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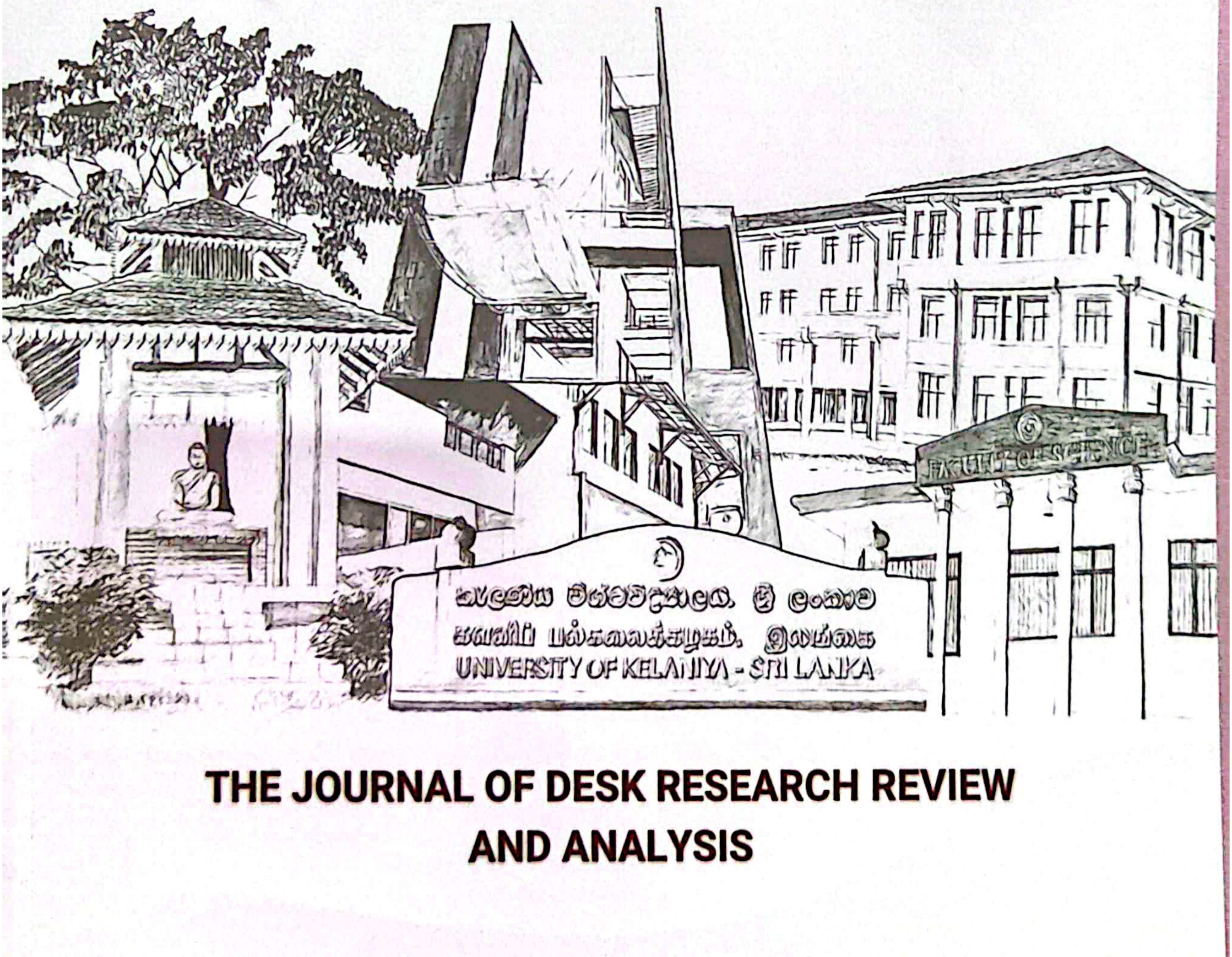

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LIPOLYTIC MICROORGANISMS AND THEIR WIDE RANGE OF MICROBIAL EXTRACELLULAR LIPASES: DIVERSE BIOTECHNOLOGICAL APPLICATIONS

