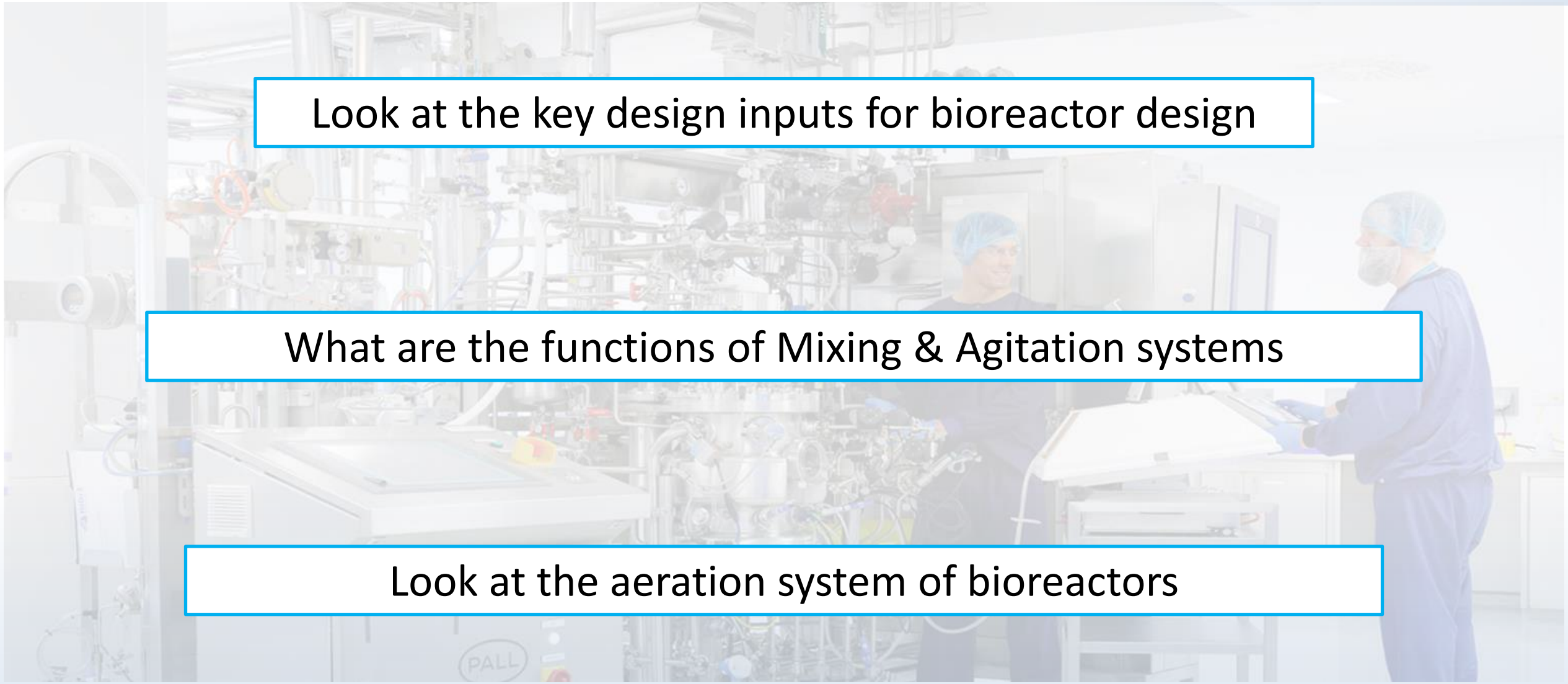
Level 8 Cell Culture Processing (BIO08045)
Lecture 7 – "Bioreactor Design"

- Dermot O' Sullivan
- Dermot.osullivan@nibrt.ie

Learning Objectives



Look at the key design inputs for bioreactor design

What are the functions of Mixing & Agitation systems

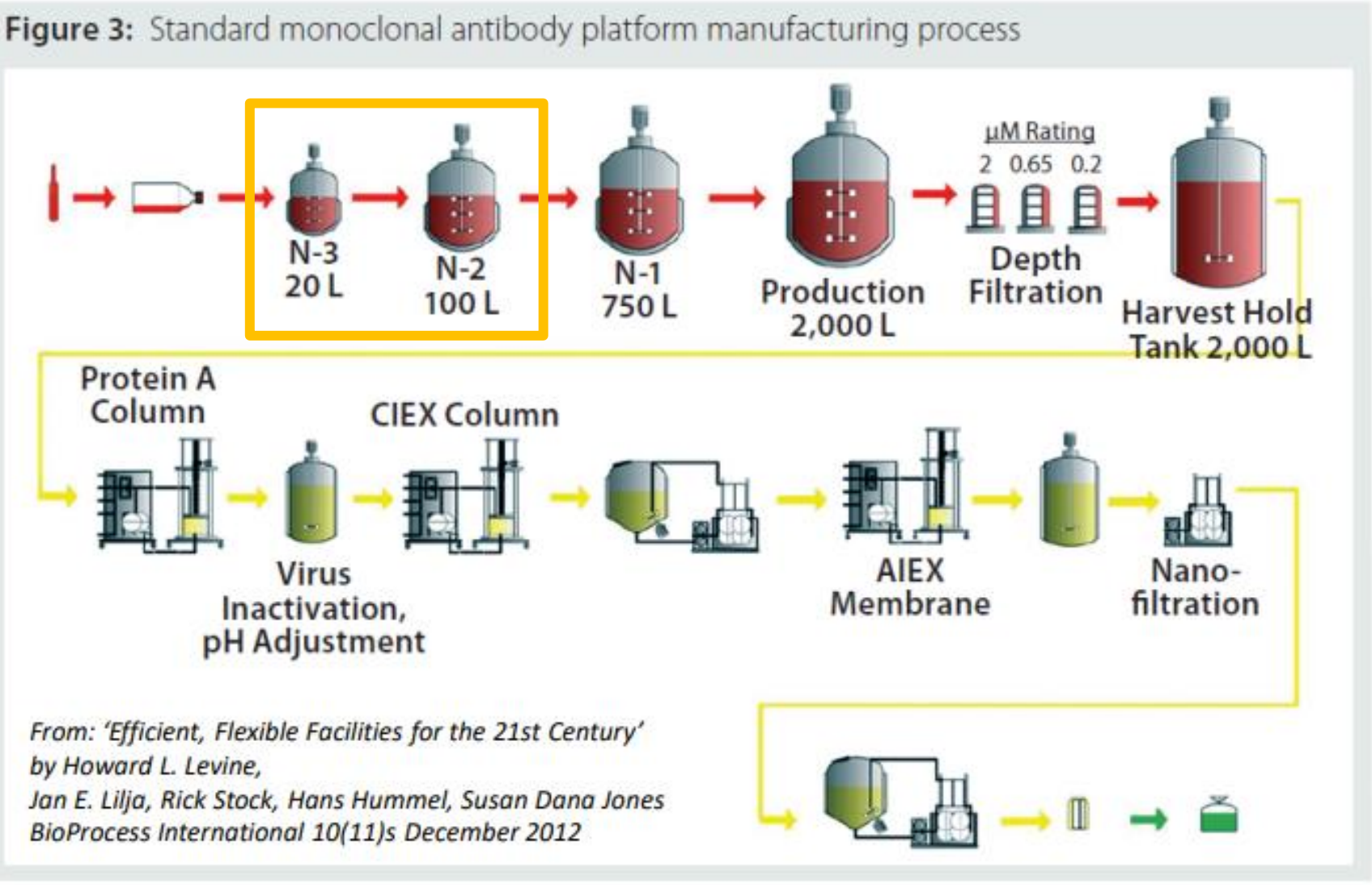
Look at the aeration system of bioreactors

Lecture Topics

- Bioreactor Design Considerations
- Bioreactor Design
- Mixing and Agitation in Bioreactors

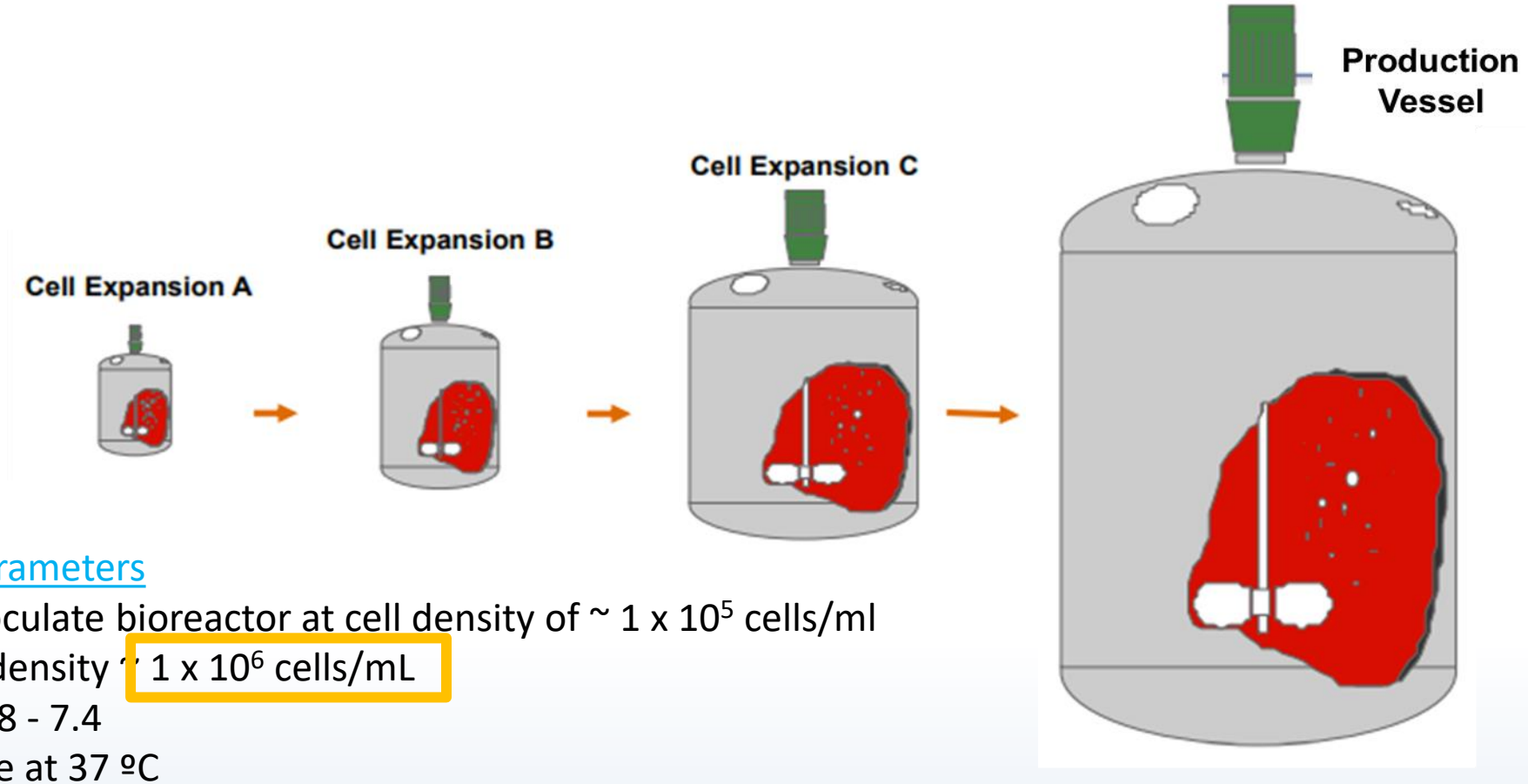
Scale-up of MAb Production

Figure 3: Standard monoclonal antibody platform manufacturing process



From: 'Efficient, Flexible Facilities for the 21st Century'
by Howard L. Levine,
Jan E. Lilja, Rick Stock, Hans Hummel, Susan Dana Jones
BioProcess International 10(11)s December 2012

