UNIVERSITY OF LEEDS

SCHOOL OF CHEMICAL AND PROCESS ENGINEERING

CAPE1310

Process, Biochemical and Reaction Engineering Fundamentals

Reaction Engineering &

Biochemical Engineering

Lecture Handout for Semester 2

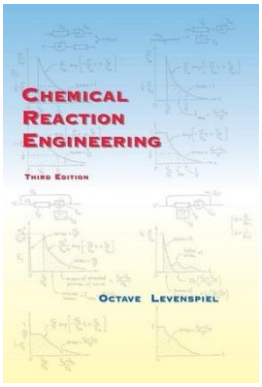
Academic year 2024/25

Prepared by

Dr Manoj Ravi

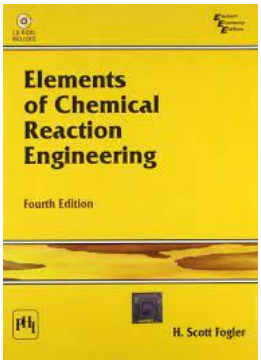
Reading List

Reaction Engineering



Chemical Reaction Engineering, 3rd edition, Octave Levenspiel

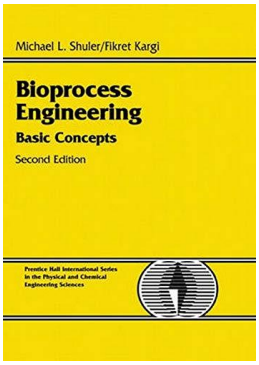
(available online via Reading list – Minerva module page)



Elements of Chemical Reaction Engineering, 3rd edition, Scott Fogler

Biochemical Engineering

Bioprocess Engineering Basic Concepts, 2nd edition, Michael Shuler and Fikret Kargi



SEMESTER 2 MODULE CONTENT

Reaction Engineering

- Chapter 1: Introduction to Chemical Reaction Engineering
- Chapter 2: Chemical Reaction Kinetics
- Chapter 3: Ideal Reactors
- Chapter 4: Mass Balance on Ideal Reactors
- Chapter 5: Experimental Determination of Rate Law

Reaction Engineering – Simulation-based learning

- Chapter 6: Simulation Exercise on Chemical Reactors

Reaction Engineering – Laboratory practical

- Chapter 7: CSTR Lab Practical
  (Also refer to assessment brief – 20% of module mark)

Biochemical Engineering

- Chapter 8: Introduction to Biochemical Engineering
- Chapter 9: Biology Basics (for self-reading; this content *will not be assessed*)
- Chapter 10: Enzyme kinetics
- Chapter 11: Microbial Growth and Bioreactors
- Chapter 12: Biochemical Product recovery

MODULE DELIVERY

- In-person lectures (please attend all sessions): explanation of concepts, mathematical derivations and solving numerical questions
- Numerical questions for homework
- Lab-based and simulation-based learning activities to complement understanding of fundamental theoretical concepts
- Questions? Please don't wait until exams period – Email m.ravi@leeds.ac.uk for direct queries and/or meeting appointments

ASSESSMENTS

- Individual report or recorded presentation (20% of module mark): Lab sessions will be held on a small group basis. Submission is due 2 weeks after lab practical.
- Written exam (35% of module mark) in the Semester 2 exam period (June 2025). Exam format: 16 MCQs worth 1 mark each (8 questions on reaction engineering & 8 questions on biochemical engineering) and detailed questions worth 30 marks each on reaction engineering and biochemical engineering.

CHAPTER 1. INTRODUCTION TO CHEMICAL REACTION ENGINEERING

Chemical reaction engineering is at the heart of any typical chemical process. As chemical engineers, we must be able to design a chemical reactor for a process.

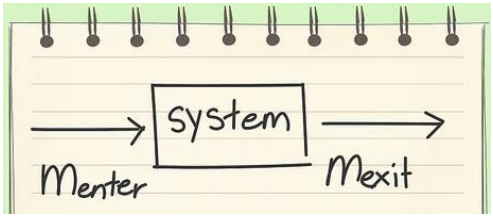
Chemical reactions can be of very different types; these are typically classified using the following terms:

- Homogeneous reactions: class of chemical reactions that takes place in a single phase (solid, liquid or gas)
    - Example: Sulphur dioxide oxidation: $2SO_2$ (g) + O_2 (g) ⇔ $2SO_3$ (g)
- Heterogeneous reactions: reaction has the presence of at least two distinct phases (solid-gas, liquid-gas, solid-gas)
    - Example: Iron rusting: 4Fe (s) + $3O_2$ (g) → $2Fe_2O_3$ (s)
- Catalytic reactions: involve the use of a material that accelerates the rate of reaction. The catalyst is not consumed in the reaction (although it often takes part in the reaction forming intermediates and then being regenerated on product formation)
    - Example: Iron in ammonia synthesis from nitrogen and hydrogen

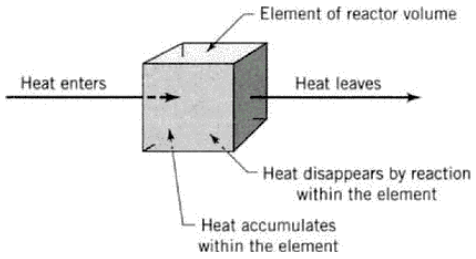
Pillars of chemical reaction engineering

Developing a strong competence in chemical reaction engineering requires fundamental understanding of the following six concepts, which you will learn in several modules:

- Mass balance (CAPE1310 & 1330): accounting for material entering a reactor, converting within the reactor, accumulating within the reactor and leaving the reactor



- Energy balance (CAPE1330): accounting for heat entering and leaving a reactor, heat accumulation within the reactor, and heat formation or disappearance due to the reaction occurring within the reactor (exothermic reactions produce heat while endothermic reactions consume heat)



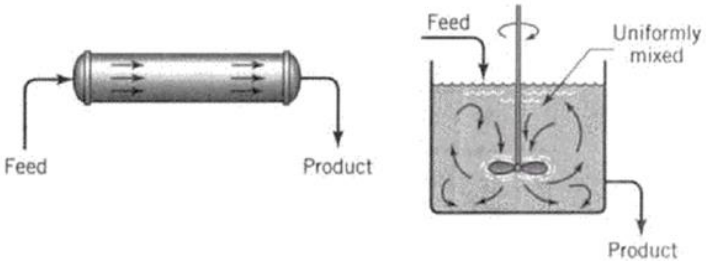
- Stoichiometry (CAPE1310 & 1330): Addresses the question of how many moles of a reactant reacts to yield how many moles of a product; example: 1 mole of nitrogen reacts with 3 moles of hydrogen to yield 2 moles of ammonia

$$1N_2 + 3H_2 \rightarrow 2NH_3$$

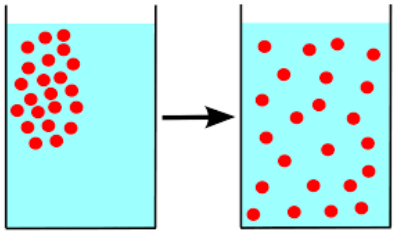
- Rate laws /kinetics (CAPE1310): relating rate of reaction to concentration and temperature

$$-r_A = kC_A^n$$
$$k = k_0 e^{-E/RT}$$

- Contacting (CAPE1310): how reactants are contacted for the reaction to take place



- Diffusion (CAPE1330): transport of material by molecular motion; considering rate of diffusion is particularly important in heterogeneous reactions



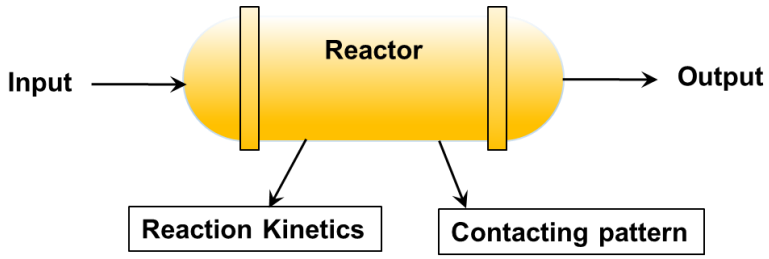
Reaction engineering teaching in the programme

CAPE1310 Reaction Engineering	CAPE2350 Reaction Engineering
Reactors for single reaction	Reactors for multiple reactions
Irreversible reactions	Reversible reactions
Constant volume reaction systems	Variable volume reaction systems
Isothermal reactor operation	Adiabatic reactor operation
Ideal reactors: Batch, CSTR, PFR	Ideal reactors: Batch, Semi-batch, CSTR, PFR & PBR

The performance equation

The mathematical expression for the output of a reactor for a given input depends on the reaction kinetics and the contacting pattern. This is called the performance equation:

$$output = f(input, kinetics, contacting)$$



Kinetics of a reaction refers typically to the rate of a reaction, which is solely a function of the properties of the reacting materials and reaction conditions (example: temperature, pressure, concentration).

CHAPTER 2. CHEMICAL REACTION KINETICS

Production capacity

The quantity of material produced (or used) per unit time is known as the production capacity or production rate (P); typical units: mol/s or kg/s

The annual production capacity of different chemicals vary depending on their global needs. Reactor and process design will take into account these scales of production.

Product	Annual production
Sulphuric Acid	230 million tonnes
Ammonia	200 million tonnes
Paracetamol	115000 tonnes
Bicalutamide	10 tonnes

Rate of reaction

The reaction rate represents how fast a chemical component is converted into another by a chemical reaction

$$A \rightarrow R$$

r_R is the rate of formation of R;
$-r_A$ is the rate of consumption or disappearance of A

Chemical reactions can proceed at widely different rates. For example, while the reaction for producing gasoline from crude petroleum occurs in the order of a second, wastewater or sewage treatment reactions proceed much slowly, in the order of days. The largely different reaction rates means the design of reactors will be largely different in these cases.

Defining rate of reaction

There are several definitions for reporting the rate of a reaction. Depending on the specific reaction, one of these definitions may be more appropriate than the others.

The reaction rate can be defined as moles of a reactant consumed or moles of a product formed per unit time per unit of a property that describes the scale of the reactor system. If the rate of change of number of moles of a product 'i' is expressed as $\frac{dN_i}{dt}$, then the rate of reaction can be defined as the moles of product:

i. Based on volume of reacting fluid: $r_i = \frac{1}{V}\frac{dN_i}{dt} = \frac{\text{moles } i \text{ formed}}{(\text{volume of fluid})(\text{time})}$
ii. Based on mass of solid/catalyst in fluid-solid systems: $r_i' = \frac{1}{W}\frac{dN_i}{dt} = \frac{\text{moles } i \text{ formed}}{(\text{mass of solid})(\text{time})}$
iii. Based on interfacial surface in two-fluid systems or based on surface of solid in gas-solid systems: $r_i'' = \frac{1}{S}\frac{dN_i}{dt} = \frac{\text{moles } i \text{ formed}}{(\text{surface})(\text{time})}$
iv. Based on volume of solid in gas-solid systems: $r_i''' = \frac{1}{V_s}\frac{dN_i}{dt} = \frac{\text{moles } i \text{ formed}}{(\text{volume of solid})(\text{time})}$
v. Based on volume of reactor, if different from the rate based on volume of fluid: $r_i'''' = \frac{1}{V_r}\frac{dN_i}{dt} = \frac{\text{moles } i \text{ formed}}{(\text{volume of reactor})(\text{time})}$

While the formulae above describe the rates of formation of a product, expressions for rate of consumption of a reactant can be derived by using 'moles *i* reacted' instead of 'moles *i* formed'

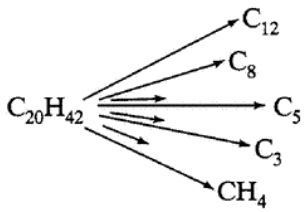
Question: Which of the above definitions of reaction rate would be most appropriate to use for the following chemical reactions:

i. Oxidation of sulphur dioxide to sulphur trioxide
ii. Synthesis of ammonia from nitrogen and hydrogen over an iron catalyst
iii. Chlorination of liquid benzene using gaseous chlorine
iv. Burning of a coal particle in air

Question: Chemical vapour deposition is a process used in the microelectronics industry to deposit thin films of constant thickness on silicon wafers. This process is of particular importance in the manufacturing of large-scale integrated circuits. One of the common coatings, Si_3N_4, is particularly resistant to oxidation and is manufactured in a factory with a production capacity of 5 kg product per day. The factory uses a 20-litre reactor for the process. What is the rate of production of Si_3N_4 expressed as r''''($\frac{\text{moles produced}}{m^3 \text{ reactor} \cdot \text{ s}}$)?

Question: Fluid catalytic cracking reactors (FCC) are the backbone of the petroleum industry. In these reactors, crude oil comprising of long chain hydrocarbons are cracked to shorter molecules. The FCC technology will continue to be relevant for a sustainable future where fossil-derived crude oil is replaced with bio-oils, for example those derived from municipal solid waste.

As a simplification of FCC, suppose that the feed consists of just C_{20} hydrocarbons, which are cracked as follows:



A typical reactor has an internal diameter of 4-10 m and is 10-20 m high and contains about 50 tons of ρ= 800 kg m^{-3} porous catalyst. The reactor is fed about 38000 barrels of oil per day (6000 $m^3.day^{-1}$, density ρ= 900 kg m^{-3}). If 60 mol% of the vaporized feed is cracked in the unit, what is the rate of reaction, expressed as -r'($\frac{\text{moles reacted}}{kg\ \text{catalyst}\ .\ s}$) and as -r''' ($\frac{\text{moles reacted}}{m^3\ \text{catalyst}\ .\ s}$)?

Stoichiometry and reaction rate

For recap of stoichiometry, refer to Lecture 1 of CAPE1330 Mass balance (Semester 1)

In a generic chemical reaction $aA + bB \rightarrow rR + sS$,

a, b, r and *s* are the stoichiometric coefficients.

The stoichiometric coefficients of a correctly balanced chemical reaction will help establish the mole ratio between reactants consumed and products formed. This will also help identify if any one of the reactants is a limiting reactant.

In the example of ammonia synthesis, $1N_2 + 3H_2 \rightarrow 2NH_3$

The stoichiometric coefficients inform us that 1 mole of nitrogen reacts with 3 moles of hydrogen to yield 2 moles of ammonia.

A reactant is limiting if it is present in less than its stoichiometric proportion relative to every other reactant.

Therefore, if we start with an equimolar feed stream of nitrogen and hydrogen for ammonia synthesis, hydrogen would be the limiting reactant.

The reaction rates for chemical species involved in a reaction are related through their stoichiometric coefficients as follows: for the reaction $aA + bB \rightarrow rR + sS$

$$\frac{-r_A}{a} = \frac{-r_B}{b} = \frac{r_R}{r} = \frac{r_S}{s}$$

In the example of ammonia synthesis, realize that

$$\frac{-r_{N_2}}{1} = \frac{-r_{H_2}}{3} = \frac{r_{NH_3}}{2}$$

Question: You had earlier considered the manufacture of Si_3N_4 in a factory with a production capacity of 5 kg product per day. The factory used a 20-litre reactor for the process. Assuming all the reactants are completely consumed to form the products as per the below reaction:

$$3SiH_4\ (g) + 4NH_3\ (g) \rightarrow Si_3N_4\ (s) + 12H_2\ (g)$$

Compute the required mass of each of the reactants to meet the daily production rate of the factory.

Reaction rate law

The rate law is an algebraic equation for reaction rate as a function of the properties of the reacting materials and reaction conditions.

For a reaction of the type, $aA \rightarrow rR$

the rate law is expressed as

$$-r_A = kC_A^n$$

where $-r_A$: rate of consumption or disappearance of species A
k: reaction rate constant; function of reaction conditions (temperature)
n: reaction order w.r.t. species A; n = 1: 1st order reaction, n = 2: 2nd order reaction,….

The kinetic rate law of a reaction is independent of the type of reactor being used for the reaction.

Important: Reaction order need not be equal to the stoichiometric coefficient! The order of a reaction can only be determined experimentally and not from the balancing of a chemical reaction equation.

For the oxidation reaction of NO to NO_2, ($2NO + O_2 \rightarrow 2NO_2$), the rate law is experimentally determined as:

$$-r_{NO} = kC_{NO}^2 C_{O_2}$$

The overall order for the reaction is 3, with the reaction being 2nd order with respect to NO and 1st order with respect to O_2.

Elementary reactions refer to reactions where one or more chemical species react through a single reaction step to form the product(s). In other words, it is a single-step reaction with no observed intermediates. Examples include cis-trans isomerization reactions and decomposition reactions such as:

- $CuCO_3(s) \rightarrow CuO(s) + CO_2(g)$
- $N_2O_4(g) \rightarrow 2NO_2(g)$

For elementary reactions, the rate law can be derived from stoichiometric coefficients of reactants in the balanced equation.

Non-elementary reaction includes multiple sub-steps and proceeds through a series of transition states. The order of the reaction for such reactions may not be equal to the stoichiometric coefficient of the balanced reaction equation.

For example, the reaction $H_2 + Br_2 \rightarrow 2HBr$ has the experimental rate law as:

$$r_{HBr} = \frac{k_1 C_{H2} {C_{Br2}}^{1/2}}{k_2 + C_{HBr}/C_{Br2}}$$

This is because this reaction proceeds through the reaction scheme shown below, which involves the generation of hydrogen and bromine free radicals:

$$\mathrm{Br_2} \rightleftarrows 2\mathrm{Br}\cdot \qquad \text{Initiation and termination}$$
$$\mathrm{Br}\cdot + \mathrm{H_2} \rightleftarrows \mathrm{HBr} + \mathrm{H}\cdot \qquad \text{Propagation}$$
$$\mathrm{H}\cdot + \mathrm{Br_2} \rightarrow \mathrm{HBr} + \mathrm{Br}\cdot \qquad \text{Propagation}$$

The units of the rate constant k depends on the order of the reaction n. When the reaction rate is expressed as mol L^{-1} s^{-1} and the concentration as mol L^{-1}, realize that the following units of rate constant can be derived from the rate law:

Reaction order 'n'	Units of 'k'
0	mol L^{-1} s^{-1}
1	s^{-1}
2	mol^{-1} L s^{-1}

The rate law can also be written in terms of pressure (and not concentration) for gas-phase reactions.

In the ideal gas law pV=nRT, the molar concentration (C) can be substituted for n/V.

Therefore, substituting p=CRT in $-r_A = kC_A^n$

gives $$-r_A = k\left(\frac{p_A}{RT}\right)^n = k' p_A^n$$

where $k' = \frac{k}{(RT)^n}$ and p_A is the partial pressure of A.

Recall basis of ideal gas law – SI units of the different terms as follows:

p in Pa; V in m^3, n in mol, T in K → R = 8.314 Pa m^3 mol^{-1} K^{-1} or 8.314 J mol^{-1} K^{-1}

Question: The decomposition of hydrogen iodide to hydrogen and iodine gas has the following rate equation:

$$-r_{HI} = 0.005 C_{HI}^2, \ \frac{mol}{cm^3.min}$$

If the concentration is to be expressed in mol/liter and time in hours, what would be the value and unit of the rate constant?

Question: The reaction rate for the high temperature pyrolysis of propane gas (A) is reported as:

$$-\frac{dp_A}{dt} = 3.66{p_A}^2,\ \frac{atm}{h}$$

(a) What are the units of the rate constant?
(b) What is the value of the rate constant for this reaction if the rate equation is expressed as

$$-r_A = -\frac{1}{V}\frac{dN_A}{dt} = k{C_A}^2,\ \frac{mol}{m^3.s}$$

Arrhenius equation

The Arrhenius law for the reaction rate constant is given as:

$$k = k_0 e^{-E/RT}$$

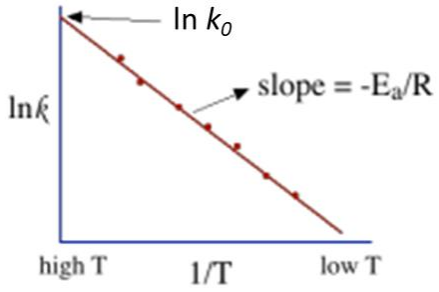
where k: reaction rate constant
k_0: pre-exponential factor
E: activation energy (J/mol)
R: gas constant (J/mol.K)
T: temperature (K)

The equation gives the mathematical basis for the common knowledge that chemical reactions occur more rapidly at higher temperatures. As the temperature rises, molecules move faster and collide more vigorously, greatly increasing the likelihood of bond cleavages and rearrangements.

The Arrhenius equation can be written in the logarithmic form as follows:

$$lnk = lnk_0 - \left(\frac{E}{R}\right)\frac{1}{T}$$

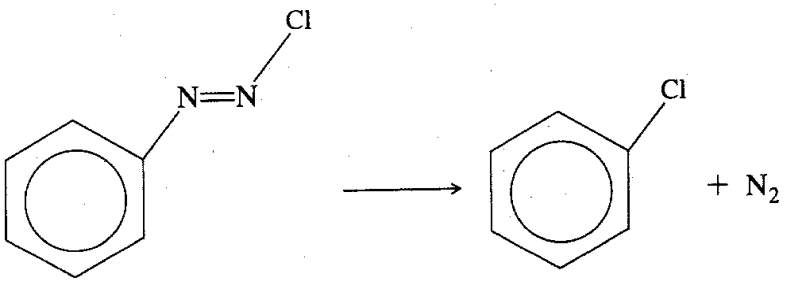
The graphical representation of the above equation is shown below



Arrhenius law written at two temperatures provided E stays constant:

$$ln\frac{r_2}{r_1} = ln\frac{k_2}{k_1} = \frac{E}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

Question: Calculate the activation energy for the decomposition of benzene diazonium chloride to give chlorobenzene and nitrogen:



using the following information for this first-order reaction:

k (s^{-1})	0.00043	0.00103	0.00180	0.00355	0.00717
T (K)	313.0	319.0	323.0	328.0	333.0

Question: Milk is pasteurized if it is heated to 63°C for 30 min, but if it is heated to 74°C it only needs 15 seconds for the same result. Find the activation energy of this sterilization process.

