

Lipases, its sources, Properties and Applications: A Review

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Abstract: This review paper provides an overview regarding the main aspects of microbial lipases production. The most important microbial lipase-producing strains are reviewed as well as the main substrates, including the use of agro-industrial residues. Current process techniques (batch, repeated-batch, fed-batch, and continuous mode) are discussed. Finally, some future perspectives on lipase production are discussed with special emphasis on lipase engineering.

Keywords: Review, Lipase, Substrates.



1. INTRODUCTION

Lipases (triacylglycerol acylhydrolases EC 3.1.1.3) are a class of hydrolase which catalyze the hydrolysis of triglycerides to glycerol and free fatty acids over an oil–water interface. In addition, lipases catalyze the hydrolysis and transesterification of other esters as well as the synthesis of esters and exhibit enantioselective properties. The ability of lipases to perform very specific chemical transformation (biotransformation) has made them increasingly popular in the food, detergent, cosmetic, organic synthesis, and pharmaceutical industries [1], [2], [3], [4]. Lipases have emerged as one of the leading biocatalysts with proven potential for contributing

to the multibillion dollar underexploited lipid technology bio-industry and have been used in situ lipid metabolism and ex situ multifaceted industrial applications [5]. The number of available lipases has increased since the 1980s. This is mainly a result of the huge achievements made in the cloning and expression of enzymes from microorganisms, as well as of an increasing demand for these biocatalysts with novel and specific properties such as specificity, stability, pH, and temperature [6], [7]. Lipases are produced by animals, plants, and microorganisms. Microbial lipases have gained special industrial attention due to their stability, selectivity, and broad substrate specificity [8], [9]. Many microorganisms are

known as potential producers of extracellular lipases, including bacteria, yeast, and fungi [10]. Fungal species are preferably cultivated in solid-state fermentation (SSF), while bacteria and yeast are cultivated in submerged fermentation [8]. The importance of lipases can be observed by the great number of published articles recently. In fact, over the last few years, there has been a progressive increase in the number of publications related to industrial applications of lipase-catalyzed reactions, performed in common organic solvents, ionic liquids, or even in non-conventional solvents. The present review is focused on lipase production discussing the main microorganisms, substrates, and process operations used in this specific field.

2. MICROBIAL SOURCES OF LIPASES

Lipases are ubiquitous in nature and are produced by several plants, animals, and microorganisms. Lipases of microbial origin represent the most widely used class of enzymes in biotechnological applications and organic chemistry. A review of the most recent (from 2004 to the present) potential microorganisms for lipase production in both submerged and solid-state fermentations are reported in Table 1.

Bacteria

Among bacterial lipases being exploited, those from *Bacillus* exhibit interesting properties that make them potential *Candidates* for biotechnological applications. *Bacillus subtilis*, *Bacillus pumilus*,

Bacillus licheniformis, *Bacillus coagulans*, *Bacillus stearothermophilus*, and *Bacillus alcalophilus* are the most common bacterial lipases. In addition, *Pseudomonas* sp., *Pseudomonas aeruginosa*, *Burkholderia multivorans*, *Burkholderia cepacia*, and *Staphylococcus caseolyticus* are also reported as bacterial lipase producers (Table 1).

Ertugrul *et al.* [11] isolated 17 bacterial strains that could grow on media based on OMW and selected the most promising strain for lipase production. After screening in tributyrin agar medium, a strain of *Bacillus* sp. was identified as the best lipase producer. After the medium optimization, the intracellular activity found was 168 U mL⁻¹. Kiran *et al.* [12] isolated 57 heterotrophic bacteria from the marine sponge *Dendrodoris nigra*, of which 37% produced a clear halo around the colonies on tributyrin agar plates for lipase production. Particularly, the strain *Pseudomonas* MSI057 exhibited large clean zones around the colonies. Then, this strain was selected for further studies, and after optimization, a maximum lipase activity was found as 750 U mL⁻¹.

Carvalho *et al.* [13] isolated a bacterium strain from petroleum-contaminated soil and codified as Biopetro-4. After investigation of several inducers on lipase activity, the maximum value obtained was 1,675 U mL⁻¹ after 120 h of fermentation. Abada [10] produced lipase from a strain of *B. stearothermophilus* AB-1 isolated from air and obtained a maximum lipase activity of 1,585 U mL⁻¹ in 48 h of fermentation. Takaç and Marul

[14] isolated microbial cultures from soil enriched by periodic sub-culturing of samples in nutrient broth containing 1% (v/v) tributyrin. The isolation process was performed by serial dilution samples on tributyrin agar (TBA) plates. *Bacillus sp.* was selected towards producing the largest opaque halo. Active colonies were re-streaked on TBA agar for purification.

Shariff *et al.* [15] isolated a thermophilic bacterium, *Bacillus sp.* strain L2 from a hot spring in Perak, Malaysia. An extracellular thermostable lipase activity was detected through plate and broth assays at 70 °C after 28 h of fermentation. In most cases, bacteria are preferably cultivated in SmF, due to the high water activity required by the microorganisms (higher than 0.9). There are few exceptions for bacteria grown in SSF. However, when the bacteria are well adapted to this solid medium, the production is normally high.

Mahanta *et al.* [16] obtained a maximum lipase activity of 1,084 U gds⁻¹ using a solvent tolerant *P. aeruginosa* PseA strain. Alkan *et al.* [17] produced an extracellular lipase by *B. coagulans* and obtained a maximum lipase activity of 149 U gds⁻¹ after 24 h of fermentation. Fernandes *et al.* [18] obtained a maximum lipase activity of 108 U gds⁻¹ after 72 h of fermentation by *B. cepacia*.

Fungi

Most commercially important lipase-producing fungi are recognized as belonging to the genera *Rhizopus sp.*, *Aspergillus sp.*, *Penicillium sp.*,

