Cell Culture Systems

Course 7: Scale Up Culture

Small Scale Culture

- Most tissue culture is performed on a small scale where relatively small numbers of cells are required for experiments.
- At this scale cells are usually grown in T flasks ranging from 25cm^2 to 175cm^2 .
- **Typical cell yields from a T175 flask range from 1x10^7 for an attached monolayer culture**

What are small cultures?

- Experiments require **multiple samples**

- To observe **cell growth** and to perform **substrate or product assays**

- Culture volumes – **100 ml**

- Conducted in **T-flasks** or **spinner flasks** in Incubator

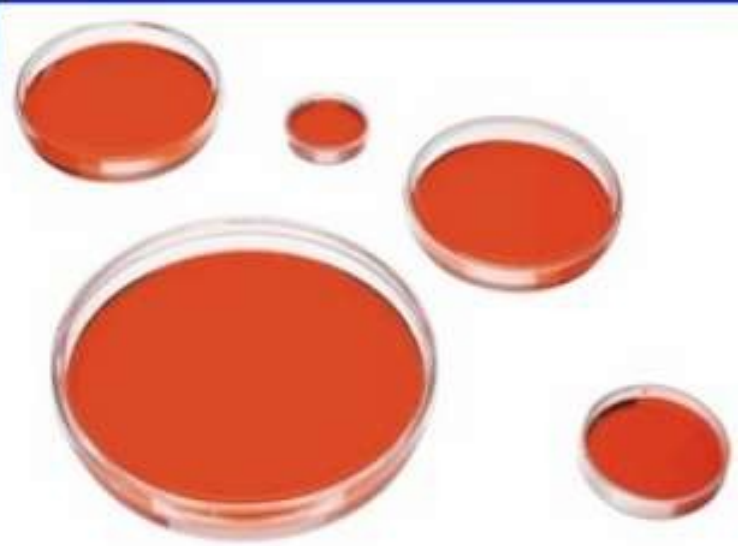
Culture Vessels

- Flasks
  - Plastic flasks are available with a range of growing areas, a variety of shapes, with several different neck designs
  - Flasks surfaces are specially treated for growing anchorage-dependent cells

Description	Growth area (cm^2)	Recommended working volume (mL)	Cell yield*
T-25	25	5 – 10	2.5×10^6
T-75	75	15 – 25	7.5×10^6
T-150	150	30 - 50	15×10^6
T-175	175	35 - 60	17.5×10^6
T-225	225	45 - 75	22.5×10^6

*Cell line dependent. Based upon a density of 1×10^5 cells/cm^2.

Culture Vessels



- **Cell culture dishes**
  - Cell culture dishes offer the best economy and access to the growth surface
  - Cell culture dishes surfaces are specially treated for growing anchorage-dependent cells

Description	Growth area (cm^2)	Recommended working volume (mL)	Cell yield*
35	8	1 - 2	0.8×10^6
60	21	4 - 5	2.1×10^6
100	55	10 - 12	5.5×10^6
150	148	28 - 32	14.8×10^6

*Cell line dependent. Based upon a density of 1×10^5 cells/cm^2.

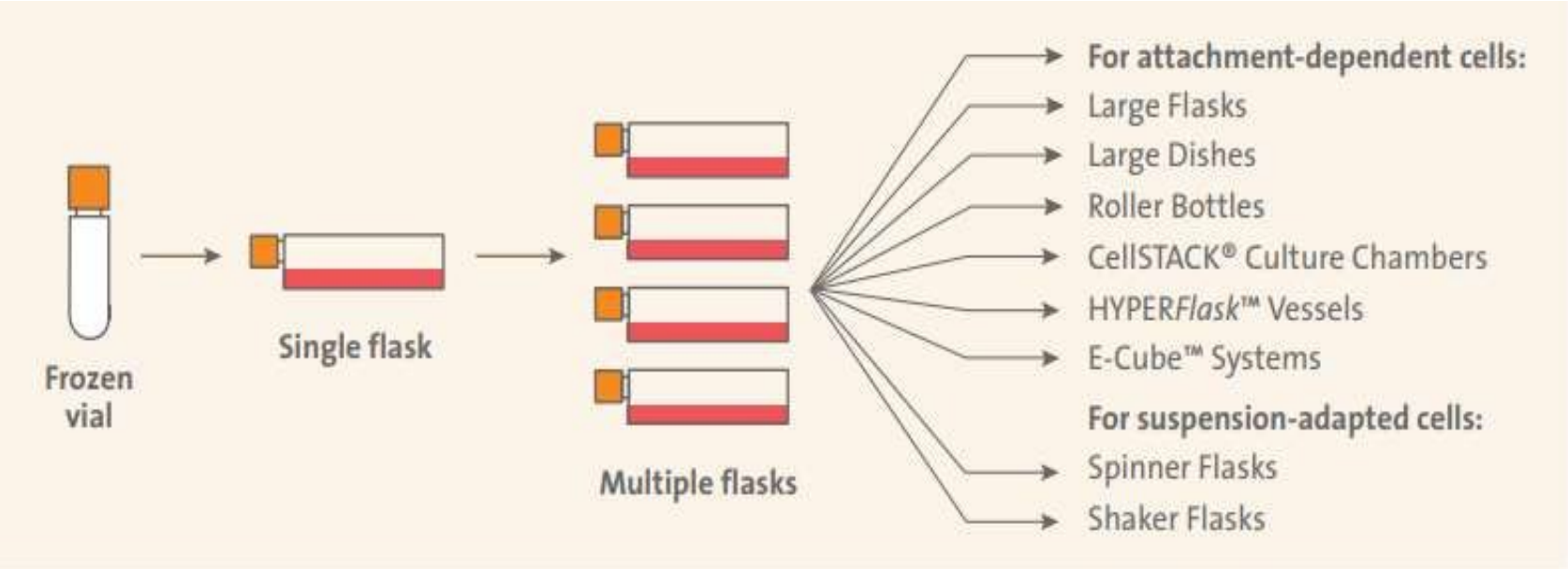
Small Scale Culture

- It is not practical to produce much larger quantities of cells using standard T flasks, due to **the amount of time required for repeated passaging of the cells, demand on incubator space and cost.**

Scale-Up Process

- Scale-up involves **the development of culture systems in stages from (small scale) laboratory to (large scale) industry.**
- The methodology adopted to increase the scale of a culture depends on the proliferation of cells and is broadly divided into two categories.

Scale Up Process



Factors in Scaling-Up

For appropriate scale-up, the physical and chemical requirements of cells have to be satisfied.

Physical parameters:

i. Configuration of the bioreactor.

ii. Supply of power.

iii. Stirring of the medium.

