Leaving Cert Biology

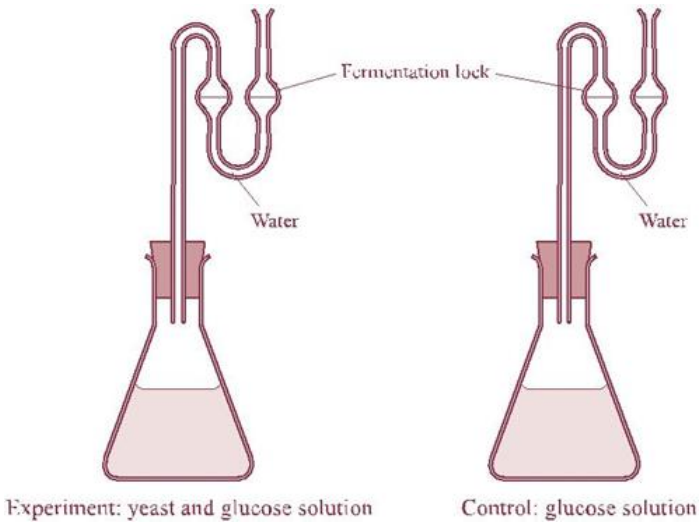
Prepare and Show the Production of Alcohol by Yeast

Experiments

Prepare and Show the Production of Alcohol by Yeast

Materials/Equipment

Yeast
Glucose
Distilled water
Sodium hypochlorite solution (15% w/v)
Potassium iodide solution (10% w/v)
2 Conical flasks (250 cm^3)
Graduated cylinder (250 cm^3)
2 Beakers (250 cm^3)
2 Beakers (50 cm^3)
Thermometer
Filter paper
Timer
Labels
2 Syringes (5 cm^3)
2 Rubber bungs
2 Fermentation locks
Weigh boats
Incubator (25˚C – 30˚C)
Water bath (50˚C – 60˚C)
Funnel



Production of Alcohol by Yeast

Procedure

1. Familiarise yourself with all procedures before starting.

To produce alcohol using yeast

2. Prepare 500 cm3 of a 10% w/v glucose solution.
3. Into each of the two conical flasks, add 250 cm3 of the 10% w/v glucose solution.
4. To one, add 5 g of yeast and swirl. Label this ‘yeast + glucose solution’.
5. The second flask acts as the control (has no yeast). Label as ‘control’.
6. Attach a fermentation lock (half-filled with water) to each flask.
7. Place both flasks in the incubator at 30 °C overnight.

To show the presence of alcohol: Iodoform test for alcohol

8. Remove both flasks from the incubator and filter the contents of each into separate beakers and label as before.
9. Transfer 3 cm3 of the yeast and glucose filtrate into a test tube and label.
10. Transfer 3 cm3 of the control filtrate into another test tube and label.
11. To each test tube, add 3 cm3 of the potassium iodide solution and 5 cm3 of the sodium hypocholorite solution.
12. Warm gently for 4 – 5 minutes in the water bath.
13. Allow to cool and observe any change(s).
14. Record and compare results.
15. Replicate the investigation or cross reference your results with other groups.

Result

Flask	Original colour of filtrate	Final colour of filtrate	Other change(s)
Yeast and glucose solution			
Control (no yeast)			

Conclusion/Comment

SKILL ATTAINMENT

PREPARE AND SHOW THE PRODUCTION OF ALCOHOL BY YEAST

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Use the graduated cylinder to measure the volumes of glucose solution
Use the syringe for measurement of small volumes
Use the electronic balance
Attach the fermentation locks to the conical flasks
Filter the suspension
Set the incubator
Set and maintain the water bath
Use the timer

Observation
Observe bubbles of carbon dioxide being liberated
Observe the effect of filtering
Observe colour changes during the iodoform test
Observe the presence/absence of yellow crystals

Recording
Write up the procedure
Record any colour changes during the iodoform test
Record the presence/absence of yellow crystals

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Clean up after the practical activity

Background information

Alcohol production by fermentation is a well established technology which has long been practised throughout the world. Continuous alcohol production with the use of immobilised yeasts, gives rise to more efficient fermentation.

Alcohol is produced by yeast fermentation of the sugars of various plants. Yeasts ferment simple sugars (monosaccharides) into carbon dioxide and ethanol under anaerobic conditions. Yeasts are single-celled fungi. The genus *Saccharomyces* is the one most commonly used due to its efficient alcohol production and tolerance of high alcohol levels. Some yeasts can live until the alcohol concentration reaches 18%.

Iodoform test

Alcohol groups when treated with potassium iodide (KI) and sodium hypochlorite (NaOCl) readily yield iodoform (CHI_3).

There are three stages in this reaction:

a) Oxidation:

aqueous sodium hypochlorite oxidises the potassium iodide to potassium hypoiodite, this then oxidises the alcohol to an aldehyde.

$$C_2H_5OH \rightarrow \begin{matrix} CH_3CH \\ O \end{matrix}$$

ethanol → ethanal

b) Substitution:

potassium hypoiodite then iodinates the aldehyde to tri-iodoethanal (iodal).

$$CH_3CHO \rightarrow \begin{matrix} CI_3CH \\ O \end{matrix}$$

ethanal → iodal

c) Hydrolysis:

aqueous sodium hypochlorite always contains sodium hydroxide, which converts the iodal to tri-iodomethane (iodoform) and sodium methanoate

$$\begin{matrix} CI_3CHO \\ + \end{matrix} \quad NaOH \rightarrow \begin{matrix} CHI_3 \\ HCOONa \end{matrix}$$

iodal + sodium hydroxide → iodoform + sodium methanoate

Formation of solid iodoform (yellow crystals) is a positive result.
Iodoform is a disinfectant and can be used as an external antiseptic.

Advance preparation

- Set the incubator.
- Set the water bath.
- Check the temperatures of the incubator and water bath with a thermometer.
- Prepare the following solutions: glucose solution (10% w/v), sodium hypochlorite solution (15% w/v), potassium iodide solution (10% w/v).

Helpful hints

- Immobilised yeast may be used instead of dried yeast - this eliminates the need to filter.
- In the iodoform test the sodium hypochlorite used must contain some sodium hydroxide. Use commercial bleach and add sodium hydroxide if necessary.
- Use pure ethanol to observe positive result in the iodoform test.
- Use a cork borer when inserting tubing into bungs.
- Using long necked conical flasks and setting the incubator at 25 °C would help to prevent overflow during fermentation.
- A Bunsen valve can be used instead of a fermentation lock during fermentation.

To construct a Bunsen valve

a) Insert a short piece of glass tubing into a single holed bung.
b) Attach approximately 4 cm of rubber tubing to the glass tubing.
c) Using a scalpel blade, carefully cut a small vertical slit in the rubber tubing. This allows the gas to escape but will prevent air from entering the flask.
d) Use a Hoffman clip to seal the end of the rubber tubing (above the slit).