REPUBLIC OF AZERBAIJAN

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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

PHYSIOLOGICAL REGULATION OF LIPOLITIC ENZYME SYNTHESIS IN MICROMYCETES ISOLATED FROM OIL-POLLUTED SOILS

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INTRODUCTION

The degree of actuality and study of the topic. In modern times, environmental protection and ensuring ecological balance are more relevant than ever. Already, the world's think tanks are sounding the alarm that the continuation of human power over nature in this way will not last long. Thus, the inability of the earth's biological resources to withstand this increase against the backdrop of an increasing human population leads to greater exposure to anthropogenic impact on natural ecosystems and, as a result, to pollution of the environment, primarily soil and water ecosystems, with various pollutants. Undoubtedly, the most serious pollutants of both ecosystems are petrol and petroleum products and different oils. First, nature (soil), to put it mildly, is a material spatial component of the existence of human civilisation, and society without nature cannot exist in any case. For this reason alone, human society is obliged to protect nature, and it is no coincidence that the search for technologies for improving the state of the environment is one of the main issues that both the modern world and the Republic of Azerbaijan prioritize. The bioremediation of oil-contaminated soils and biological treatment of wastewater using micromycetes isolated from those areas that we are working on is of this type, and is a solution technology dedicated to environmental protection, combining ecology, soil science and microbiology, with broad multidisciplinary significance, environmentally friendly and economically efficient. For this purpose, lipolytic enzymes obtained from micromycetes and strains with high lipolytic activity were used.

Thus, "*lipolytic enzymes (triacylglycerol hydrolases EC 3.1.1.3) are biocatalysts of serious industrial importance - enzymes, which catalyze the reactions of transesterification, interesterification, aminolysis, alcoholysis, acidolysis and synthesis of long-chain fatty acids (ester synthesis), and have high scientific-methodological and practical application properties in various fields of medicine, pharmacology, biotechnology and industry; in the food industry, leather processing, detergent-synthetic agents, biodiesel production, as well as in the biological treatment of wastewater and bioremediation of soils*

contaminated with oil (oils)"[1]. "*Several sources - plants, animals and microorganisms - come to our attention as producers of enzymes*"[2] that, according to modern statistical data, "*it is emphasized that plants and animals have a 15% share in the global market for the production of various enzymes (including lipolytic enzymes), and microorganisms have an 85% share*"[3]. This once again proves the idea that microorganisms, especially fungi, are a more valuable object of research. Thus, the market value of lipolytic enzymes obtained from microorganisms *"was 425 million US dollars in 2018, and this figure is predicted to exceed 590 million dollars in the next decade. The role of micromycetes in this development is undoubtedly irreplaceable*"[4]. In short, "*lipolytic enzymes obtained from micromycetes differ from other producers in that the enzyme synthesis is extracellular; lower substrate selectivity due to the breakdown of various types of fats; ease of cultivation of micromycetes, like bacteria; "The high activity and stability (being more tolerant* to *pH and temperature fluctuations) and the lower number of pathogenic species among micromycetes compared to bacteria make them better alternatives*"[5, 6].

Some studies have been conducted around the world to study fungal lipases. Thus, although there are some research works on "*application of lipolytic enzymes"*[7] obtained by fungi in various

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[1] Mehta, A., Bodh, U., & Gupta, R. Fungal lipases: a review// Journal of Biotech Research, -2017. 8, -p.58-77.

[2] Nimkande, V.D., & Bafana. A. A review on the utility of microbial lipases in wastewater treatment// Journal of Water Process Engineering, -2022. 46, -p. 102591.

[3] https://www.grandviewresearch.com/industry-analysis/enzymesindustry .

[4]Chandra, P., Enespa, Singh, R., & Arora, P.K. Microbial lipases and their industrial applications: a comprehensive review// Microbial cell factories, -2020. 19, -p. 1-42. https://doi.org/10.1186/s12934-020-01428-8

[5] Dab, A., Hasnaoui, I., Mechri, S. et al. Biochemical characterization of an alkaline and detergent-stable lipase from Fusarium annulatum Bugnicourt strain CBS associated with olive tree dieback// Plos one, -2023. 18(5), e0286091. https://doi.org/10.1371/journal.pone.0286091

[6] Kumar, A., Verma, V., Dubey, V.K., et al. Industrial applications of fungal lipases: a review// Frontiers in Microbiology, -2023. 14, 1142536.

[7]Cesário, L.M., Pires, G.P., Pereira, R.F.S., et al. Optimization of lipase production using fungal isolates from oily residues// BMC biotechnology, -2021. 21, -p. 1-13.

directions, "*obtaining lipolytic enzyme biopreparations from micromycetes*"[8], and "*optimization of enzyme synthesis*"[9], unfortunately, this opinion cannot be said for the Republic of Azerbaijan. Such an extensive study of lipolytic enzymes of this type, that is, obtained purely from micromycetes, has not been conducted so far. To date, few, fragmentary studies have been conducted in the Republic on lipolytic enzymes obtained purely from micromycetes, but these research works did not provide information on the application of such enzymes. That is, large-scale studies have never been conducted on this topic and the specific application direction of the enzyme.

Despite such a wide range of applications of lipase, even in the world, the study of this enzyme is not at the proper level. There have always been serious scientific debates on this topic, namely, on the mechanism of physiological-biochemical and microbiological regulation of the synthesis of lipolytic enzymes in micromycetes, in the search for and application of better strains in the bioremediation of oil-contaminated soils and biological treatment of wastewater. True, some studies have been conducted in some developed countries in the world on the field of application of the enzyme, and strains with high activity are used in the treatment of polluted waters and bioremediation of oil-contaminated soils. However, since these strains and enzyme preparations are adaptive to the physical-geographical and ecological conditions of the areas in question, they do not show the same effectiveness either in our region or in other regions, and this type of work is new for our republic.

Object and subject of the research. The object and subject of the research are micromycetes and their lipolytic enzymes isolated from oil-contaminated soils of the Republic of Azerbaijan.

Purpose and objectives of the research. The purpose of the presented dissertation work was to evaluate the lipolytic activity of

[8] Fatima, S., Faryad, A., Ataa, A. et al. Microbial lipase production: A deep insight into the recent advances of lipase production and purification techniques// Biotechnology and applied biochemistry, -2021. 68(3), -p. 445-458.

[9] Fahim, Y.A., El-Khawaga, A.M., Sallam, R.M., et al. A review on Lipases: sources, assays, immobilization techniques on nanomaterials and applications// BioNanoScience, -2024, -p. 1-18.

micromycetes distributed in oil-contaminated soils of the Republic of Azerbaijan and to determine their application areas.

Following the set goal, the following tasks were considered appropriate:

- Assessment of the species composition of the mycobiota of oil-contaminated soils of the Republic of Azerbaijan, creation of a collection of pure cultures of micromycetes;
- Comparative study of the phytotoxic activity of micromycetes isolated from the studied areas and oil-contaminated soils;
- Screening of micromycetes isolated from the study areas based on lipolytic activity;
- Finding optimal conditions for intensive enzyme synthesis in strains selected as active producers as a result of screening, and studying the physiological basis of enzyme synthesis in them;
- Purchasing enzyme preparations from active producers and specifying their application areas.

