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Review article

A review on thermo-alkalophilic bacterial lipase production and its applications

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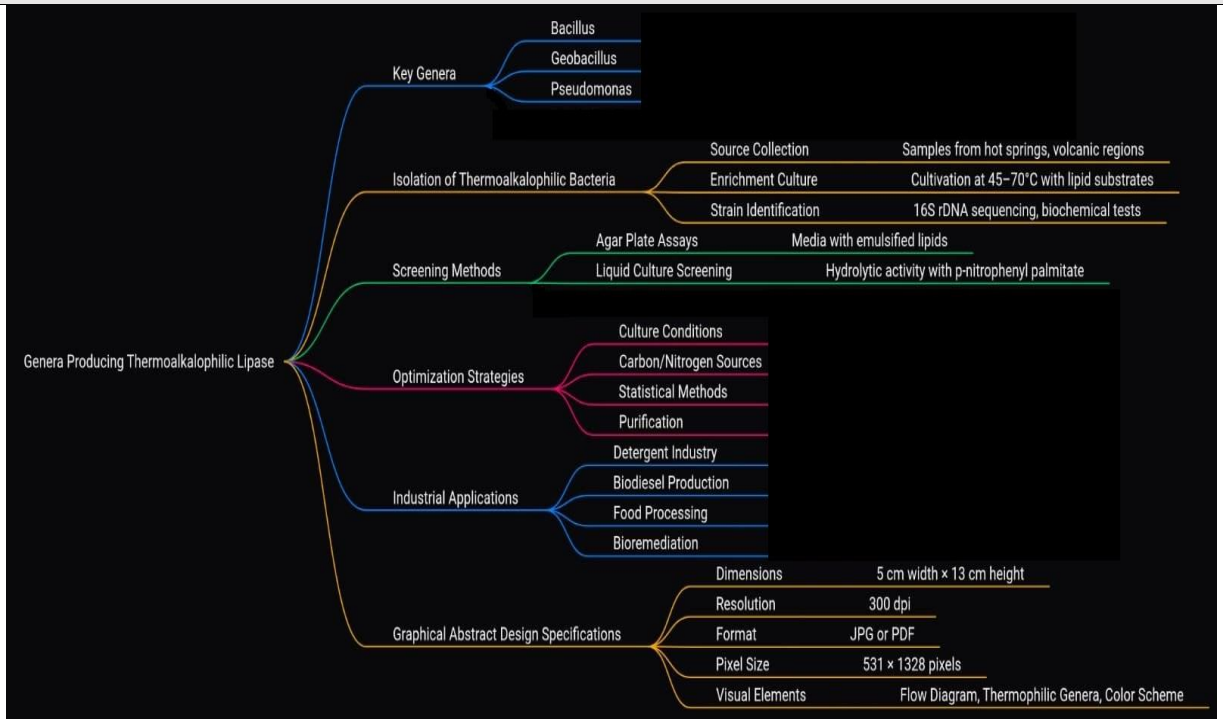
ABSTRACT

Bacterial lipases are dynamic enzymes that catalyze the hydrolysis and synthesis of ester bonds in triglycerides, resulting in the production of free fatty acids, glycerol, and mono- or diglycerides. The versatility of their mechanisms allows them to be used in diverse industrial and biotechnological applications. This review paper provides an overview of the function and structure of lipase enzymes. Thermo-alkalophilic bacterial lipases are characterized by their ability to maintain stability and catalytic efficiency under extreme environmental conditions, including high temperatures and alkaline pH. Also, this paper explained thermo-alkalophilic bacterial lipase production. The most important thermo-alkalophilic bacterial lipase-producing genera, habitat, adaptation, and physiological characteristics are presented. Bacterial lipases hold significant advantages over lipases derived from other sources, making them highly desirable for industrial applications. Isolation, screening, and identification of thermo-alkalophilic bacteria are discussed, as well as optimization and application of their lipase in many aspects of life. Engineering approaches, such as mutagenesis and immobilization, have been employed further to improve the thermal and pH stability of bacterial lipases, making them even more suitable for industrial applications in harsh environments.

Graphical abstract



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1. Introduction

Lipases are enzymes classified under the hydrolase family, primarily known for catalyzing the hydrolysis of triglycerides into glycerol and free fatty acids. This catalytic property makes them indispensable in various industrial and biotechnological applications, including food, detergent, pharmaceutical, and biofuel industries. Unlike other hydrolases, lipases demonstrate a unique property known as "interfacial activation," which enables them to function effectively at the lipid-water interface, enhancing their catalytic efficiency [1]. In addition to hydrolysis, lipases can catalyze esterification and transesterification reactions under specific conditions, such as in non-aqueous environments, broadening their industrial relevance [2].

