Core practical 12: Investigate the rate of growth of microorganisms in liquid culture

Objectives

- To understand how microorganism growth rate in liquid culture can be measured
- To be able to culture microorganisms with due regard for safe practice

Safety

- The culture of yeast is a possible biological hazard as there is potential for contamination. A single yeast culture flask should be set up for the class.
- If samples are taken this should be over a period of no more than 12 hours.
- Wear eye protection.
- Use aseptic techniques when transferring the yeast to or from the culture flask.
- Disinfect the bench with 1% Virkon® or equivalent. Leave the disinfectant on the bench for about 10 minutes. This should be done before and after working.
- Ensure that all equipment is returned to the designated point for sterilisation or disposal.

Specification links

- Practical techniques 1, 3, 8, 9, 12
- CPAC 1a, 2a, 3b, 4a, 4b, 5a

Procedure

You will use an optical method to study the growth of yeast in a liquid culture. The more yeast cells in a culture, the more turbid the culture. This means that less light can pass through the sample. A colorimeter can be used to measure turbidity in samples taken at regular intervals. The absorbance of light is measured. This is sometimes also called optical density or OD. If using a light sensor and datalogger a light is shone through the culture, and the light coming through to the other side is measured using a light meter or sensor which is connected to a datalogger and recorded automatically over time.

Steps 4 to 6 provide the data required to draw an indirect growth curve for the culture and calculate the exponential growth rate constant. Steps 7 to 12 are required to calculate yeast cell numbers (as cell densities) which can be used to produce a direct growth curve showing cell numbers.

It is important to follow aseptic technique throughout to avoid contamination of your cultures, of yourself and of the surrounding environment. The method below includes some precautions but they are not exhaustive, requirements will depend to some extent on the method used.

1. Wash your hands with soap and disinfect

Notes on procedure

- It is important that students have practised the required aseptic techniques before the practical task. Teachers should be familiar with microbiology techniques. Ensure that good practice (e.g. flaming of culture flask neck, use of sterile materials, stoppering of culture flask with non-absorbent cotton wool, autoclaving of equipment and culture flask) is carried out.
- Plan the timing of this practical activity carefully, especially if students will be using a colorimeter.
- A single culture flask should be set up for the whole class and inoculated with yeast during the task introduction. Incubate at room temperature (no greater than 25 °C).
- Two different methods are described: measuring changes in turbidity using either a) a colorimeter or b) a light sensor plus datalogger.

If using a colorimeter

- The absorbance of samples should be measured initially and then at timed intervals, ideally every 20–30 minutes for the first couple of hours and then approximately hourly, completing at least six measurements during a period of 5–12

your bench area, leaving it to soak for 10 minutes if using Virkon®, before wiping it down. Light a Bunsen burner set to a safety flame on the disinfected working area.

2. Your teacher will carry out this step to produce a single culture flask for the whole class.
   - Use a sterile clear-sided 500 cm^3 conical flask containing 250 cm^3 of 0.5% glucose solution as the culture vessel. This should be stoppered with cotton wool and should contain a sterile magnetic stirring 'flea'.
   - Using aseptic technique to prevent contamination, add 1.25 g of yeast to inoculate your culture vessel. Swirl to mix.
3. Place the flask on a magnetic stirrer and stir continuously. Cover the cotton wool stopper loosely with aluminium foil and incubate at room temperature.

If using a colorimeter

4. Fill a cuvette with glucose culture medium and use this as a blank to set the absorbance of the colorimeter to zero. Keep the glucose solution (the blank) in a refrigerator between measurements.
5. As soon as the yeast suspension has been mixed, use aseptic technique to measure a 3 cm^3 sample into a clean measuring cylinder. Transfer the sample immediately into a cuvette (which should be just over half full) and measure the absorbance. Record time and absorbance in a table. Return the stoppered yeast culture to the stirrer.
6. Repeat steps 4–6 at least five times over the next 12 hours. Take samples at intervals of 20–30 minutes over the first 2 hours. Do not remove any samples after 12 hours of culturing.

If using a light sensor and datalogger

7. Place a cool light source to one side of the yeast culture. Place a light sensor at the opposite side, very close to the culture flask.
8. Place a carboard box, with a cut-out to let in light from the light source, over the flask, stirrer and light sensor. This will protect the sensor from ambient light.
9. Connect the light sensor to a datalogger and leave to record continuously for between 10 and 24 hours.

hours. A culture period of 9–10 hours will probably be needed to reach the stationary phase, but sufficient growth should occur within the first 5 hours to enable the exponential growth rate to be estimated. A yeast culture could be set up in a morning lesson and then sampled throughout the day by different groups of students. Ensure adequate supervision of sampling if this takes place outside of normal lesson times.

