

# Biology Experiment Booklet



*R. Gallagher*

Experiment:	Page:
Food Tests	3
To prepare and examine plant cells using a light microscope	5
To prepare and examine animal cells using a light microscope	6
To investigate the effect of pH on the rate of enzyme activity	7
To investigate the effect of temperature on the rate of enzyme activity	8
To prepare an enzyme immobilisation & examine its applications	9
To investigate the effect of heat denaturation on enzyme activity	11
To investigate the influence of light intensity on the rate of photosynthesis	12
To prepare and show the production of alcohol by yeast	13
To demonstrate osmosis	14
To isolate DNA from a plant tissue	15
To investigate the growth of leaf yeast using agar plates	16
To prepare and examine the transverse section of a dicot stem	17
To dissect, display and identify the parts of a heart	18
To investigate the effect of exercise on the pulse rate	19
To investigate the effect of IAA growth regulator on plant tissue	20
To investigate the effect of water, oxygen and temperature on germination	22
To use starch agar or skimmed milk plates to show digestive activity during germination	23

# Food Tests

## Test for Starch

### Method:

1. A drop of iodine was placed on white bread or pasta.
2. A drop of iodine was placed on some water (control)
3. Both drops were then analysed (colour change)

### Result:

- Iodine + bread/pasta (starch): Colour change: Brown → black
- Iodine + water (control): Colour change: No colour change, remains brown.

## Test for Reducing Sugar

### Method:

1. Reducing sugar (glucose powder) was dissolved in a little water and added to Benedicts solution in a test tube.
2. Benedicts solution was added to water in a separate test tube (control)
3. Both test tubes were placed in a heated water bath (not boiling) and the colour change were observed.

### Result:

- Benedicts solution + reducing sugar: Colour change: Blue → brick red.
- Benedicts solution + water: Colour change: No colour change, remains blue.

## **Test for Fats/ Lipid**

### **Method:**

1. Rub some butter/oil on brown paper and leave dry.
2. Pour water (control) on brown paper and leave dry.
3. Analyse the observation.

### **Results:**

- A translucent spot forms in the brown paper rubbed with fat.
- Nothing forms in the brown paper with oil.

## **Test for Protein**

### **Method:**

1. Make up biuret solution (sodium hydroxide + copper sulfate)
2. Pour some milk into the biuret solution.
3. Pour water into a separate biuret solution.
4. Observe the colour change

### **Results:**

- Biuret solution + milk: Colour change: Blue → Purple
- Biuret solution + water: Colour change: No colour change, remains blue.

# To prepare and examine plant cells using a light microscope

## Preparing the slide:

1. Remove outer, dry scaly leaves of an onion.
2. Use a tweezers to pull a strip of thin, translucent epidermis from the onion.
3. Place a the epidermis on a microscope slide.
4. Add a few drops of iodine using a dropper, to stain the nucleus (dark red).
5. Using a mounting needle slowly add a cover slip at a 45° angle (reduce air bubbles)
6. **Note:** the purpose of the cover slip is to protect the microscope lens.
7. Examine using course focus wheel and then fine focus wheel.

## Result:

- The nucleus is dark brown.
- The cell walls can also be seen.

# To prepare and examine animal cells using a light microscope

## Preparing the slide:

1. Scrape the inside of your mouth with a cotton bud.
2. Spread the smear of cheek cells on a glass slide.
3. Add a few drops of methylene blue using a dropper, to stain nucleus dark blue.
4. Using a mounting needle slowly add a cover slip at a 45° angle (reduce air bubbles).
5. **Note:** the purpose of the cover slip is to protect the microscope lens.
6. Examine using course focus wheel and then fine focus wheel.

## Result:

- The nucleus is dark blue.

