Measurement of anaerobic respiration in yeast

This involves mixing hydrated yeast with an organic substrate such as glucose, and then sealing the surface of the liquid with oil to prevent air from entering.

Initially the yeast will respire the glucose aerobically using up all dissolved oxygen. The yeast then starts to respire anaerobically. A few drops of Janus Green indicator solution is added to the mixture to indicate when all oxygen has been removed, a blue / green to pink colour change is produced as the dissolved oxygen decreases.

To measure the rate of respiration once anaerobis is reached, the outlet from the apparatus is sealed and the movement of coloured liquid in a manometer is measured as an estimate of the rate of CO_2 production.

In the trial experiment shown below, the manometer fluid consistently moved 1 cm in approximately three and a half minutes. This timing is suitable for a class practical. The construction of the apparatus and the procedure used are shown in the steps below.

Apparatus

Boiling tube attached to glass manometer (construction shown below). Water bath (40 °C).

A 1 m length of borosilicate glass tubing was used in this apparatus, 3 mm inside diameter bore and 1.5 mm wall thickness (Timstar TU 16690/91 6 mm O.D).

- Hydrated baker's yeast (10% w/v suspension, left standing for 15 minutes).
- Glucose solution (1% w/v).
- Janus Green B (Diazine Green) solution, 0.03% w/v aqueous solution (see *Recipe Card* 11).
- Cooking oil.

Some suppliers of Janus Green B (5 grams, September 2009)

Griffin Education:	Janus Green	19168-0050 5 g
Scientific & Chemical Supplies Ltd:	Janus Green	JA005 5 g
Sigma Aldrich:	Janus Green B	201677-5G
VWR:	Janus Green B	1 * 5 g ALFAA17391.06

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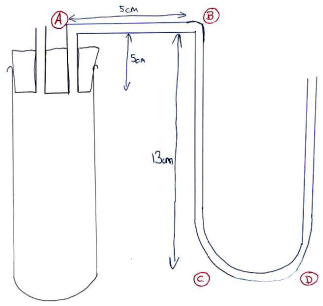
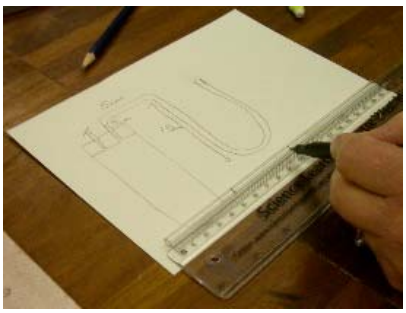
Glass working to make the manometer

Step 1:
Make a drawing of the apparatus, measuring where the bends should go to fit the boiling tube. The bends are marked with the letters A to D.



Step 2:
Use a permanent pen to mark where the bends should be placed in the Borosilicate glass tubing.



Step 3:
Starting with bend B from the diagram, turn the glass tubing in the hottest part of a roaring flame until it softens, then let it fall in the flame until it reaches the correct angle. Then take it out of the flame and let it cool and harden.

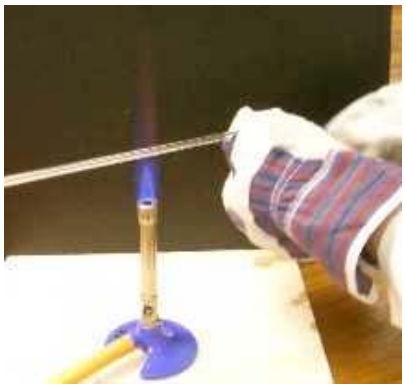
B1

B2

Step 4:
Make Bend C in the same way as bend 2, you will need to turn the glass like a handle.

In these pictures, bend B is in between the two hands of the glass worker.

C1



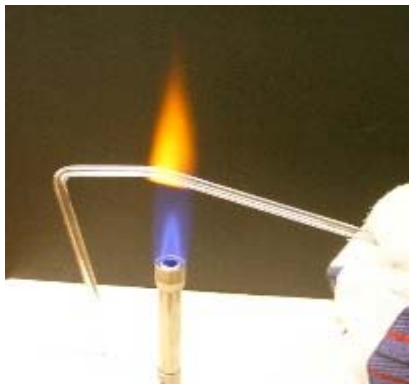
C2

C3

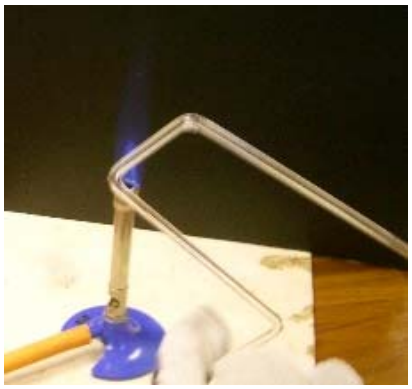
C4

Step 5:
Make bend D in the same way. Push the softened glass into the correct position at the end.