Lipases are ubiquitously found in all domains of life, including animals, plants, fungi, and microorganisms. In animals, lipases play a critical role in fat digestion and lipid metabolism, while plant lipases are involved in seed germination and oil mobilization. Fungal lipases, such as those from *Rhizopus* and *Aspergillus* species, are widely used in food processing and biotechnology due to their high specificity and stability [3]. However, the most versatile and industrially significant lipases are produced by microorganisms, particularly bacteria and yeast, due to their adaptability and efficient enzyme production systems [2].

The growing interest in bacterial lipases can also be attributed to their vast array of applications. In detergents, lipases facilitate the breakdown of grease and oil stains, even under alkaline washing conditions [4]. In biodiesel production, they catalyze the transesterification of triglycerides, enabling the synthesis of cleaner, renewable energy sources [5]. Furthermore, in pharmaceutical applications, bacterial lipases are employed in the synthesis of enantiomerically pure intermediates, which are crucial for drug development [6].

2. Structure

Lipases are enzymes specialized in catalyzing the hydrolysis of ester bonds in triglycerides, releasing glycerol and free fatty acids. Their active sites contain a conserved catalytic triad, typically composed of serine, histidine, and either aspartate or glutamate residues. These components facilitate nucleophilic attacks on ester bonds, making lipases highly efficient in their catalytic functions [7].

3. Key bacterial genera producing lipases

Prominent bacterial genera known for high lipase production include:

3.1. Bacillus

This genus is one of the most extensively studied due to its ability to produce thermostable and alkaline-tolerant lipases. For example, *Bacillus cereus* lipases have been widely used in biodiesel production and detergents [5].

3.2. Pseudomonas

Lipases from *Pseudomonas aeruginosa* are known for their broad substrate specificity and stability in organic solvents, making them ideal for pharmaceutical and cosmetic applications [8].

3.3. Burkholderia

Lipases from *Burkholderia cepacia* are commonly employed in bioremediation and waste treatment due to their ability to degrade complex fats and oils [9].

4. Thermo-Alkalophilic Bacteria

4.1. Habitat and adaptation

Thermo-alkalophilic bacteria are specialized microorganisms that thrive in extreme environments such as hot springs, alkaline lakes, and industrial effluents. These habitats are characterized by high temperatures and elevated pH levels, conditions that are typically inhibitory to most organisms. Additionally, their proteins and enzymes possess structural modifications, such as increased hydrogen bonding and salt bridges, which enhance thermostability and resistance to denaturation in alkaline conditions [7, 2].

The adaptation mechanisms of thermo-alkalophilic bacteria are also reflected in their enzyme systems, which retain activity and stability under extreme conditions. These adaptations include the presence of highly conserved amino acid sequences that contribute to structural rigidity and mechanisms to minimize oxidative stress. Such features make these bacteria invaluable for industrial applications, where robust performance in harsh environments is often required [10, 11].

4.2. Genera of Interest

Among the thermo-alkalophilic bacterial genera, *Bacillus*, *Thermomyces*, and *Geobacillus* stand out for their biotechnological relevance. These bacteria are renowned for their ability to produce thermostable and alkali-tolerant enzymes, such as lipases, proteases, and amylases, which are vital in various industrial processes. *Bacillus* species, for instance, have been extensively studied for their robust enzyme production abilities, especially lipases and proteases that remain functional at high temperatures and pH levels. Similarly, *Geobacillus* species are known for their efficiency in degrading complex polymers, making them suitable for waste management and biofuel production. On the other hand, *Thermomyces lanuginosus* is a prominent producer of thermostable xylanases and lipases, widely used in the food and biofuel industries [7, 4].

4.3. Physiological characteristics

4.3.1. Heat-resistant spores

These spores allow the bacteria to survive and flourish in environments with extreme temperatures, ensuring their persistence under conditions that would typically denature cellular components in other organisms [2]. The resistant nature of the spore has opened up potential for spores to be used as delivery vehicles for therapeutics [12].

4.3.2. Thermostable cell membranes

The cell membranes of thermo-alkalophilic bacteria are highly thermostable, containing increased proportions of saturated fatty acids and ether-linked lipids. These adaptations enhance membrane rigidity, preventing thermal denaturation and maintaining cellular integrity in high-temperature environments [7, 2]. The stability of the mem-

brane's structure and composition helped it withstand damage from reactive oxygen species. Thermostable membrane's composition can influence the effectiveness of antioxidant systems, both enzymatic and non-enzymatic, which are crucial for neutralizing reactive oxygen species [13].

