**Investigation into the effect of a named factor on the rate of dehydrogenase activity in extracts of chloroplasts**

**The effect of ammonium hydroxide on the time taken for chloroplasts to decolourise DCPIP**

**Learning objectives**

* To develop practical skills a, b and c,
* Demonstrate competencies 2a; 2b and 4a.
* Monitor the rate of dehydrogenase activity in a chloroplast suspension using a blue dye called DCPIP. DCPIP goes from blue to colourless when it accepts electrons released by the chlorophyll.

**Method**

You are provided with the following:

* spinach leaves
* access to a blender
* measuring cylinder
* muslin (or material for filtering)
* filter funnel
* 3 beakers
* ice
* isolation medium (cold)
* DCPIP solution (cold)
* distilled water (cold)
* ammonium hydroxide solution (cold)
* test tubes
* test-tube rack
* syringes (1cm3 and 5 cm3 )
* piece of aluminium foil
* lamp
* marker pen
* timer.
* Colorimeter

You should read these instructions carefully before you start work.

1. Put about 50 cm3 of isolation medium into a beaker.
2. Tear 8 spinach leaves into small pieces and put the pieces into the isolation medium in the beaker. Do not put pieces of the midrib or the leaf stalk into the beaker.
3. Half fill a large beaker with ice and place a small beaker on top of the ice.
4. Put 3 layers of muslin over the top of the filter funnel and wet it with the isolation medium. Rest the filter funnel in the small beaker on the ice.

**These steps have been done for you by our technicians**

1. Pour the spinach and isolation medium into the blender and blend for about 15 seconds. Pour the blended mixture back into the beaker.
2. Pour a little of your blended mixture through the muslin in the filter funnel. Carefully fold and squeeze the muslin to assist the filtering process. Repeat until most of the blended mixture has been filtered. Label this filtrate which is in the small beaker on ice as ‘chloroplast suspension’.
3. Label five test tubes A, B, C, X and Y. Stand these five tubes in the ice in the large beaker. Position the lamp about 10 cm from the beaker so that all tubes are illuminated. Turn on the lamp.
4. Set up tubes A and B as follows:

**Tube A**

Put 5 cm3 DCPIP solution + 1 cm3 water + 1 cm3 chloroplast suspension in the tube. Immediately wrap the tube completely in aluminium foil to exclude light.

**Tube B**

Put 5 cm3 DCPIP solution + 1 cm3 water + 1 cm3 isolation medium in the tube.

Tubes A and B are control experiments. Leave both tubes until the end of your investigation.

1. Set up tube C as follows:

**Tube C**

Put 6 cm3 water + 1 cm3 chloroplast suspension in the tube.

Tube C is for you to use as a standard.

Set up the colorimeter (The wavelength of the colorimeter should be set at 680) and use the mixture in tube C to set the absorbance to zero.

1. Set up tube X as follows:

**Tube X**

Put 5 cm3 DCPIP solution + 1 cm3 water in the tube.

Add 1 cm3 chloroplast suspension to tube X, quickly mix the contents and start the timer. After exactly 2 minutes measure the absorbance of the mixture in the colorimeter.

1. Repeat step 10 four more times.
2. Set up tube Y as follows:

**Tube Y**

Put 5 cm3 DCPIP solution + 1 cm3 ammonium hydroxide in the tube.

Add 1 cm3 chloroplast suspension to tube Y, quickly mix the contents and start the timer. After exactly 2 minutes measure the absorbance of the mixture in the colorimeter.

1. Repeat step 12 four more times.
2. Record your data in a suitable table.
3. At the end of your investigation, record the colour of the mixtures in tubes A and B.

Risk Assessment

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| Hazard | Associated Risk |
| Take care with the very bright lights (>1000 lumens) needed for this practical to work.  . | The light will not damage the retina, but the long lasting afterimage can worry. Students should be cautioned not to look directly at the light. |
| Take care with water next to electrical connections | Keep water away from electrical connections |

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|  | Apparatus and techniques |
| AT a | use appropriate apparatus to record a range of quantitative measurements (to include mass, time, volume, temperature, length and pH) |
| AT b | use appropriate instrumentation to record quantitative measurements, such as a colorimeter or potometer |
| AT c | use laboratory glassware apparatus for a variety of experimental techniques to include serial dilutions |
|  | Competencies |
| 2. Applies investigative approaches and methods when using instruments and equipment | a. Correctly uses appropriate instrumentation, apparatus and materials (including ICT) to carry out investigative activities, experimental techniques and procedures with minimal assistance or prompting.  b. Carries out techniques or procedures methodically, in sequence and in combination, identifying practical issues and making adjustments when necessary.  . |
| 4. Makes and records observations | a. Makes accurate observations relevant to the experimental or investigative procedure. |