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MICROBIAL LIPASES AND THEIR SIGNIFICANCE IN THE PROTECTION OF THE ENVIRONMENT

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Abstract: Microbial lipases represent an extremely versatile group of enzymes that are capable of performing a variety of important reactions. They belong to the class of serine hydrolases and act at the interface generated by a hydrophobic lipid substrate in a hydrophilic medium. Their synthesis and secretion by microorganisms is influenced by many factors like temperature, pH, ions, carbon and nitrogen sources and dissolved oxygen concentration. Microbial lipases are used in leather, detergent, pulp and paper industry, sewage treatment, biodiesel and biodegradable polymers production and bioremediation. Due to various properties lipases are helpful tools in biotechnology and environment protection fields.

Lipazy pochodzenia mikrobiologicznego i ich znaczenie w ochronie środowiska

Streszczenie: Lipazy bakterii i grzybów stanowią różnorodną grupę enzymów przeprowadzających reakcje: hydrolizy, *trans*-estryfikacji triacylogliceroli, enancjoselektywna syntezę i hydrolizę różnych estrów. Pod względem budowy należą do klasy hydrolaz serynowych i charakteryzują się zdolnością katalizy międzyfazowej, czyli przeprowadzania reakcji w układach typu lipid-woda. Różnorodne zdolności katalityczne lipaz umożliwiają zastosowanie ich w wielu procesach biotechnologicznych i ochronie środowiska. Enzymy te coraz częściej wykorzystuje się we wstępnej obróbce skór, przemyśle papierniczym, w produkcji detergentów i surfaktantów, w syntezie biodegradowalnych materiałów opakowaniowych, produkcji biopaliw jako alternatywnego źródła energii oraz oczyszczaniu ścieków.

Key words: application, lipases, microorganisms
Słowa kluczowe: mikroorganizmy, lipazy, zastosowanie

1. Introduction

Lipases occur widely in nature but only microbial lipases constitute an important group of biotechnologically valuable enzymes, which have become very attractive for industrial and environmental applications. Because of a great variety of catalytic activities, ease of genetic manipulations, rapid growth of microorganisms on inexpensive media as well as more convenient and safer production, they are more useful than enzymes derived from plants or animals. Moreover, most of them can be produced in large quantities and are quite stable under non-natural conditions such as high temperature and nonaqueous organic solvents employed in many applications [54]. Microbial lipases can be produced by fermentation of biobased materials but most of the commercially used lipases are produced in synthetic media by recombinant strains of bacteria, yeast and fungi [21, 56]. Particularly, bacteria from the genera *Pseudomonas*, *Staphylococcus*, *Burkholderia*, *Bacillus*, *Micrococcus*, *Acinetobacter*, *Chromobacterium* and *Streptomyces* have been exploited for the production of these enzymes [14, 35, 45, 49, 57]. The main producers of fungal lipases are species of filamentous fungi *Aspergillus*, *Rhizopus*, *Penicillium*, *Mucor*, *Geotrichum*, *Humicola* and *Fusarium*. Lipases from *Candida*, *Yarrowia* and *Pichia* have also been reported [4, 23, 47, 50, 56].

Microbial lipases find promising applications in organic chemical processing, detergent formulations, synthesis of biosurfactants, the oleochemical, dairy and agrochemical industries, paper manufacture, nutrition, cosmetics, perfumery and biocatalytic resolution of pharmaceuticals [21, 53]. Furthermore, novel lipase applications have been successfully established as a in solution to many environmental problems. For example, technologies of *in vitro* synthesis of biopolymers instead of biostable and nonbiodegradable polyolefins used as packaging materials and the synthesis of biofuels as an alternative source of energy have been implemented. Microbial lipases are also helpful in the breakdown of fats in domestic sewage and anaerobic digesters [24, 64].

