Part one

INTRODUCTION

The bioprocess study makes evident the principles that are the foundation of living systems. In the first part of this chapter, different kind of bioprocesses (specially the aerobic bioprocesses) will be analyzed, together with the most interesting parameters and a general overview on the cell metabolism. In the second part, the most usual bioreactor types with some particularities will be shown. Finally, a general overview on the bioprocess measuring systems will be presented in addition with the information organization modalities and other some consonant possibilities on these.

1.1. BIOPROCESSES

Bioprocesses are complex transformations of organic/anorganic chemical substances (substrates), introduced in the culture medium, into products of economic interest (Moser, 1985).

These transformations (conversions) represent an ensemble of biochemical (metabolic) reactions performed by living cells. The cells execute these conversions in order to acquire some metabolic products (substances which are necessary for the cellular components synthesis) or to obtain the growth or maintenance energy. At the same time, the cells are directed towards specific transformations of substrates into useful products by means of appropriate procedures (Leveau, Bouix, 1984).

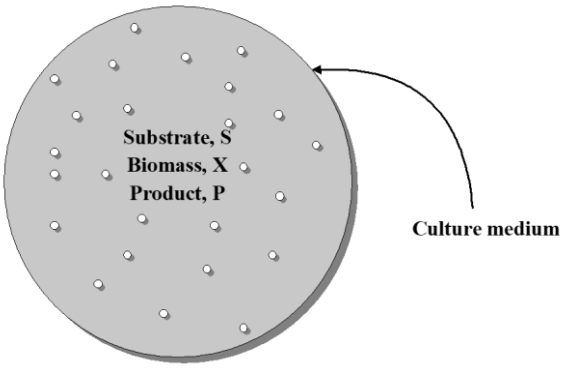

Fig. 1.1.1 Bioprocess conception

The substrates are transformed either in new structure components (cell growth system) or/and useful substances (biotransformation/biosynthesis cycle) (Nagai, 1979).

The main product of some bioprocesses is the growing cell quantity, but in most cases the formation of some metabolic products (intracellular/extracellular) is taken into consideration.

The main bioprocess types are:

- Aerobic bioprocesses (i.e. the oxygen can be considered as a second substrate);
- Anaerobic bioprocesses.

In this work, only the aerobic bioprocesses, more complex transformations than the anaerobic ones, will be considered.

The cell's metabolism (i.e. the **bioprocess**) implies the transformations through biochemical specific reactions of initial medium components in a main product and more secondary products (Leveau, Bouix, 1984, Kingsburg, 1989).

Bioprocesses take place into **a culture medium**, an aqueous solution, where the substrates are solved or mixed ready for being transformed by the cells, also introduced in this mixture through inoculation.

From a physical point of view, the culture medium consists of the following phases (Moo Young, 1985):

- Solid phase: cells and insoluble medium components;
- Liquid phase: water with soluble substrates or products;
- Gas phase: air (oxygen, nitrogen), carbon dioxide and other metabolic gases.

Due to the cell elementary chemical composition (Moo Young, 1985), the culture medium must include all substances needed for cell growth and maintenance (Bocker, Recknagel, 1983). Therefore, the main medium components are either organic/inorganic products or/and complex natural compounds (Aiba *et al*., 1973):

- *Carbon & energy sour*ce: glucose, zaharose, molasses, cellulose, n-paraffin;
- *Nitrogen source;*
- *Phosphorus source*;
- *Oxygen source* (for aerobic bioprocesses): air, air enriched with oxygen, etc.

- *Metals source (as microelements):* inorganic salts in very-small quantities.

The bioprocess can be considered as a **biosystem,** which involve *cells-medium-bioprocess installation* (this last part is usually a reactor) and is characterized by outstanding complexity and interdependencies (Moser, 1985).

For obtaining a high productivity in a bioprocess, an optimal evolution of the physic and biochemical parameters, which assure the cultivation conditions, is needed (Atkinson, Mavituna, 1985, Kompen, Sodelberg, 1985). in agreement with cell growth and other metabolic requirements.

The main cultivation parameters are (Brauer, 1985, Schugerl, 1985, Blenke, 1985, Schugerl, Sittig, 1987):

- constant temperature (T) during the bioprocess evolution, specific for the cultivated cell: usual domain 20°C - 40°C;
- pressure (p): domain 0 – 0.5 at.;
- stirrer speed (n) (only for stirred reactors) and aeration (Q);
- pH: domain 3 – 10;
- dissolved oxygen (pO_2);
- redox potential (rH);
- minim foam level.

The bioprocess evolution is always described by the following specific biochemical parameters (Charles, 1985):

- Substrate concentration (S);
- Cell concentration/biomass (X);
- Product concentration (P).

There are many distinct (on-line/off-line) methods to determine the above concentrations (Kossen, 1994, Moser, 1985, Aiba, 1973, Moo-Young, 1985, Schugerl, 1985). Depending on specific cultivation circumstances, a particular method can be used, preferably for the automatic controls ability an on-line variant. Unfortunately, if for S and X concentrations there are some on-line monitoring methods, for the product concentration the off-line methods are usual.

A good bioprocess start-up involves the medium sterility. Many sterilization methods are presented in the scientific literature (Winkler, 1983, Aiba, 1973) and are performed through thermal or chemical effects or using radiation.

