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Stability Studies Of Microencapsulated Chitinase, Screened From Marine Bioluminescnce Bacteria- Vibrio Alginolyticus.

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ABSTRACT: Marine bioluminescence bacteria, Vibrio alginolyticus was tested for its enzyme secreting potential. The marine origin enzyme tests were done chiefly for amylase, chitinase, lipase and protease out of which the bacteria showed higher potential activity for the enzyme chitinase. The zone clearance test was done to check for the enzyme activity. The highest value recorded for the chitinase activity was clearance of 2.2 centimeters by the bacteria and proved to be the most potent, among the three other enzymes. These were further studied for its media, pH and temperature optimization, Chitinase activity was better in BOSS broth in pH 5.0 and at temperature 26°C. As chitinase was highly sustaining in these optimal conditions they were furthermore subjected to enzyme immobilization in sodium alginate and calcium chloride beads. The microencapsulated enzyme was compared with the uncoated chitinase enzyme and observed for its activity in different pH and temperature for 12 months. The pH ranges analyzed are 3.5, 7.0 and 9.0 and the temperature in which they were maintained was 4°C, 37°C and 60°C.

Keywords: marine bioluminescence bacteria, Vibrio alginolyticus, chitinase activity, microencapsulation, pH optimization, temperature optimization

INTRODUCTION: Immobilization of enzyme by micro encapsulation is a crucial technique. The microcapsule is fabricated in such a way that the enzyme fits inside which has a polymeric network and has a semi-permeable membrane. The size of which is spherical and of few millimeters in diameters. Both soluble and insoluble enzymes can be encapsulated. The vesicles are generally made of liposomes and polymersomes for the coating. For internal polymeric network structures sol-gels, hydrogels, inorganic and organic hybrid materials, powered by polyelctrolytes are used. Material science in its advanced studies have good microcapsule development with an extended morphological stability, with

the physiochemical permeability well designed, reduced or less enzyme leakage and biocompatibility of the enzymes **(Christina et al., 2014).**

The microencapsulated enzymes when compared to the non coated, are tough and withstands great environmental stress. These enzymes can be used multiple times and also both the enzymes and products can be easily recovered, continuous enzyme activity and are good bioreactors designs. Also for further studies over the same immobilized enzyme is also highly possible **(Homaei et al., 2013)**.

MATERIALS AND METHODS: To increase the stability of the highly potential chitinase enzyme that was extracted from Vibrio alginolyticus were immobilized. Microencapsulation was done using calcium chloride and sodium alginate along with chitin was stabilized. The stability studies for sodium alginate beads was analyzed with different ranges of pH and temperature was done. Initially the chitinase assay done for the immobilized enzyme was 23 U/ml units. These were then subjected to further parameters. These were compared with the uncoated chitinase enzyme and the values of which was recorded every month for a brief period of 12 months.

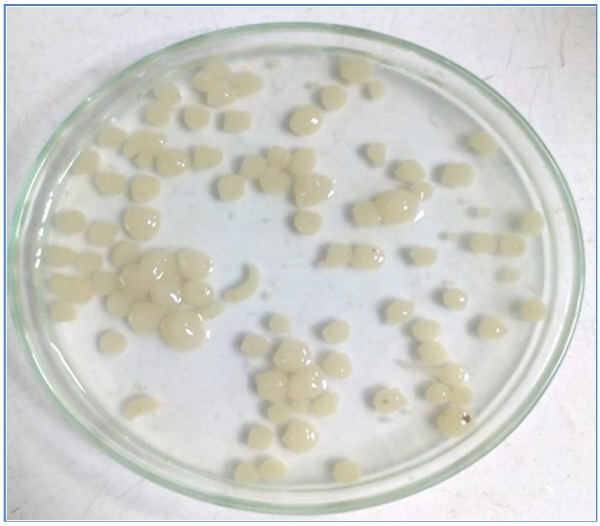


FIGURE 1: Microencapsulation of chitinase from Vibrio alginolyticus

Effect of temperature on stability of microencapsulated chitinase: To investigate the microencapsulated enzyme with its optimum temperature the assay was carried out at different temperatures. The enzyme was examined without any additives and substrates. At pH 5.5 the microencapsulated enzymes were maintained at 4^0C, 37^0C and 60^0C in 0.1 Molar sodium acetate used as a buffer, the activity of the encapsulated with its relative activity noted timely in a water bath controlled thermally for estimation of the residual activity **(Ram et al., 2007)**

