National Public Health Service for Wales
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Session 1

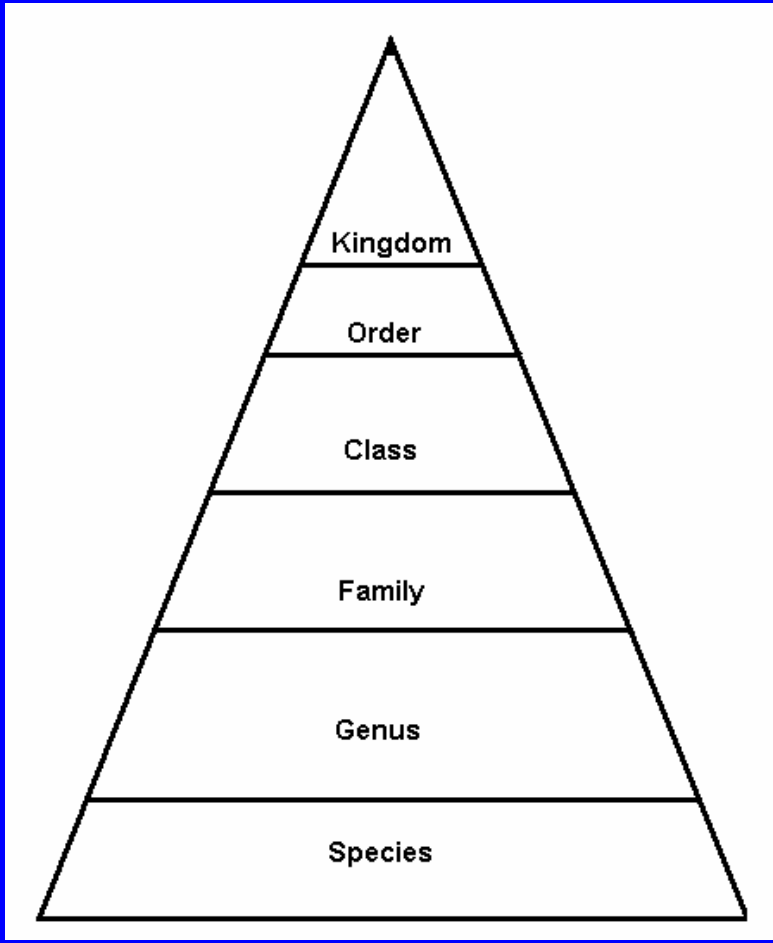
Fundamentals of Microbiology

Session overview

- Classification
- Microbial nomenclature
- Microbial growth
- Microbial death
- Spore formation

Classification

The Five Kingdom system is used to classify all organisms



Differentiation between organisms is based upon

- cellular structure (morphology)
- metabolism (biochemistry)
- reproduction
- DNA

Five basic types of micro-organism:
algae, protozoa, fungi, bacteria and **viruses**

The first four fall within three of the kingdoms:
Fungi, Protista and **Monera**

Viruses do not fall within the Five Kingdom scheme

Gram reaction (Gram positive and Gram negative)

- Developed in 1884 by Danish physician Christian Gram
- Only used for bacteria
- It is the first step in identifying unknown bacteria
- It is based upon a differential stain-***Gram positive*** cells retain the stain and ***Gram negative*** do not
- The difference is due to differences in cell wall composition

Microbial nomenclature

- All organisms have two names-the first signifies the ***genus*** and the second signifies the ***species***

