

**Biocatalysis**

Code: 100997  
ECTS Credits: 6

Degree	Type	Year	Semester
2500502 Microbiology	OT	4	2

## Contact

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## Teaching groups languages

You can check it through this [link](#). To consult the language you will need to enter the CODE of the subject. Please note that this information is provisional until 30 November 2023.

## Prerequisites

There are no official prerequisites. In any case, part of the contents of the 1st year subjects: Biochemistry and Integrated Laboratory 1, as well as part of the content of the Integrated Course 3 and Instrumental techniques of the second year, are necessary to be able to correctly follow the subject.

## 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.

Use of software to study the structures of enzymes and modulators, as well as enzyme kinetics.

## Competences

- Act with ethical responsibility and respect for fundamental rights and duties, diversity and democratic values.
- Apply knowledge of theory to practice
- Design experiments and interpret the results
- Interpret, on a molecular scale, microbial mechanisms and processes.
- Make changes to methods and processes in the area of knowledge in order to provide innovative responses to society's needs and demands.
- Obtain, select and manage information.
- Recognise the different levels of organization of living beings, especially animals and plants, diversity and bases of regulation of vital functions of organisms and identify mechanisms of adaptation to the environment.
- Take account of social, economic and environmental impacts when operating within one's own area of knowledge.
- Take sex- or gender-based inequalities into consideration when operating within one's own area of knowledge.
- Use bibliography or internet tools, specific to microbiology or other related disciplines, both in English and in the first language.

## Learning Outcomes

1. Act with ethical responsibility and respect for fundamental rights and duties, diversity and democratic values.
2. Analyse the functional mechanisms of hydrosaline and acid-base equilibrium in the animal organism.
3. Apply knowledge of theory to practice
4. Calculate and interpret the kinetic parameters of enzyme reactions, by means of graphic methods using computer programmes.
5. Design experiments and interpret the results
6. Design, execute and evaluate a basic protocol for obtaining and purifying an enzyme.
7. Evaluate the suitability of methods for determining enzyme activities and establish the experimental trial conditions.
8. Explain the underlying structural causes and the principal mechanisms of enzyme catalysis and its regulation.
9. Explain the underlying thermodynamic causes of enzyme catalysis.
10. Identify the principal mechanisms of enzyme inhibition, know their biological significance and calculate and interpret the corresponding constants.
11. Make changes to methods and processes in the area of knowledge in order to provide innovative responses to society's needs and demands.
12. Obtain information on the structural basis of enzymes and their mechanisms from the principal databases.
13. Obtain, select and manage information.
14. Take account of social, economic and environmental impacts when operating within one's own area of knowledge.
15. Take sex- or gender-based inequalities into consideration when operating within one's own area of knowledge.
16. Use bibliography or internet tools, specific to microbiology or other related disciplines, both in English and in the first language.
17. Use enzyme databases to study enzyme activity, biological functions and applications.

## Content

Theoretical content.

Unit 1. Introduction to biocatalysis.

Concept of biocatalysis. Market and use of biocatalysts. Prejudices in the use of enzymes. Historical perspective. Waves of innovation in biocatalysis. Advantages and disadvantages of biocatalysts. Different types of biocatalysis processes. Cellular and enzymatic systems: properties. Factors to consider in a biocatalytic process: source of the biocatalyst and optimization of the process.

## Unit 2. Properties, classification and nomenclature of enzymes.

General properties of enzymes: Concept and biological, chemical and practical significance. Definitions. Enzyme-substrate complex. Decreased activation energy. Transition state. Enzymatic cofactors. Nomenclature and classification of enzymes. Databases with enzyme information.

## Unit 3. Methods of determination of enzymatic activity and of obtaining enzymes.

Production and characterization of enzymes. Sources of enzymes. Techniques for the extraction of enzymes. Methods of determination of enzymatic activity. Direct and indirect, continuous and discontinuous assays. Initial rate: concept, determination, representation. Units of enzymatic activity. Effect of enzyme concentration.

