RAMA UNIVERSITY

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FACULTY OF ENGINEERING & TECHNOLOGY

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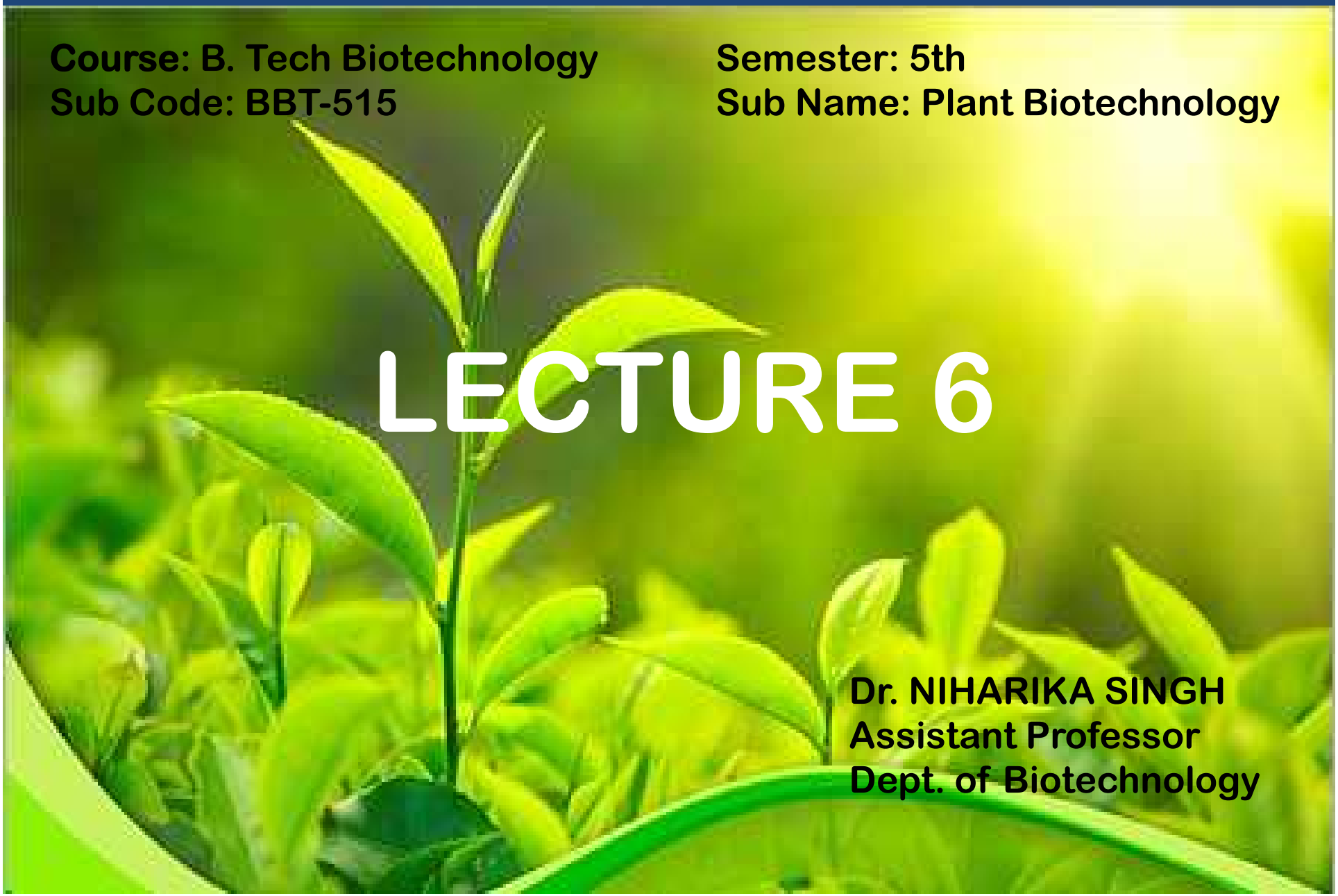
Course: B. Tech Biotechnology
Sub Code: BBT-515

Semester: 5th
Sub Name: Plant Biotechnology



LECTURE 6

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Regeneration

- The process whereby a part of a plant can be turned into a whole new plant.
- Regeneration is possible because plant cells can be made totipotent using hormones.
- Differentiated tissue: stems, leaves, roots, etc.
- Undifferentiated (embryonic) cells are totipotent: can become a whole new plant by differentiating into a whole new plant.

Steps involved in Tissue Regeneration

1) **Sterilization:** Tissue must be sterile- completely free of any microoganisms; done using aseptic technique
2) **Differentiation:** Starting tissue is called an ex-plant., differentiated cells (these cells have developed to be part of specialized tissue (root, leaf, stem, ovary, cotyledon, etc.).
3) **Transfer of explants** are plated on a sterile petridish containing hormones and nutrients that promote the explant cells to develop into callus.
4) **Callus development:** a mass of undifferentiated cells developed into seedlings.
5) **Transfer of callus cells** to petridishes: Individual cells (or clumps of cells) of the callus are transferred aseptically to a different petri dish containing sterile medium that encourages the undifferentiated callus cells to become shoots and roots.

