Universitat Autònoma de Barcelona

2022/2023

Laboratory VI

Code: 100975
ECTS Credits: 3

Degree	Type	Year	Semester
2500502 Microbiology	OB	3	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: No
Some groups entirely in Spanish: No

Other comments on languages

Lectures and assessments are given in catalan and spanish, exclusively, at the discretion of the teaching team. Teaching materials are offered in English.

Teachers

Andromeda Celeste Gomez Camacho

Daniel Yero Corona

Nuria Vigues Frantzen

Prerequisites

Students are advised to review the scientific-theoretical contents on which this subject is based.

It is also appropriate that this subject is taken simultaneously or subsequently to other subjects scheduled for the second semester of the third year of the Bachelor's Degree in Microbiology.

To take this course, students must have passed the safety and biosafety tests available in the corresponding educational space of the Campus Virtual or Moodle.

The information to pass these tests is in the communication area of the Microbiology degree. Furthermore, it is essential for students to follow the work rules as directed by teachers and to accept and follow the rules of operation of the teaching laboratories of the Faculty of Biosciences.

For security reasons, if any of the tests have not been passed or the student does not bring a lab coat and safety glasses, they will not be allowed to enter the laboratory.

Objectives and Contextualisation

This is a compulsory third year corecourse of the Bachelor's Degree in Microbiology, which introduces students to the use of a set of basic microbiology techniques for experimentation in an industrial Microbiology and Environmental Microbiology laboratory.

The knowledge gained in this course will enable students to acquire skills in other theoretical or practical subjects that make up the degree in Microbiology and are scheduled to be taken simultaneously or subsequently.

The specific objectives to be achieved are defined as follows and will allow students to:

1. Isolate and select microorganisms of industrial interest.

2. Handle a microorganism to convert it into a producer of a product of industrial interest using molecular methods.

3. Develop a process for obtaining a product of industrial interest.

4. Determine the microbiological quality of the air and surfaces in industrial facilities.

5. Determine the microbiological quality of water.

6. Describe the disinfectant effect of chemicals.

7. Convert industrial waste into products with a high protein content.

8. Select and evaluate the activity of microorganisms involved in bioremediation of soils.

9. Demonstrate the activity of bacteria inhibiting the growth of fungal pathogens.

Competences

- Adapt to new situations.
- Apply knowledge of theory to practice
- Apply the principles of risk assessment and prevention in the laboratory, and biosafety regulations on microorganisms and manipulation of different biological systems.
- Apply tools based on microorganisms to assess the environmental impact of human activity, and to recover contaminated environments.
- Assess the quality and/or microbiological safety of foods, water, drugs, cosmetics and other natural or artificial products.
- Characterise the causal agents of microbial diseases in humans, animals and plants in order to diagnose and control them, perform epidemiological studies and be aware of present-day problems with these diseases and strategies to combat them.
- Design and control processes of microbial origin and participate in productive processes in which microorganisms intervene.
- Design and use disinfection and sterilisation treatments and also methods for assessing their effectiveness.
- Develop critical reasoning skills in the field of study and in relation to the social context.
- Display a capacity for analysis, synthesis, organisation, planning and decision-making.
- Display sensibility towards environmental, health and social matters.
- Know and apply safety and quality regulations in microbiology.

Learning Outcomes

1. Adapt to new situations.
2. Analyse and monitor the population dynamics. of microorganisms in applied processes.
3. Apply biosafety regulations in the laboratory.
4. Apply disinfection treatments and evaluate their efficiency.
5. Apply in the laboratory the principles of risk assessment and prevention.
6. Apply knowledge of theory to practice
7. Apply methods for evaluating the potential for disinfection of chemical products.
8. Assess the effect of disinfectants on microorganisms and work surfaces.
9. Calculate the yield of microorganisms grown in different substrates.