Geotrichum sp., *Mucor sp.*, and *Rhizomucor sp.* Lipase production by fungi varies according to the strain, the composition of the growth medium, cultivation conditions, pH, temperature, and the kind of carbon and nitrogen sources [19]. The industrial demand for new sources of lipases with different catalytic characteristics stimulates the isolation and selection of new strains. Lipase-producing microorganisms have been found in different habitats such as industrial wastes, vegetable oil processing factories, dairy plants, and soil contaminated with oil and oilseeds among others [20]. Vishnupriya *et al.* [21] studied the lipase production by *Sterptomyces grisesus* and obtained maximum enzyme activity of 51.9U/ml. Colen *et al.* [22] isolated 59 lipase-producing fungal strains from Brazilian savanna soil using enrichment culture techniques. An agar plate medium containing bile salts and olive oil emulsion was employed for isolating and growing fungi in primary screening assay. Twenty one strains were selected by the ratio of the lipolytic halo radius and the colony radius. Eleven strains were considered and, among them, the strain identified as *Colletotrichum gloesporioides* was the most productive. In another work, Cihangir and Sarikaya [19] isolated a strain of *Aspergillus sp.* from soil samples from the different regions of Turkey and obtained an expressive activity of 17 U mL⁻¹. In SmF, Teng and Xu [23] investigated the lipase production by *Rhizopus chinensis* and obtained, at the optimized experimental conditions, a maximum

lipase activity of 14 U mL⁻¹. Bapiraju *et al.* [24] optimized the lipase production by the mutant strain of *Rhizopus sp.* and the optimum activity was 29 U mL⁻¹. Kaushik *et al.* [25] studied the production of an extracellular lipase from *Aspergillus carneus* and obtained a maximum activity of 13 U mL⁻¹. In SSF, Kempka *et al.* [26] investigated the lipase production by *Penicillium verrucosum* and the optimum activity was about 40 U gram of dry substrate⁻¹ (gds). Vargas *et al.* [27] studied the lipase production by *Penicillium simplicissimum* and obtained an activity of 30 U gds⁻¹. Both *P. verrucosum* and *P. simplicissimum* were isolated from the babassu oil industry. A quantitative comparison between SmF and SSF is difficult due to the difference in the methods used for determining the lipase activity. However, some qualitative information presented in the literature can be of interest. For example, extracellular lipases were obtained using *Rhizopus homothallicus* with lipase activities of 1,500 U gds⁻¹ and 50 U mL⁻¹, by SSF and SmF, respectively [28]. Azeredo *et al.* [29] obtained lipase activities of 17 U gds⁻¹ and 12 U mL⁻¹ for SSF and SmF, respectively, by cultivation of *Penicillium restrictum*. Recently, some works reporting the use of immobilized whole biomass of filamentous fungi have also been published. The immobilization is advantageous since it can avoid biomass washout at high dilution rates. Also, high cell concentration in the reactor could be achieved and the separation of biomass from the medium is favored [30]. Wolski *et al.* [31]

reported the use of response surface methodology to optimize the lipase production by submerged fermentation using immobilized biomass of a newly isolated *Penicillium sp.* At the optimized experimental conditions, the authors reached a lipase activity around 21 U mL⁻¹, higher than the activity obtained by the same microorganism before immobilization. Yang *et al.* [32] studied the repeated-batch lipase production by immobilized mycelium of *Rhizopus arrhizus* in submerged fermentation. The lipase productivity increased from 3 to 18 U mL⁻¹ h⁻¹, changing the process from batch to repeated-batch mode. Ellaiah *et al.* [33] used the whole immobilized biomass of *Aspergillus niger* to produce lipase and obtained similar activities for both free and immobilized biomass cultivations (4 U mL⁻¹). Elitol and Ozer [30] immobilized the whole cell of *R. arrhizus* and the rate of lipase production was constant through several repeated batch experiments.

Yeast

According to Vakhlu and Kour [34], the main terrestrial species of yeasts that were found to produce lipases are: *Candida rugosa*, *Candida tropicalis*, *Candida antarctica*, *Candida cylindracea*, *Candida parapsilopsis*, *Candida deformans*, *Candida curvata*, *Candida valida*, *Yarrowia lipolytica*, *Rhodotorula glutinis*, *Rhodotorula pilimornae*, *Pichia bispora*, *Pichia mexicana*, *Pichia sivicola*, *Pichia xylosa*, *Pichia burtonii*, *Saccharomycopsis crataegenesis*, *Torulaspora*

globosa, and *Trichosporon asteroides*. The genes that encode lipase in *Candida sp.*, *Geotrichum sp.*, *Trichosporon sp.*, and *Y. lipolytica* have been cloned and over-expressed [35]. Although lipases from *C. rugosa* and *C. antarctica* have been extensively used in different fields, there are several recent publications reporting the production of lipases by other yeasts, as shown in Table 1.

Potumarthi *et al.* [36] collected marine soil samples near an oil extraction platform in the Arabian Sea. After the isolation of the colonies, they were transferred to plates containing 2% tributyrin and incubated at 35 °C for 3–4 days. The colonies that showed the largest hydrolysis halos zone were selected. The most effective strain for lipase production was identified as *Rhodotorula mucilaginosa* (MTCC 8737) by its phenotypic characteristics. Kumar and Gupta [37] isolated 15 yeasts from petroleum and oil sludge areas in Delhi (India). The isolates were purified and checked for their lipolytic potential. Among these yeast strains, one strain was selected for further studies, based on the largest halo of lipolysis. On the basis of sequence homology, this strain was found to belong to *Rhodotorula mucilaginosa* genus and share 99% homology with the already existing database.

Ciafardini *et al.* [38] have discovered that freshly produced olive oil is contaminated by a rich microflora, capable of conditioning the physicochemical and organoleptic characteristics of the oil, through the production of enzymes. Among the

microorganisms that were isolated from this oil, several strains of yeasts were identified as *Saccharomyces cerevisiae*, *Candida wickerhamii*, *Williopsis californica*, and *Candida boidinii*, of which *S. cerevisiae* and *W. californica* showed good potential to produce lipase. The lipase activity in *S. cerevisiae* was noted to be intracellular, and extracellular in *W. californica*. The three-phase olive oil extraction process generates a dark-colored effluent, usually termed olive oil mill wastewater (OMW). D'Annibale *et al.* [39] investigated the valorization of OMW by its use as a possible growth medium for the microbial production of extracellular lipase. Among the 12 strains tested, the most promising strain was *C. cylindracea*. *Candida sp.* is the most potential lipase producer from yeasts reported in the literature. He and Tan [40] used the response surface methodology to optimize culture medium for lipase production by the strain *Candida sp.* 99-125. After optimization, the authors reported the optimum lipase activity as 6,230 and 9,600 U mL⁻¹ in shaken flasks and in a 5-L bioreactor, respectively. In a 30-L bioreactor, Tan *et al.* (2003) reached a maximum lipase activity of 8,300 U mL⁻¹, thus showing that lipase activity values are highly influenced by the microorganism, substrates, and the operational conditions. In contrast to the high activities reached in the above-mentioned works, Rajendran *et al.* [41] reported the optimum lipase activity of 3.8 U mL⁻¹ by *C. rugosa*.

