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BRIEF REVIEW ON ENZYME ACTIVITY

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ABSTRACT

Currently, enzymes are largely employed in industry, analytical procedures, and as medicine. They are highly specific catalysts for presenting in their structure an active site in which a particular substrate is converted into a product. The successful use of enzymes, mainly in industry, depends on a balance between the amount of enzyme needed, operation conditions, and reaction yields. Nevertheless, the development of the immobilization technique allowed reducing the amount of enzymes needed in a process due to catalyst recycling. The simplest math model for quantifying the enzyme activity is $v = [(S.V_{max}) \div (K_M + S)]$. The activity is affected by pH, temperature, agitation, presence or absence of inhibitors, activators, and/or stabilizers.

KEYWORDS: Enzymes, kinetics, immobilization.

INTRODUCTION

The evolution of enzyme technology can be divided into four phases, i.e., **empirical** (from 6000 B.C. to 1800), **descriptive** (from 1801 to 1898), **descriptive and quantitative** (from 1900), and **planned application** (from 1950).

During the **empirical** phase, products such as cheese, wine, beer, bread, and leather were produced in large scale without knowledge of enzyme involvement.

During the **descriptive** period, the enzyme activity began to be evidenced by observing that aqueous extracts of cereals, baker's yeast and mammal stomachs (or intestines) had the capability to digest starch (amylase activity), sucrose (invertase activity), and meat (proteolysis activity), respectively.

During the **descriptive and quantitative** phase, in which the discovery of new enzymes and their catalytic mechanisms occur, the math modeling of enzyme activity became the main goal of enzymologists. Michaelis-Menten introduced the first math model for quantifying enzyme activity in 1913, which was modified by Briggs and Haldane in 1926. Sumner, in 1926, crystallized the urease from soybean and observed its proteic nature. As the time passed the proteic nature of enzymes became clear as the number of isolates, and new enzymes were characterized and developed.

Along the **planned application** phase, the industrial uses of enzymes increased because of the improved knowledge on their molecular structure, mechanism of catalysis, quantification of activity, immobilization, and refinement of analytical techniques and equipment.

Nowadays, there is no doubt that the main biological catalysts are proteins, although the great majority of proteins do not present a catalytic activity. Recently, a class of ribonucleic acids was found having a catalytic activity. They were named ribozymes. However, their main role is restricted to the cleavage of messenger ribonucleic acids – inside the nucleus – before they are translated into proteins in the ribosome.[1]

Due to the interactions among the chemical groups located at the lateral chains of the amino acids comprising the peptide chain, the macromolecule acquires a tridimensional shape. It has a hole (active site) in which a substance (substrate) can be transformed into another compound (product).[2]

Specificity

Enzymes catalyze a reaction accepting only one substance as substrate. When it accepts two or more substrates, one of them is always preferred (Table 1).

The enzyme specificity is determined by the active site, a particular domain of the peptide molecule. There are two main theories seeking to explain the enzyme-substrate interaction. One – proposed by Fischer in the beginning of the 20th century – states that the substrate-enzyme interaction would resemble a key-lock mechanism, in which the key (substrate) fits into the lock (active site), since two conditions are satisfied: the substrate and the active site have complementary structures and compatible polarity and size. The other – proposed by Koshland in the 1960 – states that the substrate nears the enzyme molecule induces on it some structural modifications that favor the enzyme-substrate fitting.[3]

Nowadays, it is accepted that the active site can be subdivided into two particular regions, one called bond site (responsible for enzyme specificity) and the other called catalytic site (in which a sequence of well-defined 1st and 2nd order reactions occur transforming the substrate into the product or vice-versa).[2][3]

Table 1: Rate of hydrolysis of disaccharides by a glucoamylase from *Aspergillus niger*.[4]

Disaccharide	α-linkage	Rate of hydrolysis (mg glucose/unit.h)	Relative rate
MALTOSE	1,4	$2.3x10^{-1}$	100
NIGEROSE	1,3	$2.3x10^{-2}$	6.6
ISOMALTOSE	1,6	$0.83x10^{-2}$	3.6

Although the disaccharides differ only in the type of α-linkage (Table 1), the glucoamylase shows a high specificity for maltose.

ENZYME ACTIVITY

When enzymes are used in industrial processes and analytical procedures, the precise evaluation of the enzyme activity becomes important.

Focusing on the industrial scale, the decision to use or not an enzyme in a process must be based on issues such as **1)** the amount of enzyme required for carrying out the process properly, **2)** the duration of the reaction, **3)** the amount of substrate to be converted, **4)** the conditions under which the reaction occurs, and **5)** the overall cost of the process.

