

2

- 1 A group of students investigated the growth of different varieties of yeast.

The students learned that the rate of respiration in a yeast culture is proportional to the biomass of the culture. Respiration rate can be used as a measure of the growth of a yeast culture.

Respiration rates can be measured using the redox indicator TTC.

- During respiration, hydrogen ions are removed from glucose to reduce hydrogen carriers such as NAD and FAD.
 - A redox indicator can be used as a hydrogen carrier in experimental conditions instead of NAD or FAD.
 - The colour change of the redox indicator can be measured using a colorimeter.
- (a) The students carried out a preliminary experiment using a redox indicator to monitor the growth of a yeast culture over time.

The yeast was grown in a liquid culture in a conical flask, as shown in Fig. 1.1.

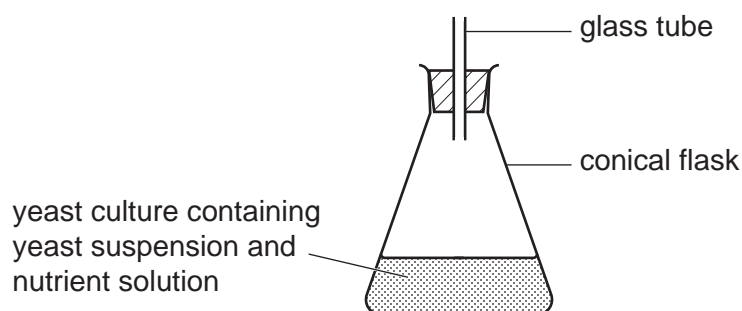


Fig. 1.1

- Different masses of yeast were added to a fixed volume of distilled water to give different concentrations of yeast in suspension.
 - Each yeast suspension was added to a separate flask of nutrient solution containing glucose.
 - A redox indicator was added to each flask and the flasks were incubated at a constant temperature for a fixed period of time.
 - The colour of each suspension was monitored over the incubation period using a colorimeter.
 - A colorimeter passes a beam of light through a coloured filter into a solution and measures the light absorbance of that solution.
 - A standard solution is used to set the colorimeter scale to zero (0) before taking any measurements.
- (i) State the independent variable **and** the dependent variable in this investigation.

independent

dependent

[2]

3

(ii) Identify **two** variables that the students have standardised in their investigation.

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..... [2]

(iii) Suggest a suitable control for this investigation.

..... [1]

(iv) Samples were taken from the flask at intervals and the absorbance was measured in the colorimeter.

As the yeast respire, the redox indicator TTC changes from colourless to pink.

Sketch a graph on Fig. 1.2 to show the expected change in absorbance over time during the incubation of yeast. Label the axes.



Fig. 1.2

[2]

Question 1 continues on page 6

(c) The students found that compressed yeast gave the highest rate of respiration.

The students then carried out two further experiments to find the best conditions for growth of compressed yeast.

In both experiments absorbance was measured in arbitrary units (a.u.). The higher the absorbance the greater the respiration rate. Respiration is proportional to the growth rate of the yeast.

In the first experiment they investigated the effect of changing pH and incubation time at a constant temperature of 30 °C.

The results of the first experiment are shown in Fig. 1.3.