## Unit 4. Analysis of enzyme kinetics.

Enzyme kinetics. Reactions with one substrate. Effect of substrate concentration: Michaelis-Menten equation. Pre-steady and steady-states: concepts. Steady state hypothesis: treatment of Briggs-Haldane. Enzymatic reactions with more than one enzyme-substrate intermediate complex. Significance of the parameters  $k_{cat}$ ,  $K_M$  and  $k_{cat}/K_M$ . Determination of kinetic parameters. Methods with linear representations: Lineweaver-Burk, Eadie-Hofstee and Hanes-Woolf. Other methods. Michaelis-Menten equation for reversible reactions: Haldane relationship.

## Unit 5. Inhibition of enzyme catalysis.

Inhibition of enzymatic catalysis: types of inhibitors. Reversible inhibitors: competitive inhibition, acompetitive and mixed inhibition (includes non-competitive inhibition). General model. Graphic analysis of the different types of inhibition. Determination of the inhibition constants. Concept of  $IC_{50}$  and its relation with the inhibition constants. Inhibition by excess substrate. Discrimination between competing substrates. Pseudo-irreversible inhibitors and irreversible inhibitors. Affinity labels. Suicide inhibitors. Use of enzyme inhibitors as drugs.

## Unit 6. Analysis of enzyme kinetics in reactions with more than one substrate.

Reactions with more than one substrate: Cleland notation. Sequential ordered mechanism, statistical sequential mechanism, double displacement mechanism (ping-pong). Mathematical treatment and graphical analysis. Methods for determining the type of mechanism. Isotopic exchange and isotopic effect.

## Unit 7. Kinetics of transient states.

Characteristics of rapid kinetic methods. Mixing methods: continuous flow, stopped flow and quenched-flow. Relaxation methods: temperature jump (T-jump), pressure jump (P-jump). "Bursts" and "lags". Analysis of the "Burst" of a reaction: determination of the concentration of active centers. Application of the fast reaction kinetics to the nitrogen assimilation process.

## Unit 8. Effect of pH and temperature on enzymatic reactions.

Action of the temperature on enzyme kinetics. Representation of Arrhenius. Enzymes of extremophile organisms. Effects of pH on 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 the micro environment on the  $pK$ . Examples.

## Unit 9. Cooperativity and Allostereism.

Ligand binding to proteins. Concept and types of cooperativity. Analysis of cooperativity. Union of oxygen to hemoglobin. Cooperativity models. Model of Monod, Wyman and Changeux. Explanation of the homotropic

cooperative effects by the MWC model. Allosteric enzymes. K-systems and V-systems. Koshland, Nemethy and Filmer model. Determination of the cooperative model that follows a certain enzyme. Example of enzyme with allosteric regulation: aspartate carbamyl transferase.

#### Unit 10. Enzymatic specificity.

The active center, specificity and three-dimensional structure. Definition of active center. Characteristics of the active center. Theories about the coupling between the enzyme and the substrate. Fisher's theory (key-lock). Koshland theory (induced-fit). Hexokinase as an example of induced coupling. Hypothesis of three-point union. Hypotheses involving tension. Stabilization of the transition state. Evidence supporting the theory of the transition state. Catalytic antibodies and their applications.

#### Unit 11. Study of the active center.

The active center. Identification of the binding and catalytic centers. Labelling with a part of the substrate. Use of artificial substrates. Chemical modification with specific irreversible inhibitors. Affinity labels. Suicide inhibitors, examples with pharmacological interest. Directed mutagenesis. Serine proteases: subtilisin. Comparison of mutagenesis and chemical labeling. Investigation of the three-dimensional structure of proteins: X-rays, NMR, molecular modeling. Restriction endonucleases. "Editorial" and error correction mechanisms: aminoacyl-tRNA synthetases.