Research methods. Taking soil samples from the study areas, sowing those samples, isolating micromycetes, and growing them into pure culture were carried out according to the classical methods currently accepted in microbiology, mycology, and soil science used in the world and our republic. The initial screening of micromycetes based on lipolytic activity was carried out using Tween-20 in a solid-phase fermentation medium, and the main screening was carried out according to the titrimetric method. Also, the accuracy of the devices and equipment we used during the studies, as well as the purity of the reagents, was at the required level. Performing all experiments in four repetitions during the studies both created confidence in the accuracy of the experiments and allowed for statistical processing of the results.

The main provisions of the dissertation defense:

- The formation of phytotoxic activity of the soil is formed due to both the source of pollution and the fungi that inhabit it;
- The high phytotoxic activity of micromycetes allows them to be used for water purification for technical purposes only;
- Quantitative and qualitative changes in the composition of the nutrient medium affect only the quantity, not the quality, of enzyme synthesis in micromycetes;

• The effect of carbon source on the synthesis of lipolytic enzymes in micromycetes is the basis for suggesting that its synthesis occurs inductively and is controlled by catabolite repression.

Scientific novelty of the research. In the conducted studies, the fungal biota of oil-polluted soils of the Republic of Azerbaijan was studied according to its species composition. As a result, 129 micromycete strains were isolated from 96 samples taken from oil-polluted areas around 24 oil wells located in the Balakhani, Binagadi, Sabail and Surakhani regions, which were selected as the study area, and were isolated into pure culture. During the identification of micromycete, it was determined that they were collected in 38 species belonging to 3 divisions (*Ascomycota, Mortierellomycota, Mucoromycota*), 13 families, and 16 genera, some of which are found for the first time like Azerbaijan (*Orbilia oligospora* and *Pirella circinan*), and some in the oil-polluted research areas (21 species).

During the study of the phytotoxic activity of oil-contaminated soils and fungi isolated from them, it became clear that both oil and oil products, as well as fungi inhabiting them, participate in the formation of the phytotoxic activity of soils, and fungi also differ from each other in terms of phytotoxic activity. Thus, weak phytotoxic activity is observed in fungi such as *Aureobasidium pullulans* (3%)*, Torula herbarum* (2%) and *Pirella circinan* (3%). In comparison, high phytotoxic activity is observed in fungi such as *Fusarium oxysporum* (56%)*, F. verticillioides* (48%)*,* and *Penicillium chrysogenum* (44%). Most of the fungi have moderate phytotoxic activity (7-29%), and fungi belonging to the genus *Trichoderma* have stimulated activity.

It has been established that not all fungal strains isolated from oil-contaminated soils have lipolytic activity, but *Aspergillus niger* AA-17, *Pencillium glabrum* AA-76 and *Rhizopus stolonifer* AA-82 differ from others in that they actively synthesize lipolytic enzymes.

Aspergillus niger AA-17 fungus, the composition of the existing Czapek medium was modified both quantitatively and qualitatively, creating a new medium, which determined that the fungus inductively synthesizes lipase under those conditions and allowed the activity to be increased to 51% compared to the original medium.

Aspergillus niger AA-17, *Pencillium glabrum* AA-76 and

Rhizopus stolonifer AA-82 were selected as active producers of lipolytic enzymes. The fungi tested in a laboratory model for the treatment of oil-contaminated soils, and it was determined that the use of the fungus *Aspergillus niger* AA-17 gave better results. At the same time, the effectiveness of the use of this strain in the treatment of technical wastewater was also experimentally confirmed.

Theoretical and practical significance of the research. The results obtained in determining the species diversity of micromycetes distributed in oil-contaminated soils are practical material that can be used in the future in the preparation of an atlas of micromycetes distributed throughout the country or a local mycobank and textbooks.

The strains isolated during the research have the potential to be used in the bioremediation of oil-contaminated soils and the biological treatment of technical wastewater. For this purpose, the preparation of scientific-methodological-practical regulations of the process in laboratory conditions also makes it possible to apply the results of the current research work, more precisely, the prepared biopreparations, in field work of this type. At the same time, our natural strains can also be used in the future to obtain recombinant strains for increasing the synthesis of lipolytic enzymes on a genetic basis.

Publication, approbation and implementation of the dissertation. A scientific work related to the topic of the dissertation has been published, including 5 scientific articles, theses or conference materials**.** The materials of the dissertation were presented at the IV Republican Conference on "Ecology: Problems of Nature and Society" (Baku, 2023), the International Conference on "Science and Education" (Russian Federation, Penza, 2023 and 2023), the XXVI Republican Scientific Conference of Doctoral Students and Young Researchers (Baku, 2024), the I International Scientific-Practical Conference on "Innovative Biotechnology for Environmental Protection: From Theory to Practice" (Belarus, Minsk, 2024), the Scientific-Practical Conference on "The Role of the National Leader Heydar Aliyev in Improving the Environment in Azerbaijan" (Baku, 2024), the International Conference on "Actual Problems of Microbiology, Biotechnology and Biodiversity" (Kazakhstan, Astana, 2024).

Organization where the dissertation work was performed. The dissertation work was performed at the Microbiological Biotechnology Laboratory of the Institute of Microbiology of MSERA.

Volume and structure of the dissertation: The dissertation consists of an introduction, a literature review (Chapter I), materials and methods (Chapter II), an experimental part (Chapters III and IV), a final analysis of the research, main results, practical recommendations, a list of used literature and abbreviations. All this consists of a total of 235060 characters.

CHAPTER I
GENERAL CHARACTERISTICS OF FUNGI ISOLATED IN OIL-POLLUTED SOILS AND THE PHYSIOLOGICAL BASIS OF THE SYNTHESIS OF LIPOLITIC ENZYMES IN THEM

Sections 1.1 and 1.2 of the dissertation deal with oil-polluted soils of the Republic of Azerbaijan, their general characteristics and physical, chemical and biological remediation of oil-polluted soils, section 1.3 deals with the study of the fungal biota of oil-polluted areas in Azerbaijan and the enzymatic activity of micromycetes isolated from those areas, and section 1.4 deals with the importance of lipolytic enzymes, the physiological regulation of enzyme synthesis in micromycetes, and the analysis of literature data on lipolytic enzymes obtained from various sources and their application areas.

CHAPTER II
MATERIAL AND METHODOLOGY OF RESEARCH

2.1. General characteristics of the research areas

The studies were conducted between 2022 and 2024 in the oil-contaminated soils of the Binagadi, Sabail, Balakhani, and Surakhani districts of Baku, as well as the Absheron Peninsula. A total of 96 soil samples were collected from around 24 oil wells (N – 352690, 360725, 362685, etc.) located in these areas. Four samples were taken from each oil well: one sample was collected from the area where oil was

discharged, while the remaining three samples were taken from a depth of 0–20 cm at distances of 1-1.5 m, 25 m, and 100 m from the well, respectively.

2.2. Methods and approaches used for analysis

Taking samples from the study area, isolating fungi and growing them into pure culture using the "*known methods accepted in microbiology and mycology were mainly carried out and pure cultures were obtained*"[10,11]. The purity of the cultures was monitored with a microscope (OMAX 40X-2500X LED Digital Lab Trinocular Compound Microscope). Malt extract agar (MEA), Saburo agar, Czapek agar and potato agar were used as nutrient media.

Identification of the isolated strains was carried out using "*known determinants based on cultural-morphological and physiological characteristics*"[12, 13, 14, 15, 16].

During the screening of fungi for lipolytic activity, cultivation was carried out in liquid Czapek medium at the temperature of 28 °C under deep cultivation conditions (200 cycles/min) for 5 days.