## To investigate the effect of pH on the rate of enzyme activity

**Enzyme:** Catalase

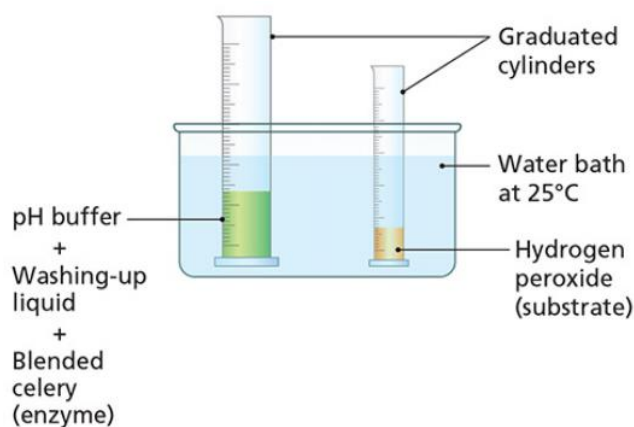
**Enzyme source:** Celery

**Substrate:** Hydrogen Peroxide

**Products:** Water and Oxygen gas

### Method:

1. Blend some celery and filter the juice.
2. Pour the celery juice into the graduated cylinder.
3. Add washing up liquid and a pH buffer.
4. Place some hydrogen peroxide into a test tube.
5. Put both graduated cylinder and test-tube into a water bath and leave until temperature is constant for both.
6. Pour the hydrogen peroxide into the catalase and pH buffer solution.
7. Immediately measure the volume of foam produced every minute (rate) using a stopwatch.
8. Repeat experiment with different pH buffers.
9. Graph results.



### Result:

- There is an optimum pH range for celery. Anything outside this range results in the enzyme becoming denatured - no foam produced.

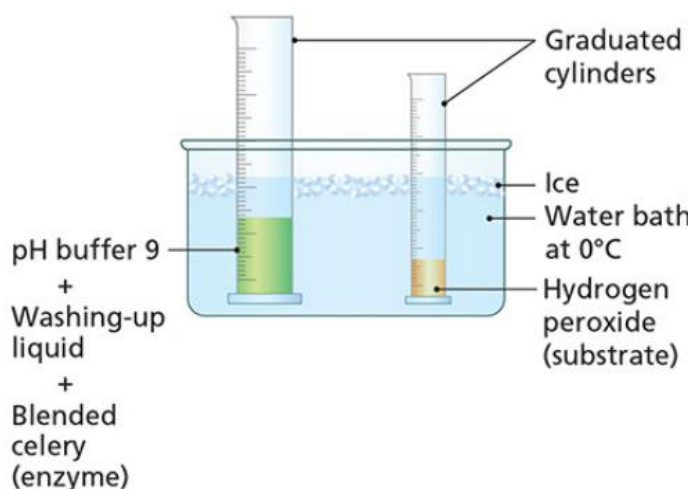
## To investigate the effect of temperature on the rate of enzyme activity

**Enzyme:** Catalase

**Enzyme source:** Celery

**Substrate:** Hydrogen Peroxide

**Products:** Water and Oxygen gas



### Method:

1. Blend some celery and filter the juice.
2. Pour the celery juice into the graduated cylinder.
3. Add washing up liquid and a pH buffer.
4. Place some hydrogen peroxide into a test tube.
5. Put both graduated cylinder and test-tube into a water bath and leave until temperature is constant for both.
6. Pour the hydrogen peroxide into the catalase and pH buffer solution.
7. Immediately measure the volume of foam produced every minute (rate) using a stopwatch.
8. Repeat experiment with different temperatures.
9. Graph results.