Conversion of a reactant

You learnt about fractional conversion in CAPE1330 Mass balance (Semester 1)

For a generic reaction, A → R,

The **fractional conversion** of a reactant, A, is represented as X_A

$$fractional\ conversion\ of\ a\ reactant = \frac{moles\ reacted}{moles\ fed}$$

Suppose that N_{A0} is the initial amount (moles) of reactant A and N_A is the final amount (moles) of reactant A present,

then the conversion of A is given by

$$X_A = \frac{N_{A0} - N_A}{N_{A0}}$$

$$or\ X_A = 1 - \frac{N_A}{N_{A0}}$$

Equivalently, the moles of 'A' i.e. N_A can be expressed in terms of initial number of moles N_{A0} and the fractional conversion X_A as follows:

$$N_A = N_{A0}(1 - X_A)$$

For a constant volume system, i.e. where the volume of the reaction system (V) doesn't change with time, the fractional conversion can be expressed in terms of concentration:

$$X_A = 1 - \frac{N_A}{N_{A0}} = 1 - \frac{N_A/V}{N_{A0}/V} = 1 - \frac{C_A}{C_{A0}}$$

Note that if the reaction volume changes during the course of the reaction, $X_A = 1 - \frac{C_A}{C_{A0}}$ cannot be used.

The above definition of fractional conversion is suited to a batch reactor system because it was defined in terms of number of moles (N_A and N_{A0}). The same can also be extended for continuous reactor systems, where we have a molar flow rate i.e. number of moles per unit time:

$$X_A = \frac{F_{A0} - F_A}{F_{A0}}$$

Or

$$F_A = F_{A0}(1 - X_A)$$

where F_{A0} is the molar flow rate (moles/time) of reactant A entering the reactor and F_A is the molar flow rate (moles/time) of reactant A leaving the reactor.

Question: Soap consists of the sodium and potassium salts of various fatty acids such as oleic, stearic and palmitic acids. The saponification reaction for the formation of soap from aqueous sodium hydroxide and glyceryl stearate is a constant volume reaction given as:

$$3NaOH + (C_{17}H_{35}COO)_3C_3H_5 \rightarrow 3C_{17}H_{35}COONa + C_3H_5(OH)_3$$

If the initial mixture consists of NaOH at a concentration of 10 mol dm^{-3} and of glyceryl stearate at a concentration of 2 mol dm^{-3}, (a) which of the reactants is the limiting reactant and what is the concentration of glycerine ($C_3H_5(OH)_3$) when the conversion of NaOH is (b) 20% and (c) 90%?

Question: A gas mixture consisting of 50 mol% methane and 50 mol% inert gas at 10 atm (1013 kPa) enters a reactor at 422.2 K at a volumetric flow rate of 6 dm^3 s^{-1}. Calculate (a) the concentration of methane at the reactor inlet in mol dm^{-3} and (b) concentration of methane in the reactor outlet if the conversion of methane in the constant volume rector is known to be 75%.

CHAPTER 3. IDEAL REACTORS

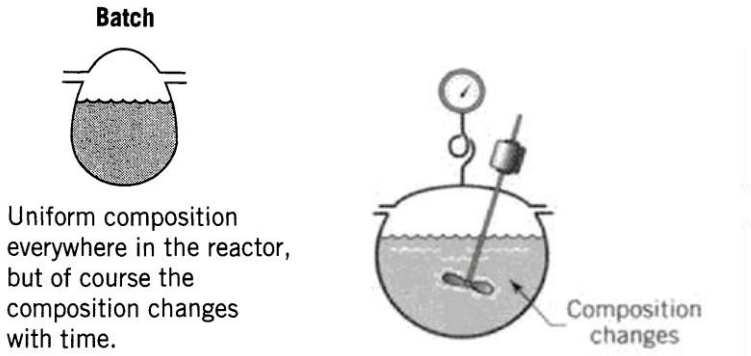
There are three main ideal reactor types:

(i) Batch reactor
(ii) Mixed flow reactor or Continuous Stirred Tank Reactor
(iii) Plug flow reactor

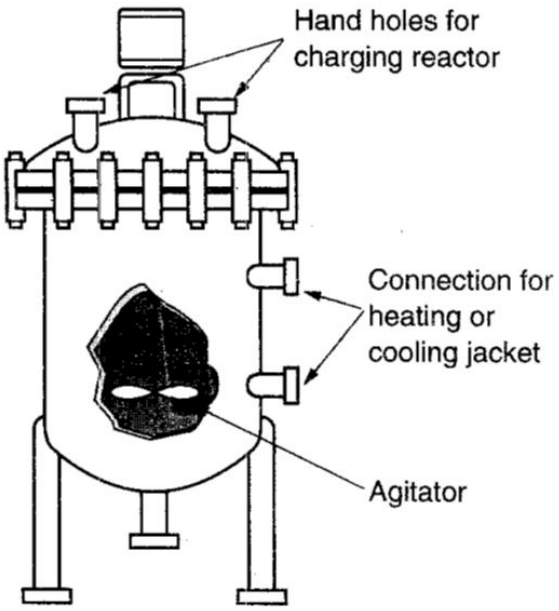
Batch reactor

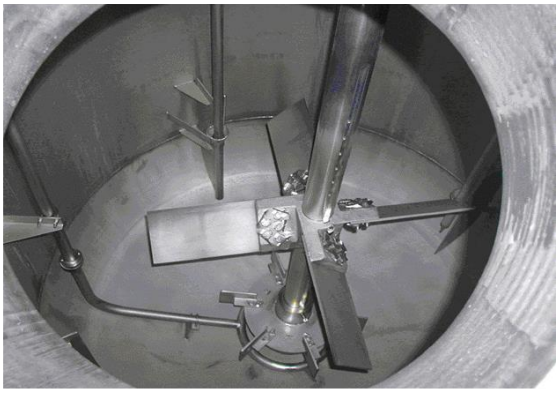
A batch reactor has neither inflow nor outflow of reactants or products while the reaction is being carried out (thermodynamically referred to as a closed system – CAPE1320).

Reactants are mixed together and the reaction is allowed to proceed for a given time in a closed vessel system. The concentration inside the reactor changes with time.



Schematic of an industrial batch reactor:





When is a batch reactor or batch process typically used?

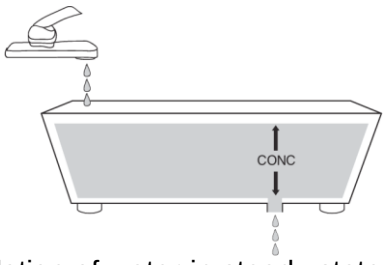
- Small-volume production
- Flexibility for multiproduct (multi-process) operation
- Frequent shutdown and maintenance cleaning required
- Lower capital cost
- Laboratory testing
- Market risk and product regulation

Continuous reactors

Continuous reactors are best suited for indefinitely long production runs of a product typically required on a large scale.

Continuous reactors are targeted to be operated in a 'steady-state', i.e., while there is a continuous flow of material entering and leaving the reactor, the properties at any point within the system – such as temperature, pressure and concentration – doesn't change with time.

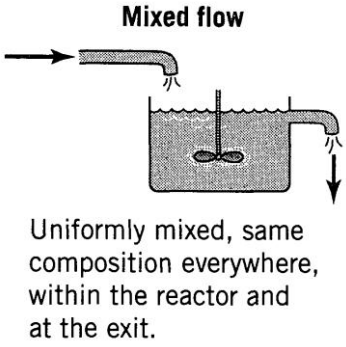
Some real-life examples of steady-state processes:



Bath tub: When the tap and bottom plug are open, under steady state, the water level in the tub 'CONC' will not change with time. Under these conditions, the rate of water flowing into the tub from the tap will equal the rate of water draining through the bottom plug. In other words, the rate of change of accumulation of water in steady state operation is zero.

Realize that a water fountain operating at steady state also works the same way.

Mixed flow reactor or Continuous Stirred Tank Reactor (CSTR)

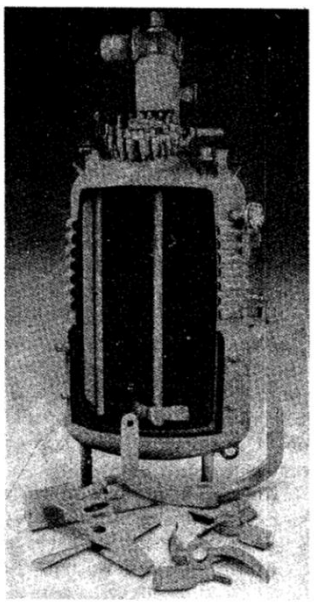


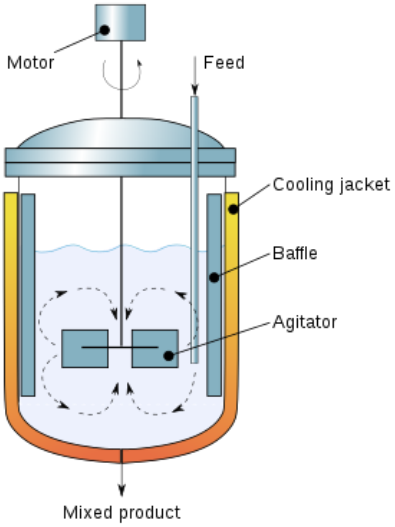
In a CSTR, there is a constant inflow of reactants and a constant outflow of products and unreacted reactants (if any). Except during the start-up and shut-down phases, this continuous reactor is operated under steady-state, where properties, such as concentration, do not change with time.

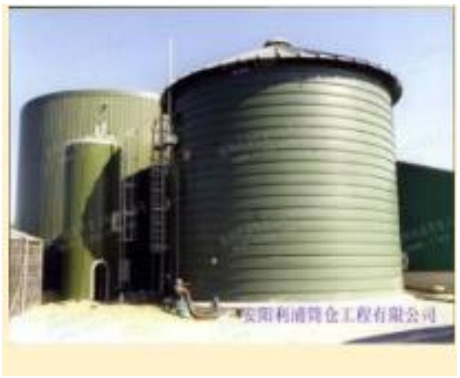
A CSTR is typically used for large-scale liquid-phase reactions where intense agitation is required. Typical CSTR reactors are often glass-lined and include a heating/cooling jacket, a motor for mixing and baffles. CSTRs can be used for reactions over a wide temperature and pressure range (usually up to 300 °C & 300 bar).

In an ideal CSTR, we assume that there is perfect mixing within the reactor. Hence, the concentration or composition is the same everywhere inside the reactor, which is equal to the concentration or composition at the reactor exit.

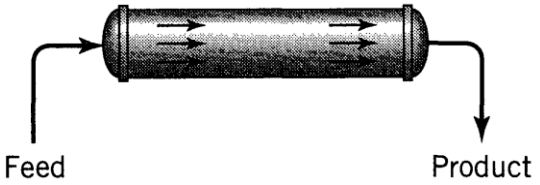
Advanatages of a CSTR include cheap construction costs and good temperature control (due to good mixing). The disadvantages include the development of deadzones (when mixing is not done properly) and potential bypass of reactants through to the outlet.

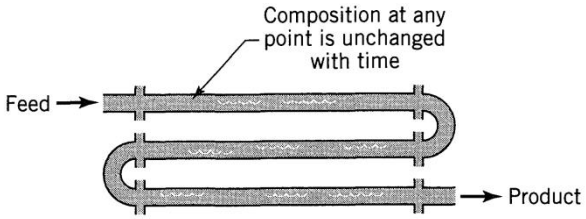






Plug Flow Reactor (PFR)





Plug flow reactors, also referred to as tubular reactors, consist of a cylindrical tube with openings on each end for reactants and products to flow through. In a PFR, there is a constant inflow of reactants and a constant outflow of products and unreacted reactants (if any). Except during the start-up and shut-down phases, this continuous reactor is operated under steady-state, where properties, such as concentration, do not change with time.

As the name suggests, the flow of feed and products in this reactor can be visualised as plugs of material flowing through the reactor. The reaction occurs as the feed flows through the reactor tube; the amount of reagent reacted increases along the length of the reactor tube. The length needs to be chosen such that the desired conversion can be achieved. In an ideal PFR, the concentration varies along the length of the reactor but there is no radial variation in concentration.

PFRs are often used for gas-phase reactions and can also be packed with a catalyst for catalytic reactions. Common industrial uses of tubular reactors are in gasoline production, oil cracking, synthesis of ammonia, biomethane production and oxidation of sulphur dioxide.

PFRs enable higher conversion per unit volume than CSTRs (this will be derived in Chapter 4). One disadvantage with PFR is the difficulty to control reactor temperature with hot spots potentially being formed within the reactor when used for exothermic reactions.

Question: For each of the chemical processes mentioned below, suggest which of the following reactor types are best suited and why: (i) batch reactor or (ii) CSTR or (iii) PFR

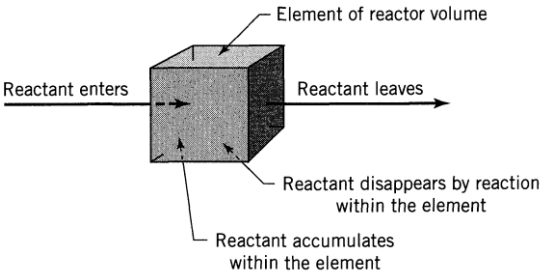
(a) Cracking of ammonia to nitrogen and hydrogen over a solid catalyst
(b) Endothermic gas-phase conversion of propanol to acetone and hydrogen
(c) Manufacture of the medical drug propranolol
(d) Large-scale wastewater treatment plants

CHAPTER 4. MASS BALANCE ON IDEAL REACTORS

> Recall the general balance equation from CAPE1330 Mass balance (Semester 1)
>
> Input + generation – output – consumption = accumulation

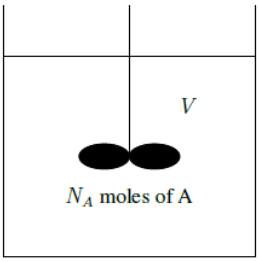
In this chapter, we will develop the performance equations for the three ideal reactor types introduced in Chapter 3. The starting point is a molecular balance expressed for a reactant:

$$\begin{pmatrix}\text{rate of reactant flow into element of volume}\end{pmatrix} = \begin{pmatrix}\text{rate of reactant flow out of element of volume}\end{pmatrix} + \begin{pmatrix}\text{rate of reactant loss due to chemical reaction within the element of volume}\end{pmatrix} + \begin{pmatrix}\text{rate of accumulation of reactant in element of volume}\end{pmatrix}$$



Each term in the above equation has units of $\left(\frac{quantity}{time}\right)$. For reactors, we will use the units of $\left(\frac{moles}{time}\right)$. We will now apply this mass balance equation to each ideal reactor type.

Batch reactor



Consider a reactant 'A' (usually the limiting reactant) reacting in the batch reactor. Let 'V' be the volume of the reacting fluid. In a batch reactor, no fluid enters or leaves the reactor during reaction.

Therefore, the general mass balance:

$$In\ flow = Out\ flow + Rate\ of\ Consumption + Rate\ of\ Accumulation$$

simplifies to:

$$(rate\ of\ consumption\ of\ reactant\ A\ due\ to\ chemical\ reaction) = -(rate\ of\ accumulation\ of\ reactant\ A\ in\ reactor)$$

These two terms can be mathematically expressed as follows:

$$Rate\ of\ consumption\ of\ reactant\ A\ by\ reaction\ \left(\frac{moles}{time}\right) = (-r_A).V$$

where

$$(-r_A).V = \left(\frac{moles\ A\ reacting}{(time)(volume\ of\ fluid)}\right)(volume\ of\ fluid)$$

Secondly,

$$Rate\ of\ accumulation\ of\ reactant\ A\ in\ reactor\ \left(\frac{moles}{time}\right) = \frac{dN_A}{dt} = \frac{d[N_{A0}(1-X_A)]}{dt}$$
$$= -N_{A0}\frac{dX_A}{dt}$$

Hence, substituting the two terms in the mass balance,

$$(-r_A).V = N_{A0}\frac{dX_A}{dt}$$

Rearranging,

$$dt = \frac{N_{A0}}{(-r_A).V}dX_A$$

Integrating,

$$t = N_{A0}\int_0^{X_A}\frac{dX_A}{(-r_A).V}$$

This is the general performance equation showing the time required to achieve a conversion X_A in a batch reactor. The volume of reacting fluid V and the reaction rate $(-r_A)$ remain under the integral sign, because in general, they both change as the reaction proceeds.

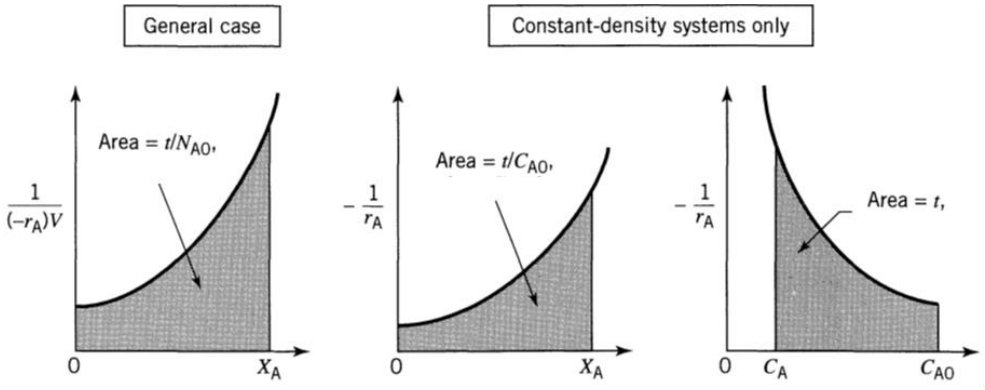
For most liquid reactions and also gas reactions that run at constant temperature and density, the volume of the reacting fluid V will not change during reaction. It can hence be pulled out of the integral in the above equation to give the following

$$t = C_{A0}\int_0^{X_A}\frac{dX_A}{(-r_A)} = -\int_{C_{A0}}^{C_A}\frac{dC_A}{(-r_A)}$$

Realize how the expression has be described in terms of dC_A above.

For constant pressure gas-phase reactions that involves a change in the number of moles (eg. A → 3B), the volume of the fluid changes during reaction → Use general performance equation

The graphical representation of the performance equations for batch reactors are:



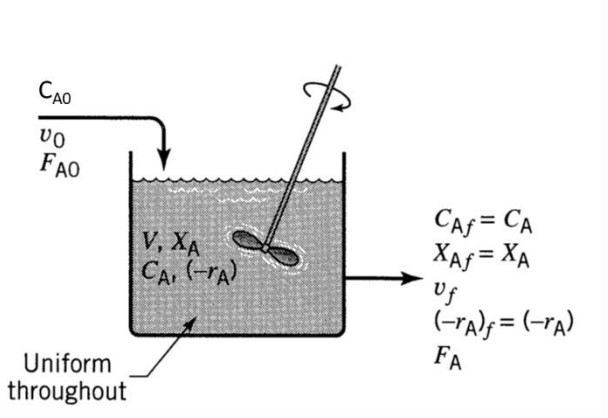
Question: The elementary, irreversible liquid-phase hydration of butylene oxide produces butylene glycol.

$$C_4H_8O + H_2O \rightarrow C_4H_{10}O_2 .$$

The reaction is conducted using water as the solvent, so that water is in large excess (i.e. assume the concentration of water is constant = 56 mol/L). The initial concentration of butylene oxide is 0.25 mol/L. The reaction rate constant is 8.3 x 10^{-4} L/mol-min at 323 K, the temperature at which the reaction is run in a batch reactor. Determine the final concentration of butylene oxide after 45 min of reaction time.

Hint: $\int_o^X \frac{dX}{(1-X)} = ln\left(\frac{1}{1-X}\right)$

Continuous Stirred Tank Reactor (CSTR)



The performance equation for a mixed flow reactor or CSTR is developed, like for the batch reactor, starting from the mass balance on reactant A.

$$In\ flow = Out\ flow + Rate\ of\ Consumption + Rate\ of\ Accumulation$$

Since we are concerned with the steady-state operation of CSTR, the accumulation term = 0

In the CSTR figure showed above, $F_{A0} = v_0 C_{A0}$, where F_{A0} is the molar flow rate of reactant 'A' in the inlet (mol/s) and v_0 is the volumetric flow rate of reactant 'A' in the inlet (m^3/s)

We can now describe the in flow, out flow and consumption terms in the mass balance for reactant 'A' as follows:

$$In\ flow\ of\ A\ \left(\frac{moles}{time}\right) = F_{A0}$$

$$Out\ flow\ of\ A\ \left(\frac{moles}{time}\right) = F_A = F_{A0}(1 - X_A)$$

$$Consumption\ of\ A\ by\ reaction\ \left(\frac{moles}{time}\right) = (-r_A).V$$

The mass balance for reactant 'A' then simplifies to:

$$F_{A0} = F_{A0}(1 - X_A) + (-r_A).V$$

$$F_{A0}X_A = (-r_A).V$$

$$\frac{V}{F_{A0}} = \frac{X_A}{(-r_A)}$$

The performance equation relates in a simple way the four terms $X_A, (-r_A), V, F_{A0}$ thus, knowing any three allows the fourth to be found directly.

$$\frac{V}{v_0} = \frac{C_{A0}X_A}{(-r_A)}$$

Note that the term on the left hand side $\frac{V}{v_0}$, has dimensions of [time]. This ratio is called 'space time' τ in flow reactors. Just as the reaction time t is the natural performance measure for a batch reactor, space time τ is the performance measure for flow or continuous reactors.

$$\tau = \frac{V}{v_0} = \frac{C_{A0}X_A}{(-r_A)}$$

Space time τ is defined as the time required to process one reactor volume of feed measured at specified conditions. Therefore, a CSTR having a space time of 5 minutes means that every 5 min one reactor volume of feed at specified conditions is being treated by the reactor.

