

MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
ODESA I. I. MECHNIKOV NATIONAL UNIVERSITY
Department of Microbiology, Virology and Biotechnology

WORKING PROGRAM OF THE EDUCATIONAL DISCIPLINE

**PHYSICAL AND CHEMICAL METHODS OF RESEARCH OF
BIOLOGICAL OBJECTS**

Higher education level::	second (Master)
Branch of knowledge:	G «Engineering, Manufacturing and Construction»
Specialty:	G21 Biotechnologies and Bioengineering
Educational professional program:	«Biotechnologies and Bioengineering»

1. Description of the discipline

Name of indicators	Branch of knowledge, Specialty, Specialization, Education Level	Characteristics of the discipline	
		<i>Full-time education</i>	<i>Correspondenc e education</i>
Total amount of credits – 4.5 hours – 135 content modules – 4	Branch of knowledge <i>G Engineering, Manufacturing and Construction</i> Specialty <i>G21 Biotechnologies and Bioengineering</i> Higher education level: <i>Second (<u>Master</u>)</i>	<i>Obligatory</i>	
		<i>Year:</i>	
		first	-
		<i>Semester</i>	
		first	-
		<i>Lectures</i>	
		22 hrs	-
		<i>Practical, seminar</i>	
		-	-
		<i>Laboratory</i>	
		22 hrs	-
		Final control form: exam	

2. Aim and Tasks of the Discipline

The aim of the discipline is to familiarize students with the basic physicochemical methods used in modern biotechnology and in conducting omics research.

Tasks:

1. To provide knowledge about the principles of the most modern optical research methods and the spectrum of their application in conducting a variety of biotechnological research.
2. To familiarize students with the basic principles of genomics, transcriptomics, proteomics and metabolomics, their role in systems biology and with basic methods used in conducting omics research.
3. To provide students with in-depth knowledge of the principles of chromatography and practical skills in its implementation.
4. To familiarize students with the basic theoretical principles of metabolomics, methods of chromatography-mass spectrometry and NMR spectroscopy, as well as with the practical application of metabolomics approaches in conducting biotechnological research.
5. Provide practical skills in analyzing metabolomic experiment data and identifying substances of biotechnologically valuable microorganisms
6. Provide in-depth knowledge of the principles of basic molecular biological research methods and practical skills in their use.
7. Provide students with knowledge of the most modern research methods in microbiology and ways to solve the problem of "uncultivable" microorganisms.
8. Introduce the principles of planning biotechnological research at the most modern level, selection of materials, reagents, devices and equipment for specific research tasks.

The process of studying the discipline is aimed at forming elements of the following **competencies**:

Integral: the ability to solve complex tasks and problems of biotechnology and bioengineering, which involves conducting research and/or implementing innovations and is characterized by uncertainty of conditions and requirements.

As a result of the discipline study a student has to

know:

- Basic principles of modern physical and chemical research methods used in biotechnology;
- range of applications of the most modern physical and chemical methods in biotechnological research;
- basic concepts of systems biology, as well as basic principles and methods of genomics, transcriptomics, proteomics and metabolomics;
- the most modern methods of cultivating microorganisms.

Be able:

- select research methods for a specific task when conducting biotechnological experiments;
- select materials, reagents, devices and equipment;
- use modern chromatographic and molecular biological research methods;
- analyze metabolomic experiment data.

3. Content of the Discipline

Module 1. Modern optical methods for studying biological objects

Topic 1. Modern methods for visualizing microobjects

Microscopy. Main types of light and electron microscopy. Similarities and differences between them. Comparison of light and electron microscopes. Methods of ultra-high resolution microscopy. Structured Illumination Microscopy – SIM. Stimulated Emission Depletion – STED. Single-Molecule Localization Microscopy – SMLM. Their principles and examples of application in biotechnology. Sample preparation methods for electron microscopy. Sample preparation stages for transmission

electron microscopy. Methods of obtaining thin sections. Ultramicrotomes. Dyes for electron microscopy. Freeze-Etching method. The principle of transmission electron microscopy. The main structural components of a transmission electron microscope. Examples of the use of transmission electron microscopy for the study of eukaryotic and prokaryotic cells. Analysis of micrographs and identification of the main internal components of the cell. The principle of operation of a scanning electron microscope. Examples of the use of scanning electron microscopy in biotechnological research. Cryo-electron microscopy - cryo-EM as one of the most modern methods of visualization of microscopic objects.

