ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 03, pp. 27683-27686, March, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

IMMOBILIZATION STUDIES ON MULTI-ENZYMES PRODUCED BY A SOIL ISOLATE *AEROMONAS JANDAEI*

***Nagesh Malik and Anupama Maity**

V.E.S. College of Arts, Science and Commerce, Department of Microbiology- Sindhi Society Chembur, Mumbai-400071

ARTICLE INFO

Article History:
Received 27th December, 2015
Received in revised form
14th January, 2016
Accepted 24th February, 2016
Published online 16th March, 2016

Key words:

Multi-enzymes, *Aeromonas jandaei,* Immobilization, Matrices.

ABSTRACT

The present study was carried out to immobilize the multi-enzymes produced by a soil isolate *Aeromonas jandaei*. The three enzymes viz. amylase, lipase and protease produced by this isolate were partially purified by dialysis and were immobilized using 3 matrices- agar, agarose and alginate at 3 different concentrations. A washout of enzymes was seen at the lower concentrations of the matrices. At 3 % concentration the enzyme substrate interaction was reduced as low activity was seen. 2 % agar and agarose gave excellent enzyme retention and activity. 1 and 2 % alginate beads however dissolved in the reaction medium; the enzyme thus immobilized could not be retained for reuse. However 3 % alginate did not dissolve and was retrievable. 2 % agar was said to be the best choice for immobilization as it is cheaper than agarose and can be reused. Also when the enzyme is not to be reused and requires to be released into the reaction medium, alginate could be a preferable choice and can be used as a delivery/ transport medium for the enzymes.

Citation: Nagesh Malik and Anupama Maity, 2016. "Immobilization studies on multi-enzymes produced by a soil isolate *Aeromonas jandaei*", *International Journal of Current Research,* 8, (03), 27683-27686.

INTRODUCTION

Many enzymes secreted by microorganisms are available on large scale and there is no effect on their cost if they are used only once in the process. In addition, many more enzymes are such that they affect the cost and could not be economical if not reused. Therefore, reuse of enzymes led to the development of immobilization techniques. (Dubey, 2006). It involves the conversion of water soluble enzyme protein into a solid form of catalyst by several methods like adsorption, entrapment, encapsulation etc. It is only possible to immobilize microbial cells and various enzymes by similar techniques (Bull *et al.,* 1983). Thus, immobilization is "the imprisonment of an enzyme in a distinct phase that allows exchange with, but is separated from bulk phase in which the substrate, effecter or inhibitor molecules are dispersed and monitored "(Trevan, 1980).

MATERIALS AND METHODS

The partially purified enzymes were subjected to immobilization by entrapment in calcium alginate beads, agar and agarose.

Corresponding author: Nagesh Malik,
V.E.S. College of Arts, Science and Commerce, Department of Microbiology- Sindhi Society Chembur, Mumbai-400071

Three concentrations of the matrices were used and the entrapped enzyme was subjected to their respective assays to check for the activity of the enzyme.

Materials

(i) Partially purified enzyme-1.0 ml in each of the matrices.
(ii) Matrices used
- Sodium alginate- 1, 2 and 3 %.
- Agar- 1, 2 and 3 %.
- Agarose- 1, 2 and 3 %.

(iii) Buffers
- Amylase- Phosphate buffer (pH 6.8).
- Protease- Sodium calcium acetate buffer (pH 8.0).
- Lipase- Tris-Cl buffer (pH 8.0).

(iv) Others
- Cold Calcium Chloride- 2, 3 and 4 %.
- Pipettes to make beads.
- Erlenmeyer Flasks (100ml).
- Small petri plates.

Method

The matrices were added to 2.0 ml of respective buffers. For agar and agarose the mixture was boiled to dissolve the agar

and agarose. Sodium alginate was added to 4.0 ml of the respective buffers and dissolved by heating lightly. Once the agar and agarose was sufficiently cooled, 1.0 ml of each of the enzymes was added to each concentration of the matrices. The mixture was rapidly agitated and immediately poured into small petri plates to obtain a uniform mould for better handling. When the sodium alginate had completely cooled each of the enzymes were added to each of the concentrations of sodium alginate and thoroughly mixed. Beads were prepared by dropping the sodium alginate-enzyme mixture into corresponding concentrations of cold calcium chloride solution. As the agar and agarose set and the alginate beads were prepared they were removed from their containers and immersed in their respective buffers in which they were prepared, for 24 hrs. The agar and agarose gels were cut into smaller pieces to increase the surface area for enzyme substrate interaction. The immobilized enzymes were subjected to their respective enzyme assays and the results were tabulated. The enzyme solutions in which the immobilized enzymes were stored were also subjected to enzyme analysis to check if a wash out of enzymes from the matrices had taken place.

