

An insight into microbial lipases and their environmental facet

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Abstract Lipases are serine hydrolases that catalyze the hydrolysis and synthesis of esters formed from glycerol and long-chain fatty acids, by acting at the oil–water interface. Lipases from microbial sources have received heightened attention for an array of industrial applications, and these enzymes have been well exploited in the environmental sector as well. In this article, we present an overview of microbial lipase, including the microorganisms from which it could be produced; the application of recombinant DNA technology tools to produce lipase with enhanced properties, the effective use of waste materials as substrates for lipase production; the usage of statistical tools to efficiently optimize the production medium; lipase purification strategies; and the immobilization of the enzyme on a variety of support materials. The next section of the article provides a gist of its application in diversified spheres and focusses exclusively on the environmentally relevant ones. Lipase-catalyzed esterification, transesterification, and interesterification reactions, an emerging area of green chemistry; lipase-mediated *in vitro* biopolymer synthesis and degradation; and the application of lipase for remediating fat and oil constituents in wastewater are dealt with in-depth. When its full potential is harnessed, the enzyme could play a pivotal role in environmental management.

Keywords Microbial sources · Recombinant lipases · Immobilization · Biopolymers · Bioremediation

Introduction

With the advent of whole-cell and enzymatic biocatalysts, chemical catalysts have been superseded to a significant extent. Factors such as high selectivity, specificity, ability to act under mild conditions, and nil residual effect have made enzymes the most sought-after catalysts for a plethora of reactions. Around 75 % of the industrially used enzymes are hydrolytic in nature, and lipases (triacylglycerol acylhydrolases) (EC. 3.1.1.3) belonging to the superfamily of serine hydrolases are quite conspicuous among them.

Lipases unanimously conform to a common structural organization, viz., the alpha/beta hydrolase fold (Ollis et al. 1992; Nardini and Dijkstra 1999). The substrate-binding site is located inside a pocket on top of the central β -sheet that is typical of this fold. The catalytic triad of serine, histidine, and aspartic acid residing at 105-224-187 positions of the amino acid sequence constitutes the active site of all serine hydrolases (Uppenberg et al. 1994a, b). This active site is shielded by a mobile lid, and whether this lid is closed or open, it determines the enzyme's inactive or active state. At the oil–water interface, the lid opens, giving the substrate access to the active site. Unlike esterases, lipases do not follow Michaelis–Menten kinetics. The size of the enzyme has been reported to vary from 19.4 kDa (Kawasaki et al. 2002) to above 300 kDa for oligomeric forms, with subunits of around 50 kDa (Salameh 2006).

Lipases catalyze the hydrolysis of esters formed from glycerol and long-chain fatty acids into di-, monoacylglycerols, fatty acids, and glycerol. The general scheme for reaction catalyzed by lipase is illustrated in Fig. 1. True lipases differ from esterases owing to the fact that they require an oil–water interface and do not hydrolyze substrates dissolved in the bulk liquid. It is tough to accurately

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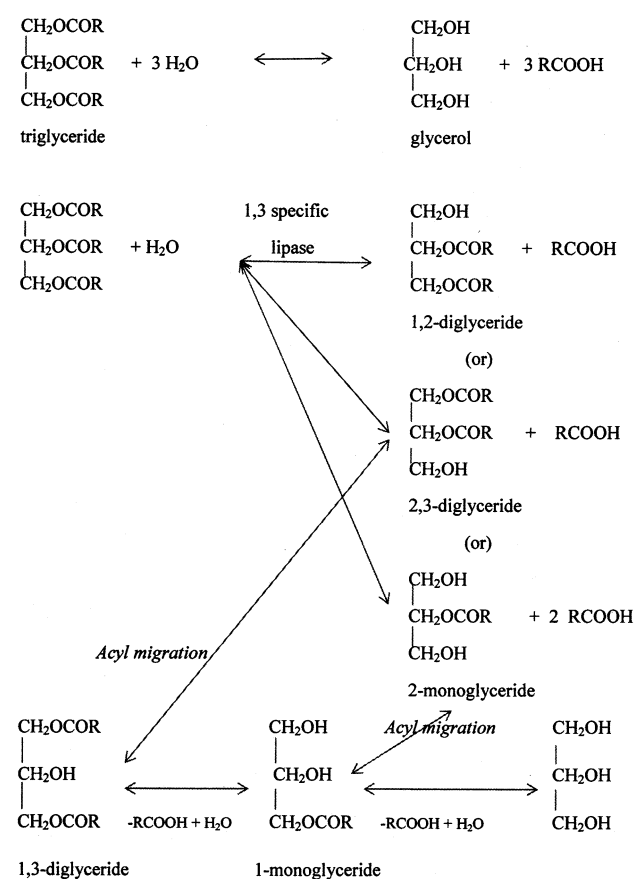


Fig. 1 General scheme for reactions catalyzed by lipases

measure the amount of interface and also the parameters of interfacial tension, surface viscosity, surface potential, etc. The emulsification of the substrate by using surface active amphipathic molecules such as detergents can have a profound impact on the measured enzyme activity.

Apart from catalyzing ester hydrolysis in the aqueous environment, lipases also catalyze the reverse reactions of esterification, interesterification, and transesterification in nonaqueous and microaqueous milieu. They are chemo-, regio-, and enantioselective and are applied widely in industrial arenas. They are also an environmentally relevant class of enzymes, contributing toward wastewater treatment, biopolymer synthesis, etc. The enzyme may be procured from plant, animal, or microbial sources, with microbial source being the most agreeable on account of enzyme stability, substrate specificity, lower production cost, and ease of manipulation. Hardly 2 % of the immense microbial biodiversity has been harnessed for enzyme production, thus justifying the search for new lipases with improved properties. In this article, we present a comprehensive view of microbial lipases, with special emphasis on how lipase-catalyzed hydrolytic and synthetic reactions could be exploited for environmental applications.

