Turning waste into valuable products: utilization of agro-industrial oily wastes as the low-cost media for microbial lipase production

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Abstract. The cost-effective production of lipase by microorganisms are currently needed. The bioconversion of agro-industrial oily wastes as an alternative oily substrate for lipase production in the form of solid-state fermentation (SSF) or submerged fermentation (SmF) would hold a potential role in future biotechnology. There are some studies revealed that yeast, fungi, and bacteria are the choice for lipase production such as from the genera Aspergillus, Penicillium, Rhizopus, Candida, Geotrichum, Stenotrophomonas, Lasiodiplodia, and Yarrowia, as well as Staphylococcus, Burkholderia, Enterococcus, Pediococcus, Pseudomonas, and Bacillus utilizing several residues such as oil cakes and oily wastewater. In this review, the microbial lipase production conducted by many scientists utilizing agro-industrial oily wastes as the substrates is summarized.

1. Introduction

In developing countries, agricultural and oil residues are produced in large quantities. However, the utilization of these wastes is still limited. Lipids, including fats, oils, and greases, are the abundant organic matters found in some agro-industrial and oily wastes [1]. These organic compounds can cause problems in the environment since the biological processes are strongly influenced by high concentrations of oil and fat [2].

Recently, the agro-industrial oily wastes are utilized in biotechnological processes, especially microbial growth [3, 4]. The lipidic wastes with abundant biomolecules could serve as a rich media with significant residual nutrients for microbial cells and lipase production [5]. The agro-industrial oily residues could be in the state of solid and liquid. Therefore, two types of fermentation technique, i.e., the solid-state fermentation or SSF, and submerged fermentation or SmF could be useful for lipase production [1, 6]. Several attempts in SSF and SmF utilizing the oily wastes for microbial lipolytic enzyme production have been successfully conducted [1, 5-19]. The oily agro-industrial residues were
used as an alternative substrates by SSF and SmF and the results show positive benefit from using the wastes as a means of valorization and low-cost operation [20].

In fact, over the last decade, the number of published articles related to lipase-producing microorganisms isolated from oil-contaminated sources, lipase production optimization, and its application of lipase in the field of biofuel production, waste treatment, and waste valorization have been increased progressively. The below sections highlight numerous contributions to lipase production utilizing the agro-industrial oily wastes as the substrate made by many scientists applying different approaches.

2. Review of Literature

Lipases (Triacylglycerol hydrolases, EC 3.1.1.3) are lipolytic enzymes that involve in the catalysis of hydrolysis of triglycerides into free fatty acids and glycerol. In the presence of organic solvents, it also catalyzes the synthesis of esters [21-22]. Lipase can reverse the reaction leading to esterification. It facilitates the formation of glycerides in the presence of water. Lipid has the characteristic of low solubility in water; therefore, lipase function the catalysis at the interface in between the substrate and the water, where enzymes are dissolved [23]. In addition, the lipolytic activities are performed mostly in long-chain fatty acids with the feature of acyl chains number is more than ten C atoms [22, 24].

Besides, lipases can perform transesterification and esterification reactions when present in non-aqueous media with high specificity and enantioselectivity for these reactions [25-30].

Based on the value in industries and enzyme market, lipases are known as the third-largest group after proteolytic and carbohydrases. This enzyme also performs a vital role in the food industry, baking, detergent formulation, pharmaceutical industries, leather processing, paper manufacturing, bioremediation, waste treatment, biocontrol agent, and biofuel production [31-33]. The use of lipase enzymes in bioapplication to replace chemical catalysts benefits from being more environmentally friendly and safer [25, 34, 35].

2.1. Source of lipases

Lipase enzymes from plants have been widely isolated and have been identified in plant tissue, such as seeds and plant saps [36]. Lipase enzymes are also produced by animals and widely isolated from insects, fish, and mammals. In mammals, lipases are found in the pancreas and the surface of the gastric mucosa's mucous cells. Whereas in insects, it is found mostly in plasma, salivary glands, venom, muscles, and abdomen [37-38]. Among the lipase producers, microorganism enzymes have more advantages than enzymes derived from plants or animals. Microorganisms have a pattern of rapid growth, genetically modifiable, and easy for controlling the environmental factors. Microbial lipases have broad substrate specificity where it shows high stability in organic solvents, low production costs, and high enantioselectivity [39-41].

