Course: Enzyme Technology.

Course Code: 15EBTP204

Course Outcomes (COs):

At the end of the course the student should be able to:

1. Follow guidelines, SOP's, safety measures and calibrations while handling equipments during experimentation.
2. Perform the enzyme technology laboratory procedures for determination of enzyme activity, specific activity and parameters affecting the enzyme.
3. Refer journal articles to Design experiments for enzyme immobilization and scrutinize the influences of various metal ions on enzymes and communicate the results in written reports according to standard guidelines.
4. Work in team to Review literature to design enzyme isolation and assay procedures and communicate the results in written reports according to standard guidelines.

Course Articulation Matrix: Mapping of Course Outcomes (CO) with Program Outcomes

Course Title: Enzyme Technology Lab										Semester:4 - Semester					
Course Code:15EBTP204										Year: 2019-20					
Course Outcomes / Program Outcomes	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Follow guidelines, SOP's, safety measures and calibrations while handling equipments during experimentation								**H**					**H**		
Perform the enzyme technology laboratory procedures for determination of enzyme activity, specific activity and parameters affecting the enzymes				**H**											
Refer journal articles to Design experiments for enzyme immobilization and scrutinize the influences of various metal ions on enzymes and communicate the results in written reports according to standard guidelines		**M**		**H**									**H**		
Work in team to Review literature to design enzyme isolation and assay procedures and communicate the results in written reports according to standard guidelines		**M**		**H**					H	**H**					

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Degree of compliance **L**: Low **M**: Medium **H**: High

Competency addressed in the Course and corresponding Performance Indicators

Competency: 2.1	Demonstrate an ability to identify and characterize an engineering problem
PI Code: 2.1.2	Identify engineering systems, variables, and parameters to solve the problems
Competency: 2.2	Demonstrate an ability to formulate a solution plan and methodology for an engineering problem
PI Code: 2.2.2	Identify, assemble and evaluate scientific information and resources.
Competency: 4.1	Demonstrate their ability to conduct investigations of technical issues consistent with their level of knowledge and understanding
PI Code: 4.1.1	Define a problem to carry-out investigation with its scope and importance.
PI Code: 4.1.2	Identify and apply relevant experimental procedure /bioinformatics tools /databases for a defined problem
PI Code: 4.1.3	Use appropriate analytical instruments /software tools to carry-out the experiments
PI Code: 4.1.4	Correlate the experimental outcomes with underlying theoretical concepts and principles
Competency: 4.2	Demonstrate their ability to design experiments to solve open ended problems
PI Code: 4.2.1	Design and develop experimental flow-charts and specify appropriate equipments for the given open ended problem
Competency: 4.3	Demonstrate an ability to critically analyze data to reach a valid conclusion
PI Code: 4.3.1	Use appropriate procedures, tools and techniques to collect and analyze data
PI Code: 4.3.2	Critically analyze data for trends and correlations, stating possible errors and limitations
PI Code: 4.3.3	Represent data in tabular and graphical forms for data analysis
PI Code: 4.3.4	Synthesize information and knowledge about the problem from the raw data to reach appropriate conclusions
Competency: 8.1	Demonstrate an ability to recognize ethical dilemmas
PI Code: 8.1.1	Identify situations of unethical professional conduct and propose ethical alternatives
Competency: 10.1	Demonstrate an ability to comprehend technical literature and document project work.
PI Code: 10.1.2	Produce clear, well-constructed, and well-supported written engineering documents

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Competency: 13.2	Demonstrate an ability to perform experimentation with accuracy and reproducibility.
PI Code: 13.2.1	Perform calibration and verification for obtaining accurate and reproducible data
PI Code: 13.2.2	Follow standard operating procedures adhering to laboratory guidelines.
competency: 9.3	**Demonstrate success in a team-based project**
PI Code **9.3.1**	Present results as a team, with smooth integration of contributions from all individual efforts

Experiment wise Plan

List of experiments/jobs planned to meet the requirements of the course.

