DNA Biotechnology and Enzymes
Unit 2~ Lesson 1
The Biotechnology Industry

Background

Biotechnology is a process (or a technology) that is used to create products like medicines by using micro-organisms, plants and animals. The biotechnology industry has seen some major breakthroughs during the past few decades that have had a major effect on the medicines and technologies used to treat life threatening diseases throughout the world such as Alzheimer’s disease, various cancers, Diabetes, AIDS and Multiple Sclerosis. As well as improving our healthcare systems, biotechnology is used in the production of various foods. Pesticides made using biotechnology are being used to improve our food supply thus making it safer and also reducing the need to use chemical pesticides. Biotechnology applications have led to a cleaner environment as they produce less waste and use less energy and water. DNA fingerprinting is also a process that involves biotechnology which has also dramatically improved criminal investigation and forensic science.

Why is biotechnology so important in the fight against infectious diseases?

- Biotechnology is used to study the genetic material of disease causing bacteria and viruses, known as pathogens and will determine what is causing the disease.
- The information obtained from studying these pathogens will be used to develop tests which will detect and identify the disease.
- These tests are tremendously important, as the quicker a pathogen is detected; the quicker the response will be to develop vaccines and drugs to cure the infection.

Questions:

What factors do you think have led to the rise in the number of infectious diseases in the past few decades?

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How do you think the biotechnology industry has helped to treat these diseases?

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DNA Biotechnology – Enzymes

What is an enzyme? An enzyme is a biological catalyst that is used to speed up reactions in our bodies without being changed at the end of the reaction. Most enzymes are proteins. In these types of reactions, the molecules at the start of the process are known as a substrate and the enzyme will convert the substrates into different molecules known as the products. Each enzyme is extremely specific for their substrate. The reaction can be best described using the “Induced Fit” hypothesis illustrated in the diagram below.

The diagram above demonstrates the following:

- The substrate approaches the enzyme active site.
- The enzyme changes shape as the substrate binds and forms the Enzyme/substrate complex.
- Products are formed as shown in the enzyme/products complex.
- The products leave the active site of the enzyme.

Optimum Conditions for enzyme activity

In order for enzymes to work they need to survive in an environment that suits their optimum requirements. The optimum conditions are as follows:

- **pH** – All enzymes have an optimum pH. Most function between a pH of 6 and 8.
- **Temperature** – Enzymes work within a temperature range that is suited to the particular organism. The optimum temperature for human enzymes is between 35 and 40°C. They will degrade quickly at temperatures higher than this.
- **Salt Concentrations** – Most enzymes cannot tolerate extremely high salt concentrations.
Enzyme Denaturation

As described above, enzymes need to exist in their optimum environments in order for them to function properly. Any alteration in their optimum conditions like an increase or decrease in temperature and pH will lead to denaturation of the enzyme. When an enzyme is denatured, it loses its shape. This is because of the hydrogen bonds breaking within the molecule due to high temperatures or extreme changes in the pH. Once denaturation occurs, the enzyme will not reform their original shape.

Task Time
Extracting the Enzyme Catalase from Liver

Background

As mentioned above, denaturation of an enzyme occurs due to changes in the surrounding environment like changes in pH and temperature. The following experiment demonstrates denaturation of an enzyme using an enzyme called Catalase which is found in liver and hydrogen peroxide (H₂O₂). In this experiment, you will extract the enzyme catalase from fresh liver, use it to break down hydrogen peroxide and test the activity of catalase under different conditions.

The catalase breaks down the hydrogen peroxide to oxygen and water.

The reaction is:

\[ \text{H}_2\text{O}_2 + \text{catalase} \rightarrow \text{H}_2\text{O} + \text{O}_2 + \text{Heat} + \text{Catalase} \]

Note that the catalase is not changed by the reaction and that heat is created meaning this reaction is exothermic.

The liver needs enzymes in order for it to break down substances in the body. In this experiment, catalase will be extracted from the liver and tested under different conditions to demonstrate the effects of changes in the environment on enzyme activity.

