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# Microbial Lipases: Between Production, Purification, and Their Biotechnological Applications

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## ABSTRACT

The breaking down of lipid into glycerol and long chain fatty acids at the oil water interface is catalyzed by the enzymes known as lipases. Some enzymes are particularly interesting since they have a wide range of applications and can be utilized as catalysts in numerous biochemical processes. In non-aqueous and micro-aqueous environments, lipases catalyzed a variety of bioconversion processes, including as esterification and inter-esterification. All living things, including plants, animals, and microbes, can production lipases. However, lipase from microorganism is mostly preferred due to their low of cost production and genetic modification is simple. Nutritional and physicochemical factors affect the biocatalysts that microorganisms secrete. Lipase production was improved by optimizing the bioprocess parameters. Microbial lipases have also drawn increased interest for a variety of uses in the detergent, food, cosmetics industries, and environmental bioremediation. This review offers information on how to produce microbial lipases for possible biotechnological applications.

***Keywords:*** lipase, enzyme, microbial, biotechnological, application.

## 1. Introduction

All living organisms have the biocatalysts known as enzymes. The distinctive characteristics of the biological activity of enzyme is found in the accuracy with which they act, requiring the least amount of energy to catalyze a specific reaction. Every stage of metabolism and biological reactions depend mainly on enzymes. In most living organisms, lipids are digested, transported, and processed by lipases, a subclass of esterase. They are adaptable and allow for various bioconversion reactions in both unicellular and multicellular organisms (Ali *et al.,* 2023).

Serine hydrolases, also known as lipases, are widely distributed and are members of the triacylglycerol ester hydrolysis family (EC3.1.113)(Javed *et al.,* 2018; Moraleda-Muñoz & Shimkets, 2007). They are known as carboxylesterases, additionally, they can catalyze the synthesis of long chain triglycerides into monoacylglycerol, diacylglycerol, fatty acid, and glycerol (Figure1). They also show ammonolysis, esterification, interesterification, alcoholysis activity, which contributes to a variety of industries(Karadzic *et al.,* 2006; Rajendran *et al.,* 2009) .

Because of the extensive range of catalytic activities that microbial lipase can carry out and the high yield of their production, Microbial lipases are most valuable than those obtained from animals and plants. The ease with which they can be genetically modified, the lack of seasonal fluctuations in their availability, the regularity of their supply, the increased stability, safety, and convenience, and the extremely high growth rates of their microorganisms(Mendes *et al.,* 2012; Reetz, 2013).

Most of microbial lipases are extracellular, so the composition of the medium has a significant impact on lipase production moreover physicochemical variables like temperature, pH, and dissolved oxygen. Since lipases are inducible enzymes, the carbon source has consistently been reported as the main determinant to produce lipase activity. This enzyme is typically generated in the presence of lipids like inducers (Tweens, glycerol, bile salts, hydrolysable esters, and fatty acids) or oils (Gupta *et al.,* 2004; R. Sharma *et al.,* 2001)

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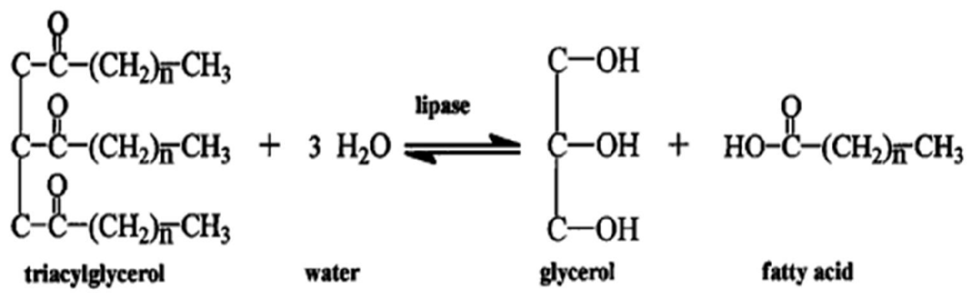


**Fig. 1:** Hydrolytic and Synthetic Action of Lipase (Thomson *et al.,* 1999)

For optimal development and production, carbon source seems to be important for achieving a high lipase yield. Nitrogen supplies and crucial micronutrients should be given significant thought. There are many other alternative media, including synthetic medium, such as oils, sugar, and complex components such yeast extract, peptone, malt extract, as well as agroindustrial residues that include all the elements required for microbe development (Treichel *et al.,* 2010).

