Computer Application in FT by Amit Kumar

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^{o}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 connections required for the aseptic transfer of a spore suspension to a seed tank

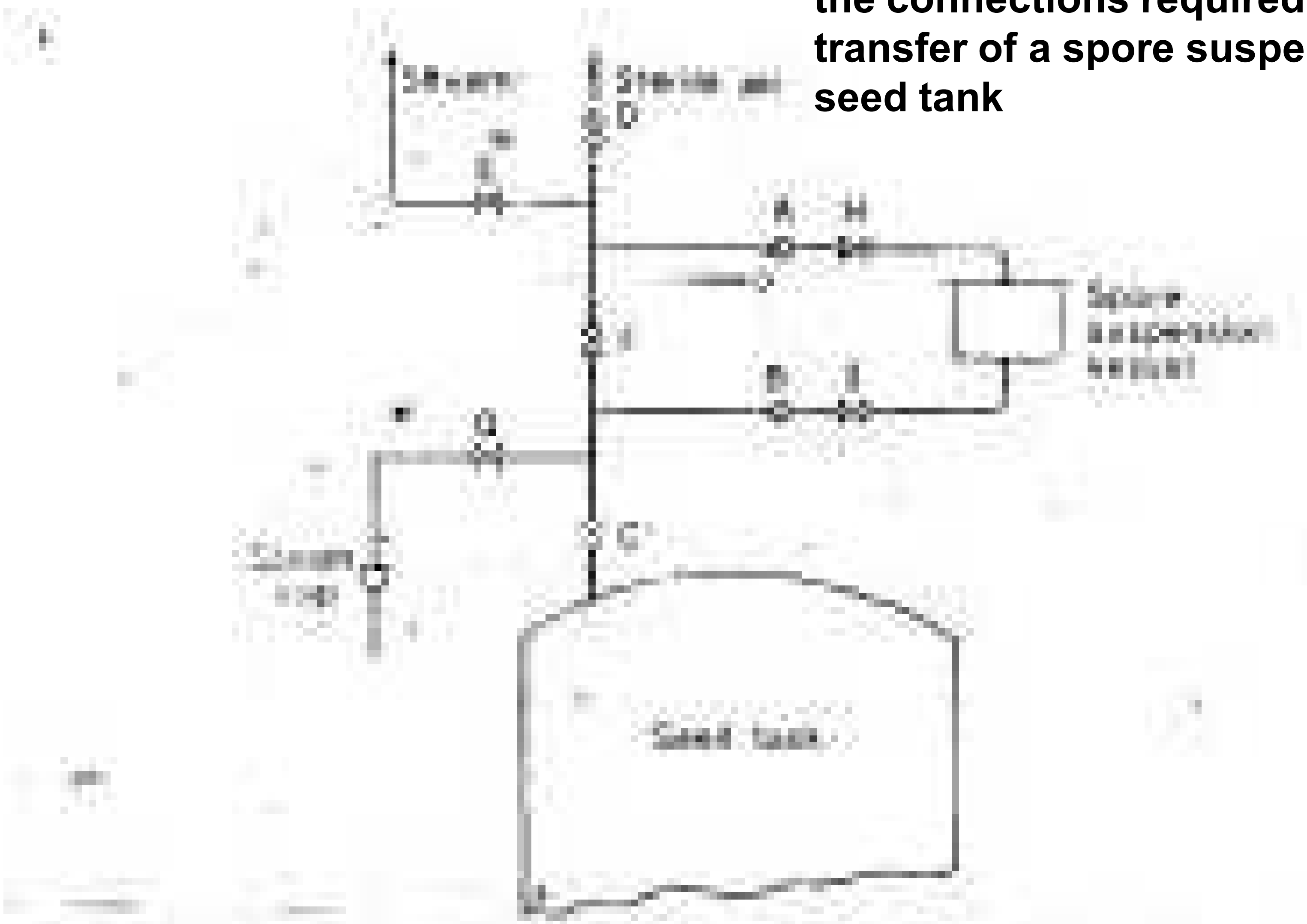
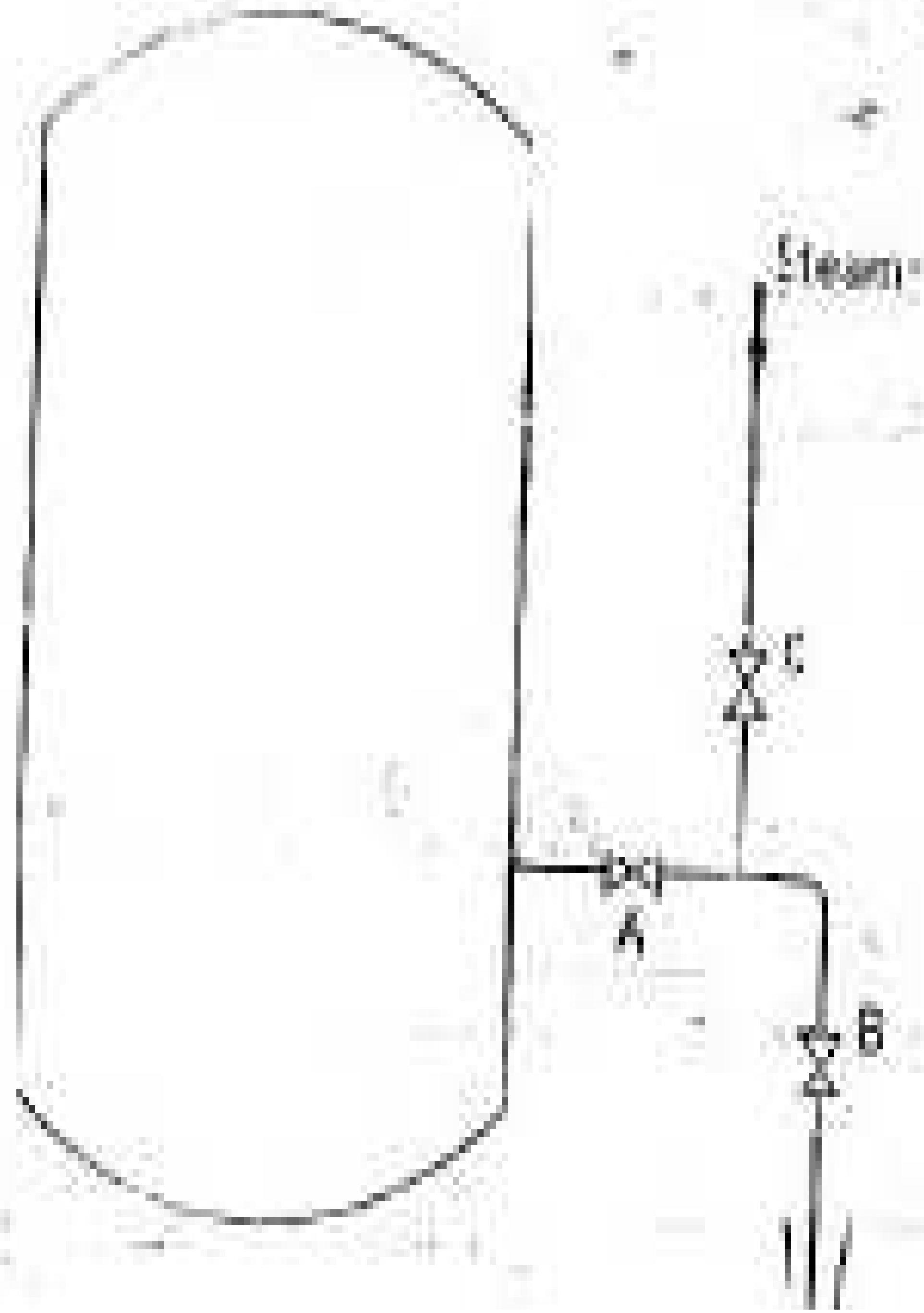


FIG. 10.3. The aseptic transfer of a spore suspension into a seed tank.[10]

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



Sampling Point

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.