Once the decision for using an enzyme is taken, and considering that the enzyme has a cost, the impacts of this cost on the overall process must be evaluated. Thereby, the increase in product yield and/or the value added to the final product, as well as the reduction of the overall energy consumption, could minimize the effects of enzyme cost on the overall process cost.

Thereby, it is clear that the success of the enzyme process depends on the optimization of three factors, i.e., amount of enzyme needed, operation conditions (pH, temperature, agitation etc.), and reaction yield.

Theoretically, as the enzymes are catalysts not consumed during the reaction, a small amount could transform into huge amount of substrate. However, an optimized and finite correlation among the amount of catalyst, its initial activity and the amount of substrate to be converted

into product must be found. Moreover, the duration of industrial processes can take one hour (such as the mashing of malted barley in brewing), three hours (such as the saccharification of the liquefied starch with glucoamylase in syrup production), or about twenty hours (such as the hydrolysis of lactose by lactase in milk or whey).[5] Of course, the enzyme technology aims to perform quick reactions at a low cost, albeit respecting the restrictions imposed by the operational conditions and the scale of the process.

Quantification of the Enzyme Activity

Let us consider the simplest model for an enzyme-catalyzed reaction:[6]

$$E + S \underset{k_2}{\overset{k_1}{\leftrightarrows}} ES \xrightarrow{k_3} E + P$$

Where: E = enzyme concentration; S = substrate concentration; ES = enzyme-substrate complex concentration; k_1, k_3 = 1st order reaction rate constants (t^{-1}); P = product concentration; k_2 = 2nd reaction rate constant ($M^{-1}.t^{-1}$).

At first, it must be stressed that the formation of ES (enzyme-substrate complex) is an obligatory step to any type of enzyme-catalyzed reaction. The ES, depending on the reaction conditions, can form the product (forward reaction) or not (reward reaction).

An enzymatic reaction can be divided in three phases (Figure 1). During **phase I** (**reaction onset**), there is the accumulation of the enzyme-substrate complex (ES) without product formation and significant substrate consumption. The existence of the ES was predicted by Brown in 1892, reinforced by Henry in 1902 and experimentally demonstrated in 1936.[3] The presumption of ES existence allowed Michaelis and Menten (1913) to postulate the first mathematical model for quantifying enzyme activity. Briggs and Haldane improved it in 1926. **Phase II** begins as soon as the ES concentration reaches a maximum value, remaining invariable for a period. The duration of the steady state regarding the ES concentration depends on the relative concentrations between the enzyme and the substrate. Along phase II, the substrate is consumed and the product accumulates in the reaction medium. Along the **phase III** – when the ES concentration is no longer constant –, the substrate consumption and product formation occur slowly.

The quantification of the enzyme activity considers the conditions observed in phases II and III.