# To prepare an enzyme immobilisation and examine its applications

**Enzyme:** Sucrase

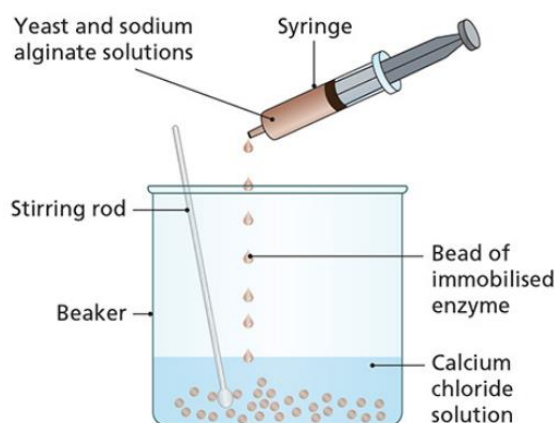
**Enzyme source:** Yeast

**Substrate:** Sucrose

**Product:** Glucose

## Method: Preparation of immobilised enzyme

1. Sodium alginate + water is mixed with yeast (sucrase) to form a gel.
2. This mixture is dropped slowly, using a syringe held up high into a solution of calcium chloride to harden the gel beads.
3. The gel beads are left for 15 minutes in the calcium chloride to harden.

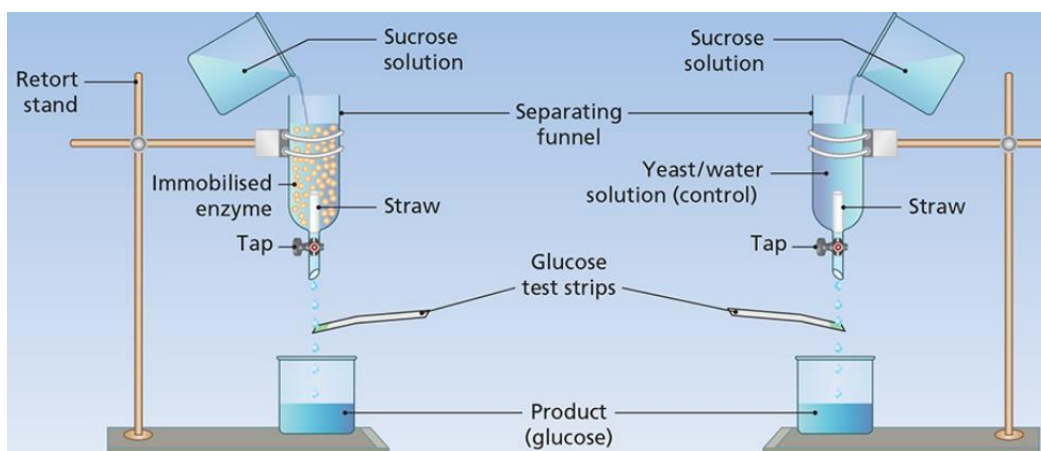


## Method: Examining the application of immobilised enzymes

1. Place the hardened beads into a dropping funnel. Place a straw inside to prevent clogging the tap.
2. Place free yeast and water into a separate dropping funnel.
3. Pour sucrose solution into both dropping funnels (same volume).
4. Open the tap of dropping funnel and collect the product(s).
5. Place a glucose test strip underneath and time how long it takes to show a positive result.
6. Compare both solutions.

**Results:**

- The free yeast showed a result for glucose faster than the immobilised yeast because it takes longer for the sucrose to penetrate the alginate beads and for the glucose to emerge.
- The free yeast beaker was a lot murkier as it contained both yeast and the product (glucose).
- The immobilised yeast beaker was clear as it only contain the product glucose.



# To investigate the effect of heat denaturation on enzyme activity

**Enzyme:** Catalase

**Substrate:** Hydrogen Peroxide

## Method:

1. Blend some celery and filter the juice.
2. Boil the celery juice and place into a graduated cylinder.
3. Add washing up liquid and a pH buffer.
4. Place some hydrogen peroxide into the solution and observe the result.
5. **Note:** A control is unboiled celery.

