BIOTECHNOLOGY FOR ENGINEERS
Core Course for
B.Eng.(Chemical Engineering)
Semester I (2010/2011)

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Course Contents

TOPIC 1: Sterilisation

- Introduction to sterilization
- Medium sterilization
- Thermal death kinetics of microorganisms
  - Effect of sterilization on quantity of nutrients
  - Sterilization of culture media
- Batch sterilization
  - Design aspects
  - Del factor during heating and cooling
  - Calculation methods

Course Contents

TOPIC 1: Sterilisation

- Continuous sterilization
- Sterilization of air
  - Methods of air sterilization
  - Theory of fibrous filter
- Sterilization of fermenter

TOPIC 2: Bacterial Growth

- Growth curve and the phases involved
- Batch culture
  - Material balance for batch culture
  - Kinetics of batch culture–Monod Model
- Continuous culture
  - Material balance for continuous culture
  - Kinetics of continuous culture–Monod Model

TOPIC 2: Bacterial Growth

- Cell growth
  - Cell growth measurement
  - Cell count–stoichiometric of cellular growth
- Continuous growth–Ideal Chemostat

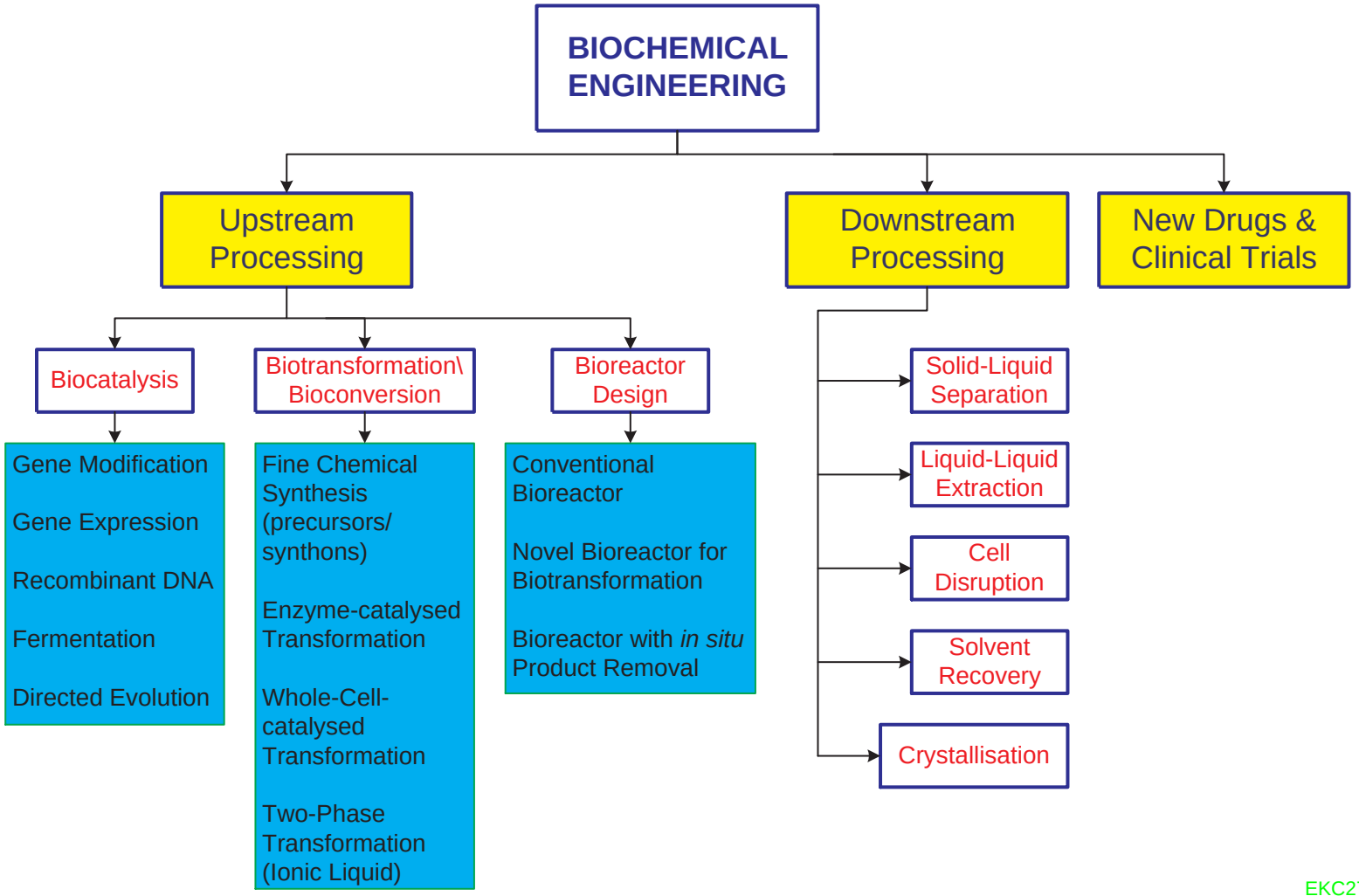
TOPIC 3: Effect of Environmental Conditions

- Effect of different conditions towards growth kinetics
  - Effect on temperature
  - Effect on pH
  - Effect on oxygen concentration
- Heat generation by microbial growth

TOPIC 4: Enzyme-Catalysed Reactions (Prof. Azlina)

- Enzyme Kinetics
  - Reactions involving enzymes
  - Michaelis-Menten Model
  - Enzyme inhibition
- Immobilised-Enzyme system

Biochemical Engineering Overview



Sterilisation

Introduction

- It differentiates a biochemical process from a chemical process.
- Pure bacterial (or any organisms) culture requires a “contamination-free” environment to grow
- Therefore, a container use to grow this organism should be free from contaminants (eg. bacteria/fungi from other species)

Sterilisation

- Fermentation can only proceed with the following:
  1. a microorganism
  2. a medium
  3. a fermenter
  4. nutrients (other additives)
  5. air, for aerobic process

Sterilisation

- A sterile environment requires all of the above
- Else, contaminants will dominate the whole scene which will lead to NO PRODUCT.
- Contamination by a foreign organism may result:
  1. contamination of the final product
  2. medium would be consumed unnecessarily to support growth of contaminating organism
  3. contaminated product will overweigh the desire product

Sterilisation

- Contamination by a foreign organism may result:
  4. contaminated product may interfere with the recovery of the desired product
  5. unsterile air in aerobic fermentation may result in the spoilage of the fermentation product.

Sterilisation

- To overcome the above problems is to carry out sterilisation using any of these methods:
  1. sterilisation of medium
  2. employing as pure inoculum as possible
  3. sterilisation of fermenter
  4. sterilisation of pipes, valves, etc. which come in contact with the fermentation process
  5. sterilisation of all materials to be added to fermenter
  6. sterilisation of air

Sterilisation

- To overcome the above problems is to carry out sterilisation using any of these methods (cont'd):
  7. disinfecting the fermenter and contact parts with non-toxic disinfectant
  8. maintaning aseptic conditions in the fermenter during fermentation
  9. maintaning the optimum/desired pH which discourages the growth of certain contaminants/undesired organisms.

Sterilisation

- Sterilisation can be carried out using:
  1. HEAT from the steam
  2. RADIATION from UV light
  3. CHEMICAL
  4. FILTRATION

Medium Sterilisation

- Medium or media (singular) is an aqueous component which consists of source of nutrients and vitamins for bacterial growth.
- It should be free from any organisms before it can be used for bacterial/fungi inoculation.
- Medium contamination can lead to:
  1. other type of organism to use the nutrients
  2. this can lead to changes in chemical structure of the nutrients
  3. change in pH

Sterilisation

- Medium contamination can lead to (cont'd):
  4. foam formation
  5. formation of metabolic products–leads to growth of fermentation
  6. alter the oxidation/reduction potential of the medium
  7. destroy/alter/degrade fermentation product

Sterilisation

- Steam/heat sterilisation is the most common method used
- Several techniques can be applied:
  1. boiling in water
  2. passing live steam
  3. autoclaving (in pressurised vessel)
- It can either be **batch** or **continuous** or **HTST**

Sterilisation

- Synthetic media–do not require much sterilisation compared to crude media
  - synthetic–(or defined) is a type of medium which consists of known chemicals component (phosphate, chloride, nitrate etc.)
  - crude–(or complex such as Luria-Bertani (LB)) is a type of nutrient which mainly consists of unknown amount of nutrients. The medium normally contains yeast extract, peptone and glucose/glycerol.
- Synthetic media may require only a small amount of heating for sterilisation

Sterilisation

- Crude media are likely to contain fungal spores and sometimes traces of bacterial cells, therefore, requires prolong heating
- However, excessive heating may cause protein denaturing or degrade the sugar
- pH should also be adjusted to neutrality (7.0) before sterilisation then adjust back to the required pH with pre-sterilised acid/alkali
- If special nutrient/vitamin is required during the fermentation, it can be filter-sterilised prior to inoculation

Thermal Death Kinetics of Microorganisms

- Thermal heatr̃ate of destruction of microbes by steam/moist heat
- it is described as;

$$-\frac{dN}{dt} = kN \tag{1}$$

Sterilisation

- or upon integration gives,

$$\ln \frac{N_0}{N_t} = \int_0^t k dt \qquad (2)$$

where;
N = no. of live organisms present
t = sterilisation time period
k = first order thermal death kinetics rate constant

Sterilisation

- The **negative** sign indicates as t increases, the number of organisms present, N tends to decrease
- Further integration of the equation leads to;

$$\ln \frac{N_0}{N_t} = kt$$

- The ratio $\frac{N_0}{N_t}$ is the inactivation factor while the reverse of the ratio describes the survival factor.
- The graphical representations of the function are discussed separately
- The k value depends entirely on the type of cells used as well as the physiological form of the cells.

Sterilisation

- Effect of sterilisation on quality of nutrients:
  - The interactions (between nutrients) can cause deleterious effect to the medium
  - Discoloration (due to Milliard browning) is a common case when reducing sugar reacts with amino acids
  - Therefore, carbohydrate components should be separated from the rest of the medium

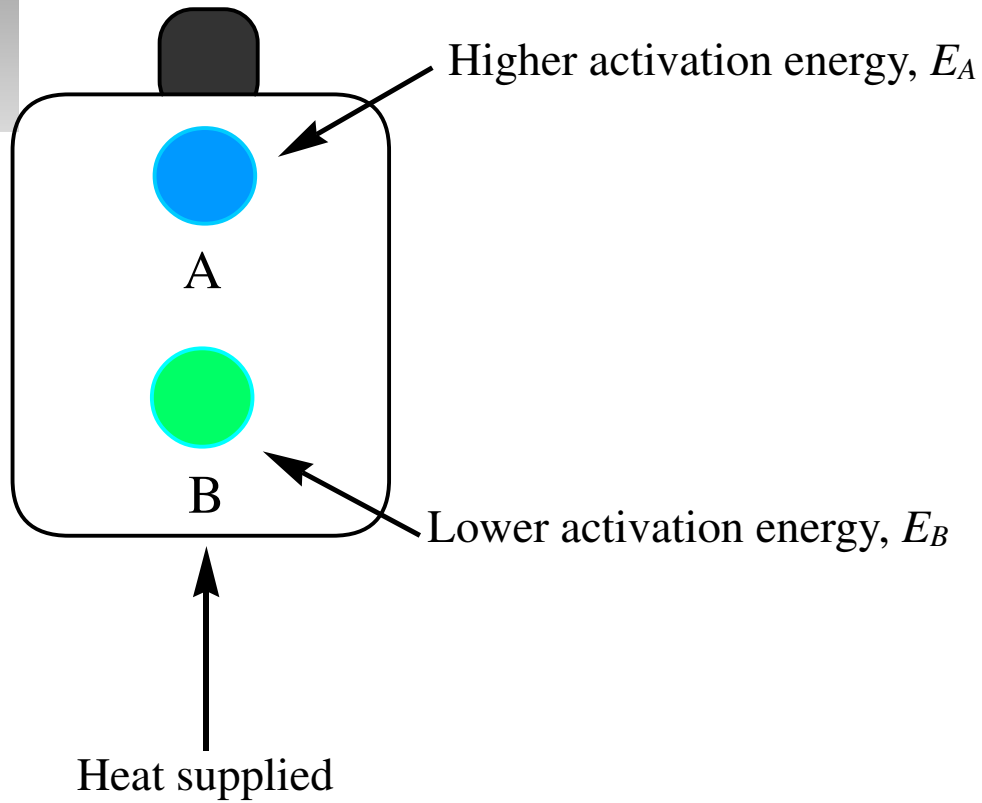
Sterilisation

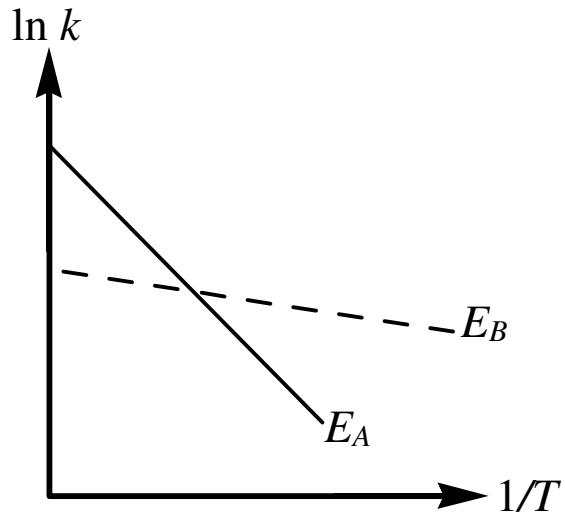
- Sterilisation of culture media:
  - Different cells/spores have different thermal death effects which can only be described using the first order equation as well as the temperature related equation–Arrhenius equation given by;

$$k = Ae^{-\frac{E}{RT}}$$

TEST YOURSELF: How do you relate the above equation with the given graph?

