Immobilisation techniques & Secondary Metabolite Production

Immobilization Techniques of Plant Cells

- Immobilization means trapping or fixing plant cells in a particular place so that they cannot move freely in the culture medium.
- The cells remain **alive and active**, but they are held inside or attached to a support material.
- In normal suspension culture, plant cells float freely in liquid medium.
- **In immobilized culture, the cells are:**
  - enclosed in gel beads,
  - attached to solid surfaces,
  - trapped inside porous materials,
  - or held between membranes.

Why Immobilization is Done

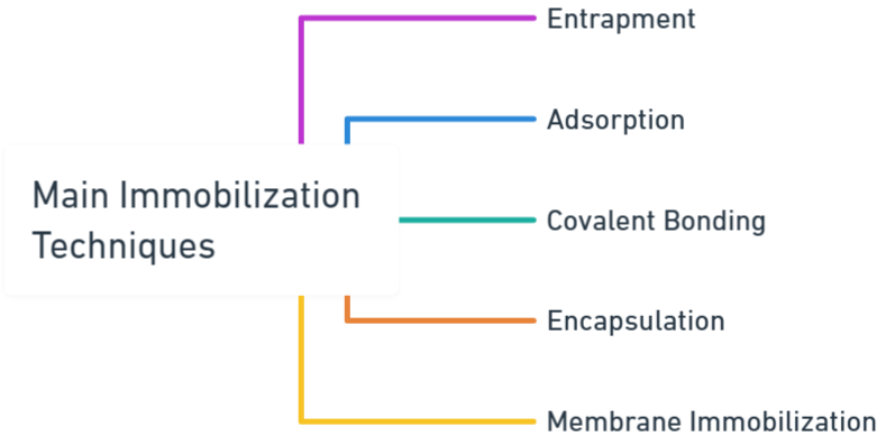
- To make plant cells easier to handle.
- To improve production of useful chemicals, especially secondary metabolites.
- To protect cells from physical damage caused by shaking.
- To allow repeated use of the same cells.
- To make separation of products easier from the culture medium.

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Basic Principle

- Plant cells are mixed with or placed onto a supporting material.
- This material holds the cells in one place.
- Nutrients from the medium can still reach the cells.
- Oxygen and other necessary substances can also move in.
- Metabolites produced by cells can move out into the medium.

Main Immobilization Techniques



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A. Entrapment

- In this method, plant cells are **trapped inside a gel or matrix**.
- The cells do not come out easily, but nutrients can pass through the gel.

Common materials used

- sodium alginate, agar, gelatin, etc.

Example of alginate entrapment

- Plant cells are mixed with sodium alginate solution.
- This mixture is dropped into calcium chloride solution.
- Calcium ions make alginate harden and form **small beads**.
- These beads contain living plant cells inside them.

Advantages:

- Easy and common
- Cells stay alive
- Good protection
- Reusable

Disadvantages:

- Slow nutrient/oxygen flow
- Beads may break
- Large cell masses reduce oxygen

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B. Adsorption

- In this method, plant cells are **attached to the surface** of a solid material.
- The attachment may happen naturally because of weak physical forces.

Supports:

- glass, plastic, cellulose, ceramics

How it works

- Cells are placed in contact with the support.
- They stick to the surface.
- Once attached, they continue growing and producing metabolites.

Advantages:

- Simple and cheap
- Good nutrient flow
- Easy product recovery

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Disadvantages:

- Cells may detach
- Uneven cell distribution
- Not for all cells

C. Covalent Bonding

- In this technique, cells are attached to a support material by **strong chemical bonds**.
- Chemical agents are used to bind cells to the carrier.

Important point

- This technique is more common in enzyme immobilization than in plant cell culture.
- It is used less for plant cells because harsh chemicals may damage the living cells.

Advantages

- Strong attachment
- Less chance of cells getting detached

Disadvantages

- Can harm cell viability
- Difficult method
- Not preferred for delicate plant cells

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D. Encapsulation

- Encapsulation means **surrounding plant cells with a semi-permeable covering**.
- It is similar to entrapment, but special capsule-like structures are formed.
- Nutrients enter the capsule and metabolites leave it.

Advantages

- Better protection of cells
- Good control over cell environment

Disadvantages

- Preparation is more complex
- Capsule stability may be a problem

E. Membrane Immobilization

- Cells are kept **behind a membrane** or between membranes.
- The membrane allows passage of nutrients and products, but cells remain inside.

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Advantages

- Good containment of cells
- Easy collection of secreted metabolites

Disadvantages

- Membrane clogging can happen
- Requires specialized setup

Characteristics of an Ideal Immobilization Matrix

- Non-toxic to cells
- Allows movement of nutrients, oxygen, and products
- Strong and stable
- Cheap and easy to prepare
- Does not react with products
- Supports long-term cell survival

Factors Affecting Success

- Type of plant cells
- Type of matrix
- Size of beads/support
- pH and temperature
- Oxygen supply
- Nutrient availability
- Cell density

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Examples of Secondary Metabolite Production Using Immobilized Cells

A. Catharanthus roseus

- Produces indole alkaloids such as ajmalicine

B. Coptis Japonica

- Produces berberine

C. Lithospermum erythrorhizon

- Produces shikonin

D. Coleus species

- Produces rosmarinic acid

E. Panax species

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- Produces ginsenosides

Free Cells vs Immobilized Cells

Free Cells

- Move freely in medium
- Multiply easily
- May get damaged by shaking
- Difficult to separate

Immobilized Cells

- Fixed in one place
- Easy to handle
- Often produce more metabolites
- Easy product recovery

Application of Immobilization in Secondary Metabolite Production

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Secondary Metabolites (Simple Meaning)

- Special compounds made by plants
- Not needed for basic growth
- Important for defense, protection, and adaptation
- **Examples:** alkaloids, flavonoids, terpenoids, phenolics, essential oils

Why Use Immobilized Cells

- Produce more metabolites
- Cells experience stress → increases production
- Can be reused many times
- Easy to use in bioreactors
- Products often released into medium

How Immobilization Helps

- Protects cells from damage
- Maintains high cell density
- Supports long-term production

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