# Microscale biology: fermentation in a pipette

**At SSERC, we've been developing a suite of experiments on a smaller scale. This article will explore a small-scale fermentation that takes place in the bulb of a pipette. The benefits of microscale experiments have been widely discussed in chemistry [1] and are equally applicable to biology.**

Microscale activities are a source of variety across the practical curriculum, building skills in precision technique. When working with smaller volumes of reagents, there is a reduction in waste and use of materials, resulting in reduced costs.

## A question of sustainability: re-use of plastic pipettes

The microscale biology experiments featured in this article rely on the use of plastic pipettes. While financially inexpensive (approximately 3p each), plastic waste should be minimised where possible. Worley *et al* [1]. explores the reuse of plastic pipettes and how this can be the subject of an investigation for pupils; ultimately, the book concludes that sufficient washing of the pipettes should mean they can be reused rather than thrown away.

## Microscale fermentation

At Levels 4, 5 and 6, the Biology curriculum explores fermentation. The National 5 Biology course specification outlines that fermentation involves the breakdown of a substrate, typically glucose, in the absence of oxygen within the cell cytoplasm. In yeast cells, glucose molecules are broken down to pyruvate, which is then irreversibly converted to ethanol, carbon dioxide and two molecules of ATP (Figure 1).

In the experiment described in this article, this equation can be investigated practically. A yeast suspension is incubated with a sugar substrate within the bulb of a plastic pipette. At SSERC, we have purchased 0.2 cm³ plastic pipettes (from eBay); however, a 1 cm³ plastic pipette can be converted into a micropipette using a simple procedure outlined by RSC Education in Chemistry [2]. The micropipette, containing yeast and sugar within the bulb, is then immersed in a test tube of universal indicator (bicarbonate indicator could also be used). The progress of fermentation is observed in 2 ways:

1) The production of carbon dioxide can be observed as bubbles of gas produced from the end of the micropipette, which can be counted to determine a rate.
2) The production of carbon dioxide changes the colour of the universal indicator from green to yellow because of increasingly acidic conditions. The time taken for this colour change can be measured.

Glucose (substrate) → (Enzymes) Carbon dioxide + Ethanol + energy (products)

**Figure 1** - Fermentation reaction in yeast.

**Materials required (per pair):**

- Test tube rack.
- 2 test tubes containing universal indicator.
- Empty test tube.
- Small volume of paraffin oil.
- Weigh boat.
- 100 cm³ beaker filled with water at 35°C.
- 1x 0.2 cm³ plastic pipette (or a 1 cm³ converted plastic pipette [2]).
- 2 small nuts that fit over the pipette stem.
- Blue roll for any spills.

**Materials shared by class:**

- Beaker of 5% yeast – made fresh; best to leave for around 30 minutes at 35°C to promote active fermentation.
- Beaker of 10% glucose.
- Water bath at 35°C.

**Method:**

An overview of the method can be seen in Figure 2. This has been adapted from a published method by Chan, K. [3].

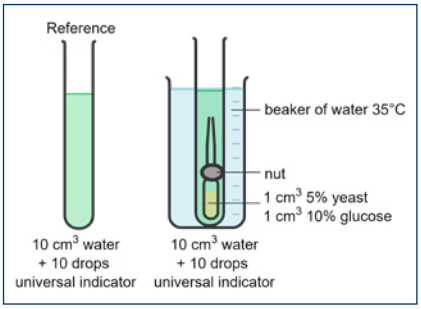


**Figure 2** - Overview of method.

1) Place the 2 test tubes of universal indicator into the beaker of water at 35°C - this acts as a mini-water bath for learners. One of the test tubes serves as a "reference" to show the colour of the indicator in the absence of fermentation. The second will be used in step 5.
2) Combine 1 cm³ 5% yeast and 1 cm³ 10% glucose in the empty test tube and incubate in the beaker of water at 35°C.

>>

3) After 5 minutes, transfer the test tube contents to a weigh boat – this makes it easier to draw up into the small pipette bulb.
4) Draw up the yeast + sugar mixture into the bulb of a 0.2 $cm^3$ plastic pipette. Ensure the mixture fills the bulb but doesn't extend into the stem of the pipette.
5) Place two small nuts over the pipette stem (Figure 3) and then lower the pipette (bulb-side down) into the test tube of universal indicator. The nuts are required to weigh down the pipette – any suitable alternative can be used.
6) Add 2-3 drops of paraffin oil to form a layer on top of the universal indicator, to prevent gas exchange. Return the test tube to the beaker of water at 35°C.
7) Start the stopwatch and record how long it takes for the universal indicator to turn yellow (Figure 4). Compare the indicator colour with the "reference".
8) After 2 minutes for equilibration, count the number of carbon dioxide bubbles produced from the end of the pipette in 1 minute. Record this value in a suitable results table.

**Figure 3** - A 0.2 $cm^3$ plastic pipette with 1 $cm^3$ 5% yeast and 1 $cm^3$ 10% sugar mixture held in the bulb. Two nuts are placed over the stem as weights.

**Figure 4** - The fermentation reaction proceeds within the bulb of the pipette, immersed in universal indicator. A reference tube containing indicator only is used for colour comparison. After 5 minutes, the indicator has changed colour due to production of carbon dioxide. A bubble of carbon dioxide can be observed at the end of the pipette.

## Results

A sample of expected results are shown below. <<

| Glucose concentration (%) | Time taken for indicator to change colour (s) | Number of carbon dioxide bubbles produced/min |
|---|---|---|
| 8 | 385 | 8 |
| 10 | 346 | 12 |
| 20 | 225 | 32 |


**References**

[1] Worley, B. and Paterson, D. (2022), Understanding chemistry through microscale practical work, ASE.
[2] RSC Education in Education (2018), Apparatus and techniques for microscale chemistry, https://edu.rsc.org/resources/apparatus-and-techniques-for-microscale-chemistry/4013407.artice.
[3] Chan, K.H. (2016), A simple microscale setup for investigating yeast fermentation in high school biology classrooms, The American Biology Teacher, 78(8), 669-675.