IB Biology Investigations

Volume 2 (Higher Level)

COPY MASTERS

(For use with the IB Diploma programme)

(Fourth edition)

Author: Paul Billiet

Series editor: David Greig

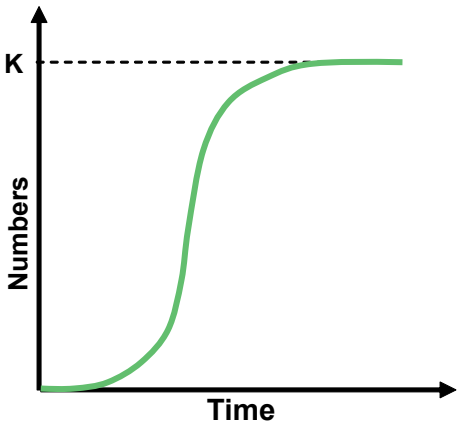
Table of Contents

Standard Level

Higher Level

Syllabus reference: Sub-Topic C.5 Skill

Population growth of simple organisms in ideal conditions can be compared to a model of population growth. The predicted growth curve from these models is the sigmoid-shaped, logistic curve. If we provide a simple unicellular organism, such as baker's yeast (*Saccaromyces cerevisiae*) with ideal conditions do we get the same shaped curve?



Materials

- microscope
- 2 × 100cm³ conical flasks (sterilised)
- haemocytometer
- electronic balance
- physiological saline (0.9% NaCl)
- pasteur pipette
- 2 × 10cm³ pipette + pumps
- cotton wool
- incubator at 30°C
- 10% glucose solution
- fresh baker's yeast

Method

1. Preparation of the yeast stock suspension: Add 1g of yeast to 10cm³ of physiological saline solution in a conical flask. Swirl to mix and leave for 10 minutes to hydrate.
2. Swirl the yeast stock suspension to mix. Take 1 drop of suspension and add to 10cm³ of 10% glucose solution in a flask and mix the contents by swirling the flask. Close the flask using cotton wool. Keep this flask at 30°C for the duration of the experiment. Set up a second flask of glucose solution and a drop of yeast stock suspension in the same way.

Use of the haemocytometer

The haemocytometer is mostly used to count blood cells but it is also useful to count small cells such as yeast cells.

The counting grid

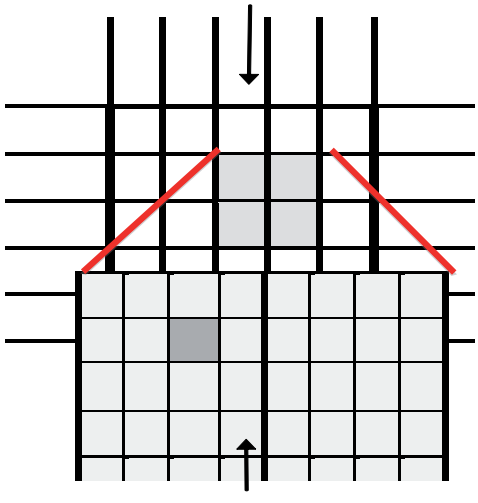
The counting grid can be seen on the centre of the bar in the middle of the slide. On either side of the bar there is a groove. The central bar is slightly lower than the rest of the slide. When the cover slip is laid over it, **a gap of 0.1mm depth** is left between the bar and the cover slip. Etched onto the centre of the bar is a counting grid that appears in the form of a cross.

In the centre of the counting grid is a square (outlined in **bold** in the diagram opposite). This square has a surface of 1mm × 1mm. This square is made up of 25 smaller squares, **0.2mm × 0.2mm each**.

Each of the 25 squares is made up of 16 smaller squares. Four of these squares are shaded in the diagram above. These four squares appear in the diagram opposite. Each of the 16 squares has a surface area of **0.05mm × 0.05mm**. These squares are the smallest squares that you will see. Examine the counting grid under the ×10 objective lens of the microscope.

Calculate the **volumes** of the different sized squares.