Cell Expansion : Production



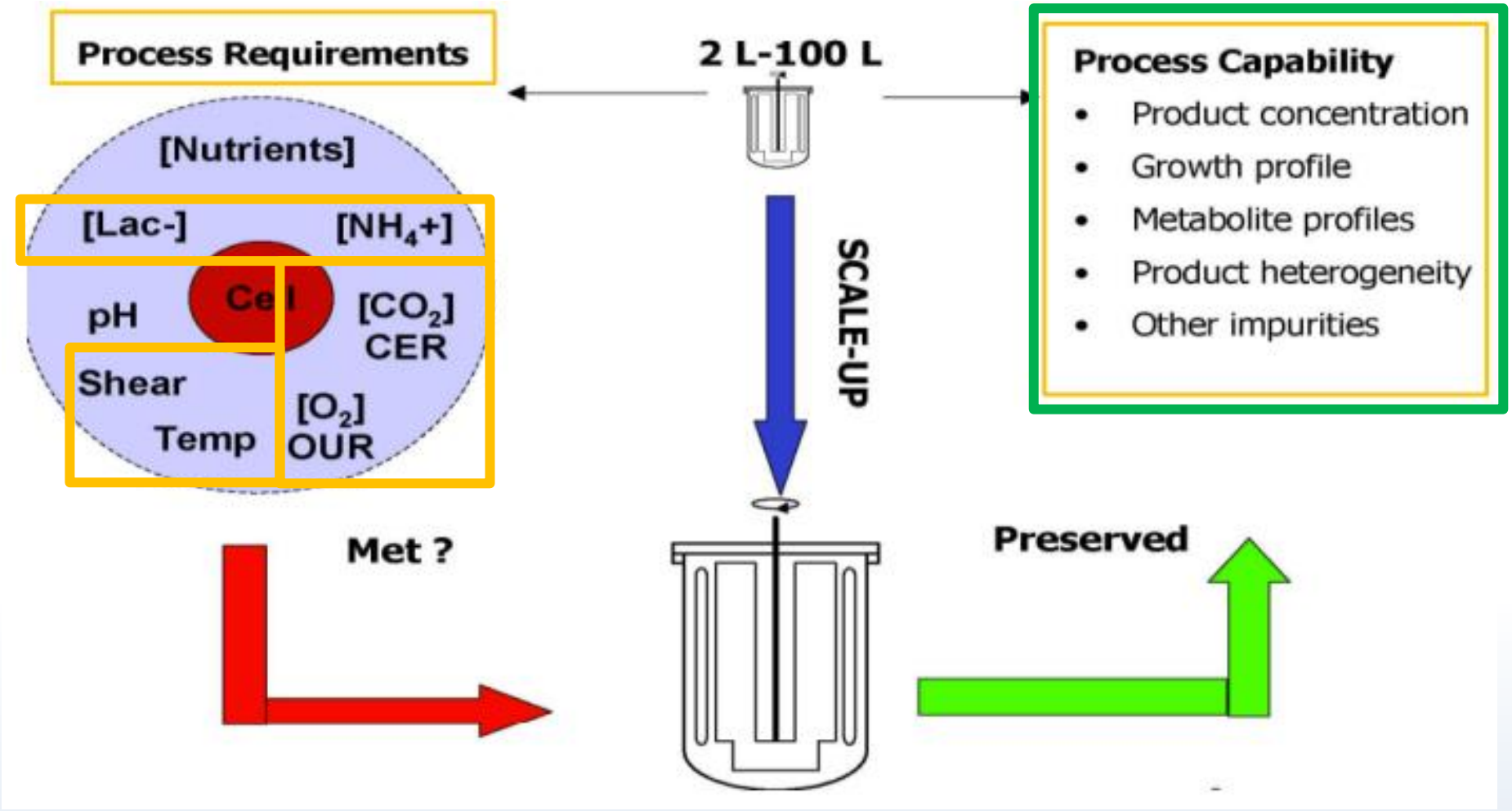
- Process Parameters

a) Typically inoculate bioreactor at cell density of ~ 1 x 10^5 cells/ml
b) Transfer at density ~ 1 x 10^6 cells/mL
c) pH kept ~ 6.8 - 7.4
d) Temperature at 37 ºC
e) DO – variable
f) Sparge rate ~ 0.1 vvm
g) Head pressure control ~ 2 – 4 psi

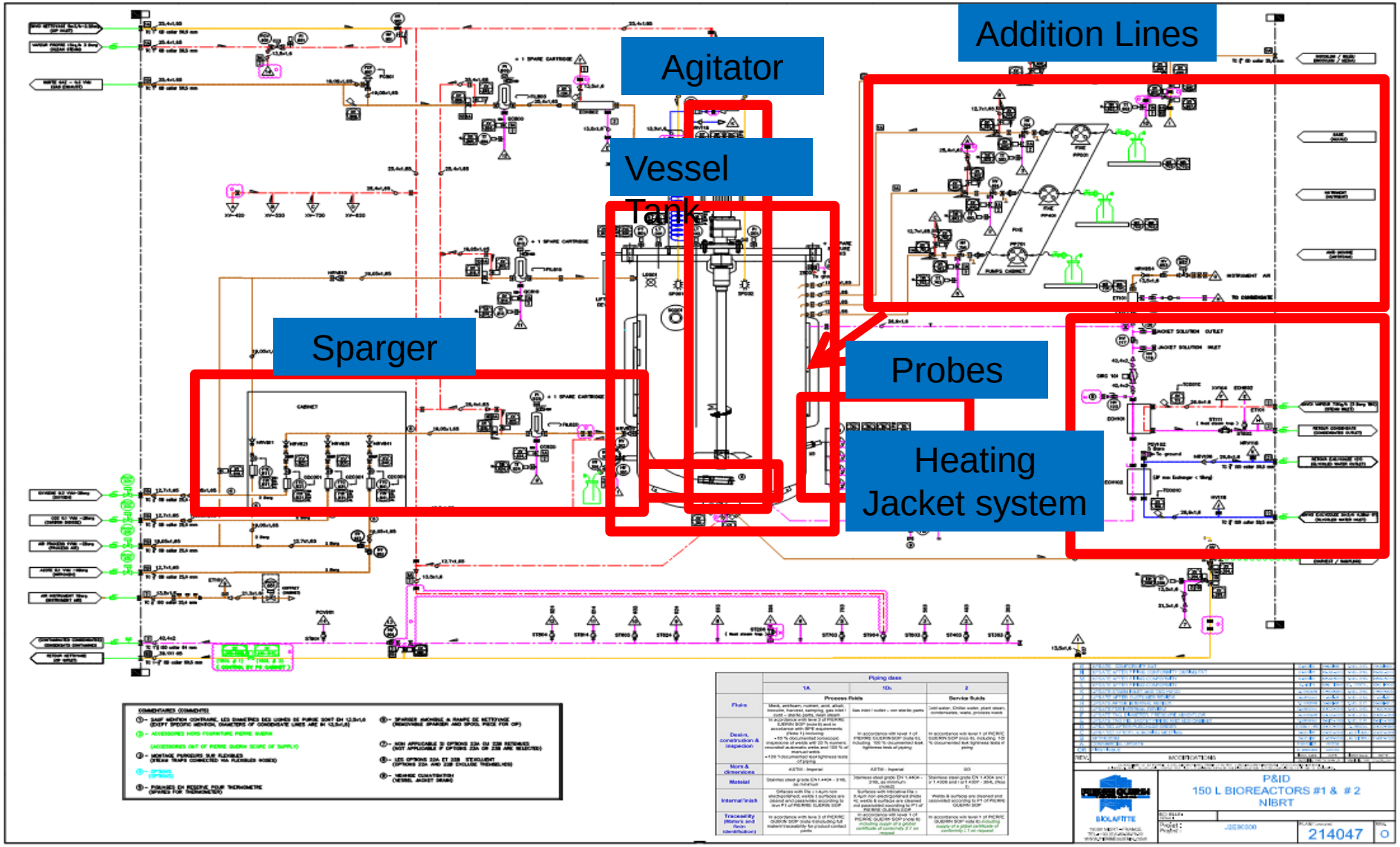
Bioreactor - Key Design Inputs

1. Microbial (E-coli) vs. Mammalian (CHO, BHK, NSO) cell-line.
2. Type of biologic product to be processed e.g. Mab (monoclonal antibodies), rhEPO (recombinant human erythropoietin), insulin etc.
3. The Quantity of Product to be produced – kgs. of biologic p.a.
4. The productivity (volumetric, specific, global) targets.
5. Mode of operation – batch, fed-batch, perfusion/continuous.
6. Yield / Conversion Rates.
7. **Quality of Product required:**
   a) Its overall purity levels.
   b) The sequence of steps involved in the production process.
   c) Is post-translational modification such as glycosylation required?
   d) What activity level does the final product need to have (in vitro, in vivo)?

Typical Bioreactor Design Scale-up Factors

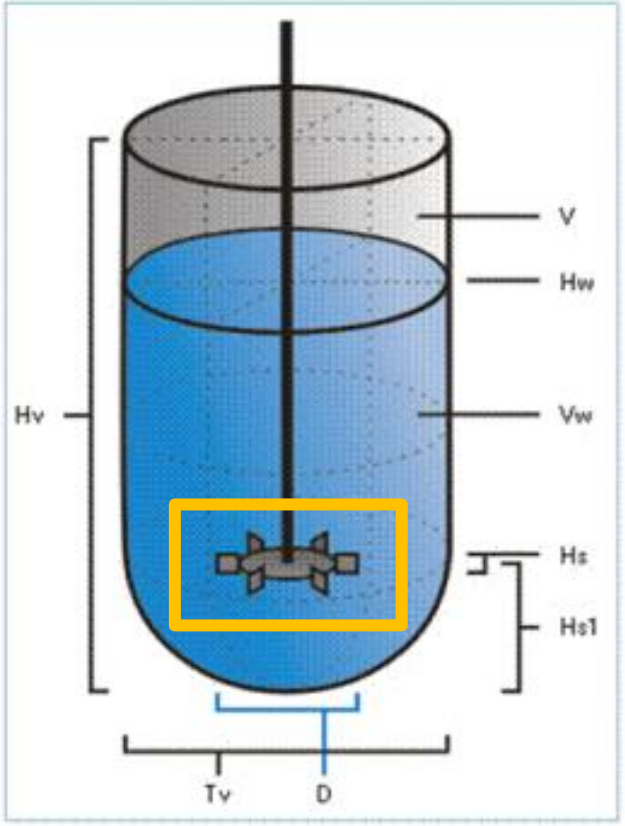


Bioreactor P&ID Piping & Instrumentation Diagram



Bioreactors

Bioreactor Design Parameters

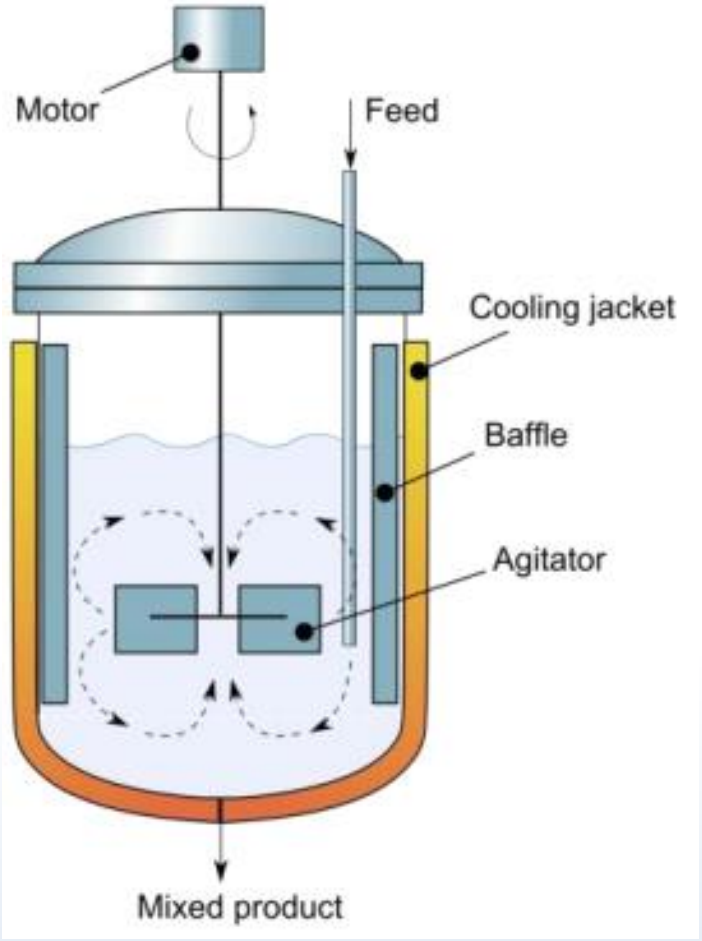


From bioreactordesign.org

V:	the total volume of the vessel (m^3)
H_v:	the height of the vessel (m)
T_v:	the diameter of the vessel (m)
V_w:	the working volume of the vessel (m^3)
H_w:	the level of the liquid (m)
D:	the diameter of the stirrer (m)
N_{stir}:	the number of stirrers
Hs1:	position of stirrer 1 from the bottom of the vessel (m)
Hs:	the height of the stirrer blade (m)
$N_{baffles}$:	the number of baffles in the vessel
Np:	the power number of the stirrer
start rpm:	the starting speed of the calculation in rounds per minute
delta rpm:	steps of increasing the speed of the stirrer during calculation in rounds per minute

Bioreactors

- Design Criteria:
  - Working Volume
  - Agitation – duration & speed
  - Aeration System
  - Heat Transfer Capability
  - Temperature Control
  - Exhaust
  - Perfusion



Bioreactors

- **Bioreactor system – key issues for specification**
  - **Bioreactor vessel**
    - —Volume, H/D ratio, freeboard (foaming)
- **Agitation**
  - Marine impeller or Rushton turbine
  - Top or bottom mounted
  - Double mechanical seal or mag-drive
  - Vent heater and/or condenser
  - Sampling ports – steam through or septum type

Bioreactors – Key Equations

Oxygen transfer rate > oxygen uptake rate

$$OTR_{fermentor} \geq OUR_{organism}$$

$$k_L a\left(C^* - C\right) \geq q_{O_2} X$$

Where:

