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IMMOBILIZATION AND ESTIMATION OF ACTIVITY OF YEAST CELLS BY ENTRAPMENT TECHNIQUE USING DIFFERENT MATRICES

Nelluri Kanaka Durga Devi * and Atluri Satya Sai Nagamani

KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada - 520010, Andhra Pradesh, India.

Keywords:

Free yeast cell, Immobilized yeast cell, Sodium alginate, Gelatin, Glutaraldehyde

Correspondence to Author:
Dr. Nelluri Kanaka Durga Devi

Assistant Professor,
KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada - 520010, Andhra Pradesh, India.

E-mail: nelluriss@rediffmail.com

ABSTRACT: Background: It becomes clear that living processes were based on the action of enzymes. Cell immobilization serves as a useful tool for the immobilization of intracellular enzymes. The purpose of study is to improve economically important processes by attaching the yeast cells to matrices. **Methods:** In this analytical study by using mixed models of matrices like (sodium alginate alone, sodium alginate and gelatin, sodium alginate and agar, sodium alginate and glutaraldehyde) to determine the effective combination for immobilizing cells without any damage. **Results:** It was noted that the glucose content in both cells increased which indicates the production of carbon dioxide and alcohol and then the values started falling down this is because the osmotic concentration of the sugar gets so great that the yeast is unable to get enough water for growth. **Conclusion:** Glucose utilization was analyzed in free and immobilized cells on four different matrices for verifying their performance and the improvement of immobilized yeast cells over free yeast cells. Use of mixed matrices enhanced the biological activity of cells thus improved their performance. Immobilization of yeast cell showed technical and economic advantages over free cell system.

INTRODUCTION: Enzymes are macromolecular biological catalysts. Enzymes accelerate, or catalyze, chemical reactions. The molecules at the beginning of the process upon which enzymes may act are called substrates and the enzyme converts these into different molecules, called products [1, 2]. Almost all metabolic processes in the cell need enzymes in order to occur at rates fast enough to sustain life. The set of enzymes made in a cell determines which metabolic pathways occur in that cell.

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The technique of cell immobilization is an outgrowth of enzyme fermentation, because of their specific catalytic activity and their high performance under mild physiological conditions. Nevertheless, enzyme immobilization process suffers from certain disadvantages which are the cost of pure enzyme, the difficulty of recycling them by extraction and problems of product contamination by leaking from the immobilized enzymes [3]. To circumvent these disadvantages, the more readily available microbial enzymes, together with the cells containing them are bound to carriers by various methods, often with remarkable improvements in enzyme activity and half-life.

The present study is not about carrying out general method of immobilization this study deals with various aspects such as immobilization of whole cells by entrapment technique using different

mediums and estimating their activity differently by measuring the glucose absorbed by the cells which promote their growth and is responsible for production of its byproducts.

MATERIALS AND METHODS:

Materials: The organism used in present study is *Saccharomyces cerevisiae* and a commercial grade baker's yeast was used. Sodium alginate with other chemicals like gelatin, agar, and glutaraldehyde used were purchased from commercial sources and were of analytical grade. Calcium chloride issued for the formation of beads. The sucrose solution serves as a source of glucose for Saccharomyces cells. The flasks and beakers used in the experiment were maintained in sterile conditions so that, they can maintain consistency in results. For the determination of Glucose presence, the amount of glucose present was measured by glucose strips.

Choice of Support for Immobilisation: The support materials used for immobilization range from elegantly produced spheroids of inorganic oxide to sand and bricks and great ingenuity has gone into devising methods of binding biocatalysts on them [4, 5]. Commercial success has been achieved where the support material has been chosen for the flow characteristics. Cost, non-toxicity and immobilization method tailored to give maximum biocatalystic activity while retaining the desirable flow characteristics

Methods:

1. Cell Immobilization by Entrapment in Sodium Alginate: Add 1 gm of sodium alginate to 25 ml distilled water in a beaker. Mix thoroughly. Mix 5 gm of dried yeast in 25 ml distilled water in a beaker and Stir well. Prepare 100 ml of 1.5 gm calcium chloride solution in the large beaker. Add the yeast suspension to sodium alginate solution and mix thoroughly with glass rod. Draw all of the mixture into 20 ml syringe. From a height of 10 cm release the mixture from syringe [6], one drop at a time, into calcium chloride solution. Beads containing yeast cells will form. Leave the beads to harden for at least 10 min. Filter the beads through a sieve and rinse with distilled water.