Going back to the performance equation derived for a CSTR,

$$\tau = \frac{V}{v_0} = \frac{C_{A0}X_A}{(-r_A)}$$

Note that X_A and $(-r_A)$ are measured at exit stream conditions (which is assumed to be the same as inside the reactor)

For a constant volume reaction system, i.e. a reaction in which the volume of the reacting fluid V does not change during reaction, we know that,

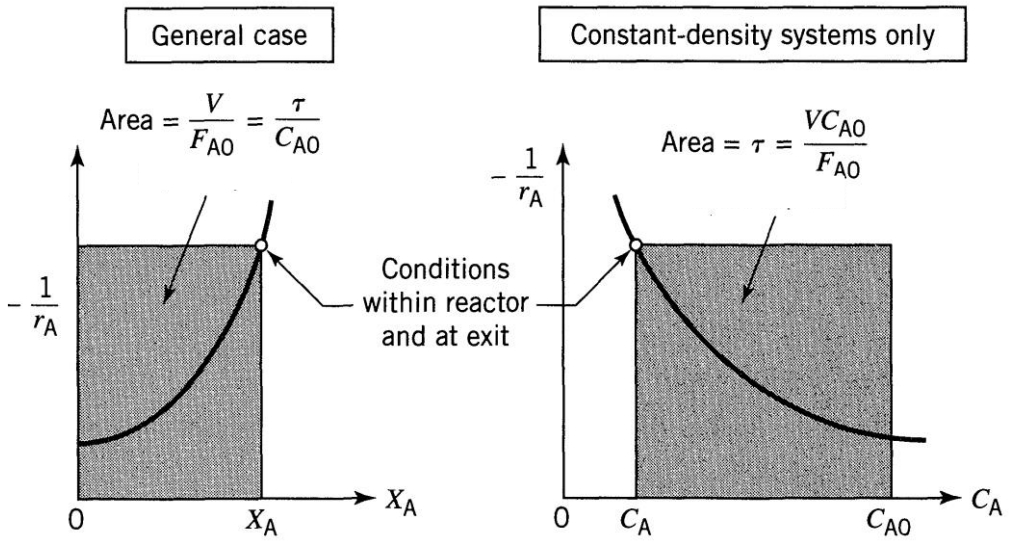
$$X_A = 1 - \frac{F_A}{F_{A0}} = 1 - \frac{C_A}{C_{A0}}$$

$$C_{A0}X_A = C_{A0} - C_A$$

Substituting for $C_{A0}X_A$ in the performance equation, the following expression can be obtained:

$$\tau = \frac{V}{v} = \frac{C_{A0} - C_A}{(-r_A)}$$

The graphical representation of the performance equations for mixed flow reactors (CSTR) are shown below:



Question: A reactant 'A' undergoes an isothermal gas-phase decomposition reaction as follows: A → B + C. The laboratory measurements below show the chemical reaction rate as a function of conversion. The temperature of reaction was 422 K and the total pressure 10 atm, with the initial feed containing an equimolar mixture of reactant A and inert gases entering the reactor with a flow rate of 6 dm^3s^{-1}.

X	$-r_A$ (mol/dm^3 · s)
0.0	0.0053
0.1	0.0052
0.2	0.0050
0.3	0.0045
0.4	0.0040
0.5	0.0033
0.6	0.0025
0.7	0.0018
0.8	0.00125
0.85	0.00100

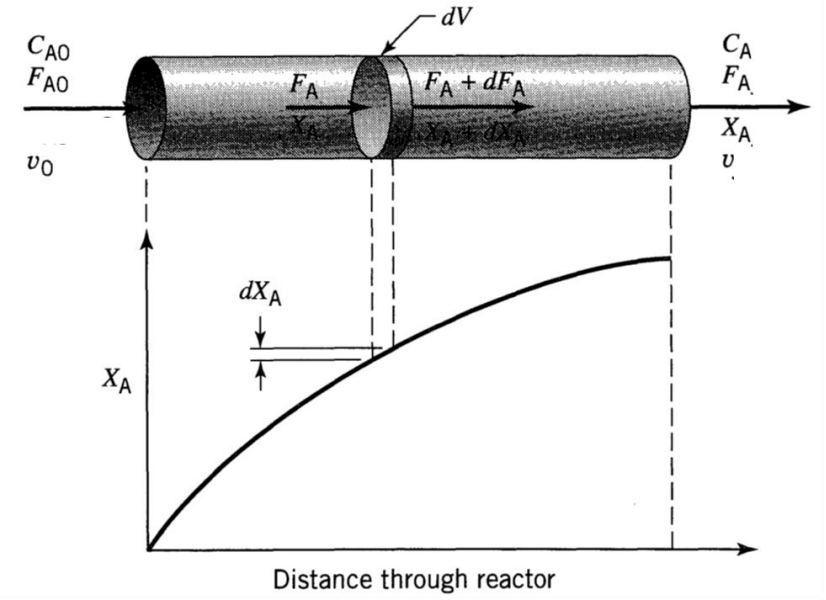
(a) Calculate the volume necessary to achieve 80% conversion in a CSTR using the performance equation.

(b) Use the kinetic data table to develop a plot of $-\left(\frac{1}{r_A}\right)$ v/s conversion X and determine the volume of CSTR necessary to achieve 80% conversion from this graphic.

Question: The hydrolysis of acetic anhydride to form acetic acid is carried out in a 1250 litre CSTR. The feed concentration is 2.5 M acetic anhydride and 50 M water. The volumetric flow rate of the feed is 15 litre.s^{-1}. The reaction is first order in acetic anhydride and first order in water with the rate constant = 0.0075 l.mol^{-1}.s^{-1}. What is the conversion of acetic anhydride?

Plug Flow Reactor (PFR)

In a plug flow reactor, the composition of the fluid varies from point to point along the flow path (equivalently the conversion increases with the distance down the tube as shown in the figure). Consequently, the mass balance for a reaction component must be made for a differential element of volume dV.



Again for a reactant A, the molecular balance is:

$$In\ flow = Out\ flow + Rate\ of\ Consumption + Rate\ of\ Accumulation$$

As we did with the CSTR, we are interested in the steady-state operation of a PFR, so the accumulation term = 0

For the differential element of volume dV shown in the figure,

$$In\ flow\ of\ A\ \left(\frac{moles}{time}\right) = F_A$$

$$Out\ flow\ of\ A\ \left(\frac{moles}{time}\right) = F_A + dF_A$$

$$Consumption\ of\ A\ by\ reaction\ \left(\frac{moles}{time}\right) = (-r_A).dV$$

Substituting these terms in the mass balance for reactant 'A':

$$F_A = F_A + dF_A + (-r_A).dV$$

Since, $F_A = F_{A0}(1 - X_A)$, we write, $dF_A = d[F_{A0}(1 - X_A)] = -F_{A0}dX_A$

Substituting the above expression for dF_A in the mass balance for 'A' we get,

$$F_{A0}dX_A = (-r_A).dV$$

$$\frac{dV}{F_{A0}} = \frac{dX_A}{-r_A}$$

This is the equation for the differential section of volume dV, which must be integrated to obtain the expression for the reactor as a whole.

$$\int_0^V \frac{dV}{F_{A0}} = \int_0^{X_A} \frac{dX_A}{-r_A}$$

$$\frac{V}{F_{A0}} = \int_0^{X_A} \frac{dX_A}{-r_A}$$

Note in the above expression, the reaction rate $(-r_A)$ cannot be pulled out of the integral, as it is dependent on the conversion (or concentration).

The same expression can be rewritten for space time τ of a PFR as follows:

$$\tau = \frac{V}{v_0} = \frac{C_{A0}V}{F_{A0}} = C_{A0}\int_0^{X_A} \frac{dX_A}{-r_A}$$

This PFR performance equation can be used to determine the size (i.e. volume) of a reactor required for a specified conversion of a reactant entering the reactor at a specified flow rate.

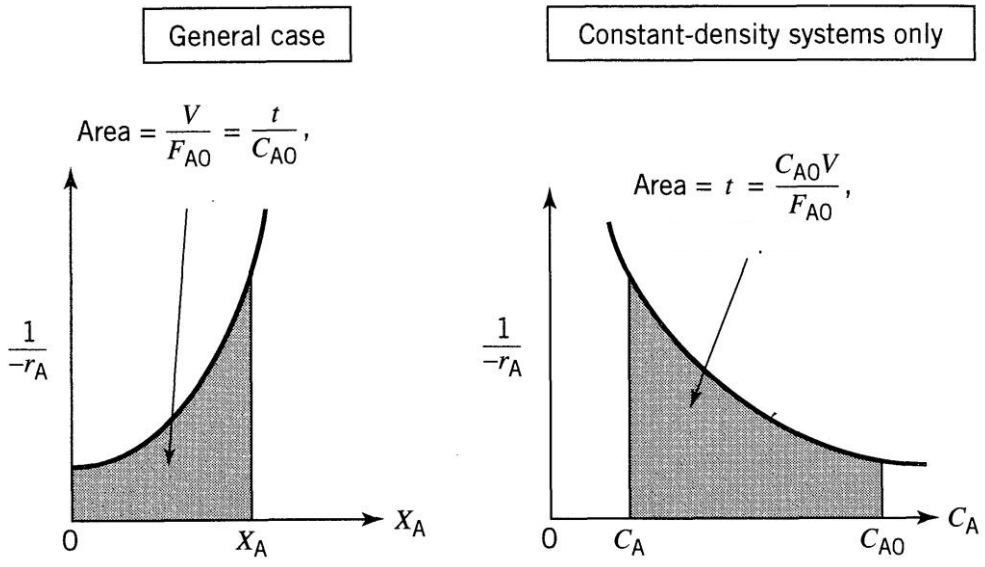
When we compare the above expression for space time τ of a PFR to that of a CSTR, we see that the difference in the equations is the reaction rate $(-r_A)$ being constant in a CSTR throughout the reactor volume while it varies in a PFR.

As we derived in the case of a CSTR, the expression for space time τ of a PFR for constant-density systems can be written in terms of concentration:

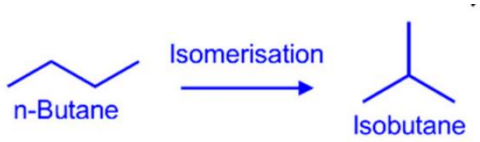
$$X_A = 1 - \frac{C_A}{C_{A0}} \; and \; dX_A = -\frac{dC_A}{C_{A0}}$$

$$\tau = \frac{V}{v_0} = \frac{C_{A0}V}{F_{A0}} = -\int_{C_{A0}}^{C_A} \frac{dC_A}{-r_A}$$

The graphical representation of the performance equations for plug flow reactors (PFR) are shown below:



Question: The isomerization of straight-chain hydrocarbons to their corresponding branched-chain isomers is an important step (called reforming) in manufacture of motor fuels because of the more favourable properties of the branched isomers.



The gaseous isomerisation of butane performed with a suitable catalyst has a rate constant of 0.0015 min^{-1} at 300 K. The activation energy of the isomerisation reaction is 105 kJ mol^{-1}. The actual reaction is to be carried out at 400 K and 9 atm pressure in a chemical processing facility with a production rate of 454 kg butane per hour. The reaction is to be carried out in parallel tubes of length 3 m and 1 inch (0.0254 m) diameter. How many tubes need to be installed in the production capacity to meet the desired production rate if the conversion of n-butane is 90%?

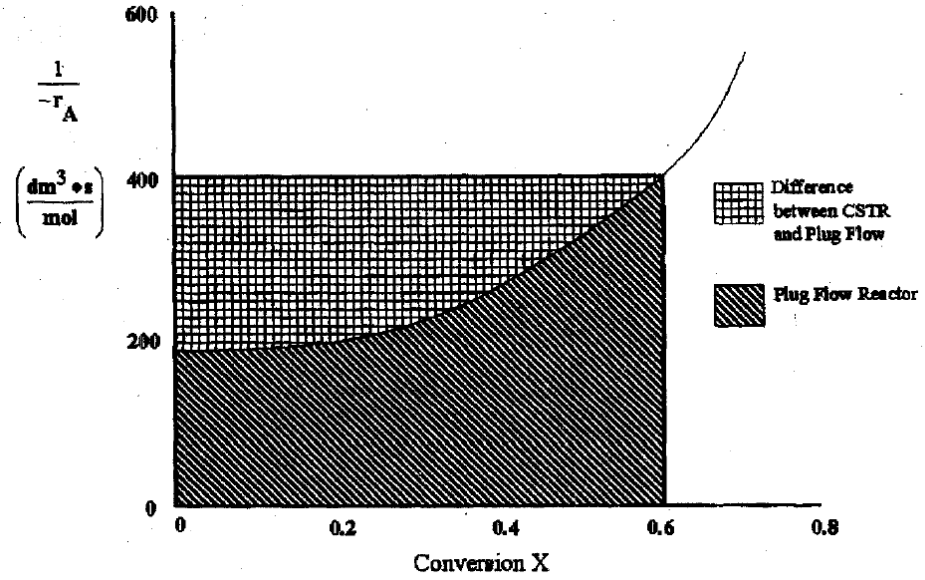
Question: A reactant 'A' undergoes an isothermal gas-phase decomposition reaction as follows: A → B + C. The laboratory measurements below show the chemical reaction rate as a function of conversion. The temperature of reaction was 422 K and the total pressure 10 atm, with the initial feed containing an equimolar mixture of reactant A and inert gases entering the reactor with a flow rate of 6 dm^3/s.

X	$-r_A$ (mol/dm^3 · s)
0.0	0.0053
0.1	0.0052
0.2	0.0050
0.3	0.0045
0.4	0.0040
0.5	0.0033
0.6	0.0025
0.7	0.0018
0.8	0.00125
0.85	0.00100

(a) Calculate the volume necessary to achieve 80% conversion in a PFR using the performance equation. How does this compare to the volume of CSTR calculated for the same reaction earlier?

(b) On a plot of $-\left(\frac{1}{r_A}\right)$ v/s conversion *X,* show the volume of PFR necessary to achieve 80% conversion.

Generally, for all positive order reactions, a smaller PFR volume is required than the CSTR volume to achieve the same conversion for a reaction. This is graphically shown for an example below.



The reason for a smaller PFR compared to CSTR for positive order reactions can also be realized intuitively. In the case of a CSTR, the reactor operates at a lower reaction rate (governed by the exit concentration). In a PFR, the reaction rate varies along the length of the reactor with higher rates of reaction observed at the start of the reactor (due to higher reactant concentration at the start of the reactor).

CHAPTER 5. EXPERIMENTAL DETERMINATION OF RATE LAW

In the previous chapter, we saw that if the rate expression $(-r_A)$ is known, we can substitute it in the appropriate performance equation of a batch reactor or CSTR or PFR to determine the size (i.e. volume) of the reactor required to achieve a specified conversion. In this chapter, we focus on ways of obtaining and analysing reaction rate data to formulate the rate law for a specific reaction.

Two common types of reactors are used to obtain reaction rate data: (i) batch reactor and (ii) flow reactors. In this chapter, we will restrict ourselves to the use of a batch reactor for obtaining reaction rate data. As we have already seen, a batch reactor is simply a container to hold the contents while they react. The batch reactor, which is best suited for small-scale laboratory experimentation, is usually operated isothermally and at constant volume. In reaction rate experiments with a batch reactor, concentration or pressure is usually measured and recorded at different times during the course of the reaction.

There are two procedures for analyzing kinetic data, the (i) differential and (ii) integral methods.

Differential method of rate analysis

The differential method of analysis is applicable when the reaction rate is essentially a function of the concentration of only one reactant; for example, in the case of decomposition reactions of the type A → products

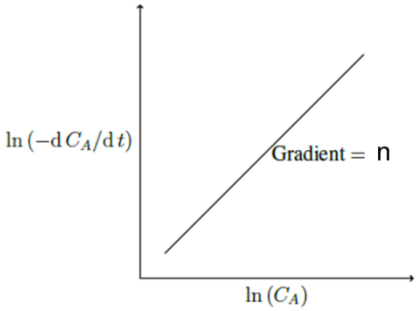
The rate law takes the form $-r_A = kC_A^n$ where n is the reaction order

When the reaction is performed in a constant-volume batch reactor, we can substitute the above rate law in the performance equation of the batch reactor:

$$-r_A = -\frac{1}{V}\frac{dN_A}{dt} - \frac{dC_A}{dt} = kC_A^n$$

$$\ln\left(-\frac{dC_A}{dt}\right) = lnk_A + nlnC_A$$

Following from the above equation, a plot of $\ln\left(-\frac{dC_A}{dt}\right)$ versus lnC_A will have a slope = reaction order (as shown below)



Using this method will require determining the derivate at various time points from the C_A versus time data. The derivative can be calculated using numerical differentiation formulae or differentiation of a polynomial fit to the collected data.

Question: The decomposition of N_2O_5 is studied in a constant volume batch reactor at 340K. Starting with an initial reactant concentration of 0.16 M, the concentration of N_2O_5 in the batch reactor for the first 4 minutes of the reaction is measured as follows:

Time, min	1	2	3	4
C_{N2O5}, M	0.113	0.08	0.056	0.04

Based on this data, use the differential method of analysis to determine the reaction order and rate constant.

Integral method of rate analysis

The integral method uses a trial-and-error approach to determine the reaction order from the collected kinetic data. We first guess the reaction order and integrate the differential equation describing the performance of a batch reactor. If the order assumed is correct, the concentration-time relationship obtained after integration will give a satisfactory fit of the concentration-time data. If the fit is not satisfactory, a different reaction order would have to be considered, until a reasonable fit is found.

Case (a): Irreversible Unimolecular-Type First-Order Reactions (Type: A → products)

To test first-order rate equation:

$$-r_A = -\frac{dC_A}{dt} = kC_A$$

Separating and integrating,

$$-\int_{C_{A0}}^{CA} \frac{dC_A}{C_A} = k\int_0^t dt$$

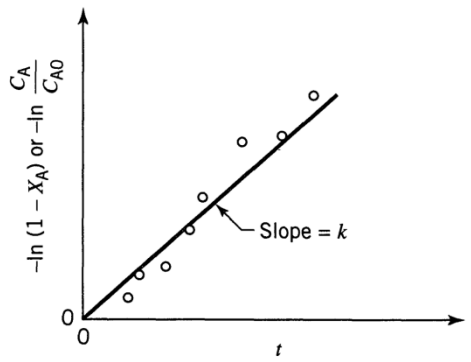
Giving,

$$-\ln\left(\frac{C_A}{C_{A0}}\right) = kt$$

In terms of conversion,

$$-\ln(1 - X_A) = kt$$

Therefore, for this reaction type, a plot of $-\ln(1 - X_A)$ or -ln $\left(\frac{C_A}{C_{A0}}\right)$ versus time will be a straight line through the origin as shown in the figure below. If the measured experimental data, when plotted on these axes, does not yield a straight line, another reaction order would have to be tested.



So if you are conducting an isothermal reaction in a batch reactor, X_A will increase with time but rate constant k should remain unchanged (as k is only a function of temperature).

Case (b): Irreversible Unimolecular-Type Second-Order Reactions (Type: A → products)

To test second-order rate equation:

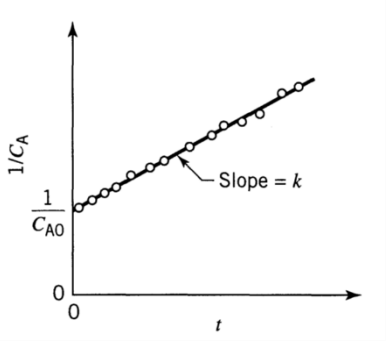
$$-r_A = -\frac{dC_A}{dt} = kC_A{}^2$$

Separating and integrating,

$$-\int_{C_{A0}}^{CA} \frac{dC_A}{C_A{}^2} = k\int_0^t dt$$

Giving,

$$\frac{1}{C_A} - \frac{1}{C_{A0}} = kt = \frac{1}{C_{A0}}\left(\frac{X_A}{1-X_A}\right)$$



Case (c): Irreversible Bimolecular-Type Second-Order Reactions (Type: A + B → products)

The corresponding rate equation is:

$$-r_A = -\frac{dC_A}{dt} = -\frac{dC_B}{dt} = kC_AC_B$$

The amount of reactants 'A' and 'B' that would have reacted after time *t* are equal and is given by $C_{A0}X_A$. Therefore, rate equation can be rewritten as:

$$-r_A = C_{A0}\frac{dX_A}{dt} = k(C_{A0} - C_{A0}X_A)(C_{B0} - C_{A0}X_A)$$

Denoting the initial molar ratio of reactants as M, where $M = \frac{C_{B0}}{C_{A0}}$

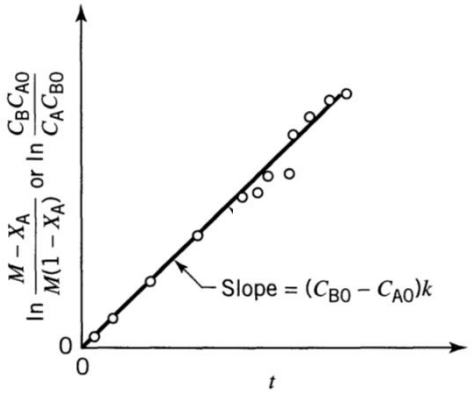
$$-r_A = C_{A0}\frac{dX_A}{dt} = kC_{A0}{}^2(1-X_A)(M-X_A)$$

or

$$\int_0^{X_A} \frac{dX_A}{(1-X_A)(M-X_A)} = C_{A0}k\int_0^t dt$$

After breakdown into partial fractions, integration, and rearrangement,

$$ln\frac{M - X_A}{M(1 - X_A)} = C_{A0}(M - 1)kt$$



Note that the above expression and graphical representation can only be used for cases where M ≠ 1 i.e. initial molar concentrations of the reactants are not equal. However, if $C_{A0} = C_{B0}$, the reaction can be treated similar to Case (b) earlier i.e. second-order A→ products

Question: The decomposition of N_2O_5 is studied in a constant volume batch reactor at 340K. Starting with an initial reactant concentration of 0.16 M, the concentration of N_2O_5 in the batch reactor for the first 4 minutes of the reaction is measured as follows:

Time, min	1	2	3	4
C_{N2O5}, M	0.113	0.08	0.056	0.035

Based on this data, use the integral method of analysis to determine the reaction order and rate constant.