4.3.3. Alkaline-resistant enzyme systems

Thermo-alkalophilic bacteria possess enzyme systems specifically adapted to function in alkaline conditions. These enzymes often exhibit structural modifications, such as an increased number of disulfide bonds and salt bridges, which enhance their stability in high-pH environments [5]. Lipases active and stable in alkaline media are valuable in various industrial processes, particularly in detergents, where they enhance cleaning performance [14].

5. Thermostability and alkaline tolerance of bacterial lipase

Thermo-alkalophilic bacterial lipases are characterized by their ability to maintain stability and catalytic efficiency under extreme environmental conditions, including high temperatures and alkaline pH. These properties make them highly desirable for industrial applications, such as detergent formulations, biofuel production, and food processing. Lipases from extremophilic bacteria are naturally adapted to be effectively active and thrive under such harsh conditions, which often denature typical enzymes [15].

Thermostability in lipases is primarily attributed to their unique structural features. Studies on *Bacillus thermocatenulatus* and *Geobacillus thermoleovorans* have revealed that these structural adaptations allow lipases to function effectively at temperatures ranging from 60°C to 80°C [16]. This makes them particularly suitable for processes like biodiesel synthesis, where high reaction temperatures are often required to reduce viscosity and improve conversion rates [5].

Alkaline tolerance in bacterial lipases is equally critical, especially in industries where high-pH conditions prevail, such as detergents and wastewater treatment. *Bacillus subtilis* lipases, for example, exhibit optimal activity in the pH range of 8 to 11. This adaptation is facilitated by the stability of ionic bonds in the enzyme's active site, which resist denaturation under alkaline conditions [17].

Studies have also examined methods to enhance thermostability and alkaline tolerance through genetic engineering and directed evolution. For example, mutagenesis experiments on Bacillus licheniformis lipase have led to increased resistance to thermal and alkaline stress, significantly expanding the enzyme's application range [10].

6. Advantages of bacterial lipases

Bacterial lipases hold significant advantages over lipases derived from other sources, making them highly desirable for industrial applications.

6.1. Thermostability and pH tolerance

Many bacterial lipases, particularly those from *Bacillus* and *Pseudomonas* species, are highly thermostable and can retain activity across a wide range of pH levels. These properties are crucial for industrial processes that require enzymes to function under harsh conditions, such as high temperatures in detergent formulations or alkaline environments in bioremediation [18, 15].

6.2. High catalytic efficiency

Bacterial lipases are known for their exceptional catalytic efficiency, enabling them to catalyze reactions rapidly and with high specificity. Their ability to break down a wide range of substrates, including long-chain fatty acids and complex triglycerides, makes them ideal for applications in food processing, biodiesel production, and pharmaceutical synthesis [10, 19].

6.3. Extracellular production

Unlike lipases from plants and animals, bacterial lipases are often secreted extracellularly, simplifying the extraction and purification processes. This reduces production costs and increases scalability, which is a key advantage for large-scale industrial applications [20].

6.4. Genetic modifiability

The genetic makeup of bacteria can be easily manipulated to enhance the properties of lipases, such as improving thermal stability, altering substrate specificity, or increasing production yield. This adaptability makes bacterial lipases highly versatile and customizable for specific industrial applications, such as the development of enzymes for biofuel production or environmentally friendly cleaning agents **[5].**

6.5. Sustainability and eco-friendliness

The use of bacterial lipases contributes to sustainable industrial practices by reducing reliance on harsh chemical processes. Their biodegradable nature minimizes environmental impact, and their ability to work under mild conditions reduces energy consumption in processes such as wastewater treatment and biopolymer synthesis [21, 6].

6.6. Wide Application Potential

Bacterial lipases are highly versatile, with applications spanning industries such as food (cheese flavor enhancement, fat modification), pharmaceuticals (drug synthesis, enantioselective reactions), and energy (biodiesel production). Their robust nature ensures their functionality across diverse industrial sectors, highlighting their indispensable role in modern biotechnology [15].

7. Functional characteristics and enzymatic mechanisms of bacterial lipase

Bacterial lipases are active enzymes that catalyze the hydrolysis and synthesis of ester bonds in triglycerides, resulting in the production of free fatty acids, glycerol, and mono- or diglycerides. The versatility of their mechanisms allows them to be used in diverse industrial and biotechnological applications.