Category: Demonstration		**Total Weightage: 10.00**		**No. of lab sessions: 2.00**
Expt./ Job No.	**Experiment / Job Details**	**No. of Lab Session(s) per batch (estimate)**	**Marks / Experiment**	**Correlation of Experiment with the theory**
1	Biochemical Measurements: Molarity, Normality, Molality, Moles, weight/volume measurements, percent solution, concentration Units. pH measurements and Buffer preparation, SOP's for instruments and safety guidelines	1.00	5.00	
	□Learning Outcomes: □The students should be able to: 1. Follow the defined SOP's for equipments and follow the safety measures 2. Reflect the Basic Biochemical concepts of calculations and Buffer preparations			Biochemical Foundation & Biomolecules
2	Molecular weight determination by SDS PAGE And Staining the gel using CBB or silver staining.	1.00	5.00	
	□Learning Outcomes: □The students should be able to: 1. Prepare the SDS-PAGE gel and run the samples 2. Determine the molecular weight of the enzyme by verification with standards			Purification of enzymes

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Category: Exercise		**Total Weightage: 30.00**		**No. of lab sessions: 6.00**
Expt./ Job No.	**Experiment / Job Details**	**No. of Lab Session(s) per batch (estimate)**	**Marks / Experiment**	**Correlation of Experiment with the theory**
1	Determination of activity of amylase enzyme	1.00	5.00	
	Learning Outcomes: The students should be able to: 1. Perform the enzyme assay by executing enzyme-substrate reaction. 2. Plot the standard graph and perform statistical calculations of data. 3. Calculate enzyme activity with defined units and corelate the activity to the source			Enzymatic techniques.
2	Estimation of protein content of amylase and specific activity	1.00	5.00	
	Learning Outcomes: The students should be able to: 1. Determine the concentration of enzyme protein by standard Biochemical method 2. Plot the standard graph and perform statistical calculations of data. 3. Calculate specific activity with defined units and corelate to the protein concentration of the source			Enzymatic techniques.
3	Effect of temperature on enzyme activity	1.00	5.00	
	Learning Outcomes: The students should be able to: 1. Execute an experiment to analyze the influence of different temperatures on enzyme catalyzed reaction. 2. Plot the graph showing effect of temperature on enzyme activity and analyze the nature of curve 3. Corelate the effect of temperature on the enzyme from selected source based on literature			Enzyme Techniques
4	Effect of pH on enzyme activity	1.00	5.00	
	Learning Outcomes: The students should be able to: 1. Execute an experiment to analyze the influence of different pH on enzyme catalyzed reaction			Enzyme Techniques

	2. Plot a graph showing the effect of pH on enzyme activity and analyze the nature of the graph 3. Correlate the effect of pH on enzyme from selected source from the literature			
5	Effect of substrate concentration on enzyme activity	1.00	5.00	
	□Learning Outcomes: □The students should be able to: 1. Conduct an experiment to analyze the influence of different substrate concentrations on enzyme activity 2. Plot a graph to show the effect of substrate concentration on enzyme activity 3. Find and calculate the kinetics Km and Vmax of enzyme and corelate to the substrate affinity principle through the literature			Enzyme Techniques
6	Effect of enzyme concentration on enzyme activity	1.00	5.00	
	□Learning Outcomes: □The students should be able to: 1. Conduct an experiment to analyze the influence of different enzyme concentration on enzyme activity. 2. plot a graph showing the influence of enzyme concentration on enzyme activity 3. Discuss the effect of enzyme concentration on enzyme from selected source from the literature			Enzyme Techniques
Category: Structured Enquiry		**Total Weightage: 20.00**		**No. of lab sessions: 4.00**
Expt./ Job No.	**Experiment / Job Details**	**No. of Lab Session(s) per batch (estimate)**	**Marks / Experiment**	**Correlation of Experiment with the theory**
1	Design and conduct an experiment to determine the kinetic parameters of immobilized enzyme.	2.00	10.00	
	□Learning Outcomes: □The students should be able to: 1. Characterize the given Problem statement to Clearly state the objectives and parameters by Assessing scientific information from literature to develop a solution plan for enzyme immobilization			Enzyme Immobilization

	2. Design experimental flow-charts and Perform all biochemical calculation to develop the experimental design and discuss correlating to the theoretical concepts 3. Conduct the experiment based on the designed protocol using instrumental methods to collect the data and analyze & interpret the results statistically 4. Plot relevant graphs and Make precise conclusions from obtained results correlating to theoretical concepts 5. Submit a report of the reproducible data on SE documenting complete methodology according to standard format			
2	Design and conduct an experiment to determine the influence of effectors on enzyme activity	2.00	10.00	
	□Learning Outcomes: □The students should be able to: 1. Characterize the given Problem statement to Clearly state the objectives and parameters by Assessing scientific information from literature to develop a solution plan for enzyme activation or inhibition by effectors 2. Design experimental flow-charts and Perform all biochemical calculation to develop the experimental design and discuss correlating to the theoretical concepts. 3. Conduct the experiment based on the designed protocol using instrumental methods to collect the data and analyze & interpret the results statistically 4. Plot relevant graphs and Make precise conclusions from obtained results correlating to theoretical concepts 5. Submit a report on SE documenting the reproducible data ad complete methodology according to standard format			Enzyme Kinetics and Enzyme Inhibitions.
Category: Open Ended		**Total Weightage: 20.00**		**No. of lab sessions: 2.00**
Expt./ Job No.	**Experiment / Job Details**	**No. of Lab Session(s) per batch (estimate)**	**Marks / Experiment**	**Correlation of Experiment with the theory**
1	Design and conduct an experiment to extract the enzyme from a source and design a method to determine its enzyme activity.	2.00	20.00	
	□Learning Outcomes: □The students should be able to:			Introduction to enzymes Enzymatic techniques.

