SEMESTER 7
C15 APPLIED BIOTECHNOLOGY
Paper Code (BBT 7001 [A])
(Credits: Theory-4, Practicals-2)
THEORY Lectures: 20

Course Objective(s)

- To study microbial growth and its maintenance.
- To develop understanding different bioreactors.
- To provide knowledge biochemistry of different industrial chemicals

Course Outcome(s):

- Learn basics of fermentation technology, bioreactors, production optimization, and downstream processing.
- On successful completion of the course the students should have understood the basics of fermentation technology and learnt the concept of different metabolite production by microbes in industrial setup.

THEORY

Unit-I Microbial Cell Growth and Death Kinetics: (10 Lectures)
Screening and Improvement of industrially important microorganisms, Microbial Growth and Death Kinetics, Media for Industrial Fermentation, Air and Media Sterilization. IKS: Contribution of Indian Scientists.

Unit-II Operation and Control of Bioreactors: (10 Lectures)
Types of Fermentation Processes: Analysis of batch, fed-batch and continuous bioreactors, stability of microbial bioreactors, analysis of mixed populations, specialized bioreactors-pulsed,

fluidized, photo bioreactors, etc. Measurement and Control of bioprocess parameters. Downstream processing, Whole cell immobilization and their industrial applications.

Unit-III Fermentation Technology: (10 Lectures)
Industrial production of chemicals: Ethanol, Acids (citric, acetic and gluconic acid), Solvents (glycerol, acetone, butanol), Antibiotics (penicillin, streptomycin, tetracyclin), Semi-synthetic antibiotics, Amino acids (lysine, glutamic acid), Single cell protein.

Unit-IV Applications of Bioprocess Engineering: (10 Lectures)
Agitation and aeration: requirement in industrial processes, concept of volumetric oxygen transfer coefficient and its determination (Kla), Factors affecting Kla values; Uses of microbes in mineral beneficiation and oil recovery. Introduction to food technology; Elementary idea of canning and packaging, Sterilization and pasteurization of food products.

PRACTICALS

1. To plot Microbial growth curve for shake flask culturing using turbidity method.
2. Prepare a standard curve of reducing sugar by 3,5-Dinitrosalicylic acid method
3. To produce invertase enzyme and find its activity from Baker's Yeast
4. Preparation of standard curve of Ethanol
5. Quantitative estimation of ethanol produced during Yeast fermentation
6. Production of Penicillin and assaying its activity
7. To get familiarized with the lab scale fermenter (bench top fermenter)
8. To determine dissolved oxygen concentration in tap and aerated water.
9. To determine the volumetric transfer coefficient (K la)
10. Estimation of BOD in a given waste water sample.
11. Centrifugation studies during settling of yeast cells.
12. Yeast cell disruption by mechanical methods.

SUGGESTED BOOKS

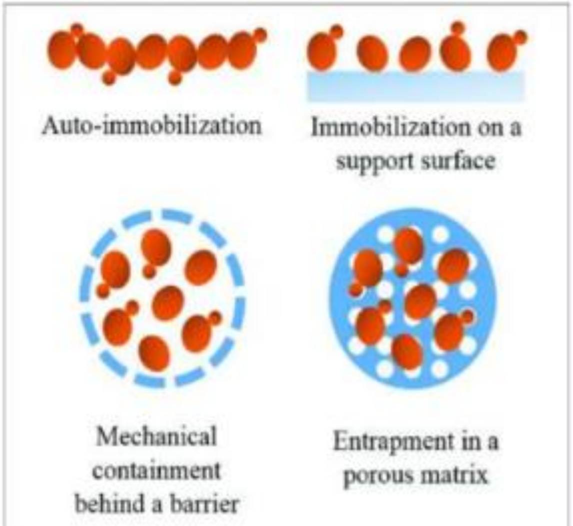
1. Bioprocess Engineering, Shular M &Kargi F, Prentice Hall
2. Biochemical Engineering Fundamentals, Bailey JE &Ollis DF
3. Bioprocess Engineering Principles, Doran, PM, Academic Press, California

The immobilization of whole cells can be defined as “the physical confinement or localization of intact cells to a certain region of space; without loss of desired biological activity.”