Topic 2. Modern methods of optical-spectral analysis

A variety of optical-spectral methods used in biotechnology. Spectrophotometry, turbidimetry, calorimetry, nephelometry. Features of similarities and differences between them. Application of these types of optical-spectral methods of analysis in biotechnological research. The Bouguer-Lambert-Beer law, which underlies optical-spectral methods of analysis. Determination of concentrations of substances according to the Bouguer-Lambert-Beer law. The concept of optical density. Features of determining the presence and concentrations of proteins and nucleic acids using a spectrophotometer. Absorption maxima of some antimicrobial compounds of lactic acid bacteria. Study of the composition of exometabolites of lactic acid bacteria of marine origin with antimicrobial (antibacterial, antifungal) activity using spectrophotometry. Fluorolog FL 3-22 Horiba Jobin Yvon, 450 spectrophotometer and examples of its use in biotechnological research. Rules and principles of analyzing the spectra of metabolites of microorganisms. Basic concepts and principles of flow cytometry. Principle and spectrum of application of Raman spectroscopy. Principles and general concepts of fluorometry and luminometry. Possibilities of their application in biotechnological research.

Module 2. Methods and basic principles of metabolomics

Topic 3. Modern methods of chromatography and mass spectrometry

Liquid and gas chromatography. Types of stationary and mobile phases for them. Ion-exchange chromatography and its use for purification of recombinant proteins. In-depth understanding of high-performance liquid chromatography (HPLC) and its application in biotechnological research. Rules for preparing samples and mobile phase for HPLC. Types of columns for HPLC. Analysis of HPLC chromatograms in the study of bacteriocins, organic acids and sugars. General principles of mass spectrometry. Chromato-mass spectrometry as a powerful modern complex of methods. Types of ionization sources and mass analyzers. Continuous and pulsed mass analyzers. Structure of mass spectrometers using the example of MALDI-TOF. Principle of MALDI-TOF. Use of MALDI-TOF in the study of protein molecules and in proteomics. The mechanism of mass spectrometry with electrospray ionization and its application in biotechnological research. Positive and negative ionization methods. Quadrupole mass filters (QMF). Their varieties and principles of operation. The principle of operation of a four-quadrupole mass analyzer. Mass spectrometer with an ion trap. Three-dimensional and two-dimensional ion trap. Canonical Mathew equation. Orbitrap with Fourier transformation. The principle of operation of the Orbitrap mass analyzer. Mass spectrum, rules and methods for its decoding. Analysis of mass spectra using the Data Analysis program. Search for main adducts of molecular ions. Databases for identifying metabolites by exact mass: Dictionary of Natural Products, mzCloud, Human Metabolome Database, Metabolica and others.

Topic 4. Metabolomics and its role in systems biology

Systems biology and the omic strategies that form its basis. General principles of genomics, transcriptomics and proteomics. Basic methods of omic approaches. Theoretical principles of metabolomics. Definition and history of metabolomics. Metabolomics as a phenotyping tool and its role in systems biology. Nuclear magnetic resonance spectroscopy. Its principles and general principles. Features of sample preparation for NMR spectroscopy. Advantages and disadvantages of this

metabolomics method compared to mass spectrometry. Analysis of metabolite spectra obtained using NMR spectroscopy and determination of their structure. Examples of the use of NMR spectroscopy in biotechnological research. Lack of a direct connection between the genome and metabolome of cells and living organisms. Genomic-metabolomic analysis. Its general principles.

