Universitat Autònoma de Barcelona

2022/2023

Biocatalysis

Code: 100764
ECTS Credits: 6

Degree	Type	Year	Semester
2500250 Biology	OT	4	2

Contact

Name: Josep Antoni Biosca Vaque
Email: josep.biosca@uab.cat

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

Other comments on languages

The classes of theory and problems will be in Catalan, but most of the graphic material and the bibliography will be in English.

Prerequisites

There are no official prerequisites. In any case, some of the contents of the 1st and 2nd year courses, Biochemistry and Biosignalling and metabolism, are necessary to follow the subject correctly.

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

Competences

- Act with ethical responsibility and respect for fundamental rights and duties, diversity and democratic values.
- Apply statistical and computer resources to the interpretation of data.
- Be able to analyse and synthesise
- Be able to organise and plan.
- Carry out functional tests and determine, assess and interpret vital parameters.
- Make changes to methods and processes in the area of knowledge in order to provide innovative responses to society's needs and demands.
- Obtain information, design experiments and interpret biological results.
- Students must be capable of applying their knowledge to their work or vocation in a professional way and they should have building arguments and problem resolution skills within their area of study.
- Students must be capable of collecting and interpreting relevant data (usually within their area of study) in order to make statements that reflect social, scientific or ethical relevant issues.
- Students must be capable of communicating information, ideas, problems and solutions to both specialised and non-specialised audiences.
- Students must develop the necessary learning skills to undertake further training with a high degree of autonomy.
- Students must have and understand knowledge of an area of study built on the basis of general secondary education, and while it relies on some advanced textbooks it also includes some aspects coming from the forefront of its field of study.
- 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.
- Understand and interpret the physicochemical bases of the basic processes of living beings
- Understand the processes that determine the functioning of living beings in each of their levels of organisation.

Learning Outcomes

1. Analyse a situation and identify its points for improvement.
2. Apply statistical and computer resources to the interpretation of data.
3. Assess the suitability of the methods for determining enzyme activities and analyse the effect of the test conditions.
4. Be able to analyse and synthesise.
5. Be able to organise and plan.
6. Calculate and interpret the kinetic parameters of enzyme reactions, by means of graphic methods using computer programmes.
7. Correctly analyse data on ligand-macromolecule affinity constants and binding points.
8. Critically analyse the principles, values and procedures that govern the exercise of the profession.
9. Describe the fundamental physicochemical principles of enzyme catalysis.
10. Describe the structural bases and the principal mechanisms of enzyme catalysis and how they are regulated.
11. Identify the principal mechanisms of enzyme inhibition, know their biological significance and calculate and interpret the corresponding constants.
12. Obtain information on the structural basis of enzymes and their mechanisms from the principal databases.
13. Propose new methods or well-founded alternative solutions.
14. Students must be capable of applying their knowledge to their work or vocation in a professional way and they should have building arguments and problem resolution skills within their area of study.
15. Students must be capable of collecting and interpreting relevant data (usually within their area of study) in order to make statements that reflect social, scientific or ethical relevant issues.
16. Students must be capable of communicating information, ideas, problems and solutions to both specialised and non-specialised audiences.
17. Students must develop the necessary learning skills to undertake further training with a high degree of autonomy.

18. Students must have and understand knowledge of an area of study built on the basis of general secondary education, and while it relies on some advanced textbooks it also includes some aspects coming from the forefront of its field of study.
19. Take account of social, economic and environmental impacts when operating within one's own area of knowledge.
20. Take sex- or gender-based inequalities into consideration when operating within one's own area of knowledge.
21. Use enzyme databases to study enzyme activity, 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. Dirct and indirect, continous and discontinous 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 kcat, KM and kcat/KM. 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 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 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. Exemples.

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. Enzymeswith 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 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 in a 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".

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 dateswill 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			
Problems solving classes	5	0.2	7, 2, 3, 6, 11, 5, 21

Sessions of laboratory work	12	0.48	2, 3, 6, 11, 5, 21
Theory classes	35	1.4	20, 19, 8, 1, 3, 9, 10, 11, 12, 13, 5, 21
Type: Supervised			
Group tutorials	2	0.08	3, 9, 10, 4
Type: Autonomous			
Problem resolution	20	0.8	7, 2, 3, 6, 11, 4, 5, 21
Study	50	2	7, 9, 10, 11, 12, 4, 5, 21
Teamwork delivered through the virtual campus platform	11	0.44	12, 4, 5, 21

Assessment

Evaluation

Partial theory exams. Individual evaluation (6/10).

- 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 30%. 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 both, quiz and 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 (1.5/10). 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 laboratory sessions 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 worked at 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 8.5 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.

