PROTOCOL

PHOTOSYNTHESIS

sserc

Check Out Our Colorimetric Assay for Quantitative Results

Page

MATERIALS

The materials listed below are "per pair" of students. Knotted wrack seaweed has been chosen as the plant in this protocol; however, if there is not a ready supply of seaweed in your area, *Egeria najas* is an excellent alternative.

AIM

The aim of this experiment is to investigate the effect of light intensity on the rate of photosynthesis.

METHODOLOGY AT A GLANCE

In this experiment, hydrogencarbonate indicator is used to monitor the consumption of carbon dioxide during photosynthesis by Knotted Wrack. As carbon dioxide concentration decreases, the lower acidity results in a colour change that can be conveniently measured using a colorimeter.

MATERIALS REQUIRED (PER PAIR)

- Bijou bottles x 6
- Fresh knotted wrack seaweed
- Hydrogencarbonate indicator (air saturated) - 2 Universal bottles full.
- Scissors
- Tweezers
- Ruler
- Cup - about 1/2 full with distilled water
- Neutral density filters (25%, 50% and 71% light transmission)
- Black paper
- Colorimeter
- Cuvettes x 7
- Cuvette holder
- Dropping pipettes (3 cm3) x 3
- Indelible pen
- Access to fluorescent tube light
- Stopwatch

HEALTH & SAFETY

When starting an experiment with a class, you must ensure you are comfortable and familiar with the risk assessment. What are the hazards, the level of risk and control measures put in place? Is the current risk assessment appropriate for your class? Do you need to make adjustments. This page of the SSERC website provides a Risk Assessment template and information about Dynamic Risk Assessments.

HAZARD	RISK	CONTROL MEASURES
Seaweed collected from the shore	Risk of allergic reactions are small for this particular plant; risk around possible contamination of plant due to cat/dog fouling, sewage or rat urine and possible infection as a result.	The site chosen for samples must be chosen carefully, taking account of possibility of contamination. See section 3.11 of Materials of Living Origin.
Hydrogen-carbonate indicator	No significant hazard for classroom use; however, technician preparation involves higher concentrations and therefore higher risk. See full details here.	For control measures for technicians, click here.

METHOD

This protocol provides a quantitative method using colorimetry. It can be adapted as a qualitative method using this colour chart.

STEP 1

Cut 5 pieces of seaweed, each measuring 2cm in length. Store the seaweed samples in distilled water until they are ready to use in Step 3.

STEP 2

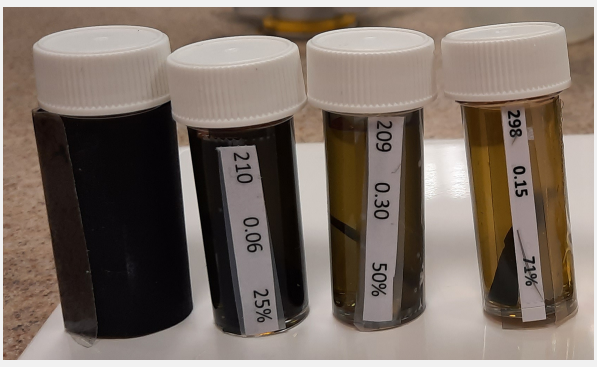
Line up 6 empty Bijou bottles. Rinse the first bottle with approximately 2-3 ml of hydrogencarbonate indicator and transfer the indicator to the second bottle. Repeat until all 5 bottles have been rinsed.

STEP 3

Place a sample of seaweed into 5 of the rinsed Bijou bottles. Fill all Bijou bottles with hydrogencarbonate indicator and write your initials on the lid.

STEP 4

Place one filter over each of four Bijou bottles. This will allow 71%, 50%, 25% or 0% light to be transmitted through to the seaweed.

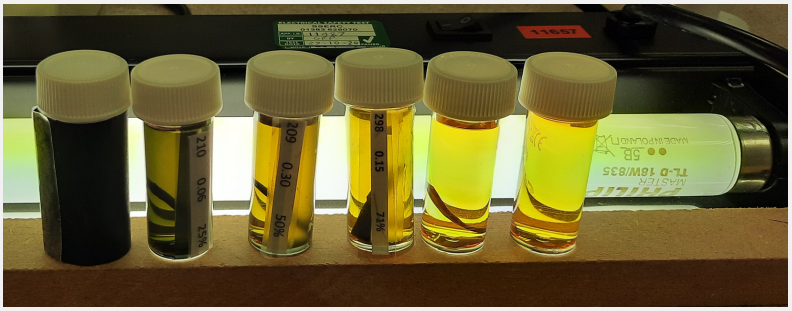


STEP 5

The 2 remaining bottles - one containing seaweed and indicator and the other containing just indicator - will not be covered.

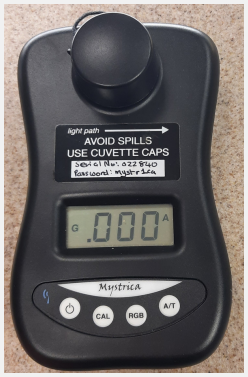
STEP 6

Place all 6 Bijou bottles in front of the fluorescent tube and note the time. Leave the sample in front of the lamp for 20 minutes.