Factors in Scaling-Up

Chemical parameters:

i. Medium and nutrients.

ii. Oxygen.

iii. pH and buffer systems.

iv. Removal of waste products.

Scale Up Process

- When considering scaling up a cell culture process there are a whole range of parameters to consider which will need to be developed and optimized if scale-up is to be successful.
- These include problems associated with **nutrient depletion, gaseous exchange, particularly oxygen depletion, and the build up of toxic by-products such as ammonia and lactic acid.**

Scale Up Process

1. Scale-up in monolayer

2. Scale-up in suspension

Scale-Up in Monolayer

- The monolayer culture are anchorage-dependent.
- Therefore, for the scale-up of monolayer cultures, it is necessary to **increase the surface area of the substrate in proportion to the number of cells and volume of the medium.**

Advantages

i. Change of medium and washing of cells easy.

ii. It is easy to perfuse immobilized monolayer cells.

iii.The cell product formation (pharmaceutically important compounds e.g. interferon, antibodies) is much higher.

iv.The same set up and apparatus can be repeatedly used with different media and cells.

Disadvantages

i. Tedious and costly.

ii. Require more space.

iii. Growth of cells cannot be monitored effectively.

iv. Difficult to measure control parameters (O_2 pH, CO_2 etc.)

Roller Bottle Culture:

- A round bottle or tube is rolled around its axis (by rollers) as the medium along with the cells runs around inside of the bottle.
- As the cells are adhesive, they attach to inner surface of the bottle and grow forming a monolayer.

Figure 4. Roller Deck

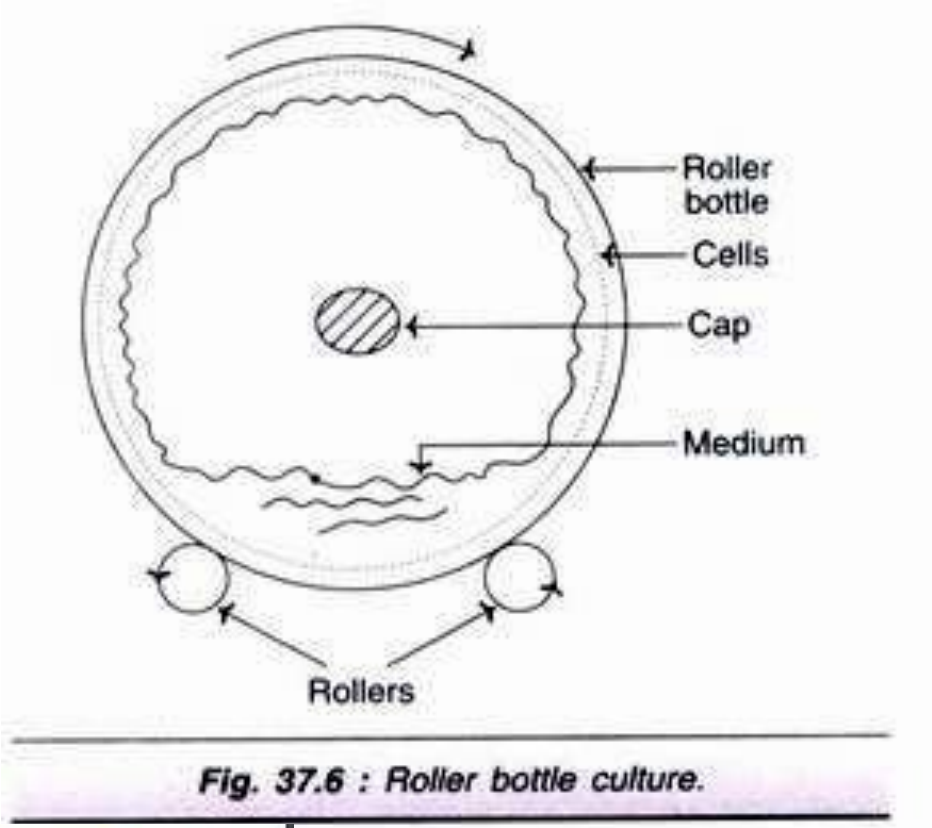


Fig. 37.6 : Roller bottle culture.

Roller Bottle Culture:

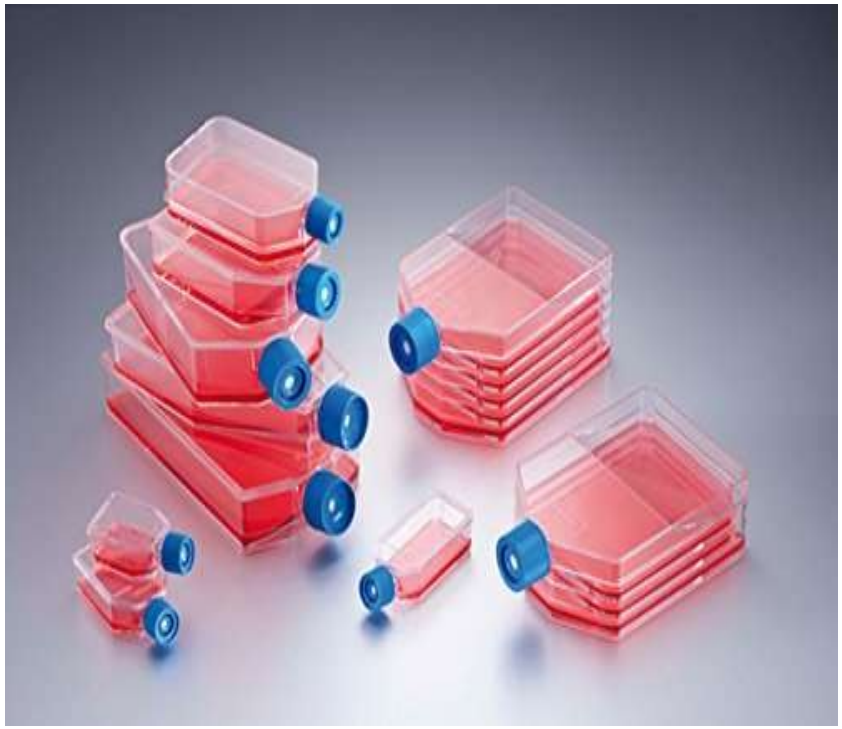
Roller bottle culture has certain advantages.