Sterilisation





Sterilisation

- Sterilisation of culture media (cont'd):
  - Sterilisation of mixed cultures with different sterilisation time–leads to different extents of viable cells
  - Consider a mixed culture, C which consists of cultures A and B
  - Using

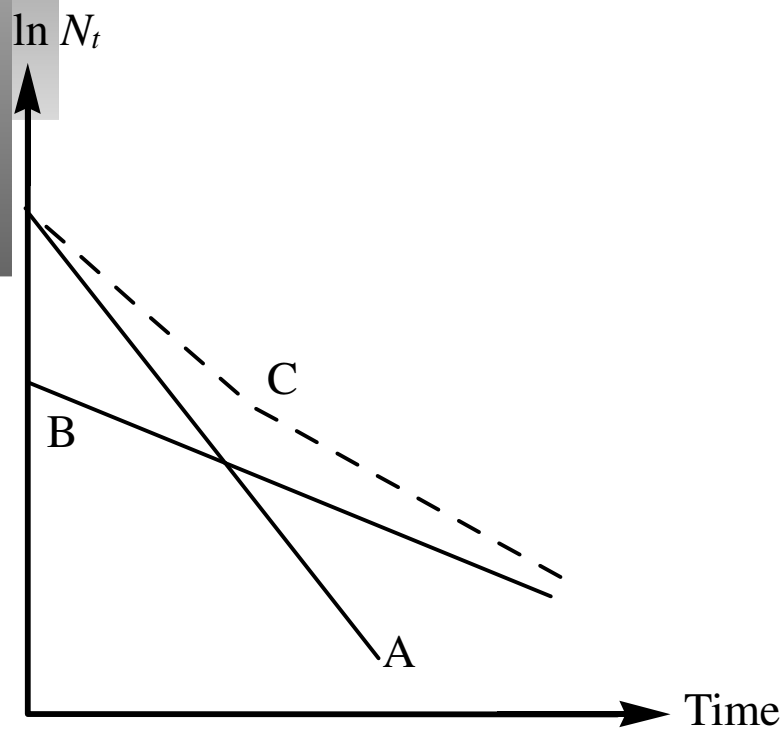
$$\ln \frac{N_0}{N_t} = kt$$

$$\Rightarrow \ln N_t - \ln N_0 = -kt$$

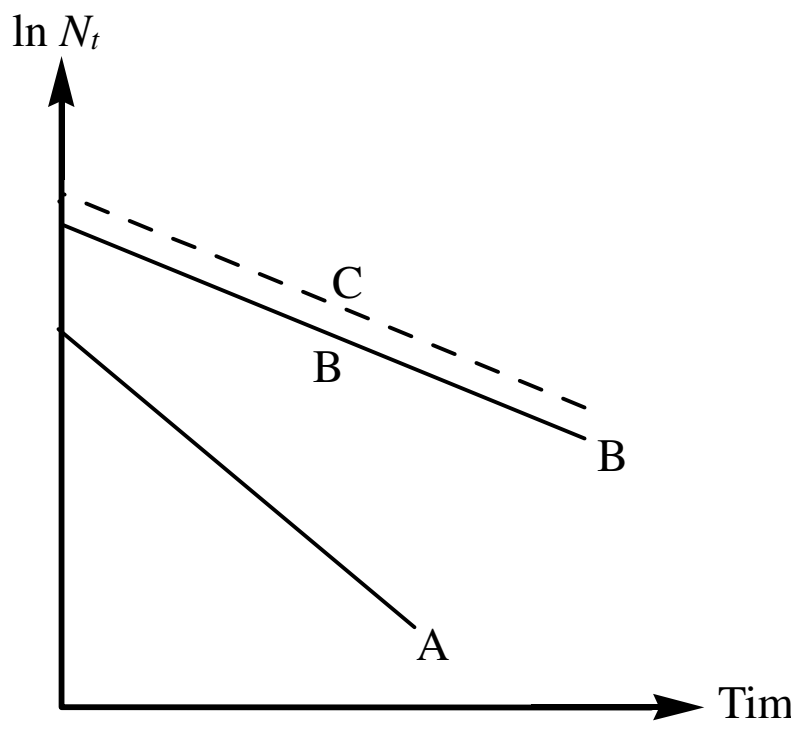
Sterilisation

- Sterilisation of culture media (cont'd):
  - Plots of each culture depending on the cellular viability are as follows:
    1. Figure (a): the more sensitive organism is dying in the early stages when curve C is following line A. When more amount of A is dead, the curve follows the path of B
    2. Figure (b): the more sensitive organism is LESS in number, it dies rather quickly, therefore, the total number of cells will always equal to the number of cells of the resistant organism–B. Hence, curve C almost follows that of B.

Sterilisation



(a) High propotion of sensitive culture A



(b) High propotion of sensitive culture B

Batch Sterilisation

- Sterilisation can be carried out in two different configurations (i) batch and (ii) continuous
- For a batch method–items to be sterilised are loaded in a sterilizer (autoclave) and steam is injected according to the desired programme and later discharge upon completion for further utilisation
- It is the popular technique use in most biotech processes, however, it has ONE disadvantage–destruction of nutrients

Sterilisation

- The advantages include:
  - LOW initial cost of investment
  - LESS chances of contamination after sterilisation–sterilisation can be carried out in same vessel/fermenter itself
  - LESS mechanical failure–since the control is carried out manually
  - EASY handling of high proportion of solid media

Sterilisation

- Design aspects:
  - The determination of whether a sterilisation is complete or not can be related to Arrhenius equation;

$$k = Ae^{\frac{E}{RT}}$$

OR seldom written as;

$$\frac{d(\ln k)}{dT} = \frac{E}{RT^2}$$

which upon integration give a constant k_0 which later equals to A, the Arrhenius constant.

Sterilisation

- Design aspects (cont'd):
  - Using the equation, a set of experiments at various temperatures and k is evaluated.
  - This is later combined with the thermal death equation;

$$\ln \frac{N_0}{N_t} = kt$$

leads to;

$$\ln \frac{N_0}{N_t} = Ate^{-\frac{E}{RT}} \qquad (3)$$

- Design aspects (cont'd):
  - The above equation acts as a "design criterion" called Del factor denoted as;

$$\nabla = \ln \frac{N_0}{N_t} \tag{4}$$

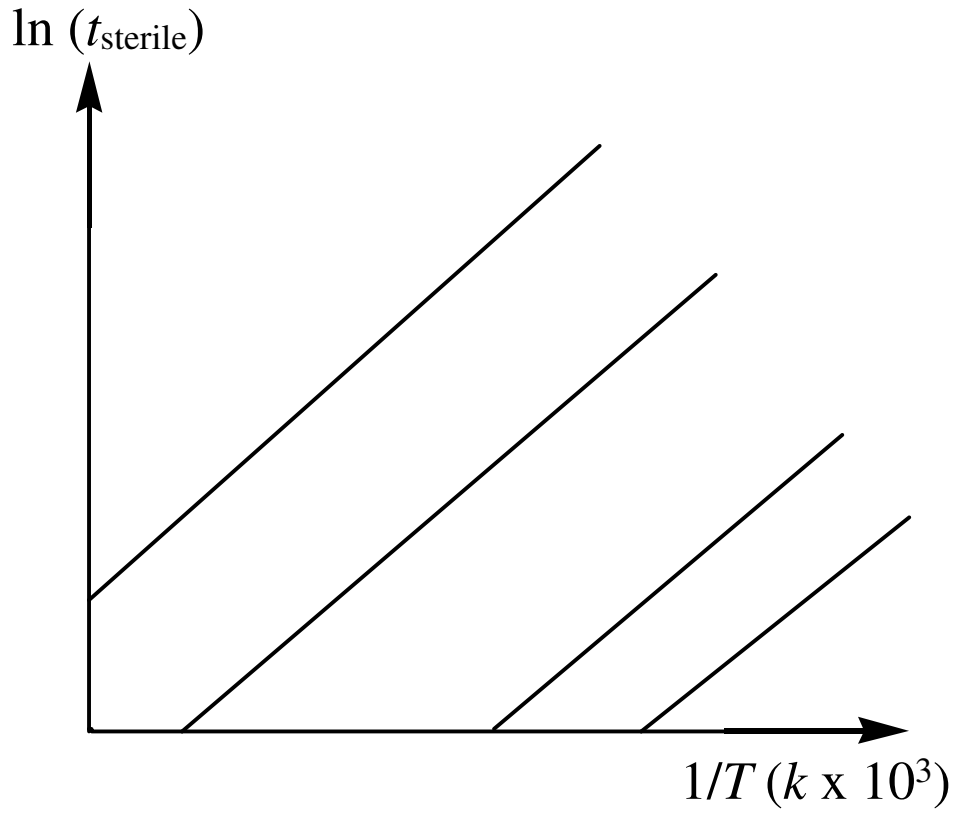
which later gives;

$$\nabla = Ate^{-\frac{E}{RT}} \tag{5}$$

and upon rearrangement leads to;

$$\ln t = \left(\frac{E}{R}\right)\left(\frac{1}{T}\right) + \ln \frac{\nabla}{A} \tag{6}$$

- Design aspects (cont'd):
  - The plot of the equation at different ∇ is given below:



Sterilisation

- Design aspects (cont'd):
  - Equation (6) acts as the design criterion called the *Del Factor*
  - It is defined as the measure of fractional reduction in living organisms count over the initial number present
  - The factor is based on certain heat and time regime
  - Consider the spores of *Bacillus stearothermophilus* which is the most heat resistant microorganism with
    - activation energy, $E = 283$ kJ/mol
    - Arrhenius constant, $A = 1 \times 10^{36.2}$ s^{-1}

Sterilisation

- Design aspects (cont'd):
  - Using the given information and $N_t = 10^{-3}$, the ∇ factor can be easily determined; with

$$N_t = 10{-}3$$

and

$$\nabla = \ln \frac{N_0}{N_t}$$

if the unsterile culture contains $N_0 = 10^{11}$ viable cells, hence

$$\nabla = \ln \frac{10^{11}}{10^{-3}} = \ln 10^{14} = 32.2$$

Sterilisation

- Design aspects (cont'd):
  - The value 32.2 is considered as the overall ∇ factor.
  - Cells normally destroy at 121°C temperature and 0.1 MN/m^{2}gauge
  - This is called the holding period since the medium is held at 121°C
  - Some of the cells will also get destroyed during the heating up to 121°C which is called the heating period
  - Some will get destroyed during the cooling period from 121°C to room temperature