The determination of lipolytic activity is carried out according to the appropriate method, and then the lipase activity (LS) is calculated according to the "*formula*"[17] given below :

$$LS = AT \times (50/B)$$

[10]Netrusov, A.I., Egorova, M.A. Zakharchuk, L.M. и др. Praktikum po microbiologii/ M.: Изд-во «Akademiya», -2005. -p. 608.

[11]Bilai V.I. Methods of experimental mycology/ Kiev: Наукова думка, -1982. -p. 500 c.

[12] Bilai, V.I. Koval E.Z. Aspergilly. - Kyiv: Наукова думка, -1988, -p. 204.

[13] Bilai, V.I. Фузарии/ В.И. Билай. - Kyiv: Наукова думка, -1977, -p. 443.

[14] Ellis, MB, Ellis, JP Microfungi on Land plants. An identification Handbook. - London: Helm, -1987, -p. 819.

[15] Kirk, P.M., Cannon, P.F., Minter D.W., et al. Dictionary of the fungi. 10th edition. -UK, -2008, -p. 747.

[16] Sutton, D. Determiner of pathogenic and conditionally pathogenic fungi / D. Sutton, A. Fothergill, M. Rinaldi. - M.: Mir, -2001. -p. 468.

[17] Laboratory practice on the technology of enzyme preparations/ M. Light and food. prom., -1980. -p. 75-76.

where A is the difference in titre between the experimental and control solutions, T is the titre of the alkali, and B is the amount of enzyme (g/ml ^{3}).

The enzyme activity unit is the amount of enzyme that hydrolyses 1 μmol of oleic acid from a 40% olive oil emulsion for 1 hour at pH 7.0 and 37°C. The enzyme activity is expressed in μmol.min $^{-1}$ ml $^{-1}$ (μ/ml).

The phytotoxic activity of micromycetes and the bio-stimulating activity of micromycetes belonging to the genus *Trichoderma* are also *"based on known methods and approaches*"[18, 19] was mainly carried out.

Based on the biodegradation of oil by micromycetes, the initial selection was based on the *"Hanson method*" [20, 21] was mainly carried out.

The oil tolerance of fungi was determined by adding agar and 1%, 5% and 10% crude oil to Bushnell-Hans nutrient medium. One Petri dish was not added with oil and the growth of the fungus there was taken as a control and was incubated at 28 ^{0}C for 7 days, placed in a thermostat. *"After development, the growth of the fungi was evaluated in % relative to the control (taken as 100%)*"[22].

The method we used in our previous studies in wastewater treatment, "*It has been slightly modified*"[23].

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[18] Varejão, E.V., Demuner, A.J., Barbosa, L.C., & Barreto, R.W. The search for new natural herbicides–Strategic approaches for discovering fungal phytotoxins// Crop Protection, -2013. 48, -p. 41-50.

[19] Bakhshalieva K.F., Huseynova G.N., Gudratova F.R., Mohommadi S.D.M., Efendi U.A. Species diversity of mushrooms common in Azerbaijan and their phytotoxic activity// Series: Natural and technical sciences, -2023, 4 (2), -p. 7-13.

[20] Hanson, K.G., Desai, J.D., & Desai, A.J. A rapid and simple screening technique for potential crude oil degrading microorganisms// Biotechnology techniques, -1993. 7, -p. 745-748.

[21] Benguenab, A., & Chibani, A. (2021). Biodegradation of petroleum hydrocarbons by filamentous fungi (Aspergillus ustus and Purpureocillium lilacinum) isolated from used engine oil contaminated soil// Acta Ecologica Sinica, -2021. 41 (5), -p. 416-423.

[22] Gaur, V.K., Tripathi, V., & Manickam, N. Bacterial- and fungal-mediated biodegradation of petroleum hydrocarbons in soil// In Development in Wastewater Treatment Research and Processes, -2022 -p.407-427. Elsevier.

[23] Ahmadli, A. Biodegradation of petroleum hydrocarbons by fungi strains of Aspergillus sp.-17, Rhizopus sp.-81, Penicillium sp.-94 isolated from oil-

To find the optimal medium, Czapek's nutrient medium (pH 7.0 at 25°C) was used as a control and baseline. A 5-day culture of *A. niger* AA-17 strain was used for inoculation.

Carbon source. During the studies, Czapek medium was used as the base medium, with glucose, fructose, maltose, lactose (30 g/l) and 28 g/l of each, supplemented with olive oil (2 g/l).

Nitrogen source. Nitrogen sources such as sodium nitrate, ammonium nitrate, peptone, and yeast extract were used during cultivation. Their amounts in the medium were calculated based on nitrogen.

Surfactants. Tween 20 was added to the nutrient medium as a surfactant at a concentration of 1; 2; 3; 4; 5 g/l.

The effect of environmental pH on enzyme synthesis was evaluated in the range of 5-9 and temperature in the range of 20-40 ^{0}C.

All experiments in the studies were performed in at least 4 replicates and the results were "*statistically*"[24] processed.

CHAPTER III
EVALUATION OF OIL-POLLUTED LANDS OF AZERBAIJAN BASED ON FUNGAL BIOTA (A CASE STUDY OF THE ABSHERON PENINSULA)

3.1. Assessment of the species composition of the fungal biota of oil-contaminated areas

As a result of the analysis of 96 samples taken from around 24 oil wells located in the study areas, 129 micromycete strains belonging to true fungi (Fungi) were isolated into pure cultures. When determining the species composition of these cultures, it was determined that they belonged to 16 genera and 38 species (Table 1). As can be seen, 66.66%

contaminated soils of Azerbaijan// In BIO Web of Conferences, -2024. v. 100, -p. 02007. EDP Sciences. https://doi.org/10.1051/bioconf/202410002007

[24] Methods of statistical processing of medical data: Methodological recommendations for residents and postgraduates of medical institutions, scientific workers / A.G. Kochetov, O.V. Лянг., В.П. Masenko, and others. – M.: РКНПК, -2012. -p. 42.

of the strains isolated into pure cultures belonged to three genera: *Aspergillus, Penicillium* and *Mucor*, which are the dominant species in the studied areas, form the core of the micromycete biota in the area and maintain their vital activity despite the high concentration of oil.

Table 1

Distribution of strains isolated from the studied areas by genera

Genus	Starin number	Share in total	Total
Penicillium	43	33,33%	66,66%
Aspergillus	29	22,48%	
Mucor	14	10,85%	
Rhizopus	8	6,2%	33,34%
Talaromyces	5	3,87%	
Trichoderma	5	3,87%	
Fusarium	4	3,1%	
Curvularia	3	2,32%	
Others	18	10,85%	

Regarding the distribution of fungi by individual genera, it became clear that 12 species belonging to the genus *Penicillium* (*P.aurantiogriseum, P.canescens, P.chrysogenum, P.citrinum, P.cyclopium, P.decumbens, P.glabrum, P.janthinellum, P.jensenii, P.lanosum, P.oxalicum* and *P.restrictum*), 6 belonging to the genus *Aspergillus* (*A.flavus, A.niger, A.ochraceus, A.versicolor, A.ustus* and *A.terreus*), 3 belonging to the genus *Mucor* (*M.circinelloides, M.hiemalis* and *M.indicus*), *Talaromyces* 2 species belonging to the genus (*T.funiculosus* and *T.purpureogenus*), 2 species belonging to the genus *Fusarium* (*F.oxysporum* and *F.verticillioides*), 2 species belonging to the genus *Trichoderma* (*T.koningii* and *T.longibrachiatum*) and 2 species belonging to the genus *Rhisopus* (*Rh.arrhizus* and *Rh.stolonifer*) have been identified. Each of the remaining 9 genera is represented by 1 species.

