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Mini Review

Marine thraustochytrids a potential source for lipase: Challenges in industrial applications

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Marine microorganisms still belong to untapped natural resources and can harness a rich source of lipase enzymes with the potential industrial applications. Thraustochytrids are heterotrophic fungus like protists that can dissolve organic matters via enzymes. It has been less explored so far. Lipase is one of the most widely used enzymes, crucial to many biotechnological and industrial processes, including the food, paper, and oleochemical industries, as well as in applications related to pharmaceuticals. However, its application is relatively expensive and challenging due to its instability and aqueous solubility. Immobilization is a commonly employed strategy to enhance lipase activity, and it has proven to be a successful approach. In comparison to free lipase, immobilized lipase on nanomaterials (NMs) has demonstrated superior properties, including greater pH and temperature stability, a longer stable duration, and the ability to be recycled. However, under specific circumstances, protein loading is comparatively decreased and lipase immobilization on NMs might also occasionally result in activity loss. The overall performance of immobilized lipase is influenced by the NMs types and properties. This review addresses thraustochytrids potential for lipase production, emerging extraction techniques employing nanomaterials, and the significance of various techniques for lipase immobilization. The immobilized lipases' potential for several applications has also been taken into account.

Keywords: Immobilized lipase, Nanomaterials; Extraction; Lipase activity

Introduction

Lipases catalyze the hydrolysis of lipids, play a crucial role in various biological processes and have garnered significant attention for their industrial applications. In recent years, lipases sourced from marine thraustochytrids have emerged as promising candidates due to their unique characteristics and adaptability to extreme environments. Thraustochytrids are marine protists known for their ability to produce high levels of lipids and lipase enzymes[1]. According to Marchan *et al.*[2], heterotrophic thraustochytrids breaks down dissolved organic materials more efficiently by enzymatic means than closely related autotrophic microalgae. Thraustochytrids are known to secrete a variety of hydrolytic exoenzymes, such as protease, lipase, cellulase, amylase, and xylanase. These enzyme functions include the breakdown of macromolecules like urease, polysaccharide hydrolase, and protease. Their ability to break down complex organic matter is facilitated by their enzymatic repertoire, which is essential for recycling nutrients. *Thraustochytrium* sp. also produces alkaline lipases that are able to hydrolyze long-chain triglycerides, finding various applications in the detergent, cosmetic, and food industries[3]. Some species in this group have the extraordinary ability to break down crude oil and tar balls[4]. These lipases being derived from marine resources could exhibit remarkable stability and activity under conditions such as high salinity, pressure, and temperature, making them particularly valuable for industrial applications, especially in the production of biodiesel, food processing, and pharmaceuticals.

Remarkably, a number of thraustochytrid strains have been effectively grown in order to produce

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useful functional chemicals. Omega-3 fatty acids, mainly docosahexaenoic acid (DHA), are among their important products. For DHA, thraustochytrids provide a sustainable substitute for conventional sources such as krill and wild fish[5]

These heterotrophs can accumulate carotenoid pigments and lipids, especially omega-3 fatty acids like DHA and EPA, along with other saturated and unsaturated fatty acids, in addition to their enzymatic function[6].

Thraustochytrids closely related to primitive fungi and protozoa. However, with the advancement of molecular techniques, the organisms were placed among heterokonts and classified to the family Thraustochytridae of the order Thraustochytrida in the class Labyrinthulomycetes. Currently, 12 genera of thraustochytrids have been found to be culturable which include *Aplanochytrium*, *Aurantiochytrium*, *Botryochytrium*, *Japonochytrium*, *Labyrinthulochytrium*, *Monorhizochytrium*, *Oblongichytrium*, *Parietichytrium*, *Schizochytrium*, *Sicyoidochytrium*, *Thraustochytrium* and *Ulkenia*[7].

Thraustochytrids, which are isolated from mangroves, are mainly obtained from decaying leaves and seawater where soluble carbohydrate permeated from the leaves may be used by the heterotrophic organisms, and the flocculent debris may be ingested directly. Microorganisms, including thraustochytrids, are able to degrade refractory organic materials to produce protein- and lipid rich compounds, suggesting their survival strategy in the mangrove habitat[8].

