Edexcel (B) Biology A-level

6.1 - Microbial techniques

Flashcards

Why are aseptic techniques important when culturing microorganisms?

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To produce uncontaminated culture so results are reliable & repeatable.

List the basic aseptic techniques.

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- Wipe surfaces with antibacterial cleaner.
- Set up Bunsen burner nearby. Convection currents prevent microbes from entering culture.
- Flame inoculating loop & neck of bottles before use.
- Minimise time that vessels containing bacteria are open.
- Sterilise all equipment e.g using an autoclave.
- Wear protective clothing.

Outline how to culture microorganisms.

Outline how to culture microorganisms.

1. Transfer bacteria to agar plate using sterile inoculating loop or pipette.
2. Tape lid on at 2 ends then invert the dish & incubate. In school lab conditions, ensure dish is not airtight & do not incubate above 25℃ to avoid growth of pathogens.

Explain the difference between a spread plate and a streak plate.

Explain the difference between a spread plate and a streak plate.

Spread plate: distribute microorganisms evenly with a sterile spreader.

Streak plate: aim to obtain single colonies by rotating the plate to build layers of the culture on at least 3 separate streaks.

Describe the 3 types of nutrient medium.

Describe the 3 types of nutrient medium.

Usually contain nitrogen, carbon & minerals. Often enriched with protein from extract of yeast, blood or meat. May be **liquid broth** or **solid agar**.

Selective mediums contain highly specific nutrient balance. Only certain microorganisms grow.

Give the advantages of using a broth medium.

Give the advantages of using a broth medium.

- Can provide anoxic & oxic conditions depending on the depth, which helps to identify microbes / determine their optimum conditions.
- Can grow a very large volume of bacteria.

Give the advantage of using agar as the medium.

Give the advantage of using agar as the medium.

Can obtain a single, discrete pure colony for study.

Name the 4 phases of a bacterial growth curve.

Name the 4 phases of a bacterial growth curve.

1. Lag phase
2. Log phase
3. Stationary phase
4. Death phase

What happens during the lag phase?

What happens during the lag phase?

Microorganisms need to adjust to the environment before reproducing so population size only increases slowly.

What happens during the log phase?

What happens during the log phase?

After every round of division, population size doubles (exponential growth).

What happens during the stationary phase?

What happens during the stationary phase?

Reproduction rate = Death rate, so population size stabilises at its maximum.

What happens during the death phase?

What happens during the death phase?

Microorganisms die due to buildup of toxic waste products & lack of nutrients.

Name 3 methods to estimate the growth of a bacterial culture.

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- Cell count.
- Turbidity measurement (type of colorimetry to measure opacity).
- Dilution plating.

Explain how to conduct a cell count.

Explain how to conduct a cell count.

1. Dilute broth sample with equal volume of trypan blue to stain dead cells blue.
2. Use a calibrated haemocytometer with volume 0.1mm^3. Count the cells in each of the sets of squares & calculate mean.
3. Number of bacterial cells = number counted x 10^4 per cm^3.

Suggest advantages and disadvantages of using a cell count.

Suggest advantages and disadvantages of using a cell count.

+ Only counts living cells
- Slow
- Expensive equipment
- Large margin for human error

Explain how to conduct a turbidity measurement.

Explain how to conduct a turbidity measurement.

1. Use colorimeter. Measure absorbance or % transmission of samples with known microorganism count.
2. Plot calibration curve: absorbance/ % transmission (y-axis), number of microorganisms (x-axis).
3. Record absorbance/ % transmission of unknown sample. Interpolate graph.

Suggest advantages and disadvantages of using a turbidity measurement.

Suggest advantages and disadvantages of using a turbidity measurement.

+ Quick.
+ Can be conducted in the field.
- Expensive equipment.
- Counts both living & dead cells.
- Requires calibration curve from known samples.
- Assumes equal density of cells across culture.

Explain how to conduct dilution plating.

Explain how to conduct dilution plating.

1. Grow a colony from a single microorganism.
2. Perform serial dilution with distilled water to see single colonies.
3. Prepare a lawn plate & count colonies.
4. Number of cells = number of colonies x dilution factor.

Suggest advantages and disadvantages of dilution plating.

Suggest advantages and disadvantages of dilution plating.

+ No complex or expensive equipment needed.
+ Only counts living cells.
- Incubation period needed (slow).

Outline the calculation for an exponential rate constant.

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Arrhenius equation: $k = Ae^{-EA/RT}$

k = rate constant

A = frequency factor

e = mathematical number with the value 2.718…

E_A = activation energy

R = gas constant

T = temperature