Lipases can be produced from microbes on a large scale, and some essential lipases are produced by bacterial genera including Burkholderia, Streptococcus, Chromobacterium, Achromobacter, Alcaligenes, Bacillus, Arthrobacter, and Pseudomonas. Bacterial lipases have been employed in the production of several commercial lipase products, including Pseudomonas sp. such as Lipomax and Lumafast for detergent formulations. Other lipases such as Amano P, Chiro CLEC-PC, Chirazyme L-1, P-30, and PS have great potential in organic synthesis. Fungal lipases have been studied since the 1950s. The genera of lipase-producing fungi including Magnusiomyces, Saprochaete, Ophiostoma, Alternaria, Eurotrium, Mucor, Rhizomucor, Rhizopus, Aspergillus, Humicola, Rhizopus, Beauveria, Candida, Fusarium, Acremonium, and Penicillium. The yeast genera such as Saccharomyces, Pichia, Yarrowia, and Hansenula have also produce lipase [42-44].

2.2. Extracellular lipases

Most isolated microorganisms perform the natural lipid hydrolysis by extracellular lipase activities in the presence of oil or fat in the substrates. Various extracellular lipase-producing microbial strains were collected from oily habitats for further isolation procedures. The different sources include palm oil mill contaminated soil [44, 45], palm kernel cake [19], oil-contaminated soil [46-47], olive oil mill wastewater [48], olive oil, soybean oil, de-oiled seedcake milk cream [49], dairy effluent [50], dry-salted
They demonstrated an excellent lipase production with potential applications in the industrial field. Some microbial species were further characterized to produce extracellular lipase enzymes by fermentation processes using the low-cost substrates such as Jatropha curcas oil, molasses, coconut oil mill waste, and palm kernel cake [14, 19, 45, 53].

2.3. Cell-bound lipases

The membrane-bound or cell-bound lipases in the yeast cells or fungal mycelia are attractive since it could be applied directly in the fermentation processes. This type of lipases can save the laborious steps of the isolation, purification, and immobilization procedures. The cell-bound lipases are naturally immobilized and very high in its stability. The mycelia and cell membrane act as the matrix that naturally immobilize to protect the enzymes. Also, the preparation of cell-bound lipases by cultivation is more straightforward compared to the conventional extracellular lipases. Moreover, the recovery could be performed by one step of filtration [54-56].

To date, few filamentous fungi have been explored to produce cell-bound lipases in their mycelia, such as Rhizopus sp. [57], Aspergillus sp. [55], Penicillium sp. [56], and Mucor sp. [58]. Yeasts also can produce whole-cell biocatalysts to perform in the biodiesel production. Some cell-bound lipases from yeast cells can be produced in the cheap medium and could be applied in a whole-cell reaction [45]. Geotrichum sp. was reported as to act with high activity in hydrolysis and synthesis in the form of extracellular and cell-bound lipases [59]. Srimhan et al. [60] isolated Rhodotorula mucilaginosa P11189, a yeast strain from the oil-contaminated soil and sites. Besides, the cell-bound or membrane-bound and the intracellular lipases (whole-cell catalysts) have been widely applied in the food industries, pharmaceuticals, biodiesel, and chemical synthesis. The schematic representation of extracellular, cell-bound, or membrane-bound, and intracellular lipases is presented in Figure 1 [61-62].

3. Methods

3.1. Agro-industrial oily wastes as the substrate for lipase production

The agro-industrial oily wastes contain a high concentration of fat, oil, and grease (FOG). Industries such as food processing, fish processing, and vegetable oil refining report high FOG levels in their effluents. Also, the wastewater from the kitchen, restaurant, and food factory present high oil content. The term FOG are relatively broad, including oil from animal and vegetable, petroleum hydrocarbons, fatty acids, phenolic compounds, surfactants, and naphthenic acids [63]. When present in wastewater in high concentration, FOG naturally will be in the upper layer, around the flocs, and decrease the rate of oxygen transfer. FOG in wastewater are presented in the several forms based on size differences, i.e., free, dispersed, emulsified, and dissolved [63-64]. This high oil content is potential for further utilization in microbial cells and lipase production.