10. Design strategies for bioremediation and biorecovery based on model systems developed in the laboratory.
11. Determine levels of atmospheric microbial contamination.
12. Develop critical reasoning skills in the field of study and in relation to the social context.
13. Display a capacity for analysis, synthesis, organisation, planning and decision-making.
14. Display sensibility towards environmental, health and social matters.
15. Establish the optimal conditions for products of microbial origin.
16. Evaluate biological activity in microbial products.
17. Evaluate the microbial load of surfaces.
18. Experimentally determine and interpret the kinetic parameters that define microbial growth.
19. Experimentally determine the parameters for defining sterilisation treatments.
20. Isolate and characterise potential biological control agents based on microorganisms for the control of pests and diseases.
21. Isolate and cultivate microorganisms of interest in biotechnology.
22. Know and apply safety and quality regulations in microbiology.
23. Select microbial communities for the treatment of contaminants.
24. Use bioindicators to assess environmental impacts.
25. Use continuous- and discontinuous-operation bioreactors.
26. Use microorganisms to evaluate the degree of contamination of the medium and recover it.
27. Use suitable instruments for the monitoring and control of processes based on microorganisms.

Content

The course is divided into the following sections:

Section 1: Industrial Microbiology

Section 2: Environmental Microbiology

Each section occupies approximately 24 hours.

Section 1: Industrial Microbiology

Content: This section consists of seven sessions focusing on the isolation and the use of microorganisms to obtain products of industrial interest. Lab work in this section will be performed under the safety and biosafety regulations indicated at the beginning of each session.

1. Isolation and selection of microorganisms of industrial interest-MI1

2. Screening for activity-MI2

3. Isolation of genes of industrial interest-MI3

4. Cloning of overexpression vector-MI4

5. Development of-production method MI5

6. Assessment-MI6

Section 2: Environmental Microbiology

Content: This section consists of eight sessions focusing on detection of microbial environmental contamination, the use of microorganisms in bioremediation and recovery of waste, as well as in plague control and plant diseases.

1. Waste management

- Bioremediation in contaminated soil-MA1

- Production of single-cell protein starting from waste-MA2

2. Control of environmental pollution

- Air and surface quality in industrial facilities-MA3

- Microbiological quality of water-MA4

- Disinfection of surfaces-MA5

3. Biological control

- Isolation of bacteria from the phylosphere.

- Determination of their potential as natural enemies of plant pathogens -MA6

The contents per session that will be taught in each of the modules are as follows:

Section 1: Industrial Microbiology

Content	Session	Activities
MI1	1	- Enrichment and isolation of *Actinomycetes* from soil samples
	2	- Enrichment and isolation of *Bacillus* from soil samples - Identification, counting and isolation of *Actinomycetes* clones
	3	- Identification, counting and isolation of *Bacillus* clones
MI2	3	- Detection of the antibiotic activity of *Actinomycetes*-plating - Detection of the enzymatic activity of *Actinomycetes*-plating
	4	- Detection of the antibiotic activity of *Bacillus*-plating - Detection of the enzymatic activity of *Bacillus*-plating
	5	- Evaluation of the enzymatic activity of *Actinomycetes* - Detection of the enzymatic activity of *Actinomycetes*-double layered plating
	6	- Detection of the enzymatic activity of *Bacillus*-double layered plating
	7	- Evaluation of the antibiotic activity of *Bacillus* - Evaluation of the enzymatic activity of *Bacillus* - Evaluation of the antibiotic activity of *Actinomycetes*

		- Evaluation and discussion of results
MI3	1	- Purification of genomic DNA of *Bacillus* - PCR to amplify genes of industrial interest
	2	- Agarose gel to determine the DNA amplification of the gene of industrial interest
MI4	2	- Restriction enzyme digestion of the *E. coli* expression vector - Restriction enzyme digestion of DNA fragments containing the gene of industrial interest - Dephosphorylation and purification of the linearised *E. coli* expression vector
	3	- Ligation of the gene of industrial interest - Transformation of the ligation reaction
	4	- Detection of productive transformants
MI5	5	- Inoculation of microorganisms producing enzymatic activities of industrial interest
	6	- Batch experiment: reinoculation of the culture and growth curve monitoring of microorganisms producing enzymatic activities of industrial interest
MI6	6	- Evaluation of the production and activity of enzymes of industrial interest produced by microorganisms
	7	- Evaluation and discussion of results