$k_L a$ = oxygen mass transfer coefficient

qO_2 = specific oxygen utilization rate

X = biomass concentration

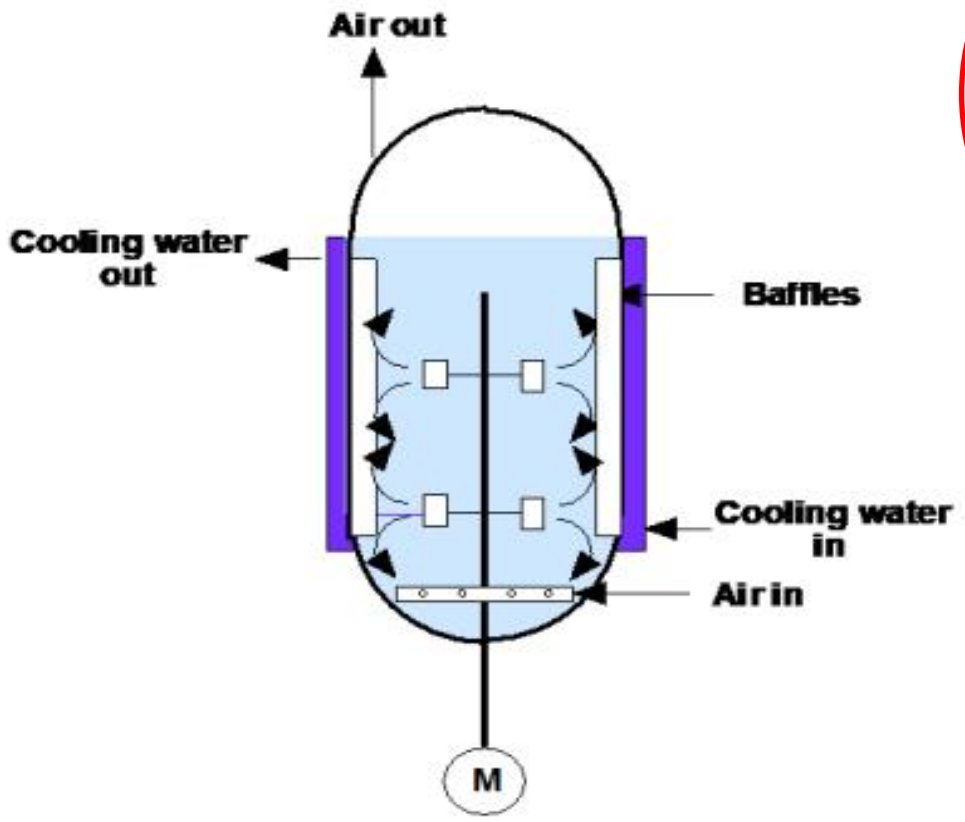
C* = saturated oxygen concentration

C = bulk oxygen concentration

Lecture Topics

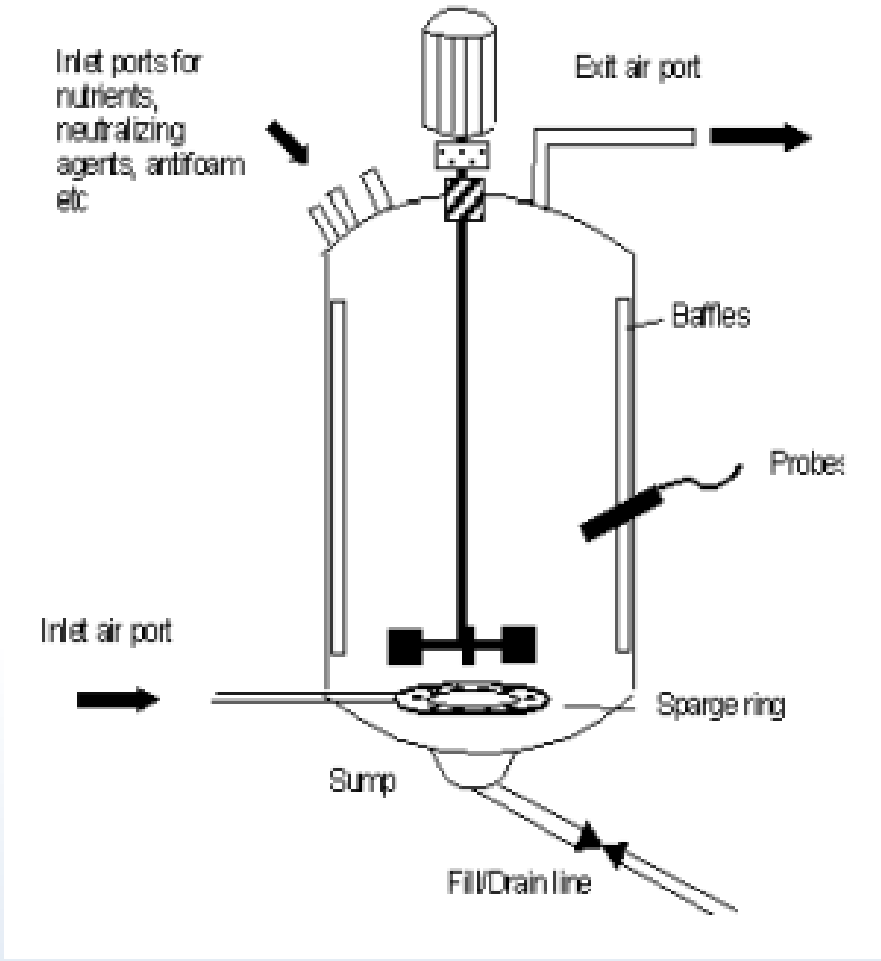
- Bioreactor Design Considerations
- Bioreactor Design
- Mixing and Agitation in Bioreactors

Typical Stirred Tank Bioreactor Design



Stirred tank bioreactor (STBR)

- Basic bioreactor contains
  - a) **Agitation system:** agitator and baffles
  - b) Oxygen delivery system: inlet air port, **sparger and exit air port**
  - c) **Foam control** system
  - d) **Temperature control** system
  - e) **pH control** system
  - f) **Sampling ports** (ports for adding nutrients, cells etc)
  - g) **Cleaning and sterilization system**
  - h) **Sump and dump line** for emptying of the reactor

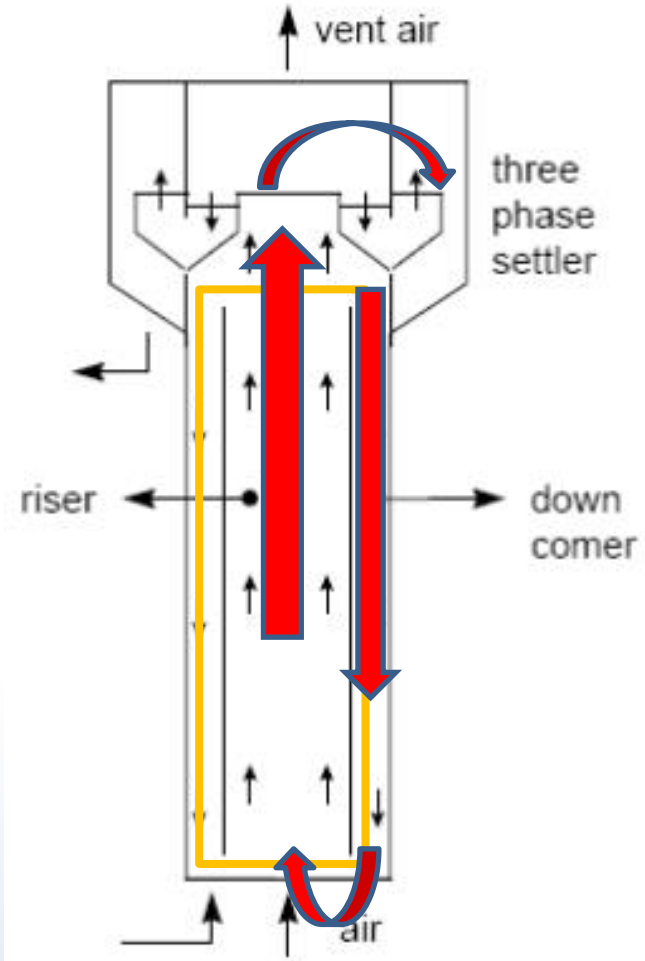


Advantages / Disadvantages of STBR

- Advantages of STBR
  a) Suitable for suspension, immobilized and encapsulated cells
  b) Multi-functional - may be used for multiple products
  c) Relatively low installation costs
  d) Ease of operation & maintenance
  e) May be operated in batch, fed-batch or continuous modes
  f) May be combined with cell recycle and perfusion systems to increase productivity
  g) Ease of validation, lot-to-lot variation reduced, robust design

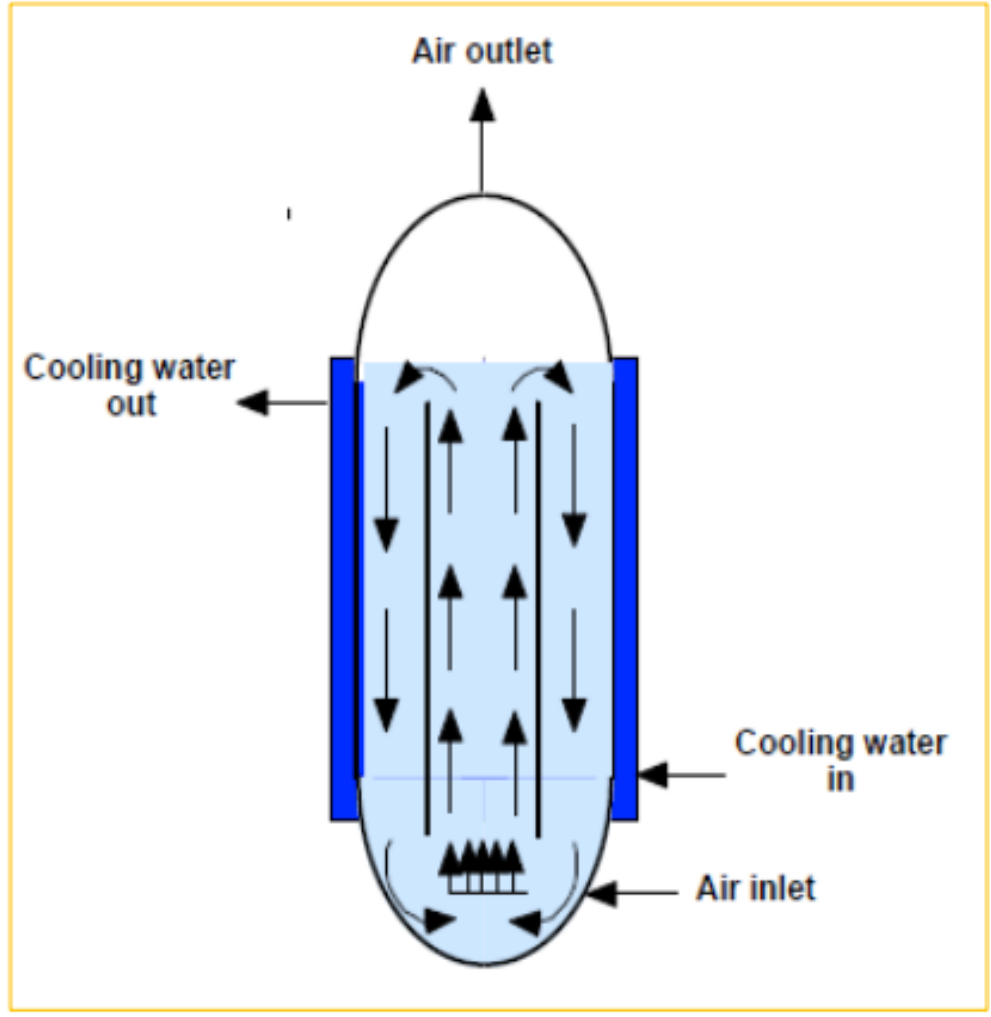
- Disadvantages of STBR
  a) Mass transfer limited - requires sparging (low O_2 solubility)
  b) Scale-up - difficult to maintain constant oxygen transfer rates
  c) Relatively low cell densities in suspension ≤2-3 x10^6/mL
  d) Aggregation of cells
  e) Shear sensitivity of cells
  f) Integration with DSP requires suitable scaling

Airlift Bioreactor Designs



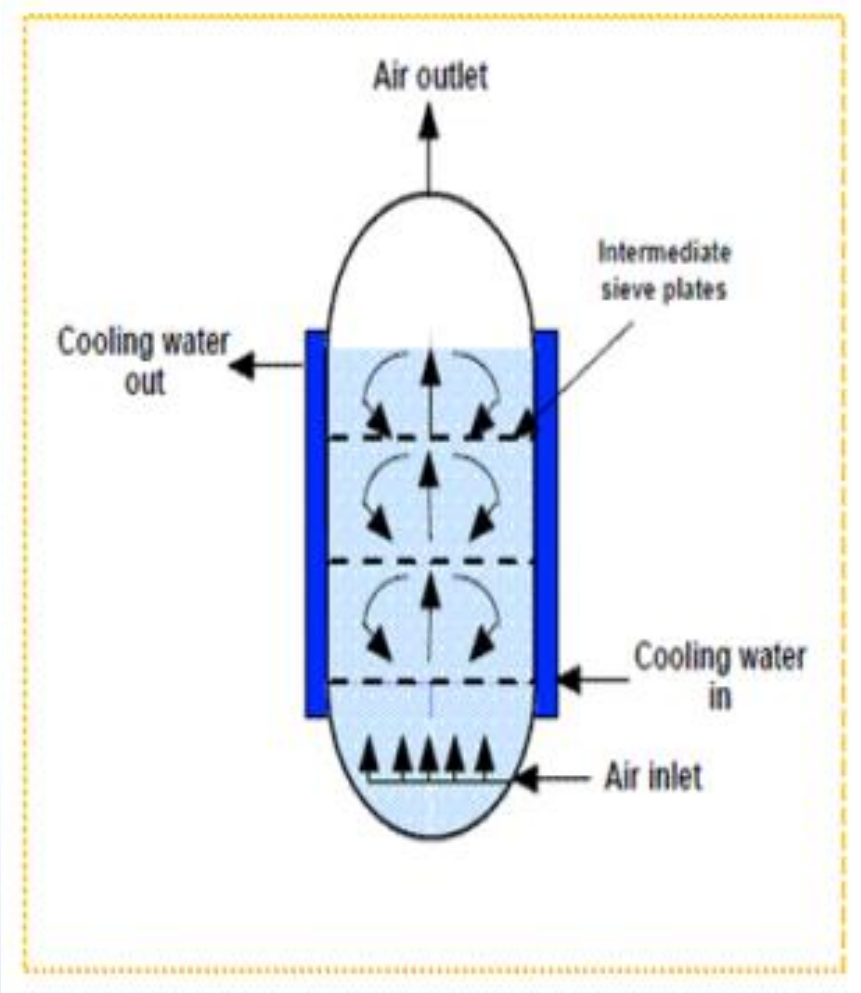
Airlift Loop Bioreactors

- Bubbles rising in the centre draft tube causes mixing to occur
- This carries fluid and cells up the draft tube
- Gas leaves the liquid at surface and degassed liquid descends in a loop downwards.
- Less shear and good oxygen transfer rates



Bubble Column Reactor

- Bubble columns disperse gas through the bioreactor using perforated plates to enhance gas dispersion and mixing.
- Provide a low-shear environment, although cells often accumulate at the surfaces of bubbles and bubble bursting at the surface can damage or destroy cells.
- Energy-efficient (low power input required to transfer a given amount of oxygen, relative to stirred-tank bioreactors).



