DESIGN AND DECONSTRUCT: STAGE 2 BIOLOGY – STUDENT B

DECONSTRUCT

Question: "What factors affect enzyme activities in washing detergents?"

Why are enzymes present in washing detergents?

Enzymes with active sites complimentary to the shape of the stain molecules are used in washing detergent to break down stains in fabric. Each enzyme has specificity for one type of reaction, meaning specific enzymes are targeted to specific types of stains. Once stains are broken down into smaller pieces, they are more easily removed.

The type of stain used in my experiment will affect which type of enzyme is required to break it down.

What enzymes are present in washing detergents?

Enzyme	Breaks down	Safety	Cost (per 100mL)	Availability
Protease	Proteins	Causes allergy or asthma symptoms or breathing difficulties if inhaled. Causes serious eye irritation.	$34	National Centre of Biotechnology Education
Lipase	Fats and oils	Causes allergy or asthma symptoms or breathing difficulties if inhaled.	$34	National Centre of Biotechnology Education[1]
Amylase	Starches	Causes allergy or asthma symptoms or breathing difficulties if inhaled.	$34	National Centre of Biotechnology Education[2]
Cellulase	Cotton fibres on surface of fabric, releasing substance	Causes allergy symptoms, prolonged contact causes minor irritation in skin and eyes.	$29	ENZYMES.BIO Industry Group Co., Ltd

All of these enzymes have a similar expense and availability and have been found to have a similar risk level. None of these enzymes seemed to be significantly superior for use in this experiment, so I decided to choose which type of stain, and then decide which enzyme type to use depending on what it requires.

What types of stains are common?

Stain type	Pigmentation level	Difficulty of quantity control	Difficulty of removal	Enzyme used to remove
Blood	High	Difficult	Difficult	Protease
Red Wine	High	Easy	Difficult	Protease
Grease	Low	Easy	Difficult	Lipase
Coffee	Medium	Easy	Easy	Protease
Grass	Low	Difficult	Difficult	Amylase

My requirements for the stain were:

- Highly pigmented – so degree of removal is easy to determine
- Amount easily controlled – ensure consistency over all trials
- Difficult to remove – ensures some stain will be left in the final measurement. If all stain washed completely off in every trial, no useful data would be obtained.

Highlighting of the table allowed me to view whether these requirements were met, showing that red wine is most suited as it fulfills all criteria. Protease enzymes will be required to remove the red wine stains.

What factors affect enzyme activity?

Temperature of water: when the water temperature is too low, low kinetic energy will produce less collisions, but if temperature is too high, enzymes will denature.

pH of water: when the water is not at the enzyme's optimum pH, they will denature.

Concentration of enzymes: when concentration of enzymes is increased, the rate of reaction will also increase as more reactions are able to occur.

Frequency of collisions: when the particles are stirred, they will collide more often. Not all collisions create successful reactions, so increased the number of collisions will increase the number of successful collisions,

[1] University of Reading, *Lipase (Lipex)*, accessed 25th June 2022, https://www.ncbe.reading.ac.uk/lipase-lipex/

[2] University of Reading, α-amylase- Stainzyme, accessed 25th June, <https://www.ncbe.reading.ac.uk/%ce%b1-amylase/>.

increasing the rate of reaction. In order to simulate a washing machine, I should mix the water. I will use a shaking incubator to consistently do this.[3]

Concentration of substrates: when concentration of substrates is increased, the rate of reaction will also increase as there are more substrates for enzymes to bind to.

I will focus on the effect of water temperature on enzyme activity in washing detergent as I expect it to have a noticeable effect on enzyme activity, and it can easily be controlled. I would like to discover the optimum temperature for the specific enzyme I choose demonstrated through the findings of this experiment.

What type of fabric will work best to experiment on?

Cotton: easily stained, and relatively easy to remove

Silk: easily stained, and difficult to remove – cannot be washed using a washing machine

Polyester: not easily stained due to synthetic fibres, very difficult to remove once stained

I decided that cotton was most suited to this experiment as it stains very well, stains are able to be removed to some extent, and it is tough enough to withstand a washing machine.

How can stain removal be measured quantitatively?

- Comparison of stain colour to a scale to determine estimated average. Unreliable due to human error.
- Spectrophotometer. Reliable and accurate due to highly precise calibration. This tool is more suited to the experiment as it will obtain high quality data.