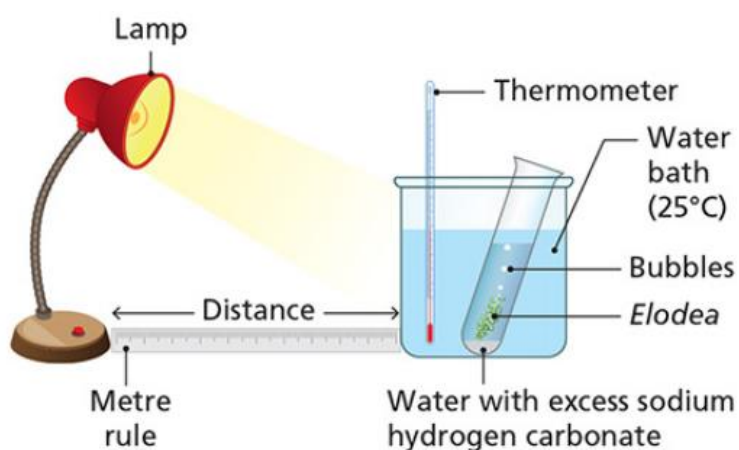
## Result:

- No foam was produced in the boiled celery as the catalase enzyme is denatured - lost its shape and function.

## To investigate the influence of light intensity on the rate of photosynthesis

### Method:

1. Add excess sodium bicarbonate to a test tube of water to saturate the water with carbon dioxide.
2. Cut a section of Elodea and place into test-tube.
3. Place test-tube in a water bath (constant temperature).
4. Start with the lamp 1m from the elodea.
5. Count the number of bubbles of oxygen coming from the cut end of the stem per minute.
6. Move the lamp closer and continue to get the rate of bubble (oxygen) production.



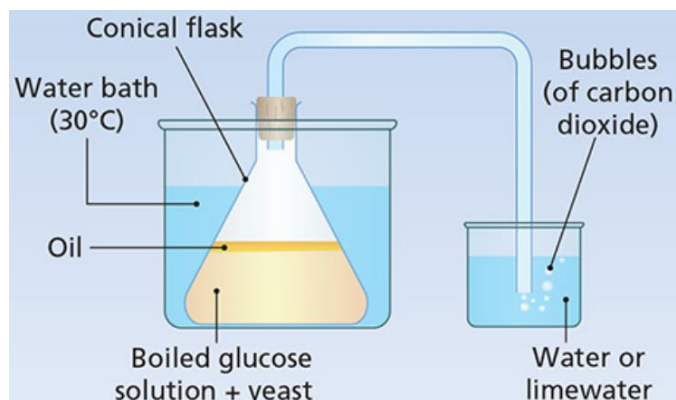
### Results:

1. As you move the lamp closer to elodea, the rate of oxygen production increases.
2. There comes a point where the rate of oxygen production hits a maximum (light saturated)

# To prepare and show the production of ethanol by yeast

## Method: Production of Alcohol

1. Boil glucose solution in conical flask (anaerobic conditions).
2. Allow solution to cool and add dried yeast.
3. Cover solution with a layer oil.
4. Place fermentation lock containing limewater over the conical flask.
5. Place conical flask and fermentation lock into a waterbath overnight.



6. **Note:** The control is same experiment with no yeast.

## Results:

- Limewater turns milky
- Bubbling occurs in conical flask

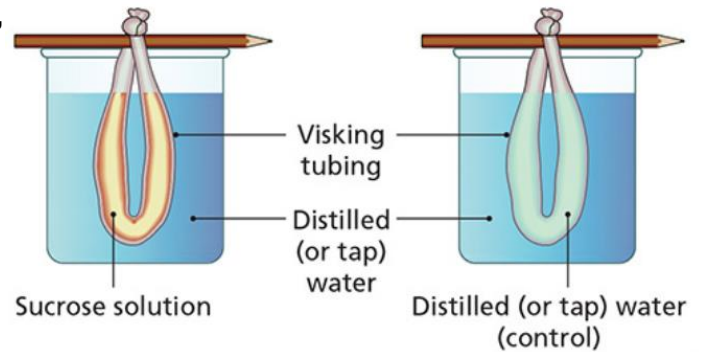
## Method: Iodoform test to show the presence of ethanol

1. Filter solution (to remove yeast cells)
2. Add potassium iodide to the filtrate.
3. Add sodium hypochlorite solution.
4. Heat and then allow to cool.
5. Yellow crystals will form if ethanol is present.

