Immobilization Of Yeast Cells For Alcohol Production

Introduction

Immobilization in biotechnology is the technique used for the physical or chemical fixation of cells, organelles, enzymes, or other proteins (e.g. *monoclonal antibodies*) onto a solid support, into a solid matrix or retained by a membrane, in order to increase their stability and make possible their repeated or continued use. Therefore it is expected that the microenvironment surrounding the immobilized cells is not necessarily the same experienced by their free-cell counterparts.

Immobilization of microbial cells in biological processes can occur either as a natural phenomenon or through artificial process. While the attached cells in natural habitat exhibit significant growth, the artificially immobilized cells are allowed restricted growth. Since the time first reports of successful application of immobilized cells in industrial applications, several research groups worldover have attempted whole-cell immobilization as a viable alternative to conventional microbial fermentations. Using immobilized cells, different bioreactor configurations were reported with variable success. The study on the physiology of immobilized cells and development of noninvasive measuring techniques have remarkably improved our understanding on microbial metabolism under immobilized state. We have presented an overview of this field.

Immobilization Methods

Many methods namely adsorption, covalent bonding, crosslinking, entrapment, and encapsulation are widely used for immobilization. The most extensively studied method in cell immobilization is the entrapment of microbial cells in polymer matrices. The matrices used are agar, alginate, carrageenan, cellulose and its derivatives, collagen, gelatin, epoxy resin, photo cross-linkable resins, polyacrylamide, polyester, polystyrene and polyurethane. Among the above matrices, widely used ones are polyacrylamide, alginate, and k -carrageenan.

Applications of Immobilization

Antibiotic production by immobilized microbial cells

Organic acids production by immobilized cells

Production of enzymes by immobilized cells

Biotransformations by immobilized microbial cells

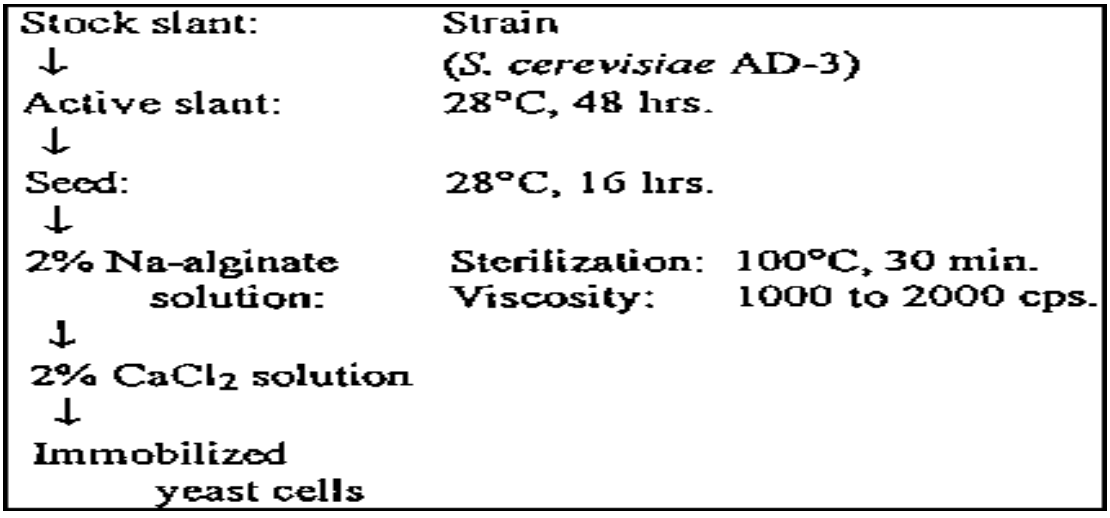
Production of alcohols by Immobilized cells

Nojima reported for the first time a large-scale continuous alcohol fermentation system by immobilized living cells of yeast. The yeast cells were mixed with photo-crosslinkable resin, and were polymerized by light sources. A pilot-plant-unit, producing 250 litres of alcohol/day, was operated for 8000 h continuously. Nagashima *et al*. operated a pilot plant of 4.0 kl capacity, using alginate-entrapped cells of yeast for alcohol fermentation for a period of 4000 h with a constant alcohol production rate of 8.5 to 9.0% by volume. Using Ca-alginate-entrapped cells of *S. cereviceae*, Jamuna and Ramakrishna reported rapid fermentation of high concentration sugar solution, thereby obtaining 20% (w/v) alcohol in 30 h.

Preparation of Immobilized yeast cells

The calcium alginate gel-entrapping method is preferred because of its high enzymatic activity, simple manner of preparation, and stability. Preparation of a uniform calcium alginate gel, necessitate maintaining the viscosity of the mixture of calcium alginate and yeast cells between 1000 and 2000 cps. The addition of a nonionic surfactant and an unsaturated fatty acid at the time of gelling is also found to improve cell retention and enzyme activity. Preparation of immobilized yeast cells is outlined in the following figure.