Topic 5. Applied metabolomics

Conceptual approaches of metabolomics. Targeted analysis, metabolite profiling, metabolomics, metabolic fingerprinting. Targeted and untargeted approaches in metabolomic studies. Methodological approaches used in targeted and untargeted analyses. Areas of application of metabolomics: medical research, agriculture, ecology, fundamental biological research. Schemes of metabolomic experiments in medical research. Processing of metabolomic experiment data. Examples of using metabolites as targets for therapeutic purposes. Examples of sample preparation in the study of antimicrobial compounds of microorganisms by metabolomic analysis. Variety of sample preparation methods. Difference in methods for isolating extracellular and intracellular metabolite products. Types of extractants used for metabolite isolation. Comparison of metabolite profiles of healthy and diseased individuals. Metabolic phenotypes of normal individuals. Relationship between a specific urinary metabolite and disease. Relationship of metabolites to lifestyle.

Module 3. Modern reaction methods for studying biological objects

Topic 6. Modern biochemical research methods

Electrophoresis and its importance in biotechnological research. In-depth information on the principle of electrophoresis of nucleic acids and SDS-PAGE. Types of gels used for electrophoresis. Features of working with polyacrylamide gels used for electrophoresis of proteins and nucleic acids. Using protein electrophoresis to assess the presence, concentration and purity of recombinant proteins. Using protein electrophoresis to study antimicrobial peptides and proteins – bacteriocins. The spectrum of applications of protein electrophoresis in biotechnological research. The UniProt database and its use for searching for information on certain protein molecules. Electrophoresis as the basis of blotting. The principle of blot hybridization. Types of blot hybridization. Southern blotting, Northern blotting, Western blotting, Eastern blotting, Far Western blotting. Stages of their implementation. Range of application of blot hybridization in biotechnological research

Topic 7. Molecular biological research methods. Polymerase chain reaction

In-depth principles and features of polymerase chain reaction (PCR). Detailed rules and principles of calculations for preparing a reaction mixture for PCR with various biotechnological purposes. Comparison of classical PCR and real-time PCR. The value of classical PCR for genetic engineering. The use of PCR to identify biotechnologically valuable strains. Reverse transcription PCR and its use in transcriptomics. Principles and features of the main types of PCR. RAPD-PCR. Immunoquantitative PCR (iqPCR). Nested PCR. Asymmetric PCR. Molecular colony method (PCR colony). Multiplex PCR. Touchdown PCR. Application of different types of PCR in biotechnological research.

Topic 8. Modern methods of sequencing nucleic acids and proteins

In-depth principles of DNA sequencing. History of the invention of sequencing methods and their role in the Human Genome Project. The importance of sequencing methods for genomics and proteomics. Whole-genome sequencing and sequencing of 16S rRNA and 18S rRNA genes. Three generations of DNA sequencing methods. Principle of the Sanger sequencing method (Sanger dideoxy procedure). Sanger sequencing in combination with sequencing gel electrophoresis and capillary electrophoresis. Principle of the Maxam-Hilbert procedure (Maxam-Hilbert sequencing). Principles of pyrosequencing. Mechanism of sequencing by synthesis (Illumina). Stages of Illumina sequencing. Principles of sequencing by ligation (SOLID sequencing). In-depth principles and concepts of nanopore sequencing. Three generations of sequencing of protein molecules. Advanced principles of protein

sequencing using the Edman method. Stages of Edman sequencing. Advanced principles of determining amino acid sequences of protein molecules using tandem mass spectrometry and next generation sequencing (NGS). General principles of bioinformatic analysis of microbial genomes to determine their biotechnological potential. Analysis of bacterial genomes using the antiSMASH bacterial version program. GenBank database and its use in biotechnological research.

Module 4. Modern methods of cultivating microorganisms

Topic 9. The problem of "unculturable" microorganisms and microchip technology

Modern problems of microbiology and related sciences. History of microbiological research methods. The problem of "unculturable" microorganisms. The number of microorganisms on the planet that are not cultivated in laboratory conditions. Microbial "dark matter". Miniaturization of classical methods of growing microorganisms. Microchips and their varieties. Microbe Observation and Cultivation Array (MOCA). Microfluidic Streak Plate (MSP). Highly automated analysis of individual cells and study of enzymatic reactions using microchips. Use of microchip technology for isolating individual bacterial cells from complex consortia. Micromachine devices. Ichip. Micro-Petri-dishes. "Lobster traps". SlipChip. Principles of their use for the isolation and cultivation of microorganisms. Specific examples of applications of micromachine devices in scientific research. Discovery of the antibiotic teixobactin using Ichip technology. Microfluidic technologies. Single emulsion droplet microfluidics. Double emulsion droplet microfluidics. Their principles and spectrum of applications in biotechnological research.