Similarly, to other heterotrophic protists, thraustochytrids utilize both DOM and particulate organic matter (POM) as nutrient sources. This indicates that the plants (e.g., phytoplankton, sea grass and algae) seawater and sediment of coastal habitats globally may be ideal substrates for the attachment of thraustochytrids[9].

Despite the immense potential of lipases from marine thraustochytrids, their industrial application faces several challenges. One significant hurdle is the limited understanding of the enzymes' biochemical properties and the lack of efficient large-scale production methods. Additionally, the industrial use of lipases is hindered by issues related to enzyme stability, substrate specificity, and the cost-effectiveness of their production. Immobilization techniques, which involve fixing enzymes onto solid supports, offer a promising avenue to address these challenges. Immobilization enhances enzyme stability, facilitates easy recovery and reuse, and often allows for better control of reaction conditions. However, the selection of suitable immobilization techniques and supports remains a critical aspect that requires careful consideration to optimize the performance of thraustochytrid-derived lipases in various industrial processes. Overcoming these challenges is essential for unlocking the full potential of thraustochytrid lipases and advancing their integration into diverse industrial applications.

This review explores the production of lipase enzyme from the marine thraustochytrids via bioprocess parameters engineering. The enzyme is immobilized with nanomaterial for the enhancement of its activity, stability and reusability. The novelty of the review is to employ marine thraustochytrids for the lipase production, which is a recent advancement when compared to other lipase producing microorganisms like bacteria, fungi and pseudomonas.

Furthermore, the application of lipase for sustainability and environmental consideration and other industrial purposes was also explored. It is important to note that currently, there are very few reports on lipases from thraustochytrids.

Lipase production using thraustochytrids

Thraustochytrids have been majorly employed for omega fatty acids, carotenoids and lipase production. Lipase is an important enzyme which is mostly produced via fungi as they produce it extracellularly. An account of lipase production via thraustochytrids and fungi has been given in Table 1

Media components essential for thraustochytrids growth

Thraustochytrids requires glucose-yeast extract-peptone media containing glucose (5%), yeast extract (2%), peptone (2%) and artificial sea water (50%v/v). Since these are marine microorganisms' sea water or salt is required for better growth. The incubation conditions could be lower temperature such as 20°C as are better adapted to marine sea surface temperature and agitation is also required for exponential growth phase[10]. Usually, for the production of lipase from thraustochytrids natural sea water (NSW) was used[5]. For enhanced lipase production ASW (Artificial Sea water) can be used which comprises of 30g of NaCl, 10.8g of $MgCl_2$ $6H_2O$, 5.4g of $MgSO_4$ $7H_2O$ and 1.0g of $CaCl_2$ $2H_2O$ which reflects closely composition of seatwer[19].

Table 1 — Lipase production by thraustochytrids and fungi

Organism	Production media	Inducers	Parameters		Enzyme activity (U/mL)
			pH	Temp. (°C)	
Thraustochytrids					
Thraustochytrium[3,10]	GYEP media	Olive oil, Tributyrin	-	20	0.2
Thraustochytrium Schizochytrium[11]	GYEP media	3.4% crude sea salt, 0.5% Olive oil	6	-	42.4±3.7*
Aurantiochytrium Schizochytrium S31[11]	GYEP media	Tween 80	5	25	38
Thraustochytrium Schizochytrium[12]	Modified Vishnaic media	Tween 80	7.3	-	25
Fungus					
Aspergillus niger MTCC2594[13]	wheat bran and gingelly oil cake	Addition WB: GOC 3:1	-	30	384.34
Penicillium wortmanii[14]	Mineral medium	5% (w/v) Olive oil	7	45	12.5
Aspergillus niger[15]	Basal medium	Rice husk, red gram husk, cotton seed cake	6	40	24.38**
Aspergillus niger[16]	Rice bran and Jatropa seed cake	1% of NaCl and 0.5% Tween 80	7.7	37	282
Aspergillus ibericus[17]	Malt extract media	Olive pomace, Wheat bran	-	30	20.8±1.0
Penicillium restrictum[18]	Basal medium with babassu cake	2% Olive oil	5.8		30.3

[*IU/mL] [**U/gds]

Factors influencing lipase production via thraustochytrids

Carbon source

Generally, lipase-encoding genes are activated for the production of microbial lipase. An important factor in production across different microbial sources is carbon supply. Mostly, glucose serves as carbon source for microbial species but interestingly, Tween 80 which acts as an inducer also improved the biomass, lipid and the production of lipase from thraustochytrids[20]. Thraustochytrids are known to use numerous carbon sources and glucose is most common for cultivation, other substrates like glycerol and galactose also result in distinct growth and product profile[11].

