Universitat Autònoma de Barcelona

2019/2020

Biocatalysis

Code: 100867
ECTS Credits: 6

Degree	Type	Year	Semester
2500252 Biochemistry	OB	2	2

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

Teachers

Mohammed Moussaoui

Prerequisites

There are no official prerequisites. In any case, some of the contents of the 1st and 2nd year subjects (first semester) are necessary to be able to follow the subject correctly. In particular, those of the following subjects: Thermodynamics and Chemical Kinetics, Organic Chemistry of Biochemical Processes, Biochemistry I, Biochemistry II, Chemistry and Protein Engineering, Basic and Advanced Instrumental Techniques.
For some activities, a basic level of reading comprehension of English is required.

Objectives and Contextualisation

The subject Biocatatalization focuses on the study of enzymes. The knowledge of enzymes is key in the Biochemistry and related sciences given their role as catalysts of biological reactions and applications in biotechnological processes. The subject analyzes enzymes from different perspectives: 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 the biotechnological and biomedical applications.
Specific objectives of the subject:
Knowledge of the general characteristics, classification and testing methods of enzymatic activity.
Analysis of enzymatic kinetics and determination and meaning of kinetic parameters.
Knowledge of enzymatic 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

- Demonstrate understanding and use of the mechanisms of biological catalysis based on the structure of biological catalysts and chemical reactions.
- Interpret experimental results and identify consistent and inconsistent elements.

- Manage bibliographies and interpret the information in the main biological databases, and also know how to use basic ICT tools.
- Manage information and the organisation and planning of work.
- Read specialised texts both in English and ones own language.
- 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.

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. Explain the fundamental physicochemical principles of enzyme catalysis.
4. Explain the structural bases and the principal mechanisms of enzyme catalysis and how it is regulated.
5. Interpret experimental results and identify consistent and inconsistent elements.
6. Manage information and the organisation and planning of work.
7. Obtain information from databases on the structure, activity, and biological functions of enzymes and their applications.
8. Read specialised texts both in English and ones own language.
9. Use ICT for communication, information searching, data processing and calculations.

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.

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

Methodology

The subject of Biocatalysis consists of theoretical classes, problem solving classes and use of computer applications, resolution and delivery of group problems and tutorials. The training activities of the subject are complemented by the practical contents of training in the field of enzymes taught in the course Integrated Laboratory 4. The following describes the organization and teaching methodology that will be followed in these types of training activities.

Theory classes:

The content of the theory program will be taught mainly by the teacher in the form of master classes with audiovisual support. The presentations used in class by the teacher will be available in the Virtual Campus of the subject before the start of each of the topics of the course. These expository sessions will be the most important part of the theory section. It is recommended to have the material published in the Virtual Campus to be able to follow the classes more comfortably. In order to consolidate and clarify the contents explained in class, it is advisable to consult regularly the books recommended in the Bibliography section and the links and resources indicated in the different topics, which contain information related to the processes explained in class.

Classes of problem solving and use of computer applications:

In these sessions the class group will be divided into two groups (A and B). The students should check the group to which they belong and attend the corresponding classes. There will be 10 problem sessions that will be devoted to solving problems related to the contents of the theory program and the use of computer applications related to enzymes.

It is intended that these classes serve to consolidate the contents previously worked in the theory classes and also to know some of the experimental strategies, the interpretation of scientific data and the resolution of problems based on real experimental situations.

Resolution and delivery of teamwork:

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 resolution of problems.

The methodology of this activity will be the following:

At the beginning of the course the students will be organized in groups of four people, registering the groups through the Virtual Campus before the deadline indicated by the teacher (see Programming of the subject).
The groups will work the problems indicated for this activity outside of class time.
The works will be delivered through the Virtual Campus. 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.

Tutorials

Individual tutorials will be held at the request of the students. 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.

Statements of the problems or cases to work to the classes of problems. It will include the problems of group work.

Programming and information on teaching activities (classroom classes, tutorials, evaluations, ...).

Activities

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Problem solving classes and the use of computer applications	10	0.4	9, 1, 2, 5, 8, 7
Theory classes	35	1.4	1, 2, 3, 4, 8, 7
Type: Supervised			
Group tutorial	0	0	1, 2, 3, 4
Type: Autonomous			
Analysis and problem solving	20	0.8	9, 1, 2, 6, 5, 8, 7
Study	57	2.28	9, 3, 4, 6, 5, 8
Teamwork resolution of problems and delivery through the Campus Virtual	24	0.96	9, 1, 2, 6, 5, 8, 7

Assessment

Partial tests of theory and problems. Individual evaluation (8.5 / 10)

- The evaluation of this activity will be carried out through two written tests in which the degree of achievement of the theoretical concepts and the resolution of problems must be demonstrated.
- Each of the tests will have an overall weight of 4.25 out of 10. The first will be scheduled in the middle of the semester and the second at the end of the semester in the period of time corresponding to the final evaluation. In both cases the tests will include the contents of the theoretical sessions and resolution of practical cases and problems.
-To pass the course, it will be necessary for the student to obtain a minimum score of 4 points out of 10 in each of these tests.
In the event that a score lower than 4 points is obtained in any of the partial tests, a test of recovery of the corresponding contents must be carried out. People who despite having passed the partial tests want to improve their qualification may also perform this recovery test. It must be taken into account, however, that the fact of carrying out this recovery test will imply the rejection of the qualification obtained in the partial tests.

Resolution of problems worked in group and use of computer applications. Group evaluation (1,5 / 10). This activity is not recoverable.

The work prepared in groups of 4 people will be delivered through the Virtual Campus. 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.
For the 2019-2020 academic year, the people registering the subject for the second time may decide to keep the grade of the group evaluation obtained during the 2018-2019 academic year, if this is greater than 5, or to carry out this evaluation activity.

Global evaluation of the subject.

The global evaluation of the subject will include the qualifications of the two partial tests and the resolution of problems worked in group and the use of computer applications. 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 and a minimum grade of 4 out of 10 in the two partial tests. If in any of the partial tests the grade is lower than 4 points, the maximum final grade of the subject will be 4 points out of 10.
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 they can obtain in the subject will be 8.5 points out of 10.

To participate in the recovery, 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.

Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Resolution of problems and practical cases and use of computer applications	15%	0	0	9, 1, 2, 6, 5, 7
Two partial examinations of theory and problems	85%	4	0.16	1, 3, 4, 6, 5, 8

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.

- 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.

- 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.

Generic 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.

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

- "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 4a ed. of the2013 english publication from Pearson Education.

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

Translated from the 7a ed. of the 2012 english publication from 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