

Biocatalysis

Code: 100956
ECTS Credits: 6

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Degree	Type	Year
2500253 Biotechnology	OT	4

Contact

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Teaching groups languages

You can view this information at the [end](#) of this document.

Prerequisites

There are no official prerequisites. In any case, part of the contents of some subjects of 1st year and 3rd year are necessary to be able to follow the subject correctly. In particular, those of the following subjects: Biochemistry, Chemistry and Engineering of Proteins and Basic and Advanced Instrumental Techniques

Objectives and Contextualisation

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 specific software to study the structure of enzymes and modulators, as well as the enzyme kinetics.

Learning Outcomes

1. CM32 (Competence) Plan a process for obtaining biotechnological products.
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3. KM34 (Knowledge) Describe the properties of microorganisms with potential application in different biotechnological processes.
4. SM33 (Skill) Interpret the kinetic parameters of enzymatic reactions, by means of graphical methods and using computer programmes.
5. SM33 (Skill) Interpret the kinetic parameters of enzymatic reactions, by means of graphic methods and using computer programmes.

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 Allosterism.

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

There will be five problem solving sessions, in which problems of enzyme purification, determination of kinetic parameters in the absence and presence of inhibitors, as well as characterization of mechanisms of inhibition and elucidation of bi-substrate reaction mechanisms will be addressed.

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 two/three people) of students established at the beginning of the course. The works must be delivered before a specific date through the Virtual Campus.

PRACTICAL LABORATORY

They are organized in 2 sessions of 4 hours in the laboratory, a session of one hour at the Chemical Analysis Service and a session of three hours in a computer room.

Program: Characterization of an enzyme overexpressed in yeast (*Saccharomyces cerevisiae*). Analysis of the stereospecificity of the reaction for different substrates using gas chromatography. Determination of kinetic parameters in steady state conditions, using specific "software". There will be also an introduction to a software to study the tridimensional structure of proteins.

Activities and Methodology

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Problems solving classes	5	0.2	
Sessions of laboratory work	12	0.48	
Theory classes	35	1.4	
Type: Supervised			
Group tutorials	2	0.08	
Type: Autonomous			
Delivery of team work through the "Campus virtual"	11	0.44	
Problem resolution	20	0.8	

The subject of Biocatalysis consists of theoretical classes, group work (delivered through the virtual campus platform), problem classes and practical laboratory sessions. The following describes the organization and teaching methodology that will be followed in these activities.

Theoretical 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 platform 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 in the Virtual Campus in printed form to be able to follow the classes more comfortably. It is advised that students consult regularly the recommended books in the Bibliography section of this teaching guide to consolidate and clarify, if necessary, the contents explained in class. It is also advisable that students use the links indicated in 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, through the organization of students in working groups in which all members must actively participate in the writing and presentation of the work.

The methodology of this activity will be the following:

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

The delivery statements 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 devoted to solving the types of problems most related to the contents of the theory program. It is intended that these classes serve to consolidate the contents previously worked in theory classes and also for the student to become familiar with some of the experimental strategies, with the interpretation of scientific data and the resolution of problems based on real experimental situations.

The statements of the problems will be delivered through the Virtual Campus in advance to the kind of problems in which they will be treated.

Classes of practical work:

There will be two 4-hour sessions, a one-hour session and a three-hour session, with the following content:

- 1.- Determination of the activity of the Bdh1p enzyme in yeast extracts (which overexpress this enzyme). Calculation of activity in U / mL of extract, against different substrates.
- 2.- Determination of the kinetic parameters for the enzyme Bdh1p versus acetoin. Preparation of reaction mixtures with different substrates. Determination of initial rates against 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 connected to a gas chromatograph.

4.- Use of a computer programs to determine the kinetic parameters of Bdh1p. Analysis of different inhibition patterns. Use of a computer program to study the structure of enzymes.

Tutorials

There will be a tutorial session of the class group before partial tests 1 and 2 and, at the request of the students, individual tutoring. In case the number of applications is high, additional classroom tutorials will be carried out, which will be announced in a timely manner through the Virtual Campus. The objective of these sessions will be to solve doubts, review basic concepts and guide the sources of information consulted.

Material available in the Virtual Campus of the subject:

Presentations used by the teacher in theory classes. Deliveries. Statement of problems. Protocol of laboratory classes. Calendar of teaching activities (classroom classes, tutorials and evaluations).

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.

Assessment

Continuous Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Elaboration of the memory of practical work in the laboratory	15	8	0.32	
Problem-solving exam	10	2	0.08	SM33
Teamwork delivered through the Virtual Campus Platform	15	0	0	CM32, KM34
Theory partial examinations	60	5	0.2	

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.

Bibliography

Bibliography

Specific titles

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Web links

They will be updated in the Virtual Campus of the subject.

Software

Software

The software that will be used during the course will be:

[COPASI](#). It is a program for the simulation and analysis of biochemical and dynamic networks.

[PYMOL](#). It is a molecular visualization program.

[JSME](#) i [CHEMSKETCH](#). Software that allows the drawing of the structures of chemical compounds.

Language list

Name	Group	Language	Semester	Turn
(PAUL) Classroom practices	441	Catalan	second semester	morning-mixed
(PLAB) Practical laboratories	441	Catalan/Spanish	second semester	afternoon
(TE) Theory	44	Catalan	second semester	morning-mixed