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# AGRO-INDUSTRIAL BIOMASS AS LOW-COST SUBSTRATE FOR MICROBIAL LIPOLYTIC PRODUCTION – REVIEW

**Gopika Nair M [1], V Priya[2*]**

[1]Research Scholar, Department of Botany, PSG College of Arts & Science, Coimbatore, India- 641014

[2*]Assistant Professor, Department of Botany, PSG College of Arts & Science, Coimbatore, India- 641014

**Corresponding author*:** V. Priya Phone: 9786066613

Email ID: priya_v@psgcas.ac.in

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**ABSTRACT**

For decades enzymes have been the most efficient biological catalysts accelerating chemical reactions that drive to yield immensely valuable end products and applications in industrial sectors. Due to their sustainable approach among many enzymes that established their prominence, lipase stands out as a highly significant enzyme in today's commercial sector owing to its versatility in applications such as the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper industries. Industries prioritize microbial-origin enzymes due to their notable characteristics, including high stability, specific substrate affinity, shorter processing times, and lower energy requirements. Meeting the high demand for microbial lipase enzymes becomes challenging due to the elevated costs of enzyme production. To address this issue, the utilization of agricultural and industrial wastes is employed as a solution to lower production costs. This review focuses on the use of affordable agro-industrial residues as substrates and assesses their effectiveness in the production of microbial lipase.

**KEYWORDS**

Lipase; Microbial lipase; Cost-effectiveness; Agro-industrial residues

## INTRODUCTION

Microbial enzymes have emerged as essential catalysts in driving innovation and promoting sustainability across diverse industrial sectors. The increasing importance of microbial enzymes is driven by the need for more sustainable and eco-friendly solutions in various industries, advancements in biotechnology, and the recognition of the efficiency and specificity of these enzymes in a wide range of applications. Due to their

versatility and efficiency, microbial enzymes are employed in various industrial sectors like food and beverage, pharmaceuticals, agriculture, textile, paper and pulp, personal care and cosmetics, bioremediation purposes, etc. The anticipated rise in the global industrial enzymes market, from $7.4 billion in 2023 to an expected $10.2 billion by 2028, suggests a surge in demand for these enzymes (Aashish, 2023). The ability to manipulate microbial enzymes through genetic engineering and various techniques provides extensive control and customization, rendering them a versatile and efficient option for a multitude of industrial applications. This adaptability fosters the creation of enzymes with customized characteristics and enhanced performance across a broad spectrum of processes. Microbial-originated amylases, proteases, cellulases, lipases, pectinases, and xylanases, among others, have solidified their roles in significant industrial sectors such as food, biotechnology, agriculture, pharmaceuticals, and more. Persistent research endeavors are propelling ongoing progress in unlocking the complete potential of microbial enzymes.

Lipases are generally defined as the enzymes that catalyze the hydrolysis of carboxyl ester bonds in water-insoluble glycerides in an emulsion to release fatty acids and glycerol. Lipases facilitate the breakdown of lipids into free fatty acids, and in a reversible process, they also serve as catalysts for esters synthesis and transesterification (Tsuchiya et al., 1999). Lipases are enzymes found widely in nature, with microbial (bacterial, fungal, and yeast), animal, and plant origins (Nwuche and Ogbonna., 2011). Nevertheless, lipases derived from microbial sources are predominantly favored due to their lower production costs and ease of genetic manipulation (Adetunji and Olaniran., 2021). A particular advantage of microbial-derived lipases, in contrast to those from other sources, is their ability to function without the need for colipases, unlike certain pancreatic lipases present in mammals (Bello et al., 2017). Also due to their high substrate specificity, reduced processing time, minimal energy requirements, and high stability, microbial lipases have garnered significant interest in light of the rapid advancements in enzyme technology (Hasan et al., 2006). Bacteria, fungi, and yeast are the most prolific sources of lipase among microorganisms. The microorganisms commonly employed for lipase production encompass *Penicillium* sp., *Candida rugose, Aspergillus niger*, *Rhizopus* sp., *Pseudomonas* sp., *Bacillus*, *Pseudomonas burkholderia*, *Streptomyces*, *Arthrobacter*, and *Archomobacter* (Ali et al., 2023). Fungi are often the preferred choice due to their tendency to secrete enzymes extracellularly, making it easier to extract enzymes from the fermentation environment (Karimi et al., 2018).