Instrumentation and Process Control

- 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.

Instrumentation and Process Control

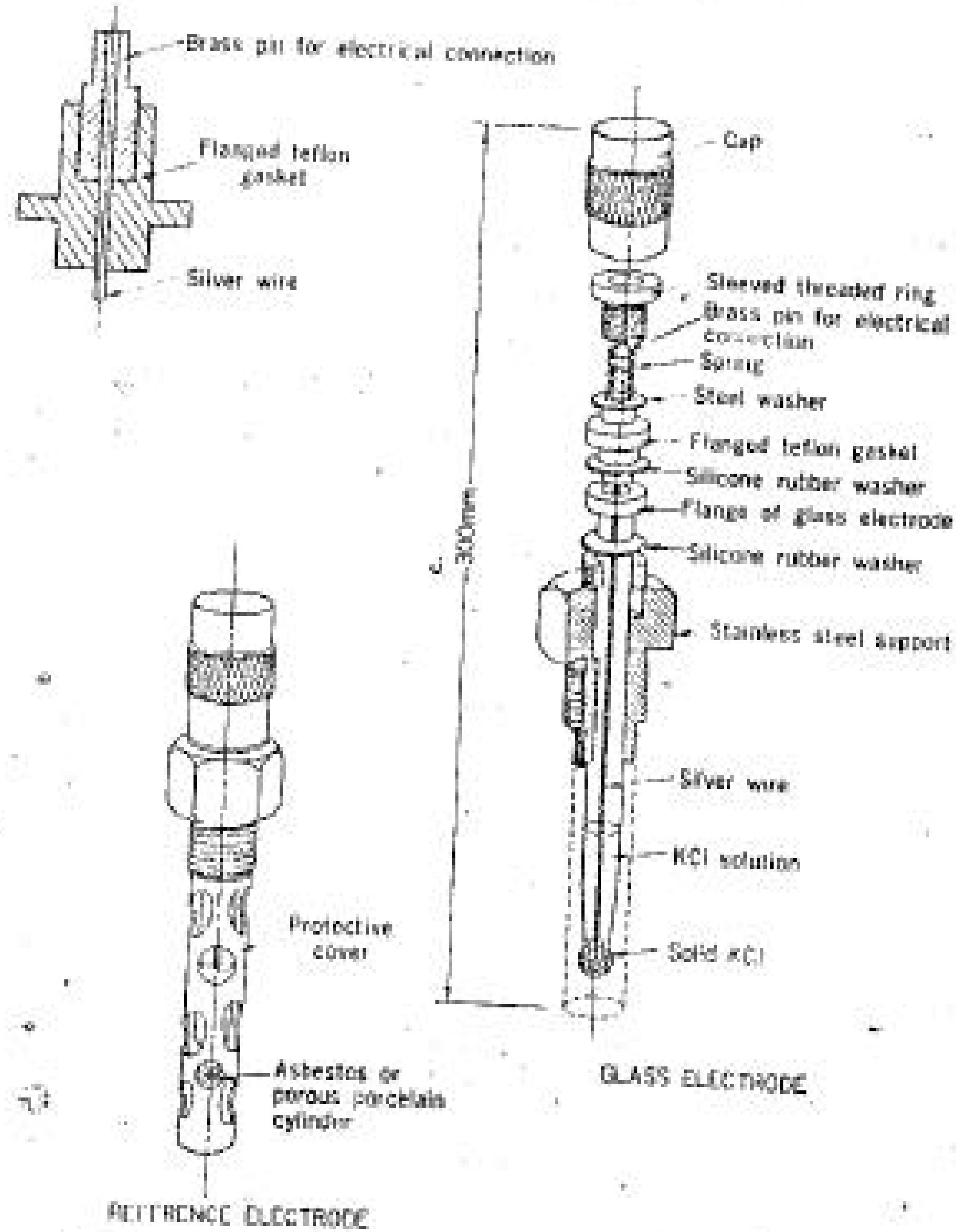
- The pH can be maintained at the desired value by their automatic addition (acid or alkaline) in response to changes recorded by the pH electrode.
- Temperature is monitored via resistance thermometers and thermistors linked to automatic heating or cooling systems.
- Levels of dissolved O2 and CO2 are 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.

Control of pH

- 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.

Control of pH

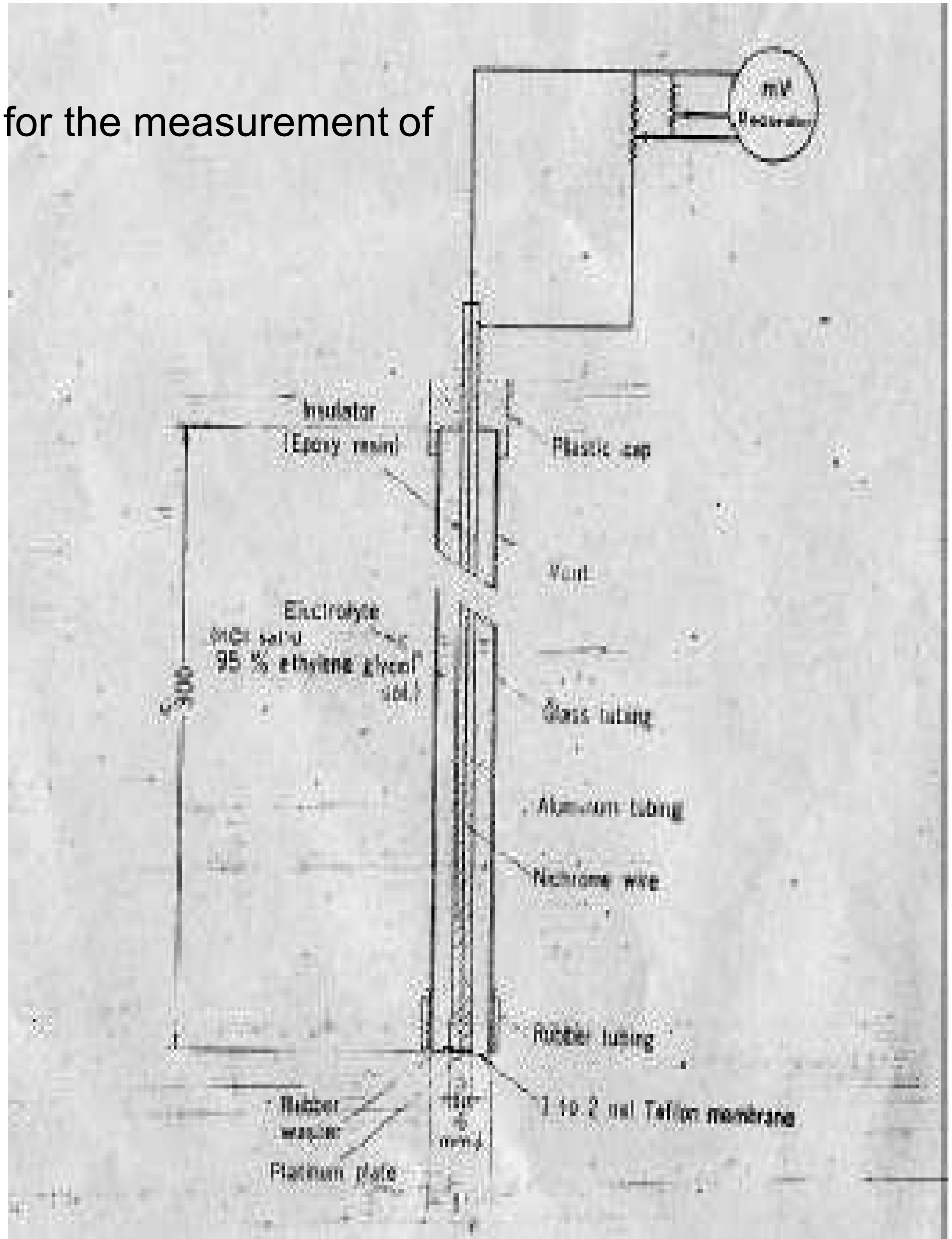
- Both electrodes were protected by steel sleeves provided with several holes, to allow free passage of the broth.
- In the glass and reference calomel electrodes manufactured by Toa Electronics, Ltd., Tokyo, the glass membrane fused on the electrode surface contains a rare metal, such as Li or La to decrease the internal resistance 10 30 to 40 mega-ohm.