Sterilisation

- Design aspects (cont'd):
  - This can be simplified into;

$$\nabla_{\text{overall}} = \nabla_{\text{heating}} + \nabla_{\text{holding}} + \nabla_{\text{cooling}}$$

- ∇ factor during heating and cooling:
  - From Equation (6), it is obvious that when the temperature increases, ∇ factor also increases
  - It is possible to get ∇ at any temperature $\Rightarrow$ holding period
  - But temperature is not constant during heating-up and cooling-down periods

Sterilisation

- ∇ factor during heating and cooling (cont'd):
  - Using;

$$\nabla = \ln \frac{N_0}{N_t} = \int_0^t k dt \tag{7}$$

  - With the above equation, the heating-up and cooling-down periods can be determined, provided the temperature and time is known
  - However, it is highly unlikely to describe the function of t an T in the form of linear, hyperbolic or exponential
  - Therefore, graphical integration is advisable

Sterilisation

- ∇ factor during heating and cooling (cont'd):
  - An experiment to get a profile of temperature w.r.t time is conducted
  - A graph of time, t against temprature, T is then plotted
  - The temperature, T_1 during a time, t_1 is noted as an average temperature at the beginning of t_1 until the end of t_1 i.e T_1 is marked at the midpoint of t_1
  - Consider the culture of *B. stearothermophilus* with Activation energy, $E = 283$ kJ/mol and Arrhenius constant, $A = 1 \times 10^{36.2}$ s^{-1}

- ∇ factor during heating and cooling (cont'd):
  - Therefore,

$$\nabla_1 = k_1 t$$

$$\nabla_2 = k_2 t$$

$$\nabla_3 = k_3 t$$

etc.
which gives the summation of individual ∇ of;

$$\nabla_{\text{total}} = \nabla_1 + \nabla_2 + \nabla_3 + \cdots$$

Sterilisation

- ∇ factor during heating and cooling (cont'd):
  - Hence,

$$\nabla_{\text{heating}} = \sum_{i=1}^{n} \nabla_i \tag{8}$$

  - Then ∇ at the given temperature can be determined by substituting in the Arrhenius equation

$$k = Ae^{-\frac{E}{RT}}$$

  - A new graph can be plotted with Arrhenius values, k against time, t and the area under the graph can be calculated

Sterilisation

- ∇ factor during heating and cooling (cont'd):
  - Refer to the separate table of time-temperature data of batch sterilisation. ∇ during heating period from 100-121°C within the time of 1-15 min is 8.7 or

$$\nabla_{\text{heating}} = 8.7$$

  - Holding time (121°C):

1. Holding time can be calculated using

$$\nabla_{\text{overall}} = \nabla_{\text{heating}} + \nabla_{\text{holding}} + \nabla_{\text{cooling}}$$

when ∇_{overall} is determined from the given $N_t = 10^{-3}$ and $N_0 = 10^{11}$

- ∇ factor during heating and cooling (cont'd):
  - Holding time (121°C) (cont'd):

2. If

$$\nabla_{\text{cooling}} = 10$$

thus,

$$32.2 = 8.7 + \nabla_{\text{holding}} + 10$$

hence,

$$\nabla_{\text{holding}} = 13.5$$

3. The specific death rate $k = 2.54$ min^{-1},

$$t = \frac{\nabla}{k} = \frac{13.5}{2.54} = 5.3\text{min}$$

Sterilisation

- ∇ factor during heating and cooling (cont'd):
  - Alternative method–Richard's Rapid Calculation
    1. Destruction of spores is at a considerable amount only at temperature $> 100°$C
    2. Heating period is only become effective beyond 100°C up to 121°C
    3. Similarly, for cooling period, the destruction of spores will only take place until the temperature drops up to 100°C (below which, it will not be significant)
    4. Using the same conditions of *B. stearothermophilus*
       - Arrhenius constant, $A = 9.51 \times 10^{37}$ min^{-1}
       - Activation energy. $E = 283$ kJ/mol

Sterilisation

- ∇ factor during heating and cooling (cont'd):
  - Alternative method–Richard's Rapid Calculation

5. The ∇ factor at 100°C is neglected and ∇ at other temperatures are based on the value at the previous temperature which occurred 1 min earlier (since temp. raise is 1°C/min)

$$\nabla_{(T)} = \nabla_{(T-1)} + k_{(T)} \times 1.0$$

6. $k_{(T)}$ is the thermal sterilisation occurred at temp. T in 1 min.

Sterilisation

- ∇ factor during heating and cooling (cont'd):
  - Alternative method–Richard's Rapid Calculation

7. The method bears on two assumptions:
   - spores destruction takes place only beyond 100°C
   - cooling & heating cycles are linear
8. When temp. during heating rises in t_1 min (for 25 min) from 100°C to 121°C instead of 21 min, ∇_{heating} will be proportionately changed;

$$\nabla_{\text{heating}} = 9.601 \times \frac{t}{21}$$

- ∇ factor during heating and cooling (cont'd):
  - Alternative method–Richard's Rapid Calculation Therefore,

$$(\nabla_{\text{heating}}) = 9.601 \times \frac{25}{21} = 11.43$$

9. If the cooling takes place rapidly in 15 min instead of 21 min, thus,

$$\nabla_{\text{cooling}} = 9.601 \times \frac{15}{21} = 6.86$$

10. Once these two values are determined, the holding factor, ∇_{holding} can be calculated.

Continuous Sterilisation

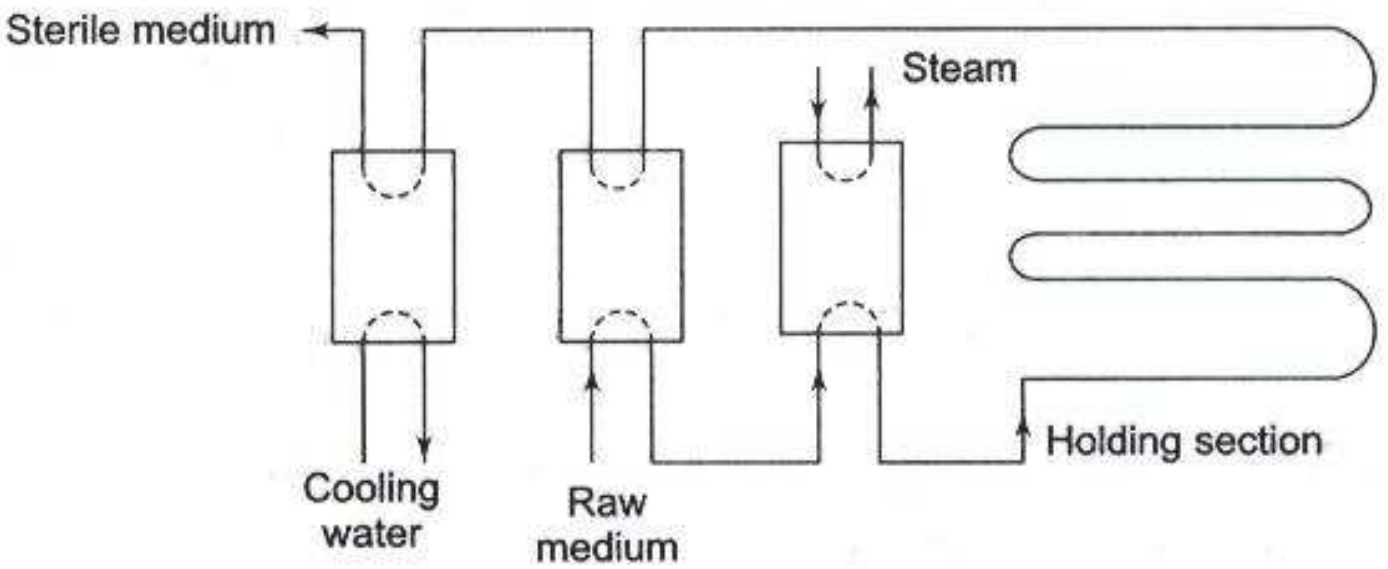
- There will be continuous inflow and outflow of material–best if capacity of operation is high
- Advantages include:
  1. capacity/throughput can be higher
  2. medium quality can be better maintained
  3. can be controlled (avoid human error)
  4. cost of running/operation–LESS
  5. short sterilisation time
  6. the only option for HTST operation
  7. holding capacity of steam–LESS
  8. ease in process scale-up

Sterilisation

- Disadvantages include:
  1. HIGH initial capital investment
  2. aseptic transportation along the line
- Two method of continuous sterilisation:
  1. continuous plate heat exchange
  2. continuous steam injection and flash cooling

Sterilisation

- Continuous plate heat exchange



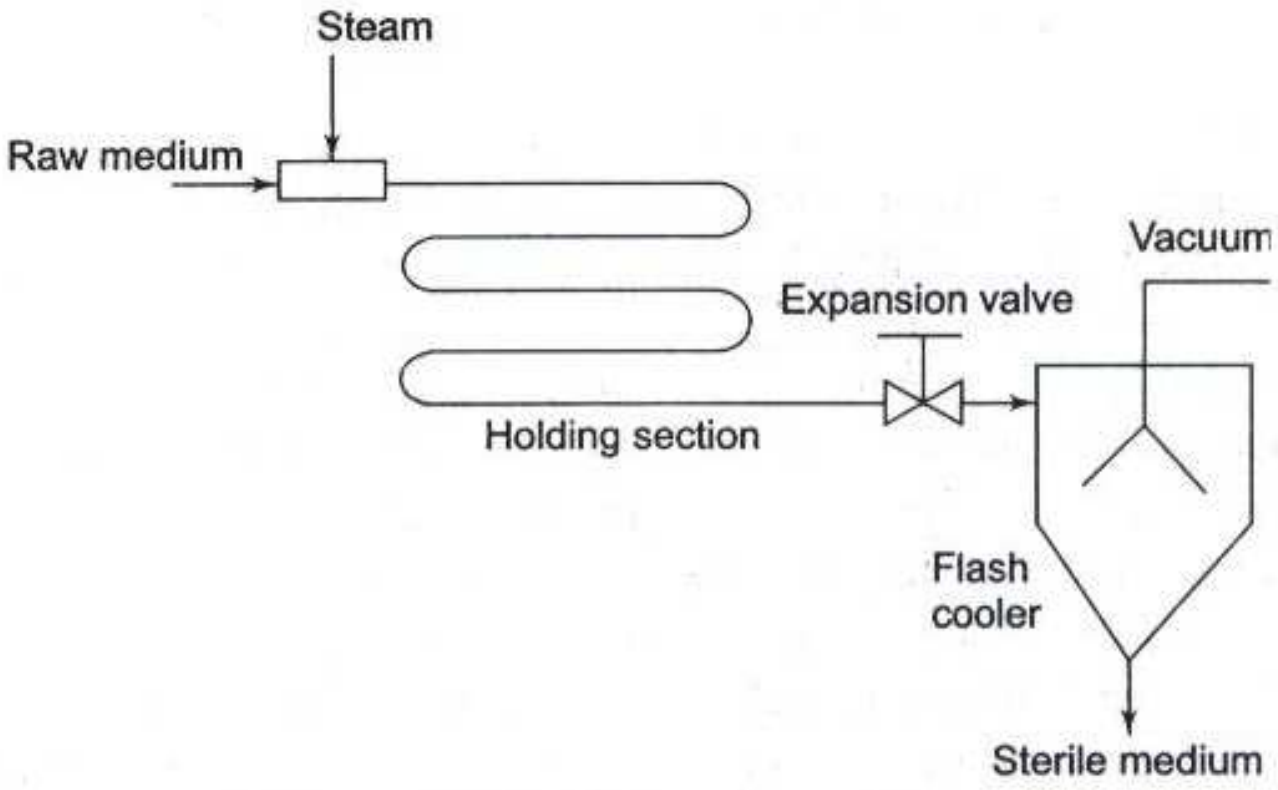
(a) Plate heat exchanger

Sterilisation

- For a continuous plate heat exchange
  1. Incoming unsterile medium is preheated by heat exchanger with the outgoing sterile medium.
  2. Subsequently, it is heated with steam in the heat exchanger and passed through the holding section
  3. The holding section maintains the medium according the required holding time requirement, flow rate of medium and length of holding section