## To demonstrate osmosis

### Method:

1. Place sucrose solution into visking tubing (semi-permeable membrane), tie it and get the mass.
2. Place water (control) into visking tubing, tie it and get the mass.
3. Place both visking tubing into water as shown in diagram and leave for one hour.
4. Remove visking tubing, dry them and reweigh.
5. Record the difference in mass and appearance of visking tubings.



### Results:

- The visking tubing with sucrose increased in mass due to osmosis whereas the control did not change in mass.
- The sucrose visking tubing was more turgid than before.

## To isolate DNA from a plant tissue

### Method:

1. Chop onion to increase surface area and add to a test-tube.
2. Add washing-up liquid to the test-tube to breakdown the cell membrane.
3. Add salt to the solution to clump the DNA together.
4. Place test-tube into a heated water bath to denature enzymes that would digest the exposed DNA.
5. Cool the DNA in an ice bath to slow the breakdown of DNA.
6. Blend the DNA to breakdown the cell wall.
7. Pour filtrate through coffee filter paper to collect the DNA.
8. Add protease to DNA solution to breakdown proteins surrounding DNA.
9. Pour in alcohol slowly as DNA is insoluble in alcohol and the white strands can be seen clearer.

### Results:

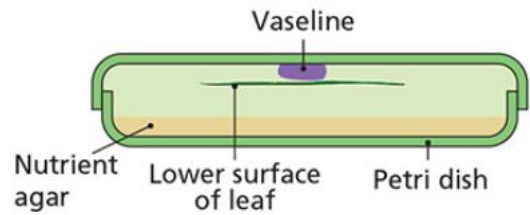
- White strands of DNA are seen in the alcohol.

# To investigate the growth of leaf yeast using agar plates

Plant: Ash leaves

Nutrient medium: Malt agar

## Method:



1. Cut a small branch containing some leaves.
2. Use gloves and wash the bench with disinfectant.
3. Sterilise a forceps by placing under flame of Bunsen burner, to transfer the leaf.
4. Place vasoline on the inside lid of the petri dish.
5. Open the lid quickly and attach the leaf to the vasoline.
6. **Note:** Control is an opened agar plate.
7. Seal both dishes with tape.
8. Incubate both plates overnight. Place the dishes upside-down for 24 hours when incubating.
9. Dispose of the agar plates and yeast by sterilising in an autoclave for 15 minutes.

## Result:

- Dish with the leaf contains pink yeast colonies on the surface of the malt agar.
- No growth on the control dish.



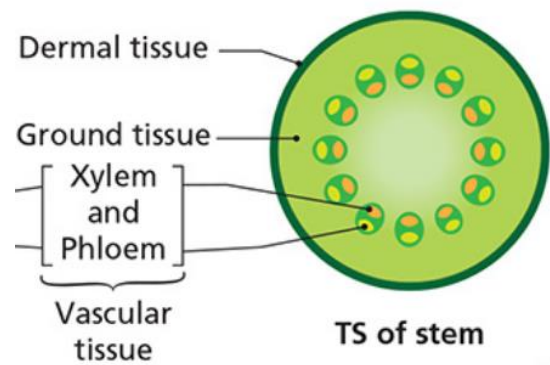
## To prepare and examine the transverse section of a dicot stem

### Method:

1. Cut out a short section of celery stem using a backed blade.
2. Store these cut sections in a clock glass of water.
3. Transfer the thinnest sections onto a microscope slide using a forceps.
4. Add a few drops of water and a cover slip at a 45° angle.
5. Observe under low power and then increase to high power of the microscope.

### Result:

Vascular bundles are seen in a circle as shown in the diagram.