3.2. Determination of phytotoxic activity

During the determination of the phytotoxic activity of the fungi

recorded in the studies, it became clear that species such as *Fusarium oxysporum, F. verticillioides, P. chrysogenum, P. citrinum* and *P. cyclopium* are characterised by relatively high rates (40-56%). (Table 2) . In fungi such as *Aureobasidium pullulans, Pirella circinan* and *Torula herbarum,* phytotoxic activity is weak (2-3%). The phytotoxic activity of the rest is 7-29% and is characterized by moderate phytotoxic activity. In 2 species of the genus *Trichoderma*, not only phytotoxic activity is observed, but also an increase in the germination capacity of seeds is observed to a certain extent (Fig. 1).

Table 2

Phytotoxic activity of the fungi obtained in research

N	Scientific name of the species	Distance*	PhA	OCS
1	2	3	4	5
1	*Alternaria atra*	100	18	-
2	*Aspergillus flavus*	P*	25	+
3	*Aspergillus niger*	P	23	+
4	*Aspergillus ochraceus*	100	17	+
5	*Aspergillus terreus*	P	18	+
6	*Aspergillus ustus*	1-1,5	22	+
7	*Aspergillus versicolor*	1-1,5	20	+
8	*Aureobasidium pullulans*	25	3	+
9	*Cladosporium herbarum*	25	11	-
10	*Curvularia lunata*	100	23	+
11	*Fusarium oxysporum*	P	56	-
12	*Fusarium verticillioides*	P	48	-
13	*Mortierella alpina*	25	15	+
14	*Mucor circinelloides*	1-1,5	23	+
15	*Mucor hiemalis*	P	22	+
16	*Mucor indicus*	1-1,5	17	-
17	*Orbilia oligospora*	25	12	-
18	*Paecilomyces variotii*	1-1,5	7	-
19	*Penicillium aurantiogriseum*	1-1,5	27	-
20	*Penicillium canescens*	1-1,5	25	-

Continuation of table 2

1	2	3	4	5
21	*Penicillium chrysogenum*	P	44	+
22	*Penicillium citrinum*	P	40	+
23	*Penicillium cyclopium*	1-1,5	41	+
24	*Penicillium decumbens*	25	26	+
25	*Penicillium glabrum*	100	19	-
26	*Penicillium janthinellum*	100	21	-
27	*Penicillium jensenii*	25	23	-
28	*Penicillium lanosum*	1-1,5	29	-
29	*Penicillium oxalicum*	100	24	+
30	*Penicillium restrictum*	25	19	-
31	*Pirella circinan*	100	3	-
32	*Rhizopus arrhizus*	25	11	-
33	*Rhizopus stolonifer*	100	9	-
34	*Talaromyces funiculosus*	25	22	-
35	*Talaromyces purpureogenus*	1-1,5	18	-
36	*Torula herbarum*	100	2	+
37	*Trichoderma koningii*	25	-	-
38	*Trichoderma longibrachiatum*	25	-	-

Qeyd. *Distance- distance from the sampling point to the oil well (m), P-soil sample taken from a soil oil pond; PhA- phytotoxic activity; OCS- Finding (+) and absence (-) of oil-contaminated soils in previous studies

It should be noted that while 2 of the recorded fungal species (*Orbilia oligospora* and *Pirella circinan*) are new to Azerbaijani nature, 21 species are recorded for the first time in oil-contaminated soils under Azerbaijani conditions.

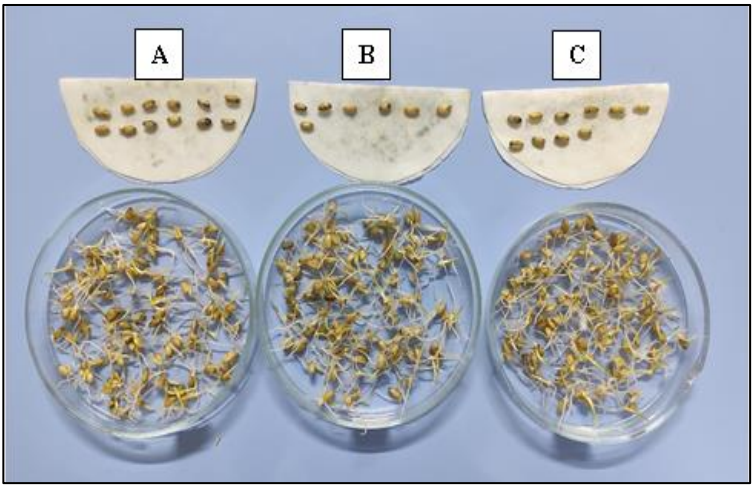

Figure 1. Determination of stimulation activity.
A – control, B – *T. koningii*, C – *T. longibrachiatum*

3.3. Annotated list of species

An annotated list of fungal species recorded in studies was compiled based on their current taxonomic classification and names. The taxonomic classification of the recorded fungi is provided in the third tab.

Table 3

Distribution of species in taxa. (N/S- number of species; N/St- number of strains)

Division	Class	Order	Family	Genera	N/S	N/St
Ascomycota	Pezizo-mycotina	Pleosporales	Pleosporaceae	Alternaria	1	2
				Curvularia	1	3
		Hypocreales	Hypocreaceae	Aspergillus	6	29
				Trichoderma	2	5
			Nectriaceae	Fusarium	2	4
		Dothideales	Saccotheciaceae	Aureobasidium	1	2
		Cladosporiales	Cladosporiaceae	Cladosporium	1	3
		Orbiliales	Arthrobotryaceae	Orbilia	1	1
		Eurotiales	Thermoascaceae	Paecilomyces	1	3
				Talaromyces	2	5
			Aspergillaceae	Penicillium	12	43
			Torulaceae	Torula	1	2
Mortierellomycota	Mortierello-mycotina	Mortierellales	Mortierellaceae	Mortierella	1	3
Mucoromycota	Mucoro-mycotina	Mucorales	Mucoraceae	Mucor	3	14
			Thamnidiaceae	Pirella	1	2
			Rhizopodaceae	Rhizopus	2	8
				Total:	38	129

18

CHAPTER IV

PHYSIOLOGICAL REGULATION OF LIPOLITIC ENZYME SYNTHESIS IN FUNGI FROM OIL-POLLUTED SOILS

4.1. Evaluation of the influence of physiological factors on lipolytic enzyme synthesis in selected active producers and identification of the optimal environment

The investigation of microorganisms in Azerbaijan that produce lipolytic enzymes remains incomplete. Consequently, we assessed fungi isolated from oil-contaminated soils for their lipolytic activity. The findings indicated that not all fungal strains exhibited extracellular lipolytic activity (Table 4).