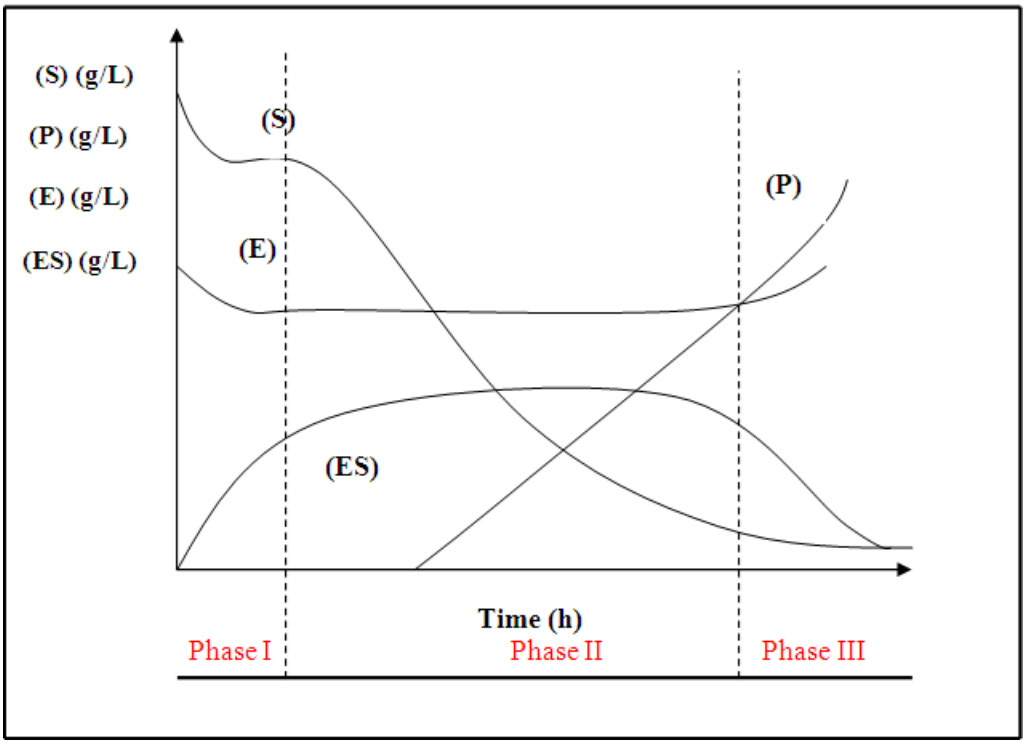

Figure 1: Variation of enzyme (E), substrate (S), product (P) and enzyme-substrate complex (ES) concentrations in relation to reaction time.

The starting point for establishing the enzyme kinetic equation is the determination of the variation in the initial concentration of substrate in relation to the reaction time at a fixed amount of enzyme (E_0). This is made by measuring the amount of substrate consumed along the period, followed by plotting the data in a (S) =f (t) graph (Figure 2). The inclination of the linear part of each curve related to a determined initial substrate concentration (S_1, S_2, S_3,……S_n) represents the initial reaction rate of the enzymatic reaction (v_1, v_2, v_3,…..v_n). By plotting v versus S, the result is a hyperbola, whose curvature tends asymptotically to a maximum v (V_{max}), i.e., the reaction rate becomes invariable in relation to the substrate concentration (Figure 3). Under controlled reaction conditions, the V_{max} becomes a kinetic constant, which describes the enzyme catalysis quantitatively. The phenomenon of saturation is seldom observed in non-enzymatic reactions. The asymptotic zone of the hyperbola would correspond to the condition in which the amount of substrate is enough to saturate the enzyme molecules present into the reaction medium completely, i.e., the ES concentration into the reaction medium is constant.

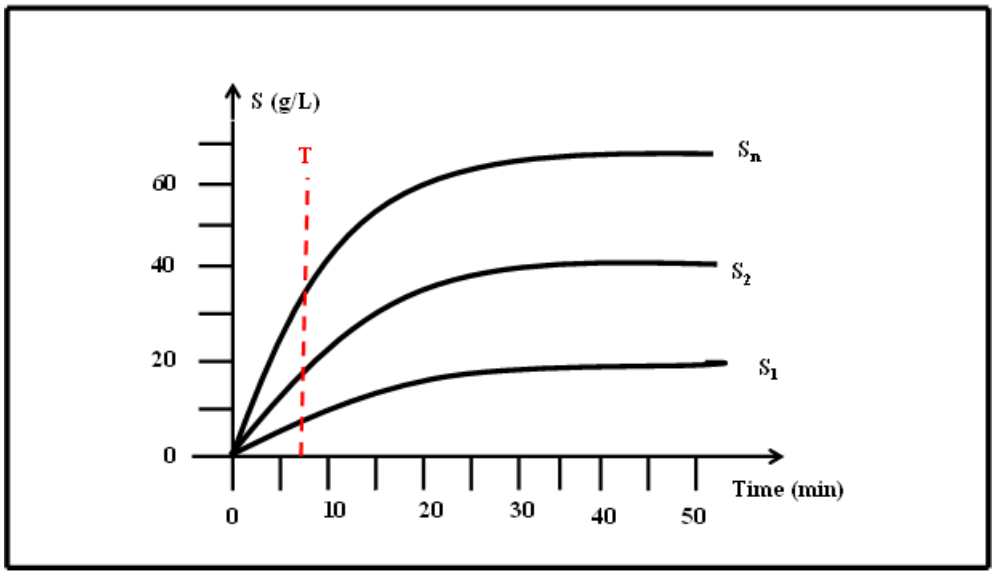

Figure 2: Variation of the substrate concentration *versus* time. The initial enzyme concentration (E_0) was constant. At the interval 0→T, the substrate consumption varies linearly with time, i.e., the reaction rate is constant. Therefore, the initial substrate concentration (S_1, S_2….S_n) was enough to saturate all the enzyme (E_0).