Sterilisation

- Continuous steam injection and flash cooling



(b) Steam injection flash cooling continuous sterilization

Sterilisation

- For a continuous steam injection and flash cooling
  1. Steam is directly and continuously injected along with the medium
  2. Therefore, heating time and heating section are negligible
  3. Holding time is based on the length of the holding pipe–where sterilisation is taking place
  4. Steam and sterilised medium under pressure–passed through the expansion valve into vacuum chamber–steam is removed under vacuum

Sterilisation

- For a continuous steam injection and flash cooling (cont'd)
  5. Sterile medium passes into preheat exchanger–give out heat to the incoming unsterile medium which then passes through the cooling zone

Sterilisation

Sterilisation of Air

- Air is important as a source of oxygen in aerobic fermentation
- For large scale fermentation, air need to be efficiently sterilised
- For a cubic metre of air contains approx. $4 \times 10^3 - -20 \times 10^3$ particles with max. possibility of 12×10^3. The dust particles are approx. 0.6 microns

Sterilisation

- Methods of air sterilisation:
  1. Heating
  2. UV rays OR other electromagnetic waves
  3. germicidal spray
  4. by filtration

Sterilisation

- Heating air is possible BUT not economical–due to its POOR thermophysical properties (lower heat transfer coeff.)
- UV ray is an effective technique in killing air-borne microbes–only applicable in small area
- Germicide can also reduce the amount of bacteria via spraying with phenol, ethylene oxide or formalin–can sterile air in a small size room

Sterilisation

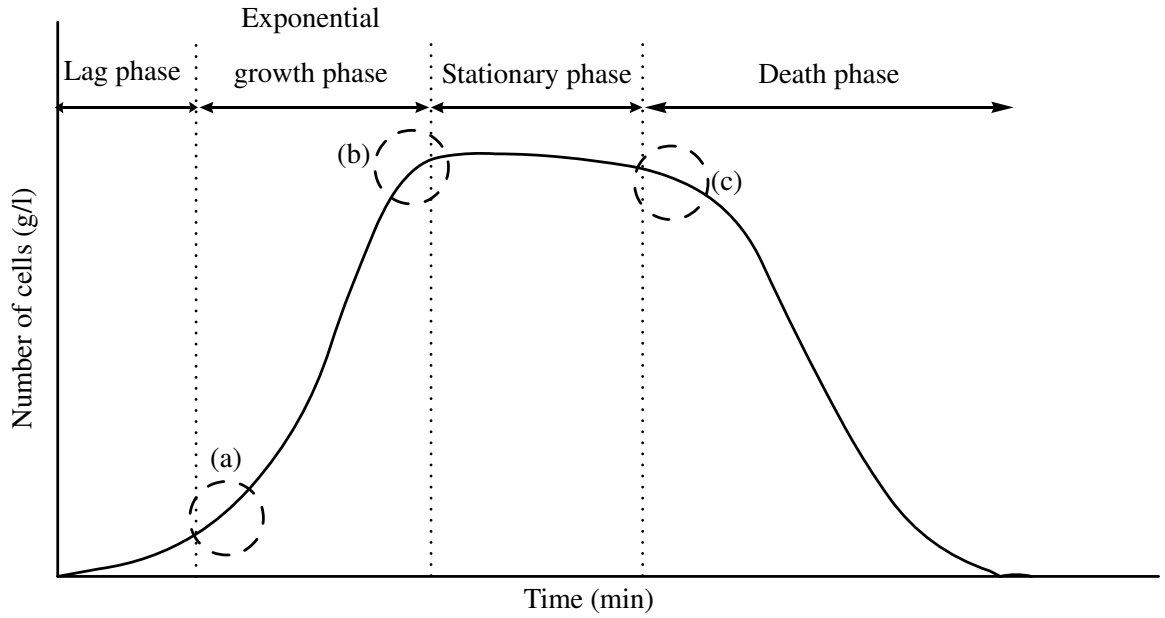
- Filtration is an effective method and practical–filter is used to remove microorganisms form the air provided that:
  - pores of a filter need to be smaller than the size of microbes–absolute filters
  - pore size is bigger than the size of microbes–fibrous filter (cotton, glass-wool, slag, steel-wool etc.)

Sterilisation

- Theory of fibrous filter:
  - The mechanisms applied are: inertial impaction, interception, diffusion, settling by gravitational force and electrostatic force
  - The comparisons are tabulated and given separately

Batch Culture

- Batch culture requires enough nutrient to maintain the growth



Bacterial Growth

- The figure shows an increase of cell at the start of the cultivation (fermentation) process
- Due to the presence of enough nutrient for the cell to grow
- The amount of nutrient decreases as it being consumed by the cell.
- Other side products such as carbon dioxide or ethanol is also formed simultaneously.

Bacterial Growth

- In batch cultures, the cell properties such as;
  - size of cells
  - internal nutrient
  - metabolic function

  vary considerably during the above growth phases.
- No apparent increase of the amount of cell at the start of cultivation, this is termed as the **lag phase**.

Bacterial Growth

- After this period (can be between 10 to 15 mins) the number of cells increases exponentially thus, this stage is called the **exponential growth phase**;
  - the cell properties tend to be constant
  - last for a short period of time
- The next stage is the **stationary phase** where the population of cell achieves it maximum number. This is because:
  - all nutrient in the closed system has been used up by the cell.
  - lack of nutrient will eventually stop the cell from multiplying.

Bacterial Growth

- The rate of cell growth is given by;

$$r_X = \mu x$$

where r_X is the volumetric rate of biomass production with units such as kg.m^{-3}.s^{-1}, x is the viable cell concentration with units of kg.m^{-1} and μ is the **specific growth rate** with units of s^{-1}.

- by following the above equation, the growth is said to follow the **first-order autocatalytic reaction**.

Bacterial Growth

- Similarly, the rate can be written as;

$$r_X = \frac{dx}{dt}$$

and thus;

$$\frac{dx}{dt} = \mu x$$

which upon rearrangement gives;

$$\mu = \frac{1}{x}\frac{dx}{dt}$$

Bacterial Growth

- Integrating the equation with the initial conditions of $(x, t) = (x_0, 0)$ gives;

$$x = x_0 e^{\mu t}$$

- Taking natural log on both sides of the equation leads to;

$$\ln x = \ln x_0 + \mu t$$

- A plot of $\ln x$ versus time, t gives straight line with a slope μ.

Bacterial Growth

- Since the equation is only valid if μ is unchanging, therefore the plot can be used to asses whether the specific growth rate is constant for a particular cell growth.
- Cell growth rates are normally expressed in terms of **doubling time**, t_d.
- When $x = 2x_0$ at $t = t_d$ thus the equation becomes;

$$2x_0 = x_0 e^{\mu t_d}$$

$$e^{\mu t_d} = 2$$

Bacterial Growth

- Taking natural log on both sides;

$$\mu t_d = \ln 2$$

or

$$t_d = \frac{\ln 2}{\mu}$$

Bacterial Growth

- The relationship of cell growth and substrate/nutrient concentration can be expressed in terms of Monod Model given by;

$$\mu = \frac{\mu_{max}[S]}{K_S + [S]}$$

- The function gives a hyperbolic curve upon plotting the data point.

Bacterial Growth

- The final stage of cell cultivation is the **death phase**. The decrease of the number of cell occurs exponentially which happens when the cell breaks open (lysed).
- The rate of death normally follows the first-order kinetics given by;

$$\frac{dN}{dt} = -k_d^{'} N$$

Bacterial Growth

- upon integration leads to

$$N = N_s e^{-k_d' t}$$

  where N_s is the concentration of cells at the end of the stationary phase and at the beginning of the death phase and k_d' is the first order death rate constant.

- Both stationary and death phase, it is important to recognise that there is a distribution of properties among the cells in a population. A summary of the different phases of cell growth is given in the table overleaf.

Bacterial Growth

Growth phase	Rate of growth	Comments
Lag	Zero	Inoculum adapting with the changing condition (temperature, pH)
Acceleration	Increasing	Trivial
Exponential	Constant	Population growth changes the environment of the cells
Retardation	Decreasing	The effect of changing conditions appear
Stationary	Zero	One or more nutrients are exhausted to the threshold level of the cell

Bacterial Growth

Growth phase	Rate of growth	Comments
Decline	Negative	The duration of stationary phase and the rate of decline are strongly dependent on the kind of organisms
Death phase	Negative	Cells lyse due to lack of nutrient

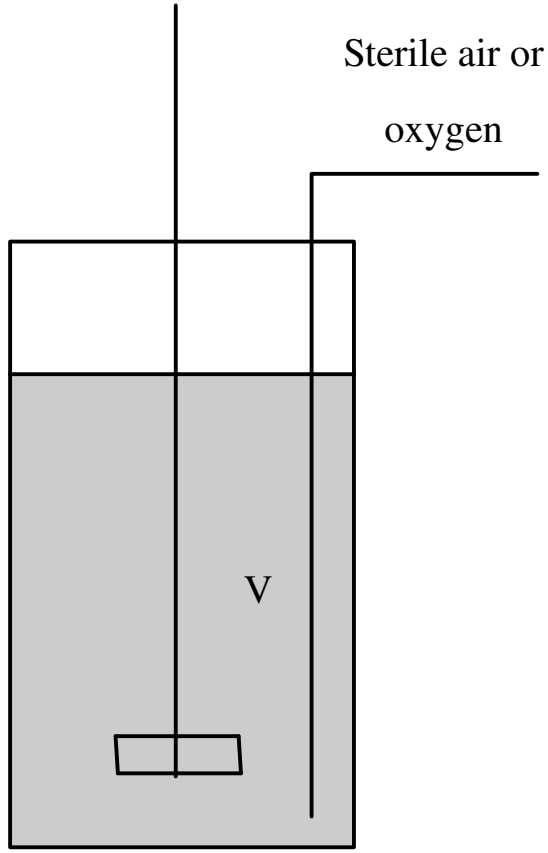
Bacterial Growth

- The balance of a batch reactor is given by the rate of accumulation of product equals to the rate of formation of the product due to chemical reaction or can be simply written as;

$$\begin{aligned} \frac{d}{dt}\left(V_R \cdot c\right) &= V_R \cdot r \\ \frac{dc}{dt} &= r \end{aligned} \tag{9}$$

where c is the amount of the component and r is the reaction rate. V_R in the first line of the equation is the total volume of the culture in the reactor.

Bacterial Growth



Bacterial Growth

- The balance of a batch reactor is given by the rate of accumulation of product equals to the rate of formation of the product due to chemical reaction or can be simply written as;

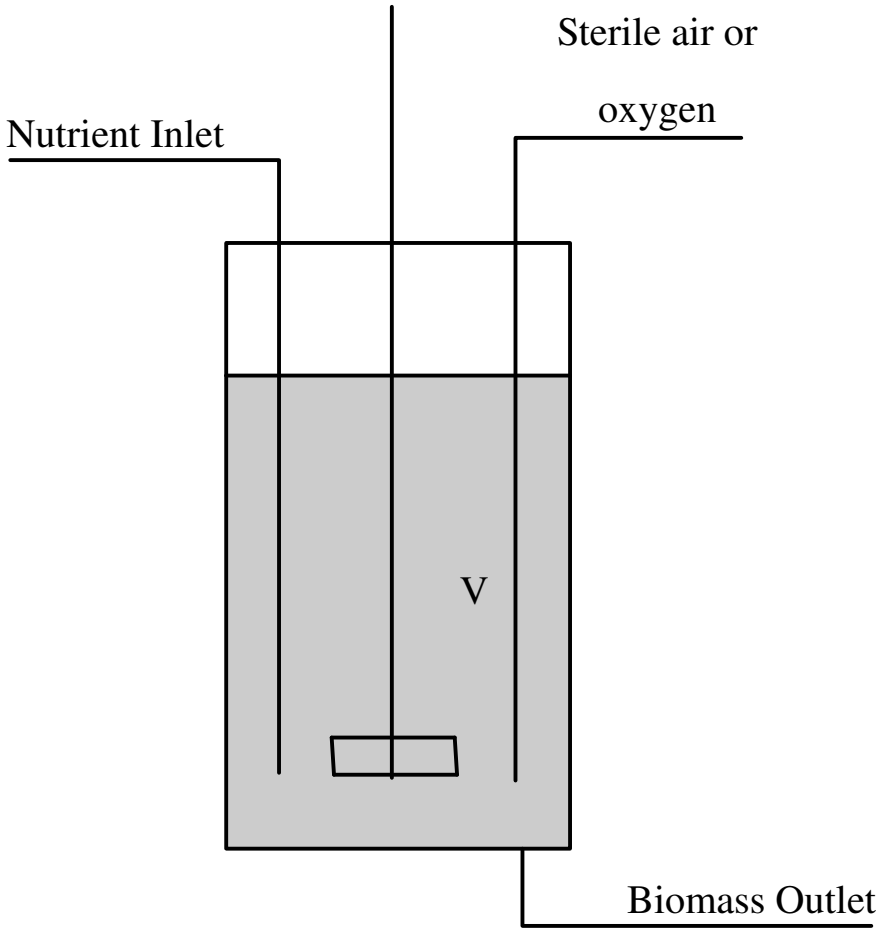
$$\begin{aligned} \frac{d}{dt}\left(V_R \cdot c\right) &= V_R \cdot r \\ \frac{dc}{dt} &= r \end{aligned} \tag{10}$$

where c is the amount of the component and r is the reaction rate. V_R in the first line of the equation is the total volume of the culture in the reactor.

