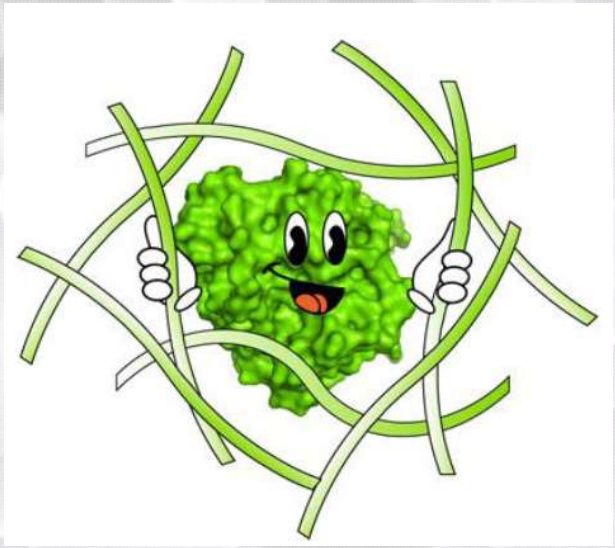
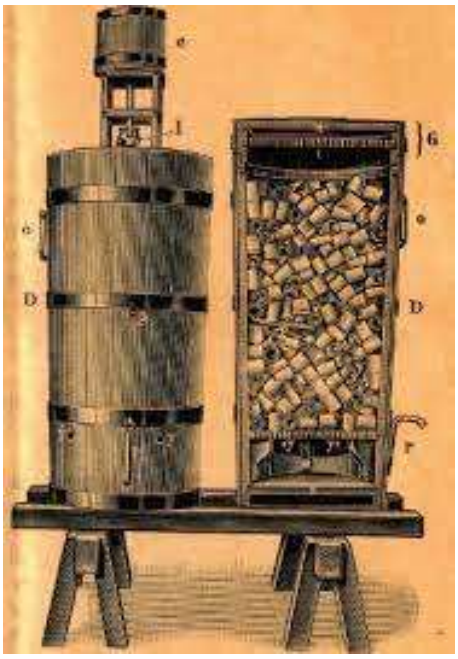
Chapter V. cell Immobilization

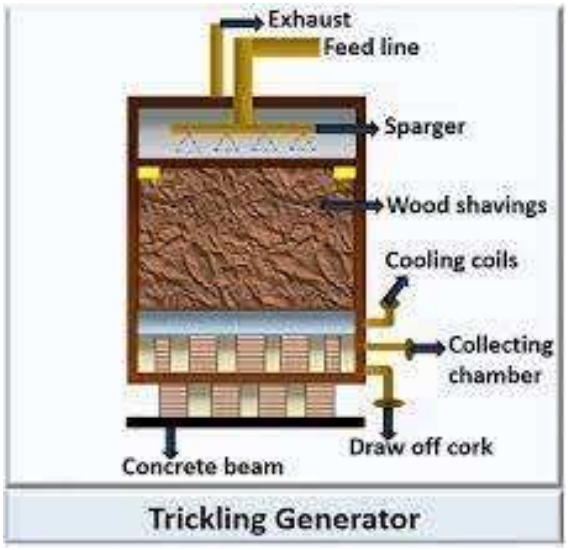


Definitions

- Immobilized cells, also known as controlled biological catalysts, are defined as high-density cells physically confined to a solid phase, or within pellets or capsules, where cell movement is limited during their use as biological agents.
- This definition excludes cells in a chemostat or those recovered by centrifugation in a batch culture and reintroduced into fermentation.
- The concept of cell immobilization was utilized long before it became recognized as potentially useful in industry.
- Examples of immobilized cells can be found in natural habitats such as soil, seawater, the digestive tract, dental plaque, or in the Orléans process of vinegar production, where cells are immobilized on wood chips, as well as in the treatment of wastewater by filtration.

Orléans process of vinegar production





Benefits

- Increased reaction speed by raising the number of cells present in the reactor.
- Prevention of microbial loss at the end of the reaction, allowing for the possibility of reusing the cells.
- Facilitation of cell separation from the fermentation medium, which enables timely termination of the reaction or simplifies clarification operations at the end of the reaction.
- Higher yields of secondary metabolites.