Table 1. Microorganisms cited in the recent literature as potential lipase producers

Microorganism	Source	Reference
<i>Acinetobacter radioresistens</i>	Bacterial	Li <i>et al.</i> [42]
<i>Pseudomonas sp.</i>	Bacterial	Kiran <i>et al.</i> [12]
<i>Pseudomonas aeruginosa</i>	Bacterial	Ruchi <i>et al.</i> [43] Mahanta <i>et al.</i> [16]
<i>Staphylococcus caseolyticus</i>	Bacterial	Volpato <i>et al.</i> [44]
'Biopetro-4'	Bacterial	Carvalho <i>et al.</i> [13]
<i>Bacillus stearothermophilus</i>	Bacterial	Abada [10]
<i>Burkholderia cepacia</i>	Bacterial	Fernandes <i>et al.</i> [18]
<i>Burkholderia multivorans</i>	Bacterial	Gupta <i>et al.</i> [2]
<i>Serratia rubidaea</i>	Bacterial	Immanuel <i>et al.</i> [45]
<i>Bacillus sp.</i>	Bacterial	Ertugrul <i>et al.</i> [11], Shariff <i>et al.</i> [15], Nawani and Kaur [46]
<i>Bacillus coagulans</i>	Bacterial	Alkan <i>et al.</i> [17]
<i>Bacillus subtilis</i>	Bacterial	Takaç and Marul [14]
<i>Rhizopus arrhizus</i>	Fungal	Tan and Yin [47], Yang <i>et al.</i> [32]
<i>Rhizopus chinensis</i>	Fungal	Teng <i>et al.</i> [48] Wang <i>et al.</i> [49], Teng and Xu [23], Sun and Xu [50]
<i>Aspergillus sp.</i>	Fungal	Cihangir and Sarikaya [19]
<i>Rhizopus homothallicus</i>	Fungal	Diaz <i>et al.</i> [28]
<i>Penicillium citrinum</i>	Fungal	D'Annibale <i>et al.</i> [39]
<i>Penicillium restrictum</i>	Fungal	Azeredo <i>et al.</i> [29] Palma <i>et al.</i> [51]
<i>Penicillium simplicissimum</i>	Fungal	Vargas <i>et al.</i> [27], Cavalcanti <i>et al.</i> [52]

<i>Penicillium verrucosum</i>	Fungal	Pinheiro <i>et al.</i> [53] Kempka <i>et al.</i> [26]
<i>Geotrichum sp.</i>	Fungal	Yan and Yan [54], Burkert <i>et al.</i> [55]
<i>Geotrichum candidum</i>	Fungal	Burkert <i>et al.</i> [56]
<i>Aspergillus carneus</i>	Fungal	Kaushik <i>et al.</i> [25]
<i>Rhizopus sp.</i>	Fungal	Bapiraju <i>et al.</i> [24], Martinez-Ruiz <i>et al.</i> [57]
<i>Aspergillus niger</i>	Fungal	Dutra <i>et al.</i> [8], Mala <i>et al.</i> [58], Falony <i>et al.</i> [59]
<i>Rhizopus oryzae</i>	Fungal	Cos <i>et al.</i> [60], Surribas <i>et al.</i> [61]
<i>Colletotrichum gloesporioides</i>	Fungal	Colen <i>et al.</i> [22]
<i>Candida utilis</i>	Fungal	Grbavcic <i>et al.</i> [3]
<i>Candida rugosa</i>	Fungal	Rajendran <i>et al.</i> [41], Boareto <i>et al.</i> [62], Puthli <i>et al.</i> [63], Zhao <i>et al.</i> [64], Benjamin and Pandey [65]
<i>Candida cylindracea</i>	Fungal	Kim and Hou [66], D'Annibale <i>et al.</i> [39] <i>Candida sp.</i> Yeast He and Tan [40]
<i>Rhodotorula mucilaginosa</i>	Yeast	Potumarthi <i>et al.</i> [36]
<i>Rhodotorula mucilaginosa</i>	Yeast	Kumar and Gupta [37]
<i>Yarrowia lipolytica</i>	Yeast	Lopes <i>et al.</i> [67], Alonso <i>et al.</i> [68], Kar <i>et al.</i> [69], Fickers <i>et al.</i> [70], Amaral <i>et al.</i> [71], Dominguez <i>et al.</i> [72]
<i>Aureobasidium pullulans</i>	Yeast	Liu <i>et al.</i> [73]
<i>Saccharomyces cerevisiae</i>	Yeast	Ciafardini <i>et al.</i> [38]
<i>Williopsis californica</i>	Yeast	Ciafardini <i>et al.</i> [38]

3. SUBSTRATES FOR LIPASE PRODUCTION

Microbial lipases are mostly extracellular and their production is greatly influenced by medium composition besides physicochemical factors such as temperature, pH, and dissolved oxygen. The major factor for the expression of lipase activity has always been reported as the carbon source, since lipases are inducible enzymes. These enzymes are generally produced in the presence of a lipid such as oil or any other inducer, such as triacylglycerols, fatty acids, hydrolysable esters, Tweens, bile salts, and glycerol [74], [20]. Lipidic carbon sources seem to be essential for obtaining a high lipase yield. However, nitrogen sources and essential micronutrients should also be carefully considered for growth and production optimization. These nutritional requirements for microbial growth are fulfilled by several alternative media as those based on defined compounds (synthetic medium) like sugars, oils, and complex components such as peptone, yeast extract, malt extract media, and also agroindustrial residues containing all the components necessary for microorganism development. A mix of these two kinds of media can also be used for the purpose of lipase production. The main studies available in the literature since 2005 covering these subjects are presented below, divided by the kind of medium used.

Synthetic Medium

Generally, high productivity has been achieved by culture medium optimization. Optimization of the concentration of each compound that constitutes a cultivation medium is usually a time-consuming procedure. The classical practice of changing one variable at a time, while keeping others constant was found to be inefficient, since it does not explain the interaction effects among variables and their effects on the fermentation process [75], [76], [77]. An efficient and widely used approach is the application of Plackett–Burman (PB) designs that allow efficient screening of key variables for further optimization in a rational way [77]. An alkaline lipase from *B. multivorans* was produced after 15 h of cultivation in a 14-L bioreactor. The medium optimization was carried out to lead to an increase of 12-fold in lipase production. Initially, the effect of nine factors, namely, concentrations of glucose, dextran, olive oil, NH₄Cl, trace metals, K₂HPO₄, MgCl₂, and CaCl₂ and inoculum density were studied using the Plackett–Burman experimental design. These components were varied in the basal medium containing olive oil as inducer and yeast extract as nitrogen source. After the screening of the most significant factors by the PB design, the optimization was carried out in terms of the olive oil, glucose, and yeast extract concentrations, inoculum density, and fermentation time. The optimal medium composition for the lipase production was determined to be (percent w/v): glucose 0.1, olive oil 3.0, NH₄Cl 0.5, yeast extract 0.36, K₂HPO₄ 0.1, MgCl₂ 0.01, and CaCl₂ 0.4 mM

