Mohamed Seddik Benyahia University of Jijel
Faculty of Nature and Life Sciences
Department of Applied Microbiology and Food Sciences
Master I Applied Microbiology
Course: Industrial Microbiology (IM)

Lab. 5 Production of protease by immobilized cells

Introduction

From the 1960s onward, the idea of immobilizing "biological catalysts," first enzymes and later microorganisms, quickly led to industrial applications such as immobilized-enzyme electrodes (biosensors), adsorbed bacteria in vinegar production, and bacterial beds for pollution control.
From a theoretical standpoint, immobilizing microbial cells offers the following advantages:
Increased reaction speed by raising the number of cells present in the reactor.
Prevention of microorganism loss at the end of the reaction, enabling their reuse.
Simplified cell/liquid separation, allowing either termination of the reaction at the desired time or easier clarification operations at the end of the reaction.

Objective:

The objective of this lab exercise is to learn how to immobilize bacterial cells by extrusion into a sodium alginate gel.

Required Materials:

- Freshly prepared bacterial culture in nutrient broth
- Sterilized sodium alginate solution (1 g in 45 ml of distilled water)
- Sterile normal saline solution
- Nutrient medium in erlenmeyer flask
- Distilled water
- Sterilized and refrigerated 0.05 M calcium chloride ($CaCl_2$) solution
- Water bath with agitation
- Sterile tubes (centrifugation)
- Sterile beakers
- Syringes (2.5 ml)
- Sterile tips
- Automatic micropipette
- Centrifuge
- Vortex mixer
- Magnetic stirrer
- Sterile filters (Whatman)
- Sterile funnel

Experimental Protocol

I. Preparation of Bacterial Cells for Immobilization
- Bacterial cells cultivated in 50 ml of nutrient broth at 37°C for 24 hours are harvested by centrifugation at 4000 g for 3 minutes.
- The pellet containing bacterial cells is washed once with normal saline and then resuspended in 5 ml of sterile normal saline.

II. Immobilization of Bacterial Cells in 2% Sodium Alginate Gel
- All operations must be performed aseptically.
- Mix 5 ml of the bacterial suspension with 45 ml of 2% sodium alginate solution (previously sterilized by autoclaving) and homogenize aseptically using a magnetic stirrer.

- Transfer the resulting mixture into a sterile syringe. Gelation is performed as follows: drop the suspension, drop by drop, into a sterilized and cooled 0.05 M $CaCl_2$ coagulation solution. The formed beads are gently stirred for one hour to ensure complete Na^+-Ca^{2+} ionic exchange.
- Wash the beads twice with sterile distilled water to remove excess calcium.
- The sodium alginate beads can be stored in normal saline at 4°C until further use.
- Inoculate the sterile nutrient medium with 2 g of immobilized cells.
- Incubate at 37°C with shaking for 24 hours
- Let the culture to stand for 2 min at room temperature then measure the protease activity of the supernatant according to the protocol described in lab 4.