From a technological point of view (Chisti, 1989, Tolbert *et al.* 1982) there are three main bioprocess modes of operation:

- *Batch* cultivation (..);
- *Fed-batch* cultivation (..);
- *Continuous* cultivation (Bliem, Katinger, 1988), Prokop, Rosenberg, 1989):

The standard evolution curve of a batch culture is shown in fig. 1.1.2. The intermediate phases are characteristic to each process with living cells (Moser, 1985, Kargi, 1985):

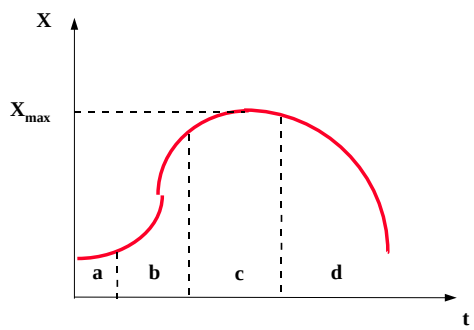

Fig. 1.1.2 Standard representation of batch culture

a) Lag phase or the cell adaptation to the new medium conditions;
b) Exponential growth phase
c) Stationary (steady state) phase;
d) Decreasing phase.

In addition, there are some cases (depending on the final product kinetics), where conjugate metabolic routes (conforming to well-controlled sequencing) can be applied, or simultaneous cultivation of two or more microorganisms is to be used (Schugerl, 1985)

There are some complex phenomena, specific to living systems, which define important concepts for the bioprocess control:

- Inhibitory substrate concentration effect (Kobayashi, 1972): each cell can use the substrate until a certain level of concentration and for concentrations superior to this value the metabolic reactions are inhibited and finally blocked. Due to its low solubility, there is no inhibition by dissolved oxygen concentration
- Growth associated/non-associated product kinetics (Gaden, 1959, Chisti, 1989): the growth associated product is mostly obtained during the exponential phase of growth, where the cell multiplication is maxim, but the non-growth-associated product is accumulated after this characteristic phase.

1.2. BIOREACTORS

1.2.1. Overview

The specific problems of aerobic bioprocesses are correlated with the **bioreactor** conception (Chisti, 1989). Bioreactors are vessels in which raw materials are biologically converted into specific products, using microorganisms, plant or animal cells or individual enzymes. Microorganisms and cells are regularly involved in trying to maintain their environment to provide optimum growing conditions. Bioreactors support this natural process by supplying suitable conditions such as optimum temperature, pH, sufficient substrate, nutritional salts, vitamins and oxygen (for aerobic microorganisms), enabling cells to grow and form metabolites (Schugerl, 1991). It is generally known that the reactor performance (hydrodynamics and mass/energy transfer characteristics) is depending on the configuration, the internal geometry and the liquid medium proprieties. The reactor performance is to be established in agreement with the cell's growth/maintenance and product formation requirements (Chisti, 1989).

Microbial processes are either aerobic or anaerobic and each type demands a different set of conditions. Aerobic reactors can be subdivided into submerged and surface reactors. Submerged reactors are more suitable than surface devices for mass production and have therefore become more popular over the last few decades. In a high-performance submerged reactor turbulence must be created to ensure high specific production rates. This turbulence is generated by energy dissipation.

In high performance submerged reactors, high specific productivity in respect to the volume of the medium is attained only with highly specific power inputs. As this energy is dissipated, it releases heat which must be removed from the system Submerged reactors must be sterilizable and well equipped with devices for removing heat, instruments and control units, making them more expensive to produce and operate than the surface reactors.

In the aerobic bioprocesses, the oxygen is the least soluble nutrient from the medium and can become a limiting substrate for cellular growth (Bailey, Ollis, 1977). Hence, the reactors must assure the oxygen transfer rate required by the cells metabolic reactions. Moreover, the reactor must provide the heat transfer too, taking into consideration the endo-thermic / exothermic phases (Moser, 1985).
Furthermore, other condition imposed to bioreactor refers to sterility maintenance, especially for long continuous cultivation (Schugerl, 1985).

The main global concept about the bioreactor performance is this installation must be optimally designed and operated so that the

cultivated cells can find all the needed environmental conditions. Hence the optimized bioreactor design is achieved based on bioprocess specificity (Charles, 1985).

Taking into consideration the geometric variants, the study of the bioreactor hydrodynamic model and mass transfer capabilities in accordance with the bioprocess specificity is the first level for the technological research in this field. After chousing the optimal variant (geometry and variation domain for the operation variables) not less important for an industrial reactor is the *scaling up* study of model bioreactors. Often the *scaling down* techniques must also be applied for achieving an optimal reactor design and operation. At the same time an adequate control strategy for the whole biosystem is to be parallel developed.

1.2.2. Bioreactors types

There are a number of ways in which turbulence can be produced by energy dissipation. From this point if view two principal bioreactor types are now used in the bioprocessing operation (Moo-Young, 1985):

- Stirrer reactor;
- Pneumatic reactor.

In the stirrer reactor energy is generated by the rotational or axial motion of mechanically moving agitators, kinetic energy being transferred to the liquid and then dissipated in it. Though the most used until now there are important reasons for developing other reactor types, the following ideas being of increase importance (Schugerl, 1991):

1. Construction of very large reactors (in this case the stirrer reactor requires high power level, high power and cooling water costs, introduces problems with heat removal).
2. Reduction in specific investment costs and in specific energy costs.
3. Reduction in substrate loss.
4. Increase in substrate conversion.

For all these reasons the use of airlift reactors, in which the energy is obtained from expansion of the compressed air, is now in progress.