Effect of pH on stability of microencapsulated chitinase: Using 0.1 Molar Sodium acetate as buffer pH assay for various pH was done for pH 3.0-6.0 and for pH 7.0-8.0, 0.1 M phosphate buffer is used in order to find the optimal pH for the microencapsulated beads. After incubating the immobilized enzymes were exposed to several pH of 3.5, 7.0 and 9.0 at 55^0C. The samples were drained in terms of the relative activity then the residual activity was often determined and it is calculated as the proportion at specific pH of activity percentage to the optimum pH activity
(Ram et al., 2007).

RESULTS AND DISCUSSION:

Stability studies of microencapsulated beads: Microencapsulation of enzymes are done for many purposes. Mostly done for the stability studies of the immobilized enzyme. Restriction of enzyme manipulation or any discharge of the product. Now as the chitinase enzyme has been encapsulated they are tested on different pH and temperature to fulfill the stability studies purpose. For 12 months the various pH and temperature range on the encapsulated enzymes were studied and the values were assessed periodically for every month. At 4°C, 37°C and 60°C temperature range and at pH 3.5, 7.0 and 9.0 it was tested. These ranges of both temperature and pH ranges for the chitinase enzymes were compared with the uncoated chitinase enzyme and the results were marked with SD (Standard Deviation) values.

i) Stability Studies of Microencapsulated Beads at Various Temperatures:

a) Stability study of microencapsulated beads at 4^0C: At 4°C, in the first month, the microencapsulated chitinase had its highest value recorded 23 U/ml which was as same as the initial value tested, which has not lost its potential, this when compared with the uncoated showed 20 U/ml, yet stable. From second month the immobilized showed very slow decrease in its enzyme activity, the uncoated when compared with had a gradual decrease in its activity. In 10 months the uncoated activity completely became inactive whereas the coated did not reduced into half of its initial value, recorded 15 U/ml.

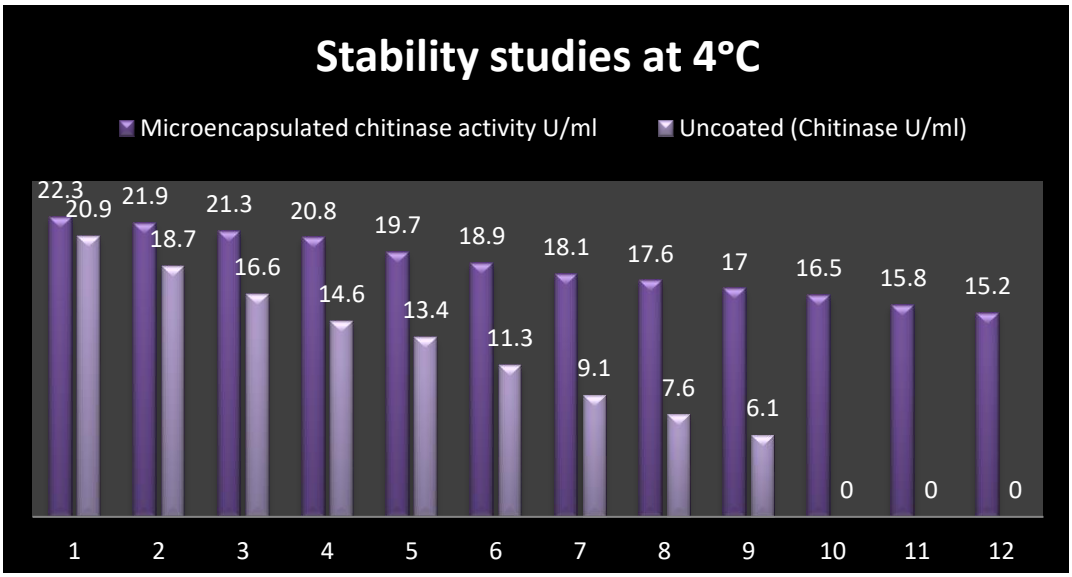
b) Stability study of microencapsulated beads at 37°C: In the very first month of the study the coated enzyme had a lesser value from the initial value at 37°C, which was 20 U/ml wherein the uncoated had almost similar values too. From the second month onwards both coated and uncoated started to decline