Fungal lipases are versatile in their enzymatic properties and substrate specificity, which make them very attractive for industrial applications (Singh and Mukhopadhyay, 2012). Among microbial sources, filamentous fungi are good extracellular lipase producers and the extraction and purification process is relatively easy. They are regarded as prominent for industrial applications owing to their best stability as well as broad substrate specificity (Treichel et al., 2010). The production of lipase by microbial strains varies depending on the strain and growth medium composition, such as the nitrogen and carbon source, and the temperature (Maheshwari et al., 2000). The lipolytic microorganisms are reported to be isolated from several sources like oil-contaminated soil and water samples, pasteurized milk, fresh and melted butter, cheese, industrial and nonindustrial sewage containing fats, and oil-processing factories across various environments.

Even though the microbial sources are abundant the production cost of the enzyme is high which thereby limits the production of the lipase enzyme. From an industrial point of view, around 30–40% of the production cost of enzymes is estimated to account for the cost of the growth medium (Joo et al., 2003). To rectify this, using agro-industrial residues as substrates in the microbial growth medium is considered to amplify enzyme production. This review concentrates on the agro-industrial residues used to produce lipase from various microbial strains thus resulting in a cost-effective lipase production.

**Agro-industrial residues as a substrate for microbial lipase synthesis**

The production of dry extract lipase from *Aspergillus niger* by solid-state fermentation using agro-industrial wastes like rice bran and Jatropha seed cake as a substrate resulted in a 4290 kg/year production capacity (Khootama et al., 2018). In the lipase production by newly isolated *Aspergillus* sp. from Amazon boreal forest soil Grape seed oil allowed an enzyme activity 2 fold higher than Cotton seed oil (CSO). The characterization revealed the enzyme is highly active at an optimum pH of 7.5 and temperature of 45°c (Tacin et al., 2019). Optimization studies of lipase production by *Aspergillus niger* on agro wastes like rice bran and Jatropha seed cake for 5 days

yielded dry lipase extract with the highest activity unit (176 U/ml enzyme) on 1% of olive oil as inducer and 282 U/ml enzyme on 1% of NaCl – 0.5% of Tween 80 as extractant (Putri et al., 2019). The use of residues from the industrial processing of palm oil as a carbon source and inducer for microbial lipase production as a cost-effective process was highly effective in the lipase production by *Aspergillus niger* C BRMCTAA 82 as the enzyme activity level was up to 15.41 IU/mL were achieved under SSF with pH 4.0 and 6.5 and at temperatures between 37 and 55 °C. The produced enzyme was thermally stable up to 60°c (Silveira *et al*., 2016). The ternary mixture of Wheat bran, soybean meal, and cottonseed meal showed 320 U $g^{-1}$ of lipase production by *Aspergillus niger* at 72 hours along with other enzymes like CMCase and α-amylase (Ohara et al., 2018). The highest lipolytic activity by *Aspergillus niger* isolated from an oil sample of a vegetable refining company was achieved with a mixture of rice bran and glycerol (19.844 U $g^{-1}$). The medium with rice bran only resulted in 13.267 U·$g^{-1}$ of enzyme production stating that the enzyme could be produced without any additional carbon source (Costa et al., 2017). *Aspergillus niger* mutant 11T53A14 with the best lipolytic activity using wheat bran as substrate was selected and three industrial by-products from corn oil refining (soapstock, stearin, and fatty acids) were evaluated as substitutes to the olive oil, and the highest lipase activities using soapstock, stearin, and fatty acids were 62.7 U/gds, 37.7 U/gds, and 4.1 U/gds, respectively (Damaso et al., 2008). The successful production of lipase by *Aspergillus niger* using coconut pulp waste as substrate was achieved by adding spore concentration inoculum $1x10^6$ spore.$mL^{-1}$ in acidic condition at 30ºC for 7 days and resulted in 72.5 mL of crude enzyme with lipase activity 10.83 U.$mL^{-1}$ and protein content 0.074 mg/$mL^{-1}$ (Suyanto et al., 2019). The groundnut cake proved to have the potential to be used as an alternative cost-effective substrate for lipase production as it supported maximum lipase production (74.117 U/ml) in Arctic sediment isolate *Stenotrophomonas maltophilia* followed by coconut cake (61.911 U/ml), neem cake (58.737 U/ml), wheat bran (49.614 U/ml) and rice bran (28.165 U/ml) (Neethu et al., 2015).