Table 4

Evaluation of isolated fungi for lipolytic activity

№	Genera	Number of strains (active),	Share in total, %	Activity (μ/ml)
1	*Alternaria*	2 (1)	50	6340
2	*Aspergillus*	29 (23)	79	5330-11300
3	*Aureobasidium*	2 (2)	100	4580-6500
4	*Cladosporium*	3 (2)	67	1700-3420
5	*Curvularia*	3 (1)	33	8050
6	*Fusarium*	4 (3)	75	2100-4500
7	*Mortierella*	3 (3)	100	3670-7960
8	*Mucor*	14 (12)	86	2560-7950
9	*Orbilia*	1 (1)	100	5830
10	*Paecilomyces*	3 (2)	67	5750-8239
11	*Penicillium*	43 (32)	74	3420-10170
12	*Pirella*	2 (1)	50	7140
13	*Rhizopus*	8 (8)	100	4680-10220
14	*Talaromyces*	5 (3)	60	3880-8420
15	*Torula*	2 (2)	100	3490-5320
16	*Trichoderma*	5 (3)	60	2340-2643
Total		129	100	1700-11300

Specifically, the minimum percentage of strains with lipolytic activity per genus was 33.3%, while the maximum reached 100%. Notably, there was no clear correlation between the percentage of strains and their levels of activity. For example, although the highest activity was observed in strains of the genus Aspergillus, the largest number of strains with lipolytic activity was found in the genus Rhizopus.

At the conclusion of this phase of research, we identified three strains as active producers: *A. niger*-17, *P. glabrum*-81, and *Rh. stolonifer*-94. This selection was based on their activity indices, which were higher than those of all the other evaluated strains.

4.2. Evaluation of the influence of physiological factors on the synthesis of lipolytic enzymes in selected active producers and selection of optimal environment

It is known that environmental factors also affect the synthesis of lipolytic enzymes in micromycetes and the catalytic activity of the synthesized enzyme, and by changing the quantitative and qualitative indicators of these parameters, it is possible to significantly increase enzyme synthesis.

Studies were conducted on the physiological basis of synthesis regulation and optimization of the environment for maximum synthesis using the fungus *Aspergillus niger* AA-17, which has the highest activity.

Aspergillus niger AA-17, cultivated in Chapek medium (final pH 7.0) under deep cultivation conditions at a temperature of 28 ^{0} C, was used as a control. Under these conditions, the activity of the strain per ml of nutrient medium was 8.2 μ/ml.

Carbon source. The role of carbon sources in the synthesis of the enzyme is quite large. In our studies, among the substances used as different carbon sources, the highest indicator that increased the synthesis of the enzyme was maltose + olive oil (28 + 2 g / l), the lowest indicator (6.0) was lactose (30 g / l). When maltose + olive oil was used, the activity increased by 17% and was equal to 9.6 bv / ml. The activity of the culture grown in the medium using only maltose was (8.3), glucose (8.6), fructose (7.9). The use of olive oil caused an increase in the indicators by inductive effect, the increase was more than 15% when

using maltose and maltose + olive oil, it varied between sucrose + olive oil (8.8), fructose + olive oil (8.3), glucose + olive oil (8.7), lactose + olive oil (7.3). The final results are shown in the diagram below (Fig. 2). As can be seen, the use of olive oil in all cases leads to an increase in enzyme activity, which is characteristic of enzymes synthesized inductively.

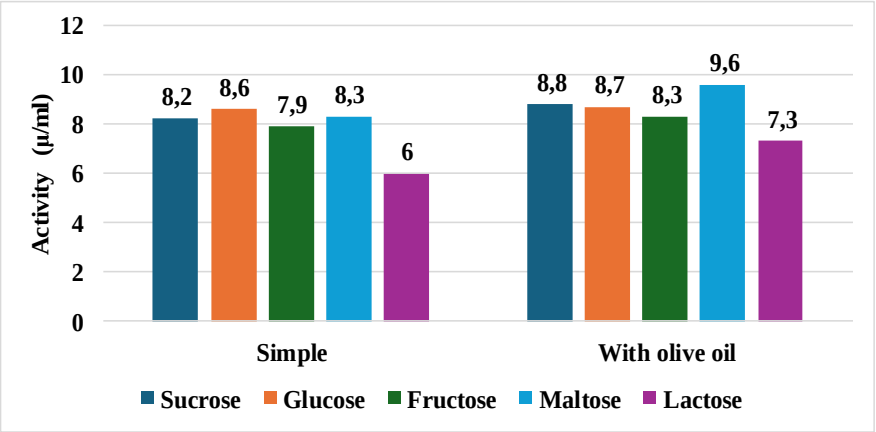

Figure 2. Effect of carbon source on activity.

Nitrogen source. In the studies conducted in this regard, namely, the effect of nitrogen sources (sodium nitrate, ammonium nitrate, peptone, yeast extract) on the synthesis of lipolytic enzymes in the fungus *Aspergillus niger* AA-17, it became clear that high lipolytic activity was obtained when peptone (4 g/l) was used. In this case, the activity increased by 16% and was 11.2 µ/ml. By changing the amount of peptone, namely 1, 2, 3, 4, 5 (g/l), the synthesis of the enzyme was optimized, and it was found that the activity obtained with peptone taken in an amount of 4 g/l increased to 11.2 µ/ml. While the activity at a peptone concentration of 4% was equal to 11.2 µ/ml, the highest level, a decrease in enzyme synthesis was observed when the concentration was increased to 5%, this decrease is also evident at other concentrations (Fig. 3).

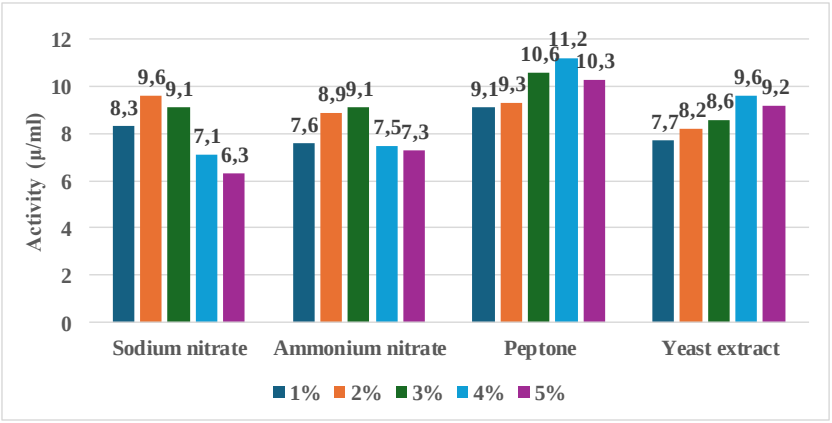

Figure 3. Effect of nitrogen source on activity

Surfactants. To enrich the composition of the nutrient medium, as well as to increase the enzymatic activity of the strain, Tween 20 was added to the medium as a surfactant in the amount of 1; 2; 3; 4; 5 g/l. The results of the studies and literature data coincided, that is, the addition of a small amount of this type of substance to the nutrient medium increased the synthesis of the enzyme by 5-7%. Thus, no effectiveness was observed when increasing the amount of this substance. Optimally, Tween 20 added to the nutrient medium in the amount of 2 g/l increased the synthesis of the enzyme by 6%. At this time, the final activity of the strain was 11.9 µ/ml.

pH. The optimal pH for strains of *A. niger* fungus has been determined differently in different studies. This is because, like many factors, the optimal pH is strain specific. For example, Mida H Mayel et al. in their study emphasized that "*the optimal pH for A. nidulans was 7, and for A. niger -LC 269109 it was 6*"[25]. In another study, the optimal pH for "*both A. niger MH078571.1 and A. niger MH079049.1*

[25] Mayel, M.H., Nwuche, C.O., Chimaobi, V.S., Eze, S.O.O., & Chilaka, F.C. Comparative Studies on the Kinetic Properties of Lipases Purified from Aspergillus nidulans and Aspergillus niger LC 269109// Biochem Mol Biol, -2020. 6 (2), -p. 10. https://doi.org/10.36648/2471-8084.6.2.10

micromycete strains was found to be 8"[26].