Various inducers like olive oil, triolein, sodium lactate, oleic acid and palmitic acid are known to increase the production of lipase. Palmitic acid was the best inducer for lipase production by *Candida rugosa* upto five-fold when compared to glucose[21]; however, it must be tried on thraustochytrids as well. It is noteworthy that these are readily available inducers and are reasonably priced. These are frequently used in addition to plant-based oils to improve lipase production[20].

Nitrogen source

Different organic and inorganic nitrogen sources significantly increase lipase production across a range of microbial species, indicating that nitrogen is a critical component regulating lipase production. It has been demonstrated that adding yeast extract to the lipase culture medium is more effective in stimulating lipolytic activity in *Thraustochytrium* sp.[11] Similar to this, Thraustochytrid sp. has been used to produce lipase by combining peptone with corn steep liquor[21] and urea is also used as a promising nitrogen source for production of lipase from *Thraustochytrium* sp.[22].

Temperature

In the shake flask method, the ideal temperature is crucial for enzyme secretion. For Thraustochytrids 20°C is where lipase biomass concentrations are highest[23]. Based on scientific observations, lipase synthesis is enhanced by 3-fold with little temperature increase of up to 30°C[3].

pH

Generally, pH for the production of lipase from thraustochytrids are higher in acidic pH. It has been demonstrated that with more acidic medium pH-6 there is 3-fold increase in the production of lipase[3].

Usually the enzymes are intracellular, hence, its extraction has a major implication in the overall development of the downstream bioprocess. Being protein, lipase stability is the utmost important aspect to be careful about while doing extraction from within the cells.

Conventional extraction methods

Mechanical approaches

Bead milling, high-speed homogenization, and high-pressure homogenization are examples of mechanical techniques that enable the application of external forces, such as solid and liquid shearing, to disrupt cells in alternative ways. Other mechanical techniques include heat, as in thermolysis and autoclaving, or involve energy transfer using methods like ultrasonication, microwaves, and lasers. Pulsed electric fields can also be used as current[24]. To determine the most suitable method for thraustochytrid cell disruption, factors like the nature of biomolecules, scalability, input energy, cell wall composition and biomass concentration should be considered.

Bead milling / Bead beating

Bead milling involves the grinding and dispersion of particles into macro or nano sizes using specialized machines, this method involves disruption of cells through the application of force generated by collisions between the cells and beads. A rotating shaft in the grinding chamber facilitates these collisions, the efficacy of cell lysis through bead milling is primarily influenced by the bead load and their diameter, selecting glass or ceramic beads allow for multiple operations within the chamber with zirconium and glass being used as high and low-viscosity media respectively[25].

High-speed homogenization

High-speed homogenization (HSH) is a highly efficient and straight forward technique utilized for disrupting thraustochytrid cells, leveraging dynamic cavitation assistance. The biomass slurry undergoes stimulation within a precise apparatus comprising a stator-rotor assembly, where the gap is typically maintained as small as 100-300 µm[26]. Typically constructed from stainless steel, the HSH equipment features stators and rotors designed in a versatile manner. The recommended operational time is specified as 30 and 60 seconds at speed of 10000 and 140000 rpm. Using a high-shear mixer in *Aurantiochytrium* sp. wet biomass, HSH has been used to disrupt cells and extract lipids simultaneously. Notably, 80% of the lipids were removed in 10 minutes at a speed of 15,000 rpm[27].

Microwave irradiation

Microwave irradiation (MI) is a sustainable technique commonly used in organic synthesis. MI represents a straight forward and scalable approach for disrupting thraustochytrid cells. Electromagnetic disruption of cell walls occurs through MI, which interacts with dielectric and polar molecules generating heat as a consequence. Parameters such as 90 seconds duration, 800 W power and a frequency of 2450 MHz were optimized for the heating, drying and disruption of thraustochytrid using MI[12].

