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A REVIEW ON THE MICROBIAL LIPASES FOR ADVANCED INDUSTRIAL APPLICATIONS

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ABSTRACT

The lipids hydrolysis is an enzyme catalyzed reaction which involves the formation of low molecular weight compounds. In processing Industry of several products, lipase is among one of the best commercial enzyme. For Lipase production, the industrially important enzymes are extracted from bacterial as well as fungal sources. However, several animals and plants can also be the source of Lipase. For development of fermentation processes, suitable thermostability, optimum pH, other physical and chemical parameters are critical. Fungi are used for high quality and quantity of Lipases but temperature stability is the only problem with it i.e. beyond 40^{o}C it is not heat stable. Though, exceptions are always there. On the other hand, thermophilic bacterium, *Bacillus sp.* strain L2 produces extracellular thermostable lipase enzymes. Previously, a Bacterial species have been reported, which produce thermostable enzyme. This lipase enzyme has a capacity to withstand 70^{o}C temperature. For Future perspective, there is need to separate microorganism species that are capable to grow at high temperature. Furthermore, their enzymes can be thermostable up to temperature of 95-100^{o}C range. The objective of this document was literature review of lipase producing microbes by defining several substrate, its profile related to thermostability and advanced industrial application.

KEYWORDS: Lipase; Thermostability; *Bacillus sp.* strain L2; Hydrolysis; Characterization

INTRODUCTION

First scientist who report lipase activity in 1848 was “Claude Bernard”.[1] Later on, lipid hydrolyzing enzymes classified into eight different classes on the basis of different fatty acids types produced by enzymatic reactions.[2] Economically, primary storage complex i.e. Lipids are the components of large number of important crops especially oilseed. It includes soybean, rapeseed and maize. Moreover, oil-rich fruits such as olive or oil palm can also be considered as its part. Fatty acids are abundantly formed by Lipids. Lipids mainly consist of different components. It includes fatty acids, Glycerophospholipids, Glycerolipids , Sterol lipids, Sphingolipids, Saccharolipids, Polyketides, and Prenol lipids. Fatty acids are formed from hydrocarbon chain that have hydroxyl group at its ends. Glycerophospholipids usually better known as phospholipids. Glycerolipids are formed by esterification of three hydroxyl group of glycerol. Sterol lipids consist of cholesterol and its derivatives. Sphingolipids are the diverse compounds. Polyketides consists of polymers of acetyl and propionyl subunits. Prenol lipids are made up of five unit of carbon. Its precursors are dimethylallyl diphosphate and isopentenyl diphosphate. Trends of lipid processing industry are increasing from recent century. In it, the lipid set up consists of lipid-converting enzymes which consist of Fatty oils hydrolysis (acid) for glycerol and free fatty acids production, by acid passaging process.[3] Lipase (hydrolases ester of triacylglycerol, having EC 3.1.1.3) also known as a liplolytic enzyme. This process of enzyme hydrolyze (breaks down) the ester linkages of lipids and other associated products (in an endo manner) and further synthesize free fatty acids as given in Figure 1.[4] Depending on enzyme source, the action, properties and hydrolysis products of enzymes can be somewhat different.

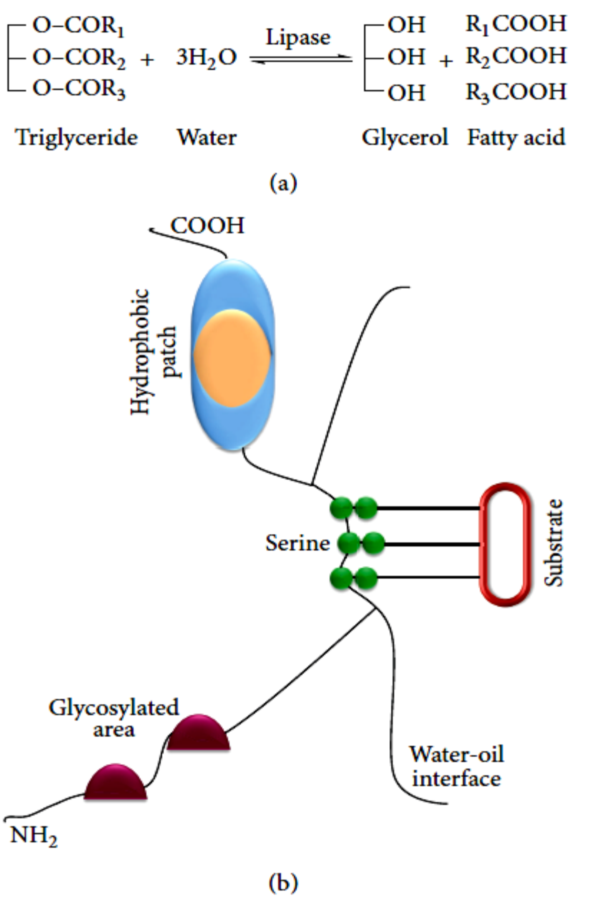

Figure 1: (a) Hydrolytic action of lipase. (b) Lipase molecule with its features.

Microbes Associated With Lipase Production

On Lipase production and characterization numerous reports have been published from different sources .[3][4][5] Industrial enzymes production from plants and animals is limited due to many factors. Although plants, animals and micro-organisms, all are involved in this enzyme production. Plant materials consist of low concentrations of lipase. Furthermore, large enzyme quantity is required for lipids processing industries. On the other hand, in the animal origin enzyme by- product form. Moreover, they are required in meat industry; therefore we have limited supply of enzyme from this source. However, to meet essential requirements of industrial market, microbial lipases from different sources produced in bulk quantities. The reason for this is that best quality lipase produced by using diverse microbial source. Enzymes associated with microbes have characteristics feature for different applications of enzyme catalyzed reactions.

Lipase Producing Bacteria

Bacterial species of *Bacillus* are used to produce lipases. Moreover *Pseudomonas*, *Staphylococcus* and *Burkholderia* are also used. [6] Commonly ideal bacteria for lipase production are Bacterial species of *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus coagulans*, *Bacillus stearothermophilus*, and *Bacillus alcalophilus*. Moreover, they are found to be industrious and productive.[7] Thermal stabilities require during fermentation processes can be fulfilled by extreme thermophilic bacterial species of *Thermoanaerobacter thermohydrosulfuricus* and *Caldanaerobacter subterraneus* and psychrophlilic bacteria species of *Aeromonas sp.* and *Psychrobacter sp.* They are usually selected and utilized. *Bacillus sp.* produced most thermostable lipase enzyme in industry.[8][9] Highly thermostable lipases are also obtained in *Bacillus sp.* strain L2 thermophilic bacteria.[10]

Lipase Producing Fungi

Many lipase producing yeast, fungi and actinomycetes strains were isolated from their natural habitat i.e. soil. In developing countries, *Aspergillus* and *Rhizopus spp.* were primarily studied for the reason that they have omnipresent nature, amplitude ecological distributions and nutritional contents basis requires by these organisms. [9] These organisms are not very rigorous and they are easily found everywhere. Fungal sources lipase enzyme e.g. *Aspergillus spp.* are easily available and this highly industrial important enzyme production source has gained much attention. Moreover, they are stable and also suitable for genetic alterations and manipulations wherever required. Several species of *Aspergillus* genus e.g. *A. niger*, and *A. carneus* have been commonly in use for production of lipase.[11] *Penicillium spp.*[12] such as *Penicillium citrinum, Penicillium restrictum, Penicillium simplicissimum* was recently reported for lipase production. Lipases were also obtained from a few thermophilic fungus spp. of *Humicola lanuginose*, *Myceliophthora thermophila*, *Mucour spp.* and *Thermomyces lanuginosus* and *Sporotrichum* (*Chrysosporium*) *thermophile*.[13] For Lipase production, some species of yeast such as *C. rugosa*, *Pichia bispora Pichia maxicana*, *Saccharomycopsis lipolytica* have been used and they are industrially very important species.[14]

Reactions Catalyzed By Lipases

According to Divakar and Manohar,[15] the reactions catalyzed by lipases are grouped into several types. Suitable substrates lipases are involve in enzyme catalyzing reactions of hydrolysis, esterification, and transesterification as shown in Figure 2.[16]

I) Hydrolysis

In it, ester bond breakage as an important reaction is carried out especially in excessive water availability. This technology is used in production of different glycerides i.e. monoglycerides, diglycerides, and other fatty acids, use in dairy products for flavouring (as an agent). Moreover, it is also used in detergents for household laundry purposes.

