

Fungal Lipase Production by Solid-State Fermentation

Luis Beltrán Ramos-Sánchez^{1*}, Mario César Cujilema-Quitio¹, Maria Caridad Julian-Ricardo¹, Jesus Cordova² and Patrick Fickers^{3,4}¹Department of Chemical Engineering, University of Camagüey "Ignacio Agramonte Loynaz" Circunvalación norte, Km 5.5, 74650 Camagüey, Cuba²Department of Chemistry, University of Guadalajara, Blvd. M. García Barragán 1421, CP 44430, Guadalajara, Jalisco, Mexico³Biotechnology and Bioprocess Unit, Université libre de Bruxelles, Av. F.-D. Roosevelt, 50, CP165 / 61, 1050 Brussels, Belgium⁴Microbial Processes and Interactions, Gembloux Agrobiotech, University of Liège, Passage des déportés, 2, 5030 Gembloux, Belgium

Abstract

Lipases are one of the most promising enzymes in the chemical and biopharmaceutical industries. Numerous applications have been reported including fine chemistry, detergents formulation and biodiesel synthesis. Lipases are commonly produced by a wide variety of yeasts and filamentous fungi in submerged fermentation or solid-state fermentation. Filamentous fungi and yeasts usually behave more efficiently in solid-state fermentation and show greater productivities when compared to submerged fermentation. Although filamentous fungi adequately growth in solid-state fermentation, there are some limitations for cultivating them by this process. Here, a review is made about lipase production in solid-state fermentation. This includes the analysis of solid-state fermentation as a promising technology, characterization of growth media and ambient factors such as moisture, pH and temperature.

Keywords: Lipase production; Solid-state fermentation; Yeast; Filamentous fungi; Bioreactor

Introduction

Lipases are serine hydrolases that catalyze in nature the hydrolysis of ester bonds of tri-, di- and mono- glycerides into fatty acids and glycerol. They are defined as triacylglycerol acyl hydrolases (E. C. 3.1.1.3) and differ from the closely related esterase's (E. C. 3.1.1.1) due to their ability to hydrolyze triglycerides at a lipid-water interface [1]. This phenomenon, the so-called "interfacial activation", was first proposed to distinguish lipases from esterases [2]. Unfortunately, this criterion was not sufficient to characterize the behavior of several lipases [3]. They were subsequently redefined as long chain fatty acid triglyceride hydrolases in apposition of esterases that are able to hydrolyze only short chain (i.e. less than 10 carbon atoms) fatty acid triglycerides.

The rising interest in lipase production mainly lies on the wide industrial applications of the enzyme [4-6]. They recognize a wide variety of substrates and may catalyze many different reactions, such as hydrolysis or synthesis of esters bonds, alcoholysis, aminolysis, peroxidations, epoxidations and interesterifications. The great number of recently published papers concerning different aspects of lipase production and applications also demonstrates the increasing importance of these enzymes [7].

Fungi and bacteria produce most lipases used in biotechnological applications. Filamentous fungi are an interesting source of lipases because they produce extracellular enzymes [8]. Lipases can be produced by Submerged Fermentation (SmF) or by Solid-State Fermentation (SSF) processes, although fungi are better adapted than yeast and bacteria to grow on SSF [8]. Despite SmF is widely used in the enzyme industry and has advantages in process control and production yields, SSF represents an interesting alternative to produce industrial enzymes at lower costs [8,9]. SSF has many preferences to SmF for microbial enzyme production including: superior production yields and productivities, lower operating costs, less demands for asepsis control, cheaper fermentation media, higher oxygen distribution, fewer operational troubles, simpler equipments and control systems, lower energy consumption [6,10].

The aim of present paper is to review literature concerning fungal lipase production by SSF in order to characterize different aspects that could be used to improve the process.

Solid-state Fermentation as a Proper System for Fungal Culture

SSF could be defined as a fermentation process in which microorganisms grow on a solid material, natural or synthetic, in absence or in a very limited amount of free liquid phase [11,12]. Bioreactors for this process could be operated in batch or in continuous mode (CSSF). In this later case, there is a flow of the solid phase within the reactor [13,14]. There are specific challenges to operate CSSF bioreactors that are not faced in classical continuous SmF. Consequently, CSSF processes are currently scarce in industry [14]. However, improved design procedures and sensors development promise a better future for CSSF at industrial scale.

