- Types of Agitation systems in bioreactors—

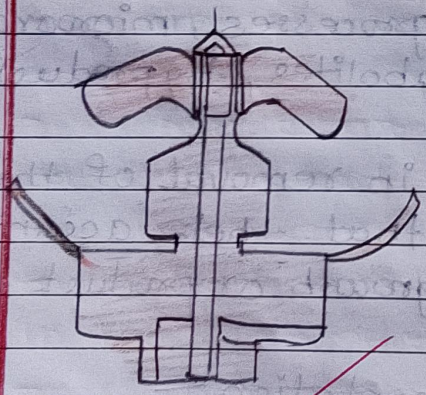
1). Mechanical Agitation—

- Impellers —

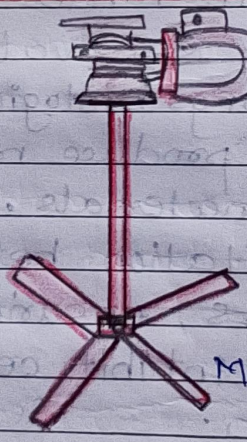
- These are most common type of agitators used in bioreactors.
- design of impeller can vary, including types such as Rushton turbines, pitched-blade turbines or marine propellers, depending on desired flow & shear characteristics.

- functions —

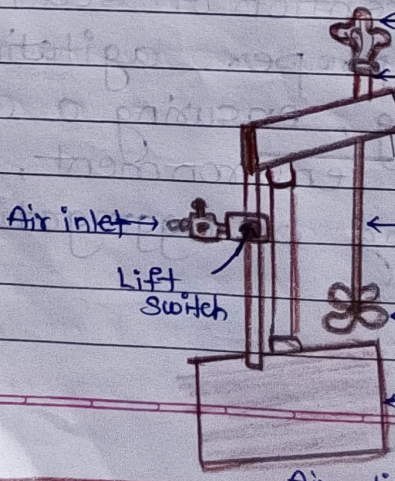
- Impellers physically stir the medium, generating shear & turbulance.
- Choice of impeller depends on types on type of cells, desired shear stress, & mixing efficiency.



Magnetic stirrer (agitation)



Mechanical Agitator



Air-lift agitator

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2) Air-lift Agitation —

• operation —

- In this system air is injected into bioreactor causing culture medium to circulate due to rising of air bubbles.

• function —

- This is less mechanical & used when lower shear stress is required.
- It often employed in cultures that are sensitive to shear forces, such as plant or animal cell culture.

3) Pneumatic Agitation —

• Operation —

- Pneumatic systems use gas flow (usually air or oxygen) to induce mixing in bioreactor.

• function —

- This system works well for cultures that requires a gentle form of agitation to avoid damaging delicate cells.
- Also beneficial in minimizing mechanical stress on cells.

4) Magnetic stirring —

• Operation —

- Is a less common but effective method used for small-scale bioreactors, where a magnetic field moves a stir bar inside culture vessel.

• function — Magnetic stirring is typically used for lab-scale applications or reactors with small volumes, as it offers precision control over agitation rates without introducing mechanical wear.

6) Explain fed & Continuous batch fermentation.

→(A) Fed-batch fermentation —

- fed-batch fermentation is a bioprocessing technique where nutrients are added to a bioreactor during cultivation, while the product remains in the reactor until the end of process, unlike batch fermentation where all nutrients added initially.
- fed-batch fermentation is a modified version of batch fermentation. it is the most common mode of operation in bioprocess industry.
- Microorganisms are inoculated & grown under batch regime for a certain amount of time, then nutrients are added to the fermenter in increments throughout the remaining duration of fermentation to feed them.
- The entire culture suspension is removed at the end of each batch.
- Because of the addition of fresh nutrients extensive biomass accumulation normally occurs in the exponential growth phase.
- Therefore fed-batch fermentation is very useful for bioprocess aiming for high biomass density or high product yeild.

• How It works ?

- Initially fermentation process starts like a batch fermentation, where microorganisms are inoculated & grown in a closed system with a fixed volume of medium.

