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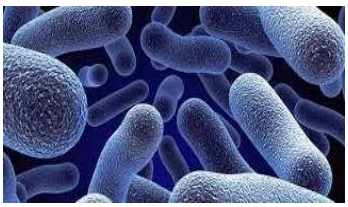
Ministry of Higher Education and Scientific Research
Djillali Liabes University of Sidi Bel Abbes
Faculty of Technology
Department of Energy and Process Engineering

BIOREACTORS

Master II: Environmental Process Engineering



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Associate professor A

Bioreactors

Preface

This handout serves as a supplementary resource for the 'Bioreactors' module and is intended for master's students in environmental process engineering, as well as all specialties related to reactor calculations. The emphasis is on the calculation and dimensioning of biological reactors. The sources for most of the materials presented are provided in the reference section for the benefit of students who wish to delve deeper.

This document is composed of four main chapters following the framework prescribed by the Ministry of Higher Education and Scientific Research, which are outlined as follows:

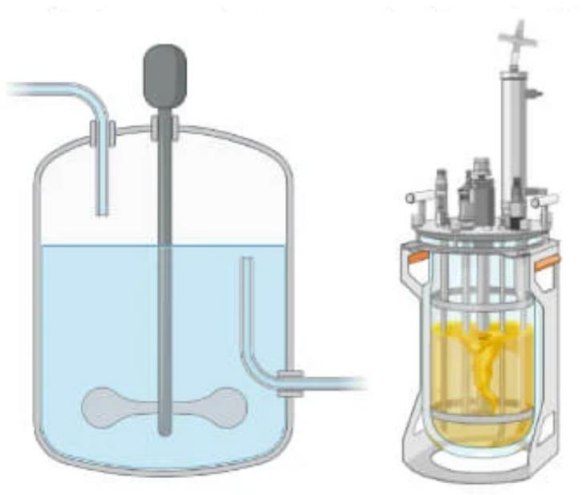
Chapter 1: Reaction Rate Modeling in Biological Systems - This chapter covers Microbial Kinetics: Monod Model, Enzyme Kinetics, and Inhibition of Enzymatic Reactions.

Chapter 2: Design and Analysis of Bioreactors - This chapter provides detailed information on Types of Bioreactors, Basic Concepts, Batch Bioreactors, Continuous Stirred-Tank Reactors, and Piston Bioreactors, followed by a comparison of batch and continuous bioreactors.

Chapter 3: Sterilization - This chapter discusses both physical and chemical sterilization methods.

The final chapter is dedicated to presenting the principle of mass transfer for aeration and agitation.

In conclusion, through this humble document, I hope that students from various disciplines will gain a deep understanding of the calculation of biological reactors."



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Summary

Introduction

Microbiology is a scientific discipline dedicated to the study of microscopic organisms, including microbes and protests. Due to their extremely small size, these organisms elude detection by the naked eye, necessitating the use of microscopes and other specialized equipment to observe and understand them.

The diversity of microorganisms is vast, encompassing a wide range of organisms, such as bacteria, viruses, fungi, archaea, and protests. Each of these groups possesses distinct characteristics and plays a crucial role in various fields, including ecosystems, human health, the food industry, biotechnology, and many other sectors.

Microbiology is a highly significant field within science because it enables us to grasp the workings of these imperceptible organisms and their influence on our world. Furthermore, it plays a pivotal role in various sectors, including medicine (studying infectious diseases), agriculture (employing microorganisms for food production), industry (manufacturing chemicals and medicines), and environmental protection (facilitating waste decomposition and nutrient cycling).

- Microorganisms are capable of carrying out a wide variety of biochemical reactions resulting in the production of biomass (cellular bodies) and in the degradation, transformation, or production of organic or mineral substances.

- For their life (maintenance or upkeep), for their growth (development and multiplication), and for the expression of their properties (mobility, luminescence, etc.), microorganisms require energy and nutrients.

  The necessary energy is derived from the environment, either directly in the form of light energy or indirectly in the form of chemical energy through the oxidation of organic or mineral substances.

Biochemistry allows us to understand the "chemical processes" by which living organisms, from the simplest like bacteria and viruses to the most complex like insects, mammals, and notably, humans. It serves as the junction where the disciplines of chemistry and biology converge.The primary aim of modern biochemistry is to achieve a comprehensive understanding of how biomolecules and their interactions, when integrated with molecular-level data, give rise to the structures and biological processes observed within cells, ultimately leading to a broader comprehension of organisms as a whole.

Molecular biology, also known as biomolecular biology, is a scientific discipline at the intersection of genetics, biochemistry, and physics, with its focus on understanding the mechanisms of cellular function at the molecular level.

According to Michel Morang, molecular biology is 'the collection of techniques and discoveries that have enabled the molecular analysis of the most intimate processes of life, those that ensure its continuity and reproduction.' Virtually all biomolecules capable of catalyzing chemical reactions within cells are enzymes.

- Enzymes accelerate reactions by millions of times compared to their absence. An enzyme works by lowering the activation energy of a chemical reaction, thereby increasing the reaction rate. The enzyme itself remains unchanged during the reaction. The initial molecules are the substrates, and the molecules formed from these substrates are the products of the reaction. Nearly all metabolic processes within the cell require enzymes to occur at a sufficient rate to sustain life. Enzymes catalyze over 5000 different chemical reactions.
- Metabolic processes involve a sequence of physical, chemical, and biological transformations that substances experience when they enter or are created within living organisms.

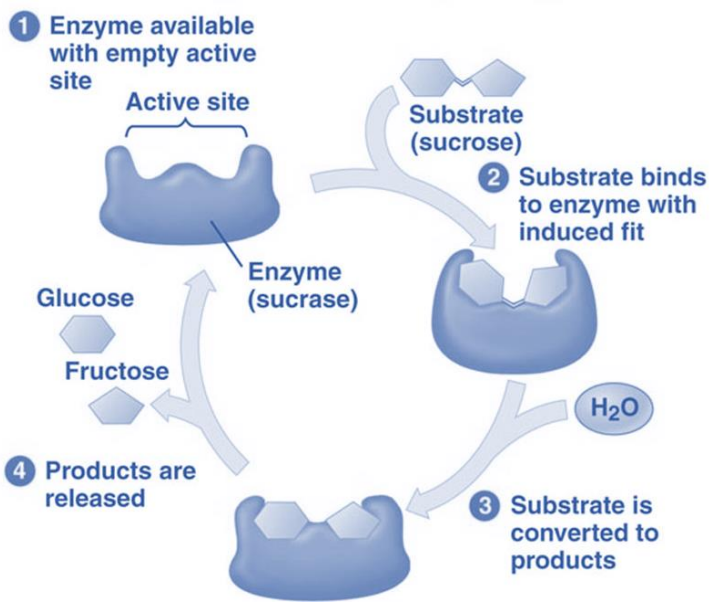
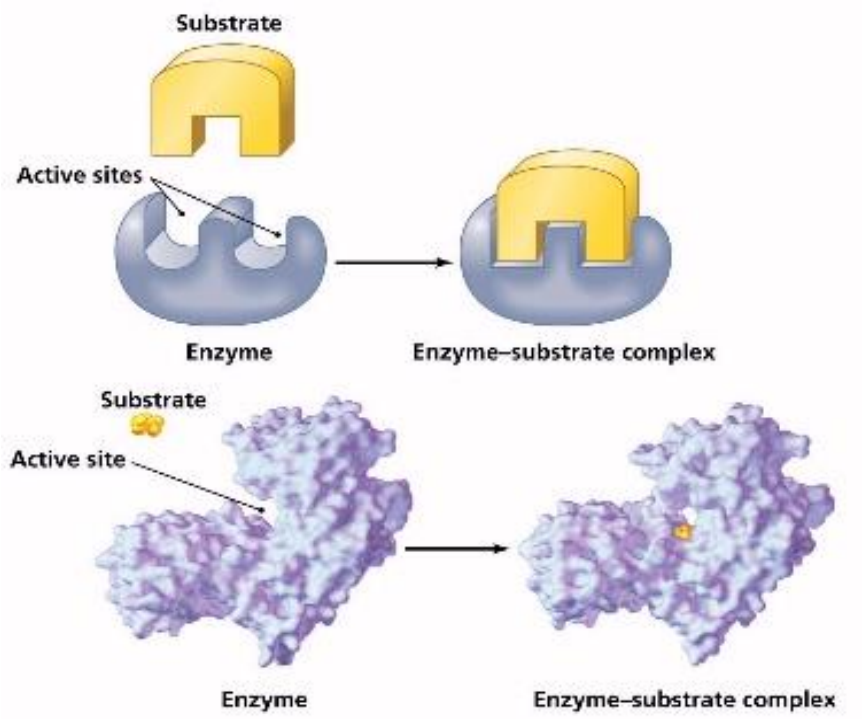


Figure 1: Enzyme life cycle

Chapter I

Modeling Reaction Rates in the Biological System



Chapter: Modeling Reaction Rates in the Biological System

Biological reactors are typically constructed following the same models as chemical reactors; these are vessels or chambers made of glass (for laboratory models) or stainless steel. They are designed to carry out enzymatic reactions (enzymatic reactors) or cell-based reactions (fermenters or cell culture reactors). Biological reactors are also referred to as bioreactors.

The bioreactor allows for the control of culture conditions (temperature, pH, aeration, etc.), and as a result, it enables the collection of more reliable information. Industrial bioreactors facilitate the production of various products, including beer, yogurt, vaccines, antibiotics, antibodies, vitamins, and organic acids. A fermenter is typically built based on the model of a bioreactor, with the addition of an aeration system.

However, the term "fermenter," which is sometimes used without any distinction from "bioreactor," helps differentiate the type of culture (bacteria or yeast for fermenter and animal cells for bioreactor). Bioreactors are designed in such a way that they must fulfill four major functions:

- Good mass transfer
- Effective heat transfer
- Maintenance of sterility
- Monitoring of parameters and control management"

1. Enzyme

1.1. Role of enzymes

Enzymes are biomolecules, which means they are molecules synthesized by living organisms. They play a role in the biochemical reactions that occur inside a living organism, thus in metabolism. They accelerate these chemical reactions, acting as biocatalysts.

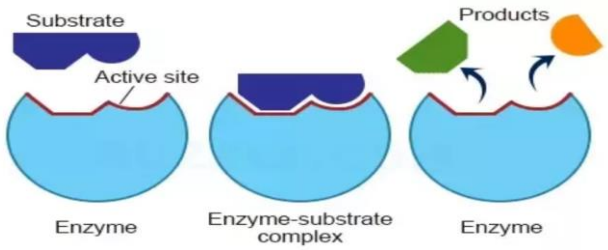


Figure 2 : Enzyme-substrate complex

Metabolism is the set of biochemical reactions that take place inside a living organism to allow it to stay alive, reproduce, develop, and respond to stimuli from its environment. Two types of biochemical reactions are distinguished:

a. Breakdown (catabolism)

b. Assembly (anabolism) of molecules.

Enzymes are involved in these biochemical reactions. Enzymes are highly efficient catalysts. The duration of the reaction is 0.001 seconds.

1.2.Mode of Action of Enzymes

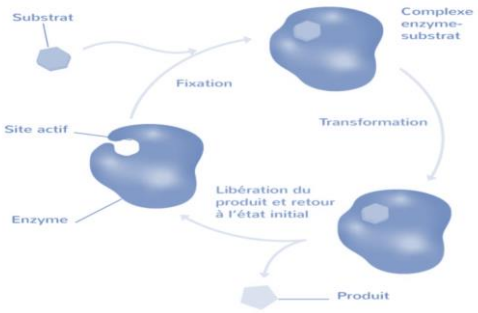
An enzyme catalyzes the conversion of a substrate (S) into a product (P).

Catalysis: This refers to the acceleration of a chemical reaction.

Substrate: It denotes a molecule that undergoes a biochemical reaction catalyzed by an enzyme.

Product: It is the molecule resulting from a biochemical reaction catalyzed by an enzyme.

The enzyme binds the substrates at a specific site called the active site. Once the enzyme-substrate complex is formed, the chemical reaction takes place, and then the enzyme releases one or more products. Since the enzyme is not altered by the reaction, it is immediately available to bind another substrate.
An enzyme can transform a thousand substrate molecules per second.



1.3.Activation energy: catalysis

An enzyme is a catalyst that increases the speed of a reaction by lowering the activation energy required for a reaction to occur.

The presence of enzymes means that more chemical reactions can occur in a given period of time compared to if they were not present, thus increasing the reaction rate. You

can see in Figure 3 below that the energy input for a reaction is much higher without enzymes than with the enzyme. Imagine this single reaction happening thousands of times, and you'll understand why it is more energetically efficient to use an enzyme in biological reactions. Many essential reactions occurring in our cells are simply too slow to occur on their own. For example, if the enzymes involved in respiration did not function properly, we would not be able to release enough energy in our cells to survive. ***Without enzymes, we would not survive!***

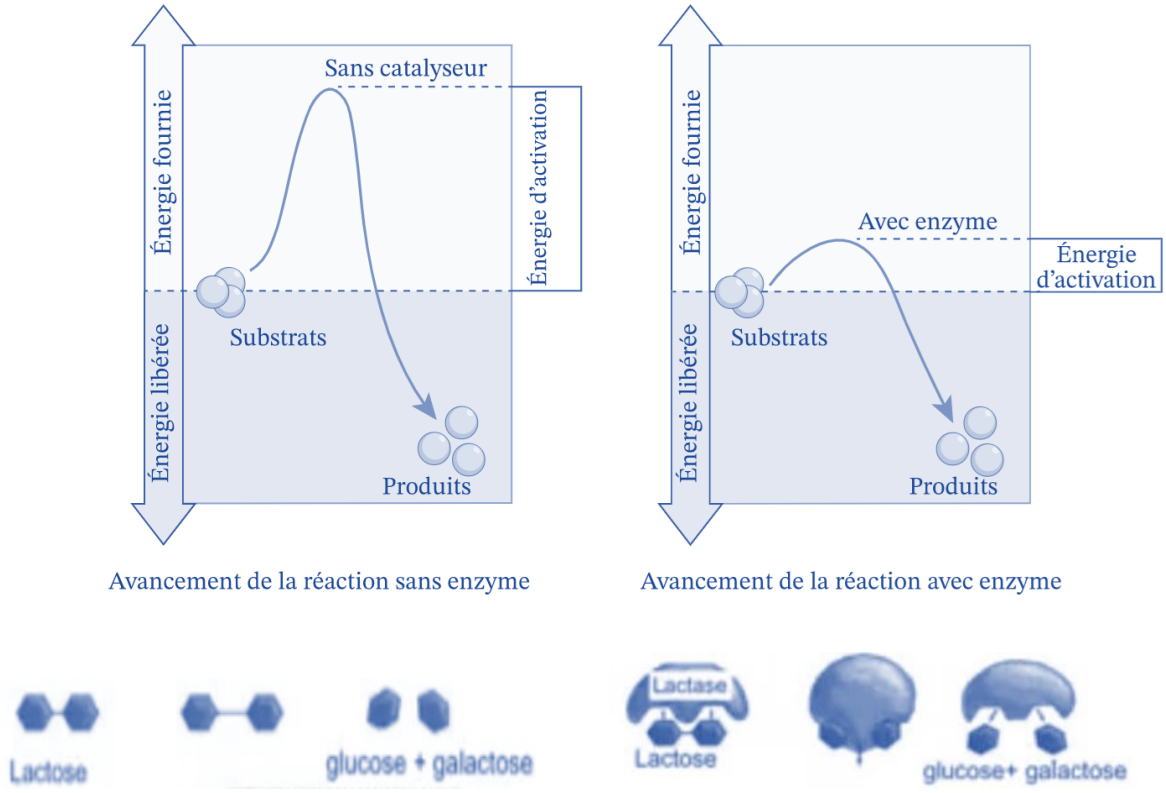


Figure 3: Two diagrams showing the activation energy (the energy required for a reaction to occur) with and without an enzyme.

- With the enzyme, the activation energy is much lower, allowing the reaction to occur more quickly.

1.4.Enzymatic structure

Enzymes are proteins comprised of amino acids linked together in one or more polypeptide chains. This sequence of amino acids in a polypeptide chain is called the primary structure. The part of the enzyme where the substrate binds is called the active site since that's where the catalytic "action" happens).

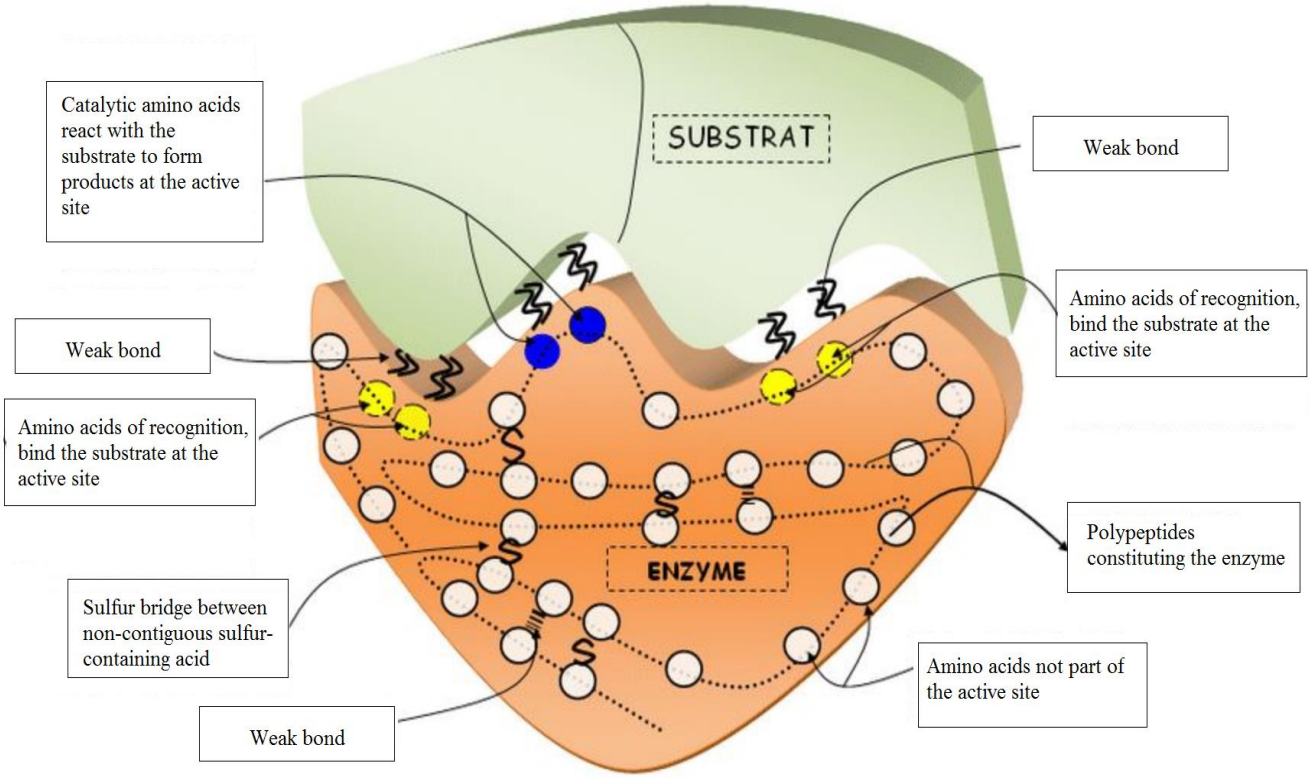


Figure 4: Enzymatic structure

1.5.Distinctive characteristics of enzymes

Each enzyme exhibits a dual specificity: substrate specificity and reaction specificity it catalyzes.

Substrate Specificity: Enzymes vary in their degrees of specificity. Some enzymes exhibit absolute specificity, transforming a single substrate into a single product (e.g., glucokinase phosphorylates only glucose). Others have broader specificity, catalyzing a specific reaction but with a class of substrates (e.g., hexokinase phosphorylates various hexoses, including glucose).

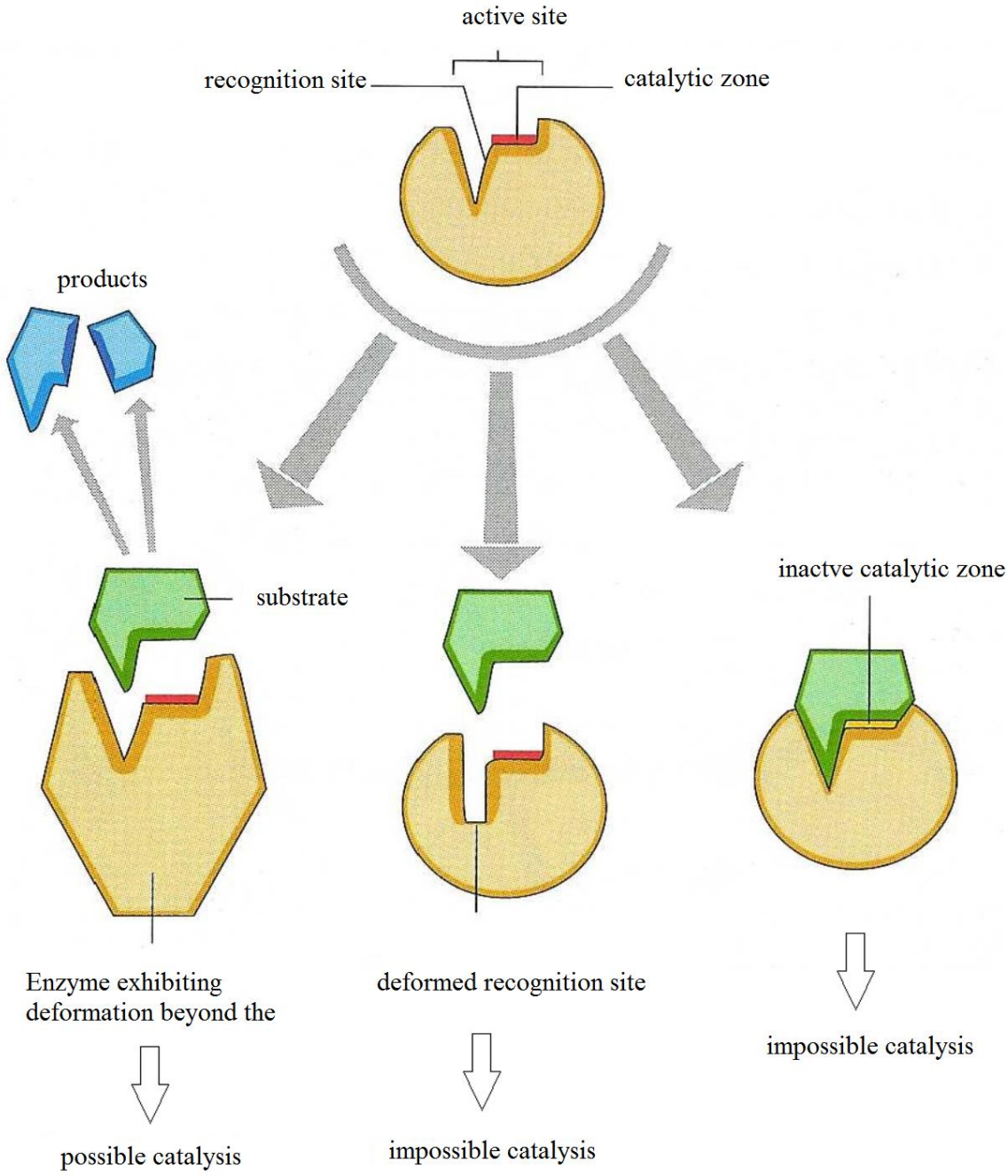


Figure 5: Enzyme specify

Reaction Specificity: For a given substrate, enzyme specificity remains constant. An enzyme catalyzes only one type of reaction among all possible reactions. For example, glucose can be phosphorylated to glucose 6-phosphate (by glucokinase) or isomerized to mannose (by glucose epimerase). Glucokinase and glucose isomerase share substrate specificity but possess different reaction specificities.

Enzymatic Specificity and Active Site: An enzyme's dual specificity is explained by the spatial configuration of its active site.

- **The active site** is a crevice lined with amino acids that are distant in primary structure but brought close together through the folding of the enzyme into its tertiary structure, forming the active site. Some amino acids are involved in substrate binding (substrate recognition site), while others participate in catalysis (catalytic site).
- **Substrate Recognition Site**: Determines substrate specificity and is composed of certain amino acids that complementarily associate with the substrate, facilitated by weak interactions (ionic bonds, hydrogen bonds, hydrophobic interactions).
- **Catalytic Site:** Determines reaction specificity and contains amino acids directly involved in catalysis (substrate-to-product transformation). In most cases, these amino acids possess ionic or reactive side chains (His, Arg, Lys, Asp, Glu, Cys, Ser, Tyr)."