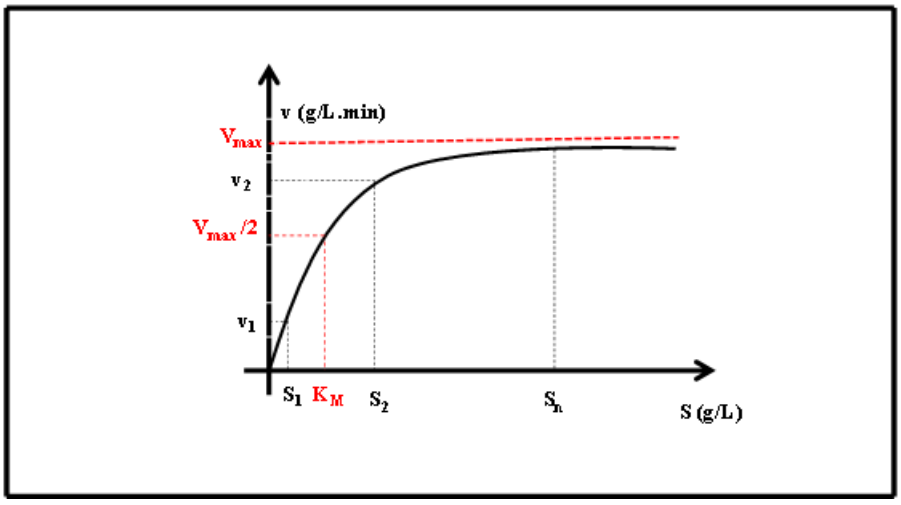

Figure 3: Reaction rate (v) *versus* initial substrate concentration. At the substrate concentration over S_2, the curve tends to a maximum value of reaction rate (V_{max}), which indicates that all enzyme (E_0) is saturated with substrate.

Considering (ES → E + P) as the limiting step of the overall reaction, then

$(- dS/dt) = v = k_3.(ES)$ (1)

However, remembering that at any time along the reaction, the whole enzyme is distributed as

$E_0 = (E) + (ES)$ (2)

Where E_0 = total amount of enzyme in the reaction medium; E = amount of enzyme not bound to the enzyme-substrate complex (ES).

When all enzyme molecules are bound to substrate molecules, then (E) = 0, and the equation (2) becomes

$E_0 = (ES)$ (3)

Replacing (3) in (1):

$(-dS/dt) = v = k_3.E_0$ (4)

Equation (4) leads to the conclusion that when the enzyme is saturated with substrate, the reaction follows a "pseudo-zero order" kinetic, i.e., apparently the reaction rate is independent from the substrate concentration. In other words, the reaction rate is directly proportional to the whole enzyme concentration. Under this condition, the k_3 is called "turnover number" (k_{cat}), which indicates the number of substrate molecules transformed per unit of time by a single enzyme molecule when the enzyme is the rate-limiting factor. Under the saturation condition, $k_{cat}.E_0$ represents the maximum rate of the reaction catalyzed by the enzyme (V_{max}). Therefore,

$(-dS/dt) = v = V_{max}$ (5)

Integrating Eq. (5):

$(S) = (S_0) - V_{max}.t$ (6)

Therefore, when the enzyme is saturated by the substrate, the substrate consumption decreases linearly along the period, i.e., the reaction occurs at a constant and maximum rate.

Figure 3 – at low substrate concentration ($0 \rightarrow S_1$) - clearly shows that v varies linearly in relation to S, or in algebraic terms:

$(-dS/dt) = v = k'.(S)$ (7)

Where k' is a 1^{st} order rate constant.

Integrating Eq. (7):

$LnS = LnS_0 - k'.t$ (8)

Therefore, Eq. (8) shows that the substrate concentration decreases exponentially with time, when the reaction becomes under unsaturated and substrate concentration decreases.

Along phase II, the (ES) remains invariable for a time interval (Figure1). This means that

$d(ES)/dt = 0$ (9)

Consequently,

$k_1.(E).(S) = k_2.(ES) + k_3.(ES)$ (10)

Rearranging Eq. (10):

$(E) = [\mathbf{K_M}.(ES)] \div (S)$ (11)

Where $\mathbf{K_M} = (k_2 + k_3)/k_1$.

Replacing Eq. (11) in Eq. (2) [this is valid insofar as the conditions under which the reaction is carried out preserve the full enzyme catalytic activity]:

$(ES) = \{[(S).E_0] \div [(S) + \mathbf{K_M}]\}$ (12)

Replacing Eq. (12) in Eq. (1):

$v = V_{max}.(S)/[(S) + \mathbf{K_M}]$ (13)

Eq. (13) describes completely the hyperbolic curve shown in Figure 3. The terms V_{max} and K_M – the so called kinetic constants – characterize an enzyme, when the catalysis is carried out under defined conditions (pH, temperature, agitation etc.). These constants are often calculated by the Eq. (14):

$1/v = (1/V_{max}) + (1/S).(\mathbf{K_M}/V_{max})$ (14)

Equation (14) represents a straight line from which the kinetic constants are calculated.