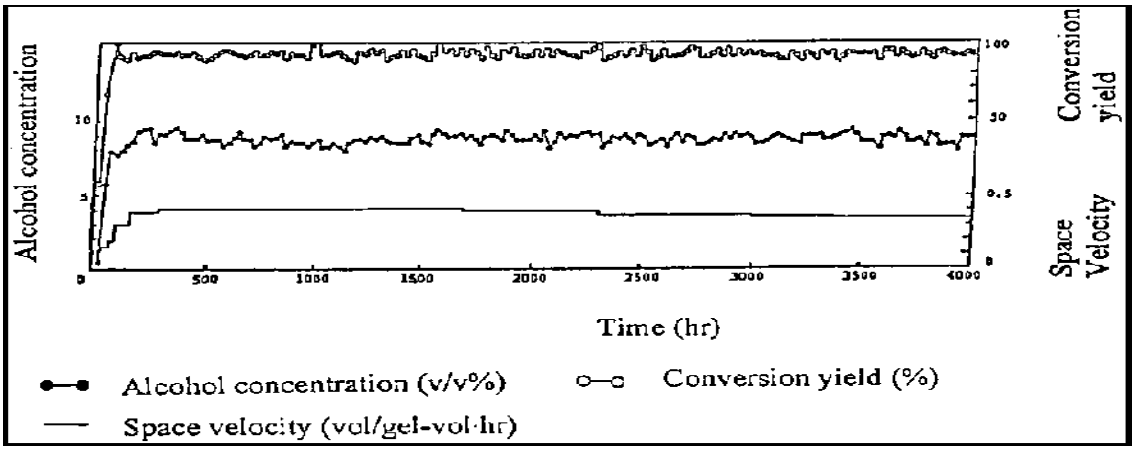
Figure: Protocol for the production of Immobilized yeast cells



Continuous plant operation using Immobilized yeast cells

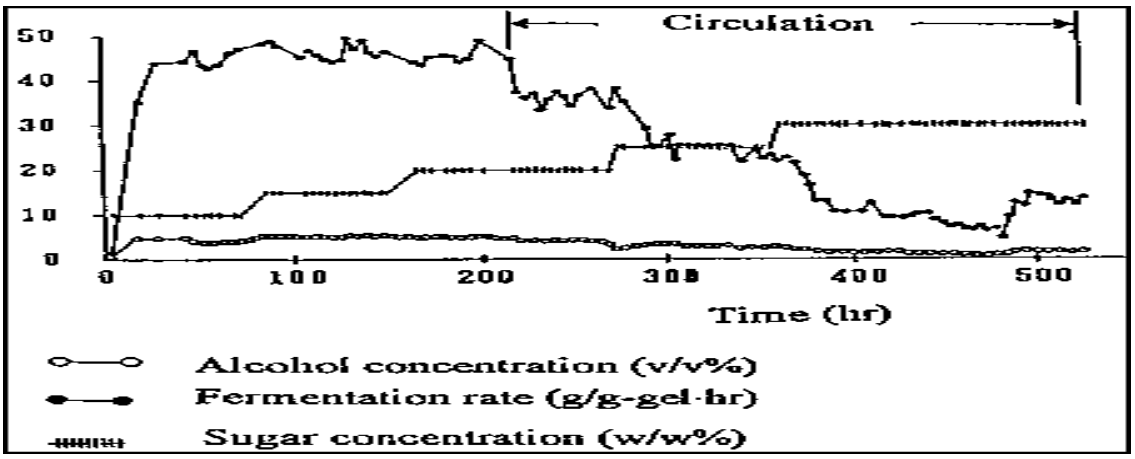
The spherical gel method can be employed for the preparation of calcium alginate gels since this method does not require the use of specialized equipment. Spherical gels are readily obtained by adding sodium alginate solution to calcium chloride solution using a nozzle. No special granulation apparatus is used when the equipment was assembled, but a gel-dropping nozzle was provided at the top of the fermentor. The fermentor was filled with a calcium chloride solution prior to fermentation, and sodium alginate solution was added dropwise to form granules. The culture medium was then supplied to the fermentor to initiate the fermentation. This procedure is found to simplify the gel preparation process. Continuous fermentation tests were conducted using two 1-KL fermentors arranged in series. The results are shown in following figure.

Figure: Continuous fermentation using Immobilized yeast cells



If immobilized continuous methods of alcohol production are used, stable yeast activity needs to be maintained.

Figure: Time profile of fermentation using an Immobilized fermentation system



Alcoholic inhibition of yeast reduces while the fermentation rate increases. In addition, this method enables raw material to be used at a high concentration, reducing the volume of waste liquor. In the instance of use of raw material at a high concentration there is a necessity of a relatively longer retention time within the fermentor.