Lipase production by microbial strains is depended on medium composition, i.e., carbon source and nitrogen sources; and cultivation conditions such as pH, temperature, and shaking [39]. These carbon sources are the main factor for lipase activity expressions since lipases are an inducible enzyme. Lipase can be produced in the presence of oils, Tweens, triacylglycerols, hydrolyzable esters, bile salts, fatty proteins, and some sugars.
acids, or glycerol [1]. At present, the use of low-cost production media is attractive to reduce the production cost of lipase enzymes. Oily organic compounds are found in the wastes from the kitchen, palm oil mill, vegetable oil processing, and other industrial processes, which could be utilized as low-cost media to stimulate growth and lipase production by microorganisms at low prices.

3.2. Lipase production by fermentation techniques

The utilization of wastes as the media for enzyme production may also have a significant impact on reducing waste treatment costs and the adverse effect on the environment. Rigo et al. [65] studied the soybean meal as an alternative medium for extracellular lipase production by *Penicillium* spp. The agro-industrial residual cakes of oil extraction could also be an excellent source of carbon and nitrogen of enzyme production medium in the form of solid-state fermentation (SSF) [66]. Some studies in using agro-industrial wastes for lipase production have been conducted. These wastes include Jatropha cake for lipase production by *Pseudomonas aeruginosa* PseA [67]; the toxic and alkaline castor bean waste from biodiesel production by *Penicillium simplicissimum* [68]; mustard seed oil cake by *Aspergillus terreus* [8]; palm fiber by *Aspergillus niger* [3]; Jatropha de-oiled seed cake by *Candida parapsilosis* [69]; palm kernel oil cake and mixed with sesame oil cake by *Aspergillus ibericus* MUM 03.49 [18]; palm kernel cake (PKC) by *Rhizopus* sp. [19]; canola cake by *Yarrowia lipolytica* [17]; and coconut kernel cake by *Lasiodiplodia theobromae* VBE-1 [6]. Neethu et al. [5] employed *Stenotrophomonas maltophilia* to produce lipase from agro-industrial wastes such as rice bran, groundnut cake, neem, wheat bran, and coconut cake. The results showed that groundnut cake improved enzyme production at optimum level at a low cost.

Another type of fermentation for lipase production, i.e., the submerged fermentation (SmF) by utilizing oily liquid waste or wastewater have been conducted. The production of lipase in a culture supplemented with soybean molasses was performed by de Morais et al. [15] using *Candida rugosa* and *Geotrichum candidum*. The medium could increase enzyme activities of both yeast and mold species. The palm oil industrial waste, palm alkaline soap stock, was utilized as the substrate in SmF by *Aspergillus niger* lipase production [3]. Palm oil mill effluent (POME), an effluent from palm oil mill industries contained a high amount of nutrients for carbon, nitrogen, mineral and salt sources which can support microorganisms to grow [70]. The yeast *Candida cylindracea* was first reported to produce lipase at a considerable level from POME [1]. *Yarrowia lipolytica* is the yeast species that could convert POME to microbial lipid and cell-bound lipase for further application in environmentally friendly biodiesel production [16]. Besides the agro-industrial wastes, the used vegetable and cooking oils are also potential as the low-cost alternative substrate in SmF. The marine-isolated strain *Pseudomonas otitidis* was performed in lipase production under SmF using the cooked sunflower oil (CSO) with response surface methodology with 1,980 U/mL lipase production was achieved [11]. Waste cooking oil (WCO) was explored as the carbon source for yeast strain, *Yarrowia lipolytica* M53 [71], as well as *Yarrowia lipolytica* W29 (ATCC 20460) [4]. Waste cooking oil was also used as the basal media for lipase production by a bacterial strain, *Bacillus subtiliss* under SmF and supplementation of nitrogen source, inducer, and Ca$^{2+}$ ion [72].