For plant and animal cells cultivation other specific reactor types can be applied.

a) Stirrer reactor

For the time being the stirrer bioreactors are the most used fermentors in the bioindustry (Brauer, 1985). They can assure optimal conditions for both discontinuous and continuous bioprocesses (Schugerl, 1985) whether special problems due to shear forces don’t occur.

The stirrer reactors are characterized by remarkable flexibility, so that the same geometry can be used with minim adaptations for different aerobic bioprocesses, only by adequately chousing the operating parameters levels.

Mechanically stirred reactor consists of a cylindrical container with a height/diameter ratio of about 1-3 or more. They are often fitted with baffles to prevent vortexing on the medium. Generally, four baffles with the diameter of 0.1 from the vessel diameter are arranged symmetrically to the shaft with a minimum wall clearance of 0.02 from the vessel diameter. Heat is either added or removed by a jacket or helical coil. Mixing and gas dispersion are achieved by the stirrer positioned along the cylindrical axis of the container.
The main part of this reactor type is the stirrer, which must accomplish the following tasks (Brauer, 1985):

- The energy transfer to the liquid;
- The dispersion of gas into the liquid;
- The oxygen transfer from the gaseous phase to the liquid phase;
- The mixing of all the medium components.

Function of different specific bioprocesses, many types of stirrers can be chosen taking into consideration the needs imposed by the cultivation, but in accordance with the criterion of a minim energy use, too.

The sterilized air is introduced into the bioreactor through the air sparger, usually a perforated ring, conveniently placed under the stirrer (well-defined diameters ratio and distance between them).

The mixing efficiency means a turbulent liquid flow, characterized by high level of the Reynolds number ($N_{Re}>10^3$). At the same time the oxygen transfer rate (OTR), a very important performance parameter for aerobic bioprocesses, depends both on the stirrer speed and the airflow, so that this dependence is to be quantitatively expressed for using in a control structure, too.

b) Pneumatic reactors

In this type of reactor compressed air is expanded and dispersed through a gas distributor (sparger). As the density of the air is considerably lower than that of the medium, gas bubbles rise up and liquid is entrained with them. The power supplied by the compressed air disperses the air and mixes the medium at the same time (Schugerl, 1991)

Bubbles coalescence, which often occurs in such reactors, is not required in aerobic bioprocesses as the oxygen transfer is decreased. The energy dissipation rate should be kept as uniform as possible throughout the reactor to prevent coalescence. Fermentation media may promote coalescence, but at a lower level than pure liquids (water).

The most used pneumatic reactors are:

- Airlift reactors (Chisti, 1989, Schugerl, 1985, Blenke, 1985);
- Column reactors (Chisti, 1989);
- Tower reactors (Chisti, 1989).

In the airlift reactor the driving force for the liquid flow is provided by the density difference between the aerated liquid in the riser zone and the non-aerated liquid in the downcomer zone, this in turn being a function of difference in gas hold-up.

From all airlift bioreactors those with internal circulation are the most used. There are also bioreactors with external flow (Schugerl, Siltig, 1987), but in these cases the hydrodynamics and mass transfer are hard to design and optimize.

The airlift reactors with internal flow have many advantages in comparison with the stirrer reactors. Hence, they perform an adequate mixing and a fast mass transfer with less power consumption; the reduced level of shear forces by comparison of stirrer reactors is another advantage for applying them in case where the danger of damage the cells integrity is great enough. Moreover, lower construction investment and more operation robustness characterize the airlift reactors. But they are less flexible than the stirrer reactors, so that a specific design for each aerobic bioprocess is taken into consideration (Chisti, 1989).

Conforming to literature references (Kossen, 1984), in the aerobic bioindustry, the stirrer reactors are used for obtaining 93% from the biotechnological products value and the pneumatic reactors stand only for 7 %.

a) Bioreactors for plant and animal cells

There are two main groups of animal cells, those who can grow freely in the culture medium and those who grow only immobilized on micro-carriers; the difference between them in cultivation conditions is done by the specific physiology. As a general propriety, valid also for plant cells, all these cell types are very much affected by the shear stress and often by the gas bubbles presence.

Due to the special cultivation conditions, imposed by the animal and plant cells characteristics, the design of bioreactors for productive processes with these cells has

developed as a distinct field (Katinger, Scheirer, 1985).

At the mean time the usual variation domain for the cultivation parameters (stirrer speed, aeration flow, temperature, pH, dissolved oxygen concentration) is always more limited than that for the microorganisms, condition who demands a more precise parameters control.
For animal cells cultivation another gaseous substrate besides oxygen is needed-the carbon dioxide-and consequently there are more problems regarding the interfacial transfer of two gases diminished only by the slower dynamics of whole cellular metabolic reactions.

In accordance with these ideas the main types of bioreactors used for the time being for cultivating animal and plant cells are:

- Stirrer reactors;
- Pneumatic reactors;
- Vibromixer reactors;
- Hollow fiber reactors and other special types.

Stirrer bioreactors (Katinger, Scheirer, 1985, Brunswick, 1982, Cheng, Papontsakis, 1986, Bliew, Katinger, 1988, Prokop, Rosenberg, 1989) have a smaller aspect ratio than those for applications with microorganisms and use impellers which specific geometry, which produce low shear stress. Thow a turbulent flow may not be obtained, the stirrer speed is always reduced.

1.3. MEASURING SYSTEM FOR BIOPROCESS PARAMETERS

Bioprocess control needs knowledge about biosystem state and evolution.