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Abstract

Lipases are economically important, effective biocatalysts widely used in various industries. These enzymes can catalyse the hydrolysis of esters formed from glycerol and long-chain fatty acids. Lipases can be extracted from plant and animal sources, even though they can be synthesised via chemical processes. Currently, microbial enzymes are being used for industrial purposes. There are 150 industrial enzymes with hydrolytic action, including lipase, which is the third largest group of industrial enzymes. Microbial lipases are utilised as biocatalysts in various biotechnological applications due to their stability in organic solvents, broad substrate specificity without a cofactor, and higher enantioselectivity. Microbial lipases are more crucial in biotechnological applications due to their ease of production and environmentally sustainable production ability. Among them, extracellular microbial lipases play a crucial role in the enzyme production industry because of their ease of production and purification. The ability of microorganisms to adapt to extreme environmental conditions has been advantageous in industrial microbiology. The organisms in those environments utilise various substances as their primary energy source, so they possess specific enzymes that break down these energy sources. To utilise microbial lipases in industry, they must withstand harsh environments. The current review provides key information about the diversity of lipolytic microorganisms. These various microbial sources can be utilised to produce lipases, including different types of microbial lipases, their classification, general structure, catalytic activity, and their wide range of biotechnological applications. In addition to the enzyme, this review discusses lipolytic microorganisms, which can be applied to wastewater treatment.

Keywords: Biotechnological Applications, Extracellular Lipases, Hydrolysis, Lipolytic Activity, Microorganisms

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Introduction

Lipase enzymes have a wide range of applications in various industrial processes. Enzymes are becoming increasingly important, with microbial lipases, in particular, holding immense economic value due to their ability to catalyse a wide range of chemical processes in both aqueous and non-aqueous conditions. Microorganisms can produce extracellular and intracellular lipases. Most microbial lipases are extracellular. Lipases can perform hydrolysis of triacylglycerols by converting them into free fatty acids, monoglycerides, diglycerides, and partial acylglycerols. Most lipolytic bacteria can be found in oil-contaminated sites for an extended period. They can produce a variety of lipolytic enzymes, including esterases, true lipases, and phospholipases. A variety of true lipases are produced from both gram-positive and gram-negative bacteria. A more significant part of bacterial lipases is produced by gram-negative bacteria, and the genus *Pseudomonas* contains at least seven lipase-producing species. Many microbes belonging to *Acinetobacter*, *Bacillus*, *Burkholderia, Idiomarina, Natronococcus, Pseudomonas, and Staphylococcus* genera have been described as having lipolytic activity. In addition to bacteria, some fungi and yeast possess the ability to produce lipolytic enzymes. Fungi can produce lipolytic enzymes belonging to various classes, which exhibit broad stability across a pH range of 4.00 to 11.00. The properties of enzymes produced by microorganisms can vary significantly from one organism to another. According to the newest lipolytic enzyme classification, they can be divided into 19 families based on protein sequence and physiological properties of the enzyme. Microbial lipase production and studies related to microbial lipase have increased in the last few decades due to their versatile applications in various sectors, including wastewater treatment, the detergent industry, biodiesel production, plastic biodegradation, lather degreasing, the pulp and paper industry, the food industry, and the pharmaceutical industry. Not only lipases but also lipolytic bacteria are helpful in many industrial processes.

In the environment, lipids are the main component which blocks the drains. Therefore, lipids can cause considerable problems in waste treatment plants. As a solution, wastewater is nowadays pre-treated with lipase-producing bacterial consortia before being introduced into treatment plants. The production of biodiesel using immobilised lipolytic microorganisms is another cost-effective and accessible method than the use of synthetic lipases for biodiesel production. The global microbial lipase market is experiencing rapid growth due to the unique characteristics of microbial lipases, including enantioselectivity, broad substrate specificity, and regioselectivity. Aside from that, any enzyme used in industry should be able to withstand extreme environmental conditions. To address this issue, several studies have been conducted to identify effective, stable, and novel lipases. This review discusses the use of stable and novel lipases for future applications in the growing world.

Industrially important lipolytic enzyme-producing microorganisms

The first discovered lipase was obtained from an animal source, pancreatic lipase, discovered by Claude Bebard in 1848 (de Romo, 1989). Lipases from the *Aspergillus flavus* and *Penicillium oxalicum* were the first microbial lipases discovered in 1935 (Verma et al., 2021). Microbial lipases are more beneficial than animal and plant lipases due to their ease of manipulation, purification, multipurpose applications, the ability for large-scale production, higher activity, stability, and the power of continuous output (Ali *et al.,* 2023). Furthermore, lipases from extreme environmental microorganisms produce unique characteristics, such as thermo-tolerant and cold-active lipases, which are more suitable for industrial applications (Verma *et al.,* 2021).

Synthesising lipase enzymes by a chemical method is challenging and costly. However, most researchers are seeking more stable microbial lipases for commercial applications (Kovaliov *et al.,* 2018). Bacteria, fungi and yeast are the most abundant sources of lipases. Among them, bacteria are the most significant sources of lipases, which are stable, catalyse hydrolytic reactions, and have higher activity (Bharathi & Rajalakshmi, 2019; Panizza et al., 2013). Fungi and yeast species also produce lipases; however, the structure and sequencing of yeast lipases are poorly understood. Many yeast species can produce commercially important lipases; for example, yeast belonging to the ascomycetous class produces commercially important lipases (Gupta *et al.*, 2015). Furthermore, various fungal species, such as *Rhizopus* sp., *Aspergillus* sp., *Penicillium* sp., *Geotrichum* sp., *and Mucor* sp., can produce commercially important lipases. The strain, growth medium composition, cultivation conditions, pH, temperature, and type of carbon and nitrogen sources all affect the amount of lipase produced by filamentous fungal species (Sarikaya, 2004).