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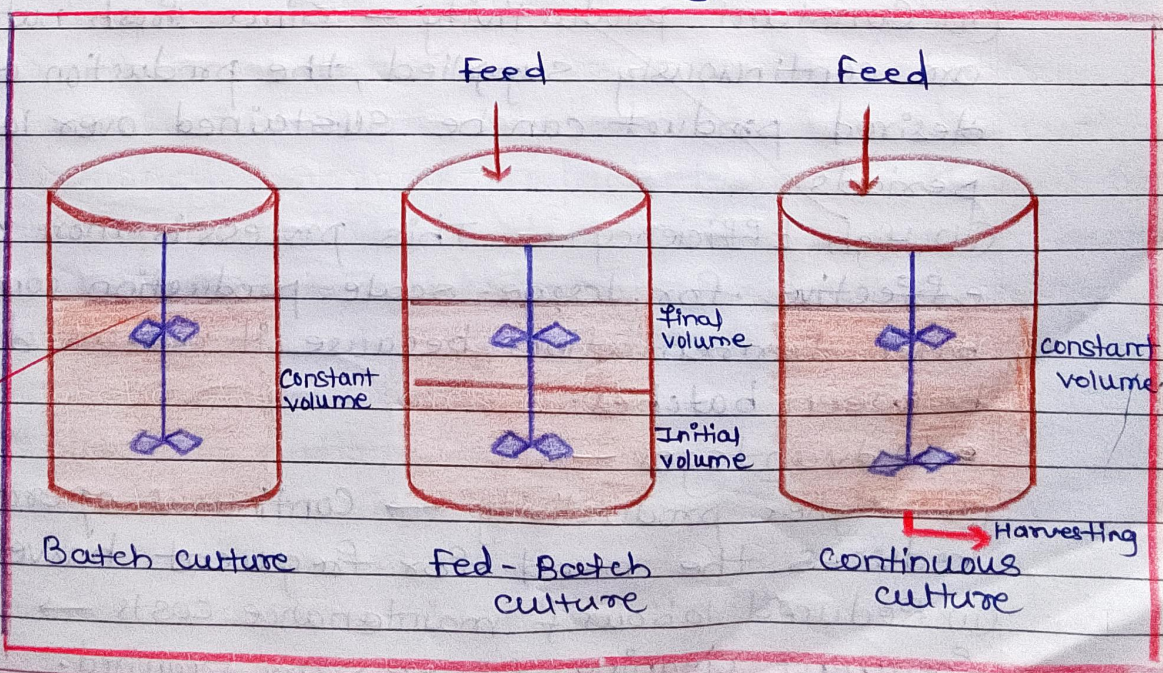
- As the culture grows & consumes initial nutrients, fresh nutrients are added in a controlled manner, either continuously or intermittently, to maintain optimal conditions for microbial growth & product formation.
- The volume of fermentation broth increases gradually as fresh medium is added, but the product is not removed until end of fermentation cycle.

• Advantages —

(i) Extends culture's productive duration.
(ii) Can be used to switch genes on or off by changing substrate.
(iii) Can be manipulated for maximum productivity using different feeding strategies.

• Disadvantages —

(i) Allows production of inhibitory agents & toxins
(ii) Provides another point of ingress for contamination



(B) Continuous fermentation —

- Continuous fermentation is a process where microorganisms are cultured in a bioreactor, & fresh nutrient-rich medium is continuously added while the culture liquid (containing microorganisms & product) is simultaneously removed at the same rate.
- fermentation carried out in open system & nutrients added and product removed at steady-state throughout process.
- Maintains microorganisms at exponential phase of growth.
- pH, temperature, & oxygen conc. as well as nutrient & product levels should be kept constant.

• Key features –

(i) Steady-state operation — system maintains constant conc. of microorganisms & nutrients.

(ii) Constant productivity – Since fresh nutrients are continuously supplied, the production of the desired product can be sustained over long periods.

(iii) High Efficiency — This process is more cost-effective for large-scale production compared to batch fermentation because it reduces downtime between batches.

• Advantages —

(i) Higher productivity → Continuous operation reduces the need for frequent harvesting.

(ii) Reduced labour & maintenance costs → Less frequent cleaning & setup are required.

(ii) Stable product quality → Consistent growth conditions lead to uniform product quality.

• Disadvantages —

(i) Complex control systems → Requires precise monitoring & regulation of parameters like pH, temperature & nutrient levels.

(ii) Risk of contamination → Since system operates continuously, any contamination can quickly affect the entire process.

• Applications —

1) Industrial production of biofuels, chemicals & pharmaceuticals.
2) Wastewater treatment.
3) Food & beverage industries (eg. brewing, yogurt production).

* Dimensional Ratios of outer shell of CSTR —

– CSTR especially when it includes an outer shell, such as in jacketed CSTRs (used for heating or cooling), dimensional ratio of outer shell plays a critical role in heat transfer, mechanical integrity & efficient mixing.

• dimensional Ratios —

1) Diameter Ratio (Do/Di) —

Do → outer shell diameter
Di → inner vessel diameter

– for standard jackets: 1.1 — 1.3

2) Height Ration (Ho/Hi)
Ho → Height of outer shell
Hi → Height of inner vessel
- Typical range about 1.0

3) wall Gap (Jacket clearence) —
- The annular space between inner & outer shell about the range of 10-50 mm.
• Design Considerations —
1) Heat Transfer efficiency.
2) Pressure & flow rates
3) Thermal expansion
4) fabrication & cost.