Each of these squares has a surface area of 0.2mm x 0.2mm

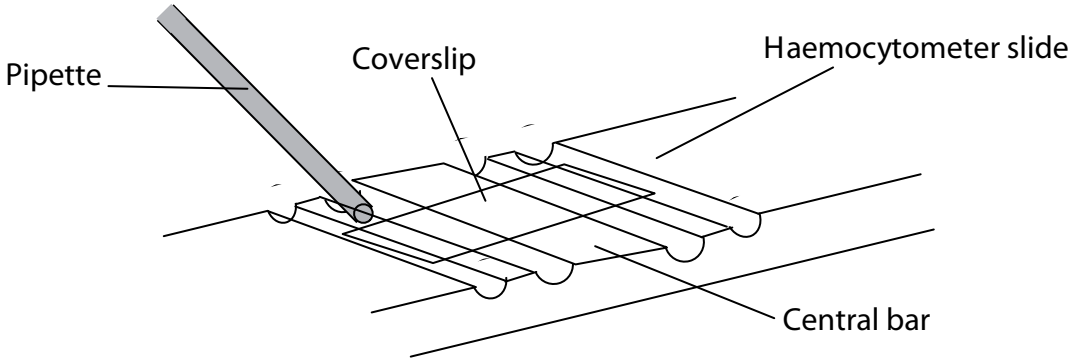


Each of these squares has an area of 0.05mm x 0.05mm

Setting up the haemocytometer

Place the coverslip on the haemocytometer.

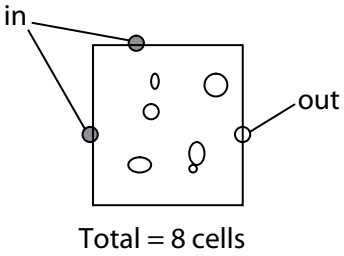
Pipette a drop of the yeast stock suspension onto the haemocytometer. Do this by holding the pipette on the edge of one of the grooves, at the edge of the central bar (see below).



Slowly push the solution out so that it travels along the groove and across the central bar. Wipe off any excess liquid.

Running a test count to familiarise yourself with counting yeast cells

1. Count the number of yeast cells found in one of the smallest squares.
2. Yeast cells that fall on the edges of the square should be counted "in" on two of the edges. For example, the cells falling on the top and left hand side (the shaded cells). Those on the other edges are not counted (the unshaded cells) *See opposite.*
3. For the yeast cells that are budding, count the buds as individual cells.
4. Now remove the cover slip and rinse the haemocytometer and cover slip with distilled water. Dry the haemocytometer using the lens tissue.



You are ready for your first count: Day 0

1. Take one of the prepared flasks from the oven. Swirl the flask. Using a clean Pasteur pipette take a few drops of the culture solution.
2. Set up the haemocytometer as before and count the number of yeast cells within a counting area made up of a group of four of the smallest squares.
3. Repeat the count in two other areas of the same size. Record the average count per area and the average number of cells per mm^3.
4. Repeat the counts using the second flask. Note that the initial dilution of the suspension is not taken into account.
5. Repeat the counts at regular times, once a day. Continue for a period of at least 15 days.
6. Represent the results in a graph of incubation time against number of yeast cells per mm^3.
7. Mark the different stages of growth observed on the graph and explain each of the stages observed.
8. Does the growth curve of the yeast population fit the predicted of the model?

Simulating exponential growth in a spread sheet

Estimates for the cell cycle of Saccaromyces cerevisiae vary from 80 to 100 minutes under ideal conditions. Taking 90min as a mean value, set up a spread sheet showing generations, time and yeast cell numbers.

Type in the first two rows for generation and time and select these cells. Move the mouse to the bottom right corner of the selected cells, click left and pull down. This will give you a series for as many minutes as you wish (16 generations is 24h).

Suppose that your average count for the yeast cells at the beginning of your experiment is 8 cells per grid on the haemocytometer. Enter this in the first cell under Cell count. Then type in the formula = "click" on cell C2 then *2. Press enter and it will calculate the number you would expect per cell after one generation (90min).

Select this cell and pull down on the bottom right hand corner again to repeat the calculation.

Assuming the volume above the grid on the haemocytometer being used is 0.00025mm^3 (this is true for the Thoma haemocytometer). Calculate the number of yeast cells per mm^3 entering the equation =C2*4000 press enter and pull down on the bottom right of the cell again.

Then plot a graph of time or generations against numbers of yeast cells per mm^3.

