

**Practical 2 - M(a)/S(b) The effect of nitrate concentration on the production of biomass by algae.**

**This practical focuses on Analysis** – Evaluation and Conclusions. You will be developing other assessed skills throughout the practical.

**Intended learning outcomes**

By the end of this practical and its write-up you should be able to:

- Draw conclusions based on the key features of the data obtained
- Explain whether the experimental evidence supports the hypothesis
- Describe the relationship between nitrate concentration and biomass production
- Give a scientific explanation of the relationship between photosynthesis, nitrate concentration and the production of biomass.
- Optional describe the relationship between nitrate concentration, photosynthesis and protein production.
- Comment on the experimental design and suggest improvements.

**Safety information**

	You should wear eye protection throughout this practical.
	Potassium nitrate is an <b>oxidising agent</b> .
	Calcium chloride is an <b>irritant</b> .
	Iron (II) sulphate is <b>harmful</b> .
 	Zinc sulphate is <b>harmful</b> and <b>dangerous to the environment</b> .
	Manganese chloride is <b>harmful</b> .
	Molybdenum trioxide is <b>harmful</b> .
 	Copper sulphate is <b>harmful</b> and <b>dangerous to the environment</b> .
	Cobalt nitrate is <b>harmful</b> .
Bleach may have been used for sterilising materials and may be <b>harmful, oxidising</b> or <b>corrosive</b> .	

### Background information

- In the light dependent reaction of photosynthesis, electrons are excited using energy from light. These high-energy electrons are passed through chains of electron carriers into NADP, which becomes reduced NADP, also using hydrogen ions from photolysis of water.
- In the light dependent reaction of photosynthesis carbon dioxide is assimilated and used in the synthesis of 3 carbon sugars. Reduced NADPH and ATP from the light dependent reactions are used during these reactions.
- The 3 carbon sugars and mineral ions are used in reactions that enable plants to synthesise all the organic molecules needed to produce new cellular material.
- The new cellular material includes polysaccharide, protein, lipid and nucleic acid that add to the biomass of the plants and thus provides a way of measuring growth in relation to photosynthesis.
- Unicellular algae grow relatively quickly and their biomass can be measured using a balance.

You will investigate the hypothesis that; An increase in **nitrate concentration** increases the **production of biomass** by a unicellular green alga.

Nitrate concentration in this case is varied by different masses of a sodium nitrate.

Experimental set	concentration of sodium nitrate/g dm <sup>-3</sup>
control	0
1	5
2	10
3	15
5	20

## Method

### Preparations

- 1 Wash your hands and then wipe your work area with disinfectant.
- 2 Wash a 250 cm<sup>3</sup> container with sterilising solution.
- 3 Connect a length of clear plastic tubing to a diffusion block. Insert a small piece of cotton wool into the other end of plastic tubing to filter the air supply. The open end of the tubing can be used if a diffusion block is not available.
- 4 Connect the plastic tubing to an aquarium pump.
- 5 If an aquarium pump is not available a water filter pump can be used. In this case two pieces of plastic tubing are needed. One piece of tubing is connected to the diffusion block and is long enough to pass through the cotton wool plug so the diffusion block (or open end) reaches almost to the bottom of the container. The other piece of tubing is connected to the water pump and is long enough for the open end to pass through the cotton wool plug but does not reach the liquid.
- 6 Collect about 250 cm<sup>3</sup> of boiled pond water.

### Preparation of culture and making observations

- 1 Pour 200 cm<sup>3</sup> of boiled pond water into the container. Keep the remaining pond water in a closed, labelled sterile bottle.
- 2 Add a known mass of sodium nitrate to the pond water and plug loosely with cotton wool. Swirl gently until all the sodium nitrate is dissolved.
- 3 Remove the cotton wool plug and add 2 cm<sup>3</sup> of an algal culture to the pond water.
- 4 Mark the level of the liquid in the container and weigh the container and its contents. (Optional – remove 1 cm<sup>3</sup> of mixture and test for protein using Biuret test)
- 5 Place a light source at a known distance from the container at room temperature.
- 6 Place the diffusion block into the liquid in the container and loosely plug in place using fresh cotton wool. Turn on the pump. Alternatively place the plastic tubing connected to the diffuser into the liquid and the tubing connected to the water suction pump into the top of the container and plug loosely with cotton wool. Connect to the water pump and turn on.
- 7 Observe any changes in the culture over several days. If the liquid level falls, top up to the mark with sterile pond water.
- 8 After 2 weeks turn off the air supply, remove the plastic tubing and cotton wool.
- 9 Reweigh the bottle and its contents. (Optional- remove 1 cm<sup>3</sup> of mixture and test for protein using Biuret test)

**Preparing a calibration curve and estimating protein content—Optional.**

- 1 Weigh 3 g of albumin powder or other protein.
- 2 Dissolve in 30 cm<sup>3</sup> of distilled water in a test tube.
- 3 Prepare a series of solutions of known protein concentration using the proportions of albumin solution and water in the table below.

