Level 9 Cell Culture Processing (BIO08045)

Lecture – "Bioreactor Modes of Operation – Part II"

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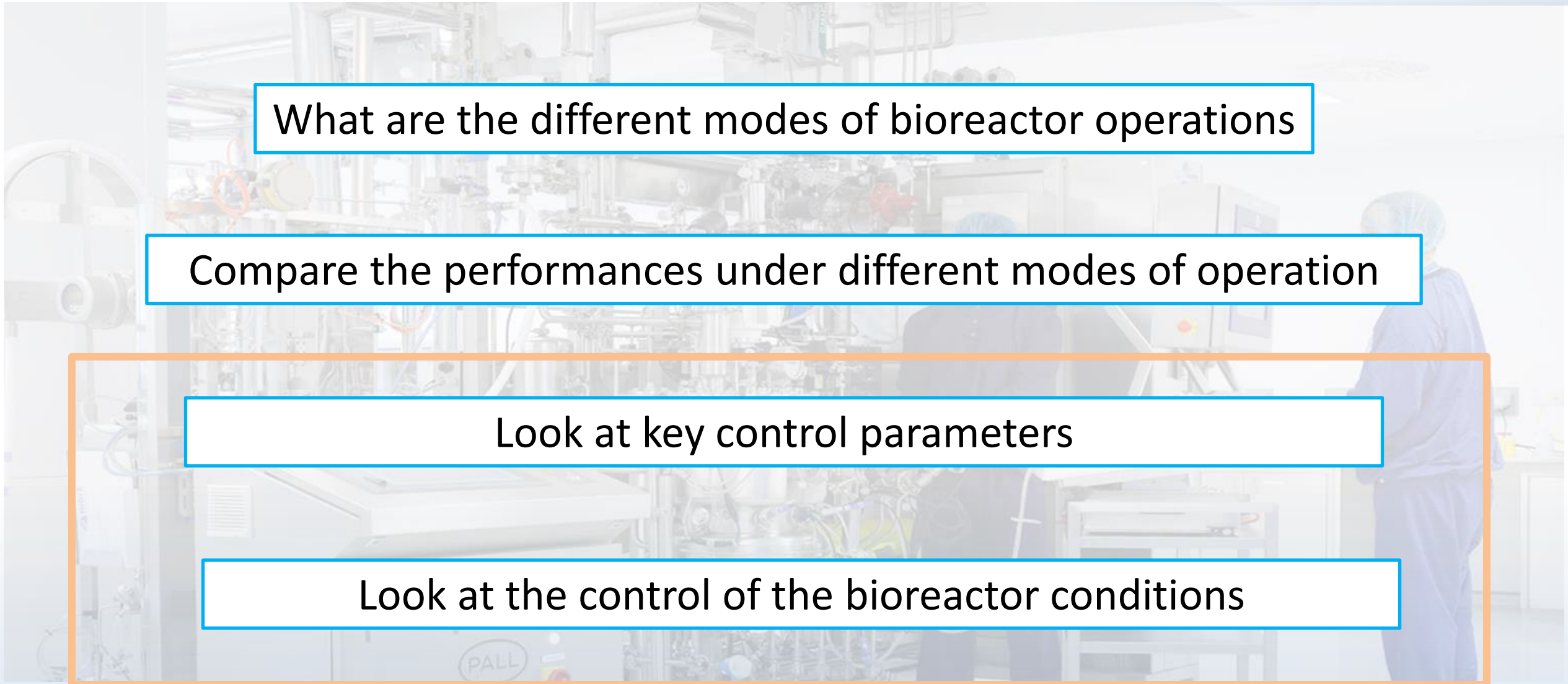
Learning Objectives



What are the different modes of bioreactor operations

Compare the performances under different modes of operation

Look at key control parameters

Look at the control of the bioreactor conditions

Reading Material

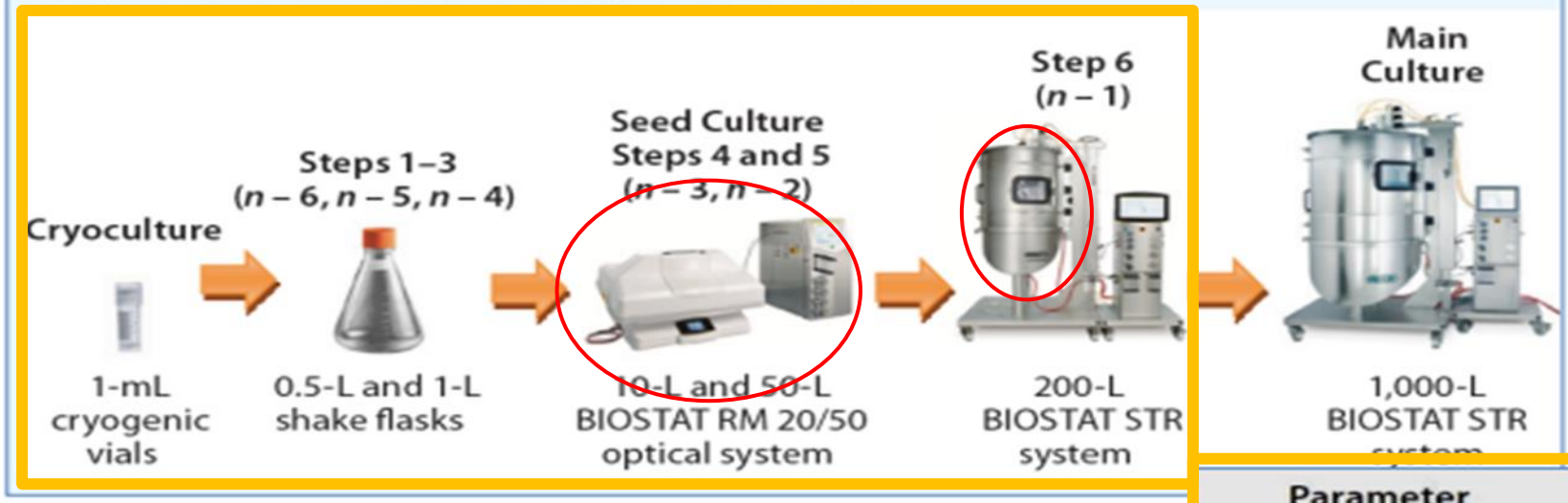
- *"Recent advances in large-scale production of monoclonal antibodies and related proteins"*. Shukla, A.A. and Thommes, J. 2010 Trends in Biotechnology Vol.28 No.5 p. 253-261
- *"Fed-Batch Cell Culture Process Optimization: A Rationally Integrated Approach"*. Jiang, Z. et al. BioProcess International 10(3) March 2012 p. 40-45
- *"Fed-Batch Mammalian Cell Culture in Bioproduction"*. Whitford, W.G. BioProcess International April 2006 p.30-40

Lecture Topics

- Control of Bioreactor Operation Parameters
- Temperature Control
- pH Control
- Bioreactor Gas Control
- Cell Density Monitoring

Single Use STR at 1000L Scale

Figure 1: Seed train of the 1,000-L fed-batch cell culture run starts with one cryogenic vial before six consecutive cell-expansion steps using single-use shaker flasks and bioreactors. A 17-day fed-batch production process followed in a BIOSTAT STR 1000 system. The entire duration from cryogenic vial to 1,000-L harvest on day 17 took 35 days.



Cultivation conditions of the 1,000-L production process comprising set points for pH, pO_2, and temperature and including ranges of gas flow rates and stirrer speeds as part of the pO_2 control cascade.

From: Reglin, R. Et al (2014) Verification of New Flexsafe STR Single-Use Bioreactor Bags: Using a CHO Fed-Batch Monoclonal Antibody Production Process at 1,000-L Scale. Bioprocess International 12(8s):53-57

Parameter	Set Point/ Range
Temperature	36.8 °C
pH	7.15
pO_2	60%
Stirrer speed	65–85 rpm
N_2	0–20 Lpm
Air flow	0–15 Lpm
O_2	0–60 Lpm

Single Use STR at 1000L Scale

Figure 2: Viable cell density (VCD) and viability of high–cell-density fed-batch processes in a BIOSTAT B 5-L glass vessel and BIOSTAT STR 50 and BIOSTAT STR 1000 Flexsafe bags; 5-L data include a standard deviation of cell density and viability for a total of four runs.

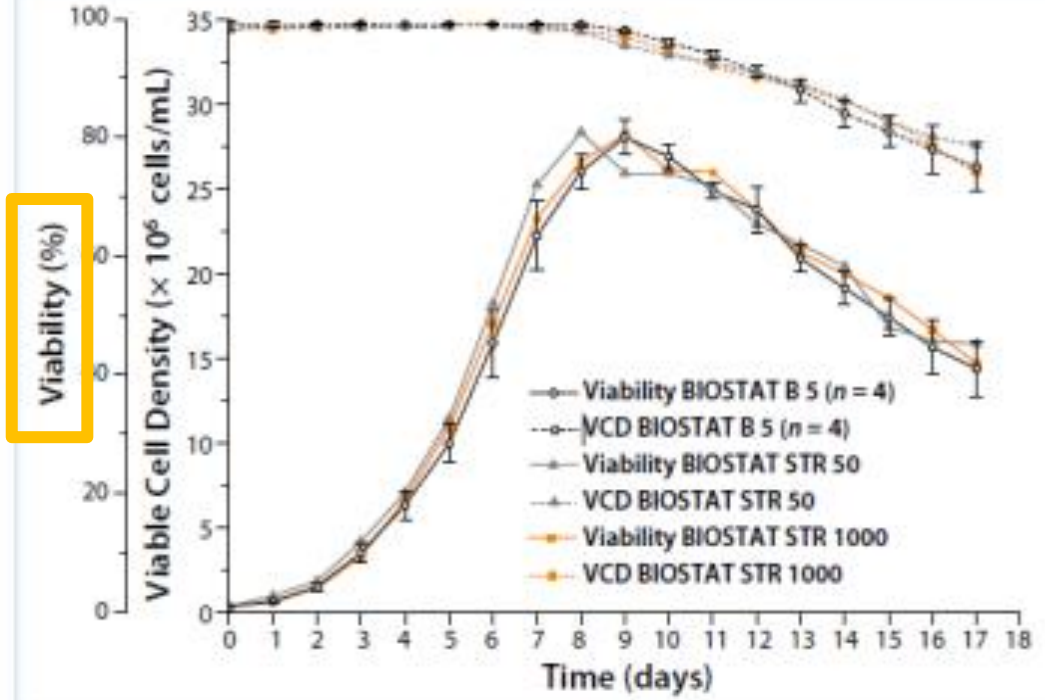
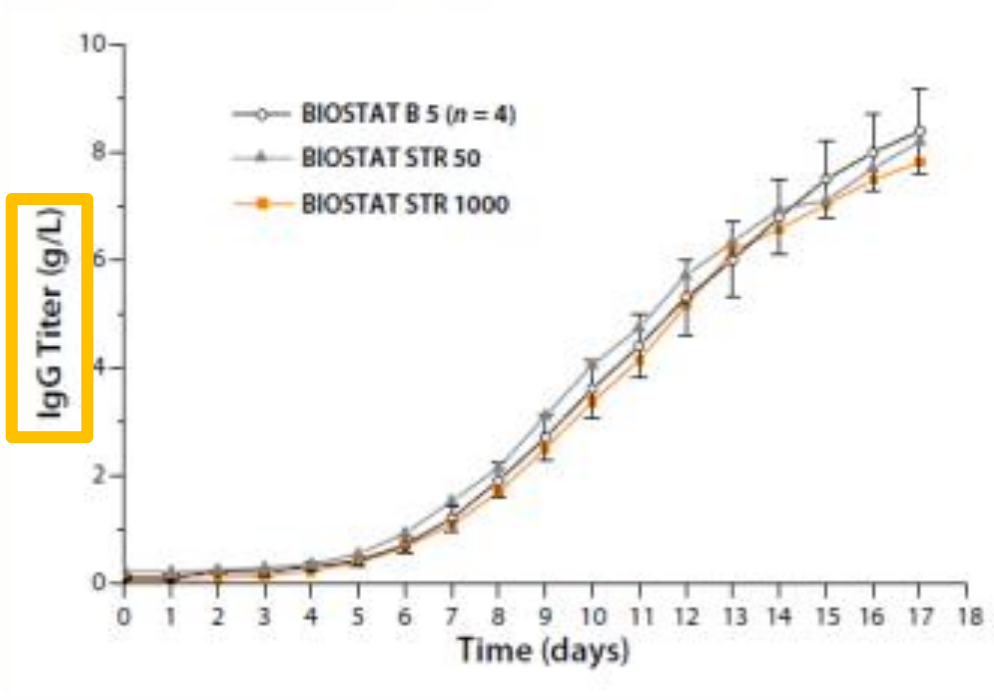


Figure 3: Monoclonal antibody (MAb) titer from high–cell-density fed-batch processes in a BIOSTAT B 5-L glass vessel and BIOSTAT STR 50 and BIOSTAT STR 1000 Flexsafe bags; 5-L data include a standard deviation of product titer for a total of four runs.



