

Methods of Cell Immobilization and Its Applications

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ABSTRACT: Immobilization is the method of entrapping/attaching the microbial or plant cells in a suitable matrix. Different methods such as encapsulation, gel entrapment, covalent bonding, cross linking and adsorption is carried out to prepare immobilized cells. When compared to enzyme immobilization, cell immobilization is regarded as a convenient method because of its low cost and improved stability. The cost of isolation and purification is very high in enzyme immobilization. These immobilized cells are mainly used in the areas of biotechnology and industrial production. This paper reviews the various methods of immobilization and application of immobilized cells in waste treatment and biodiesel production.

KEYWORDS: Immobilization, cells, waste treatment and biodiesel production.

I. INTRODUCTION

Recently, immobilized whole cell has been regarded as an alternative method of enzyme immobilization. Immobilization of enzymes is a tedious and time consuming process. Moreover, the cost of isolation and purification of enzymes is high which is not necessary in whole cell immobilization.^[1] Usage of free cell suspension in waste water treatment offers a multitude of disadvantages such as substrate inhibition, low stability, lower degradability of the cell in the presence of high concentration of pollutants and elutriation of microorganisms from the treatment systems.^[2] The main advantage that immobilization confers to whole cells are low sensitivity to temperature and pH when compared to free enzymes.^[3]

Immobilization of whole cells has been gaining importance in the area of waste water treatment. When compared to free cell suspension systems, the immobilized microbial cells offer numerous advantages such as high mechanical strength, high metabolic activity, and resistance to toxic chemicals because of more biomass concentrations and diffusion barriers within the biofilm against the toxic compounds.^[4, 5] During continuous operations of laboratory scale bioreactors, immobilized microbial cells are able to maintain the microbial population in carriers without any loss.^[6]

II. EXISTING METHODOLOGY

1. METHODS OF CELL IMMOBILIZATION:

1.1 Gel Entrapment:

Gel entrapment is the method of trapping the cells in the interstices of polymeric network such as natural or synthetic polymer. Entrapment can be achieved by means of addition of one or a combination of gelling or cross linking agent. The polymeric solution loaded with cells is extruded through needle into a hardening solution to form beads. It is

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considered as one of the easiest, simplest and safest methods of immobilization.^[7] The gelation of polyelectrolyte solution occurs in the presence of a multivalent ion of opposite charge.^[8]

Example:

Cell suspension of *Alcaligenes sp. d₂* is mixed with 4% sodium alginate solution in the ratio of 1:2. Then the mixture is extruded into 0.2 M CaCl₂ solution using syringe or suitable bead forming device to form alginate beads.^[9]

1.2 Cells containment behind a barrier:

The microbial cell containment behind a barrier can be achieved by the attachment of cells on a preformed membrane, by encapsulating the cells in microsphere or by immobilization onto an interface between two immiscible liquid. This type of immobilization is useful to separate the cells and the desired product from the effluent and when the minimum transfer of compounds are required.^[10]

Example:

Hollow fibre membrane reactor: The hollow fibre membrane reactor is designed by inserting the polysulfone (PSf) into polyether sulfone (PESf) fibre. This dual layer fibre is covered by the silicone rubber tube which serves as the shell of the reactor. The whole set up of the reactor resembles shell and tube heat exchanger. It consists of three compartments: annular space (immobilized cells), shell (feed inlet) and lumen part (permeate outlet). Feed was introduced at the shell side of the reactor and the inoculum was introduced at the annulus space using valves. Permeate from the lumen part is collected at regular intervals.^[11]

1.3 Encapsulation:

Encapsulation is the process of forming a continuous membrane around cells to be immobilized that denote the core of the system^[12] in which the inner matrix is protected by means of the outer membrane. Liquid form of active substance is the core material and polymeric wall is the outer membrane.^[13] Microencapsulation of cell can be done by three methods such as emulsification, interface technique and coacervation.

a) Emulsification:

Emulsification is the process of dispersing the cell-loaded polymeric solution into an immiscible liquid phase using a low pressure nozzle device to form an emulsion phase (droplet of polymeric solution in immiscible liquid). Finally the microencapsulated beads can be produced by cooling the emulsion to form a membrane.^[14]

Example:

The cells (wet weight) to be immobilized are mixed with the aqueous polymeric solution (agar and agarose) and the suspension is dispersed into the oil phase by continuous stirring using magnetic stirrer to form the droplets. After the droplet formation, the mixture is cooled in an ice bath and continuously stirred until solidification of polymer occurs. Then the microencapsulated beads are collected by means of centrifugation and the oil and the aqueous phase is removed using the aspirator.^[15]

b) Interfacial polymerization:

The organic phase consists of an active substance (microbes) and an oil soluble monomer is dispersed into the surfactant to form the droplets. Water soluble shell monomer is added to this dispersion to initiate the reaction with the oil soluble shell monomer. As a result of reaction between the two monomers, the polymeric shell is formed at the interface of each droplet.^[16]

c) Coacervation:

Coacervation is the process of forming continuous polymeric membrane around the liquid core. The liquid core present inside the polymeric membrane is called as coacervate.^[17] There are two types of phase separation process such as simple and complex coacervation. In simple coacervation only one polymer is used for the preparation of microcapsules whereas in complex coacervation two oppositely charged polymers soluble in aqueous media are used. Microencapsulation through coacervation is carried out by dispersing the core material into an aqueous polymeric solution. To this dispersion stabilizing agent is added to preserve the individuality of the droplets. Then the coacervating agent is added to get the desolvated polymer and it is cooled to about 5-20°C. Finally, crosslinking agent is added to harden the droplets.^[18]