Brass pin for electrical connection
Flanged teflon gasket
Silver wire
Cap
Sleeved threaded ring
Brass pin for electrical connection
Spring
Steel washer
Flanged teflon gasket
Silicone rubber washer
Flange of glass electrode
Silicone rubber washer
Stainless steel support
Silver wire
KCl solution
Solid KCl
c. 300mm
Protective cover
Asbestos or porous porcelain cylinder
GLASS ELECTRODE
REFERENCE ELECTRODE

Dissolved Oxygen

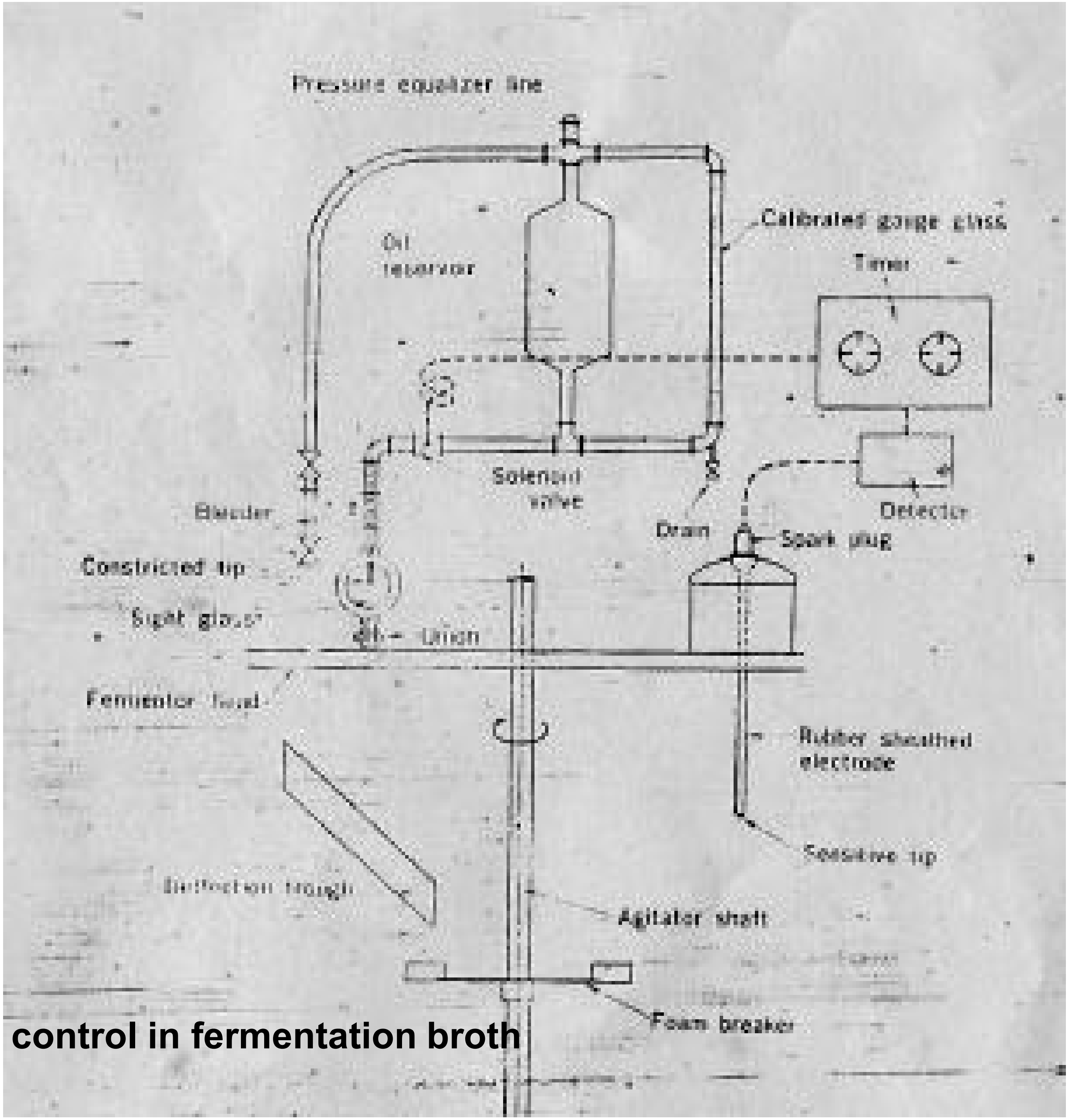
- Sensors of Dissolved Oxygen (D.O.) with a Teflon Membrane
- D.O. in the fermentation broth could be measured by removing samples intermittently and determining the oxygen in solution.
- The introduction of Teflon membranes which can be sterilized with steam has improved D.O. sensors (voltametric types)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

