

Biology Guardian

Chapter # 1

CELL STRUCTURE AND FUNCTIONS

Lecture # 2

By

Abid Ali Mughal

**Assistant Professor/Head of Biology Department
Islamabad Model College for Boys H-9, Islamabad**

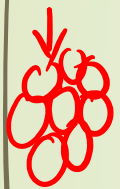
biologyguardian@gmail.com

Cell Fractionation

Fraction $\frac{1}{2}$, $\frac{1}{4}$

Principle

Tissue



Homogenization

the formation of homogenous mass of cell

shaking → Enzyme (pectinase for plant tissue
or Protease " animal "
+ suitable pH
+ suitable temp

Centrifugation

(cell homogenate)
cell suspension



Homogenous
mass of
cells

Cell fractionation is the combination of various methods used to separate a cell organelle and components based upon size and density.

Purpose / Importance

It is very useful for electron microscopy of cell components. (cell organelle)

Cell Fractionation

DNA ← heavy
light

Homogenization

Centrifugation

Rotation of a sample at high speed
A technique which is used to separate
diff. sized component according to size & density

Differential centrifugation

Density gradient Centrifugation

- ❑ Cell fractionation is the combination of various methods used to separate a cell organelle and components based upon size and density.

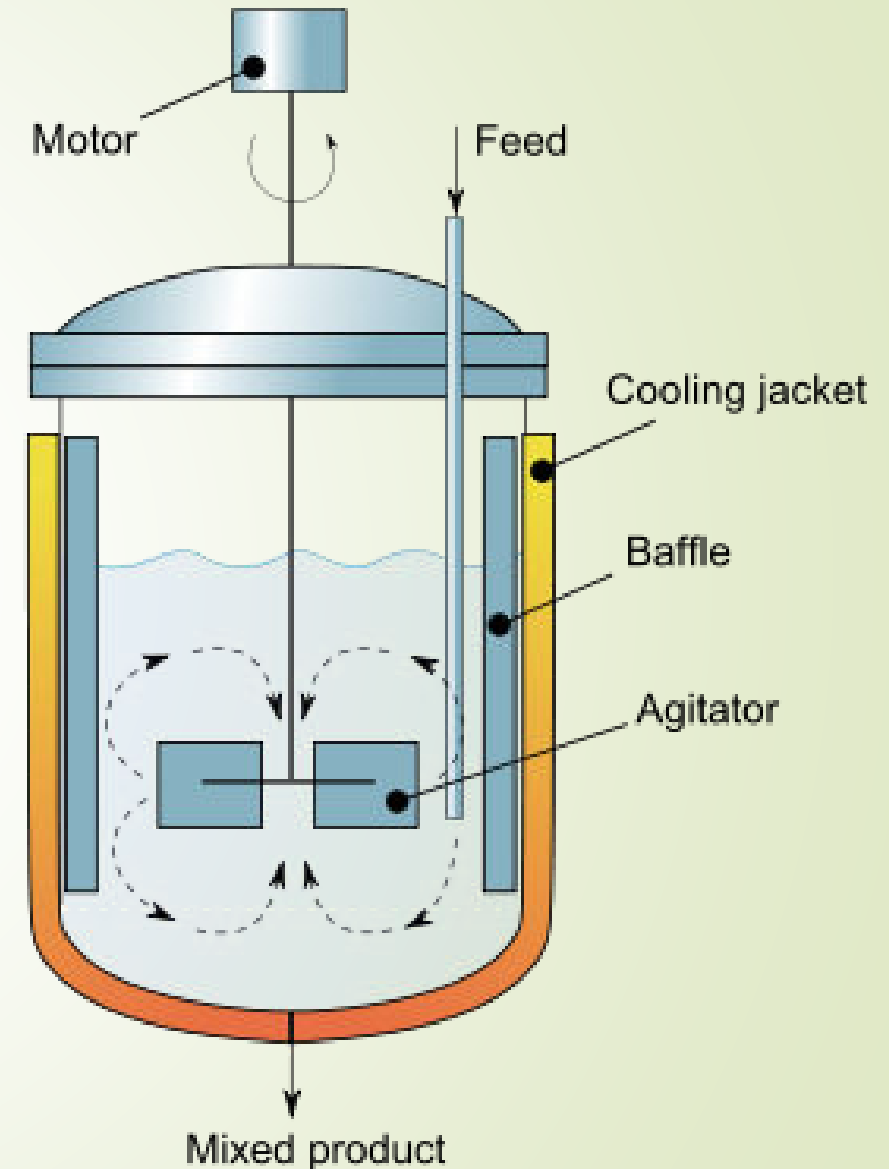
Purpose

- ❑ It is very useful for electron microscopy of cell components.

Cell Fractionation

Homogenization

- ❑ It is the formation of a homogenous mass of cells (cell homogenate or cell suspension).
- ❑ It involves the grinding of cells in a suitable medium with correct pH, ionic composition, temperature and in the presence of certain enzymes that can break the cementing substance of cells.
- ❑ For example pectinase which digest middle lamella among plant cells.
- ❑ This can be done in a cell homogenizer (food mixer/blender).
- ❑ This procedure gives rise a uniform mixture of cells i.e., cell homogenate. The resulting mixture is then centrifuged.

