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BIOREACTORS

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Preface

This course booklet is intended for first-year Master's students specializing in Microbial Biotechnology. It offers the essential fundamental knowledge required for understand and study the subject of bioreactors

The handout, developed in accordance with current ministerial guideline, offers students the opportunity to deepen their previously acquired knowledge. It provides a structured and detailed content that facilitates the understanding of key concepts. The content is organized into four chapters:

Chapter I: focuses on the fundamentals chemical kinetics (reaction types, rates reaction, order, half-life) as well as the description and evolution of the reaction mixture. It introduces key concepts such as reaction progress, conversion rate, and degree of advancement, along with the expression of concentrations and reaction rates as functions of reaction progress.

Chapter II: present the main types of chemical reactors (Batch reactor, continuous stirred tank reactor and Plug Flow Reactor), along with the corresponding material balances for each type.

Chapter III: covers enzyme kinetics and presents the various types of enzymatic reactors, including the material balance equations associated with each system.

Chapter IV: focuses on the different types of fermenters (bioreactors) and provides the material balance for each type.

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Chapter I: Chemical Kinetic

I.1. Chemical reactor

A reactor is an enclosure or vessel in which a chemical reaction takes place. It represents only a small part of the equipment used in the overall process, but it has a very significant impact on the entire process. The design of a reactor requires knowledge of many physical and chemical factors, including:

1. The thermodynamic and kinetic data of the chemical reaction (the feasibility of the reaction (ΔG) and the enthalpy change ΔH);
2. The kinetic data (knowing the speed and order of the reaction);
3. Material and heat transfer data, essential for understanding how substances and energy move within the reactor;
4. Hydrodynamic data related to fluid flow and phase interactions (physical process; knowing the flow velocity, mixing, etc.);
5. The input and output data (nature of the reagent, operating conditions, concentrations, flow rates, residence times, the nature of the products, conversion rates, and yields) and output data (nature of the products, reactant conversion rate, yield, etc.);
6. The geometric structure of the reactor, which influences performance, efficiency, and stability.

I.2. Classification of chemical reaction

There are several types of reactions, the most important are listed in the table I.1:

Table I.1. Types of reactions

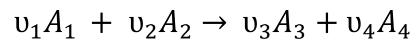
Reactions	Characteristics
Phase present	**Homogeneous**: (**Exp**: gas phase oxidation of nitrogen monoxide) $2NO_{(g)} + O_{2(g)} \rightarrow 2NO_{2(g)}$ **Heterogeneous:** -Gas/liquid (**Exp**: CO_2 absorption by ethanolamine -Gaz/solide :(**Exp**: pyrite roasting: $4FeS_{2(s)} + 11O_{2(g)} \rightarrow 2Fe_2O_{3(s)} + 8SO_{2(g)}$ -Liquid/solid: $CaO_{(s)} + H_2O_{(L)} \rightarrow Ca(OH_2)_{(aq)}$ -Gas/liquid/solid:$CaCO_{3(s)} + 2HCl_{(aq)} \rightarrow CaCl_{2(aq)} + CO_{2(g)} + H_2O_{(l)}$
Stœchiometry	**Unique (simple)**: **Exp** synthesis of NH_3 :$N_2 + 3H_2 \rightarrow 2NH_3$ **Multiple** (existence de secondary reactions (several steps)) **Exp**: Chlorination of benzene $C_6H_6 + Cl_2 \rightarrow C_6H_5Cl + HCl$ et $C_6H_5Cl + Cl_2 \rightarrow C_6H_4Cl_2 + HCl$
Equilibrium	**Irreversible:** is a chemical reaction that goes in one direction. **Exp**: polymerization of ethylene ⟶ polyethylene (plastic material) **Equilibrated:** is a reaction where the forward and reverse reaction occur simultaneously. **Exp** : esterification $Acid + Alcohol \Leftrightarrow Ester + Water$
Thermicity	**Exothermic (ΔH<0):** is a reaction that releases energy. **Exp :** $CaO + H_2O \rightarrow Ca(OH)_2 \ (H = -1155\frac{Kj}{Kg} de\ CaO)$ **Athermic (ΔH = 0)**:is a reaction in which no noticeable heat is released or absorbed. **Exp :** esterification : $R - COOH + HO - R_2 \Leftrightarrow R_1 - COO - R_2 + H_2O$ **Endothermic (ΔH > 0):** is a reaction that absorbed energy. **Exp:** $CaCO_3 \leftrightarrow CaO + CO_2$
Regime	**Chemical**: evolution depends on chemical conditions (concentration, flow rate, etc.

Physical : evolution depends on physical conditions (T, P, transfer phenomenon, etc.

I.3. Rate of reaction

I.3.1. Reaction with unique stœchiometry (or simple reaction):

Suppose a single-phase reaction:



$$\upsilon_1 A_1 + \upsilon_2 A_2 \rightarrow \upsilon_3 A_3 + \upsilon_4 A_4$$

υ_1, υ_2, υ_3 et υ_4 represent the stoichiometric coefficients, where υj is positive (+) for products and negative (-) for reagents.

A chemical equation can therefore generally be written in the form:

$$\sum_j \upsilon_j A_j = 0 \qquad (I.1)$$

For a closed system of homogeneous composition and volume V,

The rate of a reaction (r) is a measure of the specific rate of chemical transformation. It is defined as the change in number of moles of reagents (or products) per unit of **time** (dn/dt), per unit of an extensive property, which depends on the problem being treated. This extensive property can be volume, mass, surface area, etc., though the quantity is generally expressed in moles.

Thus, the rate of a homogeneous reaction is defined as follows:

$$r = \frac{1}{V.\upsilon_1} d\frac{n_{A_1}}{dt} = \frac{1}{V.\upsilon_2} d\frac{n_{A_2}}{dt} = \frac{1}{V.\upsilon_3} d\frac{n_{A_3}}{dt} = \frac{1}{V.\upsilon_4} d\frac{n_{A_4}}{dt} \qquad (I.2)$$

Volumic rates, rates of formation or disappearance:

Suppose a single-phase reaction: $\boldsymbol{aA} + \boldsymbol{bB} \rightarrow \boldsymbol{pP}$

- Rate of formation (or apparition) of product **P**:

$$r_{f,P} = +\frac{1}{V}.\frac{dn_P}{dt} > 0 \qquad \left(\frac{mol}{m^3.s}\right) \qquad (I.3)$$

- Rate of **disappearance** of reagent **A**:

$$r_{d,A} = -\frac{1}{a.V}.\frac{dn_A}{dt} > 0 \qquad \left(\frac{mol}{m^3.s}\right) \qquad (I.4)$$

The **negative signs** represent the **disappearance** of reactants, and **positive signs** represent the **formation** of products (Figure I.1).

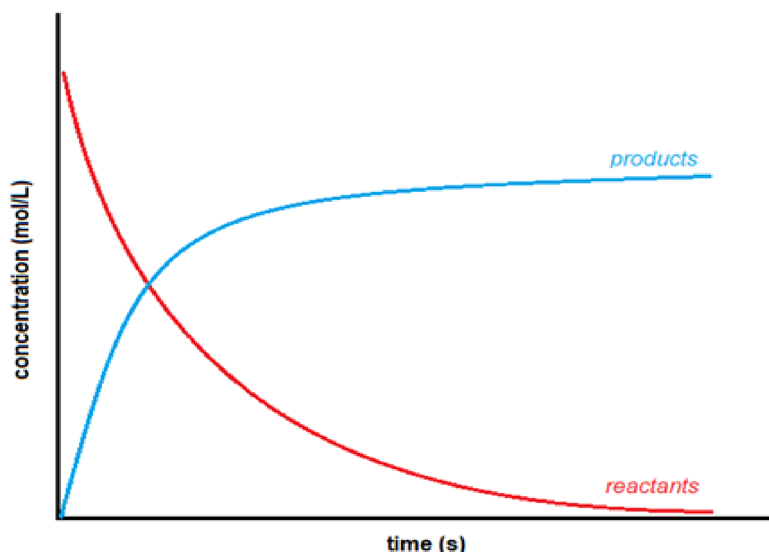


Figure I.1: The evolution of products and reagents concentration with time.

Relationship between the rates of formation and disappearance

$$aA + bB \rightarrow pP$$

$r_{d,A}\ (>0)$ $r_{d,B}\ (>0)$ $r_{f,P}(>0)$

$$r = \frac{1}{a}.r_{d,A} = \frac{1}{b}.r_{d,B} = +\frac{1}{p}.\frac{dn_P}{dt}$$

Then, the rates of reaction of all materials are related by

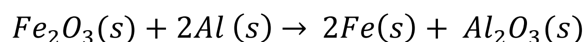
$$r_{f,P} = \frac{p}{a}.r_{d,A} = \frac{p}{b}.r_{d,B} \qquad (I.5)$$

The rate of the reaction is related to the **rate of disappearance** of the reactants and the **rate of formation** of the products. It is given by the following expression:

$$\mathbf{r} = \frac{-1}{(a)}\frac{1}{V}\frac{dn_A}{dt} = \frac{1}{(a)}\mathbf{r_{d,A}} \quad \text{donc} \quad \mathbf{r_{d,A}} = a.\mathbf{r} > 0 \qquad (I.6)$$

$$r = \frac{1}{p}.\frac{1}{V}\frac{dn_P}{dt} = +\frac{1}{p}.\mathbf{r_{f,P}} \quad \text{donc} \quad \mathbf{r_{f,P}} = \mathbf{p}.\mathbf{r} > 0 \qquad (I.7)$$

Exemple :



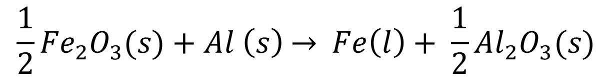
$$Fe_2O_3(s) + 2Al\,(s) \rightarrow 2Fe(s) + Al_2O_3(s)$$

This reaction can be expressed in different ways:

$$\sum \upsilon_i A_i = 0 \Rightarrow \quad 2Fe(l) + Al_2O_3(s) - Fe_2O_3(s) - 2Al\,(s) = 0$$

In this reaction, the stoichiometric coefficients are as follows: $\upsilon_{Fe_2O_3} = -1$, $\upsilon_{Al} = -2$, $\upsilon_{Fe} = +2$ et $\upsilon_{Al_2O_3} = +1$

We can also write this reaction in the following way:



$$\frac{1}{2}Fe_2O_3(s) + Al\,(s) \rightarrow Fe(l) + \frac{1}{2}Al_2O_3(s)$$

The stoichiometric coefficients are then Witten as: $\upsilon_{Fe_2O_3} = -\frac{1}{2}$, $\upsilon_{Al} = -1$, $\upsilon_{Fe} = +1\ et$ $\upsilon_{Al_2O_3} = +\frac{1}{2}$

The rate reaction is expressed as follows:

$$r = \frac{1}{\upsilon_j} r_j(A_j) = \frac{1}{(1)} r_1(Fe_2O_3) = \frac{1}{(2)} r_2(Al) = \frac{1}{(+2)} r_3(Fe) = \frac{1}{(+1)} r_4(Al_2O_3)$$

$$r = -\frac{1}{V}\frac{dn_{(Fe_2O_3)}}{dt} = -\frac{1}{2.V}\frac{dn_{(Al)}}{dt} = \frac{1}{2.V}\frac{dn_{(Fe)}}{dt} = \frac{1}{V}\frac{dn_{(Al_2O_3)}}{dt}$$

Note :

- In the case of a **homogeneous** reaction occurring in a closed reactor, the extensive variable is the **volume** of the reaction mixture.

$$r_j = \frac{1}{V}\frac{dn_J}{dt} \qquad \frac{moles\ j\ formed}{(volume\ of\ fluid)(time)} \qquad (I.8)$$

- In the case of a **heterogeneous** reaction, the extensive variable is the active surface area of the catalytic material (s)

  $s = a_P.M,\ a_P{:}\ is\ the\ active\ surface\ area\ (m^2.Kg^{-1})$

$$\grave{r}_j = \frac{1}{S}\frac{dn_J}{dt} \qquad \frac{moles\ j\ formed}{(surface)(time)} \qquad (I.9)$$

- In the case of a catalytic reaction, the extensive variable is **the mass** of the catalyst (w). $w = \rho_{p.V_p}$, ρ_p: the bulk density (or particle density) of a catalyst particle.

$$\grave{\grave{r}}_j = \frac{1}{w}\frac{dn_J}{dt} \qquad \frac{moles\ j\ formed}{(mass\ of\ solid)(time)} \qquad (I.10)$$

- Based on the unit volume of reactor, if different from the rate based on unit volume of the fluid,

$$r_j''' = \frac{1}{V_r}\frac{dn_J}{dt} \qquad \frac{moles\ j\ formed}{(volume\ of\ solid)(time)} \qquad (I.11)$$

In homogeneous systems, the volume of fluid in the reactor is often identical to the volume of reactor. In heterogeneous systems, all the above definitions of reaction rate may be used, with the choice of definition for the specific situation. The relationship between these different rates is given as follows:

$$r_j.V = r'_j.s = r''_j.w = V_r.r_j''' \qquad (I.12)$$

I.3.2. Multiple stoichiometry reaction (or complex reaction):

When **j** components are involved in **multiple independent reactions** (with i=1,2,3,..), meaning that **several reactions occur simultaneously**, the general stoichiometric equation can be written as:

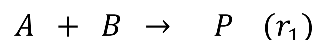
$$\sum_i \upsilon_{ij} A_j = 0 \qquad (I.13)$$

Where: υ_{ij} is the stoichiometric coefficient of species Aj in reaction i.

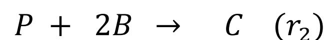
The chemical transformation rate Rj, also called the reaction rate of species Aj in the set of reactions, is expressed as:

$$\boldsymbol{R_j} = \sum_i \upsilon_{ij} \mathbf{r_i} \qquad (I.14)$$

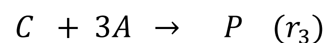
<u>Example :</u>



$$A + B \rightarrow P \quad (r_1)$$



$$P + 2B \rightarrow C \quad (r_2)$$



$$C + 3A \rightarrow P \quad (r_3)$$

There are three reactions i =1, 2, 3 and four chemical species (j=A, B, C, P)

$\boldsymbol{R_A} = -r_1 - 3r_3$ (The net production rate for A)
$\boldsymbol{R_B} = -r_1 - 2r_2$
$\boldsymbol{R_C} = r_2 - r_3$
$\boldsymbol{R_P} = r_1 - r_2 + r_3$

I.4. Rate laws

The reaction rate is a state function that depends on the local instantaneous composition as well as on state variables such as pressure (p) and temperature (T), Determining this function

requires knowledge of the fundamental properties of reaction mechanisms and the methods used to construct them, in order to establish appropriate kinetic laws for the design of process units. For this reason, we provide below a brief overview of the key concepts.

a) Irreversible reaction :

Consider the following irreversible reaction: $aA + bB \rightarrow pP$

The experimental study of concentration changes makes it possible to establish a simple relationship between the reaction rate and the reactant concentrations. This empirical relationship is known as the **Guldberg et Waage** rate equation.

$$\boldsymbol{r = k[A]^{\alpha}.[B]^{\beta}} \qquad (I.15)$$

Where:

k : rate constant.

α, β : The partial orders of the reaction with respect to A and B, respectively. The overall order of the reaction is the sum of the partial orders. **$n = \alpha + \beta$**.

[A] and [B] : the concentrations of species A and B at time t.

The reaction rate can generally be expressed in the form:

$$r = k \prod_{j=1}^{n} C_j^{\alpha_j} \qquad (r = k \prod_{j=1}^{n} P_j^{\alpha_j} \; for\ a\ gas\ phase\ reaction)$$

Where:

n : number of active species.

α_j : partial order

k : rate constant which depends on temperature.

Cj: Concentration of the reactants

Note:

In an **elementary reaction**, the partial orders are equal to the stoichiometric coefficients ($\alpha_j = \upsilon_j$).

$$aA + bB \rightarrow pP$$

$$\boldsymbol{r = k.C_A^a.C_B^b}$$

b) Reversible reaction

For a reversible reaction ($aA + bB \underset{(k_2)}{\overset{(k_1)}{\rightleftarrows}} pP$).

The rate of the reaction can typically be written as:

$$r = r_1 - r_2 \longrightarrow r = k_1 \prod_{j=1}^{n} C_j^{\alpha_j} - k_2 \prod_{j=1}^{n} C_j^{\alpha'_j} \qquad (I.16)$$

At thermodynamic equilibrium, we have a $r_1 = r_2$. *therefore:*

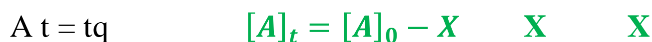
$$k_1 \prod_{j=1}^{n} C_j^{\alpha_j} = k_2 \prod_{j=1}^{n} C_j^{\alpha_j} \Rightarrow \frac{k_1}{k_2} = \prod_{j=1}^{n} C_j^{\alpha'_j - \alpha_j} = K_C \qquad (I.17)$$

K_C *: equilibrium constant*

I.4.1. *Expression of the rate law and order of a reaction*

- **Zero-order reaction** (*α= 0*)

Consider the following reaction: $aA \leftrightarrow bB + cC$



We have: $\begin{cases} r = -\frac{1}{a}\frac{d[A]}{dt} = +\frac{1}{b}\frac{d[B]}{dt} = +\frac{1}{c}\frac{d[C]}{dt} & (I.18) \\ r = k[A]^{\alpha} = k[A]^{0} = k & (I.19) \end{cases}$

(1) = (2) we will have : $k[A]^0 = k = -\frac{1}{a}\frac{d[A]}{dt}$

Before performing the integration, we separate the variables:

$$-d[A] = k.a.dt \Rightarrow \int_{[A]_0}^{[A]_t} d[A] = -k.a \int_0^t dt \Rightarrow [[A]]_{[A]_0}^{[A]_t} = [-a.k.t]_0^t$$

$$\mathbf{[A]_t = [A]_0 - a.k.t} \qquad (I.20)$$

This is a linear equation of the form Y=β – ɣ.X

If we plot $[A]_t$ as a function of time, we obtain a straight line with a y-intercept of $[A]_0$ and a negative slope of «-αk ».

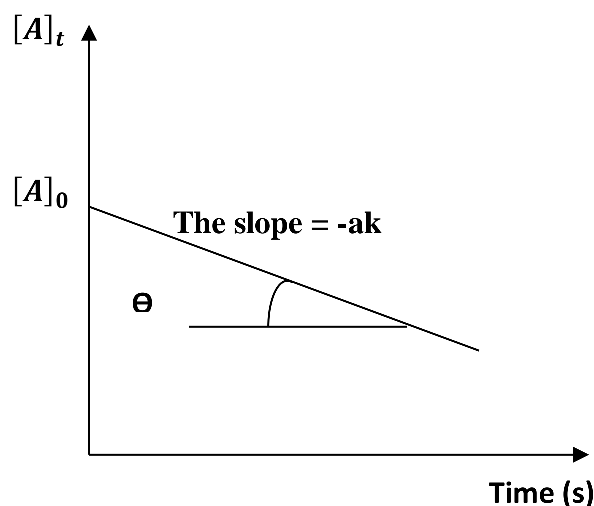


Figure I.1: Concentration [A] as a Function of Time

The unit of rate constant k corresponds to : $k = \frac{[A]_t - [A]_0}{t}$ **mol. l^{-1}.s^{-1}**

➢ ***First order reaction (α = 1):***

Consider the following reaction: $aA \leftrightarrow bB + cC$

We have: $$\begin{cases} r = -\frac{1}{a}\frac{d[A]}{dt} = +\frac{1}{b}\frac{d[B]}{dt} = +\frac{1}{c}\frac{d[C]}{dt} & (I.21) \\ r = k[A]^{\alpha} = k[A]^{1} = k\ [A] & (I.22) \end{cases}$$

$$k[A]^1 = -\frac{1}{a}\frac{d[A]}{dt}$$

Before performing the integration, we separate the variables:

$$-\frac{d[A]}{[A]} = ak.dt \Rightarrow \int_{[A]_0}^{[A]_t} \frac{d[A]}{[A]} = -\int_0^t ak.dt$$

$$[Ln[A]_t]_{[A]_0}^{[A]_t} = [-k.a.t]_0^t \Rightarrow Ln\frac{[A]_t}{[A]_0} = -k.a.t$$

$$\mathbf{Ln[A]_t = Ln[A]_0 - k.a.t} \qquad (I.23)$$

The plot of **Ln $[A]_t$** as function of **time** gives **a straight line** with a y-intercept of « **Ln $[A]_o$** » and a negative slope of «-ak ».

We can also plot **Ln $\frac{[A]_0}{[A]_t}$** as a function of time and we still obtain a straight line passing through the origin (o) with a slope equal « **α.k** ».

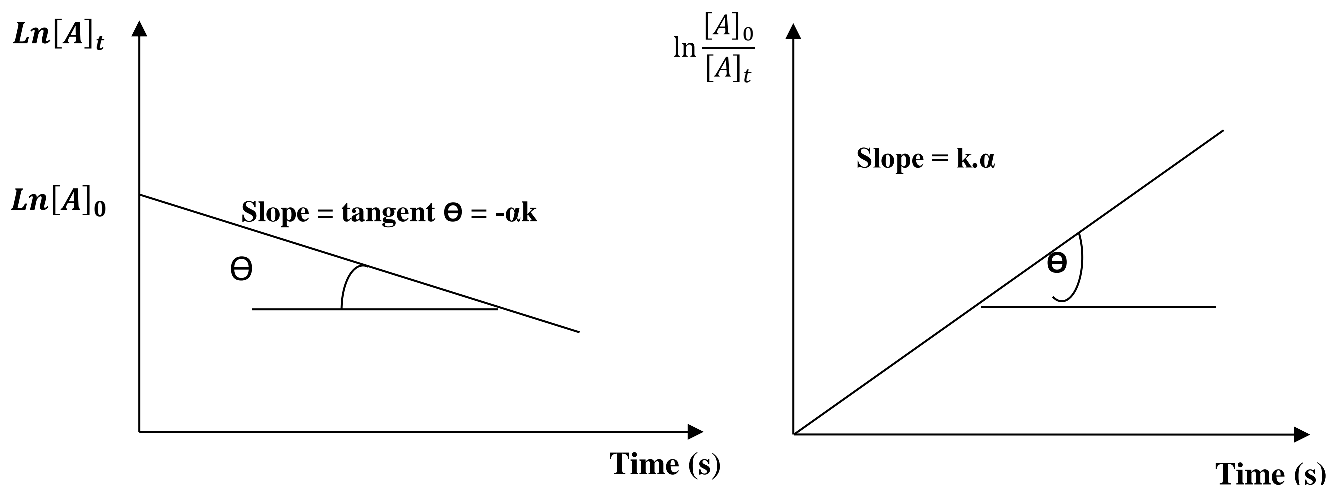


Figure I.2: Linear representation of first kinetics and determination of the apparent rate constant

For a **first** order reaction, the rate constant k has the dimensions of $[time]^{-1}$ (s^{-1}, min^{-1} or h^{-1}).

- ***Second order reaction (α= 2)***

Consider the following reaction: $\boldsymbol{aA + bB \rightarrow pP}$

In the case of [A] = [B]

In the same way as for zero and first order reactions, we obtain:

$$\text{We have: } \begin{cases} r = -\frac{1}{a}\frac{d[A]}{dt} = +\frac{1}{b}\frac{d[B]}{dt} = +\frac{1}{c}\frac{d[C]}{dt} & (I.24) \\ r = k[A]^{\alpha} = k[A]^{2} & (I.25) \end{cases}$$

$$k[A]^2 = -\frac{1}{a}\frac{d[A]}{dt}$$

Before performing the integration, we separate the variables:

$$\int_{[A]_0}^{[A]_t} \frac{d[A]}{[A]^2} = -k.a\int_0^t dt \Rightarrow \left[-\frac{1}{[A]}\right]_{[A]_0}^{[A]_t} = [-k.a.t]_0^t$$

$$\frac{1}{[A]_t} = \frac{1}{[A]_0} + k.a.t \qquad (I.26)$$

The plot $\frac{1}{[A]_t}$ as a function of time give **a straight line** with a y-intercept of « $1/[A]_0$ » and a positive slope of «a.k ». Similarly, by plotting $(\frac{1}{[A]_t} - \frac{1}{[A]_0})$ versus time, we obtain a straight line passing the origin with a slope of "**a.k**".

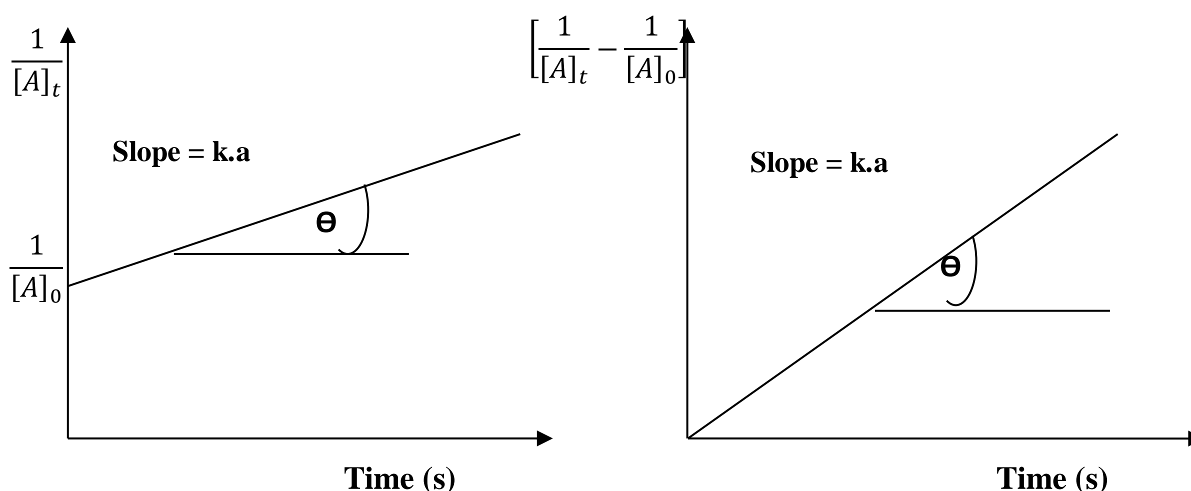


Figure I.2: Linear representation of second kinetics and determination of the apparent rate constant

For a second-order reaction, the rate constant (k) has units of is **L. mol^{-1}. s^{-1}.**

I.4.2. Half life time

The half-life corresponds to the time required for the amount (or concentration) of each chemical species to change by half of its total variation between the initial and final states. It is defined as the time at which the initial concentration of the reactant is reduced by half.

$$[A]_{t_{\frac{1}{2}}} = \frac{[A]_0}{2} \Rightarrow t = t_{1/2} \qquad (I.27)$$

The half-life is a very important parameter because:

1. It makes it possible to evaluate the time required for the completion of the studied chemical transformation.
2. It also helps us choose the appropriate method to monitor a given transformation:

   If $t_{1/2}$ is too short (i.e., if the reaction rate is high), it becomes impossible to use a titration technique, since performing such a technique requires a certain amount of time. Moreover, measuring instruments (such as pH, meters, conductimeters, or spectrophotometers) have a finite response time, meaning that the measurement result is not instantaneous.

   Therefore, the reactions should not be too fast. The duration of the measurement should be at least ten times shorter than the half-life of the transformation.

- ***Half life time for the zero order reaction:***

We have: $\begin{cases} [A]_t - [A]_0 = -k.a.t \\ t = t_{\frac{1}{2}} \Rightarrow [A]_{t_{\frac{1}{2}}} = \frac{[A]_0}{2} \end{cases}$

$$\frac{[A]_0}{2} - [A]_0 = -k.a.t_{\frac{1}{2}}$$

$$\boldsymbol{t_{\frac{1}{2}} = \frac{[A]_0}{2.a.k}} \qquad (I.28)$$

In a zero order reaction, the half-life $(t_{\frac{1}{2}})$ is proportional with the initial concentration of the reactant $[A]_0$.

- ***Half life time for the first order reaction***

We have: $\begin{cases} Ln[A]_t - Ln[A]_0 = -k.a.t \\ t = t_{\frac{1}{2}} \Rightarrow [A]_{t_{\frac{1}{2}}} = \frac{[A]_0}{2} \end{cases}$

$$\mathrm{Ln}\frac{[\mathrm{A}]_0}{2} - \mathrm{Ln}[\mathrm{A}]_0 = -\mathrm{k}.\alpha.\mathrm{t}_{\frac{1}{2}}$$

$$\mathrm{t}_{\frac{1}{2}} = \frac{\mathrm{Ln}\ 2}{\mathrm{k}.\alpha} \qquad (I.29)$$

For the first order reaction, the half-life time is independent for the initial concentration. In a first order reaction, the half life $\mathbf{t}_{\frac{1}{2}}$ does not depend on the initial concentration of the reactant $[\mathbf{A}]_{\mathbf{0}}$.

. Half-life for the second order reaction

We have : $\begin{cases} \frac{1}{[\mathrm{A}]_t} = \mathrm{k.a.t} + \frac{1}{[\mathrm{A}]_0} \\ t = t_{\frac{1}{2}} \Rightarrow [A]_{t_{\frac{1}{2}}} = \frac{[A]_0}{2} \end{cases}$

$$\frac{1}{\frac{[\mathrm{A}]_0}{2}} = \mathrm{k.a.t} + \frac{1}{[\mathrm{A}]_0}$$

$$\mathrm{t}_{\frac{1}{2}} = \frac{1}{\mathrm{k.a.}[\mathrm{A}]_0} \qquad (I.30)$$

In a second order reaction, the half-life time $\mathbf{t}_{\frac{1}{2}}$ is ***inversely proportional*** with the initial concentration $[\mathbf{A}]_{\mathbf{0}}$.

In summary: the comparative characteristics of zero, first, and second-order reactions are presented the table I.2.

Table I.2: Comparative characteristics of zero, first, and second-order reactions.

Reaction order	Linear forme	Half-Life ($t_{1/2}$)	Unite of the rate constant k
0	$[A]_t = [A]_0 - \alpha kt$	$t_{\frac{1}{2}} = \frac{[A]_0}{2\alpha k}$ ($t_{1/2}$ is proportional with $[A]_0$)	mol. l^{-1}.s^{-1} (concentration).(temps)$^{-1}$
1	$Ln[A]_t = Ln[A]_0 - \alpha\ kt$	$t_{\frac{1}{2}} = \frac{\text{Ln } 2}{\alpha k}$ $t_{1/2}$ doses not depend on the $[A]_0$)	s^{-1} (temps)$^{-1}$
2	$\frac{1}{[A]_t} = \frac{1}{[A]_0} + \alpha\ kt$	$t_{\frac{1}{2}} = \frac{1}{\alpha k.[A]_0}$ $t_{1/2}$ is inversely proportionnel to $[A]_0$).	l. mol^{-1}. s^{-1} (concentration)$^{-}$.(temps)$^{-1}$

I.5. Method for determining the order of a reaction

I.5.1. Integrated rate law method

This method involves directly substituting the experimental data (measured at different times) into the integrated rate equations corresponding to zero, first and second order reactions, and calculating the rate constant k.

If the values of k obtained are consistent (i.e., remain approximately constant), then the chosen equation correctly describes the reaction progress. Otherwise, a different rate expression must be tested.

I.5.2. Half- Life method

- If the half-life is proportional to the initial concentration $[A]_0$, the reaction is of zero order ($\alpha = 0$).
- If the half-life is independent of $[A]_0$, then the reaction is of first order ($\alpha = 1$).
- If the half-life is inversely proportional to $[A]_0$, then the reaction is of second order ($\alpha = 2$).

When experimental data are given in a table, plot t1/2 as a function of [A]0 to determine the relationship.

The reaction order can also be identified directly if the variation of half-life with initial concentration is stated in the problem.

I.5.3. Method of isolation

When a reaction involves multiple reactants, the concentrations of all reactants except one are kept constant. The reaction rate is then measured as a function of the concentration of the isolated reactant.

Example: consider the reaction $A + B \rightarrow P$

The rate law is assumed to be $r = k[A]^{\propto}.[B]^{\beta}$

To determine the order with respect to A, we keep [B] constant and in large excess. In this case, $[B]^{\beta}$ becomes constant, and the rate law simplifies to:

$$r = \grave{K}.[A]^{\propto} \quad avec\ \grave{K} = K.[B]^{\beta}$$

We then vary [A] and measure the rate to find α

Once the order with respect to this reactant is determined, the procedure is repeated for the second reactant, and so on.

I.5.4. Graphical method

If a set of experimental data is provided, the rate law of the reaction can be determined by plotting the data in different ways to identify the reaction order:

- First, plot the concentration of the reactant **[A]** versus time **t**. If this yields a straight **line**, the reaction is **zero** order.
- If the plot is a curve, then plot **ln[A]** versus time ***t***. If this gives a straight line, the reaction is **first order**.
- If that too is not linear, plot (**1/[A]**) versus time **t**. If this results in a straight **line**, the reaction is **second order**.