- If the OD rises above an absorbance value of 2, the accuracy will be lost. It may therefore be necessary to dilute later samples to bring them within the range. This dilution must then be accounted for in calculating the absorbance: multiply the reading by the dilution factor.

If using a light sensor and datalogger

- With continual datalogging the timing issues can be avoided as there is no need to take samples. Once the culture flask has been set up there is no need to remove the cotton wool stopper, so contamination and safety risks are reduced. In this case the culture can be left for up to 24 hours, provided that the culture flask is not opened during this time and is transferred directly to the autoclave or pressure cooker.
- The exponential growth rate constant calculated from this method will be negative (because light levels decline). The sign of the constant can be ignored.

Extension activity

- This allows students to calibrate results by determining the cell density of the initial microorganism population. If using the datalogger method there will be more time for students to carry out the extension activity.
- Once cell density of the initial culture has been determined, students can calculate the number of microorganisms that will result after a given time period during the logarithmic growth phase.

  Using the formula: $N_t = N_0 \times 2^{kt}$

  Where:
  - N_t is the number of organisms at time t
  - N_0 is the number of organisms at time 0
  - k is the exponential growth rate constant
  - t is the time the colony has been growing.

Extension – Relating the absorbance or light readings to the density of yeast cells in culture

10. The initial density of cells in the culture (for 1.25 g of yeast in 250 cm^3 solution) can be estimated using a haemocytometer and manufacturer's instructions.
11. Alternatively find the area of your microscope's field of view using a slide of mm graph squares on acetate. Count the squares visible using the ×4 objective. Now calculate the area of the field of view for the ×40 objective which is 0.01 of the area of the ×4 objective (i.e. the square of 4/40 as this is area).
12. Stain the yeast suspension with a few drops of 0.1% methylene blue. Using a 1 cm^3 plastic pipette, place one drop of this yeast suspension on to a microscope slide and cover with a coverslip. View under the ×40 objective and count the yeast cells that you can see within the one field of view.
13. Calculate the volume of one pipette drop by measuring the volume of 10 drops. This gives the volume under the area of the coverslip. The volume under one field of view of the ×40 objective can then be calculated (divide the area of the ×40 field of view by the area of the coverslip then multiply this figure by the volume of one drop).
14. Work out the initial yeast population cell density (cells/mm^3). This is average cell count in one field of view divided by the volume of that field of view.
15. If many cells overlap when viewed under ×40 objective, then counts will not be accurate and dilution of the yeast suspension may be required.
16. **Wash your hands and disinfect surfaces before leaving the laboratory**.

Answers to questions

1. Calibrate OD readings using either dilution plating or direct counting (using a haemocytometer). Produce a calibration curve for a range of OD readings and use the graph to estimate the number of microorganisms present.
2. The method is valid because most of the change in turbidity will be a result of microorganism cells in suspension. However, the method does not distinguish between live cells and other particles in suspension such as dead cells, spores or cell fragments, which reduces the validity.
3. The magnetic stirrer keeps cells in suspension and ensures that nutrients are evenly distributed. It also oxygenates the culture to allow aerobic respiration.

Sample data

Time/hours	OD culture 1	OD culture 2	OD culture 3
0.00	0.06	0.04	0.05
0.75	0.05	0.07	0.07
2.25	0.19	0.28	0.25
3.75	0.66	0.87	0.85
5.25	1.27	1.10	1.30
6.75	1.47	1.16	1.32
8.25	1.53	1.18	1.40
9.75	1.65	1.23	1.55
11.25	1.78	1.30	1.72
15.00	1.97	1.29	1.90

Table A Changes in optical density against time in three cultures of yeast *Saccharomyces* sp. incubated at 25 °C.

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Safety

- The culture of yeast is a possible biological hazard as there is potential for contamination. A single yeast culture flask will be set up for the class.
- If samples are removed this should be over a period of no more than 12 hours.
- Wear eye protection.
- Use aseptic techniques when transferring the yeast to or from the culture flask.
- Disinfect the bench with 1% Virkon® or equivalent. Leave the disinfectant on the bench for about 10 minutes. This should be done before and after working.
- Ensure that all equipment is returned to the designated point for sterilisation or disposal.

All the maths you need

- Recognise and make use of appropriate units in calculations.
- Recognise and use expressions in decimal and standard form.
- Estimate results.
- Use calculators to find and use power, exponential and logarithmic functions.
- Use an appropriate number of significant figures.
- Substitute numerical values into algebraic equations using appropriate units for physical quantities.
- Use logarithms in relation to quantities that range over several orders of magnitude.
- Translate information between graphical, numerical and algebraic forms.
- Plot two variables from experimental or other data.
- Calculate rate of change from a graph showing a linear relationship.
- Draw and use the slope of a tangent to a curve as a measure of rate of change.

