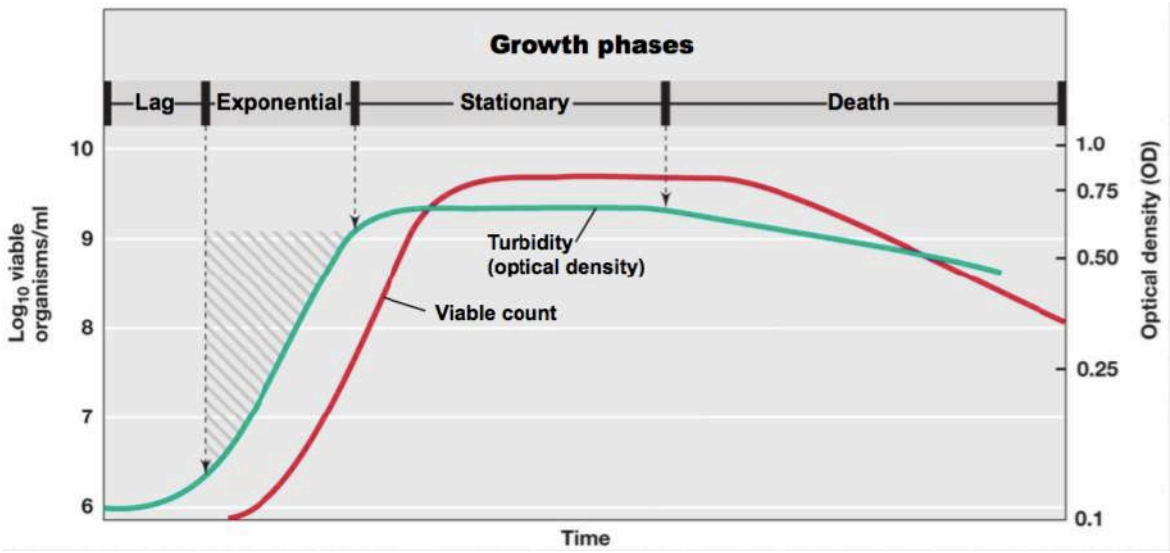
The Growth Curve

- Cell division increases the number of cells in a population
- Population growth is studied by analysing the growth curve
    - Monitoring growth of microorganisms then manipulating the environmental parameters to determine effect e.g. nutrients or temperature
- In liquid medium cells are cultured in a closed system or batch culture with no new fresh medium. Over time:
    - Nutrient concentrations decline
    - Waste components increase
- The growth of organisms dividing by binary fission is plotted as the logarithm of the number of viable cells versus incubation time



To determine the specific growth rate of a bacterial population, it is essential to know cell concentrations at varied time points.

Lag Phase

- Cells synthesising new components (to adapt to new medium)
- V slow growth or no growth at all
- Lag phase (time) can vary in length (can vary between different species)
- Taken cells from another culture/plate and placed them in a fresh environment ∴ need to begin to adjust
    - E.g. adjust cellular components, transport proteins to take up the new nutrients

Exponential (log) Phase

- Begin to grow, take up nutrients and divide
- The rate of growth is constant → dividing at the same rate
- The population is most uniform (physiologically) → all cells are mostly alike e.g. similar protein profiles
- It represents "balanced" growth
    - I.e. cells constituents are manufactured at a constant rate relative to each other
- If a nutrient or environmental condition changes, "unbalanced" growth results
    - Might get another lag phase before they take off again

Stationary Phase

- The total number of viable cells is constant

    - Occurs because metabolically active cells stop reproducing or because the reproductive rate is equal to death rate
    - Occurs because nutrients are limited, oxygen availability is limited or toxic wastes have accumulated
        - Something has occurred that's preventing growth e.g. no more phosphate
- A starvation response occurs resulting in physiological change and ∴ morphological changes
    - E.g. endospore formation (struggling) or decrease in cell size (reductive division → rod shaped cell will halve and become 2 cocci shaped cells)
    - Also, viable but not culturable, meaning cells are metabolically active but cannot be cultured
- (About a week)