#### Unit 12. Mechanisms of enzymatic catalysis.

Mechanisms of catalysis. Introduction to the mechanisms of enzymatic action. Acid-basic catalysis. Covalent catalysis. Pyridoxal phosphate. Catalysis with metal ions. Mechanisms of alcohol dehydrogenase and carbonic anhydrase. Environmental effect: electrostatic catalysis. The lysozyme Mechanism of subtilisin. Superoxide dismutase. Effects of proximity and orientation. Channeling intermediaries. Multifunctional enzymes. Enzymes with additional non-enzymatic functions "moonlighting enzymes".

#### Unit 13. Cofactors and ribozymes.

Cofactors and ribozymes. Catalytic activity of RNA. Type of ribozymes. The ribosome is a ribozyme. Biological meaning of ribozymes. Applications of ribozymes.

#### Unit 14. Regulation of enzymatic activity.

Regulation of enzyme activity. Modification of the enzyme concentration. Regulation of the synthesis and degradation of enzymes. Degradation mechanisms. Variation of the enzymatic speed in function of the concentration of substrate, product and cofactors. Activation by precursor and retro inhibition. Functional meaning of cooperativity and allosterism. Hormonal control. Isozymes. Polymerization-depolymerization. Binding to other proteins. Irreversible covalent modification. Reversible covalent modification. Enzymatic cascade systems.

#### Unit 15. Biomedical and biotechnological applications of enzymes.

Enzymes in clinical biochemistry and biotechnology. Enzymes as therapeutic agents. Enzyme indicators of pathologies. Plasma enzymes. Factors that affect the levels of plasma enzymes. Examples of enzymes with diagnostic interest. Aminotransferases. Creatine kinase. Lactate dehydrogenase. Indicators of myocardial infarction. Enzymes as reagents in clinical biochemistry. Enzymes and inborn errors of metabolism, examples. Enzymes in the industry. Large scale production of enzymes. Applications: drugs, food industry, detergents, textile industry. Immobilized enzymes. Enzymes as biosensors.

#### Unit 16. Directed evolution.

Methods to improve biocatalysts. Design and synthesis of new catalysts. Directed evolution. Generation of mutants. Selection and screening of the desired enzymatic activity. Re-design of enzymes to modify their thermostability and enantioselectivity. Adaptive evolution in the laboratory.

Problems.

The problems that are proposed refer to the analysis of enzyme activity and determination and interpretation of kinetic parameters. The statements of the problems will be delivered through the Virtual Campus.

Delivery of works through the tool of the "Virtual Campus":

Two works will be proposed through the Virtual Campus, which must be worked out by the teams (of three/four people) of students established at the beginning of the course. The works must be delivered before a specific date through the Virtual Campus.

## **Methodology**

The subject of Biocatalysis consists of theoretical classes, delivery of works by the virtual campus, classes of problems and classes of practices. The following describes the organization and teaching methodology that will be followed in these activities.

Theory classes:

The content of the theory program will be taught mainly in the form of master classes with audiovisual support. The presentations used in class will be available to students in the Virtual Campus of the subject before the start of each of the topics. These expository sessions will be the most important part of the theory section. It is recommended that students have the material published on the Virtual Campus in printed form in order to be able to follow the classes more comfortably. It is advisable for students to regularly consult the books recommended in the Bibliography section of this teaching guide in order to consolidate and clarify, if necessary, the contents explained in class. It is also advisable for students to use the links provided on the Virtual Campus, which contain videos and animations related to the processes explained in class.

Resolution and delivery of group work:

This activity aims to work on the competence of teamwork, by organizing students into work groups in which all members must actively participate in the writing and presentation of the work.

The methodology of this activity will be as follows:

At the beginning of the course, students will be organized in groups of two or three people, registering the groups through the Virtual Campus before the deadline indicated by the teacher. The groups will work on the topics indicated for this activity outside of class hours. The works will be delivered through the Virtual Campus. The grade obtained will be applicable to all members of the working group to which the student belongs.