Ultrasonication

Sonic wave sequences are rapidly compressed and decomposed during the ultrasonication process. This continuous process creates cavitation inside the cell, which results in the formation of liquid vapor referred as microbubbles. The movement of the liquid molecules is caused by acoustic waves. Depending on the ultrasound's intensity, microbubbles compress and then collapse, producing heat, high pressure, shockwaves, free radicals, and eventually the disintegration of cell walls[28].

Sonication is a successful technique for extracting intracellular bioactive substances, such as lipolytic enzymes, alcohol dehydrogenase, fructosyltransferase, galactosidase, and alkaline phosphatase[29]. Sonication time can be adjusted to find the ideal sonication duration for maximizing lipase activity and protein release. The following formula can be used to quantify the enzyme released:

$$R=Rm[1-e^{-kt}]$$

Where K is the sonication disruption constant, t is the sonication period, and R and Rm are the maximum enzyme activity that can be released and the released enzyme activity, respectively.

Emerging extraction techniques for lipase

Table 2 gives an account of advanced extraction techniques for lipase.

Polymer/salt aqueous two-phase partitioning system

Since chemical costs dominantly influence separation and purification processes, the adoption of a cost-effective polymer/salt aqueous two-phase system (ATPS) has become a prevalent choice for commercial application[38]. In the PEG/salt ATPS, the selection of a phase-forming salt plays a crucial role in facilitating the separation and extraction of target molecules between the phases[39]. To ensure extraction efficiency various phase forming salts are employed in the partitioning process. These salts possess the capability to modulate hydrophobic interaction among biomolecules within ATPS. The utilization of different salts alters the enzyme’s surrounding environment, leading to variations in partition behavior[30].

Generally, in an aqueous two-phase system (ATPS), it is necessary for the two phases to have equal osmotic pressure and be electrically neutral. The introduction of salt to the polymer solution leads to an uneven distribution of cations and anions

Table 2 — Emerging advanced extraction methods for lipase enzyme

Source	Material 1	Material 2	PF	Yield (%)
Rhizopusmicrosporus[30]	PEG-(2000) (20%)	$(NH_4)_2SO_4$ (12%)	-	92.3
Polymer/Polymer				
Burkholderia pseudomallei[31]	PEG-(8000)	Dextran T500	2.44-fold	92.1
Thermo-separating polymer/Alcohol/salt				
Burkholderia cenocepacia ST8[32]	EOPO 3900	Phosphate	14-fold*	99
Alcohol/Salt				
Burkholderia pseudomallei[33]	2-Propanol (16%)	K_3PO_4 (16%)	13.5-fold	99
Surfactant-Alcohol				
Cucurbita moschata[34]	Triton X-100 (24%)	Xylitol (20%)	16.4	97.4
Leucosporidium scottii L117[35]	TritonX-114	McIlvaine buffer	4.77	88.94
Rhizopusniveus[36]	PEG-(4000) (32.5%)	Na_2Co_3 (18.4%)	19.3-fold	92.8
Penicillium cyclopium[372]	PEG-(4000) (15%)	K_3PO_4 (70%)	9-fold	95.7

[*Kp: Partition coefficient; PF: Extraction performance factor]

between the phases, determined by their affinity for the aqueous phase. This asymmetrical distribution generates an interfacial potential, facilitating the portioning of biomolecules, especially proteins. The effectiveness of salt in inducing phase separation is illustrated in the lyotropic series, a classification of ions based on their capacity for salting-out or salting-in[40].

Polymer / polymer aqueous two-phase partitioning system

The polymer/polymer system is an additional type of aqueous two-phase system (ATPS) in which polymers are a component referred as a phase forming polymer used for separation which not only provides good stability to proteins but also makes them compatible in high water content. For example, PEG 4000 (polymer) and dextran (polymer) were commonly used for extraction of enzymes[29]. Because this system has a high-water content (>90%), it not only gives proteins stability but also improves compatibility. Beyond a critical concentration, two mutually incompatible polymers typically form two-phase systems in aqueous solutions[41].