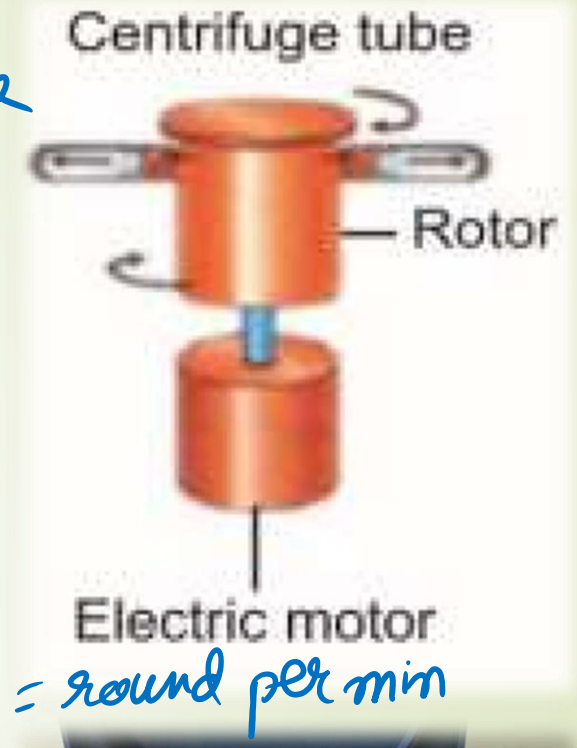


Cell Fractionation

Centrifugation

- ❑ Centrifugation is the process to separate substances on the basis of their size and densities under the influence of centrifugal force.
- ❑ It is done by the machine called centrifuge.
- ❑ This machine can spin the tubes. Contents are kept in tubes that are much like the test tubes. Spinning the tubes exerts a centrifugal force on the contents.

washing machine
← | →



rpm = round per min

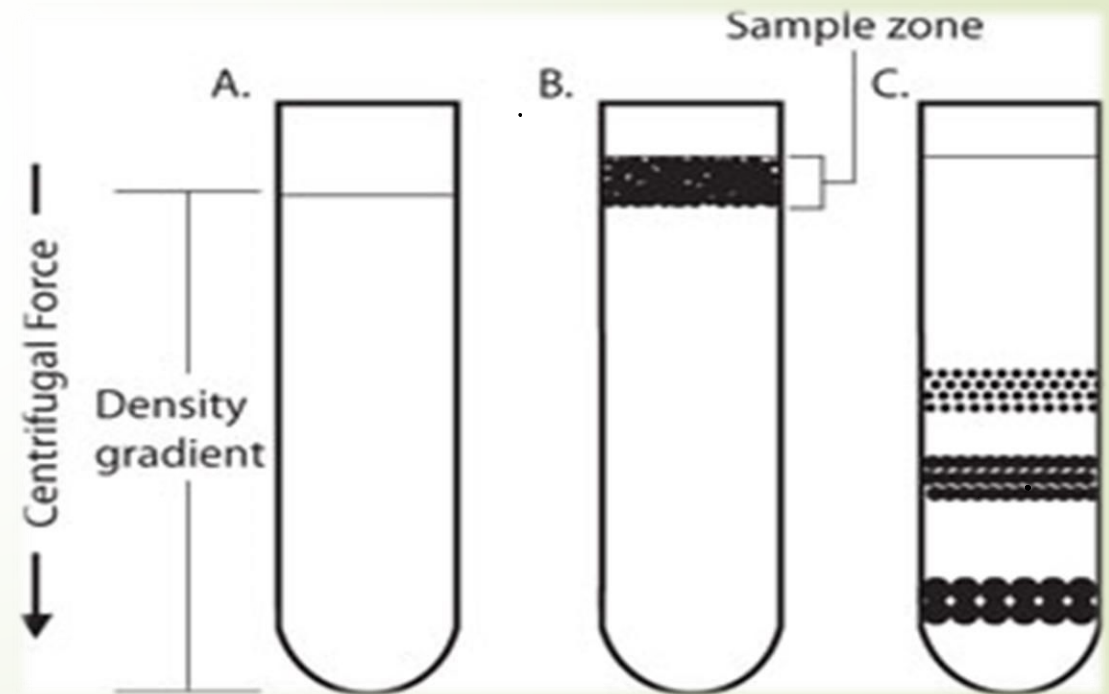


Centrifugation

Density gradient centrifugation

- In density gradient centrifugation, the components of different sizes and densities are separated in the tube containing ionic medium according to their size and densities.

- Only a single speed is used.
- Components are separated in different layers or sediments.
- The upper sediments have smaller and less dense components than lower sediments.



Centrifugation

Differential centrifugation

- ❑ In differential centrifugation the sedimentation rate for a particle of a given size and shape measure how fast the particle “settles” or sediments.
 - ❑ The faster the rotation of the centrifuge, the smaller the particles will sediment.
 - ❑ A series of increasing speeds can be used.
 - ❑ At each step, the content which make sediment in the bottom of the tube are called pellet and
 - ❑ those that remain suspended above the sediment in the form of liquid are called supernatant.
- After each speed, the supernatant can be drawn off and centrifuge again.

