

# Biological washing powders and temperature

Investigate and describe the use of biological washing powders that contain enzymes

For Cambridge IGCSE Biology 0610 (and 0970, which has the same content). Teacher answers start on a new page.

Name .....

Date .....

## Aim

To investigate the effect of temperature on how quickly a biological washing powder removes an egg yolk stain from cloth.

## Background

Biological washing powders contain enzymes that digest stains. **Proteases** break down protein stains (such as blood or egg) into amino acids. **Lipases** break down fat and grease stains into fatty acids and glycerol. Egg yolk contains both protein and fat. The small, soluble products wash out of the clothes.

Biological powders clean well at low temperatures, such as 30 to 40 °C, so less energy is needed to heat the water. At very high temperatures the enzymes are denatured: the shape of the active site changes, the substrate no longer fits, and the powder removes stains no better than a non-biological powder.

In this investigation, the same stain is put on identical pieces of cloth, which are placed in the same biological washing powder solution at different temperatures. The time taken for the stain to disappear measures how fast the enzymes work. Two controls are used: the stain in water only, and the stain in powder solution that has been boiled and cooled so that its enzymes are denatured. The boiled powder still contains detergent, so the stain may fade a little, but it is not removed.

## Hypothesis

The stain will disappear fastest at about 40 °C, because enzymes work best at a suitable temperature; it will be removed more slowly at lower temperatures and at 60 °C because the enzymes work slowly in the cold and are denatured when it is too hot.

## Variables

|                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Independent</b> | Temperature of the washing powder solution (20, 30, 40, 50 and 60 °C)                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Dependent</b>   | The time taken for the stain to disappear (/ s), up to a maximum of 1800 s (30 minutes)                                                                                                                                                                                                                                                                                                                                                                                                     |
| <b>Control</b>     | <ul style="list-style-type: none"><li>Type and mass of biological washing powder (1.0 g in 50 cm<sup>3</sup> of water)</li><li>Volume of solution (50 cm<sup>3</sup>) in each beaker</li><li>Size and type of cloth (white cotton, 5 cm × 5 cm) and number of cloths in each beaker (1)</li><li>Size, amount and age of the stain (5 drops of egg yolk dried for 24 hours)</li><li>Stirring (five stirs every minute)</li><li>pH of the solution (tap water from the same source)</li></ul> |

## Equipment

- Biological washing powder (containing protease and lipase)
- Balance, to measure to 0.1 g

- White cotton cloth cut into 5 cm × 5 cm squares, stained with 5 drops of egg yolk from a dropping pipette, then dried for 24 hours
- 5 beakers (100 cm<sup>3</sup>) for the powder solutions, and 5 thermostatically controlled water baths (or 250 cm<sup>3</sup> beakers of water kept at the right temperature)
- Measuring cylinder (50 cm<sup>3</sup>)
- Thermometers, -10 °C to +110 °C
- Kettle, ice and tap water to set up and adjust the water baths at 20, 30, 40, 50 and 60 °C
- 5 glass rods for stirring
- Forceps
- Stop-clock reading to 1 s
- Unstained white cloth, to compare the colour
- Marker pen or glass-marking pencil for labelling beakers
- Eye protection and gloves
- Powder solution that has been boiled for 5 minutes and cooled (prepared by the teacher)

## Risk assessment

| Hazard                                            | Risk                                                                                             | Precaution                                                                                                                        |
|---------------------------------------------------|--------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| Biological washing powder (enzymes; irritant, MH) | Dust or solution can irritate the skin, eyes and lungs, and enzymes may cause allergic reactions | Wear eye protection and gloves, avoid breathing in the powder, do not shake the container, and wash hands after use.              |
| Hot water                                         | Scalds from water at 60 °C                                                                       | Use a thermometer, do not heat beyond 60 °C, and let spills cool before wiping them up. Do not carry hot beakers across the room. |
| Raw egg yolk                                      | Bacteria such as Salmonella                                                                      | Wash hands after handling the stained cloth, and clean the bench afterwards.                                                      |

