IMMOBILIZED AND FREE A- AMYLASE ENZYME- AN OVERVIEW

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ABSTRACT

Purified enzyme **ex**tracts of white Rajma (*Phaseolus vulgaris* HUR 15) and Kulath (*Dolichos biflorus*) were subjected to entrapment method of immobilization.Enzyme was entrapped in calcium alginate beads.The basic idea behind the entire process was to find out whether the enzyme, a -amylase is stable in calcium alginate beads or not; if yes, then to what extent; and what are the kinetic characteristics of the immobilized enzyme?

Keywords: Entrapment, a -amylase, sodium alginate, Phaseolus, Dolichos biflorus,

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Introduction

The major concern in an enzymatic process is the instability of enzyme underrepetitive or prolonged use and inhibition by high substrate and product concentration.Immobilization is a very effective alternative in overcoming problems of instability andrepetitive use of enzymes. Entrapment method of immobilization is advantageous overother methods as they do not involve chemical modification of the enzyme (Garg,M., 2011; Gangadharan*et.al.,* 2009).Gangadharan *et.al.,* (2009) immobilized bacterial a-amylase for effective hydrolysis of raw and soluble starch. The a-amylase produced by *Bacillus amyloliquefaciens* ATCC 23842 was immobilized in calcium–alginate beads and used for effective hydrolysis of soluble and raw potato starch which was comparable to the free enzyme.

Immobilization from a-amylases has also been done in India to a certain extent. Sahukhan *et.al.,* (1993) performed immobilization of a-amylase from *Myceliophthora thermophila* D-14 (ATCC 48104). In his research, three immobilization methods were involved; covalently bound to CNBr-activated Sepharose, entrapped within crosslinked poly-acrylamide gels and Calcium alginate beads. Of the three methods, Calcium alginate beads proved to be the best carrier for immobilization.

In the present research, purified enzyme extracts of *Phaseolus vulgaris* HUR15 and *Dolichos biflorus*were subjected to entrapment method forimmobilization. In this approach, enzyme molecules are held or entrapped within calcium alginate gels. This is one of the most widely used applications.

Methodology

Preparation of Sodium Alginate:

100ml water was kept on stirrer. In stirring conditions, 3% sodium alginate wasadded to the water. Stirring was allowed for 10-15 minutes. Then the solution was left for ½ hour so that any bubbles present in the solution disappear. Then at room temperature, 2ml enzyme extract was added to the sodium alginate solution.

Calcium Chloride solution:

0.2M CaCl2 solution was prepared. This solution was kept in freezer for ½ hour tomaintain chilled conditions.

Preparation of beads:

The sodium alginate- enzyme solution was filled in a 10ml syringe. This solutionwas added drop wise to chilled CaCl2 solution. Small rounded beads about 2cm in diameter were obtained. These beads are the immobilized a-amylase enzyme beads.Kinetic characterization of the immobilized enzymes was performed using these beads. Inthe subsequent experiments, these beads will be added to DNS extract instead of freeenzyme. These beads can be recovered after each experiment. And the subsequentexperiment can be performed using the same beads.

Kinetic Characterization of the immobilized enzyme:

The kinetic characteristics were calculated using the immobilized enzyme. Tofind out these kinetic characteristics, DNS method was applied as mentioned earlier in Garg and Dobriyal 2012 (Miller, 1959). The only difference was that in place of free enzyme, the immobilized enzyme beadswere added to the mixture. Enhanced characteristics were determined.

Comparison:

Aclose comparison between the kinetic characteristics of immobilized as well asfree enzyme was done.

Results:

Immobilization of both enzymes was done on calcium alginate matrix. Shinytransparent beads of 2cm diameter with slightly yellowish-brown colorationwere obtained in case of kulath. Beads of ultra-filtered kulath extract (~30kDa) were alsoprepared which were shiny yellow in coloration.In case of rajma opaque white color beads of 2cm diameter wereobtained.

Kinetic characteristics of these immobilized enzymes beads were calculated, and itwas found out that the characteristics were highly enhanced. Although much change inoptimum pH was not observed, still we observed a remarkable increase in enzymeactivity (Table 1). Optimum pH of free kulath enzyme came out to be 6.1 and that of immobilized kulath enzymecame out to be 6.3.