Lipolytic enzyme-producing Bacteria

Bacterial lipases could be intracellular or extracellular, but some bacterial types only produce intracellular membrane-bound lipases. For example, *the B. clausii* species produces only intracellular lipases (Lee & Park, 2008). Extracellular lipase-producing bacteria release lipases into the external environment through various secretory systems (Angkawidjaja & Kanaya, 2006). Extracellular bacterial lipases were first discovered in 1901 *in Bacillus prodigiosus, Bacillus pyocyaneus*, and *Bacillus fluorescens* (K.E. E. Jaeger et al., 1994).

Most lipolytic bacteria are Gram-negative, but some Gram-positive bacteria can produce lipases. The *Bacillus* species are gram-positive lipolytic bacteria, and two mesophilic strains, *B. subtilis* and *B. pumilus*, have the smallest true lipase (20 kDa) (Arpigny & Jaeger, 1999). *B. thermocatenulatus* and *B.stearothermophilus* form lipases with identical properties. Their molecular mass is approximately 45 kDa, and they are highly active at pH 9.0 and 65 °C (Schmidt-Dannert *et al.*, 1996). *Streptococcal* enzymes are larger, with a molecular weight of approximately 75 kDa (van Oort *et al.*, 1989). Most bacterial lipases are used in the detergent industry and the synthesis of biodiesel due to their ability to withstand harsh environments. These bacterial lipases should be purified for use in industries, because purity affects the stability, enzyme activity and shelf life of the enzyme (Tomlinson *et al.*, 2018).

General structure and catalytic activity of Lipases

The first lipase structures were discovered in *Rhizomucor miehei* (Brady *et al.*, 1990). Microbial lipases have molecular weights ranging from 20 to 60 kDa, but they are all members of the α/β hydrolase fold protein family (Nardini & Dijkstra, 1999). Hydrolases are a type of enzyme whose activity is determined by the catalytic triad of Serine (Ser), Histidine (His), and Aspartate (Asp) (Faouzi *et al.*, 2015). All three amino acid residues are found in the α/β hydrolases in the order Ser-Asp-His; the Ser residues are all placed in a conserved catalytic Glycine (Gly)-X-Ser-X-Gly sequence. This conserved pentapeptide motif is found in all serine hydrolases and is vital for the catalytic activity of this class of lipases (Schreck & Grunden, 2014). An oxyanion hole is a fundamental feature seen in several enzyme structures (Kumari & Gupta, 2013).

Some microbial lipases possess a structure known as an oxyanion hole. The presence of hydrogen bonds with two amino acids that form the lipase oxyanion hole stabilises the tetrahedral intermediate produced during the catalytic mechanism of lipases (Casas Godoy *et al.*, 2012). Lipases are classified into three kinds based on the preference of the "oxygen anion hole" in catalysing various substrates (Albayati *et al.*, 2020). Most lipases consist of an unstable domain called the "lid structure" (Sarmah et al., 2018). Lipase mechanism actions are mainly described using two steps. There are interfacial activation and catalysis. Lipases have two conformations: 'closed' and 'open'. These two forms are in equilibrium, and the equilibrium favours the closed conformation in solution (Bora *et al.*, 2013).

According to some studies, specific lipases possess a lid structure that features both hydrophilic and hydrophobic faces (Ken Ugo *et al.*, 2017). The hydrophilic face is in touch with the aqueous environment. In contrast, the hydrophilic face is hidden in the protein structure's core. When the interface is introduced (triglycerides), its hydrophobic face becomes exposed. Then the enzyme will switch to a "closed" conformation. Once triglyceride is released into the environment, a signal is sent to the active site, exposing the hydrophobic face (in an open conformation) and allowing the substrate to enter the active site. This will lead to catalysis (Lotti & Alberghina, 2007). Lipase activity depends on the environment or solvent in which it is present and the presence of a suitable conformation. It means the efficacy of interfacial activation depends on the solvent in which they are present. (Priyanka *et al.*, 2019).

Classification of bacterial lipolytic enzymes.