	1. Characterize the given Problem statement and define to Clearly state the objectives and parameters by Assessing scientific information from literature to develop a solution plan for enzyme isolation from defined source 2. Design experimental flow-charts and Perform all biochemical calculation to develop the experimental design and discuss correlating to the theoretical concepts 3. Conduct the experiment based on the designed protocol using instrumental methods to collect the data and analyze & interpret the results statistically 4. Synthesize information from precise conclusions of obtained results correlating to theoretical concepts 5. Submit a report on OEE documenting detailed methodology according to standard format	

Enzyme Technology lab (15EBTP204) Rubric 2019-20 ISA (80 M)

Expt. No	PI code	Excellent 90-100%	Good 60-90%	Average 40-60%	Poor <40%
E1 (5) *D*-Biochemical calculations	13.2.2 (5)	Follow all the SOP's of lab and equipments	Follow most of the SOP's of lab and equipments	Follow few of the SOP's of lab and equipments	Little awareness on SOP's of lab and equipments
E2 (5) D- SDS PAGE	8.1.1(3)	Demonstrate complete understanding and Knowledge on unethical professional conduct during experiment conduct	Demonstrate some understanding and Knowledge on unethical professional conduct during experiment conduct	Less understanding and Knowledge on unethical professional conduct during experiment conduct	No clear Knowledge on unethical professional conduct during experiment conduct
	13.2.2 (2)	Follow all the SOP's of lab and equipments	Follow most of the SOP's of lab and equipments	Follow few of the SOP's of lab and equipments	Little awareness on SOP's of lab and equipments
E3 Determination of activity of amylase enzyme**,**	4.1.2 (2) 4.1.3 (1) 4.1.4 (1) 4.3.1 (1)	Conduct the experiment with complete involvement and accuracy	Conduct the experiment with complete involvement and with	Conduct the experiment with less accuracy using analytical	Conduct the experiment without understanding and no

E4 Estimation of protein content of amylase and specific activity, **E5** Effect of temperature on enzyme activity, **E6** Effect of pH on enzyme activity, **E7** Effect of substrate concentration on enzyme activity, **E8** Effect of enzyme concentration on enzyme activity **(5)*6=30 E**		using analytical tools by applying relevant procedures. Analyze and Interpret the obtained data statistically and correlate to the theoretical concepts.	approximation using analytical tools by applying relevant procedures. Analyze and Interpret the obtained data statistically with poor correlation to the theoretical concepts	tools by applying relevant procedures. Analyze and Interpret the obtained data with no statistical analysis and no correlation	accuracy of procedures. Poor data interpretation
E9 Design and conduct an experiment to determine the kinetic parameters of immobilized enzyme. **E10** Design and conduct an experiment to determine the influence of effectors on enzyme activity **(10)*2=20 SE**	2.2.1 (1) 2.2.2 (2)	Completely Define the problem statements and clearly define all objectives. Complete Review scientific information to solve the problem	Complete Definition of problem statements and no proper objectives. Some Review scientific information to solve the problem	Incomplete Definition of problem statements and objectives. Very few Review scientific information to solve the problem	No proper Definition of problem statements. No proper Review scientific information to solve the problem
	4.2.1 (2) 4.3.2 (2) 4.3.3 (2)	Design the experiments by selecting correct standards and suitable methods. Interpret the	Design the experiments by selecting improper standards and but methods are suitable.	Design the experiments without complete clarity of the methods. Interpret the	Fail to Design a proper experiments. Poor data interpretation. Improper graphs

