg DM) than in HD bioreactor (268.94 g/kg DM), probably due to the influence of the fermentation temperature, which was not controlled in HD. Better results could have been attained if the system was adapted with temperature control. A novel prototype is being developed in our laboratory. For column bioreactors the temperature was controlled by a water bath at 30°C. The HD bioreactor worked at room temperature, which was approximately 25°C. It is also important to point out other factors that could affect the metabolism of the fungus and citric acid production such as heat and oxygen transfer that are the main scale-up problems of SSF [9].

Seven points of biomass were considered and analytically determined at 0, 24, 48, 72, 96, 120 and 144 h of fermentation. The system Fersol1 determines the equation coefficients by successive approach. From the values of OUR and CO_2 production, obtained experimentally, the system determined a biomass yield ($Y_{X/O}$) of 4.37 g of biomass/g of consumed O_2 and a biomass maintenance coefficient (m_X) of 0.0162 g of consumed O_2/(g of biomass.h) for HD bioreactor. For column fermenters the biomass yield was 2.93 g of biomass /g of consumed O_2 and the maintenance coefficient was 0.0053 g of consumed O_2/ g of biomass h. Biomass yield was higher in HD bioreactor than in column fermenters due to the proportional higher levels of forced aeration rates [9].

Fig. 3 and Fig. 4 present the evolution of O_2 and CO_2 percentages during fermentation such as estimated biomass and analytical determined biomass for both types of bioreactor. The production of CO_2 did not exceed 0,4% and 2,2% for HD and column fermenters, respectively. This results show that in HD bioreactors the limitation of growth was excessive and, probably, the strategy of retarding aeration in 20 hours was not favorable to this system. This fact could also be shown by the biomass production during fermentation, which was only 0.87 g/100 g DM for HD bioreactor. However, in column fermenters biomass production reached 2.2 g/100 g DM.

In column fermenters, CO_2 production was detected after 24 hours, when biomass and citric acid concentrations started to raise. Maximal CO_2 production was observed at 36 hours of fermentation. Growth was associated with metabolite production. After 50 h of fermentation in HD bioreactor CO_2 production reached its maximum. At this point, citric acid production was about 30 g/kg DM. The difference between estimated and analytical determined biomass in HD bioreactor, mainly in 72 h of fermentation, was an indicative that indirect method of biomass determination, using on-line monitoring of CO_2 production, can probably correct the errors presented in biomass determination by analytical methods [9].

Case 2: Respirometry analysis of biomass production during biopigments production by SSF

Solid-state fermentation (SSF) was carried out to establish relation between growth, respirometric analysis and biopigments production from *Monascus* sp. in columns and in a drum-type bioreactor with forced air (Fig. 5 and Fig. 6). In these reactors, the best aeration rate for biopigment production was 1 ml of air, per gram of wet substrate, per minute. The outlet air composition was determined using gas chromatography (GC), while the pigments produced were measured by spectrophotometry after extraction with ethanol. An ergosterol-dosage method was used to estimate biomass production; in this method, the ergosterol was extracted and measured by liquid cromatography (HPLC). The results showed that although pigments were a secondary metabolite, its production was proportional to the biomass produced that was estimated by ergosterol analysis, and therefore could be used to estimate biomass formed in the natural support (rice). Specific velocities for pigment and biomass production were estimated by a sigmoid model applied to the data and also with the aid of Fersol1. Under ideal conditions in column fermentation, a maximum specific growth velocity of 0.039 h^{-1} and a specific pigment production velocity of 27.5AU/g biomass h was obtained, at 140 h, with 500AU/g dry fermentate after 12 days.

The specific product formation velocity in the bioreactor was 4.7AU/g h, at 240 h fermentation, and the total pigment production was 108.7AU/g dry fermentate after 15 days [16].

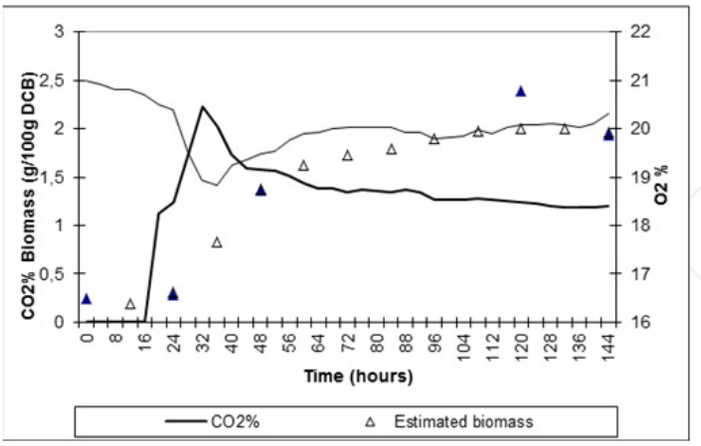

Figure 3. Evolution of kinetic parameters of citric acid production by SSF of cassava bagasse by *Aspergillus niger* LPB 21 in column bioreactor (CB). Source: [9]

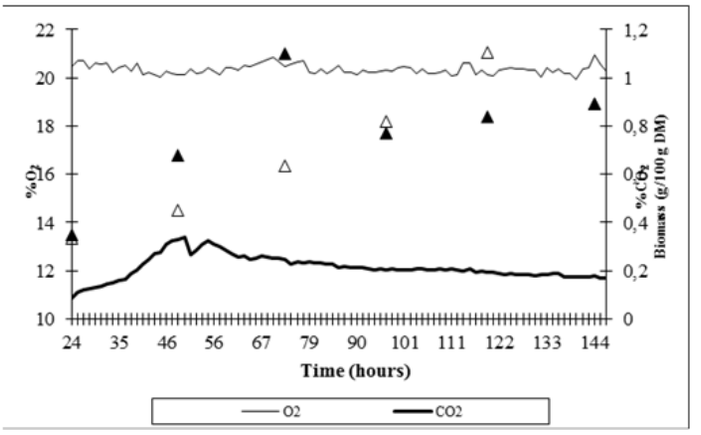

Figure 4. Evolution of kinetic parameters of citric acid production by SSF of cassava bagasse by *Aspergillus niger* LPB 21 in HD bioreactor. Source: [9]

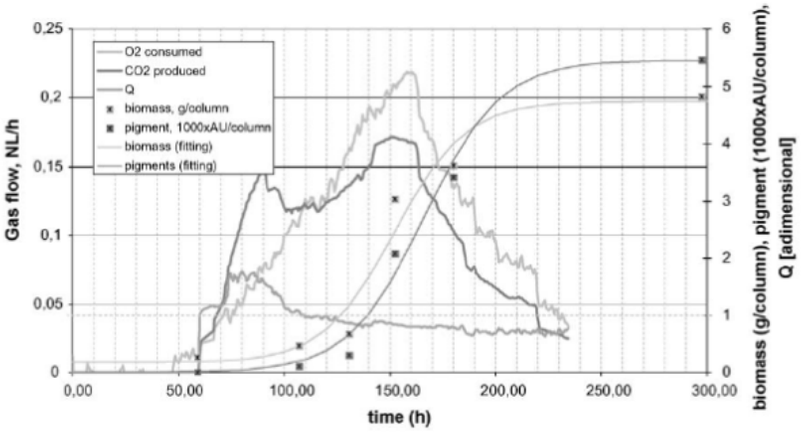

Figure 5. Results of the respirometric analysis in columns, using rice as substrate: O_2 consumption, production of CO_2, pigments (as SPABS), biomass and respiratory quotient *Q* in the course of time. Source: [16].