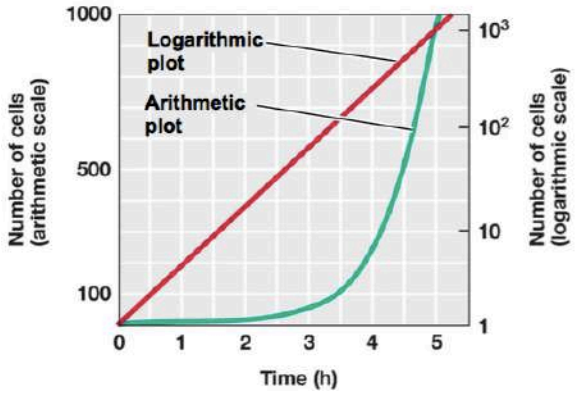
Death Phase

- No cell growth occurs (synchronous culture)
- Possibly cells are "viable but non-culturable" or VBNC
- Possible that cells die from apoptotic cell death (suicide)
    - Cells will lyse and die to release nutrients for other cells to use and survive

Mathematics Of Microbial Cell Growth

Time (h)	Total number of cells	Time (h)	Total number of cells
0	1	4	256 (2^8)
0.5	2	4.5	512 (2^9)
1	4	5	1,024 (2^{10})
1.5	8	5.5	2,048 (2^{11})
2	16	6	4,096 (2^{12})
2.5	32	.	.
3	64	.	.
3.5	128	10	1,048,576 (2^{20})

(a)



Generation Time (or doubling time)

- The time required for a population to double in size
- Varies depending on bacterial species and environmental conditions (e.g. temperature and media)
- Some bacteria double in 10 mins and others take much longer
- Some eukaryotes double in several days
- Need time and method of counting cells
- Exponential growth is best plotted on logarithmic scale → easier to calculate generation time

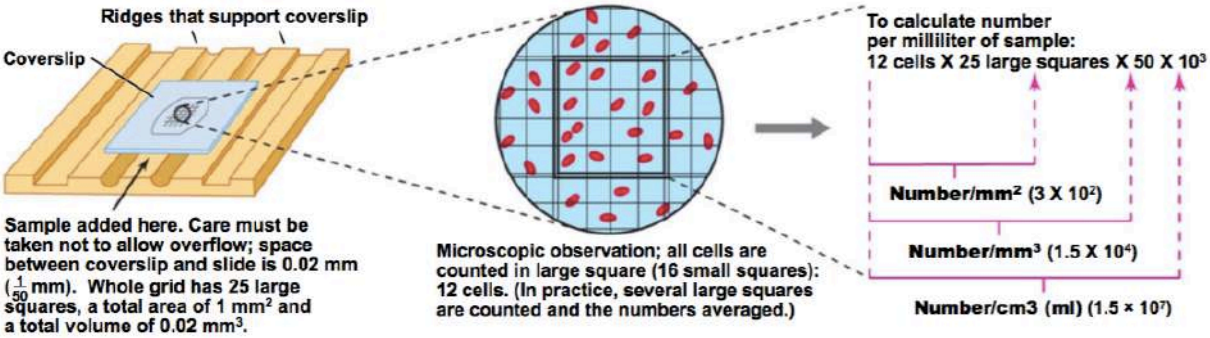
How To Determine "Generation Time"

- Population data are plotted for number of cells (log axis)
- The time to double the population is extrapolated from the plot (practical class)
- Can only calculate generation time in linear exponential phase

Measurement Of Microbial Growth

- Measurement of cell numbers
    - 1. Direct counting (use a microscope and counting chamber)
    - 2. Electronic counting (Coulter counter or flow cytometer)
    - 3. Culture techniques
    - 4. Cell mass

Direct Counting

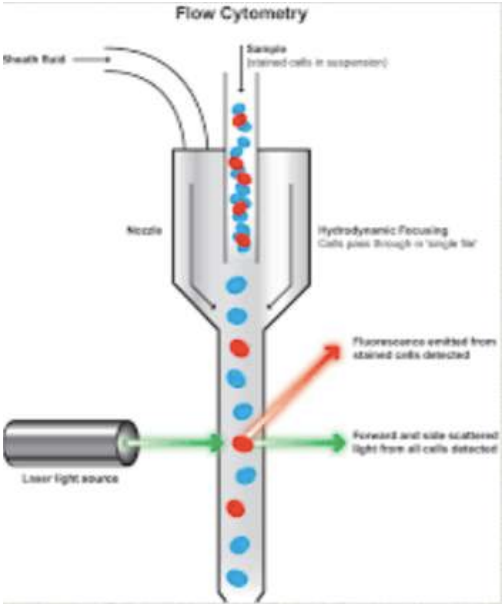


- Disadvantages:
    - Time consuming
    - Can't differentiate dead from live cells although can use stains to solve this (some stains are only picked up by live cells)