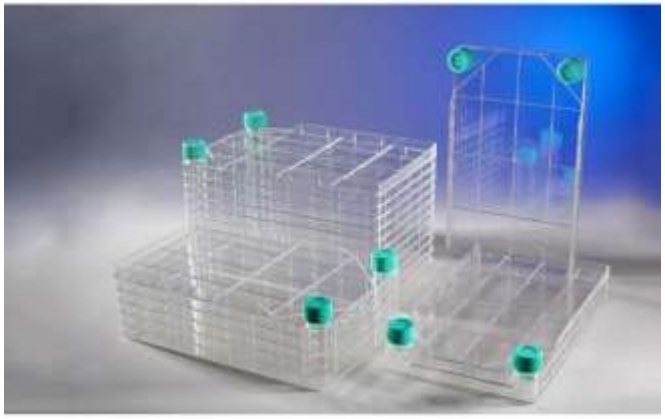
- i. The medium is gently and constantly agitated.
- ii. The surface area is high for cell growth.
- iii. Collection of the supernatant medium is easy.
- There are limitations in roller culture.
- i. Monitoring of cells is very difficult.
- ii. Investment is rather high.

Multi-surface Culture

- It is composed of rectangular petri dish-like units with huge surface area (1,000-25,000 cm^2). The units are interconnected at two adjacent corners by vertical tubes.
- The medium can flow between the compartments from one end.

Fig. 37.7 : A diagrammatic representation of Nunc cell factory.





Scale-Up in Suspensions

Spinner Flask Culture

- This is the method of choice for suspension lines including hybridomas and attached lines that have been adapted to growth in suspension e.g. HeLa S3. Spinner flasks are either plastic or glass bottles with a central magnetic stirrer shaft and side arms for the addition and removal of cells and medium, and gassing with CO2 enriched air.

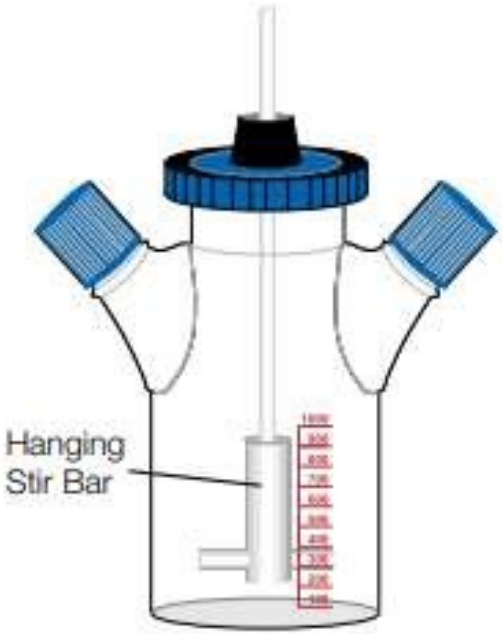
Spinner Flask Culture

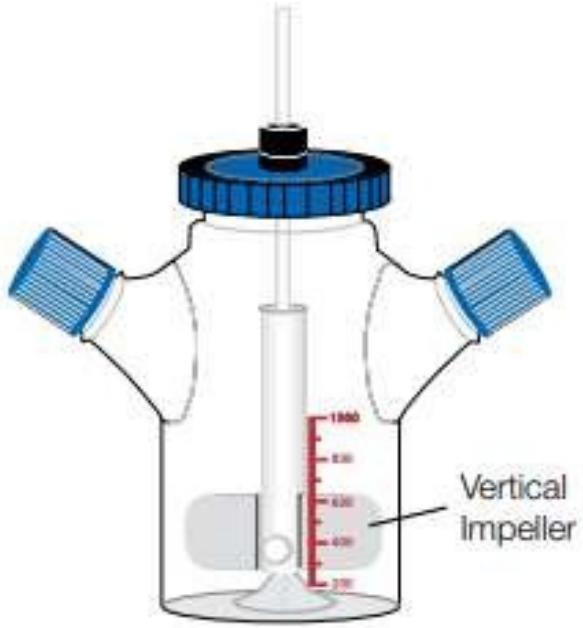
- Inoculated spinner flasks are placed on a stirrer and incubated under the culture conditions appropriate for the cell line. Cultures should be stirred at 100-250 revolutions per minute.

Spinner Flask Culture

- Spinner flasks have two basic designs; the medium is agitated (i.e., stirred) by a **hanging stir-bar assembly** or with a **vertical impeller.** The vertical impeller provides better aeration.
- The total culture volume in a spinner flask **should not exceed half of the indicated volume of the spinner for proper aeration** (e.g., a 500 mL spinner should never contain more than 250 mL of culture).