		obtained data statistically and correlate to the theoretical concepts. Analysis all the results in the form of graphs and tables	Interpret the obtained data statistically with poor correlation to the theoretical concepts. Analysis most of the results in the form of graphs and tables	obtained data with no statistical analysis and no correlation. Analysis few of the results in the form of graphs and tables.	
	9.3.1 (1)Team work	Each student demonstrates the role and responsibilities in the team work.	Some contribution of responsibilities in the team work.	Very few responsibilities in the team work.	Poor participation in the team
Poor data interpretation **E11(20M) OEE-** Design and conduct an experiment to extract the enzyme from a source and design a method to determine its enzyme activity.	2.1.2 (2) 2.2.2 (3)	Identify all parameters required for the experiment. Review scientific information to solve the problem	Identify most of the parameters required for the experiment. Some Review scientific information to solve the problem	Identify few parameters required for the experiment. Very few Review scientific information to solve the problem	Poor parameters identification. No proper Review
	4.1.1 (2) 4.2.1(3) 4.3.1(3) 4.3.2(2) 4.3.4 (2)experimental conclus	Define the OEE experiment with relevance. Design the experiments by selecting correct standards and suitable methods. Follow all the defined Procedures and techniques for data analysis. Analyze & Interpret the obtained data	Define the OEE experiment with some relevance. Design the experiments by selecting improper standards and but methods are suitable. Follow most of the defined Procedures and techniques for data	Define the OEE experiment without relevance. Design the experiments without complete clarity of the methods. Follow few defined Procedures and techniques for data analysis	No properly defined OEE experiment. Fail to Design a proper experiments. No proper Procedures and no data analysis. No clear Written conclusion

		statistically and correlate to the theoretical concepts. Write precise conclusion correlating the original sample concentrations	analysis. Analyze & Interpret the obtained data statistically with poor correlation to the theoretical concepts. Write conclusion with poor correlation to the original sample concentrations	Analyze & Interpret the obtained data with no statistical analysis and no correlation. Write conclusion with no correlation to the original sample concentrations	
	10.1.2 (3) Report	Prepare a concise report according to the format including tables, calculations, graphs and statistical analysis	Prepare a concise report according to the format including most of the tables, calculations, graphs and statistical analysis	Prepare a report with few of the tables, calculations, graphs and statistical analysis	Report preparation not according to the required format

KLE Technological University
Creating Value
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Earlier known as
B. V. B. College of Engineering & Technology

Department of Biotechnology

ENZYME TECHNOLOGY LABORATORY

Structured Enquiry 1 & 2

***Student's Names*:**	***USN:***
Nagaruchika k h	01fe17bbt023
Jovita deodhar	01fe17bbt016
Kannika gejjihalli	01fe17bbt017
Naheeda H	01fe17bbt024

Structured Enquiry 1

Effect of different concentrations of Sodium Chloride inhibitor or activator on amylase enzyme

Introduction:

Activator: **Enzyme activators** are molecules that bind to enzymes and increase their activity. They are the opposite of enzyme inhibitors. These molecules are often involved in the allosteric regulation of enzymes in the control of metabolism. Enzyme activators are molecules that bind to enzymes and increase their activity. They are the opposite of enzyme inhibitors. These molecules are often involved in the allosteric regulation of enzymes in the control of metabolism. An example of an enzyme activator working in this way is fructose 2,6-bisphosphate, which activates phosphofructokinase 1 and increases the rate of glycolysis in response to the hormone insulin. In biochemistry, activation, specifically called bioactivation, is where enzymes or other biologically active molecules acquire the ability to perform their biological function, such as inactive proenzymes being converted into active enzymes that are able to catalyze their substrates into products. An enzyme may be reversibly or irreversibly bioactivated; A major mechanism of irreversible bioactivation is where a piece of the protein is cut off by protein cleavage, causing the enzyme to stay active. On the other hand, a major mechanism of reversible bioactivation is where a cofactor is placed on the enzyme, causing it to only stay active while the cofactor stays on.

Method: Allosteric method of activation. In biochemistry, allosteric regulation (or allosteric control) is the regulation of a protein by binding an effector molecule at a site other than the enzyme's active site.The site to which the effector binds is termed the allosteric site. Allosteric sites allow effectors to bind to the protein, often resulting in a conformational change involving protein dynamics. Effectors that enhance the protein's activity are referred to as allosteric activators, whereas those that decrease the protein's activity are called allosteric inhibitors.Allosteric regulations are a natural example of control loops, such as feedback from downstream products or feed forward from upstream substrates. Long-range allostery is especially important in cell signalling. Allosteric regulation is also particularly important in the cell's ability to adjust enzyme activity.In lowering the activation energy of a reaction, enzymes decrease the barrier to starting a reaction. It's important to note, however, that the change in energy remains the same between the start and end of a chemical reaction.