- This is a binomial system-devised by Linnaeus

- Genus name can be abbreviated, species name should not be

  e.g. *Campylobacter jejuni* can be abbreviated to
  C. jejuni

  not *Campylobacter j.*

Microbial growth

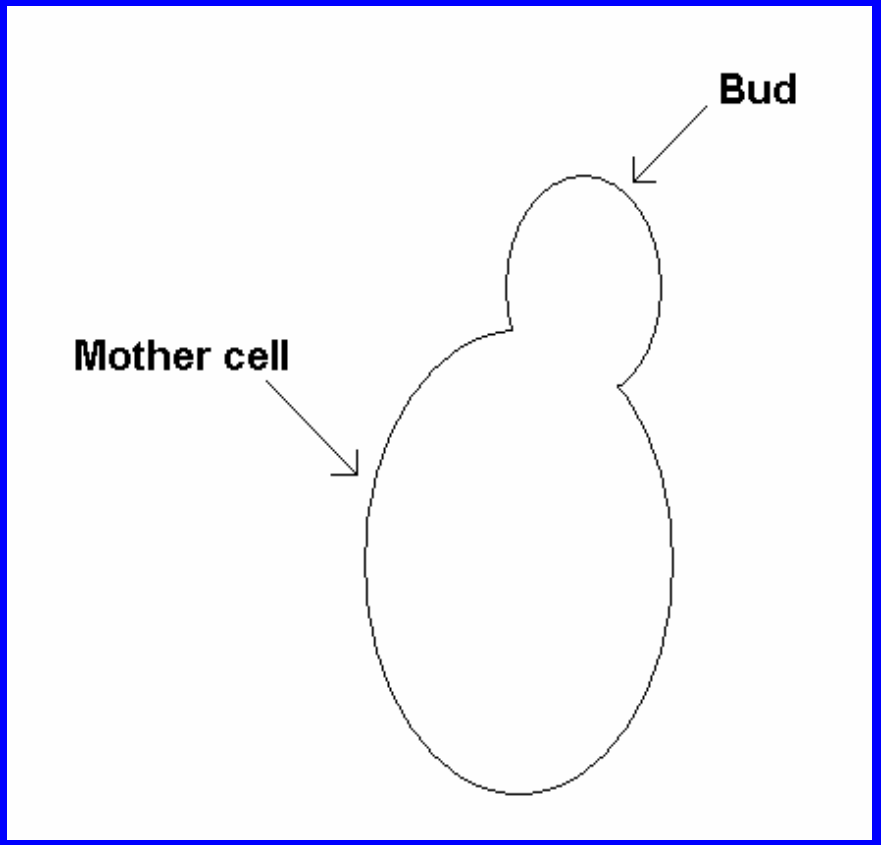
Growth is the process by which:

- individual cells increase in size and mass and
- the population increases in number

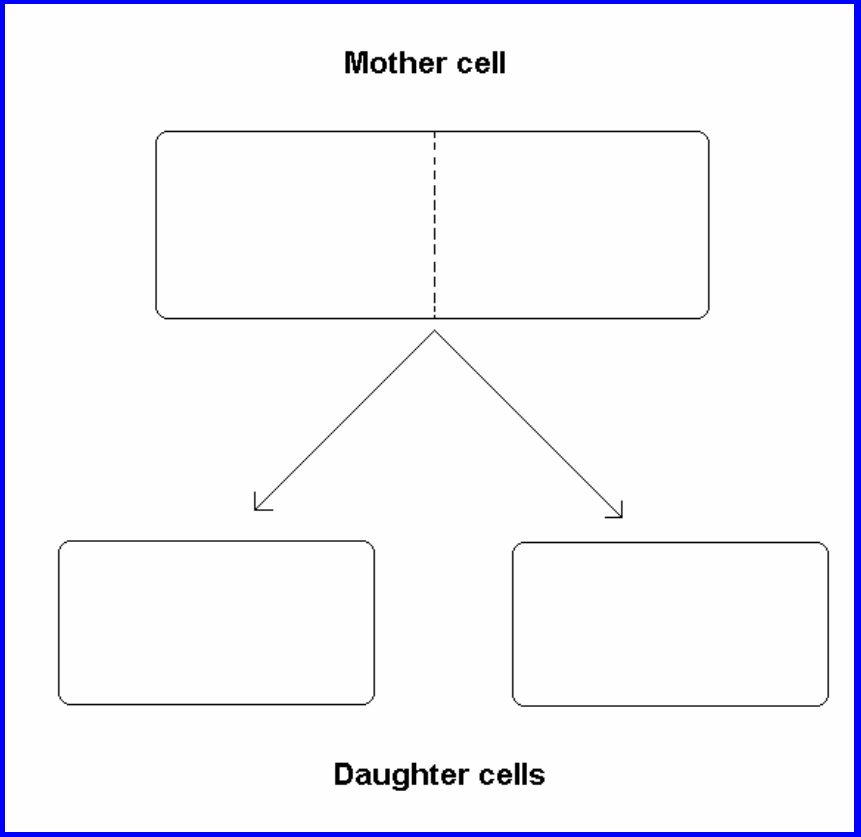
Basically ***metabolism*** and ***reproduction***