Alcohol/salt based aqueous two-phase partitioning system

The target biomolecules often accumulate in either the polymer-rich phase or the salt-rich phase in both polymer/salt and polymer/polymer systems. It is an extremely complex method to recover biomolecules from phase-forming chemicals/polymers[31]. It is commonly known to entail laborious purification procedures like ultrafiltration and crystallization, which are frequently required to extract the required proteins from these traditional aqueous two-phase systems. These systems typically require additional sophisticated processes or the back-extraction of the target biomolecule into the salt-rich bottom phase in order to remove the phase-forming components from the target product. Furthermore, proteins (enzymes) that are poorly soluble in aqueous solutions frequently accumulate in polymer/salt combinations, which leads to the depletion of enzymes. Furthermore, according to Li and group[32], recycling bottom phase producing salt is a difficult operation that uses a lot of chemicals and polymers, which pollutes the environment and raises operating expenses. As a result, micellar chemicals, alcohols, and surfactants have been used to create sophisticated and enhanced ATPS.

Surfactant/alcohol based aqueous two-phase partitioning system

A revolutionary approach to aqueous two-phase systems (ATPS) based on surfactants and alcohols, particularly sugar alcohols, is a recent innovation. Particularly than other traditional ATPS, this method shows the ability to maintain the biological activity of enzymes. Two phases can be produced by the system: a recyclable bottom alcohol phase and a top phase that is rich in surfactants[33]. Membrane-associated proteins have been solubilized using non-ionic surfactants without sacrificing their biological function. A soluble protein-surfactant mixed micelle is created during solubilization when the non-ionic surfactant displaces more lipid molecules in contact with the hydrophobic region of the protein[42].

Thermoseparating polymer-based aqueous two-phase system

Thermoseparating polymers (TSPs), which have a high-water content (70–90%w/w), have been used to create an improved and sophisticated aqueous two-phase system (ATPS). This mixture offers a mild environment in which fragile biomolecules, mostly enzymes, can be separated. In order to minimize the possibility of salting out and protein precipitation, as well as to avoid enzyme denaturation, thermoseparating requires less phase-forming components for the creation of two phases[43].

Additionally, a high interfacial contact area promotes effective mass transfer, which speeds up and refines phase separation at ambient temperature. Furthermore, thermoseparating generally uses safer, non-flammable, non-toxic, and environmentally beneficial components. According to laboratory data, thermostability ATPS not only makes phase separation and biomolecule portioning simple, but it can also manage huge quantities and cut down on further purification stages, which makes large-scale purification predictable and dependable[44]. Thus, thermostable ATPS appears as a viable solution to the industrial need for a separation method that is highly efficient, large-scale, affordable, and has a short processing time[45].

Lipase activity enhancement with nano-materials immobilization

Activated carbon from palm raceme was successfully employed as support material for the immobilization of lipase enzyme. A novel innovative method entailed employing an alanine-based deep eutectic solvent to augment enzymatic activity. A range of AC (activated carbon) samples were subjected to varying conditions, and upon reaching the optimal treatment parameters (impregnation mass ratio of 0.5, 150 min, and carbonization temperature of 400°C), the enzymatic activity escalated to 162.5%. Additionally, by modifying the incubation temperature (60°C) and water content (20-40%), the relative enzyme activity increased by of three-seven folds. These results indicated that the immobilizing technique employing AC and DES is a perfect option for possible application, especially in biotechnological applications[34].

Employing physical adsorption and subsequent cross-linking with glutaraldehyde (GA), *Candida antarctica* lipase (CAL-B) was successfully immobilized on silica nanoparticles, producing cross-linked immobilized lipase (CLIL). The optimal conditions were achieved with 1.28 mg/mL lipase concentration, 25°C temperature, 120 min adsorption period, 0.01% GA (V/V) in 7.5 mL, and 120 min cross-linking. The maximum recovery activity was seen with 87.82±0.07%, CLIL[38]. Microscopic analysis (SEM and CLSM) confirmed that lipase was successfully immobilized on the surface of silica nanoparticles. The combined physical adsorption and cross-linking approach proved to be a simple, affordable method that increases the activity and stability of lipase, offering potential applications for lipase industrialization[46]. This study suggested that the CLIL obtained by cross-linking after adsorbing lipase on silica nanoparticles is a feasible method.

Immobilization on magnetic nano-particles was studied where, aldehyde groups were added to the surface of nano-sized Fe3O4 by modifying it with 3-aminopropylethoxysilane (APTES) and glutaraldehyde (GA) through chemical co-precipitation. As a surfactant, sucrose esters-11 were used for "interface activation." Effective immobilization of *Rhizopus oryzae* lipase on the carrier led to a significant increase in activity. In comparison to the free enzyme, the immobilized lipase exhibited hydrolysis activity 9.16 times and 31.6 times higher when p-nitrophenol butyrate and p-nitrophenol palmitate were utilized as substrates. Additionally, the immobilized enzyme showed improved esterification activity and thermal stability when compared to the free enzyme, suggesting that it has great potential for industrial application[47].