Construction of Bioreactors

Lab-Scale <10L

Glass vessels

Sterile by autoclaving

Pilot to industrial scale >10L up to 25,000L

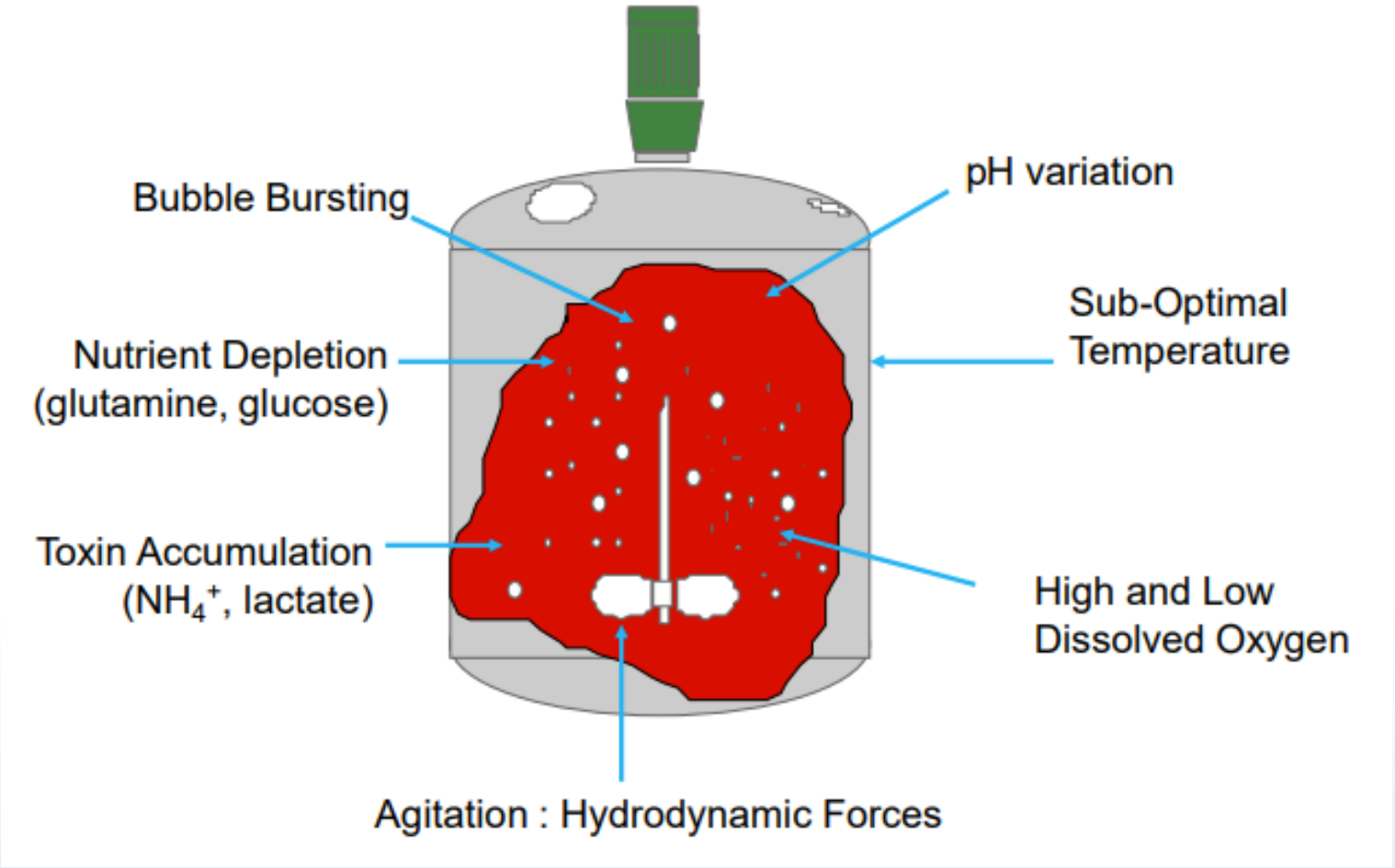
316L stainless steel construction

Electro-polished welded joints

Automated clean-in-place (CIP) systems

Sterile-in-place (SIP) using steam under pressure

Factors affecting cell viability & productivity in a bioreactor



Lecture Topics

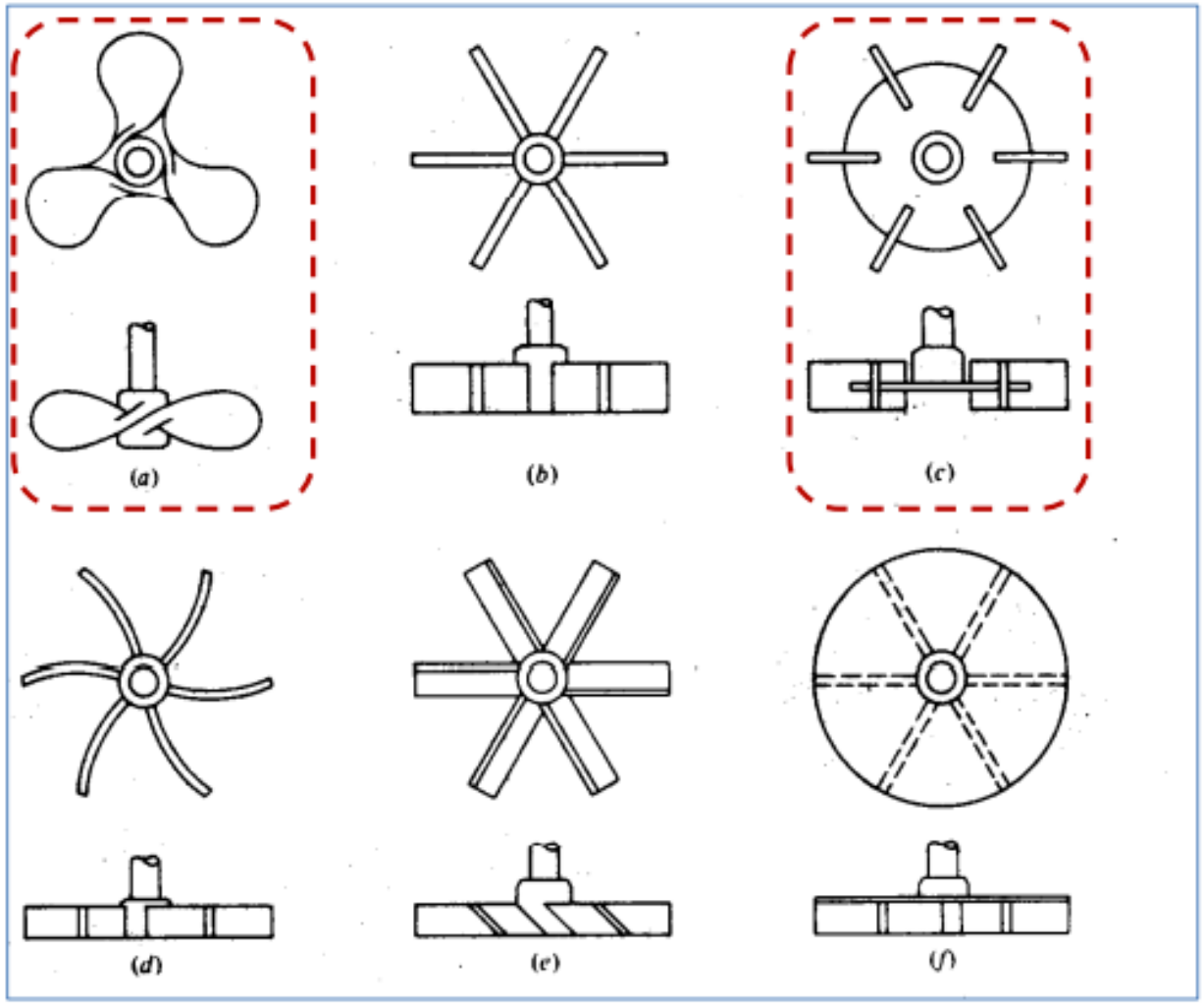
- Bioreactor Design Considerations
- Bioreactor Design
- Mixing and Agitation in Bioreactors

Mixing in Stirred Tanks

- Reactions/separations depend on 'good' mixing of all reagents.
- Aim to ensure uniform distribution of all elements of the system
  a) temperature & concentration
  b) blending and dissolution
  c) dispersion of multiple phases (gas, liquid and/or solid)
  d) heat transfer
  e) type of mixing device
  f) power required to mix

Mixing in Stirred Tanks

Marine impellor



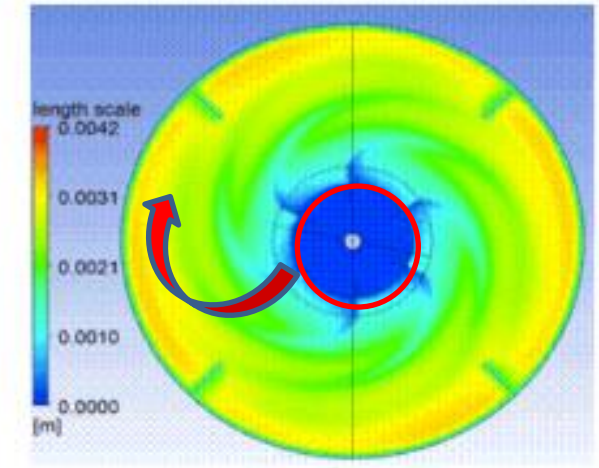
Rushton impellor

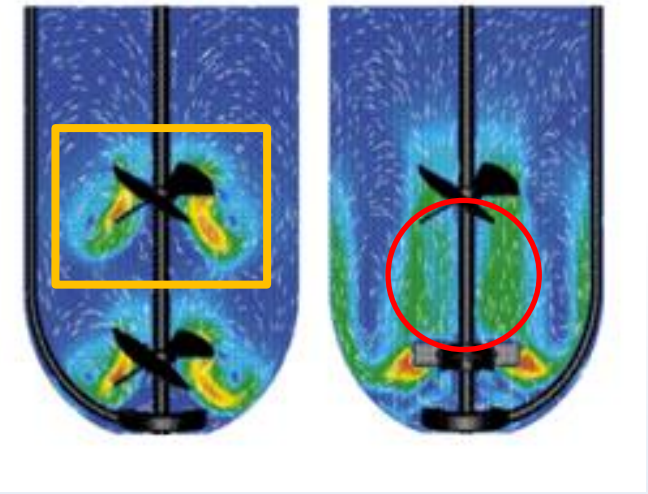
Mammalian cells and agitation

- The ‘fragility’ of mammalian cells in bioreactors has long been debated and cells can be damaged by various forces acting in stirred culture.
- The major damaging force is from **bubbles bursting** on the surface of cell.
- **Hydrodynamic shear** force resulting from motion of stirrer is of less importance.
- Stirring speeds are generally 150 – 200 rpm which is considerably less than for microbial cells (up to 350 rpm).
- To ensure adequate mixing at low speeds, the bottom of small glass bioreactors is usually round.