Electronic Counting

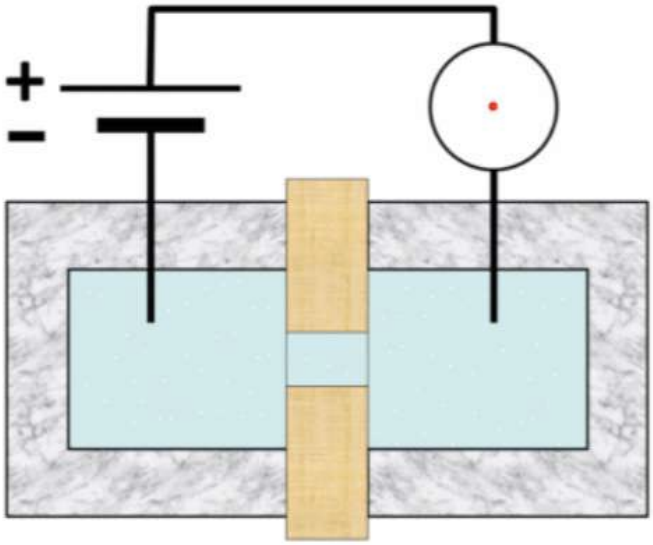
Flow Cytometer

Cells run through a laser, laser detects cell and counts it = quickly



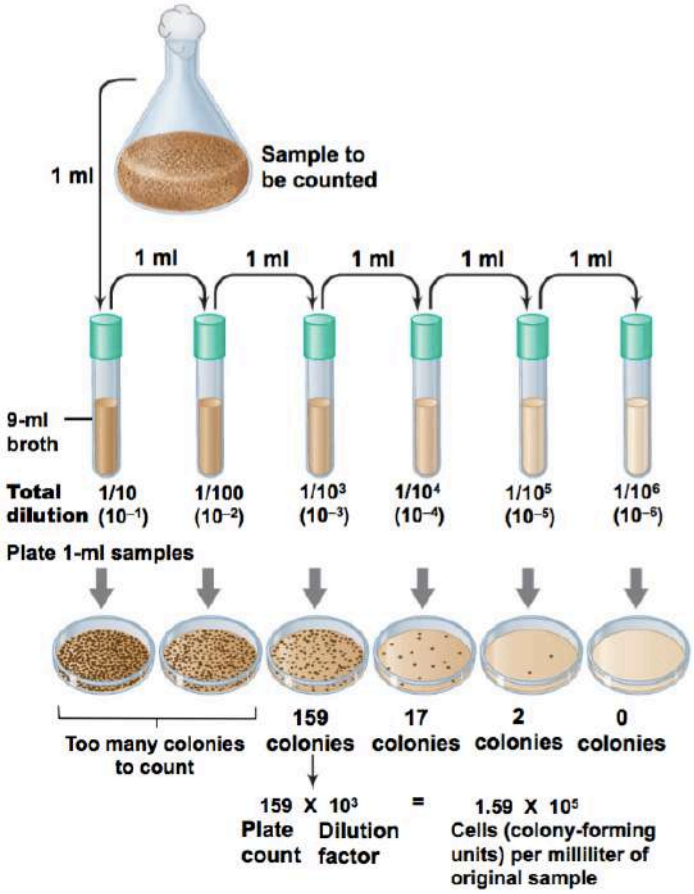
Coulter Counter

Cell will move through chamber = change in current = counts cell = quickly



Culture Techniques

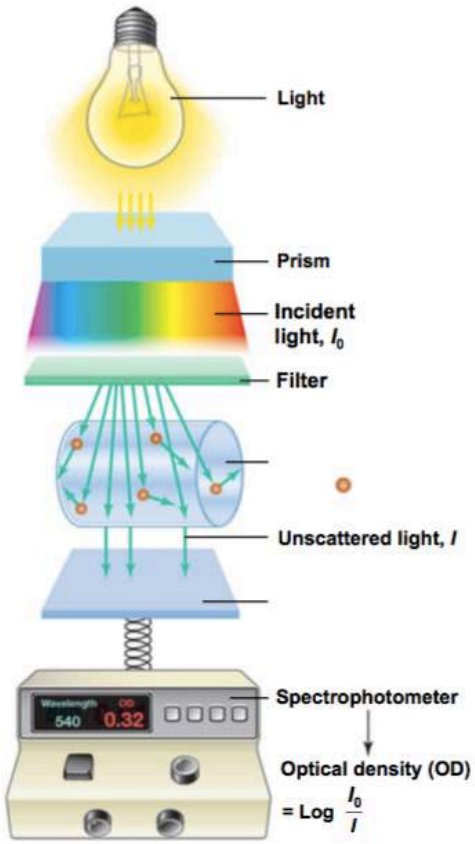
- Detection of viable cultures usually requires culturing the microorganism
    - Assumption: each colony represents 1 cell
    - Pour plate (practical class)
    - Spread plate (practical class)



