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The enzyme amylase breaks down starch into simple sugars. A student investigated the effect of pH on the activity of amylase. To do this, the student varied the pH by using different pH buffer solutions and added each buffer to equal volumes of amylase and starch solutions. Every 30 seconds a drop of the mixture was tested for starch using iodine that had been dropped into wells in a dimple tray. Iodine turns blue/black if starch is still present. The time taken for the iodine to no longer turn blue/black was recorded for each pH tested. The results can be seen below:

pH Buffer used	Time taken for iodine to no-longer turn blue/black (s)
4	120
6	60
8	150
10	Always turned blue/black

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State the pH at which the rate of reaction was greatest. Explain your answer

[2 marks]

pH 6 [1]

Starch broken down the fastest/shortest time for iodine to stop reacting [1]

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State the pH where amylase may have been denatured

[1 mark]

pH 10

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In order to ensure this was a fair test, the student needed to control the temperature. Suggest how she may have done this

[1 mark]

Waterbath/use different ratios of hot to cold water and maintain the temperature [1]

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Give **two** other variables that should be controlled in this experiment

[1 mark]

1. volume of starch/amylase [1], time to test the samples [1]

2. concentration of starch/amylase [1] same number of drops of iodine [1]

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Suggest how the student could improve the reliability of their investigation

[3 marks]

repeat the test [1]

calculate the average (time) for each repeat [1]

discarding/ignoring any anomalies [1]

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In a separate experiment, another student investigated the effect of temperature on the action of amylase.

Before he began the experiment, the student gave the following hypothesis:

“The higher the temperature, the faster the activity of amylase”

The hypothesis was only partially correct. Explain why

[2 marks]

amylase/enzyme activity slows/stops after the optimum temperature [1]

amylase enzyme is denatured [1]

the active site has changed shape/substrate or starch no longer fits the active site [1]