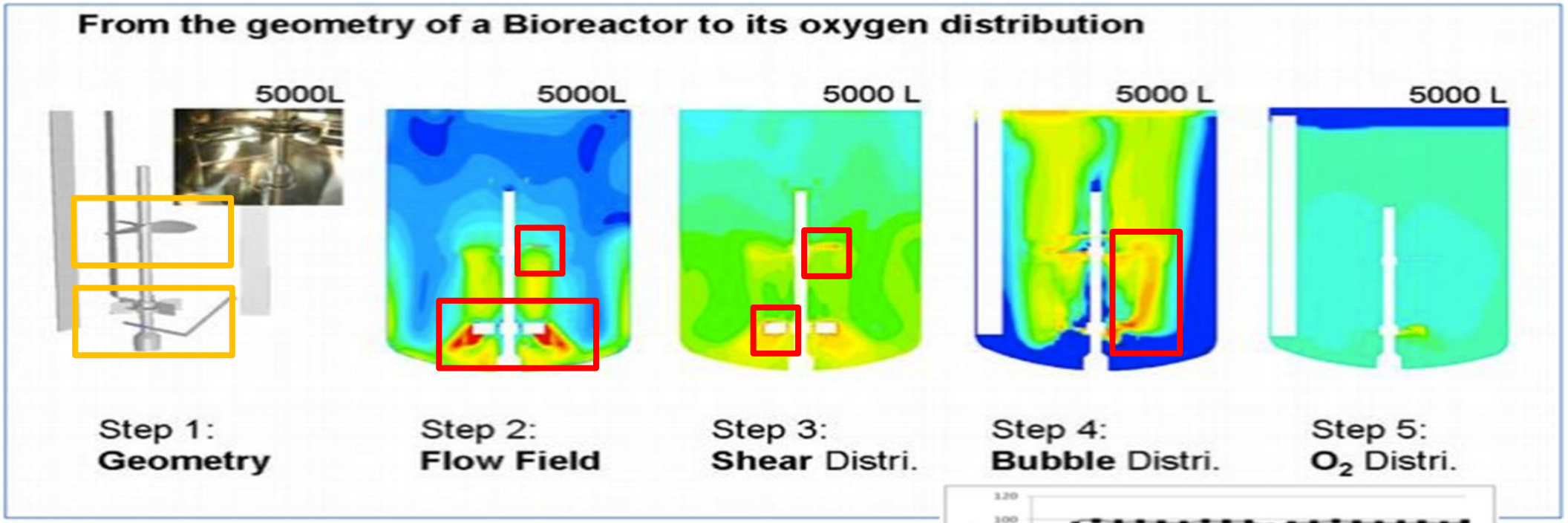
Bioreactor Agitation

- Mechanical agitation achieves the following:
  1. Disperses media nutrients throughout the cell culture solution.
  2. Disperses gas bubbles throughout the bioreactor.
  3. Increases the residence time of bubbles in the cell culture.
  4. Shears large bubbles to form smaller bubbles better oxygen transfer.
  5. Improves heat transfer and dissipation.





Bioreactor Agitation



http://www.morbidelligroup.ethz.ch/research/PRP/BenjaminNeunstoecklin/CFD.png

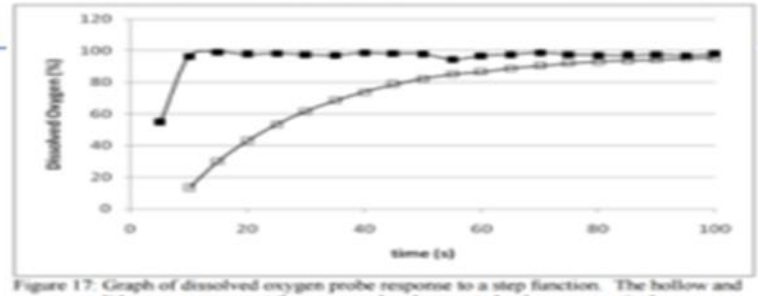


Figure 17: Graph of dissolved oxygen probe response to a step function. The hollow and solid squares represent the measured and corrected values, respectively.

29

From: Ryan Z. Davis 2010 Design and Scale-Up of Production Scale Stirred Tank Fermentors - thesis Utah State University

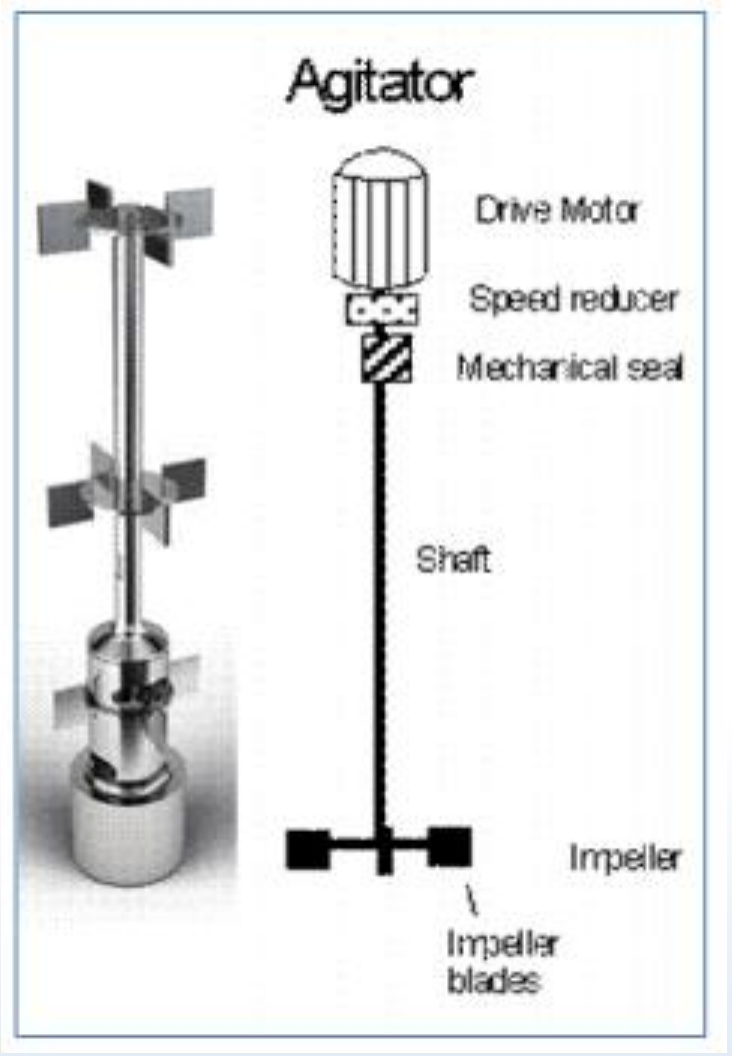
http://digitalcommons.usu.edu/cgi/viewcontent.cgi?article=1533&context=etd

Bioreactor Agitation

- Agitation systems may consist of an agitator and baffles
  - Agitator consists of drive motor, speed control, shaft and impeller
  - A control system comprising a revolution counter (RPM) and a power monitoring system to monitor the power levels drawn by the drive motor.
- The agitator may be either top or bottom mounted, direct or magnetic drive.
  - **Bottom mounted** agitators require a shorter shaft but require higher maintenance due to damage of seals in drive shaft
  - **Top mounted** agitators are not submersed in culture resulting in reduced risk of contamination.

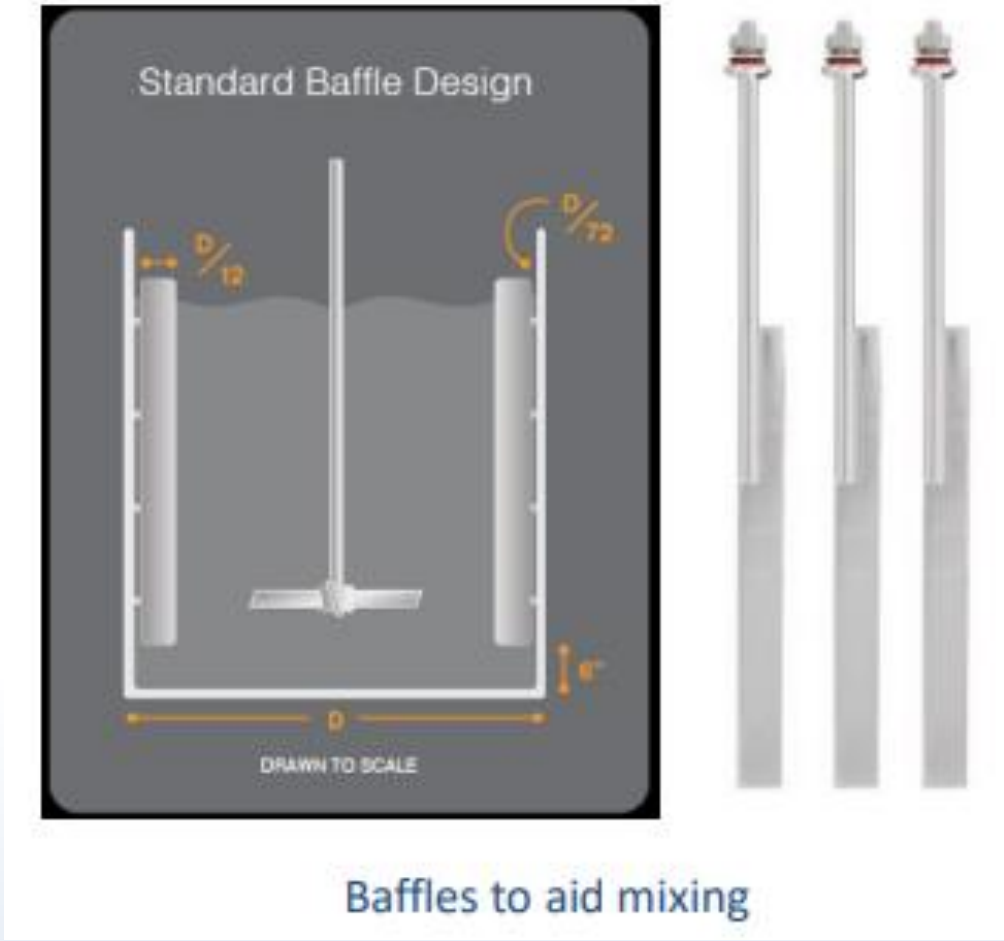
Agitation in bioreactor

- Agitation provides mixing of liquid and cells and liquid and gas leading to increased mass transfer rates
- Agitation provides the appropriate shear conditions required for breaking up bubbles
- Agitation system consists of an agitator (usually bottom mounted on large vessels) and baffles
  - **Agitator** consists of drive motor, speed control, shaft and impeller
  - **Baffles** (indentations in bioreactor) are used to break the liquid flow to increase turbulence and mixing efficiency



Bioreactor Agitation

- Agitation systems may consist of an agitator and baffles.
- Agitator consists of drive motor, speed control, shaft and seals and impeller.
- A control system comprising a revolution counter (RPM) and a power monitoring system to monitor the power levels drawn by the drive motor.

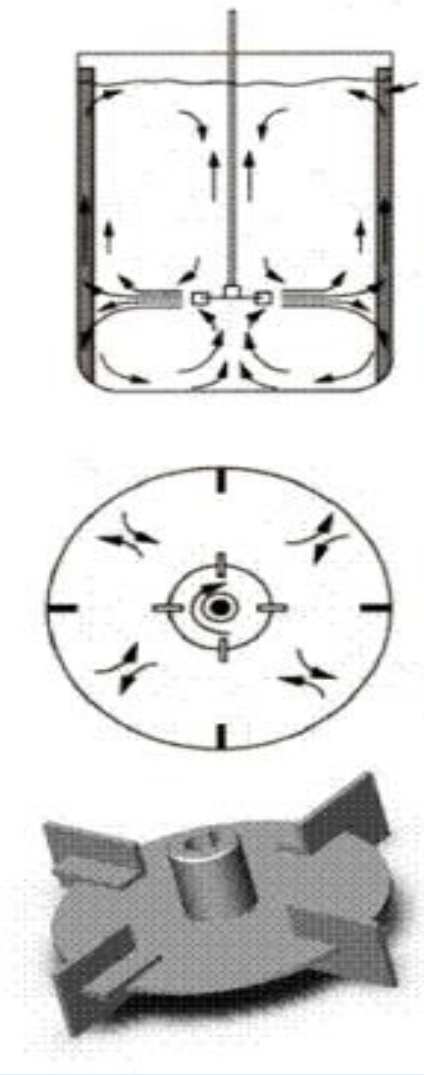


Baffles to aid mixing

Bioreactor Agitation

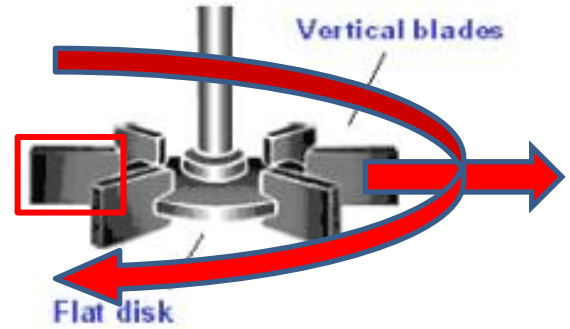
Radial flow impeller (Rushton Impeller)

- Radial flow impellers have blades which are parallel to the vertical axis of the stirrer shaft and tank.
- Radial flow drives fluid radially from the impeller in a direction perpendicular to the impeller shaft and tank walls. The fluid travels to the walls of the tank where it divides into two streams, one flowing up to the top of the tank and the other flowing down to the bottom.
- Rushton impellers provide more vigorous mixing with 4 major streams of fluid flow. This turbulent and vigorous flow regime encourages aeration of the culture but creates high shear rates. Radial flow impellers are therefore, the impeller of choice for fermentation where oxygen uptake rates are much higher than mammalian cell culture and where cell lines are not sensitive to shear stresses



Mammalian cells and Agitation

- Microbial bioreactors have radial flow impellers e.g. Rushton impeller
- For mammalian cells impeller will generate vertical and horizontal flow
- Axial flow (pitch impeller blades) which limits shear damage
- Also very popular is the marine type impeller
- Bubbles lead to foaming: usually add antifoam agents e.g. Pluronic F-68 or simethicone

