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Industrial applications of microbial lipases

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Abstract

Lipases are a class of enzymes which catalyse the hydrolysis of long chain triglycerides. Microbial lipases are currently receiving much attention with the rapid development of enzyme technology. Lipases constitute the most important group of biocatalysts for biotechnological applications. This review describes various industrial applications of microbial lipases in the detergent, food, flavour industry, biocatalytic resolution of pharmaceuticals, esters and amino acid derivatives, making of fine chemicals, agrochemicals, use as biosensor, bioremediation and cosmetics and perfumery.

Keywords: Lipases; Biocatalysts; Industrial application

1. Introduction

Enzymes are considered as nature's catalysts. Most enzymes today (and probably nearly all in the future) are produced by the fermentation of biobased materials [1]. Lipids constitute a large part of the earth's biomass, and lipolytic enzymes play an important role in the turnover of these water-insoluble compounds. Lipolytic enzymes are involved in the breakdown and thus in the mobilization of lipids within the cells of individual organisms as well as in the transfer of lipids from one organism to another [2]. Microorganisms have earlier been found to produce emulsifying agents or biosurfactants to help solubilize lipids [3]. Several thousand enzymes possessing different substrate specificities are known, however only comparatively few enzymes have been isolated in a pure form and crystallized, and little has been known about their structure and function. The advent of protein engineering techniques makes their application to important industrial enzymes, such as proteases and lipases used in detergents, amylases and glucose isomerase used in starch processing and in the bioprocessing of raw materials or in the synthesis of organic chemicals are very efficient [4].

The particular benefits offered by enzymes are specificity, mild conditions and reduced waste. It may be possible, by choosing the right enzyme, to control which products are produced, and unwanted side reactions are minimized due to specificity of enzymes that appear in the waste stream. The plant using enzymatic reactions can be built and operated at much lower capital and energy cost. Enzyme-based processes tend to have lower waste treatment costs. Enzymes however are biodegradable, and since they usually are dosed at 0.1–1.0% of the substrate, the contribution of the enzyme to the BOD in the waste stream is negligible [5].

Microbial enzymes are often more useful than enzymes derived from plants or animals because of the great variety of catalytic activities available, the high yields possible, ease of genetic manipulation, regular supply due to absence of seasonal fluctuations and rapid growth of microorganisms on inexpensive media. Microbial enzymes are also more stable than their corresponding plant and animal enzymes and their production is more convenient and safer [6].

Only about 2% of the world's microorganisms have been tested as enzyme sources. Bacterial strains are generally more used as they offer higher activities compared to yeasts [7] and tend to have neutral or alkaline pH optima and are often thermostable. Genetic and environmental manipulation to increase the yield of cells [8], to increase the enzyme activity of the cells by making the enzyme of interest constitutive, or by inducing it, or to produce altered enzymes [9], may be employed easily using microbial cells because of their short generation times, their relatively simple nutritional requirements, and since screening procedures for the desired characteristic are easier.

Some industrially important enzymes have been isolated from plants. Hydroxynitrile lyases (HNLs) have been purified from various species of higher plants. Release of HCN from

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Table 1
Enzymes produced by *Bacillus* sp.

Enzymes	Reference
Pyrophosphatase	[161,162]
Cellulase	[163–165,72]
Ferrochelatase	[166]
Serine protease	[167,168]
Alkaline protease	[169–171]
Neutral proteases	[172,173]
Amylase	[174–176,72]
Lipase	[177–181]
Esterase	[182]
Xylanase	[183]
Phytase	[184,185]
Keratinase	[186,187]
Elastase	[188]
Beta-glucanase	[189]
Pullulanase	[190]

cyanogenic glycosides is due to the cleavage of the carbohydrate moiety by beta-glucosidases to yield the corresponding alpha-hydroxynitrile, which dissociates spontaneously into HCN and a carbonyl compound, or by action of an alpha-hydroxynitrile lyase (HNL). HNLs have great potential to be used as biocatalysts for the synthesis of optically active alpha-hydroxynitriles which are important building blocks in the fine chemical and pharmaceutical industries [10]. (*R*)- as well as (*S*)-cyanohydrins are now easily available as a result of the excellent accessibility, the relatively high stability and the easy handling of hydroxynitrile lyases (HNLs) [11].

Many plant lipases have been isolated now which can be used for the production of important lipases. A cDNA clone encoding a lipase (lipolytic acyl hydrolase) expressed at the onset of petal senescence has been isolated by screening a cDNA expression library prepared from carnation flowers (*Dianthus caryophyllus*). Over-expression of the clone in *Escherichia coli* yielded a protein of the expected molecular weight that proved capable of deesterifying fatty acids from *p*-nitrophenylpalmitate, tri-linolein, soybean phospholipid, and Tween in both in vitro and in situ assays of enzyme activity [12]. Many companies market digestive enzymes prepared from plant and fungal lipases. Doctor's Holistic Market manufactures Chiro-Zyme, the digestive plant enzymes formula containing lipase from *Aspergillus niger* and *Rhizopus oryzae* [13].

Bacillus species have been found to produce a number of enzymes other than lipases that may be utilized industrially. Some of the industrially important enzymes produced by *Bacillus* sp. are listed in Table 1.

2. Historical background

The presence of lipases has been observed as early as in 1901 for *Bacillus prodigiosus*, *B. pyocyaneus* and *B. fluorescens* [14] which represent today's best studied lipase producing bacteria now named *Serratia marcescens*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*, respectively. Enzymes hydrolyzing triglycerides have been studied for well over 300 years and the ability of the lipases to catalyse the hydrolysis and also the synthesis of esters has been recognized nearly 70 years ago [15].

In 1856, Claude Bernard first discovered a lipase in pancreatic juice as an enzyme that hydrolysed insoluble oil droplets and converted them to soluble products. Lipases have traditionally been obtained from animal pancreas and are used as a digestive aid for human consumption either in crude mixture with other hydrolases (pancreatin) or as a purified grade. Initial interest in microbial lipases was generated because of a shortage of pancreas and difficulties in collecting available material.

Lipases differ greatly as regards both their origins (which can be bacterial, fungal, mammalian, etc.) and their properties, and they can catalyse the hydrolysis, or synthesis, of a wide range of different carboxylic esters and liberate organic acids and glycerol. They all show highly specific activity towards glyceridic substrates.

3. Industrially used enzymes

Lipolytic enzymes are currently attracting an enormous attention because of their biotechnological potential [16]. They constitute the most important group of biocatalysts for biotechnological applications. The high-level production of microbial lipases requires not only the efficient overexpression of the corresponding genes but also a detailed understanding of the molecular mechanisms governing their folding and secretion. The optimization of industrially relevant lipase properties can be achieved by directed evolution. Furthermore, novel biotechnological applications have been successfully established using lipases for the synthesis of biopolymers and biodiesel, the production of enantiopure pharmaceuticals, agrochemicals, and flavour compounds [17].

Some of the industrially important chemicals manufactured from fats and oils by chemical processes could be produced by lipases with greater rapidity and better specificity under mild conditions [18,19]. The chemo-, regio- and enantiospecific behavior of these enzymes has caused tremendous interest among scientists and industrialists [20].

Lipases from a large number of bacterial, fungal and plant and animal sources have been purified to homogeneity [20]. Lipases isolated from different sources have a wide range of properties depending on their sources with respect to positional specificity, fatty acid specificity, thermostability, pH optimum, etc. [21]. One could probably find a lipase from nature that would be suitable for desired application. Use of industrial enzymes allows the technologist to develop processes that more closely approach the gentle, efficient processes in nature.

Numerous species of bacteria, yeasts and molds produce lipases (Table 2). Taxonomically close strains may produce lipases of different types.