Continuous Culture

- In a continuous culture system, nutrients are supplied to the cell at a constant rate.
- To maintain a constant volume of biomass in the reactor, an equal volume of cell culture is removed.
- This will allow the cell population to reach a *steady-state* condition.
- The reactor configuration of a continuous process is given overleaf.

Bacterial Growth



Bacterial Growth

- The air is pumped into the culture vessel through a sterile filter. Bubbling of air provides:
  - supplying air for the growth of aerobic culture
  - it also circulate and agitate the culture
  - pressurise the head space of the culture vessel such that to provide a force during the removal of the media (and cells) from the vessel for analysis (OD, cell viability etc.).
- it is highly difficult to control the delivery of the nutrient and the removal of the cell so that equal amounts of medium is maintain in the vessel.

Bacterial Growth

- This can be tackled by changing the configuration of the reactor into a *semi-continuous* or *fed-batch* type reactor.
- The rate of flow of medium into a system of continuous culture is known as the **dilution rate**.
- When the number of cells in the culture vessel remains constant over time, the **dilution rate** is said to equal the rate of cell division in the culture, since the cells are being removed by the outflow of medium are being replaces by an equal number through cell division in the culture.

Bacterial Growth

- Similar to that of the batch cultivation, the material balance for a continuous culture can be written as;

$$\frac{d}{dt}\left(V_R \cdot c\right) = F_o c_o - F_i c_i - V_R \cdot r \qquad (11)$$

in order to maintain the volume within the vessel;

$$F_i = F_o = F$$

Bacterial Growth

- Thus,

$$\begin{aligned} \frac{d}{dt}(V_R \cdot c) &= F(c_o - c_i) - V_R \cdot r \\ \Rightarrow \qquad \frac{dc}{dt} &= \frac{F}{V_R}(c_o - c_i) - r - c\frac{dV_R}{dt} \end{aligned} \tag{12}$$

- For a reactor without a recycle system,

$$\frac{dV_R}{dt} = 0$$

therefore,

$$\frac{dc}{dt} = \frac{F}{V_R}(c_o - c_i) - r \tag{13}$$

let the term $\frac{F}{V_R}$ denote as D, the final equation leads to,

$$\frac{dc}{dt} = D(c_o - c_i) - r \tag{14}$$

where D is the **dilution rate** of a CSTR cultivation system.

Batch growth

- Material balance:
  - consider the **cell** concentration in the fermenter;

$$\frac{d}{dt}(V_R x) = V_R r_x$$

$$\Rightarrow V_R \frac{dx}{dt} = V_R r_x$$

since $r = \frac{\mu_{max}[S]}{K_S + [S]}$, thus;

$$\Rightarrow \frac{dx}{dt} = \left(\frac{\mu_{max}[S]}{K_S + [S]} \right)$$

Batch growth

- Material balance:
  - at steady-state, $\frac{dx}{dt} = 0$ thus,

$$\Rightarrow \frac{\mu_{max}[S]}{K_S + [S]} = 0$$

  - this clearly shows that when there is no growth in the bioreactor, there will be no cell to divide, thus no new cells to be produced.

Batch growth

- Material balance:
  - consider the **substrate/nutrient** concentration in the fermenter;

$$\frac{d}{dt}(V_R[S]) = -V_R r_x$$

  - at steady-state condition, $\frac{d[S]}{dt} = 0$ which leads to $[S] = 0$ as well.

Continuous growth

- Material balance:
  - the balance on the continuous stirred-tank fermenter/reactor is similar to that of the batch, provided that there are inlet and outlet to and from the fermenter respectively.
  - consider the **cell** concentration, x;

$$\frac{d}{dt}(V_R x) = F_i x_i - F_o x_o + V_R r_x$$

but

$$r_x = \mu x$$

Continuous growth

- Material balance:
  - thus;

$$\frac{d}{dt}(V_R x) = F_i x_i - F_o x_o + V_R \mu x$$

upon expansion and let $\mu = \frac{\mu_{max}[S]}{K_S + [S]}$ gives,

$$V_R \frac{dx}{dt} = F_i x_i - F_o x_o + V_R \left(\frac{\mu_{max}[S]}{K_S + [S]} \right) x$$

Continuous growth

- Material balance:
  - upon rearranging;

$$\frac{dx}{dt} = \frac{F_i}{V_R}x_i - \frac{F_o}{V_R}x_o + \left(\frac{\mu_{max}[S]}{K_S + [S]}\right)x$$

  - for a constant volume in a bioreactor,

$$F_i = F_o = F$$

Continuous growth

- Material balance:
  - thus,

$$\frac{dx}{dt} = \frac{F}{V_R}x_i - \frac{F}{V_R}x_o + \left(\frac{\mu_{max}[S]}{K_S + [S]}\right)x$$

let $\frac{F}{V_R} = D$, which represents the **dilution rate** of the fermenter, hence,

$$\frac{dx}{dt} = Dx_i - Dx_o + \left(\frac{\mu_{max}[S]}{K_S + [S]}\right)x$$

Continuous growth

- Material balance:
  - at a steady-state condition, $\frac{dx}{dt} = 0$;

$$\Rightarrow Dx_i - Dx_o + \left(\frac{\mu_{max}[S]}{K_S + [S]}\right) x = 0$$

  - since the cells must be grown in a sterile environment, therefore;

$$x_i = 0$$

and

$$x_o = x$$

Continuous growth

- Material balance:
  - then the equation becomes;

$$Dx = \left(\frac{\mu_{max}[S]}{K_S + [S]} \right) x$$

$$\Rightarrow D = \mu_{max} \left(\frac{[S]}{K_S + [S]} \right)$$

where;

$$D_{max} = \mu$$

Continuous growth

- Material balance:
  - at a *washout* steady-state, as the dilution rate, D of the continuous fermenter increases, the concentration of substrate, $[S]$ also increases, where

$$D > D_{max}$$

at $x = 0$.

Continuous growth

- Material balance:
  - the feed substrate, $[S]$ will be such that

$$[S] \gg K_S$$

and D_{max} becomes approximately equals to the maximum specific growth rate, μ_{max},

$$D_{max} \approx \mu_{max}$$

Continuous growth

- Material balance:
  - solving for the substrate concentration leads to;

$$[S] = \frac{K_S D}{\mu_{max} - D}$$

Continuous growth

- Material balance:
  - consider the **substrate** concentration, $[S]$;

$$\frac{d}{dt}(V_R[S]) = F_i[S]_i - F_o[S]_o - \frac{1}{Y_{X/S}} V_R r_x$$

where $Y_{X/S}$ is defined as;

$$Y_{X/S} = \frac{mass\ of\ biomass/cells\ produced}{mass\ of\ substrate\ used}$$

Continuous growth

- Material balance:
  - expanding and rearranging the terms gives;

$$\frac{d[S]}{dt} = D([S]_i - [S]_o) - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max}[S]}{K_S + [S]}\right)x$$

  - at steady-state condition, $\frac{d[S]}{dt} = 0$, therefore,

$$\Rightarrow D([S]_i - [S]_o) - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max}[S]}{K_S + [S]}\right)x = 0$$

Continuous growth

- Material balance:
  - let the outlet substrate concentration, $[S]_o = [S]$ therefore,

$$\Rightarrow D([S]_i - [S]) - \frac{1}{Y_{X/S}} \left(\frac{\mu_{max}[S]}{K_S + [S]} \right) x = 0$$

and upon substitution of the term $[S]$ into the above equation gives;

$$x = Y_{X/S} \left([S]_i - \frac{K_S D}{\mu_{max} - D} \right)$$

Effects of Environment on Cell Growth

- There are 3 main environmental factors that can give effect to the cell growth;
  1. Temperature
  2. pH
  3. Oxygen
- Effect of Temperature
  - Temperature[a] can change the configuration of cell constituents, especially proteins and membrane components. There is a 2-fold increase in the specific growth rate, μ for every 10°C rise in temperature. For certain type of cells the optimal temperature is listed below:

[a] Courtesy of Dr. W.S. Long

Effects of Environment on Cell Growth

- Effect of Temperature
  - psychrophiles ($T_{opt} < 20^{\circ}\mathrm{C}$)
    - Bacteria that grow at temperature in the range of -5 to 30°C.
    - Optimum temperatures between 10 to 20°C.
    - Microbes have enzymes which catalyse best when the conditions are cold.
    - Cell has membranes that remains fluid at these lower temperatures.

Effects of Environment on Cell Growth

- Effect of Temperature
  - psychrophiles ($T_{opt} < 20^{\circ}\mathrm{C}$)
    - Examples of this type of organism: algae that live near the poles of the Earth at temperature below 0°C, bacteria that spoil milk, meat, vegetables and fruits even when they are stored in a fridge–it only slows down the the rate of spoilage of food and cannot stop the growth of these microbes.

Effects of Environment on Cell Growth

- Effect of Temperature
  - mesophiles ($T_{opt} = 20 - 50^{\circ}\mathrm{C}$)
    - Microbes that grow at optimal temperatures in the range of 20 to 40°C.
    - These type of organisms can be found in warm-blooded creatures e.g. humans. Pathogenic bacteria is one of the kind as well as symbiotic bacteria.

Effects of Environment on Cell Growth

- Effect of Temperature
  - thermophiles ($T_{opt} > 50°\mathrm{C}$)
    - Bacteria that live at temperatures exceed 50°C.
    - It can tolerate at very harsh conditions such as decomposing materials, hot springs (temp. between 80 to 85°C) and deep in the oceans by thermal vents bubbling up from the hot rocks below the Earth's crust.

Effects of Environment on Cell Growth

	Temperature (°C)		
Group	**Minimum**	**Optimum**	**Maximum**
Thermophiles	40 to 45	55 to 75	60 to 80
Mesophiles	10 to 15	30 to 45	35 to 47
Psychrophiles			
– Obligate	-5 to 5	15 to 18	19 to 22
– Facultative	-5 to 5	25 to 30	30 to 35

Effects of Environment on Cell Growth

- Above the temperature given above, the growth rate decreases and thermal death may occur.
- When the cells cannot sustain high temperature, thermal death rate exceeds the growth rate i.e. viable cells will drop. According to the Arrhenius equation;

$$\begin{aligned} \mu &= A e^{-\frac{E_a}{RT}} \\ \Rightarrow \quad k_d^{'} &= A^{'} e^{-\frac{E_d}{RT}} \end{aligned} \tag{15}$$

Effects of Environment on Cell Growth

- A typical values for E_d for thermal destruction of microorganism are high, small increase of temperature have a significant effect on $k_d^{'}$ and the rate of death.
- - E_a: Activation energy for growth
  - E_d: Activation energy for thermal death
- Temperature also affects product formation and yield coefficient.

Effects of Environment on Cell Growth

- The optimal temperature for growth and product formation differ;
  - when $T > T_{opt}$, the maintenance requirement of cell increases.
  - m_s or m_p increases with increasing temperature with value of E between 15 to 20 $kcal \cdot mol^{-1}$ and thus decreases $Y_{X/S}$.(m_s and m_p are the maintenance coefficient for substrate and product respectively).

