MICROSCALE FERMENTATION

Supports delivery of N5 Cell Biology KA6

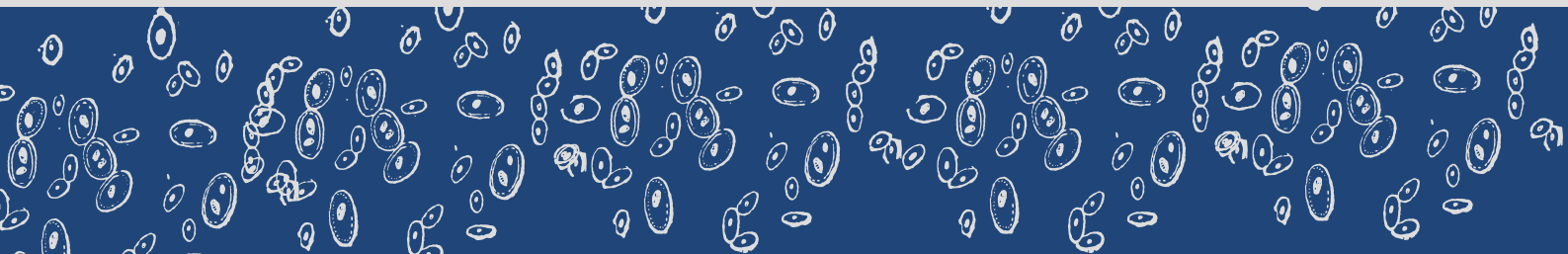
Fermentation involves the breakdown of a substrate, typically glucose, in the absence of oxygen within the cell cytoplasm. In yeast cells, glucose is broken down to pyruvate molecules, which are then irreversibly converted to ethanol and carbon dioxide. The breakdown of each glucose molecule via the fermentation pathway yields only 2 molecules of ATP.

glucose (substrate) → enzymes → **carbon dioxide + ethanol + energy** (product)

In this experiment, a yeast suspension is incubated with a sugar substrate within the bulb of a plastic pipette. The plastic pipette is converted into a micropipette using a simple procedure. The micropipette, containing yeast and sugar, is immersed in universal indicator. The progress of fermentation is observed in 2 ways:

1. the production of carbon dioxide can be observed as bubbles 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 as a result of increasingly acidic conditions.

Using microscale approaches reduces the volume of reagents required, minimising costs and waste. It offers a more sustainable approach to observe fermentation in living organisms.



HEALTH & SAFETY

There are no significant hazards in this experiment. A yeast suspension made from Baker's yeast is made and can be disposed of down the sink as this is not cultured. The yeast suspension must be made fresh.

MATERIALS & METHOD

AIM

To investigate the effect of respiratory substrate on fermentation rate in yeast.

INDEPENDENT VARIABLE

Type of respiratory substrate

DEPENDENT VARIABLE

Fermentation rate, by observing carbon dioxide gas bubbles in 1 minute and by monitoring the change in colour of universal indicator.

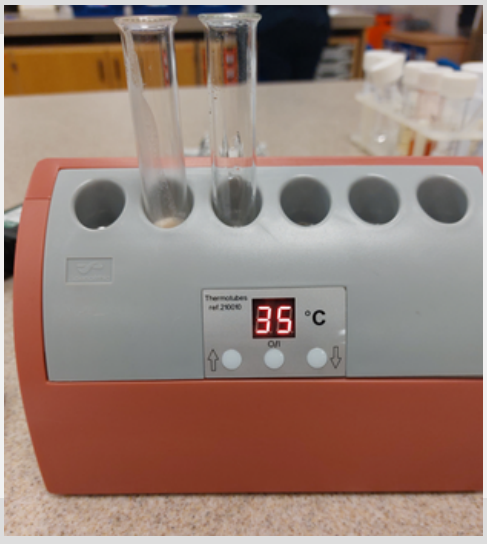
VIDEO TUTORIAL OF THE METHOD

MATERIALS REQUIRED (PER PAIR)

- 10cm³ 15% Baker's yeast suspension
- 5cm³ 10% glucose
- 5cm³ 10% starch
- 5cm³ 10% maltose
- Paraffin oil
- optional: Weigh boats (can be easier to fill the pipette bulb by decanting reagents into weigh boats first)
- Waterbath at 35°C
- Test tubes
- 3x 1cm³ plastic pipettes
- Ice cream tub filled with water ~40°C
- Test tube rack
- Water
- Universal indicator
- 2 small nuts that fit around the pipette - used to weigh the pipette down inside a test tube.
- Blue roll