Sources of microbial lipases

Lipase activities from several bacterial genera have been documented in the literature, with *Bacillus* and *Pseudomonas* being the most prominent ones. Among *Bacillus* spp, lipases with thermal tolerance have been reported from thermophilic bacteria such as *B. stearothermophilus* (Kambourova et al. 2003) and *B. thermoleovorans* (Leea et al. 2001). The crystal structure of lipase from one such thermophilic organism, *B. stearothermophilus*, has been shown to contain a unique zinc-binding site, to which the organism's increased thermal stability might be attributed (Tyndall et al. 2002). Certain strains of *B. subtilis* (Olusesan et al. 2011) and *B. coagulans* (Kumar et al. 2005) also produce lipases with thermo-tolerant attributes. The presence of charged residues and the formation of salt bridges play a key role in thermostability. The accumulation of more than two mutations had a dramatic impact on *Geobacillus thermodenitrificans* EstGtA2 lipase activity at high temperatures, suggesting an important role of conserved salt bridge-forming residues in thermostability (Charbonneau and Beauregard, 2013). Alkaline lipases are widely produced by the members of *Bacillus* genera, including *B. licheniformis* (Chakraborty and Raj 2008). Organic solvent tolerance is yet another useful trait, desirable for catalyzing synthetic reactions in nearly anhydrous conditions and has been observed in *B. sphearicus* (Tamilarasan and Kumar 2012). Enantioselective lipases find applications in producing optically pure pharmaceuticals, and *B. cereus* is capable of secreting such enzymes (Chen et al. 2007). Lipase activity has generally been proven to be calcium-dependent, and the lipase from *B. pumilis* is an exception to this norm (Kim et al. 2002). *Aneurinibacillus*, a genus that can be differentiated from *Bacillus* based on 16S rRNA sequence, also produces a thermostable and organic solvent-tolerant lipase (Masomian et al. 2013).

Several species of *Pseudomonas* exhibit extracellular lipolytic activities, with *P. fluorescens* (Kojima and Shimizu 2003), *P. aeruginosa* (Ito et al. 2001), *P. cepacia*, and *P. luteola* (Litthauer et al. 2002) being a few of the documented members. Organic solvent tolerance (Rahman et al. 2005a, b), specificity for monoacyl glycerides (Sakiyama et al. 2001), and polyunsaturated fatty acid bonds (Kojima and Shimizu 2003) are noteworthy characteristic features observed in some of these lipases. Various efflux pumps—encoding sequences, such as AcrA/B/C/D/F—are related to organic solvent tolerance and are present in the genomic DNA sequences of *P. cepacia* complex strains (Ramos et al. 2002). Certain *Staphylococci* such as *S. aureus* (Sarkar et al. 2012) and *S. xylosus* (Bouaziz et al. 2011) are also capable of lipid hydrolysis. The desirable features of thermal and organic solvent stability are possessed by a few members (Bouaziz et al. 2011) of this

genus, and quite interestingly, certain strains of halophilic *Staphylococci* produce an enzyme with remarkable tolerance to high salt concentrations (Daoud et al. 2013).

Apart from the above-mentioned bacterial genera, *Aeromonas caviae* (Velu et al. 2012), *Acinetobacter calcoaceticus* (Pratuangdejkul and Dharnstithi 2000), *Microbacterium luteolum* (Joseph et al. 2012), *Lactobacillus plantarum* (Regalla et al. 2002), *Thermus thermophilus* (Fucinos et al. 2005), and *Serratia marcescens* (Abdou 2003) are a few other bacteria with substantial lipolytic capability. Some of these isolates are especially useful, since they exhibit extremophilic properties of either heat (Fucinos et al. 2005; Velu et al. 2012) or cold tolerance (Pratuangdejkul and Dharnstithi 2000).

The lipolytic activity of various fungal and yeast strains has also been extensively explored over the past few years. Of the fungal genera, lipases from *Aspergillus spp.* have been purified and characterized by several researchers, with *A. niger* and *A. carneus* being the most widely reported species (Mhetras et al. 2009; Zheng-Yu et al. 2007). *Penicillium chrysogenum* (Tan et al. 2004), *Mucor hiemalis* (Ülker and Karaoglu 2012), *Rhizopus oryzae* (Hiol et al. 2000), and *Rhizopus chinensis* (Sun and Xu 2009) are also noteworthy sources of fungal lipases that have been subjected to intensive research. Members of *Fusarium* and *Geotrichum* genera are also lipolytic (Liu et al. 2009a, b; Cai et al. 2009). *Antrodia cinnamomea* is another less commonly reported fungus with lipolytic activity (Shu et al. 2006). Another unusual documented case is the lipase from the plant pathogenic fungus *Metarhizium anisopliae* (Silva et al. 2009). Yeasts have also been adequately studied for their lipolytic activity, with *Yarrowia lipolytica* being the most significant one (Yadav et al. 2011; Yu et al. 2007). Lipolytic activities in *Cryptococcus sp.* and *Pseudozyma hubiensis* (Bussamara et al. 2010; Kamini et al. 2000) have also been reported.

Molecular studies

Lipases have been subjected to molecular-level characterization. N-terminal amino acid residues have been identified, and parts of lipase-encoding genes have also been cloned and sequenced, based on which the homology, or lack of it, to previously reported lipases can be deduced. For instance, when the 30 N-terminal amino acid residues of *Staphylococcus simulans* lipase were sequenced, they were found to be identical to *S. aureus* PS54 (SAL PS54) lipase. However, cloning and partial sequencing of the lipase-encoding gene revealed some differences from SAL PS54 sequence (Sayari et al. 2001). Genes encoding *S. aureus* and *S. xylosus* lipases have also been similarly cloned and sequenced (Horchani et al. 2009; Mosbah et al. 2005).