Table 1:Comparison between enzyme activities of crude and immobilized kulathenzyme at different pH concentrations.

pH	Enzyme activity (IU/ml) of crude enzyme.	Enzyme activity (IU/ml) of immobilized enzyme.
3.6	5.55	4.44
4	6.11	6.67
5	18.87	18.05
6.1	27.22	22.78
6.3	26.11	37.22
6.7	18.87	44.44
7	3.39	38.89
8	<0.20	16.96
8.6	<0.20	17.19
9	<0.20	2.08

Above table shows a close comparison between optimum pH of free andimmobilized enzyme. Most enzymes are stable in pH range 6 to 8 (Welker and Campbell,1967). This table further puts light on this statement. Uptill pH 6.1, we could not observemuch increase in enzyme activity. Drastic increase in enzyme activity was observed afterpH 6.3. We observed that at pH 7 enzyme activity increased more than 11 times (~11.5times). Although we could not observe much increase in optimum pH; Free enzymeshowed an optimum pH of 6.1 (Garg and Dobriyal, 2011) while the immobilized enzyme showed an optimum pH of 6.7. We also observed that the immobilized enzyme was stableat much higher pH values than the free enzyme. While the free enzyme showed noactivity at pH 8, the immobilized enzyme showed an activity of 16.96 IU/ml at pH 8.

An important point of consideration was that at pH 3.6, the enzyme activity ofimmobilized enzyme was slight lower than that of free enzyme. Free enzyme showed enzyme activity of 5.55 IU/ml while immobilized enzyme showed enzyme activity of4.44 IU/ml. This might be so because immobilized enzyme takes higher time foroptimization than the free enzyme.

Certain similar results were seen in case of optimum temperature. Optimumtemperature of free enzyme was 45°C (Garg and Dobriyal, 2010) while optimum temperature ofimmobilized enzyme was 50°C. This simply proves the increase in thermalstability. Around 9 times increase in enzyme activity was observed at 70° C (Graph).While the free enzyme showed very less activity at 80°C, the immobilized enzymeshowed a high activity of 10.74IU/ml at 80°C (Table 2 given below).

Table 2: Comparison between enzyme activities of crude and immobilized kulath enzyme at various temperatures.

Temperature (°C)	Enzyme activity (IU/ml) of crude enzyme.	Enzyme activity (IU/ml) of immobilized enzyme.
0	**<0.20**	**0.28**
4	**0.42**	**0.55**
10	**5**	**5.55**
20	**11.11**	**14.44**
30	**15.55**	**17.22**
40	**22.22**	**22.78**
45	**25.55**	**28.94**
50	**21.67**	**>44.4**
60	**22.19**	**>44.4**
70	**4.44**	**38.89**

Kinetic characterization of immobilized a-amylase of white rajma also revealedsimilar results. Optimum pH of immobilized white rajma extract came out to be 6.5. Thiswas same as that of free enzyme (Garg and Dobriyal, 2012). Enzyme activity of immobilized enzyme increased 20 times at pH 8. While the free enzyme showed no activity at pH 8.6, theimmobilized enzyme showed an activity of 8.89 IU/ml at pH 8.6. Thus, it was finallyproved that characteristics are

enhanced in immobilized enzyme. While the pH stabilityrange for free enzyme is 4 to 7, the pH stability range for immobilized enzyme is observed to be 4 to 9 (Table 3; Graph).

Table 3: Comparison between enzyme activities of crude and immobilized white rajma enzyme at various pH concentrations.

pH	Enzyme activity (IU/ml) of crude enzyme.	Enzyme activity (IU/ml) of immobilized enzyme.
4	2	1.67
5	2.22	2.22
6.1	2.78	6.78
6.3	2.78	10.5
6.5	2.89	35.55
6.7	2	30.55
7	1.55	29.72
7.5	1.11	21.39
8	0.55	10.89
8.6	<0.20	8.89