Industrial applications of lipases

Major industrial applications of lipases are presented in Table 3.

Table 3 — Industrial applications of lipases

Application	Description
Detergent Industry[48]	Lipase remove lipid-based stains, improve efficacy of laundry detergents, breakdown fatty acids and triglycerides in stains
Biofuel production[49]	Lipase employed in biodiesel production by catalyzing the transesterification of triglycerides found in vegetable oil or animal fats into biodiesel and glycerol
Textile industry[50]	Lipase contributes in removal of unwanted fibres from fabrics, biostoning process which enhances the softness of the fabrics
Leather Industry[51]	Lipase utilized in leather processing for dehairing, degreasing and bating for removal of non-collagenous proteins from animal hides.
Waste management[52]	Lipase utilized in the treatment of industrial effluents, organic waste such as composting or biogas production where they employed in breakdown of organic matter.
Pharmaceuticals[53]	Lipase plays an important role in pharmaceuticals for the synthesis of pharmaceutical intermediates production of enantiopure drugs and formulation.
Bioremediation[54]	Lipase utilized in bioremediation for degradation of lipid rich pollutant such as oil spills and industrial effluents.

Synthesis of biodiesel

Population expansion is posing problems to the world's energy supply, requiring the development of alternate methods for generating energy as they mostly rely on gasoline, conventional transportation system raise environmental problems and pollution.

As a carbon-neutral fuel biodiesel namely fatty acid methyl esters and fatty acid ethyl esters is a product of conversion of oil/fat with short chain alcohol. So, as compared with traditional approaches, enzymatic conversion utilizing lipase as biocatalyst especially immobilized lipases are becoming the preferred method of producing biodiesel due to their process efficiency and being sustainable attracting a lot of interest in biotechnology research[55].

The procedure for producing biodiesel have been considerably improved by the use of nanomaterials for lipase immobilization serving as the perfect supporting material. It offers the advantages such as increased mass transfer, improved enzyme loading and enhanced dispersibility. Recent developments include the application of several nanomaterials for immobilization of lipase like carbon nanotubes, nanoflowers, metal organic frameworks, as well as magnetic nanoparticles, nano silicon and nanometal particles. Immobilization of lipase with nanomaterials improves catalytic performance, stability, recycling efficiency and processability of nanobiocatalyst. This indicates a shift towards innovative and sustainable approaches in the quest for efficient energy solution for biodiesel production[46]. Lipase from *Candida rugosa* was covalently immobilized on Fe3O4 to produce biodiesel from soyabean oil[56]. After immobilization process, the temperature and pH stability was enhanced and catalysed 86% conversion of oils[57].

Pharmaceutical industry

Lipases have attracted the interest of pharmaceutical companies due to their therapeutic advantages. These enzymes are being explored for their potential in treating various condition like obesity, anxiety, inflammation, pain, and cardiovascular disease, is now being investigated. Lipases have practical applications in synthetic processes; they are also used in the organic chemistry and pharmaceutical sectors to prepare optically active chemicals, like pure alcohols, amines, and carboxylic acids. Furthermore, lipases are involved in the chiral intermediate molecule synthesis that results in the anticancer medication Polixatel (Taxol 1), which is specifically used to treat ovarian cancer. Furthermore, novel methods for label-free triacyl glyceride detection have been developed, such as an LSPR-based biosensor uses lipase that has been immobilized on silver nanoparticles. Additionally, a commercial version of *Candida rugosa* lipase has been used in development of an amperometric triglyceride (TG) biosensor[58].

Food industry

The aim of the 21st-century in nutraceutical sector is to create affordable products with eco-friendly practices. Particularly in the last ten years, enzymology has become increasingly important in the food industry. Because of their effectiveness and stability, immobilized lipases are frequently used in the food industry. These enzymes play an important role in the processing of important food ingredients including fats and oils, as well as in processes like hydrolysis, esterification, and inter-esterification. The placement, orientation, and structural configuration of these elements affect the flavor, color, and melting characteristics of food products. Through hydrolysis, lipases modify these characteristics and convert undesirable fats into valuable fats that are utilized in the manufacturing of flavored milk chocolate and other dairy products.