There are also several instances of recombinant lipases, tailor-made in order to cater to specific industrial needs and overexpressed in heterologous hosts. Among bacteria, *E. coli* cells have conveniently been used to express lipases from *Staphylococcus warneri* (Kampen et al. 2001), *S. xylosus* (Mosbah et al. 2006), *Geobacillus sp.* (Ebrahim-pour et al. 2011), *G. thermocatenulatus* (Vélez et al. 2013), *G. thermoleovorans* (Soliman et al. 2007), and *Burkholderia cepacia* (Wang et al. 2009). Among eukaryotes, the yeast *Pichia pastoris* has served as a versatile expression system for lipases from fungi such as *Galactomyces geotrichum* (Fernández et al. 2006), *R. oryzae* (Guillén et al. 2011), *Fusarium graminearum* (Nguyena et al. 2010), and *Streptomyces fraediae* (Zhang et al. 2008); yeasts such as *Y. lipolytica* (Song et al. 2006), *Candida antarctica* (Liua et al. 2012), and *C. parapsilosis* (Brunel et al. 2004) and even bacteria such as *Bacillus sp.* (Sabri et al. 2009). Less commonly, recombinant lipases have also been expressed in the yeast, *Saccharomyces cerevisiae* (Florczak et al. 2013), and the fungi, *Streptomyces sp.* (Bielen et al. 2009) and *Trichoderma sp.* (Qin et al. 2012). Even the human lysosomal acid lipase has been cloned and expressed in cells of the yeast *Schizosaccharomyces pombe* (Ikeda et al. 2004). Such recombinant enzymes are produced by the heterologous host as inclusion bodies in the cytoplasm, which are dissolved by treating with chaotropic agents during the purification process and proper refolding of the enzyme is necessary to restore activity.

Studies on directed evolution have been performed with lipases. The lipase from *Bacillus pumilis* has been subjected to DNA shuffling, generating variants with improved applicability as biocatalysts (Akbulut et al. 2013). A thermostable variant of *Y. lipolytica* lipase has been identified by error-prone PCR and screening of the library in a high-performance yeast expression system (Bordes et al. 2011). In other studies, the metagenomic approach has been applied to characterize lipases (Zheng et al. 2013).

The use of agricultural residues and industrial effluents as environmentally friendly and economically viable substrates for lipase production

The chemical composition of the culture medium plays a pertinent role in influencing lipase production. Several substrates have been experimented with and this includes an array of waste materials. Exploiting such materials offers the twin benefits of offsetting the environmental pollution associated with their disposal and also helps conserve valuable resources. Lipases are generally known to be inducible enzymes, and the presence of residual lipid constituents in certain waste streams enables their utilization as viable substrates for enzyme production.



Table 1 Utilization of waste materials as substrates for lipase production

Substrate	Type of fermentation	Microorganism	References
<i>Industrial effluent and solid waste</i>			
Palm oil mill effluent	Submerged	<i>Candida cylindracea</i>	Salihu et al. (2011)
Olive mill effluent	Submerged		Brozzoli et al. (2009), D'Annibale et al. (2006)
Grease waste	Solid state	<i>Penicillium chrysogenum</i>	Kumar et al. (2011)
<i>Oil cakes</i>			
Deoiled jatropha seed cake	Solid state	<i>Pseudomonas aeruginosa</i>	Bose and Keharia (2013), Joshi and Khare (2013)
Groundnut and mustard oil cakes	Solid state	<i>Micrococcus roseus</i>	Joseph et al. (2011)
Sal (<i>Shorea robusta</i>) deoiled seed cake smf	Submerged	<i>Aeromonas sp</i>	Mahdi et al. (2012)
<i>Fibrous agro residues in combination with other substrates</i>			
Soybean meal and rice husk	Solid state	<i>Aspergillus niger</i>	Colla et al. (2010)
Gingelly oil cake and wheat bran	Solid state		Mala et al. (2007)

Lipid-rich wastewaters emanating from oil refineries, slaughter houses, and dairy industries have proven to be potential substrates facilitating lipase production. *Candida cylindracea* cultured in palm oil mill effluent (Salihu et al. 2011) and olive mill effluent (Brozzoli et al. 2009; D'Annibale et al. 2006) showed appreciable levels of lipase production. Similarly, grease waste has been used as a substrate for lipase production by *P. chrysogenum* under solid-state fermentation (Kumar et al. 2011). Microbial consortium derived from wastewater sludge, when cultured in a medium containing lipid-rich solid industrial waste under thermophilic conditions, showed good lipase production (Santis-Navarro et al. 2011).

Among the agricultural residues, oil cakes have been used to a large extent. Deoiled Jatropha seed cake has been utilized for culturing *P. aeruginosa* both under solid-state and submerged fermentations (Bose and Keharia 2013; Joshi and Khare 2013). Groundnut and mustard oil cakes have been proven to be good substrates for the production of psychrophilic lipase from *Micrococcus roseus* under semisolid-state fermentation (Joseph et al. 2011). Sal (*Shorea robusta*) deoiled seed cake extract has also been assessed for lipase production using *Aeromonas sp.* under submerged fermentation (Mahdi et al. 2012). Next to oil cakes, lignocellulosic fibrous residues such as wheat bran and rice bran have been utilized in several instances (Colla et al. 2010; Mala et al. 2007), either alone or in combination with other substrates for nutrient augmentation. Table 1 illustrates the utilization of such waste materials as cost-effective substrates for lipase production.

The use of statistical tools for optimizing lipase production

Production media have traditionally been optimized by varying one factor at a time (OFAT), which turns

cumbersome when a large number of variables are involved and this methodology also faces the limitation of not indicating the interaction effects of the different variables tested. This led to the application of statistical tools in media optimization. The significant variables influencing lipase production are usually screened through the Plackett–Burman (PB) design, and the optimal concentrations and interaction effects of these variables are inferred from the response surface methodology (RSM).

Plackett–Burman (PB) experimental design has been applied to evaluate the medium components for lipase production through submerged fermentation of microorganisms such as *Rhizopus arrhizius* and *Candida rugosa* (Rajendran et al. 2008; Rajendran and Thangavelu 2009). PB, in combination with RSM, has resulted in optimizing the medium and increasing the enzyme yield from a multitude of yeasts, fungi, and bacteria. *Rhizopus delemar* (Acikel et al. 2010), *A. carneus* (Kaushik et al. 2006), *Aspergillus awamori* (Basheer et al. 2011), *Geotrichum sp.* (Burkert et al. 2004), *C. cylindracea* (Salihu et al. 2011), *Burkholderia sp.* (Gupta et al. 2007), *Stenotrophomonas sp.* (Hasan-Beikdashti et al. 2012), etc., are noteworthy among them. Box–Behnken and central composite designs of RSM have been applied in the above cases. Such statistical optimizations of process parameters have resulted in improved lipase production and enhanced feasibility of process scale-up and commercialization.