gradually with its enzyme activity. Until the end of the year both the cases showed activity, but it was reduced. Comparing to the lower values of the coated to the uncoated was half measured to its enzyme activity10.9 U/ml and 5.8 U/ml respectively.

c) Stability study of microencapsulated beads at 60^{0}C: Nearly 20 U/ml was recorded for the coated chitinase activity at 60°C, which was quite similar to the uncoated chitinase activity. Until the third month the uncoated showed gradual decrease in its activity and stopped its enzyme activity at fourth month showing that the enzyme are completely inactive. The coated however sustained until the end of the twelveth month but had gradually reduced values exhibiting lower enzymatic activity.

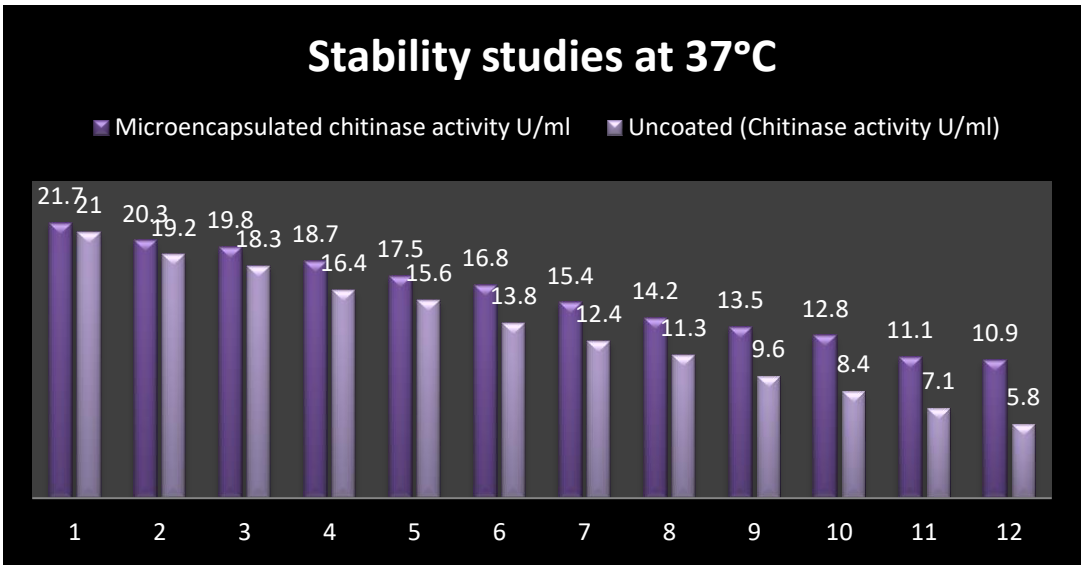
a)Tables and graph showing values of stability study of microencapsulated beads at 4^{0}C:

STABILITY AT 4^{0}C				
Month	Microencapsulated chitinase activity (U/ml)	SD	Uncoated (Chitinase U/ml)	SD
1	22.3	3.4	20.9	2.8
2	21.9	2.8	18.7	4.3
3	21.3	2.5	16.6	4.6
4	20.8	2.9	14.6	3.6
5	19.7	3.1	13.4	3.9
6	18.9	4.2	11.3	2.7
7	18.1	3.6	9.1	3.1
8	17.6	2.8	7.6	2.5
9	17.0	4	6.1	2.1
10	16.5	3.3		0
11	15.8	3.1		0
12	15.2	2.6		0