II) Esterification

As an anhydrous solvent, this enzyme catalyzed reactions take place in low water surroundings. This process takes place in controlled conditions and results in a high yield of esterified products. Examples include production of primary and secondary aliphatic and terpenic alcohols i.e. oleic acid esters. Other examples include geranyl and menthyl esters production from butyric acid and geranol or lauric acid and menthol respectively.

III) Transesterification

In this reaction, acid moiety exchange take place between two or more compounds. In it, the reaction is called acidolysis if free acid is a acyl donor, while in the reaction, if an ester is acyl donor then reaction is called interesterification. Furthermore, if nucleophile is an acyl acceptor then the reaction is called alcoholysis.

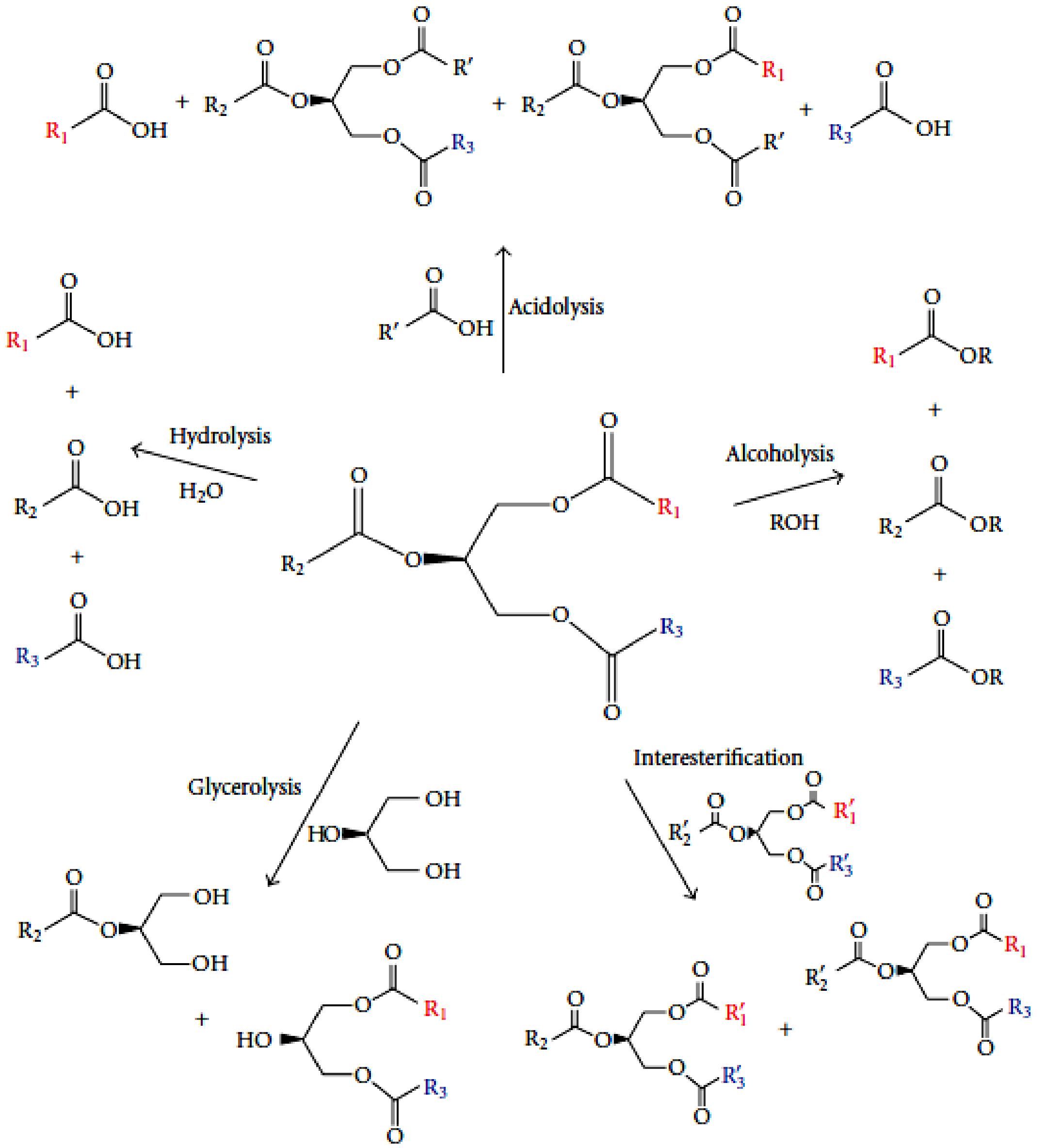

Figure 2: Reactions catalyzed by Lipase.

The Production Process of Lipase

For large scale lipase production, two most important methods are required. These are given: (a) Solid state fermentation (SSF)[17] and (b) Submerged fermentation (SmF).[18] Submerged fermentation (SmF) process was preferred technique for different enzymes production initially due to ease in use and control of various physical and chemical properties. On the other hand, due to different reasons, solid state fermentation (SSF) process is the key and chosen method for lipase production in industry, now-a-days. According to Kumar and Ray,[5] the factors involve in its preference are as follows:

1) It gives improved quality of production.
2) Ease throughout the procedure.
3) It is cost effective.
4) It saves energy and form maximum product i.e. maximum yield.

In Table 1, recent literature on production of lipases has given. A scheme on bioprocess is summarized in Figure 3.[19]