Regarding K_M, three aspects must be stressed: 1) from Eq. (13), when $K_M = (S)$, then $v = 0.5V_{max}$; 2) it is considered a referential for setting the operational substrate concentration. When (S) is at least 100 times lower than K_M, the reaction occurs in an unsaturated substrate concentration, whereas when (S) is at least 100 times higher than K_M, the reaction occurs in substrate saturation; 3) it is considered a characteristic of an enzyme when the catalysis occurs under defined conditions.

Along phase III, the (ES) is not constant, but the substrate continues to be transformed into product, although at a lower rate (Figure 1). To evaluate this phase, Eq. (13) must be integrated, resulting in

$t = [(S_0 - S) - \mathbf{K_M}.Ln(S/S_0)] \div V_{max}$ (15)

Defining substrate conversion (Y) as:

$Y = (S_0 - S)/S_0$ (16)

Replacing Eq. 16 in Eq. 15:

$t = [Y.S_0 - \mathbf{K_M}.Ln\ (1 - Y)] \div V_{max}$ (17)

The practical aspect of Eq. (17) is that the duration of the reaction can be estimated from a desired substrate conversion.

Expression of the Enzyme Activity

The enzyme activity can be expressed by several manners. For example, the hydrolysis of sucrose by invertase is expressed as total-reducing sugars formed per minute, the collagen hydrolysis by collagenase is expressed as the viscosity diminution of a standard collagen solution per minute, and the amylase activity expressed as a SKB unit.[5][7] However, it is recommended to express the enzyme activity using International Unit (U), which is defined as the amount of enzyme that catalyzes the formation of 1 μmole of product per minute in fixed conditions. The specific activity is defined as U/mg protein (if the enzyme preparation is impure), or U/μmol enzyme (for pure enzymes).

FACTORS AFFECTING ENZYME ACTIVITY

An enzyme macromolecule can be seen as formed by two main zones. The small zone is represented by the microenvironment around the active site – in which the substrate transformation occurs –, whereas the big zone corresponds to the bulk of the molecule, which guarantees an adequate active site conformation for the catalysis. The best reaction conditions are those in which the overall molecule structure remains undisturbed.

The factors affecting the enzyme activity can be classified as physical-chemical (pH, temperature, ionic strength, water activity etc.), chemical (activators, inhibitors, stabilizers etc.), and physical (pressure, shear forces, attrition etc.).[6]

Physical-Chemical Factors

pH

The pH affects reaction rate, position of equilibrium, degree of ionization, dissociation, and/or solubility of reacting molecules. In the case of enzymes, the pH affects their stability and kinetic constants (K_M and V_{max}). The enzyme activity is affected by pH due to changes in

the ionization pattern of ionic groups located in the lateral chains of amino acids constituents of the protein primary structure. The bell-shaped activity *versus* pH curve is an indication of pH influence on the enzyme molecular structure conformation. Setting the denaturing pH value is enough to plot the enzyme activity and stability in relation to pH in a same graph (Figure 4).

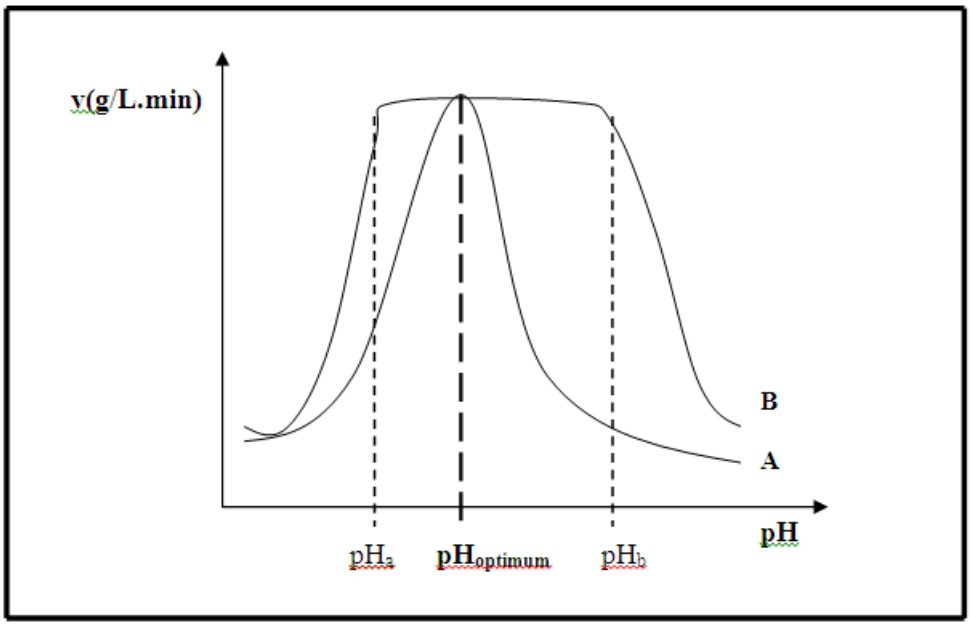

Figure 4: Effects of pH on the reaction rate catalyzed by an enzyme. Curves A and B represent the variation of enzyme activity and stability in relation to pH, respectively. Between pH_a and pH_b, the enzyme is fully stable.

