

Experimental data

Background

In the Skills of scientific experimentation, investigation and enquiry section of the new Highers in Biology and Human Biology (see Course Support Notes for Higher Biology Course and the Course Support Notes for Higher Human Biology Course) learners are expected, inter alia, to:

- work with quantitative and qualitative data, discrete and continuous data and sampled data;
- deal with experimental data presented in tables, pie and bar charts, line graphs, lines of best fit, graphs with semi-logarithmic scales, graphs with error bars and information presented as box plots;
- analyse and interpret typically two interconnected tables, charts, keys, graphs or diagrams or a single source of graphical information with two to three patterns, trends, conditions, variables or sets of results.

The Biology Team in SSERC has on a number of occasions been approached to see if we might make a range of data sets available which could then be used by learners by way of practice. In this article we present a number of data sets which might be used together with some explanation of how the data have been generated. The raw data sets are available on the SSERC

website through the resources section for Higher Biology or Higher Human Biology.

Photosynthesis/respiration

We have published a number of reports on the use of carbon dioxide probes to measure respiration and photosynthesis rates in plants [1-4] and the basic protocol which we use for experiments to measure such rates is available via the website [5]. The basic experimental set-up is shown in Figure 1.

The data shown in Figure 2 have been collected over a total of some 9000 seconds and during that time the lamp has been switched on and off on a number of occasions. ▶



Figure 1 - Experimental set-up for measuring respiration and photosynthesis rates in plants. Basil leaves (ca. 5 g) are placed into a 'reaction chamber' together with a CO₂ gas sensor (Product code VR105512) from Instruments Direct Services Ltd. The sensor is linked to a computer via a Vernier Go!Link interface. Data were collected at 4 second intervals for some 9000 s with the lamp being switched on during the periods 360-1300 s, 2040-2912 s, 3860-4680 s, 5740-6760 s, and 7928-8796 s. At other times the apparatus was in darkness. Plots of data are shown in Figures 2 and 3.

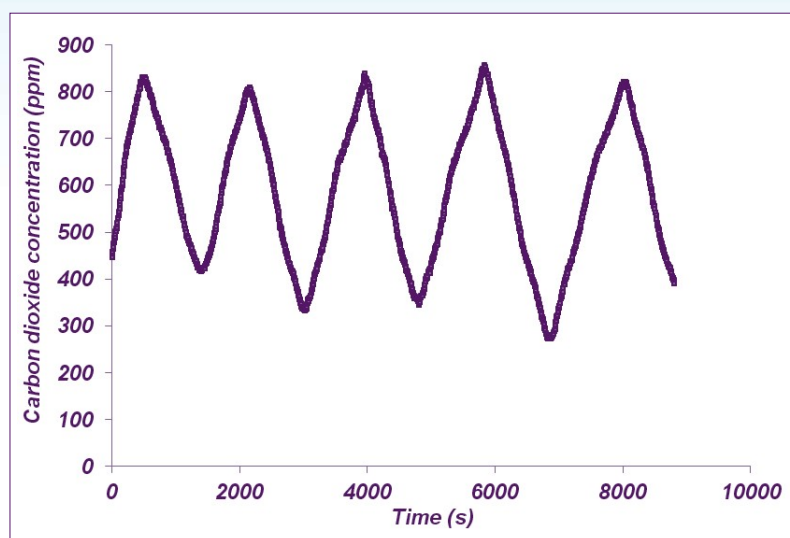


Figure 2 - The rate of change of carbon dioxide concentration in basil leaves. Data were obtained using a Vernier VR105512 probe.



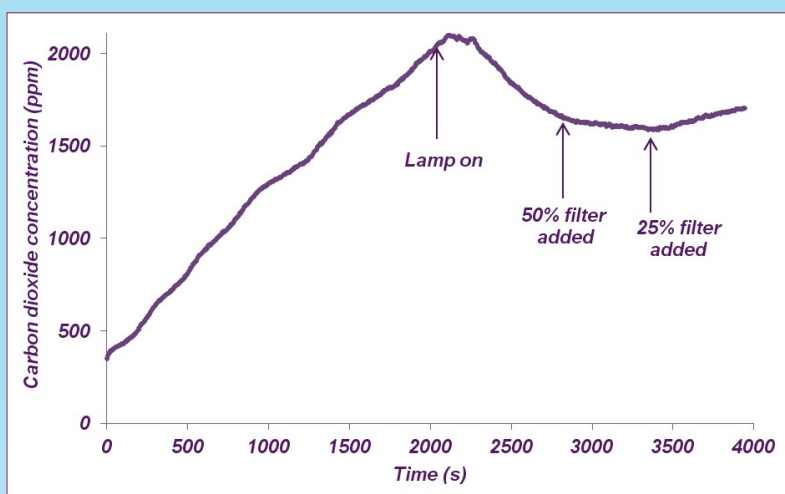


Figure 3 - Carbon dioxide concentration in a container containing basil leaves (ca. 5 g). Data were collected at 4 second intervals for some 4000 s. Between 0-2010 s the leaves were in darkness. At 2000 s the lamp was switched on. A 50% neutral density filter was added at 2720 s and this was replaced by a 25% neutral density filter at 3320 s.

