Research Plan

Project ID:

Project Title: Effect of pH on Amylase Activity — Starch Iodine Test

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a. Introduction

Enzymes are biological catalysts whose activity depends strongly on environmental conditions, particularly pH. Amylase catalyzes the hydrolysis of starch into simpler sugars. This project will investigate how different pH values affect the rate of starch breakdown by amylase, using the iodine-starch colorimetric test as an indicator of remaining starch.

b. Selection of Problem and Background Information

Understanding how pH affects enzyme activity is fundamental in biochemistry and has applications in food science, clinical diagnostics, and biotechnology. Amylase is active in different pH environments depending on its source (e.g., salivary vs. pancreatic). The iodine test produces a blue-black complex with starch; loss of color indicates starch degradation. Using this simple, safe assay will allow quantification of enzyme activity as a function of pH.

c. Objective

Research Problem / Question: How does the pH of the reaction medium affect the rate at which amylase hydrolyzes starch?

What will be found out:

- The pH at which amylase activity (starch hydrolysis rate) is maximal.
- Relative activity (rate) of amylase across a pH range (acidic to alkaline).
- Approximate kinetics (initial rate comparison) under controlled temperature.

Variables:

- **Independent:** pH of starch solution (e.g., pH 4.0, 5.5, 7.0, 8.5, 10.0).
- **Dependent:** Rate of starch disappearance measured as decrease in iodine-starch color intensity (qualitative: time to loss of color; quantitative: absorbance at 620 nm if spectrophotometer available).
- **Controlled:** Volume and concentration of starch, enzyme concentration (amylase), temperature, incubation time, mixing procedure, bottle size.

Control in the Study: A reaction mixture without amylase (same pH series) will serve as a negative control to confirm that starch disappearance is enzyme-dependent.

d. Hypothesis

If amylase is incubated with starch at different pH values, then there will be an optimal pH at which the enzyme activity (rate of starch hydrolysis) is maximal; activity will decrease at pH values far from this optimum.

e. Procedure

Design of Study: The assay will use identical starch substrate solutions adjusted to the required pH values using appropriate buffers. Constant-

volume aliquots of amylase will be added to each buffered starch solution and incubated at a controlled temperature. At fixed time intervals, aliquots will be withdrawn and mixed with iodine reagent; the color intensity (blue-black) will be recorded visually and, if available, measured spectropho- tometrically (absorbance at 620 nm). Trials will be repeated (triplicates) for statistical reliability.

Materials Required:

- Soluble starch solution (e.g., 1% w/v).
- Commercial amylase enzyme (powder or liquid; record activity units).
- Buffer solutions to cover desired pH range (e.g., citrate buffer pH 4.0–6.0, phosphate buffer pH 6.0–8.0, carbonate buffer pH 9.0–10.0).
- Iodine solution (I_2/KI) for starch test.
- Test tubes, micropipettes (or measuring pipettes), cuvettes (if spectrophotometer used).
- Water bath or incubator to maintain constant temperature (e.g., 37°C).
- Spectrophotometer (optional) set at 620 nm or colorimeter/visual comparison charts.
- Stopwatch, marker labels, safety gloves, goggles.

Stepwise Procedure:

1. Prepare 1% soluble starch solution and split into equal volumes for each pH condi- tion.
2. Adjust starch aliquots to target pH values using pre-made buffers; verify with pH paper or meter.
3. Equilibrate buffer-starch mixtures at the chosen temperature (e.g., 37°C) for 5 min- utes.
4. Prepare amylase solution with known concentration; keep on ice until use.
5. Start reaction by adding equal volume of amylase to each starch-buffer tube; mix gently and start the timer.
6. At predefined times (e.g., 0, 1, 2, 5, 10, 15 minutes), withdraw small

aliquots and immediately add iodine reagent to stop reaction and develop color.

7. Record color intensity visually (photograph samples under consistent lighting) and/or measure absorbance at 620 nm.
8. Repeat each condition in triplicate.
9. Run negative controls (starch + buffer, no enzyme) for each pH.

Proposed Data Tables (with sample/dummy values):

Table A: Time-course Absorbance (A_{620}) — pH 4.0 (triplicate average)

Time (min)	Absorbance at 620 nm (A_{620})
0	
1	
2	
5	
10	
15	

Table B: Initial Rate Proxy (A_{620}/min between 0–2 min) across pH values (dummy)

pH	A_{620}/min (0–2 min)	Relative Activity (%)	Interpretation
4.0			
5.5			
7.0			
8.5			
10.0			

Table C: Time to Loss of Blue-Black Color (visual endpoint) — dummy

pH	Time for No Iodine-Blue (min)
4.0	
5.5	
7.0	
8.5	
10.0	

f. Risk and Safety

- Work uses safe reagents (yeast-derived or commercial amylase, starch, buffers, io- dine). Iodine should be handled with gloves and avoided contact with skin or eyes.
- Wear gloves, goggles, and lab coat when handling reagents.
- Dispose of iodine-containing waste according to school laboratory rules.
- Maintain caution with hot water baths to avoid burns.

g. Data Analysis

- Plot absorbance (A_{620}) versus time for each pH to obtain reaction curves.
- Estimate initial rates by calculating slope of absorbance decrease between 0–2 min- utes (A_{620}/min) as a proxy for enzyme activity.
- Normalize rates to the maximum observed rate to compute relative activity (%).
- Identify pH of maximum activity (enzyme optimum) and discuss the shape of the pH-activity profile.
- Perform basic statistics (mean, standard deviation) on triplicates and, if appropriate, an ANOVA to test for significant differences between pH conditions.
- Present results as graphs (reaction curves and pH vs relative activity

bar plot) and a table summarizing initial rates and interpretations.

REFERENCE

1. Nelson, D. L., & Cox, M. M. *Lehninger Principles of Biochemistry*. (For enzyme fundamentals.)
2. Berg, J.M., Tymoczko, J.L., & Stryer, L. *Biochemistry*. (For enzymatic mechanisms and pH effects.)
3. Science Buddies, "How Does pH Affect Enzyme Activity?" (Practical protocol and safety notes).