Reproduction for bacteria and fungi tends to be asexual-***budding*** or ***fission***

Budding



Fission



- Growth on solids is in the form of ***colonies***-visible to the naked eye
- Within solid foods ***micro-colonies*** will appear
- In liquids, growth appears as increasing ***turbidity***
- Moulds grow as a filament where cells stay attached (described as ***hyphae***), but not all the cells will grow-only those at the tip of the filament

Two types of growth system-***open*** and ***closed***

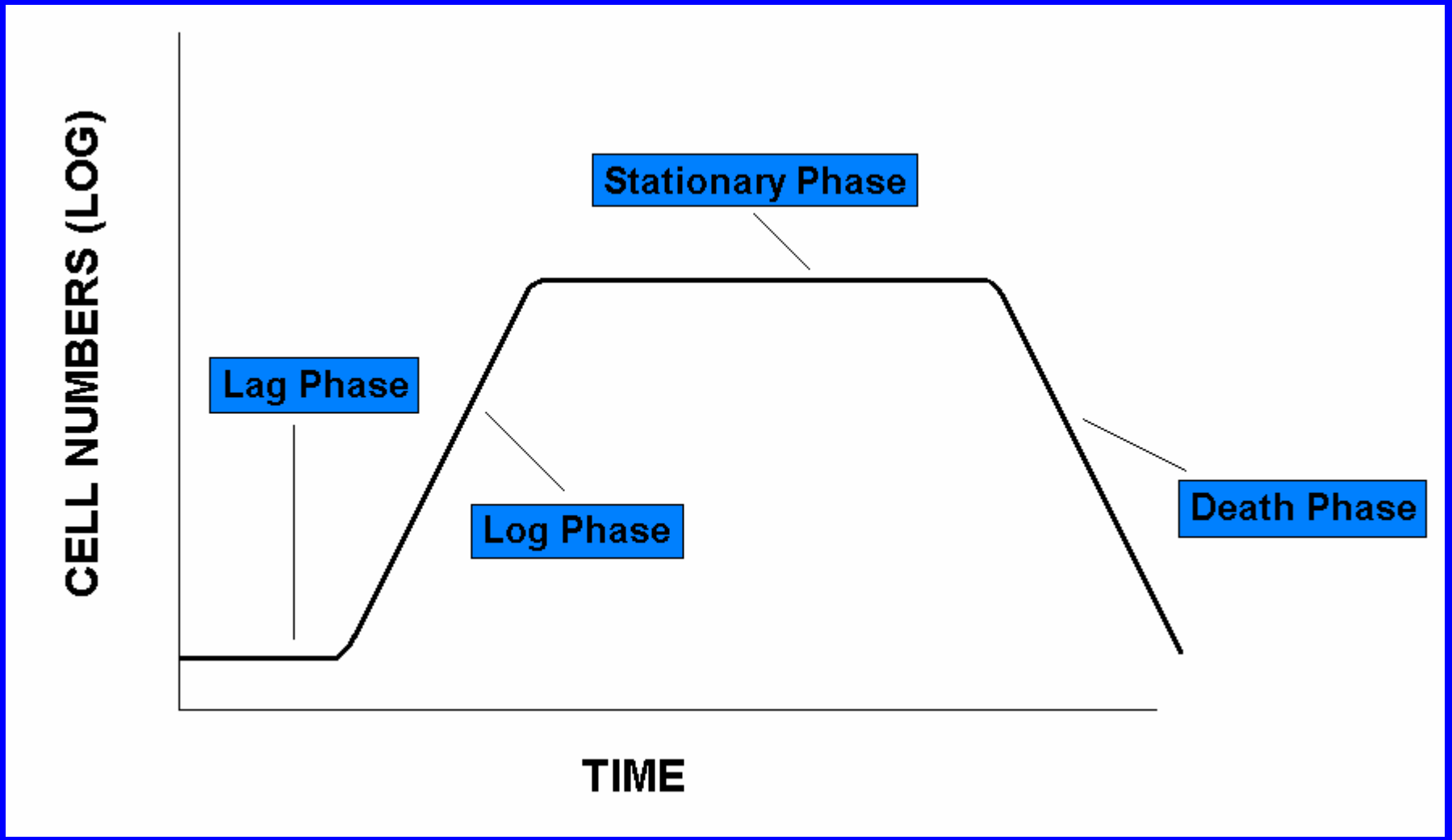
- Within a ***open system*** nutrients are constantly replenished and population growth will not stop