RESULTS AND DISCUSSION

Immobilization

Three types if matrices were used to check the feasibility of enzyme immobilization. The study was conducted to check which of the matrices were better for immobilization. The result for each of the matrix was however not compared to the other as the system differed in the agar, agarose and the alginate polymer. Agar and agarose can be compared as the system is the same. The partially purified amylase enzyme was immobilized using 1,2and 3 % of agarose, agar and sodium alginate. The enzyme activity obtained when the immobilized enzyme was placed in the reaction mixtures is shown in Fig.- 1. It can be seen that 1% agarose gave maximum enzyme activity (0.73 U ml $^{-1}$) as compared to 2% (0.68 U ml $^{-1}$) and 3%(0.6 U ml $^{-1}$). In the case of agar 2% gave highest enzyme activity (0.74 U ml $^{-1}$) followed by 3 % (0.7 U ml $^{-1}$). 1% alginate gave the highest enzyme activity (0.74 U ml $^{-1}$) as compared to the other 2 concentration. The lower enzymes activities for the higher concentration of alginate might be because the interaction between the substrate in the reaction mixture and the immobilized enzyme must be limited. Also when the 1% alginate beads were added to the reaction mixture, they completely disintegrated thus releasing the entire immobilized enzyme into the reaction mixture. Resulting in better enzyme substrate interaction and higher product formation, which in this case is breakdown of starch into maltose units. The immobilized enzyme was stored in phosphate buffer pH 6.0 at 20 °C to prevent drying of the matrix. The buffers were also checked for enzyme activity to check if there has been washout of the enzyme from the matrix. Fortunately no enzyme activity was recorded, indicating that the enzyme was retained in the matrices.

The results obtained for immobilization of lipase enzyme are as shown in Fig. 2. Tris chloride buffer having pH 7.0 was used as the wetting solution. The immobilized enzyme was stored at 40 °C. It is seen that 1% agarose gave no enzyme activity at all which could be due to washing out of the enzyme while it was stored in its wetting solution. 2% (0.24 U ml^{-1}) and 3 % agarose (0.15 U ml^{-1}) showed comparatively better activity. But lower activity was obtained for 3 % which indicated that the enzyme - substrate interactions were impeded. Similar results were obtained for the agar immobilized enzyme too. When alginate immobilized enzyme was checked for enzyme activity a slightly different result was obtained. The beads of 1 and 2 % disintegrated in the reaction mixture so maximum enzyme activity was obtained as all the immobilized enzymes were released into the reaction mixture. However better enzyme activity was obtained for 3% (0.29 U ml^{-1}) even when the beads remained intact. The low enzyme activity of the lower concentrations was possibly due to the wash out of the enzyme into the wetting solution. To confirm, the wetting solutions were used as enzyme source and were assayed.

The wetting solutions did show enzyme activity as shown in Table - 1. Thus it can be explained that in spite of disintegration of the alginate beads in the reaction mixture the activity was as low as the enzyme content in the polymer was already lower than the initial concentration due to wash out of the enzyme. For 1 % agarose and agar complete wash out of the enzyme took place resulting in zero to negligible activity when the immobilized matrix was assayed and comparatively higher activity when the wetting solution was assayed.

On studying the immobilization of partially purified protease, it can be seen from Fig.-3 that excellent immobilization of the enzyme took place. 1% concentration of agar and agarose showed lesser activity probably due to wash out of the enzyme in the wetting solutions. 2% showed good activity in all the 3 matrices. 1 and 2 % alginate beads disintegrated in the reaction mixture and released the enzyme into the medium. To confirm the washout of enzyme in the lower concentration of the matrices, the wetting solution, which in the case of protease was sodium calcium acetate (pH 6.0), were subjected to the enzyme assay. The results obtained were as tabulated in Table - 2. 2 % alginate even though partial wash out was observed a higher activity was obtained. 3% alginate showed a lower activity as the enzyme substrate interaction was hindered suggesting that 3% alginate would not be ideal for immobilization.

