INSTRUMENTATION AND CONTROL OF BIOPROCESSES

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Summary

Optimal cell growth and metabolite production, as well as biocatalyst activity, is achieved only through a narrow range of environmental conditions. Accordingly, in order to develop, optimize and assure the most efficient biological reactor operation, it is crucial that the state of the cell or enzyme environment be monitored and controlled. Furthermore, cell response to the environment must also be determined. For such

purposes, three different functions should be dealt with: analysis of experimental data, (see also Chemical methods of analysis; Physical methods of analysis), which is not covered here, measurement, and control. The bioreactor (sometimes called fermenter) is the main part of any biochemical process in which microbial, mammalian or plant cell systems are employed for the manufacture of a wide range of useful biological products. This review focuses on its control and instrumentation.

1. Introduction

A well-stirred liquid tank, one of the most commonly employed bioreactors, approximates reasonably well to the idealized state of perfect mixing. It ensures a very short mixing time and a high gas-liquid mass transfer on a small scale. These particular culture conditions are mostly achieved in so-called high performance bioreactors. Modern commercially available laboratory bioreactors (volume less than 20 L) normally suffice as high performance bioreactors. The latter, minimizes the influence of bioreactor dynamics on overall process performance (through careful control of environmental conditions). This is very important in microbial physiological studies under conditions closely approaching those found in industrial reactors to be able to distinguish between the dynamics of cell reactions and the dynamics of the reaction vessel. In such bioreactors cells are subjected to an unchanging environment when circulated throughout the liquid medium. Hence, the response to environmental variations imposed is only a consequence of the microbial behavior. Moreover, under such conditions, the properties of the broth are the same everywhere, consequently only one point of measurement is needed to establish any given culture condition, for example, temperature.

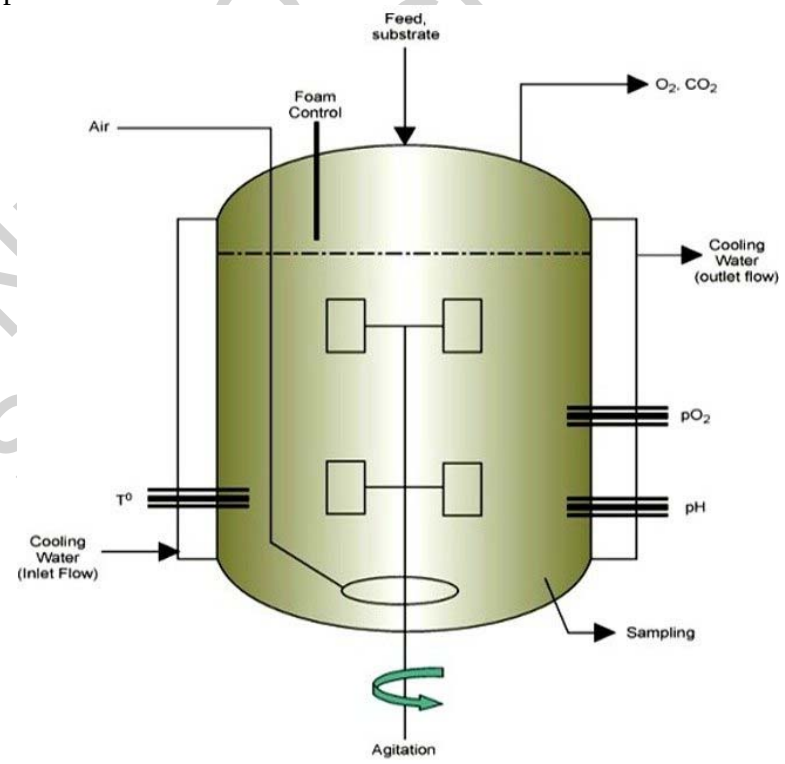

Figure 1: A standard stirred tank bioreactor

A standard stirred-tank bioreactor is normally supplied with a means of measuring temperature, agitator speed, pH, the incoming air-flow rate and dissolved oxygen concentration (Figure 1). This basic instrumentation can be complemented with several other sensors capable of determining the pressure drop, dissolved CO_2 concentration, biomass, off-gas analysis, foam control, power and torque, and liquid flow feeding. Most of these measurements are followed continuously, on-line.