## 2. Historical background

Enzymes are proteins that can catalyze a variety of chemical and biological reactions inside or outside the cell. Enzymes have highly specific natural catalysts to a variety of substrates that operate under environmental factor such as pH, pressure, temperature with conversion rates that are high(Huang *et al.,* 2019; Pàmies & Bäckvall, 2003) . In 1856, Claude Bernard discovered the lipase in pancreatic juice, which hydrolyzed insoluble oil droplet and turned them into soluble compound (Challa *et al.,* 2019). Following this, in 1901, lipase productions were noted in the bacteria *Bacillus pyocyaneus*, *Bacillus fuorescens*, and *Bacillus prodigiosus*. In the present day, *Pseudomonas aeruginosa*, *Serratia marcescens,* and *Pseudomonas fluorescent* species of bacteria have been found to produce lipases on a large scale(Homaei *et al.,* 2013) . In 1994, *Aspergillus oryzae* expressed lipolase, the first industrially produced recombinant lipase from the fungus *Termomyces anugiwnosus* (Blamey *et al.,* 2017) . In the past, either crude or purified lipase was derived from animal pancreas and utilized as a dietary supplement for the digestive tract. For the synthesis of several unique chemical compounds, it has been widely used in biocatalytic processes (Casas-Godoy *et al.,* 2012; Prasad & Roy, 2018).

## 3. Source for microbial lipase

Due to their higher stability and greater availability compared to animal and plant lipases, microbial lipases are widely distributed in nature and have significant commercial value (Borrelli & Trono, 2015). Most bioengineering applications involve naturally occurring or recombinant microbial lipases (Mayordomo *et al.,* 2000) .The abundance of microbial resources provided by nature, combined with the ability of microbes to adapt to harsh environments like those found in the hot springs, contaminated soils, volcanic vents, dead sea, offers extraordinary potential to produce lipase with unique properties (Elend *et al.,* 2007; Sánchez-Porro *et al.,* 2003).

Microbial biotechnologists have recently concentrated their attention on the industrial application of lipases derived from microorganisms, and various microbial strains have been screened and described for their ability to produce enzymes. *Pseudomonas* sp, *Rhizopus* sp, *Candida rugose, Aspergillus niger,* and *Penicillium* sp are the microorganisms most frequently used for lipase production (Bhagya Lakshmi & Audipudi, 2021; Pang *et al.,* 2021). Numerous microorganisms from various environmental conditions that belong to various genera of bacteria, yeast, and fungi have been isolated and screened. These microorganisms are potential sources of lipases (Fatima *et al.,* 2021; Pandey *et al.,* 1999).

### 3.1. Filamentous Fungi

Certain of the most commercially significant lipase producing fungi have been recognized as relating to the genera *Aspergillus* sp, *Rhizopus* sp, *Rhizomucor* sp, *Penicillium* sp, *Mucor* sp, and *Geotrichum* sp. According to Cihangir and Sarikaya.(2004) ,lipase production via filamentous fungus varies based on the composition of the growth medium, the strain, temperature, pH, and the type of nitrogen and carbon sources. The isolation and selection of new strains is prompted by the industrial need for novel source of lipase with a variety of catalytic properties. According to Sharma *et al*. (2001), There are numerous places where lipase producing microorganisms have been found including soil polluted with oil, industrial waste, processing plants, vegetable oil, and dairy plants. In (Table 1), there are descriptions of various recent research on fungi's ability of producing lipases.

**Table 1:** Fungal sources of lipse from diverse enviroments

| Microbial Source | Isolated from | Application | Reference |
|---|---|---|---|
| ***Penicillium roqueforti*** | Organic debris | Uses in ethyloleate synthesis | Soares *et al.,* (2022) |
| ***Acremonium implicatum*** | Buds of Panax notoginseng | Identifies antibacterial action against phytopathogens | Han *et al.,* (2020) |
| ***Chaetomium globosum*** | Green algea | various bioactive compounds to be created | Kamat *et al.,* (2020) |

### 3.2. Yeast

Vakhlu and Kour (2006), list the following yeast species as the primary terrestrial species that have been found to produce lipase: *Candida tropicalis*, *Candida rugosa, Candida antarctica, Candida parapsilopsis, Candida curvata, Rhodotorula pilimornae, Rhodotorula glutinis, , Trichosporon sp.,* and *Yarrowia lipolytica*, the genes encoding lipase have been cloned and overexpressed (Wang *et al.,* 2007).