There are mainly three points of view, for describing the measuring systems of bioprocess variables:

a) According to *the type of measured variable*: **physical** and **chemical** variables (i.e. biochemical/biological variables) (Aiba *et al*., 1973, Fiechter *et al*., 1987);
b) According to *the place of determination*: **in-line, on-line, off-line** (Linek *et al*., 1985);
c) According to *the type of determination*: **direct** and **indirect**, the indirect determined variables are obtained from other medium property measurements by simple data correlation. There are also estimated (calculated) variables obtained by using currently measured variables in conjunction with known equations (e.g. OTR – oxygen transfer rate – is estimated by using the dissolved O_2 concentration in a potential type equation).

The *off-line systems* correspond to laboratory analyses of the medium samples collected from the bioreactor at predetermined time periods.

The *in-line systems* are set up for those cases when it is not allowed to make measurements inside the reactor. Moreover, there are continuous measuring systems (or with a short sample period) which can be integrated in the general control structure (Anders *et al*., 1992).

Table 1. Measuring system classification

Measuring systems for physical variables	Measuring systems for chemical variables
Temperature	pH
Pressure	redox
Power consumption for mechanical stirrer	Dissolved oxygen
Foam	Dissolved carbon dioxide
Gas & liquid flow	O_2/CO_2 in the outflow gas
turbidity (OD)	Carbon source concentration (sugar)
Viscosity	Nitrogen source concentration
Liquid volume	ion concentration of Mg^2, K^+, Ca^{2+}, Na^+, Fe^{3+}, SO_4^{2-}, PO_4^3
	DNA, RNA
	NAD, NADH
	ATP, ADP, AMP

The *on-line systems* are the most recommended (Sonnleitner, Fiechter, 1992; Schugerl, 1992) as the process information can be transferred to the control system in real time.

Usually, the following parameters are used for evaluation and comparison of different measuring systems: *response time*, *sensibility*, *accuracy* and *stability* (Polakovic, Mandenius, 1994; Leung *et al*., 1991). Meanwhile, for the on-line systems, the sensors must be sterilized and thus, the measuring structures don't interfere with the biosystem.

Table 1 shows the description of the measuring instruments used in the bioprocess (cf. Aiba *et al*., 1973). Table 2 presents the Aiba's concept extension of *gateway sensors* – with their utilization into bioprocesses.

Table 2. Calculated variables types vs. sensor devices

Sensor device	Calculated variables
pH	Acid product formation
Dissolved oxygen concentration	OTR (Oxygen Transfer Rate)
Oxygen concentration in the outflow gas; gas flows	OUR (Oxygen Uptake Rate)
Carbon dioxide concentration in the outflow gas; gas flows	CPR (Carbon dioxide Production Rate)
OUR; CPR	Respiratory Quotient (RQ = OUR/CPR)
Carbon dioxide concentration and feed rate	Cell concentration and yield

1.3.1. Direct measurable variables

1.3.1.1. Direct physical determinations

The success of a fermentation depends upon the existence of defined and optimal environmental conditions for biomass and product formation. Different physical and chemical parameters require to be kept constant (or conforming to an optimal evolution trend) during the process, i.e. any deviation from a specified optimum might be corrected by a control system.

The standard direct physical determinations are showed below:

- Temperature;
- Pressure (over pressure);
- Agitator shaft power and rate of stirring;
- Foam;
- Gas and liquid flow;
- Weight.

1.3.1.1.1. Temperature (Brunswick, 1992)

Temperature determination is important in relation with bioprocess evolution as well as other process operations (i.e. sterilization, concentration, and purification). The temperature measurement is made through mercury-in-glass thermometers, bimetallic thermometers, pressure bulb thermometers, thermocouples, metal-resistance thermometers or thermistors.
The measurement accuracy is about ± 0.25 %

The temperature determination in different fermenter measurement points is useful to determine the global thermal balance.

1.3.1.1.2. Pressure (over pressure) (Brunswick, 1992)

Pressure measurements may be needed for several reasons, the most important of them is the safety. Industrial and laboratory equipment is designed to withstand a specified working pressure plus a factor of safety. Also, the measurement of pressure is important in media sterilization. Moreover, the pressure will influence the solubility of gases and contribute to the maintenance of sterility, when a positive pressure is present.

The standard measuring sensor is the Bourdon tube. When a vessel (or pipe) is to be operated under aseptic conditions, a diaphragm gauge can be used.

Alternatively, the pressure could be measured remotely using pressure bellows connected to the core of a variable transformer or a piezoelectric transducer.

1.3.1.1.3. Agitator shaft power and rate of stirring (Brunswick, 1992)

A variety of sensors can be used to measure the power consumption of a fermenter. On a large-scale vessel a wattmeter attached to the agitator motor will give a fairly good indication of power uptake. This measurement technique becomes less accurate as there is a decrease in scale to pilot scale and finally, to laboratory fermenters, the main contributing factor being friction in the stuffing box. Torsion dynamometers can be used in small-scale applications.

In all bioreactors it is important to monitor the rate of rotation (rpm) of the stirred shaft. The tachometer used for this purpose may employ electromagnetic induction, voltage generation, light sensing or magnetic force as detection mechanisms. The type of signal will determine the final choice of tachometer, which is required for recording and/or process control for regulating the motor speed. In most cases (for small laboratory fermenters) the standard practice is to use an a. c. slip motor that is coupled to a thyristor command.