With the increased awareness of environment and cost issues, biotechnology is gaining ground rapidly due to the various advantages that it offers over conventional technologies. The field of industrial enzymes is now experiencing major R&D initiatives, resulting in both the development of a number of new products and in improvement in the process and performance of several existing products. According to a report from Business

Table 2
Isolation of Lipases from various microorganisms

Lipase producing Microorganisms	Reference
Bacillus sp.	[36,191,42,35]
Bacillus subtilis	[192,193]
Bacillus thermoleovorans	[194]
Bacillus thermocatenulatus	[180]
Bacillus coagulans	[181]
Pseudomonas sp.	[195]
Pseudomonas aeruginosa	[196,197]
Pseudomonas fluorescens	[198]
Pseudomonas fragi	[199]
Enterococcus faecalis	[200]
LactoBacillus plantarum	[201]
Staphylococcus haemolyticus	[202]
Staphylococcus aureus	[203,204]
Staphylococcus warneri	[205]
Staphylococcus xylosus	[206]
Penicillium cyclopium	[207,208]
Penicillium simplicissimum	[209]
Aspergillus niger	[210]
Aspergillus oryzae	[211]
Botrytis cinerea	[212]
Chromobacterium viscosum	[213]
Streptomyces flavogriseus	[214]
Trichosporon asteroides	[215]
Trichosporon laibacchii	[216]
Rhizopus sp.	[92]
Rhizomucor miehei	[217]
Geotrichum candidum	[218,219]
Pichia burtonii	[220]
Candida cylidracae	[221]
Acinetobacter sp.	[51]
Fusarium solani	[222]

Communications Company, Inc. the global market for industrial enzymes was estimated at $2 billion in 2004 (Table 3). Volume growth of industrial enzymes is between 4% and 5% AAGR (average annual growth rate), which is accompanied by decreasing prices, due to the increase in the number of smaller players competing in the market. As a result, the market is expected to rise at an AAGR of a little over 3% over the next 4 years, and the total industrial enzyme market in 2009 is expected to reach nearly $2.4 billion [22].

The industrial enzyme market is divided into three application segments: technical enzymes, food enzymes, and animal feed enzymes (Fig. 1). The growth of animal feed enzymes is somewhat higher, expected to be close to 4% AAGR, helped in large part by increased use of phytase enzyme to fight phosphate

Table 3
Global enzyme markets by application sectors, through 2009 ($ millions)

	2002	2003	2004	2009	%AAGR 2004–2009
Technical enzymes	978.2	1009.2	1040.0	1222.0	3.3
Food enzyme	701.0	720.0	740.0	863.0	3.1
Animal feed enzyme	210.8	215.6	220.0	267.0	3.9
Total	1890.0	1945.0	2000.0	2352.0	3.3

Source: Business Communications Company, Inc.

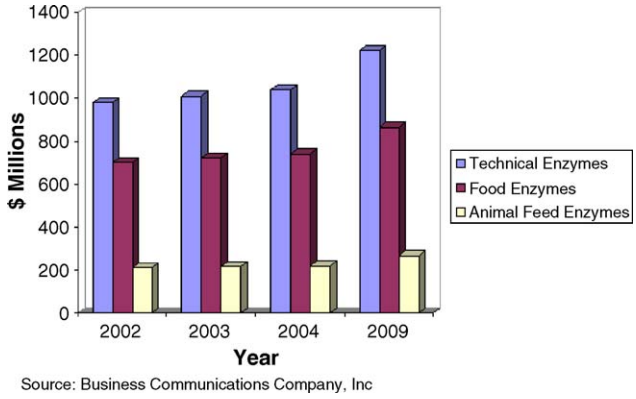

Fig. 1. Global enzyme markets by application sectors, through 2009 ($ millions).

pollution. Technical enzymes for detergent and pulp and paper manufacturing among others are the largest segments, with a 52% share. Growth will parallel the overall market. The confectionery and sweetener segment is the largest sector in food applications and is expected to grow at a healthy AAGR of around 3%. Overall, the enzymes in various food application sectors will be showing healthy growth, with an AAGR of 3.1% [22].

Among technical enzymes carbohydrases and proteases are the principal enzymes used in food and animal feed. Proteases, amylases, lipases, and cellulases are used in cleaning compounds including; laundry detergents, dishwashing detergents, and other cleaners. The production of chemicals is the third most important market. Alcohol fermentation makes up most of the market segment, pharmaceuticals (steroids and antibiotics), amino acids, proteins, lipids (triglycerides, phospholipids), textile, leather, followed by fur applications. The major part of this application is cotton and cellulosic textiles, which use mainly cellulases and amylases. The last major market is pulp and paper, which accounts for about 6%. The most notable use is that of xylanase for the prebleaching of pulp instead of the chemicals, especially chlorine, helps to reduce bleach requirements and thus reduces pollution problems. Amylase is also used in pulp and paper industry [23].

Protease and amylase lead the market with current shares of 25% and 20%, respectively. Both markets are expected to grow at approximately the same AAGR of 2.8% through the forecast period. Geographically, the North America market is currently leading with 36% of total market share and will continue to do so through the forecast period. New and emerging applications have helped to drive demand and the industry is responding with a continuous stream of innovative products [22]

A rapid growth of industries has greatly enhanced the problem of water-pollution. Industries like textile, paper, synthetic drug, insecticides and tanneries discharge large volume of highly objectionable wastes into the main water sources. The systematic industrial use of enzymes and microorganisms dates back well over 100 years. The first deliberate uses of isolated enzymes in commercial processes would appear to be the introduction of pancreatic proteases to treat hides by Rohm in 1908, and the use of proteases to chill-proof beer by Wallterstein in 1911, invertase was the first enzyme immobilized and commercially used

by Tate and Lyle in 1908 [4]. Enzymes have been used in the leather industry for many years and also have been introduced into modern textile industries. The main applications of enzymes in the leather industry are proteases which help in the dehairing of the animal hides and lipases are used for degreasing [24].

The utilization of gene technology and of new production technologies have made industrial enzymes with improved properties or better cost performance available. The benefits to the customers are considerable: cost savings in the application process, improved product quality, and in most cases also a significantly reduced impact on the environment. Gene technology offers several benefits to the enzyme industry. This technology enables the use of safe, well documented host organisms easy to cultivate, the microbial production of enzymes of animal and plant origin, the realization of enhanced efficiency and high product purity, and also the production of enzymes with improved stability and activity [25].

Directed evolution methods are now widely used for the optimization of diverse enzyme properties, which include biotechnologically relevant characteristics like stability, regioselectivity and in particular, enantioselectivity. In principle, three different approaches are followed to optimize enantioselective reactions: the development of whole-cell biocatalysts through the creation of designer organisms; the optimization of enzymes with existing enantioselectivity for process conditions; and the evolution of novel enantioselective biocatalysts starting from non-selective wild-type enzymes [26].

It is widely accepted that up to 99.8% of the microbes present in many environments are not readily culturable. 'Metagenome technology' tries to overcome this problem by developing and using culture-independent approaches. From the outset, metagenome-based approaches have led to the accumulation of an increasing number of DNA sequences, retrieved from uncultured microbes. These genomic sequences are currently exploited for novel biotechnological and pharmaceutical applications and to increase our knowledge on microbial ecology and physiology of these microbes. Using the metagenome sequences to fully understand how complex microbial communities function and how microbes interact within these niches represents a major challenge for microbiologists today [27]. In an effort to isolate novel genes from enormous and largely unexploited gene pools in uncultured microorganisms and/or those that are difficult to culture, the metagenomic library approach has recently been used successfully [28–30]. This type of approach does not require the cultivation of diverse microorganisms from environmental samples, which is often difficult or impossible and can result in an enrichment of dominant strains under a specific selective condition. Thus, more global microbial genetic information can be provided from total microorganisms than from culturable subpopulations or enrichment cultures [31].

The construction and screening of metagenomic libraries constitute a valuable resource for obtaining novel biocatalysts. Metagenomic library have been constructed in *E. coli* using fosmid and microbial DNA directly isolated from forest top soil and screened for lipolytic enzymes. Eight unique lipolytic active clones were obtained from the metagenomic library on the basis of tributyrin hydrolysis [32].

