BIOCHEMICAL ENGINEERING

A Concise Introduction

by

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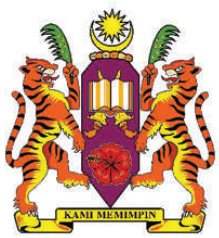

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Preface to the First Manuscript

Biochemical Engineering has been offered as one of the elective courses to the Universiti Sains Malaysia's Chemical Engineering undergraduates since 1998 under the topic of Bioprocess Engineering. The change of name from Bioprocess to Biochemical Engineering shows that the School of Chemical Engineering is very much aware of the current development of the area that combines biology and biochemistry with engineering and technology. The course might have changed its name, however, the core ingredients of Biochemical Engineering remain intact. New findings that evolve through research and development have been included so that the students are up-to-date with the most recent technology within the field.

This Lecture Notes Series has been used to cover Biochemical Engineering course that was usually offered to the Fourth Year, Chemical Engineering undergraduates. The lectures combine the topics which were handled by both authors as well as previous few academics before us, to name a few; Associate Professor Ghasem Najapour (currently a lecturer in Iran), Dr. Long Wei Sing (currently a Humbolt Fellow in Germany) and Dr. Jyoti Prasad Chaudhury (currently a lecturer in India). We greatly appreciate their help and guidance towards compiling this manuscript. Not to forget, the second author whom entirely involved during the first arrangement of the course outline as well as its syllabus nearly a decade ago, and without her support and encouragement, this manuscript would not have come into existence.

It is hoped that this manuscript would be of use to the undergraduate students who are taking the course as an elective or other similar courses that have some elements of Biochemical Engineering. It could also be an additional reference to the postgraduate students undertaking research work that relate either entirely or only a small fraction of Biochemical Engineering field. This manuscript summarises and simplifies into a concise form most of the details the topics that discussed in lengthy paragraphs within the main Biochemical Engineering textbooks.

The manuscript could be easily downloaded form the website of the School of Chemical Engineering, Universiti Sains Malaysia under the address:

`http://chemical.eng.usm.my/notes/HEKARL/notes/ekc471_notes.pdf`

Both authors would like to welcome any comments from the readers both students and academics alike so that the contents of this manuscript could be greatly improved. Your help and cooperation are very much appreciated.

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About the Authors

MASHITAH MAT DON, PhD. obtained her Bachelor Degree, B.Sc.(Hons.) in Botany in 1988 from the University of Malaya. Right after her first degree, she remained with the University of Malaya for her Master Degree (MPhil.) in the area of Biotechnology. In 1992, she joined the Forest Research Institute of Malaysia, (FRIM) for the period of 3 years where she was actively involved with the research and development focusing on the exploration of Malaysia's tropical forest for the production of pharmaceutical and agrochemical products. Most of her research while at FRIM, were based upon applying the core engineering and biological disciplines to the real life problems. Two main areas which include; microbial fermentation technology and process modelling have been her major work within the field of Biochemical Engineering. She left FRIM in 1995 and joined the School of Chemical Engineering, Universiti Sains Malaysia where she was appointed as the Programme Chairman of Bioprocess and Environmental Group for the period of 2 years. Being a Programme Chairman at the time, she was assigned to compile a syllabus of Bioprocess Engineering Course to be introduced as an elective within the Bachelor Degree at the School of Chemical Engineering. She was one of the pioneers in establishing Bioprocess Engineering to the undergraduate students which through years of revisions has changed its name to Biochemical Engineering until this present days. She has written many research articles for journals and proceedings both locally and internationally while working with FRIM as well as with Universiti Sains Malaysia and has also graduated a number of postgraduate students since then. She received her Doctorate Degree, (PhD.) in 2005 from the University of Malaya in the area of Biochemical Engineering and has recently been elected as an Associate Professor at the School of Chemical Engineering, Universiti Sains Malaysia.

MOHAMAD HEKARL UZIR, PhD. obtained his Bachelor Degree, B.Eng.(Hons.) in Chemical Engineering in 1999 from the University of Leeds, United Kingdom. After graduation, he joined Universiti Teknologi PETRONAS, (UTP) as a Trainee Lecturer until July 2000. He then left UTP and joined Universiti Sains Malaysia where he received the Fellowship Scheme to pursue for the higher degree courses. He received his Master Degree, (MSc.) in Advanced Chemical Engineering in 2001 from the University of London and a Diploma of Imperial College, (DIC.) from Imperial College of Science, Technology and Medicine, London. He completed his Doctorate Degree, (PhD.) in 2005 in the area of Biochemical Engineering from the University of London where he was attached as a Research Associate at the Advanced Centre for Biochemical Engineering, (ACBE) University College London, United Kingdom. He is now a Senior Lecturer at the School of Chemical Engineering, Universiti Sains Malaysia.

Contents

Chapter 1

Batch and Continuous Cultures

1.1 Batch culture

There are a number of biochemical processes that involve batch culture/growth of cell. This type of culture requires enough nutrient to maintain the growth. A typical growth profile is given in the figure below.

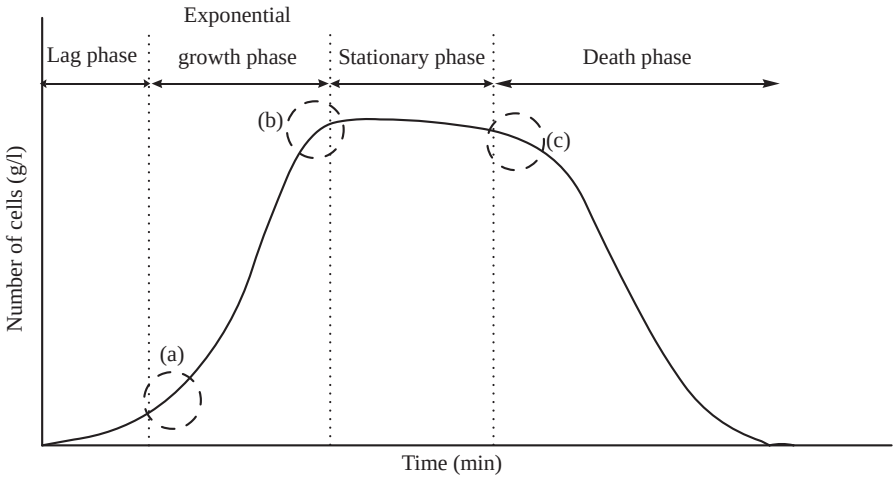

Figure 1.1: Growth curve of a batch culture. (a) Acceleration phase, (b) Retardation phase and (c) Declining phase.

The figure shows an increase of cell at the start of the cultivation (fermentation) process. This is due to the presence of enough nutrient for the cell to grow. At the same time 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.

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**. After this period (can

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

Table 1.1: Summary of the growth phases shown in Figure 1.1.

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.

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;

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

which 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. In 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 Table 1.1.

Material balance for a batch cultivation

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;

$$\begin{aligned} \frac{d}{dt}\left(V_R \cdot c\right) &= V_R \cdot r \\ \frac{dc}{dt} &= r \end{aligned} \tag{1.1}$$

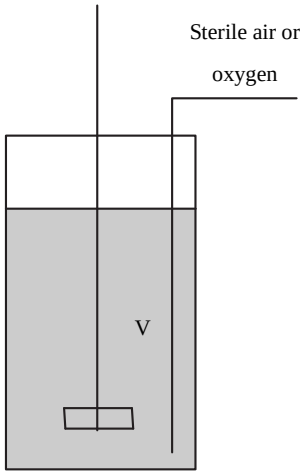

Figure 1.2: A batch reactor configuration.

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. The configuration of simple batch reactor is given in Figure 1.2

1.2 Continuous culture

Batch and continuous culture systems differ in that, in a continuous culture system, nutrients are supplied to the cell at a constant rate and in order 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 in Figure 1.3.

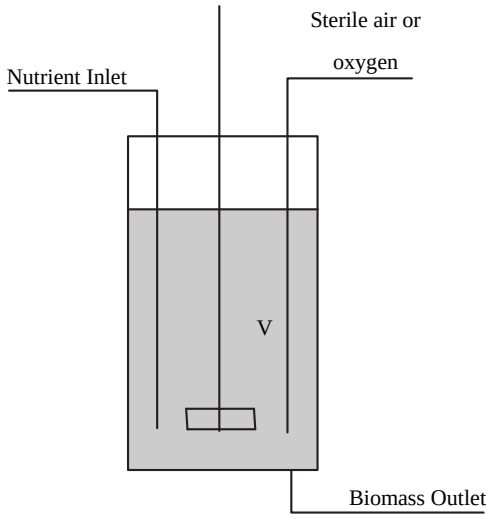

Figure 1.3: A continuous stirred tank reactor (CSTR) configuration.

Similar to the batch cultivation, 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.).

However 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. 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.

1.2.1 Material balance for a continuous cultivation

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

$$\frac{d}{dt}(V_R \cdot c) = F_o c_o - F_i c_i - V_R \cdot r \tag{1.2}$$

in order to maintain the volume within the vessel;

$$F_i = F_o = F$$

thus,

$$\begin{aligned} \frac{d}{dt}(V_R \cdot c) &= F(c_o - c_i) - V_R \cdot r \\ \Rightarrow \quad \frac{dc}{dt} &= \frac{F}{V_R}(c_o - c_i) - r - c\frac{dV_R}{dt} \end{aligned} \tag{1.3}$$

for a reactor without a recycle system,

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

therefore,

$$\frac{dc}{dt} = \frac{F}{V_R}(c_o - c_i) - r \tag{1.4}$$

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

$$\frac{dc}{dt} = D(c_o - c_i) - r \tag{1.5}$$

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

1.3 Advantages and disadvantages of different modes of operation of the stirred tank reactor

By far, the stirred tank reactor is the most common type of bioreactor used in industry. A summary of the advantages and disadvantages of different kinds of stirred tank reactor is given in Table 1.2.

Type of operation	Advantages	Disadvantages
Batch	**Versatile**: can be used for different reaction everyday **Safe**: can be properly sterilised. Little risk of infection or strain mutation. Complete conversion of substrate is possible	**High labour cost**: skilled labour is required **Much idle time**: Sterilisation, growth of inoculum, cleaning after fermentation **Safety problem**: when filling, emptying, cleaning
Continuous, steady-state (chemostat)	**Works all the time**: low labour cost, good utilisation of reactor **Often efficient**: due to the autocatalytic nature of microbial reactions, the productivity can be high. Automation may be very appealing. Constant product quality.	**Often disappointing**: promised continuous production for months fails due to (a) infection, e.g. a short interruption of the continuous feed sterilisation. (b) spontaneous mutation of microorganism to non producing strain. **Very inflexible**: can rarely be used for other productions without substantial retrofitting. **Downstream**: all the downstream process equipment must be designed for low volumetric rate, continuous operation.
Semi-batch (fed-batch)	Combines the advantages of batch and continuous operation. Excellent for control and optimisation of a given production criterion.	Some of the advantages of both batch and continuous operation but the advantages far outweigh the disadvantages, and fed-batch is used to produce both biomass (baker's yeast) and important secondary metabolites (e.g. penicillin).

Table 1.2: A summary of advantages and disadvantages of different modes of operation of the stirred tank reactor. [Adapted from Neilsen and Villadsen, (1994)].

Chapter 2

Growth Rate: The Kinetics of Cell Growth

In a usual way, the kinetics of any cellular growth can be simply described by *unstructured* models. The net rate of the biomass growth is given by μx, where x represents the biomass per unit culture volume and μ is the *specific growth rate* of the cells with the units of reciprocal time. This can be written as;

$$r_x = \mu x \tag{2.1}$$

and using a similar equation for the continuous stirred tank reactor in equation (1.5) at steady-state for the cell balance;

$$\begin{aligned} \frac{dx}{dt} &= r_x - D(x_o - x_i) \\ r_x - D(x_o - x_i) &= 0 \end{aligned} \tag{2.2}$$

rearranging this gives;

$$\begin{aligned} Dx_i &= Dx - \mu x \\ &= (D - \mu)x \end{aligned} \tag{2.3}$$

since the inlet stream of the continuous culture should be sterile, therefore, $x_i = 0$ and

$$D = \mu$$

from the above equation. This shows that the cell population in a vessel can be maintained at a certain level higher than zero given that the above criteria is achieved.

2.1 Monod growth kinetics

The growth of most of the bacterial cells is in the form of hyperbolic curve. A simple growth model describing such a curve was first proposed by Monod in 1942 by linking the specific growth rate and the concentration of the nutrient used by the cells. The model is similar to that of the Langmuir isotherm and the famous Michaelis-Menten model of enzyme-catalysed reactions. It is given by;

$$\mu = \frac{\mu_{max}[S]}{K_s + [S]} \tag{2.4}$$

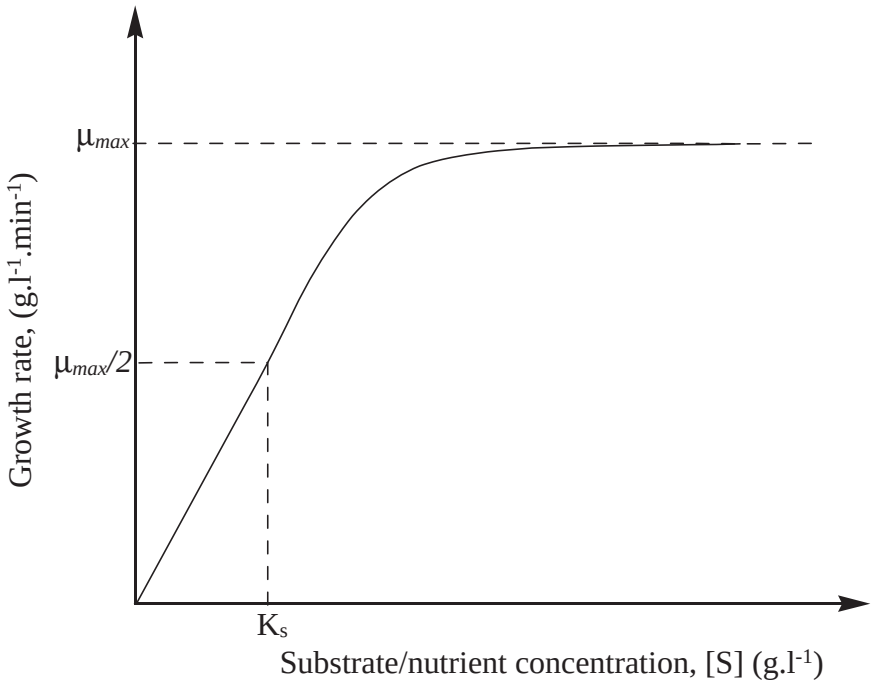

Figure 2.1: Growth curve resulted from equation (2.4) of the Monod model.

Even when there are many substrates, one of these substrates is usually limiting, that is, the rate of biomass production depends exclusively on the concentration of this substrate. At low concentrations, $[S]$ of this substrate, μ is proportional to $[S]$ but for increasing values of $[S]$, an upper values of μ_{max} for the specific growth rate is gradually reached.

where μ_{max} is the maximum growth rate when there is enough substrate supplied to the cell and the value exceeds the limiting substrate concentration, K_s;

$$[S] \gg K_s.$$

The corresponding curve resulted from equation (2.4) is given in Figure (2.1).

The model described by Monod requires the yield factor, $Y_{X/S}$ which is based on the stoichiometric parameters. Thus, the substrate balance is now can be written as;

$$\frac{F}{V}([S]_i - [S]) - \frac{1}{Y_{X/S}}\mu x = 0 \tag{2.5}$$

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

$$Y_{X/S} = \frac{mass\ \ of\ \ biomass/cells\ \ produced}{mass\ \ of\ \ substrate\ \ used} \tag{2.6}$$

substituting equation (2.4) into (2.5) gives;

$$D([S]_i - [S]) - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max}[S]}{K_s + [S]}\right)x = 0 \tag{2.7}$$

while the biomass (cell) balance at steady-state is given as;

$$\left(\frac{\mu_{max}[S]}{K_s + [S]}\right) x - Dx = 0 \tag{2.8}$$

The transient state of the system is obviously given by;

$$\begin{aligned} \frac{d[S]}{dt} &= D([S]_i - [S]) - \frac{1}{Y_{X/S}} \left(\frac{\mu_{max}[S]}{K_s + [S]}\right) x \\ \frac{dx}{dt} &= \left(\frac{\mu_{max}[S]}{K_s + [S]}\right) x - Dx \end{aligned} \tag{2.9}$$

Example 1: By referring to a system of equations (2.9), write a model describing a batch culture system and state what parameters that need to be changed. Give the conditions when *washout* steady-state occurs in a CSTR system.

Answer 1: Equation (2.9) describes the continuous condition of a stirred tank. This is apparent from the dilution rate, D appeared in the system which originally given by, $\frac{F}{V_R}$. For a batch system, there will be no feeding of substrate as well as the outlet of biomass/product, i.e. $D = 0$, thus equation (2.9) reduces into;

$$\begin{aligned} \frac{d[S]}{dt} &= -\frac{1}{Y_{X/S}}\left(\frac{\mu_{max}[S]}{K_s + [S]}\right) \\ \frac{dx}{dt} &= \left(\frac{\mu_{max}[S]}{K_s + [S]}\right)x \end{aligned} \tag{2.10}$$

In a CSTR system, the balances for substrate and biomass are given by equations (2.7) and (2.8)

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

and

$$\left(\frac{\mu_{max}[S]}{K_s + [S]}\right)x - Dx = 0$$

at a *washout* steady-state, as the dilution rate in a CSTR increases, the concentration of substrate also increases and from equation (2.7);

$$D_{max} = \mu_{max}\left(\frac{[S]}{K_s + [S]}\right) \tag{2.11}$$

when $D > D_{max}$ at $x = 0$, where *washout* steady-state will occur. The feed substrate will such that $[S] \gg K_s$ and D_{max} becomes approximately equals to the maximum specific growth rate;

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

Question 1: Find the amount of cell and the corresponding substrate for a sterile feed ($x_i = 0$) at a steady-state condition of a chemostat model. Using these steady-state terms, find the values of the cell and the substrate when $\mu_{max} = 1.0h^{-1}$, $Y_{X/S} = 0.5$, $K_s = 0.2gl^{-1}$ and $[S]_i = 10gl^{-1}$.

Question 2: Find the maximum output rate of cell production per unit volume of bioreactor, D_{max} for a CSTR system. (*Hint: Use the differential terms* $\left(\frac{d\frac{F}{V_R}x}{dD}\right)$).

Microorganism	**Limiting substrate**	K_s**(mg**$\cdot l^{-1}$**)**
Saccharomyces	Glucose	25
Escherichia	Glucose	4.0
	Lactose	20
	Phosphate	1.6
Aspergillus	Glucose	5.0
Candida	Glycerol	4.5
	Oxygen	0.042–0.45
Pseudomonas	Methanol	0.7
	Methane	0.4
Klebsiella	Carbon dioxide	0.4
	Magnesium	0.56
	Potassium	0.39
	Sulphate	2.7
Hansenula	Methanol	120.0
	Ribose	3.0
Cryptococcus	Thiamine	1.4×10^{-7}

Table 2.1: K_s values for several microorganisms (only the *genus* part of the name is given). [Adapted from P.M. Doran, Academic Press (1995)]

Chapter 3

Measurement of Cell Growth

Methods of measurement of cell mass involve both direct and indirect techniques. This can be referred from the diagram and table supplied in Figure 3.1 and Table 3.1 respectively. As shown in Figure 3.1, the method of measurement can be divided into 2 sections;

1. measurement of cell mass
2. measurement of cell numbers.

For the measurement of cell mass:

- Direct method
- Indirect method
- Turbidity method

For the measurement of cell number:

- Direct microscopic method
- Electronic counting chamber
- Indirect viable cell counts

The details of the above methods are described in the diagram overleaf.

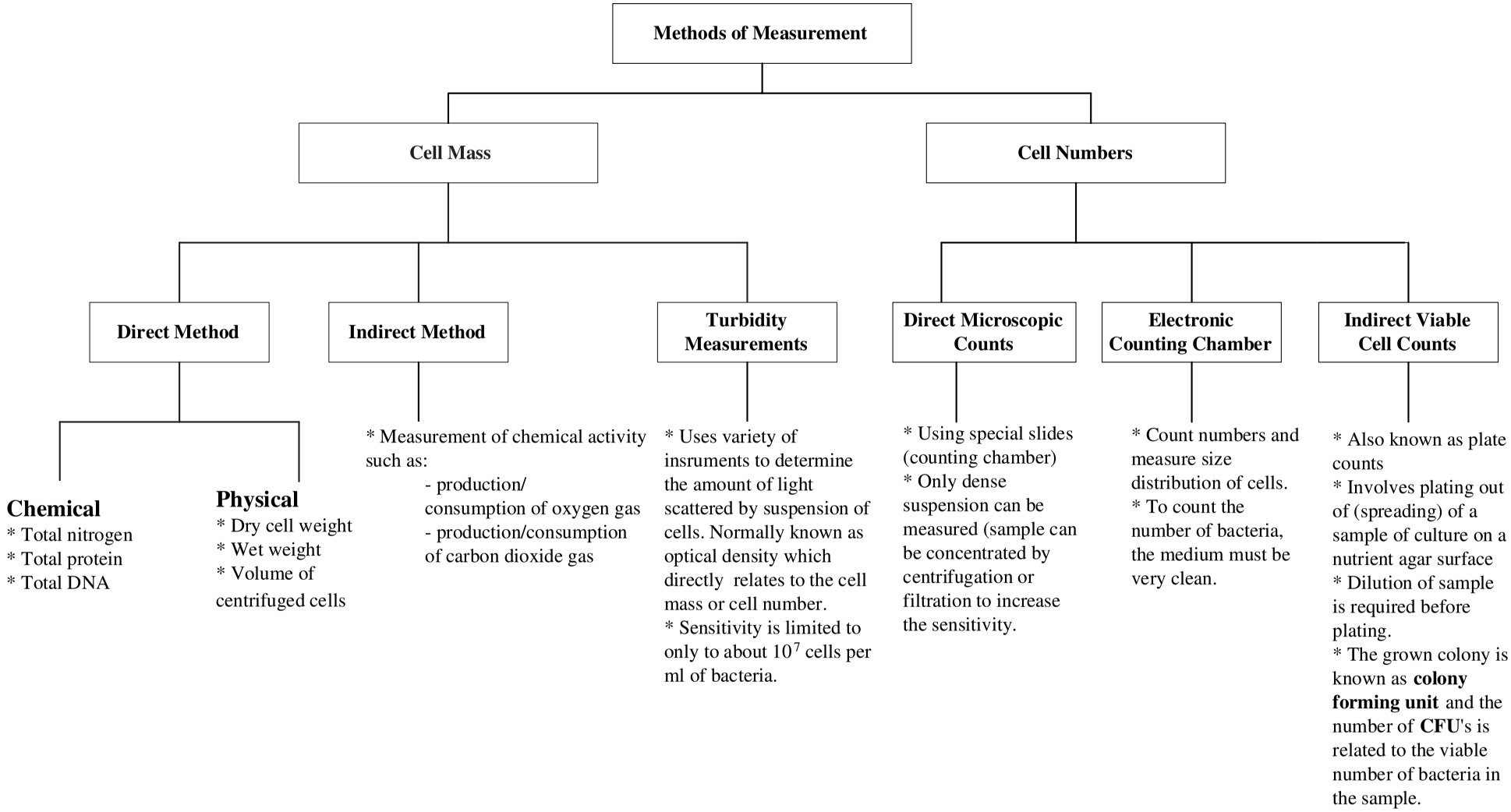

Figure 3.1: Methods used to measure cell growth.