Estimation of Yeast Activity: Mix another 4 gm of yeast in 25 ml distilled water. Pour this yeast into a separating funnel labelled "Free Yeast". Pour the beads into another separating funnel labelled "Immobilized Yeast. Prepare 100 ml of 1 gm sucrose solution with water warmed to about 40 ºC. Pour 50 ml sucrose solution into the yeast in each of the separating funnel. Using glucose strips test samples from each funnel for glucose [7]. Repeat the test at five min intervals. Record result: Run off the remaining product from each funnel into beakers. Compare the turbidity of the solutions.

2. Cell Immobilization by Entrapment in Sodium Alginate and Gelatin: Add 1 gm of sodium alginate to 25 ml distilled water in a beaker and mix thoroughly. Add 1 gm of gelatin to 25 ml distilled water and stir well. Mix 5gm of dried yeast in 25 ml distilled water in a beaker and mix thoroughly. Prepare 100 ml of 1.5 gm calcium chloride solution in the large beaker. Add the gelatin solution to sodium alginate solution and stir well. Add the yeast suspension to sodium alginate [8] and gelatin mixture and mix thoroughly with glass rod. Draw all of the mixture into 20 ml syringe. From a height of 10 cm release the mixture from syringe, one drop at a time, into calcium chloride solution. Beads containing yeast cells will for, Leave the beads to harden for at least 10 min and Filter the beads through a sieve and rinse with distilled water.

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3. Cell Immobilization by Entrapment in Sodium Alginate and Agar: Add 1 gm of sodium alginate to 25 ml distilled water in a beaker and mix thoroughly. Add 1 gm of agar to 25 ml distilled water and stir well. Mix 5 gm of dried yeast in 25 ml distilled water in a beaker and mix thoroughly. Prepare 100 ml of 1.5 gm Calcium chloride solution in the large beaker [9, 10]. Add the agar solution to sodium alginate solution and stir well.

Add the yeast suspension to sodium alginate and agar mixture and mix thoroughly with glass rod. Draw all of the mixture into 20 ml syringe. From a height of 10 cm release the mixture from syringe, one drop at a time, into calcium chloride solution. Beads containing yeast cells will form. Leave the beads to harden for at least 10 min. Filter the beads through a sieve and rinse with distilled water.

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4. Cell Immobilization by Entrapment in Sodium Alginate and Glutaraldehyde: Add 1 gm of sodium alginate to 25 ml distilled water in a beaker and mix thoroughly. Add 1 gm of glutaraldehyde to 25 ml distilled water and stir well. Mix 5 gm of dried yeast in 25 ml distilled water in a beaker and mix thoroughly. Prepare 100ml of 1.5 gm calcium chloride solution in the large beaker. Add the glutaraldehyde solution to sodium alginate solution and stir well. Add the yeast suspension to sodium alginate and glutaraldehyde mixture [11, 12, 13] and mix thoroughly with glass rod. Draw all of the mixture into 20 ml syringe. From a height of 10 cm release the mixture from syringe, one drop at a time, into calcium chloride solution. Beads containing yeast cells will form. Leave the beads to harden for at least 10 min. Filter the beads through a sieve and rinse with distilled water.

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RESULTS AND DISCUSSION:

TABLE 1: THE AMOUNT OF GLUCOSE PRESENT IN CELLS BY ENTRAPMENT IN SODIUM ALGINATE

S. no.	Time (min)	Free Yeast- presence of Glucose		Immobilized Yeast-presence of Glucose	
		Original	Duplicate	Original	Duplicate
1	5	100	150	270	200
2	10	250	280	500	400
3	15	150	130	250	200
4	20	100	90	100	70
5	25	50	40	50	30

TABLE 2: THE AMOUNT OF GLUCOSE PRESENT IN CELLS BY ENTRAPMENT IN SODIUM ALGINATE AND GELATIN

S. no.	Time (min)	Free Yeast- presence of Glucose		Immobilized Yeast-presence of Glucose	
		Original	Duplicate	Original	Duplicate
1	5	250	270	250	290
2	10	600	530	500	480
3	15	500	420	1000	900
4	20	100	170	550	430
5	25	60	80	250	220