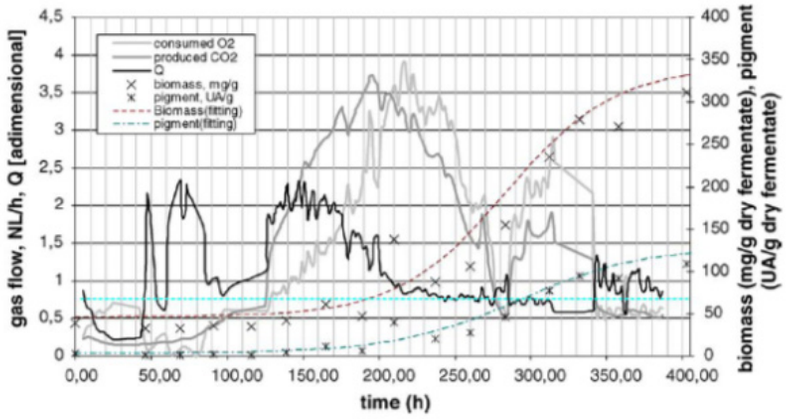

Figure 6. Results of the respirometric analysis in drum-type horizontal bioreactor using rice as substrate–oxygen uptake, CO2 and pigment production (as SPABS), biomass production and respiratory quotient in the course of time. Source: [16]

2.2. Fersol$_2$

2.2.1. Description of Fersol$_2$

The biomass synthesis is one of the most important patterns present in a fermentation bioprocess. In submerged fermentation process, this parameter can be normally measured through direct methods such as cell counting, dry biomass or optical density determination [18].

Fersol$_2$ was reported to monitor fermentation parameters and estimate biomass growth during solid state fermentation. In SSF, biomass growth kinetic behavior is very difficult to analyse because of the heterogeneous composition and the difficulty in separating the biomass from the solid substrate [19-21]. Regarding the software solution, the O_2 consumed and/or CO_2 produced seems to be more adequate because of on-line measurement possibility and fast results [21]. This method could be considered as a direct measurement of process kinetics, although in true sense it is still an indirect measure of biomass synthesis [22].

To monitor and control bioprocesses such as liquid cultures, submerged and solid state fermentations is necessary to measure the biomass, the mass of microorganism, as well as its evolution during the process. One way to measure is estimating the biomass by respirometry: measuring how much oxygen is being consumed and the carbon dioxide is being produced it is possible to estimate the mass of microorganisms. Until some years ago, our research group performed it through a gas chromatograph coupled to a data acquisition system (Fersol$_1$, described in 2.1) which made the process very expensive.

Fersol$_2$ system is a low cost alternative due the incorporation of current resources of informatics. The use of sensors for different variables detection such as temperature, flow, percentages of oxygen, and carbon dioxide, linked to a data acquisition system developed for monitoring, and subsequent, control these processes. With the measured data it is possible to use another software tool improved for the estimation of biomass growth and the determination of some kinetic parameters from process data. The parameters such as the specific growth rate, maintenance and production coefficients, which characterize the process,

allowing a more thorough analysis of its performance. For validate Fersol$_2$ system, Sturm [23] reported some experiments carried out in different bioprocesses developed by our research group involving different microorganisms, in order to better embrace the possible processes. Monitoring processes with fungi, bacteria, algae and plant cells, allowed evaluating the possible validity of the system, facing the different behaviors of several microorganisms used in fermentation, both submerged and solid state fermentation and also in cell cultures.

The complete system Fersol$_2$ is a real time acquisition system to measure environmental variables of bioprocesses such as fermentation processes controlling these parameters and helping to monitor the process in real time. Fersol$_2$ was developed under C Sharp (Microsoft) in DotNet (Microsoft) platform, which works together with Laquis, for data acquisition. but it could be developed under another platform (software) commercially available. This system is capable to monitor bioprocesses environmental parameters such as O_2, CO_2, temperature, flow rate, humidity, pH depending on the sensors which will gather data and the controllers to adjust set points of the variables.

As a basis for the development of this system was taken the Fersol$_1$ software using the methodology reported by Rodriguez-León et al [1] described in the subitem 2.1 above.

As a basic requirement, this interface allows to analyze on a single screen all major parameters calculated, as well as an indication of any change to the parameters presented on the same screen. There is no information loss when the total number of reads exceeds a certain limit imposed on each screen line. On this same screen you can open, save, change and display properties of each file reads, file input and output can be saved as a text file or as a CSV (comma separated values) according to user needs, allowing the opening of data for analysis in other programs such as Microsoft Excel, for example.

As Fersol$_2$ used as a basis, Fersol$_1$, it had limitations on the generation of graphics, especially regarding the resolution and scale, hampering the analysis graphs generated by these and yet it was impossible to download these graphics to other external resources to the program. During the development of the Fersol$_2$ was researched new graphics library, besides the C language offered by C-Sharp®, because this also showed some limitations as to transfer to other external programs, which have been solved and are fully functional in the current version of program. The acquisition program is in development phase and has the possibility of calculation of gaseous masses, flow aeration, process temperatures and relative humidity. The decision to use an industrial network, Modbus, is due to the possibility of expanding the system, incorporating other sensors or actuators, improving automation in the same conditions [23, 25].

The project has been used in bioprocesses experiments such as phytases production by solid state fermentation (SSF) [26]. Also, a test conducted with data from submerged fermentation showed the methods used, resulting in very close values of the estimated and calculated by other means [23].

The decision to modify the structure of the system has brought increased speed, stability and proper use of resources of the lab computers, so that the change should bring more benefits than difficulties with regard to possible future work related to this system.

Part of the research work is related to the application process through sensors attached to the industrial network, quite immune to noise and wrong signals that could be captured, so the tables of values generated by the system should be more reliable, with minor discrepancies with compared to theoretical values or expected.

These new features in process instrumentation such as solid state fermentation (SSF) may generate input tables for processing samples with much larger numbers than in the past, because instead of collecting new sample every two hours, for example, may reap every second, or even fraction of seconds thereof. It is essential to update this software so you may handle this amount of data in reasonable time and without loss of information useful for subsequent monitoring and control of manufacturing a certain product.

The Fersol2 has the main interface design showed in Fig. 7. Using the graphics resources of the chosen programming language, the new program's main interface was designed.

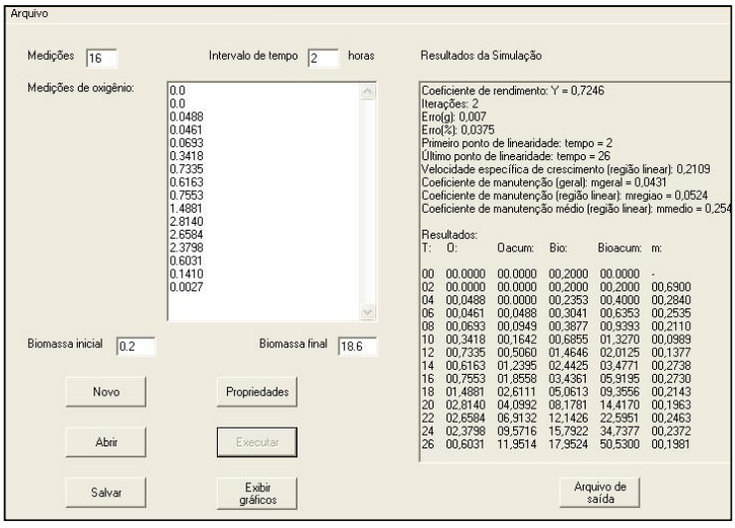

Figure 7. Fersol2 interface (*in portuguese*).