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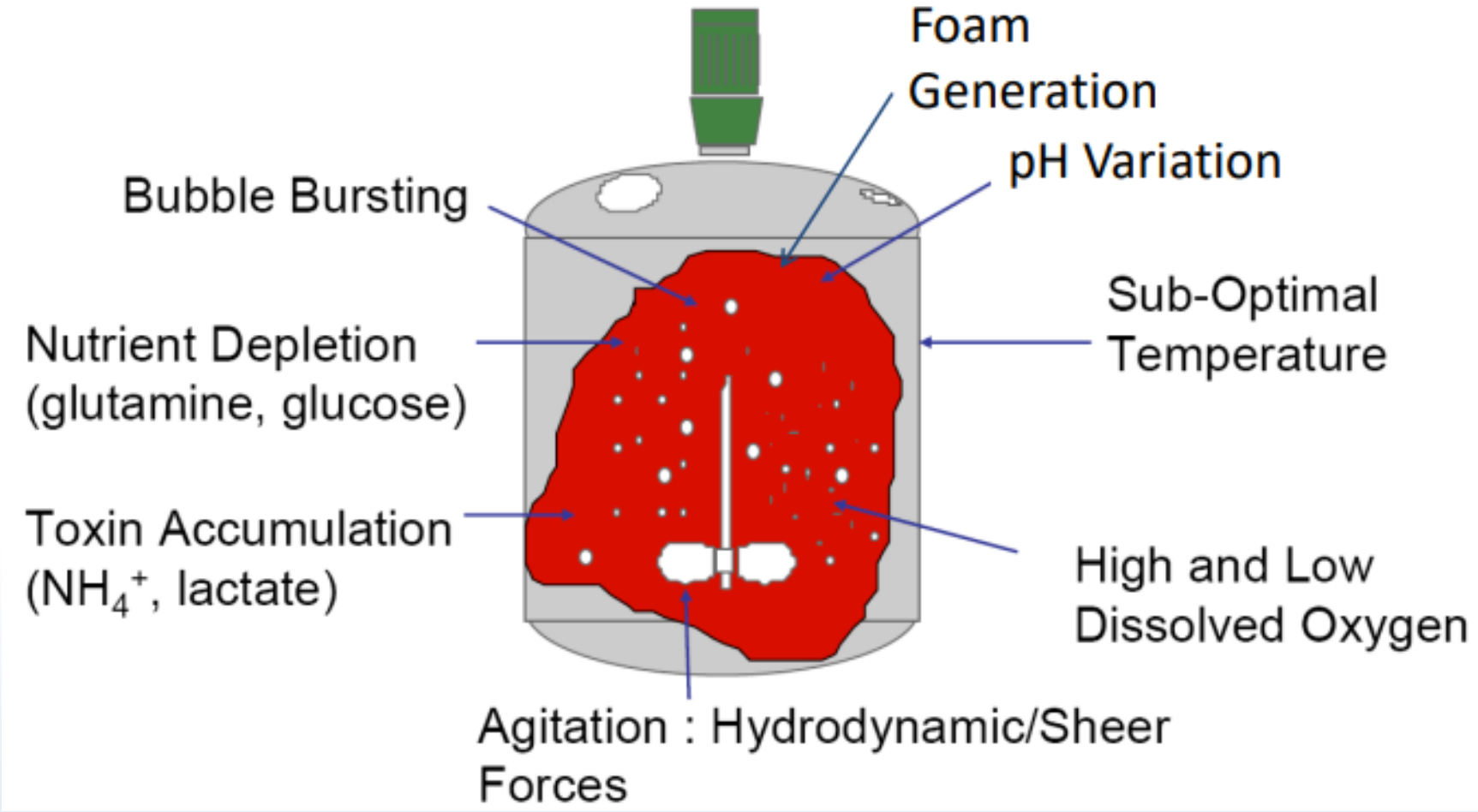
Factors affecting cell growth and productivity



Bioreactor Monitoring and Controls

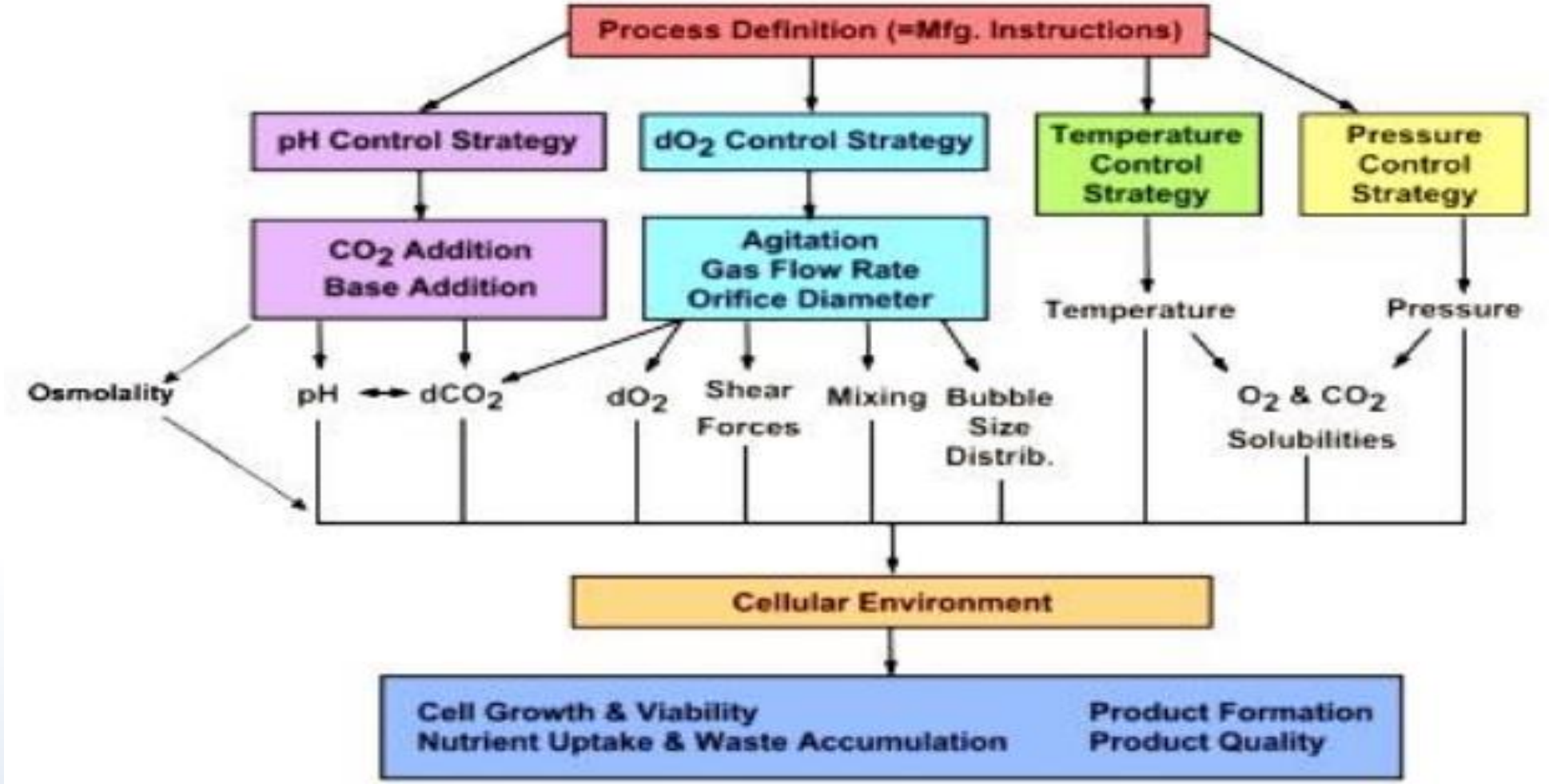
Principal Operating Parameters

1. Temperature.
2. pH.
3. Rate of Mixing
4. Oxygen Demand.
5. Gas concentrations (pO2, pCO2)
6. Cell number & viability
7. Headspace Pressure.
8. Feeding Profile.
9. Shear Control.
10. Foaming Control.
11. CIP / SIP Operations.
12. Power/energy inputs.

Critical Control Parameters in Bioreactors

- **The overall aim: to provide a controlled environment for the optimal growth of cells free from contamination and the maximum production of product:**
  1. Supply of nutrients
  2. pH
  3. Temperature control
  4. Dissolved oxygen
  5. Agitation rates
- There is a complex interplay between these parameters and changes in ones will almost invariably lead to changes in others.

Factors Affecting Growth

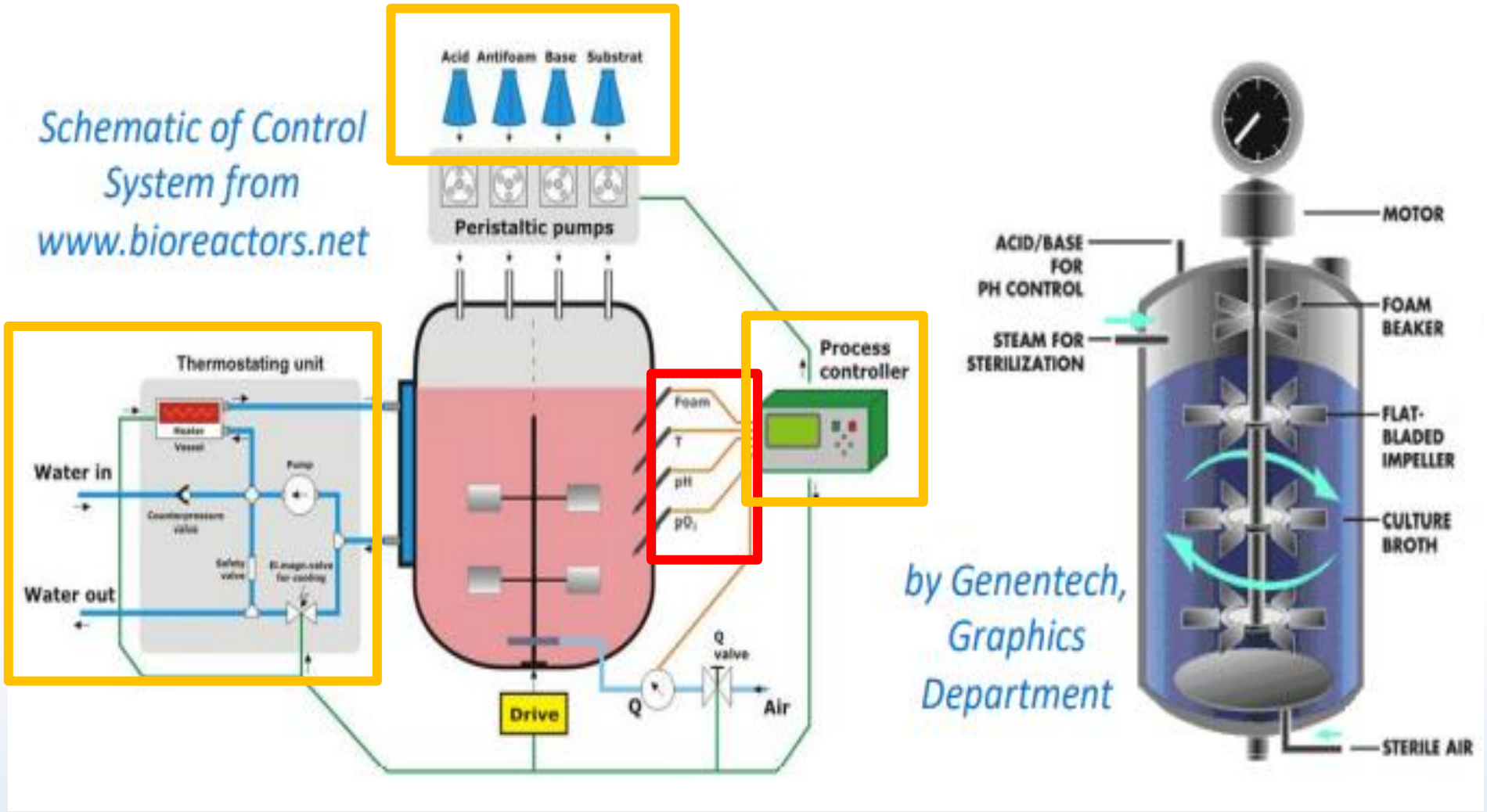


http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2958569/ Feng Li et al. Cell culture processes for monoclonal antibody production. MAbs. 2010 Sep-Oct; 2(5): 466–477. PMCID: PMC2958569

Instrumentation and Control

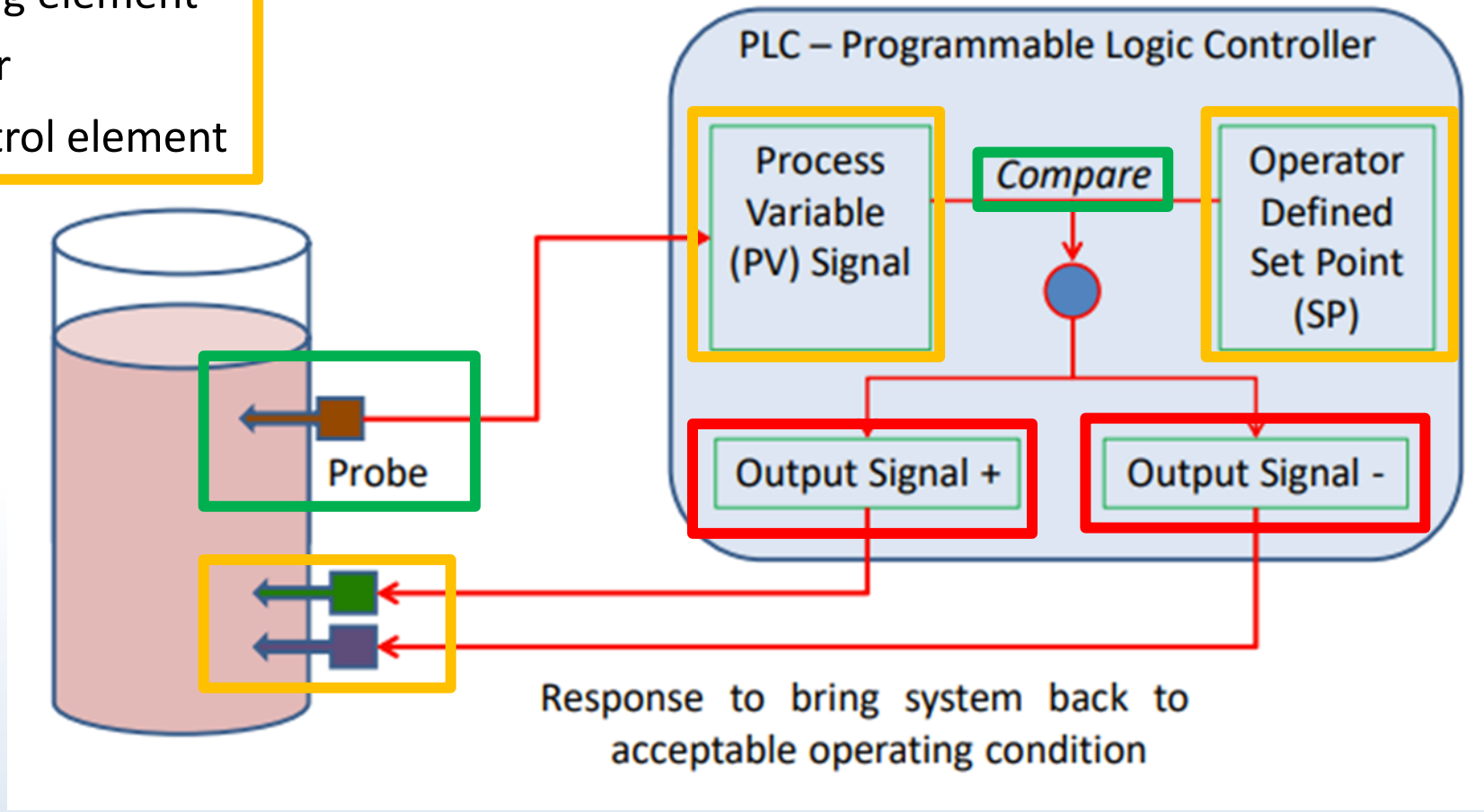
- A **process control loop** consists of four basis components:
  1. A process variable
  2. A measuring element
  3. A controller
  4. A final control element
- The measuring element senses a process property such as flow, pressure, temperature, etc., and generates a corresponding output signal.
- The controller compares the measurement signal with a predetermined desired value (set point) and produces an output signal to counteract any differences between the two.

Bioreactors



Process Control Loop

- Process control loop - 4 parts:
  1. A process variable
  2. A measuring element
  3. A controller
  4. A final control element



Online Monitoring and Control

- The following are the principal bioreactor parameters that are typically monitored on-line:
  1. Temperature of the culture using thermocouples, thermistors or RTD's.
  2. pH of the culture using electrochemical pH electrodes.
  3. Culture volume using load cells on the bioreactor legs or weigh scales.
  4. Cell density/concentration using both online and offline technology.
  5. Media feed rates using flowmeters or rotameters.