The first industrial use of immobilized enzymes was reported in 1967 by Chibata and coworkers, who developed the immobilization of Aspergillus oryzae aminoacylase for the resolution of synthetic racemic D-L amino acids Tosa *et al*. (1966). A general method for the immobilization of cells with preserved viability was reported by Kjell Nilsson *et al*. (1983). Other major applications of immobilized enzymes are the industrial production of sugars, amino acids, and pharmaceuticals Tanaka *et al*. (1993). Gashaw Mamo and Amare (June 1997) reported that agar, alginate, agarose immobilized cells of thermophilic Bacillus species produced 7.0, 6.2 and 10.5 U ml-1 of thermostable amylase in shake flasks respectively. Alginate entrapped cells released high levels of amylase (10.5Uml-1) than freely suspended cells (8.0 Uml-1). We have also found alginate to be efficient as there is no heat involved in the process of entrapment.

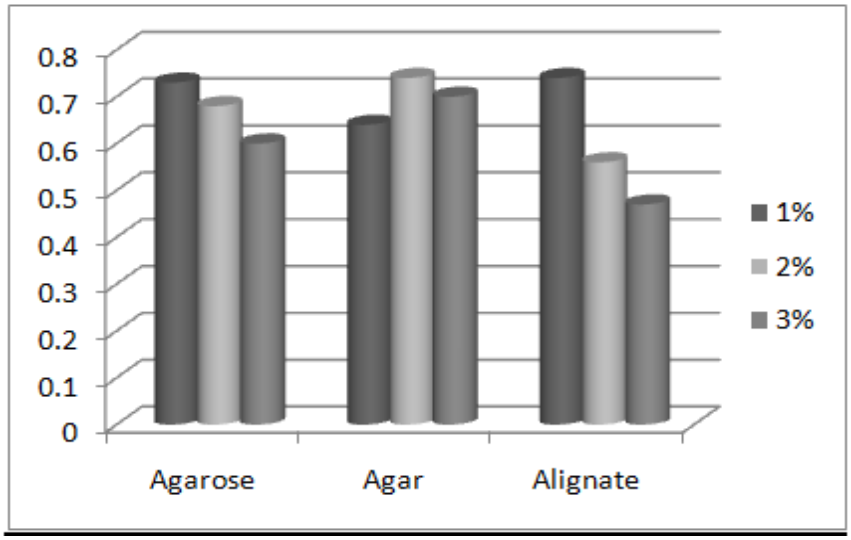

Fig. 1. Immobilization of partially purified amylase using three types of matrix

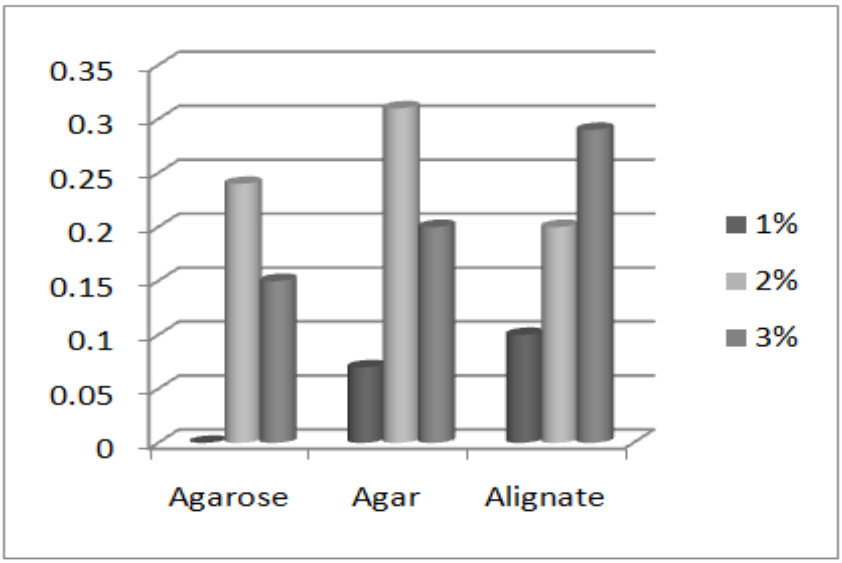

Fig. 2. Immobilization of partially purified lipase using three types of matrix

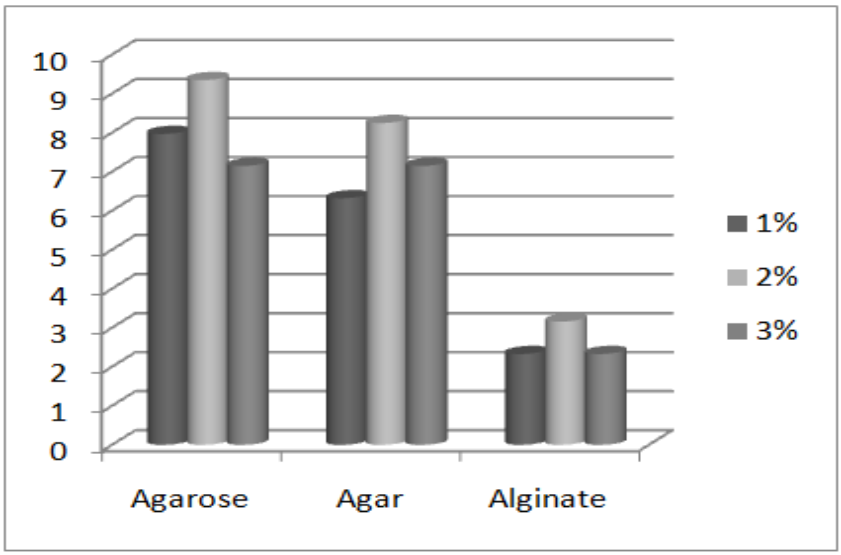

Fig. 3. Immobilization of partially purified protease using three types of matrix

Table 1. Lipase activity of the matrix wetting solutions

Matrices	1%	2%	3%
Agarose	0.22 U ml $^{-1}$	-	-
Agar	0.19 U ml $^{-1}$	-	-
Alginate	0.083 U ml $^{-1}$	0.063 U ml $^{-1}$	-

Table 2. Protease activity of the wetting solutions

Matrices	1%	2%	3%
Agarose	2.61 U ml $^{-1}$	-	-
Agar	0.825 U ml $^{-1}$	-	-
Alginate	0.69 U ml $^{-1}$	0.28 U ml $^{-1}$	-

Agarose is a purer form of agar which makes it more costly and may result in increase in the production costs. Thus 2 % agar would be a suitable immobilization matrix when the immobilized enzyme is to be retained and retrieved for reuse. Higher concentration of 3 % did not facilitate maximum enzyme - substrate interaction even though the matrices were more sturdy and resisted breaking/disintegration. Higher activities can be obtained by the addition of greater amounts of enzyme in the matrices. A point worth noting is that even though agarose and agar made good matrices it may be a bit problematic as the enzyme has to be added to the molten polymer. Care should be taken that the polymer is cool enough so that the enzyme can be added without causing heat deactivation of the enzymes and warm enough to ensure proper and complete mixing to obtain a homogenous matrix enzyme mixture. 