Volume of albumin solution / cm <sup>3</sup>	Volume of water / cm <sup>3</sup>	Concentration of albumin solution / mg dm <sup>-3</sup>
10	0	
8	2	
6	4	
4	6	
2	8	
0	10	

- 4 Carry out Biuret test on each of the solutions.
- 5 Calibrate a colorimeter using the tube without any albumin solution.
- 6 Take a reading of each tube and record the light absorbance.
- 7 Allow the cells to settle in each of the samples tested. Decant the coloured liquid into a suitable tube and read the light absorbance.

**Calculations**

- 1 Record the initial and final mass of the bottle and its contents.
  - 2 Calculate the increase in biomass over time.
  - 3 Record your increase in biomass on a class results table.
  - 4 When all the results have been recorded in the class results table, calculate the mean increase in biomass.
- *Optional calibration curve and protein measurement.*
    - 1 plot a curve of protein concentration against light absorbance.
    - 2 use the calibration curve to find the concentration of protein in your culture at the beginning and end of the investigation.
    - 3 calculate the increase in protein

If a colorimeter is not available the colours obtained from the culture can be compared to a set of standard tubes. These are made in the same way as described for colorimeter measurement, using specimen tube which can be sealed. To improve accuracy in this case, a larger range of concentrations should be made.

### Write-up

- Plot a graph to show the mean increase in mass for each nitrate concentration
- (Optional - add error bars to your graph.)
- Optional estimation of protein content
  - On the same axis as biomass, plot a graph of protein content for each nitrate concentration.
  - Compare the curves for protein content and biomass
- Draw conclusions from the investigation considering:
  - detailed description of relationships between nitrate concentration and biomass,
  - a scientific explanation of the results and conclusions,
  - the extent to which the data supports the hypothesis, commenting on any other factor that may have influenced the biomass.
- Make an evaluation considering:
  - the limitations of the methods used,
  - anomalous values if any,
  - replication and range of values of independent variable,
  - effectiveness of control of variables,
  - the confidence with which conclusions should be drawn,
  - potential improvements and further predictions

## Lesson Plan

### The effect of nitrate concentration on the production of biomass by algae.

#### Analysis – Evaluation and Conclusions.

##### Context

A practical investigation set in the context of 9700 Syllabus - aspects of the transfer of light energy during photosynthesis to produce complex organic molecules. It also has some overlap to Option S with regard to the technique of culturing and producing biomass.

It is anticipated that students will have completed an AS practical course so that they will have good basic practical skills. It is also anticipated that they will have been given learning opportunities before this so that they know how to identify the variables and to evaluate an experimental design.

##### Key aims of lesson

This practical is designed to develop the skill of Analysis – Evaluation and Conclusions. Students will be developing other assessed skills throughout the practical.

##### Intended learning outcomes

By the end of this practical and its write-up the student should be able to:

- Draw conclusions based on the key features of the data obtained
- Explain whether the experimental evidence supports the hypothesis
- Describe the relationship between nitrate concentration and biomass production
- Give a scientific explanation of the relationship between photosynthesis, nitrate concentration and the production of biomass.
- Optional - describe the relationship between nitrate concentration, photosynthesis and protein production.
- Comment on the experimental design and suggest improvements.

##### Resources required

White board or flipchart and suitable pens or blackboard and chalks

Practical materials specified on the Technical Information sheet

Some spare copies of the student worksheet

##### Planned activities (timings can be altered to suit shorter or longer lessons)

Timings/ minutes	Teacher / Student Activities
end of previous lesson	<b>Preparation</b> – first two pages of student worksheet given out for students to read in preparation for the practical lesson. Students should identify the control variables and note how they are being standardised in preparation for evaluating the method after the investigation.
0-4	<b>Introduction</b> to the aims, intended outcomes and shape of the lesson - teacher led oral presentation