2. Characteristics of lipases

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) catalyze the hydrolysis and transesterification of triglycerides, enantioselective synthesis, and the hydrolysis

of a variety of esters. They belong to the class of serine hydrolases and contain the consensus sequence G-X_1-S-X_2-G as the catalytic moiety, where G = glycine, S = serine, X_1 = histidine and X_2 = glutamic or aspartic acid. All known lipases span a molecular weight range of 19 to 60 kDa and exhibit a characteristic folding pattern α/β-hydrolase fold. The core of enzymes is composed of a central β-pleated sheet consisting of up to eight different b strands (β1-β8) connected by up to six α helices (A-F). Distinctive feature of the lipases is covering of their active site by a lid-like structure composed of one or two a helices. The lid moves allowing the active site to become accessible for the substrate [3, 41, 59].

The majority of the microbial lipases is secreted extracellularly and does not require any cofactors. Lipases isolated from bacteria and fungi posses a wide range of properties depending on their sources with respect to positional and fatty acid specificity, thermostability and pH optimum. Most of lipases attack the fatty acids at 1 or 3 carbon position of glycerol or both the positions but not the fatty acid at the 2 position of the glycerol molecule. However, through random acyl migration, the 2-fatty acid monoglyceride undergoes rearrangement pushing the fatty acid to the 1 or 3 position of the glycerol molecule [53].

Interestingly, under natural conditions lipases catalyse the hydrolysis of ester bonds at the interface between an insoluble substrate phase and the aqueous phase in which the enzyme is dissolved. Kinetics of lipases cannot be described with the Michaelis-Menten model since this model is valid only in the case of one homogenous phase. The interfacial catalysis proceeds in two stages. Firstly, the physical adsorption of the enzyme at the lipid interface connected with the enzyme activation through opening of the lid blocking the active site takes place. Upon opening, the exposed substrate-binding site and the inside of the lid form a large hydrophobic patch, which is supposed to interact with the hydrophobic substrate interface. Secondly, the formation of the enzyme/substrate complex followed by substrate hydrolysis regenerates and releases the adsorbed enzyme. However, some of the lipases do not show interfacial activation because of shorter aminoacid sequence of the lid oligopeptide as observed for *Burkholderia glumae* or lack of this structure in *Bacillus subtilis* lipase [5, 62, 63].

3. Applications of lipases

Enzymes are major contributors to clean industrial products and processes. Various industries have replaced old processes using chemicals that have detrimental effects on the environment and equipment with new processes that use enzymes under less corrosive conditions. The applications in which enzymes, among them lipases, are used are many and diverse.

Since most of the industrial processes in which lipases are employed function at temperatures exceeding 45°C, thermal stability of lipases related to their structure is the most desirable characteristic. Among features stabilising enzyme structure are: low frequency of valine, isoleucine and threonine, many disulphide bonds, hydrophobic and aromatic interactions, ion-pair networks, increase core hydrophobicity, low level of surface amino acids prone to deamidation (Gln, Asn) and oxidation (Cys, Met) [61]. Thermostable lipases stable at 55–75°C have been isolated from many sources, including *Pseudomonas fluorescens*, *Geobacillus* sp., *Kurtzmanomyces* sp. I-11 [1, 26, 32, 56]. Although few lipases, for example of *Burkholderia cepacia* and *Pyrococcus horikoshii*, are able to operate at 90–100°C, their half-lives are reported to be short [48, 65]. Cold-adapted lipases are largely distributed in microorganisms existing at low temperatures in Antarctic, Polar and Alpine regions, deep see and frozen food. Such enzymes probably are structurally modified by an increasing flexibility of the polypeptide chain enabling an easier accomodation of substrates at low temperature. A very low proportion of arginine residues as compared to lysine, a low content in proline residues, a small hydrophobic core, a very small number of salt bridges and of aromatic-aromatic interactions, as well as a large proportion of arginine on protein surface make lipases active at low temperature [25]. Cold-active enzymes exibit higher catalytic activities at low temperatures than do their mesophilic and thermophilic counterparts, for example *Acinetobacter* sp. strain no. 6 lipase with optimum at 20°C [58].