Twenty-four years ago, scientists Arpigny and Jaeger discovered that bacteria could produce different classes of lipolytic enzymes, including carboxylesterases, true lipases and phospholipases (Arpigny & Jaeger, 1999). They present the bacterial classification based on protein sequence and physiological properties. The 3D structure of lipolytic enzymes exhibits a characteristic α/β hydrolase fold and a defined order of α-helices and β-sheets. Initially, Arpigny and Jaeger classified bacterial lipases into eight families (I-VIII) based on the diversity of amino acid sequences and the biological properties of lipolytic enzymes (Arpigny & Jaeger, 1999; Jaeger et al., 1994). Among all those families, family I is the broadest family, and it is called "true lipases". Another name for the family of lipolytic enzymes is true lipases (Ramnath *et al.*, 2017).

Further studies conducted by Hausmann and Jaeger in 2010 revealed the further expansion of bacterial lipolytic enzyme classification. The study revealed that the classification could be further divided into 19 families and eight true lipase subfamilies based on phylogenetic criteria, conserved sequence motifs, and biological functions (Kovacic *et al.*, 2019). Recently, this classification was further divided into 35 families and 11 actual lipase sub-families (Hitch & Clavel, 2019).

Production of Microbial Lipases.

Microorganisms usually produce these enzymes in the presence of a carbon source such as triacylglycerols, fatty acids, hydrolysable esters, Tweens, bile salts, and glycerol (Gupta *et al.*, 2004). Lipidic carbon sources are crucial for high lipase yield, while nitrogen and micronutrients are essential for growth and production optimisation. Alternative media, such as sugars, oils, peptone, yeast extract, malt extract, and agro-industrial residues, fulfil these nutritional requirements (Treichel *et al.*, 2010). The production of lipases from microorganisms, such as bacteria and fungi, requires specific reaction variables, including temperature, pH, substrate, and production medium, which are crucial for isolating and developing commercially valuable products. Numerous fermentation techniques can be used for the production of lipases (Faryad *et al.*, 2020). Fermentation is a well-known method that can be used to convert complex substrates to simpler molecules. The two primary techniques for producing enzymes are solid-state fermentation (SSF) and submerged fermentation (SmF), each with distinct benefits over the other (Rigo et al., 2009).

Microorganisms are suspended in a liquid production medium with precisely controlled amounts of dissolved nutrients as part of the submerged fermentation procedure. Due to its homogeneity and ease of use, it has advantages over various strategies (Aravindan *et al.*, 2007). The SSF procedure is conducted in the absence of moisture and utilises a solid substrate. Therefore, to initiate the proper growth of microorganisms, the substrate should contain the necessary nutritional components and the optimal amount of moisture (Faryad *et al.*, 2020). Another method of lipase production is cell immobilisation. The physical or chemical entrapment of biological agents on a solid matrix, such as an alginate matrix, is a characteristic of the immobilisation approach. The production of lipases from microbes is commonly used to protect microbial cells from extreme environmental conditions, such as temperature fluctuations, varying pH levels, and shear stress (Oliveira *et al.*, 2018).

Applications of microbial lipases and lipolytic organisms

Wastewater treatment

The use of lipases from various sources to treat wastewater from lipid-processing companies, restaurants, and slaughterhouses is a novel method in enzyme biotechnology that prepares the wastewater for standard biological treatment (Adetunji & Olaniran, 2021). Several researchers have studied the potential for treating wastewater containing oil and grease by using physicochemical and biological methods (Abdulsalam *et al.,* 2011; Wong *et al.,* 2007). Oil and Grease constituents could lead to various issues in treatment plants and potentially reduce plant performance. Several problems these constituents might cause include slowing down sedimentation due to the growth of filamentous microorganisms, bulking sludge development, clogging, and foul odours. Therefore, pre-treatment of those oil and grease-containing effluents can reduce the above issues (Karigar & Rao, 2011). Bacterial consortia have been developed to provide optimal inoculum for treating high-intensity oil and grease wastewater. (Mongkolthanaruk and Dharmsthiti, 2002; Prasad and Manjunath, 2011). One example of the interaction between lipase-secreting yeast, such as *Candida cylindracea,* which can assimilate glycerol, and bacteria, such as *Burkholderia arboris*, illustrates the fundamental importance of yeast-bacteria symbiosis in the biodegradation of oil and grease (O&G) (Kanmani *et al.,* 2015).

Not only microorganisms, but also their enzymes, are used to treat wastewater containing fat. In an activated sludge system, the lipases perform an essential role. Lipids in an activated sludge system comprise 30-40 % of the total volume. The effect of adding a lipase-rich enzyme pool to an activated sludge system under high-fat-containing conditions has been studied (Wang *et al.,* 2022). However, the continuous addition of enzymatic preparations is very costly; therefore, scientists suggested using them as a backup strategy during periods of high fat content in the effluent (Damascene *et al.,* 2008). Immobilised enzymes and whole-cell biocatalysts can also remediate fat and oil. Immobilised lipase has contributed to the hydrolysis of O&G components in food manufacturing effluent without pre-treatment; the reductions in COD and O&G were 49% and 45%, respectively. With immobilised lipase pre-treatment, the reduction was 65% and 64%, respectively (Jeganathan *et al.,* 2007).