Significance:

The reaction can be carried out at a faster rate.
Enzyme activity will be increased.
It decreases the K_m value , which gives the enyme high affinity towards the substrate.

Method: Dinitrosalicylic acid (DNS) method for the estimation of reducing sugars.

Principle: Amylase convert Reducing sugars to maltose which is measured by DNS method. When a metal ion in a particular concentration is added to the enzyme and substrate containing medium, it affects the product formation. If it is a activator it increases the product formation which gives intense color and hence higher O.D value than the control(enzyme+buffer+starch).If the metal ion is a inhibitor it decreases the product formation ,which gives less intense color and hence lower O.D value than the control.

Materials:

- Sodium Chloride.
- Amylase Enzyme.
- Phosphate Buffer (pH 7.0).
- Substrate (1% Starch).
- 5. DNS Reagent.

Standard: Sodium Chloride (NaCl)

Stock Conc.: 500mM

Working conc. Range: 100mM to 500mM

Reagent Preparations:

1. Sodium Chloride (NaCl) : 500mM of NaCl

   Moles= Given Mass/ Molecular Mass

   $500x10^{-3}$= a/58.5

   a= 0.147 g in 5ml ➔ a= 147 mg in 5ml

   From stock, prepare working of different concentrations.

   For 400mM,

$C_1V_1=C_2V_2$ ➔ $500x10^{-3}$ xV_1= $400x10^{-3}$ x 0.1 ➔ V_1 = 0.08 ml of stock, volume it up to 0.1ml

Similarly, for 300mM take 0.06ml of stock , for 200mM take 0.04ml and for 100mM take 0.02ml and volume all of them to 0.1ml with distilled water.

2. Phosphate buffer(0.1M) -20ml

   NaH2PO4 – 119.98gm in 1000 ml 1M

   ➔0.2399 g in 20ml gives 0.1M

   Na2HPO4 – 141.96 g in 1000 ml water gives 1M

   ➔0.2839 g in 1000 ml water gives 0.1M

   Add monobasic to dibasic and make the pH to 7

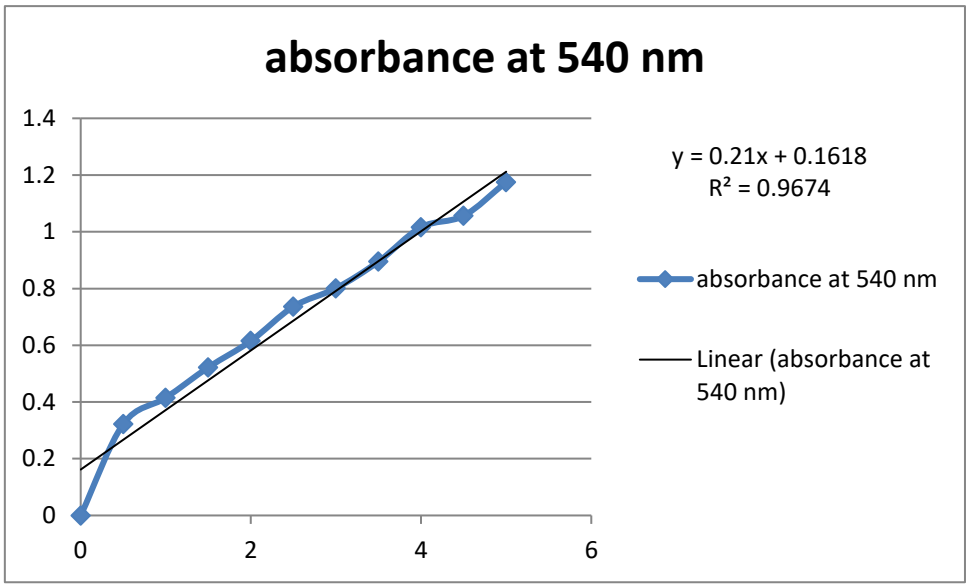
Methods: Procedure

1. Take 7 test tubes label from T1 to T7.
2. To all test tubes add 0.3 ml buffer,0.5ml starch and 0.1 ml inhibitor but, add 4ml of buffer in blank, and do not add inhibitor to the control and pre-incubate at 37 degree Celsius for 5min.
3. Add 0.1 ml enzyme to all test tubes except the blank and incubate for 10 min.
4. Add 1ml DNS to all test tubes and keep the TT in boiling water bath for 10 min.
5. Take O.D at 540 nm.
6. Plot the bar graph of Conc of Inhibitor v/s Enzyme Activity and linear graph of Conc of Inhibitor v/s % inhibition.