SSF offers a quite different environment than SmF for cell growth and metabolism. In that regard, SSF is well adapted for yeasts and filamentous fungi cultivation since they can grow at low moisture content by contrast to bacteria [15]. Technologically, SSF presents several advantages compared to SmF [16]. Oxygen transfer limitations often encountered in SmF are eluded by a much more enlarged surface (external and/or internal) of the solid support for mass transfer [12,17]. In addition, cells and especially aerial hyphae are exposed directly to the gas phase, increasing significantly the overall oxygen transfer. Since SSF is performed at low water activity, it permits to prevent bacterial contaminations that require a high water activity to grow [18]. Moreover, the control of the optimal water activity in regards to a specific fungal species together with the utilization of an adequate cell inoculum, also permit to avoid or at least to reduce contamination by other fungal species. Beside these advantages, SSF also present several drawbacks such as heat generation by the cellular metabolic activity.

***Corresponding author:** Luis Beltrán Ramos-Sanchez, Department of Chemical Engineering, University of Camagüey "Ignacio Agramonte Loynaz" Circunvalación norte, Km 5.5, 74650 Camagüey, Cuba, Tel: +53-32-261192; E-mail: luis.ramos@reduc.edu.cu

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One way to dissipate this heat consists in the mechanical agitation/mixing of the solid medium [19]. However, this action could be harmful for filamentous fungi. It requires, thus a great compromise between cell survival and heat dissipation for process viability. This problem is not encountered for yeasts, thus offering a good opportunity for producing enzymes such as lipases with enhanced possibilities of competing in enzyme market.

SSF versus SmF for Lipase Production

SSF has many advantages over SmF for microbial enzyme production namely high yield and productivities, lower operating costs, low-cost fermentation media (raw biomass, agro industrial residues), higher oxygen distribution, fewer operational troubles, simpler equipment and control system [10,20].

A comparative study on alkaline lipase production by a strain of *Aspergillus fumigatus* under SSF and SmF was made in which the maximum concentration of the enzyme was comparable in both cases [21]. However, lipase produced by SSF was stable over a period of 15 days whereas lipase production in SmF decreased from day 5. Other authors reported lipase produced by SSF to be 24% more active and 64% more thermally stable than lipase produced by SmF using a strain of *Rhizopus homothallicus* [22].

Although most of the industrial enzymes are currently produced by submerged fermentations, SSF appears a promising technology due to the advantages it offers, such as lower cost of production [12,23-25]. Compared to the cost of culture medium in SmF, SSF of agro-industrial solid wastes is inexpensive, and therefore this process is industrially and economically attractive [16,21]. Coradi et al. [20] reported a cost of lipase production by *Trichoderma viride* in SSF ten times lower than in SmF. For instance, total capital investment needed for a production scale of 100 m³ of lipase concentrate per year is 78% higher for SmF compared to SSF [17]. These results confirm the interesting potential of SSF for producing lipase at low cost.

Industrial Applications of Lipases

Lipases are essential components in the modern industrial process due to their ability to catalyze, depending on the thermodynamic conditions, hydrolysis reactions as well as synthesis reactions such as esterification and transesterification (Figure 1) [26]. Remarkably, in the last decade, yeast lipases were also applied in the manufacture of pharmaceuticals, pesticides, biosensors and in waste management [27]. Furthermore, the genes encoding lipases in *Candida* sp., *Geotrichum* sp., *Trichosporon* sp. and *Yarrowia lipolytica* have been cloned and over-expressed [7,28]. The most important lipase producers among yeasts used in industrial processes are *Candida* species namely *C. antarctica*, *C. cylindracea*, *C. lipolytica* and *C. rugosa* [7,29,30]. *C. rugosa* lipase is one of the enzymes most frequently used in bio-transformation and has the great advantage of being considered as safe for food applications. The crude enzyme extract contains at least five isoenzymes, denominated Lip1 to Lip5. Despite they share high sequence homology, they have different catalytic characteristics, substrate specificities and thermal stabilities [31]. CAL B from *C. antarctica* is the second most studied yeast lipase, having several applications, such as synthesis of flavors and fragrance esters, surfactants, biodiesel, waxes, acylated flavonoids and modified glycerides as well as kinetic resolution of racemic esters and amines [32]. Its stability has also been improved using ionic liquids or no aqueous solvents for enzymatic synthesis [31]. *Y. lipolytica* lipase produces high value compounds, being used for bio-transformation of steroids and synthesis of pharmaceutical intermediates and fine

chemicals [28]. Lipases from yeast are used in many different industrial processes as illustrated in the following sections.

C. antarctica lipases were used for esterification of free fatty acids in the absence of organic solvent and for transesterification of fatty acid methyl esters in hexane with isopropylidene glycerol [33]. Immobilized lipases from *C. antarctica*, *C. cylindracea* and *G. candidum* were used for the esterification of functionalized phenols for synthesis of lipophilic antioxidants [34]. *C. rugosa* lipase is used by Nippon Oils & Fats (Tokyo, Japan) for the preparation of highly pure unsaturated fatty acids (oleic, linoleic, linoleic, etc.) [35].