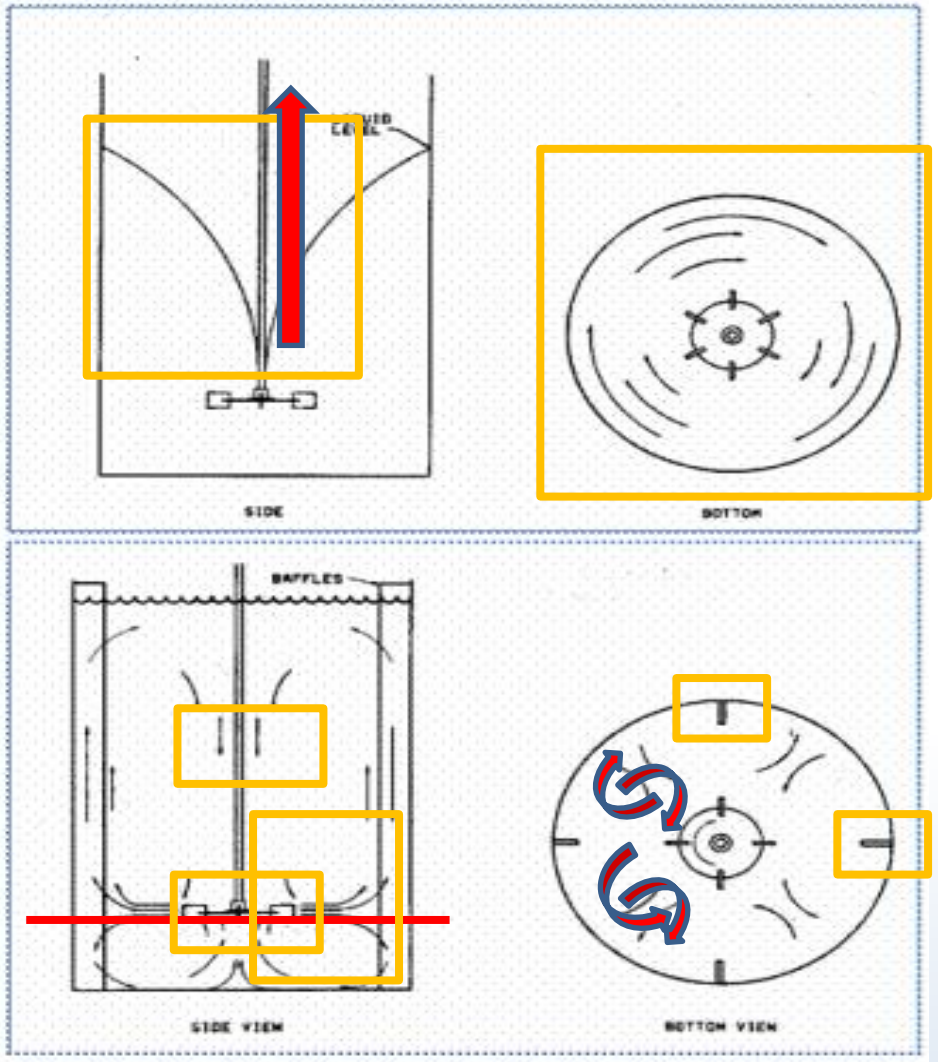


Radial flow impeller

Axial flow impeller

Mixing in Stirred Tanks

Standard impeller - Rushton turbine



Bioreactor Agitation – Rushton Impellor

$$\frac{D_a}{D_t} = \frac{1}{3} \qquad \frac{H}{D_t} = 1 \qquad \frac{J}{D_t} = \frac{1}{12}$$

$$\frac{E}{D_a} = 1 \qquad \frac{W}{D_a} = \frac{1}{5} \qquad \frac{L}{D_a} = \frac{1}{4}$$

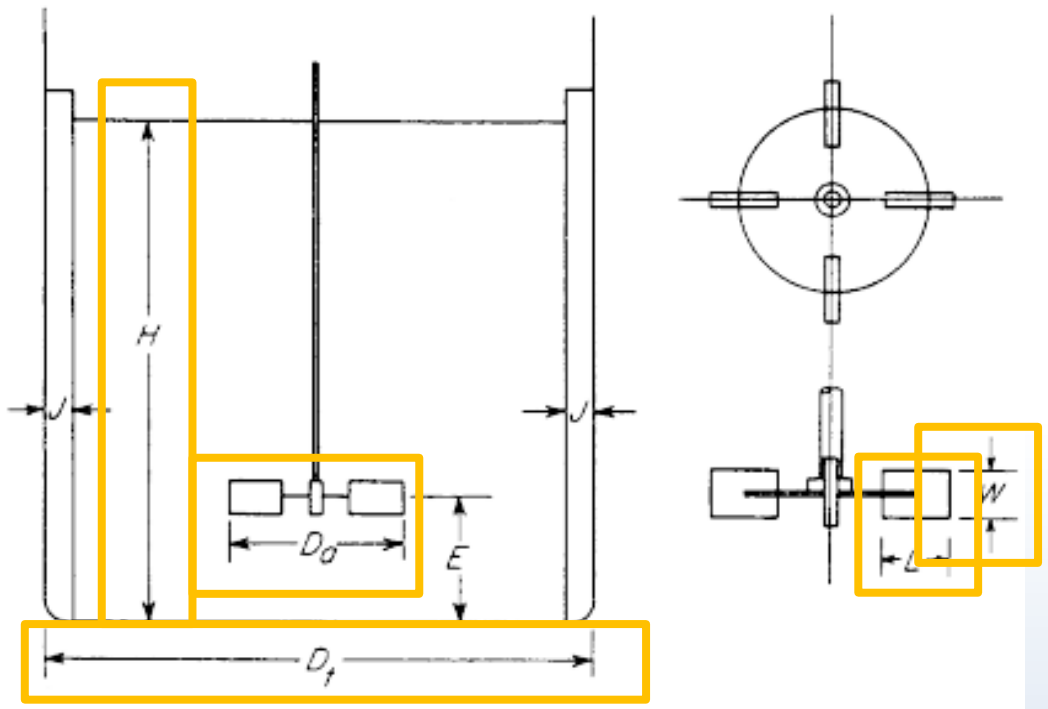
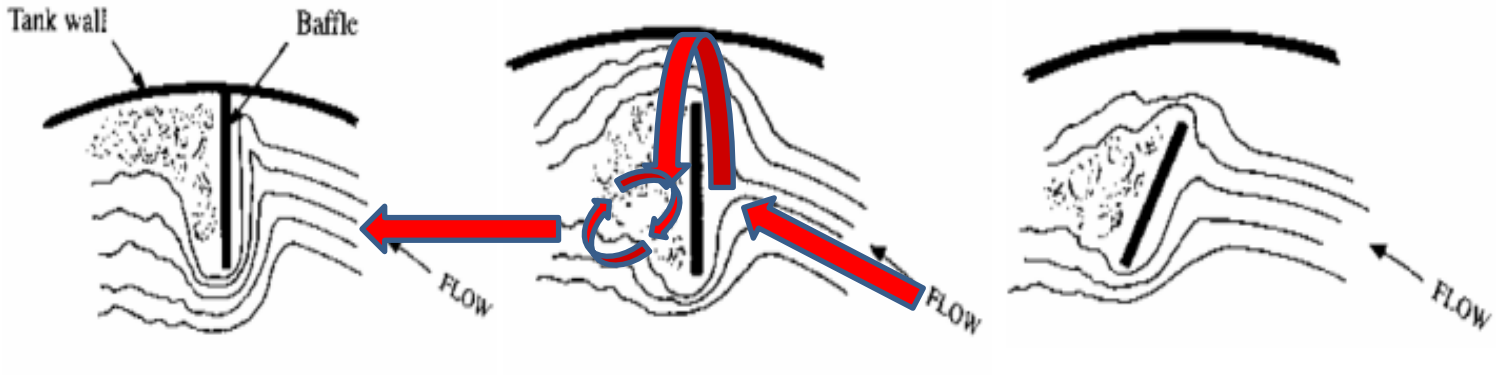


Figure 9-9 Measurements of turbine. (*After Rushton et al.*[32])

Bioreactor Agitation

- **Baffles** (indentations in bioreactor) are used to break the liquid flow to increase turbulence and mixing efficiency.

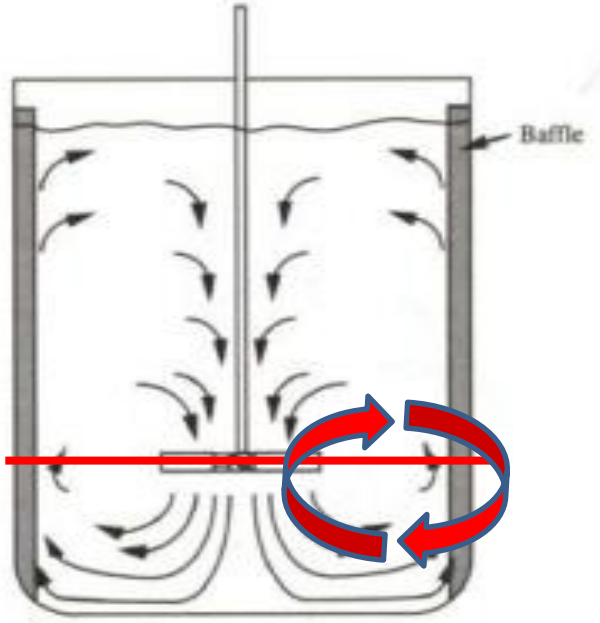


- These are used to prevent a vortex in mammalian cell culture axial flow agitation systems or to increase mixing in fermenters.

Bioreactor Agitation

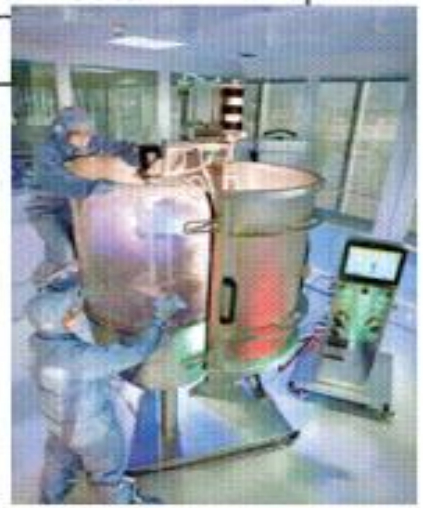
Axial flow hydrofoil impeller (Marine impeller)

- Axial flow hydrofoil impellers have blades which make an angle of less than 90 degrees to the plane of rotation and promote axial top to bottom motion.
- Axial impellers provide a much gentler mixing regime while still maintaining good bulk fluid mixing and low shear, axial impellers are therefore, the impellers of choice for mammalian cell culture.

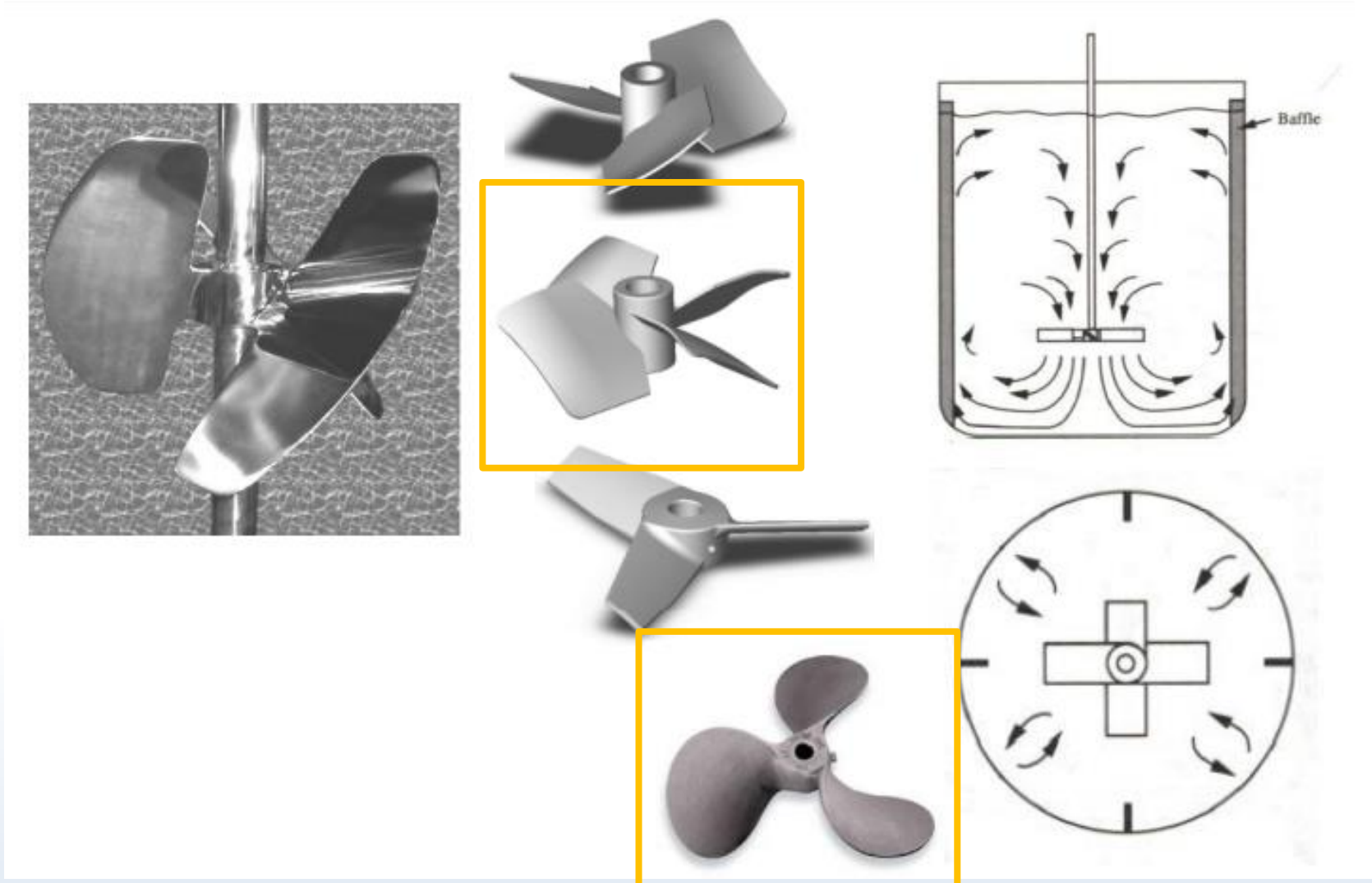


Design elements of a SUT Bioreactor

Table 2 -- Single Use Bioreactor Design Elements	
Tank height (at working volume)	1.5 tank diameter
Impeller diameter	0.33 tank diameter
Impeller number of blades	3
Impeller blade pitch	45°
Impeller blade height	0.5 impeller diameter
Impeller clearance from tank bottom	1 impeller diameter
Impeller clearance from tank side	0.5 impeller diameter
Impeller power number (calculated)	2.1

