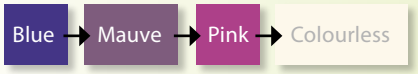
Quantifying respiration rate using resazurin

Within the biology curriculum, at National 5 and Higher [1], it is suggested that learners might use the colour changes of resazurin dye as a measure of respiration rate, or dehydrogenase activity, in yeast.

Resazurin dye, sometimes known, as *Alamar Blue*™, was first used to quantify the bacterial content of milk and is now also used as an indicator of cell viability in mammalian cell cultures. Because resazurin changes colour when it is reduced, it will change colour in the presence of respiring cells. The oxidation of respiratory substrates such as glucose, catalysed by dehydrogenase enzymes, produces hydrogen ions which in cells will normally transfer to and reduce co-enzymes such as NAD. If resazurin is present in the surrounding medium it will also be reduced by hydrogen and undergo the following colour changes:



oxidised *partially reduced* *reduced*

Various protocols using resazurin to illustrate dehydrogenase (or respiratory) activity in yeast have been available for some time [2]. Based on these, and in response to requests from teachers for data sets that can be easily accessed by students, the Biology Team at SSERC has considered two ways of using resazurin and yeast cultures to generate quantifiable data. Here we outline these protocols briefly; more technical detail of the methods, suggestions for investigations and additional data sets can be found on the SSERC website [3].

Figure 1

1) Using resazurin and a colour chart to investigate respiration rate in yeast

As a starting point we set up the following:

- Test tube A - 3 cm^3 boiled 2.5% yeast suspension, 3 cm^3 5% glucose solution, 3 cm^3 0.01% resazurin.
- Test tube B - 3 cm^3 2.5% yeast suspension, 3 cm^3 5% glucose solution, 3 cm^3 0.01% resazurin.
- Test tube C - 3 cm^3 2.5% yeast suspension, 3 cm^3 5% glucose solution, 3 cm^3 distilled water.

Figure 1 shows the colour changes after incubation for 20 minutes at 35°C.

Test tubes containing 3 cm^3 2.5% yeast suspension, 3 cm^3 5% glucose solution and 3 cm^3 0.01% resazurin, were incubated at 35°C and colour changes were recorded every 3 minutes over a period of 30 minutes and used to produce a colour chart (Figure 2). Resazurin dye will change colour, as indicated from left to right in the chart, as it becomes increasingly reduced. The numerical values can be related to respiratory/ dehydrongenase activity in yeast with 10 being equivalent to no activity.

Further investigations were set up in which yeast concentration, glucose concentration and temperature were varied.

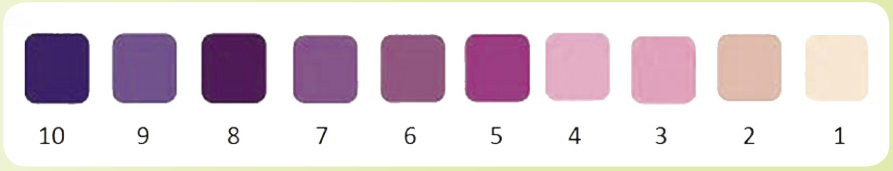


Figure 2 *- Resazurin reduction colour chart.*

A typical set of results using 2.5% yeast suspension, 5% glucose solution and 0.01% resazurin is shown in Figure 3.

Using this basic method, students might carry out investigations into respiratory rate in yeast by altering variables such as temperature, respiratory substrate, substrate concentration, type of yeast and yeast concentration.