## Method

1. Label five 100 cm<sup>3</sup> beakers 20, 30, 40, 50 and 60 °C. Weigh 1.0 g of biological washing powder into each and add 50 cm<sup>3</sup> of water to each. Stir until the powder has dissolved.
2. Set up water baths at 20, 30, 40, 50 and 60 °C. Check each temperature with a thermometer.
3. Stand each labelled beaker of powder solution in the water bath at the same temperature. Wait until a thermometer in the solution shows that temperature.
4. Put a stained cloth square on a white tile and compare the colour with an unstained piece, so you know what 'no stain' looks like.
5. Working with a partner, use forceps to put one stained cloth into each of the five beakers at the same time. Start the stop-clock.
6. Stir each solution five times every minute, using a separate glass rod for each beaker.
7. Look at each cloth every 30 seconds and compare it with the unstained cloth. Record the time when the yellow stain can no longer be seen. If the stain is still visible after 1800 s (30 minutes), record '> 1800'.
8. During the test, check the water bath temperatures and add hot or cold water if needed.
9. Repeat the whole investigation a second time at each temperature, using fresh stained cloths and fresh solution.
10. Control 1: at the same time as a 40 °C test, put a stained cloth into 50 cm<sup>3</sup> of water only at 40 °C and test it in the same way.

- L1 . Control 2: warm 50 cm<sup>3</sup> of the boiled and cooled powder solution to 40 °C, put a stained cloth into it and test it in the same way.
- L2 . Calculate the mean time at each temperature. Wash your hands and clean the bench.

## Results

| Results table: time for the egg yolk stain to disappear |                                         |                                         |               |
|---------------------------------------------------------|-----------------------------------------|-----------------------------------------|---------------|
| temperature of solution / °C                            | time for stain to disappear, test 1 / s | time for stain to disappear, test 2 / s | mean time / s |
| 20                                                      |                                         |                                         |               |
| 30                                                      |                                         |                                         |               |
| 40                                                      |                                         |                                         |               |
| 50                                                      |                                         |                                         |               |
| 60                                                      |                                         |                                         |               |
| 40 (water only, no powder)                              |                                         |                                         |               |
| 40 (powder solution boiled and cooled first)            |                                         |                                         |               |

## Graph

Line graph of mean time for stain to disappear / s on the y-axis (for example 400 to 1400, 2 cm for 200 s; the axis does not have to start at 0) against temperature of solution / °C on the x-axis (20 to 60, 2 cm for 10 °C). Plot each point as a cross (×) and draw a smooth curve of best fit. The curve will have a minimum (the fastest removal) near 40 °C. Do not plot the two control results.



**Exam questions** 10 questions · 26 marks

**1** State the independent variable and the dependent variable in this investigation. **[2 marks]**

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**2** State three variables that must be kept the same in each test so that it is a fair test. **[3 marks]**

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**3** A stained cloth was put in powder solution that had been boiled and then cooled to 40 °C. The stain faded slightly but was not removed. Explain this result. **[2 marks]**

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**4** At 50 °C the times taken for the stain to disappear were 600 s and 660 s. Calculate the mean time. **[2 marks]**

Answer = .....

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- 5** The table shows the mean time for the stain to disappear at different temperatures. State the temperature at which the powder worked best, give a reason, and say what this shows about the enzymes in the powder. **[3 marks]**

| Mean time for the stain to disappear |               |
|--------------------------------------|---------------|
| temperature of solution / °C         | mean time / s |
| 20                                   | 1170          |
| 30                                   | 750           |
| 40                                   | 465           |
| 50                                   | 630           |
| 60                                   | 1380          |

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- 6** Explain the results at 20 °C and at 60 °C in the table in question 5. **[3 marks]**

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- 7** Egg yolk contains protein and fat. Name the type of enzyme that digests each of these and state the products of digestion of the protein. **[3 marks]**

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- 8** The student judged the end-point by eye. Explain one problem with this, and suggest how it could be reduced. **[2 marks]**

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**9** Give two advantages of using a biological washing powder, rather than a non-biological powder, to clean clothes with food stains. **[2 marks]**

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**10** Describe how you could use a similar method to find the effect of pH on the action of a biological washing powder. **[4 marks]**

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## Teacher answers

Practical skill 17: Biological washing powders and temperature. The example results are practice data, not real measurements.

### Example results

| Example results (practice data)              |                                         |                                         |               |
|----------------------------------------------|-----------------------------------------|-----------------------------------------|---------------|
| temperature of solution / °C                 | time for stain to disappear, test 1 / s | time for stain to disappear, test 2 / s | mean time / s |
| 20                                           | 1140                                    | 1200                                    | 1170          |
| 30                                           | 720                                     | 780                                     | 750           |
| 40                                           | 450                                     | 480                                     | 465           |
| 50                                           | 600                                     | 660                                     | 630           |
| 60                                           | 1320                                    | 1440                                    | 1380          |
| 40 (water only, no powder)                   | > 1800                                  | > 1800                                  | > 1800        |
| 40 (powder solution boiled and cooled first) | > 1800                                  | > 1800                                  | > 1800        |