Lipase purification

Recovery of lipase from the production medium routinely entails ultrafiltration and ammonium sulfate precipitation followed by purification to homogeneity in ion exchange and gel filtration chromatographic columns. Alternatively, aqueous two-phase extraction (ATPS) and reverse micellar extraction (RME) have been used for enzyme recovery.

Alcohol—salt-based ATPS—has been used to recover lipase from *Burkholderia pseudomallei* (Ooi et al. 2009). An extractive fermentation using ATPS has also been tried out for the simultaneous cell cultivation and downstream processing of lipase derived from the same species (Ooi et al. 2011). Such an extractive fermentation technique employing a thermoseparating reagent has been tried out for recovering *B. cepacia* lipase as well (Show et al. 2012). RME is yet another unconventional route to single-step recovery and purification of lipase. The cationic detergent cetyltrimethylammonium bromide (CTAB) and the Aerosol OT (bis 2-ethylhexyl) sodium sulfosuccinate system have been used under optimized conditions for RME of lipase (Gaikaiwari et al. 2012; Nandini and Rastogi 2009).

Among the chromatographic techniques, expanded bed adsorption can serve as a single-step procedure for purifying enzymes directly from particulate containing fermentation broths, as bed expansion increases void volume. This method has been used for purifying lipase from *P. cepacia* by adsorption on to Amberlite 410 ion exchange resins (Padilha et al. 2009). Affinity and pseudoaffinity chromatographic techniques involving ligand–receptor interactions are also useful for lipase purification. Affinity-based isolation of *Burkholderia glumae* lipase through steric chaperone interactions has been reported (Pauwels and Gelder 2008). Biomimetic affinity purification using synthetic ligands and hydroxyapatite chromatography has resulted in single-step purification of *C. antarctica* lipases (Dimitrijevic et al. 2012; Yao et al. 2011).

Lipase immobilization

Lipases have been immobilized on a variety of supports, permitting repetitive usage of the enzyme, increased stability, and easier product recovery. Such immobilized enzymes and whole cells have been used in a wide range of applications. Adsorption, covalent bonding, and entrapment are the methods commonly used for immobilization, and an appropriate technique that retains enzyme activity and is strong enough to prevent enzyme leakage has to be selected for a particular application.

Candida spp. lipases have extensively been immobilized on diversified materials such as pretreated textile (Adachi et al. 2006); nonwoven fabrics of polypropylene, polyethylene terephthalate, and viscose fiber (Li et al. 2011); macroporous silica monoliths (He et al. 2010); silica-PEG gel (Yang et al. 2010); treated chitosan membranes (Orrego et al. 2010); ternary blend film comprising of chitosan, polylactic acid (PLA), and polyvinyl alcohol (Badgular et al. 2013); chitosan tethered poly(acrylonitrile-co-maleic acid) surface (Ye et al. 2005); natural kaolin (Rahman et al. 2005a, b); and Amberlite IRC-50 and Al₂O₃ (Minovska

et al. 2005). Immobilization of *R. oryzae* lipase on silica aerogels (Kharrat et al. 2011), and *Thermomyces sp.* lipase on regenerated cellulose, glass fiber, and polyvinylidene fluoride grafted with 1,4-diaminobutane and activated with glutaraldehyde (Chen et al. 2012) are a few other instances of immobilized fungal lipases.

Among bacterial lipases, *Pseudomonas spp.* lipases have been covalently immobilized on porous polymethylacrylamide cross-linked with N,N'-methylene bisacrylamide (Wu and Tsai 2004) and bacterially produced exopolysaccharide (Dimitrijevic et al. 2011). Zirconia particles (Wang et al. 2012), alginate—k-carrageenan hybrid matrix (Abdulla and Ravindra 2013), Celite carriers (Liu et al. 2009a, b), polyglutaraldehyde activated styrene—divinylbenzene copolymer (Dizge et al. 2009), synthetic macroporous alkylated glycidyl epoxy copolymers (Bhushan et al. 2008), etc., have served as support materials for immobilization of lipases from other bacteria including *Burkholderia sp.* and *Arthrobacter sp.*

Applications of lipases

Microbial lipases constitute a key group of industrial enzymes. Their rampant applications in fat and oleochemical industry, textile industry, detergent industry, food industry (dairy products, bakery products, confectionaries, tea processing, and flavor development), diagnostic and medical fields, synthesis of fine chemicals and pharmaceuticals, biodiesel production, synthesis of biodegradable polymers, and in bioremediation have been extensively reviewed and documented (Hasan et al. 2006). Three crucial spheres of environmentally appropriate applications alone are discussed here:

Lipase-catalyzed esterification, transesterification, and interesterification reactions: an emerging area of green chemistry

Lipases are known to carry out hydrolysis of ester bonds in aqueous environments (Liu et al. 2008; Ramani et al. 2010). Their intrinsic property also permits them to catalyze the reverse reactions of esterification, transesterification, and interesterification in nonaqueous and microaqueous milieu.

Lipases from *P. aeruginosa* (Ji et al. 2010), *A. niger* (Romero et al. 2012), and *Ralstonia sp.* (Yoo et al. 2011) are potential candidates for biodiesel production. *A. oryzae* whole-cell biocatalysts expressing *Geobacillus thermocatenulatus* (Adachi et al. 2013) and *Fusarium heterosporum* lipase (Yoshida et al. 2012), and *S. cerevisiae* expressing *Candida sp.* lipase (Liu et al. 2013) have also been demonstrated to be useful in biodiesel production.

