Study effect of pH and Heavy metals on enzyme activity

Effect of pH

Changes in pH have influence on enzymes. The most favorable pH value is known as the optimum pH. This is the point that the enzyme is most active. This is graphically illustrated in figure.

Extremely high or low pH values generally result in complete loss of activity for most enzymes. pH is also a factor in the stability of enzymes. As with activity, for each enzyme there is also Changes in pH have influence on enzymes. The most favorable pH value is known as the optimum pH. This is the point that the enzyme is most active. This is graphically illustrated in figure.

Extremely high or low pH values generally result in complete loss of activity for most enzymes. pH is also a factor in the stability of enzymes. As with activity, for each enzyme there is also a region of pH optimal stability.

Changes in pH have influence on enzymes.

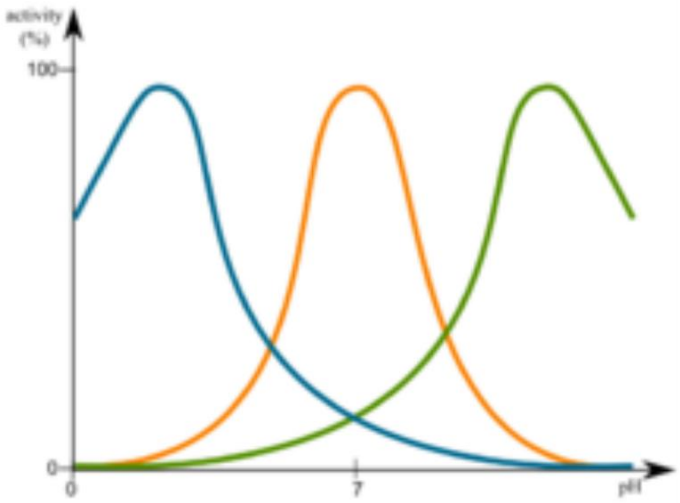
Including temperature and pH there are other factors, such as ionic strength, which can influence the enzymatic reaction. Each of these physical and chemical parameters must be considered and optimized in order for an enzymatic reaction to be accurate and reproducible.

Enzymes typically are most active in a pH range of 5-9. This is due to the fact that proteins function in an environment that reflects this pH. There are a variety of reasons as to why proteins have a narrow pH range. A variety of amino acid residues as well as the carboxyl and amide termini of proteins have a pKa range in the range of intracellular pH. As a result, a change in pH can protonate or deprotonate a side group, thereby changing its chemical features. For example, carboxyl termini, under deprotonated, could potentially lose an interaction with a adjacent subunit, changing the enzyme conformation. In conclusion, this conformation could cause a decrease in substrate affinity. A more drastic pH change can change the protein folding, thereby completely deactivating the enzyme or cause irreversible proteolysis.

However, pH change can potentially be utilized by enzymes for regulation or protein function.

1. The binding of the substrate to enzyme.
2. The ionization states that the amino acid residues of the catalytic site of the enzyme have.
3. The ionization state of the substrate.
4. The variation in protein structure (More significant at extreme pH values).

The rates of many enzymatic reactions adhere to a bell shaped curve when they are a function of pH



Graph of enzyme activity as a function of pH. Green- high pH enzyme; Blue- low pH enzyme; Orange- neutral pH enzyme.

These curves reflect the ionization state of the amino acid residues that must have a specific ionization state for enzymatic activity to take place. The observed pK's (maxima point) often hints at the identity of the amino acid residues which are essential for enzymatic activity. For instance, an observed pK of ~4 suggests that either an Asp of a Glu is essential to the enzyme. pK of ~6 can hint towards a His residue whereas pK of ~10 hints toward a Lys residue.

However, it is crucial to remember that the micro-environment in which the enzyme is in also affects its activity. For example, an Asp residue in a non-polar environment or in close proximity to another Asp residue would attract protons more strongly than in any other environment and this have a higher pK value.

Moreover, pH effects on an enzyme could cause denaturation of the enzyme rather than protonation or deprotonation of specific catalytic residues.

