ID: 4043

Application of Biotechnology in Water Treatment: Immobilization of Microorganisms by Inclusion

Belalia Fatiha[1*], Harichane Alya[1], Merakchi Akila[2]
[1]Institute of Science, Morsli Abdullah University Center, Tipaza, Algeria
[2]Institute of Technology, Akli Mohand Oulhadj University, Bouira, Algeria
*Corresponding author's email: belalia.fatiha@cu-tipaza.dz

ABSTRACT

The work concentrated on a method for immobilizing microorganisms, including natural polymer-based support, a crucial tool in biotechnological processes. Bacteria have been immobilized in calcium alginate beads; the ideal gelification concentrations have been determined based on the stability of the beads concerning gel dissolution, cellular enlargement in the surrounding medium and nutrient diffusion issues. The number of live cells within the bead was monitored by dissolving the gel in a sterilized tampon with a pH of 7.

Keywords: alginate, deads, immobilization, microorganisms.

1 Introduction

The biological polymers that are extracted from brown algae are new materials in the fields of biotechnology, biomedicine, and macromolecular chemistry. Its ability to produce gels with a dense consistency is used to encapsulate or immobilize living cells, enzymes, and drugs. The use of biodegradable, biocompatible, and non-toxic polymers for living organisms [1, 2] for the immobilization and encapsulation of biologically active molecules allows for the avoidance of several issues that arise with synthetic macromolecules [3]. The use of alginate hydrogels for immobilization has been extensively documented in the literature [4]. The work aims to manufacture variable-sized alginate particles with higher stability that can permanently immobilize drugs, cells, enzymes, or even bacteria. The process of making these microcapsules involves the gelification of the polymer by extrusion.

2 Experimental

2.1 Cell immobilization

These operations are carried out in aseptic settings. After bacterial suspensions are homogenized with the sodium alginate solution, immobilization occurs. The cellular suspension is introduced into a 5 ml sterile serine. The syringe was fixed to a foil to ensure the suspension's distribution into drops. The precipitates in the coagulation solution ($CaCl_2.6H_2O$) immediately gelled. After that, the beads were gently shaken for an hour to complete the Na^+ and Ca^{2+} exchange. After that, the beads were washed twice in sterile water to determine the excess calcium content [1]. We follow the same procedures for the witness (blank trial without cells). The gel must dissolve before measuring the number of live cells immobilized in the beads. The viability of the cells should not be affected by this gel destabilization.

2.2 The viability of the cells in the gel dissolving solutions

After the beads dissolve, it becomes necessary to determine the percentage of cells that survive during the procedure. 10 ml of tampon (Na_2HPO_4+ citric acid) with cell suspensions incubated at 37°C (pH of 7). Determine the total number of bacteria (viable units) based on the number of colonies discarded in the agars and the culture results for 10 Colonies (Fig.1). The following table shows the amount of time needed for an ensemble of beads to dissolve in a quantity of solution while stirring (Table 1) [5].

DOI: 10.21467/abstracts.170 ISBN: 978-81-970666-1-0 (eBook)

Table 1- The viability of the cells in the gel dissolving solutions

Alginate gel solutions	Duration of dissolution (min)		Quantification (UFC)		
	Witness	Gel + E.coli	E.coli	Witness	Gel+E.coli
Sterilized buffer solution (pH of 7)	25	90	8.10^6	0	4.10^6

(a) (b)

Figure1: (a) E.Coli colonies on nutrient PCA agar. (b) E.coli immobilized in alginate beads

3 Results and Discussion

The results show that immobilization could shield the cells from phase-specific toxicity by preventing direct cell-to-environment contact; however, the loss of cells in the environment could not be prevented, as evidenced by the two-fold decrease in bacterial population over two hours [6]. Florence Majerus [7] noted an analogous slowdown in growth when plant cells were immobilized in an alginate gel. The author suggests that factors that may be involved in this growth limitation include the structure of the alginate gel and its charge, which is carboxylic groups; on the other hand, P. Boyaval (1985) investigated Lactobacillus Helveticus immobilization in calcium alginate sheets [8].

4 Conclusions

First and foremost, the main goal of our work was to value polysaccharides so that they might be used in many applications. The biopolymer known as alginate is obtained by extracting brown algae and assesses an impressive swelling property. This work has made it possible to demonstrate that alginate particles may be prepared in the form of millimeter-sized beads using a straightforward formulation process without the need for an organic solvent. A type of bacterial cell is immobilized to develop and assess the efficacy of these beads. This is a useful strategy for stabilizing and facilitating the use of cellular systems. It appears that the inclusion of cells in the polymer matrix has the effect of slowing down growth. To this end, immobilization in alginate gel may result in a net inhibition of the growth of some cells, such as the E. coli bacteria. According to Florence Majerus [7], this growth slowing may be explained by the intervention of several barriers brought about by the presence of gel.

• The physical order: a mechanical opposition to cellular proliferation.
• Nutritional order: restricting the transfer of gas and substrates.

Reference

[1] Oren A., Gurevich P., Gemmel R.T., Teske A., Halobaculum gomorrense gennov sp nov, a novel extremely halophilic archaeon from the Dead Sea. Int. J. Syst. Bacteriol. 45(1995).747–754

[2] Park H., Park K., Hydrogels in bioapplications. In hydrogels and biodegradable polymers for bioapplications; American Chemical Society: Washington. (1996). 2-9

[3] Gabriel E., Propriétés de rétention et de libération de micronutriments par des réseaux protéiques: étude du système gélifié à lactoglobuline/fer. (2003).

[4] Pronova . General Technical Information II. Alginate application. (2000)

[5] Mahmoud A., Yataghene A., Amrane A., Maachiet R., Biosurfactant production by free and alginate entrapped cells of Pseudomonas fluorescens. Springer: J Ind Microbiol Biotechnol. 35(2008)1303-1308. Algeria.

[6] Belalia F., Djelali N.E., Investigation of swelling /adsorption behavior of calcium alginate beads. Rev. Roum. Chim., 61(10) (2016) 747-754

[7] Florence Majerus., Production d'alcaloïdes par des suspensions de Catharanthus roseus. Mise en œuvre de cellules immobilisées dans un gel d'alginate.(1986) Paris.

[8] Boyaval P., Lebrun A., Goulet J., Étude de l'immobilisation de Lactobacillus helveticus dans des billes d'alginate de calcium. (1985): 185-199.

ISBN: 978-81-970666-1-0

Series: AIJR Abstracts

DOI: 10.21467/abstracts.170