Topic 10. Biofilm chips and other methods for studying biofilms

Modern definition of biofilm and stages of its formation. Positive and negative significance of biofilms, including for biotechnology. Method for studying biofilms in plates using crystal violet dye (Crystal violet test). Examples of studying the effect of bacteriocins and other metabolites of lactic acid bacteria on biofilms of bacteria that cause food spoilage and opportunistic bacteria. Use of biofilm chips (BiofilmChip) for studying bacterial biofilms. Principle of operation of biofilm chips. Stages of creating chips for studying biofilms. Examples of studies conducted using biofilm chips.

4. Topics of seminar classes

Seminars are not provided.

5. Topics of practical classes

Practical classes are not provided.

6. Topics of laboratory classes

№	Topic name	Amount of hours	
		Full-time education	Correspondence education
1	Topic 1. Purification of green fluorescent protein (GFP) using ion-exchange chromatography with the BioLogic LP system (Bio-Rad, USA) Prepare the buffers necessary for ion-exchange chromatography. Filter and degas them. Perform sample preparation of green fluorescent protein. Perform anion-exchange chromatography using the step-by-step elution method using a Bio-Scale Mini Macro-Prep High Q Cartridge column and the BioLogic LP system (Bio-Rad, USA). Select the obtained fractions and determine the concentration of GFP in them using spectrophotometry.	6	

2	Topic 2. Analysis of data from a metabolomic experiment to identify the composition of extracellular metabolites of marine microorganisms Using the Data Analysis program, analyze the chromatogram and mass spectra obtained using high-performance liquid chromatography in combination with electrospray ionization mass spectrometry and a quadrupole mass analyzer. Identify one of the four most common adducts (M+Na, M+NH ₄ , M+K, M+ACN+H) for each substance represented by a peak in the mass spectrum. Having determined the exact molecular mass, identify the compounds using the Dictionary of Natural Products on DVD database.	6	
3	Topic 3. Analysis of GFP purity by protein electrophoresis Prepare polyacrylamide gels for protein electrophoresis. Perform sample preparation of GFP fractions obtained as a result of ion exchange chromatography. Load samples on gels and perform protein electrophoresis. Fix and stain the resulting gels, decolorize their background. Obtain the corresponding electrophorograms.	6	
4	Topic 4. Performing classical PCR to identify the main genera of lactic acid bacteria Isolate DNA from lactobacteria cultures by thermal lysis. Check the presence, concentration and purity of DNA using spectrophotometry. Create recipes for reaction mixtures containing primers for the main genera of the LAB: <i>Lactobacillus</i> , <i>Enterococcus</i> , <i>Pediococcus</i> , <i>Lactococcus</i> , <i>Leuconostoc</i> . Prepare reaction mixtures and carry out amplification with the required parameters. Analyze the results of classical PCR by performing horizontal electrophoresis in agarose gel and 1X TAE buffer.	4	
	Totally	22	

7. Methods of Education

The course will be taught in the form of lectures (22 hours) and laboratory classes (22 hours), independent work of students (91 hours).

The main training of students is carried out in lectures and laboratory classes, and also relies largely on independent study of the discipline. When teaching the discipline, the following teaching methods are used: lectures, explanations, conversations, instructions, discussions, discussion of problem situations, illustrations (including multimedia presentations), demonstrations; test exercises; individual performance of laboratory work.

8. Forms of Control and Assessment Methods

1. Current control:

- performance and defense of laboratory work;
- oral asking questions;
- independent work of the applicant with a note, recommended literature in preparation for lectures and laboratory classes;

2. Periodic control:

Control work on the content module is carried out in the form of testing after completing the study of the educational material of each content module. Control works include test questions, each of which has four answer options and only one of them is correct. For each correct answer to one question, a student receives 0.5 points. There are 20 questions in the control works.

