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Lipolytic enzymes: Producers, industrial application, prospects of production in the Republic of Azerbaijan

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Abstract

Lipolytic enzymes (triacylglycerol hydrolases E.C. 3.1.1.3), especially lipases are biocatalysts that hydrolyse biopolymers of oil into glycerol and fatty acids of great practical importance in industry, as well as transesterifiers and biocatalysts used in oil synthesis. These substances of serious industrial importance are obtained from different sources (plant, animal and microorganism) and used for different purposes. Examples include waste disposal, medicine, biotechnology, bioremediation, wastewater treatment, detergents, vegetable and animal oils, dairy products, and biodiesel production. As we mentioned, even though lipolytic enzymes are acquired from various sources, these enzymes obtained from micromycetes are practical because the enzyme system is extracellular, and they are easy to obtain and relatively inexpensive. However, the research done and carried out in this field is still not at the required level, and the demand for a physiological, microbiological and biochemical study of these enzymes obtained from fungi remains relevant for the modern era. This review article is dedicated to the possibilities of application and production of lipolytic enzymes in some fields in the Republic of Azerbaijan, taking as an example the world practice of recent years and based on the work done in this field in our Republic. As an example of these problems, we can point to the bioremediation of oil-contaminated soils and the biological cleaning of wastewater. so the problems in these areas are relevant and important to be solved not only in our country but also in the world, against the background of environmental protection and the inevitability of water shortages in the future due to the depletion of water resources.

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Introduction

Lipases are biocatalysts that play a crucial role in various biochemical reactions, including hydrolysis, transesterification, and long-chain fatty acid synthesis (Fig. 1). Specifically, these enzymes facilitate the breakdown of triacylglycerols into glycerol and fatty acids, with the potential for interesterification, acidolysis, alcoholysis, and aminolysis. Due to their versatile nature, lipases have garnered significant attention in both academic and business settings as catalysts for a wide range of applications (Pérez *et al.*, 2019).

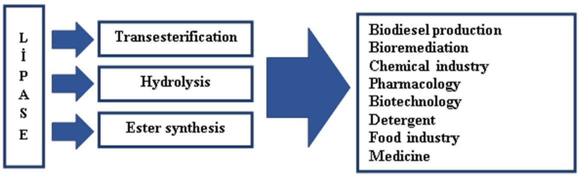

Fig. 1. Reactions catalyzed by lipolytic enzymes and areas of application

These enzymes operate under moderate temperature, pH, and pressure conditions with high substrate selectivity and catalysis rates. This makes them more cost-effective and efficient than chemical catalysts. Lipolytic enzymes' versatility has always made them the focus of industrial enterprises. However, there are also major problems with their use. For example, their acquisition requires high capital (excluding microorganisms), low stability, difficulties in their reuse, etc. (Filho *et al.*, 2019).

Enzymes sourced from a range of living organisms, including bacteria, fungi, yeasts, plants, and animals, possess significant economic and industrial value (Bharathi and Rajalakshmi, 2019; Panyachanakul *et al.*, 2020). While lipase production is seen across various organisms, microorganisms, specifically bacteria and fungi, are often the first to come to mind as primary producers. This is because their acquisition is relatively less expensive and faster than other sources, and micromycetes exhibit extracellular enzyme synthesis (Melani *et al.*, 2020; Subash *et al.*, 2013).

Microbial lipases

In 2018, the value of lipolytic enzymes of microbial origin in the global enzyme market was 425 million US dollars, and according to the estimated statistical indicators, these indicators are expected to be 590.2 million US dollars in 2023 with a growth of 6.8% (Chandra *et al.*, 2020). Many microorganisms, such as bacteria, fungi, and yeasts, are considered producers of lipolytic enzymes, and lipolytic enzymes of microbial origin are used in many areas of industry (Adetunji and Olaniran, 2021).

Bacterial lipases: A lot of work has been done in the direction of studying the lipolytic activity of bacteria spread in oil-contaminated soils around the world. For example, the lipolytic activity of many bacteria belonging to the genera *Pseudomonas* and *Bacillus* has been studied and information about their fields of application has been given. At this time, the growth and physiological characteristics of the *Pseudomonas aeruginos* bacterial strain were studied, and the influence of carbon and nitrogen sources, pH and temperature of the environment on enzyme synthesis was studied (Ilesanmi *et al.*, 2020). The present study aimed to characterize the capability of bacteria for degrading oil and oil-based products. As part of the investigation, various fungal strains were evaluated, with *Geobacillus* sp. D4, *Geobacillus* sp. D7 and *Anoxybacillus geothermalis* D9 are identified as the most active strains. The results of the study provide valuable insights into the ability of these bacterial strains to degrade oil and oil-based products and may be useful for future research in this field (Yusoff *et al.*, 2020).