	A	B	C	D
1	Generation	Time / min	Cell count	Cell concentration / numbers mm^{-3}
2	0	0		
3	1	90		
4				
5				
6				
7				
8				
9				
10				

	A	B	C	D
1	Generation	Time / min	Cell count	Cell concentration / numbers mm^{-3}
2	0	0	8	
3	1	90	=C2*2	
4	2	180		
5	3	270		
6	4	360		
7	5	450		
8	6	540		
9	7	630		
10	8	720		

	A	B	C	D
1	Generation	Time / min	Cell count	Cell concentration / numbers mm^{-3}
2	0	0	8	
3	1	90	16	
4	2	180	32	
5	3	270	64	
6	4	360	128	
7	5	450	256	
8	6	540	512	
9	7	630	1024	
10	8	720	2048	

	A	B	C	D
1	Generation	Time / min	Cell count	Cell concentration / numbers mm^{-3}
2	0	0	8	=C2*4000
3	1	90	16	
4	2	180	32	
5	3	270	64	
6	4	360	128	
7	5	450	256	
8	6	540	512	
9	7	630	1024	
10	8	720	2048	

Some points for consideration

- Suggest various factors that might influence the rate of growth of the yeast cells.
- A similar curve is seen when plotting the average numbers of yeast cells in a counting area against the incubation time. Why were you asked to plot the number of cells per mm^3?
- In what other ways could the data obtained be analysed, for example, could other graphs have been drawn?

Research

What industrial processes use information similar to that obtained in this investigation?

IB Biology Investigations

Volume 2 (Higher Level)

TEACHING NOTES

(For use with the IB Diploma programme)

(Fourth edition)

Author: Paul Billiet

Series editor: David Greig

SL

Syllabus Correlation Table (Core)

Core Topic
Practical Number
Sub topic

		Syllabus reference		Title
1	1A	1.1	**Skill:** Use of a light microscope to investigate the structure of cells and tissues, with drawing of cells. Calculation of the magnification of drawings and the actual size of structures and ultrastructures shown in drawings or micrographs. (Practical 1)	CELLS
	1B	1.4	**Skill:** Estimation of osmolarity in tissues by bathing samples in hypotonic and hypertonic solutions. (Practical 2)	ESTIMATING THE WATER POTENTIAL OF PLANT TISSUES
	1C		**Aim**: Dialysis tubing experiments can act as a model of membrane action. Experiments with potato, beetroot or single-celled algae can be used to investigate real membranes.	MEMBRANE INTEGRITY IN CABBAGE LEAF CELLS
	1D	1.6	**Skill:** Identification of phases of mitosis in cells viewed with a microscope or in a micrograph.	THE CELL CYCLE IN PLANT TISSUES
			Skill: Determination of a mitotic index from a micrograph.	
2	2A	2.1	**Skill:** Identification of biochemicals such as sugars, lipids or amino acids from molecular diagrams.	DIALYSIS: SEPARATING MOLECULES BY SIZE
	2B	2.2	**Application:** Use of water as a coolant in sweat.	WATER AS A COOLANT
	2C	2.3	**Skill:** Use of molecular visualization software to compare cellulose, starch and glycogen.	POLYSACCHARIDE MOLECULES
	2D	2.5	**Skill:** Experimental investigation of a factor affecting enzyme activity. (Practical 3)	MEASURING THE RATE OF REACTION OF AN ENZYME CONTROLLED REACTION
	2E	2.5, 2.8	**Application:** Use of anaerobic cell respiration in yeasts to produce ethanol and carbon dioxide in baking.	IMMOBILISING YEAST ENZYMES
	2F	2.8	**Skill:** Analysis of results from experiments involving measurement of respiration rates in germinating seeds or invertebrates using a respirometer.	RESPIRATION RATES OF AN INVERTEBRATE
	2G	2.9	**Skill:** Separation of photosynthetic pigments by chromatograph. (Practical 4)	LEAF PIGMENTS, THEIR EXTRACTION AND SEPARATION
	2H		**Skill:** Drawing an absorption spectrum for chlorophyll and an action spectrum for photosynthesis	USING SPECTROSCOPY TO STUDY LEAF PIGMENTS
	2I		**Skill:** Design of experiments to investigate the effect of limiting factors on photosynthesis.	METHODS TO MEASURE THE RATE OF PHOTOSYNTHESIS
3	3A	3.1	**Skill:** Use of a database to determine differences in the base sequence of a gene in two species.	USING A PROTEIN DATABASE
	3B	3.5	**Skill:** Design of an experiment to assess one factor affecting the rooting of stem-cuttings.	FACTORS AFFECTING ROOTING IN PLANTS
4	4A	4.1	**Skill:** Setting up sealed mesocosms to try to establish sustainability. (Practical 5)	ECOLOGICAL SUCCESSION IN A MICROBIAL ECOSYSTEM
	4B		**Skill:** Testing for association between two species using the chi-squared test with data obtained by quadrat sampling.	PLANT ASSOCIATION TEST
5	5A	5.3	**Application:** Recognition features of bryophyta, filicinophyta, coniferophyta and angiospermophyta	DICHOTOMOUS KEY FOR PLANT PHYLA
			Skill: Construction of dichotomous keys for use in identifying specimens.	
6	6A	6.1	**Application:** Use of dialysis tubing to model absorption of digested food in the intestine.	DIALYSING TUBING GUT
	6B	6.2	**Skill:** Recognition of the chambers and valves of the heart and the blood vessels connected to it in dissected hearts or in diagrams of heart structure.	THE ANATOMY OF THE HEART
	6C	6.4	**Skill:** Monitoring of ventilation in humans at rest and after mild and vigorous exercise. (Practical 6)	VENTILATION AND EXERCISE

