

3.2.2 Rates, microscopy and the root-tip squash

Name: _____ Class: _____ Date: _____ Total: 23 marks

KEY WORDS graticule • stage micrometer • mitotic index

Objective

Handle the measurement practicals of Units 1–2: enzyme initial rates (CP4), the microscope and graticule (CP5), the root-tip squash and mitotic index (CP6), plant-section drawings (CP7) and fibre tensile strength (CP8). Original practice for the Unit 3 assessed skills.

1 Key knowledge and worked examples

■ Initial rate from a time course

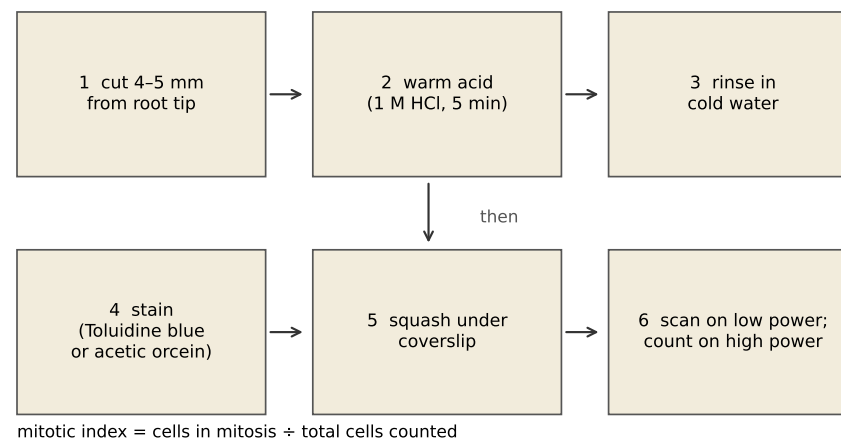
Mix enzyme and substrate at a controlled temperature and pH; sample at fixed intervals; test each sample (iodine for starch). Plot product or substrate against time and read the **initial rate** from the steep start of the curve – or use $1/\text{time}$ to a fixed endpoint as a rate **proxy**, valid only when the endpoint and starting concentrations are identical.

$$\text{rate proxy} = 1/t = 1/40 \text{ s}^{-1} = 0.025 \text{ s}^{-1}$$

■ The graticule converts eyepiece units to real lengths

An eyepiece **graticule** is a ruler in the eyepiece; its divisions mean different real lengths at each magnification, so calibrate it against a **stage micrometer** at the magnification you use. At $\times 400$, if one graticule division = $2.5 \mu\text{m}$, a cell 12 divisions long is $30 \mu\text{m}$.

■ Root-tip squash, then count



Mitotic index = cells in mitosis ÷ total cells counted. Percentages of cells per stage $\times$ total cells gives counts: 80 % of 98 cells = 78.4, reported as 78 – a fraction of a cell cannot be observed.

■ Drawings earn marks by convention

Plan diagrams show tissues, no individual cells, no shading. Cell drawings use clean lines with straight label lines. Record magnification or a scale bar for every drawing. Fibre strength: same length and treatment of fibres, increasing masses until break, compare the mass at break.

2 Practice

2.1 State the four stages of mitosis in order, and name the stage a sample has reached if 80 % of its cells sit condensed on the equator with no separated chromatids. [3]

2.2 A graticule division is 2.5 μm at $\times 400$. A cell spans 14 divisions. Calculate its length, and the length it would span if the same cell were viewed at $\times 100$ where one division equals 10 μm . [3]

2.3 Of 450 cells counted in a squash, 36 are in mitosis. Calculate the mitotic index and, for a 20-hour cell cycle, the time spent in mitosis. [3]

2.4 Explain why iodine is tested on removed samples rather than added into the starch-
amylase mixture. [2]

3 Exam-style questions

3.1 Describe how a root sample is prepared and examined to find the percentage of cells in each stage of mitosis, naming the stain you would use. [4]

3.2 Amylase acts on starch at 25 $^{\circ}\text{C}$ and at 60 $^{\circ}\text{C}$. The endpoint (first negative iodine test) comes at 160 s and 20 s respectively. Calculate both rate proxies, and explain what the comparison does and does not tell you about enzyme structure. [4]

3.3 Fibres extracted from a nettle stem are mounted with masses added until each breaks: untreated fibres break at a mean of 310 g, alkali-treated at 145 g, with ranges of ± 40 g and ± 25 g. Describe an improvement that would tighten these comparisons, and state one conclusion the data supports and one it does not. [4]

Solutions

Original-task marking: M1 method, A1 accuracy, B1 reasoning.

2.1 Prophase, metaphase, anaphase, telophase. Metaphase.

2.2 $14 \times 2.5 \mu\text{m} = 35 \mu\text{m}$. At $\times 100$: $35/10 = 3.5$ divisions.

2.3 Index = $36/450 = 0.08 = 8\%$. Time = $0.08 \times 20 \text{ h} = 1.6 \text{ h}$.

2.4 Iodine would stop or slow the reaction it is measuring, so each sample is a separate small portion tested once, keeping the main mixture's time course intact.

3.1 Cut the terminal 4–5 mm of the root tip; place in warm dilute acid to soften/macerate the tissue; stain with a named stain, e.g. Toluidine blue or acetic orcein; squash under a coverslip and scan, then count on high power the cells in each stage across fields of view.

3.2 $1/160 = 0.00625 \text{ s}^{-1}$ and $1/20 = 0.050 \text{ s}^{-1}$. The 60°C rate is $8 \times$ higher, showing faster activity at this temperature for this enzyme up to that point. It does not establish the temperature optimum or that the enzyme is undamaged – rate at 60°C alone cannot show the denaturation point, which needs a wider temperature range up to and beyond it.

3.3 Improvement: repeat with more fibres of matched length and diameter from the same stem region, reporting the mean with range or SD, mounting masses in equal steps with the same clamping. Supports: alkali treatment reduces tensile strength – the mean break mass is less than half, outside the ranges' overlap. Does not support: any claim about flexibility, commercial fibre quality, or other treatments/species not tested.