3% alginate would have been ideal as there is no heat involved, and unlike 1 and 2 % it does not disintegrate in the reaction mixture, but 3% alginate prevents complete enzyme – substrate interaction. However this can be overcome by longer reaction durations, and higher enzyme being incorporated into the matrix. The characteristics of the matrix are of paramount importance in determining the performance of the immobilized enzyme system. Ideal support properties include physical resistance to compression, hydrophilicity, inertness toward enzymes ease of derivatization, biocompatibility, resistance to microbial attack, and availability at low cost Trevan (1980); Brodelius and Mosbach (1987); Buchholz and Klein (1987). In our work we have found 2 % agarose being the best choice for immobilization followed by agar when the immobilized enzyme is to be retrieved back and reused. However if the enzyme is not required and requires only a delivery medium, 2 % alginate would be an excellent choice.

REFERENCES

Brodelius, P. and Mosbach, K. 1987. Immobilization Techniques for Cells/Organelles.

Buchholz, K. and Klein, J. 1987. Characterization of Immobilized Biocatalysts. In: Methods in Enzymology volume 135, (Mosbach, K., ed.), Academic Press, London, pp. 3–30.

Bull A. T. Holt G. and Lilly M.D. 1983. Biotechnology: International Trends and perspectives, pp84, Oxford and IBH publication. Co. PVT. Ltd, New Delhi

Dubey R. C. 2006. in "A Textbook of Biotechnology", S. Chand and company Ltd. pp514. , New Delhi.

Gashaw Mamo and Amare G. 1997. Biotech. Tech. 11: (6), pp 447-450

Kjell Nilson 1983. European J App Microbiol. and Biotech. 17: pp319-326

Tanaka, A., Tosa, T., and Kobayashi, T. 1993. Industrial Application of Immobilized

Tosa, T., Mori, T. Fuse, N., and Chibata, I. 1966. Studies on continuous enzyme Biocatalysts, Marcel Dekker, New York, NY. Enzymologia. 31, pp214–224. reactions.

Trevan M.D. 1980. Immobilised Enzymes: An Introduction and Application in Biotechnology, Chichester, John Wiley. In: Methods in Enzymology, volume 135, (Mosbach, K., ed.), Academic Press, London, pp. 173–454.

****