Equipment

- bench disinfectant, paper towels or cloth
- Bunsen burner

either

- colorimeter set to absorbance (with filter of around 600 nm wavelength)
- colorimeter cuvettes
- 5 cm^3 measuring cylinders or 2000 µl automatic pipette

or

- light source, light sensor and datalogger

for the yeast culture (one for whole class):

- dried baker's yeast (1.25 g)
- 250 cm^3 of culture medium (0.5% glucose solution) in a 500 cm^3 sterile conical flask
- small amount of glucose culture medium to blank the colorimeter
- sterilised magnetic stirrer
- non-absorbent cotton wool and aluminium foil

for counting cell densities:

- microscope, with slide and coverslip
- dropper pipette
- mm^2 graph paper photocopied on to acetate

Diagram

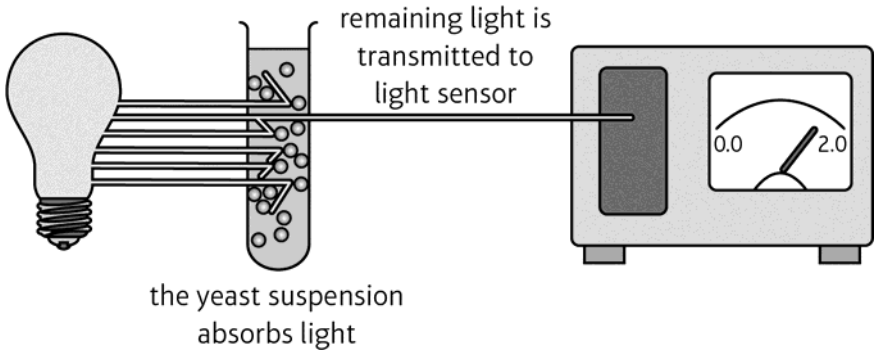

fig A Turbidity measurements can be used to measure a population of microorganisms indirectly. With a colorimeter, the higher the absorbance of light, the more yeast cells are present. Using a light sensor and datalogger, the higher the transmitted light reading, the fewer yeast cells are present.

Procedure

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It is important to follow aseptic technique throughout to avoid contamination of your cultures, of yourself and of the surrounding environment. The method below includes some precautions but they are not exhaustive; requirements will depend to some extent on the method used.

1. Wash your hands with soap and disinfect your bench area, leaving it to soak for 10 minutes if using Virkon®, before wiping it down. Light a Bunsen burner set to a safety flame on the disinfected working area.
2. Your teacher will carry out this step to produce a single culture flask for the whole class.
   - Use a sterile clear sided 500 cm^3 conical flask containing 250 cm^3 of 0.5% glucose solution as the culture vessel. This should be stoppered with cotton wool and should contain a sterile magnetic stirring 'flea'.
   - Using aseptic technique to prevent contamination, add 1.25 g of yeast to inoculate your culture vessel. Swirl to mix.
3. Place the flask on a magnetic stirrer and stir continuously. Cover the cotton wool stopper loosely with aluminium foil and incubate at room temperature.

If using a colorimeter

4. Fill a cuvette with glucose culture medium and use this as a blank to set the absorbance of the colorimeter to zero. Keep the glucose solution (the blank) in a refrigerator between measurements.
5. As soon as the yeast suspension has been mixed, use aseptic technique to measure a 3 cm^3 sample into a clean measuring cylinder. Transfer the sample immediately into a cuvette (which should be just over half full) and measure the absorbance. Record time and absorbance in a table. Return the stoppered yeast culture to the stirrer.
6. Repeat steps 4–6 at least five times over the next 12 hours. Take samples at intervals of 20–30 minutes over the first 2 hours. Do not remove any samples after 12 hours of culturing.

If using a light sensor and datalogger

7. Place a cool light source to one side of the yeast culture. Place a light sensor at the opposite

side, very close to the culture flask.

8. Place a carboard box, with a cut-out to let in light from the light source, over the flask, stirrer and light sensor. This will protect the sensor from ambient light.
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Extension - Relating the absorbance or light readings to the density of yeast cells in culture

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11. Alternatively find the area of your microscope's field of view using a slide of mm graph squares on acetate. Count the squares visible using the ×4 objective. Now calculate the area of the field of view for the ×40 objective which is 0.01 of the area of the ×4 objective (i.e. the square of 4/40 as this is area).
12. Stain the yeast suspension with a few drops of 0.1% methylene blue. Using a 1 cm^3 plastic pipette, place one drop of this yeast suspension on to a microscope slide and cover with a coverslip. View under the ×40 objective and count the yeast cells that you can see within the one field of view.
13. Calculate the volume of one pipette drop by measuring the volume of 10 drops. This gives the volume under the area of the coverslip. The volume under one field of view of the ×40 objective can then be calculated (divide the area of the ×40 field of view by the area of the coverslip then multiply this figure by the volume of one drop).
14. Work out the initial yeast population cell density (cells/mm^3). This is average cell count in one field of view divided by the volume of that field of view.
15. If many cells overlap when viewed under ×40 objective, then counts will not be accurate and dilution of the yeast suspension may be required.
16. **Wash your hands and disinfect surfaces before leaving the laboratory**.