Tabulation:

TT No.	Vol. of Buffer (ml)	Vol. of Substrate (ml)	Vol. of Inhibitor (ml)	Conc. of Inhibitor (mM)		Vol. of Enzyme (ml)		Vol. of DNS (ml)		O.D. at 540nm
Blank	0.4	0.5	0.1	500	Pre Incubate for 5 min at 37ºC	0	Incubate for 10 min at 37ºC	1	Boiling water bath for 10 min	0
Control	0.4	0.5	0.0	0		0.1		1		0.07
1.	0.3	0.5	0.1	100		0.1		1		0.13
2.	0.3	0.5	0.1	200		0.1		1		0.11
3.	0.3	0.5	0.1	300		0.1		1		0.09
4.	0.3	0.5	0.1	400		0.1		1		0.07
5.	0.3	0.5	0.1	500		0.1		1		0.05

Enzyme Activity from Standard Maltose Calibration Chart:



From maltose calibration curve y=0.21x

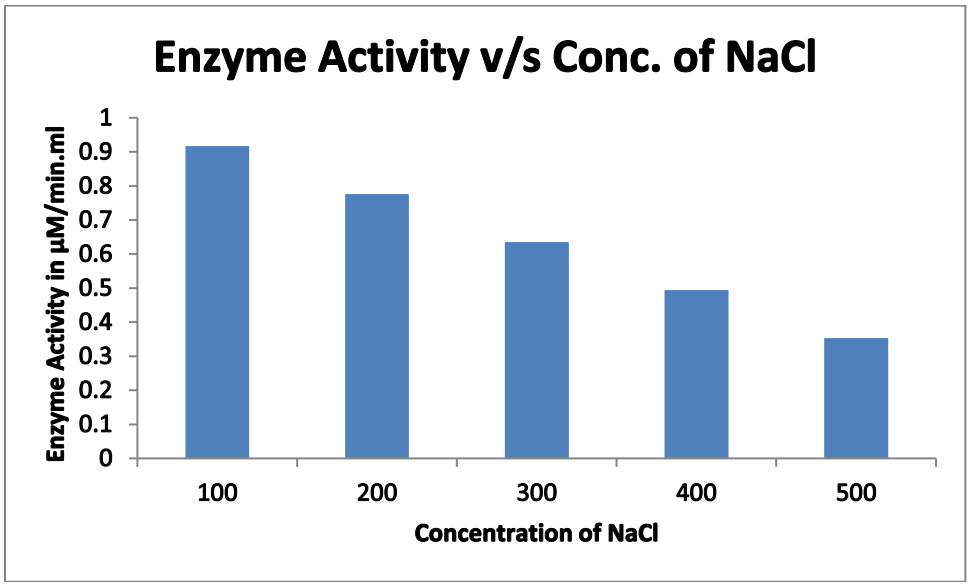
Control: y=0.07 x=0.33 µmoles/ml

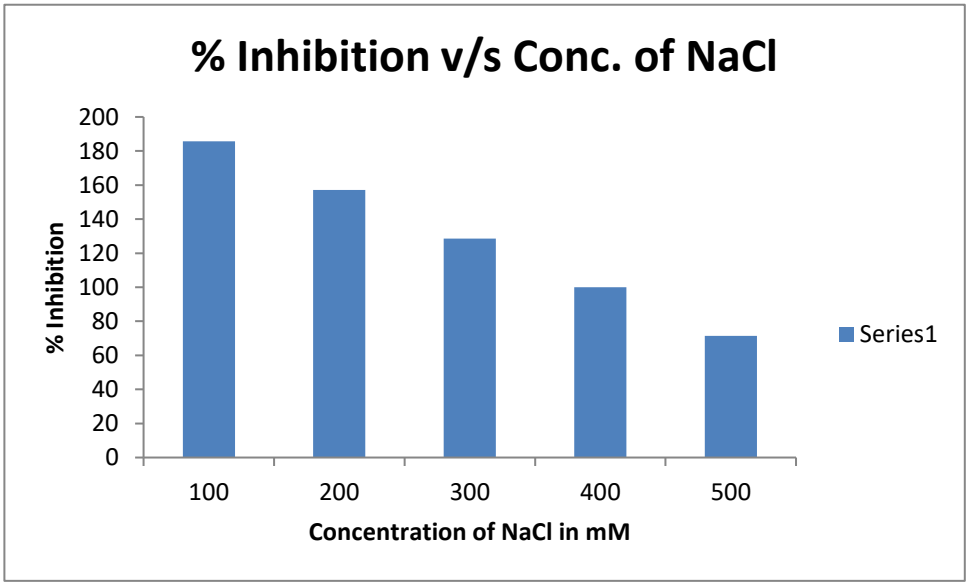
1. y=0.13 x=0.6190 µmoles/ml EA=0.6190 µM/min.ml