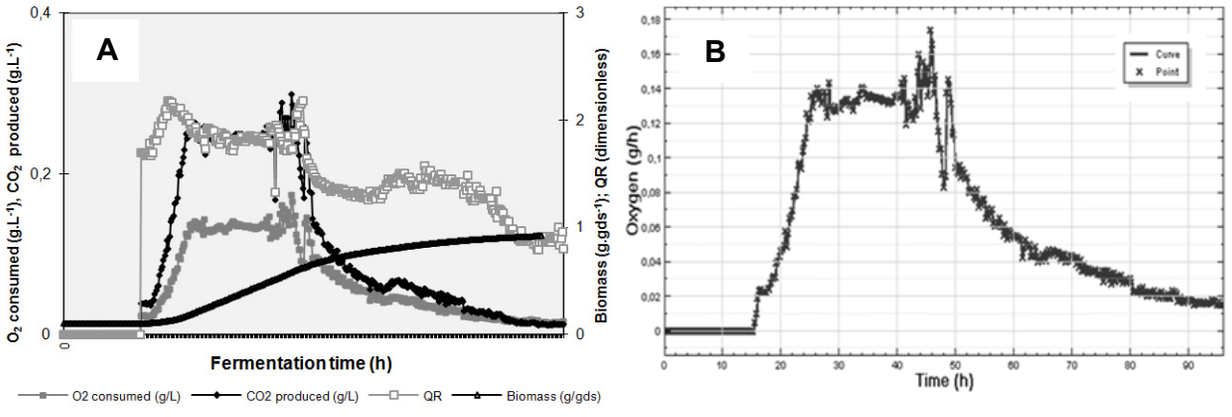

Figure 8. Example of graphs generated by the Fersol2 data acquisition system. (A) profile of O_2 consumption, CO_2 produced, biomass and respirometric quocient (QR) data transferred from Fersol2 to Microsoft® Office Excel and a graph plotted during a solid state fermentation by a fungal strain; (B) Oxygen consumption showed in Fersol2 software.

The Table 1 shows the components of Fersol$_2$ system and their descriptions.

Components	Description
PC with the software	The industrial network Modbus [18] was used and the platform of development Laquis® (LCDS Company) [24]
Air pump	Air supply when the bioprocess require O_2 (aerobic systems) or CO_2 (photosynthetic process)
Air distribution system	Air distribution in different bioreactors, when several experiments must be done simultaneously
Humidifiers	Air passes through flasks containing sterile water to keep moisture content of bioreactors and avoid drying during air distribution.
Water bath with controlled temperature	Normally used for keep Raimbault column or column-type bioreactors commonly used in solid state fermentation processes
Bioreactors	Applied for different types of bioreactor such as column-type bioreactors, Raimbault columns, tray-type bioreactor, drum bioreactor, submerged fermentation bioreactors, cell culture bioreactors are some examples of bioprocesses already reported using Fersol$_2$.
Filter	Air sterilization in input of bioreactor and avoid environmental air contamination in output of bioreactor systems
Flow sensor	The sensor model is Aalborg GFM [27]. Two sensors are used, one with a response in the range 0-100 mL/min (laboratory sacle) and the other in the range of 0-200 L/min (large scale). For use in drum-type reactors, both with a capacity of 2-10 kg, model with larger scale showed better adaptation, mainly by major differences in the need for aeration between the types of bioreactors. The sensors operate by the principle of thermal dispersion. To minimize errors due to pressure fluctuations, this sensor uses a method for disposing between two capillaries, where flow laminar, then calculating the value of actual flow, taking into account the law ideal gas and without the need for auxiliary measurements temperature before and from the sensor, which would be indispensable considering that the pressure of gas varies, either by pumping conditions, or by changes in pressure drop of the reactor itself during the process. The accuracy of this sensor is 1.5% at least against the full scale value.
Controllers display	Shows in real-time data measured by the sensors. Digital visor.
Cylindrical sensors base (Support)	The gas sensors and humidity are adjusted in a PVC tube where the air is applied through the flow sensor. Inside the tube is formed an “air” in the exhaust gases, where the respective sensors measure its concentration. For measurement or temperature control, the Pt100 has been adapted as directly as possible in touch with the process, in order to minimize possible errors.

Transmitter	A transmitter RHT-DM 4–20 mA 150 mm (Novus) measured relative air humidity (%) the outlet process temperature (ºC). This device works as a relative humidity probe. It is installed together with the cylindrical sensors base.
Thermistor sensor	A thermistor sensor is installed together with the cylindrical sensors base.
O_2 sensor	Two sensors are used for measuring gas percentage, in the process input, another installed in the output. The difference between the percentages of these sensors resulted in percentage consumed by microorganisms in the process. The sensors used are of the O_2-A2 model provided by Alpha Sense® [28]. Used in conjunction with an amplifier circuit, signal present between 4-20 mA signal industry standard. The signal varies according to the percentage of oxygen changes from 15% to 25% respectively. With the use of controllers for starting signal. Resolution was established in hundredths of a percentage. These sensors operate through a process called metal-air battery [29], where the oxidation reaction on a metal electrode generates an electrical signal proportional to the percentage of oxygen in the air.
CO_2 sensors	For later determination of the mass of CO_2, two sensors were used similar to those described above, one installed at the entrance and another at the output of the process. The model GMT 221 was provided by Vaisala [30] by interfering between two beams of infrared wavelengths slightly different. The result is generated by a signal output rate of 4-20 mA, with a range of activity between 0 and 20% CO_2. The resolution of this signal is of 0.006%, but the controller that this is connected has a resolution of 0.01%, thus limiting the latter value.
Humidity sensor	In order to monitor the change in relative humidity at the outlet of processes, we used a probe RHT-DM, supplied by the manufacturer Novus [31], with the measurement condition from 0 to 100%, with a signal 4-20mA output, the two wires, supporting a working temperature between -20ºC and 80°C. The basic operation of this sensor is a capacitive probe.
Temperature sensor	For the measurement of temperature and, in some processes its control was using a Pt100 sensor. This choice was based on a wide range of using this type of sensor, and its quick response associated with large linearity. In order to get a signal, this sensor is connected to a controller model N1100 [32], which allow the reading resolution of one-tenth of a degree Celsius, with a maximum error of 0.2% of measurement.

Source: Based on [23]

Table 1. Components and Descriptions of Fersol$_2$ data acquisition system

2.2.2. Applications of Fersol₂ system already reported

Several studies involving bioprocesses have been reported using the Fersol₂ data acquisition system. Some examples are presented below:

Case 1: Relation of enzyme production and fungal growth in column-type bioreactor for SSF

Spier et al [26] reported the relation between phytase production and fungal growth during solid state fermentation in column-type bioreactor monitored by Fersol₂ data acquisition system (Fig. 9). The biomass measured by indirect quantification by ergosterol concentration presented high correlation (R2=0.988) with the biomass estimated by the data generated by the Fersol₂ system [23]. Fig. 9 shows a representative image of Fersol₂ system connected to the column type bioreactor, including adaptation to sensors, controllers and computer system for data acquisition schematic form. Besides (B) shows digital display panel showing the parameters been monitored on-line.