Research has demonstrated the potential of using several sources of pure immobilized lipases to improve the flavors and fragrances of fruits. For example, pentyl nonanoate is produced by immobilized lipase from *Rhizomucor miehei* when it is immobilized on anionic exchange support, which enhances fruit scent[59] Similarly, to produce Geranyl butanoate, which enhances cherry flavor, pure immobilized lipase isolated from Candida Antarctica in polyurethane foam[60]. The food processing industry uses immobilized lipases produced by a variety of microbial strains, including as *Aspergillus Niger* and Candida Antarctica, to enhance the aroma of fruits like pineapple, melon, and plum. Moreover, it has been reported that immobilized lipases on synthetic materials like polyvinyl alcohol and natural polymers like chitosan can synthesize chemicals like citronellol laureate, which enhances the flavor of lemons. These illustrations highly highlight the versatile application of immobilized lipase in food industry[61].

Detergent industry

Enzymes are widely used in the manufacturing of detergents in developed countries. This method conserves energy by enabling lower washing temperatures, which assists in reducing the environmental effect of detergent production. Lipases are important to the detergent industry; each year, over a thousand tons of lipases are added to approximately thirteen billion tons of detergent. Immobilization methods, which are essential in the detergent industry, offer creative ways to improve the activities of enzymes. Compared to their native counterparts, immobilized lipases have remarkable characteristics such higher stability and activity, chemical resistance, specificity, selectivity, and inhibitor sensitivity. Because this immobilization ensures stability at greater washing temperatures, it overcomes an immense difficulty facing by the detergent industry. Thus, immobilized lipases support the detergent sector by assisting in the removal of fats and oils, color retention and stain removal[62].

Textile industry

The textile industry, a long-standing sector that makes important contributions to the economies of many nations, has noticed an increase in the use of enzymes, especially lipases. Enzymes exhibit distinguished properties and confer environmental benefits fascinating the transition towards its increased utilization. The textile industry depends heavily on lipases, which are essential industrial enzymes that are sold commercially. They are used to improve the permeability of the fabric by aiding in exclusion of size lubricants which results in improved coloring stability. Lipase-containing formulations that work well are used to design denim and other fiber fabrics demonstrated how lipase from pig pancreas may be immobilized using a glutaraldehyde combination on zirconia-coated alkyl-amine crystal beads. The physical characteristics of the enzyme were changed throughout the immobilization procedure from those of its native form, leading to improved washing of cotton fabric. The selected immobilized material showed the highest level of enzyme activity. Since the textile business demands high pH and temperature levels, free enzymes frequently degrade because of their proteinous nature. On the other hand, immobilized enzymes demonstrate the ability to adapt to and maintain stability in such harsh conditions, withstanding numerous cycles[63].

Importance of lipase in bioremediation

Lipases are used extensively in the food, cosmetic, and dyeing sectors, but remain unexplored about their potential in environmental applications, especially bioremediation. By converting pollutants into non-toxic chemicals, it treats soil that has been contaminated with oily compounds. lipases derived from solid-state fermentation (SSF) offer potential in contaminant treatment. By enabling the direct application of the full solid matrix including microorganisms, bio compounds, and the culture medium to the soil, the SSF technique provides benefits in the treatment of contaminants. Because of this, there is no longer a need for a precipitation and recovery step, which significantly lowers the entire process costs[64].

Lipases are essential for enhancing the bioremediation of oily effluents, which are released from many sources and comprise oils, fats, and proteins. *Acinetobacter* sp., *Mycobacterium* sp., and *Rhodococcus* sp. lipases have been successfully used to reduce oil spills of PAHs, n-alkanes, and aromatic hydrocarbons. On the other hand, lipases from *Pseudomonas aeruginosa* have shown the ability to break down castor oil, and *Pseudomonas* sp. lipase has been used in the bioremediation of soil contaminated with industrial waste oil. It has also been demonstrated that *P. aeruginosa* aids in the bioremediation of crude oil-contaminated wastewater, resulting in a weekly drop in waste toxicity of around 80% and a progressive decline in oil concentrations[65].