# To dissect, display and identify the parts of a heart

## Method:

1. Put on gloves and goggles.
2. Wash heart.
3. Place heart on dissecting board and identify the front and back of heart.
4. Using a scapel, cut an X to the left and to the right of heart.
5. Using a forceps lift the cut flaps to expose the right ventricle, right atria and and tricuspid valve.
6. Repeat to expose the left ventricle, left atria and and bicuspid valve.
7. Cut a small section at base of aorta and pulmonary artery to expose 'semi-lunar valves'
8. Dispose of heart and sterilise equipment.

## To investigate the effect of exercise on the pulse rate

### Method:

1. In a standing position determine your pulse rate at rest and record result.
2. Jog on the spot for 1 minute and immediately count your pulse rate straight after. Record result.
3. Compare results.

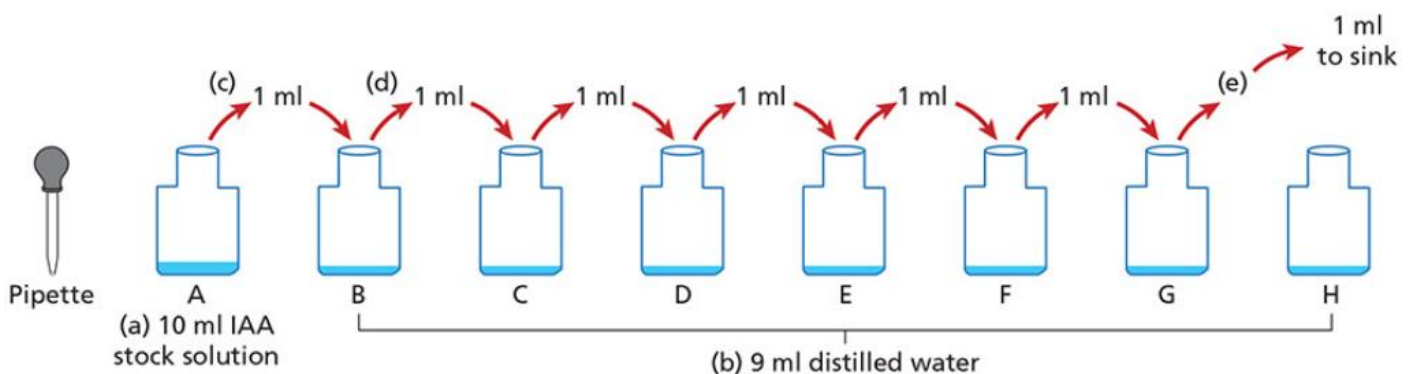
### Result:

The pulse rate increases with exercise.

## To investigate the effect of IAA growth regulator on plant tissue

### Method: Serial dilution

1. Label 6 petri dishes, droppers and bottles as follows with several different concentrations, distilled water (control).
2. Using a syringe add 10mL of IAA solution to the first bottle
3. Using another syringe add 9mL of distilled water to each of the next 5 bottles.
4. Using a dropper, remove 1mL of IAA solution from the first bottle and add it to the second bottle. Place a cap on and mix the second bottle.
5. Using a different dropper remove 1mL of solution from the second bottle and add it to the third bottle. Place a cap on and mix the third bottle.
6. Using a different dropper each time, repeat this procedure up to final bottle. This is called a serial dilution.
7. Leave final bottle as it is only distilled water (control).



### Method: Investigate the effect of IAA on plant tissue

1. Fit a circular acetate grid inside the lid of each petri dish.
2. Place six radish seeds across the middle of a grid line.
3. Cover the seeds with filter paper.
4. Add 2mL of each bottle to its matching dish using the appropriate dropper.
5. Place some cotton wool on top of the filter paper.