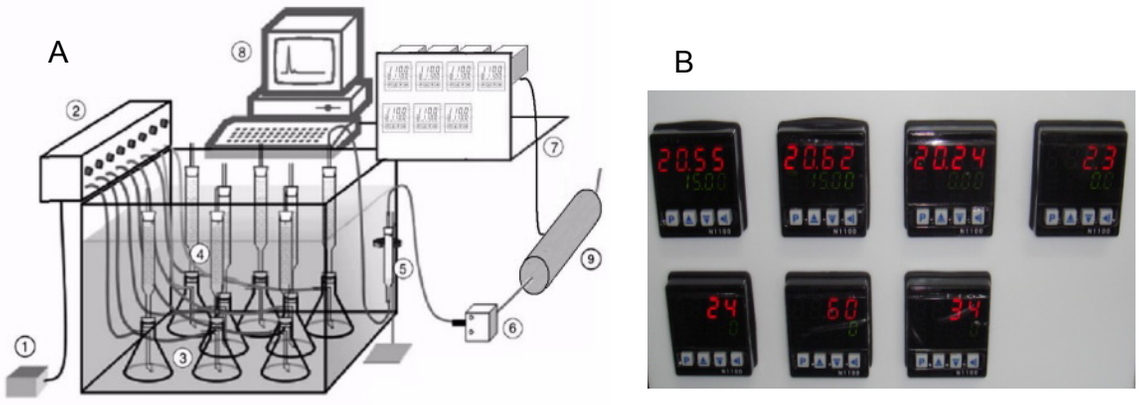

Figure 9. (A) Fersol₂ system. Source: [26]; (B) Fersol₂ control panel. Source: [23]

Case 2: Analysis of vegetable cell culture growth and respiration in a bubble immersion bioreactor

Fersol₂ was applied for monitoring vegetable cells culture in a bubble immersion bioreactor (BIB) model for temporary immersion [33] showed the variation of the percentage of oxygen, considering the difference between input and output system, where it is possible to check the breathing cycle and photosynthesis. This cycle of respiration showed values of 10 hours 42 minutes and photosynthesis during 13 hours 4 minutes. The time for the supply of artificial light was 16 hours, which showed a small delay with respect to light exposure and it is associated in achieving reaction of photosynthesis. The application of Fersol₂ system in this study was to assist in raising relevant information to determine a mathematical model of the reactor under study. The results were related to the flow of great use to design of the model, including showing some of the limitations of the real reactor [23].

Case 3: Microalgae growth

In study with microalgae there was observed one cycle determined in oxygen consumption and hence the gas production dioxide. Whereas microalgae perform photosynthesis, indicating a negative O_2 consumption can be interpreted as the production of gas, a constant

rate. This shift in the signal O_2 consumption occurs in some periods, given the flow and mass of microalgae present in the process. In the same way, the behavior of production of carbon dioxide shows a cyclic behavior compared with oxygen, as determined by correlation between these elements in the metabolism of microalgae [23, 34].

2.3. Other systems already developed

2.3.1. Softsensors

The term "softsensor" is a combination of the words "software", once the models are usually computer programs, and "sensors", because the models are delivering similar information as their hardware counterparts. A softsensor enables bioprocess information acquisition in real time, such as data on the specific growth rate, the biomass concentration and/or the product concentration, via indirect measurements using software sensors. Considering the process control point of view, the biomass concentration is especially interesting to characterize the bioprocess [35]. A softsensor represents the association of hardware sensor(s) with an estimation algorithm, which is integrated into the monitoring, optimization and control systems. Its performance depends on the quality of the measured input data and the adequacy of the process description [36].

Softsensors can be distinguished in two different classes: model-driven and data-driven. The model-driven softsensors is most commonly based on First Principle Models. First Principle Models describe the physical and chemical background of the process. These models are developed primarily for the planning and design of the processing plants, and therefore usually focus on the description of the ideal steady-states of the processes which is only one of their drawbacks which makes it difficult to base softsensors on them.. On the other hand, data-driven softsensors are based on the data measured within the processing plants, and thus describe the real process conditions. Comparing to the model-driven softsensors, data-driven softsensors are more reality related and describe the true conditions of the process in a better way. According to Kadlec et al (2009) [37], the most popular modelling techniques applied to data-driven softsensors are the Principle Component Analysis (PCA) in a combination with a regression model, Partial Least Squares, Artificial Neural Networks, Neuro-Fuzzy Systems and Support Vector Machines (SVMs).

2.3.2. Some applications of softsensors in bioprocess

The automated control of bioprocess variables is difficult due to the complex nature of biotechnology processes, besides the lack of industrially viable sensors for online measurement of these variables.

The applications of fuzzy logic and artificial neural network approaches enable the optimization and control of small or large scale fermentation processes, especially where limited knowledge about the process is available. In recent years, the artificial neural network methodology has become one of the most important techniques applied for biomass estimation [35].

Arazo-Bravo et al [38] applied neuro-fuzzy FasArt and FasBack for the modelling and control of a penicillin production batch process. A softsensor for the prediction of the biomass, viscosity and penicillin production delivers the necessary information for the control mechanisms of the FasBack adaptive controller. The holistic control model is trained and evaluated using simulated process data. The trained model is then able to deliver satisfactory results for the real process control.

"Simple softsensors can also be based on titration techniques [39]." The consumption rates of base (or acid) were used as input for softsensors for substrate and biomass concentrations. The data acquisition and control software was written in LabVIEW 5.1 (National Instruments).The authors successfully demonstrated this titrimetric technique to: (a) Control of aerobic fermentation - estimation of biomass and substrate (phenol) concentrations; (b) Control of (anaerobic) acidification reactors - estimation of metabolite concentrations; (c) Control of (anaerobic) acidification reactors - estimation of inhibitory effects. The results show the versatility of software sensors based on titrimetric techniques and demonstrate the potential for process control in applications in which more sophisticated sensors are not available or affordable.

The use of a visual programming environment LabVIEW to program custom control functions for bioprocess research have already been presented [40]. The time taken for a bioprocess scientist to program new functions compared well with typical times expected for experienced programmers using conventional languages. Experienced LabVIEW programmers develop applications significantly faster. For the development of the system, three aspects of the study were carried out. First, the supervisory control program was written using LabVIEW to encode the feed control algorithm and drivers to communicate with equipment in the plant. A continuous culture was used to define the upper and lower limits to the range of specific growth rates which gave high growth yield from the carbon source as well to determine the value of the growth yield and the relationship between growth yield and specific growth rate in this range. The third aspect was the control system implemented for the production of yeast biomass at constant specific growth rate in a fed-batch bioprocess. The package described by the authors was flexible, easy to use and was ideally suited to developing new applications for control of bioprocesses. It was demonstrated with the development of a system to control specific growth rate in a fed-batch culture.

Knowledge based systems for supervision and control of a bioprocess was presented and applied to data of an industrial antibiotic fermentation [41]. In this paper an approach towards the automatic generation of fuzzy rules was generated describing the relationship between the kinetics of the preculture and the antibiotic yield of the main culture. The fuzzy-C-means algorithm was used for process classification (Software DataEngine, MIT GmbH, Aachen, Germany). For the selection of rules the software WINROSA (MIT GmbH) was applied. Fuzzy membership functions were tuned using the software tool FuzzyOpt (SEI GmbH, Ilmenau, Germany). In order to rate and select rules and finally to optimize

parameters of membership functions of fuzzy variables different criteria are discussed in relation to the aim of the knowledge based control. Results are presented with respect to process monitoring. Genetic algorithms proved suitable for optimization procedures due to the existence of multiple local optima.