Lipolytic bacteria immobilised on different matrices were used in a grease trap system for restaurant effluent. When the O&G concentration reaches 5,000 mg/L, the matrix-based trap system eliminates large amounts of O&G and COD (Nisola *et al.,* 2009). Oil and grease-containing wastewater can be converted into precious products by microbial lipase. The lipid contents trapped in taps and other equipment can be recovered, and the recovered lipids can be used in the production of biodiesel through lipase-catalysed esterification or transesterification reactions (Montefrio *et al.,* 2010). Lipase and protease products have been used as a cleaning-in-place approach in dairy businesses to eliminate milk fouling deposits from stainless steel panels (Montefrio *et al.,* 2010). A crucial aspect of bioremediation is that the microbe and enzyme must be able to survive and function efficiently in the field. To address this, new microorganisms and extremophilic enzymes with temperature, pH, or organic solvent tolerance must be identified and applied through comprehensive and gradual screening programs (Ayilara & Babalola, 2023). Several lipase-producing Microorganisms isolated from contaminated environments have been used alone or in consortia to carry out oil and grease bioremediation. (Table 1)

Table 01: Several lipase-producing Microorganisms isolated from contaminated environments have been used alone or in consortia to treat wastewater.

References	(Sarmurzina *et al.*, 2013)	(Hendrasarie N *et al.*,, 2023)	(Zinjarde *et al.*, 1998),(Oswal *et al.*, 2002)	(Zhao *et al.*, 2024)	(M P Prasad and K Manjunath, 2011)
Isolated Site	Industrial grease trap wastewater	Restaurant wastewater.	Oil-polluted marine water.	Crude oil contaminated soil.	Fat and oil-rich wastewater is collected from palm oil mills.
Effect & Action Mechanism	Secretes lipases with high substrate specificity and is helpful in the hydrolysis of triglycerides.	Enhance enzymatic activity, particularly lipase, which can transform complex oil and grease structures into fatty acids and glycerol, playing a key role in the degradation of long-chain oil structures.	Degrade alkanes and lipids, but it avoids the breakdown of lignin and other palm structural components and reduces the amount of fats in palm oil mill effluent. The treated material can be sediment using an appropriate flocculating agent.	It can utilise waste cooking oil (WCO) as a raw material for the synthesis of lipopeptide biosurfactants.	Depending on the lipase source and reaction conditions, it catalyses both hydrolysis and interesterification reactions.

Efficiency of Degrading Lipid-Rich Wastewater	80% decrease in lipid content after 48 hours.	97.42% removal of oil and grease present in restaurant wastewater after 24 hours.	95% COD reduction at a 2-day hydraulic retention period.	75.88 % utilisation rate of waste cooking oil content. Complete lipid degradation occurs after 72 hours.	Within 12 days of incubation, the lipid content decreased from 25,000 mg/L to about 80 mg/L, and the average BOD value decreased from 3200 mg/L to less than 40 mg/L.
Microorganisms/Consortia	*Pseudomonas aeruginosa*	*Lactobacillus* bacterial consortia (*Bacillus sp., Pseudomonas sp., and Lactobacillus sp.)*	*Yarrowia lipolytica* NCIM 3589	*Bacillus subtilis* YZQ-2	*B. subtilis, B. licheniformis, B. amyloliquifaciens, S. marsescens, P. aerginosa, and S. aureus*

Detergent industry

One of the essential and significant applications of lipases is their use as detergent additives in laundry detergents and household products. In most developed countries, enzymes are used for the production of detergents. Removing fat and oil patches from fabrics is challenging, but lipases present in detergents help to hydrolyse fat and oil (Mehendale *et al.,* 2022). Lipase used in detergent production must be robust and compatible under stressful circumstances at a wide temperature range for use in the detergent industry (Thermophiles and the Applications of Their Enzymes as New Biocatalysts, 2019). According to estimates, approximately 1,000 tons of lipases are added to around 13 billion tons of detergent annually (Mehta *et al.,* 2017).

The use of lipase enzymes in detergent production offers several benefits, including reducing washing time and extending the lifespan of fabrics (Ken Ugo *et al.,* 2017). Lipases for detergent manufacturing need to meet the following criteria: (i) ability to hydrolyse fats of various compositions (lower specificity to the substrate); (ii) ability to withstand relatively harsh washing conditions (pH 9.0-11.0, 30-60°C); and (iii) ability to withstand with harmful surfactants (linear alkyl benzene sulfonates) and enzymes (proteases), both of which are significant components of several detergent formulations (Sharma et al., 2001).