HL

Syllabus Correlation Table (AHL)

AHL Topic
Practical Number
Sub topic

		Syllabus reference		Title
7	7A	7.1	**Skill:** Utilization of molecular visualization software to analyse the association between protein and DNA within a nucleosome.	THE NUCLEOSOME
8	8A	8.1	**Skill:** Distinguishing different types of inhibition from graphs at specified substrate concentration.	INHIBITORS OF UREASE
9	9A	9.1	**Skill:** Measurement of transpiration rates using potometers. (Practical 7) **Skill:** Design of an experiment to test hypotheses about the effect of temperature or humidity on transpiration rates.	TRANSPIRATION IN PLANTS
	9B		**Aim:** Measurement of stomatal apertures and the distribution of stomata using leaf casts, including replicate measurements to enhance reliability are possible experiments.	STOMATA: THE SITE OF TRANSPIRATION IN PLANTS
	9C	9.1 & 9.2	**Skill:** Drawing the structure of primary xylem vessels in sections of stems based on microscope images. **Skill:** Identification of xylem and phloem in microscope images of stem and root.	CELL TYPES AND TISSUES IN THE PETIOLE OF CELERY
	9D	9.4	**Skill:** Drawing of half-views of animal-pollinated flowers.	THE STRUCTURE AND ADAPTATIONS OF FLOWERS
	9E		**Skill:** Drawing internal structure of seeds. **Skill:** Design of experiments to test hypotheses about factors affecting germination.	SEED STRUCTURE AND VIABILITY
10	10A	10.2	**Skill:** Use of a chi-squared test on data from dihybrid crosses.	USING CHI^2 TEST TO ANALYSE INHERITANCE PATTERNS
11	11A	11.3	**Application:** Consequences of dehydration and over hydration.	HOMEOSTASIS AND RED BLOOD CELLS

SL HL

Syllabus Correlation Table (Options)