Lipase market potential and significance

The lipase market is predicted to grow at a Compound Annual Growth Rate (CAGR) of 6.2% from 2017 to 2025, surpassing the USD 797.7 million mark globally. The creation of new and improved lipase versions via molecular techniques is a noteworthy development in lipase research that has occurred recently. This involves combining directed enzyme evolution with logical enzyme design to provide lipases particular and desirable characteristics lipases as hydrolases and water-soluble enzymes, are essential for catalyzing the breakdown of insoluble triacylglycerol's to glycerol, diacylglycerols, monoacylglycerols, and free fatty acids as a result of this enzymatic activity[66]. Because they can catalyze a wide range of processes, such as hydrolysis, interesterification of esters, and ester synthesis, lipases demonstrate their versatility. Remarkably, they exhibit various transformation features that are Regio-, enantio-, and stereo-selective transformation properties[67]. Since lipases are the only enzymes that become active when they are adsorbed onto an oil–water interface, they are excellent catalysts for dynamic reactions. This adaptability has resulted in prospective uses across a range of industries, such as the production of paper, oleochemicals, dairy products, agrochemicals, detergent formulations, bio-surfactant synthesis, and organic chemical, nutrition, cosmetics and pharmaceutical processing[68].

Future prospective and challenges

Lipase may catalyze a wide range of processes, including hydrolysis, amino lysis, interesterification, transesterification, and acidolysis, it has steadily emerged as the biocatalyst of interest. However, one of the main obstacles to lipase's utilization in industrial applications is its cost. Nanoparticle-assisted immobilization approaches challenges and enable lipase to be reused in various applications. Both natural polymers and nanoparticles are promising supports for lipase immobilization; however, nanoparticles dominate natural polymers in terms of size, surface area, and enzyme loading capacity, making them an important tool for the development of immobilized enzymes. Lipase immobilization with nanoparticles also improves activity and kinetic parameters and increases extraction efficiency. Consequently, with the goal to minimize overall process costs and contribute to green technology, future research in this field should focus on applying molecular biology and genetic engineering techniques to produce modified microbial lipase with natural polymers that reduces the use of nanoparticles. Furthermore, the creation of novel supports made from easily accessible, reasonably priced waste biomass and/or naturally occurring materials may present a competitive advantage for lipase's industrial uses.

Distinguishing nanomaterials from conventional immobilized enzymes allows for the development of a novel nano biocatalyst. Thus, for the effective development of stable and active biocatalysts that offer a wide range of applications, the proper selection of nanomaterial, enzyme, and immobilization approach is always essential.

The application of nano biocatalyst in various branches of industries, medicine and bioremediation but nano material immobilized with Lipase enzyme for bioremediation and other applications is still needed to be lightened as lipases are essential for biodegradation of oil containing waste water, saline water and other water impurities present in wide range of environment.

Conclusion

The primary obstacle for enzymatic production is the expense of lipase. Less catalytic activity, stability and productivity are the major challenges for industrial applications of lipases for which immobilization could be the best solution. Immobilization technology facilitates lipase to be reused and allows for continuous production. Lipase is commonly immobilized through adsorption and covalent coupling mechanisms, with entrapment being infrequently employed due to the necessity for direct contact between the lipase's active center and the interface. Nanomaterials are an emerging source used for the immobilization of various enzymes, variety of nanoparticles, nanotubes, and nanofibrous membranes are used in lipase immobilization, having distinct effect on the immobilized lipase's performance due to their nano dimensions which make it preferable over natural polymers enhancing the reusability of enzyme and increasing catalytic performance and stability. Desired immobilization of lipase with nanomaterials results into the enhancement of lipase activity when immobilized with various nano particles and develop biohybrid system plays crucial role and have been emphasized. Lipase immobilized on nanomaterials exhibits diverse potential applications, including but not limited to biosensors, oil hydrolysis, pharmaceutical synthesis, and biodiesel production, owing to its desirable functionalities. As knowledge accumulates regarding lipase immobilization on nanomaterials, there is an anticipation of developing more controlled and specific systems designed for higher efficacy utilization of lipase.

The utilization of nanomaterials is still limited, as evidenced by their high production costs, aggregation tendencies, non-uniformity, and ignorance of immobilized enzyme aggregates which necessitates further technological advances.

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