Spinner Flask Culture



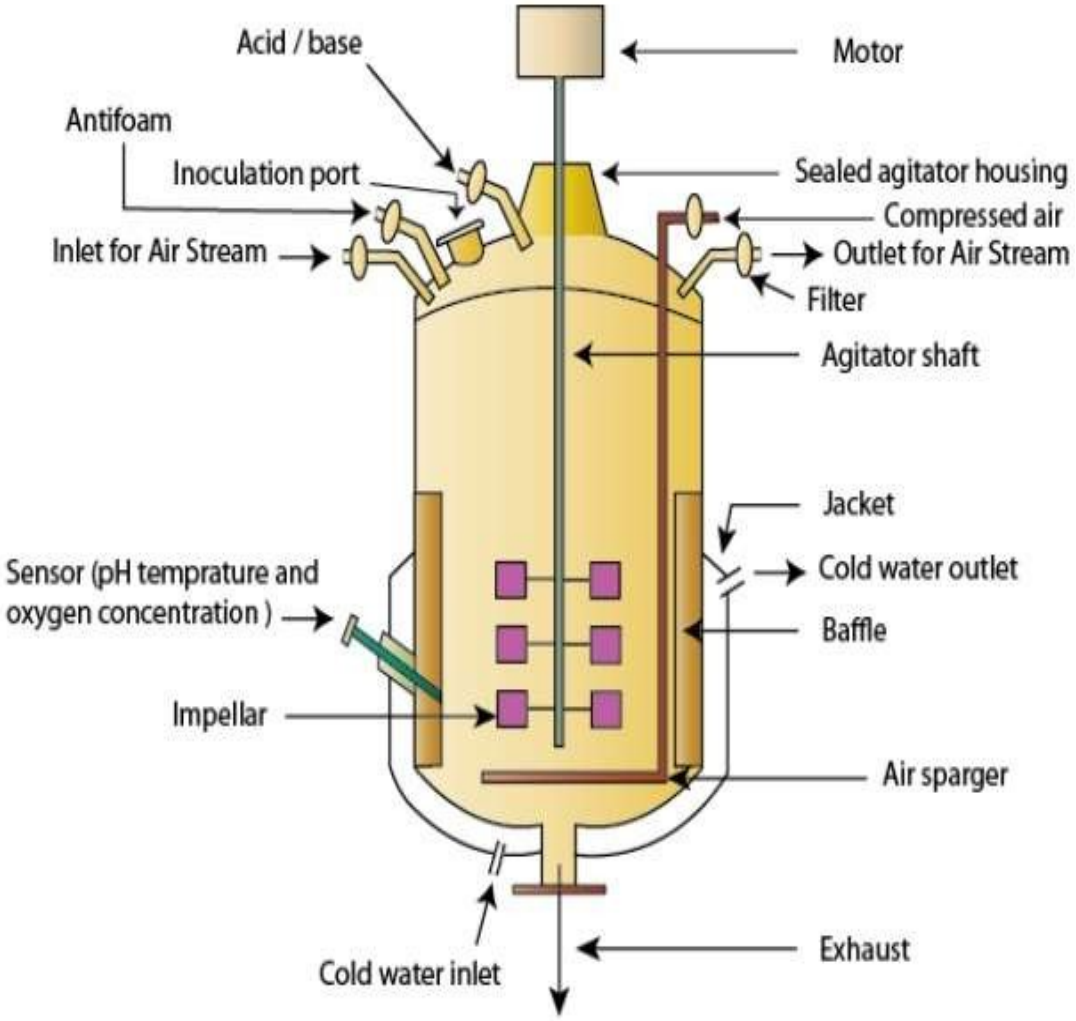


BIOREACTORS

INTRODUCTION

- Bioreactor – can be described as a vessel which has provision of cell cultivation under sterile condition & control of environmental conditions e.g., pH, Temperature, Dissolved oxygen etc.
- It can be used for the cultivation of microbial plant or animal cells.
- This process can either be aerobic or anaerobic.
- The bioreactors are commonly cylindrical, ranging in size from litres to cubic metres, and are often made of stainless steel.

DIAGRAM OF A TYPICAL BIOREACTOR



SPECIFICATIONS OF A BIOREACTOR

A typical bioreactor consists of following parts:

- **Agitator** – used for the mixing of the contents of the reactor which keeps the "cells" in the perfect homogenous condition for better transport of nutrients and oxygen to the desired product(s).

- **Baffle** – used to break the vortex formation in the vessel, which is usually highly undesirable as it changes the center of gravity of the system and consumes additional power.

- **Sparger** – In aerobic cultivation process, the purpose of the sparger is to supply adequate oxygen to the growing cells.

- **Jacket** – The jacket provides the annular area for circulation of constant temperature of water which keeps the temperature of the bioreactor at a constant value

What are the **parameters** that control adequate culture growth?

- **Agitation –**
- **Bubble bursting** on culture surface resulting from culture aeration
- **Stirring speed is low** – rotation of a **suspended bar by a magnetic stirrer**

- **are not suitable in larger volumes**

- **Impellers** – vertical and horizontal movement

Parameter - **Agitation**

- Maximum stirring rates for **suspension – 100-150 rpm**
- **Microcarrier** - < 40 rpm (suspension and anchorage dependent cells)
- **Round** bottom (**animal** cells) and **flat bottomed** vessels (**bacterial** cells)

Parameter – **Temperature control**

- **Thermocirculator** – pumps heated water around an outer jacket
- **Larger fermenters** – pumps water through coiled pipes within culture

- **Circulating warm air**

- **Low volume fermenters** – External heating pads

Parameter – pH control

- **Optimal pH - 7.4** – for maximum growth
- **Enriched Co2 atmosphere** decreases pH fluctuations
- 1-litre culture @ 2x106 cells/ml @ **gas flow of 100 ml/min**

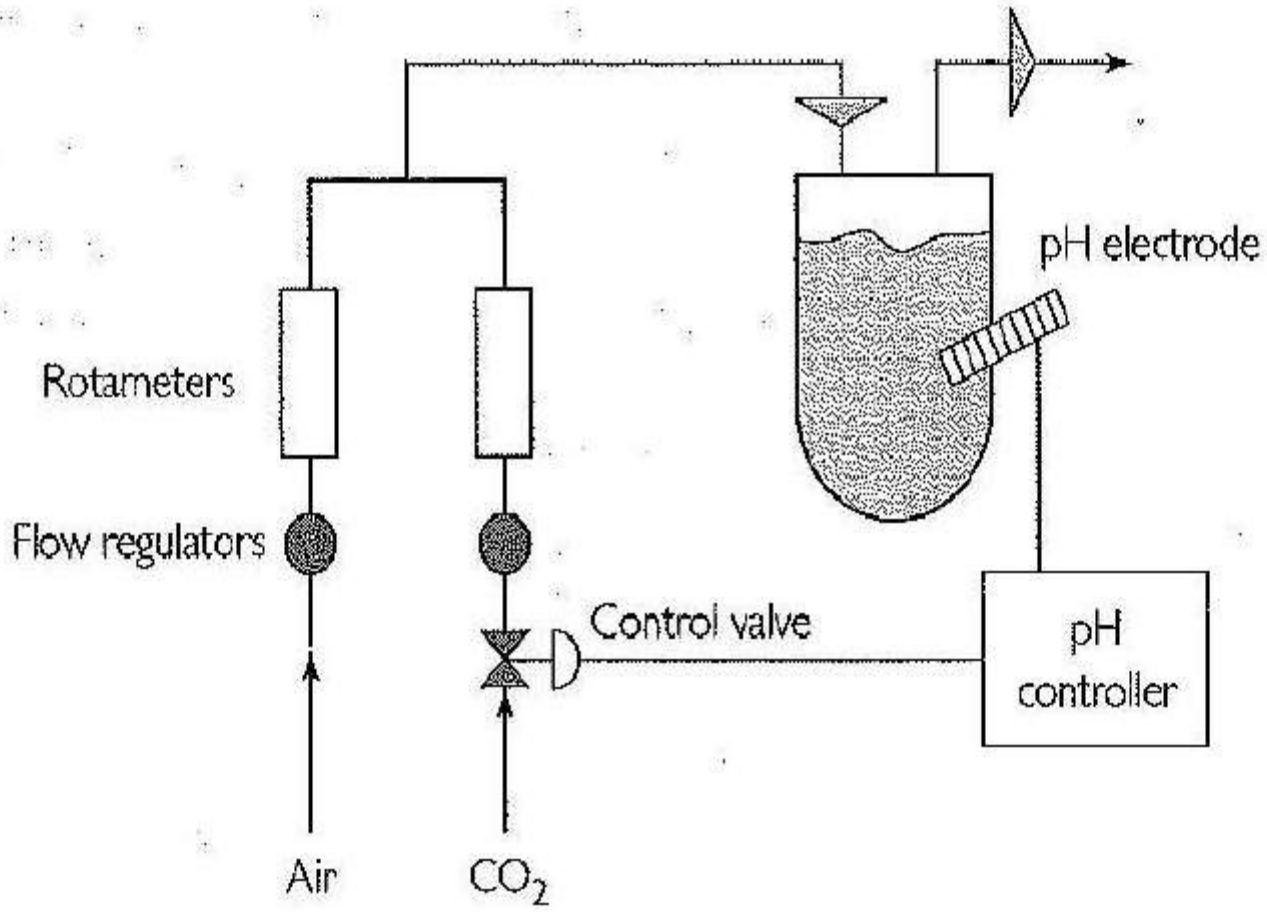


Figure 9.6

CO_2 control of culture pH in a bioreactor (from Kilburn, 1991).

