**ACTIVITY: Fermentation**

**Activity idea**

In this activity, students set up an alcoholic fermentation, prepare and view a slide of bacteria responsible for monolactate fermentation and answer questions about a video clip on gut bacteria fermentation.

By the end of this activity, students should be able to:

* explain the meaning of the terms ‘aerobic’ and ‘anaerobic’
* describe the bacterial fermentation process used in yoghurt production
* describe the yeast (fungal) fermentation process used in wine making
* describe the bacterial fermentation process that occurs in the part of the human gut known as the large intestine
* demonstrate a basic understanding of some of the gut bacteria research being conducted by scientists at Plant & Food Research.

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**Introduction/background**

Fermentation is a process by which the living cell is able to obtain energy through the breakdown of glucose and other simple sugar molecules without requiring oxygen. It is an anaerobic (without oxygen) process.

Within the cell cytoplasm, glucose or other simple sugars are broken down in a series of steps to produce the 3-carbon compound known as pyruvate. During this process, some of the energy locked up in the sugar molecules is transferred to ATP molecules, the energy currency of the cell.

The pyruvate can then undergo further breakdown to produce lactic acid (monolactate fermentation), ethanol (alcoholic fermentation) or short-chain fatty acids (saccharolytic fermentation). It is the type of cell that determines which of these fermentation processes occurs, for example:

* human muscle cell – lactic acid
* yeast cell – ethanol
* gut bacterial cell – short-chain fatty acids such as acetate, propionate and butyrate.

If the human muscle cell is operating with oxygen freely available (aerobic conditions), the pyruvate is transferred to the cell mitochondria and converted through a sequence of steps into CO2 and water. The energy locked up in the pyruvate is transferred to ATP molecules as this process takes place. If oxygen is unavailable (anaerobic conditions), pyruvate is converted to lactic acid.

Yeast cells convert the pyruvate to ethanol even although oxygen might be available.

Gut bacteria live in an anaerobic environment and convert the pyruvate into short-chain fatty acids (see [here](https://www.sparknotes.com/biology/cellrespiration/glycolysis/section3/).)

Since the final products of the fermentation process are dependent on the type of microorganisms that carry out the fermenting, this activity alerts students to the fact that there are 3 main types of fermentation. It is a common misconception that ethanol is the main end product of fermentation.

**What you need**

* Glucose (5g per group)
* Active yeast (1g per group)
* Limewater
* Conical flask 100mL
* Measuring cylinder 50mL
* Boiling tube
* Access to a balance
* Warm water 30–40°C
* Cotton wool
* Stopper to fit a boiling tube
* Microscope giving up to x400 magnification
* Microscope slides
* Yoghurt containing live probiotic bacteria
* Bunsen burner
* Access to the video clip [Function of the large intestine](https://www.sciencelearn.org.nz/videos/1096-function-of-the-large-intestine)
* Student worksheet: [Investigating fermentation](#investigating)
* Student worksheet: [Function of the large intestine](#function)

**What to do**

1. Give out copies of the student worksheet [Investigating fermentation](#investigating) and assist students to gather the materials they need and complete the experiment.

1. Discuss the findings:

***Fermentation of glucose using yeast (alcoholic fermentation)***

The fermentation process with yeast yields ethanol and carbon dioxide as products. The carbon dioxide, when passed over and mixed with limewater, causes the limewater to turn milky.

The equation for this reaction is:

CO2(g) + Ca(OH)2(aq) → CaCO3(s) + H2O(l)

carbon dioxide limewater calcium carbonate water

[cloudy/milky solution]

The odour of the glucose/yeast solution when made up is different from the odour after a period of fermentation. The presence of ethanol in the ‘brew’ is responsible for this.

***Viewing fermenting bacteria in yoghurt (monolactate fermentation)***

Compared with a typical animal cell such as an epithelial cell from the lining of the mouth, bacterial cells are exceptionally small. The image on the right indicates this.