High performance bioreactors are equipped with numerous *in situ* sensors. For controlling operating variables, a flexible direct digital control (DDC) system is used rather than classical single-purpose controllers. With a precise control of many operating variables ensured, these variables can therefore be assumed to be culture parameters. Due to tight control of the culture parameters and the near ideal homogeneity of the medium, it is possible to perform highly reproducible experiments, which is of paramount importance for fundamental understanding of the underlying cellular reactions that determine overall process performance. Along with sensors for standard culture parameters, modern high performance bioreactors are normally equipped with *in situ* advanced sensors, for example, fluorescence sensors, and on-line analyzers, like on-line Flow Injection Analysis of nutrients.

Many culture variables cannot be measured on-line however, or the effort at implementing the required methodology for on-line analysis is too great compared with any advantage it may afford. Off-line analysis is therefore still very important in physiological studies. This holds especially true for measurement of biotic variables, such as measurement of intracellular metabolites, yet off-line analysis is also preferred for a number of components of the medium.

2. Common Instruments for Process Automation

The following section briefly discusses instrumentation commonly used in the monitoring and control of bioreactors.

2.1. Temperature

Thermocouples are the most widely used temperature sensors since they are cheap, provide rapid response, and may be used to measure both high and low temperatures. They are less accurate (± 0.5 ºC to ± 2.2 ºC) than other sensors however, and must be calibrated periodically. Thermocouples consist of two wires of different metals that are welded at one end. Connecting the free terminals to a transducer closes the electric circuit. If a temperature difference exists between the free terminals and the welded end, an electric current will flow through the circuit. These sensors are classified according to the metals used: J, iron-constantan; T, copper-constantan; K, chromel-alumel; etc. Temperature ranges and ambient conditions (reducing or oxidizing) would determine the specific type to use.

The Resistance Temperature Detector (RTD) is a popular device as it provides more precise temperature measurement (± 0.1 ºC) than thermocouples, and does not need periodic calibration. These devices are more expensive and are suitable over a narrower temperature range than thermocouples, though. Its operating principle is based on the

change in electric resistance metals experience with temperature. Platinum RTDs (Pt 100) are widely used.

2.2. Gas Flowrate

Differential pressure producers are frequently found in industrial applications given their reliability and easy of use. Their accuracy ranges from ± 0.8 percent to ± 5 percent. The basic principle is that any obstruction (concentric orifice, Venturi, Pitot tube, etc.) in the fluid stream generates a pressure loss that is related to the flowrate of the stream. The main limitation of such devices is that the cost in energy from the loss of pressure caused can be considerable. Further, a differential pressure transducer is needed to be able to include the measurement in a digital control system.

Another commonly found air velocity measurement instrument is the turbine anemometer. Here, a rotating device is placed in the path of the fluid, where its rotational speed is proportional to the fluid velocity and provides accurate flow measurement over wide ranges. Thermal anemometers can also be used to measure air velocity efficiently, in which a thin wire immersed in the fluid is heated by an electric current. The velocity of the fluid is related to the heat dissipated from the wire. If anemometers are used for flow rate measurements, full calibration is required since the velocity given depends on the anemometer's transversal position within the duct.

Gas flow rate sensors providing analog output require periodic calibration.

2.3. Liquid Flowrate

There are many ways of measuring liquid flowrates ranging from those based on simple hydraulic properties to those based on radioactive effects. These instruments are usually sensitive to noise; hence interference from other electric devices must be controlled. Differential pressure producers (orifice plate, Venturi, Pitot tube) are low cost, but they are difficult to calibrate at high flow rates and need a transducer to generate an analog output.