Question: Hydrogen peroxide decomposes by first-order kinetics, and in a batch reactor 50% of A is converted in a 5-minute run. How much longer would it take to reach 75% conversion?

Hint: Start with the equation for first-order reaction derived under integral method of analysis (Case (a))

Question: Repeat the previous problem for second-order kinetics.

CHAPTER 6. SIMULATION EXERCISE ON CHEMICAL REACTORS

This chapter will help reinforce the fundamental concepts you have learnt about the three types of reactors (Batch reactors, CSTR and PFR) by engaging with interactive simulations. This is a fun learning activity that makes you think and reflect on the concepts you have learnt.

You will use the interactive simulations available on reactorlab.net. Specifically, you will use the following simulations:

Batch reactor: https://reactorlab.net/web_labs/web_lab_13/

Continuous Stirred Tank Reactor (CSTR): https://reactorlab.net/web_labs/web_lab_14/

Plug Flow Reactor (PFR): https://reactorlab.net/web_labs/web_lab_15/

In each of these simulations, you can vary several parameters, such as reaction temperature, initial concentration of the reactant, reactor volume etc. to see the impact it has on the final conversion achieved. In these simulations, you are modelling reactions of the type:

$$A \rightarrow \text{products}$$

where the order of the reaction in reactant 'A' can be chosen as -1, 0, 1 or 2.

After inputting the values, click on 'Run' and the software will display the final conversion (in %) and final concentration of the reactant 'A' (in mol/m^3) on the right. Suitable graphical display of the simulation will also be shown. You can clear your results by clicking on 'Reset'. However, clicking on 'Reset' will **not** reset the values of the parameters to the default values. They will remain on the last set of values you used to run the simulation.

Batch reactor Simulation exercise

Question (i). What is the conversion of the reaction achieved in the batch reactor with the default parameter values as follows:

Temperature (K)	Reactant concentration (C_{A0}, mol/m^3)	Reactor Volume (V, m^3)	Reaction time (s)	Rate constant, k	Activation energy (E_a, kJ/mol)	Reaction order, n
300	10	100	100	0.01	60	1

Question (ii). Next, you perform the same reaction under the same conditions but in a bigger batch reactor of volume 200 m^3. What is the conversion of the reaction achieved after 100 seconds in this bigger reactor? How does it compare to the conversion you got in the previous question? Can you reason your findings?

Question (iii). Let us go back to using a batch reactor of volume 100 m^3. We are now interested in determining the evolution of the reaction over time. What is the conversion of the reaction in this batch reactor after 100 s, 200 s and 300 s? What is the trend you observe of conversion v/s time? Can you reason why this trend is observed?

Temperature (K)	Reactant concentration (C_{A0}, mol/m^3)	Reactor Volume (V, m^3)	Reaction time (s)	Rate constant, k	Activation energy (E_a, kJ/mol)	Reaction order, n
300	10	100	100, 200, 300	0.01	60	1

Question (iv). Next, let us evaluate the effect of reaction rate constant 'k' on the conversion achieved in the batch reactor. We are currently working with a first order reaction. So what will the units of rate constant 'k' be? What is the conversion of the reaction when k = 0.02 and k = 0.03 (other parameters as in table below)? How does this compare to the case of k = 0.01? How do these three conversion values compare to the values you got in Question (iii) earlier? Can you explain the similarities in values?

Temperature (K)	Reactant concentration (C_{A0}, mol/m^3)	Reactor Volume (V, m^3)	Reaction time (s)	Rate constant, k	Activation energy (E_a, kJ/mol)	Reaction order, n
300	10	100	100	0.01, 0.02, 0.03	60	1

Question (v). The next task is to compare the conversion achieved in the batch reactor for a first-order reaction with that of a second-order reaction. For the same reactor volume and the other conditions remaining unchanged (as in table below), what is the conversion for a 2nd order reaction? Is it lower or higher than what was achieved for a 1st order reaction? Can you explain why it is higher or lower?

Temperature (K)	Reactant concentration (C_{A0}, mol/m^3)	Reactor Volume (V, m^3)	Reaction time (s)	Rate constant, k	Activation energy (E_a, kJ/mol)	Reaction order, n
300	10	100	100	0.01	60	1, 2

CSTR Simulation exercise

Question (vi). Input the following default parameter values for the CSTR simulation. Based on the given data, what is the value of space time τ in seconds? What is the conversion achieved in the CSTR under these conditions?

Temperature (K)	Reactant concentration (C_{A0}, mol/m^3)	Reactor Volume (V, m^3)	Volumetric flow rate (v_0, m^3/s)	Rate constant, k	Activation energy (E_a, kJ/mol)	Reaction order, n
300	10	100	1	0.01	60	1

Question (vii) Next, you perform the same reaction under the same conditions (table below) but in a smaller CSTR of volume 50 m^3. What is the conversion achieved now? How does it compare to the conversion you got with the bigger CSTR in Question (vi)? Can you reason your findings? Also, how are the findings different to Question (ii), where you investigated the effect of batch reactor volume on conversion?

Temperature (K)	Reactant concentration (C_{A0}, mol/m^3)	Reactor Volume (V, m^3)	Volumetric flow rate (v_0, m^3/s)	Rate constant, k	Activation energy (E_a, kJ/mol)	Reaction order, n
300	10	50	1	0.01	60	1

Question (viii) Let us go back to using the CSTR of volume 100 m^3. We are now interested in determining the impact of doubling the volumetric flow rate of the reactant on conversion. How does the CSTR conversion change when volumetric flow rate is changed from 1 to 2 m^3/s? How does this conversion compare to what you got in the previous question? Can you reason the similarity in conversion values?

Temperature (K)	Reactant concentration (C_{A0}, mol/m^3)	Reactor Volume (V, m^3)	Volumetric flow rate (v_0, m^3/s)	Rate constant, k	Activation energy (E_a, kJ/mol)	Reaction order, n
300	10	100	2	0.01	60	1

PFR Simulation exercise

Question (ix). When using the same default reaction conditions as we used for the CSTR (Question (vi) and in table below), what final conversion is achieved for the same reaction in a PFR? Is the conversion higher or lower than that obtained in a CSTR? Why is it so? Can you understand this through a graphical representation of the performance equation of a CSTR and PFR?

Temperature (K)	Reactant concentration (C_{A0}, mol/m^3)	Reactor Volume (V, m^3)	Volumetric flow rate (v_0, m^3/s)	Rate constant, k	Activation energy (E_a, kJ/mol)	Reaction order, n
300	10	100	1	0.01	60	1

Question (x). For the same reaction, can you compute the size of the PFR (or reactor volume) needed to get the same conversion as you got with the 100 m^3 CSTR in Question (vi)?

Temperature (K)	Reactant concentration (C_{A0}, mol/m^3)	Reactor Volume (V, m^3)	Volumetric flow rate (v_0, m^3/s)	Rate constant, k	Activation energy (E_a, kJ/mol)	Reaction order, n
300	10	?	1	0.01	60	1

Question (xi). In this question, you have to evaluate the impact of initial reactant concentration (C_{A0}) on the final conversion achieved in a PFR for a 1st order and 2nd order reaction. First, consider the case of a 1st order reaction. How does the final conversion change when initial concentration is halved from 10 to 5 mol/m^3?

Temperature (K)	Reactant concentration (C_{A0}, mol/m^3)	Reactor Volume (V, m^3)	Volumetric flow rate (v_0, m^3/s)	Rate constant, k	Activation energy (E_a, kJ/mol)	Reaction order, n
300	5, 10	100	1	0.01	60	1

Next, repeat the same thing for a 2nd order reaction. How does the final conversion change when initial concentration is halved from 10 to 5 mol/m^3? How are the findings different from what you observed in the case of 1st order reaction? Can you use the performance equation to explain your findings?

Temperature (K)	Reactant concentration (C_{A0}, mol/m^3)	Reactor Volume (V, m^3)	Volumetric flow rate (v_0, m^3/s)	Rate constant, k	Activation energy (E_a, kJ/mol)	Reaction order, n
300	5, 10	100	1	0.01	60	2

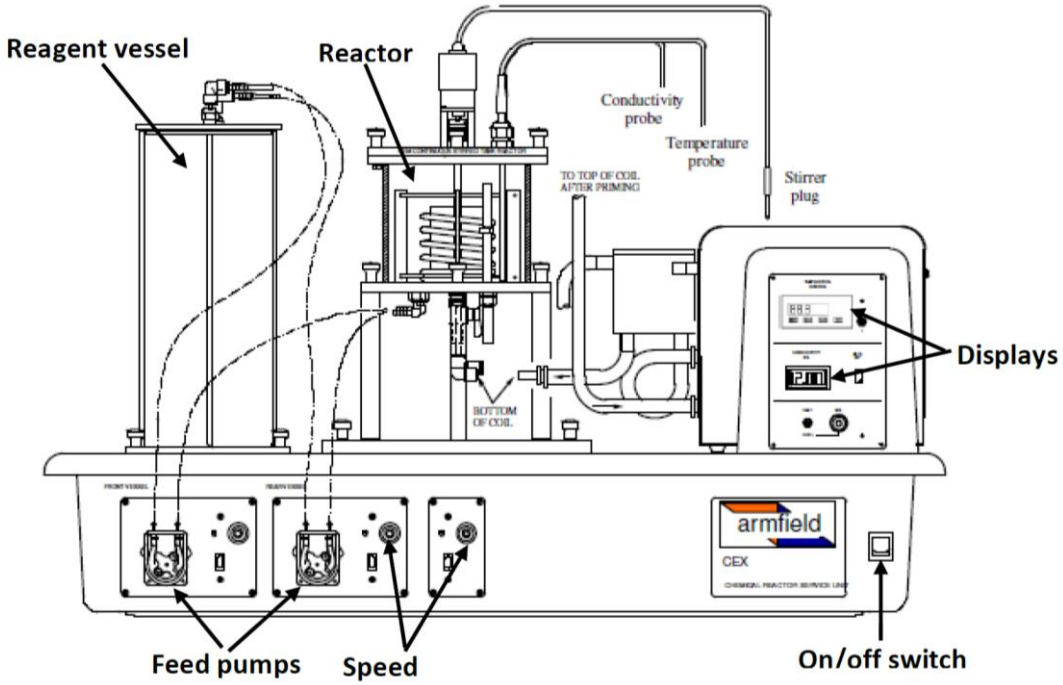
CHAPTER 7. CSTR LAB PRACTICAL

Objective

To operate a continuous stirred tank reactor (CSTR) and study a homogeneous liquid-phase reaction through careful experiment design and analysis of resulting data.

Description of apparatus

The figure below is a labelled drawing of the experimental apparatus – the Armfield CEM-MkII CSTR unit. Two feed pumps are used to pump the chemical reagents from the two feed tanks into the reactor separately through connectors in the base of the reactor. The reaction temperature is controlled by circulating heating or cooling liquid through the stainless-steel coil inside the reactor, which provides the heat transfer surface for either heating or cooling the chemical reactants. A variable speed agitator/mixer is used to provide efficient mixing. The progress of the reaction, i.e. the conversion of the reactants to products, is tracked by measuring the changes of ionic conductivity of the reaction solution using a conductivity probe.



Reaction Chemistry

In this lab practical, the following reaction will be studied:

$$NaOH + CH_3COOC_2H_5 \rightarrow CH_3COONa + C_2H_5OH$$

Sodium hydroxide + ethyl acetate → sodium acetate + ethyl alcohol

0.1 M aqueous solutions (initial reactant concentration) of NaOH and $CH_3COOC_2H_5$ will be provided.

Lab safety

Before coming to the lab, inform yourself of the hazards and operational risk associated with performing this experiment. These can be found on Minerva in the 'Lab safety' folder under 'Assignment – CSTR Lab practical'.

It is essential to wear protective clothing, gloves and safety glasses when handling the chemicals used in this experiment: Refer to the Material Data Sheets for more details.

When in any doubt about safe laboratory practice, always ask the teaching staff in the laboratory!

General methodology

- Make up 5.0 litre batches of 0.1 M sodium hydroxide and ethyl acetate wearing the required personal protective equipment. (This will generally be done for you)
- Remove the lids of the reagent vessels in the apparatus and carefully fill to a level approximately 50 mm from the top. Refit the lids.
- Adjust the set point of the temperature controller to the required temperature, e.g. 30 °C.
- Set the pump speed flow control to deliver the required flow rate of reactants.
- Set the agitator speed controller to the required value.
- Switch on both pumps and the agitator motor and start the data logger program to record ionic conductivity data.

Interpretation of results

To understand how the conductivity data can be used to calculate conversion in the CSTR, let us follow the following nomenclature for concentrations:

$C_{A\mu}$: NaOH concentration in the feed vessel (mol dm^{-3})

C_{A0}: initial NaOH concentration in the mixed feed stream (mol dm^{-3})

C_{A1}: NaOH concentration inside CSTR which is same as in reactor outlet (mol dm^{-3})

$C_{A\infty}$: NaOH concentration inside CSTR at maximum possible conversion (mol dm^{-3})

C_B: ethyl acetate concentn. (with same subscripts as above: $C_{B\mu}$, C_{B0}, C_{B1}, $C_{B\infty}$) (mol dm^{-3})

C_C: sodium acetate concentn. (with same subscripts as above: $C_{C\mu}$, C_{C0}, C_{C1}, $C_{C\infty}$)(mol dm^{-3})

And the following nomenclature for volumetric flow rates:

v: total volumetric feed rate (dm^3 s^{-1})

v_A: volumetric feed rate of sodium hydroxide (dm^3 s^{-1})

v_B: volumetric feed rate of ethyl acetate (dm^3 s^{-1})

Therefore, the initial concentration of the two reactants in the mixed feed is related to the concentration in the feed vessel and volumetric flow rates as follows:

$$C_{A0} = \left(\frac{v_A}{v_A + v_B}\right) C_{A\mu}$$

$$C_{B0} = \left(\frac{v_B}{v_A + v_B}\right) C_{B\mu}$$

Also realize that the following expressions for the concentration of the product, sodium acetate, are true:

$$C_{C\infty} = C_{A0} - C_{A\infty}$$

$$C_{C1} = C_{A0} - C_{A1}$$

Through the above expressions, we can also write down the expressions for the final concentration of the reactant NaOH:

$$C_{A\infty} = 0 \qquad for\ C_{A0} < C_{B0}$$

$$C_{A\infty} = C_{A0} - C_{B0} \qquad for\ C_{A0} \geq C_{B0}$$

We are particularly interested in the concentrations of NaOH (A) and sodium acetate (C) because both sodium hydroxide and sodium acetate contribute to the measured conductivity of the reaction solution while ethyl acetate and ethanol do not. This is because of the presence of sodium ions in sodium hydroxide and sodium acetate.

Let us follow the following nomenclature for conductivity (Λ):

Λ_0: initial ionic conductivity (Siemens)

Λ_1: ionic conductivity at measured experimental time (Siemens)

Λ_∞: ionic conductivity at maximum possible conversion (Siemens)

Conductivity is an additive property and since only sodium hydroxide (A) and sodium acetate (C) contribute to the measured conductivity, we can write

$$\Lambda = \Lambda_A + \Lambda_C$$

Writing down the same expression at maximum possible conversion:

$$\Lambda_\infty = \Lambda_{A\infty} + \Lambda_{c\infty}$$

Meanwhile at the start of the reaction,

$$\Lambda_0 = \Lambda_{A0}\ (since\ C_{C0} = 0)$$

The ionic conductivity of sodium hydroxide and sodium acetate at a given concentration and temperature (T > 294 K) are expressed through the following relationships:

$$\Lambda_{A0} = 0.195[1 + 0.0184(T - 294)]C_{A0}$$

$$\Lambda_{A\infty} = 0.195[1 + 0.0184(T - 294)]C_{A\infty}$$

$$\Lambda_{C\infty} = 0.070[1 + 0.0284(T - 294)]C_{C\infty}$$

We are now interested in calculating the concentrations of sodium hydroxide and sodium acetate represented as C_{A1} and C_{C1} respectively, from the measured conductivity data.

Based on the above expressions,

$$\frac{\Lambda_{A\infty}}{C_{A\infty}} = \frac{\Lambda_0}{C_{A0}};\ thus\ \Lambda_{A\infty} = \frac{\Lambda_0}{C_{A0}}.C_{A\infty}$$

Substituting for $\Lambda_{A\infty}$ in $\Lambda_\infty = \Lambda_{A\infty} + \Lambda_{C\infty}$, we get,

$$\Lambda_{c\infty} = \Lambda_\infty - \frac{\Lambda_0}{C_{A0}}.C_{A\infty}$$

Similarly,

$$\frac{\Lambda_{A1}}{C_{A1}} = \frac{\Lambda_0}{C_{A0}}; thus\ \Lambda_{A1} = \frac{\Lambda_0}{C_{A0}}.C_{A1}$$

Substituting for Λ_{A1} in $\Lambda_1 = \Lambda_{A1} + \Lambda_{C1}$, we get,

$$\Lambda_{C1} = \Lambda_1 - \frac{\Lambda_0}{C_{A0}}.C_{A1}$$

Similarly,

$$\frac{\Lambda_{C\infty}}{C_{C\infty}} = \frac{\Lambda_{C1}}{C_{C1}}\ or\ \frac{C_{C\infty}}{C_{C1}} = \frac{\Lambda_{c\infty}}{\Lambda_{c1}}$$

From expressions forC_{C1} and $C_{C\infty}$ written earlier, $\frac{C_{C\infty}}{C_{C1}} = \frac{C_{A0}-C_{A\infty}}{C_{A0}-C_{A1}}$

Therefore,

$$\frac{C_{C\infty}}{C_{C1}} = \frac{C_{A0} - C_{A\infty}}{C_{A0} - C_{A1}} = \frac{\Lambda_{c\infty}}{\Lambda_{c1}}$$

Substituting expressions for $\Lambda_{C\infty}$ and Λ_{c1} from above,

$$\frac{C_{A0} - C_{A\infty}}{C_{A0} - C_{A1}} = \frac{\Lambda_\infty - \frac{\Lambda_0}{C_{A0}}.C_{A\infty}}{\Lambda_1 - \frac{\Lambda_0}{C_{A0}}.C_{A1}}$$

Solving the above equation for C_{A1}, we get,

$$C_{A1} = C_{A0} + (C_{A\infty} - C_{A0})\left(\frac{\Lambda_0 - \Lambda_1}{\Lambda_0 - \Lambda_\infty}\right)$$

Since $\frac{C_{C1}}{C_{C\infty}} = \frac{C_{A0}-C_{A1}}{C_{A0}-C_{A\infty}}$, we can write the expression for C_{C1} as

$$C_{C1} = C_{C\infty}\left(\frac{\Lambda_0 - \Lambda_1}{\Lambda_0 - \Lambda_\infty}\right)$$

Thus, by measuring the ionic conductivity, the concentrations of reactants and products can be deduced.

The conversion of sodium hydroxide is given as

$$X_A = \frac{C_{A0} - C_{A1}}{C_{A0}}$$

Mass balance on CSTR

We can write the mass balance on the CSTR to evaluate the reaction rate constant for this second-order reaction.

$$In\ flow = Out\ flow + Rate\ of\ Consumption + Rate\ of\ Accumulation$$

Since we are concerned with the steady-state operation of CSTR, the accumulation term = 0

In accordance with the nomenclature introduced earlier, v is the volumetric flow rate of the feed and C_{A0} is the concentration of NaOH in the entering feed. We can now describe the inflow, out flow and consumption terms in the mass balance for reactant NaOH as follows:

$$In\ flow\ of\ A\ \left(\frac{moles}{time}\right) = vC_{A0}$$

$$Out\ flow\ of\ A\ \left(\frac{moles}{time}\right) = vC_{A1}$$

For a second-order reaction with equimolar feed of sodium hydroxide and ethyl acetate,

$$Consumption\ of\ A\ by\ reaction\ \left(\frac{moles}{time}\right) = (-r_A).V = kC_{A1}^2.V$$

The mass balance for reactant 'A' then simplifies to:

$$vC_{A0}\ = vC_{A1} + kC_{A1}^2.V$$

which can be re-written for the reaction rate constant as,

$$k = \frac{v}{V}.\left(\frac{C_{A0} - C_{A1}}{C_{A1}^2}\right)$$

or,

$$k = \frac{(v_A + v_B)}{V}.\left(\frac{C_{A0} - C_{A1}}{C_{A1}^2}\right)$$

Hence, the rate constant of this reaction can be experimentally determined.

CHAPTER 8. INTRODUCTION TO BIOCHEMICAL ENGINEERING

Biochemical engineering refers to the technology involved in the manufacturing of products using biological cells/tissues/enzymes etc. Therefore, this topic requires the extension of chemical engineering principles to systems using biological materials/catalysts.

Biochemical engineers are interested in translating lab discoveries in life sciences to real processes and products (for example, producing the COVID-19 vaccine at scale).

Biochemical processes are an essential feature of several industries in the food, chemical and pharmaceutical sector. Biochemical processes use microbial, animal or plant cells, or components of cells such as enzymes, to manufacture products of value.

Some examples of biochemical processes:

- Synthesis of pharmaceuticals: antibiotics, insulin, vaccines etc.
- Synthesis of commodity chemicals such as acetic acid, acetone and ethanol from biomass
- Synthesis of speciality chemicals: enzymes and vitamins
- Manufacture of food and beverage products

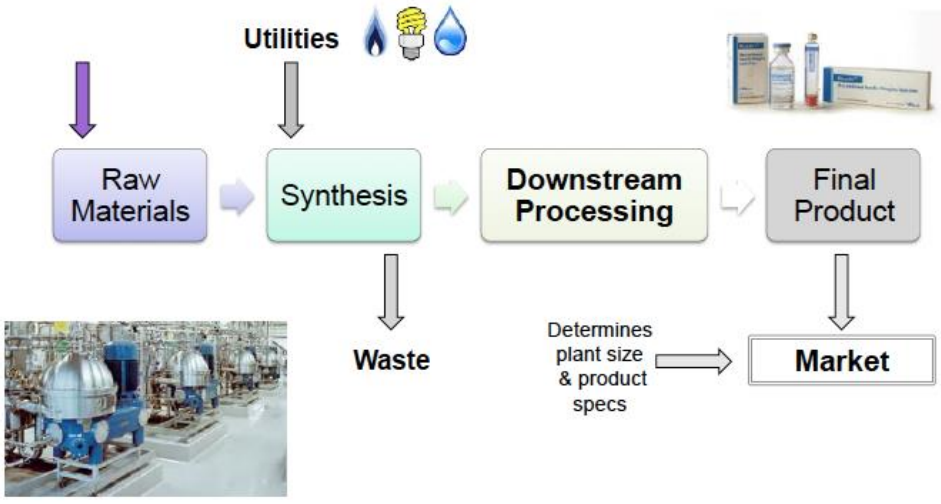
The rapid progress in biotechnology makes biochemical engineers all the more important in the future. Think of all that has been accomplished in the recent past:

- Molecular-level manipulation of DNA & personalised medicine
- Biologically manufactured fuels
- Microorganisms to degrade pollutants

A simplistic block diagram of a biochemical processes is sketched below. A bioprocess is typically divided into upstream and downstream processing.