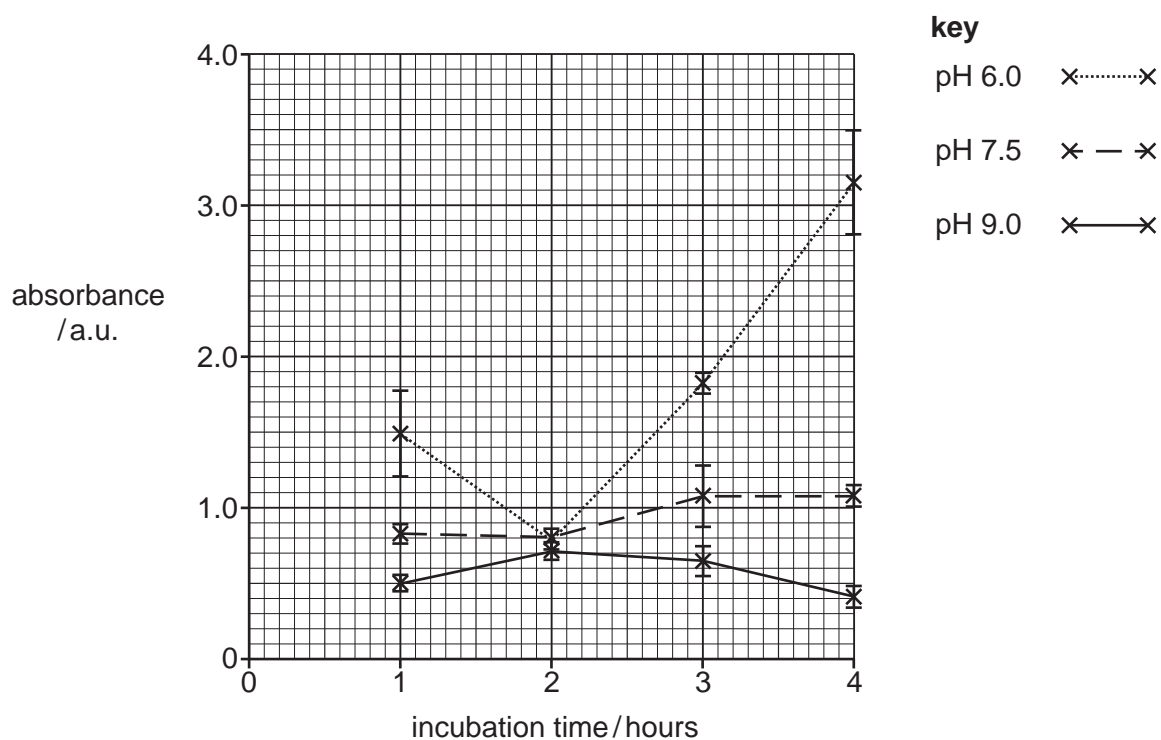


Fig. 1.3

In the second experiment the students investigated the effect of changing pH and temperature at a constant incubation time of 4 hours.

The results of the second experiment are shown in Table 1.1.

Table 1.1

temperature /°C	pH 6.0		pH 7.5		pH 9.0	
	absorbance /a.u.	S_M	absorbance /a.u.	S_M	absorbance /a.u.	S_M
22	2.28	+/- 0.60	1.10	+/- 0.28	1.40	+/- 0.72
30	3.16	+/- 0.28	0.50	+/- 0.04	0.94	+/- 0.02
40	1.10	+/- 0.52	0.40	+/- 0.04	0.54	+/- 0.04
50	0.48	+/- 0.08	0.40	+/- 0.04	0.28	+/- 0.02

- (i) State what the standard error (S_M) shows.

.....
 [1]

- (ii) The graph in Fig. 1.3 shows the 95% confidence intervals for the data.

$$95\% \text{ confidence interval} = +/- 2 \times S_M$$

State what this indicates about the data.

.....

 [1]

- (iii) After completing these two experiments the students concluded that the growth rate of yeast is highest when incubated at 30°C and pH 6.0 for 4 hours.

State **two** ways in which the data support this conclusion.

.....

 [2]

[Total: 18]

2 Populations of European ash trees, *Fraxinus excelsior*, are susceptible to a chronic tree disease called ash dieback. Since 2012, this disease has spread through Europe causing large-scale loss of woodland.

- Ash dieback is caused by a fungal pathogen.
- Symptoms include stem lesions, death of growing shoots and wilting of leaves.
- To limit the spread of the disease, 693 hectares of ash woodland were cleared in the UK between 2012 and 2015. Clearing the woodland involved uprooting the trees and burying them.

Table 2.1 shows the numbers of new cases of ash dieback in a variety of different environments in the UK between 2013 and 2015.

Table 2.1

environment	number of new cases of ash dieback		
	2013	2014	2015
woodland	18	36	56
plant nurseries	16	8	5
private gardens	2	5	0
farmland	13	10	2
roadside	3	5	16
total	52	64	79

- (a) (i) Calculate the percentage change in the number of new cases of ash dieback in plant nurseries, between 2013 and 2015.

Show your working and give your answer to the nearest whole number.

.....% [2]

(ii) Describe **and** explain the differences between the changes in the numbers of cases of ash dieback from 2013 to 2015 in the environments shown in Table 2.1.

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..... [2]

Question 2 continues on page 10

- (b) It was noticed that, in all woodlands infected with ash dieback, there were some trees that were not affected. Investigations were carried out across Europe to identify the genes responsible for tolerance to ash dieback, using a range of techniques including microarrays.

Using the results of this research scientists hope to be able to replant forests with ash trees tolerant to ash dieback.

- Microarrays are used to detect the expression of thousands of genes at the same time.
- A slide is printed with thousands of spots in defined positions.
- Each spot contains single-stranded DNA of known sequence, which acts as a probe to detect gene expression.
- mRNA samples are collected from healthy ash trees and from ash trees showing symptoms of ash dieback.
- Each sample of mRNA is converted to complementary DNA (cDNA) and fluorescently labelled.
- Different colour fluorescent dyes are used for the samples taken from the healthy and diseased trees.
- The two samples are added to the slide and allowed to hybridise onto the DNA on the microarray slide.
- The microarray slide is then scanned to measure the expression of each gene.