3.1. Leather industry

Leather industry contributes to one of the major industrial pollution problems. The pollution causing chemicals are: lime, sodium sulphide, salts, solvents (trichloroethylene, white spirit, marlophen 89) and surfactants (nonyl phenol ethoxylate, alkyl alcohol ethoxylate). The formation of pollution is significantly higher in the pre-tanning than the post-tanning operations. In order to overcome the hazards caused by the tannery effluents the use of enzymes as an alternative tool has been applied in pre-tanning operations such as soaking, dehairing, bating, degreasing and offal treatment [27].

Lipases are enzymes that specifically degrade fat and so cannot damage the leather itself. They hydrolyse not only the fat on the outside of the hides and skins but also the fat inside the skin structure. Alka-

line lipases are applied during soaking and/or liming preferably in combination with the relevant protease. The combination of alkaline lipases and proteases applied in enzyme-assisted liming process speeds up the reaction led with the standard chemicals. The role of protease is opening up the membranes surrounding the fat cell, making the fat accessible to the lipase. It makes fat more mobile and the breakdown products emulsify the intact fat, so that in many cases proper degreasing with surfactants is not necessary. The composition of enzymes breaks down fat and proteinaceous matter such as dermatan sulphate, thus facilitating the opening up of the structure and removal of hair [21].

Degreasing is the next step in the leather treatment. This process is generally carried out using aqueous emulsification with detergents or by solvent extraction. It is well known that organic solvents like kerosene, petrol, perchloroethylene, trichloroethylene and surfactants such as nonyl phenol ethoxylate are highly unsafe and hazardous to the workers and heavily pollute the environment. When using lipases the original surfactant dosage can be reduced by at least 50% in the case of both sheepskins and pigskins. For bovine hides lipases allow tensides to be replaced completely. Furthermore, the application of lipases, which are projected as alternatives for solvents and detergents, allow the recovery of valuable by-products [42].

The additional advantage of using lipases is more uniform colour and a cleaner appearance of the leather manufactures. Moreover, good opening of the fibre structure by lipases improves the subsequent uptake of the waterproofing chemicals during the production of hydrophobic and waterproof and low-fogging leathers. Lipases can also be applied in an acid process, for example for pickled skin or wool-on and fur, or semiacid for wetblue.

In the last decade, the leather industry has successfully applied commercial bioproducts containing microbial lipases. Among them are Forenzym SK and LM (bacterial lipases and proteases) for soaking and dehairing, Novolime (microbial lipase and protease blend) for liming, Forenzym BT (pancreatic tripsin and bacterial lipase and protease) for bating, Forenzym WG-L (bacterial acid lipase), Forenzym DG (bacterial lipase), Greasex (microbial alkaline lipase) and NovoCor A (microbial acid lipase) for degreasing [60].

3.2. Paper and pulp manufacture

Certain types of wood, especially from pines, contain lipophilic extractives in which resin acids, triglycerides, steryl esters, terpenes, terpenoids and waxes constitute the major part. These compounds cause production problems in pulp and paper manufacturing when released together with carbohydrates and lignin into the process waters during mechanical pulping [51]. Dispersed as colloidal droplets, ranging in size from 0,15 to 0,4 μm, and sterically stabilised by polysaccharides, these substances cause numerous problems in pulp and paper manufacture, including deposits in and on equipment, adverse effect on water absorption by the pulps, holes and tearing, discoloration and hydrophobic spots on the paper. As individual compounds the most troublesome components of resins are triglycerides, fatty acid esters of glycerol, because of the greatest negative effect on paper tensile strength [33]. The presence of extractives can also result in odour and taste problems in food packaging materials. Some of wood extractives together with organic solvents may also be detrimental to the environment when released into the wastewaters. Current methods for controlling pitch include traditional methods such as debarking and wood seasoning before pulping, which allow pitch to deteriorate by the action of indigenous microorganisms, and adding talc or other chemicals to the pulp to coat the resin and deactivate its surface [36]. Seasoning takes a long time and often leads to discoloration and the chemical pulping leaves a large proportion of free sterols, fatty acids and their derivatives [28]. Alternatively, biological pitch control by microbial pre-treatment i.e. spraying selected microorganisms onto wood chips or logs has been developed. The drawbacks of this method are rather long treatment time and the necessary adjustment of conditions, as well as decreased pulp yield and paper optical quality [6, 20].