Turbine meters are small electric turbines that generate an electromotive force (emf), which is proportional to the mean fluid velocity. These instruments are reliable, precise and can be used at different measurement ranges, although they are expensive and may only be used with clean liquids. More suited for dirty liquids are magnetic sensors, since they are non-invasive, but which are very expensive.

The instrument generates a magnetic field that is perpendicular to the liquid flow. If the liquid is a conductor, an emf is produced that is proportional to the fluid's velocity. Less expensive Doppler sensors, can also be used to measure flowrates of dirty liquids. Here, a continuous ultrasonic wave (0.5 to 10 MHz) emitted by the instrument is reflected by the bubbles and suspended solids in the liquid stream. This reflection is related to the liquid's flowrate. Radioactive sensors are based on the tracking of radioactive trace species. These are very precise instruments and applicable to any kind of fluid, but they are very expensive and dealing with radioactive species is difficult.

2.4. Off-Gas Analysis

Analysis of exhaust gases (CO2 and O2) provides information on the physiological state and respiration rate of the culture. On-line measurement of CO2 and O2 can be performed by gas chromatography (GC), or special purpose gas analyzers. The sampling air must be dried and the flowrate regulated before entering the instrument. The main advantages of GC are that many compounds can be monitored with the same instrument over a wide range of values.

Gas analyzers, on the other hand, are more precise and provide a faster response (few seconds). Several such instruments exist. Paramagnetic analyzers, available for CO2 and O2, are very precise, do not require periodic calibration, present low interference to other gases and have long lifetimes, but they are expensive.

Infrared instruments, available for CO2 only, are precise and have a long lifetime, though they are expensive and need occasional calibration. Electrochemical analyzers, only for O2, are low cost instruments that provide good precision, however a fuel cell must be changed periodically (between 6 months and 2 years).

2.5. pH

The sensing element consists of a pair of electrodes in contact with the liquid sample, and a temperature compensation device. One of the electrodes is used as a reference, while the other is sensitive to the pH of the sample. The pH sensitive electrode (usually made of glass) contains a buffer solution at a constant pH. The glass behaves as a membrane that separates the sample from the buffer solution; a potential proportional to the pH difference is generated.

The reference electrode is built to be ion insensitive, and is commonly made of an inert material containing a solution of Ag/AgCl or Hg/HgCl. pH electrodes ought to be cleaned frequently, depending on the nature of the sample; fats and oils are particularly harmful. Some devices are available (water jet, ultrasonic) for automatic cleaning.

If pH control is critical to bioreactor operation, measurement redundancy is recommended. Using three sensors would be wise because when one gives a very different reading from the other two it means that it needs cleaning.

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Web Sites for FIA/SIA freely available databases:

http://www.flowinjection.com

http://www.fia.unf.edu

Web Site with recent developments in NIR technology: http://www.OceanOptics.Com

Biographical Sketches

Ricardo Pérez-Correa was born in Santiago, Chile, on February 14, 1958. He received his B.Sc. and M.Sc. in chemical engineering at the Universidad de Chile, Santiago, Chile, in 1980 and 1982, respectively, and a Ph.D. in chemical engineering in 1987 from Imperial College, University of London. From 1987 until 1991 he was an assistant professor in the Chemical Engineering Department, Universidad de Chile, Santiago. Since 1991 he has been with the Chemical and Bioprocess Engineering Department, Pontificia Universidad Católica de Chile, Santiago. In 1999, Dr. Pérez-Correa was appointed associate professor in Chemical Engineering. His research interests are mainly in dynamic modeling, simulation and control of processes and bioprocesses.

Eduardo Agosin Trumper was born in October 2, 1955 in Santiago, Chile. He received his B.Sc. in Agricultural Sciences and M.Sc. in Food Science and Nutrition from the Catholic University of Louvain-La-Neuve, Belgium, in 1979. He graduated as a Doctor-Engineer in 1985 from the Center of Agroindustrial Biotechnology, Institut National Agronomique Paris-Grignon, Paris, France. From 1985 till 1990, he worked as an associate Professor at the Institute of Nutrition and Food Technology, INTA,

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