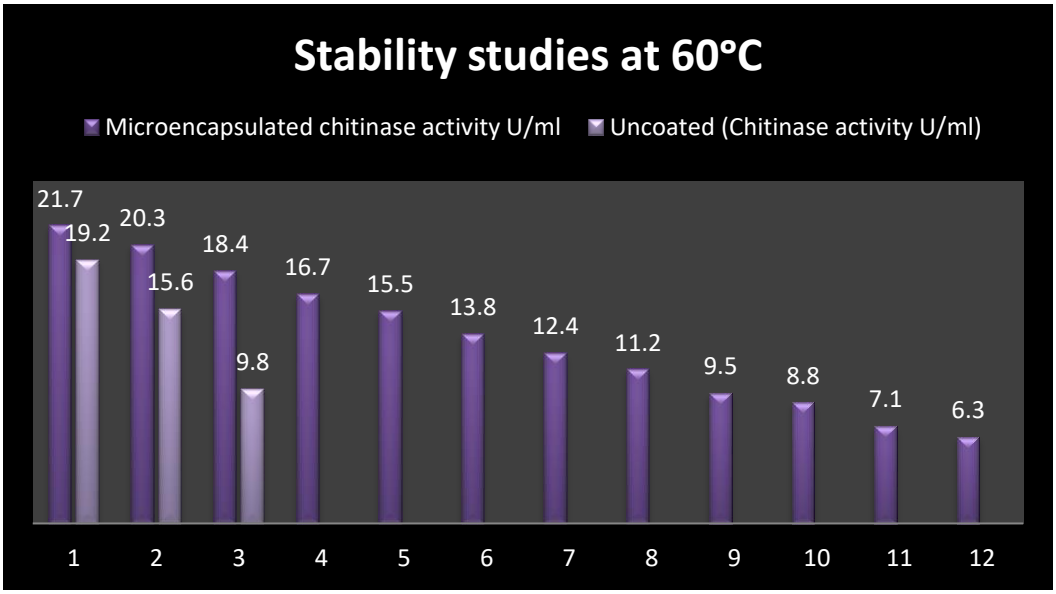
STABILITY AT 37^{0}C				
Month	Microencapsulated chitinase activity (U/ml)	SD	Uncoated (Chitinase U/ml)	SD
1	21.7	4.1	21.0	3.5
2	20.3	3.8	19.2	3.7
3	19.8	3.2	18.3	2.9
4	18.7	2.9	16.4	2.3
5	17.5	2.7	15.6	4.1
6	16.8	3.2	13.8	4.6
7	15.4	3.0	12.4	2.5
8	14.2	3.7	11.3	2.8
9	13.5	2.6	9.6	3.4
10	12.8	2.2	8.4	3.6
11	11.1	3.3	7.1	3.2
12	10.9	1.9	5.8	2.7

b) Tables and graph showing values of stability study of microencapsulated beads at 37°C



c) Tables and graph showing values of stability study of microencapsulated beads at 60^{0}C

STABILITY AT 60^{0}C				
Month	Microencapsulated chitinase activity (U/ml)	SD	Uncoated (Chitinase U/ml)	SD
1	21.7	3.5	19.2	4.1
2	20.3	3.6	15.6	2.9
3	18.4	2.7	9.8	3.6
4	16.7	2.5	0	0
5	15.5	4.1	0	0
6	13.8	4.3	0	0
7	12.4	3.4	0	0
8	11.2	3.8	0	0
9	9.5	2.5	0	0
10	8.8	2.9	0	0
11	7.1	3.2	0	0
12	6.3	3.8	0	0



ii) Stability studies of microencapsulated beads at various pH:

a) Stability study of microencapsulated beads at pH 3.5: At the acidic pH 3.5 when the microencapsulated enzymes where checked for their activity 20 U/ml was recorded for the very first month and the uncoated with 18 U/ml. A gradual decline in the chitinase enzyme value was recorded for the coated and a complete declined values for uncoated from the second month onwards. In the seventh month the uncoated had stopped showing its enzyme activity and the coated however sustained till the twelfth month with highly reduced value of 5.6 U/ml.