The result of our studies was that the strain showed the highest activity at pH 6.5. At this time, the activity of the strain increased to 5% and the final activity was equal to 12.39 μ/ml. However, at an indicator higher than 7.5, both the biomass of the micromycete and its enzymatic activity decrease, and at pH 9, the enzyme activity almost completely decreases. At indicators lower than 7.5, the increase in the alkalinity of the nutrient medium also leads to a decrease in the enzyme activity and the biomass of the strain, since at pH 5 this indicator reaches its minimum limit (fig. 4).

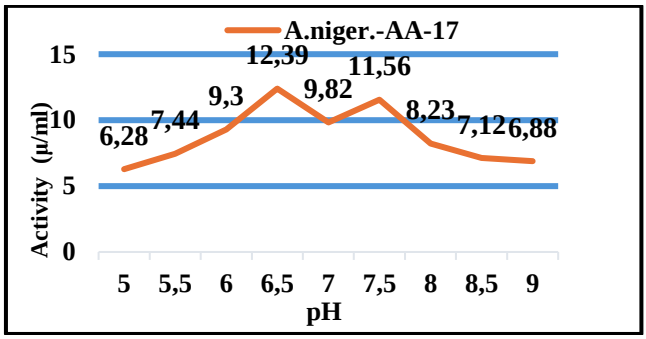

Figure 4. Determination of the optimal pH.

Temperature. To determine the optimal temperature, culture solutions of the fungus cultivated at different temperatures were used. Thus, at a temperature of 20° C, the growth of the fungus was weak, and its enzymatic activity was low. At a temperature of 24° C, these indicators continued to increase and finally reached their maximum indicator (12.39 μ/ml) at 28^0 C. At temperatures higher than 28^0 C, the activity began to decrease, and at 40^0 C, the development of the fungus was almost not observed (fig. 5).

[26] Alabdalall, A.H., Al-Anazi, N.A., Aldakheel, L.A., Amer, F.H., Aldakheel, F.A., Ababutain, I.M., & Al-Khaldi, E.M. Application and characterization of crude fungal lipases used to degrade fat and oil wastes// Scientific Reports, -2021. 11 (1), -p. 19670. https://doi.org/10.1038/s41598-021-98927-4

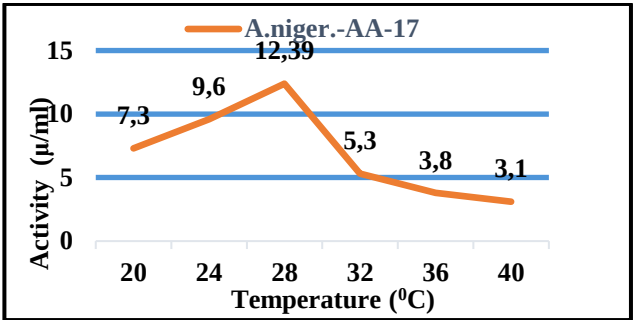

Figure 5. Determination the optimal temperature.

After optimization, Modified Chapek medium at 28 ^{0}C with a pH of 6.5 (maltose+olive oil (28.0+2.0 g), peptone- 4.0 g, K_2HPO_4 - 1.0 g, $MgSO_4 \cdot 7H_2O$- 0.5 g, KCl- 0.5 g, $FeSO_4 \cdot 7H_2O$- 0.01 g and Tween 20- 1 g) was determined as the optimal medium.

4.3. Prospects for the use of lipolytic enzymes in the Republic of Azerbaijan.

As you know, enzymatic activity in fungi and bacteria is strain specific. That is, the lipolytic activity of different strains belonging to the same species is different. There are many reasons for this; ecological conditions, physical-geographical location, the substrate from which the strain was isolated, the nutrient medium in which it was cultivated and its composition, etc. Therefore, studying the activity of more strains in different nutrient media, will create conditions for finding better producers.

4.3.1. Bioremediation. When determining the ability of micromycetes to biodegrade oil according to the Hanson method, three main indicators are considered: "*the first is the change in the color of the indicator from blue to colorless, the second is the decrease in the amount of oil in the flask, and the third is the growth of mycelium*"[27]. Thus,

[27] Benguenab, A., & Chibani, A. Biodegradation of petroleum hydrocarbons by filamentous fungi (Aspergillus ustus and Purpureocillium lilacinum) isolated from used engine oil contaminated soil// Acta Ecologica Sinica, -2021. 41(5), -p. 416-423. https://doi.org/10.1016/j.chnaes.2020.10.008

although one or more of these indicators were observed in one way or another in all strains used (*Aspergillus niger* AA-17, *Rhizopus stolonifer* AA-81, *Penicillium glabrum* AA-94), all three indicators were quite high in the *Aspergillus niger* AA-17 strain (Table 5). This allows us to note that all three strains can be used for bioremediation work in oil-contaminated soils due to their oil biodegradation ability, the higher indicators of the *Aspergillus niger* AA-17 strain compared to the others made the use of this strain and the enzyme biopreparation obtained from it alone more expedient.

Table 5

Determination of oil biodegradability

Strains	I/D*	A/O	M/G
A.niger AA-17	+++	+++	+++
Rh.stolonifer AA-81	++	++	+++
P.glabrum AA-94	+++	+++	++
Control	-	-	-

*Note. (I/D) Indicator discoloration. -: no color change, +: weak, ++: moderate, +++: discoloration; (M/G) Mycelium growth. -: no growth observed, +: weak, ++: moderate, +++: strong; (N/M) Amont of oil. -: no change in quantity, +: weak, ++: moderate, +++: completely hydrolyzed

After 14 days of development, the biomass of the strains was measured, and it was found that the highest biomass was produced by *the Aspergillus niger* AA-17 (0.285 g/l) strain (Table 6). As can be seen, the greatest growth was observed at a 1% oil concentration, since all three strains showed a growth of 50-75%. As the oil concentration increased, the growth percentage of the strains decreased, and finally, at a 10% oil concentration, the growth percentage of micromycetes compared to the control samples was less than 10%. This is also known from the literature data that micromycetes can be active in soils contaminated with oil up to 5-7%, and a concentration above the indicated limit seriously hinders the development of micromycetes and makes it impossible to carry out bioremediation work in these soils.

Table 6

Tolerance of strains to different concentrations of oil

Strains	Oil concentration		
	1%	5%	10%
A.niger AA-17	++++	+++	+
R.stolonifer AA-81	++++	+++	+
P.glabrum AA-94	++++	++	+

For this purpose, in subsequent studies, specific quantitative indicators of oil degradation in the soil were studied for all three strains. Thus, a laboratory model of the area was built under laboratory conditions through modelling and the amount of oil in the soil over time was determined by chemical analyses. Under these conditions, the *Aspergillus niger* AA-17 strain degraded 14% of the crude oil in the soil after 15 days of incubation (Table 7). After 30 days of incubation, the strain degraded 40% of the crude oil, and after 90 days of development, the strain degraded 63% of the oil. In the latter, the final amount of crude oil was 0.29 g. The amount of crude oil taken at the initial stage was taken from soils slightly contaminated with oil. Based on the results obtained, it can be said that this indicator is quite good, and the strain is effective.