Temperature

Temperature has a generalized effect on reaction rates, interfering with reagent solubility, enzyme stability, and kinetic constants. Two opposite mechanisms – activation (constant rate increases with the temperature increases) and denaturation (thermal unfolding of quaternary and tertiary structures of the enzyme) – occur simultaneously as the reaction temperature increases. In general, up to 50-60^{o}C, the increase in reaction rates (activation) overruns thermal denaturation. Except for thermophilic enzymes, at temperatures above 60^{o}C the denaturation predominates and the reaction rate slows and ceases around 80-90^{o}C. The optimum temperature of an enzyme is a temperature at which the greatest amount of substrate changes in time units. Assigning an optimum temperature to any enzyme is inadequate unless the time of the reaction is also expressed.

The optimum temperature for any enzyme not only changes in relation to time, but may also change in relation to changes in pH, concentration and purity of enzyme preparation. Heat liability increases markedly as an enzyme preparation becomes more purified. It is clear, furthermore, that water is essential for heat inactivation, since highly purified enzymes are more stable in the dry than in the moist state. The effects of temperature on the shelf life of an enzyme also deserve to be considered because an enzyme extract submitted to different temperatures for different periods will have different residual activities. Modifications introduced in the molecular structure of the enzyme – either by chemical procedure (protein engineering) or by genetic modification of the source of enzyme (molecular biology) –, aiming to increase its thermal resistance is an approach strongly encouraged by enzyme producers.

Miscellaneous

Other factors – such as ionic strength and water activity (A_w) – can more or less affect enzyme activity depending on the particular type of enzyme, and the intensity and duration of their action along the reaction. The ionic strength is related to the concentration of ions in the reaction medium, which can affect the solubility of enzymes and reagents, and the ionization of ionic groups at the lateral chains of amino acids constituting the primary structure of proteins. The A_w of the reaction medium can interfere with the activity and catalytic mechanism of an enzyme. Hydrolytic enzymes, for instance, at low A_w have the catalytic rate decreased or their mechanism of action changed to the transfer pattern (the enzyme catalyzes the formation of the bond which normally it disrupts).[8]

Chemical Factors

Chemical factors, diversely of physical-chemical or physical factors, act on a specific region of the enzyme molecule, mainly at the active site domain.

Activators

The activator is a compound that increases enzyme activity. It can be bound to the enzyme molecule (prosthetic group) or dissolved in the reaction medium, which, in turn, bounds to the enzyme at the moment of catalysis (coenzyme).

Stabilizers

The enzyme, in presence of its substrate, is stabilized in relation to temperature. For example, liquid enzyme preparations are added with modified substrates. Thereby, amylase and

protease can be stabilized by adding, respectively, modified starch and peptides into the solution. There are cases in which the activator can also act as a stabilizer. The most notorious example is the ion Ca^{2+} on amylase activity.[9]

Inhibitors

Inhibitor is a substance that decreases the enzyme reaction rate through binding specifically to some domain of the enzyme molecule. Normally, inhibitors act on the active site or on another site and do not damage the tertiary and/or quaternary structure of the protein.

In an industrial point of view, the presence of an inhibitor in the reaction medium is always undesirable regardless of its nature. Thereby, a good practice is to avoid it.

Enzyme inhibition is broadly classified into two types: irreversible and reversible. Irreversible inhibition usually involves the destruction or modification of one or more functional groups of the enzyme. The reversible inhibition – in which the inhibitor bounds to the enzyme through non-covalent bonds and can be released in the presence of an appropriate amount of substrate in the reaction medium – can be divided into three main types: 1) **competitive**: the substrate and inhibitor molecules compete for the active site of the enzyme. Depending on the relative amounts of both substances, the inhibitory effect can prevail or not on the enzyme catalysis; 2) **noncompetitive**: the substrate and the inhibitor molecules do not compete for the same domain of the enzyme molecule; 3) **uncompetitive**: the inhibitor bounds to the enzyme only after the substrate is inserted into the active site, i.e., the inhibitor bounds directly into the enzyme-substrate complex.