Two different approaches have been previously used to isolate novel lipase genes. Henne et al. [33] isolated novel genes conferring lipolytic activity in *E. coli* transformed with metagenomic libraries constructed with temperate environmental soil samples. As an alternative approach, Bell et al. [34] used a PCR method for the direct isolation of novel lipase genes from metagenomes to avoid potential difficulties in achieving the expression of a lipase in a heterologous host.

4. Use of thermostable/alkalophilic enzymes

The importance of thermostable lipases for different applications has been growing rapidly. Most of the studies realized so far have been carried out with mesophilic producers. Many lipases from mesophiles are stable at elevated temperatures [35]. Proteins from thermophilic organisms have been proved to be more useful for biotechnological applications than similar proteins from thermophiles due to their stability [36].

Biocatalyst thermostability allows a higher operation temperature, which is clearly advantageous because of a higher reactivity (higher reaction rate, lower diffusional restrictions), higher stability, higher process yield (increased solubility of substrates and products and favourable equilibrium displacement in endothermic reactions), lower viscosity and fewer contamination problems [37]. These advantages surmount certain drawbacks arising from more stringent requirements for materials, harder post-reaction inactivation, and restrictions in the case of labile substrates or products. Thermostable biocatalysts are therefore highly attractive [38].

Thermostable enzymes can be obtained from mesophilic and thermophilic organisms; even psycrophiles have some thermostable enzymes. Thermophiles represent an obvious source of thermostable enzymes, being reasonable to assume that such character will confer their proteins a high thermal stability. This is certainly so, as can be appreciated in the case of several biotechnologically relevant enzymes from the hyperthermophilic archaebacteria *Pyrococcus furiosus* and *Thermotoga* sp. [39–41].

Thermostable lipases from microbial sources are highly advantageous, for biotechnological applications, since they can be produced at low cost and exhibit improved stability [42]. In recent years there has been a great demand for thermostable enzymes in industrial fields. Thus thermostable lipases from various sources have been purified and characterized [35–44].

The advantages of running bioprocesses at elevated temperatures are:

- higher diffusion rates;
- the increased solubility of lipids and other hydrophobic substrates in water;
- decreased substrate viscosities;
- increased reactant solubilities;
- higher temperature faster reaction rates;
- the reduced risk of microbial contamination.

Alkalophilic thermophiles have great potential in detergent and leather industries.

Thermophiles are a valuable source of thermostable enzymes with properties that are often associated with stability in solvents and detergents, giving these enzymes considerable potential for many biotechnological and industrial applications [45,46]. One of these enzymes is a thermophilic and thermostable lipolytic enzyme that has been applied to the synthesis of biopolymers and biodiesel and used for the production of pharmaceuticals, agrochemicals, cosmetics, and flavours [46].

Alkaliphiles have also made a great impact in industrial applications. Biological detergents contain alkaline enzymes, such as alkaline cellulases and/or alkaline proteases, which have been produced from alkaliphiles. The current proportion of total world enzyme production destined for the laundry detergent market exceeds 60%. It has also been reported that alkali-treated wood pulp could be biologically bleached by xylanases produced by alkaliphiles. The alkaliphiles are unique microorganisms, with great potential for microbiology and biotechnological exploitation. Alkaline enzymes should find additional uses in various fields of industry, such as chiral-molecule synthesis, biological wood pulping, and more production of sophisticated enzyme detergents. Furthermore, alkaliphiles may be very good general genetic resources for such applications as production of signal peptides for secretion and promoters for hyperproduction of enzymes [47].

5. Applications of lipases

Microbial lipases constitute an important group of biotechnologically valuable enzymes, mainly because of the versatility of their applied properties and ease of mass production. Microbial lipases are widely diversified in their enzymatic properties and substrate specificity, which make them very attractive for industrial applications. In the industrial segment, lipases and cellulases are anticipated to post the best gains. It is expected that in the next few years lipases will benefit from their versatility and continued penetration into the detergent and cosmetics markets. Cellulases, which share lipases' versatility, will continue to be used to emulate the stone-washing of denim while making substantial gains in the pulp and paper industry as bleaching and lignin-removal agents. Lipases and cellulases, like most specialty and industrial enzymes, will increasingly be produced via recombinant DNA technology [48]. Lipases are used in two distinct fashions. They are used as biological catalysts to manufacture other products (such as food ingredients) and by their application as such (in making fine chemicals).

Following proteases and carbohydrases, lipases are considered to be the third largest group based on total sales volume. The commercial use of lipases is a billion-dollar business that comprises a wide variety of different applications [49]. The majority of the enzymes in current industrial use are of microbial origin and are produced in conventional aerobic submerged fermentations, which allows greater control of the conditions of growth than solid-state fermentations [50]. Lipases have received increased attention recently, evidenced by the increasing amount of information about lipases in the current literature. The renewed interest in this enzyme class is due primarily to investigations of their role in pathogenesis and their increasing use in biotechnological applications. Lipases are valued biocatalysts because they act under mild conditions, are highly stable in organic solvents, show broad substrate specificity, and usually show high regio- and/or stereoselectivity in catalysis [51].

The usefulness of bacterial lipase in commerce and research stems from its physiological and physical properties.

A large amount of purified lipase could become available, i.e. ease of mass production.
Bacterial lipases are generally more stable than animal or plant lipases.
Lipases are active under ambient conditions and the energy expenditure required to conduct reactions at elevated temperatures and pressures is eliminated that reduces the destruction of labile reactants and products.
Thermophilic microorganisms and enzymes stable at high temperatures and adverse chemical environments are of advantage in industrial uses.
Due to specificity of enzymes, unwanted side products that normally appear in the waste stream are reduced or eliminated.
The use of enzymes, can decrease the side reactions and post-reaction separation problems.
Lipase catalysed processes offer cost-effectiveness too, in comparison with traditional downstream processing.
Lipases remain active in organic solvents in their industrial applicability.
When immobilized lipases are used under typical 'industrial' conditions, reactor temperatures as high 70 °C are possible for prolonged periods.

5.1. Lipases in fat and oleochemical industry

Lipases are part of the family of hydrolases that act on carboxylic ester bonds. The physiologic role of lipases is to hydrolyse triglycerides into diglycerides, monoglycerides, fatty acids, and glycerol. In addition to their natural function of hydrolyzing carboxylic ester bonds, lipases can catalyse esterification, interesterification, and transesterification reactions in nonaqueous media. This versatility makes lipases the enzymes of choice for potential applications in the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper industries [52].

Some fats are much more valuable than others because of their structure. Less valuable fats can be converted into more useful species using blending of chemical methods but these tend to give quite random products. Lipase catalysed transesterification of cheaper oils can be used, for example to produce cocoa butter from palm mid-fraction [53].

The lipase catalysed transesterification in organic solvents is an emerging industrial application such as production of cocoa butter equivalent, human milk fat substitute "Betapol", pharmaceutically important polyunsaturated fatty acids (PUFA) rich/low calorie lipids, "designers fats or structured lipid" and production of biodiesel from vegetable oils [224,54].

Mucor miehei (IM 20) and *Candida antarctica* (SP 382) lipases were used for esterification of free fatty acids in the absence of organic solvent or transesterification of fatty acid methyl esters in hexane with isopropylidene glycerols [55].

Interesterification and hydrogenation are techniques which have been useful in the preparation of glyceride products for use in the manufacture of butter and margarine. In the conventional interesterification reaction, interesterification is conducted in the presence of a catalyst such as sodium, sodium methylate, or the like. However, the conventional reaction is not selective with respect to esterification of a fatty acid substrate at a reactive position with glycerine. On the other hand, an interesterification process conducted in the presence of lipase as a catalyst [56] is known, however, this process requires the presence of water to activate the lipase. The presence of water causes hydrolysis of interesterified glycerides with resultant decreases in yield of the glyceride product. Therefore, a need continues to exist for a method of improving the yield of glyceride products by an interesterification reaction [57].

