Amylase: pH and reaction time

Student activity • practical and reasoning

YEAR 10 / LESSON 12 / BIOLOGY + SCIENCE (DOUBLE AWARD)

The question

How does buffer pH affect the time to lose detectable starch?

Compare five buffer pH values at one controlled temperature. Each pair investigates an assigned pH three times, then pools class evidence.

Equipment

- Eye protection; two reaction tubes plus control tubes, rack, 2 cm^3 syringes, 1 cm^3 measure and pipette
- Spotting tile or white well plate, timer and thermometer
- Supplied 1% starch, pilot-checked amylase solution, assigned buffer pH 4–8, dilute iodine and water
- Access to 30 °C thermostatic bath; no flame
- Suitable pH paper or calibrated probe for a spare mixture

Wear eye protection; iodine stains and can irritate eyes. Avoid contact with buffer and enzyme solutions. Hot water and glass can burn: use racks and holders at the warmer baths. Students do not handle enzyme powder or saliva.

Course coverage: Biology only practical: 2.14B. Shared explanation: 2.13.

Plan, model or apparatus sketch

Draw and label the apparatus or biological model; identify one limitation.

Method and reasoning

Read the whole sequence before starting

YEAR 10 / LESSON 12 / BIOLOGY + SCIENCE (DOUBLE AWARD)

1. Wear eye protection. Work at the assigned buffer pH 4, 5, 6, 7 or 8, keeping all tubes in a 30 °C bath. Record actual temperature and buffer identity. Prepare starch-positive and water-negative iodine references.

2. Measure 2 cm^3 starch into one tube. In another combine 2 cm^3 supplied amylase and 1 cm^3 of the assigned buffer. Equilibrate both for five minutes at 30 °C; do not add indicator to the enzyme reaction.

3. Combine the solutions, mix gently and start the timer immediately. Keep the reaction tube in the bath. Use a clean pipette to add one drop to a fresh iodine well every 20 seconds.

4. Record the last blue-black sample time and the first sample that retains the negative-reference colour. Never return iodine to the reaction tube. Stop at 300 s if no endpoint occurs and write >300 s.

5. Repeat twice with fresh solutions. Run a no-enzyme control with 2 cm^3 water replacing amylase. Keep temperature, volumes, stocks and sampling constant. Check a separate spare mixture with suitable pH paper or a probe; record its pH without contaminating the reaction.

6. Calculate 1/t in s^{-1} for completed runs and plot activity against mixture pH. Keep >300 s observations as bounds, not zero rates. Compare repeat spread and identify the highest sampled activity without claiming an exact optimum.

Cleanup

- Collect iodine and buffer mixtures as directed by the school’s disposal instructions.
- Rinse equipment promptly, return it safely, clean the bench and wash hands.

Record your evidence

Blank spaces are for your own work

YEAR 10 / LESSON 12 / BIOLOGY + SCIENCE (DOUBLE AWARD)

Reference and task data

Record your own times, including last-positive and first-negative bounds. Example calculation only: an endpoint at 100 s gives 1/t = 0.010 s^{-1}. A result >300 s gives an upper bound below about 0.0033 s^{-1}; do not enter 300 as an exact time or plot a zero rate. Use the same enzyme batch throughout; its optimum depends on its source.

Trial	Mixture pH	Last + / s	First – / s	1/t / s^{-1}
1				
2				
3				
No enzyme				

Show your calculations, labelled model or correction here.

Explain and evaluate

Use evidence and show calculation steps where relevant

YEAR 10 / LESSON 12 / BIOLOGY + SCIENCE (DOUBLE AWARD)

1. Identify the independent and dependent variables.

2. Why hold temperature constant?

3. Calculate 1/t for an endpoint of 125 s.

4. How should >300 s be recorded?

5. Why check a spare mixture rather than put indicator into the reaction?

6. How could you find an optimum more precisely?