Using a spectrophotometer, a quantifiable colour measurement of the stained fabric can be obtained.[7] Although these are expensive, access to a spectrophotometer would greatly increase the accuracy and reliability of results as it eliminates the risk of human error. Single-beam spectrophotometers measure the transmittance and absorbance properties of a material as a function of a wavelength, determining the concentration using this measurement. They produce a percentage of transmittance, allowing the colour difference between the swatch before and after to be measured quantitatively. This prevents factors such as difference in human colour perception and lighting from affecting the accuracy of results.

What type of red wine will work best to experiment on?

- Pinot Noir – light stain, inexpensive
- Gamay – light stain, inexpensive
- Cahors – very dark stain, expensive and rare
- Merlot – dark stain, inexpensive

Although any red wine would work, Merlot is most suited to the experiment as it is highly pigmented, and readily available in Australia.

How will I control the temperature of the water?

Lab incubators allow temperatures between 15°C and 75°C to be set and maintained. Using a lab incubator would guarantee consistent and accurate temperature throughout the entire cycle.

DESIGN

Question: "How does water temperature affect the rate of protease enzymes in laundry detergents when removing red wine stains from cotton?"

VARIBLES: Independent variable: the temperature of the solution (°C) that the fabric is soaked in to remove the stain, measured using a thermometer and regulated using an incubator. The temperatures measured will be 10, 25, 40, 55, and 70°C as the enzymes typically function optimally at temperatures between 20 and 60°C. These temperatures provide a wide range, going 10°C above and below the usual optimum temperatures in order to observe the effect of low kinetic energy and denaturation on enzyme activity in laundry detergents.

Dependent variable: the rate of enzyme activity (µmole/min/mg) will be measured using the spectrophotometer, which provides a percentage of stain removed. The percentage of the initial and final colour will be measured, providing qualitative data when these results are compared.

Controlled variables:

pH of solution (pH): a suboptimum pH can cause the denaturation of enzymes, decreasing the rate of reaction. This must be kept constant to obtain accurate results. For this reason, I will use distilled water.

volume of solution (mL): this must be kept constant to ensure accuracy as the volume of solution affects the concentration of particles, hence affecting the number of collisions and rate of reaction.

volume of enzymes (g): a consistent amount of protease must be used as a higher volume of enzymes would increase the rate of reaction, affecting the results' accuracy.

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[3] Labwit Scientific, 'Shaking Incubators', accessed 2nd July 2022, <http://www.labwit.com.au/shaking_incubators.html>.

type of fabric: the fabric type affects how easily the stain is removed, so keeping this consistent will improve the results accuracy and reliability.
Type of enzymes: the purpose of the experiment is to determine the effect of temperature on a particular type of enzyme; therefore, it must stay consistent
Type of stain: the type of stain must stay consistent to ensure its difficulty of removal stays consistent to accurately measure the enzyme's activity.
Amount of stain (mL): this must be kept equal to ensure its difficulty of removal stays consistent. This will be controlled by leaving the fabric swatches in the beakers with consistent volumes of wine for the same length of time.
Time of reaction (s): this must stay consistent as the longer an enzyme has to complete the reaction, the more stain will be removed.
Amount of protein in wine (g/mL): the amount of protein present in the red wine stain will impact the difficultly in removing the stain. This will be controlled by taking each sample of wine from the same bottle.
Frequency of collisions due to stirring: this affects enzyme activity, so it must be kept consistent to ensure accuracy. I will control this by using a shaking incubator – this will produce consistent mixing, simulating the environment of a washing machine.

Uncontrolled variables:

Protease inhibitors: other substances used in the experiment may contain inhibitors of protease enzymes, slowing the rate of reaction. Due to my lack of knowledge regarding whether the substances used contain inhibitors, this cannot be controlled.

AIM: to investigate the optimum working temperature of the enzyme protease, which is determined by measuring the effectiveness of protease enzymes in removing stains in different environments of temperatures.

HYPOTHESIS: I hypothesise that the optimum temperature will be around 40^{O}C as the particles will have enough kinetic energy for plentiful collisions, but enzymes will not become denatured because the temperature is too high.

SAFETY CONSIDERATIONS: I will need to take care around glassware to ensure no breakages occur, as they can be dangerous. If breakages do occur, a teacher should immediately be notified. I will also need to be cautionary around the concentrated protease powder as they can be harmful, as explained above.