TABLE 3: THE AMOUNT OF GLUCOSE PRESENT IN CELLS BY ENTRAPMENT IN SODIUM ALGINATE AND AGAR

S. no.	Time (min)	Free Yeast- presence of Glucose		Immobilized Yeast-presence of Glucose	
		Original	Duplicate	Original	Duplicate
1	5	500	440	300	250
2	10	1000	900	500	450
3	15	1000	850	500	530
4	20	600	400	250	200
5	25	250	200	100	130

TABLE 4: THE AMOUNT OF GLUCOSE PRESENT IN CELLS BY ENTRAPMENT IN SODIUM ALGINATE AND GLUTARALDEHYDE

S. no.	Time (min)	Free Yeast- presence of Glucose		Immobilized Yeast-presence of Glucose	
		Original	Duplicate	Original	Duplicate
1	5	250	300	150	120
2	10	600	550	330	250
3	15	1000	900	500	530
4	20	500	450	500	450
5	25	300	200	280	200

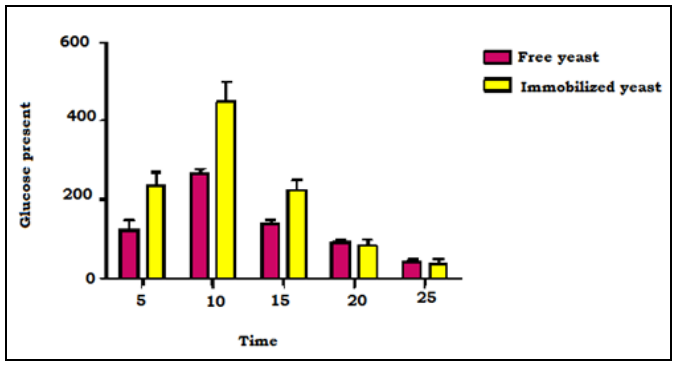

FIG. 1: PRESENCE OF GLUCOSE BY ENTRAPMENT OF CELLS IN SODIUM ALGINATE

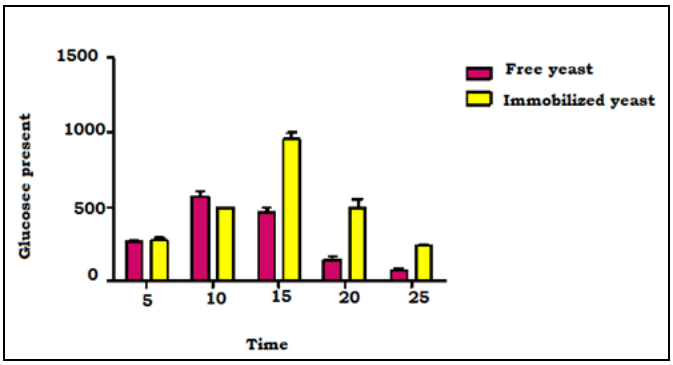

FIG. 2: PRESENCE OF GLUCOSE BY ENTRAPMENT OF CELLS IN SODIUM ALGINATE AND GELATIN

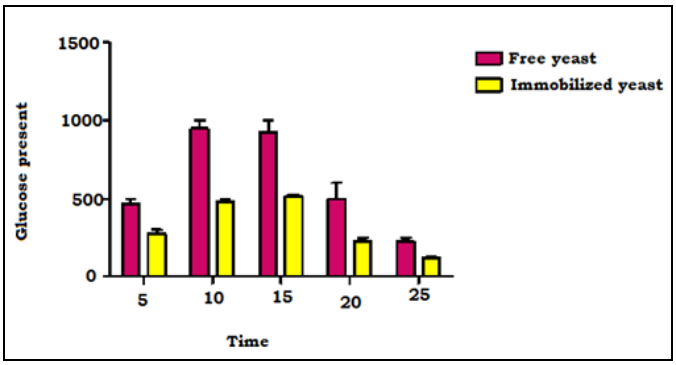

FIG. 3: PRESENCE OF GLUCOSE BY ENTRAPMENT OF CELLS IN SODIUM ALGINATE AND AGAR

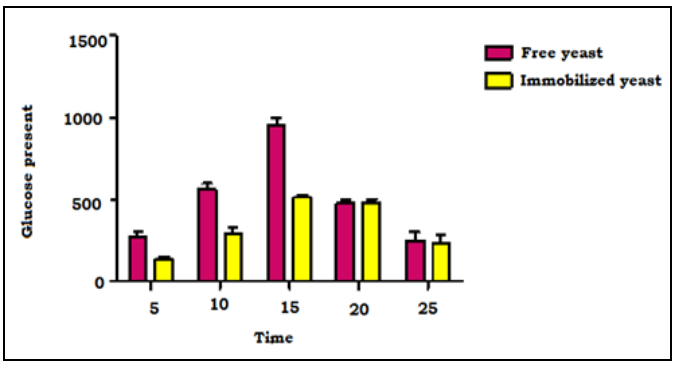

FIG. 4: PRESENCE OF GLUCOSE BY ENTRAPMENT OF CELLS IN SODIUM ALGINATE AND GLUTARALDEHYDE

DISCUSSION: The range of microbial cells used in immobilization roughly equals that used in fermentation processes. The aim is to improve economically important processes by attaching the microbial cells to matrices.

In the present study attempts were made to carry out whole cell immobilization of *Saccharomyces species* by gel entrapment in different matrices. The different matrices selected were sodium alginate, sodium alginate and gelatin mixture, sodium alginate and agar mixture, sodium alginate and glutaraldehyde mixture. In Cell immobilization by entrapment in sodium alginate method, the immobilized beads were formed by mixing yeast suspension in sodium alginate solution. The beads obtained by this method are spherical and were stable. From the results in **Table 1**, it was observed that the glucose content in both immobilized and free yeast reached a threshold value and started falling down. In Cell immobilization by entrapment in sodium alginate and gelatin method, reported that gelatin was used at a concentration of 20% for cell immobilization, hence we employed this concentration of gelatin for entrapment. However it was observed that the bead formation does not takes place. To promote the bead formation entrapment was done by using sodium alginate and gelatin mixture. Then the beads were obtained.

From the results in **Table 2** it was observed that the glucose content in both immobilized and free yeast reached a threshold value and started falling down. In Cell immobilization by entrapment in sodium alginate and agar method, the survey of literature indicated that agar is widely used for immobilizing