Online Monitoring and Control cont/d.

- The following are the principal bioreactor parameters that are typically monitored on-line: cont/d.

6. Vessel pressure using pressure recorders.
7. Dissolved oxygen (DO) concentration in the culture using Mettler-Toledo Thornton or Broadley-James sensors or equivalent.
8. Partial pressure of carbon dioxide in the culture using Mettler-Toledo Thornton or Broadley-James sensors.
9. Protein concentration using OD280 optical density monitoring or equivalent.

Offline Monitoring and Control.

- **The following are typical offline monitoring tests that may be performed for process control:**
  - Cell density using either of the following:
    - Dry-weight measurements.
    - Cell counting techniques e.g. hemocytometer.
  - pH offline sample testing.
  - Concentration of substrates using enzymatic analysis techniques.
  - Concentration of product using spectrophotometry e.g. absorbance
    - – optical density (OD280 at 280 nm wavelength) based on turbidity measurements. Can also use HPLC analysis for protein concentration.

Bioreactor Monitoring and Controls : Key Considerations

- Monitoring the dissolved oxygen (DO) and dissolved carbon dioxide (CO_2) levels of the culture and providing adequate O_2 supply through sparging from the bottom of the bioreactor.
- Regulating the CO_2 levels to optimise cell performance and control pH levels adequately.
- pH monitoring and adding either CO_2 or base such as Sodium Bicarbonate ($NaHCO_3$) or acid such as citric acid as needed to balance the pH. pH changes arise from metabolite generation in the media e.g. carbon dioxide, lactate, ammonia etc.
- Temperature monitoring and control for optimal cellular growth and product synthesis.

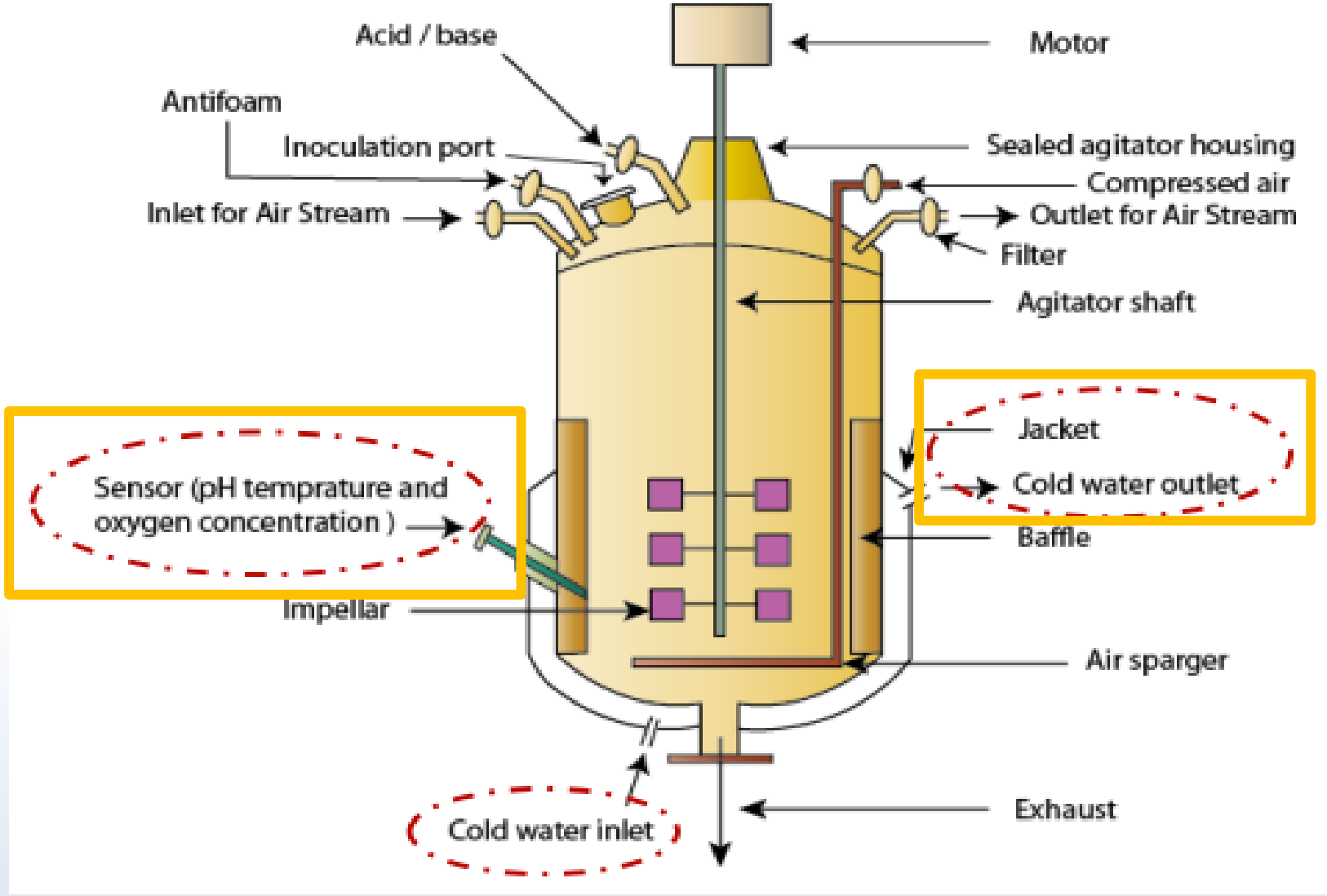
Bioreactor Monitoring and Controls : Key Considerations cont/d

- Adequate mixing to ensure sufficient supply of O_2 and nutrients to the cells and also to prevent the accumulation of toxic metabolic by-products.
- Balance of thorough agitation to disperse bubbles versus the risk of damaging the cells through excessive agitation and shear effects.
- Control of foaming in the bioreactor through the addition of anti-foaming chemicals or mechanical foam-breaker to minimise damage risk to vent filters and back-pressure control. Also nitrogen sparging to break up foam.

Lecture Topics

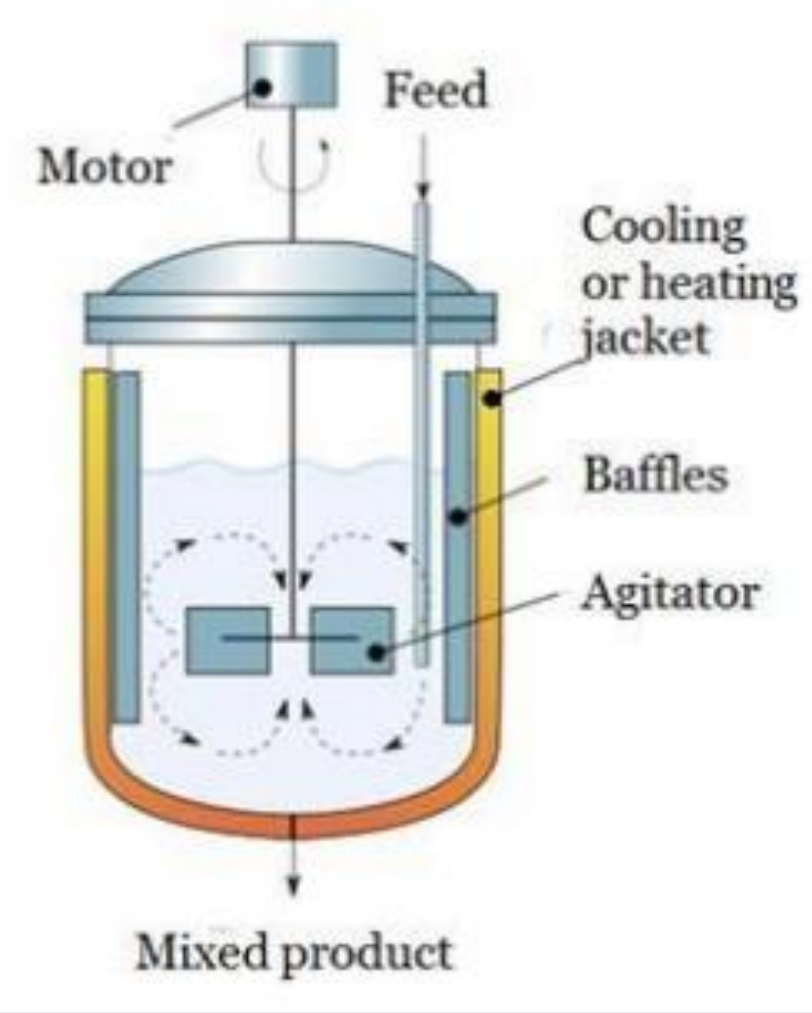
- Control of Bioreactor Operation Parameters
- Temperature Control
- pH Control
- Bioreactor Gas Control
- Cell Density Monitoring

Temperature control



Temperature control

- Temperature control system consists of temperature probes (thermometer)and heat transfer system
- The main elements are as follows:
  - An external heating/cooling jacket system surrounding the bioreactor body with a hot water and cooling water supply and return system.
  - The means for monitoring the internal culture medium temperature and for regulating the cooling water or hot water supply control systems to maintain the target levels



Temperature Control

- The growth of cells is based on many chemical reactions on which temperature has a great effect. Above a certain cut-off temperature growth rates start to decline rapidly due to protein denaturation.
- Temperature for optimal cell growth and temperature for optimal product formation may differ.
  - e.g. cultures genetically engineered to grow at one temperature may be induced with a higher or lower optimum temperature for production.
- Higher temperatures (above 40°C) may cause cell viability issues.
- Temperature is generally well maintained and can be controlled to ±0.5 °C.

Temperature Control in Bioreactors

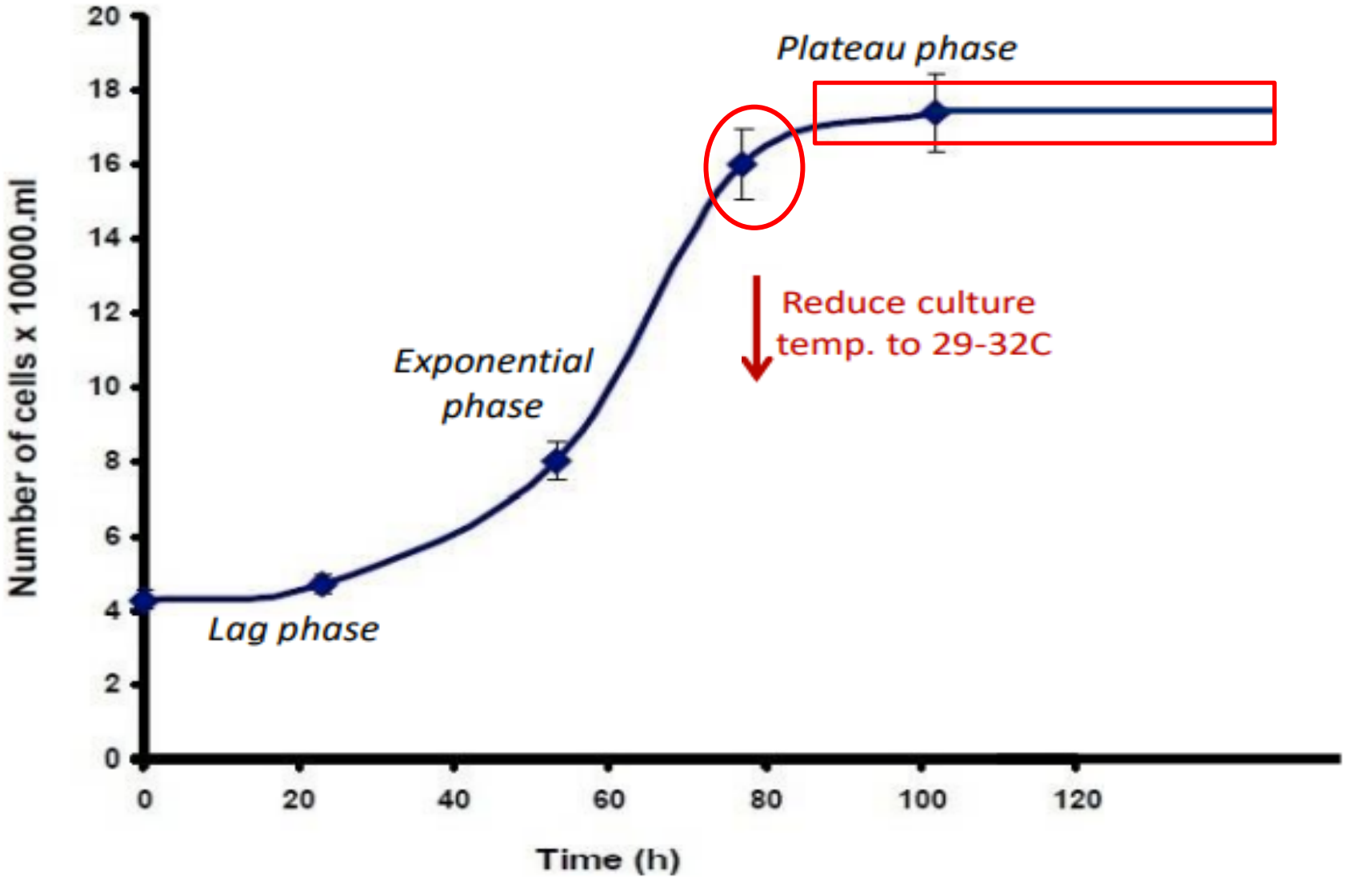
Temperature Control is desirable to ensure the following:

a) That the cells operate in an optimal temperature environment of 36°C +/- 0.5°C.