I.6. Temperature dependence of the rate equation

For many reactions, particularly elementary reactions, the rate expression can often be written as the product of a temperature-dependent term and a composition- dependent term:

$$r_j = f_1(temperature).f_2(Composition) \text{ or } r_j = k.f_2(Composition)$$

In such cases, the temperature dependent term, represented by the rate constant k, is generally well represented by Arrhenius equation:

$$K = Aexp^{\frac{-E_a}{RT}} \qquad (I.31)$$

Where:

- A: is the frequency factor or pre-exponential factor.It characterizes the probability of two reactants molecules will collide with the proper orientation to react. It depends on molecular geometry and steric factors.
- E: is the activation energy, representing the minimum energy barrier that must be overcome for a successful reaction to occur.
- R: is the universal gas constant.
- T is the absolute temperature in kelvin.

If the rate constants are known at two different temperatures: k_1 at T_1 and k_2 at T_2, we can write:

$$k_1 = Aexp^{\frac{-E_a}{RT_1}} \quad (I.32)$$

$$k_2 = Aexp^{\frac{-E_a}{RT_2}} \quad (I.33)$$

$$\frac{I.32}{I.33}: \quad \frac{k_1}{k_2} = \frac{Aexp^{\frac{-E_a}{RT_1}}}{Aexp^{\frac{-E_a}{RT_2}}}$$

$$ln\frac{k_1}{k_2} = \frac{E_a}{RT_2} - \frac{E_a}{RT_1} = \frac{E_a}{R}.\frac{T_1 - T_2}{T_1T_2}$$

We can deduce that:

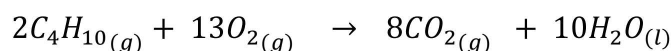
$$E_a = R(\frac{T_1T_2}{T_1-T_2}).ln\frac{K_1}{K_2} \quad (I.34)$$

I.7. Concept of conversion rate and reaction progress (Extent of reaction)

The reaction phase in which a chemical transformation takes place, generally consists of two type of components:

- **Active components**: these are the chemical species that **participate** in the reaction, denoted as $A_1, A_2, \ldots, A_j$
- **Inert components**: these are species that do **not participate** in the chemical reaction and remain unchanged, they are denoted as $\mathbf{A_I}$.

<u>Exp</u> : combustion of butane in air (oxygen + nitrogen)



$$2C_4H_{10\,(g)} + 13O_{2\,(g)} \rightarrow 8CO_{2\,(g)} + 10H_2O_{(l)}$$

The active components are:

$$A_1 = C_4H_{10} \; ,A_2 = O_2 \; ,A_3 = CO_2, \; A_4 = H_2O \; et \; \boldsymbol{A_I = N_2} \; .$$

We will now define the concepts of **conversion rate** and **degree of advancement** of reaction for two types of systems, namely:

a) Closed system (batch or semi batch reactors): A closed system consists of a reactive mixture, with a uniform composition, which evolves over time due to the chemical reactions, but does not exchange matter with the surroundings. There is no inflow or outflow. The mass or molar presence is defined by:

- The number of substance (n_j);
- The molar concentration (Cj) ;
- The molar fraction (or the molar proportion (xj (for a liquid) and y for gas):

  The molar concentration defined as: $C_j = \frac{n_j}{V}$

Where: V: volume of the defined phase (which may vary in the case of a gas phase).

- *The mol fraction of components Aj defined as* **:** $x_j = \frac{n_j}{n}$

n: the number of moles present in the reaction phase (including active species and inert compounds at time t.) $n = n_1 + n_2 + \cdots + n_I$ (where n_I is the number of moles of inert compounds).

At the **reference state (denoted by subscript zero)**, under pressure P_0 and temperature T_0, the mole numbers are n_{j0} for the reactive species and n_I for the inert components, which are constant by definition).

The total number of moles ofactive components at the reference state is given by :

$$n_0 = n_{10} + n_{20} + \cdots$$

We can then define the **partial mole fractions** of each species j as: $y_j = \frac{n_j}{n_0}$

Another useful quantity in the **gas phase** is the **ratio of inert compounds to active species at the reference state,** which is expressed as**:** $I = \frac{n_I}{n_0}$

b) Open system (continuous flow reactors):

An open system exchanges matter with surroundings (Figure I.4) : through feeding (inlet) and withdrawal (outlet).

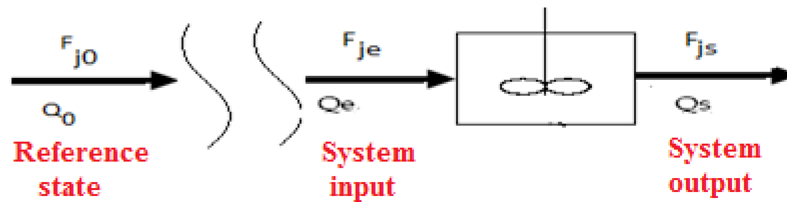


Figure I.4: Open system.

Q_0, Q_e, Q_s : reference, inlet, and outlet volumetric flow rates of the reactor ($m^3 . s^{-1}$).

F_{j0}, F_{je}, F_{js}: reference, inlet, and outlet molar flow rates of the reactor ($mol. s^{-1}$).

At the reference state $\xi = X = 0$.

There is both an inlet and an oulet flow. To describe the system, we use a spatio- temporal function Cj (t, x), which represents the concentration of species j as a function of time and position in spece:

- Molar flow rates: $\mathbf{F} = \frac{\text{quantity}}{\text{time}} \quad \frac{mol}{s}$ $\qquad (I.35)$

- Volumetric Flow rate : $\mathbf{Q} = \frac{\text{volume (rectionnel)}}{\text{time}} \left(\frac{\text{m3}}{\text{s}} \text{ or } \frac{\text{L}}{\text{s}}\right)$ $\qquad (I.36)$

The relation between molar flow (F) and volumetric flow (Q): $F = Q.C_A$

I.7.1. Closed system

a. Case of a simple reaction:

Consider the following simple reaction:

$$aA + bB \rightarrow pP$$

We assume that A is the limiting reactant, i.e. the reactant that is completely consumed first during a chemical reaction).

Let:

$\mathbf{n_{A0}}$ **:** the number of moles of species A present in the system at t=0 ;

$\mathbf{n_A}$ **:** the number of moles of species A remaining in the system at any time t.

$\boldsymbol{n_{A_0} - n_A}$**:** the number of moles of species A that have disappeared (**i.**e. reacted or been transformed)

- **Reaction progress (ξ) :**

The molar extent of reaction, denoted by ξ(ksi), for a system in evolution is defined by the following relation :

$$\xi\,(t) = \frac{n_j - n_{j0}}{\upsilon_j}\ (mol) \tag{I.37}$$

υ_j : stoichiometric coefficient

If ξ is known, the instantaneous number of moles of each component can be determined based on the initial number of moles and the extent of reaction using the following equation:

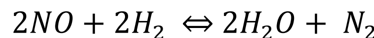
$$n_j = n_{j_0} + \upsilon_j\,\xi_{j_0} \qquad (\text{a t=0, } \xi=0)$$

In the case of the previous equation : $aA + bB \rightarrow pP$

$$\xi = \frac{n_A - n_{A_0}}{-a} = \frac{n_B - n_{B_0}}{-b} = \frac{n_P - n_{P_0}}{p}$$

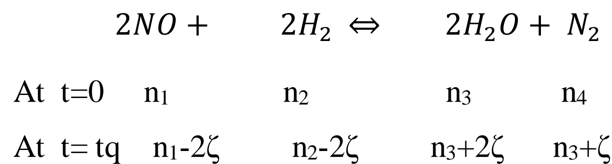
Example:

Let us consider the following reaction between nitric oxide and dihydrogen



$$2NO + 2H_2 \Leftrightarrow 2H_2O + N_2$$

It takes 2 moles of NO and 2 moles of H_2 to produce 1 mole of N_2 and 2 moles of H_2O.



	$2NO +$	$2H_2 \Leftrightarrow$	$2H_2O +$	N_2
At t=0	n_1	n_2	n_3	n_4
At t= tq	n_1-2ζ	n_2-2ζ	n_3+2ζ	n_3+ζ

ξ(t) is called the extent of reaction at time *t*, and it is defined by:

$$\xi\,(t) = \frac{n_j - n_{j0}}{\upsilon_j} \quad \text{or} \quad n_j = n_{j_0} + \nu_j\,\xi_{j_0}$$

Note: The extent of reaction is a global quantity: it is **identical for all species** in the reaction, whether it is calculated from reactants or products.

- **Generalized extent of reaction (χ)**

The generalized extent of reaction, denoted (χ), is a dimensionless number that is not associated with any specific component of the reaction.

$$\chi = \frac{n_j - n_{j_0}}{\upsilon_j n_0} = \frac{\xi}{n_0} \tag{I.38}$$

n_0 : total number of moles of reactive species at time t = 0 ($n_0 = \sum_{j=1}^{n} n_{j_0}$)

The generalized extent of reaction (χ) varies between **0** and a limiting value (χ_{Lim}) determined by the complete consumption of the limiting reactant.

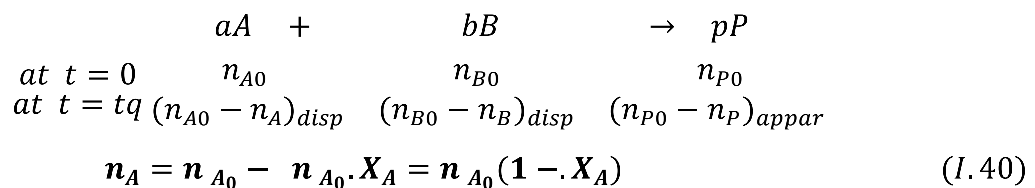
$$n_j = n_{j_0} + \upsilon_j.\ \chi.n_0$$

For $n_A = 0$ it follow that $\chi = \chi_{lim} \Rightarrow \boldsymbol{\chi_{lim}} = \frac{\mathbf{n_{A_0}}}{-\upsilon_A.\mathbf{n_0}}$

- **The conversion rate of reactant A (X_A)**

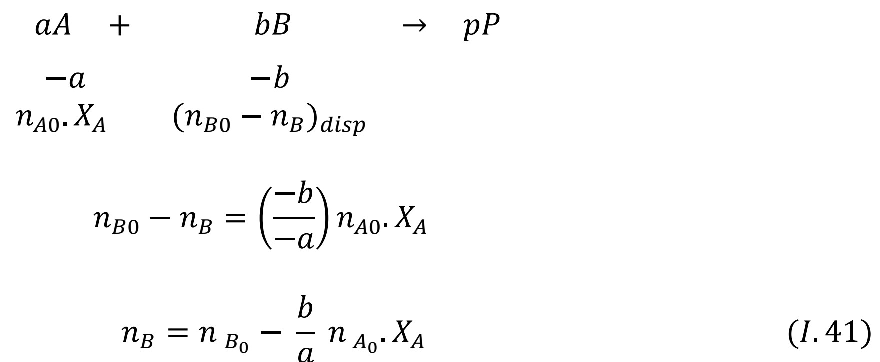
Also called the transformation rate, or simply conversion) is defined as the ratio of the number of moles of A that have reacted to the initial number of moles of A present in the system. It is expressed as:

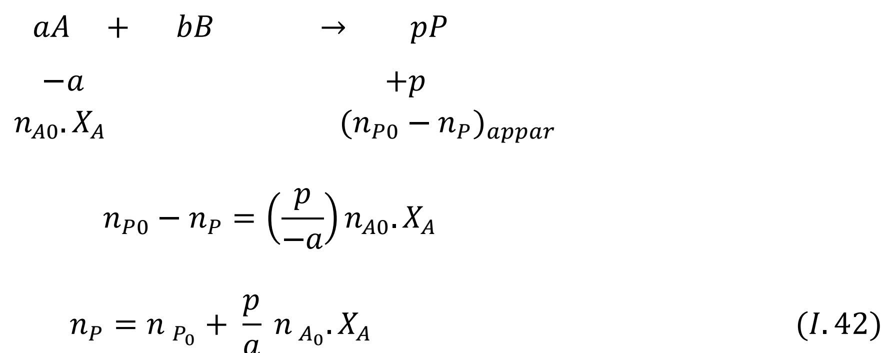
$$\mathbf{X_A} = \frac{\mathbf{n_{A_0} - n_A}}{\mathbf{n_{A_0}}} \qquad (I.39)$$



$$\boldsymbol{n_A = n_{A_0} - n_{A_0}.X_A = n_{A_0}(1 -.X_A)} \qquad (I.40)$$

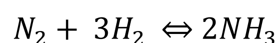
The other mole numbers can be expressed as a function of X_A.





Exercise:

Consider a closed reactor initially containing 1 mole of nitrogen (N_2) and 1 mole of hydrogen (H_2). The following reaction may occur in the reactor:



At a given time, the contents of the reactor are**:** 0,8 mol de N_2, 0,4 mol de H_2 et 0,4 mol de NH_3. What are, at this moment, the values of the extent of reaction and the conversion rate of the limiting reactant?

- **Solution**

Values of the progress reaction:

$$\xi = \frac{n_{N_2} - n_{0,N_2}}{-1} = \frac{0,8 - 1}{-1} = 0,2\ mol$$

$$\xi = \frac{n_{H_2} - n_{0,H_{20}}}{-3} = \frac{0,4 - 1}{-3} = 0,2\ mol$$

$$\xi = \frac{n_{NH_3} - n_{0,NH_3}}{2} = \frac{0,4 - 0}{2} = 0,2\ mol$$

Values of the conversion rates:

The limiting reactant is H_2 $\left(\frac{n_{H_2}}{\upsilon_{H_2}} = \frac{0.4}{3} < \frac{n_{N_2}}{\upsilon_{N_2}} = \frac{0.8}{1}\right)$

Then $X_{H_2} = \frac{n_{0_{H_2}} - n_{H_2}}{n_{0_{H_2}}} = \frac{1-0,4}{1} = \mathbf{0,6\quad (60\%)}$

b. Case of multiples reaction :

For multiple reactions, the overall reaction system can be written in the following form:

$$\sum_j \upsilon_{ij} A_j = 0 \qquad (I.43)$$

$$n_j = n_{j_0} + \sum_i^m \upsilon_{ij} \xi_i \qquad (I.44)$$

$$n_j = n_{j_0} + \boldsymbol{n_0} \sum_i^m \upsilon_{ij} X_i \qquad (I.45)$$

i and j represent respectively the reaction number (or equation index), and j represents the chemical species.

I.7.2. Open system

a. Case of the simple reaction:

Let us consider the following simple reaction : $aA + bB \rightarrow pP$ (Where A is the limiting reactant).

- **Reaction progress (ξ) (extent of reaction):**

The molar extent of reaction, ξ(ksi) for an evolving system is defined by the following relation:

$$\xi = \frac{F_j - F_{j_0}}{\upsilon_j} \tag{I.46}$$

it is expressed in mole/l ($\xi = 0$ pour t=0)

υ_j: Stoichiometric Coefficient.

The molar flow rate Fj of each component can be determined as a function of ξ at any point in the steady-state flow.

$$F_j = F_{j_0} + \upsilon_j \xi \qquad \text{(a t=0, } \xi\text{=0)}$$

At the inlet of the reactor : $F_{je} = F_{j_0} + \upsilon_j \xi_e$

At the outlet of the reactor: $F_{js} = F_{j_0} + \upsilon_j \xi_s$

The combination of the inlet and outlet flow rates gives:

$$F_{js} = F_{je} + \upsilon_j(\xi_s - \xi_e) \tag{I.47}$$

- **Generalized extent reaction (χ)**

The generalized extent reaction is defined as follows:

$$\chi = \frac{F_j - F_{j_0}}{\upsilon_j F_0} = \frac{\xi}{F_0} \tag{I.48}$$

F_0 is the total molar flow rate of the reactive species at the reference state:

$$F_0 = \sum_j F_{j0} \tag{I.49}$$

For an open system, we have:

At the inlet of the reactor : $F_{je} = F_{j_0} + \upsilon_j F_0 X_e$

At the outlet of the reactor: $F_{js} = F_{j_0} + \upsilon_j F_0 X_s$

The combination of the inlet and outlet flow rates gives:

$$F_{js} = F_{je} + \upsilon_j F_0 (X_s - X_e) \tag{I.50}$$

Note: In the case where the reference state is the same as the reactor inlet, we have:

$F_{je} = F_{j_0}$, ξe =0, Xe =0 (this is because the reaction has not yet started- therefore, no conversion or extent of reaction has occurred at this stage).

$n_A = n_{A0}$, $X_e = \frac{n_A - n_{A_0}}{\upsilon_j n_0} = 0$), therefore we can write: $F_{js} = F_{j0} + \upsilon_j F_0 X_s$

- **Conversion rate of the limiting reactant X_A**

The conversion rate for an open system is given by the following expression:

$$\mathbf{X_A} = \frac{\mathbf{F_{A_0} - F_A}}{\mathbf{F_{A_0}}} \quad (I.51)$$

$$\text{Thus}: F_A = F_{A_0} - F_{A_0}.X_A = F_{A_0}(1 - X_A) \quad (I.52)$$

The other molar flow rates of this reaction can be expressed as a function of X_A.

$$F_B = F_{B_0} - \frac{b}{a}F_{A_0}.X_A \quad (I.53)$$

$$F_P = F_{P_0} + \frac{p}{a}F_{A_0}.X_A \quad (I.54)$$

b. Case of multiple reaction

For a multiple reaction: $\sum_j \upsilon_{ij}\mathbf{A_j} = \mathbf{0}$

$$F_j = F_{j_0} + \sum_i^m \upsilon_{ij}\xi_i \quad (I.55)$$

$$F_j = F_{j_0} + \boldsymbol{F_0} \sum_i^m \upsilon_{ij} X_i \quad (I.56)$$

i and j are the number of reaction and components respectively.

ξ_i and X_i are the extent of reaction and generalized extent respectively for the i[ème] reaction

I.8. Expression of the reaction mixture volume as a function of the extent of reaction:

- **In the case of gas phase: (simple reaction, closed system)**

When the volume of reacting mixture changes proportionately with conversion, as in the case of gas phase reaction, this implies working volume changes with time.

Let us consider an ideal gas:

$PV_j = n_jRT \quad \Rightarrow \quad V_j = \frac{n_jRT}{P}$,

V_j: *volume occupied by* n_j *moles of species j at ime t* .

On a aussi **:** $V_I = \frac{n_IRT}{P}$, V_I: *volume occupied by* n_I *moles of inerte*

The total volume of the reaction mixture, $\mathbf{V_T}$**,** is given by the following expression:

$$\boldsymbol{V_T = V_I + \sum V_j} \qquad (I.57)$$

$$\boldsymbol{V_T} = \frac{n_IRT}{P} + \sum_{j=1}^{n} \frac{n_jRT}{P} = \frac{RT}{P}\left(n_I + \sum_{j=1}^{n} n_j\right)$$

The expression of $\mathbf{n_j}$ as a function of the generalized extent of reaction χ is given as follows:

$$n_j = n_{j_0} + \upsilon_j.\, \chi.n_0$$

$$\boldsymbol{V_T} = \frac{RT}{P}.\left(n_I + \sum_{j=1}^{n}\left(n_{j_0} + \upsilon_j.\, \chi.n_0\right)\right)$$

$$\boldsymbol{V_T} = \frac{RT}{P}.\left(n_I + \sum_{j=1}^{n} n_{j_0} + \chi.n_0 \sum_{j=1}^{n} \upsilon_j\right)$$

$\boldsymbol{V_T} = \frac{RT}{P}.\left(n_I + n_0 + \chi.n_0 \sum_{j=1}^{n} \upsilon_j\right)$ *with* $\sum_{j=1}^{n} n_{j_0} = n_0$ *at initial state*

$$\sum_{j=1}^{n} \upsilon_j = \Delta\upsilon$$

$$\boldsymbol{V_T} = \frac{RT}{P}.(n_I + n_0 + \chi.n_0\Delta\upsilon) \qquad (I.58)$$

At reference state:

$$\boldsymbol{V_0 = V_I + \sum V_{j0}}$$

with : $V_{j0} = \frac{n_{j0}RT_0}{P_0}$ et $V_{I0} = \frac{n_{I0}RT_0}{P_0}$

$$\boldsymbol{V_0 = \left(n_I + \sum n_{j0}\right)\frac{RT_0}{P_0} = (n_I + n_0)\frac{RT_0}{P_0}}$$

The ratio : $\frac{\boldsymbol{V_T}}{\boldsymbol{V_0}} = \frac{\frac{RT}{P}.(n_I + n_0 + \chi.n_0\Delta\upsilon)}{\boldsymbol{(n_I - n_0)\frac{RT_0}{P_0}}} = \frac{\boldsymbol{RTP_0}}{\boldsymbol{RPT_0}}\left[\frac{n_I + n_0}{n_I + n_0} + \frac{\boldsymbol{Xn_0\Delta v}}{n_I + n_0}\right]$

$$\boldsymbol{\frac{V_T}{V_0} = \beta(1 + \mathcal{E}X)}$$

Where:

β : Physical expansion Factor : $\beta = \frac{T.P_0}{T_0.P}$

Ɛ ; Chemical expansion Factor : $\boldsymbol{\varepsilon} = \frac{n_0 \Delta \upsilon}{n_I + n_0} = \frac{\Delta \upsilon}{I+1}$, $I = \frac{n_I}{n_0}$

So we get:

$$V_T = \beta . V_0 (1 + \mathcal{E} . \chi) \qquad (I.59)$$

The total volume of the reaction mixture can also be expressed as a function of the conversion rate X_A.

$$V_T = \beta . V_0 (1 + \mathcal{E}_A . X_A) \qquad (I.60)$$

Where : $\varepsilon_{A=}\ \varepsilon . \frac{n_{A0}}{-\upsilon_j n_0}$

Note:

In the case of the multiple reactions, we write: $\varepsilon_i = \frac{\Delta \upsilon_i}{1+I}$: Chemical expansion factor of the i^{ème} reaction. $\Delta \upsilon_i = \sum_{j=1}^{n} \upsilon_{ij}$

$$V_T = V_0 . \beta . (1 + \sum_{i=1}^{m} \varepsilon_i X_i)$$

- **In the case of liquid or condensed phase:**

  In the case of liquid phase, the reaction volume is equal to:

  $V_T = V_I + \sum_j V_j = n_I v_I + \sum_j n_j v_j$

v_I : partial molar volume occupied by one mole of inert (m^3/mole).

v_j : partial molar volume occupied by one mole of A_j (m^3/mole).

V_j : volume occupied by n mole of A_j

We replace $\mathbf{n_j}$ with its expression as a function of the extent of reaction.

$$n_j = n_{j_0} + \upsilon_j . \chi . n_0$$

$$V_T = n_I v_I + \sum_j (n_{j_0} v_{j} + v_j . \upsilon_j . \chi . n_0)$$

$$V_T = n_I v_I + \sum_j n_{j_0} v_j + \chi . n_0 \sum_j v_j . \upsilon_j$$

$$\sum_j n_{j_0} v_j : volume\ occupied\ at\ t = 0\ by\ all\ reactants$$

$V_0 = n_I v_I + \sum_j n_{j_0} v_j$, V_0: the volume occupied by the mixture of reaction at t=0 when χ =0.

$V_T = V_0 + \chi . n_0 \Delta v_j$ with $\Delta v_j = \sum_{j=1}^{n} v_j . \upsilon_j$

$$V_T = V_0 \left(1 + \frac{n_0}{V_0} . \chi . \Delta v_j\right)$$

Let $\varepsilon = \frac{n_0}{V_0} . \Delta v_j = C_0 . \Delta v_j$

$$V_T = V_0 \left(1 + \mathcal{E} . \chi\right) \qquad (I.61)$$

Chapter II: Classification of Chemical Reactors and Material Balance

II.1. Reactor definitions

Reactors are devices vessels used to carry out chemical reactions under controlled conditions. They are essential components in chemical and biochemical processes, where raw materials are transformed into desired products. The classification of reactors can be based on several criteria. Figure II.12 illustrate the main criteria for reactor classification.

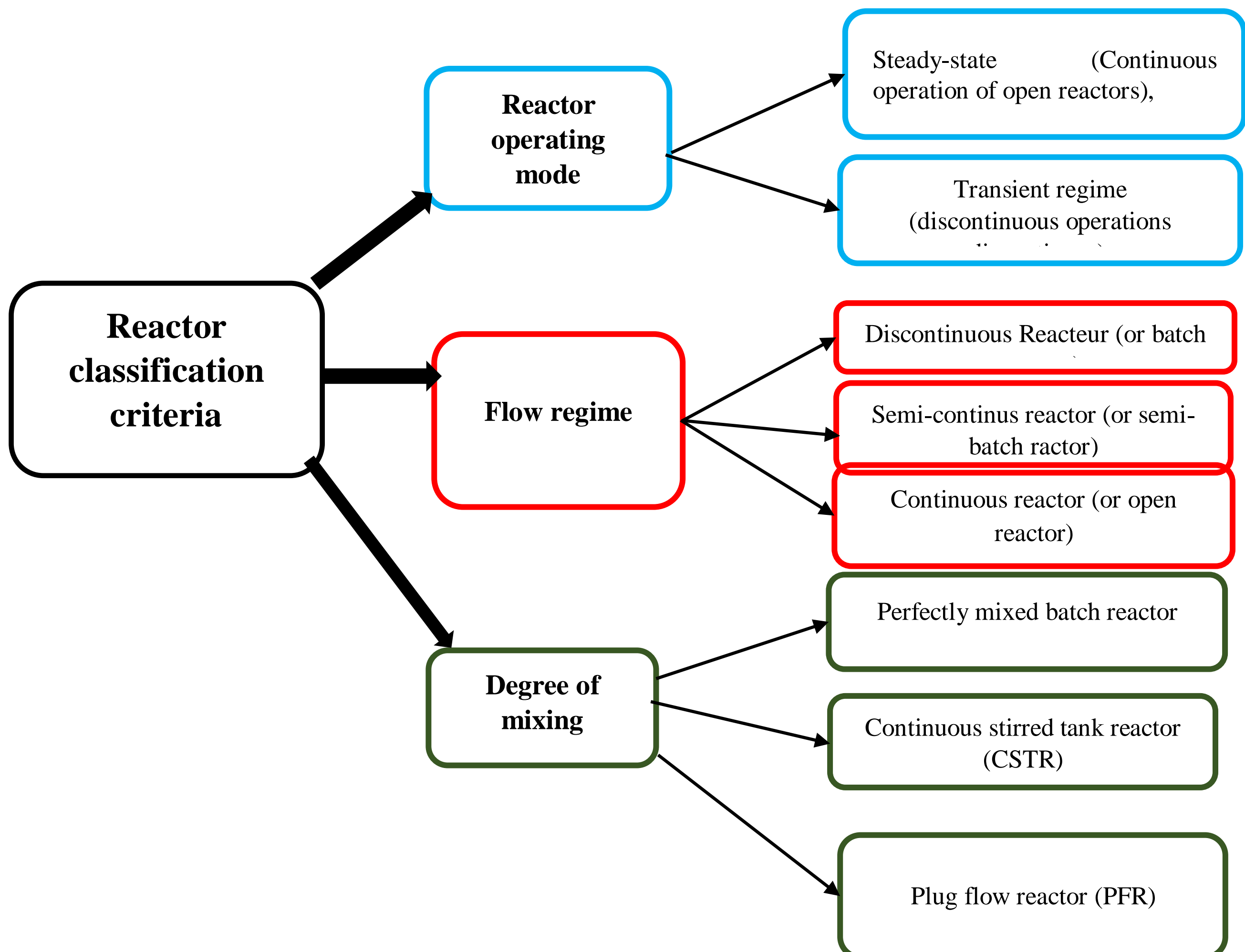


Figure II.1: The main criteria for the reactor classification

II.1.1. Batch reactor

A batch reactor is a reactor that **does not exchange matter with the surroundings** during its operation. It is characterized by:

- ***Discontinuous (batch) operation:*** the reactants are loaded at the beginning, and the products are removed at the end of the reaction.

- ***Uniform properties throughout the reactor:*** variables such as composition, temperature, and concentration are considered uniform in all parts of the reactor (i.e., the concentration of each species is the same everywhere inside the vessel). This implies perfect mixing and no spatial gradients inside the reactor.
- ***The reactor operates under transient (unsteady-state) conditions***: meaning that the composition changes over time. Mathematically, this is expressed as:

$$\left(C_j = f(t) \Rightarrow \frac{dn_j}{dt} \neq 0 \; or \; \frac{dC_j}{dt} \neq 0\right)$$

Where:

C_j is the concentration of species j,

$\frac{dn_j}{dt} \neq 0$ this indicates that the amount of species j changes with time.

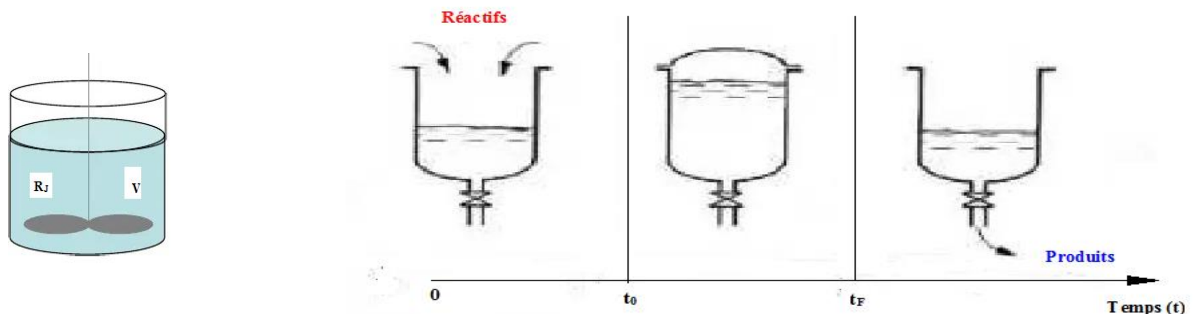


Figure III.1: Batch reactor (fonctioning steps)

II.1.2. Semi-batch reactor

In a semi batch (or semi – open) reactor, a reactant or a product is continuously fed or withdrawn from the system. These reactors are generally well-mixed but have a variable volume. This type of reactor is characterized by operation under **transient** conditions.

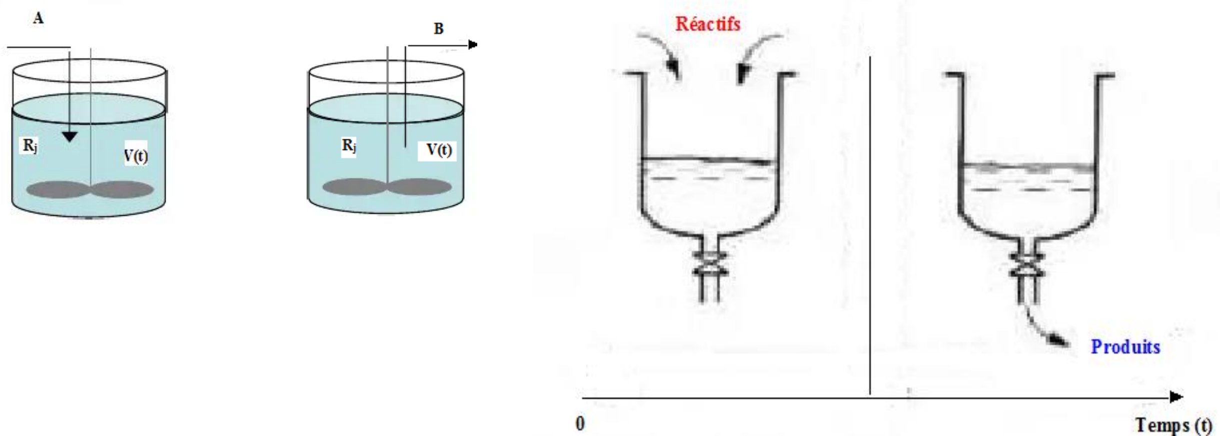


Figure III.2: semi-batch reactors

II.1.3. Open reactors

In the case of continuous reactors, a distinction is made between the continuous stirred-tank reactor (CSTR) and the plug flow reactor (PFR).

a) Continuous stirred tank reactor (CSTR)

The continuous stirred-tank reactor, also called a uniform open reactor operating under steady-state conditions, consists of a perfectly stirred vessel into which all reactants are continuously fed, and products are continuously removed. The main assumptions of this model are:

- Homogeneous mixing at the molecular level;
- Uniform temperature throughout the reactor;
- Constant volume and density over time;
- All operating parameters are independent of time (steady state);
- The concentration and temperature of the outlet stream are identical to those inside the reactor.

Continuous stirred-tank reactors (CSTRs) consist of the following components (Figure II.4) :

- A tank reactor ;
- An agitation system to mix the reactants (such as a pump or high-speed reactant injection);
- Inlet and outlet pipes for adding reactants and allowing the flow of products.