The statements of the deliveries will be published through the Virtual Campus where the delivery dates will also be indicated.

Problem solving classes:

There will be 5 problem sessions that will be dedicated to solving the types of problems most related to the contents of the theory program. These classes are intended to consolidate the contents previously worked on in the theory classes and also to familiarize the student with some of the experimental strategies, with the interpretation of scientific data and problem solving based on real experimental situations.

Problem statements will be delivered through the Virtual Campus in advance of the class of problems to be addressed.

Classes of practical work:

There will be 2 4-hour sessions at the laboratory, a one-hour session at the Chemical Analysis service and a three-hour session in a computer room, with the following content:

- 1.- Determination of the activity of the enzyme Bdh1p in yeast extracts (which overexpress this enzyme). Calculation of the activity in U / mL of extract, against different substrates.
- 2.- Determination of the kinetic parameters by the enzyme Bdh1p against acetoin. Preparation of reaction mixtures with different substrates. Determination of initial velocities versus acetoin and determination of kinetic parameters with a spreadsheet.
- 3.- Separation of substrates and products of the reaction mixtures by extraction with ethyl acetate. Characterization of the substrates and products of the Bdh1p reaction by separating them in a chiral column located in a gas chromatograph.
- 4.- Use of a computer program for the determination of the kinetic parameters of Bdh1p. Analysis of different inhibition patterns. Use of a computer program to study the structure of enzymes.

Tutoring sessions.

There will be a tutoring session of the class group before the partial tests 1 and 2 and, at the request of the students, individual tutoring. In the event that the number of applications is high, they will be made. In addition, classroom tutorials that would be announced in a timely manner through the Virtual Campus. The aim of these sessions will be to resolve doubts, review basic concepts and guide on the sources of information consulted.

Material available in the Virtual Campus of the subject:

Presentations used by the teacher in theory classes. Deliveries. Problem statements. Protocol of the practical classes. Calendar of teaching activities (classroom classes, tutorials and assessments).

Annotation: Within the schedule set by the centre or degree programme, 15 minutes of one class will be reserved for students to evaluate their lecturers and their courses or modules through questionnaires.

## Activities

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Laboratory practices	12	0.48	3, 4, 17, 5, 10, 13
Problem solving classes	5	0.2	4, 17, 6, 10
Theory classes	35	1.4	1, 15, 14, 17, 6, 10, 11, 13, 7, 8, 9
Type: Supervised			
Group tutorials	2	0.08	10, 13, 8, 9
Type: Autonomous			
Problem resolution	20	0.8	4, 17, 6, 10, 13, 7, 16
Study	50	2	2, 4, 10, 13, 7, 8, 9, 16
Teamwork delivered through the Virtual Campus Platform	11	0.44	17, 13, 7, 16

## Assessment

This subject includes two types of assessment: continuous and unique.

### Continuous assessment.

The objective of the continuous assessment is to encourage the student's effort throughout the course, allowing them to evaluate their degree of follow-up and understanding of the subject.

Partial theory tests. Individual evaluation (6/10)

- The evaluation of this activity will be carried out through two written tests in which the student must demonstrate his degree of achievement of the theoretical concepts.

- Each of the tests will have an overall weight of 30%. The first will be scheduled in the middle of the semester and the second at the end of the semester. Both tests will include "test" questions and short questions related to theory classes.

Problem test. Individual evaluation (1/10)

On the day of the second partial theory test, three problems of the types covered through the problem classes will have to be solved. The result of this test will have an overall weight of 10%.

Submissions via the Virtual Campus. Group evaluation (1.5/10).