Effects of Environment on Cell Growth

- Temperature also affects the rate limiting step of biochemical mechanisms;
  - during fermentation, the rate of biochemical reaction increases at higher temperature (reaction rate higher than the diffusion rate).
  - therefore, diffusion becomes the rate limiting step. This is normally occur in immobilised cell system with pore diffusional resistance.
    - Molecular diffusion: E = 6$kcal \cdot mol^{-1}$
    - Biochemical reaction: E = 10$kcal \cdot mol^{-1}$
  - diffusional limitations must be carefully considered at high temperature.

Effects of Environment on Cell Growth

- The plot of growth rate versus temperature of the group of microorganisms given in the previous Table is shown in Figure 1.

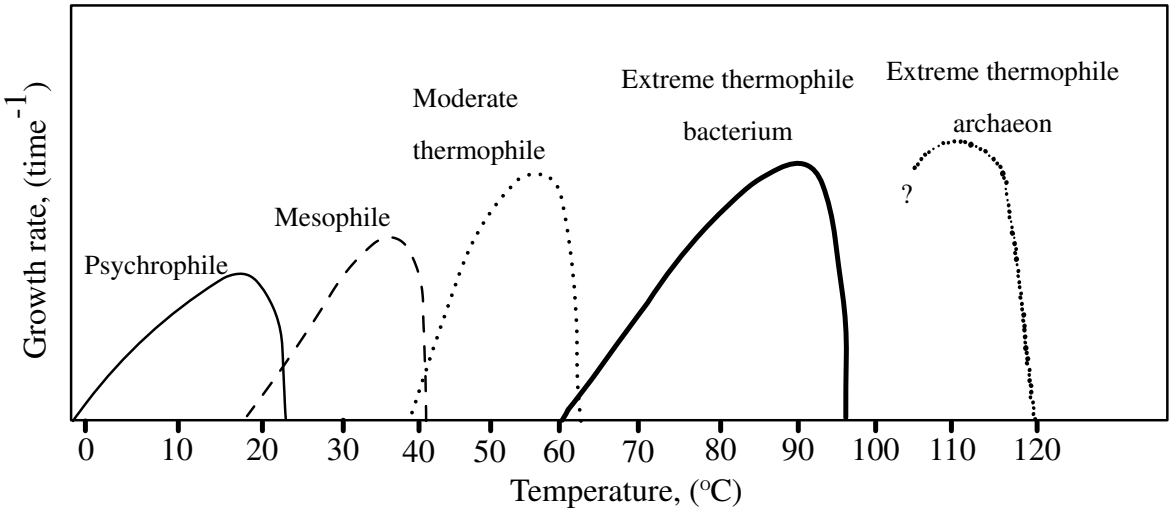


Figure 1: Growth rate versus temperature for five environmental classes of procaryotes.

Effects of Environment on Cell Growth

- Effect of pH
  - The influence of pH on cellular activity is determined by the sensitivity of the individual enzymes to changes in the pH.
  - Enzymes are normally active only within a certain pH interval and the total enzyme activity of the cell is therefore a complex function of the environmental pH.
  - Consider the influence of pH on a single enzyme, which is taken to represent the cell activity.

Effects of Environment on Cell Growth

- Effect of pH

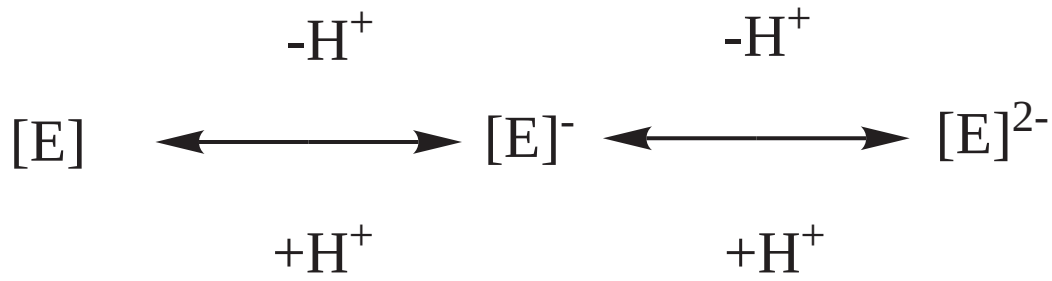


Figure 2: Enzyme forms with changing of pH.

Effects of Environment on Cell Growth

- Effect of pH
  - Where $[E]^-$ is taken to be the active form of enzyme while the two other forms are assumed to be completely inactive, with K_1 and K_2 being the dissociation constants for the free acids $[E]$ and $[E]^-$ respectively.
  - The fraction of active enzyme $[E]^-$ is calculated to be;

$$\frac{[E]^-}{[E]_{tot}} = \frac{1}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}} \tag{16}$$

Effects of Environment on Cell Growth

- Effect of pH
  - The enzyme activity is taken to be

$$k = k_e[E]^{-}.$$

If the cell is determined by the activity of the enzyme considered above, the maximum specific growth rate, μ_{max} becomes;

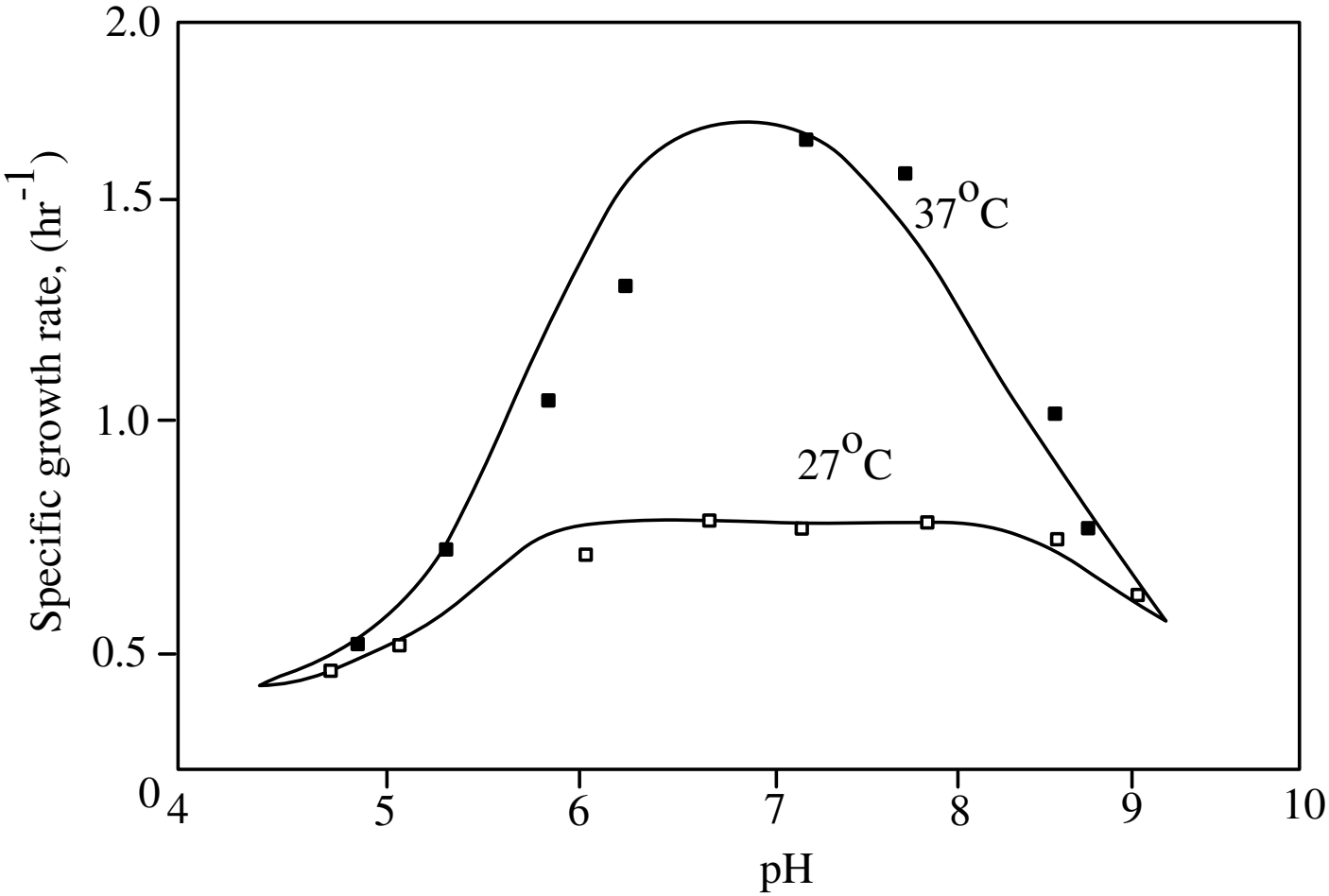
$$\mu_{max} = \frac{k[E]_{tot}}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}} \qquad (17)$$

Effects of Environment on Cell Growth

- Effect of pH
  - This model has been found to fit well with the specific activity data for a few microorganisms and the fitting for *E. coli* cell as well as values of the fitted parameters is given in Figure 3 and Table 1 respectively.

Effects of Environment on Cell Growth

- Effect of pH



Effects of Environment on Cell Growth

- Effect of pH

Parameter	27°C	37°C
k	0.82	1.90
K_1	1.5×10^{-5}	5.0×10^{-6}
K_2	1.0×10^{-9}	3.0×10^{-9}

Table 1: Parameter values for the fitting of equation (17 to the specific growth data).

Effects of Environment on Cell Growth

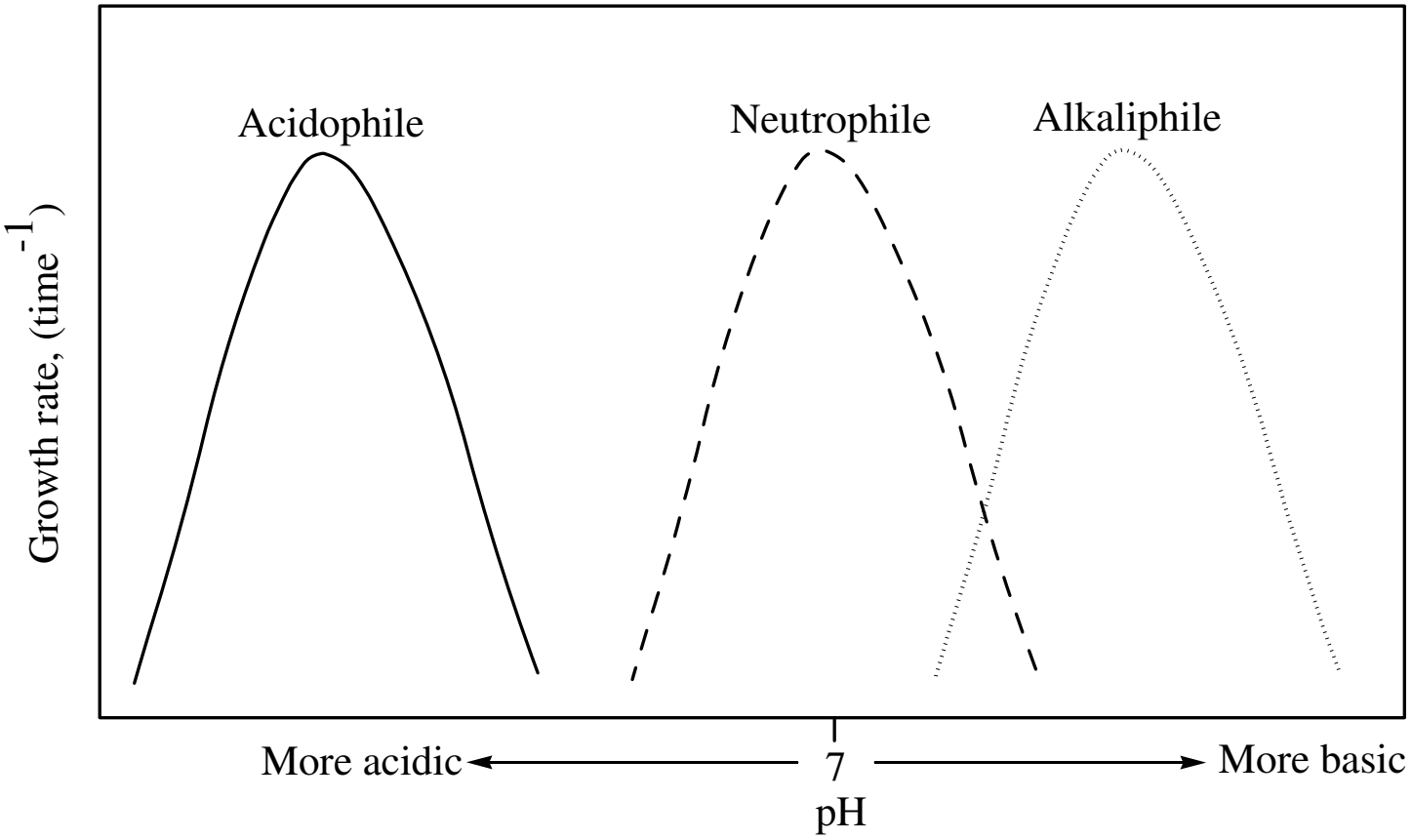
- Effect of pH
  - The range of pH over which the microorganism grows is defined by the 3 main categories;
    1. minimum pH: below which the microorganisms cannot grow
    2. maximum pH: above which the microorganisms cannot grow
    3. optimum pH: at which the microorganisms grow best.