4-9	<p><b>Context</b> - review light independent reactions of photosynthesis and light independent reactions as a means of producing small organic molecules that can be used to synthesise larger molecules. The requirement for mineral ions for some molecules, emphasising role of nitrate for proteins and nucleic acids. The need for these molecules for growth and reproduction of plants. Teacher-led questioning, student responses / discussion, students building a multi-coloured learning outline on the board.</p>
10-14	<p><b>Introduction to method</b> - teacher demonstration of assembling the diffuser and pump system to be used. If groups are to be working together using different nitrate concentrations – allocation of concentrations.</p> <p>If optional protein measurements are to be carried out - demonstration of colorimeter if necessary. Within a group, each individual can carry out Biuret test on one or more concentrations of protein.</p>
15 – 45	<p><b>Carrying out the practical</b> - students carry out the practical work, entering their results into a table on the board and tidying away apparatus as soon as they have finished.</p>
46 - 50	<p><b>Optional measurement of protein content.</b> Students carry out Biuret test must be carried out on the initial culture and the coloured liquid decanted into a specimen tube for storage in a refrigerator</p>
51-60	<p><b>Drawing together the threads</b> - teacher-led class discussion on the design of the experiment, identifying independent and dependant variables. These should be and discussed in relation to methods of measurement and any limitations that might affect the results. Variables that might influence the results should also be identified and how these are being controlled discussed. A summary on an OHP or flip chart should be built up for reference at the end of the investigation.</p>
<b>Lesson 2</b> 0-5	<p><b>End of previous lesson</b> - students reminded about the practical and reminded to bring the student work sheets to the lesson.</p>
0-4	<p><b>Introduction</b> – reminder of the aims, intended outcomes and shape of the lesson – teacher led oral presentation using the summary OHP or flip chart from the first session.</p>
5 – 25	<p><b>Carrying out the practical</b> – students carry out the weighing of the sample entering their results into a table on the board and tidying away apparatus as soon as they have finished.</p>
26 – 40	<p><b>Optional preparation of standard protein solutions.</b> – students make a series of protein solutions and carry out Biuret test. Biuret test carried out on sample from culture. Colorimeter readings taken or colour of samples compared to colours of known concentrations. The preparation of the standard series can be carried out at the end of the first session and the tubes stored in a refrigerator if necessary.</p>
26 – 60 or 41 – 60	<p><b>Drawing together the threads</b> –Page 3 of the student work sheet handed out. Teacher-led class discussion on the results and their meaning - teacher led introduction to write-up, which should include class-work, finished off as homework as necessary, (graphing results, conclusions, evaluation of design referring to limitations and any improvements</p>

### Useful Information

- In the light dependent reaction of photosynthesis, electrons are excited using energy from light. These high-energy electrons and hydrogen ions from photolysis of water are used to reduce NADP and synthesise ATP from ADP and inorganic phosphate.
- Reduced NADP and ATP are used in the light independent reactions to synthesise glyceraldehyde phosphate (GP), a 3-carbon sugar that feeds into a number of metabolic pathways that lead to the synthesis of simple organic monomers, such as amino acids, hexoses and more complex molecules such as nucleotides and fatty acids.
- Amino acids and nucleic acids require a supply of nitrogen for their synthesis, which is obtained from nitrate. The more protein and nucleic acids synthesised the greater the rate of growth, leading to an increase in biomass.
- Unicellular algae such as *Chlorella sp.* are easy to culture and grow relatively fast. They can be cultured commercially as a source of single cell protein. Some parts of the world grow *Chlorella sp.* in sewage lagoons as a means of reducing pollution from nitrate. The dried cells may be used in animal food. They are also being studied as a means of reducing pollution by toxic metals as they accumulate ions such as aluminium.
- Conclusions – students might be expected to relate the increase in mass to an increase in photosynthesis and the production of more protein/cellular material, leading to more cell division and an increased population. The more nitrate available the greater the production of new biomass. At higher nitrate concentrations may see less increase as other factors are limiting so the results may not fully support the hypothesis. Also students might suggest that sodium influences plant growth.
- The Independent variable is nitrate concentration; the dependent variable is the increase in biomass (or protein content).
- Other variables are:
  - Light – controlled by a constant light source.
  - Carbon dioxide concentration – controlled by the air flow.
  - Other organisms – controlled by using sterile pond water and pure culture. Also the use of semi-sterile conditions during the experimental set up.
  - Volume of pond water – controlled by topping up the container.
  - Quantity of chlorella – controlled by using same volume of pure culture.
  - Exposure of organism to nitrate supply, light carbon dioxide – mixing due to air flow prevents settling of cells and mixes with the required nutrients.
  - Temperature – varies with room temperature
- Possible improvements may include:
  - Temperature; Students may suggest a means of controlling this more effectively, e.g. temperature controlled room, water bath.
  - Concentrations of carbon dioxide- students may suggest this is a limiting factor and use sodium hydrogen carbonate as a source;
  - Light availability; - students may suggest increased light illumination.
  - Volume of chlorella used – students may suggest a greater volume to obtain results faster;