Voltametric probe for the measurement of dissolved oxygen



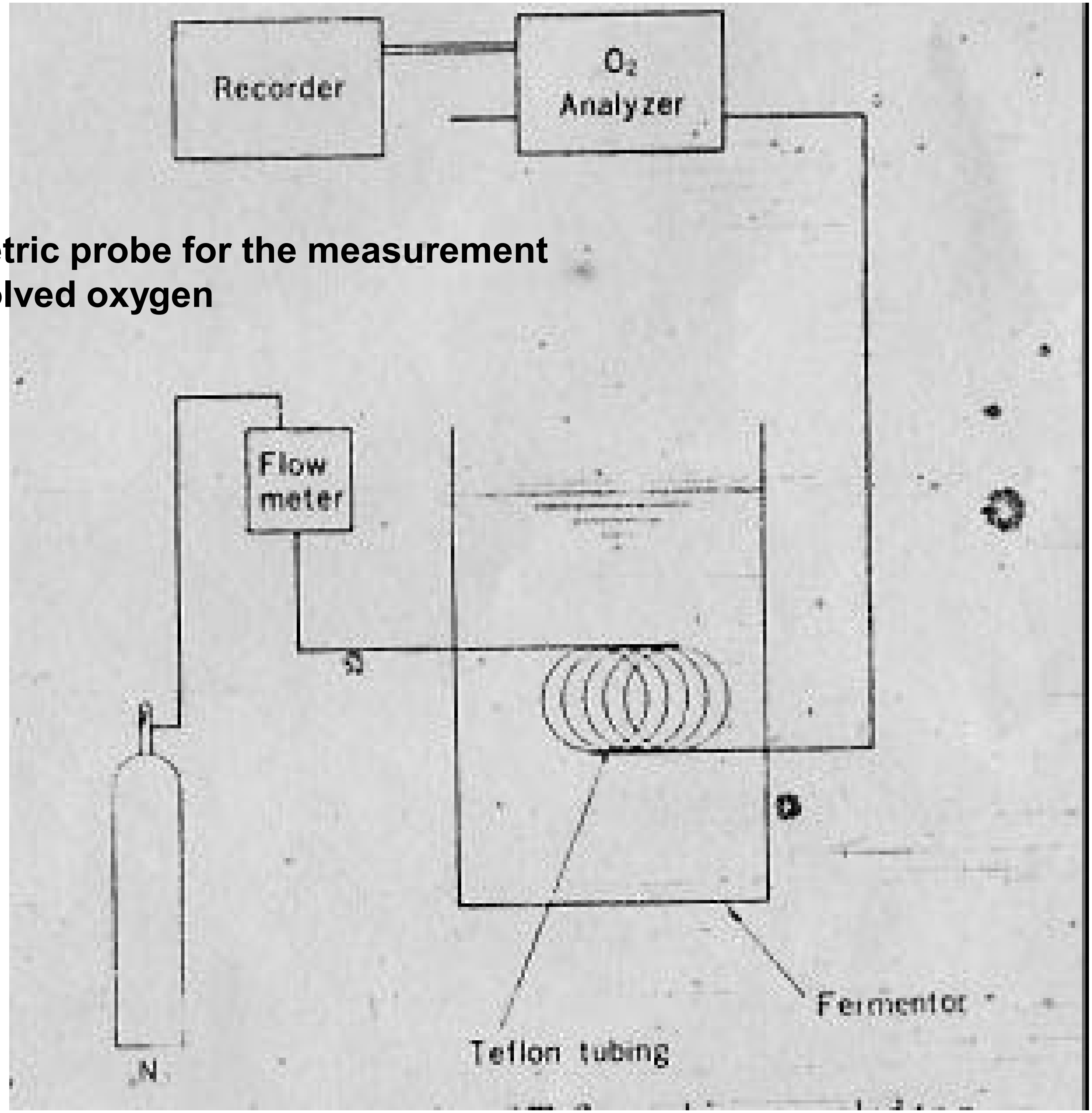
Foam Control

- A mechanical foam disintegrator attached to the agitator shaft often supplements the action of antifoam agents.
- In an antifoam system; if the foam contacts a rubber sheathed electrode, an electric unit actuates a solenoid valve to allow the passage of a sterile antifoam agent into the fermentor.
- A deflection trough is provided to ensure uniform distribution of antifoam agent.
- The action of antifoam agent is supplemented by the centrifugal effect of the foam breaker.
- The amount of antifoam entering fermentor is usually controlled by a timer in the circuit to the solenoid valve.



Foaming control in fermentation broth

Voltametric probe for the measurement of dissolved oxygen



On-line Analysis of Other Chemical Factors

- For good control of process, all chemical factors which can influence growth and product formation ought to be continuously monitored.
- This ideal situation has not yet been achieved but a number of techniques are currently being developed.
- **Ion-specific Sensors**
- They developed to measure NH4+ Ca2+, K+, Mg2+, PO3-, S02-, etc.
- Response time of these electrodes ranges from 10 seconds to several minutes depending on the concentration of the ion species, the composition of the sample.
- However, none of these probes is steam sterilizable.

Enzyme and Microbial Electrodes

- Enzyme or microbial cell electrodes can be used in some analyses.
- A suitable enzyme or microbial cell, which produces a change in pH or forms oxygen in the enzyme reaction is chosen and immobilized on a membrane, held in close contact to a pH or oxygen electrode.
- Unfortunately, the oxygen demand of the enzyme may restrict the maximum substrate concentration, which might be detected in a medium.
- Enfors (1981) –overcame this problem of glucose determination by co-immobilizing glucose oxidase and catalase.

Enzyme and Microbial Electrodes

- It has been possible to use a ferrocene derivative as an artificial redox carrier to shuttle electrons from glucose oxidase to a carbon electrode, thus making the device largely independent of oxygen concentrations.
- Enzyme electrodes are also commercially available to monitor cholesterol, triglycerides, lactate, acetate, oxalate, methanol, ethanol, creatine, ammonia, urea, amino acids, car-bohydrates and penicillin.
- sterilized penicillinase electrodes was prepared by assembling sterile components or by standing assembled components in chloroform before placing in a fermenter.

Near infra-red Spectroscopy

- Hammond and Brookes (1992) developed near infra-red spectroscopy (NIR;460-1200nm) for rapid, continuous and batch analysis of components of fermentation broths.
- They used NIR absorbance bands to simultaneously estimate fat (in the medium), techoic acid (biomass) and antibiotic (the product) in antibiotic production process.
- Fat analysis has been made possible with a fiber optic sensor placed in situ through a port in the fermenter wall.
- The assay time for an antibiotic has been reduced from 2 hours to 2 minutes.
- The method has also been developed to measure alkaline protease production in broths,
- It has been used to measure glucose, lactic acid and biomass in a lactic acid fermentation.

Mass Spectrometers

- It can be used for on-line analysis, it has a response time of less than 5 seconds for full-scale response and taking about 12 seconds for a sample stream.
- It allows for monitoring of gas partial pressures (O2 , CO2, CH4. etc.) dissolved gases (O2, CO2, CH4, etc.) and volatiles (methanol, ethanol, acetone, simple organic acids, etc.).