Large amounts of lipases, nearly 1000 tons per year, are used in the detergent industry for removal of oil-based stains [29]. For this purpose, lipases from *C. cylindracea*, *Y. lipolytica* and *C. antarctica* are preferred because they can operate at low temperatures and alkaline pH, compared with bacterial and other fungal lipases [36,37]. Miyoshi Yushi, a company in Nagoya (Japan), produces sizable amounts of soap through lipase-based hydrolysis of oils and fats with *C. rugosa* lipase [35]. Enzymatic synthesis of surfactants has been carried out at moderate temperatures (60-80°C) with excellent regioselectivity. Unichem International (USA) has manufactured isopropyl myristate, isopropyl palmitate and 2-ethyl palmitate, as emollient for personal care products using *C. cylindracea* lipase [38].

Lipases are used for flavor development and improving quality of foods. *C. parapsilosis* and *C. antarctica* lipases were used to synthesize hydroxamic acids, a food additive, and short chain flavor thioester, respectively. Additionally, *C. rugosa* lipase was used in the cheese manufacture [27]. The flavor development in concentrated milk and creams by microbial lipases was investigated, finding that each lipase developed a characteristic flavor. *C. rugosa* lipases were found as the most suitable for this purpose [39]. Several structural lipids were synthesized by yeast lipases and included in infant formula and nutraceuticals [29,30]. *C. antarctica* lipase is employed as a robust biocatalyst for esterification reactions, obtaining high conversions of flavoring esters of short-chain [40]. *C. cylindracea* lipase is used in bakery products manufacturing [41].

Biocatalytic processes are used to prepare chiral intermediates for pharmaceuticals. (S)[1-(acetoxyl)-4-(3-phenyl) butyl] phosphonic acid diethyl ester, a key chiral intermediate required for 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 using *Geotrichum candidum* lipase [42]. CAL-B lipase was used for the enzymatic resolution of a racemic mixture of 2-pentanol, a key chiral intermediate required for synthesis of anti-Alzheimer's drugs [42]. *C. cylindracea* lipase was applied to the resolution of 2-bromopropionic acids and 2-chloropropionic acids which are starting materials for the synthesis of phenoxy propionic herbicides [43]. *C. cylindracea* and *C. antarctica* lipases have been used to resolve the enantiomers of flurbiprofen, naproxen, ibuprofen, suprofen and baclofen [43]. Both enzymes were successfully used to synthesize lobucavir, hepatitis B antiviral and ribavarin antiviral [44]. These lipases are also suitable for transesterification reactions, displaying both wide substrate specificity and regioselectivity [45].

Croda Universal Ltd. (Sweden) uses *C. cylindracea* lipase to synthesize wax esters (esters of fatty acids and fatty alcohols) as additives for personal care products. According to the manufacturer, the overall production cost is slightly higher than that of the conventional method, however the cost is justified by the improved quality of the final product [42]. *C. rugosa* lipase was selected to hydrolyze triacylglycerol for clinical