Transesterification resolution of (R, S)-1-phenylethanol by lipase from *Pseudomonas stutzeri* (Cao et al. 2012), enzymatic transesterification of vegetable oils with methanol by *Streptomyces sp.* lipase (Mander et al. 2012), transesterification of corn and soybean oils with ethanol and butanol by synthetic resin-bound truncated *C. antarctica* lipase (Hughes et al. 2012), and transesterification of palm oil by whole cells of *Rhodotorulla mucilagenosa* (Srimhan et al. 2011) have also been recorded. Such lipase-mediated transesterification reactions are useful in biodiesel production from vegetable oils. Not only is biodiesel an important renewable energy, but also the use of enzymatic biocatalysts in the production process makes the fuel more environmentally acceptable. For example, sodium hydroxide, potassium hydroxide, sulfuric acid, and supercritical fluids used to catalyze biodiesel production could be substituted by lipases.

The extracellular lipase secreted by *Burkholderia multivorans* has efficiently catalyzed the synthesis of ethyl butyrate esters that find extensive applications in the food and fragrance industries (Dandavate et al. 2009). Lipase from *Acinetobacter sp.* has mediated the synthesis of the flavor ester ethyl caprylate. The compound has a “fruity-flowerly” fragrance and is used in various fruity flavors such as peach, apple, banana, and pineapple. It serves as a flavor-enhancing compound in fermentation industry and is commonly associated with wines and whiskey (Ahmed et al. 2010). Lipase from *Amycolatopsis mediterranei* has brought about synthesis of the flavor ester isoamyl acetate, one of the most important flavor and fragrance compounds used in the food, beverage, cosmetics, and pharmaceutical industries because of its characteristic banana flavor (Dheeman et al. 2011).

Production of structured lipids with dietary significance is yet another important arena of research. Fatty acid composition of several edible oils has been favorably modified by lipase-catalyzed interesterification reactions. Olive oil enriched with medium-chain-length fatty acids (Nunes et al. 2011) and sardine oil enriched with n-3 polyunsaturated fatty acids (Chakraborty et al. 2010; Chakraborty and Raj 2009) constitute such examples.

Lipase-catalyzed biopolymer synthesis and degradation

Synthesis and degradation of biopolymers helps maintain homeostasis in biological systems. In vivo biopolymer production by several bacterial strains, especially under conditions of limiting nitrogen and excess carbon in the growth medium, has been well documented (Khanafari and Sepahei 2007; Sandhya et al. 2013). It is possible to synthesize these biopolymers in vitro by enzyme-mediated processes, and lipases play a predominant role in this. Such

enzymatic polymerizations are quite specific (regio- and enantioselective) when compared to their chemical counterparts, enabling precise control over the polymer structure, and are carried out under milder conditions. Traces of chemical catalysts in the product can be avoided, which makes them particularly amenable to biomedical applications. Oxyacids and their esters, dicarboxylic acids or their derivatives and lactones, are the monomer combinations used as substrates for the polymerization reaction. Polyhydroxyalkanoate (PHA), PLA, and polycaprolactone (PCL) are important classes of biopolymers that can be synthesized in vitro using tailored lipases. Such in vitro biopolymer syntheses have been reviewed by certain researchers (Hiraishi and Taguchi 2009; Kobayashi and Uyama 2002; Sandoval et al. 2010).

Ring-opening polymerization (ROP) of ϵ -caprolactone (CL) can result in low-molecular-weight polyesters with unique multiphase morphology. However, mechanistic limitations exist in such reactions, and these include the limit for methoxy-poly(ethylene glycol) initiator esterification and slower monomer conversion in concentrated solutions, factors that have been investigated by researchers (Panova and Kaplan 2003). Ultrasonic irradiation has been shown to greatly improve *C. antarctica* lipase B-mediated ROP of ϵ -CL to poly-6-hydroxyhexanoate in the ionic liquid 1-ethyl-3-methylimidazolium tetrafluoroborate. Sonication improved the monomer conversion by 63 % and afforded a polymer of narrower molecular weight distribution and a higher degree of crystallinity (Gumel et al. 2012). In other studies, lipase-catalyzed synthesis of poly-CL has been carried out in supercritical carbon dioxide and the influence of operating conditions on polymer chain size and polydispersity index was evaluated (Santos et al. 2012). Novel copolymers based on ω -pentadecalactone have also been enzymatically synthesized by a combination of ROP and polycondensation. These new biopolymers have potential application in the manufacture of drug-loaded biodegradable microspheres for modified release drug delivery (Thompson et al. 2006).

In another significant observation, the lipase from *Candida sp.* has catalyzed the synthesis of aliphatic polyester poly(butylene sebacate) from diethyl sebacate and 1,4-butanediol in the absence of organic solvents. Poly(butylene sebacate) is a remarkable member of the polyester family. It can be used as biodegradable thermoplastics and biocompatible medical materials. As a plasticizing agent, it can be converted to various forms, such as leatheroids, wrapper films, and fibers. It can substitute conventional thermoplastics on account of its thermal stability and good mechanical strength (Liu et al. 2011).

Lactate-based polymers are highly valuable in biomedical and food industries. Synthesis of poly-L-lactide by lipase-mediated ROP of L-lactide in the presence of

supercritical carbon dioxide has been achieved. Lipase B from *C. antarctica* (CALB) was employed for this reaction, and semi-crystalline polymers with a molecular weight up to 12,900 g/mol were attained (García-Arrazola et al. 2009). Whole-cell biocatalysts displaying CALB have also been utilized for the synthesis of enantiomeric ethyl lactate from ethanol and lactic acid. The synthesis efficiency was temperature-dependent and reached 74 % at 50 °C (Boskhomdzhev et al. 2010). Enzymatic synthesis of β -D-galactosyl-L-lactic acid ethyl ester (GLAEE), which is difficult to synthesize via traditional chemical routes, has been reported. Polymerization of GLAEE to yield a unique biopolymer β -D-galactoside-co-L-lactic acid with the aid of commercial lipase Novozyme 435 has also been executed (Jia and Wang 2007). A variety of sugar-hydroxyl acid copolymers can be synthesized using the same approach, leading to the development of a new class of biopolymers.