[2]. Kumar and Gupta [37] compared the medium optimization for the yeast *T. asahii* by both one variable at a time and statistical approach. A Plackett–Burman design for seven independent variables (glucose, olive oil, yeast extract, malt extract, MgCl₂, and CaCl₂ concentrations and time) was applied to select the most significant factor. All variables significantly influenced lipase production, apart from glucose and CaCl₂ concentrations. Response surface methodology indicated that the requirement of malt extract and yeast extract varied with the type of lipase inducer used. The use of high malt and yeast extract concentrations favored lipase production when corn oil was used as substrate. On the other hand, the use of Tween 80 as inductor inhibited the lipase production. Conversely, during kerosene induction both malt and yeast extracts requirements were minimal. Wang *et al.* [49] optimized the fermentation medium for lipase production by *R. chinensis*. In order to improve the productivity of lipase, the effects of oils and oil-related substrates were assessed by orthogonal test and response surface methodology. The optimized medium for improved lipase activity consisted of peptone, olive oil, maltose, K₂HPO₄, and MgSO₄·7H₂O. Rajendran *et al.* [41] used the Plackett–Burman statistical experimental design to evaluate the fermentation medium components. The effect of 12 medium components was studied in 16 experimental trials. Glucose, olive oil, peptone, and FeCl₃·6H₂O were found to have more significant influence on lipase production by *C. rugosa*. Takaç

and Marul [14] improved the lipase production by *B. subtilis* using different concentrations of lipidic carbon sources such as vegetable oils, fatty acids, and triglycerides. One percent of sesame oil afforded the highest activity with 80% and 98% enhancements with respect to 1% concentrations of linoleic acid and triolein as the favored fatty acid and triglyceride, respectively. The same authors tested the use of glucose as carbon source and verified that it presented an impressive effect on lipase production. Abada [10] produced lipase from *B. stearothermophilus* AB-1. The authors observed that the use of xylose, tryptophan, alanine, phenylalanine, and potassium nitrate as supplement lead to the highest lipase production. Ruchi *et al.* [43] carried out media optimization through response surface methodology for cost-effective production of lipase by *P. aeruginosa*. The effects of 11 media components (peptone, tryptone, NH₄Cl, NaNO₃, yeast extract, glucose, glycerol, xylose, arabic gum, MgSO₄, and NaCl) were assessed by a Plackett–Burman design, and the most significant factors (arabic gum, MgSO₄, tryptone, and yeast extract) optimized by the response surface methodology. After optimization, the lipase production was increased 5.58-fold, yielding an activity of 4,580 U mL⁻¹. Kaushik *et al.* [25] used the response surface approach to investigate the production of an extracellular lipase from *A. carneus*. Interactions were evaluated for five different variables (sunflower oil, glucose, peptone, agitation rate, and incubation period) and a 1.8-fold

increase in production was reported at optimized conditions. Lin *et al.* [78] investigated the influence of different culture conditions, temperature, pH, carbon, nitrogen, mineral sources, and vitamins on the production of lipase by *Antrodia cinnamomea* in submerged cultures. Nine carbon sources, 14 nitrogen sources, six mineral sources, and five vitamins were investigated. The authors found that 5% glycerol, 0.5% sodium nitrate, and 0.1% thiamine provided the best results. The lipase production reached 54 U mL⁻¹ after 17 days of incubation. He and Tan [40] used the response surface methodology to optimize the culture medium for lipase production with *Candida sp.* 99-125. In the first step, a Plackett–Burman design was used to evaluate the effects of different components in the culture medium (soybean oil, soybean meal, K₂HPO₄, KH₂PO₄, (NH₄)₂SO₄, MgSO₄, and Spam 60). Soybean oil, soybean meal, and K₂HPO₄ concentrations have a significant influence on lipase production. Results were optimized using central composite designs and response surface analysis. The optimized condition allowed lipase production to be increased from 5,000 to 6,230 U mL⁻¹ in a shaken flask system. The lipase fermentation in a 5-L vessel reached 9,600 U mL⁻¹. The use of pure synthetic medium in solid-state fermentation has been hardly presented in the literature. For example, Martinez-Ruiz *et al.* [57] reported the production of lipase from *Rhizopus sp.* using perlite (as inert support) supplemented with urea, lactose, olive oil,

K₂HPO₄, KH₂PO₄, MgSO₄·7H₂O, polyvinyl alcohol, and an oligoelement solution. The activity reached was 75 U gram of inert support⁻¹.

Agroindustrial Residues

Over the recent years, research on the selection of suitable substrates for fermentative process has mainly been focused on agroindustrial residues, due to their potential advantages. Utilization of agroindustrial wastes provides alternative substrates and may help solving pollution problems, which otherwise might be caused by their disposal. The nature of the substrate is the most important factor affecting fermentative processes. The choice of the substrate depends upon several factors, mainly related to cost and availability. Thus, process optimization may involve the screening of several agroindustrial residues. Many reports of SSF have been recently published, in which emphasis is given to the application of agricultural by-products for the production of fine chemicals and enzymes, including lipases. Vargas *et al.* [27] investigated the lipase production by *P. simplicissimum* using soybean meal as substrate supplemented with low-cost substrates: soybean oil, wastewater from a slaughterhouse (rich on oil and fat), corn steep liquor, and yeast hydrolyzed. Soybean meal without supplements appears to be the best medium of those tested for lipase production. Alkan *et al.* [17] studied the effect of several agroindustrial residues (wheat bran, rice husk, lentil husk, banana waste, watermelon waste, and melon waste) on lipase

production by SSF using *B. coagulans*. The best results were obtained using solid waste from melon supplemented with NH_4NO_3 and 1% olive oil. Kempka *et al.* [26] produced lipase from *P. verrucosum* by SSF using soybean meal, sugar cane molasses, corn steep liquor, yeast hydrolyzed, yeast extract, sodium chloride, soybean oil, castor oil, corn oil, olive oil, and peptone. Soybean meal was the best substrate. Mahanta *et al.* [16] reported the lipase production by SSF with *P. aeruginosa* PseA using *Jatropha* seed cake supplemented with different carbon (starch, maltose, glucose) and nitrogen (peptone, NH_4Cl , NaNO_3) sources. *Jatropha* seed cake without supplementation showed a lipase activity of 625 U gds⁻¹. When supplemented with maltose, the activity reached values of 976 U gds⁻¹, while with NaNO_3 the activity was 1,084 U gds⁻¹. Mala *et al.* [50] developed a SSF for lipase production by *A. niger* MTCC 2594 using wheat bran and gingelly oil cake as substrates and supplement, respectively, and the results showed that addition of gingelly oil cake to wheat bran increased the lipase activity by 36% and the activity was 384 U gds⁻¹. Dutra *et al.* [8] monitored the biomass growth of *A. niger* in SSF for lipase production using the digital image processing technique. The strain of *A. niger* was cultivated in SSF using wheat bran as support, which was enriched with 0.91% of ammonium sulfate. The addition of several vegetable oils (castor, soybean, olive, corn, and palm oil) was investigated to enhance lipase production. A