Parameter – pH control

- Direct **acid** or **alkali** addition
- Net acidic production (**lactic acid**) from cellular metabolism – alkali (**NaHCO3**)
- **HCl** is added
- Computer-controlled pump or gas valve to a pre-set pH value
- **Rotameters** indicate rate of gas flow – controlled by **flow regulators** (**fig-9.6**)

Requirements for a bioreactor for animal cell culture

1) well-controlled environment (T, pH, DO, nutrients, and wastes)
2) supply of nutrients
3) gentle mixing (avoid shear damage to cells)
4) gentle aeration (add oxygen slowly to the culture medium, but avoid the formation of large bubbles which can damage cells on contact).
5) removal of wastes

Scale-up

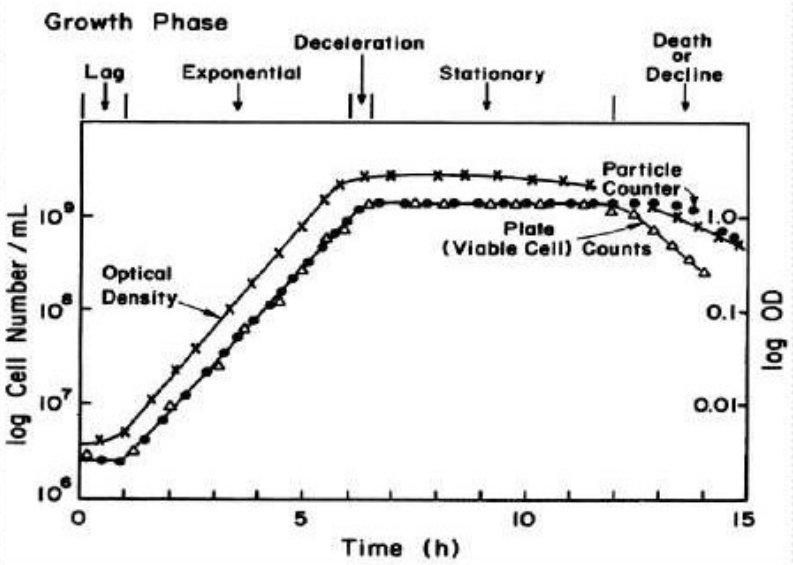
- Start with small volume reactors
  - T flasks, shaker flasks (5-25 mL)
- Intermediate scale
  - Small, highly controlled bioreactors (1-5 L)
- Production scale
  - Large reactors (20-1,000 L)

Types on the basis of mode of operation

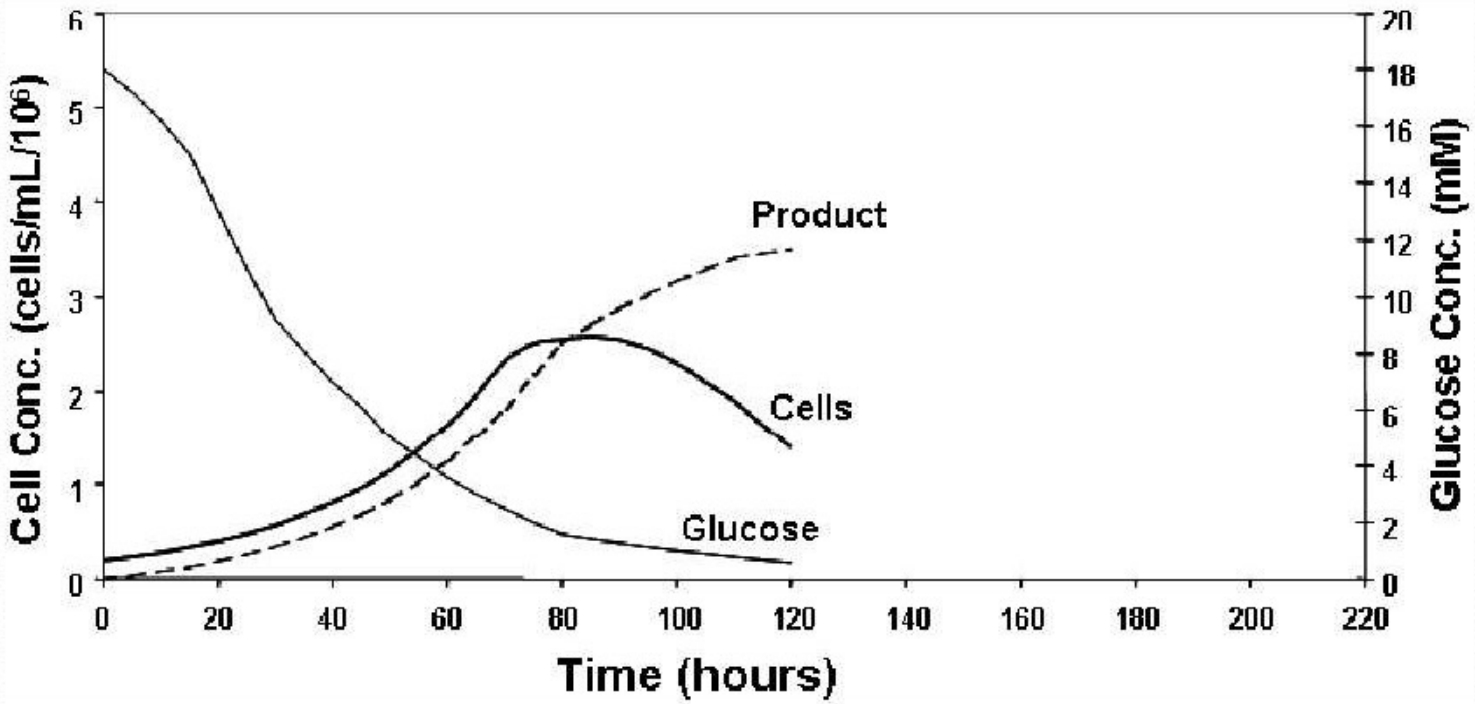
- Batch
- Fed Batch
- Continuous

Batch Culture

- A closed culture system which contains an initial, limited amount of nutrient. The inoculated culture will pass through a number of phases following a growth curve. The growth curve contains four distinct regions as
  - Lag Phase
  - Exponential Phase
  - Stationary Phase
  - Death Phase