- Range of nitrate concentrations - students may suggest a greater range and/or smaller intervals between the concentrations. Higher nitrate concentrations should be tested to test hypothesis fully.
- Weighing more frequently to find rate of growth/ population increase;
- Source of nitrate - students may suggest another nitrate salt to eliminate the possible effect of sodium on growth or another source of nitrogen such as an ammonium salt;

### Technical Information

#### The effect nitrate concentration on biomass production in algae.

As this investigation takes several days for the results to be obtained it will require a number of students to share results. Ideally at least two students or groups of students should investigate each nitrate concentration and use a means value in their calculations. If resources do not allow for this then one set of values should be sufficient.

The **apparatus and materials** required for this are listed below.

The amount of apparatus listed is for **one student or one group of students** if they are to work in groups.

For some of the chemicals, it is convenient to make up more than is required in order to give sufficient quantities for accurate measurement.

- 1 A supply of pond water. This can be previously sterilised by boiling and kept in a sterile container. If pond water is not available a complete mineral culture solution can be used. A formula for *Chlorella* is supplied
- 2 Sterilising solution. Household peroxide or hypochlorite bleach diluted as indicated by the manufacturer is suitable.
- 3 1 plastic container. Washed, clear plastic drinks containers are suitable.
- 4 1 plastic specimen tube or other container with a lid that can hold 50cm<sup>3</sup> of liquid.
- 5 Plastic tubing – about 50cm
- 6 Diffusion block
- 7 Aquarium air pump or water pump. Most aquarium pumps can drive up to 4 diffusers. If a water pump is used up to 4 can be connected in series. Pumps can be turned off overnight but should be allowed to run for at least 4 hours each day to ensure sufficient air supply.
- 8 2 cm<sup>3</sup> of a pure culture of a unicellular alga e.g. *Chlorella* sp.
- 9 1 desk lamp, which could be a proper articulated lamp, or a bulb-holder safely screwed to a small wooden base.
- 10 Non-absorbent cotton wool
- 11 Marker pen
- 12 Disinfectant for wiping bench
- 13 Sterile 1 cm<sup>3</sup> syringe or Pasteur pipette.
- 14 Sterile 250 cm<sup>3</sup> measuring cylinder or measuring beaker
- 15 Access to a top pan weighing balance
- 16 Optional – Biuret reagents and tubes for testing.

**Chlorella growth medium- pH 6.8**

Component	g/dm <sup>-3</sup>
Potassium nitrate(KNO <sub>3</sub> )	1.25
Potassium hydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	1.25
Magnesium sulphate (MgSO <sub>4</sub> .7H <sub>2</sub> O)	1.00
Calcium chloride (CaCl <sub>2</sub> )	0.084
Boric acid (H <sub>3</sub> BO <sub>3</sub> )	0.014
Iron (II) sulphate (FeSO <sub>4</sub> 7H <sub>2</sub> O)	0.050
Zinc sulphate (ZnSO <sub>4</sub> )	0.088
Manganese chloride (MnCl <sub>2</sub> .4H <sub>2</sub> O)	0.014
Molybdenum trioxide (MoO <sub>3</sub> )	0.007
Copper sulphate (CuSO <sub>4</sub> .5H <sub>2</sub> O)	0.016
Cobalt nitrate (Co(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O)	0.005
EDTA	0.500

Information about algae and algal cultures can be obtained from;

<http://www.saps.plantsci.cam.ac.uk/docs/algalballs.doc>.

<http://www.botany.ubc.ca/cccm/>

[info@fba.org.uk](mailto:info@fba.org.uk)

**Safety Precautions/Risks.**

Potassium nitrate = O	
Calcium chloride = H (Irritant only)	
Iron (II) sulphate = H	
Zinc Sulphate = H, N	 
Manganese Chloride = H	
Molybdenum trioxide =H	
Copper sulphate = H, N	 
Cobalt Nitrate = H	

If household peroxide or hypochlorite bleach is used for sterilisation, manufacturers instructions should be followed and appropriate safety precautions taken.

A risk assessment should be carried out as a matter of course.