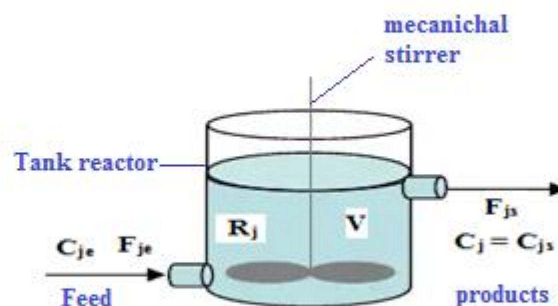


Figure II.5: Individual reactor

CSTRs are frequently used in industrial processes, particularly in homogeneous liquid-phase flow reactions that require constant stirring. However, they are also used in the pharmaceutical sector and in biological processes such as cell cultures and fermentation tanks. CSTRs can be used individually (Figure II.5) or in series (II.6) (cascade configuration).

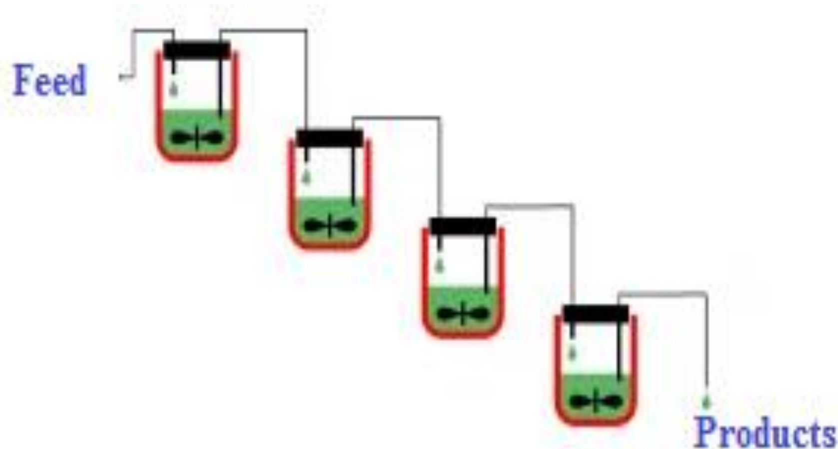


Figure II.6: CSTR cascade

b) Plug Flow Reactor (PFR)

A plug flow reactor (PFR) (Figure II.7) may be used for both liquid-phase and gas-phase reactions and for both laboratory-scale investigations of kinetics and large-scale production. The reactor itself may consist of an empty tube or vessel, or it may contain packing or a fixed bed of particles (catalyst particles).

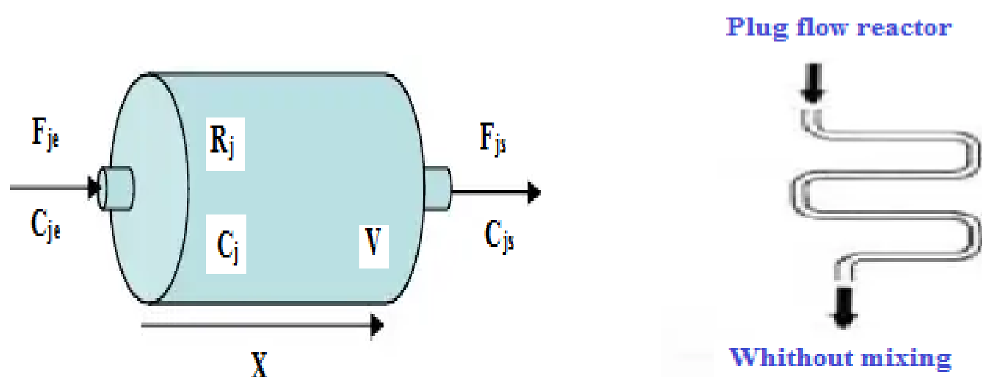


Figure II.7: Plug flow reactor.

A Plug flow reactors (PFR) is similar to a CSTR in being a flow reactor, but is different in its mixing characteristics. It is different from a batch reactor in being a flow reactor, but similar in the progressive change of properties, with position replacing time.

In PFR, the reaction mixture progresses continuously through the reactor in parallel, independent slices that do not exchange matter-much like a piston moving through a cylinder. Axial diffusion is negligible.

All characteristics of this reactor are presented follow:

- The flow through the vessel, both input and output streams, is continuous, but not necessarily at constant rate; the flow in the vessel is piston flow (PF);
- The system mass inside the vessel is not necessarily fixed;
- There is no axial mixing of fluid inside the vessel (i.e., in the direction of flow);
- There is complete radial mixing of fluid inside the vessel (i.e., in the plane perpendicular to the direction of flow); thus, the properties of the fluid, including its velocity, are uniform in this plane;
- The density of the flowing system may vary in the direction of flow;
- The system may operate at steady-state or at unsteady-state;
- There may be heat transfer through the walls of the vessel between the system and the surroundings.

The characteristics of the PFR, as described in the seven points above, give rise to the following consequences

1. Each fluid element has the same residence time **t** implying the absence of residence time no spread in **t**.
2. Properties may change continuously in the direction of flow.
3. In the axial direction, each portion of fluid, no matter how large, acts as a closed system in motion, not exchanging material with the portion ahead of it or behind it.
4. The volume of an element of fluid does not necessarily remain constant through the vessel; it may change because of changes in **T**, **P** and $\mathbf{n_t}$, the total number of moles

II.2. Comparison between different types of reactors

Each type of reactor has its advantages and disadvantages.

- A continuous stirred tank reactors (CSTR) can deliver large quantities of product per unit time and operate over extended periods, but is less suitable for reactions with slow kinetics. For this type of synthesis, batch reactors are generally preferred.
- Plug flow reactors tend to take up less space and offer higher conversion rates. However, they are rarely used for highly exothermic reactions due to rapid temperature rises that are difficult to control. Furthermore, plug flow reactors (PFR) have higher operating and maintenance costs than CSTRs.
- The interior of the reactor (CSTR) is accessible allowing for the implementation of process analytical technology (PAT)
- Multiple CSTR units can be easily connected for cascade operation or integrated into more complex flow systems alongside PFRs and other reactor types.
- Overall yield per unit volume is generally lower than that of tubular flow reactors (PFR).

Note:

The characteristic equations of these two reactors (batch and plug flow) are identical: achieving the same conversion of a reactant requires the same residence time. However, in the case of the batch reactor, additional downtime must be taken into account for operations such as filling, emptying, and cleaning, which are often labor-intensive and environmentally burdensome.

For this raison, in large-scale processes involving high production volumes, the plug flow reactors (PFR) is generally preferred. In contrast, the batch reactor is typically reserved for the synthesis of small quantities of high-value products., such are pharmaceuticals or specialty chemicals.

II.3. Material balance over a reactor volume

The starting point for all design is the material balance expressed for any reactant (or product). Thus, as illustrated in Fig III.5, we have

$$\begin{pmatrix} rate\ of\ reactant \\ Flow\ \mathbf{Into} \\ element\ of\ volume \end{pmatrix} + \begin{pmatrix} reate\ of\ reactant \\ flow\ \mathbf{out}\ of \\ element\ of\ volume \end{pmatrix}$$

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

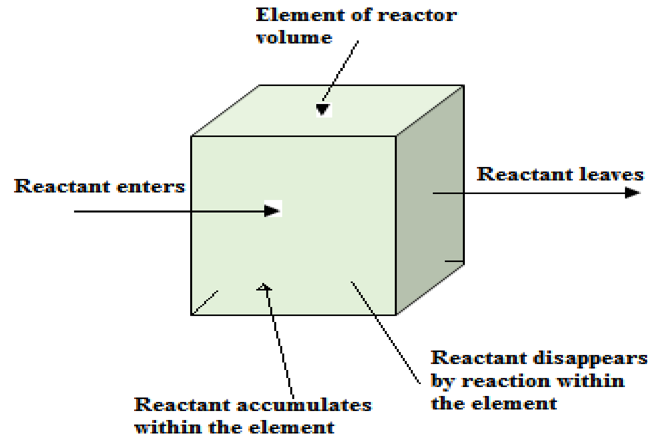


Figure III.5: Material balance for an element of volume of the reactor.

Where the composition within the reactor is uniform, i.e, independent of position), the material balance can be applied over a differential volume element and then integrated over the entire reactor for the appropriate flow and concentration conditions. For the various reactor types the obtained equation can be simplified accordingly, and its integrated from provides the basic performance equation for that type of unit.

Input + **Disappearance (Prod)** = **Output** + **Accumulation** (II.1)

$$F_{je} + R_j.V = F_{JS} + \frac{dn_j}{dt} \quad (II.2)$$

Where:

F_{je} : inlet molar flow of specieses j (mol/s) ($F_{je} = Q_e C_{je}$)

F_{js} : outlet molar flow of species j (mol/s) ($F_{js} = Q_s C_{js}$)

R_j : the effective rate of transformation of species j involved in **i** reactions

In the case of multiple reaction: $$R_j = \sum_i^m \upsilon_{ij} r_i \quad (II.3)$$

In the case of simple reaction: $$R_j = \upsilon_j r = r_j \quad (II.4)$$

rj: Volumetric reaction rate of species j (mole/s.m^2).

υ_j: Stoichiometric coefficient.

V : volume of reactor (m^3).

II.3.1. Ideal batch reactor

In a batch reactor, since the composition is uniform throughout at any instant of time, we way make the accounting about the entire reactor.

Note that no fluid enters or leaves the reaction mixture during reaction. Eq.II.1, which was written for component A, becomes:

Disappearance (Prod) = Accumulation

$$\text{So :} \quad \boldsymbol{R_j.V = \frac{dn_j}{dt}} \tag{II.6}$$

Its describe the material balance equation for a closed reactor is written as follows:

With : $\boldsymbol{R_J = \sum_i^m \upsilon_{ij} r_i}$

$$\text{Thus :} \quad \boldsymbol{V} \sum_i^m \upsilon_j r_i = \frac{dn_j}{dt} \tag{II.7}$$

Case of simple reaction

Consider a simple reaction **:** $\boldsymbol{A \Rightarrow p}$

The material balance on the key reactant A is written as:

$$\boldsymbol{R_A.V = \frac{dn_A}{dt}}$$

$$R_A = \upsilon_A.\mathbf{r} = \boldsymbol{r_A} \; ; \; \left(\boldsymbol{r} = \frac{1}{\upsilon_j}.\frac{dn_j}{dt} = \frac{1}{\upsilon_j}.\boldsymbol{r_j}\right)$$

$$r_A V = \frac{dn_A}{dt} \Rightarrow \frac{dn_A}{V.r_A} = dt \tag{II.8}$$

$$\textit{we can also writte:} \; \frac{dn_A}{V.r_A} = dt \Rightarrow \frac{dVC_A}{V.r_A} = dt$$

1) Expression of residence time as a function of molar concentration

To calculate the t_s the equation (II.8) should be integrate.

$$\int_{n_{A0}}^{n_{Af}} \frac{dn_A}{V.r.\upsilon_A} = \int_0^{t_s} dt \Rightarrow t_s = \int_{n_{A0}}^{n_{Af}} \frac{dn_A}{V.r.\upsilon_A}$$

$$t_s = \int_{C_{Ae}}^{C_{As}} \frac{dC_A}{r.\upsilon_A} = \int_{C_{Ae}}^{C_{As}} \frac{dC_A}{r_A} \tag{II.9}$$

Residence time t_s: *is the time the mixture (reactant A) must remain in the reactor for the conversion to increase from X_{AE} to X_{AS} (or from C_{Ae} to C_{As}, n_{Ae} to n_{As}*

1. Expression of residence time as a function of conversion X_A

We have : $n_A = n_{A_0} - X_A n_{A_0} \Rightarrow n_A = n_{A_0}.(1 - X_A)$

The material balance equation (II.8) is written as follows: $r.\upsilon_A.V = \frac{dn_A}{dt} = \frac{d(n_{A_0} - X_A n_{A_0})}{dt}$

$$t_s = \int_{n_{A0}}^{n_{Af}} \frac{dn_A}{V.r.\upsilon_A} = \int_{n_{A0}}^{n_A} \frac{d(n_{A_0} - X_A n_{A_0})}{V.r.\upsilon_A} = n_{A_0} \int_0^{X_A} -\frac{d(X_A)}{V.r.\upsilon_A}$$

$$t_s = n_{A_0} \int_0^{X_A} \frac{d(X_A)}{-r.\upsilon_A.V}$$

At t = 0, $X_{Ae} = 0$

With : $(\upsilon_A = -1)$

Then

$$\mathbf{t_s = n_{A_0} \int_0^{X_A} \frac{d(X_A)}{r.V}} \qquad (II.10)$$

If V is constant: (case of liquid phase) ($V=V_0$)

$$t_s = \frac{n_{A_0}}{V} \int_0^{X_A} \frac{d(X_A)}{r} = C_{A_0} \int_0^{X_A} \frac{d(X_A)}{r}$$

If V is not constant:

$$V_T = \beta.V_0(1 + \varepsilon_A.X_A)$$

$$t_s = n_{A_0} \int_0^{X_A} \frac{d(X_A)}{r.\beta.V_0(1 + \varepsilon_A.X_A)} = \frac{n_{A_0}}{V_0} \int_0^{X_A} \frac{d(X_A)}{r.\beta.(1 + \varepsilon_A.X_A)}$$

$$\mathbf{t_s = C_{A_0} \int_0^{X_A} \frac{d(X_A)}{r.\beta.(1+\varepsilon_A.X_A)}} \qquad (II.11)$$

2. 1. Expression of residence time as a function of reaction extent

We have: $n_A = n_{A_0} + \upsilon_A \xi$

The material balance equation (II.8) is written as follows:

$$r.\upsilon_A V = r_A V = \frac{dn_A}{dt} = \frac{d(n_{A_0} + \upsilon_A \xi)}{dt}$$

$$t_s = \int_{n_{A0}}^{n_{Af}} \frac{dn_A}{V.r.\upsilon_A} = \int_{n_{A0}}^{n_A} \frac{d(n_{A_0} + \upsilon_A \xi)}{V.r.\upsilon_A} = \int_0^{\xi} \frac{\upsilon_A d(\xi)}{V.\upsilon_A \mathbf{r}}$$

$$\mathbf{t_s = \int_0^{\xi} \frac{d(\xi)}{r.V}} \qquad (II.12)$$

A t = 0, ξ= 0

With : $\mathbf{r_A = \upsilon_A r = -r \quad (\upsilon_A = -1)}$

Application N°1:

The reaction is carried out in the liquid phase: $A \Rightarrow p$ (First-order with respect to the reactant A : $\mathbf{r = KC_A}$ with $\mathbf{k=0{,}5\ min^{-1}}$) in the closed uniform reactor (initially containing $\mathbf{5\ m^3}$ of a solution of A in an inert solvent, with a concentration $\mathbf{C_{A0}=3 mol.L^{-1}}$.

1- How does the concentration of reactant A vary over time?
2- How long does it take to reach a 90% or of 99% conversion?

Solution:

1. Expression of the concentration of reactant A as a function of time:

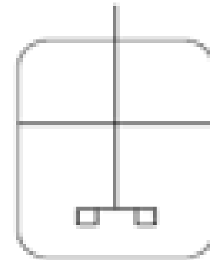
Material balance on reactant A in a perfectly mixed closed reactor (batch reactor)

The material balance for the key reactant A is written as:

Disappearance of A = Accu of A

$$R_A.V = \frac{dn_A}{dt}$$

$$\upsilon_A \mathbf{r}.\mathbf{V} = \frac{dn_A}{dt} \Rightarrow -r.V = \frac{dn_A}{dt} \quad (\upsilon_A = -1)$$



The reaction takes place in the liquid phase, so we assume that $V=V_0$=constant.

$$-r.V = \frac{dV.C_A}{dt} \Rightarrow -k.C_A = \frac{dC_A}{dt} \qquad (1)$$

$$-k.dt = \frac{dC_A}{C_A} \Rightarrow -Kt = Ln\left(\frac{C_A}{C_{A0}}\right)$$

$$C_A = C_{A0}.e^{-kt}$$

2. Determination of the time required to achieve 90% and 99 % of the conversion rate.

We have: $n_A = n_{A_0}.(1-X_A) \Rightarrow \frac{n_A}{V} = \frac{n_{A_0}}{V}.(1-X_A)$

Thus : $C_A = C_{A_0}.(1-X_A) \quad (2)$

We replace the expression (2) in (1), we find:

$$-k.C_{A_0}.(1-X_A) = \frac{dC_{A_0}.(1-X_A)}{dt}$$

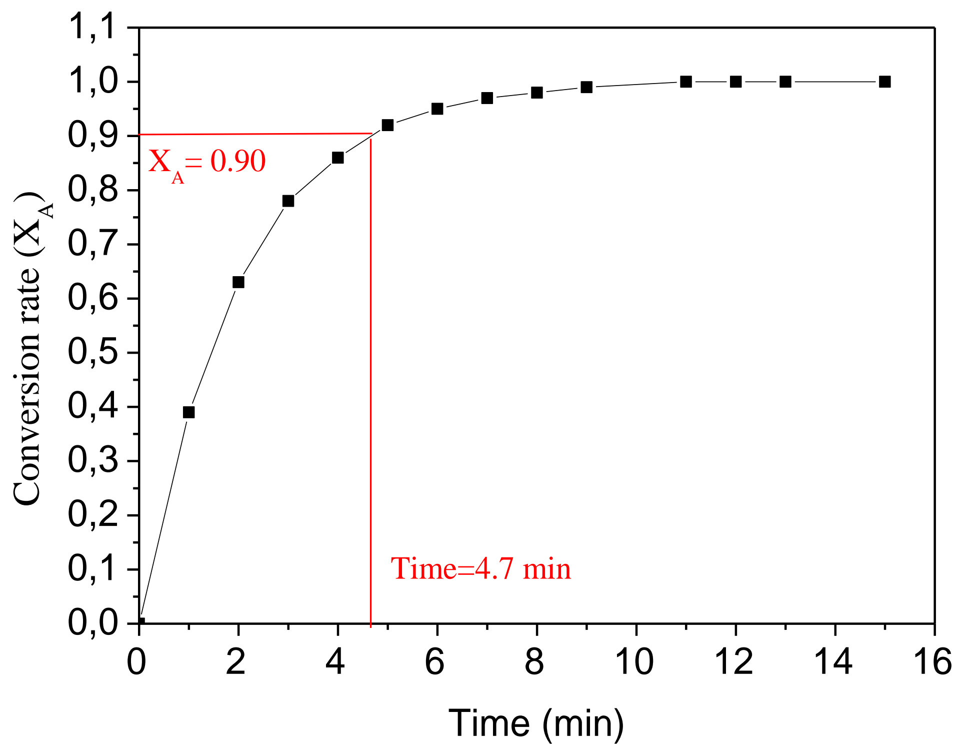
$$-k.dt = \frac{-C_{A_0} dX_A}{C_{A_0}.(1-X_A)} \Rightarrow k.dt = \frac{dX_A}{(1-X_A)}$$

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

$$\left[-lin(1-X_{A(t)})\right] = kt$$

$X_A(t) = 1\text{-}e^{-kt}$

Time(min)	XA (t)=1 - e^{-kt}
0	0,00
1	0,39
2	0,63
3	0,78
4	0,86
5	0,92
6	0,95
7	0,97
8	0,98
9	0,99
11	1,00
12	1,00
13	1,00
15	1,00



According to the figure: XA = F(t), we can note that, to achieve a conversion rate of 90%, a time of 4.7 min is required and to achieve a conversion of 99%, a time of 9 min is required

II.3.2. Steady-state mixed flow reactor

Depending on the degree on internal mixing of reactants and products during a chemical reaction, there are two limiting cases:

- **The perfectly stirred reactor (Continuous Stirred-Tank Reactor, CSTR)**
- **The plug flow reactor (PFR)**

a) Continuous stirred-tank reactor (CSTR) :

This reactor is known as a well-mixed reactor or homogeneous reactor. It is characterized by a perfectly uniform composition and temperature of the reaction mixture throughout its entire volume V_R. There is an immediate change in the concentration of the reactant upon entering the reactor, shifting from its inlet value to its outlet value : **Cj = Cjs** (*The product stream withdraw from the reactor has the same instantaneous composition as the reaction mixture*).

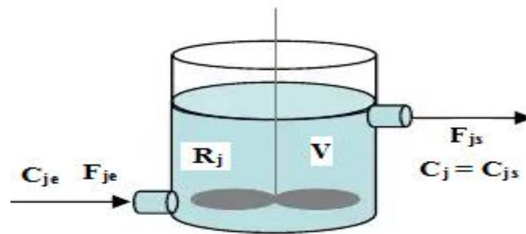


Figure II.6: Steady-state mixed flow reactor

The material balance equation gives as:

Inlet + **Disappearance (Prod)** = **Outlet** + **Accumulation**

$$F_{je} + R_j.V = F_{JS} + \frac{dn_j}{dt}$$

As Cj = Cjs, it is therefore assumed that the reactor operates under **steady-state** conditions.: $\frac{dn_j}{dt}=0$

Then: $$F_{je} + R_j.V = F_{JS} \qquad (II.13)$$

Case os simple reaction

Consider a simple reaction : $\boldsymbol{A \Rightarrow p}$

The material balance on the key reactant A is written as:

$$R_j = \sum_{i}^{m} \upsilon_j r_i = \upsilon_j r_i$$

$$F_{Ae} + \upsilon_A r V = F_{AS} \qquad (II.14)$$

Space time τ: this time is specific to open reactors. It is defined as the time required to process a volume of fluid equal to the volume of the reactor. It is given by the *ration of the reaction mixture volume "V_R" to the reference volumetric flow* rate (inlet) "Q_0".

$$\tau = \frac{V_R}{Q_0} \qquad (II.15)$$

a) ***Expression of space time as function of molar concentration:***

$$F_{je} = Q_e C_{je} \quad et \quad F_{js} = Q_s C_{js} \qquad (II.16)$$

Under **steady – state** conditions, the material balance for the component A over the entire reactor (equation II.14) is written as:

$$Q_e C_{Ae} + \quad r_A V_R - \quad Q_s C_{As} = 0$$

Note:

In a perfectly mixed open reactor operating under steady-state conditions, the outlet conditions are identical to those within the reactor (the concentration of species in the reactor is the same as at the outlet). Therefore, we can write : $\mathbf{r_A = r_{AS}}$.

Then : $$Q_e C_{Ae} + \mathbf{r_{As}} V_R - \quad Q_s C_{As} = 0 \qquad (II.17)$$

In the presence of inert components: $\mathbf{F_{Ie} = F_{Is}}$

- **Case where ρ = constant ⇒ QAe = QAs = Q0 (liquid phase)**

It is assumed that the flow rate Q_0 is constant at both the inlet and the outlet: $Q_e = Q_s = Q_0$

$$Q_0 \left(C_{Ae} - C_{As}\right) = -r_A V_R \qquad \Rightarrow \frac{V_R}{Q_0} = \frac{(C_{Ae} - C_{As})}{-r_A}$$

$$\tau = \frac{V_R}{Q_0} = \frac{(C_{Ae} - C_{As})}{-r_A} \qquad (II.18)$$

- **Case where ρ≠constant ⇒ QAe ≠ QAs ≠ Q0 (case of gas phase)**

$$V_R = \frac{(C_{Ae}.Q_e - C_{As}.Q_s)}{-r_A} \qquad (II.19)$$

b) Expression of space time as function of conversion X_A :

We have:

$$\begin{cases} F_{Ae} + \upsilon_A.r.V_R = F_{AS} = F_{Ae} + r_A.V_R \\ F_{Ae} = F_{A_0} - X_{Ae}F_{A_0} \\ F_{As} = F_{A_0} - X_{As}F_{A_0} \end{cases}$$

Then : $F_{A_0} - X_{Ae}F_{A_0} + r_A(X_{As}).V_R = F_{A_0} - X_{As}F_{A_0} \Rightarrow F_{A_0}(X_{As} - X_{Ae}) = -r_A(X_{As}).V_R$

Or: $F_{A0} = Q_0C_{A0}$ So: $Q_0C_{A0}(X_{As} - X_{Ae}) = -r_A(X_{As})V_R$

Thus: $\tau = \frac{V_R}{Q_0} = \frac{C_{A0}(X_{As} - X_{Ae})}{-r_A(X_{As})}$

In inlet of the reactor: $X_{Ae}=0$ then:

$$\tau = \frac{V_R}{Q_0} = \frac{C_{A0}X_{As}}{-r_A(X_{As})} = \frac{C_{A0}X_{As}}{r(X_{As})} \qquad \text{(II.20)}$$

c) Expression of space time as function of generalized advancement X:

$$\begin{cases} F_{Ae} = F_{A_0} + \upsilon_A X_e F_0 \\ F_{As} = F_{A_0} + \upsilon_A X_s F_0 \end{cases}$$

Then : $F_{A_0} + \upsilon_A X_e F_0 + r_A(X_s).V_R = F_{A_0} + \upsilon_A X_s F_0$

$$\upsilon_A F_0 (X_e - X_s) + r_A(X_s).V_R = 0$$

$$(Where: F_0 = Q_0.C_0 \text{ and } \upsilon_A = -1)$$

Then : $-Q_0.C_0 (X_e - X_s) + r_A(X_s).V_R = 0$

$$\frac{C_0 (X_e - X_s)}{r_A(X_s)} = \frac{V_R}{Q_0} = \tau$$

At the inlet of the reactor : Xe = 0, thus:

$$\tau = \frac{C_0 .X_s}{-r_A(X_s)} = \frac{C_0 .X_s}{r(X_s)} \qquad (II.21)$$

Application N°2:

For a liquid-phase reaction of the type: **A+….→ Products**, an experimental CSTR of volume 1.5 L is used to measure the rate of reaction at given temperature. If the steady- state feed rate is 0.015 L/s, the feed concentration (C_{A0}) is 0.8 mol. L^{-1}, and A is 15% converted on flow through the reactor, what is the value of ($-r_A$)?.

Solution:

The materials balance for the CSTR is expressed as follows:

Input + **Disappearance (Prod)** = **Output** + **Accumulation**

$$F_{je} + R_j.V = F_{JS} + \frac{dn_j}{dt} \qquad \text{with: } \frac{dn_j}{dt} = 0$$

$$F_{Ae} + \upsilon_A r V_R = F_{AS}$$

$$F_{Ae} + r_A V = F_{AS} \Rightarrow Q_0.C_{A0}(1 - X_{Ae}) - Q_0.C_{A0}(1 - X_{AS}) = -r_A V_R$$

$$\text{Then: } Q_0.C_{A0}.X_{AS} = -r_A V_R \qquad with\ X_{Ae} = 0$$

So: $-r_A = \frac{Q_0.C_{A0}X_{AS}}{V_R} = \frac{0.015.\ 0.8.0.15}{1.5} = 0.0012\ mol.L^{-1}s^{-1}$

b) plug flow reactor:

In a plug flow reactor, the composition of the fluid varies from point to point along a flow path; consequently, the material balance for a reaction component must be made for a differential element of volume dv (Figure II.7). In a plug reactor, the material balance compound A is given by II.1:

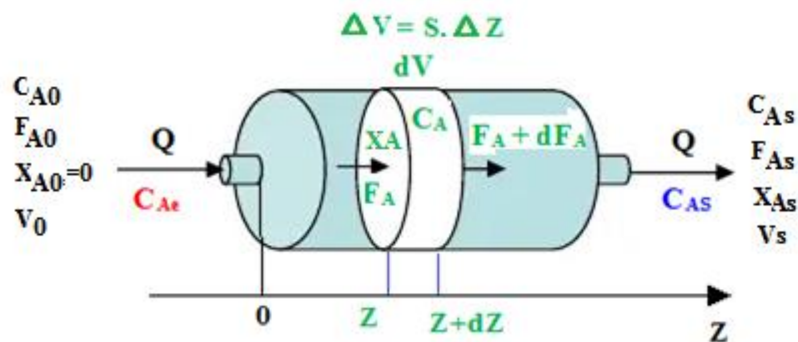


Figure II.7: Steady-state plug flow reactor.

Inlet + **Disappearance (Prod)** = **Oulet** + **Accumulation**

It is therefore assumed that the plug flow reactor operates under **steady-state** conditions.

Thus : $Accu = 0 \Rightarrow \frac{dn_j}{dt} = 0$

Input + **Disappearance (Prod)** = **Output**

$$F_j\big|_z + R_j.dV = F_j\big|_{z+dz} \quad \Rightarrow F_j + \upsilon_j r_j dV = F_J + dF_J \qquad (II.22)$$

d) *Case of unique réaction*

Let us write the material balance for reactant A over the section dV :

$$F_A + R_j.dV = F_A + dF_A$$

$$\upsilon_A r_A.dV = dF_A \quad For : (\upsilon_A = -1)$$

$$-r.dV = dF_A \qquad (II.23)$$

F_A et $F_A + dF_A$ are the inlet and outlet flow of the differential volume dV.

- ***Expression of space time us fonction of the molar concentration***

We have : $F_A = Q_0 C_A$ *then*: $-r.dV = d(Q_0 C_A) = Q_0 . dC_A$

So: $$\frac{d(C_A)}{r} = -\frac{dV}{Q_0}$$

The space time Ʈp for a plug flow reactor is given by the following equation:

$$\tau_p = \int_0^{V_R} \frac{dV}{Q_0} = \frac{V_R}{Q_0} = -\int_{C_{A0}}^{C_{AF}} \frac{d(C_A)}{r} \qquad (II.24)$$

- ***Expression of space time us a function of the conversion X_A***

First : $-r.dV = dF_A$ *with:* $\upsilon_A = -1$

As known: $F_A = F_{A_0} - X_A F_{A_0} = F_{A_0}(1 - X_A)$

Then: $-r.dV = -F_{A0} dX_A$

$$\frac{dX_A}{r} = \frac{dV}{F_{A0}}$$

We have $F_{A0} = Q_0 C_{A0}$, therefore :

$$\frac{dX_A}{r} = \frac{dV_R}{Q_0 C_{A0}} \quad \Rightarrow \int_0^{X_A} \frac{dX_A}{r} = \int_0^{V_R} \frac{dV}{Q_0 C_{A0}}$$

$$\tau_p = \int_0^{V_R} \frac{dV_R}{Q_0} = \frac{V_R}{Q_0} = C_{A0} \int_0^{X_A} \frac{d(X_A)}{r} \qquad (II.25)$$

e) **Expression of space time as a function of generalized advancement X :**

We have : $-r.dV = dF_A$

As known: $F_A = F_{A_0} + \upsilon_A . F_0 . X$

Then : $-r.dV = \upsilon_A.F_0.dX$

$$-\frac{dX}{r} = \frac{dV}{\upsilon_A.F_0}$$

Since $F_0 = Q_0C_0$ therfore :

$$-\int_{X_e}^{X_s}\frac{dX}{r} = \int_0^{VR}\frac{dV}{\upsilon_A.Q_0C_0} \Rightarrow -C_0\int_{X_e}^{X_s}\frac{dX}{r}. = \int_0^{VR}\frac{dV}{\upsilon_A.Q_0}$$

$$\tau_p = \int_0^{V_R}\frac{dV_R}{Q_0} = \frac{V_R}{Q_0} = -\upsilon_A.C_0\int_{X_e}^{X_s}\frac{d(X)}{r} \text{ avec } \upsilon_A = -1$$

$$\tau_p = \frac{V_R}{Q_0} = C_0\int_{X_e}^{X_s}\frac{d(X)}{r} \qquad (II.26)$$

The most important results obtained in this chapter for ideal reactor models, are summarized in table II.1.