A system that can automatically select the moment when the feeding of inverted sucrose should start in the Cephalosporin C batch production process, was implemented using fuzzy methodology [42]. The quantities of sugars, cell mass and Cephalosporin C correspond to variables not monitored continually, but quantified through the analysis of samples taken periodically from the bioreactor. By monitoring the percentage of CO_2 in the outflow gases, it was possible to observe a point of maximum evolution when the microorganism growth phase finishes. Therefore, the moment when the feeding should begin was characterized by a transition from increasing (positive variations) to decreasing (negative variations) CO_2 evolution rates. A fuzzy controller was designed that operates on three reasoning levels, attention, action and protection. The corresponding algorithm was implemented in C language. The results obtained indicated that the algorithm is robust for the tested conditions, allowing a safe automatic operation.

The structure and the functions of the advanced knowledge-based BIOGENES© control system for the control of a fed-batch *S. cerevisiae* cultivation have been described [43]. The BIOGENES knowledge-based control system (KBCS) was built using the industrial control system programming tool GENESIS ™ for Windows, from ICONICS Inc., for creation of the basic control level, and the expert system shell Clips 6.04, from NASA'S Johnson Space center, for creation of the advanced KB-level. The KB-level of the BIOGENES© was able to identify the metabolic state of the yeast and supervisory process control for the fed-batch process. In addition, with the BIOGENES© KBCS, the authors also developed a softsensor for biomass concentration estimation.

Solid-state fermentation is a complex process, including a combination of chemical, biological and transport phenomena. The development of reliable, real-time, and high-performance systems to control the fermentation process is essential [44].

Jiang et al [45] demonstrates that Fourier transform near-infrared (FT-NIR) spectroscopy combined with support vector data description (SVDD) is an efficient method to develop one-class classification model for the rapid monitoring of SSF. The physical and chemical changes in solid-state fermentation (SSF) of crop straws were monitored without the need for chemical analysis. SVDD algorithm was employed to build a one class classification model, and some parameters of SVDD algorithm were optimized by cross-validation in calibrating model. All algorithms were implemented in Matlab 7.1 (Mathworks, USA) under Windows XP. Result Software (AntarisTM II System, Thermo Scientific Co., USA) was used in NIR spectral data acquisition. The SVDD algorithm in the work was developed by Tax et al. (1999) and the SVDD Matlab codes were downloaded from http://homepage.tudelft.nl/n9d04/dd_tools.html for free of charge. Others were developed by authors themselves which were the modification of the algorithm described by Tax et al [46].

Baeza, Gabriel and Lafuente [47] presented the development and implementation of a Real-Time Expert System (RTES) for the supervision and control of a wastewater treatment pilot plant with biological removal of organic matter and nutrients. The hardware architecture contains different supervision levels, including two autonomous process computers (plant control and analysers control) and a PLC, being the expert system the top supervisory level. The expert system has been developed using a commercial, industrially validated RTES-development software G2Ô (by Gensym Corp). It actuates as the master in a supervisory setpoint control scheme and it is based on a distributed architecture. This system has been running continuously for 600 days. The supervisory Expert System has shown an excellent performance to manage the pilot wastewater treatment plant. The system developed detects and controls all the wrong and special operations, for example pump failure, feeding problems, probes malfunction, equipment maintenance, analysers control and maintenance.

Flow Injection Analysis (FIA) systems with an integrated biosensor could indeed be important tools in bioprocess monitoring. To facilitate the optimal use of a FIA system, the prerequisites are stable, sensitive and robust hardware with features for data analysis and evaluation.

A versatile automated continuous flow system (VersAFlo) was developed [48] for bioanalytical applications, providing a platform to employ biosensors for continuous analysis of bioprocesses with precise control of flow, volume and defined events has been developed. The system was based on National Instruments LabVIEW and was verified for online analysis of IgG employing a heterogeneous immunoassay in a competitive flow-ELISA mode. Also, the production of recombinant protein is a growing field and the requirements on bioprocess monitoring and control in such processes are crucial.

Di Sciacio and Amicarelli [35] proposed a biomass concentration estimator for a biotechnological batch process based on Bayesian regression with Gaussian process. A real bioprocess was designed and exemplified for a *Bacillus thuringiensis* δ-endotoxins production process on the basis of experimental data from a set of various batch fermentations. The authors concluded that this Bayesian non-parametric framework is sufficiently flexible to represent a wide variety of bioprocess data, and makes possible interpreting the prior distribution, computing the posterior, and the full predictive distributions, as well as, the mean predictions and the predictive uncertainties.

In industrial applications, there are some softwares that only work like a HMI (human machine interface), collecting data and displaying on the computer screen only. These systems do not perform control over the process variables. The software called SCADA (supervisory control and data acquisition) does both, acquisition and control functions, acting on the variables directly and changing process values. Some examples of suppliers of these systems are LabVIEW, by National Instruments, Elipse SCADA, by Elipse, andHMI / SCADA Software, by Advantech. These systems may be accessed in the following website links: http://www.ni.com/labview/, http://www.elipse.com.br/ and http://www.advantech.com/products/automation-software/sub_1-2mlc9t.aspx, respectively.

2.4. Biosensors

2.4.1. Concept of biosensor

Biosensors are bioelectronic devices able to detect rapidly chemical species and/or biological (analyte), both qualitatively and quantitatively. This type of detector allows varied tasks such as: on-line control at industrial level; automation of biochemical and chemical plants; environmental analysis in real-time; in vivo analysis; detection and quantification of relevant biological substances and detection of chemical warfare, among other applications. Biosensors` devices can be of continuous use (most of them) or disposable (i.e: blood glucose meters).

Biosensors are composed essentially by: biological element; transducer; and electronics. The biological element or sensor element has the property of selective recognition and interaction with the interest analyte. The surface of the sensor element is usually covered with biological material, i.e: antibodies, nucleic acids, proteins, organelles, cells, among others. The interaction between the biological element and the analyte results in the modification of one or more physico-chemical properties (pH, electron transfer, mass transfer, heat transfer, the release of ions or gases) which are detected and measured by the transducer. The main objective is to produce an electronic signal. This signal must be proportional in magnitude and/or frequency to the concentration of a particular analyte or a group of analytes interacting with the biosensor. Finally, electronics consists of an amplifier of electrical signals and system data processing.

Therefore, a biosensor combines the specificity of an active biological component to an analyte of interest, with a sensitive transducer, which converts the biological signal into an electrical signal proportional to the concentration of the analyte, which can be further processed and interpreted [49].

2.4.2. Immobilization of biological material: The most important step of the development of a biosensor

The most important step in the development of the biosensor is the immobilization of the biological material, and this must be done in a way that the binding site to the molecule of interest becomes clear. There are several ways to perform the immobilization: occlusion; microencapsulation; physical adsorption; covalent binding; attachment with polyethylenimine (PEI); acrylamide membrane; protein A.

Some of the desired properties after immobilization are: physico-chemical and mechanical stability of the components; short time of response when interacting with analytes of interest; good selectivity and sensibility; low limit of detection (which means low concentrations of the analyte are able to promote a response from the biosensor) and accuracy [50].

2.4.3. Classification

Biosensors can be classified according to the type of biological material of its sensor, or according to the type of transducer employed.