MATERIALS:

- Cotton t-shirt. Commonly worn and will stain easily.
- Ruler. This ensures swatches are consistent sizes.
- Fabric scissors. These will allow clean, precise cuts.
- 5x 500mL beaker. Their large size will allow enough space for the fabric to lay flat.
- 50mL merlot red wine. The brand will not affect the accuracy of the results as long as it is kept consistent.
- 100mL measuring cylinder. This ensures accurate measurements.
- 10mL pipette. Allows easy control when pouring the red wine.
- Spectrophotometer. This produces an accurate and valid measurement.
- 5x 250mL beakers. These are large enough to prevent spillages when stirring the solution.
- 500mL distilled water. A neutral pH ensures enzymes are not denatured by the water's pH, slowing the rate of reaction.
- 5x lab incubators. These provide consistent temperatures for the reactions to occur at, creating reliable data.
- Electric scales. As long as these are calibrated accurately, this will produce a more accurate measurement.
- 5g protease powder. Enzyme in powder form is easier to control the amount used in the solution and is cheaper than alternatives.
- 5x petri dish. This is convenient when measuring protease powder.
- Tongs. Used as a safety precaution to prevent burns and smashed glassware.
- Marker. Allows me to keep track of beakers and avoid confusion.
- Stopwatch. This will ensure accurate timing, which is an important factor to control.

METHOD:

1. Using the ruler, measure five 5x5cm squares on the t-shirt and cut them out with scissors. This size allows them to fit into the beakers.
2. Lay the five swatches flat at the bottom of separate 500mL beakers. Laying these flat ensures the fabric is consistently stained all over.

3. Measure 50mL of red wine in a measuring cylinder, and, using a pipette, pour 10mL into the centre of each beaker. Let sit for 24 hours, and then, if not already dry, leave until all swatches are dried before completing the experiment. Ensure swatches remain in the beaker for the same length of time to achieve consistent staining. Allowing the swatches to dry ensures the swatches have stained well, ensuring the wine stain is more difficult to remove than if it was still wet. Allowing swatches to dry for a consistent length of time will help regulate the stain's difficulty of removal.
4. Once dry, measure the stain of each swatch using the spectrophotometer. This will obtain a precise initial colour measurement. This measurement will be used later when determining the colour change. Using a spectrophotometer provides more accurate and meaningful data than the other alternatives.
5. Fill five more 100mL beakers with 100mL of distilled water. Place these beakers in the lab incubators and set to temperatures of 15, 30, 45, 60, and 85. Measure the temperature of water in the beakers to ensure the required temperature has been reached before proceeding. The incubators will adjust the water to a certain known temperature, allowing accurate results as the temperature stays consistent. These temperatures are a range of those common in washing machines.
6. Using the electric scales, measure 1g of protease powder into each of the five petri dishes. The powder must be diluted in a solution with a 1:100 ratio, so 1g of powder and 100mL of water will produce this.
7. Remove the water-filled beakers from the incubators, using tongs for the 65 and 75^{O}C incubators. The tongs are a precautionary tool to prevent burns.
8. Add the powder to these beakers and stir until dissolved and a solution is formed. The powder must form a solution to complete the reaction.
9. Pour the solutions into the beakers containing the fabric, ensuring the fabric remains flat. Label each beaker according to the temperature, and place back inside the respective incubators. Set a timer for 60 minutes to allow the reaction to take place. The fabric must lay flat to ensure consistent surface area for reactions over each trial, ensuring reliability.
10. Once the time is done, remove the fabric from the beaker, rinse with tap water, and dry. Rinsing and drying will allow the swatch to become its true colour when measured.
11. Using the spectrophotometer, measure the final percentage of transmittance of each fabric swatch to determine their colour change. This measurement obtains quantitative data necessary for the experiment's results.
12. Repeat steps 1-11 twice more. Increasing the number of trials increases the accuracy as an average can be obtained.
13. Calculate the percentage of stain removed in each trial using the formula $percentage\ removed = initial\ percentage - final\ percentage$

RECORDING RESULTS:

Temperature (°C)	Percentage of stain removed in Trial 1 (%)	Percentage of stain removed in Trial 2 (%)	Percentage of stain removed in Trial 3 (%)	Average percentage of stain removed (%)
15				
30				
45				
60				
85				

The results can be graphed using the independent variable (water temperature) on the x-axis, and the dependent variable (percentage of stain removed) on the y-axis.