4. Results and Discussion

4.1. Lipase production using oily agro-industrial wastes

The summary of lipase-production using oily sources and oily wastes as the substrates is presented in Table 1.

| Type of fermentation | Substrates          | Microorganisms             | Lipase production optimization method | Optimum conditions and main parameters | Maximum lipase activity | Ref  |
|----------------------|---------------------|-----------------------------|--------------------------------------|----------------------------------------|-------------------------|------|
| Solid-State Fermentation (SSF) | Soybean meal | *Penicillium* P58 and P74 | Urea and soybean oil supplementation | Soybean oil 1 g/100 g and urea 3 g/100 g | Up to 200 U/g | [65] |
| Castor bean waste | *Penicillium simplicissimum* | Plackett-Burman followed by central composite rotatable design (CCRD) | Inoculum level at 10$^7$ spores/g; particle size: 0–1180 lm; and initial moisture at 48.5% | 155.0 U/g | [68] |
| Type of fermentation | Substrates | Microorganisms | Lipase production optimization method | Optimum conditions and main parameters | Maximum lipase activity | Ref |
|----------------------|------------|----------------|--------------------------------------|----------------------------------------|-------------------------|-----|
| Winterization residue (WR) and raw sludge (RS) from the oil-refining facility | The native microbial community obtained from the wastes | N.A | N.A | 120,000 UA/g | 7 |
| Mustard oil cake (MoC) | *Aspergillus terreus* | One-factor-at-a-time (OFAT) | Initial pH of 6.0, temperature at 30 °C, 96-h incubation period, lactose as carbon source, and ammonium persulphate as nitrogen source and 80 % moisture content | 1566.67 U/mL | 8 |
| Groundnut cake | *Stenotrophomonas maltophilia* | One-factor-at-a-time (OFAT) | Groundnut cake, temperature at 28 °C, initial pH 6.0, and 60% moisture | 74.117 U/mL | 5 |
| Coconut kernel-cake | *Lasiodiplodia theobromae* VBE-1 | One-factor-at-a-time (OFAT), Response surface methodology (RSM)-central-composite experimental design | Initial pH 8.0, temperature at 30 °C, 66.4% moisture content, coconut oil (0.985% (v/v)), and 1.5% (w/v) Triton X-100 | 698 U/g | 6 |
| Palm fiber | *Aspergillus niger* | Response surface methodology | Initial pH at 4.0 and 6.5 and temperatures in between 37 and 55 °C | 15.41 IU/mL | 3 |
| Jatropha de-oiled seed cake | *Candida parapsilosis* | One-factor-at-a-time (OFAT) | 40 g substrate load, 66% moisture, inoculum level at 10⁴, initial pH 8.0 and temperature at 37 °C, olive oil and yeast extract | 1056.66 ± 2.92 U/g | 69 |
| Palm kernel oil cake (PKOC) | *Aspergillus ibericus* MUM 03.49 | Central composite design | 0.45 g of PKOC per g total substrate, 57% moisture, 6 days incubation | 460 ± 38 U/g | 18 |
| Palm kernel cake (PKC) | *Rhizopus* sp. | One-factor-at-a-time followed by face-centered central composite design | Inoculum level at 2% (v/w), olive oil as substrate at 2% (v/w), peptone at 0.6% (w/w), ethanol at 2% (v/w), 70% moisture, initial pH 10.0, temperature at 45 °C, and 72 h incubation. | 58.63 U/g | 19 |