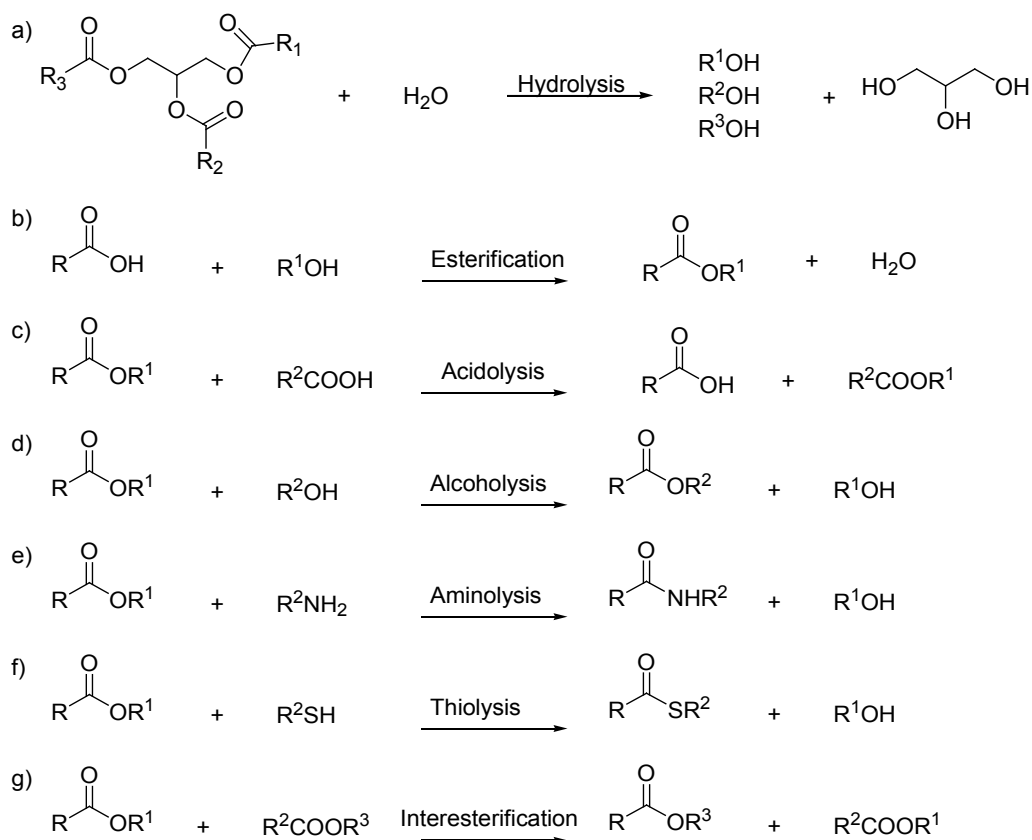


Figure 1: Reactions catalyzed by lipases. a) hydrolysis, b) esterification, c) acidolysis, d) alcoholysis, e) aminolysis, f) thiolyis, g) interesterifications

lipid analysis and developed as a biosensor [38,43]. *C. rugosa* lipase was also used to synthesize lovastatin, a drug lowering serum cholesterol level. The asymmetric hydrolysis of 3-phenylglycidic acid ester, which is a key intermediate in the synthesis of diltiazem hydrochloride, a coronary vasodilator, was carried out with *S. marcescens* lipase [46].

Lipases from different sources are able to catalyze biodiesel synthesis but those from *C. antarctica* and *C. rugosa* are the most used [45]. The Novozym 435 (CAL-B) lipase is able to convert at high yield used olive oil which contained high levels of free fatty acids and water content into biodiesel [45]. Simple alkyl ester derivatives of restaurant grease waste were prepared using immobilized lipases from *Thermomyces lanuginosus* and *C. antarctica* [47].

Lipases are utilized in activated sludge and other aerobic waste processes, where thin layers of fats must be continuously removed to allow oxygenation. Effective breakdown of solids, clearing and prevention of fat blockage or filming in waste systems are important in many industrial operations such as degradation of organic debris using a commercial mixture of lipase, cellulase, protease and amylase or sewage treatment, cleaning of holding tanks, septic tanks, grease traps. *C. rugosa* lipase is a good option to hydrolyze residual fats [37]. *C. rugosa* lipase was also used by Nippon Paper Industry to hydrolyze 90% of wood triglycerides as a pitch control method [43,48].

Regulation of Lipase Production Concerning the Medium Composition

Yields in lipase synthesis in SSF vary according the strain, the growth medium (solid support), culture medium (carbon and nitrogen

sources) and cultivation conditions (pH, temperature and moisture). The main important lipases from filamentous fungi produced in SSF belong to the genera *Aspergillus*, *Rhizopus* and *Penicillium* while those from yeast origin belong to *Candida* and *Yarrowia* (Table 1). Most of the lipases produced by yeast and filamentous fungi are extracellular and their production is modulated by the culture conditions. Expression of lipase encoding genes is regulated by the nutrients from the culture medium and/or by the solid substrate used. Lipids such as triglycerides, fatty acid or long chain fatty acid esters are requested for high lipase production yields. Oleic acid (cis-9-Octadecenoic acid) was reported as the most suitable inducer for the production of the main extracellular Lip2p lipase in *Y. lipolytica* [51,52]. However, pure oleic acid is not convenient for the development of industrial processes owing to its excessive cost. With the aim of developing a cost-friendly process, different oleic acid esters and triglycerides have been tested for their ability to support lipase synthesis. Olive oil, mainly constituted by triolein as the main triglyceride, and methyloleate led to lipase activities equal to 1130 and 1190 U ml⁻¹, respectively [53]. These results are in the range of that obtained with pure oleic acid (i.e. 1045 U ml⁻¹). By contrast, ethyloleate led to a significant decrease of lipase production (195 U ml⁻¹). Expression of the *yLIP2* lipase-encoding gene, and thus Lip2p synthesis appeared also to be markedly influenced by oleic acid concentration [51]. By contrast, carbon sources such as glucose and glycerol were found to repress lipase production [54].