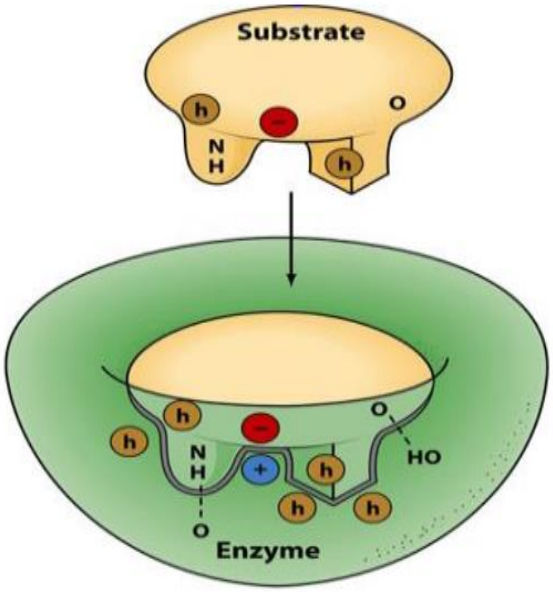


Figure 6: Geometric and electronic complementarity between enzyme and substrate

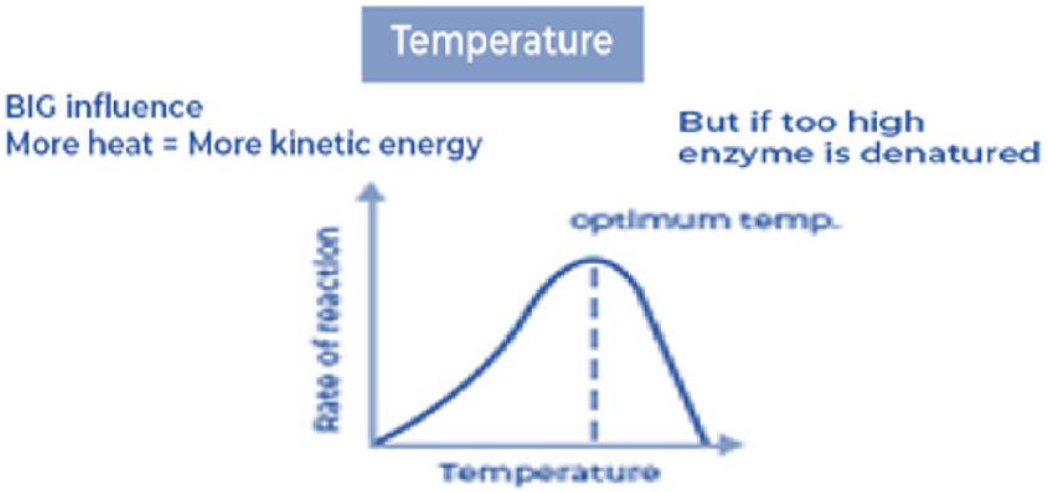
Hydrophobic groups are represented by an 'h' in a circle, and the dashed lines represent hydrogen bonds.

1.6.Enzymatic activity

Enzymatic activity is the amount of substrate converted by the enzyme in moles per unit of time. It measures the quantity of active enzyme present in a mixture at a given moment. The factors that affect the activity of an enzyme include temperature, pH, substrate and enzyme concentrations, and the presence of inhibitors.

1.6.1. Temperature

- The kinetic energy of molecules also increases with increasing temperature. This means that there are more random collisions between molecules per unit of time in a fluid.
- Because enzymes catalyze reactions by randomly colliding with Substrate molecules, increasing the temperature speeds up the reaction, resulting in more product formation.
- As the temperature rises, more bonds, particularly the weaker Hydrogen and Ionic bonds, will break due to strain. When bonds within the enzyme are broken, the Active Site changes shape.
- However, increasing the temperature increases the Vibrational Energy of molecules, specifically enzyme molecules, putting strain on the bonds that hold them together.
- As temperature rises, the shapes of more enzyme molecules' Active Sites become less complementary to the shape of their Substrate, and more enzymes are denatured. This reduces the rate of reaction.
- Because the Active Site is no longer complementary to the shape of the Substrate, it is less likely to catalyze the reaction. The enzyme will eventually become denatured and cease to function.
- In summary, as temperature rises, the rate of reaction rises initially due to increased Kinetic Energy. However, the effect of bond breaking will increase over time, and the rate of reaction will begin to slow.

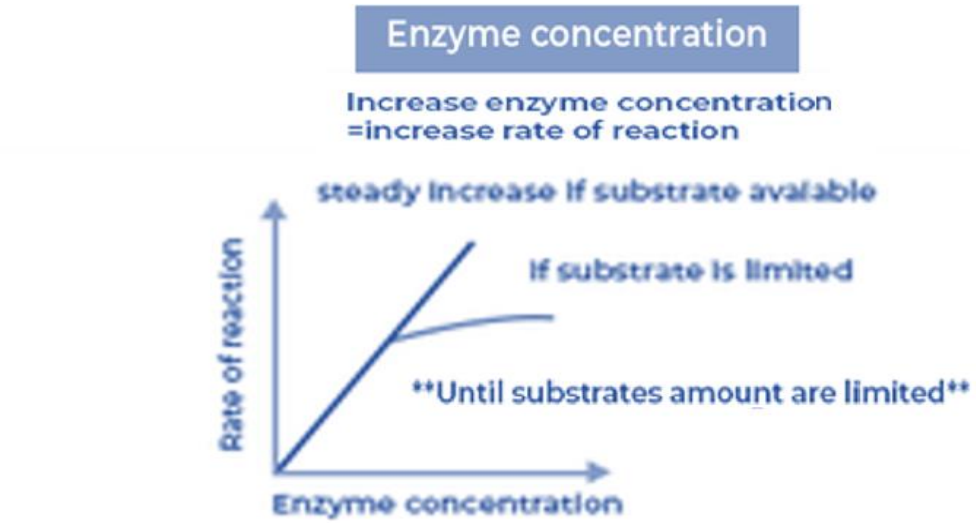


1.6.2. Concentration

- The rate of an enzyme-catalyzed reaction is affected by changing the concentrations of the enzyme and the substrate. Controlling these factors in a cell is one way for an organism to regulate enzyme activity and thus metabolism.
- Changing the concentration of a substance affects the rate of reaction only if it is the limiting factor: that is, it is the factor that prevents a reaction from proceeding at a faster rate.
- If it is the limiting factor, increasing concentration will increase the rate of reaction up to a point where it will no longer affect the rate of reaction. This is because it will no longer be the limiting factor and will be replaced by another factor limiting the maximum rate of reaction.
- As a reaction progresses, the rate of reaction decreases as the Substrate is depleted. In an experimental situation, the highest rate of reaction, known as the Initial Reaction Rate, is the maximum reaction rate for an enzyme.

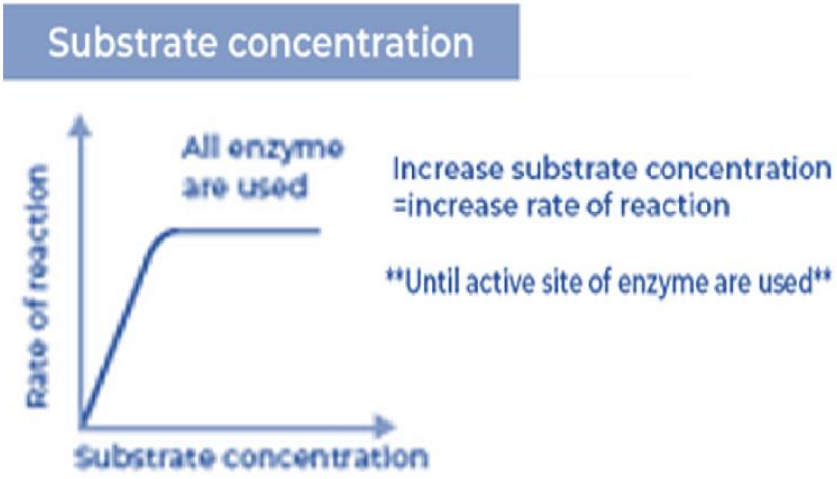
a. Enzyme Concentration

- Transient bonds formed between enzymes and their substrates catalyze reactions by lowering the activation energy and stabilizing the transition state.
- Given an abundance of substrates and necessary cofactors, enzymatic reactions with higher enzyme concentrations will reach equilibrium before those with lower enzyme concentrations.
- Simply put, a higher enzyme concentration means that there are more enzyme molecules available to process the substrate. Because of the high levels of the enzyme-substrate complex, the reaction has a faster initial catalytic rate, which gives it a head start in the shift toward reactant-product equilibrium.



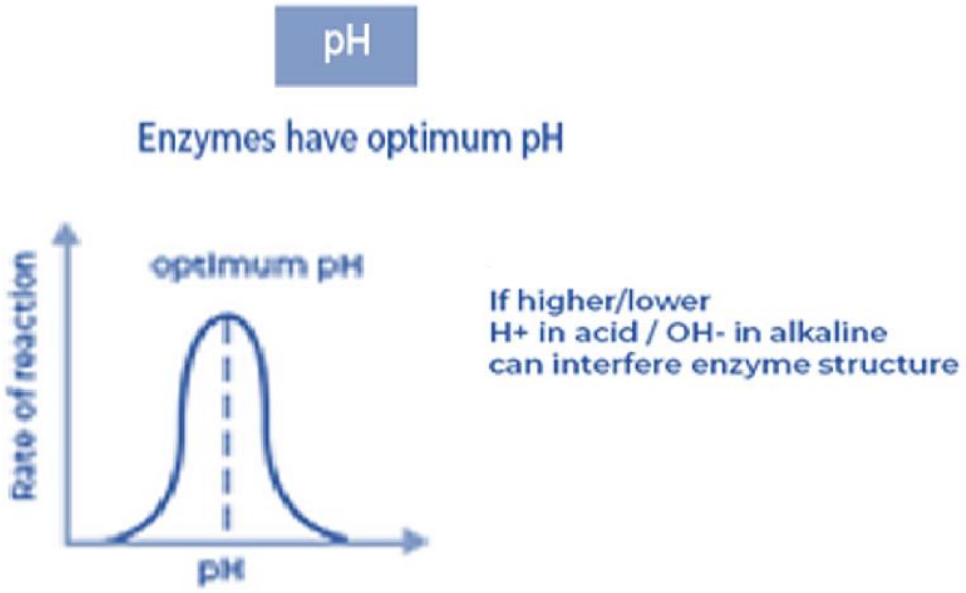
b. Substrate Concentration

- When a geometrically and electronically complementary substrate can access the enzyme's catalytic or active site, catalytic activity occurs. The active residues transiently bond with the substrate, catalyzing the substrate's transformation into a product.
- As a result, the greater the number of substrate-occupied active sites, the greater the catalytic activity and the faster the shift toward enzyme-product equilibrium.
- The majority of enzymes follows the Michaelis-Menten kinetics, which describes the relationship between enzyme activity and substrate concentration in two stages. The relationship between the two is initially linear and plateaus as the number of unbound active sites decreases.



1.6.3. pH Value

- Proteins, like enzymes, contain electrical charges due to the sequence of their amino acid residues because they are made up of a chain of amino acids.
- The majority of the amino acids in the chain serve as the foundation for intramolecular interactions that give the enzyme its three-dimensional structure. Few others serve as functional residues at the active site of the enzyme.
- Overall, the amino acids determine substrate specificity and limit enzyme activity to a narrow pH range. Most enzymes work best in slightly acidic or basic environments.
- However, because some enzymes are native to extremely acidic or basic environments, they are most active in these pH ranges.



1.6.4. Inhibitor or Effector

- Many enzymes rely on non-substrate and non-enzyme molecules to regulate or initiate catalysis. Certain enzymes, for example, rely on metal ions or cofactors to establish catalytic activity.
- Many enzymes, such as allosteric enzymes, rely on effectors to activate their catalytic activities and promote or inhibit their subsequent binding to substrates.
- Similarly, inhibitors may bind to the enzyme or its substrate in order to inhibit ongoing enzymatic activity and prevent subsequent catalytic events.

- When inhibitors form strong bonds to the enzyme's functional group, the effect on enzyme activity is irreversible, rendering the enzyme permanently inactive.
- Reversible inhibitors, as opposed to irreversible inhibitors, only render enzymes inactive when bound to the enzyme.
- Competitive inhibitors compete with substrates for binding to the enzyme functional group residues at the catalytic sites. Other types of inhibitors bind to the non-substrate binding allosteric site rather than the catalytic site.
- It is non-competitive if an inhibitor binds to the enzyme concurrently with the enzyme-substrate binding. An inhibitor is uncompetitive if it only binds to a substrate-occupied enzyme**.**

2. Modeling Reaction Rates in the Biological System

Due to the lack of satisfactory theories to explain biological systems, biologists typically rely on modeling tools and simulation to attempt to understand the behavior of these systems. One study considers biological systems as 'complex systems characterized by non-linearities, i.e., emergence, which also means 'the whole is not equal to the sum of its parts.' The overall behavior of a biological system is an emergent behavior arising from interactions among many local components (such as DNA, proteins, cells)… Each component has its simple behavioral rules, while a group of components can exhibit complex emergent behavior (i.e., adaptability, evolution, security, self-maintenance). The biological system is inherently distributed without a central controlling element."

"This summary effectively encapsulates the challenges that modeling and simulation tools face when dealing with biological phenomena and systems: non-linearities, openness, understanding of self-organizing and emergent phenomena, etc.

The modeler is a specialist in the strategy of constructing and using models: they are proficient in a wide variety of techniques and methods; they draw inspiration from the biological problem to propose a model (rather than the other way around); they engage in understanding the biological aspects of what they model

The modeler creates models by proposing them. A model is a symbolic representation of certain aspects of a real-world phenomenon. It is not an end in itself but rather a tool in the modeler's toolkit. It is closely tied to experience and/or observation.

An effective model must, above all, be operational, meaning it should address the initial objectives, be interpretable in biological terms, and be translatable into simple terms accessible to everyone.

In this chapter, we utilize two widely adopted kinetic models for catalytic microorganisms: the Monod model and the Michaelis-Menten model. (More models are descripted in ***annex 1***)

2.1.Microbial Kinetics: Monod Model

A substrate is considered limiting if there is a cause-and-effect relationship between the depletion of this substrate and the cessation of growth. It is not the only possible cause of the cessation of microbial growth: other factors may include the accumulation of toxins produced by individuals, variations in pH induced by a fermentation product (such as lactic acid or acetic acid), or the depletion of oxygen.

This fundamental concept, introduced early by MONOD (1935, 1936), is a distinguishing characteristic of the model that sets it apart from classical growth models.

2.1.1. The experimental context

2.1.1.1 Growth phenomenon

The model proposed by MONOD (1941) is used to account for the growth of microorganisms. Originally, the goal was to model this growth in a non-renewed medium, which in laboratory jargon corresponds to the analysis of 'batch cultures,' a term that is more elegantly rendered by Quebecois as 'cuvée cultures' (e.g., MAYER et al. 1982)."

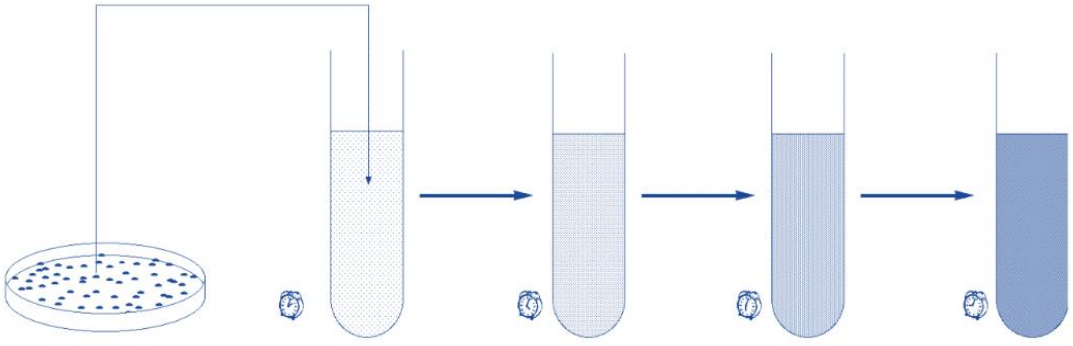


Figure 7: microorganism growth – MONOD experiment

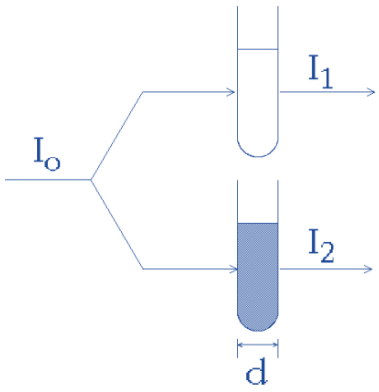
A typical experiment, schematically represented above, involves inoculating a culture medium by diluting an isolated bacterial colony on an agar medium (Petri dish). Over time, turbidity appears in the medium, followed by increasingly pronounced opalescence, a characteristic sign of population growth. Due to its amplifying effect, the growth of microorganisms is a ubiquitous technique in microbiology (MONOD 1949).

2.1.1.2 Measuring biomass

The measurement of the opacity of the culture medium is a highly regarded technique for measuring biomass, which is the dry bacterial mass present per unit volume (ML-3). It is indeed possible to use a law, similar to the Beer-Lambert law, expressing the proportionality between the bacterial contribution to the absorbance of the medium A and the biomass B present in solution.

$$A = \text{Log}\frac{I_o}{I_2} - \text{Log}\frac{I_o}{I_1} = \text{Log}\frac{I_1}{I_2} = \alpha\ d\ B$$

Where Io represents the intensity of the incident ray, I1 is the intensity of the ray transmitted in the absence of biomass, I2 is the intensity of the ray transmitted in the presence of biomass, d is the optical path length, and a is a proportionality coefficient.



Absorbance is conventionally reported with an optical path length of 1 cm to define the optical density (OD) of the medium:

$$DO = \frac{1}{d}\ \text{Log}\frac{I_1}{I_2} = \alpha\ B$$

This empirical relationship has been experimentally verified by numerous authors (MONOD 1941, TOENNIES and GALLANT 1949, SCHAECHTER et al. 1958, LUEDEKING et PIRET 1959, SPAUN 1962, DEAN et ROGERS 1967, KOCH 1970, GRIFFITH et MELVILLE 1974).

- Several mathematical models have been proposed to account for this growth curve.
- Monod's model is the oldest, the most well-known, and still the most widely used. It is an empirical model that effectively captures phases 2 and 3, closely related to the Michaelis-Menten law for enzymatic reactions. It is expressed as follows:

$$\frac{dX}{dt} = r_{max} \quad \text{avec :} \quad r = r_{max}\left(\frac{[S]}{K_S + [S]}\right)$$

K_S signifies a threshold concentration below which the growth rate becomes highly dependent on substrate concentration. This constant corresponds to the value of S, which is generally small but often higher than the values required by discharge standards.

$$r = \frac{r_{max}}{2} \cdot K_S$$

21.1.3. Evolution of a bacterial culture

A bacterial culture grows until the depletion of the nutrient medium. Figure 1, established under constant conditions (temperature, pH, etc.), represents the variation in the concentration of the bacterial culture (biomass: X) and that of the substrate (S) on which it grows over time in a batch reactor. Several phases follow one another:

- ***Latent phase (Phase 1)***

During this adaptation phase, the cell synthesizes the enzymes necessary for metabolizing the substrate, especially. This phase is important when the water is not initially seeded with adapted microorganisms. During this phase, there is no cell reproduction.

- ***Exponential growth phase (Phase 2)***

During this phase, the growth rate, dX/dt, increases proportionally to X: $\frac{dX}{dt} \cdot \frac{1}{X} = r_m$

with r_{max} being the maximum growth rate (typical of the culture and the substrate), maintained as long as there is sufficient substrate.

- ***Slowdown Phase (Phase 3)***

This phase corresponds to the depletion of the culture medium with the disappearance of one or more elements necessary for bacterial growth. X continues to increase, but dX/dt decreases. In some cases, the slowdown phase can also be attributed to the accumulation of inhibitory products originating from bacterial metabolism itself.

- ***Stationary Phase (Phase 4)***

X reaches its maximum value, Xmax. Overall growth stops, even though the cells maintain metabolic activity. They gradually deplete their intracellular reserves, and their mortality balances the synthesis.

- ***Decline Phase (or Endogenous Phase) (Phase 5)***

The concentration of living cells decreases due to an increase in mortality. This mortality is a result of enzymatic autolysis of the cells.

These different phases and the equations that govern them are applicable in both aerobic and anaerobic environments. The values of the various coefficients depend, of course, on the nature of the microorganisms, the substrate, and various parameters (temperature, pH, presence or absence of dissolved O2).

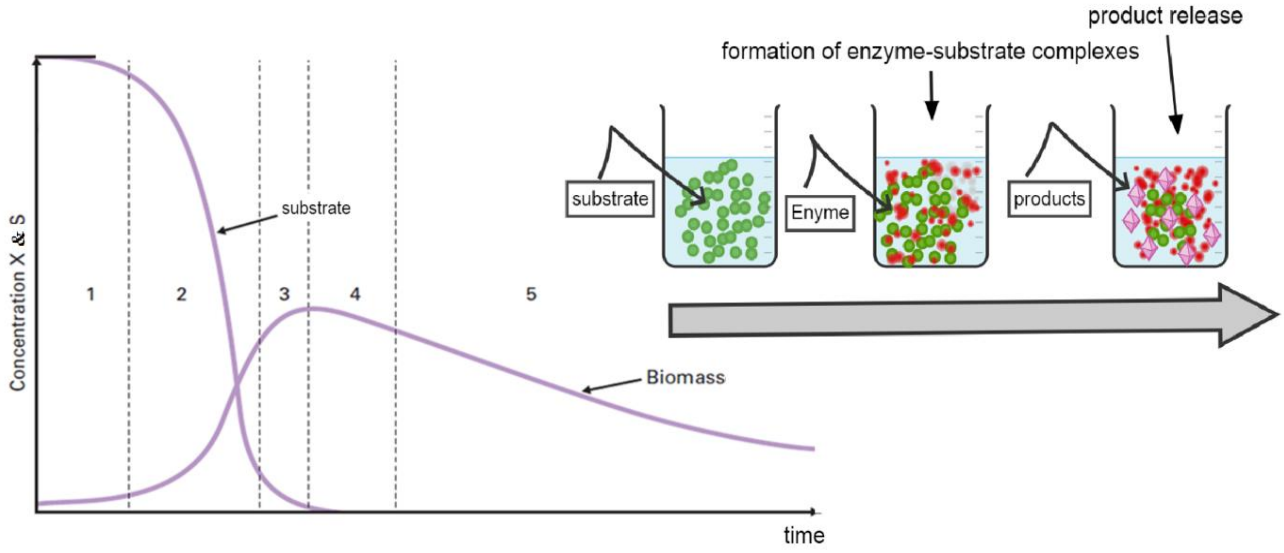


Figure 8: Evaluation of a bacterial culture

2.1.1.4. Production in a liquid bioreactor

Data concerning the growth state parameters, substrate consumption, and product production

A substrate S in the medium - let's call it SL - can potentially become the limiting factor for the specific growth rate. The most classic model for this specific limitation effect is Monod's model:

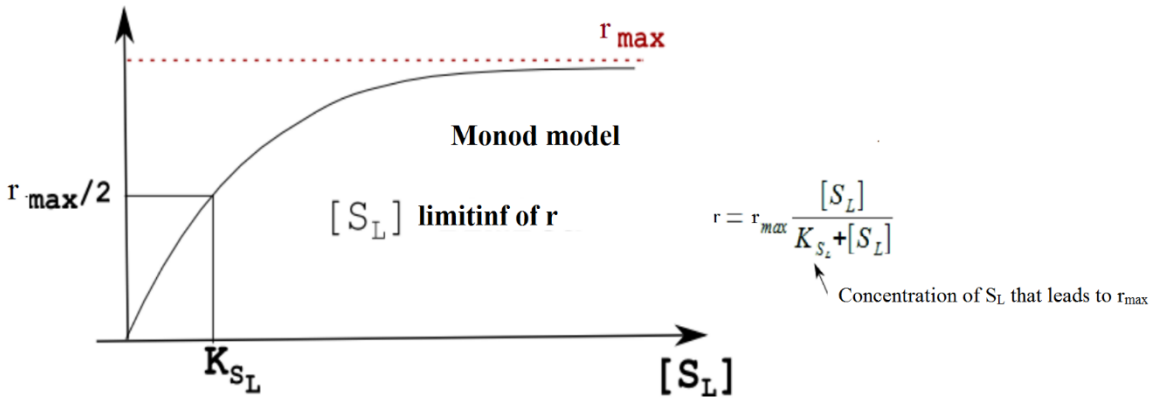


Figure 9: Saturation curve – MONOD model

The analogy with the Michaelis-Menten-Henry equation is evident. The cell is a 'large enzymatic catalyst' that uses nutrients as substrates and catalyzes its own creation.