| Canola cake and soybean meal | *Yarrowia lipolytica* IMUFJR50682 | Fractional Factorial Design and Central Composite Rotatable Design | Canola cake as substrate, temperature at 44 °C and pH 7.7 Soybean meal as substrate, temperature at 37 °C and pH 7.0 | 72.6 ± 2.4 U/g and 93.9 ± 2.9 U/g | 17 |
| Submerged Fermentation (SmF) | Tuna-processing waste | *Staphylococcus epidermidis* CMSTP12 | One-factor-at-a-time (OFAT) | Defatted fish meat at 2.5%, glucose at 2%, sodium chloride at 4%, and Tween 20 at 0.8% | 21.1 ± 0.327 U/mL | 73 |
| Palm oil mill effluent (POME) | *Candida cylindracea* ATCC 14830 | One-factor-at-a-time (OFAT) | Peptone at 0.45% (w/v), Tween 80 at | 20.26 U/mL | 1 |
| Type of fermentation | Substrates | Microorganisms | Lipase production optimization method | Optimum conditions and main parameters | Maximum lipase activity |
|----------------------|------------|---------------|----------------------------------------|---------------------------------------|------------------------|
| Soybean meal and soybean oil | *Burkholderia* sp. ZYB002 | One-factor-at-a-time (OFAT) | Ammonium sulfate at 1 g N/L, initial pH at 5.0 | 3353 ± 27 U/L | \[16\] |
| Fish waste (fish waste protein hydrolysate and fish waste oil) | *Enterococcus faecium* MTCC 5695 and *Pediococcus acidilactici* MTCC 11361 | Factorial design and Box-Behnken design using response surface methodology (RSM) | MTCC 5695: 5% v/v fish waste oil, 0.15 mg/mL fish waste protein hydrolysate, incubation 24 h, and MTCC 11361: 4% v/v fish waste oil, 0.15 mg/mL fish waste protein hydrolysate and incubation for 24 h | 1715 U/mL and 493 U/mL | \[10\] |
| Olive mill wastewater | *Yarrowia lipolytica* | Supplementation with nutrients | Olive mill wastewater, ammonium sulfate at 0.6 % w/v, yeast extract at 0.1 % w/v, maltose at 0.5 % w/v, olive oil at 0.3 % w/v, and peptone at 0.1 % w/v | 850 IU dm⁻³ | \[9\] |
| Cooked sunflower oil (CSO) | *Pseudomonas otitidis* | Plackett–Burman design and RSM | Incubation for 75 h, temperature at 35 °C, pH 7.5, dextrose at 1.25 g/L, and 4 % substrate concentration | 1,980 U/mL | \[11\] |
| Waste frying sunflower oil (WFO) | *Bacillus cereus* ASSCRC-P1 | 2-level Plackett–Burman design and followed by Box-Behnken design (BBD) | Waste frying oil at 80 g/L, KH₂PO₄ at 2 g/L, K₂HPO₄ at 2 g/L, peptone at 5 g/L, beef extract at 5 g/L, CuSO₄ at 0.001 g/L, NaCl at 0.5 g/L, MnSO₄ at 0.002 g/L, 2 ml/l triton X-100 and inoculum level 50 ml | 340 U/mL | \[12\] |
| Soybean molasses | *Candida rugosa* and *Geotrichum candidum* | Central Composite Planning (CCP) | Soybean molasses at 200 g/L, temperature at 27 ± 1 °C, pH 3.5. Incubation time for 12 h (C. rugosa) and 24 h (G. candidum) | 12.3 U/mL and 11.48 U/mL | \[15\] |
| Palm alkaline soapstock | *Aspergillus niger* | N.A | N.A | 10.46 IU/mL | \[3\] |
### 4.2. Effect of agro-industrial oily wastes media composition and culture condition on lipase production