Upstream bioprocessing

Upstream bioprocessing is the first step where cells or microbes are grown in bioreactors. The growth of cells or microbes requires the presence of a nutrient-rich growth media to be present in the bioreactor. A bioreactor is a device that supports a biologically active environment containing organisms or biochemically active substances derived from organisms.



Downstream bioprocessing

Downstream processing refers to the technology involved in the recovery and purification of products from bioreactors.

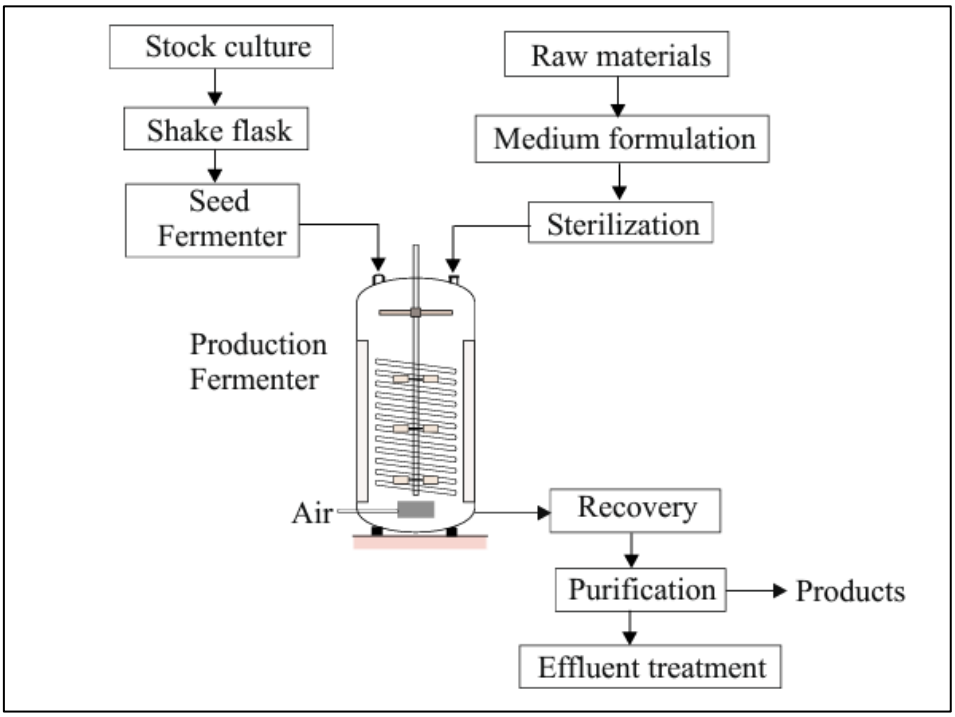
Fermentation

Fermentation is a typical upstream process which involves the chemical breakdown of a substance (typically an organic compound) by cells, such as bacteria, yeast etc.

Cells can be visualised as micro-factories and can therefore, convert raw material into products of value. For example, the manufacture of yoghurt and cheese from milk involves the use of bacterial cells while yeast (a fungus) is used to make bread and alcoholic beverages from wheat and barley.

The cell to be used in a fermentation process is chosen based on its ability to produce the desired product. Firstly, a seed stock of cells is put into a small amount of growth media in a shake flask. The media gives the required nutrition for the cells to grow. When the population of cells has grown and consumed most of the nutrients, it is moved into a larger vessel with more growth media (seed fermenter). Once a large and healthy enough cell population is achieved, it is transferred into a bioreactor or fermenter (see figure below) that is fed with plenty of fresh media. Under well-monitored conditions in the bioreactor, cells will continue to grow and manufacture the desired product. The fermentation conditions that are typically monitored include temperature, pressure, pH, oxygen level and nutrient levels.

Once the fermentation is complete (raw material in media is fully consumed), the product would have to be harvested i.e. separated from the mixture in the bioreactor. As described earlier, this recovery and purification phase of the process is called downstream processing.



Bioprocess flowsheet

There are four phases to a bioprocess flowsheet (3 of them related to upstream processing and 1 related to downstream processing):

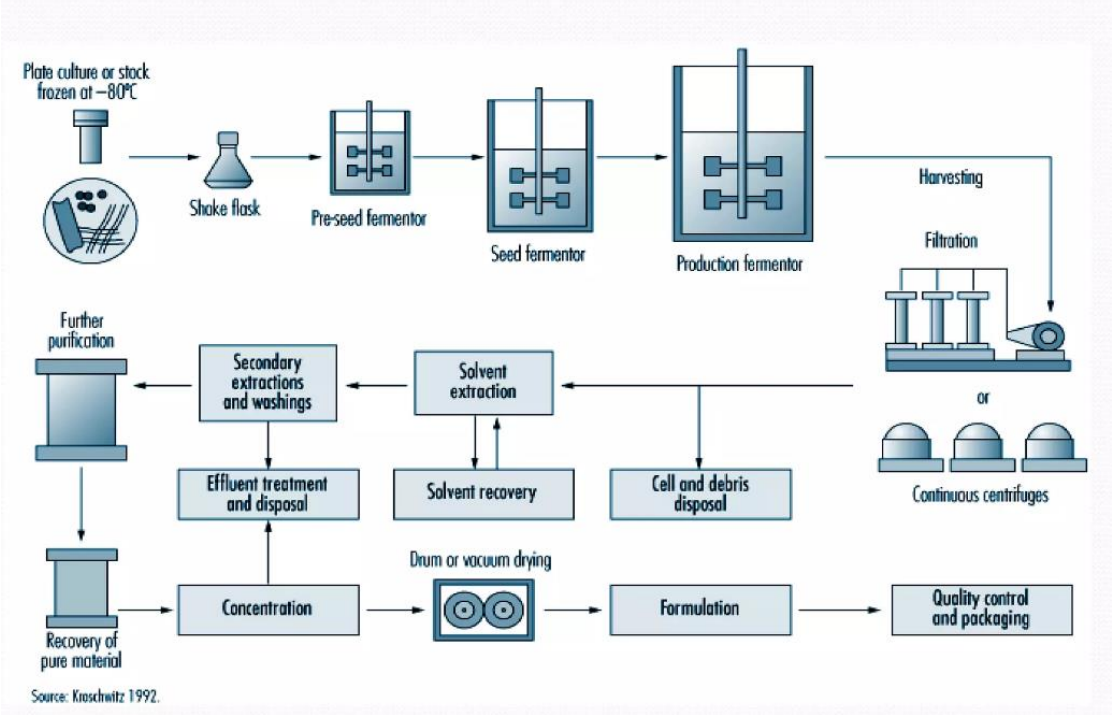
Phase 1: Cellular System: Choosing a cell line for the biochemical process.

Phase 2: Cellular Kinetics: Defining the growth/kinetic activity of the cellular system.

Phase 3: Bioreactor or fermenter design: Critical attributes include sterile conditions, agitation, temperature, scale-up.

Phase 4: Proposing the downstream process: A series of steps to remove contaminants, and isolate, purify and polish the final bioproduct.

Example biochemical process: Insulin production



The production of recombinant human insulin from a genetically modified bacterial culture is typically done using the proinsulin method and *E.coli* bacteria. Proinsulin is the prohormone precursor to insulin.

Phase 1 & 2: *E.coli* is chosen as the cellular system as it has simple and well-understood genetics. It is also easy to manipulate (Human insulin gene has to be added to modify *E.coli* plasmid DNA). Initial culture of proinsulin producing bacteria is grown under appropriate growth conditions.

Phase 3: The grown bacteria is placed in a bioreactor filled with growth media containing carbon, nitrogen and phosphorous sources to promote further bacterial growth and proinsulin production.

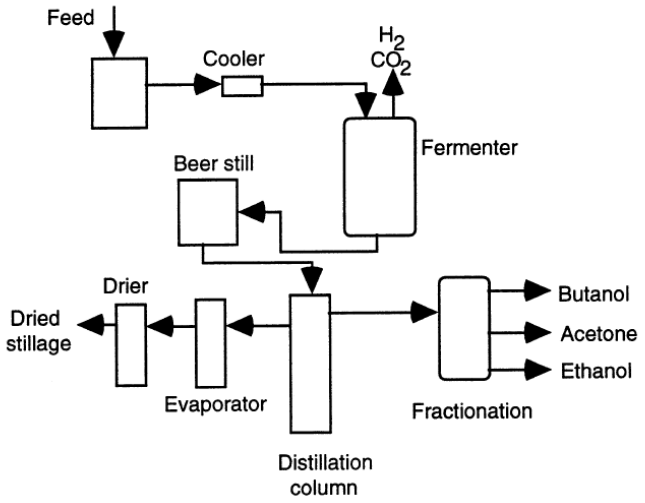
Phase 4: First step in downstream processing is the isolation of bacterium containing proinsulin inclusion bodies, typically achieved by filtration and centrifugation. Then the proinsulin has to be separated from rest of cell debris by cell disruption, further centrifugation and purification steps.

Example biochemical process: Acetone-butanol production

Acetone and butanol have wide applications as industrial solvents and are also used in the production of detergents. While they are conventionally manufactured from petrochemical intermediates, there is increasing interests to produce these chemicals from a sustainable feedstock. This can be rendered possible by biochemical engineering.

Clostridium species are used for acetone–butanol production. *C. acetobutylicum* can ferment a large number of carbohydrates such as glucose, lactose, fructose, galactose, xylose, sucrose, maltose, and starch. The fermentation products include acetone, butanol, and ethanol.

Starch, molasses, cheese whey, Jerusalem artichoke, and lignocellulosic hydrolyzates can be used as raw material for acetone–butanol fermentations. Molasses can be used as carbon source (6% sucrose) with the addition of nitrogen and phosphate. Lignocellulosics hydrolyzates (wood, paper, crop residues) contain glucose, galactose, mannose, and pentose sugars, most of which are fermentable by *C. acetobutylicum* to acetone and butanol. Varying ratios of acetone and butanol are obtained depending on the nature of the carbon source (feedstock).



In the simplied block diagram shown above, starch is used as raw material. *C. acetobutylicum* enzymatically hydrolyzes gelatinized corn starch to glucose and maltose. The grain mesh is first gelatinized at 65°C and then sterilized at 105°C. The cooked mash is cooled down to 35°C and is pumped to fermenters where the batch fermentation period is usually 2–2.5 days. First, rapid growth and production of acetic/butyric acids and carbon dioxide and hydrogen occur. In a second phase, growth ceases, and the organisms convert acetic and butyric acids to acetone and butanol.

To recover acetone and butanol, at the end of the solvent phase the broth is transferred to a beer still that concentrates solvents. Solvents are then separated by fractionation, and the stillage is dried.

Production by fermentation is not yet economically attractive due to low levels of product concentrations (0.7–1.5%) and high cost of product recovery. However, fermentation may be the preferred method for production if a shortage of oil products exists, or as demands for environmentally friendly processes further increase.

CHAPTER 9. BIOLOGY BASICS

This chapter is a condensed version of Chapter 2 of the textbook 'Bioprocess Engineering Basic Concepts' 2nd Edition by Shuler and Kargi.

Types of cells

Cells are the basic building blocks of all living things. For example, our human body is composed of trillions of cells. Living cells can be quite diverse, occupying a wide variety of habitats and taking up widely ranging sizes and shapes. Furthermore, the temperature, pH, and moisture requirements for growth also vary from one kind of cell to another. Cells that grow well at low temperatures (below 20°C) are called *psychrophiles*, and those that are suited to temperatures greater than 50°C are called *thermophiles*.

Likewise, some cells require oxygen for their growth and metabolism, while for some other types of cells, the presence of oxygen will inhibit growth. The former type is called *aerobic* while the latter is classified as *anaerobic*.

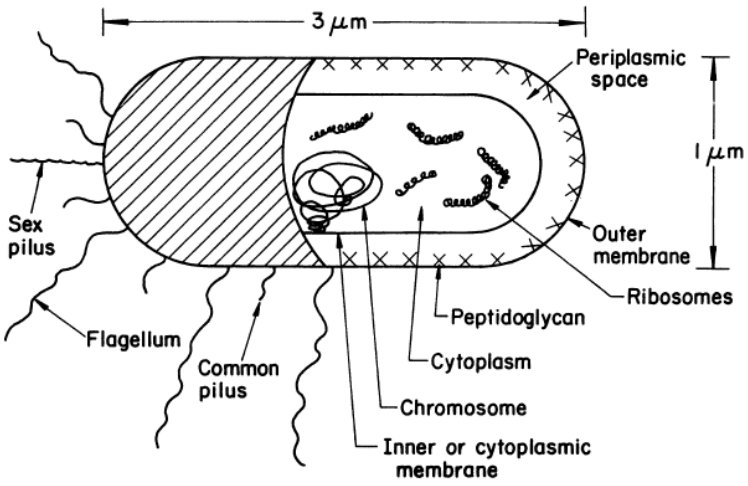
The nomenclature of microorganisms is usually done using a binary system. Example: *E. coli*, which is a common gut organism. *E.* stands for *Escherichia*, which represents the genus, which is a group of related species. *Coli* is the specific species, which includes organisms that are substantially alike.

There are two primary cell types: *prokaryotic and eukaryotic*. Procaryotes have a simple structure with a single chromosome. Prokaryotic cells have no nuclear membrane and no organelles. Eukaryotic cells have a true nuclear membrane and contain mitochondria, endoplasmic reticulum, golgi apparatus, and a variety of specialized organelles.

Procaryotes

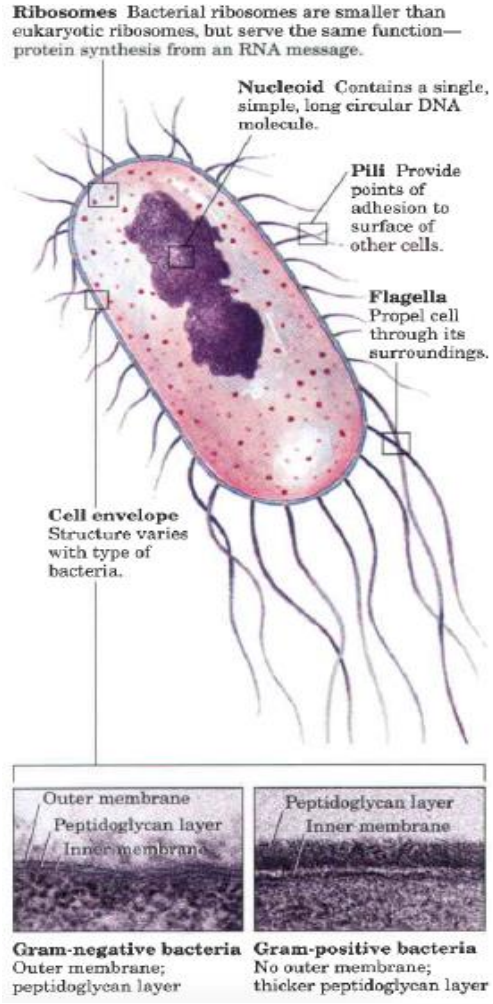
Most prokaryotes vary from 0.5 to 3 micrometers in radius. The prokaryotes can be divided into two major groups: the *eubacteria* and *archaebacteria*.

Eubacteria are further classified based on the gram stain. The staining procedure first requires fixing the cells by heating. The basic dye, crystal violet, is added; all bacteria will stain purple. Next, iodine is added, followed by the addition of ethanol. *Gram-positive* cells remain purple, while *gram-negative* cells (schematic figure below) become colourless.



A typical gram-negative cell is *E.coli*. It has an outer membrane supported by a thin peptidoglycan layer. Peptidoglycan is a complex polysaccharide with amino acids and forms a chain-like fence. A second membrane (the inner or cytoplasmic membrane) exists and is separated from the outer membrane by the periplasmic space.

Gram-positive cells do not have an outer membrane. Rather they have a very thick, rigid cell wall with multiple layers of peptidoglycan.

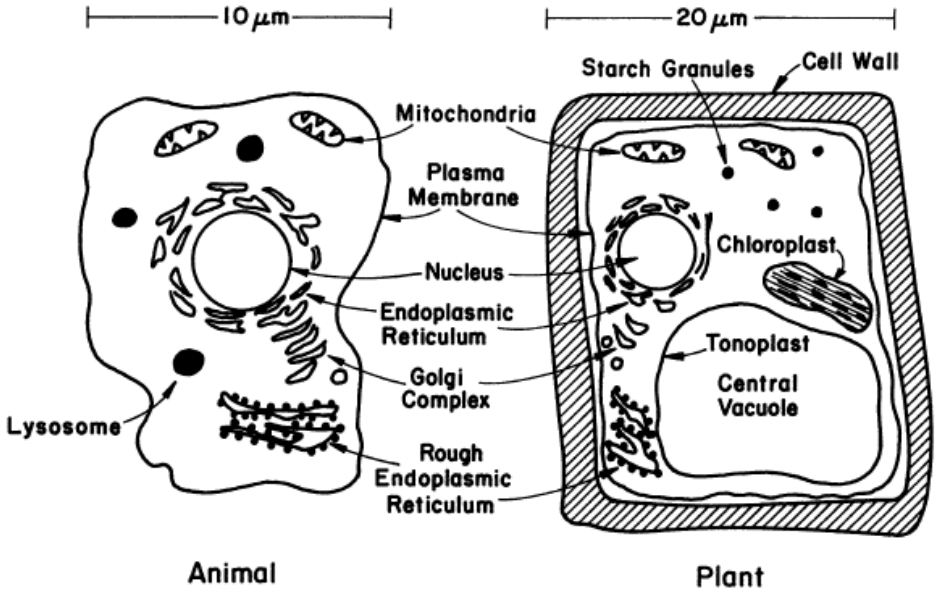


Unlike eubacteria, archaebacteria do not have a peptidoglycan and the lipid composition of the cytoplasmic membrane in eubacteria is very different compared to archaebacterial. The archaebacteria usually live in extreme environments and possess unusual metabolism. Methanogens, which are methane-producing bacteria, belong to this group.

Eukaryotes

Fungi (yeasts and molds), algae, protozoa, and animal and plant cells constitute the eukaryotes. Eukaryotes are five to ten times larger than prokaryotes in size.

In terms of cell wall and cell membrane structure, eukaryotes are similar to prokaryotes. Plant cell wall is composed of cellulose fibers embedded in pectin aggregates, which impart strength to the cell wall. Animal cells do not have a cell wall but only a cytoplasmic membrane. For this reason, animal cells are very shear-sensitive and fragile.

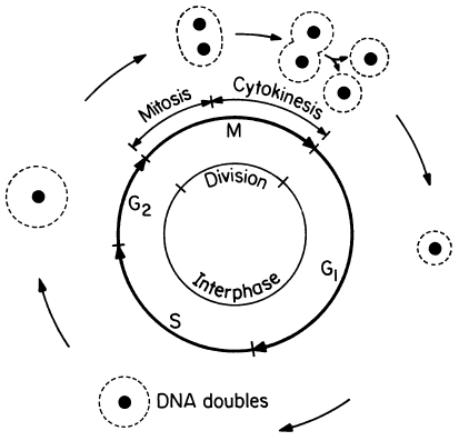


- The nucleus of eukaryotic cells contains chromosomes as nuclear material (DNA molecules with some closely associated small proteins) surrounded by a membrane.
- The mitochondria are the powerhouses of a eukaryotic cell, where respiration and oxidative phosphorylation take place. The mitochondria contain a complex system of inner membranes called cristae. A mitochondrion has its own DNA and protein-synthesizing machinery and reproduces independently.
- The rough endoplasmic reticulum contains ribosomes on the inner surfaces and is the site of protein synthesis. The smooth endoplasmic reticulum is more involved with lipid synthesis.
- Lysosomes are very small membrane-bound particles that contain and release digestive enzymes.
- Golgi bodies are very small particles composed of membrane aggregates and are responsible for the secretion of certain proteins.
- Vacuoles are membrane-bound organelles of low density and are responsible for food digestion, osmotic regulation, and waste-product storage.
- Chloroplasts are relatively large, chlorophyll-containing, green organelles that are responsible for photosynthesis.

Cell division and reproduction

Asexual cell division in eukaryotes involves several major steps, such as DNA synthesis, nuclear division, cell division, and cell separation. Sexual reproduction in eukaryotic cells involves the conjugation of two cells called gametes. The single cell formed from the conjugation of gametes is called a zygote. The zygote has twice as many chromosomes as compared to the gamete.

The cell-division cycle is divided into four phases. The M phase consists of *mitosis* where the nucleous divides, and *cytokinesis* where the cell splits into separate daughter cells. All of the phases between one M phase and the next are known collectively as the *interphase*. The interphase is divided into three phases: G_1, S, and G_2. The cell increases in size during the interphase period. In the S phase the cell replicates its nuclear DNA.



Cell construction

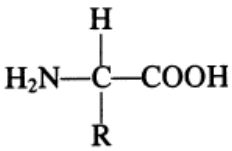
Living cells are composed of high-molecular-weight polymeric compounds such as proteins, nucleic acids, polysaccharides, lipids, and other storage materials (fats, glycogen). These biopolymers constitute the major structural elements of living cells.