2.2 Enzyme Kinetics: Michaelis-Menten Model

There are different enzymatic mechanisms depending on the enzyme in question. Michaelian enzymes operate as follows: a substrate S binds to an enzyme E to form an intermediate ES, called the enzyme-substrate complex, and then this intermediate dissociates to produce a product P with the regeneration of the enzyme E. An important clarification: each active site behaves independently of the others, whether they are physically separated (one active site per molecule) or not (multiple active sites per molecule).

Of course, there are many other enzymatic mechanisms. Allosteric enzymes, in particular, necessarily have more than one active site per molecule (at least two), and the kinetic characteristics of one active site will vary depending on the state of the other sites on the same molecule (whether they are bound to a substrate or not).

Each mechanism is associated with specific kinetic characteristics (the evolution of catalytic speed over time). To conduct a kinetic study, it is necessary to measure the instantaneous rate of the reaction at different times, carefully selecting the initial conditions. From these measurements, curves representing the reaction kinetics can be plotted, allowing for the determination of certain characteristic values.

The Michaelis-Menten equation is a mathematical expression describing the kinetic parameters of a chemical reaction catalyzed by a Michaelian enzyme."

The kinetics of an enzymatic reaction is the change in the concentration of molecules (substrates and reaction products) over time. Recording this kinetics involves that these molecules possess physicochemical properties that allow for quantitative detection:

- Measurement of absorbance (Beer-Lambert relationship)
- Measurement of fluorescence emission
- Counting the number of disintegrations after radioactive labeling

2.2.1. Overall Rate of Product Formation

Let's consider the simplest mechanism that describes:

$$E + S \underset{k_{-1}}{\overset{k_1}{<====>}} ES \underset{k_{-2}}{\overset{k_2}{<====>}} E + P$$

- Substrate binding to the free enzyme
- Formation of the non-covalent ES complex (Michaelis-Menten complex)
- Formation of the product P after the release of the enzyme

The rate of the reaction is the change in substrate or product concentration over time. The minus sign indicates that the entity is decreasing."

$$v = -\frac{d[S]}{dt} = \frac{d[P]}{dt}$$

- It is preferable, from the perspective of precision, to measure the rate of product formation rather than the rate of substrate disappearance. Indeed, one can measure a small increase in product concentration more accurately (since it starts from zero) than

a small decrease in substrate concentration (which is 'huge' at the beginning of the reaction).

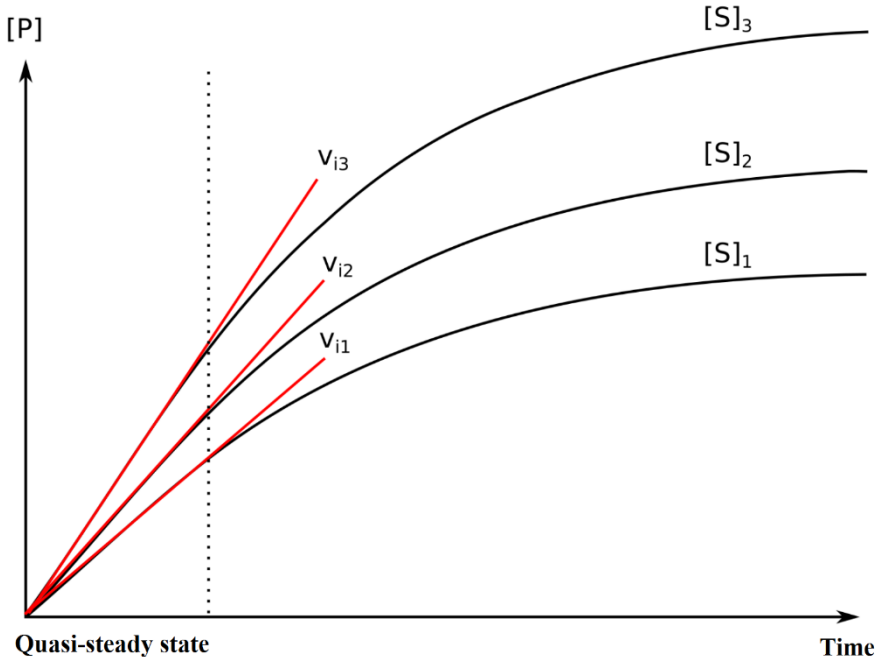


Figure 10: Product formation rate

- The equation for the overall rate related to the product concentration is the sum of the rates of product formation and disappearance:

$$v = \frac{d[P]}{dt} = (k_2 \,.\, [ES]) - (k_{-2} \,.\, [E].[P])$$

2.2.2. The Henri-Michaelis-Menten relationship

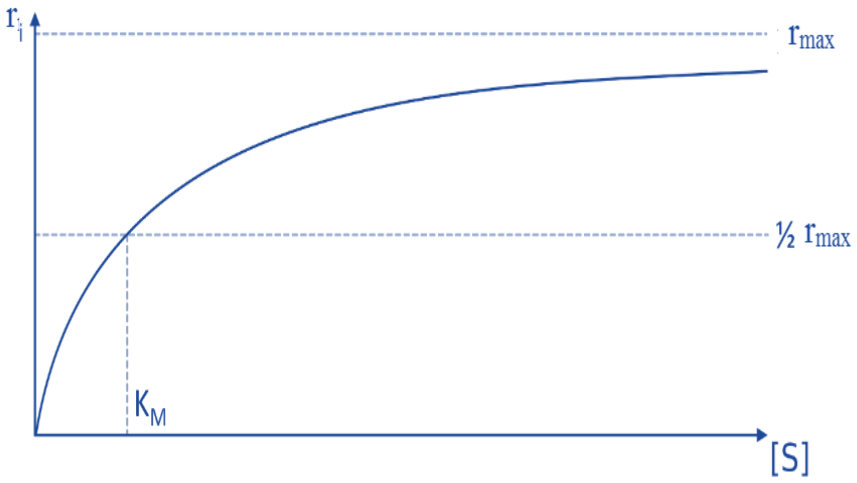
$$r_i = r_{Max} \cdot \frac{[S_0]}{K_S + [S_0]}$$

catalytic term — binding term

- r_i: initial reaction rate
- r_{max}: maximum reaction rate
- K_m: Michaelis-Menten constant
- [s]: substrate concentration

Leonor Michaelis (1875 1949)

- r_{max} is the catalytic term: it's the asymptote of the saturation curve.



Maud Menten (1879 - 1960)

Figure 11: kinetic curve

• The term ($[S_0] / K_S + [S_0]$): binding term is the saturation function (hyperb olic curve).

The Michaelis-Menten equation describes the kinetic curve of r = f[S]:

- For low substrate concentrations, when [S] << Km, r = [S] r_{max}/Km, and the rate is directly proportional to the substrate concentration.
- For high substrate concentrations, when [S] >> Km, r = r_{max}, and the rate is independent of the substrate concentration.
- The significance of the Km constant is evident. When [S] = Km, r = r_{max}/2.
- Km is the substrate concentration required for the enzyme to reach half of its maximum rate (1/2) r_{max}.

Note: The reaction rate can change if we change the enzyme to obtain the same product from the same substrate. The maximum reaction rate is proportional to the initial enzyme concentration. This form of relationship can be applied by a two-step mechanism:

- Rapid and stable formation of a complex
- Decomposition of the complex into product-enzyme.

The Michaelis-Menten constant represents the enzyme's affinity for the substrate. It is greater when the chemical rate constant is lower.

2.2.3. Graphical determination of the values of Km and rmax

To improve the precision of the graphical determination of these two constants, there are graphical representations that linearize the results, enabling extrapolations that are more accurate.

There are four graphical methods for determining the kinetic parameters of the Michaelis-Menten law under given experimental conditions: Lineweaver-Burk (LB: double inverses), Eadie-Hofstee (EH), Hanes, and Cornish-Bowden-Eisenthal (CBE).

All four methods require experimental data for the initial reaction rate as a function of substrate concentration at a fixed enzyme concentration.

- **Lineweaver-Burk method**

In biochemistry, the Lineweaver–Burk plot (or double reciprocal plot) is a graphical representation of the Michaelis–Menten equation of enzyme kinetics, described by Hans Lineweaver and Dean Burk in 1934.

The reciprocal of the Michaelis-Menten equation is very useful for the analysis of kinetic data:

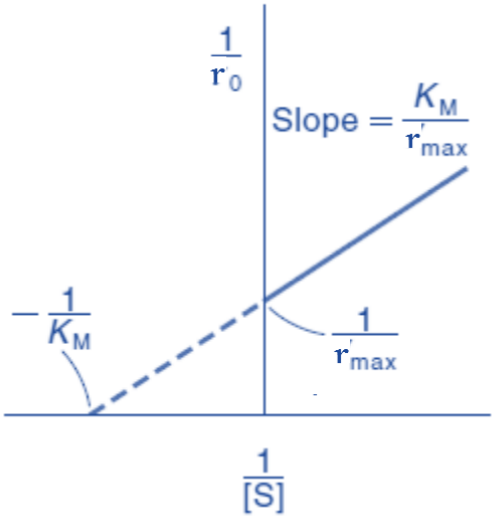
$$\frac{1}{r_o} = \frac{K_m + [S]}{r_{max} \cdot [S]}$$

which can also be written as:

$$\frac{1}{r_o} = \frac{K_m}{r_{max} \cdot [S]} + \frac{[S]}{r_{max} \cdot [S]}$$

and simplifies to:

$$\frac{1}{r_o} = \frac{1}{r_{max}} + \frac{K_m}{r_{max}} \cdot \frac{1}{[S]}$$



The advantage of this mathematical transformation is that it allows you to plot a graph of 1/r vs. 1/[S], where the curve is actually a straight line for enzymes following the Michaelis-Menten relationship between reaction rate and substrate concentration.

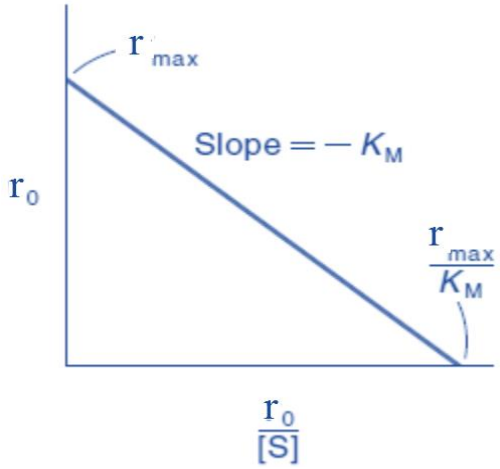
- **Eadie-Hofstee method**

The Eadie-Hofstee plot was used historically for rapid identification of important kinetic terms like Km and Vmax, but has been superseded by nonlinear regression methods that are significantly more accurate and no longer computationally inaccessible. It is also more robust

$$r = r_{max}\frac{[S]}{[S]+K_m} \longrightarrow r[S]+rK_m = r_{max}[S] \longrightarrow \frac{r[S]}{[S]}+\frac{rK_m}{[S]} = r_{max} \longrightarrow \boxed{r = r_{max}-\frac{rK_m}{[S]}}$$

against error-prone data than the Lineweaver–Burk plot, particularly because it gives equal weight to data points in any range of substrate concentration or reaction velocity

- A graph of r as a function of r/[S] forms a straight line.



- **Hanes method**

$$r = r_{max}\frac{[S]}{[S]+K_m} \longrightarrow r([S]+K_m) = r_{max}[S]$$

which leads to $$\frac{K_m+[S]}{r_{max}} = \frac{[S]}{r} \longrightarrow \frac{[S]}{r} = \frac{K_m}{r_{max}}+\frac{[S]}{r_{max}}$$

- A graph of [S]/r as a function of [S] forms a straight line.

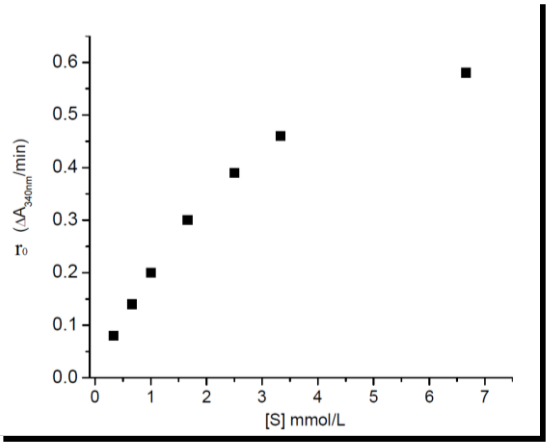
➢ **Cornish-Bowden-Eisenthal method**

$$r = r_{max}\frac{[S]}{[S]+K_m} \longrightarrow r_{max}[S] = r\,[S] + r\,K_m \longrightarrow \boxed{r_{max} = \frac{K_m}{[S]} + r}$$

- Each pair of coordinates (r, [S]) yields unique values for the y-intercept (r) and slope (r/[S]) in a graph of r_{max} as a function of Km. For each coordinate (r, [S]), the y-intercept and slope values define a unique line, and the lines will intersect at an identical point corresponding to the enzyme's r_{max} and Km.

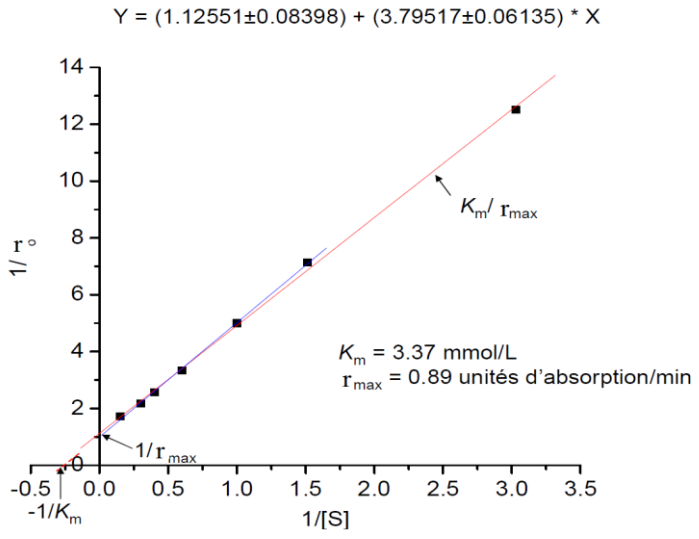
Application

Consider the following kinetic data:

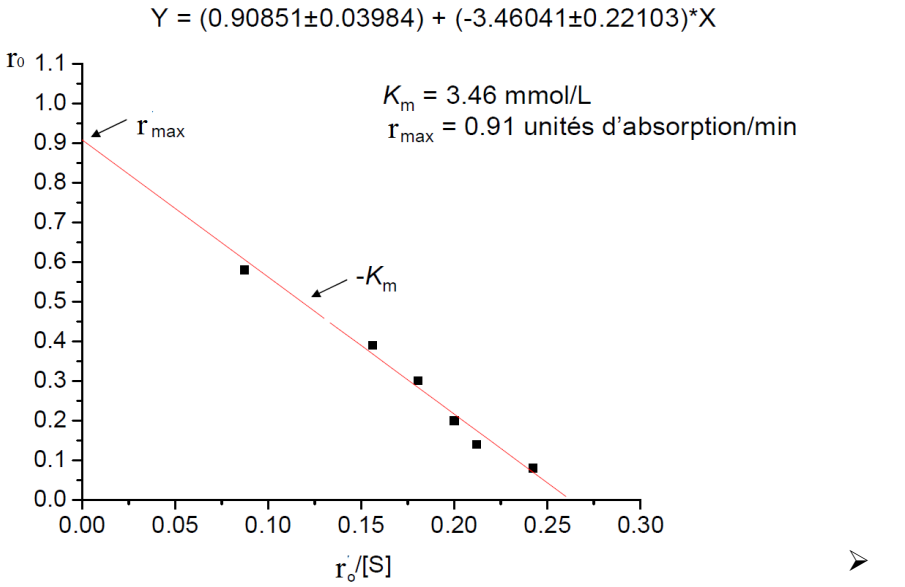


- Determine the kinetic parameters graphically: r_{max} and Km

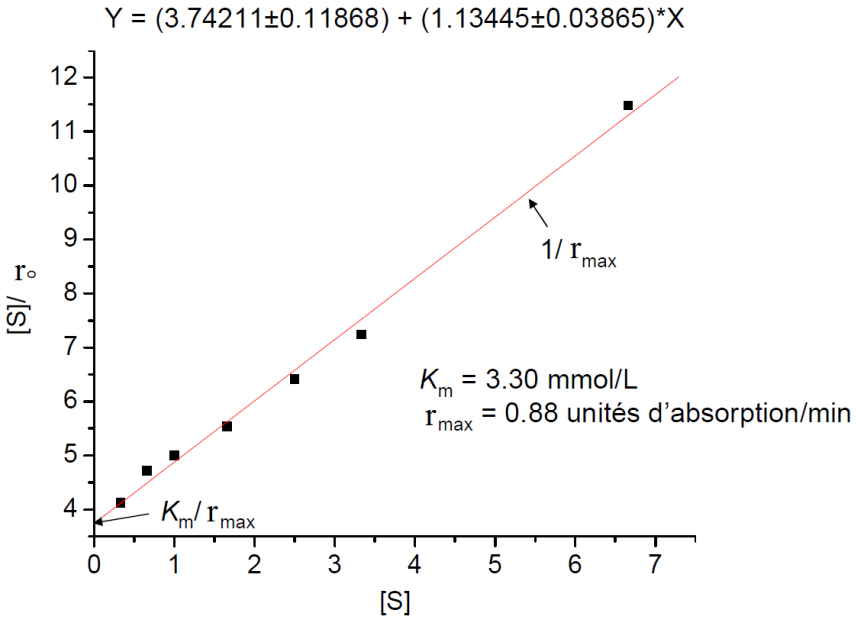
➢ **Lineweaver-Burk method**



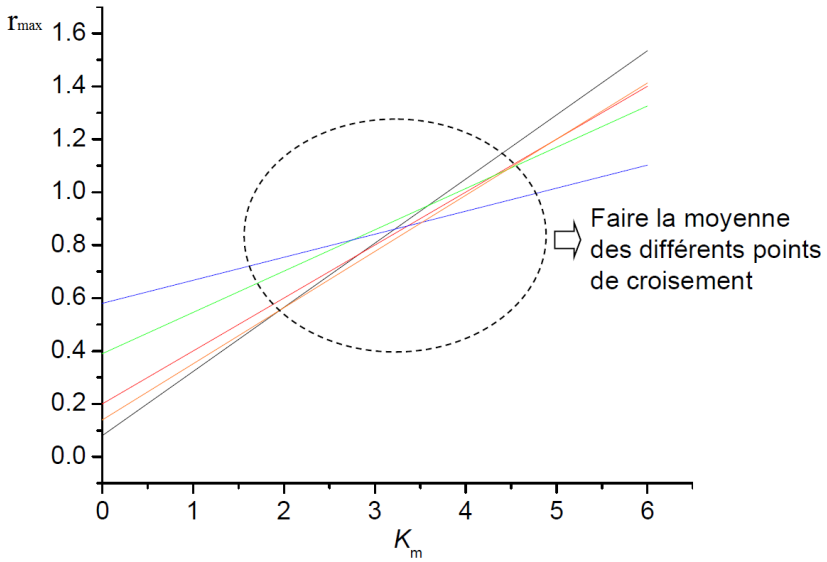
- **Eadie-Hofstee method**



- **Hanes method**



➢ **Cornish-Bowden-Eisenthal method**



2.3 Inhibition of Enzymatic Reactions and activators

Inhibitors are a class of compounds, which decrease or reduce the rate of an enzyme-catalyzed reaction. By studying enzyme inhibition, researchers have understood the nature of functional groups at the active site and the mechanism of specificity. Enzyme activity is usually regulated by the phenomenon called feedback mechanism where the end product is responsible for inhibiting the enzyme's activity. Infact, the amount of products formed is controlled by enzyme inhibition.

In irreversible inhibitions, inhibition gradually elevates with respect to time. When the irreversible inhibitor concentration exceeds the enzyme concentration, it results in complete inhibition.

2.3.1. Reversible Inhibition

Reversible inhibition can be of three types

- Competitive inhibition
- Non-competitive inhibition, and
- Uncompetitive inhibition

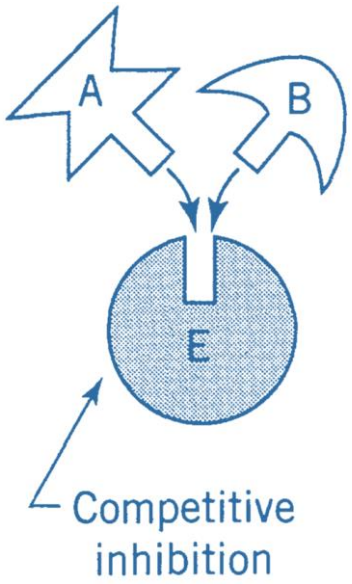
2.3.1.1. Competitive Inhibition

In this case, both the substrate S and the inhibitor I compete. Competitive inhibition can be reversed by increasing the concentration of the substrate.

$$E + S \underset{k_{-1}}{\overset{k_1}{<\!\!-\!\!>}} ES \overset{k_3}{<\!\!-\!\!>} E + P$$

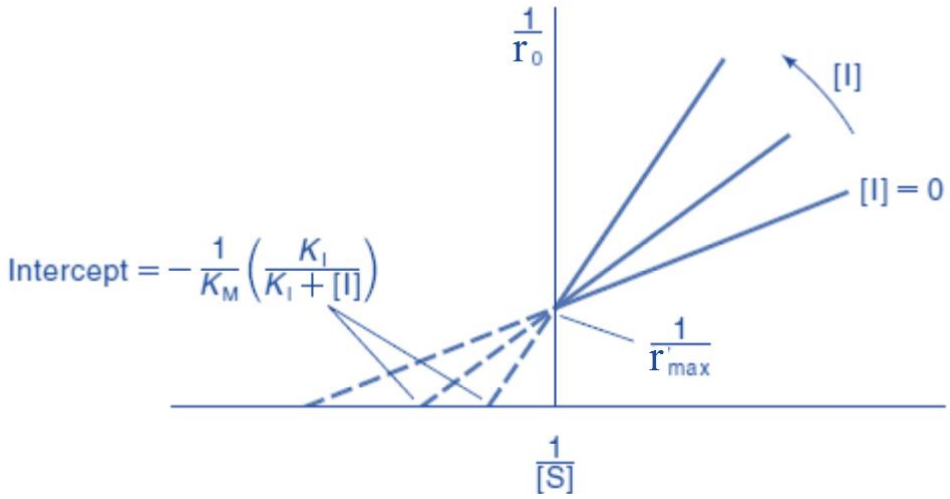
$$+$$

$$I <\!\!-\!\!> EI$$



The Line weaver Burk equation would be:

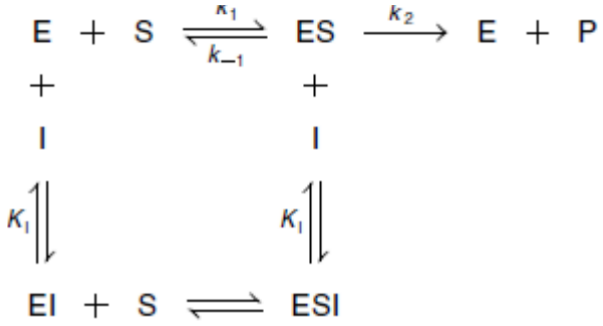
$$r_o = \frac{r_{max}}{1 + (K_m/[S]) \times (1 + [I]/K_i)}$$



2.3.2.2. Noncompetitive inhibition

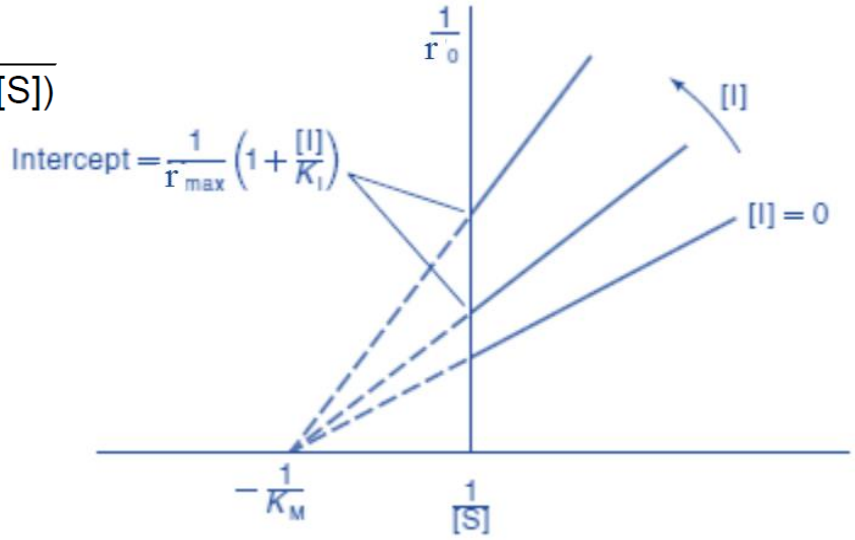
In non-competitive inhibition, the inhibitor may bind with both the free enzyme as well as the enzyme-substrate complex. However, the inhibitor binds with enzyme at a site, which is distinct from the substrate-binding site. The binding of the inhibitor however does not affect the substrate binding, and vice versa.