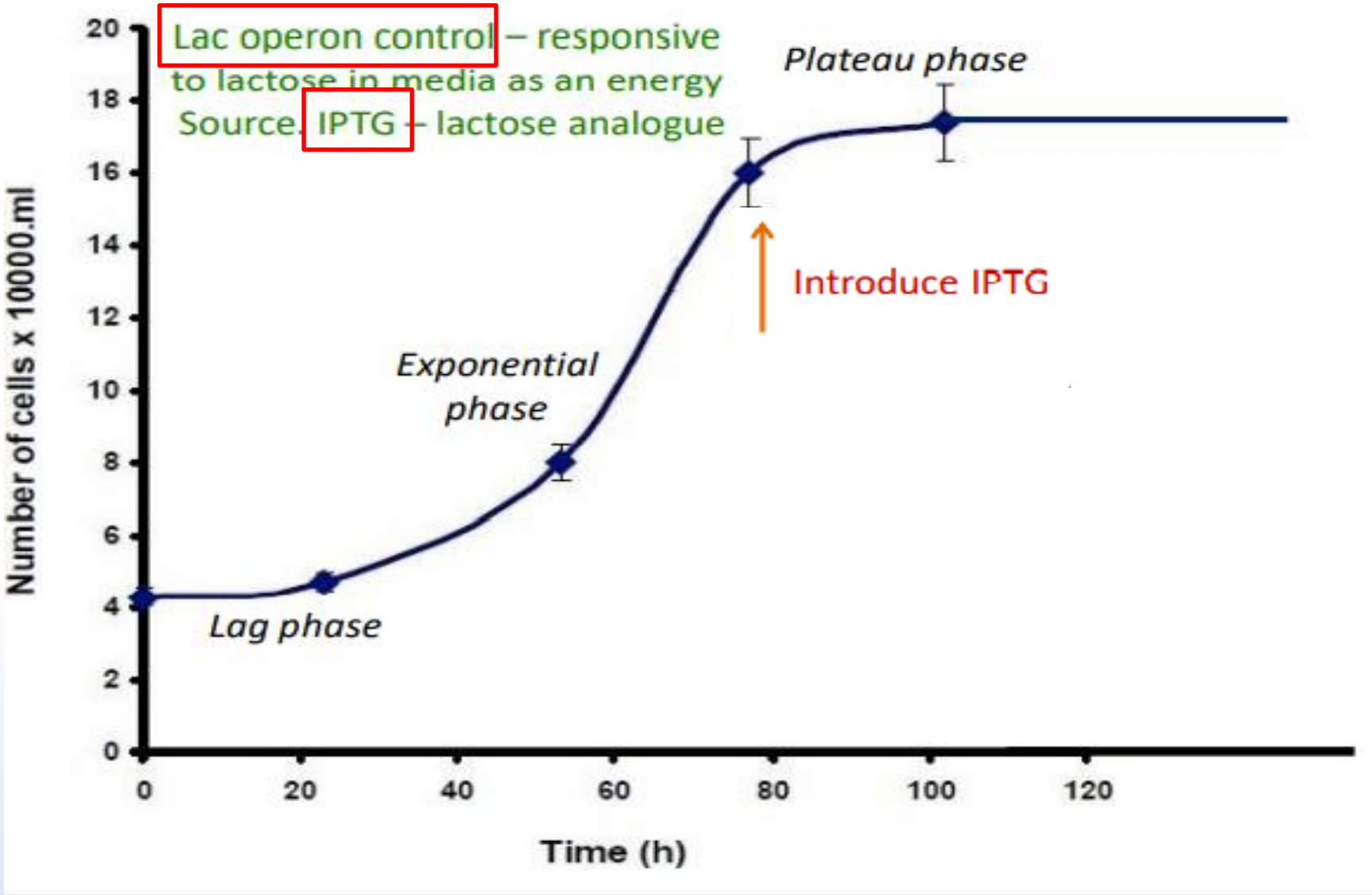
b) That all of the additional heat entering into the system from cellular metabolic activity and from the energy of agitation is effectively removed.

c) Corrective action if the temperature strays outside its normal range which will lead to increased cell death rate and a considerable loss in performance.

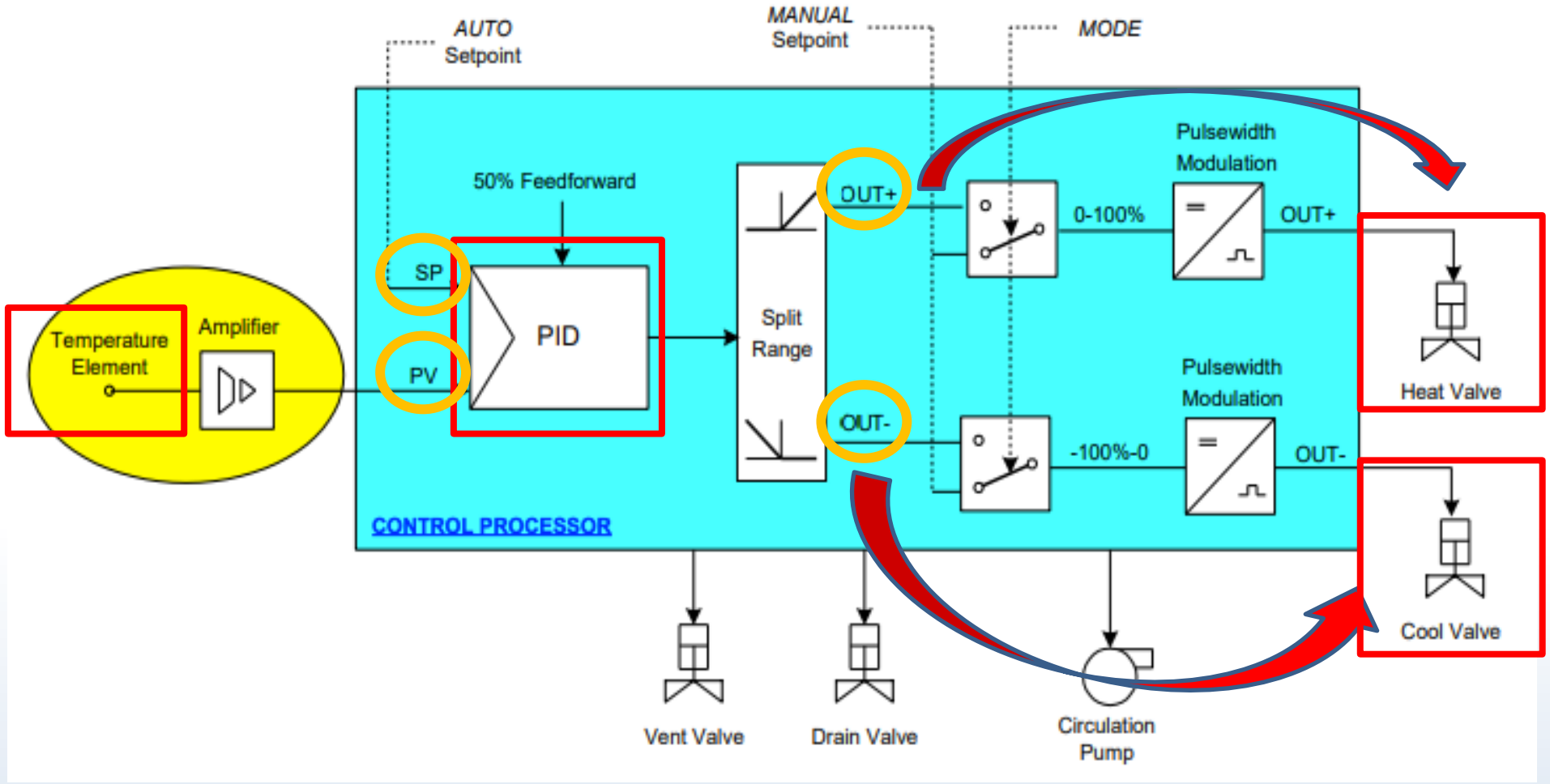
Activation of Expression – Temperature Switch

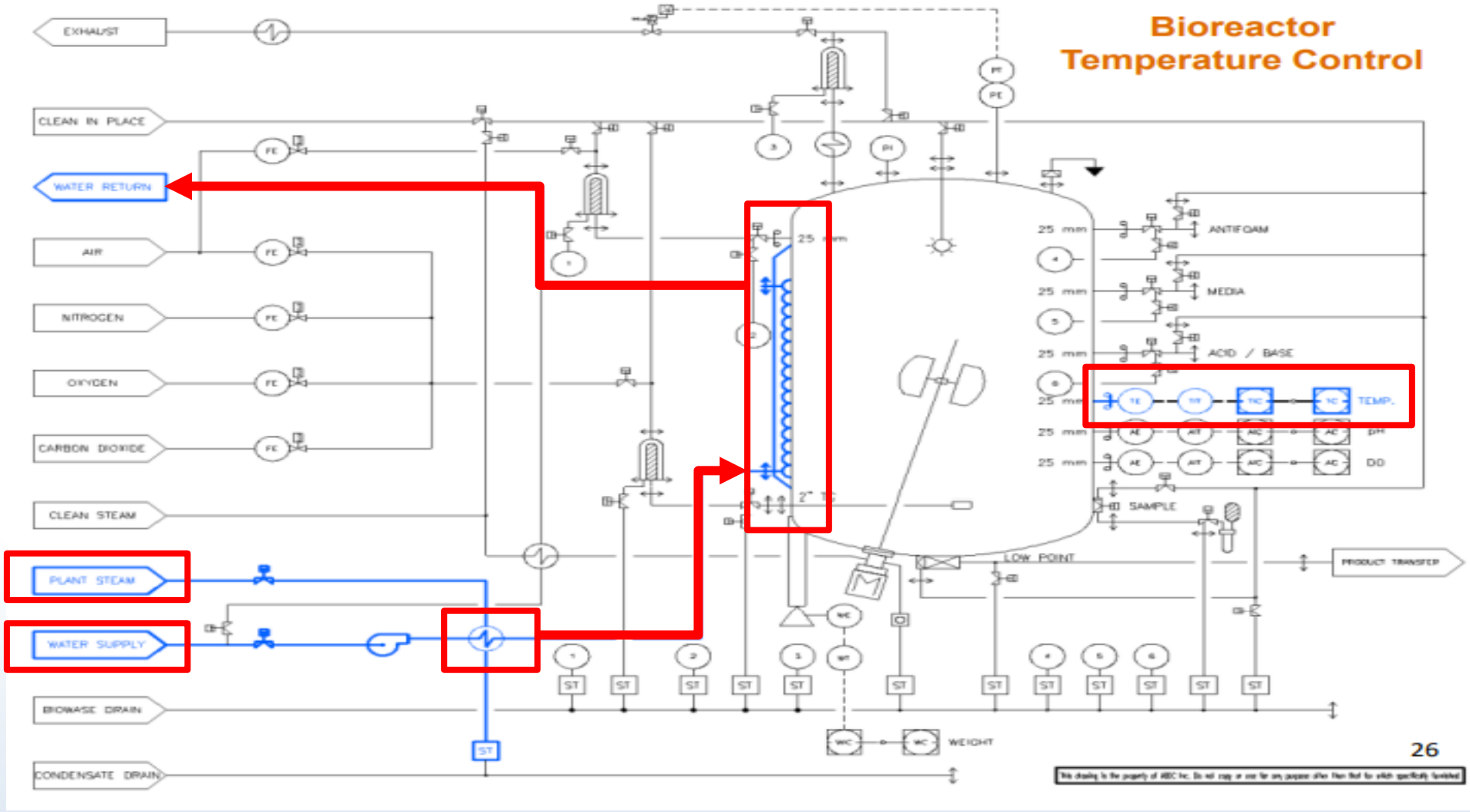


Activation of Expression – Metabolite Switch

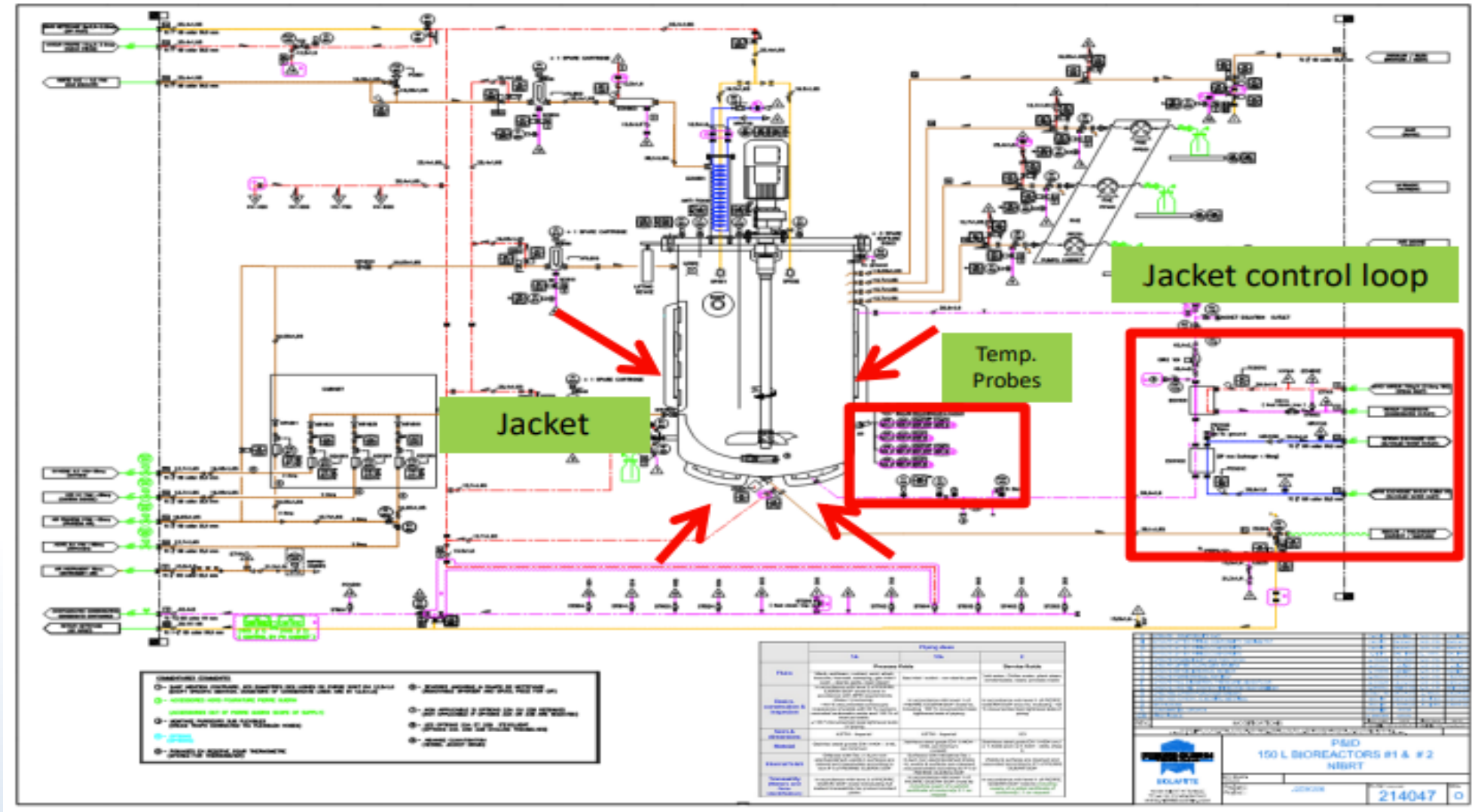


Temperature Control Loop




Bioreactor
Temperature Control
EXHAUST
CLEAN IN PLACE
WATER RETURN
AIR
NITROGEN
OXYGEN
CARBON DIOXIDE
CLEAN STEAM
PLANT STEAM
WATER SUPPLY
BIOWASTE DRAIN
CONDENSATE DRAIN
ANTIFOAM
MEDIA
ACID / BASE
TEMP.
pH
DO
SAMPLE
LOW POINT
PRODUCT TRANSFER
WEIGHT
26