maximum lipase activity was obtained using 2% of castor oil. Sun and Xu [50] reported that a combined substrate of wheat flour with wheat bran supported both good biomass and enzyme production by *R. chinensis* in SSF. Azeredo *et al.* [29] investigated the effects of different carbon sources, mainly carbohydrates and lipids, to support growth and lipase production by *P. restrictum* in SSF. Small trays containing 10 g of ground babassu cake as the basal medium were supplemented with different carbon sources (babassu oil, olive oil, oleic acid, tributyrin, starch, and glucose). In all tested media, the carbon source concentration was calculated to give a C/N ratio of 13.3. The use of olive oil led to higher lipase activities. Diaz *et al.* [28] obtained extracellular lipases from SSF and SmF by *R. homothallicus*. The SmF culture medium consisted of corn steep liquor, peptone, K_2HPO_4 , KH_2PO_4 , and MgSO_4 while in SSF the medium was composed of sugarcane bagasse as support, supplemented with olive oil, lactose, urea, K_2HPO_4 , and MgSO_4 . Cell cultures in SmF yielded a maximum extracellular lipase activity of 50 U mL⁻¹ after 22 h of fermentation and in SSF cell cultures yielded a maximum lipase activity of 1,500 U gds⁻¹ after 12 h of fermentation. Falony *et al.* [59] tested the lipase production by *A. niger* in SSF using wheat bran as support and supplemented with synthetic medium composed of glucose, Na_2HPO_4 , KH_2PO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, CaCl_2 , $(\text{NH}_4)_2\text{SO}_4$, NH_2CONH_2 , and olive oil. Cavalcanti *et al.* [52] investigated the lipase production by SSF

in fixed-bed bioreactor using babassu cake supplemented with sugar cane molasses. Pinheiro *et al.* [53] investigated the lipase production by *P. verrucosum* in submerged fermentation using a medium based on peptone, yeast extract, NaCl, and olive oil and an industrial medium composed of corn steep liquor, yeast hydrolysate, NaCl, and olive oil. When comparing both tested media, the best results were obtained using the one based on peptone, yeast extract, NaCl, and olive oil. Potumarthi *et al.* [36] employed molasses as sole carbon source for lipase production by *R. mucilaginosa* MTCC 8737. A maximum lipase activity was verified using 1% of molasses. The increase in molasses concentration resulted in lower lipase activity, probably due to the enhancement on the medium viscosity. Volpato *et al.* [44] used the Plackett–Burman statistical design and the central composite design in order to optimize culture conditions for lipase production by *S. caseolyticus* strain EX17 growing on raw glycerol, which was obtained as a by-product from the enzymatic synthesis of biodiesel. The lipase activity was lower when compared to the literature but results were very interesting, since it was shown that the excess of raw glycerol obtained from biodiesel process can be used for lipase production, which has potential application in the enzymatic biodiesel synthesis and other fields. Immanuel *et al.* [45] investigated the production of extracellular lipase in submerged fermentation of *Serratia rubidaea*. The tryptone was replaced by low-cost equivalents such as yeast

extract, skim milk power, casein protease, peptone, beef extract, and urea. The carbon sources tested on lipase production were sucrose, fructose, lactose, galactose, and starch. The effect of surfactants as inducers of lipase production was also evaluated using Tween 20, Tween 80, polyethylene glycol 300, and Triton 100. The triglycerides tested were olive oil, coconut oil, gingelly oil, tributyrin, and cod olive oil. Casein, starch, Tween 20, and gingelly oil were the most suitable nitrogen sources, carbon sources, surfactant, and lipids, respectively. Yan and Yan [54] tested a combination of different experimental designs to optimize the production conditions of cell-bound lipase from *Geotrichum sp.* A single factorial design showed that the most suitable carbon source was a mixture of olive oil and citric acid and the most suitable nitrogen source was a mixture of corn steep liquor and NH_4NO_3 . Burkert *et al.* [55] studied the effects of carbon source (soybean oil, olive oil, and glucose) and nitrogen source concentrations (corn steep liquor and NH_4NO_3) on lipase production by *Geotrichum sp.* using the methodology of response surface reaching a lipase activity of 20 U mL^{-1} . D'Annibale *et al.* [39] evaluated the suitability of OMW as a growth medium for lipase production in SmF using *Penicillium citrinum* NRRL 1841, a versatile strain able to produce lipase on different kinds of OMW. Lipase production by *P. citrinum* in OMW-based media was significantly stimulated by nitrogen addition, with ammonium chloride proving to be the most effective source. In contrast, the

addition of vegetable oils (olive, corn, and soybean oil) did not significantly affect lipase production. Bapiraju *et al.* [24] optimized the lipase production by the mutant strain *Rhizopus sp.* BTNT-2 in SmF. The optimum substrates for lipase production were potato starch as a carbon source, corn steep liquor as a nitrogen source, and olive oil as lipid source. Production Processes Fermentative processes have been conducted in batch, repeated-batch, fed-batch, and continuous mode. The mode of operation is, to a large extent, dictated by the characteristics of the product of interest. This section will consider recent applications of batch, repeated-batch, fed-batch, and continuous processes to lipase production in SmF and SSF.