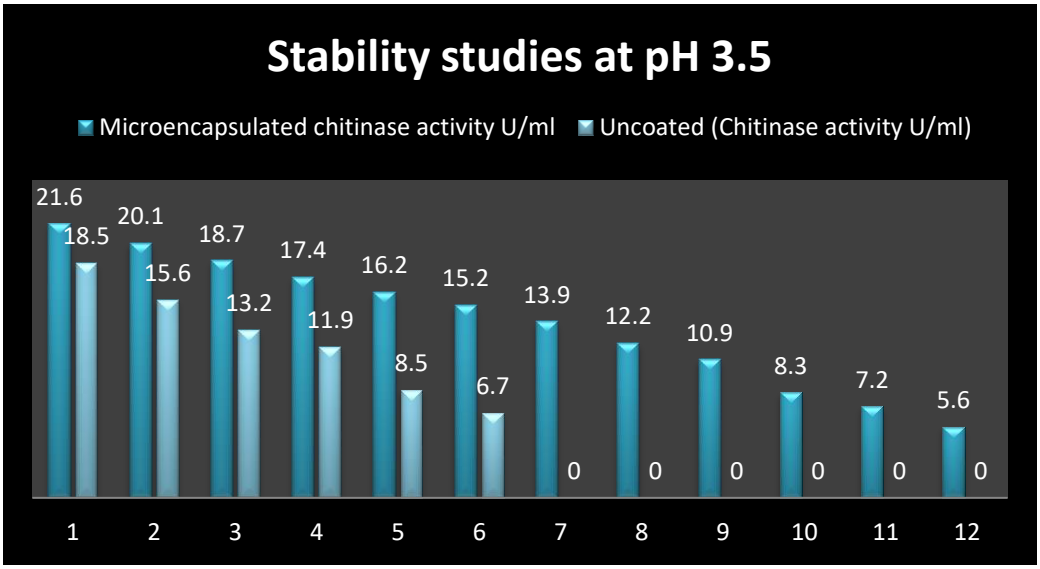
b) Stability study of microencapsulated beads at pH 7.0: The enzyme activity at pH 7 showed 22 U/ml value in the first month for the coated and the uncoated with 21.5 U/ml. Then on from the months that followed a gradual and a drastic change of values were recorded for the uncoated and the coated respectively. The activity of the enzymes stopped for the uncoated at 10 months showing 6.6 U/ml values and the coated showed 11 U/ml value at the end of the year. At neutral pH only the uncoated has lost its activity.

c) Stability study of microencapsulated beads at pH 9.0: At 9.0, the alkaline pH the microencapsulated enzyme showed 20 U/ml and the uncoated with 16 U/ml values recorded. A slight decline over the months was noted for the coated whereas the uncoated showed a sharp drastic declined values. The

Seventh month showed nil activity for the uncoated and the coated enzyme activity sustained until twelve months having 7.3 U/ml value.