Three fungal strains *Aspergillus flavus*, *Trichoderma harzianum*, *Penicillium chrysogenum* were able to grow and produce lipase in both culture media; wheat bran-olive oil, and wheat bran-castor oil cake in which *Aspergillus flavus* showed maximum lipase activity of 121.35 U/gds which was five and nine times the lipase produced by *Trichoderma harzianum* and *P. chrysogenum* under a neutral pH and 96 hours of fermentation period (Toscano et al., 2013). The lipase production of *Aspergillus ibericus* maximized with olive pomace (OP) and wheat bran (WB) as substrates and a low C/N ratio of 25.2 and 10.2% (w/w) lipids in the substrate, by the mixture of OP: WB (1:1) and supplemented with 1.33% (w/w) $(NH4)_2SO_4$ (Oleivera et al., 2017).

Mango seed and peel, wastes from the industrial processing of the fruit, were evaluated for lipase production by *Yarrowia lipolytica* under submerged fermentation and the mango tegument with yeast extract showed promising results for lipase production achieving around 3500 U/L (Pereira et al., 2019). The lipase production by *Yarrowia lipolytica* isolated from the estuary, showed the highest activity of 72.6 ± 2.4 U/g when using canola cake and olive oil as a substrate. The same isolate produced lipase from soybean meal after 14 h of fermentation reached the highest value of 93.9 ± 2.9 U/g using olive oil as substrate (Souza et al., 2017).

Among a group of forty fungal strains screened for exogenous lipase activity, *Penicillium chrysogenum* was selected for its best enzymatic activity which was then undergone comparative study with various substrates. Wheat bran showed the highest enzyme activity of 460 units per gram of dry fermented substrates (U/ gds), but the combination of wheat bran and black gram husk (1: 1) resulted in a higher enzyme yield of 650 U/ gds after 7 days of incubation with 40 % of initial moisture content of the substrate at pH 9 (Rajeswari et al., 2011). Agro-industrial wastes such as sesame oil cake, groundnut oil cake, and coconut oil cake were used for the solid-state fermentation for lipase production by *Candida rugosa* NCIM 3462 and maximum lipase activity of 22.40 U/g substrate was obtained using sesame oil cake which was 1.8 times the maximum activity obtained during the initial screening of variables by 'one-factor-at-time' approach. Employing RSM (Response Surface Methodology) for the optimization revealed that the temperature and substrate-to-moisture ratio were 32.3 C and 1:3.23 g/ml (Rajendran and Thangavelu, 2013).

Among different residues used as substrates such as rice bran, wheat bran, canola seed oil cake, sunflower hulls, and peanut shells, the canola seed oil cake showed the highest lipase activity in *Penicillium fellutanum.* A maximum lipase activity of 521 units/gram dry substrate (U/gds) was obtained after 48 h of reaction time in a media containing 10 g canola seed oil cake as a substrate supplemented with 2% olive oil as inducer and 50% moisture content at an initial pH of 4.0 using 2 mL inoculum at 30°C (Amin and Bhatti, 2014). The enzyme

profile of *Pleurotus ostreatus* showed the highest activity of lipase on canola oil seed cake as a substrate under SSF conditions among other substrates like cotton-oilseed cake, linseed-oil cake, sesame-oilseed cake, rice bran, and wheat bran. Under optimized conditions of moisture level, 50.0%; pH, 4.0; temperature, 30 °C, and olive oil, 2.0% after 72 h of incubation period the enzyme activity was improved up to 1.6 folds with respect to the original enzyme activities (Rehman *et al*., 2019). A 1:2 mixture of castor oil cake and sugarcane bagasse supplemented with 1 % (v/w) olive oil showed the best results for lipase production in *Trichoderma harzianum* under SSF (Coradi et al., 2013).

To achieve maximum lipase production by *Penicillium roqueforti* ATCC 10110 through SSF using cocoa bran residues (5g) as substrate fermentation time (0 to 120 h) and palm oil (PO) percentage (0 to 50%) were optimized through analysis of one factor at a time (OFAT), with lipase activity as the response. For a 72 h incubation, the highest enzyme activity achieved using SSF without adding PO was $14.67 \pm 1.47$ U $g^{1}$, whereas, with PO (30%), it was $33.33 \pm 3.33$ U $g^{1}$, thus demonstrating a 44% increase in enzyme activity (Araujo et al., 2022).

Large-scale production of *Aspergillus niger* lipase was found to be enhanced by using solid-state fermentation method and utilizing agro-industrial waste rice bran and Jatropha seed cake for 5 days with variations of inducer and extractant. Also, 1% of olive oil was found to be the best inducer, yielding dry lipase extract with the highest activity unit (176 U/ml enzyme) (Putri et al., 2020).