Section 2: Environmental Microbiology

Content	Session	Activities
MA1	4	- Enrichment and isolation of hydrocarbon-degrading bacteria from contaminated soil samples - Determination of the microbial activity of contaminated soil samples

	8	- Reading total heterotrophs - Detection of total hydrocarbon degrading microorganisms - Detection and enumeration of microorganisms degrading polycyclic aromatic hydrocarbons - Detection of n-hexadecane degrading microorganisms
	9	- Enumeration of total hydrocarbon degrading microorganisms - Enumeration of n-hexadecane degrading microorganisms - Evaluation and discussion of results
MA2	1	- Inoculation of bioreactors with microorganisms in media with different concentrations of sugars - Initial sampling of the culture: viable cell plating, sugars, OD - Analysis of samples: viable cell plating, OD
	2	- Sampling of the culture: viable cell plating, sugars, OD - Analysis of samples: viable cell counting, growth rate of the culture, doubling time, OD, determination of sugar consumption
	3	Final sampling of the culture: - Analysis of the samples: - Cell counting and graphs - Determination of sugar consumption - Determination of the biomass of the culture - Calculations - Evaluation and discussion of results
MA3	5	- Detection ofmicroorganisms in air samples - Detection of microorganisms in surface samples
	7	- Observation and counting of microorganisms of air and surface samples - Evaluation and discussion of results
MA4	5	- Plating of viable microorganisms from water samples - Detection and counts of coliform bacteria and *Escherichia coli*

		- Intestinal Enterococcal detection and counting - Detection and counts of *Clostridium perfringens*
	6	- Evaluation and test to confirm the presence of *Escherichia coli* - Evaluation and test to confirm the presence of *Clostridium perfringens*
	7	- Evaluation of the presence of *Escherichia coli* in water samples - Evaluation and test to confirm the presence of faecal enterococci
	8	- Count of viable microorganisms from water samples - Evaluation and discussion of results
MA5	6	- Detection of microorganisms in disinfected surface samples
	8	- Observation and counting of microorganisms from disinfected surfaces - Evaluation and discussion of results
MA6	5	- Isolation of epiphytic bacteria from leaves of different plant species
	6	- Preparation of axenic cultures of leaf epiphytic bacteria
	7	- Conducting the biological control test of fungi by leaf epiphytic bacteria - Evaluation of the results obtained from the antagonistic potential of leaf epiphytic bacteria on a phytopathogenic fungus - Evaluation and discussion of results

Methodology

This subject will be taught in three small groups of students (maximum 24 students per session). Attendance of the classes is obligatory to be able to acquire the competences of the subject. If a student, for justified and unforeseeable reasons (such as a health problem, death of a relative up to second degree, accident, enjoying the status of elite athlete and having a competition or sports activity that they are obliged to attend, etc.), has not attended a session/practice session, they will have to speak with the teacher in charge and present the corresponding proof as soon as possible (official medical certificate stating explicitly the inability to attend the session/practice session, police report, justification from the competent sports organisation, etc.). In the case of a strike, if a student decides to exercise their right to strike, they must notify the subject coordinator within a maximum period of 48 hours after the day of the strike. In no event shall the absence exceed 20% of the

programmed activities. The maximum absence in each of the sections (industrial or environmental microbiology) is set at a maximum of 10% in order to be assessed. If this value is exceeded, the subject will be classified as Non-assessable.

Students will have a Handbook of the subject before the beginning of the practical sessions. At each practical session it is compulsory for the student to wear their own lab coat, laboratory glasses, and have a permanent marker, calculator and the Handbook of the subject that will be available on the Campus Virtual, Moodle platform, or where indicated by the teachers. It is also necessary to have a notebook (Miquelrius, Abacus or Oxford) with stiched pages, where each student will note the laboratory observations. To undertake the practical sessions the students will work in pairs and under the supervision of the teacher. At the beginning of and/or during each session the teacher will make brief theoretical explanations of the content of the practicals and procedures to be carried out by the students, as well as the specific safety measures and the treatment of the different chemical and biological waste generated.

