Edexcel B Biology A-Level

Core Practical 12

Investigate the rate of growth of microorganisms in liquid culture

Aseptic technique is used to **avoid contamination** of the sample from outside substances, such as microorganisms. This is important to get **reliable** and **repeatable** data.

One way of measuring bacterial growth is through **colorimetry**. This is a quick and relatively simple way of measuring growth. A disadvantage of measuring growth in this way is that it doesn't provide a direct count and also counts non-viable bacteria - to provide an indication of number of bacteria, colorimetry readings have to be related to bacterial count via a **calibration curve** produced using a **haemocytometer**.

Aseptic Technique

- Wipe down surfaces with **antibacterial cleaner** both **before and after** experiment.
- Use a **Bunsen burner** in the work space so that **convection currents** draw microbes away from the culture.
- **Flame the wire hoop** before using to transfer bacteria.
- **Flame the neck of any bottles** before use to prevent any bacteria entering the vessel (air moves out so unwanted organisms don't move in).
- Keep all vessels containing bacteria **open for the minimum amount of time.**
- **Close windows and doors** to limit air currents.

Equipment

- Bench disinfectant
- Paper towels
- Bunsen burner
- Colorimeter
- Cuvettes
- Measuring cylinders
- Yeast suspension
- Microscope
- Slides and coverslip
- Pipette
- Graph paper photocopied to acetate

Method

1. Use a cuvette filled with **glucose culture medium** to set the absorbance of the colorimeter to **zero**.
2. Fill a measuring cylinder with **yeast suspension**. Transfer it into a cuvette. Measure the absorbance.
3. Repeat steps 1 and 2 at least **five times over twelve hours**.
4. To relate colorimetry readings to a direct cell count, use a **haemocytometer**, or determine your microscope's field of view with a piece of graph paper photocopied

onto acetate. Stain the yeast suspension with methylene blue, add a drop to the slide and count the visible yeast cells.

5. Calculate the volume of one drop by measuring the volume of ten drops. This information will enable you to calculate an **overall density** and therefore cell count.

Risk Assessment

Hazard	Risk	Safety Precaution	In emergency	Risk Level
Disinfectant	Flammable	Keep away from naked flame	Put out fire; seek assistance	Low
Biohazard	Contamination ; infection	Use disinfectant; wash hands with soap after dissection; do not incubate at human body temperature; do not open agar plate post incubation	Seek assistance	Low/medium (depends on likeliness of bacteria sample used to cause infection)
Naked flame	Fire hazard; burns	Keep away from flammable materials; tie up long hair	Put out fire; seek assistance;run burns under cold water immediately	Low

Graph

- Plot a graph of absorbance against time.

Conclusion

- Absorbance increases when **number of bacteria increases**. This makes the suspension more opaque, transmission decreases and absorbance increases.
- Therefore, absorbance follows the same trend as the bacterial growth curve:
    - **Lag** (bacteria don't grow at maximum rate as they adapt to new environmental conditions)
    - **Log** (exponential growth - bacteria grow at theoretical maximum rate)
    - **Stationary** (as resources begin to be used up, bacterial growth is equal to bacterial death so number doesn't change)
    - **Death** (many more bacteria are dying and so number decreases)