Table 7

The ability of strains with high lipolytic activity to degrade oil in soil (100 g) at 28^0 C.

1*	2	3	4	5	6	7
A.niger AA-17	18	15	100	8	6,9	14
A.niger AA-17		30		8	4,8	40
A.niger AA-17		90		8	2,9	63
A.niger AA-17		15		25	24,5	2
A.niger AA-17		30		25	23,4	6
A.niger AA-17		90		25	20,9	17
Rh.stolonifer AA-81		15		8	7,3	9
Rh.stolonifer AA-81		30		8	5,3	34
Rh.stolonifer AA-81		90		8	3,7	54
Rh.stolonifer AA-81		15		25	24,7	1
Rh.stolonifer AA-81		30		25	24,1	4
Rh.stolonifer AA-81		90		25	21,2	16
P.glabrum AA-94		15		8	7,6	5
P.glabrum AA-94		30		8	5,5	33
P.glabrum AA-94		90		8	4,1	49
P.glabrum AA-94		15		25	24,8	1
P.glabrum AA-94		30		25	24,4	3
P.glabrum AA-94		90		25	21	16

Note. *1- Strain; 2- Moisture (%); 3- Time (day); 4- Initial soil addition (g); 5- Initial oil addition in soil (g/kg) 6- Final oil in soil (g/1kg); 7- Index (%)

In the second stage, we introduced 25 grams of oil per kilogram of clean soil, simulating soils that are moderately contaminated with oil. Under these conditions, the effectiveness of the same strain was observed to be 2% after 15 days of incubation, 6% after 30 days, and 17% after 90 days. A significant decrease in the strain's activity is anticipated as the incubation period progresses. This suggests that an increase in oil concentration complicates the bioremediation process. Consequently, a model specific to highly contaminated soils with oil was not developed in the subsequent stage.

The same experiment was subsequently conducted using the *Rhizopus stolonifer* AA-81 and *Penicillium g*labrum AA-94 strains. In

this instance, the Rhizopus stolonifer AA-81 strain was able to degrade 9% of the initial crude oil amount after 15 days of incubation, 34% after 30 days, and 54% after 90 days. In comparison, the *Penicillium glabrum* AA-94 strain achieved degradation rates of 5%, 33%, and 49%, respectively, under identical conditions. Throughout these experiments, it became clear that the *Aspergillus niger* AA-17 strain was the most effective in degrading crude oil. Consequently, the optimization of enzyme synthesis was focused exclusively on this strain.

Later, a relatively pure enzyme preparation obtained from *the Aspergillus niger* AA-17 strain was used in the bioremediation of oil-contaminated soils. This time, a model of the area was again built in laboratory conditions. Unlike the previous study, this time the preparation was mixed with water, and the soil was washed with that solution. The use of the preparation gave the expected results and was more effective than the direct use of the strain itself. The experiments were conducted based on the difference in enzyme concentration based on the initial amount of oil. In this case, 0.1%, 0.5%, 1%, 1.5% and 2% enzyme concentrations were used. In this case, taking a higher concentration of the enzyme increased the percentage of oil degradation, which was at its highest level at 2%, but reached the most optimal level at 1% concentration. Because, increasing the enzyme concentration from 1% to 1.5% gave only a 1% increase in degradation time, and increasing it to 2% gave a 2% increase, respectively. This is not economically viable, as doubling the amount of enzyme in this case only results in a 2% increase (Table 8).

Table 8

Degradation of oil in soil using an enzyme preparation obtained from *A. niger* AA-17 strain

*E/Q (%)	Temperature	Time (day)	ISA (gr)	IO (q/1kq)	FO (q/1kq)	In-dex
0,1	28^0 C	10	100	8	6,9	14
0,5					6,3	21
1					5,0	37
1,5					4,9	39
2					4,8	40
0,1		20			4,3	46
0,5					3,8	52
1					2,8	65
1,5					2,6	67
2					2,5	69
0,1		30			3,5	56
0,5					3,0	63
1					1,8	78
1,5					1,7	79
2					1,6	80

Note. *E/Q- Enzyme quantity; Time (day); ISA- Initial soil addition (g); IO- Initial oil addition in soil (g/kg) FO- Final oil in soil (g/1kg); 7- Index (%)

4.3.2. Wastewater treatment. The treatment of wastewater, oil and grease layers observed near the coastline of water bodies polluted with oil and grease using lipolytic enzymes obtained from fungi is an innovative, environmentally friendly technology that has no additional negative impact on human health and the environment. The use of this technology is practically not practiced in the Republic of Azerbaijan. Extensive research has been conducted in the world to find producers that can be used in this direction, and information has been provided on the use of a number of fungal strains [28].

[28] Kumar, A., Verma, V., Dubey, V.K., Srivastava, A., Garg, S.K., Singh, V.P., & Arora, P.K. Industrial applications of fungal lipases: a review// Frontiers in Microbiology, - 2023. 14, -p. 1142536. https://doi.org/10.3389/fmicb.2023.1142536

For this purpose, screening of the fat-degrading ability of active strains was carried out. Among the active strains, *Aspergillus niger* AA-17 was found to be the most effective. The strain had the highest performance (Table 9). Thus, this strain was better than the others in terms of its ability to biodegrade both oils.

Table 9

Determination of the oil-degrading ability of fungi

Substrat / Strain	Olive oil			Fried fish oil		
	I/D*	R/O	M/G	I/D	R/O	M/G
A.niger AA-17	+++	+++	+++	+++	+++	++ +
Rh.stolonifer AA-81	++	++	+++	+	+	+
P.glabrum AA-94	+++	+++	++	+	+	++
Control	-	-	-	-	-	-

***Note.** I/D -*Indicator discoloration*. -: no change in color, +: weak, ++: medium, +++: complete decolourization; R/O -*Reduction of oil*. -: no reduction, +: weak, ++: medium, +++: complete reduction and M/G - *Mycelial growth*. -: no visual growth, +: weak, ++: medium, +++: strong.

FINAL ANALYSIS OF RESEARCH

The contamination of soil and water ecosystems by oil and its byproducts has been a focus of research for many years, especially in traditional oil-producing countries such as the Republic of Azerbaijan. Despite extensive studies, this issue continues to be relevant today, with millions of hectares of land worldwide having been, and still being, polluted by oil to varying degrees.

The issue of ecosystem pollution from oil and oil products poses a significant challenge in the Republic of Azerbaijan. Currently, the affected land area is estimated to be between 10,000 and 30,000 hectares. Moreover, several aquatic ecosystems in Azerbaijan are also contaminated with oil and its derivatives. Ongoing research aims to address this pollution and facilitate the restoration of these ecosystems for future use. Although some promising results have emerged from these studies, the issue has not yet been fully resolved. One contributing factor is that the proposed methods and approaches have not given sufficient emphasis to the use of biological agents, particularly fungi, either through direct application or indirectly via substances derived from them, such as enzymes.

With this in mind, the fungal biota of oil-polluted soils in Azerbaijan was examined to analyze its species composition. Consequently, strains isolated from soils collected around 24 oil wells in the Balakhani, Binagadi, Sabail, and Surakhani regions were assessed for their phytotoxic activity and the production of lipolytic enzymes. Notable active producers were selected, and the physiological aspects of enzyme synthesis in these fungi were studied. The research highlighted the promising application of enzyme preparations obtained from these strains in the bioremediation of oil-contaminated soils and the biological treatment of industrial wastewater. These findings resulted in the development of key conclusions and practical recommendations.