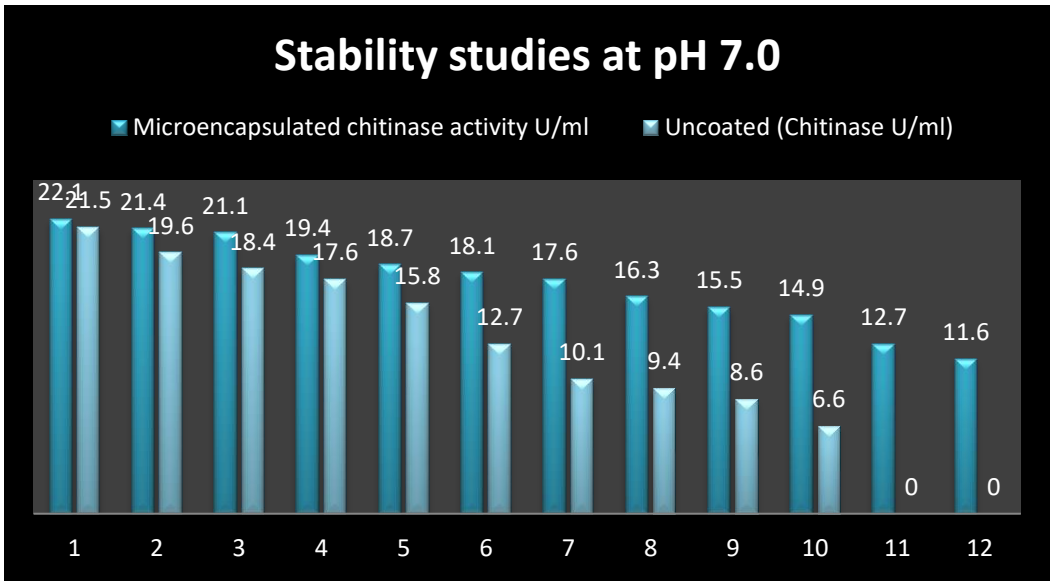
STABILITY AT 3.5 pH				
Month	Microencapsulated chitinase activity U/ml	SD	Un coated (Chitinase U/ml)	SD
1	21.6	2.1	18.5	4.5
2	20.1	2.5	15.6	3.7
3	18.7	3.3	13.2	3.5
4	17.4	3.6	11.9	2.9
5	16.2	4.5	8.5	3.4
6	15.2	3.9	6.7	2.8
7	13.9	3.5	0	0
8	12.2	2.8	0	0
9	10.9	2.6	0	0
10	8.3	3.4	0	0
11	7.2	3.8	0	0
12	5.6	4.1	0	0

a) Tables and graph showing values of microencapsulated stability studies at pH 3.5



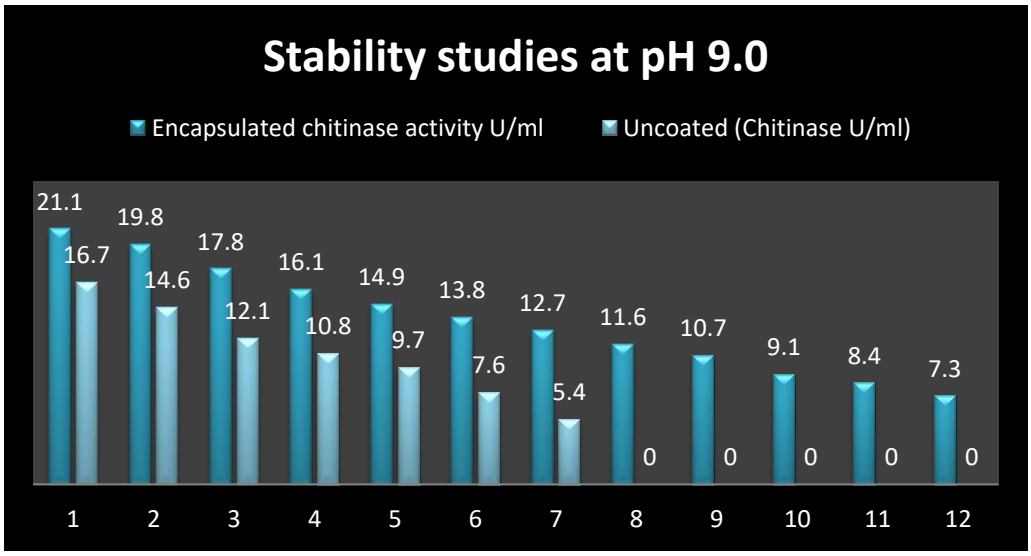
STABILITY AT 7.0 pH				
Month	Microencapsulated chitinase activity U/ml	SD	Un coated (Chitinase U/ml)	SD
1	22.1	3	21.5	2.8
2	21.4	3.4	19.6	3.9
3	21.1	3.8	18.4	2.3
4	19.4	4.6	17.6	2.9
5	18.7	4.4	15.8	2.2
6	18.1	3.5	12.7	2.7
7	17.6	3.8	10.1	3.3
8	16.3	3.2	9.4	3.1
9	15.5	3.5	8.6	3.6
10	14.9	2.8	6.6	4.2
11	12.7	2.7	0	0
12	11.6	2.2	0	0

b) Tables and graph showing values of stability study of microencapsulated beads at pH 7.0