In addition to these biopolymers, cells contain other metabolites in the form of inorganic salts (e.g., NH_4^+, PO_4^{3-} , K^+, Ca^{2+}, Na^+, SO_4^{2-}), metabolic intermediates (e.g., pyruvate, acetate), and vitamins. The elemental composition of a typical bacterial cell is 50% carbon, 20% oxygen, 14% nitrogen, 8% hydrogen, 3% phosphorus, and 1% sulfur, with small amounts of K^+ ,Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and vitamins.

A living cell can be visualized as a very complex reactor in which more than 2000 reactions take place. These reactions (metabolic pathways) are interrelated and are controlled in a complicated fashion.

Amino acids and proteins

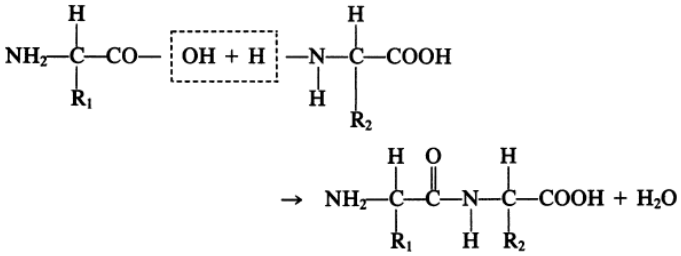
Proteins are the most abundant organic molecules in living cells, constituting 40% to 70% of their dry weight. Proteins are polymers built from amino acid monomers. Amino acids contain at least one carboxyl group and one amino group, but they differ from each other in the structure of their R groups or side chains (see amino acid chemical formula below).



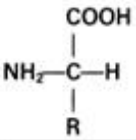
Proteins have diverse biological functions, which can be classified in five major categories:

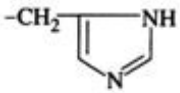
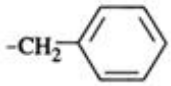
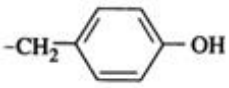
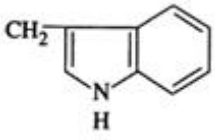
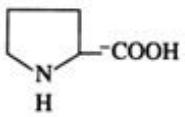
- Structural proteins: glycoproteins, collagen, keratin
- Catalytic proteins: enzymes
- Transport proteins: haemoglobin, serum albumin
- Regulatory proteins: hormones (insulin, growth hormone)
- Protective proteins: antibodies, thrombin

The proteins are amino acid chains. The condensation reaction between two amino acids results in the formation of a *peptide bond* (see reaction scheme below). The peptide bond is planar. Peptides contain two or more amino acids linked by peptide bonds.



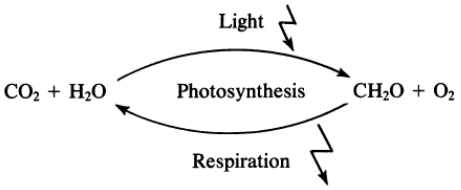
Polypeptides usually contain fewer than 50 amino acids. Larger amino acid chains are called *proteins*. Many proteins contain organic and/or inorganic components other than amino acids; such proteins are called conjugated proteins Haemoglobin is a conjugated protein and has four heme groups, which are iron-containing organometallic complexes.

Chemical Structure of 21 Amino Acids of the General Structure 

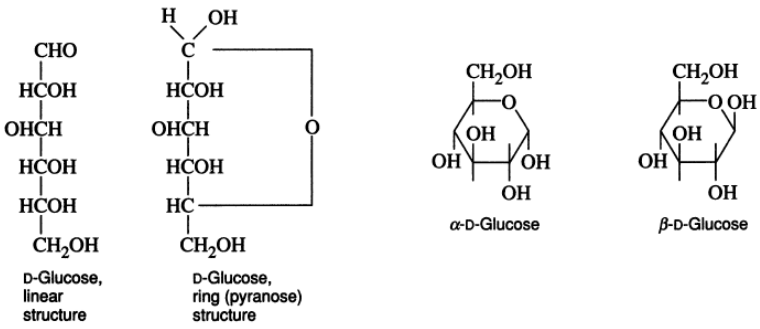
R Group	Name	Abbreviation	Symbol	Class
—H	Glycine	GLY	G	Aliphatic
—CH_3	Alanine	ALA	A	
—$CH(CH_3)_2$	Valine	VAL	V	
—$CH_2CH(CH_3)_2$	Leucine	LEU	L	
—$CHCH_3CH_2CH_3$	Isoleucine	ILU	I	
—CH_2OH	Serine	SER	S	Hydroxyl or sulfur containing
—$CHOHCH_3$	Threonine	THR	T	
—CH_2SH	Cysteine	CYS	C	
—$(CH_2)_2SCH_3$	Methionine	MET	M	
—CH_2COOH	Aspartic acid	ASP	D	Acids and corresponding amides
—CH_2CONH_2	Asparagine	ASN	N	
—$(CH_2)_2COOH$	Glutamic acid	GLU	E	
—$(CH_2)_2CONH_2$	Glutamine	GLN	Q	
—$(CH_2)_3CH_2NH_2$	Lysine	LYS	K	Basic
—$(CH_2)_3NHCNHNH_2$	Arginine	ARG	R	
	Histidine	HIS	H	
	Phenylalanine	PHE	F	Aromatic
	Tyrosine	TYR	Y	
	Tryptophan	TRP	W	
	Proline	PRO	P	Imino acid
—CH_2—S—S—CH_2—	Cystine	—		Disulfide

Carbohydrates: Mono- and Polysaccharides

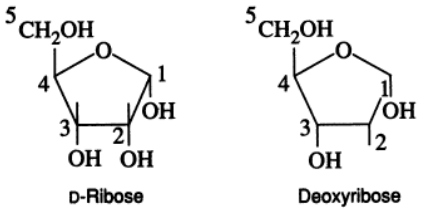
Carbohydrates play key roles as structural and storage compounds in cells. Carbohydrates are represented by the general formula $(CH_2O)_n$, where $n \geq 3$. In the case of plant cells, carbohydrates are synthesized through photosynthesis as shown below:



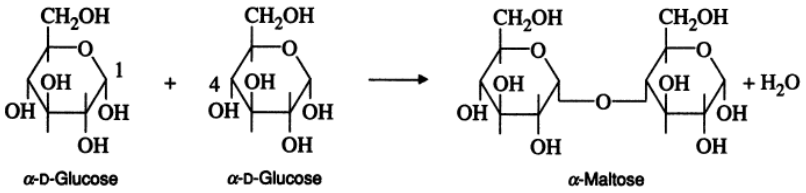
Monosaccharides are the smallest carbohydrates and contain three to nine carbon atoms. Common monosaccharides are either aldehydes or ketones. For example, glucose is an important monosaccharide, which can adopt a linear or ring structure, as shown below:



A particularly important group of monosaccharides are D-ribose and deoxyribose. These are five carbon ring-structured sugar molecules and are essential components of RNA and DNA.



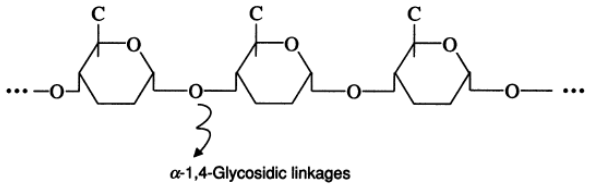
Disaccharides are formed by the condensation of two monosaccharides. For example, maltose is formed by the condensation of two glucose molecules via α-1,4 glycosidic linkage.



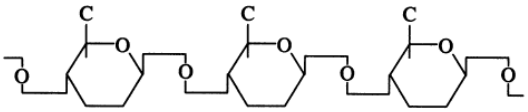
Likewise, lactose, which is found in milk and whey, is a disaccharide of β-D-glucose and β-D-galactose.

Polysaccharides are formed by the condensation of more than two monosaccharides by glycosidic bonds. The polysaccharide processing industry makes extensive use of enzymatic processing and biochemical engineering.

For example, amylose is a polysaccharide that is water insoluble and constitutes about 20% of starch. Amylose is a straight chain of glucose molecules linked by α-1,4 glycosidic linkages.



Similarly, cellulose is a long, unbranched chain of D-glucose with a molecular weight between 50,000 and 1 million daltons. However unlike in amylose, the linkage between glucose monomers in cellulose is a β-1,4 glycosidic linkage.



Lipids and Fats

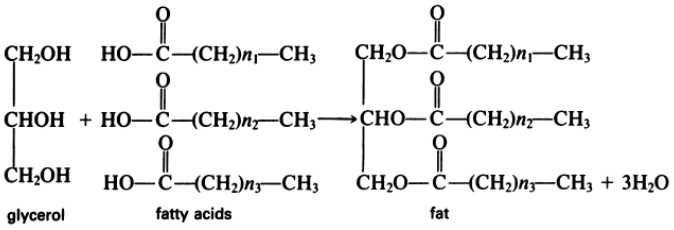
Lipids are hydrophobic biological compounds that are insoluble in water, but soluble in nonpolar solvents, such as benzene. Fats are lipids that can serve as biological fuel-storage molecules.

The major component in most lipids is *fatty acids*, which are made of a straight chain of hydrocarbon (hydrophobic) groups, with a carboxyl group (hydrophilic) at the end. A typical fatty acid can be represented as: $CH_3 – (CH_2)_n – COOH$

The value of *n* is typically between 12 and 20. Unsaturated fatty acids contain double —C=C— bonds, such as oleic acid.

$$CH_3—(CH_2)_7—HC{=}CH—(CH_2)_7—COOH$$

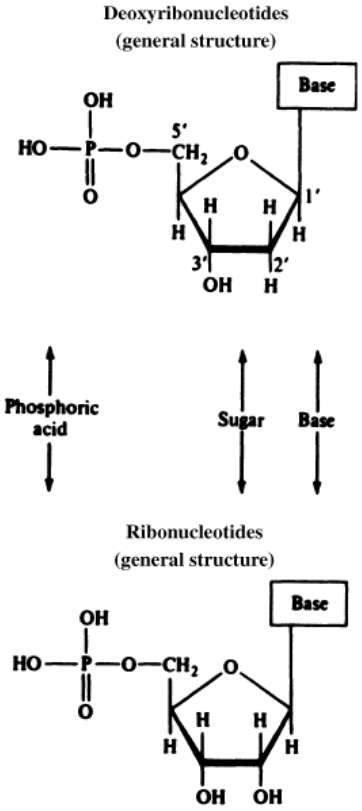
Fats are esters of fatty acids with glycerol. The formation of a fat molecule can be represented by the following reaction:



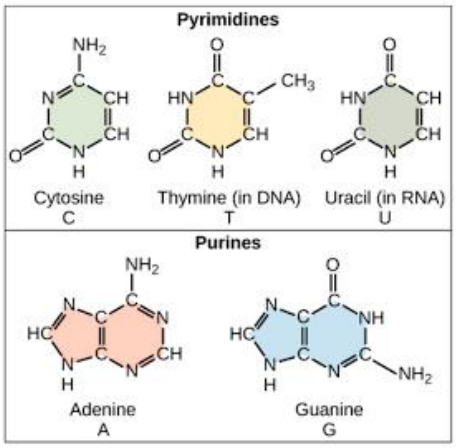
Nucleic acids: DNA and RNA

Nucleic acids play the central role in reproduction of living cells. *Deoxyribonucleic acid* (DNA) stores and preserves genetic information. *Ribonucleic acid* (RNA) plays a central role in protein synthesis. Both DNA and RNA are large polymers made of their corresponding nucleotides.

Nucleotides are the building blocks of DNA and RNA and also serve as molecules to store energy and reducing power. The three major components in all nucleotides are phosphoric acid, sugar (ribose or deoxyribose), and a base (purine or pyrimidine) (see figure below)



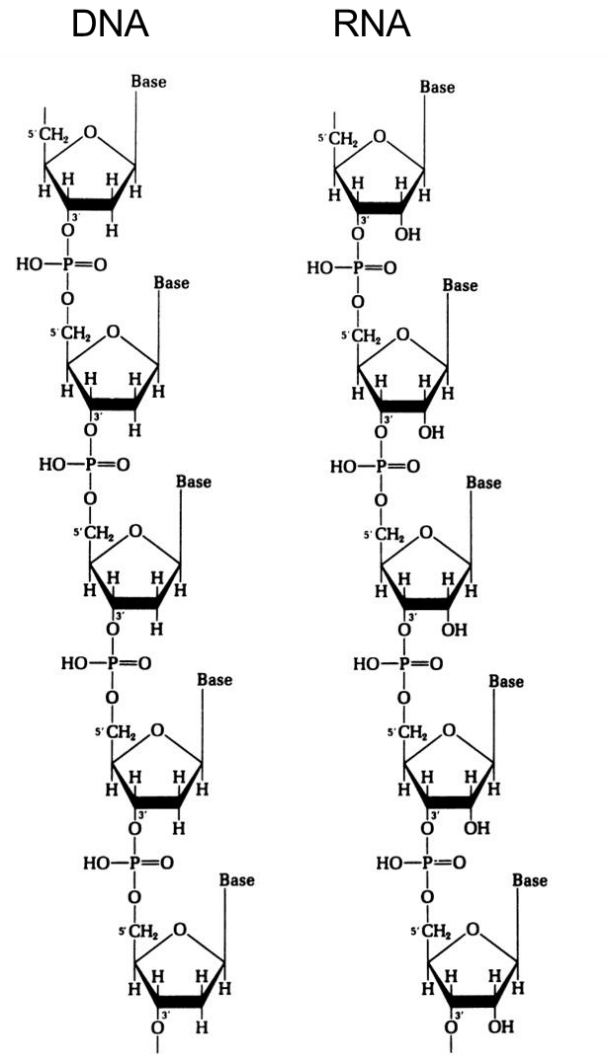
Speaking of the bases in nucleotides, there are two major purines present in nucleotides: adenine (A) and guanine (G), and three major pyrimidines, which are are thymine (T), cytosine (C), and uracil (U). Deoxyribonucleic acid (DNA) contains A, T, G, and C, and ribonucleic acid (RNA) contains A, U, G, and C as bases. It is the base sequence in DNA that carries genetic information for protein synthesis.



The polynucleotides (DNA and RNA) are formed by the condensation of nucleotides. The nucleotides are linked together between the 3' and 5' carbons' successive sugar rings by phosphodiester bonds. The structures of DNA and RNA are shown below.

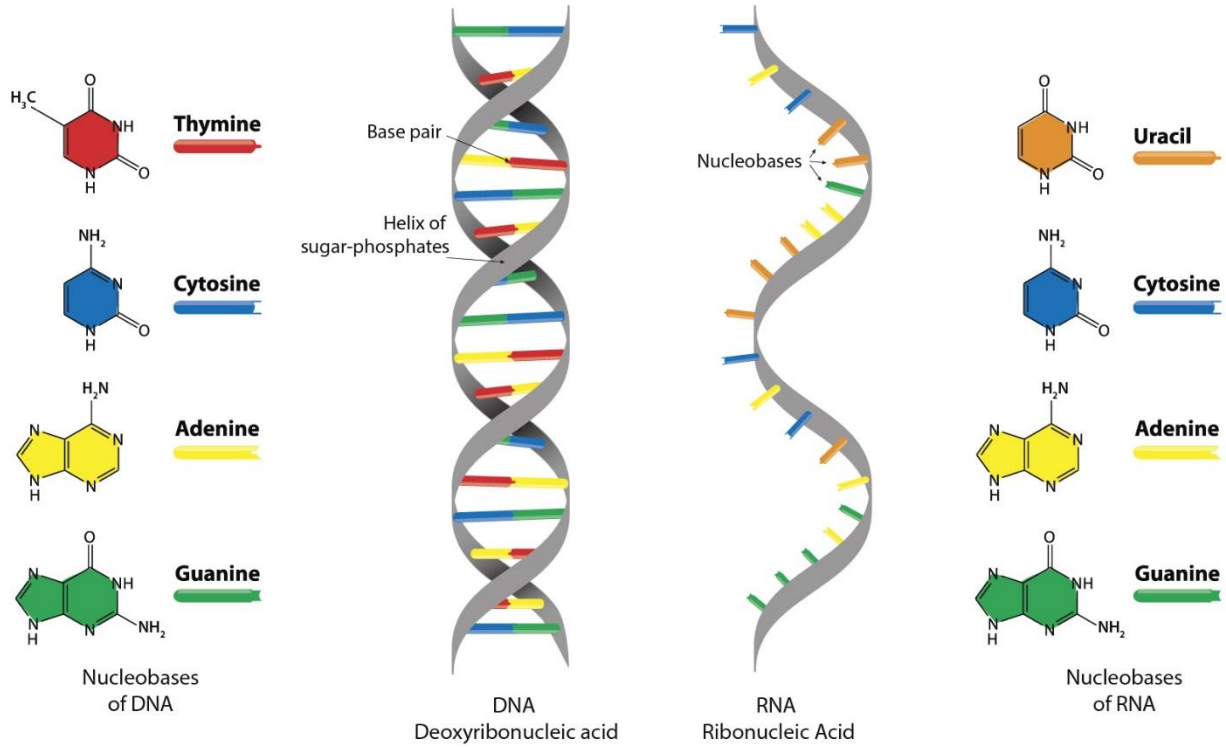
DNA is a very large thread-like macromolecule and has a double-helical three-dimensional structure. The sequence of bases (purines and pyrimidines) in DNA carries genetic information, whereas sugar and phosphate groups perform a structural role. In the double-helical structure, two helical polynucleotide chains are coiled around a common axis. The main features of double-helical DNA structure are as follows:

- The phosphate and deoxyribose units are on the outer surface, but the bases point toward the chain center.
- The diameter of the helix is 2 nm.
- The two chains are held together by hydrogen bonding between pairs of bases. Adenine is always paired with thymine (two H bonds); guanine is always paired with cytosine (three H bonds). This feature is essential to the genetic role of DNA.
- The sequence of bases along a polynucleotide is not restricted in any way, although each strand must be complementary to the other. The precise sequence of bases carries the genetic information.



The major function of DNA is to carry genetic information in its base sequence. The genetic information in DNA is transcribed by RNA molecules and translated in protein synthesis. The templates for RNA synthesis are DNA molecules, and RNA molecules are the templates for protein synthesis. The formation of RNA molecules from DNA is known as DNA transcription, and the formation of peptides and proteins from RNA is called translation.

Certain RNA molecules function as the genetic information-carrying intermediates in protein synthesis (*messenger,* m-*RNA*), whereas other RNA molecules [*transfer* (t-*RNA*) and *ribosomal* (r-*RNA*)] are part of the machinery of protein synthesis.



RNA is a long, unbranched macromolecule consisting of nucleotides joined by 3'–5' phosphodiester bonds. An RNA molecule may contain from 70 to several thousand nucleotides. RNA molecules are usually single stranded

Cell nutrients

Nutrients required by cells to function and grow can be classified in two categories:

- *Macronutrients* needed in concentrations larger than 10^{-4} *M*: Carbon, nitrogen, oxygen, hydrogen, sulfur, phosphorus, Mg^{2+}, and K^{+}
- *Micronutrients* needed in concentrations of less than 10^{-4} *M:* Trace elements such as Mo^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+}, Ca^{2+}, Na^{+}, vitamins and growth hormones.

There are two major types of growth media, which are *defined* and *complex* media. Defined media contain specific amounts of pure chemical compounds with known chemical compositions. A medium containing glucose, $(NH_4)_2SO_4$, KH_2PO_4, and $MgCl_2$ is a defined medium. Complex media contain natural compounds whose chemical composition is not exactly known. A medium containing yeast extracts, peptone, molasses, or corn steep liquor is an example of a complex medium.

CHAPTER 10. ENZYME KINETICS

This chapter is a condensed version of Chapter 3 of the textbook 'Bioprocess Engineering Basic Concepts' 2nd Edition by Shuler and Kargi.

Enzymes represent the largest class of proteins. Over 2000 different kinds of enzymes are known. Enzymes are highly specific in their function and have extraordinary catalytic power. The advantages of using enzymes as catalysts include (i) high specificity meaning fewer undesired side products, (ii) Gentle reaction conditions: mostly aqueous reaction media at ambient temperature and (iii) rate of enzyme-catalysed reaction is usually much faster compared to the same reaction being catalysed by non-biological catalysts.

Enzymes are named by adding the suffix *-ase* to the end of the substrate (i.e. reactant molecule) it interacts with, such as urease when the substrate is urea, or the reaction it catalyzes, such as alcohol dehydrogenase for the alcohol dehydrogenation reaction.

Some common applications of enzymes:

- Catalase in the textile industry to decompose hydrogen peroxide into nascent oxygen and water for bleaching action.
- Amylase in bread making to convert complex sugars, such as starch, to simple sugars, such as maltose.
- Xylanase during pulp treatment to remove lignin in paper manufacture.

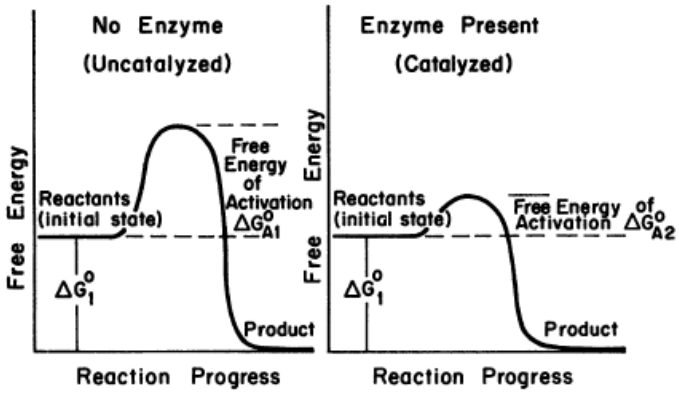
Name	Example of Source	Application
Amylase	*Bacillus subtilis, Aspergillus niger*	Starch hydrolysis, glucose production
Glucoamylase	*A. niger, Rhizopus niveus, Endomycopsis*	Saccharification of starch, glucose production
Trypsin	Animal pancreas	Meat tenderizer, beer haze removal
Papain	Papaya	Digestive aid, meat tenderizer, medical applications
Pepsin	Animal stomach	Digestive aid, meat tenderizer
Rennet	Calf stomach/recombinant *E. coli*	Cheese manufacturing
Glucose isomerase	*Flavobacterium arborescens, Bacillus coagulans, Lactobacillus brevis*	Isomerization of glucose to fructose
Penicillinase	*B. subtilis*	Degradation of penicillin
Glucose oxidase	*A. niger*	Glucose → gluconic acid, dried-egg manufacture
Lignases	Fungal	Biopulping of wood for paper manufacture
Lipases	*Rhizopus,* pancreas	Hydrolysis of lipids, flavoring and digestive aid
Invertase	*S. cerevisiae*	Hydrolysis of sucrose for further fermentation
Pectinase	*A. oryzae, A. niger, A. flavus*	Clarification of fruit juices, hydrolysis of pectin
Cellulase	*Trichoderma viride*	Cellulose hydrolysis

The commercial large-scale manufacture of high fructose corn syrup (HFCS), a sweetener used in place of table sugar, from corn starch relies on the use of three enzymes as shown below. The global market for HFCS was around $6 billion in 2019.