### 3.3. Bacteria

Among bacterial lipases being utilized, those from *Bacillus* demonstrate unique characteristics that make them potentially suitable applicants. The most prevalent bacterial lipases are those produced by *Bacillus licheniformis*, *Bacillus alcalophilus, Bacillus pumilus, Bacillus coagulans,* and *Bacillus subtilis*. Additionally, reports of the production of bacterial lipase by *Pseudomonas* species, *Pseudomonas aeruginosa, Burkholderia multivorans, Burkholderia cepacian* (Treichel *et al.,* 2010). In (Table 2), There are several recent research on the production of lipases by bacteria.

**Table 2:** Bacterial sources of lipase from diverse environments

| Microbial source | Isolated from | Application | Reference |
|---|---|---|---|
| ***Rhodotorula glutinis*** | Isolated in Antarctica | Has the ability to fast grow, produce lipids, and be employed in a variety of industrial applications | Daniela Maza *et al.,* (2020) |
| ***Enterobacter cloacae*** | Acidic condition | Potential for medical applications of enantioselective esterification | Asitok *et al.,* (2023) |
| ***Anoxybacillus gonensis UF7*** | Hot spring | Biodiesel production | Altinok *et al.,* (2023) |
| ***Serratia nematodiphilia*** | Pulp effluent and Paper | Making of paper and increasing brightness, intensity, and the pulping rate | Intwala & Barot, (2022) |
| ***Escherichia coli*** | Acidic condition | Grow in moderate acidic condition | Xu *et al.,* (2021) |
| ***Bacillus amyloliquefaciens*** | Environment | Enhanced production of lipases in solid-state fermentation | Mazhar *et al.,* (2023) |
| ***Escherichia coli*** | Acidic condition | grow in moderate acidic condition | Xu *et al.,* (2021) |

## 4. Screening methods of lipase producer

For discovering microorganisms that produce lipases, a variety of lipase enzyme screening techniques have been suggested and employed. These techniques involve cultivating microbial strain on liquid medium or on solid media containing various substrates. Lipase screening can be broadly divided into two types: indirect and direct approaches.

**4.1. Direct method**

Lipolytic bacteria can be screened using solid medium with add of substrates or indicator. It is a helpful fast screening technique to assess each microorganism's capacity to produce lipase enzyme (Mateos-Díaz *et al.,* 2012).

**4.2. Indirect method**

Many quantitative techniques, including the spectrophotometric, molecular screening techniques, titrimetric approach, and chromatographic method, can be used to assess a microorganism's ability to produce lipase(Bharathi & Rajalakshmi, 2019).

**5. Production of lipase from microorganism**

Most of the microbial lipase is extracellular. It is common practice to use submerged fermentation and solid-state fermentation systems to extract lipase from microorganisms .

**5.1. Submerged fermentation system**

Liquid fermentation system is another name for a submerged fermentation system. According to Costa *et al*.( 2017), using this technique, microorganisms are grown in liquid medium while utilizing provided nutrients to create compounds. Furthermore, SMF-produced lipases can be recovered and purified in a straightforward manner. Lipase is mostly produced by bacterial strains using the SMF system.

**5.2. Solid state fermentation system**

It is utilized in a variety of industries, including the food and pharmaceutical sectors, to manufacture chemicals from microbes on solid substrate media (Borrelli & Trono, 2015). SSF primarily utilized to generate mycelial, yeast, and fungal lipases using a variety of solid substrates. For instance, Sethi *et al*.(2016), used mustard oil as a substrate for *Aspergillus* sp to produce lipase in both SSF and liquid fermentation, with the SSF method demonstrating the most effective outcome. Along with the lower production costs, the benefits of generating lipase enzyme using SSF have been highlighted. Large-scale fungi-produced lipase manufacturing can be economically alternative by solid state fermentation. (Colla *et al.,* 2015).

**6. Factors influencing microbial lipase production.**

The numerous physicochemical and nutritional factors influencing the production of microbial lipase are illustrated in (Figure 2) as well as discussed below.