Learners could be asked one or more of the following:

- 1) In the absence of light why is there a rise in $[CO_2]$?
- 2) The $[CO_2]$ starts to fall when the lamp is switched on - why should that be? (Interestingly the fall in $[CO_2]$ starts to become noticeable some 200 s after the lamp is switched on - why might there be this 'lag phase'?)
- 3) Are the rates of rise and fall in $[CO_2]$ the same in each segment of the plot? What does this tell us about how robust the protocol is?

Amongst the topics covered in the *Sustainability and Interdependence* in Higher Biology is the suggestion that learners might 'Carry out experimental investigations on limiting factors in photosynthesis'. Using carbon dioxide probes to monitor the rate of change of $[CO_2]$ as a function of light intensity is a relatively straightforward procedure and we have produced data sets for learners to analyse. The procedure involves the insertion of one or more neutral density filters between the lamp and the reaction chamber shown in Figure 1. Neutral density filters reduce light intensity across the visible range by a relatively constant amount (see [4] for further details).

In Figure 3 above we show the change in $[CO_2]$ under a variety of conditions of light intensity.

Learners could be asked one or more of the following:

- 1) What is the rate of change of $[CO_2]$ under conditions of 100% light, 50% light, 25% light and 0% light?
- 2) What is the explanation for the rise in $[CO_2]$ when light intensity is low (0% and 25%)?

Enzyme kinetics

β -glucosidase

In the most recent issue of the SSERC Bulletin [6] we provided a

protocol for using β -glucosidase in enzyme assays. In the same article we also gave some data sets which might be suitable for analysis by learners in support of their Assignment. We have placed the protocol on the SSERC website and separated out the associated data sets.

Dopa Oxidase

We have previously published [7] protocols and preliminary data for the enzyme dopa oxidase. We present here further data sets for this enzyme when extracted from both banana and germinating mung beans. Briefly, in our standard protocol enzyme extract (in this case from banana) and distilled water are placed in a cuvette and this solution is used to zero the colorimeter at the observation wavelength (in the data that are presented here we have used a Mystrica colorimeter and used the blue diode (ca. 450 nm)). In a new cuvette, substrate (L-dopa), distilled water and enzyme extract are mixed and the absorbance data recorded every 5 s (see Figure 4).

The effect of enzyme concentration can be readily shown by varying the amount of enzyme extract added and this is illustrated in Figure 5.

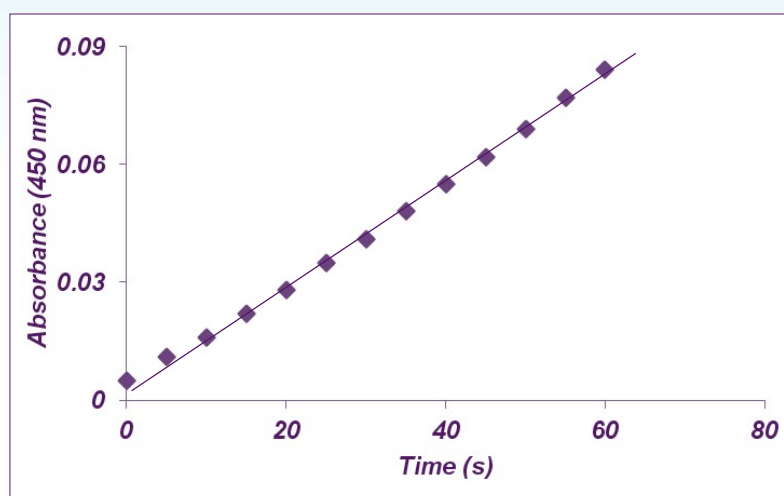


Figure 4 - Absorbance changes measured in a 1 cm cuvette containing dopa oxidase extract (10 μ l) from banana, distilled water (0.99 cm^3) and L-dopa (2 cm^3 , 1.25×10^{-2} mol dm^{-3}). Initial rate of reaction can be calculated from the slope of the solid line shown.