Batch Processes

Most papers reporting lipase production use batch mode in shaken flasks. However, there are a considerable number of studies focusing on the use of bubble, airlift, and stirring bioreactors. Main characteristics and application of these bioreactors will be reviewed in this section, as the use of flasks in batch mode for lipase production has already been presented in previous sections of this work. Kar *et al.* [69] investigated the influence of extracellular factors (namely, methyl oleate dispersion in the broth, dissolved oxygen variations, and pH fluctuations) on lipase production by *Y. lipolytica* in a 20-L batch bioreactor in different scale-down apparatus, which have been designed to mimic the environmental behavior of each factor in

laboratory conditions. These systems allow reproducing the hydrodynamic phenomena encountered in large-scale equipment for the three specified factors on microbial growth, extracellular lipase production, and the induction of the gene LIP2 encoding for the main lipase of *Y. lipolytica*. Among the set of environmental factors investigated, the dissolved oxygen fluctuations generated in a controlled scale-down reactor has led to the more pronounced physiological effect, decreasing the LIP2 gene expression level. The other environmental factors observed in a partitioned scale-down reactor, i.e., methyl oleate dispersion and pH fluctuations, led to a less severe stress interpreted only by a decrease in microbial yield and hence in the extracellular lipase-specific production rate. Potumarthi *et al.* [36] studied the influence of media and process parameters (aeration and agitation) on fermentation broth rheology and biomass formation in 1.5-L stirred tank reactor for lipase production using *R. mucilaginosa* MTCC 8737. A maximum lipase activity of 72 U mL⁻¹ was obtained during 96 h of fermentation at 2 vvm, 200 rpm, pH 7, and 25 °C. Lipase yield with respect to substrate, YP/S, was 25.71 U mg⁻¹; with respect to biomass, YP/X, 10.9 U mg⁻¹; and biomass yield on substrate, YX/S, was 2.35 mg mg⁻¹. Gupta *et al.* [2] obtained an alkaline lipase from *B. multivorans* within 15 h of growth in a 14-L bioreactor. An overall 12-fold enhanced production (58 U mL⁻¹) was achieved after medium optimization. Fickers *et al.* [70] reported the development of a process for

the extracellular lipase secreted by *Y. lipolytica*. The enzyme production was carried out in a 2,000-L bioreactor that led to a lipase activity of approximately 1,100 U mL⁻¹ after 53 h of fermentation. Puthli *et al.* [63] investigated the fermentation kinetics for the synthesis of lipase by *C. rugosa* in a batch system in a 2-L triple impeller bioreactor. These studies illustrated the influence of gas-liquid mass transfer coefficient on the cell growth and hence on the lipase production. To maintain sufficient oxygen concentration for the optimum cell growth and lipase activity, fermentation has been carried out at 600 rpm and at different aeration rates. Gas flow rate of 50.34 cm³ s⁻¹ yielded optimum production of lipase. He and Tan [40] obtained a maximum lipase activity of 9,600 U mL⁻¹ in a 5-L bioreactor with *Candida sp.* Burkert *et al.* [56] compared the lipase production by *Geotrichum candidum* in both 3-L stirred tank and airlift bioreactors. In the stirred reactor, the optimum conditions of agitation and aeration for lipase production were 300 rpm and 1 vvm, leading to an activity of 20 U mL⁻¹ in 54 h of fermentation. For the airlift bioreactor, the best aeration condition was 2.5 vvm, which yielded similar lipase activity after 30 h of fermentation. In the absence of mechanical agitation, lipase yields around 20 U mL⁻¹ were achieved in a shorter time, resulting in a productivity about 60% higher compared to that obtained in the stirred reactor. D'Annibale *et al.* [39] investigated the lipase production in shaken flasks, in a stirred tank (3 L), and in a bubble

column reactor (3 L). The lipase production was 1,230, 735, and 430 U L⁻¹ for shaken flasks, stirred reactor and bubble column, respectively. Alonso *et al.* [68] studied the lipase production in a 2-L stirred tank reactor at different agitation speeds and air flow rates. The most pronounced effect of oxygen on lipase production was determined by stirring rate. A maximum lipase activity was detected in the late stationary phase at 200 rpm and air flow rate of 0.8 vvm, when the lipid source had been fully consumed. Higher stirring rates resulted in mechanical and/or oxidative stress, while lower speeds seemed to limit oxygen levels. An increase in the availability of oxygen at higher air flow rates led to faster lipid uptake and anticipation of enzyme release in culture medium. The same trend verified for lipase production in submerged fermentation is valid for solid-state fermentation: most of the works are reporting the lipase production in tray bioreactors (conical flasks), using a few grams of substrate [26], [27], [8], [17], [29], [59]. Different from SmF, where there are variations in bioreactor configuration, SSF is mostly restricted to a packed-bed configuration. We have not found reports focusing on the use of a rotating drum, intermittently agitated, or fluidized bioreactor. Cavalcanti *et al.* [52] used a 30-g packed-bed bioreactor to improve productivity and scaling-up of lipase production using *P. simplicissimum* in solid-state fermentation. The influence of temperature and air flow rate on enzyme production was assessed by statistical experimental design, and an

empirical model was fitted to experimental data. Higher lipase activities could be achieved at lower temperature levels and higher air flow rate values. A maximum lipase activity (26.4 U gds^{-1}) was obtained at 27°C at an air flow rate of 0.8 L min^{-1} . Diaz *et al.* [28] reported the lipase production in a 50-g packed-bed bioreactor at 40°C and 50 mL min^{-1} of air. A maximum lipase activity of $1,500 \text{ U gds}^{-1}$ was achieved after 12 h of fermentation. Mala *et al.* [58] reported the lipase production in a 1-kg tray bioreactor. The production was lower when compared to that obtained in a 100-g tray bioreactor.

Repeated-Batch Processes

The repeated-batch processes combines the advantages of fed-batch and batch processes, mainly making possible to conduct the process by long periods and improving the productivity compared to the batch process. The work of Yang *et al.* [32] investigated the lipase production by immobilized mycelium from *R. arrhizus* in submerged fermentation using repeated-batch fermentations. The time to replace the volume, the volume of the replaced medium, and the optimal composition of the medium were optimized. Immobilized cells showed high stability for repeated use. Nine repeated batches were carried out in flasks for 140 h and six repeated batches in a 5-L bioreactor. The lipase productivity increased from $3.1 \text{ U mL}^{-1} \text{ h}^{-1}$ in batch fermentation to $17.6 \text{ U mL}^{-1} \text{ h}^{-1}$ in repeated-batch fermentation. Li *et*

al. [45] used repeated fed-batch strategy to produce lipase from *Acinetobacter radioresistens*. A constant cell concentration was shown to be a prerequisite to extend the number of repeated cycles, and adequate cell growth rate was critical for obtaining high lipase yield. In the previous cited work, the authors also verified that the pH control presented a high influence on lipase production. Dissolved oxygen constant feeding, on the other hand, could be manipulated to allow adequate growth rate for efficient lipase production. The lipase productivity reached $42,000 \text{ U h}^{-1}$ in a 2.5-L bioreactor. Benjamin and Pandey [65] carried out experiments in batch and repeated-batch (fed-batch type) for lipase production using immobilized *C. rugosa* cells in packed-bed bioreactor. A maximum enzyme activity (17.9 U mL^{-1}) was obtained when the fermentation was carried out in repeated-batch mode using a feed medium containing arabic gum and caprylic acid, keeping the flow rate of the feed at 0.4 mL min^{-1} and allowing each cycle to run for 12 h. Fed-Batch Processes The fed-batch processes are characterized by the addition of one or more nutrients to the bioreactor during the process, maintaining the products inside the bioreactor until the final of fermentation. The fed-batch processes are amply employed to minimize the effects of the cell metabolism control and, mainly, prevent the inhibition by substrate or metabolic products. Zhao *et al.* [64] scaled up from 5- to 800-L fed-batch bioreactors for the high cell density fermentation of *C. rugosa* lipase in the constitutive *Pichia pastoris*