  e.g. biofilms in the pipework of food factories will have constant access to fresh nutrients

- Within a ***closed system***, nutrients and other factors will become limited and growth will eventually cease

  e.g. in a bottle of milk the nutrients will run out

There are four distinct growth phases for a unicellular organism in a closed, liquid culture



Growth phases

- **Lag phase**

  No growth occurs because the cells are adapting to the new environment

  Length of period varies, dependent on culture conditions

- **Log phase**

  Cells are growing exponentially or logarithmically-the population doubles within a certain time-depends on exact conditions, but can be as little as 20 mins

  This is called the ***generation time***

- **Stationary phase**
  A nutrient depletion or toxic by-product build up stops the cells from metabolising or reproducing-the cell numbers remain static

- **Death phase**
  In this phase the cells start to die and the viable population decreases

  Population death is also exponential-not all the cells die at once

Methods for measuring growth

- ***Colony counts***
  aerobic colony count for all viable bacteria
  selective counts for particular organisms/species

- ***Direct counts***
  using a microscope-viability can be determined with special stains

- ***Absorbance measurement***
  for clear liquid cultures only

Microbial death

Death can be defined as the inability of a cell to both metabolise and reproduce due to irreversible cell damage

Death of cells leads to loss of ***viability***-normally defined as the ability to grow and reproduce

i.e. producing visible colonies on a plate or producing turbidity in liquid

However, lack of growth is not always the same as death

Some species may enter a ***viable but non-culturable phase***-cells do not reproduce in culture but may retain viability and if pathogenic, the ability to infect

Examples are *Listeria* and *Campylobacter*

This is possibly a stress reaction and may occur in aquatic environments

Still just a theory!