- Reduced susceptibility to attack by phages and contaminants.
- Protection against certain inhibitory effects in the environment.
- Increased biological and physical stability of cells due to supportive protection.
- High stability of plasmids in cells that contain them.

Disadvantages

- Cells may separate from the support, potentially contaminating the product.
- Cell proliferation can damage the support matrix, leading to the release of cells.
- Metabolism may be altered by immobilization (e.g., oxygen transfer).
- The mechanical and chemical stability of certain supports may be insufficient (e.g., shearing, dissolution, decomposition).

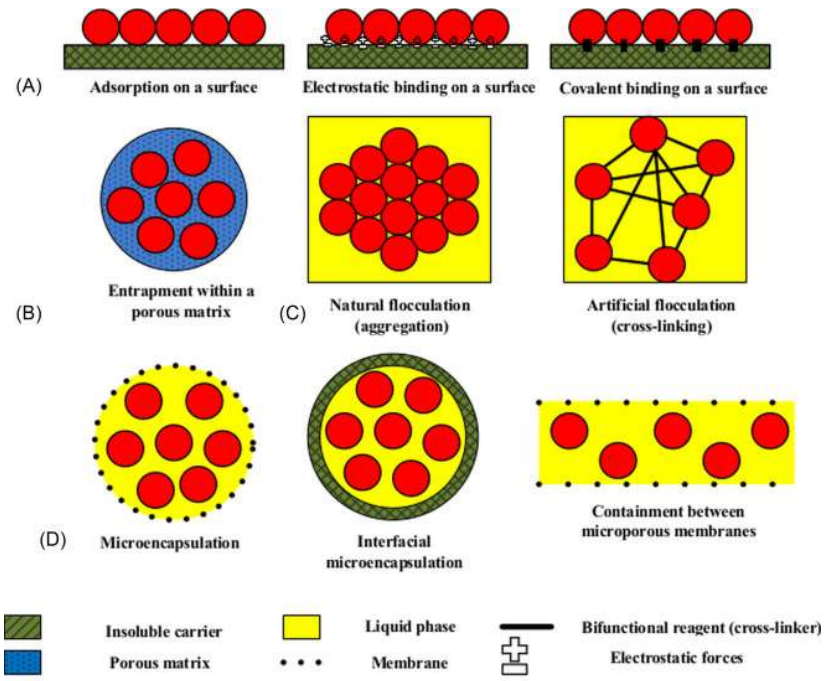
Matrixes and supports

- Permeable to substrates and product solutions.
- Inert and resistant to chemical and microbial degradation.
- Economical and requires minimal preparation steps.

Examples

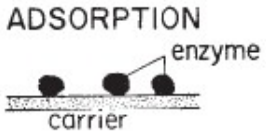
- Natural gels (sodium alginate, carrageenan, etc.)
- Synthetic gels (Polyacrylamide, Polyvinyl PVA, Polyethylene glycol, etc.)

Cell immobilization methods



(A) Immobilization on a surface of a solid carrier
(B) Entrapment within a porous matrix
(C) Cell flocculation (aggregation)
(D) Mechanical containment behind a barrier

1. Adsorption



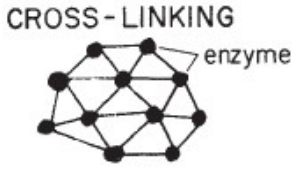
- This technique involves attaching cells to the surface of a solid support.
- Cells and support materials are simply left in contact under specific physicochemical conditions.
- Various forms of support exist; for instance, polymer microbeads (0.2 mm in diameter) provide a large contact surface.