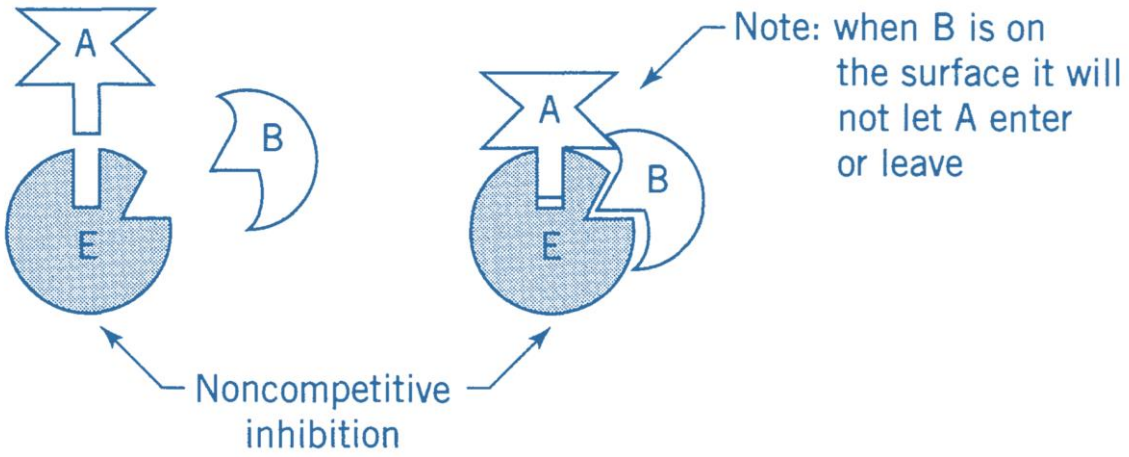
The reactions are:



The line weaver burk equation would be:

$$r_o = \frac{r_{max}}{(1+[I]/K_i)\times(1+K_m/[S])}$$

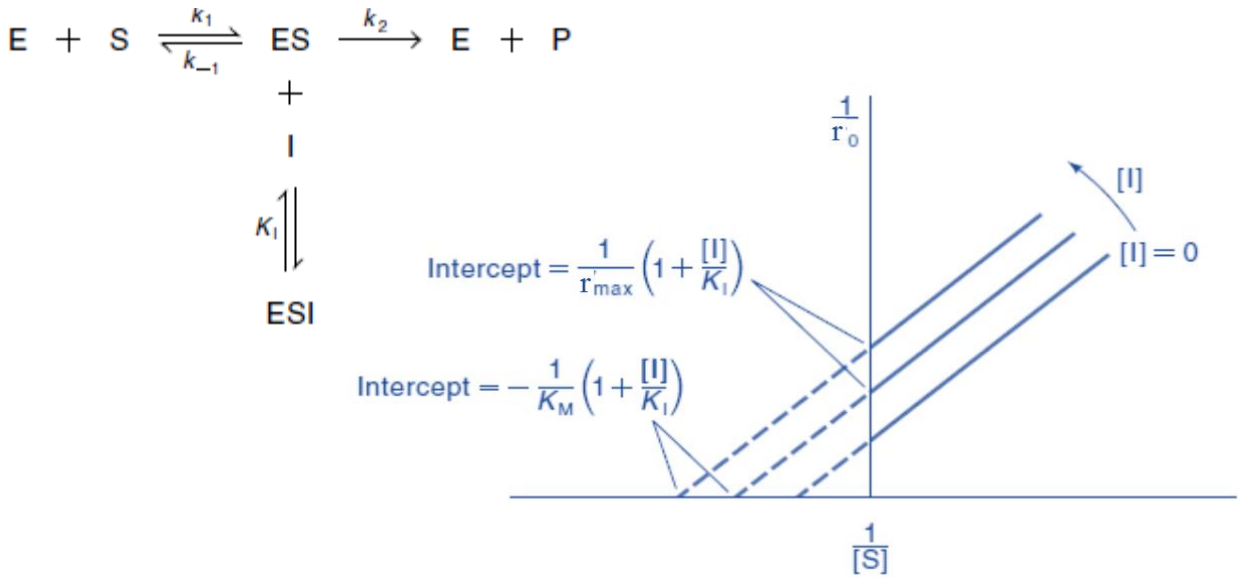




r_{max} reduced by 1+ [I] / KI factor but Km is unchanged

2.3.2.3. Uncompetitive Inhibition

In Uncompetitive Inhibition, an inactive ESI complex is formed when Inhibitor reversibly binds to the enzyme– substrate [ES] complex. Here in this case inhibitor does not bind to the free enzyme.



Here in the above reaction, ESI complex does not form a product because 'I' does not interfere with the formation of ES. Again, unlike competitive inhibition, uncompetitive inhibition cannot be reversed by increasing the substrate concentration.

Table below represents a quiq summary of linear Kenitic

Table1: Quick summary of linear kinetic plots the most used

	Lineweaver-Burk	**Woolf-Hanes**	**Eadie-Hofstee**
Equation	*Reciprocal of MMH Equation*	*LB x [S]*	*LB x r_0 r_{max}*
y-axis	1/ r_0	[S]/ r_0	r_0
x-axis	1/[S]	[S]	r_0 /[S]
y-intercept	1/ r_{max}	Km/ r_{max}	r_{max}
x-intercept	-1/Km	-Km	r_{max} /Km
slope	Km/ r_{max}	1/ r_{max}	-Km

2.3.3. Enzyme Activators

- Activators are species that increase enzymatic activity without being directly involved in the reaction catalyzed by the enzyme.
- These species can be necessary for the catalytic activity of the enzyme, such as prosthetic groups or cofactors, or they can enhance the specific activity of an already active enzyme (e.g., inorganic ions).
- At a saturated and constant substrate concentration, increasing concentrations of the activator increase the initial reaction rate; a limiting rate is reached at high activator concentrations.
- In some cases, such as activation by a prosthetic group, the following sequence of reactions can serve as a kinetic model:

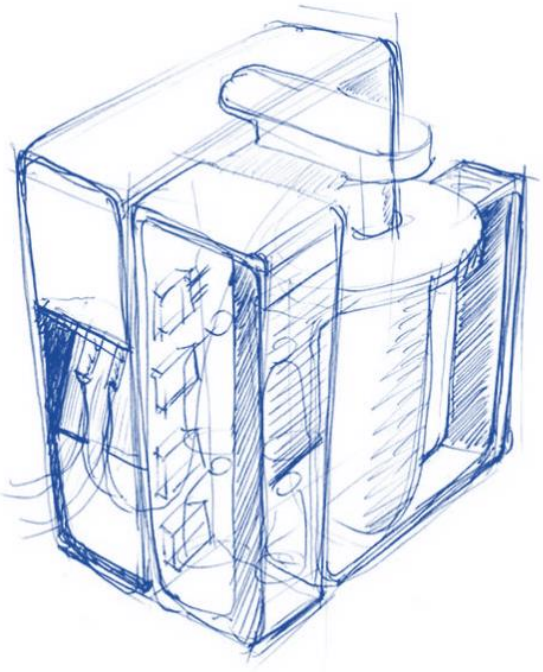
$$A + E_{inactive} \longrightarrow E_{active}$$

$$S + E_{active} \longrightarrow SE_{active} \longrightarrow E_{active} + P$$

- Parallel reaction sequences must be considered if the enzyme is catalytically active in the absence of the activator.
- Except for prosthetic groups or cofactors, activators are generally not specific, and several species can have the same activating effect on an enzyme (e.g., amylase is activated by a variety of anions, including Cl-)."

Chapter II

Design and Analysis of Bioreactors



II. Design et analyse des bioréacteurs

Bioreactor can be defined as a vessel, deployed to utilize the activity of a biological catalyst to achieve a desired chemical transformation. It is the heart of any biochemical process in which enzymes, microbial, mammalian or plant cell systems are used for manufacture of a wide range of useful biological products. A bioreactor system comprises a bioreactor, sensors, control system and software to monitor and control the conditions inside the bioreactor. Bioreactors are extensively used for the production of pharmaceuticals, food bio-based materials (such as poly-lactic acid), food processing, fermentation, bio-fuels and in waste treatment, etc…

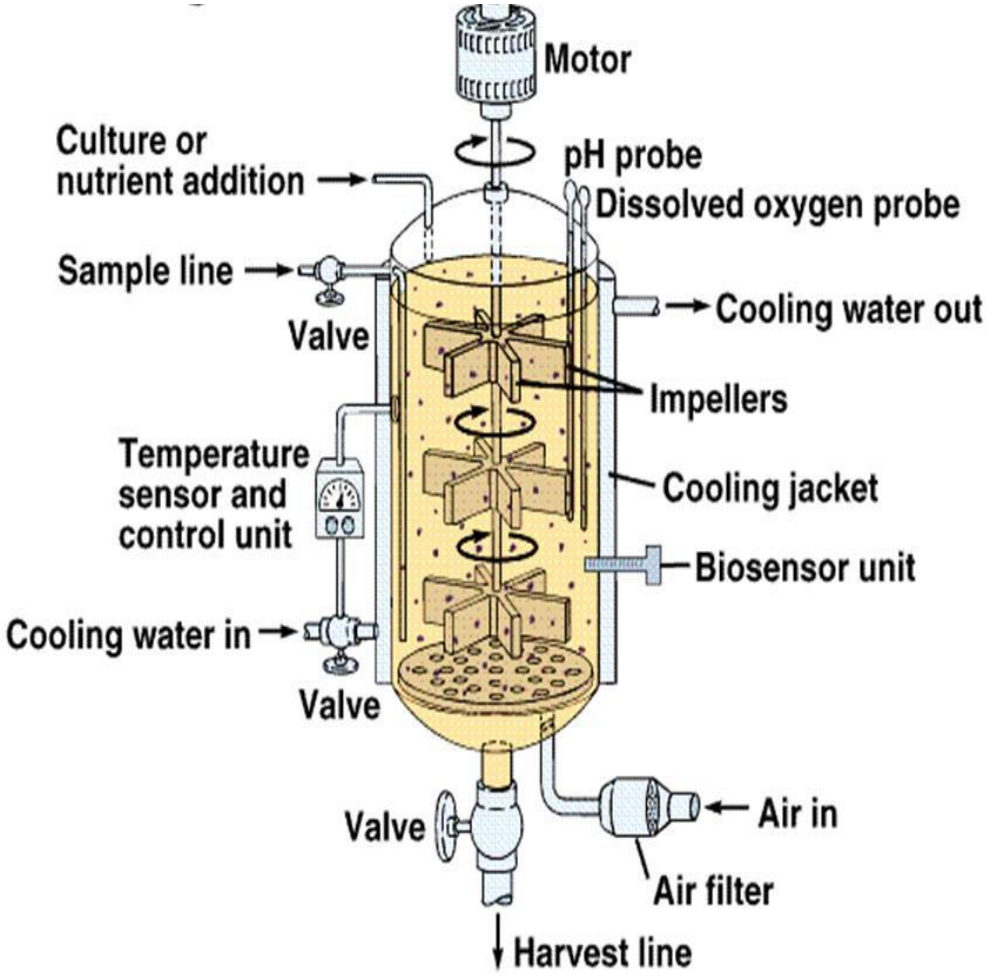


Figure 12: Bioreactor parts

A bioreactor should provide the following:

(i) Agitation (for mixing of cells and medium),

(ii) Aeration (aerobic fermenters; for O_2 supply),

(iii) Baffles (to prevent vortex formation and to improve aeration efficiency),

(iv) Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level, etc.,

(v) Sterilization and maintenance of sterility,

(vi) Withdrawal of cells/medium (for continuous fermenters).

Modern fermenters are usually integrated with computers for efficient process monitoring, data acquisition, etc.

These bioreactors are commonly cylindrical, ranging in size from litres to cubic metres, and are often made of stainless steel.

To design a bioreactor, some objectives have to be defined. The decisions made in the design of the bioreactor might have a significant impact on overall process performance.

Knowledge of reaction kinetics is essential for understanding how a biological reactor works. Other areas of bioprocess engineering such as mass and energy balances, mixing, mass transfer and heat transfer are also required.

The performance of any bioreactor depends on many functions, such as those listed below:

- Biomass concentration
- Nutrient supply
- Sterile conditions
- Product removal
- Effective agitations
- Product inhibition
- Heat removal
- Aeration
- Correct shear conditions
- Metabolisms/microbial activities

There are three groups of bioreactor currently in use for industrial production:

1. *Non-stirred, non-aerated system*: about 70% of bioreactors are in this category.
2. *Non-stirred, aerated system*: about 10% of bioreactors.

3. *Stirred and aerated systems*: about 20% of the bioreactors in industrial operation.

The main function of a properly designed bioreactor is to provide a controlled environment to achieve optimal growth and/or product formation in the particular cell system employed.

Frequently the term "fermenter" is used in the literature to mean "bioreactor". The performance of any bioreactor depends on many functions including:

• Biomass concentration must remain high enough to show high yield.

• Sterile conditions must be maintained for pure culture system.

• Effective agitation is required for uniform distribution of substrate and microbes in the working volume of the bioreactor.

• Heat transfer is needed to operate the bioreactor at constant temperature, as the desired optimal microbial growth temperature.

• Creation of the correct shear conditions. High shear rate may be harmful to the organism and disrupt the cell wall; low shear may also be undesirable because of unwanted flocculation and aggregation of the cells, or even growth of bacteria on the reactor wall and stirrer.

II.1. Types of bioreactors

There are mainly three types of reactions involved in fermentation process namely batch, continuous, and semi-continuous (or fed-batch), which depend on the feeding strategy and the medium used in the bioreactor.

Traditional stirred tank reactors (STRs) in batch and continuous configurations have been in use for centuries and remain widely employed in the chemical and bioprocessing industry due to their simplicity. Additionally, there are other specialized bioreactor designs with unique operational features, including photo-bioreactors, rotary drum reactors, mist bioreactors, membrane bioreactors, packed and fluidized bed bioreactors, as well as bubble column and air lift bioreactors.

These specialized bioreactors have been developed for specific applications in various processes.

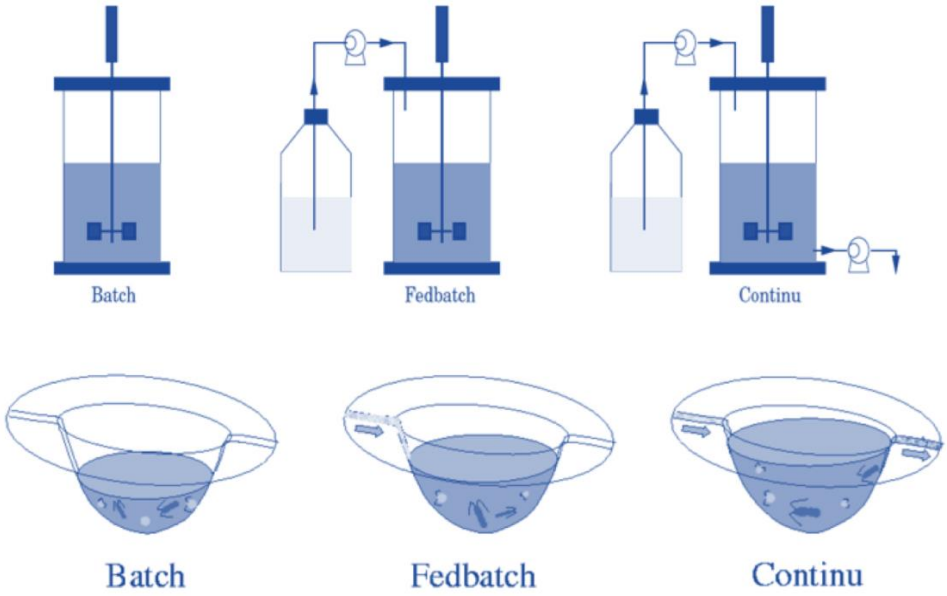


Figure 13: bioreactors types

Table 2: Advantages and disadvantages of batch, semi-batch, continuous reactor

	Advantages	**Disadvantages**
Batch	Simple equipment; suitable for small production volumes along with multi-product flexibility	Downtime for loading and cleaning; reaction conditions change with time
Semi-batch or Fed-batch operation	Control of environmental conditions e.g. substrate concentration (inhibition), induction of product formation; most flexible for selecting optimal conditions; most frequently used in biotechnological processes and in fine chemical industry	Requires feeding strategy e.g. to keep constant temperature or substrate concentration
Continuous	High productivity; better product quality due to constant conditions; good for kinetic Studies	Requires flow control, longevity of catalyst necessary, stability of organisms

➢ ***Other types of bioreactors and their applications***

There are different types of bioreactors, each with its own characteristics and specific applications in biotechnology and industry. Here are some of these types of bioreactors and their main applications.

Table 3: Some important applications of bioreactors

Type of bioreactor	**Applications**
Stirred tank fermenter	Antibiotics, citric acid, Exopolysaccharides, cellulose, Chitinolytic enzymes, Laccase, Xylanase, Pectic, and pectate lyase, Tissue mass culture, Lipase, Polygalacturonases, Succinic acid
Bubble column fermentor	Algal culture, Chitinolytic enzymes
Airlift fermentor	Antibiotics, Chitinolytic enzymes, Exopolysaccharides, Gibberelic acid, Laccase, Cellulase, Lactic acid, Polygalacturonases, Tissue mass culture
Fluid bed fermentor	Laccase
Packed bed fermentor	Laccase, Hydrogen, Organic acids, Mammalian cells,
Photobioreactor	Wastewater treatment, water quality management, remediation of contaminated soil
Membrane bioreactor	Alginate, Antibiotic, Cellulose hydrolysis, Hydrogen production, Water treatment, VOCs treatment

II.3. Basic Concepts

The bioreactor is the heart of any biochemical process as it provides an environment for microorganisms to obtain optimal growth and produce metabolites for the biotransformation and bioconversion of substrates into desirable products. The reactors can be engineered or manufactured based on the growth requirements of the organisms used.

In Reactor Engineering, the balances are generally written on a macroscopic scale, considering the compounds (reactants and products):

• Writing in mass or moles is equivalent.

• Writing in molar flow rate is the most practical, as the stoichiometry of the reaction can be directly taken into account.

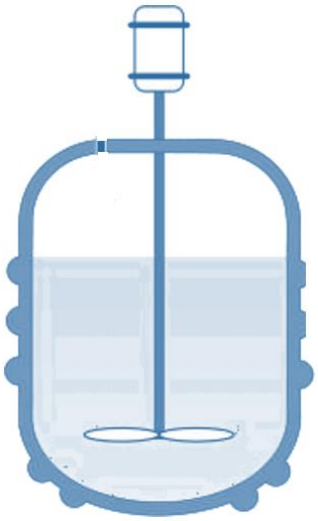
Input - Output +Generation = Accumulation

$$F_{iE} + \nu_i rV = F_{iS} + \frac{dn_i}{dt}$$

i= substrate

II.3.1. Batch Bioreactors

In the batch process, following sterilization, microorganisms are introduced into the sterile culture medium. Throughout this incubation period, the composition of the medium undergoes changes over time, including variations in cell population, substrate levels, which encompass nutrient salts and vitamins, and product concentrations. The fermentation proceeds for a predetermined duration, culminating in the collection of the final product. To facilitate aerobic growth, the medium is aerated to ensure a continuous supply of oxygen, and any gaseous by-products like CO_2 are expelled.



$$\nu_i rV = \frac{dn_i}{dt} \quad (F_{iE}\ et\ F_{iS} = 0)\ \text{Avec}\ \ n_i = n_{i0} + \nu_i n_0 . X$$

$$\nu_i rV = \frac{d(n_{i0} + \nu_i n_0 . X)}{dt}$$

$$\nu_i rV = \nu_i n_0 \frac{dX}{dt}$$

$$dt = n_0 \frac{dX}{rV} \rightarrow \int_o^{t_s} dt = n_0 \int_{X_E}^{X_S} \frac{dX}{rV}$$

Ou encore $t_s = n_0 \int_{X_E}^{X_S} \frac{dX}{rV}$

$$r = \frac{dC_s}{dt} = \frac{r_{\max} C_S}{K_m + C_S}$$

$$K_m \ln \frac{C_{s0}}{C_s} + \left(C_{s0} - C_s\right) = r_{\max} t$$

- A batch bioreactor is normally equipped with an agitator to mix the reactant, and the pH of the reactant is maintained by employing either buffer solution or a pH controller.
- A foam breaker may be installed to disperse foam.

II.3.2. Continuous Bioreactors

In continuous mode operations of a bioreactor, a continuous influx of fresh medium is introduced, and simultaneously, products and the culture are extracted at an equivalent rate. This ensures the maintenance of constant nutrient and cell concentrations throughout the entire process. Continuous processes are commonly employed for high-volume production, reactions utilizing gas, liquid, or soluble solid substrates, and for processes involving microorganisms with a high degree of genetic stability. Examples of typical products produced through continuous processes encompass vinegar, baker's yeast, and treated wastewater. The chemostat serves as a well-known example of a continuous process reactor.

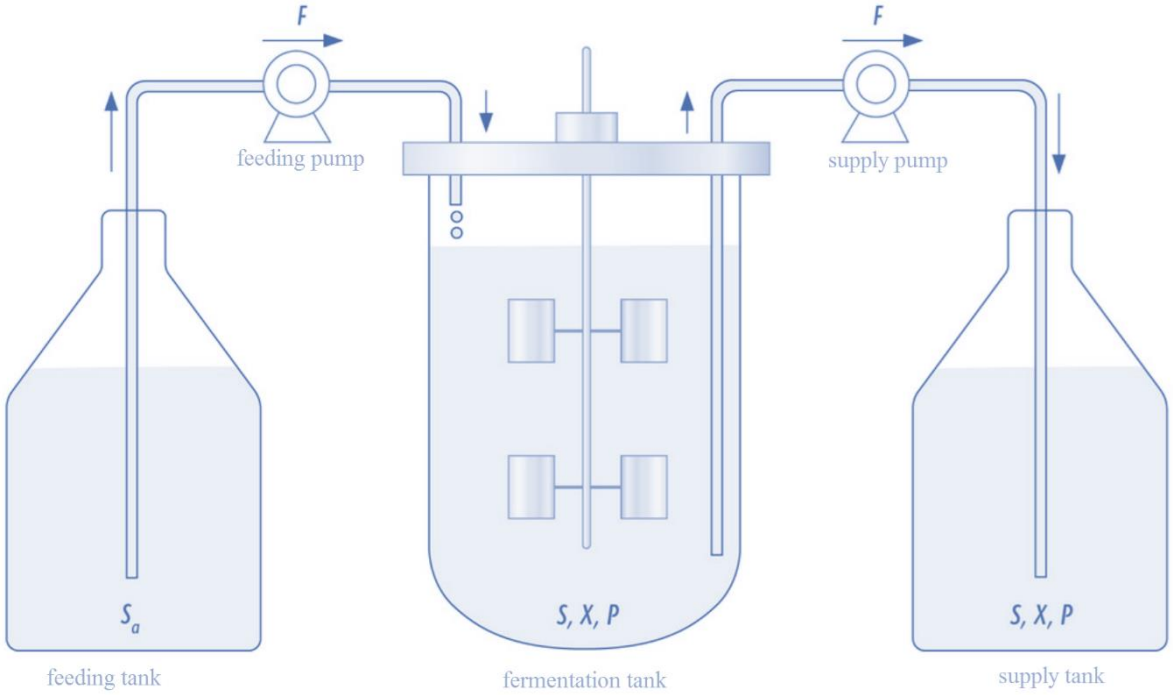
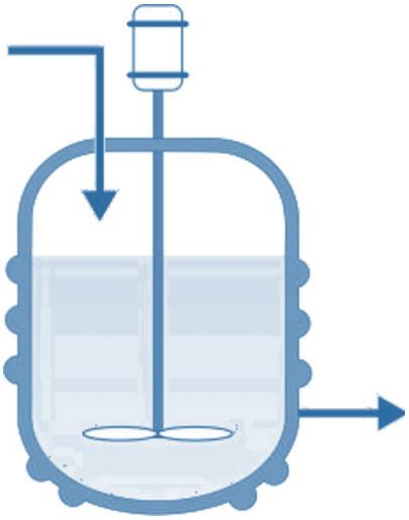


Figure 14: Functioning illustration of a bioreactor in continuous mode

II.3.2.1. Continuous Stirred Tanks

An apparatus in which both the introduction and removal of material occur simultaneously. The agitation of the reaction mixture is perfect to the extent that the conditions inside the reactor match those at the outlet.



$$F_{iE} + \nu_i r V = F_{iS} + 0$$

At the inlet: $F_{iE} = F_{i0} + \nu_i F_0 . X_E$

At the outlet: $F_{iS} = F_{i0} + \nu_i F_0 . X_S$

$$\frac{X_S - X_E}{V_R} = \frac{r}{Q_0 C_0}$$

$\frac{V_R}{Q_0} = C_0 \frac{X_S - X_E}{r}$; $\tau = \frac{V_R}{Q_0}$

$(C_{s0} - C_{ss}) / \tau = \frac{r_{max} C_S}{K_m + C_S}$

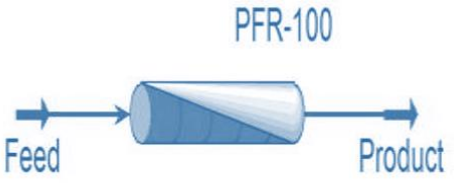
Or

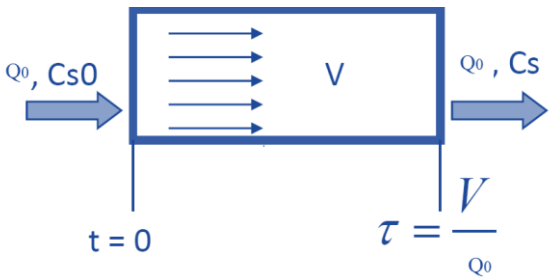
$$\frac{r_{max} C_S}{K_m + C_S}$$

II.3.2.2. Piston Bioreactors

In a plug-flow reactor, the substrate enters one end of a cylindrical tube with is packed with immobilized enzyme and the product steam leaves at the other end.