Method	Application	Comments
Direct microscopic count	Enumeration of bacteria in milk or cellular vaccines	Cannot distinguish living from dead cells
Viable cell count	Enumeration of bacteria in milk, foods, soil, water, lab culture etc.	Very sensitive if plating conditions are optimal
Turbidity measurement	Estimations of large numbers of bacteria in clear liquid media and broth	Fast and nondestructive, but cannot detect cell densities less than 10^7 cells per ml
Measurement of total nitrogen (N) or protein	Measurement of total cell yield from very dense cultures	Only practical applied in research lab
Measurement of biochemical activity, such as O_2 uptake rate, CO_2 production rate ATP production etc.	Microbiological assay	Requires a fixed standard to relate chemical activity to cell mass and/or cell numbers
Measurement of dry weight or wet weight of cells or volume of cells after centrifugation	Measurement of total cell yield in cultures	Probably more sensitive than total nitrogen or protein measurements

Table 3.1: Tabulated methods and their applications in laboratory and industries.

Chapter 4

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

4.1 Effect of Temperature

Temperature[1] 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:

- psychrophiles ($T_{opt} < 20$°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.
  - 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 slow down the the rate of spoilage of food and cannot stop the growth of these microbes.
- mesophiles ($T_{opt} = 20 - 50$°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.
- thermophiles ($T_{opt} > 50$°C)
  - Bacteria that live at temperatures exceed 50°C.

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

	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

Table 4.1: Classification of microorganisms in terms of growth-rate dependence on temperature. [Adapted from Bailey and Ollis (1986), McGraw-Hill].

– It can tolerate at very harsh conditions such as, decomposing material, 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.

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;

$$\begin{aligned} \mu &= Ae^{-\frac{E_a}{RT}} \\ \Rightarrow \quad k_d^{'} &= A^{'}e^{-\frac{E_d}{RT}} \end{aligned} \tag{4.1}$$

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.

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).

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: $E = 6kcal \cdot mol^{-1}$
  - Biochemical reaction: $E = 10kcal \cdot mol^{-1}$
- diffusional limitations must be carefully considered at high temperature.

The plot of growth rate versus temperature of the group of microorganisms given in Table 4.1 is shown in Figure 4.1.

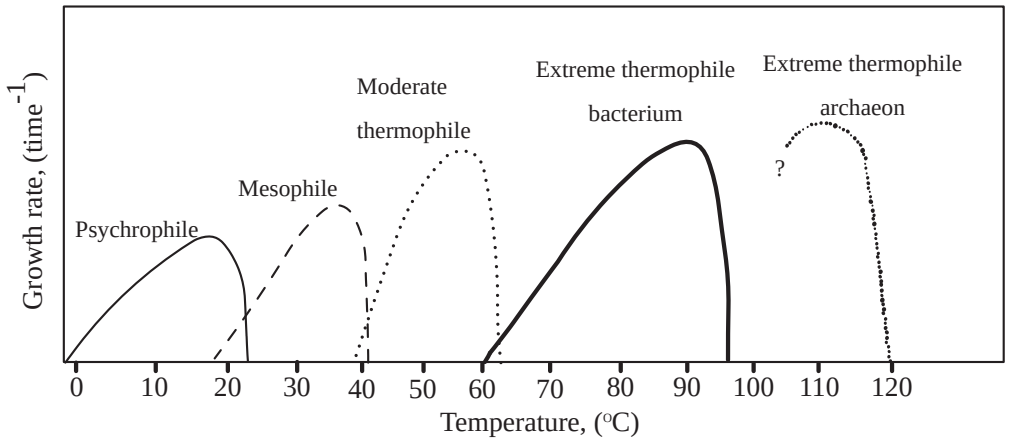

Figure 4.1: Growth rate versus temperature for five environmental classes of procaryotes.

4.2 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. For instance, consider the influence of pH on a single enzyme, which is taken to represent the cell activity.

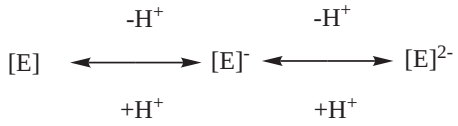

Figure 4.2: Enzyme forms with changing of pH.

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. Thus, the fraction of active enzyme $[E]^-$ is calculated to be;

$$\frac{[E]^-}{[E]_{tot}} = \frac{1}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}} \tag{4.2}$$

and 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;

$$\mu_{max} = \frac{k[E]_{tot}}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}} \tag{4.3}$$

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 4.3 and Table 4.2 respectively.

The range of pH over which the microorganism grows is defined by the 3 main categories;

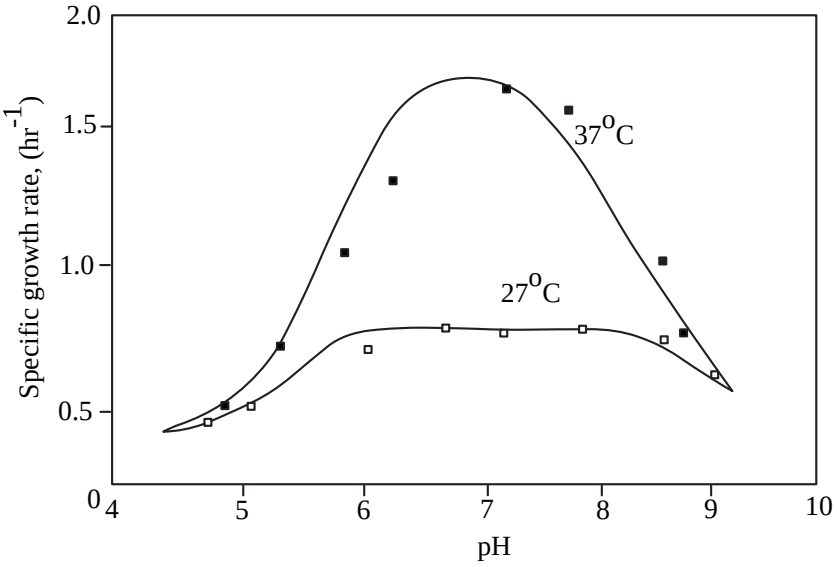

Figure 4.3: Influence of pH on the maximum specific growth rate of *E. coli*. '■', for growth at 37°C and '□' for growth at 27°C. [Adapted from Neilsen and Villadsen, (1994), Plenum Press.]

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 4.2: Parameter values for the fitting of equation (4.3 to the specific growth data).

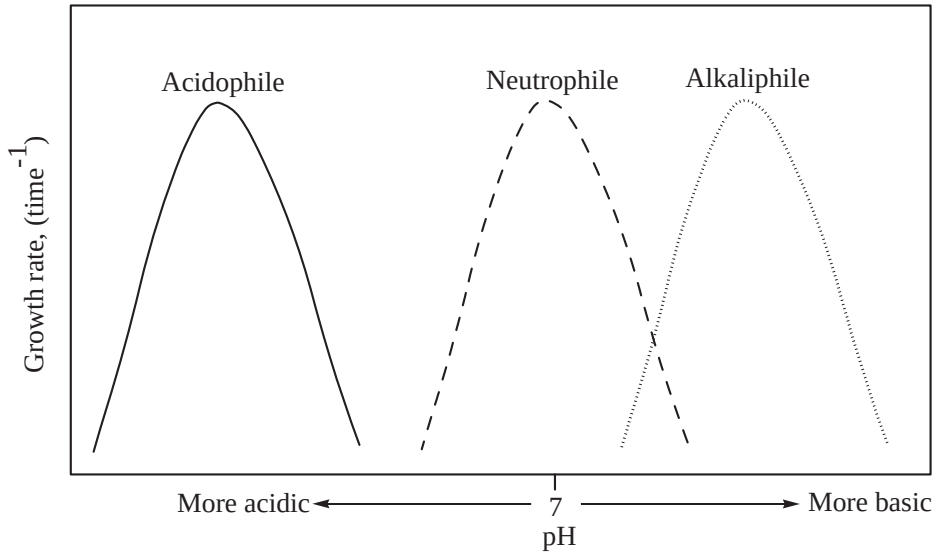

Figure 4.4: Growth rate versus pH for three environmental classes of procaryotes. Most free-living bacteria grow over a pH range of about three units. Note the symmetry of the curves below and above the optimum pH for growth.

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.

For most bacteria there is an orderly increase in growth rate between the minimum and the optimum and a corresponding 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 4.4.

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*. A few types of procaryotes are given in Table 4.3.

4.3 Effect of Oxygen

Dissolved oxygen[2] (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.

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

		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
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

Table 4.3: Minimum, maximum and optimum pH for growth of certain procaryotes.

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·l^{-1}, the growth rate of the organism is 50% of its maximum, even if large amount of nutrient (glucose) is present. 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.

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 $\text{mg}_{O_2}\cdot\text{l}^{-1}\text{h}^{-1}$ and;

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

4.3.1 Oxygen Uptake Rate (OUR)

Oxygen uptake rate is given by;

$$OUR = q_{O_2} X = \frac{\mu X}{Y_{X/O_2}} \tag{4.4}$$

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 ($g_{dcw} \cdot l^{-1}$)

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;

$$\begin{aligned} OUR &= N_{O_2} \\ \Rightarrow \frac{\mu X}{Y_{X/O_2}} &= k_L a(C^* - C_L) \end{aligned} \tag{4.5}$$

since the terms (μX) is the rate of cell growth with respect to time, hence;

$$\frac{dX}{dt} = k_L a(C^* - C_L) \tag{4.6}$$

As depicted in equation (4.6), 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.

4.3.2 Heat Generation by Microbial Growth

During bacterial growth, the 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. Therefore, the yield factor due to the heat produced, $Y_\triangle$ can be written as;

$$Y_\triangle(g_{cell} \cdot kcal^{-1}) = \frac{Y_s}{(\Delta H_s - Y_s \Delta H_c)} \frac{(g_{cell} \cdot g_{substrate}^{-1})}{(kcal \cdot g_{substrate}^{-1})} \tag{4.7}$$

Such an equation is derived based on the approximate energy balances over the two different pathways shown in Figure 4.5 given that 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, H_2O and CO_2.

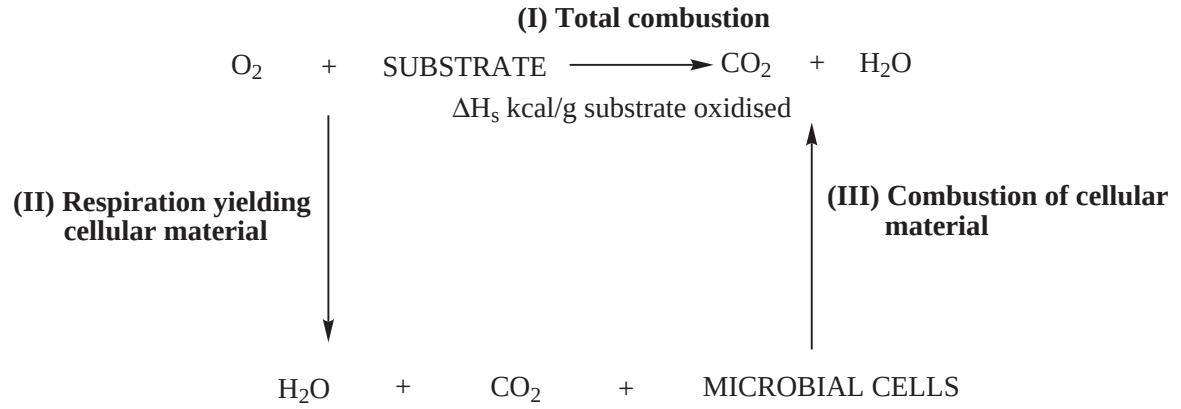

Figure 4.5: Approximate heat balance in substrate consumption.

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).
Example 2: Estimate the heat of combustion of *Pseudomonas fluorescens* growing in glucose medium.
Answer 2: The reaction for cell combustion is given as;

$$\mathrm{CH_{1.66}N_{0.20}O_{0.27}(CELLS) + 1.28O_2 \rightarrow CO_2 + 0.10N_2 + 0.83H_2O}$$

It is assumed that such a reaction produces carbon dioxide, water and nitrogen. By assuming that the heat of combustion of oxygen is 104kcal per mole of $\mathrm{O_2}$, the heat released by combustion of bacteria can be estimated using the inverse of equation (4.7);

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

$$\Rightarrow \frac{(1.28)(104)}{[12+(1.66)(1)+(0.02)(14)+(0.27)(16)]}\left(\frac{(\mathrm{mol\ O_2})(\mathrm{kcal\cdot mol^{-1}\ O_2})}{g}\right) = 6.41\mathrm{kcal\cdot g^{-1}}$$

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 $5.8kcal \cdot g_{dcw}^{-1}$.

In general, it can be seen that group of hydrocarbons produces more heat compared to the partially oxygenated species, for instance, $\Delta H_c(\mathrm{CH_4}) > \Delta H_c(\mathrm{CH_3OH})$ and $\Delta H_c(n-alkanes) > \Delta H_c(glucose)$. The comparison of the growth factors between various bacteria is tabulated above.

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.

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
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

Table 4.4: Comparing yield coefficients of different bacteria grown on different carbon sources. [Adapted from Bailey and Ollis, McGraw-Hill, (1986)].

The basis for thermodynamic calculations is the definition of Gibbs free energy in the ith reaction component;

$$G_i = G_i^0 + RT\ln(c_i) \tag{4.8}$$

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. This is done by assigning values for the standard Gibbs free energy level of CO_2, H_2O and molecular nitrogen, 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. Therefore, equation (4.8) changes to;

$$\Delta G_{ci} = \Delta G_{ci}^0 + RT\ln(c_i) \tag{4.9}$$

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;

$$\Delta G_{c,j} = \sum_{i=1}^{N} \alpha_{ji}\Delta G_{ci} + \sum_{i=1}^{L} \gamma_{ji}\Delta G_{ci} + \sum_{i=1}^{M} \beta_{ji}\Delta G_{ci}; \quad j = 1, \ldots, J \tag{4.10}$$

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.

- 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 (4.9) can be omitted since its contribution to the overall change in free energy in a reaction is negligible. Therefore the only standard free energies reduces equation (4.10) into;

$$\Delta G_{c,j}^0 = -D_j = \sum_{i=1}^{N} \alpha_{ji}\Delta G_{ci}^0 + \sum_{i=1}^{L} \gamma_{ji}\Delta G_{ci}^0 + \sum_{i=1}^{M} \beta_{ji}\Delta G_{ci}^0; \quad j = 1, \ldots, J \tag{4.11}$$

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

$$\Delta G^0_{c,j} = \Delta H^0_{c,j} - T\Delta S^0_{c,j} \tag{4.12}$$

where $\Delta H^0_{c,j}$ represents the enthalpy of reaction which equals to the generation of heat for the reaction. Similar to equation (4.11), the enthalpy balance for jth reaction can be set up as;

$$\Delta H^0_{c,j} = -Q_j = \sum_{i=1}^{N} \alpha_{ji}\Delta H^0_{ci} + \sum_{i=1}^{L} \gamma_{ji}\Delta H^0_{ci} + \sum_{i=1}^{M} \beta_{ji}\Delta H^0_{ci}; \quad j = 1, \ldots, J \tag{4.13}$$

with Q_j representing the amount of heat generated by the jth reaction. 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;

$$r_Q = \sum_{j=1}^{N} Q_{s,j} r_{s,j} + \sum_{j=1}^{J} Q_j r_j + \sum_{j=1}^{M} Q_{p,j} r_{p,j} \tag{4.14}$$

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;

$$r_Q = \sum_{j=1}^{J} Q_j r_j \tag{4.15}$$

Equations (4.15) or (4.14) and (4.13) are important especially in estimating the amount of heat generated by growth processes. Such values are important in designing the necessary cooling capacity in bioreactors.

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

$$\Delta H^0_{ci} = 115\kappa^*_i \tag{4.16}$$

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;

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

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;

$$0.600\mathrm{CH_{1.62}O_{0.53}N_{0.15}} + 0.400\mathrm{CO_2} + 0.649\mathrm{H_2O} \rightarrow \mathrm{CH_2O} + 0.090\mathrm{NH_3} + 0.384\mathrm{O_2}$$

2. anaerobic growth with stoichiometric equation;

$$0.122\mathrm{CH_{1.62}O_{0.53}N_{0.15}} + 0.582\mathrm{CH_3O_{0.5}} + 0.297\mathrm{CO_2} + 0.05\mathrm{H_2O} \rightarrow \mathrm{CH_2O} + 0.018\mathrm{NH_3}$$

given that the heat of combustion of;

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

Answer 3:

1. Using equation (4.13), the heat generated when *Saccharomyces cerevisiae* is grown aerobically;

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

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

2. Similarly, for the anaerobic growth of yeast;

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

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

It is clear from the above results, that the heat generated in the aerobic process is much higher than in the anaerobic process. Such a 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. 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 $0.25\mathrm{hr}^{-1}$, the specific rate of heat production can be calculated using;

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

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

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

such growth is known as *diauxic growth* and can be described by the given stoichiometric equations:

$$CH_{1.6}O_{0.5}N_{0.15} + 2.06CH_3O_{0.5} + 2.20CO_2 + 1.59H_2O \rightarrow 5.26CH_2O + 0.15NH_3 + 1.13O_2$$

and

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

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**.

Chapter 5

Viable Cell Growth: The Stoichiometry of Microbial Reactions

5.1 Medium Formulation and Yield Factors

During the growth of cells, substrates that provide energy and raw materials are needed for the synthesis of additional cell mass. Generally, in a biochemical process, the cell environment should contain elements required in order to form additional cell mass and the free energy from the substrate consumed should exceed the free energy of cells and metabolic products formed. This is summarised in Figure 5.1.

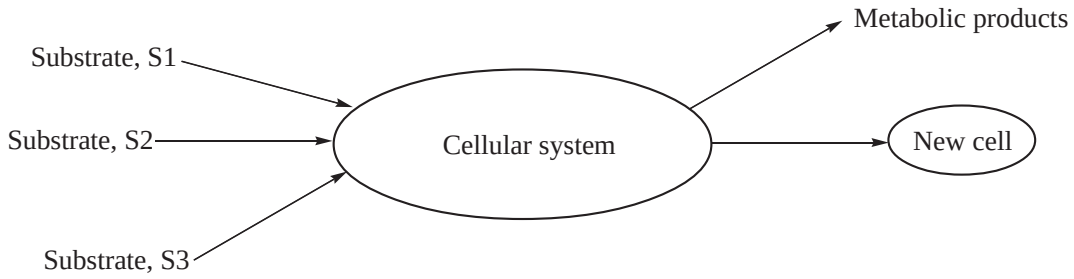

Figure 5.1: Simplified diagram showing formation of additional cell mass and side products.

The figure provides the whole picture of the macroscopic perspective of the stoichiometric constraints use for the growth process. Such a process does not involve any particular reaction pathways that the system employs to effect the overall growth reaction.

In order to obtain the amount of every element that took part in a growth process, the cellular content of all elements should be known. These include carbon, nitrogen, oxygen and hydrogen (C, N, O and H respectively). For the same strain that grows in different environment–composition of the elemental basis of the cell mass is consistent. However, the formulation of the medium is highly complicated due to:

- some substrate elements are released in products and not assimilated into cell material.
- Consideration of the limiting rate and limiting stoichiometry.
- Specific nutrients may be limiting/specific products may be inhibitory–due to metabolic properties of cell strain.

The two limiting factors above may not occur at the same time as the other. One type of nutrient may limit the the rate of cell growth, but it may also cause by the depletion of a different compound that stops the cell to grow. Such difficulties can only be observed by conducting an experimental analysis which can distinguish between the rate limiting and stoichiometric limiting.

In order to obtain a balance amount of cell formed from the amount of substrate used, a ratio between the cell and nutrient is used. It is normally termed as the yield factor, $Y_{X/S}$ which is given by;

$$Y_{X/S} = \frac{\Delta X}{\Delta S}$$

and has the unit of the cell and nutrient/substrate concentrations.

Example 4: Calculate the yield factor for the production of acetic acid using *Acetobacter aceti* bacteria. The chemical equation for the conversion of acetic acid is given below;

$$C_2H_5OH(\textbf{ETHANOL}) + O_2 \rightarrow CH_3CO_2H(\textbf{ACETIC ACID}) + H_2O$$

Acetobacter aceti is added into a vigorously aerated medium containing 10gl_l ethanol. After some times, ethanol and acetic acid concentrations become 2gl_l and 7.5gl_l respectively.

1. What is the overall/observed yield of acetic acid produced from the batch culture.
2. Determined the percentage of the observed yield in relation to the theoretical yield.

Answer 4:

1. By using a basis of 1 litre, the overall yield over the entire culture period is given as;
$$Y_{P/S} = \frac{7.5\text{g}}{(10-2)\text{g}} = 0.94\text{g}_{product} \cdot \text{g}^{-1}_{substrate}$$
2. The theoretical yield is based on the mass of ethanol actually used for synthesis of acetic acid, thus, from the stoichiometric equation given above;
$$Y_{P/S} = \frac{1\text{gmol of acetic acid}}{1\text{gmol of ethanol}} = \frac{60g}{46g} = 1.30\text{g}_{acid} \cdot \text{g}^{-1}_{ethanol}$$
and the percentage of observed yield can be calculated by putting;
$$\frac{observed\ yield}{theoretical\ yield} = \frac{0.94}{1.30} \times 100 = 72.3\%$$

5.2 Material Balance of Cell Growth

The method of obtaining a balanced growth equation is very much similar to the equation describing chemical reactions. One needs to establish a chemical formula for dry cell material–if the elemental composition of a particular strain growing under conditions is known, the ratios of subscripts in the empirical cell formula $C_\alpha H_\beta O_\gamma N_\delta$ can be determined.

In order to get a unique bacterial cell formula and corresponding formula weight, one should employ a formula which contains 1 gram-atom of carbon; therefore, $\alpha = 1$ with other unknown (β, γ and δ) fixed, such that the formula is consistent with the known relative elemental weight content of the cell.

- 1 *C-mole* of cells is defined as the quantity of cells containing 1 gram-atom of carbon-12 (12.011) corresponding to the cell formula weight with the carbon subscript α as unity (1).