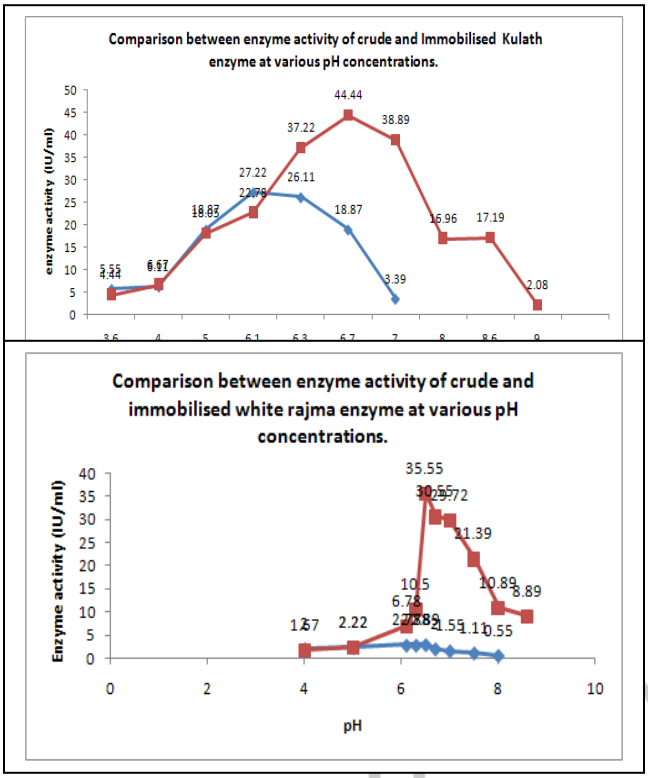
Coming to temperature, optimum temperature of immobilized white rajma a-amylase enzyme came out to be 60°C, against temperature optimum of 48°C for free enzyme (Garg and Dobriyal, 2012). Enzyme activity increased 24 times at 70°C temperature. Theimmobilized enzyme was found to be stable at 80° C; enzyme activity corresponding to8.42 IU/ml. Thus, high thermostability of immobilized enzyme was proved.

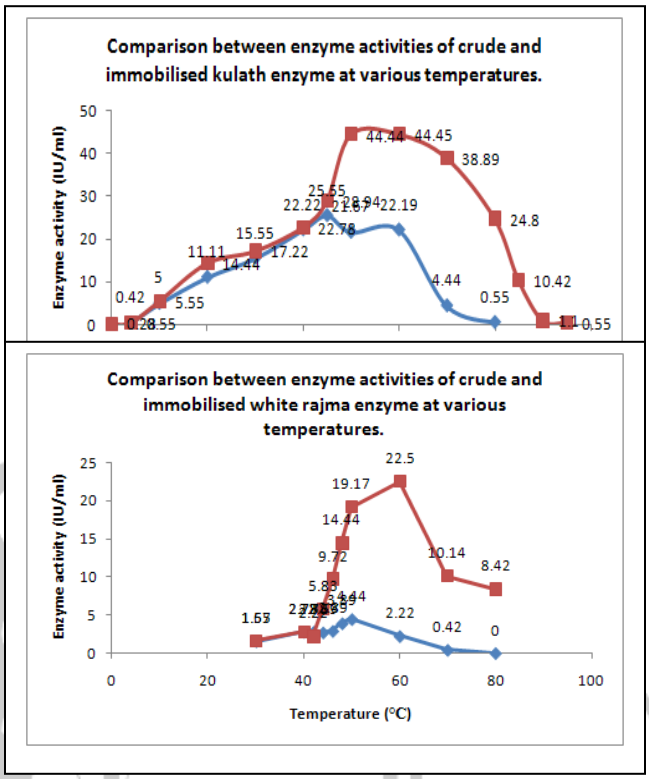
Table 4: Comparison between enzyme activities of crude and immobilized rajma enzyme at various temperatures.

Temperature (°C)	Enzyme activity (IU/ml) of crude enzyme.	Enzyme activity (IU/ml) of immobilized enzyme.
30	1.55	1.67
40	2.78	2.78
42	2.78	2.22
44	2.69	5.83
46	2.89	9.72
48	3.89	14.44
50	4.44	19.17
60	2.22	22.5
70	0.42	10.14
80	<0.20	8.42

Re-utilization of immobilized beads was not possible more than twice as duringenzyme assay, when the beads came to boil they almost dissolved, thus leaving only the contaminated solution for taking out the O.D. values. But since, immobilized beads could be reused 2 times, therefore usage ofimmobilized enzyme in comparison to

free enzyme is easy and user friendly.Further preparation of immobilized beads which could be used more than twice remains a topic of further research.





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