- An ideal plug-flow reactor can approximate the long tube, packed-bed and hollow fiber or multistage reactor
- Continuous operation without stirring





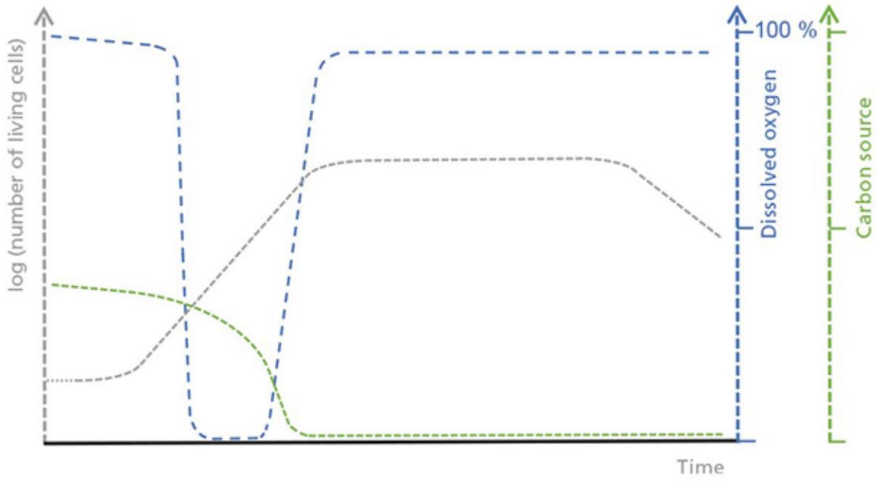
$$F_i = F_{i0} + \nu_i F_0 . X \rightarrow \nu_i r = \frac{\partial (F_{i0} + \nu_i F_0 . X)}{\partial V_R}$$

$$\rightarrow r = F_0 \frac{\partial X}{\partial V_R} \rightarrow \frac{r}{F_0} = \frac{\partial X}{\partial V_R}$$

$$K_m \ln \frac{C_{s0}}{C_s} + (C_{s0} - C_s) = r_{max} \tau$$

II.3.3. Comparison of Batch Bioreactors and Continuous Bioreactors

Table 4: Comparison of Batch and Continuous Bioreactors

<table>
<tr><td colspan="2">Batch: where no extra feeding is used from beginning to end of the process</td></tr>
<tr><td>The advantages</td><td>The disadvantages</td></tr>
<tr><td>- Short duration
- Less chance of contamination as no nutrients are added
- Separation of batch material for traceability
- Easier to manage</td><td>- Product is mixed in with nutrients, reagents, cell debris and toxins
- Shorter productive time
- Can involve storage of batches for downstream processing</td></tr>
<tr><td colspan="2">Schematic illustration of the correlations between living cell concentration, dissolved oxygen, and the limiting carbon source in batch operation.</td></tr>
<tr><td colspan="2"> 
</td></tr>
<tr><td colspan="2">➢ In the initial lag phase, the living cell count only increases slowly, which leads to a moderate but steady uptake of the carbon source. Oxygen consumption increases during the exponential growth phase until it exceeds possible oxygen input. Once the carbon source is depleted, the stationary phase starts and is followed by a dead phase, during which the living cell count drastically decreases.</td></tr>
<tr><td colspan="2">Continuous: where the feed rate of a growth-limiting substance keeps either cell density constant (a chemostat) or cell density determines the feed rate of the substrate (turbidostat). Cell retention can offer another, very productive option (perfusion). The incoming feed rate matches the rate of removal of harvest. The balanced nature of the feeding allows a steady state to be achieved, which can last for days to months. This state is good for studying microbial metabolism or long-term production</td></tr>
<tr><td>- Allows the maximum productivity
- Time for cleaning, sterilisation and handling of the vessel are all reduced
- Provides a steady state for metabolic studies when many elements sum to zero</td><td>- difficult to keep a constant population density over prolonged periods
- The products of a continuous process cannot be neatly separated into batches for traceability
- Increased risk of contamination and/or genetic changes</td></tr>
</table>

- More comparisons in ***annex 2***

Chapter II

Sterilization

IV- Sterilization

Sterilization is the process of complete or partial destruction or deactivation of microorganisms such as bacteria, viruses, fungi, and spores, as well as other microscopic life forms, with the goal of preventing their growth and spread. Sterilization is commonly used in the fields of healthcare, food safety, biological research, and the pharmaceutical industry, among others.

Bioreactor sterilization is a critical process in biotechnology and bioprocessing industries. Bioreactors are vessels or containers used to cultivate and grow microorganisms, cells, or tissues for various purposes, including the production of pharmaceuticals, biopharmaceuticals, enzymes, and biofuels. Sterilization of bioreactors is essential to maintain aseptic conditions and prevent contamination of the culture.

Traditional manufacturing methods often relied on the natural competition among microorganisms, where a highly concentrated inoculum of the desired microorganism and metabolites harmful to environmental microorganisms were introduced to achieve a successful culture of the desired microorganisms. However, increasing sanitary and product quality standards have emphasized the need to carefully manage the microorganisms involved in these processes to prevent contamination. As a result, sterilization, a procedure that eliminates all microorganisms present, has become a critical initial step in the majority of bioreactor-based productions.

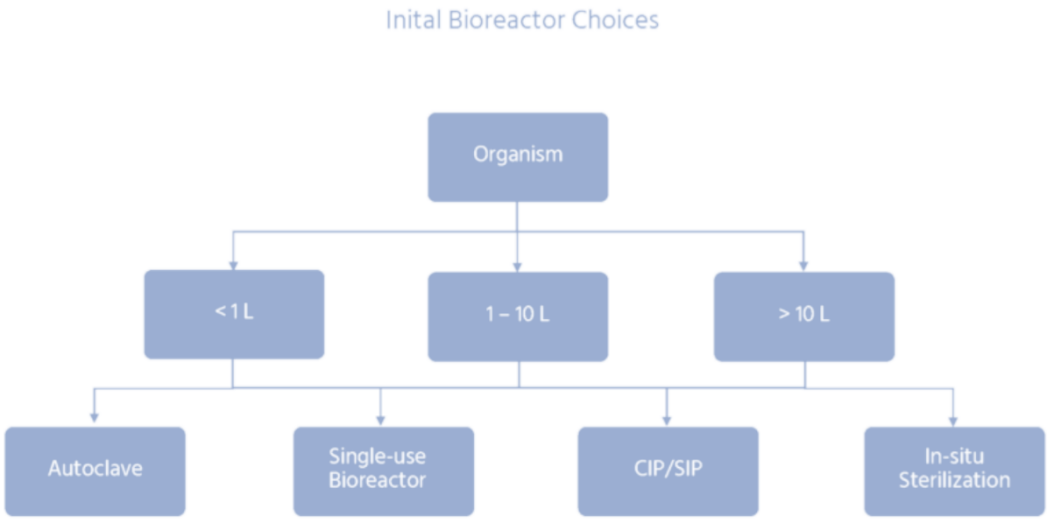


Figure 15: Bioreactor choices

To maintain a sterile environment, culture chambers and all associated equipment, including pipelines, probes, and filters, undergo sterilization either between each production batch or during the production process itself. The method of sterilization used may vary depending on the material or equipment.

In-Place Sterilization (SIP): Some bioreactors are equipped with SIP systems that allow for the sterilization of the entire bioreactor system, including the vessel and associated components, in situ (in place). This process often involves the circulation of steam or hot water through the system.

Steam-in-Place (SIP): Steam-in-place is a specific method where steam is used to sterilize the bioreactor vessel and its associated components. It is commonly employed for larger bioreactor systems.

There are several methods of sterilization, each tailored to specific needs. Some of the most common methods of sterilization classified to two types include:

III.1. Physical Sterilization

Here are some common physical methods for bioreactor sterilization:

- ***Autoclaving:*** Autoclaves are commonly used to sterilize bioreactor components that can withstand high temperatures (usually above 121°C) and high pressure for a specific period. These components may include stainless steel vessel parts, piping, and certain types of sensors. Steam at high temperatures and pressures effectively kills microorganisms, including spores.
- ***Depyrogenation:*** Used in products that can degrade when exposed to steam or humidity but can withstand high temperatures. Metal instruments and needles are often sterilized this way.
- ***Gamma Irradiation:*** For single-use or disposable bioreactor systems, gamma irradiation can be used to sterilize the entire system before use. This method is often preferred for single-use bioprocessing equipment.

- ***Sterile Filtration:*** Filtration through porous membranes of specific sizes allows microorganisms to be retained, while sterile fluids or gases feeds entering or leaving the bioreactor. This method is often used to sterilize heat-sensitive liquids, such as culture media.

The choice of sterilization method depends on factors such as the type of bioreactor, the materials it's composed of, the process being conducted, and regulatory requirements. Maintaining sterility throughout the bioprocessing cycle is crucial to ensure the production of high-quality bioproducts and to prevent contamination that could compromise the integrity of the culture or the final product.

III.2. Chemical Sterilization

Chemical Sterilization: Heat-sensitive components of a bioreactor system, such as tubing, filters, and some sensors, may be sterilized using chemical methods. Chemical sterilization can be accomplished using substances like hydrogen peroxide vapor, peracetic acid, or other suitable chemical agents.

The sterilization of empty fermenters with gaseous chemical agents is possible (beta-propiolactone, ethylene oxide), but pressurized steam is universally employed for sterilizing fermenters and culture media because it is more practical and cost-effective to use.

One of the primary concerns when conducting this type of sterilization is the toxic effect of residual chemicals remaining in the product and the operator's safety associated with exposure to a sterilant.

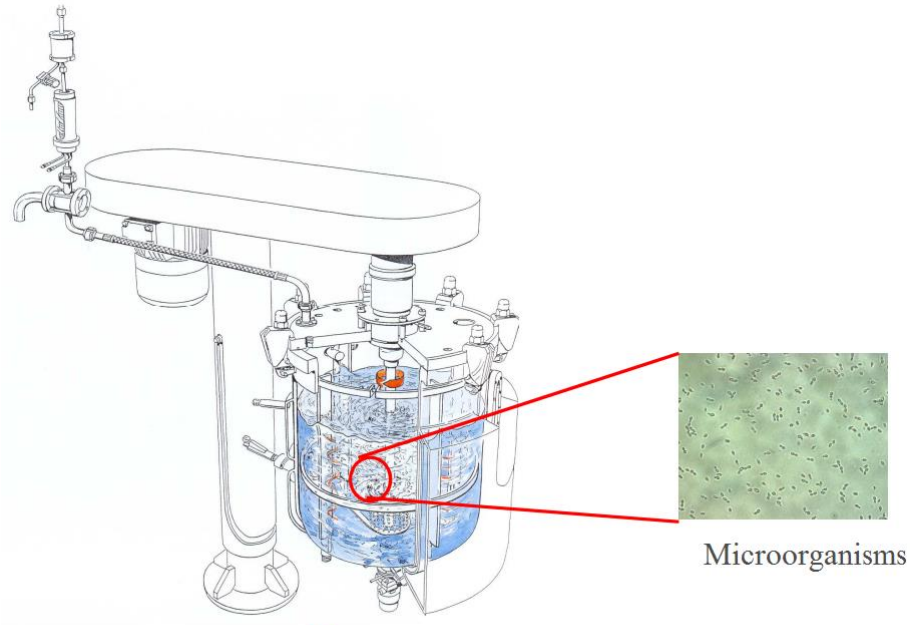
III.3. Single-Use Bioreactor

It is possible to eliminate the sterilization-related risks in the enclosure by using single-use bioreactors. These polymer-based bioreactors (currently used for high-value-added productions) are sterilized by the manufacturer and only need to be connected to non-disposable fixed equipment, which still needs sterilization (see Figure 10). These disposable bioreactors also eliminate potentially hazardous maintenance operations between batches. At the end of production, bioreactors contaminated with pathogenic microorganisms or GMOs are disposed of in the infectious healthcare waste stream (DASRI) or inactivated and disposed of in the non-hazardous industrial waste stream.

Figure 16: Single use bioreactor

Chapter II

Mass Transfer in bioreactor



V- Transfert de matière dans les bioréacteurs

To facilitate the separation of enzymes from liquid media after a reaction, they can be immobilized either on the surface of non-porous particles or within porous particles. This process is known as enzyme immobilization. There are three main methods of immobilization:

- Adsorption immobilization
- Covalent binding immobilization
- Inclusion immobilization

The rate laws take the same form as those for non-immobilized enzymes, but the rates (r and rmax) are expressed, in the case of surface immobilization, in $mol \cdot m^{-2} \cdot s^{-1}$ of the liquid-solid contact surface or in $mol \cdot kg^{-1} \cdot s^{-1}$ of the support mass. If the enzyme is enclosed within porous particles, the rates (r and rmax) are expressed in $mol \cdot m^{-3} \cdot s^{-1}$ of the solid phase or in $mol \cdot kg^{-1} \cdot s^{-1}$ of the support.

However, when the particles are spherical or cylindrical in shape, the conversion between these units is straightforward and depends on the size and density of the solid particles."

The concentrations of substrate and product to be considered in kinetic equations are the concentrations at the liquid-solid interface in the case of surface immobilization or the concentrations within the liquid phase filling the pores of solid particles in the case of inclusion immobilization.

In both immobilization scenarios, the phenomenon of diffusion at the liquid-solid or gas-solid interface must be taken into account. The liquid or gas phase contains the substrate, while the solid phase contains the enzyme, which can be in the form of blocks, membranes, films, or beads. Various substances present in the liquid phase need to be transported (transferred) to the immobilized enzyme. This material transport represents the rate-limiting step in the kinetics, and it becomes rate-limiting only when it crosses the solid-liquid interface. This diffusion phenomenon is described by **Fick's law**."

The mass flux J is defined as the amount of substance (or moles) that crosses a surface area (cm^2) during a unit of time (s). The first law of Fick states that:

$$\mathbf{J = - D\ grad}\left[S\right]$$

D: diffusion coefficient or molecular diffusivity of the solute in the solvent ($cm^2 \cdot s^{-1}$).

If the enzyme is immobilized inside the solid (membrane), 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 effective diffusivity is referred to as D_{eff}.

$$J = - D_{eff} \frac{d[S]}{dx}$$

V.1. Aeration and agitation

Make uniform suspension of microbial cells in homogeneous nutrient medium and provide sufficient oxygen.

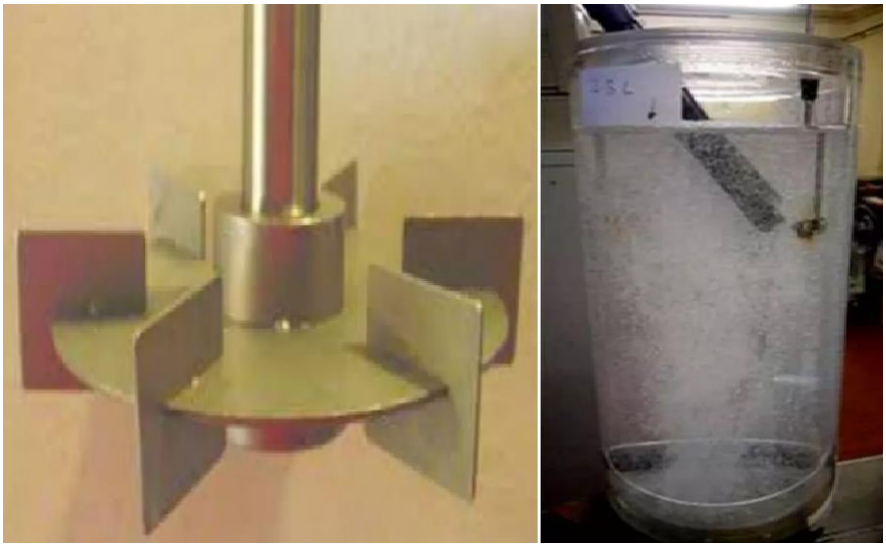


Figure 17 : Aeration / agitation system

V.1. 1. Aération : transfert de matière gaz-liquide

An aeration system is one of the very important parts of a bioreactor. It is important to choose a good aeration system to ensure proper aeration and oxygen availability throughout the culture.

It contains two separate aeration devices (sparger and impeller) to ensure proper aeration in a bioreactor.

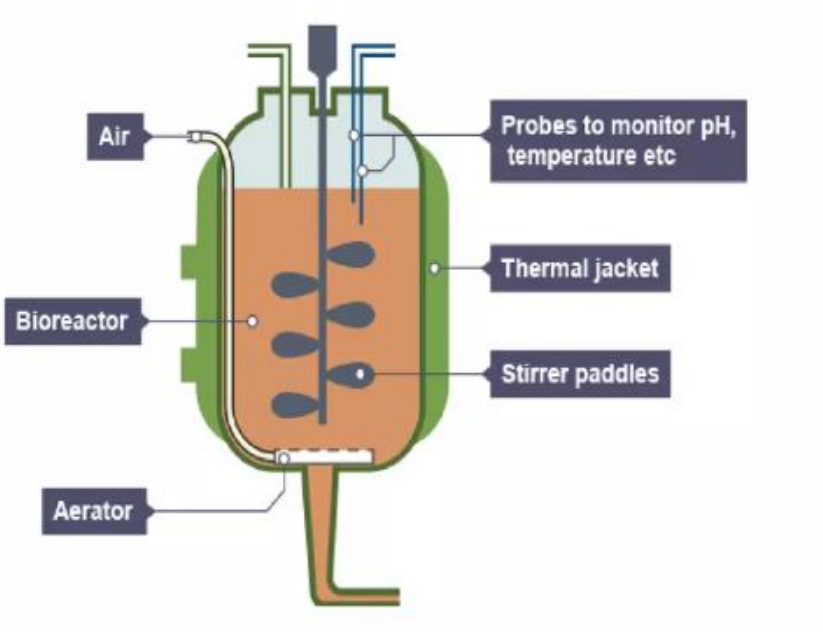


Figure 18 : Aeration in bioreactor

The stirring accomplishes two things:

- It helps to mix the gas bubbles through the liquid culture medium and
- It helps to mix the microbial cells through the liquid culture medium, which ensures the uniform access of microbial cells to the nutrients.

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

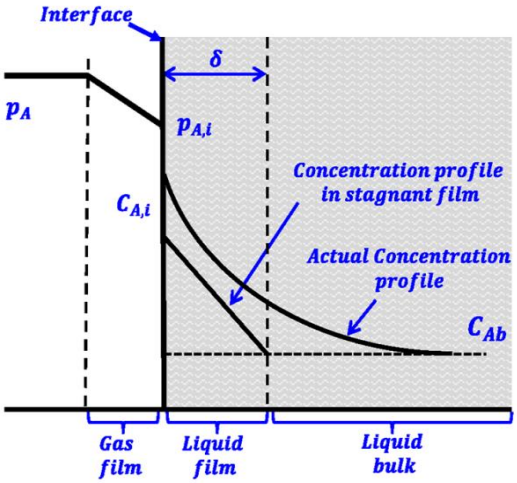


Figure 19 : Gas – liquide transfer

Taking into account the previous considerations, the following equation is accepted to express the transfer rate (dCL/dt) of a gas into a liquid:

$$\frac{dC_L}{dt} = K_L \times a \times \left(C^* - C_L\right)$$

Where C* = gas concentration in the liquid phase at saturation: (mole.L^{-1})

C_L = gas concentration in the liquid phase at time t: (mole.L^{-1})

K_L = transfer coefficient (specific to the gas and liquid): (cm/h)

a = interface area between gas and liquid: (cm^2/cm^3)

This rate (velocity) of transfer is expressed in mole.L^{-1}.h^{-1} and is commonly referred to by the abbreviation OTR (Oxygen Transfer Rate).

It can be observed that the transfer rate will be higher the further the gas concentration is from saturation. Conversely, it will be zero at saturation ($C^* = C_L \rightarrow C^* - C_L = 0$), which is logical.

Since K_L is a typical constant for the studied situation, it is not possible to influence it. It actually represents the speed at which gas molecules can cross the space that separates them from the liquid.

Définitions importantes :

OTR : oxygen transfer rate (capacité de transfert physique)

OUR : oxygen uptake rate (capacité d'absorption biologique)

$$\frac{dC_L}{dt} = k_l a.\underbrace{(C_L^o - C_L)}_{OTR} \underbrace{q_{o_{2X}}}_{OUR}$$

Pour une culture microbienne avec un taux de croissance µ, une concentration en biomasse X et un coefficient de rendement YOX, la demande en oxygène (OUR) est donnée par :

$$OUR = \frac{\mu . X}{Y_{xo}}$$

Oxygen uptake rate (OUR)

Oxygen demand primarily depends on biomass characteristics and its population. Biomass involvement is expressed as Q_{O2} (specific O_2 assimilation rate), which corresponds to the amount of O2 consumed during fermentation per unit of biomass and per unit of time (mole.g^{-1}.h^{-1}). Its value is, of course, dependent on the microorganism's nature (species) and its physiological state.

In the case of batch fermentation, this rate depends on the growth phase in which the population is located.

The total biological oxygen demand (OUR) can thus be written as Q_{O2}. X, with X representing the microbial population.

Apart from any biological activity, the substrate itself can be an oxygen consumer if it is highly reduced and easily oxidizable. Oxygen demand for non-biological oxidations is considered negligible in most fermentations using standardized substrates. However, this is not always the case (see: difference between BOD5 and COD in wastewater treatment).

The following graph illustrates the evolution of key parameters related to oxygen demand and supply in the case of batch fermentation in an aerated bioreactor.