7.1. Interfacial activation

Bacterial lipases exhibit interfacial activation, a unique mechanism where the enzyme becomes active upon encountering a lipid-water interface. In their inactive form, the catalytic site is shielded by a hydrophobic lid. Upon interaction with the lipid interface, the lid undergoes conformational changes, exposing the catalytic triad for substrate binding. This mechanism enables bacterial lipases to effec-

tively hydrolyze triglycerides, which are otherwise insoluble in aqueous environments [1].

7.2. Catalytic triad

The catalytic triad, consisting of serine, histidine, and either aspartic or glutamic acid, is essential to the enzymatic activity of bacterial lipases. These residues work synergistically to break ester bonds in triglycerides. The serine residue acts as a nucleophile, forming a covalent intermediate with the substrate, while histidine and aspartic acid aid in proton transfer to complete the reaction [22].

7.3. Enantioselectivity

Enantioselectivity is a distinguishing feature of bacterial lipases, allowing them to selectively interact with one enantiomer of a chiral substrate. This property is crucial in the pharmaceutical industry, where enantiomerically pure compounds are required for drug synthesis. For example, lipases from *Pseudomonas fluorescens* are commonly used to produce optically active intermediates for anti-inflammatory drugs and beta-blockers [23].

This selectivity is achieved through the unique structural configuration of the enzyme's active site, which recognizes specific molecular geometries. Such precision makes bacterial lipases indispensable in stereoselective reactions, including the production of speciality chemicals and agrochemicals. Their enantioselectivity also finds applications in the synthesis of flavors and fragrances [24].

7.4. Substrate specificity

Bacterial lipases exhibit an impressive range of substrate specificities, enabling them to act on various triglycerides and other ester-containing compounds. This property is beneficial in modifying fats and oils, such as creating structured lipids for nutritional purposes in the food industry [25].

7.5. Activity in non-aqueous media

One of the most remarkable features of bacterial lipases is their ability to retain activity in non-aqueous media. This property significantly expands their utility, allowing them to perform esterification and transesterification reactions in organic solvents. Such reactions are critical in the synthesis of biodiesel, flavors, and speciality chemicals, where water would inhibit the desired reaction [26].

7.6. Thermal and pH stability

Bacterial lipases are well known for their stability under extreme conditions, such as high temperatures and varying pH levels. For example, lipases from *Bacillus stearothermophilus* retain their activity at temperatures exceeding 70°C, making them ideal for processes like detergent formulations and industrial biocatalysis that require high thermal resistance [11].

The broad pH tolerance of bacterial lipases further enhances their versatility. Many lipases can operate effectively in alkaline conditions, which is crucial for applications in detergents and wastewater treatment. Lipases from *Pseudomonas fluorescens* are especially noted for their stability in both acidic and basic environments, allowing their use in diverse industrial processes [10].

The robust nature of these enzymes is attributed to their structural stability and intrinsic properties. Engineering approaches, such as mutagenesis and immobilization, have been employed further to improve the thermal and pH stability of bacterial lipases, making them even more suitable for industrial applications in harsh environments [2].

8. Sequential steps for thermo-alkalophilic bacterial lipase production

8.1. Isolation of thermo-alkalophilic bacteria From extreme environments

Isolation of thermo-alkalophilic bacteria typically begins with the collection of environmental samples, followed by enrichment culture techniques. Samples are incubated in nutrient media adjusted to high temperatures (50–80°C) and alkaline pH (8–12) to promote the growth of thermo-alkalophilic bacteria while inhibiting non-adapted species. Enrichment media often include specific carbon or nitrogen sources that favor the growth of lipase-producing bacteria, such as triglycerides or oils [2, 22].

8.2. Screening for lipase producers

The screening of isolated strains for lipase production involves qualitative and quantitative assays. A typical qualitative method is the use of tributyrin agar plates, where lipase activity is indicated by the formation of clear zones around bacterial colonies due to the hydrolysis of tributyrin. For quantitative screening, enzyme activity is typically measured using spectrophotometric assays with chromogenic substrates, such as p-nitrophenyl palmitate, which release a colored product upon hydrolysis. These assays are performed under conditions that mimic industrial environments, such as high temperatures and alkaline pH, to identify robust lipase producers [25].

8.3. Bacterial identification

Advanced molecular techniques, such as 16S rRNA sequencing, are used to identify the isolated strains, linking their phylogenetic classification to specific enzymatic properties. This step is critical for targeting species with known industrial potential, such as *Bacillus stearothermophilus* and *Geobacillus thermoleovorans* [11, 10].