1.3.1.1.4. Foam (Brunswick, 1992)

The formation of foam is a difficulty in many types of bioprocesses, which can create serious problems if not controlled. It is a common practice to add an antifoam agent to a fermenter when the culture starts foaming above a certain predetermined level. The methods used for foam sensing and antifoam additions will depend on process and economic considerations.

A standard foam sensing consists in a probe, which is inserted through the top plate of the fermenter. Normally, the probe is a stainless steel rod, which is set at a defined level above the broth surface. When the foam rises and touch the probe tip, a current is passed through the circuit of the probe (i.e. the foam acts as an electrolyte).

A number of mechanical antifoam devices have been made, including discs, propellers, brushes attached to the agitator shaft above the surface of the broth. Hence, the foam is broken down when it is thrown against the walls of the bioreactor.

Unfortunately, most of the mechanical devices have to be used in conjunction with an antifoam agent.

1.3.1.1.5. Flow measurement (for gases and liquids) (Brunswick, 1992)

One of the simplest methods for measuring gas flow introduced into a fermenter is by means of a variable aria meter, i.e. rotameter, which consists of a vertically mounted gas tube with an increasing bore and enclosing a free-moving float. The position of the float is indicative of the flow rate. The accuracy depends on having the gas at a constant pressure, but errors of up to ± 10% of full-scale deflection are quoted. The errors are greatest at low flow rates.

Ideally, rotameters should not be sterilized and are therefore normally placed between a gas inlet and a sterile filter.

The use of oxygen and carbon dioxide gas analyzers for effluent gas analysis requires the provision of a very accurate gas-flow measurement if the analyzers are to be used effectively. These instruments have a ± 1% full scale accuracy and work on the principle of measuring a temperature difference across a heating device placed in the path of the gas flow.

The measurements of flow rates of sterile liquids present a number of problems, which have to be overcome. On a laboratory scale flow rates may be measured manually using a sterile burette connected to the feed pipe and timing the exit of a measured volume. The use of rotameters is also available.

In batch and fed-batch culture fermenters a cheaper alternative is to measure flow rates indirectly by load cells (see below, the weight section). The fermenter is attached to load cells, which monitor the increases, and decreases in weight of the vessel at regular time intervals. Provided the specific gravities of the liquids are known it is possible to estimate flow rates in different feed pipes.

1.3.1.1.6. Weight (Brunswick, 1992)

A load cell offers a convenient method of finding the weight of an object.

A load cell is essentially an elastic body, usually a solid or tubular steel cylinder, its compressive strain under axial load being measured. The cell is calibrated by measuring compressive strain over the appropriate range of loading. A convenient method of measuring the strain is by means of electrical resistance strain gauges that are cemented to the surface of the cylinder. The changes of resistance with strain are proportional to load and are determined by appropriate electrical apparatus.

It is therefore possible to monitor feed rates from medium reservoirs, acid and bases utilization for pH control and the use of antifoam agent for foam control: the changes in weight in a known time interval can be used as a measure of liquid flow rates.

1.3.1.2. Direct chemical determinations

The regular chemical determinations are presented below:

- pH;
- Redox potential;
- Dissolved oxygen concentration (pO_2);
- Dissolved carbon dioxide concentration;
- Exit-gas analysis;
- On-line analysis of other chemical factors (ion-specific sensors, enzyme electrodes, microbial electrodes, mass spectrometers, fluorimeters).

1.3.1.2.1. pH (Brunswick, 1992)

In batch culture, the pH of an actively growing culture will not remain constant for very long. Hence, in most processes there is a need for pH measurement and control during the fermentation if maximum yield of a product is to be obtained. The pH may be further controlled by the addition of appropriate quantities of ammonia or sodium hydroxide if too acid, or sulfuric acid if the change is to an alkaline condition. Normally, the pH drift is only in one direction.

pH measurement is carried out using a combined glass reference electrode that will withstand repeated sterilization at temperature of 120°C and pressures of 138kN/m^2. The electrode is connected via leads to a pH-meter.

1.3.1.2.2. Redox potential (rH) (Brunswick, 1992)

The redox potential is a measure of the oxidation-reduction potential of a biological system and can be determined as a voltage, the value in any system depending on the equilibrium of:

$$Reduced\ form \Leftrightarrow Oxidized\ form + electron(s)$$

The measuring electrode consists of gold, platinum or iridium probe, which is welded to a copper lead. The interpretation of results presents difficulties since the microorganisms can be at different redox potential by comparison with the broth.

1.3.1.2.3. Dissolved oxygen concentration (pO_2) (Brunswick, 1992)

In most aerobic fermentations it is essential that the dissolved oxygen concentration does not fall below a specified minimal level.

In small fermenters (1 dm^3) the most used electrodes are galvanic and have a lead anode, silver cathode and employ potassium hydroxide, chloride, bicarbonate or acetate as electrolyte. These electrodes are suitable for monitoring very slow changes in oxygen concentrations, but they are very sensitive to temperature fluctuations. Moreover, the electrodes have a limited life because of corrosion of the anode.

Polarographic electrodes are more commonly used in pilot or production bioreactors. For an increase of precision, they are both pressure and temperature compensated.

1.3.1.2.4. Dissolved carbon dioxide concentration (Brunswick, 1992)

The measurement of dissolved carbon dioxide concentration is possible with an electrode, since a pH or voltage change can be detected because of the gas going into solution. The first available electrode consisted of a combined pH electrode with a bicarbonate buffer surrounding the bulb and ceramic plug. Unfortunately, this electrode was not steam sterilizable. A new version (which can be steam sterilized) involves a thin layer of aqueous bicarbonate over the glass sensing membrane of the pH electrode.