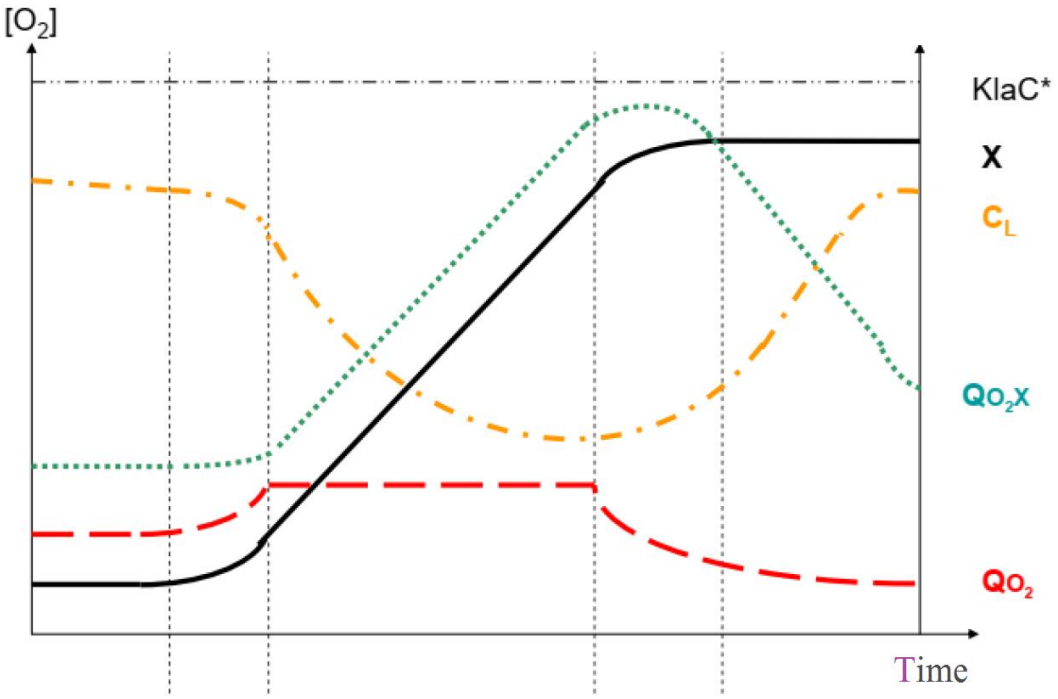


Figure 20: Oxygen demand parameters in a batch aerated bioreactor

Oxygen Transfer rate

The supply of O_2 is ensured by injecting air, or even pure oxygen, into the reactor. However, two major difficulties arise: firstly, the low solubility of oxygen in water, which limits the amount available at any given time, and secondly, the presence of several factors that slow down the transfer of O_2 molecules to the cells.

The gap between oxygen demand and supply can be illustrated with a numerical example. Considering the solubility of O_2 in water to be around +/-8 ppm (8 mg/L) at typical incubation temperatures (20 - 30°C) and referring to the table 5.2 above, it is evident that the oxygen requirements for one minute of fermentation far exceed the available quantity. This means that without aeration, a culture concentrated at 10 g of biomass per liter can consume all the oxygen in the reactor in less than a minute. It should be noted that the biomass's function will be disrupted well before anoxia sets in because oxygen acts as a limiting factor as soon as its concentration falls below the critical concentration (see Monod equation, with O_2 as a substrate).

The first problem, therefore, concerns the solubility of O_2 in the aqueous phase of the substrate. Indeed, this solubility is quite limited and even lower at higher substrate

temperatures. Most industrial fermentations use mesophilic or even thermophilic biomass, and thermal regulation is always favored to maintain the flora at its optimum temperature.

Optimizing Oxygen Transfer

We have two factors that we can manipulate to increase the rate of oxygen transfer from gas bubbles to microorganisms: kL.a and (C_L-C_{L0}).

$$Q = kL.a\ (C_L - C_{L0})$$

Increase (C_L-C_{L0})

- Adjust the solute pressure (increase the oxygen percentage in the air) (pure O_2 x 5)
- Increase the air pressure in the fermentor (2 bars: quantity x 2)

Increase kL.a

- Influence of salt (limited usability)
- Influence of organic compounds (ethanol, limited usability)
- Influence of detergent; favorable at low concentration, unfavorable at high concentration

Increase a

- Increase a through agitation and shear force"

V.1. 2. Agitation: mass transfer by forced convection

In a problem of forced mass convection, the fluid motion is still governed by the conservation of total mass and the theorem of momentum quantities. The fundamental equations for local balance of these two quantities therefore remain unchanged, considering a globally isochoric mixture.

Agitation is a major criterion in bioreactor design. This typically involves maintaining the fermentation temperature. Since fermentation reactions can be highly exothermic (releasing heat), it is essential to thoroughly address this criterion.

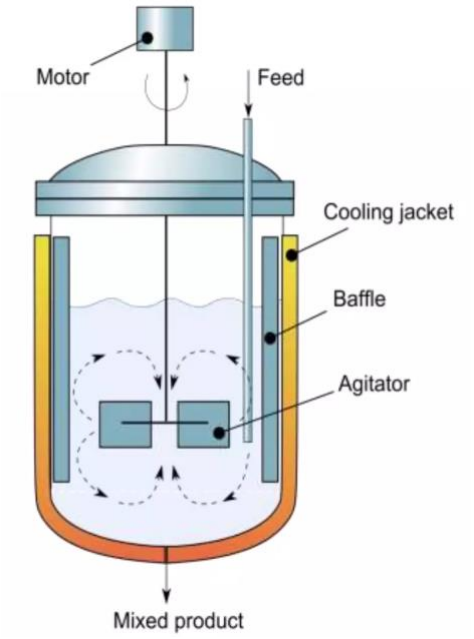


Figure 21: Agitation in bioreactor

Special designs of bioreactor can be used for organisms, which loosely fit into a fourth category. These may be algae, anaerobic bacteria or fungi growing on a solid substrate. Adaptation to the vessel design, geometry and mixing is necessary in these cases. The organisms are often associated with agriculture and biofuel applications (green biotechnology).

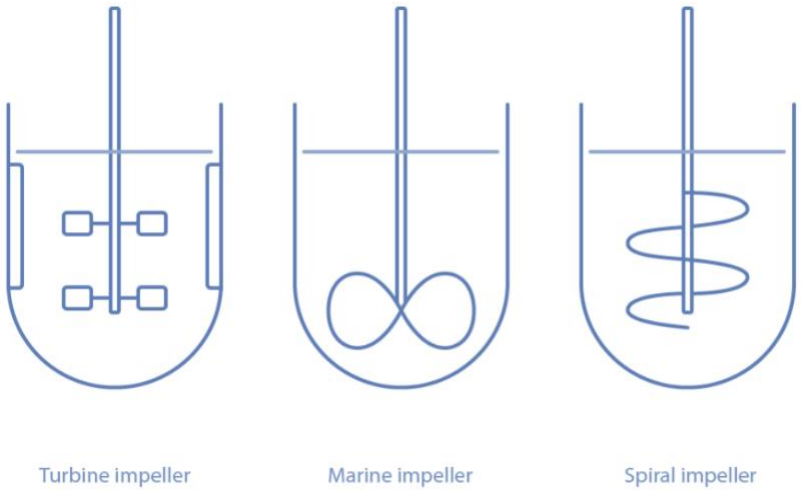


Figure 22 : Impeller designs in stirred tank bioreactor

The Illustration bellow presents the most common impeller designs used in stirred-tank bioreactors:

- Turbine impeller for microbial bioreactors;
- Marine impeller for cell culture bioreactors;
- Spiral impeller for solid substrates.

For mechanical agitation in standard tanks, there are two types of agitation devices with different properties:

Flow motion generated by different impellers types: (a) radial; (b) axial; (c) mixed.

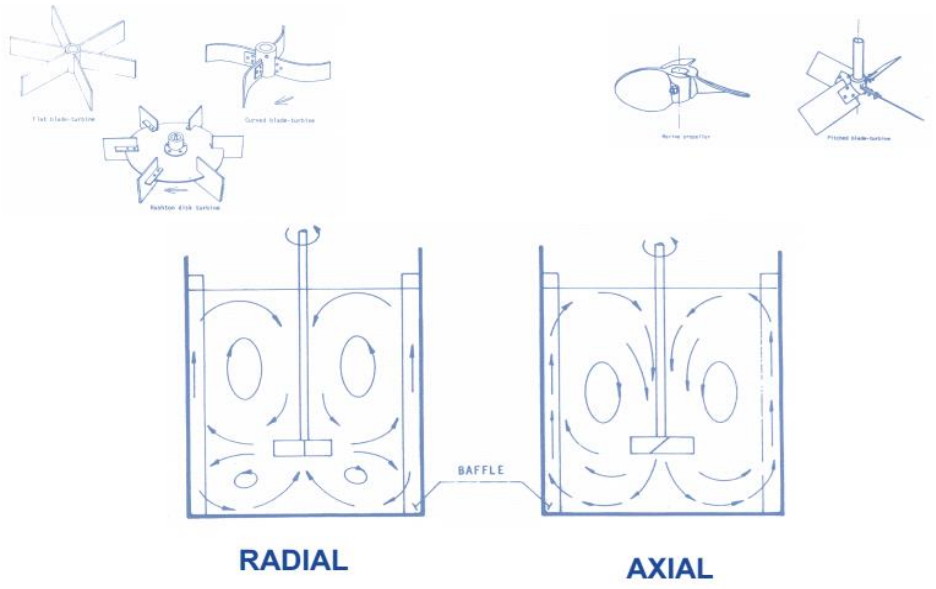


Figure 23: Agitation types in bioreactor

Radial-flow impeller (shear-inducing)

A Rushton turbine is often referred to as a disk turbine.

The disk design ensures that most of the motor power is consumed at the tips of the agitator and thus maximizing the energy used for bubble shearing.

Axial-flow impeller"

For bioreactors that require aeration, air circulation serves a dual purpose (aeration and agitation). This is known as pneumatic agitation or airlift. It is less 'traumatic' for very

delicate cell suspensions and is well suited for aerobic processes. Oxygenation gas alone creates turbulence and maintains cells in homogeneous suspension while ensuring proper mass transfer. The bioreactor's geometry is carefully designed to optimize oxygen transfer (the tank's bottom geometry is conical).

Furthermore, the volume of the culture medium represents only a portion of the total tank volume. Approximately 1/5th to 1/4th of the total free volume is reserved to account for the increase in volume due to air injection, agitation, and foam formation during fermentation."

The agitation accomplishes these things:

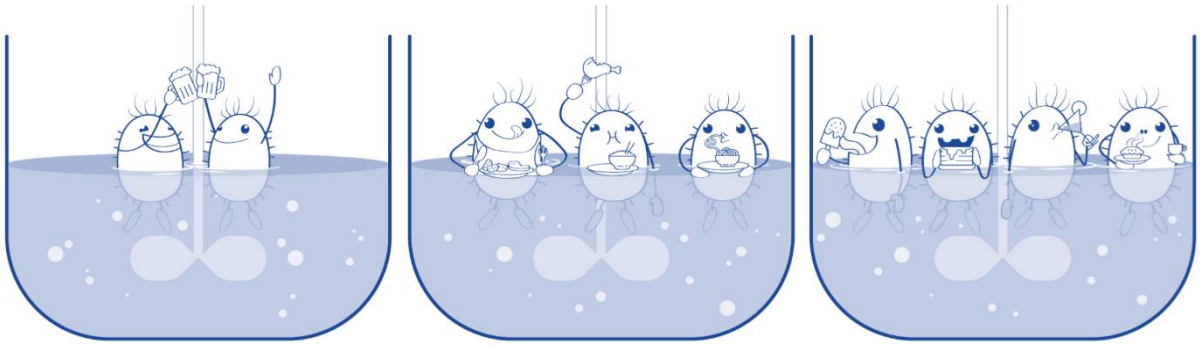
- Bulk fluid and gas phase mixing
- Air dispersion
- O2 transfer
- Heat transfer
- Suspension of solid particles
- Maintenance of uniform environment throughout the vessel.

Mixing Time

The mixing time, t_M, is the time it takes to make the liquid phase homogeneous in concentration following a disturbance

Figure 24: Mixing time evolution in bioreactor

Tutorials



Exercise 1

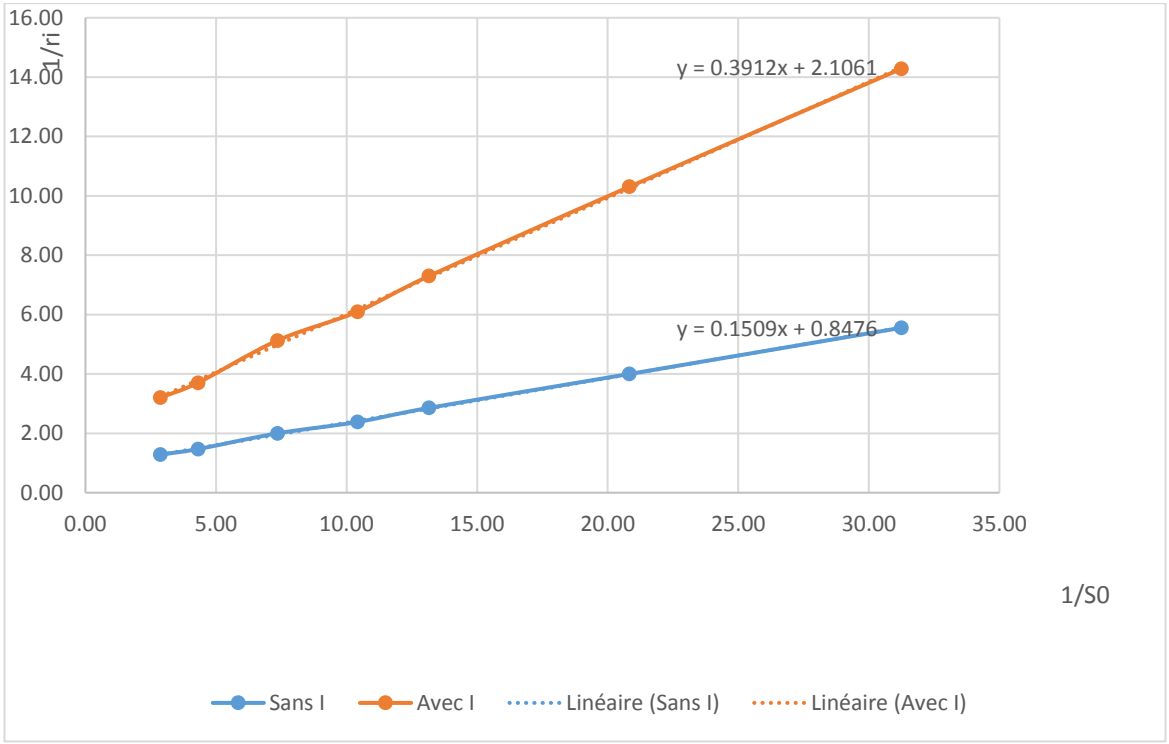
We monitor the enzymatic reaction in the absence and presence of inhibitor I. For various initial substrate concentrations, we obtain the following initial rates:

$[S]_0$ (mM)	ri (M/min)	
	Sans inhibiteur	[I] = 9 mM
0,032	0,18	0,070
0,048	0,25	0,097
0,076	0,35	0,137
0,096	0,42	0,164
0,136	0,50	0,195
0,232	0,68	0,270
0,348	0,78	0,312

1. Determine the kinetic parameters (rmax and KM) in the absence and presence of the inhibitor.
2. Specify the type of inhibition and calculate the inhibition constant KI.

Solution 1

S0	**ri sans inhib**	**ri avec inhib**		**1/S0**	**1/ri ss inhib**	**1/ ri avec inhib**
0,032	0,18	0,07		31,25	5,56	14,29
0,048	0,25	0,097		20,83	4,00	10,31
0,076	0,35	0,137		13,16	2,86	7,30
0,096	0,42	0,164		10,42	2,38	6,10
0,136	0,5	0,195		7,35	2,00	5,13
0,232	0,68	0,27		4,31	1,47	3,70
0,348	0,78	0,312		2,87	1,28	3,21



KM/ rmax avec I	**0,39**	
KM app	0,186	
KM/rmax ss I	0,15	
KM	0,18	
kMapp = (1+ I/KI)*Km	1+ I/KI =kMapp/kM	1,04
	kI (mM)	22,5

1/rmax avec I	**2,11**
rmax avec I	0,47
1/rmas ss I	0,85
rmax ss I	1,18

Inhibition type is : non compétitif car $K_M \approx\approx K_{Mapp}$

Exercise 2

Consider the enzymatic reaction: S -> products

Temps (min)	[S0] mM			
	10	30	100	150
0	0	0	0	0
2,5	0,9	1,9	3,2	3,6
5	1,8	3,9	6,4	7,1
7,5	2,5	5,6	9,6	10,7
12,5	3,7	7,6	15,2	17,6

Determine the kinetic parameters.

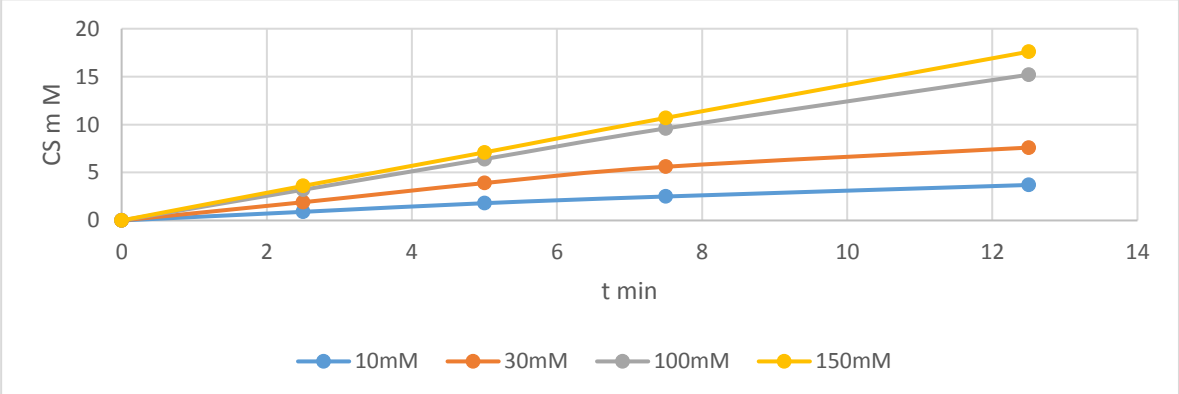
We want to continuously process a 1 mole/liter solution of S in a perfectly stirred enzymatic reactor under steady-state conditions with a constant volume, using a flow rate of 1 liter/minute.

Using the previously found kinetic parameters:

Calculate the volume required to achieve 85% conversion of S.

Solution 2

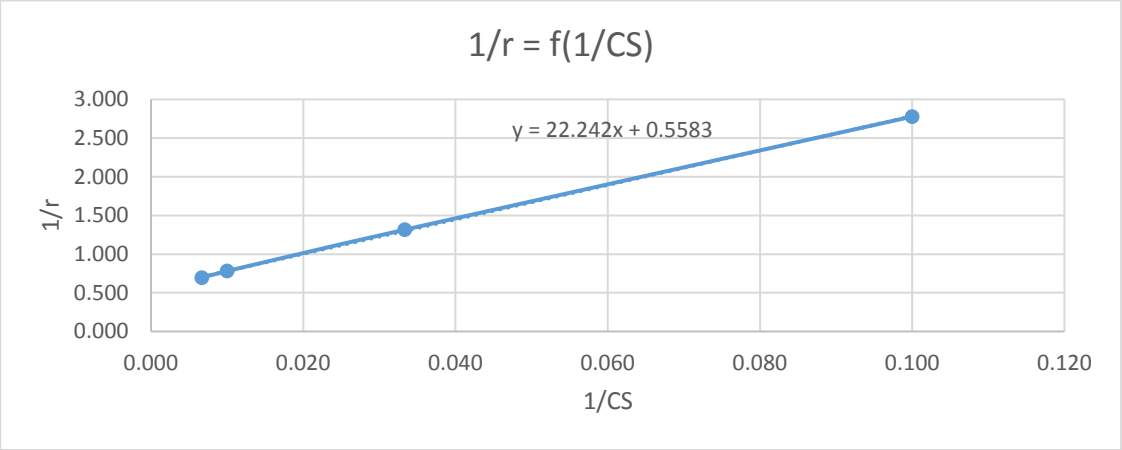
1. Determination of kinetic parameters: Plotting reaction curves



From these curves, we draw tangents to determine the rates of each concentration:

CS	10	30	100	150
r	0,36	0,76	1,28	1,44
1/CS	0,100	0,033	0,010	0,007
1/r	2,778	1,316	0,781	0,694

a. curve 1/ r = f (1/ CS), S : substrate



KM/rmax = 22,24 1/rmax = 0,558 donc rmax = 1,8 mM/l KM = 39,85 Mm

1. Volume ?

$$\tau = \frac{V_R}{Q_0} = \frac{X_{sS} - X_{sE}}{r} = \frac{X_{sS} - X_{sE}}{r_{max} \cdot \frac{c_s}{K_M + C_S}} \; avec \; c_s = c_{s0}(1 - X_{SS}) \;\; soit \; V : cst$$

$$V_R = Q_0 . \frac{X_{sS} - X_{sE}}{r_{max} \cdot \frac{c_S}{K_M + c_S}}$$

$$V_R = 1. \frac{0{,}85\,(39{,}85 + 1{,}8(1 - 0{,}85))}{1{,}8\,(1 - 0{,}85)} = 126{,}30\; l$$

Exercise

The compound 1-anilino-8-naphthalene sulfonate (ANS) is an inhibitor of acetylcholinesterase. To determine the nature of the inhibition, we measured the initial hydrolysis rates of acetylcholine at various concentrations of this substrate, in the absence of ANS (exp A), and in the presence of ANS (2.05 x 10^-4 M) (exp B). The following table represents these experiments (A & B). The rates are expressed in µM of acetylcholine hydrolyzed per minute per mg of protein.

[Acétylcholine] (mM)	Vitesses de la réaction	
	A	B
0,165	66	40
0,220	82	45
0,330	107	52
0,450	130	56,5
0,550	143	59
0,660	156	61

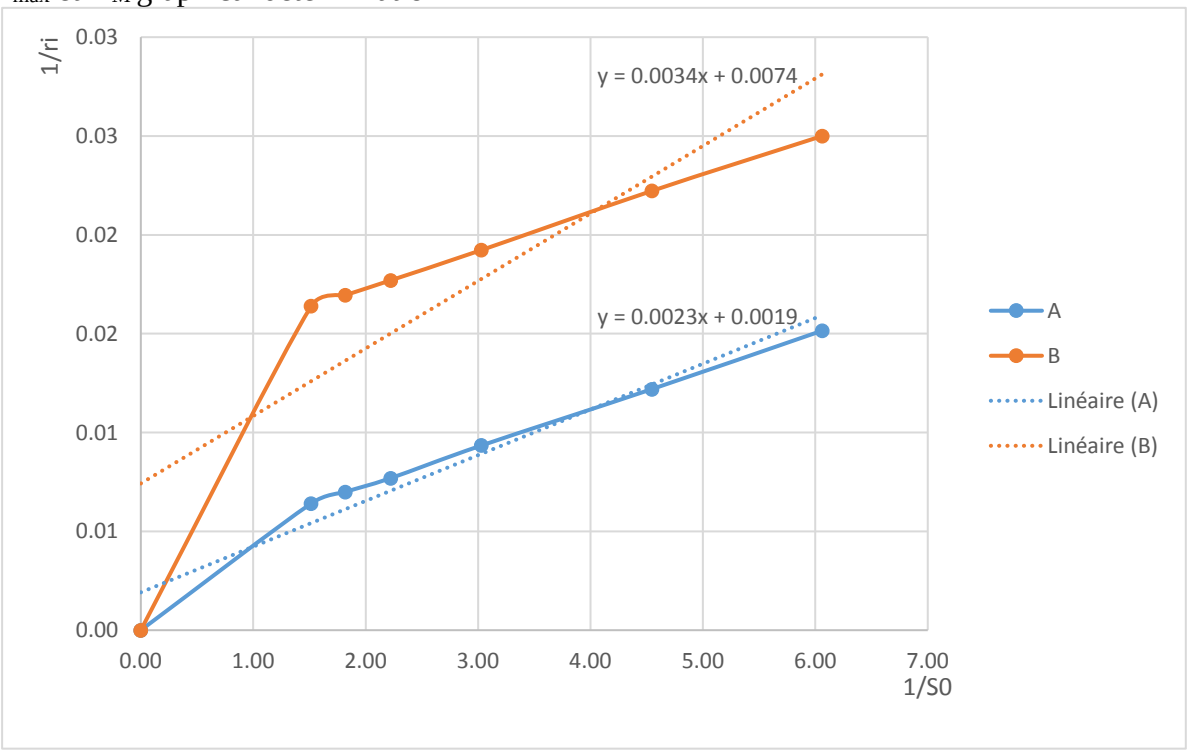
Specify the type of inhibition and calculate the inhibition constant KI.