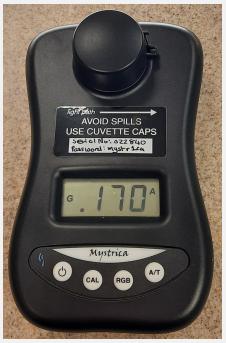
STEP 7

Thoroughly mix the contents of each Bijou bottle. Switch on the colorimeter and select the Green Diode ("G") and Absorbance ("A"). Place a cuvette containing distilled water into the sample holder and press "CAL" to zero the colorimeter.



STEP 8

Use a fresh pipette to transfer the indicator solution from the Bijou bottle which did not contain any seaweed into a fresh cuvette. Measure the absorbance and note it down. This reading must be subtracted from all other readings.



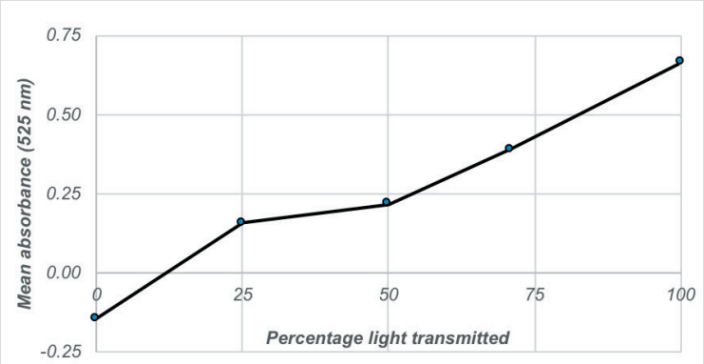
STEP 9

Using a new cuvette each time, measure and record the absorbance of the remaining indicator solutions from the Bijou bottles, starting with 0% light transmitted. Top images shows results after 25 minutes (left to right: 0%, 25%, 50%, 71%, 100% light transmitted; most right was indicator only)

RESULTS

Anticipated results for this protocol are included below. The first set of results were derived at SSERC by the Biology team and were originally published in this SSERC Bulletin. The second set of results were generated by teacher delegates on a recent SSERC professional learning course.

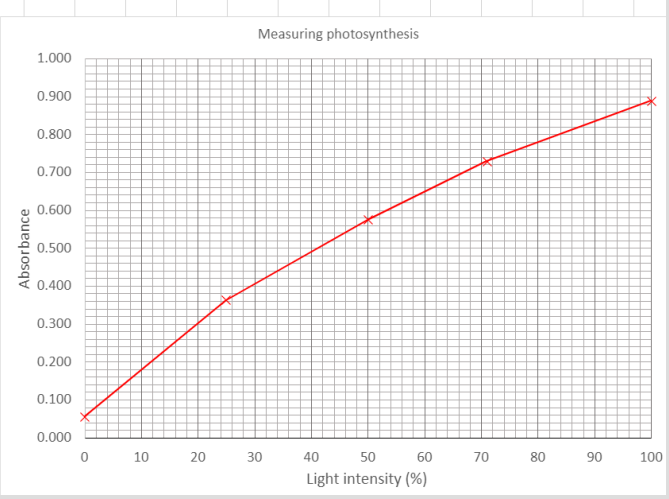
The effect of light intensity on the rate of photosynthesis in samples of knotted wrack was investigated. The data shown are the absorbance values of hydrogencarbonate indicator solution after illumination for a period of 60 minutes. The absorbance of a sample of indicator in the absence of knotted wrack was measured to be 0.43 and this value has been subtracted from the mean values to yield the absorbance value in the column named "corrected mean absorbance".



	Absorbance (525 nm) of hydrogencarbonate indicator						
Light Intensity (%)	Run 1	Run 2	Run 3	Run 4	Run 5	Mean of runs 1-5	Corrected mean absorbance
0.0	0.28	0.28	0.29	0.29	0.29	0.29	-0.14
25	0.59	0.57	0.58	0.60	0.59	0.59	0.16
50	0.63	0.65	0.64	0.65	0.66	0.65	0.22
71	0.82	0.85	0.85	0.83	0.87	0.84	0.39
100	0.8*	1.13	1.06	1.08	1.11	1.10	0.67

This set of data was obtained on a professional learning course by teacher delegates. Three different groups shared their data and averages were calculated and plotted.

Light intensity (%)	Absorbance			
	1	2	3	Average
0	0.08	0.03	0.06	0.056
25	0.35	0.41	0.33	0.363
50	0.55	0.53	0.64	0.574
71	0.68	0.74	0.77	0.729
100	0.94	0.92	0.8	0.888



REFERENCES

This classroom friendly protocol was adapted from SSERC bulletin 268.

SSERC (2019), Photosynthesis using seaweed: opportunities for quantitative studies, *SSERC bulletin*, **268**, page 8-10

CONTACT

Any questions about this protocol? Contact Annie McRobbie (annie.mcrobbie@sserc.scot)