**6.1. Carbon sources**

Source lipase-coding genes are routinely induced to create microbial lipase. Carbon sources play an important part in lipase stimulation in all types of microbial sources. When using mustard seed oil as the carbon source for lipase synthesis, *Aspergillus terreus* showed a decent yield (Sethi *et al.,* 2013). The use of a mixture of sugarcane bagasse and olive oil cake as a carbon source increases the production of lipase in fungal strains. Compared to alternative carbon sources, olive oil cakes showed improved lipase production (Zarevúcká, 2012). Tween 80 utilization has improved the recovery of lipase produced by *Acinetobacter* sp (Li *et al.,* 2001). Higher lipases are also frequently employed in addition to vegan oils because of their inexpensive and easily accessible yield(Messias *et al.,* 2009) .

**6.2. Nitrogen sources**

Production of lipase is significantly influenced by nitrogen. The increased synthesis of lipases from various microbial species has been significantly influenced by a variety of organic and inorganic sources of nitrogen (Das *et al.,* 2017; Oliveira *et al.,* 2015) . Additionally, lipase was made using *Aspergillus* species and a mixture of peptone and other nitrogen extracts (Orozco Colonia *et al.,* 2019).

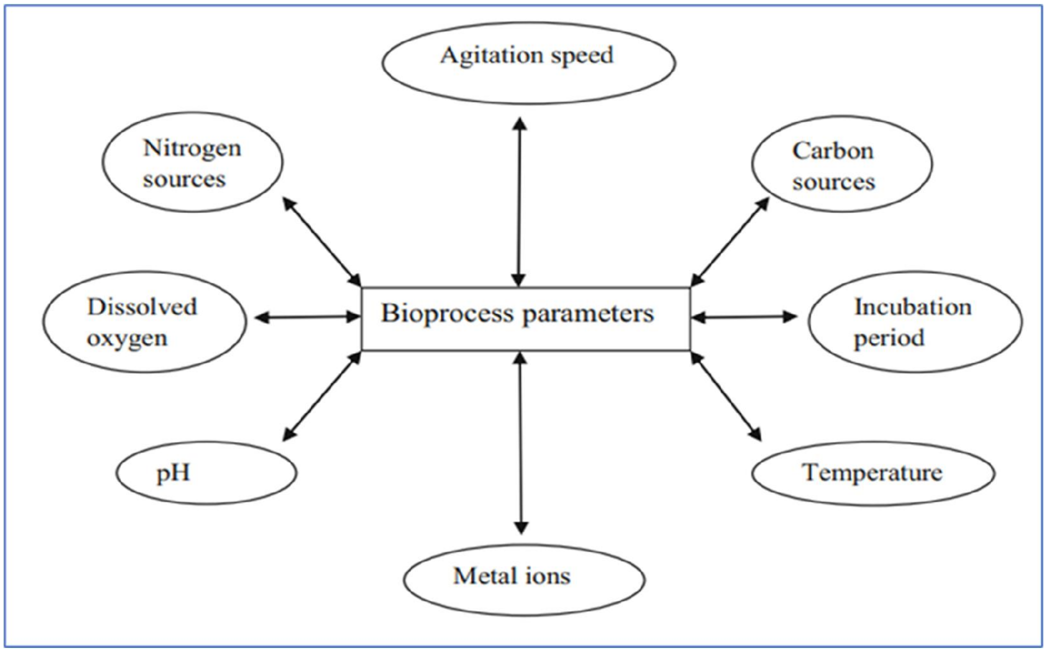


**Fig. 2:** Schematic digram depicting bioprocess parameters that infuence microbial lpase producton*(Adetunji & Olaniran, 2021)*

### 6.3. Temperature

At a temperature of 37 C, a higher lipase biomass concentration was reported, indicating the significance of optimal temperature for the secretion of enzymes via the shake flask procedure(Bharathi & Rajalakshmi, 2019) . According to research, the production of lipases is increased when temperatures rise by as little as 38 C. The product of lipase is decreased at low temperatures, and the activity of enzymes is impacted by high temperatures. (de Souza *et al.,* 2019).

### 6.4. pH

Bacterial lipases often have a pH that is either alkaline or neutral. At neutral and alkaline pH levels, research has shown that yeast and bacterial cells produce more lipase(Bharathi & Rajalakshmi, 2019) . At an acidic pH, however, fungal lipase synthesis was greater. At pH 4 of the reaction medium, lipase activity and product increased, according to Turati *et al.,* (2019).