Table II.1: equations corresponding for each reactor

Inlet + Disappearance (Prod) = Oulet + Accumulation	
Batch reactor	
Material Balance Simple reaction A → Products	$\boldsymbol{R_A.V = \frac{dn_A}{dt}}$
Expression of residence time as a function of molar concentration (**C_A**)..	$t_s = \int_{C_{Ae}}^{C_{As}}\frac{dC_A}{r_{(C_A)}.\upsilon_A} = \int_{C_{Ae}}^{C_{As}}\frac{dC_A}{r_{A(C_A)}}$
Expression of residence time as a function of conversion rate (**X_A**).	**If V is constant : (liquid phase) ($V=V_0$)** $t_s = C_{A_0}\int_0^{X_A}\frac{d X_A}{\upsilon_A.r_{(X_A)}} = C_{A_0}\int_0^{X_A}\frac{d X_A}{r_{(X_A)}}$ **If V does not constant :** $V_T = \beta.V_0(1+\varepsilon_A.X_A)$ $t_s = C_{A_0}\int_0^{X_A}\frac{d(X_A)}{r_{(X_A)}.\beta.(1+\varepsilon_A.X_A)}$
Expression of residence time as a function of generalized extent reaction (**χ**).	$t_s = C_0\int_0^{XS}\frac{d\chi}{r}$ à (V=cte)
Expression of residence time as a function of molar extent reaction (**ξ**).	$t_s = C_0\int_0^{XS}\frac{d\xi}{n_0.r} = \int_0^{XS}\frac{d\xi}{V..r} =$ à
Steady state mixed flow reactor	
Material Balance Simple reaction A → Products	$\boldsymbol{F_{Ae} + R_A.V = F_{Ae} + \upsilon_A rV = F_{AS}}$

Expression of space time (Ʈ) as a function of molar concentration(C_A). .	**where ρ = constante ⇒ $Q_{Ae} = Q_{As} = Q_0$ (liquid phase)** $\tau = \frac{V_R}{Q_0} = \frac{(C_{Ae}-C_{As})}{-r_{A(C_{AS})}}$ or $\tau = \frac{(C_{Ae}-C_{As})}{r_{(C_{AS})}}$ **as ou ρ≠constante ⇒ $Q_{Ae} \neq Q_{As} \neq Q_0$ (cas d'une phase gazeuse)** $V_R = \frac{(C_{Ae}.Q_s - C_{As}.Q_s)}{-r_{A(C_{AS})}}$ or $V_R = \frac{(C_{Ae}.Q_s - C_{As}.Q_s)}{r_{(CAs)}}$ then $\tau = \frac{V_R}{Q_0}$
Expression of space time as a function of conversion rate **(X_A).**	$Ʈ = \frac{V_R}{Q_0} = \frac{C_{A0} X_{As}}{r_{(X_{As})}}$
Expression of space time as a function of generalized extent reaction **(χ).**	$Ʈ = \frac{C_0 . X_s}{r_{(X_s)}} = \frac{\upsilon_A C_0 . X_s}{r_{A(X_s)}}$
Expression of space time as a function of molar extent reaction **(ξ).**	$Ʈ = \frac{\xi_s}{V . r_{(X_s)}} = \frac{\upsilon_A . \xi_s}{V . r_{A(X_s)}}$
Plug flow reactor	
Material Balance Simple reaction A → Products	$R_A . dV = \upsilon_A . \boldsymbol{r} . \mathbf{dV} = \boldsymbol{dF_A}$
Expression of space time as a function of molar concentration .	$\tau_p = \frac{V_R}{Q_0} = -\int_{C_{Ae}}^{C_{As}} \frac{d(C_A)}{r_{(C_A))}}$
Expression of space time as a function of conversion rate **(X_A).**	$\tau_p = \frac{V_R}{Q_0} = C_{A0} \int_0^{X_A} \frac{d(X_A)}{r_{(X_A))}}$
Expression of space time as a function of generalized extent reaction **(χ).**	$\tau_p = \frac{V_R}{Q_0} = C_0 \int_{X_e}^{X_s} \frac{d(X)}{r_{(X)}}$
Expression of space time as a function of molar extent reaction **(ξ).**	$\tau_p = \frac{V_R}{Q_0} = \int_{X_e}^{X_s} \frac{d(\xi)}{V . r_{(\xi)}}$

Application N°3:

The following irreversible chemical reaction is considered: $A \rightarrow B$

This reaction is first order with respect to A, with a rate constant is k =0.3 min^{-1}. The initial concentration of A is C_{A0} = 2mol/L. this reaction was studied in three reactors in order to compare their behavior. These reactors were:

- A batch reactor
- A continuous stirred-tank reactor (CSTR)
- A plug flow reactor (PFR)

1. Write the mass balance equation for each reactor
2. Derive the relationship between conversion X_A and residence time (for each reactor)
3. Calculate the residence time needed to achieve a conversion of 80%. If the reactor draining time is 2 minutes, calculate the duration of one production cycle.

4. If the inlet flow rate is Q_0=0.5 L/min, determine the volume of the reactor needed to reach the desired conversion.
5. Compare the three reactors in terms of efficiency time or volume required to reach 80% conversion.

Solution:

As known, the general materials balance equation is:

Input + **Disappearance (Prod) =** **Output** + **Accumulation**

- ***Batch reactor***

1. *Mass balance equation*

In batch reactor: Input= output = 0, then:

Disappearance (Prod) = Accumulation

The material balance on the limiting reactant A is written as:

$$R_A . V = \frac{dn_A}{dt} = r_A V = \upsilon_A . r . V = -r . V \qquad (\upsilon_A = -1)$$

$$\frac{dn_A}{V . r_A} = dt \Rightarrow \quad \frac{dVC_A}{V . r_A} = dt = \frac{dC_A}{r_A} = \frac{dC_A}{\upsilon_A . r}$$

2. *Relationship between conversion X and residence time*

We have : $C_A = C_{A_0} - X_A C_{A_0} \Rightarrow C_A = C_{A_0} . (1 - X_A)$

The material balance equation is written as follows: $-r = \frac{dC_A}{dt} = \frac{d(C_{A_0} - X_A C_{A_0})}{dt}$

$$t_s = \int_{C_{Ae}}^{C_{As}} \frac{dC_A}{r_{(C_A)} . \upsilon_A}$$

$$t_s = \int_{n_{A0}}^{n_{Af}} \frac{dC_A}{-k . C_A} = \int_{C_{A0}}^{CA} \frac{d(C_{A_0} - X_A C_{A_0})}{-k . (C_{A_0} - X_A C_{A_0})} = C_{A_0} \int_0^{X_A} \frac{d(1 - X_A)}{-k . (1 - X_A)}$$

$$t_s = \frac{1}{-k} \int_0^{X_A} \frac{-d(X_A)}{(1 - X_A)}$$

$$t_s = \frac{-1}{k} \ln(1 - X_A)$$

3. *Residence time (t_s)*

$$t_s = \frac{-1}{0.3} \ln(1 - 0.8) = 5.36\, min$$

$$t_{cycle} = t_s + t_{vidange} = 5.36 + 2 = 7.36\, min$$

4. *The volume of the reactor:*

$$V = t_s . Q_0 = 5.36 * 0.5 = 2.18L$$

- **Stirred-tank reactor (CSTR)**

1. *Mass balance equation*

In Stirred-tank reactor (CSTR): Accumulation = 0 the reactor operates under steady state conditions) , then:

Inlet + Disappearance (Prod) = Outlet

The material balance on the limiting reactant A is written as:

$$F_{Ae} + R_A.V = F_{Ae} + \upsilon_A r V = F_{AS}$$

$$(V=V_R) \text{ and } F_{Ae} = C_{Ae}.Q_e, \quad F_{As} = C_{As}.Q_s$$

$$Q_e C_{Ae} + r_A V_R - Q_s C_{As} = 0$$

with:

$r_A = r_{AS}$: the rate of reactant A at the reactor outlet. $\upsilon_A = -1$

$$Q_e C_{Ae} + r_A V_R - Q_s C_{As} = 0$$

2. *Relationship between conversion X and residence time*

It is assumed that the flow rate Q_0 is constant at both the inlet and the outlet (liquid phase):

$$Q_e = Q_s = Q_0$$

Then : $Q_0 (C_{Ae} - C_{As}) = -r_A V_R \Rightarrow \tau = \frac{V_R}{Q_0} = \frac{(C_{Ae} - C_{As})}{-r_A}$

3. *Space time τ :*

We have : $\tau = \frac{V_R}{Q_0} = \frac{(C_{Ae} - C_{As})}{k.C_{As}} = \frac{C_{A0}\,(X_{AS})}{k.C_{A0}(1 - X_{AS})} = \frac{0.8}{0.3.2.(1-0.8)} = 13.33min$

4. *The volume of the reactor:*

$$V = \tau . Q_0 = 13.33\ .0.5 = 6.67L$$

- **A plug flow reactor (PFR)**

1. *Mass balance equation*

The reactor (PFR) operates under steady state conditions) , then: Accumulation = 0

Input + Disappearance (Prod) = Output

The material balance on the limiting reactant A is given by:

$$F_A|_z + R_A.dV = F_A|_{z+dz}$$

$$F_A + \upsilon_A r.\mathrm{dV} = F_A + dF_A$$

$$-r.\mathrm{dV} = dF_A \qquad \upsilon_A = -1$$

2. ***Relationship between conversion X and residence time***

We have : $F_A = F_{A_0} - X_A F_{A_0}$ *and* $r = \mathrm{k}.C_A = \mathrm{k}.C_{A0}.(1 - X_A)$

$$-\mathrm{k}.C_A.\mathrm{dV} = -F_{A0} dX_A$$

$$\text{So:} \quad \frac{d\mathrm{X_A}}{\mathrm{k}.C_{A0}.(1-X_A)} = \frac{dV}{\mathrm{F_{A0}}}$$

As: $\mathrm{F}_{A0} = \mathrm{Q_0 C_{A0}}$ thus :

$$\text{So:} \frac{d\mathrm{X_A}}{\mathrm{K}.C_{A0}.(1-X_A)} = \frac{dV}{\mathrm{Q_0 C_{A0}}} \Rightarrow \frac{1}{k}\int_0^{X_A} \frac{d\mathrm{X_A}}{(1-X_A)} = \int_0^{V_R} \frac{dV}{\mathrm{Q_0}}$$

After integration: $\tau_p = \frac{V_R}{Q_0} = -\frac{1}{k}.\ln(1 - X_A)$

3. ***Space time*** τ_p***:***

$$\tau_p = -\ln(1 - 0.8) = 5.36\, min$$

4. ***Reactor volume***

$$V_R = \tau_p.Q_0 = 5.36\,.0.5 = 2.67\, L$$

5. ***Comparison between the three reactors:***

- Batch reactor: flexible and simple to operate, but its average productivity decreases when the 2-minute emptying/ refilling time is included.
- CSTR: continuous operation but less efficient requires a larger reactor volume to achieve the same conversion.
- PFR: most efficient, achieves the target conversion in shorter time with a smaller reactor volume.

Chapter III: Enzymatic reactors

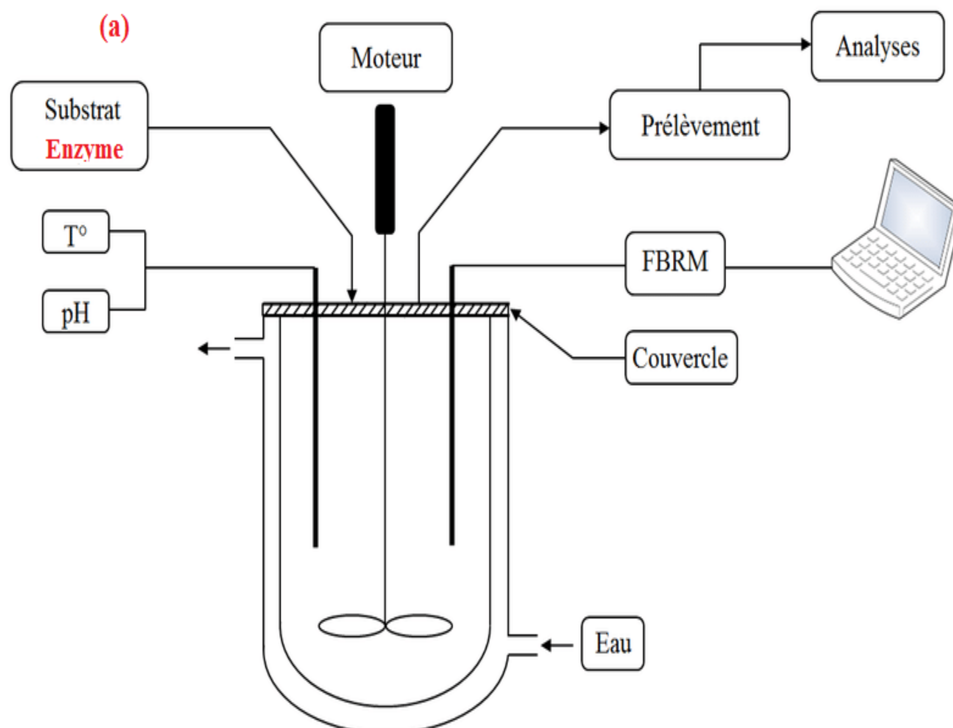
III.1. Definition

Bioreactors are vessels in which biological processes take place. Their design must take into account the physiology of the microorganisms or enzymes involved, as well as the objectives of bioconversions and fermentations.

The production processes of many widely consumed food products include at least one step involving microorganisms or enzymes:

When this step involves **microorganisms** that grow by consuming pert or a reactant called a substrate and transforming into various products, the reactor used is called a **fermenter** (**Figure III.1.(a)**);

On the other hand, when the process involves a biochemical reaction catalyzed by **enzymes** that convert a substrate into a product, it is carried out in an **enzymatic reactor** (Figure III.1.(b)).



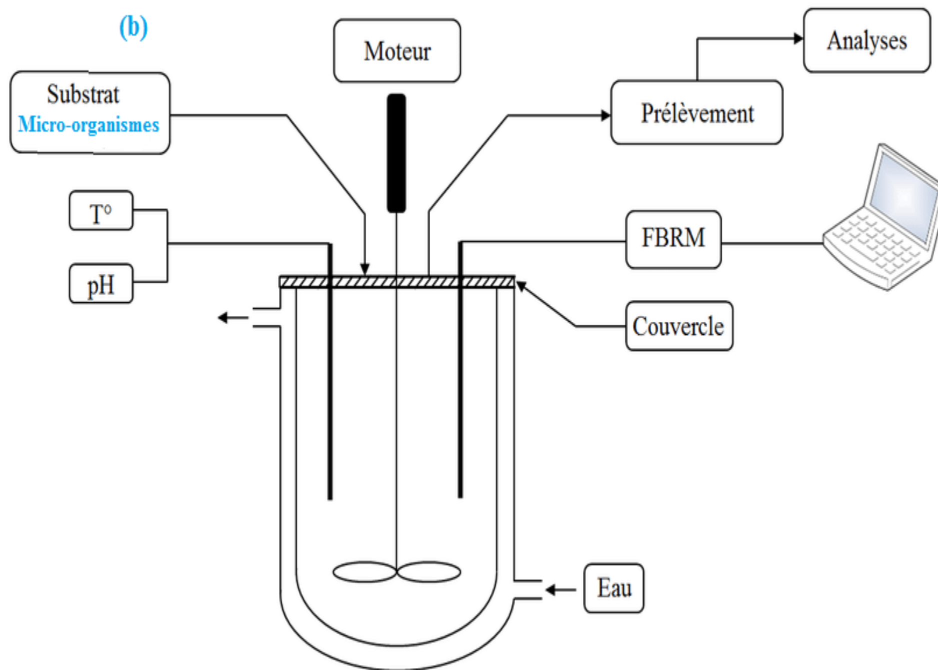


Figure III.1: Bioreactors; (a): Fermenter, (b): enzymatic reactor

The construction technologies for these two types of reactors are therefore different. Fermenter require materials that are resistant to heat sterilization and must be completely airtight to maintain sterile conditions. However, despite these technological distinction differences, both enzymatic reactors and fermenters involve similar underlying phenomena, such as reaction kinetics, and heat and mass transfer.

III.2. Enzymatic reaction

Almost all chemical reactions occurring in biological systems are catalyzed by enzymes, a biological protein that accelerate the conversion of substrates into products without being consumed during reaction (equation III.1).

$$Substrate \underset{}{\overset{Enzyme}{\rightleftarrows}} Product \qquad (III.1)$$

To quantify an enzyme in terms of molar concentration, its molar mass must be known-something that is only possible for a limited number of purified enzymes. In practice, the rate of the catalyzed reaction is often used to estimate the amount of enzyme through its activity.

The occurrence of an enzymatic reaction requires the presence of the following element (Figure III.2).

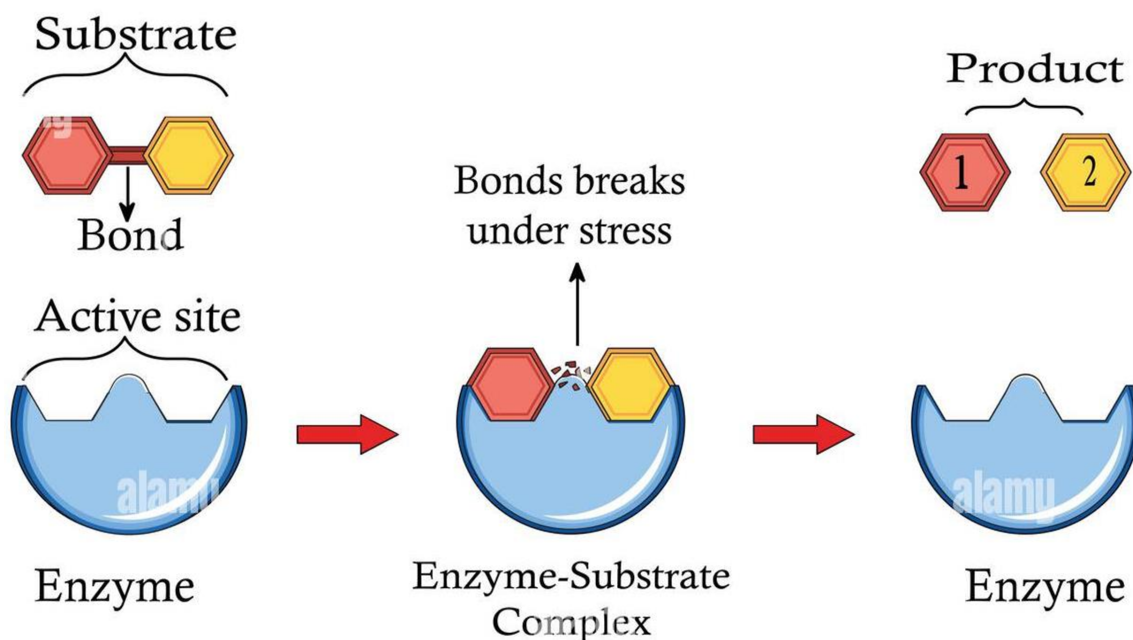


Figure III.2: A diagram illustrating the structure of an enzyme, including its active site to which a substrate molecule binds to form an enzyme-substrate comples. Once the reaction is complete, the products are realeased from the active site.

- **Substrate (S)**

A molecule that participate in an enzymatic reaction and is reversibly transformed under the catalytic action of enzyme.

- **Product (P)**

A product is a molecule that appears during a reaction catalyzed by an enzyme. It is the new compound formed from the transformation of the substrate.

- **Enzyme (E)**

An enzyme is protein that catalyzes many reaction, particularly biochemical reactions, including many necessary for the maintenance of life. The catalytic action is usually very specific, and may be affected by the presence of other substances both as inhibitors and as coenzymes (organic compound that activate the primary enzyme to a catalytically active form).

The role of the enzyme in catalyzing biochemical reactions is to reduce the activation energy required for the reaction to occur, as illustrated in the figure III.3.

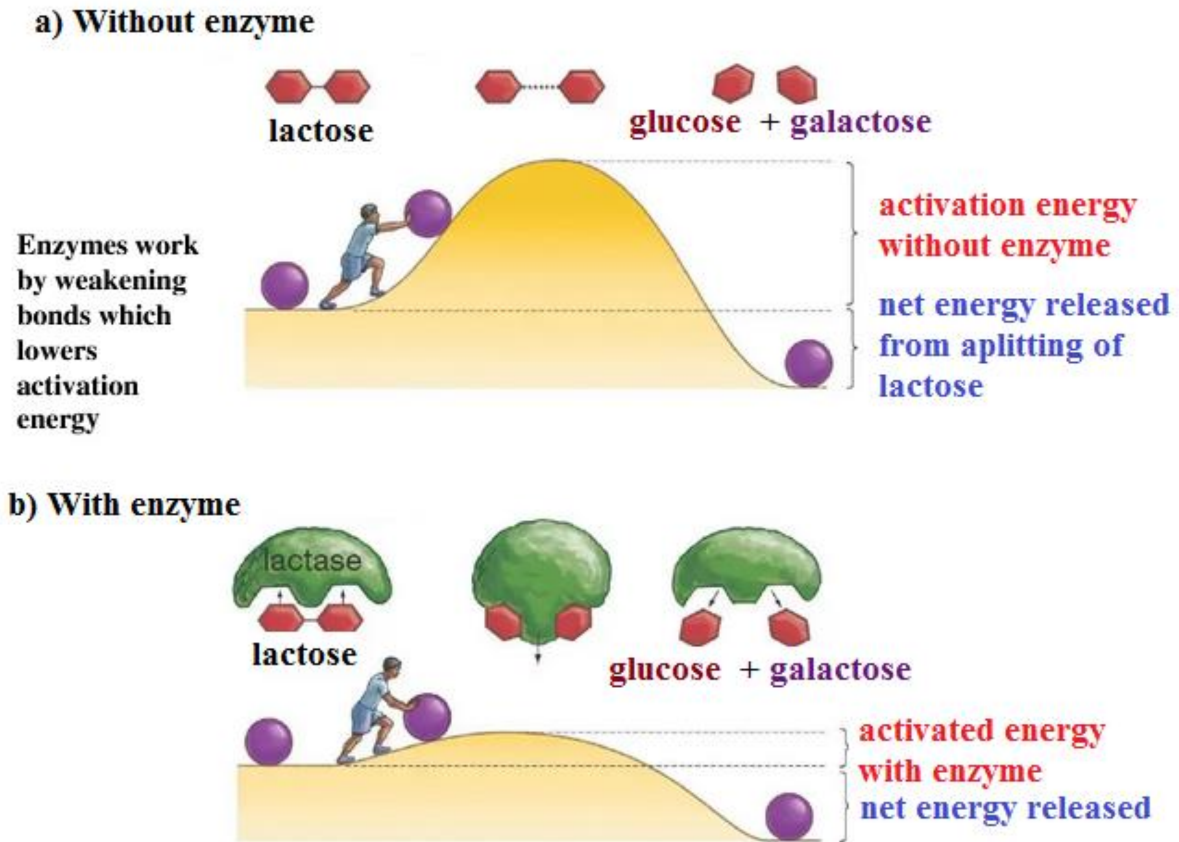


Figure III.3: Effect of enzymes on activation energy during the hydrolysis of lactose.

Enzymes are commonly grouped according to the type of reaction catalyzed. Six classes of enzymes have been identified:

- Oxidoreductases: catalyze various types of oxidation-reduction reactions;
- Transferases: catalyze the transfer of functional groups, such as aldehydic or acyl groups;
- Hydrolases: catalyze hydrolysis reactions;
- Isomerases: catalyze isomerization;
- Ligases: which, with ATP (Adenosine triphosphate) as a cofactor, lead for the formation of bonds between carbon and other atoms, including carbon, oxygen, nitrogen, and sulfur.
- Lyases: catalyze the addition of chemical groups onto bonds.

Enzymes are highly specific to reaction they catalyze (Figure III.1). This specificity is due to the presence of a three-dimentional region known as the active site, which selectively

recognizes and binds to a particular substrate (S). this binding enables the enzyme to act on specific chemical reactions with great precision.

Enzymes may exist in two physical forms:

- Soluble enzymes, which operate in a homogeneous medium;
- Insoluble enzymes, immobilized on a solid support, creating a heterogeneous reaction medium.

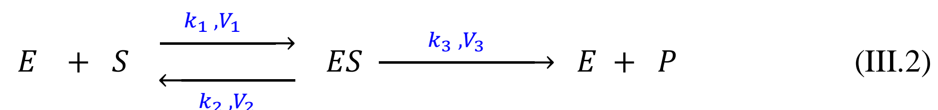
As a result, two different mechanisms are distinguished, governed by two different types of kinetics:

- Homogeneous enzymatic kinetic, governed by the laws of homogeneous catalysis.
- Heterogeneous enzymatic kinetic, governed by the laws of heterogeneous catalysis.

III.3. Homogeneous enzymatic kinetic

We will focus on enzymes that exhibit Michaelian behavior, meaning they are present at a low concentration relative to that of the substrate. If this condition is not met, the reaction can no longer be described using Michaelis Menten kinetics.

In 1913, Michaelis and Menten established the following equation to describe the rate of an enzymatic reaction:



$$E + S \underset{k_2, V_2}{\overset{k_1, V_1}{\rightleftharpoons}} ES \xrightarrow{k_3, V_3} E + P \quad \text{(III.2)}$$

Michaelis assumed that the reaction between E (enzyme) and S (substrate) involves the formation of an intermediate complex, ES.

Additionally, he considered that the reaction between E and S to be an equilibrium process. According to the classical interpretation by these two authors (Michaelis and Menten), the enzymatic reaction can be divided into two main steps:

1. First, there is a rapid and reversible reaction between the enzyme **(E)** and the substrate **(S)** to form the enzyme-substrate complex **(ES)**, with a rate constant k_1 for the forward reaction and k_2 for the reverse reaction. The enzyme-substrate (ES) association is stabilized by weak energy bonds, such as hydrogen bonds, hydrophobic interactions, and ionic bonds.

$$E + S \underset{k_2, V_2}{\overset{k_1, V_1}{\rightleftharpoons}} ES \quad \text{(III.3)}$$

They assumed that no products are present at the beginning of the reaction ($k_1 > k_3$ and $k_2 > k_3$).

2. The ES complex then breaks down into the reaction product P, while regenerating the enzyme. This is the rate limiting step of the reaction, which occurs with a rate constant k_3.

$$ES \xrightarrow{k_3, V_3} E + P \qquad (III.4)$$

The kinetic study of an enzymatic reaction consists of analyzing the overall reaction rate and the influence of various parameters that may affect it. The expression for the initial reaction rate (Vi) of the overall enzymatic reaction is given by:

$$V_i = -\frac{d[S]}{dt} = \frac{d[P]}{dt} \qquad (III.5)$$

We also have: $-\frac{d[S]}{dt} = v_1 - v_2 = k_1[E][S] - k_2[ES] \qquad (III.6)$

$$v_i = \frac{d[P]}{dt} = v_3 = k_3[ES] \qquad (rate\ of\ the\ rate\ limiting\ step) \qquad (III.7)$$

Replacing equations (6) and (7) into equation (5), we get:

$$k_1[E][S] - k_2[ES] = k_3[ES]$$

$$k_1[E][S] = [ES](k_2 + k_3)$$

$$\frac{[E][S]}{[ES]} = \frac{(k_2 + k_3)}{k_1} = k_M \qquad (III.8)$$

- The ratio $\frac{(k_2 + k_3)}{k_1}$ corresponds to the dissociation constant of the ES complex, known as the Michaelis- Menten constant, k_M (expressed in moles/liter). It is importante to note that the constant $\mathbf{k_M}$ defines the affinity of the enzyme for its substrate. So a high K_m indicates low affinity, meaning a higher substrate concentration is required to reach half of the maximum reaction rate.
- According to equations (6) and (7), determining the rate of product formation (P) requires knowledge of the instantaneous concentration of the complex [ES], which is generally difficult- if not possible- to measure directly (intermediate reaction).
- Similarly, the instantaneous concentration of free enzyme [E] is just as difficult to measure as [ES]. Therefore, it is preferable to express the reaction rate in terms of the initial enzyme

concentration $[E]_0$, which is a known and controllable parameter in enzymatic kinetics experiments.

In the mechanism described in equation (2), the enzyme exists in two forms: either as free enzyme (E), or as part of the enzyme-substrate complex (ES). Thus, we can write the conservation of enzyme concentration with the following equation:

$$[E]_0 = [E] + [ES] \Longrightarrow [E] = [E]_0 - [ES] \qquad (III.9)$$

Replacing equation (9) in the equation (8) we get :

$$\frac{([E]_0 - [ES])[S]}{[ES]} = \frac{([E]_0[S] - [ES][S])}{[ES]} = k_M$$

$$[ES]k_M + [ES][S] = [E]_0[S] \Longrightarrow [ES] = \frac{[E]_0[S]}{k_M + [S]} \qquad (III.10)$$

Replacing the expression of [ES] in the equation (6), we get:

$$V_i = V_3 = k_3[ES] = k_3 . \frac{[E]_0 . [S]}{k_M + [S]}$$

$$v_i = v_{max} . \frac{[S]}{k_M + [S]} \qquad (III.11)$$

this is the Michaelis Menten equation:

v_{max} ($mol.\ L^{-1}.s^{-1}$): the maximum reaction rate, defined as : ($v_{max} = k_3[E]_0$) ;

[S] ($mol.\ L^{-1}$): substrate concentration,

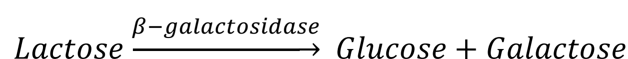
k_M: ($mol.\ L^{-1}$): the Michaelis et Menten constant, which corresponds to the substrate concentration at which the reaction rate is half of v_{max}.

k_3 (or kcat): the catalytic constant, a kinetic constant that reflects the number of co substrate molecules converted to product per enzyme molecule per unit time, under conditions of substrate saturation.

V_{max} is the maximum rate of an enzymatic reaction, reached when all available enzyme molecules are saturated with substrate. In other words, under these conditions, the entire enzyme population exists in the enzyme-substrate (ES) complex form.

Exemple of Michelis –Menten enzyme: β-galactosidase (lactase)

La β-galactosidase, also known as lactase, is an enzyme that catalyzes the hydrolysis of lactose (a disaccharide) into its two monosaccharide components:



$$Lactose \xrightarrow{\beta-galactosidase} Glucose + Galactose$$

This type of reaction is characterized by ;

- It is a single-substrate, **non-allosteric**, **uninhibited**, and **soluble enzyme** → therefore, it follows Michelis-Menten kinetic very well.
- The measure of the initial reaction rate (V_0) at different lactose concentrations, gives us a hyperbolic curve.
- If the curve were sigmoidal (S-shaped), it would be characteristic of an allosteric enzyme like aspartate transcarbamoylase, and would not follow Michaelis –Menten kinetics.

III.3.1. Michaelis representation: vi = F([S])

When plotting the initial reaction velocity (Vi) as a function of the substrate concentration [S] (see figure III.4), a hyperbolic curve is obtained, with v_{max} as one of its asymptotes. This curve can be divided into three distinct regions:

- **Part 1:** [S] << k_M (low substrate concentration):

In this case, the Michaelis – Menten equation simplifies to:

$$v_i = \frac{v_{max}}{k_M}[S] = constante.[S]$$

Here, the reaction is first-order with respect to substrate. The rate is directly proportional to the substrate concentration.

- **Part 2:** [S] = k_M (intermediate substrate concentration):

This corresponds to the point of the curve, where the reaction order is between 0 and 1.In this zone, the concentration at which : $V_i = \frac{V_{max}}{2}$

(represents the substrate concentration at which the enzyme is half-sartured.

- **Partie 3:** [S] >> k_M (high substrate concentration):

Under these conditions : $v_i = v_{max}$. the reaction becomes zero-order with respect to the substrate: the rate is constant and independent of substrate concentration.

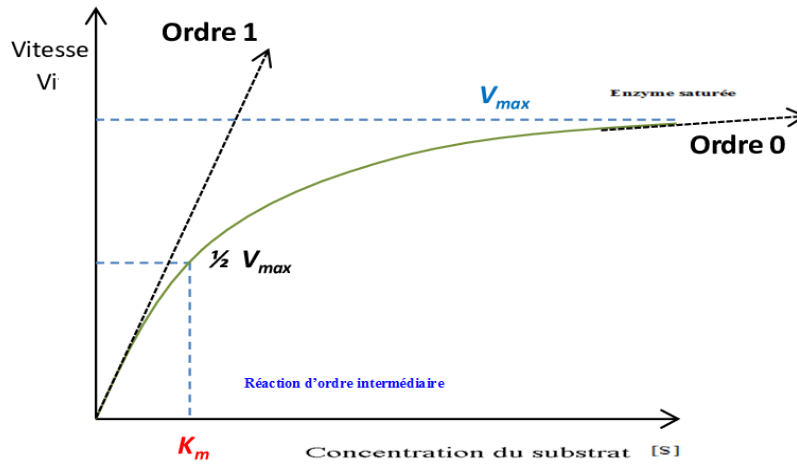


Figure III.4: Evolution of enzyme activity (reaction rate) with substrate concentration

The parameters k_M and v_{max} can be determined by linearizing the Michaelis-Menten equation. One common method for this is **Lineweaver Burk method**, which consists of calculating $1/v_i$ according to:

$$\frac{1}{v_I} = \frac{k_M}{v_{max}} \cdot \frac{1}{[S]} + \frac{1}{v_{max}} \qquad (III.12)$$

This is the linear form of the Michelis- Menten equation. Ils plot $\frac{1}{v_I} = f(\frac{1}{[S]})$ is illustrated in figure III.5. The y-intercept is $1/v_{max}$, the slope is k_M/v_{max} and the x-intercept is $(-1/K_M)$.