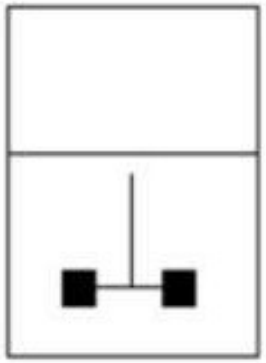


Batch Curve

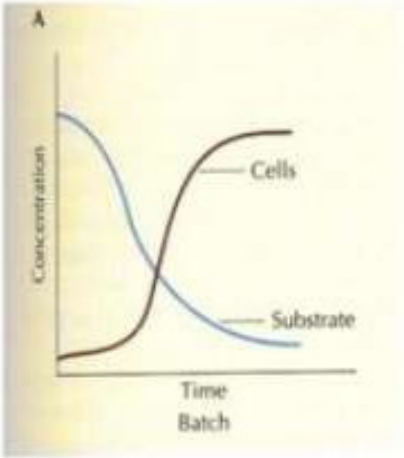

Cell Conc. (cells/mL/10⁶)
Glucose Conc. (mM)
Time (hours)
Product
Cells
Glucose
0
1
2
3
4
5
6
0
2
4
6
8
10
12
14
16
18
20
0
20
40
60
80
100
120
140
160
180
200
220

I. Batch fermentation

In batch fermentation, the sterile growth medium is inoculated with the appropriate microorganisms, and the fermentation proceeds without the addition of fresh growth medium.



Batch

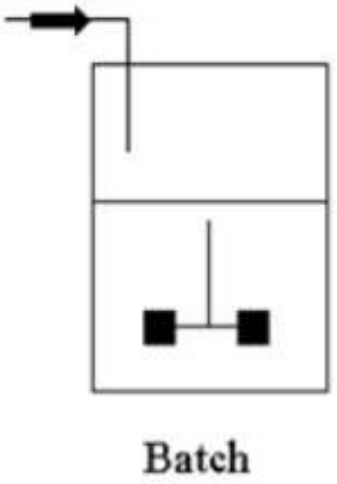


(1) The composition of culture medium, the concentration of microorganisms, the internal chemical composition of the microorganisms and the amount of metabolite are all change during fermentation.

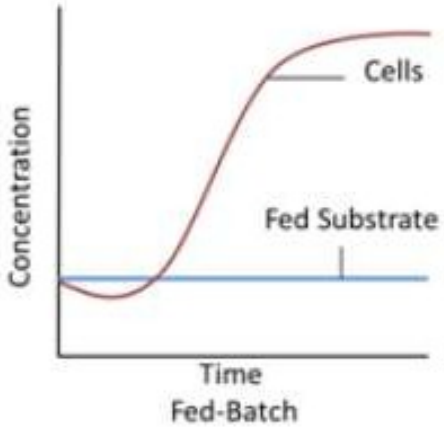
(2) All 6 phases of bacterial growth cycle are observed.

II. Fed-Batch fermentation

In fed-batch fermentation, fresh growth medium is added continuously during fermentation, and no growth medium is removed until the end of the process.



Batch



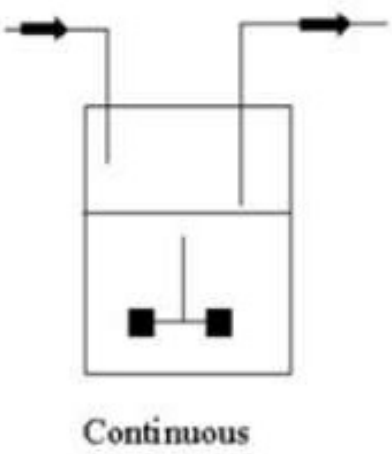
(1) The continuous addition of medium prolongs both the log and stationary phases, thereby increasing the biomass and the amount of metabolites.

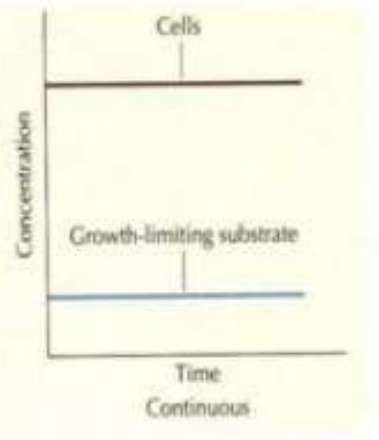
(2) However, microorganisms in stationary phase often produce proteolytic enzymes (proteases) to degrade proteins.

(3) A fed-batch fermentation strategy can increase the yield from 25% to more than 1,000% compared with batch fermentation.

III. Continuous fermentation

In continuous fermentation, fresh growth medium is added continuously during fermentation, but there is also continuous removal of an equal volume of medium containing suspended microorganisms.





(1) A steady-state condition of the total number of cells and the total volume in the bioreactor is maintained.

(2) A single reaction can be maintained for a much longer period.

(3) However, potential drawbacks are loss recombinant plasmid constructs in some cells, difficulty in maintenance of an industrial scale and variation from batch to batch.

Types of bioreactors/fermenters

There are 3 fundamental classes of bioreactors

- Stirred-tank reactors (STRs),
- Bubble columns and
- Airlift reactors.

A. STRs:

(1) STRs have internal mechanical agitation and are the most commonly used.

(2) STRs are usually constructed from stainless steel or glass for laboratory-scale.

(3) Heat generated from metabolism of the growing cells or the energy input by agitation is a limitation of the size of a bioreactor.

B. Bubble columns:

(1) It relies on the introduction of air or another gas for agitation.

(2) The smaller air bubbles introduced under high pressure near the bottom become larger ones as they rise through the column, leading to uneven gas distribution.

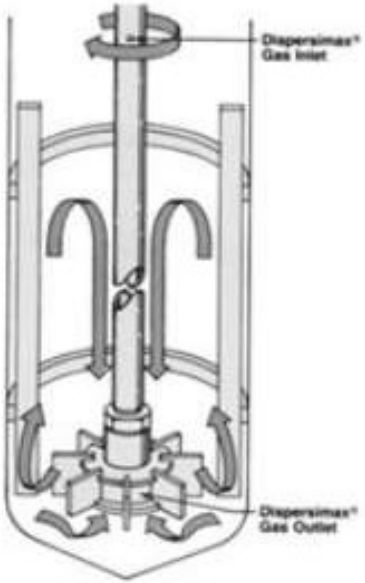
C. Airlift reactors:

(1) There are two main types: internal-loop reactors and external-loop reactors.

