Universitat Autònoma de Barcelona

2019/2020

Biocatalysis

Code: 100956
ECTS Credits: 6

Degree	Type	Year	Semester
2500253 Biotechnology	OT	4	0

Contact

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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

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.

Competences

- Apply the principal techniques for the use of biological systems: recombinant DNA and cloning, cell cultures, manipulation of viruses, bacteria and animal and plant cells, immunological techniques, microscopy techniques, recombinant proteins and methods of separation and characterisation of biomolecules.
- Describe the molecular, cellular and physiological bases of the organisation, functioning and integration of living organisms in the framework of their application to biotechnological processes.

- Design and implement a complete protocol for obtaining and purifying a biotechnological product.
- Design continuation experiments for problem solving.
- Interpret experimental results and identify consistent and inconsistent elements.
- Learn new knowledge and techniques autonomously.
- Obtain information from databases and use the software necessary to establish correlations between the structure, function and evolution of macromolecules.
- Read specialised texts both in English and ones own language.
- Reason in a critical manner
- Search for and manage information from various sources.
- Search for, obtain and interpret information from the principal databases on biology, bibliography and patents and use basic bioinformatic tools.
- Think in an integrated manner and approach problems from different perspectives.
- Use ICT for communication, information searching, data processing and calculations.
- Use analytical methodologies for assaying the biological activity of cellular components, especially enzymes, both in vitro and in vivo.
- Work individually and in teams

Learning Outcomes

1. Assess the suitability of the methods for determining enzyme activities and analyse the effect of the test conditions.
2. Calculate and interpret the kinetic parameters of enzyme reactions, by means of graphic methods using computer programmes.
3. Design continuation experiments for problem solving.
4. Design, execute and evaluate a basic protocol for obtaining and purifying an enzyme.
5. Explain the fundamental physicochemical principles of enzyme catalysis.
6. Explain the structural bases and the principal mechanisms of enzyme catalysis and how it is regulated.
7. Identify the principal mechanisms of enzyme inhibition, know their biological significance and calculate and interpret the corresponding constants.
8. Interpret experimental results and identify consistent and inconsistent elements.
9. Learn new knowledge and techniques autonomously.
10. Obtain information on the structural basis of enzymes and their mechanisms from the principal databases.
11. Read specialised texts both in English and ones own language.
12. Reason in a critical manner
13. Search for and manage information from various sources.
14. Think in an integrated manner and approach problems from different perspectives.
15. Use ICT for communication, information searching, data processing and calculations.
16. Use enzyme databases to study enzyme activity, biological functions and applications.
17. Use knowledge of living organisms and their enzyme systems to design processes and obtain biotechnological products.
18. Use these techniques to identify, clone, and express genes and proteins that are useful for designing and obtaining enzymes.
19. Work individually and in teams

Content

Topic 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.

Topic 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.

Topic 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. 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-stationary and steady-states: concepts. Stationary state hypothesis: treatment of Briggs-Haldane. Enzymatic reactions with more than one enzyme-substrate intermediate complex.

Unit 5. Determination of kinetic parameters.

Determination of kinetic parameters. Methods with linear representations: Lineweaver-Burk, Eadie-Hofstee and Hanes-Woolf. Other methods. Significance of the kcat, KM and kcat / KM kinetic parameters. Michaelis-Menten equation for reversible reactions: Haldane relationship.

Unit 6. 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 IC50 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 7. 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.

Topic 8. Kinetics of ephemeral or 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) Analysis of the "Burst" of a reaction: determination of the concentration of active centers "Bursts" and "lags".

Topic 9. 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 union and catalysis. Effects of the micro environment on the pK.

Topic 10. 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.

Topic 11. 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.

Topic 12. 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. The alcohol dehydrogenase. Restriction endonucleases. "Editorial" and error correction mechanisms: aminoacyl-tRNA synthetases.

Topic 13. 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. Enzymeswith additional non-enzymatic functions "moonlighting enzymes".

Topic 14. 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.

Topic 15. 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.

Topic 16. 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.

Topic 17. 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 whichproblems 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 group work (through the Virtual Campus platform)

During the course two deliveries will be made (through the virtual campus platform) of topics related to subjects covered in class. They can be done by groups of two or three people.

PRACTICAL LABORATORY

They are organized in 2 sessions of 4 hours, a session of one hour and a session of three hours.

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.

Methodology

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).