2.4.3.1. According to the biological component

The biosensors can be classified as: chemoreceptors; enzymatic sensors; immunosensors; microbiological sensors.

Chemoreceptors use proteins as sensing elements. The interaction with the analyte leads to conformational changes of the proteins and depending on the degree of this event, a different signal will be generated, transduced and transmitted. However, this type of sensor has a difficult handling and connection to the transducer, and has a lower specificity when compared to other types of sensors, like enzymatic sensors or immunosensors.

Enzymatic biosensors use enzymes, usually immobilized, as biological component. Enzymes are biological catalysts of high specificity and sensitivity, therefore, this class of biosensor is of great importance in biological processes. However, they have relatively low stability, and thus, a strict control over the environment conditions such as pH, temperature and pressure is necessary for its employment.

Imunosensors use globular proteins of serum, the immunoglobulins, as biological element. These proteins are antibodies, which mean they bind to antigens with high affinity and specificity. However, their high molecular weight makes difficult the adaptation to the transducer. In addition, background reactions may occur. To avoid these undesirable reactions, secondary antibodies must be added and the base on which the antibody is attached must be blocked.

The microbial biosensor uses immobilized microorganisms, which specifically recognize an organic compound. Thus his metabolic activity is altered, and such change is detected by the transducer.

2.4.3.2. According to the transducer

After interacting with the biological element, the signal is detected and sent for processing. This role is performed by an element called transducer. The signals can assume several forms, since there is a great variety in the biological components used. The transducers are classified according to the type of physico-chemical stimulus received. The main types are electrochemical, optical, calorimetric, and piezoelectric.

The electrochemical transducers are based on the movement of electrons and diffusion of electroactive species. There are three different types: amperometric, potentiometric and conductimetric [51].

The amperometric transducers are based on the measurement of electric current, generated from reactions of oxidation and reduction of electroactive species. The system is composed typically by three electrodes: a working electrode, in which will occur the reaction of interest; a reference electrode, which sets the potential applied to the first electrode; and a counter electrode, which provides current to the working electrode. In practice, for some applications only the first two are sufficient. The two major drawbacks of this class of

sensor: they are sensitive to background noise, and the relatively high potential required can oxidize other compounds than the interest analyte, and so, generating a higher electrical current, and a false result. Some innovations have attempted to overcome these problems, such as the use of diffusion limiting membranes to maintain the concentration of substrate below the degree of saturation of the enzyme and the use of mediators [52]. The construction of chemically modified electrodes by the development of immobilization techniques of both enzymes and mediators has opened a new amperometric class of transducers, in which mediators can be incorporated into electrodes by adsorption, occlusion in polymer films, covalent bonding or simply mixed in carbon slurry [53]. This type of biosensor is largely available today at the market, because they are based on REDOX enzymes (oxiredutases). These enzymes have a well established market, and can act on fatty acids, sugars, amino acids, aldehydes and phenols.

The potentiometric biosensor, in general, has a reference electrode (inert) and a working electrode (preferably ion-selective). Both electrodes are put in contact with the sample; a constant electric current is generated. If the sample is a broth in which will occur an enzymatic reaction, a difference of potential is developed between the electrodes, due to the production or consumption of strong polar ions by the enzymes during the catalysis. These reactive species are detected by the ion selective electrode, and then a quantifiable signal will be generated and transmitted [54]. The ion-selective electrodes are fast, sensitive, cheap and the measurement is simple, because only pH measurement is required.

In conductimetric biosensors, changes are observed in conductance measurements resulting from products of catalytic reactions. The operating principle involves a pair of micro-electrodes separated by an electrolyte solution containing the enzyme and the sample to be detected. The electric field is generated by applying a difference of potential between the microelectrodes, where there are variations in the concentrations of polarized species. Many enzymatic reactions produce a change in conductivity, but only a few provide a signal with stable magnitude. This type of transducer has not been widely used, due to the difficulty to perform measurements with simple devices. Also, the signal is very dependent from the temperature and usually the dilution of the sample is required.

The optical methods to transduce signals are: absorption, fluorescence, phosphorescence, and polarization interference, which can be used in solid state sensors. Optical transducers can be associated with an immobilized biological component in the presence or absence of an indicator. Its principle is based on the fact that some enzymatic reactions alter the optical property of certain substances and the light emitted by this element or its response to biological lighting can be conveniently transmitted via optic fibers and monitored in optical equipments [55].

Some biological reactions are accompanied by the release of energy enabling them to be quantified calorimetrically, relating the amount of heat generated with the amount of

substrate consumed or with the amount product formed. The calorimetric biosensors allow this monitoring, but have some important disadvantages: high costs, complexity, low specificity to the analyte. Another problem: changes of enthalpy occur not only in the biochemical reactions, but also in the mixture, dilution and solvation of the components. Heat is also exchanged with the environment. All these factors may contribute to possible errors of analysis.

Some types of crystals (ie: anisotropic crystals, those lacking a center of symmetry) generate an electrical signal when subjected to mechanical stress. Similarly, if subjected to an electrical signal, they undergo mechanical deformation proportional to the signal. This is called the piezoelectric effect. Thus, with the applying of an oscillating electric potential, the crystal will vibrate. These vibrant cristals can be used as devices to generate electrical currents. The vibration frequency is affected by the mass of adsorbed material in its surface, which can be related to changes in a reaction. Piezoelectric materials that can be used in sensors include ceramic materials such as barium and plumb titanates, as well as "natural" materials such as quartz and tourmaline. Some organic polymers, such as polyvinylidene fluoride (PVDF), also form crystals with piezoelectrical properties.

Tables 2 and 3 summarize the classification of biosensors, its principle of operation, advantages and disadvantages, some of them already cited in the text:

Transductor type	**Principle of operation**	**Advantages**	**Disadvantages**
Chemoreceptors	Interaction between proteins and the analyte	Simpler and cheaper than other sensors	Difficult conection with the transducer; low specificity for a particular analyte
Enzymatic	Catalysis of the substrate (analyte) by immobilized enzymes	High specificity	Low stability
Immunosensors	Specific binding of the analyte to an antibody	High specificity and afinity	Difficult conection with the transducer; background reactions
Microbiological	Specific recognition of an analyte by an immobilized microrganism	Easy to isolate; less sensitive to pH and temperature variations	Higher response time; lower selectivity

Table 2. Biosensors´ classification – according to the biological component

Transducer type	Principle of operation	Advantages	Disadvantages
Amperometric	Generation of electric current	Wide range of application for REDOX enzymes	Background noise and low selectivity (except for modified designs)
Potentiometric	Generation of a diference of potential	High selectivity, low time of response	Less reliable than amperometric; more complex than conductimetric
Conductimetric	Generation of a condutance change	Very cheap and simple design	Low range of application; low reliability
Optical	Alteration of optical properties of the medium	Low cost; perspective for new uses in the future	Only applicable when optical changes occur
Calorimetric	Detection of generated heat	Wide range of application; high reliability	High cost; high complexity; low specificity
Piezoelectric	Generation of electric current due to mechanical stress	Very high time of response; low complexity	Low reliability

Table 3. Biosensors´ classification – according to the type of transducer

2.5. Applications of biosensors

The application of biosensors is very broad and diversified, being used in several areas:

- Food industry (the determination of glucose in instant coffee and sulfite in foods);
- Pharmaceutical Industry (determination of ascorbic acid, epinephrine and dopamine on drugs);
- Medicine (quantification of urea in urine and glucose in human serum);
- Environmental Engineering (environmental control and the determination of pesticides);
- Bioprocess Engineering (determination of substrates and products of fermentation processes, ie:pentoses, hexoses, organic acids, aminoacids, lipids, proteins; determination of gases concentrations, ie: oxigen and carbon dioxide and even quantification of celular concentration).