Due to the study of the enzymatic activity of bacteria belonging to the genus *Bacillus*, which is widespread in Azerbaijan and exhibits high eurybiont characteristics and cosmopolitanism, it was found that many species belonging to this genus have lipolytic, β-glucanase, cellulolytic, amylolytic, proteolytic and pectinolytic activity. Later, brief information about the areas where they are used was given (Akhundova *et al.*, 2020). These enzymes obtained from bacteria are used in many fields of industry, so it is not practical to mention them individually. Therefore, it is more appropriate to summarize these data and present them in the following table (Table 1).

Fungal lipases: Although many indicators make lipolytic enzymes obtained from microorganisms superior to plant and animal lipases, among microorganisms, fungi differ from others (bacteria and microscopic algae) in the fact that enzyme synthesis is extracellular, etc. considered as a better producer due to reasons (Geoffry and Achur, 2018).

Table 1. Fields of application of lipolytic enzymes obtained from some bacteria

Species	Strain	Application	Reference
Geobacillus stearothermophilus	FMR12	Washer detergent	(Abol-Fotouh *et al.*, 2021)
Aeribacillus pallidus	-	Wastewater treatment	(Ktata *et al.*, 2020)
Kocuria flava	ASU5 (MT919305)	Biodiesel production	(Najjar *et al.*, 2021)
Pseudomonas yamanorum	LP2	Hydrolysis of oil	(Komesli *et al.*, 2021)
Bacillus sp.	VITL8	Utilization of wastes	(Balaji *et al.*, 2020)
Streptomyces sp.	A3301	Biodegradation of plastic	(Panyachanakul *et al.*, 2020)
Bacillus aerius	24 k	Textile	(El-Fiky *et al.*, 2022)

Table 2. Some areas of use of lipolytic enzymes obtained from fungus

Species	Application	Reference
Arthrographis curvata & *Rhodosporidium babjevae*	Biotechnology	(Aamri *et al.*, 2020)
Rhizopus oryzae & *Candida rugosa*	Biodiesel production	(Yang *et al.*, 2021)
Aspergillus niger MH078571.1 & *A.niger* MH079049.1	Utilization of oil	(Alabdalall *et al.*, 2021)
Aspergillus fumigatus	Synthesis of ethyl acetate and ethyl lactate	(Mehta *et al.*, 2020)
Candida rugosa	Medicine	(Sood *et al.*, 2023)
Aspergillus sclerotiorum	Wastewater treatment	(De Moura Dickel *et al.*, 2023)
Pseudomonas spp. & *Aspergillus* spp.	Bioremediation	(Yusne and Razak, 2023)
Candida albicans	Washer detergent	(Safdar *et al.*, 2023)
Penicillium citrinum	Hydrolysis of vegetable oil	(Lima *et al.*, 2019)

Fungi with lipolytic activity are mainly found in oil-contaminated soil (Fashogbon *et al.*, 2021; Wadia and Jain, 2017), aquatic environments (Mendes *et al.*, 2019), on various plants (Rangel Fonseca Bessa *et al.*, 2021; Spencer *et al.*, 2020), food (Humaid *et al.*, 2020), etc. from such substrates, extracted into pure culture from extreme conditions (Angelin and Kavitha, 2022), enzyme activities were determined and waste disposal, oil hydrolysis, biodiesel production, medicine, synthetic detergents, food industry, bioremediation, etc. the possibilities of use in such areas have been studied (Melani *et al.*, 2020). Fields of use of lipolytic enzymes obtained from mushrooms are shown in Table 2.

The enzymes referred to in this context are sourced from various genera of fungi. The enzymes obtained from micromycetes belonging to the genera *Aspergillus* (Kavitha *et al.*, 2021), *Rhizopus* (Yu *et al.*, 2016), and *Penicillium* are highly effective in breaking down lipids. Due to their high level of lipolytic activity, these enzymes are widely used in medicine, biotechnology, and food industry. They offer numerous opportunities for medical and economic applications (Ortellado *et al.*, 2021).