Nitrogen sources used to supplement the culture medium also greatly influence lipases production. Various mineral and organic nitrogen sources were tested for their capacity to support cell growth and lipase production [51]. Mineral nitrogen such as ammonium chloride

Strain	Solid support / substrate	Lipase activity	References
Yeast			
<i>Candida rugosa</i>	Coconut oil cake	88 U g ⁻¹	[65]
	Sesame oil cake	32 U ml ⁻¹	[85]
	Groundnut oil cake	57 U ml ⁻¹	[85]
	Rice Bran	37 U g ⁻¹	[80]
<i>Yarrowia lipolytica</i>	Sugarcane bagasse/wheat bran	9 U g ⁻¹	[90]
	Palm kernal cake	19 U g ⁻¹	[84]
Mold			
<i>Aspergillus niger</i>	Wheat bran	650 U g ⁻¹	[50]
	Wheat bran/oleic acid	49 U g ⁻¹	[6]
<i>Aspergillus fumigatus</i>	Defatted rice bran	9 U ml ⁻¹	[21]
<i>Aspergillus carneu</i>	Sunflower	13 U g ⁻¹	[69]
<i>Rhizopus homothalicus</i>	Sugarcane bagasse/olive cake	43 U ml ⁻¹	[81]
<i>Rhizopus oryzae</i>	Sugarcane bagasse	215 U g ⁻¹	[16]
<i>Penicillium brevicompactum</i>	Babassu oil cake	49 U g ⁻¹	[75]
	Castor meal	88 U g ⁻¹	[75]
<i>Penicillium restrictum</i>	Babassu oil cake	30 U g ⁻¹	[74]
<i>Pennicillium sp</i>	Soybean meal	140 U g ⁻¹	[79]
<i>Rhizomucor pusillus</i>	Sugarcane bagasse/olive cake	21 U ml ⁻¹	[81]
	Sugarcane bagasse	5 U g ⁻¹	[81]
<i>Rhizomucor rhizopodiformis</i>	Sugarcane bagasse	3 U g ⁻¹	[81]

Table 1: Strain used in solid-state fermentation for lipase production. Lipase activity, unless stated otherwise, is expressed in unit per gram of dry solid support

or ammonium sulfate had no influence on either cell growth or lipase production. By contrast, a significant increase in lipase productivity was observed upon addition of organic nitrogen sources such as urea or casein hydrolysates. For instance, tryptone N1 (Organotechnie, La Courneuve, France) allowed a 166-fold increased lipase production compared to the non-supplemented medium. However, cultures in the presence of casamino acid, which result from a complete hydrolysis of casein, led to a low lipolytic activity. These results suggest that specific peptides constituting tryptone N1 could regulate lipase production [51]. This hypothesis was confirmed by testing separately different tryptone constituting peptides [55].

Recently, lipase production by *Y. lipolytica* in SSF using different agro-industrial residues was reported [56]. When soybean cake and its sludge were used, lipase activity reached 139 U g⁻¹ after 14 h of fermentation whereas a lipase activity of 102 U g⁻¹ was obtained after 28 h of fermentation when cottonseed cake was used as substrate without any supplement [56].

C. rugosa is able to produce three lipase isoenzymes, namely Lip1, Lip2 and Lip3. Their production is modulated in yield (enzymatic activity) and in quality (isoenzyme synthesis) by the culture conditions and medium composition. Glucose and glycerol repress lipase synthesis [57,58] whereas oleic acid, ester of oleic acid and olive oil enhance its synthesis [59]. At low concentrations, oleic acid seems to favor the synthesis and secretion of Lip2 and Lip3 lipase isoenzymes while Lip1 is only produced at high oleic acid concentration [60]. Lipase production was also reported in the presence of carbon sources such as sterols [61], hexadecane [61], carboxylic acids [59], vegetable oils [62] and dodecanol [63]. By contrast, no lipase production could be detected in the presence of alkane, despite this carbon source could support cell growth [58]. Corn steep liquor [64] was reported has a suitable nitrogen source for lipase production in *C. rugosa* together with urea [65] and peptone [66].