Large scale business with world production of palm oil at 6.5 million t pa and butter fat at 5.5 million t pa. Cocoa butter fat is a high value product because TAGs are high in stearates, which give a melting point of 37 °C. So cocoa butter fat melts in the mouth to give cooling sensation, smooth 'mouth appeal' effect for e.g. chocolate. Palm oil TAGs are high in palmitate and they give a melting point of 23 °C so is oil at room temperature and is a low value product. Conversion of palm oil into cocoa-butter fat substitute can be achieved by interesterification and is now a commercial process. Using interesterification a cheap raw material is converted into a cocoa butter equivalent, which is high value and used in food, confection and cosmetics industries. Commercial production plants use *Mucor* IME obtained by precipitating the enzymes with acetone then mixing slurry with inorganic support such as alumina to which the enzyme binds by ion-exchange [58].

The adsorption of lecithin, together with lipase onto a carrier, was effective for conducting the interesterifying reaction efficiently for edible oils and fats. Palm oil was blended with canola or soy-bean oil, and were modified by enzymatic selective interesterification to improve the handling properties at low temperature [59]. Immobilized *M. miehei* lipase in organic solvent catalysed the reactions of enzymatic interesterification for production of vegetable oils such as; corn oil, sunflower oil, peanut oil, olive oil and soybean oil containing omega-3 polyunsaturated fatty acids [60].

The catalytic performance of the immobilized lipase was evaluated by determining the composition change of fatty acyl groups and triacylglycerol (TAG). The interesterification process resulted in the formation of new TAGs, mainly tripalmitin and dipalmitostearin, both of which were absent in the original oil. These changes in TAG composition resulted in an increase in slip melting point, from the original 25.5 °C to 36.3, 37.0, and 40.0 °C in the modified POo with 30, 50, and 70% stearic acid, respectively [61].

The use of lipases to carry out industrial hydrolysis of tallow has a number of advantages. The heat required is much less, only enough to melt the tallow, about 50 °C, so the consumption of fossil fuels to make steam and stainless steel is much less. Also because of the low temperature, there is less degradation of unsaturated fatty acids, so pure, natural fatty acids can be obtained without distillation, even from highly unsaturated oils. Because of their nutritional value, undegraded poly-unsaturated fatty acids may be important to preserve in the production of food additives such as mono- and diglycerides. Finally, depending on the specificity of the lipase and the raw material, partial hydrolysis could yield a concentrated or purified mixture of fatty acids and/or partial glycerides with unique properties not found in bulk fatty acids from total hydrolysis of tallow [62]. Diacylglycerols are the main components of new cooking oils (ex: Econa, Kao corp.). These oils are proposed to slow the increase of blood triglycerides to help prevent the accumulation of body fat and high blood cholesterol levels [63].

Lipolysis is the "constructive" consequences of the ability of lipase to hydrolyse lipids so as to obtain fatty acids and glycerol, both of which have important industrial applications. For instance, fatty acids are used in soap production [64]. Glycerides are the major components of depot, or storage, fats in plants and animal cells. Those that are solid at room temperature are known as oils. Lipases catalyse the hydrolysis of fats and oils, and other carboxylic acid esters. MacRae et al. [65] has reported that oil, which being solid at room temperature in crude condition could be converted into fluid conditions by substitution of about 4–50% of its palmitic acid content. Lipozyme (immobilized *Mucor meihei* lipase) have been used to catalyse glycerolysis of melted tallow to synthesize monoglycerides [66].

The scope for the application of lipases in the oleochemical industry is enormous. Fats and oils are produced world wide at a level of approximately 60 million t pa and a substantial part of this (more than 2 million t pa) is utilized in high energy consuming processes such as hydrolysis, glycerolysis and alcoholysis. The conditions for steam fat splitting and conventional glycerolysis of oils involve high temperatures of 240–260 °C and high pressures (methanolysis is currently performed under slightly milder conditions). The resulting products are often unstable as obtained and require re-distillation to remove impurities and products of degradation. In addition to this, highly unsaturated heat sensitive oils cannot be used in this process without prior hydrogenation.

The saving of energy and minimization of thermal degradation are probably the major attractions in replacing the current chemical technologies with biological ones. However, in spite of their apparent superiority, enzymic methods have not as yet attained a level of commercial exploitation commensurate with their potential. There have been several communications about relatively small-scale enzymic fat splitting processes for the production of some high value polyunsaturated fatty acids and the manufacture of soap. For instance Miyoshi Oil & Fat Co., Japan, reported the commercial use of *Candida cylindracea* lipase in the production of soaps [67]. The company claimed that the enzymic method yielded a superior product and was cheaper overall than the conventional Colgate–Emery process.

5.2. Production of biodegradable polymers

Lipases have become one of the most important groups of enzymes for its applications in organic syntheses. Lipases can be used as biocatalyst in the production of useful biodegradable compounds. 1-Butyl oleate was produced by direct esterification

of butanol and oleic acid to decrease the viscosity of biodiesel in winter use. Trimethylolpropane esters were also similarly synthesized as lubricants. Lipases can catalyse ester syntheses and transesterification reactions in organic solvent systems has opened up the possibility of enzyme catalysed production of biodegradable polyesters. Aromatic polyesters can be synthesized by lipase biocatalysis [68].

5.3. Use of lipase in textile industry

Lipases are used in the textile industry to assist in the removal of size lubricants, in order to provide a fabric with greater absorbency for improved levelness in dyeing. Its use also reduces the frequency of streaks and cracks in the denim abrasion systems. Commercial preparations used for the desizing of denim and other cotton fabrics, contains both alpha amylase and lipase enzymes [69].

Rakuto Kasei Israel, Ltd. is a producing enzymes for the textile industry. They supply enzymes for desizing, stone washing of Denim and Jeans, Catalase as Peroxide kille (after bleaching), enzymatic wash, bio polishing of knitted goods, all other chemicals for jeans treatment stone washing and silicone treatment [70].

In the textile industry, polyester has certain key advantages including high strength, soft hand, stretch resistance, stain resistance, machine washability, wrinkle resistance and abrasion resistance. Synthetic fibers have been modified enzymatically for the use in the production of yarns, fabrics, textiles, rugs and other consumer items. It relates to modification of the characteristics of a polyester fiber so that such polyesters are more susceptible to post-modification treatments. The use of polyesterase (closely related to lipase) to improve the ability of a polyester fabric to uptake chemical compounds, such as cationic compounds, fabric finishing compositions, dyes, antistatic compounds, anti-staining compounds, antimicrobial compounds, antiperspirant compounds and/or deodorant compounds [71].

PCT Publication No. WO 97/43014 (Bayer AG) describes the enzymatic degradation of polyesteramide by treatment with an aqueous solution comprising an esterase, lipase or protease. JP 5344897 A (Amano Pharmaceutical KK) describes a commercial lipase composition which is dissolve in solution with an aliphatic polyester with the result that the fiber texture is improved without losing strength. Polymers of aliphatic polyethylene are also disclosed which can be degraded by lipase from *Pseudomonas* spp. PCT Publication No. 97/33001 (Genencor International, Inc.) discloses a method for improving the wettability and absorbance of a polyester fabric by treating with a lipase [71].

5.4. Lipases in detergent industry

The most commercially important field of application for hydrolytic lipases is their addition to detergents, which are used mainly in household and industrial laundry and in household dishwashers. The cleaning power of detergents seems to have peaked; all detergents contain similar ingredients and are based on similar detergency mechanisms. To improve detergency, modern types of heavy duty powder detergents and automatic dishwasher detergents usually contain one or more enzymes, such as protease, amylase, cellulase and lipase [72].

Enzymes can reduce the environmental load of detergent products, since they save energy by enabling a lower wash temperature to be used; allow the content of other, often less desirable, chemicals in detergents to be reduced; are biodegradable, leaving no harmful residues; have no negative impact on sewage treatment processes; and do not present a risk to aquatic life [73].