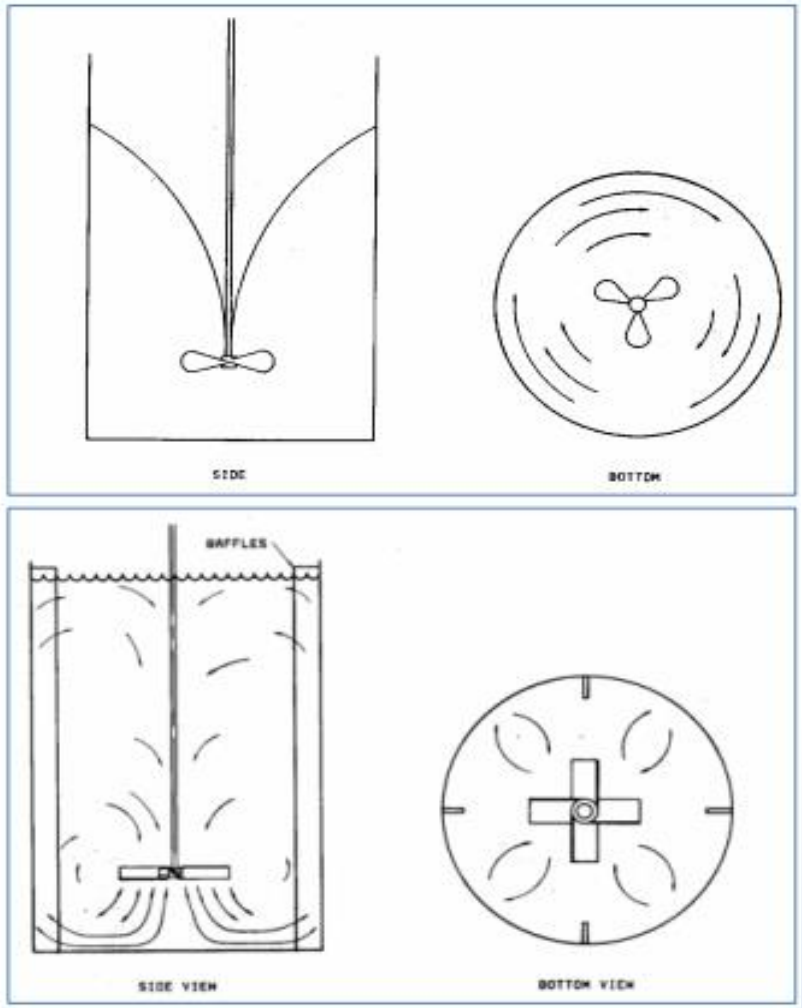


Mixing in Stirred Tank Bioreactors



Mixing in Stirred Tanks

Axial turbine



Mixing in Stirred Tanks

Cytotechnology (2006) 50:9–33
DOI 10.1007/s10616-006-9005-8

ORIGINAL PAPER

Reactor engineering in large scale animal cell culture

Alvin W. Nienow

Use dual, up-pumping, wide-blade axial flow hydrofoil impellers of diameter of 0.4 to 0.5 of the vessel diameter with clearance between them of 0.33 to 0.5 T with a sparger below the lower impeller. Here, the lower impeller ensures good air dispersion and the upper one efficient liquid blending close to the top. Use baffles to ensure required power input, good vertical mixing and air dispersion are all achieved.

T = vessel diameter

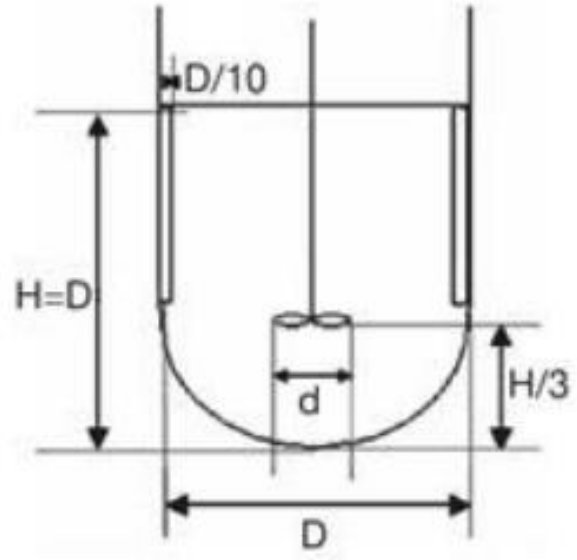
Mixing in Stirred Tanks

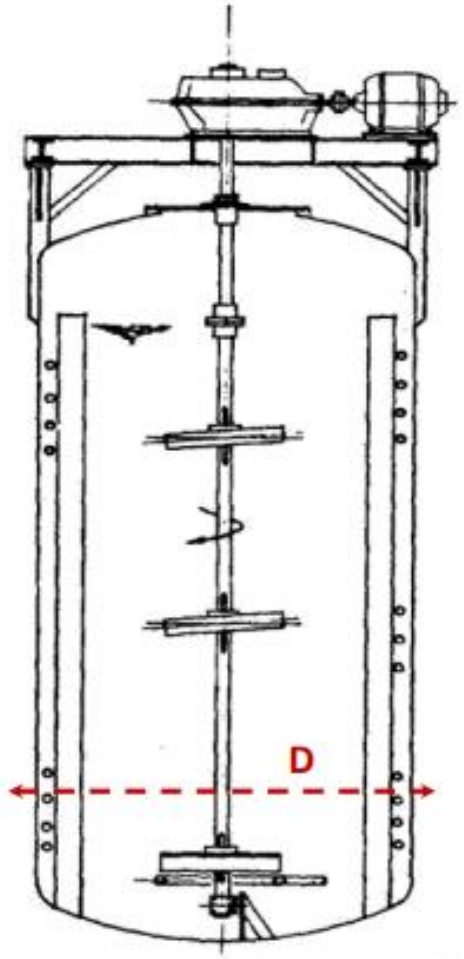
Standard configuration

Multiple impellers

axial flow impellers – D apart

radial flow impellers – 1.5 D apart





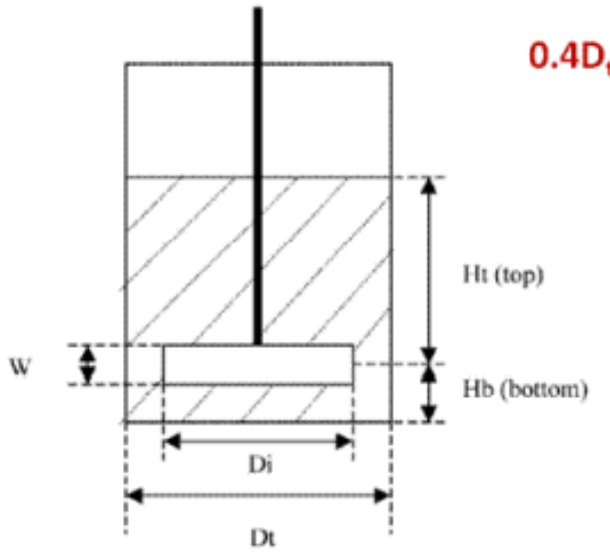
Mixing in Stirred Tanks

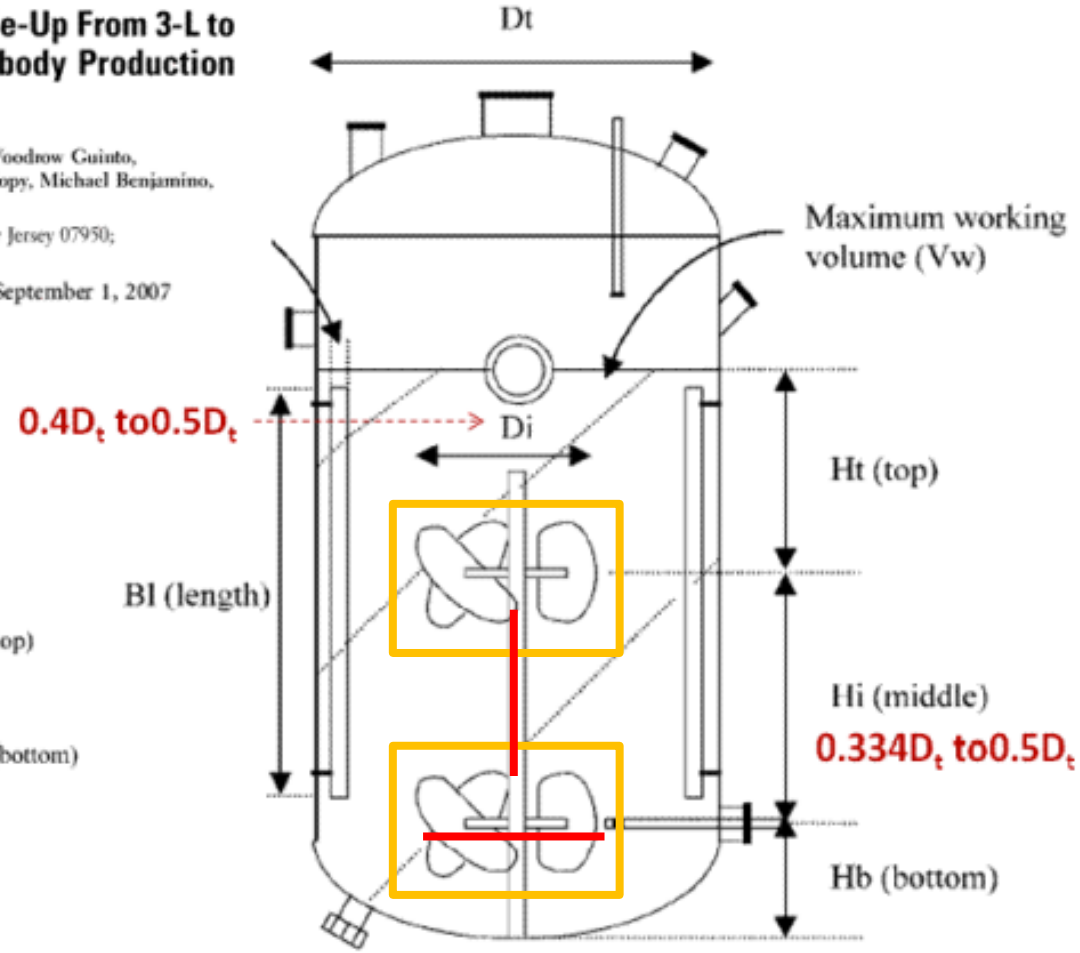
Fed-Batch Bioreactor Process Scale-Up From 3-L to 2,500-L Scale For Monoclonal Antibody Production From Cell Culture

Jeng-Dar Yang, Canghai Lu, Brad Stasny, Joseph Henley, Woodrow Guinto, Carlos Gonzalez, Joseph Gleason, Monica Fung, Brett Collopy, Michael Benjamino, Jennifer Gangi, Melissa Hanson, Elisabeth Ille

Immunomedics, Inc. 300 American Road, Morris Plains, New Jersey 07950;

Biotechnology and Bioengineering, Vol. 98, No. 1, September 1, 2007



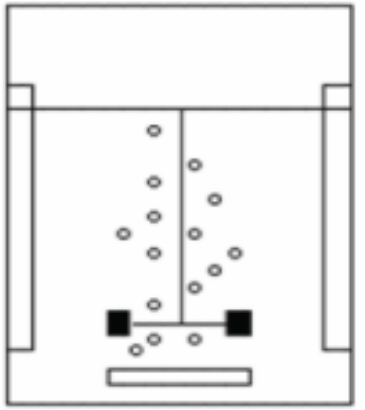


Mixing in Stirred Tanks: Bioreactor Vessel Dimensions

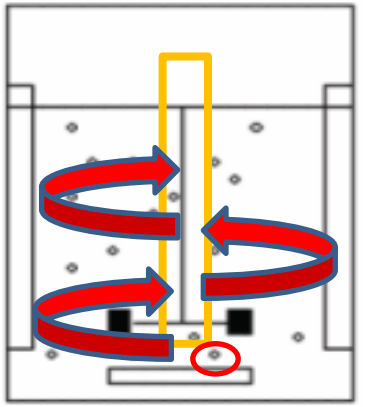
Variable	3 Litre	75 Litre	300 Litre	2500 Litre
V_s (L) – tank volume	00	75.00	300.00	2500.00
V_w (L) – working volume	2.00	50.00	225.00	2000.00
D_i (in) – impeller diameter	4.30	7.00	11.50	24.00
D_t (in) – tank diameter	6.30	14.00	23.00	10.40
H_t (in) – distance from upper impeller to top of liquid	5.60	4.20	5.60	36.00
H_i (in) – distance between impellers	0.00	10.50	17.30	24.80
Baffle no.	1.40	7.20	12.40	4.00
B_w (in)	0.00	4.00	4.00	3.90
B_l (in) – height of liquid column	0.00	18.50	29.40	61.00
$L = H_t + H_i + H_b$ (in)	7.00	21.90	35.30	71.20
D_i / D_t	0.68	0.50	0.50	0.50
L / D_i	1.60	3.10	3.10	3.00

Agitation rate and aeration

- Impellers generate shear force which plays major role in bubble generation.
- **Slow impeller speed**: bubbles will not be sheared into smaller bubbles and will tend to rise directly to the surface.
- **Fast impeller speed**: smaller bubbles will be generated, and these bubbles will move throughout the reactor increasing gas hold up and bubble residence time.