Production of biodiesel

Biodiesel, commonly referred to as biofuel, is composed of esters derived from long-chain fatty acids and short-chain alcohols (Kalscheuer *et al.*, 2006). Currently, biodiesel catalysts are classified as alkali, acid, or enzyme. Among these, the use of enzymes which are both environmentally friendly and economical. Lipases are enzymes that catalyse the trans-esterification process, converting fatty acids and short-chain alcohols into methanol (Lotti *et al.*, 2015). The major difficulty with biofuel manufacturing is the chemical synthesis process, which utilises an acid or base as a catalyst. The main disadvantages of this technique are its high energy requirements, soap production, and the generation of environmentally toxic acid- or alkali-based effluent (Noureddini *et al.*, 2005). Utilising microbial enzymes as biocatalysts to produce biodiesel can help overcome these challenges.

Multiple strategies are now being employed to produce biodiesel via lipase-mediated transesterification of oils. Immobilisation is one of them. Immobilisation is a technological procedure that binds enzymes within or on the outermost layer of a solid support, resulting in a heterogeneous enzyme system (Tan *et al.*, 2010). Another method that could be used for biofuel manufacturing via lipases is the whole-cell approach. Although the production of lipases using microorganisms is relatively straightforward, the purification and extraction of the enzyme are complex processes. Therefore, scientists found that the whole-cell approach is more convenient for biofuel production (Fukuda *et al.*, 2001).

Novel lipase-producing *B. subtilis* strains that are isolated from oil-contaminated wastewater have an excellent ability for both the remediation of vegetable oil effluents and for producing biofuels from non-edible plant oils (Rana *et al.*, 2019). Production of biodiesel at low temperatures and the use of biocatalysts have a benefit over chemical synthesis because biodiesel generation at low temperatures decreases the cost and energy consumption of the process (Rana *et al.*, 2019), another study showed that *Microbacterium* sp. could be a valuable source of lipase enzyme, which is employed in the transesterification processes for biodiesel generation (Tripathi *et al.*, 2014).

Biodegradation of Plastics

Polyethene-terephthalate (PET), Polyamides (PA), Polystyrene (PS), Polyvinyl chloride (PVC), Polycaprolactone (PCL), Polyurethane (PUR), and many more types of plastics are being used in everyday life. Most of these synthetic polyester plastics (PoE) are non-biodegradable. Those PoE are disposed of in landfills, which leads to environmental pollution. Therefore, this issue needs an urgent solution (Tserki *et al.*, 2006). As a solution to this issue, bioplastics have been introduced as a clean and green technology, allowing microorganisms to utilise these bioplastics. Thus, plastic degradation mediated by microbial enzymes has been regarded as a potential answer to this global issue (Tokiwa *et al.*, 2009).

Various strategies for the breakdown of polyester-based materials have been researched over time. Lipase-assisted technology exhibits broad substrate specificity and exceptional potential to catalyse the hydrolysis and depolymerisation of polyesters (Amin *et al.*, 2022). *P. protegens* BC2-12, *P. protegens* CHA0, *P. protegens* Pf-5, *P. fuorescens* A506 and Pf0-1, *and P. chlororaphis* are lipase-making bacteria which can biodegrade polyurethanes (PUR) (Chandra *et al.*, 2020). PueB lipase from Gram-negative *P. chlororaphis* was one of the first enzymes discovered to act on PUR (Danso *et al.*, 2019).

PueB lipase from Gram-negative *P. pelagia* (PpelaLip) is used in the in silico genome mining technique (Aiman *et al.*, 2018). *Pseudomonas* sp. significantly degraded polyurethane. *P. aeruginosa* has been shown to produce a large number of extracellular lipases, which aid in the breakdown of aromatic-aliphatic polyesters and polyester amides (Wilkes & Aristilde, 2017).

Leather industry

The leather products sector makes an important contribution to the development of countries that manufacture high-quality leather. The leather industry is one of the sectors that contributes significantly to environmental pollution. Pre-tanning, tanning, and post-tanning are the three crucial steps in the leather industry. It has been observed that the tanning stage, which specialises in turning raw animal hides into leather, is said to be the most environmentally harmful of the three, which account for around 80% of all industrial emissions (Roy, 2012). Pollution occurs due to the use of several chemicals in the leather industry. Some of them are sodium sulphide, salts, solvents (trichloroethane, wine spirit, marlophen), lime, and surfactants (Jayanthi *et al.*, 2019).