Solution:

To determine the type of inhibitor, one should first determine the values of rmax and K_M for comparison. For this purpose, the **double inverse** method is employed

1/S0	1/ri A	1/ ri B
6.06	0.02	0.03
4.55	0.01	0.02
3.03	0.01	0.02
2.22	0.01	0.02
1.82	0.01	0.02
1.52	0.01	0.02

r_{max} et K_M graphical determination



1/rmax B	0.0135	**Km/ rmax B**	0.0019
rmax B µM/min.mg	74.0741	**Km app** µM	0.1407
1/rmas A	0.0035	**Km/rmax A**	0.0019
rmax A µM/min.mg	285.7143	**Km** µM	0.5429

NOTE : Neither rmax nor K_M are the same, so the inhibitor is of the type: Non-competitive (same slope for both lines)

K_I= ?

$K_{Mapp} = K_M / (1+ (I /K_I))$ $K_I = I / ((K_M / K_{Mapp}) -1) = 7.175\ 10^{-5}$ M

Exercice :

The hydrolysis of sucrose (S) catalyzed by an enzyme E was studied by measuring the initial rate (r0) for a series of initial concentrations of sucrose (CS0) at specific temperature and enzyme conditions. The following results are extracted from the publication by CHASE et al. in 1962.

C_{S0} mol L^{-1}	**0.0292**	**0.0584**	**0.0876**	**0.117**	**0.146**	**0.175**	**0.234**
r_0 mol L^{-1} s^{-1}	0.182	0.265	0.311	0.330	0.349	0.372	0.371

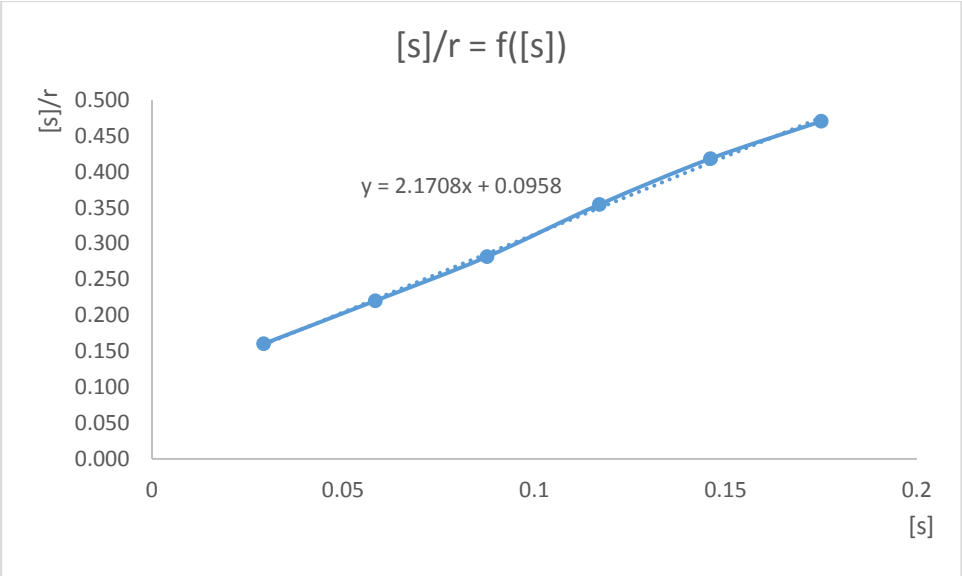
Using the Michaelis-Menten model, determine the values of rmax and the constant KM.

Solution

Méthode de hans :

$$\frac{[S]}{r} = \frac{K_m}{r_{max}} + \frac{[S]}{r_{max}}$$

CS	0.0292	0.0584	0.0876	0.117	0.146	0.175
r0	0.182	0.265	0.311	0.33	0.349	0.372
CS/r0	0.160	0.220	0.282	0.355	0.418	0.470



1/rmax	2.170
r max mol/l.s	0.460
KM/rmax	0.095
KM mol/l	0.044

Exercice:

The enzymatic decomposition reaction of a substrate S (CSo=0.6 M, CEo=0.008 M) is conducted in a 10 L piston reactor. For varying flow rates, the corresponding CS values were recorded. The rate equation for this type of reaction is given below. Here are the CS values:

C_S , mol/L	0,35	0,16	0,4	0,1
Q_o , L/h	10	5	3,5	2

- Determine the rate equation for this reaction, assuming it follows the Michaelis-Menten model.
- Deduce the volume of a single stirred reactor capable of achieving 78% conversion for a feed stream containing the substrate and enzyme (Qo=15 L/h, CSo=0.05 mol/L, CEo=0.008 mol/L).

solution

$$r = \frac{r_{max}.S}{K_M + S}$$

$$ts = \int_S^{So} \frac{dS}{-rS} = \int_S^{So} \frac{dS}{\frac{r_{max}.S}{K_M + S}} = \frac{1}{r_{max}} \int_S^{So} \frac{K_M + S}{S} dS = \frac{1}{r_{max}} \left(\int_S^{So} \frac{K_M}{S} dS + \int_S^{So} dS \right)$$

$$\tau = \frac{1}{r_{max}} \left(K_M \ln \frac{So}{S} + (So - S) \right)$$

Ou la forme linéarisée :

$$\frac{So - S}{\ln \frac{So}{S}} = -KM + r_{max} \frac{\tau}{\ln \frac{So}{S}}$$

Des données du tableau suivant, on trace la droite dont la pente (rmax) et l'ordonnée à l'origine est KM

Cs M	0,35	0,16	0,4	0,1
Qo, L/h	10	5	3,5	2
Temps de passage, h	1	2	2,8571429	5
So-S	0,25	0,44	0,2	0,5
ln So/S	-1,39	-0,82	-1,61	-0,69
t/ln So/S	-0,72	-2,44	-1,78	-7,21
So-S/lnSo/S	-0,18	-0,54	-0,12	-0,72

Les valeurs des paramètres de l'équation de vitesse sont donc : KM=0.1308 M et rmax=0.0855 M/h

Et l'équation de lavitesse est donc :

$$r = \frac{0.0855.S}{0.1308 + S}$$

V_R= 88.2 L

Exercise:

Glucoamylase hydrolyzes maltose into glucose according to the reaction:

Maltose + H2O ----------> 2 glucose

We want to carry out a continuous enzymatic reactor involving glucoamylase, an enzyme with the following kinetic parameters determined at 40°C (Michaelis-Menten enzyme):

rmax = 0.02 moles of maltose min^-1 L^-1

KM = 35 mmol/L

We aim to continuously process a maltose solution at 1 mole/liter with a flow rate of 1 L/min while achieving 95% conversion of maltose.

For each of the cases below, perform a mass balance (constant flow rate and reactor volume, steady-state) and calculate the reactor volume required to achieve the desired conversion:

- In the case of a perfectly stirred reactor
- In the case of a piston reactor.

The hydrolysis of sucrose (S) catalyzed by an enzyme E was studied by measuring the initial rate (r0) for a series of initial concentrations of sucrose (CS0) at a specific temperature and enzyme conditions. The following results are extracted from the publication by CHASE et al. in 1962.

C_{S0} mol L^{-1}	0.0292	0.0584	0.0876	0.117	0.146	0.175	0.234
r_0 mol L^{-1} s^{-1}	0.182	0.265	0.311	0.330	0.349	0.372	0.371

Based on the Michaelis-Menten model, determine the value of rmax and the Michaelis constant.

SOLUTION

Linear regression of the data given, according to equation 10.3-2, results in $V_{max} = 0.46$ mol $L^{-1}s^{-1}$, and $K_m = 0.043$ mol L^{-1}. Figure 10.2 shows the given experimental data plotted according to equation 10.3-2. The straight line is that from the linear regression; the intercept at $1/c_{S_0} = 0$ is $1/V_{max} = 2.17$ mol^{-1} L s, and the slope is $K_m/V_{max} = 0.093$ s.

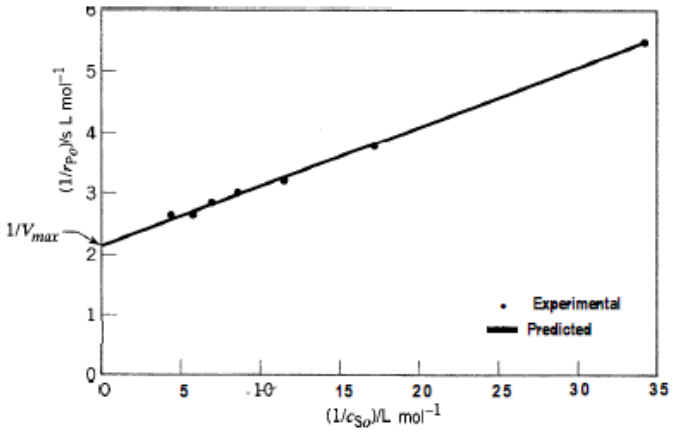


Figure 10.2 Lineweaver-Burk plot for Example 10-1

Exercise

We monitor the hydrolysis kinetics of A by E1, respectively in the absence of an inhibitor and in the presence of E2, E3, or E4.

The values of the obtained initial velocities are as follows:

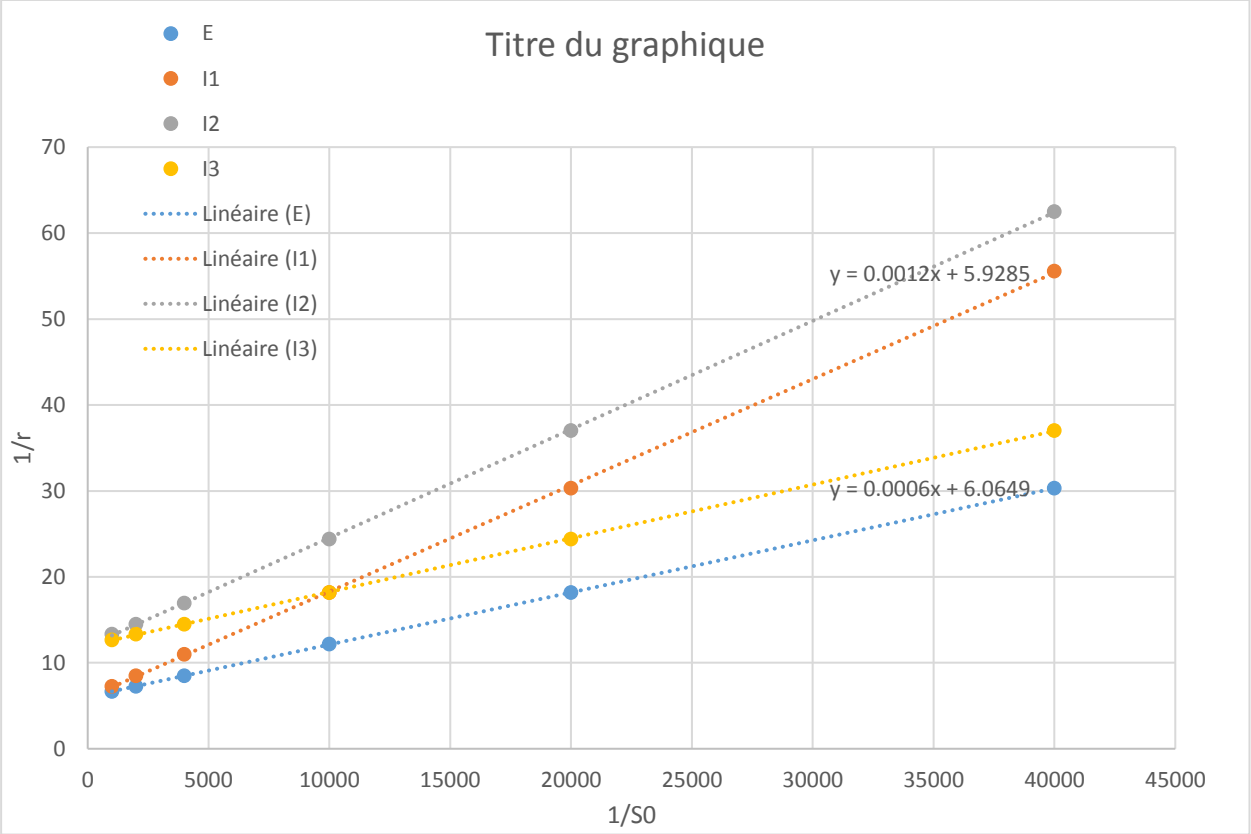
$[S_0]$ (M)	ri ($\mu M.min^{-1}$)			
	Sans I	[E2] = $3\ 10^{-4}$ M	[E3]= 0,26 M	[E4] = 0,17 M
0	0	0	0	0
$2,5\ 10^{-5}$	0,033	0,018	0,016	0,027
$5\ 10^{-5}$	0,055	0,033	0,027	0,041
$1\ 10^{-4}$	0,082	0,055	0,041	0,055
$2,5\ 10^{-4}$	0,118	0,091	0,059	0,069
$5\ 10^{-4}$	0,138	0,118	0,069	0,075
$1\ 10^{-3}$	0,150	0,138	0,075	0,079

1. Determine r_{max} and K_M using the representation of your choice in the absence of inhibitors.
2. Determine the kinetic parameters $r_{max\ app}$ and K_{Mapp} in the presence of inhibitors.
2. Calculate the inhibition constants KI.
3. Explain the type of inhibition observed for each of the inhibitors in this exercise.

Solution

1&2. The kinetic parameters rmax and KM in the absence and presence of inhibitors.

1/S0	1/rE	1/rI1	1/Ri2	1/Ri3
40000	30,3030303	55,5555556	62,5	37,037037
20000	18,1818182	30,3030303	37,037037	24,3902439
10000	12,195122	18,1818182	24,3902439	18,1818182
4000	8,47457627	10,989011	16,9491525	14,4927536
2000	7,24637681	8,47457627	14,4927536	13,3333333
1000	6,66666667	7,24637681	13,3333333	12,6582278



The linear form of the Michaelis & Menten equation is:

$$\frac{1}{r} = \frac{Km}{r_{max}} \cdot \frac{1}{[S_0]} + \frac{1}{r_{max}}$$

	E	**I1**	**I2**	**I3**
$\frac{1}{r_{max}}$	6,0649	5,9285	11,92	12,004
r_{max}	0,165	0,169	0,084	0,083
$\frac{Km}{r_{max}}$	0,0006	0,0012	0,0006	0,0013
Km	$9{,}89.10^{-5}$	$2{,}02.10^{-4}$	$1{,}08.10^{-4}$	$5{,}00.10^{-5}$
Type d'inhibition		compétitive	Non compétitive	incompétitive
Expression de K_I A partir de l'équation de vitesse		$\frac{K_M . [I_0]}{K_M^{app} - K_M}$	$\frac{V_M^{app} . [I_0]}{V_M - V_M^{app}}$	$\frac{V_M^{app} . [I_0]}{V_M - V_M^{app}}$
K_I		0,0003	0,2696	0,1721

Explanation of the observed inhibition type for each inhibitor:

1. The enzyme of reaction E has nearly the **same maximum rate** (rmax) as I1, so enzyme I1 is **competitive** with E.

2. The enzyme of reaction E has almost **the same Michaelis constant** (Km) as I2, so enzyme I2 is **non-competitive** with E.

3. For increasing inhibitor concentrations ([E] < [I3]), **the lines are parallel**, so enzyme I3 is **uncompetitive** with E

Exercise

Glucoamylase hydrolyzes maltose into glucose according to the reaction:

Maltose + H2O ----------> 2 glucose

We want to carry out a continuous enzymatic reactor involving glucoamylase, an enzyme (Michaelian Enzyme) with the following kinetic parameters determined at 40°C:

rmax = 0.02 moles of maltose (min)^(-1) (liter)^(-1)

KM = 35 mmol/liter

We aim to continuously process a maltose solution at 1 mole/liter with a flow rate of 1 liter/min while achieving 95% conversion of maltose.

For each case below, calculate the reactor volume required to achieve the desired conversion:

- In the case of a perfectly stirred reactor.
- In the case of a piston reactor.

Solution

Volume du réacteur

Maltose + H2O ----------> 2 glucose

L'état physique de cette réaction est liquide donc il n'y aura pas de changement de volume et donc : [S] = [S0]. (1-X), et la vitesse de réaction selon le modèle de Michaelis Menten : $r = \frac{r_{max}.[S]}{K_M+[S]}$

1. dans le cas d'un réacteur parfaitement agité

$$\tau = \frac{V_R}{Q_0} = \frac{C_0(X_S - X_{E)}}{r} \; avec \; r = \frac{0{,}02.[S]}{35.10^{-3} + [S]}$$

$$V_R = Q_0\left(\left(\frac{K_M.X}{r_{max}.(1-X)}\right) + \frac{C_0}{r_{max}}\right.$$

AN: V_R = 83,25 L

2. dans le cas d'un réacteur piston

$$\tau = \frac{V_R}{Q_0} = C_0.\int_{X_E}^{X_S}\frac{dX}{r} = C_0.\int_{X_E}^{X_S}\frac{dX}{\frac{r_{max}.[S]}{K_M+[S]}}$$

$$V_R = Q_0C_0\left[-\frac{K_M}{r_{max}}\ln(1-X_S)\right]_0^{0,95} + \left[\frac{X_S}{r_{max}}\right]_0^{0,95}$$

AN: V_R = 52,74 L

Exercise

We are studying an enzymatic reaction (A ---> B) catalyzed by an enzyme E by monitoring the kinetics of the appearance of B:

	$[A]_0$ mM				
t min	1	2	5	10	20
0	0	0	0	0	0
1	1,5	2,4	3,8	4,8	5,6
2	2,7	5,2	8	10	12
3	3,4	6,8	12	15	18
4	4,3	7,9	19	18	23
5	5	9	22	22	27
6	5,8	10	24	24	30

- Plot the kinetics.

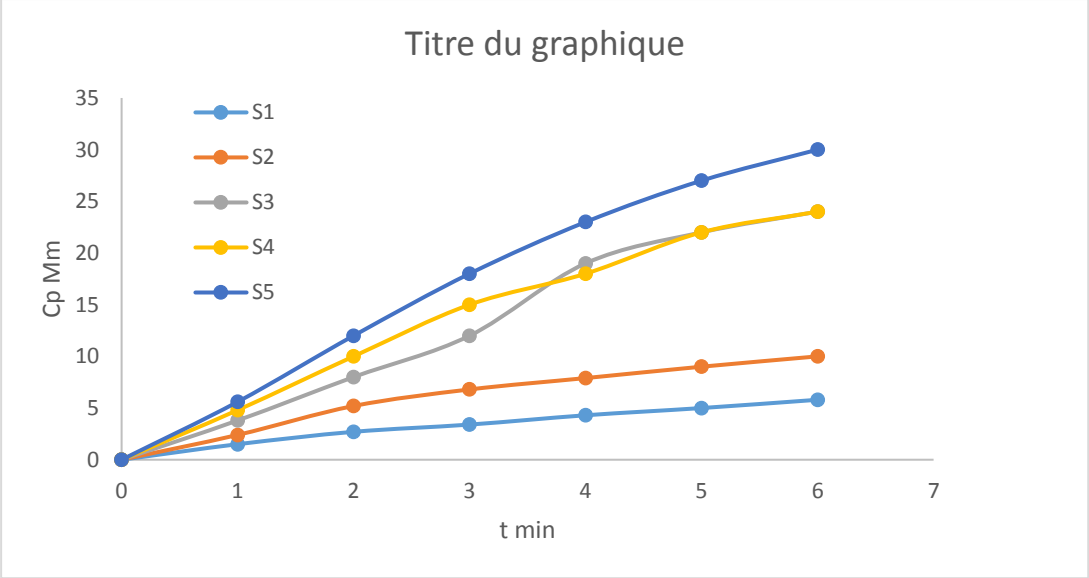
This same reaction was studied in the presence of two other enzymes. The table below represents the initial velocities for each enzyme.

	r_0 en mM/min		
$[A]_0$ mM	E1 [I] = 0	E2 [I] = 3,5mM	E3 [I] =6,5mM
1	……..	1,05	0,59
2	……..	1,68	1,08
5	……..	2,65	2,15
10	……..	3,35	3,22
20	……..	3,91	4,27

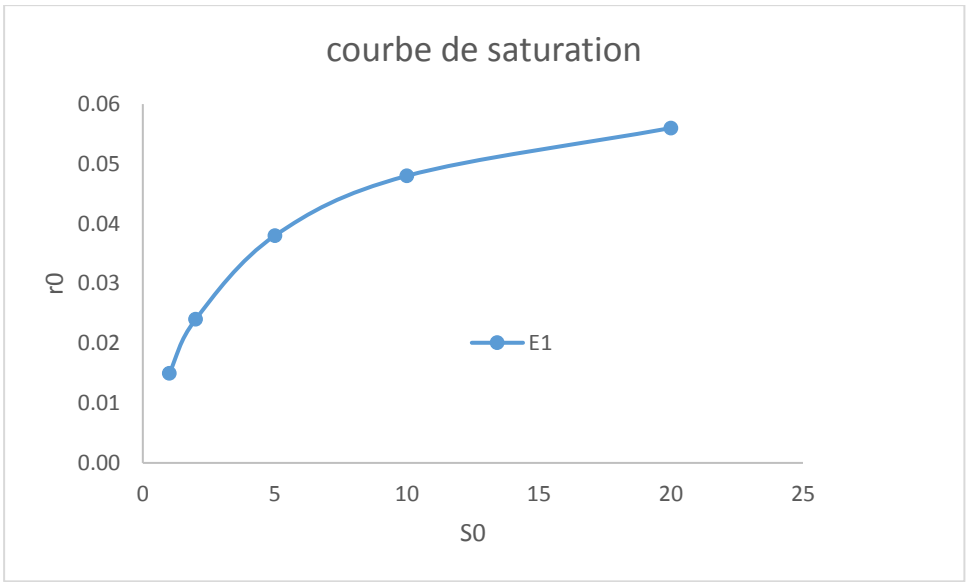
- Complete the table.
- Determine the kinetic parameters of the enzyme in the absence of inhibitors using the saturation curve.
- Determine the kinetic parameters of the enzyme in the absence and presence of inhibitors using the double reciprocal method (Explain the method and provide the principles of the other two).

- Compare the values of the kinetic parameters of enzyme E1 for both determination methods. Comment on your results.
- Conclude the type of inhibitors E2 and E3 and justify.
- Calculate KI.

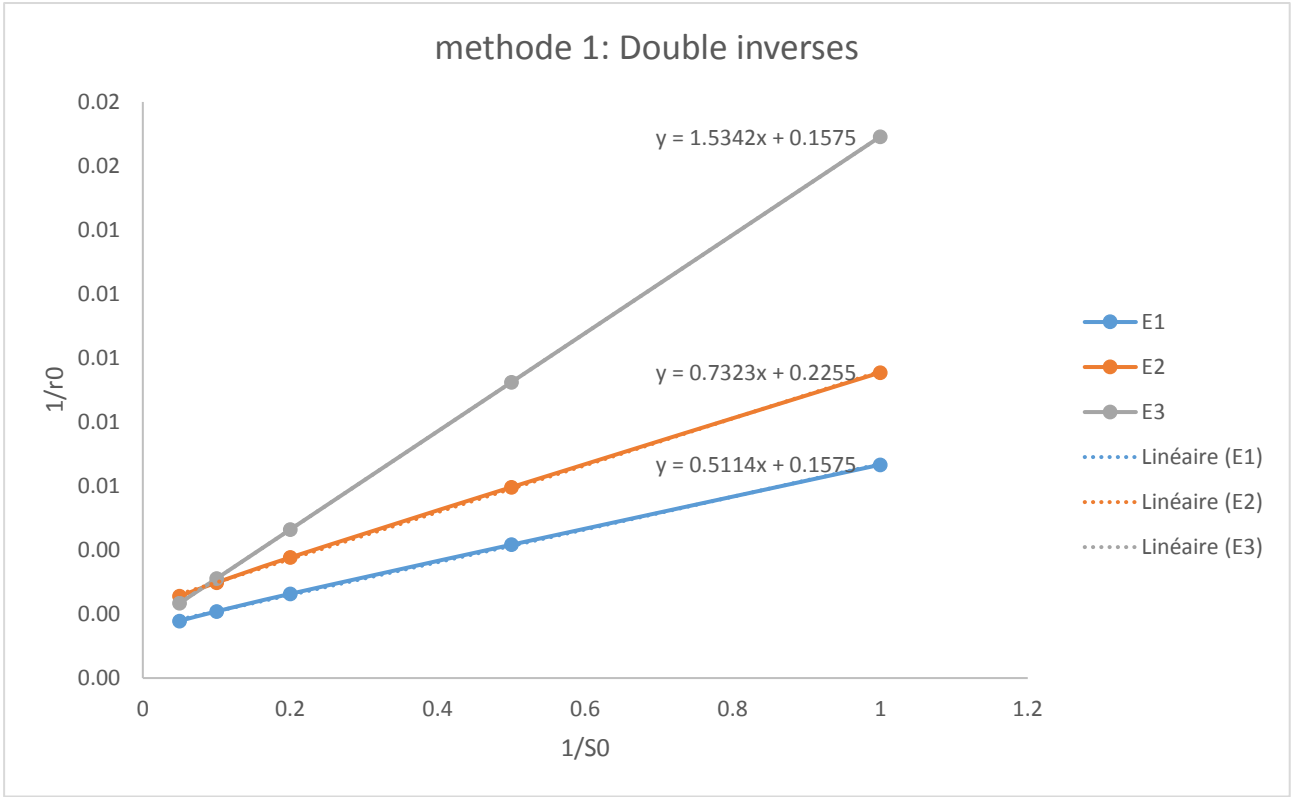
Solution



s	r0		
	E1	E2	E3
1	1,50	1,05	0,59
2	2,40	1,68	1,08
5	3,80	2,65	2,15
10	4,80	3,35	3,22
20	5,60	3,91	4,27
		r max	5,6 Mm/min
		Km	3,1 mM



1/S	1/r0		
	E1	E2	E3
1	0,67	0,95	1,69
0,5	0,42	0,60	0,92
0,2	0,26	0,38	0,46
0,1	0,21	0,30	0,31
0,05	0,18	0,26	0,23



Parameters	E1	with inhi	E2	E3	justificatif
1/rmax	0,1575	1/rmax app	0,2255	0,1575	
KM/rmax	0,5114	KM/rmax app	0,7323	1,5342	
r max	6,349	r max app	4,435	6,349	E1 E3 the same rmax
KM	3,247	KM app	3,247	9,741	E1 E2 the same K_M
Type of inhibiteur			Non compétitives	compétitivess	
K_I			rmaxapp = rmax.(1+ [I]/KI) KI= 3,54 mM	Kapp = KM.(1+ [I]/KI) KI= 1,75mM	

Exercise:

An enzyme catalyzes the isomerization of a double bond in an unsaturated fatty acid.