STEP 1

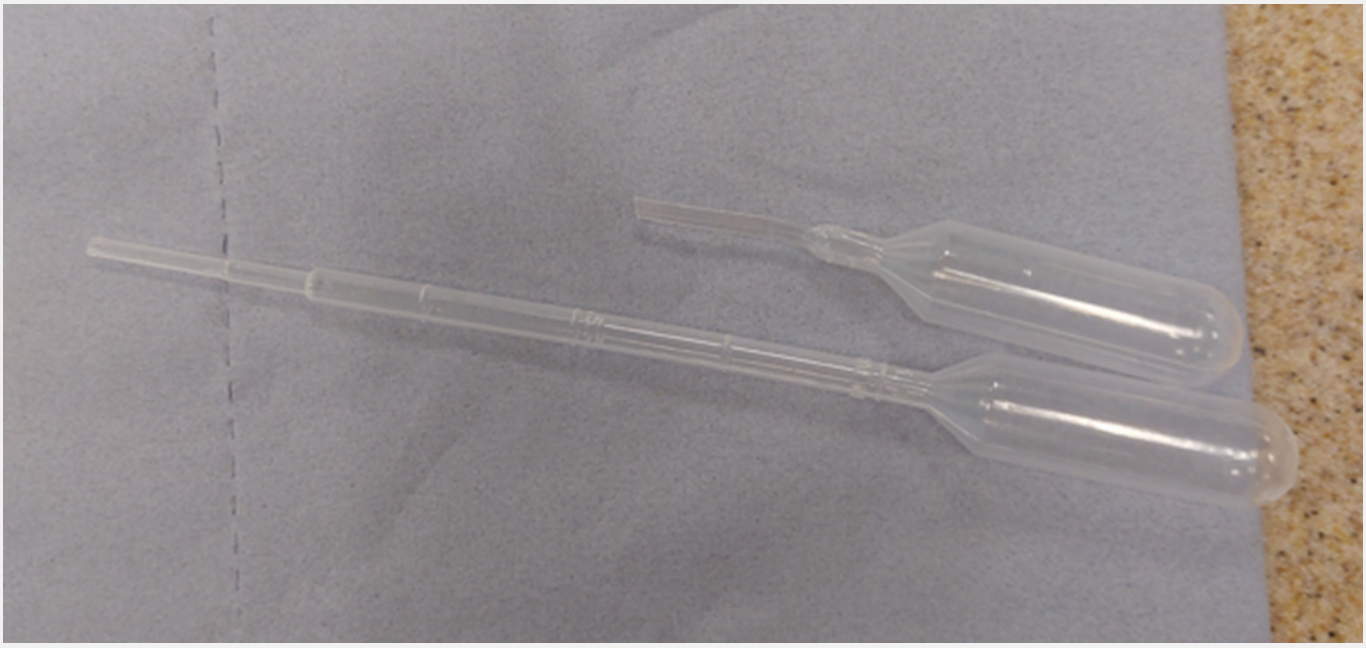
To a test tube, add 2cm³ yeast suspension and 2cm³ 10% glucose solution. Incubate at 35°C.



STEP 2

Make a micropipette from a 1cm ^{3} plastic pipette (make three overall, one for each sugar substrate). An alternative option is to buy 0.2ml plastic mini pipettes.

Instructions for making these are available from the Royal Society of Chemistry website.



STEP 3

Add water at 40°C to an ice cream tub (or similar). Place a test tube rack into the tub. This allows learners to participate in the experiment from their desk instead of moving back and forth to a classroom waterbath.