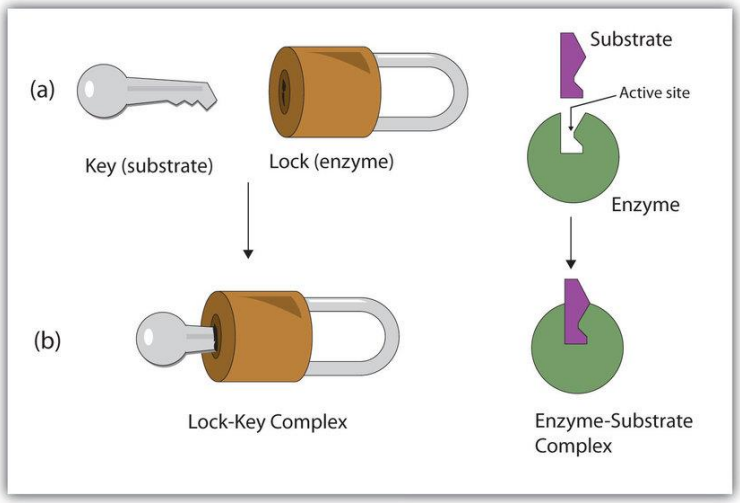
$$\text{Corn starch} \xrightarrow{\alpha\text{-amylase}} \text{Thinned starch} \xrightarrow{\text{Gluco-amylase}} \text{Glucose} \xrightarrow{\text{Glucose isomerase}} \text{HFCS}$$

Enzymes are very effective biological catalysts. Each enzyme's molecule contains an *active site* to which its specific substrate is bound during catalysis.

Enzymes lower the activation energy of the reaction by binding the substrate and forming an enzyme–substrate complex. Enzymes do not affect the free-energy change or the equilibrium constant. The catalytic action of an enzyme from an activation energy perspective is shown below:



Enzyme kinetics is often described using a lock-and-key mechanism model. The interaction between the enzyme (E) and the substrate (S) is usually by weak forces. In most cases, van der Waals forces and hydrogen bonding are responsible for the formation of enzyme-substrate (ES) complexes. After the ES complex has been established, the product (P) can be formed and released, regenerating a free enzyme active site (E).



Michaelis-Menten model for enzyme kinetics

The mathematical model describing the kinetics of a single substrate enzyme-catalyzed reaction is referred to as the Michaelis-Menten kinetics.

The starting basis of the Michaelis-Menten kinetic model is that an enzyme has a fixed number of active sites to which substrates can bind. At high substrate concentrations, all these active sites may be occupied by substrates, or in other words, the enzyme is said to be *saturated*.

The reaction scheme for enzyme kinetics is:

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

which involves a reversible step for enzyme–substrate complex (ES) formation and an irreversible dissociation of the (ES) complex to yield the product and regenerate the enzyme.

Based on above reaction scheme, rate of product P formation is:

$$r_P = \frac{dC_P}{dt} = k_2 C_{ES}$$

The rate of change of concentration of the complex ES can be written as:

$$\frac{dC_{ES}}{dt} = k_1 C_E C_S - k_{-1} C_{ES} - k_2 C_{ES}$$

The free enzyme concentration i.e. concentration of enzyme not binding with a substrate molecule, denoted as C_E can be computed as:

$$C_E = C_{E0} - C_{ES}$$

where C_{E0} is the initial total enzyme concentration.

Assuming a rapid equilibrium between the enzyme and the substrate to form the (ES) complex, we can write:

$$\frac{k_{-1}}{k_1} = \frac{C_E C_S}{C_{ES}}$$

Substituting $C_E = C_{E0} - C_{ES}$ in the above expression, we can write the following expression for C_{ES}:

$$C_{ES} = \frac{C_{E0} C_S}{\left(\frac{k_{-1}}{k_1}\right) + C_S} = \frac{C_{E0} C_S}{K_m + C_S}$$

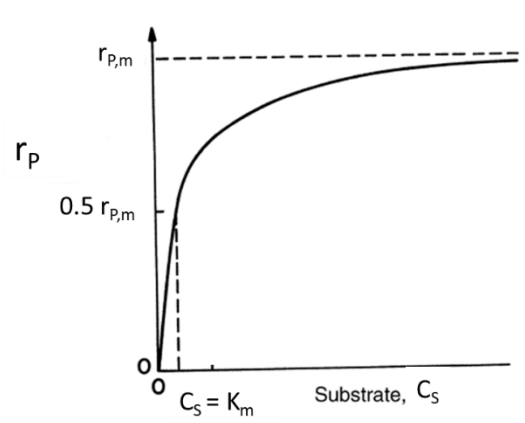
where $K_m = k_{-1}/k_1$

Substituting the above expression for C_{ES} in the expression for rate of product formation:

$$r_P = \frac{dC_P}{dt} = k_2 \frac{C_{E0} C_S}{K_m + C_S} = \frac{r_{P,m} C_S}{K_m + C_S}$$

Where $r_{P,m} = k_2 C_{E0}$ and corresponds to the maximum forward velocity (or rate) of the reaction

- $r_{P,m}$ changes if more enzyme is added, but the addition of more substrate has no influence on $r_{P,m}$
- K_m is often called the Michaelis–Menten constant and is derived by assuming rapid equilibrium in the first step. A low value of K_m suggests that the enzyme has a high affinity for the substrate.
- K_m corresponds to the substrate concentration giving the half-maximal reaction rate (can you derive this?)



The graphical dependence of rate of product formation, v on substrate concentration C_S is shown above.

Determining rate parameters of Michaelis-Menten kinetics

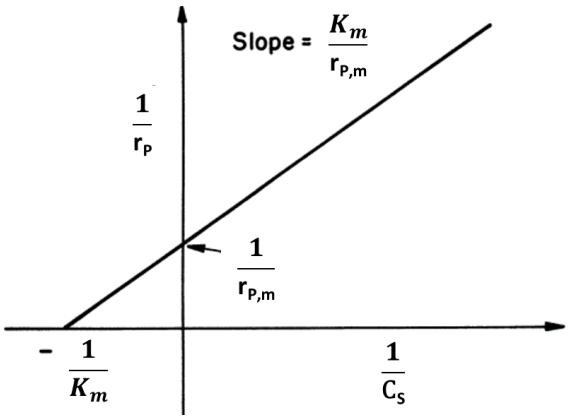
The determination of rate parameters (K_m and $r_{P,m}$) are often done in laboratory experiments using a batch reactor, which is charged with a known amount of substrate C_{S0} and enzyme C_{E0}. During the time course of the reaction, substrate concentration C_S and rate of reaction or rate of product formation r_P are measured.

The Michaelis-Menten parameters can then be evaluated as follows:

The expression $r_P = \frac{r_{P,m}C_S}{K_m+C_S}$ can be written in a linear form as:

$$\frac{1}{r_P} = \frac{1}{r_{P,m}} + \frac{K_m}{r_{P,m}}\frac{1}{C_S}$$

Double reciprocal plot: A plot of $1/r_P$ versus $1/C_S$ yields a linear line with a slope of K_m/V_m and y-axis intercept of $1/r_{P,m}$, as depicted below. Thus, plotting collected experimental data in this form helps determine K_m and $r_{P,m}$.



Interpretation of K_m and $r_{P,m}$: While K_m is an intrinsic parameter, $r_{P,m}$ is not. K_m is solely a function of rate parameters and is expected to change with temperature or pH. However, $r_{P,m}$ is a function of the rate parameter k_2 and the initial enzyme concentration C_{E0}. As C_{E0} changes, so does $r_{P,m}$.

Question: The rate of an enzymatic reaction observed with an initial substrate concentration of 0.001 M is 31 μM.min^{-1}. The value of the Michaelis–Menten constant is 0.54 mM. What is the maximum rate of reaction?

Question: The initial rates at various urea C_S concentrations for the urease-catalyzed hydrolysis reaction are as follows (for all experiments, initial concentration of urease is constant):

C_S (M)	$2.5*10^{-6}$	$4*10^{-6}$	$1*10^{-5}$	$2*10^{-5}$	$4*10^{-5}$	$1*10^{-4}$	$2*10^{-3}$	$1*10^{-2}$
r_P (μmol $m^{-3}{\cdot}min^{-1}$)	28	40	70	95	112	128	139	140

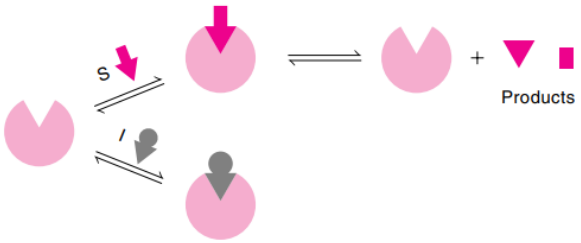
Plot the presented data on MS-Excel as (i) a Michaelis-Menten hyperbolic curve and (ii) the linear double-reciprocal plot. Compute $r_{P,m}$ and K_m.

Inhibited enzyme kinetics

Certain compounds may bind to enzymes and reduce their activity. These compounds are known as enzyme inhibitors (I). Enzyme inhibitions may be irreversible or reversible.

Irreversible inhibitors such as heavy metals (lead, cadmium, mercury, and others) form a stable complex with enzyme and reduce the enzyme activity. Reversible inhibitors may dissociate more easily from the enzyme after binding. The three major classes of reversible enzyme inhibitions are competitive, non-competitive, and uncompetitive inhibitions.

Competitive inhibitors are usually substrate analogues and directly compete with the substrate to bind with the active site of the enzyme as shown below.



The competitive enzyme inhibition reaction scheme can be depicted as:

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

$$+$$

$$\mathrm{I}$$

$$\Big\Downarrow K_I$$

$$\mathrm{EI}$$

Again assuming rapid equilibrium for ES and EI complexes, we can write the following expressions:

$$K_m = \frac{k_{-1}}{k_1} = \frac{C_E C_S}{C_{ES}} \; and \; K_I = \frac{C_E C_I}{C_{EI}}$$

$$C_E = C_{E0} - C_{ES} - C_{EI}$$

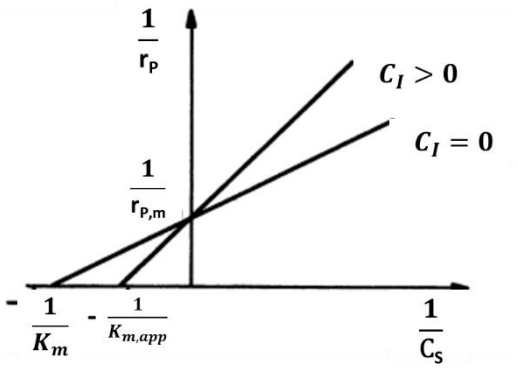
From this, derive the following expression for rate of product formation:

$$r_P = \frac{r_{P,m} C_S}{K_m[1 + \frac{C_I}{K_I}] + C_S} = \frac{r_{P,m} C_S}{K_{m,app} + C_S}$$

The rate equation for competitive inhibition can also be linearized and plotted on a double reciprocal plot. Below is the comparison with the enzyme kinetics without any inhibition:

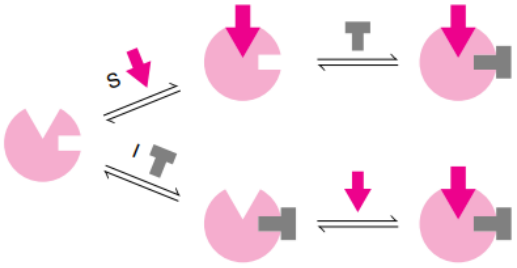
$$\frac{1}{r_P} = \frac{1}{r_{P,m}} + \frac{K_{m,app}}{r_{P,m}} \frac{1}{C_S}$$

The maximum rate of reaction $r_{P,m}$ is the same for uninhibited and competitively inhibited enzymatic reaction. However, the value of the Michaelis-Menten constant under competitive inhibition conditions is larger than the value for the same reaction when it is uninhibited. This is apparent from the relation: $K_{m,app} = K_m[1 + \frac{C_I}{K_I}]$



Fun activity: Play around with the interactive graphs of enzyme kinetics with competitive inhibition available here.

Non-competitive inhibitors are not specifically substrate analogues. Such inhibitors may bind to the enzyme whether or not the substrate has already been bound as shown in the reaction scheme below.



$$\begin{array}{ccccc} \mathrm{E} + \mathrm{S} & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & \mathrm{ES} & \xrightarrow{k_2} & \mathrm{E} + \mathrm{P} \\ + & & + & & \\ \mathrm{I} & & \mathrm{I} & & \end{array}$$

$$\begin{array}{ccc} k_{-3} \Vert k_3 & & k_{-3} \Vert k_3 \\ \mathrm{EI} + \mathrm{S} & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & \mathrm{ESI} \end{array}$$

Uncompetitive inhibitors bind to the ES complex only and have no affinity for the enzyme itself.

The reaction scheme for uncompetitive inhibition is as follows:

$$\begin{array}{ccccc} \mathrm{E} + \mathrm{S} & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & \mathrm{ES} & \xrightarrow{k_2} & \mathrm{E} + \mathrm{P} \\ & & + & & \\ & & \mathrm{I} & & \end{array}$$

$$k_{-3} \Vert k_3$$

$$\mathrm{ESI}$$

We can derive the reaction rate expressions for non-competitive and uncompetitive inhibition, similar to how we derived for the case of competitive inhibition. However, as these are slightly more complicated, these derivations are not covered in this module and will not be assessed. Should you be interested in these derivations, please refer to the following links:

Derivation of enzyme kinetics for non-competitive inhibition

Derivation of enzyme kinetics for uncompetitive inhibition

Question: You had solved this question earlier: **'**The rate of an enzymatic reaction observed with an initial substrate concentration of 0.001 M is 31 µM.min^{-1}. The value of the Michaelis–Menten constant is 0.54 mM. What is the maximum rate of reaction?' Now consider the case where a reversible inhibitor (C_I = 0.1 mM) is present in the reaction mixture, which competitively inhibits the enzymatic reaction. Under these inhibition conditions, the value of the Michaelis–Menten constant is 0.70 mM. Determine (i) value of K_I (ii) maximum rate of reaction and (iii) rate of reaction for the same initial substrate concentration of 0.001 M

CHAPTER 11. MICROBIAL GROWTH AND BIOREACTORS

This chapter is a condensed version of Chapter 6 of the textbook 'Bioprocess Engineering Basic Concepts' 2nd Edition by Shuler and Kargi.

Microorganisms can grow (replication and change in size) under a variety of physical, chemical, and nutritional conditions. In a suitable nutrient medium, organisms extract nutrients from the medium and convert them into biological compounds. Microbial growth is an autocatalytic reaction and described as:

Substrate (S) + cells (C) → extracellular products (P) + more cells (nC)

The net specific growth rate of microbial growth is defined as:

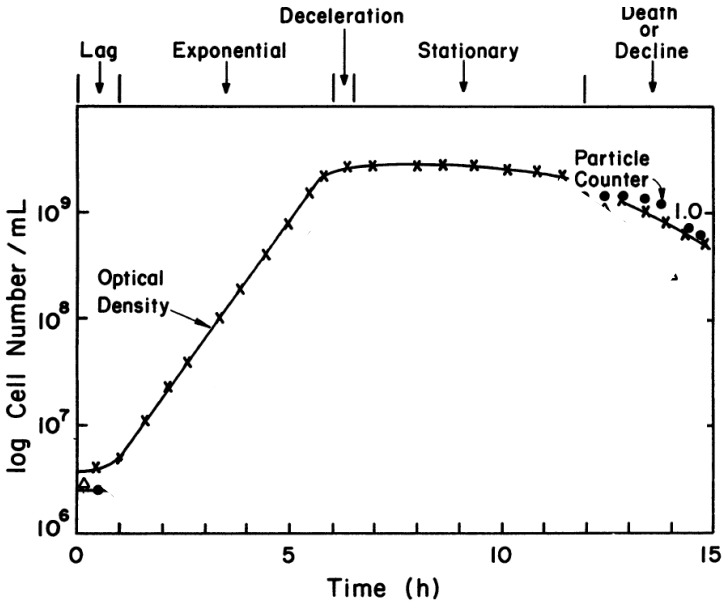
$$r_{net} = \frac{1}{C_C}\frac{dC_C}{dt} = r_g - r_d$$

where C_C is cell mass concentration (*g*/l), *t* is time (h), and r_{net} is net specific growth rate (h^{-1}). The net specific growth is the difference between a gross specific growth rate, r_g (h^{-1}), and the rate of loss of cell mass typically due to cell death, r_d (h^{-1}).

Batch growth

Batch growth refers to culturing and growing cells in a vessel with an initial charge of medium that is not altered by further nutrient addition or removal. (same as a batch reactor)

When a liquid nutrient medium in a batch reactor is inoculated with a seed culture, the microbial organisms grow by taking up dissolved nutrients from the medium. A typical batch growth curve includes the following phases: (1) lag phase, (2) logarithmic or exponential growth phase, (3) deceleration phase, (4) stationary phase, and (5) death phase.



(1) Lag phase: The lag phase occurs immediately after inoculation and is a period of adaptation of cells to a new environment. when they are transferred to a new medium. Depending on the composition of nutrients, microorganisms reorganize their molecular constituents – new enzymes are synthesized, the synthesis of some other enzymes is repressed, and the internal machinery of cells is adapted to the new environmental conditions.

Low concentration of some nutrients and growth factors can cause a longer lag phase. To minimize the duration of the lag phase, cells should be adapted to the growth medium and conditions before inoculation.

(2) The exponential growth phase is also known as the logarithmic growth phase. In this phase, the cells have adjusted to their new environment. Cells multiply rapidly and cell mass and cell number density increase exponentially with time. This is a period of balanced growth, in which all components of a cell grow at the same rate i.e. the average composition of a single cell remains approximately constant during this phase of growth.

Since the nutrient concentrations are large in this phase, the growth rate is independent of nutrient concentration. The exponential growth rate is first order:

$$\frac{dC_C}{dt} = r_{net}C_C, \qquad C_C = C_{C0}\, at\ t = 0$$

Integrating,

$$ln\frac{C_C}{C_{C0}} = r_{net}t \quad or \quad C_C = C_{C0}e^{r_{net}t}$$

where C_C and C_{C0} are cell concentrations at time *t* and *t* = 0 respectively.

The time required to double the microbial mass τ_d is given by the expression:

$$\tau_d = \frac{\ln 2}{r_{net}} = \frac{0.693}{r_{net}}$$

(3) The deceleration growth phase follows the exponential phase. In this phase, growth decelerates due to either depletion of one or more essential nutrients or the accumulation of toxic by-products of growth.

(4) The stationary phase starts at the end of the deceleration phase, when the net growth rate is zero or when the growth rate is equal to the death rate.

$$r_{net} = 0 = r_g - r_d$$

(5) At the end of the stationary phase, because of either nutrient depletion or toxic product accumulation, the death phase begins. The rate of death usually follows first-order kinetics:

$$\frac{dC_C}{dt} = -r_dC_C \quad or \quad C_C = C_{C,S}\, e^{-r_dt}$$

where $C_{C,S}$ is the concentration of cells at the end of the stationary phase and r_d is the first order death-rate constant.

In both the death and stationary phases, it is important to recognize that there is a distribution of properties among individuals in a population. With a narrow distribution, cell death will occur nearly simultaneously; with a broad distribution, a sub-fraction of the population may survive for an extended period. It is this sub-fraction that would dominate the re-establishment of a culture from inoculum derived from stationary or death-phase cultures. Thus, using an old inoculum may select for variants of the original strain having altered metabolic capabilities.

Yield coefficients

Yield coefficients used to describe microbial growth kinetics are defined based on the amount of consumption of a substance.

Based on consumption of substrate: $Y_{C/S} = -\frac{\Delta C_C}{\Delta C_S}$ and $Y_{P/S} = -\frac{\Delta C_P}{\Delta C_S}$

Based on oxygen consumption: $Y_{C/O_2} = -\frac{\Delta C_C}{\Delta O_2}$

For organisms growing aerobically on glucose, $Y_{C/S}$ is typically 0.4 to 0.6 g/g for most yeast and bacteria, while Y_{C/O_2} is 0.9 to 1.4 g/g. Anaerobic growth is less efficient, and the yield coefficient is reduced substantially.

Question: A strain of *Penicillium chrysogenum* was grown in a batch culture on glucose and the following data were obtained. Calculate (a) the maximum net specific growth rate and (b) the yield coefficient $Y_{C/S}$.