## 7. Lipase purification

Purification of lipases includes several stages depend on whether the enzyme is extracellular or internal. In either case, the generated proteins are further salted off using $(NH_4)_2SO_4$ precipitation procedures(Yang *et al.,* 2016). Dialysis and chromatography are then used to the precipitated protein. According to the source of microorganism and the size of the proteins, different chromatography procedures are used. Lipases are typically purified using chromatography techniques.

The resultant crude fractions are then tested for the presence of enzyme using a variety of assay procedures and additional separation techniques such as DEAE-Sepharose, gel filtration chromatography and ion exchange. (Jing *et al.,* 2012; Unni *et al.,* 2016).

## 8. Potential biotechnological applications of microbial lipases

Due to their flexibility in terms of enzymatic properties and substrate specificity, microbial lipases comprise an important category of biotechnologically significant enzymes. Lipases are choice for several uses in the detergent, food, leather, textile, pharmaceutical, paper industries, and cosmetics, among others, due to these characteristics (Figure 3)(Hasan *et al.,* 2006; Priyanka *et al.,* 2019) .

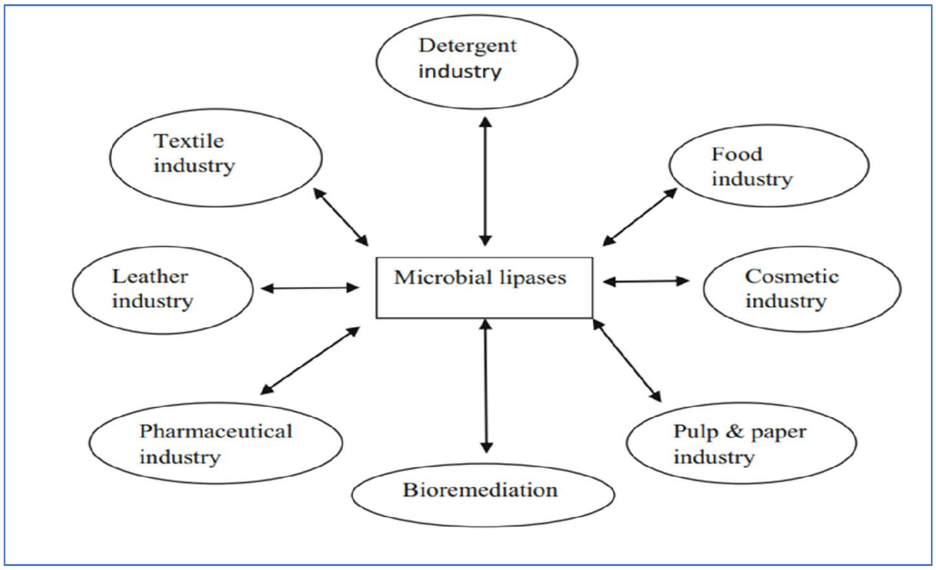


**Fig. 3:** Schematic illustration of potential biotechnological application of microbial lipase (Adetunji & Olaniran, 2021)

### 8.1. Role of Lipase in Biosensor

A growing number of researchers are interested in using lipases in biological assays or biosensors. Because of their broad substrate specificity and higher commercial availability, lipases are commonly utilized. Lipases can serve multiple roles in biosensors. As enzymatic substrates or inhibitors, they can be used (Herrera-López, 2012; Sandoval & Herrera-López, 2018). The use of lipases as enzymatic biosensors is crucial in analytical techniques used in a variety of fields, including biodegradable polymers, environmental science, oleochemicals, the food industry additionally, as diagnostic tools for determining the amounts of triglycerides and cholesterol in blood samples. (Sandoval & Herrera-López, 2018). The enzyme activity of lipases can be inhibited through multiple inhibitors. Different lipase inhibitors from various sources were identified in certain recent research . For example, the lipase from *Bacillus cereus* is inhibited by cetyltrimethyl ammonium bromide D2(Dutta & Ray, 2009) *Yarrowia lipolytica* is inhibited by cadmium-II (Kumari & Gupta, 2012) and *Mycobacterium* TB is inhibited by cobalt (Deb *et al.,* 2006) . Additionally, lipase can be utilized to create various biosensors or bioassays by including it into optical and electrochemical biosensors (Pohanka, 2019).