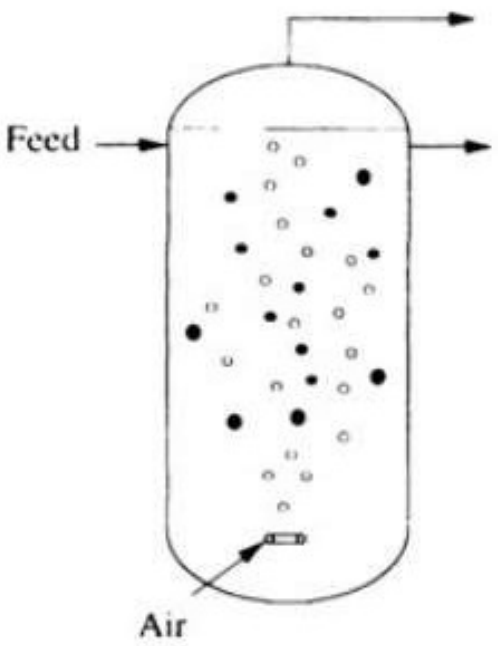
(2) The motion of an introduced air causes density differences within the different parts of the bioreactor.

(3) Internal-loop airlift reactors are simple in design, but both the volume and the circulation rate are fixed once they are constructed.

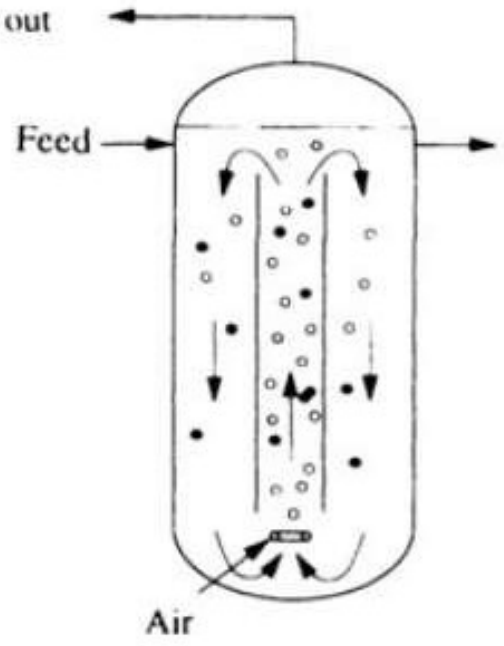
Types of bioreactors/fermenters



Stirrer Tank Reactor

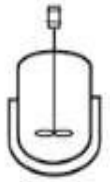


Bubble column reactor

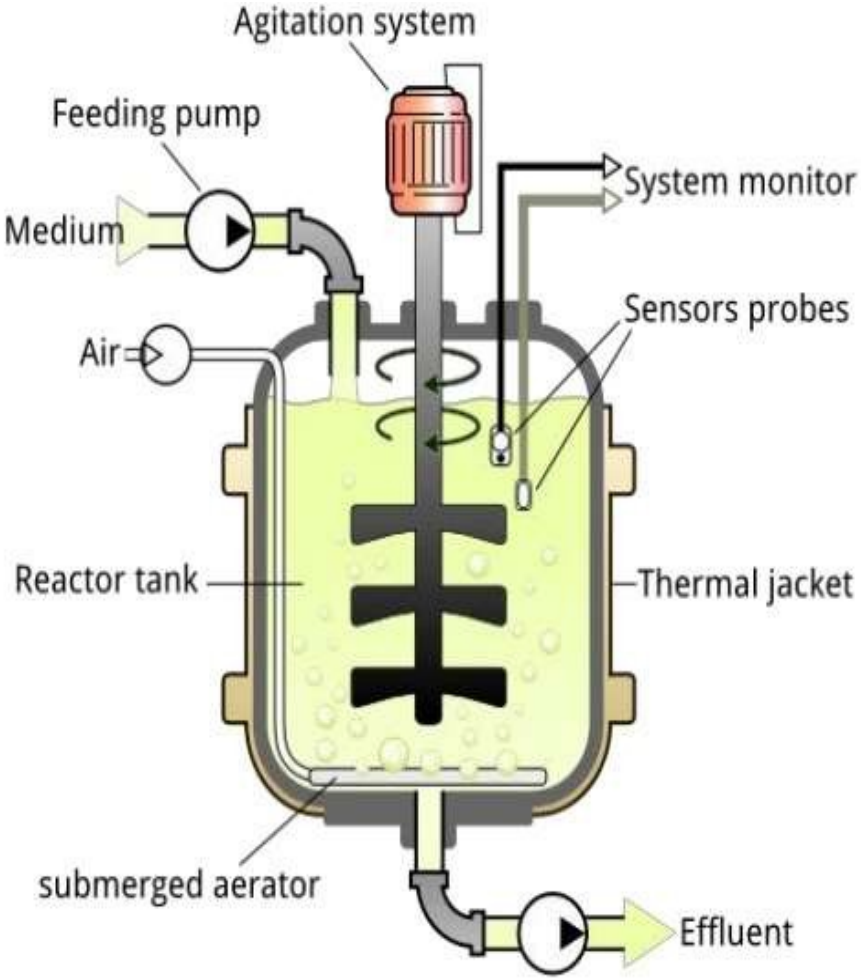


Air lift reactor

CONTINUOUS STIRRED TANK BIOREACTORS



- A continuous stirred tank bioreactor consists of a cylindrical vessel with motor driven central shaft that supports one or more agitators (impellers).
- The shaft is fitted at the bottom of the bioreactor.
- The number of impellers is variable and depends on the size of the bioreactor i.e., height to diameter ratio, referred to as aspect ratio.
- The aspect ratio of a stirred tank bioreactor is usually between 3-5. However, for animal cell culture applications, the aspect ratio is less than 2.
- The diameter of the impeller is usually 1/3 rd of the vessel diameter.
- The distance between two impellers is approximately 1.2 impeller diameter. Different types of impellers (Rustom disc, concave bladed, marine propeller etc.) are in use.



General structure of a continuous stirred-tank type bioreactor

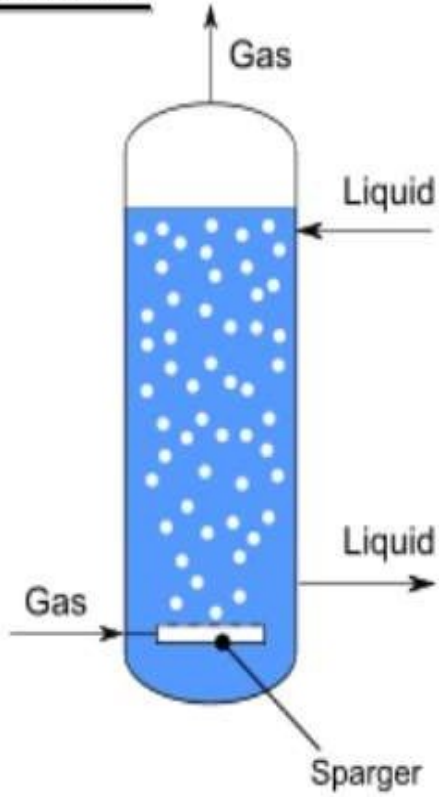
- In stirred tank bioreactors or in short stirred tank reactors (STRs), the air is added to the culture medium under pressure through a device called sparger.
- The sparger may be a ring with many holes or a tube with a single orifice.
- The sparger along with impellers (agitators) enables better gas distribution system throughout the vessel.
- The bubbles generated by sparger are broken down to smaller ones by impellers and dispersed throughout the medium.
- This enables the creation of a uniform and homogeneous environment throughout the bioreactor.