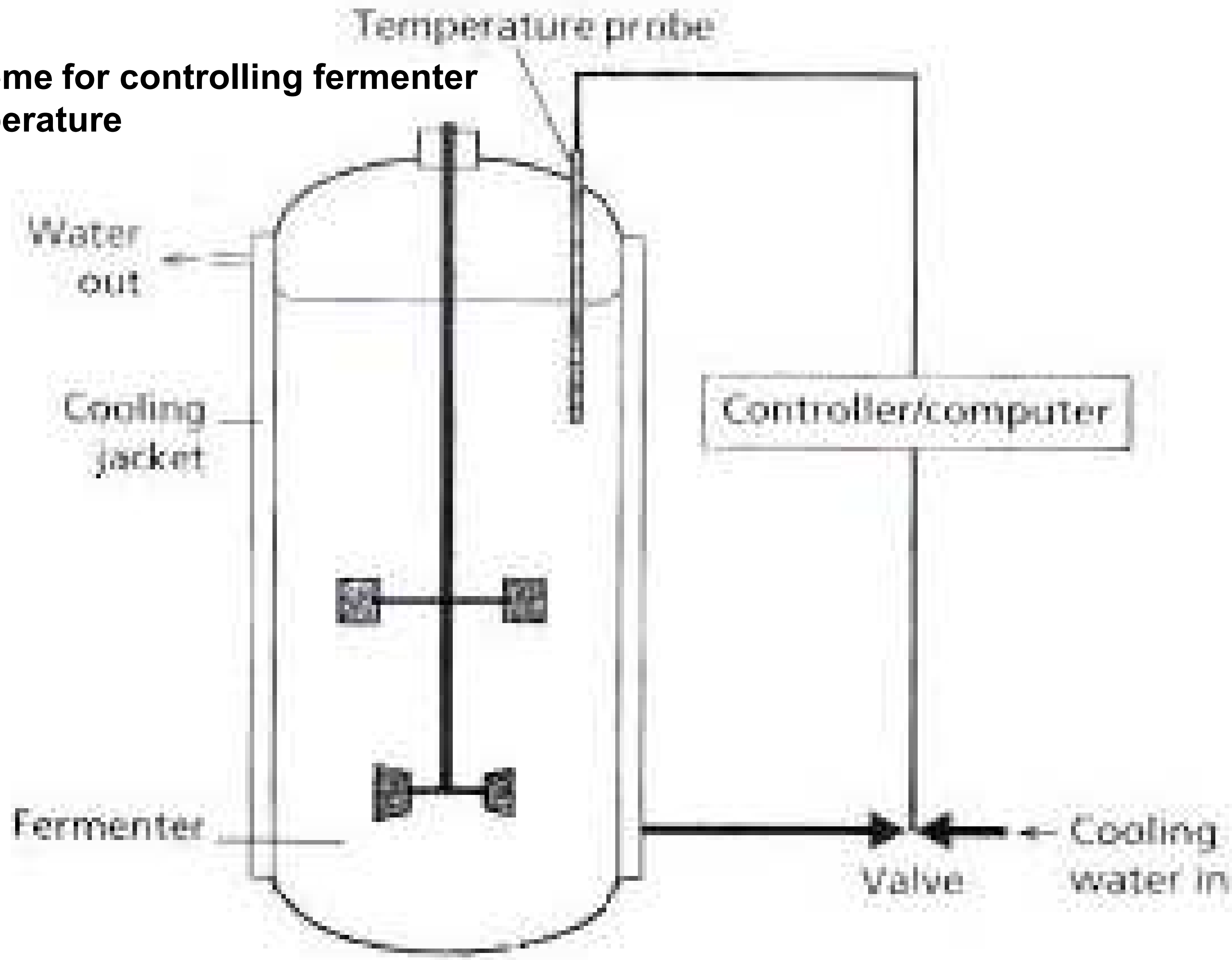
Control Systems

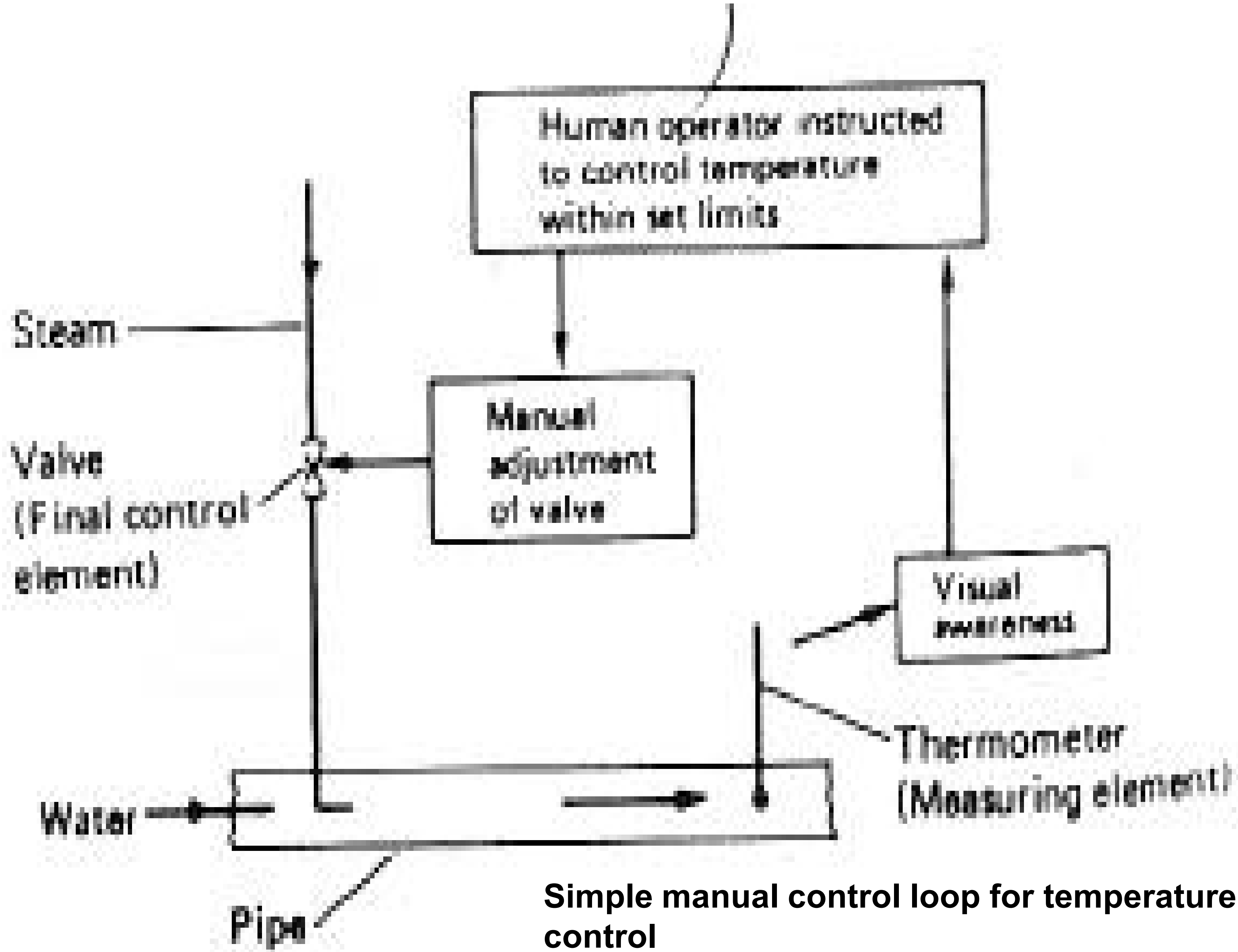
- The basic principle of control involves a sensory sys-tem linked to a control system and feedback loop.
- Sensors are used to measure and record the events within the bioreactor.
- In conjunction with process control, they maintain the difference between the measured and desired values at a minimum level.
- Overall control can be manual or automated; newer systems have integral and derivative control systems.
- Data recorded from the sensors and control decisions are downloaded to a computer where appropriate calculations can be per-formed to determine production of biomass and product, overall oxygen and carbon dioxide transfer rates, nutrient utilisation, power usage, etc.