Lipase (Patent # 6,265,191, issued 07/24/2001) is immobilized on surfaces to facilitate oil removal from the surfaces and to alter wettability of the surfaces of fabric. The lipase is isolatable from a *Pseudomonas* organism such as *Pseudomonas putida* ATCC 53552 or from an organism expressing a coding region found in or cloned from the *Pseudomonas*. Lipase sorbed on fabric forms a fabric-lipase complex for oil stain removal. The lipase may be sorbed on fabric before or after an oil stain, and the lipase is active to hydrolyse an oil stain on dry fabric or fabric in laundering solutions. The sorbed lipase has enhanced stability to denaturation by surfactants and to heat deactivation, is resistant to removal from fabric during laundering, retains substantial activity after drying fabric at an elevated temperature, and retains activity during fabric storage or wear. Redeposition of oil and oil hydrolysis by-products during laundering of fabric is retarded by the lipase. Oil hydrolysis by-products are removable during laundering of fabric at a basic pH or in the presence of a surfactant [74]

GatewayProClean Inc. USA offers two laundry programs that utilize enzyme technology, both a liquid and a solid. Enzymes break down soils into simpler forms that can easily be removed by the cleaner. Lipase is used in detergent formulations to remove fat-containing stains such as those resulting from frying fats, salad oils, butter, fat-based sauces, soups, human sebum or certain cosmetics. The enzyme hydrolyses triglycerides into mono- and diglycerides, glycerol and free fatty acids, all of which are more soluble than the original fats [75].

The problematic area in textile cleaning is the removal of fatty stains. As early as 1988 the company Novo Nordisk developed a lipase, an enzyme capable of dissolving fatty stains. This enzyme is produced naturally by a selected strain of the fungus Humicola, but quantities are too low for commercial application. Traditional methods to increase yield proved unsuccessful, so the gene coding for this lipase was cloned and inserted into the fungus *Aspergillus oryzae*. This fungus now produces the enzyme in commercially relevant yields so that it can be used in detergents, allowing better washing performances and energy savings. Improved industrial enzymes in unlimited quantity lipase fights fatty stains [76].

In 1994, Novo Nordisk introduced the first commercial lipase, Lipolase, which originated from the fungus *T. lanuginosus* and was expressed in *A. oryzae*. In 1995, two bacterial lipases were introduced—Lumafast from *Pseudomonas mendocina* and Lipomax from *Pseudomonas alcaligenes*, both produced by Genencor International, AU-KBC Research Center, Life Sciences, Anna University, Chennai, India [53]. Lipases used as

detergents also include those from *Candida* [77] and *Chromobacterium* [78]. Laundering is generally carried out in alkaline media, lipases active under such conditions are preferred [79–81], for example, the *A. oryzae* derived lipase. Alkaline Lipase produced by *Acinetobacter radioresistens* had an optimum pH of 10 and was stable over a pH range of 6–10; therefore have great potential for application in the detergent industry [82].

Other enzymes are currently widely used in household cleaning products. A great deal of research is currently going into developing lipases, which will work under alkaline conditions as fat stain removers.

Godfrey and West [223] reported in 1996 that about 1000 t of lipases are sold every year in he area of detergents. Lipases are stable in detergents containing protease and activated bleach systems. Fuji et al. [83] described that lipase is an enzyme which decomposes fatty stains into more hydrophilic substances that are easier to remove than similar non-hydrolysed stains.

The other common commercial applications for detergents is in dish washing, a bleaching composition [84], decomposition of lipid contaminants in dry cleaning solvents [85], liquid leather cleaner [86], contact lens cleaning [87], clearing of drains clogged by lipids in food processing or domestic/industrial effluent treatment plants [88], degradation of organic wastes on the surface of exhaust pipes, toilet bowls, etc. [89], removal of dirt/cattle manure from domestic animals by lipases and cellulases [85], washing, degreasing and water reconditioning by using lipases along with oxidoreductases, which allows for smaller amounts of surfactants and operation at low temperatures [90]. The lipase component causes an increase in detergency and prevents scaling.

5.5. Lipases in food processing, flavour development and improving quality

In the present day, fat and oil modification is one of the prime areas in food processing industry that demands novel economic and green technologies. Tailored vegetable oils with nutritionally important structured triacylglycerols and altered physicochemical properties have a big potential in the future market. Microbial lipases which are regiospecific and fatty acid specific, are of immense importance and could be exploited for retailoring of vegetable oils. Cheap oils could also be upgraded to synthesize nutritionally important structured triacylglycerols like cocoa butter substitutes, low calories triacylglycerols and oleic acid enriched oils. Lipase mediated modifications are likely to occupy a prominent place in oil industry for tailoring structured lipids since enzymation modifications are specific and can be carried out at moderate reaction conditions [91].

Lipases have also been used for addition to food to modify flavour by synthesis of esters of short chain fatty acids and alcohols, which are known flavour and fragrance compounds [92]. Psychrotrophic Gram-negative bacteria, such as *Pseudomonas* species, pose a significant spoilage problem in refrigerated meat and dairy products due to secretion of hydrolytic enzymes, especially lipases and proteases. This study characterized the enzymes produced by strains of *P. fluorescens* isolated from pasteurized milk [93].

Lipases have earlier been used in production of leaner meat such as in fish. The fat is removed during the processing of the fish meat by adding lipases and this procedure is called biolipolysis. The lipases also play an important role in the fermentative steps of sausage manufacture and to determine changes in long-chain fatty acid liberated during ripening. Earlier, lipases of different microbial origin have been used for refining rice flavour, modifying soybean milk and for improving the aroma and accelerating the fermentation of apple wine [94].

5.6. Resolution of racemic mixtures

Lipases can be used to resolve the racemic mixtures and to synthesize the chiral building blocks for pharmaceuticals, agrochemicals and pesticides. Some lipases retain their activity in nonpolar organic solvents. Thus can be used in the hydrolysis of water-insoluble esters, such as in the resolution of racemic mixtures through stereospecific hydrolysis. The resolution of stereoisomers by enantio selective hydrolysis or esterification, have been developed [95].

Chirality is a key factor in the efficacy of many drugs; thus, the production of single enantiomers of drug intermediates has become increasingly important in the pharmaceutical industry. Chiral intermediates and fine chemicals are in high demand from the pharmaceutical and agrochemical industries for the preparation of bulk drug substances and agricultural products. There has been an increasing awareness of the enormous potential of microorganisms and enzymes for the transformation of synthetic chemicals with high chemo-, regio- and enantioselectivity [96].

Lipase from *C. antarctica* (Novozyme (R) 435) has been used for the kinetic resolution of racemic flurbiprofen by the method of enantioselective esterification with alcohols [97].

Baclofen is chemically (*RS*)-beta-(aminomethyl)-4-chlorobenzene propanoic acid. It is used in the therapy of pain and as a muscle relaxant. It produces two isomers. Lipase from *C. cylindracea* has been used as a catalyst for resolving racemic mixture [98].

Lipase-catalysed stereoselective acetylation of racemic 7-[*N*, *N*′-bis-(benzyloxy-carbonyl)*N*-(guanidinoheptanoyl)]-alpha-hydroxy-glycine 24 to corresponding *S*-(−)-acetate 25 was demonstrated. *S*-(−)-acetate 25 is a key intermediate for total chemical synthesis of (−)-15-deoxyspergualin 23, an immunosuppressive agent and antitumor antibiotic [99].

Biocatalytic processes were used to prepare chiral intermediates for pharmaceuticals. These include the following processes. (*S*)[1-(acetoxyl)-4-(3-phenyl)butyl]phosphonic acid diethyl ester 21, a key chiral intermediate required for total chemical synthesis of BMS-188494 (an anticholesterol drug) was prepared by stereoselective acetylation of racemic [1-(hydroxy)-4-(3-phenyl)butyl]phosphonic acid diethyl ester 22 using *G. candidum* lipase [100].