#### 4.2.1. Effect of nitrogen sources

Organic and inorganic nitrogen sources hold the important role in lipase production. The inorganic nitrogen is easy to be utilized by the cells, whereas organic nitrogen is essential for cell growth and amino acid supply for cell metabolism and protein synthesis. For instance, Rigo et al. [65] used soybean meal for lipase production by *Penicillium* P58 and P74. Soybean oil and urea were added into the growth medium for lipase production yielding up to 200 U/g. Mustard oil cake (MoC) with the supplementation of ammonium persulfate produced 1566.67 U/mL lipase by *Aspergillus terreus* [8]. Rhizopus sp. performed lipase production at 58.63 U/g by utilizing palm kernel cake (PKC) supplemented by 0.6 % (w/w) peptone [19]. The lipase from *Candida cylindracea* ATCC 14830 was optimized in palm oil mill effluent (POME) medium supplemented by peptone 0.45 % (w/v) with the result of the lipase production at 20.26 U/mL [1]. Also, utilizing POME supplemented by ammonium sulfate gave 3353 ± 27 U/L lipase yield by *Yarrowia lipolytica* TISTR 5151 [16]. Another experiment is lipase production by *Yarrowia lipolytica* using olive mill wastewater as the substrate which was supplemented with ammonium sulfate (0.6 % w/v) and peptone (0.1 % w/v). After optimization, the lipase achieved 850 IU dm⁻³ [9]. Another report is lipase production optimization by *Lasiodiplodia theobromae* VBE-1 lipase production in a medium containing coconut kernel cake and coconut oil as the inducer. This medium gave the highest lipase production at 698 U/g.

#### 4.2.2. Effect of carbon sources

Cells are known to have carbon as a fundamental component. Many organic compounds in the form of triglycerides and ester-based detergents could induce lipase production in a system. Lipase production is relied on the carbon source type and its concentration. In this case, the oil in the wastes act as the carbon source to support microbial lipase production. In some cases, supplementation of additional carbon source is needed to support an optimum result of lipase production. For example, *Aspergillus terreus* produced lipase in mustard oil cake (MoC) containing medium with lactose as the carbon source, and it reached the maximum (1556.67 U/mL) within 96 h [8]. Venkatesagowda *et al.* [6] optimized *Lasiodiplodia theobromae* VBE-1 lipase production in a medium containing coconut kernel cake and coconut oil as the inducer. This medium gave the highest lipase production at 698 U/g.

#### 4.2.3. Effect of temperature and pH

Certain microorganisms can produce lipase, which is affected by various factors. Each lipase-producing microorganism needs specific fermentation conditions, especially temperature and pH. Proper selection of the temperature during the fermentation process, particularly in the initial phase of fermentation, is essential for fast cells reproduction. pH can trigger the shape of proteins by the disruption of the bonds in the protein structure. It is also responsible in the metabolic pathways. Enzyme fermentation needs the best pH condition to support the best enzyme action. It could be in an acidic, neutral, or basic pH which bend the protein into the fit shape to allow best fermentation processes. Sethi *et al.* [8] optimized the lipase production in mustard oil cake (MoC) by *Aspergillus terreus* using initial pH 6.0 and temperature at 30 °C which was effectively increased lipase production up to 1566.67 U/mL. Another experiment lipase production by *Stenotrophomonas*
maltophilia in groundnut cake medium could increase the lipase production at 74.117 U/mL with fermentation temperature 28 °C and pH 6.0 [5]. Initial pH at 8.0 and temperature at 30 °C gave a maximum effect on lipase production by Lasiodiplodia theobromae VBE-1 up to 698 U/g in coconut kernel cake medium [6]. The initial pH at 8.0 and temperature at 37 °C for lipase production fermentation by Candida parapsilosis using Jatropha de-oiled seed cake could increase the lipase production up to 1056.66 ± 2.92 U/g [69]. Rhizopus sp. gave maximum lipase production at 58.63 U/g in palm kernel cake (PKC) medium with an initial pH 10.0 and 45 °C within 72 h of fermentation [19]. Different medium also needs different temperature and pH value condition. For example, the canola cake medium was utilized for lipase production by Yarrowia lipolytica IMUFRJ50682 at 44 °C and pH 7.7, whereas using soybean meal was at 37 °C and pH 7.0. The maximum lipase productions were obtained at level 72.6 ± 2.4 U/g and 93.9 ± 2.9 U/g, respectively [17]. More acidic palm oil mill effluent (POME) medium with initial pH at 5.0 gave high lipase production by Yarrowia lipolytica TISTR 5151 at 3353 ± 27 U/ [16]. The acidic condition was also applied for lipase production optimization by Candida rugosa and Geotrichum candidum at pH 3.5 and temperature at 27 ± 1 °C [15]. Ramani et al. [11] investigated the lipase production (1,980 U/mL) utilizing cooked sunflower oil (CSO) by Pseudomonas otitidis. The maximum lipase production was obtained in fermentation condition at temperature 35 °C and pH 7.5.