Consider an aerobic growth which the products are cells, carbon dioxide and water, and the initial components such as carbon and nitrogen sources; CH_xO_y and $H_lO_mN_n$ respectively–the equation can be written as follows;

$$a\mathrm{CH_XO_Y} + b\mathrm{O_2} + c\mathrm{H_LO_MN_N} \rightarrow \mathrm{CH_\beta O_\gamma N_\delta} + d\mathrm{H_2O} + e\mathrm{CO_2}$$

from the above stoichiometric equation, 4 relationships consist of the stoichiometric coefficients a, b, c and d are;

$$\begin{aligned} \mathrm{C} &: a = 1 + e \\ \mathrm{H} &: aX + cL = \beta + 2d \\ \mathrm{O} &: aY + 2b + cM = \gamma + d + 2e \\ \mathrm{N} &: cN = \delta \end{aligned} \tag{5.1}$$

The above equations is accompanied with an additional relationship obtained from experimental determination, i.e. *respiratory quotient* (RQ);

$$\begin{aligned} RQ &= \frac{moles\ of\ \mathrm{CO_2}\ \text{formed}}{moles\ of\ \mathrm{O_2}\ consumed} \\ &= \frac{e}{b} \end{aligned} \tag{5.2}$$

When equation (5.1) is combined with equation (5.2), all 5 equations with 5 unknowns can be solved for the stoichiometric coefficients.

Example 5: A production of single-cell protein from hexadecane is given by the following reaction equation;

$$\mathrm{C_{16}H_{34}} + a\mathrm{O_2} + b\mathrm{NH_3} \rightarrow c\mathrm{CH_{1.66}O_{0.27}N_{0.20}} + d\mathrm{CO_2} + e\mathrm{H_2O}$$

with $CH_{1.66}O_{0.27}N_{0.20}$ represent the biomass. Given that RQ = 0.43, determine the stoichiometric coefficients a, b, c, d and e.
Answer 5: Consider the component balance from each compound;

$$\begin{aligned} \mathrm{C} &: 16 = c + d \\ \mathrm{H} &: 34 + 3b = 1.66c + 2e \\ \mathrm{O} &: 2a = 0.27c + 2d + e \\ \mathrm{N} &: b = 0.20c \end{aligned} \tag{5.3}$$

and for RQ; $\frac{d}{a} = 0.43$. This set of simultaneous equations can be easily solved using MATLAB® or manually using linear algebra applying the matrix form of

$$\mathbf{X} = \mathbf{A^{-1}b}.$$

Converting the above 5 equations in the form of matrix;

$$\mathbf{A} = \begin{pmatrix} 0 & 0 & 1 & 1 & 0 \\ 0 & -3 & 1.66 & 0 & 2 \\ -2 & 0 & 0.27 & 2 & 1 \\ 0.43 & 0 & 0 & -1 & 0 \\ 0 & 1 & -0.2 & 0 & 0 \end{pmatrix}$$

and

$$\mathbf{b} = \begin{pmatrix} 16 \\ 34 \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

and the inverse of $\mathbf{A}$;

$$\mathbf{A}^{-1} = \begin{pmatrix} -0.2529 & 0.4863 & -0.9726 & -2.1980 & 1.4589 \\ 0.2217 & -0.0418 & 0.0836 & 0.3890 & 0.8745 \\ 1.1087 & -0.2091 & 0.4182 & 1.9451 & -0.6273 \\ -0.1087 & 0.2091 & -0.4182 & -1.9451 & 0.6273 \\ -0.5876 & 0.6108 & -0.2216 & -1.0309 & 1.8325 \end{pmatrix}$$

therefore,

$$\mathbf{X} = \mathbf{A^{-1}b}$$

$$\mathbf{X} = \begin{pmatrix} -0.2529 & 0.4863 & -0.9726 & -2.1980 & 1.4589 \\ 0.2217 & -0.0418 & 0.0836 & 0.3890 & 0.8745 \\ 1.1087 & -0.2091 & 0.4182 & 1.9451 & -0.6273 \\ -0.1087 & 0.2091 & -0.4182 & -1.9451 & 0.6273 \\ -0.5876 & 0.6108 & -0.2216 & -1.0309 & 1.8325 \end{pmatrix} \begin{pmatrix} 16 \\ 34 \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

$$\mathbf{X} = \begin{pmatrix} a \\ b \\ c \\ d \\ e \end{pmatrix} = \begin{pmatrix} 12.4878 \\ 2.1260 \\ 10.6302 \\ 5.3698 \\ 11.3660 \end{pmatrix}$$

Therefore, a complete stoichiometric cell growth equation becomes;

$$C_{16}H_{34} + 12.49O_2 + 2.13NH_3 \rightarrow 10.6CH_{1.66}O_{0.27}N_{0.20} + 5.37CO_2 + 11.37H_2O$$

Question 4: Assume that the experimental measurements for a certain organism have shown that cells can convert two-third (wt/wt) of the substrate carbon (alkane or glucose) to biomass.

1. Calculate the stoichiometric coefficients for the following biological reactions;

   - Hexadecane:

   $$C_{16}H_{34} + aO_2 + bNH_3 \rightarrow cC_{4.4}H_{7.3}N_{0.86}O_{1.2} + dH_2O + CO_2$$

   - Glucose:

   $$C_6H_{12}O_6 + aO_2 + bNH_3 \rightarrow cC_{4.4}H_{7.3}N_{0.86}O_{1.2} + dH_2O + CO_2$$

2. Calculate the yield coefficients $Y_{X/S}$ ($g_{dcw}/g_{substrate}$), Y_{X/O_2} (g_{dcw}/g_{O_2}). Comment on the differences.

5.3 Degree of Reduction

In the case of the formation of extracellular products, an additional stoichiometric coefficient is added, therefore, the element balance is not appropriate/applicable. The concept of **degree of reduction** is introduced to solve such problems. The technique is used for proton-electron balances in bioreactions. The degree of reduction for organic compounds is denoted as γ and it is defined as the number of equivalent of the available electrons per gram atom carbon.

The available electrons are the electrons that would be transformed to oxygen upon oxidation of a compound to carbon dioxide, water and ammonia.

The degree of reduction of any element in a compound is equal to the valence of this element, for instance, the valence of carbon in CO_2 is +4 and the valence of nitrogen in NH_3 is -3.

Example 6: Calculate the degree of reduction of these substrate used for bacterial/yeast fermentation;

- Methane
- Glucose
- Ethanol

Answer 6:

- For methanol, the formula is given by CH_4 therefore, the sum of the valence electron for carbon and hydrogen;

  $$1(4) + 4(1) = 8$$

  thus, the degree of reduction of methane;

  $$\gamma_{CH_4} = \frac{8}{1} = 8.$$

- Similarly, for glucose with the chemical formula of $C_6H_{12}O_6$, the degree of reduction;

  $$\gamma_{C_6H_{12}O_6} = \frac{6(4) + 12(1) + 6(-2)}{6} = 4$$

- and finally for ethanol, C_2H_5OH,

  $$\gamma_{C_2H_5OH} = \frac{2(4) + 6(1) + 1(-2)}{2} = 6.$$

The values above can be arranged in the order given below;

$$\gamma_{CH_4} > \gamma_{C_2H_5OH} > \gamma_{C_6H_{12}O_6}$$

which indicates that at a high degree of reduction, there will be a low degree of oxidation.

Chapter 6

Fed-Batch Culture

The main reason of having a fed-batch or *semi-continuous* type bioreactor is to avoid over-fed of substrate that can lead to cell inhibition and high growth rate for cell culture. The fed-batch configuration is given in Figure 6.1. The substrate feeding is continuously

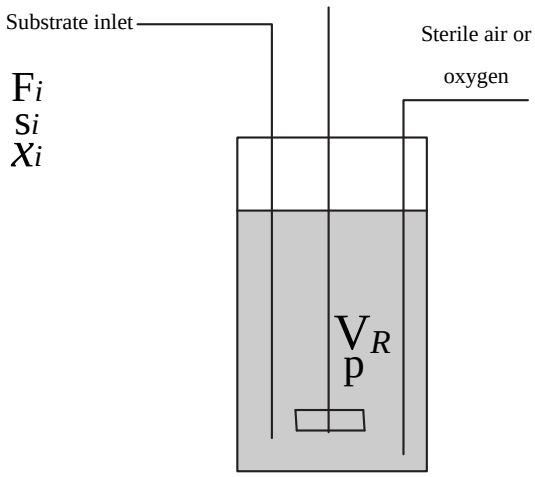

Figure 6.1: Fed-batch bioreactor configuration.

added until a maximum liquid in a fermenter is reached. When this happened or the substrate/nutrient is completely depleted, a fermenter may be allowed to continue or be partially/completely emptied depending on the process. The effluent resulted from the conversion of substrate/side-products from a particular fermentation are not removed and thus such a reactor can be used to monitor cells under LOW substrate/nutrient conditions without *washout* occurring.

6.1 Fed-Batch Model Formulation

Mathematical model of a fed-batch bioreactor can be written as;

- for the reactor volume:

$$\frac{dV_R}{dt} = F \tag{6.1}$$

  assuming that $\rho_{feed} = \rho_{liquid\ in\ fermenter}$

- for biomass:

$$\begin{aligned} \frac{d(xV_R)}{dt} &= F_i x_i + rV_R \\ V_R\frac{dx}{dt} + x\frac{dV_R}{dt} &= F_i x_i + (\mu x)V_R \\ V_R\frac{dx}{dt} &= F_i x_i - x\frac{dV_R}{dt} + \mu x V_R \end{aligned} \tag{6.2}$$

from equation (6.1) above, and also

$$\mu = \frac{\mu_{max} s}{K_s + s}$$

with $F_i = F$;

$$\begin{aligned} \frac{dx}{dt} &= \frac{F}{V_R}x_i - \frac{F}{V_R}x + \left(\frac{\mu_{max} s}{K_s + s}\right)x \\ \frac{dx}{dt} &= Dx_i - Dx + \left(\frac{\mu_{max} s}{K_s + s}\right)x \\ \frac{dx}{dt} &= (x_i - x)D + \left(\frac{\mu_{max} s}{K_s + s}\right)x \end{aligned} \tag{6.3}$$

for a *sterile feed*,

$$x_i = 0$$

hence;

$$\frac{dx}{dt} = -xD + \left(\frac{\mu_{max} s}{K_s + s}\right)x \tag{6.4}$$

- for substrate/nutrient:

$$\begin{aligned} \frac{d(sV_R)}{dt} &= F_i s_i - \frac{1}{Y_{X/S}} rV_R \\ s\frac{dV_R}{dt} + V_R\frac{ds}{dt} &= F_i s_i - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max} s}{K_s + s}\right)xV_R \\ V_R\frac{ds}{dt} &= F_i s_i - s\frac{dV_R}{dt} - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max} s}{K_s + s}\right)xV_R \end{aligned} \tag{6.5}$$

let $F_i = F$ and according to equation (6.1) above;

$$\begin{aligned} \frac{ds}{dt} &= \frac{F}{V_R}(s_i - s) - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max} s}{K_s + s}\right)x \\ \frac{ds}{dt} &= D(s_i - s) - \frac{1}{Y_{X/S}}\left(\frac{\mu_{max} s}{K_s + s}\right)x \end{aligned} \tag{6.6}$$

- for product:

$$\begin{aligned} \frac{d(pV_R}{dt} &= \frac{Y_{P/S}}{Y_{X/S}} rV_R \\ p\frac{dV_R}{dt} + V_R\frac{dp}{dt} &= \frac{Y_{P/S}}{Y_{X/S}}\left(\frac{\mu_{max} s}{K_s + s}\right)xV_R \\ V_R\frac{dp}{dt} &= \frac{Y_{P/S}}{Y_{X/S}}\left(\frac{\mu_{max} s}{K_s + s}\right)xV_R - p\frac{dV_R}{dt} \end{aligned} \tag{6.7}$$

similarly, from equation (6.1);

$$\begin{aligned} V_R\frac{dp}{dt} &= \frac{Y_{P/S}}{Y_{X/S}}\left(\frac{\mu_{max}s}{K_s+s}\right)xV_R - pF \\ \frac{dp}{dt} &= \frac{Y_{P/S}}{Y_{X/S}}\left(\frac{\mu_{max}s}{K_s+s}\right)x - p\frac{F}{V_R} \\ \frac{dp}{dt} &= \frac{Y_{P/S}}{Y_{X/S}}\left(\frac{\mu_{max}s}{K_s+s}\right)x - Dp \end{aligned} \tag{6.8}$$

6.2 Comparison Between Fed-Batch and Continuous Bioreactors

- Major difference is the liquid effluent in a fed-batch system is not continuously removed, thus washout could not occur.
- Can be set and maintain their specific growth rate and substrate concentration to an optimal level.

6.3 Advantages of Fed-Batch System

- Because cells are not removed during fermentation, therefore this type of fermenter is well suited for production of compounds produced during very LOW or zero growth.
- The feed does not need to contain all the nutrients needed to sustain growth. Feed may contain only the nitrogen source or metabolic precursor.
- Contamination is highly unlikely for fed-batch system unless it has occurred during the early stage of a fermentation.
- Can operate in a number of ways; batch to fed-batch to batch in an alternate manner. The feed for substrate/nutrient can be manipulated in order to optimise the product formation. For instance, during fermentation, the feed composition or the flowrate can be adjusted to match the physiological state of cells.

6.4 Application of Fed-Batch System

Since the system can maintain the growth/reaction at LOW nutrient/substrate concentrations, therefore;

- it can be used to get the product or cells when the nutrient/substrate is inhibitory to the cells or affect the mass transfer rate. This can be maintained by controlling the substrate feeding such as during the production of citric acid, amylase enzyme and vinegar.
- can get higher biomass.cells yield–LOW substrate concentration is important especially in the production of mammalian cells, baker's yeast and antibiotic.

- the production is dependent on a specific nutrient composition, carbon to nitrogen ratio.
- oxygen uptake rate (OUR) must be restricted in order to maintain LOW substrate concentrations.

Chapter 7

Mixing and Mass Transfer

Transport phenomena in biological systems actually influences the kinetic behaviour of cells. This can happen on different scales. A connection between the length scale and transport phenomena have to be addressed in biological systems so that the kinetics measurements and models can be developed under conditions which will resemble in some senses encountered in the large scale bioreactors. The diagram of the length scales characteristics is attached.

Normally, fluid circulations is apparent in a large scale bioreactor. Turbulence levels within the reactor gives an impact to the dissolved oxygen concentration and thus influencing the overall cellular kinetics during the growth. A method which is commonly applied to tackle such a problem is mixing. It is a physical operation that reduces non-uniformities in fluid by eliminating gradients of concentration, temperature etc. This is done by interchanging material between different locations to produce a mingling of components. For a *perfectly mixed* system, there is a random homogeneous distribution of system properties that involves:

- dispersing gas (O_2, CO_2, air etc) through liquid in the form of small bubbles (smaller bubbles leads to higher mass transfer rate).
- maintaining suspension of solid particles, for instance immobilised enzymes/cells or the cell itself.
- blending soluble components of the medium such as sugars.
- dispersing immiscible liquids to form an emulsion/suspension of fine drops
- promoting/maintaining consistent heat transfer to or from the liquid.

Mixing can be divided into 2 parts:

1. macro-mixing
2. micro-mixing

7.1 Macro-mixing

Macro-mixing is characterised by the *residence time distribution*, (RTD) for a continuous flow system. It is actually the time spent within the boundaries of the system i.e. the time between the inlet and outlet for a certain volume element (exceptional for ideal

plug flow reactor where all volume elements leave the system with different RTD). The key characteristic parameters for the RTD function, $E(t)$ are the mean residence time or space time and the variance, which can be found from the first and second moment of the distribution function given by equation (7.1) and (7.2) respectively.

$$M_n(t) = \int_{V_y} y^n f(y,t) dy \tag{7.1}$$

$$\begin{aligned} \sigma(t)^2 &= \frac{M_2(t)}{n(t)} - \langle y \rangle^2 \\ &= \frac{\int_{V_y} y^2 f(y,t) dy}{n(t)} - \langle y \rangle^2 \end{aligned} \tag{7.2}$$

The RTD can be determined by measuring the response in the outlet from the system after an ideal pulse of a tracer has been imposed on the inlet. This is described by the Dirac delta function given below;

$$\delta(t - t_0) = \begin{cases} \infty; & t = t_0 \\ 0; & t \neq t_0 \end{cases} \tag{7.3}$$

A spectral analysis of the output may reveal fine detail of the mixing pattern of the tracer in the reactor.

Modelling of macro-mixing is based on simulation of an experimentally determined RTD for a particular bioreactor. For pulse experiments in small-scale continuous lab bioreactors, the RTD is normally found to be an exponentially decaying function that can be simulated by the RTD for an ideal continuous-stirred-tank reactor (CSTR) which is given by,

$$E(t) = \frac{1}{\tau} e^{-\frac{t}{\tau}} \tag{7.4}$$

where τ = space time F/V for the reactor or can be written as $D = 1/\tau$.

For a large scale bioreactor, the RTD is not that simple and more complex models have to be applied. The two most common models used for simulation of RTD are:

- dispersion model (based on the plug flow model with back-mixing in the axial direction). Back-mixing is characterised by the dispersion coefficient represented by the *Peclet* number (Pe);

  $$Pe = \frac{uL}{D_{dispersion}}$$

  where u is the linear flowrate through the length of the reactor L with the dispersion coefficient, $D_{dispersion}$ similar to the diffusion coefficient in the *Fick's* laws. With the defined Pe number, the RTD for the dispersion model is given by;

  $$E(\theta) = \frac{1}{\tau} \sqrt{\frac{Pe}{4\pi\theta}} e^{-(1-\theta)^2 \frac{Pe}{4\theta}} \tag{7.5}$$

- tanks in series model where the flow through the bioreactor is viewed as the flow through a series of equal-sized stirred tanks and the only parameter in the model is the number of tanks, n in the chain. Therefore, the mean residence time in each tank is τ/n and the RTD for this model is given by;

  $$E(\theta) = \frac{1}{\tau} \frac{n^n \theta^{n-1}}{(n-1)} e^{-n\theta} \tag{7.6}$$

For $n = 1$, the RTD reduces to that of for one ideal CSTR and for a very large n, the RTD approaches that for an ideal plug flow reactor.

both are one-parameter models.

Despite the similarities between the RTD for the dispersion model and for the tank-in-series model, they never become identical and a direct comparison of their parameters can therefore not be made.

7.2 Micro-mixing

By nature, this type of mixing is difficult to quantify in nature, however it is generally characterised in terms of the time t_m needed for the mixing of a liquid phase to a certain degree of mixing m which is given by;

$$m = \frac{s - s_0}{s_\infty - s_0} \tag{7.7}$$

where s is the concentration at time t_m, s_0 the initial concentration and s_∞ the concentration when $t \to \infty$.

Several correlations between the mixing time in stirred tank reactors and the stirring speed N (s^{-1}) are listed in the literature. One example of correlation for unaerated system is given by;

$$t_m \propto \frac{1}{N} \tag{7.8}$$

with a proportionality constant for this is between 8 to 24. However, for aerated systems, there is no well-defined correlation between the mixing time and the stirring speed of impeller. It has been found that the mixing time decreases with increasing gas flowrate.

- for small-scale bioreactors (V<50 L): the mixing time in LOW viscosity media such as water is in the range of 1–5 s at typical operating conditions.
- for large-scale bioreactors: higher mixing times are observed (30–50 s) and for viscous media such as fermentation of filamentous microorganisms – higher mixing times are needed.

If a mixing is approximated by a first-order process, the deviation of the concentration of an admixed solute from its final value is given by;

$$\frac{d[S_\infty - s(t)]}{dt} = -k[S_\infty - s(t)]; \;\; s(0) = s_0 \tag{7.9}$$

and by integration gives;

$$\frac{s_\infty - s(t)}{s_\infty - s_0} = e^{-kt} = 1 - m \tag{7.10}$$

Rearranging equation (7.10) leads to;

$$t_m = -\frac{1}{k} \ln(1 - m) \tag{7.11}$$

7.3 Methods for Characterising Mixing

The simplest method of characterising mixing is by tracer techniques in which a tracer is added to the bioreactor and its concentration is measured as a function of time. The techniques include;

1. **conductivity** method based on electrolytes as tracer:
    - inexpensive
    - easily implemented
    - disadvantage: (most media used for fermentation are good conductors – poor sensitivity)
2. **pH method** based on acids or bases as tracer
    - easily implemented
    - measuring change of pH after addition of base(acid), therefore mixing time can be determined.
    - method can be applied under real process conditions
    - microbial activity *may* influence the results since many microorganisms produce acids as metabolic products (it is important that the mixing time is smaller than the characteristic time for acid production)
    - disadvantage: most fermentation media have a high buffer capacity and large pulses are required in order to obtain good sensitivity.
3. **fluorescence** method based on fluorophores as tracer
    - based on measurement of an inert fluorophore such as NADPH, riboflavin or coumarin.
    - possible to quantify mixing time under real process conditions
    - for many fermentation media, the background fluorescence is high and the sensitivity may therefore be poor
4. **isotope** method based on radioactive isotopes as tracer.
    - based on addition of of radioactive isotopes and measurement of radioactivity using scintillation counters
    - advantage: sensor can be placed outside bioreactor – can be easily fitted
    - disadvantage: radioactive effect caused to the product formed

Chapter 8

Oxygen Transfer

8.1 Gas-Liquid Mass Transfer

For the gas-liquid mass transfer, the interface between the gas and liquid phases is shown in Figure 8.1. This is normally modelled by the two-film theory first introduced by Whitman

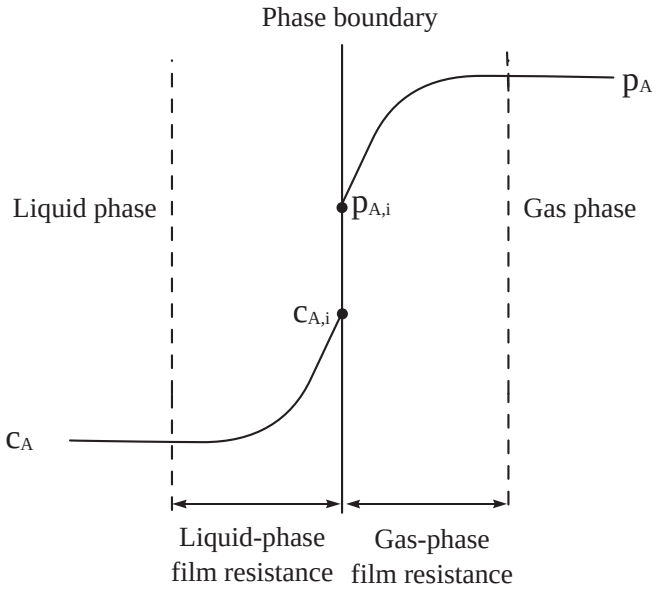

Figure 8.1: Concentration gradient for gas-liquid mass transfer.

in 1923. The flux J_A of compound A through each of the two films is described as the product of the concentration difference across the film layer, which is the linear driving force and the mass transfer coefficient. This also defines the *Fick's* Law of diffusion given by;

$$J_A = -\mathscr{D}_{AB}\frac{dC_A}{dy}.$$

Here, the flux across the gas-film is given by;

$$J_{A,g} = k_g(p_A - p_{A,i}) \tag{8.1}$$

where p_A is the partial pressure of compound A in the gas bubble and i refers to the concentration at the gas-liquid interface. Similarly, for the liquid-film, the flux given by;

$$J_{A,l} = k_l(c_{A,i} - c_A) \tag{8.2}$$

Ammonia	1.71×10^{-1}
Methane	745.5
Ethane	545.1
Carbon dioxide	29.7
Oxygen	790.6
Hydrogen	1270.0

Table 8.1: *Henry's* constants (atm·L·mol^{-1}) for some gases in water at 25°C.

where c_A is the concentration of compound A. In a dilute aqueous solutions, the concentration of the each sides of the gas-liquid interface is related by *Henry's Law*;

$$p_{A,i} = H_A c_{A,i}$$

where H_A is the *Henry's* constant with units of atm·L·mol^{-1}. A few *Henry's* constants is given in Table 8.1.