Lipase-catalysed stereoselective acetylation of racemic 7-[*N*, *N*′-bis-(benzyloxy-carbonyl)*N*-(guanidinoheptanoyl)]-alpha-hydroxy-glycine 24 to corresponding *S*-(−)-acetate 25 was demonstrated. *S*-(−)-acetate 25 is a key intermediate for total chemical synthesis of (−)-15-deoxyspergualin 23, an immunosuppressive agent and antitumor antibiotic. Enzymatic

resolution of racemic 2-pentanol and 2-heptanol by lipase B from *C. antarctica* was demonstrated. *S*-(+)-2-pentanol is a key chiral intermediate required for synthesis of anti-Alzheimer's drugs [100].

The resolution of 2-halopropionic acids, starting materials for the synthesis of phenoxypropionate herbicides is being carried out on a 100-kg scale by Chemie Linz Co. (Austria) under a license from the Massachusetts Institute of Technology. The process is based on the selective esterification of (*S*)-isomers with butanol catalysed by porcine pancreatic lipase in anhydrous hexane. Typically, >99% enantiomeric excess (ee) is obtained at 75% of the theoretical yield and the resolution is complete in several hours. Production of both (*R*) and (*S*) isomers of alpha(*sub)-phenoxypropionic acids, which are useful synthons for the preparation of enantiomerically pure herbicides and non-steroidal anti-inflammatory drugs (naproxen, ibuprofen) respectively. The required optically pure derivative can be obtained directly via the (*trans*)esterification or hydrolysis of the corresponding ester. These resolutions have been performed on a multi-kilogramme scale by several companies world-wide [53].

The advantages of using enzymes in the synthesis of organic compounds relate to their versatility, high reaction rates, and regio- and stereospecificity and the relatively mild reaction conditions involved. Stereospecificity is especially important in the synthesis of bioactive molecules, as only one of the enantiomeric forms usually manifests bioactivity, whereas the other is often toxic [101].

The usefulness of lipases in the preparation of chiral synthons is well recognized. Another instance of commercial application of lipases to the resolution of racemic mixtures is the hydrolysis of epoxy alcohol esters. The highly enantioselective hydrolysis of (*R*,*S*)-glycidyl butyrate has been developed by DSM-Andeno (the Netherlands). The reaction products (*R*)-glycidyl esters and (*R*)-glycidol, are readily converted to (*R*)- and (*S*)-glycidyltosylates, which are very attractive intermediates for the preparation of optically active beta-blockers and a wide range of other products [53].

Lipases are currently being used by many pharmaceutical companies world-wide for the preparation of optically active intermediates on a kilo-gramme scale. A number of relatively small biotechnological companies, such as Enzymatix in the UK, specialise in biotransformations and offer a whole variety of intermediates prepared via lipase mediated resolution. Regioselective modifications of polyfunctional organic compounds is yet another area of expanding lipase application. Lipases have been successfully applied in the regioselective modification of castanospermine. a promising drug for the treatment of AIDS [53].

5.7. Diagnostic tool

Lipases are also important drug targets or marker enzymes in the medical sector. They can be used as diagnostic tools and their presence or increasing levels can indicate certain infection or disease.

Lipases are used in the enzymatic determination of serum triglycerides to generate glycerol which is subsequently determined by enzyme linked colorimetric reactions. The level of lipases in blood serum can be used as a diagnostic tool for detecting conditions such as acute pancreatitis and pancreatic injury [102]. Acute pancreatitis usually occurs as a result of alcohol abuse or bile duct obstruction. Although serum trypsin level ultrasonography, computed tomography and endoscopic retrograde cholangiopancreatography are the most accurate laboratory indicators for pancreatitis but serum amylase and lipase levels are still used to confirm the diagnosis of acute pancreatitis [103].

Some new developments in diagnosing pancreatitis have been made by using lipases. Many cell types secrete lipases; hence, serum lipase activity is not specific for pancreatitis or exocrine pancreatic insufficiency (EPI). Pancreatic lipase is a more specific marker for the pancreas, which has led to the development of a test for measurement of canine pancreatic lipase. Canine pancreatic lipase has been shown to be significantly decreased in dogs with EPI. Serum pancreatic lipase immunoreactivity (PLI) concentration is highly specific for exocrine pancreatic function and is also highly sensitive for pancreatitis. The test is an immunoassay and typically requires about 5–7 days to get results. A serum feline pancreatic lipase immunoreactivity (fPLI) test was recently developed and preliminary findings suggest that this test is more sensitive than any other diagnostic tool for the diagnosis of feline pancreatitis. The current "gold standard" for diagnosing pancreatitis is pancreatic biopsy for histologic evaluation [104]. Diagnosis of chronic pancreatitis and revealing the presence of exocrine pancreatic insufficiency have also been determined by measuring serum amylase, pancreatic isoamylase, lipase, trypsinogen and elastase [105].

Aeromonas bacteria found in drinking water possess a wide variety of virulence-related genes and suggest the importance of examining as many isolates as possible in order to better understand the health risk these bacteria may present. Characterizing the virulence factors of *Aeromonas* bacteria indicate that municipally treated drinking water is a source of potentially pathogenic *Aeromonas* bacteria [106].

Higaki and Morohashi (2003) [225] have examined *Propionibacterium acnes* lipase in skin diseases and Unsei-in. Butyric acid production in axillary seborrheic dermatitis (ASD) was higher than in other dermatitis, and that in acne vulgaris (AV) was significantly higher than in controls. *P. acnes* lipase is the pathogenic factor in AV and fatty acids produced by lipase might be the pathogenic factor in ASD. Kanagawa haemolysin, slime, lipase, and colonial opacity have also been considered as virulence markers in infections by *Vibrio cholerae* [107].

Potential virulence factors (elastase, proteinase, lipase, phospholipase C, alginate) as well as surface properties (hydrophobicity, motility) were determined in 103 *P. aeruginosa* strains isolated from patients with cancer. Sixty-nine percent of the strains demonstrated higher level of lipase (20–150 U/mL); these elevated levels of enzymes were associated mainly with nontypable strains. The considerable virulence of tested *P. aeruginosa* strains was confirmed. The nontypable strains manifested the most frequent group with high level of elastase, proteinase, lipase, hydrophobicity and motility [108].

P. aeruginosa is an opportunistic pathogen and its ability to synthesize and secrete numerous different virulence factors are regarded as biological properties contributing to the pathogenicity of *P. aeruginosa*. Among the virulence factors are many enzymes, including lipases. Lipase of pathogenic bacteria such as *P. acnes* [109] *Corynebacterium acnes* and *Staphylococcus aureus* [110] has also been found to have the influence on skin rash in acne patients.

5.8. Bakery products, confectionery and cheese flavouring

Lipases are extensively used in the dairy industry for the hydrolysis of milk fat. Current applications include the flavour enhancement of cheeses, the acceleration of cheese ripening, the manufacturing of cheese like products, and the lipolysis of butterfat and cream. The free fatty acids generated by the action of lipases on milk fat endow many diary products, particularly soft cheeses, with their specific flavour characteristics. Thus the addition of lipases that primarily release short chain (mainly C4 and C6) fatty acids lead to the development of a sharp, tangy flavour, while the release of medium chain (C12, C14) fatty acids tend to impart a soapy taste to the product. In addition, the free fatty acids take part in simple chemical reactions, as well as being converted by the microbial population of the cheese. This initiates the synthesis of flavour ingredients such as acetoacetate, beta-keto acids, methyl ketones, flavour esters and lactones [100].

A whole range of microbial lipase preparations has been developed for the cheese manufacturing industry: *Mucor meihei* (Piccnate, Gist-Brocades; Palatase M, Novo Nordisk), *A. niger* and *A. oryzae* (Palatase A, Novo Nordisk; Lipase AP, Amano; Flavour AGE, Chr. Hansen) and several others [100].

Lipases also play a crucial role in the preparation of so-called enzyme modified cheeses (EMC). EMC is a cheese that is incubated in the presence of enzymes at elevated temperature in order to produce a concentrated flavour for use as an ingredient in other products (dips, sauces, dressings, soups, snacks, etc.) [100].