When cells are encapsulated in an immobilized cell system, the term “bioencapsulation” or “microencapsulation” is used

Methods of Cell Immobilization

- **Physical methods** (encapsulation, adsorption, entrapment)
- **Chemical methods** (covalent binding, crosslinking)
- **Biological methods** (cell entrapment within matrices)



Advantages of cell immobilization

- Increased stability and longevity of cells.
- Enhanced productivity and efficiency.
- Reusability of immobilized cells.
- Improved control over cell environment.
- Compatibility with continuous processes.
- Enhanced safety
- Versatility in application

Physical Methods

Encapsulation

It involves trapping cells within a semi-permeable membrane or capsule. The membrane allows small molecules like nutrients and waste products to pass through while retaining the cells inside. Examples of encapsulation materials include alginate, agarose, and polyacrylamide hydrogels

Adsorption

Here, cells adhere to the surface of a solid support material. The attachment can be reversible or irreversible, depending on the properties of the support material and the cells. Common support materials for adsorption include activated carbon, silica gel, and glass beads.

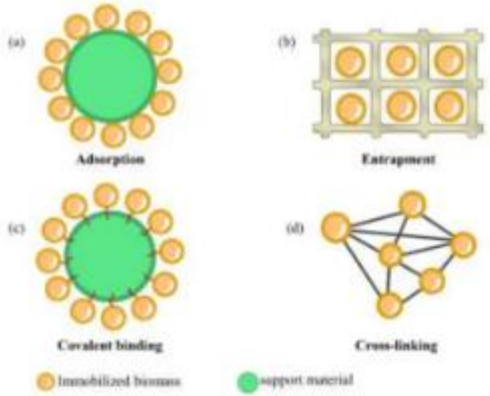
Entrapment

Entrapment entails physically trapping cells within a porous structure or matrix. The cells are immobilized within the pores of the support material, which can be a natural polymer (e.g., gelatin) or a synthetic polymer (e.g., polyvinyl alcohol). Entrapment provides a stable environment for the cells while allowing diffusion of nutrients and products.

Chemical methods

Covalent binding

This involves the **formation of stable chemical bonds** between reactive functional groups on the cell surface (e.g., amino or carboxyl groups) and **reactive groups on the support matrix** (e.g., aldehyde or epoxy groups).
Common agents - Glutaraldehyde, carbodiimides, and periodate



Crosslinking

This involves the formation of chemical bonds within or between support matrix molecules, **creating a network** that immobilizes the cells. Cells may be physically trapped within the crosslinked matrix or chemically bound to it. Crosslinking agents - Polyethyleneimine, and polyethylene glycol diacrylate

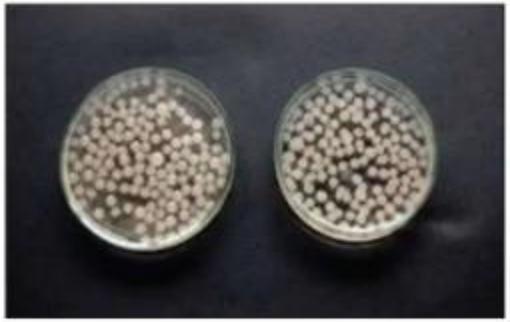
Biological Methods

Cell Encapsulation in Natural Matrices

Cells are encapsulated within naturally occurring polymers such as alginate, agarose, collagen, or chitosan. These matrices provide a biocompatible environment for the cells and support their growth and function.

Genetically engineered Matrices

It can be engineered to produce their own extracellular matrix components, such as self-assembling peptides or engineered proteins. These matrices are designed to promote cell attachment, proliferation, and differentiation.



Desirable characteristics

- High cell mass-loading capacity
- Affords easy access to nutrient media
- Is a simple and "nontoxic" immobilization procedure
- Affords high surface-to-volume ratio
- Facilitates optimum mass transfer
- sterilizable and reusable
- Facilitates easy separation of cells and carrier from media
- Is suitable for conventional reactor systems as well as cell suspension and anchorage-dependent cells
- Should be biocompatible for animal cells
- Contains immunoprotection barrier
- Should be economically viable.