6. Pour the remaining 7cm<sup>3</sup> of each solution onto the cotton wool in each dish.
7. Put the lid on each dish and keep it closed with tape on either side.
8. Place all the dishes in the incubator for 2 – 3 days. Stand the dishes vertically on their edge so that the roots grow down and the shoots grow up.
9. Using the acetate paper, measure the length of the roots and the shoots of the seedlings in each dish.
10. Calculate the average length of the roots and the shoots in each dish.

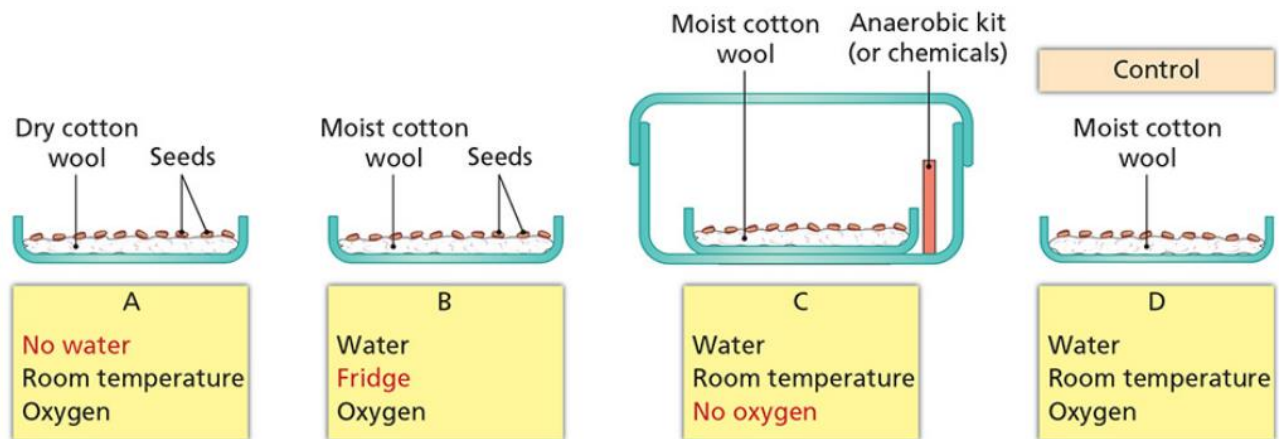
**Results:**

- IAA stimulates growth of roots and shoots up to a certain concentration
- High IAA concentration: Shoot growth
- Low IAA concentration: Root growth

# To investigate the effect of water, oxygen and temperature on germination

## Method:

1. Set up apparatus as shown.



## Results:

- Dish A: No germination due to no water present
- Dish B: No germination due to a low temperature
- Dish C: No germination due to no oxygen
- Dish D has all three factors present (**control**): Germination occurs

## To use starch agar or skimmed milk plates to show digestive activity during germination

### Method:

1. Soak broad bean seeds in water for a day to soften testa and activate enzymes.
2. Wash the bench with disinfectant to ensure microorganism do not affect result.
3. Boil two of the seeds for five minutes, to denature enzymes (**control**)
4. Use a backed blade to split the four seeds in half.
5. Sterilise the half seeds in alcohol for 10 minutes, then rinse them in water.
6. Flame a forceps using a Bunsen burner, allow cool.
7. Barely open a petri dish A and using the forceps place the half seeds, face down on the starch agar.
8. Re-flame the forceps and barely open a second petri dish, B. Using the forceps place the boiled half seeds, face down on the starch agar (**control**).
9. Leave the covered dishes in a warm place for two days.
10. Remove the half seeds and add dilute iodine solution to the dishes.

### Result:

- Iodine goes blue black in the presence of starch. The entire dish containing the unboiled seeds went blue black except for underneath the seeds (remains brown) due to the seeds enzyme (amylase) digesting the starch to make maltose.
- The entire dish containing the boiled seeds went blue black including underneath the seeds due to the seeds enzyme (amylase) being denatured due to boiling.

### Alternative:

- Repeat experiment with skimmed milk and use the biuret solution instead.