In order to achieve good performance and to acquire the competences corresponding to this subject, it is essential that students undertake a comprehensive reading of the Handbook for the subject, becoming familiar with the practices that will be carried out in each session, as well as with the methodology that they will have to apply in each case.

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			
Practical Laboratory Classes	48	1.92	1, 2, 5, 3, 6, 7, 4, 8, 20, 21, 9, 22, 12, 11, 19, 18, 10, 15, 23, 14, 13, 24, 25, 26, 27, 16, 17
Type: Supervised			
Tutoring	1	0.04	1, 2, 5, 3, 6, 7, 4, 8, 20, 21, 9, 22, 12, 11, 19, 18, 10, 15, 23, 14, 13, 24, 25, 26, 27, 16, 17
Type: Autonomous			
Comprehensive reading of the practical sessions laboratory handbook	5	0.2	2, 5, 3, 7, 4, 8, 20, 21, 9, 11, 19, 18, 10, 15, 23, 24, 25, 26, 27, 16, 17
Drafting of laboratory notebook	6	0.24	13
Preparation of oral presentations	3	0.12	12, 13
Study	12	0.48	2, 5, 3, 7, 4, 8, 20, 21, 9, 11, 19, 18, 10, 15, 23, 24, 25, 26, 27, 16, 17

Assessment

The assessment of the subject will be conducted by section and will be continous. The weight of the assessment on the final grade of the subject of each section is: Section 1, 50%, Section 2, 50%. It will be necessary to obtain a grade equal to or higher than 5 in each of the practical sections separately to be able to pass the subject.

The assessment of each section will be carried out as follows:

Section 1. Industrial Microbiology

Continuous assessment of group work

Oral presentation discussion of the results of the practical sessions and participation in the practical sessions (20%)

Individual assessment of the content

Daily follow-up of the notebook and work in the laboratory (10%)

Individual questionnaire with multiple-choice questions (20%)*

* The individual questionnaire can be set at any time during the practice sessions and there may be more than one.

Section 2: Environmental Microbiology

Continuous assessment of group work

Delivery of a final report Bioreactors (3.3%)

Oral presentation discussion of the results of the practical sessions and participation in the practical sessions (6.7%)

Individual evaluation of the contents

Daily follow-up of the laboratory book (10%). Individual multiple-choice test (30%)

In each section, the student's attitude in the laboratory, punctuality, use of laboratory equipment (lab coat and goggles), compliance with safety and biosecurity regulations, and understanding and follow-up of the Handbook of the subject will be assessed. This assessment does not entail an increase in the grade, but it can mean a reduction of up to 20% of the final grade obtained in each module.

Since attendance of the activities scheduled in this subject is mandatory, absence from any of the sessions must be justified and may not exceed 20%. In any case, this 20% can not be accumulated in a single section, the maximum of each being 10% absence. If this value is exceeded, the student will be gradedas Non-assessable (*No avaluable*).

Students who do not pass the assessments for the different sections of the subject will be able to retake them on the date scheduled at the end of the semester (retakes), answering a multiple-choice test associated with the section not previously passed (20% and 30%, respectively for the Individual multiple-choice test). Retake exams will not be scheduled for the other activities.

Students who do not obtain the minimum grade required to pass each of the sections of the integrated laboratory, will not be able to pass the subject. In this case, the final maximum grade of the subject will be 4.

Because this subject is split into modules, from the second registration, students will only have to be assessed in the specific sections that have not been passed.

Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Delivery of laboratory notebook	20%	0	0	2, 5, 6, 7, 4, 8, 20, 21, 9, 22, 11, 19, 18, 10, 15, 23, 13, 24, 25, 26, 27, 16, 17
Evaluation of Section 1 Industrial Microbiology: multiple-choice test	20%	0	0	7, 4, 8, 20, 9, 11, 19, 18, 10, 23, 14, 24, 25, 26, 16, 17

Evaluation of Section 1 Industrial Microbiology: oral presentation and class participation	20%	0	0	12, 13
Evaluation of Section 2 Environmental Microbiology: delivery of a report	3,3%	0	0	2, 6, 9, 18, 10, 15, 14, 25, 27
Evaluation of Section 2 Environmental Microbiology: multiple-choice test	30%	0	0	1, 2, 5, 3, 6, 21, 22, 12, 15, 13, 27
Evaluation of Section 2 Environmental Microbiology: oral presentation and class participation	6,7%	0	0	12, 13

Bibliography

Bibliography and web links are indicated in the corresponding sections of the laboratory manual.