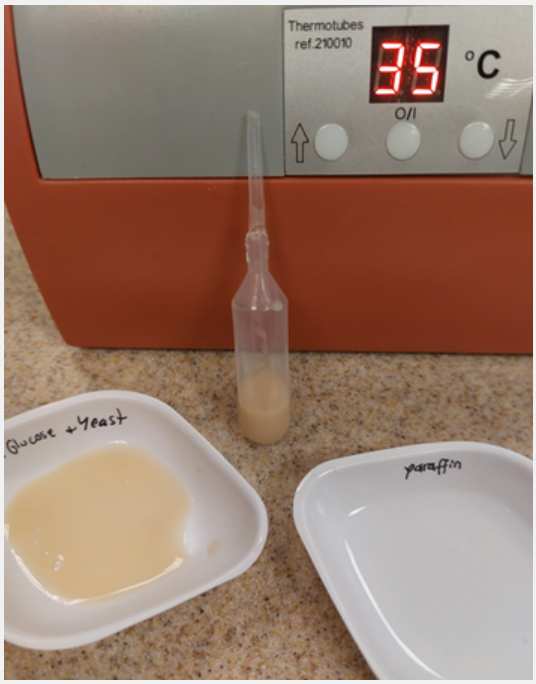
STEP 4

Add 10cm^3 water and 10 drops of universal indicator to 2 test tubes. One of these is a "control" tube for colour comparison; the other can be labelled with the first sugar to be tested, e.g. "glucose".
The important part here is to make sure there is a deep colour of green - the exact volumes are not important as long as the pipette is submerged.

STEP 5

To the micropipette, suck up approximately 1cm^3 of yeast/sugar suspension into the bulb.

This can be easier if some of the suspension is decanted into a weigh boat first.



STEP 6

Place two small nuts over the end of the micropipette to allow it to sink in the test tube of universal indicator.

STEP 7

Place the micropipette into the test tube of universal indicator labelled with the sugar being tested.

STEP 8

Once the micropipette is submerged in the universal indicator, add a small layer of paraffin oil to the top of the indicator solution in the test tube. This will exclude oxygen from the reaction.

Image above: Step 7-8 - Add the micropipette to the test tube of universal indicator and then add a layer of paraffin oil to the top to exclude oxygen and retain the carbon dioxide.

STEP 10

Start the stopwatch and record how long it takes for the universal indicator to change from green to yellow.

This happens due to the production of carbon dioxide, which produces increasingly acidic conditions.

STEP 11

Allow two minutes for equilibration, and then count the number of carbon dioxide gas bubbles produced in one minute.

STEP 12

The process can then be repeated using a yeast suspension mixed with an alternative sugar substrate. Results can be recorded in the table below.

Sugar	Number of bubbles / min	Time for universal indicator to become yellow (s)
Glucose		
Maltose		
Starch		

INDEPENDENT VARIABLES

This microscale assay can be easily adapted to monitor the effect of:

- Concentration or type of yeast (e.g. fresh or dried)
- Concentration of a particular sugar (the image below shows the results obtained, after three minutes incubation, with 8%, 10% and 20% glucose solution.
- Incubation temperature
- Incubation duration

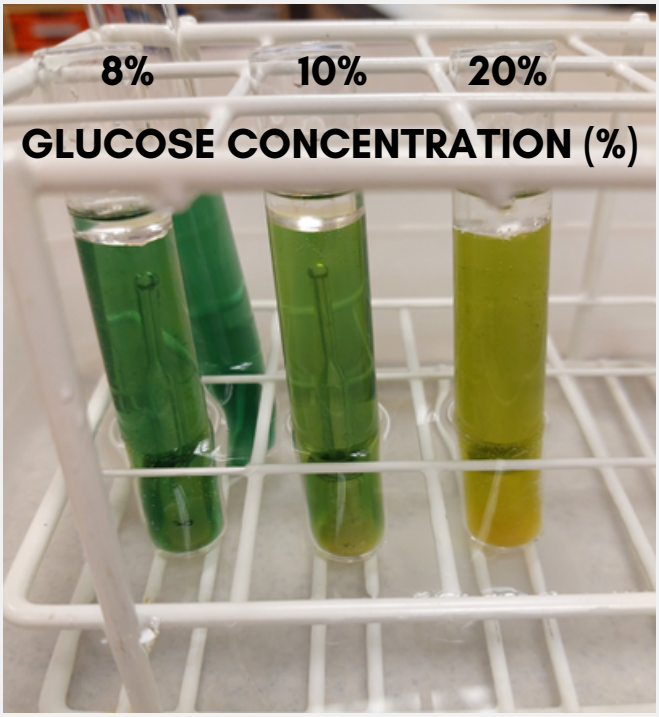


Image opposite: A yeast suspension was mixed with either 8%, 10% or 20% glucose solution and incubated at 35℃ for 3 minutes. This is the colour of universal indicator after 3 minutes.

References: This protocol was adapted from the following source - Chan, K.H. (2016), *A simple microscale setup for investigating yeast fermentation in high school biology classrooms*, The American Biology Teacher, 78 (8), p 669-675.