Biocatalysis

Code: 100956
ECTS Credits: 6

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<td>2500253 Biotechnology</td>
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**Use of languages**

- Principal working language: catalan (cat)
- Some groups entirely in English: No
- Some groups entirely in Catalan: Yes
- Some groups entirely in Spanish: No

**Contact**

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**Teachers**

Xavier Parés Casasampera

**Prerequisites**

There are no official prerequisites. In any case, some of the contents of the 1st year course, Biochemistry, are necessary to follow the subject correctly.

**Objectives and Contextualisation**

The subject Biocatalysis focuses on the study of enzymes, their properties and applications. The knowledge of enzymes is key in the context of Biochemistry, Molecular Biology and related sciences, given their role as catalysts of biological reactions and their applications in biotechnological processes. The subject analyzes enzymes from different perspectives: their activity, kinetics, mechanisms and applications. The general objective of the subject is to provide the foundations for the analysis, characterization and use of enzymes from the point of view of research and from their biotechnological and biomedical applications.

**Specific objectives of the subject:**

- Knowledge of the general characteristics, classification and testing methods of enzymatic activity.
- Analysis of enzyme kinetics and determination and meaning of kinetic parameters.
- Knowledge of enzyme inhibition and its applications, especially in the field of drugs.
- Analysis of the active center and knowledge of the methods of characterization.
- Analysis of enzymatic and regulatory mechanisms.
- Biomedical and biotechnological applications of enzymes.

**Content**

**Lesson 1**

Lesson 2
General properties of the enzymes: Concept and biological significance, chemistry and practice. Definitions
Classification of enzymes.

Lesson 3
The obtention and characterization of enzymes. Sources of collection. Techniques for the extraction of
enzymes. Methods of determining the enzymatic activity. Initial rate concept, determination, representation.
Units of enzymatic activity. Effect of enzyme concentration.

Lesson 4
Pre-steady and steady states: concepts. Hypothesis of steady state: treatment of Briggs-Haldane. Pre-steady
state. Methods of study. "Bursts" and "lags".

Lesson 5
Determination of KM and Vmax. Methods of Lineweaver-Burk and Eadie-Hofstee. Other methods to determine
the kinetic parameters. Meaning of the kinetic parameters kcat and KM. Concept of kcat / KM: catalytic
efficiency and enzymatic specificity. Michaelis-Menten's equation for reversible reactions: Haldane's
relationship.

Lesson 6
Inhibition of enzyme catalysis: types of inhibitors. Reversible inhibitors: competitive and non-competitive
inhibition; Acompetitive and mixed inhibition. General model of inhibition. Graphic analysis of the different types
Substrate excess inhibition. Discrimination between competitive substrates. Pseudoirreversible and irreversible
inhibitors. Use of inhibitors as drugs. Affinity markers. Suicide substrates as irreversible inhibitors.

Lesson 7
Reactions with more than one substrate: Cleland's notation. Double displacement mechanism (ping-pong);
Ordered sequential mechanism; Statistical sequential mechanism. Mathematical treatment and graphic
analysis. Methods for determining the type of mechanism. Isotopic exchange and isotopic effect concepts and
applications.

Lesson 8
Action of the temperature on the enzyme kinetics. Arrhenius representation. Enzymes of extremophile
organisms. Effects of pH on the enzyme kinetics. Ionization of essential residues. Influence of pH on the kinetic
parameters. Evaluation of ionization constants. Identification of the ionizable groups involved in the processes
of binding and catalysis. Effects of microenvironment on the pK. Examples

Lesson 9
Binding of ligands to proteins. Concept and types of cooperativity. Analysis of the cooperativity. Binding of
oxygen to hemoglobin. Models of cooperativity. Model of Monod, Wyman and Changeux. Explanation of the
homotropic cooperative effects by the MWC model. Allosteric enzymes. Systems K and systems V. Model of
Koshland, Nemethy and Filmer. Determination of the cooperativity model that follows an enzyme. Example of
enzyme with allosteric regulation: aspartate carbamoyltransferase.

Lesson 10
Enzymatic specificity. The active site, specificity and tridimensional structure. Definition of active site.
Characteristics of the active site. Theories on enzyme-substrate binding. The Fisher model (lock-and-key). The
Koshland model (induced fit). The hexokinase as induced fit example. Hypothesis of binding to three sites.
Hypothesis of strain effect. Stabilization of the transition estate. Evidence supporting the theory of the transition
state. Catalytic antibodies. Applications of catalytic antibodies

Lesson 11
The active site. Identification of the binding and catalytic sites. Labeling with a part of the substrate. Use of
artificial substrates. Chemical modification with irreversible specific inhibitors. Affinity labeling. Suicide
inhibitors, examples of pharmacologic interest. Site directed mutagenesis. The serine-proteases: subtilisin.
Comparison of mutagenesis and chemical labeling. Investigation of the tridimensional structure of proteins:
X-rays, NMR, molecular modeling. Evolutionary invariability of amino acid residues. The alcohol dehydrogenase.

Lesson 12

Lesson 13

Lesson 14

Lesson 15

Lesson 16

Problems
The problems that are proposed refer to some aspects of the syllabus, emphasizing the determination of kinetic parameters in different situations: presence of inhibitors, bisubstrate reactions, non-homogeneous preparations, etc. The statements of the problems will be delivered through the Virtual Campus in advance to the problem sessions in which they will be solved.

Practical sessions.
In the practical sessions different methodologies will be applied aimed at the characterization of a biocatalyst overexpressed in yeast (Saccharomyces cerevisiae). The stereo-specificity of the reaction will be determined and different computer programs will be used to determine its kinetic parameters and to study its tridimensional structure.