Bioactive hydrophilic sugar moieties can be applied for the functionalization of aliphatic biopolymer PHA, thereby augmenting its hydrophilicity and hence biodegradability. Novozyme 435 has been effectively used to synthesize one such functionalized biopolymer poly(1'-O-3-hydroxyacyl sucrose), having potential applications in biomedical and other allied industrial niches (Gumel et al. 2013).

Degradation of biopolymers has also been given due attention. The long-term kinetic curves for biodegradation of poly(3-hydroxybutyrate) (PHB), its copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate), and a PHB/PLA blend have been compared (Boskhomdzhev et al. 2009). The rate of biodegradation was analyzed in vitro in the presence of lipase, and such studies are useful in the development of PHB-based medical devices. In another study, lipase-secreting *Bacillus pumilus* isolated from the rhizosphere of mangroves has been shown to degrade poly-CL at thrice the hitherto reported speed, resulting in complete degradation in just 20 days (Motiwalla et al. 2013). In other molecular-level studies, the purification, cloning, and expression of an *A. niger* lipase for degradation of poly(lactic acid) and poly(ϵ -CL) have been carried out (Nakajima-Kambe et al. 2012). Table 2 summarizes such biopolymer synthetic and degradative reactions.

Bioremediation of oil and grease (O&G) containing wastewater

Treatment of O&G containing wastewater by physico-chemical as well as biological methods has been extensively researched (Wong et al. 2007; Abdulsalam et al. 2011), as these constituents could lead to a multitude of problems in treatment plants and seriously undermine the plant's performance. Reduction in cell-aqueous phase transfer rates, sedimentation hindrance due to the growth of

Table 2 Synthesis and degradation of biopolymers via lipase-catalyzed reactions

Biopolymer synthesized/degraded	References
Synthesis of poly(ϵ -caprolactone) with multiphase morphology	Barrera-Rivera et al. (2012)
Synthesis of poly(ϵ -caprolactone) in supercritical carbon dioxide	Santos et al. (2012)
Synthesis of ω -pentadecalactone polymers for the production of biodegradable microspheres	Thompson et al. (2006)
Synthesis of β -D-galactosyl-L-lactic acid ethyl ester (GLAEE); polymerization of GLAEE to form poly(β -D-galactoside-co-L-lactic acid) (PGLA)	Jia and Wang (2007)
Synthesis of poly-L-lactide using supercritical carbon dioxide	García-Arrazola et al. (2009)
Ultrasound-assisted synthesis of poly-6-hydroxyhexanoate	Gumel et al. (2012)
Functionalization of medium-chain-length polyhydroxyalkanoates using biologically active hydrophilic sugar moieties	Gumel et al. (2013)
Biodegradation of poly(3-hydroxybutyrate) (PHB), its copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate), and PHB/polylactic acid blend	Boskhomdzhev et al. (2009, 2010)
Degradation of poly(ϵ -caprolactone)	Motiwalla et al. (2013)
Degradation of poly(lactic acid) and poly(ϵ -caprolactone)	Nakajima-Kambe et al. (2012)

filamentous microorganisms, development of bulking sludge, clogging, and emanation of foul odors are a few representative difficulties posed by these constituents. Pretreatment of such wastewaters to bring about lipid hydrolysis makes them more amenable to conventional biological treatment and hydrolytic enzymes find promising applications in this sector. Such applications of lipases and other hydrolytic enzymes have been reviewed earlier (Cammarota and Freire 2006; Mrozik et al. 2008; Karigar and Rao 2011).

Potential microbial strains for O&G bioremediation

Several lipase-producing bacterial, fungal, and yeast strains have been employed either individually or as a consortium to bring about O&G bioremediation, and such isolates, more often than not, have been obtained from contaminated environments. Among bacteria, *Pseudomonas spp.* have served as handy tools for bioremediation and several strains of *P. aeruginosa* have been especially useful. Statistical methods have been adopted to optimize the lipase production process as well as the process of oil hydrolysis by *Pseudomonas sp.* (Gaur and Khare 2011; Verma et al. 2012). *B. stearothermophilus* isolated from slaughter house waste and a bacterial pool of *Bacillus spp.* isolated from

Table 3 Microbes applied in oil and grease bioremediation

Microorganism	References
Bacteria	
<i>Pseudomonas aeruginosa</i>	Mobarak-Qamsari et al. (2012)
<i>Bacillus sp.</i>	Bayoumi et al. (2012)
<i>Bacillus stearothermophilus</i>	Granzotto et al. (2012)
<i>Burkholderia sp.</i>	Matsumiya et al. (2007)
<i>Raoultella planticola</i>	Matsumiya et al. (2007), Sugimori et al. (2013)
<i>Microthrix parvicella</i>	Nielsen et al. (2002)
Fungi	
<i>Geotrichum candidum</i>	Asses et al. (2009)
<i>Penicillium chrysogenum</i>	Kumar et al. (2012)
<i>Penicillium restrictum</i>	Valladão et al. (2009, 2011)
<i>Rhizopus oryzae</i>	Efremenko et al. (2008)
Yeast	
<i>Candida rugosa</i>	Chakraborty et al. (2012)
<i>Yarrowia lipolytica</i>	Lanciotti et al. (2005), Gonçalves et al. (2009)
<i>Mrakia blollopsis</i>	Tsuji et al. (2013)
<i>Lipomyces starkey</i>	Yousuf et al. (2010)
Consortia	
<i>Pseudomonas aeruginosa</i> , <i>Bacillus sp.</i> and <i>Acinetobacter calcoaceticus</i>	Mongkolthananuk and Dharmsthiti (2002)
<i>Bacillus subtilis</i> , <i>Bacillus licheniformis</i> , <i>Bacillus amyloliquifaciens</i> , <i>Serratia marsescens</i> , <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i>	Prasad and Manjunath (2011)
<i>Burkholderia arboris</i> and <i>Candida cylindracea</i>	Matsuoka et al. (2009)

aged petroleum contaminated soil also hold promise for bioremediation (Bayoumi et al. 2012; Granzotto et al. 2012). Other documented bacteria genera comprise of *Burkholderia sp.* and *Raoultella planticola*, the latter being a novel isolate capable of biodegrading edible oil under acidic conditions (Matsumiya et al. 2007; Sugimori et al. 2013). In other studies, bacterial consortia have been formulated as potential inoculum for the treatment for high strength O&G wastewaters. One such bacterial consortium comprised of *P. aeruginosa*, *Bacillus sp.*, and *Acinetobacter calcoaceticus* (Mongkolthananuk and Dharmsthiti 2002), and the other one comprised of *B. subtilis*, *B. licheniformis*, *B. amyloliquifaciens*, *S. marsescens*, *P. aeruginosa*, and *S. aureus* (Prasad and Manjunath 2011).