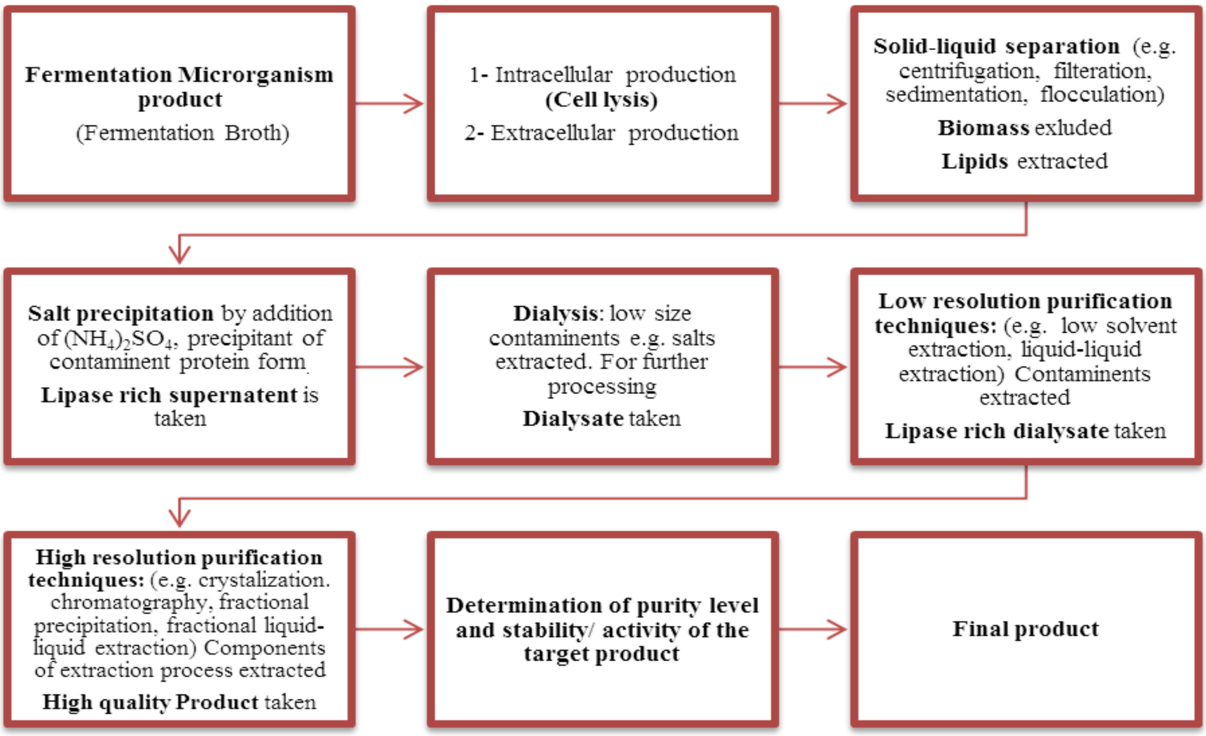

Figure 3: Schematic bioprocess for production and purification of microbial product.

Factors Involve in Production and Optimization of Lipase

For production of lipase, different factors are involved. These are: nutrient sources (i.e. nitrogen, carbon, metallic ions), pH and temperature.[20] A few of these will be given as follows

Nitrogen Sources

A variety of nitrogen sources such as organic or inorganic matters are used for lipase production. These organic or inorganic matters are corn steep liquor, soya bean meal, peptone extract, ammonium phosphate, and ammonium chloride.[21] Stimulating effect of organic nitrogen sources like peptone, yeast extract has been reported. For the maximum production of lipase, peptone is observed among one of the best candidate.[3]. In Table 2, few nitrogen sources required by microbes are shown.

Carbon Sources

Many sources of carbon are used for Lipase production. These carbon sources includes a variety of carbohydrates (i.e. fructose, glucose, maltose, galactose, sucrose, lactose, dextrose, etc), industrial and agricultural waste involving oil spills and sugarcane bagasse, canola seed oil, husk of rice, respectively.[22] Other carbon sources used by microorganisms for lipase production are given in Table (3).

Table 1: Recent literature on microbial production of lipase - An insight.

Microorganism	Substrate	Fermentation process	Incubation time for maximum activity	References
A. niger 11T53A14	Wheat bran	SSF	62.7 $U.g^{-1}$ (48 h)	[23]
Penicillium sp.	Olive oil	SmF	21.0 $U.mL^{-1}$ (120 h)	[24]
Rhizopus oryzae NRRL 3562	Coconut oil	SSF	96.2 $U.g^{-1}$ (115 h)	[25]
Bacillus subtilis OCR-4	Ground nut oil cake	SSF	4.5 $U.g^{-1}$ (48 h)	[26]
Burkholderia cepacia LTEB11	Sugarcane bagasse and sunflower seed meal	SSF	234 $U.g^{-1}$ (96 h)	[27]
Rhizopus chinensis	Wheat bran, wheat flour, andolive oil	SSF	24.4 $U.g^{-1}$ (72 h)	[28]
Pseudozyma hubeiensis HB85A	Soybean oil	SmF	5.3 $U.mL^{-1}$ (18 h)	[29]
P. chrysogenum	Grease waste and wheat bran	SSF	46 $U.mL^{-1}$ (168 h)	[5]

Metal ions

For lipase production, these metal ions play a key role. Lipase metalloenzymes property is the fundamental reasons for this. The inorganic salts are also very important for maximum lipase production. These inorganic salts includes: $MgSO_4$, $(NH_4)_2SO_4$, NaCl, K_2HPO_4, $BaCl_2$. Culture medium containing (w/v) soybean oil 4.187%, soybean powder 5.840%, K_2HPO_4 0.284%, KH_2PO_4 0.1%, $(NH_4)_2SO_4$ 0.1%, $MgSO_4$ 0.05% and Span 60 0.1% by *Candida spp.* was favourable for lipase production requirement. In addition to it, these components are critical for growth of organism and activity of lipase.

Table 2: Nitrogen sources required by microbes for production of lipase.

Microbes	Source of nitrogen	References
R. arrhizus	Yeast Extract	[30]
Aspergillus wentii, *Mucor racemosus, R. arrhizus, R. oryzae* and *R. nigricans*	Peptone	[30][31]
Rhodotorula glutinis	Ammonium Phosphate	[32]
C.cylindracea NRRL Y-17506	Ammonium Chloride	[21]
P.citrinum	Corn Steep Liquor and soybean meal	[33]

Effect of Temperature and pH on Lipase Activity

Enzyme action is time dependent process. Enzyme activity and temperature stability are important factors in enzyme action. In reaction kinetics, when temperature is increased, activity of enzyme is also increased, but further increase in temperature cause denaturation of enzyme. It can be a problem in industrial applications, where enzyme is expected to be use for long term operations. As we know that enzyme is globular protein that speeds up the chemical reaction without being consumed in it. Therefore, further increase in temperature can affect enzyme activity. It is reported that after high temperature no satisfactory enzyme activity left for product formation. It is possible that negligible activity may be present. Recently, enzymes obtained from extremophiles are found to be interestingly very important. Most inhospitable places of earth are the natural habitat of these micro-organisms. These microbes, natural habitat includes: volcanic springs. They possess enzymes with intense thermotolerance property. Thermostable lipases for different organisms such as *Bascillus spp. and Pseudomonas spp.* have been isolated. As compared to other *Bacillus* species, the thermostable enzymes obtained from thermophilic Bacillus strain A30-1 were stable. Moreover, they give maximum production when oil of corn and olive were used.[6][34] In such studies, temperature effect on action of lipase has been reported, previously.

Table 3: Carbon sources required by microbes for production of lipase.

Microbes	Sources of carbon	References
C. rugosa	Olive Oil	[35]
Bacillus sp. strain Wai 28A 45	Tripalmitin	[36]
Penicillium aurantiogriseum	Glucose and Inducer	[37]
Rhizopus Nigricans	Glucose and triglycerides	[31]
Penicillium citrinum	Olive Oil and Tween 80	[38]
Pseudozyma hubeiensis HB85A	Tween 80	[29]

The temperature optimum was recorded at 35-60°C for activity of enzyme, in such studies. The stability and activity of enzyme is also dependent on time, temperature, and effect of pH. At high temperature, enzymes are usually less stable over time. Moreover, pH value is at optimum level. Therefore, for this reason, in industrial applications, determination of optimum/favourable pH should be done under closed conditions. Therefore, in such cases, those enzymes are selected that can withstand at optimum pH and its activity is not affected. pH is an essential parameter for enzyme activity. Commonly, lipases are stable at pH i.e. ranges from 1 to 8.5.[3] Different temperature and pH suitable for microorganisms growth for lipase production are described in Table (4).

Table 4: Temperature and pH effect on lipase production.