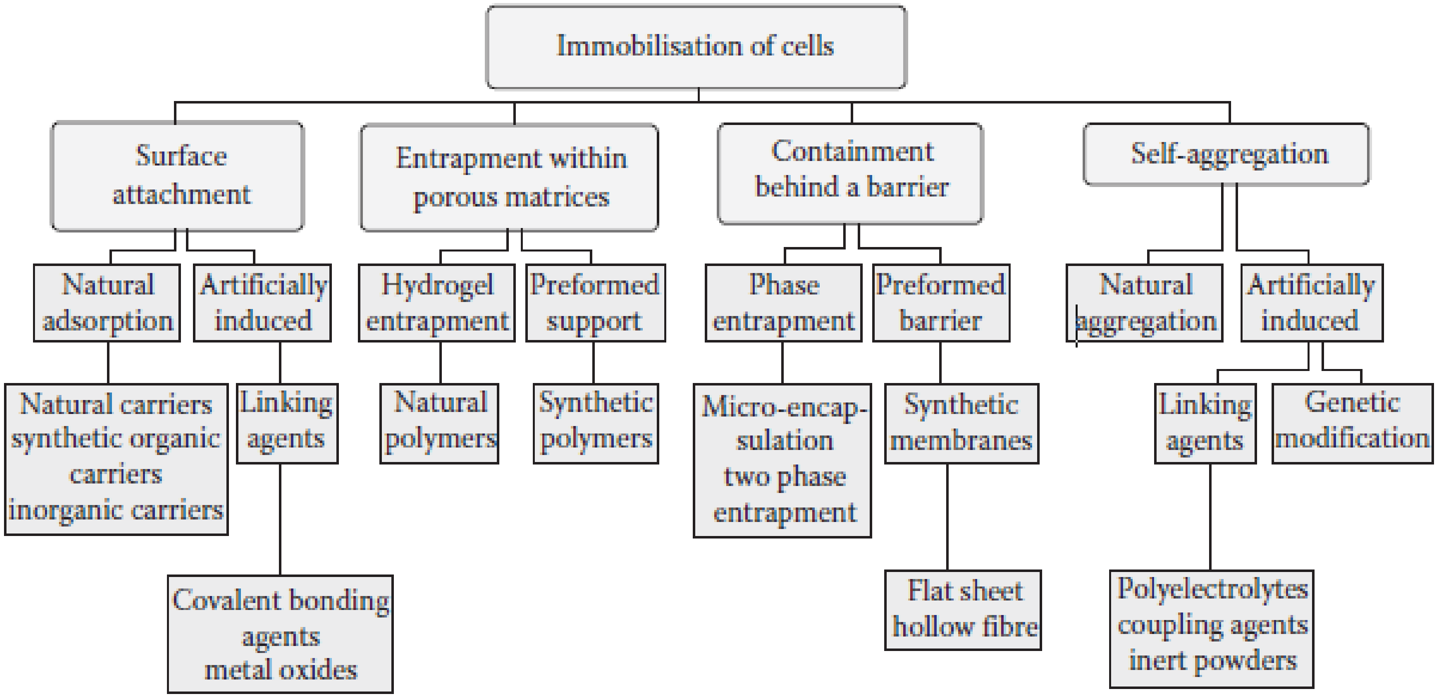


FIGURE 12.1 Classification of immobilized cell systems according to the physical localization and the nature of the microenvironment (Willaert, R.G. and Baron, G.V., *Rev Chem Eng.*, 12:1–205, 1996. With permission.).