Physical Factors

Such unspecific factors can act negatively on enzyme catalysis through purely mechanical effects, such as the shear forces originated by the agitation of the reaction medium.[10] Furthermore, the internal reactor pressure can also affect catalysis mainly in the case of the formation of a gas product.

Thermodynamic of the Enzyme Catalysis

The enzyme – as any catalyst – facilitates the progress of the reaction by lowering the energy necessary for reagent molecules to reach the transition state. However, the enzyme catalytic mechanism always requires the formation of the enzyme-substrate complex, in which case the Gibbs free energy equals that constituted by the product and the free enzyme. In other

words, the enzyme-substrate complex is a stable form of the enzyme. Thereby, the issue is that the enzyme catalysis might stop at the formation of the intermediate complex. However, experiments undoubtedly show that the enzyme catalysis progresses, never stopping at the intermediate step. An explanation can be giver by considering that the enzyme-substrate complex along time becomes structurally stressed due to electrostatic and hydrophobic incompatibilities among the chemical groups belonging to both substrate and enzyme molecules. Consequently, the complex is destabilized, resulting in product and enzyme liberation to the medium. Besides, the internal entropy of the reacting system decreases as the enzyme-substrate complex is formed insofar as the structure of the intermediate is more organized than when both enzyme and substrate molecules are free in solution. The initial entropy of the system is reestablished only when product and enzyme molecules are free in the medium.

ENZYME IMMOBILIZATION

Focusing on any intact cells, thousands of macromolecules (nucleic acids, proteins, lipoproteins, polysaccharides etc.) are disposed inside a small space (the internal cell volume), leading to an intense interaction among them. All intracellular enzymes are associated to membranous cell structures, acting as typical heterogeneous catalysts. This leads to think about a possibility to use the enzyme or any other biological component (organelles or the whole cell itself) linked to an inert support either through chemical or physical means.

The immobilization technique, as this procedure is usually called, was developed in the 1960s. Currently, it is well developed.

The main advantages of this technique are the use of continuous reactors, the increase of biomaterial stability, and the reuse of the biomaterial.

The types of immobilization methods are **1) chemical methods**: they can be divided in two different approaches, i.e., covalent binding on a solid support, and cross-linking. In the covalent binding procedure, the enzyme is linked to the support through covalent bonds. Chemical groups belonging to the lateral chains of the amino acids constituting the primary structure of the enzyme react with the superficial groups of the support, which are generated by pre-treating the support with reagents such as cyanogen bromide, epoxides, divinyl sulfone, and 3-aminopropyltriethoxysilane. Cross-linking allows aggregating enzyme

molecules in such a way that the aggregation becomes so large that they are no longer soluble. In this way, the enzyme functions both as the catalyst and as the support material. Glutaraldehyde is the most commonly used cross-linking reagent. Often cross-linking is used in combination with other immobilization methods, for example, to stabilize preparations of enzymes immobilized by physical adsorption; **2) physical methods:** the main approaches are adsorption, physical deposition for use in organic media, and entrapment. Adsorption is a method in which the biomaterial is adsorbed on the surface of the support through the electrostatic interaction among charged groups of the support and those of the biomaterial. It is a simple, smooth and not deleterious technique for almost all types of enzymes. The chemical nature of the support can be organic (Dowex and DEAE-cellulose, for example) or inorganic (bentonite, alumina, colloidal silica, among others). It has been demonstrated that enzymes can work well as catalysts in organic media with a low amount of water present (water is necessary for activating the enzyme). A practical way of preparing the enzyme for use in organic media is to immobilize it on a solid support (for example, celite and porous glass). This can be carried out by either adsorbing the enzyme directly on the support or by drying a mixture of the support and an aqueous solution of the enzyme.[11] Entrapment consists on polymerizing a suitable monomer (acrylamide) in the presence of a cross-linker (bisacrylate) and an enzyme. At the end, the enzyme molecules remain entrapped in the three-dimensional network of the polymer. Another way to promote entrapment is to dissolve an enzyme in an aqueous solution of sodium alginate (or sodium carragenan) and transfer the mixture into a solution containing Ca^{2+} that cross-links polysaccharide chains, thereby forming a hydrogel. It must be stressed that in this case, no polymerization occurs by only cross-linking the polysaccharide molecules.[12] Therefore, the two risks involving polymerization – the heat generated during polymerization and the reaction of the enzyme with the monomers and the free radicals formed –, which can inactivate the enzyme, are completely absent. A variant of this method is the entrapment in microcapsules formed by polymer membranes (polyamides, polyurethanes and polyesters) permeable to the substrate and the product, but not to enzymes. Microcapsules can be prepared through an emulsion in which small droplets of water containing the enzyme and a water-soluble monomer are formed in a bulk organic phase containing the other monomer. Polymerization occurs at the interface, resulting in enzyme entrapment. The size of the microcapsules may vary by using different conditions during emulsification; **3) membrane isolation:** flat membranes and hollow fiber devices can be used to retain enzymes in the reactor while allowing products to be removed, often in a concentrated solution. This type of continuous reactor is called