### Conclusion

In the example results the stain disappeared fastest at 40 °C (mean 465 s) and more slowly at lower and higher temperatures (1170 s at 20 °C and 1380 s at 60 °C), so the optimum temperature for the powder was about 40 °C. At low temperatures the enzyme and substrate molecules have less kinetic energy, so there are fewer collisions and the reaction is slow. At high temperatures the enzymes begin to be denatured, so the stain is removed more slowly. The stain was not removed by water alone or by boiled powder solution within 1800 s. The stain in the boiled powder faded slightly, because the detergent in the powder still works, but it was not removed, so the removal of the stain was caused by active enzymes in the powder, and not by the water, the detergent or the stirring. This supports the hypothesis and explains why biological powders are used at around 30 to 40 °C, which also saves energy.

## Errors and improvements

| Error                                                                                                                                 | Effect on the results                                                                  | Improvement                                                                                                                                                                   |
|---------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Judging when the stain has 'disappeared' depends on the person's eye (random error)                                                   | The times are not very precise, and different people could record different end-points | Agree a clear end-point beforehand, compare with an unstained cloth, and have the same person judge every time. Use a colorimeter or compare with a colour chart if possible. |
| The solution cools down (or warms up) during the test, which can last up to 30 minutes, especially at 20 and 60 °C (systematic error) | The temperature is not the one stated, so the results may be misleading                | Keep the beaker in the water bath during the test, check the temperature with a thermometer and top up with hot or cold water as needed.                                      |
| Stains on different pieces of cloth differ in size or thickness                                                                       | A thicker stain takes longer to remove, which is not due to temperature                | Use a dropping pipette to put the same number of drops on each piece, spread to the same area, and leave the cloths to dry for the same time.                                 |
| Only two tests are done at each temperature                                                                                           | An anomalous result has a big effect on the mean, so the optimum may not be correct    | Repeat at least three times at each temperature, ignore any anomalous results, and test more temperatures near 40 °C (for example 35 and 45 °C).                              |

## Mark scheme

One mark for each point unless a maximum is given. Words in brackets are not needed for the mark.

### 1. [2]

- independent variable: temperature (of the solution) (1)
- dependent variable: time taken for the stain to disappear (1)

### 2. [3]

- mass / type of washing powder (1)
- volume of the solution / volume of water (1)
- size, type of cloth / size of stain / amount of egg yolk (1)
- stirring / pH / time the stain was left to dry (1)
- Max 3

### 3. [2]

- boiling denatured the enzymes (1)
- so the enzymes could no longer break down the stain / the shape of the active site changed so the substrate no longer fits (1)
- the detergent in the powder removed a little of the stain (1)
- Max 2

### 4. [2]

- $(600 + 660) \div 2$  (1)
- 630 (s) (1)
- correct answer with no working scores 2

**5. [3]**

- 40 °C (1)
- because the stain disappeared in the shortest time / fastest (1)
- so enzymes (protease and lipase) work best at about this temperature (1)

**6. [3]**

- at 20 °C the enzymes work slowly because the molecules have less kinetic energy / fewer collisions between enzyme and substrate (1)
- at 60 °C the enzymes are denatured (1)
- the shape of the active site changes so the substrate no longer fits / fewer enzymes work (1)

**7. [3]**

- protein: protease (1)
- fat: lipase (1)
- products of protein digestion: amino acids (1)

**8. [2]**

- it is subjective / different people may judge the end-point differently, so the times are not accurate (1)
- compare with an unstained cloth / agree a clear end-point / use the same person each time (1)

**9. [2]**

- works at lower temperatures (1)
- so less energy is used / saves money (1)
- enzymes break down protein and fat stains into small soluble molecules that wash out (1)
- Max 2

**10. [4]**

- use solutions of the powder at different pH values, for example by adding buffer solutions, acid or alkali (1)
- keep the temperature (at the optimum, e.g. 40 °C) and the other variables the same (1)
- put a stained cloth in each solution and time how long the stain takes to disappear (1)
- repeat and calculate a mean for each pH (1)
- the pH with the shortest time is the optimum (1)
- Max 4

**Exam tips**

- Name the independent variable (temperature) and the dependent variable (time for the stain to disappear), and say what is kept the same.
- A boiled-powder control shows the enzymes cause the stain removal. Say that boiling denatures the enzymes, and mention the active site.
- Do not say enzymes are 'killed' at high temperature. They are denatured because the shape of the active site changes.
- Give the unit in the table heading with a slash, for example 'time / s', and do not put units in the body of the table.
- Link the enzyme to the stain: protease digests protein stains (to amino acids), lipase digests fat stains (to fatty acids and glycerol).