For the enhanced production of lipase by *Penicillium notatum,* different agricultural wastes including canola oilseed cake, sesame oilseed cake, linseed oil cake, cotton oilseed cake, rice bran, and wheat bran were used as substrates under solid-state fermentation conditions. Among these, the canola oil seed cake proved to be the best substrate, and maximum lipase activity of 5335 U/g dry substrate was observed after 96 h of incubation period using 60% moisture content, at pH 5, incubation temperature of 30°C and olive oil of 3%. The optimization of conditions led to a two-fold enhancement of lipase activity as compared to the initial enzyme activity (Rehman et al., 2011).

Using different substrates that combine solid and liquid waste; two mixtures were applied for the lipase production *Penicillium roqueforti* ATCC 10110 under SSF. Mixture I consisting of cocoa residue, solid palm oil residue, and liquid palm oil residue, and mixture II cocoa residue, mango residue, and palm oil residue liquid palm was applied. And production points of $6.67 \pm 0.34$ U $g^{-1}$ and $6.87 \pm 0.35$ U $g^{-1}$ were obtained from mixture I and II stating that the combination of liquid and solid residues in fermentation processes can be successfully done (Menesez et al., 2022).

Imperfect fungi *Geotrichum* had better potential to produce lipase 17 $U.cm^{-3}$ and 11 $U.cm^{-3}$ using corn steep liquor and soybean oil than other fungal and yeast isolates from the Brazilian rainforest (Maldonado et al., 2014).

Lipase production media of *Bacillus amyloliquefaciens* isolated from environmental samples was optimized with cheap agro-industrial wastes and best results were obtained with agro-industrial waste with optimum enzyme production as 41.20 U/mL in soya bean extract, 25.87 U/ml in rice bran, 25.83 U/mL in wheat bran, 30.03 U/mL (sunflower) and 32.23 U/mL in oat bran (Mazhar et al., 2023).

Among the total 14 bacterial strains isolated from soil samples *Bacillus subtilis* strain NL-39 which was selected based on the best lipase production when supplemented with soya bean meal gave maximum enzyme activity (34.93 U/ml) under SSF. The lipase production by other low-cost agro-industrial waste, including sunflower meal, wheat bran, oat bran, rice bran, and sugar cane bagasse was 23.83, 12.17, 10.40 10.00, and 16.23 U/ml, respectively. The lipase production by *B. subtilis* using peels of different fruits, including banana, orange, watermelon, and melon as carbon sources, was 27.17, 21.37, 10.57, and 8.43 U/ml, respectively. The corn cob produced 12.27 U/ml while waste oils of various industries produced 16.17 U/ml (Shan oil), 13.67 U/ml (automobile), 13.37 U/ml (unbranded waste cooking oil), and 6.03 U/ml (Sitara oil) of enzyme activity when used as substrate for lipase production. Among the agro-industrial wastes, fruit peels, and waste oil samples used in this study, the maximum lipase production was observed with soya bean meal, banana peel, and Shan oil waste (Mazhar et al., 2016).

*Micrococcus roseus* isolated from soil samples of Gangotri glaciers, Western Himalayas were supplied with different agro wastes as substrates for producing cold-active lipase in semisolid state fermentation. Among various substrates tested, groundnut oil cake (GOC) favored cold-active yield of lipases at 15 ± 1◦C within 48 h (Joseph et al., 2011).

Production of lipase by a novel rumen bacterium, *Pseudomonas* sp. strain BUP6 on agro-industrial residues showed higher lipase production when grown in Basal salt medium (BSM) supplemented with oil cakes. Among the five oil cakes; coconut, groundnut, cottonseed, gingelly, or soybean, groundnut cake supported the maximum lipase production (107.44 U/gds) (Faisal et al., 2014).

Optimization of lipase production by *Bacillus subtilis* PCSIR NL-38 strain with rape seed oil cake as substrates indicated that the strain showed maximum lipase production at pH 7 with $NH_4NO_3$ as inorganic nitrogen source, glucose as carbon source, $FeSO_4.7H_2O$ as salt, with 7% inoculum size and 96 hours of incubation (Abbas et al., 2017).

The utilization of olive mill wastewater for its possible application as a growth medium for lipase production by microbial strains *Geotrichum candidum* (NRRL Y-552 and Y-553), *Rhizopus arrhizus* (NRRL 2286 and ISRIM 383), *Rhizopus oryzae* (NRRL 6431), *Aspergillus oryzae* (NRRL 1988 and 495), *Aspergillus niger* (NRRL 334), *Candida cylindracea* (NRRL Y-17506) and *Penicillium citrinum* (NRRL 1841 and 3754, ISRIM 118). *C. cylindracea* NRRL Y-17506 showed the highest lipase activity on all the typologies of OMW used. Its lipase production on OMW was markedly affected by the type of nitrogen source and was induced by the addition of olive oil. The highest activity (9.23 IU $ml^{-1}$ ) of the yeast was obtained on OMW supplemented with NH4Cl (2.4 g $l^{-1}$ ) and olive oil (3.0 g $l^{-1}$ ) (Annibale et al., 2006).