membrane reactor, which can be classified as unimodular (the flat membrane is set at the bottom of the tank) and bimodular (the flat membrane is encased in a module connected in series with the reaction tank). By using a membrane with an adequate molecular cut-off, the enzyme can be soluble in the reaction medium.[13] There are two main advantages. First, the mildness of the procedure does not damage the catalyst; second, linking the catalyst to an insoluble support is completely unnecessary, so that the immobilization cost – which depends on the durability and cost of the membrane, currently less expensive than the chemical procedure for creating a covalent bond between the enzyme and the support – of the overall process is practically negligible; **4) two phase systems:**[14] the main idea of this type of immobilization is to retain the enzyme at one phase while the product is removed from the reactor at the other phase. By employing an organic/aqueous two-phase system, often the enzyme partitions to the aqueous phase, and the substrate and product partition mainly to the organic phase. The solvent should be chosen by its capacity to dissolve substrates and products and inflict a minimum damage to the catalytic capability of the enzyme. Enzyme denaturation can occur at the inter-phase, especially if the phases are mixed vigorously to achieve an effective mass transfer. Otherwise, the use of two-phase systems is a mild immobilization method. A variant of this procedure is to mix aqueous solutions of two incompatible polymers to form a two-phase system, in which the enzyme is partitioned to one of the phases, and the small molecules are distributed evenly among the phases. The interfacial tension in these systems is low, the procedure conditions are very mild, and the extent of enzyme inactivation is negligible.

Authors have described thousands of inert materials (hydrogels, silica, porous glass beads, nanoparticles of zinc oxide etc.) that can be used as supports in the immobilization technique. The supports available can be classified as follows: **1) microporous or non-porous:** glass, nylon, silica; **2) microencapsulated**: nitrocellulose, cellulose triacetate; **3) moderate cross-linking polymers:** polyacrylamides, polyvinylpyrrolidone; **4) low cross-linking polymers**: agarose, sephadex and related resins; and **5) macroporous**: alumina, silica, nickel oxide.

The choice of the immobilization method and type of support depends on the peculiar characteristics of the biomaterial and the conditions under which the immobilized biomaterial is used. There is no universal method of immobilization or type of support.

In general, the immobilization conditions can only be established empirically. The procedure consists in immobilizing the biomaterial in several supports using different techniques

followed by the evaluation of the immobilized system performance. The pair support-immobilization method that retains the highest biological activity is thus selected.

The immobilization technique presents not only advantages, but also some disadvantages. The intrinsic interaction between enzyme molecules and the support can cause perturbation in enzyme structure (steric and/or conformational hindrance). Moreover, the loss of enzyme activity can also result from diffusion (gradient of substrate and product concentration established between the bulk of the medium and the surface of the support) and partition – unbalanced distribution of charged chemical species in the medium due to the establishment of an electrostatic drive force between the microenvironment (that surrounds the enzyme molecule) and the bulk of the medium.

The immobilization technique allowed a more frequent use of biomaterials in industry, therapeutics (confection of subcutaneous capsules for controlled delivering of hormones), and lab analysis (automatic dosing equipments, enzyme electrode, biosensors and enzyme immunoassays). In industry, the use of glucose isomerase for the conversion of glucose into fructose (high fructose corn syrup), the aminoacylase for the separation of racemic mixture of amino acids, microbial lipase in triglyceride hydrolysis, and the lactase for removing lactose from milk and/or whey are stressed.[5][15]

CONCLUSION

Knowing the mechanism and the method to determine the catalytic activity is a pivotal aspect for enzyme application. The simplest equation describing the enzyme activity is $v = [(S.V_{max}) \div (\mathbf{K_M} + S)]$. Several factors – such as pH, temperature, presence or not of inhibitors, stabilizers, and activators – can interfere with enzyme activity. The success of the enzyme process depends on an optimized balance between the enzyme needed, operation conditions (pH, temperature, agitation etc.) and reaction yield. By using the immobilization technique, which allows reusing the enzyme and using continuous reactors, it is possible to decrease the overall process cost due to the possibility of using a lower amount of enzyme.

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