The biochemistry behind the enhancement of the flavour of dairy products is as follows: the acceleration of the flavours occurs when there is the formation of free fatty acids and soluble peptides and amino acids during the maturation stage of the dairy product. Lipases have been used in the improvement of flavour in coffee whiteners to produce the creamy flavour, and buttery texture of toffees and caramel [111]. Blue cheese flavour development is due to enzymes from *Pencillium roqueforti* [112].

Larios et al. [113] have shown that *C. antarctica* lipase fraction B (CAL-B) can be employed as a robust biocatalyst in esterification reactions due to the high conversions obtained in the synthesis of short-chain flavour esters in an organic solvent, although this enzyme exhibited modest enantioselectivity with chiral short-chain carboxylic acids.

Shay et al. [114] showed that fermentation of *Candida utilis* in the presence of beef extract/butteroil and lipases followed by spray drying, produced yeast, which had a beefy/blue cheese-like flavour. The improved yeast can be used in the production of better-flavoured alcoholic beverages.

The use of enzymes enable bakeries to extend shelf-life of breads, enhance and control non-enzymatic browning, increase loaf volume and improve crumb structure. Bio-Cat Inc., Enzyme Industry, Troy, VA, offer product line of enzymes that can aid in these functions and more. Lipases from *A. niger*, *R. oryzae*, *C. cylindracea* are used in bakery products [115].

Millbo S.p.a. (Italy) manufactures a wide and continuously evolving range of bakery enzymes to fulfill the needs of the bakery trades. It supplies lipases (M 300LF) which are effective in replacing partially or totally the emulsifiers, and to increase the volume in bread and bakery [116].

5.9. Cosmetics

Unichem International (Spain) has launched the production of isopropyl myristate, isopropyl palmitate and 2-ethylhexylpalmitate for use as an emollient in personal care products such as skin and sun-tan creams, bath oils etc. Immobilized *Rhizomucor meihei* lipase was used as a biocatalyst. The company claims that the use of the enzyme in place of the conventional acid catalyst gives products of much higher quality, requiring minimum downstream refining [53].

Wax esters (esters of fatty acids and fatty alcohols) have similar applications in personal care products and are also being manufactured enzymatically (Croda Universal Ltd.). The company uses *C. cylindracea* lipase in a batch bioreactor. According to the manufacturer, the overall production cost is slightly higher than that of the conventional method, but the cost is justified by the improved quality of the final product [53].

Retinoids (Vitamin A and derivatives) are of great commercial potential in cosmetics and pharmaceuticals such as skin care products. Water-soluble retinol derivatives were prepared by catalytic reaction of immobilized lipase [117].

Lipases have been used in hair waving preparation [118]. Lipases have also been used as a component of topical anti-obese creams [119] or as oral administration [120].

5.10. Lipases in tea processing

The quality of black tea is dependent great extent on the dehydration, mechanical breaking and enzymatic fermentation to which tea shoots are subjected. During manufacture of black tea enzymatic breakdown of membrane lipids initiate the formation of volatile products with characteristic flavour properties emphasize the importance of lipid in flavour development. Lipase produced by *Rhizomucor miehei* enhanced the level of polyunsaturated fatty acid observed by reduction in total lipid content [121].

5.11. Medical applications

Lipases and/or esterases (hereinafter referred to as esterases) isolated from the wax moth (*Galleria mellonella*) were found to have a bacteriocidal action on *Mycobacterium tuberculosis* (MBT) H37Rv. This preliminary study may be regarded as part

of global unselected screening of biological and other materials for detecting new promising sources of drugs [122].

Lipases may be used as digestive aids [79]. Lipases are the activators of Tumor Necrosis Factor and therefore can be used in the treatment of malignant tumors [123]. Although human gastric lipase (HGL) is the most stable acid lipase and constitutes a good candidate tool for enzyme substitution therapy [124]. Lipases have earlier been used as therapeutics in the treatment of gastrointestinal disturbances, dyspepsias, cutaneous manifestations of digestive allergies, etc. [125].

Berrobi et al. [126] have filed a patent for pharmaceutical preparations that contain hyaluronidase and/or thiomucase enzymes in addition to lipases for use in skin inflammations. Lipase from *Candida rugosa* have been used to synthesize lovastatin, a drug that lower serum cholesterol level. The asymmetric hydrolysis of 3-phenylglycidic acid ester which is a key intermediate in the synthesis of diltiazem hydrochloride, a widely used coronary vasodilator, was carried out with *S. marcescens* lipase [127].

Lipases from plants are also used in various medicines as digestive aids. A comprehensive high-potency Plant Enzyme supplement that supports digestion, Similase (digestion of protein, carbohydrates, fats, fibers and phytates) is manufactured by [128]. Digestive Plant enzymes; All-Vita NorthWest, Vitaline® Herbal Formulas, are also manufactured by Health Care Professionals, Oregon, USA [129].

5.12. Lipases as biosensors

A biosensor based on the enzyme-catalysed dissolution of biodegradable polymer films has been developed. The polymer-enzyme system; poly(trimethylene) succinate, was investigated for use in the sensor, which is degraded by a lipase. Potential fields of application of such a sensor system are the detection of enzyme concentrations and the construction of disposable enzyme based immunosensors, which employ the polymer-degrading enzyme as an enzyme label [130].

Radiolabelled polynucleotide probes have been employed extensively for the detection of complementary nucleic acids by specific hybridization. Within the last few years, various methods have been developed using enzyme-labelled probes to avoid unstable and hazardous isotopes. By screening various hydrolytic enzymes to fit the special demands, fungal lipases turned out to be the most practical [131].

Lipases may be immobilized onto pH/oxygen electrodes in combination with glucose oxidase, and these function as lipid biosensors [132] and may be used in triglycerides [133] and blood cholesterol determinations [134].

5.13. Degreasing of leather

Lipases represent a more environmentally sound method of removing fat. For bovine hides, lipases allow tensides to be replaced completely. For sheepskins, which contain up to 40% fat, the use of solvents is very common and these can also be replaced with lipases and surfactants. If surfactants are used for sheepskins, they are usually not as effective and may be harmful to the environment. Maps (India) offers a range of lipases for degreasing which work in different pH conditions; Palkodegrease, lipase for degreasing in neutral to alkaline pH conditions and Palkodegrease AL, Lipase for degreasing in acidic pH conditions [135].

Degreasing is an essential stage in the processing of fatty raw materials such as small animal skins and hides from intensively fed cattle. Conventional methods use organic solvents and surfactants, which can give rise to environmental problems such as volatile organic compound (VOC) emissions. Lipase enzymes can remove fats and grease from skins and hides, particularly those with a moderate fat content. Both alkaline stable and acid active lipases can be used in skin and hide degreasing. Lipases hydrolyse triglyceride (the main form of fat stored in animal skins) to glycerol and free fatty acids. To improve the process, alkaline stable proteases are used to encourage the degradation of fat cell membranes and sebaceous gland components. Deliming and bating are the most suitable processing stages for using lipases. Acid active lipases can be used to treat skins that have been stored in a pickled state [136].

The main advantages of using lipases are a more uniform colour and a cleaner appearance. Lipases also improve the production of hydrophobic (waterproof) leather; makers of leather for car upholstery have commented that ‘fogging’ is reduced.

Lipases offer the tanner two advantages over solvents or surfactants: fat dispersion and production of waterproof and low-fogging leathers. Alkaline lipases are applied during soaking and/or liming, preferably in combination with the relevant protease. Among other things, the protease will open up the membranes surrounding the fat cell, making the fat accessible to the lipase. The fat becomes more mobile and the breakdown products emulsify the intact fat, which will then distribute itself throughout the pelt so that in many cases a proper de-greasing with surfactants will not be necessary. This facilitates the production of waterproof and low-fogging stock. Lipases (acid) can also be applied in an acid process, e.g. for pickled skin or wool and fur, or semiacid for wetblue Wool. Novozyme, Denmark markets NovoCor ABL and NovoCor ADL, the combination of an acid lipase and an acid protease, for acid bating of fur and wool; NovoLime, a protease/lipase blend for enzyme-assisted liming of hides and skins; NovoCor AD, an acid lipase for degreasing of hides and skins [137].