A particular residue may be replaced by doing site directed mutagenesis. Doing so provides researchers with a reliable approach to identifying residues that are required for substrate binding or catalysis.

Experimental procedure :

1. Preparation of different buffers of different pH like Acetate buffer(pH 3.6 to 5.6), Tris - HCl buffer (pH 6.8 to 9) , Glycine NaOH buffer (pH8.6to 10.6) following the method in Chapter 1.

2. Enzyme alkaline phosphatase as in the previous chapter .

3. Substrate p- nitrophenyl phosphate solution following the previous chapter .

4. Protocol should be as follows :

Tube No.	pH of the buffer added	Buffer added in ml.	Substrate in specified buffer added in ml.	Enzyme in specified buffer added in ml.	Time for	1M NaOH soln .to be added in ml.	Reading at 410 nm.
1	5	0.5	0.5	0	In	1	
1'	5	0.4	0.5	0.1		1	
2	6	0.5	0.5	0	cu	1	
2'	6	0.4	0.5	0.1		1	
3	7	0.5	0.5	0		1	
3'	7	0.4	0.5	0.1	ba	1	
4	8	0.5	0.5	0		1	
4'	8	0.4	0.5	0.1	ti	1	
5	9	0.5	0.5	0		1	
5'	9	0.4	0.5	0.1	on	1	
6	10	0.5	0.5	0		1	
6'	10	0.4	0.5	0.1	At 37^{o}C	1	

5. Draw the graph of pH vs. ΔOD ie., reading at 410 nm. The pH at which reading is maximum that is the optimum pH of the enzyme substrate reaction under experiment .

Effect of Heavy metals

Bacterial alkaline phosphatase (ALP) is essential for efficient recycling of phosphorous in the soil. However, soil pollution by various heavy metals has the potential to affect soil microbial ALP activity and adversely affect soil quality. To assess the effect of various heavy metal pollutants on *E. coli* ALP, a systematic study to examine the effect of Cd^{2+}, Hg^{2+}, Cu^{2+}, Co^{2+}, Ca^{2+} (individually as well in combination) on ALP activity was observed. Results show that Hg^{2+}, Cu^{2+}, and Cd^{2+} all readily inhibited *E. coli* ALP (Hg^{2+} and Cu^{2+} were most inhibitory, followed by Cd^{2+}). However, Co^{2+} had minor activating effect on ALP. Furthermore, as expected, divalent alkaline earth metals like Ca^{2+} and Mg^{2+} activated the enzyme

Co-incubation studies suggest a cumulative inhibitory effect when Cu^{2+} and Hg^{2+} were incubated together with ALP. Interestingly, incubation of Ca^{2+} decreases the inhibitory effect of Cd^{2+}, however, no such protection was observed when Ca^{2+} was incubated with Hg^{2+}.

Experimental procedure :

1. Alkaline phosphatase solution preparation as discussed in previous chapter.

2. Substrate p nitrophenyl phosphate preparation as discussed in previous chapter.

3. $CaCl_2$, $ZnSO_4$, $MgCl_2$, $Cd(NO_3)_2$ salt solutions of 20 mM of each should be prepared.

4. Glycine - NaOH buffer of 0.01M and of pH 9 is to be prepared.

5. The following protocol can be used :

Tube No.	Buffer added in ml.	Substrate added in ml.	Metal ion added in ml.	Enzyme added in ml.	Incubation at 37^0C	1M NaOH added in ml. to stop reaction	Reading at 410nm
1	0.5	0.5	0	0	For	1	
2	0.3	0.5	0	0.1	10mins	1	
3	0.3	0.5	Ca^{2+} 0.1	0.1	Maximum	1	
4	0.3	0.5	Mg^{2+} 0.1	0.1		1	
5	0.3	0.5	Zn^{2+} 0.1	0.1		1	
6	0.3	0.5	Cd^{2+} 0.1	0.1		1	

- 6. Draw the bar diagram considering the Tube 2 (control) as 100 like the following .