8.4. Optimization

Once lipase-producing strains are identified, further optimization is carried out to maximize enzyme yield. Factors such as substrate concentration, temperature, pH, and incubation time are adjusted to determine the optimal conditions for production.

8.4.1. Temperature and pH optimization

Thermoalkalo-stable enzymes, especially bacterial lipases, are designed by nature to thrive under extreme operational conditions. The temperature and pH range for their optimal activity typically falls between 50–70°C and pH 8–10, making them exceptionally suited for industrial environments. This combination of thermal and alkaline stability is particularly crucial for industries like detergents and biofuels, where high heat and alkaline conditions prevail. For example, lipases from *Geobacillus thermoleovorans* and *Bacillus subtilis* have been extensively studied for their ability to sustain catalytic activity at elevated temperatures, ensuring uninterrupted reactions during long operational cycles [10].

8.4.2. Incubation period and inoculum size

The incubation period directly influences the growth phase of bacteria and the availability of nutrients. Extended incubation can result in nutrient depletion, accumulation of inhibitory byproducts, and eventual decline in enzyme synthesis. Conversely, a well-defined incubation period ensures that the bacterial culture remains in its exponential growth phase, where enzyme production is at its peak. For instance, studies on *Bacillus subtilis* demonstrated that a 48-hour incubation period provided maximum lipase activity under optimal growth conditions [10].

Insufficient inoculum size can lead to delayed growth and lower enzyme production due to inadequate bacterial populations. Overcrowding, on the other hand, creates competition for nutrients and oxygen, negatively impacting enzyme yields [27].

8.4.3. Carbon and Nitrogen sources

Carbon sources significantly impact the production of Thermoalkalo-stable enzymes such as lipases, as they provide the energy and building blocks required for microbial growth and enzyme synthesis. Lipase-producing bacteria, including *Bacillus subtilis* and *Geobacillus thermoleovorans*, show a preference for oils (e.g., olive oil, soybean oil) as carbon sources due to their ability to induce lipase activity. Oils serve both as inducers and substrates, promoting the synthesis of enzymes tailored for lipid hydrolysis. Glycerol, another widely used carbon source, supports microbial growth while reducing the cost of production, making it a preferred substrate in industrial processes [28].

Nitrogen sources are equally crucial in enzyme production, as they contribute to the synthesis of proteins, including enzymes. Organic nitrogen sources such as peptone, yeast extract, and casein hydrolysate are widely used for their ability to support bacterial growth and enzyme secretion. For instance, peptone provides a readily available source of amino acids and peptides, which are essential for microbial metabolism and lipase biosynthesis. In contrast, inorganic nitrogen sources, such as ammonium sulfate or nitrate, are less effective for certain bacterial strains but can complement organic sources when used in combination. Research on *Bacillus stearothermophilus* revealed that combining yeast extract with ammonium nitrate maximized lipase activity and yield [10].

8.4.4. Metal ions and cofactors

Metal ions play a crucial role in stabilizing the structure and enhancing the activity of lipases, particularly those derived from thermoalkalo-stable bacteria. Calcium ions (Ca^{2+}) are among the most commonly studied metal ions for their stabilizing effects on lipase conformation. These ions bind to specific regions on the enzyme, reducing structural fluctuations and ensuring optimal catalytic activity [11]. Similarly, magnesium ions (Mg^{2+}) have been reported to improve the binding of lipases to their substrates, thus increasing hydrolytic efficiency [29].

Cofactors, which include organic molecules and metal ions, are vital for lipases' functionality. These cofactors participate directly in enzymatic reactions by assisting in substrate binding or by stabilizing the transition states during catalysis. For instance, lipases that require cofactors such as ATP or NADH often exhibit enhanced catalytic rates and improved thermal stability [26].

8.5. Purification techniques

The process generally involves a series of steps to isolate and concentrate the enzyme while removing unwanted impurities.

8.5.1. Centrifugation and Precipitation

The first step in lipase purification often involves centrifugation to separate the cell-free supernatant containing the extracellular lipase. Following this, precipitation methods such as ammonium sulfate precipitation are used to concentrate the enzyme. This step exploits the solubility differences of proteins, allowing the lipase to be selectively precipitated. For example, in studies with *Bacillus subtilis*, 60–80% ammonium sulfate saturation resulted in significant enzyme recovery with minimal protein contaminants [27].