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Example:

Alginate-chitosan microcapsule: The cells (Baby Hamster Kidney cell) to be immobilized are dispersed into sodium alginate solution. This suspension was extruded through syringe into Chitosan solution (buffered in CaCl₂) to form alginate-chitosan microcapsule.^[19]

1.4 Adsorption/Adhesion:

The adhesion of bacterial cells on the surface of support matrix is initiated by the attraction of cells on the matrix followed by adsorption.^[20] The microbial cells can be attached on the porous or non-porous matrix. The interaction between the cells and matrix is provided by van der Waals, electrostatic, hydration, and hydrophobic forces. For the immobilization of viable cells adsorption process is well suited when compared with entrapment technique.^[21] This type of immobilization is considered as one of the easiest technique. Naturally most of the microbial cells has tendency to adhere on the surface of support matrix. Affinity of microbial cells towards the support is primarily depends on the chemical nature and age of the cells.

Example: Adsorption of fungus on the wood chip

The adsorbent on which the cells to be immobilized were kept in a cultivated medium to promote the growth/attachment of cells on the surface of adsorbent. The medium is incubated for 3 days at 30°C. Finally the adsorbent is separated from the medium and dried by vacuum freeze desiccator.^[22]

1.5 Advantages:^[23, 24, 25]

- Wash out of cells from the beads can be eliminated
- Protects cells from toxic environment
- Loading of biomass is high
- Ease of separation
- Cost effective method due to the possibility of reusing the catalyst

1.6 Disadvantages:^[26]

- Loss of cells from the support in case of immobilization by adsorption technique
- Biomass loading is limited

1.7 Application of Biocatalysts:

In recent years, immobilized whole cells have been gaining importance in the field of waste water treatment and biodiesel production. Microorganisms like bacteria, yeast and fungi are used for their degrading potential. Biodiesel is produced using microorganisms or waste generated from industries. Table 1 lists the application of immobilized cells.

Table: 1 Application of immobilized cell.

S.NO	MICROORGANISMS USED	APPLICATION	IMMOBILIZING MATRIX	REFERENCE
1.	<i>Coriolus versicolor</i>	Decolorization of kraft mill effluent	Alginate	D. Livernoche <i>et al.</i> (1981) [27]
2.	<i>Rhodospseudomonas palustris</i> and <i>Rhodospirillum molischianum</i>	Hydrogen production and treatment of effluent from sugar refinery & Straw paper mill.	Agar	M. Vincenzini <i>et al.</i> (1982) [28]
3.	<i>Aspergillus awamori</i>	Glucoamylase production	Reticulate Polyurethane foam	Elba Bon, Colin Webb (1989) [29]
4.	<i>Aspergillus niger</i>	Olive mill waste water treatment (shake flask repeated batch)	Polyurethane Sponge cubes	NikolayVassilev <i>et al.</i> (1997) [30]

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5.	<i>Trametes versicolor</i>	Decolourization and AOX removal from paper mill effluent	Alginate	Sammaiah pallerla, Robert P. Chambers (1997) [31]
6.	<i>Rhodobacter sphaeroides</i>	Hydrogen production from tofu waste water	Agar	Heguang Zhu <i>et al.</i> (1999) [32]
7.	<i>Rhizopus oryzae</i>	Biodiesel fuel production (Batch)	Polyurethane foam	Kazuhiro Ban <i>et al.</i> (2001)
8.	<i>Pseudomonas putida</i>	Biodegradation of phenolic industrial waste water (FBR)	Alginate	G. Gonzalez <i>et al.</i> (2001) [33]
9.	<i>Chlorella vulgaris, Azospirillum brasilense</i>	Removal of ammonium and phosphorous ions from synthetic waste water	Alginate	Luz E. de-Bashan <i>et al.</i> (2002) [34]
10.	<i>Paecilomyces sp. And Microbrevisluteum</i>	Removal of color and detoxification of pulp and paper mill effluent	Gravel solid support	Pratibhasingh , IndhuShekar (2004) [35]
11.	<i>Rhizopus oryzae</i>	Biodiesel fuel production (PBR)	Reticulated Polyurethane foam	Shinji Hama <i>et al.</i> (2007) [36]
12.	<i>Trichoderma Viride</i>	Biosorption of Cr VI	Alginate, Agar	Narsi R Bishoni <i>et al.</i> (2007) [37]
13.	<i>Scenedesmus sp.</i>	Ammonai-nitrogen and Orthophosphate removal	Alginate	Endongzhang <i>et al.</i> (2008) [38]
14.	<i>Klebsiella oxytoca</i>	Treatment of cyanide waste water	Alginate and Cellulose triacetate	C. Y. Chen <i>et al.</i> (2008) [39]
15.	<i>Bacillus subtilis</i>	Biosorption of copper II	Chitosan	Yun-Guo LIU <i>et al.</i> (2013) [40]

III. CONCLUSION

Thus the whole cell immobilization has proven to have immense scope for application in the field of waste water treatment and biodiesel production. The methodology for efficient immobilization is dependent on the concentration of cells and the area of application. The usage of immobilized biological material in an eco friendly method can be further adapted to other applications as a solution to human resource management.

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