% Activation= (Activity in testtube/activity of control)x100%

1. For 100mM, 884%

Bar Graphs:





Discussion of the results: According to the results procured the maximum level of activation is 100 mM concentration. Hence it is the most ideal conc for activation of amylase enzyme. We see that as the conc of NaCl increases the enzyme activity decreases thus, turning into an inhibitor.

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Structured Enquiry 2

Immobilization of amylase enzyme by calcium alginate gel beads and determination of its kinetics

Introduction: Immobilization: The term immobilized enzymes refers to enzymes physically confined or localized in a certain defined region of space with retention of their catalytic activities which can be used repeatedly and continuously. The major components of an immobilized enzyme system are, the enzyme, the matrix, and the mode of attachment. An immobilized enzyme is an enzyme that is attached to an inert, insoluble material such as calcium alginate (produced by reacting a mixture of sodium alginate solution and enzyme solution with calcium chloride). This can provide increased resistance to changes in conditions such as pH or temperature. It also allows enzymes to be held in place throughout the reaction, following which they are easily separated from the products and may be used again - a far more efficient process and so is widely used in industry for enzyme catalysed reactions. An alternative to enzyme immobilization is whole cell immobilization.

Methods of Immobilization: 1) Physical Entrapment 2) Membrane Confinement 3) Adsorption. 4) Covalent Binding.

Importance:

1) This method can provide resistance to the enzyme to changes in conditions such as temperature and pH.
2) It allows the enzyme to stay at a single place throughout the reaction.
3) Products can be easily separated an efficiently.

Significance: Immobilized enzymes are very important for commercial uses as they possess many benefits to the expenses and processes of the reaction of which include:
Convenience: Minuscule amounts of protein dissolve in the reaction, so workup can be much easier. Upon completion, reaction mixtures typically contain only solvent and reaction products.
Economy: The immobilized enzyme is easily removed from the reaction making it easy to recycle the biocatalyst.
Stability: Immobilized enzymes typically have greater thermal and operational stability than the soluble form of the enzyme.

Applications:

(1) Increased functional efficiency of enzyme

(2) Enhanced reproducibility of the process they are undertaking

(3) Reuse of enzyme

(4) Continuous use of enzyme

Principle: In this method enzymes are physically entrapped inside a porous matrix. Bonds involved in stabilizing the enzyme to the matrix may be covalent or non-covalent. The matrix used will be a water soluble polymer. The form and nature of matrix varies with different enzymes. Pore size of matrix is adjusted to prevent the loss of enzyme. Pore size of the matrix can be adjusted with the concentration of the polymer used. Agar-agar and carrageenan have comparatively large pore sizes. The greatest disadvantage of this method is that there is a possibility of leakage of low molecular weight enzymes from the matrix.

$2\ Na(alginate) + Ca^{++} \rightarrow Ca(alginate)_2 + 2Na^{+}$

Examples of commonly used matrixes for entrapment are:

(1). Polyacrylamide gels

(2). Cellulose triacetate

(3). Agar

(4). Gelatine

(5). Carrageenan

(6). Alginate

Materials: $CaCl_2$ – mol wt. 110.98
Requirement- 0.2M $CaCl_2$ solution, 110.98g in one litre gives 1 molar, so 2.2196g in 100ml gives 0.2M $CaCl_2$ required solution.
Ratio of enzyme to sodium alginate in 1:2
Starch – 1% soln – dissolve 1 g of starch in 100 ml of prepared 0.1 M sodium phosphate buffer (pH 7).Sodium alginate- 1.5 g in 50 ml of distilled water.
Enzyme- 10ml of enzyme in sodium alginate solution.
DNS Reagent

Methods: DNS method

Methods: Procedure

Bead preparation:

1. Prepare respective volumes and conc of sodium alginate,enzyme and calcium chloride.
2. Mix enzyme and sodium alginate in required proportion.
3. Drop this mixture with the help of a syringe drop-by-drop in $CaCl_2$ soln so as to form several circular beads.
4. Keep it overnight in the refrigerator at 4 C.