Microbes	pH (maximum)	Temperature (maximum)	Lipase activity	References
Bacillus sp. *Bacillus brevis*	6	30 °C	168 U/ml 5.1 U/ml	[7][30]
Pseudomonas sp. *P. simplicissimum*	7.5	25 to 30 °C	4.5 U/mg 90 U/g	[39]
Burkholderia multivorans	7	37 °C	122.3 U/ml	[40]
Aspergillus sp.	7	30 °C	25.22 U/ml	[18]
Rhizopus chinensis CCTCC M201021	6	30 °C	13.875 U/ml	[41]
Rhizopus homothallicus	6.5	40 °C	10,700 U/mg	[42]
Rhodotorula mucilaginosa MTCC 8737	7	25±2 °C	72 U/ml	[43]
Serratia marcescens	7	30 °C	-	[44]
Penicillium citrinum	7	22 °C	-	[38]
P. aurantiogriseum	7	29 °C	25 U/ml	[37]
C. cylindracea	6.5	27 °C	20.4U/ml	[39]

Advance Industrial Uses of Lipases

Lipase is known as fastest growing enzyme due to its importance. In industrial production, microbial Lipases considered among one of the best enzyme. The use of different techniques involving enzyme production from various sources of microorganisms is intensively competitive for industrial use, because it is connected with health, welfare, and prosperity of mankind. Many commercially important enzymes i.e. Lipases and their derivatives are produced from *Bacillus* spp. To use this enzyme, many industrial processes are important. These industrial processes involve industrial, environmental processes and food biotechnology manufacturing. Summarizations of major applications of lipases are presented in Figure 4.[45] Application of lipases is extend to various fields of routine life. Some of the commercial lipase available is given in Table 5.

Fats and Oils Industry

Food components i.e. fats and oils are very important. For lipids modification, many industries use lipase. It involves the glycerides alteration or replacements. This reaction of enzyme catalysis involves hydrolysis, transesterification and inter-esterification of lipids molecules and their conversion into glycerol and free fatty acids. The carboxylic ester bonds in the lipids molecules undergoes hydrolysation in a randomly manner for fatty acids and glycerol production. Many lipids processing industries enormously used for the removal of grease and fats and oils modification.[46] Microbial lipases can also use for flavour development and enhancement in dairy food products and other food items (i.e. meat, vegetables, fruit, baked foods, milk product and beer) processing. As a feed part, many lipids or waxes materials are used. In addition to it, feed nutritional significance can be enhanced by lipase addition.[47] Successfully, Lipases, as a catalyst have been used for esters synthesis. In food industry, flavouring agents are used that consists of short-chain fatty acids, also known as esters. For synthesis of ester, immobilization of Lipase on silica and microemulsion that are based on organels are made. They are extensively used.

Bakery Industry

In bakery industries, different characteristics (i.e. quantity, aroma, taste, and texture) enhancement of product is made by lipase. It performs a key role in all these important parameters. This enzyme is the major part to increase bakery products storage time i.e. shelf life. Effectiveness of lipase enzyme is great in initial firmness reduction and specific bread's volume increase. It is the best candidate for this purpose. It also helps to improve the softness of the bread. Now-a-days, in baking industry, lipolytic enzymes are focussed greatly. Recently, it has reported that lipases mainly phospholipases are important substitute of emulsifier that were traditionally used. This enzyme has ability for *in situ* production of emulsifying lipids by degradation of polar wheat lipids.[48]

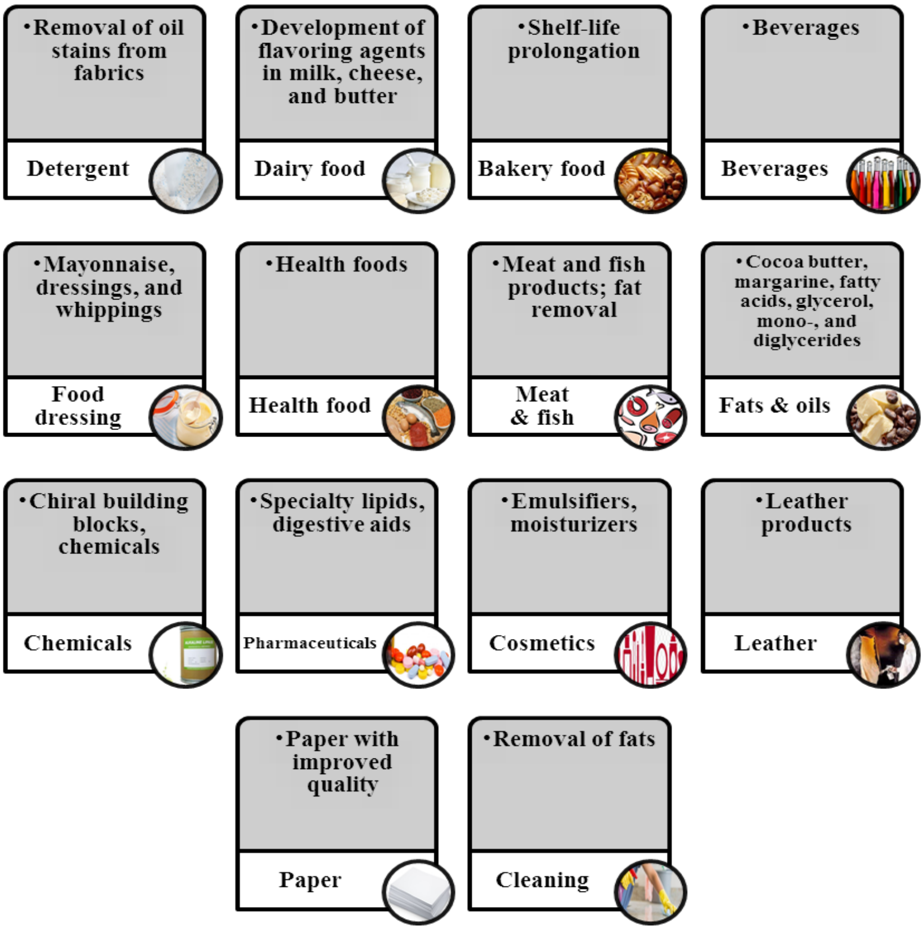

Figure 4: Microbial lipases-Industrial applications.

Dairy Industry

For hydrolysation and solubilisation of milk fats most dairy industries use lipase. Lipases increase the fatty acid chain lengths, and flavours of various cheeses. Enhancement of cheese ripening can also be done by lipases.[31] As compared to normal Cheese, enzyme modified cheese has 10% extra fat in it. For manufacturing of cheese, in industrial processing microbial production of lipase have been developed from *M. miehei*, *A. niger*, *A. oryzae* and several other species. Lipase is known as lipolytic agent. It is essentially required for lipids removal from butter, fat and cream. Unilever filed a patent in 1976 which describes hydrolytic synthetic process for cocoa butter production substitute with the help of lipase immobilization.[46]

Detergent Industry

Detergents formulation having lipases is extensively used in different countries of world. Enzymes (such as lipase, protease, amylase and cellulose) formulated detergents are now being produced in United States, Europe and Japan for washing purposes. Detergent formulation required higher pH and temperature stability. Therefore, in formulation alkaline lipases and proteases are mainly used. Lipase has valuable role in detergent quality improvement and extensively used. It works by affecting activity to hydrolyze fats; therefore it is a major additive to use in household and industrial detergency. The removal of fat detergents and soap bar become easy by this enzyme addition. Moreover, they also increase the stability and efficiency of fat removal.[34]

Food Industry

In food industry, by using lipase, gain in body weight and conversion of feed ratio have increased. Lipases, also involves to convert less desirable fats into desirable one. It readily hydrolyzes the lipid molecule into glycerol and fatty acids, which increases the digestibility of essential fats and oils. The aqueous and non-aqueous media lipolytic reactions are novel dynamics for industrially important products. Lipase catalyzed reactions form products that are used in flavour synthesis of wines, baked foods, emulsifiers, supplements and dairy products. Thus lipase, can also used as biosensor in food industry. Moreover, fats and oils can be removed from meat and fish products by these enzyme catalyzed reaction.[49]

Paper Industry

For the removing hydrophobic components of wood may be also known as 'pitch', lipase has been used. It improves the paper quality and protects against severe problems that may occur in paper manufacturing.[50] The reaction involves the conversion of waxes present in wood and triglycerides into simpler compounds by lipase action. This reaction is the key necessity of hurdle free production of paper. For this reason, the use of lipase in paper and pulp industry is enormous. As reported by Sharma and his colleague in 2001,[34] Japanese industries that are Nippon Paper Industries are using fungal lipase taken from *Candida rugosa* for hydrolysation of wood triglycerides (up to 90%). This method is found to be an effective method for pitch control during paper manufacturing.