Prospects of the use of lipolytic enzymes in the Republic of Azerbaijan

Bioremediation: Due to its geographical and ecological position, the Republic of Azerbaijan has historically suffered from the problem of oil pollution, as it has rich polycyclic aromatic hydrocarbon deposits and the area where the first oil was extracted on land. 33.3 thousand ha of the Absheron peninsula, which has an area of more than 220 thousand ha, is considered impractical, of which 10 thousand ha are contaminated with oil and oil products. Most of the pollution is observed in Binagadi, Surakhani, Sabunchu, Balakhani, and Sabayil regions (Bayramli, 2020).

Bioremediation of oil-contaminated soils of the Republic of Azerbaijan with microorganisms using fungal-bacterial association was carried out based on 10-day development by adding oil to nutrient media where microorganisms were cultivated, and it was concluded that biological remediation works carried out with bacteria or fungi alone are more effective than works carried out with bacteria-fungus association less effective (Aliyeva *et al.*, 2017). Many studies have been conducted in this direction. An example of this is the study of the ability of bacteria belonging to the genus *Arthrobacter* to decompose phenols and some aromatic hydrocarbons from oil-contaminated soil (Babashli *et al.*, 2022).

Aspergillus, *Penicillium*, *Alternaria*, *Mucor*, *Fusarium*, etc., which are considered natural bio remediators that can live even in places with high levels of pollution, in the works conducted in the Republic on the use of fungi in the bioremediation of oil-contaminated soils. There is data on the ability of fungi belonging to the genera to break down oil in net-contaminated soils (Ahmadli, 2022). Also, research studies of some fungal strains with high-quality activity were carried out by Bakhshaliyeva and others, and at this time, the active strains of *Aspergillus* sp.-17, *Penicillium* sp.-81 and *Rhizopus* sp.-94 were subjected to enzyme synthesis temperature, environmental reaction, carbon and nitrogen its relationship to its characteristics was studied and adapted to optimal conditions (Bakshaliyeva *et al.*, 2023). Although there is some research on the study of micromycetes and their enzymatic activity in oil-contaminated soils, these materials make it impossible to evaluate the results of the type of biotope and do not allow conducting of statistics of possibilities. The need to carry out wider and more systematic research remains relevant (Bakhshaliyeva *et al.*, 2017).

Wastewater treatment: Dirty water refers to water bodies contaminated with oil and oil products, as well as oils discharged in sewage. One of the main environmental problems of the Caspian Sea in modern times is oil hydrocarbon pollution. In the research conducted in 2019, these hydrocarbons were found on the surface of the sea using satellite images. It became known that the Caspian Sea is filled with oil and oil products from several sources (naturally from the seabed, mud volcanoes, during oil production and transportation, oil tankers, etc.) is contaminated (Mityagina *et al.*, 2019).

In general, there are about 200 natural and artificial lakes, big and small, on the Absheron peninsula, among which Buyukshor, Binagadi, Khojasen, Masazyr, Kurdakhani and Qırmızı lakes belong to natural lakes and have a special importance due to their depth, water capacity and size. These lakes are mainly fed by groundwater and rainwater. Therefore, the level of lakes depends on seasonal changes. The current state of the lakes is polluted with petroleum products, various chemical wastes and oils (Khalilova and Ahmadzade, 2022). On the other hand, according to the information provided by "Azersu" OJSC, 1 million m^3 of sewage is discharged from sewage lines during the day (the volume of wastewater treatment facilities in Baku city is 774.7 thousand m^3, and the volume of biological water treatment facilities is 667.5 m^3). Wastewater discharged from sewer lines contains various vegetable and animal oils used in the household. As the last stage, these waters are discharged into the Caspian Sea and various lakes of the Absheron Peninsula, causing ecological problems in the hydrosphere. According to statistical data, it is known that the total area of oil-polluted lakes on the Absheron Peninsula is more than 7 thousand hectares (https://azersu.az/az/static/9).

Biodiesel production: Biodiesel is an environmentally friendly alternative to traditional diesel obtained by transesterification of vegetable and animal fats. This process uses lipolytic enzymes of microbial origin, where lipases are used to transesterify fats in the presence of alcohol. At this time, oil esters (biodiesel) and glycerin are bought, both of which are in high demand in the domestic economy (Bhan and Singh, 2020).

Due to the geopolitical situation and the presence of sufficient oil and gas deposits, the work done in the production of bioenergy in AR is not at the required level. However, due to the richness of its climate, and physical-geographical-ecological conditions, AR, which has conditions for the cultivation of many types of plants, also has sufficient resource potential due to the remains of agricultural plants. These residues are valuable raw materials for biofuel and biodiesel production. According to the data of 2021, the total amount of agricultural residues in AR was 1,099,346 tons. According to the amount of residues, the share of cotton and barley plants in the total indicator was 47 and 14.47% (Naeimi *et al.*, 2023).