1.3.1.2.5. Exit-gas analysis (Brunswick, 1992)

The measurement and recording of the effluent gas composition is important in many fermentation studies. By observing the concentrations of carbon dioxide and oxygen in the entry and exit gases in the bioreactor, and knowing the gas flow rate it is possible to determine the oxygen uptake rate of the system, the carbon dioxide evolution rate, and the respiration rate of the microbial culture.

A paramagnetic gas analyzer can determine the oxygen concentration, taking into account the strong affinity of the oxygen for a magnetic field.

Carbon dioxide is commonly monitored by infrared analysis using a positive filtering method.

Generally, it is expensive to have separate carbon dioxide and oxygen analyzers for each fermenter. Therefore, it may be possible to couple up a group of fermenters via a multiplexer to a single pair of gas analyzers.

1.3.1.2.6. On-line analysis of other chemical factors (Brunswick, 1992)

Ion-specific sensors
Ion-specific sensors have been developed to measure NH_3^+, Ca^{2+}, K^+, $Mg^{2=}$, PO_4^{3-}, etc. However, none of these probes are steam sterilizable.

Enzyme electrodes
Enzyme electrodes may also be used in some analyses. Hence, a suitable enzyme, which produces a change in pH or determines oxygen formation in the enzyme reaction, is chosen. This enzyme is immobilized on a membrane held in close contact to a pH or oxygen electrode.

Microbial electrodes
Microbial electrodes using immobilized whole cells have been developed and used for determination of assimilable sugars, acetic acid, ethyl alcohol, vitamin B, nicotinic acid, glutamic acid and cephalosporins.

Mass spectrometers
The mass spectrometer can be used for on-line analysis since it is very versatile and has a response time less than 10 seconds for full-scale response, but is unfortunately expensive. It does allow for monitoring of gas partial pressures (O_2, CO_2, CH_4, etc.) dissolved gases (O_2, CO_2, CH_4, etc.) concentrations and volatiles (methanol, ethanol, acetone, etc.).

Fluorimeters
It is well established that fluorimetric measurements are very specific and rapid but their use in bioprocess studies is quasi-limited for the time being. Hence, the measurement of NAD (provided it remains at a constant concentration in cells) would be an ideal method for continuous measurement of microbial biomass concentration.

1.3.2. Indirectly measurable variables

The indirect measurements allow variable values estimation at each sample period, *t*. Usually, the corresponding errors exceed the errors resulted from direct measurements (i.e. the statistical error between the direct/indirect method till to 40%) (Montague *et al.*, 1988).

Commonly, the bioprocess dynamics can be depicted by the equation (Staniskis, Simutis, 1988):

$$\frac{dx}{dt} = f(x(t), u(t), p_1(t), t) + v(t) \qquad \textbf{1.3.2.1}$$

and the measure:

$$z(t) = h(x(t), u(t), p_2(t), t) + w(t) \qquad \textbf{1.3.2.2}$$

where: $x^T = (x_1,.....,x_L)$ = state variables vector (biomass concentration X, substrate concentration S, product concentration P, etc.);

$u^T = (u_1,.....,u_k)$ = control variables vector (dilution rate D, pH, temperature T, etc.);

$p_1(t)$, $p_2(t)$ = bioprocess model vector and measuring system vector;

f, h = process model vector and measure model vector (non-linear operators) dim f = L; dim h = p;

v, w = noise;

z = measure vector.

The indirect measurements are generally performed by the application of discrete methods. Hence, if it is supposed (Montague *et al.*, 1988) that the direct measurement of process variables is done at each moment K and the indirect measurement performed at each moment k, from (1.3.2.1), (1.3.2.2) it is obtained:

$$Z(K) = \begin{bmatrix} x_1 \\ x_2 \\ \dots\dots\dots\dots \\ x_L \\ h_1(x(K),u(K),p_2(K),K) \\ \dots\dots\dots\dots \\ h_{p-L}(x(K),u(K),p_2(K),K) \end{bmatrix} + \begin{bmatrix} v_1 \\ v_2 \\ \dots \\ v_L \\ v_{L+1} \\ \dots \\ v_p \end{bmatrix}$$

1.3.2.3

where: t = m*Δt, m*2Δt, the information is obtained both from direct and indirect measurements;

m = ΔT/Δt and m Δt < t < m 2Δt.

Moreover:

$$x(k+1) = \Phi(x(K),u(K),p_1(K),K,\Delta t) + w(K)$$

$$Z(K) = \begin{bmatrix} h_1(x(K),u(K),p_2(K),K) \\ \dots\dots\dots\dots \\ h_{p-L}(x(K),u(K),p_2(K),K) \end{bmatrix} + \begin{bmatrix} v_1 \\ \dots \\ v_{p-L} \end{bmatrix}$$

1.3.2.4

The measurement algorithm for discrete filtration can take the estimation of $\hat{x}$(K), if the equation (1.3.2.3), (1.3.2.4) can be determined. Commonly, this item is not reached due to the following factors:

- There is always a time dependent evolution of the bioprocesses (i.e. the parameter vectors change theirs values during the process behavior;
- The equations (1.3.2.3), (1.3.2.4 don't describe all process phenomena, but they are only a simplified representation.