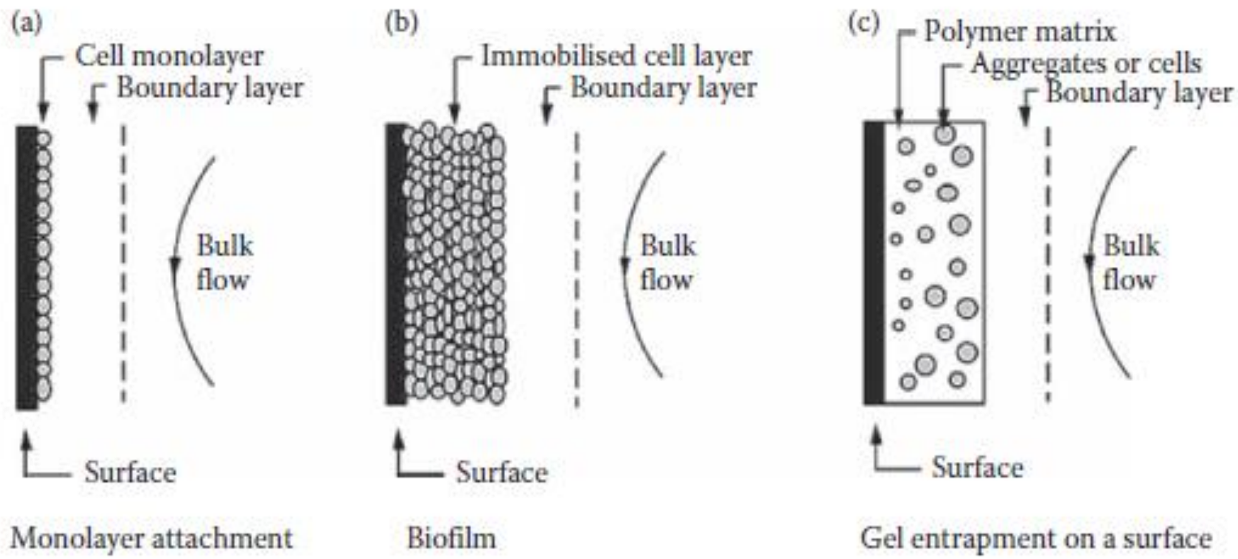


FIGURE 12.2 Cell-immobilization by adsorption/attachment to a surface: (a) adsorption of a monolayer, (b) adsorption of a biofilm, and (c) adsorption of an "artificial" biofilm (i.e., adsorption of a gel layer containing immobilized cells or cell aggregates).

TABLE 12.1
Examples of Cell-Immobilization by Attachment to a Surface

Material	Cell Type	Application
	Bacteria	
Ion exchange resin	*Bacillus stearothermophilus*	Amylase production
Coke	*Zymomonas mobilis*	Ethanol production
Seashell pieces	*Bacillus* sp. + *Aeromonas* sp. + *Alcaligenes* sp.	Decolorization and degradation of triphenyl methane dyes
	Fungi	
Celite	*Penicillium chrysogenum*	Penicillin production
Stainless steel fiber cloth	*Saccharomyces cerevisiae*	Beer production
Sugarcane bagasse	*Candida guilliermondii*	Xylitol production
Straw	*Agaricus* sp.	Laccacase production
Structural fibrous network of papaya wood	*Aspergillus terreus*	Itaconic acid production
	Animal Cells	
Surface modified polyethylene film	Midbrain cells	Neural differentiation
Poly(lactide-co-glycolide), poly(D,L-lactide)	Chondrocyte	Growth on biodegradable scaffolds (tissue engineering)
Pyrex glass, polysterene, glass beads	*Trichoplusia ni*	Recombinant protein production

Source: Adapted from Willaert, R.G. and Baron, G.V., *Rev Chem Eng.*, 12:1–205, 1996; Willaert, R. *Fermentation Microbiology and Biotechnology*, 2nd ed., pp. 287–361, 2007b. Boca Raton, FL: CRC Press.

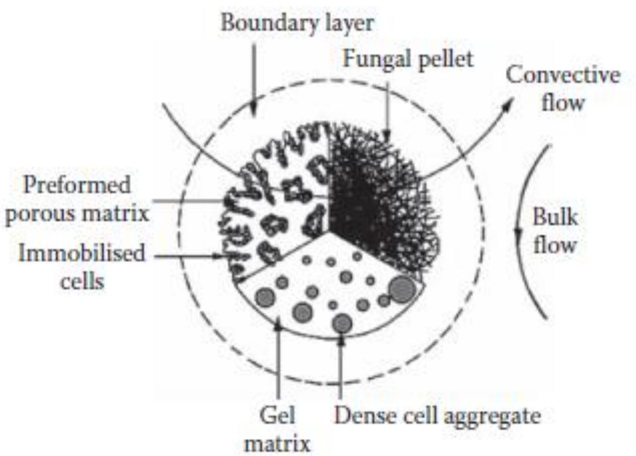


FIGURE 12.3 Spherical particle showing three immobilization methods: (1) cell immobilization in a preformed porous particle (upper left), (2) immobilization by self-aggregation (i.e., a fungal pellet), and (3) immobilization in a gel matrix (lower part).

TABLE 12.2
Gel Formation Mechanisms for Cell Entrapment

Principle of Gelation	Material
Ionotropic gelation	Alginate, chitosan
Thermal gelation	Agar, agarose, κ-carrageenan, collagen, gelatin, gellan gum, curdlan
Precipitation	Cellulose, cellulose triacetate
Polymerization with crosslinking reagent	Polyacrylamide, polymethacrylate, polyacrylamide-hydrazide
Polycondensation	Polyurethane, epoxy resin
Radical-mediated polymerization by irradiation with near-ultraviolet light	Photocrosslinkable resin prepolymers
Crosslinking through photodimerization by irradiation with visible or ultraviolet light	Photosensitive resin prepolymers
Radiation polymerization	Poly(2-hydroxyethyl methacrylate/acrylate), Poly(vinyl alcohol), poly(ethylene glycol) diacrylate/dimethacrylate
Gelation by iterative freezing and thawing or crosslinking with boric acid (and calcium alginate)	Polyvinyl alcohol

TABLE 12.4
Some Examples of Cell Immobilization in Preformed Porous Support Materials