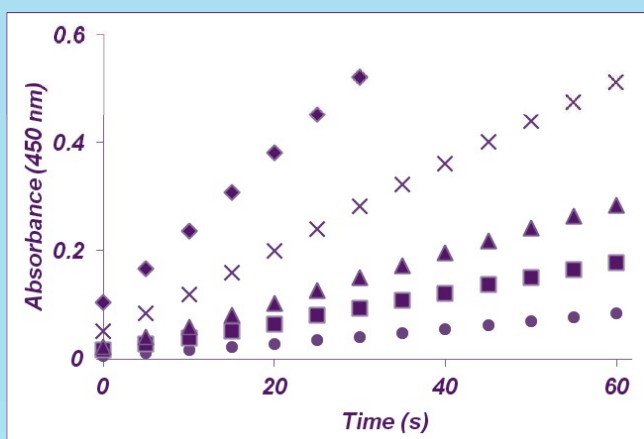


Figure 5 - Absorbance changes measured in a 1 cm cuvette containing dopa oxidase extract (● 10 µl; ■ 25 µl; ▲ 50 µl; × 100 µl; ◆ 200 µl) from banana, distilled water and L-dopa ($1.25 \times 10^{-2} \text{ mol dm}^{-3}$). Initial rate of reaction can be calculated from the slopes of the plots.

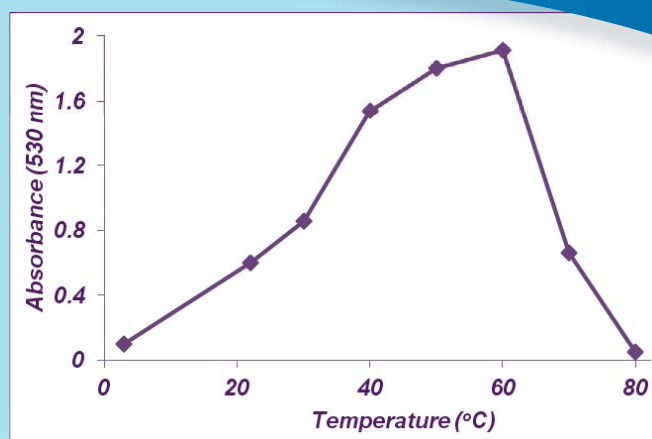


Figure 6 - The effect of temperature on the absorbance changes measured 10 minutes after mixing phosphatase extract with phenolphthalein phosphate. The reaction was stopped prior to absorbance measurement by the addition of sodium carbonate solution to a final concentration 0.5 mol dm^{-3} .

The data referred to in Figures 4 and 5 are for dopa oxidase extracts from banana. The website datasets also include data on enzyme extracted from mung beans.

Learners could be asked one or more of the following:

- 1) Why does the rate of change of absorbance increase with increasing enzyme concentration?
- 2) What is the relationship between enzyme concentration and rate of change (i.e. what do plots of rate of change as a function of enzyme concentration look like?)?

Phosphatase

In a previous publication [8] we described a protocol for the extraction and assay of phosphates from mung beans. An interesting observation [9] is that the temperature profile of phosphatase is such that a maximum is observed at around 55°C. We have re-examined these earlier observations and the data from our experiments are shown in Figure 6. More detailed protocols on how these experiments were undertaken are available on the SSERC website. Briefly, the amount of phenolphthalein produced by the enzyme after a period of 10 minutes is measured and recorded.

The effect of temperature on the absorbance changes were measured 10 minutes after mixing phosphatase extract with phenolphthalein phosphate. The reaction was stopped prior to absorbance measurement by the addition of sodium carbonate solution to a final concentration 0.5 mol dm^{-3} .

Conclusions

We hope that the various data sets shown, together with additional examples on the SSERC website, will be useful sources of information for students in support of their studies. Excel files with all the raw data are available on the SSERC website. ◀

References

- [1] Limiting factors in photosynthesis. SSERC Bulletin (2014), **246**, 2-6.
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- [5] Photosynthesis and respiration - protocol available at www.sserc.org.uk/index.php/biology-2/biology-resources/higher-biology-revised/metabolism-a-survival/3425-maintaining-metabolism.
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- [7] Dopa oxidase - a perfect enzyme? SSERC Bulletin (2013), **242**, 8-10 (adapted in part from protocols on the Mystrica website, www.mystrica.com/Enzymes/dopaoxidase).
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- [9] Phosphatase enzymes from plants: a versatile resource for post-16 students. B. Meatyard, J Biol. Ed. (1999), **33**, 109-112.

Acknowledgements

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