Hence, for measuring noises rejection, an adaptive measuring system can be taken into consideration, cf. the following picture:

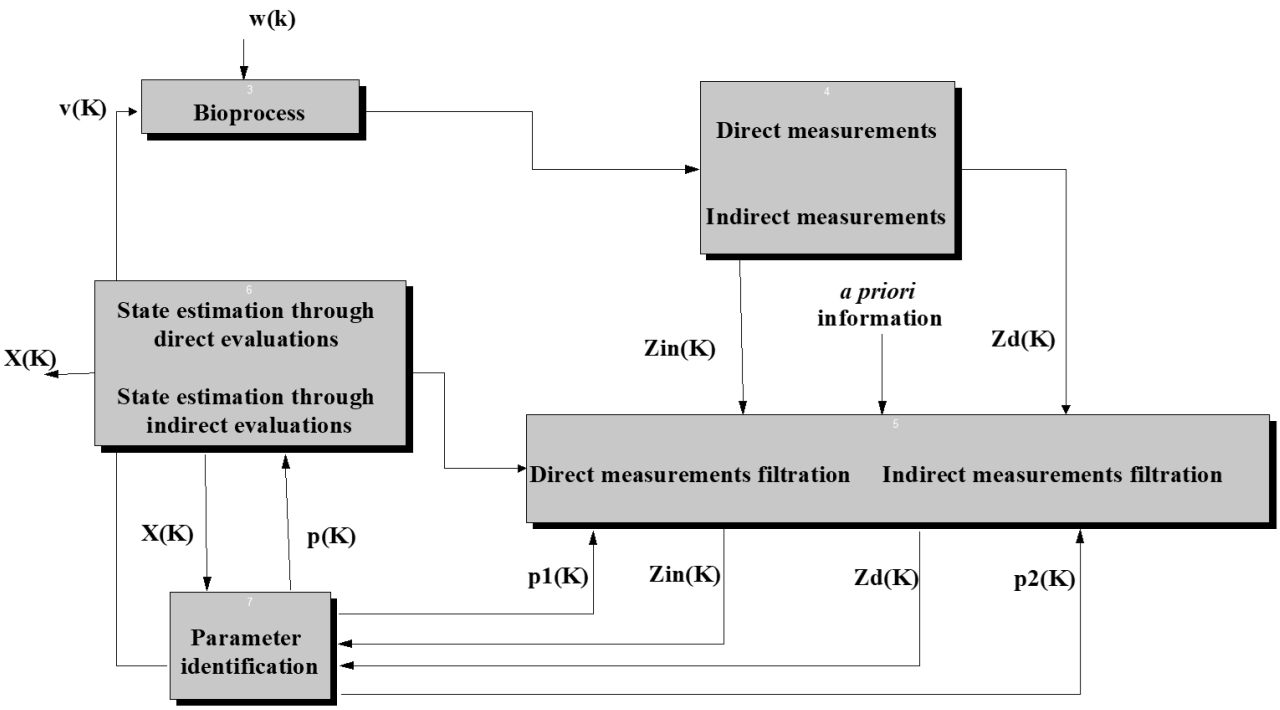

Fig. 1.3.2.1 General adaptive structure for bioprocess measurements (cf. Stanskis, Simutis, 1988)

In this case it would be considered that the direct measuring errors are approximated by a gaussian white noise E{V} = 0 and the dispersion is $G^2_{\nu i} = (K\nu_i \cdot z_i)^2$. In this case, $K\nu_I$ is a coefficient which characterizes the medium errors of measurements. The experiments have already demonstrated that the $K\nu_I$ value is contained in the domain 0.001…0.5 and depends on measuring method and state variable. Commonly, the following parameters can be measured: oxygen uptake rate (r_{O2}), pH rate evolution (r_a), carbon dioxide production rate (r_{CO2}), heat production rate (r_Q). Different kinetic equations link these parameters using the process state variables.

Hence, a continuous bioprocess can be described by the following equation set:

$$\frac{dx}{dt} = D(X_0 - X) + \mu(X,S,P,u',p_1,t)X + W_1(t)$$
$$\frac{dS}{dt} = D(S_0 - S) + \eta(X,S,P,u',p_1,t)X + W_2(t)$$
$$\frac{dP}{dt} = D(P_0 - P) + \varepsilon(X,S,P,u',p_1,t)X + W_3(t)$$

1.3.2.5

where: X_0, S_0, P_0, X, S, P = biomass concentration, limiting substrate concentration and product concentration (input/output);

μ, η, ε = specific growth rate, specific consumption rate and specific product formation rate;

u' = $[T, pH, pO_2 ...]^T$ = the environmental process vector;

p_1 = model parameter vector;

D = dilution rate;

$W_1(t)$, $W_2(t)$, $W_3(t)$ = modeling noises.

Therefore, the indirect measurement system is described by the following equation set (where $\nu_1 \ldots \nu_4$ are modeling noises):

$$r_{O_2}(t) = K_1\mu(X,S,P,u',p_1,t)X(t) + K_2X(t) + K_3\varepsilon(X,S,P,u',p_1,t)X(t) + \nu_1(t)$$
$$r_{CO_2}(t) = K_{41}\eta(X,S,P,u',p_1,t)X(t) + K_5X(t) + K_6\varepsilon(X,S,P,u',p_1,t)X(t) + \nu_2(t)$$
$$r_{al}(t) = f(U) + K_7\mu(X,S,P,u',p_1,t)X(t) + K_8X(t) + K_9\varepsilon(X,S,P,u',p_1,t)X(t) + \nu_3(t)$$
$$OD(t) = K_{10}X(t) + \nu_4(t)$$