Table 5: Sources and Industrial applications of lipases available commercially.

Types	Sources	Applications	Companies Marketing	References
Fungal Lipases	*C. antartica A/B*	Organic synthesis	Novo Nordisk (Denmark) Boehringer Mannheim Novo Nordisk, Amano, Biocatalyst	[51]
	Candida rugosa	Organic synthesis	Amano (Japan) Biocatalyst (UK) Boehringer Mannheim, Fluka, Genzyme, Sigma Novo Nordisk (Denmark) Boehringer Mannheim	
	Rhizomucor miehei	Food processing	-	
	Thermomyces lanuginosus	Detergent additive	-	
Bacterial Lipases	*P. mendocina*	Detergent additive	Genencor International (USA)	[51]
	Pseudomonas alcaligenes	Detergent additive	Genencor International (USA)	
	Burkholderia cepacia	Organic synthesis	Amano, Fluka, Boehringer Mannheim	
	Chromobacterium viscosum	Organic synthesis	Asahi, Biocatalyst (UK) Toyo Jozo (Japan) Merck (USA)	
	P. aeruginosa	Organic synthesis	Unilever (The Netherland)	
	B. glumae	Organic synthesis	Biocatalysts	
	P. fluorescens	Organic synthesis	-	

Alcohol Industry

Fermentable fatty acids are produced by the conversion of lipids with the help of lipases. Lipases are basically used for resolution of alcohols. The distinctive of character of stereospecificity of lipases is broadly used for racemic organic acid mixtures identification in immiscible biphasic systems. This process takes place via different reactions of esterification and transesterification reactions. Lipase-catalyzed transesterification reactions are used to form pure enantiomerically solution from Racemic alcohols. Lipase is a major chemical having essential role in most of the biological and chemical reactions.[34]

Biodegradation of Plastics

Biodegradable plastics are used to avoid environmental problems. Basically, it is the part of clean technology. However, biodestructible plastics are used now-a-days as an alternative to biodegradable plastics, but they both comprise of different properties. The difference is in the rate and level of degradation. In it biodegradation plastics require more treatment as compared to biodestructible one. Different strategies of biodestructibility for plastic destructibility completely devised by an Research Institute of Japan. This strategy uses the lipase ability to degrade polycaprolactone also known as aliphatic polyester. In addition to it, this method can be used to increase degradation rate by mixing aliphatic polyester compound with plastic.[52]

Biodiesel Production

Fatty acids that are monoalkyl ester may also known as Biodiesel. According to Ribeiro and his colleagues,[16] In this series of reactions, methanol or ethanol and oil or fat reaction take place in catalyst presence as a result glycerin mixture and fatty acids that is alkyl esters is generated. This is called biodiesel (Figure 5). It is beneficial as compared to petroleum diesel. Biodiesel basic characteristics consist of biodegradability. Furthermore, it has low level of combustion products that are particulates and other oxides mainly carbon and sulfur oxides. Microrganisms associated to different natural habitat of temperature e.g. mesophiles, psychrophiles, and thermophiles are involve in production of biodiesel. Thermophilic lipases such as *Rhizomucor miehei* and *Thermomyces lanuginose*, which is immobilized on ion-exchange resin and silica gel are involve in production of biodiesel by sunflower oil conversion, respectively. This conversion mainly takes place by the process of methanolysis.[53]

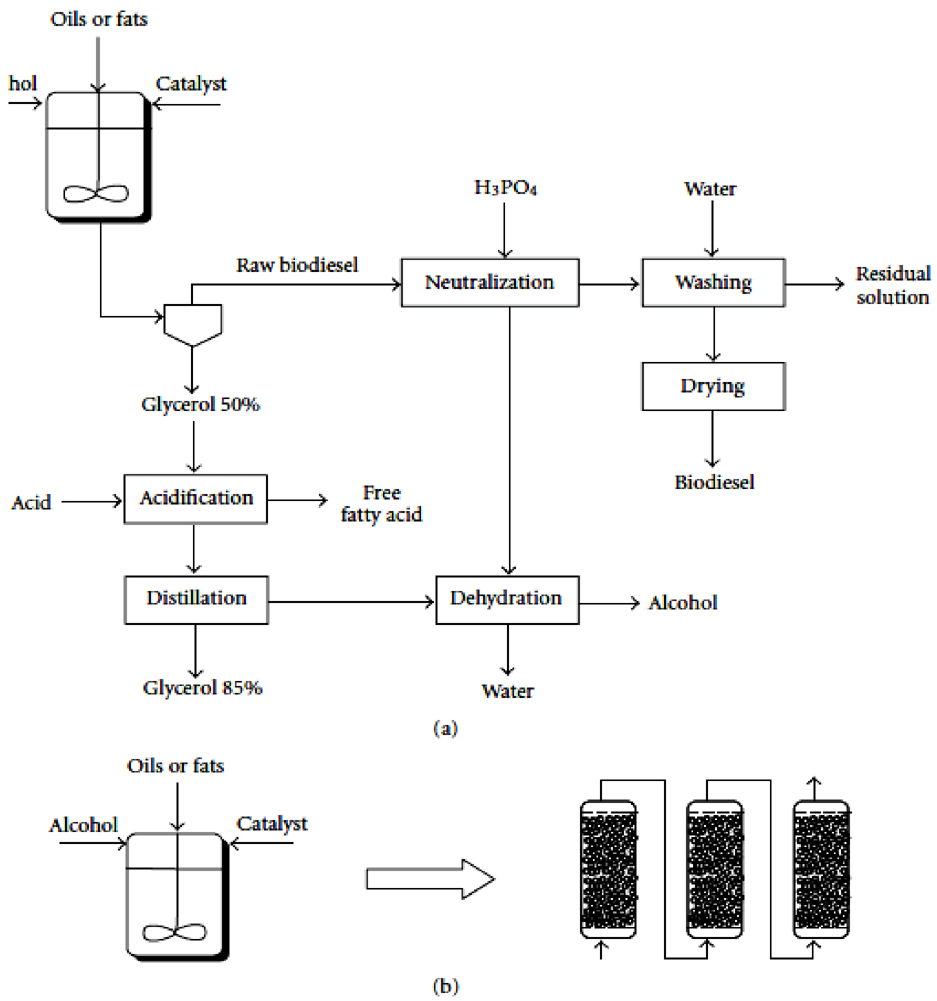

Figure 5: Biodiesel production illustrated by flowsheet. (a) Non-Enzymatic industrial process involve chemical; (b) Enzymatic process (alteration in reactor design for biocatalysis).