Microbial enzymes are potential tools for pitch control due to their specifity enabling new technologies for processing pulps and fibers. Lipases can reduce pitch problems by lowering the triglycerides content of groundwood pulp. An enzyme obtained from *Candida cylindrica*, when added to the groundwood stock chest reduced pitch problems and talc consumption considerably. The dosage of chemicals was reduced, the time of seasoning of the wood was greatly shortened, resulting in lowered bleach consumption. Additionally lipase improved pulp properties and speeded water absorption, increased the strength and the specific volume of the resulting paper [28].

Because enzymes adsorb rapidly on pulp fiber it is not possible to recycle or reuse it. On the other hand, its adsorptive properties enable application at low pulp constistencies and the enzymes remain active and attached during various treatments and washing stages [15]. The lipase process has been scaled-up in a 12-ton per day pulp trial and has been shown to remove 90% of the triglycerides in 3 h with stirring at 37°C. At industrial scale, commercial recombinant lipase expressed in *Aspergillus oryzae*, known as Resinase (Novozymes), is used [9].

3.3. Detergent industry

Nowadays, there is a tendency to use lipases in the detergent industry for removal of fatty residues in laundry, dishwashers and for cleaning of clogged drains [48]. Enzymes break down soils into simpler forms that can easily be removed by the cleaner. The importance of lipases in washing agents results not only from their high efficiency, but also their role in environmental protection. Enzymes can reduce the environmental load of detergents, are biodegradable, leave no harmful residues, have no negative impact on sewage treatment processes and, what is more important, do not present a risk to aquatic life. An ideal detergent enzyme should be stable at high pH and temperature, effective at low enzyme level and have broad substrate specificity. Moreover, it should withstand oxidising and chalating agents and resist degradation caused by other enzymes present in detergent washing formula [22].

Good candidates for detergent applications are lipases produced both by bacteria and fungi. For example, bacterial lipase from *Ralstonia pickettii* improved the removal of oil from cotton fabric by 24–27% as an additive to detergent, at 40°C as washing temperature, 20 min as washing time and 0.6% as detergent concentration. The high efficiency in olive oil removal from cotton fabric exhibited also fungal lipases from *Candida cylindracea* and *Aspergillus niger* [22]. Recently R a t h i *et al*. [48] found a novel alkaline lipase synthesized by *Burkholderia cepacia* RGP-10 with pH optimum of 11 and activity in the range of 25–100°C. The isolated lipase was highly stable towards oxidising agents, alkaline proteases, both ionic and non-ionic surfactants as well as commercial detergents which makes this enzyme a potential additive for detergent formulation.

The first commercial lipase introduced in 1994 by Novo Nordisk into detergent industry was Lipolase originated from the fungus *Thermomyces lanuginosus* and expressed in *Aspergillus oryzae*. Lipolase TM, active and stable under alkaline conditions and over a broad temperature range, has a remarkable resistance to proteolytic activity of commonly used detergent proteases. It has been reported that significant benefit of using Lipolase™ was not revealed during a single wash system but only after repeated wash and dry cycles. It resulted from the low adsorption level of the enzyme caused by the presence of surfactant molecules required for dissolution of hydrolysis products in the water phase. Additionally, application of calcium chelators keeping low calcium concentration during the wash cycle decreases activity of this calcium-dependent enzyme. The free calcium concentration cannot be increased since it decreases general detergency. Moreover, in the presence of calcium, calcium soaps are formed that are poorly water soluble and hard to remove from the fabric. Due to this inconvenience cutinases seem to be more practical in fatty soil removal. Contrary to lipases, cutinases are able to hydrolyze in the absence of calcium, although at a low adsorption rate of lipolytic enzymes are also hampered by the presence of surfactants [17].