Bioreactor Temperature Control

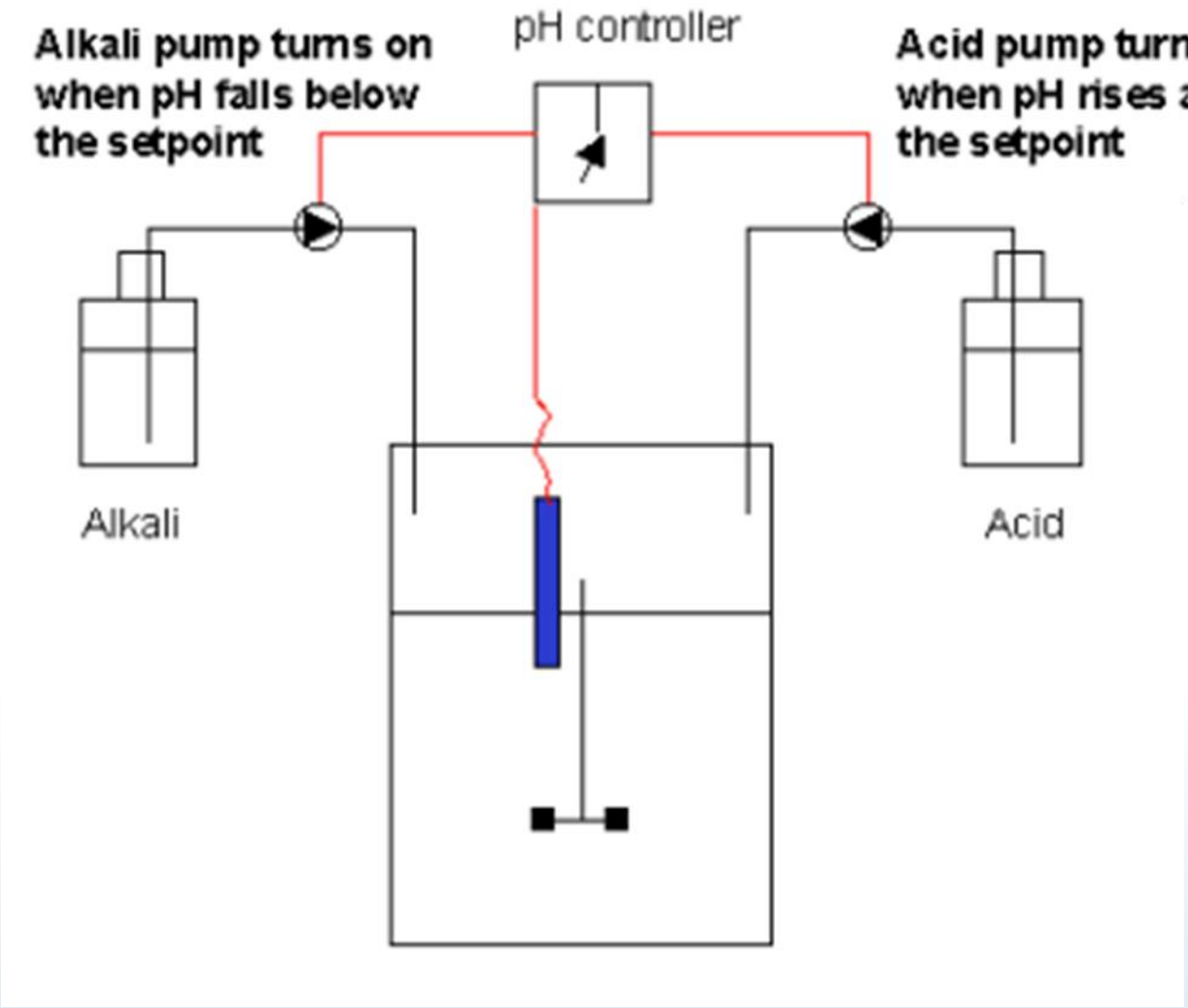


Lecture Topics

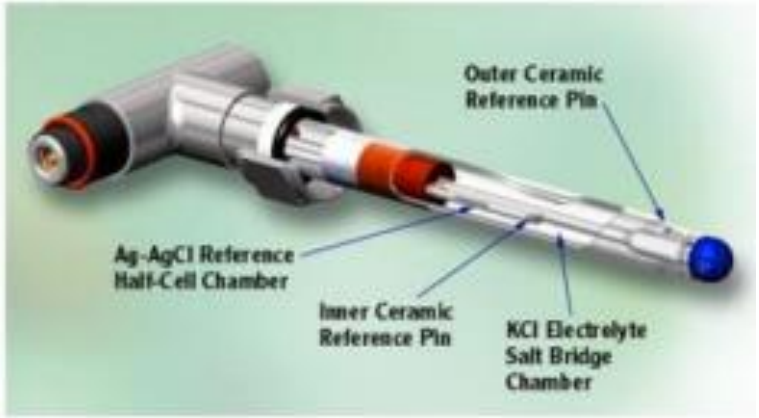
- Control of Bioreactor Operation Parameters
- Temperature Control
- pH Control
- Bioreactor Gas Control
- Cell Density Monitoring

pH control

- Vital to keep pH within a narrow range
- Measured by a sterilizable pH probe
- Acid (CO_2) or alkali (KOH) used to adjust pH
- More complex systems can use buffer to control pH



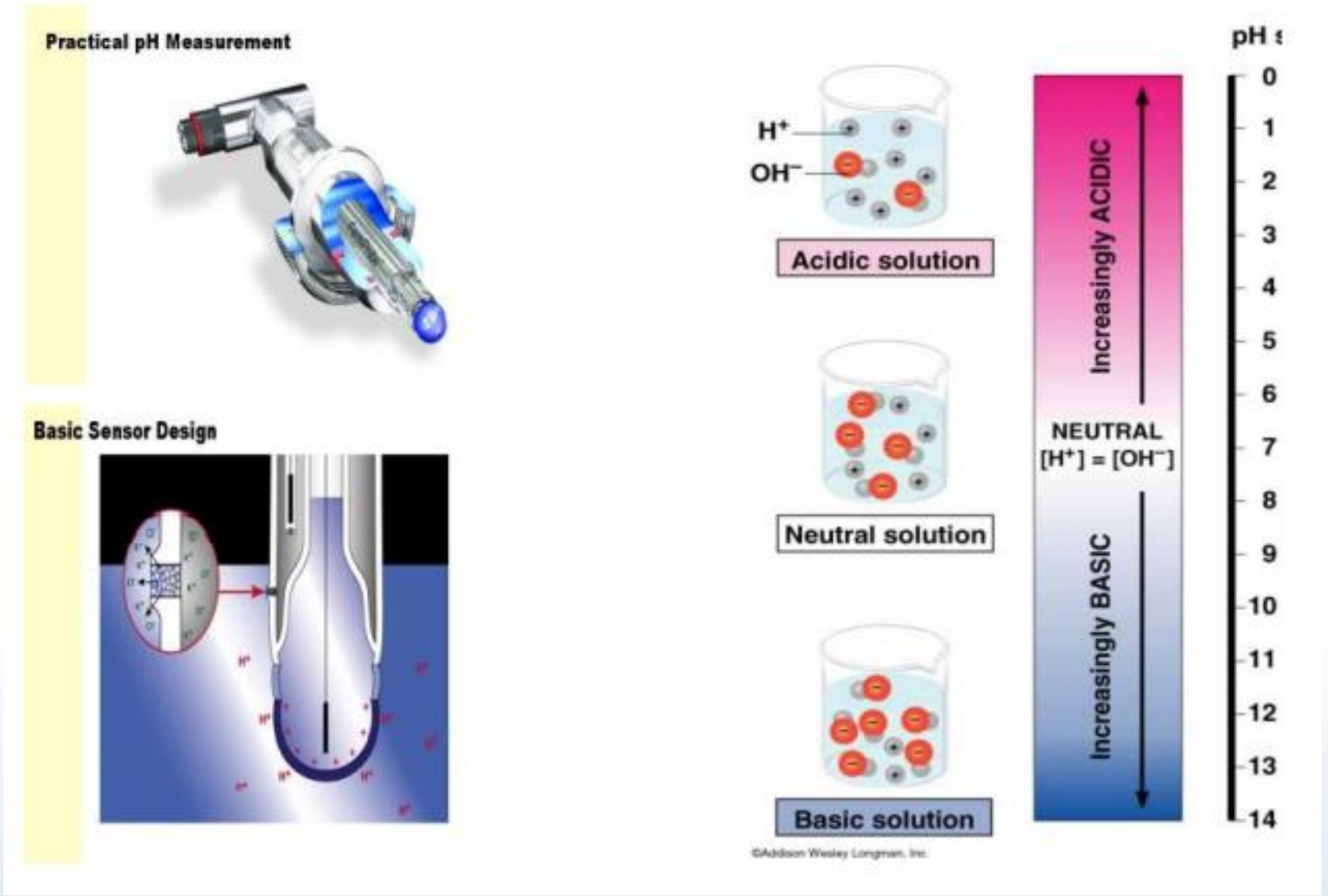
Bioreactor pH Control



pH Control is desirable to ensure the following:

- That the cells do not encounter adverse pH conditions that could either kill them off or inhibit their growth and productivity levels.
- The pH operating range is normally very tight e.g. 6.80 – 7.40 pH
- For CHO cell lines this range is even tighter at 7.0 - 7.4 pH
  - Adjustment to pH: narrow range (pH 7 - 7.4), pH generally drops as glucose → lactic acid. Drop in pH requires addition of alkali; increase requires increased sparging of CO_2

pH Control


Practical pH Measurement
Basic Sensor Design
H+
OH−
Acidic solution
Neutral solution
Basic solution
Increasingly ACIDIC
NEUTRAL
[H+] = [OH−]
Increasingly BASIC
pH
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
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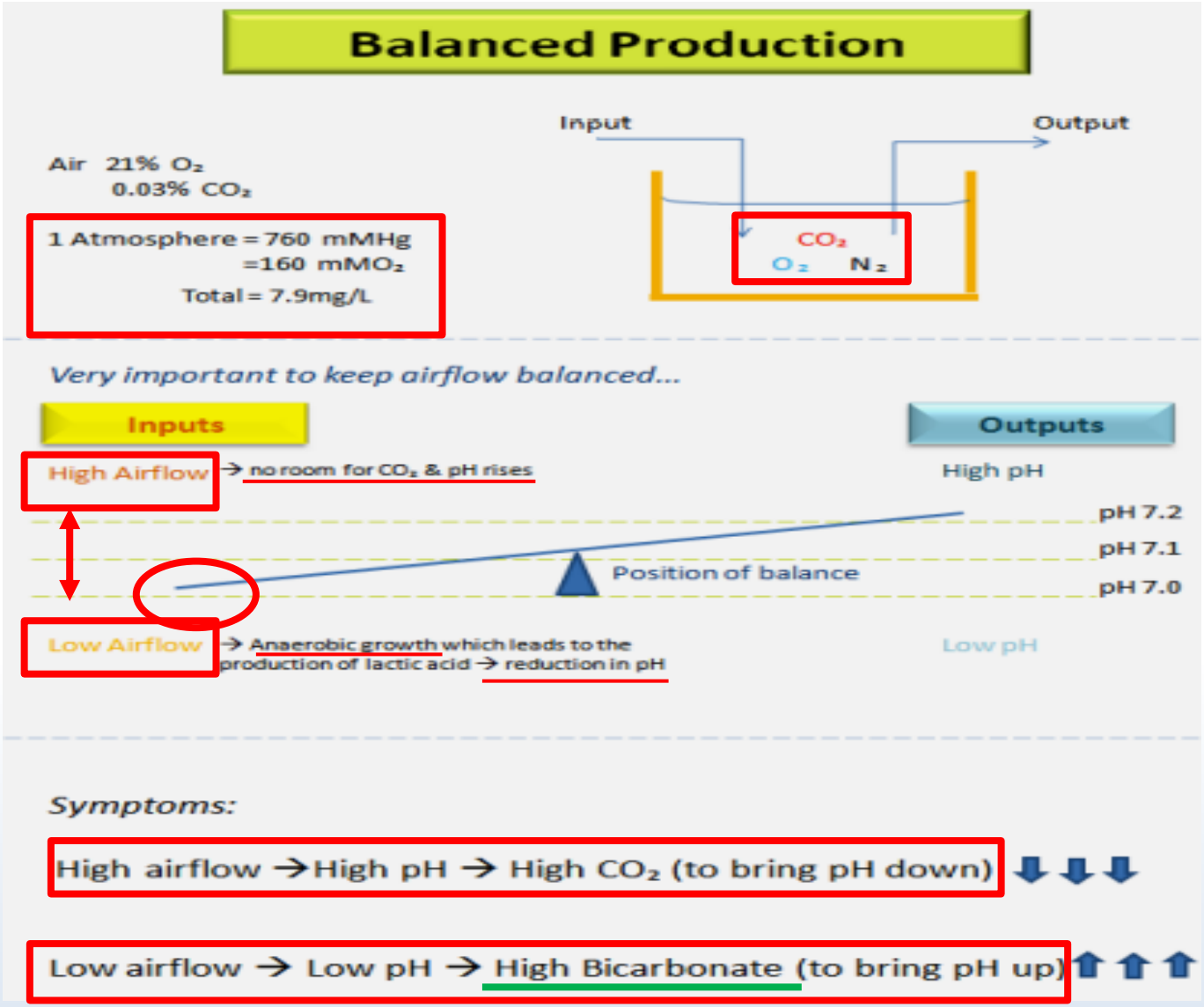
pH Control for Mammalian Cell Cultures