Effects of Environment on Cell Growth

- Effect of pH
  - For most bacteria there is an orderly increase in growth rate between the minimum and the optimum
  - Orderly decrease in growth rate between the optimum and the maximum pH–reflecting the general effect of changing $[H^+]$ on the rates of enzymatic reaction as shown in Figure 120.

Effects of Environment on Cell Growth

- Effect of pH



Effects of Environment on Cell Growth

- Effect of pH
  - Microorganisms that grow at an optimum pH well below neutrality (7.0) are called *acidophiles*.
  - Those that grow best at neutral pH are called *neutrophiles* and those that grow best under alkaline conditions are called *alkaliphiles*.
  - Obligate *acidophiles* such as *Thiobacillus* species require a low pH for growth. This is due to their dissolving membranes and the cells lyse at neutrality.
  - Several genera of Archaea such as *Sulfolobus* and *Thermoplasma* are obligate *acidophiles*.

Effects of Environment on Cell Growth

- Effect of pH
  - A few types of procaryotes are given in Table 123.

	pH		
Organism	**Minimum**	**Optimum**	**Maximum**
Thiobacillus thiooxidans	0.5	2.0–2.8	4.0–6.0
Sulfolobus acidocaldarius	1.0	2.0–3.0	5.0
Bacillus acidocaldarius	2.0	4.0	6.0
Zymomonas lindneri	3.5	5.5–6.0	7.5
Lactobacillus acidophilus	4.0–4.6	5.8–6.6	6.8
Staphylococcus aureus	4.2	7.0–7.5	9.3
Escherichia coli	4.4	6.0–7.0	9.0

Effects of Environment on Cell Growth

- Effect of pH

	pH		
Organism	**Minimum**	**Optimum**	**Maximum**
Clostridium sporogenes	5.0–5.8	6.0–7.6	8.5–9.0
Erwinia caratovora	5.6	7.1	9.3
Pseudomonas aeruginosa	5.6	6.6–7.0	8.0
Thiobacillus novellus	5.7	7.0	9.0
Streptococcus pneumoniae	6.5	7.8	8.3
Nitrobacter sp.	6.6	7.6–8.6	10.0

Effects of Environment on Cell Growth

- Effect of Oxygen
  - Dissolved oxygen[a] (DO) is an important substrate in an aerobic fermentations–limiting substrate, since O_2 is sparingly soluble gas in water (7p.p.m at standard temperature and pressure: 25°C and 1atm). When oxygen is rate limiting, the specific growth rate, μ varies with DO.
    - Below a critical oxygen concentration, the growth approaches a first-order rate.
    - Above the critical oxygen concentration, growth rate becomes independent of DO.

[a] Courtesy of Dr. W.S. Long

Effects of Environment on Cell Growth

- Effect of Oxygen
  - When dissolved oxygen level is below the critical level, then the oxygen concentration is a growth rate limiting, thus, another medium becomes the growth extent limiting.
  - This can be seen in *Azotobacter vivelandii*–at dissolved oxygen of 0.05mg$\cdot$l^{-1}, the growth rate of the organism is 50% of its maximum, even if large amount of nutrient (glucose) is present.

Effects of Environment on Cell Growth

- Effect of Oxygen
  - This, however, does not affect the amount of cells formed since the cells will keep growing whenever there is enough oxygen dissolved. The critical oxygen concentration varies with different organisms;
    - bacteria and yeast: 5–10%
    - mold cultures: 10–50%
  - The growth extent or the mass of cells formed depends on the amount of glucose, on the other hand, the growth rate depends on the amount of oxygen dissolved, DO.

Effects of Environment on Cell Growth

- Effect of Oxygen
  - The transfer of oxygen from gas bubbles to cells is limited by oxygen transfer through liquid film surrounding the gas bubbles;

$$N_{O_2} = k_L a(C^* - C_L)$$

where N_{O_2} is the oxygen transfer rate (OTR) with the units of $mg_{O_2} \cdot l^{-1} h^{-1}$ and;

- k_L: O_2 transfer coefficient ($cm \cdot h^{-1}$)
- a: gas–liquid interface area ($cm^2 \cdot cm^{-3}$)
- $k_L a$: volumetric O_2 transfer coefficient (h^{-1})
- C^*: saturated dissolved oxygen ($mg \cdot l^{-1}$)
- C_L: actual dissolved oxygen ($mg \cdot l^{-1}$)

Effects of Environment on Cell Growth

- Effect of Oxygen
  - Oxygen Uptake Rate (OUR)
    - Oxygen uptake rate is given by;

$$OUR = q_{O_2} X = \frac{\mu X}{Y_{X/O_2}} \tag{18}$$

where;

- q_{O_2}: specific rate of O_2 consumption ($mg_{O_2} \cdot g_{dcw}^{-1} \cdot h^{-1}$)
- Y_{X/O_2}: oxygen yield coefficients ($g_{dcw} \cdot g_{O_2}^{-1}$)
- X: cell concentration ($g_{dcw} \cdot l^{-1}$)

Effects of Environment on Cell Growth

- Effect of Oxygen
  - Oxygen Uptake Rate (OUR)
    - When oxygen is the rate limiting step; rate of oxygen consumed is equal to the rate of its being transferred, and assuming that there is no maintenance requirement for oxygen compared to cell growth. Therefore;

$$\begin{aligned} OUR &= N_{O_2} \\ \Rightarrow \frac{\mu X}{Y_{X/O_2}} &= k_L a(C^* - C_L) \end{aligned} \tag{19}$$

Effects of Environment on Cell Growth

- Effect of Oxygen
  - Oxygen Uptake Rate (OUR)
    - since the terms (μX) is the rate of cell growth with respect to time, hence;

$$\frac{dX}{dt} = k_L a(C^* - C_L)Y_{X/O_2} \tag{20}$$

Effects of Environment on Cell Growth

- Effect of Oxygen
  - Oxygen Uptake Rate (OUR)
    - The rate of cell growth varies linearly with the amount of dissolved oxygen, DO.
    - Thus, the concentration of oxygen in any fermentation medium should be maintained in order to obtain a stable cell growth. This can be established by;
      - using a supply of oxygen-enriched air
      - using pure oxygen under atmospheric pressure between 2 to 3 atm.

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth
  - Chemical reaction that occurs within the cells produces energy which is released as heat.
  - Cellular heat production is primarily the result of energy and growth metabolism which consequently makes the heat generated from the cells to be approximately proportional to the energy in utilising substrate.

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth
  - the yield factor due to the heat produced, $Y_{\triangle}$ can be written as;

$$Y_{\triangle}(g_{cell} \cdot kcal^{-1}) = \frac{Y_s}{(\Delta H_s - Y_s \Delta H_c)} \frac{(g_{cell} \cdot g_{substrate}^{-1})}{(kcal \cdot g_{substrate}^{-1})} \quad (21)$$

  - It is derived based on the approximate energy balances over the two different pathways shown in Figure 4

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth
  - The predominant oxidant is oxygen, the heat generation ΔH_s per gram of substrate completely oxidised minus $Y_s \Delta H_c$, the heat obtained by combustion of cells grown from the same amount of substrate, will reasonably approximate the heat generation per gram of substrate consumed in the fermentation which produces cells, $\mathrm{H_2O}$ and $\mathrm{CO_2}$.

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth

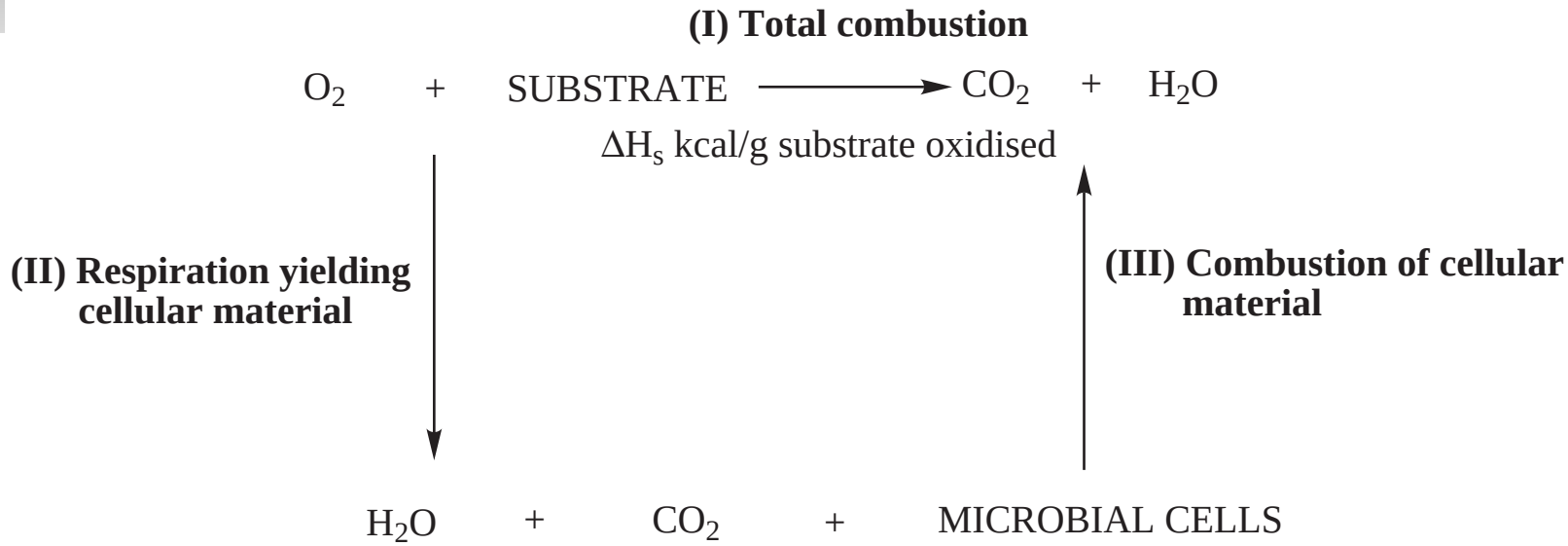


Figure 4: Approximate heat balance in substrate consumption.

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth
  - If there are no experimental data on the energy as well as the compounds used, the heat of combustion can be estimated using the energy obtained from the transfer of electrons from a compound that has reductance degree denoted by γ_s to a compounds such as carbon dioxide or methane which has zero reductance degree.
  - This gives a function of

$$K\gamma_s$$

where K is within the range of 26 to 31kcal/(electron equivalence).

Effects of Environment on Cell Growth

- **Example 2:** Estimate the heat of combustion of *Pseudomonas fluorescens* growing in glucose medium.