Option Topic	Practical Number	Sub topic	Syllabus reference	Title
A	12A	A.2	**Application:** Use of the pupil reflex to evaluate brain damage.	THE PUPIL REFLEX
	12B	A.3	**Application:** Red-green colour-blindness as a variant of normal trichromatic vision.	THE RETINA AND COLOUR VISION
	12C	A.4	**Skill:** Analysis of data from invertebrate behaviour experiments in terms of the effect on chances of survival and reproduction.	STUDYING INVERTEBRATE BEHAVIOUR
	12D	A.4	**Aim:** Data logging using an ECG sensor to analyse neuromuscular reflexes.	RECORDING AN EMG
B	13A	B.1	**Skill:** Experiments showing zone of inhibition of bacterial growth by bactericides in sterile bacterial cultures.	DO DISINFECTANTS KILL BACTERIA?
	13B		**Skill:** Gram staining of Gram-positive and Gram-negative bacteria.	THE GRAM STAIN FOR BACTERIA
	13C	B.5	**Skill:** Use of software to align two proteins.	COMPARING PROTEINS FROM DIFFERENT SPECIES
	13D		**Skill:** Use of software to construct simple cladograms and phylograms of related organisms using DNA sequences	MAKING A CLADOGRAM FROM PROTEIN SEQUENCES
C	14A	C.1	**Skill:** Use of a transect to correlate the distribution of plant or animal species with an abiotic variable.	USING INVERTEBRATE PITFALL TRAPS ON A LINE TRANSECT
	14B	C.2 &4	**Skill:** Investigation into the effect of an environmental disturbance on an ecosystem. Skill: Analysis of the biodiversity of two local communities using Simpson's reciprocal index of diversity.	MEASUREMENT OF A DIVERSITY INDEX AND A BIOTIC INDEX
	14C	C.5	**Skill:** Modelling the growth curve using a simple organism such as yeast or species of *Lemna*.	POPULATION GROWTH OF YEAST CELLS
D	15A	D.1	**Skill:** Determination of the energy content of food by combustion.	ENERGY FROM FOOD
	15B	D.4	**Skill:** Measurement and interpretation of the heart rate under different conditions.	HEART RATE AND BLOOD PRESSURE
			Skill: Interpretation of systolic and diastolic blood pressure measurements.	
	15C		**Skill:** Mapping of the cardiac cycle to a normal electrocardiogram (ECG) trace.	TAKING AND READING AN ELECTROCARDIOGRAM
	15D	D.6	**Skill**: Analysis of dissociation curves for hemoglobin and myoglobin. **Application**: Consequences of high altitude for gas exchange.	MYOGLOBIN AND HEMOGLOBIN

APPENDICES	1	6, 11 & D	The IB animal experimentation policy and the biology course safety guidelines.	INFORMED CONSENT FORM
	2	4.1	**Guidance:** Sampling should be based on random numbers. In each quadrat the presence or absence of the chosen species should be recorded.	TABLE OF RANDOM NUMBERS
	3			USING A TI CALCULATOR TO GENERATE RANDOM NUMBERS

14C POPULATION GROWTH IN YEAST

Time: 1 hour to set up and 15 minute counting periods.

Syllabus reference: Option C.5

Skill: Modelling the growth curve using a simple organism such as yeast or species of *Lemna*.

Materials

Stock solution of yeast

Dried yeast is practical to use although other kinds, for example, fresh bakers yeast, could also be used.

Physiological saline may be available in the pharmacy or a 0.9% aqueous solution of sodium chloride.

The counting grid referred to here is the **Neubauer** (improved). Other counting grids commonly used are the Malassez or Thoma. Many types exist.

Malassez: The distance between the central bar and the cover slip is 0.2mm. The surface area of the smallest squares seen is 0.05mm × 0.05mm giving a volume of 0.0005mm^3.

Thoma (new): Similar dimensions apply in the "new" grid: the distance between the central bar and the cover slip is 0.1mm. The smallest square seen is 0.05mm × 0.05mm giving a volume of 0.00025mm^3.

Method

Finding time available to carry out the counting may be difficult depending on the facilities available.The counting period should be as many days as possible. In trial runs good results were obtained during a counting period of 12-18 days. This varies depending on the type and state of the yeast cells and the handling of the yeast culture. It is important to maintain a steady temperature, as yeast cells are sensitive to temperature shock. The amount of glucose, oxygen or contamination by other microbes will also influence results.

Sterile flasks and sterile Pasteur pipettes are used at the beginning to minimise initial contamination of the culture. Sterile cotton wool from a packet can be used. After this, sterile techniques could be used, although, in the testing of this experiment good results were obtained and contamination was only observed after 18 days. If sterile techniques are to be used, the cotton wool should be passed through Bunsen flame each time it is removed and also the neck of the flask before replacing the cotton wool and sterile pipettes should be used each time. The workbench should also be cleaned with bleach for example and all manipulation carried out in an area close to a Bunsen Burner flame.