D1



D2



D3

Step 6:
Finally, make bend A, as shown below.

A

Step 7:
Use a file to score the glass, then gently break along the score line to remove any extra glass tubing.

Step 8:
You may need to put the apparatus back in the flame to get the bends to the correct shape.

Step 9:
Any open ends should be flame polished by heating them in the flame until they glow and then letting them cool.

You also need to polish the ends of a small piece of tubing; this will hold the syringe.

Step 10:
You now have all the components for the apparatus.

The rubber bung should have two holes that will fit the glass tubing snugly.

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Assembling the Apparatus

Step 1:
Lubricate the end nearest bend A using washing up liquid, then pass it through one of the holes in the rubber bung. You will also need to do this for the small glass tube.

Step 2:
Place the bung into a boiling tube and attach a syringe to the small glass tube using silicone tubing. The apparatus should look as below.

Step 3:
Use the syringe to draw up coloured liquid into the tubing.

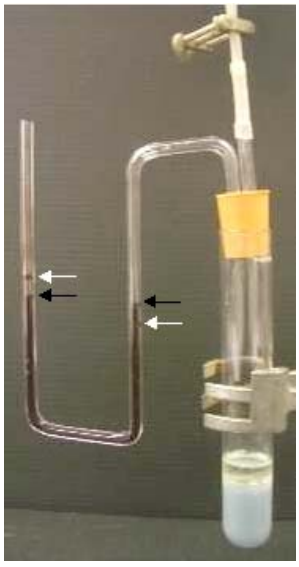
Then invert the apparatus to make a manometer.

Step 4:
Mix the following in the boiling tube.

- 5 cm^3 of yeast solution.
- 5 cm^3 of glucose solution.
- a few drops of Janus Green solution (approximately 0.5 cm^3).

Use a pipette to add a layer of oil to the surface of the yeast mixture.

Finally, apply a Hoffman clip to the silicone tubing and clamp the apparatus in an upright position.



As the experiment requires determination of the time for the movement of 1 cm of fluid in the manometer, mark the starting levels of the fluid in the two arms (shown by black arrows).

Also mark the position that the fluid will have in the two arms after it has moved 1 cm due to the CO_2 produced (shown by white arrows).

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Carrying out the measurement

Step 1:
With the Hoffman clip open, place the apparatus in a large beaker containing warm water (40 °C). The Janus Green will start off with a blue / green colour and this will gradually change to pink as oxygen is used up by the yeast respiration.

1

2

3

4

Close the clip when the Janus Green stops changing colour. This took about 7 to 8 minutes in our experiment.

It is useful to have a boiling tube with completely anaerobic Janus Green for colour comparison.

Step 2:
As CO_2 is produced, the fluid in the arm nearest the boiling tube will be pushed down. The fluid will rise in the other arm.

Time how long it takes for the manometer fluid to move 1 cm.

Step 3: Open the Hoffman clip to release the pressure out of the apparatus and reset the manometer fluid levels. Close the clip and then repeat the measurement as in step 2.

Results

In our experiment, the fluid moved 1 cm in approximately 3 minutes 20 seconds.

This time will vary with the bore of the tubing, and would be quicker if capillary tubing had been used. A smaller bore would also be more accurate because the activity of the yeast could change considerably in the time it takes to move the manometer fluid.

The timing would also be more accurate if more than 1 cm was measured, as this would reduce the lack of precision associated with determination of the point at which the fluid crossed the line. If a longer distance is to be measured then capillary tubing should be used to ensure that the total time taken for the movement does not reduce the accuracy.

Conclusion

This experiment can determine the rate of CO_2 production with reasonable accuracy. The conditions affecting the yeast could be varied in terms of temperature, pH, glucose concentration, etc. Different organic substrates could also be used and possibly other microbial organisms.

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