STABILITY AT 9.0 pH				
Month	Microencapsulated chitinase activity U/ml	SD	Un coated (Chitinase U/ml)	SD
1	21.1	4	16.7	3.6
2	19.8	4.3	14.6	2.9
3	17.8	3.2	12.1	4.1
4	16.1	2.8	10.8	2.7
5	14.9	4.6	9.7	3.6
6	13.8	4.1	7.6	4.5
7	12.7	3.9	5.4	3.7
8	11.6	3.3	0	0
9	10.7	2.8	0	0
10	9.1	2.6	0	0
11	8.4	4.1	0	0
12	7.3	3.6	0	0

c) Tables and graph showing values of stability study of microencapsulated beads at pH 9.0



CONCLUSION: The microencapsulated chitinase activity when checked for its stability over various temperatures to find the optimal range when compared to the uncoated chitinase enzyme activity for twelve months. Of all the temperatures 4°C, 37°C and 60°C the immobilized enzymes withstood all the

temperature parameters, whereas the uncoated enzyme at some point had showed zero activity over two temperatures (4°C and 60°C). The enzyme activity for the coated showed better results in the first month for 4°C recorded 22.3 U/ml, the uncoated value recorded 21.0 U/ml its highest at 37°C. At 60°C the uncoated lost its potential after the third month. The purpose of enzyme immobilization hence was met, making them withstand all the temperature ranges with however making the enzymes to be active. The coated showed highest activity at 4°C and lowest at 37°C. Making the optimum temperature for enzyme immobilization to be 4°C. **(Gan et al., 2012)** in his optimum temperature study denoted that the higher enzyme activity was observed at 40°C.

The enzyme immobilization activity for the chitinase enzyme when checked over a period of 12 months and compared with the uncoated enzymes had showed relatively greater stability over the enzyme activity. At all pH 3.5, 7.0 and 9.0 the coated exhibited its activity. The highest value recorded for the first month for the coated was at pH 7.0 with 22.1 U/ml. the lowest recorded was at pH 3.5 with 5.6 U/ml, which was at twelfth month making the neutral pH 7.0 as the optimum temperature in this study for the immobilized enzyme. With the coated enzyme they lost its potential at some point or the other over all the pH media assessed. **(Bussamara et al., 2012)** studied the optimum pH at 4.6 and 6.0 for enzyme immobilization both being the acidic medium. Wherein, this study had proved pH 7.0 the neutral pH to be optimal for the microencapsulated.

REFERENCES:

Bussamara R, Dall'agnol L, Schrank A, Fernandes KF and Vainstein MH (2012), Optimal Conditions for Continuous Immobilization of Pseudozyma hubeiensis (Strain HB85A), Lipase by Adsorption in a Packed-Bed Reactor by Response Surface Methodology, Enzyme research. 329178.

Chritina Rother and Bernd Nidetzky (2014), Enzyme Immobilization by Microencapsulation, Methods, Materials, and Technological Applications, Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology**.**

Gan Z, Zhang T, Liu Y, and Wu D (2012), Temperature-triggered enzyme immobilization and release based on cross-linked gelatin nanoparticles. PloS one. 7(10).

Homaei AA, Sariri R, Vianello F, and Stevanato R (2013), Enzyme immobilization: an update, Journal of chemical biology. 6(4), 185–205.

Ram Singh, Taranjeet Pandey and Ashok (2019), Microbial Enzymes, An Overview. 10.