Information on the use of biological catalysts of microbial origin in the production of biodiesel has not been found in the Republic. Instead, the production of biodiesel from the transesterification of plant waste by means of chemical catalysts was obtained using sunflower and corn plants in the presence of CaO. At this time, CaO catalyst was obtained using nanotechnologies and used to obtain biodiesel from vegetable oils. Also, in the aforementioned studies, methods for separating glycerol from the reaction mixture were developed (Mahmudova, 2021).

Purpose

The purpose of this research work is to determine the advantages and disadvantages of using lipolytic enzymes of fungal origin in the initial stage of wastewater treatment, bioremediation of oil-contaminated soils, and biodiesel production using local bioresources, as well as to optimize enzyme synthesis in laboratory conditions and ensure their application in industry, which will serve to increase the industrial potential of the country. On the other hand, in terms of environmental safety and economic efficiency, the use and popularization of production of enzymes in solving existing problems has been the focus of our research. Our research shows that the use of lipolytic enzymes obtained from funguses in biotechnology will give effective results, mass synthesis of enzymes to supply the domestic market at the initial stage, and then the production of enzymes according to world standards. The country's rich microbial biodiversity and personnel potential make this possible. However, it is also true that there are some shortcomings, such as adapting high technology and personnel training to world standards. Finally, this research aims at innovative solutions of such problems and increasing the competitiveness of the republic in the world by using local biodiversity.

Discussion of results

As it is known from the literature, there is not enough information about lipolytic enzymes in AR, either from fungi or bacteria. Also, it is very rare to find information on the fields of application of these enzymes. In general, the recommendations for the purchase and application of these enzymes in AR, which have a wide range of applications, can be grouped as follows. In the world, this gain is the use of strains with full lipolytic activity, along with a large number of patents, according to which serious scientific-practical results are obtained. However, for the effective use of these tools in other regions, which are suitable for the physical-geographical-ecological conditions of each region, it is up to the researchers to work in the region.

On the other hand, both in our Republic and in the world, special attention is paid to the improvement of ecological law and environmental protection. If we consider that the volume of oil-contaminated land in our Republic is the same as that of physico-chemical remediation works, there are some harmful factors (destruction of beneficial microbiota, secondary pollution, and full capital investment), and then the use of microorganisms is one of the best solutions.

At the same time, the pollution of water bodies (the same inland lakes of Absheron region, the Caspian Sea) by vegetable and animal fats, oil and oil products destroys hydrobionts there (it should be taken into account that the fauna of the Caspian Sea is distinguished by its uniqueness) and seriously affects people's health. It also causes the reduction of available freshwater resources. For this, it is more appropriate to use microbial lipolytic enzymes as an alternative to food catalysts used in wastewater treatment. Thirdly, considering the risk of exhausting fossil fuels, biodiesel production capacity is the most environmentally friendly fuel in the world. Oil residues of agricultural plants used in this process used oil resources are generated during the year in thousands of tons. This also causes serious scientific-practical and economic damage for the use of those persons and the elimination of dependence on oil. It is possible to obtain the analysis of literature data and to take the World practice as a basis.

1. Microorganisms are more effective than other solutions in treating oil-contaminated soils in Baku, the Absheron Peninsula, and the country.
2. It is more appropriate to use preparations of lipolytic enzymes of microbial origin to improve the environment, given the reduction of drinking water resources and contamination of the water environment with oils and fats.
3. AR has a wide range of resources that can be used for biodiesel production, which is the main factor for the mass production of biodiesel, and instead of chemical catalysts used as transesterifiers in the process, lipolytic enzymes of microbial origin are used due to their environmental friendliness, cheap availability, and practicality. it is economically more effective.
4. Because the lipolytic activity of microorganisms and the effectiveness of the purchased preparations are compatible with local climatic conditions, in environmental problems and in the areas intended for use, AR should use local products obtained at the expense of its own local bioresources.

Recommendations

1. Because the enzymatic activity of microorganisms is strain-specific, more use should be done in multiple microbial strains, acquired and patented.
2. The use of mushrooms selected for their high activity and the preparations obtained from them should be determined in specific areas, and scientific-methodical instructions should be prepared.
3. The mechanisms of the lipolytic activity of microorganisms should be studied, and the physiological-biochemical basis of their synthesis should be acquired by obtaining process-reducing inhibitors and stimulating inducers.

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