The rod-shaped structures are *Lactobacillus acidophilus* bacteria. Here they can be seen against a typical epithelial cell. The largest are about 1/10th of the average diameter of the epithelial cell. Average epithelial cell size is ~25μm.

1. Give out copies of the student worksheet [Function of the large intestine](#function) and have students complete it.
2. Discuss the responses.

**Investigating fermentation**

***Fermentation of glucose using yeast (alcoholic fermentation)***

1. Place 5g of glucose in a conical flask and add 50mL of warm water. Swirl the flask to dissolve the glucose.
2. Add 1g of active yeast to the flask. Gently swirl and then carefully smell the contents before loosely plugging the top of the flask with cotton wool. Note any odour.
3. Set aside for 15–20 minutes in a warm place.
4. Place a 2cm depth of limewater in a boiling tube.
5. Remove the cotton wool plug from the conical flask and carefully pour the invisible gas above the fermenting solution into the boiling tube.
6. Stopper the boiling tube and shake to mix the invisible gas with the limewater. Note what happens.
7. Carefully smell the contents of the flask and compare with the previous odour. Note your observations.

***Viewing fermenting bacteria in yoghurt (monolactate fermentation)***

1. Place a small drop of yoghurt (probiotic) into a clean microscope slide. Add a larger drop of water and carefully mix the yoghurt and water into a thin paste.
2. Using a teat pipette, carefully transfer a small amount of this paste to another clean microscope slide.
3. Evaporate the water from the paste by carefully passing the slide through a Bunsen flame. This process fixes the bacteria to the slide.
4. Allow the slide to cool and place a drop of methylene blue stain over the fixed bacteria. Set aside for several minutes. Rinse the stain off the slide with a small amount of water. Carefully dry the slide without disturbing the bacteria.
5. Place the slide on the microscope stage and view under low power and then high power (x400).
6. Sketch what you see under the high power.

**Function of the large intestine**

View the video clip [Function of the large intestine](https://www.sciencelearn.org.nz/videos/1096-function-of-the-large-intestine) and answer the following questions:

1. Name the main functions of the large intestine.
2. It has been estimated that the number of cells in the human body is a staggering 100 trillion. How does the number of bacteria found in the gut compare with this figure?
3. Why is this huge number of bacteria considered to be an organ in its own right?
4. In a section of the video, Dr Juliet Ansell states: “Gut bacteria are there to ferment undigested material...”
5. What are some of the products of this fermentation process?
6. How are these products utilised by the gut?
7. Why is it that too much protein in the diet can have a negative health impact?
8. Apart from bacteria living in the gut, there is another feature of the gut that is proving to be of great importance in stress-related diseases. What is this feature?

**Function of the large intestine – answers**

View the video clip Function of the large intestine and answer the following questions:

1. Name the main functions of the large intestine.

Compacting what is left of the food, taking water out, fermentation.

1. It has been estimated that the number of cells in the human body is a staggering 100 trillion. How does the number of bacteria found in the gut compare with this figure?

It is estimated that there are 10 times as many bacteria in the gut as there are cells in the human body.

1. Why is this huge number of bacteria considered to be an organ in its own right?

The bacteria are performing metabolic processes such as fermentation, immune response and stimulating the enteric nervous system.

1. In a section of the video, Dr Juliet Ansell states: “Gut bacteria are there to ferment undigested material...”
2. What are some of the products of this fermentation process?

Short-chain fatty acids such as acetate, propionate and butyrate.

1. How are these products utilised by the gut?

The short-chain fatty acids serve as an energy source for the bacterial cells as well as the cells lining the gut.

1. Why is it that too much protein in the diet can have a negative health impact?

Gut bacteria putrefy protein from undigested food. Some of the products of this process such as nitrogenous end products are harmful.

1. Apart from bacteria living in the gut, there is another feature of the gut that is proving to be of great importance in stress-related diseases. What is this feature?

The lining of the gut has a very rich supply of nerve cells. This enteric nervous system has been liked to a ‘second brain’ as it feeds information to the brain as well as receiving messages from the brain. There is more to ‘gut feeling’ than just a turn of phrase.