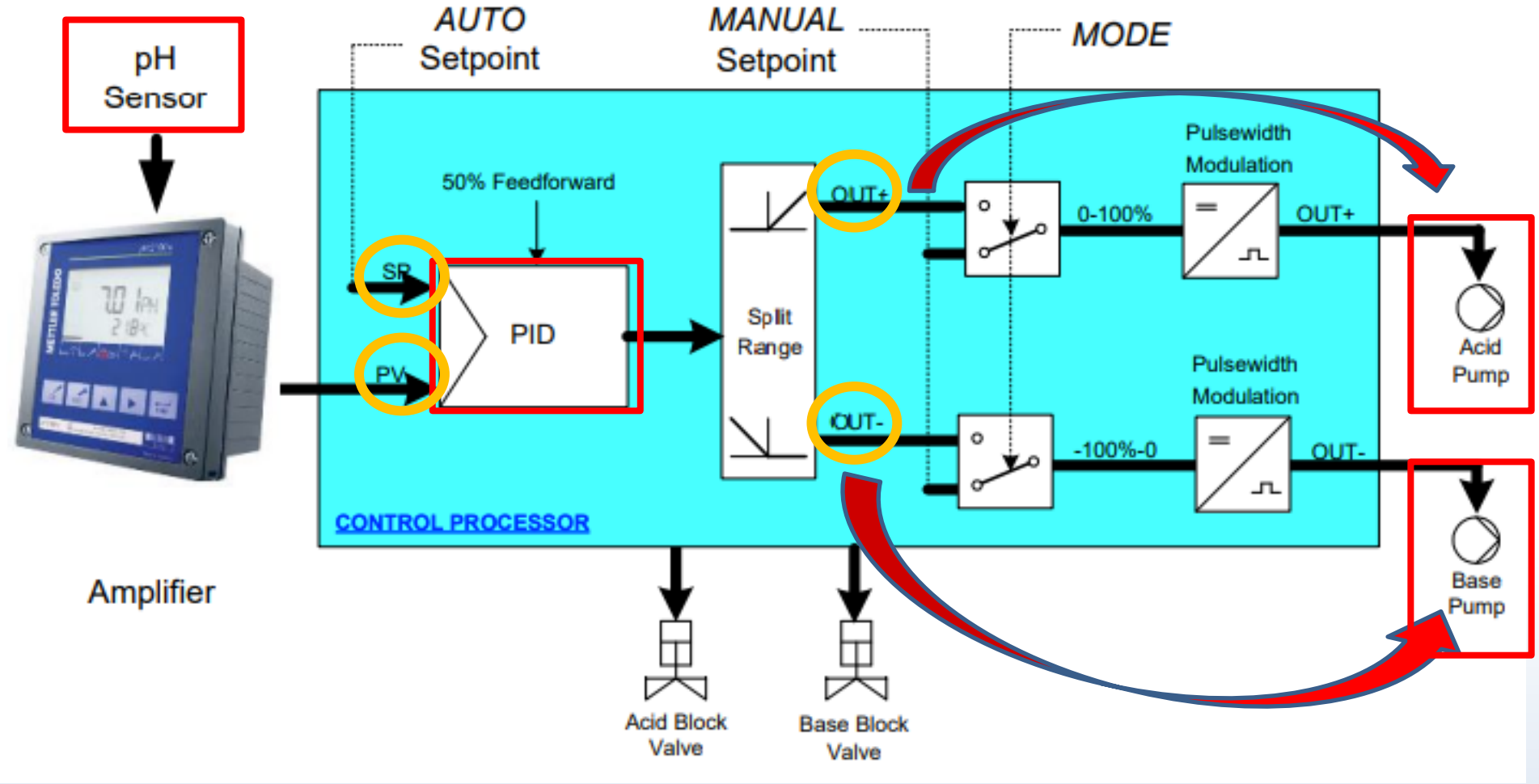


Bioreactor pH Control

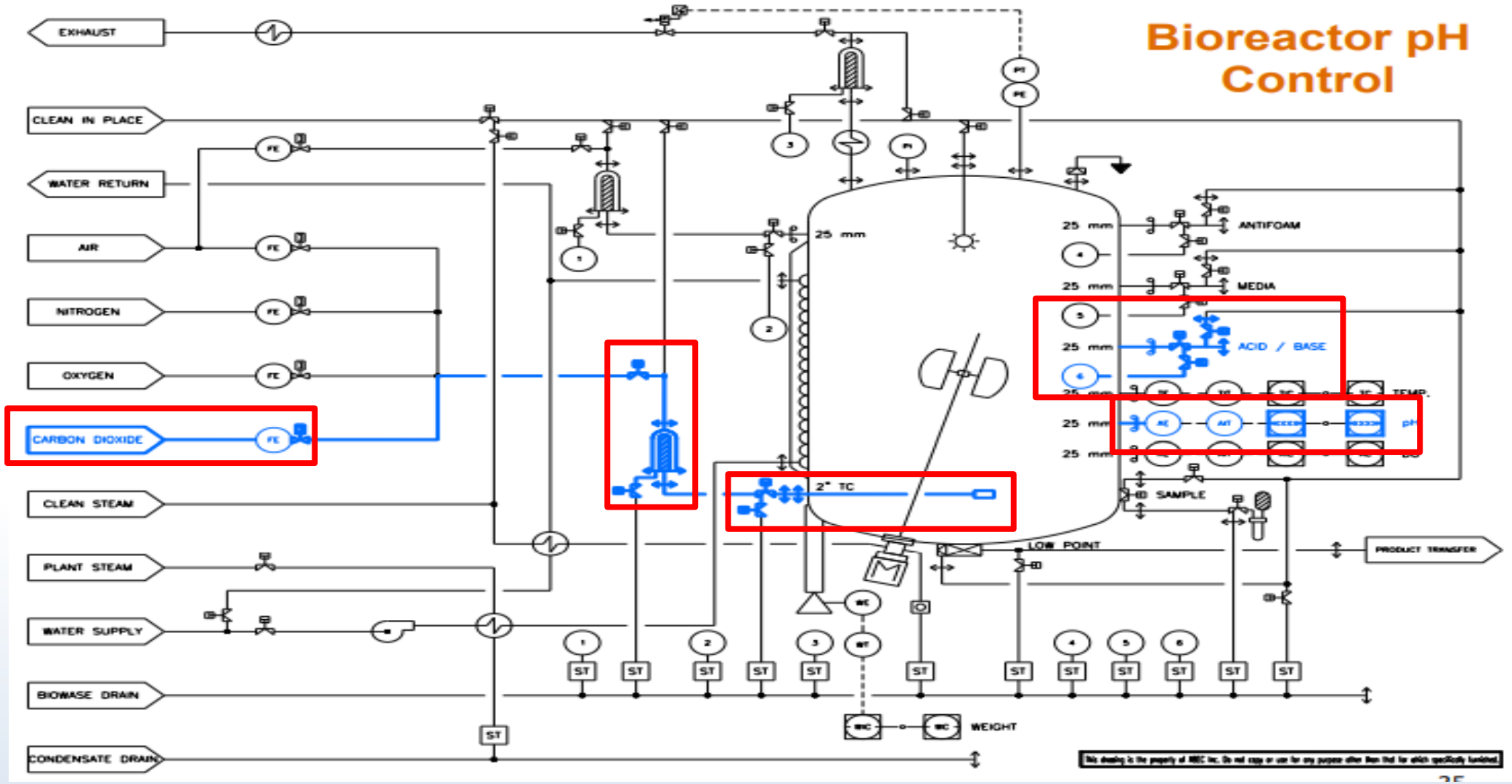
The main pH control elements are as follows:

- A pH monitoring and control system using pH electrodes to generate a control signal based on the level of pH being experienced.
- pH control is achieved either by sparging in additional CO_2 gas into the culture medium or by flowing in additional base solution such as Sodium Bicarbonate ($NaHCO_3$) or acid such as citric acid.

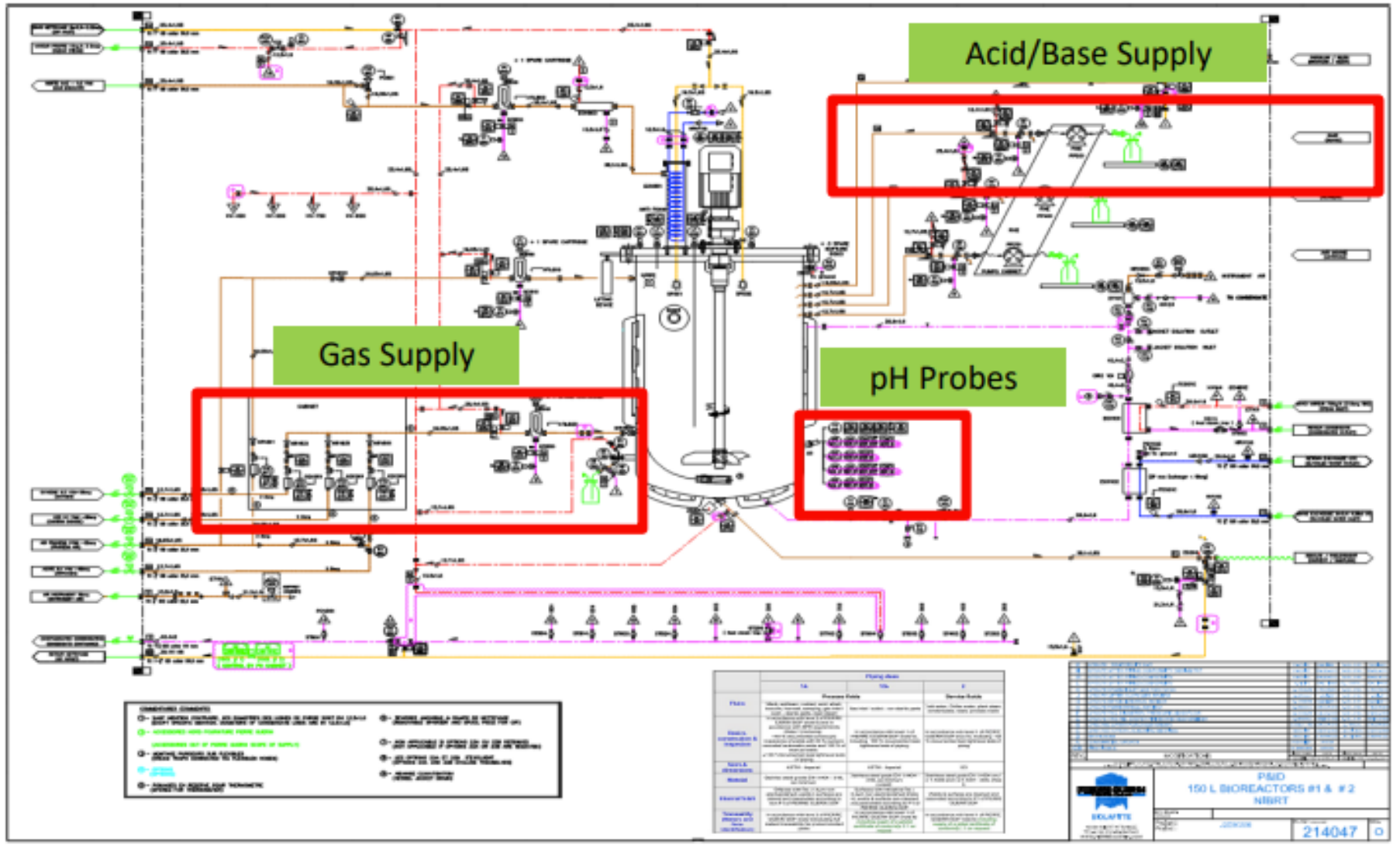
Bioreactor pH Control



Bioreactor pH Control



Bioreactor pH Control

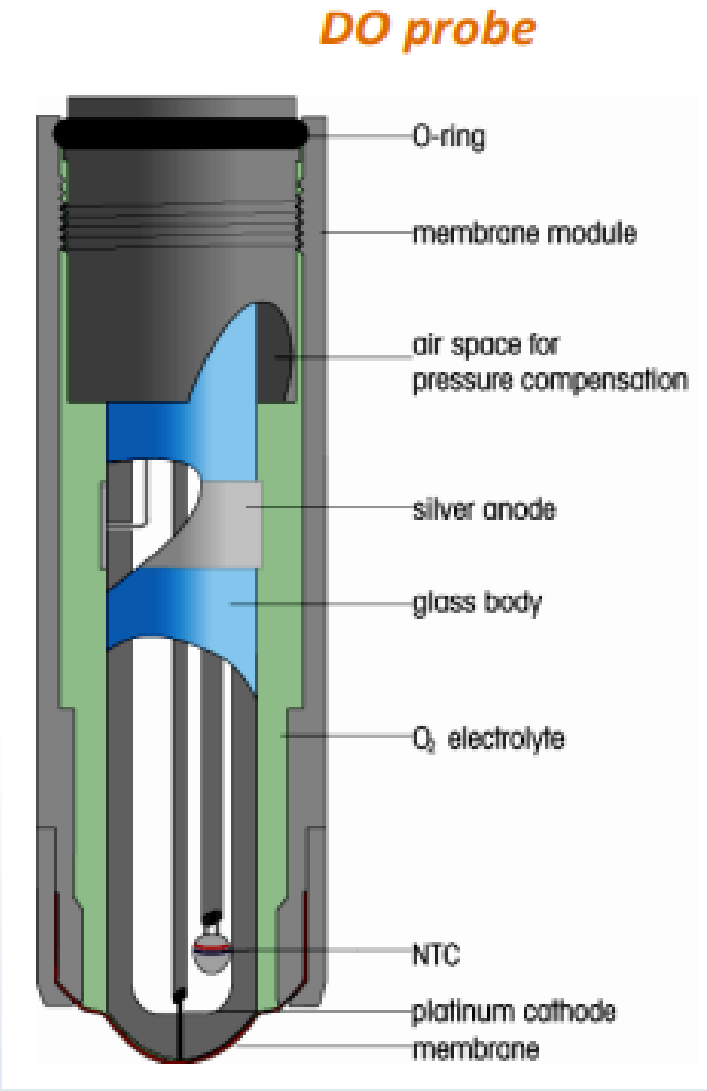


Lecture Topics

- Control of Bioreactor Operation Parameters
- Temperature Control
- pH Control
- Bioreactor Gas Control
- Cell Density Monitoring

The Importance Of Oxygen Measurement

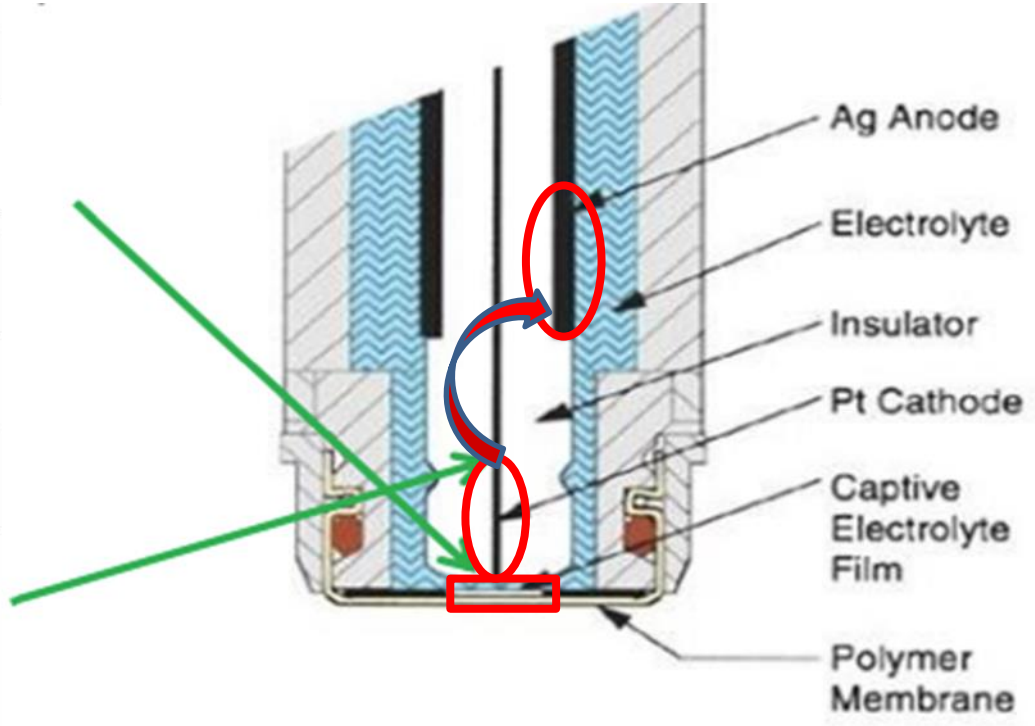
- DO (dissolved oxygen) level is controlled to ensure sufficient oxygen for cells to grow.
- Oxygen – an odorless and tasteless gas (O_2) which represents 21% of the atmosphere
  - **Sparingly soluble in culture media (7mg per litre @37°C;) yet is consumed in large amounts**
  - Sterile filtered air or oxygen normally enters the fermenter through a sparger system, and airflow rates for large fermenters rarely exceed 0.5 - 1.0 volumes of air per volume of medium per minute (v/v/m)



DO Probes

How does the DO probe work?