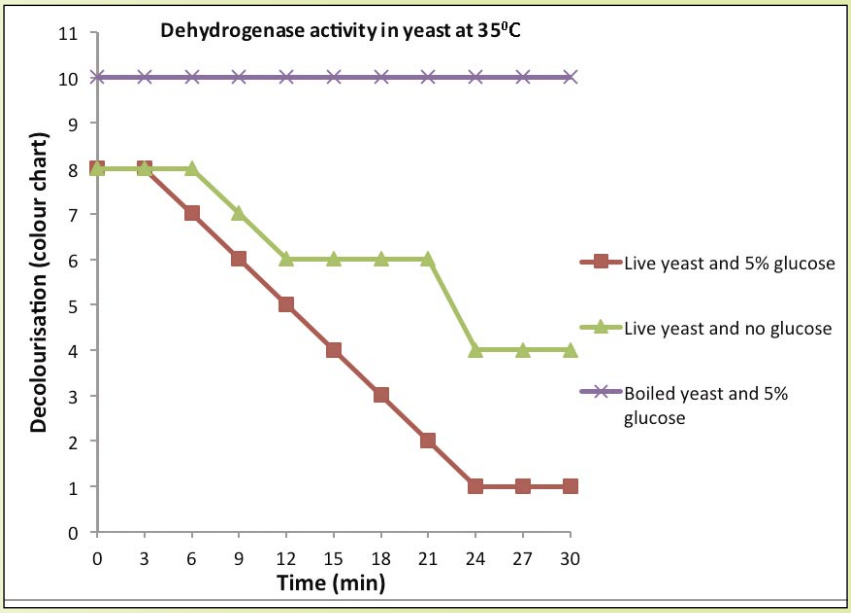


Figure 3 *- Dehydrogenase activity in yeast at 35°C.*

2) Using resazurin and a colorimeter to investigate respiration rate in immobilised yeast

The turbidity of yeast suspensions makes it impossible to obtain reliable colorimeter readings for the decolourisation of resazurin. Immobilisation of the yeast in gel beads means that the yeast can easily be separated from the surrounding solution at various points over time and colorimeter readings can be taken. By setting the colorimeter filter at 590 nm (measuring the disappearance of the resazurin's blue colour) data corresponding to the respiratory activity of the yeast can be generated.

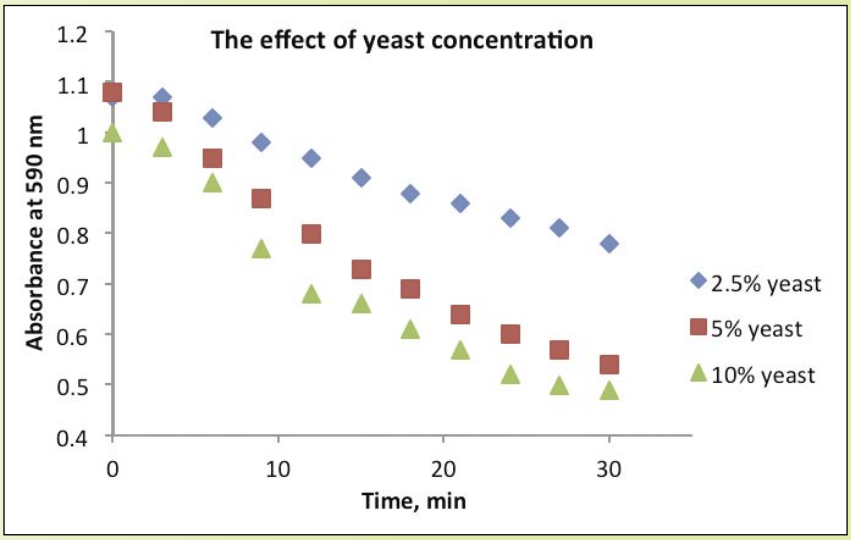


Figure 4 *- The effect of yeast concentration.*

Again students could carry out investigations into respiratory rate in yeast by altering variables such as temperature, respiratory substrate, type of yeast and yeast concentration. A typical set of results using 5% glucose solution, 0.01% resazurin and varying concentrations of yeast suspension is shown in Figure 4.

A protocol detailing the methods for carrying out the basic practical activities discussed here, together with additional data sets and the colour chart, can be found on the SSERC website (3).

References

[1] National 5, Higher Biology and Higher Human Biology Support Notes:
- http://www.sqa.org.uk/files_ccc/CfE_CourseUnitSupportNotes_N5_Sciences_Biology.pdf.
- http://www.sqa.org.uk/files_ccc/CfE_CourseUnitSupportNotes_Higher_Sciences_Biology.pdf.
- http://www.sqa.org.uk/sqa/controller?p_service=Front.search&pContentID=41454&q=Higher%20Human%20Biology.

[2] For example, the Higher Biotechnology Protocols Booklet can be accessed at: http://www.educationscotland.gov.uk/resources/nq/b/nqresource_tcm4339534.asp?strReferringChannel=educationscotland&strReferringPageID=tcm:4-615801-64&class=l1+d86716.

[3] See http://www.sserc.org.uk/index.php/biology-2/biology-resources/higher-human-biology-res/human-cells-h/3767-cellular-respiration?highlight=WyJyZXNhenVyaW4iXQ==SSERC for protocol and data sets.