In SSF, lipase production is also modulated by the solid support/medium used. Twelve solid substrates were tested in regard to lipase production in *Aspergillus niger* [67]. The highest lipase production was obtained with wheat bran (198 U g⁻¹) and gingelly oil cake (169 U g⁻¹). Addition of various nitrogen sources, carbohydrates and inducers

to the substrate did not yielded to any significant increase of lipase synthesis. This demonstrates that in some cases, the solid substrate used is sufficient to support both cell growth and lipase production at high yield. Other authors reported that lipase synthesis by *A. niger* is enhanced in the presence of Tween 80, soybean meal, urea and ammonium sulfate [68]. Lipase production in *Aspergillus carneus* was improved by a response surface approach using a one-at-a-time method [3,69]. The highest lipase productions were obtained in a medium composed of sunflower oil (1%), glucose (0.8%) and peptone (0.8%). Similarly, the highest lipases production by *Aspergillus terreus* was obtained in a medium containing corn oil (2%) and casein hydrolysate (0.1%) [70]. The presence of calcium and magnesium ions enhanced lipase secretion [70]. Beside this, the highest lipase production by *Aspergillus oryzae* was obtained in a medium containing sunflower oil (3%), glucose (2%), yeast extract (1%) and polypeptone (2%) [71]. Similarly to *Aspergillus sp*, lipases synthesis by *Rhizopus arrhizus* is inhibited by glucose whereas a 2.5 fold increase in lipase production was obtained in the presence of corn oil used as inducer [72]. For *Rhizopus oryzae* growing on rice straw, olive oil (1.38%) and soybean meal (1.27%) are the optimal inducer and nitrogen source, respectively [73]. In these conditions, lipase production reached 59 U g⁻¹. Lipase production by *Penicillium restrictum* in SSF was investigated on solid waste from the babassu oil industry. The medium was supplemented with peptone, olive oil or starch at different C/N ratios. The highest lipase production was obtained with 1% of peptone enrichment (C/N ratio of 11.7, 28 U g⁻¹) and with 2% olive oil enrichment (C/N ratio of 14.1, 30 U g⁻¹) [74]. Molasses, urea and soybean oil concentrations as well as moisture content were tested for lipase production by *Penicillium brevicompactum* in SSF using babassu cake and castor meal as solid support [75]. For babassu cake, the highest lipase activity was obtained for a moisture content of 70% and 2% of soybean oil. This result was in accordance with those obtained for *Burkholderia sp.* using olive oil [76]. Beside this, lipase production by *Penicillium citrinum* was investigated on medium containing 1% groundnut-oil refinery residues as carbon source and various nitrogen contents. Ammonium chloride (0.75%) was found to be the best nitrogen source compared to ammonium sulfate and urea. This latter yielded to a decrease of lipase synthesis by 85% [77]. Compared to oleic acid, a two-fold increased lipase activity

was obtained in the presence of this oil refinery residue due to its high carbohydrates content (35%). Production of lipase by *Penicillium simplicissimum* in SSF, using babassu cake as the basal medium, was investigated in the presence of olive oil, sugarcane molasses, corn steep liquor and yeast extract. Olive oil and corn steep liquor were found the most suited carbon and nitrogen sources for lipase synthesis [78]. Rigo et al. [79] reported that the medium supplementations with urea and soybean oil significantly increased lipase production by *Penicillium* sp. grown in SSF with soybean meal as the solid substrate [79]. All these data highlight clearly that production of fungal lipase depends of several factors such as the solid substrate used and the nature of the carbon and nitrogen sources used as supplement. Additionally, the moisture content, pH and temperature should also be taken into account for process optimization.

Solid Media, Solid Support and Ambient Factors used for Lipase Production

As stated above, one of the main characteristics of SSF is the utilization of solid substrates as a support for cell growth. For some applications, these solid supports are also the only sources of carbon and nitrogen available to sustain microbial growth. These supports could be from artificial nature or of biological origin. In the recent years, considerable researches have been carried out using agricultural wastes, which are renewable and abundantly available, to produce value-added products by SSF. Utilization of agro-industrial wastes provides alternative substrates and may help to solve pollution problems. The nature of the substrate is the most important factor affecting fermentative processes.

The choice of a substrate depends on several factors, mainly related to their cost and availability but also to the nature of the compound to produce (enzyme, metabolite, biomass) [7]. Many substrates such as rice bran [80], coconut oil cake [65], olive cake and sugar cane bagasse [81], gingelly oil cake [67], babassu oil cake [74], wheat bran [82], *Jatropha curcas* seed cake [83], niger seed oil cake [84], palm kernel cake [84], groundnut oil cake [85] and mustard oil cake [86] have been used successfully for lipase production. The most important process parameters that have been investigated and optimized up to now are the incubation time, inoculum age, inoculum size, initial moisture content, nature and concentration of carbon and nitrogen sources as well as the C/N ratio of the medium [87].