Class schedules of the subject can be obtained in the information section of the Microbiology Degree website.

Industrial Microbiology

-R.S. Burlage, R. Atlas, D. Stahl, G. Geesey and G. Sayler, (1998). Techniques in Microbial Ecology. New York, NY. Oxford University Press.

-L-M. Prescott (2002). Microbiology. Capter 42: Industrial Microbiology and biotechnology, 5th Edition, New York, NY. The McGraw−Hill Companies.

-M. Rabbani, H.M. Sadeghi, F. Moazen, M. Rahimi and G. Salehi. (2011). Cloning and Expression of Randomly Mutated Bacillus subtilis α-Amylase Genes in HB101. Biotechnology Research International doi:10.4061/2011/305956.

-R.C. Cadwell andG.F. Joyce. (1994). Mutagenic PCR. Genome Res. 3: S136-S140.

-M.J. Waites, N.L. Morgan, J.S. Rockey and G. Higton (2001) Industrial Microbiology: an introduction. London, UK. Blackwell Science Ltd.

-M.R. Ladisch, N.S., Mosier, (2009) Modern Biotechnology. John Wiley & Sons, Inc. https://doi.org/10.1002/9780470473412

-Alpha amylase activity protocol: http://www.worthington-biochem.com/aa/assay.html

-Preparation of phosphate buffers: http://openwetware.org/wiki/Phosphate_buffer

Environmental Microbiology

1.-Waste treatment

-Martin Alexander (1999) Biodegradation and Bioremediation. 2nd Edition. Cornell University, Ithaca, New York, U.S.A. AcademicPress.

-Ajay Singh, Ramesh C. Kuhad, Owen P. Ward. (2009) Advances in applied bioremediation. Berlin, Heidelberg. Springer-Verlag. https://dx.doi.org/10.1007/978-3-540-89621-0

-Ralph, M. andJi-Dong, G. (eds) (2010) Environmental Microbiology. 2nd Edition. Wiley-Blackwell. https://onlinelibrary.wiley.com/doi/book/10.1002/9780470495117

-Yates, M. V. et al. (eds) (2016) Manual of Environmental Microbiology. 4th Edition. ASM Press. doi: 10.1128/9781555818821.

2.-Control of the environmental contamination

-Wen-TsoLiu, Janet K. Caister (2010) Microbiology. Norfolk, UK. Academic Press.

-Harley–Prescott. (2002) Laboratory exercises in microbiology (5th Edition). Boston, Mass. The McGraw-Hill companies.

-Delort, A.-M. and Amato, P. (eds) (2017) Microbiology of aerosols. Wiley Blackwell. https://onlinelibrary.wiley.com/doi/book/10.1002/9781119132318

-Mohee, R. and Mudhoo, A. (eds) (2012) Bioremediation and Sustainability: Research and Applications. Wiley-Blackwell. https://onlinelibrary.wiley.com/doi/book/10.1002/9781118371220

3.-Biological control

- H.J. Benson, (2001) Microbiological applications. Laboratory manual in general microbiology (8th edition). Boston, Mass. The McGraw-Hill companies.

- B. Prapagdee, C. Kuekulvong and S. Mongkolsuk (2008). Antifungal Potential of Extracellular Metabolites Produced by Streptomyces hygroscopicus against Phytopathogenic Fungi. International Journal of Biological Sciences 4:330-337.

-Ghannoum, M. et al. (eds) (2015) Microbial Biofilms. Second Edi. ASM Press. doi: 10.1128/9781555817466.

General Microbiology

-Martín A., Béjar V., Gutierrez J.C., Llagostera M. y Quesada E. 2019. Microbiología Esencial. 1ª edición. Editorial Médica Panamericana. https://www.medicapanamericana.com/VisorEbookV2/Ebook/9788491102427

Software

No specific software is foreseen