In the event that an overall grade of less than 5 points is obtained, a recovery test must be carried out, which may be of the theoretical contents of the first and / or second part and / or of the problems. To participate in the recovery, students must have previously been assessed in a set of activities whose weight is equivalent to a minimum of two thirds of the total grade of the subject or module. Therefore, students will obtain the grade of "Non-Assessable" when the assessment activities performed have a weighting of less than 67% in the final grade. People who, despite having passed the subject, want to improve their qualification will also be able to take this recovery test. It should be borne in mind, however, that taking this test to recover from any partial or problems will involve renouncing the grade obtained above.

Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Partial exams	60	5	0.2	20, 19, 8, 1, 3, 9, 10, 11, 13, 4
Problem-solving exam	10	2	0.08	7, 3, 6, 11, 5
Teamwork delivered through the virtual campus platform	15	0	0	20, 19, 8, 3, 12, 18, 17, 16, 14, 15, 4, 5, 21
Writing and presentation of the laboratory practices	15	8	0.32	2, 3, 6, 11, 12, 4, 5, 21

Bibliography

Specific titles

- Biocatalysis. Fundamentals and applications (2004). A. S. Bommarius, B. R. Riebel. Wiley-VCH Verlag GmbH & Co. Accés on line UAB:

https://cataleg.uab.cat/iii/encore/record/C__Rb2008692__Sbommarius__Orightresult__U__X4?lang=cat&suite=de

- Biocatalysis. Biochemical Fundamentals and Applications (2018). P. Grunwald. World Scientific. 2nd Edition.

- Biomolecular kinetics. A step-by-step guide. (2017). C. Bagshaw. 1st edition. CRC Press.

http://web.a.ebscohost.com/pfi/results?vid=1&sid=1cff0d71-f937-4165-90ba-a072467c5916%40sessionmgr4006

- Biotransformations in Organic Chemistry. 6th ed. K. Faber (2011). Ed. Springer. Accés on line UAB:

https://cataleg.uab.cat/iii/encore/record/C__Rb2038210__Skurt%20faber__Orightresult__U__X4?lang=cat&suite=

- Enzyme Assays. A Practical Approach. R. Eisenthal and M. J. Danson (2002) 2nd ed. Oxford University Press. Oxford.

- Enzyme Kinetics: Principles and Methods, Third, enlarged and improved Edition. Bisswanger, H. 2017. WileyVCH Verlag GmbH & Co. KGaA. Accés on line UAB:

https://cataleg.uab.cat/iii/encore/record/C__Rb2033620__Sbisswanger__Orightresult__U__X4?lang=cat&suite=de

- Enzyme Kinetics: Catalysis & control: a reference of theory and best-practice methods. 2010. Purich, D.L.Elsevier Academic San Diego, California (recurs electrònic).

https://cataleg.uab.cat/iii/encore/record/C__Rb1856617__Spurich__Orightresult__U__X4?lang=cat&suite=def

- Enzymes: Biochemistry, Biotechnology, Clinical Chemistry. Palmer, T., Bonner, P. 2nd ed. 2007. Elsevier. Accés on line UAB:

https://cataleg.uab.cat/iii/encore/record/C__Rb1962824__Spalmer%20and%20bonner__Orightresult__U__X2?lan

- Exploring proteins, a student's guideto experimental skills and methods. Price, N.C.Ed. Oxford University Press, 2009

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

https://onlinelibrary-wiley-com.are.uab.cat/doi/book/10.1002/9781118540398

- Fundamentals of Enzyme Kinetics. A. Cornish-Bowden (2012). 4th edition. Wiley-Blackwell.

- Industrial Enzymes. Structure, Function and Applications (2007). Ed. J. Polaina and A.P. MacCabe. Springer.

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

General titles.

- "Biochemistry" (2019). Berg, J.M., Tymoczko, J.L, Gatto, Jr., Stryer, L 9ª ed. MacMillan International. New York

- "Biochemistry" (2013), Mathews, C. K., van Holde, K. E., Appling, D., Anthony-Cahill, S. 4ª ed. Pearson Education. Upper Saddle River.

- "Voet's Principles of Biochemistry" (2018). Voet D.,Voet J.G. i Pratt C.W. 5th Edition, Global Edition (2018). Wiley.

- "Fundamentos de Bioquímica. La vida a nivel molecular" (2016). Voet D., Voet J.G. i Pratt C.W. 4ª ed. Ed. Médica Panamericana. Traduït de la 4ª ed. anglesa de l'any 2013. Accés des de la UAB: https://www-medicapanamericana-com.are.uab.cat/VisorEbookV2/Ebook/9786079356972#{%22Pagina%22:%22

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

Translated from the 4th english edition 2013 published by Pearson Education.

- "Bioquímica con aplicaciones clínicas" (2013). Stryer,L., Berg, J.M., Tymoczko, J.L. 7a ed. Ed. Reverté.

Translated from the 7th english edition, 2012, published by WH Freeman and Company.

- "Lehninger Principles of Biochemistry" (2017). Nelson, D.L. and Cox, M.M. 7ª ed. Freeman, New York.

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

Links

They will be updated in the Virtual Campus of the subject

Software

Software

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

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

CHEMBIODRAW.

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