In general, biosensors are the first element of a data acquisition system in Bioprocesses, when the variables to be measured and controlled are concentrations of biomolecules. The detection of these analytes requires greater sensibility and/or specificity, but once they are properly detected, as shown at the last subsections, the processing of data is similar to processes used in other industries. Some particular examples of application are shown in the previous section, with the detailing of the data acquisition system in its whole scope.

3. Conclusions

When monitoring a process, the verification in real-time of determinant parameters for a proper functioning of this process is crucial for better understanding. If the process is still under study, or if the monitoring processes is well known, allowing evaluate smaller changes in their characteristics when occur any disorder or condition unfavorable. Data acquisition systems in bioprocesses present great importance for industrial, economical and scientific purposes.

In industrial applications high speed data acquisition systems are generally required, for example in manufacturing machines. This characteristic is necessary because of the high frequency in the variables changing, and to perform real time acquisition. In other words, the process variables change rapidly over time, such as the speed of an electric motor, or the pressure value in a duct, for instance.

In bioprocesses, although there are a large number of variables to be monitored or controlled, the speed of values changing is not so high. Thus, to make the data acquisition relating to changes in pH or respirometric values, such as oxygen consumed and carbon dioxide produced, for example, many recordings are not necessary per unit of time, as these variables are usually connected to the biomass growth, which does not change very quickly.

Many alternatives of systems are commercially available for laboratory and/or industrial control. Besides two examples of systems Fersol$_1$ and Fersol$_2$ were described, and biosensors are specified to help students, scientists and engineers to understand and to choose which type and specifications of real-time systems may be the most adequate for their necessities and applications.

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Acknowledgement

Authors would like to thank Prof. PhD José Angel Rodríguez-León (Positivo University, Brazil), Prof. PhD Dario Eduardo Amaral Dergint (UTFPR, Brazil) for technical assistant. And also CNPq for finantial support.

4. References

[1] Rodriguez-León J A, Sastre L, Echevarria J, Delgado G, Bechstedt W. A Mathematical Approach for the Estimation of Biomass Production Rate in Solid State Fermentation. Acta Biotechnologica, 1988; (8) 307-310.

* Corresponding Author

[2] Sturm W, Soccol CR, Dergint DEA, Rodríguez-León JA, Magalhães DCNV, Pandey A. Informatic in Solid-State Fermentation. In: Pandey A, Soccol CR, Larroche C. (ed.) Current Developments in Solis-State Fermentation. New Delhi: Asiatech Publishers; 2007. p.169-180.

[3] Soccol CR, Vandenberghe LPS. Overview of Solid-State Fermentation in Brazil. Biochemical Engineering Journal, 2003, (13): 205-219.

[4] Raimbault M, Roussos S, Lonsane BK. Solid State Fermentation at ORSTOM: History, Evolution and Perspectives. In: Roussos S, Raimbault M, Lonsane BK. (ed.) Advances in Solid Substrate Fermentation. New York : Springer-Verlag; 2008. p.577-612.

[5] Saucedo-Castañeda G. Contrôle du Métabolisme *de Schwanniomyces castellii* Cultivé sur Support Solide. Thèse de Doctorat. Université Montpellier II, Montpellier, France; 1991.

[6] Soccol CR. Physiologie et Métabolisme de *Rhizopus* en Culture Solide et Submergée en Relation avec la Dégradation d'Amidon Cru et la Production d'Acide L(+) Lactique. PhD Thesis. University of Compiegne France; 1992.

[7] Stertz SC, Soccol CR, Raimbault M, Pandey A, Rodriguez-Léon J. Growth Kinetics of *Rhizopus formosa* MUCL 28422 on Raw Cassava Flour in Solid State Fermentation. Journal of Chemical Technology and Biotechnology 1999; 74 1-7.

[8] Pandey A, Soccol CR, Rodriguez-León JA, Nigam P. Production of Organic Acids by Solid-State Fermentation. In: Pandey A, Soccol CR, Rodriguez-Leon JA, Nigam P. (ed.) Solid-State Fermentation in Biotechnology – Fundamentals and Applications. New Delhi: Asiatech Publishers Inc.; 2001. p. 113-126.

[9] Prado FC, Soccol CR, Vandenberghe LPS, Lisboa C, Paca J, Pandey A. Relation Between Citric Acid Production and Respiration Rate of Aspergillus niger in Solid-State Fermentation in Flasks, Trays and Horizontal Drum Bioreactors Using Treated Cassava Bagasse. Engineering in Life Sciences 2004; 4(2):179-186.

[10] Soccol CR, Prado FC, Vandenberghe LPS, Pandey A. General Aspects In Citric Acid Production by Submerged and Solid-State Fermentation. In: Pandey A. (ed.) Concise Encyclopedia of Bioresource Technology. New York: Haworth Press: 2004. p. 617-628.

[11] Brand D, Pandey A, Rodríguez-León JA, Roussos S, Brand I, Soccol CR. Relation Between COffee Husk Caffeine Degradatin and Respiration of Aspergillus sp. in Solid State Fermentation. Applied Biochemistry and Biotechnology 2002; 102, p. 169-177.

[12] Vandenberghe LPS, Soccol CR, Pandey A, Lebeault JM. On-line monitoring of citric acid production using respirometry in solid state fermentation with cassava bagasse. In: More Quality of lLife by Means of Biotechnology: proceedings of International Symposium on the Bioconversion of Renewable Raw Materials, 2000, Hannover: Germany.

[13] Vandenberghe LPS. Développement d'un Procédé pour la Production d'Acide Citrique par Fermentation en Milieu Solide à partir de Résidus de l'Agro-Industrie du Manioc. PhD Thesis. Université de Technologie de Compiègne Compiègne; 2000.

[14] Raimbault M, Alazard D. Culture Method to Study Fungal Growth in Solid Fermentation. European Journal of Applied Microbiology and Biotechnology 1980; 9 199-209.