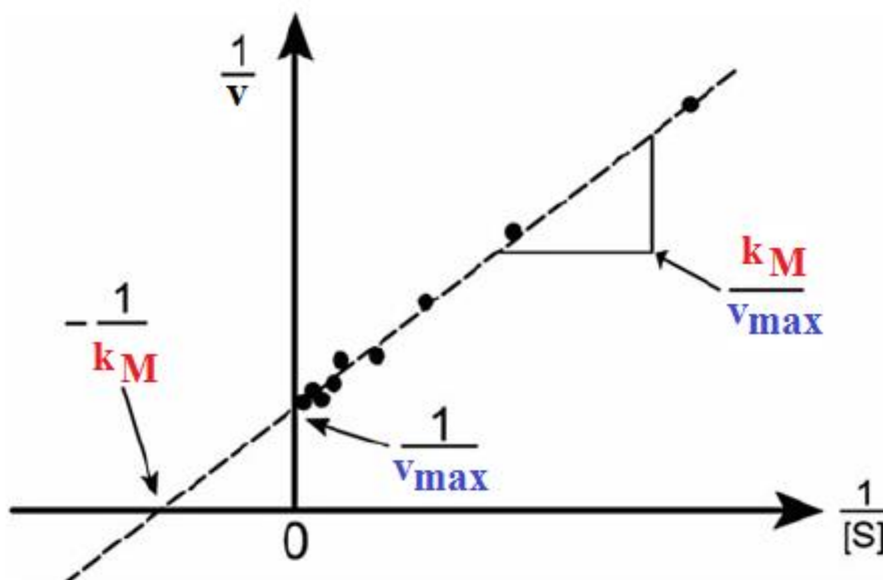


Figure III.5. linear form of the Michelis- Menten equation

Note:

Units used to characterize enzyme quantity in terms of molar concentration:

- Specific units: these are particular to a given enzyme under defined experimental conditions.
- *International Units (IU):* One IU is the amount of enzyme that catalyzes the transformation of 1 micromole of substrate per minute under specific conditions.
- *The* ***katal*** *(kat):* one katal is the amount of enzyme that converts 1 mole of substrate per second.

These units are usually referred to either:

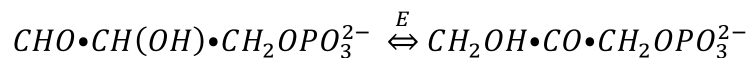
- ✓ Per volume of enzyme preparation (e.q; mL of liquid),
- ✓ Per mass of material (e.g., mg, g or Kg of solid preparation).

Example: Liquid invertase has approximately 4000 UI/mL. This means is catalyzing the hydrolysis of 1 micromole of sucrose per minute under the following conditions:

- ✓ Temperature: 55°C ;
- ✓ pH :4 ;
- ✓ Initial sucrose concentration : 0.3M.

Application:

The enzyme triose phosphate isomerase catalyzes the interconversion of D-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.



$$CHO{\bullet}CH(OH){\bullet}CH_2OPO_3^{2-} \overset{E}{\Leftrightarrow} CH_2OH{\bullet}CO{\bullet}CH_2OPO_3^{2-}$$

The following results (Table III.1) refer to the initial reaction velocity, v, with glyceraldehyde 3-phosphate (S) as substrate at a total enzyme concentration $[E]_0 = 2.22.10^{-11}$ M, pH=7.42, and T=30°C.

Table III.1: Evolution of the initial reaction velocity (v) as a function of glyceraldehyde 3-phosphate (S)concentration.

$[S]\ 10^{-3}$(mol/l)	0.071	0.147	0.223	0.310	0.602	1.47	2.6
$v.\ 10^{-7}$ (mol/l.s)	1.31	2.45	3.37	3.9	5.63	7.47	8.17

Determine the Michaelis constant K_M and the catalytic constant for the enzyme under these conditions.

Solution

Using the linear form of the michaelis Menten model:

$$v_i = v_{max}.\frac{[S]}{k_M + [S]} \quad \Rightarrow \quad \frac{1}{V_I} = \frac{k_M}{V_{max}}.\frac{1}{[S]} + \frac{1}{V_{max}}$$

This equation has the following form: $y = ax + b$

Table III.2: Lineweaver – Burk linearization of enzyme kinetics

1/[S] (10^3) (mol/l)	14,085	6,803	4,484	3,226	1,661	0,680	0,385
1/v (10^7) (mol/l.s)	0,763	0,408	0,297	0,256	0,178	0,134	0,122

The representation of $\frac{1}{V_I} = f(\frac{1}{[S]})$ is given in figure III.6.

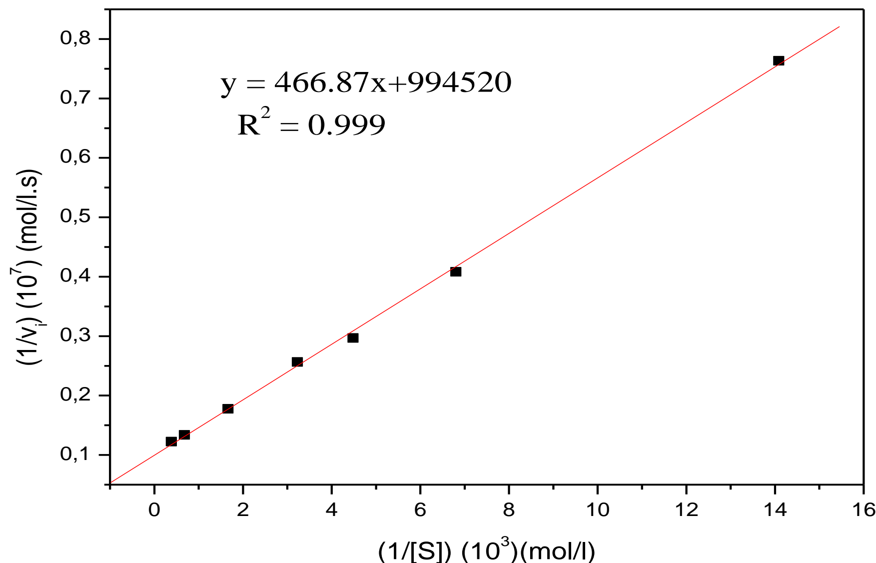


Figure III.6: Lineweaver – Burk linearization of enzyme kinetics

The plot of 1/v versus 1/[S] gives a straight line that does not pass through the origin, represented by the following equation: $\frac{1}{v} = 466.87\frac{1}{[S]} + 994520$

$$a = \frac{k_M}{V_{max}} = 466.87 \Rightarrow k_M = 1.005 * 10^{-6} * 466.87 \Rightarrow k_M = 4.69 * 10^{-4} mol/l$$

$$b = \frac{1}{V_{max}} = 994520 \quad \Rightarrow V_{max} = 1.005.10^{-6} mol/l.s$$

The catalytic constant k3 is determined from this equation: $V_{max} = k_3.[E]_0$

$$k_3 = \frac{V_{max}}{[E]_0} = \frac{1.005.10^{-6}}{2.22.10^{-10}} = \mathbf{4.52 * 10^3 s^{-1}}$$

$$k_3 = 4.52 * 10^3 s^{-1}$$

III.3.2 Factors affecting enzyme activity

Factors that affect enzymatic activity, defined as the rates of reactions catalyzed by enzyme is influenced by several factors, including:

- The nature of the enzyme, particularly the presence of inhibitors and coenzymes;
- The nature of the substrate;
- The concentration of enzyme and substrate;
- Temperature
- pH
- organic solvents;
- External factors such as irradiation (photo, sonic or ionizing) and shear stress.

1. The nature of the enzyme, particularly the presence of inhibitors and coenzymes:

Enzyme activity depends on the enzyme's structural integrity and the availability of essential cofactors. Inhibitor decrease or block enzymatic activity by interacting with the active site or another region of the enzyme, whereas coenzymes and cofactors are required for optimal catalytic function and may enhance the reaction rate.

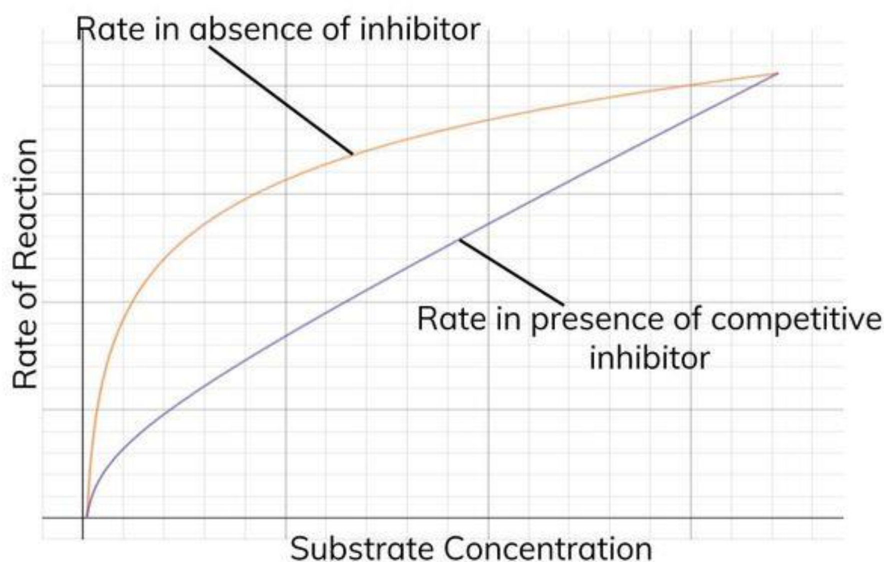


Figure III.7: Effect of competitive inhibition on enzymatic reaction rate.

2. ***The nature of the substrate:*** the chemical structure, concentration, and affinity of the substrate for the enzyme influence the reaction rate. A substrate that fits well into the enzyme's active site forms the enzyme-substrate complex more efficiently, resulting in higher enzymatic activity.

3. *Effect of temperature, pH and organic solvents on the reaction rate*

In general, enzyme activity exhibits an optimum with respect to temperature (Figure III.8). Initially, the activity increases with temperature, accordance with the Arrhenius law. So, the molecular collisions become more frequent and energetic, thereby accelerating the reaction.

However, beyond a certain temperature threshold, this positive effect is counteracted by a negative effect: he enzyme undergoes denaturation, losing its three-dimensional structure, which renders it inactive.

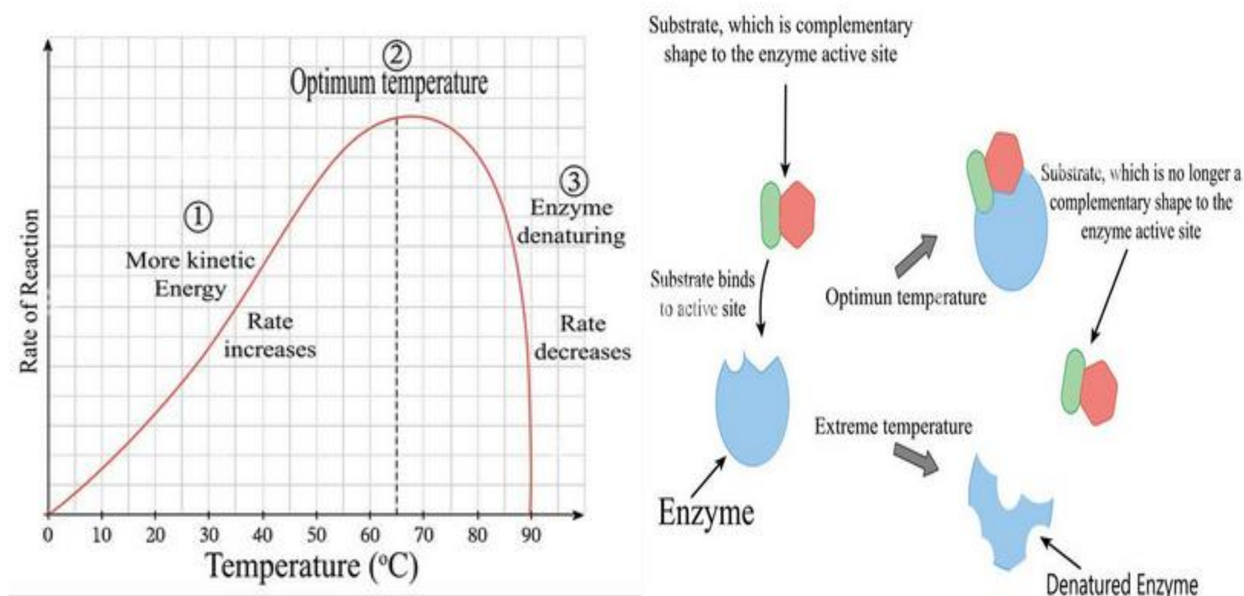


Figure III.8. Effect of temperature

Similarly, enzyme activity has an optimal pH, due to the acid-base properties of the chemical groups located at the active site. For the active site to function properly, these groups must be in the appropriate protonation state.

→ ***At low pH***, amino groups are fully protonated and exist as quaternary ammonium ions, where the nitrogen atom lacks a lone electron pair, preventing it from participating in catalysis.

→ ***At high pH***, carboxylic acid groups are deprotonated to form carboxylate ions, which no longer possess labile hydrogen atoms necessary for certain interactions. Proper functioning

of the active site requires a specific protonation state of the various functional groups involved in catalysis (Figure III.9).

Thus, changes in enzyme activity with pH reflect this requirement for specific ionization states within the active site.

These variations result from a combination of two effects:

- The ionization of amino acid residues with acid-base properties within the active site;
- The destabilization of the enzyme's tertiary structure at non optimal pH levels.

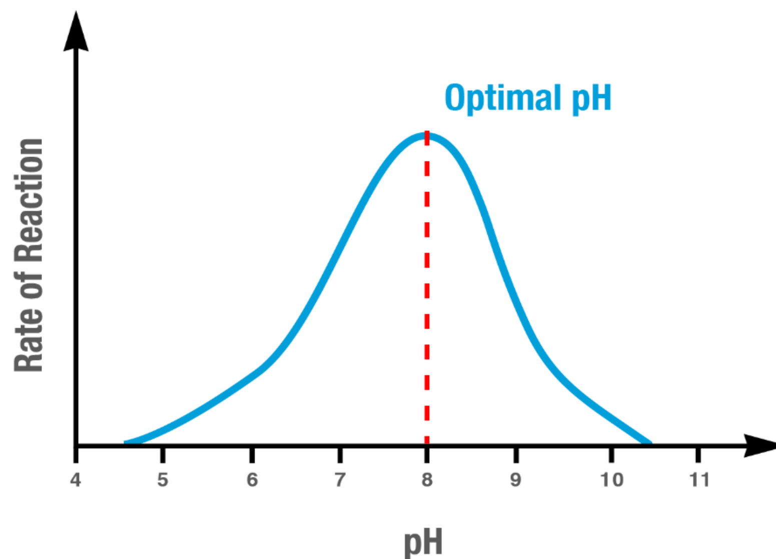


Figure III.9. Effect of pH.

Note:

Most enzymes are active at neutral pH. However, some enzymes function under acidic conditions, such as pepsin, a digestive enzyme produced in the stomach that hydrolyzes proteins at a pH between 2 and 3.5. pH not only affects the enzyme's structure and activity, but also influence the structure and solubility of the substrate, potentially altering how effectively it interacts with the enzyme

Similarly, organic solvents can effect enzyme function. Water is essential for proper enzyme function, as it forms a hydratation shell around the protein, bound by hydrogen bonds- this is known as bound water. This hydratation layer helps maintain the enzyme's three dimensional structure and is crucial for catalytic activity.

Therefore, replacing water molecules with organic solvents leads to a drastic change in protein structure, often resulting in denaturation and loss of activity.

III.4. Heterogeneous enzyme kinetics

One of the main challenge in using biocatalysts is reducing the cost associated with enzymes and while increasing their stability in organic solvents and under harsh reaction conditions. Immobilizing enzymes on suitable solid supports offers a promising solution to these challenges. Enzyme immobilization also offers additional advantages such as:

- Reusability of the catalyst;
- Higher productivity per unit reactor volume;
- Feasibility of continuous processes;
- Production of purer products.

Enzyme immobilization can be carried out using three main approaches, as illustrated in figure III.10.

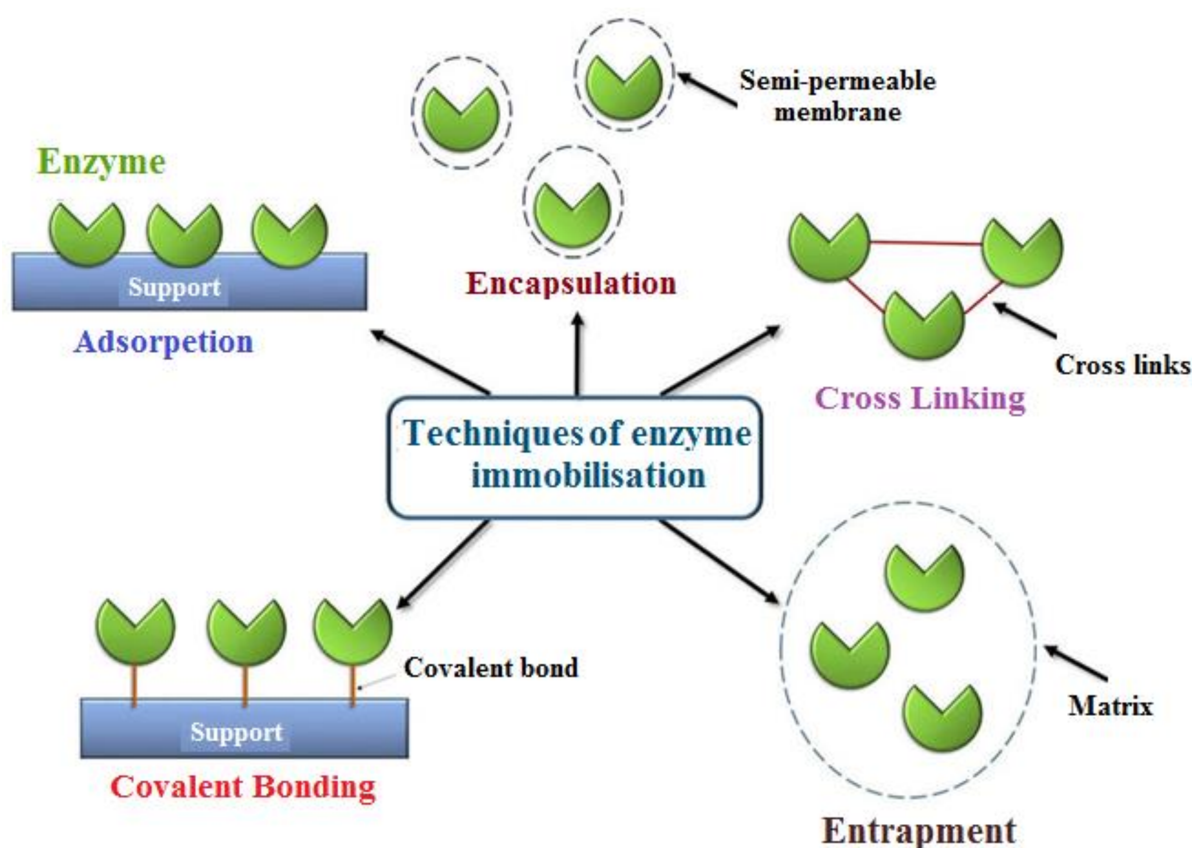


Figure III.10: Enzyme immobilization techniques

Note:

The reaction medium, we are dealing with:

- A **liquid phase,** containing the **substrate**, the **buffer**, and possibly some **effectors (inhibitor or coenzyme)**;
- A **solid phase** containing the enzyme. This solid phase may take the form of blokes, membranes, films, or most commonly beads.

III.4.1. Effects of immobilization on enzyme properties

Immobilization of the enzyme can modify the kinetic behavior of enzymes compared to the free sate due to (Figure III.11):

- Conformational changes (enzyme distortion);
- The creation of steric hindrance between the support and the active site, or between the effector and its binding site.

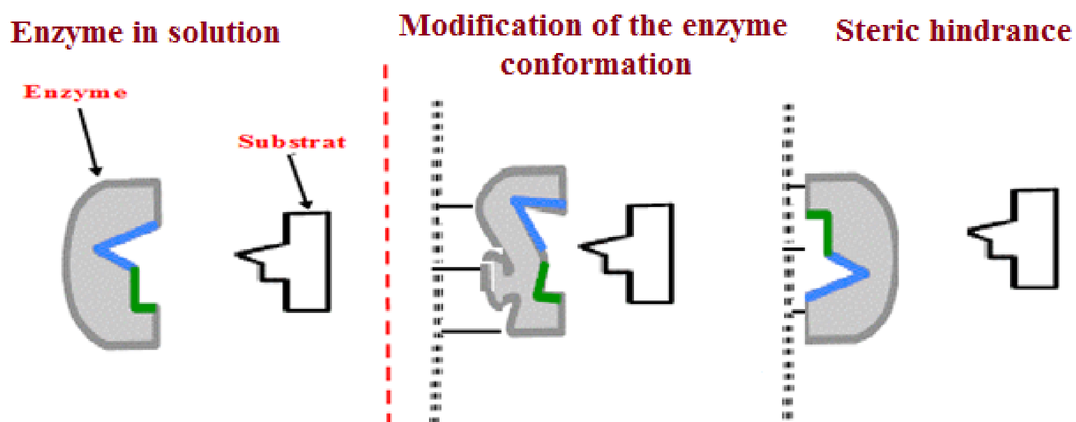


Figure III.11 : Modification des caractères des enzymes du fait de l'immobilisation.

Note:

The rate of transformation in heterogeneous catalysis is referred to as « activity ». The rate laws have the same mathematical from as those for non-immobilized enzymes. However, in the case of surface bound enzymes, the reaction rates such as the initial activity Vi and the maximum rate V_{max} are expressed differently:

- In **$mol.m^{2-}.s^{-1}$** based on the liquid-solid contact surface area,
- In **$mol.Kg^{-1}.s^{-1}$** based on the mass of the support.

III.4.2. Heterogeneity of the reaction medium

The parameters that characterize the reaction mixture (such as pH, temperature, pressure, concentration, etc.) do not have the same value at every point within the mixture. This heterogeneity is mainly due to:

- ***partitioning phenomena:*** result from interactions between the reactants (substrate, product, or effectors) and **functional** groups on the support.

- **Non uniform distribution of reactants:** molecules may accumulate differently in the solid and liquid phases.

Example:

If the support is a negatively charged membrane, a positively charged reactant will become more concentrated near the support than in the bulk solution.

III.4.3. Mass transfer limitations (diffusional resistances)

In heterogeneous systems, reaction rates can be limited not only by enzyme kinetics but also by diffusion processes. The reactants may encounter difficulty diffusing from the liquid phase to the enzyme immobilized on the support. As a result, the substrate concentration near the enzyme may differ from that in the bulk liquid phase. Consequently, the enzymatic reaction can become limited by the availability of one of the reactants (usually the substrate).

Note:

Therefore, while in a homogeneous medium, concentration changes depend only on time, whereas in a heterogeneous system, they depend on both time and position space.

When the evolution of reactant concentrations varies in space, it means that concentration gradients can develop. Two types of concentration gradients can be distinguished:

a) External mass transfer limitations (Discontinuous gradient):

These occur between the bulk liquid phase and the external surface of the support. Reactants must diffuse through a boundary layer (film) that surrounds the immobilized enzyme particles. If this diffusion is slow, it becomes the rate limiting step.

b) Internal mass transfer limitations (Continuous gradient):

These limitations occur within the pores or internal structure of the support. After crossing the external boundary, reactants must diffuse through the interior of the support to reach the enzyme molecules. Limited internal diffusion can reduce the overall reaction rate.

The figure III.12, illustrates this process: First, the substrate molecules (A_1) in the bulk liquid phase diffuse through a stagnant liquid film near the external surface of the catalyst. Then, the substrate molecules diffuse through the internal pores to reach the active sites, where adsorption, transformation, and desorption processes occur. Subsequently, the product molecules (A_2) must diffuse back to the bulk liquid through both pore and film diffusion.

When the diffusion rate of the substrates from the bulk liquid to the active sites of the immobilized enzyme is slower than the catalytic reaction rate, the observed reaction rate, i.e.,

the apparent enzyme activity, becomes lower than that of the free dissolved enzyme. The rate of substrate transport during

External mass transfer is often described as the product of a mass transfer coefficient and the corresponding driving force, namely the substrate concentration gradient. Chen a membrane is used; the external mass transfer rate is also proportional to the membrane surface area.

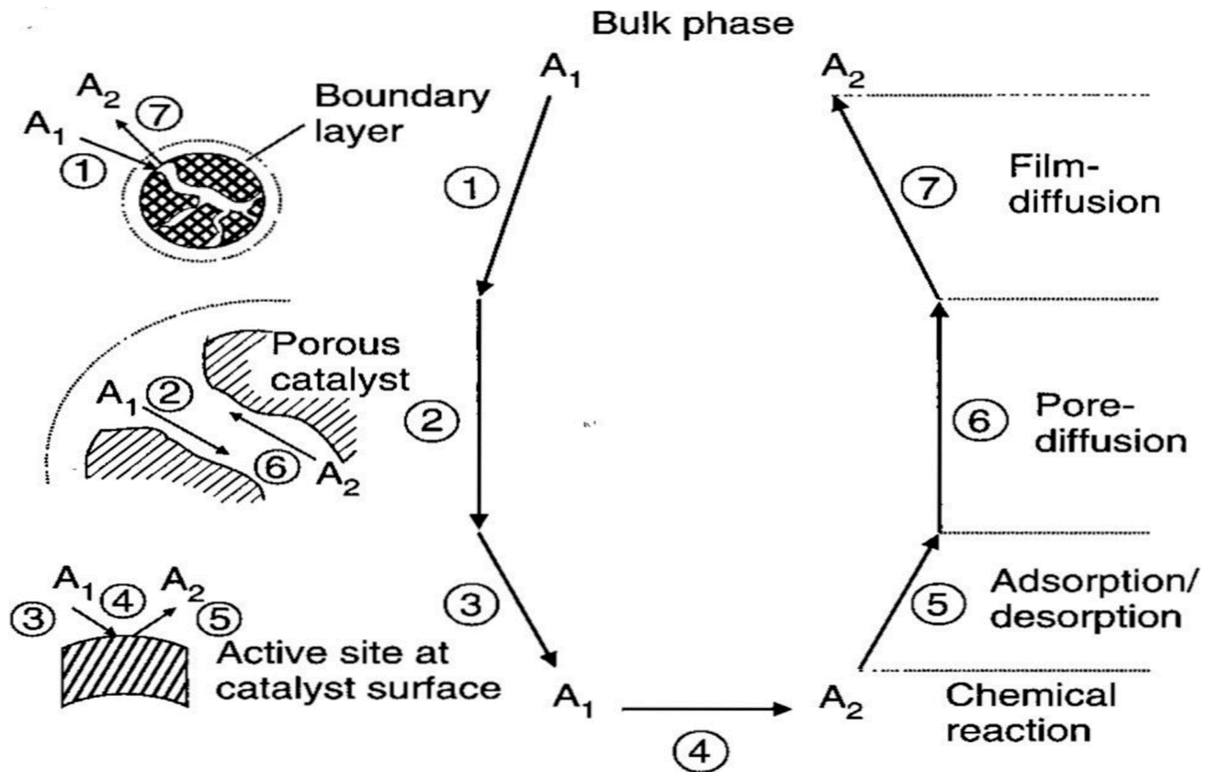


Figure III.12: Individual steps of a heterogeneous solid–liquid catalytic reaction on a porous catalyst. $\mathbf{A_1}$ represents the substrate and $\mathbf{A_2}$ denotes the product.

III.4.4. Transport in the liquid phase and at interfaces

III.4.4.1. Transport in the liquid phase

a) Molecular diffusion

In a solution, molecules are in constant thermal (Brownian) motion. When there is no bulk movement of the fluid (i.e., in a stationary medium), mass transfer occurs via molecular diffusion. A passive phenomenon resulting from a **concentration gradient**.

This phenomenon is governed by **Fick's First Law**, which describes and quantifies the diffusive flux of a solute through a medium. The law states that the flux is proportional to the negative concentration gradient, indicating that solutes move from regions of high concentration to low concentration. The mathematical expression of Fick's first law is:

$$J = -Dgrad[S] \tag{III.13}$$

Where:

- J: is the diffusion flux (amount of substance per unit area per unit time) (mol cm^{-2} s^{-1});
- D: is the diffusion coefficient (cm^2/s). It is a constant reported in the literature, and it depends on the molar mass. In general:

  D is large when M is small (e.g., : protons, small molecules)

  D is small when M is large (e.g : proteins diffuse with difficulty).

  [S] : is the substrate concentration (mol/m^3)

  grad [s]: concentration gradient at the location where the flux is determined.

$$grad\ [S] = \frac{d[s]}{dx} \qquad x: direction\ of\ the\ diffusion$$

X: is the position (m).

When the enzyme is immobilized inside the solid, the diffusivity of the substrate within the support may differ from the diffusivity of the molecule in solution due to interactions with the support. This is called the **effective diffusivity** and it is characterized by its diffusion coefficients, denoted as $\mathbf{D_{eff}}$:

Then : $$J_D = -D_{eff}\frac{d[s]}{dx} \tag{III.14}$$

b) Convection: the homogenization of a solution can be accelerated by creating mass transport in the fluid through convection. Convection involves the bulk movement of fluid, carrying solutes along with it, and can be much more efficient than molecular diffusion alone. Two types can be distinguished :

- ***Forced convection***: induced by external means, such as mechanical stirring, shaking, or pumping of the liquid;
- ***Natural convection***: occurs spontaneously due to density gradients within the fluid, often caused by heat transfer or concentration differences.

  The flux due to convection is given by the following relation:

$$J_C = C.v_f \tag{III.15}$$

Where:

J_c: convective flux due to convection ($mol.m^{-2}.s^{-1}$);

C: is a substate concentration

v_f : is the fluid rate.

The mass flux due to convection and diffusion is expressed by:

$$J = C.v_f - D_{eff}.grad[S] \tag{III.16}$$

III.4.4.2. Transports at interfaces

At the boundary between two phases (eg., liquid-liquid, liquid-gas, or liquid-solid), mass transfer can occur across the interface. This transfer is driven by a difference in chemical potential or concentration gradient between the two phases.

When the solid support is introduced into the solution at a given time, a certain amount of substrate is consumed by the enzyme catalyzed reaction. To maintain bulk solution toward the solid-liquid interface

As a result, a concentration gradient will be established until the bulk fluid and the interface.

Over time, a dynamic equilibrium, known as coupling, is established between the biochemical reaction rate and the rate of mass transfer.

At steady state, when concentration remain constant over time, the rate of substrate transport to the enzyme surface (J) becomes equal to the enzymatic reaction rate (Vs or Vi):

$$J = h.([S] - [S]_i) \quad (III.17)$$

And

$$V_i = \frac{V_{\max.} . [S]_i}{k_M + [S]_i} \quad (III.18)$$

Where:

J : is the flux of substrate toward the enzyme ($mol.m^{-2}. s^{-1}$);

vi is the rate of the enzymatic reaction at the interface ($mol.m^{-2}. s^{-1}$);

[S]: substrate concentration in the solution ($mol. L^{-1}$).

$[S]_i$: substrate concentration at the interface liquid-solid.

k_M : Mechaelis Menten constant;

h: liquid-solid mass transfer coefficient expressed in $cm. s^{-1}$.

$$V_i = J \Rightarrow h.([S] - [S]_i) = \frac{V_{\max.} . [S]_i}{k_M + [S]_i}$$

$$h.([S] - [S_I]).(k_M + [S_i]) = V_{\max.} . [S_i]$$

$$h.k_M([S] - [S]_I) + h.([S] - [S]_I).[S]_I = V_{\max.} . [S]_I$$

The previous equation is divided by $h[S]^2$.

$$\frac{h.k_M[S]}{h[S]^2} - \frac{hk_M[S]_i}{h[S]^2} + \frac{h.[S]_i[S]}{h[S]^2} - \frac{h[S]_i^2}{h[S]^2} = \frac{V_{\max.} . [S]_i}{h[S]^2}$$

$$\frac{k_M}{[S]} - \frac{k_M[S]_i}{[S]^2} + \frac{[S]_i}{[S]} - \frac{[S]_i^2}{[S]^2} = \frac{V_{\max.} . [S]_i}{h[S]^2}$$

$$\frac{k_M}{[S]} - \frac{k_M[S]_i}{[S]^2} + \frac{[S]_i}{[S]}\left(1 - \frac{[S]_i}{[S]}\right) = \frac{V_{\max.} \cdot [S]_i}{h\,[S]^2} \qquad (III.19)$$

The number of parameters can be reduced by introducing the following dimensionless variables:

$$\begin{cases} D_a = \frac{V_{max}}{h.[S]} \\ K = \frac{k_M}{[S]} \\ Y = \frac{[S_i]}{[S]} \end{cases}$$

$$K - Y.K + Y.(1 - Y) = D_a.Y$$

$$K(1 - Y) + Y(1 - Y) = D_a Y \qquad (III.20)$$

The previous equation becomes:

$\frac{(1-Y)}{D_a} = \frac{Y}{K+Y}$ Avec $0 < Y < 1$

Da, the Dam Koehler number, is a dimensionless number defined as the ratio between the maximum enzymatic reaction rate and the maximum transfer rate. It quantifies the significance of external diffusion phenomena. For a given system:

If Da<<1: Mass transfer is fast compared to the enzymatic reaction rate. The **reaction is the limiting step.** Improving enzyme activity or loading may increase overall performance.