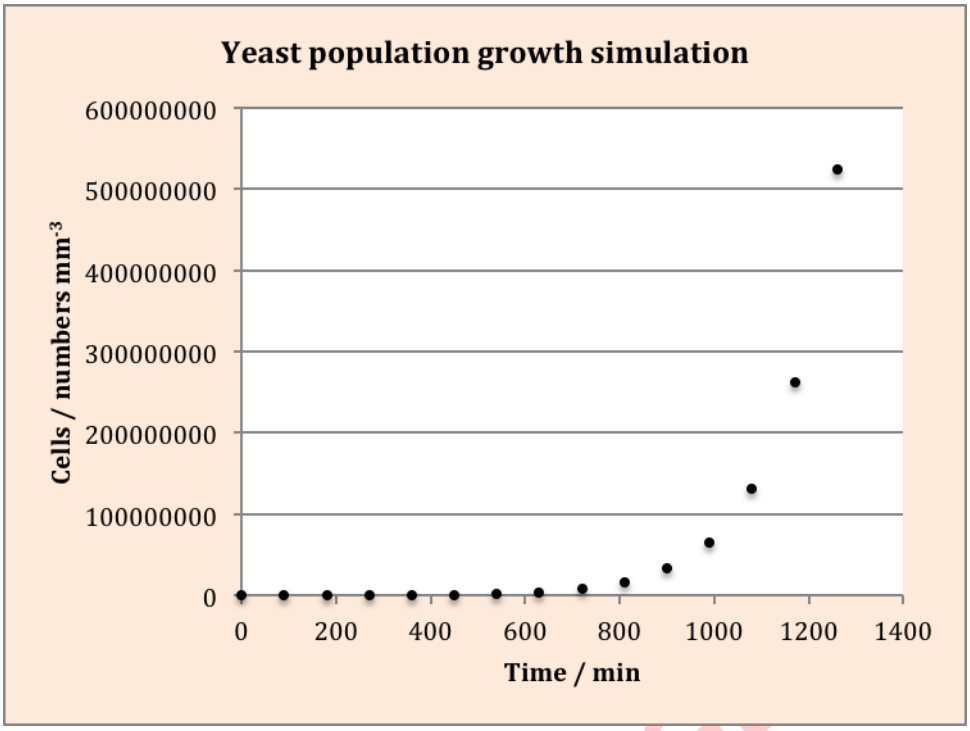
Students may need help in locating the correct squares in which to count.

Simulating exponential growth in a spread sheet

The estimates suggested for yeast cell cycle duration were taken from:

<http://mpf.biol.vt.edu/research/budding_yeast_model/pp/wt_glucose.php>

The results below show the exponential growth curve for 21h (14 generations at 90 min per generation).



Some points for consideration

- Various factors influencing the rate of growth of yeast cells: temperature, oxygen or absence of oxygen, substrate concentration, other minerals present and space.
- Plotting the numbers of yeast cells in mm^3 instead of numbers per $0.00025mm^3$ is a more practical unit to use. If either units of volume are used, this is better than a relative density i.e. 'numbers per counting area'.
- Rate of growth could be determined by plotting, for example: growth increments (the number by which the colony increased per day) **v** time unit.

To investigate further

Many variables could be investigated:

- The concentration of the glucose
- The substrate used (other sugars could be tried: fructose, lactose, sucrose, etc.)
- Incubation temperature
- The size or shape of the culture containers
- The volume of culture solution used
- Aerobic and anaerobic conditions

These are **Sample Pages** only from the fourth edition of one of the IBID Science Volumes. This Volume is part of a series of full-colour **Copy Masters** as follows:

SUBJECT	VOLUME
BIOLOGY	Standard Level Investigations with Teaching Notes
	Higher Level Investigations with Teaching Notes
	Internal Assessment Handbook
CHEMISTRY	Standard Level Investigations with Teaching Notes
	Higher Level Investigations with Teaching Notes
	Internal Assessment Handbook
PHYSICS	Standard Level Investigations with Teaching Notes
	Higher Level Investigations with Teaching Notes
	Internal Assessment Handbook

These Volumes have been prepared and published specifically to support the teaching of the **IB Diploma Sciences**. They are for sale as either as paper copies or secure pdf files and may only be used by the purchasing school under the conditions of sale.

More information may be obtained from our website
<*www.ibid.com.au*>