or cells

2. Flocculation/cross-linking

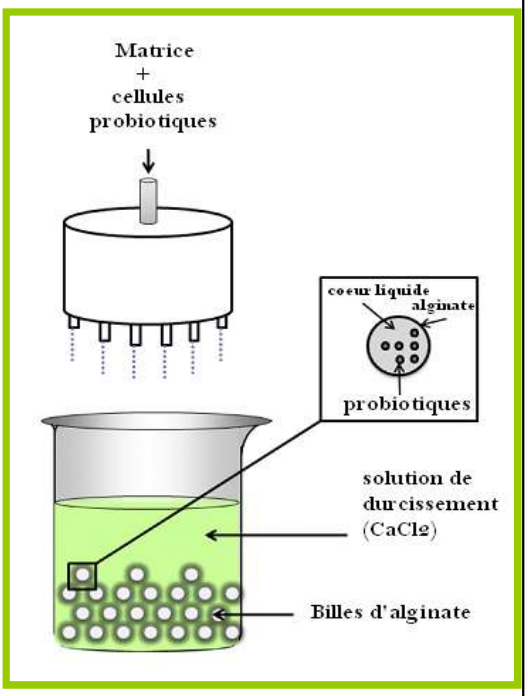
- Flocculation is a natural phenomenon in many bacteria that can also be induced artificially.
- It involves the agglomeration, or crosslinking, of microbial cells within a network held together by polymers, forming macroscopic aggregates of varying sizes known as "flakes."

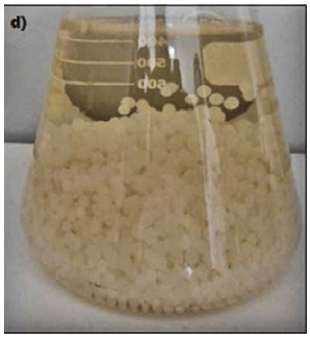
- Example of matrix: Chitosan, a positively charged colloid.



3. Inclusion

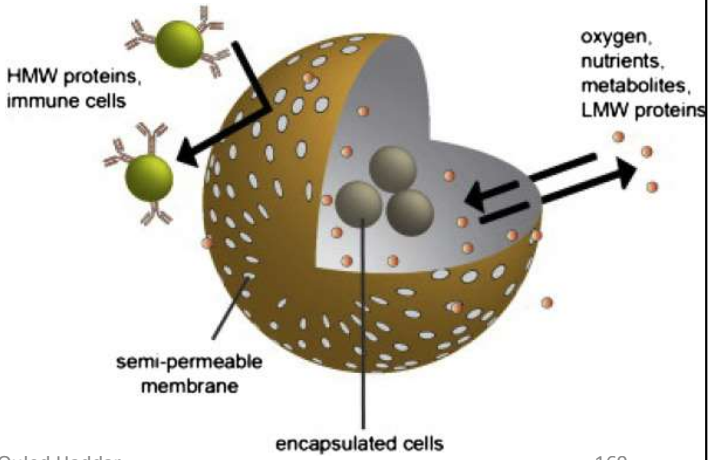
- This method involves embedding microbial cells directly into the porous matrix of a polymer, typically a gel such as sodium alginate.





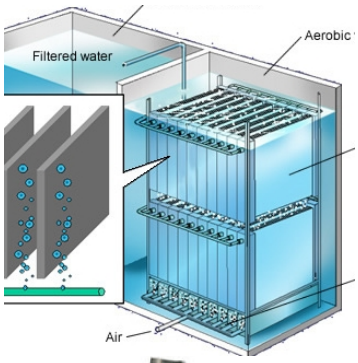
4. Encapsulation

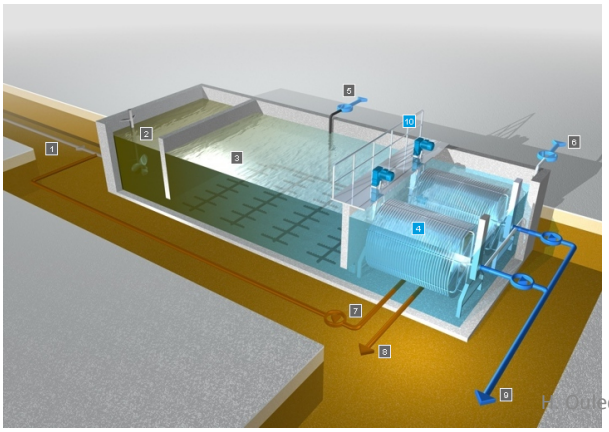
- This method involves encapsulating microbial cells within an insoluble polymer film.
- For example, cells are introduced into a calcium alginate matrix and treated with polylysine, forming a film on the surface of the beads. The beads are then incubated in citric acid to dissolve any unbound alginate.
- The resulting semi-permeable film allows nutrients and low molecular-weight metabolites

