

INSTRUMENTATION AND CONTROL

Fermentation control systems - manual and automatic control in fermentation processes. Architecture of Fermentation systems, temperature measurement and control, flow measurement and control, pressure measurement and control, measurement of pH and dissolved oxygen and related sensors, Computer applications in fermentation technology.

OPERATION, INSTRUMENTATION AND PROCESS CONTROL

Introduction

The fact that the fermentor must be operated aseptically presents a new problem in design; the transfer of the inoculum to the main fermentor and the intermittent sampling of the broth during the fermentation require special precautions

Aseptic Operation

- Pipe Lines and Valves
- The pipelines transporting materials required for aseptic use should be sterilized with steam (usually at 120°C for 20 to 30 minutes).
- Pipelines should free from steam condensate after sterilization.
- Building materials: stainless steel (with few exceptions).
- Valves have to be installed, diaphragm types are preferred as they committed less contamination risk.
- For considerations of costs, the conventional stop, angle, and globe valves are frequently used in the fermentation industry.

Aseptic Inoculation and Sampling

- The spore suspension vessel and *its* piping is first sterilized and then spores induced into the vessel.
- A sterile air is used to blow the spore suspension from the vessel to the seed tank.
- It is frequently necessary to sample the broth during fermentation; sampling point, should be steam sterilized.
- The end of the sampling pipe is immersed, for example, in 40 % formaldehyde solution.

- When a sample is required, the vessel containing the germicide is removed and steam is blown through sampling pipes long enough to sterilize the section.
- Steam is allowed to bleed for sometime then the valve is opened to allow some broth bleed for cooling and dissipate the wasted remains in the pipes, a sample is taken, valves closed

Instrumentation and Process Control

- Controlling is required to optimize productivity and product yield, and ensure reproducibility.
- The key physical and chemical parameters depend on:
 - a. the bioreactor,
 - b. its mode of operation
 - c. microorganism being used.
- Parameters are aeration, mixing, temperature, pH and foam control.
- Control and maintenance at optimum levels is mediated by sensors (electrodes), along with compatible control systems and data logging.
- Internal sensors that are in or above the fermentation medium (pH, oxygen, foam, redox, medium analysis and pressure probes) should be steam sterilizable and robust.
- Some sensors do not come into direct contact with any internal component of the bioreactor and do not need sterilization; for example, load cells, agitator shaft power and speed meters, and external sensors used to analyze samples regularly withdrawn from the bioreactor.
- Samples can be taken off-line for various analyses, such as cell counts and determination of DNA, RNA; lipids, specific proteins, carbohydrates and other key metabolites and substrates.

- Control of pH is usually a major factor as many fermentations yield products that can alter the pH of the growth media.
- Fermentation media often contain buffering salts, usually phosphates, but their capacity to control pH can be exceeded and addition of acid or alkali may be required.
- The pH can be maintained at the desired value by their automatic addition (acid or alkaline) in response to changes recorded by the pH electrode.
- Temperature is monitored via resistance thermometers and thermistors linked to automatic heating or cooling systems.
- Levels of dissolved O₂ and CO₂ are determined using O₂ and CO₂ electrodes.
- Foam is controlled through three basic methods: media modification, mechanical foam-breaking devices or the automatic addition of chemical antifoam agents.
- Success of fermentation depends on defined environmental conditions for Biomass and product formation.
- temperature, pH, degree of agitation, oxygen concentration in the medium and other factors may have to be kept constant during the process.

There are three main classes of sensor:

Sensors which penetrate into the interior of the fermenter

e.g. pH electrodes, dissolved-oxygen electrodes.

Sensors which operate on samples which are continuously withdrawn from the fermenter

e.g. exhaust-gas analysers.

Sensors which do not come into contact with the fermentation broth or gases

e.g. tachometers, load cells.

1. *In-line sensor.* The sensor is an integrated part of the fermentation equipment and the measured value obtained from it is used directly for process control.

2. *On-line sensor.* Although the sensor is an integral part of the fermentation equipment, the measured value cannot be used directly for control. An operator must enter measured values into the control system if the data is to be used in process control.
3. *Off-line sensor.* The sensor is not part of the fermentation equipment. The measured value cannot be used directly for process control. An operator is needed for the actual measurement (e.g. medium analysis or dry weight sample) and for entering the measured values into the control system for process control.