3.4. Biodiesel production

Biodiesel has become more attractive recently because of its environmental benefits and the fact that it is made from renewable resources [38]. It can be produced from many vegatable oils (soybean, canola, coconut and sunflower oils), animal fats (tallow, grease), recycled greases and used vegetable oils or algae [24]. Chemically, it is a mix of monoalkyl esters of long chain fatty acids, non-flammable, non-explosive with a flash point of 150°C. Unlike petrodiesel, it is biodegradable and significantly reduces tailpipe emissions, visible smoke, noxious fumes and odors [11].

Biodiesel is obtained during a reaction between triacyloglicerols in vegatable oils or fat and a monohydric alcohol such as methanol (transesterification) in the presence of basic catalyst (NaOH or KOH) to give the corresponding mono-alkyl esters. After the reaction, free glycerol and the residual catalyst are removed during the water washing process. Principally, methanol is used as the efficient and cheapest alcohol for transesterification. The same reaction using ethanol is more complicated, because water-free alcohol and oil with low water content are required in order to obtain glycerol separation. On the other hand, ethanol derived from agricultural products, is renewable and biologically less objectionable in the environment [11, 55]. So far, all commercial biodiesel producers used alkali-catalyzed process for the transesterification but acid and enzyme catalysis has been also proposed lately [8, 46].

Although, the enzyme-catalyzed transesterification processes are not yet commercially developed many laboratory results have been reported. For example, S a l i s *et al*. [52] tested three different immobilized lipases from *Candida antarctica* B, *Rhizomucor miehei* and *Burkholderia cepacia* towards the reaction between triolein and short chain alcohols to produce oleic acid alkyl esters (biodiesel) in solvent free conditions. They observed that secondary alcohols usually are less reactive than primary alcohols, conversely, linear and branched primary alcohols with short alkyl chains C2-C4 showed high reaction and conversion rate. Moreover, the mixture of linear and branched short chain alcohols was successfully used for oleic acid ester synthesis. In other studies, D e n g *et al*. [12] used immobilized lipase from *Candida* sp. 99–125 for the production of biodiesel through enzymatic reaction be-

tween long-chain fatty acids and methanol in a solvent system. Biofuel was also produced from two Nigerian lauric oils: palm kernel oil and coconut oils by transesterification with different alcohols using PS30 lipase as a catalyst. In the conversion of palm kernel oil to alkyl esters, ethanol gave the highest conversion of 72%, whereas methanol only 15%. With coconut oil, 1-butanol and *iso*-butanol achieved the highest conversion of 40% while only traces of methyl esters were observed using methanol [2].

Environmental benefits of using biodiesel in comparison to petroleum based fuels are significant. Biodiesel reduces emission of carbon monoxide by approximately 50% and carbon dioxide by 78.5% on a net basis. It contains less aromatic hydrocarbons than common fuels, eliminates sulfur emissions and reduces by as much as 65% the emission of particulates (small particles of solid combustion products). Biodiesel causes more NOx emissions than petrodiesel but these contaminants can be reduced through the use of catalytic converters. Moreover, it has a higher catane rating then petrodiesel and therefore ignites more rapidly when injected into the engine. The limitations include the relatively high production cost, moderate reaction yields, difficulties found during purification of the unreacted substrates and inaccessibility of feedstocks [53].

3.5. *In vitro* synthesis of biodegradable polyesters

Various and relatively easily adjustable properties of plastics make them ideal material for a variety of products and applications. However, significant amount of nonbiodegradable polymers used for packaging, sanitary and agricultural uses causes serious problem of plastic waste accumulation. One way to solve the problem of waste management is by replacing bioresistant synthetic polymers with biodegradable ones. Presently polyesters constitute the most attractive class of artificial polymers which, after use, can be degraded under environmental conditions [16].