Since the interfacial concentration is not directly measurable, therefore, the overall flux of the considered component from the gas bubble to the liquid phase is specified as an overall mass transfer coefficient multiplied by the driving force in the liquid phase;

$$J_A = K_l(c_A^* - c_A) \tag{8.3}$$

where c_A^* is the saturation concentration in the bulk liquid corresponding to gas phase;

$$c_A^* = \frac{1}{H_A} p_A.$$

At steady state;

$$J_{A,g} = J_{A,l} = J_A$$

and by inserting $p_{A,i} = H_A c_{A,i}$ and $c_A^* = \frac{1}{H_A} p_A$ into equation (8.1) leads to;

$$\frac{1}{K_l} = \frac{1}{H_A k_g} + \frac{1}{k_l} \tag{8.4}$$

where k_g is typically larger than k_l and for gases with large values of H_A, such as oxygen and carbon dioxide, the gas-phase resistance is therefore negligible. Thus, the overall mass transfer coefficient K_l is approximately equal to the mass transfer coefficient in the liquid film, that is, k_l. This value is normally used for quantification of the mass transfer despite the fact that in practice only K_l can be measured.

In order to find the mass transfer rate of compound A per unit reactor volume (volumetric mass transfer rate, N_A) that gives;

$$N_A = J_A a$$

where a is the interfacial area per unit liquid volume (m^{-1}), the flux J is multiplied by $\frac{A}{V}$ which defines a above. Therefore;

$$N_A = k_l a(c_A^* - c_A) \tag{8.5}$$

- The product of the liquid mass transfer coefficient k_l and the specific interfacial area, a is called the volumetric mass transfer coefficient, $k_l a$.

- due to difficulties in determining k_l and a, their product is normally used to specify the gas-liquid mass transfer.
- the volumetric mass transfer rate can be obtained/calculated if $k_l a$ and the driving force $(c_A^* - c_A)$ are known where the driving force can be approximated using;

$$(c_A^* - c_A) = \frac{(c_{A,inlet}^* - c_A) - (c_{A,outlet}^* - c_A)}{\ln(c_{A,inlet}^* - c_A) - \ln(c_{A,outlet}^* - c_A)} \tag{8.6}$$

8.2 Oxygen Transfer Rate

Mass transfer in biological processes is important especially for the gas-liquid system. A sparingly soluble gas such as oxygen is transferred from a rising air bubble into a liquid phase containing cells. The gas molecules must pass through a series of transport resistances, the relative magnitudes of which depend on;

- bubble hydrodynamics,
- temperature,
- cellular activity and density,
- solution composition,
- interfacial phenomena and other factors.

The resistances that involved during the transfer of gases through the cellular system include;

1. diffusion from bulk gas (interior of bubble) to the gas-liquid interface
2. movement through the gas-liquid interface
3. diffusion of the solute through the relatively unmixed liquid region (stagnant liquid film) adjacent to the bubble into the well-mixed bulk liquid
4. transport of the solute through the bulk liquid to a second relatively unmixed liquid region surrounding the cells (stagnant liquid film)
5. transport through the second unmixed liquid region associated with the cells (liquid-cell interface)
6. diffusive transport into the cellular floc, mycelia or solid particle
7. transport across cell envelope (cytoplasm) to intracellular reaction site

However, the relative magnitudes of the various mass transfer resistances depend on;

- the composition of the liquid
- rheological properties properties of the liquid
- mixing intensity
- bubble size

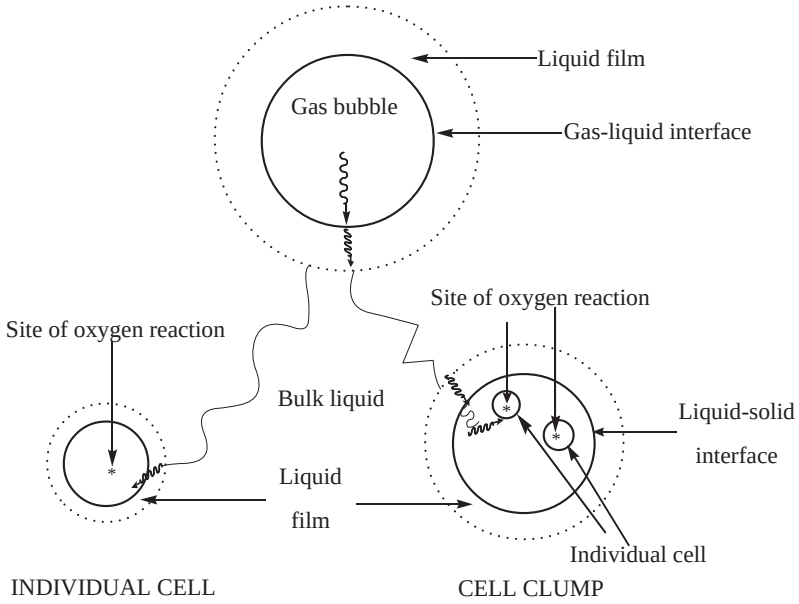

Figure 8.2: Transfer of oxygen molecules from gas bubble to cell/cell clump.

- cell clump size
- interfacial adsorption characteristic

For most bioreactors, the following analysis is valid;

1. fast transfer of within the gas bubble
2. negligible resistance at the gas-liquid interface
3. liquid film contribute major resistance to oxygen transfer
4. in a perfectly-mixed fermenter, concentration gradients in the bulk liquid are minimised and the mass transfer resistance in this region is small. (For viscous fermentation broths, high mixing speed can be difficult and therefore, oxygen transfer resistance in the bulk liquid may be important)
5. the effect of liquid film that surrounding the cell towards mass transfer can be neglected since the size is thinner than that of the gas bubbles and the cells are very much smaller than the bubbles themselves.
6. negligible resistance at the cell-liquid interface
7. for clumped cells, the intraparticle resistance is likely to be significant. This is caused by the diffusion of oxygen molecules through the solid pellet before reaching the interior cells. Magnitude of this resistance depends on the size of the clump
8. negligible intracellular oxygen transfer resistance due to small distance

8.2.1 Oxygen Consumption in Cell Growth

In most aerobic cultures, cells obtained the oxygen supply from the liquid. The rate of transfer of oxygen from gas to liquid is highly important especially when there is a high cell density in a particular fermentation–this leads to the oxygen limited growth. The rate of oxygen transfer per unit volume of fluid is given by N_A stated previously in equation (8.5) above.

The solubility of oxygen in aqueous solutions at ambient temperature and pressure is about 10 ppm and such a value can be easily consumed in aerobic cultures. Therefore, continuous supply of the gas is important. Design of fermenters for aerobic operation must take these factors into account and provide optimum mass transfer conditions.

8.2.2 Factors Affecting Cellular Oxygen Demand

The rate of oxygen consumed by cells in a fermenter determines the rate at which the oxygen must be transferred from gas to liquid. Factors that influence the oxygen demand include:

1. type of bacterial used (species)
2. culture growth phase
3. nature of carbon source

This is due to;

- the increase of cell concentration during the batch growth and

  $$\text{total rate of } O_2 \text{ uptake} \propto \text{number of cells present}$$

  and the rate of oxygen consumption per cell also varies.

- the relationship between the O_2 uptake rate and the cell concentration is given by;

  $$Q_{O_2} = q_{O_2} x \ \ (g \cdot L^{-1} \cdot s^{-1}) \tag{8.7}$$

When the level of dissolved O_2 is lower than the required value, the specific rate of oxygen uptake is now dependent on the O_2 concentration in the liquid. The plot of oxygen concentration c_{O_2} against the specific oxygen uptake rate, q_{O_2} is similar to the plot of the Monod model.

- If c_{O_2} is **above** the critical oxygen concentration, c_{crit}, q_{O_2} is will be at a constant maximum and independent of c_{O_2}.
- If c_{O_2} is **below** c_{crit}, q_{O_2} is approximately linearly dependent on oxygen concentration.

Therefore, in order to avoid oxygen limitations;

- the dissolved oxygen concentration at every point in the fermenter must be above c_{crit}. (Exact value of c_{crit} depends on the type of organism, however, under average operating conditions the values are between 5% and 10% of air saturation).

- for other type of cells that have high c_{crit}, maintaining

$$c_{O_2} > c_{crit}$$

is more difficult than culture of low c_{crit}.

From equation (8.7), when the transfer of gases achieved a steady-state point within the fermenter, the rate of oxygen transfer from the gas bubbles should be equal to the rate of oxygen consumed by the cells which left the equation as;

$$k_l a(c^*_{O_2} - c_{O_2}) = q_{O_2} x \tag{8.8}$$

1. for small $k_l a$, the ability of the reactor to deliver O_2 to the cells is limited and otherwise for high $k_l a$.

2. to increase $k_l a$ for a constant metabolic rate of cell, the stirring speed can be increased such that the thickness of the boundary layer surrounding the gas bubbles can be reduced and therefore increases in the dissolved concentration of O_2. This is clear from the supplied equation above.

3. if the rate of consumed oxygen increases at constant $k_l a$, the dissolved oxygen concentration should decrease

4. the maximum rate of oxygen transfer occurs when $(c^*_{O_2} - c_{O_2})$ is at its optimum value which means that $c_{O_2} = 0$. Hence, (8.8) reduces into (8.9) for maximum cell density;

$$x_{max} = \frac{k_l a c^*_{O_2}}{q_{O_2}} \tag{8.9}$$

5. in order to maintain $c_{O_2} > c_{crit}$ at a minimum, $k_l a$ in the fermenter should follow;

$$(k_l a)_{crit} = \frac{q_{O_2} x}{(c^*_{O_2} - (c_{O_2})_{crit})} \tag{8.10}$$

Interfacial area and bubble behaviour

The interfacial area is given by;

$$a = \frac{A}{V}$$

where A represents the total area in the gas-liquid dispersion and V is the volume of a particular liquid.

In empirical correlations for volumetric mass transfer coefficient, the specific interfacial area is always applied and based on the total volume of the gas-liquid dispersion given by;

$$a_d = \frac{A}{V} = \frac{A}{V_l + V_g} \tag{8.11}$$

and the above equation is also related by;

$$a_d = a(1 - \varepsilon) \tag{8.12}$$

where

$$\varepsilon = \frac{V_g}{V_l}$$

which defines the *gas hold-up* in the dispersion. The term a in equation (8.12) is given by;

$$a = \frac{6\varepsilon}{(1-\varepsilon)d_{mean}} \tag{8.13}$$

where d_{mean} represents the mean bubble size which can be found by applying the *Sauter diameter* or the surface-average diameter, given by;

$$d_{Sauter} = \frac{\sum n_i d_{b,i}^3}{\sum n_i d_{b,i}^2}$$

In a bioreactor, 3 main factors interact to determine the bubble size distribution;

1. **Bubble formation**: determine by the break-up into discrete bubbles of the gas stream as it is sparged into the liquid phase
2. **Bubble break-up**: determined by the competition between the stabilising effect of the surface tension and the destabilising effect of inertial forces
3. **Bubble coalescence**: fusion of bubbles, determined by the properties of the gas-liquid interface.

The value of the initial bubble diameter, $d_{b,i}$ from the *Sauter diameter* is determined by;

$$\pi d_o \sigma = \frac{\pi}{6} d_{b,i}^3 g(\rho_l - \rho_g)$$

which upon rearrangement gives;

$$d_{b,i} = \left(\frac{6\sigma d_o}{g(\rho_l - \rho_g)}\right)^{1/3} \tag{8.14}$$

where σ is the surface tension of the liquid phase, d_o is the orifice diameter and g is the acceleration of gravity.

- the equation only holds up to a certain flowrate where the bubbles start to increase with the gas flowrate.
- higher gas flowrate–swarms of bubbles and finally, continuous jet flow will be formed.
- for viscous media, liquid viscosity rather than the bubble surface tension provides the predominant resistance to bubble formation

The bubbles formed will travel around the gas-liquid dispersion which is vigorously agitated, therefore results in **turbulent flow field**–which gives maximum bubble size, $d_{b,max}$ and can be determined by balancing the shear stress and the surface tension:

1. shear stress, τ_s acting on the bubble tend to distort the bubble into an unstable shape such that the bubble disintegrate into smaller bubbles
2. the surface tension force acting on the bubbles tends to stabilise the spherical shape of the bubble
3. during the disperse phase, there is viscous resistance to deformation of the bubble

In gas-liquid dispersions, the viscous resistance is negligible compared to the surface contribution. At equilibrium;

$$\tau_s = k_1 \frac{\sigma}{d_{b,max}} \tag{8.15}$$

where k_1 is a dimensionless constant.

- If $d_b > d_{b,max}$, the shear stress acting on the bubble is larger than the surface tension – therefore, results in bubble break-up.
- To calculate $d_{b,max}$, suitable value of τ_s should be determined.

For a **turbulence flow field**, (according to the theory of turbulence) the shear stress acting on the bubbles with diameter, d_b is given by;

$$\tau = k_2 \rho_l^{\frac{1}{3}} \left(\frac{P_g}{V_l}\right)^{\frac{2}{3}} d_b^{\frac{2}{3}} \tag{8.16}$$

with $\frac{P_g}{V_l}$ refers to the power dissipation per unit volume and k_2 is a dimensionless constant. Combining equations (8.15) and (8.16) leads to the maximum stable bubble diameter;

$$d_{b,max} = k \frac{\sigma^{0.6}}{\left(\frac{P_g}{V_l}\right)^{0.4} \rho^{0.2}} \tag{8.17}$$

where k is a combination of constants k_1 and k_2. It is fairly obvious from equation (8.17) that when the power input is increased, the maximum stable diameter is reduced.

8.3 Measurement of $k_l a$ in Continuous-Stirred-Tank Bioreactor and Airlift Bioreactor

8.3.1 Continuous-Stirred-Tank Bioreactor

Mass transfer coefficients for oxygen are normally determined experimentally. The methods include:

1. Oxygen-Balance Method
2. Dynamic Method
3. Sulphite Oxidation

Oxygen-balance method

This method is based on equation (8.5) for gas-liquid mass transfer. For oxygen transfer;

$$N_{O_2} = k_l a (c^*_{O_2} - c_{O_2})$$

The steps of carrying the experiment is given as follows:

1. Measuring the content of the inlet and outlet flow of oxygen

2. The different in oxygen flow between inlet and outlet should equal to the rate of oxygen transfer from gas to liquid at steady-state, thus

$$N_{O_2} = \frac{1}{V_l}(F_{g,i}c_{O_2,g,i} - F_{g,o}c_{O_2,g,o}) \tag{8.18}$$

where V_l and F_g are the volume of media in the fermenter and the volumetric flowrate of gas respectively. The difference between the two flowrates above results in the rate at which oxygen is transferred out of the gas into the liquid represented by N_{O_2}. Since the concentration of gases is normally measured by their partial pressure given by;

$$\begin{aligned} pV &= nRT \\ \frac{n}{V} &= \frac{p}{RT} \end{aligned} \tag{8.19}$$

since $c = \frac{n}{V}$, substitute into equation (8.18) gives;

$$N_{O_2} = \frac{1}{RV_l}\left\{\left(\frac{F_g p_{O_2,g}}{T}\right)_{inlet} - \left(\frac{F_g p_{O_2,g}}{T}\right)_{outlet}\right\} \tag{8.20}$$

3. Measure the partial pressure of oxygen at the inlet and outlet streams as well as their temperatures in order to determine N_{O_2}. This value is then used together with $c^*_{O_2}$ and c_{O_2} to calculate $k_l a$. Methods of determining $c^*_{O_2}$ and c_{O_2} are given below:

   (a) **method of determining saturation constant of dissolved oxygen or oxygen solubility ($c^*_{O_2}$)**: The different of concentration given by $(c^*_{O_2} - c_{O_2})$ refers to the driving force for the oxygen transfer. The value of driving force is usually small and thus, the saturation constant have to be found accurately. A detailed method is published by Battino R. and Clever H. L. in 1966. The paper describes 5 different method which include;

   - Monomeric-volumetric method
   - Mass spectrometric method
   - Gas chromatographic method
   - Chemical method
   - High pressure/high temperature method

   (b) **method of determining dissolved-oxygen concentration (c_{O_2})**: The dissolved-oxygen concentration in media is normally measured using a dissolved-oxygen electrode. Two types of electrodes are available;

   i. galvanic electrode
   ii. polarographic electrode

   - for both types, a membrane which is permeable to oxygen separates the fermentation fluid from the electrode. Oxygen diffuses through this membrane to the cathode where it reacts to produce a current between anode and cathode proportional to the oxygen partial pressure in the broth/media.
   - the electrolyte solution in the electrode supplies ions which take part in the reactions and must be replenished at regular intervals.
   - supply of oxygen molecules from the bulk medium in the vessel to the cathode within the probe is in itself a mass-transfer process.

- this happens because there is no liquid motion in the membrane or electrolyte solution and little motion in the liquid film at the membrane interface–operation of the probe relies on diffusion of oxygen across these thicknesses.
- due to such diffusion processes, the response of the probe (electrode) to sudden changes in dissolved-oxygen level is subject to delay.
- electrode can be improved if the bulk liquid is stirred rapidly–therefore decreases the thickness of the liquid film at the membrane surface.
- the smaller the size of cathode and low rate of oxygen consumed by the probe–means that their response is quicker–so that it can be used to measure dissolved-oxygen levels in un-agitated systems.
- repeat calibration of the probe is necessary before use.
- **precaution**: do not leave probe in the fermentation broth for longer period–this can cause fouling due to cells attaching to the membrane surface.

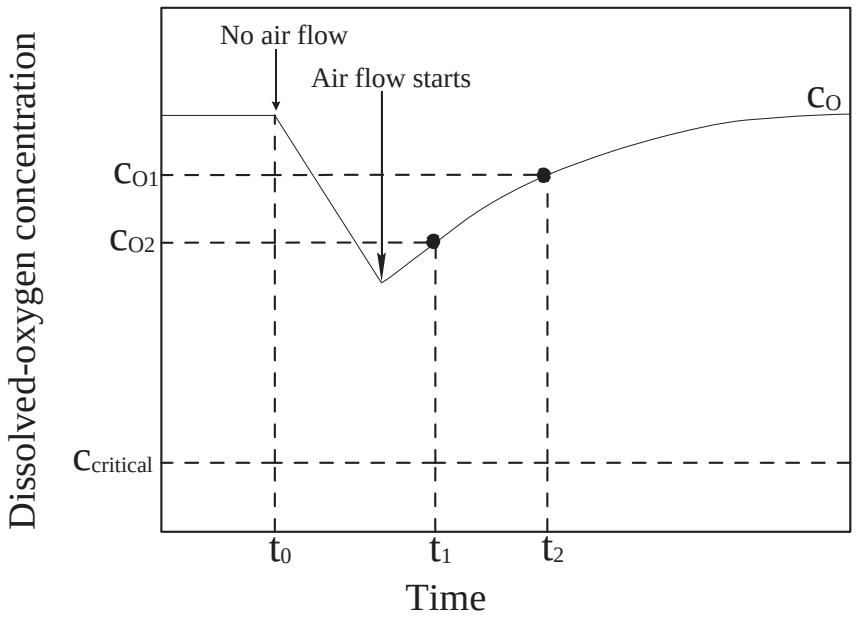

Figure 8.3: Graph for measuring dissolve-oxygen concentration with dynamic method.

Dynamic method

Suitable with the name, this technique of obtaining the mass transfer coefficient is based on the transient state of oxygen balance. The cost of equipment required to undertake this method is low (one of the advantages). Consider a fermenter containing cells in a batch configuration;

- by referring to Figure 8.3, at time $t = 0$, the broth is de-oxygenated (by sparging nitrogen gas into the fermenter or by stopping the air flow if the culture is oxygen-consuming). The drop of the dissolved oxygen concentration c_{O_2} can be apparently seen.

- when the level is about half of the critical value (precaution should be taken so that the level does not drop below this critical velue), air is pumped back into the vessel at a constant flowrate. The increase in the dissolved-oxygen concentration is monitored.

- by assuming that the re-oxygenation of the media is fast relative to the growth, the dissolved-oxygen concentration will reach a steady-state value, $\bar{c}_{O_2}$. This shows the balance between the oxygen supply and oxygen consumption in the system.

- $c_{O_{2,1}}$ and $c_{O_{2,2}}$ are two oxygen concentrations measured during the re-oxygenation at times t_1 and t_2 respectively. From these information, a transient equation for the rate of oxygen dissolved in the media can be developed.

$$\frac{dc_{O_2}}{dt} = k_l a(c^*_{O_2} - c_{O_2}) - q_{O_2} x \tag{8.21}$$

where, $q_{O_2}x$ is the rate of oxygen consumed by the cells which can be determined by considering the final steady dissolved-oxygen concentration $\bar{c}_{O_2}$. When $c_{O_2} = \bar{c}_{O_2}$,

$$\frac{dc_{O_2}}{dt} = 0$$

since there is no change in c_{O_2} with time, thus;

$$k_l a(c^*_{O_2} - \bar{c}_{O_2}) = q_{O_2} x \tag{8.22}$$

Upon substitution into equation (8.21) and rearranging gives;

$$\frac{dc_{O_2}}{dt} = k_l a(\bar{c}_{O_2} - c_{O_2}) \tag{8.23}$$

and assuming that $k_l a$ is constant with time throughout, integrating above equation leads to;

$$k_l a = \frac{\ln\left(\frac{\bar{c}_{O_2} - c_{O_{2,1}}}{\bar{c}_{O_2} - c_{O_{2,2}}}\right)}{t_1 - t_2} \tag{8.24}$$

- using the above equation, the mass transfer coefficient can be estimated by taking two points or several points of ($c_{O_{2,1}}$,t_1) and ($c_{O_{2,2}}$,t_2) from Figure 8.3.
- $\ln\left(\frac{\bar{c}_{O_2} - c_{O_{2,1}}}{\bar{c}_{O_2} - c_{O_{2,2}}}\right)$ is then plotted against $(t_1 - t_2)$ and a straight line graph with slope representing the $k_l a$ can be found.
- for this technique, an oxygen probe with fast response time is needed to measure c_{O_2} otherwise–accurate result cannot be obtained.
- the average residence time of the gas in the system can also affect the accuracy of the this method. This occur during the switching of de-oxygenation to the aeration at the start of the measurement. There is a nitrogen has hold-up within the vessel when air is re-introduced–measuring c_{O_2}, does not reflect the kinetics of simple oxygen transfer until a hold-up of air is established (longer time is needed for large vessel). For convenient, this method is only suitable for vessels with height less than 1 m.
- the technique also is not suitable for viscous media/broths for a similar reason; longer residence times of bubbles in viscous media affect the accuracy of the measurement.