If Da>>1: Mass transfer is slow compared to the enzymatic reaction rate. The system is limited by **external diffusion**. Improving mixing or increasing the mass transfer coefficient (h) is necessary.

If Da ≃ 1: Both transfer and reaction rates are of the same order of magnitude. The system is **coupled**, and optimization should consider both phenomena.

When the Damkoehler number is neither much greater nor much smaller than 1, the above second-degree equation with respect to [Si] allows for the calculation of X (or $\frac{[S_i]}{[S]}$)

$$\frac{(1 - Y)}{D_a} = \frac{Y}{K + Y} \Rightarrow Y^2 + (D_a + K - 1)Y - K = 0$$

Thus, we have a second-order equation. To solve it, we calculate the discriminant Δ:

$$\Delta = (D_a + K - 1)^2 + 4K$$

$$Y = \frac{-(D_a + K - 1) \pm \sqrt{(D_a + K - 1)^2 + 4K}}{2}$$

$$Y = \frac{1}{2}\left[-(D_a + K - 1) \pm (D_a + K - 1)\sqrt{1 + \frac{4K}{(D_a + K - 1)^2}}\right]$$

$$Y = \frac{[S_i]}{[S]} = \frac{1}{2}(D_a + K - 1)\left[-1 \pm \sqrt{1 + \frac{4K}{(D_a + K - 1)^2}}\right]$$

The signing front of the square root must be chosen so that the ratio $\frac{[S_i]}{[S]}$ is positive.

Calculating the substrate concentration [Si] at the interface makes it possible to determine the effectiveness factor Ɛ (equation below), which quantifies the influence of transfer phenomena.

$$\varepsilon = \frac{reaction\ rate\ in\ the\ presence\ of\ diffusion\ limitations}{reaction\ rate\ in\ the\ absence\ of\ diffusion\ limitations} = \frac{\frac{Y}{(K+Y)}}{\frac{1}{(K+1)}}$$

$$\varepsilon = \frac{\frac{Y}{(K+Y)}}{\frac{1}{(K+1)}} = \frac{Y.(K+1)}{(K+Y)} \qquad (21)$$

This factor is less than or equal to 1, and in general, any increase in resistance due to mass transport results to a decrease in the observed enzymatic activity. This reduction reflects the extent to which mass transfer. It depends on:

- The geometry of the support (bead, membrane, etc.);
- The substrate diffusivity
- The reaction rate.

III.5. Enzyme reactors

Enzyme reactors can be classified according to several criteria, including:

- The presence of soluble or immobilized enzymes;
- The mode of substrate feeding,
- The nature of the support, if the enzyme is immobilized

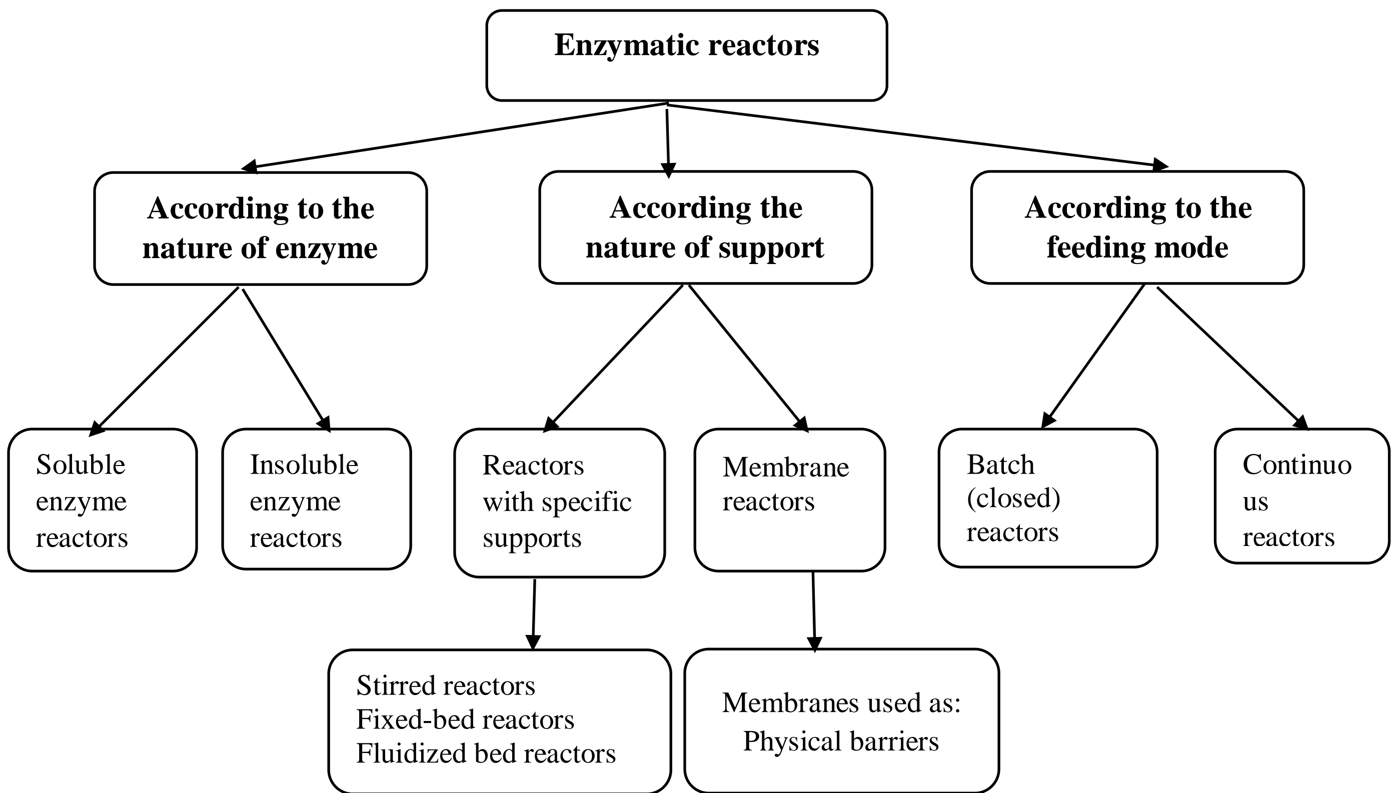


Figure III.13: Classification of enzymatic reactors

→ **Classification based on substrate feeding mode**

a) Batch operation

Batch or discontinuous bioreactors are not continuously supplied with substrate or enzyme. The substrate (solid or liquid) and the enzyme are mixed and agitated in a reaction vessel. The mixture is allowed to react for a defined period, after which the contents are removed for product recovery and, if applicable, enzyme separation.

This type of reactors is commonly used in case involving:

Soluble enzyme preparations,

Solid or colloidal substrates,

Immobilized enzymes, especially in small scale or laboratory settings.

The advantages of this type of reactor include: operational simplicity and low cost. However, it is important to consider the time required for **filling**, **emptying**, and **cleaning** the vessels between batches.

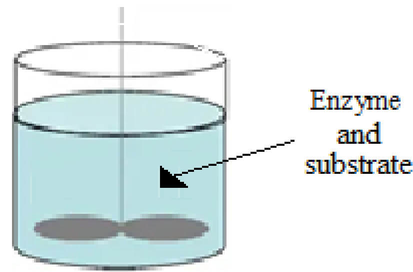


Figure III.14: Schematic diagram of a batch enzymatic reactor

b) Continuous operation

The continuously operating bioreactor, the substrate solution is continuously fed into to reactor, while an equal volume of liquid is simultaneously withdraw, keeping the reactor volume constant over time rea with substrate. If the enzyme is used in soluble form, it may be lost with outflow, requiring continuous addition to maintain activity.

This is on significantly increase operational costs. Therefore, to retain the enzyme within the system and ensure economic viability, two main strategies are often employed:

- The use of an ultrafiltration module to retaiun soluble enzymes whiles allowing the product to pass through;
- The use of immobilized enzyme, which remain fixed within the reactor and are not lost in the outflow.

Advantages: one of the main advantages is that substrate transformation can proceed indefinitely, allowing for constant product output. Additionally, the reactor operates full time, maximizing equipment usage and providing significant economic benefits in terms of productivity and cost efficiency.

In practice:

- In real world applications, it is used over varying periods of time: long-term operation can lead to changes in the biological material (e.g., mutation of microorganisms, denaturation and thus inactivation of proteins, etc.).
- The reactor’s shape is actually adapted to the form in which the enzyme is immobilized.
- The geometry of continuous reactors is generally cylindrical. The two classic ideal types are:
  - ✓ The perfectly mixed continuous reactor.
  - ✓ The plug flow reactor

III.6. Material balance on enzymatic reactors

The design and analysis of enzymatic bioreactors are based on the balance method. This approach involves writing mass balances, for a given substance or chemical species, most commonly the substrate and the product at a specific moment in time and location within the reactor (Figure III.15). The general principle is to apply the law of conservation of mass, which states:

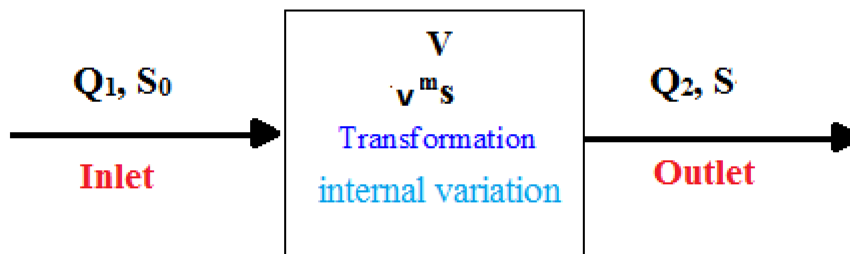


Figure III.15: Substrate inlet and outlet in a bioreactor.

Where:

$[S]_0$: Substrate concentration in the reactor inlet ($g.dm^{-3}$)

$[S]_1$: Substrate concentration in the reactor outlet ($g.dm^{-3}$)

$v^m s$: Specific substrate conversion rate per unit volume ($g.\ dm^{-3}.\ h^{-1}$ or $mol.\ dm^{-3}.\ h^{-1}$)

Q_1: Inlet flow rate ($L.h^{-1}$)

Q_2: Outlet flow rate ($L.h^{-1}$)

V: volume of the bioreactor (dm^3)

The balance equation is written as:

Inlet + disappearance by reaction = Outlet + Accumulation

$$F_{inlet} + F_{disappearance\ by\ reaction} = F_{outlet} + F_{Internal\ rate\ of\ change} \quad (III.22)$$

Inlet rate (F_{inlet}): this refers to the amount of matter entering during a given period of time, coming from outside the surface or volume where the balance is being performed. It is given by the following expression:

$$F_{Inlet} = Q_1.[S_0]$$

Transformation rate ($F_{transformation}$) : This is the amount of matter, over the same time interval, resulting from the biochemical transformation occurring within the surface or volume of the

bioreactor where the balance is being performed. This rate is positive if there is production (appearance) of a product and negative if there is consumption (disappearance) of a substrate.

It is equal to the product of the specific substrate conversion rate per unit volume ($v^m s$) and the volume (V) of the bioreactor:

$$F_{transformation} = -V.v^m s$$

The out let rate (F_{out}): this is the amount of substance leaving the system over a given time interval, through the surface or volume where the mass balance is applied. It is given by the following expression:

$$F_{Outlet} = Q_2.[S]$$

The internal rate of change ($F_{Internal\ rate\ of\ change}$) : this corresponds to the change in the amount of substrate over time, due to the operating conditions of the bioreactor. It is expressed as follows:

$$F_{Internal\ rate\ of\ change} = \frac{d[S]_j.V_j}{dt}$$

Where:

V_j : volume of the bioreactor at point j.

The overall expression of the balance in the general case is written as:

$$Q_1.[S]_0 - V.v^m s = Q_2.[S] + \frac{d[S]_j.V_j}{dt} \quad (III.23)$$

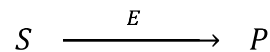
Note:

To solve this equation, it is necessary to define the operating mode of the system. This means that assumptions can be made regarding how the bioreactor operates (e.g., ideal operating modes), which allows us to explicitly express the term $\frac{d[S]_j.V_j}{dt}$) .

Additionally, a simple kinetic law for substrate consumption, such as Michaelis-Menten kinetics, can be chosen.

III. 7. Ideal Bioreactors with Michaelis-Menten kinetics:

Let us consider an enzymatic reactor in which the following reaction takes place:



Whith a Michaelis Menten kinetics : $v_m s = v^m_{max} s \cdot \frac{[S]}{k_M + [S]}$

III.7.1 Batch or discontinuous reactors

In batch enzymatic reactors, there is no exchange of matter with the outside. Therefore, the inlet and outlet flow rates are zero:

$$F_{Inlet} = F_{Outlet} = 0.$$

- The enzyme and substrate are added at the beginning. The reaction is allowed to proceed.
- The products are collected at the end.
- The mixture is perfectly mixed (ideal behavior), so the substrate concentration is uniform throughout the vessel and is equal to the substrate concentration [S] at time t.

The internal rate of change can then be written as follows: $\frac{d[S].V}{dt}$ (instead of $\frac{dS]_j.V_j}{dt}$)

We also have: $\frac{d[S].V}{dt} = \frac{[S].dV}{dt} + \frac{V.d[S]}{dt}$

The variation of volume with respect to time is zero. As a result, the internal rate of change simplifies to: $F_{Internal\ rate\ of\ change} = \frac{V.d[S]}{dt}$

Since **V** is constant, it can be taken out of the derivative:

$$-V.v^m s = \frac{V.d[S]}{dt}$$

$$-v^m s = \frac{d[S]}{dt}$$

To find the expression of substrate concentration as a function of time, it is sufficient to substitute into the previous equation the substrate consumption kinetics: The Michaelis Menten Kinetics

$$v_m s = v^m_{max} s \cdot \frac{[S]}{k_M + [S]}$$

$$-v^m_{max} s \cdot \frac{[S]}{k_M + [S]} = \frac{d[S]}{dt} \Rightarrow (k_M + [S]).d[S] = v^m_{max} s \, .[S].dt$$

$$k_M d[S] + [S]d[S] = v^m_{max}s\,.\,[S].\,dt$$

$$-\frac{d[S]}{[S]}.\,k_M - d[S] = v^m_{max}s\,.\,dt$$

$$-k_M \int_{[S]_0}^{[S]} \frac{d[S]}{[S]} - \int_{[S]_0}^{[S]} d[S] = v^m_{max}s \int_0^t dt$$

$$k_M Ln\frac{[S]_0}{[S]} + [S]_0 - [S] = v^m_{max}s.\,t \qquad (III.24)$$

The substrate concentration can be replaced as a function of the conversion rate (X_A) and the initial substrate concentration $[S]_0$.

We have : $X_S = \frac{[S]_0 - [S]}{[S]_0} \Rightarrow [S] = [S]_0.\,(1 - X_S)$

$$k_M Ln\frac{1}{1 - X_S} + X_S[S]_0 = v^m_{max}s.\,t$$

$$-k_M Ln(1 - X_S) + X_S[S]_0 = v^m_{max}s.\,t \qquad (III.25)$$

Note:

For a given initial substrate concentration $[S]_0$, the following parameters can be calculated using the above equation:

- The reaction time required to achieve a desired conversion rate,
- The bioreactor volume
- The kinetic parameters of the enzyme used, under the following conditions: no inhibition, irreversible reaction, and a constant $v^m_{max}s$ (maximum specific rate per unit of support or volume). If the enzyme is immobilized, we write:

$$v_m s = \boldsymbol{\varepsilon}\,.\,v^m_{max}s\,.\,\frac{[S]}{k_M + [S]}$$

III.7.2. Continuous Reactor

a) Continuous Stirred Tank Reactor- CSTR)

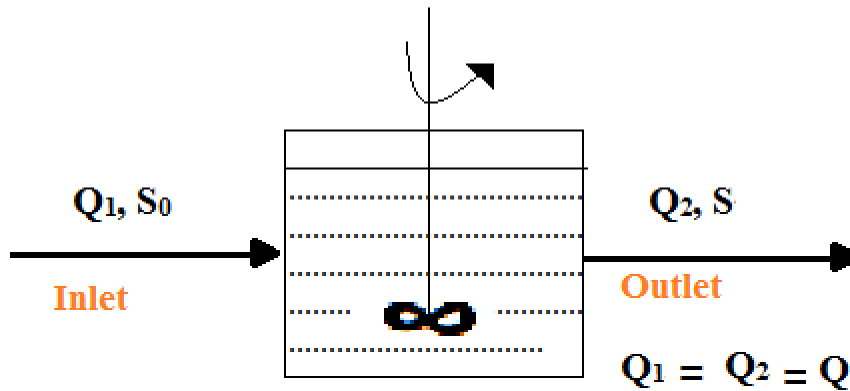


Figure III.16 : Perfectly Mixed continuous reactor

The balance equation is written a: **inlet** + **Prod** = **outlet** + **Accu**

$$F_{inlet} + F_{transformation} = F_{outlet} + F_{Internal\ rate\ of\ change}$$

$$F_{Internal\ rate\ of\ change} = \frac{d[S].V}{dt} = \frac{[S].dV}{dt} + \frac{V.d[S]}{dt}$$

Since the reactor is perfectly mixed, the substrate concentration is uniform throughout the entire vessel and is equal to the substrate concentration at a given time t.

The variation of volume whith respect to time is zero ($\frac{dV}{dt} = 0$). As a result, the internal rate of change is reduced to:

$$F_{Internal\ rate\ of\ change} = \frac{V.d[S]}{dt} \qquad (III.26)$$

If we also assume that the reactor operates under steady- state conditions (i.e., the concentration does not change over time), then [S] = constant, and thus $\frac{d[S]}{dt} = 0$

The balance equation is then written as:

$$Q.[S]_0 - V.v^m s = Q.[S] + 0$$

$$Q.([S]_0 - [S]) = V.v^m s$$

$$Q.([S]_0 - [S]) = V.v_{max}^m s.\frac{[S]}{k_M + [S]}$$

$$([S]_0 - [S]).(k_M + [S]) = v_{max}^m s.\frac{V}{Q}[S]$$

$$([S]_0 - [S]).k_M + [S].([S]_0 - [S]) = v_{max}^m s.\frac{V}{Q}[S]$$

$$\frac{([S]_0 - [S])}{[S_0]}.\frac{[S]_0}{[S]}.k_M + [S]_0.\frac{([S]_0 - [S])}{[S]_0} = v_{max}^m s.\frac{V}{Q}$$

$X_A.\frac{[S]_0}{[S]}.k_M + [S]_0.X_A = v_{max}^m s.\frac{V}{Q}$

We have $\boldsymbol{X_S = \frac{[S]_0-[S]}{[S]_0} \Longrightarrow X_S = 1 - \frac{[S]}{[S]_0}}$

$$\frac{X_S}{(1 - X_S)}.k_M + [S_0].X_S = v_{max}^m s.\frac{V}{Q} \quad (III.27)$$

c) Plug flow reactor-PER

Unlike the perfectly mixed continuous reactor, the concentration of the various reactive species varies depending on the location within the bioreactor.

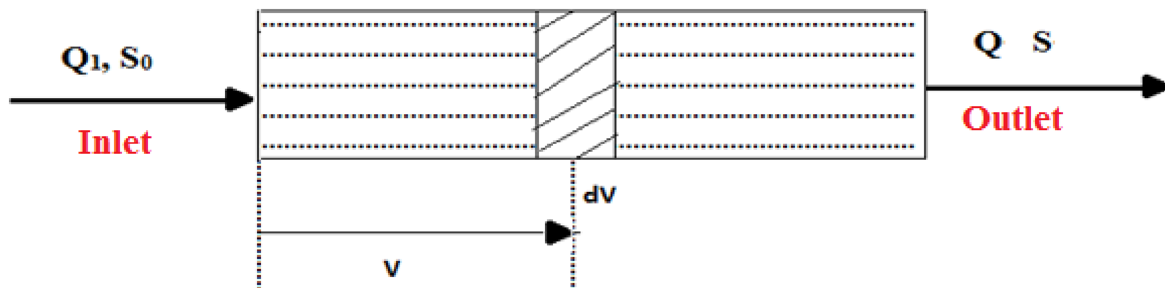


Figure III.17: plug flow bioreactor: representation of a differential volume dV in which the reaction rate remains constant

$$F_{inlet} + F_{transformation} = F_{outlet} + F_{Internal\ rate\ of\ change}$$

$$V_{Inlet} = Q.[S]_v$$

$$F_{Internal\ rate\ of\ change} = -v^m s.dV$$

$F_{Outlet} = Q.([S_V] + d[S])$ = Q. ($[S]_v + \frac{d[S]}{dV}.dV)$

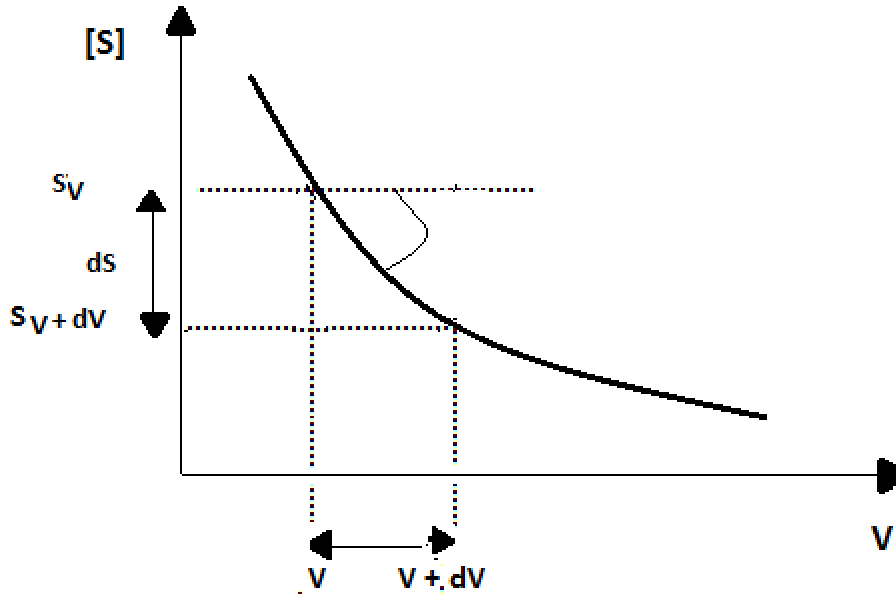


Figure III.18: Variation of substrate concentration in the volume element dv of a plug flow bioreactor.

The material balance is written as follows:

$Q.[S]_v - v^m s.dV$ =Q. ($[S]_v + \frac{d[S]}{dV}.dV)$

For the Michaelis Menten kinetics, for the element dv, we have:

$$-v_{max}^m s \cdot \frac{[S]}{k_M + [S]} \ .dV = Q.d[S]$$

$$-v_{max}^m s \cdot [S] \ .dV = Q.d[S](k_M + [S])$$

$$-v_{max}^m s \cdot [S] \ .dV = k_M Q.d[S] + Q.d[S][S]$$

$$-v_{max}^m s \cdot dV = k_M Q.\frac{d[S]}{[S]} + Q.d[S]$$

$$-v_{max}^m s \cdot \int_0^V dV = k_M Q.\int_{[S]_0}^{[S]} \frac{d[S]}{[S]} + Q.\int_{[S]_0}^{[S]} d[S]$$

$$v_{max}^{m} s \,.V = k_M Q.Ln\frac{[S]_0}{[S]} + Q.(\,[S]_0 - [S])$$

$$v_{max}^{m} s \,.\frac{V}{Q} = k_M.Ln\frac{[S]_0}{[S]} + (\,[S]_0 - [S])$$

$$v_{max}^{m} s \,.\tau = k_M.Ln\frac{[S]_0}{[S]} + (\,[S]_0 - [S]) \qquad (III.29)$$

$\tau = \frac{V}{Q}$; represents the residence time of the substrate.

Expression of the conversion rate

$v_{max}^{m} s \,.\tau = k_M.Ln\frac{[S]_0}{[S]} + (\,[S]_0 - [S])$

$$\boldsymbol{X_S = \frac{[S]_0 - [S]}{[S]_0} \Longrightarrow \frac{[S]}{[S]_0} = 1 - X_S}$$

$$v_{max}^{m} s \,.\tau = -k_M.Ln(1 - X_S) + X_S\,[S]_0 \qquad (III.30)$$

III.8. Reactor design for optimal enzyme performance

Reactor design plays a crucial role in ensuring optimal enzyme performance. Key design considerations include:

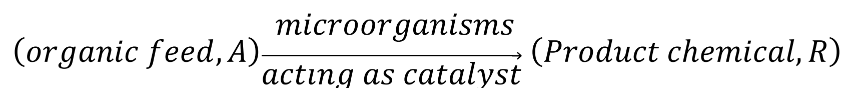
- Reactor geometry and configuration ;
- Mixing and mass transfer characteristics;
- Temperature control and heat transfer;
- Enzyme immobilization and support materials.

Chapter IV: Fermenters

IV.1. Fermentation

Fermentation is a classic method of bioprocessing that involves the action of microorganisms, such as bacteria and fungi, to alter the nutritional profile of foods through both anaerobic and aerobic conversion of macronutrients. These microorganisms ameliorate the physical properties of food, leading to different effects on the nutritional and sensory characteristics of the final product. In general, fermentations are reactions wherein a raw organic feed is converted into product by the action of microbes .

Microbial fermentations can be represented by:



$$(organic\ feed, A) \xrightarrow[acting\ as\ catalyst]{microorganisms} (Product\ chemical, R)$$

Fermentation processes utilize microorganisms to convert solid or liquid substrates into various products. The substrates used exhibit great diversity, any material capable of promoting microbial growth can be considered a potential substrate. Similarly, fermentation-derived products show tremendous variety. Commonly consumed fermented products include bread, cheese, sausage, pickled vegetables, cocoa, beer, wine, citric acid, glutamic acid soy sauce.

Most commercially useful fermentation may be classified as either solid-state or submerged cultures. In solid-state fermentations, the microorganisms grow on a moist solid with or no' free' water, although capillary water may be present. Solid state and submerged fermentations may each be subdivided into oxygen requiring aerobic process, and anaerobic process that must be conducted in the absence of oxygen.

Examples of this type of fermentation are seen in mushroom cultivation, bread-making and the processing of cocoa, and in the manufacture of some traditional foods.

IV.2. Factors influencing fermentation

A fermentation is influenced by numerous factors, including **temperature**, pH, nature and composition of the medium, dissolved O_2, dissolved CO_2, operational system (e.g. batch, fed-batch, continuous), feeding with precursors, mixing (cycling through varying environments), and shear rates in the fermenter.

The variations in these factors can affect: the rate of fermentation; the product spectrum and yields, the organoleptic properties of the product (appearance, taste, smell and texture); the generation of toxins; nutritional quality; as well as other physicochemical properties and the composition of the medium

IV.3. The different phases of bacterial growth

The growth of bacteria (or other microorganisms, as protozoa, microalgae or yeasts) in batch culture can be modeled with four different phases, as shown in Fig IV.1:

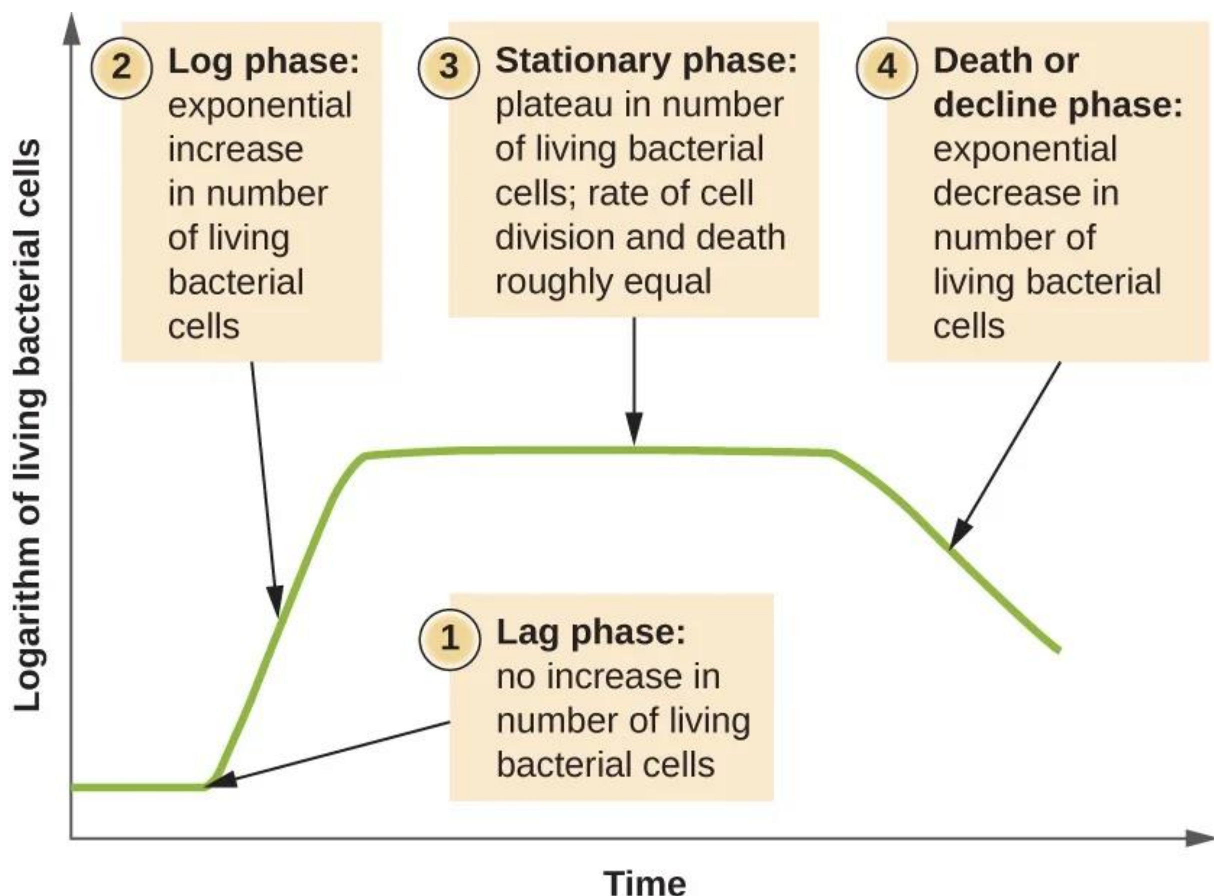


Figure IV.1. Bacterial growth curve

IV.3.1. Lag phase

During lag phase, bacteria adapt themselves to growth conditions. This period corresponds to the time, when individual bacteria cells are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, RNA, enzymes and other molecules are synthesized.

IV.1.2. Log phase or exponential phase

The log phase (sometimes called the logarithmic phase or the exponential phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. Exponential growth can be described by equation (IV.1):

$$\frac{d[X]}{dt} = \mu[X] + k_d[X] \qquad (IV.1)$$

Where:

[X]: is the biomass concentration at time

μ: is the specific growth rate (i.e. growth rate per unit cell mass);

k_d: is the specific death rate.

During exponential growth, the specific death rate is negligible and **equation IV.1** reduces to **equation IV.2**:

$$\frac{d[X]}{dt} = \mu.[X] \qquad (IV.2)$$

For a cell mass concentration X_0 at the beginning of the exponential growth phase ($[X]_0$ usually equal to the inoculum concentration in the fermenter), and taking the time at which exponential growth begins as zero, **equation IV.2** can be integrated to produce **equation IV.3**:

$$ln\frac{[X]}{[X]_0} = \mu t \qquad (IV.3)$$

μ remains constant at this phase

Using equation IV.3, the biomass doubling time, td, can be derived (**equation IV.4**):

At $t = t_{1/2} \Rightarrow [X] = \frac{[X]_0}{2}$

$$t_d = \frac{Ln\ (2)}{\mu} \qquad (IV.4)$$

Doubling times typically range over 4- 160 min. Bacteria generally grow faster than yeasts and yeasts multiply faster than moulds. The maximum biomass concentration in submerged microbial fermentations is tgenerally between 40 and 50 $kg.m^{3-}$

IV.3.3. Stationary phase

The growth phase is followed by the stationary phase, in which the size of a bacterial population phase remains constant, even though some cells continue to divide and others begin to die. During the stationary phase, the rate of bacterial cell growth is **equal** to the rate of bacterial cell death.