It is known that the ability of various types of hydrolases to degrade polyesters accompany their ability to synthetize polyesters, because enzymes, like other catalysts, affect rates of reversible reactions in both directions. To displace the equilibrium in favor of synthesis rather than hydrolysis, these reactions are performed in non-aqueous or micro aqueous media. Moreover, the lack of sensitive cofactors, which have to be recycled, makes hydrolases such as lipases particularly attractive for organic synthesis. New strategy for enzymatic *in vitro* synthesis of polyesters *via* nometabolic pathways also offers a great opportunity for using non-petrochemical renewable resources as starting substrates of functional polymeric materials and thus contributes to global sustainability without depletion of scarce resources [31].

Taking into consideration catalytic specificity of lipases, polyester synthesis could proceed *via* various polymerization modes so called ring-opening polymerization (ROP) of lactones or lactides, polycondensation of dicarboxylic acids with glycols or polycondensation of oxyacids and their esters [30].

Ring-opening polymerization is an addition polymerization in which an end of growing polymer chain can react with additional cyclic monomers to propagate the chain. Because catalytic site of lipase is a serine residue the first step is the cleavage of the lactone ring to give an acyl-enzyme intermediate. Then nucleophilic attack of water, included partly in the enzyme, onto the acyl carbon of the intermediate produce ω-hydroxycarboxylic acid. In the propagation stage, the intermediate is nucleophilically attacked by the terminal hydroxyl group of a propagating polymer. The polymerization of lactones is carried out mostly in bulk or in solution. The type of organic solvent seemed to be an important factor affecting polyester synthesis [30].

Among the most widely used lactones or lactides in ROP are 4 to 17-membered lactones such as β-propiolactones polymerized by *Pseudomonas* family lipases and *Candida cylindracea* lipase [39, 43], δ-valerolactone and ε-caprolactone polymerized by commercially available lipases originated from *Candida antarctica*, *Pseudomonas fluorescens*, *Burkholderia cepacia* and porcine pancreas lipase [29, 44] as well as 12–17 membered macrolides, for which polymerization reaction proceeded even in aqueous medium [30].

High molecular weight poly(ε-caprolactone) is a commercially available biodegradable plastic. Caprolactone was the first monomer which was reported to be subject to lipase catalyzed ring-opening polymerization. In the case of crude industrial lipases such as porcine pancreas lipase, *Burkholderia cepacia* and *Pseudomonas fluorescens* lipase, often more than 40 wt.% of the enzymes was required for the efficient production of the poly(ε-caprolactone), when less than 1 wt.% of C*andida antarctica* lipase was enough to induce the polymerization. Furthermore, the catalytic activity remained unchanged over five cycles indicating the possibility of reusing this enzyme for the polymerization. Additionally, the linear polymer was obtained when polymerization proceeded in bulk, whereas the main product obtained in organic solvents, among which toluene was the most appropriate, had cyclic structure [10].

Polycondensation is a reaction leading to the propagation of the polymer chain by a repeated condensation process proceeding with simultaneous releasing of simple molecules. The chemical catalytic processes normally require high temperature that makes the incorporation of thermally unstable moieties into the

products impossible. Also the preparation of linear polymers when using monomers having multiple functional groups could be problematic if the protection-deprotection protocols were be applied. Owing to the enzymes, it is possible to overcome some or all of these difficulties due to some unique features of lipases like enantio-, chemo-, and regioselectivity [34].

The scale-up experiment on polymer synthesis with *Candida antarctica* lipase revealed that it is possible to obtain polyester from adipic acid and 1,6-hexanediol in more than 200 kg yield. Moreover, this solvent-free system displayed potential as an environmentally clean process owing to mild conditions and no use of organic solvents and toxic catalysts [30].