1. Oxygen in solution diffuses across the gas permeable membrane into the sensor electrolyte solution.
2. Oxygen undergoes a reduction reaction at the cathode that produces a nano amp current.
3. The current produced is proportional to the oxygen present in solution



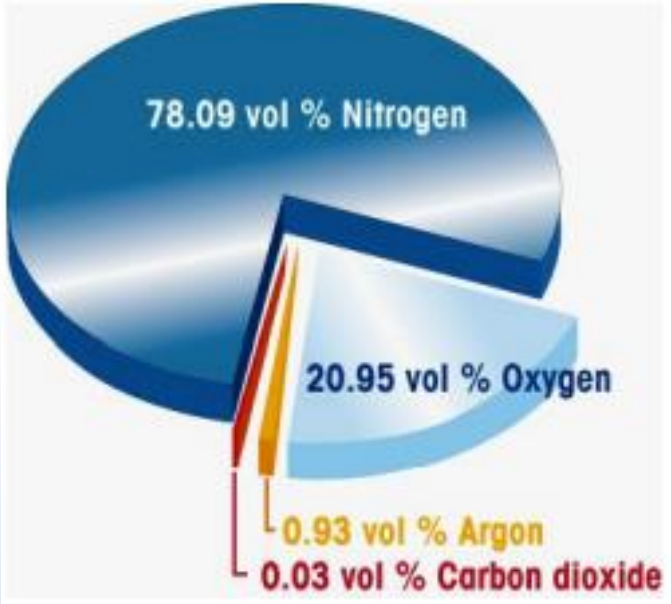
The Clark Oxygen Sensor

Partial Pressure of Oxygen

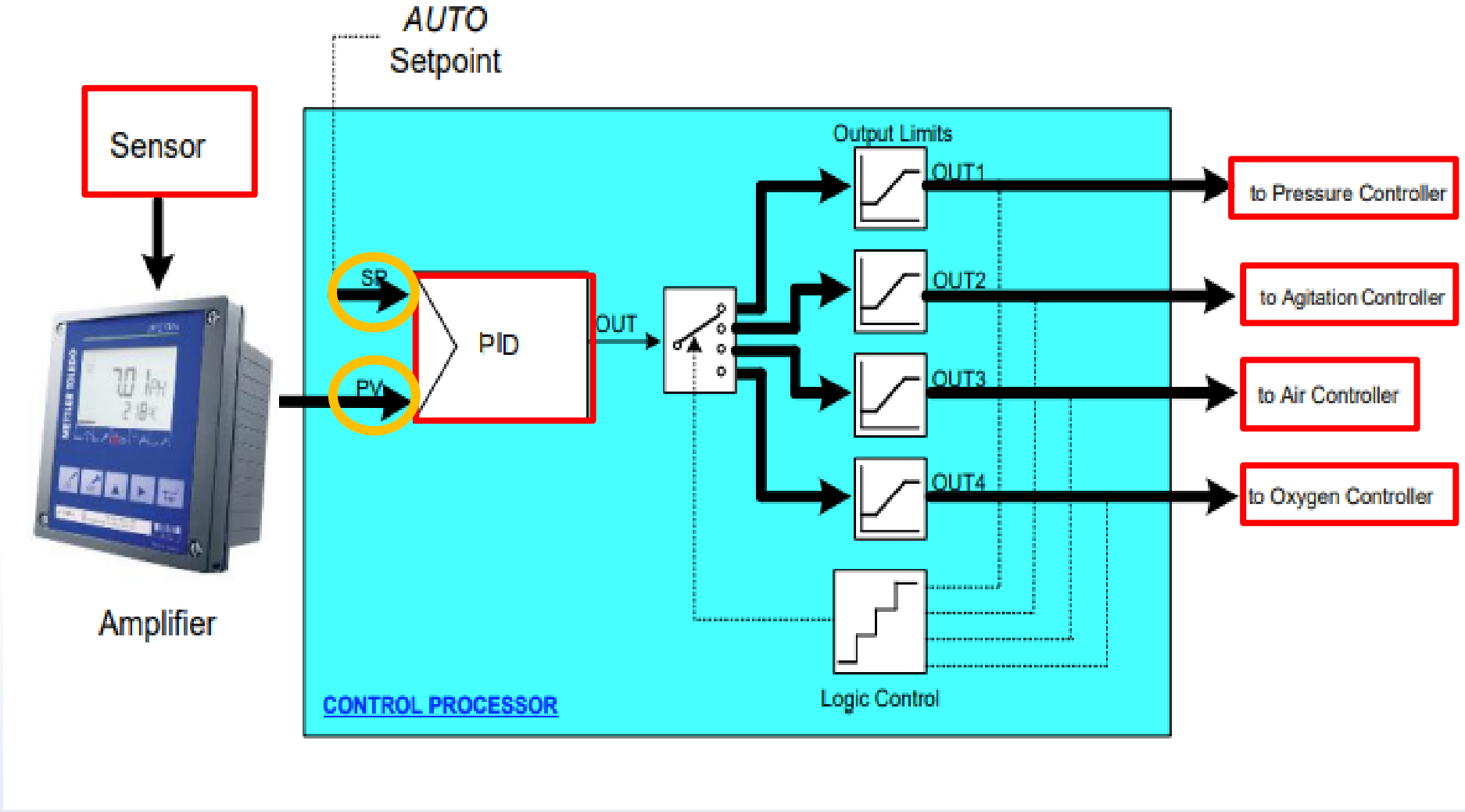
- The total pressure of a mixture of gases is equal to the sum of the partial pressures of all of the individual component gases.
  - The partial pressure is the pressure that each gas would exert if it alone occupied the volume of the mixture at the same temperature
    - Example:
      - In dry air, under 1bar
      - P(O2) = 1*0.2095 = 209.5 mbar

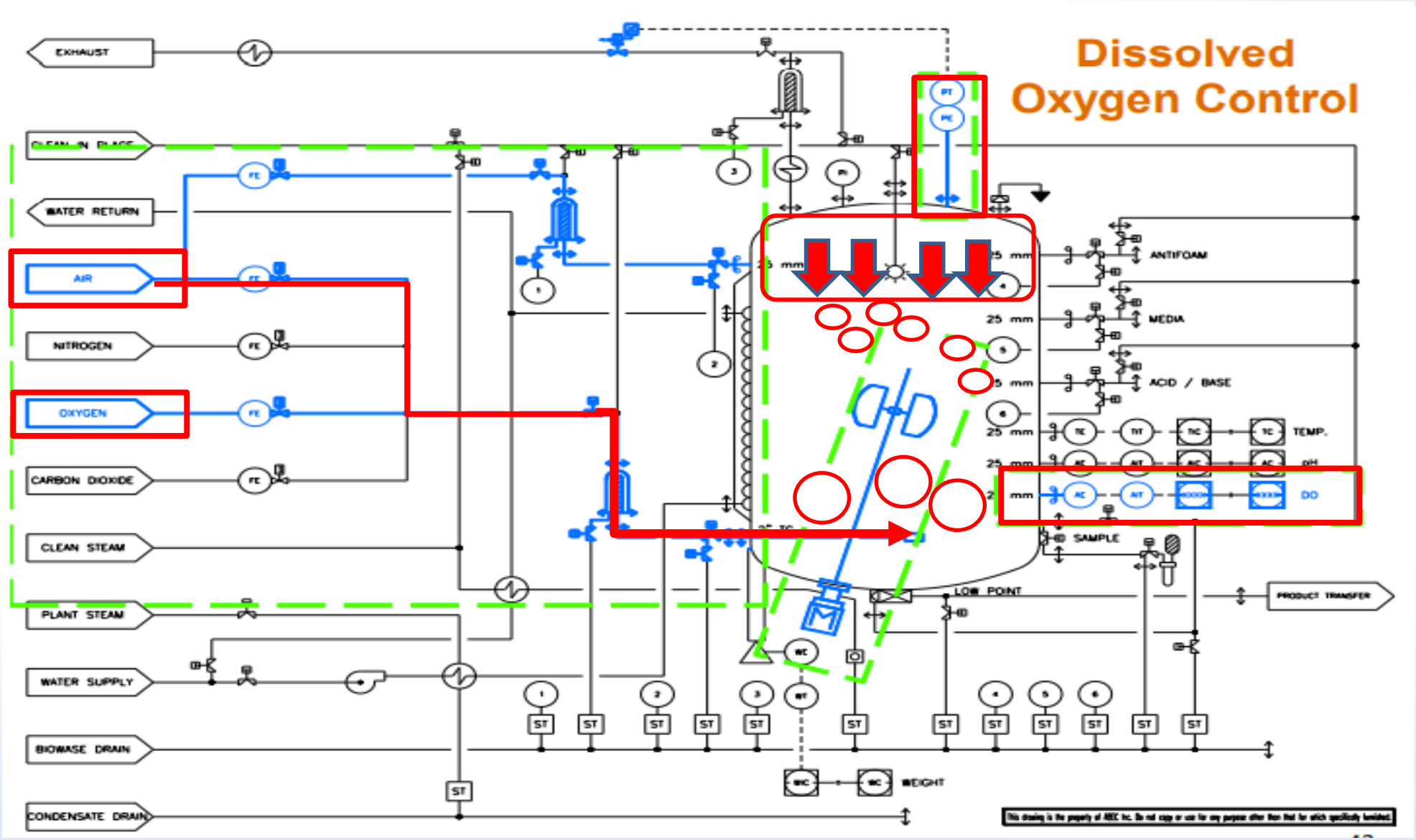
      (0.2095 because this is the proportion

      of air made up by oxygen (i.e. 20.95%)



Dissolved Oxygen Control




Dissolved
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EXHAUST
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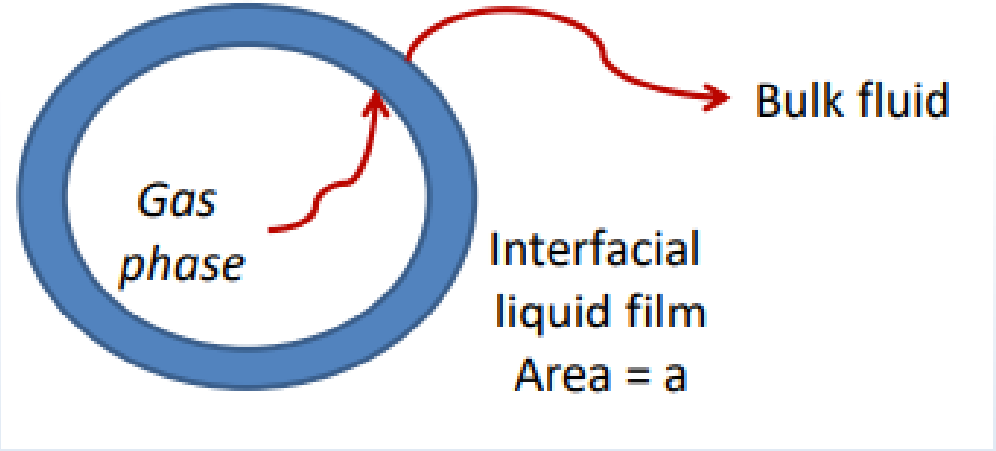
BIOWASE DRAIN
CONDENSATE DRAIN
ANTIFOAM
MEDIA
ACID / BASE
TEMP.
pH
DO
SAMPLE
LOW POINT
PRODUCT TRANSFER
WEIGHT

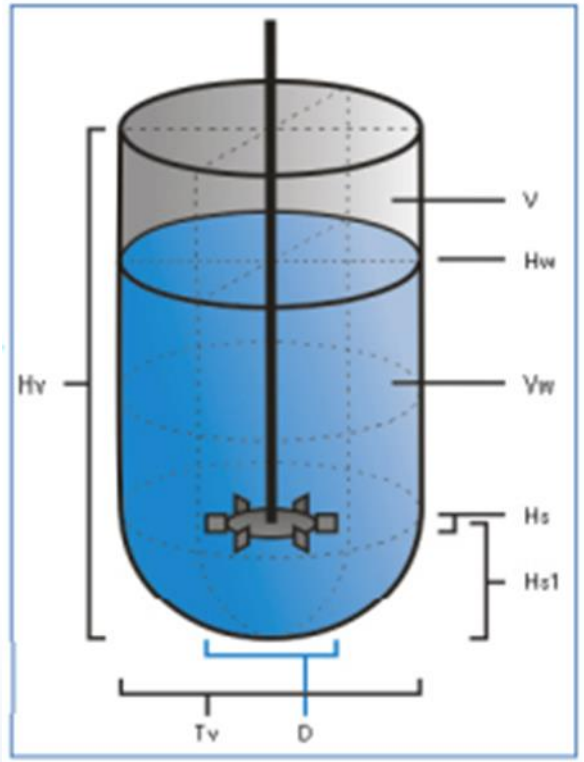
Impact of Vessel design on kLa

- The oxygen mass transfer coefficient is the rate at which oxygen is transferred from the gas phase to the liquid phase
  - kL—the liquid film transfer coefficient for oxygen
  - a—the interfacial area between the gas and the liquid
  - Rate is measured per hour (h^{-1}) or per second (s^{-1})



Impact of Vessel design on kLa

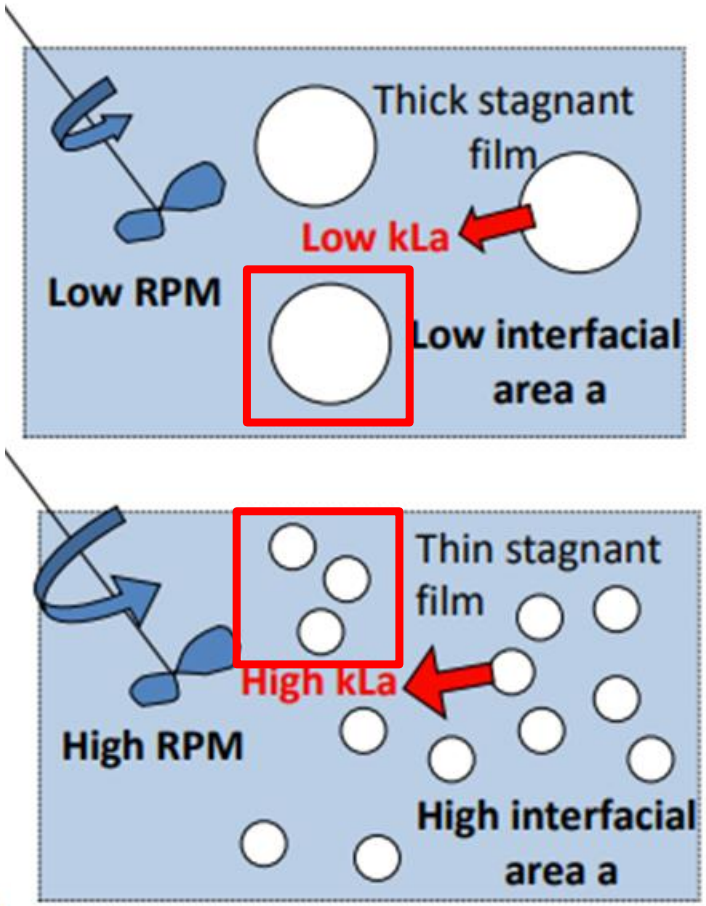
- Various aspects of the reactor design can have an effect on the kLa and thus the OTR available in a reactor.
- These include:
  - Operating volume
  - Internal volume
  - Location of impeller
  - Type of impeller
  - Number of baffles
  - Gas supply-sparger design
  - Height
  - Number of impellers
  - Impeller blade width/length
  - Baffle height/length