Time (h)	Cell concentration (g/l)	Glucose concentration (g/l)
0	1.25	100
9	2.45	97
16	5.1	90.4
23	10.5	76.9
30	22	48.1
34	33	20.6
36	37.5	9.38
40	41	0.63

Microbial Products

The products produced during microbial growth can be classified in three major categories:

1. Growth-associated products are produced simultaneously with microbial growth. The specific rate of product formation r_p is proportional to the specific rate of growth r_g. The production of a constitutive enzyme is an example of a growth-associated product.

$$r_p = \frac{1}{C_C}\frac{dC_P}{dt} = Y_{P/C}\, r_g$$

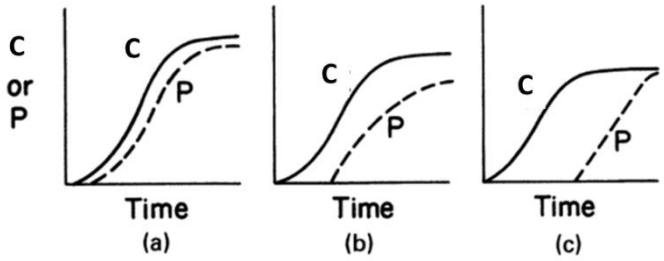
2. Non-growth-associated product formation takes place during the stationary phase when the growth rate is zero. The specific rate of product formation is constant. Many secondary metabolites, such as antibiotics (for example, penicillin), are non-growth-associated products.

$$r_p = constant$$

3. Mixed-growth-associated product formation takes place during the slow growth and stationary phases. Lactic acid fermentation, xanthan gum, and some secondary metabolites from cell culture are examples of mixed-growth-associated products.

$$r_p = \alpha r_g + \beta$$

Realize in the above equation: If α=0: non-growth-associated and if β=0: growth associated and $\alpha = Y_{P/C}$



Kinetic patterns of growth and product formation in batch fermentations: (a) growth-associated product formation, (b) mixed-growth-associated product formation, and (c) nongrowth-associated product formation.

Question: In the batch growth of *Saccharomyces cerevisiae* with glucose substrate, ethanol is formed as a product. Which of the three product categories does ethanol belong to?

Time (h)	Glucose (S), g/L	Biomass (X), g/L	Ethanol (P), g/L
0	100	0.5	0.0
2	95	1.0	2.5
5	85	2.1	7.5
10	58	4.8	20.0
15	30	7.7	34.0
20	12	9.6	43.0
25	5	10.4	47.5
30	2	10.7	49.0

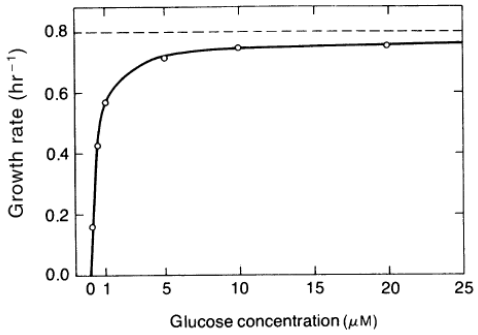
Monod model for microbial growth kinetics

The growth kinetics of a microbial population in a batch reactor is described using the Monod equation. The model is semi-empirical and describes substrate-limited growth.

These kinetics are similar to the Michaelis–Menten kinetics for enzyme reactions.

$$r_g = \frac{r_{g,m} C_S}{K_s + C_S}$$

The relationship of specific growth rate r_g to substrate concentration 'C_S' often assumes the form of saturation kinetics. The assumption is that a single chemical species 'C_S' is growth-rate limiting. For example, in the experimental graph below, the concentration of glucose (the species 'S') influences the specific growth rate of *E.coli*, and this can be described by the Monod kinetic model.



In the Monod equation, $r_{g,m}$ is the maximum specific growth rate and K_s is the saturation constant or half-velocity constant. That is $r_g = \frac{1}{2} r_{g,m}$ when $K_s = C_S$ (we saw the same relationship for K_M in Michaelis–Menten enzyme kinetics)

Here is a list of data of K_s values for different microorganisms:

Microorganism	Limiting substrate	K_s(mg·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}

Inhibited microbial growth kinetics

At high concentrations of substrate or product and in the presence of inhibitory substances in the medium, microbial growth becomes inhibited, and growth rate depends on inhibitor concentration. The inhibition pattern of microbial growth is analogous to enzyme inhibition. As classified earlier, reversible inhibition can be competitive, uncompetitive or non-competitive.

For example, in the case of competitive inhibition of microbial growth by a toxic compound 'I', the equation for specific growth rate will take the following form:

$$r_g = \frac{r_{g,m} C_S}{K_S[1 + \frac{C_I}{K_I}] + C_S}$$

Realize how similar it is to the equation derived in the previous chapter for competitive enzyme inhibition kinetics.

Question: The following data on growth of baker's yeast in a particular medium at 300 K and varying oxygen partial pressures were obtained:

P_{O_2}	Q_{O_2} (no sulfanilamide)	Q_{O_2} (20 mg sulfanilamide/mL added to medium)
0.0	0.0	0.0
0.5	23.5	17.4
1.0	33.0	25.6
1.5	37.5	30.8
2.5	42.0	36.4
3.5	43.0	39.6
5.0	43.0	40.0

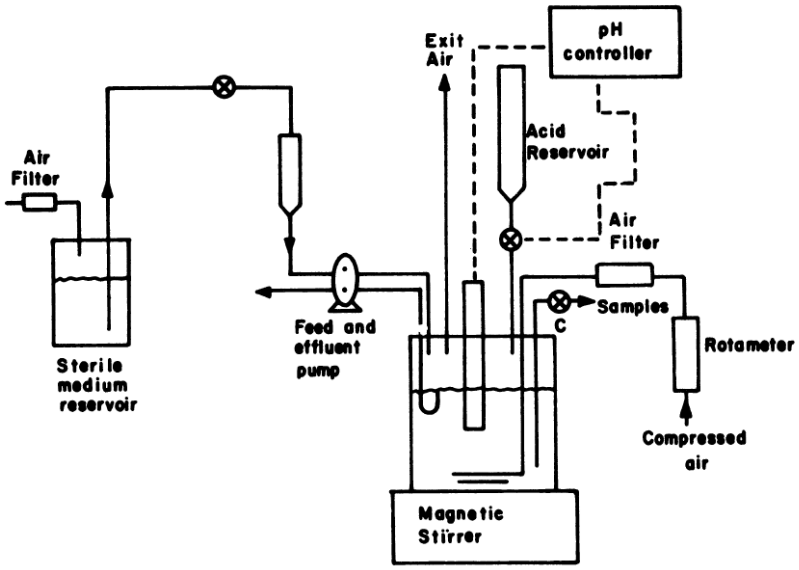
P_{O_2} = oxygen partial pressure, mmHg; Q_{O_2} = oxygen uptake rate, μL of O_2 per hour per mg of cells.

(a) Calculate the maximum oxygen uptake rate and the saturation constant.
(b) Comment on whether sulphanilamide is a competitive inhibitor for oxygen uptake.

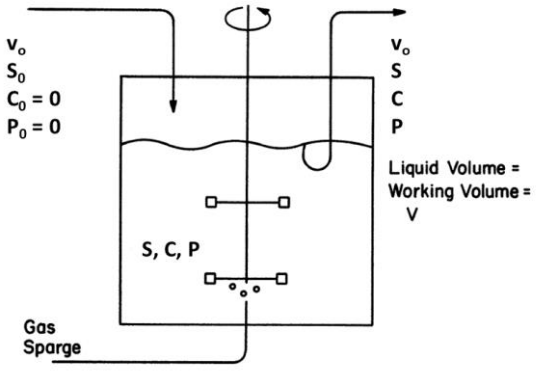
Cell growth in Continuous Culture

In a batch culture, the culture environment changes continually with time. In a continuous culture, fresh nutrient medium is continually supplied to a well-stirred culture, and products and cells are simultaneously withdrawn.

The primary type of continuous cultivation reactor is the chemostat (schematic below), which is essentially a CSTR-type reactor. Plug flow reactors can also be used, although this is usually rare. The term 'chemostat' refers to constant chemical environment. At steady state, the nutrient, product, and cell concentrations are constant.



An ideal chemostat is the same as a perfectly mixed continuous-flow, stirred-tank reactor. At steady state, fresh sterile medium is fed to the completely mixed and aerated (if required) reactor, and cell suspension is removed at the same rate. Liquid volume in the reactor is kept constant.



We can write a mass balance on the cell concentration around the chemostat as:

$$In\ flow - Out\ flow + Formation - Disapperance = Accumulation$$

$$v_0 C_{C0} - v_0 C_C + V r_g C_C - V r_d C_C = V \frac{dC_C}{dt}$$

where, v_0 is the volumetric flow rate of nutrient solution (l/h), V is the culture volume (l) (assumed constant), C_C is the cell concentration (g/l), and r_g and r_d are growth and death rate constants (h^{-1}), respectively.

Rearranging the previous expression,

$$\frac{dC_C}{dt} = DC_{C0} + (r_g - r_d - D)C_C$$

where D is the dilution rate = v_0/V. Realize that D is the reciprocal of space time.

Usually, the feed media are sterile, ie. C_{C0}=0, and if the death rate is negligible compared to the growth rate ($r_d << r_g$), at steady state (dC_C/dt=0):

$$r_g = D$$

Therefore, under these conditions, in a chemostat, the growth rate of cells is equal to the dilution rate.

Hence, a simple description of chemostat performance can be made by substituting the Monod equation for r_g.

$$r_g = D = \frac{r_{g,m} C_S}{K_s + C_S}$$

where C_S is the steady-state limiting substrate concentration (g/l).

As it was done in the case of Michaelis–Menten kinetics, a plot of $1/r_g$ (equivalently 1/D) versus $1/C_S$ can be used to estimate values for $r_{g,m}$ and K_s.

Question: What happens if D is set at a value greater than $r_{g,m}$?

Question: A chemostat study was performed with yeast. The medium flow rate was varied and the steady-state concentration of yeast cells and glucose in the fermenter were measured and recorded. The inlet concentration of glucose was set at 100 g/L. The volume of the fermenter contents was 500 mL. The inlet stream was sterile.

Flow rate F, mL/hr	Cell Conc. C_X, g/L	Substrate Conc. C_S, g/L
31	5.97	0.5
50	5.94	1.0
71	5.88	2.0
91	5.76	4.0
200	0	100

(a) Use the data to formulate the Monod rate equation to describe yeast growth

(b) Can you explain the yeast cell and substrate concentration detected when a flow rate of 200 mL/h is used?

CHAPTER 12. BIOCHEMICAL PRODUCT RECOVERY

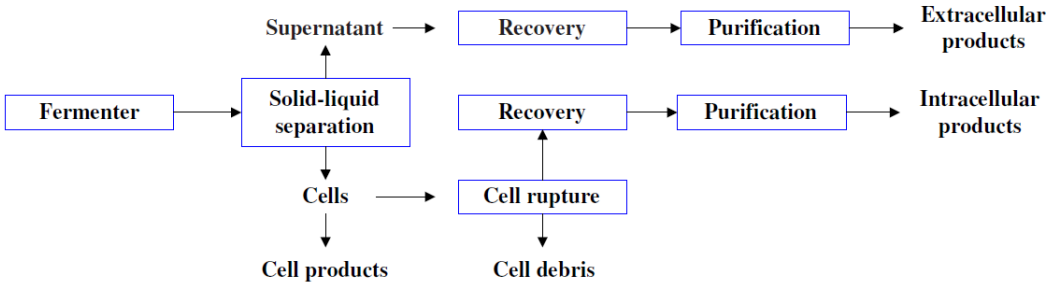
This chapter is a condensed version of Chapter 11 of the textbook 'Bioprocess Engineering Basic Concepts' 2nd Edition by Shuler and Kargi.

The steps of downstream processing to separate and recover the desired product from the contents of the bioreactor depends on the nature of the product. The unit operations that follow the bioreactor would depend on whether the product is an intracellular product, extracellular product or the cell (biomass) itself.

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

Since the chemical nature of a fermentation broth is quite complex and extremely high purity is required for some products (e.g., some pharmaceuticals), recovery and purification often require many processing steps and in many cases represent a manufacturing cost higher than that involved in producing the product.

Recovery and purification are major expenses in production of most fermentation products; often they comprise more than 50% of the total manufacturing costs, especially for an intracellular product. This is because the recovery of intracellular products requires multiple steps as shown in the schematic below: solid-liquid separation of fermentation broth, cell disruption, removal of cell debris and further purification of the intracellular product.



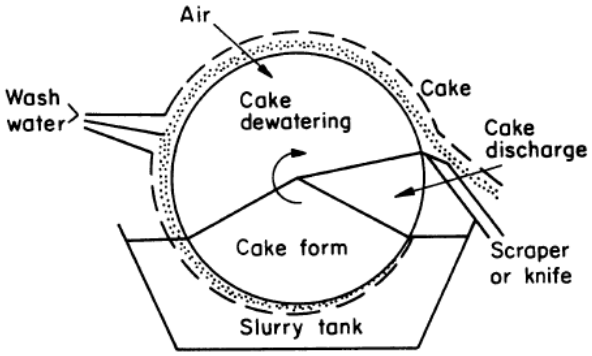
The separation of solids such as biomass, insoluble particles, and macromolecules from the fermentation broth is usually the first step in product recovery. The major methods used for the separation of cellular material (biomass) are (1) filtration, (2) centrifugation, and (3) coagulation and flocculation.

Filtration

Fermentation broth is passed through a filter medium, and a filter cake is formed as a result of deposition of solids on the filter surface.

Continuous rotary filters are widely used in the fermentation industry. The drum is covered with a layer of precoat, usually of diatomaceous earth, prior to filtration. A small amount of coagulating agent or filter aid is added to the broth before it is pumped into the filter. As the drum rotates under vacuum, a thin layer of cells adhere to the drum. The thickness of the cell layer increases in the section designed for forming the cake. The layer of solids is washed and

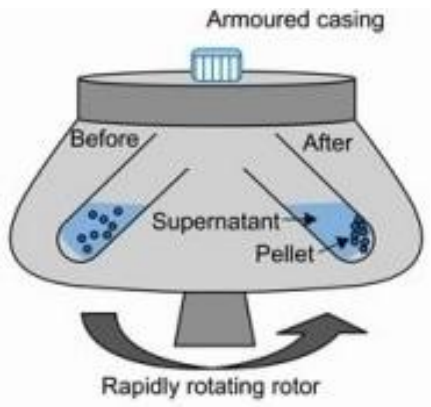
dewatered during its passage to the discharge point, where a knife blade cuts off the cake. A vacuum maintained in the drum provides the driving force for liquid and air flow.



Centrifugation

Centrifugation is used to separate particles of size between 100 and 0.1 µm from liquid by centrifugal forces. A centrifuge separates particles from solution through the use of a rotor; the rotation of the rotor about a central axis generates centrifugal force upon the particles in the suspension.

As a rotor spins in a centrifuge, particles will sediment at a rate proportional to the centrifugal force applied to it. The viscosity of the sample solution and the physical properties of the particles also affect the sedimentation rate of the particles.



Coagulation and Flocculation

Coagulation and flocculation are usually used to form cell aggregates before centrifugation, gravity settling, or filtration to improve the performance of these separation processes.

Coagulation is the formation of small flocs from dispersed colloids using coagulating agents, which are usually simple electrolytes that are relatively inexpensive.

Flocculation is the agglomeration of small flocs into larger settlable particles using flocculating agents, which are usually polyelectrolytes. These are more expensive than simple coagulating electrolytes but also tend to be more efficient. Polyelectrolytes used in flocculation are high-molecular-weight, water-soluble organic compounds; for example, polystyrene sulphate, polyacrylamide and polyethylene imide.

Cell Disruption or Rupture

After cells are separated from liquid broth, if the desired product is intracellular, then the cells need to be disrupted to release the intracellular products. There are both mechanical and non-mechanical cell disruption methods. These include:

- Ultrasonic vibration: An electronic generator is used to generate ultrasonic waves, and a transducer converts these waves into mechanical oscillations by a titanium probe immersed in a cell suspension that can disrupt the cell wall and membrane of bacterial cells.
- Dyno-mill: This method makes use of small beads (20 to 50 mesh). Cell suspension is pumped through a horizontal grinding chamber filled with about 80% beads. Within the grinding chamber is a shaft with specially designed discs. When rotated at high speeds, high shearing and impact forces from millions of beads break cell walls. The broken cells are then discharged.
- Freeze-thawing: By slowly freezing and then thawing a cell paste, the cell wall and membrane may be broken, releasing enzymes into the media. This is a commonly used non-mechanical cell disruption method.
- Chemical treatment: Enzymes such as lysozyme (a carbohydrase) can be used to lyse cell walls of bacteria. Likewise, actively growing cells can be treated with an antibiotic, such as penicillin or cycloserine, that interferes with cell-wall synthesis and can be used to cause cell disruption.

Separation of soluble products

Most microbial products, such as antibiotics, organic acids, solvents, amino acids, and extracellular enzymes, are soluble and extracellular. Various methods have been developed to recover such soluble products, including precipitation, extraction, adsorption etc.

Precipitation

The first step in the purification of intracellular proteins after cell disruption is usually precipitation. Proteins in a fermentation broth can be separated from other components by precipitation using certain salts. Examples include streptomycin sulphate and ammonium sulfate.

The two major methods used for protein precipitation are as follows:

- Salting-out by adding inorganic salts such as $(NH_4)_2SO_4$ at high ionic strength: The added ions interact with water more strongly, causing protein molecules to precipitate.
- Solubility reduction at low temperatures by adding organic solvents (T < -5°): the precipitation of proteins is achieved in this case through a reduction of the dielectric constant of the solution, which results in stronger electrostatic forces between the protein molecules and facilitates protein precipitation.

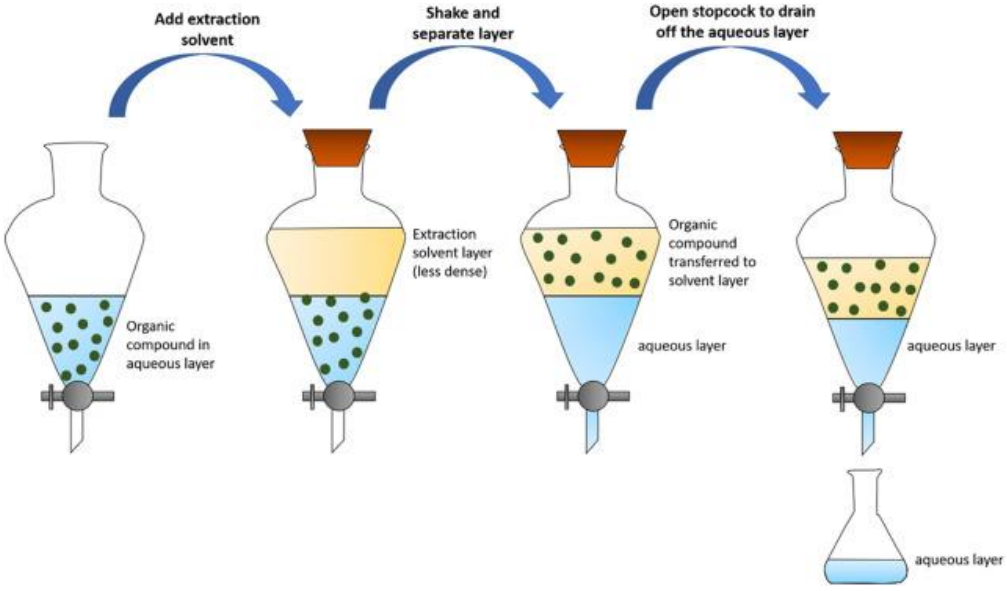
Extraction

Liquid extraction is commonly used to separate inhibitory fermentation products such as ethanol and acetone–butanol from a fermentation broth. Antibiotics are also recovered by liquid extraction (using amylacetate or isoamylacetate). Ideally, the liquid solvent used for extracting the desired substance from the fermentation broth should be nontoxic, selective, inexpensive, immiscible with the fermentation broth, and should have a high distribution coefficient for the product.

The extraction of a compound from one phase to the other is based on solubility differences of the compound in one phase relative to the other. When a compound is distributed between two immiscible liquids, the ratio of the concentrations in the two phases is known as the distribution coefficient (K_D):

$$K_D = \frac{Y_L}{X_H}$$

where Y_L and X_H are concentrations of the solute in light and heavy phases, respectively. In most cases, the light phase will be the organic solvent and the heavy phase will be the aqueous fermentation broth.



Adsorption

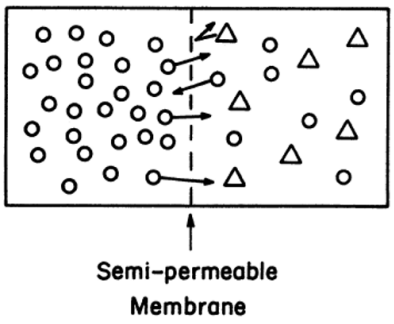
Adsorption refers to the phenomenon of sorption of solutes from liquid media onto solids. It is a commonly used method for separating soluble materials from fermentation broth. Various mechanisms may be involved in adsorption. In physical adsorption, weak forces, such as van der Waals forces, are dominant; however, in ion-exchange adsorption, strong ionic bonds are utilized.

The type of adsorbent used depends on the particular application. The most widely used adsorbent for waste-water treatment applications is activated carbon, since it has large internal surface area per unit weight. Ion-exchange resins and other polymeric adsorbents can be used for protein separations of small organics. For example, a carboxylic acid cation exchange resin is used to recover streptomycin. Adsorption capacity varies depending on adsorbent, adsorbate, physicochemical conditions, and the surface properties of the adsorbent and adsorbate.

Solute is transferred from liquid to solid phase, and an equilibrium is reached after a while in a batch operation. Various types of solid–liquid contactors have been developed for the adsorption of solutes. Among these are packed-bed and agitated-vessel contactors.

Dialysis

Dialysis is a membrane separation operation used for the removal of low molecular weight solutes such as organic acids (100 < MW < 500) and inorganic ions (10 < MW < 100) from a solution. A well-known example is the use of dialysis membranes to remove urea (MW = 60) from urine in artificial kidney (dialysis) devices.



A typical dialysis membrane separation. Low-MW component 1 (○) diffuses through membrane from high to low concentration region. High-MW component (Δ) cannot pass.

In biotechnology, dialysis can be used to remove salts from a protein solution, which is often a step in resolubilizing proteins. A schematic of a dialysis membrane is depicted above: since the membrane is selective, it separates two phases containing low-MW and high-MW solutions. The MW cutoff of a dialysis membrane is typically small and low-MW solutes move from a high to a low concentration region.