CONTINOUS CULTURE

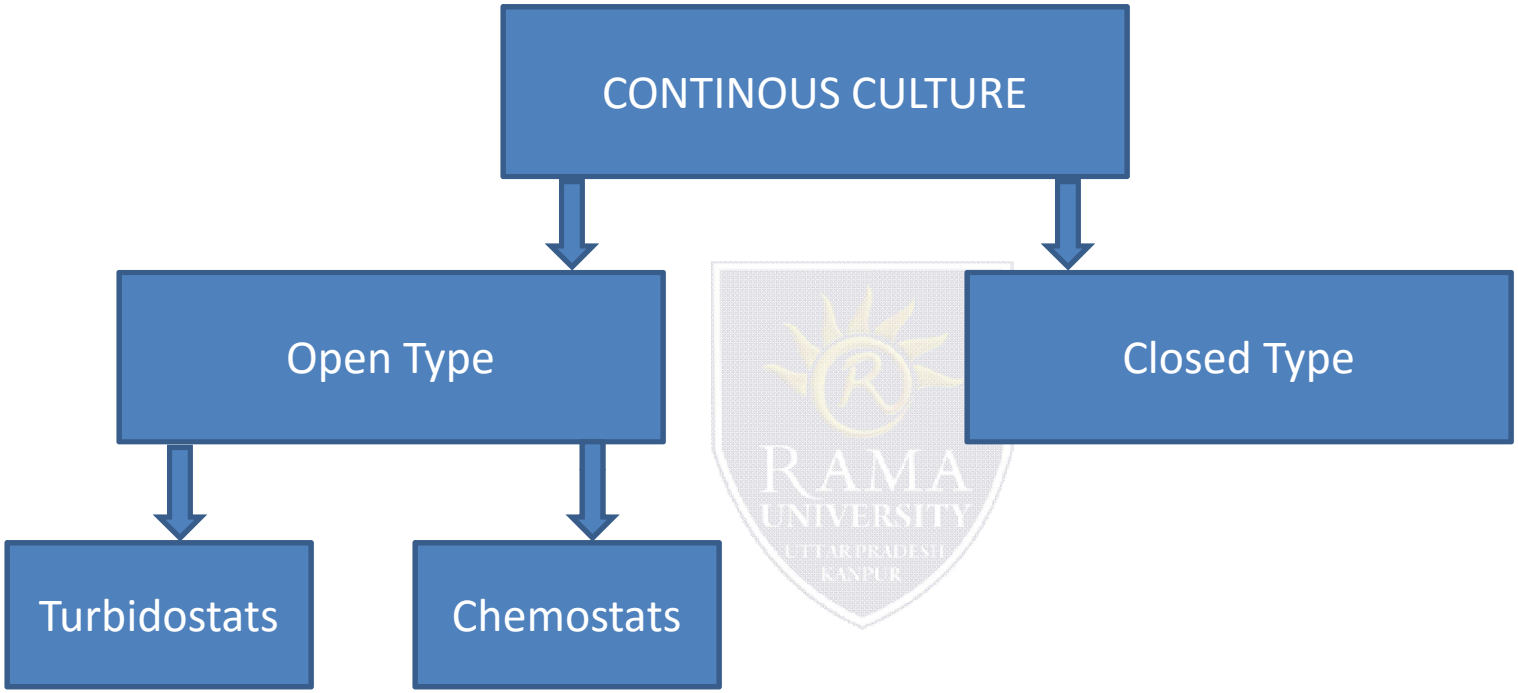
- In continuous culture system, the old liquid medium is replaced continuously by the fresh liquid medium to stabilize the physiological states of the growing cells.
- In this system, nutrient depletion does not occur due to the continuous flow of nutrients and the cells always remain in the steady growth phase.
- Cell proliferation takes place under constant condition.

- This system allows
  - ✓ **Establishment** of steady states of growth and metabolism.
  - ✓ **Study** of the changes which occur in transitions from one steady state to another.
  - ✓ **Identification** of the controlling factors.

- Such culture systems are of **two types:**

1. Open type

2. Closed type


CONTINOUS CULTURE
Open Type
Closed Type
Turbidostats
Chemostats

1. Open type

- In open type, both the cells and used medium are replaced with fresh medium thus maintaining culture at constant and submaximal growth rate.

- Open continuous cell suspension culture is of two types :

    1. Chemostat
    2. Turbidostat

Chemostat

- ✓ In this system, culture vessels are usually cylindrical or circular in shape and possess inlet and outlet pores for aeration and the introduction and removal of cells and medium.
- ✓ Such a system is maintained in steady state.
- ✓ Thus in steady state condition the density, growth rate, chemical composition and metabolic activity of the cells all remain constant.
- ✓ Such continuous cultures are ideal for studying growth kinetics and the regulation of metabolic activity in higher plants.

Turbidostat

- ✓ A turbidostat is a continuous culturing method where the turbidity of the culture is held constant by manipulating the rate at which medium is fed.
- ✓ In this system, the cells are allowed to grow upto a certain turbidity, when the predetermined volume of culture is replaced by fresh culture.
- ✓ The turbidity is measured by the changes of optical density of medium.
- ✓ An automatic monitoring unit is connected with the culture vessel and such unit adjusts the medium flow in such a way, as to maintain the optical density or pH at chosen, present level.

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