METHODS OF MEASURING PROCESS VARIABLES

Temperature

- measured by mercury-in-glass thermometers, bimetallic thermometers, pressure bulb thermometers, thermocouples, metal resistance thermometers or thermistors. Metal-resistance thermometers and thermistors are used in most fermentation.

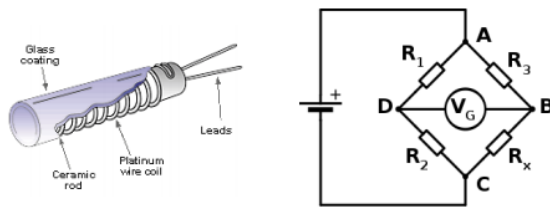
MERCURY-IN-GLASS THERMOMETERS

- A mercury-in-glass thermometer may be used directly in small bench fermenters, but its fragility restricts its use. In larger fermenters it would be necessary to insert it into a thermometer pocket in the vessel, which introduces a time lag in registering the vessel temperature. This type of thermometer can be used solely for indication, not for automatic recording.

ELECTRICAL RESISTANCE THERMOMETERS

- It is well known that the electrical resistance of metals changes with temperature variation.
- This property has been utilized in the design of resistance thermometers.
- The bulb of the instrument contains the resistance element, a mica framework (for very accurate measurement) or a ceramic framework around which sensing element is wound.
- A platinum wire of 100 Ω resistance is normally used. Leads emerging from the bulb are connected to the measuring element.

- This reading is normally obtained by the use of a Wheat stone bridge circuit and is a measure of the average temperature of the sensing element.
- This type of thermometer does have a greater accuracy ($\pm 0.25\%$) than some of the other measuring devices and is more sensitive to small temperature changes.
- There is a fast response to detectable changes (1 to 10 seconds), and there is no restriction on distance between the very compact sensing point (30 X 5 mm) and the display point of reproducible readings. These thermometers are normally enclosed in stainless-steel sheaths if they are to be used in large vessels and ancillary equipment



Control of pH

pH is a critical factor in most bioprocesses (5,6). As the impact on performance and in some cases impact on product quality has been described, an in situ monitoring and control is needed. In addition, a risk mitigation strategy is widely used by comparing in situ measurement with periodic off-line measurements. The most common form of pH sensor used for fermentation monitoring is based on the electrode design introduced by Ingold in 1947. The detailed design of a pH electrode and functional principles can be found elsewhere. A large variety of pH probes based on the electrochemical design are commercially available. pH probes can be autoclaved and cleaned in place. However, a reduction in lifetime and robustness needs to be taken into account. Steam sterilization can affect the glass membrane potential, thereby adversely affecting the robustness of these conventional pH probes (9). Also, optical pH sensor, which uses pH indicators immobilized on waveguides such as optical fibers, is available. Measurement of pH changes can be detected with a good precision by the change in absorption or in fluorescence. With the development of disposable bioreactors, the need for disposable sensors has also evolved. Several companies have developed disposable sensors for pH, DO, and dissolved CO₂

(DCO₂). In these sensors, a fluorescence dye sensitive to the analyte is immobilized in a matrix or patch, which can be miniaturized to a few millimeters. This enables monitoring and control with presterilized/ γ -irradiated systems, even of miniature system

- Sterilizable Electrodes are placed directly within the broth.
- The half-cell of the glass electrode was composed of Ag/AgCl saturated with solid KCl.
- The solid KCl increases the mechanical resistance of the glass electrode to external force by depositing, fine particles of KCl on the glass surface during heat sterilization and cooling of the electrode.
- The half cell of the reference electrode consisted of the same material as the glass electrode asbestos of porcelain cylinder being used as the junction material.
- For good insulation, both the glass and reference electrodes were mounted in Teflon jackets and silicone rubber.
- The internal resistance of the electrode was 300-500 mega-ohm.
- Both electrodes were protected by steel sleeves provided with several holes, to allow free passage of the broth.
- In the glass and reference calomel electrodes manufactured by Toa Electronics, Ltd., Tokyo, the glass membrane fused on the electrode surface contains a rare metal, such as Li or La to decrease the internal resistance 10 30 to 40 mega-ohm.

Dissolved Oxygen

- Sensors of Dissolved Oxygen (D.O.) with a Teflon Membrane
- D.O. in the fermentation broth could be measured by removing samples intermittently and determining the oxygen in solution.
- The introduction of Teflon membranes which can be sterilized with steam has improved D.O. sensors (voltametric types) considerable and now D.O. values can be determined continuously during the fermentation.