Lipase production by *Diutina rugosa* when compared with synthetic media containing glucose, peptone, yeast extract, oleic acid, and ammonium sulfate and alternative media with agro-industrial residues, such as molasses, corn steep liquor (CSL), and olive mill waste (OMW); highest enzyme production was reported on the latter medium. Also, the lowest UPC (media cost per unit of lipase produced) was obtained when the agroindustrial residues were combined and used at the improved concentrations (Freitas *et al*., 2021). Residual biomasses babaçu mesocarp and rice husk showed immobilization efficiencies greater than 98% on lipase enzyme produced by *Thermomyces lanuginosus* (TLL) (Lira et al., 2021).

The enzyme activity of *Bacillus subtilis* strains LB1a and LB5a indicated that cassava wastewater as a substrate was better than the synthetic medium for enzymes like amylases and proteases. However, the lipolytic activity in cassava wastewater was always lower than in the synthetic medium during the whole fermentation process for both microbial cultures (Barros et al., 2013).

The production of lipase with poultry fat hydrolysing ability by *Candida viswanithii* was done economically and cheaply using mixed wheat bran plus spent barley grain (1: 1, w/w) supplemented with 25.0% (w/w) olive oil which thereby increased the production up to 322.4%, compared to the initial conditions (Almeida et al., 2016).

Agro- industrial wastes including tofu dregs, coconut dregs, and corn bran were used for the solid-state fermentation of extracellular lipase from *Aspergillus niger.* Lipase with the highest activity level (8.48 U/mL) obtained using a tofu dreg substrate was immobilized and assayed by a biodiesel synthesis reaction which resulted in a 48.3% yield (Aliyah et al., 2016). The production of biofuel using thermostable bacterial lipase from hot spring bacteria *Bacillus licheniformis* out of olive oil cake a low-cost agricultural residue yielded a 66.5% yield of biodiesel (Sahoo et al., 2018).

An alkaline lipase was produced from solid-state fermentation of soybean flour by lipase producer strain of *Enterobacter aerogenes* which enhanced the washing efficiency when incorporated in detergent formulations (Mitra et al., 2010).

Lipase from *Aspergillus niger* MYA 135 by submerged fermentation using a combination of agro-industrial raw materials and waste products as medium components showed biodiesel formation with the addition

of butanol. A three-stepwise addition of butanol, a biodiesel conversion of 57.32 and 95.89% was reached in the presence of free and immobilized biomass (Regner et al., 2019).

On substituting tributyrin with SDOCE (Sal (Shorea robusta) deoiled seed cake extract) lipase production by bacterial isolate *Aeromonas* sp. S1 isolated from the dairy industry showed a 24-fold (195 $UmL^{-1}$) compared with the initial 8.13 U $mL^{-1}$ lipase activity. The isolate when checked with the dairy wastewater treatment showed a reduction in chemical oxygen demand by 93%, oil, and grease (O&G) by 75%, and total suspended solids (TSS) by 47% after 96 h of treatment (Mahdi et al., 2012).

The lipase production by *Aspergillus melleus* under solid-state fermentation showed the highest enzyme activity in canola seed oil cake amongst other agro-industrial residues. The isolated lipase (alkaline) characteristics indicated that the enzyme could be used in soaps and detergent manufacturing industries (Amin et al., 2014).

Three kinds of low-cost agro-industrial wastes sugarcane molasses, waste cooking oil, and crude glycerol were tested as substrates for the simultaneous production of lipase and single cell protein (SCP) in which Sugarcane molasses appeared as the most effective cheap medium, allowing the production of 16420 U/ml of lipase and 151.2g/L of single cell protein at 10-liter fermentation scale (Yan et al., 2018).

## CONCLUSION

The utilization of agro-industrial residues as substrates for large-scale enzyme production has been prevalent, primarily driven by the higher costs associated with enzyme production. The production of lipase enzymes by microorganisms such as bacteria, fungi, and yeast was observed to be maximized through the utilization of low-cost and inexpensive residues. Furthermore, when compared to commercially available synthetic mediums, the activity of the lipase enzyme was demonstrated to be enhanced in the alternative agro-residue medium. The efficiency of immobilization was also noted to be improved with its utilization, emphasizing its impact on cost-effective enzyme production and its effectiveness in large-scale industrial preparations.

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