RESULTS

1. A collection of 129 fungal strains was established from oil-polluted soils in the Republic of Azerbaijan. This collection encompasses 2 subkingdoms (Dikarya and Mucormyceta), 3 divisions (*Ascomycota*, *Mortierellomycota*, and *Mucormycota*), 13 families, 16 genera, and 38 species. Notably, 2 species—*Orbilia oligospora* and *Pirella circinan*—were identified as being naturally distributed in Azerbaijan, while 21 species were discovered in oil-polluted soils for the first time [4, 7, 12].

2. During the study of the phytotoxic activity of oil-contaminated soils and fungi isolated from them, it became clear that both oil and oil products, as well as fungi inhabiting them, participate in the formation of the phytotoxic activity of soils, and fungi also differ from each other in terms of phytotoxic activity. Thus, while some of them show weak (3-9%), some moderate (11-29%), and some strong (40-56%) activity, biostimulation (2.3-5.6%) activity was recorded in fungi of the genus *Trichoderma* [7, 12].
3. It has been established that not all fungal strains isolated from oil-contaminated soils have lipolytic activity, but *Aspergillus niger* AA-17, *Pencillium glabrum* AA- 76 and *Rhizopus stolonifer* AA-82 differ from other producers in their ability to actively synthesize lipolytic enzymes [2, 3, 4, 5, 10].
4. The *Aspergillus niger* AA-17 strain, the composition of the existing Czapek medium was modified both quantitatively and qualitatively, creating a new medium in which the fungus was found to inductively synthesize lipase and the activity it showed compared to the initial medium was increased to 51% [4, 10].
5. The strain of *Aspergillus niger* AA-17 and the enzyme biopreparation derived from it were evaluated in a laboratory model for their effectiveness in cleaning oil-contaminated soils. In soil samples containing 8 g/kg of oil, the strain successfully degraded 63% of the initial oil content after 90 days of cultivation. In contrast, the enzyme biopreparation achieved a degradation rate of 78%. These findings suggest that both the strain and the biopreparation are promising candidates for use in bioremediation efforts [1, 6, 10, 11].
6. *Aspergillus niger* AA-17 can purify 1 m^3 of water in 3 months under appropriate conditions (oxygen regime, temperature, pH, etc.) for the treatment of oil-based pollutants in technical wastewater, which has been experimentally confirmed [8, 9, 10].

PRACTICAL RECOMMENDATIONS

1. The contamination of soil with oil and petroleum products leads to alterations in both the structure and functional activity of the affected area. Consequently, the migration of oil into soil profiles results in changes to its physicochemical properties, a reduction in the availability of easily accessible nutrients, alterations in pH, and other modifications, ultimately creating adverse conditions for the survival of microbiota, plants, and soil invertebrates. Therefore, the bioremediation of such soils is more effectively achieved using agents derived from organisms capable of thriving in these environments, particularly lipolytic enzymes.
2. Modifying traditional nutrient media used for the synthesis of lipolytic enzymes in micromycetes to suit the selected producer can lead to even more effective results. Modifying traditional nutrient media used for the synthesis of lipolytic enzymes in micromycetes to suit the selected producer allows for even more effective results.
3. During bioremediation, when microorganisms are unable to carry out their physiological processes effectively at high levels of contamination and the efficacy of the enzyme-based biopreparations decreases, some physicochemical remediation processes can be performed. Once the contamination reaches the required level, it is more appropriate to first use biopreparations, followed by active strains.

LIST OF PUBLISHED SCIENTIFIC WORKS RELATED TO THE DISSERTATION TOPIC

1. Ahmadli A.A. The role of micromycetes distributed in oil-polluted areas in the process of biological restoration of soil// Materials of the IV Republican Conference "Ecology: Problems of Nature and Society", -Baku, -2023, -p. 210-211
2. Ahmadli A.A., Seidova G.M., Gasanova A.R., Omar P.M. Some

features of the synthesis of hydrolytic enzymes in fungi // International Center for Scientific Cooperation "Science and Education", Penza, -2023, No. 1, -p. 49-52
3. Mamedova A.E., Ahmadli A.A. Interrelationships of microorganisms as a method of selective isolation of fungi from soil // International Center for Scientific Cooperation "Science and Education", Penza, -2023, p. 1, No. 1, -p. 30-32
4. Bakshaliyeva K.F., Ahmedli A.A., Seyidova G.M. Evaluation of micromycetes isolated from oil-contaminated soils for lipolytic activity// Advanced Studies In Biology, -2023, v. 15, № 1, -p. 129-135. https://doi.org/10.12988/asb.2023.91697
5. Ahmadli A.A. Possibilities of using lipolytic enzymes obtained from micromycetes in biodiesel production// Materials of the XXVI Republican Scientific Conference of Doctoral Students and Young Researchers (NASCO XXVI). -Baku, -2024, -p. 37-40
6. Ahmadli A.A. Biodegradation of petroleum hydrocarbons by fungi strains of Aspergillus sp.-17, Rhizopus sp.-81, Penicillium sp.-94 isolated from oil-contaminated soils of Azerbaijan// In BIO Web of Conferences, -2024, v. 100, -p. 02007. EDP Sciences. https://doi.org/10.1051/bioconf/202410002007
7. Ahmadli A.A., Mammadova A.E., Muradov P.Z. Analysis of micromycetes found in oil-contaminated soils of Azerbaijan: species composition and biological activity// I International Scientific and Practical Conference Innovative Biotechnologies for Environmental Protection: from Theory to Practice, Minsk, -2024, - p. 114-115
8. Ahmadli A.A. The use of lipolytic enzymes obtained from micromycetes in the purification of wastewater from oil-based pollutants// Materials of the scientific-practical conference on the topic “The role of the National Leader Heydar Aliyev in the improvement of the environment in Azerbaijan”. -Baku, -2024, -p. 160-161
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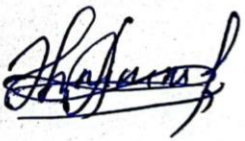
treatment// Materials of international scientific-practical conference "Current problems of microbiology, biotechnology and biodiversity" dedicated to the 20th anniversary of RCM. - Astana, -2024, -p. 206-209

10. Ahmadli A.A., Muradov P.Z. Lipolytic enzymes: Producers, industrial application, prospects of production in the Republic of Azerbaijan// Int. J. Biosci. -2024, v. 25, № 5, -p. 284-293. http://dx.doi.org/10.12692/ijb/25.5.284-293
11. Ahmadli A.A., Muradov P.Z. Methods used in remediation of oil contaminated soils// Advanced Studies in Biology, -2024, v. 16 (1), -p. 175-186. https://doi.org/10.12988/asb.2024.91935
12. Ahmadli A.A., Seyidova G.M. Study of fungal biodiversity of oil-contaminated soils of Azerbaijan and assessment of their cytotoxic activity// Natural and technical sciences, -2024, v. 2, no. 12, -p. 5-9. https://doi.org/10.37882/2223-2966.2024.12-2.02



The defense will be held on **“23” April 2025** at **11-00** at the meeting of the Dissertation council FD 1.07 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at the Institute of Microbiology MSERA

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