Control Systems

- All of this information may be used to construct a mathematical computer model of the process.
- If a deviation is discovered, appropriate alarm and correctional systems are activated, giving greater control of the fermentation.
- Any control system needs to be calibrated when first installed and then regularly checked to conform to good manufacturing practices (GMP).

Scheme for controlling fermenter temperature



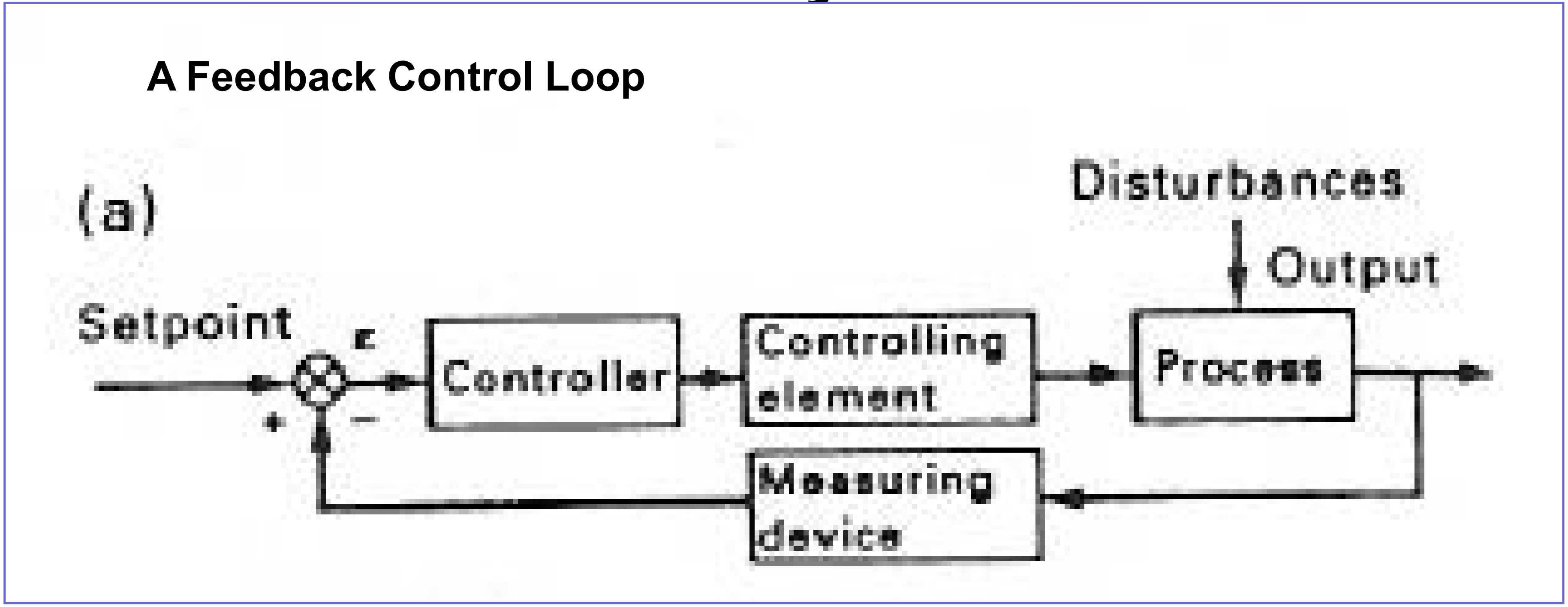


Simple manual control loop for temperature control

Control Systems

- The process parameters may be controlled using control loops.
- A control loop consists of four basic components:
- 1. A measuring element.
- 2. A controller.
- 3. A final control element.
- 4. The process to be controlled.

Control Systems



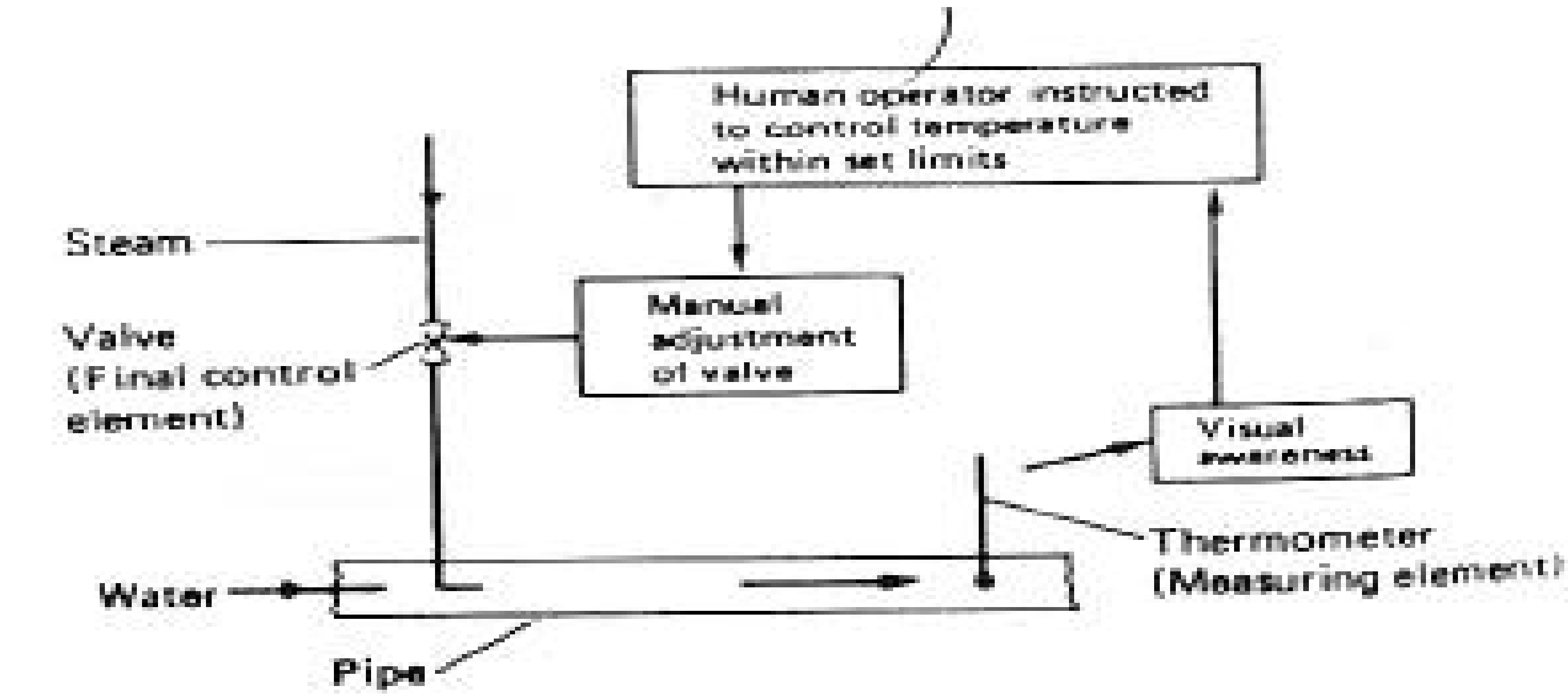
- **In the simplest type of control loop (feedback control):**
- The measuring element senses a process property (flow, pressure, temperature, etc) then generates corresponding output signal.
- The controller compares the measurement signal with the set point and produces an output signal to counteract any differences between the two.