- Assume every colony is derived from one cell
- Always use scientific notation for giving cell numbers

Cell Mass

- Increases in the total cell mass, as well as total cell number, are characteristics of cell growth
    - Changes in total cell mass can be determined by total dry weight
    - Spectrophotometry
- This is especially useful for measuring growth of a filamentous microorganism such as a fungi
- Optical density


Light
Prism
Incident light, I_0
Filter
Unscattered light, I
Spectrophotometer
Optical density (OD)
$= \text{Log } \frac{I_0}{I}$

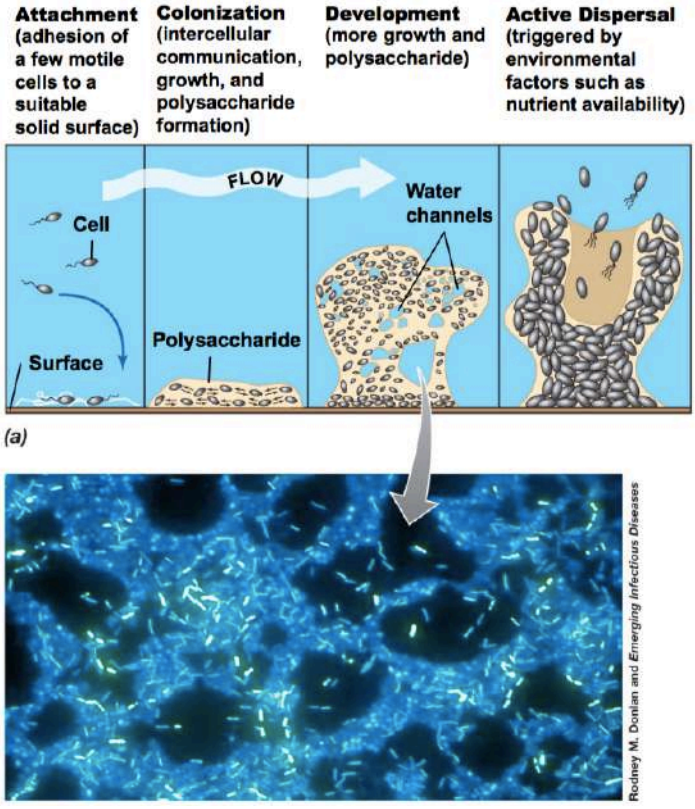
Biofilms

- Biofilms → a collection of microorganism surrounded by the slime they secrete, attached to an inert or living surface

