Unicellular: a single celled organism EG bacteria, archaea, protozoa

Growing Microorganisms

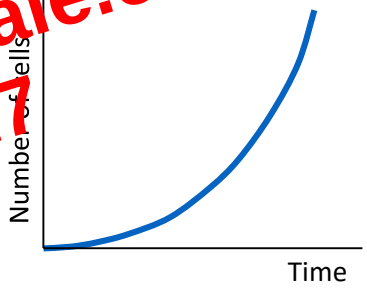
- Grown in a liquid or solid medium EG agar
- The medium must contain all of the nutrients needed for the microorganism to grow
- It must be sterilised before adding the sample
- This then needs to be incubated at an appropriate temperature and gaseous environment

On Solid Media

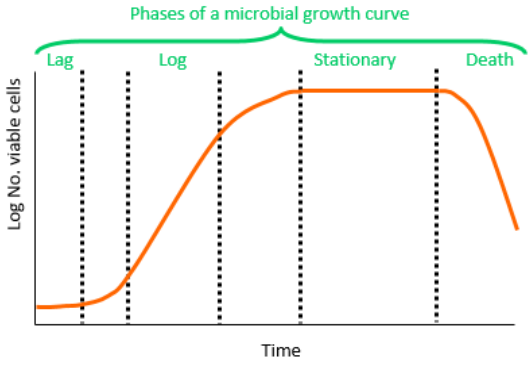
- **Streak Plate**
  - A cotton bud containing the microorganism is swiped across the agar plate
  - Used for different sections
- **Spread Plate**
  - A small volume of liquid culture is pipetted onto the surface of the agar plate
  - A sterile spreader is used to spread the culture across the plate as evenly as possible
- **Pour Plate**
  - Either a small volume of liquid culture is added to cooled molten agar, and after mixing, the agar is poured into a petri dish and allowed to solidify
  - The liquid culture could be put into an empty petri dish and cooled molten agar is poured over it and mixed

20.10.15 Microbial Growth

- Single celled organisms divide by binary fission (doubling)
- This gives an exponential increase
- A culture will grow exponentially when it has a ready supply of its nutrients



The Microbial Growth Curve



- When growing bacteria in flasks or test tubes, you will not get exponential growth all the time
- **Lag:** the organism is adapting to its new environment, the enzymes are being synthesised, cells are increasing in size but not dividing
- **Log:** cells are undergoing exponential growth with constant growth rate and mean generation time. The slow and length of the log phase depend on how well the medium meets requirements
- **Stationary:** there is no net increase in cell number, the nutrient (or oxygen) has been exhausted, the environment has changed (pH) or toxic products have accumulated
- **Death:** cells die, often exponentially, there is no energy, pH damage or damage from the product of toxic products

Batch & Continuous Culture

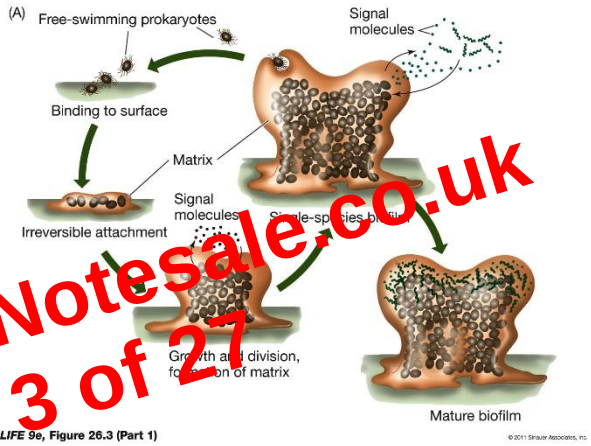
- When microbes are inoculated into a fixed volume of growth medium in a closed system it is described as a batch culture
- Continuous culture is where the microbes are permanently in the log phase of growth
    - The new culture is added at the same rate that the culture is removed from the vessel
    - This should achieve a steady state and will maximise the production of the microbe and it products
    - Important in industry
    - **Chemostat: equipment for continuous culture of microorganisms**

Microorganism Growth on Solid Medium

- You're unable to count the number of cells
- For bacteria, the colony will spread out to obtain the nutrients from agar
- The growth on a solid medium is usually slower
- Fungi grows out in a roughly circular method

Biofilms

- Microbial growth on solid material in the environment often takes the form of biofilms
- A few microbes associate with a solid surface which can support their growth (moisture, nutrients etc.)
- They attach, divide and increase in number with many also producing extra cellular polysaccharide material forming a protective matrix
- They may secrete molecules that attract other microbes, which will increase the size & complexity of the biofilm
- Some parts of the biofilm will break off and spread



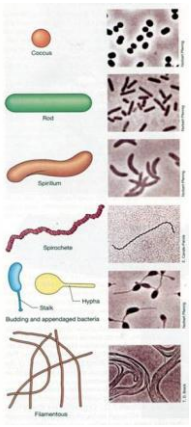
Counting in Liquid Media

- Using a counting chamber
- A glass slide with an engraved precise grid of known area is flooded with a dilution of culture, this is covered with a cover slip
- The depth between the grid and coverslip is measured
- The number of cells in several squares is counted, and then the average no. per square
- The volume of liquid in each square is calculated and you can then work out the no. of cells per ml of culture