The importance of water content in SSF has long been recognized [88,89]. This could be viewed in terms of moisture or water activity. Moisture represents the fraction of water present in the medium while water activity represents the fraction of free water in available to microorganisms [90]. The moisture content of the solid substrate used can vary due to liquid evaporation, microbial metabolism and by the water-binding characteristics of the substrate [12]. This moisture content has a significant impact on microbial growth and metabolic activities, including lipase production [90]. In general, the moisture content used in SSF processes vary between 30 and 85% [91]. The influence of initial moisture content on lipase production by *Y. lipolytica* from niger seed oil cake was studied in the range of 50 to 100% (v/w) [84]. An initial moisture content of 60% (v/w) lead to the maximal value of lipase production (18.5 U_g⁻¹). At higher moisture contents, the lower lipase production observed could be due to a decrease of the substrate porosity and hence in a decrease of the gaseous exchange that lead to suboptimal conditions for cell growth and enzyme production. By contrast, low moisture content could lead to a reduction of the nutrients solubility and availability, a reduced

degree of swelling and a higher water tension. For the production of lipase in SSF by *Y. lipolytica* from mustard oil cake, the maximum lipase activity was obtained at 50% v/w initial moisture content [86]. The effect of initial moisture content from 40 to 90% (v/w) on lipase production of *C. rugosa* using groundnut oil cake was investigated. A moisture content of 60% v/w was reported as the optimal value [85,86]. Although moisture content is a commonly used factor to optimize growth medium, it is known that microorganisms are more sensitive and specific to water activity [88].

Another important parameter to consider in SSF is the pH variation during the process since this latter is difficult to control due to the heterogeneity of the system. During cell growth, pH variation is mainly caused by the secretion of organic acids or by the assimilation of nitrogen sources such as ammonium, nitrate or urea. Each microorganism has an optimal pH range for growth and metabolic activity, including lipase production and secretion. However, they are able to grow in a wide pH range from 2.5 to 8.5. This versatility of fungi in regards to pH can be beneficially exploited to prevent or minimize bacterial contaminations [91]. Lipase production has been investigated for different pH values ranging from 3.0 to 9.0. The maximum lipase production has been observed between pH 6.0 and 7.0 [5].

Temperature control is also an important factor to consider. During SSF, it is difficult to remove the excess of heat generated by the cellular metabolism, mainly due to the low thermal conductivity of the solid medium [15]. In some cases, this heat accumulates within the solid medium, causing a decrease in microbial growth and even, in some cases cell death. The temperature at the center of the fermenting solid medium could be higher up to twenty degrees than the incubation temperature [92]. An airflow through the system is often applied to dissipate metabolic heat from the solid medium and also to supply the oxygen requirements for cultures [92]. Remarkably, optimal temperatures for lipase production have been reported in the range of 28 to 45°C [12,86].

Lipase Production using different SSF Bioreactors

Lipase production by SSF has been mainly performed in tray bioreactors (TB) and packed-bed bioreactor (PBB). TB are the simplest design for SSF [93,94]. They can be considered as a modification of a column bioreactor in which the height of the bed is short enough to prevent temperature profiles to form in the axial direction. Tray bioreactors consist of a chamber where controlled air (flow, temperature and relative humidity) is circulated around a number of trays (Figure 2). Each tray contains a layer of solid substrate, typically between 5 and

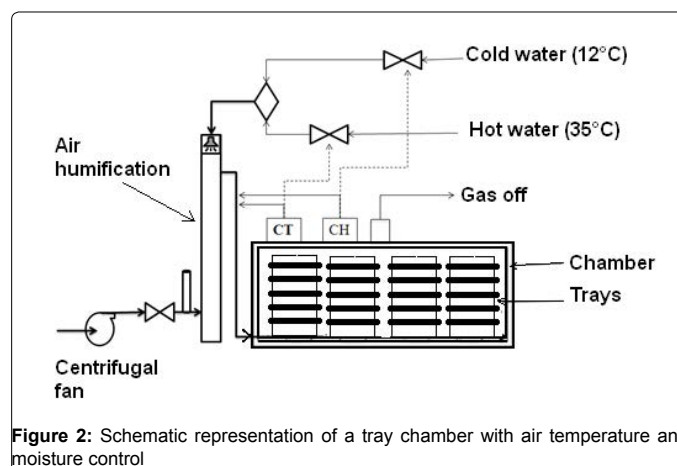


Figure 2: Schematic representation of a tray chamber with air temperature and moisture control