various microbial cells. Accordingly we attempted to immobilize our cells by gel entrapment using agar. But the beads were not formed. For the formation of beads entrapment of cells were carried in sodium alginate and agar mixture. From the results in **Table 3** it was observed that the glucose content in both immobilized and free yeast reached a threshold value and started falling down. In Cell immobilization by entrapment in sodium alginate and glutaraldehyde, the beads were not formed by using glutaraldehyde alone. The use of glutaraldehyde as a matrice or a support for immobilizing enzymes and cells is facilitated by the use of sodium alginate as a cross linking agent. This treatment with sodium alginate resulted in hardening glutaraldehyde leading to improved mechanical strength and stability. From the results in **Table 4** it was observed that the glucose content in both immobilized and free yeast reached a threshold value and started falling down.

Yeast is a fungus and needs a supply of energy for its living and growth. Sugar supplies this energy. So we add sucrose solution for the estimation of immobilized and free yeast activity. From all the **Fig. 1, 2, 3** and **4**, it was noted that the glucose content in both burettes reached a maximum value and started falling down; it may be due to the osmotic concentration of the sugar gets so great that the yeast is unable to get enough water for growth. As fresh yeast is more than 90% water, the single substance most needed for growth is water. Exactly, do not know whether yeast cells are able to take up water actively, by expenditure of metabolic energy to pump the water against the water potential gradient.

When sodium alginate alone was used as matrix the glucose absorbed by beads was little, comparatively the beads in the mixture of sodium alginate and agar has absorbed the maximum amount of glucose than any other mixtures employed in our study. More or less the absorption of glucose by beads entrapped in mixture of sodium alginate and glutaraldehyde were similar to sodium alginate and agar mixture. The beads in the mixture of sodium alginate and gelatin absorbed the glucose at high rate.

CONCLUSION: The use of immobilized whole cells in industrial processes has attracted due to advantages over traditional processes. Immobilization provides high cell concentrations and cell reuse. With the study it is aimed to find the best carrier for immobilization of yeast cells and estimation of their activity with free cells. It was noted that mixed matrices showed better result than the single support so, conducted this analytical study by using mixed models of matrices. Glucose utilization was analyzed in free and immobilized cells on four different matrices. Use of mixed matrices enhanced the biological activity of cells thus improved their performance. Using free or immobilized yeasts, the rates were of the same order.

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CONFLICT OF INTEREST: Nil

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