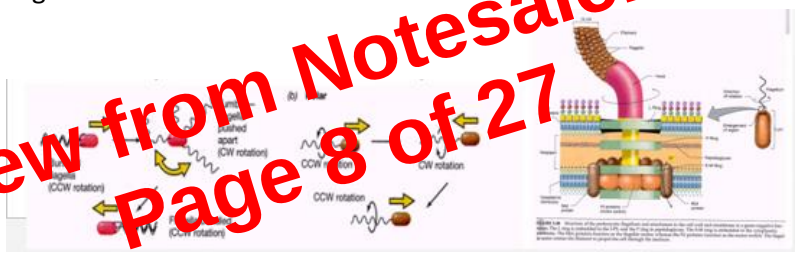
Counting on Solid Media

- Use either a pour or spread plate technique for colonies to be seen
- The original sample must be serially diluted to ensure a good number of clearly separated colonies can be counted

Bacteria (Proteobacteria/Eubacteria)



- Morphological characteristics of bacteria
  1. Cell Morphology
     - **Coccoid:** round cells EG *Staphylococcus*
     - **Rods:** elongated capsule shaped cells EG *Bacillus*
     - **Curved cells:** bent lozenges, boomerang or undulating shapes EG *Vibrio, Spriillum*
     - **Helically coiled:** EG Spirochaetes
     - **Stalked or budding forms:** EG *Caulobacter*
     - **Filamentous:** elongated cells or groups of cells EG Strepto/Actinomycetes and Cyanobacteria
  2. Colony Morphology
     - This is whether the colony is glistening or smooth
     - Feathered edges or smooth edges
     - Raised or flat
     - Stringing or solid
     - General colour characteristics
  3. Flagellum Morphology & Motility
     - Many bacteria are motile and have their own unique flagella made up of subunits of a protein known as flagellin
     - They may have a single polar flagellum or multiple peritrichous flagella
     - Each has a unique rotary motor in the cell envelope which causes the flagellum to rotate



  4. Cell Wall Characteristics
     - Cell wall biochemistry and structure has been one of the main discriminative characteristics between different groups of bacteria
     - The gram stain works because of the differences in cell wall properties between the Gram positive (+) and the gram negative (-) species
     - Sensitivity to some antibiotics (Penicillin) is related to cell wall properties
     - Unique bacterial cell walls components:
       - **Peptidoglycan (Murein):** unique to all bacteria, forms a thin sheet in which the 2 sugar derivatives are connected by peptide cross links. These chains are further cross linked with amino acids which gives them greater rigidity. This forms the main wall layer in Gram positive species
       - **Lipopolysaccharide layer (LPS):** this forms an additional outer lipid bilayer to the walls of Gram positive bacteria, the space in between is known as the periplasm
       - **Teichoic acids:** acidic polysaccharides are unique to Gram positive bacteria and give their walls an overall negative charger

- Vomiting
- Fever

- **Intoxications**
  - Caused by a toxin being released by bacteria growing on food
  - Ingestion of live bacteria is not required
  - The toxins are exotoxins
  - Enterotoxins
    - These cause the release of water and sodium ions to pass from the enterocytes (cells lining the gut) into the gut lumen, leading to diarrhoea
    - These may also stimulate vomit receptors in the gut which send impulses to the vomit centre in the brain
    - The toxin will remain in the gut
    - ***Staphylococcus aureus***
      - There is an incubation period of 30minutes to 6 hours
      - Causes nausea, vomiting, diarrhoea and abdominal pain
      - Can last from a few hours to 3 days
      - There needs to be $5x10^6$ organisms/gram of food or 1 ng of the toxin/gram of food
      - Contaminates via the skin, nose throat
      - Common from cooked meat, dairy products & canned foods
  - Neurotoxins
    - These pass through the lining of the gut and then enter the blood stream
    - They are then transported to all regions on the body
    - They bind to nerve endings in tissue causing paralysis

Organism	Time to Onset of Symptoms in Hours	Symptoms
Staphylococcus aureus	1–6	Nausea, vomiting, and diarrhea
Clostridium botulinum	18–36	Dizziness, double vision, swallowing, and breathing problems
Bacillus cereus	1–6 10–12	Vomiting, abdominal pain, diarrhea, and nausea

  - ***Campylobacter***
    - C. jejuni and C. coli are the species that cause most infections
    - They are a major cause of food poisoning
    - Symptoms are visible within 2-5 days and lasts the same amount of time, but can linger for weeks
    - They produce flu-like symptoms, abdominal pain, nausea
    - The animal gut acts as the reservoir EG pigs, cattle, sheep etc.
    - They become infected through contaminated water, milk or meat
    - There can be cross contamination or poor hygiene during preparation EG not cooking meat thoroughly
  - ***Escherichia coli***
    - Most are non-pathogenic, normal gut flora
    - EHEC or VTEC *E. coli* 0157:H7 are the most important causes of food poisoning
    - There is an incubation period of 3-4 days
    - Causes abdominal pain, diarrhoea with bleeding, internal bleeding
    - Has a duration of about 2-9 days