Fungal cultures with lipolytic activity have also been practically applied for bioremediation. Submerged and static cultures of *Geotrichum candidum* applied in olive mill wastewater treatment and solid culture of *P. chrysogenum* applied in bioremediation of waste cooking oil exemplify this statement (Asses et al. 2009; Kumar et al. 2012). Yeasts have been efficaciously used in remediation of wastewaters from oil mills, and *Y. lipolytica* has found massive applications in olive mill wastewater treatment (Gonçalves et al. 2009; Lanciotti et al. 2005). Extremophilic

strains always have an edge, and the antarctic yeast *Mrakia blollopsis* has been fruitful in the low-temperature remediation of milk fat curdle (Tsuji et al. 2013). Yeast–bacteria symbiosis is also valuable in O&G biodegradation as shown in the association between lipase-secreting *Burkholderia arboris* and glycerol-assimilating *C. cylindracea* (Matsuoka et al. 2009). Table 3 depicts the microbial strains that have played a leading role in O&G bioremediation.

Effect of enzymatic pre-hydrolysis on anaerobic digestion of various industrial wastewaters

Upflow anaerobic sludge blanket (UASB) reactor, packed bed reactor (PBR), membrane bioreactor (MBR), and sequencing batch reactor (SBR) are the reactor types widely employed for remediating O&G containing wastewater (Chakraborty et al. 2012; Masse et al. 2003; Jegannathan et al. 2007a, b). Anaerobic digestion is preferred over aerobic treatment for high strength wastewaters. The efficacy of anaerobic treatment is often enhanced when such treatment is preceded by lipase-catalyzed hydrolysis.

Turbidity, volatile suspended solids, and COD removal in dairy wastewater treated in UASB reactor were greatly

enhanced when it was pretreated with 0.1 % babassu cake containing *Penicillium restrictum* lipase (Cammarota et al. 2001). The benefits of hydrolysis become evident especially while treating strong wastewaters containing 1,000 mg/l O&G (Leal et al. 2006). Hydrolytic pretreatment also has a profound impact on the microbial community in the bioreactor. Lipase-rich enzyme preparation produced by *Penicillium sp.* has been shown to alter the microbial communities in UASB and horizontal-flow anaerobic immobilized biomass (HAIB) reactors. Molecular analysis of the bacteria and archaea domains revealed significant differences in the microbial profiles in experiments conducted with and without pre-hydrolysis (Cammarota et al. 2013). Enzymatic extract prepared from *P. aeruginosa* has also improved the treatability of synthetic dairy wastewater in a batch bioreactor, resulting in accentuated COD removal and biogas production (Mobarak-Qamsari et al. 2012). There are several such instances where hydrolytic enzymes have been successfully used as adjuvants in the anaerobic treatment of dairy wastewaters (Leal et al. 2002).

Enzymatic pre-hydrolysis has also been helpful in remediating slaughter house wastewater. Lipase-rich solid enzymatic preparation (SEP) produced by the fungus *P. restrictum* positively influenced poultry wastewater treatment in UASB reactor (Valladão et al. 2009, 2011). Wastewater from the swine meat industry has also been hydrolyzed with an SEP, and the hydrolysis efficiency has been compared with a commercial enzyme Lipolase 100T from Novozymes (Rigo et al. 2008a). The comparative hydrolytic efficacies of these enzymes for wastewaters from the bovine meat industry have also been investigated (Rigo et al. 2008a, b) and SEP proved to be superior in both the cases. The effectiveness of enzymatic pre-hydrolysis has also been compared with alkaline hydrolysis for the treatment of slaughter house wastewater. Sodium hydroxide and three lipases of plant, animal, and bacterial origin were tried, and the pancreatic lipase PL-250 increased the free long-chain fatty acid (LCFA) concentration maximally. The bacterial lipase LG-1000 was also efficient in reducing the average size of fat particles, but high doses >1,000 mg/l were required (Masse et al. 2001). In yet another study involving a commercial *C. rugosa* lipase, the pretreated effluent produced about four times more biogas than the crude effluent (Pereira et al. 2006).

The effect of pretreatment using an enzymatic mixture comprising of lipase–protease–carbohydrase in 1:2:1 ratio on solubilization and volatile fatty acid (VFA) production in the fermentation of food waste has been probed (Kim et al. 2005). Increase in VFA production was three times when compared to the control fermenter, with n-butyrate and acetate being the major forms. The favorable role of enzymatic hydrolysis in controlling the oily and greasy substances in recycled fiber pulping wastewater has also been assessed (Liu et al. 2012).

The role of lipases in activated sludge systems

Lipids are noxious constituents in activated sludge systems as they contribute to 30–40 % of the wastewater COD and flair up the growth of filamentous microorganisms. Hence, their transformation to innocuous components is desired. For a sound description of the process involved in the transformation of lipids in such systems, a conceptual model has been suggested. It involves the adsorption/desorption of both triacylglyceride and LCFA onto surfaces of sludge flocs, hydrolysis of triacylglycerides by lipases and the uptake of LCFA by bacteria (Dueholm et al. 2001). This model could assist in the design and evaluation of activated sludge experiments with lipids.