Sulphite oxidation method

This method is based on the oxidation of sodium sulphite to sulphate in the presence of a catalyst such as copper ions, Cu^+. Such a method has been found to give higher values of $k_l a$ compared to other techniques.

8.3.2 Airlift Bioreactor

The configuration of the airlift bioreactor is shown in Figure 8.4. The method of determining the mass transfer coefficient can be done in 2 ways;

1. Dynamic method
2. Sulphite oxidation method

Sulphite method

- The method is based on the modified sulphite method used by Vilaça *et al.* (1999).
- Throughout the work, the temperature was controlled at 37°C.

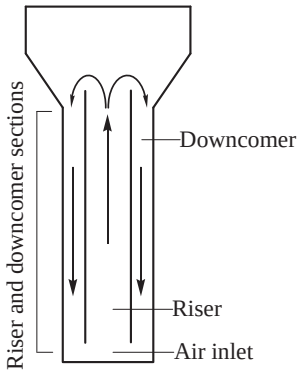

Figure 8.4: Airlift bioreactor configuration.

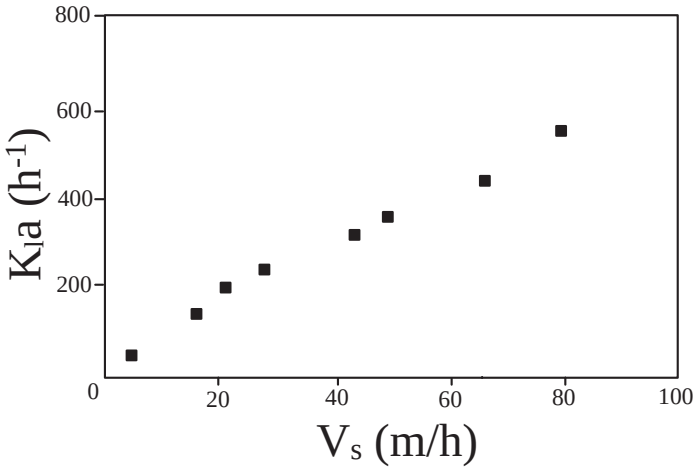

Figure 8.5: The volumetric oxygen transfer coefficient in the airlift bioreactor using sulphite method.

- The oxygen probe was installed in the riser section during the runs and the air flowrate was set in a range of different values from 0.1 to 1.5 vvm, fixed for each run.
- And the correlation is given by;

$$k_l a = 14.32 V_s^{0.84} \tag{8.25}$$

  obtained from the graph shown above.
- It is impossible to distinguish differences in the oxygen transfer coefficient in the riser and the downcomer sections (see Figure 8.4) using the sulphite method.

Dynamic method

- The determination of $k_l a$ using the dynamic method was described by Ruchti *et al.* (1981).
- The volumetric oxygen transfer coefficient was measured in two different position, in the riser, and in the downcomer and the air flowrate was varied between 0.1 and 1.5 vvm.

Riser section	Downcomer section
$k_l a = 0.75 {V_s}^{0.96}$	$k_l a = 0.67 {V_s}^{0.96}$
$(R^2 = 0.997)$	$(R^2 = 0.950)$

Table 8.2: $k_l a$ correlations for riser and downcomer sections in an airlift bioreactor according to Vilaça *et al.*.

- The study was divided into 2 parts, the mass transfer coefficient for the riser section and the that for the downcomer section. The correlations taken at 37°C are tabulated in Table 8.2.
- Since air was introduced into the bioreactor directly in the riser, this section is supposed to have a better mass transfer, leading to $k_l a$ values higher than in the downcomer for a given temperature.
- It was observed that there were more bubbles leaving the liquid in the upper section of the reactor than going down in the downcomer section.
- Bello *et al.* (1985) have reported that the oxygen concentration inside the bubbles in the downcomer is lower than the one in the riser.

Chapter 9

Liquid Mixing

A general concept of mixing has been mentioned in Section 7. The main reason of applying mixing in fermentation processes is to reduce the uniformities in fluid by elimination the gradients of concentration, temperature and other properties. Such process can be accomplished by interchanging material between different locations to produce a mingling of components. For a perfectly mixed system, there is a random homogeneous distribution of system properties which involves:

- blending soluble components of the medium
- dispersing gases, for instance, air through the liquid in the form of small bubbles
- maintaining suspension of solid particles (cells or immobilised enzymes)
- dispersing immiscible liquids to form emulsion/fine drops
- promoting heat transfer to or from liquid

If mixing does not maintain a uniform suspension of biomass, substrate concentrations can quickly drop to zero in areas where cells settle out of suspension.

As mentioned in the last point above, bioreactors must be capable of transferring heat to or from the broth quickly enough such that the desired temperature is maintained. The usual way in achieving mixing of components is by mechanical agitation using impeller. The **theory of mixing** has been discussed at length in Section 7.

9.1 Types of Mixing and Stirrers

Mixing:

- mixing is usually carried out in a stirred tank reactor which has been fitted with baffles. The normal shape of a reactor is cylindrical that can avoids unreachable region such as sharp corners and pockets. This also discourages formation of stagnant regions.
- mixing is achieved using an impeller mounted in the tank for use with Newtonian fluids–ratio of tank diameter : impeller diameter is about 3:1.

Impeller:

- impeller is normally positioned overhead on a centrally located stirrer shaft and driven rapidly by the stirrer motor.

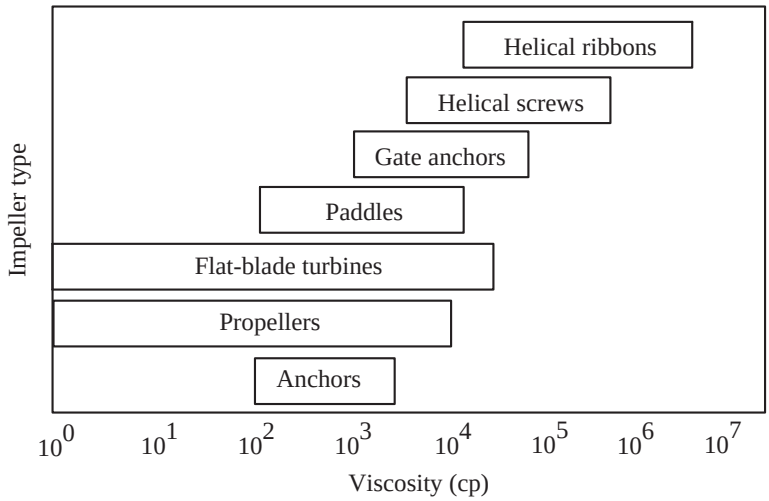

Figure 9.1: Viscosity ranges for different impellers.[Adapted from Doran P., Academic Press, (2004).]

- liquid is forced away from the impeller, circulates through the vessel and periodically returns to the impeller.
- depth of liquid in the tank should be no more than 1.0–1.25 times the tank diameter.
- some impellers have flat blades, propeller type and helical screw.
- choice of impeller depends on;
    1. viscosity of liquid (refer Figure 9.1)
    2. sensitivity of systems to mechanical shear.
- for *LOW* to *MEDIUM* viscosity liquids–propeller and flat-blade turbines are recommended.
- frequently used impeller for industrial fermentation–6-flat-blade disc-mounted turbine (Rushton turbine) is used.
- a diagram of different types of impellers is attached.

Baffles:

- strips of metal mounted against the wall of the tank–installed to reduce vortexing and swirling of liquid.
- it is attached to the tank by means of welded brackets–4 equally-spaced baffles are usually sufficient to prevent vortex formation.
- optimum baffle width depends on the impeller design and fluid viscosity–of order (1/10 to 1/12) the tank diameter.
- can be mounted away from wall with clearance of about 1/50 the tank diameter.
- this will prevent sedimentation and development of stagnant zones at the inner edge of the baffle during mixing of viscous cell suspensions.

9.2 Types of Flows in Agitated Tanks

Flow pattern in a stirred-tank depend on:

1. the impeller type/design
2. properties of fluid
3. size and geometric proportions of vessel, baffles and impeller

The flow can be divided into 3 different patterns:

1. circular flow
2. radial flow
3. axial flow

For circular flow:

- rotational in action
- simple flow
- movement of liquid in streamline fashion–gives little mixing between fluid at different heights in the tank
- can lead to vortex development
- at high speed–vortex may reach the impeller so that gas from the surrounding atmosphere is drawn to the liquid
- generally disadvantageous and should be avoided
- can be prevented by installing baffles that interrupt such a flow and create turbulence

For radial flow:

- this type of flow is produced from impellers that have blades which are parallel to the vertical axis of the stirrer shaft and tank
- example: 6-flat-blade disc turbine impeller
- liquid is driven radially from the impeller against the walls of the tank–divided into 2-stream, one flowing upwards to the top of the tank and the other flowing downwards toward the base of the tank
- both streams reach the central axis of the tank and are drawn back to the impeller
- this type of flow also produces circular flow and it has to be avoided with baffles

For axial flow:

- this type of flow generally produced by impeller with blades that have an angle of less than 90° to the plane of rotation and promote axial top to bottom motion
- propellers are type of impeller that give axial flow in a stirred-tank

- fluid leaving the impeller is driven downwards until it is deflected from the base of the tank
- it then spreads out over the floor and flows up along the wall before being drawn back to the impeller
- such impellers are particularly useful when strong vertical currents are required especially when dealing with mixing of solid particles–strong axial flow leaving the impeller will avoid the solid from settling at the bottom of the tank

9.3 The Mechanism of Mixing

An effective mixing of fluid can be achieved when;

- sufficient velocity of fluid which can carry material into most parts of the tank
- turbulent flow for perfect mixing

These factors are a combination of physical processes that include;

- distribution (process which comprises of macromixing)
  - during the mixing of dye, the process of transporting the dye to all regions of vessel by bulk distribution currents is termed *distribution*
  - the process can be relatively show
  - for large vessel, the size of the circulation paths is also large therefore leads to long period of time for the flow to traverse–this inhibits rapid mixing
  - *distribution* is the the **slowest** step in mixing process
  - for high rotational speed impeller–the process will be turbulence (this only happens when fluid travels randomly or erratically in the form of cross-currents)
  - kinetic energy from the turbulence fluid is directed into regions of rotational flow known as *eddies*
- dispersion (either macro– or micro– mixing)
  - process of breaking up bulk flow into smaller eddies
  - it facilitates rapid transfer of material throughout the vessel
  - the size of eddies that limits the degree of dispersion is give approximately by the *Kolmogorov* scale of mixing or also known as *scale of turbulence*, γ;

    $$\gamma = \left(\frac{\nu^3}{\varepsilon}\right)^{1/4}$$

    where γ is the characteristic dimension of the smaller eddies, ν represents the kinematic viscosity of the fluid and ε is the local rate of turbulence energy dissipation per unit mass of liquid
- diffusion (process which comprises of micromixing)
  - molecular diffusion is generally regarded as slow process

- over small distances, the process can accomplish quite rapidly
- for eddies of diameter 30 to 100 μm, homogeneity is achieved in about 1 s for low viscosity fluids
- if power input to a stirred-tank can produce eddies of the given dimensions– mixing on a molecular scale is then accomplished

9.4 Power Requirement for Mixing

In order to drive the impeller for mixing, electrical power is usually used. For a given stirrer speed, the power required depends on the resistance offered by the fluid to rotation of the impeller. The average power consumption per unit volume for industrial bioreactors;

- 10 kWm^{-3} (small vessel)
- 1–2 kWm^{-3} (large vessel)

Friction from the motor (gearbox and seals) also contribute to loss of energy transmitted to the fluid, thus, the electrical power consumed by the motor is greater than the mixing power–depends on the efficiency of the drive.

Methods of calculating power requirements during mixing can be divided into 2 types:

1. un-gased Newtonian fluids
2. un-gased non-Newtonian fluids
3. gased fluids

9.4.1 Un-gased Newtonian Fluids

For non aerated fluids, the mixing power depends on;

- stirrer speed
- impeller diameter and geometry
- fluid properties (density, viscosity etc.)

These can be expressed in terms of the dimensionless numbers, for instance, Reynolds number, Re and Power number, N_p;

$$Re = \frac{\rho D v}{\mu} \tag{9.1}$$

and

$$N_P = \frac{P}{\rho N_i^3 d_i^5}. \tag{9.2}$$

where P is the power, ρ is the fluid density, N_i refers to the stirrer speed, d_i represents the diameter of the impeller (while D represents pipe diameter), v is the fluid velocity and μ gives the viscosity of the fluid.

- These two correlations has been determined experimentally for different range of impellers and agitated tank configurations. **This is given in an attached paper**.

- When the power number of a particular impeller is found, the power required can be determined by rearranging equation (9.2) which gives;

$$P = N_P \rho N_i^3 D_i^5 \tag{9.3}$$

The two correlations also depend on the flow regimes within the tank (either in the region of laminar flow, transition flow or turbulent flow):

- for **laminar regime**, ($Re < 10^1$) is for a number of impellers, ($Re \geqslant 10^2$) is for stirrers with very small wall-clearence such as the anchor and helical-ribbon
- in this regime, the power number is inversely proportional to the Reynolds number;

$$N_P \propto \frac{1}{Re}$$

or

$$P = k\mu N_i^2 D_i^3$$

where k is the proportionality constant

- power required for laminar flow is independent of the density of the fluid however it is directly proportional to fluid viscosity
- for **transition regime** between laminar and turbulent flow, both density and viscosity affect power requirements
- there usually a gradual transition from laminar to fully-developed turbulent flow in agitated tanks (flow pattern and Re range for transition depend on system geometry
- for **turbulent regime**, the power number is independent of Re, thus;

$$P = N'_P \rho N_i^3 D_i^5$$

where N'_P is the constant value of the power number in the turbulent regime.

- N'_P for turbine impeller is significantly higher than for most other impellers–this shows that turbines transmit more power to the fluid than other designs
- the power required for turbulent flow is independent of the viscosity of the fluid, but proportional to the fluid density
- for most small impellers in baffled tanks, turbulent regime is fully developed at $Re > 10^3$ or 10^4
- for tanks without baffles, turbulent is not fully developed until $Re > 10^5$

Impeller type	k $(Re = 1)$	N'_P $(Re = 10^5)$
Rushton turbine	70	5–6
Paddle	35	2
Marine propeller	40	0.35
Anchor	420	0.35
Helical ribbon	1000	0.35

Table 9.1: Constants k and the Power number in turbulent regime, N'_P used in the equations for laminar and turbulent regime respectively. [Values adapted from Doran P.M., Academic Press, (2004)]

9.4.2 Un-gased non-Newtonian Fluids

For non-Newtonian fluids, the power estimation is more difficult. Viscosity of such fluids varies with shear conditions, the impeller Re used to correlate power requirements must be re-defined.

A few correlations has been developed based on the apparent viscosity μ_a given by

$$\mu_a = K\dot{\gamma}^{n-1}$$

which gives Re;

$$Re = \frac{N_i D_i^2 \rho}{\mu_a} \tag{9.4}$$

thus, substituting μ_a leads to

$$Re = \frac{N_i D_i^2 \rho}{K\dot{\gamma}^{n-1}} \tag{9.5}$$

where n is the flow behaviour index, K is the consistency index and $\dot{\gamma}$ is the shear rate. Problem arises in determining $\dot{\gamma}$ and an approximate relation has been used for stirred tanks;

$$\dot{\gamma} = kN_i$$

where k is a constant with magnitude dependent on the geometry of the impeller. Substituting this into equation (9.5) leads to;

$$Re = \frac{N_i^{2-n} D_i^2 \rho}{k^{n-1} K} \tag{9.6}$$

A plot of the Power number against the Reynolds number for the non-Newtonian fluids for Rushton turbine in a baffled tank is attached separately.

9.4.3 Gased Fluids

Apparently, when gas is sparged in a liquid, the hydrodynamic of the liquid around the impeller (or any fluids) immediately changes. This reduces the power requirements of the mixing when the density of the fluid decreases.

Large gas-filled cavities developed behind the stirrer blades in gased liquids will reduce the resistance to the fluid flow and decrease the drag coefficient of the impeller.

The ratio of the gased to un-gased power requirement is given below;

$$\frac{P_g}{P_0} = 0.10 \left(\frac{F_g}{N_i V}\right)^{-0.25} \left(\frac{N_i^2 D_i^4}{g W_i V^{2/3}}\right)^{-0.20} \tag{9.7}$$

where P_g is the power with the gas sparging, P_0 refers to the power consumption without the gas sparging, F_g is the volumetric gas flowrate, N_i is the stirrer speed, V is the liquid volume , D_i is the impeller diameter, g refers to the gravitational acceleration and W_i is the impeller blade width.

The average deviation between the two systems is approximately 12%–with sparging, the power consumption could be reduced to as little as 50% of the un-gased value which also depends on the flowrate of the gas.

Chapter 10

Kinetics of Substrate Utilisation, Product Formation and Biomass Production in Cell Cultures

1. The kinetics of substrate consumption in cellular growth and enzyme-catalysed reaction and their relationship with bioreactor modelling
    - unstructured batch growth models
    - structured kinetic models
        - compartmental models
        - metabolic models
2. The kinetics of product formation
    - Unstructured models
    - Structured product formation kinetics models
3. Microbial and enzyme kinetics models and their applications in bioreactor design
    - Continuous-stirred-tank bioreactor
    - Plug-flow-tubular bioreactor with immobilised enzyme

10.1 The kinetics of substrate consumption in cellular growth and enzyme-catalysed reaction and their relationship with bioreactor modelling

The growth of cell with the presence of enough substrate can be described using other form of model equations. This can be referred in Table 10.1. These models are proposed in such a way that they could give better fits to experimental data points.

Model type	Model equation
Tessier	$\mu = \mu_{max}\left(1 - e^{-\frac{S}{K_s}}\right)$
Moser	$\mu = \frac{\mu_{max}}{1+K_s S^{-\lambda}}$
Contois	$\mu = \mu_{max}\frac{S}{Bx+S}$

Table 10.1: Models used to described cellular growth

- for *Tessier* and *Moser*, the equations are rendered algebraic solution much complex compared to the *Monod* model
- however for the *Contois* model, it contains the apparent Michaelis constant that is proportional to the biomass concentration, x. The last term from this model will diminish the maximum growth rate as the population density increases leading to;

$$\mu \propto \frac{1}{x}$$

- the specific growth rate could be inhibited by medium constituents such as substrate or product. For a case of substrate inhibition, the term μ is given by;

$$\mu = \frac{\mu_{max} S}{K_i + S + \frac{S^2}{K_p}}$$

- for a case that exhibits product inhibition such as yeast fermentation, the specific growth rate is written as;

$$\mu = \mu_{max}\frac{S}{K_i + S}\frac{K_p}{K_p + P}$$

- it is also possible that two or more substrates may simultaneously be growth-limiting, thus, a model that can describe such a system is given by;

$$\mu = \mu_{max}\frac{S_1}{K_1 + S_1}\frac{S_2}{K_2 + S_2}\cdots$$

When the growth passes the lag phase, as that shows in Figure 1.1 it follows the transient form of equation given below. This is known as the exponential phase, where cells multiplied rapidly and the number of living cells doubles regularly with time.

$$\frac{dx}{dt} = \mu x \tag{10.1}$$

which upon integration with $x = x_0$ and $t = t_{lag}$ leads to;

$$\ln\frac{x}{x_0} = \mu(t - t_{lag}) \tag{10.2}$$

or can be written in a simplified form;

$$x = x_0 e^{\mu(t-t_{lag})} \qquad t > t_{lag}$$

When the number of cells doubles, $x = 2x_0$, substitution of the new x into equation (10.2) and with further simplification gives;

$$\bar{t}_d = \frac{\ln 2}{\mu} \tag{10.3}$$

where $\bar{t}_d$ is the time required for the cells to double its population.

- for steady-state growth in a fermenter, only a single parameter μ is required to characterise the population during exponential batch growth
- growth is balanced during this stage of batch cultivation
- deviations from exponential growth arise–nutrient level/toxin concentration in the fermenter achieves a value which can no longer support the maximum growth rate.
- lack of nutrient in a growth medium during exponential growth can be represented by a differential form of equation (rate of nutrient consumed is proportional to the mass of cells);

  $$\frac{dn}{dt} = -k_n x \tag{10.4}$$

  where n is the amount of nutrient.
- then by assuming that the exponential growth continues unabated until the stationary phase is reached and taking the time when the growth begins at $t = 0$;

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

  where x_0 is the amount of cells when the exponential growth starts. Substitute this into equation (10.4) and integrating gives;

  $$n_0 = \frac{k_n}{\mu}(x_s - x_0) \tag{10.5}$$

  where x_s represents the cells population when the nutrient is exhausted when it is at the stationary state. n_0 is the amount of nutrient at $t = 0$. Rearranging (10.5) leads to the maximum population when a particular nutrient has depleted.

  $$x_s = x_0 + \frac{\mu}{k_n} n_0$$

If there is a particular toxic contaminating the growth, the basic equation (10.1) changes to;

$$\frac{dx}{dt} = kx(1 - f(q)) \tag{10.6}$$

where $f(q)$ is the function describing the toxin concentration.

- for a linear rate of toxin concentration given by, $f(q) = bc_{tox}$, with c_{tox} is the toxin concentration, therefore, equation (10.6) is replaced by,

  $$\frac{dx}{dt} = kx(1 - bc_{tox}) \tag{10.7}$$

  where b is the proportionality constant.

- the concentration of toxin itself is described by;

$$\frac{dc_{tox}}{dt} = qx \tag{10.8}$$

which upon integration leads to;

$$c_{tox} = q\int_{t_0}^{t} x dt \tag{10.9}$$

and substitution into equation (10.7), the growth rate is then given by;

$$\frac{dx}{dt} = kx\left(1 - bq\int_{t_0}^{t} x dt\right) \tag{10.10}$$

- since the growth rate is given by $\frac{dx}{dt} = \mu x$, thus the specific-growth is then, $\mu = \frac{1}{x}\frac{dx}{dt}$. Similarly, from equation (10.10), the effective specific-growth rate can be written as;

$$\mu_{\mathit{eff}} = \frac{1}{x}\frac{dx}{dt} = k\left(1 - bq\int_{t_0}^{t} x dt\right) \tag{10.11}$$

which represents the instantaneous specific-growth rate at any time t.

