

## Investigating the use of biological washing powders that contain enzymes

## Transcript

Shortly after enzymes were discovered in the early twentieth century, scientists began to consider their practical uses. One of the earliest industrial applications of enzymes is in biological washing powders.

Washing powders contain a range of enzymes, including amylase, proteases and lipases. These help to break down some of the molecules found in blood, food and plant-based stains. Enzymes are proteins that catalyse biological reactions without themselves being changed during the process.

All enzymes have an active site. This has a complementary shape to that of a specific substrate, to which it is able to bind. The formation of an enzyme-substrate complex is crucial to the breakdown of the substrate into products.

This investigation compares the effectiveness of washing powders by measuring their ability to break down starch, protein and oil.

Agar plates are used in this experiment. These can be prepared by heating a solid agar powder in water, and allowing the mixture to cool slightly before pouring into Petri dishes.

For the purposes of this investigation, three pairs of agar plates are prepared. This will make it possible for two different washing powder solutions, which are named brand A and brand B, to be tested. Each agar pair contains an additional substance. One pair contains milk powder, which contains a white protein called casein. The second pair contains starch powder and iodine solution to show the presence of starch. This plate is blue-black in colour, and the final pair contains vegetable oil, and a small volume of alkali called sodium hydrogen carbonate, and universal indicator solution. Because of its extra liquid content, this plate will contain agar that has a noticeably different consistency from the other two.

Cutting small wells into the plates will provide a space into which the two different washing powders can be placed. For this purpose, a cork borer is used. However, to ensure it is aseptic before making contact with the agar, it is first passed through a Bunsen burner flame. Four wells are made for each washing powder. One of these, in the centre, will act as the control, and is labelled with a 'C.' The other three wells will allow for the collection of repeats.

The control washing powder solutions are prepared by heating a small volume in a test tube placed in a water bath at 95°C for at least five minutes. The high temperature denatures enzymes in the washing powder solutions, which removes the effect of the independent variable in this investigation. A denatured enzyme is unable to catalyse the breakdown of a substrate into products, because its active site has changed shape. This prevents the formation of enzyme-substrate complexes.

A dropping pipette is used to add the washing powder solutions to three of the four wells on each side of the plates. Care is taken to ensure that the volume of washing powder added to the wells is just enough to fill them, without spilling over the sides. This is repeated twice more for each plate, using the same washing powder solution. It is important to ensure that a different pipette is used to transfer the second washing powder solution, to avoid cross-contamination.

Finally, a sample of each of the boiled washing powder solutions is placed into the control wells on each plate, which have been labelled 'C.'

After the washing powder solutions have been put into every well, the plates are then placed into a tray, and then stored in a place where they will not be disturbed. After allowing enough time for the plates to incubate, remove them from the tray.

The results should show that halos, or rings, of colour change have taken place around some of the wells containing the washing powder solutions. These regions are called the zone of digestion. To investigate the effectiveness of the two types of washing powder, the diameter of the zone of digestion around the well will be measured.

For the starch-agar plate, iodine solution has been added. As the starch is gradually broken down by the amylase in the washing powder to simple sugars, the blue-black colour will become colourless.

For the protein-agar plate, the white milk protein casein, is digested by the proteases in the washing powder to form amino acids. This decolourises the milk.

As the oil is broken down into fatty acids and glycerol by the lipases in the washing powder, the fatty acids neutralise the sodium hydrogen carbonate. This causes the indicator to decolourise.

The zone of digestion is proportional to the distance from the centre of the well to the outer limit of the circle that has changed colour, or that has been decolourised.

Firstly, for the starch-agar plate A, measure the diameter of each of the zones of digestion using a rule, and record your results. Then repeat with plate B. Next, measure and record the zones of digestion on the protein-agar plates. Finally, measure and record the zones of digestion on the oil-agar plates.

As would be expected, the control wells show no zone of digestion. This is because the enzymes in the washing powder have been denatured by boiling.

Because three measurements were taken for each washing powder on each type of plate, a mean for each set of values can be calculated.

The collected data can be used to plot three bar charts that show the mean diameter of the zone of digestion for each washing powder solution. The results show that both washing powders A and B are very similar at digesting starch, protein and oil.

This experiment showed how the enzyme activity of two different biological washing powders can be compared. Biotechnologists can use the results of similar experiments to help optimise the activity of enzymes at lower temperatures. This reduces the energy expenditure of washing machines.