expression system. The fermentation conditions for both lab and pilot scale were optimized. The exponential feeding combined with pH control succeeded in small-scale studies, while a two-stage fermentation strategy, which shifted at 48 h by fine tuning the culture temperature and pH, was considered effective in pilot-scale fermentation. A lipase activity of approximately 14,000 U mL⁻¹ and a cell wet weight of ca. 500 g L⁻¹ at the 800-L scale were obtained. Surribas *et al.* [61] compared different fed-batch cultivation strategies for the production of *Rhizopus oryzae* lipase from *P. pastoris* in a 20-L bioreactor. Several drawbacks have been found using a methanol non-limited fed-batch. Oxygen limitation appeared at early cell dry weight and high cell death was observed. A temperature limited fed-batch has been proposed to solve both problems. However, a methanol non-limited fed batch resulted in better productivities. Finally, a medium with low concentration of salt was used to overcome cell death problems. A temperature-limited fed-batch was applied thereafter to solve oxygen transfer limitations. This combined strategy resulted in lower productivities when compared to a methanol non-limited fed-batch. However, the cultivation was extended for a longer time, and a 1.3- fold purer final product was obtained mainly due to cell death reduction. Kim and Hou [66] cultivated *C. cylindracea* NRRL Y-17506 to produce extracellular lipase from oleic acid as a carbon source. The highest lipase activity obtained in flask culture was 3 U mL⁻¹ after 48 h

of fermentation. Fed-batch cultures (intermittent and stepwise feeding) were carried out in a 7.5-L bioreactor to improve cell concentration and lipase activity. For the intermittent feeding, the final cell concentration was 52 g L⁻¹ and the lipase activity was 6.3 U mL⁻¹ after 138.5 h of fermentation. Stepwise feeding was carried out to simulate an exponential feeding and to investigate the effects of specific growth rate on cell growth and lipase production. The highest final cell concentration obtained was 90 g L⁻¹ when the set point of specific growth rate was 0.02 h⁻¹ and the highest lipase activity was 23.7 U mL⁻¹ at 179.5 h. High specific growth rate decreased extracellular lipase production in the latter part of fed-batch cultures, due to build-up of over-supplied oleic acid. Ikeda *et al.* [79] developed a fed-batch fermentation process to enable the production of large amounts of recombinant human lysosomal acid lipase in *Schizosaccharomyces*. A feedback fed-batch system (5 L bioreactor) was used to determine the optimal feed rate of a 50% glucose solution used as carbon source. At the time of the initial consumption of glucose in the batch-phase culture, the nutrient supply was automatically initiated by means of monitoring the respiratory rate change. The obtained profile of the feed rate was applied to the feed forward control fermentation. Finally, the cells were grown up to 50 g dry cell weight and the lipase expression was 16 U mL⁻¹. Ito *et al.* [80] investigated the lipase production by a two-step fed-batch culture (2 L bioreactor) of an organic

solventtolerant bacterium. The two-step lipase production comprising a growth phase in fed-batch mode and a production phase, in which lipase was induced by the addition of 5% stearic acid, was carried out. In the growth phase, the maximum cell concentration at 16 h was 30.2 g L⁻¹ dry cell, and lipase activity was 96 U mL⁻¹ after 35 h, which is approximately 40 times higher than the production level obtained in flask culture. Gordillo *et al.* [81] studied the lipase production by *C. rugosa* in a 6-L bioreactor using two different fed-batch operational strategies to maximize lipase activity: constant substrate feeding rate and specific growth rate control. A constant substrate feeding rate strategy showed that a maximum lipase activity (55 U mL⁻¹) was reached at low substrate feeding rates, whereas lipase tends to accumulate inside the cell at higher rates of substrate addition. In the second fed-batch strategy studied, a feedback control strategy has been developed based on the estimation of state variables (cells and specific growth rate) from the measurement of indirect variables, such as carbon dioxide evolution rate by mass spectrometry. An on-off controller was then used to maintain the specific growth rate at the desired value, adjusting the substrate feeding rate. A constant specific growth rate strategy afforded higher final lipase activity (117 U mL⁻¹) at low specific growth rates. With a constant specific growth rate strategy, lipase production by *C. rugosa* was enhanced 10-fold compared to a batch operation. Continuous Processes. Montesinos *et al.* [82] investigated the

production of extra- and intracellular lipases in continuous cultures of *C. rugosa* using pure or carbon source mixtures. Lipase productivity in continuous cultures increased by 50% compared to data obtained from batch fermentation and was dependent on the dilution rate applied. Maximum yields relative to consumed substrate were obtained with oleic acid at low dilution rates. The authors found that during nitrogen limitation, lipase activity was suppressed. Results obtained were compared to previous data from batch and fed-batch cultures for the purpose of selecting the best process strategies for the lipase production with *C. rugosa*. The best lipase yields were obtained in fed-batch fermentation using oleic acid. Jensen *et al.* [83] studied the production of extracellular enzymes by the fungus *Thermomyces lanuginosus* in chemostat cultures at a dilution rate of 0.08 h⁻¹ in relation to different ammonium concentrations in the feed medium. Under steady-state conditions, three growth regimes were recognized and the production of several enzymes from *T. lanuginosus* was recorded under different nutrient limitations ranging from nitrogen to carbon/energy limitations. The range and the production of carbohydrate hydrolyzing enzymes and lipase increased from regime I (NH₄Cl ≤ 600 mg L⁻¹) to regime III (NH₄Cl ≥ 1,200 mg L⁻¹), whereas production of protease was the highest in regime II (600 mg L⁻¹ < NH₄Cl < 1,200 mg L⁻¹). Mathematical Modeling of Lipase Production Some basic steps are required to scale-up lipase production processes.