- the rate of the specific-growth tends to decrease rapidly as observed from the equation below;

$$\frac{d\mu_{\mathit{eff}}}{dt} = -kbqx$$

and

$$\frac{d}{dt}\left(\frac{d\mu_{\mathit{eff}}}{dt}\right) = -kbq\frac{dx}{dt}$$

- when growth suddenly stops, $\frac{dx}{dt} = 0$, thus from equation (10.10);

$$1 - bq\int_{t_0}^{t} x dt = 0$$

hence,

$$\frac{1}{bq} = \int_{t_0=0}^{t} x dt \tag{10.12}$$

When the toxin concentration, c_{tox} reaches a value where $c_{tox} = \frac{1}{b}$, then the growth will cease. Dilution of toxic medium or addition of a certain chemical (non-nutritive) substance to react with the toxin should allow further growth, therefore increase in maximal stationary phase biomass concentration, x_s. For cells that stop growing due to nutrient exhaustion, dilution and addition of other chemicals would not change x_s.

10.2 Unstructured Batch Growth Models

A brief discussion on modelling of bacterial growth has been discussed in the beginning of the course using the simplest approach of the form;

$$\frac{dx}{dt} = f(x).$$

Such a model does not need to neglect any changes occurring in the medium during growth. The model which represents dx/dt as μx was used according to *Malthus's law* with a constant μ.

Inhibition was than introduced assuming that it is proportional to the squared of the amount of cells;

$$\frac{dx}{dt} = kx(1 - \beta x) \qquad x(0) = x_0 \tag{10.13}$$

Integrating (10.13) gives,

$$x = \frac{x_0 e^{kt}}{1 - \beta x_0(1 - e^{kt})} \tag{10.14}$$

At a stationary state when $dx/dt = 0$, the curve representing the amount of cells would give $x_s = 1/\beta$.

If the production rate of an inhibitor/toxin is proportional to the population growth rate, therefore;

$$\frac{dc_{tox}}{dt} = \alpha \frac{dx}{dt}$$

with α as the constant of proportionality, with the initial condition given as $c_{tox}(0) = 0$, integrating the above differential form would give;

$$c_{tox} = \alpha(x - x_0).$$

Other class of unstructured models approaching stationary population level can be formed based on limiting nutrient exhaustion with constant yield factor $Y_{X/S}$ and $\mu = \mu(s)$ as the assumptions.

Most of the equations described above failed to describe the declining phase after the stationary period of nutrient exhaustion. A form of integral function has been introduced as an addition to μx which is given by;

$$\int_0^t K(t, r)x(r)dr \tag{10.15}$$

- the population history is represented by $K(r, t)$ for a particular growth rate.
- using such a term, all past values of the population density can influence the growth rate at time t.
- for K independent of t, it can represent the influence of a component of the culture which concentration rate can be written as;

$$\frac{dc}{dt} = |K(t)x(t)| \tag{10.16}$$

- on the other hand, if K is a constant equivalent to K_0 and the population history above is added to equation (10.13), hence;

$$\frac{dx}{dt} = kx(1 - \beta x) + K_0 \left| \int_0^t x(r)dr \right| \qquad x(0) = x_0 \tag{10.17}$$

The unstructured growth models have several disadvantages;

- they show no lag phase and give no detailed information into the variables which influence growth
- it gives no attempt to utilise/recognise knowledge about cellular metabolism and regulation

10.3 Structured Kinetic Models

The unstructured models above do not recognise nor represent the composition of cell. For a situation which cell population changes significantly and in which these composition changes influence kinetics of cells–structured models should be used.

The biophase variables employed in structured models are typically the mass, x_j or molarity, c_j per unit volume of biophase. This can be written in the differential form as;

$$\frac{d}{dt}\left(\frac{1}{\rho_c}V_R x c_j\right) = \frac{1}{\rho_c}V_R x r_{f,j} + \frac{1}{\rho_c}\Phi_x c_j \tag{10.18}$$

where;
ρ_c = mass density of cell
$r_{f,i}$ = molar rate of formation of component j
Φ_x = mass of cells added to the reactor
V_R = culture of volume
x = cell mass concentration
c_j = moles j per unit volume cells

One assumption used based on the last term in equation (10.18) is that any cells added or removed from the reactor have the same state as the cell population in the reactor. If this condition is not met, the balance equation must be modified.

And assuming that the density of the cell, ρ_c and volume of culture V_R are time invariant, equation (10.18) may be written in the form of;

$$\frac{dc_j}{dt} = r_{f,j} - c_j\left(\frac{1}{x}\frac{dx}{dt}\right) + c_j\frac{\Phi_x}{x_{V_R}} \tag{10.19}$$

For a batch reactor configuration, the term, Φ_x is zero since no cell is being fed and the quantity denoted by $\left(\frac{1}{x}\frac{dx}{dt}\right)$ can be represented by the specific growth rate μ which leaves equation (10.19) in the form of;

$$\frac{dc_j}{dt} = r_{f,j} - \mu c_j \tag{10.20}$$

10.3.1 Compartmental models

1. the simplest structured models
2. compartmentalisation of components/section into small sizes
    - synthetic components such as RNA and precursors
    - structural components such as DNA and proteins
3. this could also be defined as the assimilatory component and a synthetic component
4. class of transport and reaction occurring in a cell population include;
    - molecular collision
    - chemical reactions

- diffusion
- RNA turnover
- protein synthesis
- increase in cell number
- completion of batch process
- spontaneous mutation

which process has a characteristic relaxation time (time to reach steady-state) after a perturbation.

What is important in modelling such systems?

1. **relationship between the time scale of changes in cells' environment, τ_E and the spectrum of relaxation times of cellular processes.**
2. **those cellular processes that respond very fast to environmental changes (small relaxation time/τ_E ratio) may be assumed to be in quasi steady-state.**
3. **for large relaxation time compared to τ_E, cellular system may be assumed to be frozen at the initial state (for example: significant genetic changes are not usually expected during a single batch cultivation).**

A simple two-compartmental model that produces several aspects of batch growth dynamics has been developed and the postulates are given below:

1. the synthetics portion is produced by uptake of external nutrient S with yield coefficient Y. The rate of cell synthetic component formation is first order in total cell density x and nutrient mass concentration
2. the structural-genetics cell component is produced from component 1 at a rate proportional to $\rho_1\dot{\rho}_2$
3. doubling of component 2 is necessary and sufficient for cell division–the cell density is proportional to the density of component 2 in the culture
4. biomass–comprised entirely of components 1 and 2

This can be observed in the system of equation below:

$$\begin{aligned}
\frac{ds}{dt} &= -\frac{1}{Y}k_1 sx \\
\frac{dx}{dt} &= k_1 sx \\
\frac{d\rho_1}{dt} &= k_1 s(\rho_1+\rho_2) - k_2\rho_1\rho_2 - \mu\rho_1 \\
\frac{d\rho_2}{dt} &= k_2\rho_1\rho_2 - \mu\rho_2
\end{aligned} \tag{10.21}$$

also noting that;

$$\mu = k_1 s \tag{10.22}$$

and

$$\rho_1 + \rho_2 = \rho_c = constant \tag{10.23}$$

with the final equation in system (10.21) can be re-written as;

$$\frac{df_2}{dt} = -k_1 s f_2 + k_2 \rho_c f_2 (1 - f_2) \tag{10.24}$$

where f_2 is the ratio of ρ_2/ρ_c.

- The first 2 equations from system (10.21) can be used to write s in terms of x.
- Result from this can be substituted into $\frac{dx}{dt}$ to obtain a form identical to equation (10.13).
- An integration of this will give the logistic equation (10.14).

Such a model has been used to simulate batch growth using a stationary, nutrient-exhausted inoculum that corresponds to $\rho_1 = 0$. Simulation results in several frequently observed features of batch microbial cultures, include:

- existence of lag phase–cell size increases
- exponential growth phase–cell size is maximum
- change in composition of cells during growth cycle–since these changes are evident during exponential phase
- stationary phase with relatively small cells

10.3.2 Metabolic models

- this type of models incorporate some aspects cell metabolism
- more biological detail incorporated in a model, it will become more specific to a particular organism/process
- the more detailed the model becomes, the more one should know *a priori* about the organism

10.4 Product Formation Kinetics

10.4.1 Unstructured model

Simplest type of product formation kinetics arise when there is a simple stoichiometric connection between product formation and substrate utilisation or cell growth. The rate of product formation can be written as;

$$r_{f_p} = -Y_{P/S} r_{f_s} \tag{10.25}$$

it can also be expressed as;

$$r_{f_p} = Y_{P/X} r_{f_x} \tag{10.26}$$

For anaerobic fermentation such as *Lactobacillus delbrukii*, the kinetics of product formation is famously expressed by the Leudeking-Piret kinetics given by;

$$r_{f_p} = \alpha r_{f_x} + \beta x \tag{10.27}$$

Such a form is normally used in fitting product formation data. The first term in equation (10.27) refers to the energy used for growth and the second term refers to the energy used for maintenance.

Example 7: Consider a batch fermentation model given by a logistic model below;

$$\frac{dx}{dt} = kx(1 - \beta x)$$

and product formation is given by Leudeking-Piret kinetics of the form;

$$\frac{dp}{dt} = \alpha \frac{dx}{dt} + \beta x$$

and substrate utilisation is given by;

$$\frac{ds}{dt} = -\frac{1}{Y_{X/S}} \frac{dx}{dt} - \frac{1}{Y_{P/S}} \frac{dp}{dt} - kx$$

Solution: For substrate consumption:

Substitute $\frac{dp}{dt}$ into $\frac{ds}{dt}$ gives;

$$\frac{ds}{dt} = -\left(\frac{1}{Y_{X/S}} + \frac{\alpha}{Y_{P/S}}\right) \frac{dx}{dt} - \left(\frac{\beta}{Y_{P/S}} + k\right) x$$

which reduces into

$$\frac{ds}{dt} - \gamma \frac{dx}{dt} - \eta x$$

where

$$\gamma = \left(\frac{1}{Y_{X/S}} + \frac{\alpha}{Y_{P/S}}\right)$$

and

$$\eta = \left(\frac{\beta}{Y_{P/S}} + k\right)$$

Integrating $\frac{dx}{dt}$ gives a Riccati type equation of the form;

$$x = \frac{x_0 e^{kt}}{1 - \beta x_0 (1 - e^{kt})}$$

which upon rearrangement gives;

$$\ln\left(\frac{\frac{x(t)}{x_s}}{1 - \frac{x(t)}{x_s}}\right) = kt - \ln\left(\frac{x_s}{x_0} - 1\right) \tag{10.28}$$

where β is substituted for the stationary cell population, x_s.

A graph of $\ln\left(\frac{x(t)/x_s}{1-x(t)/x_s}\right)$ against the time, t is then plotted which gives the gradient, k and the y-intercept of $\ln\left(\frac{x_s}{x_0}-1\right)$ that can be used to determine x_0.

For product formation:

Using,

$$\frac{dp}{dt} = \alpha\frac{dx}{dt} + \beta x$$

with x given by the Riccati equation and upon integration gives;

$$p(t) - p_0 - \beta\left(\frac{x_s}{k}\right)\left(1 - \frac{x_0}{x_s}(1-e^{kt})\right) = \alpha(x(t)-x_0)$$

The constant β is found at the stationary phase of batch culture;

$$\beta = \frac{\frac{dp}{dt}|_{stationary\,phase}}{x_s}$$

and α is determined by a plot of $p(t) - p_0 - \beta\left(\frac{x_s}{k}\right)\left(1 - \frac{x_0}{x_s}(1-e^{kt})\right)$ against $(x(t)-x_0)$.

10.4.2 Structured product formation kinetic modelling

- only little development for the structured kinetics of product formation
- models are usually developed based on metabolite production
- most are based on antibiotic fermentation such as *Claviceps purpurea*
- the model is divided into 4, the cellular growth, the extracellular and intracellular phosphate levels and the product formation. These are given below:

  For cellular growth:

  $$\frac{dx}{dt} = k_1[1 - e^{-K_1 p_i}]x - k_2x^2$$

  For extracellular phosphate:

  $$\frac{dp}{dt} = -k_3\frac{p}{p+K_2}x + (Y_{P/X} + p_i)k_2x^2$$

  For intracellular phosphate:

  $$\frac{dp_i}{dt} = k_3\frac{p}{p+K_2} - k_1(Y_{P/X} + p_i)(1 - e^{-K_1 p_i})$$

  For product formation:

  $$\frac{da}{dt} = k_4\frac{K_3x}{K_3 + p_i^2}$$

10.5 Microbial and enzyme kinetic models and their applications in bioreactor design

10.5.1 Plug-flow-tubular bioreactor with immobilised enzyme

Plug Flow Turbular (Bio)Reactor

- it is an alternative to CSTR system
- no mixing required
- fluid entering the reactor passes through as a discrete "plug" and it does not interact with neighbouring fluid element
- can be achieved by supplying high fluid flowrate which could minimise back-mixing and variation of fluid velocity
- the diagram of a plug-flow-tubular reactor is given in Figure 10.1.

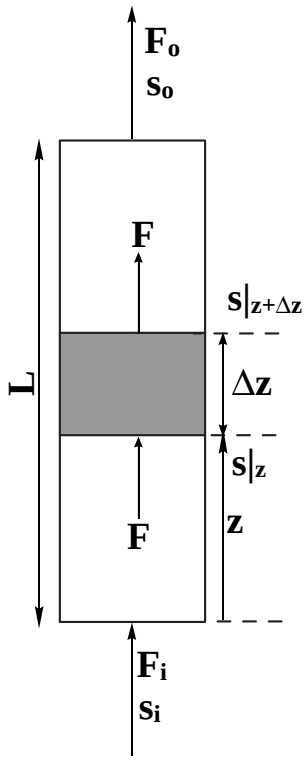

Figure 10.1: Flow diagram of a continuous plug-flow-tubular reactor.

- liquid in PFTR flows with constant velocity, thus identical residence time in reactor
- during a flow and reaction, concentration gradients for substrate and product will develop in the direction of flow

Consider an enzyme reaction using a PFTR:

- consider a small section of the reactor of length Δz as shown in Figure 10.1
- this section is of length z from the feeding point.

- balancing the above component from z to $z + \Delta z$ (infinitely small area) gives;

$$\frac{d(sV_R)}{dt} = Fs|_z - Fs|_{z+\Delta z} - r_e V_R$$
$$V_R \frac{ds}{dt} = Fs|_z - Fs|_{z+\Delta z} - \left(\frac{V_{max} s}{K_m + s}\right) V_R \tag{10.29}$$

where the rate of enzyme reaction is given by the Michaelis-Menten model of the form

$$r_e = \frac{V_{max} s}{K_m + s}$$

but $V_R = A\Delta z$; thus,

$$\frac{ds}{dt} = \frac{F(s|_z - s|_{z+\Delta z})}{A\Delta z} - \left(\frac{V_{max} s}{K_m + s}\right) \tag{10.30}$$

at steady-state condition;

$$\frac{F(s|_{z+\Delta z} - s|_z)}{A\Delta z} = -\frac{V_{max} s}{K_m + s}$$
$$\frac{F}{A\Delta z}(s|_{z+\Delta z} - s|_z) = -\frac{V_{max} s}{K_m + s} \tag{10.31}$$

and $\frac{F}{A}$ is the superficial velocity, u through the column;

$$u\left(\frac{s|_{z+\Delta z} - s|_z}{\Delta z}\right) = -\frac{V_{max} s}{K_m + s} \tag{10.32}$$

taking limit when $\Delta z \to 0$ on the left hand side of equation (10.32) leads to;

$$\lim_{\Delta z \to 0} \frac{s|_{z+\Delta z} - s|_z}{\Delta z} = \frac{ds}{dz}$$

and (10.32) becomes;

$$u\frac{ds}{dz} = -\frac{V_{max} s}{K_m + s} \tag{10.33}$$

- integration of equation (10.33) with boundary conditions $s = s_i$ at $z = 0$ gives;

$$L = \frac{u}{V_{max}}\left(K_m \ln \frac{s_i}{s_o} + s_i - s_o\right) \tag{10.34}$$

- rearranging equation (10.34) leads to the residence time of the fluid in a particular PFTR;

$$\tau = \frac{L}{u} = \frac{1}{V_{max}}\left(K_m \ln \frac{s_i}{s_o} + s_i - s_o\right) \tag{10.35}$$

Equation (10.34) would give the reactor length, while equation (10.35) gives the residence time required to achieve conversion of substrate from s_i to s_o at flowrate, u.

The system is however impractical for use with enzymes unless they are immobilised and retained inside a vessel. For immobilised enzyme, reactions are obviously affected by diffusion and equation (10.33) should include a mass transfer effect, η_T;

$$u\frac{ds}{dz} = -\eta_T \frac{V_{max} s}{K_m + s} \tag{10.36}$$

where η_T is defined by;

$$\frac{\text{actual reaction rate with diffusion}}{\text{reaction rate without diffusion}}$$

This form of equation cannot be integrated explicitly since the term η_T is a function of s and s also varies with the distance z in a plug-flow tubular reactors.

Immobilised enzymes

- This is one type of heterogeneous reaction which considers **only** the concentration gradient. Temperature effect in for biological based reactions is relatively low and the effect can be generally neglected.
- Reactions normally involve solid-phase catalysts that consist of macroscopic flocs, clumps and pellets.
- If cells or enzymes do not produce such clumps of solid surfaces, they can be induced to do so using immobilisation technique.
- Immobilisation can be done in various methods such as;
  - entrapment within gels (agarose, alginate and carrageenan)
  - porous solid material (ceramics, porous glass and resin beads)
- In both methods above, site of reaction are distributed throughput the particles–catalyst particle of higher activity can be formed by increasing the loading of cells or enzyme per volume of matrix.
- Advantages:
  - continuous operation with the same material (recycle of catalyst)
  - enhance stability of certain enzymes by increasing their half-life

For a spherical catalyst of radius R, immersed in a well-mixed liquid with reactant/substrate S, the concentration gradient is given in Figure (10.2) below.

- in a bulk liquid away from the particle, the substrate concentration is uniform with, $C_{S,b}$
- towards the centre of the particle, substrate is consumed by reaction with C_S and decreases within the particle–for uniform distribution of enzyme/cells throughout the particle, the profile would be symmetrical with a minimum at the centre.
- *external mass transfer*: transfer of liquid from the bulk concentration to the surface of the sphere–therefore, create a gradient at the surface of the particle. (Specified by (1) in Figure (10.2))
- *internal mass transfer*: transfer of liquid from the surface of the particle towards the centre of the sphere. (Specified by (2) in Figure (10.2))

Because concentrations vary in solid catalysts, local rates of reaction also vary depending on position within the particle:

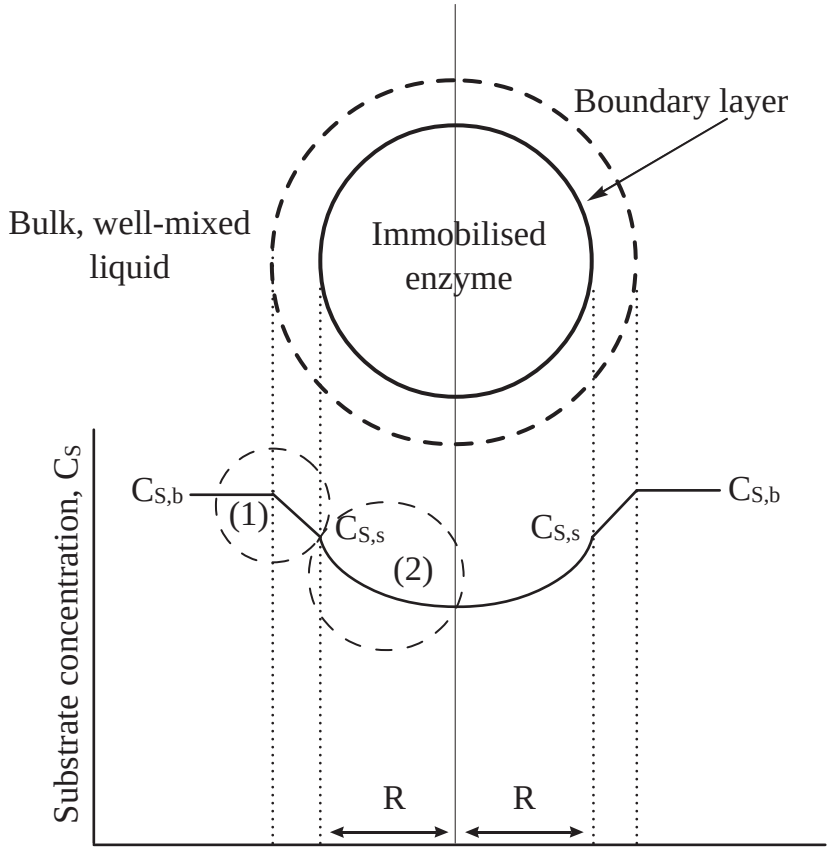

Figure 10.2: Substrate concentration profile for an immobilised-enzyme particle.

- for *first-order* reactions, reaction rate changes with position if substrate is depleted.
- each enzyme/cell responds to substrate concentration at its location with rate of reaction determined by the kinetic parameters of the catalyst. This is known as *intrinsic rate* of reaction.
- the actual (true) rate of reaction is difficult to measure in solid catalyst without altering the reaction conditions–but the overall reaction rate for the entire catalyst can be measured.
- in a closed system, rate of disappearance of substrate from bulk liquid **must** equal the overall rate of conversion, this is known as the *observed rate*.