IV.3.4. Death phase

The death phase (decline phase), bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions.

IV.4. Fermenter

A fermenter, also called a bioreactor, is an essential piece of equipment in many industries. It is a vessel, typically made of glass or stainless steel, designed to create a controlled environment where microorganisms (such as yeasts, bacteria, or fungi) can grow. The main purpose of a fermenter is to cultivate these microorganisms in order to:

- **Produce biomass:** that is, to generate a large quantity of microorganisms themselves, such as yeast for baking or brewing.
- **Produce metabolites:** these are substances produced by the microorganism, such as antibiotics, vitamins, enzymes, or hormones.
- **Bioconversion:** using microorganisms to transform one substance into another- for example, in waste treatment.

A fermenter is a culture vessel, with a variable volume ranging from a few liters to several cubic meters in the case of industrial units. The tank is hermetically sealed. A fermenter generally consists of several components that work together to support the fermentation process.
The main components of this system are:

1. **Fermenter vessel:** most fermented containers are made of glass and stainless steel to reduce pressure and corrosion. It provides a workable environment for production.
2. **Heating and cooling apparatus:** the cooling jacket and silicon in a reactor help to remove excess heat, while internal coils provide heat during fermentation.
3. **Feed ports:** the silicon tubes are available for adding nutrients and acid/alkali for fermentation.
4. **Foam control:** the foam produced during the fermentation process has many side effects like it decreases efficiency, and productivity, degrading product quality, and many more. So, the foam detector is placed in a reactor, adding some anti-foam is used to deform the fermenter.
5. **Valves:** in the fermenter, valves regulate the liquid flow. Most of the reactor contains at least three valves in it.
6. **Sparger:** it is used in introducing sterile air to a fermentation vessel. It also aids in providing the vessel with the correct aeration.

7. **Impeller:** the role of an impeller in a fermenter is to distribute microbial cells in a nutrient media evenly and also to reduce the bubbles produced with the help of impeller blades.
8. **Computer:** modern automated and semi-automated software programs are used for collecting data, monitoring, and controlling the process, such as Ambr® Clone Selection software application used for cell line screening and Software called Biostat® T CHO introduces users to bioprocess engineering and controls a bioreactor as well.
9. **Baffles:** baffles are the metal stripes attached to the wall of the container to prevent vertex and improve aeration in the fermenter.
10. **Regulator:** this device is used to control and maintain the temperature, pH, nutrients, oxygen concentration, and product concentration.

All these components are carefully designed and integrated to create an environment that maximizes the productivity of the microorganisms involved.

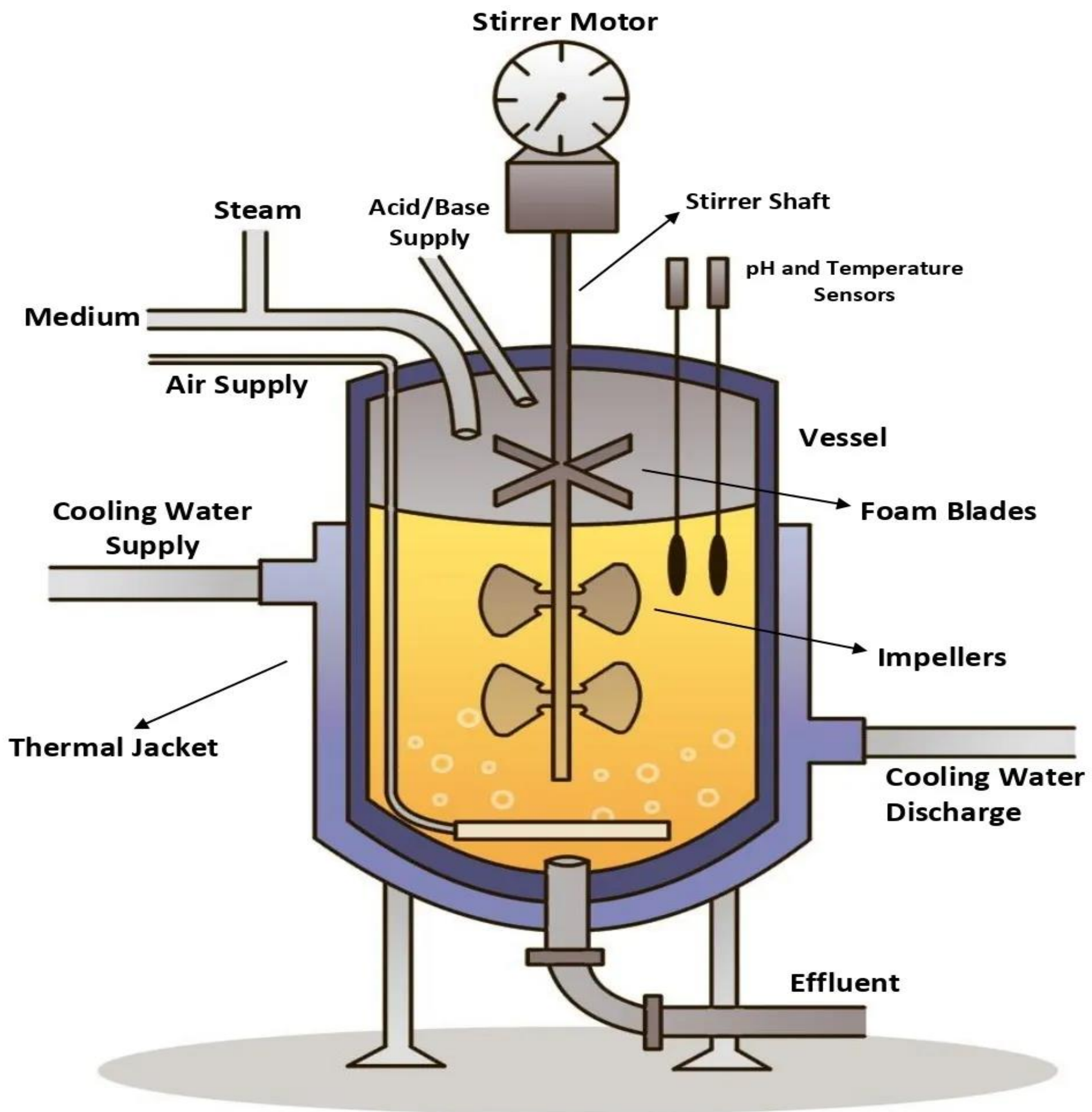


Figure IV.2: Fermenter

IV.5. Overview of the formulation of fermentation parameters

The overall reactions of cell growth and biotransformation can be described in kinetic terms and quantified using the parameters mentioned above.

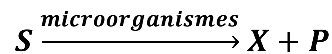
IV.5.1. Microbial kinetic

Bacterial growth kinetics describes the evolution of a bacterial population over time in a given environment.

Importance:

- *In microbiology:* understand of infection dynamics, fermentations, or biotechnology.
- *In industry:* optimizing production of metabolites (e.g., antibiotics, probiotics).
- *In public health:* control of bacterial proliferation in food or the environment.

Microbial kinetics (or microbial growth kinetics) can be described based on the followedsimplified bioconversion reaction:



$$S \xrightarrow{microorganismes} X + P$$

Where:

S: substrate concentration (g/L)

X: concentration of biomass (microbial cells) (g/L)

P: concentration of product (g/L)

The reaction rates associated with the three main components are generally expressed in $g.L^{-1}.h^{-1}$. They are denoted as follows:

r_S: the substrate consumption rate (S);

r_X: the biomass rate, also called the growth rate (X);

r_P: the product formation rate (P).

IV.5.2. Growth rate (μ)

The growth rate of a microbial population expresses the speed at which bacteria multiply. It refers to the number of divisions occurring per unit of time. The growth rate is therefore defined as the variation in the number of bacteria or their biomass per unit of volume or time. The growth rate can be described by the following differential equation:

$$r_X = \frac{d[X]}{dt} = \mu.[X] \Rightarrow [X] = \frac{1}{\mu}.\frac{d[X]}{dt} \quad (IV.5)$$

Where:

[X]: represents the biomass concentration (g/L), and μ is the specific growth rate (h^{-1})

However, under real culture conditions, the specific growth rate μ is generally not constant, as it depends on the availability of nutrients, particularly the limiting substrate. To account for this dependence, Monod proposed an empirical relationship between μ and the concentration of the limiting substrate S:

$$\mu = \mu_{max}.\frac{[S]}{[S] + K_s} \quad (IV.6)$$

$$r_X = \mu_{max} \cdot \frac{[S]}{[S] + K_s} \cdot [X] \qquad (IV.7)$$

Where:

μ_{max}: is the maximum specific growth;

Ks: is the half saturation constant (corresponding to the substrate concentration at which $\mu = \mu_{max}/2$).

By combining this expression with the initial growth equation, we obtain a more realistic model of microbial growth, widely used in bioprocess engineering to simulate and optimize fermentation process.

Estimation of μ_{max} and Ks:

The Monod equation is used to determine the values of μ_{max} (maximum specific growth rate) and Ks (half saturation constant). To facilitate experimental estimation, the equation can be linearized, yielding a linear relationship between $1/\mu$ and $1/[S]$.

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{1}{\mu_{max}} \cdot \frac{1}{[S]} \qquad (IV.8)$$

The graphical representation of this relationship (Figure IV.3) produces a straight line, where the **y** intercept and the slope allow for the calculation of $\mathbf{1/\mu_{max}}$ and $\mathbf{Ks/\mu_{max}}$, respectively.

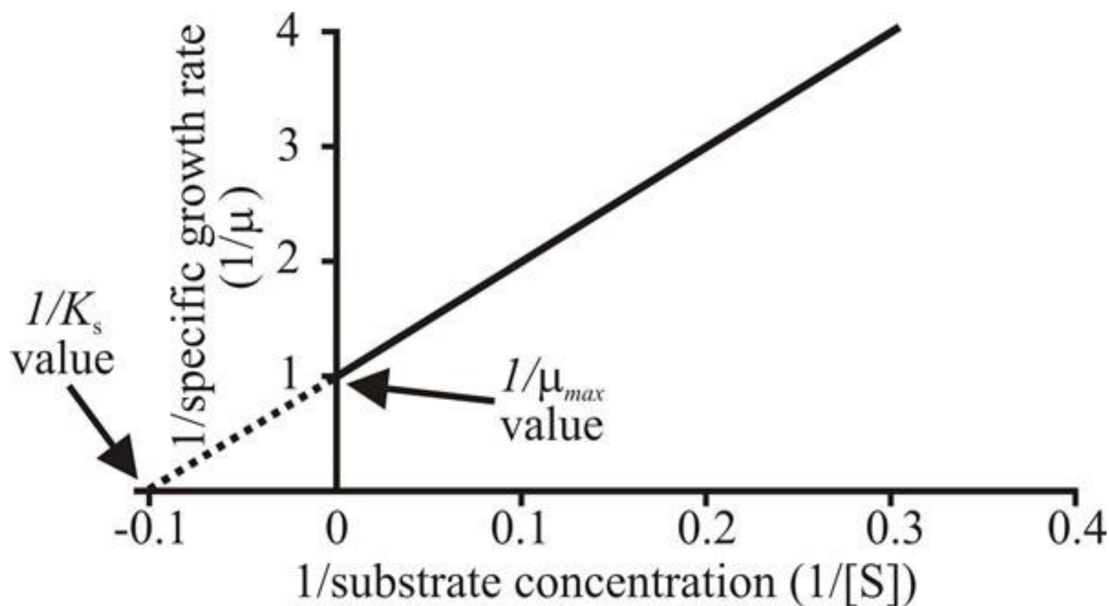


Figure IV.3: Estimation of μ_{max} and K_S

IV.5.3. Number of generations

The number of generations represents how many times a bacterial population has doubled during a given period of time. It can be calculated using different formulas:

At time t during the fermentation process, the number of generations N is given by:

1. Based on initial and final cell count (or biomass):

$$N = \frac{\log(N_t) - \log(N_0)}{\log 2} \qquad (IV.9)$$

Where:

N: is the number of generations;

N_0: is the initial number of cells (or biomass);

N_t : is the number of cells at time t.

<u>Based on specific growth rate (μ) and time</u>

$$N = \frac{t}{t_d} = \frac{t.\mu}{ln(2)} \qquad (IV.10)$$

Where:

t: duration of the growth phase (in hours).

This formula is used when the specific growth rate and the elapsed time are known.

IV.5.4. Biomass yield

The biomass yield ($Y_{X/S}$) is a key parameter in bioprocesses. It represents the efficiency with which a microorganism converts a substrate into biomass. It is expressed by the following equation:

$$Y_{X/S} = \frac{\Delta[X]}{\Delta[S]} = \frac{d[X]}{d[S]} \qquad (IV.11)$$

Where:

$Y_{X/S}$: biomass yield on substrate (g of biomass/g of substrate);

Δ[X]: biomass produced (g/L);

Δ[S]: substrate consumed (g/L).

IV.5.5. Product yield

The product yield ($Y_{P/S}$) is an indicator of how efficiently the substrate is converted into the desired product. It represents the amount of product formed (exp: metabolite, enzyme, organic

acids,..) relative to the amount of the substrate consumed. It is expressed by the following equation:

$$Y_{P/S} = \frac{\Delta[P]}{\Delta[S]} = \frac{d[P]}{d[S]} \qquad (IV.12)$$

Where:

$Y_{P/S}$: Product yield on substrate (g of product/g of substrate);

Δ[P]: Product produced (g/L);

Moreover, the substrate consumption rate and the product formation rate are given by the following equations:

$$r_S = -\frac{d[S]}{dt} = -\frac{1}{Y_{X/S}}.\mu.[X] = -\frac{r_X}{Y_{X/S}} \qquad (IV.13)$$

$$\begin{cases} r_x = \mu.dX \\ dS = \frac{dX}{Y_{X/S}} \end{cases}$$

$$r_P = \frac{d[P]}{dt} = Y_{P/S}.\frac{d[S]}{dt} = \frac{Y_{P/S}}{Y_{X/S}.}r_X \Rightarrow r_P = r_X.Y_{P/X} \qquad (IV.14)$$

$Y_{P/X}$: is the product yield on biomass

$$Y_{P/X} = \frac{[P]-[P]_0}{[X]-[X]_0} = \frac{\frac{[P]-[P]_0}{[S]-[S]_0}}{\frac{[X]-[X]_0}{[S]-[S]_0}} = \frac{Y_{P/S}}{Y_{X/S}} \qquad (IV.15)$$

$$r_S = -\frac{r_X}{Y_{X/S}} = -\frac{r_P.Y_{P/S}}{Y_{X/S}} = \frac{r_P.Y_{P/S}}{\frac{Y_{P/S}}{Y_{P/X}}} \qquad (IV.16)$$

IV.5.6. Metabolic coefficient q:

The metabolic coefficient q measures the specific rate of substrate consumption by biomass at a given time during fermentation.

$$q_S = \frac{1}{[X]}\frac{d[S]}{dt} = -\frac{r_S}{[X]} \qquad (IV.17)$$

Where:

q: metabolic coefficient (g of substrate consumed /g of biomass / hours ($g.g^{-1}.h^{-1}$)).

X: biomass concentration (g/L).

dS/dt: rate of substrate consumption (g/L/h). Since the substrate is consumed, dS/dt is usually negative, but we often use the absolute value of q to focus on the rate.

$q_P = \frac{r_P}{X}$: the specific product formation rate, expressed in g of product formed per g of biomass per hour ($g.g^{-1}.h^{-1}$). It describes the productivity of the biomass in terms of product synthesis.

IV.6. General mass balance equation

The basic form of a mass balance used for any system, including fermenters is:

$$Inlet + Generation\ (or\ consumption) = Outlet + Accumulation$$

- *Mass balance equation with respect to the substrate S:*

$$Q_E[S]_0 - r_s\, V = Q_S[S] + \frac{d(V.[S])}{dt} \qquad (IV.18)$$

- *Mass balance equation with respect to biomass X:*

$$Q_E[X]_0 + r_X\, V = Q_S[X] + \frac{d(V.[X])}{dt} \qquad (IV.19)$$

- *Mass balance equation with respect to product P:*

$$Q_E[P]_0 + r_P\, V = Q_P[P] + \frac{d(V.[P])}{dt} \qquad (IV.20)$$

Where:

V: volume of the liquid

Q: volumetric flow rate

$[S]_0$, $[X]_0$ and $[P]_0$: initial concentrations of the substrate, biomass, and product, respectively

[S], [X] and [P]: concentrations of the substrate, biomass, and product at time t, respectively

IV.6.1. Batch fermentation (Discontinuous)

The Batch fermentation (discontinuous Non –fed) is a process carried out in a closed system, where all necessary nutrients are supplied at the start of the cultivation. During fermentation, no additional (culture) medium is added, except for neutralizing agents or antifoam, which are introduced in very small amount. Similarly, no part of the culture is removed until fermentation

is complete. The process continues until the nutrients are depleted or inhibitory by – products build up to levels that halt microbial growth or activity.

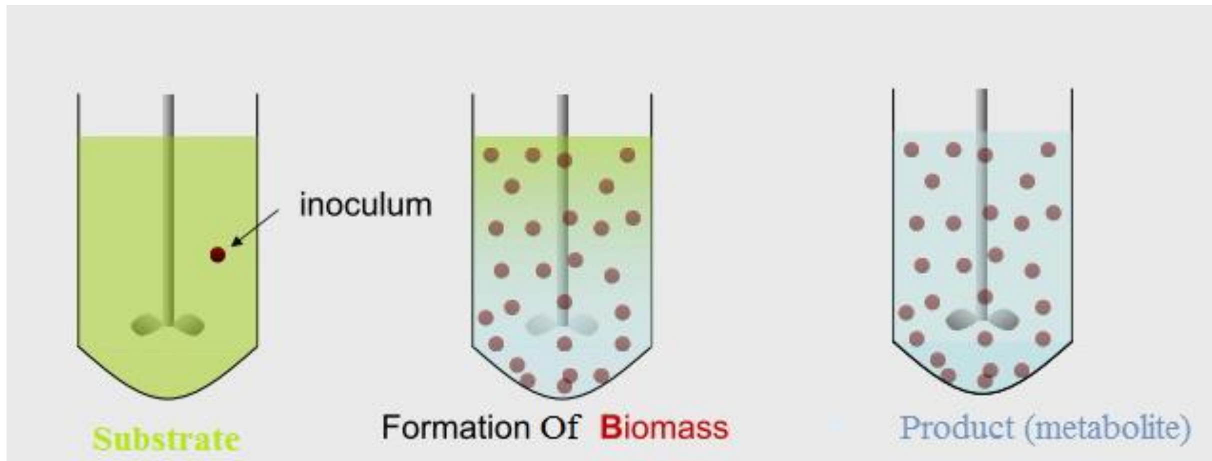


Figure IV.4: Batch fermentation

1. *Mass balance on the bath fermenter*

The reactor operates in closed mode:

- No inlet of material: $\mathbf{Q_E = 0}$;
- No outlet of material: $\mathbf{Q_S = 0}$;
- The volume remains constant during the entire fermentation process.

This type of fermentation is called batch or unfed batch, because all components (substrate, biomass, etc.) are added at the beginning, and the system is then left to ferment without any addition or removal until the end.

2. *Mass balance equation with respect to the substrate S:*

$$-r_S = \frac{d[S]}{dt}$$

3. *Mass balance equation with respect to biomass S:*

$$r_X = \frac{d[X]}{dt}$$

4. *Mass balance equation with respect to product P:*

$$r_P = \frac{d[P]}{dt}$$

The biomass growth rate is proportional to the biomass concentration present:

$$\frac{d[X]}{dt} = \mu.[X]$$

During the optimal exponential phase:

$$\mu = \mu_{max}$$

$$\frac{d[X]}{dt} = \mu_{max}.[X]$$

$$\frac{d[X]}{[X]} = \mu_{max.}dt$$

$$\int_{[X]_0}^{[X]} \frac{d[X]}{[X]} = \mu_{max.} \int_0^t dt$$

$$\ln \frac{[X]}{[X]_0} = \mu_{max.}t$$

$$[X] = [X]_0.e^{\mu_{max.}t} \qquad (IV.21)$$

$$r_s = \frac{r_x}{Y_{X/S}};\ r_x = \mu_{max}.[X]\ \text{et}\ r_P = Y_{P/X}.r_X \qquad (IV.22)$$

$$\begin{cases} Y_{X/S} = \frac{\Delta[X]}{\Delta[S]} \Rightarrow \Delta[S] = \frac{\Delta[X]}{Y_{X/S}} \Rightarrow [S] = [S]_0 + \frac{[X]-[X]_0}{Y_{X/S}} = [S]_0 + \frac{[X]}{Y_{X/S}} - \frac{[X]_0}{Y_{X/S}} \\ Y_{P/X} = \frac{\Delta[P]}{\Delta[X]} \Rightarrow \Delta[P] = Y_{P/X}.\Delta[X] = Y_{P/X}.([X] - [X]_0) \end{cases} \qquad (IV.23)$$

$$\begin{cases} [S] = [S]_0 + \frac{[X]_0.e^{\mu_{max}.t}}{Y_{X/S}} - \frac{[X]_0}{Y_{X/S}} = [S]_0 + \frac{X_0}{Y_{X/S}}.(e^{\mu_{max}.t} - 1) \\ \Delta[P] = Y_{P/X}.([X] - [X]_0) = Y_{P/X}.[X]_0(e^{\mu_{max}.t} - 1) \end{cases} \qquad (IV.24)$$

The time required for the substrate concentration to decrease from S_0 to S during its consumption in fermentation can be expressed by the following formula:

$$t = -\int_{S_0}^{S} \frac{d[S]}{r_s} \qquad (IV.25)$$

r_s: is the fermentation reaction rate, generally modeled by the Monod equation.

The time required for the cell concentration to increase from $[X]_0$ to $[X]$ is given by the following formula:

$$t = \int_{X_0}^{X} \frac{d[X]}{r_X} \qquad (IV.26)$$

r_X: represents the cell growth rate.

The time required to reach a final product concentration P is given by the following equation:

$$t = \int_{[P]_0}^{[P]} \frac{d[P]}{r_P} \qquad (IV.27)$$

r_p: represents the rate of the formation of product (metabolite).

To determine the productivity of a batch fermentation, it is necessary to consider the total duration of the process, which includes, in addition to the actual fermentation time, the time required for preparation, filling, possible sterilization, emptying, and possibly cleaning of the reactor. These “auxiliary” times can range from 3to 24 hours. The total duration of the process is given by:

$$t_{total} = \frac{1}{\mu_{max}} ln \frac{[X]_f}{[X]_0} + t_{aux} \qquad (IV.28)$$

Where:

$[X]_0$: initial biomass concentration;

$[X]_f$: final biomass concentration;

t_{taux}: auxiliary time (preparation, sterilization, cleaning, etc).

The overall productivity of the batch process is then given by:

$$Q = \frac{[X]_f}{\frac{1}{\mu_{max}} ln \frac{[X]_f}{[X]_0} + t_{aux}} \qquad (IV.29)$$

Q: global biomass productivity (mass. volume^{-1}. time^{-1}).

IV.6.2. Feed batch fermentation

This is a modification from of batch fermentation. The process adds substrate periodically as the fermentation progress to ensure that the substrate is always present at the optimum concentration. The feed rate Q is adjusted so that the substrate concentration remains constant in the fermenter (d[S]/dt =0) and corresponds to a stage of the logarithmic phase of cell growth. Indeed, we must take into account the variation in the reactor volume and, consequently, the change in the dilution rate.

Substrate can be added in various ways:

- Starting with a small volume of medium and continuously adding diluted substrate: in this case, the volume increases significantly while the biomass concentration changes very little;

- Starting with a large volume and continuously adding concentrated substrate: in this case, the volume increases only slightly and can be considered constant, while the biomass concentration increases.

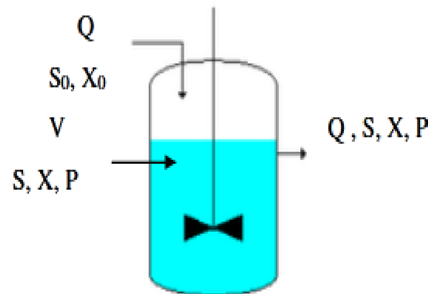


Figure IV.5 : Feed batch fermenter

1. *Mass balance equation with respect to the substrate S:*

$$Q_E[S]_0 - r_s\,V = \frac{d(V.[S])}{dt} = \frac{[S]d(V)}{dt} + \frac{Vd[S]}{dt} \quad \left(with\frac{d[S]}{dt} = 0\right) \qquad (IV.30)$$

$$r_s = \frac{-r_X}{Y_{X/S}} = \frac{-\mu.[X]}{Y_{X/S}}$$

$$r_X = \frac{d[X]}{dt} = \mu.[X] \qquad \mu: growth\,rate$$

$$Q_E[S]_0 - \frac{-\mu.[X]\,V}{Y_{X/S}} = \frac{[S].d(V)}{dt} \qquad (IV.31)$$

$Y_{X/S}$: the biomass yield per substrate consumed.

2. *Mass balance equation with respect to biomass X:*

$$r_X\,V = \frac{d(V.[X])}{dt} = \mu.[X].V = V\frac{d[X]}{dt} + [X].\frac{dV}{dt} \qquad (IV.32)$$

Case1: Constant volume ($V = V_0$)

If the reactor volume remains constant: $\frac{dV}{dt} = 0$

$$\frac{d[X].V}{[X].V} = \mu dt \Rightarrow \int_{[X]_0V_0}^{[X]V} \frac{d[X].V}{[X].V} = \mu\int_0^t dt \Rightarrow Ln\left(\frac{[X]V}{[X]_0V_0}\right) = \mu.t$$

$$t = \frac{1}{\mu}ln\frac{[X]}{[X]_0} \qquad (IV.33)$$

Case 2 : Variable volume with constant biomass concentration

When the reactor volume increases while the biomass concentration remains constant:

$$\frac{d[X]}{dt} = 0$$

$$\frac{d[X].V}{[X]} = \frac{[X]dV}{[X]} = V.\mu.dt \Rightarrow \mu = \frac{dV}{Vdt}$$

$$\mu = \frac{dV}{Vdt} = \frac{Q}{V} = D \qquad (IV.34)$$

Where $D = \frac{Q}{V}$ et $Q = \frac{dV}{dt}$

Q: volumetric flow rate

D: dilution rate

3. *Mass balance equation with respect to product P:*

$$r_P\, V = \frac{d(V.[P])}{dt} \qquad (IV.35)$$

Where:

V: volume of the liquid;

Case 3: Variable volume and biomass increase (Fed batch reactor)

The increase in the liquid volume inside the bioreactor is given by:

$$dV = Qdt$$

Where:

Q: feed flow rate;

V: reactor volume.

In a fed-batch reactor, both the fermentation volume and the total biomass amount ([X]. V) increase with time, and its expressed according to the following expression:

$$\frac{d([X].V)}{dt} = r_X\, V = \mu.[X].V$$

$$\int_{X_0V_0}^{XV} \frac{d([X].V)}{[X].V} = \int_0^t \mu.dt \Rightarrow$$

$$[X].V = [X]_0 V_0 . e^{\mu.t} \tag{IV.36}$$

Therefore, the substrate balance equation can be written as follows:

$$Q[S]_0 - \frac{\mu.[X].\ V}{Y_{X/S}} = \frac{[S].d(V)}{dt} = Q.[S]\ \Rightarrow Q([S]_0 - [S]) - \frac{\mu.[X]_0 V_0 . e^{\mu.t}}{Y_{X/S}} = 0$$

$$\mathrm{Q} = \frac{\mu.[\mathrm{X}]_0 \mathrm{V}_0 . \mathrm{e}^{\mu.\mathrm{t}}}{\mathrm{Y}_{\mathrm{X/S}}.([\mathrm{S}]_0 - [\mathrm{S}])} \tag{IV.37}$$

$$\int_{V_0}^{V} dV = \int_0^t Q dt \Rightarrow V = V_0 + \int_0^t Q dt$$

$$V = V_0 + \frac{\mu.[\mathrm{X}]_0 \mathrm{V}_0 .}{\mathrm{Y}_{\mathrm{X/S}}.([\mathrm{S}]_0 - [\mathrm{S}])} \int_0^t \mathrm{e}^{\mu.\mathrm{t}}\ dt \tag{IV.38}$$

The combination of equations (IV.36) and (IV.38) gives the biomass concentration at time t:

$$XV = [X]_0 V_0 . e^{\mu.t} \Rightarrow [\mathrm{X}] = \frac{[X]_0 V_0 . e^{\mu.t}}{V} = \frac{[X]_0 V_0 . e^{\mu.t}}{V_0 + \frac{\mu.[\mathrm{X}]_0 \mathrm{V}_0 .}{\mathrm{Y}_{\mathrm{X/S}}.([\mathrm{S}]_0 - [\mathrm{S}])} \int_0^t \mathrm{e}^{\mu.\mathrm{t}}\ dt}$$

$$X = \frac{[X]_0 . e^{\mu.t}}{1 + \frac{\mu.[\mathrm{X}]_0 .}{\mathrm{Y}_{\mathrm{X/S}}.([\mathrm{S}]_0 - [\mathrm{S}])} \int_0^t \mathrm{e}^{\mu.\mathrm{t}}\ dt}$$

$$X = \frac{[X]_0 . e^{\mu.t}}{1 + \frac{[\mathrm{X}]_0}{\mathrm{Y}_{\mathrm{X/S}}.([\mathrm{S}]_0 - [\mathrm{S}])} (\mathrm{e}^{\mu.\mathrm{t}} - 1)} \tag{IV.39}$$

IV.6.3. Continuous reactor

a) Perfectly mixed fermenter (Chemostat):

In a continuous fermenter, the constant supply of substrate allows for sustained exponential type growth. Under steady state conditions, the concentrations of biomass, substrate, and products as well as the growth rate, remain constant.

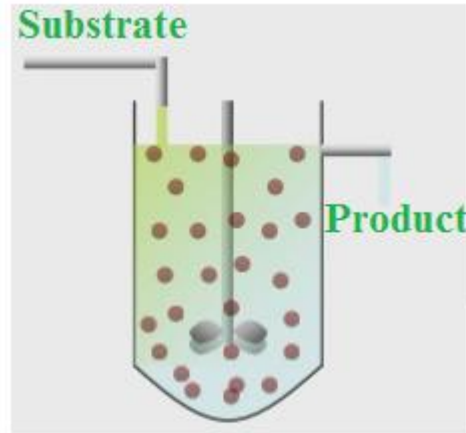


Figure IV.6: Continuous fermenter

1. *Mass balance equation with respect to the substrate S:*

$$Q_E[S]_0 - r_s V = Q_S[S] \quad (with Q_E = Q_S = Q) \qquad (IV.40)$$

$$Q([S]_0 - [S]) = r_s V$$

$$D([S]_0 - [S]) = r_s \qquad (IV.41)$$

$$r_s = \frac{r_X}{Y_{X/S}} = \frac{\mu.[X]}{Y_{X/S}} \, and \, D = \frac{Q}{V}$$

$$r_X = \frac{d[X]}{dt} = \mu.[X]$$

$Y_{X/S}$: the biomass yield per substrate consumed.

2. *Mass balance equation with respect to biomass X:*

$$Q_E[X]_0 + r_X V = Q_S[X] \qquad (IV.42)$$

$$Q([X] - [X]_0) = r_X V$$

$$D([X] - [X]_0) = r_X = \mu.[X]$$

Then

$$\tau_{Chemostat} = \frac{1}{D} = \frac{([X] - [X]_0)}{r_X} = \frac{[X]}{r_X} \quad ([X]_0 = 0) \qquad (IV.43)$$

The mean residence time at which the dilution rate equals the growth rate is $\tau = \frac{1}{D} = \frac{X}{r_X}$

3. *Mass balance equation with respect to product P:*

$$Q_E[P]_0 + r_P V = Q_S.[P] \qquad (IV.44)$$

$$D([P] - [P]_0) = rp \qquad (IV.45)$$

Note:

Equilibrium is only maintained if the dilution rate D is lower than a critical value D*. In fact, when D>D*, wash out occurs and X→0.

The critical dilution rate can be derived from an equation based on the Monod model:

$$D^* = \frac{\mu_{max}.[S]_0}{K_S + [S]_0}$$

b) Plug flow reactor (PFR)

A continuous culture can be carried out in a plug flow reactor. In this type of fermenter, the biomass and product concentrations increase from the inlet to the outlet, while the substrate concentration decreases. Moreover, the absence of axial mixing requires a continuous biomass inlet (via feed or recycling).