To send animal skin for the tanning process, excess fat in the skin should be removed. This step, known as leather degreasing, is part of the pre-tanning process. (Rosa *et al.*, 2017). The tannery effluents released during leather processing cause hazardous effects on humans and animals. Microbial enzymes are used in pre-tanning operations as a solution to overcome those hazardous problems (Kamini *et al.*, 1999). Lipases cannot damage the leather; instead, they only degrade the fat layer. This lipase can remove fat inside the skin structure. Alkaline lipases are used to speed up the liming process (Hasan *et al.*, 2006).

Degreasing is crucial in processing fatty raw materials, such as small animal skins and hides from specific animals. (Moujehed *et al.*, 2022) In many cases, degreasing is performed using aqueous emulsification with detergents or solvent extraction. The solvents used in this process are kerosene, petrol, trichloroethylene and surfactants. Those compounds can contribute to environmental pollution, and they are also highly hazardous to individuals working in the leather industry. (Dixit *et al.*, 2015) The leather sector utilised commercial microbial lipases during the last few years. Some studies have revealed effective microbial lipases that can be utilised in the leather industry (Table 2).

Table 02: Commercially available lipases and other lipases that can be used in the leather industry that contain microbial lipases.

Microbial lipases	Applications in the Leather Industry.	Reference
Microbial Lipase from *Yarrowia lipolytica* (LIP2 lipase)	Efficient in degreasing sheepskins and lowering the quantity of hazardous compounds.	(Moujehed *et al.*, 2022)
Geobacillus thermoleovorans DA2 (Thermo-alkaliphilic lipase)	As an environmentally friendly degreasing chemical, it lowers lipid content and improves leather quality.	(Abol Fotouh *et al.*, 2016)
Microbial Lipase from *Rhizopus nodosus*.	Utilised to degrease suede leather products made from wooled sheep hides.	(Muthukumaran & Dhar, 1982)
Lipase from *Aspergillus niger*	Used for degreasing leather.	(Adetunji & Olaniran, 2021)
NovoCor ABL and NovoCor ADL, NovoLime (a combination of microbial	Used for acid-bating of wool and fur.	(Fact *et al.*, 2020)

acid lipase and acid proteases)		
Lipases from *Aspergillus tamarii* MTCC5152	The enzyme has a 92% fat solubility and can be used in tanning and fleshing applications.	(Dayanandan *et al.*, 2013)
Greasex (microbial alkaline lipase) and NovoCor A (microbial acid lipase)	Used for degreasing leather.	(Thanikaivelan *et al.*, 2004)
Forenzym BT (pancreatic trypsin, bacterial lipase, and protease)	Used for baiting.	(Thanikaivelan *et al.*, 2004)
Forenzym WG-L (bacterial acid lipase), Forenzym DG (bacterial lipase), Forenzym SK (bacterial lipase)	Used for soaking and dehairing.	(Thanikaivelan *et al.*, 2004)

Pulp and paper industry

Certain kinds of timber, mainly pines, contain lipophilic extractives, the majority of which are resin-acidic substances, triglycerides, esters, terpenes, terpenoids, and waxes. Once these chemicals, carbohydrates, and lignin are released into the process fluids during mechanical pulping, they trigger production-related problems in the pulp and paper industry (Rundlöf *et al.*, 2000). Since the 1990s, microbial enzymes have been used in the pulp and paper industry to modify raw starch and control pitch (Bajpai, 1999). Pitch creates unfavourable conditions in the paper production process, but the microbial lipases employed for eliminating pitch have no negative impact on the environment or paper quality (Houde *et al.*, 2004). Lipase for wastepaper deinking can enhance pulping rate and brightness, reduce chemical consumption, increase equipment life, lower pollution, save energy and time, and minimise the cost. Lipase from *Pseudomonas* species (KWI-56) is added to a deinking composition to increase whiteness and decrease remaining ink stains (Nathan & Rani, 2020).

Oil biodegradation

Researchers have discovered that monitoring soil microbial lipase activity is crucial for determining the effectiveness of diesel oil degradation in recently contaminated soils (Riffaldi *et al.*, 2006). Oil spills in coastal areas can be degraded by fungi, which might enhance ecorestoration and enzymatic oil processing in industrial applications (Hasan *et al.*, 2006). The breakdown of petroleum-based hydrocarbons in colder environments, such as Alpine soils, is typically triggered by naturally occurring microbes adapted to cold environments, which can degrade these types of pollutants (Margesin *et al.*, 2003). According to a study carried out by Deive and Sanroma (2010, Shake bottles and a bench-scale bioreactor have both been used to study the biodegradation of waste cooking oil and its use as a booster of lipase synthesis in *Yarrowia lipolytica*. The discovery of effective methods for biodegrading waste oils and fats would be beneficial. Studies on this subject focus on utilising waste oil as a source material to produce biodiesel. They discovered that microbial lipases can be utilised to facilitate these processes (Adamczak *et al.*, 2009). Vegetable oils can be biodegraded by a variety of microbes, including both aerobic and anaerobic ones, but the chemical composition of the oil can influence this process (Pereira *et al.*, 2003).