Mass balance for a heterogeneous reaction is based on a number of assumptions:

- particle is isothermal
- mass transfer occurs by diffusion only
- diffusion can be described using *Fick's Law* with constant effective diffusivity
- particle is homogenous
- substrate partition coefficient is unity
- particle is at steady-state
- substrate concentration varies with a single spatial variable

For a **shell (particle is not a solid sphere)** mass balance of substrate, C_S, the equation is given by;

$$\frac{\partial(C_S V_p)}{\partial t} = A_r J_r|_r - A_r J_r|_{r+\Delta r} - r_{enz} V_p \tag{10.37}$$

From *Fick's Law*;

$$J_r = -\mathscr{D}_{s,eff} \frac{dC_S}{dr}$$

where $\mathscr{D}_{s,eff}$ is the effective diffusivity. Substitution into equation (10.37) with LHS expansion gives;

$$V_p \frac{\partial C_S}{\partial t} = -A_r \mathscr{D}_{s,eff} \frac{dC_S}{dr}\bigg|_r + A_r \mathscr{D}_{s,eff} \frac{dC_S}{dr}\bigg|_{r+\Delta r} - r_{enz} V_p \tag{10.38}$$

rearranging gives;

$$V_p \frac{\partial C_S}{\partial t} = A_r \left(\mathscr{D}_{s,eff} \frac{dC_S}{dr}\bigg|_{r+\Delta r} - \mathscr{D}_{s,eff} \frac{dC_S}{dr}\bigg|_r \right) - r_{enz} V_p \tag{10.39}$$

For shell type structure;

$$V_p = 4\pi r^2 \Delta r \quad \text{(for spherical shell volume)}$$

and

$$A_r = 4\pi r^2$$

Therefore, equation (10.39) expands into;

$$4\pi r^2 \Delta r \frac{\partial C_S}{\partial t} = 4\pi r^2 \left(\mathscr{D}_{s,eff} \frac{dC_S}{dr}\bigg|_{r+\Delta r} - \mathscr{D}_{s,eff} \frac{dC_S}{dr}\bigg|_r \right) - r_{enz} 4\pi r^2 \Delta r \tag{10.40}$$

with further simplification;

$$r^2 \frac{\partial C_S}{\partial t} = \frac{\left(\mathscr{D}_{s,eff} \frac{dC_S}{dr} r^2 \Big|_{r+\Delta r} - \mathscr{D}_{s,eff} \frac{dC_S}{dr} r^2 \Big|_r \right)}{\Delta r} - r_{enz} r^2 \tag{10.41}$$

Taking limit for the middle terms

$$\lim_{\Delta r \to 0} \frac{\left(\mathscr{D}_{s,eff} \frac{dC_S}{dr} r^2 \Big|_{r+\Delta r} - \mathscr{D}_{s,eff} \frac{dC_S}{dr} r^2 \Big|_r \right)}{\Delta r} = \frac{\partial}{\partial r} \left(\mathscr{D}_{s,eff} \frac{dC_S}{dr} r^2 \right)$$

therefore equation (10.41) becomes;

$$r^2 \frac{\partial C_S}{\partial t} = \frac{\partial}{\partial r} \left(\mathscr{D}_{s,eff} \frac{dC_S}{dr} r^2 \right) - r_{enz} r^2 \tag{10.42}$$

expansion of the middle terms and rearranging leads to;

$$\frac{\partial C_S}{\partial t} = \frac{1}{r^2} \mathscr{D}_{s,eff} \left(r^2 \frac{\partial}{\partial r} \left(\frac{\partial C_S}{\partial r} \right) + 2r \frac{\partial C_S}{\partial r} \right) - r_{enz} \tag{10.43}$$

At steady-state condition;

$$\frac{\partial C_S}{\partial t} = 0$$

hence it reduces into an ordinary differential equation;

$$\frac{1}{r^2}\mathscr{D}_{s,eff}\left(r^2\frac{d}{dr}\left(\frac{dC_S}{dr}\right)+2r\frac{dC_S}{dr}\right)=r_{enz} \tag{10.44}$$

rearranging the above equation gives;

$$r^2\frac{d}{dr}\left(\frac{dC_S}{dr}\right)+2r\frac{dC_S}{dr}=\frac{r^2}{\mathscr{D}_{s,eff}}r_{enz} \tag{10.45}$$

Assuming that the reaction only follows the *first-order* rate equation, where;

$$r_{enz}=k_1C_S$$

therefore equation (10.45) can be integrated with boundary conditions of

$$C_S=C_{S,s} \qquad \text{at} \quad r=R$$

and

$$\frac{dC_S}{dr}=0 \qquad \text{at} \quad r=0$$

into the form given by (*integration can be easily done using the technique of Laplace Transform*);

$$C_S=C_{S,s}\frac{R}{r}\frac{\sinh\left(r\sqrt{\frac{k_1}{\mathscr{D}_{s,eff}}}\right)}{\sinh\left(R\sqrt{\frac{k_1}{\mathscr{D}_{s,eff}}}\right)} \tag{10.46}$$

or it can be written in the form of;

$$\frac{C_S}{C_{S,s}}=\frac{R}{r}\left(\frac{e^{r\sqrt{\frac{k_1}{\mathscr{D}_{s,eff}}}}-e^{-r\sqrt{\frac{k_1}{\mathscr{D}_{s,eff}}}}}{e^{R\sqrt{\frac{k_1}{\mathscr{D}_{s,eff}}}}-e^{-R\sqrt{\frac{k_1}{\mathscr{D}_{s,eff}}}}}\right)$$

For reactions following *zeroth-order* kinetics, where;

$$r_{enz}=k_0$$

and the substrate balance equation at steady-state is given by;

$$\mathscr{D}_{s,eff}\left[\frac{d}{dr}\left(\frac{dC_S}{dr}\right)r^2+2r\frac{dC_S}{dr}\right]-k_0r^2=0$$

where upon integration with boundary conditions;

$$C_S=C_{S,s} \qquad \text{at} \quad r=R$$

and

$$\frac{dC_S}{dr}=0 \qquad \text{at} \quad r=R_0$$

the final expression relating substrate concentration and the radius of sphere is given by;

$$\frac{C_S}{C_{S,s}}=1+\frac{1}{C_{S,s}}\frac{k_0R^2}{6\mathscr{D}_{s,eff}}\left[\left(\frac{r}{R}\right)^2-1+\frac{2R_0^3}{R^2}\left(\frac{1}{r}-\frac{1}{R}\right)\right]$$

- According to above equation, k_0 varies with cell or enzyme density in the catalyst (immobilised form).
- The rate of reaction is independent of substrate concentration.
- Therefore, substrate depletion should be accounted by assuming that the depletion occurs at radius R_0.
- The range of radius where the rate of reaction is zero due to substrate exhaustion is given between;

$$0 < r \leqslant R_0$$

- everywhere else inside the particle;

$$r > R_0$$

  the volumetric rate of reaction is constant and equal to k_0 irrespective of substrate concentration.

- it is impossible to determine C_S using the final expression above since R_0 is impossible to determine.
- assuming that the substrate concentration exists throughout the particle;

$$C_S > 0$$

  which make

$$R_0 = 0$$

  the expression then reduces into;

$$\frac{C_S}{C_{S,s}} = 1 + \frac{1}{C_{S,s}} \frac{k_0}{6\mathscr{D}_{s,eff}} (r^2 - R^2)$$

- to avoid the centre of particle from lack of substrate, calculating the the maximum radius of particle when substrate just depleted at the centre point, thus $C_S = 0$ when $r = 0$ leads to;

$$R_{max} = \sqrt{\frac{6\mathscr{D}_{s,eff} C_{S,s}}{k_0}}$$

Thiele Modulus and Effectiveness Factor

In order to view the definition of Thiele Modulus represented by Φ, using the previous equation (10.45):

$$r^2 \frac{d}{dr}\left(\frac{dC_S}{dr}\right) + 2r\frac{dC_S}{dr} = \frac{r^2}{\mathscr{D}_{s,eff}} r_{enz}$$

Dividing by r^2 and rearranging gives;

$$\frac{d^2C_S}{dr^2} + \frac{2}{r}\frac{dC_S}{dr} - \frac{r_{enz}}{\mathscr{D}_{s,eff}} = 0 \tag{10.47}$$

Consider a *first-order* reaction kinetics where;

$$r_{enz} = k_1 C_S$$

thus, (10.47) becomes;

$$\frac{d^2C_S}{dr^2} + \frac{2}{r}\frac{dC_S}{dr} - \frac{k_1 C_S}{\mathscr{D}_{s,eff}} = 0 \tag{10.48}$$

THIS PART WILL BE COVERED BY DR. MASHITAH MAT DON

EXAMPLES

QUESTION 1:

The isomerisation of 5×10^{-2}mol·dm^{-1} bulk concentration of glucose to fructose is conducted at 313K in a batch reactor using immobilised glucose isomerase. The reaction exhibits reversible Michaelis-Menten kinetics and is characterised by K_m value of 2×10^{-3} mol·dm^{-1}. The determined effectiveness factor η of 0.7 reveals an appreciable contribution of mass transport to the measured reaction rate. Calculate the substrate concentration at the solid-liquid interface under these conditions. (*Hint: The reaction occurs at the solid surface, therefore, neglect internal mass transfer*)

SOLUTION 1:

For a batch reaction, the effectiveness factor, η is given by;

$$\eta = \frac{\text{actual reaction rate with diffusion (observed reaction rate)}}{\text{reaction rate without diffusion}}$$

External mass transfer is modelled using flux equation;

$$N_s = k_s(C_{S,b} - C_S)$$

where

k_s = mass transfer coefficient

$C_{S,0}$ = substrate concentration at the bulk fluid

C_S = substrate concentration at the interface

Therefore, for a reaction occurring at the interface, the rate of substrate consumption is given by;

$$\frac{dC_S}{dt} = k_s(C_{S,b} - C_S) - r_{enz}$$

and r_{enz} is given by the Michaelis-Menten model;

$$\frac{V_{max}C_S}{K_m + C_S}$$

hence;

$$\frac{dC_S}{dt} = k_s(C_{S,b} - C_S) - \frac{V_{max}C_S}{K_m + C_S}$$

and at steady-state;

$$k_s(C_{S,b} - C_S) = \frac{V_{max}C_S}{K_m + C_S}$$

while the rate of reaction without mass transfer is given by;

$$\frac{dC_{S,b}}{dt} = \frac{V_{max}C_{S,b}}{K_m + C_{S,b}}$$

Since;

$$\eta = \frac{\text{observed reaction rate}}{\text{reaction rate without diffusion}}$$

thus;

$$\eta = \frac{\frac{V_{max}C_S}{K_m+C_S}}{\frac{V_{max}C_{S,b}}{K_m+C_{S,b}}}$$

Dividing both nominator and denominator by $C_{S,b}$ results in;

$$\eta = \frac{\frac{\frac{C_S}{C_{S,b}}}{\frac{K_m}{C_{S,b}}+\frac{C_S}{C_{S,b}}}}{\frac{1}{\frac{K_m}{C_{S,b}}+1}}$$

Given that;

$C_{S,b} = 5 \times 10^{-2}\text{mol}\cdot\text{dm}^{-1}$

$K_m = 2 \times 10^{-3}$ mol·dm^{-1}

$\eta = 0.7$

Substitute into η and let $\frac{C_S}{C_{S,b}} = x$ gives;

$$0.7 = \frac{\frac{x}{\frac{0.002}{0.05}+x}}{\frac{1}{\frac{0.002}{0.05}+1}}$$

$$x = 0.8235$$

Since $x = \frac{C_S}{C_{S,b}}$ thus;

$$C_S = 0.8235 \times 5 \times 10^{-2}$$
$$= 0.00412\text{mol} \cdot \text{dm}^{-1}$$

QUESTION 2:

During the growth of *E. coli* in a batch reactor, the pattern can be modelled using the Monod expression of the form;

$$\mu = \frac{\mu_{max}S}{K_s + S}$$

where;

μ = specific growth rate

μ_{max} = maximum specific growth rate

K_s = Monod constant

S = substrate concentration

For the above process, show how the time required to reach the maximum population of cells can be estimated.

If the concentration of cells at the start of the exponential growth is 0.08g/L and the essential substrate concentration is 36g/L. Find the time where the maximum population of cells is reached given that $\mu_{max} = 0.55\text{h}^{-1}$, $K_s = 1.2$g/L and $k = 1.4\text{g}_{substrate}/(\text{g}_{cells}\cdot\text{h})$

SOLUTION 2:

Substrate consumption during the growth can be described by;

$$\frac{dS}{dt} = -kX$$

and the cell growth is given by;

$$\frac{dX}{dt} = \mu X$$

where

$$X = \frac{1}{\mu}\frac{dX}{dt}$$

Substitute X into $\frac{dS}{dt}$ and integrating gives;

$$\frac{dS}{dt} = -k\left(\frac{1}{\mu}\frac{dX}{dt}\right)$$

$$\int dS = -\frac{k}{\mu}\int dX$$

But

$$\mu = \frac{\mu_{max}S}{K_s + S}$$

hence;

$$\int dS = -\frac{k}{\frac{\mu_{max}S}{K_s+S}}\int dX$$

rearranging gives;

$$\int_{S_0}^{0} \frac{S}{K_s + S} dS = -\frac{k}{\mu_{max}}\int_{X_0}^{X_S} dX$$

Integrating both sides leads to;

$$X_S = X_0 + \frac{\mu_{max}}{k}\left(S_0 + K_s \ln\frac{K_s}{K_s + S_0}\right)$$

The average μ can is determined by dividing the specific growth rate with the total substrate in the reactor, which gives;

$$\mu_{avg} = \frac{\int_{S_0}^{0} \mu\, dS}{\int_{S_0}^{0} dS}$$

$$\mu_{avg} = \frac{\mu_{max}\int_{S_0}^{0} \frac{S}{K_s+S} dS}{\int_{S_0}^{0} dS}$$

$$\mu_{avg} = \frac{\mu_{max}\left[S_0 + K_s \ln\frac{K_s}{K_s+S_0}\right]}{S_0}$$

Therefore, the new cell growth expression should be in the form of;

$$\frac{dX}{dt} = \mu_{avg} X$$

and integrating,

$$\int_0^t dt = \frac{1}{\mu_{avg}}\int_{X_0}^{X_S} \frac{1}{X} dX$$

gives;

$$t = \frac{1}{\mu_{avg}} \ln\frac{X_S}{X_0}$$

substituting μ_{avg} previously lead to;

$$t = \frac{S_0}{\mu_{max}\left[X_0 + \frac{\mu_{max}}{k}\left(S_0 + K_s \ln \frac{K_s}{K_s+S_0}\right)\right]} \ln \frac{X_S}{X_0}$$

Consider the amount of cell produced at the given substrate concentration:
Using;

$$X_S = X_0 + \frac{\mu_{max}}{k}\left(S_0 + K_s \ln \frac{K_s}{K_s + S_0}\right)$$

$$X_S = 0.08 + \frac{0.55}{1.4}\left(36 + 1.2 \ln \frac{1.2}{36 + 1.2}\right)$$

$$X_S = 12.604$$

and using the expression for μ_{avg};

$$\mu_{avg} = \frac{0.55\left[36 + 1.2 \ln \frac{1.2}{36+1.2}\right]}{36}$$

$$\mu_{avg} = 0.487$$

thus, the time taken for the cell population to reach a maximum value;

$$t = \frac{1}{\mu_{avg}} \ln \frac{X_S}{X_0}$$

$$t = \frac{1}{0.487} \ln \frac{12.604}{0.08}$$

$$t = 10.34\text{h}$$

QUESTION 3:

Enzyme is immobilised in 8 mm diameter agarose beads at a concentration of 0.018 kg protein per metre cube gel. Ten beads are immersed in a well-mixed solution containing 3.2×10^{-3} kg m^{-3} substrate. The effective diffusivity of substrate in agarose gel is 2.1×10^{-9} m^2 s^{-1}. Kinetics of the enzyme can be approximated as first order with specific rate constant 3.11×10^5 s^{-1} kg$^{-1}_{protein}$. Mass transfer effects outside the particles are negligible. Plot the steady-state substrate concentration profile as a function of particle radius.

SOLUTION 3:

Given that
The bead diameter, $D = 8$ mm
Therefore, $R = 4\times10^{-3}$ m
Effective diffusivity of substrate, $\mathscr{D}_{s,eff} = 2.1\times10^{-9}$ m^2 s^{-1}
Concentration of substrate at the surface of beads, $C_{S,s} = 3.2\times10^{-3}$ kg m^{-3}

Calculating the volume of each bead;
Volume of solid sphere,

$$V_b = \frac{4}{3}\pi R^3$$

$$V_b = \frac{4}{3}\pi(4 \times 10^{-3})^3$$

$$V_b = 2.68 \times 10^{-7}\text{m}^3$$

Therefore, for ten beads within the vessel, gives, $V_{b,T} = 2.68 \times 10^{-6}\text{m}^3$

And the amount of enzyme immobilised with the beads $= 2.68\times10^{-6}\times0.018 = 4.83\times10^{-8}$kg

The specific rate constant $k_1 = 3.11\times10^5$ s^{-1} kg$^{-1}_{protein}$, therefore, for 4.83×10^{-8}kg of protein;

$$k_1 = 0.015\,\text{s}^{-1}$$

Using equation describing the substrate concentration at different radius of bead;

$$C_S = C_{S,s}\frac{R}{r}\frac{\sinh\left(r\sqrt{\frac{k_1}{\mathscr{D}_{s,eff}}}\right)}{\sinh\left(R\sqrt{\frac{k_1}{\mathscr{D}_{s,eff}}}\right)}$$

calculating

$$R\sqrt{\frac{k_1}{\mathscr{D}_{s,eff}}} = 4 \times 10^{-3}\sqrt{\frac{0.015}{2.1 \times 10^{-9}}} = 10.69$$

thus,

$$\sinh(10.69) = 2.20 \times 10^4$$

Substitute values in the main equation for C_S and calculate C_S at different values of r leads to a graph given below:

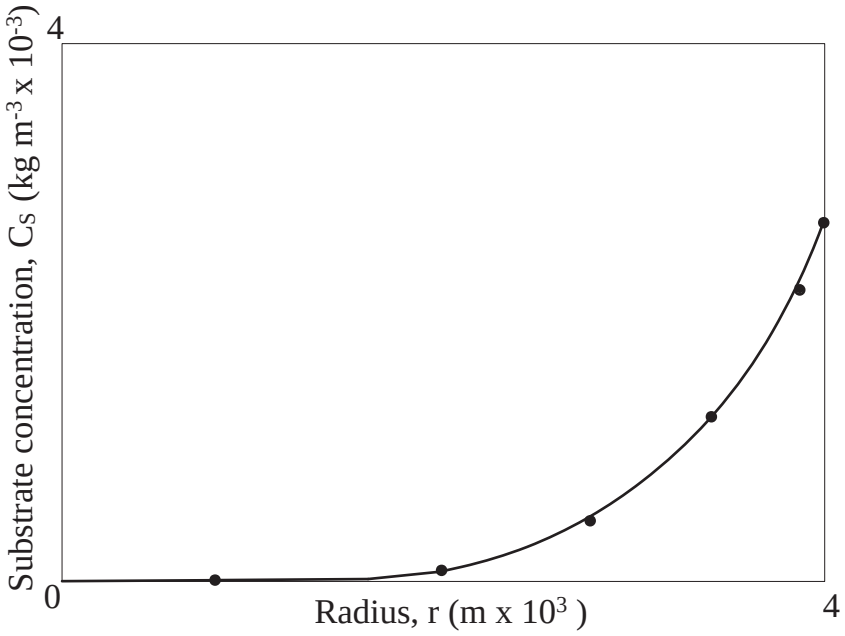

Figure 10.3: Substrate concentration profile.

QUESTION 4:

Immobilised lactase in used to hydrolyse lactose in dairy waste to glucose and galactose. Enzyme is immobilised in resin particles and packed into a 0.5 m^3 column. The total

effectiveness factor for the system is close to unity. K_m for the immobilised enzyme is 1.32 kg·m^{-3} V_{max} is 45 kg·m^{-3}h^{-1}. The lactose concentration in the feed stream is 9.5 kg·m^{-3}, a substrate conversion of 98 % is required. The column is operated with plug flow for a total of 310 day per year.

1. At what flowrate should the reactor be operated?
2. How many tonnes of glucose are produced a year?

SOLUTION 4:

1. For 98% substrate conversion;

$$\frac{S_o}{S_i} = 0.02$$
$$S_o = 0.02 \times 9.5$$
$$S_o = 0.19\text{kg} \cdot \text{m}^{-3}$$

(*For S_o and S_i, refer to the plug-flow-tubular reactor diagram in the notes*)

Since the flowrate is given by;

$$F = \frac{V}{\tau}$$

where τ is given by;

$$\tau = \frac{K_m}{V_{max}} \ln \frac{S_i}{S_o} + \frac{S_i - S_o}{V_{max}}$$

Substitute all values into the above equation gives;

$$\tau = 0.32\text{h}$$

Therefore the flowrate the reactor should operate;

$$F = \frac{0.5}{0.32} = 1.56\text{m}^3\text{h}^{-1}$$

2. The rate of conversion of substrate into product if given by;

$$F(S_i - S_o)$$

thus

$$\text{Rate} = 1.56(9.5 - 0.19) = 14.5\text{kg} \cdot \text{h}^{-1}$$

For mole yearly basis, this converts into 315 kmol y^{-1} (*Use molecular weight of lactose as 342*). The hydrolysis process in governed by the chemical equation;

$$\text{LACTOSE} + \text{H}_2\text{O} \rightarrow \text{GLUCOSE} + \text{GALACTOSE}$$

It is obvious that 1 mol of lactose produces 1 mol of glucose, therefore, 315 kmol of lactose will give 315 kmol of glucose per year and in terms of tonnes (*Molecular weight of glucose is 180*);

$$\text{Glucose produced} = 56.7\text{tonnes} \cdot \text{y}^{-1}$$

Chapter 11

Sterilisation

11.1 Introduction

Sterilisation refers to any process that effectively kills or eliminates transmissible agents such as fungi, bacteria, viruses, prions and spores forms from a surface, equipment, foods, medications or biological culture medium. Sterilisation can be achieved through application of heat, chemicals, irradiation, high pressure or filtration. There are two types of sterilisations:

1. physical sterilisation
    - heat sterilisation
    - radiation sterilisation
2. chemical sterilisation
    - ethylene oxide
    - ozone
    - chlorine bleach
    - glutaraldehyde
    - formaldehyde
    - hydrogen peroxide
    - peracetic acid
    - prions

11.1.1 Applications

Foods

The first application of sterilisation was through a thorough cooking to affect the partial heat sterilisation of foods and water. Cultures that practice heat sterilisation of food and water have longer life expectancy and lower rates of disability. Canning of foods by heat sterilisation was an extension of the same principle. Ingestion of contaminated food and water remains a leading cause of illness and death in the developing world, particularly for children.

Medicine and Surgery

Generally, surgical instruments and medications that enter an already sterile part of the body such as blood or beneath the skin must have a high sterility assurance level. Examples of such instruments include scalpels, hypodermic needles and artificial pacemakers. This is also essential in the manufacture of parenteral pharmaceuticals.

Heat sterilisation of medical instruments is known to have been used in ancient Rome, but mostly disappeared throughout the Middle ages resulting in significant increases in disability and death following surgical procedures.

Preparation of injectable medications and intravenous solutions for fluid replacement therapy requires not only a high sterility assurance level, but well-designed containers to prevent entry of adventitious agents after initial sterilisation.

11.1.2 Heat Sterilisation

Steam sterilisation

A widely used method for heat sterilisation is the autoclave. Autoclaves commonly use steam heated to 121°C or 134°C. To achieve sterility, a holding time of at least 15 minutes at 121°C or 3 minutes at 134°C is required. As items such as liquids and instruments packed in layers of cloth may take longer to reach the required temperature than the steam solid instruments additional sterilising time is usually required. After sterilisation, autoclaved liquids must be cooled slowly to avoid boiling over when the pressure is released.

Proper autoclave treatment will inactivate all fungi, bacteria, viruses and also bacterial spores, which can be quite resistant. It will not necessarily eliminate all prions.

For prion elimination, various recommendations state 121-132°C (270°F) for 60 minutes or 134°C (273°F) for at least 18 minutes. The prion that causes the disease

- Introduction to sterilisation
- High temperature sterilisation
- Different types of sterilisation techniques
    1. Inoculation table
    2. Media for stock cultures
    3. Microbiological media
    4. Glass wears/petri dish
- Dry heat sterilisation
- Sterilisation with filtration
- Electron beam sterilisation
- Chemical sterilisation
- Batch and Continuous sterilisations

Chapter 12

Bioreactor Design

- Bioreactor types
- Reactor concepts
- Designing a biocatalyst reactors
    1. Introduction
    2. Batch bioreactor
    3. Continuous-flow stirred-tank reactor (CSTR)
    4. Cascade CSTR
    5. Plug-flow bioreactor
    6. Comparison of enzyme reactors
    7. Design of real bioreaectors

Chapter 13

Downstream Processing in Biochemical Engineering

13.1 Introduction to downstream processing

- Downstream processing refers to separation and purification processes of fermentation/enzyme reaction into desired products.
- It accounts for up to 60% of the total production costs, excluding of the purchased raw materials.
- Fermentation can be cells themselves, components within fermentation broth or those trapped in cells.
- This is summarised in the table below:

Types	Products	Concentrations (g/l)
Cell itself	Baker's yeast, single cell protein	30
Extracellular	Alcohols, organic acids,amino acids	100
	enzymes, antibiotics	20
Intracellular	Recombinant DNA proteins	10

Table 13.1: Examples of bioprocessing products and their typical concentrations.