8.5.2. Chromatographic Techniques

After precipitation, chromatographic techniques are employed for further purification. Ion-exchange chromatography separates proteins based on charge differences, while gel filtration chromatography separates them based on molecular size [26].

8.5.3. Ultrafiltration and Electrophoresis

Ultrafiltration is another technique used to concentrate lipase and remove small molecules. To assess purity, SDS-PAGE electrophoresis is commonly employed, revealing the molecular weight of the enzyme and ensuring that contaminating proteins are minimized [30].

9. Lipase characterization

9.1. Thermal stability

Thermo-alkalophilic lipases exhibit exceptional stability at elevated temperatures, typically within the range of 50°C to 70°C, with some even maintaining activity beyond 80°C. This high thermal stability is attributed to their robust molecular structure, which resists denaturation at extreme temperatures [11].

9.2. pH stability

These lipases work efficiently across a broad pH spectrum, especially in alkaline environments. Most thermo-alkalophilic lipases exhibit optimal activity between pH 8 and 10, and many maintain stability over a range of pH 4 to 11. For example, lipases from *Thermomyces lanuginosus* and *Geobacillus stearothermophilus* are noted for their strong performance under alkaline conditions [10, 2].

10. Applications or trends of lipase

10.1. Detergent industry

Lipases enhance the cleaning power of detergents by breaking down oil and grease stains. Their stability in alkaline conditions makes them ideal for laundry and dishwashing applications. This capability is particularly effective for treating stubborn oil and grease stains on fabrics. Their enzymatic action complements other detergent components, reducing the need for harsh chemicals and thereby offering an eco-friendlier alternative for cleaning [2, 4].

10.2. Food industry

Lipases play a crucial role in the dairy industry, especially in cheese ripening. They hydrolyze milk fat into free fatty acids, which serve as precursors for various aromatic compounds. These compounds enhance the characteristic flavors of aged cheeses like Parmesan, Gouda, and Roquefort. Microbial lipases, particularly those from Rhizomucor and Candida species, are frequently used due to their high activity and specificity, ensuring consistent flavor profiles [4, 31].

10.3. Pharmaceutical industry

Lipases are extensively utilized in the pharmaceutical industry to produce enantiomerically pure compounds, which are crucial in drug formulations. Their capacity to selectively catalyze reactions involving specific enantiomers guarantees the creation of optically pure intermediates. For instance, lipases are employed in the synthesis of chiral drugs such as ibuprofen and naproxen. The high enantioselectivity of lipases from Pseudomonas fluorescens and Candida antarctica has rendered them essential for these applications [29, 6].

10.4. Biodiesel production

Lipases play a pivotal role in biodiesel production by catalyzing the transesterification of triglycerides into fatty acid methyl esters and glycerol. This reaction involves converting oils or fats into biodiesel using alcohols such as methanol or ethanol. Compared to traditional chemical catalysts, lipases provide several advantages, including mild reaction conditions, high specificity, and reduced formation of by-products. Lipases from species like *Candida antarctica* and *Pseudomonas fluorescens* have demonstrated exceptional efficiency in biodiesel production processes [10, 26].

10.5. Bioremediation

Lipases play a key role in the bioremediation of lipid-rich industrial effluents, such as those produced by food processing, dairy, and oil refining industries. These enzymes break down fats and oils into glycerol and free fatty acids, considerably lowering the chemical oxygen demand (COD) and biological oxygen demand (BOD) of wastewater. For example, lipases from Bacillus subtilis and Pseudomonas aeruginosa have been effectively used in treating oily wastewater, supporting sustainable waste management practices [5, 17].

11. Conclusion and prospects

Lipases from thermo-alkalophilic bacteria are outstanding biocatalysts with broad industrial uses, thanks to their stability and activity under harsh conditions. Their capacity to endure high temperatures and alkaline settings makes them essential in sectors like detergents, food processing, pharmaceuticals, and biofuels. Progress in strain isolation, fermentation techniques, and enzyme engineering has greatly boosted lipase production and effectiveness, leading to more cost-efficient and sustainable industrial processes. However, challenges remain in scaling up production, lowering costs, and enhancing enzyme stability during storage and use. Future research should aim to discover new bacterial strains, improve fermentation methods, and engineer lipases with greater specificity and durability. Overcoming these obstacles will allow thermo-alkalophilic bacterial lipases to continue advancing industrial biotechnology and addressing environmental issues. Their environmentally friendly and sustainable applications highlight the increasing importance of enzymatic processes in creating a greener future.

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