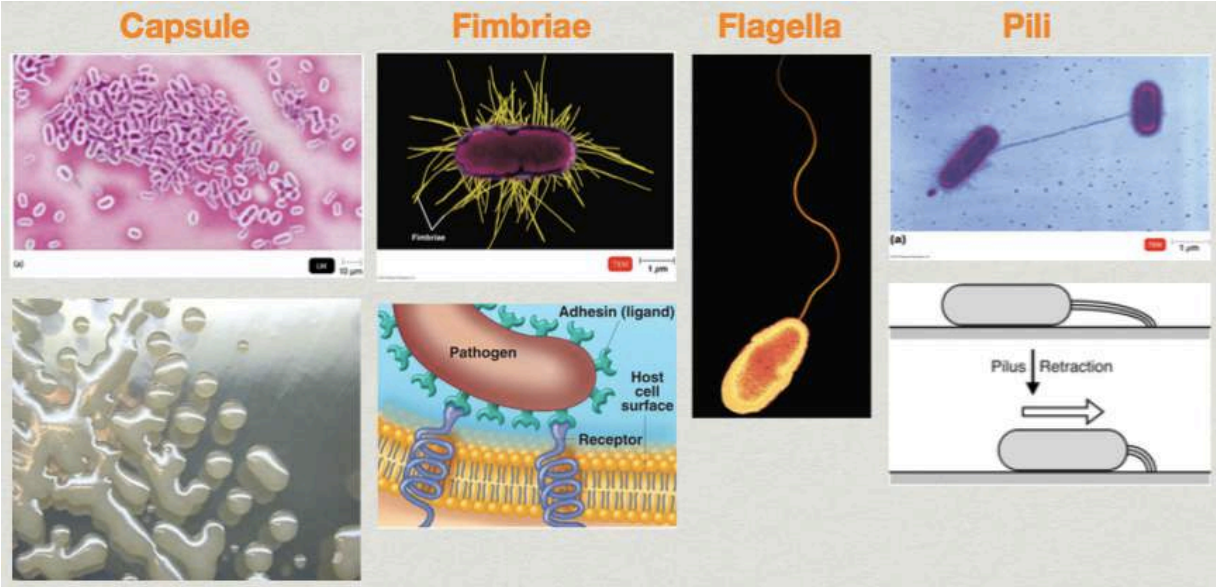
- Biofilms tend to be more resistant = harder to sterilise
- Stringy material = polysaccharide 'slime'
- Degrade enamel by fermentation
- Medical devices → usually Staph. Aureus
    - Device needs to be removed bc hard to treat/fix
- Usually mixed microbial species
    - E.g. dental plaque has > 700 species of bacteria and archaea
    - Can have monocultured biofilms, not often
- Default mode of growth

Biofilm Formation

1. Reversible adhesion → adhere but can swim away
2. Irreversible adhesion → agent (fimbrae, pili or polysaccharide) been ↑ = attachment
3. Colonisation → growth and produce slime. Communication between cells
4. Development → can develop into interesting structures, 'holes' allows water and nutrients to flow through, keeping biofilm hydrated and with sufficient nutrients
5. Active dispersal → cells can be released to colonise a new place



Linking Bacterial Structures to Biofilm



Capsule

- Thick polysaccharide allows cells to grow and form 'biofilm' structures
- Stickiness

Fimbriae

- Important during initial adhesion stage from reversible to irreversible
- Attachment to living and non-living surfaces
- 'Lock and key' and ligand interaction

Flagella

- Helps organism migrate to the cell surface to being association
- Also involved in allowing attachment to the surface

Pili

- Twitching motility, assists colonisation of surface
- Important in formation of thick films esp. organism psuedemonis erodgenosa (lung infection)

There are other structures involved in biofilm formation; these are just the most common

Why Form Biofilms?

Resist phagocytosis by protozoa and immune cells	Macrophage → attach to bacteria cells and kill them Protozoa → attack and eat cells But forming a biofilm = becomes too big for other cells to engulf and eat
Collect nutrients from flowing water	Bacteria is fixed in a stationary position, with water flowing over → collecting nutrients = efficient feeding
Nutrient exchange	If you have a multi-species biofilm, one organism might being to degrade a large compound, take what they need for energy and excrete the waste products Another organism can use this waste for energy, and this cycle can continue Bacteria can work together cooperatively to break down difficult molecules and use their products
Resist penetration of toxic molecules (e.g. antibiotics)	Antibiotics/other toxins might diffuse though, but only to outer layers Cells in centre and protected, and therefore survive Stickiness of cells can grab compounds, preventing them having any effect

	Charge can also repel toxins = resistance
Genetic exchange	Bacteria replicate using binary fission = not much genetic diversity through sexual reproduction Bc bacteria are all so close together, they can transfer DNA between each other. Some can therefore pick up new genes/DNA from another bacteria = new function Methods = translation, transduction and conjugation

Affects of Biofilms

System	Impact of Biofilm
Swimming pools	Pathogen survival and cosmetic degradation Can be difficult to treat with chlorine bc toxin resistant = disease Can produce acids which corrode pipes/pool lining
Drinking water pipes	Pathogen survival & pipe corrosion
Food processing	Pathogen survival & contamination
Dental plaque	Acid production = caries &/or gingivitis Bacteria on teeth take sugar consumed and convert it to acid = corrodes enamel
Toilet bowls	Cosmetic degradation
Medical devices	Failure of device - source of pathogen Difficult to treat with antibiotics = need to remove device  Biofilm on voice prosthesis implant
Infection	Failure of treatment (e.g. antibiotics) Difficult to treat with antibiotics bc cannot penetrate through to all bacteria = once treatment stops, organism comes back  *P. aeruginosa* biofilm contaminated lung