Activities

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Problems solving classes	5	0.2	15, 1, 2, 4, 7, 12, 19
Sessions of laboratory work	12	0.48	9, 15, 1, 13, 2, 3, 8, 12, 19, 16
Theory classes	35	1.4	9, 13, 5, 6, 11, 10, 14, 12, 19, 18, 17, 16
Type: Supervised			
Group tutorials	2	0.08	13, 14, 12
Type: Autonomous			
Elaboration of the memory of the practical work in the laboratory	11	0.44	9, 15, 1, 13, 2, 3, 7, 8, 11, 10, 14, 12, 19, 16
Problem resolution	15	0.6	15, 1, 13, 2, 4, 7, 8, 10, 12, 19
Study	40	1.6	9, 13, 5, 6, 11, 10, 14, 12, 19, 16

Assessment

Evaluation

Partial theory exams. Individual evaluation (5/10).

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

- Each of the tests will have a global weight of 25%. The first will be scheduled in the middle of the semester and the second at the end of the semester. The two tests will include short questions related to the theory classes.

Problems examination. Individual evaluation (1/10).

The day of the second partial test, three problems will have to be solved. These problems will be similar to the ones explained in the classes of problems. The result of this test will have a global weight of 10%.

Deliveries through the Virtual Campus. Group evaluation (2.5/10). This activity is not recoverable.

Two deliveries related to the content given in the theory classes will be carried out during the course. The works prepared in groups of 2-3 people will be delivered through the Virtual Campus Platform. For the assessment will be taken into account not only the correct resolution of the work but also its approach and presentation. The entire group will receive the same rating.

If deemed necessary, the teacher may request that a questionnaire concerning the group's work be filled in individually. Although the results of this questionnaire will not have, in the first place, a specific weight in the qualification 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 grade obtained by the group will not be applied or it may be reduced.

Attendance at practical classes and realization of the corresponding memory. Group evaluation (1.5 / 10). This activity is not recoverable.

The student must bring the appropriate material such as gown, protective glasses and the practice script (previously workedat home). The attitude of the student in the laboratory, as well as his work, will be evaluated. The student will deliver a practice report on the day set by the teacher in which the questions have been answered. The evaluation of the attitude will suppose 25% of the note and the evaluation of the presented memory, the remaining 75% of the total of the note.

Global evaluation of the subject.

The global evaluation of the subject will include the qualifications of the two partial tests of theory, the test of problems, the qualification of the teamwork deliveries and the qualification of the laboratory practices. On a total of 10 points it will be necessary to obtain a global grade equal to or greater than 5 points for the total evaluation of the subject.

The persons who, for just cause and having received the prior authorization of the professor, do not belong to any work group have not been able to demonstrate the passing of some competences and learning results of the subject. In this case, the maximum grade that can be obtained in the subject will be 7.5 points out of 10.

To pass the course requires that the theory note + the problem note + the teamwork note + the practical note add up to a minimum of 5 points out of 10 possible.

Recovery test.

To participate in the recovery test, students must have been previously evaluated in a set of activities the weight of which equals a minimum of two thirds of the total grade of the subject or module. Therefore, the students will obtain the "Not Evaluable" qualification when the evaluation activities carried out have a weight lower than 67% in the final grade.

On the day of the recovery test, there will be a recovery test of the first partial, another of the second partial and another of problems. Students can attend any of them. In the case of wanting to improve the qualification, the students can also be presented in any of the tests: the realization of this new test (or tests) supposes the resignation to the first qualification.

Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Laboratory practices and memory elaboration	15	20	0.8	9, 15, 1, 13, 2, 3, 4, 7, 8, 11, 10, 14, 12, 19, 16
Problem-solving exam	10	2	0.08	1, 2, 4, 7, 12
Teamwork delivered through the Virtual Campus Platform	25	4	0.16	9, 15, 1, 10, 19, 16
Theory partial examinations	50	4	0.16	13, 3, 5, 6, 7, 8, 14, 12, 18, 17

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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- Structure and Mechanism in Protein Science. A guide to Enzyme Catalysis and Protein Folding (1998). A. Fersht. W.H. Freeman & Company.

Generic Titles

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- "Biochemistry" (2011). Voet, D., and Voet, J.G. 4th ed. Ed.Wiley. London

- "Bioquímica" (2013). Mathews, C.K., van Holde, K.E., Appling, D., Anthony-Cahill, S. 4th ed. Addison / Wesley. McGraw-Hill / Interamericana. Madrid.

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- "Lehninger Principios de Bioquímica" (2014). Nelson, D.L. and Cox, M.M. 6th ed. Omega. Barcelona.

Web links

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