Cosmetics Industry

Lipases are essential ingredients of personal care products which are most commonly used. Recently, Unichem International has produced different chemical compounds. These chemical compounds includes: isopropyl myristate, isopropyl palmitate, and 2-ethylhexyl palmitate. The difference between the enzymatic and non-enzymatic process for production of cosmetic esters has given in Figure 6. [54] These compounds are used in several products like skin, sun-tan creams, and bath oils etc. Moreover, Wax esters are produced by *C. cylindracea* lipase in batch fermentation.[55]

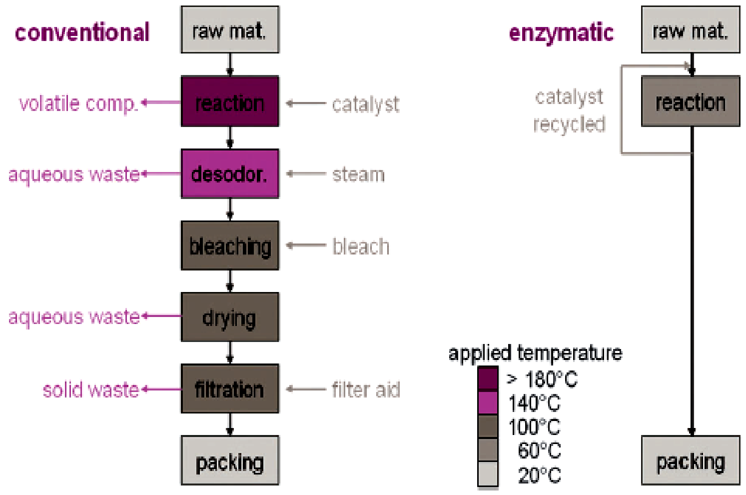

Figure 6: Conventional i.e. non-enzymatic and enzymatic esterification of cosmetic fatty acid esters production.

Agrochemical Industry

Lipases are extensively used for herbicides production in agrochemical industry. Novel herbicide that is Indanofan is discoverd. It is used in paddy fields for grass weeds. In 1999, its racemic mixture was commercialized, but when herbicidal activity tested only enantiomer that activate itself was (S)-enantiomer. For its synthesis, resolution reaction by enzyme catalysis is involved which further consists of inversion techniques that take place chemically. Figure 7 illustrates an outline of yields and use of different lipases sources for fermentation. From it, esters of low molecular weight forms. Moreover they are also involve in different reaction catalysed by lipases e.g. synthesis, interesterification, transesterification and hydrolysis of fats and oils.[56]

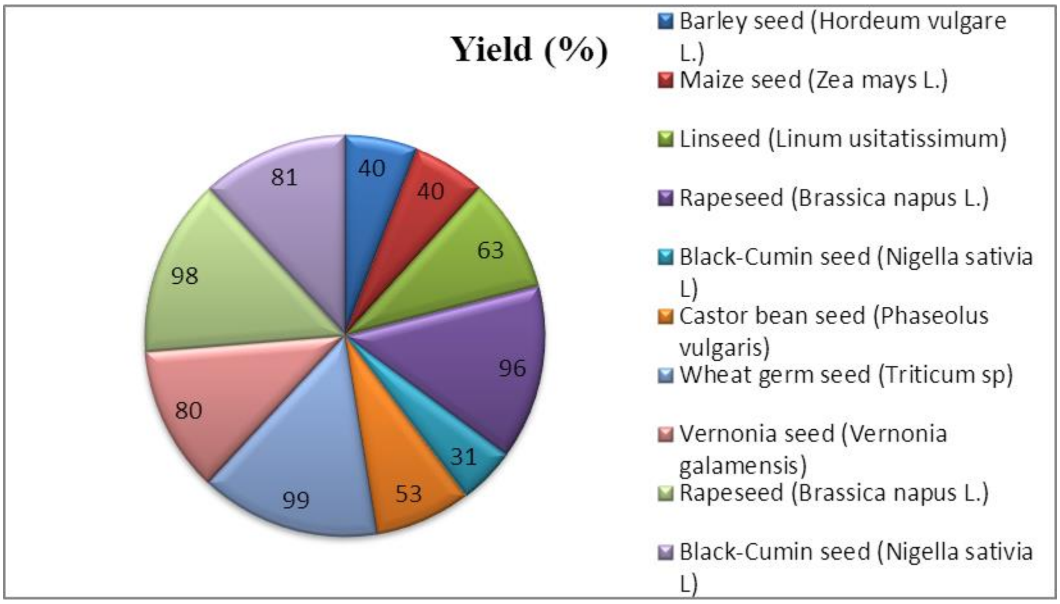

Figure 7: Some lipases and their applications.

Pollutants Bioremediation

Microorganisms, animals and plants derived lipids are degraded by lipase. Lipases are used for bioremediation of organic pollutants such as oil spill and treatment of waste effluents. Lipase used organic pollutants such as oil spill as substrate. The reaction involve the simple hydrolysis process i.e. triacylglycerols to glycerols and free-fatty acids. It is basically involve in oil spills control. The activity of lipase is an indicator parameter of soil hydrocarbon degradation testing. In biphasic oil-water system, *Candida rugosa* lipases use for hydrolysis of triolein as shown in figure 8.[57]

Different Pharmaceutical compounds are produced by microbial lipases which are further used for improvement of PUFAs from the source of plant and animal lipids. Moreover, a range of pharmaceuticals products are form by mono and diacylglycerides. In addition to it, PUFAs have many metabolic benefits due to which they are widely used as food additives, pharmaceuticals and nutraceuticals. In the field of medicine, liposomes are actively used for drugs action optimization to targeted transportation and anatomical barriers.[58]

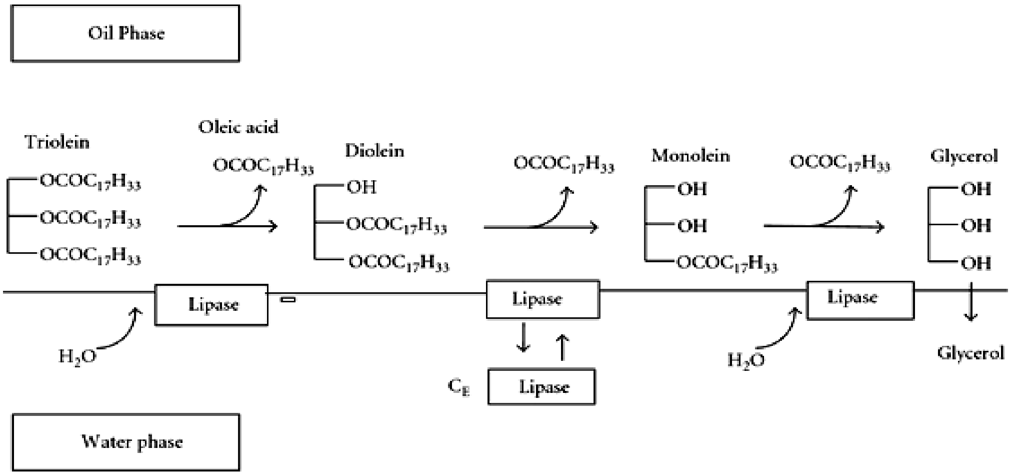

Figure 8: In biphasic oil-water system, mechanism proposed for hydrolysis of triolein by *Candida rugosa* lipase. C_E = Enzyme concentration in the bulk of water phase.