The first one has been widely discussed in the literature and includes the choice of a suitable microorganism and substrates for lipase production. The second step is related to the choice of the bioreactor configuration for the process development and the study, at laboratory scale, of how the manipulated variables affect the performance. The third step deals with the development and validation of mathematical models as a tool for scale-up, process control, and optimization. Finally, the analysis of technical and economical viability of the process is required. In this section, it is focused on the main aspects regarding the mathematical models for lipase production reported in the last 10 years, and how these models have been used as a tool for process scale-up or process improvement. Rajendran *et al.* [41] proposed an unstructured kinetic model to simulate the experimental data. The logistic model, the Luedeking–Piret model, and the modified Luedeking–Piret model were found to be suitable to efficiently predict cell mass, lipase production, and glucose consumption, respectively, with high correlation coefficient (R^2). From the estimated values of the Luedeking–Piret model parameters, α and β , it was found that the lipase production by *C. rugosa* is growth-associated. Haider *et al.* [84] modeled and optimized the lipase production by a soil microorganism using artificial neural network (ANN) and genetic algorithm (GA) techniques, respectively. The ANN model, based on back propagation algorithm, showed to be highly

accurate in predicting the system with a correlation coefficient value close to 0.99. Optimization using GA, based on the ANN model, resulted in the following values of the media constituents: 9.991 mL L⁻¹ oil, 0.100 g L⁻¹ MgSO₄, and 0.009 g L⁻¹ FeSO₄. A maximum (7.69 U mL⁻¹) of lipolytic activity at 72 h of culture was obtained, using the ANN–GA method, which was found to be 8.8% higher than the maximum values predicted by a statistical regression-based optimization technique response surface methodology. Boareto *et al.* [62] proposed an efficient hybrid neural phenomenological model (HNM) for the lipase production process by *C. rugosa*. The experimental data used corresponded to fed-batch operation with constant substrate feed rate. ANNs were trained to represent the aqueous and intracellular lipase activity and were further associated with a reduced version of the mechanistic model of the proposed HNM. When compared to experimental data, the HNM exhibited higher accuracy. The HNM can be used in process monitoring using on-line measurements of CO₂ and substrate feed rate to infer enzyme activities and also substrate and biomass concentrations. Ghadge *et al.* [85] investigated the effect of hydrodynamic flow parameters and interfacial flow parameters on the activity of lipase in a bubble column reactor. Lipase solution was subjected to hydrodynamic flow parameters in 0.15 and 0.385 m bubble column reactors over a wide range of superficial gas velocity. The flow parameters were estimated using

an in-house computational fluid dynamic code based on the $k-\varepsilon$ approach. The extent of lipase deactivation in both columns was found to increase with an increase in hydrodynamic and interfacial flow parameters. However, the use of the same values of the parameters, the extent of deactivation was different in the two columns. The rate of deactivation was found to follow a first-order kinetics. An attempt was made to develop rational correlations for the extent of deactivation as well as for the deactivation constant. The rate of deactivation was found to be depending on the average turbulent normal stress and interfacial flow parameters, such as bubble diameter and bubble rise velocity. Becker and Markl [86] modeled olive oil degradation by the thermophilic lipolytic strain *Bacillus thermotovorans* IHI-91 in chemostat and batch culture, to obtain a general understanding of the underlying principles and limitations of the process and to quantify its stoichiometry. Chemostat data were successfully described using the Monod chemostat model extended by terms for maintenance requirements and wall growth. Oleic acid accumulation observed during batch fermentation can be predicted using a model involving growth-associated lipase production and olive oil hydrolysis. Simulations confirmed that this accumulation was the cause for sudden growth cessation occurring in batch fermentations with higher olive oil starting concentrations. Further, an oscillatory behavior, as observed in some chemostat experiments, was predicted using the latter model.

Gordillo *et al.* [81] developed a simple structured mathematical model coupled with a methodology to estimate biomass amounts, specific growth rate, and substrate amounts and applied to the production of *C. rugosa* lipase in batch, fed-batch, and continuous operations with a 10-fold increase in productivity relative to batch operation. Montesinos *et al.* [87] proposed a simple structured mathematical model coupled with a methodology to estimate state variables and parameters and applied to *C. rugosa* batch and continuous lipase production. Process modeling was carried out using Advanced Continuous Simulation Language. Once model parameters were determined, the whole model was validated, showing satisfactory results. For estimation of the best strategy to improve lipase productivity, different simulations of batch, fed-batch, and continuous cultures were performed. The maximum enzyme productivity was predicted in continuous culture at moderate dilution rates and substrate feeding of 8 g L^{-1} . However, the highest predicted lipase activity was reached in fed-batch cultures with a prefixed substrate feeding in order to maintain constant—at their optimal values—the relation substrate/biomass or the specific growth rate. The fed-batch process mode matched the simulation results.

Conclusions

Critical analysis of current literature shows that microbial lipases are one of the most produced

enzymes. This review showed that many researchers worldwide direct their activities to the screening of new lipase-producing microorganisms and, subsequently, on the optimization of the medium composition and operational variables. All these efforts are justified by the great versatility of lipase applications. There has been much development on lipase production by bioprocesses, mainly using submerged fermentation such as the screening of high lipase producers, successful substitution of synthetic medium by agroindustrial residues, scale-up of process, different process operation modes, strategies of bioreactor operation, and the use of mathematical models as a tool for process optimization. Though the screening of microorganism producers of lipases has afforded satisfactory results to the present, based on our experience, we believe that the use of engineering lipases will predominate in the near future since the production of engineering lipases will allow attainment of enzymes with new remarkable characteristics for a specific application. The use of agro-industrial residues as substrates for lipase production favors, undoubtedly, the reduction of production costs associated to substrates. However, systematic studies should be carried out to check whether the global production cost is lowered, including the downstream step. Of course, in some cases, the difficulties imposed by the use of residues in the purification of enzyme produced make its use unfeasible. The fed-batch operation mode has provided the best results in terms of lipase activity

and productivity. In all cases cited in this review, the feeding rate was not optimized. An interesting alternative could be the use of an optimization tool such as the dynamic optimization or optimum control. This would allow the time domain to be partitioned in N-subintervals for which the feeding rate could be optimized. The application of a dynamic optimization tool makes it possible to find the optimal profile of feeding rate that maximizes the production or productivity. It may be important to emphasize that the use of an optimization tool would require a mathematical model able to represent the process in a satisfactory and reliable way. The results discussed in this review clearly demonstrate that SSF, as long as the cultivation volume is kept to a very small scale, yields good results in terms of process productivity. However, SSF is difficult to scale-up due to existing gradients (mainly in packed-bed bioreactor) in temperature, pH, moisture, oxygen, substrate, and inoculum. Considering the difficulties in handling the large volume bioreactors related to the lipase production by SSF, perhaps, it could be more practical to use SSF for the production at small scale; meanwhile, other bioreactor configurations are investigated, such as rotating drum, intermittently agitated, or fluidized bed bioreactors. These configurations allow a better uniformity of medium, decreasing considerably the gradients, though agitation can cause microorganisms death.

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