[15] Pintado J, Torrado A, González MP, Murado MA. Optimization of Nutrient Concentration for Citric Acid Production by Soilid-State Culture of Aspergillus niger on Polyurethane Foams. Enzyme Microbial and Technology, 1998; 23, p. 149-156.
[16] Carvalho JC, Pandey A, Oishi BO, Brand D, Rodriguez-León JA, Soccol CR. Relation Between Growth, Respirometric Analysis and Biopigments Production from Monacus by Solid-State Fermentation. Biochemical Engineering Journal 2006; 29, p. 262-269.
[17] Vandenberghe LPS, Soccol CR, Pandey A, Lebeault J-M. Solid-State Fermentation for the Synthesis of Citric Acid by *Aspergillus niger*. Bioresource Technology 2000; 74 175-178.
[18] Sturm W, Dergint DEA, Soccol CR, Pandey A. Instrumentation and Control in SSF. In: Pandey A; Soccol CR; Larroche C (ed.) Current Development in Solid-State Fermentation. v.1. New York: Springer Science + Business Media, 2008. p. 146-168.
[19] Singhania RR, Soccol CR, Pandey A. Recent advances in solid-state fermentation. Biochemical Engineering Journal 2008; 44(1):13–18.
[20] Pandey A, Soccol CR, Mitchell D. New developments in solid state fermentation: I bioprocesses and products. Process Biochemistry 2000; 35:1153–1169
[21] Pandey A, Soccol CR, Rodríguez-León JA, Nigam P. Solid state fermentation in biotechnology: fundamentals and applications. New Delhi: Asiatech Publishers Inc, 2001.
[22] Sturm W, Soccol CR, Dergint DEA, Rodríguez-León JA, Magalhães DCNV. Informatics in solid state fermentation. Chapter 8. In: Ed. Pandey, A.; Soccol, C.R.; Larroche, C. Current Development in Solid-State Fermentation. Delhi: Springer Asiatech Publishers, 2008. p.168-178.
[23] Sturm, W. Development of Software Tools for Acquisition of Data and Evaluation of Results in Fermentation Processes in Solid and Submerged and Cell Culture. PhD Thesis. Federal University of Paraná, Curitiba; 2009. 104p. (*In Portuguese*).
[24] LCDS Company. Leão Consultoria e Desenvolvimento de Sistemas LTDA (Lion Consultant and Systems Development, Brazil. http://www.lcds.com.br/ (accessed 21 April 2012).
[25] Sturm W. Industrial Sensors – Theorical Concepts and Practical Aplications. Rio de Janeiro: Papel Virtual Editora, 2004. (*In Portuguese*).
[26] Spier MR, Woiciechowski AL, Letti LAJ, Scheidt GN, Sturm W, Rodriguez-León JA, de Carvalho JC, Dergint DE, Soccol CR. Monitoring fermentation parameters during phytase production in column-type bioreactor using a new data acquisition system. Bioprocess Biosystems Enginering 2010; 33:1033-1041.
[27] Aalborg. Low Cost Mass Flow Meters. GFM Model Flow Sensor. http://www.aalgorg.com. (accessed: 10 May 2010).
[28] Alphasense. Technical Specification: Oxygen Sensor O2A2. Data Sheet. Disponível em: http://www.alphasense.com . Acesso 25 July 2009.
[29] Alphasense. How Oxygen Sensors Work. Alphasense Application Note AAN009. http://www.alphasense.com (accessed 05 May 2008).
[30] Vaisala. CO_2 Sensors model GMT221. Technical Data. Disponível em: http://www.vaisala.com . Acesso: 10/05/2006.
[31] Novus, Brazil. Products Catalog. http://www.novus.com.br (accessed 10 May 2008).

[32] Novus, 2008b. Operation Handbook N1100. http://www.novus.com.br. (accessed: 10 May 2008).
[33] Soccol CR, Scheidt GN, Mohan R. Biorreator do tipo imersão por bolhas para as técnicas de micropropagação vegetal. Universidade Federal do Paraná. Patente (DEPR. 01508000078); 2008.
[34] Sydney EB. Respirometric balance and analysis of four microalgaes: Dunnaliela tertiolecta, Chlorella vulgaris, Botryococcus braunii and Spirulina platensis. 2009. Dissertação (Mestrado em Master BIODEV) - Université de La Méditerranée Aix Marseille II, 2009.
[35] di Sciascio F, Amicarelli AN (2008). Biomass estimation in batch biotechnological processes by Bayesian Gaussian process regression, Computers and Chemical Engineering, 32, 3264–3273.
[36] Gjerkes H, Malensek J, Sitar A, Golobic I. (2011) Product identification in industrial batch fermentation using a variable forgetting factor , Control Engineering Practice,2001, 19 1208–1215
[37] Kadlec P, Gabrys B, Strandt S. Data-driven Soft Sensors in the process industry, Computers and Chemical Engineering, 2009 33 795–814.
[38] Arazo-Bravo MJ, Cano-Izquierdo JM, Gmez-Snchez E, Lpez-Nieto MJ, Dimitriadis YA, Lpez-Coronado J. (2004). Automatization of a penicillin production process with soft sensors and an adaptive controller based on neuro fuzzy systems. *Control Engineering Practice*, *12*(9), 1073–1090.
[39] Feitkenhauer H, Meyer U. (2004).Software sensors based on titrimetric techniques for the monitoring and control of aerobic and anaerobic bioreactors, Biochemical Engineering Journal, 17, 147–151
[40] Gregory ME, Keay PJ, Dean P, Bulmer M, Thornhill NF. A visual programming environment for bioprocess control..Journal of Biotechnology, 1994, 33 233-241.
[41] Guthke R, Schmidt-Heck W, Pfaff M. Knowledge acquisition and knowledge based control in bioprocess engineering, Journal of Biotechnology, 1998, 65, 37–46.
[42] Sousa JR, Almeida PIF. Design of a fuzzy system for the control of a biochemical reactor in fed-batch culture, Process Biochemistry 2001, 37 461–469.
[43] Hrnĉiřík P, Náhlík J, Vovsík J. The BIOGENES System for Knowledge-based Bioprocess Control, Expert Systems with Applications, 2002, 23 145-153.
[44] Ödman P, Johansen CL, Olsson L., Gernaey, K.V.; Lantz, A.E. (2009) On-line estimation of biomass, glucose and ethanol in Saccharomyces cerevisiae cultivations using in situ multi-wavelength fluorescence and software sensors, J. Biotechnol., 144 102–112.
[45] Jiang H, Liu G, Xiao X, Mei C, Ding Y, Yu S. Monitoring of Solid-state fermentation of wheat straw in a pilot scale using FT-NIR spectroscopy and support vector data description. Microchemical Journal, 2012, 102, 68–74.
[46] Tax DMJ, Duin RPW. Support vector domain description, Pattern Recogn. Letter, 1999, 20, 1191–1199.
[47] Baeza J, Gabriel D, Lafuente J. An expert supervisory system for a pilot WWTP Environmental Modelling & Software, 1999 14, 383–390.

[48] Kumar MA, Mazlomi MA, Hedström M, Mattiasson B. (2012) Versatile automated continuous flow system (VersAFlo) for bioanalysis and bioprocess control. Sensors and Actuators B, 161, 855– 861.
[49] Salgado AM. Desenvolvimento e Aplicação de Sensores e Sistemas de Monitoração de Biomassa, Etanol e de Substrato por Modelo. Tese de Doutorado, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil; 2001.
[50] Walker JM, Gingold EB. Biologia Molecular y Biotecnologia. 2ª Edição, Zaragoza; 1997.
[51] Thévenot DR, Toth K, Durst RA, Wilson GS. Electrochemical Biosensors: Recommended Definitions and Classification. Biosensors & Bioelectronics, v. 16, p. 121-131; 2001.
[52] Perez EF. Desenvolvimento de um Biosensor Amperométrico para Oxalato. Dissertação de Mestrado. Universidade Estadual de Campinas, Campinas, São Paulo; 2000.
[53] Romani A, Minunni M, Mulinacci N, Pinelli P, Vincieri FF. Comparison among Differential Pulse Voltammetry, Amperometric Biosensor, and HPLC/DAD Analysis for Polyphenol Determination. Journal Agriculture Food Chemistry, v.48, p. 1197-1203; 2000.
[54] Arya SK, Datta M, Malhotra BD. Recent Advances in Cholesterol Biosensor. Biosensors and Bioelectronics, v. 23, p.1083–1100, 2008.
[55] Hall EAH. Amperometric Biosensor. Open University Press, London, p.351; 1990.