4.2.5. Effect of inoculum level. The density of microbial inoculum is also an essential factor for lipase production. The inoculum level at low density could affect lipase's lower production since there is low microbial biomass. In contrast, the high level of inoculum may produce too much biomass and inadequate production of lipase. The highest lipase production using castor bean waste was achieved at 155.0 U/g from Penicillium simplicissimum with an inoculum concentration of 10⁷ spores/g [75]. Balakrishna et al. [69] investigated the lipase production optimization by Candida parapsilosis utilizing Jatropha de-oiled seed cake. The optimum lipase production at 1056.66 ± 2.92 U/g with 10⁵ inoculum size was reached. Another experiment of lipase production using palm kernel cake (PKC) by Rhizopus sp. gave maximum lipase production at 57.63 U/g by initial inoculum size 2% (v/v) [19]. Bacillus subtilis at 5 % (v/v) inoculum gave the highest lipase production at 4.96 U/mL using waste cooking oil and olive oil as carbon sources [72].

4.2.6. Effect of incubation time. Biomass and enzyme productions are closely associated as well as very important to the process. The incubation time of lipase production by microorganisms ranged from 24-48 h for bacteria and 72-120 h for yeast and fungi. The experiment of lipase production by fungal species, Aspergillus terreus, using mustard oil cake was optimum after 96-h incubation achieving 1566.67 U/mL [8]. The experimental design was applied for optimizing lipase production by Burkholderia sp. ZYB002. After 24 h-incubation, 22.8 U/mL enzyme productions was reached [74]. Another bacterial species, i.e., Enterococcus faecium MTCC 5695 and Pediococcus acidilactici MTCC 11361, produced maximum lipase activity after 24 h of fermentation time in fish waste oil and fish waste protein hydrolysate media [10]. Ramani et al. [11] investigated the optimization of lipase production in cooked sunflower oil (CSO) medium by Pseudomonas otitidis for 75 h. The optimum enzyme production at 1,980 U/mL was achieved. A short period of optimum lipase production was reached by Candida rugosa within 12 h in soybean molasses containing medium. In contrast, Geotrichum candidum needed 24 h to achieve maximum lipase production in the same condition [15]. A more extended period of lipase production was reached by Aspergillus ibericus MUM 03.49 in palm kernel oil cake (PKC) medium, a production of 460 ± 38 U/g of lipase was obtained after 6 days fermentation [18]. Another fungal species, Rhizopus sp., produced optimum lipase production in palm kernel cake within 72 h of fermentation at 58.63 U/g [19].

4.2.7. Effect of surfactant. Lipase reaction occurs at the interface in between substrate and enzyme. It depends on the lipid substrates in membrane bilayers and monolayers, oil-in-water emulsions, micelles, and vesicles [75]. In lipase activity measurement, it is not easy to perform this reaction since the enzyme acts on insoluble substrates. It needs high speed of shaking and surfactants to facilitate enzyme-substrate interaction both in solution and at interface. Surfactant can enhance the accessibility and availability of the substrates to the enzyme active sites. Triton X-100, Tween 80, or Gum Arabic as surfactant agents
could be added to the culture media and can enhance lipase activity [73]. Gum Arabic is popular to be used as an emulsifier which exhibits the lowest hydrophile-lipophile balance (HLB) value at 11.9. Triton-X 100 and Tween 80 have 13.6 and 15.0, respectively. A lower HLB value indicates lower water solubility of a compound. With lower solubility, it can be incorporated into the oil layer at the water-in-oil emulsion interface better than the high one. Therefore, it can perform an ideal oil-water emulsion stabilizer. Gum Arabic is an effective surfactant for the synthesis of R. mucilaginosa lipase [77]. Tween 20 could enhance the lipase production by Staphylococcus epidermidis CMST Pi 2 [78].