3. Final control - exam. The final grade is the sum of points for current, periodic and final control, which is an exam. The exam is conducted in written form. Control and evaluation of educational activities in the discipline is carried out on a 100-point scale.

**GENERAL CRITERIA FOR ASSESSING THE ACADEMIC ACHIEVEMENTS OF
HIGHER EDUCATION STUDENTS**

National scale assessment	Theoretical study	Practical study
	A student	
Excellent	fully owns the educational material, freely, independently and with good reasoning presents it during oral presentations and written answers; deeply and comprehensively reveals the content of theoretical issues, using normative, mandatory and additional literature; draws independent conclusions, identifies cause-and-effect relationships; independently finds additional information and uses it to implement the tasks set before him. The applicant is able to highlight the essential features of the studied using operations of synthesis, analysis, identify cause-and-effect relationships, form conclusions and generalizations, freely operate with facts and information.	deeply and comprehensively reveals the essence of practical tasks, using normative, mandatory and additional literature; can reasonably choose a rational way to complete the task and evaluate the results of their own practical activity; performs creative tasks and initiates new ways of completing them; freely uses acquired theoretical knowledge when analyzing practical material; shows a creative approach to completing tasks during independent work.
Good	has a sufficiently complete command of the educational material, presents it reasonably during oral presentations and written answers, using normative and mandatory literature; when presenting some questions, there is a lack of sufficient depth and argumentation, applies knowledge to solve standard situations; independently analyzes, generalizes and systematizes educational information, but individual minor inaccuracies and minor errors are allowed.	correctly completed most of the practical tasks according to the example; has stable task performance skills.
Satisfactory	owns educational material at a reproductive level or reproduces a certain part of educational material with elements of logical connections, knows the basic concepts of educational material; has difficulties in highlighting the essential features of the studied material; in identifying cause-and-effect relationships and formulating conclusions.	can use knowledge in standard situations, has elementary, unstable skills in completing the task. Correctly completed half of the practical tasks. The applicant has difficulties in highlighting the essential features of the studied matter; in identifying cause-and-effect relationships and formulating conclusions.

Unsatisfactory with reassembly capability	has a superficial and fragmentary grasp of the educational material (without argumentation and justification); unsystematically singles out random features of the studied material; is unable to perform the simplest operations of analysis and synthesis; make generalizations, conclusions; makes significant errors when answering.	insufficiently reveals the essence of practical tasks, while allowing significant inaccuracies, correctly completed individual practical tasks with the help of a teacher, lacks developed skills and abilities.
Unsatisfactory with mandatory re-study of the discipline	does not know educational material.	performs only elements of the task, requires constant assistance from the teacher.

Rating Scale: National and ECTS

Total points for all types of learning activities	ECTS Scale	National Scale
90-100	A	Excellent
85-89	B	Good
75-84	C	
70-74	D	
60-69	E	Satisfactory
35-59	FX	Unsatisfactory with reassembly capability
0-34	F	Unsatisfactory with mandatory re-study of the discipline

9. Learning and Methodological Support

The working program of the discipline, syllabus, multimedia presentations, methodological instructions for conducting laboratory classes, which are posted on the website of the Faculty of Biology <http://biologywiki.onu.edu.ua>