ADVANTAGES OF STRS

- There are many advantages of STRs over other types. These include the efficient gas transfer to growing cells, good mixing of the contents and flexible operating conditions, besides the commercial availability of the bioreactors.

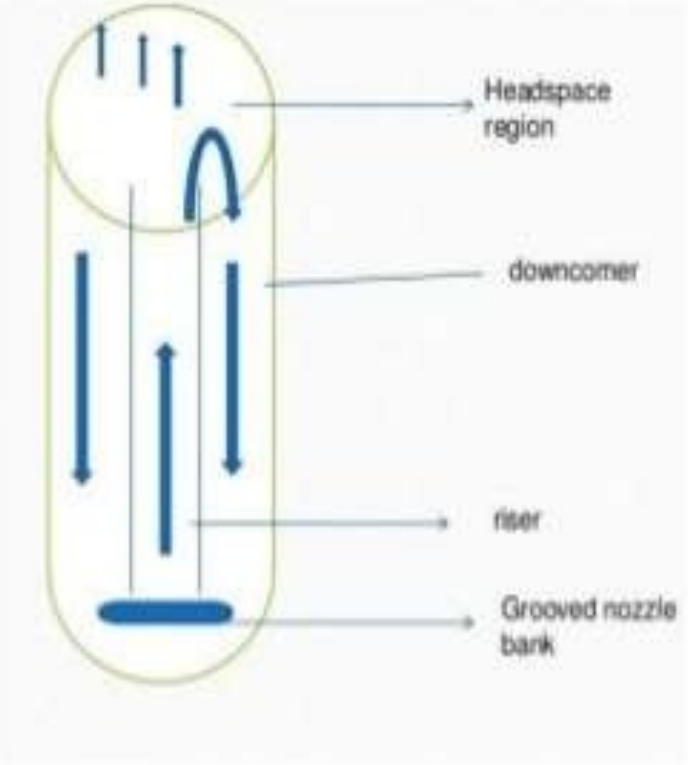
BUBBLE COLUMN BIOREACTORS

- In the bubble column bioreactor, the air or gas is introduced at the base of the column through perforated pipes or plates, or metal micro porous spargers.
- The flow rate of the air/gas influences the performance factors —O2 transfer, mixing.
- The bubble column bioreactors may be fitted with perforated plates to improve performance.
- The vessel used for bubble column bioreactors is usually cylindrical with an aspect ratio of 4-6 (i.e., height to diameter ratio).



AIRLIFT BIOREACTORS

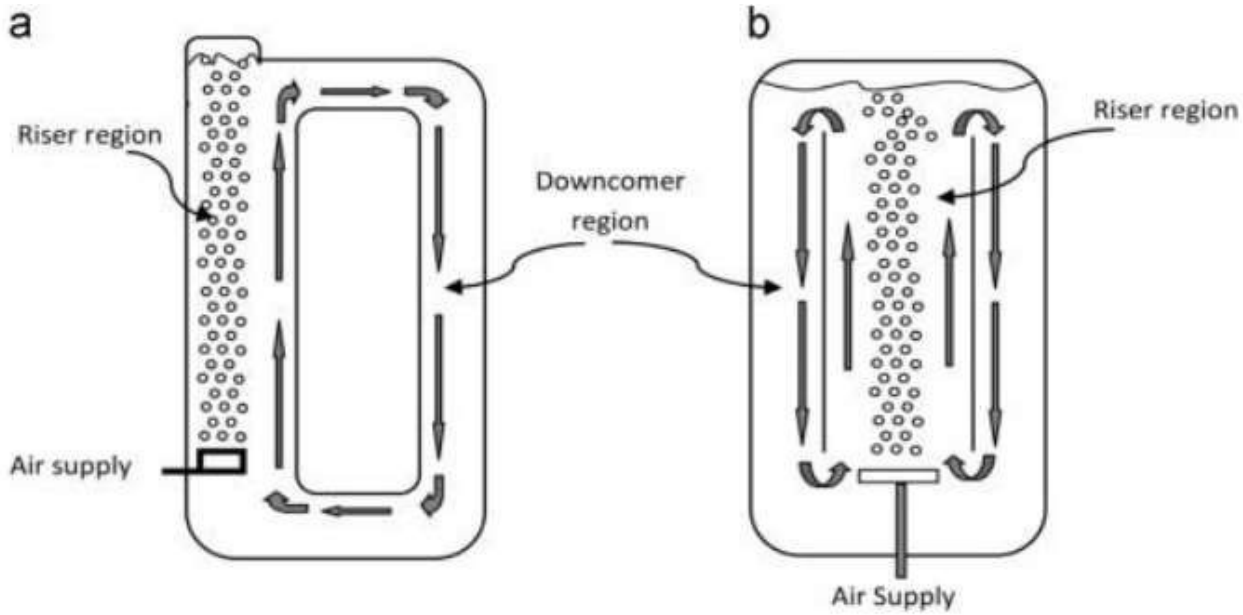
- In the airlift bioreactors, the medium of the vessel is divided into two interconnected zones by means of a baffle or draft tube.
- In one of the two zones referred to a riser, the air/gas is pumped. The other zone that receives no gas is the down comer.
- The dispersion flows up the riser zone while the down flow occurs in the down comer.



TYPES OF AIRLIFT BIOREACTORS

There are two types of airlift bioreactors.

- **Internal-loop airlift bioreactor** has a single container with a central draft tube that creates interior liquid circulation channels.
- These bioreactors are simple in design, with volume and circulation at a fixed rate for fermentation.
- **External loop airlift bioreactor** possesses an external loop so that the liquid circulates through separate independent channels.
- These reactors can be suitably modified to suit the requirements of different fermentations.
- In general, the airlift bioreactors are more efficient than bubble columns, particularly for more denser suspensions of microorganisms.
- This is mainly because in these bioreactors, the mixing of the contents is better compared to bubble columns.



Schematic of airlift bioreactor with (a) external recirculation and (b) internal recirculation