Control Systems

- The final control element receives the control signal and adjusts the process by changing a valve
- opening or pump speed and causing the controlled process
- property to return to the set point.

Manual Control

- A Simple example of control is manual control of a steam valve to regulate the temperature of water flowing through a pipe.
- Labor, cost and should be kept minimum and use automated control as much as possible.

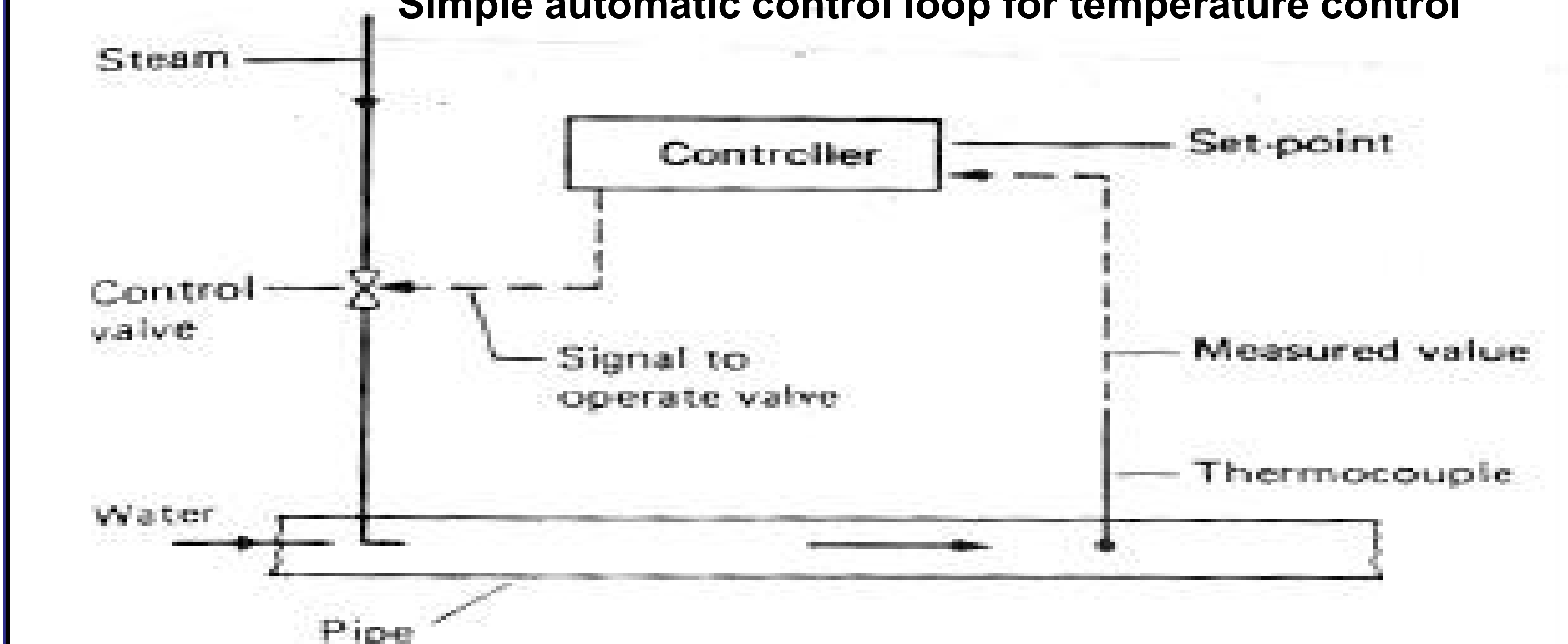


Simple manual control loop for temperature control

Automatic Control

- When an automatic control loop is used, certain modifications are necessary.
- The measuring element must generate an output signal which can be monitored by an instrument.
- E.g. the thermometer is replaced by a thermocouple, which is connected to a controller which in turn will produce a signal to operate the steam valve.

Simple automatic control loop for temperature control



Computer Applications in Fermentation Technology

- Three distinct areas of computer function were recognized:
- **1. Logging of process data.**
- Data logging is per-formed by the data acquisition system which has both hardware and software components.
- There is an interface between the sensors and the computer. The software should include the computer program for sequential scanning of the sensor signals and the procedure of data storage.
- **2. Data analysis (Reduction of logged data).**
- Data reduction is performed by the data-analysis sys-tem, which is a computer program based on a series of selected mathematical equations.
- This analyzed information may then be put on a print out, fed into a data bank or utilized for process control.

Computer Applications in Fermentation Technology

- **3. Process control.** Process control is also performed using a computer program.
- Signals from the computer are fed to pumps, valves or switches via the interface.
- In addition the computer program may contain instructions to display devices or teletypes, to indicate alarms, etc.
- There are two distinct fundamental approaches to computer control of fermenters.
- 1) Fermenter is under the direct control of the computer software. This is termed Direct Digital Control (DDC).
- 2) The use of independent controllers to manage all control functions of a fermenter and the computer communicates with the controller only to exchange information.
- This is termed Supervisory Set-Point Control (SSC).