Safety Precautions:

- Protective clothing must be worn i.e. gloves, aprons and safety glasses.
- Hydrogen peroxide is corrosive and will bleach your clothes and irritate your eyes so use carefully and with caution.
- Liver may carry salmonella and E.coli so wash hands thoroughly after handling.
Materials you will need:
- Fresh Liver
- Distilled water
- Hydrogen peroxide
- Boiling water
- Salt
- Vinegar
- Baking soda
- A dropper
- A blender
- Test-tubes

Procedure:

1) Cut the liver up into small pieces, about 1-2 centimetres in size.
2) Place the liver into the blender and add an equal volume of water.
3) Blend the liver and water until it becomes a smooth mixture.
4) Keep the liver extract cool.
5) Place some of the liver extract into a test-tube and carefully add some hydrogen peroxide. CAUTION: Hydrogen peroxide is corrosive.
6) The liver extract will start to bubble and fizz. This is due to the catalase breaking down the harmful hydrogen peroxide into oxygen and water. Make a note of the results and use this as your positive control.
7) Take some of the liver extract and place into four separate test-tubes. Label them 1-4.
8) To test the effects of acid, treat test-tube 1 with vinegar before testing. Add a few drops of hydrogen peroxide. Record the results.
9) To test the effects of a base, treat test-tube 2 with the baking soda. Add a few drops of hydrogen peroxide. Record the results.
10) To test the effects of heat, treat test-tube 3 with boiling water and add a few drops of hydrogen peroxide. Record the results.
11) To test the effects of high salt content, treat test-tube 4 with some salt. Again add a few drops of hydrogen peroxide and record the final result.

Results

<table>
<thead>
<tr>
<th>Liver treated with:</th>
<th>Effects Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid</td>
<td></td>
</tr>
<tr>
<td>Baking Soda</td>
<td></td>
</tr>
<tr>
<td>Boiling Water</td>
<td></td>
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<tr>
<td>Salt</td>
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</tbody>
</table>
Discussion:

Discuss your results with your lab partner and teacher. Do you think the experiment was effective in demonstrating the effects of changes in environmental conditions of enzyme activity?

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Questions:

1. What do your results demonstrate about the effects of changes in the environment for enzyme activity?

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2. Another part of this experiment you can try is relighting a glowing splint. By placing a glowing splint in one of the test-tubes after the addition of hydrogen peroxide the splint will relight. Why do you think this is?

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3. What is hydrogen peroxide changed into when reacted with catalase?

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4. Is catalase changed during the reaction? Explain your answer.

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Lesson 2~ Digestive Enzymes

Background

Digestive enzymes are responsible for the breakdown of food in our bodies. Different enzymes with different functions are produced within different areas of the digestive tract. These enzymes work to break down the complex substances into simpler ones so that they can be absorbed. For example protease enzymes are secreted in the stomach and pancreas and digest proteins. Proteins consist of long chains of amino acids and protease enzyme cuts them up into peptides, smaller chains of amino acids, and eventually to individual amino acids which can be absorbed in the small intestine. Many types of fruit and vegetables contain essential digestive enzymes. These enzymes are important as they work in harmony with our own body’s enzymes to assist in the breakdown of food and other processes.

An example of one of these enzymes is bromelain found in pineapples. Bromelain is a proteolytic enzyme i.e. it breaks down proteins.

It also helps to break down blood clots by digesting the blood clotting protein fibrin. As well as thinning our blood, bromelain helps to break down mucus in our bodies.

Task Time
The Pineapple and Jelly Experiment

Background

Have you ever wondered why jelly manufacturers warn you against putting pineapple or Kiwi fruit into the jelly before it sets? As described above, pineapple contains the proteolytic enzyme bromelain which will breakdown and digest proteins. This experiment will investigate the proteolytic properties of bromelain using jelly. Jelly is made up of gelatin, a protein extracted from animal tissue.

Safety precautions

- This experiment involves pouring boiling water, be careful not to scald yourself!

Materials you will need:

- Fresh pineapple
- Jelly
- Boiling water
- Two containers
Procedure:

1) Make the jelly according to the instructions on the packet. Pour half into one container and the other half into the second container.
2) Cut up the fresh pineapple. Add some of the pineapple to the first container and none to the second.
3) Leave both jellies to set, checking on them every so often to see what is happening.
4) Examine both jellies after they have been left to set for a while. What do you notice about them?

Discussion:

Discuss the results with your teacher and lab partner and answer the following questions.

Questions:

What results did you observe for the jelly that contained the pineapple?

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What effect did the pineapple have on the jelly?

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