- **Answer 2:** The reaction for cell combustion is given as;

$$\mathrm{CH_{1.66}N_{0.20}O_{0.27}(CELLS)} + 1.28\mathrm{O_2} \rightarrow$$

$$\mathrm{CO_2} + 0.10\mathrm{N_2} + 0.83\mathrm{H_2O}$$

  - Assuming that such a reaction produces carbon dioxide, water and nitrogen. By assuming that the heat of combustion of oxygen is 104kcal per mole of O_2, the heat released by combustion of bacteria can be estimated using the inverse of equation (21);

- **Answer 2:**
  -

$$\frac{1}{Y_\Delta}$$

$$\Rightarrow \frac{(1.28)(104)}{[12 + (1.66)(1) + (0.02)(14) + (0.27)(16)]}$$

$$\left(\frac{(\text{mol } \text{O}_2)(\text{kcal} \cdot \text{mol}^{-1} \text{ O}_2)}{g}\right)$$

$$= 6.41\text{kcal} \cdot \text{g}^{-1}$$

Effects of Environment on Cell Growth

- **Answer 2:**
  - In an actual dry cells measurement, the weight includes about 10% of ash, therefore, the heat of combustion of cell, $\Delta \mathrm{H_c}$ is only 90% of the value calculated above, i.e. approximately $5.8 kcal \cdot g_{dcw}^{-1}$.

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth
  - It can be seen that group of hydrocarbons produces more heat compared to the partially oxygenated species, for instance, $Y_\Delta(\mathrm{CH_4}) < Y_\Delta(\mathrm{CH_3OH})$ and $Y_\Delta(n-alkanes) < Y_\Delta(glucose)$.
  - The comparison of the growth factors between various bacteria is tabulated in the next slide.

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth

Substrate	Y_s, ($\mathbf{g}_{cell}$·$\mathbf{g}_{substrate}$)	Y_{O_2}, ($\mathbf{g}_{cell}$·$\mathbf{g}_{\mathrm{O_2\ consumed}}$)	Y_Δ, ($\mathbf{g}_{cell} \cdot kcal$)
Malate	0.34	1.02	0.30
Acetate	0.36	0.70	0.21
Glucose equivalents, (molasses, starch, cellulose)	0.51	1.47	0.42

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth

Substrate	Y_s, ($\mathbf{g}_{cell}\cdot\mathbf{g}_{substrate}$)	Y_{O_2}, ($\mathbf{g}_{cell}\cdot\mathbf{g}_{\mathrm{O_2\ consumed}}$)	Y_Δ, ($\mathbf{g}_{cell}\cdot kcal$)
Methanol	0.42	0.44	0.12
Ethanol	0.68	0.61	0.18
Isopropanol	0.43	0.23	0.074
n-Paraffins	1.03	0.50	0.61
Methane	0.62	0.20	0.061

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth
  - The heat produced from cellular growth can also be related to the Gibbs free energy ΔG.
  - Some of the free energy present in the substrate dissipates to the surrounding environment.
  - This is apparent in an aerobic processes, the heat generated may be substantial and to keep the temperature constant, bioreactors are equipped with either external or internal cooling facilities.

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth
  - The basis for thermodynamic calculations is the definition of Gibbs free energy in the ith reaction component;

$$G_i = G_i^0 + RT \ln(c_i) \tag{22}$$

  - Where G_i^0 is the Gibbs free energy at standard conditions and c_i is the concentration of the reaction component in moles per litre.
  - In dealing with microbial growth, only free energy of certain components are required/interested thus, arbitrary energy level is introduced.

Effects of Environment on Cell Growth

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  - This is done by assigning values for the standard Gibbs free energy level of $\mathrm{CO_2}$, $\mathrm{H_2O}$ and molecular nitrogen, $\mathrm{N_2}$ to zero.
  - This reference point is chosen since no living systems can have Gibbs free energy for growth from combustion of any of these 3 compounds.
  - Equation (22) changes to;

$$\Delta G_{ci} = \Delta G^0_{ci} + RT \ln(c_i) \qquad (23)$$

Effects of Environment on Cell Growth

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  - Where the subscript c refers to combustion. Using the given equation for combustion, the change of Gibbs free energy for intracellular reactions, J can be calculated;

$$\Delta G_{c,j} = \sum_{i=1}^{N} \alpha_{ji} \Delta G_{ci} + \sum_{i=1}^{L} \gamma_{ji} \Delta G_{ci} + \sum_{i=1}^{M} \beta_{ji} \Delta G_{ci} \qquad (24)$$

$$j = 1, \ldots, J$$

Effects of Environment on Cell Growth

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  - Where N, L and M refers to substrate, biomass and metabolic product respectively, while α, β and γ defines the stoichiometric coefficients of a particular growth equation.
  - It follows the conditions:
    - if $\Delta G_{c,j} < 0$, the reaction runs spontaneously in the forward direction.
    - if $\Delta G_{c,j} = 0$, the reaction is in equilibrium.
  - To calculate the energy dissipation, the last term in equation (23) can be omitted since its contribution to the overall change in free energy in a reaction is negligible.

Effects of Environment on Cell Growth

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  - The only standard free energies reduce equation (24) into;

$$\Delta G^0_{c,j} = -D_j = \sum_{i=1}^{N} \alpha_{ji} \Delta G^0_{ci} + \sum_{i=1}^{L} \gamma_{ji} \Delta G^0_{ci} + \sum_{i=1}^{M} \beta_{ji} \Delta G^0_{ci} \quad (25)$$

  - With D_j representing the amount of energy dissipated to the surrounding environment when jth reaction proceeds. This is given by;

$$\Delta G^0_{c,j} = \Delta H^0_{c,j} - T \Delta S^0_{c,j} \quad (26)$$

Effects of Environment on Cell Growth

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  - Where $\Delta H^0_{c,j}$ represents the enthalpy of reaction which equals to the generation of heat for the reaction. Similar to equation (25), the enthalpy balance for jth reaction can be set up as;

$$\Delta H^0_{c,j} = -Q_j = \sum_{i=1}^{N} \alpha_{ji} \Delta H^0_{ci} + \sum_{i=1}^{L} \gamma_{ji} \Delta H^0_{ci} + \sum_{i=1}^{M} \beta_{ji} \Delta H^0_{ci}; \quad j = 1,...,J \tag{27}$$

  - With Q_j representing the amount of heat generated by the jth reaction.

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth
  - Multiplying this equation with the rate of the individual reactions, the specific rate of the heat generation in each intracellular reaction can be found and therefore the total specific heat generated by the growing cells by adding all the specific rates of all reactions;

$$r_Q = \sum_{j=1}^{N} Q_{s,j} r_{s,j} + \sum_{j=1}^{J} Q_j r_j + \sum_{j=1}^{M} Q_{p,j} r_{p,j} \qquad (28)$$

Effects of Environment on Cell Growth

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  - For reactions involving transport of species across cellular membrane such as substrate diffusing in and product diffusing out of cells, they do not contribute to the overall heat generation and thus the above equation reduces into;

$$r_Q = \sum_{j=1}^{J} Q_j r_j \tag{29}$$

  - Equations (29) or (28) and (27) are important especially in estimating the amount of heat generated by growth processes.

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth
  - A correlation was then proposed by Roels in 1983 to determine the heat of combustion of several compounds and it is given by;

$$\Delta H_{ci}^{0} = 115\kappa_i^* \tag{30}$$

  - With the units of kJ per C-mole and κ_i^* is defined as the degree of reduction of the ith compounds calculated on the basis of N_2 being the nitrogen source; i.e. the multiplier for nitrogen λ_N is zero. κ_i^* in the above equation is calculated using;

$$\kappa_i^* = 4 + \alpha_i - 2b_i.$$

Effects of Environment on Cell Growth

- **Example 3:** Calculate the heat generated during the growth of Baker's yeast (*Saccharomyces cerevisiae*) in two different conditions;
  1. aerobic growth with stoichiometric equation (without the formation of ethanol) in a defined medium;

$$0.600\mathrm{CH_{1.62}O_{0.53}N_{0.15}} + 0.400\mathrm{CO_2} + 0.649\mathrm{H_2O}$$

$$\rightarrow \mathrm{CH_2O} + 0.090\mathrm{NH_3} + 0.384\mathrm{O_2}$$

Effects of Environment on Cell Growth

- Given that the heat of combustion of;
  2. anaerobic growth with stoichiometric equation;

$$0.122\mathrm{CH_{1.62}O_{0.53}N_{0.15}}+0.582\mathrm{CH_3O_{0.5}}+0.297\mathrm{CO_2}+0.05\mathrm{H_2O}$$

$$\rightarrow\mathrm{CH_2O}+0.018\mathrm{NH_3}$$

  - *Saccharomyces cerevisiae*, $\Delta H^0_{c,cell}$ = 560kJ/mole
  - Glucose, $\Delta H^0_{c,glucose}$ = 467kJ/mole
  - Ammonia, $\Delta H^0_{c,ammonia(g)}$ = 383kJ/mole
  - Ethanol, $\Delta H^0_{c,ethanol}$ = 683kJ/mole

Effects of Environment on Cell Growth

- **Answer 3:**
  1. Using equation (27), the heat generated when *Saccharomyces cerevisiae* is grown aerobically;

$$-\mathrm{Q}_{\mathrm{aerobic}}=0.600(560)-467-0.090(383)=-165.5\mathrm{kJ/mole}$$

$$\mathrm{Q}_{\mathrm{aerobic}}=165.5\mathrm{kJ/C-mole\,glucose}$$

  2. Similarly, for the anaerobic growth of yeast;

$$-\mathrm{Q}_{\mathrm{anaerobic}}=0.122(560)+0.582(683)-467-0.018(383)=-8.1\mathrm{kJ/mole}$$

$$\mathrm{Q}_{\mathrm{anaerobic}}=8.1\mathrm{kJ/C-mole\,glucose}$$

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth
  - It is clear from the above results, that the heat generated in the aerobic process is much higher than in the anaerobic process.
  - Large amount of heat is produced when the yeast is grown aerobically is not reflected in a correspondingly large biomass yield.
  - This shows that the enthalpy originally present in glucose is wasted in the aerobic process but for the anaerobic process, the enthalpy of glucose is retrieved back in ethanol.

Effects of Environment on Cell Growth

- Heat Generation by Microbial Growth
  - It is also apparent that the cooling requirement in aerobic process is much higher compared to anaerobic processes.
  - If baker's yeast is grown aerobically, at a specified growth rate of 0.25hr^{-1}, the specific rate of heat production can be calculated using;

$$r_Q = \sum_{j=1}^{M} Q_{p,j} r_{p,j}$$

$$= 165.5 \times \frac{0.25}{0.600} = 69\text{kJ} \cdot \text{C} - \text{mole}^{-1}\text{biomass} \cdot \text{hr}^{-1}$$

Effects of Environment on Cell Growth

- **Question 3:** Heat generation during the batch growth of *Saccharomyces cerevisiae*: During the batch growth of *S. cerevisiae*, there is a high glucose concentration at the start of the fermentation, and ethanol is produced. When the glucose is exhausted, the yeast may continue to grow on ethanol, but the specific growth rate is lower and two distinct growth phases are consequently observed:
  - when yeast metabolises glucose
  - it metabolises ethanol

Effects of Environment on Cell Growth

- **Question 3:** Such a growth is known as *diauxic growth* and can be described by the given stoichiometric equations:

$$\mathrm{CH_{1.6}O_{0.5}N_{0.15}} + 2.06\mathrm{CH_3O_{0.5}} + 2.20\mathrm{CO_2} + 1.59\mathrm{H_2O}$$

$$\rightarrow 5.26\mathrm{CH_2O} + 0.15\mathrm{NH_3} + 1.13\mathrm{O_2}$$

and

$$\mathrm{CH_{1.6}O_{0.5}N_{0.15}} + 0.59\mathrm{CO_2} + 1.81\mathrm{H_2O} \rightarrow 1.59\mathrm{CH_3O_{0.5}} + 0.15\mathrm{NH_3} + 1.35\mathrm{O_2}$$

Effects of Environment on Cell Growth

- **Question 3:** Given that the reaction/growth rate for the first equation with glucose, $\mu_{glucose} = 0.35\text{hr}^{-1}$ and for the second equation with ethanol, $\mu_{ethanol} = 0.15\text{hr}^{-1}$. Calculate the heat of production in each of the two reactions using the heat of combustion given in **Example 3**.