Process Control

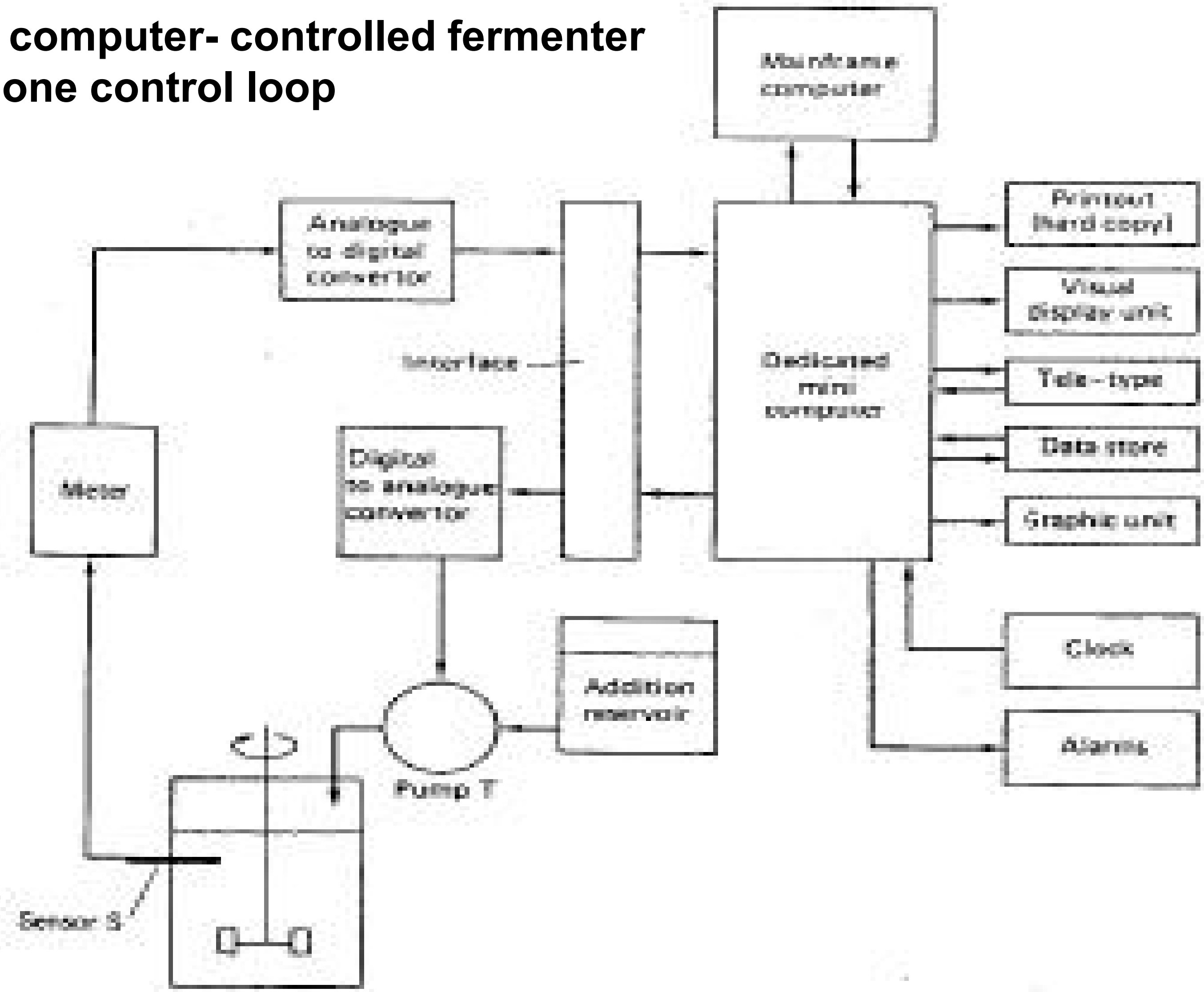
- Three levels process control that might be incorporated into a system were recognized.
- Each higher level involves more complex programs and needs a greater overall understanding of the process.
- The first level of control, involves sequencing operations, such as manipulating valves or starting or stopping pumps, instrument recalibration, on-line maintenance and fail-safe shut-down procedures.
- In most of these operations the time base is at least in the order of minutes, so that high-speed manipulations are not vital.
- Two applications in fermentation processes are sterilization cycles and medium batching.

Process Control

- The second level of control involves process control of temperature, pH, foam control, etc. where the sensors are directly interfaced to a computer, Direct Digital Control (DDC).
- When this is done separate controller units are not needed.
- The computer program determines the set point values and the control algorithms, such as PID, are part of the computer software package.
- Computer failure can cause major problems unless there is some manual back-up facility.

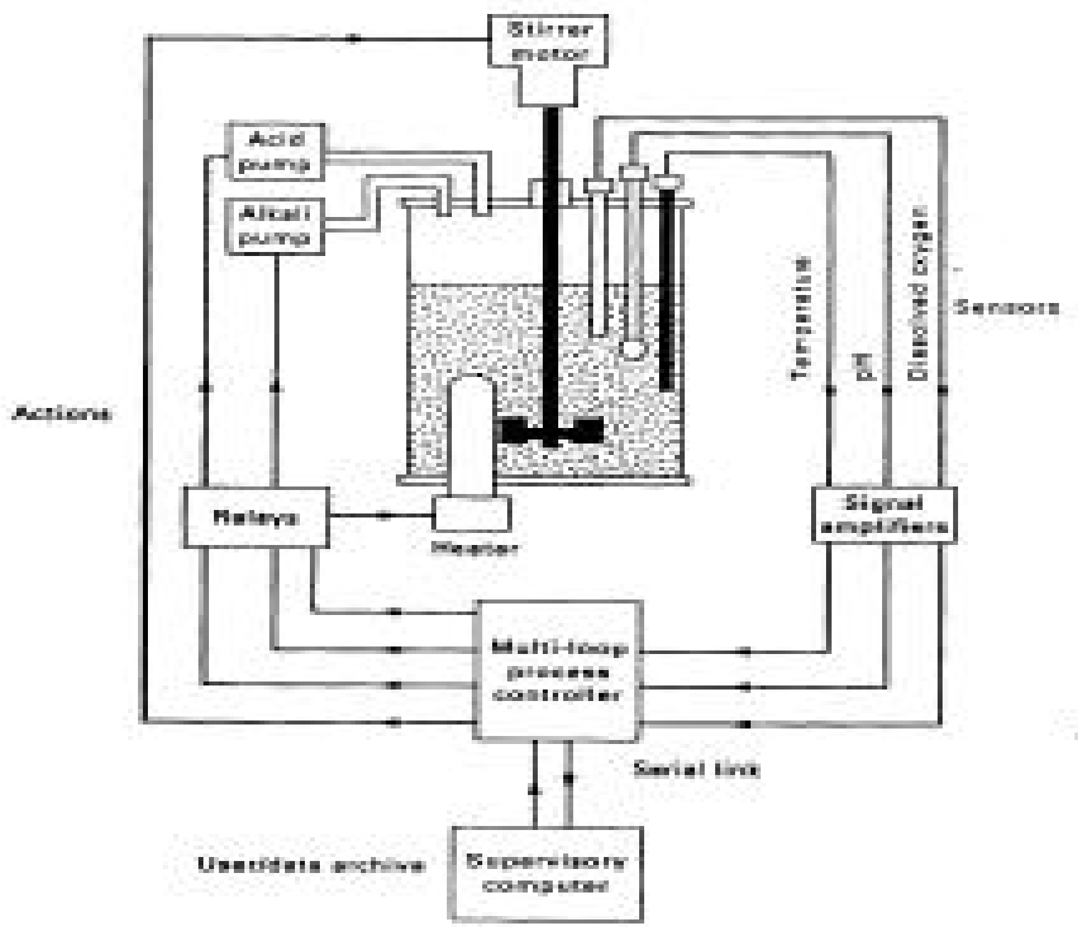
Process Control

Layout of computer- controlled fermenter with only one control loop



Process Control

- The alternative approach is to use a computer in a pure supervisory role, All control functions are per-formed by an electronic controller using a system illustrated in the figure:



Process Control

- In such a system the linked computer only logs data from sensors and sends signals to alter set points when instructed by a computer program or manually.
- This system is known as Supervisory Set-Point Control *(SSC) or Digital Set-Point Control (DSC).*
- When SSC is used the modes of control are limited to proportional, integral and derivative because the direct control of the fermenter is by an electronic controller.
- In the event of computer failure the process controller can be operated independently.

Process Control

- The third (advanced) level of control is concerned with process optimization.
- This will involve understanding a process, being able to monitor what is happening and being able to control it to achieve and maintain optimum conditions.
- Firstly there is a need for suitable on-line sensors to monitor the process continuously.
- A number are now available for dissolved oxygen, dissolved carbon dioxide, pH, temperature, biomass (the bug meter, NADH fluorescence, near infra-red spectroscopy) and some metabolite (mass spectroscopy and near infra-red spectroscopy).

Process Control

- Secondly, it is important to develop a mathematical model that adequately describes the dynamic behavior of a process.
- This approach with appropriate on-line sensors and suitable model programs has been used to optimize bakers' yeast production, an industrial antibiotic process and lactic acid production.