Food industry

In recent years, the food manufacturing industry has increasingly sought novel, cost-effective, and eco-friendly technologies, particularly in the realm of fat and oil modification (Aravindan *et al.*, 2007). When processing fish meat, lipases are added to remove fat; this process is known as biolipolysis. Additionally, lipases play a crucial role in the fermentation phases of the sausage-making process, as well as in regulating changes in the long-chain fatty acids produced during the ripening process (Seitz, 1974).

Microbial lipases are also used in the dairy industry for the hydrolysis of milk fat. Some of the uses of microbial lipases include enhancing the flavour of cheese, increasing the rate of cheese ripening, lipolysis of butterfat and milk, and producing cheese-like products (Jooyandeh *et al.*, 2009). During lipid hydrolysis, it releases short-chain fatty acids, which help enhance the flavour of milk-based products. Moreover, hydrolysis of medium-chain fatty acids enhances the soapy taste (Hasan *et al.*, 2006). The following biochemistry is responsible for the enhanced flavour of dairy products. When free fatty acids, soluble peptides, and amino acids are formed during the dairy product's maturing stage, the flavours improve faster. Lipases have been utilised to enhance the flavour of coffee whiteners and impart a creamy, buttery texture to toffees and caramel (Beloqui *et al.*, 2008). By using enzymes, bakeries can improve crumb structure, boost and regulate non-enzymatic browning, raise loaf volume, and prolong the shelf life of bread. The enzyme industry provides a range of enzymes that can help with these purposes. Bread and pastry products contain lipases from *A. niger, R. oryzae, and C. cylindricalceae* (Hasan *et al.*, 2006).

Pharmaceutical industry

Microbial lipases can be used to enrich polyunsaturated fatty acids (PUFAs), which are gained from animal and plant lipids (Carvalho *et al.*, 2003). Also, a variety of pharmaceuticals are produced using their mono and diacylglycerides (Ray, 2012). The production of nutraceuticals is another key aspect of this industry. In addition to their routine nutritional value, nutraceuticals (nutritional ingredients) offer a range of health benefits. (Reyes *et al.*, 2022) Some biotechnological applications, such as immobilisation, are used to extract and add these nutraceuticals to common food items. Lipases from *C. Antarctica* and *Lactobacillus ruteri* were used in immobilisation. (Linko & Wu, 1996).

Conclusion and recommendations

Enzymes play a key role in industrial processes as catalysts. Depending on the substrate, enzymes can catalyse any biological or chemical reaction relevant to that substrate. Various types of enzymes are found abundantly in nature as biological catalysts. Lipolytic enzymes can be biologically produced using microorganisms. Those microorganisms can produce intracellular or extracellular lipases. These extracellular lipases are suitable for the bulk production of lipases, which are required for various industries. According to the nature of the isolated microorganism site, the properties of the extracted lipase enzymes can vary. For example, lipases isolated from extreme environments can produce more stable enzymes under extreme conditions, such as high temperatures or acidic pH levels. Although lipases can be produced through chemical methods, there is an increasing demand for microbial lipases. Recently, there has been a significant increase in the commercial use of microbial lipases. In recent years, researchers have carried out numerous studies to identify effective new microbial lipases. Multiple studies were conducted to introduce innovative biotechnological processes utilising novel lipolytic microorganisms. This review provides updated information on the role of microorganisms in enzyme production, industrially important lipolytic enzyme-producing microorganisms, the classification of bacterial lipolytic enzymes, the general structure and catalytic activity of lipase, and the applications of microbial enzymes in various industries, including wastewater treatment, detergent production, and biodiesel production.

As future aspects of enzyme technology, novel microbial lipases can be isolated with broad lipase characteristics. The research focuses on several key areas related to lipase applications. This includes the development of immobilised microbial lipase on various carriers for treating wastewater and other industrial applications, the creation of genetically engineered lipases that are more effective than natural ones, and the combination of lipases with other enzymes for use in the leather industry. Additionally, the development of new bacterial consortia is being explored to enhance the biodegradation of fats and oils. These efforts aim to produce more efficient and effective lipases in future.

Additionally, optimising lipase-catalysed reactions for scaling up processes will be the focus of future studies. Research on the isolation and production of novel lipases with unique characteristics is currently ongoing using bioinformatics tools and recombinant DNA technology. Several studies are focused on genetically modifying and improving microbial lipases to enhance their thermostability, catalytic activity, and solvent tolerance, which are essential for meeting industrial standards. Additionally, studies on commercial lipases can be improved by giving better consideration to the site of isolation, enzymology, biochemistry, and their applications across various industries.

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