10. The Recommended Literature

Main

1. Blanco-Cabra N., López-Martínez M. J., Arévalo-Jaimes B. V., Martín-Gómez M. T., Samitier J., and Torrents E. A new BiofilmChip device for testing biofilm formation and antibiotic susceptibility. *Biofilms and Microbiomes*. 2021. V. 7:62. <https://doi.org/10.1038/s41522-021-00236-1>
2. Davidson S.-L. and Niepa T. H. R. Micro-technologies for assessing microbial dynamics in controlled environments. *Frontiers in Microbiology*. 2022. V. 12:745835. doi: 10.3389/fmicb.2021.745835
3. Klassen A., Faccio A.T., Canuto G. A. B., da Cruz P. L. R., Ribeiro H.C., Tavares M. F. M., Sussulini A. Metabolomics: definitions and significance in systems biology. In: *Metabolomics: from fundamentals to clinical applications. Advances in experimental medicine and biology* (Eds. Sussulini A.). 2017. V. 965, Berlin: Springer, Cham. https://doi.org/10.1007/978-3-319-47656-8_1.
4. Kuang Q., Purhonen P., and Hebert H. Two-dimensional crystallization procedure, from protein expression to sample preparation. *BioMed Research International*. 2015:693869. <http://dx.doi.org/10.1155/2015/693869>

5. Merlich A., Galkin M., Choiset Y., Limanska N., Vasylieva N., Ivanytsia V., Haertlé T. Characterization of the bacteriocin produced by *Enterococcus italicus* ONU547 isolated from Thai fermented cabbage. *Folia Microbiol.* 2019. V. 64 (4). P. 535-545. <https://doi.org/10.1007/s12223-019-00677-4>
6. Savaryn J. P., Toby T. K., Kelleher N. L. A researcher's guide to mass spectrometry-based proteomics. *Proteomics*. 2016. V. 16(18). P. 2435-43. doi: 10.1002/pmic.201600113.
7. Senger R. S., Kavuru V., Sullivan M., Gouldin A., Lundgren S., Merrifield K. et al. Spectral characteristics of urine specimens from healthy human volunteers analyzed using Raman chemometric urinalysis (Rametrix). *PLoS ONE*. 2019. V. 14(9): e0222115. <https://doi.org/10.1371/journal.pone.0222115>
8. Spaggiary L., Pedretti N., Ricchi F. et al. An untargeted metabolomic analysis of *Lacticaseibacillus (L.) rhamnosus*, *Lactobacillus (L.) acidophilus*, *Lactiplantibacillus (L.) plantarum* and *Limosilactobacillus (L.) reuteri* reveals an upregulated production of inosine from *L. rhamnosus*. *Microorganisms*. 2024. V. 12: 662. URL: <https://doi.org/10.3390/microorganisms12040662>

Additional

1. Limanska N. V., Totyskyi V. M., Sergieieva Zh. Yu., Ivanytsia T. V., Vasylieva N. Yu., Krylova K. D., Ivanytsia V. O., Tovkach F. I. Molecular biological methods for studying microorganisms: a textbook. Odesa: Odessa National University, 2014. P. 179 (In Ukrainian).
2. Sivolob A. V. Molecular biology: a textbook. Kyiv: Publishing and printing center "Kyiv University", 2008. P. 384 (In Ukrainian).
3. Totyskyi V. N. Genetics. A textbook with the seal of the Ministry of Education and Science. Odesa: Astroprint, 2008. P. 712 (In Ukrainian).
4. Kurnaz I.A. Techniques in genetic engineering. Boca Raton: CRC Press, 2015. 305 p. URL: <https://fcen.uncuyo.edu.ar/catedras/techniques-in-genetic-engineering.pdf>
5. Lorenz T.C. Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. *Journal of Visualized Experiments*. 2012. V. 63, P. 1-15. URL: <https://pubmed.ncbi.nlm.nih.gov/22664923/>

15. Electronic Information Resources

<https://www.theclarklab.org/microscopy-for-the-modern-era/>
<https://www.magnusopto.com/blog/post/microscopy-techniques-a-comprehensive-guide-for-scientists-and-students.html?srsId=AfmBOoqB956DDEJfytpz5plyEurxSJAxH3spyry2JulcnKiUUIIm6SN8>
<https://www.azosensors.com/article.aspx?ArticleID=3231>
<https://youtu.be/Sn3dNMv-67k?si=Q7vO4sgdBeDs-G9N>
<https://www.ebi.ac.uk/training/online/courses/metabolomics-introduction/what-is>
<https://dnatech.ucdavis.edu/illumina-high-throughput-sequencing>
<https://youtu.be/eCj0cRtJvJg?si=F3Pm-eW3aa5KSRBn>