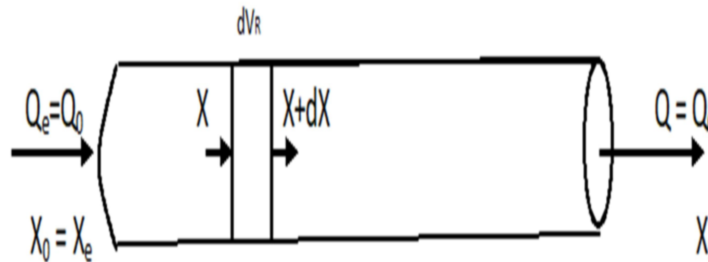


Figure IV.7: Plug flow fermenter (PFR)

The mathematical analysis of a plug flow system is similar to that of a batch system.

1) Mass balance equation with respect to biomass X:

$$Q.[X] + r_X\, dV = Q([X] + d[X]) \quad \Rightarrow \quad r_X\, dV = Q.d[X] \qquad (IV.46)$$

$$\mu_{max}.[X].dV = Q.d[X] \Rightarrow \int \frac{dV}{Q} = \frac{1}{\mu_{max}} \int_{[X]_0}^{[X]} \frac{d[X]}{[X]}$$

$$\tau_{piston} = \frac{1}{\mu_{max}} \int_{[X]_0}^{[X]} \frac{d[X]}{[X]}$$

$$\tau_{piston} = \frac{1}{\mu_{max}}.Ln\frac{[X]}{[X]_0} \qquad (IV.47)$$

2) Mass balance equation with respect to the substrate S:

$$Q.[S] + r_S\, dV = Q([S] + d[S]) \quad \Rightarrow \quad r_S\, dV = Q.d[S] \qquad (IV.48)$$

$$\int \frac{dV}{Q} = \int_{[S]_0}^{[S]} \frac{d[S]}{r_S}$$

$$\tau_{piston} = \int_{[S]_0}^{[S]} \frac{d[S]}{r_S} \qquad (IV.49)$$

3) Mass balance equation with respect to the product P:

$$Q.[P] + r_P\, dV = Q([P] + d[P]) \quad \Rightarrow \quad r_P\, dV = Q.d[P] \qquad (IV.50)$$

Application:

The antibiotic Tylosin was produced in a CSTR using *Streptomyces fradiae* in a 5-liter laboratory fermenter. For different substrate flowrates the concentrations of product and biomass were measured.

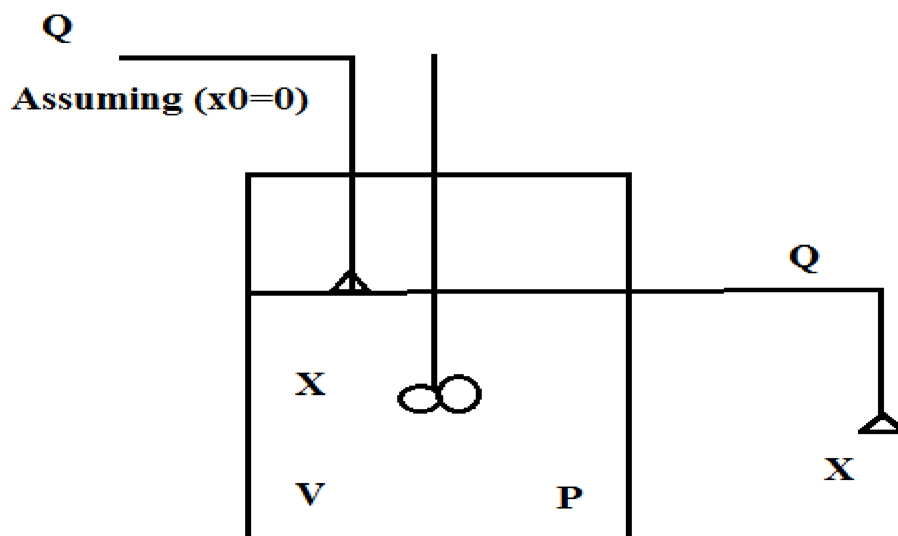
Table IV.1:

Variation of biomass and tylosin concentration with flow rate;

Low rate flow (mL/h)	**50**	**100**	**150**	**200**	**350**
Biomass concentration (g DW/L)	39	29	28	22	17
Tylosin concentration (g/L)	0.7	0.4	0.2	0.1	0.01

Determine the specific growth (**µ**) rate and the specific production rate (**q_P**)**,** using the above experimental data.

Solution



Convert flowrate to liters per hour

$$Q = \frac{Flowrate\ (\frac{mL}{h})}{1000}$$

Calculate dilution rate

$$D = \frac{Q}{V}$$

The rate of inlet is the rate at which the product species is formed by the reaction. The rate of outlet is the rate at which a species leaves the system.

Assuming that the system is operating as steady state condition, the mass balance equation is given follow:

Rate of inlet to control region = rate of outlet from control region

Mass balance of biomass X:

$$Q_E[X]_0 + r_X\, V = Q_S[X]$$

$$Q([X] - [X]_0) = r_X\, V$$

$$\boldsymbol{Q}.[\boldsymbol{X}] = \boldsymbol{V}.\boldsymbol{\mu}.[\boldsymbol{X}] \quad \text{with } X_0=0$$

Mass balance of product P(tylosin):

$$Q_E[P]_0 + r_P\, V = Q_S[P] \qquad with\text{:}\ \ [P]_0 = 0$$

$$Q.P = rp\,.V$$

$$\boldsymbol{Q}.[\boldsymbol{P}] = \boldsymbol{q_p}\,.[\boldsymbol{X}].\boldsymbol{V} \Rightarrow \boldsymbol{q_p} = \frac{\boldsymbol{Q}.[\boldsymbol{P}]}{[\boldsymbol{X}].\boldsymbol{V}}$$

At steady state → μ = D

Table IV.1:

Variation of biomass and tylosin concentration with flow rate;

Low rate flow (mL/h)	**50**	**100**	**150**	**200**	**350**
Biomass concentration (X) (g drey weight/L)	39	29	28	22	17
Tylosin concentration (g/L)	0.7	0.4	0.2	0.1	0.01
D=μ=Q/v	0.01	0.02	0.03	0.04	0.002
$\boldsymbol{q_p} = \frac{\boldsymbol{Q}.\boldsymbol{P}}{\boldsymbol{X}.\boldsymbol{V}}$	$1.8.10^{-4}$	$2.7.10^{-4}$	$2.14.10^{-4}$	$1.8.10^{-4}$	$4.12.10^{-4}$

IV.7. Submerged culture fermenters

The major types of submerged-culture bioreactor are (**Figure IV.**8) :

- Stirred tank fermenter;
- Bubble column;
- Airlift fermenter;
- Fluidized bed fermenter;
- Trickle bed fermenter.

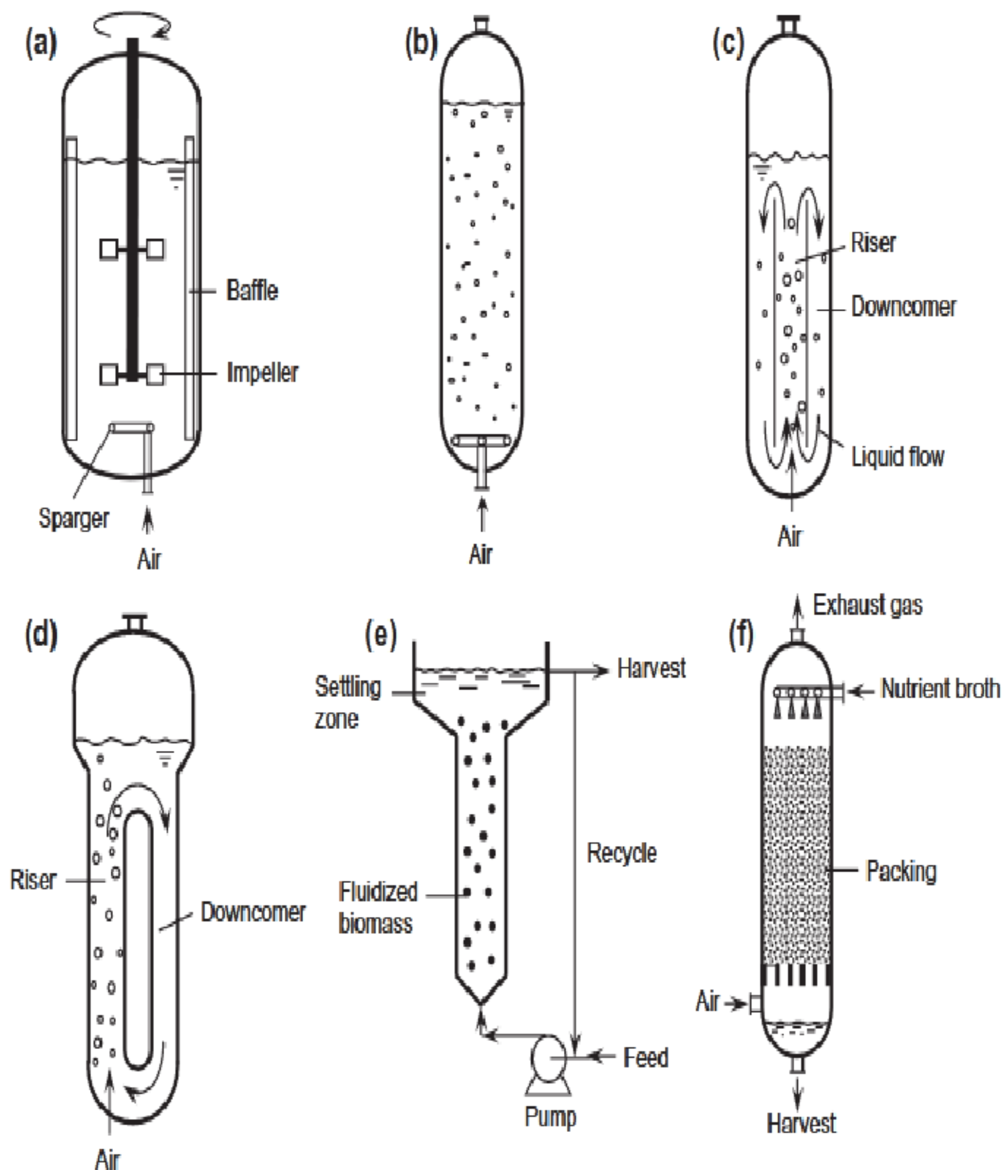


Figure IV.8: Types of submerged culture fermenter. (**A**) stirred-tank fermenter, (**B**) Bubble column, (**C**) internal-loop airlift fermenter, (**D**) External-loop airlift fermenter, (**E**) fluidized-bed fermenter, (**F**) trickle-bed fermenter.

IV.7.1. Stirred tank fermenter (see Fig. IV.8(A) and Fig IV.9).

This is a cylindrical vessel with a working height to diameter ratio (aspect ratio) of 3-4. A central shift supports three to four impellers, placed about 1 impeller-diameter apart. Various types of impeller, that direct the flow axially (parallel to the shaft) or radially (outwards from the shaft) may be used (**Figure IV.9**)

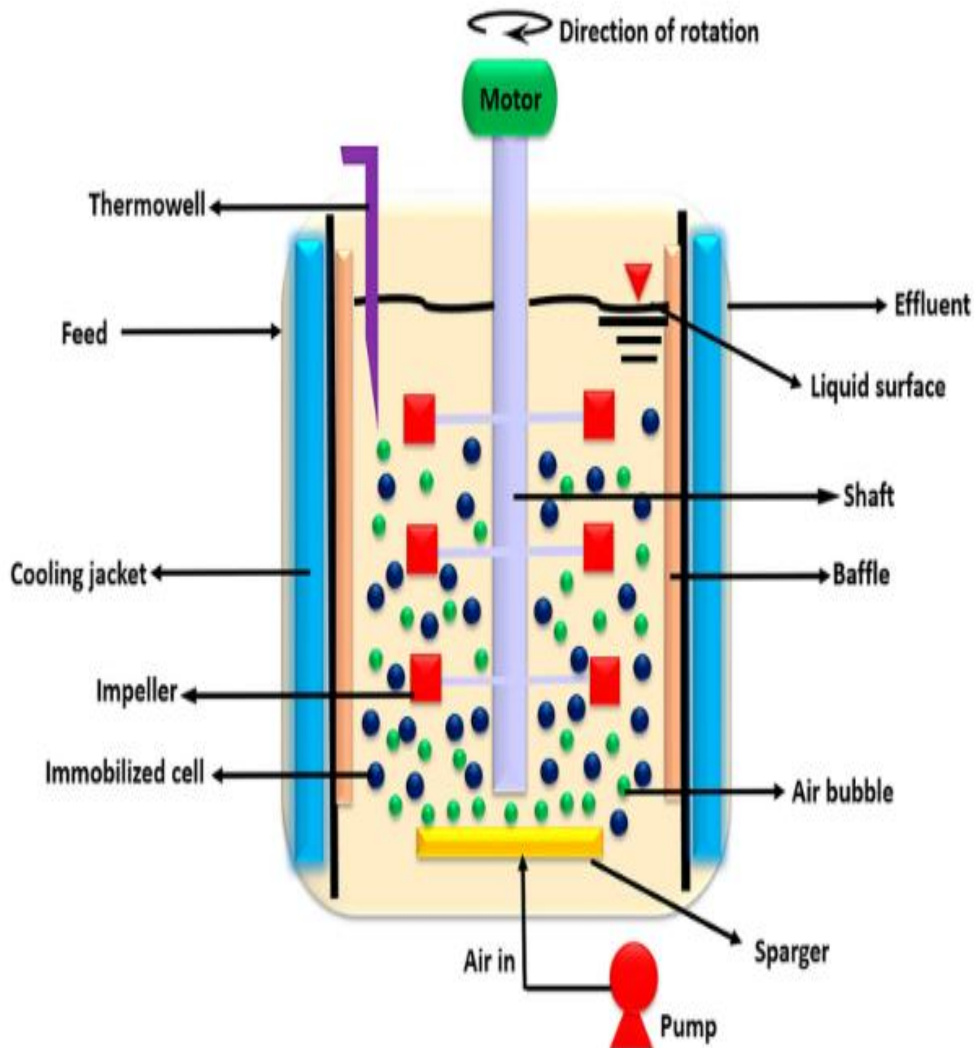


Figure IV.9: Stirred tank fermenter

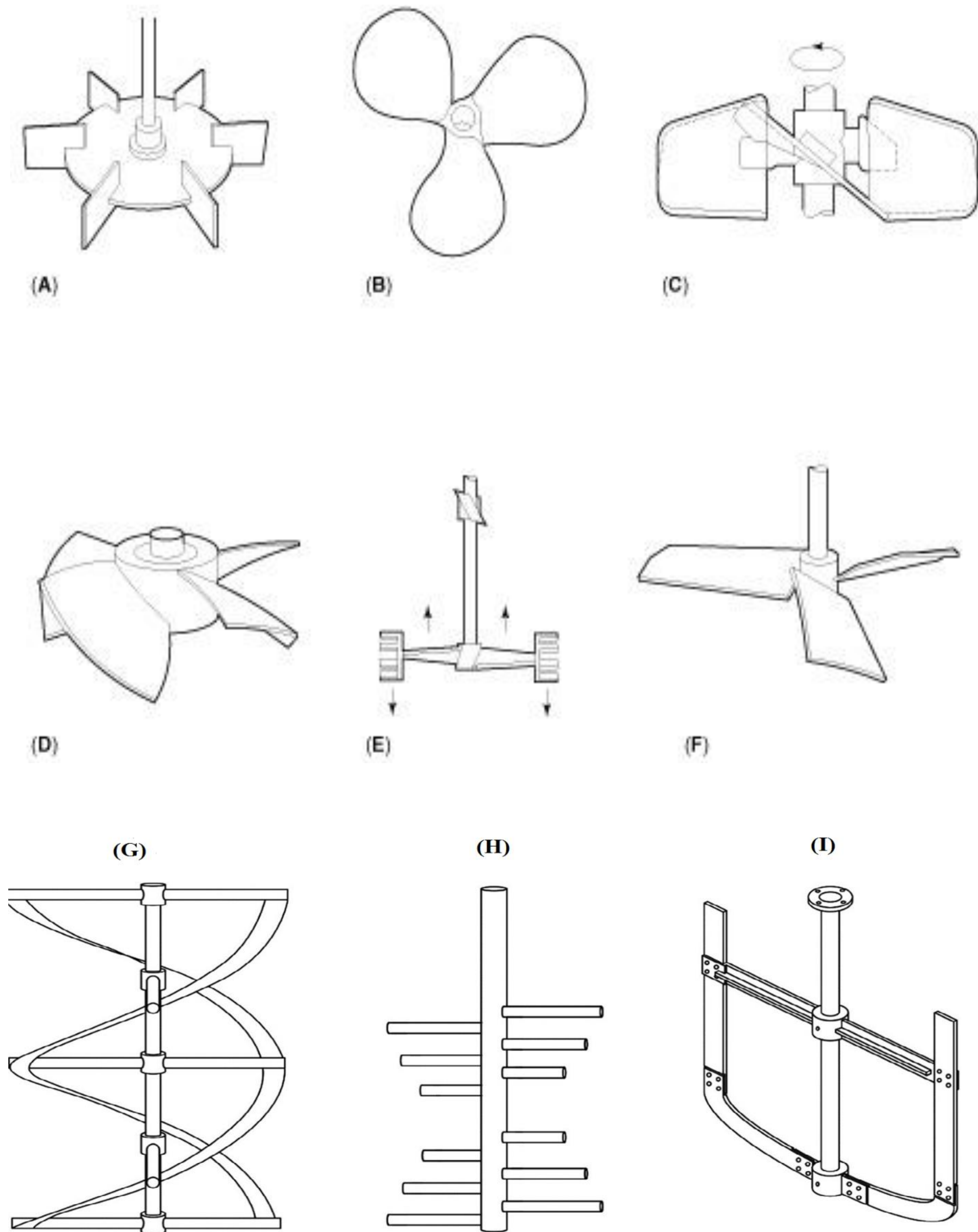


Figure IV.10: Impellers for stirred-tank fermenters. **(A)** Rushton disc turbine (radial flow), **(B)** Marine propeller (axial flow), **(C)** Lightning hydrofoil (axial flow), **(D)** prochem hydrofoil (axial flow), **(E)** intermig (axial flow), **(F)** Chemineer hydrofoil (axial flow), **(G)** Helical ribbon impeller, **(H)** Peg mixer, **(I)** Anchor impeller.

IV.7.2. Bubble column bioreactor (Fig IV.8 (B) and Fig.IV.11)

This is a vertical vessel filled with liquid where air or gas is bubbled from the bottom to mix the culture and supply oxygen to microorganisms for growth or production of biological products. Although simple, it is not widely used because of its poor performance relative to other systems. It is not suitable for very viscous broths or those containing large amounts of solids.

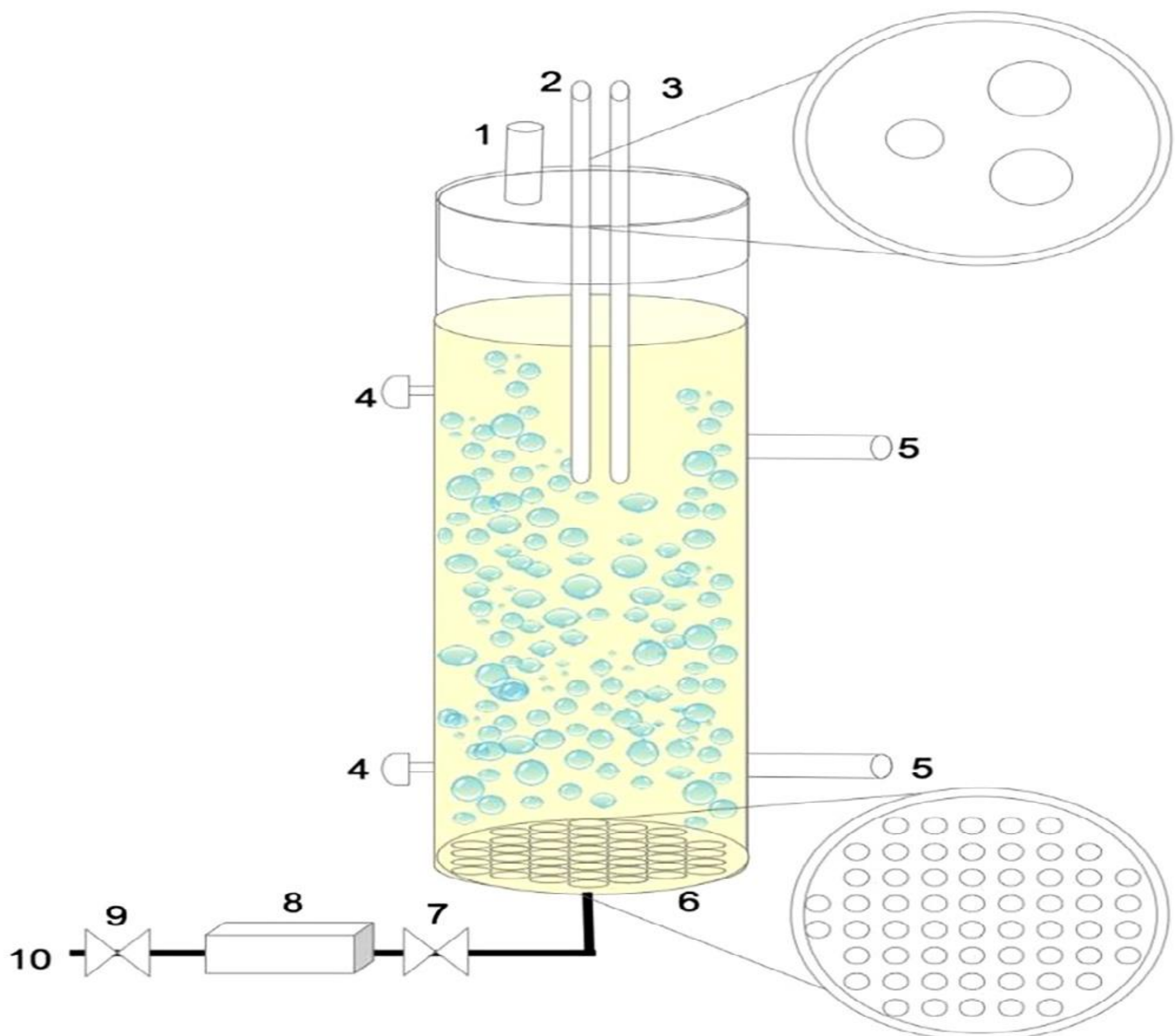


Figure IV.11: Bubble column bioreactor (1. Gas outlet, 2. pH electrode with temperature meter, 3. Oxygen probe, 4. Sample collection, 5. Translators pressure, 6. Sparger, 7. Gas filter, 8. Rotameter, 9. Pressure regulator and 10. Gas inlet).

IV.7.3. Airlift fermenters (Fig IV.8 © and (D) and Fig. IV.12).

Airlift fermenters exist in two types: internal-loop and external-loop designs.

- ***In the internal-loop design***, the aerated riser (where the liquid moves upward) and the unaerated down comer (where the liquid flows downward) are contained within the same vessel.
- ***In the external-loop design***, the riser and the down comer are separate tubes, connected near the top and the bottom.

Liquid circulates between the riser (upward flow) and the down comer (down ward flow). The working aspect ratio (hauteur/diameter) of airlift fermenters is 6 or greater. Generally, these are very capable fermenters, except for handling the most viscous broths. Their ability to suspend solids and transfer O_2 and heat is good. The hydrodynamic shear is low. The external loop design is relatively little-used in industry.

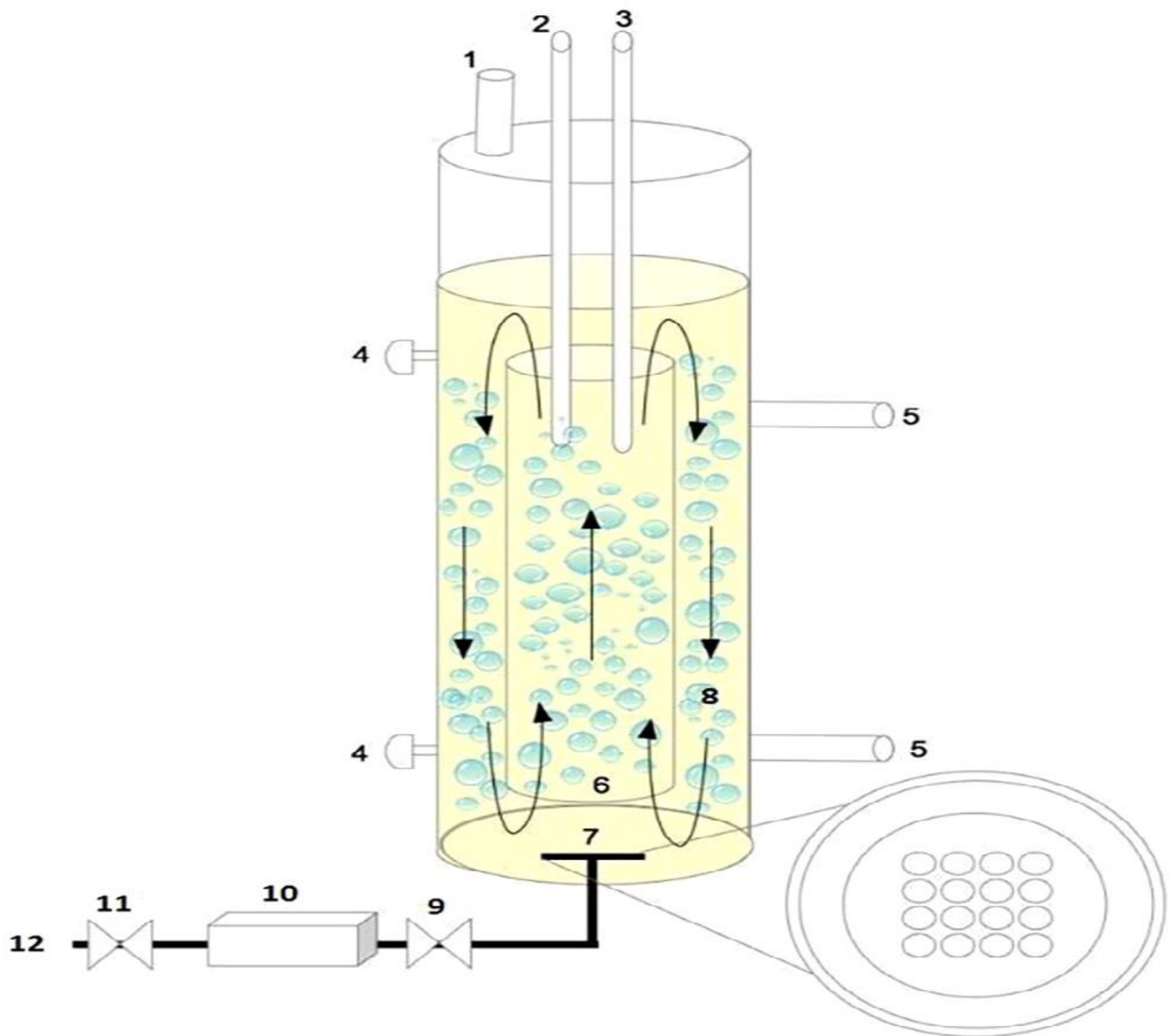


Figure IV.12: Gas-lift bioreactor (1. Gas outlet, 2. pH electrode with temperature meter, 3. Oxygen probe, 4. Sample collection, 5. Translators pressure, 6. Riser, 7. Sparger, 8. Downcomer, 9. Gas filter, 10. Rotameter, 11. Pressure regulator and 12. Gas inlet).

IV.7.4. Fluidized bed fermenter (Figure VI.8 (F))

These are similar to bubble columns, but they have an enlarged top section. Fresh or recirculated liquid is continuously pumped into the bottom of the vessel, at a velocity high enough to keep the solids in suspension.

These fermenters need an external pump. The expanded top section slows down the upward flow locally, preventing the solids from being washed out of the bioreactor.

IV.7.5. Trickle-bed fermenter (Fig. IV.8(F) and Fig IV.13)

These fermenters consist of a cylindrical vessel packed with support material (woodchips, rocks, plastic structures). The support has large open spaces that allow the flow of liquid and gas and provide surfaces for microorganisms to grow (on the solid support).

A liquid nutrient broth is sprayed onto the top of the support and trickles down the bed. Air may flow upward, countercurrent to the liquid flow.

These fermenters are used in vinegar production, as well as in other processes. They are suitable for low viscosity liquids with few suspended solids.

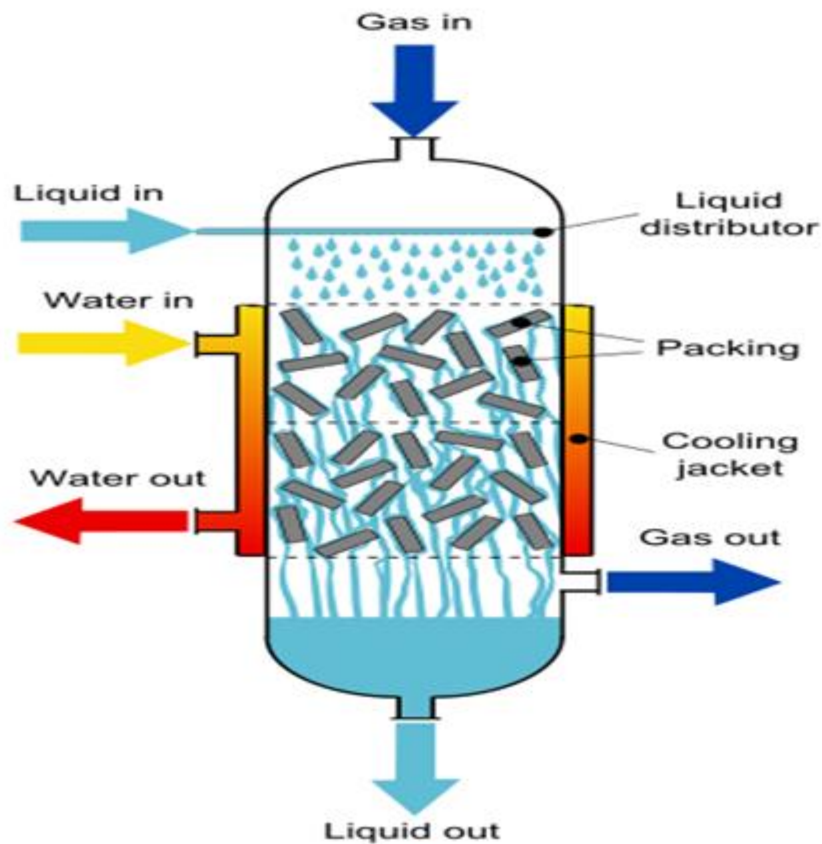


Figure IV.13: Trickle-bed fermenter

IV.8. Sterilization Methods

Autoclaving (steam under pressure)

- Small to medium volumes (laboratory or pilote scale).

- The fermenter and its accessoires are exposed to high temperature, high pressure stream (120°C for 15-20 minutes).
- Simple and effective (for small fermenters)
- Difficult for a large industrial fermenter

In situ sterilization by steam (SIP: Sterilization In Place)

- Large industrial vessels
- Saturated steam is circulated through the fermenter, pipes and heat exchangers.
- Typical temperature: 120- 130°C, duration;15-60 minutes, depending on volume and configuration.
- No disassembly required, ensures of both vessel and piping.

Chemical Sterilization

- For heat sensitive components (pumps, membranes, filters).
- Uses disinfectant or sterilizing solutions, such as: (ethanol, sodium hypochlorite, hydrogen peroxide), often followed by a rinse with sterile water.

References

[1] J. Villermaux « Génie de la réaction chimique » TEC et DOC – Lavoisier, 1993

[2] D. Schweich « Génie de la réaction chimique » TEC et DOC – Lavoisier, 2001

[3] O. Levenspiel « Chemical reaction engineering » Third Edition John Wiley & Sons, 1999.

[4] Riba, J.-P. (s.d.). Réacteurs enzymatiques et fermenteurs. Techniques de l'Ingénieur, Ref. J2 670. https://www.techniques-ingenieur.fr

[5] J. P. Guiraud « Microbiologie alimentaire » Dunod, Paris, 2003.

[6] G. Coutouly « Génie enzymatique » Masson et Doin, Paris, 1991

[7] G. Charles, Jr. Hill, Thatcher W.Root « Introduction to chemical engineering kinetics & reactor design » John Wily & sons, 2014.

[8] Y. Chisti, «Fermentation technology». In: industrial Biotechnologiy: Sustainable Growth and Economic Success », Soetaert, W, Vandamme, E.J, eds, Wiley-VCH, New York, 2010, 149-171.

[9] Marcela Sofía Pino, Rosa M. Rodríguez-Jasso, Michele Michelin, Adriana C. Flores-Gallegos, Ricardo Morales-Rodriguez, José A. Teixeira, Héctor A. Ruiz, « Bioreactor design for enzymatic hydrolysis of biomass under the biorefinery Concept », Chemical Engineering Journal, 347, 2018, 119-136.