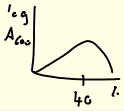
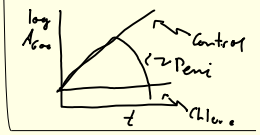
Bacterial Practicals

- **Random notes**
  - 1 molecules in E.coli is at a oncentration of 10^-9 M
- **Control of gene expression**
  - **Diauxic growth** is when a culture of bacteria is grown on a mixture of two sugars (lactose and glucose, for example)
    - The bacteria grow well to begin with
    - Then stop growing
    - Then start up again
    - Each phase is due to the use of *one* of the sugars, due to the fact that one inhibits the formation of the enzymes necessary to metabolise the other
  - Glucose assay
    - Coupled enzyme assay
      - Consists of glucose oxidase, peroxidase and a colour reagent, ABTS (colourless) and a buffer
      - Glucose + O2 --> Gluconolactone + H202
      - Reduction of H2O2 coupled with oxidation of ABTS, which goes green
      - Reaction goes all the way - measuring *extent* instead of *rate*
    - Protocol
      - Add solution to the mixture
      - Incubate for 30 minutes
      - Read absorbance at **436 nm**
  - Beta-galactosidase assay
    - ONPG is hydrolysed into something whose **anion** is yellow by the enzyme
    - Therefore, must be at a pH above the pK of the phenolic -OH
    - That's why we quench the reaction with an alkali
    - Protocol
      - Wash cells to remove residual lactose (this would corrupt the test because the enzyme would also be digesting the lactose)
      - Add cells to warm OPNG/CTAB
        - CTAB is a mild detergent which lyses the cells
      - Incubate for 10 mins, and quench using alkali
      - Spin and pellet bacteria - then read supernanant at **420 nm**
  - Results
    - Protocol
      - Every 15 minutes
        - Take A600 reading
        - Proportional to cell number
      - Every 30 minutes
        - Separate bacteria from medium
        - Do beta-galactosidase assay on bacteria
        - And glucose assay on medium
    - Shows that
      - Originally, bacteria continue growing exponentially on glucose exclusively - beta-galactosidase level stays the same
      - When glucose runs out, short induction period, during which bacterial growth slows down and enzyme is produced
      - Then, continues growing exponentially, but slower than before
      - The cell will not make beta galactosidase until it absolutely has to
      - Need a lack of glucose and and presence of lactose
      - A Lac repressor binds to DNA in low lactose
      - RNA Pol does bind to the Lac gene even when glucose is abundant, but its progress is slow
      - cAMP (present when glucose is low) binds a CAP protein upstream from the RNA Pol, and this highly increases the rate of transcription
    - Presenting
      - All on single curve
      - Two scales
        - A600 scale logarithmic (because exponential growth)
        - Beta-galactosidase on the other one, linear scale
      - Divide beta galactosidase by 600 to get number per amount of cell
- **Bacterial growth**
  - See standard curve under "antibiotics"
  - Often exhibit three phases
    - **Lag phase**, when cells have been put into new medium
      - Don't seem to be growing
      - May be inducing enzymes allowed them to metabolise the medium
    - **Exponential phase**
      - Cells grow exponentially
      - Best "doubling time" E. Coli can do is 20 minutes
    - **Stationary phase**, when growth stops
      - Usually because one or more nutrients have run out
      - Or toxic metabolic products are accumulating
- **Bacteriophage lambda**
  - Two life-styles
    - Lytic mode
      - Virus enteres the cell and immediately sets about reprogramming it to make more viruses
      - It's done, and the host host cells are lysed
    - Lysogenic mode
      - Cells are infected, but the virus hides in the bacterial chromosme
      - There is lies domant, and gets replicated with the host cell
        - What keeps it quiet is a viral protein called a cI repressor
        - It is the one protein that *is* synthesised in the dormant phase
        - It keeps the rest of the viral DNA silent
      - Such a carrier is aclled a **lysogen**
      - Something triggers the dormant genes back to life, and all the above happens
      - Can have mutant lambda in which temperature brings phage back to life
        - Afterwards, stays alive whatever the temperature
        - CSH25 E. Coli are lysogenic for this kind of lambda
      - 

        - Takes about 40 minutes for bacteriophage to be assembled in cell and cell lysed
  - Plaque assays
    - Allow us to measure the number of phages
    - Principle
      - Take "lawn" of E-coli bacteria growing
      - Put a few virus particles on it
      - Where a virus lands, the bacterium will lyse releasing hundreds of new virus particles
      - These infect neighbouring bacteria, which lyse in their tuurn, and so on - a "cleared area" or "plaque" form against he backgrond of cloudy lawn
      - Each plaque represents the presence of just *one* founder phage particle
    - Protocol
      - Add **chloroform** to kill the remaining bacteria and release phages
      - Put into "SM" solution (containing Mg2+ and gelatine) which keeps the phage particles in good shapre
      - Prepare serial 10-fold diluations of these bacteria in SM mix, **using a new pipette tip each time**
      - Spot on a nutrient plate with a lawn of E-coli partitioned 8-ways
      - Precautions
        - Make sure bubbles don't form on the pippete when spotting - otherwise, aerosols - flies everywhere
        - Don't jiggle plate around, or it'll mix
      - Always have a control
    - Calculation
      - Count the number of plaques in the most concentrated solution possible = number of p.f.u. (plaque forming units)
      - See how many that would have been in 1ml
      - Use absorbance measurements to deduce how many bacteria were in 1ml originally
        - Can deduce how absorbance is correlated to number of bacterias using serial dilutions of a sample with known aborbance
      - Work out p.f.u./bacteria
- **Antibiotics**
  - Two types
    - **Bacteriostatic** stops growth - e.g. **chloroamphenicol**
      - Growth slows down to very low
      - Interferes with protein synthesis (targets ribosomes)
      - Cell walls stop growing without actual lysis happening
      - Could have re-plated some of these cells without antibiotics and seen if *those* grew
    - **Bactericidal** kills the bacteria, e.g. **penicillin**
      - Growth continues, then drops, as soon as the cell replicates and new cell wall needs to be made
      - Prevents the cross-bridges that strengthen the cell wall
      - Beta-lactam ring binds to PBP (penicillin-binding proteins) which are transpeptidases responsible for the cross-linking
      - Other antibiotics will bind to PBPs within the septum region - cells grow as string, or to those that regulate shape
      - Bacteria can develop enzymes that break down the beta-lactam ring. Drug companies include **augmentin** which inhibits these enzymes
    - Other examples
      - Rifampicin inhibits transcription (v. specific)
      - Sulphenilamides inhibit folic acid synthesis (we can't synthesise it, so does't affect us)
  - 

- **Disinfectants**
  - Also kil bacteria

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