- Normally in bioprocessing, the product of interest is the cell itself.
- Cells are separated from the fermentation broth, then washed and dried.
- For extracellular products, when cells are separated, the products in the dilute aqueous medium required to be recovered and purified.
- For intracellular products, they can be released by rupturing the cells and then recovered and purified.
- For enzyme reactions, the procedures are the same as that of the extracellular products.
- This is simply shown in the diagram below:

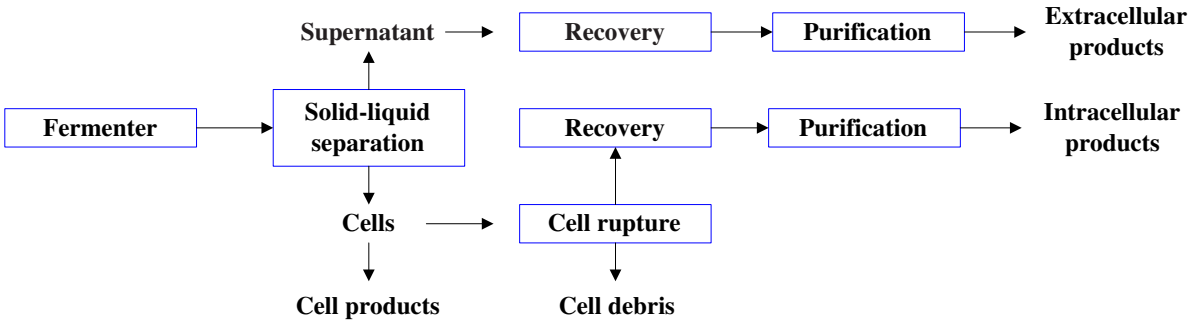

Figure 13.1: Major process steps in downstream processing.

- Unique characteristics of bioseparation products include:
  1. products are in dilute concentration in an aqueous medium
  2. products are usually temperature sensitive
  3. variety of products to be separated
  4. products can be intracellular, often insoluble inclusion bodies
  5. physical and chemical properties of products are similar to contaminants
  6. high purity and homogeneity may be needed for human health care.

13.2 Solid-Liquid Separation

- First procedure/step in downstream processing
- Solid particles to be separated consist of cellular mass;
  - with specific gravity between 1.05–1.1 (not much greater than the broth)
  - shape may be spheres, ellipsoid, rods, filaments or flocculent.
  - typical sizes of cells vary widely:
    1. bacterial cells (0.5–1μm)
    2. yeast cells (1–7μm)
    3. fungal hyphae (5–15μm) in diameter and (50–500μm) in length
    4. suspension animal cells (10–20μm)
    5. suspension plant cells (20–40μm)
  - there are 2 techniques for solid-liquid separation:
    1. filtration/ultrafiltration
    2. centrifugation

13.2.1 Filtration/Ultrafiltration

- It is a technique where a mixture of solid and liquid is separated by forcing through a filtering medium on which solids are deposited.
- Categories of filtration depend on:
  1. filtering medium used

2. range of particle sizes removed
3. pressure differences
4. principle of filtration: conventional filtration, microfiltration, ultrafiltration, reverse osmosis.

- For a **conventional filtration**:
  - size of particles: $d_p = 10\mu$m
  - effective for dilute suspension, large and rigid particles
  - 2 types of filters used:
    1. pressure
    2. vacuum
  - for cell recovery:
    1. filter press: normally used for small scale separation of bacteria and fungi from broth
    2. rotary drum filter: used for large-scale separation, common filter medium includes canvas, wool, synthetic fabrics, metal or fibre glass

- A number of factors will influence the choice of the most suitable filtration technique/equipment and they include;
  1. properties of filtrate–viscosity and density
  2. nature of solid particles–size, shape, size distribution and packing characteristics
  3. solid:liquid ratio
  4. need for recovery of the solid/liquid fraction or both
  5. scale of operation
  6. need for batch/continuous operation
  7. need for aseptic conditions
  8. need for pressure/vacuum suction to ensure an adequate flow rate of the liquid

- Assuming a laminar flow across a filter, the rate of filtration can be expressed as a function of pressure drop;

$$\frac{1}{A}\frac{dV_f}{dt} = \frac{\Delta p}{\mu(L/k)} \tag{13.1}$$

where:

A = area of filtering surface

k = D'Arcy's filter cake permeability

L = thickness of filter cake

$\frac{dV_f}{dt}$ = rate of filtration

Δp = pressure drop

- The rate of filtration is proportional to the pressure drop and inversely proportional to the filtration resistance where;

$$\frac{L}{k} = R_M + R_C$$

where;

R_M = resistance due to filter medium

R_C = resistance due to the cake

- R_M is relatively small compared to R_C
- R_C is proportional to the filtrate volume;

$$\frac{L}{k} \approx R_C = \frac{\alpha \rho_c V_f}{A} \tag{13.2}$$

where;

α = specific cake resistance

ρ_c = mass of cake solids per unit volume of filtrate

- Substituting equation (13.2) into (13.1) and integrating leads to the time taken when the filtration rate is constant at a certain pressure drop, Δp.

$$t = \frac{\mu \alpha \rho_c}{2\Delta p} \left(\frac{V_f}{A} \right)^2 \tag{13.3}$$

1. the resultant equation clearly shows that the higher the viscosity of a solution, the longer it takes to filter a given amount of solution.
2. an increase of the cake compressibility increases the filtration resistance α, thus increases the difficulty of the filtration.
3. for solutions of high viscosity (non-Newtonian liquid) and highly compressible filter cakes, pre-treatment is required;
   - heating to denature existing proteins
   - adding electrolytes to promote coagulation and flocculation
   - adding *filter aids* to increase porosity and reduce compressibility of cakes

- Filter aids:
  - common practice especially when filtering bacteria or gelatinous suspensions
  - *Kieselguhr*–diatomaceous earth is widely used
  - it contains voidage ≈ 0.85–improve the porosity of filter cake and thus gives a faster flow rate upon mixing with the initial flow rate
  - amount of filter aid to be applied should be determined experimentally
  - methods of applying:
    - thin layer of slurry of *Kieselguhr* is applied to the filter to form a precoat prior to broth filtration

Figure 13.2: Flush plate and frame filter assembly. The cloth is shown away from the plates to indicate flow of filtrate in the grooves between pyramids.

    - ∗ appropriate quantity of filter aid is mixed with the harvested broth and filtration is started to build up a satisfactory filter bed
    - ∗ initial raffinate is returned to the remaining broth prior to starting the true filtration
    - ∗ for vacuum drum filters with advancing filter blades, initial built up of thick precoat filter occurs

- Batch filters:

    1. Plate-frame filters
        - consist of plates and frames arranged alternately
        - it is a pressure-type filter
        - the diagram is shown below
        - plates are covered with filter cloths/filter pads
        - slurry is fed to the filter press through continuous channel formed by the holes in the corners of the plates and frames
        - the filtrate runs down grooves in the filter plates and discharged through outlet taps to a channel.
    2. Pressure leaf filters
        (a) Vertical metal-leaf filters:
            - consist of number of vertical porous metal leaves
            - solid from slurry gradually build upon the surface of the leaves and filtrate is removed from the plates via horizontal hollow shaft (can be rotated during filtration)
            - solids are removed at the end of a cycle by blowing air through the shaft and into filter leaves

        (b) Horizontal metal-leaf filters

Figure 13.3: Staked-disc filter: Metafilter pack.

- metal leaves are mounted on a vertical hollow shaft within pressure vessel
- only the upper surfaces of the leaves are porous
- filtration continues until the cake fills the space between disc-shape leaves OR when operational pressure becomes excessive
- solid cake can be discharged by releasing the pressure and spinning the shaft with a drive motor

(c) Staked-disc filters

- consist of a number of precision-made rings, stacked on a flutted rod
- rings are normally made from stainless steel
- assembled staked are placed in a pressure vessel which can be sterilised if necessary
- the packs are normally coated with a thin layer of *Kieselguhr* which acts as a filter aid
- during operation; filtrate passes between discs and it is removed in the grooves of the fluted rods
- solids are deposited on the filter coating
- for cleaning, solids are removed from the rings by applying back pressure via the fluted rods
- this type of filter is commonly used for polishing liquids such as beer

- Continuous filters

1. Rotary vacuum filters
   - commonly used by large industries which need continuous liquid processing
   - it consists of: rotating, hollow, segmented drum covered with a fabric/metal filter which is partially immersed in a trough containing the broth to be filtered.

Figure 13.4: Rotary vacuum drum filter showing a string discharge filter operation. Section 1 to 4 are filtering, section 5 to 12 are de-watering and section 13 is discharging the cake with the string discharge. Section 14, 15 and 16 are ready to start a new cycle. A, B and C represent dividing members in the annular ring [Adapted from Principles of Fermentation Technology, 1984].

- interior of the drum is divided into a series of compartments to which the vacuum is normally applied as the drum slowly revolves ($\approx$ 1 rpm)
- just before filter cake is discharged, air pressure may be applied to help ease the filter cake off the drum
- there are a number of filter cakes manufactured;
  (a) string discharge
    * normally used if fibrous filter cake is produced by fungal mycelia
    * the cake can be easily separated with string discharge
    * long lengths of string 1.5m apart are threaded over the drum and round 2 rollers
    * the cake is lifted free from the upper part of the drum when the vacuum pressure is released and carried to the small rollers where it falls free
  (b) scraper discharge
    * normally used for collecting yeast cells
    * filter cake is removed by an accurately positioned knife blade
    * since knife is close to drum, there may be gradual wearing of the filter cloth on the drum
  (c) scraper discharge with precoating of the drum
    * filter drum is coated with filter aid, 2 to 10 cm thick (to avoid blockage of filter cloth)
    * built up cake on the drum is cut away by the knife blade
    * cake is removed together with a thin layer of filter coat

Figure 13.5: Cake discharge on a drum filter using strings.

Figure 13.6: Cake discharge on a drum filter using a scraper.

Figure 13.7: Cake discharge on a precoated drum filter.

13.2.2 Centrifugation

- Alternative method when filtration is ineffective (in the case of small particles)
- Require more expensive equipment and cannot be scaled to the same capacity as filtration equipment
- Some centrifuges can be used for separating 2 immiscibly liquids and breaking emulsions
- Two basic types of centrifuges (large-scale):
  1. tubular centrifuge
  2. disk centrifuge
- Tubular centrifuge:
  - hollow cylinder rotating element in a stationary casing
  - suspension usually fed through bottom and clarified liquid is removed from top leaving solid deposit on the bowl's wall
  - accumulated solids are recovered manually from bowl
  - for a typical tubular centrifuge: bowl size: 2 to 5 in diameter, 9 to 30 in. height, max. speed 15,000 to 50,000 rpm
- Disk centrifuge:
  - often used for bioseparation
  - operate continuously
  - consists of a short, wide bowl (8 to 20 in.) diameter which turns on a vertical axis

Figure 13.8: Two basic types of centrifuges (a) tubular and (b) disk.

  - closely spaced cone-shaped disc in the bowl decrease the distance that a suspended particle has to be moved to be captured on the surface and increases the collection efficiencies
  - feed enters the bowl at the bottom, flows into channels and upward past the disks
  - solid particles are thrown outward and the clear liquid flows toward the centre of the bowl and discharge through annular slit
  - slit can be removed intermittently/continuously

- Theory of centrifugation:
  - based on Stoke's Law
  - the rate of sedimentation of spherical particles suspended in a fluid of Newtonian viscosity characteristics is proportional to the square of the diameter of the particles:

$$v = \frac{d^2\omega^2 r(P_p - P_i)}{18\mu} \tag{13.4}$$

  - the equation is only applicable when $Re < 1.0$ or

$$\frac{dv\rho}{\mu} < 1.0$$

    which is always the case for biological solutes
  - during the centrifugation process, the acceleration provided by the centrifugal force

$$a = \omega^2 r$$

    increases the rate of settling process

13.3 Liquid-liquid Extraction

13.3.1 Introduction

- It is a unit operation based on differential solubility of a consolute in two immiscible solvents.
- It is also known as solvent extraction.
- The application is rather popular due to its inherent flexibility and its suitability for processing heat sensitive-products.
- The operation is highly dependence on the the solubility of the chemical used that leads to the strengths and limitation of the operation.
- Possible choices of solvents results in the unmatched versatility of extraction as a mass transfer operation.
- Characteristics of the operation of particular relevance to bioseparations are the effectiveness of extraction at low temperatures and the tendency of organic solvents to denature most proteins irreversibly.
- Extraction in the pharmaceutical industry has been used primarily in the isolation of antibiotics from fermenter broth, in the preparation of natural and synthetic vitamins, and the preparation of drugs from naturally occurring materials.
- Contrary to extraction, applications in most other industries, very dilute aqueous solutions are usually fed into the process.
- Extraction may be carried out in batch or staged-batch sequence such in mixer-settler unit, however, most pharmaceutical extractions have been carried out in differential units.
- Centrifugal extractors, in particular of which the Podbielniak extractor is perhaps the best known example have been related intimately to the development of commercial antibiotic production processes.
- Fractional extraction processes involve two or more consolute components and are designed to enrich the extract in the desired product component and to isolate the product from the aqueous feed solution.
- Conventional liquid-liquid extraction is not normally useful for the isolation of protein products because of the irreversible denaturation of these macromolecules that typically occurs in organic solvents.
- Immiscible aqueous-phase solutions can be prepared by the addition of polymers or polymers with an addition of salts to water.

13.3.2 Fundamental of Liquid-Liquid Extraction

- When a given solute is exposed to 2 different solvents, it will be more soluble in one of them.

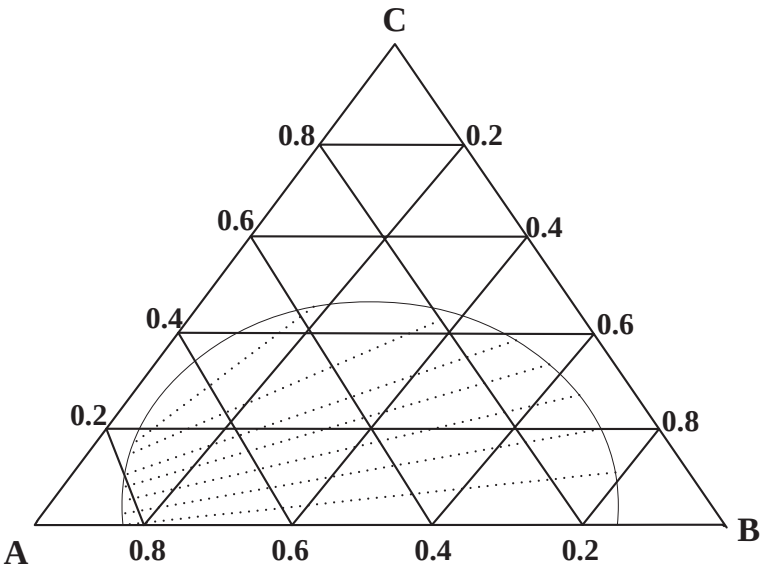

Figure 13.9: Phase envelope at a given temperature showing solubility data for a typical ternary two phase mixture.

- The unit operation of liquid-liquid extraction exploits this differential solubility of a consolute component in 2 immiscible, or partially immiscible solvents.
- A number of factors are considered in order to obtain an optimum extraction which include;
    1. thermodynamic equilibrium constraints
    2. rates of mass transfer from phase to phase
    3. hydrodynamics
    4. relative floe rate
    5. choice of solvent
    6. processing equipment
    7. economics of proposed process

Thermodynamic equilibrium constraints

- Thermodynamics actually sets the fundamental limitations of proposed extraction
- It is the quantitative measure of the relative solubilities of the consolute in the 2-liquid phase which is given by;

$$K = \frac{C_i}{C_j} \tag{13.5}$$

- In petrochemical/chemical extractions, the solubility data are typically represented in triangular coordinates at a given temperature.
- The compositions in the two phases are represented by the points on the phase envelope joined by the tie line passing through the feed composition.
- The above triangle representation is not typically useful for pharmaceutical extraction because of the extreme dilutions of the solutions.

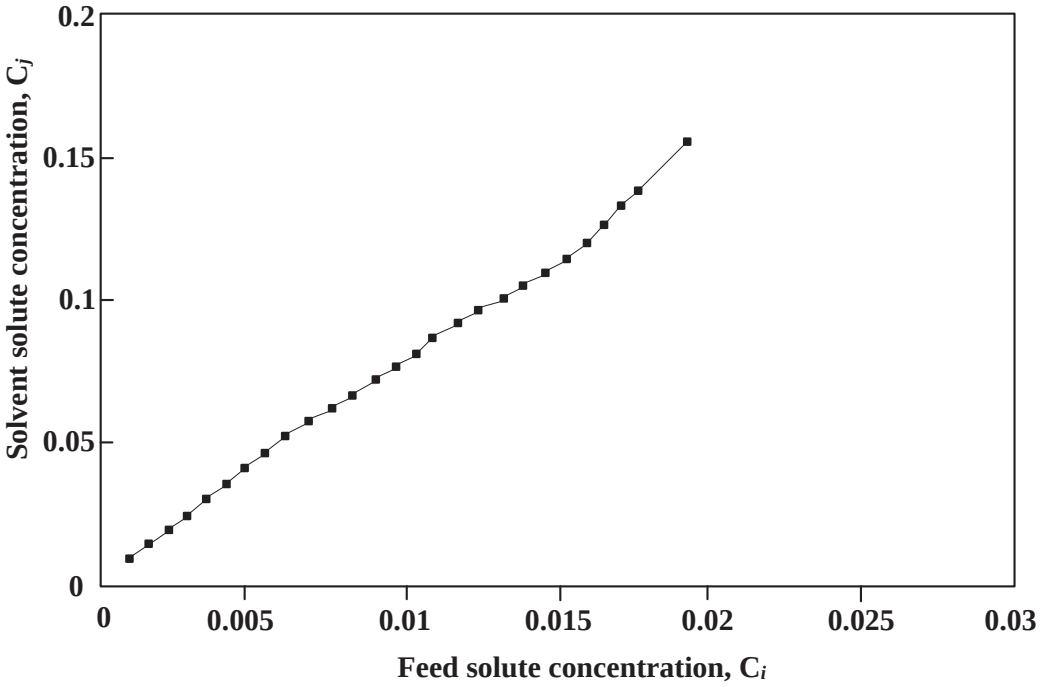

Figure 13.10: Equilibrium curve showing solute concentration in the solvent compared with solute concentration in the feed.

- Data are instead represented as plots of concentration, mole, or mass fraction in the phase versus that in the other phase.
- Partition coefficient of a component is a function of composition, temperature, pH and ionic strength.
- Manipulation of these variables can be used to separate the purified product from the extracting solvent, as well as to optimise the extraction process.
- In the case of of biological products, careful consideration must be given to product stability under the conditions of the extraction.

13.4 Cell Rupture/Disruption

- Disruption of cellular materials is usually difficult due to the strength of the cell walls and high osmotic pressure inside
- Techniques of rupturing cells have to be powerful and mild such that the desired components are not damaged
- Cells can be ruptured either mechanically or chemically (biologically)
- Mechanical methods:
    1. Liquid shear (homogenisation)
        - it is actually a positive displacement pump with an adjustable orifice valve
        - used normally for large scale cell disruption

- pump pressurises the cell suspension to approximately 400 to 500 bar then releases it through a spacial discharge valve–creating very high shear rates
- cooling to 4°C is necessary to compensate for heat generated during the adiabatic compression and homogenisation steps

2. Solid shear (pressure extrusion)
   - using frozen microorganisms at temperature of -25°C through small orifice
   - normally lab scale procedure
   - disruption is due to combination of liquid shear through narrow orifice with the presence of ice crystals
   - 90% cell disruption is able to achieved with a single passage of *S.cerevisiae* using a throughput of 10kg yeast cell paste per hour
   - ideal for microbial products which are very temperature labile
3. Agitation with abrasives
   -
4. Freeze-thawing
5. Ultra-sonicator

- Chemical (biological) methods:

1. Detergent
2. Osmotic shock
3. Alkali treatment
4. Enzyme treatment

13.5 Product Recovery

13.5.1 Extraction

13.5.2 Adsorption

13.6 Purification

13.6.1 Chromatography

- Separation of various compounds by the flow of a liquid in a porous, solid, sorptive medium.
- Separation occurs by the principle of different migration properties of solutes in a particulate adsorptive medium-components are separated in the form of distinct bands.
- The operation is time dependent-components are separated at increasing time interval of fluid flow.
- Separation is based on different adsorptive characteristics of solute materials on absorbent particles.

- Fluid flow carries the least adsorptive material to the far end of the column, and the most adsorptive material is retained on top of the column.
- Different bands are obtained at different locations of the column depending on the adsorptive characteristics of the solute compounds.
- The forces between solute molecules and adsorbent particles vary depending on the type of chromatography. The types of chromatographic methods are given below:

    1. **Adsorptive chromatography**: It is based on the adsorption of solute molecules onto solid particles, such as alumina and silica gel, by weak van der Waals forces and stearic interaction.
    2. **Liquid-liquid partition chromatography**: It is based on the different partition coefficients (solubility)of solute molecules between an adsorbed liquid phase and passing solution.
    3. **Ion-exchange chromatography**: It is based on the adsorption of ions (electrically charged compounds)on ions-exchange resin particles by electrostatic forces.
    4. **Gel filtration chromatography**: It is based on the penetration of solute molecules into small pores of packing particles on the basis of molecular size and shape of the solute molecules.
    5. **Affinity chromatography**: It is based on the specific chemical interaction between solute molecules and ligands (a functional molecule covalently linked to a support particle)bound on support particles. Ligand-solute interaction is very specific, such as enzyme-substrate interaction, which may depend on covalent, ionic forces or hydrogen-bond formation. Affinity binding may be molecular size and shape specific.
    6. **Hydrophobic chromatography**: It is based on hydrophobic interactions between solute molecules such as proteins and functional groups like alkyl residues on support particles.
    7. **High-pressure liquid chromatography**: It is based on the general principles of chromatography, with the only different being high liquid pressure applied to the packed column. Due to high-pressure liquid (high liquid flow rate) and dense column packing. HPLC provides fast and high resolution of solute molecules.

13.6.2 Precipitation

13.6.3 Electrophoresis

13.6.4 Membrane Separation

13.7 Solvent Recovery

13.8 Drying

13.9 Crystallisation