Limiting factors for a broader use of synthetic aliphatic polyesters derived from diols and diacids are their poor mechanical properties and low melting temperature. It has been proven that the incorporation of rigid aromatic structures into aliphatic copolyesters was helpful for the synthesis of polymers with improved mechanical and thermal properties. In this process there were many critical factors affecting molar mass of growing polyester such as relative position of the two functional groups on a benzene ring, the type of diol and reduced pressure. Additionally, L i n k o *et al.* [37] demonstrated the inability of commercially available crude lipase of *Rhizomucor miehei* to propagate polyester chain when terephtalic acid (1,4-benzene dicarboxylic acid) instead of isophtalic acid (1,3-benzene dicarboxylic acid) was used as substrate. In this case, only immobilized Novozyme-435 lipase (*Candida antarctica* lipase) was found to be an efficient biocatalyst. Also the influence of different factors on various multi-component copolyesters synthesis using Novozyme-435 lipase was investigated [19]. The results indicated that the flexibility in the methodology permits the enzymatic introduction of various functionalities in the polymeric system for any specific application for example in plant therapy for controlled delivery of pesticides or insecticides in agriculture.

As mentioned above, the important feature of enzyme catalysis is its reversible nature depending on reaction conditions. Studies conducted with aliphatic polyesters subjected to hydrolytic degradation by various lipases have shown that, under certain conditions, previously synthesized polymer could be decomposed to give oligomers with fixed molecular weight. Contrary to enzymatic degradation, acid-catalyzed degradation leads to random bond cleavage of polymer. Moreover, after removal of the solvent, mixture of the oligomers could be easily polymerized again in the presence of the same lipase. These data indicate that the lipase-dependent polymerization and degradation processes could be controlled by the presence or absence of the solvent, providing a new methodology of plastics recycling [30].

3.6. Wastewater treatment

Domestic and restaurant wastewaters as well as wastewater from dairies are rich in biodegradable organic molecules and usually contain high levels of lipids and proteins. Excessive amount of oil and grease can form oil films on water surfaces preventing the diffusion of oxygen from air into water causing problems in the aeration system and leading to the development of undesirable filamentous microorganisms as *Thiothrix*, *Beggiatoa* and *Nocardia*. Aggregates formed by oil and other particles present in wastewater can cause blockage of water drainage lines. Moreover, these substances reduce the cell-aqueous phase transport rate through the formation of a lipid coat around biological flock. They also hinder flocculation and sedimentation, the reason of microbial biomass decrease. To overcome these difficulties it is necessary to reduce the concentration of fat and oils or to eliminate these materials from wastewaters. Commonly used mechanical and physicochemical methods of oil and grease removal are expensive and low efficient while the addition of microbial lipases to lipid-rich wastewater before its released into the environment may significantly accelerate the biodegradation processes in wastewaters [7, 13].

Reports on the production of lipases by microorganisms and their potential future application for wastewater treatment have been published. Among many described fungal strains producing the hydrolytic enzymes, *Penicillium restrictum* is the most promising. It was identified as a not only lipase but also glucoamylase and protease producer both in submerged and solid state fermentation. Lipase activity of this strain depended on C/N ratio and the highest (30.3 U/g initial dry weight) was observed after 24 h of culturing in the presence of 2% olive oil [18]. From bacterial lipolytic enzymes particularly useful is lipase of *Pseudomonas aeruginosa* LP602. This enzyme added to lipid-rich restaurant wastewater reduced the lipid concentration to less than 10 mg/ml and the lipid fraction was degraded by 70% during the first 24 h [13]. The studies on biodegradation of lipid-rich wastewater were also conducted using mixed bacterial consortium composed of lipase producing strains *Pseudomonas aeruginosa* and *Acinetobacter calcoaceticus* LP009LP602, and protease and amylase producer *Bacillus* sp. B304. The mixed culture was very effective in lipid degradation. Whithin 12 days under aerobic conditions, lipid content was reduced from 20,000 mg/l to less than 20 mg/l [40].

4. Summary

Microbial lipases are currently attracting attention because of their application in environmental protection. Factors posing limitations include relatively high

cost of lipases production and lack of individual enzymes with the catalytic specifities and properties required in a wide variety of processes. The decreasing cost of their mass production, persistent screening for new microorganisms and characterization of their lipolytic enzymes will open new ways to solve many urgent environmental problems.

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