See lecture 7 Bioreactor Design & Engineering

Effect of Agitation on kLa

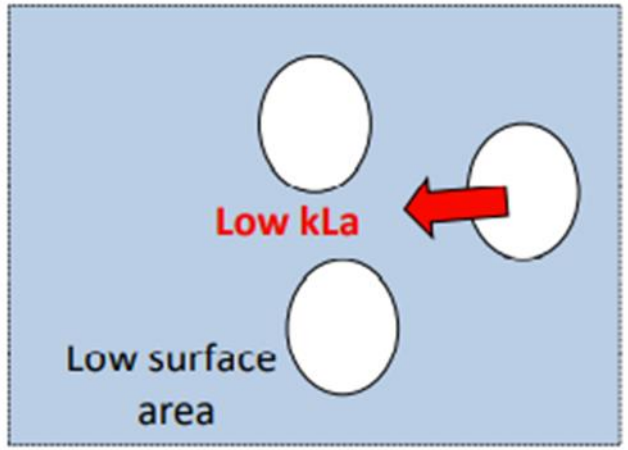
- An increase in agitation results in an increase in kLa
  - Agitation serves to disperse gas throughout the vessel
  - Agitation breaks up gas bubbles to form smaller bubbles with greater surface area

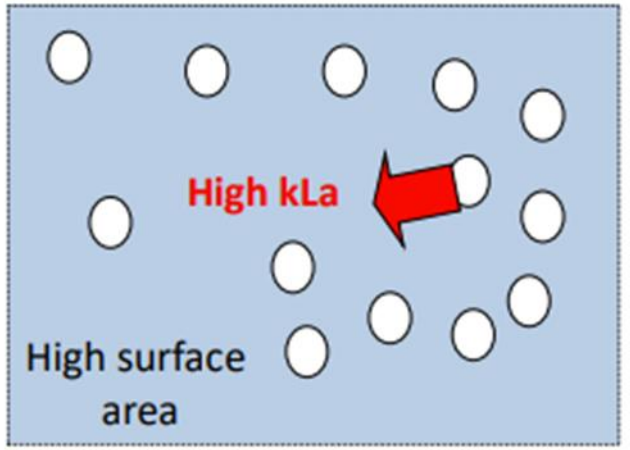


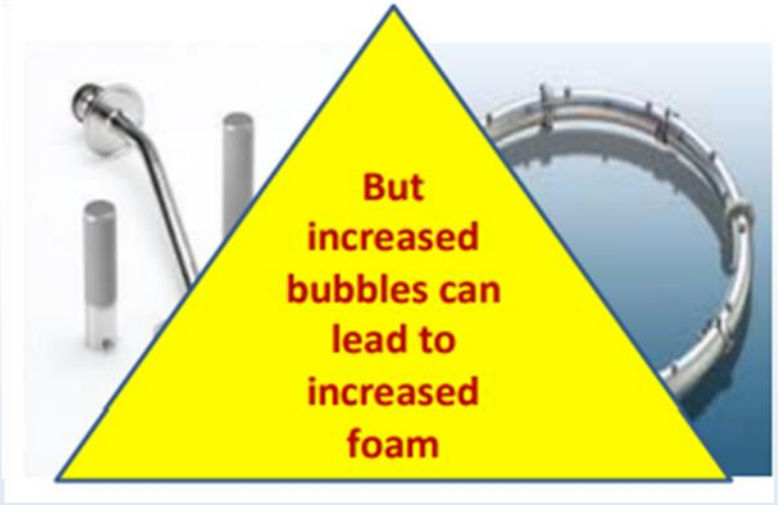
But increased agitation can lead to increased shear forces

Effect of Bubble Size on kLa

- Bubble size can be decreased by increasing agitation or by using a sparger with smaller holes—a sintered sparger
- A decrease in bubble size results in an increase in surface area (a) and an increase in kLa







Effect of Temperature on kLa

- As temperature is raised oxygen becomes less soluble in water

Temperature	Saturation O_2 Conc. (mg/L)
25°C	8.10
37°C	6.99

Foaming Problems

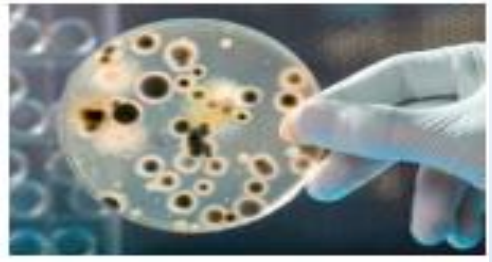
- If foam levels increase and escape from the reactor through the exhaust, it can wet and block the exhaust air filters.
- Filters blocked by foam decrease outgoing air flow. This means that:
  - Air will not pass through exhaust filters
  - Pressure may build or air flow into vessel may fall
  - DO_2 reduces to a critical level
  - Lactic acid production begins – reduced kLa
  - Cell viability affected – shear effect
  - Product quality potentially affected
- A filter bypass exhaust valve may be installed to open at high pressures to maintain airflows at expense of sterility control.

Foam in a Reactor

Cell cultures foam extensively for a variety of reasons:

- vessel volume
- culture medium contains proteins and amino acids
- cell lysis promotes foaming
- agitation and aeration

- Filters that get wet due to over foaming also become a potential entry point for contaminating micro-organisms.



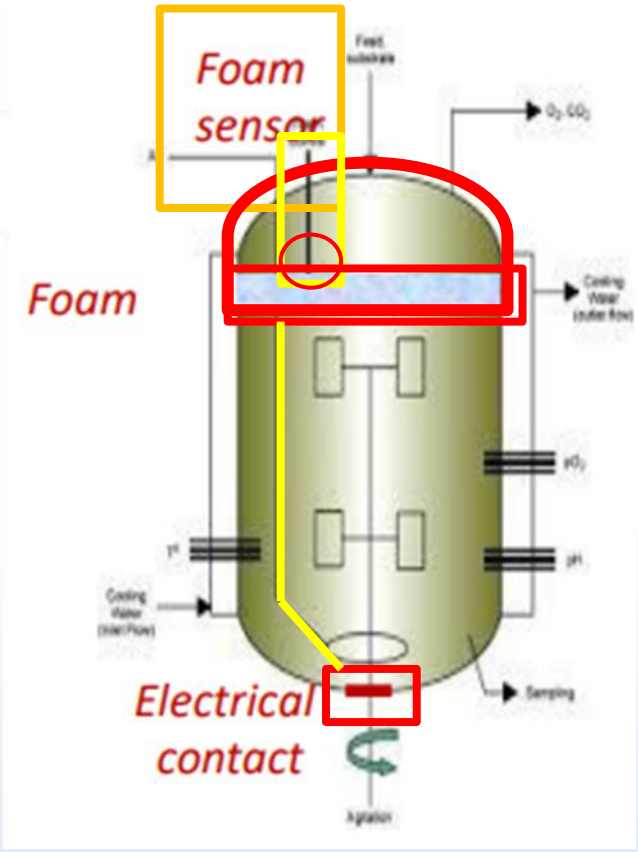
Foaming Problems

- If there is no bypass exhaust valve, blocked filters decrease outgoing air flow which may result in a pressure build up in the reactor

- A “bursting/rupture disc” is required on a reactor to relieve pressure in these situations. Bursting discs are non-reclosing devices that are designed to burst or rupture at a pre-determined pressure in order to relieve dangerous levels of pressure or a vacuum

Foam Control

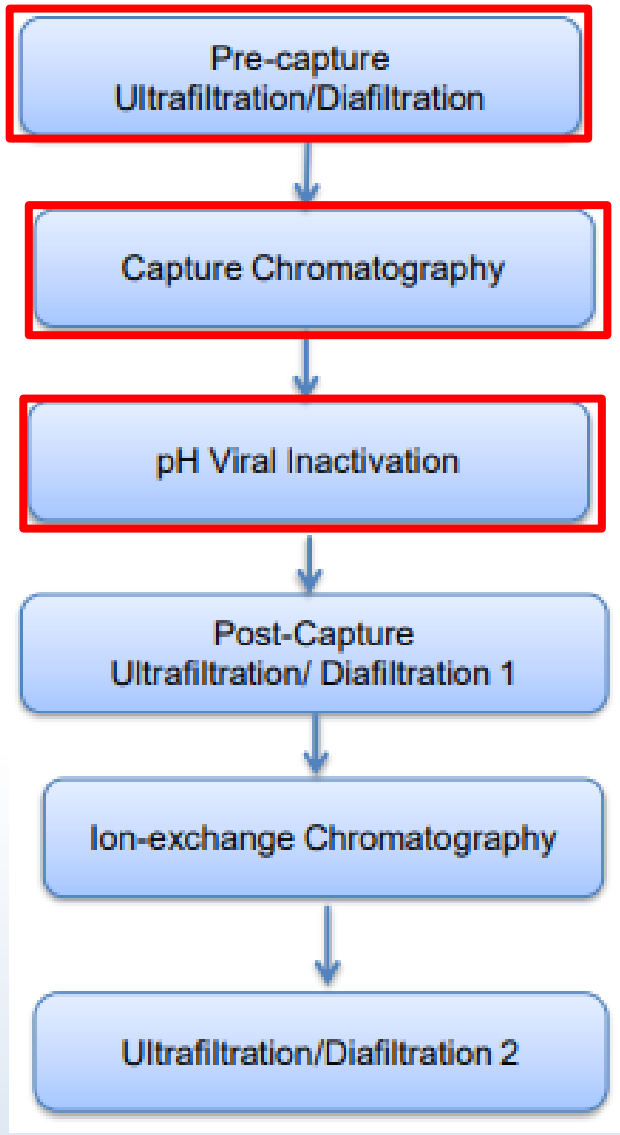
- Bioreactors are typically operated at a maximum working volume of 0.75 of the total volume to prevent foam-over and to allow for volume increase due to aeration and agitation.
- Foam can be controlled by either:
  - mechanical breakers (crude approach)
  - anti-foam chemical agents



AntiFoam Control

- Antifoam addition should be controlled and only added as required. Excess antifoam can have a major impact in downstream processing by fouling downstream membranes

- Antifoam can also have an effect on the kLa in a reactor



Antifoam affecting kLa

- Antifoam agents reduce the surface tension in foam and as a result the foam collapses.
- A reduction in surface tension = smaller bubbles
- This should increase $k_L a$ ↑ (the surface area is increased by having smaller bubbles)
- However, because of the viscosity of the antifoam agent, the interface mobility of the bubbles is significantly decreased by the antifoam agent, resulting in a decrease in ↓k_L a (transfer coefficient).
- The decrease in k_L offsets the increase in a, resulting in a net drop in the mass transfer coefficient.

∴ Antifoam reduces $K_L a$

k_L is the rate at which O_2 is transferred from the gas phase to the liquid phase

a is the interfacial area between the gas and the liquid

k_L is the liquid film transfer coefficient for O_2

Lecture Topics

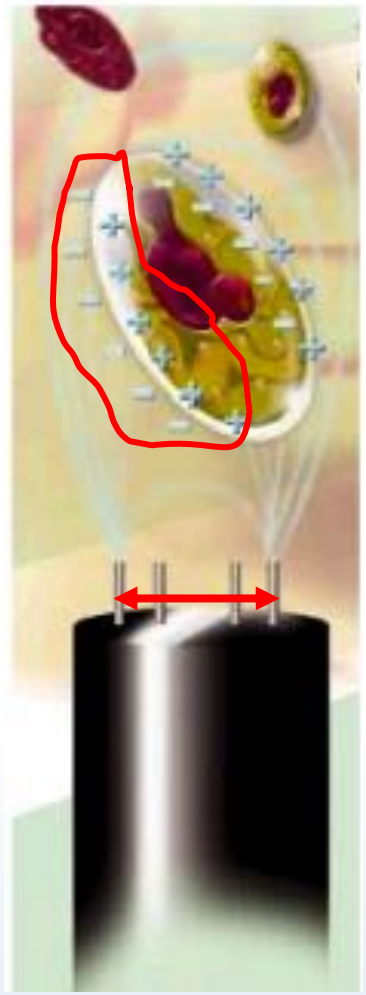
- Control of Bioreactor Operation Parameters
- Temperature Control
- pH Control
- Bioreactor Gas Control
- Cell Density Monitoring

Bioreactor Cell Density Control

- **Cell density control is desirable to ensure the following:**
  1. That the optimum cell density is achieved for overall system productivity.
  2. That the media supply is balanced to cell density – this prevents feast-famine cycles.
  3. Stable metabolic state to ensure stable product formation.
- **The main elements are as follows:**
  1. A means for monitoring actual viable cell density in culture medium e.g. Aber online or equivalent and Cedex offline or equivalent (or Coulter counter) technologies.
  2. Biomass removal system (biomass pump) to regulate the culture cell density.

The Aber Viable Cell Monitor

- Online measurement of Viable Cells (VC/mL).
- Capacitance Measurement - picofarads.
- Adjustments of on-line signal to off-line measurement of cell density using algorithm from the Cedex system.



Cedex Cell Counting System

- Sample taken up by syringe, mixed with a precise amount of Trypan Blue and incubated in the syringe to allow the dead cells to absorb the Trypan Blue
- Syringe then pumps cell mixture into the flow chamber in single steps
- A picture is taken each time the syringe pumps the cells through the flow chamber and the picture is analyzed

Summary

- Bioreactors - provide controlled environments for the aseptic growth of cells and sterile product formation.
- Modes of operation include batch, fed-batch and perfusion systems with perfusion offering the best performance w.r.t. Cell density and product formation.
- Critical parameters to be controlled are temperature (36+0.5°C), pH (6.8 to 7.4), DO and cell number & viability
- Use process control loops to monitor and control conditions in real time

Check out....

- *"Bioreactor Operational Excellence: Best Practices from Scale-up to Control"* By Brian J. Stamper and Cillian McCabe, BioProcess Research and Development, Eli Lilly and Company
- http://www.pharmamanufacturing.com/articles/20 09/045.html?page=1

Questions?

Sample Questions

- Write a detailed note on process control loops for bioreactors using pH as the exemplar.
- Effective oxygenation of bioreactors is probably the most critical success factors for bioreactor operations. Explain the factors at play in determining the success or failure?
- Write a note on temperature control in mammalian cell bioreactors. Include detail on the temperature values used, the impact of exceeding those values, and how the target values are maintained.