Impeller slow



Impeller fast

Mammalian Cells and Oxygen

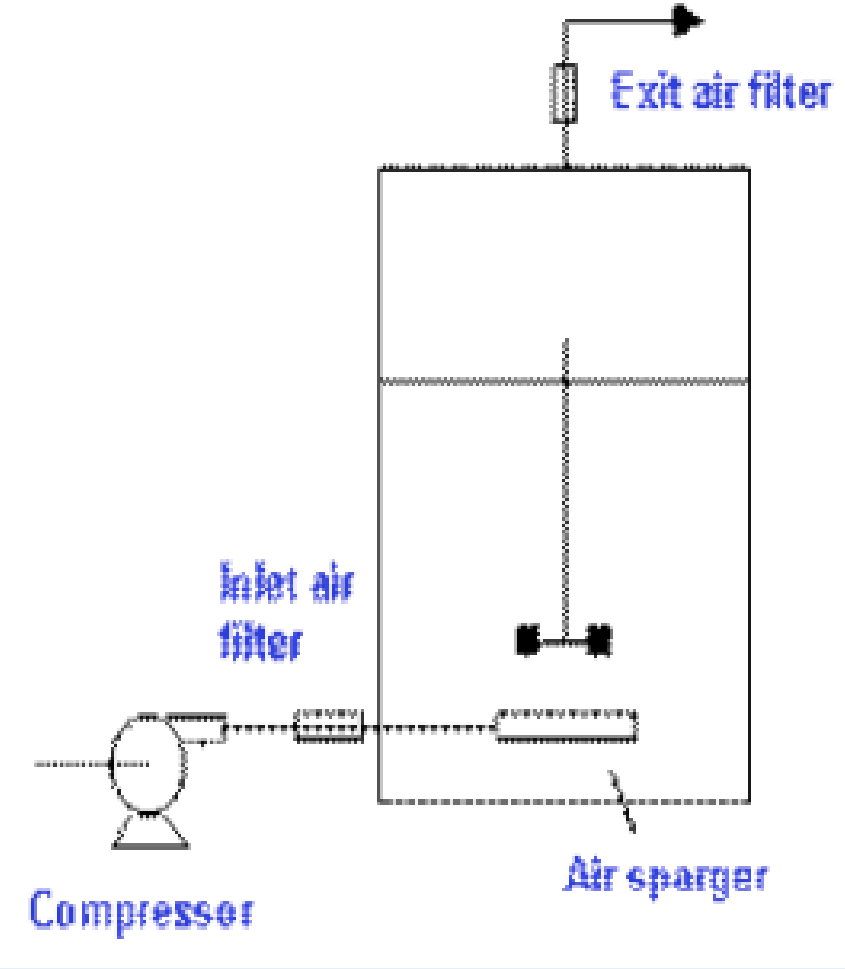
- **Supply of O_2 to satisfy cell metabolism is one of major problems associated with culture scale-up.**
- **O_2 consumption rate of mammalian cells varies from 0.06 - 0.6 mmol/litre/hr:**
  - Small flasks O_2 demand satisfied by gas diffusion from head space but as culture volume increases, the surface:volume ratio decreases and cultures >1L require additional O_2.
- **O_2 transfer involves transfer of O_2 from gas phase to liquid phase: measured as kLa:**
  - If cells utilize O_2 faster than it can be supplied, dissolved O_2 conc. in media decreases and cells die.

Oxygen in bioreactor

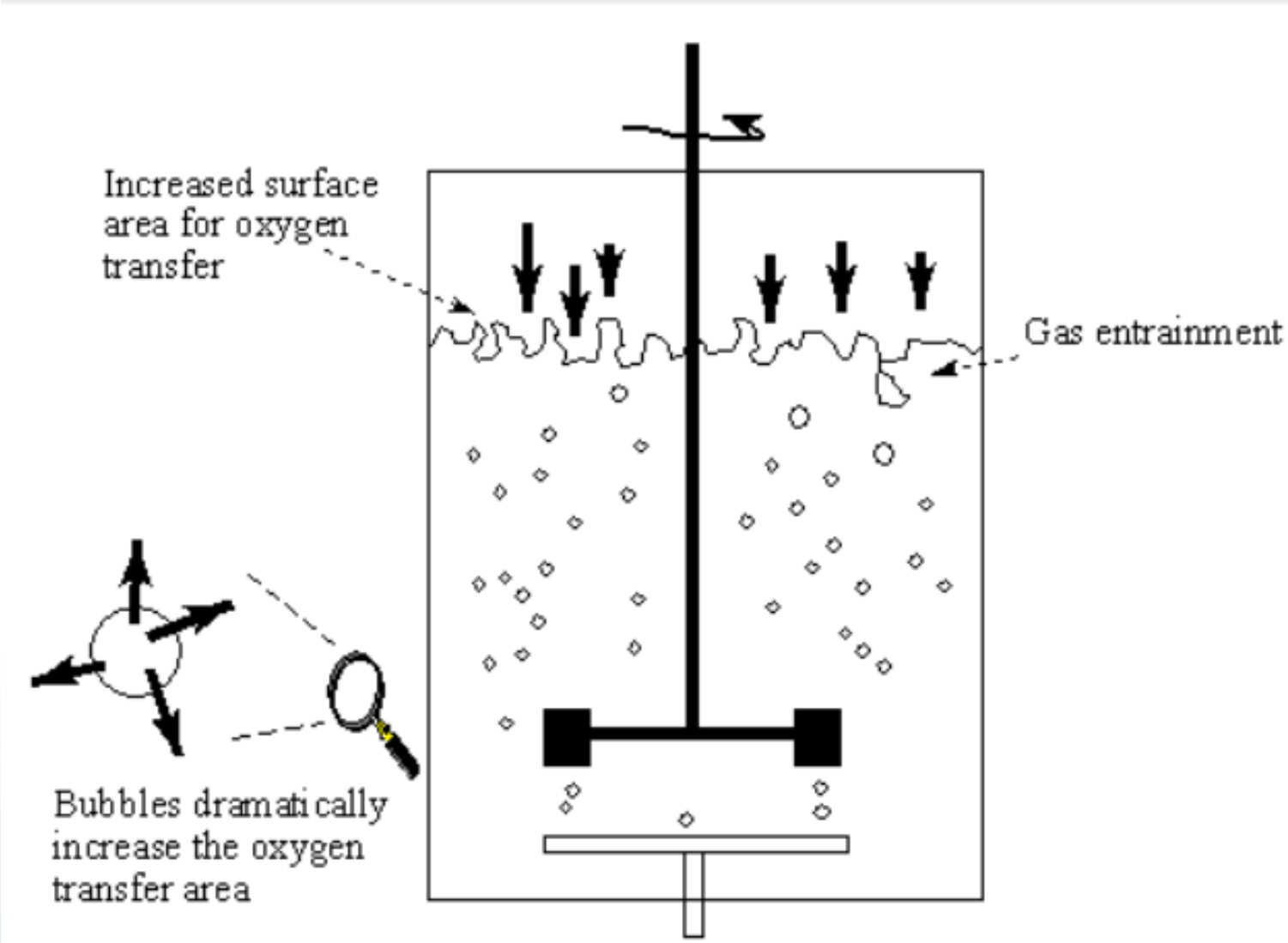
- To avoid O_2 depletion, Oxygen Transfer Rate (OTR) across liquid surface must be increased above the Oxygen Utilisation Rate (OUR).
- Need a constant supply of oxygen but:
  a) Solubility of O_2 in an air-saturated aqueous solution is low (0.22 mM at 37°C).
  b) This is the maximum conc. of O_2 in culture media and is referred to as 100% air saturation.
  c) Growth of many cells is optimal at dissolved O_2 concentration below max O_2 solubility, typically 20-50% air saturation.
- Levels of dissolved O_2 (DO) measured using an O_2 probe : electrode with gas-permeable membrane which reacts to O_2 levels.

Aeration in bioreactor

- Oxygen delivery system consists :
  a) Compressor: forces air into bioreactor
  b) Inlet air sterilization system: sterilise air into system - use membrane filter
  c) Air sparger: aeration device, holes in metal ring - breaks air into small bubbles
  d) Exit air sterilization system: prevent exit organisms into environment

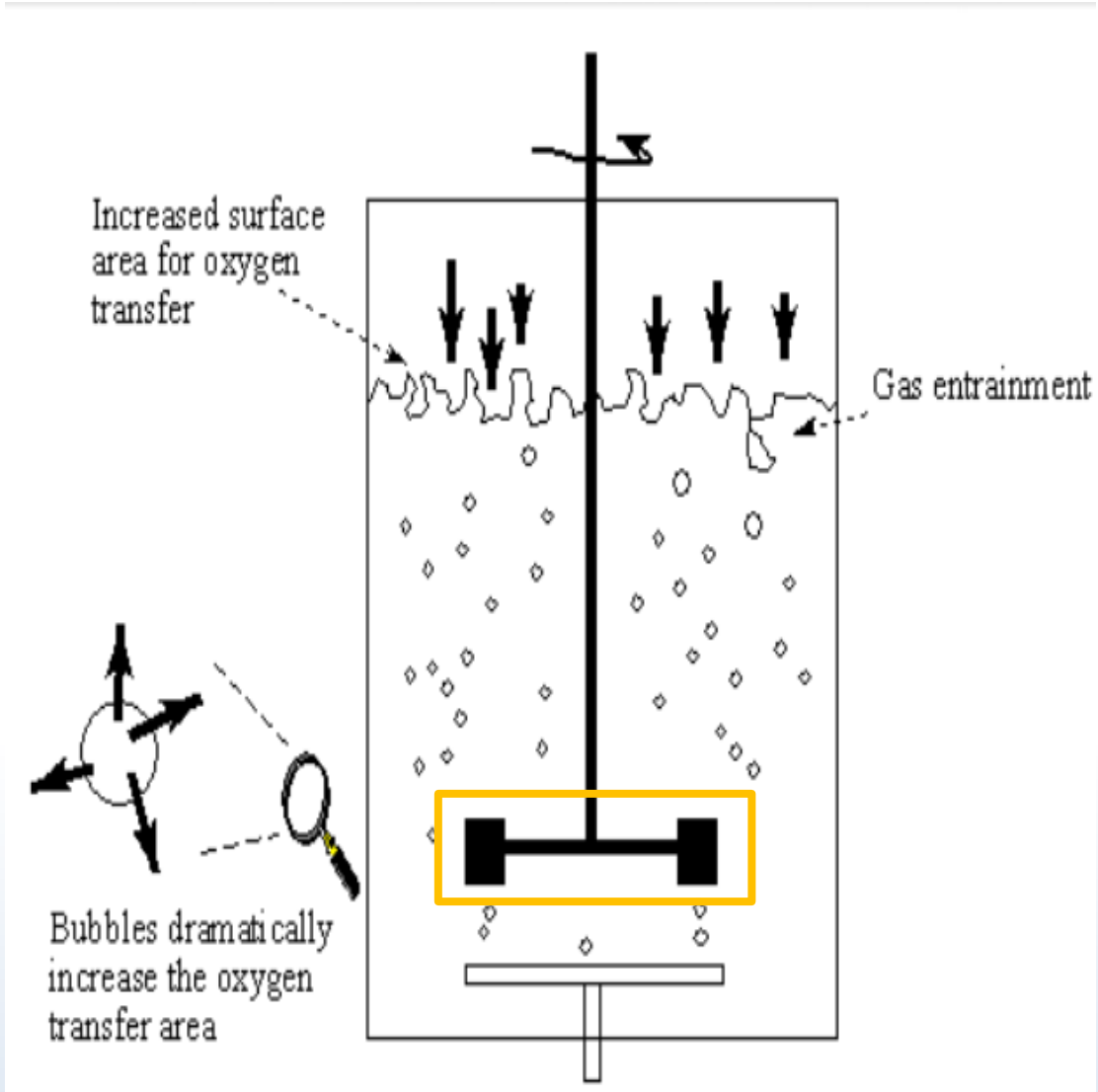


Sparging and agitation



Sparging and agitation

- For liquid volumes greater than 3 litres, air sparging is required for effective oxygen transfer.
- Introduction of bubbles into the culture fluid by sparging, leads to a dramatic increase in the oxygen transfer area.
- Agitation is used to further break up bubbles and increases kLa



Summary Points

- Design of the bioreactor is dependent on the cell type – anchorage dependent or independent?
- Industrial scale biomanufacturing generally prefers and most commonly uses anchorage independent cells e.g. CHO / BHK.
- Stainless steel bioreactors have been the system of choice to date, but the trend is shifting towards disposable systems.
- Key design considerations include:
  - Aseptic operation
  - Agitation and aeration
  - Temperature control
  - pH control

Questions?

Sample Questions

- Mixing and agitation is a critical activity in any bioreactor. How is this achieved in a typical stirred tank reactor? What additional modifications to the reactor structure that can enhance the agitation process?
- Write a detailed note on aeration of mammalian cell bioreactors.
- Describe the construction of a typical stirred tank bioreactor for mammalian cell culture. How might it differ from microbial fermentors?