1.3.2.6

A Kalman procedure is imposed in these circumstances (i.e. on-line variable estimation based on 1.3.2.3 process model):

$$\hat{X}(K+1) = X(K+1/K)(K+1) \bullet$$
$$\bullet (Z(K+1) -$$
$$- h(\hat{X}(K+1/K), u(K+1), p_2(K), K+1))$$

1.3.2.7

The one-level prediction Kalman algorithm is depicted below:

$$\hat{X}(K+1/K) = \Phi(\hat{X}(K), u(K), p_1(K), K, \Delta T)$$

1.3.2.8

Moreover, the algorithm for *a priori* computation of covariance errors matrix (CEM) is:

$$P_x(K+1/K) = S_a \frac{\partial\Phi[\hat{X}(K), u'(K), p_1(K), K, \Delta T]}{\partial\hat{X}(K)} \cdot P_x(K) \cdot \frac{\partial\Phi^T[\hat{X}(K), u(K), p_1(K), K, \Delta T]}{\partial\hat{X}(K)} + Q(K)$$ **1.3.2.9**

and the one for CEM estimation:

$$P_x(K+1) = P_x(K+1/K) - P_x(K+1/K)\frac{\partial h^T[\hat{X}(K+1/K), u(K+1), p_2(K), K+1]}{\partial \hat{X}(K+1/K)} \cdot$$
$$\cdot [\frac{\partial h[\hat{X}(K+1/K), u(K+1), p_2(K), K+1]}{\partial \hat{X}(K+1/K)} \cdot P_x(K+1/K) \cdot$$
$$\cdot \frac{\partial h^T[\hat{X}(K+1/K), u(K+1), p_2(K), K+1]}{\partial \hat{X}(K+1/K)} + S(K+1)]^{-1} \cdot$$
$$\cdot \frac{\partial h[\hat{X}(K+1/K), u(K+1), p_2(K), K+1]}{\partial \hat{X}(K+1/K)} \cdot P_x(K+1/K)$$

1.3.2.10

Furthermore: =

$$P_x(K+1) = \frac{\partial h^T[\hat{X}(K+1/K), u(K+1), p_2(K), K+1]}{\partial \hat{X}(K+1/K)} \cdot S^{-1}(K+1)$$

1.3.2.11

where: S_a = the ageing factor;

S(K), Q(K) = the corresponding matrices of measuring noise covariance and modeling noise covariance.

The initial conditions X(0) and $P_X(0)$ are the best (initial) estimations of the state variable vector and the uncertainty degree of X(0) selection, respectively. The parameters p_1 and p_2 are time dependent. Hence, the state variables can be estimated. A commonly used procedure is the Least Squares Method. So, the equation 1.2.3.4 is developed in Taylor series:

$$X_1(K+L) = \Phi_1(p_1(K)) + W(K)$$
$$Z_1(K) = h_1(p_2(K)) + V(K)$$

1.3.2.12

and the estimation of $p_1(K)$ is obtained with recursive LSM:

$$p_1(K) = \hat{p}_1(K-1) +$$
$$+ \mathrm{K}(X_1(K-1) - \Phi_1(K)\hat{p}_1(K-1))$$
$$\mathrm{K}(K) = P(K-1)\Phi_1^T(K)X_1(K)P(K-1)\Phi_1^T(K) +$$
$$+ \frac{1}{S_0^2}$$
$$P(K) = S_0^2(I - \mathrm{K}(K)\Phi_1(K)P(K-1))$$

1.3.2.13

A similar algorithm can be used for p_2 estimation. Consequently, $p_1(0)$ and $p_2(0)$ can be determined following the off-line identification, and P(0) will define the preliminary error identification of $p_1(0)$ and $p_2(0)$. A second method makes possible the simultaneous estimation of process state and model parameters. Hence, we suppose that $p_1(K)$ and $p_2(K)$ are fixed within a sample period; the variable state vector will be:

$$X_c(K+1) = \begin{bmatrix} X(K+1) \\ p_1(K+1) \\ p_2(K+1) \end{bmatrix} = \begin{bmatrix} \Phi[X(K), u(K), K, \Delta T] \\ p_1(K) \\ p_2(K) \end{bmatrix} + \begin{bmatrix} W(K) \\ W_{p_1}(K) \\ W_{p_2}(K) \end{bmatrix}$$

1.3.2.14

and the algorithm (1.3.2.7) ÷ (1.3.2.11) can be applied in this case, too.

CONCLUSIONS

1. General bioprocess presentation make evident the living systems specificity, i.e. non-linearity, multi-variability and powerful interdependence between process variables. In these conditions, different cultivation procedures impose specific control optimizations being able to optimize the ensemble bioprocess – bioreactor.
2. The instrumentation development for the measuring of bioprocess variables is a basic condition for future control strategy development. The actual limitations of measuring systems are:
    - The performant measuring instruments are generally too expensive and are not included in the standard bioreactor equipment.
    - Generally, only the environmental cell medium is monitored and so, the intermediate level for metabolic reaction rates is not known.
    - The enzymatic activity determination is accomplished off-line.

    Consequently, the bioprocess control structure will progress depending on measuring systems development. The control based on analytical (*a priori*) model is limited due to the scarcity of the corresponding measuring systems, able to supervise the intracellular phenomena and to monitor the interactions between bioprocess variables. To surpass these difficulties the implementation of intelligent techniques represents a more applicable solution, even if only the advanced phenomena cognition can establish the right solution for the optimal bioprocess control.