DNS method:

1. Prepare free enzyme, buffer, starch solutions as per requirement.
2. Dry and weigh the beads. Calculate the amount of beads required for given concentration.
3. Add 0.5 ml of buffer to each of the tubes and blank. Add 0. ml of buffer in blank.
4. Add 0.5 ml of starch in all the test tubes and pre-incubate for 5 min.
5. Add beads of respective weight to each of triplicates and 0.03 ml of free enzyme to the control. Incubate for 10 min.
6. Add 1 ml of DNS to all the test tubes. Remove the beads. Keep it for boiling for 5 min.
7. Take O.D. value at 540nm.

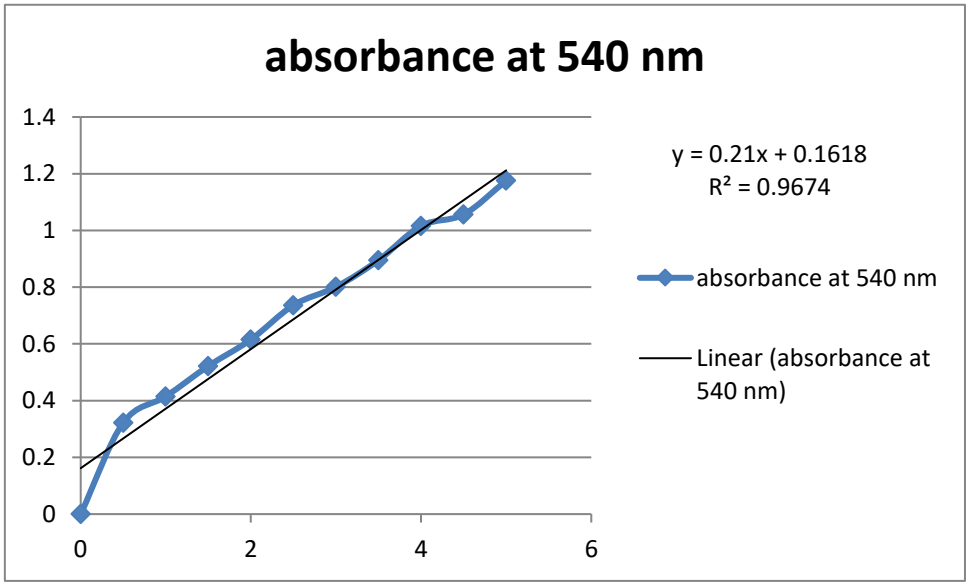
Tabulation

TT no.	**Buffer pH 7.0**	**0.2-2% Starch**		**Enzyme Beads**		**DNS Reagent**		**OD at 540nm**
Blank	**0.4ml**	**0.5 of 1%**		**---------**		**1.0ml**		**0**
Control	**0.4ml**	**0.5 of 1%**		**0.1ml**		**1.0ml**		**0.1345**
T1	**0.4ml**	**0.5**		**225mg**		**1.0ml**		**0.2543**
T2	**0.4ml**	**0.5**		**225mg**		**1.0ml**		**0.3456**

T3	**0.4ml**	**0.5**	**Pre incubate the tubes for 10min at 37°C**	**225mg**	**Incubate the tubes for 10mins at 37 °C**	**1.0ml**	**Keep the tubes in boiling water bath for 10mins and cool the tubes.**	**0.4563**
T4	**0.4ml**	**0.5**		**225mg**		**1.0ml**		**0.5423**
T5	**0.4ml**	**0.5**		**225mg**		**1.0ml**		**0.6336**
T6	**0.4ml**	**0.5**		**225mg**		**1.0ml**		**0.6793**
T7	**0.4ml**	**0.5**		**225mg**		**1.0ml**		**1.3325**
T8	**0.4ml**	**0.5**		**225mg**		**1.0ml**		**1.6435**
T9	**0.4ml**	**0.5**		**225mg**		**1.0ml**		**1.4573**
T10	**0.4ml**	**0.5**		**225mg**		**1.0ml**		**1.6284**

Graphs and calculations:

Enzyme activity



From maltose calibration curve y=0.21x

Control: y=0.04 x=0.19 µmoles/ml

1. y=0.2543 x=1.7946 µmoles/ml EA=1.7946 µM/min.ml

Results: The enzyme activity for free enzyme is lower than the immobilized enzyme.

Discussion of the results: we see that with same amount of substrate conc. The enzyme activity for immobilized enzyme is very much higher than the activity of the free enzyme.