### 8.2. Role of Lipases in Biodiesel Production

To produce biodiesel (mono-fatty acid alkyl esters-FAAE), lipases can be utilized to trans esterify and esterify fats and oils (Cavalcante *et al.,* 2021; Goswami, 2022) . Although its application is well-established in the mentioned industries (leather, food, textile, etc.), lipase application in biodiesel synthesis is still in its nascent stages. Some researchers are utilizing free lipase to produce biodiesel (Alexandre *et al.,* 2022; Mendes *et al.,* 2023). However, most of the research advise using lipase that has been immobilized for this purpose (Abdulmalek & Yan, 2022) . To produce biodiesel, a variety of methods are optimized since immobilized lipase is crucial. The selection of the lipases depends on their origin and formulation because microbial lipases are widely utilized to produce biodiesel (Abdulmalek & Yan, 2022; Alexandre *et al.,* 2022; Altinok *et al.,* 2023) .The significant properties of microbial lipases include their lower production inhibition, high activity, short reaction time, and thermal tolerance. Lipases are produced by microorganisms such *Candida antarctica* (Pollardo *et al.,* 2017) , *Rhizopus arrhizus* (Sharma *et al.,* 2018) , and *Bacillus aerius* (Narwal *et al.,* 2015).

### 8.3. Role of Lipases in Bioremediation

Petroleum, insecticides, fertilizers, pesticides, plastics, and other xenobiotic chemicals that contain hydrocarbons are only a few examples of the xenobiotic molecules that are continually affecting our natural environment. The removal of these pollutants has been achieved utilizing a variety of techniques. These practices aren't efficient or environmentally friendly. Enzyme-based bioremediation is an environmentally friendly and an easily adaptable approach to get removal of these dangerous substances from our natural ecosystem compared to chemical and physical methods. However, the use of these enzymes in bioremediation has been limited by difficulties with manufacturing and prohibitive costs .Thus, the utilization of microbial enzymes for bioremediation is becoming increasingly important on a worldwide basis. Moreover, microbial enzymes are better able to convert pollutants into harmless materials and reduce environmental pollution. *Pseudomonas* sp was recently isolated from petroleum oil-contaminated sites and examined for lipase production by Jacob *et al.,* (2022). *Pseudomonas* sp can be used to degrade petroleum oil polluted soils, according to the study's authors. Multiple research studies demonstrate that microbes that breakdown hydrocarbons produce lipolytic enzymes like lipase (Jacob *et al.,* 2022). Similarly, because micronanoplastics negatively impact the health of organisms in all biological systems, it is crucial to remediate the increasing amount of MNPs in all systems. several research have documented the usage of plastic resistant bacteria strains like *Bacillus amyloliquefaciens* 1 and 2 at waste disposal sites and fungi such *Aspergillus clavatus*. All these microorganisms use various strategies to break down plastic as a source of carbon. Lipases are produced by a variety of bacterial and fungal species, including *Rhizopus delemar, Candida cylindracea, Pseudomonas fluorescens, Pseudomonas aeruginosa, Penicillium simplicissimum,* and *Candida cylindrace*a (Pathak & Navneet, 2017; Wang *et al.,* 2019).

## 9. Conclusions and Future Prospects

The current review demonstrates the versatility of lipases as biocatalysts for many bioconversion processes. Microbial lipase has a broad spectrum of applications. The majority lipase screened from microbes are secreted extracellular that can be isolated with high the purity. Microbial lipases from new microorganisms have been the focus of recent research. These enzymes are getting a lot of interest because of the rapid development of enzyme technology and their practical application in numerous industries. Major advancements have also been made in the purification and production of microbial lipase from various microbial sources. The current review offers current data on microbial lipase application in the leather, food, textile, paper, cosmetic, and detergent sectors as well as on the manufacture of lipase from various microbiological sources and from both traditional and contemporary purifying procedures. The current review also demonstrates the application of lipases in biosensors, their production of biodiesel, and their role in bioremediation.

The future research will focus mainly on optimization of lipase-catalyzed reactions methods in consideration of actual production. Microbial lipases are increasingly in demand for application in a variety of industries due to their capacity to significantly improve numerous biotechnology-based production processes. Using recombinant DNA technology, bioinformatics techniques, and other microbiological sources, researchers are focusing on screening and creating novel lipases have unique features. The specificity, productivity, and thermostability of current microbial lipases would be enhanced using novel methods. Additionally, the commercial applicability of lipase can be enhanced by adopting new research concentrating on the biochemistry, source, and application of this enzyme in various applications in industry.

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