Two deliveries related to the content given in the theory and problems classes will be made during the course. The works prepared in groups of 2-3 people will be delivered through the Virtual Campus. For the assessment, not only the correct resolution of the work but also its approach and presentation will be taken into account. The whole group will receive the same grade. If deemed necessary, the teacher may request that a questionnaire regarding the group's work be completed individually. Although the results of this questionnaire will not initially have a specific weight in the grade of the subject, in case of detecting negative evaluations of a person by the rest of the members of their group that show that they have not participated in the work, the qualification obtained by the group will not be applied or may be reduced.

Attendance to practical classes and completion of the corresponding report. Group evaluation (1.5/10).

The student must bring the appropriate material such as gown, protective glasses and the practice script (previously worked at home). The attitude of the student in the laboratory will be evaluated, as well as their work. The student will deliver a practice report on the day signed by the teacher in which he will have answered the questions raised. The evaluation of the attitude will represent 25% of the grade and the evaluation of the report presented, the other 75% of the total grade.

Recovery test .

Students who have not obtained a grade equal to or greater than 5, will have to take the recovery exam, in which they can choose between theory 1st partial and/or theory 2nd partial and / or problems.

The activities of deliveries of works through the virtual campus and the assistance and presentation of the memory of the practical classes are not recoverable.

### Examination-based assessment.

Theory (60% of the overall grade)

Individual evaluation through:

A final test, which will be carried out at the same time as the second partial exam of the subject, in which the subject will be that of the whole subject. In this test there will be multiple choice questions and short questions. The weight of this test will be 60% of the overall grade.

Problems (10% of the overall grade).

A final test, which will be carried out at the same time as the second partial exam of the subject, in which three problems of all the subject matter of the subject must be solved. The weight of this test will be 10% of the overall grade.

Submissions through the Virtual Campus: (15% of the overall grade).

The content and rules of this section are the same as those described under the heading of continuous assessment.

Attendance to practical classes and completion of the report. Group assessment (15% of the overall grade).

The content and rules of this section are the same as those described under the heading of continuous assessment.

In all cases, in addition to knowledge, the acquisition of written communication skills will be taken into account.

Recovery test.

Students who have not obtained a grade equal to or greater than 5, will have to take the recovery exam, in which they can choose between taking a theory exam of the whole course and/or problems.

Global evaluation of the subject.

In case of continuous evaluation, the overall evaluation of the subject will include the qualifications of the two partial theory tests and the problem test, as well as the delivery of group work and the attendance and presentation of the memory of the practical classes. Out of a total of 10 points, it will be necessary to obtain a global grade equal to or greater than 5 points to pass the subject.

In the case of single assessment, the overall evaluation of the subject will include the grades of the final theory and problem test, as well as the delivery of group work and attendance and presentation of the report of the practical classes. Out of a total of 10 points, it will be necessary to obtain a global grade equal to or greater than 5 points to pass the subject.

Students who cannot attend an individual assessment test for justified reasons (such as illness, death of a first-degree relative or accident) and provide the corresponding official documentation to the teacher or degree coordinator, will be entitled to take the test in question on another date.

## Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Problem-solving exam	10	2	0.08	4, 6, 10, 7
Teamwork delivered through the Virtual Campus Platform	15	0	0	17, 13, 7, 12
Theory partial examinations	60	5	0.2	1, 15, 14, 6, 10, 11, 7, 8, 9
Writing and presentation of the laboratory practices	15	8	0.32	2, 3, 4, 17, 5, 10, 13, 12, 16

## Bibliography

### Specific titles

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- Evaluation of enzyme inhibitors in drug discovery. R. A. Copeland (2013). 2nd ed. Wiley Interscience. John Wiley & Sons.

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## Links

They will be updated in the Virtual Campus of the subject

## Software

### Software

The programs that will be used during the course are:

COPASI.

COPASI is a program for the simulation and analysis of biochemical and dynamic networks.

<http://copasi.org/>

PYMOL.

It is a molecular visualization program.

<https://pymol.org>

JSME and CHEMSKETCH.

Software that allows the drawing of the structures of chemical compounds.