Fig. 2.1 shows a typical microarray slide.

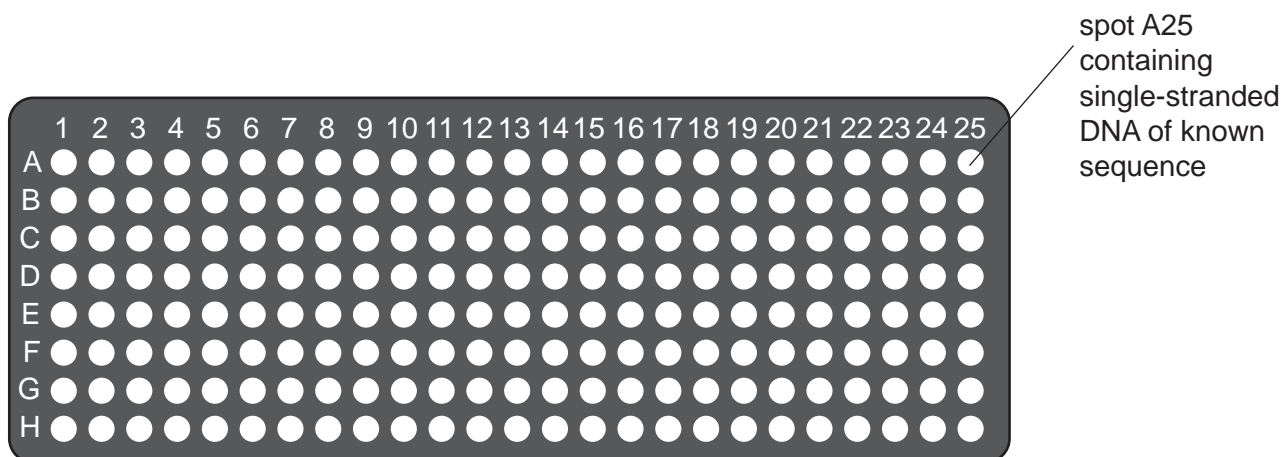


Fig. 2.1

- (i) Explain how the cDNA in samples from the ash trees hybridises onto the microarray slide.

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..... [1]

- (ii) Suggest how the level of each gene expression is determined.

.....
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..... [1]

Question 2 continues on page 12

(c) In a study, trees showing varying degrees of damage caused by ash dieback were assessed and samples were taken.

The trees used in the study were at four locations across Europe.

- Location 1 had 2200 ash trees. Some 10-year-old trees were identified and 44 were sampled.
- Location 2 had 600 ash trees. Some 14-year-old trees were identified and 20 were sampled.
- Location 3 had 379 ash trees and 19 trees of varying ages were sampled.
- Location 4 was the entire woodland in a small European country and 23 ash trees of varying ages were sampled.
- Healthy ash trees growing next to very heavily diseased trees in location 4 were identified and 96 of these trees were sampled.

(i) Explain why it was important that samples from healthy trees were taken from trees growing next to very heavily diseased trees.

.....

 [1]

(ii) Describe how the study ensured that a diverse range of ash trees was selected for sampling.

.....

 [2]

The scientists who carried out the study assessed the degree of damage caused by ash dieback in each ash tree using a six-point scale, as shown in Table 2.2.

Table 2.2

scale point	0	1	2	3	4	5
degree of damage to each tree	little	mild	moderate	high	severe	very severe

- (iii) Suggest why a numeric scale was used to record the degree of damage to the trees caused by ash dieback.

.....

 [1]

The samples collected in the study were analysed by using microarrays. The scientists found that there was variation in one nucleotide location in the ash genome. This nucleotide variation was correlated to the tolerance of ash trees to ash dieback.

Table 2.3 shows a comparison of the base sequence of part of the genome of the European ash tree, *F. excelsior*, showing either low or high tolerance to ash dieback, together with the base sequences of the same part of the genome from other species of ash tree and their tolerance level.

Table 2.3

species of ash tree	tolerance to ash dieback	base sequence
<i>F. excelsior</i>	low	TTGAAAACA
<i>F. excelsior</i>	high	TTGAAAGCA
<i>F. mandshurica</i>	high	TTGAAAGCA
<i>F. americana</i>	high	TTGAAAGCA
<i>F. ornus</i>	high	TTGAAAGCA

- (d) (i) State which base is associated with tolerance to ash dieback.

.....
 [1]

- (ii) Suggest how a change in one nucleotide base could result in a change of tolerance to a pathogen.

.....

 [1]

[Total: 12]

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