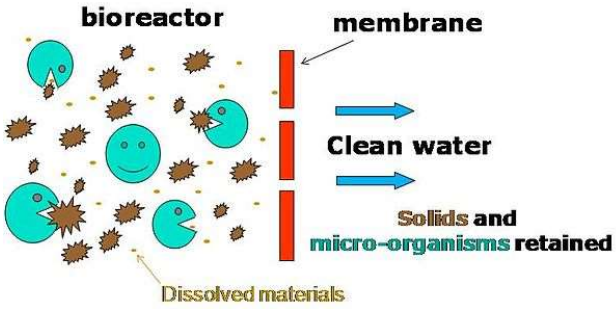


5. Retention by membrane processes

This method uses membrane processes to retain microorganisms in a specific section of the reactor, allowing for high cell concentrations. A physical barrier, such as a microfiltration membrane or hollow fiber (membrane à fibres creuses), confines the cells







Applications

Column of immobilized cells for industrial application

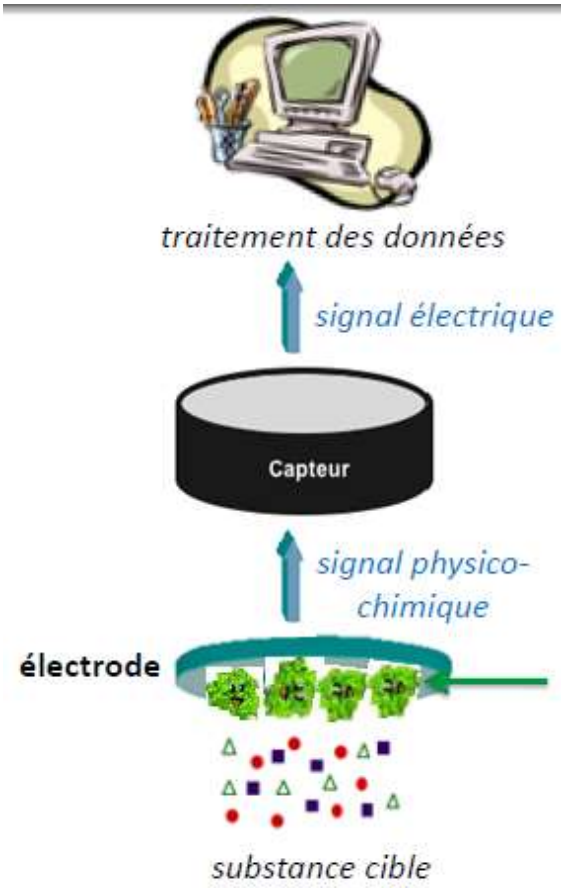
Applications

- Food industry (vinegar, sourdoughs, preservation)
- Environment (biosensors, WWTP, bioremediation)
- Pharmaceutical industry
- Production of enzymes
- Other metabolites (antibiotics, organic acids, alcohol, etc.)

Lactic acid bioproduction by immobilized cells of *Lactobacillus*

- An example of an industrial bioprocess based on immobilized cells is the production of lactic acid. In this process, *Lactobacillus* cells are immobilized within a polymer matrix, such as calcium alginate, and used in a continuous fermentation system.
- The immobilization of cells allows for higher cell densities, stability, and reuse of the biocatalyst, leading to more efficient lactic acid production.
- This process is commonly used in the food, pharmaceutical, and chemical industries, where lactic acid serves as a preservative, pH regulator, and precursor for biodegradable plastics like polylactic acid (PLA).

Biosensors



Catalyst (cell or enzyme) immobilized