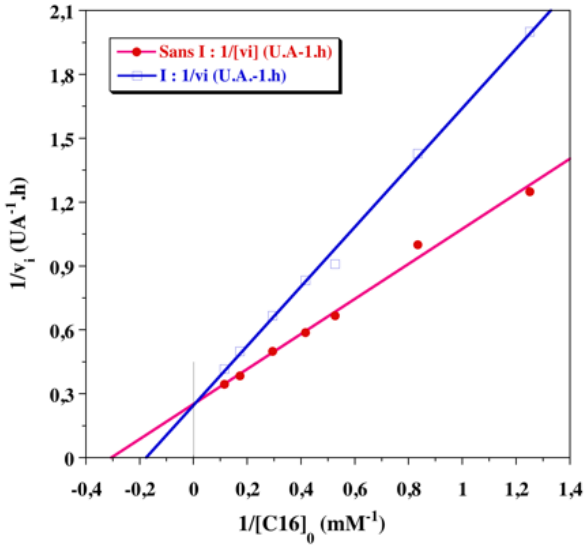
We are studying the reaction catalyzed by this enzyme, in the absence and presence of an inhibitor I. We monitor the enzymatic reaction by measuring the change in absorbance due to the appearance of the product. For various substrate concentrations, we obtain the following initial velocities:

$[S]_0$ (mM)	r_0 (U.A.h^{-1}) $[I_0]$ = 55 µM	r_0 (U.A.h^{-1})
0,8	0,5	0,8
1,2	0,7	1,1
1,9	1,1	1,5
2,4	1,2	1,7
3,4	1,5	2,0
5,8	2,0	2,6
8,7	2,4	2,9

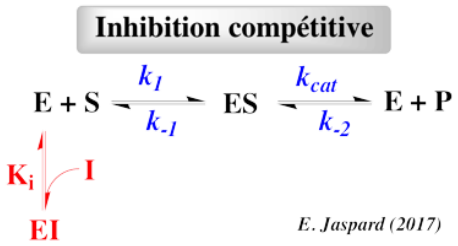
- Explain the method for obtaining initial velocities.

- Determine the kinetic parameters (rmax and KM) and the kinetic parameters in the presence of the inhibitor using a graphical method of your choice.
- Concentrations should be expressed in molarity. Specify the type of inhibition and calculate the inhibition constant KI

Solution:



Il s'agit d'un inhibiteur compétitif.



A partir des valeurs déterminées graphiquement, on calcule : K_I = (K_M x [I]) / (K_M^{app} - K_M) = 73,3 µM

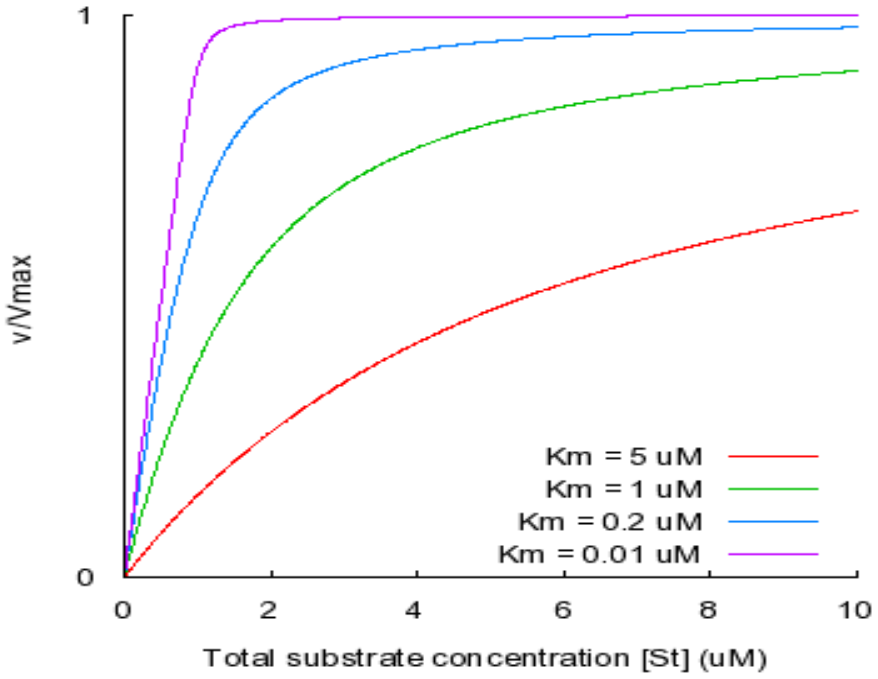
Paramètres	Sans I	[I_0] = 55 µM
1/r_M ou 1/r_M^{app} (UA^{-1}.h)	0,26	
r_M ou r_M^{app} ($mM.h^{-1}$)	0,22	
-1/K_M ou -1/K_M^{app} (mM^{-1})	- 0,31	- 0,18
K_M ou K_M^{app} (mM)	3,2	5,6
Type inhibition	-----	Compétitive
K_I (µM)	-----	73,3

Exercise

Let's assume that the concentration of a substrate S is 10^-4 M. Also, suppose that enzyme A has a Km value of 10^-3 M for this compound, and enzyme B has a Km value one hundred times smaller, i.e., Km = 10^-5 M, for the same compound.

- Which enzyme will modify the substrate X first (in other words, which enzyme has the highest affinity for substrate X)?

Solution



Applications

Problem 1/

Substrate A and enzyme E flow through a mixed flow reactor (V = 6 liter).

From the entering and leaving concentrations and flow rate find a rate equation to represent the action of enzyme on substrate.

C_{E0}, mol/liter	C_{A0}, mol/liter	C_A, mol/liter	v, liter/hr
0.02	0.2	0.04	3.0
0.01	0.3	0.15	4.0
0.001	0.69	0.60	1.2

Problem 2:

At room temperature sucrose is hydrolyzed by the enzyme sucrase as follows:

$$\text{sucrose} \xrightarrow{\text{sucrase}} \text{products}$$

Starting with sucrose (C,, = 1 mol/m3) and sucrase (CEO = 0.01 mol/m3) the following data are obtained in a batch reactor (concentrations are calculated from optical rotation measurements)

C_A, mol/m^3	0.68	0.16	0.006
t, hr	2	6	10

Find a rate equation to represent the kinetics of this reaction.

Problem 3

In a number of separate runs different concentrations of substrate and enzyme are introduced into a batch reactor and allowed to react. After a certain time the reaction is quenched and the vessel contents analyzed.

From the results found below find a rate equation to represent the action of enzyme on substrate.

Run	C_{E0}, mol/m^3	C_{A0}, mol/m^3	C_A, mol/m^3	t, hr
1	3	400	10	1
2	2	200	5	1
3	1	20	1	1

Problem 4/

Carbohydrate A decomposes in the presence of enzyme E. We also suspect that carbohydrate B in some way influences this decomposition. To study this phenomenon various concentrations of A, B, and E flow into and out of a mixed flow reactor (V = 240 cm3).

C_{A0}, mol/m^3	C_A, mol/m^3	C_{B0}, mol/m^3	C_{E0}, mol/m^3	v, cm^3/min
200	50	0	12.5	80
900	300	0	5	24
1200	800	0	5	48
700	33.3	33.3	33.3	24
200	80	33.3	10	80
900	500	33.3	20	120

(a) From the following data find a rate equation for the decomposition.

(b) What can you say about the role of B in the decomposition?

(c) Can you suggest a mechanism for this reaction?

Problem 5/

Enzyme E catalyzes the decomposition of substrate A. To see whether substance B acts as inhibitor we make two kinetic runs in a batch reactor, one with B present, the other without B. From the data recorded below

Run 1. $C_{A0} = 600$ mol/m^3, $C_{E0} = 8$ gm/m^3, no B present

C_A	350	160	40	10
t, hr	1	2	3	4

Run 2. $C_{A0} = 800$ mol/m^3, $C_{E0} = 8$ gm/m^3, $C_B = C_{B0} = 100$ mol/m^3

C_A	560	340	180	80	30
t, hr	1	2	3	4	5

(a) Find a rate equation to represent the decomposition of A.

(b) What is the role of B in this decomposition?

(c) Suggest a mechanism for the reaction.

Problem

To calculate the time required (residence time) to transform 100 moles of glucose into ethanol in a bioreactor, we can assume, for simplicity, that the bioreactor is a batch reactor (closed reactor) with a constant volume equal to 0.01 m^3. This simplifying assumption allows us to estimate the time t required to reach a final conversion rate Xf of 0.3. According to this assumption, the time t (residence time) is given by:

$$t = N_{A0} \int_0^{X_f} \frac{dX}{V.r}$$

Where:

NA0 is the number of moles of glucose at the beginning of fermentation.

V is the volume of the bioreactor.

X is the conversion rate.

Xf is the final conversion rate of the reaction.

r is the reaction rate of fermentation.

1. Write the material balance equation for reactant A (glucose) with an initial concentration CA0 that decomposes according to the reaction, assuming no volume variation in the uniform closed reactor. Find the expression for the residence time t as given above.

2a - Calculate the time t in minutes if the reaction rate r is first-order and is of the form:

$$r = k\, C_{A0}\, (1\text{-}X)$$

Where k = 1 h-1 is the reaction rate constant, and CA0 = 104 mole/m3 is the initial concentration.

2b - Calculate the time t in minutes if the reaction is enzymatic and follows the form:

$$\boldsymbol{r} = \frac{k\, C_A}{1+K_2\, C_A}$$

With

$C_A = C_{A0}(1-X)$

$k = 1h^{-1}$, $C_{A0} = 10^4$ mole/m^3, et $K_2 = 10^{-5}$ m^3/mole

Problem

In a semi-continuous enzymatic reactor, the hydrolysis of a sucrose solution by invertase is carried out. Determine the time elapsed when the reactor volume becomes 10 times the initial volume. Given:

- The enzyme follows the Michaelis-Menten kinetics.
- Feed concentration of substrate S0 = 50 g/L
- Substrate concentration in the reactor Sc = 10 g/L
- Initial reactor volume V0 = 1 liter
- Feed rate Q = 5 L/h
- KM = 0.5 g/L
- Maximum specific rate of hydrolysis rmax = 2.5 g/h/mg of enzyme
- Enzyme concentration E = 2 mg/L

MORE EXERCICE in French language in Annex 3

Annex

Annex 1

The following table lists some other non-structured models encountered in the literature. Most of these are more precise than the Monod equation and allow for describing other phases of the growth curve (aside from the exponential phase).

All these models are established for batch culture and can potentially be modified and adapted for semi-continuous and continuous cultures.

Table summarizing the list of main models used in the study of fermenter cultures.

Modèle de	Equations	Descriptions
Monod (1949)	$\mu = \mu_m \cdot \frac{S}{K_s + S}$	Modèle hyperbolique K_S : contante de saturation
Teissier (1936)	$\mu = \mu_m \cdot [1 - \exp(-S/K_S)]$	Exprime le contrôle de la diffusion du substrat
Moser (1958)	$\frac{1}{\mu} = \frac{1}{\mu_m} + \frac{K_s}{\mu_m} \cdot \frac{1}{S^n}$	Analogie avec la cinétique des enzymes allostériques
Contois (1959)	$\mu = \mu_m \cdot \frac{S}{K_1.X + S}$	Modèle hyperbolique K_1 : contante
Powell (1967)	$\mu = \mu_m \cdot \frac{S}{(K_2 + K_3.D) + S}$	K_2 et K_3: contantes D : diffusion du substrat
Edwards et Wilke (1968)	$\mu = \mu_m .(1 - \frac{X}{X_f}).(a_1 + 2.a_2.t + 3.a_3.t^2 + ...)$ avec X_f = biomasse finale	Fonction polynomiale du temps a_1, a_2, a_3,... : constantes
Herbert (1967)	$\mu = \mu_m \cdot \frac{S}{K_s + S} - K_d$	K_d : taux de mortalité
Edwards (1970)	$\mu = \mu_m \cdot \frac{S}{K_s + S} \cdot e^{-S/Ki}$	inhibition par le substrat K_i : constante d'inhibition
Edwards (1970)	$\mu = \mu_m \cdot [e^{-S/Ki} - e^{-S/Ks}]$	inhibition par le substrat selon l'équation de Teissier
Andrews (1968)	$\mu = \mu_m \frac{S}{K_S + S + S^2/K_i}$	inhibition par le substrat K_i : constante d'inhibition
Luong (1987)	$\mu = \mu_m \cdot \frac{S}{K_s + S} \cdot [1 - \frac{S}{S_m}]^n$	S_m : concentration limite > il n'y a plus de croissance
Hinshelwood (1946)	$\mu = (\mu_m - K_i.P) \cdot \frac{S}{K_s + S}$	inhibition par le produit K_i : constante d'inhibition
Aiba (1968)	$\mu = \mu_m \cdot \frac{S}{K_s + S} \cdot e^{-P/Ki}$	inhibition par le produit K_i : constante d'inhibition
Nagatani (---)	$\mu = \mu_m \cdot \frac{S}{K_s + S} \cdot (\frac{K_i}{K_i + P})$	inhibition par le produit K_i : constante d'inhibition
Levenspiel (1980)	$\mu = \mu_m \cdot \frac{S}{K_s + S} \cdot [1 - \frac{P}{P_m}]^n$	P_m : concentration limite > il n'y a plus de croissance

Annex 2

Comparison of batch bioreactors and continuous bioreactors

Batch Bioreactors	**Continuous Bioreactors**
Batch fermentation is a type of industrial fermentation in which products are arvested in batches while stopping the process at the end of each batch.	Continuous fermentation is another type of industrial fermentation process in which fermentation occurs over a longer period while adding nutrients at the beginning and at regular intervals between the process and harvest.
Fresh Media	
In batch fermentation, fresh medium is added at the beginning.	In continuous fermentation, fresh media are added at regular intervals.
Product Harvest	
In batch fermentation, once the fermentation is complete, the product is harvested.	In continuous fermentation, products and biomass are harvested at regular intervals multiple times during the process.
Process Termination	
In batch fermentation, when one batch is ready, the process is terminated.	In continuous fermentation, the process continues for a long time until harvest is performed multiple times.
Fermentation Configuration	
The configuration of batch fermentation is not altered from the outside once it is initiated.	The configuration of continuous fermentation can be modified during the fermentation process.
Conditions Inside the Fermenter	
In batch fermentation, conditions will not remain constant.	In continuous fermentation, conditions are maintained constant."

Turnover Rate	
In batch fermentation, the turnover rate is low.	In continuous fermentation, the turnover rate is high.
Utilization of Nutrients in the Bioreactor	
In batch fermentation, nutrients are utilized by microorganisms at a slower rate.	In continuous fermentation, nutrients are rapidly consumed by microorganisms.
Microbial Growth	
In batch fermentation, microbial growth occurs in the lag, log, and stationary phases.	In continuous fermentation, microbial growth is in the exponential phase all the time.
Type of System	
Continuous fermentation is an open system.	Batch fermentation is a closed system.
Bioreactor Cleaning	
The bioreactor is cleaned after harvesting one batch in batch fermentation.	The bioreactor does not require cleaning as addition and harvesting are continuous.
Bioreactor Size	
Larger bioreactors are used for batch fermentation.	Smaller bioreactors are used for continuous fermentation.
Environmental Conditions Inside the Bioreactor	
In batch fermentation, environmental conditions are less similar to the natural environment.	In continuous fermentation, conditions are closer to the natural environment."
Relevance	
Batch fermentation is suitable for the production of secondary metabolites.	Continuous fermentation is suitable for the production of primary metabolites.

Contamination Risk	
The risk of contamination is lower in batch fermentation.	There is a higher risk of contamination in continuous fermentation.
Initial Cost	
The initial cost will be lower for batch fermentation setup.	The initial cost will be higher for continuous fermentation installation.

Annex 3

Fiche 1 : Etude de la cinétique d'une réaction enzymatique

Exercice 1

On réalise sur une préparation pure en enzyme la mesure de l'apparition du produit en fonction du temps pour une concentration en substrat de 0,1 $mol.L^{-1}$ dont voici les résultats :

Temps (min)	0	0,5	1	2	3	4	5
[Produit] ($\mu mol.L^{-1}$)	0	109	205	405	489	534	555

1) Tracer le graphique de la concentration de produit en fonction du temps.
2) Déterminer la vitesse initiale de la réaction enzymatique.
3) On mesure la vitesse initiale Vi pour différentes concentrations de substrat (voir tableau ci-dessous). Déterminer graphiquement les constantes cinétiques de cette enzyme et déterminer sa vitesse initiale pour une concentration en substrat égale à 0,1 $mol.L^{-1}$.

[S] ($mol.L^{-1}$)	0,0125	0,025	0,05	0,1
Vi ($\mu mol.L^{-1}minute^{-1}$)	133	167	190	Compléter

Exercice 2

On se propose d'étudier les caractères cinétiques de ces deux enzymes vis à vis de leur substrat commun : le glucose.

La vitesse initiale de la réaction a été mesurée pour des concentrations différentes en substrat à 20°C et à pH=7. Les résultats expérimentaux sont reproduits dans le tableau suivant :

Sachant que la concentration en enzyme utilisée pour les deux séries d'expériences est la même :

$[Glucose]_i$ en $mol.L^{-1}$	**V_i avec la glucokinase en $\mu mol.L^{-1}.min^{-1}$**	**$[Glucose]_i$ en $mol.L^{-1}$**	**V_i avec l'hexokinase en $\mu mol.L^{-1}.min^{-1}$**
$5,0.10^{-3}$	1,61	$5,0.10^{-3}$	0,490
$6,7.10^{-3}$	2,00	$6,7.10^{-3}$	0,575
$10,0.10^{-3}$	2,67	$10,0.10^{-3}$	0,607
$20,0.10^{-3}$	2,93	$20,0.10^{-3}$	0,806
$50,0.10^{-3}$	4,17	$50,0.10^{-3}$	0,893

1) Déterminer les valeurs de K_M et de V_m pour ces deux enzymes.
2) Comparer les deux K_M. Conclure.
3) Comparer les deux V_m. Conclure.
4) Sachant que la glycémie normale est d'environ 5 $mmol.L^{-1}$, indiquer si chacune de ces deux enzymes agit dans les conditions d'obtention de la vitesse maximum.
5) Quelle serait l'influence d'une augmentation importante de la glycémie?

Fiche 2: IHNIBITEURS

Exercice 1

Une enzyme E catalyse la réaction d'hydrolyse : A + H_2O <===> B + C
On suit la cinétique d'apparition du produit C, pour différentes concentrations en substrat A. Les valeurs obtenues (en µmoles de C par tube) sont présentées dans le tableau suivant :

Temps (min)	[S0] (mM)			
	10	30	100	150
0	0	0	0	0
2,5	0,9	1,9	3,2	3,6
5	1,8	3,9	6,4	7,1
7,5	2,5	5,6	9,6	10,7
12,5	3,7	7,6	15,2	17,6
17,5	4,4	9,1	18,3	--------

- Représentez les cinétiques et déterminez la vitesse initiale de chacune d'elle.
- Pourquoi ne se préoccupe-t-on pas de la concentration de l'eau ?
- Tracez la courbe de saturation ($r_0 = f(s_0)$) et commentez-la.
- Déterminez les paramètres cinétiques de l'enzyme à partir de cette représentation.
- Déterminez les paramètres cinétiques à l'aide de la représentation des doubles inverses.
- Expliquez la différence entre les valeurs déterminées à partir de ces deux représentations.

Exercice 2

On suit la cinétique d'hydrolyse de **A** par l'**E1**, respectivement en absence d'inhibiteur et en présence d'**E2**, d'**E3** ou d'**E4**. Les valeurs des vitesses initiales obtenues sont les suivantes :

$[S_0]$ (M)	ri ($\mu M.min^{-1}$)			
	Sans I	[E2] = $3\ 10^{-4}$ M	[E3]= 0,26 M	[E4] = 0,17 M
0	0	0	0	0
$2,5\ 10^{-5}$	0,033	0,018	0,016	0,027
$5\ 10^{-5}$	0,055	0,033	0,027	0,041
$1\ 10^{-4}$	0,082	0,055	0,041	0,055
$2,5\ 10^{-4}$	0,118	0,091	0,059	0,069
$5\ 10^{-4}$	0,138	0,118	0,069	0,075
$1\ 10^{-3}$	0,150	0,138	0,075	0,079

1. Déterminez r_{max}, et K_M à l'aide de la représentation de votre choix en absence des inhibiteurs.
2. Déterminez les paramètres cinétiques r_{max}^{app} et K_M^{app} en présence des inhibiteurs.
3. Calculez les constantes K_I.
4. Expliquez le type d'inhibition observé pour chacun des inhibiteurs de cet exercice.

Fiche 3: REACTEURS HOMOGENES

Exercice 1

Dans un bioréacteur, on hydrolyse le sucrose à la température ambiante par catalyse enzymatique selon la réaction suivante

$$\text{sucrose} \xrightarrow{\textit{sucrase}} \text{produit}$$

Avec un débit volumique constant Q de 25 litre/ mn et une concentration initiale C_{Ao} en sucrose de 10 M, on obtient un taux de conversion X_A de 90 % de sucrose.

La vitesse r d'hydrolyse du sucrose est décrite par la loi de Michaelis-Menten de la forme :

$$r_A = \frac{k_1 C_A}{1 + K_M C_A} \frac{(\text{mol})}{(\text{litre})\,(\text{min})}$$ avec C_A concentration de sucrose

ou $k_1 = 0{,}2\ \text{min}^{-1}$ et $K_M = 1{,}0\ (\text{mol/liter})^{-1}$,

1- Ecrire le bilan matière du sucrose qui se décompose selon la réaction; sans variation de volume dans chacun des réacteurs suivant:

- Réacteur fermé uniforme
- Réacteur ouvert parfaitement mélangé en régime permanent
- Réacteur en écoulement piston en régime permanent

Calculer le temps de séjour ou de passage pour chacun des trois réacteurs. Conclure.

Exercice 2

La glucoamylase hydrolyse le maltose en glucose selon la réaction

$$\text{Maltose} + H_2O \text{ ----------> } 2\ \text{glucose}$$

On souhaite réaliser un réacteur enzymatique continu, impliquant la glucoamylase , enzyme (Enzyme michaelienne) ayant les paramètres cinétiques, déterminés à 40 °C, suivants: r_{max} = 0,02 mole de maltose $(\text{mn})^{-1}$ $(\text{litre})^{-1}$ et K_M = 35 mmole /litre. On veut traiter en continu la solution de maltose à 1 mole/litre avec un débit de 1 litre/mn en ayant 95 % de conversion de maltose. Pour chaque cas, ci-dessous, faire un bilan massique (débit, volume du réacteur constants et régime permanent) et calculer le volume du réacteur à mettre en œuvre pour obtenir la conversion souhaitée.

1. dans le cas d'un réacteur parfaitement agité
2. dans le cas d'un réacteur piston.

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