Analysis of results

1. Plot a graph of absorbance or light reading against time.
2. Identify the lag and exponential (log) phases of the growth curve on your graph.
3. From the exponential (log) phase of the growth curve, estimate the time taken in hours for the population size to double.
4. Calculate the exponential growth rate constant using the formula:

$$k = \frac{\log_{10}OD_1 - \log_{10}OD_0}{\log_{10}2 \times t}$$

Where:

- OD_0 is the absorbance or light reading at the start of the log phase
- OD_1 is the absorbance or light reading after time t (use a reading towards the end of log phase)
- t is the time the culture has been growing.

5. If you have time, re-plot the absorbance–time graph using a semi-logarithmic scale. This can be done using appropriate graph paper, or more easily by entering the results into a spreadsheet and using the graphing functions. In popular drawing packages, this may be achieved by drawing the graph then clicking on the axis and selecting 'logarithmic scale'. Examine the two graphs and compare them with the examples in Student Book Section 6.1.3, fig A.

Learning tip

- When we talk about the growth of microbe populations we are referring to the increase in the number of cells. The growth rate of microorganisms can be expressed in terms of the mean growth rate constant (k). This is equivalent to the number of generations per unit time. Growth can also be described using the time taken for one generation. As bacterial populations grow by binary fission, this is the same as the time taken for the population (or optical density) to double.

Questions

1. The simple optical method for examining growth gives no measure of the actual numbers of cells. How can this method be improved to provide such information?
2. Comment on the validity of using turbidity measurements to follow the growth of populations of microorganisms.
3. Why was the magnetic stirrer used?

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Objectives

- To understand how microorganism growth rate in liquid culture can be measured
- To be able to culture microorganisms with due regard for safe practice

Safety

- Wear eye protection.
- The microorganisms are a potential biological hazard. Use aseptic techniques when working with the yeast cultures.
- Disinfect the bench with 1% Virkon® or equivalent. Leave the disinfectant on the bench for about 10 minutes. This should be done before and after working.
- Follow current best practice for handling microorganisms and sterilising equipment before and after use. An autoclave or pressure cooker will be required for effective sterilisation of equipment and guidelines for safe use should be carefully followed.

Equipment per student/group	Notes on equipment
bench disinfectant	1% Virkon® or equivalent to be left for about 10 minutes on the bench
paper towels or cloth	to dry off the bench
Bunsen burner	one per group
colorimeter set to absorbance with a filter of wavelength of around 600 nm colorimeter cuvettes	Set the colorimeter to approximately 600 nm wavelength or use an orange/red filter. Small volume cuvettes (normally 4 cm^3). Sterilise cuvettes in an autoclave after use.
light sensor or light meter datalogger cool light source cardboard box	This is an alternative to the colorimeter. The datalogger method has both practical and safety advantages and should be used if possible. Ensure that the light source will not produce heat, is within its latest PAT test range, and is positioned so that it is not likely to fall over Set to continuous recording The box will shade the sensor from ambient light. Cut out an area to allow light from the light source to enter. Place the box over the flask, stirrer and light sensor.
dried baker's yeast (not fast acting)	1.25 g for yeast culture plus 1.25 g to make up an identical suspension for counting initial yeast cells density.

250 cm^3 of 0.5% glucose solution in a sterile 500 cm^3 clear conical flask	Use only glucose solution as the medium. A single culture flask will be set up as a culture vessel for the whole class to measure. The flask should be stoppered with non-absorbent cotton wool covered with aluminium foil. Ensure that the flask used can fit into the sterilising equipment available at the school. Flasks containing incubated cultures must not be opened before they have been fully sterilised by steam at 121 °C for 15 minutes in an autoclave/pressure cooker
small bottle of 0.5% glucose solution for use as a blank	store in the fridge between sampling events
magnetic stirrer	The flea should be sterilised in 70% ethanol for 5 minutes before and after use. Remove from the flask after use using another magnet and re-sterilise. Do not autoclave as this will demagnetise it.
non-absorbent cotton wool	to stopper the culture flask
aluminium foil	to cover the stopper
5 cm^3 measuring cylinder or 2000 µl automatic pipette	Two per group. Measuring cylinders must be sterilised in an autoclave after use. Provide a disposal container for pipette tips.
microscope	with ×4 and ×40 objectives
mm^2 graph paper photocopied on to acetate attached to a microscope slide	a 2 cm strip of the acetate such be fixed to the slide using sticky tape
slides and coverslips	
dropper pipette	
0.1% methylene blue stain	

Notes