Survivor curves

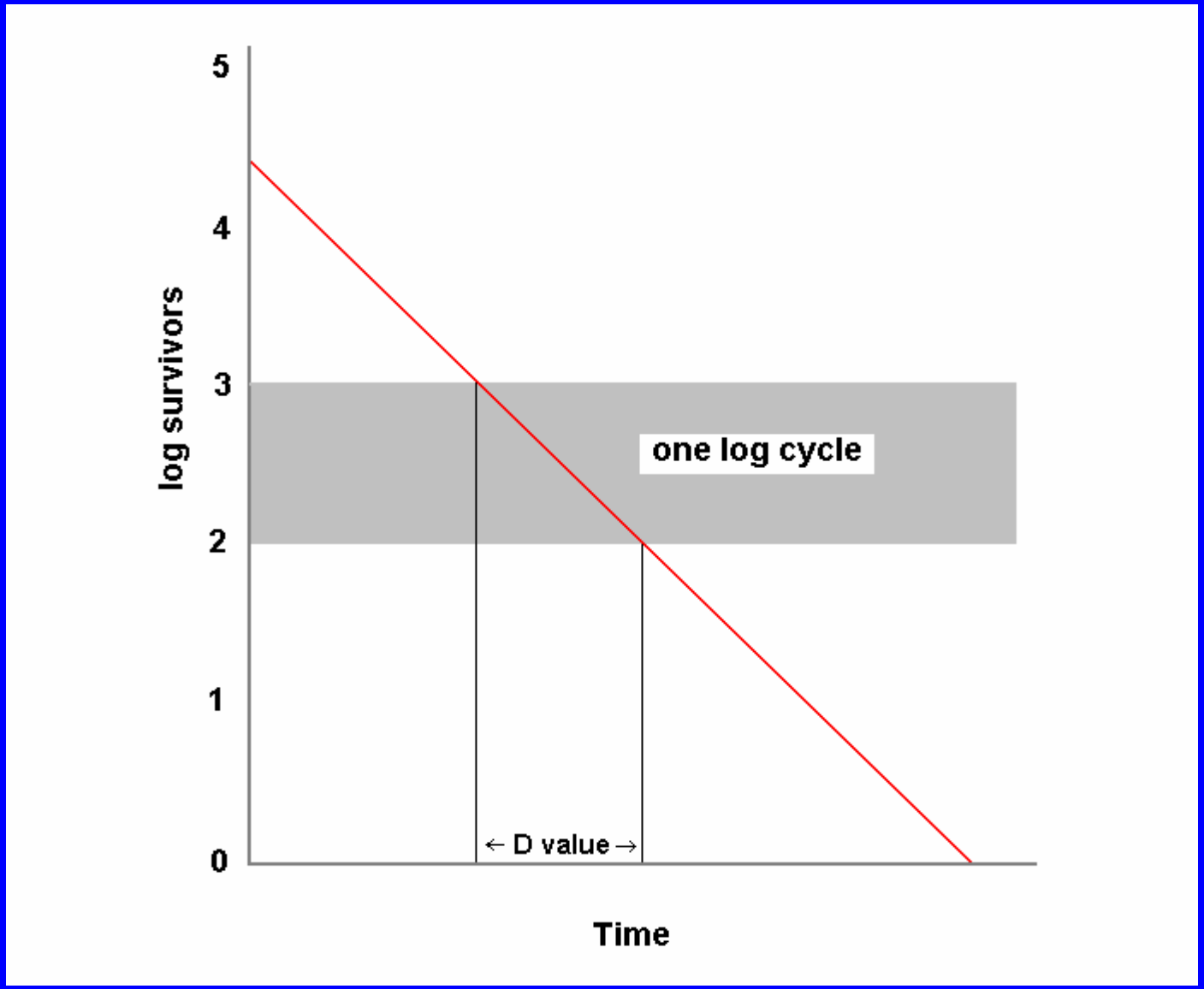
When the population (rather than the individual cell) is considered, microbial death can be given a mathematical basis and various parameters can be calculated

D values give the time taken to reduce the population by one log (e.g. 1000 to 100)

What does this mean?

It basically gives a measure of the resistance to factors such as heat and radiation

Survivor curve



Other more complex parameters can be calculated from D values e.g. Z and F values

Why is this relevant?

D and other values are the basis for all food processing operations-pasteurisation, sterilisation and canning

e.g. Cans undergo a 12D cook for *Clostridium botulinum*- in other words the cook is aimed at reducing *Cl botulinum* spores by 12 logs

For example, this means a reduction from 10^{6} to 10^{-6} spores per gram

Spore formation

For moulds, spore formation is a method of reproduction

Moulds will release millions of spores, which will then move through air or liquid, and if they land on a suitable surface they will germinate and produce ***vegetative*** mould cells

It is solely a ***reproductive*** function

Some bacteria also produce spores-however, this is a ***survival*** mechanism

Bacterial spores are known more correctly as ***endospores***, since they are produced within the cell and only released upon the death of the cell

Endospores are dormant and will only germinate to produce a vegetative cell when conditions are suitable for bacterial growth

Bacterial spores are very resistant to heat, alkali, acid, dryness and can survive for years

Session summary

- Classification and nomenclature are important to ensure consistency of identity and naming
- Growth is an increase in cellular mass and population
- Unicellular growth in closed liquid systems occurs in four phases
- Death curves can be used to calculate appropriate heat treatment regimes for foodstuffs
- Bacterial endospore formation is an important survival mechanism