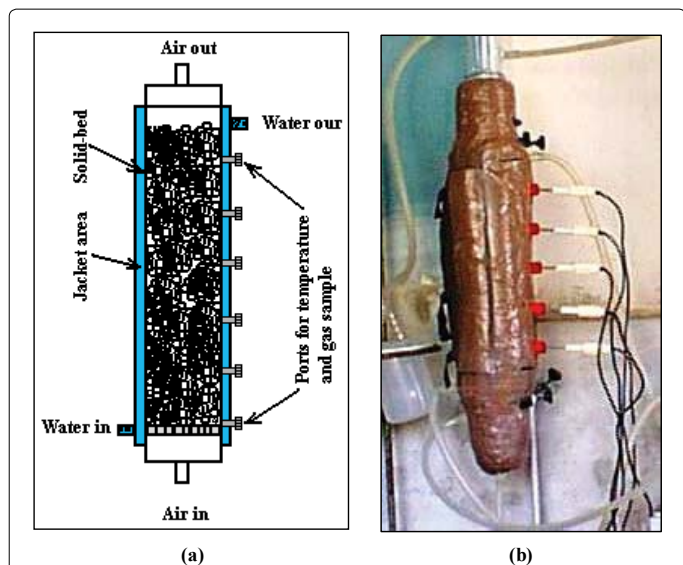


Figure 3: Packed-bed bioreactors used at different stages of process development: (a) schematic representation; (b) Real lab-scale bioreactor [7]

Strain	Bioreactor type	Scale (g)	Lipase activity (Ug ⁻¹)	Time (h)	References
Yeast					
<i>Yarrowia lipolytica</i>	TB	25	26	96	[84]
<i>Candida rugosa</i>	TB	12	11	120	[85]
	TB	10	88	96	[65]
Mold					
<i>Penicillium Sp.</i>	TB	10	140	48	[79]
<i>Penicillium simplicissimum</i>	TB	10	90	72	[78]
<i>Penicillium chrysogenum</i>	TB	17	65	72	[98]
<i>Aspergillus niger</i>	TB	33	1934	96	[99]
	TB	20	63	48	[6]
	TB	10	623	72	[9]
	TB	100	598	72	[9]
	TB	1000	523	72	[9]
<i>Rhizopus chinensis</i>	TB	30	350	36	[101]
	TB	10	142	96	[100]
<i>Rhizopus homothallicus</i>	PBB	20	826	12	[102]
	PBB	50	1500	13	[103]
<i>Rhizopus microsporus var. tuberosus</i>	PBB	20	74	20	[104]
<i>Rhizopus microsporus var. chinensis</i>	PBB	20	72	20	[104]
<i>Rhizopus oligosporous</i>	TB	10	48	48	[105]
	TB	8	77	120	[106]
<i>Rhizopus oryzae</i>	TB	5	236	72	[16]

Table 2: Lipase activity achieved in tray (TB) and packed-bed bioreactors (PBB)

15 cm deep. Trays usually have an open top and perforated bottom to favor exchange with the gaseous phase. Intermittent mixing of the solid substrate by hand could be carried out but in general this will occur only once per day. The main drawback of TB is their low volumetric efficiency compared to column bioreactor [95]. Indeed, the thickness of the bed must remain low while the space between the trays must remain large enough to ensure an optimal gas and heat transfer. There have been no significant advances in tray design over the last decade.

PBB typically involves a static bed on top of a perforated plate through which conditioned air is blown (Figure 3). In some PBB design, air is blown through a perforated rod inserted into the center of the

bed. Over the last 25 years, PBB has received much experimental and modeling attention. The main attractive characteristic is that it has no mechanical (i.e. moving) parts, thus reducing the cost of construction, operation and maintenance. In PBB processes, axial dynamical temperature profiles could be observed [96]. Since the water-carrying capacity of the air increases with temperature, these axial temperature gradients promote evaporation within the bed even if water-saturated air is used to aerate the column. Despite, this evaporation phenomenon can remove up to 65% of the heat generated by the cellular metabolism, it lowers the moisture of the solid substrate and thus limit the cellular growth [97]. In PBB, moisture regulation is almost impossible since the bed is unmixed. Therefore, it is desirable to increase heat removal by other means such as water jacket as shown in Figure 3.

In the literature, reports on large-scale production are very scarce and most of them are in the scale of few grams of solid support [11]. At small scale, comparison of the yields of lipase productions obtained for different bioreactors design is not significant. Indeed, the process at that scale is not influenced by axial temperature profiles that form within the solid substrate. Therefore, the differences in lipase productions, presented in Table 2, seem to be more dependent on strains and growth conditions than other factors. The data shown in Table 2 indicate that lipase productions are below level of 500 Ug⁻¹ in 71% of the cases while less that 10% are over 1500 Ug⁻¹. This indicates that very high productions and productivities could be reached under controlled SSF process. However at higher scale, a decrease in process productivity should be expected. Nevertheless, Edwinoliver and coll. reported only a slight decrease in lipase production when scaling up the process from 10 g to 1000 g [9].

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