4.2.8. Effect of trace element. The lipolytic enzyme requires specific metal ions such as Mg$^{2+}$, Fe$^{2+}$, Ca$^{2+}$, Cu$^{2+}$, Co$^{2+}$, Na$^+$, K$^+$, Mn$^{2+}$, and Zn$^{2+}$ as the cofactor to increase the enzyme activity through attachment with the carboxylate side chain groups of aspartyls and the enzyme's glutamyl residues. Various trace elements were added and examined on lipase production by Staphylococcus epidermidis CMST-Pi 1, and the results revealed that all the supplied trace elements increased lipase production at 17.5 U/ml. Zinc sulfate-induced most lipase production (26.5 U/ml), whereas EDTA, increased lipase production at 18.63 U/ml [78]. Effect of trace elements on lipase production showed that only calcium chloride and magnesium sulfate have an inducing effect on lipase production by Staphylococcus cohnii AP-CMST in anchovy processing waste [79].

4.3. Strategies for improvement and optimization of lipase production

The optimization of lipase production is needed to achieve the maximum yield of enzyme and minimum cost of production. Choosing agro-industrial oily waste with abundant organic compounds and inducer is preferable and an efficient optimization and downstream processing methods are necessary [9, 12, 76]. As presented in Table 1, there are two common methods for lipase production optimization, i.e., the conventional and statistical methods of optimization. One variable at a time (OVAT) or one factor at a time (OFAT) is the conventional procedures of optimization and is described as altering one variable/factor at a time while all other parameters are kept constant [5, 8, 9, 16, 17, 65, 69, 71-73]. The conventional method does not measure the interaction among the factors. Therefore, a statistical experimental approach which can provide bioprocess plan and interactions among variables in the systems could be an answer to this problem. The statistical experiment are usually applied in the response surface methodology (RSM) and further developed in several types of design of experiments (DoE) such as Plackett-Burman design (PBD) [11-12, 68, 74]; central composite rotatable design (CCRD) [17, 19, 68]; central composite planning (CCP) [15]; Box-Behnken design (BBD) [10]; central composite design (CCD) [6, 18, 19]; and face-centered central composite design (FCCCD) [1]. Also, Taguchi method was developed as one of DoE [4]. The term of RSM is a collection of mathematical and statistical techniques for building the empirical model. RSM has the objective of optimizing a response (output), which is affected by several independent variables (input).

A combination of one-factor at a time (OFAT) and response surface methodology (RSM) was found to enhance the lipase production by some microorganisms effectively. RSM could be employed to study the interaction of the parameters which are obtained from the OFAT or Taguchi method. Also, RSM can overcome the limitation of OFAT in lipase production optimization. For example, the lipase from Candida cylindracea ATCC 14830 was optimized under the submerged fermentation of palm oil mill effluent (POME) by using a combination of OFAT, two-level Plackett–Burman (PB) design, response surface methodology (RSM) based on the face-centered central composite design (FCCCD) [1]. The optimum lipase production from Lasiodiplodia theobromae VBE-1 was achieved using OFAT and the RSM-central-composite design (CCD) using coconut kernel cake in solid-state fermentation [6]. OFAT, followed by a face-centered central composite design, was applied in the optimization of Rhizopus sp. Lipase production utilizing palm kernel cake (PKC) as the substrate [19].

5. Conclusion and future directives

Agro-industries oily wastes are commonly generated from food processing, fish processing, vegetable oil refining industries, and kitchen, restaurant, and food factories, which present high oil content in the wastewater. The abundant oil content in the agro-industrial oily wastes can be utilized as an alternative substrate for microbial lipase production by low-cost solid-state and submerged fermentation
techniques. The potential lipase producing microorganisms could be isolated from these oil-contaminated sites for further lipase production optimization process utilizing the wastes. Some factors, such as the oil content, nitrogen sources, carbon sources, pH, temperature, inoculum level, and incubation time, significantly affect the lipase production. Therefore, the physicochemical analysis on the oily wastes to obtain the information from its organic and inorganic compounds might be a critical point before further supplementation by another compound in the agro-industrial oily wastes media. Thus, efficient lipase production from wastes can reduce the enzyme production cost.

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