Pharmaceutical Industry

Anti-inflammatory drugs that have non-steroidal properties consist of Profens (an active enantiomer form) i.e. Ketoprofen and ibuprofen. Lipid catalyzation are involve in kinetic resolution (by using hydrolysis and esterification) to synthesize Pure (R)-Ketoprofen and (s)-ibuprofen. In addition to in situ racemization, synthetic reactions are catalyzed by lipases. The kinetic enzymatic esterification of *rac* Ketoprofen and *rac*-Ibuprofen is illustrated in figure 9.[59] These are ultimate life saving drugs. Furthermore, immobilized lipases are involved in successful production of nutraceuticals.[58]

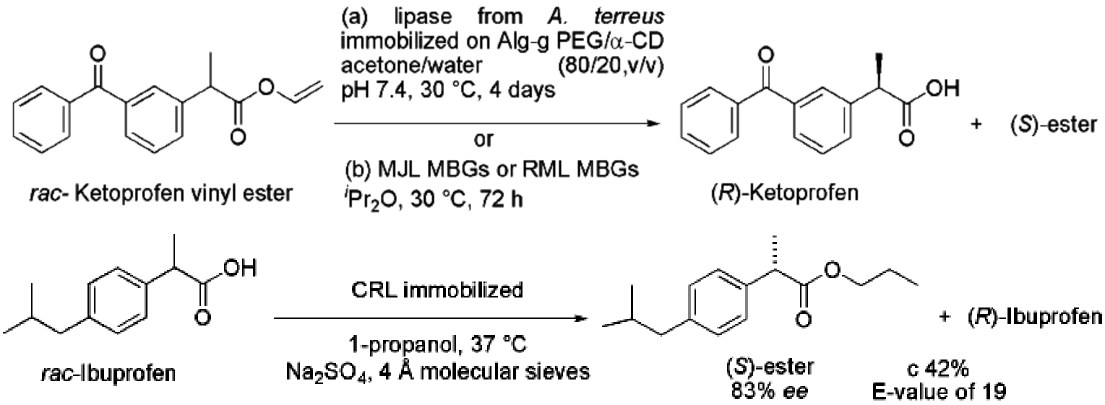

Figure 9: Kinetic enzymatic esterification of *rac* Ketoprofen and *rac* Ibuprofen, a nonsteroidal drug with anti inflammatory activity. MBGs= Micro-emulsion-based organogels.

CONCLUSION

This review illustrates the importance of bacterial and fungal microbes in the production of lipase. From an industrial point of view, mostly organisms of the genus *Bacillus* have been utilized for the production of thermostable lipases. It has been reported that the enzymes produced from bacterial sources can withstand heat inactivation up to a temperature of 70°C. The fungal species especially, *A. niger, and A. carneus* are used for the production of lipase, but enzyme from fungal sources is not so capable of producing the thermostable variety and can withstand a temperature of only upto 40°C. On the other hand, among the bacterial species, *Bascillus spp.* is the most promising strain to produce highly thermostable lipase. The maximal lipase stability from this organism's activity has been recorded at temperature up to 30°C at pH 7.

REFERNECES

1. De-Romo AC. Tallow and the time capsule: Claude Bernard's discovery of the pancreatic digestion of fat. Hist Philos Life Sci, 1989; 11(2): 253-74.
2. Arpigny JL, Jaeger KE. Bacterial lipolytic enzymes: classification and properties. Biochem J, 1999; 343: 177-183.
3. Salihu A, Alam MZ. Production and applications of microbial lipases: A review. Sci Res Essays, 2012; 7(30): 2667-2677.
4. Gopinath SCB, Anbu P, Lakshmipriya T, Hilda A. Strategies to Characterize Fungal Lipases for Applications in Medicine and Dairy Industry. BioMed Res Int, 2013; 2013(154549): 1-10.
5. Kumar, Ray J. Fungal Lipase Production by Solid State Fermentation-An Overview. J Anal Bioanal Tech, 2014; 6:1.
6. Wang Y, Srivastava KC, Shen GJ, Wang HY.Thermostable alkaline lipase from a newly isolated thermophilic Bacillus strain, A30-1 (ATCC 53841). J Ferment Bioeng, 1995; 79: 433-8.
7. Ertugrul S, Donmez G, Takac S. Isolation of lipase producing *Bacillus* sp. from olive mill wastewater and improving its enzyme activity. J Hazard Mat, 2007; 149: 720-724.
8. Nawani N, Kaur J. "Studies on lipolytic isoenzymes from a thermophilic *Bacillus sp.*: Production, purification and biochemical characterization". Enzym Microbiol Technol, 2007; 40: 881-887.
9. Thakur S. Lipases, its sources, Properties and Applications: A Review. Int J Sci Eng Res, 2012; 3: 1-29.
10. Shariff FM, Leow TC, Mukred AD, Salleh AB, Basri M, Rahman RNZRA. "Production of L2 lipase by *Bacillus sp.* strain L2: Nutritional and physical factors". J Microbiol Biotechnol, 2007; 47: 406-412.
11. Dutra JCV, Terzi SC, Bevilaqua JV, Damaso MCT, Couri S, Langone MAP, *et al.*, "Lipase production in solidstate fermentation monitoring biomass growth of *Aspergillus niger* using digital image processing". Appl Biochem Biotechnol, 2008; 147: 63-75.
12. Annibale AD, Sermanni GG, Federici F, Petruccioli M. "Olive-oil wastewaters: A promising substrate for microbial lipase production". Bioresour Technol, 2006; 97: 1828-1833.
13. Maheshwari R, Bharadwaj G, Bhat MK. Thermophilic Fungi: Their Physiology and Enzymes. Microbiol. Mol Biol Rev, 2000; 64(3): 461-488.
14. Vakhlu J, Kour A. "Yeast lipases: Enzyme purification, biochemical properties and gene cloning". Electronic J Biotechnol, 2006; 9: 1-17.