Traditionally the treatment of animal skins in the leather manufacturing industry has used lime and sodium sulphide mixtures to dissolve hair present on the skins. This method is both polluting and unpleasant. The removal of residual fats and protein debris that are associated with the hide and the hair by chemical processes, such as liming, is not efficient [94]. It has become common practice to utilize a mixture of lipases and proteases for this purpose (known in technical jargon as the bating process) [5,138]. The enzyme loosens and removes the hair on the skins, which can then be filtered off. The end product is of a higher quality when compared to leather manufactured using traditional methods. *Rhizopus nodosus* lipase was used for the degreasing of suede clothing leathers from wooled sheep skins [139].

5.14. Waste/effluent/sewage treatment

Lipases are utilized in activated sludge and other aerobic waste processes, where thin layers of fats must be continuously removed from the surface of aerated tanks to permit oxygen transport (to maintain living conditions for the biomass). This skimmed fat-rich liquid is digested with lipases [88] such as that from *C. rugosa*. Effective breakdown of solids and the clearing and prevention of fat blockage or filming in waste systems are important in many industrial operations. Examples include: (i) degradation of organic debris—a commercial mixture of lipase, cellulase, protease, amylase, inorganic nutrients, wheat bran, etc. is employed for this purpose; (ii) sewage treatment, cleaning of holding tanks, septic tanks, grease traps, etc.

Effluent treatment is also necessary in industrial processing units, such as abattoirs, the food processing industry, the leather industry, the poultry waste processing [111]. Both *P. aeruginosa* LP602 cells and the lipase were shown to be usable for lipid-rich wastewater treatment [140]. Fats in wastewater treatment plants that contains mainly triglycerides is hydrolysed by immobilized lipase [141].

A product of Oasis Environmental Ltd., WW07P contains a specially formulated range of adapted high-performance microorganisms developed for use in the biological wastewater treatment with a high content of greases, fats, and oils. It also contains surface tension depressants and penetrants which loosen and liquefy heavy grease deposits, thereby assisting in their biodegradation [142].

Bacterial lipases are involved in solution of such environmental problems as the breakdown of fats in domestic sewage and anaerobic digesters [111]. The first stage in the degradation and recycling of primary sewage sludge and particulate organic matter is the solubilization and enhanced hydrolysis of complex polymeric organic carbon structures associated with the anaerobic sulphidogenic environment. During the process of anaerobic digestion, macromolecules are broken down into simpler low molecular weight compounds in the presence of extracellular enzymes. The increased sulphide concentration generated during the sulphate reduction process stimulates the enzymes (proteases, lipases and glucosidases) leading to enhanced solubilization of primary sewage sludge [143].

Simple alkyl ester derivatives of restaurant grease were prepared using immobilized lipases as biocatalysts. The lipase from *Pseudomonas cepacia* was found to be the most effective in catalysing the methanolysis and ethanolysis of grease [144]. A mixture of industrial cellulase, protease, and lipase, in equal proportion by weight, reduced total suspended solids (TSS) by 30–50% and improved settling of solids in sludge. An increase in solid reduction was observed with increasing enzyme concentration [145]. Lipases of plant, bacterial and animal (pancreatic) origin have shown to hydrolyse and/or reduce the size of fat particles in slaughterhouse wastewater (SHW). Four pretreatments to hydrolyse and/or reduce the size of fat particles in slaughterhouse wastewater (SHW) were tested. Pancreatic lipase appeared more efficient with beef fat than pork fat, possibly because beef fat contains less polyunsaturated fatty acids than pork fat. The bacterial lipase LG-1000 was also efficient in reducing average fat particle size [146].

5.15. Oil biodegradation

Biodegradation of petroleum hydrocarbons in cold environments, including Alpine soils, is a result of indigenous cold-adapted microorganisms able to degrade these contaminants. Seven genotypes involved in the degradation of *n*-alkanes (*P. putida* GPo1 alkB; *Acinetobacter* spp. alkM; *Rhodococcus* spp. alkB1, and *Rhodococcus* spp. alkB2), aromatic hydrocarbons (*P. putida* xylE), and polycyclic aromatic hydrocarbons (*P. putida* ndoB and *Mycobacterium* sp. strain PYR-1 nidA) was determined in 12 oil-contaminated (428–30,644 mg of total petroleum hydrocarbons [TPH]/kg of soil) [147].

According to Vasileva-Tonkova and Galabova [148] bacterial monocultures isolated from lubricant-contaminated wastewater of an electric power station showed positive response in bioaugmented clean-up of wastewater contaminated with hydrocarbons and organic polymers using hydrolytic enzymes.

Margesin et al. [149] have found that monitoring of soil microbial lipase activity is a valuable indicator of diesel oil biodegradation in freshly contaminated, unfertilized and fertilized soils. Fungal species can be used to degrade oil spills in the coastal environment, which may enhance ecorestoration as well as in the enzymatic oil processing in industries [150].

5.16. Pulp and paper industry

The pulp and paper industry processes huge quantities of lignocellulosic biomass every year. The technology for pulp manufacture is highly diverse, and numerous opportunities exist for the application of microbial enzymes. Historically, enzymes have found some uses in the paper industry, but these have been mainly confined to areas such as modifications of raw starch. The enzymatic pitch control method using lipase have been in use in a large-scale paper-making process as a routine operation since early 1990s [151].

Lipase for wastepaper deinking can increase the pulping rate of pulp, increase whiteness and intensity, decrease chemical usage, prolong equipment life, reduce pollution level of waste water, save energy and time and reduce composite cost. The addition of lipase from *Pseudomonas* species (KWI-56) to a deinking composition for ethylene oxide–propylene oxide adduct stearate improved whiteness of paper and reduced residual ink spots [152].

5.17. Use of lipases in production of biodiesel

The limited (and fast diminishing) resources of fossil fuels, increasing prices of crude oil, and environmental concerns have been the diverse reasons for exploring the use of vegetable oils as alternative fuels [153]. The biodiesel fuel from vegetable oil does not produce sulphur oxide and minimize the soot particulate one-third times in comparison with the existing one from petroleum. Because of these environmental advantages, biodiesel fuel can be expected as a substitute for conventional diesel fuel [154].

Immobilized *P. cepacia* lipase was used for the transesterification of soybean oil with methanol and ethanol [155]. Fatty acid ethyl esters have also been prepared from castor oil using *n*-hexane as solvent and two commercial lipases, Novozym 435 and Lipozyme IM, as catalysts [156]. Novozyme 435 have also been used to catalyse the transesterification of crude soybean oils for biodiesel production in a solvent-free medium [157].

Simple alkyl ester derivatives of restaurant grease were prepared using immobilized lipases from *Thermomyces lanuginosa* and *C. antarctica*, as biocatalysts [144]. Fatty acids esters were produced from two Nigerian lauric oils, palm kernel oil and coconut oil, by transesterification of the oils with different alcohols using PS30 lipase as a catalyst. In the conversion of palm kernel oil to alkyl esters (biodiesel), ethanol gave the highest conversion of 72%. Some of the fuel properties compared favourably with international biodiesel specifications [158].

Despite its importance, studies on the mechanisms of production of microbial lipases and the role of lipidic substances used as inducers in lipase production are scarce [159]. Lipases represent an extremely versatile group of bacterial extracellular enzymes that are capable of performing a variety of important reactions, thereby presenting a fascinating field for future research [160]. The understanding of structure–function relationships will enable researchers to tailor new lipases for biotechnological applications [49].

Synergisms are expected from interchange of experiences between crystallographers, biochemists, geneticists, and enzyme kineticists, and food, chemical, and biochemical engineers. Extensive and persistent screening for new microorganisms and their lipolytic enzymes will open new, simple routes for synthetic processes and consequently, new ways to solve environmental problems.

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