* Working Volume of CSTR —
- The working volume is actual volume of fluid inside CSTR where chemical reaction occurs.
- It is typically less than total geometric volume of reactor to allow for head space.
• formula -

$$T = \frac{V}{Q}$$

T (residence time) min or sec.
V (working volume) (m^3 or L)
Q (volumetric flow rate) (m^3/min or L/s)

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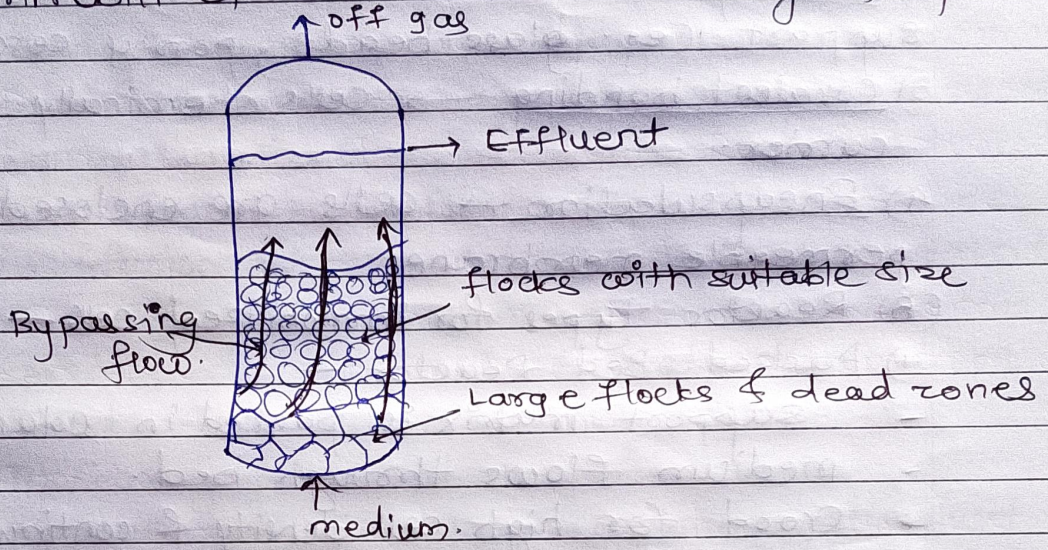
* Immobilized Cell Bioreactor Design & operation



- ICB is a device where cell are physically restricted, often within a matrix or support allowing for high cell densities & continuous processes.
- This Technology enhances productivity, simplifies product operation & protects cells from harsh conditions.
- Is a type of bioreactor where microbial or mammalian cells are fixed or entrapped in a solid matrix or on a support surface, rather than being freely suspended in liquid medium.
- This process is coidely used in bioprocessing for continuous product formation, stability & reusability of cells.

• Design —

(A) Immobilization Methods —

1) Entrapment → cells are trapped within gel matrix (eg. alginate, agarose)

2) Adsorption → cells adhere to surface of solid support (eg. glass beads, porous ceramics)
3) Covalent bonding → cells chemically bonded to carrier.
4) Encapsulation → cells are enclosed in semi-permeable membrane.

(B) Reactor types for immobilized cells —

1) Packed Bed Reactor —
- support matrix is packed in column.
- medium flows through bed.
- Good for high cell density & continuous operation

a) Fluidized Bed Reactor —
- small immobilized cells beads are suspended by upward flow of medium.
- offers better mass transfer & mixing

3) Stirred Tank Reactor with immobilized cells —
- Modified version of CSTR with beads or carriers suspended or packed.
- Needs gentle stirring to prevent bead damage.

• Operation —

1) start up →
- load immobilized cell matrix in reactor
- fill with sterile nutrient medium.
- Begin slow flow of substrate & monitor for stability.

2) Continuous or semi-continuous feeding —
- substrates (eg glucose, amino acids) are continuously fed.
- products harvested from outlet stream.

3) Cell Retention & Reuse –
- Cell remain within matrix & do not wash out.
- Enables long term operation without frequent re-inoculation.
4) Mass Transfer considerations –
- Diffusion of nutrients & oxygen must reach the inner cells.
- product diffusion out of matrix should be efficient.
5) Monitoring & Control –
- parameters like pH, temperature, dissolved oxygen, & flow rates are tightly controlled.
- Monitoring prevents bead clogging, contamination or cell death.

• Advantages –
1) High cell density & productivity
2) Continuous operation possible.
3) Lower risk of contamination due to reduced cell handling
4) Reuse of immobilized cells reduces cost.
5) Greater stability of enzyme or metabolite production.

• Limitations –
1) Mass transfer limitations → especially in dense beads.
2) Bead / channel clogging in packed systems.
3) Cell leakage from matrix over time.

• Applications –
1) Pharmaceuticals → Antibiotic or enzyme production.

2) food industry → fermentation of wine, beer, vinegar
3) Environment → waste water treatment using immobilized bacteria
4) Biofuels → ethanol or biogas production.