15. Divakar S, Manohar B. Use of lipase in the industrial production of esters. In: Polaina J, MacCabe AP. (Eds.), Industrial Enzym, 2007; Wil VCH Verlag: 283-300.
16. Ribeiro BD, Castro AM, Coelho MAZ, Freire DMG. Production and Use of Lipases in Bioenergy: A Review from the Feedstocks to Biodiesel Production. Enzym Res, 2011; 2011: 1-17.
17. Mukhtar H, Hanif M, Rehman AU, Nawaz A, Haq IU. Studies on the lipase production by *Aspergillus niger* through solid state fermentation. Pak J Bot, 2015; 47(SI): 351-354.
18. Colla LM, Rizzardi J, Pinto MH, Reinehr CO, Bertolin TE, Vieira-Costa JA. Simultaneous production of lipases and biosurfactants by submerged and solid-state bioprocesses. Bioresour Technol, 2010; 101: 8308-8314.
19. Ventura SPM, de Barros RLF, Barbosa JMP, Soares CMF, Lima AS, Coutinho JAP. Production and purification of an extracellular lipolytic enzyme using ionic liquidbased aqueous two-phase systems. Green Chem, 2012; 14 (2012): 734-740.
20. Treichel H, de Oliveira D, Mazutti MA, Di Luccio M, Oliveira VJ. A Review on Microbial Lipases Production. Food biooprocess Technol, 2010; 3: 182-196.
21. Brozzoli V, Crognale S, Sampedro I, Federici F, Annibale AD, Petruccioli M. Assessment of olive-mill wastewater as a growth medium for lipase production by *Candida cylindracea* in bench-top reactor. Bioresour Technol, 2009; 100: 3395-3402.
22. Amin M, Bhatti HN, Zuber M, Bhatti IA, Asgher M. Potential use of agricultural wastes for the production of Lipase by *Aspergillus melleus* under solid state fermentation. J Anim Plant Sci, 2014; 24(5): 1430-1437.
23. Damaso MCT, Passianoto MA, Freitas SC, Freire DMG, Lago RCA, Couri S. Utilization of agroindustrial residues for lipase production by solid-state fermentation. Braz J Microbiol, 2008; 39(4): 676-681.
24. Wolski E, Menusi E, Mazutti M *et al.*, Response surface methodology for optimization of lipase production by an immobilized newly isolated *Penicillium* sp. Ind Eng Chem Res, 2008; 47(23): 9651-9657.
25. Garlapati VK, Vundavilli PR, Banerjee R. Evaluation of lipase production by genetic algorithm and particle swarm optimization and their comparative study. Appl Biochem Biotechnol, 2010; 162(5): 1350-1361.
26. Singh M, Saurav K, Srivastava N, Kannabiran K. Lipase production by *Bacillus subtilis* OCR-4 in solid state fermentation using ground nut oil cakes as substrates. Current Res J Bio Sci, 2005; 20102(4): 241-245.
27. Salum TFC, Villeneuve P, Barea B *et al.*, Synthesis of biodiesel in column fixed-bed bioreactor using the fermented solid produced by *Burkholderia cepacia* LTEB11. Process Biochem, 2010; 45(8): 1348-1354.
28. Sun SY, Xu Y. Solid-state fermentation for “wholecell synthetic lipase” production from *Rhizopus chinensis* and identification of the functional enzyme. *Process Biochem,* 2008; 43(2): 219-224.
29. Bussamara R, Fuentefria AM, de-Oliveira E, Broetto L, Simcikova M, Valente A, Vainstein MH. Isolation of a lipase-secreting yeast for enzyme production in a pilot-plant scale batch fermentation. Bioresour Technol, 2010; 101: 268-275.
30. Rajendran A, Thangavelu V. Statistical experimental design for evaluation of medium components for lipase production by *Rhizopus arrhizus* MTCC 2233. LWT - Food Sci Technol, 2009; 42:985-992.
31. Ghosh PK, Saxena RK, Gupta R, Yadav RP, Davidson S. Microbial lipases: production and applications. Sci Prog, 1996; 79(2): 119-157.
32. Papaparaskevas D, Christakopoulos P, Kekos D, Macris BJ. Optimizing production of extracellular lipase from *Rhodotorula glutinis*. Biotechnol Lett, 1992; 14: 397-402.
33. Sztajer H, Maliszewska I. The effect of culture conditions on lipolytic productivity of *Penicillium citrinum*. Biotechnol Lett, 1989; 11: 895-898.
34. Sharma R, Chistib Y, Banerjeea UC. Production, purification, characterization, and applications of lipases. Biotechnol Advances, 2001; 19 (2001):627-662.
35. Benjamin S, Pandey A. Optimization of Liquid media for lipase production by *Candida rugosa*. Bioresour Technol, 1996; 55: 167-170.
36. Janssen PH, Monk CR, Morgan HW. A thermophilic, lipolytic *Bacillus* sp. and continuous assay of its *p*-nitrophenyl-palmitate esterase activity. FEMS Microbiol Lett, 1994; 120: 195-200.
37. Lima VMG, Krieger N, Sarquis MIM, Mitchell DALP, Ramos, Fontana JD. Effect of Nitrogen and Carbon Sources on Lipase Production by *Penicillium aurantiogriseum.* Food technol Biotechnol, 2003; 41(2): 105-110.
38. Maliszewska I, Mastalerz P. Production and some properties of lipase from *Penicillium citrinum.* Enzym Microb Technol, 1992; 14: 190-193.
39. Haba E, Bresco O, Ferrer C, Marques A, Busquets M, Manresa A. Isolation of lipase-secreting bacteria by deploying used frying oil as selective substrate. Enzym Microbiol Technol, 2000; 26: 40-44.
40. Gupta N, Sahai V, Gupta R. Alkaline lipase from a novel strain Burkholderia multivorans: Statistical medium optimization and production in a bioreactor. Process Biochem, 2007; 42(4): 518-526.
41. Teng Y, Xu Y. “Culture condition improvement for wholecell lipase production in submerged fermentation by Rhizopus chinensis using statistical method”, Bioresour Technol, 2008; 99: 3900-3907.
42. Diaz JCM, Rodrıguez JA, Roussos S, Cordova J, Abousalham A, Carrier F, Baratti J. Lipase from the thermotolerant fungus *Rhizopus homothallicus* is

more thermostable when produced using solid state fermentation than liquid fermentation procedures. Enzym Microb Technol, 2006; 39: 1042-1050.

43. Potumarthi R, Subhakar C, Vanajakshi J, Jetty A. Effect of Aeration and Agitation Regimes on Lipase Production by Newly Isolated *Rhodotorula mucilaginosa* MTCC 8737 in Stirred Tank Reactor Using Molasses as Sole Production Medium. Appl Biochem Biotechnol, 2008; 151: 700-710.
44. Long Z, Xu J, Pan J. Significant Improvement of *Serratia marcescens* Lipase Fermentation, by Optimizing Medium, Induction, and Oxygen Supply. Appl Biochem Biotechnol, 2007; 142:148-157.
45. Vulfson EN. Industrial applications of lipases. In: Woolley P, Peterson SB, editors. Lipases—their structure, biochemistry and applications. 1994; *Cambridge Univ Press*: 271-88.
46. Ray A. Application of Lipase in Industry. Asian J Pharm Tech, 2012; 2(2): 33-37.
47. Luciane MC, Aline MMF, Rizzardi J, Bertolin TE, Reinehr CO, Costa JAV. Production and Characterization of Lipases by Two New Isolates of *Aspergillus* through Solid-State and Submerged Fermentation. BioMed Res Int, 2015; 725959: 1-9.
48. Collar C, Martilnoz JC, Andrew P, Armero E. Effect of enzyme association on bread dough performance: A response surface study. Food Sci Technol Int, 2000; 6: 217-226.
49. Silva WOB, Mitidieri S, Schrank A, Vainstein MH. Production and extraction of an extracellular lipase from the entomopathogenic fungus *Metarhiziumanisopliae*. Process Biochem, 2005; 40: 321-326.
50. Jaeger KE, Reetz TM. Microbial lipases from versatile tools for biotechnology. Trends Biotechnol, 1998; 16: 396-403.
51. Gunasekaran V, Das D. Lipase fermentation: progress and prospects. Ind J Biotechnol, 2005; 4: 437-445.
52. de Castro HF, Anderson WA. Fine chemicals by biotransformation using lipases. Quimica Nova,1995; 18(6): 544-554.
53. Al-Zuhair S. Production of biodiesel by lipase-catalyzed transesterification of vegetable oils: A kinetics study. Biotechnol Prog, 2005; 21: 1442-1448.
54. Thum O. Enzymatic Production of Care Specialties Based on Fatty Acid Esters. Tenside Surfactants Deterg, 2004; 41: 287–290.
55. Hoq MM, Yamane T, Shimizu S, Funada T, Ishida S. J Am Oil Chem Soc, 1985; 62: 1016–1021.
56. Barros M, Fleuri LF, Macedo GA. Seed Lipases: Sources, Applications and Properties – A Review. Braz J Chemical Eng, 2010; 27(1) : 15 – 29.
57. Hermansyah H, Wijanarko A, Gozan M, *et al*., Consecutive reaction model for triglyceride hydrolysis using lipase. J Technol, 2007; 2: 151-157.
58. Linko YY, Wu XY. Biocatalytic production of useful esters by two forms of lipase from *Candida rugosa*, J Chem Technol Biotechnol, 1996; 65: 163-170.
59. Hu, Wang CN, Zhang W, Zhang S, Meng Y, Yu X. Immobilization of Aspergillus terreus lipase in self-assembled hollow nanospheres for enantioselective hydrolysis of ketoprofen vinyl ester. J Biotechnol, 2015; 194: 12-18.