The impact of the addition of lipase-rich enzyme pool on an activated sludge system under fat shock loads has been researched. Continuous addition of enzymatic preparation can become cost-prohibitive, and the study suggested it as an emergency measure at times of fat overloads in the effluent. Such a measure resulted in efficient COD removal in the test reactor for 270 days without any operational problems (Damascene et al. 2008). The utilization of a SEP produced by *P. restrictum* in activated sludge systems treating dairy wastewater with high levels of O&G has been scrutinized and found to be effective with 13 % higher COD removal, 40 % lower accumulation of O&G in flocs, 1.7 times higher biomass concentration, and 1.3 times higher specific oxygen uptake rate (Rosa et al. 2006).

In certain interesting studies, extraction of lipases and proteases from activated sludge using the nonionic detergent Triton X-100, EDTA, and cation exchange resin has been attempted (Gessesse et al. 2003). In other experiments, the filamentous bacterium *Microthrix parvicella* has been demonstrated to be a specialized lipid consumer, being able to take up LCFA under anaerobic conditions and subsequent usage of the stored material for growth when nitrate or oxygen is available as electron acceptors (Nielsen et al. 2002).

Immobilized enzyme and whole-cell biocatalysts in O&G remediation

Immobilized lipase has been instrumental in bringing about hydrolysis of O&G contents in pet food industry wastewater. COD and O&G reduction were 49 and 45 %, respectively, without pretreatment and 65 and 64 %, respectively, with immobilized lipase pretreatment (Jeganathan et al. 2007a, b). *C. rugosa* lipase immobilized in calcium alginate beads has been used for hydrolyzing pet food industry wastewater (Jeganathan et al. 2006). *C. rugosa* lipases have also been immobilized in polyethersulphone membrane (Chakraborty et al. 2012). Lipase-producing bacteria immobilized on



different matrices have been incorporated in a grease trap system for restaurant wastewater. When the influent O&G concentration exceeded 5,000 mg/l, the matrix-based trap system showed higher O&G and COD removal (Nisola et al. 2009). *R. oryzae* cells entrapped in polyvinyl alcohol cryogel have been used for the treatment of complex food industry wastewater (Efremenko et al. 2008). The immobilized cells possessed concurrent lipolytic, amylolytic, and proteolytic activities.

Converting O&G wastewater into high-value products: the economic advantage

The lipid constituents may be recovered from grease traps or from the wastewater itself by flotation, centrifugation, or filtration. Extraction procedure involving zeolite and a natural mixture of clays and diatomaceous earth can also be used to recover lipids. Such recovered lipids can then be used for biodiesel production through lipase-catalyzed esterification or transesterification reactions. One particular study revealed that the free fatty acid (FFA) content of the recovered O&G could be increased to 15 % over a 20-day period by lipase-catalyzed esterification process, following which alkali-catalyzed biodiesel production was carried out (Montefrio et al. 2010). In another study, *Lipomyces starkey*-mediated conversion of olive mill wastewater into lipids suitable for biodiesel production has been demonstrated (Yousuf et al. 2010).

Olive mill wastewater has also been exploited for generating high-value products such as industrially important enzymes through fungal fermentative processes. Enzymes, such as lipase, laccase, Mn-dependent peroxidase, pectinase, and also exopolysaccharides, *have been produced*. A process based on the acidogenic fungus *Aspergillus niger* has been used to increase the phosphorus content of OMW (Crognale et al. 2006). The yeast *Y. lipolytica*, by use of specific enzymatic pathways from hydrocarbonoclastic bacteria, is also being developed into a microbial factory capable of producing industrially valuable compounds such as wax esters, carotenoids, PHA, and free hydroxylated fatty acids (Sabirova et al. 2011). Palm oil mill effluent has been utilized for PHA production and nutrient removal in a fed-batch reactor, and 66 % PHA production with the removal of total organic carbon and nitrate by 19 and 3 %, respectively, were achieved (Din et al. 2013).

Other novel treatment approaches

Co-composting of oiled bleaching earth with waste sludge has been practiced as an alternative way of bioremediation, and lipases play prominent roles in stabilization of the waste

(Piotrowska-Cyplik et al. 2013). A combination of microwave irradiation and lipase treatment has been applied for biodegrading lipid-rich wastewater (Saifuddin and Chua 2006). Lipase and protease products have been tried for removing milk fouling deposits from stainless steel panels, as a cleaning-in-place strategy in dairy industries (Boyce et al. 2010). Recombinant DNA technology has also been exploited in bioremediation. To combine the advantage of the oleaginous yeast *Y. lipolytica* with the high activity of fungal lipases, effective lipase-displaying arming yeast was constructed using the flocculation functional domain of *S. cerevisiae* as the protein anchor. When applied into an activated sludge bioreactor, it resulted in 96.95 and 97.6 % oil and COD removal, respectively (Song et al. 2011).

Concluding remarks

Lipolytic microorganisms and lipases constitute one of the most important groups of biocatalysts for environmental applications, as reinforced by this article. They lack sequence homology, exhibit catalytic promiscuity, and hence are truly versatile. In lieu of the recent research progress, our understanding of this unique enzyme has broadened, making its application more practically feasible, but it is still not complete. Several constraints are yet to be overcome. The prohibitive cost of commercial lipases is one dimension of the problem. The cost often stems from feedstock price and the complex downstream processing steps resulting in low enzyme yield. The use of agro industrial residues and industrial effluents as substrates can help offset the cost and serve as a pro-environment measure too. Simple and innovative purification strategies such as liquid–liquid and reversed micellar extraction need to be looked into. Immobilization methods permitting continuous use of the enzyme can also bring down the cost. For bioremediation, the microbe and the enzyme should be robust enough to survive and function effectively in the field condition, which is another challenge. New microbes and enzymes with extremophilic properties in terms of temperature, pH, or organic solvent tolerance need to be identified through intensive screening programs. Alternatively, tailored lipases could be fabricated through metagenomics, site-directed mutagenesis, and cloning and expression of the gene in heterologous hosts. These measures could pave the way for lipolytic microbes and their enzymes playing a more proactive, affordable, and realistic role in environmental protection.

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