Material	Microorganism	Application/Product
	Natural Organic Polymers	
Bacteria		
Cotton towel	*Propionibacterium acidipropionici*	Propionic acid production
Microalgae		
Luffa sponge	Chlorella sorokiniana	Removal of heavy metals (Cd, Cr, Ni)
Fungi		
Cellulose carrier	*Rhizopus niveus*	Wax ester production
Luffa sponge	*Saccharomyces cerevisiae*	Ethanol production
	Synthetic Organic Polymers	
Bacteria		
Silicone carrier (ImmobSil®)	*Lactobacillus rhamnosus*	Exopolysaccharide production
Fungi		
Polystyrene foam	*Phanerochaete chrysosporium*	Peroxidase production
Polyurethane foam	*Agaricus* sp.	Laccacase production
	Aspergillus niger	Citric acid production
	Yarrowia lipolytica	Oil degradation
Mammalian cell		
Polyester fibrous matrix	Hybridoma cells	Monoclonal antibody production
Polyethylene terephthalate	Human trophoblast	Tissue engineering
	Anorganic Materials	
Bacteria		
Kiezelguhr (Celite)	*Xanthomonas campestris*	Xanthan gum production
Fungi		
Ceramics	*Saccharomyces cerevisiae*	Ethanol production
Kiezelguhr (Celite)	*Penicillium chrysogenum*	Penicillin production
Porous glass	*Saccharomyces cerevisiae*	Beer maturation
	Metallics	
Bacteria		
Alumina pellets	*Zymomonas mobilis*	Ethanol production
Stainless steel knitted mesh	*Zymomonas mobilis*	Levan and ethanol production
Fungi		
Stainless steel fiber cloth	*Saccharomyces cerevisiae*	Beer production
Stainless steel knitted mesh	*Phanerochaete chrysosporium*	Peroxidase production

Source: Adapted from Willaert, R.G. and Baron, G.V., *Rev Chem Eng.*, 12:1–205, 1996; Baron, G.V. and Willaert, R.G., *Fundamentals of Cell Immobilization Biotechnology*, pp. 229–44, 2004. Dordrecht, The Netherlands: Kluwer Academic Publishers; and Willaert, R. *Fermentation Microbiology and Biotechnology*, 2nd ed., pp. 287–361, 2007b. Boca Raton, FL: CRC Press.

TABLE 12.3
Some Applications of Cell Entrapment in Hydrogels

Hydrogel Material	Organism	Application
	Bacterial Cells	
Alginate	*Bifidobacterium longum*	Lactic acid production from whey
	Lactococcus lactis	Cell growth and release
	Streptococcus thermophilus	Inoculation of milk
Alginate-whey protein	*Lactococcus lactis*	Nisin production
Cellulose acetate phtalate	*Bifidobacterium pseudolongum*	Probiotics production
NPAE alginate	*Lactobacillus rhamnosus*	Probiotics production
PVA cryogel	*Bacillus agaradhaerens*	β-Cyclodextrin production
	Microalgae	
Calcium alginate	*Chlorella vulgaris*	Removal of the pollutants: nitrogen and phosphorus, heavy metals (Cd, Cr, Cu, Fe, Pb, Ni), biocides
κ-Carrageenan	*Scenedesmus acutus*	Removal of heavy metals: cadmium, chromium, zinc
Chitosan	*Scenedesmus bicellaris*	Removal of nitrogen and phosphorus
	Fungi	
Calcium alginate	*Saccharomyces cerevisiae*	Ethanol production
Polyethylene oxide	*Candida versatilis* and *Zygosaccharomyces rouxii*	Bioflavor of soy sauce
	Mammalian Cells	
Barium alginate	Engineered NIH/3T3 cells	Continuous release of interleukin 12 for cancer therapy
Calcium alginate	Engineered HEK 293 EBNA	Angiostatin release for cancer therapy
	Rat bone marrow cells	Cell proliferation in a 3D scaffold
Gelatin-HPA	MDCK and NIH/3T3 cells	Scaffold and carrier for tissue engineering
RGDS chitosan	Rat osteosarcoma cells	Regeneration of bone-like tissue

Source: Adapted from Willaert, R.G. and Baron, G.V., *Rev Chem Eng.*, 12:1–205, 1996; Willaert, R. *Fermentation Microbiology and Biotechnology*, 2nd ed., pp. 287–361, 2007b. Boca Raton, FL: CRC Press.

Note: PVA, polyvinyl alcohol; RGDS, Arg-Gly-Asp-Ser; [c]NPAE, *N*-palmitoylaminoethyl; [d]HPA, hydroxyphenylpropionic acid.